STUDIES ON THE POTENTIAL DIFFERENCE ACROSS THE GASTRIC MUCOSA

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by

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Abstract

There is a potential difference across the gastric mucosa, the luminal aspect being negative with respect to the serosal aspect. It is lower in the antrum than in the body of the stomach, but details of the anatomical distribution are not known. The potential difference is thought to be a measure of the integrity of the mucosa. However, it has not yet been shown to be lower in patients with gastritis than in normal subjects. In this thesis attempts have been made to fill those gaps, and the effects of drugs thought to damage the mucosa, and of those used in the treatment of gastric ulcer, have been studied.

A new technique of measurement under direct vision at endoscopy wasused to show that the potential difference is low not only in the antrum, but also at the high lesser curve, and invariably lower there than at the high greater curve. This finding may be of importance in view of the known predilection of ulcers for the antrum and the high lesser curve. The same technique, combined with histological examination of biopsies taken from the sites of measurement, was exploited to demonstrate that gastritis is associated with a significantly lower than normal potential difference.

Some evidence is presented to suggest that the fall in the potential difference produced by aspirin occurs during absorption of the drug in the stomach. Other anti-inflammatory drugs studied did not lower the potential difference, and a modification of the technique used is suggested for the assessment of their effects. This modified technique was used to demonstrate that, unlike gefarnate, carbenoxolone protects the mucosa from the fall in the potential difference produced by aspirin.

Measurement of the potential difference should be further exploited in the evaluation of the effects of drugs on the gastric mucosa.

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HISTORICAL INTRODUCTION

CHAPTER I

DISCOVERY AND DEVELOPMENT OF TECHNIQUE

In 1834 Donné introduced a platinum needle into the stomach of a rabbit and another one into the gall-bladder. When the two electrodes were connected to a galvanometer, a potential difference was recorded. In a series of similar experiments, mainly in rabbits, he showed that the deflection of the galvanometer needle was invariably in the same direction. The author believed that the electrical phenomenon he had discovered was caused entirely by chemical reactions:-

"Il ne faut, en effet, rien voir de vital dans le phénomène; c'est une action purement chimique." On the other hand, he quotes Matteucci as having exactly the opposite view:-

"C'est donc dans la vie et par la vie que ces états électriques existent et se produisent." Matteucci came to that conclusion after finding that asphyxia or death led to the abolition of the potential difference.

Thus, controversy started almost immediately after discovery. The existence of Donné's potential difference was never in doubt. It was confirmed again and again in different animals by subsequent investigators. They all asked questions about the distribution, the location, the mechanism of production of this phenomenon, and its role in gastric physiology. The answers, however, were not forthcoming. For decades there was no agreement between the findings of any two investigators. There are two main reasons why the results of different workers were not comparable. The first is that they were all measuring the resultant of two

potential differences, depending on where the reference electrode was introduced. Thus, Donné himself had his reference electrode in the gall-bladder. Swyngedauw, who was the first to measure the gastric potential difference in man (1928a, 1928b, 1928c), used either the mouth or the skin of the little finger as his reference point, although he was well aware of the fact that both the buccal mucosa and the skin have significant potential differences across them.

The second cause for the lack of comparability of the findings of different investigators was summarized by Ravin et al in 1950:-

"For over fifty years investigators have been measuring gastric potentials employing a variety of electrodes and measuring instruments. The results are, in many instances, conflicting, because techniques have varied." Most of the early workers used metal electrodes, thus, introducing a variable junction potential. The ideal electrode, apart from producing no junction potential, needs to be stable, of low resistance, and non-polarizable. Furthermore, early galvanometers, whilst being sensitive enough were of low resistance and, therefore, tended to draw current and lower the potential difference they were supposed to be measuring.

In spite of the complex technical problems of measurement of direct current biological potentials, an excellent attempt was made by Bohlen in Biedermann's laboratory as early as 1894. He used the non-polarizable zinc-zinc acetate electrode which had recently been invented by du Bois-Reymond. His reference electrode was placed directly in contact with the serosa of the stomach, and he was, therefore, probably the first worker to measure with reasonable accuracy the gastric potential difference. He showed that the mucosal aspect was negative with respect to the serosal

aspect in the frog, the rabbit, the guinea-pig and the rat. He confirmed that anoxia and haemorrhage lowered the potential difference, and that death abolished it. He showed that secretion of acid by the gastric mucosa is associated with a fall in the potential difference, a finding that has since been confirmed repeatedly.

It has often been claimed that Alvarez was the first to measure the human gastric mucosal potential difference in 1922. Like many others, he found the technical difficulties challenging. Although he started work in this field in 1913, it took him nine years to produce consistent results in the anaesthetized cat. His only published human work was about potentials recorded over the intact abdomen of a thin old woman. He tried again and again, but even by 1948 he had to admit that the tracings he obtained with an intragastric electrode in man "were erratic, probably because of constant changes in the acidity of the gastric mucosa." He did, however, with the help of Mahoney in 1922, make a major contribution by developing a small calomel electrode that was suitable for use in this field. Swyngedauw, on the other hand, produced excellent tracings of the human gastric potential difference in 1928. He carefully avoided all metal junctions, and used non-polarizable calomel electrodes. He did, however, use saturated sodium chloride bridges, thereby introducing a small liquid junction potential. He was well aware of the limitations of his technique which involved the use of a reference electrode in contact with the skin or the buccal mucosa:-

"Pour déterminer d'une façon certaine le potential devéloppé au niveau de la muqueuse gastrique en période sécrétoire, il faudrait plonger une première électrode dans le sang des vaisseaux

gastriques, et une autre dans la lumière de l'organe."

Adair and Goodman were the first to use equipment designed to almost completely eliminate liquid junction potentials. Since potassium and chloride ions diffuse at almost exactly the same rate, the use of saturated potassium chloride bridges provides a liquid junction potential of only a fraction of a millivolt. It is interesting to note that this pioneer work was done in man in 1936, before similar techniques were applied to laboratory animals. Titaev in 1938 used similar equipment with great success in man and in the dog. He managed to show that the potential difference was much lower at the pyloric end of the stomach than at the fundus. In 1955, Goodman and his colleagues produced a further improvement. Instead of using a low resistance galvanometer (400 ohms) as Adair and Goodman had previously done, they introduced the use of a very high resistance (10 megohms) millivoltmeter, thereby ensuring that the current flowing through the system was negligible.

The final refinement in human work in this field was the introduction of intravenous reference electrodes. Although the desirability of doing so had been mentioned by Swyngedauw in 1928, and Adair and Goodman had actually used the technique in 1936, it was not until 1965 that reproducible results were published by Andersson and Grossman in five human volunteers. The validity of using an intravenous reference electrode was demonstrated by Geall et al in 1968. In nine patients undergoing laparotomy, they showed that the potential difference recorded was the same whether the reference electrode was in a peripheral vein or in contact with the serosa.

CHAPTER II

THE ANATOMICAL DISTRIBUTION AND SITE OF ORIGIN OF THE POTENTIAL DIFFERENCE

In 1938, Titaev made the important observation that the gastric potential difference is much lower in the antrum of the rabbit than in the fundus. The obvious implication is that the results of different workers, or of the same worker in different subjects or animals, are not comparable unless the exploring electrode is at the same site in the stomach.

The anatomical distribution of the potential difference in man was studied by Andersson and Grossman (1965) in five volunteers, and by Geall and his colleagues (1970) in fourteen subjects. The results are shown in Table I. The mucosal surface from the oesophagus to the duodenum is electrically negative with respect to blood, and the size of the potential difference is greatest in the acid-secreting part of the stomach.

TABLE I

Anatomical distribution of potential difference (mV.) in man

	Duodenum	Antrum	Corpus	Oesophagus
Andersson and Grossman (1965)	-7.4	-34.6	-43.6	-15.0
Geall et al (1970)	-6.2	-24.9	-35.5	-10.1

A similar distribution obtains in the dog (Dennis et al, 1959), the cat (Obrink and Waller, 1965) and the rat (Cummins and Vaughan, 1963). The figures for the dog are shown in Table II. The only exceptions seem to be the dogfish and the skate, which have a

negligible gastric potential difference (Hogben, 1959).

TABLE II

Anatomical distribution of the potential difference in the dog

	Duodenum	Antrum	Corpus
P.D. (mV.)	-11	-48	-64

Many of the early investigators have suspected that the gastric potential difference originates in the mucosa. Confirmation of this idea came after the pioneer work of Delrue, who showed in 1930 that the isolated frog gastric mucosa is capable of remaining alive and of secreting acid in response to histamine. This membrane has since been repeatedly shown to have the same potential difference across it as the whole stomach wall in vivo. Crane, Davies and Longmuir (1948), for example, found a potential difference of -35mV. across the isolated gastric mucosa of the summer frog.

Since the mammalian gastric mucosa cannot be kept alive and fully oxygenated for long, a different technique had to be used to demonstrate that the potential difference originates in the mucosa. Rehm (1946) did measurements in vivo in the five anaesthetized dogs. He showed that the mean potential difference obtained with one electrode on the mucosa, and one in the submucosa, was -72.3mV. This figure was very similar to the one across the wall of the stomach, which was -73.2mV. The potential difference between the submucosa and the serosa was less than 1mV. Furthermore, 95% alcohol applied to the mucosa depressed the potential difference, whereas it had no effect when applied to the serosa.

The actual cells generating the potential difference are thought to be the oxyntic cells. Durbin (1963) used the technique

of heavy blotting to remove at least 90% of the surface epithelial cells of the isolated frog gastric mucosa. The oxyntic cells are left intact, as shown by a normal rate of acid secretion. Durbin showed that the short-circuit current of the preparation was unchanged after such treatment. The microelectrode studies of Villegas (1962), again in the isolated frog gastric mucosa, provide further evidence. Electrophoresis of carmine allowed him to identify the exact site at which he was making his measurements. He found that the potential difference of -29mV. across the oxyntic cells consisted of two steps, one of -18mV. between the serosal aspect and the interior of the cell, and one of -11mV. between the interior and the luminal aspect. Thus, it seems that, in the acid-secreting part of the stomach, the potential difference originates from the very cells that secrete acid. The site of origin of the potential difference in the antrum is not known.

CHAPTER III

THE MECHANISM OF PRODUCTION OF THE POTENTIAL DIFFERENCE

The demonstration by Matteucci in 1834 that asphyxia and death will abolish the gastric mucosal potential difference has been confirmed repeatedly by other workers, showing that life is essential for the generation of the potential difference. However, the use of the intact animal could not provide the controlled conditions necessary for the elucidation of the mechanism involved in its production. What was needed was a preparation that would allow the simultaneous measurement of electrical and other phenomena, such as ionic fluxes and hydrogen ion secretion.

The first and most convenient preparation was discovered by Delrue in 1930. He demonstrated that the isolated gastric mucosa of the frog could be kept alive for several hours, and that by placing it between two chambers, acid secretion could be measured accurately under different experimental conditions. It is, therefore, not surprising that much more is known about the electrophysiology of the gastric mucosa of the frog than of mammals.

The electrical properties of the isolated gastric mucosa of the frog were studied by Crane, Davies and Longmuir in 1948. They measured the potential difference whilst currents of different magnitudes were passed through the mucosa, and were thus able to calculate the resistance of the membrane. In the summer frog, the mean potential difference was -35mV., and the mean resistance 210 Ohm-Cm^2. They showed that the mucosa obeyed Ohm's law, and that the maximum current registered when it was short-circuited was 0.07 milliampère.

Further progress had to await the technique devised by Ussing and Zerahn in 1951 to demonstrate that the potential difference across the skin of the frog is generated by active transport of sodium ions. The isolated skin was placed between two celluloid chambers containing identical Ringer solutions. A potential difference of 100mV. was recorded, the inner aspect being positive. The membrane was short-circuited, and the short-circuit current measured. Using ^{24}Na, the authors measured the influx of sodium ions. In separate experiments under the same conditions, they showed the efflux of sodium ions to be very small. They thus showed that there was active transport of sodium. They went further to demonstrate that the short-circuit current was equal to the net flux of sodium. Whilst those experiments did not prove that the potential difference was generated solely by active transport of sodium, the evidence produced by the authors was impressive.

The technique of Ussing and Zerahn has been exploited by Hogben (1951, 1955) to identify the ion responsible for electrical activity in the isolated gastric mucosa of the frog. The membrane was bathed by identical solutions on the two sides, and the potential difference across it was reduced to zero by passing an external current through it. Since there are no electrical and concentration gradients under such conditions, the short-circuit current represents active transport of ions across the membrane. Hogben measured hydrogen ion secretion at the same time, and using isotopic tracers for sodium, potassium, chloride and bicarbonate, measured fluxes of those ions in both directions. He found the net flux of sodium ions to be negligible. Thus, active transport of sodium could not be responsible for the electrical activity of the mucosa of the frog in contrast with the skin of the same

animal. The situation with potassium fluxes is more complex, since the high intracellular concentration makes it difficult for observations to be made under conditions of equilibrium. Nevertheless, the net flux of potassium was found to be very small compared with the short-circuit current, making it unlikely that active transport of potassium ions could play an important part in the electrical activity of the membrane. On the other hand, fluxes of chloride ions were found to be large, and the net flux from the serosal aspect to the mucosal aspect of the membrane was of the same order as the short-circuit current. In a series of forty-eight one-hour experiments in which ^{36}Cl and ^{38}Cl were used to measure simultaneous unidirectional chloride fluxes, Hogben found that

Net Cl flux - H^+ secretion = short-circuit current.

It thus appears that active transport of chloride ions is the mechanism responsible for the electrical activity of the frog gastric mucosa. Hogben (1952, 1955) also found that there was a smaller, but nevertheless appreciable, active transport of bicarbonate ions in the direction opposite to that of chloride. The significance of this finding is not yet clear. A possible explanation is that there is partial exchange diffusion between chloride and bicarbonate ions.

It has not been possible to carry out similar detailed work on the mammalian stomach. It is difficult to keep the isolated mucosa alive and well oxygenated for long. However, some observations have been possible with the help of a special chamber devised by Rehm in 1944. This could be applied tightly to the mucosal aspect of a segment of the stomach of an anaesthetized dog without interfering with its blood supply. In this way it was possible to measure

potential difference, hydrogen ion secretion and net fluxes of ions simultaneously. Using this preparation, Dennis et al (1955) demonstrated active transport of chloride ions into the lumen of the canine stomach. They were, of course, unable to prove that this was responsible for the electrical activity of the mucosa.

Work with some mammals has suggested the possibility that active transport of sodium rather than chloride ions is the main factor responsible for the potential difference. Cummins and Vaughan (1963) measured potential difference and short-circuit current using the isolated wall of the rat stomach in a chamber. They showed that replacement of chloride by sulphate in the chamber had no effect, whereas replacement of sodium by choline abolished electrical activity. As pointed out by Durbin (1967), the lack of effect of chloride withdrawal does not mean very much in such a short-lived preparation, since the tissue is likely to be able to use its own stores of chloride during the experiment. Furthermore, dependence of electrical activity on the presence of sodium ions does not necessarily imply that active transport of sodium is the mechanism producing the electrical activity. This was demonstrated by Sernka and Hogben in 1969. They used isolated rat and guinea-pig gastric mucosae, performing experiments similar to those of Hogben on the frog gastric mucosa. They demonstrated active transport of chloride, sodium and potassium ions. They further showed that the presence of sodium ions is essential for active transport of chloride, whereas substitution of chloride by isethionate has no effect on the transport of sodium.

One criticism that can be levelled against investigators who have used isolated mammalian stomach walls and mucosae is that those preparations may well have been suboptimally oxygenated

during the experiments. Most workers have produced no concrete evidence to the contrary. Sernka and Hogben showed that their preparations did not produce lactic acid, but they could not explain why their isolated rat gastric mucosa secreted hydrogen ions only at the rate of 2.7μEq./sq. cm./hour, when the in vivo rate is 48μEq./sq. cm./hour. The work of Flemstrom (1971) on the isolated frog gastric mucosa at different oxygen tensions is, therefore, particularly relevant. He showed that there was active transport of sodium ions in this preparation at a pO_2 of 300mm. of mercury, but not at a pO_2 of 700mm. He produced some evidence to show that the mucosa was hypoxic at the lower pO_2 in spite of the absence of lactic acid formation. It would seem possible, therefore, that active transport of sodium in the mammalian gastric mucosa is merely a reflexion of hypoxia.

The relationship between the mucosal potential difference and hydrogen ion secretion is of considerable theoretical interest, as well as of practical importance to all investigators in this field. It has been known since the time of Bohlen (1894) that initiation of secretion is accompanied by a fall in the potential difference. This phenomenon has been demonstrated in man and several experimental animals. Rehm (1953) showed a fall in the potential difference across the chambered canine mucosa from -70mV. to -50mV. after a subcutaneous injection of 2mg. histamine. If the chamber was previously filled with 0.1M HCl, the fall in the potential difference was very much smaller. This suggests that at least part of the decrease in potential difference is caused by the creation of a diffusion potential between hydrochloric acid in the tubules and the mucosal solution. This, however, cannot be the only link between acid secretion and electrical activity.

Rehm has shown (1945, 1956) that if a current is passed from an external source to enhance the mucosal potential difference, secretion of hydrogen ions is increased whilst net chloride flux is decreased. The author's own explanation of the "Rehm effect" is that there are two electrogenic pumps, the one for hydrogen ions working in the direction opposite to that for chloride ions. Some evidence for such a hydrogen ion pump was obtained by Heinz and Durbin in 1959. They used the chambered isolated frog gastric mucosa. When all the chloride in both chambers was replaced by sulphate, the potential difference and short-circuit current were reduced to zero. The mucosa was still capable of secreting at about two-thirds the maximal rate with normal solutions. When secretion started, a reversed short-circuit current appeared. It was proportional to the rate of secretion and it disappeared when acid secretion was inhibited by thiocyanate.

It is clear that chloride transport, which is responsible for the maintenance of the gastric mucosal potential difference at least in the frog, and hydrogen ions secretion, are both active processes requiring the expenditure of energy. The link between the two, however, remains an enigma. Metabolic inhibitors, such as cyanide and dinitrophenol affect both of them (Heinz and Durbin, 1957). Yet thiocyanate will inhibit hydrogen ion secretion without affecting the short-circuit current, and acetazolamide and other carbonic anhydrase inhibitors will abolish the short-circuit current in the histamine-stimulated mucosa without affecting the rate of hydrogen ion secretion (Durbin and Heinz, 1958). The isolation of a microsomal adenosine triphosphatase from the gastric mucosa of the bullfrog by Durbin and Kasbekar (1965) is of great theoretical interest. It is stimulated by chloride ions and

inhibited by both thiocyanate and dinitrophenol. This enzyme is quite likely to be involved in the active transport of chloride. The fact that it is also stimulated by bicarbonate ions reminds one of the theory of Hogben (1952), according to which partial exchange between chloride and bicarbonate ions at the mucosal surface generates the potential difference. Sachs et al (1965) have also isolated an adenosine triphosphatase from the microsomes, and later (Sachs et al, 1972) from a vesicular system from frog gastric mucosa. This is sometimes stimulated by histamine, and the authors propose that it is involved in the secretion of hydrogen ions. It is not at all clear yet whether the two groups of workers have in fact isolated the same enzyme, and whether this enzyme is involved with both electrical activity and hydrogen ion secretion.

CHAPTER IV

THE PERMEABILITY OF THE GASTRIC MUCOSA AND ITS RELATIONSHIP TO THE POTENTIAL DIFFERENCE

"Why does the living stomach of a warm-blooded animal not digest itself?" This is a question that has been asked again and again over the years. Numerous theories have been formulated, none of them entirely satisfactory. Hollander (1954) had the idea of a two-component mucous barrier. The first line of defence, according to this concept, is a layer of mucus, which is continuously being replaced. This is a dynamic barrier acting in two ways. It neutralizes acid, and it retards the penetration of chemical irritants. The second component consists of the surface epithelial cells themselves. Those are continuously being shed and rapidly replaced. Hollander (1952) believed in the Pavlovian idea that the parietal cells secrete hydrochloric acid at a constant concentration of about 165mN whatever the rate of secretion, and thought that acidity was regulated by a second, non-parietal component. In Heidenhain pouch dogs, he showed that stimulation with histamine causes the concentration of acid to rise with rate of secretion until a plateau is reached at 165mN. However, if mucus is washed out of the pouch before the experiment, this relationship no longer holds. Furthermore, if atropine is used as well as histamine, high concentrations of acid are obtained at low secretion rates. Hollander conceded that "reabsorption and ionic interchange" could also play a part, but insisted that neutralization by the non-parietal component of gastric secretion was very much more important.

Teorell, on the other hand, proposed that acidity was regulated

by reabsorption through the mucosa. In anaesthetized cats (Teorell, 1939) he demonstrated rapid disappearance of hydrogen ions from an instilled solution. He thought that the gastric mucosa acted purely as a "dialyzing membrane", and predicted that the concentration of acid placed in such a membrane would fall exponentially (Teorell, 1947). In his experiments, the concentration of acid in the stomach did fall exponentially, but at a much slower rate than he predicted or found when he used a model. He had, in fact, demonstrated a barrier to the reabsorption of hydrogen ions.

The significance of the gastric mucosal barrier was not fully appreciated until Code rekindled interest in the subject by demonstrating the presence of a barrier to sodium ions in the stomach of the anaesthetized dog (Code et al, 1963). The authors excluded the antrum from the rest of the stomach by means of a ligature, and, using ^{22}Na, showed that absorption of sodium from an instilled solution was slow. In the presence of acid, absorption of sodium was negligible. This work was extended by Davenport, Warner and Code in 1964, under more rigidly controlled conditions. They took advantage of the fact that the Heidenhain pouch dog does not secrete much acid into the pouch under resting conditions. They followed the disappearance of sodium and hydrogen ions from the pouch, and by using ^{24}Na, measured bidirectional fluxes of sodium. They thus confirmed that presence of a barrier to sodium and hydrogen ions, and went further to show that the barrier could be broken by the chemical irritant, eugenol. In the presence of eugenol, net fluxes of sodium and hydrogen ions increased seven to thirteen-fold. The most striking increases were in the flux of sodium into the pouch, and that of hydrogen in the opposite

direction, the two occuring at rates that correlated well with each other.

Over the succeeding years, Davenport exploited the use of the Heidenhain pouch dog to study several agents capable of breaking the gastric mucosal barrier. He showed (Davenport, 1964) that acetylsalicylic acid and acetic, butyric and propionic acids increase the permeabilityto sodium and hydrogen ions in an acid medium, but not when buffered with Tris. He demonstrated (Davenport, 1965a and 1967a) that salicylic and acetylsalicylic acids in 1mM HCl were as efficient at breaking the barrier as when placed in 100mM HCl, although bleeding tended to occur only when the higher concentration of acid was used. Pepsin did not seem to be playing an important role. Using albumin labelled with ^{51}Cr, he showed (Davenport, 1966) that exudation of plasma contributed about one-third of the large amount of extra fluid appearing in the pouch when the barrier was broken. Another agent that broke the barrier was ethanol at a concentration of 14% or more (Davenport, 1967b). Ethanol differed from the weak organic acids in that it did not require the presence of an acid medium to have its effect. The increase in permeability to sodium and hydrogen ions is accompanied by histologically demonstrable, reversible damage to the mucosa (Dinoso et al, 1971).

Hollander's original idea of a gastric mucosal barrier has had to be modified to a certain extent as a result of the work of Code, and of Davenport. The latter's concept of the barrier is as follows (Davenport, 1970):-

Whilst mucus is certainly capable of neutralizing acid, it is a very poor barrier in that it offers little resistance to the passage of sodium and hydrogen ions. The only barrier of importance

consists of the cellular layer itself. It keeps the back-diffusion of hydrogen ions and the movement of sodium ions in the opposite direction to a minimum. The barrier can be disrupted temporarily by several substances during absorption. Ethanol is absorbed at any pH, whilst weak acids, including acetylsalicylic acid, need to be in an acid environment, where they are unionized and lipid-soluble, for absorption. Following disruption of the barrier, back-diffusion of hydrogen ions takes place, causing damage to the mucosa, an outpouring of fluid, and sometimes haemorrhage.

An increase in the passive permeability of the gastric mucosa, as happens when the mucosal barrier is broken, is synonymous with a rise in the conductance of the mucosa. If one assumes that the mechanism responsible for the generation of the potential difference is not altered by the agent breaking the barrier, it is clear that a rise in conductance will be associated with a fall in the absolute value of the potential difference. This is indeed what has been observed by numerous investigators. Thus, Davenport, Warner and Code (1964) found an exponential relationship between transmucosal potential difference and flux of sodium ions into the gastric lumen when eugenol was used to break the barrier. Chvasta and Cooke (1972) found the same relationship between potential difference and flux of sodium ions into the gastric lumen or of hydrogen ions in the opposite direction when they used acetylsalicylic acid. More recently, Flemstrom and Marsden (1973) actually measured potential difference, short-circuit current and resistance of the isolated frog gastric mucosa during exposure to acetylsalicylic acid. They confirmed the expected fall in resistance. However, both the potential difference and the short-circuit current fell before they could detect any change in the

resistance. It would seem, therefore, that, at least in this preparation, acetylsalicylic acid lowers the potential difference by a direct inhibition of the electrogenic transport mechanism as well as by increasing the passive permeability of the mucosa to ions. The relationship between ionic fluxes and transmucosal potential difference may well be more complex than suggested by theoretical considerations, but this does not detract from the fact that the potential difference is a measure of the integrity of the mucosa.

CHAPTER V

PRACTICAL APPLICATION OF POTENTIAL DIFFERENCE AND IONIC FLUX MEASUREMENTS

Early attempts to use measurements of potential difference as a tool in clinical investigation proved to be disappointing. Goodman tried to diagnose benign and malignant diseases of the stomach by means of an analysis of the waves in potential difference tracings, and of the response of the potential difference to ingested milk (Goodman, 1942; Goodman et al, 1955). His findings, however, could not be confirmed by other workers. Thus, Ingram and Richards (1953) found the milk response to be too variable to be of clinical significance, and did not find wave pattern analysis to be helpful in diagnosis.

More recent developments in technique have allowed some progress to be made in the use of potential difference and ionic flux measurements in several fields.

Anatomical Studies

Greenwood et al (1965) found that there was a very sudden and dramatic fall in the potential difference recorded when the exploring electrode was withdrawn from the stomach into the oesophagus of the dog. This change corresponded exactly to the position of the cardiac sphincter, even in dogs in which a hiatus hernia had been created surgically beforehand. This technique was used by the same group (Helm et al, 1965) to find the exact position of the gastro-oesophageal junction in man. The results obtained were as good as those found with pressure measurements. Histological evidence that the site of maximum change in the

potential difference in man is in fact the junction between gastric and oesophageal mucosae was produced by Meckeler and Ingelfinger (1967).

The site of the gastroduodenal junction cannot be accurately defined by means of pressure measurements, or by the use of a pH electrode. Andersson and Grossman (1965) showed that there was a sharp rise in the potential difference when the exploring electrode is pulled out of the duodenum into the antrum, and recommended this technique for the identification of the junction.

The Abnormal Gastric Mucosa

In Heidenhain pouch dogs, stimulation of secretion with histamine after exposure of the mucosa to eugenol yields only small amounts of acid, whereas if the hydrogen ions are prevented from back-diffusing by trapping them with glycine under the same conditions, acid secretion can be shown to be occurring at the expected rate (Davenport, Warner and Code, 1964). This finding prompted Davenport (1965b) to ask whether "the apparent hyposecretion of acid by patients with gastric ulcers (is) a consequence of a broken barrier to diffusion of hydrogen ions into the gastric mucosa?"

Measurement of ionic fluxes across the gastric mucosa of man is fraught with difficulties. Unlike the Heidenhain pouch of the dog, the resting human gastric mucosa secretes significant amounts of acid, and gastric emptying is taking place all the time. Furthermore, some neutralization of acid by mucus and other secretions occurs. Chapman et al (1968) instilled 200ml. isotonic hydrochloric acid with a non-absorbable marker for fifteen minutes into the stomachs of normal subjects and patients with gastric ulcers. Using the method of Hunt (1951), the volume secreted and

the volume passing through the pylorus were calculated. The actual rate of back-diffusion of hydrogen ions was worked out on the basis of Hollander's two-component hypothesis. The authors demonstrated a more rapid fall in concentration of hydrogen ions and rise in sodium ion concentration in three out of seven patients with gastric ulcers as compared with seven controls. When insorption of hydrogen ions was calculated, however, no significant difference could be found between the two groups.

More recently, Chapman et al (1972) have used the technique of trapping secreted hydrogen ions during stimulation with pentagastrin to determine the rate of back-diffusion of hydrogen ions. They found the latter to be four times faster in gastric ulcer patients than in duodenal ulcer patients. Healing of the gastric ulcer in two patients did not improve the gastric mucosal barrier.

Bile Salts and Gastric Mucosal Disease

The idea that bile reflux into the stomach could play a part in the pathogenesis of gastric ulceration occurred to du Plessis (1965) as a result of his study of bile-acid conjugates in gastric aspirates. The concentration was higher than in normals in nine out of fourteen patients with benign lesser curve gastric ulcers. Furthermore histological study of partial gastrectomy specimens showed evidence of gastritis always extending from the pylorus proximally at least to the level of the ulcer in the majority of patients with gastric ulcers, whereas gastritis was uncommon in duodenal ulcer patients. Radiological evidence of reflux was obtained by Capper (1967) in twenty-four of twenty-nine gastric ulcer patients, whilst no reflux could be demonstrated in any of twenty-three normal controls.

Confirmation of excessive bile reflux into the stomach with a benign lesser curve ulcer was obtained in an elegant study by Rhodes et al in 1969. They used C^{14}-labelling of the bile salt pool for measurement of bile salt concentration in gastric aspirates. Concentrations were much higher in ten ulcer patients than in controls. Healing of the ulcer is not always associated with a decrease in bile reflux (Black et al, 1971). Rhodes (1972) suggested that the basic abnormality in patients with benign lesser curve ulcers is a motility defect which allows bile to reflux into the stomach and damage the mucosa.

Both potential difference and ionic flux measurements have shown that bile salts can break the gastric mucosal barrier in man. Geall et al (1970) observed a fall in the mean potential difference of six healthy subjects from -39mV. to -28mV. after instilling into the stomach 25ml. of duodenal aspirate. The duodenal aspirate must, of course, have had other substances such as pancreatic enzymes in it apart from bile salts. Ivey et al (1970) used a pure bile salt mixture at a concentration (5.5mM.) similar to that in gastric aspirates in patients with gastric ulceration. They exploited the instillation technique of Chapman et al (1968), and found that back-diffusion of hydrogen ions increased more than four-fold after instillation of the bile salt mixture. The authors (Ivey et al, 1971) repeated the same experiment in atropinized subjects in the hope that the decrease in acid secretion could unmask back-diffusion. Although they found it impossible to calculate the flux of hydrogen ions into the mucosa, they observed an even greater net flux after the bile salt mixture than in their previous study.

Drugs that damage the gastric mucosa

A large number of commonly used analgesic and anti-inflammatory

drugs produce symptoms of indigestion. Aspirin in particular has been extensively studied. It causes acute erosions and haemorrhage (Douthwaite and Lintott, 1938), occult blood loss (Jabbari and Valberg, 1970), exfoliation of gastric epithelial cells (Croft, 1963), possibly chronic atrophic gastritis (Edwards and Coghill, 1966), and probably chronic gastric ulceration in man (Chapman and Duggan, 1969; Gillies and Skyring, 1969). St. John and his colleagues (1973) have demonstrated the production of chronic gastric ulcers in the rat as a result of long-term ingestion of aspirin. Other drugs that have been implicated as causes of gastric mucosal damage and ulceration are phenylbutazone (Kern et al, 1957), indomethacin (Emmanuel and Montgomery, 1971) and corticosteroids (Kern et al, 1957). Many other retrospective studies concerning the association between those drugs and gastric ulceration have been carried out on populations consisting mainly of patients with rheumatoid arthritis. Apart from the difficulties inherent in all retrospective studies, the situation here is further complicated by the fact that patients with rheumatoid arthritis frequently use several drugs. Furthermore, there is as yet no good evidence to show that those patients have a normal gastric mucosa to start with.

Overholt and Pollard (1968) used the instillation technique to demonstrate increased permeability of the gastric mucosa to sodium ions in man when the instilled solution contained 20mM. acetylsalicylic acid. However, they did not take acid secretion into consideration, and probably for that reason failed to show an increase in permeability to hydrogen ions. In order to circumvent the problems caused by acid secretion and neutralization, Smith et al (1971) took advantage of the fact that permeability to lithium

ions is proportional to that of hydrogen ions (Chung et al, 1970a; 1973). They showed that 20mM. acetylsalicylic acid solutions produced an increase in permeability of the gastric mucosa to lithium, and therefore to hydrogen ions, in man. It has also been shown that 650mg. of aspirin suspended in 50ml. of normal saline will lower the gastric mucosal potential difference in man from -40mV. to -30mV. (Geall et al, 1970).

Very little work has been done on anti-inflammatory agents other than aspirin. Chvasta and Cooke (1972) found a small decrease in the potential difference and a small increase in the permeability to hydrogen and sodium ions in Heidenhain pouch dogs with indomethacin, but not with phenylbutazone or corticosteroids. Indomethacin did not have the same effect on the mucosa of canine denervated antral pouches (Cooke and Kienzle, 1974). Murray et al (1973) found that prednisone, indomethacin and phenylbutazone had no signficant effect on the gastric mucosal potential difference in man.

Drugs Used in the Treatment of Gastric Ulcers

The only drug that has been shown to promote the healing of gastric ulcers is carbenoxolone (Doll et al, 1968; Cocking and McCaig, 1969). The mode of action of this drug remains unknown (Sircus, 1972). It has been shown to increase the life span of gastric epithelial cells (Lipkin, 1970) and the rate of secretion of mucus (Cross et al, 1972), and to inhibit the activity of pepsin (Henman, 1970).

The first attempt to find out whether carbenoxolone exerts its therapeutic effect by strengthening the gastric mucosal barrier was made by Cross et al (1972). They measured the increase in gastric mucosal permeability in Heidenhain pouch dogs caused by

bile before and after irrigating the pouch with a solution of carbenoxolone for one hour. They found an increase in the resistance of the mucosa to penetration by hydrogen but not be sodium ions.

Ivey and Gray (1973) attempted a similar experiment in normal human volunteers. They found that carbenoxolone given for three weeks at a dose of 100mg. three times a day had no effect on the increase in permeability to hydrogen and sodium ions caused by 10mM taurocholic acid solution. Instillation of a solution containing 100mg. carbenoxolone into the stomach fifteen minutes before the taurocholic acid solution also had no effect.

CHAPTER VI

UNANSWERED QUESTIONS

Both the permeability of the gastric mucosa and the potential difference provide an indication of its functional integrity. In man, measurement of permeability is made difficult by the fact that secretion, neutralization and gastric emptying are taking place at the same time as passive ionic movements. On the other hand, measurement of the potential difference is simple, and it lends itself to the study of the gastric mucosa, normal and abnormal, in a variety of experimental situations.

Anatomical Studies

By passing an electrode through the biopsy channel of a gastroscope, it should be possible to place the tip accurately in contact with the mucosa at various sites in the stomach, and thus to map the distribution of the potential difference. It is already known that there is a difference between the antrum and the body of the stomach, but questions such as whether there is a difference between the greater and the lesser curve can only be answered by carrying out measurements under direct vision.

The Abnormal Gastric Mucosa

The permeability of the gastric mucosa is greater in gastric ulcer patients than in those with duodenal ulcers, even after healing of the gastric ulcer (Chapman et al, 1972). This suggests that gastritis is associated with an increased permeability, and one would, therefore, expect to find a lower than normal potential difference in patients with gastritis. A study of the correlation between histology and potential difference is needed to confirm this.

Whilst the conventional technique of measuring the potential difference could provide the answer, measurement under direct vision with biopsies taken from the same sites would obviously produce more accurate information.

Drugs that Damage the Gastric Mucosa

Aspirin has been shown to lower the potential difference in man (Geall et al, 1970). It is generally believed that this effect is mediated during the absorption through the gastric mucosa of the drug. However, there have been no potential difference studies during which intragastric pH, on which absorption of aspirin depends, (Hogben et al, 1957), and plasma salicylate levels have been monitored. The effect of intravenous salicylate has also not been studied.

As far as other drugs, such as phenylbutazone, indomethacin and corticosteroids, are concerned, there has been only one study in man (Murray et al, 1973). Since the results were different, at least in the case of indomethacin, from those obtained in the dog (Chvasta and Cooke, 1972), this study needs repeating.

Drugs Used in the Treatment of Gastric Ulcers

By measuring the fall in potential difference caused by a standard dose of an irritant agent before and after treatment with drugs such as carbenoxolone and gefarnate, it should be possible to assess whether they can protect the gastric mucosa. The only work done to date along those lines (Ivey and Gray, 1973) has used permeability measurements, and has produced results that conflict with those obtained in animals (Cross et al, 1972).

S E C T I O N II

TECHNIQUES

CHAPTER VII

MEASUREMENT OF THE GASTRIC MUCOSAL POTENTIAL DIFFERENCE

The technique used for measurement of the potential difference was essentially as described by Andersson and Grossman (1965). Radiometer (Copenhagen) measuring and recording instruments were used throughout. Electrolyte bridges, which will be called exploring electrode and reference electrode for convenience, were prepared locally in the laboratory.

Exploring Electrode

The exploring or gastric electrode consisted of a polyethylene tube 150cm. long with an internal diameter of 3mm., completely filled with saturated potassium chloride in 3% agar. Each electrode was prepared immediately before use. The tube was filled with the mixture whilst it was hot, care being taken to avoid the formation of air bubbles. Subsequent cooling allowed the formation of a gel inside the tube. Marks were made at 5cm. intervals on the tube.

Reference Electrode

The reference or intravenous electrodes consisted of finer polyethylene tubes (internal diameter 1.14mm.) approximately 100cm. long, also containing saturated potassium chloride in 3% agar. They were made in batches of ten to twenty. As soon as the electrolyte mixture had solidified, the ends of the electrode were sealed. Each electrode was threaded through a needle (Bard I Cath) for subsequent insertion into a vein. From then on it was kept in a sealed polyethylene bag until the time of use. Batches of electrodes were sent away for sterilisation by gamma radiation.

Calomel Electrodes

The actual electrodes used were calomel half-cells (K100, Radiometer, Copenhagen), which are stable and non-polarizable. Electrical continuity between each calomel electrode and the corresponding electrolyte bridge was provided by a small beaker containing saturated potassium chloride solution.

Millivolt Meter

A pH meter (Titrator TTT2, Radiometer, Copenhagen) was used as a direct current millivolt meter. This has a very high input resistance (2×10^{12} ohms) and therefore draws little current from the source.

Recording Equipment

Potential difference was recorded on paper by means of a Servograph Pen Drive REA310 with pH meter interface REA100 (Radiometer, Copenhagen). A full-scale deflection of 100mV. was used throughout. The chart speed used was 2mm. per minute in most cases.

Procedure

After an overnight fast, the subject was asked to swallow the gastric or exploring electrode until the right distance between tip and incisor teeth was achieved. Using a no-touch technique, the sealed ends of the reference electrode were cut off, and the electrode was introduced into a vein in the left antecubital fossa via the needle, which was then removed and discarded. The free ends of the electrodes were placed in the beakers containing saturated potassium chloride, into which were also dipped the ends of the calomel electrodes. The zero-line was obtained by placing the two ends of a polyethylene tube containing saturated potassium

chloride in 3% agar in the two beakers. Recording of the gastric mucosal potential difference was started only after this line had been steady for at least ten minutes.

Figure 1 shows an experiment in progress.

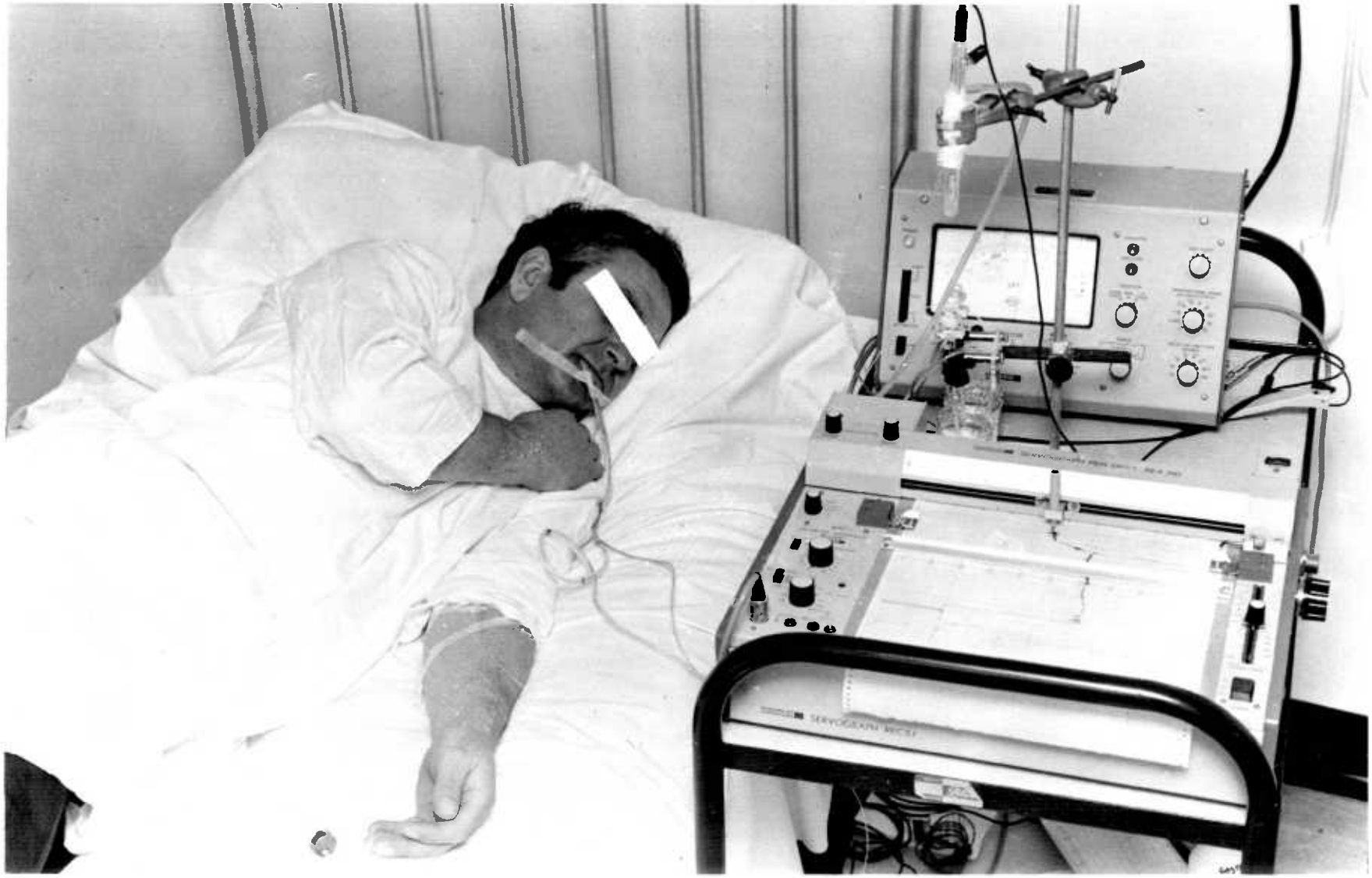


Figure 1 : Measurement of gastric mucosal potential difference in progress.

CHAPTER VIII

THE POTENTIAL DIFFERENCE ACROSS THE SKIN

The use of an intravenous reference electrode is simple, and the procedure is not at all time-consuming. However, the preparation, sterilization, and storage of the electrodes do present some practical problems. For example, although the ends of each electrode and the bag in which it is kept are sealed, the electrolyte mixture tends to dry after some time, leaving crystals and air bubbles. It is, therefore, not surprising that many investigators in the past have used skin electrodes instead. Obviously such a technique cannot measure the actual gastric mucosal potential difference. It could be used to compare the latter in different subjects or groups of subjects, if one can assume that those subjects have the same potential difference across the skin. It could also be used to compare the potential differences in different parts of the stomach, or to study the effect of various drugs, if the assumption is made that the skin potential difference remains steady during the period of study. In order to find out whether such assumptions can be made, the potential difference between the skin and the blood was recorded for thirty minutes in five subjects.

Procedure

A reference electrode was inserted in a vein, and the free end placed in one of the two beakers containing saturated potassium chloride as described for the measurement of the gastric mucosal potential difference. The subject was then asked to place a finger in the other beaker, and the potential difference was recorded for thirty minutes.

Results

The skin was electrically positive with respect to the blood. In all the subjects the potential difference fell gradually over a period of twenty minutes, after which it became relatively steady. The potential difference for each subject at five minute intervals is shown in Table III. The gradual fall in the mean for the five subjects is shown in Figure 2.

TABLE III

Time-course of skin potential difference (mV.)

Time (Mins.)	0	5	10	15	20	25	30
Subject 1	53	37	31	22	15	18	20
Subject 2	49	37	31	28	27	27	26
Subject 3	45	40	28	23	20	18	17
Subject 4	53	43	40	35	36	37	36
Subject 5	43	37	34	30	27	26	26
Mean	48.6	38.8	32.8	27.6	25.0	25.2	25.0
S.E.M.	2.0	1.2	2.0	2.4	3.6	3.5	3.3

Discussion

In the small group studied, the skin potential difference varied markedly from subject to subject. Hence if the skin is used as the site of reference for measurement of the gastric mucosal potential difference, inter-subject comparisons are not possible. Furthermore, by using such a technique, one should observe a gradual fall in the recorded potential difference as the skin potential difference falls over the first twenty minutes. Andersson and Grossman (1965) measured the post-bulbar duodenal potential difference twice in twenty-six subjects, withdrawing the exploring electrode into the stomach between the two measurements.

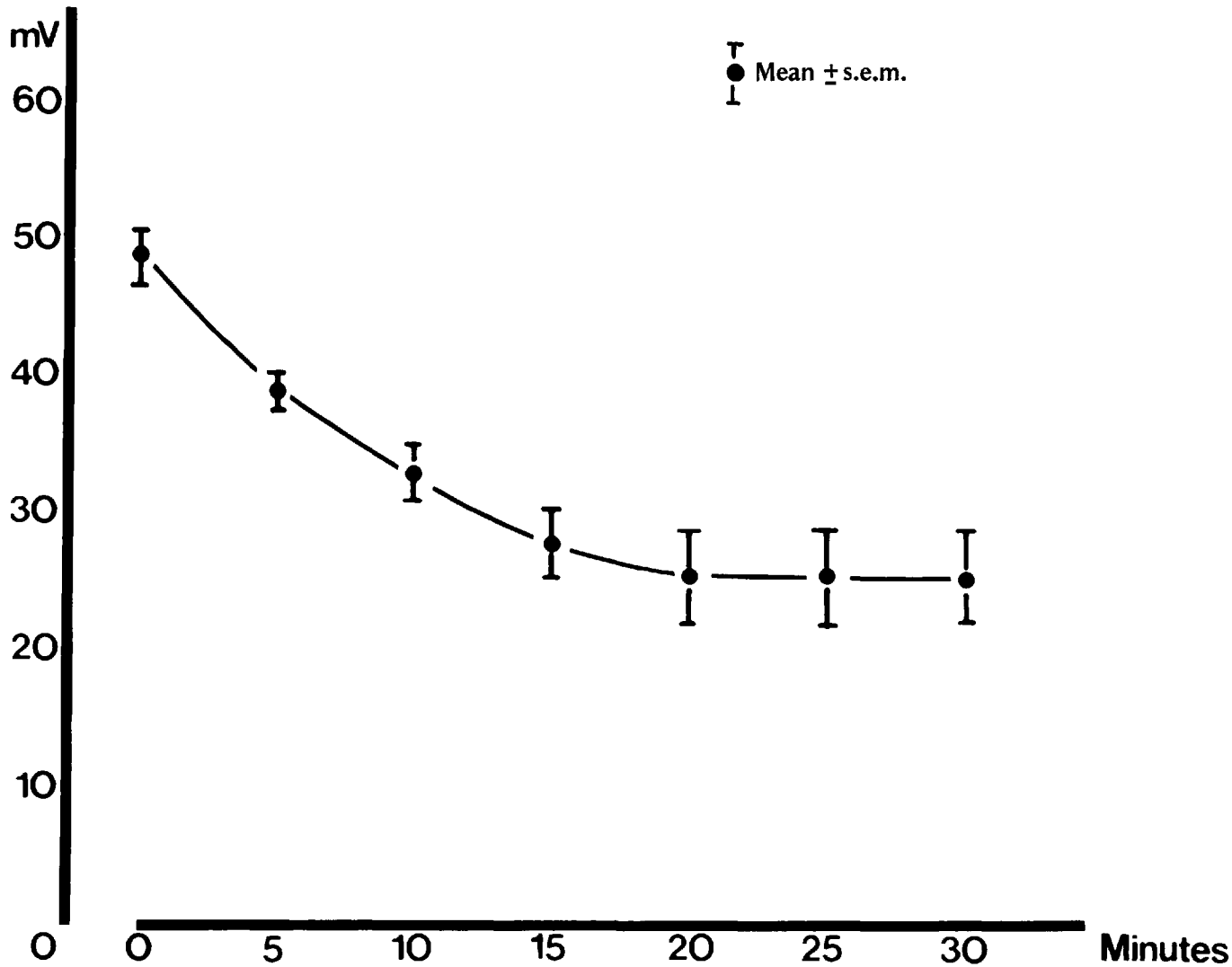


Figure 2 : Potential difference across the skin in five subjects.

-42-

They could not explain why there was a statistically significant fall of 2.4mV. between the two readings. The results obtained here suggest that the use of the skin as the site of reference was almost certainly responsible for this observation. Such changes in the recorded potential difference, being independent of the state of the gastric mucosa, preclude the use of skin electrodes in studies designed to measure the effects of drugs on the mucosa, or to compare the potential differences in different parts of the stomach.

An ingenious method of abolishing the skin potential difference by means of an intradermal injection of saline has been described by Archampong and Edmonds (1972). Unfortunately, the duration of the effect of a saline injection was unpredictable, and in some cases it was no more than ten minutes. During the present studies, experiments lasted well over ten minutes. Hence intravenous reference electrodes were used throughout.

CHAPTER IX

MEASUREMENT OF THE POTENTIAL DIFFERENCE UNDER DIRECT VISION

The potential difference at various sites in the stomach was measured under direct vision at endoscopy in a number of subjects. This experiment was carried out only on patients who needed a diagnostic endoscopy, informed consent having been obtained beforehand. By including patients with gastric ulcers, and those with duodenal ulcers or X-ray negative dyspepsia, it was ensured that both abnormal and normal gastric mucosae would be represented in this series.

Equipment

The measuring equipment was exactly the same as was used in the conventional technique for the measurement of the gastric mucosal potential difference, except that the exploring electrode was replaced by a finer polyethylene tube (internal diameter 1.40mm.) containing saturated potassium chloride in 3% agar. When this electrode was passed through the biopsy channel of the endoscope (GIFD2, Olympus), and the light source was switched on, no potential difference was created between the two ends of the electrode. Hence it was assumed that the endoscope did not interfere with the measurement of the potential difference.

Procedure

After an overnight fast, the subject was premedicated with intravenous diazepam in a dose of 20-30mg. Atropine was not used. A reference electrode was inserted in an antecubital vein in the left arm. Diagnostic endoscopy was performed with the subject on his or her left side. As much fluid as possible was sucked out

of the stomach before making any potential difference measurements. The fine exploring electrode was passed through the biopsy channel of the endoscope, and the tip was gently pressed against the mucosa. The free ends of the electrodes were placed in the beakers containing saturated potassium chloride as described previously, and the potential difference recorded for one minute. Measurements were made in the duodenal bulb, the prepyloric region, the incisura, the low greater curve, the high lesser curve and the high greater curve. A biopsy was then taken from each of those sites.

Difficulties

The first few attempts at measuring the potential difference under direct vision produced erratic tracings. Keeping the tip of the exploring electrode in contact with the mucosa proved to be the most difficult part of the procedure. A restless subject who would not keep still made it impossible to do so. Intermittent contact leading to wide fluctuations in the recorded potential also obtained when the stomach was very active and contracting vigorously. If the electrode was pressed too firmly against the mucosa, the latter was sometimes scratched, as evidenced by the appearance of a small amount of blood. This invariably led to a fall in the recorded potential difference.

In spite of those difficulties, it was found that with care and patience it was possible to obtain satisfactorily steady tracings in the majority of subjects. Preliminary studies showed that results were reproducible. In the group of subjects studied, no attempt was made to record at any one site for more than one minute, or to make duplicate measurements. In this way it was possible to complete the experiment in an average of twenty minutes after having carried out diagnostic endoscopy.

CHAPTER X

OTHER TECHNIQUES

pH Measurements

It is well known that the absorption of aspirin in the stomach is pH dependent (Hogben et al, 1957). The effect of aspirin on the gastric mucosal barrier is also dependent on pH (Davenport, 1964). It was therefore felt that the pH of gastric contents had to be monitored in all experiments in which the effect of aspirin on the potential difference was being measured.

In the earliest experiments, small volumes of gastric contents were aspirated at intervals via a side-tube attached to the exploring electrode. This procedure proved to be unsatisfactory, since it tended to cause large artefacts in the potential difference tracing. It thus became possible to measure the pH only before instillation of the drug into the stomach, and at the end of the experiment. This procedure was therefore abandoned, and an intragastric pH electrode (Beckmann glass electrode) was used instead. It was attached to the potential difference electrode and the side-tube. At intervals, the recording of the potential difference was momentarily stopped, and the pH recorded, using the same pH meter and pen writer.

Measurement of Plasma Salicylate

In a number of experiments involving the use of aspirin, plasma salicylate levels were measured at intervals during the test. The method used was adapted from that of Routh et al (1967). The use of whole blood produced inconsistent results, and therefore plasma obtained by centrifuging heparinized blood for two minutes at 2000r.p.m. immediately after venepuncture was used throughout.

Two ml. plasma were pipetted into a bottle containing 30ml. dichloroethane, 1ml. water and 0.6ml. 5N Hcl. A mechanical shaker was used for shaking the bottle for fifteen minutes, after which the contents were filtered. Twenty-five ml. of the organic phase was transferred to a bottle containing 6ml. of 4% sodium bicarbonate solution. The bottle was shaken for five minutes, and then the contents centrifuged. Two ml. aliquots of the resulting aqueous phase were transferred into each of two quartz cuvettes. One ml. water was added to one cuvette (reference cell) and 1ml. 2.5N sodium hydroxide solution to the other (sample cell). Spectral absorbance of the sample was read on a Beckmann DU spectrophotometer after having set the reference at zero absorbance at 319mU.

Duplicate samples of plasma were used throughout. Plasma from the same subject, obtained before the ingestion of aspirin, was used for making up standard solutions containing 2, 5, 8 and 10mg. salicylate per 100ml.

Almost exactly the same standard curves were obtained whether plasma or water was used (see Figure 3), showing that extraction of salicylate from plasma was complete. Results obtained with duplicate damples of plasma were so close together that it was not felt necessary to test reproducibility any further.

In all experiments on the effect of aspirin on the gastric mucosal potential difference, a standard dose of 600mg. aspirin in the form of two Solprin tablets was used. In a preliminary experiment, plasma salicylate levels at 10, 20, 30, 45 and 60 minutes were measured in four healthy fasting subjects after ingestion of two Solprin tablets. The results are shown in Table IV and in Figure 4.

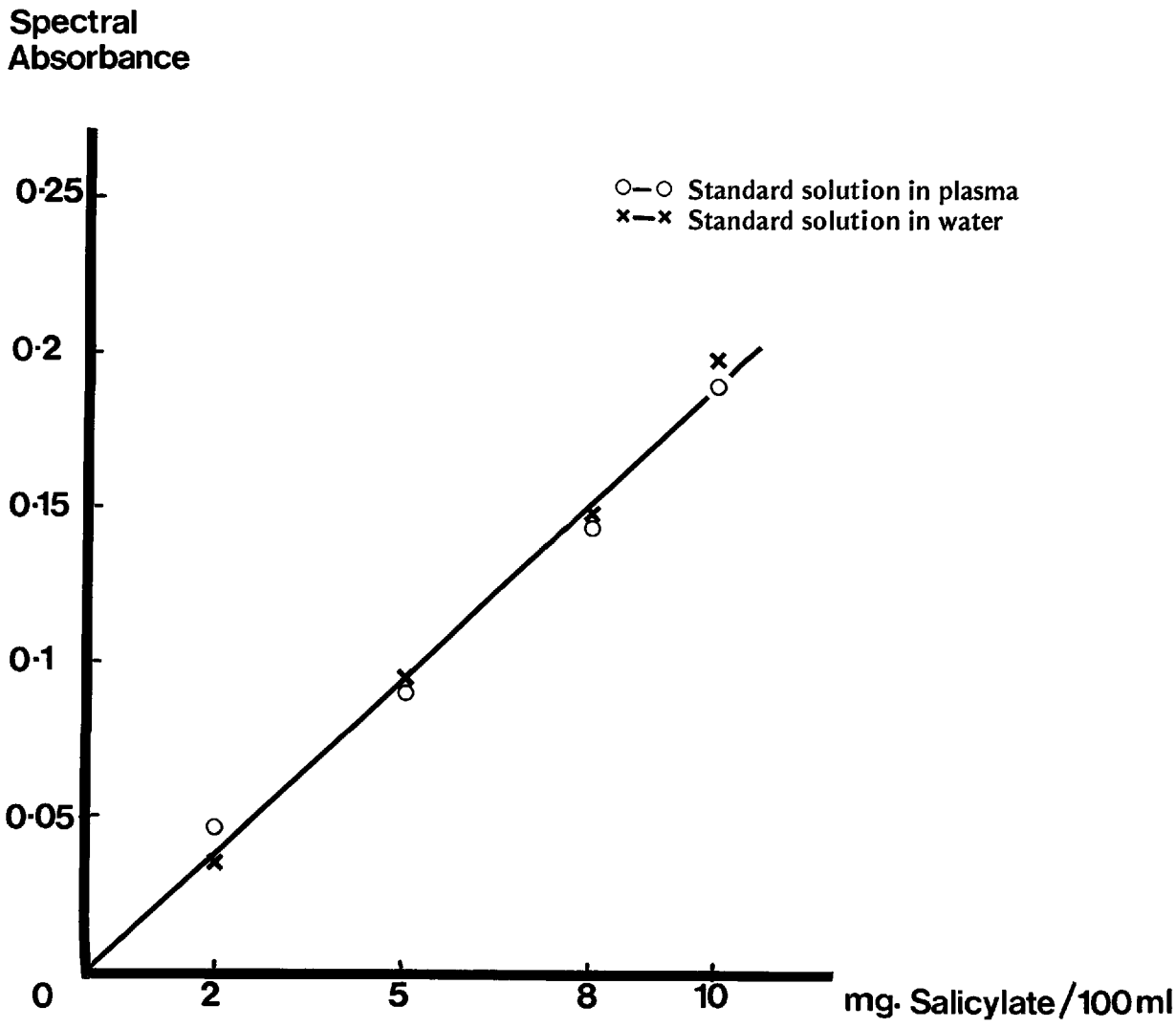


Figure 3 : Standard curve for measurment of plasma salicylate levels.

TABLE IV

Plasma salicylate levels (mg./100ml.) after ingestion of 600mg. aspirin

Minutes / Subjects	10	20	30	45	60
1	5.0	6.0	6.25	5.6	3.3
2	2.0	5.0	5.6	5.8	6.15
3	3.25	5.65	6.15	6.15	6.4
4	2.1	5.0	7.2	6.1	5.85
Mean	3.1	5.4	6.3	5.9	5.4
S.E.M.	0.7	0.2	0.3	0.1	0.7

In three out of four subjects, the peak level was obtained at thirty minutes, and in one the level continued to rise very slowly after thirty minutes. There was wide variation between individual results at ten and sixty minutes, and much less variation over the twenty to forty-five minute time interval. This finding was used to determine the best times to take blood samples in order to obtain the peak plasma salicylate level during experiments on the effect of aspirin on the potential difference.

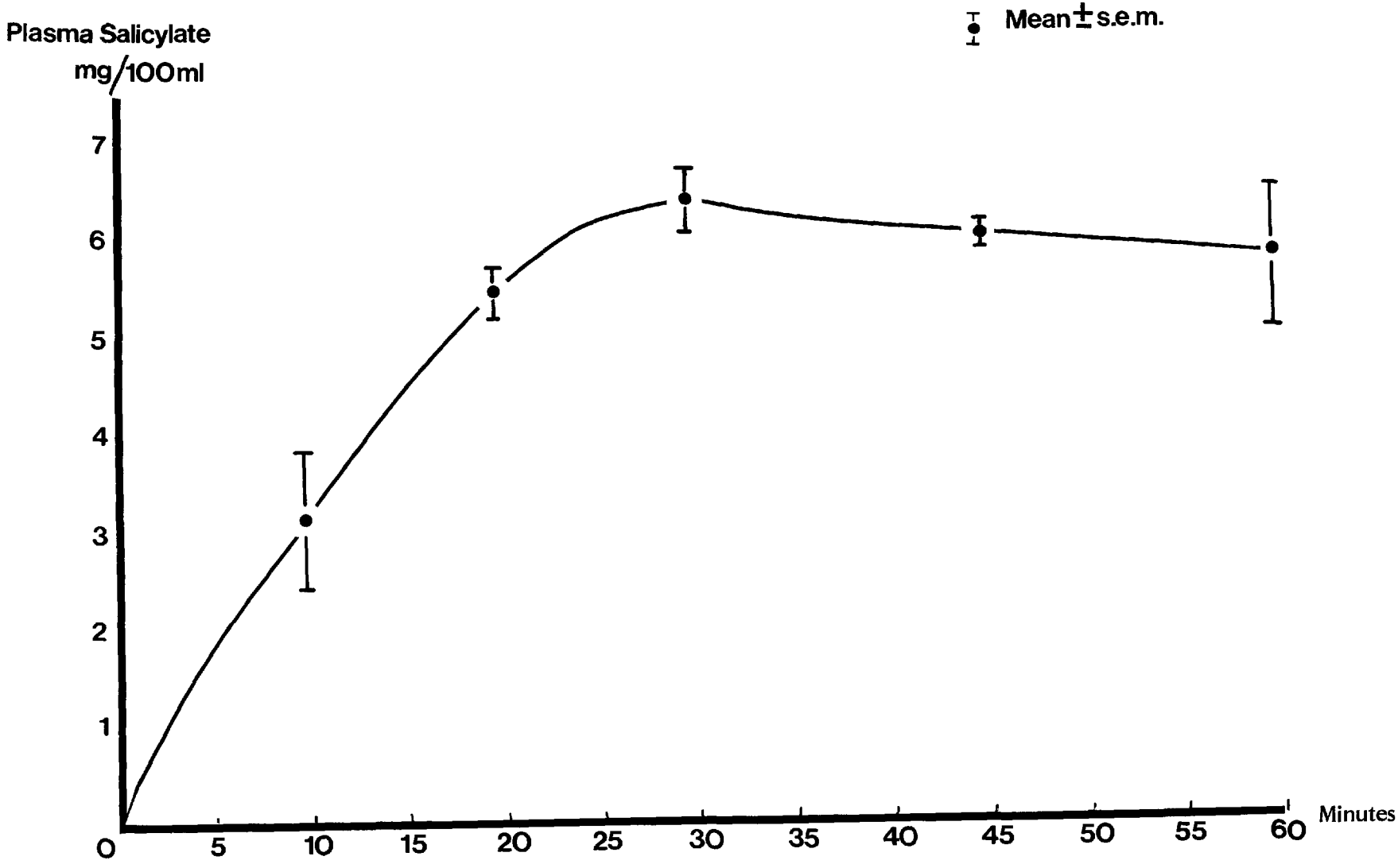


Figure 4 : Plasma salicylate levels in four normal subjects after ingestion of 600 mg. aspirin.

S E C T I O N III

EXPERIMENTAL WORK ON THE NORMAL AND THE ABNORMAL GASTRIC MUCOSA

CHAPTER XI

STUDIES USING CONVENTIONAL TECHNIQUES

The rate of back-diffusion of hydrogen ions through the gastric mucosa is four times faster in gastric ulcer patients than in duodenal ulcer patients (Chapman et al, 1972). Furthermore, healing of the gastric ulcer in two patients did not improve the gastric mucosal barrier, suggesting that the impaired barrier is associated with the gastritis rather than with the ulcer itself. One would therefore expect to find a lower than normal potential difference across the abnormal gastric mucosa.

At a first glance, this hypothesis could be tested very simply by measuring the potential difference in a group of normal controls and in groups of patients with gastric ulcers or histologically proven gastritis. The problem arises, however, as to where to measure, since it is well-known that the potential difference is not the same in all parts of the stomach (Andersson and Grossman, 1965). Preliminary studies were therefore carried out to determine the best position of the tip of the gastric electrode that would give steady and reproducible readings. In four presumed normal volunteers, it was found that if the electrode was passed orally to the antrum of the stomach, and then gradually withdrawn with the subject lying on his left side, the highest potential difference was recorded when the tip of the electrode was 5-10cm. below the cardia. The procedure was repeated at a later date, and Table V shows that good reproducibility was obtained. Whether or not the tip of the electrode was screened radiologically did not affect the reproducibility.

TABLE V

Highest gastric mucosal potential difference recorded in four normal subjects on two separate occasions

Subject	P.D. (-mV.)	
	1st Measurement	2nd Measurement
1	45	45
2	49	44
3	42	43
4	43	42

Subjects

Five groups of subjects were studied.

(1) Twelve presumed normal subjects.

(2) Eighteen patients with classical lesser curve gastric ulcers. Diagnostic endoscopy was carried out in every case.

(3) Five patients with extensive gastritis diagnosed at endoscopy and histologically from biopsies.

(4) Three patients with pernicious anaemia.

(5) Eleven patients with long-standing rheumatoid arthritis. Neither endoscopy nor biopsy was carried out on any of these patients.

Procedure

The equipment used and the technique are described in Section II, Chapter VII. Informed consent was obtained, and after an overnight fast the subject was asked to swallow the exploring electrode until the tip was at least 70cm. from the incisor teeth. The intravenous electrode was inserted, and thereafter the subject was kept on his or her left side. The potential difference was recorded continuously on paper whilst the exploring electrode was withdrawn 5cm. at a time. The level

of the highest potential difference was accepted only after it had been steady for at least ten minutes.

Results

The results are shown in Figure 5 and also in Table A1 in the Appendix. The mean for the patients with gastric ulcers was -32.4mV., as compared with a value of -45.7 for the normal subjects. Statistical analysis using Student's 't' test showed this difference to be significant ($p < 0.001$).

The mean for the patients with gastritis, -33.6mV. was also significantly lower ($p < 0.01$) than that for the normal group.

The group of patients with pernicious anaemia was too small for statistical analysis. However, all three patients had potential differences lower than -37mV., the lowest level recorded in the group of normal subjects.

One patient with rheumatoid arthritis had a very high potential difference of -54mV. In spite of that, the mean for the whole group, -37.7mV., was significantly lower ($p < 0.01$) than that for the normal group.

Discussion

The groups of patients with gastric ulcers and gastritis used in this study had significantly lower gastric mucosal potential differences than the group of presumed normal subjects. This lends support to the finding of Chapman et al (1972), namely that patients with gastric ulcers or gastritis have a broken gastric mucosal barrier. However, the evidence presented here is incomplete. It is not known whether the potential difference recorded when the tip of the exploring electrode is at a certain distance from the incisor teeth reflects the activity of a small area of mucosa at that level, or of a whole rim of mucosa round the circumference

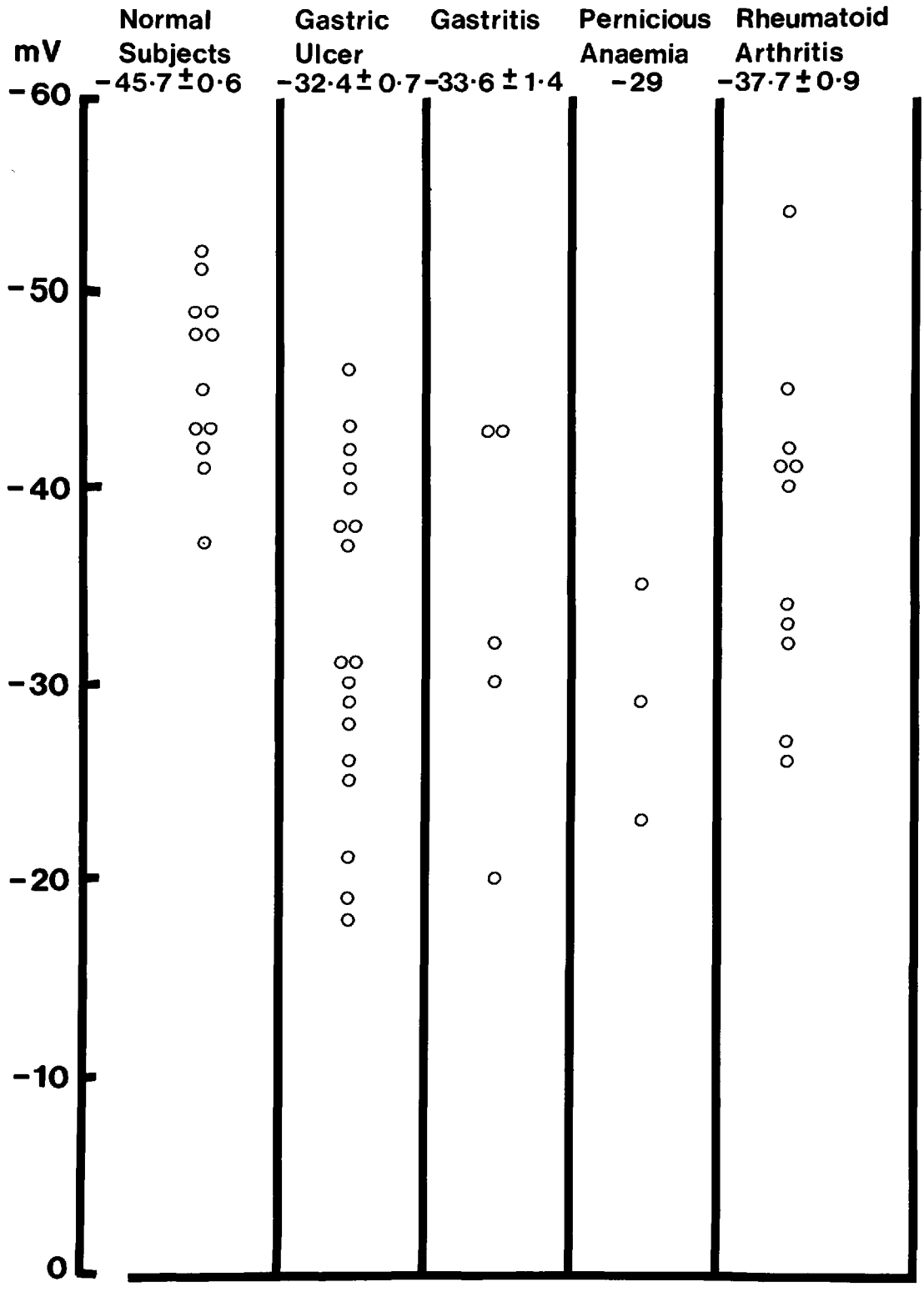


Figure 5 : Potential difference in the body of the stomach in normal subjects and four groups of patients. The mean ± s.e.m. are shown at the top of each column.

of the stomach. Hence the use of the conventional technique does not allow one to correlate potential difference accurately with histology. Since gastritis is more severe at the lesser curve than at the greater curve, specially in patients with gastric ulcers, one really needs to measure the potential difference at the lesser curve and to obtain biopsies for histology from the same site. Hence the need to complement this study with one in which the potential difference is measured under direct vision during endoscopy.

The finding that patients with pernicious anaemia have lower than normal potential differences is not surprising, since the gastric mucosa in those patients is atrophied. Andersson and Grossman (1966) also found lower (or more positive) potential differences in three patients with pernicious anaemia. Unfortunately those authors used skin electrodes, making it impossible to give absolute numberical values to the potential differences they recorded.

The group of patients with long-standing rheumatoid arthritis were also found to have significantly lower than normal potential differences. Since they were all on anti-inflammatory drugs, and had been for some time, it is not possible to say whether their impaired gastric mucosal barrier was associated with their disease or with the drugs used for treatment.

CHAPTER XII

STUDIES INVOLVING MEASUREMENT UNDER DIRECT VISION AT ENDOSCOPY

The anatomical distribution of the gastric mucosal potential difference in man has been worked out by Andersson and Grossman (1965) and by Geall et al (1970), using conventional techniques. Their results are shown in tabular form in Chapter II, Section I. In a preliminary experiment using the same techniques, similar results were obtained when the exploring electrode was gradually withdrawn from the duodenum into the body of the stomach in seven volunteers, and from the body of the stomach into the oesophagus in another seven subjects. The results, shown in Figures 6 and 7, confirm that the potential difference is low in the duodenum and the oesophagus, intermediate in the antrum, and high in the body of the stomach. They do not, however, give any indication of the potential difference at specific sites. Thus it is not known, for example, how the potential difference at the lesser curve compares with that at the greater curve at the same level in the stomach. In order to answer such questions, the potential difference was measured under direct vision at endoscopy.

The other aim of this project was to obtain further evidence in favour of an association between an abnormal gastric mucosa and a lowered potential difference. The use of conventional techniques has provided some evidence in favour of such an association. As mentioned in the previous chapter, however, such techniques do not lend themselves to an accurate study of potential difference and histology at the same sites.

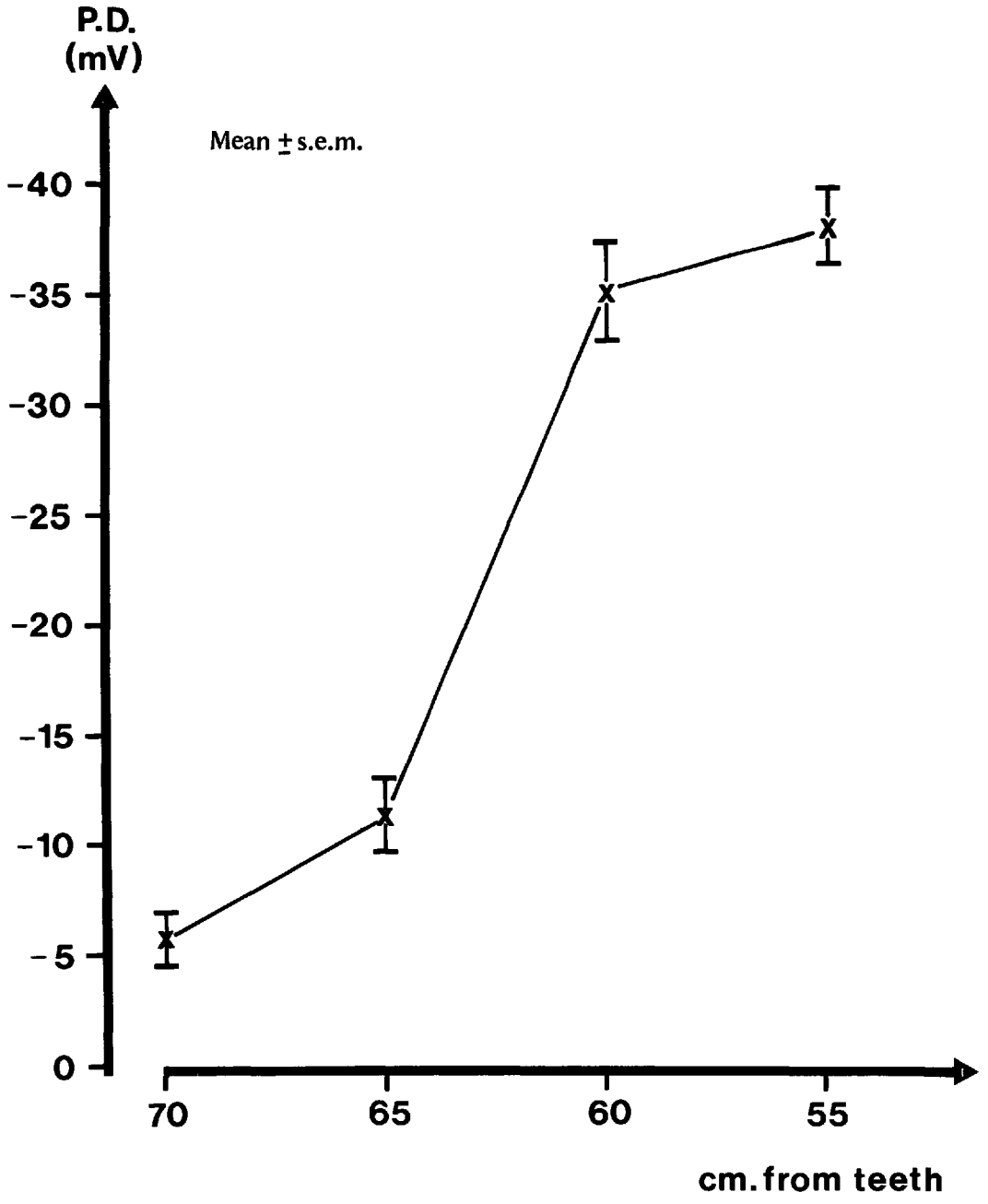


Figure 6 : Potential difference recorded as electrode is withdrawn from duodenum into body of stomach in seven subjects.

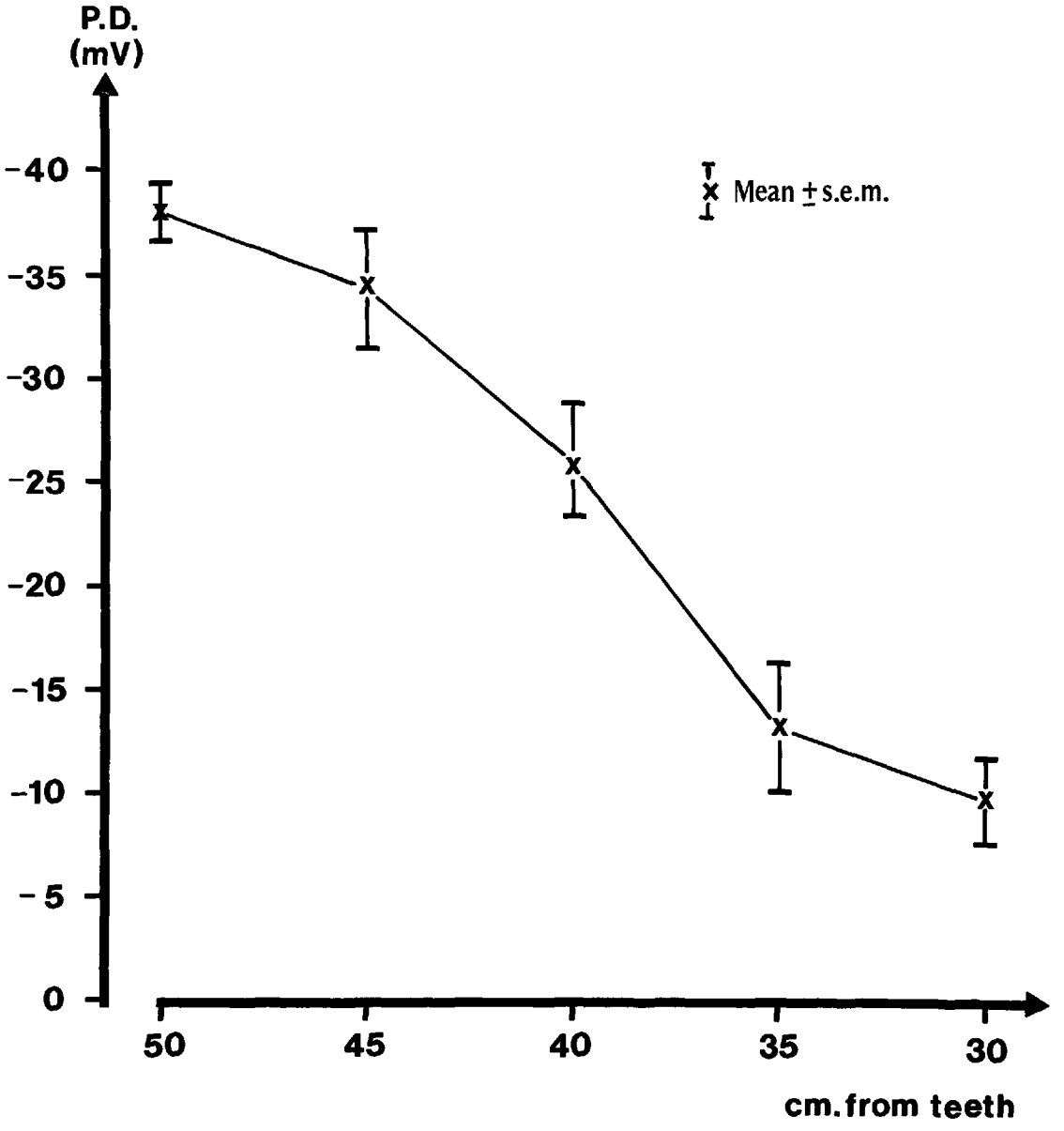


Figure 7 : Potential difference recorded as electrode is withdrawn from body of stomach into oesophagus in seven subjects.

Procedure

The equipment and technique used are fully described in Chapter IX, Section II. During diagnostic endoscopy, a note was made as to whether there was appreciable reflux of bile-stained fluid into the stomach. After sucking out the resting juice, the potential difference was measured in the duodenal bulb, at the pre-pyloric region, the incisura, the low greater curve, the high lesser curve and the high greater curve. A biopsy was then taken from each of those sites.

Histology

Each biopsy specimen was immediately fixed in buffered formol saline. After routine processing and orientation, it was embedded in paraffin wax and sections were cut at four or five levels. Each section was stained with haematoxylin and eosin. All the slides were examined and reported upon by Dr. P. Fitzpatrick in the absence of any knowledge of the potential differences recorded. The recommendations of Whitehead (1973) were followed. An estimate of the number of parietal cells present was also made, the biopsies being graded 0, 1, 2 and 3.

Subjects

It was not considered ethical to submit normal subjects to endoscopy. Only patients who needed a diagnostic endoscopy were included in the series. In order to have some subjects with a normal gastric mucosa, a number of patients with duodenal ulceration, and a number with X-ray negative dyspepsia were included. Informed consent was obtained some days before the procedure.

Twenty-five subjects were studied, but three had to be rejected because the potential difference recordings were too

erratic. The endoscopic diagnosis for the remaining twenty-two was as follows:-

Gastric ulcer	-	5
Duodenal ulcer	-	4
Pyloric ulcer	-	2
Hiatus hernia	-	1
Normal	-	10

Results

A. Subjects without bile reflux

In eighteen subjects there was no appreciable reflux of bile. This was too small to divide into several sub-groups according to severity of gastritis. Hence it was decided to divide it into two sub-groups only, one with normal or near-normal histology, and one with moderate or severe gastritis. For simplicity, the former will be called 'normal' and the latter 'gastritis'.

The division was made according to the histology of the high lesser curve biopsy for two reasons:-

(1) The mucosal potential difference at the high lesser curve in the normal human stomach has never yet been measured.

(2) Whenever there was gastritis at the high lesser curve, it was invariably present in the antrum. Furthermore, in this series it was also present at the high greater curve, although it was generally less severe there than at the high lesser curve. All the patients with a 'normal' high lesser curve mucosa also had a normal or 'near-normal' greater curve mucosa.

Twelve patients had a normal mucosa at the high lesser curve and six had gastritis. The latter sub-group included two of the patients in whom no ulcer was found at endoscopy, and four of the five patients with gastric ulcers. The fifth patient with a gastric ulcer was atypical in that the ulcer was on the posterior wall of the stomach.

A photomicrograph of the high lesser curve mucosa from one patient in each sub-group is shown in Figure 8.

(a) Subjects with a normal gastric mucosa

The distribution of the potential difference in the normal stomach is shown in Figure 9. The mean and S.E.M. for the twelve subjects are shown for each site.

The individual results for the high lesser curve and the high greater curve are given in Table VI. The potential difference at the high greater curve was invariably higher than at the high lesser curve, making statistical analysis unnecessary.

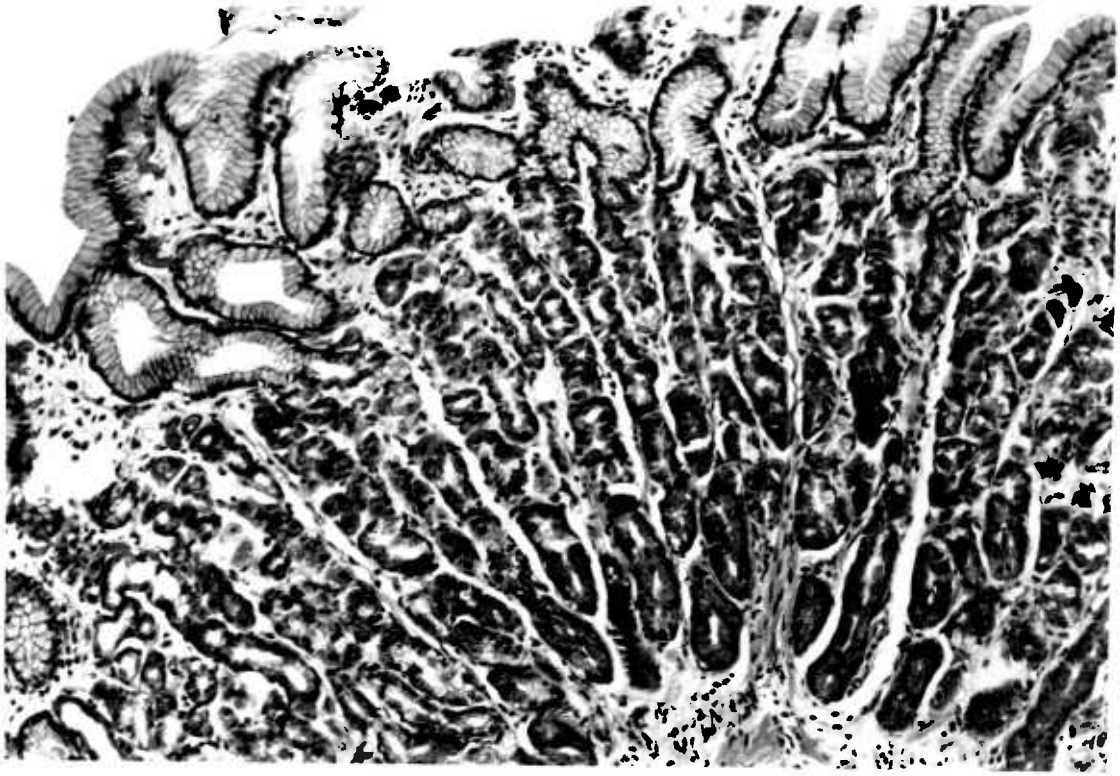
There was no significant difference between the number of parietal cells at those two sites, the grades being 2.9 ± 0.08 and 2.6 ± 0.15 (mean $\pm$ S.E.M.) respectively.

(b) Subjects with gastritis

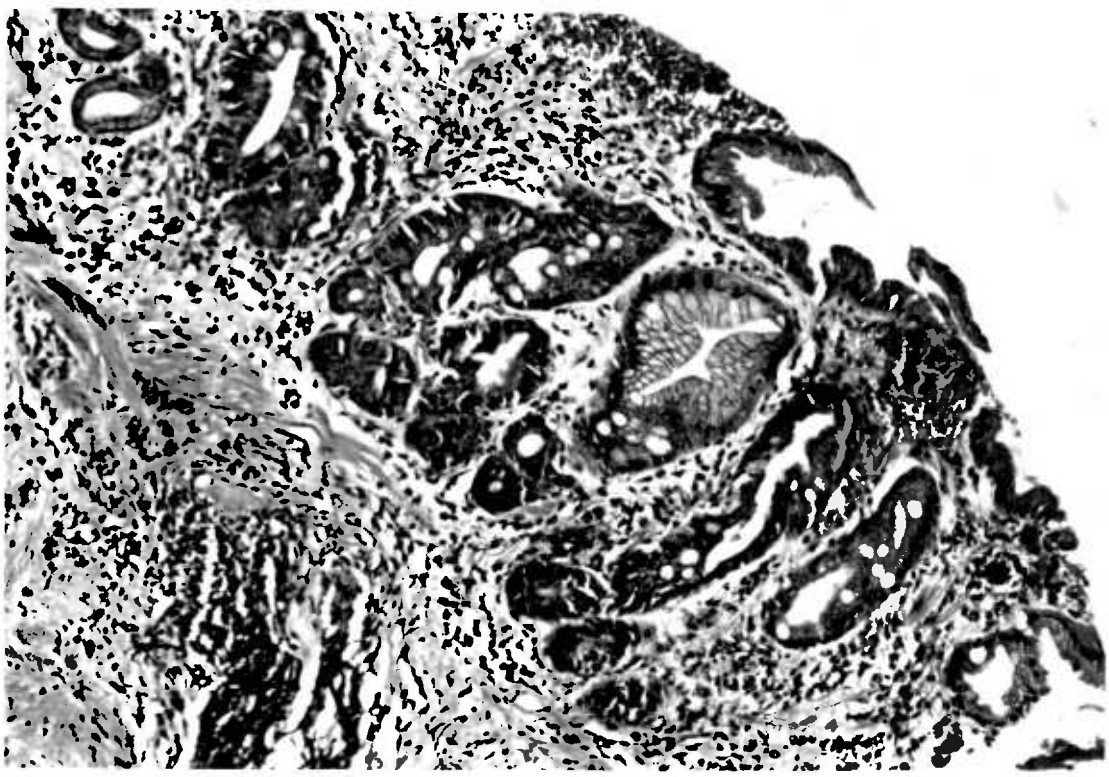
The distribution of the potential difference for the six patients with gastritis is shown in Figure 10. The mean and S.E.M. for each site is shown.

The individual results for the high lesser curve and the high greater curve are given in Table VII.

Figure 8 : Photomicrographs of high lesser curve gastric mucosa (Haematoxylin & eosin x 100)



(a) Normal



(b) Chronic atrophic gastritis with intestinal metaplasia

Figure 9 : Potential difference (mean ± s.e.m. in mV) in the duodenal bulb and at five sites in the stomach in twelve subjects with a normal high lesser curve mucosa.

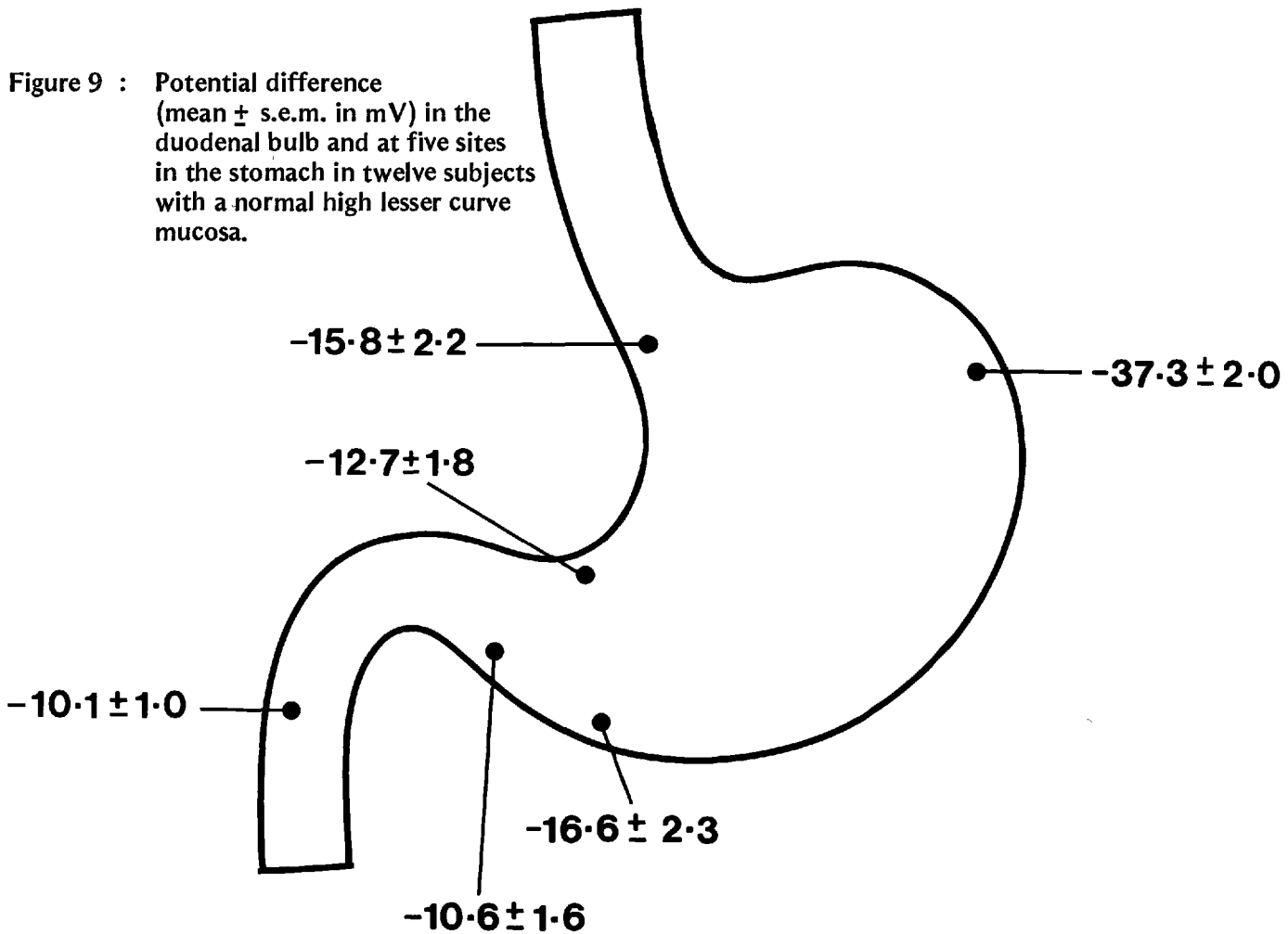


TABLE VI

Potential difference at the high lesser curve and high greater curve in subjects with normal gastric mucosa

SUBJECT	POTENTIAL DIFFERENCE (-mV.)	
	HIGH LESSER CURVE	HIGH GREATER CURVE
1	19	45
2	15	33
3	11	32
4	11	38
5	9	30
6	20	33
7	21	41
8	22	48
9	16	37
10	8	32
11	32	49
12	5	29
MEAN	15.8	37.8
S.E.M.	2.2	2.0

Figure 10 : Potential difference (mean ± s.e.m. in mV) in the duodenal bulb and at five sites in the stomach in six subjects with gastritis at the high lesser curve.

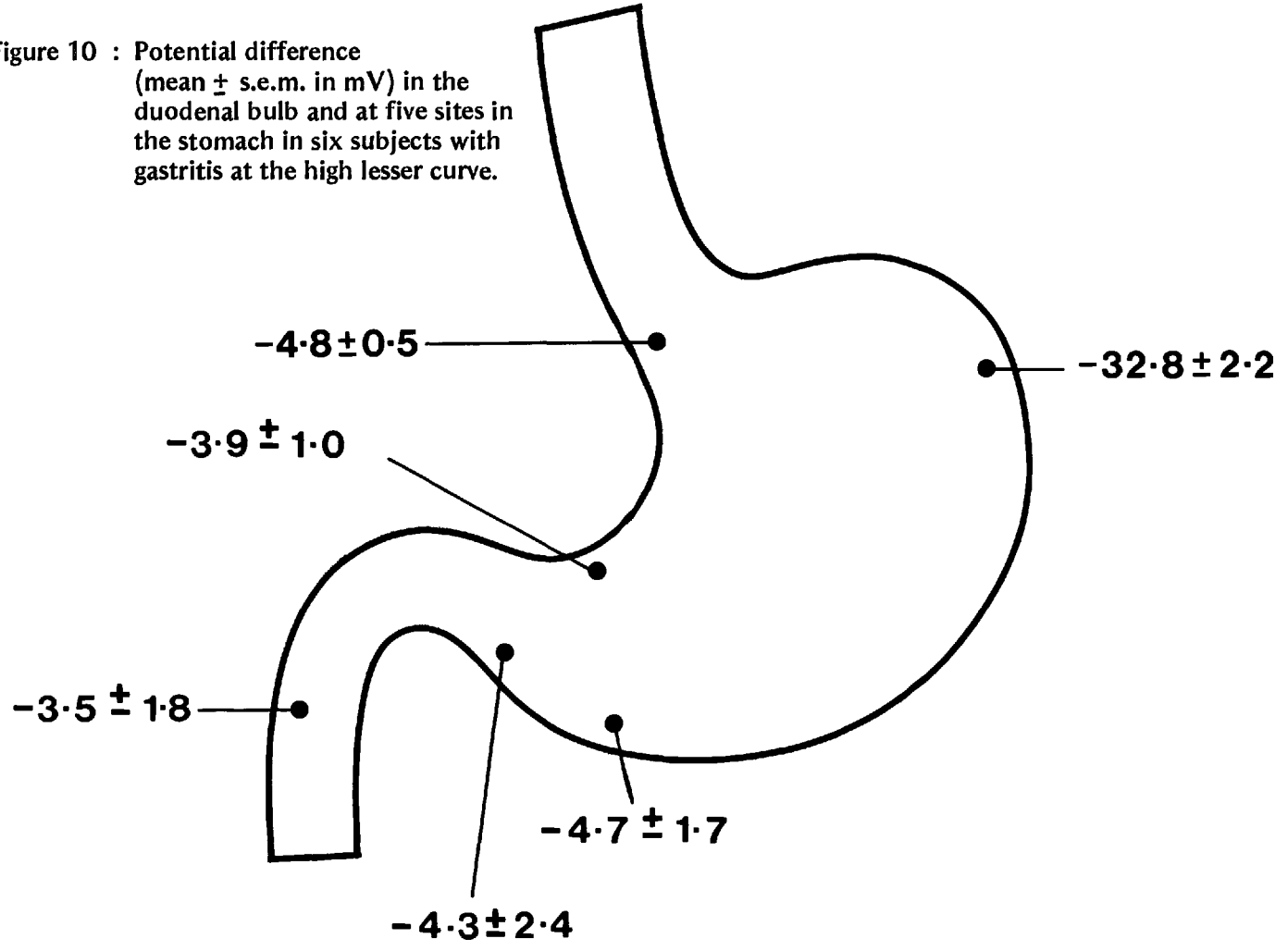


TABLE VII

Potential difference at the high lesser curve and high greater curve in subjects with gastritis

SUBJECT	POTENTIAL DIFFERENCE (-mV.)	
	HIGH LESSER CURVE	HIGH GREATER CURVE
1	3	23
2	5	36
3	5	35
4	6	32
5	6	32
6	4	39
MEAN	4.8	32.8
S.E.M.	0.5	2.2

Once again the potential difference was invariably lower at the high lesser curve than at the high greater curve. The mean potential difference at the high lesser curve, -4.8mV., was much lower than in the normal group, where it was 15.8mV. A student's 't' test showed that this difference was statistically significant ($p < 0.01$).

The potential difference at the high greater curve was also lower in this group (-32.8mV.) than in the normal group (-37.8mV.), but statistical significance was not reached.

The parietal cell grading of the high lesser curve mucosa for the normal group and the group with gastritis is shown in Table VIII.

The difference between the means of 2.6 and 1 was statistically significant ($p < 0.001$).

TABLE VIII

Parietal cell grade of high lesser curve mucosa

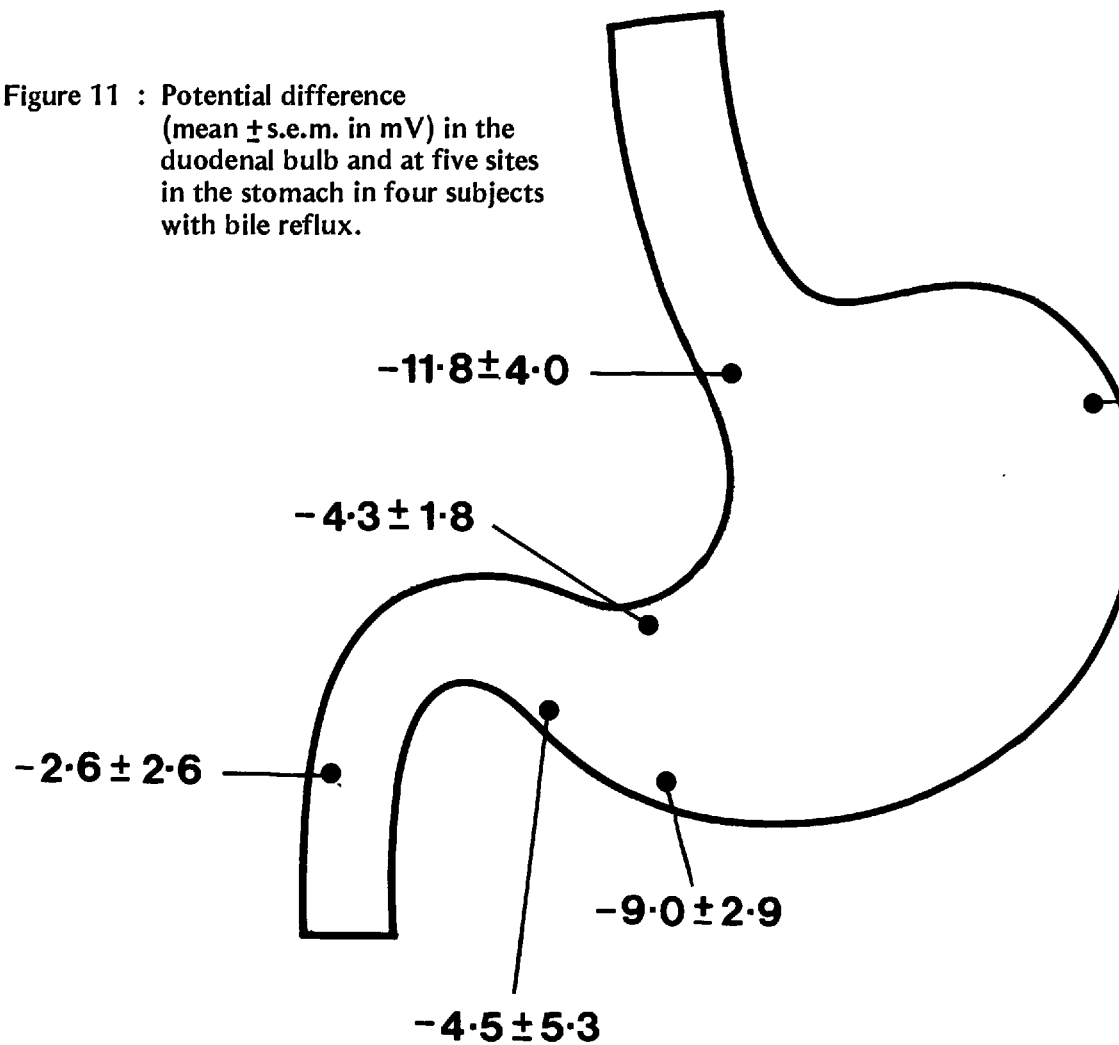
NORMAL SUBJECT	PARIETAL CELL GRADE	GASTRITIS SUBJECT	PARIETAL CELL GRADE
1	3	1	0
2	2	2	2
3	3	3	1
4	3	4	0
5	2	5	2
6	3	6	1
7	3		
8	3		
9	2		
10	2		
11	2		
12	3		
MEAN	2.6	MEAN	1
S.E.M.	0.15	S.E.M.	0.37

B. Subjects with bile reflux

In four subjects there was obvious reflux of bile-stained fluid into the stomach. Since the subjects were on their left sides, the fluid was lying in a pool in contact with the greater curve. The gastric mucosa was histologically normal at all sites in two cases, and there was moderate or severe gastritis in the other two.

The potential difference was very low at all sites whether there was gastritis or not. This was particularly striking at the high greater curve, where the figures recorded were very

Figure 11 : Potential difference (mean ± s.e.m. in mV) in the duodenal bulb and at five sites in the stomach in four subjects with bile reflux.



much lower than even those in the group with gastritis, but without reflux of bile.

The results for this group are shown in Figure 11.

Discussion

The anatomical distribution of the potential difference in the normal stomach

It has been shown by various investigators in the past that the gastric mucosal potential difference is higher in the body of the stomach than in the antrum (see Section 1, Chapter II) in man as well as several other species. This finding has been confirmed here in a preliminary study.

The use of the endoscope has made it possible to go further. In the group of twelve subjects with a normal gastric mucosa, the potential difference in the antrum was found to be much lower than at the high greater curve as expected. In addition, the potential difference at the high lesser curve was also found to be much lower than at the high greater curve.

The question arises, at this stage, as to why the low potential difference at the high lesser curve is not recorded when conventional techniques are used. It can be shown that if a potential difference is set up across an area of the wall of a hollow sphere, an electrical field is set up inside the sphere in such a way that equipotential lines curve away from the source towards the centre of the sphere. In an object having the shape of the stomach, one would expect the high potential difference at the high greater curve to set up equipotential lines as shown diagrammatically in Figure 12. In view of the shape of those lines, the tip of an exploring electrode would need to be very close indeed to the lesser curve in order not to be unduly influenced by the high

EQUIPOTENTIAL LINES IN THE STOMACH

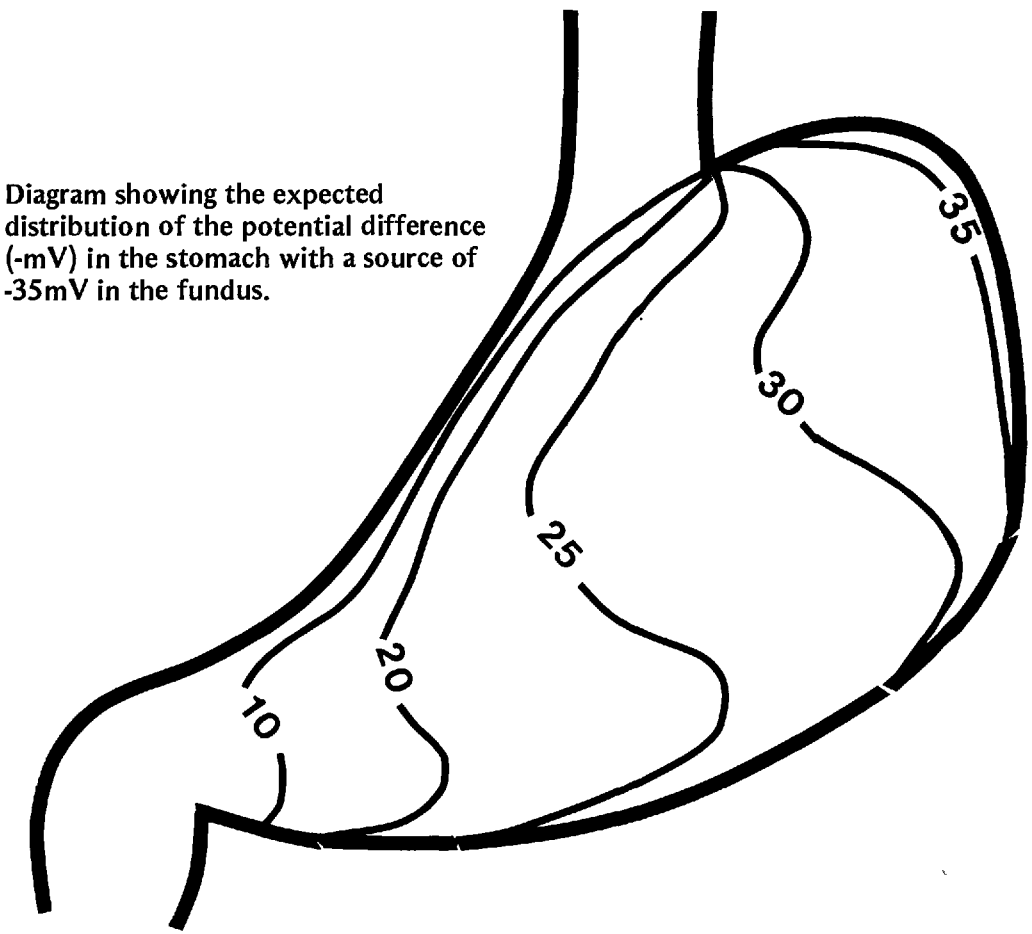


Figure 12 : Diagram showing the expected distribution of the potential difference (-mV) in the stomach with a source of -35mV in the fundus.

greater curve. There is as yet no experimental evidence to back this theory (formulated by Mr. A. Goddard).

It is not at all clear why the potential difference should be so much lower at the high lesser curve than at the high greater curve. As mentioned in Section I, Chapter II, the potential difference is thought to originate from the parietal cells. In the present study, no significant difference was found between the numbers of parietal cells at the high lesser curve and at the high greater curve. Further work, including microelectrode studies at various sites, needs to be carried out before this question can be answered.

Dyck et al (1969) have shown that hydrogen ions are lost from the denervated, separated, antral pouch of the dog at a rate fourteen times faster than from an equal area of the denervated fundic pouch. Sodium ions appeared nineteen times faster into the antral pouch than into the fundic pouch. Although similar experiments cannot be carried out in man, the results of potential difference measurements fit in with the idea that the antral mucosa is a weaker barrier than the mucosa of the body of the stomach. The authors suggest that this weakness of the barrier is related to the vulnerability of the antral mucosa, and to the high incidence of peptic ulceration in that part of the stomach. It seems possible that the low potential difference found in this study at the lesser curve is similarly associated with the frequency of peptic ulcers there.

Gastritis and the potential difference

Classical lesser curve gastric ulcers are associated with gastritis affecting the antrum and the lesser curve of the body of the stomach to an extent depending on how high on the lesser

curve the ulcer is (Du Plessis, 1965). The greater curve of the body of the stomach is also usually affected, although to a lesser extent (Gear et al, 1971). Chapman et al (1972) have depended on this association to demonstrate that the abnormal gastric mucosa is more permeable than the normal. The fact that the permeability did not change after healing of the ulcer suggested that it was not the ulcer itself, but rather the gastritis, which is known to persist after healing (Gear et al, 1971), that is associated with the enhanced rate of ionic interchange. Measurement of ionic fluxes in man, apart from being complex and depending on a number of assumptions (see Section I, Chapter V), necessarily involves the whole gastric mucosa. It does not therefore allow a proper comparison between the permeabilities at any specific site of normal and abnormal gastric mucosae.

Potential difference measurements with conventional techniques go one step further in that they are made in one particular part of the stomach. However, the same criticism applies to a certain extent, since the measurements are not made at one site where a biopsy can be taken for histology.

In the present study, measurement was made and biopsy taken from the same sites. The six patients with high lesser curve gastritis had significantly lower potential differences at the high lesser curve than the twelve with a normal mucosa.

The potential difference in the antrum was also markedly lower in those patients than in the normal group. This is not surprising, since all the patients with high lesser curve gastritis also had antral gastritis. However, since the patients were not divided into groups according to histology of the antral mucosa, this finding should not be emphasized.

In the group of patients with gastritis, the only site where the potential difference was reasonably high was the high greater curve. It was still, however, somewhat lower than in the normal group, though not significantly so. Although gastritis was present at the greater curve in all the six patients with lesser curve gastritis, it was generally not as severe as at the lesser curve, and in some cases it was mild.

In the group of patients with gastritis and low potential differences, there were significantly fewer parietal cells at the high lesser curve than in the normal group. This finding was expected, since, at least in patients with high lesser curve ulcers, of whom there were four in this series, there is an extension of pyloric type mucosa beyond the antrum (Whitehead, 1973). Whether it is the gastritis itself, or the associated paucity of parietal cells, that causes the potential difference to be low, cannot be determined from the evidence presented here.

The assocation between gastritis and low potential difference supports Davenport's theory (1965b) that "the apparent hyposecretion of acid by patients with gastric ulcer (is) a consequence of a broken barrier to diffusion of hydrogen ions into the gastric mucosa." Back-diffusion of hydrogen ions may not, however, be the only cause of the hyposecretion. The fact that gastritic mucosa contains fewer parietal cells than the normal suggests that there may be true hyposecretion as well.

Bile reflux and the potential difference

Bile salts have been shown to be capable of breaking the gastric mucosal barrier. They increase the back-diffusion of hydrogen ions (Ivey et al, 1970, 1971) and lower the gastric

mucosal potential difference (Geall et al, 1970) when instilled into the stomach. For this reason the subjects in the present study were divided into two groups according to whether there was reflux of bile-stained fluid into the stomach. All four subjects with bile reflux had low potential differences at all the sites. This was particularly striking at the greater curve, where the bile-stained fluid had pooled. Since this group consisted of two subjects with gastritis and two with a normal gastric mucosa, the results could not be compared formally with those of either of the two sub-groups without bile reflux. A larger number of patients needs to be studied in order to have enough subjects with bile reflux to divide into two further sub-groups according to whether the mucosa is normal or not.

S E C T I O N IV

STUDIES ON THE EFFECTS OF DRUGS THAT DAMAGE THE GASTRIC MUCOSA

CHAPTER XIII

ASPIRIN

Of all available analgesic drugs, the one most commonly used is aspirin. Against its beneficial effects must be weighed its important side-effects, of which damage to the gastric mucosa is the most important. It often causes gastric erosions and haemorrhage (Douthwaite and Lintott, 1938). Symptoms of indigestion occur even more frequently, and occult blood loss occurs in virtually all subjects who ingest the drug (Jabbari and Valberg, 1970). Whether it can cause chronic damage to the human gastric mucosa has not been definitely established although Edwards and Coghill (1966) have provided circumstantial evidence suggesting aspirin ingestion as one of the factors involved in the aetiology of chronic atrophic gastritis. Furthermore, retrospective studies by Gillies and Skyring (1969) and by Chapman and Duggan (1969) have suggested that there may be a link between aspirin ingestion and gastric ulceration.

Aspirin is one of the agents originally shown by Davenport 1964, 1965a, 1967a) to be capable of breaking the gastric mucosal barrier. He felt that this is the first step in the chain of events leading to damage of the mucosa. It is followed by back-diffusion of hydrogen ions, and then by exfoliation of gastric epithelial cells as demonstrated by Croft (1963), sometimes leading to actual erosions and haemorrhage.

Studies in man by Overholt and Pollard (1968) and by Smith et al (1971) have confirmed an increase in gastric mucosal permeability following exposure to aspirin. As expected, this increase in permeability is accompanied by a fall in the mucosal

potential difference (Geall et al, 1970; Murray et al, 1973).

Whilst it is generally assumed that the fall in potential difference is associated with absorption of aspirin in the stomach, there have been no studies to confirm this. The object of this project was to confirm the effect of aspirin on the gastric mucosal potential difference in normal subjects as well as in patients with an abnormal gastric mucosa, and to try and shed some light on the mechanism of this effect.

Subjects

Three groups of subjects were used in this study after having obtained their informed consent.

Group I consisted of twelve patients with gastric ulcers and one with generalized gastritis. All subjects in this group had their diagnosis confirmed by endoscopy and biopsy.

Group II consisted of six presumed normal subjects.

Group III consisted of three presumed normal subjects.

Procedure

The equipment and methods are described in Section II, Chapter VII. After an overnight fast, the exploring electrode and side-tube were passed orally to about 80cm. The intravenous electrode was inserted and potential difference recording was started. With the subject lying on his left side, the exploring electrode was gradually withdrawn and eventually secured in such a position that the maximum potential difference was obtained. This was generally 5-10cm. below the cardia.

When the base-line had been steady for at least fifteen minutes, each subject was tested with 40ml. water instilled into

the stomach via the side-tube. In none of the subjects did this procedure cause a large artefact.

When the base-line was steady again, the subjects in Groups I and II were tested in the same way with 600mg. aspirin in the form of two Solprin tablets in 40ml. water. Potential difference was recorded for fifty to sixty minutes.

The three subjects in Group III were tested with an intravenous injection of a solution containing 900mg. lysine acetylsalicylate (Aspégic, Laboratoire Egic), which is equivalent to 500mg. acetylsalicylic acid. Potential difference was recorded for one hour, after which the subjects were tested with oral aspirin in the same way as the other two groups.

Blood samples were collected at twenty minute intervals during the experiment from all subjects in Groups I and III for the measurement of plasma salicylate as described in Section II, Chapter IX.

Results

Group I The instillation of the test solution containing aspirin into the stomach always resulted in a marked fall in the potential difference. This effect started within a few minutes, and generally reached its maximum in fifteen or twenty minutes, after which recovery took place and was virtually complete within an hour. An example is shown in Figure 13. The base-line potential difference (P.D.) and the maximum fall in potential difference (Δ P.D.) for the thirteen subjects in Group I are shown in Table IX. In order to be able to make inter-subject comparisons, Δ P.D. has been expressed as a percentage of P.D. (%ΔP.D.) in the next column.

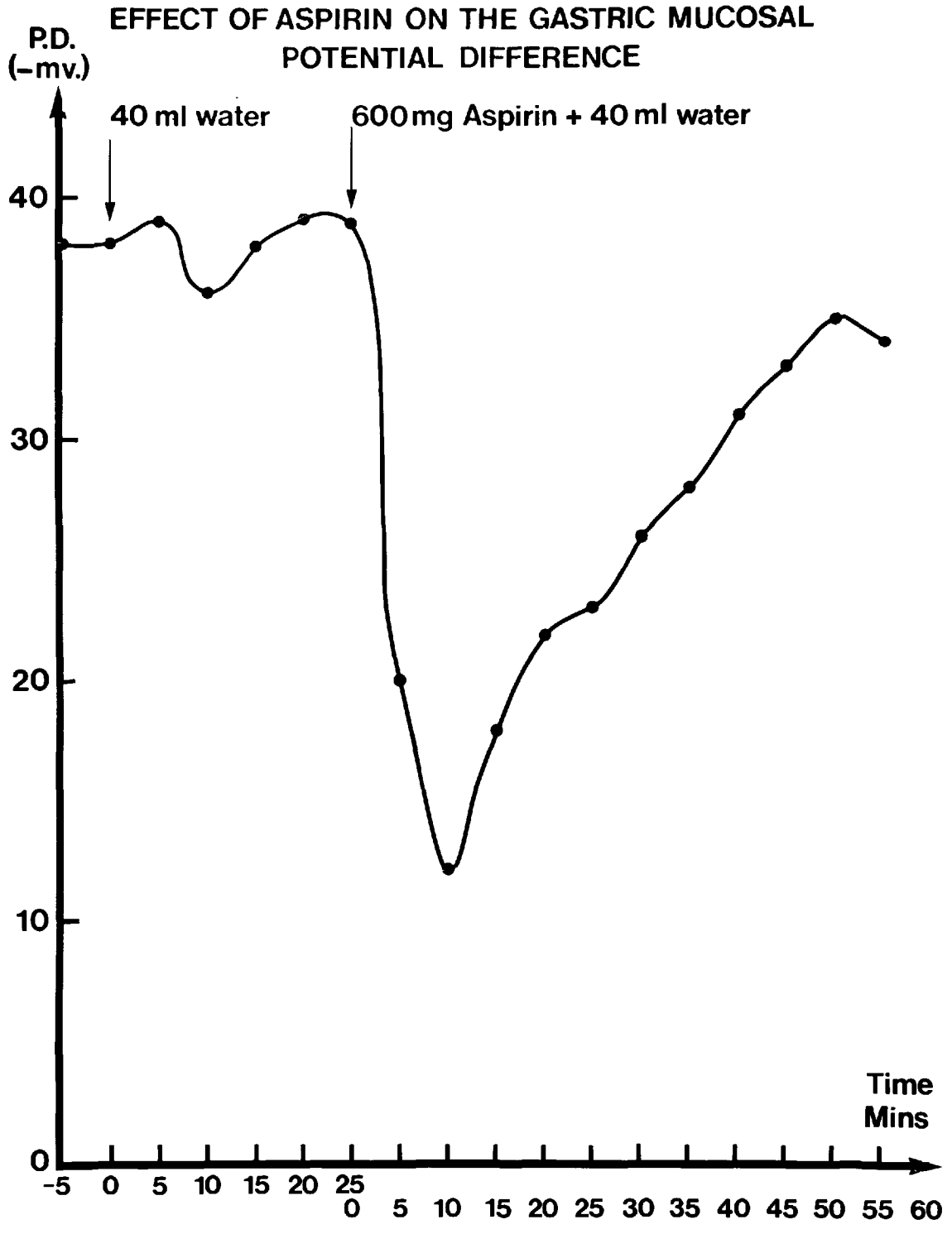


Figure 13 : Example of the change in the potential difference after instillation of the aspirin test solution into the stomach.

TABLE IX

Effect of 600mg. aspirin on potential difference, and highest plasma salicylate level for subjects in Group I

SUBJECT	P.D. (-mV.)	Δ P.D.	%Δ P.D.	PLASMA SALICYLATE (mg./100ml.)
1	31	7	23	2.1
2	30	13	43	3.8
3	27	16	41	4.15
4	31	15	48	4.3
5	40	27	67.5	4.75
6	18	8	44	3.7
7	26	16	61.5	5.5
8	40	28	70	4.75
9	45	28	62	4.1
10	37	24	64	3.6
11	46	22	52	3.25
12	38	18	53	3.4
13	28	14	50	4.85
MEAN	33.6		52.2	4.02
S.E.M.	2.3		3.6	0.25

The highest plasma salicylate level attained during the one hour after instillation of the test solution is also shown in Table IX. In Figure 14, %ΔP.D. has been plotted against plasma salicylate level. The regression equation was

$$\%\Delta P.D. = 9.9 \times \text{plasma salicylate} + 12.4$$

The correlation coefficient was 0.66

Group II The results for the normal subjects in this group are shown in Table X. The base-line potential difference was higher than for Group I, the means being -47.7mV. and -33.6mV. respectively. %ΔP.D. was similar for the two groups although the scatter was wider in Group I than in Group II.

Group III The effect of intravenous lysine acetylsalicylate on the potential difference of the three normal subjects in this group is shown in Table XI. ΔP.D. was unremarkable in all three subjects. There was a small fall in the potential difference in two subjects and a small rise in one. The highest plasma salicylate level attained during the one hour after the injection was comparable with the levels for Group I subjects after instillation of aspirin into the stomach.

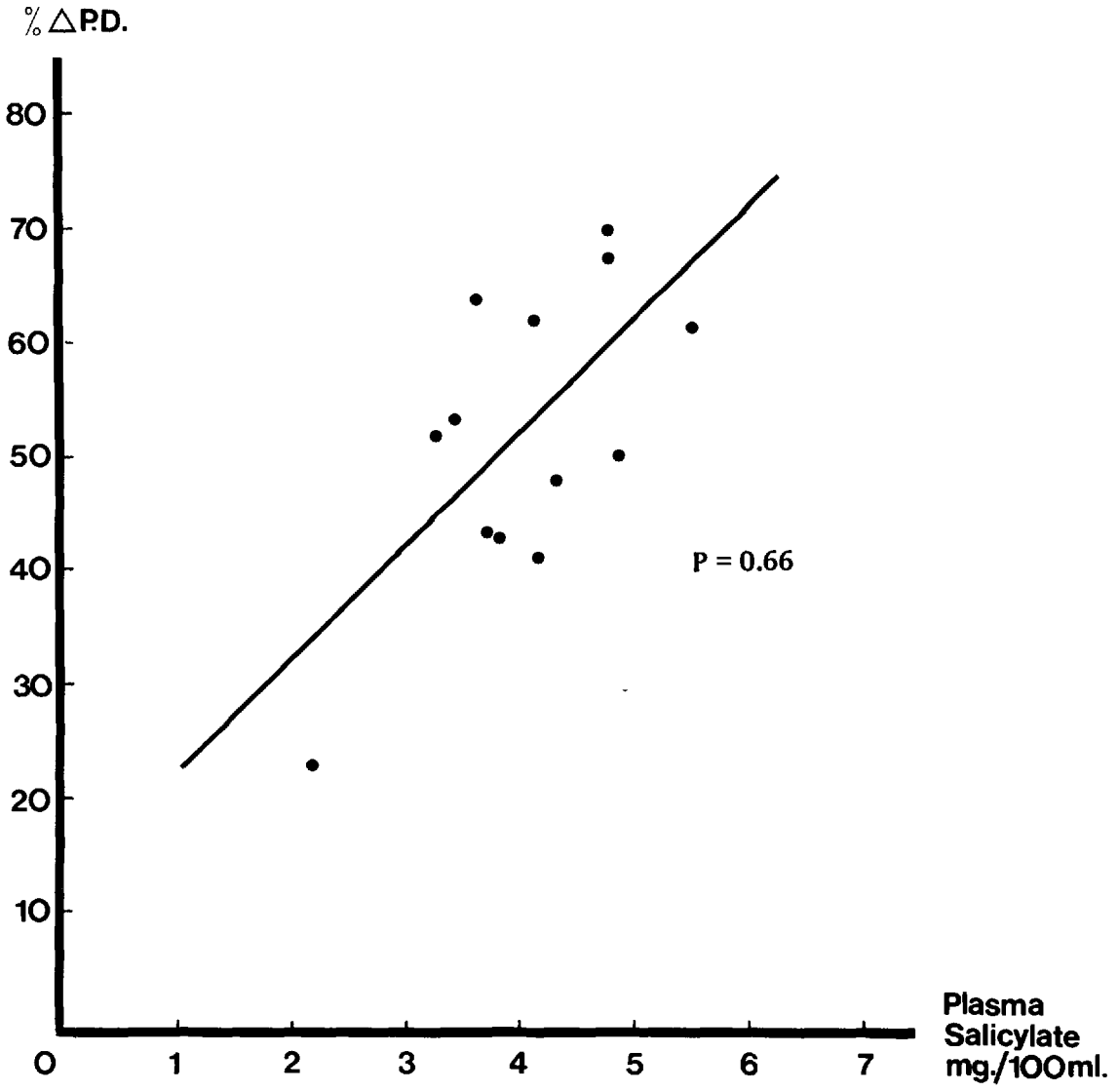


Figure 14 : Relationship between the percentage fall in the potential difference and the highest plasma salicylate level.

TABLE X

Effect of 600mg. aspirin on the potential difference for subjects in Group II

SUBJECT	P.D. (-mV.)	ΔP.D.	%ΔP.D.
1	43	20	47
2	49	28	57
3	45	24	53
4	37	21	57
5	52	20	38
6	60	32	53
MEAN	47.7		50.8
S.E.M.	3.2		3.0

TABLE XI

Change in P.D. and plasma salicylate level after intravenous injection of 900mg. lysine acetylsalicylate for subjects in Group III

SUBJECT	P.D. (-mV.)	Δ P.D.	PLASMA SALICYLATE (mg./100ml.)
1	51	3 (Fall)	4.3
2	35	7 (Rise)	4.1
3	45	7 (Fall)	4.9
MEAN	43.7		4.4

All three subjects subsequently responded to instillation of 600mg. aspirin in 40ml. water into the stomach with the usual marked fall in potential difference as shown in Table XII.

TABLE XII

Effect on P.D. of 600mg. aspirin instilled into the stomach of subjects in Group III

SUBJECT	P.D. (-mV.)	Δ P.D.	%Δ P.D.
1	50	26	52
2	42	23	55
3	40	31	78
MEAN	44		61.7

Discussion

The base-line potential differences for the normal subjects in Groups II and III were higher than those for the patients with gastric ulcer or gastritis in Group I. This is in keeping with the results described in Section III, Chapter XI.

Instillation of a solution containing aspirin into the stomachs of the normal subjects in Group II produced a consistent and marked fall in the potential difference, confirming the findings of previous investigators (Geall et al, 1970; Murray et al, 1973). The findings in the present study show that the abnormal gastric mucosa responds in the same way to aspirin. There was a fall in the potential difference in all the patients in Group I, and although the scatter was wider than for Group II, the average was similar in those two groups.

The use of $\%\Delta$P.D. needs to be commented upon. Most previous investigators have used the actual maximum fall in the potential difference (Δ P.D.) as a measure of the effect of the drug or agent being tested. This is not unreasonable when only normal subjects are being considered. However, when subjects with an abnormal gastric mucosa, and therefore with markedly different base-line potential differences, are being compared, problems of interpretation arise. To take a hypothetical example, a modest fall in the potential difference of a subject with a base-line level of -50mV. would appear enormous when compared with the most dramatic fall for a subject with a base-line level of -20mV. Some other investigators have used the nadir of the potential difference tracing instead of ΔP.D. This, however, would obviously distort the results in the opposite direction, exaggerating the apparent effect of the drug when the base-line potential difference

is low. In order to make inter-subject comparisons possible, it was therefore decided to follow the example of Rehm (1950) and use %ΔP.D.

It is possible to measure gastric absorption of aspirin in man, as has been shown by Hogben et al (1957), and Cooke and Hunt (1970). However, the technique interferes with the continuous recording of the potential difference. Hence, during potential difference studies one has to fall back on measurement of plasma salicylate levels. The latter can, of course, only give an approximate indication of gastric absorption, since the small intestine also absorbs aspirin, and since metabolism and excretion are taking place at the same time as absorption. Nevertheless, gastric absorption in an acid medium is rapid in the rat (Schanker et al, 1957) and even more so in man (Hogben et al, 1957). The latter found rates of absorption of 50-81% in forty minutes in subjects lying on their left sides. Although during that time about half of the gastric contents had escaped into the intestine, the rate of absorption there is much slower at 21% (Schanker, 1960).

The decision to use the group of patients with an abnormal gastric mucosa for a study of the correlation between %ΔP.D. and plasma salicylate level was based on the following:-

1. There is a wide range of base-line potential differences among such subjects (see Section III, Chapter XI).
2. Preliminary studies had shown that the effect of aspirin on the potential difference also varied from subject to subject in such groups.
3. It has been shown by Siurala et al (1969) that there is a wider range in the rates of gastric absorption of aspirin among such subjects than among normal subjects.

Although the correlation was not perfect, it does suggest that the fall in the potential difference is associated with absorption of aspirin. Another possible explanation for this correlation is that it is the actual presence of salicylate in the plasma rather than the process of absorption that causes the fall in the potential difference. This seems very unlikely, but nevertheless it needs to be considered in view of the confusing literature concerning the effects of parenteral salicylates.

Damage to the gastric mucosa and haemorrhage have been reported in rats by Brodie and Chase (1967) after intraperitoneal administration of a solution of aspirin. The same group (Overholt, Brodie and Chase, 1969) also found an increased gastric mucosal permeability after such an injection. In man, Grossman et al (1961) demonstrated occult blood loss after intravenous administration of salicylates, a finding that could not be confirmed by Winkelman and Summerskill (1961). Ivey et al (1972) could find no change in gastric mucosal permeability after infusing large doses of sodium acetylsalicylate intravenously.

Sodium acetylsalicylate solutions have to be infused very slowly, and are therefore unsuitable for use in potential difference studies. Various other salicylates are therapeutically active, an example being choline salicylate (Broh-Khan, 1960). Like aspirin, those drugs are rapidly metabolized to salicylate. It was decided in this study to use one such compound, lysine acetylsalicylate, which is commercially available for parenteral administration. The effect of intravenous injection of this drug on the potential difference was found to be negligible in spite of the adequate rise in the plasma salicylate level. This confirms the findings of Ivey et al (1972).

The results described in this chapter all point to the potential difference being lowered during absorption of aspirin through the gastric mucosa. The actual mechanism of production of this change, and of gastric mucosal damage, remains unknown. Martin (1963) has produced an ingenious theory according to which a drug like aspirin would enter the gastric mucosal cells with ease, since it is in an acid medium in the gastric lumen, and therefore largely unionized. Once in the neutral environment of the cells, however, it becomes ionized and therefore diffuses out much more slowly. According to Martin it is this accumulation of the drug in the cells that causes the damage. In order to put this theory to the test, one needs to carry out studies that would combine a technique to show the accumulation of aspirin in the cells, such as autoradiography, with potential difference measurements, and perhaps also with electron microscopy to demonstrate damage to the cells (Hingson and Ito, 1971).

CHAPTER XIV

OTHER DRUGS: PHENYLBUTAZONE, INDOMETHACIN AND CORTICOSTEROIDS

Apart from aspirin, which nearly all patients with rheumatoid arthritis take at some time, other commonly prescribed anti-inflammatory drugs are phenylbutazone and indomethacin. Corticosteroids are also not too infrequently used, and are prescribed for a number of other inflammatory conditions such as ulcerative colitis and asthma.

All those drugs have been implicated as aetiological factors in gastric ulceration and damage to the gastric mucosa. Patients on phenylbutazone frequently develop dyspepsia, and sometimes erosive gastritis and haemorrhage (Roth, 1964). An increased incidence of peptic ulcer has been reported by Kern et al (1957). Indomethacin is another well-known cause of dyspepsia, and the retrospective studies of Emmanuel and Montgomery (1971) suggest that it can cause gastric ulceration. Both drugs produce gastric mucosal erosions and haemorrhage in the rat (Wilhelmi and Menasse-Gdynia, 1972).

An increased incidence of gastric ulcers among patients on corticosteroids has been found by several groups of investigators, including Kern et al (1957) and Emmanuel and Montgomery (1971). Those ulcers, and the ones found in patients on other anti-inflammatory drugs, were atypical in that they were on the greater curve of the antrum, on the posterior wall or in the fundus.

Nearly all studies linking gastric ulceration with anti-inflammatory drugs other than aspirin have been carried out on groups of patients with rheumatoid arthritis. It is quite likely that those patients are unduly prone to develop gastric ulcers

anyway (Kern et al, 1957). Furthermore, nearly all of them also take aspirin, a drug which is known to damage ~~to~~ the gastric mucosa. It is therefore possible that the anti-inflammatory drugs affect particularly the already abnormal gastric mucosa of the patient with rheumatoid arthritis, or that there is some form of synergism between effects of those drugs and aspirin on the mucosa. The failure of Kirsner et al (1956) to find an increased incidence of peptic ulcers among ulcerative colitis patients on large doses of corticosteroids favours such possibilities.

Very little work has been done to find out whether anti-inflammatory drugs other than aspirin are capable of breaking the gastric mucosal barrier. In the Heidenhein pouch of the dog, Chvasta and Cooke (1972) found that indomethacin, at pH 1.0 or 7.3, caused a significant increase in ionic permeability and lowering of the potential difference. Phenylbutazone and corticosteroids did not have the same effect. Studies on man in the same laboratory (Murray et al, 1973) did not show a fall in the potential difference with any of those three drugs.

In the present study, the effect on the potential difference of phenylbutazone, indomethacin, prednisolone and its soluble derivative, prednisolone disodium phosphate, was measured. As far as possible, commercially available preparations were used. Since Predsol tablets (Glaxo Laboratorites) contain citrate as well as prednisolone disodium phosphate, the effect of a citrate solution was assessed separately.

Subjects and Test Solutions

Group I - Phenylbutazone was used only on subjects who were already on the drug or had been on it before. All six subjects

suffered from rheumatoid arthritis. There is no commercially available suspension or solution of phenylbutazone, and the drug forms a very poor suspension in water. Hence the test solution was made up by dissolving 100mg. phenylbutazone sodium powder in 40ml. dilute sodium hydroxide solution, so that the final pH was 9.0.

Group II - Five subjects were tested with a commercially available suspension containing 50mg. indomethacin, diluted with water to 40ml.

Group III - Three subjects were tested with 20mg. prednisolone in the form of four tablets shaken with 40ml. water to form a suspension.

Group IV - Three subjects were tested with 20mg. prednisolone disodium phosphate in the form of four Predsol tablets dissolved in 40ml. water.

Group V - Three subjects were tested with 600mg. sodium citrate dissolved in 40ml. water.

Procedure

The equipment and methods are described in Section II, Chapter VIII. The procedure was the same as in the last chapter, except that pH was monitored throughout the experiments by means of an intragastric electrode. The effect of 40ml. of water was observed, followed by that of the test solution. Potential difference was recorded for one hour after instillation of the test solution, after which 600mg. aspirin in 40ml. water was instilled and recording continued for a further hour.

Results

Group I - The effect of phenylbutazone on the potential difference of the six subjects with rheumatoid arthritis is shown in Table XIII. There was no fall in the potential difference in any of the subjects. In fact, there was a rise in all but one of them, the mean %ΔP.D. being 26%. All the subjects showed a marked fall in P.D. after aspirin as shown in the last column, the mean fall being 60%. The intragastric pH, which was close to 9.0, the pH of the instilled solution, at the start of the test period of one hour, invariably fell below 2.0 within five minutes.

TABLE XIII

Effects of 100mg. phenylbutazone and 600mg. aspirin on P.D.

SUBJECT	PHENYLBUTAZONE			ASPIRIN
	P.D.(-mV.)	ΔP.D.(Rise)	%ΔP.D.(Rise)	%ΔP.D.(Fall)
1	40	10	25	76
2	42	0	0	46
3	41	11	27	58
4	33	19	58	74
5	54	8	15	61
6	35	11	31	45
MEAN	40.8		26	60
S.E.M.	3.0		7.8	5.4

Group II - The results of the effects of indomethacin and aspirin on the gastric mucosal potential difference are shown in Table XIV. One subject did not show an appreciable fall in the potential difference after aspirin, and was therefore not included in the series. Of the remaining four, subjects 1 and 2 had histologically proven gastritis, and the other two were normal.

All the subjects showed a rise in P.D. after indomethacin, the mean rise being 25.8%.

TABLE XIV

Effect of 50mg. indomethacin and 600mg. aspirin on P.D.

SUBJECT	INDOMETHACIN			ASPIRIN
	P.D.(-mV.)	ΔP.D.(Rise)	%ΔP.D.(Rise)	%ΔP.D.(Fall)
1	32	6	19	19
2	32	8	25	32
3	38	8	21	51
4	45	17	38	53
MEAN	36.8		25.8	39.3
S.E.M.	3.1		4.3	8.1

The two patients with gastritis had a high intragastric pH throughout the test, whereas the two normal subjects maintained low pH values. Those are shown in Table XV.

TABLE XV

pH of gastric contents after instillation of indomethacin suspension

Subject	pH before indomethacin	pH after indomethacin			
		5 minutes	20 minutes	40 minutes	60 minutes
1	6.2	6.5	6.2	6.1	6.3
2	7.1	7.1	7.0	7.0	6.9
3	1.4	1.6	1.2	1.2	1.1
4	1.8	2.7	2.2	2.2	2.2

Group III - The second subject in this group had a duodenal ulcer, the other two being normal. Prednisolone produced no appreciable effect in any of the three subjects. The results are shown in Table A2 in the Appendix. The pH for each subject came down to less than 2.0 within twenty minutes of instilling the test solution. Aspirin produced a marked fall in the potential difference in all three subjects, %ΔP.D. being 55, 64 and 39% respectively.

Group IV - The first two subjects in this group had duodenal ulcers, the third being normal. Prednisolone disodium phosphate produced an appreciable fall in the potential difference in two out of three subjects, as shown in Table XV. In all the subjects the pH was less than 2.0 within five minutes of instilling the test solution. All three subjects responded normally to aspirin, the mean %ΔP.D. being 59%.

TABLE XVI

Effects of 20mg. prednisolone disodium phosphate and 600mg. aspirin on P.D.

SUBJECT	PREDNISOLONE DISODIUM PHOSPHATE			ASPIRIN
	P.D.(-mV.)	ΔP.D.(Fall)	%ΔP.D.(Fall)	%ΔP.D.(Fall)
1	44	3	7	54
2	49	14	29	66
3	41	16	39	57
MEAN	44.7		25	59

Group V - The first subject had a gastric ulcer, the other two being normal. There was a fall in P.D. in all three after sodium citrate, as shown in Table XVII. The mean %ΔP.D., 23.3%, was similar to the one obtained after prednisolone disodium phosphate in Group IV. The pH was below 2.0 in all three subjects.

TABLE XVII

Effects of 600mg. sodium citrate and 600mg. aspirin on P.D.

SUBJECT	SODIUM CITRATE			ASPIRIN
	P.D.(-mV.)	ΔP.D.(Fall)	%ΔP.D.(Fall)	%ΔP.D.(Fall)
1	38	11	29	48
2	41	10	24	58
3	43	6	17	54
MEAN	40.7		23.3	53.3

Discussion

None of the three drugs, phenylbutazone, indomethacin and prednisolone, produced a fall in the gastric mucosal potential difference. This confirms the findings of Murray et al (1973). Both phenylbutazone and indomethacin are, like aspirin, weak acids with the same pKa of 4.5 (Chvasta and Cooke, 1972). They are therefore expected to exert their maximum effects on the gastric mucosa in an acid medium, in which they are unionized and lipid-soluble. In the group tested with phenylbutazone, the pH was low in all the subjects. In the indomethacin group, two subjects had a high pH, and two a low pH. The response of the gastric mucosa to the drug was the same in all four subjects. The absence of a fall in the potential difference after exposure of the gastric mucosa to those two drugs even at a low pH is disappointing.

Had it been otherwise, measurement of the potential difference could have been the basis of a simple screening test for drugs that damage the gastric mucosa. The cause of the modest, but consistent rise in the potential difference produced by those drugs is unknown, and would need further studies, and possibly in vitro experiments, to elucidate.

Prednisolone did not affect the potential difference at all, confirming previous findings in the dog and in man. The soluble Predsol tablets produced a fall in the potential difference in all the subjects tested, and in two out of three this fall was substantial. However, sodium citrate, which is contained in those tablets, produced a fall of the same magnitude. Hence it is very likely that it was the citrate rather than prednisolone disodium phosphate that caused the fall in the potential difference. For that reason, this study, which appeared promising at first, was not extended.

It seems very likely, from the results obtained here, that the anti-inflammatory drugs studied affect the gastric mucosa by a mechanism other than the one involved in aspirin-induced mucosal damage. In this context, it is interesting that Max and Menguy (1970) found that cortisone reduced the rate of renewal of gastric epithelial cells, whereas aspirin increased the rate of exfoliation. Those investigators administered cortisone over a period of several days, and it is possible that this mode of administration is necessary before one can detect an effect of a corticosteroid or other anti-inflammatory drug on the gastric mucosa. Thus Chung et al (1970b) found that chronic administration of aspirin and prednisolone in patients with rheumatoid arthritis had a greater effect on gastric mucosal permeability than administration of aspirin on its own.

Work done in the same laboratory on dogs (Silen, 1972) has shown that the effect of aspirin or sodium taurocholate on gastric mucosal permeability is significantly enhanced after a course of prednisolone. In the present study, $\%\Delta P.D.$ after aspirin was much higher, with a mean of 60%, in the six patients with rheumatoid arthritis than in the six normal subjects in the last chapter, where the mean $\%\Delta P.D.$ was 50.8% (see Table XI). The difference was not statistically significant. Further studies need to be carried out, in which subjects, preferably with a normal gastric mucosa, would act as their own controls, and the effect of aspirin on the potential difference would be measured before and after a course of various anti-inflammatory drugs.

S E C T I O N V

STUDIES ON DRUGS USED IN THE TREATMENT OF GASTRIC ULCER

CHAPTER XV

GEFARNATE

Gefarnate, or genaryl farnesylacetate, is a synthetic ester of the terpenic compound farnesylacetic acid. Animal experiments by several groups of investigators including Murari (1964) and Wissmer and Adami (1965) have shown that it gives protection against acute gastric ulceration induced by restraint, phenylbutazone, prednisolone and other agents. It has been claimed to promote ulcer healing in man, and although most of the work done was in the form of uncontrolled (Wissmer and Adami, 1965) or limited controlled trials (Newcomb et al, 1970), the drug became widely used in the treatment of gastric ulcers. Langman et al (1973) have carried out the only controlled clinical trial comparing gefarnate with carbenoxolone, which is known to be effective. They found gefarnate to be inferior to carbenoxolone, but not ineffective. The big advantage of gefarnate was the lack of side-effects.

The drug has no effect on gastric secretion, and the mode of action remains unknown. There has been no work done to find out whether it affects gastric mucosal permeability or potential difference, and whether it strengthens the gastric mucosal barrier against assault by aspirin or other agents such as bile salts. In the present study, the gastric mucosal potential difference (P.D.) and the effect of a standard dose of aspirin on it (%ΔP.D.) were measured in a group of patients before and at the end of a course of gefarnate. Since intragastric pH has a profound effect on gastric absorption of aspirin, and also affects the action of

aspirin on gastric mucosal permeability (Davenport, 1964) and therefore on potential difference, pH was monitored during the experiments.

Subjects

The group of subjects tested consisted of nine patients with classical benign lesser curve gastric ulcers and two with generalized gastritis. Endoscopy and multiple biopsies were carried out on all of them. They were treated with gefarnate 300mg. daily in divided doses for four weeks. Tests were carried out immediately before, and at the end of the course of treatment.

Procedure

The equipment and methods are described in Section II. The procedure was the same as in Section IV, Chapter XII. Potential difference wasrecorded until the base-line was steady for at least fifteen minutes. Then 40ml. water was instilled into the stomach. Unless the water produced a large artefact, it was followed, when the base-line was steady again, by the test solution of 600mg. aspirin in the form of two Solprin tablets in 40ml. water. Potential difference was recorded for fifty to sixty minutes after instillation of the test solution.

In the first few tests carried out, an attempt was made to aspirate small samples of gastric contents for measurement of pH. This proved technically unsatisfactory, and later on an intragastric Beckmann glass electrode was used for monitoring pH.

Results

The base-line potential difference for the eleven subjects before and at the end of their course of gefarnate is shown in Table XVIII.

TABLE XVIII

Gastric mucosal potential difference (-mV.)

SUBJECT	BEFORE GEFARNATE	AFTER GEFARNATE
1	40	36
2	29	29
3	30.5	45
4	39	35
5	45.5	53
6	43	40
7	46	41
8	26	39
9	14.5	22
10	37	36
11	25	23
MEAN	34.1	36.4
S.E.	3.0	2.7

There was no significant difference between the means of -34.1mV. before and -36.4mV. after treatment.

The fall in the potential difference produced by aspirin, expressed as a percentage of the base-line potential difference, is shown in Table XIX. Two subjects were excluded from the series because of large artefacts in the potential difference tracing after the instillation of water or of the test solution. There were large differences between the percentage drop after aspirin before and after treatment with gefarnate in three out of the nine subjects, but the means of 53.6 and 55.3% did not differ significantly.

pH was monitored in six of the subjects during the tests before

and after the course of gefarnate. Whatever the pH at the beginning of the experiment, it approximated to 4.5., the pH of the test solution, immediately after instillation. Thereafter, in five out of the six subjects, it came down rapidly over the next five minutes or so, and then remained remarkably steady until the end of the test period. In subject 9 the pH rose back to the pre-aspirin level, and then varied very little. Since it is mathematically wrong to take arithmetic means of pH values, the pH at the end of the test period has been used for comparison. This is shown in Table XX.

TABLE XIX

Percentage drop in potential difference after aspirin

SUBJECT	BEFORE GEFARNATE	AFTER GEFARNATE
1	65	33
3	54	42
4	46	40
5	24	53
6	65	70
7	62	56
8	61.5	69
10	64	69.5
11	41	65
MEAN	53.6	55.3
S.E.	4.7	4.7

TABLE XX

Intragastric pH

SUBJECT	BEFORE GEFARNATE	AFTER GEFARNATE
5	1.7	1.1
7	1.0	1.8
8	1.9	1.7
9	6.8	5.9
10	1.6	1.2
11	1.3	1.2

Wilcoxon's non-parametric test shows that there was no significant change in pH after treatment with gefarnate.

Discussion

There was no significant rise in the gastric mucosal potential difference after treatment with gefarnate. Even when those patients whose ulcers had healed at the end of the course of treatment were considered as a separate group, no significant change in the potential difference was observed. This is not surprising since gastritis tends to persist after healing of a gastric ulcer (Gear et al, 1971).

Aspirin produced the usual fall in the potential difference in all the subjects, and there was no signficant change in the effect of aspirin after treatment with gefarnate. There were no consistent changes in pH that could have masked such a change.

There has been no previous work on the effect of gefarnate on the gastric mucosal barrier for comparison with the results obtained here. The controlled clinical trial carried out by Langman et al (1973) showed that gefarnate is inferior to carbenoxolone.

The authors came to the conclusion that gefarnate is active to a certain extent only after comparing their results with those of a previous trial in which a placebo was used. Hence some doubt still remains as to whether the drug is effective in the treatment of gastric ulcers. The findings in the present study do not necessarily imply that gefarnate is ineffective. They are, however, consistent with the view that it does not strengthen the gastric mucosal barrier.

CHAPTER XVI

CARBENOXOLONE

The triterpene carbenoxolone is a synthetic derivative of glycyrrhetinic acid, which is itself the aglycone of one of the constituents of liquorice root, glycyrrhizic acid. It was first shown to be effective in the treatment of gastric ulcer in a controlled trial by Doll et al in 1962. This has since been confirmed repeatedly. In twenty-one out of twenty-two trials reviewed by Sircus (1972), the drug was found to be effective. The only one in which no benefit was observed was uncontrolled and contained only eight patients.

In spite of an enormous amount of work published on the subject, the mode of action remains unknown. Bank et al (1967) and Cocking and MacCaig (1969) found a reduction in acid output after a course of treatment. Neither group of investigators, however, found the expected increase in acid output in the patients on placebo after healing of the ulcer, and the latter actually found a substantial decrease. Henman (1970) found that the drug reduced peptic activity in pylorus-ligated rats, and suggested that this could be of importance in the treatment of ulcers.

Most workers feel that it is not the aggressive forces, acid and pepsin, that are affected by carbenoxolone, but rather the defence mechanisms of the stomach. An increase in the amount of mucus secreted in the stomach has been observed by several groups of investigators, including Rhodes et al (1972) and Steer and Colin-Jones (1975). Since it is the cellular layer itself that is of importance in Hollander's two-component barrier, the finding of Lipkin (1970, 1971) that carbenoxolone produces a

marked increase in the life span of gastric mucosal cells in rats is of great interest. In man, protection of the gastric mucosa against histological damage, caused by ingestion of a salty Japanese pickle, has been demonstrated (MacDonald et al, 1967; MacDonald and Stoller, 1973).

Work on the effect of carbenoxolone on the gastric mucosal barrier has produced conflicting results. Cross and Rhodes (1972), using Heidenhain pouch dogs, found that the drug did not affect back-diffusion of hydrogen ions directly, but did protect the mucosa against the increased back-diffusion of hydrogen ions after exposure to bile salts. Thompson et al (1974), using the same experimental animal, found that carbenoxolone did reduce the back-diffusion of hydrogen ions directly. Work on man by Ivey and Gray (1973) showed no change in ionic fluxes after treatment, and no protection against bile salts. The same conclusion was reached by Bennett et al (1974). These authors measured potential difference and the effect of aspirin on it in normal subjects before and after treatment with carbenoxolone, and found no change. Colin-Jones and Taylor (1973), on the other hand, measured ionic fluxes in gastric ulcer patients and found a decrease after treatment.

The present study, which was carried out concurrently with the one on gefarnate, involved only a small number of normal subjects, and concentrated on patients with an abnormal gastric mucosa.

Subjects

Tests were carried out on two groups of subjects before and at the end of a course of treatment.

Group I consisted for four presumed normal volunteers. They were treated with carbenoxolone 300mg. daily in divided doses for one week.

Group II consisted of fourteen patients with gastric ulcers, and three with gastritis. The diagnosis was confirmed in all cases by endoscopy and biopsy. They received carbenoxolone 300mg. daily in divided doses for one week, after which the dose was halved and treatment continued for a further three weeks.

Procedure

The equipment and methods are described in Section II. The procedure was identical with the one adopted in the gefarnate study described in the last chapter. Apart from measuring potential difference and the effect of a standard dose of aspirin on it, and monitoring pH, plasma salicylate levels were measured twenty, forty and sixty minutes after instillation of the test solution. At the beginning of the study, the method of Trinder (1954) was used. However, it yielded poor duplicates, and was abandoned for that of Routh et al (1967). This is described in Section II.

Results

Group I - The base-line potential difference before and after carbenoxolone is shown in Table XXI. There was no substantial change in any of the four subjects.

TABLE XXI

Gastric mucosal potential difference (-mV.)
(Normal Subjects)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
1	54	50
2	43	39
3	45	37
4	37	42

There was a marked fall in the percentage drop in the potential difference after aspirin in the first subject after the course of carbenoxolone, as shown in Table XXII. No change was observed in the other three subjects.

pH was not markedly different after treatment, as shown in Table XXIII. The intragastric electrode was not available when the first subject was tested.

TABLE XXII

Percentage drop in potential difference after aspirin
(Normal Subjects)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
1	68	33
2	47	44
3	53	52
4	57	57

TABLE XXIII

Intragastric pH
(Normal Subjects)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
2	1.8	1.9
3	1.8	1.7
4	1.4	2.1

Plasma salicylate levels were measured in the last three subjects, and the highest levels obtained were somewhat lower after treatment in two subjects than before, as shown in Table XXIV.

TABLE XXIV

Plasma salicylate (mg./100ml.)
(Normal Subjects)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
2	3.7	3.7
3	5.9	4.7
4	4.7	4.2

Group II

(a) Base-line potential difference. There was no significant change in the base-line potential difference after treatment with carbenoxolone, the means being -34.6mV. before, and -35.1mV. after treatment. The individual results are shown in Table XXV. Comparison with Table XVIII in the last chapter shows that there was no signficant difference in the base-line potential difference before treatment between the gefarante group and the carbenoxolone group, the means being -34.1 and -34.6mV. respectively.

(b) Effect of aspirin on potential difference. Three subjects had to be excluded from the series because of large artefacts produced by instillation of water or of the test solution. The percentage drop in the potential difference after aspirin for the remaining fourteen subjects before and after treatment with carbenoxolone is shown in Table XXVI. This was lower after treatment than before in twelve out of the fourteen subjects. A paired t-test showed that the difference between the means, 51.3% and 37.6%, was statistically significant ($p < 0.01$).

TABLE XXV

Gastric mucosal potential difference (-mV.)
(Patients)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
1	37.5	49.5
2	40	25
3	42	39
4	30.5	25
5	20	20
6	52	51
7	40	45
8	43	42
9	31	34
10	30	32
11	27	21
12	39	43
13	25	31
14	35	43
15	31	38
16	47	43
17	18	15
MEAN	34.6	35.1
S.E.	2.2	2.6

TABLE XXVI

Percentage drop in potential difference after aspirin (Patients)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
1	67	47
2	54	23
3	52	51
4	57	34
6	61.5	37
7	47.5	49
8	47	43
9	23	21
10	43	37.5
12	66	56
13	41	41
14	67.5	49
15	48	37
17	44	0
MEAN	51.3	37.6
S.E.	3.3	3.9

(c) pH. Monitoring of intragastric pH during the test period was carried out in nine subjects. The pattern was similar to that found in the gefarnate group, as described in the last chapter. The pH at the end of the test period is shown in Table XXVII. There was a substantial rise in the pH after treatment in only one subject. Wilcoxon's non-parametric test showed no significant difference before and after treatment.

Subjects 9 and 17, who were the only two with a high intragastric pH after carbenoxolone, also produced the lowest percentage drop in the potential difference. The scatter of pH was not wide enough for a correlation between percentage drop in potential difference and pH to be established.

TABLE XXVII

Intragastric pH
(Patients)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
8	1.6	1.5
9	4.2	3.9
10	1.8	1.5
11	2.1	1.3
13	1.3	1.5
14	1.6	1.6
15	1.5	1.6
16	1.3	1.1
17	1.4	6.3

(d) Plasma salicylate levels. Good duplicate results were obtained in six subjects in this group before treatment, and in ten after treatment. In five subjects, results were obtained before and after treatment. Those are shown in Table XXVIII.

There was a marked fall in the plasma salicylate level in one of those subjects after treatment with carbenoxolone, but there was no significant difference between the means of 3.61 and 3.23mg./100ml.

TABLE XXVIII

Plasma salicylate levels before and after treatment with carbenoxolone

SUBJECT	PLASMA SALICYLATE (mg./100ml.)	
	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
9	2.1	2.05
10	3.8	4.2
13	4.15	4.5
15	4.3	4.2
17	3.7	1.2
MEAN	3.61	3.23
S.E.M.	0.39	0.67

When the percentage drop in the potential difference after aspirin was plotted against the highest plasma salicylate level measured during the test, two separate regression lines were obtained, as shown in Figure 15. The correlation coefficient of the line representing patients before treatment was 0.97, and that of the line representing the patients after treatment with carbenoxolone was 0.95. There was no significant difference between the slopes of the lines, but the percentage drop in the potential difference was 8.3% lower after treatment with carbenoxolone than before for any plasma salicylate level. Analysis of covariance (Armitage, 1971) shows this difference to be significant ($p < 0.02$).

Discussion

The base-line potential differences of the patients with gastric ulcer or gastritis (Group II) were similar to those of the gefarnate

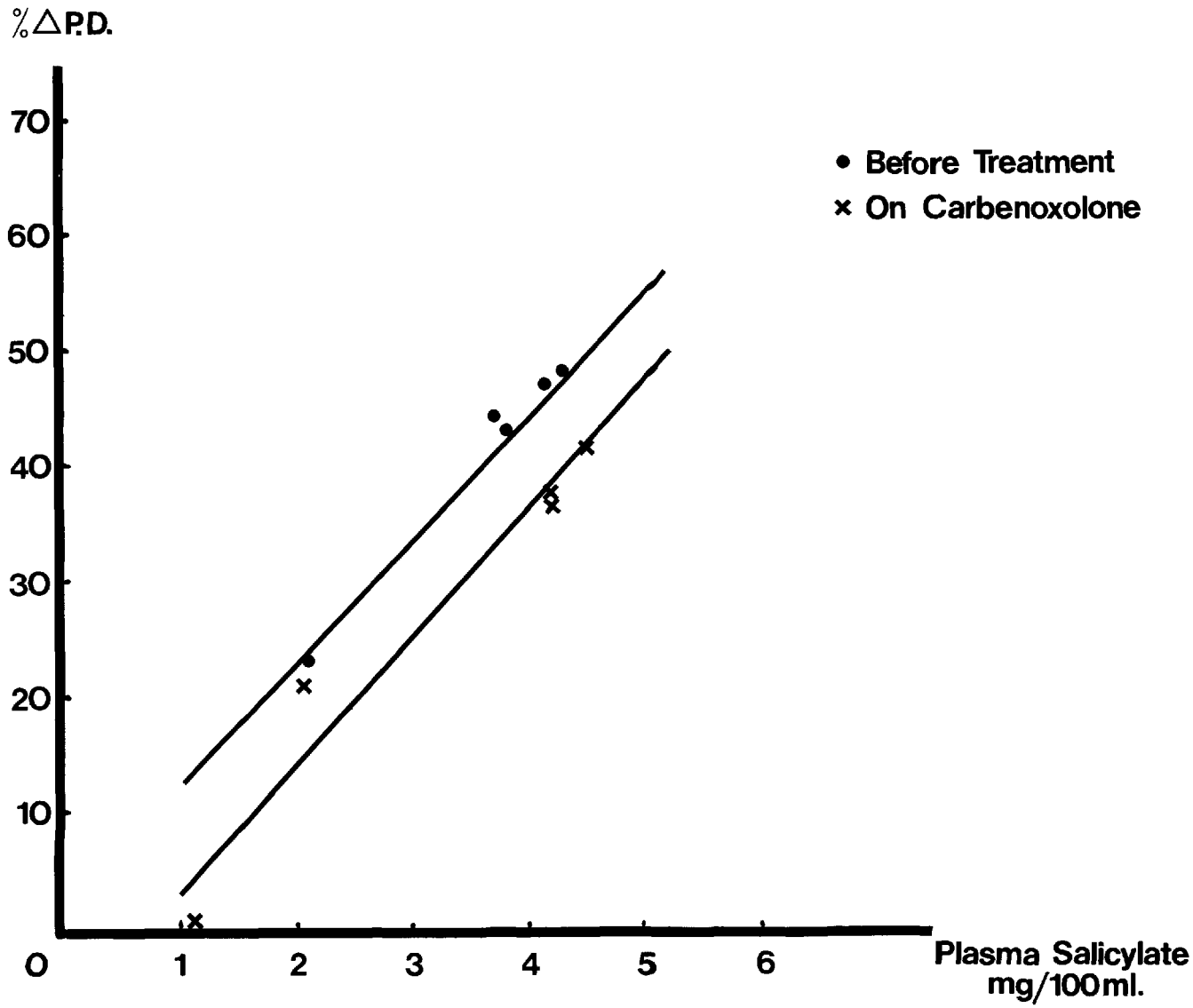


Figure 15 : Relationship between the percentage fall in the potential difference and the highest plasma salicylate level in five subjects before and after treatment with carbenoxolone.

group described in the last chapter, showing that those two groups are comparable.

Carbenoxolone did not affect the base-line potential difference in the normal subjects or in the group of patients. This is in keeping with the findings of Cross and Rhodes (1972) in the dog, of Ivey and Gray (1973) who studied ionic fluxes in normal subjects, and of Bennett et al (1974) who found no change in potential difference after carbenoxolone in normal subjects.

In the group of normal subjects in the present study there was a substantial fall in the percentage drop of the potential difference after aspirin only in one out of four subjects treated with carbenoxolone. Unfortunately, this was the subject in whom pH was not monitored and salicylate levels were not measured. The failure of carbenoxolone to affect the aspirin-induced fall in the potential difference in three out of four subjects could be due to the fact that the normal subjects were treated for one week only - it was considered unethical to expose normal subjects to a full course of treatment in view of the well-known side-effects of the drug. On the other hand, those results fit in with the findings of Ivey and Gray (1973) and of Bennett et al (1974).

The effect of carbenoxolone on the gastric mucosal barrier on subjects with an abnormal gastric mucosa has not been studied before. The results obtained here show that it produces a significant reduction in the effect of aspirin on the gastric mucosa. The percentage drop in the potential difference was used as a measure of the effect of aspirin for reasons discussed in Section IV, Chapter XIII. Even if the actual drop of the potential difference was used for comparison, it can be seen from Table XXIX that there was a reduction in the effect of aspirin in eleven out of fourteen subjects after treatment with carbenoxolone.

TABLE XXIX

Drop in potential difference (mV.) after aspirin (Patients)

SUBJECT	BEFORE CARBENOXOLONE	AFTER CARBENOXOLONE
1	25	23.5
2	22	6
3	22	20
4	17.5	8.5
6	32	19
7	19	22
8	20	18
9	7	7
10	13	12
12	26	24
13	10	13
14	24	21
15	15	14
17	8	0

Since Bank et al (1967) and Cocking and MacCaig (1969) have found a reduced output of acid after treatment with carbenoxolone, it could be argued that the results obtained here are explainable simply on the basis of a higher intragastric pH after the course of treatment. This would result in a decrease in the absorption of aspirin and a smaller effect on the gastric mucosa. However, only in one out of the nine patients in whom pH was monitored was there an appreciable rise in pH after treatment.

In the five patients in whom salicylate levels were obtained before and after treatment, carbenoxolone did not produce a

significant change in the levels. Excellent correlation was obtained between percentage drop in potential difference and highest plasma salicylate level measured both before and after treatment. This was at least in part due to the fact that there were only a small number of patients involved. Furthermore, as mentioned before, plasma salicylate levels can only give an approximate indication of gastric absorption of aspirin. Nevertheless, the results obtained suggest that carbenoxolone protects the abnormal gastric mucosa from aspirin by a mechanism other than slowing down its absorption.

The present study could form the basis of a screening test on drugs thought to be capable of protecting the gastric mucosa. Calcraft et al (1973) have already made a start by showing that amylopectin sulphate diminishes the effect of bile salts on ionic fluxes and potential difference across the gastric mucosa of dogs with Heidenhain pouches. This effect needs to be confirmed in patients with an abnormal gastric mucosa.

General Discussion

As a result of work on sodium and hydrogen ion fluxes across the gastric mucosa, initiated by Code and continued by Davenport (Davenport, 1970), it has become generally accepted that permeability is a measure of the integrity of the mucosa. Since measurement of ionic permeability in man is technically difficult and involves a number of assumptions inevitably leading to inaccuracies in the results, many investigators have turned to the gastric mucosal potential difference as an alternative. In spite of a large amount of work carried out on the subject, the exact significance of the potential difference remains unknown. Nevertheless, a relationship between potential difference and ionic permeability has been clearly established (Davenport et al, 1964; Chvasta and Cooke, 1972). Incomplete knowledge of the mechanism of production of the potential difference, and of its "raison d'être", should not therefore prevent one from using it as a measure of the integrity of the gastric mucosa.

In this thesis, the anatomical distribution of the potential difference has been studied in both the normal and the abnormal stomach. Evidence is produced to show that there is a lower than normal potential difference across the gastritic mucosa, a finding that lends further support to the concept that the potential difference is a measure of the integrity of the mucosa. The effects of commonly used drugs that damage the mucosa, and of those that protect it, have been studied.

Preliminary studies on the potential difference across the skin showed that it varied in magnitude from subject to subject.

Furthermore, it was inconstant in any one subject over a period of thirty minutes. Hence it was felt that the use of the skin as a reference point for the measurement of the gastric mucosal potential difference was unsatisfactory. Even the technique of abolishing the skin potential difference by means of an intradermal injection of saline (Archampong and Edmonds, 1972) was unacceptable, since the effect often lasted no longer than ten minutes. Intravenous reference electrodes were therefore used throughout in the studies described in this thesis.

Measurement of the gastric mucosal potential difference in fourteen subjects confirmed previous findings (Andersson and Grossman, 1965; Geall et al, 1970) that it is lower in the antrum than in the body of the stomach. Further mapping of the distribution of the potential difference was made possible by developing the technique of measurement under direct vision at endoscopy. In a group of twelve subjects shown to have a histologically normal mucosa, the potential difference was found to be invariably lower at the high lesser curve than at the high greater curve. In the dog, the low potential difference across the antral mucosa is associated with a markedly increased ionic permeability when compared with fundal mucosa (Dyck et al, 1969). It is obviously impossible to measure permeability in different parts of the normal human stomach. All the same, it has been suggested that the lower potential difference in the antrum may explain the predilection of ulcers for this part of the stomach. In view of the findings here, the same suggestion could be made about the high lesser curve. These findings obviously need to be confirmed by other investigators.

Although it has been thought for some years that the potential difference across the gastritic mucosa is lower than that across

the normal mucosa, the only experimental work on the subject has never been published (Ivey and Clifton, 1974). In a study involving five subjects with histologically proven gastritis and eighteen with gastric ulcers, the potential difference was found to be significantly lower in both those groups than in twelve normal volunteers. This finding was confirmed in a study in which measurements were made under direct vision at endoscopy and biopsies were taken from the same sites for histology. The potential difference at the high lesser curve was significantly lower in six subjects with gastritis than in twelve with a normal mucosa. The association between a low potential difference and gastritis suggests that the potential difference is indeed a measure of the integrity of the mucosa. One needs to remember, however, that the potential difference originates from the parietal cells, and that there are fewer parietal cells in the gastritic than the normal mucosa. Further work needs to be done on a larger number of subjects before the potential difference can be fully correlated with the severity of gastritis and the number of parietal cells.

Patients with rheumatoid arthritis are often treated with drugs that may be ulcerogenic, including aspirin, phenylbutazone, indomethacin and corticosteroids. Furthermore, they may start off with an abnormal gastric mucosa, as suggested by Kern et al (1957). One would therefore expect them to have a more permeable mucosa and a lower potential difference than the normal. The potential difference in eleven subjects with long-standing rheumatoid arthritis was found to be significantly lower than in twelve normal volunteers. This is in agreement with the results of Ivey and Clifton (1974), who found an increased gastric mucosal permeability in such patients. On the other hand, Murray et al (1974) found the

potential difference to be the same in a group of patients with rheumatoid arthritis as in normal volunteers. Further study is obviously necessary before this conflict can be resolved.

Aspirin is known to be capable of lowering the gastric mucosal potential difference in normal subjects (Overholt and Pollard, 1968), but the mechanism is unknown. This effect was confirmed in healthy volunteers, and shown to apply to patients with an abnormal gastric mucosa as well. Reasonable correlation was obtained between the maximum effect of aspirin on the potential difference and the highest plasma salicylate level measured after instillation of the test solution in the stomach. On the other hand, plasma salicylate levels of the same order, produced by intravenous injections of lysine acetylsalicylate in three subjects, had no appreciable effect on the gastric mucosal potential difference. Those results suggest that it is not the salicylate in the blood, but rather its passage through the gastric mucosa, that affects the latter. Those results support the hypothesis put forward by Martin in 1963, according to which the drug passes easily from the acid environment in the lumen, where it is unionized and lipid-soluble, into the cells; once it is in the neutral environment in the cells, it becomes ionized and diffuses out very slowly; the resulting accumulation inside the cells produces the damage. Since the actual absorption of aspirin in the stomach was not measured, the evidence presented in this thesis is obviously incomplete. Further studies need to be carried out. Those would ideally combine a technique that would demonstrate accumulation of aspirin in the cells, such as autoradiography, with potential difference measurements, and with electron microscopy to show the damage to the cells.

It was hoped that measurement of the potential difference would provide a simple method of screening drugs for their damaging effects on the gastric mucosa. Studies carried out with phenylbutazone, indomethacin and prednisolone, produced disappointing results. None of those drugs lowered the potential difference. The soluble commercial preparation of prednisolone disodium phosphate (Predsol) was the only drug that produced a consistent fall in the potential difference. However, a solution of sodium citrate, which is a constituent of Predsol, had almost exactly the same effect.

Murray et al (1973) have also found that phenylbutazone, indomethacin and corticosteroids do not lower the gastric mucosal potential difference in man, although earlier work in the same laboratory had shown that indomethacin increased mucosal permeability in the dog (Chvasta and Cooke, 1972). It is clear that, at least in man, other techniques need to be used to demonstrate the damaging effect on the gastric mucosa of most drugs other than aspirin. Chung et al (1970b) have found that chronic administration of aspirin and prednisolone in patients with rheumatoid arthritis had a greater effect on gastric mucosal permeability than administration of aspirin alone. Similarly, in the dog, the effect of aspirin or sodium taurocholate on permeability of the gastric mucosa is significantly enhanced by a course of prednisolone (Silen, 1972). A study in which the effect of aspirin on the gastric mucosal potential difference is measured in groups of normal subjects before and after courses of various anti-inflammatory drugs should produce interesting results.

This technique of measuring the effect of a standard dose of aspirin on the potential difference before and after a course of

treatment with another drug was used in this thesis to study the action of gefarnate and carbenoxolone on the gastric mucosa. In eleven patients with gastric ulcer or gastritis, gefarnate produced no change in the potential difference or in the effect of aspirin on the potential difference. On the other hand, carbenoxolone, which has been shown to be superior to gefarnate in the treatment of gastric ulcer (Langman et al, 1973), produced a significant decrease in the effect of aspirin on the potential difference. That this protective effect on the gastric mucosa was not mediated by any change in the pH of gastric contents was demonstrated by monitoring the pH in nine of the seventeen patients studied. It is also unlikely that carbenoxolone exerted its action by slowing down absorption of aspirin, since plasma salicylate levels during the experiments were not significantly lower after the course of carbenoxolone. Since plasma salicylate levels were measured in five patients only, and since those levels do not reflect gastric absorption of aspirin accurately, this study needs to be extended.

The carbenoxolone study was carried out on seventeen patients with gastric ulcer or histologically proven gastritis. Bennett et al (1974) used normal volunteers in a similar study, and did not find any change in the effect of aspirin on the potential difference after a two-week course of carbenoxolone. Similar results were obtained here when four normal subjects were given carbenoxolone for one week. The discrepancy between the responses of the normal subjects and the patients may be explained by the fact that the latter were given a longer course of treatment, namely four weeks. An alternative explanation is that the normal and the abnormal gastric mucosae respond differently to the drug.

Further study is clearly indicated.

The results of the study of the effect of carbenoxolone on the abnormal gastric mucosa are encouraging. The technique used could prove to be a useful tool in the investigation of drugs claimed to be effective in the treatment of gastric ulcer or gastritis.

Summary

The discovery of the gastric mucosal potential difference, and the development of techniques used for its measurement in the experimental animal and in man, are reviewed. Its origin, anatomical distribution, and physiological significance, and its relationship to the ionic permeability of the mucosa, are discussed. Previous work on practical applications of potential difference measurements is reviewed.

Many investigators have used the skin as the reference point for the measurement of the gastric mucosal potential difference. A preliminary study involving five subjects showed the potential difference across the skin to be variable. The technique of Andersson and Grossman, involving the use of an intravenous reference electrode, and an exploring electrode passed blindly into the stomach, was therefore used.

A new method of measurement of the potential difference under direct vision at endoscopy is described. This technique, and the one of Andersson and Grossman, were used in a number of studies on the normal and the abnormal gastric mucosa in man:-

(1) It was confirmed that the potential difference is higher in the body of the stomach than in the antrum.

(2) Measurement of the potential difference under direct vision showed that it was invariably lower at the high lesser curve (-15.8 ± 2.2mV.) than at the high greater curve (-37.3 ± 2.0mV). The implications of this finding, with particular reference to the predilection of ulcers for the

high lesser curve and the antrum, where the potential difference is also low, are discussed.

(3) The potential difference, measured with the technique of Andersson and Grossman, was significantly lower in five subjects with gastritis (-33.6 ± 1.4mV.) and in eighteen subjects with gastric ulcers (-32.4 ± 0.7mV.) than in twelve normal volunteers (-45 ± 0.6mV.).

(4) The association between gastritis and a lower than normal gastric mucosal potential difference was more firmly established by making measurements under direct vision at endoscopy and taking biopsies from the same sites. The potential difference was significantly lower at the high lesser curve in six subjects with gastritis (-4.8 ± 0.5mV.) than in twelve subjects with a normal mucosa (-15.8 ± 2.2mV.). Further work involving a larger group of subjects needs to be done before potential difference can be properly correlated with severity of gastritis and other factors such as the number of parietal cells present.

(5) Eleven patients with rheumatoid arthritis were found to have a significantly lower potential difference (-37.7 ± 0.9mV.) than twelve normal volunteers (-45.7 ± 0.6mV.). This finding is discussed with special reference to the possible damaging effects of anti-inflammatory drugs on the gastric mucosa. Other investigators have produced conflicting results, and further work is necessary.

(6) It was confirmed in six normal volunteers that exposure of the gastric mucosa to aspirin produces a temporary fall in the absolute value of the potential difference. Thirteen patients with gastric ulcers or gastritis responded in a similar way. Reasonable correlation was obtained between the maximum effect of aspirin on the potential difference and the highest plasma salicylate level measured. Intravenous injection of a salicylate in three subjects had no effect. The results suggest that the effect of aspirin on the mucosa occurs during gastric absorption of the drug, but further work needsto be carried out to prove this.

(7) Phenylbutazone, indomethacin and prednisolone did not lower the potential difference. This is in agreement with the findings of other workers. It is suggested that the effects of those drugs on the mucosa could be demonstrated by measuring the effect of a standard dose of aspirin on the potential difference before and after a course of those drugs in groups of volunteers.

(8) A course of gefarnate had no effect on the potential difference, or on the fall in the potential difference produced by a standard dose of aspirin, in eleven patients with gastric ulcers or gastritis.

(9) Carbenoxolone, which is a more effective drug than gefarnate in the treatment of gastric ulcer,

also had no consistent effect in a similar study involving four normal volunteers. This is in agreement with the findings of other investigators.

(10) When carbenoxolone was given to seventeen patients with gastric ulcer or gastritis, the potential difference was not altered. However, the percentage fall in the potential difference produced by a standard dose of aspirin decreased significantly, from 51.3 $\pm$ 3.3% to 37.6 $\pm$ 3.9%. This effect was mediated without a significant change in the pH of gastric contents or in plasma salicylate levels.

This technique could form the basis of amethod of screening drugs thought to be of use in the treatment of gastric ulcer or gastritis.

Participation

The whole of this thesis is my own work with the exception of histological examination of gastric mucosal biopsies, which was carried out by Dr. P. Fitzpatrick.

The work was suggested by Dr. D. G. Colin-Jones, who, with Dr. J. E. Lennard-Jones, provided advice and encouragement throughout.

The studies presented in support of this thesis were done with the guidance and advice of Dr. D. G. Colin-Jones.

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APPENDIX

TABLE A1

Highest P.D. (-mV.) recorded in five groups of subjects

NORMAL VOLUNTEERS		GASTRIC ULCER		GASTRITIS		PERNICIOUS ANAEMIA		RHEUMATOID ARTHRITIS	
Subject	P.D.	Subject	P.D.	Subject	P.D.	Subject	P.D.	Subject	P.D.
1	41	1	38	1	32	1	23	1	40
2	43	2	29	2	43	2	35	2	42
3	51	3	46	3	43	3	29	3	41
4	43	4	18	4	20			4	33
5	49	5	38	5	30			5	54
6	42	6	28					6	32
7	45	7	31					7	41
8	37	8	40					8	27
9	52	9	25					9	45
10	48	10	37					10	34
11	48	11	42					11	26
12	49	12	19						
		13	26						
		14	30						
		15	31						
		16	21						
		17	43						
		18	41						
MEAN	45.7		32.4		33.6		29		37.7
S.E.M.	0.6		0.7		1.4				0.9

TABLE A2

Effect of 20mg. prednisolone on P.D.

SUBJECT	TIME (MINUTES)													
	-5	0	5	10	15	20	25	30	35	40	45	50	55	60
1	48	48	47	45	45	44	46	45	45	47	46	47	48	49
2	42	43	40	40	41	42	40	40	40	42	41	40	40	41
3	48	49	48	48	50	50	50	49	43	47	51	52	49	42

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Trichobezoar, gastric polyposis, protein-losing gastroenteropathy and steatorrhoea

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Trichobezoar, gastric polyposis, protein-losing gastroenteropathy and steatorrhoea

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SUMMARY A mentally subnormal patient presenting with oedema was found at gastroscopy to have a large trichobezoar and multiple gastric polyps. The serum concentrations of albumin and IgG were low in the absence of proteinuria, and the gastrointestinal clearance of radiochromium after the intravenous administration of radiochromic chloride was increased. These findings are compatible with increased gastrointestinal loss of plasma proteins. In addition, the patient had steatorrhoea. All of these abnormal findings were markedly improved after operative removal of the bezoar.

A trichobezoar is a mass of hair, wool, and similar material found in the stomach, and less commonly in the intestine, of some patients with abnormal appetites. The adolescent girl with long, flowing hair and the mentally subnormal feature prominently among those afflicted.

Complications are frequent. Gastric ulceration, haemorrhage, perforation, and intestinal obstruction are among the better known. The association between bezoar and gastric polyposis, though well documented, is not so widely appreciated (Davies, 1921; Charache, Polayes, Behr, Murata, and Dimetriades, 1957; Valberg, McCorriston, and Partington, 1966). In one case, one of the polyps had become malignant (Charache *et al*, 1957).

Ankle swelling as a presenting symptom was first mentioned by Davies in 1921. Plasma proteins were not measured. Wine, in 1957, described a patient with gastric trichobezoar, oedema, and hypoproteinaemia. An insight into the pathophysiology of this complication was obtained by Valberg and his colleagues in 1966. Using ^{131}I-labelled human albumin and polyvinylpyrrolidone, they demonstrated excessive gastrointestinal protein loss in one patient, and their results, though incomplete, suggested that it occurred in another patient as well. Gastrointestinal protein loss returned to normal after operative removal of the bezoar.

The patient reported here presented with oedema only. He was found on investigation to have a large trichobezoar associated with gastric polyposis, protein-losing gastroenteropathy, and steatorrhoea.

Received for publication 11 June 1973.

Method

Gastrointestinal protein loss was estimated by the clearance technique of Van Tongeren and Reichert (1966). Forty microcuries of $^{51}CrCl_3$, diluted in physiological saline acidified to pH2, was injected intravenously. Faeces were collected for seven days, each specimen being kept separately. Blood samples were taken 15 minutes, 30 minutes, and 12 hours after the injection, and thereafter at daily intervals. Plasma radioactivity was measured with a well counter (Packard Auto-Gamma spectrometer—model 300B) and expressed as a fraction of the dose per litre. The radioactivity of each specimen of faeces was measured in a sensitive large-volume gamma counter (J. & P. Engineering, Reading, Limited) and expressed as a fraction of the dose per specimen. The rate of clearance of plasma into the gastrointestinal tract, in litres per day, is obtained by dividing the cumulative faecal radioactivity by the area under the curve of plasma radioactivity against time.

Serum immunoglobulin levels were measured by the method of Mancini, Carbonara, and Heremans (1965). Urinary indican was estimated by the method of Curzon and Walsh (1962).

Case Report

The patient was a 20-year-old male, mentally subnormal as a result of the congenital rubella syndrome. Swelling of the ankles was first noticed in March 1972 in the institution where he has been resident for several years. By the end of September 1972, he had pitting oedema of both legs up to the

knees. There was a mass in the epigastrium and right hypochondrium extending down to the umbilicus. Minimal ascites and a small pleural effusion on the right were the only other positive findings. The cardiovascular system, in particular, was normal.

Urine testing on several occasions showed no significant albuminuria. Blood urea was 36 mg/100 ml. Liver function tests were normal. Haemoglobin level was 10·9 g/100 ml. Serum iron was 66 μg/100 ml. Serum vitamin B_{12} and red cell folate were normal.

The diagnosis was made at gastroscopy. Apart from the trichobezoar, a large number of polyps were seen in the stomach. They were most numerous in the antrum, where the surface of some of them was ulcerated. Two smaller polyps were seen in the duodenum. Multiple biopsies of the gastric polyps showed that they were benign. They were histologically different from adenomata. Although it was not possible to exclude a congenital origin, an inflammatory one was considered more likely (Dr A. McIver).

The results of other investigations are shown in table I, and the barium meal appearance in figure 1.

Before Operation		*Eight Weeks after Operation*
Total serum proteins	g/ 3·1	6·3
Serum albumin	100 1·3	3·2
Serum globulin	ml 1·8	2·9
IgG	590	1400 (normal 900-1300)
IgA	mg/100 ml 220	280 (normal 160-400)
IgM	180	310 (normal 60-180)
Gastrointestinal clearance of ^{51}Cr-labelled protein	180 ml plasma/day	34 (normal <20)
Five-day faecal fats	14·1 g/day	5·7
Urinary indican	100 mg/day	—

Table I *Results of investigations carried out before and after removal of the trichobezoar*

The patient started vomiting on 17 October 1972. Two days later, after parenteral fluid and electrolyte replacement, laparotomy was carried out by Mr T. Rowntree. The presence of the polyps was confirmed (fig 2). The bezoar removed was a perfect cast of the stomach, with short tails protruding into the duodenum and the oesophagus (fig 3). The extension into the oesophagus is a very unusual feature. The liver appeared normal.

Recovery was uneventful. The parents consented to the readmission of the patient eight weeks after the operation. Oedema had by then disappeared completely. The results of investigations are compared with the preoperative ones in table I. A repeat gastroscopy showed the polyps to be still present (fig 4). They were somewhat smaller and no longer ulcerated.

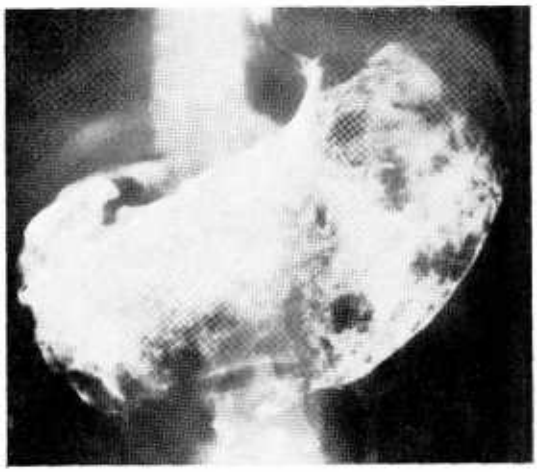

Fig 1 *Barium meal showing the trichobezoar in the stomach.*

Fig 2 *Trichobezoar being removed from the stomach. One large and several smaller polyps can be seen on the exposed mucosa.*

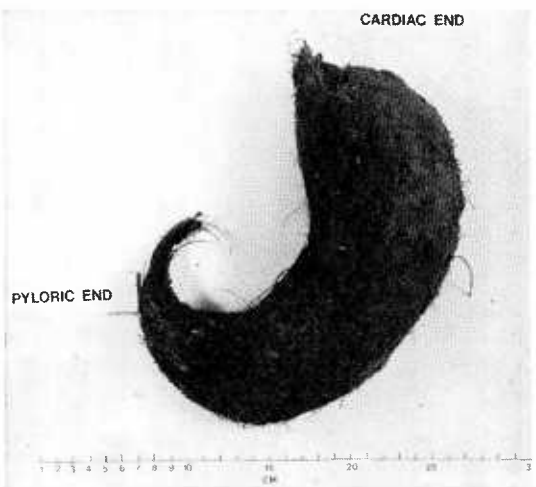

Fig 3 *Trichobezoar removed from the stomach of the patient.*

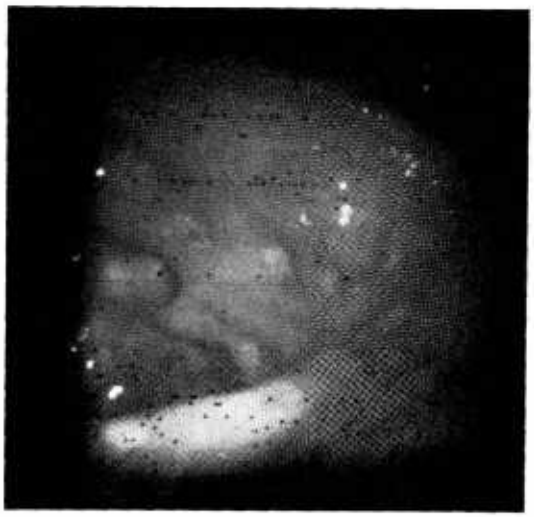

Fig 4 *Polyps as seen at gastroscopy eight weeks postoperatively.*

Discussion

Oedema and hypoproteinaemia in the absence of a grossly deficient diet, proteinuria, or overt liver disease should automatically raise the suspicion of protein-losing gastroenteropathy. In this condition the IgG level is usually low, presumably because this protein, like albumin, has a low fractional turnover rate (Waldmann, 1970). A low IgG level was found in our patient, making a diagnosis of protein-losing gastroenteropathy all the more likely. The high rate of clearance of plasma into the gastrointestinal tract confirmed the diagnosis.

The superiority of a technique measuring the clearance of plasma proteins has already been pointed out by Waldmann (1970). Unlike other methods, it relates gastrointestinal loss to the plasma pool.

Removal of the bezoar caused a return towards normality of all the variables measured. This is in keeping with the findings of previous workers, namely, disappearance of oedema and return of serum proteins, where measured, to normal. We interpret the postoperative improvement as evidence for a causal relationship between the bezoar and the protein-losing state. As for the mechanism involved, one can only speculate that the constant irritation of the gastric mucosa leads to the oozing of plasma into the gastric lumen. A possible additional factor is the presence of gastric polyps, which are known to be capable of causing protein loss (Dich, Paaby, and Schwartz, 1961). The polyps were still present postoperatively. This may explain why the rate of clearance of plasma did not return completely to normal.

The patient of Davies (1921), who presented with oedema and was found to have a gastric trichobezoar, also suffered from persistent diarrhoea. Faecal fats were not measured. Steatorrhoea does not appear to have ever been considered as a complication of gastric bezoars. It was present in our patient before, but not after, the removal of the bezoar. A duodenal biopsy taken at gastroduodenoscopy showed the presence of normal villi, making coincidental coeliac disease very unlikely. Culture of duodenal contents aspirated eight weeks postoperatively showed no evidence of bacterial overgrowth. Unfortunately, this was not performed preoperatively, and the bezoar was not cultured. It is of interest that steatorrhoea has been observed in other causes of protein loss in the stomach. In his series of 10 such patients, Jarnum (1963) found three with raised faecal fats. Two of those three were achlorhydric, and bacterial overgrowth in the small intestine as a cause of the steatorrhoea is a distinct possibility (Sherwood, Goldstein, Haurani, and Wirts, 1964). The trichobezoar of our patient had the usual highly offensive smell. It is possible that bacterial contamination of the bezoar resulted in bacterial overgrowth in the small intestine and caused his steatorrhoea. The raised urinary indican supports this hypothesis.

We are grateful to Dr P. Todd, to Mr T. Rowntree, and to the Nuclear Medicine Department, and in particular to Mr R. Mardell, and to the nursing staff of the Metabolic Ward for their cooperation.

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PROTECTION OF THE HUMAN GASTRIC MUCOSA FROM ASPIRIN BY CARBENOXOLONE

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The relative impermeability of the gastric mucosa to sodium and hydrogen ions is a measure of its integrity. Thus damage to the mucosa by aspirin, for example, is reflected by an increase in the back-diffusion of hydrogen ions from lumen to serosa and in the movement of sodium ions in the opposite direction (Davenport, 1965). It is possible to demonstrate that aspirin increases the permeability of the gastric mucosa in man as well as in experimental animals (Overholt and Pollard, 1968), but the technique is difficult.

An alternative method is to utilize the potential difference (p.d.) across the mucosa as an indication of its integrity. Changes in the permeability of the gastric mucosa are accompanied by changes in the transmucosal potential difference. Indeed, it has been shown that there is an exponential relationship between the potential difference and the permeability to sodium ions (Davenport, Warner and Code, 1964; Cook and Kienzle, 1974). Since measurement of the potential difference in man is relatively easy, the fall produced by a standard dose of aspirin was chosen in this study to compare the protective actions of carbenoxolone and gefarnate on the gastric mucosa.

Subjects

Tests were carried out on three groups of subjects before and at the end of a course of treatment.

Group I consisted of nine patients with gastric ulcers, and two with gastritis. They were treated with gefarnate 300 mg daily in divided doses for 4 weeks.

Group II consisted of four normal volunteers who received carbenoxolone 300 mg daily in divided doses for 1 week.

Group III consisted of 14 patients with gastric ulcers, and three with gastritis. They received carbenoxolone 300 mg daily in divided doses for 1 week, after which the dose was halved for a further 3 weeks.

All subjects in Groups I and III had their diagnosis confirmed by endoscopy and biopsy.

Methods

Potential difference: Potential difference was measured by the method of Andersson and Grossman (1965). The reference electrode for intravenous insertion consisted of a fine polyethylene tube (internal diameter 1·14 mm) containing 3 per cent agar saturated with potassium chloride. This was sterilized by gamma-radiation. The intragastric electrode was a similar tube (i.d. 3 mm) containing the same material. Attached to it was another tube which allowed instillation of test solutions into the stomach. Potential difference was measured with balanced calomel half-cells (K100, Radiometer, Copenhagen) and recorded on paper (Servograph Pen Drive REA 310, Radiometer, Copenhagen) via a pH meter (Titrator TTT2, Radiometer, Copenhagen).

pH: In some subjects intragastric pH was monitored by means of a Beckmann glass electrode attached to the potential difference electrode.

Plasma salicylate levels: The extraction method of Routh *et al.* (1967) was used for measuring plasma salicylate levels in some of the subjects during the test.

Procedure

After an overnight fast, the subject was kept in the sitting position whilst the exploring electrode and side-tube (with or without the pH electrode) were passed orally to about 80 cm. The subject then lay on his left side, and the reference electrode was introduced into a peripheral vein via a sterile needle. The circuit was completed and the exploring electrode was withdrawn gradually until the highest potential difference was

recorded. The tip of the electrode was then usually 5–10 cm below the cardia.

When the base-line potential difference (p.d.) had been steady for at least 15 min, 40 ml of water was instilled into the stomach. When the base-line was steady again, the test solution of two Solprin tablets, containing 600 mg of aspirin in 20 ml of water, was instilled, followed by a further 20 ml of water. Potential difference was recorded for 1 hour, by which time it had usually fallen to its lowest level, and recovery was well on the way (*see Figure 1*).

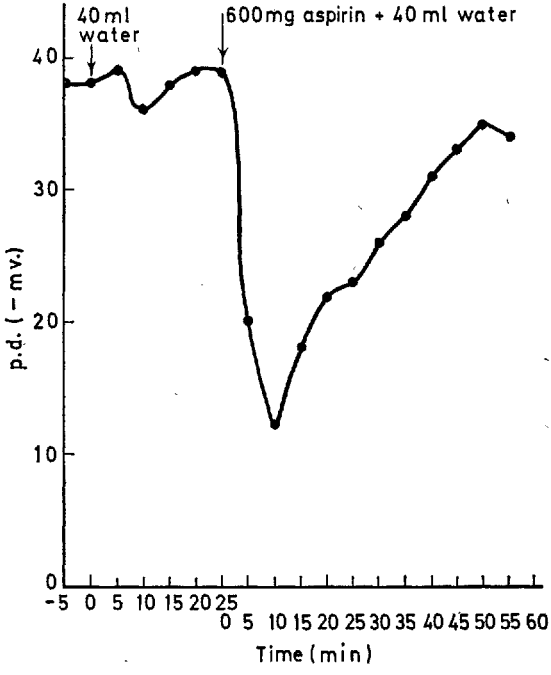

Figure 1. Effect of aspirin on the gastric mucosal potential difference.

When a pH electrode was used, the recording of potential difference was temporarily stopped whilst pH was recorded at intervals during the test. Plasma salicylate levels were measured

from blood samples taken 20, 40 and 60 min after the instillation of aspirin.

Results

Group I

Table I shows the base-line potential difference for the 11 subjects of this group before and after their course of gefarnate. The mean p.d. after treatment, −36·4 mV, was not significantly different from that before treatment, −34·1 mV.

TABLE I

GASTRIC POTENTIAL DIFFERENCE (−mV)

Subject	*Before gefarnate*	*After gefarnate*
1	40	36
2	29	29
3	31	45
4	39	35
5	46	53
6	43	40
7	46	41
8	26	39
9	15	22
10	37	36
11	25	23
Mean ± S.E.	34·1 ± 3·0	36·4 ± 2·7

The maximum fall in the p.d. after instillation of aspirin, expressed as a percentage of the base-line level (Δp.d.), was taken as a measure of the effect of aspirin on the mucosa. The results for this group are shown in Table II. Two cases were excluded because of large artefacts in the p.d. tracing after the instillation of water or of the test solution. There was no significant difference between the means of 53·6 and 55·3 per cent before and after treatment with gefarnate.

TABLE II

PERCENTAGE DROP IN POTENTIAL DIFFERENCE AFTER ASPIRIN

Subject	*Before gefarnate*	*After gefarnate*
1	65	33
3	54	42
4	46	40
5	24	53
6	65	70
7	62	56
8	62	69
10	64	69
11	41	65
Mean ± S.E.	53·6 ± 4·7	55·3 ± 4·7

Group II

The base-line levels of p.d. before and after a 1-week course of carbenoxolone are shown in Table III. There is no important change in the p.d. of any of the four subjects, although there was a fall in Δp.d. in one subject (Table IV).

TABLE III

GASTRIC POTENTIAL DIFFERENCE (−mV)
(NORMAL SUBJECTS)

Subject	*Before carbenoxolone*	*After carbenoxolone*
1	43	39
2	45	37
3	37	42
4	54	50

Group III

(a) *Base-line potential difference:* As shown in Table V, treatment of this group of patients with carbenoxolone for

4 weeks produced no significant change in p.d., the mean values being −34·6 mV, before and −35·1 mV after treatment.

TABLE IV

PERCENTAGE DROP IN POTENTIAL DIFFERENCE AFTER ASPIRIN (NORMAL SUBJECTS)

Subject	*Before carbenoxolone*	*After carbenoxolone*
1	47	44
2	53	52
3	57	57
4	68	33

TABLE V

GASTRIC POTENTIAL DIFFERENCE (PATIENTS)

Subject	*Before carbenoxolone*	*After carbenoxolone*
1	38	50
2	40	25
3	42	39
4	31	25
5	20	20
6	52	51
7	40	45
8	43	42
9	31	34
10	30	32
11	27	21
12	39	43
13	25	31
14	35	43
15	31	38
16	47	43
17	18	15
Mean ± S.E.	34·6 ± 2·2	35·1 ± 2·6

(b) *Potential difference:* Technically satisfactory results were obtained in 14 of the 17 subjects in this group, and are shown in Table VI. The drop in p.d. was 51·3 ± 3·3 per cent (mean ± S.E.) before and 37·6 ± 3·9 per cent after a 4-week course of carbenoxolone. A paired *t*-test shows this change in Δp.d. to be significant ($P < 0{\cdot}01$).

TABLE VI

PERCENTAGE DROP IN POTENTIAL DIFFERENCE AFTER ASPIRIN (PATIENTS)

Subject	*Before carbenoxolone*	*After carbenoxolone*
1	67	47
2	54	23
3	52	51
4	57	34
6	62	37
7	48	49
8	47	43
9	23	21
10	43	38
12	66	56
13	41	41
14	68	49
15	48	37
17	44	0
Mean ± S.E.	51·3 ± 3·3	37·6 ± 3·9

(c) *pH:* The intragastric pH immediately after instillation of aspirin was, in all cases, close to 4·5, the pH of the test solution. In the majority of cases, it changed rapidly over the next 5–10 min, after which it remained stable. Averaging the pH values throughout the 1-hour test period, including the first few minutes, would have been mathematically wrong. For the purpose of this study, it was felt that the pH at the end of the test period was adequate, since there were no important fluctuations during the last 50 min of the test. The pH before and

after a 4-week course of carbenoxolone for the nine subjects in whom measurements were made is shown in Table VII. There were no important differences, with the exception of one subject (No. 17) in whom the pH was much higher after the course of treatment.

TABLE VII

INTRAGASTRIC pH

Subject	*Before carbenoxolone*	*On carbenoxolone*
8	1·6	1·5
9	4·2	3·9
10	1·8	1·5
11	2·1	1·3
13	1·3	1·5
14	1·6	1·6
15	1·5	1·6
16	1·3	1·1
17	1·4	6·3

(d) *Plasma salicylate levels:* Plasma salicylate levels were measured in 13 subjects from both Groups I and III before their course of treatment, and in 10 subjects from Group III after a 4-week course of carbenoxolone. The means of the highest levels measured for the untreated and the treated groups are almost exactly equal (4·02 ± 0·25 and 4·04 ± 0·44 mg/100 ml).

When Δp.d. was plotted against the highest plasma salicylate level measured during the test, two separate regression lines were obtained (*see Figure 2*). The correlation coefficient of the line representing the untreated subjects was 0·66, and that for the line representing the treated subjects 0·91. There was no significant difference between the slopes of the lines, but Δp.d. was 14·7 per cent lower in the carbenoxolone-treated group than in the untreated group for any plasma salicylate level. Analysis of covariance (Armitage, 1971) shows this difference to be highly significant ($P < 0·001$).

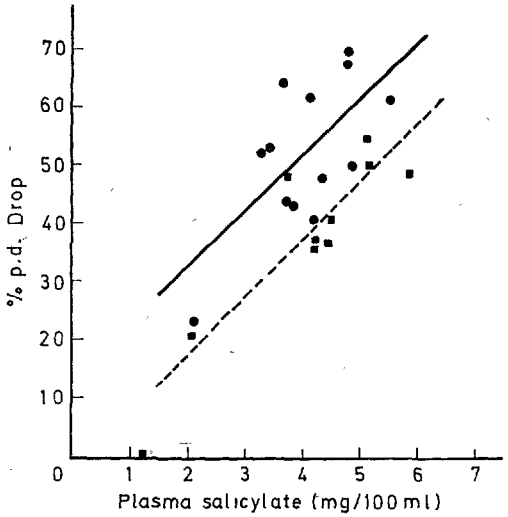

Figure 2. The regression lines are drawn for the relationship between percentage p.d. drop and the highest plasma salicylate. Before treatment, ●——● ($r = 0{\cdot}66$); after carbenoxolone ■ - - - ■ ($r = 0{\cdot}91$).

Discussion

The group of patients treated with gefarnate (Group I) was comparable with the group treated with carbenoxolone (Group III) in that there was very little difference between their baseline potential differences as shown in Tables I and V. Those were lower than for normal subjects (*see* Table III), and failed to rise to normal levels after treatment with either gefarnate or carbenoxolone (*see* Tables II and VI). Such a finding was not unexpected, since it is well-known that gastritis very frequently persists when gastric ulcers are treated (Gear, Truelove and Whitehead, 1971).

In studies at present being undertaken in which the potential difference has been measured under direct vision and compared with histology, a reduced potential difference has consistently been found when gastritis has been present. Even after healing, the site of the previous ulcer has a low p.d. of approximately −4 mV. (A. Hossenbocus, P. Fitzpatrick and D. Colin-Jones, unpublished observations).

Gefarnate is not as effective as carbenoxolone in the treatment of gastric ulcers (Langman, Knapp and Wakely, 1973). In this study, it produced no change in the effect of aspirin on the transmucosal potential difference. This does not, of course, mean that gefarnate cannot influence the gastric mucosa by other mechanisms.

Carbenoxolone, on the other hand, produced a significant decrease in the effect of aspirin on the potential difference (Table VI), whether the results for Δp.d. are expressed as a percentage fall, or in millivolts. Since the p.d. is an indication of mucosal integrity this finding fits in with earlier work in which ionic fluxes across the mucosa in gastric ulcer patients were shown to be affected by a period of treatment with carbenoxolone (Colin-Jones and Taylor, 1973).

Since the absorption of aspirin in the stomach is greater the lower the pH of the gastric contents (Hogben *et al.*, 1957; Cooke and Hunt, 1970) and it has been suggested that acid production is diminished after treatment with carbenoxolone (Bank *et al.*, 1967), it could be argued that the change in the effect of aspirin after treatment with carbenoxolone merely reflects a rise in pH. The pH measurements in this study (*see* Table VII) do not offer any quantitative evidence about the effect of carbenoxolone on acid production. However, they do show that there was no important change in the pH during the test period in eight out of nine subjects treated with carbenoxolone.

It was not possible to measure the gastric absorption of aspirin during the tests performed in this study. There is no difference between the highest plasma salicylate level attained after a standard dose of aspirin in untreated subjects and in subjects treated with carbenoxolone. As shown in *Figure 2*, there is reasonable correlation between the fall in the potential difference after aspirin and the highest plasma salicylate level. Those results suggest that carbenoxolone protects the gastric mucosa against aspirin without influencing its absorption.

Only one out of four normal subjects treated with carbenoxolone for 1 week showed a reduction in Δp.d. (Table IV). This finding is in agreement with the work of Ivey and Gray (1973) who found that treatment with carbenoxolone for 3

weeks did not protect the gastric mucosa against taurocholate in five normal volunteers. The protective effect of carbenoxolone on abnormal gastric mucosa has been demonstrated in this study, but we cannot answer the question as to whether carbenoxolone similarly affects presumed normal gastric mucosa. The failure to show an effect of carbenoxolone in the normal subjects could be due to insufficient numbers or too short a treatment period. Further studies are clearly needed.

Summary

The effect of a standard dose of aspirin on the gastric transmucosal potential difference was used in this study to compare the protective actions of gefarnate and carbenoxolone. Neither drug had any effect on the base-line potential difference. Carbenoxolone, but not gefarnate, produced a significant decrease in the effect of aspirin on the gastric mucosa of patients with gastric ulcers or gastritis. This effect is not mediated through a change in the pH of gastric contents. It is also unlikely that it is mediated through a decrease in the absorption of aspirin. The effect of carbenoxolone on the normal human mucosa was similar to that on the abnormal mucosa in only one out of four subjects. The implications are discussed.

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