The acute and chronic effects of cigarette smoking upon various aspects of gastric secretion.

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ABSTRACT

This study examined the inter-relationship between cigarette smoking (acute and chronic) on the one hand and gastric acid secretion, duodenogastric reflux and pyloric loss on the other.

Using gastric intubation and a histamine secretogogue, pure gastric secretion (Vg), pyloric loss (PL) and duodenogastric reflux (DGR) were measured in smokers and non-smokers with and without duodenal ulcer disease. The subjects were studied at basal, $1/8^{th}$ maximal, 1/4 maximal and maximal stimulation. Acute cigarette smoking significantly reduced Vg in both controls (C) (n = 8) and duodenal ulcer subjects (DU) (n = 14); there was no change in either pyloric loss or duodenogastric reflux. The fall in Vg coincided with the period in which there is known to be a rise in the plasma levels of toxic tobacco products.

In both groups of chronic smokers (C, n = 68; DU, n = 177) maximal Vg was significantly greater than in non-smokers (C, n = 46; DU, n = 36). Under basal conditions Vg was lower in the smokers (C, n = 25; DU, n = 91) compared to non-smokers (C, n = 20; DU, n = 15) but this did not reach significance.

The rate of pyloric loss was constant in any one individual, independent of secretory or clinical state. However, pyloric loss was higher in those with a greater

secretory capacity: namely, smokers and duodenal ulcer patients.

Duodenogastric reflux was greatest under basal conditions and significantly reduced at maximal stimulation, this was true for both groups (C & DU). This was thought to represent increased activity of the pylorus under conditions of maximal stimulation. Duodenogastric reflux was greater in those with duodenal ulcers compared to controls at both basal and maximal secretion. Analysis showed that this difference may in part reflect the disease process.

Chronic cigarette smoking had no direct effect on pyloric loss or duodenogastric reflux, any effect there was, was mediated through changes in secretory capacity.

Dose-response analysis revealed no evidence of increased sensitivity of the parietal cells in either duodenal ulcer patients or chronic smokers. The increased secretory capacities of these groups is simply due to an increased parietal cell mass.

It is suggested that the toxic products of tobacco smoke cause an acute reduction in Vg which eventually leads to an increase in parietal cell mass and hence Vg.

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I must also acknowledge the work done on gastric secretion by my predecessors. Without their hard efforts I would not have been able to answer the questions that stimulated this research. None of this would have been possible without the uncomplaining patients and volunteers who submitted to gastric secretion tests.

Finally I wish to thank Deborah for tolerating my many hours spent with my computer and providing encouragement when it was needed most.

DECLARATION

The suggestion for this work came from a discussion with Professor Michael Hobsley on the remarkable lack of agreement amongst workers who had studied the acute effects of smoking upon gastric secretion; this in the light of the agreement from three major studies into the chronic effects of smoking.

It soon became apparent that this apparently simple study would require a detailed analysis of the acute and chronic changes in non-smokers and smokers with and without duodenal ulcer disease; it is this work that forms the basis of this thesis.

The design and running of the acute studies was the sole work of this the author. The new theoretical approach to the raw data from basal, sub-maximal and maximal studies to provide dose-response data, and information about transpyloric fluid shifts was also the sole work of the author. This work provided the essential framework that enabled interpretation of the acute and chronic studies. In all the author performed some 60 out of the 347 studies used in this work. Although the author did not routinely perform the analysis of gastric juice he was fully conversant with all the experimental techniques used.

It is believed that this thesis makes three original contributions to the understanding of the effect of smoking upon gastric secretion. First, that the dose

response relationships of true gastric secretion in controls and patients with duodenal ulcers are identical; they simply lie upon different parts of the same spectrum of gastric activity. This spectrum appears to based upon gastric capacity, reflected by the size of the parietal cell mass. Secondly, that acute smoking has an effect which in the long term moves an individual along this spectrum from a position of relatively low activity to a higher one. This leads to a potential imbalance in the defensive and offensive factors in duodenal ulcer genesis and so may allow an ulcer to develop.

Thirdly,that there is a complex interrelation between pyloric loss and duodenogastric reflux; pyloric loss is determined to large extent by parietal cell mass whereas duodenogastric reflux is affected by both pyloric loss and the presence of the duodenal ulcer diathesis.

"a custom lothsome to the eye, hateful to the nose, harmful to the brain, dangerous to the lungs ... and by causing over quick digestion, fill the stomach full of crudites."

James I, 1604

Counterblaste to Tobacco

INTRODUCTION

SMOKING AND PEPTIC ULCER DISEASE

1/.Epidemiology

Peptic ulcer remains an important disease; in an endoscopic survey of 358 supposed healthy controls, peptic ulceration was found in 6 cases, a point prevalence of 1.68% (Ihamaki et al 1979). More importantly it has been estimated that about 10% of the population can be expected to develop a peptic ulcer during their lifetime (Kurata and Haile 1984). The majority of these people, about 85%, will develop duodenal ulceration (Ihamki et al 1979). Tobacco smoking although showing a decline in popularity, is still a popular habit; in 1986 over 78,000 tonnes of cigarettes were consumed in the United Kingdom alone (Tobacco Advisory Council - personal communication). It has for many years been the advice of doctors that patients with duodenal ulcer disease should give up smoking. This has been based on good epidemiological evidence showing an association between the two; the demonstration of a possible mechanism of cause and effect is though still required.

i). Incidence

The exposure to environmental ulcerogens is thought to be important in the development of an ulcer diathesis. Many such factors, suspected of

having a causative role in the pathophysiology have been studied; however for many the evidence has found to be lacking. There is however strong epidemiological evidence for a positive association between smoking and peptic ulcer disease.

An early study into the relationship between smoking and peptic ulcer disease was published by Barnett in 1927 (Barnett 1927). He found that the habit of cigarette smoking was more common in ulcer patients than controls. The difference was not thought significant and he concluded that there was no association between smoking and peptic ulcer disease. However the majority of subsequent studies have shown a significant relationship between smoking and peptic ulcer disease. In a review of six major studies into the prevalence of peptic ulcer disease in smokers and non-smokers it was found that the results were strikingly consistent; peptic ulcers were almost twice as common in smokers than non-smokers - mean prevalence 1.9 : 1 (Harrison et al 1979).

A recent epidemiological survey calculated the attributable risk of cigarette smoking and alcohol ingestion combined, to the development of a duodenal ulcer. Attributable risk is amount of disease in a population that can be accounted for by a specific risk factor, such as smoking. It was shown that in men 75% of duodenal ulcer disease could be abolished by removing the exposure to smoking or alcohol (alone or in combination).

This is of an order similar to that observed between cigarette smoking and the development of lung cancer (Piper et al 1984).

A prospective endoscopic survey of over 1200 outpatients found that the prevalence of peptic ulcers in smokers was 1.8 times that of non-smokers. There was a significant dose relationship between the number of cigarettes smoked and duodenal ulcer occurrence but this was not so in the case of gastric ulcer (Ainley et al 1986).

ii). Treatment

Smoking has a deleterious effect on both the healing and relapse rates of duodenal ulcer. One hundred and thirty five patients with proven duodenal ulcers were studied; healing was assessed endoscopically. Whilst on H_2 antagonists there was a significantly lower healing rate in smokers; 63 per cent as compared to 95 per cent in non-smokers (Korman et al 1983). It is interesting to note that there was a positive correlation between cigarette consumption and the failure to heal; by four weeks 89% of light smokers (< 9/day) but only 40% of heavy smokers (> 30/day) had healed. At the end of the 12 month study period the ulcer had recurred in 84 per cent of the smokers but in only 53 per cent of non smokers (Korman et al 1983).

iii). Secretion

A major factor in the pathogenesis of duodenal ulceration is the volume and acidity of gastric juice. The chronic effect of smoking on gastric acid secretion has been studied in an effort to elucidate the mechanism by which smoking promotes ulceration.

Following an initial report that maximal gastric secretion was increased in duodenal ulcer patients that smoked compared to non smokers (Whitfield and Hobsley 1979); three major studies have shown an increased capacity to secrete acid in smokers compared to non-smokers. The pentagastrin stimulated gastric acid secretion was measured in 136 patients with duodenal ulceration and in 90 controls; it was significantly raised in heavy smokers (Parente et al 1985). In a similar study the basal and peak acid outputs were measured in 201 healthy controls; both were significantly raised in male smokers, however only the peak acid output was elevated in female smokers (Massarrat et al 1986). The findings of both these studies were confirmed when maximal gastric secretion, corrected for pyloric loss and duodenogastric reflux, was measured in 201 patients with duodenal ulcers and 122 controls. Maximal gastric secretion was raised in smokers compared to non-smokers in all groups and in only one, male controls, was this not significant. This increase in gastric secretion showed a positive correlation to the total number of cigarettes smoked (Whitfield and Hobsley 1987).

Although all this evidence supports the theory that smoking is a causal agent in the pathogenesis of duodenal ulceration, the mechanism by which this occurs is still unclear. The assumption has been that chronic cigarette smoking over a period of years; leads to an increased maximal gastric secretory capacity; this in turn increases the incidence of duodenal ulceration. This must mean that the parietal cell mass of an individual has increased its ability to secrete acid; this can only occur in one of two ways. First the actual number of cells in the parietal cell mass could be increased. Secondly, the individual parietal cell could increase its maximal secretory capacity.

i). Increased parietal cell mass

This is the mechanism which many believe explains the different secretory capacity of duodenal ulcer patients and controls. Several studies support this claim.

Fourteen males with duodenal ulcers were compared with 11 age-sex matched controls; both groups were normo-secretors. Pentagastrin stimulation was used, and the dose producing half the observed maximal response (D_{50}) was calculated for both acid and pepsin secretion. The D_{50} was the same for the ulcer patients and the controls. The conclusion was that there was no increased

sensitivity of the parietal cells in the ulcer group (Aly and Emas 1982). This is in agreement with earlier work (Wormsley and Mahoney 1967).

The maximal acid output, in response to histamine (Card and Marks 1960) and pentagastrin (Cheng et al 1977), was correlated with the parietal cell count in post-gastrectomy specimens. There was a strong positive correlation between the two; and this with the above evidence supports the contention: that the increased secretory capacity in ulcer patients simply reflects an increase in the parietal cell population.

ii). Increased parietal cell sensitivity

An increase in the maximal secretory capacity may also be due in part to an increased sensitivity of the individual parietal cells. It may be that chronic smoking increases the parietal cell sensitivity at all levels of stimulation, not just at maximal stimulation. This would cause smokers to produce more acid than non-smokers in response to a wide range of "physiological" sub-maximal stimuli. This in turn would render them more liable to duodenal ulceration and explain the increased maximal secretory capacity. This is to some extent supported by experimental work. The change in gastric secretion, in response to graded doses of pentagastrin, was measured in both controls and duodenal ulcer subject s. It was claimed that the resulting dose-response curves showed that duodenal ulcer patients not only have

a greater acid secretory capacity, but are also more sensitive to pentagastrin (Isenberg et al 1975).

3/. Hypothesis

i). Gastric acid secretion

The crux of the matter is whether acute smoking has an acute effect on gastric secretion that can explain the findings of the chronic studies. The effect of acute smoking on gastric secretion has been studied in an attempt to shed light on this problem. The effect of acute smoking upon gastric secretion was first studied in the 1920's and the results of these early studies (Gray 1929, Schnedorf and Ivy 1939) are as much at variance with each other as are the present day studies (Fletcher et al 1985, Parente et al 1985). Indeed, depending on the study, smoking has been shown to decrease, increase or have no effect upon gastric secretion. The disagreements amongst early studies can be attributed to problems with methodology and a failure to understand the complexities of gastric secretion. However, even modern studies have failed to answer the question; "Does acute smoking affect gastric secretion?".

ii). The pylorus

It is thought that it is the delivery of un-buffered acid to the duodenum that is pathogenic; and not per se the increased intra-gastric acidity. It is a commonly

held view that the loss, through the pylorus, is abnormal in duodenal ulcer patients when compared to controls. Increased delivery of acid to the duodenum would lead to mucosal breakdown and so ulceration. This increased delivery could be due to an increase in the volume or in the acidity of the gastric juice. Acute cigarette smoking may increase gastric secretion and so the acid delivery to the duodenum. This could explain both the increased maximal gastric secretion and incidence of duodenal ulcers in chronic smokers. Smoking may of course only affect the pylorus, causing it to relax and so increase duodenal acidity without directly affecting acid secretion. Although pyloric loss and duodenogastric reflux have been studied in relation to smoking, the work has in the main, been qualitative. No major quantitative studies exist that study the acute and chronic effects of smoking on the movement of fluid across the pylorus.

It is therefore proposed to study the acute changes in the volume and acidity of gastric secretion, pyloric loss and duodenogastric reflux; in response to acute cigarette smoking. The dose-response curves and the chronic changes in all aspects gastric secretion; in both smokers and non-smokers with and without duodenal ulcers, will also be studied. Thus the acute changes can be analysed in the light of the chronic changes; and hopefully enable further light to be shed on the

mechanism by which gastric acid secretion is increased in smokers.

HISTORICAL REVIEW

THE NATURE OF GASTRIC JUICE

1/. The early years

The nature of gastric secretion has interested man since early times. Hippocrates thought digestion was essentially similar to the preparation of food for cooking (Walder 1962a); Galen stated that the stomach, intestines and liver were involved in a complex process to turn all food into blood (Walder 1962b). Van Helmont proposed "An acid ferment in the stomach responsible for digestion" but spiritual agencies known as "Archaevi" were invoked to back up the stomach's actions (Van Helmont 1648). This mechanism of digestion was still being supported by Stahl in 1737, almost 100 years later. There was some elaborate work done on the salivary glands by Wharton in 1656, following this Sylvius (1679) proposed saliva and pancreatic juice as the main digestive juices. In work that was published after his death in 1679, Borelli proposed that the stomach was a vascular mill (Borelli 1680). This debate prompted Hunter's pithy remarks on the stomach (quoted by Robertson 1931)

> "it is not either a mill, a fermenting vat nor a stewing pan; but a stomach gentleman, a stomach."

This view was not held by all; Reaumur, Spallanzani, and many others studied the stomach intensely.

i). Animal work

Viridet was one of the earliest workers in this field. His studies of gastric secretion involved the sacrificing of a variety of animals such as dogs, cats and pigs; these were sacrificed in either the fasting or post prandial state, and their stomach contents analyzed. It was Viridet who in 1692 showed the stomach contents to be acid; noting that the stomach contents turned "solutio heliotropii" red. He then postulated that the regurgitation of this acid fluid was the pathological basis of heartburn; an astute observation!

Reaumur in 1752 studied his pet kite, a member of the buzzard family, who like all birds of prey will vomit indigestible objects. He used metal tubes which were packed with sponge to absorb a large amount of gastric juice; this would then be vomited back up once the meal had been digested and so allow the study of digestion in living animals. He showed that stomach contents turned "blue paper" red thus indicating acidity. He then went on to perform the first in vitro work on digestion; but failed to complete this work as his "subject" died, presumably from exhaustion, soon afterwards.

Spallanzani in 1783 extended the animal work and in particular he studied the three degrees of fermentation: the vinous, the acid and the putrid. Since Spallanzani could detect no evidence of fermentation nor of putrefaction; but actually showed gastric juice to have antiseptic qualities, he favoured the acid principle. He

sent gastric juice from a crow to Scopoli who performed the first recorded analysis of gastric contents. He reported "the salt in this fluid is not common salt, but sal ammoniac...This salt is neither acid nor alkaline." (Robertson 1931).

ii). Human studies

Animal studies were closely followed by work on humans; these experiments were crude and sometimes dangerous, but from them has developed the exact modern science of gastric physiology.

Vomiting, either self induced or caused by an emetic was one of the earliest means of analysing human gastric juice. Reuss in 1760 neutralised his stomach with alkali, ate a test meal of meat and vegetables and then took an emetic. He noted that the gastric juice so produced was acidic (Macquart 1786). This was quite a common, albeit extremely hazardous, method of experimentation. Stevens presented his Inaugural Thesis to Edinburgh University in 1777; he was the first to apply Reaumur's work to humans and to perform in vitro digestion successfully. He studied a Hungarian circus performer who could regurgitate at will such objects as pebbles; in place of these he gave him specially constructed perforated silver balls to swallow. He then repeated all of Reaumur's work but judging by the technique used it is unlikely that he knew of Reaumur's work (Robertson 1931).

John Hunter as a result of his post mortem work on humans and fish suggested that digestion was due to "..something secreted by the coats of the stomach which is thrown into its cavity and there animalfies the food or assimilates it to the nature of blood.." (Hunter 1786). He also noted that there was acid in the stomach even though no vegetative matter had been ingested; perhaps the first report of basal acid secretion in humans. Although he made no suggestion about the mechanisms that might be involved he noted that the acid was increased by some diseases but decreased by others (Hunter 1786).

Beaumont in 1833 stated that the main problem was not the analysis of the juice but a failure to obtain it in sufficient quantities. It was for this reason that Alexis St Martin became the most famous gastric secretion subject; in 1822 he sustained injuries to his chest and stomach from a musket accidentally discharged at a distance of less than one yard. He recovered after a long illness but was left with a gastric fistula; and it was through this fistula, nearly three years after the accident that Beaumont was able to study gastric function. Beaumont inserted a tube through the fistula tract and noted; ".. On introducing the tube the fluid began to flow.. ". This was from a stomach free from food. Technical problems prevented the use of such pouches in animal studies until the operation was perfected in 1881 by Heidenhain (Heidenhain 1883).

A novel manner to examine human gastric contents was used by Enderlin in 1843 and Smith in 1875: the gastric contents of criminals were examined shortly after execution. It is interesting to note that one had a hearty last meal whereas the other only managed a glass of wine!

iii). The nature of the acid

By the end of the 18th century gastric juice was known to digest food, although its exact nature was a subject of much bitter controversy. The first attempt to determine the nature of the acid was made by Macquart in 1786; he found various salts and acids such as acetic, lactic and phosphoric acids in the gastric juice of calves and sheep. Young in 1803 also attempted to identify the acid. His experiments on frogs led him to conclude that "the acid....is to be referred to their gastric juice"; however, poor chemical analysis led him to conclude that the acid was phosphoric acid. It was over 30 years later that William Prout, in a communication to the Royal Society of Medicine proved that free hydrochloric acid was the only acid present (Prout 1824). It was not surprising that most scientists found it difficult to accept that the human stomach could secrete such a strong acid. There followed nearly 30 years of argument between the "Prout school" on one hand and those who favoured weaker acids, such as lactic and phosphoric acid, on the other. The debate was finally settled in

favour of hydrochloric acid by Bidder and Schmidt in 1852, two years after Prout's death. They confirmed Prout's work that the excess chloride was sufficient to account for the entire acidity of the gastric juice. This equation we now express as :

[Total Chloride] = [Neutral Chloride] + [Titratable Acid].

2/. The development of the gastric secretion test

Once the nature of the acid had been settled it then became a problem as to how best to measure gastric secretion quantitatively; this required a safe means of obtaining the juice as well as an accurate method of analysis.

i). The early naso-gastric tube

a). Design

It was Van Helmont in 1648 who first made a flexible catheter from leather soaked in resin, but it was Boerhaave in 1744 who first described the use of a stomach tube. He used the tube to give the antidote to children who had swallowed hemlock, and were unable to swallow because of the convulsions. The tube was put to similar use by John Heysham, a Carlisle physician, who in 1780 gave "cuprum ammoniacum" to an epileptic with hysterical dysphagia. In 1793 John Hunter used a stomach tube made from eel skin to feed a patient with a

temporary paralysis and an inability to swallow. In 1767 Alexander Monroe III, a teacher of William Prout, suggested attaching a pump to the end of a stomach tube to aspirate ingested poisons. It was left to Physick to put this into practice, when in 1812 he aspirated laudanum from twins who had accidentally been overdosed. In 1871 Leube first used the stomach tube to analyze gastric contents; it was therefore well over a hundred years after it had been invented that the tube was first used for diagnostic purposes. By 1909 Einhorn had devised a smaller tube capable of entering the duodenum and by 1931 Robertson regarded all variations by Ryle , Rehfuss and others as modifications of this tube.

Recently the gastric tube has been modified; a second lumen has been added to allow the infusion of various markers dyes which are needed to assess accurately the volume of gastric secretion. Apart from changes in the materials used, there have been no other major changes in tube design.

b). Positioning

Any positioning of the tube was initially more by luck than anything else. It is true that various involved methods using compass needles, oscillating coils and the like; were occasionally used to detect the metal tip of the tube, but they never came into general use. The development of fluoroscopy enabled the tube to be positioned in the most dependent part of the stomach with

accuracy, and so the results were thought to be more realistic.

This procedure was time consuming and involved radiation; and there was no evidence that any benefit was to be had by using it. It was for these reasons that the water recovery test was developed and validated by Hassan and Hobsley in 1970. In this they studied 42 subjects in whom the tube was positioned by the water recovery test. All were then checked fluoroscopically, in only 21 the tube was in the 'optimal' position (the tube pointing down into antrum of the stomach). There was no significant difference between those in the optimal position and the others when recovery fractions and aspirated volumes were examined. In other words, the water recovery test became the hallmark of satisfactory tube placement.

ii). The technique

The first studies involved a single aspiration to test the gastric acidity several hours after a test meal. The repeated sampling of gastric juice only became practical with the advent of the soft flexible gastric tube. This developed into the fractional test meal that was used by Ehrenreich (1912) on the Continent and Rehfuss (1914) in the USA. Prolonged intermittent suction with aspiration every 30 to 60 minutes was the norm; but Carlson in 1915 advocated regular suction every 5 - 10 minutes to increase the recovery of basal gastric

secretion. In 1923 it was shown that continuous mechanical suction was more efficient than hand aspiration (Lim et al 1923). These two modifications increased the recovery of gastric juice but aspiration was not complete; a major source of inaccuracy persisted.

A method to calculate the completeness of the aspiration was needed. In theory the problem is relatively simple; a known volume of juice is removed from an unknown volume in a given time. All that is required is the application of the dye dilution technique. A marker of known concentration and volume is instilled into the stomach, allowed to mix well and a sample aspirated after a known time. The concentration of the marker in the aspirated sample is found and hence the actual volume of gastric secretion calculated.

This was first investigated by Mathieu in 1896. The indicator phenolsulphonphthalein (phenol red PSP) was first used as a dilution indicator by Gorham (1923). Penner, Post and Hollander in 1940 showed that when gastric emptying was inhibited phenol red recovery approached 100%. Later work showed that there were losses of up to 17% after the initial instillation; however subsequent instillations showed recovery was close to 100% (Bloom et al 1967). This initial apparent loss is due to the sequestration of PSP in the folds of gastric mucosa. The technique was modified by Hobsley and Silen in 1969, the use of a continuous infusion of phenol red through a double lumen naso-gastric tube

overcame the problem of sequestration. Other markers have been studied, $^{51}CrCl_3$ and polyethylene glycol, but they have shown no advantage over phenol red (Ivey and Schedl 1970).

The volume by which aspiration is incomplete can be equated to pyloric loss. In a plateau of secretion it is improbable that the unaspirated volume would accumulate without affecting the plateau, hence it must be lost through the pylorus.

iii). The measurement of the acid

Once the nature of the acid had been determined and Leube had performed the first gastric secretion test, it became important to be able to measure the acid quantitatively. In 1886 0.1M NaOH and litmus paper was used (Jaworski and Gluzinski 1886); this was soon superseded by the use of indicators such as phenolphthalein and congo red by Ewald in 1892 and Toepfer developed his reagent in 1894. However by 1912 Christiansen was using the pH scale and titrating to an end point; there then followed over the next 20 years discussion as to the end point. In 1931 Hollander settled on pH 7 as the end point. The next change in this field was the development of the electrometric titration method; and it is this that is in use in various forms today.

iv). The stimulation of gastric juice

Bernard and Barreswill in 1844, and Bidder and Schmidt in 1852 were amongst the first to use gastric stimulants; such as pepper or a test meal of pebbles! The early workers such as Von Leube used test meals, of which there were many sorts, to stimulate the stomach. Solid, semi solid and liquid meals ranging from standardised breakfasts to large volumes of 10% alcohol were all used at one time or another. However, they were criticised on the grounds that there was either contamination or dilution of the aspirated gastric juice.

Basal secretion was first suggested as an index by Beaumont (1883) and then later by Pavlov in 1902. In 1912 Carlson produced the first data on basal inter-digestive secretion; and this was confirmed by later work (Carlson 1923). The study of basal secretion was hampered by the low volumes obtained, and so the study of nocturnal basal secretion with its higher volumes became popular (Lim et al 1923). In 1951 it was shown that the acid output during the morning 1 hour aspiration was almost identical to the hourly rate obtained during the rather laborious 12 hour nocturnal test (Levin et al 1951).

The use of non-oral stimulants was first advocated by Ehrman in 1912 who used pilocarpine, but it had too many side effects. In 1920 Popielski first used histamine in dogs and Carnot first used it in humans in 1922 (Carnot et al 1922); however the production of quite

severe side effects limited its use and prevented maximal stimulation. The first use of an anti histamine with a single injection of histamine was in 1949 (Conrad et al 1949). Kay (1953) developed the augmented histamine test in which he showed that there was a maximal secretory response to increasing doses of histamine. In 1954 the first detailed dose-response curves for histamine in humans were produced, from this the theoretical maximal dose as well as the K_m were calculated (Adam et al 1954). In 1964 Lawrie, Smith and Forrest developed the histamine infusion test. In this test a continuous infusion of histamine combined with an antihistamine produced a constant blood level without serious systemic effects. This allowed more reliable plateaus of gastric secretion at all levels of stimulation to be produced. Thus maximal gastric secretory capacity could be calculated accurately for the first time.

3/.The pylorus

The gastroduodenal junction is marked by the confluence of the gastric muscle layers into a prominent, thickened ring called the pylorus. There are some features which distinguish this pyloric muscle from that of the stomach and duodenum (Schulze-Delrieu and Shirazi 1983).

Functional studies, both radiographic and electrical have shown the pylorus to be a high pressure zone (Fisher and Cohen 1973). Furthermore, experimental work has shown that this high pressure zone responds to physiological stimuli. Using a three channel manometer the basal pyloric pressure was measured in 28 fasting subjects; a mean value of 5mmHg was obtained. Normal saline or 0.1M hydrochloric acid (HCl) was instilled into the duodenum, each labelled with phenol red to enable measurement of duodenogastric reflux. Normal saline produced no change in pyloric pressure but 34.2% (+/- 9.3%) of the saline refluxed into the stomach. The instillation of 0.1 HCl caused the pyloric pressure to rise to 17.2 mm Hg (-/+ 1.4 mm Hg) with a marked fall in duodenogastric reflux to 1.8% (+/- 0.9%). Hence the pylorus can be said to have the physiological and to an extent the anatomical characteristics of a gastrointestinal tract sphincter (Fisher and Cohen 1973).

However the view that the pylorus is merely a propulsive unit is one that is commonly held. The distal antrum, pylorus and proximal duodenum can function as a single entity, expelling gastric contents into the duodenum (Heading 1984). Liquid gastric contents are therefore "lost" from the stomach. Flow can be in the opposite direction, resulting in a gain of duodenal contents within the stomach.

4/.Duodenogastric reflux

In 1833 Beaumont was probably the first man to document duodenogastric reflux:

" irritation of the pyloric extremity of the stomach with the end of the tube or the bulb of the thermometer, generally occasions the flow of bile into the this organ."

Nowadays the passing into the stomach of duodenal fluid, containing intestinal, biliary and pancreatic secretions, is known as duodenogastric reflux.

i). Measurement of duodenogastric reflux

Duodenogastric reflux is often seen during routine upper gastrointestinal endoscopy, however the subjective assessment of refluxed bile correlates poorly with measured bile acid concentration (Domellof et al 1980). One of the earliest attempts to measure duodenogastric reflux was by Capper, Arith and Kilby in 1966. Using a mercury weighted tube barium was introduced into the second part of the duodenum; the use of cine-radiography enabled the peristaltic waves to be timed and the extent of reflux to be assessed. Reflux was graded according to how far up the stomach the barium spread. This was of course subjective and the effect of the transpyloric tube on reflux was an unknown quantity.

Non absorbable markers, such as polyethylene glycol (PEG) and phenol red, were used to quantify the reflux

but duodenal intubation was still required. The measurement of the refluxate as part of a gastric secretion test seemed to be the ideal solution. Bile acids, bilirubin and lysolecithin were all measured; these assays are all to some extent affected by the gastric pH, interpretation of the results is thus not as straight forward as it might seem.

The use of a "physiological marker" rather than a presumed toxic component of the refluxate was first proposed by Hobsley, Gardham and Hassan in 1968. The differential sodium concentration between duodenal and gastric juice is the basis of this test. Since sodium is effectively absent from pure gastric juice but is present in high quantities in duodenal juice it acts as a marker of the refluxate.

a). The "Sodium marker"

This requires further explanation. It is known that the concentration of sodium in aspirated gastric juice falls as the secretion rate (Makhlouf et al 1966) and the hydrogen ion concentration rises (Hobsley and Silen 1970). The chloride ion concentration is always greater than the sum of the hydrogen and sodium ion concentrations. The difference of 11-27 mmol/l being made up by a fairly stable potassium ion concentration. Several theories have been developed to account for these phenomena; all of which rely on an assumption first made by Pavlov in 1910. He stated that parietal cells only

secrete hydrogen ions at a constant concentration, namely 170 mmol/l.

Teorell's back diffusion theory was proposed in 1947. He argued that after secretion into the lumen hydrogen ions moved down the concentration gradient back into the cells, exchanging on a one to one basis with sodium ions. Thus sodium diffused into the gastric lumen as hydrogen moved back into the mucosa. However more recent work has shown that back-diffusion is small in undamaged mucosa (Davenport et al 1964).

Hollander (1932) proposed a two component model, acid and alkali. In this the acid is of constant concentration but of variable volume. The alkali component is of constant volume and with a composition approximately that of interstitial fluid.

The theoretical basis of this was explored by Hobsley in 1974 who showed that the variations in gastric acidity and tonicity were better explained by duodeno-gastric reflux than alternative theories such as the "increased alkaline component" (Hirschowitz 1961). This was confirmed experimentally (Fiddian-Green et al 1979) using intravenous indocyanine green to label the bile. A maximal plateau of gastric secretion was induced and secretin given to induce duodenogastric reflux. The amount of duodenogastric reflux was calculated from the sodium concentrations (Hobsley 1974). The concentration of indocyanine green was measured. There was a good correlation between the concentration of indocyanine

green in the gastric aspirate and the calculated volume of duodenogastric reflux. Though an important part of gastric juice analysis, it is important to note that gastric intubation is still required.

Other exogenous markers such as C^{14} chenoxydecholic acid, that are excreted by the biliary system have also been used. They do provide a quantitative measurement, but only of biliary reflux.

b). Radionuclide measurement

A variation of this technique is to use Tc99m labelled iminodiacetic acid (IDA) to radio-label the bile, external scintillation scanning is then used to assess duodeno-gastric reflux. Bile reflux has been extensively studied using this method by Thomas in England and Muller-Lissner in Germany. Quantification is possible by analysis of counts over regions of interest such as the liver, duodenum and stomach. However accurate assessment of low volume intermittent reflux is not possible (Thomas 1984).

Measurements of duodenogastric reflux that are obtained from intubation techniques are criticised on the grounds that the effect of the naso-gastric tube is an unknown quantity. The evidence for this is largely anecdotal and stems from the observations of Beaumont in 1833. Recent work has shown that this criticism is invalid. Gastric emptying and duodenogastric reflux in healthy volunteers was found to be unaffected by gastric

and trans-pylori intubation) (Muller-Lissner et al 1982). In disease, gastric intubation did not affect the measurement of duodenogastric reflux in patients with peptic ulcers (Wolverson et al 1984).

ii). The pathophysiology of duodenogastric reflux

Duodenogastric reflux is physiological, occurring in all healthy controls studied (Muller-Lissner et al 1983), but the amount of refluxate is minimal (Johnson and Eyre-Brook 1984). However it is considered to be pathological in patients with duodenal ulceration (Thomas et al 1984). It is proposed that increased duodenogastric reflux leads to antral gastritis; and suppression of antral somatostatin. This increases gastrin release which in turn increases parietal cell sensitivity. The net effect is a hypersecretory state, and hence increased delivery of acid to the duodenum. This is challenged by work (Wolverson et al 1984, Muller-Lissner et al 1983) that has shown that the amount of duodenogastric reflux is no more excessive in peptic ulcer patients when compared to controls.

ADVERSE EFFECTS OF SMOKING

1/. Introduction

Christopher Columbus's crew were most probably the first Europeans to witness tobacco smoking. On November 2nd 1492 they landed on an island, now known as Cuba, and saw the natives smoking rolled up tobacco leaves. It was introduced to Europe in the early part of the 16th century by travellers from the New World. It was used mainly as a medicinal herb until Sir Walter Raleigh introduced social smoking to England in 1586.

2/. The Stomach

i). The early years

James I of England was one of the earliest and most vehement anti-smokers and in 1604 he published his famous "Counterblaste to Tobacco". In this he makes what is probably the earliest statement on the deleterious effects of smoking:

> "a custom lothsome to the eye, hateful to the nose, harmful to the brain, dangerous to the lungs....and by causing over quick digestion, fill the stomach full of crudites."

A highly subjective statement to say the least! The use of tobacco flourished and it continued to be regarded as the "Herba panacea". It was used to treat a wide range of ills from strangulated hernia to strychnine poisoning. One of the earliest medical tobaccophobes was

John Lizas, Professor of Anatomy at Edinburgh University. In a book published in 1854, he claimed tobacco caused many ills including vomiting, dyspepsia and diarrhoea. Samuel Solly in 1857 wrote to the Lancet in reply to "The great tobacco question - is smoking injurious to your health?". In this he states that immoderate use of tobacco leads to severe dyspepsia. He mentions the experience of Napoleon I, who after smoking a cigarette was quoted as saying "Oh , the swine! my stomach". J.Pidduck in the same debate stated that smoking tobacco caused gastric irritation. The medical profession was however not agreed on this matter; they were almost equally divided into those for and against smoking, as was shown by the correspondence in the Lancet.

ii). The 20th century

Danielopolu in 1925 noted that during bismuth meals smoking produced an initial increase in gastric contractility but this was followed by a gastric paralysis for at least an hour. In the same year Lickint stated that nicotine decreased the secretion of pepsin and renin. Smoking was also noted to cause pyloric spasm and so produce hyperchlorhydria; this was said to make the diagnosis of peptic ulcer more difficult. Toxic dyspepsias were in vogue; tobacco dyspepsia was accepted as a clinical entity and was mentioned in Rehfuss's monograph on the subject " Diseases of the stomach "

published in 1927. It was noted that in tobacco dyspepsia there was exaggerated vagal tone and pyloric spasm caused by sympathetic inhibition; thus imitating a duodenal ulcer. Tobacco was said to be pressor in nature, unlike all other toxic dyspepsias which were depressor in nature (Ryle 1926). In 1926 Sir Humphrey Rolleston, the eminent physician, summed up the debate on smoking and its effects on the stomach:

> "To smoke or not to smoke, that is the question, Whether a mild cigar assists the digestion, Or whether it begets a kind of quaintness."

iii). Smoking and gastric secretion

a) Early work

In 1929 Gray studied 100 patients with organic upper gastrointestinal disease; of these 63 were shown to have duodenal ulcers. In 50 of 63 (80%) duodenal ulcer patients smoking was found to cause an increased gastric secretion. Smoking before breakfast produced an increase in the volume of the fasting stomach contents, of 10 to 20 ml. There was an increase in gastric acidity, as shown by their gastric acid curves, in 25% of these patients. In all but two of the remainder there was no change in gastric acidity curves and in those two the acidity was distinctly lower.

Gray then further studied 50 of these patients, all of whom were between 25 and 45 years old, had symptoms

for at least two years and had smoked for five or more years. In these patients two fractional analyses were done prior to the smoking study; of these 70% showed the usual acid curves for duodenal ulcers as described by Rehfuss (1914). Study of the fasting stomach showed that in 60% of the group there was a distinct increase in gastric secretion with high acid figures. Smoking prior to the test meal again increased the fasting contents and also produced a high Rehfuss curve for the first hour. Gray concluded that in a third of patients with peptic ulcer disease, smoking caused hyperacidity and an increase in gastric secretion.

It is of interest to note that no mention was made of the remainder of the group, as to whether the secretion was unaffected or perhaps even depressed by smoking. This is possibly the first objective analysis of smoking and gastric secretion.

In 1934 Trowell looked at the relationship between the habit of tobacco smoking and chronic duodenal ulcer. Of the 249 hospital control patients who smoked cigarettes 32% inhaled, this habit was more than twice as common (70%) in duodenal ulcer patients with similar smoking habits. To explain these observations he postulated that duodenal ulcer patients received a greater dose of carbon monoxide and nicotine and that this might be important in ulcer development.

The experimental evidence led Crohn in 1938 to state "That tobacco, particularly in the form of cigarettes,

increases gastric secretion and produces acid hyper-secretion".

McCormick in 1938 quoted Friedrich who postulated a mechanism of ulcer formation. He asserted that the nicotine caused vasospasm of the gastric mucosa which was visible on light microscopy; and this combined with the smoking-induced gastric hyperacidity predisposed to ulcer formation.

Schnedorf and Ivy (1939) criticised most workers in this field for making statements on the effects of smoking based on little more than clinical impression. Gray was, they stated, the first serious author on smoking and gastric secretion but they criticised him for giving neither figures on acidity nor experimental details. They first studied fasting secretion in smokers, non smokers and patients with duodenal ulcer. Basal secretion was collected by continuous aspiration for nearly two hours; following an initial 30 minute control period the patient smoked 4 to 7 cigarettes for the remainder of the test. Each test was repeated 2 or 3 times. Of the forty normal subjects seventeen showed no significant change, but in 22 there was a significant decrease in gastric secretion . In the patients with duodenal ulcer there were similar results: there was no change in 8 of the 20 patients and in 11 there was a significant decrease. The effect of smoking upon gastric secretion stimulated by a test meal of bouillon and crackers was examined. In cases were smoking appeared to

have an effect it caused a decrease in intra-gastric acidity. A series of controls showed the swallowing of saliva to have little effect. It is to be noted that non smokers were made to smoke, it was claimed that this had no effect.

In 1941 Ehrenfeld and Sturtevant, in an experimental review, noted that the general feeling of many authors was that smoking caused an increase in gastric secretion; nicotine was felt by some to be the causative agent. Hurst and Stewart (1929) made much of the theory that in those patients with duodenal ulcers the parasympathetic system was in the ascendancy. These duodenal ulcer patients were said to be more sensitive to the effects of nicotine. Ehrenfeld and Sturtevant pointed out that the conclusions of Schnedorf and Ivy were at variance with this general feeling; but emphasised that their conclusions were based experimental evidence.

In an attempt to replicate these results, Ehrenfeld and Sturtevant studied 33 controls and 23 peptic ulcer patients using fractional analysis following an alcohol test meal. The patients only smoked 2 cigarettes and of the controls 76% showed an increase in gastric acidity compared to 87% of the ulcer patients. An attempt was made to study the role of nicotine by using denicotinised cigarettes. There were marked differences between ordinary and de-nicotinised cigarettes: the latter produced a lesser rise in gastric acidity, the authors were not though convinced that nicotine was the causative

agent. The results of these authors were at variance with those of Schnedorf and Ivy, whose high experimental cigarette dose was offered as a an explanation. It must be said that smoking four or more cigarettes during 90 minutes is 'abnormal' for many smokers.

Nine years later continuous gastric secretion was believed to be more accurate than duplicate fractional analysis (Hodges and Gilmour 1950). Using this technique the effect of smoking two cigarettes in 22 subjects was studied. Hodges and Gilmour argued that the smoking dose was similar to Ehrenfield and Sturtevant but the technique was similar to that used by Schnedorf and Ivy. Of the 22 subjects studied there was no change in 50%, a rise in gastric acidity in only 32% and the remaining 18% showed a decrease. They agreed with Schnedorf and Ivy that smoking within tolerance limits has little effect.

Stiegmann, Dolehide and Kaminski (1954) noted the controversial data and felt the role of nicotine could be best examined by comparing standard and filter cigarettes. A group of 98 patients of both sexes and including non-smokers(44 hospital controls and 54 peptic ulcer patients) were studied under basal conditions. Two 10 minute basal studies were made followed by 2 cigarettes and a further one hours collection. Of the controls 50% showed a rise in gastric acidity but there was a rise in 90% of the ulcer patients. In those who smoked a filtered cigarette only 25% and 60% respectively of the patients showed a rise in gastric acidity.

Moreover the greatest actual rise in acidity occurred after smoking a standard cigarette; there was a tendency for this to occur in the ulcer group. They subjected their results to a statistical analysis and concluded that smoking produced a significant rise in gastric acidity.

Cooper and Knight (1956) studied 120 duodenal ulcer patients who were cigarette smokers. They were divided into two groups: 'smokers' (smoking during the test) and 'controls' (non-smoking). Under conditions of basal stimulation, it was shown that smoking continuously for half an hour had no significant effect on gastric secretion.

By 1959 workers were applying considerable thought to experimental design. The change in basal secretion over the period of the test was studied to reduce the errors involved in experiments upon smoking and basal secretion (Piper and Raine 1959). They stated that smoking 4-6 cigarettes in one hour significantly increased gastric secretion in terms of volume , free and total acid and chloride. All patients acted as their own controls on a separate occasion. They criticised many previous studies on the grounds of failure to allow for natural variation and an inadequate period of study. They stated that studies of smoking and test meal stimulated gastric secretion were of little use unless gastric emptying was allowed for. They still were unable

to explain the differences between Steigman and colleagues (1954) and Schnedorf and Ivy (1939).

b). Modern studies

The development of the histamine-infusion test (Lawrie et al 1964) and the use of pentagastrin infusions (Wormsley et al 1966) were important. They enabled the dose response curves for various groups of patients to be calculated. A dose could then be calculated that would provide steady sub-maximal stimulation of the parietal cell mass.

Using this plateau of gastric secretion, the possible inhibitory or stimulatory effects of cigarette smoking can be studied more accurately. This was first done by in 1971 using pentagastrin (Debas et al 1971); they studied 12 healthy volunteer smokers and non smokers. Individual dose-response curves were plotted, and the doses required for a 50% plateau of stimulation calculated. All subjects, both smokers and non-smokers, smoked three cigarettes of the same brand over one hour. The test was repeated five times on each volunteer and there was no significant increase in gastric secretion in response to cigarette smoking. However they noted that the weight of evidence suggested that peptic ulcer patients do experience a rise in gastric secretion in response to cigarette smoking. They proposed nicotine as the causative agent, and suggested that its actions on the dominant parasympathetic system of a duodenal ulcer

patient could explain the increase in gastric secretion.

Using a similar technique Wilkinson and Johnson (1971) studied both normal volunteers and duodenal ulcer patients, the effect of intravenous nicotine was also examined. Smoking caused a significant decrease in acid output and concentration. Nicotine produced a fall in acid secretion in 3 of the 4 patients studied, the dose used was however different in all cases.

Both these groups of workers studied the change pepsin secretion; although Wilkinson and Johnston (1971) demonstrated a decrease as opposed to the increase shown by Debas and his colleagues (Debas et al 1971); in neither group was this significant.

Once again the difference in results was explained by a different, in this case higher, cigarette dose (Wilkinson and Johnston 1971). In reply to this the validity of performing only one test on each subject was questioned, and it was doubted if the smoking dose was indeed different (Debas and Cohen 1972).

A study of 16 smokers and 16 non-smokers claimed to show smoking had no effect on gastric secretion (Fung and Tye 1973). They studied the effect of acute cigarette smoking on the level of basal secretion; both groups were made to smoke. In both groups there was no significant difference either in the pre- or post-smoking levels of basal secretion of the two groups or in the maximal secretory capacity. This study had no true controls and

with earlier studies, the validity of the results obtained by making non-smokers smoke can be questioned.

In 1974, the 1959 work of Piper and Raines was repeated (Whitecross et al 1974) and it produced very different conclusions. They studied both volunteers and ulcer patients who smoked; it was the same experimental model used 15 years previously but the composition of the cigarettes had changed. This time smoking had no effect on gastric secretion; but bile reflux, subjectively assessed, did seem to be increased.

In a review of the subject in 1978 Wormsley came to the conclusion that:

> "although we may dislike patients smoking we have no compelling reason for stopping them".

In 13 healthy male volunteers pentagastrin was used to produce maximal stimulation; the effect of intravenous nicotine or cigarettes smoking upon the volume and acidity of gastric juice was measured (Sonnenberg and Husmert 1982). Intravenous nicotine and smoking had a similar effect: the volume and acidity of the gastric secretions fell. However, the juice was aspirated by hand and no corrections for pyloric loss or duodenogastric reflux were made; the accuracy of this study is therefore questionable.

Fletcher, Shulkes and Hardy in 1985 studied gastric secretion and mucosal blood flow. Using the technique of McCloy (1978) and with corrections for pyloric loss (Hobsley and Silen 1969) and duodenogastric reflux

(Whitfield and Hobsley 1979); they demonstrated a significant decrease in the gastric acid output of six volunteers after smoking cigarettes for one hour. It is uncertain from their work how the acid output was corrected nor were any values for pyloric loss or duodenogastric reflux given. They postulated that nicotine acting at the ganglia may produce stimulation of post ganglionic sympathetic nerves to the stomach or stimulate the release of catecholamines, both of which inhibit gastric secretion. Gastric mucosal blood flow was also reduced but there was no significant correlation between this and the fall in gastric secretion.

iv). Chronic studies

By 1985, although the acute effects of cigarette smoking on gastric secretion had been extensively studied there were few reports on the chronic effects. Those reports that did appear were preliminary abstracts (Whitfield and Hobsley 1979, Massarat et al 1982).

However within one year three separate groups of workers reported on 750 patients. In the first of these (Parente et al 1985) 136 patients with duodenal ulcers were compared against 90 controls; in heavy smokers there was a significant increase in the pentagastrin stimulated acid secretion and the fasting serum pepsinogen 1. This was true for both the controls and subjects with duodenal ulcers. They argued, in view of their own and other workers studies on the acute effect of smoking on

gastric secretion, that what acute response there might be, is negated by the chronic effects. They also suggested that the chronic effect shown may be mediated through indirect pathways, such as the vagus , producing either an increase in the secretory cell mass or an enhancement of its secretory ability.

Massarat and colleagues (1986) studied 201 patients with proven normal upper gastro-intestinal tracts in both smokers and non-smokers. They studied the basal (BAO) and the peak (PAO) acid outputs in all subjects, as well as the pepsin output in 85 patients. They found that in male smokers the BAO and the PAO were significantly greater than in the non smokers; this was also true for the PAO in female smokers. When further analysed, the product of daily cigarette consumption and smoking years was the index most closely correlated to the increase in gastric secretion.

This was also found to be so in patients with duodenal ulcers (Whitfield and Hobsley 1985). Work by Whitfield and Hobsley (1987) on 122 control subjects and 201 patients with duodenal ulcers confirmed this finding of increased maximal acid secretion in smokers. Using multiple regression analysis of stature, age and smoking habit they showed that these factors alone did not totally account for the increased secretion in duodenal ulcer patients when compared to controls.

3/. The pylorus and duodenogastric reflux

Cigarette smoking is known to be an important factor in the pathogenesis of peptic ulceration. Duodenogastric reflux is increased in patients with peptic ulceration and this has led to the hypothesis that duodenogastric reflux may be increased in chronic smokers.

One of the earliest means of studying this was by observing the reflux of a radio-opaque substance, instilled directly into the duodenum, back into the stomach (Capper et al 1966). Using a modification of this technique, cigarette smoking was studied in 13 controls and 9 dyspeptic patients. However in only three of these was the cause of the dyspepsia known: a duodenal ulcer, a gastric ulcer and antral gastritis. Smoking increased reflux in 70% (9/13) of controls and 77% (7/9) of dyspeptic patients (Read and Grech 1973). Smoking between 3 to 5 cigarettes over two hours produced an increased in duodeno-gastric reflux in 6 of 10 of dyspeptic patients with proven gastric ulcers (Dippy et al 1973). Of the remaining four, reflux was decreased in two and un-changed in the others. The assessment was highly subjective - a visual grading of the biliary contamination in gastric aspirate.

These findings have been supported by more quantitative studies. A recent study examined both

the acute and chronic effects of smoking on bile reflux in the fasting and post prandial states (Muller-Lissner 1986). The author claimed that bile reflux was greater in chronic smokers compared with non-smokers. Cigarette smoking produced an acute rise in bile salt reflux which was more marked in the fasting state.

An alternative approach has been to examine the pylorus as a regulator of duodenogastric reflux and pyloric loss. The pyloric pressure was measured in a small group, 3 controls, 3 patients with duodenal ulcer and 1 with a gastric ulcer. The basal pressure was 10.2 mmHg and this fell to 7.9 mmHg after smoking one cigarette in less than four minutes (Valinzuela et al 1976). A study into the effect of smoking upon the gastric emptying rate of a test meal supported this work (Grimes and Goddard 1978). They found that the rate of emptying of the liquid phase was significantly faster after smoking one cigarette; and suggested that this might be due to relaxation of the pylorus. However this work was not supported by recent studies from Australia, in which bile reflux was measured in 13 healthy male volunteers using quantitative assessments of various bile acids. The extent of reflux, expressed as bile acid concentration, during basal secretion was measured before, during and after smoking one cigarette. Smoking

caused no significant increase in the amount of refluxed bile acids (Yeomans et al 1981).

EXPERIMENTAL METHOD

OUTLINE

The various aspects of gastric function to which we have alluded can all be studied by simple variations of the same basic technique. The gastric studies can conveniently divided into two; those looking at the chronic changes consequent on cigarette smoking and those concerned with acute changes during smoking. The chronic studies can be divided into those involving the parietal cell mass and those providing information about the motor functions.

Studies into the effect of chronic smoking on the parietal cell sensitivity are based on dose : response work. The responses in terms of gastric secretion (volume and acidity) to four levels of stimulation were measured. Maximal and sub-maximal (1/4 and 1/8) doses of histamine, as well as no stimulation (basal secretion) were used. Smokers and non-smokers, with and without duodenal ulcers were studied. The motor functions are those involved in the transpyloric movement of fluid, namely pyloric loss and duodenogastric reflux. The inter-relation of these two is easily studied, both being measured routinely during a gastric secretion test. It is to be remembered that we are measuring the effect rather than the actual changes in motor function brought on by smoking.

The acute effects of smoking one cigarette are best measured against the background of a plateau of sub-

maximal secretion. For this a dose that stimulated 50% of the parietal cell mass was used. This gives the maximal opportunity to evaluate any changes, be it positive or negative, in the various parameters of gastric function to be studied. Smokers with and without duodenal ulcers were studied.

EXPERIMENTAL DETAIL

1/. Subject selection

All patients were collected from either the Gastro-Intestinal Endoscopy Clinic at the Middlesex Hospital or directly from surgical outpatients. In all patients the diagnosis of duodenal ulcer was confirmed by either endoscopy or barium studies. The presence of an active ulcer was considered the minimum criterion on barium meals. Severe erosive duodenitis seen on endoscopy was felt to be part of the ulcer diathesis and was considered the minimum criterion for inclusion. Concurrent gastritis or a gastric ulcer were grounds for exclusion. Any patient with a previous history of gastric surgery that would affect gastric secretory capacity was excluded. The most common cause for exclusion was vagotomy with or without a drainage procedure. Simple oversewing of a perforated duodenal ulcer, with no other procedure, did not exclude the patient. Controls came from two main groups. The first was comprised of

individuals with no history of upper gastro-intestinal tract disease who volunteered to undergo a gastric secretion test; these were in the main student volunteers who received financial remuneration for doing so. The second consisted of patients with upper abdominal symptoms who had no demonstrable abnormality on endoscopic or radiological examination; who agreed to undergo a gastric secretion test. A chronic smoker was defined as one who had smoked on a daily basis for more than one year. The definition of a non-smoker was one who had never smoked tobacco.

2/. Equipment

i). The naso-gastric tube

During this study two types of nasogastric tube were used, both variations of the same basic design. This was of a double lumen naso-gastric tube, the second narrower lumen was for instillation of phenol red and the larger for the aspiration of gastric contents. The early tube was hand made in the laboratory using a standard 14 G naso-gastric tube and a length of fine polyvinyl tubing (Portex Ltd). Using an introducer the fine tubing was passed through the side wall of the naso-gastric tube and down the lumen. At the lower end it was brought out as a loop using a similar technique. The exact details can be better appreciated by studying the design photographs (Fig 4.1, 4.2). The side wall of the loop

was sliced off to provide an exit port of considerably greater area than that afforded by the cross section of the fine tubing. The end of the tube was crimped and all the introducer sites sealed with a plastic spray (Nobecutane, Astra).

This tube had two main disadvantages. First the lower loop gave the naso-gastric tube an uneven contour, which at times made its passage through the naso-pharynx uncomfortable for the patient. Secondly the passage of the smaller tube through the wall of the tube caused narrowing. This on occasions caused gastric residue to collect and impair the aspiration of gastric juice.

The second tube was specially made (Portex Ltd). Using modern plastic extrusion techniques it is possible to make a standard naso-gastric tube with a second lumen in the wall of the tube. The side arm is connected to the lumen by plastic welding techniques. Thus removing the problem of narrowing the upper part of the main aspiration channel. Removing the wall of the naso-gastric tube overlying the second lumen creates an exit port. This tube has a smooth contour and its passage is more easily tolerated by the patient. Both tubes were weighted and marked at 10cm. intervals from the tip. The aspiration channel on both designs had a standard 6mm sleeve connector. A Luer lock connector was attached to the infusion arm (Fig 4.1, 4.2).

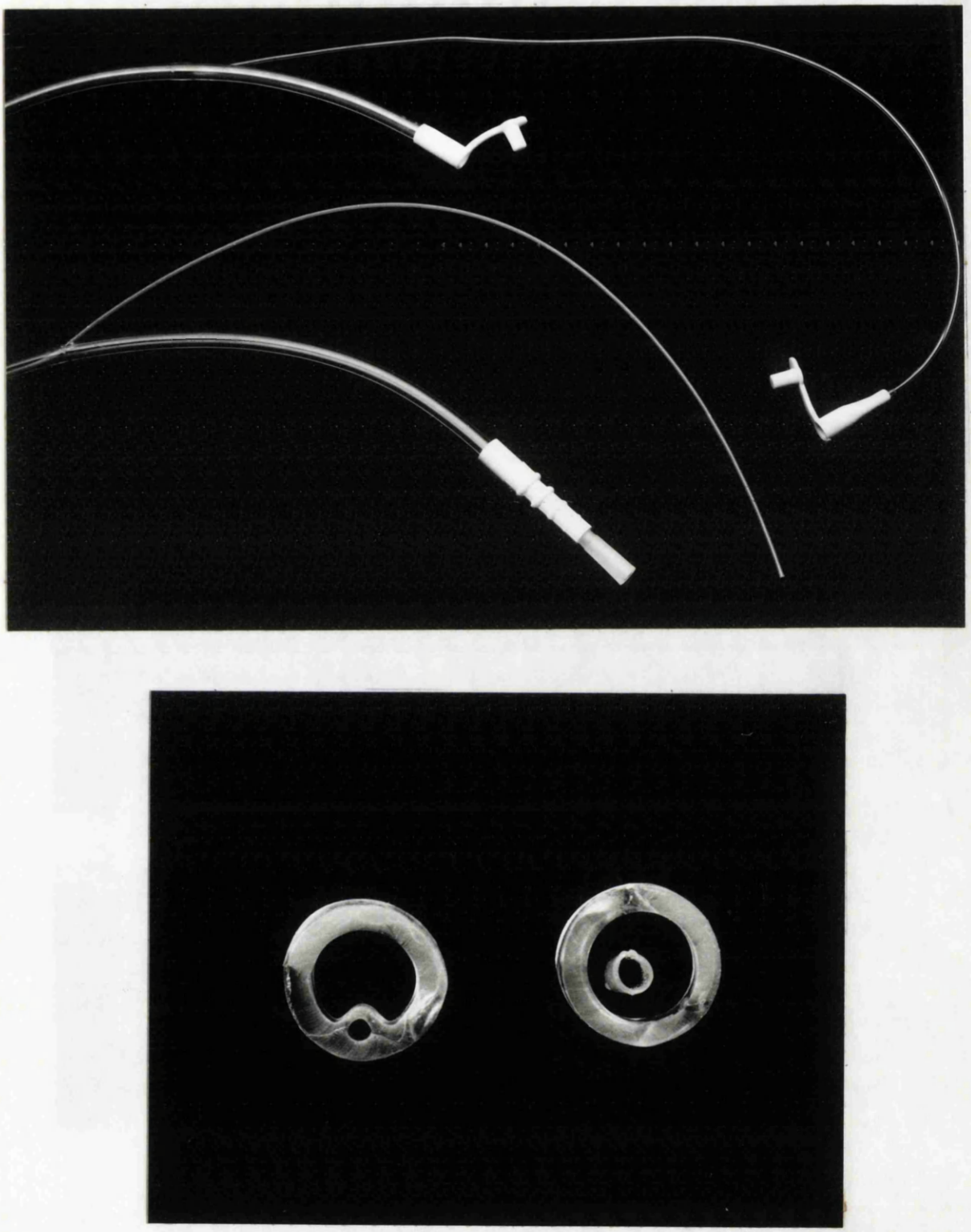


Fig 4.1

The naso-gastric tube

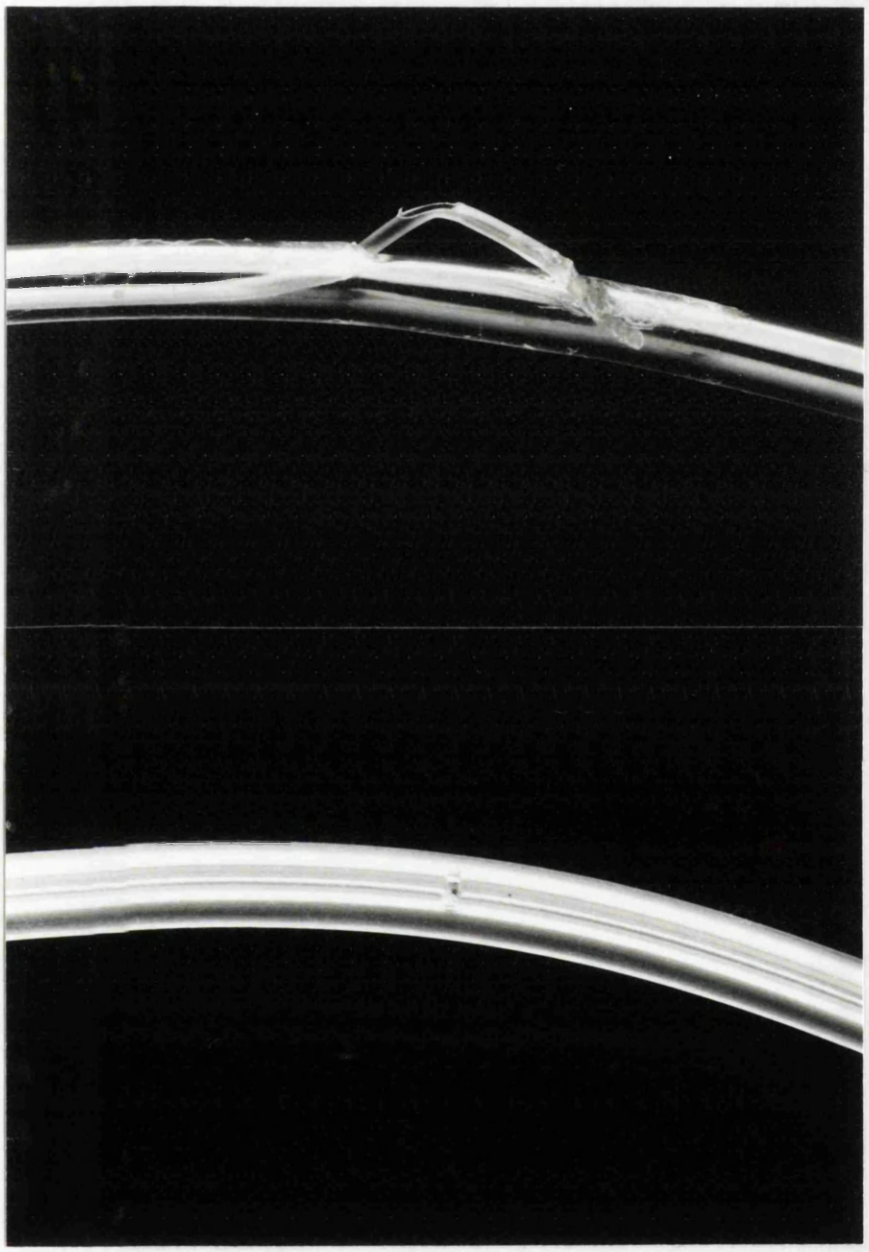


Fig 4.2

The naso-gastric tube

ii). Suction pump

A technique of continuous mechanical suction with intermittent blowback was used. The pump used was designed specifically for gastric secretion tests (Sycopel Scientific Ltd) and generated suction pressures of up to 100 mmHg. Above this pressure a safety valve causes the machine to cut out. The pump clears the tube of any blockages by blowing back at regular intervals (3 min) for fixed period of time (30 sec); this blowback pressure rarely exceeded 160 mmHg. The naso-gastric tube was connected via a sleeve connector to a wide bore tube that emptied into the collecting flask. The inlet was at the neck of the flask, the outlet was at the base on the opposite side. A side port was connected by fine tubing to the mercury manometers, allowing continual pressure readings. The outlet tubing was connected to soft rubber tubing which ran through the roller pump mechanism and so to the final collecting flask.

iii). Infusion pumps

These were used for the infusion of phenol red, histamine phosphate and of promethazine (Phenergan, May & Baker Ltd.). Three pumps of exactly the same design (Model 352, Sage Instruments) were used throughout the test; they could be set for both varying syringe size and infusion rate. A standard 60cc syringe (BD Plastipak) with an infusion rate of 10.2ml/hr was used in all the

syringe pumps. The calibration of the pumps was checked at regular intervals.

iv). Timer

The gastric juice was collected in 10 minute aliquots. To ensure that the investigator changed over between samples at the same time and received advanced warning that a collection period was ending, a specially constructed timer was used. This was an electrical device connected to a pair of coloured lamps and a buzzer; which were activated in a standard sequence.

v). Phenol red (Phenosulphathlein)

The phenol red was made up as a stock solution in the following manner. Six grammes was dissolved in a litre of distilled water and allowed to settle for one month. This allowed the optical properties of the phenol red to stabilise (Hobsley and Silen 1969). At the start of each test 60 ml was drawn up into the syringe through a CVP manometer line (Portex) secured to the syringe by a Luer lock. The whole assembly was freed of air bubbles and fitted into the syringe pump. The pump was run at maximum speed until the syringe driver was flush against the syringe driver. The pump was switched down to its normal rate and allowed to run for several minutes. A ten minute aliquot was collected into a standard 10 ml collecting flask. The flask was then capped and labelled (Pre-test standard) and the infusion pump stopped. The

equipment was now ready for the start of the test; the next stage was the preparation of the subject.

3/. Patient preparation

Prior to the test the subject must have taken nothing by mouth for at least eight hours, stopped smoking for at least 12 hours and taken no H_2 antagonists for at least 48 hours. The subject was collected from the ward at 8.00am., once in the gastric secretion laboratory the patient's weight and height were measured. A full gastric history was taken and all the information entered onto a standard collection form. The details of the test were once again explained and everything was done to ensure that the subject was as relaxed as possible.

i). Passage of the naso-gastric tube

The patient sat in an upright position and local anaesthetic gel (Lignocaine 1%) was squeezed into the nostril; the subject sniffed the gel up into his nose. This was repeated until enough gel had been instilled to provide adequate analgesia and lubrication; five minutes was usually sufficient for this agent to take effect. During this period the subject swallowed a few small mouthfuls of water, to moistens the somewhat dry throat and help swallowing when the tube was passed. The head was tilted back and the naso-gastric tube passed through

the nostril to the back of the throat. Once in this position the head was brought forwards and a small mouthful of water taken: too much water could lead to choking when the tube was passed. The tube was passed further in, the patient swallowed and the tube passed into the upper oesophagus; several swallows were sometimes required. Coughing suggested laryngeal irritation: tipping the head further forwards overcame this problem by directing the tube into the pharynx. Once the tube was in the oesophagus the subject swallowed several mouthfuls of water to ease the passage of the tube into the stomach. Aspiration of gastric juice rather than air confirmed that the tube was in the stomach and not the right main bronchus. The tube was then passed in up to its furthest mark: if resistance was met this implied that the tube had been passed in too far and was coiling up upon itself. A problem with short subjects, it can be overcome by externally assessing the length of tube required before it is passed. Once the tube was so positioned the stomach was emptied of the overnight secretions and the swallowed water.

ii). The water recovery test

This simple technique ensured that the tip of the naso-gastric tube lay in a satisfactory position, and obviated the need for fluoroscopic screening. The subject swallowed 20 ml of tap water and aspiration being immediately attempted. If between 16 and 20 ml was

obtained then the tube was said to have passed the water recovery test. The tube was then withdrawn 2.5cm and the test repeated. The tube was fixed, at the nose, at the shortest possible position at which it passed the water recovery test.

iii). Position of the subject

Once the tube was secured the patient lay in the semi recumbent position, that is with the legs flat and the hips flexed to 45°. There is no difference in recoveries between this position and the left-lateral (Hassan and Hobsley 1970); indeed subjects tended to adopt this position for short periods of time during the test for reasons of comfort alone.

iv). Intravenous access

In all tests a large bore cannula was used. Although not necessary for the histamine/promethazine hydrochloride infusions, it was considered good practice in case emergency venous access was required. A 16G cannula (Wallace) was inserted under local anaesthesia into a large forearm vein. This was connected by a three way tap to a slow-running infusion of 0.9% saline. The cannula was secured in the standard manner.

4/. Preparation of the intravenous infusions

In essence the patient received an infused dose of histamine concurrent with an anti-histamine, promethazine hydrochloride (Phenergan). The doses used were based on earlier work (Lawrie et al 1964) on the infusion test. The dose for maximal stimulation is 0.04 mg/kg/hr, for 1/4 and 1/8 stimulation the doses are 0.01 mg/kg/hr and 0.005 mg/kg/hr respectively. The usual period of histamine infusion was at least one and a half hours so a two hour infusion was prepared. The dose of histamine was calculated (0.04 x Wt(kg) x 2)mg and drawn up using an insulin syringe (U100). It was then transferred to a 60cc syringe and made up to 22ml with 0.9% saline. A four hour infusion would have required double the dose made up to 44ml. The dose of anti-histamine used was standard in all tests, 25 mg of promethazine hydrochloride (Phenergan) made up to the same volume as the histamine infusion (22 ml). The two syringes were placed in the double syringe pump and secured in position. A separate CVP manometer line was attached to each syringe and an 25G needle fixed to the other end. The syringes were primed as above (page 55). The standard infusion rate was now set and the needles inserted into the intravenous line through the rubber bung. The procedure was exactly the same for the smaller doses of histamine except that the dose of histamine was proportionately less. In the rare case of severe side

effects developing due to the histamine, the appropriate syringe could be stopped and the anti-histamine allowed to continue.

5/. The gastric secretion test

i). Outline

The broad pattern of the gastric tests is of a basal period, a sub-maximal period and a period of maximal stimulation. The details of the tests are as follows:

a) Standard

Basal	1 hr
Submaximal (1/4 or 1/8)	1 1/2 hr
Maximal	1 - 1 1/2 hr

b) Smoking

Basal	1/2 hr
Sub-maximal (1/8)	3 hr
1 cigarette smoked half way through this period	
Maximal	1 hr

The smoking study differed from the standard test in several ways. The main purpose of the smoking study was to examine the effect of smoking on a sustained sub-maximal plateau of gastric secretion. A full one hour basal period was not required and this was reduced to half an hour. The maximal period of one and a half hours was occasionally shortened to one hour without affecting the estimation of the maximal gastric secretory capacity.

In such cases the study was now only four and a half hours in duration compared to a possible five and a half hours.

ii). Collecting the sample

All gastric secretion collected up to time zero was discarded and this flask was used for all collections throughout the duration of the test. From time zero to about nine minutes the juice was collected in the flask. It was then decanted into a standard 100cc measuring cylinder and the juice collected up to exactly 10 minutes, as signalled by the timer. The outflow tube of the pump is then replaced into the collecting flask and to avoid loss of secretions during the transfer the tube is clamped by finger pressure. The volume of secretion was measured; if less than 10 ml it was returned to the flask to be pooled with the subsequent aliquot. A sample must contain 10 ml or more, otherwise analysis is not possible. The volume of the aliquot sample was noted and it was then filtered (Whatman's No 1 paper) into a numbered screw-top specimen jar. Since the total volume had been noted, only a filtered specimen was needed for analysis; and as long as this was more than 10 ml then the absolute volume was unimportant. At the end of the test all the bottles were sealed and stored at 4°C to await analysis.

iii). Conclusion of the test

At the end of the period of maximal stimulation all infusion and suction pumps were stopped, the naso-gastric tube and the intravenous lines were removed. The phenol red pump was restarted and a 10 minute sample collected, in exactly the same manner as at the start of the test (Post-test standard). The patient returned to the ward and was allowed to leave the hospital after at least four hours bed rest and a meal. All subjects were told to refrain from driving or operating machinery for 24 hours; this allowed time for the anti-histamine to wear off.

ANALYSIS

All samples were analysed within a week of collection, and usually within 48 hours. The samples were removed from the refrigerator and allowed to warm up to room temperature. Each sample was analysed for the following: phenol red; and chloride, hydrogen, potassium and sodium in ionic form. The hydrogen ion concentration (titratable acidity) was used calculate the output of acid and sodium ion concentration was used to calculate the amount of duodenogastric reflux. Chloride and potassium were measured as a check on analytical precision: if the anion/cation difference was greater than 4 mmol then the sample was re-analysed. These raw data were then fed into a BBC microcomputer and sent to a main frame computer at Imperial College. A printout was

then received giving values of maximal acid output (MAO), Vg, pyloric loss and duodenogastric reflux.

1/. Sample analysis

i). PSP concentration

A sample of the filtered aspirate was drawn up into a 1 in 200 diluter. The diluent contained ammonium hydroxide to render the sample alkaline and develop the colour. It was then passed into a flow cell within the spectrophotometer (Corning Spectrophotometer Model 256), and readings at 558 nm and 410 nm taken. The pre- and post-test samples were carefully washed out of their bottles into 10 ml flasks. They were measured as above and the means of the readings at each wavelength calculated. These were used later in the calculations.

ii). Chloride ion

This was measured electrochemically using a silver electrode chloride meter (EEl Chloride Meter Model 96, Radiometer Copenhagen), and a direct reading obtained in mmol/l.

iii). Hydrogen ion

One millilitre of aspirate was titrated against 0.1N NaOH to pH 7 using a pH meter and autotitrator (pH Meter 26, Autoburette ABU 12 Radiometer Copenhagen). The results were in millilitres of 0.1N NaOH and

multiplication gave the hydrogen ion concentration in mmol/l.

iv). Sodium and Potassium ions

These were measured in a flame photometer (FLM3 Radiometer Copenhagen) and the results given mmol/l. Since the same diluent (ammonium hydroxide) was used for the PSP readings it was possible to semi-automate the readings of phenol red, sodium ions and potassium ions.

2/. Computer analysis

The data were analysed using a specially written Fortran program run on the Imperial College main frame computer. It produces a hard copy via the laboratory terminal and adds the results to the master file stored at Imperial.

i). Calculation of pyloric loss

Blood and bile are potential contaminants of the aspirated gastric juice; they have a small absorbence at 558 nm and a peak at 410 nm. A correction for this absorbence has been calculated, although it is never more than 7% (Crawford and Hobsley 1968):

$$PSP_{corr} = PSP_{558} -(0.135 \times PSP_{410}) + 0.004$$

Using this value the aspirated volumes were corrected for pyloric loss. The phenol red standard (PSP stand) was adjusted to allow for the 1 in 200 dilution

that the aspirated sample underwent (PSP adj). Using simple ratios the total volume of gastric contents was calculated:

$$V_{tot} = PSP_{adj} / PSP_{asp} \times V_{asp}$$

Allowance must be made for the actual volume of PSP infused: in 10 minutes this is 1.7 ml. Subtracted from V.tot this gave V.cor, the volume of gastric juice corrected for incomplete aspiration and pyloric loss. The aspirated volume was similarly treated to produce V.obs, the volume of aspirated gastric juice corrected for the volume of infused marker. For reasons that have already been mentioned V.cor - V.obs can be equated to the volume of pyloric loss.

Finally the PSP standards from all of one sample batch were regressed against time; from this a corrected standard for each test was re-calculated and used to produce the final result. This produced a much greater uniformity of results.

ii). Correction of electrolyte concentrations

The infused volume of marker has the effect of reducing the electrolyte concentrations by dilution. Correction for this can be made by the fraction V.tot/V.cor, which at low volumes of secretion (< 20 ml /10 min) can be appreciable.

iii). Calculation of acid output

The corrected hydrogen ion concentration and V.obs were used to calculate the acid output as follows:

Acid output = H^{+}_{corr} x V.obs x 6 x 10^{-3}

The result is in mmol/hr.

iv). Calculation of duodenogastric reflux

This relies, as has been stated before, on the disparity between the sodium concentrations in gastric and duodenal juice. This analysis requires that the effect of swallowed saliva is assumed to be negligible. The concentration of sodium in duodenal juice is assumed to be constant at 0.143 mmol/ml. "Pure gastric juice" (Vg) is composed as follows Cl^{-} 0.170, H^{+} 0.145, Na^{+} 0.7 and K^{+} 0.17 (mmol/ml), this is based on work done by Hobsley and Whitfield (1977). The difference in electrolyte concentrations can be said to be due to reflux of duodenal contents. The volume of reflux Vr is calculated as follows:

a) Vcor = Vr + Vg

In terms of sodium output, this equation can be expressed as:

b) Vcor x $[Na^{+}]$cor = Vg($[Na^{+}]$Vg) + Vr($[Na^{+}]$Vr)

The $[Na^{+}]$cor is the concentration of sodium in the aspirated juice, and the $[Na^{+}]$Vg is that of pure gastric juice, and $[Na^{+}]$Vr is assumed to be 0.143 mmol/ml. Rearranging the equations a & b we get:

Vcor x $[Na^{+}]$cor = 0.007(Vcor - Vr) + 0.143(Vr)

which will give

$$Vr = (Vcor \times (([Na^+]cor - 0.007)/0.143)$$

This can be subtracted from Vcor (corrected for pyloric loss) to give Vg, this is the volume of gastric juice secreted from the gastric mucosa before pyloric loss and duodenogastric reflux have occurred.

v). Plateau selection

This was done by the computer, using a strict set of rules, to produce the longest possible plateau using the latest samples in the period. No plateau could start from the first period nor could it last for less than 20 minutes. The PSP recovery of each sample had to be within 15% of the mean of the proposed plateau. From the plateau the mean values of acid output, volume of gastric secretion, pyloric loss and duodenogastric reflux were calculated for each period.

3/. Statistical analysis

The raw data were collected and entered onto a standard spreadsheet (Lotus 123 Release 2.0) and stored on an IBM AT with a 30 megabyte hard disk. This provided an easily understood data format, allowed continual updating of the data and enabled the data to be analysed on a wide variety of software. The Lotus files were sent in an ASCII format to the main frame computer at Imperial College via an interfacing package "Procomm". This

enabled the main bulk of the statistical analysis to be done using the "Minitab" statistical package (Ryan et al), however less bulky files were analysed on the IBM AT using commercial software (Oxstat).

Non-parametric analysis was performed on all the data and so all populations are described by medians and 5% - 95% ranges. Although many of the populations were large and normally distributed some of the groups studied were quite small and so all were compared by the Mann-Whitney test. In several cases parametric analysis was used for paired data in large groups and the appropriate test used is indicated in the text.

Comparison of ratios, such as male/female and smoking/non-smoking, between populations were compared using the methods described by Bradford-Hill (1961).

Linear regression analysis of single and multiple variables was used to explore the relationship between various factors measured during the gastric secretion test. The regression analysis expressed the relationship between a dependent and independent variable in form $y = mx + c$. The relationship between these two variables in two populations, such as controls and those with duodenal ulcer, was studied using standard techniques to compare the slopes of the equations obtained by linear regression analysis.

The varying sizes of the populations studied means that care must be taken in the analysis of such results and this is dealt with in the relevant sections.

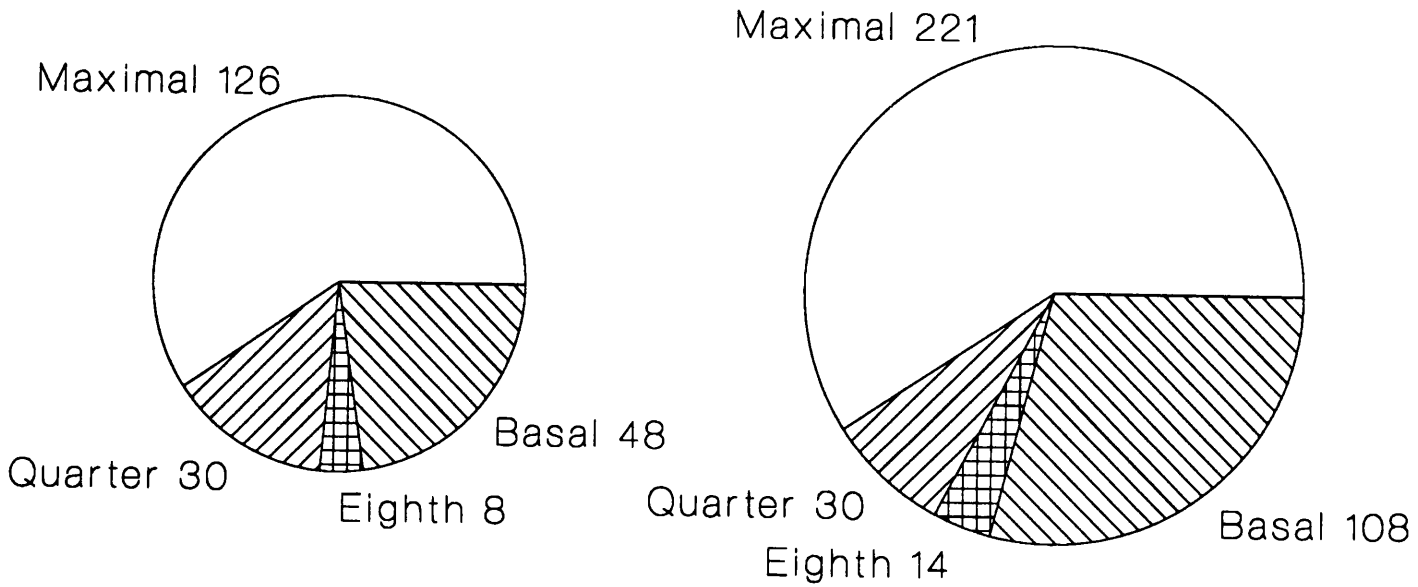
SUBJECT COMPARABILITY

1/. Study numbers (Fig 5.1)

In both groups, ie. duodenal ulcer patients and controls, taken together, there are four sections based upon the periods of stimulation used namely: basal, 1/8th maximal, 1/4 maximal and maximal. Since it was the maximal secretory capacity that was of most interest for both diagnostic and research reasons, it was these sub-groups that were the largest amongst both the controls and the duodenal ulcer patients. Secretion under basal conditions was always collected but only in the numbers indicated in the figure was the period long enough for an accurate assessment of basal secretion to be made. The numbers studied at the two sub-maximal doses were specifically for research and formed the smallest sections. The two groups and the four sections defined 8 sub-groups.

2/. Sex Ratios (Fig 5.2a, 5.2b)

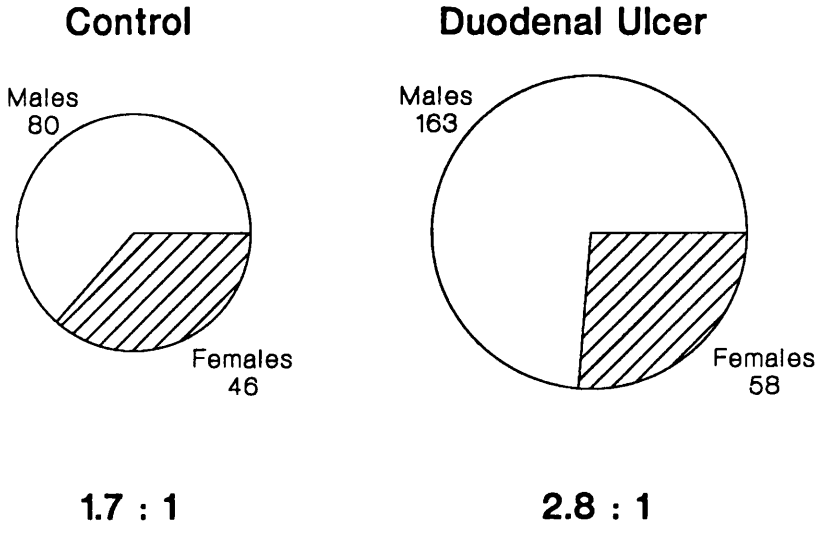
In all but one of the 8 sub-groups there were more males than females (min 1.3 : 1, max 4 : 1). The exception was the sub-group of control subjects studied at $1/8^{th}$ maximal stimulation: all but two of the eight volunteers were females.



Control **Duodenal Ulcer**

Fig 5.1
Subjects studied

Maximal Dose



Quarter Dose

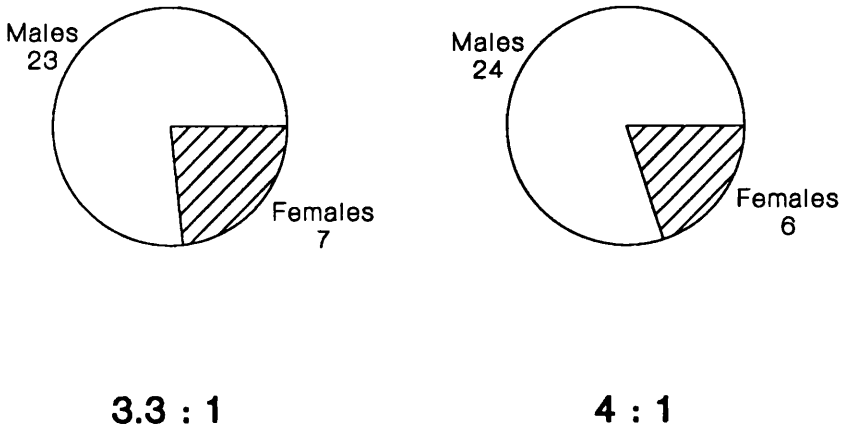
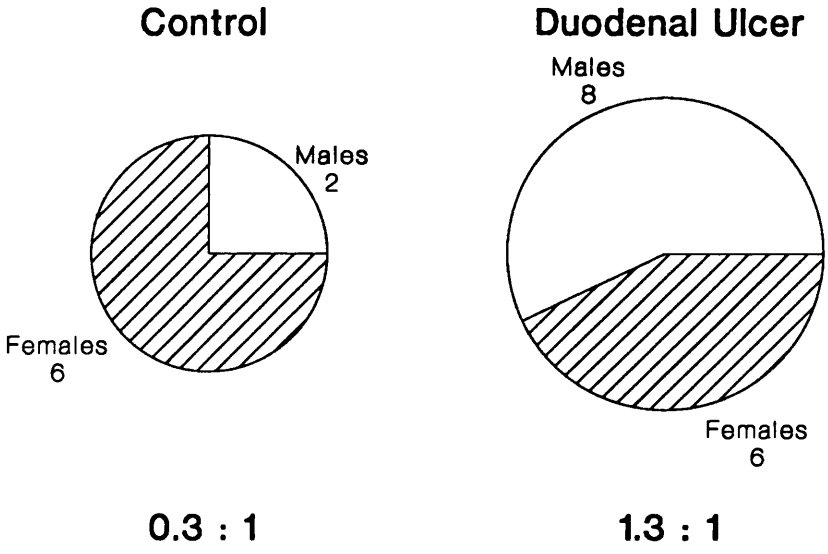


Fig 5.2a
Sex Ratios

Eighth Dose



Basal Stimulation

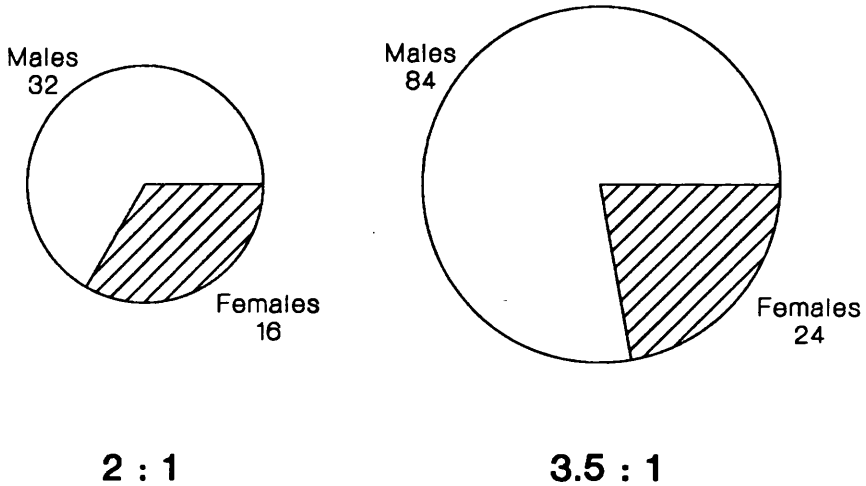


Fig 5.2b
Sex Ratios

In each of the four stimulation sections the male : female ratio was lower in the controls than in the corresponding duodenal ulcer groups (Fig 5.2a, 5.2b). Statistical analysis of these ratios revealed that in only the maximal section of secretion was this difference significant ($p<0.05$).

3/. Age of Subjects (Fig 5.3, Table 5.1)

The median ages of 7 of the sub-groups ranged from 37.5 years to 51.5 years, and none of these sub-groups differed significantly in age from the others. The eighth was the sub-group of controls who received $1/8^{th}$ maximal stimulation: their median age of 23.5 years was significantly lower than the medians of the other groups ($p < 0.05$).

4/. Weight (Fig 5.4, Table 5.2)

None of the sub-groups differed significantly in weight from the other sub-groups. The median weights of the eight sub-groups ranged from 63.0 kg. to 71.5 kg.

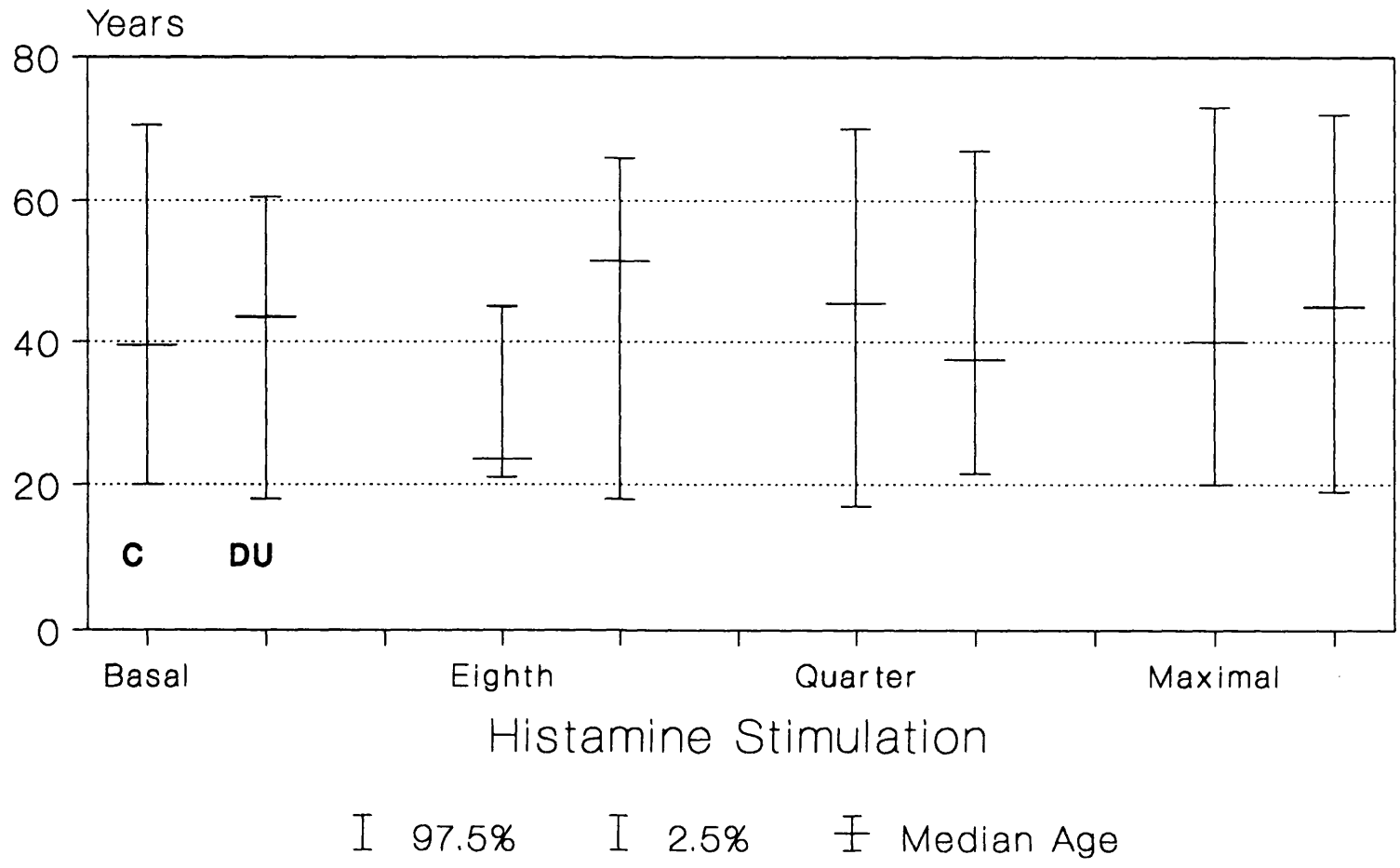


Fig 5.3
Age - all subjects

Age of Subjects

	C		DU	
Stimulation	range	median	p median	range
Maximal	(20.0 → 73.0)	**40.0**	ns **45.0**	(19.0 → 72.0)
	p	ns	ns	
Quarter	(17.0 → 70.0)	**45.5**	ns **37.5**	(21.5 → 67.0)
	p	0.05	ns	
Eighth	(21.0 → 45.0)	**23.5**	0.01 **51.5**	(18.0 → 66.0)
	p	0.02	ns	
Basal	(20.0 → 60.5)	**39.5**	ns **43.5**	(18.0 → 70.5)

Table 5.1

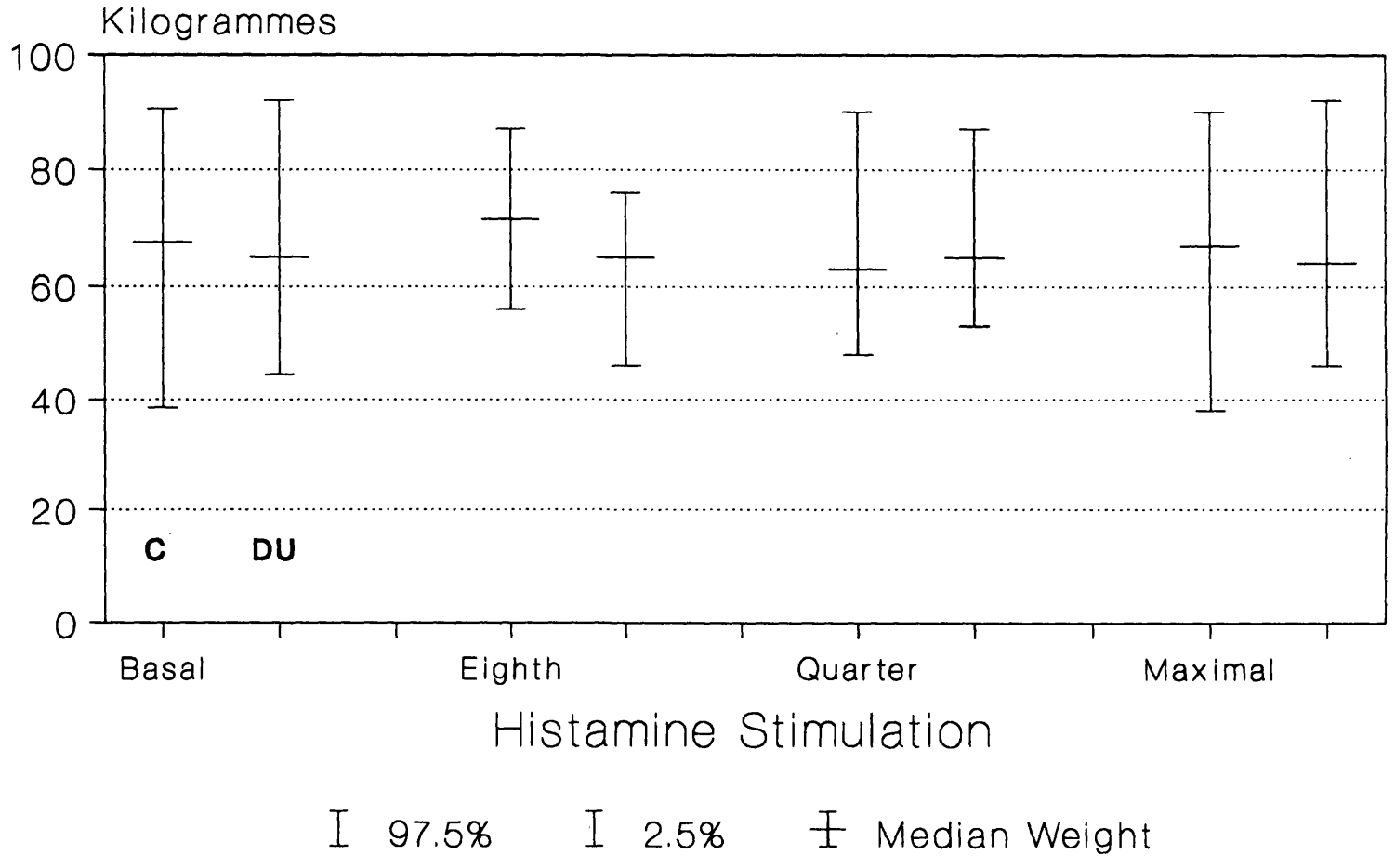


Fig 5.4
Weight - all subjects

Weight of Subjects

	C		DU	
Stimulation	range	median	p median	range
Maximal	(38.0 → 90.0)	**67.0**	ns **64.0**	(46.0 → 92.0)
	p	ns	ns	
Quarter	(48.0 → 90.0)	**63.0**	ns **65.0**	(53.0 → 87.0)
	p	ns	ns	
Eighth	(56.0 → 87.0)	**71.5**	ns **65.0**	(46.0 → 66.0)
	p	ns	ns	
Basal	(38.5 → 90.5)	**67.5**	ns **65.0**	(44.5 → 92.0)

Table 5.2

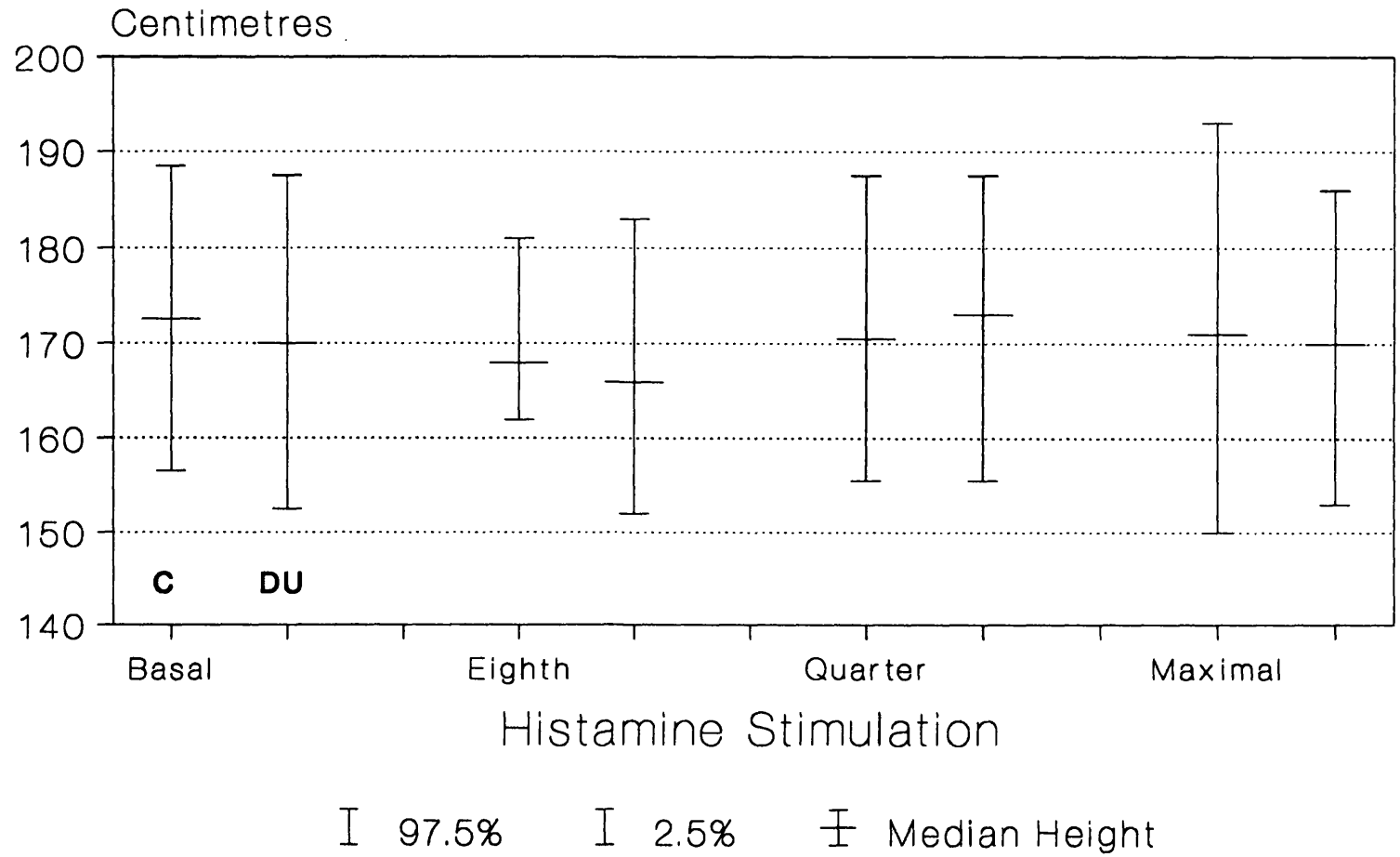


Fig 5.5
Height - all subjects

Height of Subjects

	C		DU	
Stimulation	range	median	median	range
		p		
Maximal	(150.0 → 193.0)	**171.0**	ns **170.0**	(153.0 → 186.0)
	p	ns	ns	
Quarter	(155.0 → 187.0)	**170.5**	ns **173.0**	(155.5 → 187.5)
	p	ns	ns	
Eighth	(162.0 → 181.0)	**168.0**	ns **166.0**	(152.0 → 183.0)
	p	ns	ns	
Basal	(156.5 → 188.5)	**172.5**	ns **171.0**	(152.5 → 187.5)

Table 5.3

5/. Height (Fig 5.5, Table 5.3)

The median heights for the eight sub-groups ranged from 166.0 cm. to 173.0 cm and again no sub-group differed significantly from the others.

6/. Smoking habits

i). Incidence of smoking (Fig 5.6a, 5.6b)

In the duodenal ulcer group smokers out-numbered non-smokers in three of the sub-groups by approximately 5 : 1; in the sub-group undergoing $1/8^{th}$ maximal stimulation the ratio was lower at 2.5 : 1. In the control group the smoking habit was less common with ratios in three of the four sub-groups of 1.5:1 or less; this was significant at $p < 0.005$. This was not so in the fourth (control $1/8^{th}$ maximal) sub-group who for reasons of selection were all smokers.

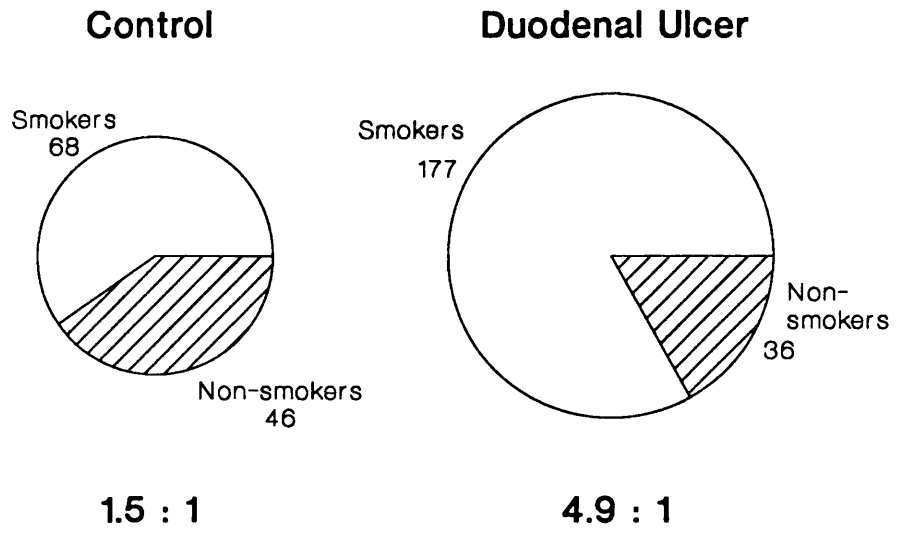
ii). Smoking factor (Fig 5.7, Table 5.4)

The median smoking factor ((cigs/d x smoking years) was similar in all the stimulation sections except those who received $1/8^{th}$ maximal stimulation. In this section the smoking factor was significantly higher in the duodenal ulcer patients receiving $1/8^{th}$ maximal than in both the duodenal ulcer patients who received 1/4 maximal stimulation ($p < 0.01$) and the

controls who received 1/8th maximal stimulation ($p < 0.05$).

Examination of the ages of these two sub-groups shows that the duodenal ulcer sub-group in the 1/8th maximal section were the oldest and the corresponding controls the youngest (Table 5.1). In all eight sub-groups the daily cigarette consumption was very similar (Table 5.5). It is thus the length of the smoking history that accounts for the difference in smoking factor rather than the daily cigarette consumption.

Maximal Dose



Quarter Dose

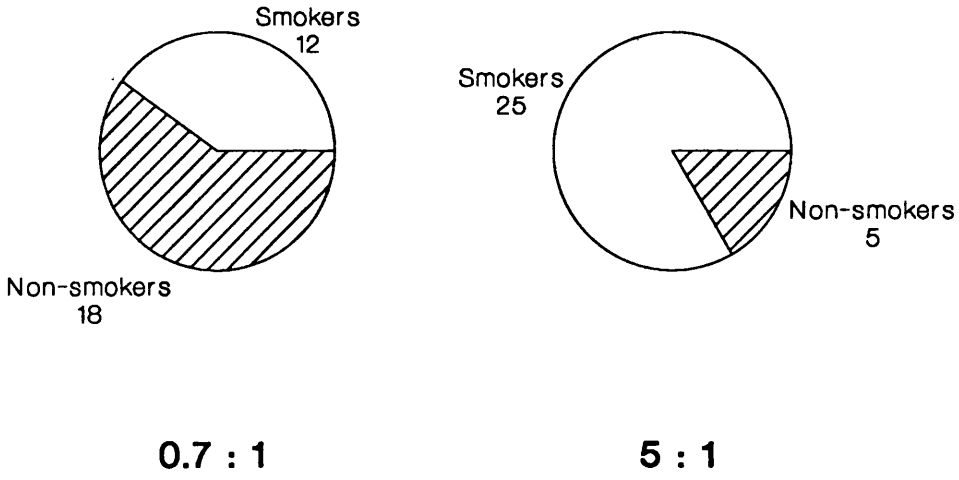
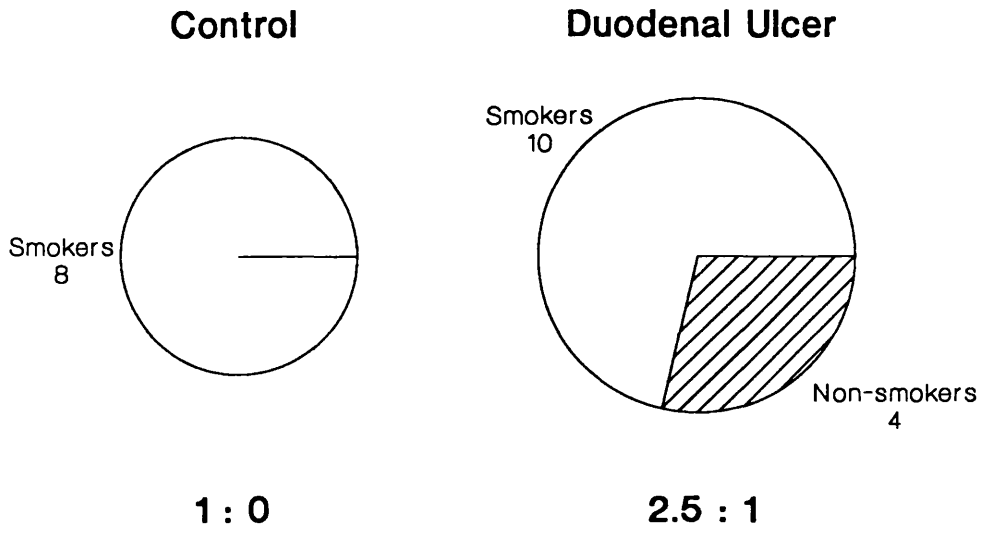


Fig 5.6a
Smoking Ratios

Eighth Dose



Basal Stimulation

Fig 5.6b
Smoking Ratios

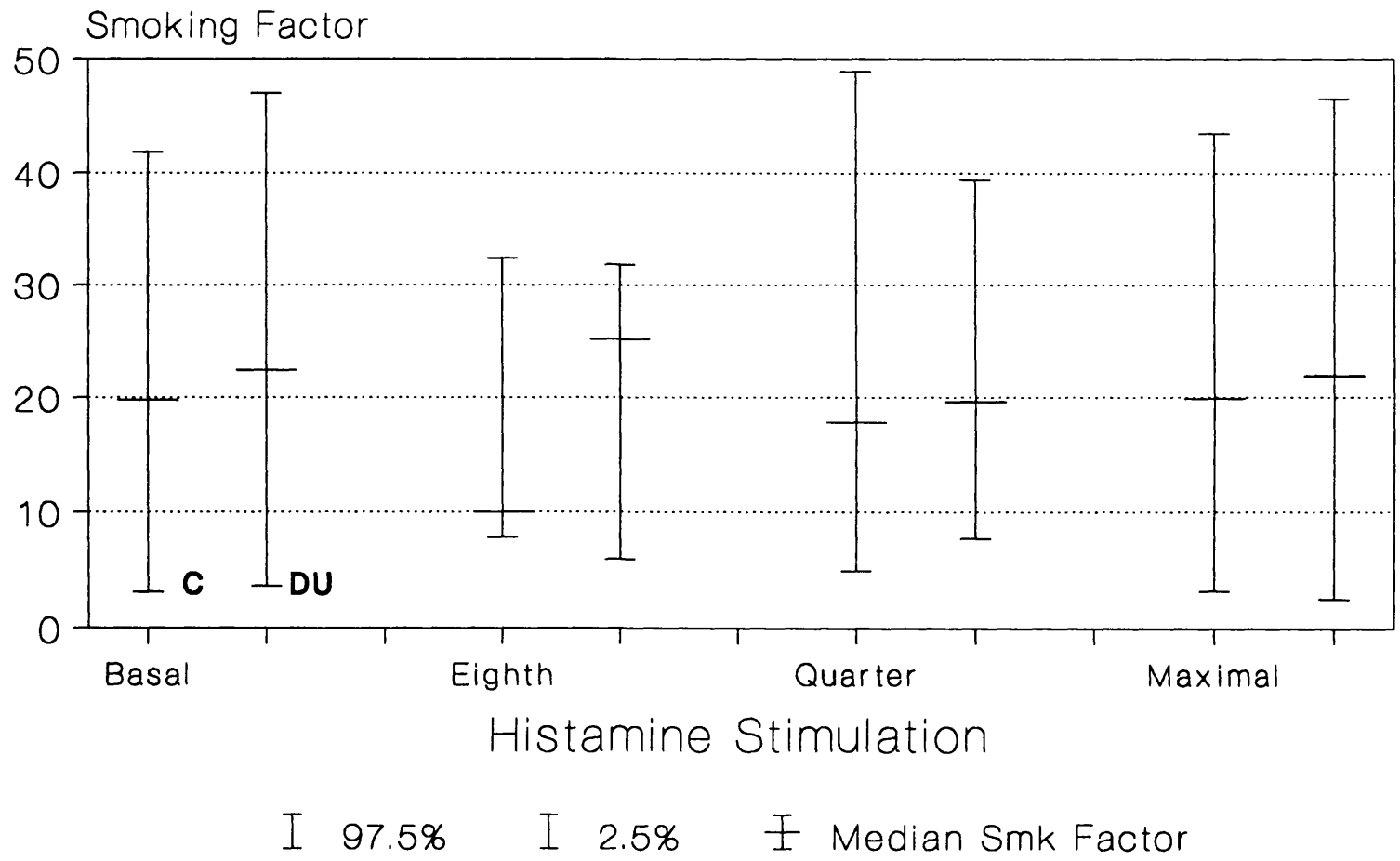


Fig 5.7
Smoking Factor - all subjects

Smoking Factor

	C		DU	
Stimulation	range	median	p median	range
Maximal	(3.2 → 43.5)	**19.9**	ns **21.9**	(2.5 → 46.5)
	p	ns	ns	
Quarter	(4.9 → 48.9)	**17.8**	ns **19.6**	(7.7 → 39.4)
	p	ns	0.01	
Eighth	(7.8 → 32.4)	**10.0**	0.05 **25.1**	(5.9 → 31.8)
	p	ns	ns	
Basal	(3.1 → 41.8)	**19.8**	ns **22.4**	(3.6 → 47.0)

Table 5.4

Cigarettes per day

		C	DU	
Stimulation	range	median	median	range
Maximal	(3.0 → 60.0)	**20.0**	**20.0**	(5.0 → 60.0)
Quarter	(4.0 → 60.0)	**19.5**	**22.0**	(10.0 → 60.0)
Eighth	(15.0 → 40.0)	**20.0**	**20.0**	(5.0 → 40.0)
Basal	(5.0 → 50.0)	**15.5**	**20.0**	(4.0 → 60.0)

Table 5.5

RESULTS

1/. Vg

In both the duodenal ulcer and the control groups there was an increase in the Vg as the stimulation increased from basal, through eighth and quarter-maximal stimulation to maximal stimulation (Fig 6.1). This increase was significant ($p < 0.01$) between all levels of stimulation except between the two levels of sub-maximal stimulation (1/4 & $1/8^{th}$ maximal).

Vg was greater in the duodenal ulcer group than in the corresponding control group at all levels of stimulation. This was significant ($p < 0.01$) except at $1/8^{th}$ maximal stimulation (Fig 6.2, Table 6.1).

2/. Dose-response analysis

The varying doses of exogenous histamine were expressed as multiples of the lowest dose (0.005 mg/kg/hr)which was given arbitrary value of one; the maximum dose of histamine had therefore a value of eight. Under basal conditions there was no exogenous histamine and it was thus assigned a "dose value" of zero. A linear plot of median Vg against dose for duodenal ulcer patients and controls is shown

(Fig 6.3).

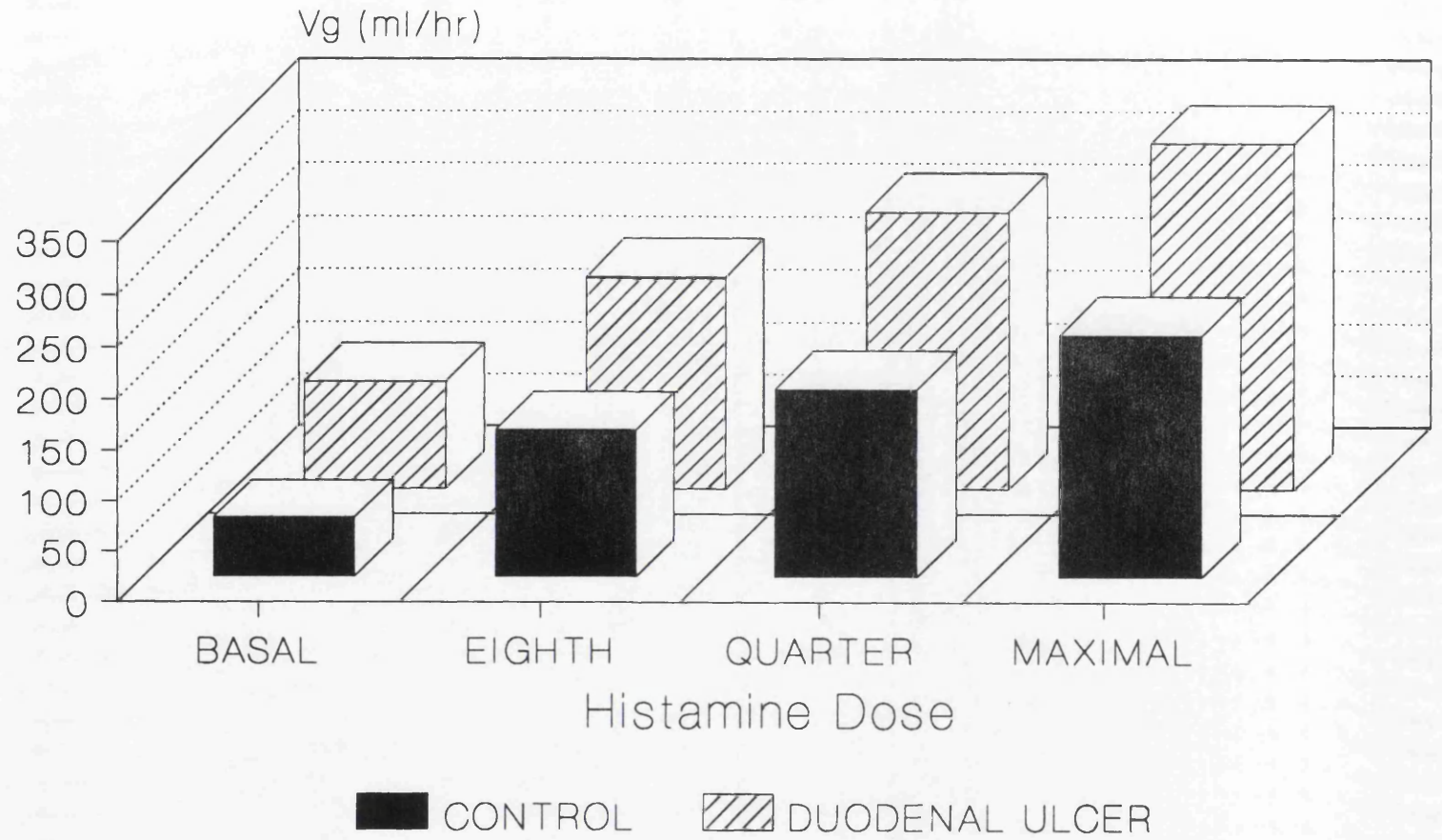


Fig 6.1
Gastric secretion over the whole range of stimulation

Gastric Secretion of Subjects
Vg. (ml/hr)

	C		DU	
Stimulation	range	median	median	range
		p		
Maximal	(78.0 → 531.6)	**237.6**	0.01	**331.8**	(141.0 → 678.6)
	p	0.01		0.01	
Quarter	(31.2 → 397.8)	**185.4**	0.01	**266.4**	(100.8 → 524.4)
	p	ns		ns	
Eighth	(78.0 → 243.0)	**145.8**	ns	**204.6**	(93.6 → 472.8)
	p	0.01		0.01	
Basal	(16.2 → 203.4)	**58.8**	0.01	**105.0**	(30.6 → 309.6)

Table 6.1

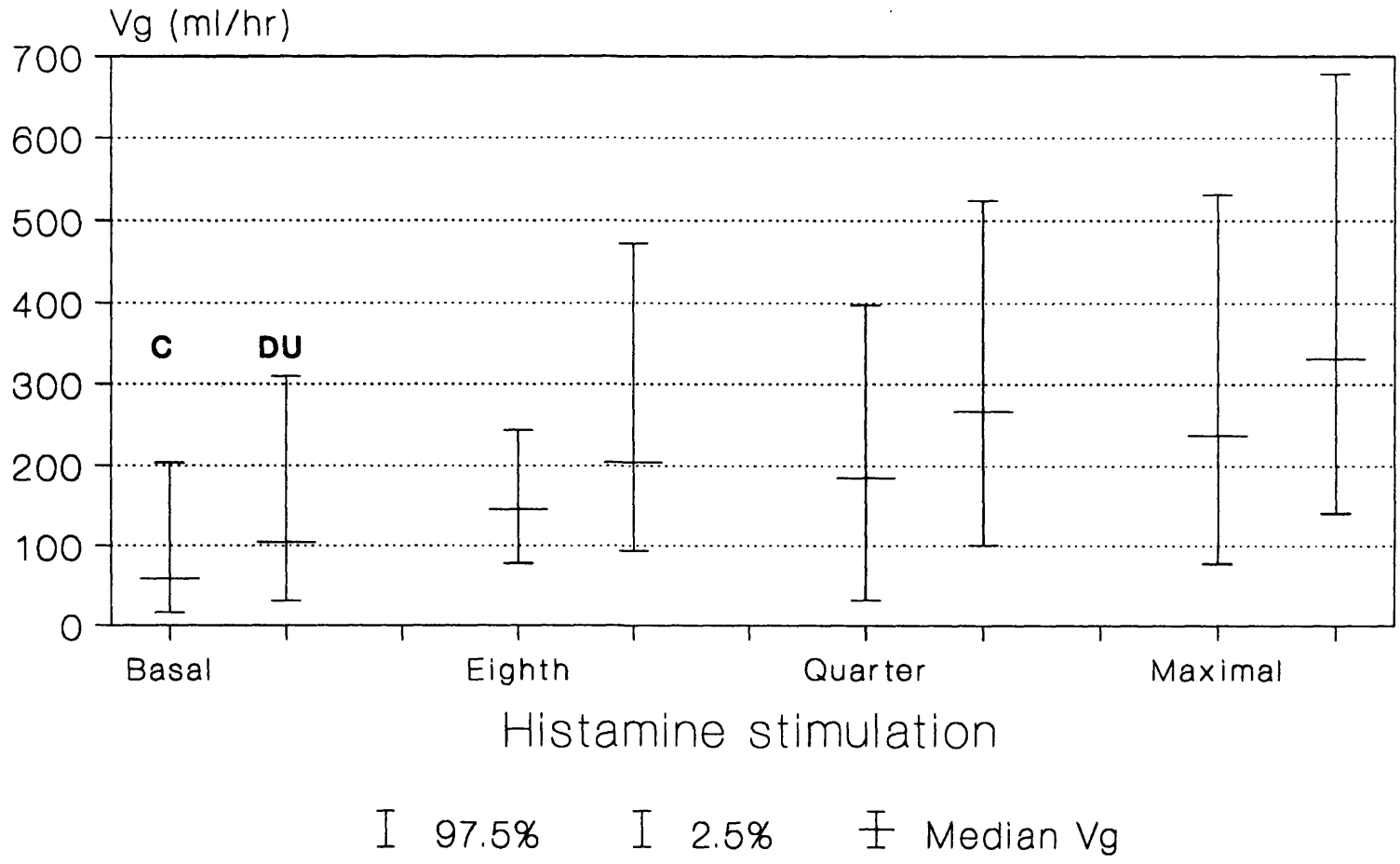


Fig 6.2
Median Vg and 95% range

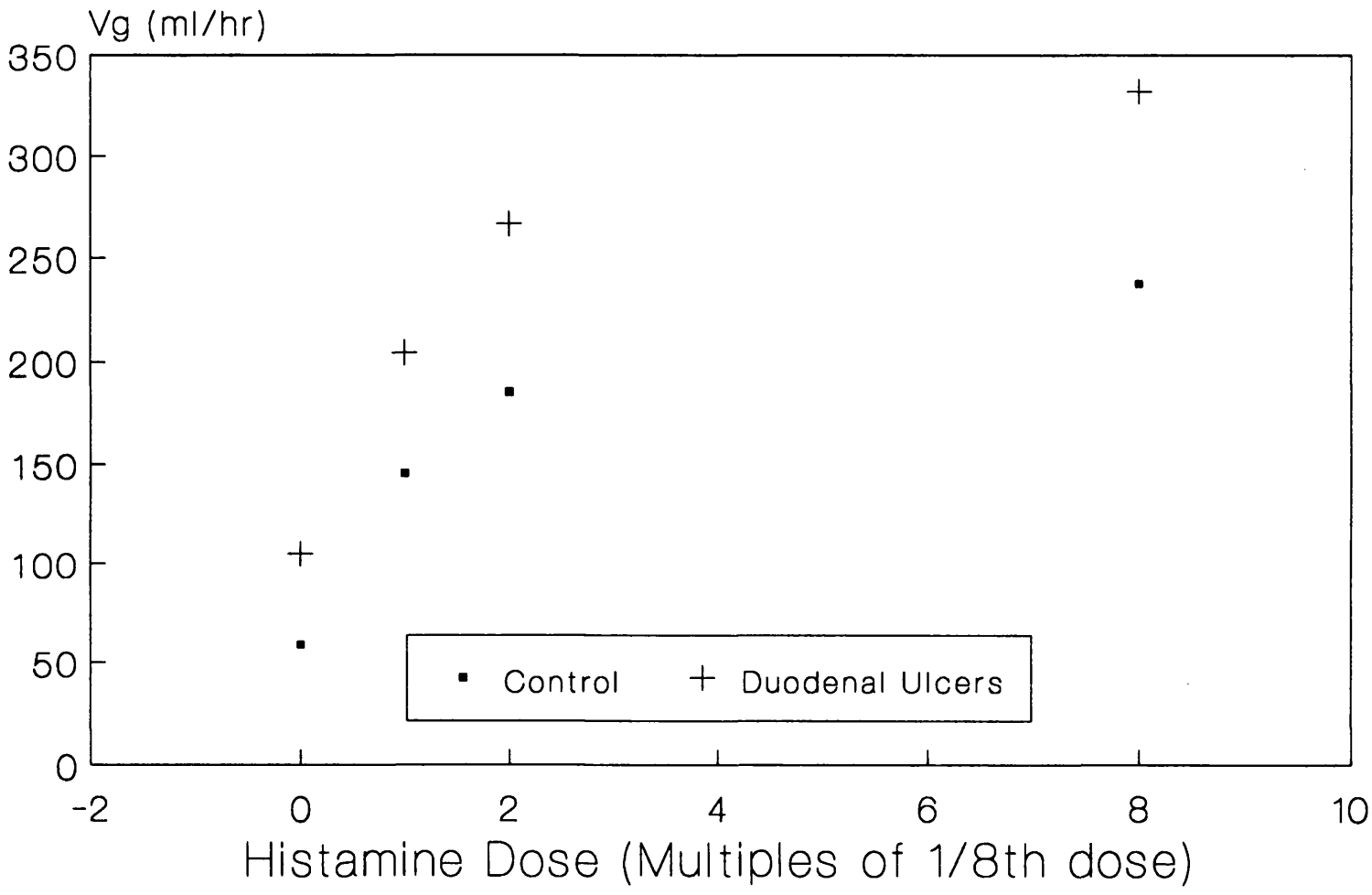


Fig 6.3
Dose-response plot (R vs D)

i). Linear transformation

The freehand curves suggest that they may be part of a rectangular hyperbola: a typical dose-response curve. If this is the case then a simple linear transformation of the hyperbolic function is possible.

From the equation;

$$r = Vmax \times d/(K_x + d)$$

3 linear transformations are possible:

1. (1/r) vs (1/d) Lineweaver-Burke plot
2. r vs (r/d) Hofstee plot
3. d/r vs d

where r = response (Vg)

d = dose (Histamine)

Vmax = theoretical maximal response

Kx = dose required to produce 50% of that response.

The application of this to the median Vg for exogenous histamine gives straight lines for both the duodenal ulcer and control groups for all three plots; and in all three there was an extremely good fit of the line to the points (Figs 6.4, 6.5 & 6.6). When similar manipulations were performed on the data with the basal secretion subtracted, a good fit was obtained in all three control plots but in only one of the three duodenal ulcer plots (equation 3).

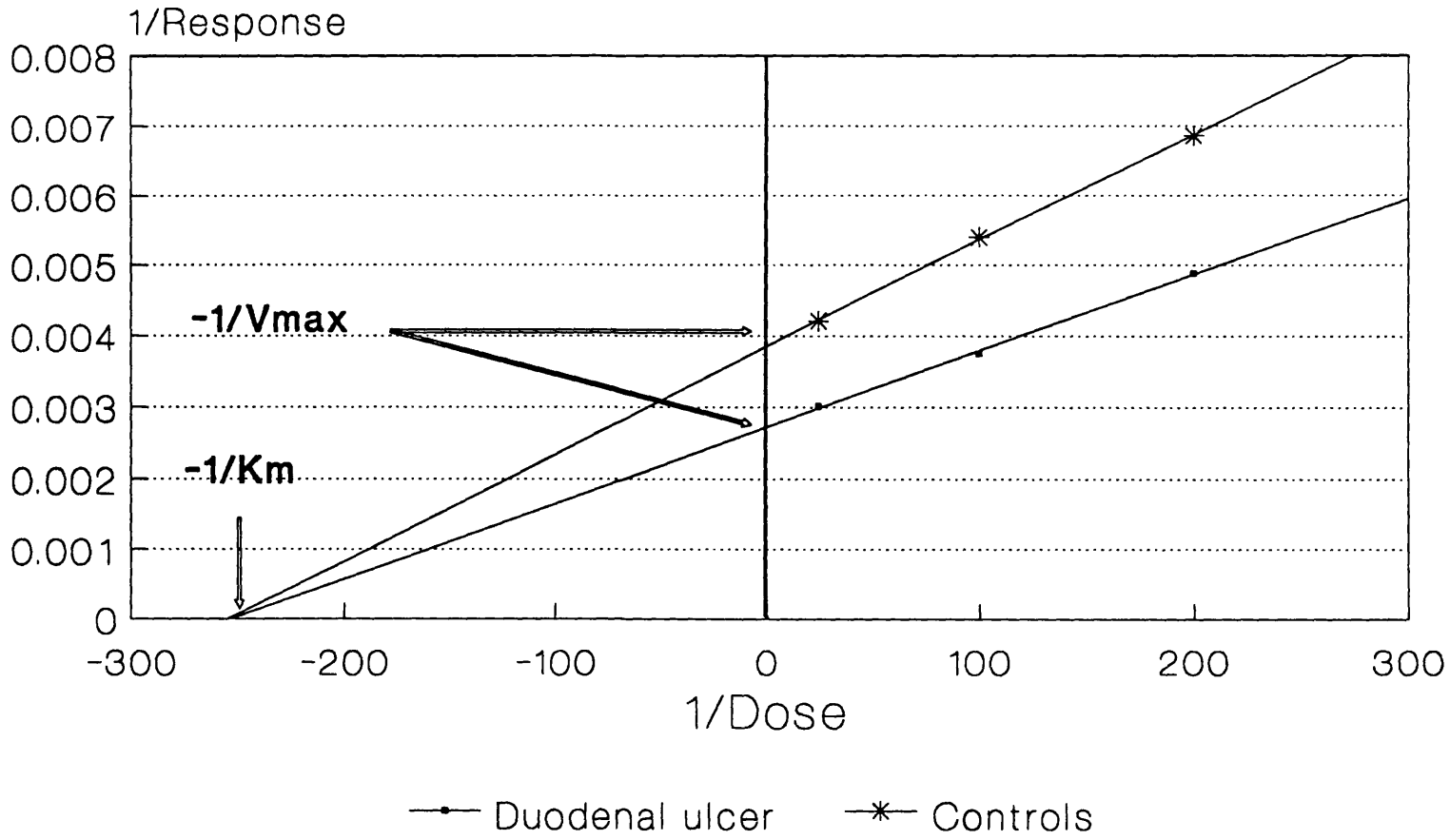


Fig 6.4
Linear transformations
Lineweaver-Burke (1/D vs 1/R)

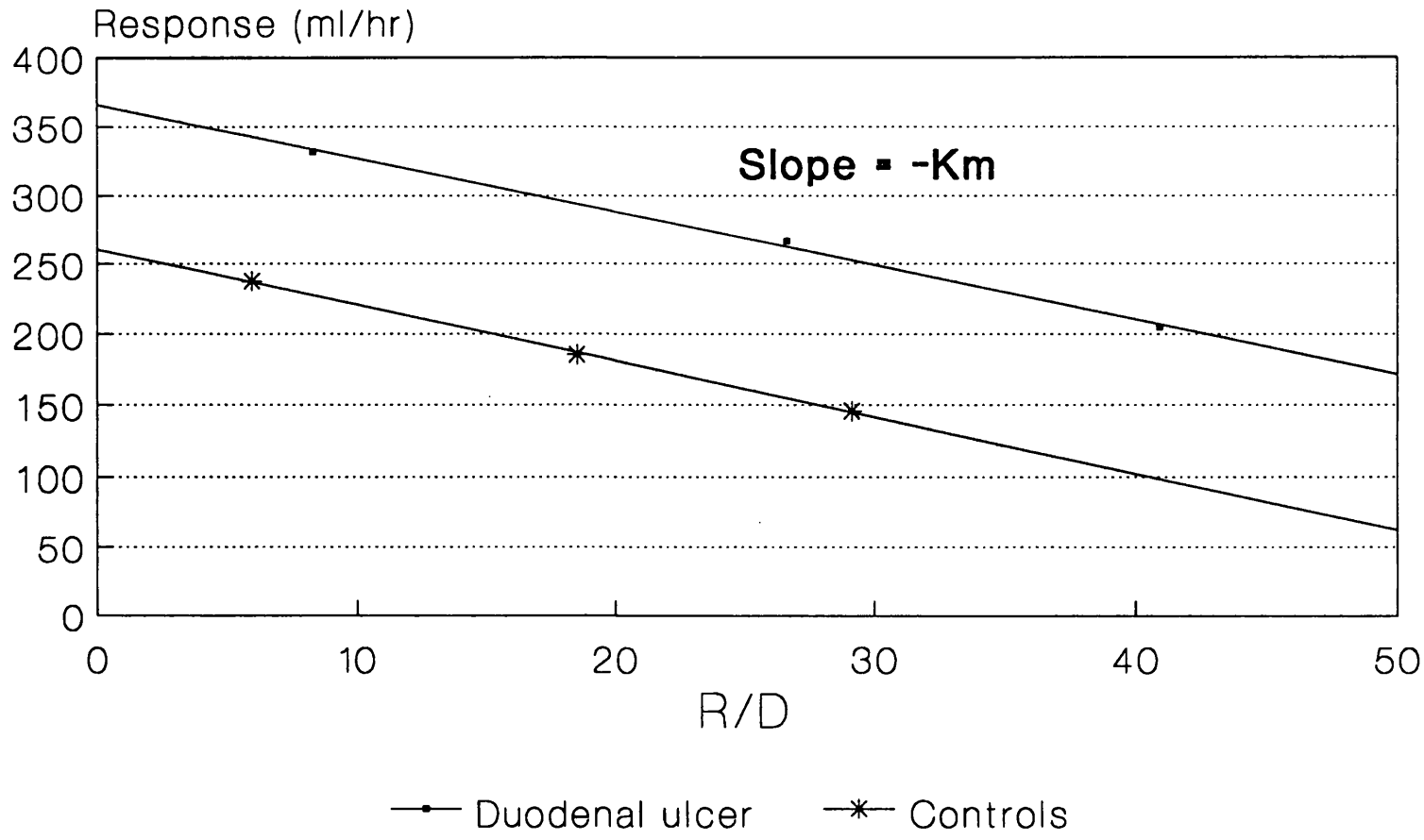


Fig 6.5
Linear transformations
Hofstee (R vs R/D)

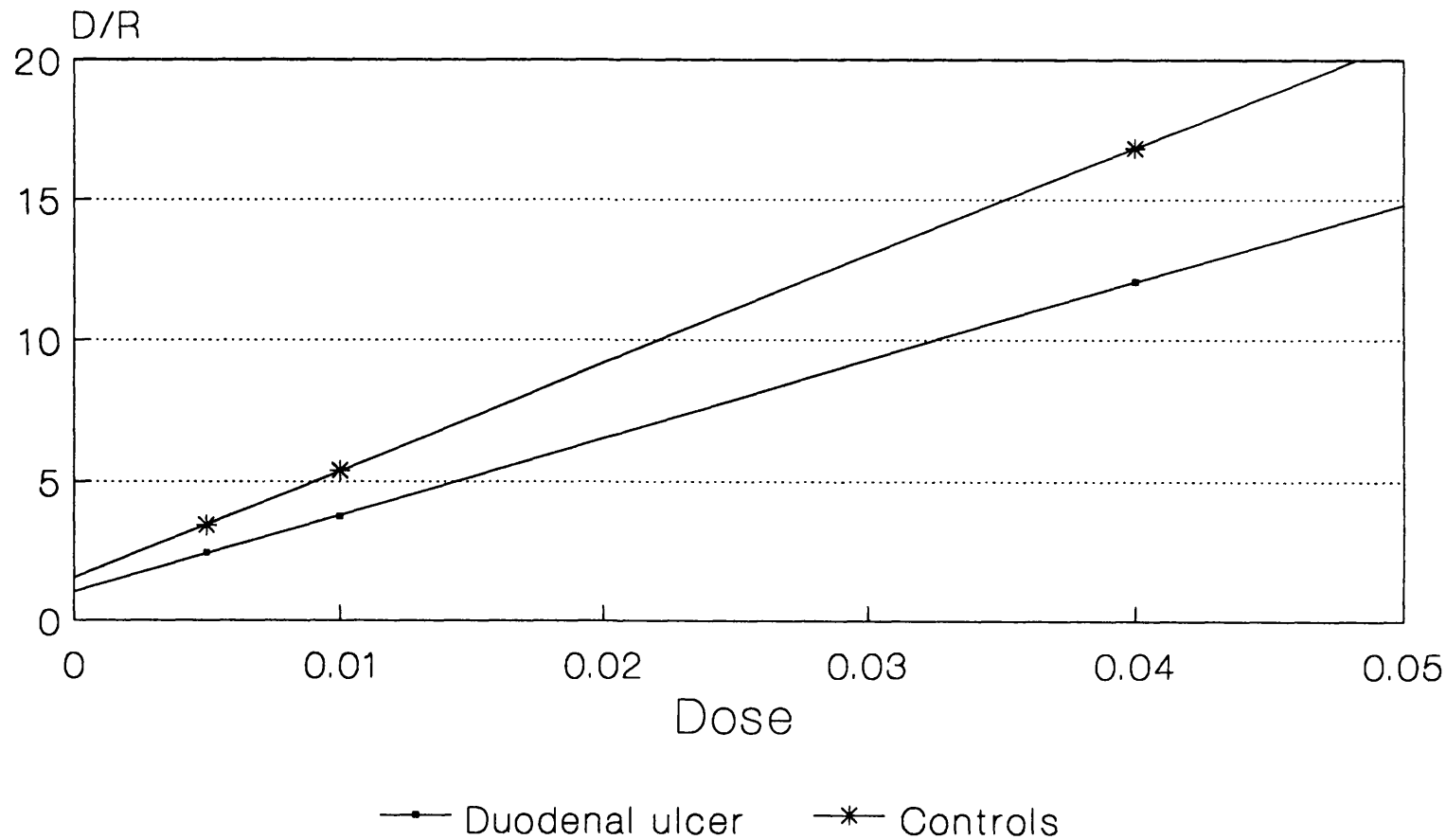


Fig 6.6
Linear transformations
(D/R vs D)

Linear transformations of dose-response data
(uncorrected for basal secretion)

	Kx x 10-3 mg/kg/hr		Vmax ml/hr	
	C	DU	C	DU
1/r vs 1/d	3.78	3.66	277	382
r vs r/d	3.95	3.88	260	365
d/r vs d	4.00	3.70	262	363
mean	**3.95**	**3.74**	**266**	**370**

(with corrections for basal secretion)

	Kx x 10-3 mg/kg/hr		Vmax ml/hr	
	C	DU	C	DU
1/v vs 1/d	5.56	*	272	*
r vs r/d	5.48	*	270	*
d/v vs d	5.22	5.47	267	376
mean	**5.42**	**5.47**	**269.5**	**376**

* = no significant linear relationship thus no values obtained.

Table 6.2

ii). Kx and Vmax

a). Basal secretion included

The values of Kx differed only marginally between the control and duodenal ulcer groups in all three transformations; and the absolute values of Kx were very similar regardless of the method of its calculation. The mean value of Kx for the controls was 3.95 x 10-3 mg/kg/hr (range 3.78 to 4.00 x 10-3 mg/kg/hr) and for the duodenal ulcer group it was 3.74 x 10-3 mg/kg/hr (range 3.66 to 3.88 x 10-3 mg/kg/hr), a variation of less than 6%. The nature of the calculation of Kx meant that it is only possible to analyse Kx statistically in one of the three transformations. In the plot r vs r/d (equation 2), Kx is the gradient of the line and the Kx calculated from this showed that there was a difference of less than 2% between the control and duodenal ulcer groups; this was not significant.

The values of Vmax were greater in the duodenal ulcer group than in the control group, a mean of 370 ml/r (range 363 ml/r to 382 ml/h) compared to 266 ml/h (range 260 ml/h to 277 ml/h). This was significant ($p < 0.01$). In both groups, control and duodenal ulcer, Vmax was about 12% greater than Vg at maximal histamine stimulation (Table 6.2).

b). Basal secretion subtracted

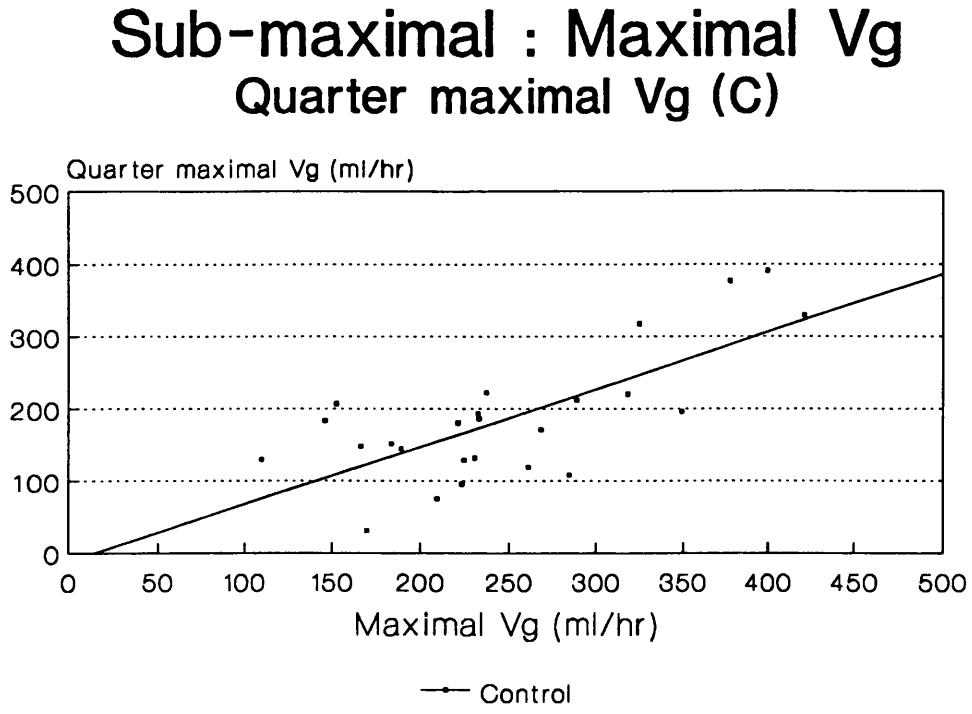
The values were calculated from the linear equations with corrections as described by Grossman (1973). In the

control group all three linear transformations gave good fits and thus Kx and Vmax were calculated. In the duodenal ulcer group there was only a good linear fit in one of the three transformations (equation 3). In the control group the mean Kx was 5.42 x 10-3 mg/kg/hr (range 5.22 to 5.56 x 10-3 mg/kg/hr) compared to the single values of Kx in the duodenal ulcer group of 5.47 x 10-3 mg/kg/hr. This value differed from the corresponding control Kx by 5%, it was not possible to test this statistically. It is to be noted that all values of Kx were higher than those calculated from the median values of Vg with basal secretion included. The mean Vmax in the control group was 269.5 ml/h (range 267 to 272 ml/h), almost the same as the value with basal secretion included: 266 ml/h. In the duodenal ulcer group the a single value of Vmax was 376 ml/h, again similar to the value obtained with basal secretion included: 370 ml/h (Table 6.2).

iii). Sub-maximal Vg as a predictor of maximal Vg

Initial inspection of the relationship between the secretion rates at sub-maximal stimulation (1/4 & 1/8th doses) and at maximal stimulation suggested that there was little difference between the control and duodenal ulcer groups. At quarter-maximal stimulation Vg was 78% of maximal in controls and 80% in the duodenal ulcer group. At eighth-maximal stimulation Vg was 61% in both groups; basal secretion was 24% of maximal Vg in

controls and 31% in the duodenal ulcer group. The relationship between the individual secretion rates at sub-maximal secretion (1/4 & 1/8th doses) and the corresponding Vg at maximal stimulation was examined (Fig 6.7, 6.8 & 6.9). At both levels of stimulation and in both groups sub-maximal Vg was a very good predictor of the maximal secretory capacity ($r > 0.72$). A similar analysis of the relationship between basal and maximal secretion showed significant correlation but with a much lower correlation coefficient ($r <= 0.37$) for both the controls and duodenal ulcer patients (Table 6.3).



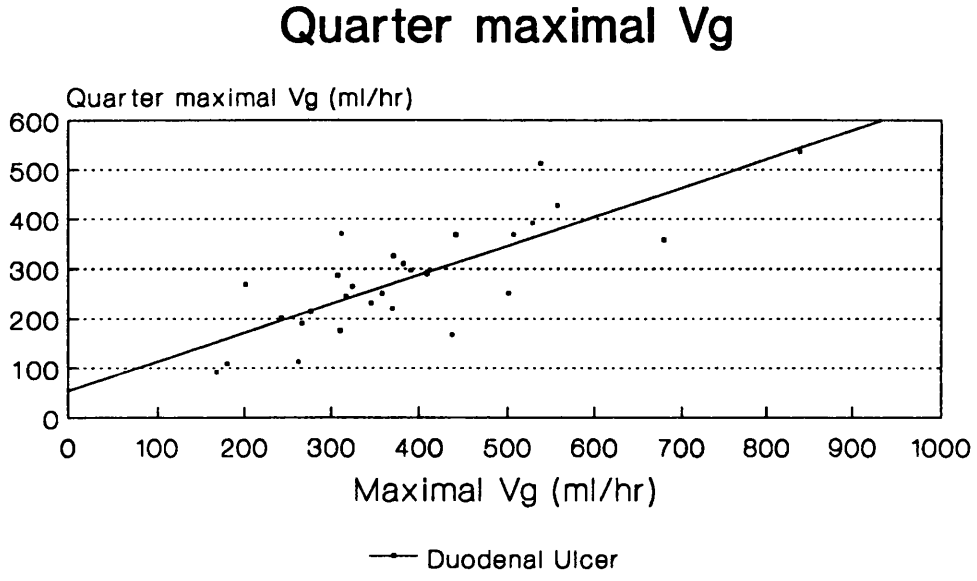
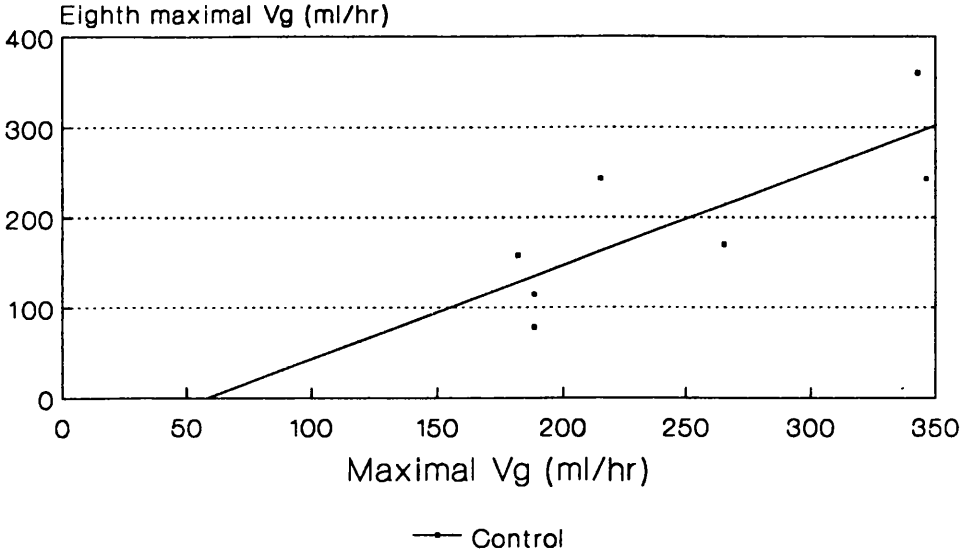


Fig 6.7
Sub-maximal to maximal Vg (1/4)

Sub-maximal : Maximal Vg

Eighth maximal Vg (C)



Eighth maximal Vg (DU)

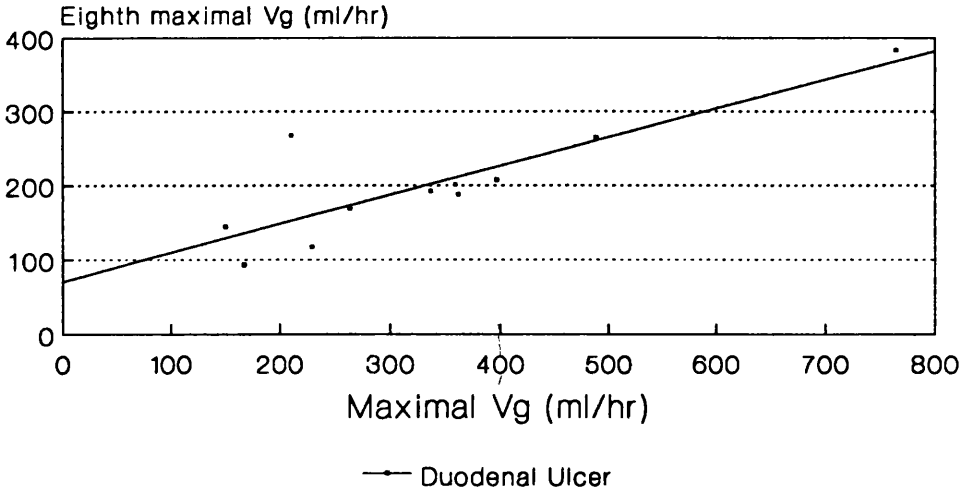
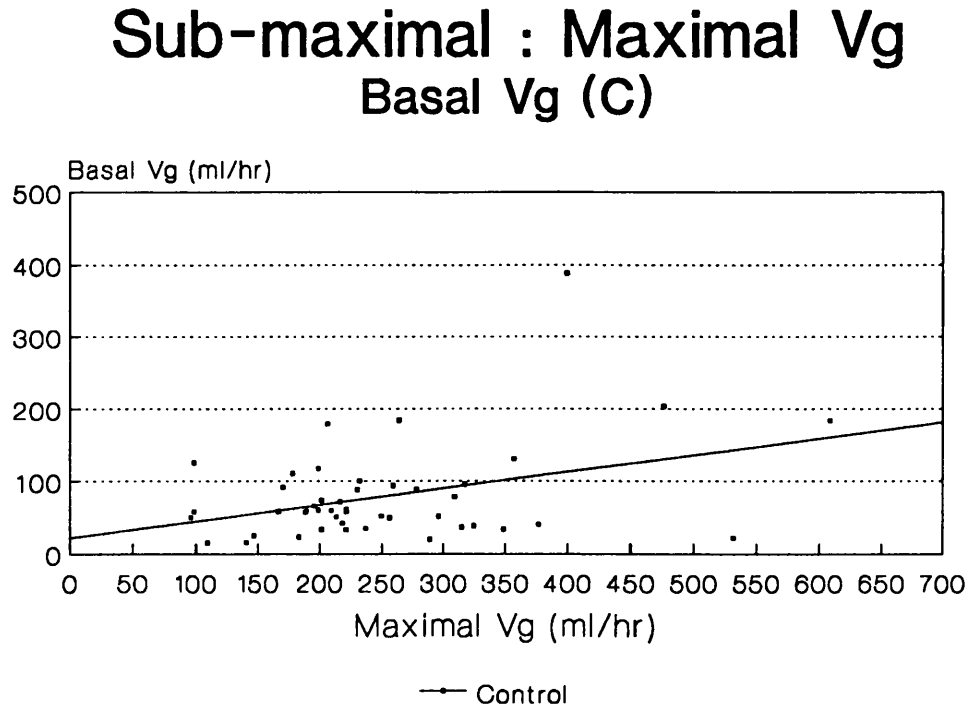


Fig 6.8
Sub-maximal to maximal Vg (1/8th)



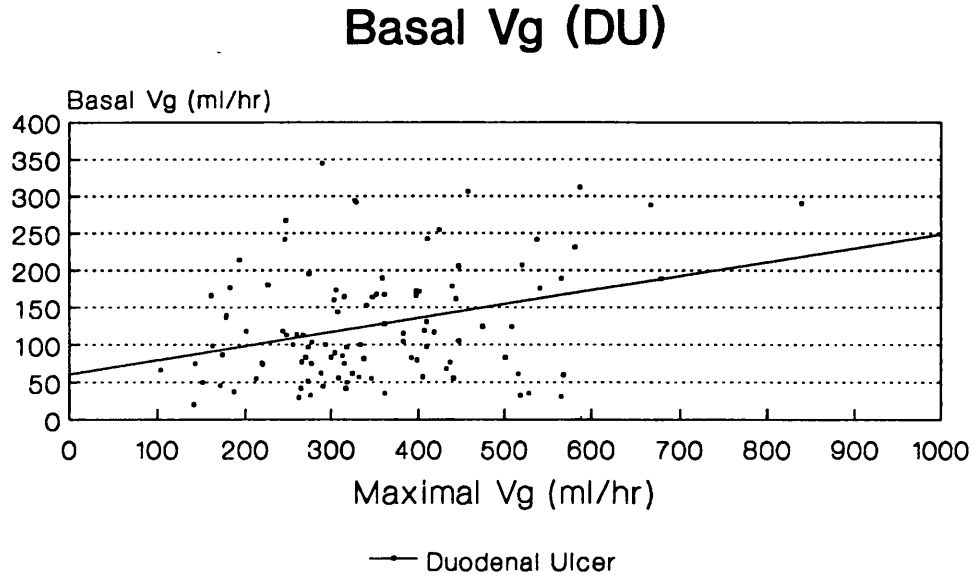


Fig 6.9
Sub-maximal to maximal Vg (Basal)

Sub-Maximal vs Maximal Vg

Regression Analysis

		n	r	T	p
Quarter	DU	29	0.79	6.77	0.001
	C	25	0.72	5.08	0.001
Eighth	DU	11	0.85	4.78	0.001
	C	7	0.79	2.89	0.035
Basal	DU	104	0.33	3.48	0.001
	C	46	0.37	2.62	0.012

Quarter maximal (Non-smokers & Smokers)

	n	r	m	c	T	p
Non-smoker	13	0.72	0.81*	-16.3	3.4	0.006
Smokers	12	0.74	0.78*	-5.8	3.5	0.006

* = n.s

Table 6.3

3/. Smoking and secretory capacity

The relationship of quarter maximal to maximal secretion was examined in smoking and non-smoking control subjects. There were 13 non-smokers and 12 smoking control subjects in the group, complete smoking histories were available for all 25 subjects.

In the non-smoking controls the median Vg at 1/4 maximal stimulation was 183.6 ml/h and this rose to 237.0 ml/h at maximal secretion, an increase of 77.4%. For the smokers the values were 176.1 ml/h and 223.8 ml/h respectively, this being a increase of 78.6%.

These two divisions were further analysed using regression analysis, it was already known that in the sub-group of controls there was a strong relationship between sub-maximal Vg and Maximal Vg (see above and table 6.3). There was a strong correlation between sub-maximal and maximal Vg in both non-smokers ($r= 0.72$) and smokers ($r=0.74$), $p < 0.006$. The x coefficient (m) was 0.78 for non-smokers and for smokers it was 0.81, the slopes of the two equations were not significantly different (Table 6.3 & Fig 6.10).

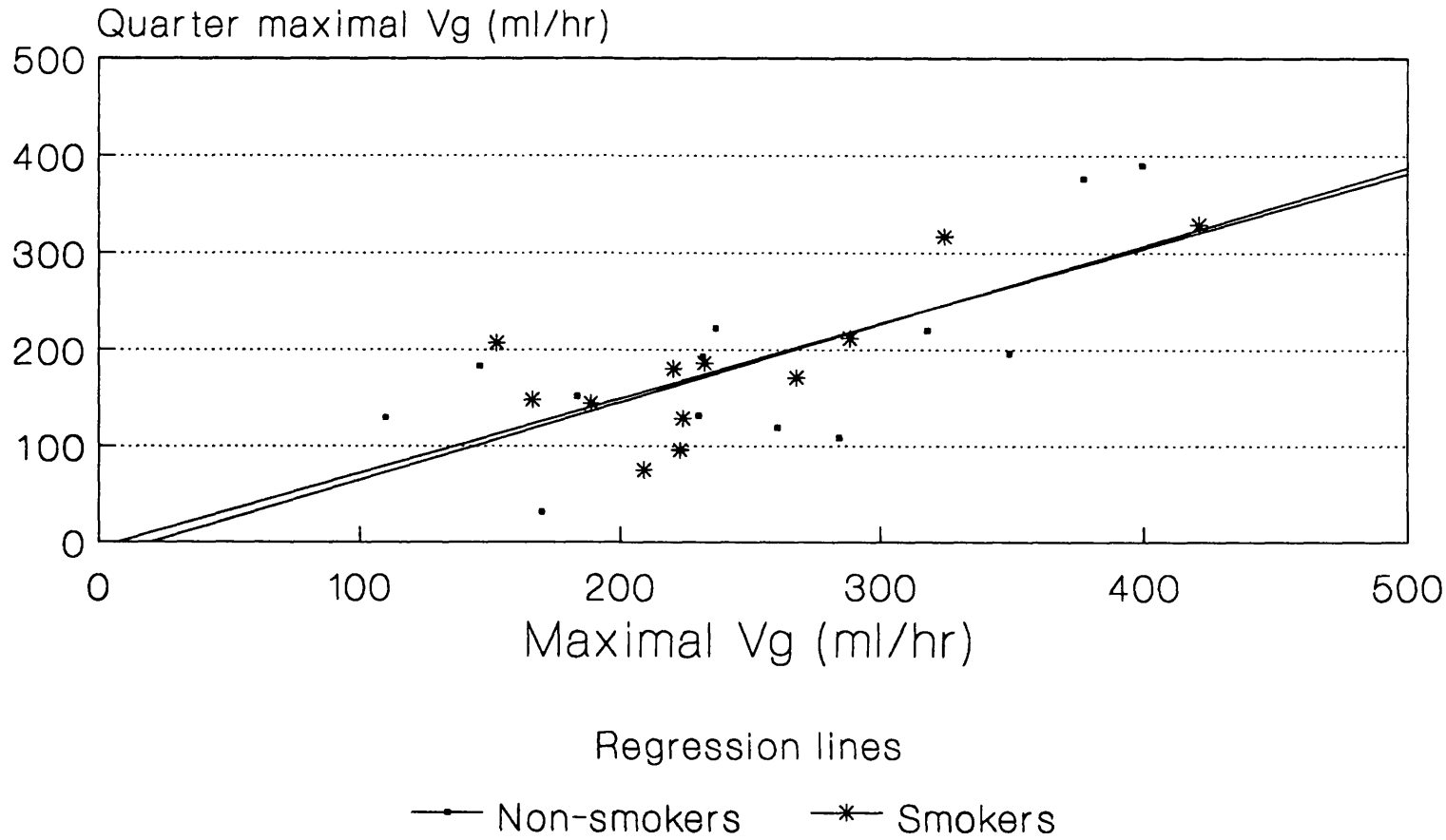


Fig 6.10
The effect of smoking upon the relationship of 1/4 to Maximal Vg in controls.

1/. Pyloric loss

Initial inspection did not reveal differences between groups as obvious as were seen with Vg. In the control group median pyloric loss ranged from +22.2 ml/h to +28.8 ml/h (n.s), there was no marked trend with the alteration in the level of stimulation. In the duodenal ulcer group median pyloric loss appeared to be greatest at maximal stimulation +47.4 ml/h, but no parallelism was seen between pyloric loss and increasing levels of stimulation. For example, the loss under basal conditions (39.6 ml/h) was not significantly different from the loss at maximal stimulation (Table 6.4).

i). Regression analysis

Within each level of secretion stimulus, the relationship between the rate of secretion and pyloric loss was explored using linear regression analysis. Within each sub-group there were significant positive correlations under basal conditions, at 1/4 maximal stimulation and at maximal stimulation for both duodenal ulcer patients and controls. However, in the 1/8th maximal section there was no correlation in either sub-group (Table 6.5). Inspection of the table further showed that only the large groups with a significant

Pyloric loss in subjects
ml/hr

	C		DU	
Stimulation	range	median	range	
Maximal	(-6.6 → 182.4)	**25.5**	**47.4**	(-35.4 → 264.0)
Quarter	(-37.2 → 141.6)	**28.8**	**22.2**	(-51.6 → 88.2)
Eighth	(-27.6 → 36.0)	**24.6**	**24.0**	(-46.2 → 54.6)
Basal	(-7.8 → 186.6)	**22.2**	**39.6**	(-12.0 → 186.0)

Table 6.4

Pyloric loss vs Vg
Linear regression

		n	r	T	p
Maximal	C	122	0.36	4.5	<0.001
	DU	209	0.56	10.0	<0.001
	C & DU	331	0.56	12.3	<0.001
Quarter	C	30	0.47	3.1	<0.05
	DU	29	0.32	2.1	<0.01
Eighth	C	8	0.08	0.2	n.s
	DU	14	0.13	0.9	n.s
Basal	C	48	0.52	4.2	<0.001
	DU	108	0.71	10.5	<0.001
	C & DU	156	0.67	11.4	<0.001

Table 6.5

positive correlation between pyloric loss and Vg, namely the basal and maximally stimulated groups, would benefit from a more detailed analysis.

ii). Pyloric loss at basal and maximal secretion

At both levels of stimulation pyloric loss was significantly greater in the duodenal ulcer patients than in controls ($p < 0.005$); it is to be noted that pyloric loss was about 80% greater in the duodenal ulcer patients than in the controls. However there was no significant difference in the rates of pyloric loss at basal or maximal stimulation within either patient group: controls or those with duodenal ulcers (Table 6.6). When loss was expressed as a fraction of Vg it was found that under basal conditions it was about 38% in both controls and duodenal ulcer patients; whereas at maximal stimulation pyloric loss was only about 12% of Vg in either group (Table 6.6, Fig 6.11). It was apparent that there was a link between increased gastric secretion and increased pyloric loss, and between the duodenal ulcer state and increased pyloric loss; but since the duodenal ulcer state is itself associated with increased gastric secretion it is difficult to know whether it is the ulcer state or the increased secretion that governs the increased pyloric loss.

Pyloric loss & Vg.cor
Basal & Maximal Secretion (ml/h)

	C	(% of Vg)	DU	(% of Vg)	p
P.loss	**25.5**	(14.3%)	**47.4**	(10.7%)	< 0.005
Maximal Vg	237.6		331.8		
p	n.s		n.s		
P.loss	**22.2**	(37.7%)	**39.6**	(37.7%)	< 0.005
Basal Vg	58.8		105.0		

Table 6.6

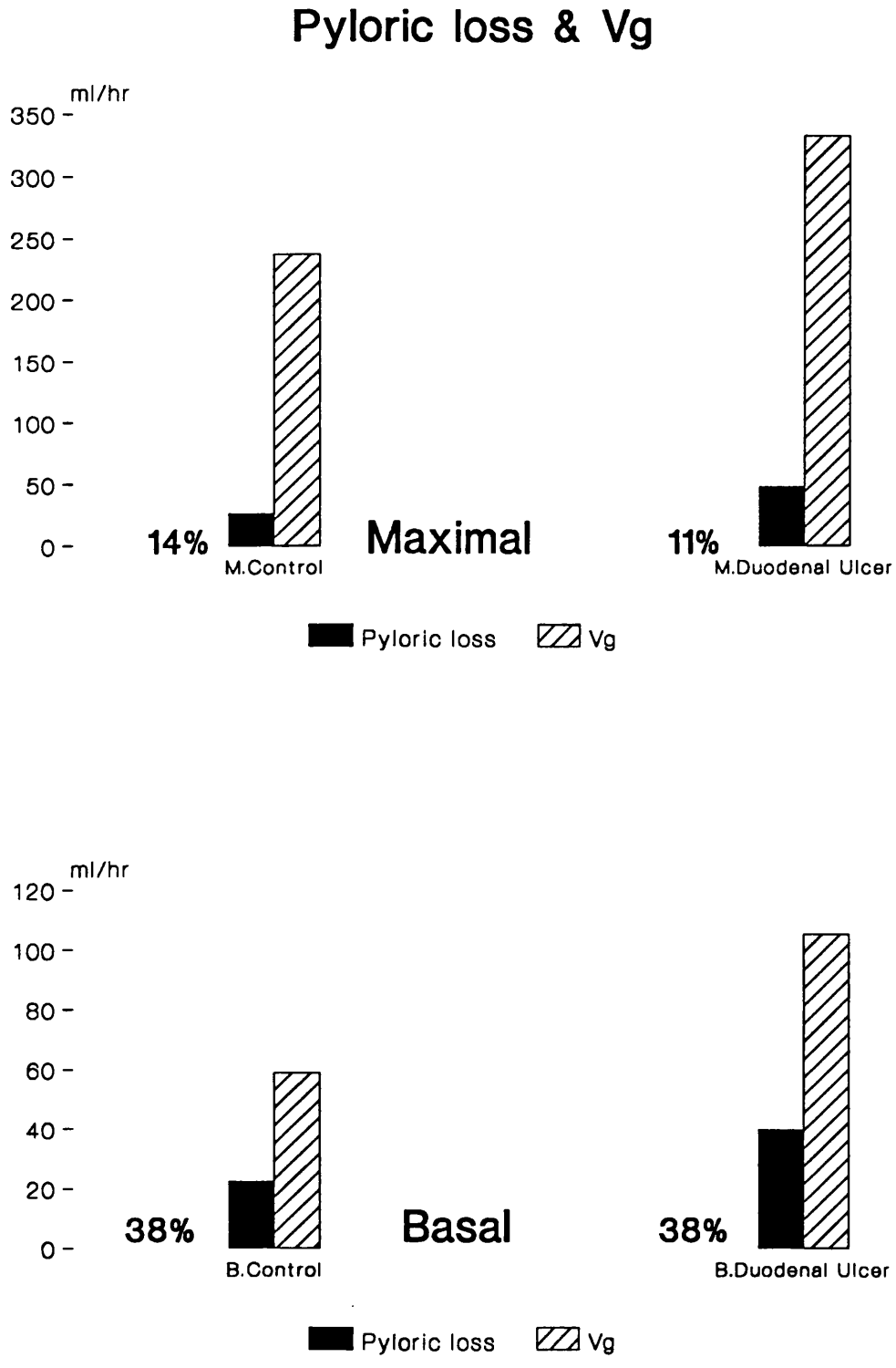


Fig 6.11
Pyloric loss as a percentage of Vg

a). Disease state

The control and duodenal ulcer groups were combined and regression analysis of pyloric loss and Vg performed for each of the basal and maximally stimulated states. Again a significant positive correlation was found between Vg and pyloric loss (Table 6.5); this finding suggested that the significant difference in pyloric loss between controls and duodenal ulcer patients was simply due to the greater Vg in these patients and not to the disease state per se. To confirm this a range of Vg values amongst the controls and duodenal ulcer patients that overlapped was found. The median Vg of each group was very similar as was the median pyloric loss; the significance of this was verified by the Mann-Whitney test.

In the basal group the median Vg for the controls (n=40) was 58.5 ml/h and for the duodenal ulcers (n=48) 61.5 ml/h, the corresponding pyloric losses were 22.2 ml/h and 27.6 ml/h (n.s). In the maximal group, Vg in the controls (n=98) was 248.4 ml/h and in the duodenal ulcer (n=71) group 247.2 ml/h, the corresponding pyloric losses were 27.6 ml/h and 33.6 ml/h (n.s). This study has failed to demonstrate any significant correlation with disease state. Although this is not proof that such a relationship does not exist, it does appear that at both levels of stimulation the pyloric loss is a function of <u>maximal Vg</u> and not of the disease state. The higher levels of pyloric loss in the duodenal ulcer group simply

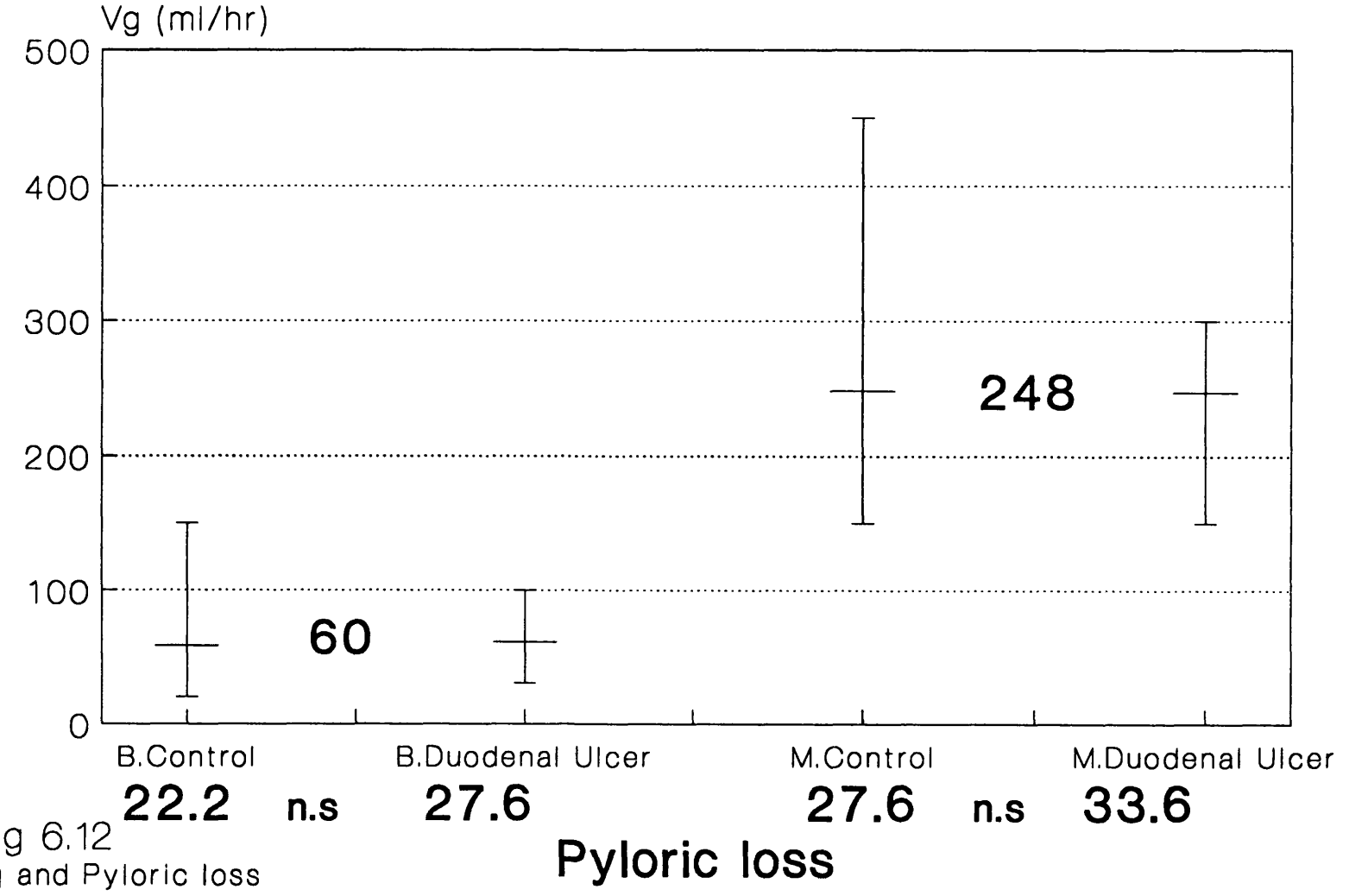


Fig 6.12
Vg and Pyloric loss
Range comparison

reflect the greater secretory capacity of patients in that group (Fig 6.12).

b). Stimulation state

Within a particular group, be it control or duodenal ulcer, there was no significant variation in pyloric loss between basal and maximal secretion, although there was a much greater Vg in the maximal state.

In the control subjects Vg was 58.8 ml/h in the basal state rising to 237.6 ml/h in the maximal state (304%)

In the duodenal ulcer patients Vg was 105.0 ml/h in the basal state rising to 331.8 ml/h in the maximal state (215%).

This information strongly suggested that within a particular group pyloric loss was fairly constant. Indeed when paired values of pyloric loss at basal and maximal secretion were examined in controls (n = 45) and duodenal ulcer patients (n = 101); it was found that there was no significant difference (paired t-test) in pyloric loss when stimulation rose from basal to maximal. This explains the fall in the proportion of PL : Vg with increased stimulation.

c). The effect of chronic cigarette smoking.

The basal and maximal stimulation groups were divided into smokers and non-smokers; the median values for pyloric loss and Vg were found for each of these sub-

groups. No allowance was made for the presence of duodenal ulcer disease since this has been shown to have no specific effect (see above).

In the maximal group there were 252 smokers with a median Vg of 321.0 ml/h and a pyloric loss of 39 ml/h; there were 79 non-smokers with a median Vg of 241.8 ml/h and a corresponding pyloric loss of 21.6 ml/h. Vg and pyloric loss were found to be significantly greater amongst the smokers compared to the non-smokers. In both the smokers and non-smokers a significant positive correlation was found between pyloric loss and Vg (table 6.7).

In the group in whom basal secretion was measured there were 121 smokers and 35 non-smokers. For the smokers the median Vg was 81.6 ml/h with a pyloric loss of 34.8 ml/h; amongst the non-smokers the values were 103.8 ml/h (Vg) and 48.6 ml/h (PL). Although Vg and pyloric loss tended to be lower for the smokers compared to the non-smokers this did not reach significance. Again a positive strong correlation was found between pyloric loss and Vg for both smokers and non-smokers (table 6.7)

Relationship between Vg and pyloric loss.

Maximal stimulation

	n	Vg ml/h.	PL ml/h.
Smokers	252	321.0	39.0
		p = 0.001	p = 0.01
Non-smokers	79	241.8	21.6
	Regression analysis		
Smokers	PL = - 43.02 + 0.306 Vg		r = 0.524
Non-smokers	PL = - 69.60 + 0.448 Vg		r = 0.669

Basal secretion

	n	Vg ml/h.	PL ml/h.
Smokers	121	81.6	34.8
		n.s	n.s
Non-smokers	35	103.8	48.6
	Regression analysis		
Smokers	PL = - 11.4 + 0.575 Vg		r = 0.75
Non-smokers	PL = - 14.76 + 0.697 Vg		r = 0.625

Table 6.7

2/. Duodenogastric reflux

i). Duodenogastric reflux and secretion state

The absolute values of duodenogastric reflux were greatest under basal conditions and tended to decrease as the level of stimulation and Vg increased (Table 6.8, Fig 6.13); this was true for both groups. At maximal stimulation duodenogastric reflux was significantly lower than under basal conditions for both controls and those with duodenal ulcers ($p < 0.001$). Duodenogastric reflux was significantly greater in the duodenal ulcer group than the controls under basal conditions ($p < 0.002$), a similar trend was found at maximal stimulation , though this did not reach significance (Table 6.9).

Initial inspection suggested a possible inverse relationship between Vg and duodenogastric reflux, reflux being lowest when Vg was at its greatest. However regression analysis showed there to be no relationship between Vg and duodenogastric reflux in any of the eight sub-groups. Even when analysed in total, regardless of disease state or level of stimulation, no relationship was found despite the large numbers of involved ($n = 487$).

ii). Disease state

The effect of disease state upon duodenogastric reflux was examined in the same manner as for pyloric loss and Vg.

At basal secretion a range of Vg was found for which there was no significant difference between controls and those with duodenal ulcers; for the controls Vg was 61.2 ml/h (n = 40) and for the duodenal ulcer group it was 75 ml/h (n = 63). The corresponding values for duodenogastric reflux were 12.3 ml/h for controls and 19.6 ml/h for the duodenal ulcer group, this difference was not significant.

At maximal stimulation Vg was 304.2 ml/h for the controls (n = 70) and 309.3 ml/h for those with duodenal ulcers (n = 168); the corresponding values for duodenogastric reflux were -4.8 ml/h and 1.8 ml/h, this was highly significant (p 0.006) (Fig 6.14).

iii). Duodenogastric reflux and the effect of chronic cigarette smoking

This was studied at maximal secretion and under conditions of basal stimulation, with sub-divisions on the basis of disease and smoking habit. In the maximal group there were 114 controls of whom 68 were smokers; the median duodenogastric reflux for the smokers was -1.8 ml/h and 1.2 ml/h for the non-smokers (n.s.). There were 206 patients with duodenal ulcer disease of whom 176 were smokers; the median reflux was 1.5 ml/h for smokers and 3.0 ml/h for non-smokers. Once again the difference was not significant (table 6.10).

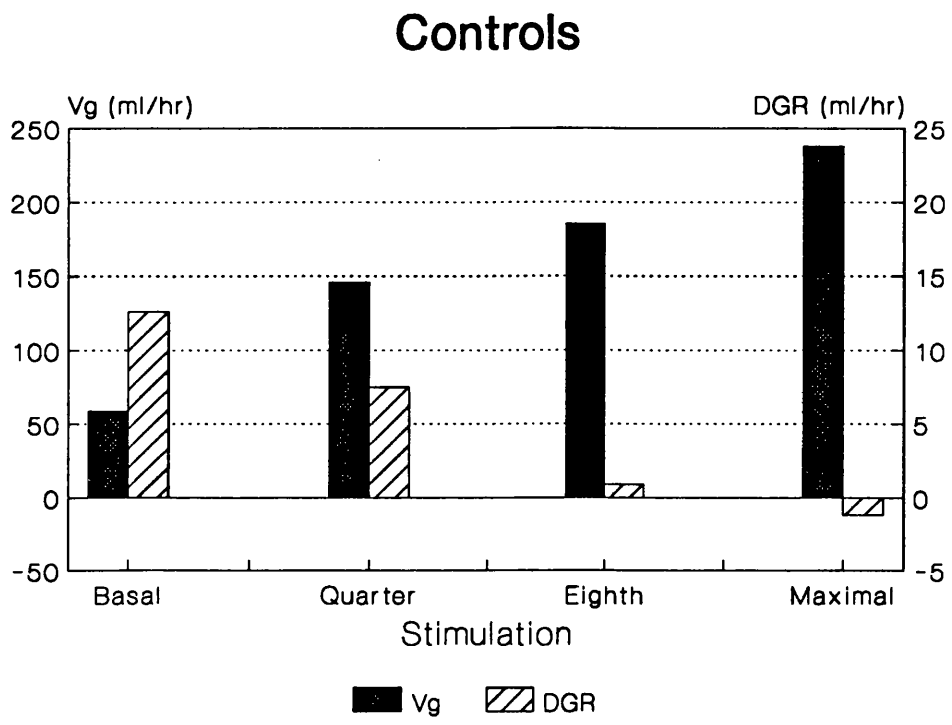
In the basal group there were 45 controls, of whom 20 were smokers; and 106 patients with duodenal ulcer

disease of whom 91 were smokers. In the control group the median reflux for smokers was 17.7 ml/h but only 11.4 ml/h in the non-smokers (n.s). In the ulcer subjects reflux in those who smoked was 22.2 ml/h but only 15.0 ml/h in the non-smokers ($p < 0.02$) (table 6.10)

Duodenogastric reflux in subjects
ml/h

		C	DU	
Stimulation	range	median		range
Maximal	(-15.0 → 65.4)	**-1.2**	**1.8**	(-19.2 → 109.2)
Quarter	(-14.4 → 78.0)	**0.9**	**2.4**	(-20.4 → 30.0)
Eighth	(-7.8 → 90.0)	**7.5**	**15.3**	(-3.0 51.0)
Basal	(0.6 → 59.4)	**12.6**	**21.0**	(-9.0 → 57.6)

Table 6.8



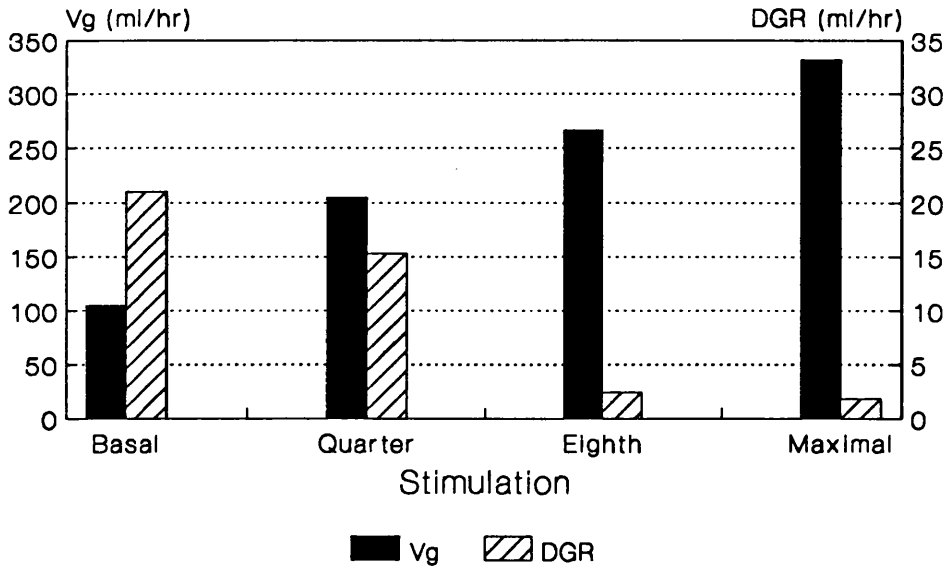


Fig 6.13
Trend of DGR with Vg

Duodenogastric reflux
Basal & Maximal Secretion (ml/h)

	C	DU	p
DGR Maximal	**-1.2**	**1.8**	n.s
p	0.001	0.001	
DGR Basal	**12.6**	**21.0**	0.002

Table 6.9

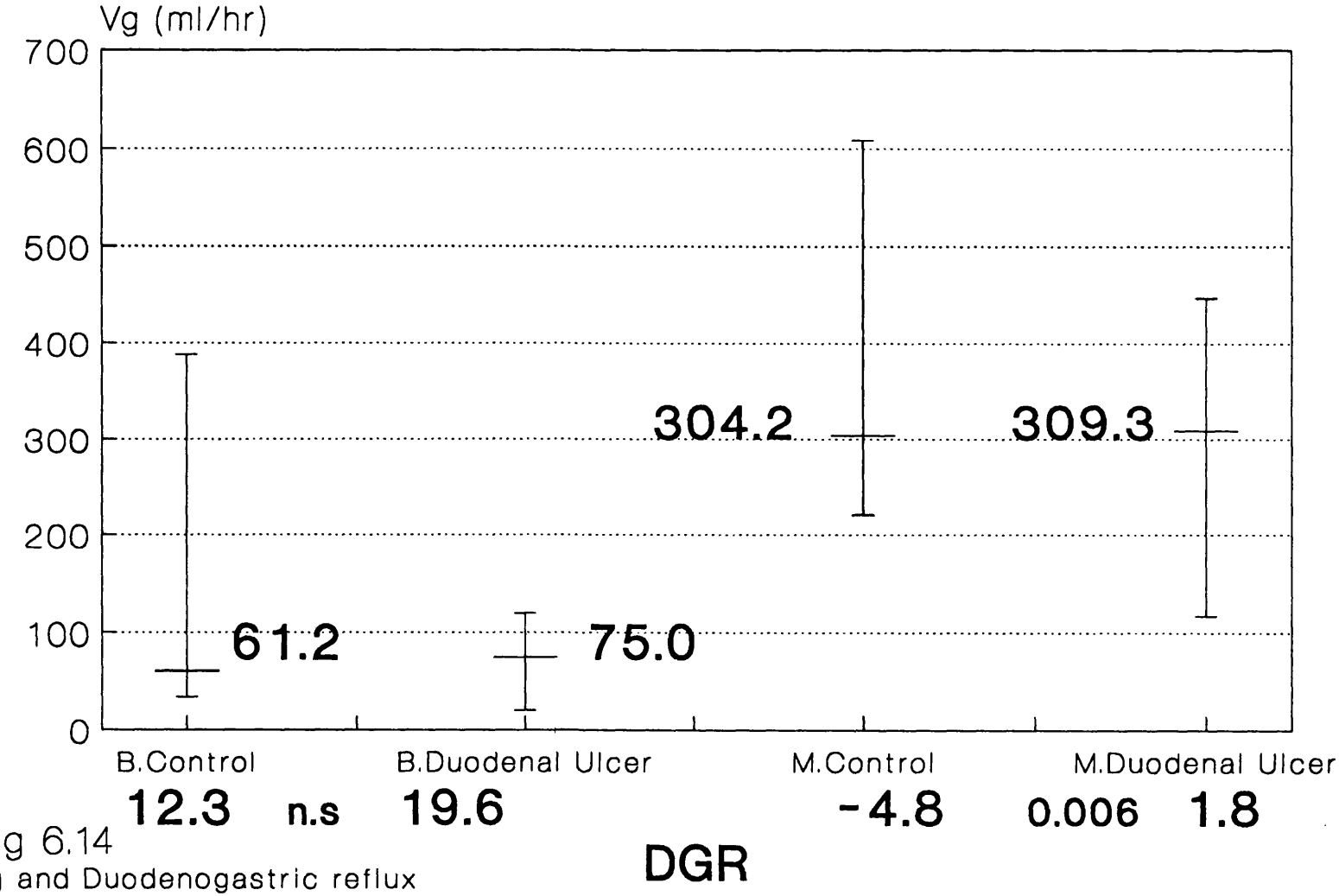


Fig 6.14
Vg and Duodenogastric reflux
Range comparison

Duodenogastric reflux and smoking.
ml/h

Basal secretion

	C	DU
Smokers	17.7	22.2
		p < 0.02
Non-smokers	11.4	15.0

Maximal secretion

	C	DU
Smokers	-1.8	1.5
Non-smokers	-1.2	3.0

Table 6.10

1/. Chronic changes

i). Basal secretion

a). Numbers studied

In the control group the numbers of smokers and non-smokers were roughly similar; except for female non-smokers of whom there were only four (Fig 6.15, Table 6.11). In those with duodenal ulcers male smokers were by far and away the largest group, numbering 73 (69%); the other groups were of similar size to the corresponding controls (Fig 6.15). There was a complete smoking history in all of the controls and in 106 of the 108 duodenal ulcer patients.

b). Stature

Stature as measured by height and weight did not show any significant differences between smokers and non-smokers in any of the sub-groups (Tables 6.12, 6.13).

c). Vg

Amongst male controls non-smokers had a significantly greater basal secretion than smokers ($p < 0.008$); a similar trend was found in female subjects, but this was not significant. Male ulcer patients who smoked had a marginally increased basal secretion but this was not significant.

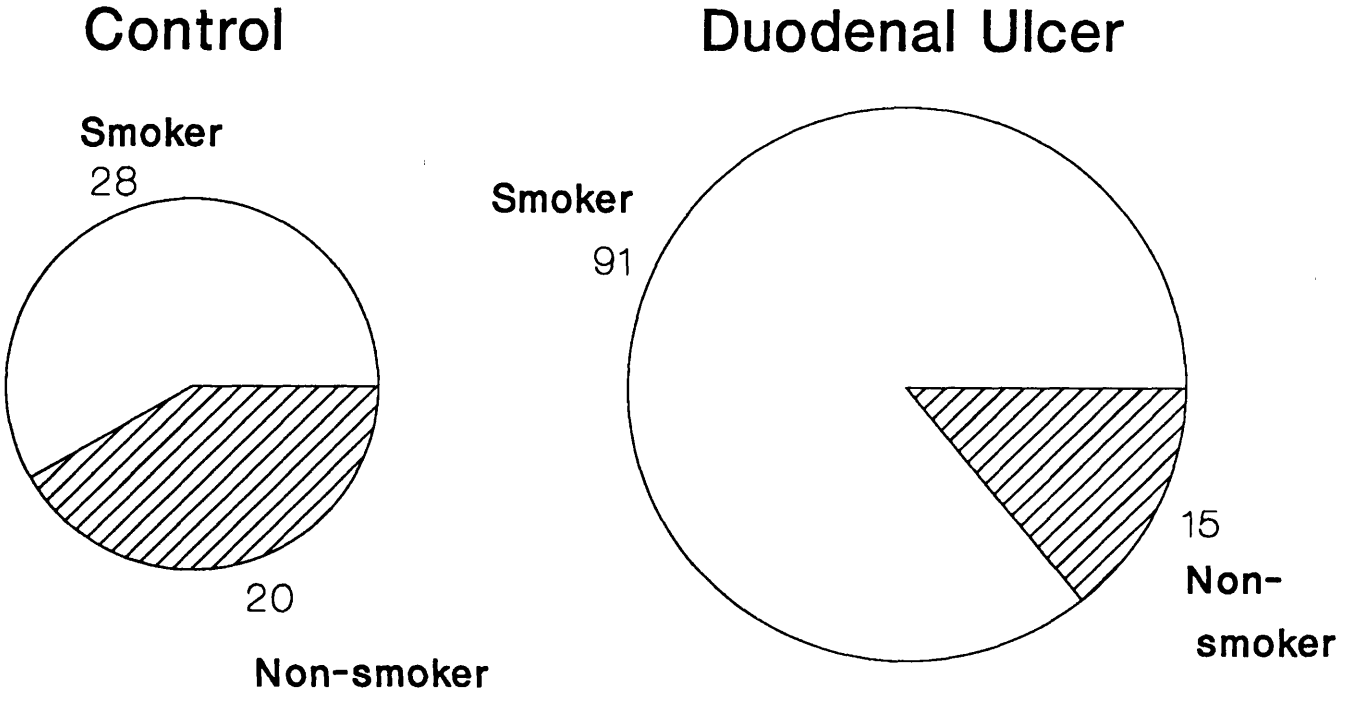
Basal Gastric Secretion

Number of Subjects

	MALE	FEMALE
Smoker	16	12
CONTROLS		
Non-smokers	16	4

	MALE	FEMALE
Smoker	73	18
DUODENAL ULCER		
Non-smoker	9	6

Table 6.11



Male	16	16	73	9
Female	12	4	18	6

Fig 6.15
Number of non-smokers and smokers under conditions of basal secretion

Basal Gastric Secretion

Weight of Subjects (kg)

	Non-smokers		Smokers
	median	p	median
Male	69.0	ns	70.0
CONTROLS			
Female	60.5	ns	58.0
Male	68.0	ns	67.0
DUODENAL ULCERS			
Female	58.0	ns	58.0

Table 6.12

Basal Gastric Secretion

Height of Subjects (cm)

	Non-smokers		Smokers
	median	p	median
Male CONTROLS	174.5	ns	175.0
Female	164.0	ns	161.0
Male DUODENAL ULCERS	170.0	ns	173.0
Female	156.5	ns	163.5

Table 6.13

Basal Gastric Secretion

Vg (ml/h)

	range	MALE median	FEMALE median	range
Non-smokers	(35.4 → 203.4)	**97.2**	**73.2**	(25.2 → 126.0)
CONTROLS	p	0.008	n.s	
Smoker	(19.8 → 111.0)	**51.6**	**54.6**	(15.6 → 78.6)
Non-smoker	(50.4 → 294.6)	**114.0**	**115.8**	(55.2 → 177.0)
DUODENAL ULCER	p	n.s	n.s	
Smoker	(30.0 → 312.0)	**117.0**	**79.2**	(33.0 → 267.0)

Table 6.14

The overall trend was one of increased basal secretion in non-smokers compared to smokers (Fig 6.17, Table 6.14).

ii). Maximal secretion

a). Numbers studied

Male smokers were the largest sub-group amongst both controls and duodenal ulcer patients. The sizes of the remaining sub-groups were roughly similar (Fig 6.16).

b). Stature

As in the basal studies there was no significant difference between the height and weight of the various sub-groups (Table 6.16, 6.17).

c). Vg

There was a trend in Vg in all four groups, maximal Vg being greater in smokers compared to non-smokers. This was significant ($p < 0.05$) in all but one group: male controls. In this group the increased Vg in smokers was not significant (Table 6.18, Fig 6.17).

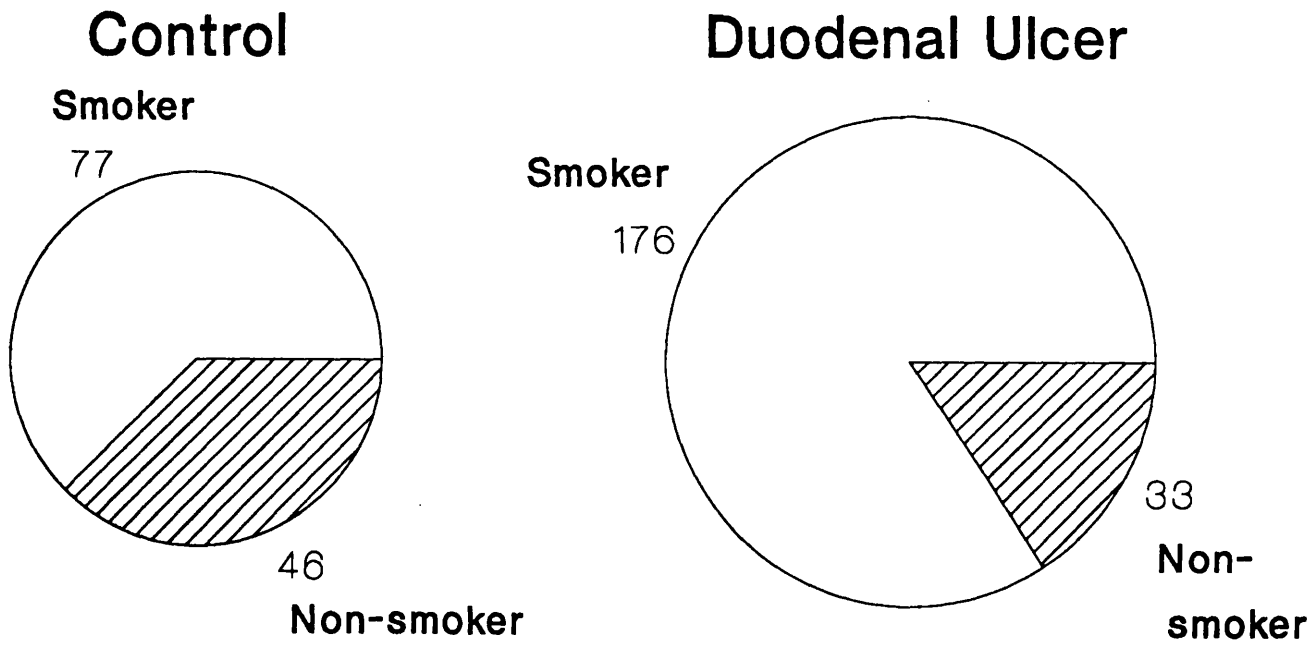
Maximal Gastric Secretion

Numbers of Subjects

	MALE	FEMALE
Non-smokers	28	18
CONTROLS		
Smoker	49	28

	MALE	FEMALE
Non-smokers	16	17
DUODENAL ULCER		
Smoker	139	37

Table 6.15



Male	49	28	139	16
Female	28	18	37	17

Fig 6.16
Number of non-smokers and smokers under conditions of maximal secretion

Maximal Gastric Secretion

Weight of Subjects (kg)

	Non-smokers		Smokers
	median	p	median
Male	72.5	ns	70.0
CONTROLS			
Female	57.5	ns	58.0
Male	68.0	ns	68.0
DUODENAL ULCERS			
Female	54.5	ns	57.0

Table 6.16

Maximal Gastric Secretion

Height of Subjects (cm)

	Non-smokers		Smokers
	median	p	median
Male	175.0	ns	175.0
CONTROLS			
Female	159.5	ns	160.0
Male	172.0	ns	173.0
DUODENAL ULCERS			
Female	157.0	ns	163.0

Table 6.17

Maximal Gastric Secretion

Vg (ml/h)

	range	MALE median	FEMALE median	range
Non-smokers	(123.0 → 439.2)	**257.7**	**171.0**	(48.0 - 262.8)
CONTROLS	p	n.s	0.005	
Smokers	(96.0 → 531.6)	**288.6**	**203.4**	(109.2 → 396.6)
Non-smokers	(193.2 → 390.6)	**285.0**	**225.6**	(158.4 → 359.4)
DUODENAL ULCERS	p	0.007	0.05	
Smokers	(141.6 → 682.2)	**380.4**	**265.5**	(171.0 → 435.6)

Table 6.18

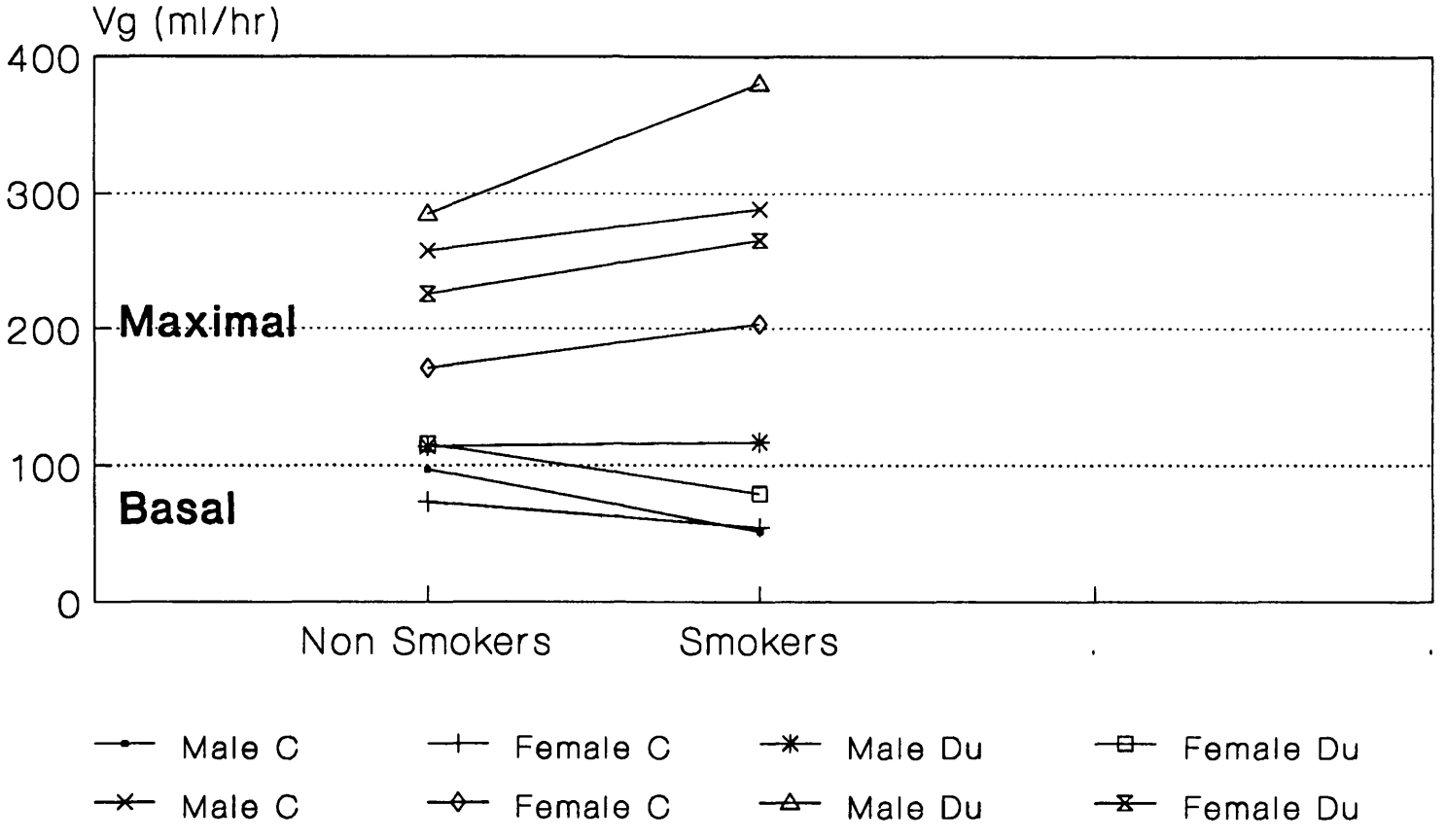


Fig 6.17
Trend of Vg in smokers and non-smokers
at basal and maximal secretion

2/. Acute changes

i). Vg

In the control group the median Vg before smoking was 145.8 ml/h, after smoking a single cigarette gastric secretion fell by 23% to 112.2 ml/h; this was significant ($p < 0.05$). In duodenal ulcer patients the median Vg prior to smoking was 237.6 ml/h, after smoking a single cigarette there was a 26% fall in Vg to a median value of 175.2 ml/h ($p < 0.02$) (Table 6.19, Fig 6.18). The median Vg for each ten minute collection period was found, and plotted against time to show the relationship of the change in Vg to cigarette smoking. In both the controls and the duodenal ulcer subjects the new lower plateau of gastric secretion was attained by the end of 11th collection period, about 20 minutes after the cigarette smoking had commenced (Fig 6.18).

ii). Pyloric loss

In the control patients pyloric loss was 16.8 ml/h before smoking and was unchanged after cigarette smoking; it did however rise in the duodenal ulcer patients from 30 ml/h to 37.5ml/h (25%) but this was not significant (Table 6.19). In both controls and duodenal ulcer subjects the plateau of pyloric loss was not as stable as that of Vg. There was no discernible change in the pattern of pyloric loss in relation to the smoking of a single cigarette (Fig 6.19).

iii). Duodenogastric reflux

In both groups there was a fall of about 50% in duodenogastric reflux after smoking a single cigarette: controls 1.3 to 0.7 ml/h and DU's 33.0 to 14.7 ml/h (Table 6.19). However in neither group did this reach significance. Again no discernible change in the pattern of duodenogastric reflux related to the smoking of a single cigarette could be discerned (Fig 6.20).

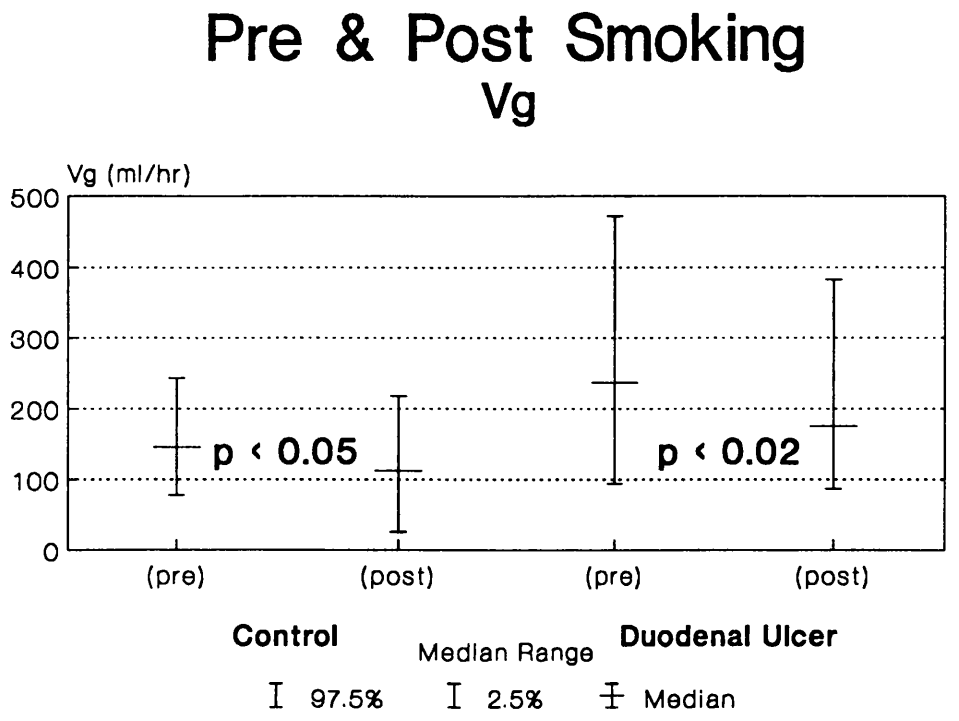
It is to be noted that the pattern of pyloric loss and duodenogastric reflux with time is different over the first hour compared with the latter two hours. This just highlights the alteration in these two measurements that occurs when the level of stimulation is changed from basal to sub-maximal stimulation. No such similar change in the pattern in either pyloric loss or duodenogastric reflux occurs after the smoking of one cigarette. This just serves to emphasise the results of the statistical analysis that showed that smoking had no significant effect upon trans-pyloric fluid shifts.

Gastric secretion

Pre & Post Smoking

		Medians Pre	p	Post	
CONTROLS					
Vg (ml/h)	(78.0 → 243.0)	**145.8**	<0.05	**112.2**	(25.8 → 218.4)
P.loss (ml/h)	(-27.6 → 37.8)	**16.8**	n.s	**16.8**	(-10.2 → 33.6)
DGR (ml/h)	(-7.8 → 90.0)	**1.3**	n.s	**0.7**	(-7.2 → 21.6)
DUODENAL ULCER					
Vg (ml/h)	(93.6 → 472.8)	**237.6**	<0.02	**175.2**	(87.0 → 383.4)
P.loss (ml/h)	(-46.2 → 159.6)	**30.0**	n.s	37.5	(-32.4 → 70.8)
DGR (ml/h)	(13.2 → 51.0)	**33.0**	n.s	**14.7**	(11.4 → 55.8)

Table 6.19



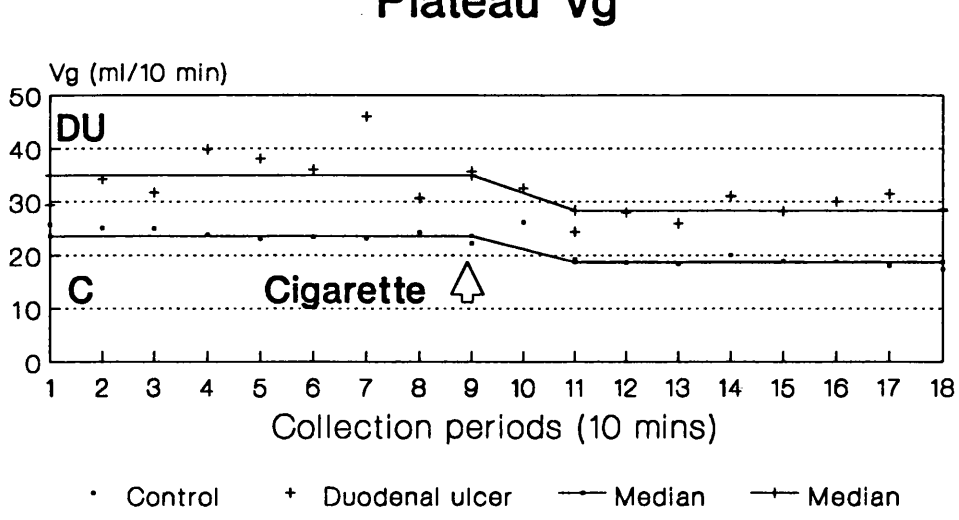
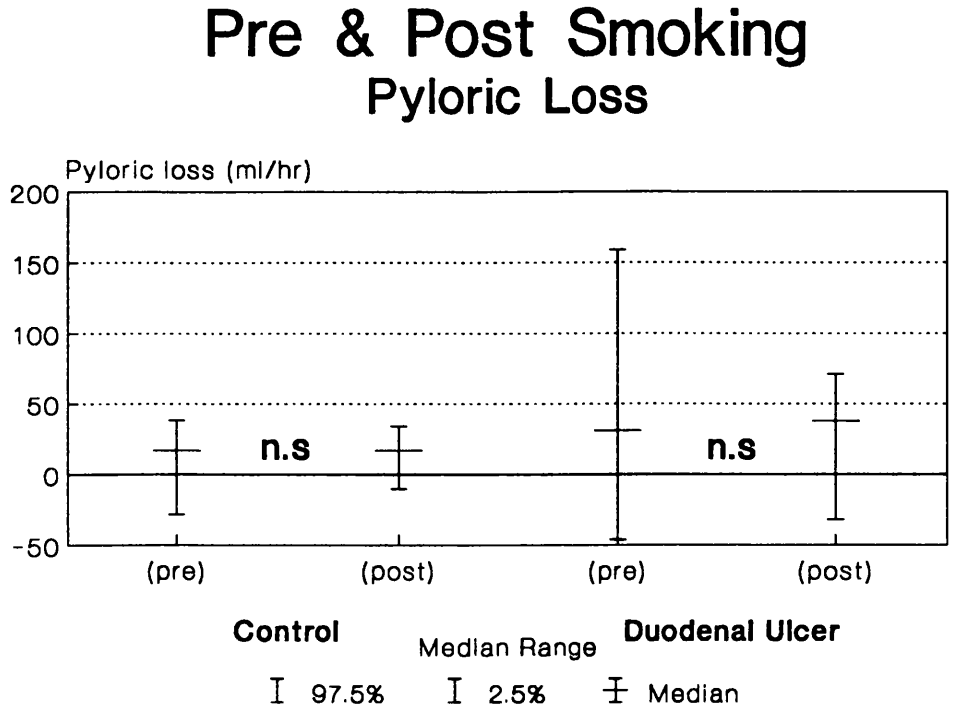


Fig 6.18
The acute effect of smoking upon Vg and its temporal relationship



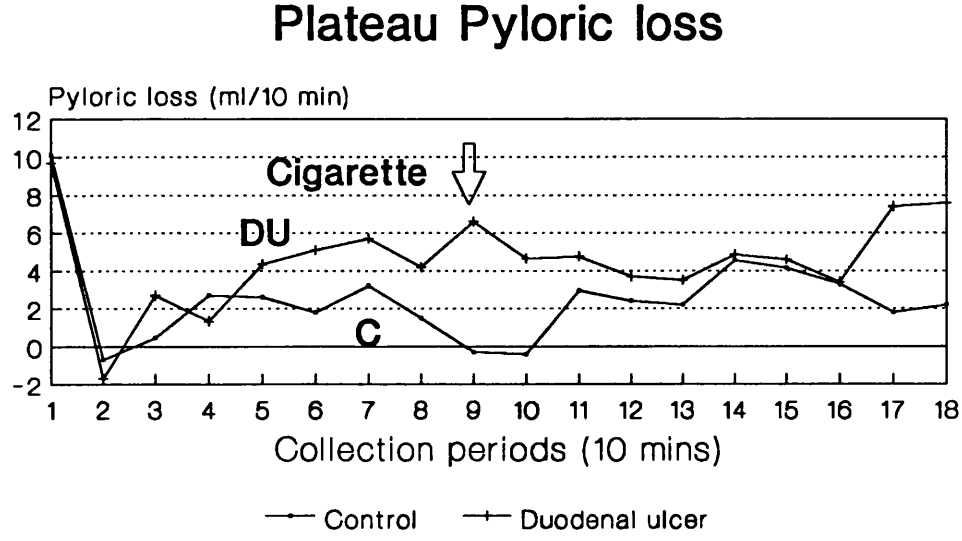
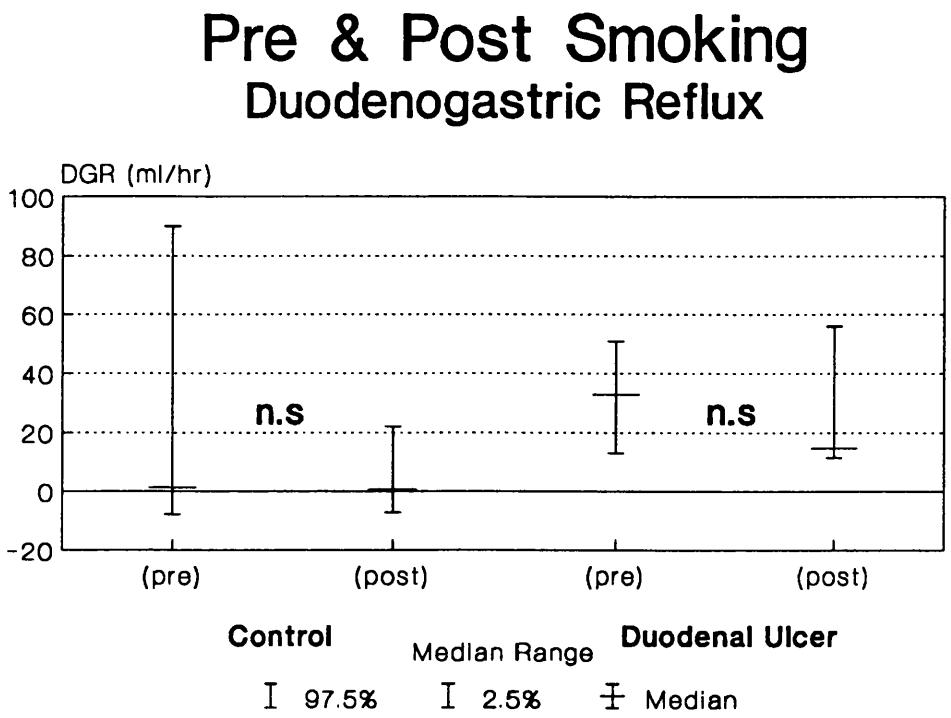


Fig 6.19
The acute effects of smoking upon PL and its temporal relationship



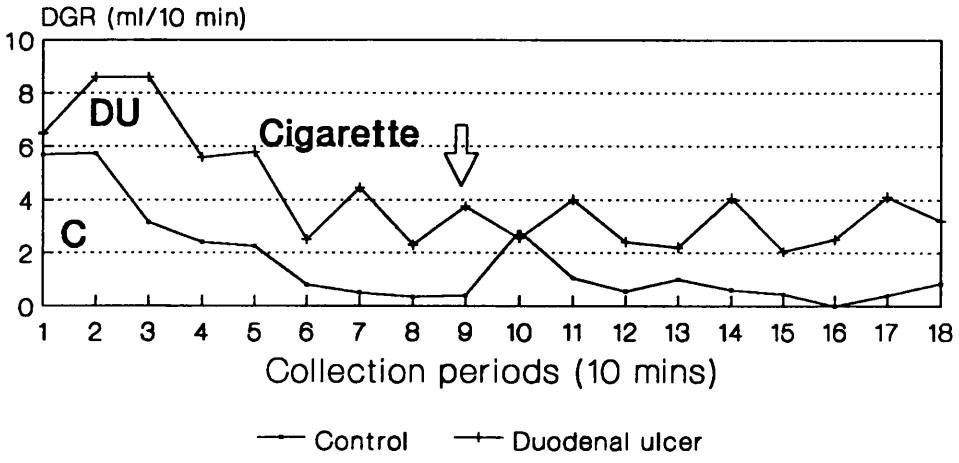


Fig 6.20
The acute effect of smoking on DGR
and its temporal relationship

DISCUSSION

1/. Subjects

i). Duodenal ulcer patients.

The type of patient referred to the hospital clinics with a diagnosis of duodenal ulcer disease has changed over the last few years. Many of these patients have initially been treated by their own doctor; a referral to hospital only being made when treatment failed or a relapse has occurred. This is of course due to the widespread treatment of 'ulcer like' symptoms by H_2 antagonists. Thus the more recently studied patients represent a more selected population, with possibly a more severe form of duodenal ulcer disease than those studied five or even ten years ago. Any effects of this would be highlighted in the most recent sub-group studied ($1/8^{th}$ maximal DU); which is also the second smallest sub-group. There is no recognised way to study this possible effect; but it must be borne in mind when analysing data and comparing it to other studies.

ii). Controls

The controls were all patients or student volunteers and the difficulty in obtaining such subjects has increased over the years. The patients considered suitable for this study are those with unrelated conditions, such as inguinal hernias or benign breast

disease. Nowadays such people are more aware of the potential risks of any medical procedure, and are more reticent to volunteer for a procedure that has no direct benefit to themselves. This is as it should be, but it does mean that the type of control subject has changed over the years. In the most recent control sub-group ($1/8^{th}$ maximal), 75% were paid student volunteers; the majority of these were female nurses. This is a reflection of the fact that amongst students, female nurses are the most impoverished smokers! This has an obvious effect on the profile of the sub-group compared to the others; this is considered in detail on the discussion on Vg.

2/. Techniques

i). Preparation

All subjects were asked to refrain from ingesting food, liquids and H_2 antagonists and cigarette smoking. Inspection of the nature and volume of the initial aspirate from the naso-gastric tube confirmed compliance with the first request. However in the main it had to be taken on trust that the patient had refrained from smoking for 12 hours, although in-patients were closely supervised to ensure that they did not smoke.

ii). Plateau stimulation

It has been the practice of this department to use an intravenous histamine infusion to stimulate gastric secretion. This gives a more reliable plateau (Lawrie et al 1964) of secretion compared to the transient peak obtained with a single injection of histamine (Kay 1953). It is claimed that a continuous infusion of pentagastrin is as accurate (Aubrey 1970) but some work has suggested that 'fade' occurs with pentagastrin (Emas and Svensson 1972). There is no evidence that fade occurs when sub-maximal doses of histamine are used to stimulate gastric secretion. Indeed it was the very stability of this plateau that allowed the effect of acid-base changes upon gastric secretion to be made (Hobsley and Silen 1966). A detailed analysis of this plateau stability can be found in Appendix I.

The use of histamine is criticised on the grounds of patient safety and comfort; indeed one series reported a 5% incidence of serious side-effects (Johnson and Robinson 1967) even after the administration of an anti-histamine. Some patients do undoubtedly feel flushed (histamine) and sleepy (anti-histamine); but in over 1400 gastric secretion tests performed in this department there have been no serious side effects.

iii). Collection periods

The minimum collection period with histamine was one hour but in the vast majority of cases secretions were

collected over 90 minutes. It has been shown that a collection period of one hour is as accurate as one of two hours (Aubrey 1970). However the selection of a plateau by the computer occurs more often if the collection period is 90 minutes (see Methods page 67). As with any computer programme it is important to realise that its failure to select a plateau does not necessarily mean that a plateau has not been reached (Whitfield and Hobsley 1979b).

DOSE-RESPONSE RELATIONSHIPS

1/. Absolute values of Vg

As expected increasing exogenous histamine produced increasing amounts of Vg; the effect of other factors that influence Vg and might have a bearing upon the dose-response relationship must be considered.

The stature of the eight sub-groups showed no significant differences when analysed in terms of height and weight. These factors have been shown to have a strong positive correlation with Vg, both when considered individually or in combination as Lean Body Mass. Height alone shows a good positive correlation ($r = 0.587$), this is almost as good as the more complicated derivation of Lean Body Mass ($r = 0.618$) (Hassan and Hobsley 1971). It is thus routine practice in this department to standardise all measurements of Vg to a height of 170cm. However since there is no significant height difference between the sub-groups this has not been done in the present study.

Age is also known to be related to gastric secretion; tending to decrease with increasing age (Hassan and Hobsley 1971); the relationship is though more complicated than this simple statement implies. Factors such as decreasing height (Marks 1961), and an increasing incidence of gastritis (Baron 1963) are associated with increasing age; these may well be

contributory factors. Age standardization is not routine as the relationship to Vg is nowhere near as clear cut nor is it anything like as large an effect as it is for height. The control group who received 1/8th stimulation were significantly younger than all the others; so the value of Vg may be slightly higher. This age factor might explain why there is less difference than one might have expected between the secretion rate of this sub-group (Control 1/8th) on one hand, and the secretion rates of DU 1/8th and Duodenal Ulcer 1/4 sub-groups on the other hand.

The smoking factor in this 1/8th control group is significantly lower than all the rest (table 5.4); and this is due to their shorter smoking history. In smokers the increase in gastric secretion is related to the length of smoking history and the daily cigarette consumption (Parente et al 1985, Massarat et al 1986) This group (1/8th C) who are all smokers will have a lower Vg than expected when compared to the other sub-groups with longer smoking histories.

It therefore seems likely that these two factors, age and smoking history probably cancel each other out. The lack of significance of the difference between 1/4 and 1/8th stimulation in both the duodenal ulcer and control sub-groups is therefore likely to be due to the smaller numbers of subjects studied, rather than any other factor.

2/. Dose-response analysis

Classically a dose-response study is performed using either the single-dose (SD) or the continuous-dose (CD) regimen. In this the same patient receives increasing doses of the same stimulant as either single doses on different days (SD) or as a stepways infusion on a single day (CD). In an experimental model it has been shown that if the stimulant used is histamine then either method can be used with equal accuracy in calculating Km and Vmax (Emas and Svensson 1972).

In this study the dose response studies were obtained in a different manner. All subjects received sub-maximal stimulation (either 1/4 or 1/8 maximal histamine) immediately followed by maximal stimulation. No person was subjected to both sub-maximal doses and the majority of basal studies were followed by maximal stimulation alone. The standard dose-response techniques allow accurate results to be obtained from small numbers. In this study, it is believed that the use of larger numbers and the accurate technique for measuring gastric secretion, compensates for any errors arising from the use of different individuals to plot different points on the dose-response curve.

i). Standard plots

The freehand plots of histamine dose against Vg show part of a typical dose-response curve (fig 6.3),

relatively linear over the mid-range and flattening out at the upper end of the range: a rectangular hyperbola. This type of plot is very limited in its use and can only indicate that the system is likely to follow simple dose-response kinetics. To test that this is indeed a rectangular hyperbola, and to study the system in more detail, it is necessary to use linear transformations.

ii). Linear Transformations

There are two parameters that characterise the dose-response relationships, K_x and R_{max}. R_{max} is the calculated maximal response of the system to an infinite dose of stimulant; K_x is the dose required to produce half of this response. Only once these are known is it possible to compare the responses of two populations, such as controls and duodenal ulcer patients, to a drug such as histamine.

The formula for the rectangular hyperbola that is typical of dose-response curves can be derived from the law of mass action (Clark 1933) and contains four functions: dose(D), response(R), R_{max} and Kx.

$$R = R_{max} (D) / (Kx + D) \qquad (1)$$

It is not practical from a few dose-response pairs to see if the data fit this equation but it is possible to represent this equation (1) in the linear form $y = mx + c$ (Equations 2,3,4).

$$R = R_{max} - Kx (R/D) \qquad (2)$$

$$D/R = (Kx/R_{max}) + (1/R_{max})D \qquad (3)$$

$$1/R = (1/R_{max}) + (Kx/R_{max})(1/D) \qquad (4)$$

It is standard practice to use one of these linear transformations in dose-response studies.

In equation (2) the dependent variable (R) appears on both sides of the equation, there will therefore be some degree of inevitable correlation. Since both variables are subject to error it is in theory no longer possible to use the method of least squares to test the linear fit. In equation 3 the independent variable (D) is found on both sides of the formula and so there is again some degree of inevitable correlation. The Lineweaver-Burke plot (equation 4) suffers from a different criticism. The use of reciprocals tends to give undue emphasis to the smallest values of R, which are indeed the ones likely to have the greatest percentage error. This is also true of equation 3.

In a mathematical model Dowd and Riggs (1965) calculated the percentage errors in K_x and R_{max}, when determined by these three equations; and compared them to the actual values in the model. It was shown that the Lineweaver-Burke plot (4) was much less reliable than the other two. Amongst the others (Equations 2,3) the plot R vs R/D (2) was slightly superior when the error in R was large and variable. It was also claimed that it exaggerates any deviation of the data from the theoretical relationship; this is known as the Hofstee plot. Thus poor data will not give as good a fit

compared to the other equations, whereas the opposite is true of the Lineweaver-Burke plot (4).

The application of the data to these three equations gave strikingly similar results for both K_x and R_{max} (Vg.max) in both controls and duodenal ulcer patients (Fig 6.4, 6.5, 6.6 and Table 6.2).

a). R_{max} (Vg_{max})

We know that the Vg at maximal stimulation (0.04 mg/kg/hr) in duodenal ulcer patients is significantly greater than in controls. It is therefore to be expected that the values of Vg_{max} will also be significantly different. In these studies Vg (0.04) at maximal stimulation was 89% of Vg.max for both controls and duodenal ulcer patients. Vg_{max} is a theoretical consideration and does not relate to the practical situation since it is the calculated response to an infinite dose of stimulant. Increasing the dose of 0.04 mg/kg/hr is unlikely to produce any significant increase in gastric secretion (Kay 1953, Lawrie et al 1964).

b). K_x

In all the equations the values of K_x were remarkably similar in both controls and duodenal ulcer patients. In all cases K_x was greater in the duodenal ulcer group than in the controls, but only by a very small amount (3-7%). Since the data come from different individuals there is not a separate K_x for each subject; statistical analysis

by the normal means is not therefore possible. However in the plot R vs R/D (Hofstee), the slope is equal to $-K_x$ and so can be tested by standard methods for comparing slopes; this showed there to be no significant difference between the two values of K_x. Since this plot is the most testing, it is likely that the difference between the values of K_x in the other two equations also failed to reach significance.

iii). The problem of basal secretion

In most dose-response studies the level of basal stimulation is ignored, although part of each response is due to basal stimulation. An elegant theoretical examination into the problems of basal secretion and dose-response analysis suggested that it should be taken into account (Grossman 1973). In this model it is assumed that the system under study obeys dose-response kinetics perfectly (equation 1); and that basal secretion is driven by a stimulus that is additive to the exogenous stimuli under study. In the model used, the subtraction of basal secretion improved the fit of the data to a standard linear transformation of the dose-response curve. Failure to do this resulted in an artificially low estimate of Vg_{max} and K_x. However in practice two more assumptions must be made when subtracting basals: first that the subtraction of basal secretion does not affect the accuracy of the raw data; and secondly that it is

applicable to both controls and duodenal ulcer patients.

The application of this to the data presented here did not produce as uniformly good results as when the basals were ignored. In only one plot, that of D/R vs D (equation 3), was there a significant correlation for both populations: controls and duodenal ulcer patients. The plot most likely to produce a 'good fit' (1/R vs 1/D) and the plot most likely to show up any deviations (R vs R/D) failed to show any significant correlation with the data from the duodenal ulcer patients.

The values of Vg_{max} (calculated from equation 3) of 267.0 ml/hr (C) and 376.0 ml/hr (DU) were very similar to the values obtained when basal secretion was ignored, 262.0 ml/hr (C) and 363.0 ml/hr (DU). In all the control groups Vg_{max} could be calculated (equations 2,3 & 4); and it was of a similar order of magnitude to those values obtained when basal secretion was ignored (Table 6.2).

The K_x for controls (5.22×10^{-3} mg/kg/hr) was marginally smaller than for duodenal ulcer patients (5.47×10^{-3} mg/kg/hr), but it is unlikely to be significantly different. If it were it would suggest that ulcer patients were less sensitive to histamine than controls; and this seems unlikely. The mean value of K_x for the controls (equations 2,3 & 4) was 5.42 mg/kg/hr, this being almost identical to the single value obtained for the duodenal ulcer patients. The overall values of Kx were higher than if basals were ignored, in keeping with

Grossman's theory (Table 6.2). The failure to attain uniformly good linear transformation of the basal-subtracted data could be explained in several ways. First, because of the low volumes involved, basal secretion is likely to have a proportionately greater error than sub-maximal and maximal secretion. This means that the subtraction of one from the other is likely to increase the error; and this is going to affect the values of sub-maximal secretion more than maximal secretion. This would cause deviation from linearity at this end of the dose-response range, which indeed was the case. Secondly, endogenous basal secretion and exogenous histamine may not be simply additive, although there is no hard evidence for this. Thirdly, basal secretion in duodenal ulcer patients is perhaps inherently different from that in control patients; it was only in this group that linear transformation was not uniformly possible. However as will be discussed below this is also unlikely. It is likely that the first suggestion is the most acceptable explanation for these discrepancies.

Initial inspection of the dose-response data suggests that they form part of a typical dose-response curve. Using any of the standard linear transformations gave uniformly good results. There was marked similarity between values of Vg_{max} for controls (277.0, 262.0 & 260 ml/hr) and for duodenal ulcer patients (378.0, 363.0 & 365.0 ml/hr). The expected difference between the values of Vg_{max} for controls and duodenal ulcer patients was also

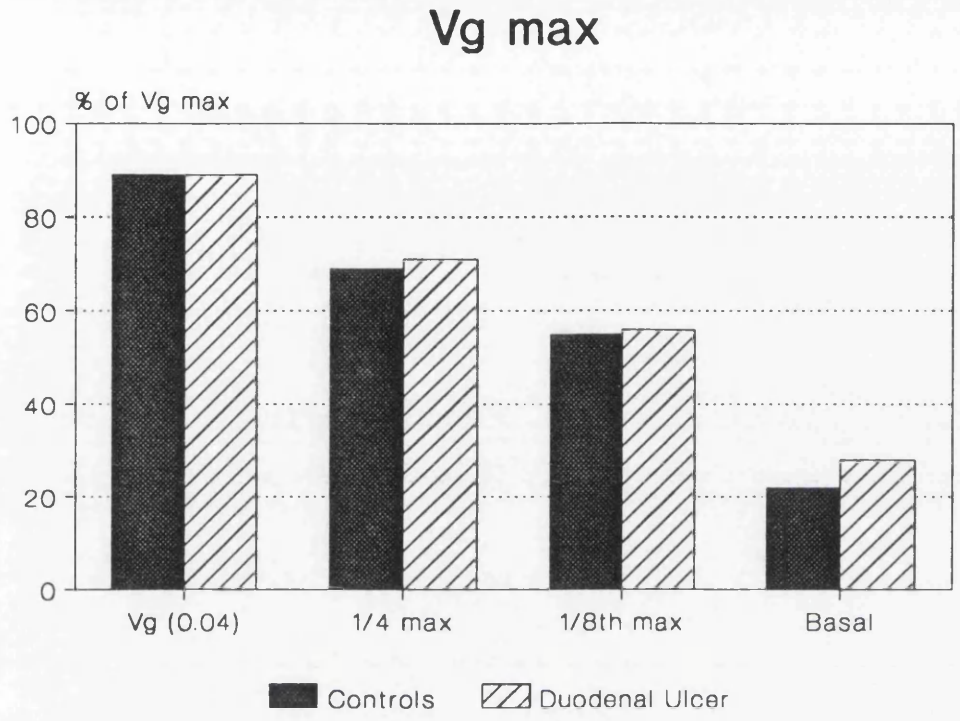
remarkably constant (101 ml/hr to 105 ml/hr). The values of K_x were strikingly similar (DU: 3.66, 3,70 & 3.88 x 10^{-3} mg/kg/hr; C: 3.78, 4.00 & 3.95 x 10^{-3} mg/kg/hr) and this strongly suggests that there is no difference in parietal cell sensitivity. Taking basal secretion into account failed to show such consistent results; this is most likely due to mathematical factors.

The values of Vg are therefore a reasonable representation of the dose-response relationships involved. Indeed, if this was not so it is unlikely that they would have stood up to more detailed analysis; and this has not been the case.

3/. The relationship of sub-maximal to maximal Vg

Given that a dose of about 3.9 x 10^{-3} mg/kg/hr produces a 50% stimulation in both groups; then other doses should cause similar percentage stimulations in both controls and duodenal ulcer patients. This is indeed so when basal and sub-maximal values of Vg are compared to Vg_{max} or $Vg_{0.04}$ (Fig 7.1).

The strong correlation between sub-maximal and maximal $Vg_{0.04}$ (Fig 6.7, 6.8, 6.9 and Table 6.3), in both controls and duodenal ulcer subjects provides strong evidence that there is no difference in the sensitivity of the two populations. If there were it would be expected that the percentage of maximal induced by a specific dose would be greater in duodenal ulcer patients



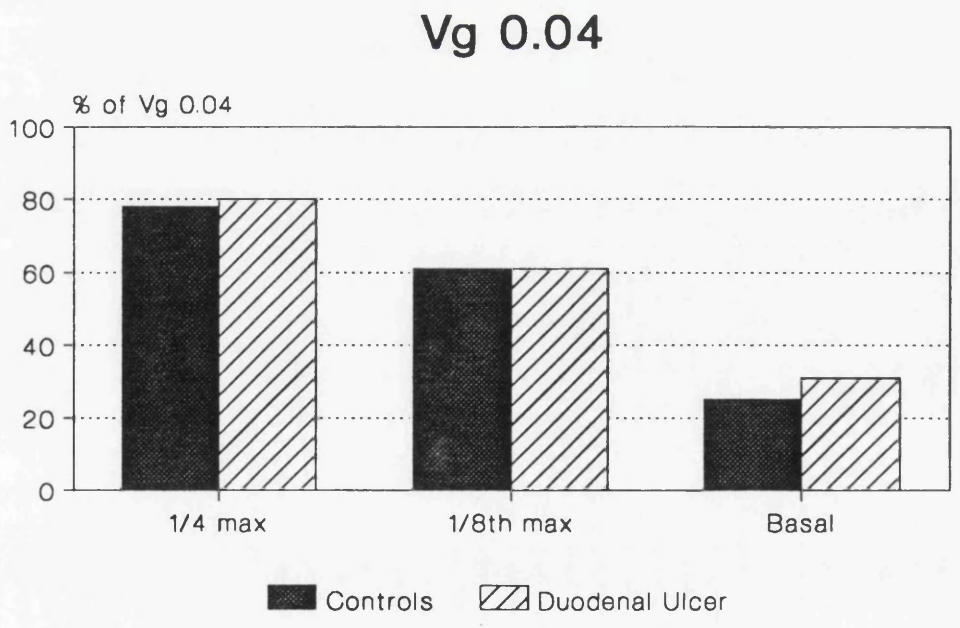


Fig 7.1
Relationship of sub-maximal Vg to actual and theoretical maximal Vg

than controls. The difference in basal secretion as a percentage of maximal is only of the order of 6%; and could easily be explained by the greater error inherent in the collections of low volumes of gastric juice. This is reflected in the results of regression analysis that reveals a correlation between basal and maximal secretion in both groups but with lower 'r values' (r = 0.37 & DU = 0.34).

4/. Theoretical Considerations

Let drug X combine with a receptor site S to form a complex SX; this produces a certain response, proportional to the amount (or concentration) of SX:

S + X <=> SX Response.

Application of the Law of Mass Action to this equilibrium permits us to develop a hyperbolic function which is identical to the classic Michaelis-Menten equation; the linear transformations used in enzyme kinetics are therefore equally applicable (Appendix II). However in the case of drug-receptor interactions it must be appreciated that there are three critical assumptions upon which this equation is based:

1/. The biological response is proportional to the receptor occupancy; known as the "occupancy assumption".

2/. One drug molecule combines with one receptor site.

3/. A negligible fraction of the total drug is combined.

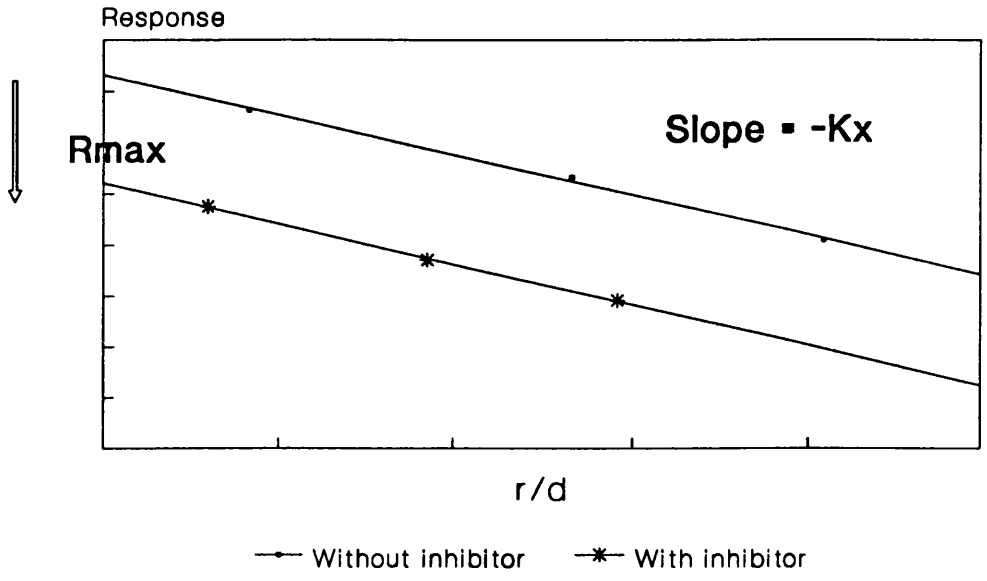
In the case of controls and duodenal ulcer patients the values of K_x and R_{max} have been calculated and can be represented graphically (Fig 7.2). Can this be interpreted in terms of the "occupancy assumption"? If the number of receptor sites available for drug interaction is reduced, by for example a non-competitive inhibitor, what is the effect? The maximal response R_{max} will be reduced in proportion to the loss of receptor sites; however those receptors that remain will have the same affinity for the drug as before, thus K_x is unchanged. This gives an identical linear plot to that of the duodenal ulcer patients and controls (Fig 7.2). This would also explain the fact that identical doses produce identical percentage stimulation in both groups.

Since we are dealing with the same drug given to different populations, any difference must be the result of variation in either the histamine receptors or in the parietal cells. If there were receptors of varying sensitivities, how would this be interpreted in terms of K_x and R_{max}? It is assumed that:

1/. The receptor is either dormant or active.

2/. Each receptor when activated contributes equally to the response.

Hofstee transformation
Presence of non-competitive inhibitor



Population of two sizes (C & DU)

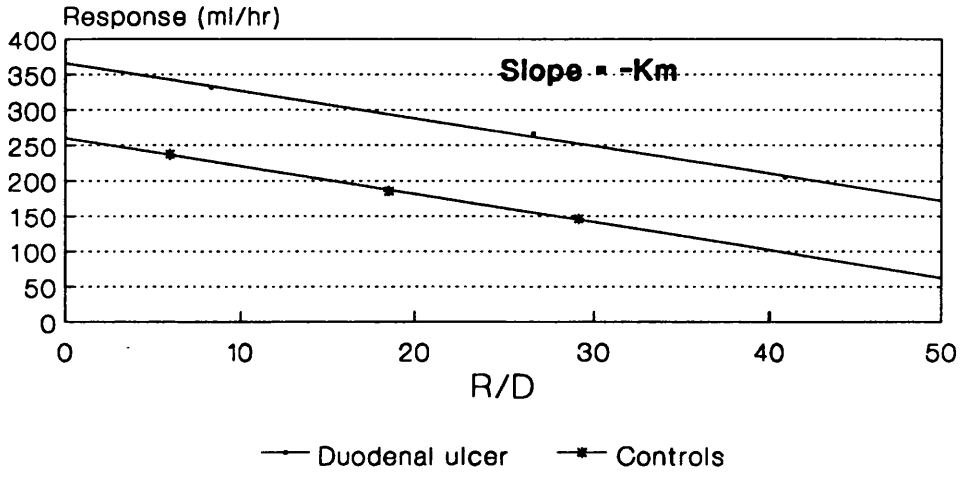


Fig 7.2
Comparison of linear transformations in inhibition and increased receptor states

3/. The threshold for stimulation varies with each receptor.

If we plot the sensitivities (log threshold-dose) against frequency a Gaussian curve is obtained (Fig 7.3). Plotting this as a cumulative frequency distribution a sigmoid curve is obtained[1], in other words a typical log-dose curve (Fig 7.3). In this the median log threshold-dose is equal to K_x and the area under the Gaussian curve to R_{max}. So for duodenal ulcer patients the area under the curve will be greater than for controls; they will however share the same median, as Kx is the same for both. Since the sub-maximal (1/4 & $1/8^{th}$ maximal) doses stimulate 80% and 60% respectively in both groups; the shape of the curves must also be the same.

In these models the results of the dose-response studies can be explained solely by a difference in the size of the parietal cell mass of the two populations. Other theories have been advanced to explain various drug receptor interactions; but there is no more evidence for their existence than there is for the more simple theories expounded above.

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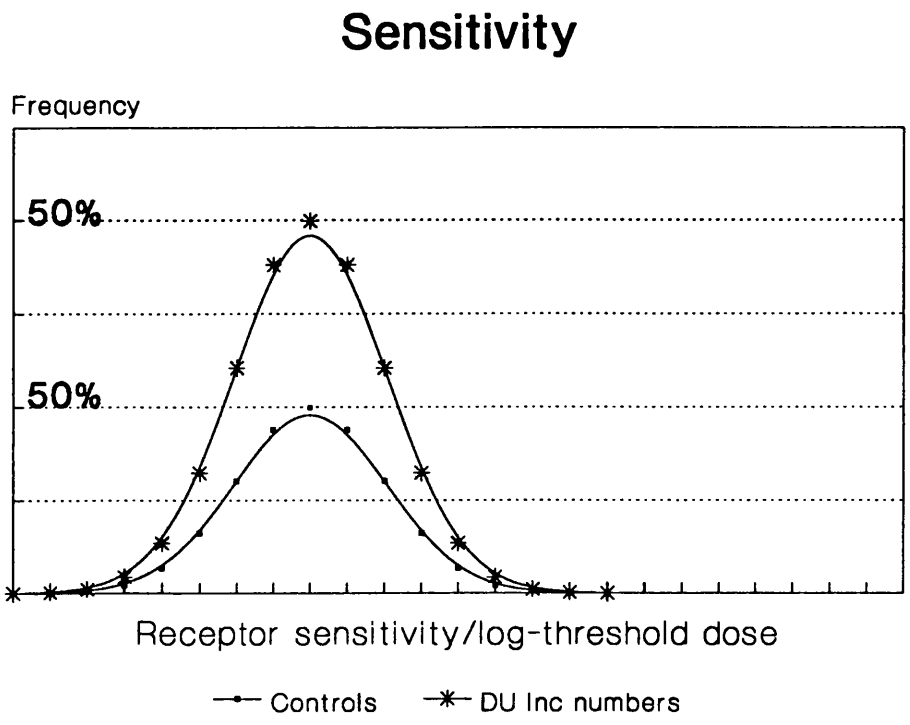
[1]. A simple dose-response curve is hyperbolic, if the log-dose is plotted against response then a linear plot is obtained. This cannot be linear from zero to infinity and it therefore is assumed to plateau at the extremes of the dose range - hence the sigmoid curve of the log-dose response plot.



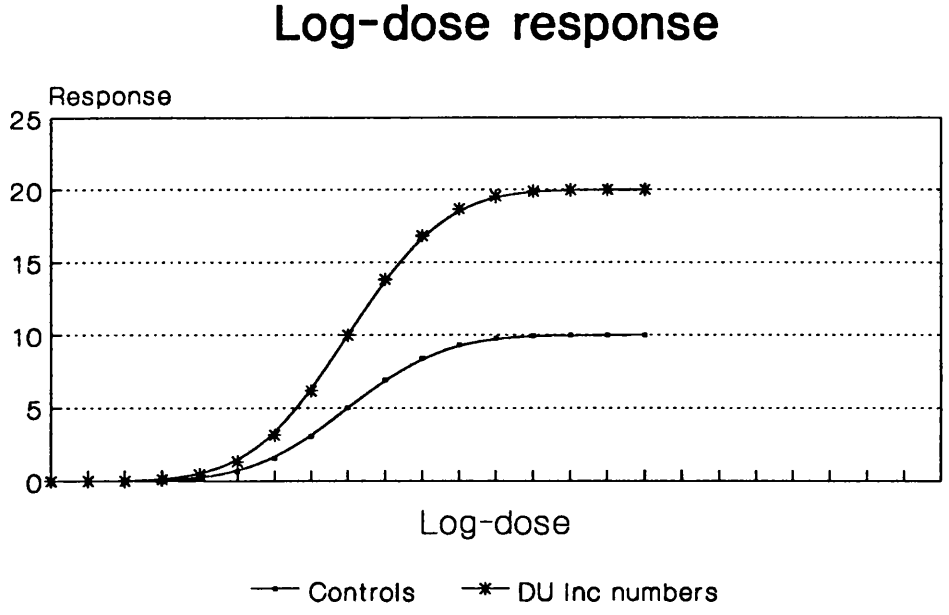


Fig 7.3
The relationship between dose-response and parietal cell distribution

5/. Size versus sensitivity of the parietal cell mass

i). Increased parietal cell mass

a). Basal secretion

In a study (Hunt et al 1963) into the explanation for the increased basal secretion in duodenal ulcer patients three causes were proposed:

1/. Supra-normal drive on a normal population of parietal cells.

2/. Hyper-excitable parietal cells responding to a normal stimulus.

3/. Increased number of parietal cells in duodenal ulcer patients.

Initial work had been to examine the proportion of basal secretion to maximal secretion by histamine (Hunt and Kay 1954); in both groups basal secretion had been found to be 25% of maximal. Studies into the effect of pharmacological (Seidelin 1961) and surgical vagotomy (Gelb et al 1961) had shown that they had an equal effect on basal and maximal secretion.

Hunt and his co-workers then re-examined their data using a measure of gastric secretion called the "parietal component": a corrected figure that allowed for the bicarbonate secretion of non-parietal cells. The percentage of basal : maximal secretion was studied in both groups and again found to be very similar: in the region of 25%. They concluded that in the light of their work, as well as that of others, the difference in basal

secretion was solely due to a difference in the size of the parietal cell mass; hyper-excitability was not thought to be an important factor.

In another study, basal secretion, corrected for pyloric loss and duodenogastric reflux, was measured in controls, patients with duodenal ulcers and vagotomy subjects (Faber and Hobsley 1977). As expected, basal secretion was significantly greater in the ulcer group than in controls. The extreme variability in basal secretion was noted, but this was attributed to physiological variation rather than to experimental error. The study of the vagotomy subjects concluded that increased vagal drive contributed to duodenal ulceration in only a minority of patients: 20 to 25%. The proportion of basal : maximal secretion was similar to other studies: 22% in controls and 31% in duodenal ulcer patients. However there was no significant relationship found between the basal and maximal secretion in individuals. This is in disagreement with work that showed a weak, but significant correlation between basal secretion and pentagastrin stimulated maximal acid output (Wormsley and Grossman 1965).

Can the dose receptor models proposed above explain all these findings? To do so the basal secretogogue must be assumed to be endogenous histamine, secreted in response to the conditions of the test. It is also reasonable to assume that the individual basal response varies widely. It appears as though varying amounts of

the endogenous secretogogue histamine are secreted in response to the same basal conditions of the test. Three individuals with the same parietal cell mass could have widely differing sensitivities to the test conditions, producing differing amounts of histamine and so of basal secretion. The relationship of each basal to maximal secretion would be different in each case. If the parietal cell mass of these three was doubled then the same variation in response would still exist and again no relationship of basal to maximal would be found. The only difference would be the absolute size of the response. The first group can be thought of as the controls and the second as the duodenal ulcer patients. The assumption also dictates that the median sensitivity to the test conditions produces a median endogenous dose of histamine that acts on two parietal cell populations of differing size. The net result is significantly differing basals, with a similar percentage of basal : maximal; but with no individual relationship between basal and maximal gastric secretion. This explains the majority of findings about basal secretion (Baron 1963, Lam et al 1980); and also explains why the subtraction of basal secretion in dose-response studies is not as simple as some suggest (Grossman 1973).

However the correlation between basal and maximal Vg in this study suggests that this is not the complete story. The correlation coefficients were much lower than for the other two levels of stimulation (1/4 & 1/8th) but

were still significant. It is probable that the individual variation to the test is not as great as some authors have suggested. So with the large numbers studied in the present study a weak but significant correlation has been found.

b). Measurement of the parietal cell mass

In 1950 Tongen showed that the total acid concentration during a triple-histamine test was roughly proportional to the parietal cell concentration. Cox in 1952 showed that duodenal ulcer patients have a larger parietal cell population than those with gastric ulcers. This work stimulated Card and Marks in 1960 to study the phenomenon using more accurate techniques. The maximal acid output (MAO) using the augmented histamine test was measured before and after gastrectomy. The parietal cells in the portion of stomach resected were counted after corrections were made for shrinkage, cell over-estimation and mucosal thickness. A significant linear correlation between MAO and parietal cell mass was found ($r = 0.95$); a marginally better fit ($r = 0.97$) was obtained with a curvi-linear fit. This was thought to be due to technical problems such as failure to account for basal secretion, inability to determine R_{max} or counting non-functional cells in patients with gastritis. This work was confirmed when the same technique was used on 12 Orientals with duodenal ulcers who were examined before and after gastric resection (Cheng et al 1977). Again a

strong correlation between parietal cell mass and MAO was found (r = 0.95) and the calculated output of acid per unit parietal cell mass was identical to that found by Card and Marks.

c). Dose-response studies.

Dose-response has been studied using two different approaches. One has been to calculate the percentage of maximal that is elicited by a certain dose of stimulant and this is compared in controls and duodenal ulcer patients. Graded doses of histamine were used on two such groups, each consisting of five individuals. The percentage response obtained at various doses was similar in both controls and duodenal ulcer patients (Hunt and Kay 1954). The response to sub-maximal doses of pentagastrin (0.001 & 0.01 microgm/kg/min) was expressed as a percentage of maximal in 13 controls and 23 patients with duodenal ulcers. There was no significant difference between controls and duodenal ulcer patients in the percentage of maximal elicited by each of the these doses (Wormsley and Mahoney 1967).

The authors of both studies concluded that there was no evidence for any increased sensitivity in the parietal cells of duodenal ulcer patients.

More commonly, standard dose response experiments are carried out. Such a study, using pentagastrin, was performed on 11 male controls and 14 males with duodenal ulcers who had similar secretory capacity (Aly and Emas

1982). The peak acid output to various doses of pentagastrin was determined and corrected for basal secretion; all values were corrected for pyloric loss. The mean values of the individual dose-response curves were used to produce a mean dose-response curve for controls and duodenal patients. Using a standard linear transformation, no significant difference was found between the K_x values for the two populations. The authors concluded that in males of similar secretory capacity, sensitivity did not play an important role.

ii). Increased sensitivity in the duodenal ulcer patient.

Two studies using pentagastrin claimed that increased sensitivity was an important factor in duodenal ulcer patients (Isenberg et al 1975 & Petersen and Myren 1975). In both the sizes of the control and ulcer groups were similar: about 20 each. No corrections were made for either pyloric loss or duodenogastric reflux, but in both basal secretion was subtracted. In both, the K_x for duodenal ulcer patients was significantly lower than controls; the authors concluded that these patients were more sensitive to pentagastrin than controls. Increased vagal tone, the presence of a circulating inhibitor or an abnormal pentagastrin metabolism were three hypotheses offered to explain this increased pentagastrin sensitivity (Isenberg et al 1975). The authors did acknowledge that the size of the parietal cell mass was still an important factor but were unable to explain the

discrepancy between their results and earlier work (Hunt and Kay 1954 & Wormsley and Mahoney 1967).
The supporters of the increased sensitivity theory have little hard evidence of a physiological mechanism that accounts for the increased R_{max} and decreased K_x in duodenal ulcer patients. Thus, for the present the weight of evidence seems to support the findings of this study. That is, that the difference in the basal, sub-maximal and maximal gastric secretion between a control and duodenal ulcer patient can be explained solely by a difference in the size of the parietal cell mass.

6/. Smoking and secretory capacity

In the two groups studied in the present investigation there were both smokers and non-smokers; but there were almost three times more smokers in the duodenal ulcer group than in the control group ($p < 0.005$). If chronic smoking had any effect upon the sensitivity of the parietal cell mass then it might have been expected to show up in these dose-response studies. An increase in sensitivity of the parietal cells of smokers would give rise to a lower value of K_x; this would exert a greater effect upon the overall value of K_x in the duodenal ulcer group, since they have more smokers. The remarkably similar values of K_x and the extremely good linear fit in all the studies suggest that this is not the case.

A more detailed study of the effect of chronic smoking upon was possible in the control 1/4 maximal sub-group. In this it was found that the same sub-maximal dose (0.01 mg/kg/hr) produced an almost identical response in terms of a fraction of the maximal possible dose, 77.5% in non-smokers and 78.7% in smokers. It is also to be noted that the linear relationship between 1/4 maximal Vg and maximal Vg is the same in non-smokers and smokers, the x coefficients being 0.81 and 0.78 respectively (Fig 6.10 and Table 6.3). This strongly suggests that in this sub-group at least smoking has no detectable effect upon the sensitivity of the parietal cells to exogenous histamine.

A similar analysis was not possible in either the duodenal ulcer 1/4 maximal sub-group or the 1/8th section as there were very few non-smokers. The basal secretion section was not subjected to such an analysis since the correlation between basal Vg and maximal Vg, although significant, was not nearly as strong (see above).

There is no other work, known to this author, that has studied the effect of chronic smoking upon the dose-response properties of the parietal cell mass. It seems reasonable to suggest that the increased secretory capacity of smokers is, as with duodenal ulcer patients, solely due to an increased parietal cell mass.

1/. Pyloric loss

i).Artefacts

The relative merits of the various dilution indicators have been discussed earlier, and will therefore not be dealt with further (see page 20).

Can the extent of pyloric loss measured during a particular study be attributed to technical problems, such as placement of the tube? There have been studies that show that tube position (Hassan and Hobsley 1970, Findlay et al 1972), tube design and subject position (Hassan and Hobsley 1970) all have no effect on the completeness of aspiration.

The volume calculated by the application of the dye dilution technique is, as has been stated already, equivalent to pyloric loss. In theory this value could range from zero up to a value equivalent to the total amount of fluid within the stomach. Indeed in all 8 sub-groups pyloric loss is a significant volume: median loss being between 10 to 40% of Vg. However in all 8 sub-groups the range has a negative component, this is clearly not meaningful and the explanation must lie in the experimental technique. If there is no pyloric loss then the recovery of phenol red would be 100%, since the volume aspirated cannot exceed the volume infused. There is a certain amount of experimental error inherent in the technique; it is thus possible for this to give rise to

apparent phenol red recoveries of more than 100%. This in turn would give rise to the calculation of a negative pyloric loss. This error seems not to be related to the volume of aspirated gastric juice. Examination of the four largest sub-groups (Maximal C & DU, Control C & DU) shows that in all but one (Maximal DU) the ranges of pyloric loss are almost identical; approximately -10 to 180 ml/hr. In the group of duodenal ulcers which secreted maximally, ie the group with the largest volumes of aspiration the range is even greater: -35 to 264 ml/hr.

ii). Pyloric loss and Vg

In any one individual the rate of pyloric loss does not change significantly over the wide range of stimulation from basal to maximal secretion. In an individual there may be a significant rise in Vg from basal to maximal secretion (300% - 400%) but pyloric loss remains constant. It seems that the rate of pyloric loss in an individual is relatively constant and is not determined by the secretory state of that individual at that time.

The small range over which median pyloric loss varies in all 8 sub-groups might, upon initial inspection, suggest that it is a fixed amount. However regression analysis has shown that in 6 of the 8 sub-groups there is a significant positive correlation between Vg and pyloric loss; this would not be so if pyloric loss was a fixed amount. The failure to show this correlation in 2 sub-groups (1/8th maximal C & DU)

is most probably due to the small numbers involved. Initial inspection would suggest that this is in complete contradiction to the findings discussed above, however there is a simple explanation. There is a significant positive correlation between Vg and pyloric loss at both basal and maximal secretion. It thus appears that those individuals with a large secretory capacity, as shown by a large basal and maximal secretion, will have a larger pyloric loss than an individual with a lesser secretory capacity.

Pyloric loss is constant regardless of the secretory state and there is significant positive correlation between basal and maximal Vg; thus when pyloric loss is correlated against maximal and basal Vg there will be one true correlation and one correlation by association result. The relationship between maximal Vg and pyloric loss is causal but the that of pyloric loss and basal Vg is casual

iii). Pyloric loss and disease

Under conditions of basal and maximal stimulation pyloric loss is significantly greater amongst duodenal ulcer patients compared to controls. Is this a specific effect of the disease process or just a reflection of the greater secretory capacity of the duodenal ulcer patient?

Groups of controls and duodenal ulcer patients with a similar median Vg were found to have similar median rates of pyloric loss; this holds true at both extremes

of stimulation: basal and maximal. It thus appears that the presence of duodenal ulcer disease has no specific effect upon pyloric loss.

It has been shown that the duodenal acid load after a peptone meal was significantly greater in duodenal ulcer patients than in controls; the implication being that this was due to the disease (Cano and Isenberg 1975). This present study suggests that the increased acid load delivered to the duodenum is greater in those with duodenal ulcers, not because of their disease per se, but because of their secretory capacity.

The conclusion must be that this relationship between maximal Vg and pyloric loss is a real one. Indeed recent work has shown that phenol red estimated pyloric loss is physiological and gives an index of the intrinsic emptying ability of the stomach (Wieman et al 1989).

In any one subject pyloric loss has a constant value peculiar to that individual and the size of this loss is related to the maximal secretory capacity of that person.

2/. Duodenogastric reflux

The measurement of duodenogastric reflux is based upon the sodium concentration of the aspirated juice and upon certain assumptions made about the electrolyte concentrations of pure gastric and duodenal juices. It is assumed that when there is no duodenogastric reflux

the concentration of sodium in the aspirated juice will be equal to that of pure gastric juice; no allowances are made for swallowed saliva (Hobsley 1974). Zero reflux is therefore to some extent an arbitrarily defined figure: it is determined by the population studied by Hobsley and zero reflux relates to zero reflux in those patients alone. As with pyloric loss there is an element of experimental error: in the case of duodenogastric reflux, any error in the crucial measurement, (Na^+) in the gastric aspirate, has its greatest effect when it approaches zero, ie. when that concentration is about 7mmol/l (see page 66). In seven of the eight sub-groups the range of calculated duodenogastric reflux has a negative component; this must be artefactual since common sense dictates that true duodenogastric reflux cannot fall below zero. The mathematical formula from which duodenogastric reflux is calculated relies upon the use of an arbitrarily defined zero and the negative values of duodenogastric reflux simply reflect the errors in this definition.

The use of a 'sodium marker' has been validated by Fiddian-Green and colleagues (1979) using indocyanine green to label bile. The modern equivalent of indocyanine green is Tc^{99m}butyliminodiacetic acid (BIDA), and using scintillation techniques duodeno-gastric reflux is assessed by the use of external counters placed over the stomach (Thomas et al 1984). Using these techniques it has been shown that gastric intubation does

not have a significant effect upon the estimation of duodenogastric reflux (Wolverson et al 1984; Muller-Lissner et al 1982). It is the belief of the writer that the technique used in this study for the assessment of duodenogastric reflux is a valid one; indeed it may be more accurate than any method using 'marked bile' which constitutes only one part of duodenal contents (Pancreatic juice and Succus entericus) and whose presentation into the duodenum is intermittent. It is therefore reasonable to accept as meaningful any significant differences found between groups in this study.

The question whether patients with duodenal ulcer disease reflux more than controls was difficult to determine. At first sight the median values of duodenogastric reflux in the duodenal ulcer and control groups did not differ from each other when maximally stimulated. However there was evidence that reflux fell as the secretion rate rose; comparing reflux at basal and maximal secretion showed this in both groups (C & DU) (Fig 6.13 & Table 6.9). In order to disentangle the effect of Vg from any possible effect of the duodenal ulcer disease itself, recourse was made to the same technique that was used in similar circumstances when considering pyloric loss (page 114). A subset from each of the control and duodenal ulcer groups was arbitrarily chosen to include all individuals with the same ranges of Vg, whether they were duodenal ulcer patients or

controls, and the median values of reflux in the two groups was compared. In those with duodenal ulcers the absolute values of duodenogastric reflux were greater than controls when groups with similar median Vg were studied. However only at maximal stimulation did this reach significance. It would seem that the failure to reach significance is artefactual and due to the selection of the groups with similar median Vg's. When the groups as a whole, regardless of median Vg, were studied the same trend was found; duodenogastric reflux was higher in the duodenal ulcer group compared with the controls. It was only under conditions of basal secretion that this was significant. It is the feeling of this author that the increased duodenogastric reflux found in duodenal ulcer patients at all levels of stimulation is real and part of the disease process; and not as with pyloric loss related to the size of the parietal cell mass. The evidence for this is weak and any effect is probably small

Duodenogastric reflux is normal, it is found in control subjects regardless of the method used (Muller-Lissner et al 1983) and this is confirmed by the results of this study. It is thus not the 'all or none' pathological event that it was once thought to be. The finding of increased duodenogastric reflux in patients with duodenal ulcer disease is in agreement with other studies showing greater reflux in duodenal ulcer patients than in controls (Thomas 1983; Dewar et al 1982).

One study claims that the incidence of duodenogastric reflux is the same in both duodenal ulcer patients and controls (Wolverson et al 1985). In this particular study the measurement of duodenogastric reflux was on an 'all or none' basis, and no attempt was made to quantify the reflux in either group. All it showed was that duodenogastric reflux occurred in both groups, and this fact is not in dispute.

The majority of workers have used external scintillation techniques to measure duodenogastric reflux and this must be borne in mind when the results of this study are compared with other work.

In an extensive review of the literature (Niemala 1985) it was stated that:

> "duodenogastric reflux is common in symptom free control subjects....but it is particularly frequent in gastric ulcer patients and in duodenal ulcer patients."

This statement is more in line with the observations of this study than with findings of Wolverson and colleagues.

3/. Transpyloric flux

The absolute amount of prograde flow through the pylorus (pyloric loss) does not differ significantly

between basal and maximal stimulation in either the control or duodenal ulcer groups:

C. 22.2 to 25.5 ml/hr

DU. 39.6 to 47.4 ml/hr.

Retrograde flow of duodenal contents (duodenogastric reflux) falls as the level of stimulation rises from basal to maximal, this is true for both groups (C & Du):

C. 12.6 to -1.2 ml/hr

DU. 21.0 to 1.8 ml/hr.

In both groups (C & DU) the total flux across the pylorus falls as the level of stimulation rises:

C. 34.8 to 24.3 ml/hr

DU. 60.6 to 49.2 ml/hr

What is the logical explanation for this finding? First, it is important to realise that pyloric loss that refluxes back into the stomach is not duodenogastric reflux, it is not even pyloric loss; it is regarded as gastric juice that has never left the stomach. It could be that an increased hydrogen ion delivery to the duodenum has caused a reduction in duodenogastric reflux. Alternatively the increase in the stimulatory state of the stomach might be linked to an increase in the efficacy of the pylorus as an anti-reflux mechanism.

There is evidence that the pylorus has the characteristics of a physiological sphincter. In vitro work has shown that the pylorus possesses shows some features that distinguish it from the adjacent muscle. It has been shown to respond differently to gastrin (Lipshutz

and Cohen 1972) and to have different neuro-mechanical properties (Schulze-Delrieu and Shirazi 1983). In vivo work has shown that there is a 1.5cm high pressure zone within the region of the anatomical pylorus (Fisher et al 1973).

The activity of this sphincter is to some extent governed by the acidity of gastric and duodenal juice. The rate of gastric emptying is decreased by acidic solutions: the more acidic the solution the slower the emptying (Hunt and Knox 1972). This effect is thought to be mediated by specific small bowel receptors which have been localised in dog studies (Minami and McCallum 1984). Initially this appears to be in contradiction to our results with regard to pyloric loss, but in the present study the stomach was being kept empty. However, studies in humans have shown that the instillation of acid into the duodenum causes a significant rise in pyloric pressure of over 200% associated with a marked fall in duodenogastric reflux (Fisher et al 1973). This is in complete accord with our observations that reflux becomes less at higher rates of secretion.

In this study within any one group, be it control or duodenal ulcer, the rate of pyloric loss did not change between basal and maximal stimulation. At basal secretion the 'fixed alkaline component' of gastric secretion, exerts a greater effect upon the acidity of intra-luminal gastric juice than it does at maximal secretion. At maximal secretion this 'alkaline

component' is swamped by the vast increase in Vg, so in going from basal to maximal secretion there is an increase in both the volume and acidity of the intragastric juice. In this study the volume rate of pyloric loss remains constant during the change from basal to maximal stimulation, however since the acidity of the intragastric juice rises so must the acidity of the pyloric loss rise. Thus the rate of acid loss through the pylorus must also rise, this increased acid load could act on specific small bowel receptors which in turn would feed back on the pylorus, and the resulting increased pyloric activity would have the effect of reducing duodenogastric reflux.

Duodenal retroperistalsis is a recognised physiological event in duodenogastric reflux (Johnson and Eyre-Brook 1984). It may well be that when the stomach changes from the fasting (basal) to the feeding (stimulated) state, retroperistalsis is reduced to allow the prograde flow of chyme into the duodenum; and the observations of this study are simply a manifestation of this rather than the proposed increased pyloric activity. A reduction in the volume of non-gastric duodenal secretions could result in there being less duodenal contents to reflux. However an increase in the stimulatory state of the stomach is likely to be linked with an increase in these secretions in anticipation of the digestive process. The author has no evidence to refute this.

be
It might that the changes in transpyloric flux are all due to the agents used in the gastric secretion test. Histamine is a physiological gastric secretogogue and may also have a direct effect upon the pylorus, duodenal motility or duodenal secretions. However to determine whether or not histamine has a direct or indirect action on the duodenum or pylorus is a difficult question to answer. The antihistamine used is chosen for action upon H_1 receptors which are non-gastric and therefore it is extremely unlikely it would have an effect upon the gastro-duodeno axis, but some non-specific action cannot be ruled out.

4/. Smoking and trans pyloric loss

i). Pyloric loss

There was a positive correlation between Vg and pyloric loss in smokers and non-smokers at both extremes of stimulation. The correlation coefficient was similar for smokers and non-smokers at maximal stimulation (Sm = 0.306, N.Sm = 0.448) and at basal secretion (Sm = 0.575, N.Sm = 0.697); it would appear that chronic cigarette smoking has little effect upon the relationship of pyloric loss and Vg.

Pyloric loss parallels Vg in smokers and non-smokers at both basal and maximal stimulation. Thus at maximal stimulation pyloric loss is greater in smokers compared to non-smokers since Vg is also higher in non-smokers.

However under basal conditions both Vg and pyloric loss were lower in smokers compared to non smokers (Table 6.7) but this was not significant. If cigarette smoking has no direct effect upon pyloric loss then these are the exact findings one would predict from a knowledge of the relationship of Vg and pyloric loss. One is forced to conclude that chronic cigarette smoking has only an indirect effect upon on pyloric loss by its effect on the secretory capacity of the parietal cell mass.

ii). Duodenogastric reflux

Duodenogastric reflux has been shown to be greater amongst duodenal ulcer patients compared to controls, and at basal secretion as opposed to maximal stimulation. These trends persisted when smokers or non-smokers were considered as separate groups. Chronic cigarette smoking did not appear to alter the overall relationship between duodenogastric reflux, disease and stimulation state (Table 6.10).

1/. Basal secretion

The control and duodenal ulcer groups were both divided up into four sub-groups: male smokers, male non-smokers, female smokers and female non-smokers.

In the control group of basal secretion all but one of these sub-groups were of similar size. The small size of the female non-smoking sub-group (4) was a reflection of the difficulties in finding females who were prepared to act as controls and were non-smokers. The validity of comparing the two female control sub-groups can therefore be questioned; however no such argument can be levelled at the male control sub-groups. In the duodenal ulcer group, the male smokers were by far and away the largest sub-group; forming 68% of the total. Again this disparity in sub-group sizes means that the comparison of both male and female smokers and non-smokers can be questioned.

The overall trend when comparing smokers to non-smokers of like sex was that smokers had a lower basal Vg. This only reached significance in the male controls ($p < 0.05$). The expected trend would be that the basal Vg would be greater in smokers compared to non-smokers, as is found when maximal gastric secretion is similarly examined. The only sub-group in which this was found was

male duodenal ulcer patients who smoked, it was also the sub-group with the highest basal secretion.

This discrepancy amongst the basal Vgs might be due to differences in stature or age between the various sub-groups. Stature, as defined by height and weight, was not significantly different between sub-groups of the same sex; thus this can not explain the findings. The differences in gastric secretion due to sex are not relevant since only sub-groups of like sex are compared.

Two other studies that contradict the findings of this present study have shown that basal acid output is greater in smokers than non-smokers. In a study of healthy controls the basal acid output of 55 male smokers was significantly greater than in 49 male non-smokers. It was marginally greater in female smokers compared to non-smokers, this did not reach significance. The authors concluded the increased gastric secretory capacity was related to the cigarette consumption (Massarrat et al 1986). In an earlier study on 176 medical students it was found that the level of basal secretion showed a significant positive correlation with daily cigarette consumption. However no such relationship was found between maximal acid output and cigarette smoking (Novis et al 1973). It is though to be noted that in neither of these studies was basal gastric secretion measured using Vg. A possible explanation of the discrepancy between this study and other work will be dealt with in a later section (see p 190 & 197).

2/. Maximal secretion

The results in this study are the most recent figures from this department and are in complete agreement with earlier studies (Whitfield and Hobsley 1987). Maximal Vg was greater in smokers compared to non-smokers in all sub-groups. It will be noted that the only group in which this difference was not statistically significant was male controls; the same sub-group in which basal Vg was significantly lower in smokers than non-smokers.

A study of both male and female controls found that peak acid output was significantly greater in smokers compared to non-smokers. This was closely related to cigarette consumption (Massarrat et al 1986). A study of smokers and non-smokers comparable for age, sex and duodenal ulcer history found a similar relationship between peak acid output and cigarette consumption (Parente et al 1985).

In several studies it has been shown that gastric secretory capacity is greater in smokers compared to non-smokers at both basal and maximal levels of stimulation. The failure of this study to support these findings under basal conditions is possibly due to the relatively small numbers of some of the sub-groups studied. On the other hand, it must be emphasised that the finding was common to all the groups studied. An alternative explanation is that in the smokers there may be a significant level of

tobacco products even after 12 hours abstinence. This could have an inhibitory effect upon the basal secretion and so lead to a low basal Vg in smokers compared to non-smokers. At maximal secretion the relatively large doses of stimulant would swamp this inhibitory effect.

It might also be argued that these results reflect a difference in basal secretion compared to histamine stimulated gastric secretion. The results of the dose-response studies strongly suggest that this is not the case; basal secretion correlated well with maximal secretion in both controls and duodenal ulcer patients. The general conclusion must be that the size or sensitivity of the parietal cell mass is increased in smokers as a result of many years of chronic smoking.

1/. Vg

Inspection of the results revealed remarkable uniformity in the change in Vg after smoking a single cigarette. In 15 out of 16 subjects studied there was a fall in Vg and in both groups (C & DU) this was of the order of 25%. This is not an inconsiderable fall and it appears that the fall is similar in both groups. Although factors such as stature,age and sex affect the absolute values of Vg they can have no effect upon the changes consequent upon smoking a cigarette; because obviously they remain constant throughout the study. Thus despite the small numbers studied the 25% fall is highly significant. The only difference between the two groups is the presence of duodenal ulcer disease; this means that there is a higher median Vg amongst the ulcer group. The effect of smoking did not appear to be greater in one group than the other; the percentage fall was the same in both groups (C & DU).

This is obviously a significant fall in Vg but the question arises as to whether the fall might be an artefact rather than due to smoking itself. Possible such factors are: a) Fade,

b) Indirect effects of smoking and

c) The smoking dose.

i). Artefact

a). Fade

Fade is a pharmacological phenomenon in which the response of a steady state system to a given stimulus decreases with time. It might be argued that the cigarette smoking had no effect and that the observed reduction in secretion simply coincided with the fade of the system. There is no evidence of fade with histamine from this unit or other studies. In this study fade was not detected in 7 patients who underwent 1/4 maximal stimulation for at least three hours (Appendix I).

In animal studies there was no evidence of fade in response to continuous infusions of histamine. The infusions lasted up to seven hours with the dose increasing every hour. No significant difference was found between the response at a given dose whether it was given alone or as part of a continuous seven hour infusion. However it was noted that there was an element of fade with intravenous pentagastrin after only two hours (Emas and Svensson 1972).

In human studies it has been shown that after 2 1/4 hours of maximal and supra-maximal histamine infusions there is no evidence of fatigue (Aubrey 1970). Three individuals underwent three hour intravenous infusions of histamine on many separate occasions; each time a different dose was used. There was no difference between the acid output at two hours and three hours (Adam et al 1954). Human and animal studies into the response of

varying doses of intravenous histamine, over a prolonged period; have failed to show that histamine displays fade.

There is thus no evidence to support the hypothesis that; the difference in Vg between the pre and post smoking periods is due to fade.

b). Smoking

It could be that the smoking indirectly affects Vg. Nausea has been shown significantly to inhibit penta-gastrin stimulated gastric secretion. Fifteen tests were performed on three subjects to assess the effect of smoking on gastric secretion. In five of these smoking-induced nausea was associated with a significant fall (65%) in acid output (Cohen et al 1971).

All subjects studied in this experiment were observed closely throughout the test; and regularly questioned about their condition. No subject complained of nausea or any other gastro-intestinal tract symptoms at any time during the sub-maximal plateau. Some discomfort at the beginning of the test; flushing and occasionally a headache at the end of maximal stimulation were not uncommon. The act of smoking with a naso-gastric tube in place is unnatural, but the very fact that no subject complained of these symptoms known to be associated with an inhibition of gastric secretion is an important point.

The fall in Vg associated with smoking a cigarette is therefore highly unlikely to be caused by an indirect

effect of smoking such as nausea. Indeed if this was a problem with such studies then one might expect that the majority of such studies would show an inhibition of gastric secretion, and this is plainly not the case.

c). Smoking dose

In this study subjects smoked one of their own cigarettes in their normal manner at their own rate. The amount of cigarette products, such as nicotine, carboxyhaemoglobin and thiocyanate, that enter the bloodstream can loosely be termed "smoking dose". This smoking dose depends on many factors such as cigarette type, smoking technique, inhalation and individual characteristics.

In an attempt to maximise this "smoking dose" and so exaggerate any effect of smoking; a wide variety of smoking patterns have been used. In some studies subjects have smoked up to seven cigarettes in an hour (Schnedorf and Ivy 1939); this is about a 1/3 of the average total daily consumption. In others the same brand, often of high strength, has been smoked by all subjects. This has meant those used to mild cigarettes were made to smoke a much stronger cigarette (McCready et al 1985). These type of smoking patterns are clearly unnatural and thus any effect they have upon gastric secretion can be disregarded as unnatural. The question of whether to smoke one cigarette or two in quick succession is more subtle.

Two cigarettes will probably produce a greater "smoking dose", but a single cigarette is a more normal smoking pattern. In the relatively unnatural circumstances of a gastric secretion test it was felt by this author that the smoking conditions should be as near to normal as possible. This meant that because of individual variations in cigarette type, inhalation and pharmaco-kinetics the "smoking dose" would be very varied. This is reflected in the variation in the percentage fall in Vg after smoking a single cigarette: 1.9% to 66.9%. The fall in Vg after smoking is real and not an artefact of the experimental system.

ii). The temporal relationship between Vg and cigarette products

Two of the most commonly studied by products of cigarette smoking are nicotine and carboxyhaemoglobin; and to a lesser extent the thiocyanate ion.

After smoking a single cigarette there is a significant nicotine and carboxyhaemoglobin boost. The extent of this boost is determined by several factors, such as the degree of inhalation, cigarette type and puffs per cigarette (Wald et al 1975). In a particular individual this carboxyhaemoglobin boost seems to be fairly constant (Wald et al 1975); and smokers tend to adjust their smoking habit to maintain a constant nicotine intake (Feyerbrand et al 1985). There is though a ten fold variation in carboxyhaemoglobin levels (Wald

et al 1978) and a wide variation in nicotine levels (Russel et al 1980, Wald et al 1975) between individuals after smoking a single cigarette. The boost reaches its peak within 10 minutes of smoking a cigarette (Armitage et al 1975, Ashton and Telford 1973) but tails off over a considerably longer period: the half-life of nicotine (Isaac and Rand 1972) and carboxyhaemoglobin (Ashton and Telford 1973) is in the region of two hours. Thus a person who smoke 20 cigarettes a day has 20 nicotine and carboxyhaemoglobin boosts a day which each last for considerably longer than it took to smoke the cigarette. There is said to be no day to day accumulation of cigarette products as they are washed out while the subject sleeps. However the mean CoHb in smokers after nearly eight hours sleep was significantly higher than in non-smokers (Castelden and Cole 1974). Twenty four hour monitoring of serum nicotine levels in chronic smokers showed that they do not fall to zero but run at about 12 ng/ml (Benowitz and Jacob 1984). The plasma thiocyanate level is significantly higher in smokers compared to non-smokers and it has a half life of between 10 - 14 days, it is for this reason that it is used a marker of chronic smoking habits (Veasey 1981). It would appear that although there is no "hangover effect" smokers have a higher basal level of smoking products than non-smokers. These chronological events relate well with the acute changes in gastric secretion seen after smoking a single cigarette. There was an almost immediate fall in Vg and

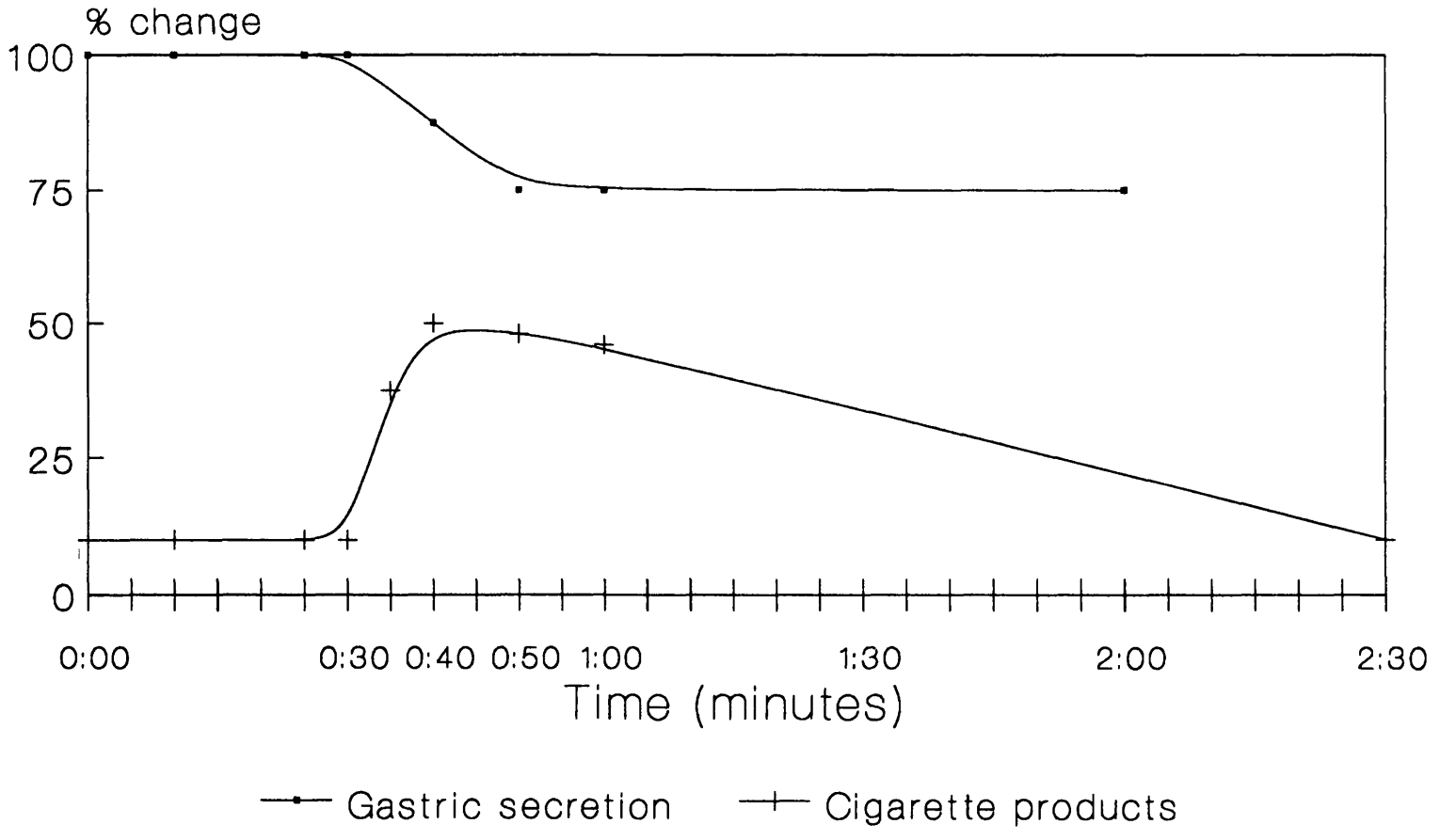


Fig 7.4
Gastric secretion (Vg) and cigarette products (CoHb and Nicotine)

the new lower plateau was reached within 20 minutes; this depressed level of Vg was sustained for the following hour or so. This is shown in idealised form in Figure 7.4. This higher basal level of tobacco products may also account for the finding of a lower basal Vg in smokers compared to non-smokers (see previous section).

iii). Other work

There has over the years been a multitude of studies claiming to study the effects of smoking upon gastric secretion (Chapter 3). Many of them can be discounted since they have used unnatural smoking protocols (see above). The use of basal or maximal gastric secretion has been used in many and these studies can also be discounted. Basal secretion occurs in response to test conditions and the plateau is therefore variable as well as subject to the errors inherent in low volume collections. Maximal stimulation overcomes these criticisms but is unable to show any stimulatory effect; and may well swamp any inhibitory effect of smoking. A sub-maximal infusion of either pentagastrin or histamine to produce about 50% stimulation is ideal. This produces a steady state in which any stimulatory or inhibitory effects can be measured. The studies that fulfil these two simple criteria are few.

The volume and acidity of aspirated gastric juice is the net result of gastric secretions, duodenogastric reflux, pyloric loss and swallowed saliva. The failure

to account for pyloric loss and duodenogastric reflux means that any true change in gastric secretion could be masked by changes in trans-pyloric fluid flux. The effect of swallowed saliva is impossible to correct for completely, since there is no marker that can be used to quantify its presence in the aspirated gastric juice. Spitting out saliva cannot remove saliva from the aspirate and so seems a pointless exercise.

A recent Australian study did make these corrections and used a 50 % plateau of stimulation; but the subjects smoked three or four cigarettes in an hour. They also expressed the corrected gastric secretion (Vg) in micromol/min rather than its correct units of ml/hr (Fletcher et al 1985). The authors showed a significant reduction in acid output following cigarette smoking. To date this appears to be the best attempt to assess the effect of smoking upon true gastric secretion.

2/. The effect of nicotine upon gastric secretion

i). In animals

Using male (Sprague-Dawley) rats the effect of nicotine hydrogen tartrate upon basal and maximal gastric secretion was studied (Thompson 1970). At both levels of stimulation, basal and maximal, a subcutaneous dose of 100 microgm/kg of nicotine produced a significant fall in the volume and acidity of gastric juice. A more recent study found that an intravenous dose of 100 microgm/kg

caused a remarkable fall in gastric secretion in 5 of 7 rats studied (Osumi et al 1980). However not all studies into the acute effects of nicotine have shown such results. In rats a single intravenous shot (500 microgm/kg) produced stimulation of gastric secretion (Osumi et al 1980); and in dogs an infusion of nicotine (100 microgm/kg/hr) over one hour had no significant effect upon the acidity of sub-maximal stimulation of gastric juice (Konturek et al 1971). In the rat studies it is only the higher doses of nicotine that appears to stimulate gastric secretion.

Studies in which chronic smoking has been simulated have produced interesting results (Thompson et al 1970). The administration of 300 microgm/kg/day of nicotine for 15 days was calculated to be equivalent to a daily consumption of 10-15 cigarettes a day. There was a significant increase in the basal levels of gastric juice and volume. The acute response to nicotine in the chronically treated rats was to cause a significant decrease in gastric juice and volume.

ii). In humans

Two out of three studies in humans have also shown similar results. A dose of nicotine acid tartrate, ranging from 1 to 4 mg, was infused in to four subjects over 15 minutes. The doses of 1,2 and 3 mg produced 26%, 16% and 60% reductions in pentagastrin sub-maximally stimulated gastric secretion. A dose of 4mg produced no

reduction in gastric acid output (Wilkinson and Johnson 1971). It is a small study and the results must be treated accordingly. In a larger study of 13 male smokers and non-smokers; infusions of nicotine significantly reduced gastric acid output. A plateau of gastric secretion was established using 0.76 microgm/kg/hr of pentagastrin; infusions of 2.5, 5.0, 7.5 and 10 microgm/kg/hr of nicotine were then given in conjunction with the pentagastrin. The nicotine produced a reduction in acid output which was dose-related. The smoking of five cigarettes reduced acid output by 63%, an equivalent response to a nicotine infusion of 5 microgm/kg/hr (Sonnenberg and Husmert 1982).

This inhibitory effect of nicotine in humans was challenged by Debas and Cohen in 1972. In a letter to the Lancet they claimed that 2mg of nicotine had no significant effect upon gastric acid output.

In the three human studies the doses used varied widely from 0.7 mg/hr (Sonnenberg and Husmert 1982) to 12 mg/hr (Wilkinson and Johnson 1971); but in all of these studies the dose of nicotine tended to be much lower (assuming a weight of 70 kg) than the in the animal studies. In all three human studies the effects of the nicotine were the same as the cigarette smoking in the same experimental setting. In two (Sonnenberg and Husmert 1982 & Wilkinson and Johnson 1971) smoking and nicotine both reduced acid output and in the other (Debas and Cohen 1972) neither nicotine or cigarette smoking had

any effect on gastric acid output. In none of these human studies was any allowance made for pyloric loss or duodenogastric reflux.

On balance it is probable that nicotine given parenterally acts has an acute inhibitory action on the gastric secretion of both rats and humans.

3/. Possible mechanisms by which gastric secretion is reduced

i). Nicotine

Nicotine is a pharmacological agent whose effects upon the body are well known; it is a major component of cigarette smoke. As has been stated it has been shown in human and animal studies to decrease gastric secretion. How might this occur? Nicotine is known to stimulate autonomic ganglia initially but eventually it causes depolarization; resulting in decreased sensitivity of the receptor to acetyl choline. This was the proposed mechanism by which nicotine was said to reduce gastric secretion (Wilkinson and Johnson 1971). Studies of parietal cell preparations have shown that there are several agents involved in the stimulation of gastric secretion: gastrin, histamine and acetyl choline. Specific receptors for gastrin, histamine and cholinergic agents have been shown to exist on the parietal cell. (Malinowska and Sachs 1984). The cholinergic and histaminic agents are potentiating stimulants of gastric

secretion (Berglundh 1977). It is therefore possible that nicotine could act by reducing the potentiating effect of the cholinergic receptors upon histamine stimulated gastric secretion; which could result in a fall in gastric secretion.

Studies in rats have suggested that nicotine may exert its effect via the central nervous system. The intra-ventricular administration of nicotine produced a rise in gastric acid output. This was thought to be mediated via the efferent vagus as the response was blocked by the intravenous administration of atropine (Osumi et al 1980). The effect of chronic nicotine administration in increasing gastric secretion was absent when rats with an abdominal vagotomy were studied. It was felt that the vagus played a role in causing the increased gastric secretion (Thompson and Angulo 1971).

There are however marked species differences in terms of the vagal influence upon gastrin and acid secretion. In dogs vagal stimulation increases gastrin levels and stimulates acid secretion (Grossman 1979). There is evidence that in man the vagus causes inhibition of acid and gastrin release through a putative hormone "Vagogastrone". Vasoactive intestinal peptide (VIP) is released by vagal stimuli and could fulfil this role (Fahrenkrugl et al 1978). Thus the pathway that in the rat stimulated gastric secretion, may in man reduce acid output.

Earlier workers who found that nicotine reduced gastric secretion proposed an alternative mechanism. It had been shown that nicotine releases intestinal serotonin; which itself reduces gastric secretion (Thompson 1968). Serotonin was also known to be an inhibitory transmitter of the amygdala region of the brain (Lee et al 1969). It was proposed that nicotine-released serotonin, via a local or central pathway, was the cause of a reduction in gastric secretion (Thompson 1970).

It has been suggested that nicotine causes a fall in gastric secretion indirectly by reducing mucosal blood flow. Two studies examined the relationship between gastric secretion and mucosal blood flow. In one intravenous nicotine was given and the change in gastric secretion and mucosal blood flow recorded. There was a significant fall in gastric secretion as well as mucosal blood flow; however the fall in secretion was greater than the drop in blood flow and it thought unlikely that the change in blood flow was the cause (Sonnenberg and Husmert 1982). A similar study used cigarette smoking rather than intravenous nicotine; again gastric secretion fell to a greater extent than did mucosal blood flow (Fletcher et al 1985). It seems unlikely that cigarette smoking or nicotine exert their effect via alterations in mucosal blood flow.

ii). Other cigarette products

Carboxyhaemoglobin and thiocyanate levels have not been studied in relation to gastric secretion in man; however the effect of anoxia and thiocyanate on a parietal cell population have been studied. Histamine acts upon the resting parietal cell to transform it to an active state and cause secretion of hydrogen and chloride ions (Carlisle et al 1978). Anoxia (Helander 1984) and thiocyanate (Hersey et al 1981) have both been shown to inhibit this process; the parietal cell transforms into the active state but there in no acid secretion.

4/. Pyloric loss and duodenogastric reflux

The most important fact about pyloric loss is that it is determined to a large extent by Vg and pyloric activity. Duodenogastric reflux is dependent not only on the level of activity of the duodenum but also that of the pylorus and stomach as well (Johnson and Eyre-Brook 1984). Many of these factors have been shown to be affected by cigarette smoking (see below); and so failure to account for them means that the effect of smoking upon gastric secretion cannot be accurately assessed. The small numbers of subjects studied means that definitive statements about pyloric loss and duodenogastric reflux cannot be made. It does though seem reasonable to comment on any trends and relate these to more detailed work done elsewhere.

It has been argued that between individuals pyloric loss was to large extent dependant on Vg; but that individual pyloric loss remained constant despite large variations in Vg. If this held true in the smoking study then a fall in Vg of 25% should have no effect upon pyloric loss. Duodenogastric reflux showed a significant rise when Vg dropped by about 300%, a 25% fall is unlikely to bring about similar changes.

There has been work that has shown that cigarette smoking can relax the human pylorus. The gastric emptying of a liquid meal was significantly faster after smoking a cigarette; relaxation of the pylorus was a suggested cause (Grimes and Goddard 1978). Smoking was also shown to cause a significant decrease in pyloric pressure (Valenzuela et al 1976). In this study duodeno-gastric reflux tended to be lower after cigarette smoking; but with a relaxed pylorus and no increase in pyloric loss a rise might have been expected. This would be analogous to the change in trans-pyloric fluid flux that is seen between basal and maximal secretion. In dog studies both the volume and bicarbonate content of pancreatic secretion fell in response to intravenous nicotine (Konturek et al 1972). In humans smoking was found to significantly decrease duodenal bicarbonate levels, this was closely related to plasma nicotine levels (Murthy et al 1977). Bile salt reflux has been shown to be unaffected (Yeomans et al 1981) or increased (Muller-Lissner 1986) by smoking.

It is obvious that the effect of smoking upon trans-pyloric fluid shifts is governed by the effect of smoking upon the whole gastro-duodenal axis; and not just the pylorus. Failure to take account of changes in pyloric loss and duodenogastric reflux is obviously a source of error.

THE POSSIBLE RELATIONSHIP OF THE ACUTE CHANGES IN Vg TO THOSE FOUND IN CHRONIC SMOKERS

It has been shown that chronic smoking leads to a chronically raised gastric secretory capacity in both controls and duodenal ulcer subjects. It has also been argued that the secretory capacity of an individual is related solely to the size of his parietal cell mass; and not due to any alteration in the sensitivity of those cells. It has also been suggested that in smokers, increased sensitivity of the parietal cells plays no part their increased gastric secretory capacity. Acute cigarette smoking has been shown to significantly reduce Vg in controls and duodenal ulcer subjects. Is there any evidence to link these acute and chronic changes in Vg that occur in relation with cigarette smoking?

1/. The effect of a reduction in Vg

Gastrin is released from the antral G cell and is involved in a complex inter-reaction with the parietal cell which results in the secretion of H^+ and Cl^- ions into the gastric lumen (Malinowska and Sachs 1984). It is argued that this hydrochloric acid acts upon the antrum to inhibit further release of gastrin and hence hydrochloric acid (Adrian et al 1981). It can therefore be argued that any factor that interferes with this

negative feedback loop could lead to a rise in gastrin levels.

It has been shown that the ingestion of antacids is associated with a rise in serum gastrin provided that alkalinization occurs (Feurle 1975); regardless of the type of antacid used.

It has also been shown in both man and animals that chronic acid inhibition has an effect upon gastrin. Ranitidine given to rats over a 10 week period produced a significant rise in plasma gastrin levels (Sundler et al 1986). Prolonged administration of H^2 antagonists has been reported to cause hypergastrinaemia (Hakanson et al 1975).

The chronic 20 cigarette a day smoker thus exposes himself to regular falls in gastric secretion associated with each cigarette smoked. This could in turn lead to interference with the normal negative feedback loop and result in rise in the circulating gastrin levels.

In eight patients with duodenal ulcer disease the serum gastrin levels were measured before, during a 30 minute smoking period and for 30 minutes after smoking had stopped. There was a significant rise of 12% in the serum gastrin levels by the end of the smoking period. There was no concurrent measurement of gastric secretion (Brandesborg et al 1978). In a further study the changes in basal gastric secretion and serum gastrin levels were measured before, during and after smoking a cigarette. The trend was of a rise in the serum levels of immuno-

output, increased parietal cell density and an overall increase in the total number of parietal cells. The chronic adminstration of histamine had no such effects (Crean et al 1969). In patients with the Zollinger-Ellison syndrome there is an abnormally high circulating gastrin level caused by a gastrin secreting tumour. In these patients there is marked gastric mucosal hyperplasia with an increased parietal cell count (Ellison and Wilson 1967). In rats the effect of saline, pentagastrin or histamine upon protein synthesis was studied. It was found that pentagastrin lead to an increase in protein synthesis within 90 minutes of administration, it was limited to certain parts of the gastro-intestinal tract; and was unrelated to its secretory properties (Johnson 1976).

It is now well established that gastrin has a trophic effect on the parietal cells independent of its secretory potential. The long term administration of antacids to rats led to an increase in the fasting serum gastrin; there was an increase in the total parietal cell count. It was argued that this was due to the trophic effects of the raised gastrin levels (Mazzacca et al 1978).

Acutely, the increased gastrin may have some effect upon gastric secretion, however it may well be blocked by the inhibitory effect of cigarette smoking that caused it in the first place. Chronically, small and repeated surges in the circulating gastrin levels may over many

years have a significant trophic effect. Thus after 20 years of smoking the size of the parietal cell mass of an individual will have increased, leading to an increased gastric secretory capacity. A finding that has been confirmed by several major studies (Whitfield and Hobsley 1987, Massarat et al 1986 and Parente et al 1985).

CONCLUSIONS

1/. It is the contention of this study that acute cigarette smoking causes a significant depression in gastric secretion. This effect is a true fall in gastric secretion as smoking has no effect upon either pyloric loss or duodenogastric reflux.

2/. The increased gastric secretory capacity of the duodenal ulcer patients compared to controls is solely due to greater numbers of parietal cells. In controls the parietal cells of chronic smokers are no more sensitive to histamine than their non-smoking counterparts.

3/. In chronic smokers there is an increase in gastric secretory capacity under conditions of maximal stimulation.

Suggestions for further work.

It is suggested that the acute inhibitory effects of cigarette smoking interfere with the normal negative feedback loop of gastrin secretion. This is in turn leads to regular boosts of the circulating gastrin levels, which

over many years exert a trophic effect upon the parietal cells; which increase in number and lead to the increased gastric secretory capacity found in smokers.

The inter-relationship of the constituents of cigarette smoke, nicotine, carboxyhaemoglobin and thiocyanate on one hand; and gastric hormones such as gastrin, serotonin and somatostatin on the other, needs further study. In particular the temporal relationship between the two; both after smoking a single one cigarette and throughout the whole day.

The gastrin levels in chronic smokers with and without duodenal ulcer disease should be compared to those of non-smokers. If this showed gastrin levels to be raised in smokers then this would be of great interest.

No work exists that has studied the dose-response properties of the parietal cell masses of smokers and non-smokers with a similar secretory capacity. It would be important to show conclusively that the chronic elevation of gastric secretion in smokers was indeed solely due to an increase in the parietal cell mass.

The exposure of rats to pulses of nicotine, carboxyhaemoglobin and thiocyanate and the monitoring of the changes in gastric secretion and local circulating levels of gastric hormones would provide valuable

information. If this was continued over many weeks then it might be possible to develop an animal model that demonstrates the acute and chronic effects of cigarette smoking on the stomach.

APPENDIX I

FADE

It has been stated elsewhere (page 193) that the histamine stimulation of gastric secretion is not subject to fade. Fade is a pharmacological phenomenon in which the response of a system to a steady stimuli decreases or 'fades' with time. To answer criticism that the experimental system used in this study produced fade, prolonged sub-maximal histamine stimulation was studied in seven subjects.

The sub-maximal dose of histamine used was one quarter of the maximal dose normally used (0.01 mg/kg/hr). The experimental procedure was exactly the same as set out in the methods section (page 47) apart from the fact that the collection periods lasted for 15 minutes rather than 10 minutes. A one hour basal collection was made and then discarded following which a three hour quarter maximal histamine infusion was started. Histamine stimulated gastric secretion takes between 30 and 60 minutes to reach a plateau and so the first hour's collection was not considered further. Not all subjects continued the study for a full three hours, however all lasted for at least 2 1/2 hours.

In each subject the median Vg over the 2^{nd} and 3^{rd} hours of the study was calculated, the Vg for each collection period of that individual was then expressed

as a percentage of this median Vg (table A.1). In a perfect situation there would be no variation in Vg throughout the 2nd and 3rd hours of the study, in other words a perfect plateau. The Vg for each collection period expressed as a percentage of the median would be 100%, however if there was fade this would not be so and the percentage would fall over the later periods.

The percentage of the median were plotted against time for each individual along with the overall group median (Fig A.1)

The graph shows quite clearly that Vg varies about the median (100%) as would be expected in an experimental system. There is no evidence of a decline in the percentage as the study progresses, in other words 'fade'. Although this is a small study it is in complete agreement with the work of others (Emas and Svensson 1972, Aubrey 1970) and the contention that histamine induced gastric secretion is not subject to fade.

	Subject						
Time	1	2	3	4	5	6	7
1.15	102.5	82.8	82.0	54.6	115.0	100.0	82.0
1.30	100.0	100.0	123.6	82.8	123.6	72.4	123.6
1.45	127.7	89.1	107.7	115.5	96.7	85.2	107.7
2.00	132.2	82.1	102.5	113.1	94.1	124.9	102.5
2.15	123.6	106.6	97.5	88.2	102.9	N/A	97.6
2.30	92.2	104.6	65.5	101.5	97.1	56.7	65.4
2.45	95.0	127.5		98.6		126.9	
3.00	69.4			107.1		149.5	

Table A.1

Vg for each collection period expressed as a percentage of the median Vg for that subject.

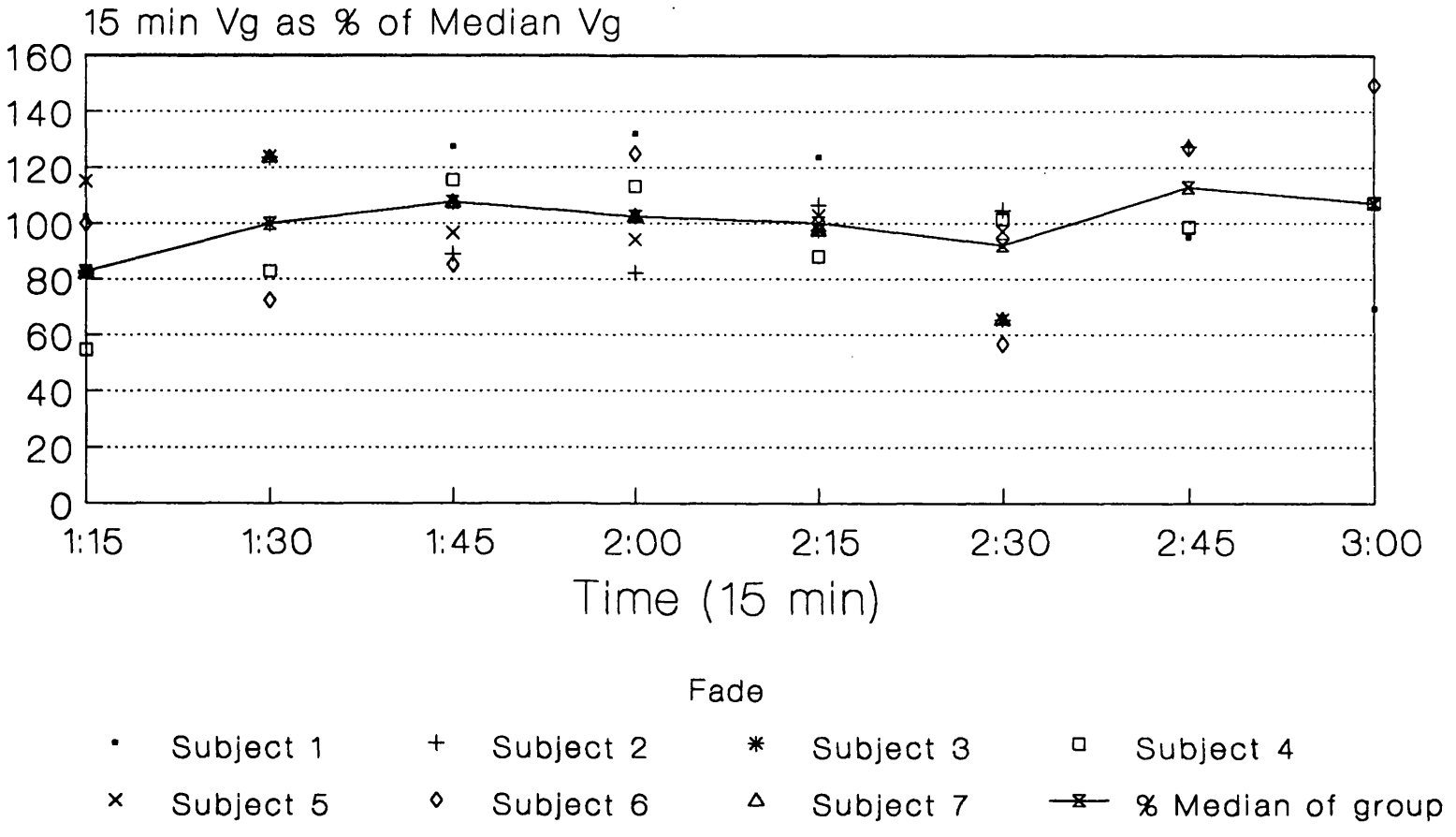


Figure A.1
Fade

APPENDIX II

The derivation of the linear equations for dose-response analysis from the Law of Mass Action

i). The hyperbolic function from the Law of Mass Action

The application of the Law of Mass Action to dose-response relationships was largely the work of A.J.Clark (1885-1941). A biological effect was assumed to be related to the combination of drug molecules with specific receptors. It was postulated that the extent of the response was directly proportional to the occupancy of the receptors; maximal occupancy producing a maximal response.

Let drug X and a receptor site S combine to produce SX which produces a response of size R is proportional to the concentration of SX.

Thus:

$$\text{Drug X} + \text{Receptor S} \underset{k_2}{\overset{k_1}{<=>}} \text{SX}$$

so $SX \times k_3 = R$

At equilibrium:

$$\frac{(S)\ (X)}{(SX)} = \frac{k_2}{k_1} = K_x$$

K_x is the dissociation constant of the complex.

Let S_T = the total receptor concentration,

so $S_T = (S) + (SX)$ and so $(S) = (S_T - SX)$.

Rearranging:

$$K_x = \frac{(S_T - SX)\ \ (X)}{(SX)}$$

thus $$\frac{(SX)}{(S_T)} = \frac{(X)}{K_x + (X)}$$

Let R_{max} = Maximal response of the system to drug D

therefore $R_{max} = k_3(S_T)$

then $$\frac{R}{R_{max}} = \frac{(SD)}{(S_T)}$$

so $$\frac{R}{R_{max}} = \frac{(D)}{K_x + (D)}$$

and so $$R = \frac{R_{max}\ (D)}{K_x + (D)}$$

This is a hyperbolic function in which R = 0 when (D) = 0 and R approaches R_{max} when (D) becomes very large.

NB. In this function when $\frac{R}{R_{max}} = \frac{1}{2}$

ie a 50% response K_x must equal (D).

ii). The derivation of the linear equations from the hyperbolic function

From $$R = \frac{R_{max}\ (D)}{K_x + (D)}$$ by inversion

we get $$\frac{1}{R} = \frac{K_x + (D)}{R_{max}\ (D)}$$

or $$\frac{1}{R} = \frac{K_x}{R_{max}\ (D)} + \frac{(D)}{R_{max}\ (D)}$$

or $$\frac{1}{R} = \frac{K_x}{R_{max}\ (D)} + \frac{1}{R_{max}}$$

This double reciprocal plot is also known as the Lineweaver-Burke plot.

From $$R = \frac{R_{max}\ (D)}{K_x + (D)}$$

we get $$\frac{R}{R_{max}} = \frac{(D)}{K_x + (D)}$$ and inverting

giving $$\frac{R_{max}}{R} = \frac{K_x}{(D)} + 1$$

or $$R_{max} = \frac{R\ K_x}{(D)} + R$$ which gives

$$R = -K_x \frac{R}{(D)} + R_{max}$$

This is the Hofstee transformation.

From $$R = \frac{R_{max}\ (D)}{K_x + (D)}$$

we get $$\frac{R}{(D)} = \frac{R_{max}}{K_x + (D)}$$ inverting

gives $$\frac{(D)}{(R)} = \frac{K_x}{R_{max}} + \frac{1}{R_{max}}\ (D)$$

This is the third of the linear transformations.

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