HUMAN GASTROINTESTINAL SURFACE pH MEASURED BY NOVEL ELECTRODE SYSTEMS

by

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To Ronald Richard Charles Rawlings,

I hope he would approve.

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DECLARATION

This thesis comprises my own original research. No part of this work has previously been submitted as a thesis in any form.

SUMMARY

Events occurring at the gastrointestinal mucosa have implications for the absorption of nutrients and the protection of the surface epithelium. In recent years, accumulating evidence suggests that the mucosal surface pH can differ from that of the bulk perfusing solution and that the surface pH is not necessarily the same in different areas of the alimentary tract. Measurements of surface pH have been performed by previous workers in animals both *in vitro* and *in vivo* and in human biopsy material. However, a comprehensive characterisation of gastro-duodenal mucosal surface pH in humans *in vivo* has yet to be published and no information exists at all about human *in vivo* jejunal mucosal pH. The purpose of this investigation was to measure gastrointestinal mucosal pH in humans *in vivo* and to compare results obtained from control patients and patients with gastrointestinal disease.

Existing measuring systems of gastrointestinal pH are specifically designed to measure luminal pH or are unsuitable for mucosal pH measurements. Consequently, to measure mucosal pH in humans *in vivo*, two novel measuring systems were employed. To measure gastro-duodenal mucosal pH, an electrode was passed through the biopsy channel of an endoscope. Using this technique, the electrode could be seen to touch the mucosa in specifiable areas when measurements were made at endoscopy. Proximal intestinal mucosal pH was measured by attaching a plastic pH electrode to a Crosby biopsy capsule. When swallowed, the capsule-electrode assembly allowed for continuous measurement of

intestinal mucosal pH and the retrieval of biopsy material for histological and biochemical analysis. A plastic pH electrode was required for use at jejunal biopsy because of the unsuitability of available glass electrodes. Two hydrogen ion-selective carriers were compared for their suitability as components in a plastic electrode for use in humans. The plastic pH electrodes were developed and constructed in the laboratory and the electrode properties were extensively characterised. The electrodes used at jejunal biopsy had comparable operational characteristics to glass pH electrodes within the physiological pH range.

Measurements of jejunal mucosal pH were obtained in 9 control, 13 coeliac and 11 irritable bowel syndrome (IBS) patients. A pH gradient ranging from near neutral in the duodenum to an acidic pH of 5.9 in the jejunum was measured in control patients. In contrast, coeliac patients had a significantly alkaline pH in the duodenum and a jejunal mucosal pH of 6.7, significantly less acid than control values. Coeliac patients on a normal diet had a near neutral mucosal pH while that of patients on a gluten free diet was still significantly elevated. In IBS patients, jejunal mucosal pH was not significantly different from control values. An elevated jejunal mucosal pH in coeliac disease may result from a loss of acid secreting villous tip cells and neutral or alkaline secretion from the increased numbers of crypt cells associated with coeliac disease. There may also be a contribution from increased tissue permeability.

Measurements of gastro-duodenal mucosal pH revealed that acid in the stomach had a significant influence on the

recorded pH. Without gastric acid (pH>3), the mucosal pH was close to neutrality from the fundus to the duodenum in control patients. In contrast, in the presence of acid (pH<3), fundal and antral mucosal pHs were significantly more acidic than neutral. Duodenal pH was unaffected by the presence of acid in the stomach. In patients with gastric ulcer, the antral surface pH was near neutral in the absence of gastric acid and remained elevated even when acid was present. In gastric ulcer, a more alkaline antral surface pH was also associated with active ulceration but less so with healing ulcers and with gastritis without ulceration. Similarly, duodenal ulcer was associated with a strikingly alkaline duodenal mucosal pH. Once again, acid in the stomach accentuated the elevation in duodenal mucosal pH when compared with control values.

It is proposed in the cytoprotection hypothesis that the mucosa can maintain a neutral surface pH in the presence of acid and that peptic ulcer patients have a reduced capacity to maintain this neutral layer. From the results obtained in this project, it is proposed that it is normal for acid to dissipate the "mucus-bicarbonate" layer and reduce mucosal pH. In addition, peptic ulceration is associated with alkalinity in the affected area and not a reduced mucosal pH as predicted. An hypothesis is presented which suggests that this alkalinity is inappropriate and may have pathological consequences for subsequent control of acid secretion, gastric emptying and pancreatic bicarbonate secretion.

ABBREVIATIONS

ATP	adenosine tri-phosphate
ERCP	endoscopic retrograde cholangio-pancreatography
GFD	gluten-free diet
IBS	irritable bowel syndrome
id	internal diameter
im	intra-muscular
iv	intra-venous
NaTPB	sodium tetraphenylborate
ND	normal diet
OCPH	p-octa-decyloxy-m-chlorophenylhydrazone-mesoxalonitrile
od	outside diameter
o-NPOE	o-nitrophenyloctylether
pCO_2	partial pressure of carbon dioxide
pK_a	negative logarithm of dissociation constant
pH	negative logarithm of hydrogen ion activity
PVC	polyvinylchloride
r	regression coefficient
TDDA	tri-n-dodecylamine
Tris	tris(hydroxymethyl)aminomethane

PREFACE

The main objective of this project was to measure the pH at the mucosal surface of the human stomach and proximal small intestine *in vivo*. The reasoning behind this objective originates from recently revived interest in mucosal events associated with gastrointestinal diseases. Measurements of mucosal surface pH may lead to a better understanding of how diseases affect the mucosal tissue. Maintenance of the pH of the mucosal surface has implications for absorption of dissociable weak-electrolytes and for the protection of the stomach and duodenum. Alterations in surface pH would therefore disrupt the normal functions of the mucosa.

However, the majority of devices used for gastro-intestinal pH measurements are inadequate for measuring mucosal surface pH. Through necessity therefore, some of the project was devoted to developing suitable systems for measuring *in vivo* mucosal surface pH during routine clinical investigations. For this reason, the format of this thesis is rather unconventional. The format is intended to lead the reader through each separate stage of the project so that a clear picture of the overall task is gradually created. This is hopefully achieved by initially describing the clinical relevance of the work, followed by an analysis of why existing measuring systems cannot adequately fulfill the requirement of measuring mucosal surface pH. This progresses into a detailed section on plastic pH electrodes which are necessary components of one

of the devised measuring systems to be used. It is after this stage that the clinical experimental work, in which mucosal surface pH is measured, is described and the findings from these studies are detailed in a clinical results section. Finally, the suitability of the selected measuring systems is discussed and the findings from the pH measurements are put into perspective with particular reference to the physiology of the gastrointestinal tract and to what extent mucosal events can be altered and possibly participate in gastroïntestinal disease.

Although an attempt has been made to separate developmental and experimental work, some overlapping of these aspects is unavoidable; the reader's forbearance would be appreciated during such instances.

1 i) The Clinical Context

The gastrointestinal mucosa is lined with mucus which, as well as acting as a lubricant, is thought to be a protective layer against tissue damaging substances. Accumulating evidence suggests that this mucus layer can have a different pH to that of the gastrointestinal bulk contents and that the surface pH is not necessarily the same in different areas of the alimentary tract. It is not known what happens to the pH of this mucus layer when disease affects the mucosa. Peptic ulceration and coeliac disease are two instances where knowledge of the mucosal surface pH may be an important factor in understanding what happens to the tissue during gastrointestinal disease. It is perhaps appropriate to examine the evidence which suggests that the surface pH might be of importance in peptic ulceration and coeliac disease.

i a) Peptic ulcer disease and gastric acid.

The search for the cause of peptic ulceration has centred on two major questions: a) is there a change in the aggressive factors thought to be responsible for ulceration and b) do the defence mechanisms responsible for protecting the mucosa alter in any way which may allow ulceration to occur? These are not necessarily exclusive mechanisms. The relationship of acid to ulceration has proved to be easier to investigate than the link between mucosal defence mechanisms and ulceration. This is only because technological developments have not kept pace with

advancing scientific ideas. What follows is a brief introduction to some of the ideas concerned with gastric acid which have been suggested as aetiological factors in the development of ulceration.

If a patient with peptic ulcer disease was asked what he thought was the possible cause of his condition, his answer may well be that he was secreting too much acid. However, when measurements of basal (1) and stimulated maximal (2) acid secretion from control and peptic ulcer patients were compared, there was considerable overlap between groups. In cases of gastric ulcer, acid secretion is often similar to or lower than normal (2,3,4,5,6) . Acid secretion decreases with age (2,3) and this, associated with the increase in incidence of gastric ulcer with age (2), may possibly explain the lower levels of acid recovered in some gastric ulcer patients. Only one third of duodenal ulcer patients have acid hypersecretion (2,3,4,5,6) and whether or not duodenal ulcer patients with high acid output had such high levels before ulceration occurred is uncertain. Evidence (2,7) suggests that when the ulcer first develops, the acid secretion is normal but increases as the disease progresses. Therefore an increased level of acid secretion is unlikely to be a prerequisite for mucosal damage although it is accepted that acid must be present at some stage, since patients with pernicious anaemia never develop peptic ulceration. While suppression of acid secretion is an effective clinical treatment for ulceration, this does not answer the more physiological question of whether excess acid prior to ulceration is

instrumental in causing the ulceration. In short, there is no clear picture of acid secretory capacity before, during or after gastric and duodenal mucosal ulceration.

As well as studies of acid secretion, there have been investigations of the acid neutralising capabilities of the stomach and small intestine. The idea that the persistence of peptic ulcer was due to an imbalance between gastric acid and the alkaline secretions of the duodenum was proposed in 1929 (8). This suggestion encouraged others to compare gastric and duodenal secretions, initially by aspirating fluid samples (9,10) and latterly by measurement with glass pH electrodes (11,12,13,14,15,16,17,18,19,20, 21).

The reports stemming from this type of study are not consistent in experimental technique and there are many conflicting results. There are discrepancies about the relationship between acid secretory capacity and duodenal pH. In one study (9) no parallel relationship between acidity in the duodenal bulb and any of the customary indices of gastric acidity could be found. However, a positive correlation between peak acid output and log mean hydrogen ion activity in the proximal duodenum has been observed in more recent studies (20).

A similarly inconclusive situation exists concerning duodenal bulb pH and neutralising ability. Some investigators suggested that the first portion of the duodenum is more acid for longer periods of time in duodenal ulcer patients (9,22,23). In contrast, no

differences in duodenal bulb acidity have been found by later workers (16,17,23). Although finding no significant differences in duodenal mean pH between patient groups, M^{c}Cloy (21) did notice that there were significantly longer periods of acidification of the duodenal bulb in ulcer patients.

The technically difficult task of maintaining electrodes or aspiration tubes in the duodenal bulb is a major obstacle to obtaining reproducible results. It is not too difficult to maintain measuring devices at fixed positions within more distal regions of the duodenum. The pH in the distal duodenum is more stable than that in the duodenal bulb. However, this is not the area where duodenal ulcers frequently occur. Correspondingly, there is a greater consensus of results which indicates that there is no difference in pH between control and duodenal ulcer patients (13,17,24,25). This does not imply that a difference does not exist in the bulb, only that neutralisation is complete in the distal part of the duodenum.

Measurements of duodenal bulb pH have not conclusively indicated whether or not there is an imbalance between gastric acid and duodenal alkalinity in duodenal ulcer disease. If there is an excess of acid entering the duodenum, it must be derived from increased acid secretion, an increased rate of gastric emptying, or a combination of the two. Considering that few patients with duodenal ulcer are hypersecretors of gastric acid, the possibility of the duodenum receiving acid at more frequent intervals may be a

more reasonable hypothesis. Compared to control patients, duodenal ulcer patients have an increased rate of gastric emptying (26,27), although this was not shown in earlier studies (28,29). An increased rate of gastric emptying could account for the longer and more frequent periods of acidity measured in duodenal ulcer patients (9,21,22).

An increase in the acidity of the duodenal bulb caused by an increased rate of gastric emptying is not what would be predicted from studies on the control of gastric emptying. Sufficient evidence exists to suggest that acid in the duodenum inhibits gastric emptying (4,30) and acid secretion (4,31). In dogs, the receptors mediating this inhibitory effect are in the first 5cm of the duodenum (32,33), although to-date, no anatomical evidence for their existence has been found. The inhibitory pathways may be neural (34,35,36,37,38,39), humoral (40,41) or both. The inhibition of gastric secretion may be mediated by secretin (41) released from the duodenum in response to acid impinging on the mucosa. If secretin regulates gastric emptying, the lower level of secretin release detected in duodenal ulcer patients (42) would perhaps explain the increased rate of gastric emptying in ulcer patients. Just why duodenal ulcer patients should have lower levels of secretin release has not yet been explained. We are left with the conclusion that while much work has been done with respect to gastric acid and peptic ulceration, little is still known about how these two aspects are related.

Secretin is also thought to be the humoral agent responsible for the control of bicarbonate secretion from Brunner's glands in the duodenum (43,44) and the secretion of bicarbonate from the pancreas (4,45). Consequently, secretin may not only control the duodenal environment by inhibiting acid secretion and gastric emptying but also by stimulating the mechanisms responsible for protecting the mucosa.

i b) Peptic ulceration and mucosal defence.

Mucosal defence against gastric acid relies on the acid being neutralised by bicarbonate. Bicarbonate is derived from pancreatic and biliary secretions into the duodenum and from mucosal secretions in the duodenum (46,47) and stomach (48,49,50,51). Measurements of pancreatic bicarbonate and gastric acidity output have once again produced conflicting results. It is agreed that bicarbonate secretion from the pancreas is more than adequate to neutralise acid output in control patients (52,53). In duodenal ulcer patients it is suggested (52) that 13 out of 15 subjects studied could neutralise peak gastric acid output. In contrast, others (53) have stated that although pancreatic output was normal in ulcer patients, maximal gastric acid was higher than in control patients. In a subsequent paper (54) it has been shown that even if bicarbonate secretion equals the amount of acid in the duodenum, this does not necessarily imply that neutralisation will be complete. In fact, neutralisation will depend on the concentration of acid and bicarbonate and on the partial pressure of CO_2 in the duodenum. Unless

all these factors are known, the value of this type of study is diminished.

The idea of the mucosa having its own protection against acid has been suggested repeatedly in the past (46,55,56) but it has only been in the last decade that the mechanism behind this protection has been studied in a concerted way. A "mucus-bicarbonate" barrier (50,57,58) which buffers intraluminal acid by maintaining a neutral pH at the mucosal surface is currently thought to be the means by which the mucosa prevents itself from being digested. Studies on frog gastric fundic mucosa *in vitro* (48) suggested that there was a low basal secretion of bicarbonate from the tissue. The guinea pig is also capable of producing basal gastric bicarbonate *in vivo* (49). The amphibian duodenum, *in vitro*, apparently secretes bicarbonate which has both an active and a passive component (59,60). It would appear that duodenal bicarbonate secretion is not restricted to amphibians for it has also been measured *in vivo* in rats, guinea pigs, cats, rabbits, dogs, pigs (61) and humans (62).

Microelectrode studies of gastric mucosal surface pH reveal that there is a gradient of increasing pH from the bathing medium to the tissue surface in rabbits *in vitro* (63), rats *in vivo* (64) and humans *in vitro* (65). The human studies are difficult to interpret because abnormal specimens were studied from patients given anti-motility and anti-secretory drugs. A similar pH gradient has been measured in rat duodenal tissue *in vivo* (66). Interestingly, the fact that the local duodenal mucosal pH

need not reflect luminal pH in dogs was reported as long ago as 1965 (67). More recently it has been shown in humans that patients secreting acid have a neutral duodenal mucosa (68) which remains so even when bathed with exogenous acid (69).

If a "mucus-bicarbonate" barrier exists in humans, an imbalance between mucosal protection factors and acid and pepsin impinging on the mucosa could result in mucosal damage (46). Ethanol, anti-inflammatory drugs and bile salts are well recognised initiators of mucosal damage (4,57,58). Their mode of action could be through breaking down the mucus lining of the mucosa and allowing acid and pepsin to attack the tissue. Such damage tends to be diffuse in nature and does not explain the localisation of peptic ulcers in certain common areas. Despite present knowledge of the mucosal protective system it is apparent that the mechanisms behind the initiation of a peptic ulcer are not fully understood.

On the assumption that an imbalance may exist between aggressive and protective mechanisms an obvious experiment is to measure the pH of the mucosa in normal and pathological conditions. If the pH at the surface of the tissue is less in ulcer patients than control patients, this would provide crucial evidence that an acid-alkali imbalance is present in peptic ulceration. To-date there have only been two studies (70,71) of gastric and duodenal mucosal pH in humans in relatively undisturbed conditions. In neither study could differences in mucosal pH be found

between normal and duodenal ulcer patients, although changes in gastric pH were noted in gastric ulcer and gastritis patients. It was for this reason that this project was undertaken to measure gastric and duodenal mucosal pH with the hope that a better understanding of mucosal defence mechanisms would emerge.

i c) Small intestinal disease and mucosal pH.

The second aim of the study was to look at how diseases of the small intestine affect mucosal surface pH *in situ*. When anastomosed to the stomach, the jejunum is highly susceptible to ulceration (72). The conclusion drawn from this observation is that, unlike the duodenum, the jejunum lacks adequate protection against gastric acid. Although ulceration of the jejunum is a rare occurrence in patients without a gastro-jejunostomy, it is still possible that diseases affecting the jejunal mucosa may alter the mucosal surface pH. Indeed, the *in vitro* pH of human jejunal tissue (73,74) is more alkaline in patients with coeliac disease than the surface mucosal pH of control patients. Coeliac disease is associated with changes in jejunal mucosal structure and absorptive function.

An altered jejunal surface pH, were this to be found *in vivo*, would help to explain certain clinical abnormalities associated with coeliac disease. Such abnormalities are typified by the following examples. The presence of steatorrhoea is a prime indication that a patient may have coeliac disease. The failure to absorb fatty acids, leading to steatorrhoea, may be the result of an abnormal mucosal surface pH. A precedent to this proposal is that fatty acid

absorption increases with decreasing luminal pH (75). Depending on the pK_a of the fatty acid, the pH at the mucosal surface will determine the extent of dissociation. With non-ionic diffusion, absorption across a lipid membrane is enhanced when the compound is in the un-ionised, or neutral, form (76,77,78). Hence, if a change in surface pH shifts the dissociation equilibrium of a fatty acid to the ionised species, the rate of absorption will be reduced and steatorrhoea will follow.

In accordance with the pH-partition theory of non-ionic diffusion described above, changes in mucosal surface pH would also explain folate malabsorption in coeliac disease (79). In a similar fashion to fatty acid absorption, folate absorption is highly pH dependent (80,81). Much of the initial *in vitro* work on jejunal surface pH (73,74,81,82) was done with reference to its influence on folate malabsorption.

Subsequently the idea of surface pH affecting the absorption of other compounds has been expanded to the field of weak-electrolytes taken in the form of drugs for the treatment of diseases unrelated to the digestive tract. The paradoxical reversal of rates of absorption of weak acids and bases in coeliac disease, compared to control patients (83,84,85), can also be attributed to the state of ionisation of the drug. The increased surface pH of coeliac tissue *in vitro* (73,74) suggests that weak bases will be preferentially absorbed while the absorption of weak acids will be reduced.

The measurement of surface pH in patients with coeliac disease and in control patients *in vivo* would show if *in vitro* observations (73,74) accurately reflect coeliac jejunum *in situ*. However, there have been very few *in vivo* studies of the pH of the distal duodenum and proximal jejunum in humans and such information as is available exclusively relates to luminal pH (18,80,86,87,88,89). For this reason, *in vivo* measurements of jejunal surface pH were made in patients with functional intestinal disorders.

1 ii) Existing pH Measuring Systems

So far, the clinical relevance of mucosal surface pH has been outlined. To test the idea that mucosal surface pH is altered in human gastrointestinal disease it is necessary to make *in vivo* measurements. However, an assessment of available measuring systems suggested none were suitable for the proposed study. To illustrate this point, what follows is an appraisal of these unsatisfactory options. It is not the intention of this introduction to review the numerous studies of gastrointestinal pH that have been published over the last hundred years or more. The history and development of gastric analysis prior to 1939 is outlined in an excellent review by Hollander and Penner (90,91,92) to which the interested reader is referred. The following review will therefore confine itself to technical developments in the last forty five years.

The use of oral or nasal intubation with aspiration of gastrointestinal content is a simple, prevalent method for examining gastric or intestinal secretions. Fluid aspiration has several disadvantages, the main one being that the pH of aspirated fluid reflects the situation prevailing in the lumen and does not allow inferences to be made about the mucosal surface pH. Aspirates can be mixtures of fluid of uncertain origin or from a specific region but with an unspecifiable degree of contamination from other areas. Continual removal of fluid may itself have an effect on subsequent secretions and this could be misleading.

An alternative which circumvents fluid removal is to place a dialysis bag containing distilled water in the stomach (93). Hydrogen ions then diffuse from the gastric secretion into the bag and dialysate hydrogen ion concentration is measured by titration after recovery from the stomach. A more complex system based on the same principle is the use of ion exchange resins (94). Resin, containing the dye azure A, will exchange molecules of the dye for hydrogen ions when they react with the resin. The azure A is absorbed into the blood and the amount of dye appearing in the urine is proportional to the amount of hydrogen ion in the gastric secretion. However, both of the above methods of measuring gastrointestinal pH again only provide a measure of the concentration of acid in the lumen.

In the methods described above, measurements of pH are made either at intermittent time intervals or only at the end of the experimental period. Continuous assessment of *in situ* pH is not possible with these systems. Consequently, any transient fluctuations in hydrogen ion secretion between the selected sampling intervals will not be recorded. In contrast, *in situ* glass pH electrodes provide fast, reliable and continuous recordings of gastrointestinal pH. The *in situ* electrode system has been used in many forms with varying degrees of success. Miniaturisation of the electrode and electrode recording equipment has been the most notable development in recent times. This has led to increasing patient tolerance of the system during investigative procedures. Apart from the

conventional glass pH electrode attached to a conducting cable, electrodes based on the principle of radiotelemetry have also been used for gastrointestinal pH measurements (95,96,97). Unlike conventional glass electrodes, the use of radiotelemetry devices has not found favour amoung research workers because they usually require lengthy pre-conditioning before use and are often unreliable.

Studies of *in situ* gastrointestinal pH using pH electrodes have been limited to the measurement of luminal content pH. Electrodes floating free in the lumen of the stomach or intestine may make contact with the mucosa at some stage, a fact recognised and prevented by past investigators (11,18,67). Even if the electrode does make contact with the mucosa the frequency and duration of contact is impossible to determine. The shape of the pH sensitive glass bulb will almost inevitably ensure that some contribution to the recorded value will come from the lumen. Thus, the recorded pH will be a composite of mucosal and luminal fluid pH. Finally, a further disadvantage of free floating electrodes is the problem associated with maintaining the electrode within the area of interest. The use of electrode combinations (16,17,19,20,98), although partially successful in determining the location of an electrode, are cumbersome and not particularly reliable.

From this brief discussion, it must be concluded that existing methods of measuring gastrointestinal pH are not suited for the measurement of mucosal surface pH *in vivo*. For the measurement of gastric and proximal duodenal mucosal pH a system where a pH electrode is taped to the

outside of an endoscope has recently been developed (Lucas & Holmes 1977, personal communication & ref 70). Development of miniaturised electrodes allowed passage of an electrode through the biopsy channel of the endoscope (71). The combination of fibre-optic flexible endoscopes and miniaturised pH electrodes is undoubtedly a useful development. This has finally allowed mucosal surface pH to be measured conveniently and with precision in specifiable areas. It has opened up new possibilities for investigating *in situ* mucosal conditions which, until now, have only been studied *in vitro* or have been implied from animal models.

The endoscope can only reach as far as the second part of the duodenum without causing severe discomfort. For this reason another means of delivering an electrode to the small intestinal mucosa was required. This was found in the form of an electrode attached to a Crosby biopsy capsule. With an electrode appropriately positioned, contact between the pH sensitive tip and the mucosa could be virtually ensured. The inflexibility and rounded pH sensitive tip of conventional glass electrodes made them unsuitable for use with the Crosby biopsy capsule system. A plastic, flexible electrode was a possible solution to these restrictions. However, plastic pH electrodes are not commercially available and therefore these had to be produced within the laboratory.

In summary, in order to make mucosal pH measurements *in situ*, two new measuring systems were required. While the endoscopy procedure for measuring mucosal surface pH has

previously been described, the Crosby biopsy capsule and electrode combination was an untested but potentially useful device for measuring small intestinal mucosal pH. Before describing these techniques in more detail, an account of the construction and characteristics of plastic pH electrodes, which were eventually used at jejunal biopsy, must first be presented.

2 i) Introduction

Although there is an extensive literature on ion-selective electrodes, most of the reported work has been orientated to the theoretical aspects and the application of these electrodes to analytical chemistry. Less frequently encountered in the literature are designs for electrodes suitable for clinical investigations. Some efforts have been made at applying electrode technology to biological and clinical problems, particularly in the areas of hydrogen (99,100,101), potassium (102,103), calcium (104), sodium (105) and methadone (106) ion determinations. All these electrodes have been successful for their particular purposes but none were refined enough for clinical applications, particularly for *in situ* work on humans. Such an electrode has to be robust, preferably re-usable, and safe for use in clinical experiments. It was the aim of this project to develop a pH electrode to these specifications. Prior to describing the construction of plastic pH electrodes it will be necessary to review briefly some theoretical aspects of ion-selective electrodes with particular emphasis on pH electrodes.

A pH electrode is an electrochemical concentration cell comprising two metal electrodes immersed in two solutions, each with a different activity of hydrogen ions. These solutions are separated from each other by a semi-permeable membrane which selectively allows hydrogen ions to pass through. As hydrogen ions move down their activity

gradient, an electrical gradient opposes the movement of the ions. The total potential energy difference across the membrane is called the electrochemical potential difference (u). u is the sum of the electrical and concentration potential energy differences across the membrane.

That is $u=W_e+W_c$

where W_e is the electrical potential energy difference and W_c is the chemical potential energy difference.

When $W_e=W_c$, then hydrogen ions will be in equilibrium across the membrane and u will be zero.

W_e is the product of E_H, the transmembrane voltage due to H^+ ions, F, the Faraday constant and n_H, the valency of hydrogen ions.

That is $W_e=n_HFE_H$

W_c is proportional to the difference between the natural logarithms of the internal and external hydrogen ion activities.

That is $W_c=RT\ln\frac{[A_H]_i}{[A_H]_o}$

where R is the universal gas constant, T is absolute temperature and $[A_H]_i$ and $[A_H]_o$ are the activities of hydrogen ions on either side of the membrane.

Therefore, the electrochemical potential difference is described by the equation:

$$u=n_HFE_H + RT\ln\frac{[A_H]_i}{[A_H]_o}$$

At equilibrium, u=0 and the equation can be rewritten as:

$$E_H=\frac{RT}{n_HF}\ln\frac{[A_H]_i}{[A_H]_o}$$

This is the Nernst equation. Since the membrane potential

depends on the activity gradient across the membrane, changes in the activity of hydrogen ion on one side of the membrane will cause the electromotive force of the concentration cell to change. Hence, by noting the relationship between changes in external hydrogen ion activity and changes in potential difference of the cell, a means of measuring the activity of hydrogen ions in a test solution is made possible.

In highly dilute solutions, the number of hydrogen ions that determine the properties of the solution (ie. activity) is equal to the number of hydrogen ions added (ie. concentration). However, as the solution becomes less dilute, the activity of hydrogen ions becomes proportional to the concentration. It is thought that the reason for this is because a hydrogen ion will attract a cloud of negatively charged particles and will repel other hydrogen ions. This attraction of unlike charges and repulsion of like charges applies to all ions in solution. Hence, the ions in solution become ordered and this order opposes the natural tendency for the entropy of the system to reach a maximum, that is, to become disordered because of the thermal energy in the system. Consequently there are fewer "active" hydrogen ions in solution as the concentration increases. An empirical relationship between the concentration of ions and their activity has been described by the Debye-Huckel equation which can be found in most texts on electrochemistry and will therefore not be described here.

In most practical circumstances, biological solutions are not highly dilute and the electrode membrane potential will be determined not by the concentration of hydrogen ions in the solution but by their activity. Consequently, calibration of pH electrodes should be performed using buffers of known hydrogen ion activity prior to measuring the pH of a test solution.

For a univalent ion such as hydrogen, a ten fold change in hydrogen ion activity represents an electrode potential change of 58mV at 20°C. Therefore, an "ideal" plastic pH electrode would have to have what is referred to as a Nernstian response ie. 58mV/pH unit change. However, most pH electrodes are not "ideal" since the recorded electrode potential is determined not only by the activity of hydrogen ions in solution but also by other, interfering ions in the same solution. To take into account these interfering ions the Nernst equation can be rewritten in a form sometimes referred to as the Nicolsky equation:

$$E_H = \frac{RT}{n_H F} \ln\{[A_H] + [K_{HJ}(A_j)^{n_H/n_J}] + \ldots\}$$

Here, all the terms are as before except:

K_{HJ} is the selectivity coefficient of an interfering ion, J, relative to the sensed ion, H.

This equation can be extended to allow for all the activities of the interfering ions in the solution under examination. The degree to which an electrode is affected by the activities of interfering ions is a function of the ion specificity of the electrode.

For liquid-membrane electrodes, ion specificity is determined by the carrier incorporated in the lipophilic solvent which makes up the ion-selective membrane. Carriers are either charged or neutral compounds which have an affinity for the ion under investigation but must be sufficiently hydrophobic to be retained in a lipid or lipophilic support membrane. Carriers within liquid-membranes function by forming a complex with the selected ion which then diffuses through the membrane (107). In contrast, the mechanism underlying ion-selective glass electrode sensitivity (107), although not fully understood, is thought to involve ion exchange at the membrane interfaces coupled with movement of cations in a lattice of fixed anion sites within the membrane.

Carriers selective for hydrogen ions have been isolated from naturally occurring sources. Certain organic weak acids, capable of uncoupling oxidative phosphorylation in mitochondria, can also increase hydrogen ion permeation through lipid bilayers (108). Another naturally occurring substance, the 3-hydroxy picolinamide residue of the antibiotic virginamycin-S (109) is a proton ionophore.

Many of these naturally occurring proton carriers are hydrophilic and are not suitable for incorporation into a lipid based membrane. However, larger molecular weight homologues of these compounds exhibit both an affinity for hydrogen ions and are hydrophobic. One of these, p-octadecyloxy-m-chlorophenylhydrazone-mesoxalonitrile (OCPH), a derivative of a naturally occurring weak acid, functions particularly well when incorporated in a solvent polymer

(99). Similarly, the octylpicolinamide homologue of the virginamycin-S proton ionophore, in the solvent o-nitrophenyloctylether, can be used in pH sensitive microelectrodes (109).

These two carriers are examples of charged ionophores. The activities of monovalent cations, other than the hydrogen ions under investigation, can create substantial interference when charged ionophores are incorporated into electrodes (110). Since organic solvents used for liquid membranes also exhibit selectivity for large , but not small, ions, discrimination over interfering ions can only be achieved if the solvent and carrier have similar ion specificity. A low selectivity due to incompatible ion specificities of solvent and carrier will result in a further reduction of the selectivity if the electrode is used in solutions in which the activities of interfering monovalent cations is high.

Extremely high selectivities can be achieved by using neutral ion-specific carriers as membrane components (107,110). Neutral carriers are composed of polar and non-polar groups and assume a stable conformation that provides a cavity specific for the uptake of the selected ion (110,111). The cavity is created by a rigid arrangement of the polar groups which have a series of coordination sites, while the non-polar groups form a lipophilic shell around the coordination centre.

Neutral lipophilic tertiary amines exhibit hydrogen ion carrier properties when incorporated in an organic solvent.

Hydrogen ion neutral-carrier macro (112) and micro (113) electrodes, based on tri-n-dodecylamine (TDDA), have selectivities almost equal to those of glass pH electrodes. By impregnating PVC with carrier containing solvent, electrodes with plastic membranes have been constructed (112). These plastic membranes were mounted in commercially available electrode "bodies" with comparatively large surface areas ($25mm^2$). Consequently, these electrodes would not be suitable for the proposed clinical investigations and it was necessary therefore to develop a new technique for constructing electrodes with TDDA incorporated in plastic.

Two proton ionophores were selected for use in plastic pH prototype electrodes in this study. OCPH, a charged weak acid, has been compared with the neutral carrier, TDDA. Each compound has been assessed as a suitable candidate for the pH electrodes. In particular, their selectivity for hydrogen ions over other monovalent cations was examined. The performance of the carriers was compared with that of glass pH electrodes which, for this study, have been used as the standard to which all comparisons are referred. Before detailing the results of these comparisons, the construction of the plastic electrodes both for laboratory and clinical use will be described.

2 ii) Source of chemicals

The ion selective carrier p-octyldecyloxy-m-chloro phenylhydrazone-mesoxalonitrile (OCPH) and the copolymer matrix were gifts from Dr. O.H. LeBlanc; GEC, Schenectady,

N.Y. USA. Tri-n-dodecylamine (TDDA) was purchased from BDH Chemicals Ltd., Poole, Dorset, England, as was tetrahydrofuran. Dichloromethane, tetraphenylborate and o-nitrophenyloctylether were obtained from Fluorochem Ltd., Glossop, Derbyshire, England. All PVC tubing used was from Portex, Hythe, Kent, England.

For the buffer solutions, citric acid, sodium bicarbonate and commercial phthalate buffers were from BDH Chemicals Ltd. Potassium hydrogen phthalate came from Riedel-de Haen Ag, Seelze-Hannover and Trizma base from Sigma Chemical Co., Poole, Dorset, England. Potassium dihydrogen orthophosphate and monopotassium phosphate were purchased from Koch-Light Laboratories Ltd., Buckinghamshire, England.

2 iii) Construction of plastic pH electrodes

For the construction of plastic pH electrodes, a solution of the appropriate hydrogen ion carrier and its corresponding membrane was prepared and stored in liquid form. Plastic membrane solutions based on the OCPH carrier were prepared by incorporating 1% w/w OCPH in a copolymer matrix previously described by Leblanc et al (99). This mixture was dissolved in excess dichloromethane. Tri-n-dodecylamine (TDDA) membrane solutions were similarly prepared. The ion-selective mixture consisted of 30 microlitres of the ion-selective carrier, TDDA, dissolved in 200 microlitres of o-nitrophenyloctylether (o-NPOE), a lipophilic solvent through which the carrier could diffuse. To this was added 1.2mg sodium tetraphenylborate (NaTPB) which created fixed anionic sites within the membrane.

Fixed anionic sites reduce interference from anions in the solution under investigation (114) and therefore increase the ion-selectivity of the electrode. The ion-selective TDDA mixture was added to 100mg PVC dissolved in excess tetrahydrofuran. Fragments of PVC tubing or powdered PVC (Lonzavyl S-704)) could be used. To obtain flexible membranes with powdered PVC, 30 microlitres of dibutyl-sebacate plasticiser were included in the membrane mixture. Solidification of the membrane mixture and prolonged storage had no apparent effect, since sensitivity to hydrogen ions was detected at least one year later, on resuspension of the mixture in fresh solvent.

The same method of casting the hydrogen ion-selective membranes was used for both ion exchangers. Sufficient solvent was allowed to evaporate until a mixture remained which was close to congealing. The end of a length of PVC tubing, 1.6mm outside diameter, was dipped in this solution and slowly withdrawn (105). A meniscus was formed over the end of the tubing which, on evaporation of the solvent, left a tough but flexible membrane. Repeated dipping of the electrode in the plastic mixture formed a membrane with a surface which was flat. A membrane that was too thin resulted in a concave surface which was undesirable. When withdrawn from a test solution, an electrode with a concave membrane would have fluid retained in the cavity formed by the membrane. When immersed in a different solution, this residual fluid would act as a diffusion barrier to hydrogen ions in the new solution and would reduce the response time of the electrode.

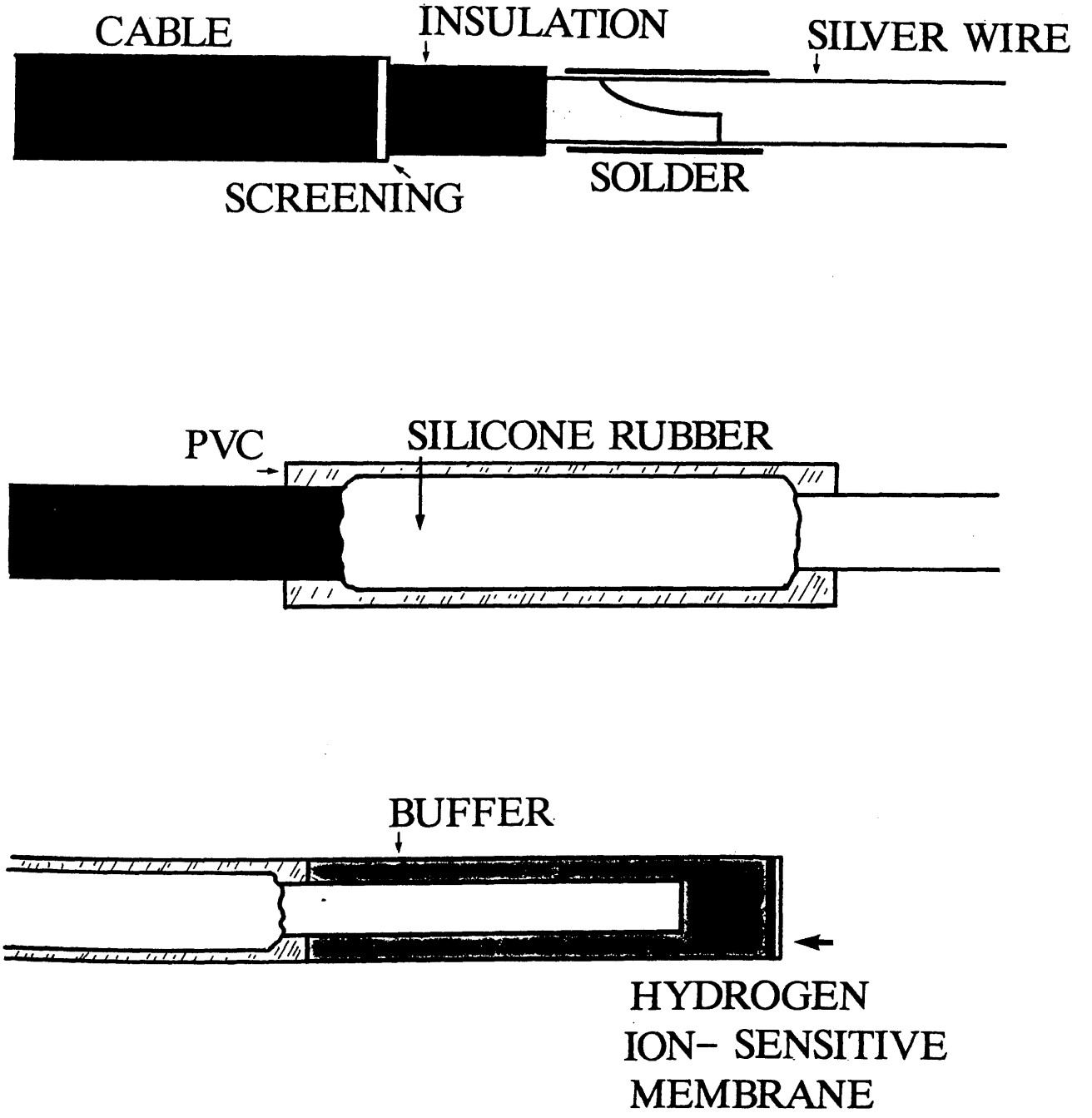
Electrodes suitable for laboratory studies were easily produced by filling a 5-10cm length of PVC tubing, upon which a membrane had been cast, with 0.1M pH7 Tris/HCl buffer (115). pH 7 buffer was used as the internal filling solution because when immersed in pH 7 buffer, the electrode should ideally have no standing potential. Most pH meters are constructed so that when an electrode is immersed in pH 7 buffer, the potential difference registered on the meter is zero millivolts. If the electrode was filled with pH 5 buffer, for example, the approximately 116mV potential difference generated between the internal and external buffer solutions would probably be too large to be offset by the average pH meter. However, this was not a problem if changes in pH were measured on a normal voltmeter but was important when the electrodes were used with pH meters in clinical experiments. The use of pH 7 buffer appeared to have no deleterious effects on electrode performance, despite the recommendation of pH 5 (99) or pH 5.6 (112) buffer as the internal solution. A chlorided silver wire soldered onto a screened cable was inserted in the open end of the tubing which could be sealed to the cable with adhesive if desired. These electrodes required no pre-conditioning and could be immediately used.

Catheter-type electrodes, suitable for <u>in vivo</u> gastro-intestinal pH measurements, had to be as thin as possible to prevent them from interfering with the movement of the Crosby biopsy capsule to which they would be attached. The electrodes also required a fluid resistant seal between the

plastic and the cable to avoid short-circuiting the electrode. The design of these electrodes was therefore more complicated (Figure 1). A 1cm length of 0.5mm silver wire was soldered to the exposed inner core of a 2 metre length of screened cable (Belden 8700, 1.27mm o.d.; Wadsworth Electronics Ltd.). A thin covering of silicone rubber adhesive (RTV 102; General Electric, UK.) was used to insulate the solder joint from the outer screening of the cable. After 24 hours, during which time the silicone rubber dried, a 1cm section of PVC tubing (2mm o.d.) was used to cover the silicone rubber and extend approximately 3mm beyond the end of the cable's protective PVC jacket. A slightly longer length of heat-shrink plastic (2.4mm o.d.; R.S.Components, UK.) was positioned over the PVC tubing and heated with a soldering iron. As the heat-shrink plastic contracted, it formed a mould for the underlying PVC tubing which melted during the heating process. When cool, the hard mould was carefully cut open with a scalpel blade exposing the now solid PVC coating which encapsulated the solder joint; this provided further insulation and strength to the joint.

To expose the silver wire, which had been totally covered by the molten PVC during the heating procedure, the PVC capsule was cut back to within 2mm of the silicone rubber. Since it was now possible for fluid to seep between the cut PVC and the silver wire, the joint was dipped in a solution of Lacomit varnish (Agar Aids Ltd.; Stansted, England) which sealed the plastic-wire junction. A 2-3mm section of silver wire was scraped clear of varnish and

Figure 1. Method of constructing a plastic pH electrode for use in clinical experiments.



chlorided for approximately 20 seconds. A 5mm piece of PVC tubing with a hydrogen ion-selective membrane on one end was filled with pH 7 Tris/HCl buffer and positioned over the chlorided silver wire. The junction between the tubing and the PVC capsule was sealed with cement prepared by dissolving fragments of PVC tubing in excess tetrahydrofuran. The opposite end of the cable was connected to a suitable plug and the electrode was then ready for use.

On the basis of the foregoing technique of electrode construction, electrodes could be modified to suit specific requirements. By inserting a ceramic plug into the end of the PVC tubing (100) prior to casting the membrane by the dip technique, electrodes with rigid sensing tips could be produced. These electrodes were ideal for passing through the lumen of naso-gastric tubes or down the biopsy channel of an endoscope. Another type of electrode had a membrane sited in the side rather than at the end of the PVC tubing. A hole, 1mm in diameter, was cut in the side of a length of PVC tubing, 3mm from the end. The membrane was cast by smearing a small volume of the membrane mixture over the side hole and allowing the excess solvent to evaporate. The end of the tubing was sealed with PVC dissolved in tetrahydrofuran and the plastic tip could be connected to a cable in the manner described above. This type of electrode could be used in parallel with other electrodes to provide a string of electrodes for measuring pH at pre-selected distances down the intestinal tract. With the membrane in the side of the electrode, it is easier to produce a

streamlined device which would not have unsecured pH tips (17) snagging the mucosa as it passed down the intestine.

Once the electrode had been constructed, all that remained was to examine the functional characteristics of these electrodes. For this purpose, the small laboratory style electrodes described earlier were used. The following section describes the experiments undertaken and the results of those experiments.

2 iv) Characterisation of plastic pH electrodes

To determine their characteristics, plastic pH electrodes were used in conjunction with a calomel reference electrode immersed in a solution of saturated KCl. The KCl reservoir and the test solution were electrically connected with an agar bridge (3mm o.d; 3% agar in 3M KCl). The calomel and pH electrodes were connected to a high impedance electrometer (Keithley 610C) and all measurements were made in millivolts at ambient temperature.

The response of the plastic electrodes to different buffering ions in solution was investigated. Solutions of citrate, phosphate, bicarbonate, tris(hyroxymethyl)amino-methane (Tris), and phthalate buffers were made in the laboratory according to the formulae in reference (115); their composition is detailed in Appendix 1. Commercially available buffer (BDH) based on potassium hydrogen phthalate (pH 4.0; 0.05M) was used for comparison with freshly made phthalate solutions.

The experimental procedure involved titrating the selected buffer between pH 3 and 11 with 0.1N HCl or NaOH. The pH change, as measured by a standard laboratory glass pH electrode with a combined reference electrode (Pye-Unicam 40/E7) connected to a pH meter (Corning EEL5), was compared with the voltage change recorded by the plastic electrode.

In the presence of bicarbonate, citrate, phosphate and Tris buffers, the potential difference generated by the ion-selective carriers was pH dependent. The change in potential difference of TDDA electrodes to changes in hydrogen ion activities was close to the theoretical maximum (58mV/pH unit) when the selected buffer was titrated within the range of pH 4-11 (Figure 2). A diminished response was measured either side of this range, particularly in the acid region. In contrast, the response of OCPH electrodes depended on the buffering ion in solution (Figure 3). In bicarbonate and phosphate buffers, a pH sensitivity of more than 50mV/pH unit was measured in the range of pH 5.5-10, while in Tris and citrate buffers the ranges over which this level of sensitivity could be measured were pH 4-7 and pH 4-6 respectively. When compared to OCPH electrodes, TDDA electrodes had a wider operative pH range independent of any buffering ions in the solutions tested so far.

The response of OCPH electrodes in laboratory made phthalate buffers was extremely unstable while no response was measured in commercial phthalate buffers (Figure 4). An

Figure 2. Response of TDDA plastic pH electrodes to citrate (x), bicarbonate (•), phosphate (□) and Tris (o) buffers.

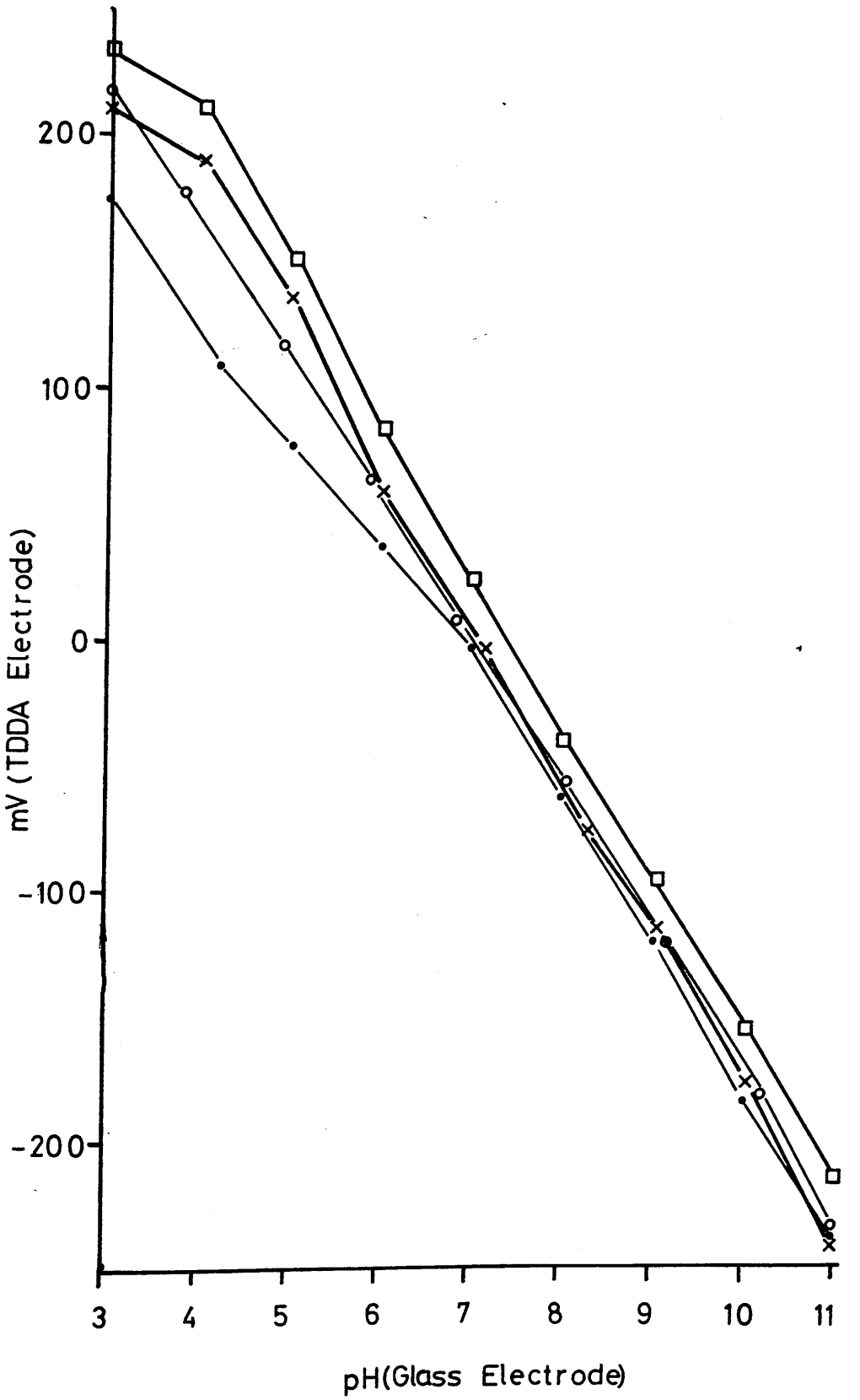


Figure 3. Response of OCPH plastic pH electrodes to citrate (X), bicarbonate (•), phosphate (□) and Tris (o) buffers.

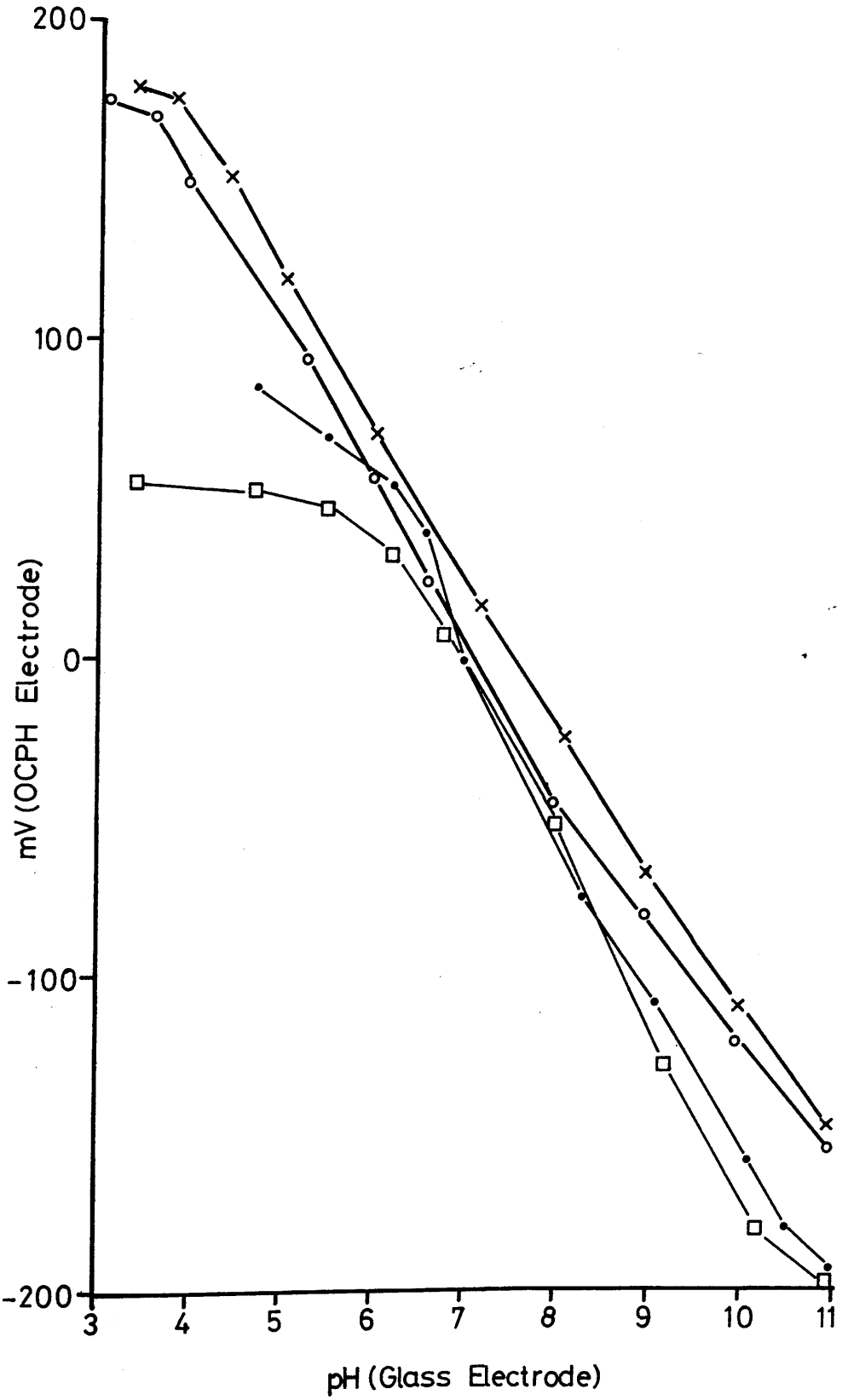
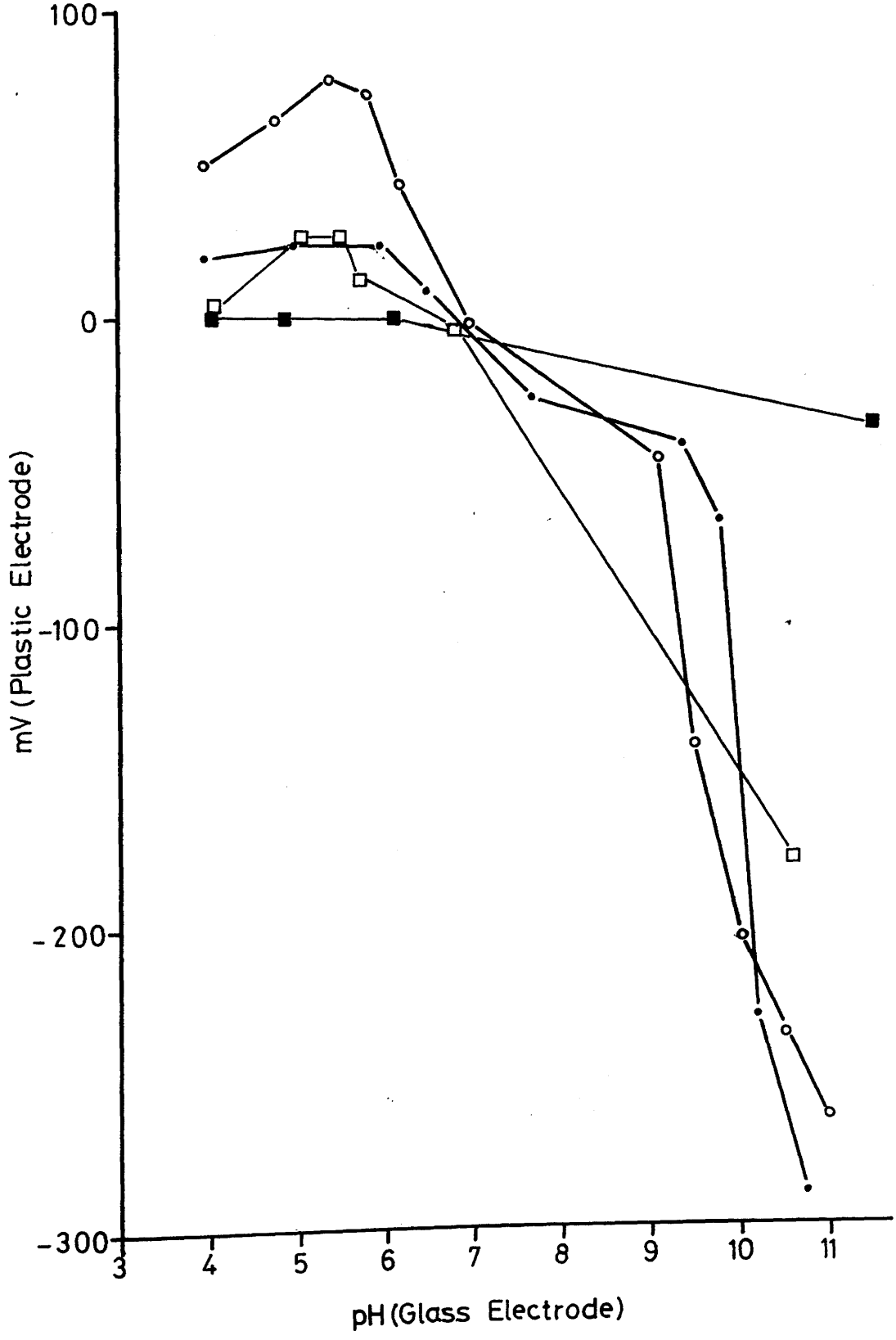


Figure 4. Response of TDDA (o) and OCPH (□) plastic pH electrodes to commercial phthalate (shaded) and laboratory made phthalate (open) buffers.



unstable response was characterised by large fluctuations in the recorded potential with time at constant pH values. The response of TDDA electrodes in both types of phthalate buffer was less unstable than OCPH electrodes. Despite this, measurements could not be made with any degree of accuracy. Evidently, unstable membrane potentials are the result of lipophilic buffer ions interacting with the lipid solvent phase of the plastic membranes (99) making accurate pH determinations impossible under these circumstances. Therefore, perfectly functioning electrodes may appear not to be working if calibrated in phthalate buffers.

Electrode resistance was measured with a Keithley (610C) electrometer, which is equipped with an ohm-meter facility, while the electrode was immersed in pH 7 Tris/HCl buffer. The measured resistance was of the order of 1-10 megohms for TDDA electrodes and 10-100 megohms for OCPH electrodes. Omission of sodium tetraphenylborate from the TDDA based plastic membranes increased resistance tenfold.

Response time was measured by transferring the pH and reference electrodes from a pH 7 to a pH 5 buffer and following the response on a paper chart recorder (Speedomax) set at a rate of 30cm/minute; the buffers, at 20° C, were stirred continuously. Sensitive electrodes typically gave 90% of their final value within one second and reached a stable potential within 15 seconds. Thus, plastic electrodes have a fast response time to changes in pH.

Electrode drift was measured by leaving the electrode in a pH 7 buffer for periods of up to 48 hours. The potential was recorded throughout and a chart recorder set at a rate of 1cm/minute monitored the potential change. The container of buffer was sealed at the start of the experiment to prevent evaporation of water and the pH of the buffer was measured with a glass electrode before and after the experiment. A representative value for drift was 3mV/24hrs which, for an electrode with a pH sensitivity of 58mV/pH unit, is 0.05 pH units/24hrs. This is a very low value of drift for which corrections could easily be made at the end of any experiment.

The selectivity coefficients were measured for hydrogen over sodium and potassium ions, the two major interfering cations of physiological significance. Electrode selectivity was determined using the fixed interference method (116). A 1N solution of the interfering cation at a pH of 11-12 was titrated with 1N HCl in the presence of the plastic electrode and a glass pH electrode. The solution, at ambient temperature, was stirred continuously as the pH was titrated to pH 9-7.

When the potential difference of the plastic electrode was plotted against the measured pH (Figure 5 and Figure 6), two regression lines were drawn, each through the linear portions of the plotted results. By extrapolating the regression lines until they intersected each other, a value of activity of the primary ion (A_a) was obtained. This activity represented the activity of the primary ion at which the contribution by the interfering ion, to the

Figure 5 Selectivity Coefficients For TDDA Electrodes

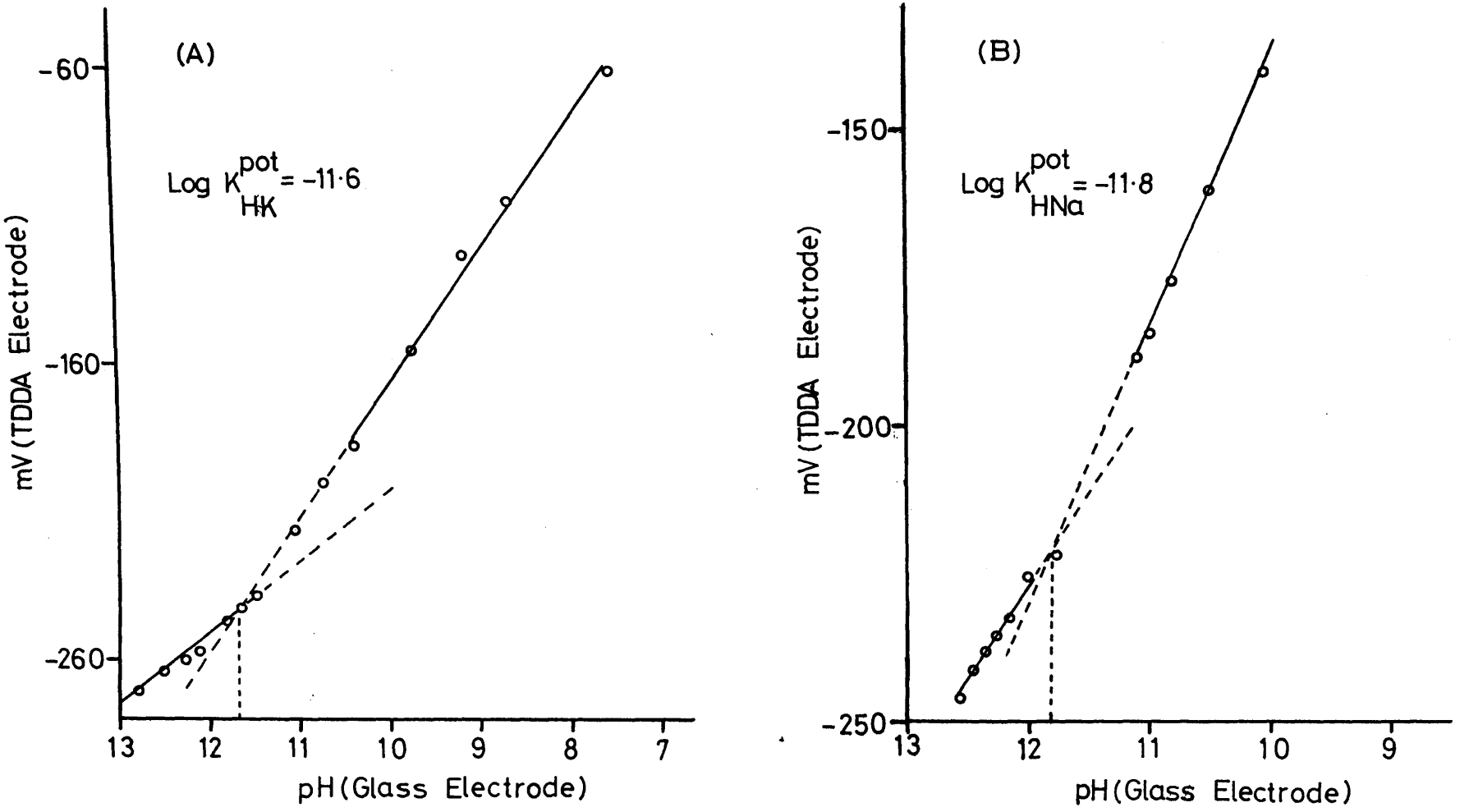
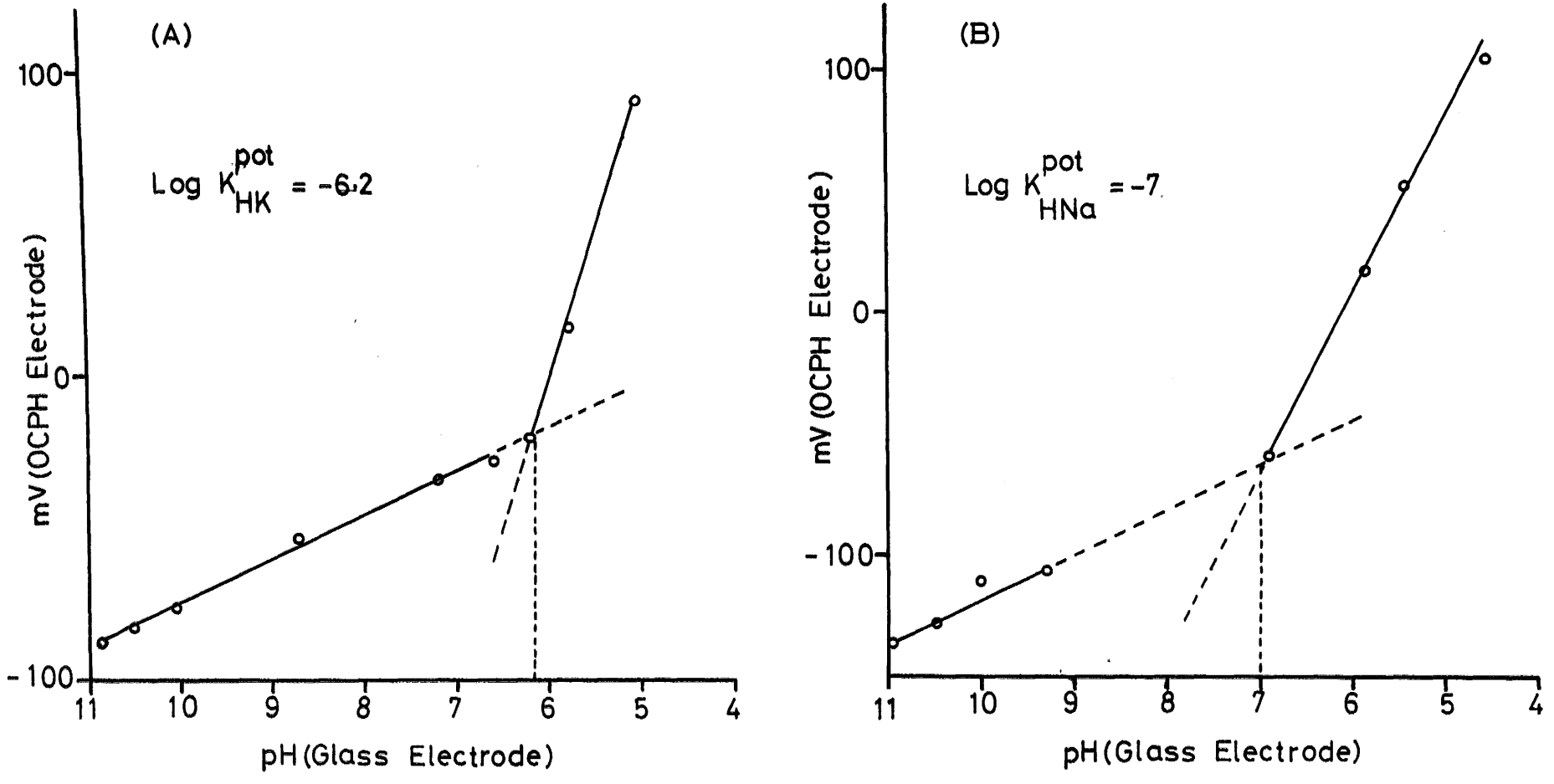


Figure 6 Selectivity Coefficients For OCPH Electrodes



generated potential, was such that any further change in potential difference due to a reduction in the concentration of the primary ion was negligible. The value of A_a is used to calculate K_{ab}, the selectivity coefficient of the primary ion, a, over the interfering ion, b, from the equation:

$$K_{ab}=A_a/(A_b)^{z_a/z_b}$$

where z is the charge on the ions. A_b, the activity of the interfering ion, b, is assumed to be equal to the concentration of b in the solution which in these experiments was 1N.

Measured selectivity coefficients for TDDA electrodes were extremely high at $10^{-11.8}$ for sodium (Figure 5B) and $10^{-11.6}$ for potassium (Figure 5A). These values are probably more accurate than previously reported (112) values. By titrating with 10mM HCl, as opposed to 1N HCl, selectivity coefficients of $10^{-10.6}$ for sodium and $10^{-10.0}$ for potassium were obtained, both remarkably similar to reported values. The discrepancies can be attributed to substantial dilution of the test solution with 10mM HCl, a factor not associated with the use of 1N HCl.

OCPH electrodes have a relatively poorer selectivity for hydrogen ions over other monovalent cations. Measured selectivity coefficients of $10^{-7.0}$ for sodium (Figure 6B) and $10^{-6.2}$ for potassium (Figure 6A) would suggest, contrary to published observations (99), that these electrodes are susceptible to interfering cations even when these are present in relatively low concentrations.

In conclusion, the functional characteristics of two hydrogen ion-selective carriers have been compared with each other and their suitability for use in plastic electrodes has been assessed. TDDA electrodes responded over a wider pH range than OCPH electrodes. The pH range of TDDA electrodes extends into the more alkaline region of physiological pH values and would be more suited to the measurement of intestinal pH than OCPH electrodes. Further, the selectivity of hydrogen ions oyer sodium and potassium ions is greater for TDDA electrodes than OCPH electrodes. In intestinal secretions where the activity of sodium ions is relatively high, TDDA electrodes are less likely than OCPH electrodes to suffer from cation interference. For these reasons, the hydrogen ion-selective carrier tri-n-dodecylamine was chosen as the ionophore to be incorporated into plastic pH electrodes for clinical investigations. The functional characteristics of TDDA electrodes are as good as glass pH electrodes within the physiological range of pH. Consequently, plastic electrodes based on the TDDA ionophore are adequate substitutes for glass pH electrodes with the added benefit of being robust yet flexible. Unfortunately, the failure of the electrodes to measure below pH 4 negated their use at endoscopy for gastric pH measurements.

In an earlier section the construction of a plastic pH electrode for use in conjunction with a Crosby capsule was described. It has been shown in this section that the characteristics of the ionophore selected for incorporation

into the plastic electrode are ideal for intestinal pH measurements. The following section describes clinical investigations using these electrodes. In addition, the method of measuring gastric and duodenal surface pH will be detailed.

CLINICAL INVESTIGATIONS

Before describing the clinical experimentation, it is important to examine some technical problems which have caused confusion in the past (117). The present section is an attempt to confirm that the experimental technique used throughout the clinical investigations is indeed valid and to show that the confusion that exists is largely derived from the misinterpretation of previously published work (12,67).

3 i) Should combined or separated electrodes be used?.

The selected methods of measuring gastrointestinal surface pH in this project required separate reference electrodes. To measure mucosal surface pH at endoscopy, the pH electrode was passed down the biopsy channel of the endoscope. This biopsy channel was not sufficiently large to accommodate a combined pH and reference electrode. For this reason, a reference electrode was placed in the buccal cavity. A buccal reference electrode was also used when intestinal surface pH measurements were made at jejunal biopsy. Although the reference electrode could have been combined with the pH electrode it would have increased the overall size of the measuring device. An increase in the size of the system was undesirable since it was likely that this would disrupt the smooth passage of the capsule-electrode assembly through the intestine.

The validity of using separate recording electrodes for gastrointestinal pH measurements has previously been investigated (12,15). The potential difference between an

electrode in the stomach and a skin reference electrode was approximately 40-60mV more negative than potentials registered when the enteric electrode was positioned in the oesophagus or duodenum (12). Thus, if a skin reference electrode is used, allowances have to be made for what is assumed to be a transmural potential difference. In contrast, the potential difference between two enteric electrodes separated by upto 15mm has a mean value of 0mV (12). Consequently, the use of a separate skin reference electrode is perhaps questionable when measuring pH in different areas of the gastrointestinal tract because of the liklihood of varying transmucosal potential differences having a distorting influence on the recordings. However, a separate enteric reference electrode would appear to have no effect on the recorded potential difference.

To make measurements of mucosal surface pH in this project, the electrodes would be separated by 1m or more, although both electrodes would be in contact with the enteric mucosa. What effect such a large separation would have on the recorded potential was uncertain.

To investigate if this could be a source of systematic error, a series of experiments were undertaken. These experiments were concerned with the following questions: a) does physical separation affect the recorded potential difference between two electrodes? b) is there any change in this potential difference when the same experiment is performed in the gastrointestinal tract? and c) could a local electrical field, possibly generated by the mucosa,

interfere with the recorded potential?

3 ii) The effect of separate recording electrodes on the measured pH.

The effect of physical separation of a glass pH electrode and its reference electrode was simulated on the bench. Three beakers containing pH 7 buffer were joined by two buffer filled 1.5m lengths of PVC tubing (2mm i.d.). The voltage between the pH and reference electrodes, when immersed in the first beaker, was compared to that obtained by moving the pH electrode to the second and third beakers. No change in potential difference between the electrodes was measured with a separation of 1.5m and at 3m separation the potential difference changed by 1mV; this represented an insignificant change in pH.

In addition to the laboratory test, the effect of electrode separation on recorded pH was investigated *in vivo*. During endoscopy, the potential difference between a reference electrode in the buccal cavity and a reference electrode placed in several regions of the stomach and duodenum was measured in ten patients. The potential differences between areas within the same patient were not consistent and a maximum variation of 39mV was measured in one patient. However, the change in potential difference from the mouth to the duodenum was not linear with distance (Table 1). An average of the potential differences recorded between electrodes in all ten patients was not significantly different from zero in all areas. Thus, electrode separation would not influence the recorded pH when the reference electrode is in the buccal cavity.

Table 1	Individual and mean potential difference measurements (millivolts) between a buccal and a second reference electrode sited at indicated points within the gastrointestinal tract.						
Patient	Duodenal Loop	Duodenal Cap	Pylorus	Antrum	Fundus	Oesophagus	Mouth
1	6.0	0	-	-21.0	4.0	-12.0	2.0
2	17.0	13.5	7.0	2.0	19.0	12.0	6.0
3	16.5	3.0	4.5	11.0	14.0	-5.0	-9.5
4	-9.5	-1.5	-6.0	-9.0	-0.5	-13.0	-
5	31.5	29.0	23.5	23.0	22.0	18.5	-
6	0.5	-2.5	1.5	-6.0	-4.0	-13.5	-14.0
7	-	0	-6.5	-21.5	-2.0	-31.5	0
8	4.0	11.0	-1.0	-27.5	-7.5	-15.0	-19.0
9	-	-6.0	-7.0	-7.0	2.5	-19.0	-9.0
10	-11.5	-7.0	-19.0	-21.0	-11.0	-2.0	-8.0
Mean±SEM	6.81±5.10	3.95±3.48	-0.33±3.93	-7.70±5.09	3.65±3.54	-8.05±4.64	- 6.44±2.99

Variations in potential difference are probably the result of changing ionic composition under the gastro-duodenal reference electrode. Changes in ionic composition will affect the junction potential of a reference electrode but since pH electrodes only respond to changing activities of hydrogen ion, changes in the activities of other ions will not affect the recorded pH under physiological conditions.

Since the gastrointestinal mucosa is a secretory tissue, localised potential differences may be generated by ion fluxes or separation of electrical charges. The effect of a local potential difference on the recorded pH was a possible source of error which had to be taken into consideration. To simulate these conditions, a pH and reference electrode were placed in a pH 7 buffer while a voltage from a variable voltage generator was applied between two inert reference electrodes in the same buffer solution. There was no change in recorded pH when a potential difference of 0-1V was applied across the two reference electrodes. Local potential differences within the gastrointestinal tract would not therefore influence the recorded pH.

As a result of these investigations, it was concluded that the pH values recorded in the surface measurements were not affected by artifacts derived from using separate recording electrodes. Further, local tissue potentials are also unlikely to affect the recorded pH. It may even be an advantage to use a system where the reference electrode is

maintained in one place. With a combined electrode, the ionic composition of the fluids with which the reference electrode comes in to contact will change as the electrode moves from the stomach to the duodenum. As suggested above, changes in ionic composition may cause variations in electrode junction potentials, thereby affecting the recorded pH. Without being able to compensate for these changes in junction potential, the accuracy of pH recordings from different areas using a combined reference electrode is questionable.

What follows is a description of the clinical procedures used for measuring gastrointestinal surface pH, beginning with a report on the patients who were used for the investigations.

3 iii) Patient groups

a) Biopsy Groups: Investigations were performed with the patients' informed consent. Jejunal surface pH measurements were made in patients referred for jejunal biopsy for the investigation of possible small bowel disease or for assessment of treatment in cases of previously diagnosed coeliac disease. A total of seventy patients were investigated but jejunal pH measurements were successfully obtained in only forty seven. The unsuccessful investigations were mostly attributable to the capsule failing to leave the stomach. From the successful investigations, three patient groups could be adequately defined. These consisted of a control group of 9 patients with no evident upper gastrointestinal reason for their

symptoms, a coeliac group of 13 patients, and 11 patients with irritable bowel syndrome (IBS) with no signs of upper gastrointestinal disease but whose symptoms improved with a high fibre diet. The remainder consisted of patients with definable but uncommon illnesses, the small number of which precluded statistical analysis, and patients with small bowel disease still eluding a conclusive diagnosis at the time of study.

Patients in the jejunal biopsy investigations consisted of mainly women with two men in each of the patient groups. All groups were matched with respect to age with an average of 32 years (range 17-58 years) for the control group, an average of 33 years (range 21-54 years) for the coeliac group and an average of 36 years (range 15-59 years) for the IBS group.

b) Endoscopy Groups: Upper gastrointestinal pH measurements were made in two series of unselected patients attending for endoscopic examination. One series of 82 subjects received an injection of atropine (atropine sulphate 0.6mg i.m.) prior to endoscopy and are referred to as the atropinised series of patients. The other series of 164 patients received no atropine and are accordingly referred to as the non-atropinised series.

Within each series, patients were sub-divided according to the eventual diagnosis. Sub-groupings by disease consisted of controls, gastritis, gastric ulcer, duodenitis, duodenal ulcer, hiatus hernia, oesophagitis and pernicious anaemia. However, there was considerable overlap

of disease groups and some categories selected here may be facets of one disease.

In addition, a separate series of pH measurements were made in 22 subjects who were undergoing endoscopic cannulation for pancreatic and gall bladder dysfunction but who otherwise had normal upper gastrointestinal function.

In the non-atropinised and the atropinised series of endoscopy patients, 55% of the patients in each series were male. Details of the age structure for each group are to be found in Appendix 2.

3 iv) Measurement of jejunal surface pH.

By combining an electrode with a Crosby capsule, histological and functional information was obtained during the same investigation. Catheter-type plastic pH electrodes based on the tri-n-dodecylamine carrier were used for this study. The flexible tip of the electrode was positioned against the metal capsule (Figure 7) and aligned so that the sensing membrane was almost perpendicular to the intestinal mucosa as the capsule passed through the proximal small bowel. Securing the capsule suction line to the electrode cable with PVC cement ensured that the overall dimensions of the assembly were not significantly increased. Not only was this reassuring for the patient but capsule transit was unimpeded and the proportion of unsuccessful biopsy investigations was unchanged.

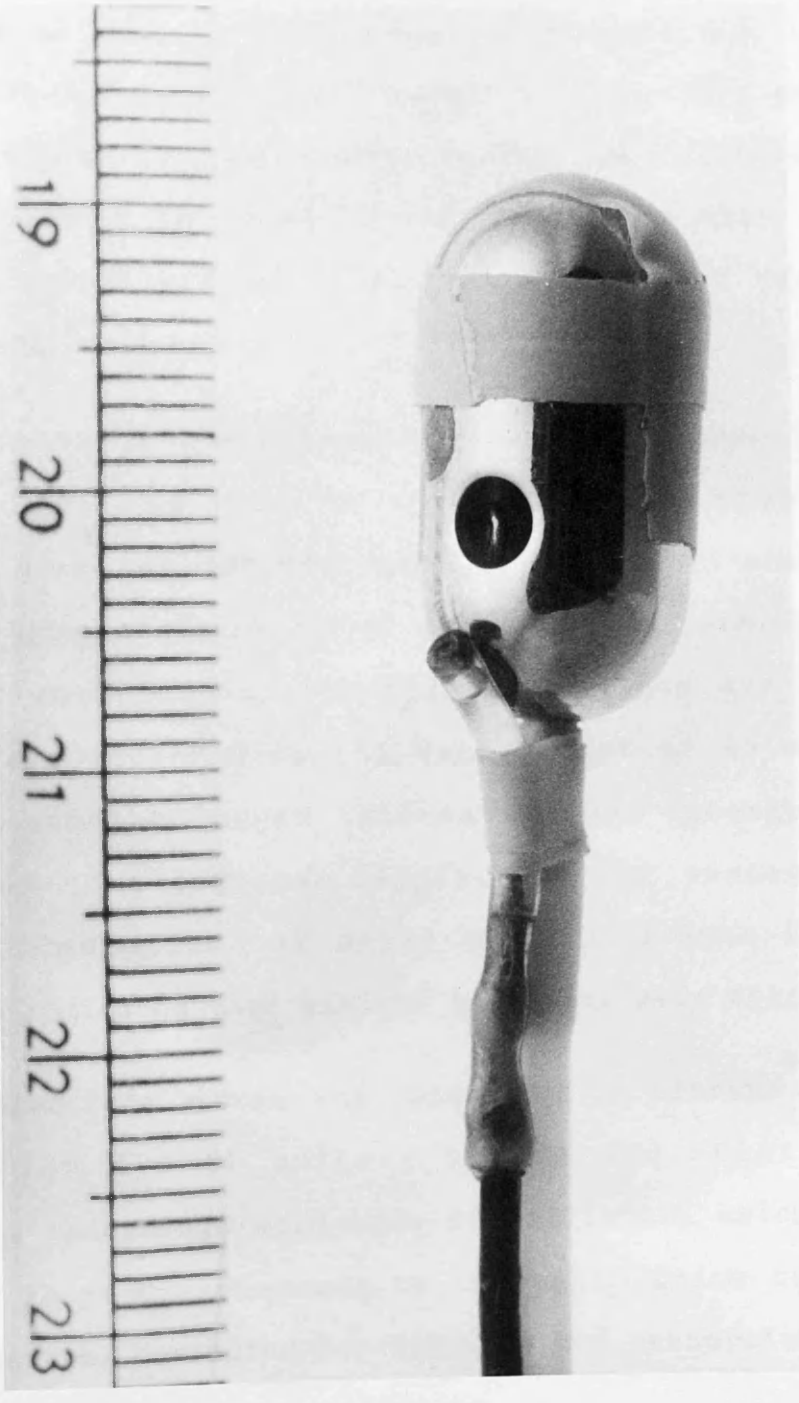
The reference electrode consisted of a Pye-Unicam (340) Ag/AgCl glass electrode with a porous ceramic plug. This was immersed in a solution of 3M potassium chloride from which an agar bridge (3M KCl in 3% agar; o.d. 1.6mm) was led to the patient's buccal mucosa. The pH and reference electrodes were coupled to the input channel of a battery powered pH meter (Knick Portamess 654) whose continuous output was monitored by a paper chart recorder (Watanabe Servocorder).

An operating system based on a data logger (Grant Squirrel SQ8) was used as an alternative recording mechanism when two patients were studied simultaneously. The data logger replaced the pH meter and pen recorder but all other experimental conditions remained the same. Results were recorded on the data logger at 15 second intervals and the logged information was subsequently transferred to a computer (Apple IIe) for storage and analysis. Comparison of paper chart and data logger recordings indicated that similar pH values were obtained.

The electrode system was calibrated in citrate (pH 5) and Tris (pH 7 & 8) buffers before and after each experiment. Recordings were made in millivolts which were converted to pH by reference to the calibration buffers and, if need be, corrected for drift on the assumption that electrode drift was linear with time.

The experimental procedure differed little from the jejunal biopsy regime routinely used in the investigation unit. After the patient had swallowed the capsule assembly,

Figure 7. Method of attaching a plastic pH electrode to a Crosby biopsy capsule. Scale is in centimetres.



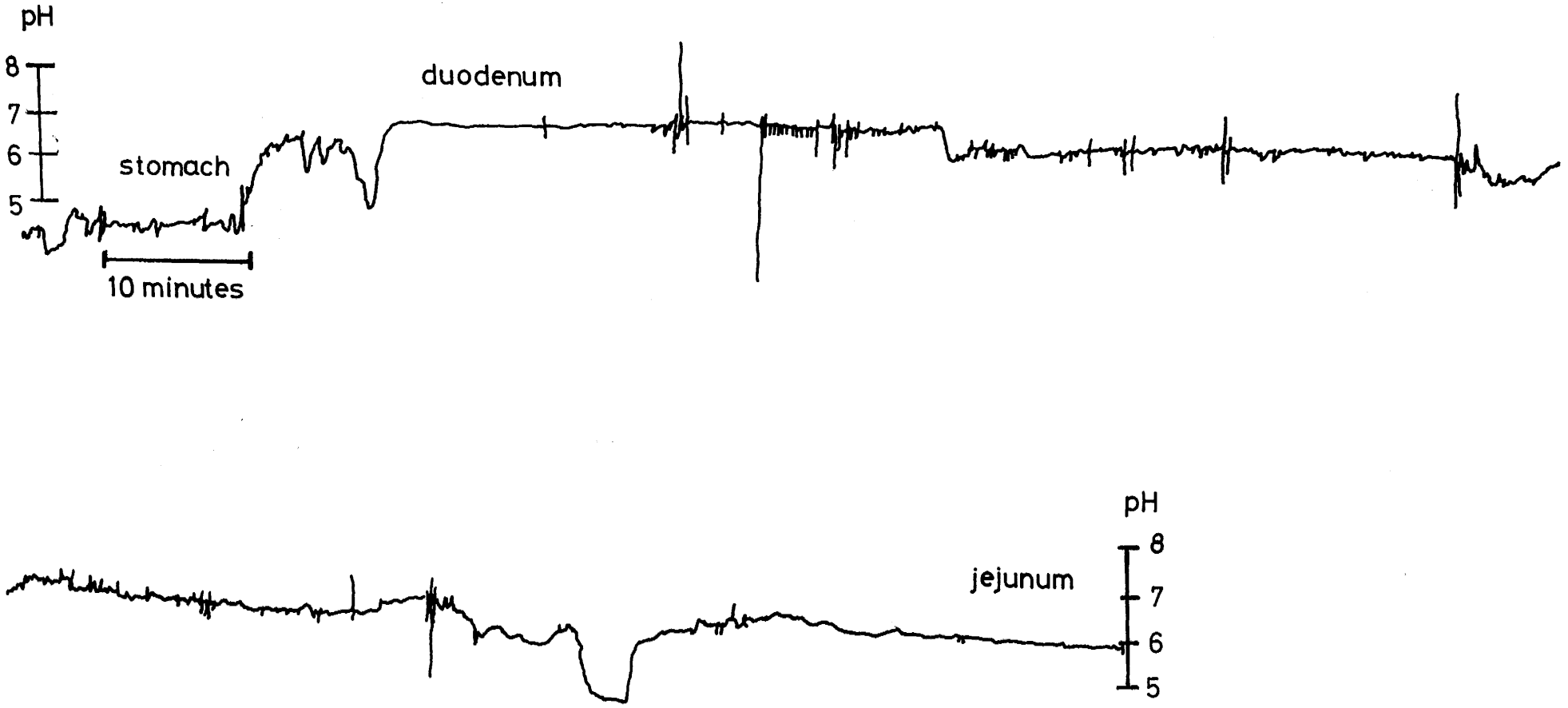
the electrodes were connected to the monitoring equipment and a continuous recording initiated. Movement of the electrode from the stomach to the duodenum (Figure 8) was verified by fluoroscopy and was always characterised by a rise in pH from acidic to near neutral values. The capsule's progress was subsequently monitored by fluoroscopy at half hourly intervals. Continuous measurements of mucosal surface pH were made until the capsule was distal to the ligament of Treitz, the anatomically accepted junction between duodenum and jejunum. At this point, a biopsy was taken whilst still recording the mucosal surface pH. In this way, the pH of the mucosa was measured as close to the site of biopsy as possible and during suction.

After retrieval, the excised tissue was removed from the capsule and the specimen was halved; one portion was placed in Bouin's fixative for histological examination, the other was weighed and stored at -5^{o}C prior to the assay of disaccharidase activities by the method of Dahlqvist (118).

3 v) Measurement of gastric and duodenal surface pH.

Flexible fibre-optic endoscopes allow convenient access to the upper gastrointestinal tract. Under direct visual control, the tip of the endoscope can be positioned in all regions of the stomach and up to the papilla of Vater in the duodenum. The endoscope used in this investigation was an Olympus GIF-1T panendoscope (forward viewing) with a biopsy channel of 3.7mm in diameter, sufficiently large to accommodate the smallest commercially available glass pH

Figure 8 A Representative Recording of Surface pH From a Patient at Jejunal Biopsy

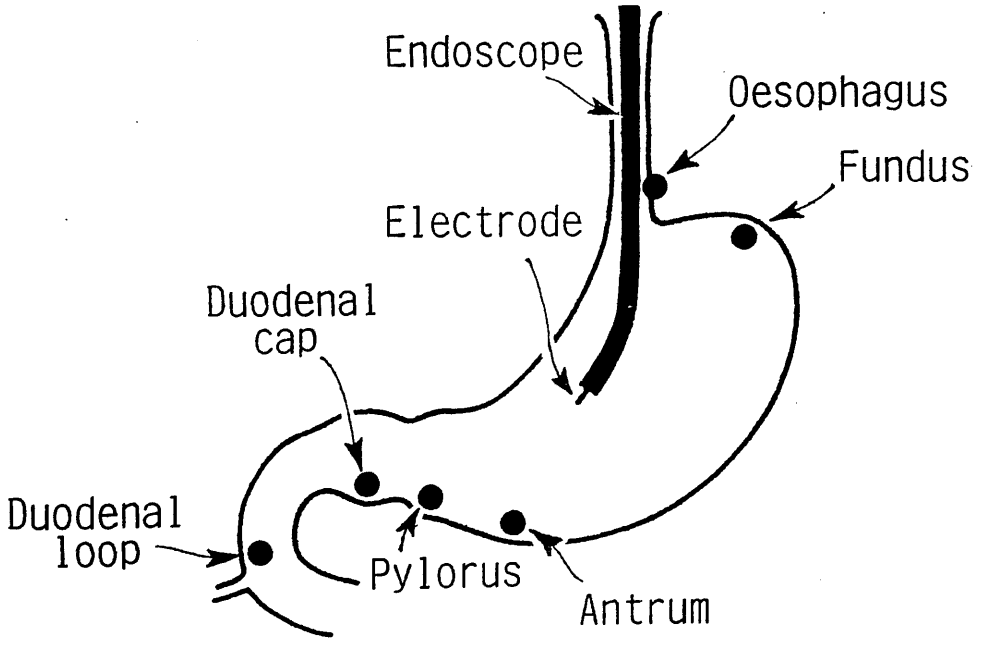


electrode.

The electrode was a catheter-type glass pH electrode (Oesophageal pH probe MI-506; Microelectrodes Inc., New Hampshire, USA) with a maximum diameter of 1.6mm. The electrode was used in conjunction with the reference electrode and pH meter used for jejunal pH measurements. The system was calibrated in pH 7 and 4 phthalate buffers prior to the endoscopy session and the electrode was zeroed in pH 7 buffer between individual investigations; on average each endoscopy session provided three or four suitable patients.

Patients were sedated with Diazemuls (KabiVitrum Ltd., UK.; 10-20mg i.v.) immediately before the endoscope was passed. The co-operating physicians examined the patient and concluded with the endoscope in the second part of the duodenum, proximal to the papilla of Vater. At this stage the pH electrode was advanced down the biopsy channel. The reference electrode was inserted into the patient's mouth and held against the buccal mucosa. The pH in six regions of the gastrointestinal tract was then measured (Figure 9). These were the second part of the duodenum (referred to as the duodenal loop), the duodenal cap, the pyloric channel, the greater curvature of the pre-pyloric antrum, the cardia of the fundus on the greater curvature, and 5cm proximal to the Z-line of the gastro-oesophageal junction. Within each region the tip of the electrode was positioned against the mucosa until a stable pH reading could be obtained. The electrode was then removed from the mucosa and allowed to

Figure 9 Positions within the stomach and duodenum from where pH measurements were made at endoscopy.



contact any surrounding luminal fluid. A second mucosal reading was made to obtain an average value of mucosal pH. The procedure was repeated in the next region until all areas had been measured.

Atropinised patients did not have the pH measured in the duodenal loop and those at ERCP (endoscopic retrograde cholangio-pancreatography) had only the duodenal cap and loop measured; the side-viewing JF-1T Olympus endoscope used at ERCP is unsuitable for gastric pH measuring. In addition to a sedative (Diazemuls), ERCP patients also received pethidine (Roche; 50mg i.v.), a short lasting analgesic.

3 vi) Statistical analysis

Examination of past and present papers on gastro-intestinal pH will reveal several different ways of expressing pH data for statistical analysis. Confusion exists (19,119) about the correct way to analyse the data because pH is a logarithmic term. When pH is converted into hydrogen ion activities the distribution of data may become skewed. It is often assumed that because this skew is present in the transformed data, it is unjustifiable to treat the pH data as a normally distributed sample. However, the response of a pH electrode, which is described by the Nernst equation, is linearly related to the log of the hydrogen ion activity of the solution under examination (120) and will be normally distributed when randomly sampled (121). Therefore it is quite justifiable to use statistics for normal distributions when analysing pH data. That is not to say that pH data cannot be skewed, since

under physiological conditions, non-normally distributed data sometimes arise. The occurrence of skew on the distribution of pH data can be dealt with using non-parametric statistics.

Statistical treatment of pH data collected from the clinical investigations in this project was as follows. Each group of pH values was tested for the presence of significant skew and kurtosis. Comparisons of all patient groups was by "Student's" t-test if no abnormality in the sample distributions was detected. Tests on the equality of variance between patient groups was performed using the F-test statistic and the appropriate t-test using either pooled or separate variances was used. If any one group of values was not normally distributed, all comparisons with that group were performed using Mann-Whitney or chi-squared non-parametric statistics. Stated values in the text are the mean value for a disease group variable and the standard error of that mean while given significance levels refer to t-tests, unless otherwise stated. Implementation of all statistical analysis was by BMDP (122) and Minitab (123) programmes available on an ICL 2976 computer.

CLINICAL RESULTS

Using the methods described in the preceding section, it was possible to measure the mucosal surface pH from the oesophagus to the proximal jejunum in humans *in vivo*. Because the study was not restricted to measuring mucosal surface pH in healthy control patients but was also extended to examine diseased patients, it is possible to assess if any changes in mucosal surface pH occur in disease states. The following analysis of results starts with the jejunal surface pH measurements and continues with a more extensive section on gastro-duodenal surface pH measurements.

4 i) Proximal small bowel surface pH.

In this study, the mucosal surface pH was continuously measured from the proximal duodenum to the proximal jejunum. The duodenal mucosal surface pH (Figure 10) measured at jejunal biopsy in a series of control patients without functional gastrointestinal disease was $6.76 \mp 0.16(9)$, not significantly different from neutrality. As the electrode progressed down the proximal intestine, the pH fell towards more acid values. The jejunal surface pH (Figure 11), measured at the site of biopsy, was 5.90 ∓ 0.10 in nine control patients and was significantly ($p<0.001$) more acid than neutrality. These results show that a pH gradient exists down the proximal small bowel of control patients, the pH becoming more acid the further along the bowel the electrode records. No inferences about the surface pH of more distal regions can be made from this study, although there is no reason why this system for

Figure 10. Duodenal surface pH measured at jejunal biopsy. Coeliac patients were either on a normal diet (ND) or a gluten free diet (GFD). Number of patients in parentheses.

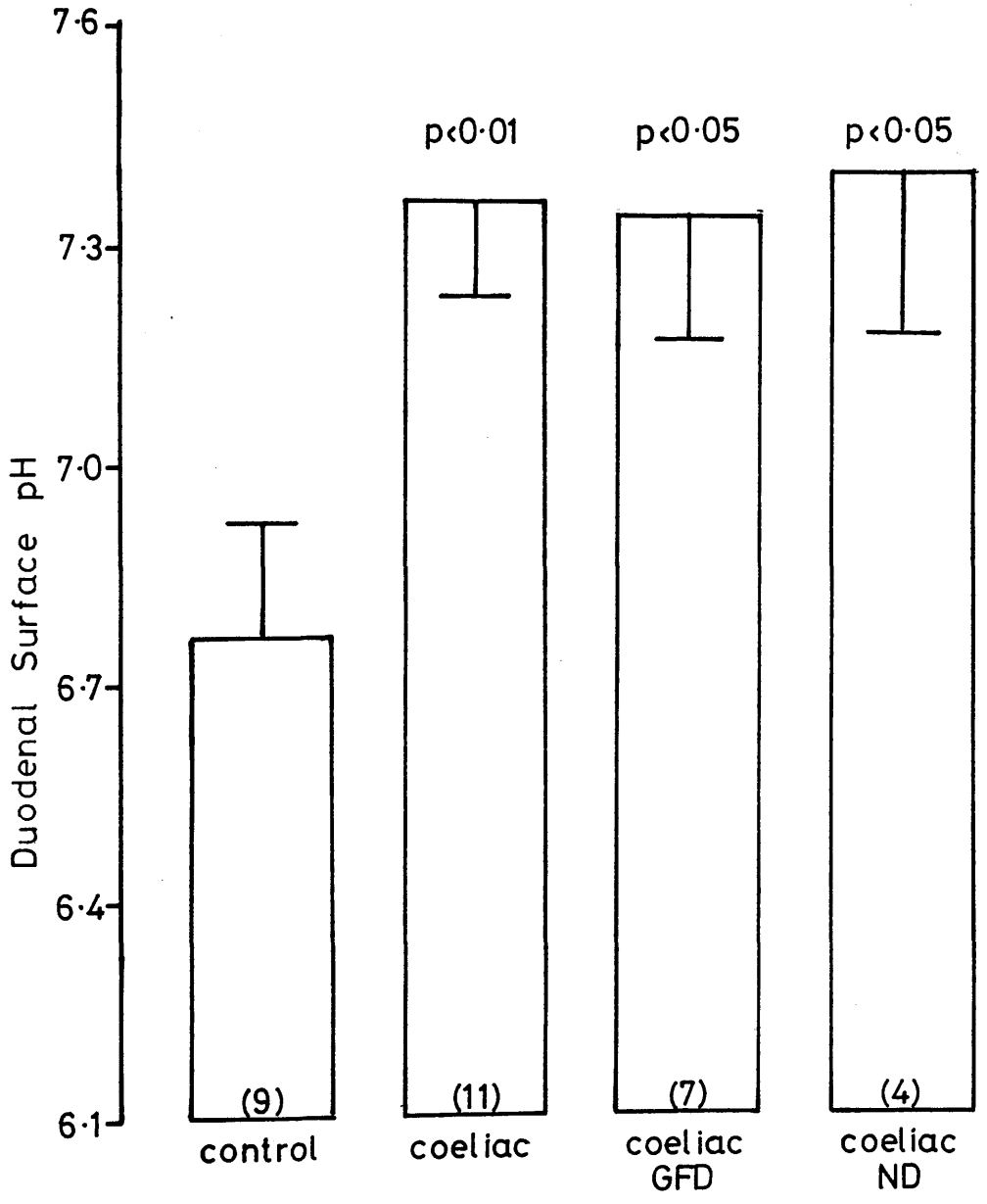
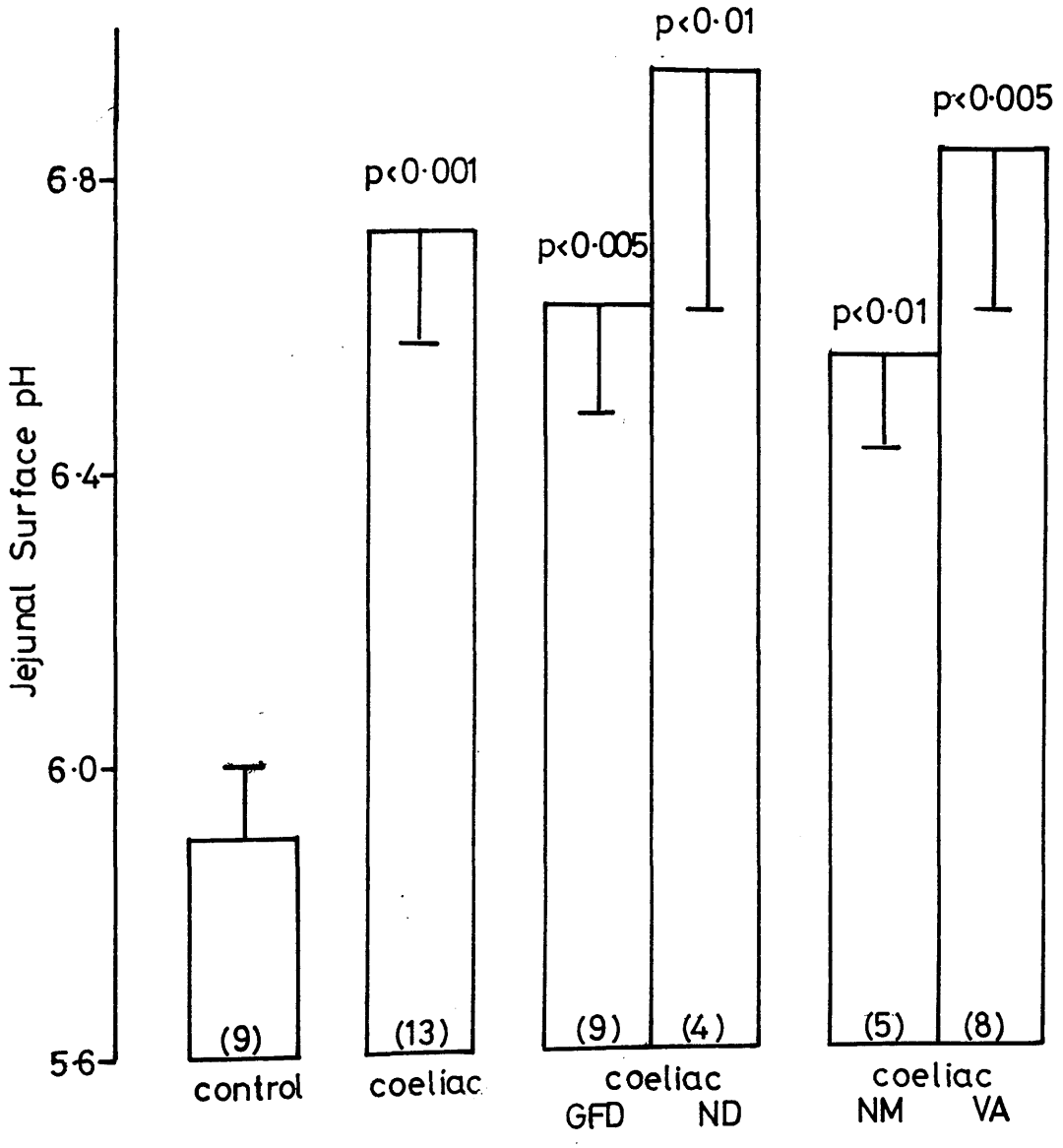


Figure 11. Jejunal surface pH measured at jejunal biopsy in control and coeliac patients. The coeliac group consists of patients on a gluten free diet (GFD) or a normal diet (ND) and patients with a normal mucosa (NM) or with villous atrophy (VA). Number of patients in parentheses.



measuring surface pH cannot be extended to measuring ileal mucosal surface pH.

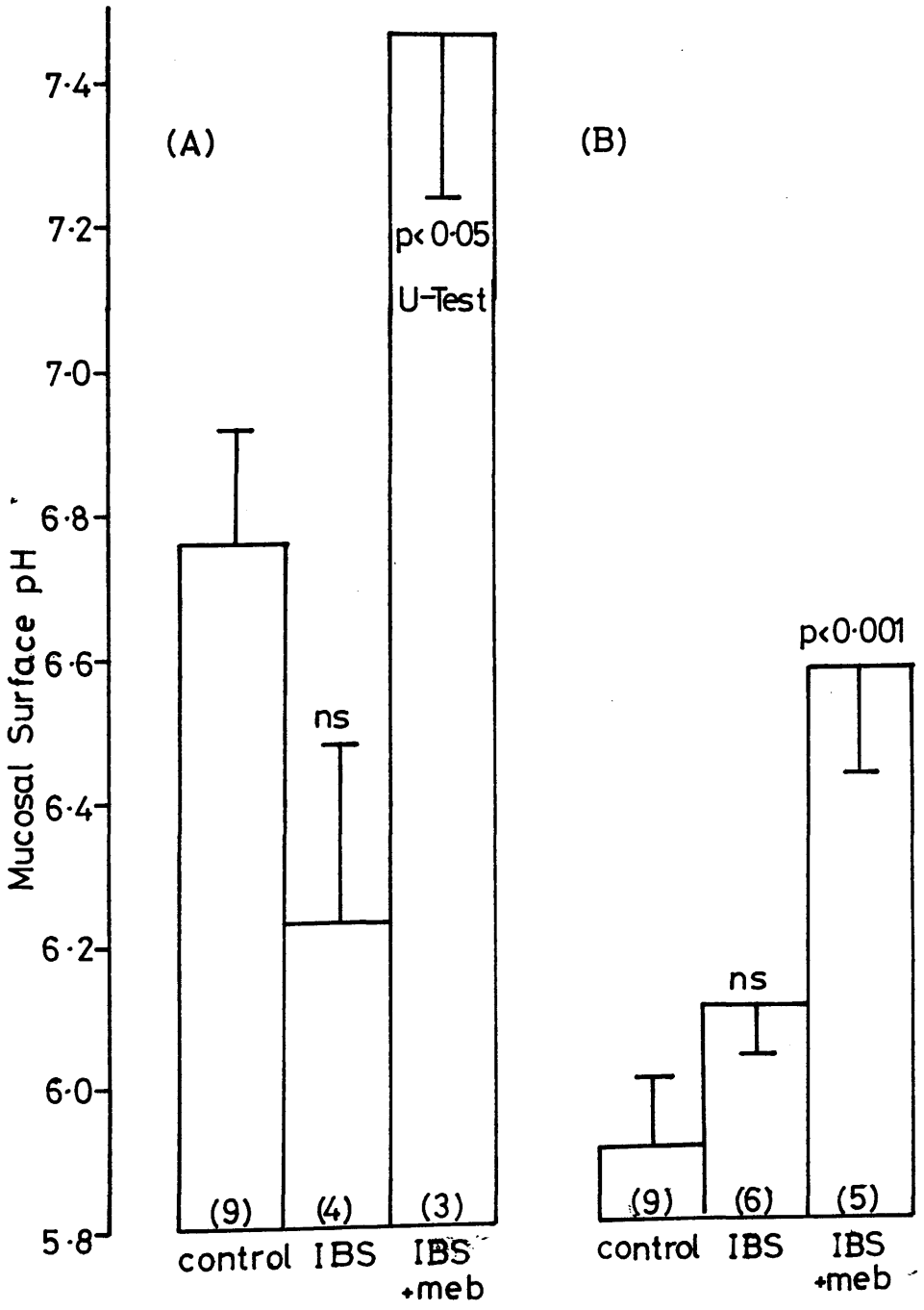
Coeliac patients are a suitable group of people for comparison with the control state since the disease is associated with intestinal derangement. The stunted jejunal villi characteristic of coeliac disease respond favourably to a gluten-free diet. This allows a distinction to be made between treated and untreated patients. The duodenal mucosal surface pH (7.36$\mp$0.13) in eleven coeliac patients was significantly ($p<0.01$) more alkaline than that of control patients (Figure 10). No difference between patients on (7.34$\mp$0.17(7)) or off (7.40$\mp$0.22(4)) a gluten-free diet could be established. In the jejunum, coeliac patients had a mean surface pH of 6.73$\mp$0.15(13) which was not significantly different from neutrality and was significantly ($p<0.001$) less acid than the control group (Figure 11). Four of the coeliac patients on a normal diet had an almost neutral surface pH of 6.95$\mp$0.33 which was significantly ($p<0.01$) more alkaline than the control group. However, the remaining nine treated coeliac patients also showed a significantly ($p<0.005$) elevated jejunal surface pH of 6.63$\mp$0.15(9) when compared with the control group.

An alternative sub-division of the coeliac patient data is by the appearance of the mucosa. Eight coeliac patients with partial or sub-total villous atrophy had a jejunal surface pH (Figure 11) of 6.84$\mp$0.22 which was more alkaline ($p<0.005$) than the control value. The remaining five

patients with a mucosa of normal appearance were still significantly ($p<0.01$) more alkaline ($6.56\mp0.13(5)$) than the control subjects. From this data it appears that alterations in surface pH persist despite coeliac patients having a structurally normal jejunal mucosa.

A third, relatively large, group of patients was identified from within the experimental population. These patients had irritable bowel syndrome (IBS) which, although not associated with tissue structural damage, is often characterised by irregularities in bowel movements and stool consistency. In the duodenum (Figure 12a), the mucosal surface pH of four IBS patients was 6.23 ∓ 0.25, not significantly different from the duodenal surface pH of control patients. Unlike control patients, there was not such a noticeable pH gradient from the duodenum to the jejunum where a mean surface pH of $6.10\mp0.07(6)$ was recorded. The jejunal surface pH was not significantly different from control values (Figure 12b). In addition to the six IBS patients on a high fibre diet, five further IBS patients were also taking mebeverine (Colofac; Duphar Laboratories Ltd.), an anti-spasmodic drug for reducing intestinal motility, prior to investigation. In this sub-group of patients, the duodenal surface pH ($7.47\mp0.23(3)$) was significantly ($p<0.05$; U-test) more alkaline than control duodenal pH values and significantly ($p<0.03$) more alkaline than the duodenal surface pH of IBS patients not receiving mebeverine. In the jejunum, the mebeverine treated group of patients (Figure 12b) had a mean jejunal surface pH of $6.58\mp0.15(5)$ which was significantly ($p<0.01$)

Figure 12. (A) Duodenal and (B) jejunal mucosal surface pH of patients with irritable bowel syndrome (IBS) either with or without mebeverine (meb) pretreatment. Number of patients in parentheses.



higher than the mean of 6.10∓0.07(6) from IBS patients not treated with mebeverine. It was also significantly ($p<0.001$) less acid than control values for jejunal surface pH. This suggests that either the local anti-spasmodic effects of mebeverine or some other property of the drug can influence the mucosal surface pH.

The measurements of mucosal surface pH have been expressed only in relation to the appearance of the jejunal tissue and with respect to disease groups. Jejunal tissue derangement can be associated with changes in functional activity of the tissue; patients with active coeliac disease invariably have lower than normal disaccharidase levels. Throughout this jejunal surface pH investigation, samples of biopsied tissue were assayed for lactase, maltase and sucrase activities (Table 2). The measured activities are an alternative index of tissue function with which surface pH values can be correlated. There were significantly ($p<0.01$) lower than normal levels of lactase, maltase and sucrase activity in the untreated coeliac patients and patients with villous atrophy. Patients on a gluten-free diet had higher ($p<0.05$) maltase and sucrase activities than patients on a normal diet. Patients with irritable bowel syndrome had normal enzyme activities and there was no effect of mebeverine on enzyme activity.

On combining data from all patients, jejunal surface pH correlated inversely with lactase ($r=0.56$; $p<0.01$), but not with maltase ($r=0.29$) or sucrase ($r=0.29$) levels. Improved correlations were found when the logarithmic values for enzyme activities were used. Significant correlation was

TABLE 2 DISACCHARIDASE ACTIVITIES OF TISSUE EXCISED AT JEJUNAL BIOPSY

	LACTASE	MALTASE	SUCRASE
Control(all)	5.14∓1.21(9)	25.96∓4.16(9)	6.57∓1.20(9)
Coeliac(all)	1.42∓0.46(13)*	15.79∓3.68(13)	4.17∓0.95(13)
Coeliac(GFD)	1.82∓0.62(9)**	20.02∓4.53(9)	5.32∓1.12(9)
Coeliac(ND)	0.50∓0.15(4)*	6.25∓3.03(4)*	1.58∓0.95(4)*
Coeliac(NM)	2.60∓0.98(5)	26.66∓6.66(5)	6.90∓1.58(5)
Coeliac(AM)	0.68∓0.19(8)*	8.99∓2.15(8)*	2.46∓0.73(8)*
Irritable Bowel	6.28∓1.08(6)	30.63∓4.70(6)	5.86∓0.90(5)
Irritable Bowel + meb	3.32∓1.32(5)	30.46∓4.64(5)	8.04∓1.02(5)

GFD=Gluten-Free Diet, ND=Normal Diet

NM=Normal Mucosa, AM=Abnormal Mucosa

meb=mebeverine.

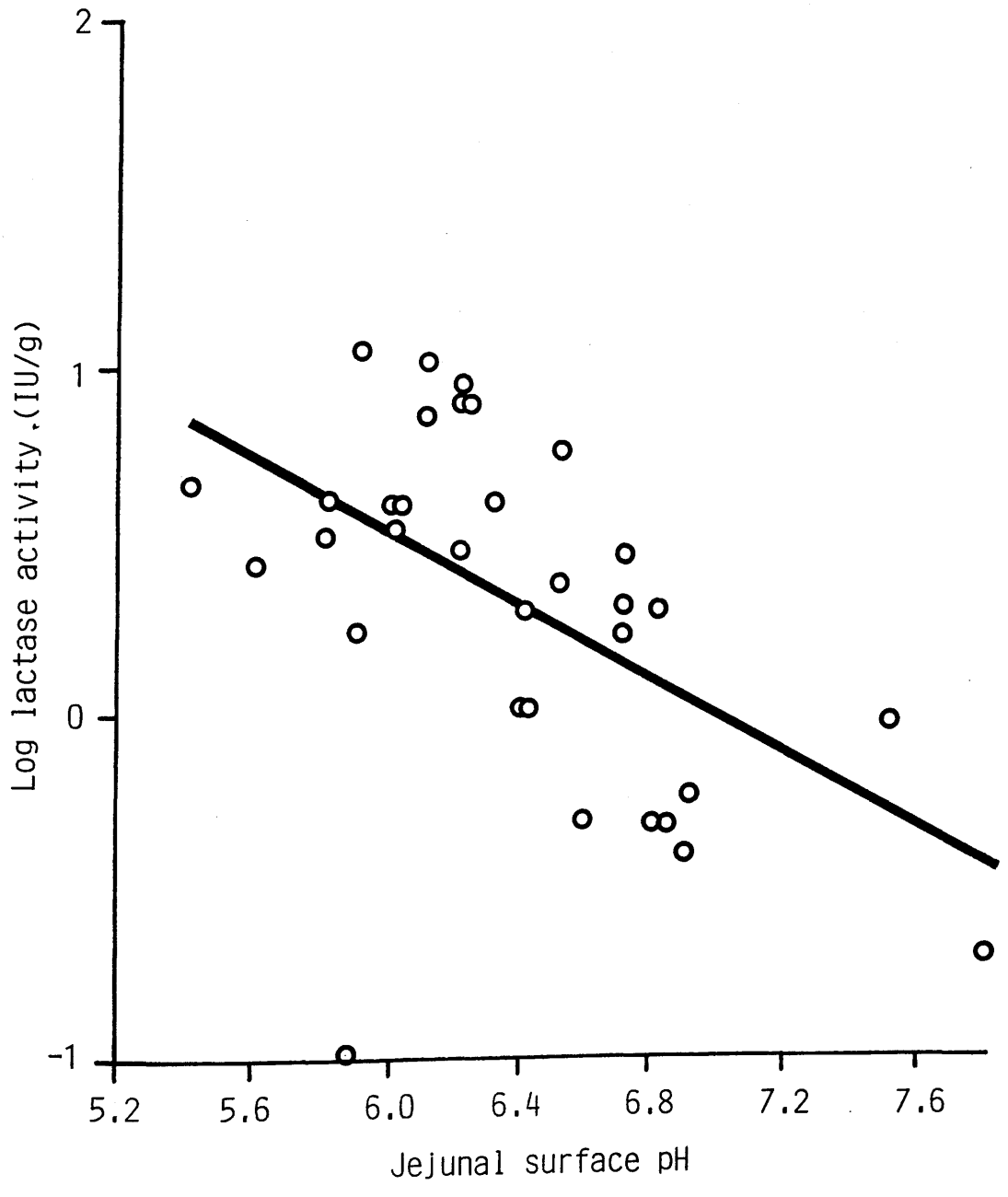
* $p<0.01$; ** $p<0.05$

observed when log values for maltase ($r=0.53$; $p<0.01$) and sucrase ($r=0.46$; $p<0.01$) were plotted against jejunal surface pH; taking the log of lactase activity (Figure 13) had little effect on the calculated correlation coefficient. No correlation was seen in the coeliac group alone. The apparent correlation of surface pH with jejunal enzyme activity does not imply that one is the result of the other. However, it does suggest that a disease which results in reduced disaccharidase activity due to tissue damage in the jejunum may also be associated with an elevated surface pH.

In addition to the three groups of patients already described, individual cases or small groups of patients with gastrointestinal disease were investigated. Alterations in surface pH were most noticeable in patients with inflammatory bowel disease. Three patients with long-standing Crohn's disease had a raised jejunal surface pH ($6.67\mp0.27(3)$), as did a patient with ulcerative colitis (pH 6.8). In comparison, two patients with dermatitis herpetiformis had rather acid surface pHs (pH 5.3 & 5.7) and a patient with post-vagotomy diarrhoea had a surface pH of 5.4. A patient with oral Crohn's which did not affect the intestine had an acidic jejunal surface pH of 5.5. Thus, the occurrence of an elevated surface pH appears not only to be associated with intestinal structural damage eg. coeliac disease, but also with inflammatory diseases such as Crohn's disease and ulcerative colitis.

Figure 13. The relationship between jejunal surface pH and Log lactase activity (International units per gram wet weight tissue (IU/g)) measured in all patients.

$r = -0.541$; $n = 33$; $p < 0.01$



This section of the study has shown that small bowel tissue derangement can result in changes of duodenal and jejunal surface pH. Can such changes in surface pH be seen in gastric and proximal duodenal diseases?

4 ii) Gastric and proximal duodenal surface pH.

Using the jejunal biopsy procedure it was possible to measure proximal intestinal surface pH but not gastric mucosal pH values. This was for two reasons: within the stomach the electrode could not be maintained against the gastric mucosa and secondly, the plastic pH electrode would not respond below pH 4. However, it is possible to measure gastric surface pH at endoscopy using a glass pH electrode. The glass pH electrode can be seen to touch the mucosa (Figure 14) and it can be maintained in the appropriate position for as long as is necessary to obtain a stable recording of pH. Unlike mucosal pH, obtaining luminal pH readings was more difficult. When patients were placed on their left side for endoscopy, any gastric juice in the stomach pooled in the fundus. Consequently, there was never any difficulty in obtaining a fundal luminal fluid measurement. In areas other than the fundus it was not always possible for the electrode to make contact with luminal fluid and the recorded pH slowly drifted upwards, providing a source of alkaline error. Consequently, some caution is required when considering luminal values.

When mucosal and luminal pH readings (Appendices 3 & 4) are compared, a difference between recorded values can be seen in all regions of the stomach and duodenum (Figure 15)

Figure 14. A glass pH electrode making contact with the duodenal mucosa to obtain a surface pH reading at endoscopy.

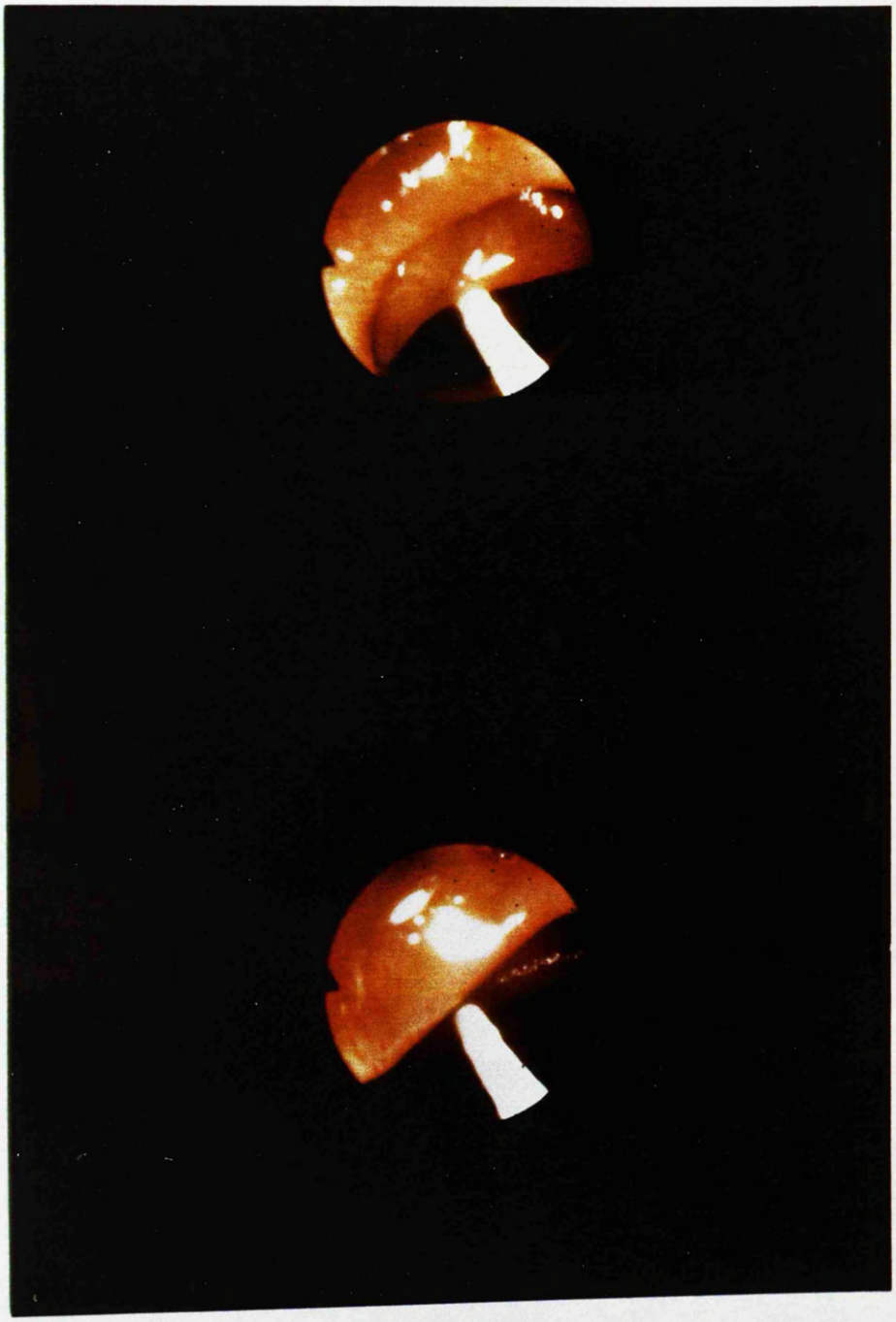
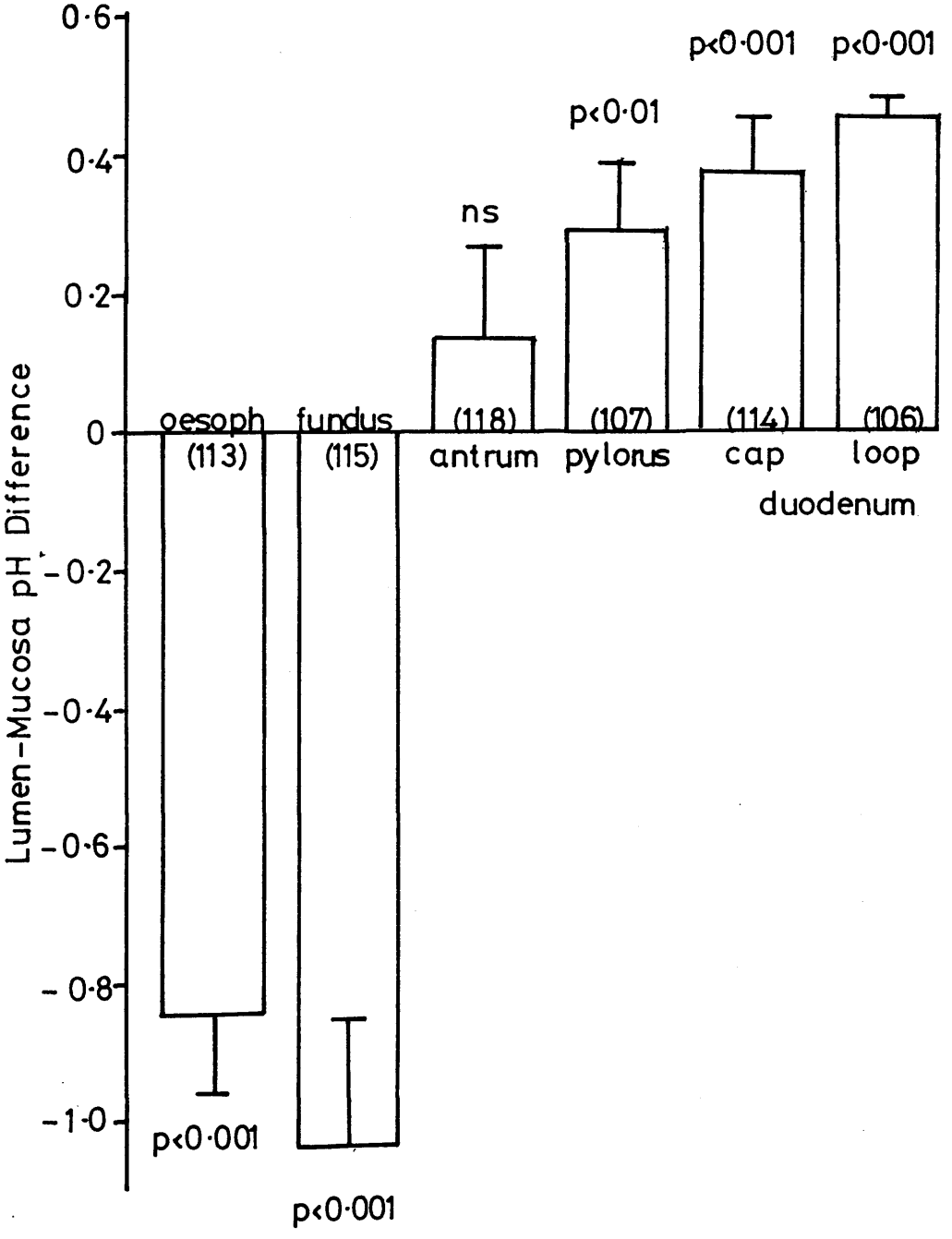


Figure 15. Lumen-mucosa pH differences measured in all investigated regions. Number of patients in parentheses. Probabilities refer to a significant deviation from zero.



and in all patient groups (Table 3,4 & 5). Thus, the mucosal surface pH can be, and invariably is, different to the pH of the fluid perfusing the tissue. A significant correlation ($p<0.01$) exists between lumen and surface pH in all regions (Table 6) although this does not necessarily imply that one is the result of the other.

The distribution of fundal luminal pH (Figure 16) spans a wide range of pH values although it appears that there is an "acid secreting" and a "resting" population within the total distribution. Acid is implicated as a causative factor of ulceration and therefore acid is most likely to provoke a mucosal response. For this reason, an alternative way of analysing the data was to sub-divide the patients into those with a fundal lumen pH above or below pH 3. The value of pH 3 was an arbitrarily selected value, although any other cut-off point could have been used. For ease of description, patients with a pH below 3 are referred to as acid secreting patients. This partitioning of the results in no way implies that the patients with a low luminal pH secrete excess acid, only that at the time of endoscopy these patients had acid in the fundus.

ii a) Patients with no upper gastrointestinal abnormalities

Twenty four patients with no gastric or duodenal abnormalities seen at the time of endoscopy were selected as a control group for comparison with patients with gastrointestinal disease. The measured surface pH in these control patients ranged from a maximum of pH 8.1 in the antrum and pylorus to a minimum of pH 0.95 in the fundus. There was a pH gradient from the fundus to the duodenum

TABLE 4 LUMEN-MUCOSA pH DIFFERENCES OF PATIENT GROUPS MEASURED AT ENDOSCOPY

Patient Group	Antrum	Pylorus
Normal	0.18∓0.24(24)	0.13∓0.20(21)
Gastritis	0.98∓0.46(6)	0.40∓0.26(6)
Gastric Ulcer	0.01∓0.38(20)	0.22∓0.21(18)
Duodenitis	-0.31∓0.36(14)	-0.06∓0.55(11)
Duodenal Ulcer	0.40∓0.27(22)	0.52∓0.23(20)*
Hiatus Hernia	-0.90∓0.62(4)	0.38∓0.14(5)
Oesophagitis	-0.33∓0.50(16)	0.41∓0.22(16)
Pernicious Anaemia	0.81∓0.15(5)**	0.74∓0.15(5)**

* $p<0.05$; ** $p<0.02$

significantly different from zero

TABLE 5 LUMEN-MUCOSA pH DIFFERENCES OF PATIENT GROUPS MEASURED AT ENDOSCOPY

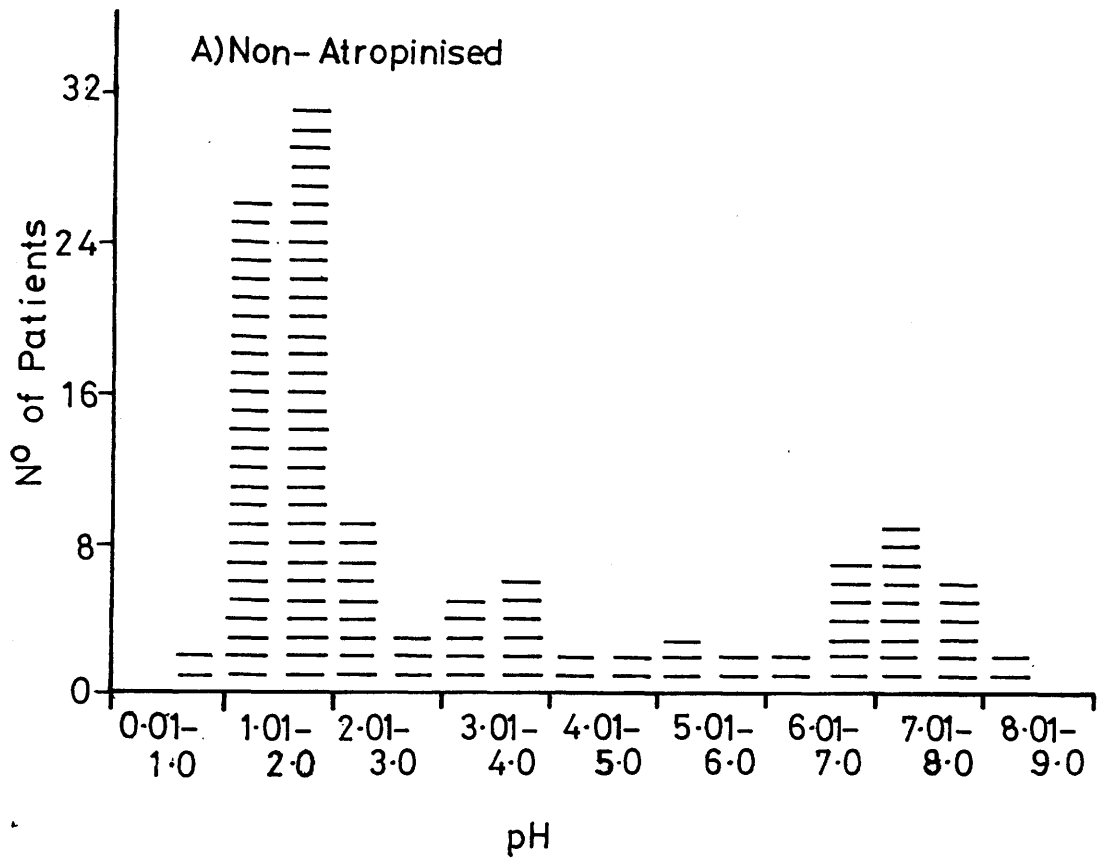
Patient Group	Duodenal Cap	Duodenal Loop
Normal	$0.60 \mp 0.12(23)^{***}$	$0.48 \mp 0.07(22)^{***}$
Gastritis	$-0.51 \mp 0.94(6)$	$0.43 \mp 0.08(4)^{**}$
Gastric Ulcer	$0.44 \mp 0.06(18)^{***}$	$0.40 \mp 0.09(18)^{***}$
Duodenitis	$0.47 \mp 0.10(14)^{***}$	$0.41 \mp 0.10(12)^{*}$
Duodenal Ulcer	$0.20 \mp 0.21(21)$	$0.40 \mp 0.08(18)^{***}$
Hiatus Hernia	$0.37 \mp 0.23(5)$	$0.39 \mp 0.15(5)$
Oesophagitis	$0.28 \mp 0.35(16)$	$0.54 \mp 0.12(13)^{***}$
Pernicious Anaemia	$0.70 \mp 0.06(5)^{***}$	$0.64 \mp 0.14(5)^{**}$

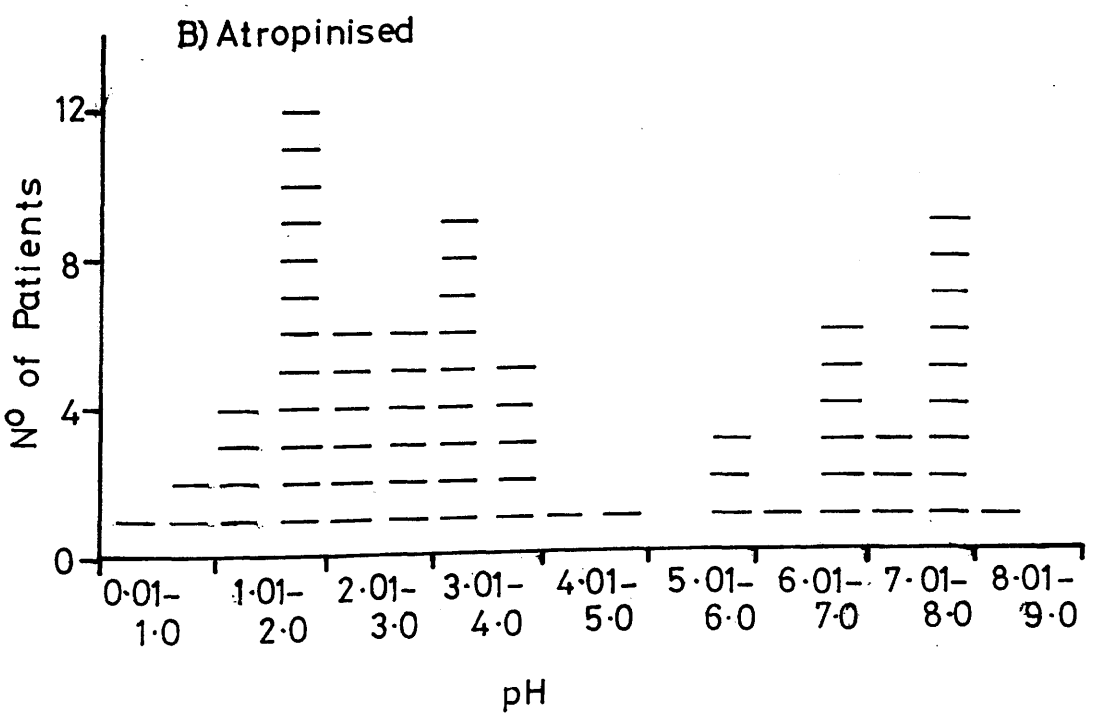
* $p<0.02$; ** $p<0.01$; *** $p<0.001$
significantly different from zero

TABLE 6 LUMEN-MUCOSA CORRELATION COEFFICIENTS

GROUP	COEFFICIENT	NUMBER	SIGNIFICANCE
Oesophagus	0.821	113	$p<0.01$
Fundus	0.608	115	$p<0.01$
Antrum	0.824	118	$p<0.01$
Pylorus	0.801	107	$p<0.01$
Duodenal cap	0.337	114	$p<0.01$
Duodenal loop	0.359	104	$p<0.01$

Figure 16. Frequency distribution histograms of fundal lumen pH in (A) non-atropinised and (B) atropinised patients investigated at endoscopy.





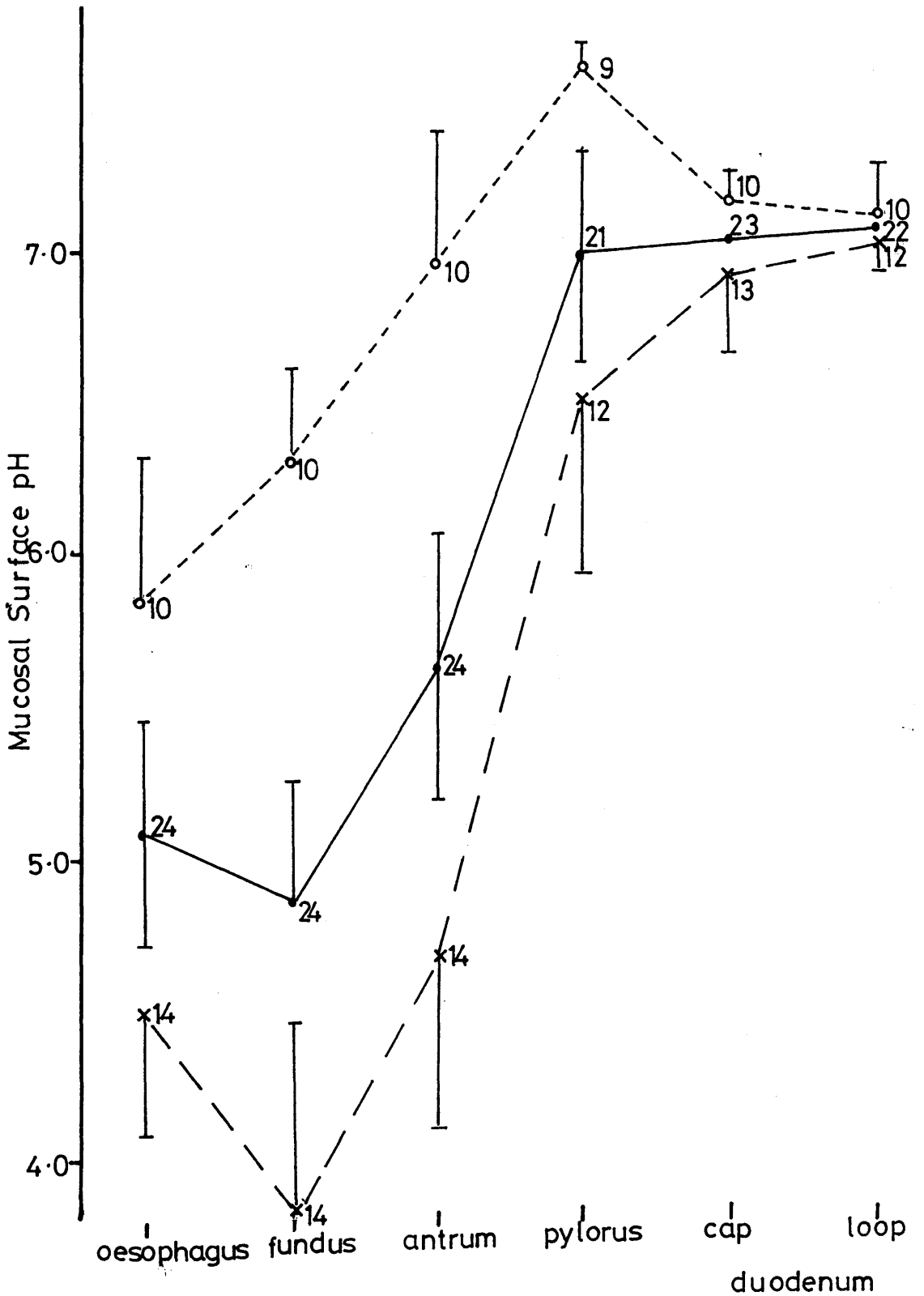
(Figure 17). The mean fundal pH of 4.86∓0.46(24) was more acid than the mean antral (5.63∓0.45(24)), pyloric (6.99∓0.35(21)) and duodenal cap (7.05∓0.16(23) and loop (7.09∓0.09(22)) surface pHs.

For control patients without acid in the fundus (fundal lumen pH 5.86∓0.59(10)), the mucosal surface pH from the fundus to the duodenum was close to neutrality. In contrast, when an acid fundal lumen (1.85∓0.14(14)) was present, the fundal (3.82∓0.63(14)) and antral (4.67∓0.58(14)) mean surface pHs were significantly ($p<0.001$ & $p<0.01$ respectively) more acidic than neutral. These results indicate that the stomach cannot maintain a neutral mucosal pH when acid is in the stomach.

However, duodenal mucosal pH was near neutral and very similar in both acid and non-acid secreting sub-groups of the control population. The luminal pH was more alkaline than the mucosa in the pylorus ($p<0.001$), duodenal cap ($p<0.01$) and duodenal loop ($p<0.05$) when no acid was present in the stomach. When acid was present, only the luminal pH in the duodenal loop was more alkaline ($p<0.01$) than the mucosal pH. Thus the duodenum is capable of maintaining a neutral surface pH while the stomach secretes acid and when the fluid in contact with the mucosa is alkaline. Whether a neutral surface pH can be maintained at the duodenal mucosa when the tissue is directly challenged with acid cannot be determined from this study.

The duodenal mucosal pH was also neutral when measured at ERCP. The purpose of this pilot study was to see what

Figure 17. Mucosal surface pH of all investigated areas of control patients (•) and in control patients with a fundal lumen pH ⩽ 3 (X) or pH > 3 (o). Numbers refer to the number of patients in each group.



effect a reduction or a complete absence of biliary bicarbonate would have on duodenal surface pH. The surface pHs in the duodenal cap (7.06∓0.07(22)) and duodenal loop (6.91∓0.09(22)) were not significantly different from pH 7. A comparison between these results and those obtained at endoscopy is problematical because of the different experimental conditions under which the results were obtained. However, it is interesting to note that the ERCP results are remarkably similar to the duodenal cap (7.05∓0.16(23)) and duodenal loop (7.09∓0.09(22)) results obtained from control subjects at endoscopy.

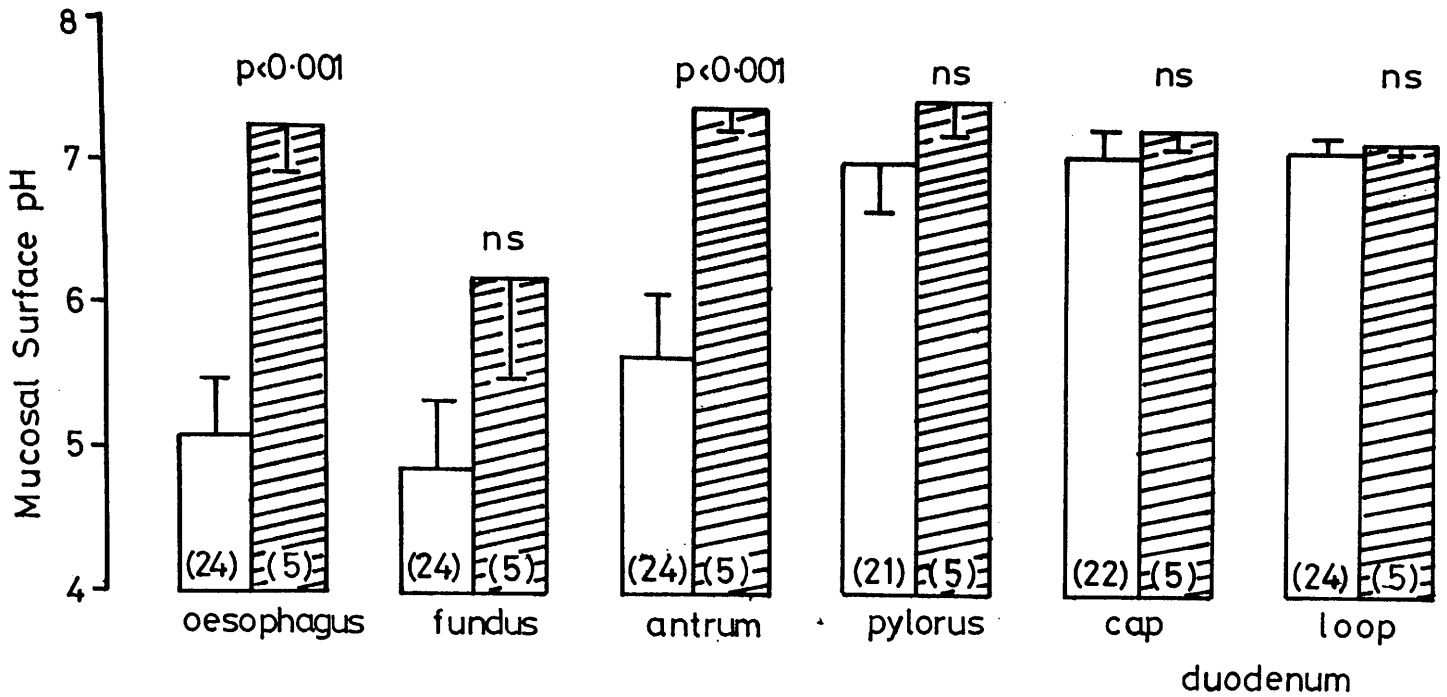
Since the patients at ERCP generally had common bile duct obstruction but little else wrong with them, ie. normal upper gastrointestinal function, the values measured in the duodenum strengthen the impression that normal duodenal surface pH is neutral. Further, bile secretion does not necessarily have to occur for the maintainence of a neutral surface pH and therefore the surface pH may probably be determined primarily through mucosal mechanisms. To investigate this further, it would be necessary to measure the duodenal surface pH in patients with both biliary and pancreatic duct obstruction to eliminate any influences exerted by pancreatic bicarbonate on duodenal mucosal pH.

The influence of acid on mucosal surface pH was always a factor which had to be considered. A separate group of five patients with pernicious anaemia were investigated to determine what effect the total absence of acid would have

on the gastric and duodenal surface pH. For comparison, the fundal lumen pH in the pernicious anaemia group (7.08∓0.16(5)) was significantly ($p<0.01$) higher than in non-acid secreting control patients (5.86∓0.59(10)). The mucosal pH of all areas measured (Figure 18) in pernicious anaemia patients was not significantly different from neutral. However, the possibility that pernicious anaemia may itself affect the mucosal pH cannot be discounted and these results should be considered with caution. A possible avenue for further investigation would be to measure the gastric mucosal pH in pernicious anaemia patients before and after bathing the tissue with acid. The ability of the mucosa to neutralise the acid could then be assessed and compared with earlier findings from control endoscopy patients.

Neural control of gastric acid and pepsinogen secretion is mediated through vagal stimulation. It is also suggested (124) that bicarbonate secretion by the mucosa is partially controlled by stimulation of vagal nerve fibres. By reducing or inhibiting vagal stimulation with atropine, it is possible to examine what effect this has on mucosal surface pH. For this part of the study, fifteen patients were selected as a control group for the atropinised series. A pH gradient from the fundus to the duodenum, similar to that seen in the non-atropinised series, was also present in the atropinised patients. Although there was an underlying trend towards more alkaline surface pH values in the atropinised series of control patients, there were no significant differences in surface pH when mean pH

Figure 18. Mucosal surface pH of all investigated areas in control (open) and pernicious anaemia (shaded) patients. Number of patients in parentheses.

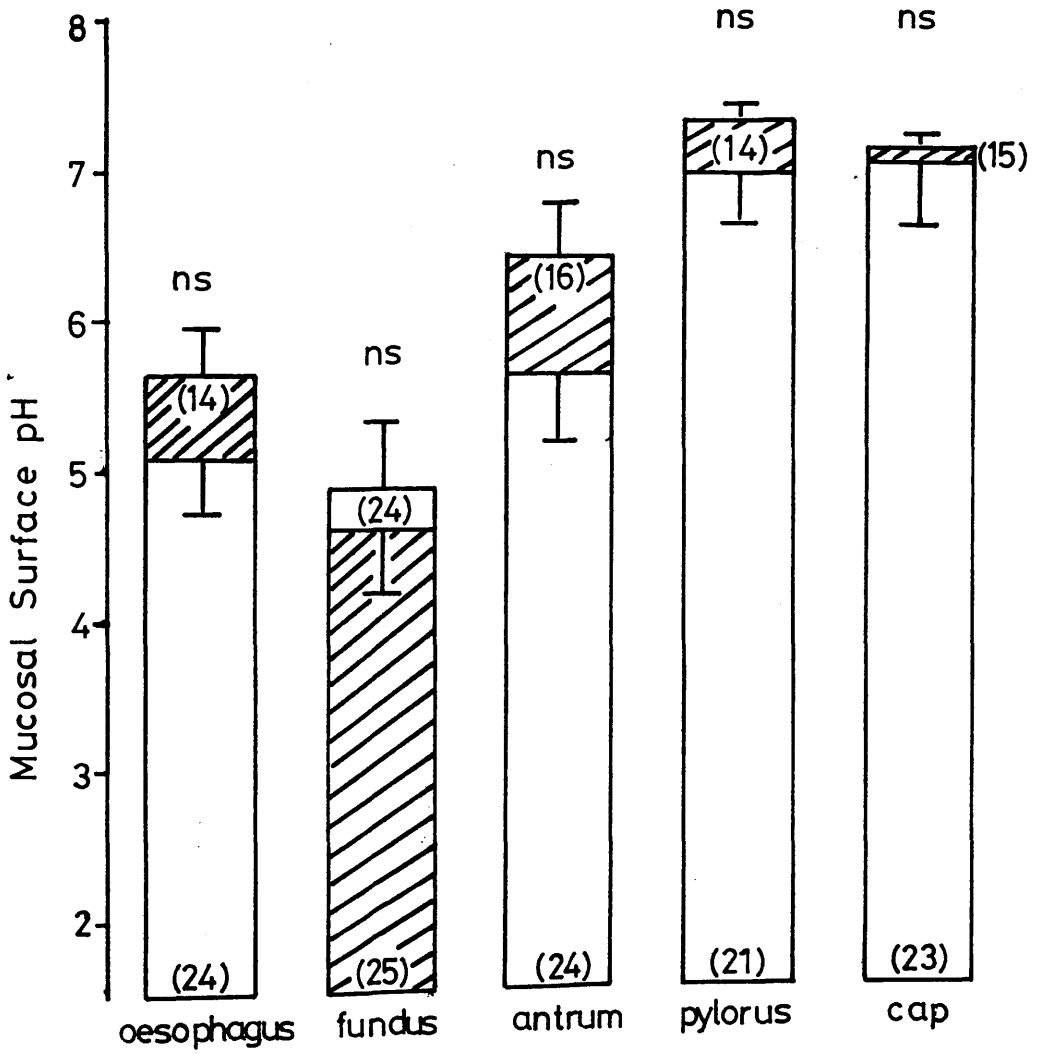


values from atropinised and non-atropinised patients were compared (Figure 19).

Out of the total of fifteen atropinised control patients, only four patients had a fundal lumen less than or equal to pH 3. Comparing patients with and without acid in the fundus, fundal surface pH was very similar in both groups. However, when no acid was present, the surface pH of the fundus in the atropinised series was lower ($p<0.001$) than in the non-atropinised series of patients. The pyloric mucosal pH seemed to be most affected by atropine. When no acid was present in the fundus, the pyloric surface pH was reduced ($p<0.01$) from $7.62\mp0.08(9)$ in the non-atropinised series to $7.19\mp0.11(9)$ when atropine was given. The reverse situation was found when acid was present in the fundus. Here, pyloric surface pH rose from $6.52\mp0.57(12)$ in the non-atropinised series to $7.58\mp0.25(4)$ in the atropinised series. The luminal pH of the pylorus and duodenal cap were more alkaline ($p<0.01$ & $p<0.02$ respectively) than the mucosa when there was no acid in the stomach. However, when acid was present, these differences could no longer be measured. This was similar to the results from the non-atropinised series. In summary, atropine, in the dose administered, could not be shown to have any concerted effect on luminal or mucosal pH in control subjects.

The preceding results have shown that although a near neutral mucosal surface pH can be measured in the duodenum, despite the presence of acid in the stomach, the gastric mucosal pH does not maintain a neutral microclimate under similar circumstances. If this is what happens in normal

Figure 19. Mucosal surface pH of control patients either with (shaded) or without (open) atropine pre-treatment. Number of patients in parentheses.



patients, what changes in mucosal pH, if any, occur in gastric and duodenal ulcer disease? To answer this question, pH results from patients with peptic ulcer disease were compared with control values.

ii b) Patients with gastric ulcer

From the total population of non-atropinised patients, twenty people had a gastric ulcer which was either active or at some stage of healing. With the notable exception of the antrum, the surface pH of all areas was very similar to that of control patients (Figure 20). In the antrum, the surface pH of 6.96∓0.15(20) in gastric ulcer patients was significantly ($p<0.01$) more alkaline than the mean antral surface pH of 5.63∓0.44(24) measured in control patients. The difference between antral surface pH in the two groups became even more significant ($p<0.005$) when only the acid secreting patients were compared (Figure 21). Here, the antral surface pH of control patients was 4.67∓0.58(14) but in the gastric ulcer group, the antral surface pH (6.83∓0.27(11)) remained elevated and was not significantly different from neutral. This near neutral surface pH could not be attributed to a higher fundal luminal pH since mean fundal luminal pHs in control (1.85∓0.14(14)) and gastric ulcer (1.69∓0.10(11)) acid secreting patients were not statistically different. This was true also for antral luminal pH values in the acid secreting groups.

When the data from gastric ulcer patients without acid in the fundus was examined, a different situation was found. Here, gastric mucosal surface pH did not differ from

Figure 20. Mucosal surface pH in control (closed circles) and gastric ulcer (open circles) patients. Numbers refer to the number of patients in each group.

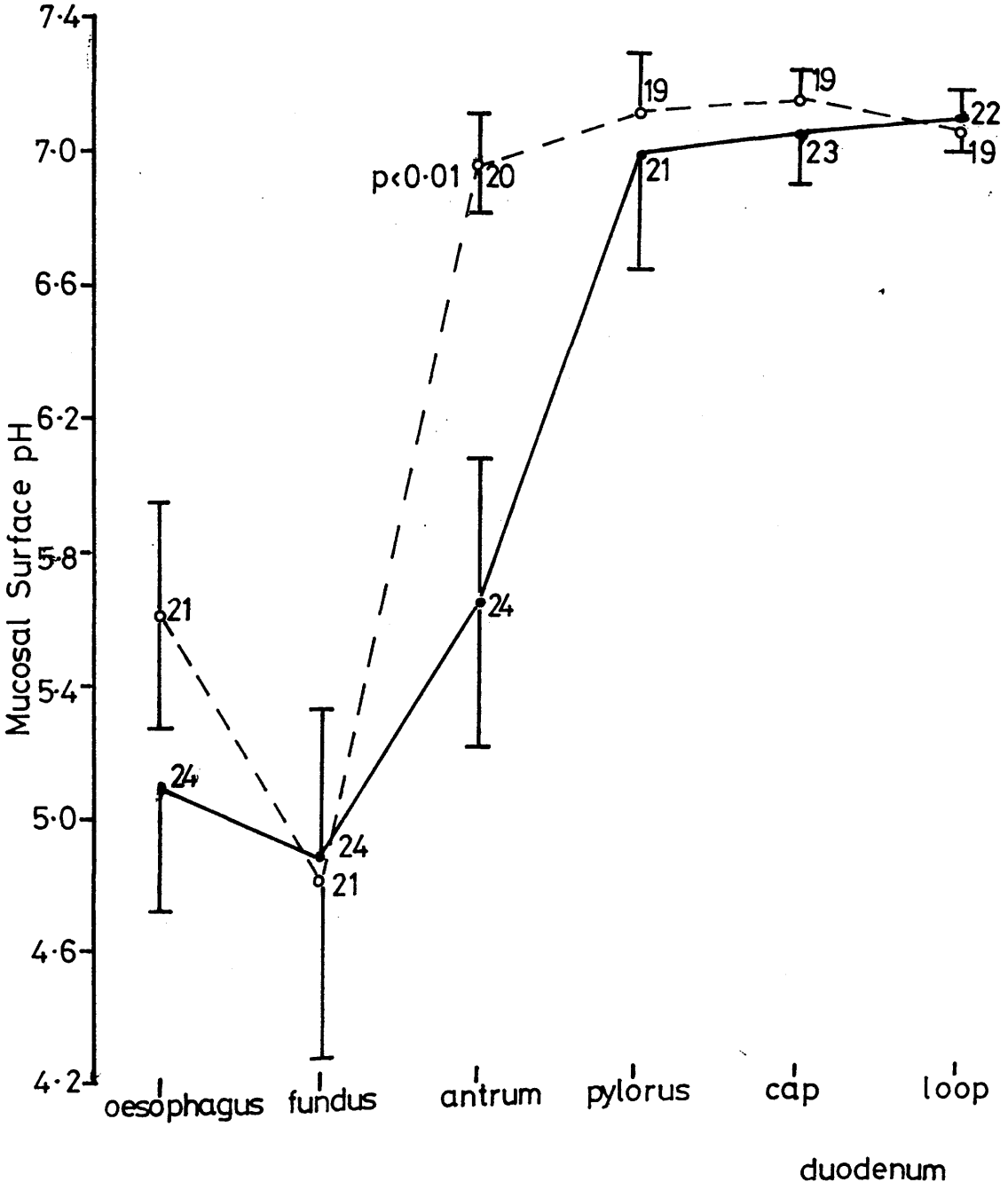
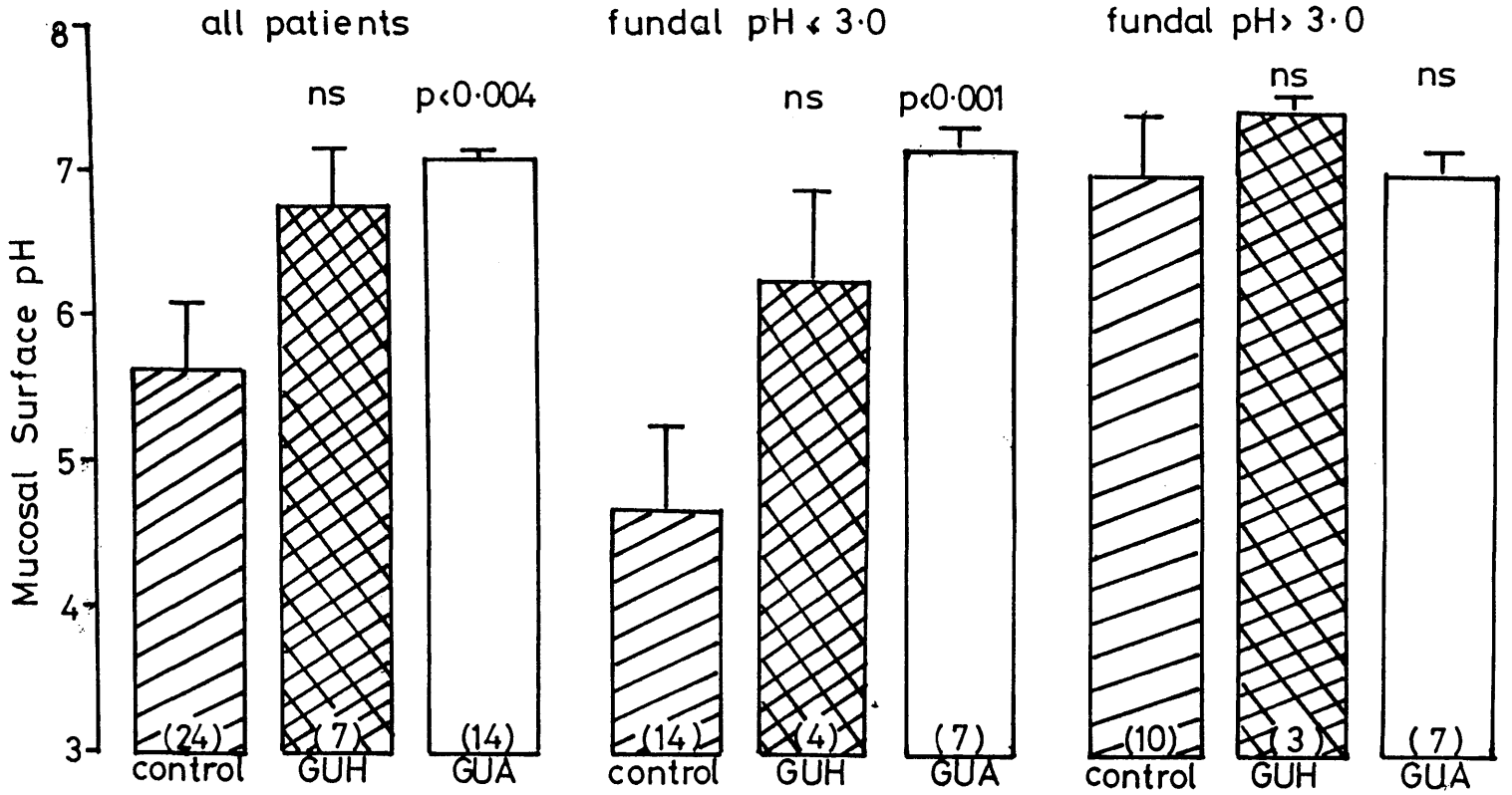


Figure 21. Mucosal surface pH in gastric ulcer patients with healed (GUH) or active (GUA) ulcers, and with a fundal lumen pH ⩽ 3.0 or pH > 3.0. Number of patients in parentheses.



pH values obtained in a comparable control group. Even in the antrum (Figure 21), mucosal surface pH in gastric ulcer patients (7.11∓0.10(10)) was similar to that of control patients (6.97∓0.43(10)). Therefore, the antral surface pH in gastric ulcer disease is broadly neutral and does not differ from that seen in normal subjects when no acid is present in the stomach. It is only when acid is present in the fundus that the striking antral differences are unmasked, with the gastric ulcer group preserving an almost neutral antral surface pH while that of control patients becomes more acid.

Another way of determining whether this antral phenomenon is related to the presence of a gastric ulcer is to sub-divide the group into patients with either active or healing ulcers (Figure 21). After sub-division the antral surface pH of patients with active gastric ulcer disease (7.07∓0.09(14)) is significantly ($p<0.004$) more alkaline than control values. In contrast, the antral surface pH of patients with healing gastric ulcers (6.75∓0.41(7)), although still elevated, is not significantly so. Once again, the presence of acid in the stomach accentuates the antral surface pH difference ($p<0.001$) between active ulcer patients (7.16∓0.16(7)) and control patients (4.67∓0.58(14)). In patients secreting acid with healing gastric ulcers (6.25∓0.62(4)) no significant differences from normal could be detected. However, without acid in the fundus, no difference in antral mucosal pH was noted between active and healing, active and control or, healing and control patient groups. Thus, an elevated antral

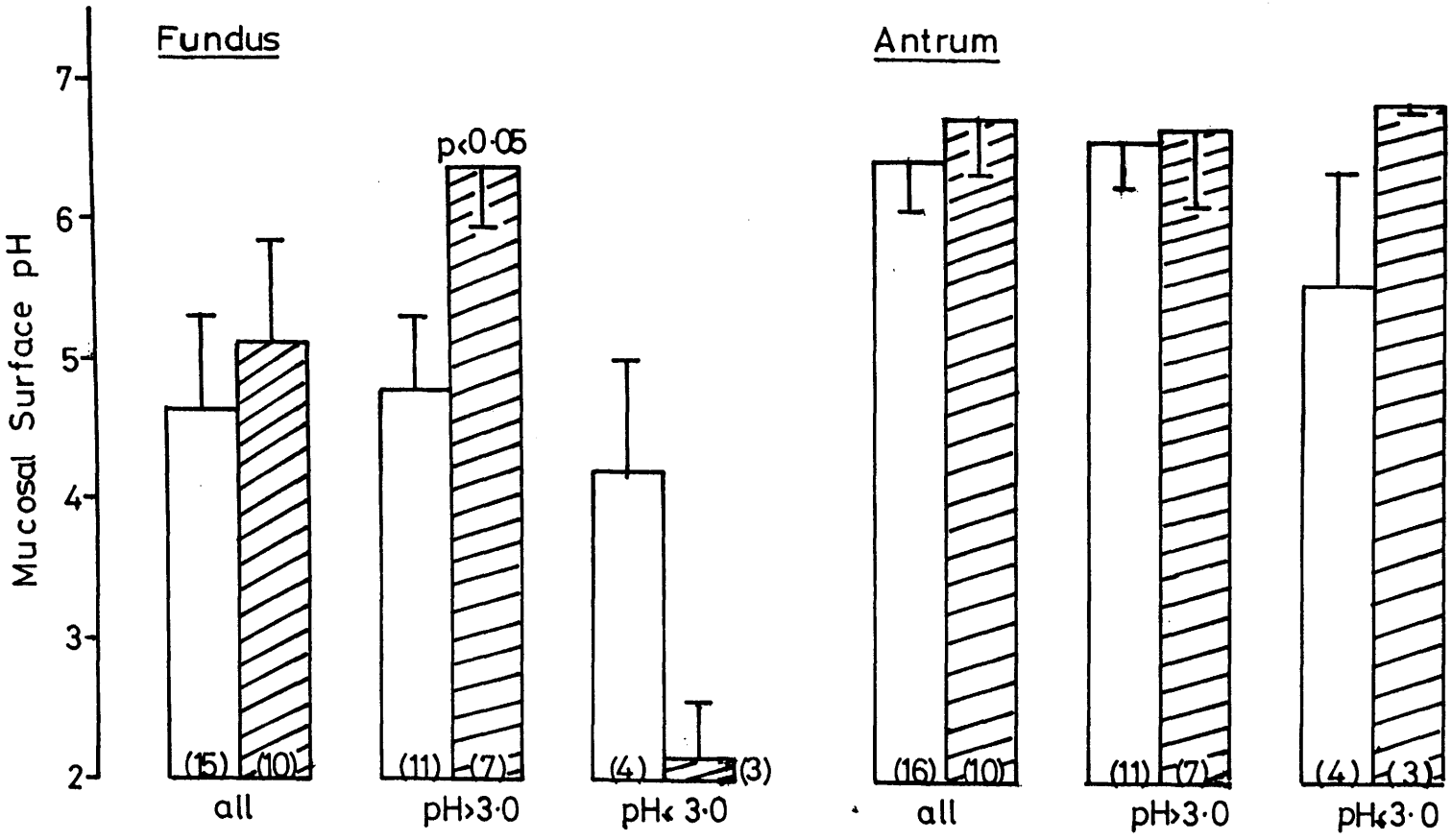
surface pH in gastric ulcer disease was associated with increased ulcer activity and the presence of acid in the stomach.

Gastric ulcers were often associated with gastritis which was either diffuse or localised around the ulcer. Six patients with gastritis only had an antral surface pH of 6.68∓0.30(6) which was not significantly different from the mean control value (5.63∓0.44(24)). But, if only acid secreting patients were considered, then patients with gastritis (6.50∓0.29(5)) had a significantly ($p<0.02$) more alkaline antral surface pH than control subjects (4.67∓0.58(14)).

If the view is taken that gastritis precedes the formation of gastric ulcer, then the evidence presented so far suggests that the antral surface pH becomes more alkaline as the disease progresses from gastritis to fully active gastric ulcer. At the onset of the disease, gastric acid must be present for the antral alkalinity to be detected but as the disease develops, lower levels of acidity are sufficient to stimulate the alkaline secretion of the antrum.

In the atropinised series of patients no significant differences were detected between control and gastric ulcer patients, whether combined into one group or sub-divided into active or healing gastric ulcer. Similarly, the presence of acid in the stomach (Figure 22) had no effect on gastric or duodenal surface pH. However, fundal surface pH of patients without acid in the stomach was higher

Figure 22. Mucosal surface pH in the fundus and antrum of atropinised control (open) and gastric ulcer (shaded) patients. The groups are sub-divided into those with a fundal lumen pH ⩽ 3.0 or pH > 3.0. Number of patients in parentheses.



($p<0.05$) in gastric ulcer patients ($6.39\mp0.45(7)$) than in control patients ($4.77\mp0.53(11)$) without gastric acid. In the antrum of these two patient sub-groups, the mean mucosal surface pHs were almost indistinguishable from each other.

In the case of gastritis patients receiving atropine, five out of the six patients had a fundal lumen above pH 3. Within this group of five gastritis patients, the oesophageal ($7.17\mp0.47(5)$) and fundal ($6.95\mp0.41(5)$) mean surface pH values were significantly ($p<0.05$, $p<0.01$ respectively) more alkaline than control values of pH (oesophagus: $5.70\mp0.38(11)$; fundus: $4.77\mp0.53(11)$). The antral surface pH ($7.33\mp0.29(5)$) of this gastritis group was not significantly different from a control pH of $6.58\mp0.35(11)$.

Atropine pre-medication appears to abolish antral pH differences between control patients and patients with gastric disease. In contrast, with atropine the fundal surface pH becomes more alkaline than normal in gastric disease when no acid is present. The reason for this apparent switch in areas affected is unclear and perhaps requires further investigation.

In summary, gastric ulcer disease is associated with a conspicuously elevated antral surface pH when acid is present in the fundus. If no acid is present, there is no discernable difference in the antral surface pH of normal and gastric ulcer patients, and is broadly neutral. There is no difference in antral surface pH between acid and non-

acid secreting gastric ulcer patients. This antral phenomenon is seen to a certain extent in gastritis patients but is abolished when atropine premedication is administered to ulcer and gastritis patients.

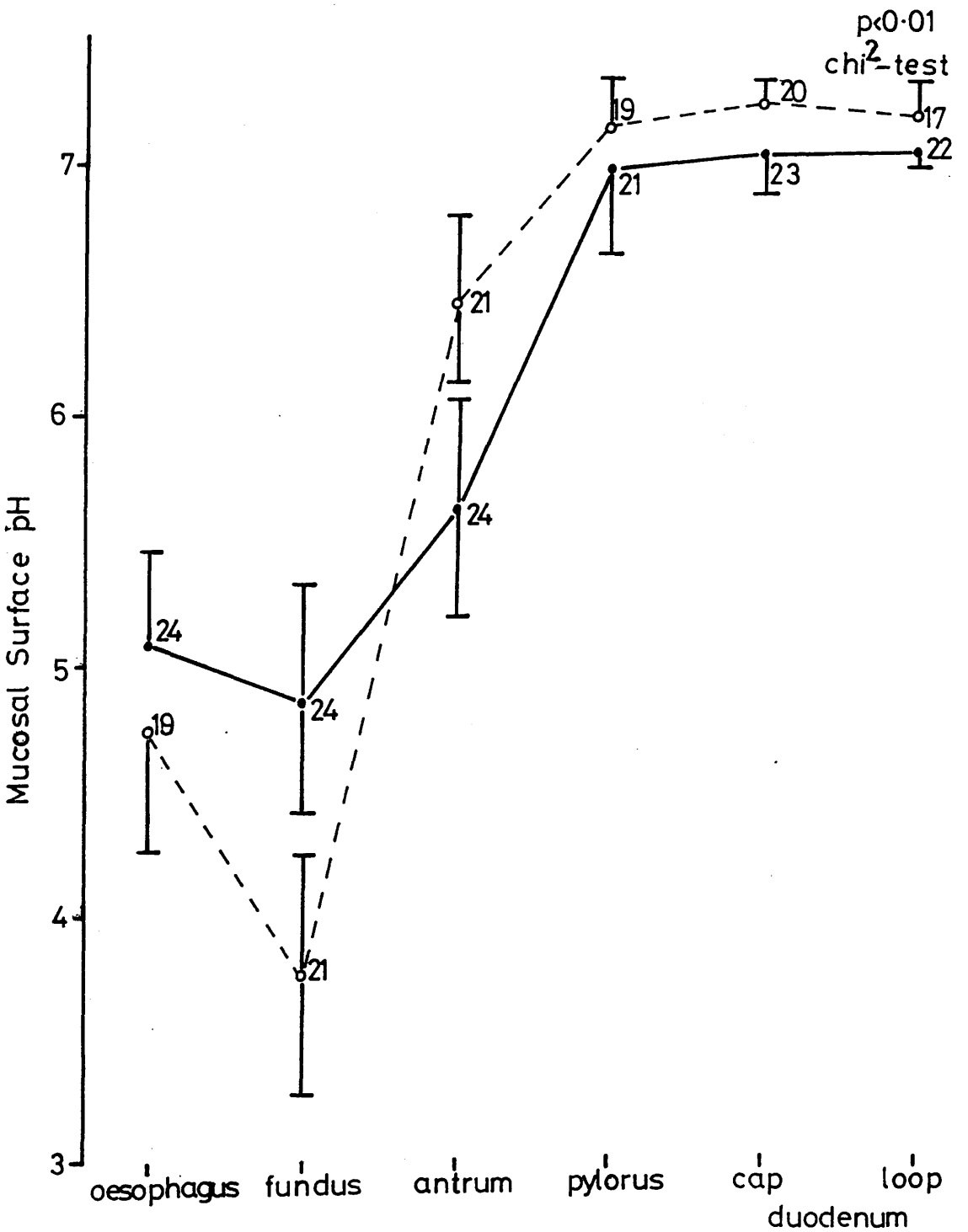
ii c) Patients with duodenal ulcer

Gastric ulcer disease and duodenal ulcer disease are often considered to be separate entities. In the light of the more alkaline surface pH measured in gastric ulcer disease, could such an example of altered surface pH be found in patients with duodenal ulcer?

To investigate this, the mucosal surface pH of twenty two patients with active duodenal ulcer disease was compared with control values (Figure 23). In the fundus, the luminal pH of ulcer patients (3.03∓0.47(21)) was similar to the control value of 3.52∓0.48(24) and no differences in the gastric mucosal surface pH were noted between the two patient groups. Therefore, duodenal ulcer disease has no effect on gastric mucosal pH. In the duodenal cap, where the majority of ulcers were situated, mucosal surface pH (7.26∓0.08(20)) was significantly ($p<0.01$) more alkaline than neutrality but was not statistically different from the control pH (7.05∓0.16(23)). However, in the duodenal loop, duodenal ulcer patients (7.20∓0.15(17)) did have a more ($p<0.01$; chi-squared test) alkaline surface pH than control patients (7.09∓0.09(22)).

When only acid secreting patients were compared, duodenal ulcer patients had a lower ($p<0.02$) fundal lumen

Figure 23. Measurements of mucosal surface pH in control (closed circles) and duodenal ulcer (open circles) patients investigated at endoscopy. Numbers refer to the number of patients in each group.



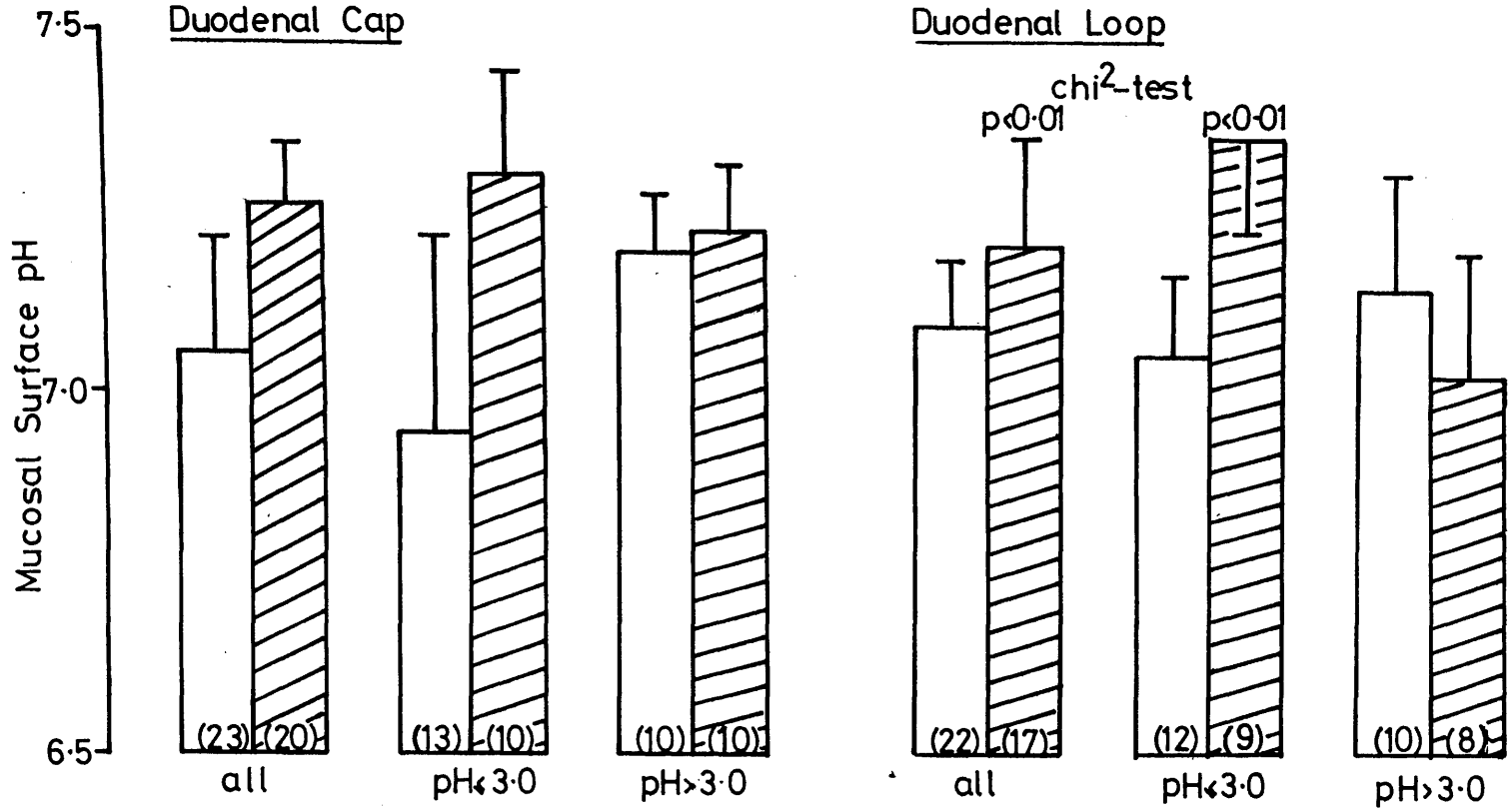
pH (1.45∓0.07(12)) than control patients (1.85∓0.14(14)). This was also true of fundal mucosal pH (duodenal ulcer: 2.32∓0.31(12); control: 3.82∓0.63(14); $p<0.05$). Once again, in the duodenum (Figure 24), no differences in cap mucosal pH were measured but duodenal loop pH in ulcer patients (7.35∓0.23(9)) was strikingly more ($p<0.01$; chi-squared test) alkaline than in control patients (7.05∓0.11(12)).

Therefore, although duodenal cap surface pH in duodenal ulcer patients is significantly alkaline it is no more alkaline than that measured in control subjects. In contrast, duodenal loop surface pH is substantially more alkaline than normal in duodenal ulcer disease. Interestingly, while acid in the fundus has virtually no effect on the duodenal loop surface pH in normal patients, there is a noticeable increase in the duodenal loop surface pH of ulcer patients.

Twelve patients who had duodenitis only were also investigated. In these duodenitis patients, the gastric and duodenal surface pHs were remarkably similar to mean control surface pH values, even when acid was present in the stomach. This implies that alkalinity can only be measured during active duodenal ulceration and not during earlier stages of the disease if indeed duodenitis leads to ulceration. This is in contrast to gastric ulcer disease where the development from gastritis through to active ulceration was associated with the appearance of a progressively more alkaline antral surface pH.

Atropine pre-medication abolished any signs of

Figure 24. Duodenal mucosal surface pH of duodenal ulcer (shaded) and control (open) patients with (pH ≤ 3.0) and without (pH > 3.0) acid in the fundus. Number of patients in parentheses.

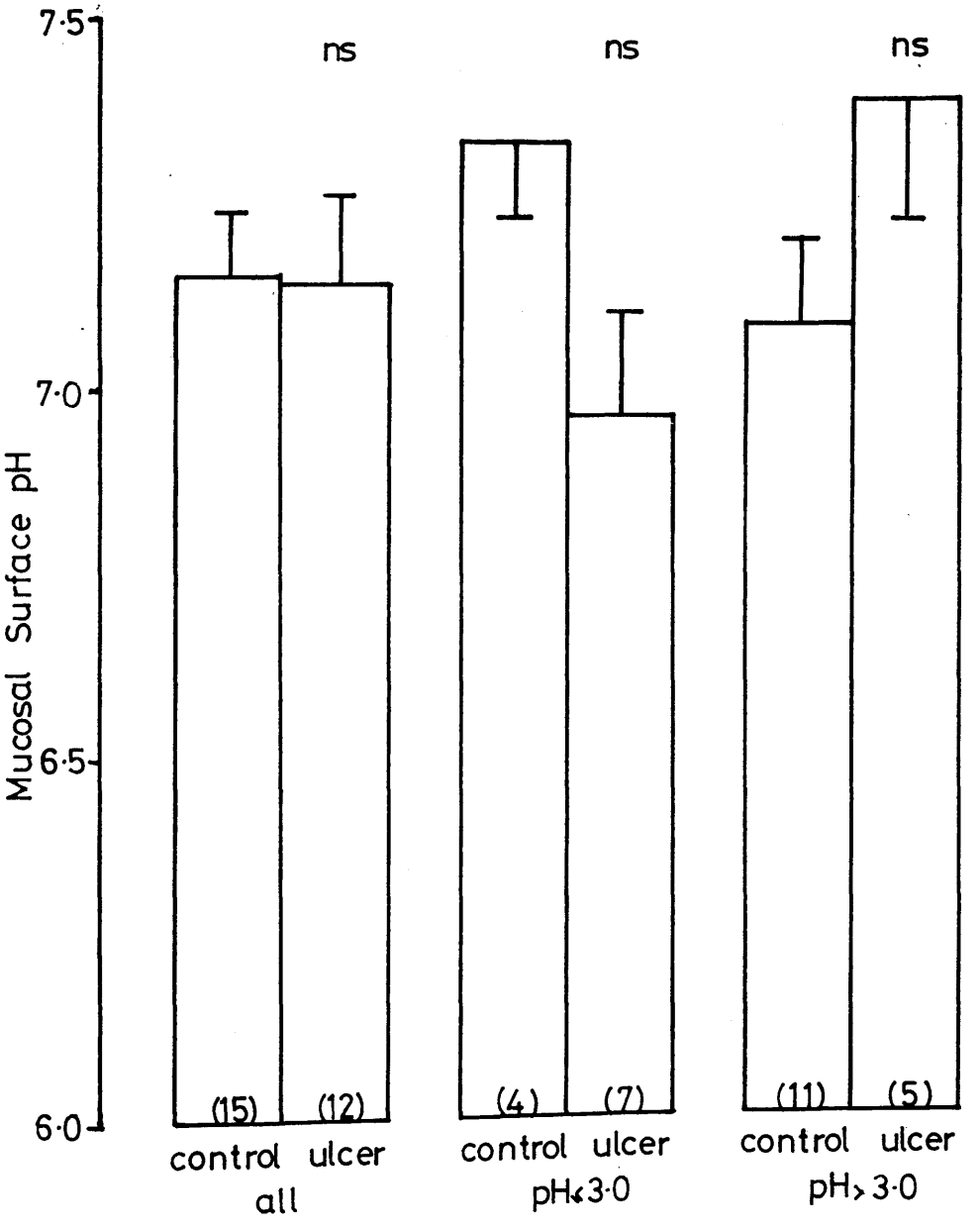


increased alkalinity in the duodenum of ulcer patients (Figure 25). The duodenal surface pH (7.14∓0.12(12)) was not very different from control values (7.15∓0.09(15)). When only acid secreting patients were considered, duodenal cap surface pH was lower in duodenal ulcer disease (6.96∓0.14(7)) than in control patients (7.33∓0.10(4)), although this could not be shown to be statistically significant. Of some interest was the fundal surface pH (2.36∓0.27(10)) of atropinised acid secreting duodenal ulcer patients. This was more acid ($p<0.02$) than the pH of 4.20∓0.80(4) measured in atropinised acid secreting control patients. This data suggests that atropine exerts its effects more in the duodenum than it does in the fundus. The above data allows one to imply that the secretion of bicarbonate by the duodenum, when an ulcer is present, is partially controlled by cholinergic mechanisms which are blocked by atropine.

Finally, as well as gastric and duodenal ulcer patients, several patients with oesophagitis and patients with hiatus hernia were investigated. However, there were no unusual pH measurements found in these patient groups. Interested readers are referred to these results in Appendices 3 & 4.

In summary, similar to gastric ulcer disease, duodenal ulcer disease can be distinguished from the normal state by the presence of an increased mucosal surface pH within or near to the area of ulceration. This alkalinity becomes even more evident when acid is in the stomach.

Figure 25. Duodenal cap surface pH of atropinised control and duodenal ulcer patients with (pH ≤ 3) or without (pH > 3.0) acid in the fundus. Number of patients in parentheses.



DISCUSSION

With the development of any new technique, various unforseen problems will arise, the majority of which are soluble. However, there will always be limitations to any system and it is important that these are recognised. It is the purpose of the following section to clarify certain assumptions which have been made and to discuss the merits and limitations of each system.

5 i) Gastro-duodenal pH measurements at endoscopy

To measure gastrointestinal surface pH, existing clinical procedures have been adapted for the intended purpose. Apart from convenience, there are two other major benefits to measuring gastro-duodenal surface pH at endoscopy. Firstly, the position of the electrode can be determined accurately and the flexibility of the endoscope allows measurements to be made within virtually any area of the stomach or duodenum. In contrast, the position of free-floating electrodes within the stomach cannot be accurately determined or controlled. The second major advantage is that the existence of any mucosal disease can be examined at the same time as the experimental measurement is made. Therefore, patients do not need to undergo any more examinations than are necessary for clinical diagnosis.

In previous reports (70,71) of endoscopic pH measurement no mention was made about the practical difficulties which may be encountered. In neither report was reference made to the pH of the luminal contents. It is indeed quite difficult to obtain a steady recording of pH

in the gastric lumen in regions other than the fundus. Only if gastric juice was present in relatively large quantities could this be achieved. Unless a fluid continuum existed between the pH electrode in the lumen and the buccal mucosa no stable pH values could be obtained. Were it possible to use a combined pH and reference electrode at endoscopy, luminal pH could be measured with greater reliability. With such a system the close proximity of the two electrodes to each other would ensure less fluctuation of the recording as interruption of fluid continuity would be less likely. This problem will not be resolved until a suitable electrode of sufficiently small diameter is designed for passage through the endoscope.

When the electrode was placed on the gastric and duodenal surface it was assumed that the value of pH recorded at this time was the pH of the mucosa. There are however, some distorting influences which must be considered. It cannot be said for certain that the electrode was in contact with the mucosa alone. The rounded tip of the electrode meant that a contribution to the recorded pH from luminal fluid impinging on the pH sensitive glass could not be discounted. Thus the recorded value would be a composite of mucosal and luminal pH. This luminal contribution to the recorded pH would be far less than that of conventional, large diameter glass pH electrodes floating free in the stomach or duodenum because of the smaller size of the electrode tip (1.4mm). However, even with the small glass electrode, the pH values for the mucosa cannot be without uncertainty. It must therefore be

assumed that the recorded pH is that of the overlying mucus layer and not that precisely at the epithelial cell surface.

From each region only two measurements of mucosal pH were made and these were averaged to give a mean value. Were it possible to 'map' the entire area diligently within the time available, the average pH might differ from the value obtained with the present procedure. In addition, the recorded values only represent the pH at the time of endoscopy. With this system it is not feasible to monitor mucosal pH over long periods of time. Without such a study, it cannot be known if the surface pH remains constant or varies with the amount of acid secreted or to what extent food or other substances may alter the mucosal pH. Patients were examined after an approximately 12 hour fast and usually in the early afternoon, all factors which would suggest that the gastric secretions were at basal levels. However, until a non-invasive means of measuring mucosal pH is devised it cannot be known what effect the mere presence of the endoscope will have on gastric and duodenal secretions.

Despite these uncertainties, endoscopy as a means of delivering an electrode to the gastrointestinal mucosa is efficient, quick and provides minor discomfort to the patient. Its use is limited to the stomach and proximal duodenum so alternative systems must still be used for measuring small intestinal mucosal pH.

5 ii) The measurement of small intestinal mucosal pH

The Crosby capsule-electrode assembly is a novel system for measuring small intestinal mucosal surface pH. Measurements can be obtained as an adjunct to jejunal biopsy without interfering with the clinical test. In return, the clinical test can be enhanced from the information provided by continuous pH monitoring. This is exemplified by pH changes signalling movement of the capsule from the stomach to the duodenum, dispensing with the need to X-ray the patient as frequently as is sometimes necessary when monitoring capsule movement. Subsequent capsule-electrode transit can only be determined by intermittent fluoroscopy.

To improve the chances of contact between the electrode's pH sensitive surface and the tissue, the electrode tip was positioned almost perpendicularly to the mucosa. If the buccal reference electrode was momentarily removed from the mucosa, sharp deflections of the pH readings into the acid range could be produced. Such deflections were also seen when increased intestinal motility was induced in patients by administering Metaclopramide hydrochloride (Maxolon, Beecham; 10-20mg i.v.). This would suggest that the electrode intermittently lost contact with the mucosa, severing coupling between reference and pH electrodes. Using this criterion, it was possible to tell when the electrode was not touching the mucosa. Even if some doubt remains about mucosal-electrode contact, the final jejunal pH reading is almost certainly representative of the mucosal pH. By drawing the mucosa

into the capsule, the electrode, being close to the capsule apperture, must touch the tissue and register the pH.

In the jejunum, surface pH can be expressed with reference to villous height, villous to crypt cell ratios, enzyme activities and related measurements. Unfortunately it was not possible to examine the duodenal tissue to determine any correlations between the duodenal pH and histological or biochemical findings. An improvement of this system would be to use a multiple biopsy system which would excise tissue from several identifiable positions along the intestine during the same investigation.

A further uncertainty was knowing how long the electrode remained in any one position. If the capsule-electrode assembly moved through the intestine at a constant rate then it would be simple to identify changes in mucosal pH with respect to time and position. However, variations in intestinal motility do not allow such assumptions to be made and X-ray hazards do not permit continuous screening of the patient. A mercury-bag attachment may assist the movement of the assembly through the intestine at a more even rate.

In summary, the Crosby-capsule electrode measuring combination has, for the first time, allowed *in vivo* measurements of human proximal small bowel surface pH to be made with minimal effort. As with any new technique there will always be improvements which can be made and some of those suggested here might make what is already a simple but highly effective procedure even more useful and

reliable. The possibility of measuring mucosal surface pH in more distal regions of the intestine remains to be investigated although there is no reason why this should pose a problem.

5 iii) An assessment of the plastic pH electrode.

It was for use with this capsule-electrode system that a plastic pH electrode was developed. A large proportion of the project was devoted to overcoming various unforseen electrode development problems. One such problem was the water-tight seal between the internal filling solution and the outer screening of the cable. The present use of silicone rubber, varnish and PVC has eliminated the sealing problem.

Many electrodes were discarded because of air bubbles appearing in the internal filling solution during storage. These eventually rendered the electrode useless by breaking the fluid contact between the silver wire and ion-selective membrane. The bubbles were subsequently found to be the result of evaporation of water through the PVC. Whether evaporation occurred through the PVC tubing or the membrane is not clear but evaporation was evident within 24hrs. A simple answer to the problem was to store the electrodes in pH 7 Tris/HCl buffer when not in use. Water evaporation from the electrode never occurred during an experiment.

The functional characteristics of the two hydrogen ion ionophores have been studied. TDDA was selected as the ion exchanger for the plastic electrodes, rather than OCPH, because of its wider operative pH range and its higher

cation selectivities. TDDA, a neutral ion-carrier, has consistently been shown to have better operating characteristics than charged ionophores, whether incorporated in plastic (112) cast in plastic onto ion-selective field effect transistors (ISFETs) (126) or used in liquid membranes for microelectrodes (113).

The formula used in preparing the TDDA membrane solution was not based on the prescribed formulation (112) but this did not affect the response obtained with these electrodes. Indeed, the pH range, cation selectivity, response time, resistance and drift of these electrodes are comparable to previously published results (112). This does not necessarily mean that the composition of a membrane solution has no effect on electrode performance. Previously described plastic pH electrodes (112,126) have been constructed from powdered PVC with an appropriate plasticiser. While the choice of plasticiser appears not to affect the slope of the electrode response, it does cause variations in electrode drift (126). The amount of drift noticed with the intestinal pH electrodes when used *in situ* in this study was never excessive and could be corrected for, but it is interesting to note that the same degree of drift never occurred in buffer solutions. This would suggest that some constituent of intestinal fluid "interferes" with the electrode. Since PVC tubing was used in the construction of the electrode membranes the plasticiser was not known. However, by using powdered PVC and selecting an appropriate plasticiser, electrode drift *in situ* might be eliminated. This awaits further

investigation.

TDDA plastic pH electrodes, with a lower limit of detection of pH 4, are not suitable for measuring gastric pH. A neutral hydrogen ion-selective carrier, which functions in the acid range (pH 1-4), has recently been developed (127). However, although useful for measuring gastric pH, it does not satisfy the requirement for a plastic pH electrode which responds over the entire pH range (pH 0-14). Until a suitable ion exchange carrier is developed, plastic pH electrodes will continue to be regarded with scepticism by researchers accustomed to working with glass electrodes.

The potential design of plastic pH electrodes will be determined by the purpose for which they are desired. Likewise, the electrode design described here could be used for measuring other ions of interest. By substituting the ion-exchanger with an appropriate alternative, intestinal measurements of potassium, sodium or virtually any other ion could be made. The electrodes could be used in conjunction with the Crosby capsule for intestinal mucosal ion measurements or, allowed to float free in the gastro-intestinal lumen, these electrodes could provide ion measurements of instilled solutions. Such studies would be useful for monitoring ion movements in secretory diarrhoea or in following the absorption of drugs eg. methadone absorption (106), where suitable ligands exist.

In conclusion, plastic pH electrodes can be used successfully for intestinal pH measurements and they may

soon be available for gastric pH measurements. With responses similar to glass electrodes they are an adequate substitute, an important point when one considers that they can be produced for a fraction of the cost of glass gastrointestinal pH electrodes. Finally, plastic electrodes can be constructed for measuring most ions of interest and, because of the versatile ways in which plastic can be used, they may be adapted for most purposes.

5 iv) Gastrointestinal disease and mucosal pH

The pH results obtained in this present study using the devised measuring systems have shown that there are changes in mucosal pH when gastrointestinal disease is present. It is the purpose of the following section to discuss these results.

From the results of the two separate clinical studies performed as part of this project, a pH profile of the mucosa of the proximal gastrointestinal tract can be described. Essentially, the mucosal pH in the stomach of normal patients is acid, becomes neutral in the duodenum, and gradually acidifies towards the jejunum. This is a simplified view, particularly in the stomach where the mucosal pH will vary with the pH of the gastric juice. The pH profile and the gastric mucosa response to gastric juice is altered when the tissue is damaged. There were markedly abnormal mucosal pH changes in the three major gastrointestinal diseases investigated: coeliac disease, gastric ulcer and duodenal ulcer. In each case the disease is

characterised by an increase in the mucosal pH of the affected area. In the jejunum, the pH of the normally acidic mucosa approaches neutrality in coeliac disease. In gastric ulcer disease, the pH of the antrum rises to neutrality, regardless of the gastric juice pH. Finally, duodenal ulcer disease is associated with a normally neutral duodenal mucosa becoming alkaline. Alkalinity is the common factor in all cases.

5 iv) a) Mucosal pH of the small intestine

A jejunal mucosal pH of 5.9 measured in control patients was remarkably similar to values recorded from human biopsy tissue (73,74). This value probably reflects a mixture of villous tip acidity and crypt cell neutral or alkaline fluid secretion. Using microelectrodes, Lucas et al (128) have measured a microclimate pH of 6.3 in rat proximal jejunum *in vitro*. pH 6.3 was also the value obtained in rat *in vivo* studies using mini glass electrodes (tip diameter = 1.5mm) (129). However, in each case, the exact location of the electrode tip in relation to the jejunal villi was unknown. In a recent paper (130) a mean pH of 6.65, the lowest recorded in these experiments, has been measured between 10 and 50um below the villous tip with antimony microelectrodes. Measurements in the crypts gave alkaline values.

Antimony electrodes are sensitive to oxygen tension (131) so results obtained with these electrodes may be suspect. A different pH profile from that measured with antimony electrodes has been measured using liquid ion

exchanger microelectrodes (H. Daniel, private communication 1986). With the liquid ion exchanger electrodes the lowest pH value recorded at the villous tips was pH 6.2 with no increase in pH as the electrode moved down into the crypt cell region. The acid region of the villi corresponds to a position adjacent to cells thought to be morphologically best adapted for digestion and absorption (132). As yet, it has not been possible to measure values as low as pH 5.3 which was proposed, on the basis of drug absorption studies (133), as the real pH at the epithelial cell surface. In summary, the present pH values obtained at biopsy agree with estimates in rat proximal jejunum.

The mechanism by which the normal jejunum acidifies the mucosa is not fully understood, although there are some indications as to how this may occur. The mechanism of acidification of the luminal bathing solution has been studied (134,135) using the everted sac technique (136). Acidification can occur in the absence of glucose in the incubating medium but is increased at glucose concentrations greater than 1mM. However, acidification is not a consequence of the active transport of hexoses since galactose and 3-O-methylglucose, although actively transported, do not increase acidification. Increased acidification results from the absorption of metabolisable sugars such as glucose and fructose, which are actively transported, and mannose, which is not. ATP, applied exogenously to the jejunal mucosa increases acidification which is abolished by aminophylline, a phosphodiesterase inhibitor. These observations (134) have led to the

conclusion that acidification results from the hydrolysis of ATP and depends on aerobic metabolism of sugars.

Acidification of the jejunal mucosa may occur through hydrogen ion secretion or bicarbonate absorption. A rise in pCO_2 of bicarbonate solutions instilled into the jejunum (88,137,138) seems to favour hydrogen ion secretion rather than bicarbonate absorption *per se*. As well as mediating bicarbonate absorption, hydrogen ion secretion enhances sodium absorption through a sodium-hydrogen exchange mechanism (137,138,139) thought to exist in the jejunal mucosa (140). However, measurements of the serosally directed sodium flux (135) do not change when acidification is inhibited by aminophylline. This suggests that sodium-hydrogen exchange may not be the only mechanism in the acidification process.

Instead, a hydrogen-potassium exchange mechanism which could account for two-thirds of acidification is postulated (135). The serosally directed potassium flux in the jejunum is the same order of magnitude as the mucosally directed hydrogen flux and both oubain and aminophylline can reduce potassium uptake to zero, paralleling their effect on acidification. Potassium uptake by jejunal mucosa is increased in the presence of metabolisable sugars (141). This does not imply that potassium uptake is coupled to active transport of sugars since the uptake of unmetabolisable actively transported sugars reduce potassium absorption (141). Similarly, hydrogen ion secretion is unaffected by the active transport of unmetabolisable hexoses but is increased when metabolisable

sugars are transported (134). This metabolic dependency of potassium absorption and hydrogen ion secretion lends further support to the existence of a hydrogen-potassium exchange mechanism, probably situated in the apical membrane of epithelial cells.

An alternative transport mechanism for hydrogen ions might occur through the translocation of metabolically produced lactic acid (130). A mucosally directed diffusion of lactic acid would be followed by the dissociation of lactic acid to lactate and hydrogen ions in the acid microclimate. Lactate would then facilitate sodium absorption through an electroneutral lactate-Na^+ cotransport system.

It has been suggested that the acid microclimate is not due to hydrogen ion secretion but to the mucus layer (142). It is postulated that mucus is an ampholyte which allows hydrogen ions to enter down their concentration gradient when the bulk solution is acid. When the perfusing solution is alkaline, it is suggested that negatively charged mucus retains hydrogen ions within the mucus layer. Thus an acid microclimate can be maintained. Removal of the acidic mucosubstance by shearing eliminates the acid microclimate. The subsequent failure of glucose at concentrations upto 14mM to reproduce this region of acidity is given as evidence that glucose stimulated hydrogen ion secretion is insufficient to maintain an acidic microclimate. However, the authors have yet to provide corroborative evidence to explain various factors like sodium dependency of acidification and the effects on

acidification of selective inhibitors of carrier mechanisms.

In conclusion, acidification of the jejunal mucosa could potentially occur through a number of transport mechanisms and any one or more may ultimately be responsible. Future investigations may eventually provide a definitive answer.

Hydrogen ion secretion is assumed to occur from the absorptive villous tips of the jejunum, corresponding to the low pH recorded close to this region (130). Removal of the villous tips, as occurs in coeliac disease, would prevent hydrogen ion secretion and a neutral or alkaline mucosal pH would be expected. The near neutral jejunal mucosal pH measured *in vivo* in coeliac disease confirms previous *in vitro* findings (73,74). The highest pH readings were obtained from untreated patients with villous atrophy, but treated patients with a structurally normal jejunal mucosa were also shown to have an elevated surface pH. It is possible that the apparent increase in jejunal surface pH is the result of two, not necessarily exclusive, mechanisms.

Firstly, coeliac disease is characterised by a flat avillous mucosa with crypt cell hyperplasia. As well as the loss of acid-secreting absorptive cells, there is reported to be an approximately three fold increase in the number of crypt cells (143). It is still unknown to what extent crypt cells contribute to intestinal secretion but areas containing crypt cells but not villi, such as the colon, do

not acidify their contents (144). Whether the crypt cells secrete bicarbonate is as yet unknown but intestinal secretion in coeliac disease is associated with an increase in tissue prostaglandin E_2 (145) which stimulates bicarbonate secretion in the human duodenum (62). Thus, fluid secreted onto the surface of the tissue by crypt cells is likely to be neutral or alkaline and may account for the observed mucosal pH in coeliac disease.

The apparent increase in jejunal surface pH in coeliac disease may also be the result of an increase in tissue permeability. Increased tissue permeability has been measured in coeliac patients using the cellobiose/mannitol test (146,147). The exudation of fluid through the paracellular pathways would contribute to the crypt cell secretion and help maintain a neutral surface pH. An increase in intestinal permeability is often present in treated coeliac patients with structurally normal jejunal mucosa (146), as determined by routine microscopy. However, recent computerised morphometry studies (148) show that subtle changes in an apparently normal looking biopsy are associated with an increase in cellobiose/mannitol absorption. This may well explain the persistently elevated surface pH measured in treated coeliac patients with normal jejunal mucosa.

Jejunal biopsy disaccharidase activity was measured for each patient and a correlation was found between lactase, maltase and sucrase activities and mucosal pH. A similar correlation between *in vitro* jejunal mucosal pH and lactase activity has been noted (149). An important aspect of

measuring disaccharidase activities by the method of Dahlqvist (118) is that the enzyme-substrate incubation occurs at pH 6, which is the optimum pH value for disaccharidase activity in tissue homogenates (150). This is appropriate for normal jejunal tissue. However, the measured activity of enzymes obtained from patients with a high surface pH does not necessarily reflect the true activity occurring at the tissue microclimate pH. Under these circumstances, the true activity may well be higher or lower than the measured activity. In retrospect, the optimum pH for disaccharidase activity should have suggested that the pH at the mucosa of healthy individuals would be approximately pH 6.

In conclusion, while other people have measured jejunal surface pH in animals *in vitro* and *in vivo* and in man *in vitro* since Lucas first applied electrodes to rat jejunum (128), this is the first instance where jejunal mucosal pH has been measured in humans *in vivo*. Measuring jejunal surface pH in humans has confirmed the findings of the *in vitro* human studies and shows that an acid microclimate is not an *in vitro* artifact. The similarity between human and rat jejunal mucosal pH means that the rat is a useful model for studying the effect of surface pH on weak electrolyte absorption and can be used for studying drug pharmacokinetics.

5iv) b) Peptic ulceration and mucosal pH

It is proposed in the cytoprotection hypothesis (50) that a neutral zone of mucus and bicarbonate exists at the

mucosal surface of the stomach and duodenum. Mucosal damage is thought to be the result of the failure of this zone to inhibit the action of acid and pepsin on the mucosa.

Bicarbonate secretion has been measured in gastric and duodenal tissue of several animal species (46,47,48,49,50,51). Several species and regional differences have been noted but there are some characteristics which appear common to all. Both gastric and duodenal bicarbonate secretion are dependent on metabolism since transport is abolished by metabolic inhibitors (59,60). Gastric secretion of bicarbonate possibly occurs through an electroneutral HCO_3-Cl^- exchange at the luminal membrane of surface epithelial cells (151). However, duodenal transport seems more complex for evidence exists for both an electroneutral HCO_3-Cl^- exchange (151) and an electrogenic transport of HCO_3 (60,151).

Instilled acid is a strong stimulus of bicarbonate secretion in the duodenum (61,62,66). The mechanism by which acid stimulates bicarbonate production may occur through several second messenger systems which have been implicated in bicarbonate secretion. Cholecystokinin (CCK), pancreatic glucagon and gastric inhibitory polypeptide (GIP) stimulate duodenal bicarbonate secretion but secretin and vasoactive intestinal polypeptide (VIP) do not (151). This effect of VIP on amphibian duodenum is in contrast to the increase in bicarbonate secretion from rat duodenum observed after intravenous infusion of VIP (152). This difference is likely due to the absence of Brunner's glands in the amphibian duodenum. The effect of CCK on gastric

mucosal bicarbonate secretion may be masked by a concomitant increase in hydrogen ion secretion (151). Other hormones investigated for their effect on gastric bicarbonate secretion include glucagon, which stimulates, and GIP, which inhibits bicarbonate production (151).

There is much evidence to suggest that formation of prostaglandins is important in the gastro-duodenal defence. Prostaglandin E_2 (PGE_2) and prostaglandin F_2 (PGF_2) are the two most prevalent prostaglandins isolated from human biopsy homogenates (153). Exogenous PGF_2 and 16,16-dimethyl PGE_2, a synthetic analogue of PGE_2, stimulate alkaline secretion in the isolated amphibian gastric mucosa (49) while 16,16-dimethyl PGE_2 causes a dose-dependent rise of luminal alkalinisation of bullfrog duodenum (59). PGE_2 also increases duodenal bicarbonate secretion in cats, rats and dogs, but not in guinea pigs or rabbits (61). Inhibitors of prostaglandin synthesis reduce or inhibit bicarbonate secretion (61,151,154), while activators of endogenous prostaglandin formation stimulate secretion (155). Prostaglandins are also known to stimulate intestinal fluid and ion secretion in humans (156).

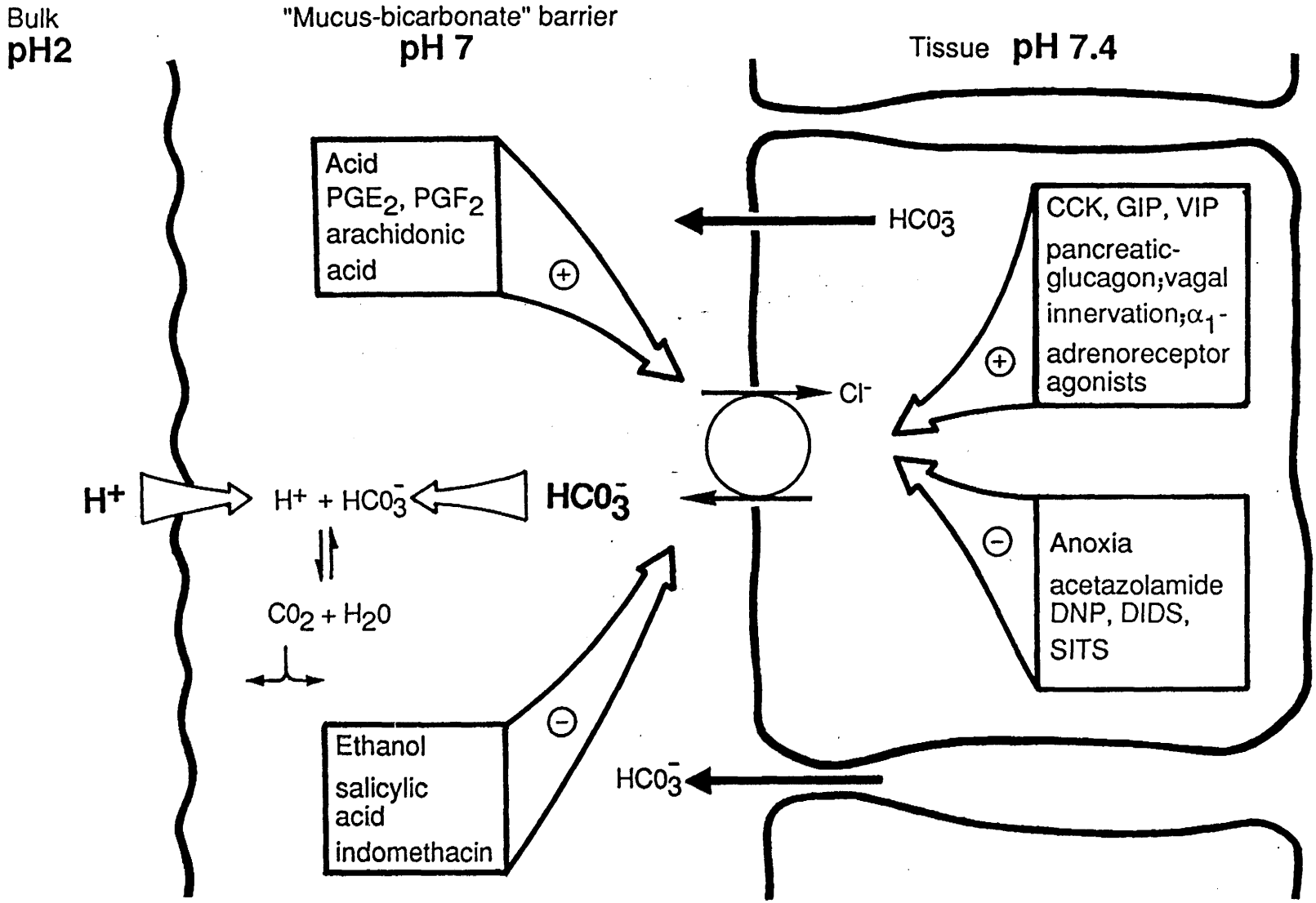
Finally, neural control of bicarbonate secretion has only recently been examined, although vagal stimulation has been known to cause duodenal alkali secretion for many years (124). Stimulation of cat vagal fibres causes an increase in gastric bicarbonate secretion (125) and electrical field stimulation of amphibian duodenal bicarbonate secretion is reduced by atropine, indicating

the existence of muscarinic receptor control of secretion (157). This muscarinic receptor control of bicarbonate secretion is perhaps another example of species differences since the muscurinic agonist carbachol has only a slight stimulatory effect on rat duodenal bicarbonate secretion (158). The adrenergic neural system also appears to be involved since $alpha_1$-adrenoceptor agonists promote duodenal bicarbonate secretion (159). These observations on the control of bicarbonate secretion are summarised in Figure 26.

All this evidence favours bicarbonate secretion into the lumen by the gastro-duodenal mucosa but does not prove that a "mucus-bicarbonate" barrier exists at the mucosal surface. To substantiate this hypothesis, a near neutral pH should be measurable at the mucosal surface under normal conditions and conversely, when the cytoprotection fails to operate, an acidic mucosal pH would be expected. Several investigations of gastro-duodenal mucosal surface pH (64,65,66,160) have indeed shown that a neutral pH can be measured. As yet, this has not been confirmed in humans *in vivo* and nothing is known about the effects of ulceration on the "mucus-bicarbonate" barrier if this indeed exists in humans. From the results of this project, some very interesting observations can be made.

In control patients, a near neutral mucosal pH was measured from the fundus to the duodenum, but only when the fundal luminal fluid was higher than pH 3. This neutral pH could not be maintained in the stomach when the mean fundal lumen pH was 1.85. A similar observation in humans has

Figure 26 **Proposed mechanisms for the control of gastro-duodenal bicarbonate secretion**



recently been reported (69). In contrast, the results from this project reveal that peptic ulceration is associated with an increased surface pH which exists despite the presence of high concentrations of acid in the stomach. In both gastric and duodenal ulceration, the mucosa of the affected area was more alkaline than that in corresponding areas of control patients. The alkalinity was found to increase with the severity of the disease and when the fundal lumen pH was low.

There are two notable points from this data. Firstly, the neutral surface pH in normal patients cannot withstand acid and secondly, in ulcer patients, a more alkaline surface pH exists. The first point is perhaps not so surprising after closer examination of published data supporting the evidence for a neutral microclimate.

Previous studies of the pH gradient across the gastric mucus layer in rat *in vivo* (64) and human *in vitro* (65) have indicated that a near neutral zone can be measured. Interestingly, this zone cannot withstand acid at a concentration of 40mM (pH 1.4). In another study of rat fundus *in vitro* (160), even acid at 20mM was sufficient to abolish the pH gradient.

Similarly, a pH-gradient at the luminal surface of the rat duodenum *in vivo* does exist when the luminal pH is as low as pH 2 (66) although this is dissipated when the acid concentration perfusing the tissue is increased to 50mM (160). An interesting point to note is the fact that a pH-gradient cannot be demonstrated in rat duodenum when an

alkaline perfusate is used (160). (The actual findings are not reported). Consequently, a neutral microclimate may only exist if bicarbonate secretion is stimulated by acid perfusates. If a pH 7.4 perfusate is alkalinised, as has been reported (61), one would expect to find a mucosal pH the same as or more alkaline than the perfusate pH. Until this is demonstrated, the existence of net basal bicarbonate secretion must be regarded with some scepticism.

The concentration of acid necessary for abolishing the duodenal pH-gradient is higher than is necessary for the same effect on gastric mucosa but then the duodenal mucosa of several animal species produces bicarbonate at a much higher rate than the whole of the gastric mucosa (151). Consequently, acidification of the juxtamucosal layer when the lumen pH is low may be the result of a breakdown of the "mucus-bicarbonate" barrier. The fact that the present data shows that this occurs quite readily in normal patients suggests that it need not be pathological as is often thought.

The second interesting fact to emerge from this study was the appearance of a more alkaline surface pH in ulcer patients which was unaffected by gastric acid. The failure of cytoprotection and the development of an ulcer does not automatically imply that an acid surface pH should be detected in ulcer patients. After all, pH was measured once ulceration had occurred, that is, once the damage had been done.

An alkaline surface pH in peptic ulceration is likely to be the result of tissue inflammation. A more definitive explanation cannot be derived from this investigation but it is possible to speculate on potential mechanisms. Alkalinity may simply be due to an increase in plasma leakage of bicarbonate. Certainly, large volumes of fluid are secreted by the gastric mucosa when tissue permeability is increased by damaging substances such as salicylic acid (161). Alternatively, the local release of prostaglandins stimulated by tissue damage will promote bicarbonate secretion. Increased duodenal prostaglandin activity has indeed been measured in duodenal ulcer patients (162) and increased levels of PGE_2 have been found in patients with gastritis (163). There may even be an increased cholinergic stimulation of bicarbonate since, in this study, an alkaline surface pH could no longer be measured in gastric and duodenal ulcer patients when atropine was administered before endoscopy.

In summary, this study has shown that a neutral mucosal surface pH exists in the human stomach and duodenum. It has also demonstrated a less well appreciated fact that acid can significantly reduce the surface pH in normal patients. Finally, contrary to popular belief, ulcer patients have a significantly more alkaline mucosal pH which is more resistant to acid. This alkaline surface pH is likely to result from tissue inflammation.

It is difficult to reconcile the hypothesis of a neutral surface pH, which can withstand gastric acid, with

what is known about the physiology of inhibition of acid secretion, gastric emptying and several other related topics. However, the findings of this study fit well with the known physiology of the gastrointestinal tract and the purpose of the following section is to elaborate on this point.

5 v) Mucosal surface pH and gastrointestinal physiology.

Events occurring at the gastrointestinal mucosa have a direct bearing on the regulatory feedback mechanisms which control absorption, secretion and motility. These feedback mechanisms occur through both neural and hormonal transmission. Receptors in the duodenal mucosa, activated by acid, are implicated in the inhibition of acid secretion via a neural pathway (37), possibly consisting of vagal afferents responsive to acid stimulation of the duodenal mucosa (38). Similarly, duodenal regulation of gastric emptying is mediated through either a neural or a hormonal feedback system activated by acid in the proximal duodenum (32). Inhibition of gastrin release by acidifying the antrum (164) is a hormonal mechanism for inhibiting acid secretion and similarly, pancreatic bicarbonate secretion is stimulated by duodenal acid infusion (165) mediated by the acid induced release of secretin (45). Consequently, there is much evidence to suggest that acid is intimately involved in regulating these normal physiological functions.

To activate these feedback mechanisms, acid is required in relatively high concentrations. For instance, gastrin release is only inhibited when the antral pH is less than pH 2.5 (164). Likewise, pancreatic bicarbonate secretion is maximally stimulated when the duodenal pH falls below pH 3 (45). If, however, the present interpretation of the cytoprotection hypothesis is correct, how these control mechanisms can operate becomes a mystery. By maintaining the mucosal surface pH at neutrality, as proposed in the cytoprotection hypothesis, acidity regulated feedback mechanisms will not be activated. The fact that they are activated proves that the cytoprotection hypothesis is essentially a biochemical model applied to a clinical problem and has no bearing on the physiology of the system. This is not to say that cytoprotection does not play an important role in protecting the mucosa from acid damage. Unfortunately, investigations of cytoprotection have tended to highlight its protective elements and have largely ignored some rather important data, perhaps because it does not fit with the current concept of mucosal protection.

It has been shown in this thesis and in published data (66,160) that a low luminal pH is sufficient to abolish an otherwise neutral mucosal surface pH. In all cases, the luminal pH was below pH 2 and this corresponds well with the pH required to activate the inhibitory mechanisms outlined above. Interpreting this data in a physiological context allows one to suggest the following.

Under conditions of basal acid secretion, a neutral

mucosal surface pH is maintained and protects the mucosa from digestion by acid and pepsin. However, when the intraluminal acid reaches a particularly high concentration, the 'mucus-bicarbonate' layer is insufficient to buffer the acid. Acid impinging on the mucosa can then stimulate inhibitory feedback control mechanisms, indicating an overloading of the system. Subsequent inhibition of acid and pepsin secretion, gastric emptying and stimulation of pancreatic bicarbonate secretion, all contribute to reducing the acid load. Thus, under this system, an equilibrium between aggressive and protective factors can be re-established.

In contrast, to assume that a neutral surface pH can be maintained is illogical. This would ultimately lead to unregulated increases in acid secretion and gastric emptying. It is highly unlikely that the mucosal protective mechanisms could withstand such an increase in acid load and the 'mucus-bicarbonate' barrier would collapse. This collapse could be detected by measuring an acid surface pH when the gastric contents were acidic; this is exactly what has been found in this study.

To speculate on the cause of peptic ulceration is beyond the scope of this thesis but the observations made in this project may explain several anomalies associated with the disease. Unlike control patients, peptic ulcer patients maintained a significantly alkaline mucosal surface pH despite the presence of an acidic fundal lumen. From the preceeding discussion on the relationship between mucosal surface pH and physiological events, an alkaline

surface pH is undesirable in the presence of a highly acidic bathing medium.

Increased gastric acid secretion and gastric emptying in duodenal ulcer disease are compatible with a failure of acid to inhibit these responses. Similarly, reduced levels of plasma secretin, which have been measured in duodenal ulcer patients (42), can be explained by the failure of acid to stimulate secretin release from the duodenum. A less than normal plasma secretin concentration will reduce pancreatic bicarbonate secretion and have less of an inhibitory effect on acid secretion. Acid is also responsible for the release of 5-hydroxytryptamine (5-HT) from the duodenal mucosa (166) and 5-HT is an inhibitor of gastric acid secretion (167). Once again an alkaline surface pH would prevent the release of 5-HT and acid secretion will continue unabated. Finally, an increase in gastric emptying in duodenal ulcer disease may also be attributed to an alkaline surface pH in the duodenum. Perfusion of the dog duodenum with alkaline solutions significantly increases motility in denervated fundic pouches (168). This suggests that alkalinity either inhibits the release of an inhibitory hormone or conversely, promotes gastric motility through the release of a stimulatory hormone.

In addition to the possible effects of alkalinisation on hormonal control mechanisms, neural inhibition of acid secretion and gastric emptying may also be reduced. As well as vagal afferent fibres originating from mucosal

chemoreceptors sensitive to acid, vagal afferent fibres responsive to instilled alkali have been isolated in the cat stomach (35,38) and duodenum (38). However, the function of alkali-sensitive receptors is unknown. The possibility that these receptors may be stimulated by an alkaline mucosal surface pH suggests a role for them in the pathogenesis of peptic ulceration.

Consequently, by maintaining an alkaline surface pH in peptic ulcer disease, acid in the antrum and duodenum would fail to inhibit subsequent gastric acid secretion and gastric emptying and also result in reduced pancreatic bicarbonate output. It can be implied therefore, that alkalinity in peptic ulcer disease drives the system. That is, the mucosal tissue is faced with an ever-increasing acid load which can only lead to further tissue damage and, by implication, increased tissue alkalinity. This is consistent with the view that peptic ulceration is often associated with increased acid output. It also complements the suggestion that an increase in acid secretion does not precede but is the result of ulceration (7).

In conclusion, the cytoprotection hypothesis, as it stands, is not compatible with the existence of regulatory feedback systems which are known to exist. In contrast, the results from this study show that acid could potentially exert its inhibitory effects by dissipating the near neutral microclimate. Finally, an increase in mucosal pH in peptic ulcer patients may explain factors such as increased acid secretion and gastric emptying measured in these patients. Therefore, while bicarbonate secretion may be

important in protecting the mucosa from acid and pepsin, inappropriate bicarbonate secretion is likely to be pathological.

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APPENDIX 1

FORMULAE FOR BUFFER SOLUTIONS

BICARBONATE BUFFER* pH 9; 0.1M.

84.54ml, 1M sodium bicarbonate + 7.74ml, 1M sodium hydroxide; made upto 1L.

CITRATE BUFFER pH 5; 0.1M.

21.01g citric acid + 200ml, 1N sodium hydroxide; made upto 1L.

PHOSPHATE BUFFER pH 5; 0.1M.

99.2ml potassium dihydrogen orthophosphate (KH_2PO_4) (0.907g/100ml)

0.8ml disodium hydrogen orthophosphate (Na_2HPO_4) (1.187g/100ml)

PHTHALATE BUFFER pH 4; 0.1M.

50ml, (20.42g/l) potassium hydrogen phthalate; made upto 1L.

TRIS BUFFER* pH 7; 0.1M.

100ml, 1M hydrochloric acid + 107.2ml, 1M Tris-(hydroxymethyl)aminomethane; made upto 1L.

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APPENDIX 2

AGES OF PATIENTS EXAMINED AT ENDOSCOPY

CLINICAL GROUP	AGE (YEARS: mean±sem)	
	NO ATROPINE	ATROPINE
Normal	51.5∓3.5	32.5∓4.1
Duodenal Ulcer	41.5∓4.2	47.6∓2.8*
Gastric Ulcer	48.6∓3.6	55.0∓3.3**
Duodenitis	38.9∓3.3+	37.3∓3.7*
Gastritis	56.2∓5.8	61.3∓2.6**
Oesophagitis	53.2∓4.1	-
Hiatus Hernia	52.0∓9.2	59.1∓7.1**
Pernicious Anaemia	52.5∓3.0	-

+ $p<0.05$; * $p<0.01$; ** $p<0.001$

APPENDIX 3

UPPER GASTROINTESTINAL SURFACE pH MEASUREMENTS

(Mean+SEM)

GROUP	OESOPHAGUS	FUNDUS	ANTRUM
Normal	5.09∓0.37(24)	4.86∓0.46(24)	5.63∓0.44(24)
Duodenal Ulcer	4.74∓0.48(19)	3.73∓0.47(21)	6.46∓0.34(21)
Gastric Ulcer	5.61∓0.34(21)	4.70∓0.44(21)	6.96∓0.15(21)*
Duodenitis	4.63∓0.46(12)	4.24∓0.64(13)	5.20∓0.57(14)
Gastritis	4.60∓0.86(6)	4.85∓1.22(6)	6.68∓0.30(6)
Oesophagitis	4.38∓0.54(16)	3.67∓0.54(16)	4.58∓0.52(16)
Hiatus Hernia	4.15∓1.12(4)	3.81∓1.48(4)	5.41∓0.96(4)
Pernicious Anaemia	7.24∓0.31(5)**	6.19∓0.71(5)	7.39∓0.18(5)**

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

UPPER GASTROINTESTINAL SURFACE pH MEASUREMENTS

(Mean+SEM)

GROUP	PYLORUS	DUODENUM (CAP)	DUODENUM (LOOP)
Normal	6.99∓0.35(21)	7.05∓0.16(23)	7.09∓0.09(22)
Duodenal Ulcer	7.16∓0.20(19)	7.26∓0.08(20)	7.20∓0.15(17)*
Gastric Ulcer	7.11∓0.18(19)	7.16∓0.08(19)	7.06∓0.07(19)
Duodenitis	7.28∓0.14(11)	7.21∓0.08(14)	7.08∓0.12(12)
Gastritis	7.13∓0.28(6)	7.13∓0.08(6)	7.00∓0.11(4)
Oesophagitis	6.20∓0.56(16)	7.13∓0.16(16)	7.14∓0.10(13)
Hiatus Hernia	7.82∓0.14(5)	7.17∓0.05(5)	7.06∓0.08(5)
Pernicious Anaemia	7.43∓0.24(5)	7.22∓0.12(5)	7.15∓0.07(5)

* $p<0.01$; Chi-squared test

UPPER GASTROINTESTINAL LUMINAL pH MEASUREMENTS

(Mean+SEM)

GROUP	OESOPHAGUS	FUNDUS	ANTRUM
Normal	4.47±0.43(24)	3.52±0.48(24)	5.81±0.55(24)
Duodenal Ulcer	3.81±0.51(20)	2.99±0.44(22)	7.68±0.26(20)**
Gastric Ulcer	4.36±0.54(21)	3.62±0.52(21)	6.95±0.43(21)
Duodenitis	4.41±0.65(12)	2.62±0.48(12)	4.89±0.68(14)
Gastritis	3.67±1.07(6)	2.56±1.03(6)	7.66±0.60(6)*
Oesophagitis	3.53±0.59(16)	2.51±0.55(16)	4.24±0.66(16)
Hiatus Hernia	3.48±1.48(4)	2.99±1.49(4)	6.31±1.47(4)
Pernicious Anaemia	7.24±0.31(5)***	7.08±0.16(5)***	8.20±0.14(5)***

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

UPPER GASTROINTESTINAL LUMINAL pH MEASUREMENTS

(Mean+SEM)

GROUP	PYLORUS	DUODENUM (CAP)	DUODENUM (LOOP)
Normal	7.12∓0.45(21)	7.65∓0.11(23)	7.56∓0.08(22)
Duodenal Ulcer	7.68∓0.26(20)	7.45∓0.23(21)	7.58∓0.16(18)
Gastric Ulcer	7.35∓0.35(19)	7.60∓0.09(19)	7.46∓0.09(19)
Duodenitis	7.23∓0.60(11)	7.68∓0.11(14)	7.49∓0.17(12)
Gastritis	7.53∓0.49(6)	6.62∓0.99(6)	7.43∓0.18(4)
Oesophagitis	6.61∓0.58(16)	7.41∓0.34(16)	7.68∓0.11(13)
Hiatus Hernia	8.20∓0.08(4)	7.54∓0.24(5)	7.45∓0.10(5)
Pernicious Anaemia	8.17∓0.14(5)	7.92∓0.12(5)	7.79∓0.17(5)

APPENDIX 4

UPPER GASTROINTESTINAL SURFACE pH MEASUREMENTS OF PATIENTS WITH ATROPINE PRE-MEDICATION

(mean+sem)

GROUP	OESOPHAGUS	FUNDUS	ANTRUM
Normal	5.64∓0.32(14)	4.62∓0.44(15)	6.43∓0.35(16)
Duodenal Ulcer	4.52∓0.36(17)*	3.35∓0.45(17)	5.56∓0.41(17)
Gastric Ulcer	5.95∓0.43(10)	5.12∓0.72(10)	6.72∓0.38(10)
Duodenitis	4.09∓0.52(12)**	4.04∓0.53(15)	5.79∓0.48(15)
Gastritis	6.56∓0.51(7)	6.06∓0.95(6)	7.29∓0.26(7)
Hiatus Hernia	6.19∓0.45(7)	6.11∓0.85(6)	5.83∓0.73(7)

* $p<0.05$; ** $p<0.02$

UPPER GASTROINTESTINAL SURFACE pH MEASUREMENTS OF PATIENTS WITH ATROPINE PRE-MEDICATION

(mean±sem)

GROUP	PYLORUS	DUODENUM(CAP)
Normal	7.34±0.11(14)	7.15±0.09(15)
Duodenal Ulcer	7.12±0.22(13)	7.14±0.12(12)
Gastric Ulcer	7.86±0.16(9)**	6.98±0.11(10)
Duodenitis	7.34±0.16(12)	7.05±0.15(16)
Gastritis	7.35±0.19(6)	7.33±0.22(6)
Hiatus Hernia	6.72±0.27(7)*	6.89±0.11(7)

* $p<0.05$; ** $p<0.02$

UPPER GASTROINTESTINAL LUMINAL pH MEASUREMENTS OF PATIENTS WITH ATROPINE PRE-MEDICATION

(mean±sem)

GROUP	OESOPHAGUS	FUNDUS	ANTRUM
Normal	5.38±0.34(14)	4.37±0.50(15)	6.93±0.42(16)
Duodenal Ulcer	4.12±0.46(17)*	3.06±0.46(17)	5.84±0.51(17)
Gastric Ulcer	5.62±0.75(10)	4.93±0.83(10)	7.45±0.47(10)
Duodenitis	3.22±0.55(12)**	2.47±0.46(15)**	6.41±0.51(15)
Gastritis	6.54±0.65(7)	5.98±1.01(6)	7.70±0.45(7)
Hiatus Hernia	6.05±0.69(7)	5.65±1.08(6)	6.37±0.86(7)

* $p<0.05$; ** $p<0.01$