GASTRIC DENERVATION AND SECRETION

A Study of gastric secretion following unilateral vagotomy in the rat and the dog, and following immunosympathectomy in the rat.

by

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ABSTRACT OF THESIS.

This thesis presentsan experimental investigation into the effects of gastric denervation on gastric secretion. It is presented in two sections and Section I is sub-divided into two parts. The first part of Section I deals with the effect of unilateral vagotomy in the rat. Unilateral vagotomy reduced the spontaneous secretion of gastric juice in the pylorus-ligated rat. The mean acid output after unilateral vagotomy was significantly smaller than the acid output in control rats, and significantly greater than the mean acid output after bilateral vagotomy. Similar differences were observed whether unilateral vagotomy was performed in the abdomen at the time of pyloric ligation, or in the neck one week prior to pyloric ligation.

Part two of Section I is concerned with the effect of unilateral vagotomy on dogs with a gastric fistula, following sham-feeding and gastrin infusions. Difference between pre- and post-unilateral vagotomy results only became apparent at the upper levels of stimulation. Explanations have been advanced for the results but the gross reduction may represent the sum of a number of different effects. It is suggested that only 'maximal stimuli' may be useful experimentally and clinically for differentiating varying degrees of partial denervation of the stomach.

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In Section II the effect of immunosympathectomy on gastric secretion is considered in the rat. The immunologically suppressed sympathetic nervous system gives rise to an increased incidence of gastric glandular ulceration in pylorus ligated rats. This is accompanied by a decreased mucus content in the gastric juice with no appreciable change in acid or pepsin output. Adrenaline treatment of these animals seems to reverse the ulcerogenic effects of sympathetic ablation and restores mucus production to normal and above normal levels.

PREFACE

The experimental work for this thesis was carried out at the Department of Experimental Surgery, Donner Building, McGill University where the author held a Simpson Smith Travelling Research Fellowship and a Medical Research Council of Canada Fellowship. The Chairman of the Department of Surgery was Professor Fraser N. Gurd, B.A., M.D. C.M., M.Sc., Dip. Surg., F.R.C.S.(C), F.A.C.S., and the supervisors of this work were Professor Donald R. Webster, O.B.E., M.D. C.M., M.Sc., Ph.D., F.R.C.S (C), F.A.C.S., Hon. F.R.C.S. (Eng.), and Doctor George K. Wlodek, M.D. C.M., M.Sc., F.R.C.S.(C), F.A.C.S.

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DESIGN OF THESIS

This thesis presents an experimental investigation into the effects of gastric denervation on gastric secretion.

Section I of the thesis is concerned with the effect of unilateral vagotomy on gastric secretion and is subdivided into two parts; the first deals with the results of unilateral vagotomy in the pylorus-ligated rat. The second part is a study of sham-feeding and gastrin infusion in the dog before and after unilateral vagotomy.

Section II is concerned with the effects of immunosympathectomy on acid, pepsin, and mucus secretion in the pylorus-ligated rat and an assessment of the resultant gastric mucosal changes.

Both parts of Section I have been discussed and summarized separately and as far as possible different facets of the problem have been presented in each part. Unavoidably there has been some repetition but the gain in simplicity has been considered of greater importance. Similarily in Section II, details of the method have been repeated in order to save the reader from having constantly to refer back.

SECTION I

INTRODUCTION

The Anatomy of the Vagus Nerves.

INTRA-ABDOMINAL COURSE.

The parasympathetic nerve supply to the stomach enters the abdomen through the oesophageal hiatus as anterior and posterior nerves. The origin, pattern and distribution of these nerves has been described in man, dog, cat, and rabbit in great detail by many authors, amongst whom must be mentioned Swan 1834, Bougery 1844, Kollman 1860, Perman 1916, Latarjet 1921, McCrea 1924, McSwiney 1931, Mitchell 1940, 1959, Jackson 1948, Shimizu 1954, and Ruckley 1964. These authors have shown that the nerves do not correspond to the right and left nerves in the neck, for there has been a considerable intermingling of fibres in the oesophageal plexus.

Descriptions vary as to the number of trunks passing through the oesophageal hiatus. Jackson 1948 and Ruckley 1964 have made studies of the normal pattern in man and their figures are quoted here. Jackson dissected fifty cadavers and found a single anterior abdominal vagus in 70% of cases and a single posterior abdominal vagus in 92% of cases. Ruckley dissected twelve cadavers and his results correspond very closely, the figures being 66% and 92% respectively. Thus the commonest pattern

is a single anterior and a single posterior abdominal vagus nerve. Variations from the normal pattern are not uncommon and Jackson found a double anterior vagus in 28% of cases and three anterior trunks in 2% of cases. Corresponding figures for the posterior vagus were 6% and 2%.

DISTRIBUTION OF THE VAGUS NERVES TO THE ABDOMINAL VISCERA.

Over the further course of the abdominal vagus nerves there is some general agreement. The anterior abdominal vagus gives off a hepatic contribution which travels as a single branch, or as two or three separate nerves, in the lesser omentum. The hepatic branches divide above the pylorus into ascending branches which go to the liver and gall bladder and a descending branch which supplies the pylorus. The main branches of the anterior abdominal vagus supply the anterior wall of the stomach excluding the pylorus. The most medial of the gastric branches is often the largest, this is the 'greater anterior gastric nerve' of Mitchell or the 'lesser curve nerve' of Latarjet. The anterior nerve also sends a small contribution to the coeliac plexus. However a fair proportion of the fibres continue onto the posterior wall of the stomach as gastric branches, supplying it as far as the pylorus. Ruckley (1964) has shown that the posterior nerve gives off hepatic branches in addition. Ruckley emphasized that only the 'gastric' branches of the vagi reach the acid secreting area of the stomach although of course the pyloric branches

may affect gastric secretion indirectly via their innervation of the gastrin secreting part of the stomach. A further noteworthy point is that when the anterior or posterior abdominal vagus is 'abnormally thin' the other nerve takes over the area left deficient and so the posterior vagus may on occasion supply the anterior wall and vice versa.

THE INTRA-MURAL DISTRIBUTION OF THE VAGUS NERVES.

There is no general agreement about the intra-mural distribution of these nerves although many investigations have been made since Dogiel (1895) investigated the enteric plexuses with the intra-vitam methylene blue technique and described the structure of the myenteric (Auerbachs) and the sub-mucuous (Meissners) plexuses. Amongst the investigators must be included Agababow (1912), Boeke (1915 and foll.). Muller (1920), Kuntz (1920 and foll.), Langley (1922), de Castro (1923), Carpenter (1924), Johnson (1925), Hill (1927), Stohr (1928 and foll.). Lawrentjew (1928), Nonidez (1944) and Hillarp (1946). As long ago as 1925 Johnson had suggested that the enteric neurones were the second neurones on the vagal pathway and the sympathetic fibres whose second neurones are said to be in the coeliac ganglia do not synapse in the bowel wall but proceed directly to their destinations. Johnson's opinion is the view that is most widely held today.

Further light was thrown on the peripheral parts of the nervous system by Gasser (1950), Sjostrand (1953), Palade (1954), Causey (1960),

and Richardson (1958, 1960) using the electron microscope. Rivilis (1965, 1967) in a light and electron microscopy study of the intra-mural distribution of the vagus in the stomach showed that the vagus nerves entered the stomach parallel to the blood vessels. These vagal bundles contained unmyelinated fibres and an occasional myelinated fibre. The bundles interlaced and then joined Auerbach's plexus. It was not possible with the Gold Chloride technique used to see whether the vagal fibres synapsed here. The myenteric plexus was composed of 5, 6, and 7 sided figures by primary bundles. Across the figures ran finer secondary and tertiary bundles and from the latter, fibres were seen supplying both muscle layers. No nerve endings were seen in the muscle. From Auerbach's plexus other bundles went between the gaps in the groups of inner circular muscle fibres and joined the much finer plexus of Meissner, whence fibres went up between the gastric pits to supply the mucosal cells.

In a few sections, possible 'terminal areas' between axon and effector cell were seen. Here the axon appeared free from Schwann cell cytoplasm on the side of the 'synaptic area' and the axon was seen to be close to the gastric cell (0.16μ away) and no 'sheath cell' intervened; only a few collagen fibres lay in the space. The effector cell demonstrated very marked invagination of its cell wall in this region, possibly to increase the 'receptor area'. The axon contained numerous vesicles

and mitochondria in this area and there were vesicles in the space between the axon and the effector cell. It was suggested that these were neuro-effector areas and that the vesicles contain acetyl-choline. Support for this view came from the work of Von Euler and Heller (1963) who isolated and identified nor-adrenaline from the vesicles of adrenergic nerves.

A Brief History of Vagotomy.

Billroth performed the first gastrectomy for cancer in 1881 and the operation later found a place in the surgical treatment of duodenal ulceration. Nevertheless gastric resection created its own problems in the nutritional disturbances that often followed (Wells and Welbourne 1951; Glazebrook and Welbourne 1952; Randal 1958). It was therefore with some eagerness that surgeons were willing to try the newer operation of vagotomy for duodenal ulcer, moreover, an operation with a lower mortality than partial gastrectomy (Hoerr 1959, 1960; Nyhus 1960).

Although vagotomy had been performed by Exner in 1911 for duodenal ulcer as a result of the work of Pavlov on the role of the vagi as the mediator of the cephalic phase of gastric secretion and although surgeons such as Bircher (1928) and Latarjet (1922) had also used it, it was not until the paper of Dragstedt and Owens in 1943 that vagotomy became a surgical entity. The first vagotomies were carried out without drainage, but later a drainage procedure was added in the form of a gastroenterostomy in

in 1945 (Dragstedt) and a pyloroplasty in 1950 (Weinberg).

Surgeons treating duodenal ulcer however were caught between the twin dilemmas of nutritional disturbances which are fairly common after partial gastrectomy and recurrent (or stomal in the case of gastrectomy) ulceration which is rare after partial gastrectomy but relatively common after vagotomy (Capper and Welbourne1955; Illingworth 1960; Kay and Cox 1962; Burge 1964; Wastell 1967; Brit. Med. Jour. editorial 1968; Goligher et al. 1968 (a) and 1968 (b).

It is important to stress here that not all surgeons are agreed about the relative lack of nutritional side-effects following vagotomy and a drainage procedure', Goligher et al 1966, 1968 (a) and 1968 (b) noted only marginally better results regarding optimal weight with such operations compared with gastric resection procedures. Cox (1966) feels that the nutritional effects of vagotomy and drainage have yet to be adequately assessed and from his own experience feels that vagotomy may not be superior to gastric resection. A prospective trial is needed in which total abdominal vagotomy and selective abdominal vagotomy can be compared. It will also be important to have a comparison of different drainage procedures when accompanied by vagotomy. Recurrent ulceration however is definitely higher after vagotomy and a drainage procedure than after a gastric resection procedure (see above authors). Regeneration from the proximal stumps of the cut vagus has often been blamed for the recurrent ulceration

and therefore ligature of the vagus as well as removal of part of the vagus was advocated to overcome this.

Incomplete Vagotomy.

In 1950 Weinstein et al. noted a return of function rather than a persistence of function based on the Hollander (1946) Insulin Test in patients who had undergone vagotomy and in whom at a second operation often only a relatively small nerve trunk was found. Rivilis (1965) in a combined secretory and light and electron microscopic study in the rat showed that this may have been brought about by 'collateral nerve sprounting', a process whereby intact nerve fibres may take over the re-innervation of adjacent denervated tissue. This process was well advanced in rats after four months after the incomplete vagotomy. It was accompanied by a significant recovery of gastric secretion compared with an acute incomplete vagotomy group.

Further force was added to the argument that incomplete vagotomy was one of the main causes of recurrent ulceration as Walters et al. (1947) had published their results estimating that 8% of patients had such a complicated nerve distribution that there was a very real danger of nerve trunks being left undivided at operation. Burge (1960) re-emphasized the risks of this as did Clark and Murray (1963). McLeod (1967) has said that 35% of vagotomies are incomplete as judged by insulin testing, and that the commonest fault is the leaving behind of a whole nerve trunk

(i. e. the main anterior or posterior vagus); this is however a higher estimate than is usually quoted in the literature.

The physiological role of the vagus in acid production and its inter-relationships with gastrin.

It has been known since the studies of Pavlov (1895) that the sight, smell and anticipation of food could cause a flow of gastric juice via vagal impulses on the gastric HCl glands and that this cephalic phase of gastric secretion could be abolished by section of the vagus nerves. However the vagus has many other roles in gastric secretion. Uvnas (1942) proposed that vagal stimulation released gastrin from the antrum and also that the cephalic phase of gastric secretion depends on a neuro-humoral mechanism in which gastrin from the antrum plays a major role. Uvnas based his views on acute experiments in cats, in which electrode stimulation of the vagi led to gastric secretion only in the presence of an intact antrum or in the presence of exogenous gastrin.

Further proof that gastrin was released by vagal activity was demonstrated by Pe Thein and Schofield (1959). They obtained acid responses to sham feeding from denervated transplanted pouches in dogs with an isolated innervated antrum. This response could be abolished or reduced when the antrum was acidified or removed.

The importance of gastrin in potentiating the direct action of the vagus on the acid secreting cells has been emphasized by Olbe (1964) who

has shown that the sham feeding response requires the presence of gastrin to produce appreciable secretion.

Dogs were sham fed after resection of the main sources of gastrin and virtually no response was obtained from a Pavlov pouch. When subthreshold doses of gastrin (or histamine) were infused a marked elevation in acid secretion ensued. Gastrin thus potentiates the direct vagal action on the HCl glands. Emas and Grossman (1967) have demonstrated that after bilateral vagotomy, the gastrin dose response curve is shifted to the right, suggesting that the bilateral vagotomy had eliminated tonic vagal impulses that normally sensitize the glands to gastrin stimulation.

Forrest (1956) has shown that local stimulants of gastrin release such as irrigation of isolated antral pouches with acetylcholine gave better responses from a denervated fundic pouch when the nerve supply of the antrum was intact than when it was denervated.

Thus we see that the direct vagal action on the HCl secreting glands requires the presence of gastrin to produce more than negligible secretion. The synergism between these two stimuli can result in a marked acid secretion even when the action of each stimulus alone produces a negligible secretory effect, emphasizing the significance of the vagal release of gastrin.

The first section of this thesis is concerned with the effects on gastric secretion that the leaving intact of a complete vagal trunk may have.

In 1948 Dragstedt had stated that the leaving intact of a single vagal trunk in man and animals may result in a failure to affect gastric secretion. Sebus and Charbon (1963) reported that the gastric secretory response to insulin hypoglycemia and to histamine in the dog was not altered by cutting 75% of the vagal fibres to the stomach. In contrast, in the rat, Shay, Komarov and Gruenstein (1949) did find a reduction in the rate of gastric secretion in the six hour pylorus-ligated rat after unilateral vagotomy. It is possible that the small reduction in the rate of secretion after acute unilateral vagotomy in the rat was simply due to excessive handling of the glandular stomach and other intra-abdominal organs at the time of nerve section.

Many studies have been carried out on the effects of total abdominal vagotomy on gastric secretion and yet only the above three references could be found concerning the effect of unilateral vagotomy. This is all the more surprising as a common form of incomplete vagotomy in the surgical patient is the leaving behind of a whole trunk. It would seem to be important to know what are the effects of unilateral vagotomy on gastric secretion in order to appreciate what may be the outcome of such a procedure in man.

In this study therefore, unilateral and also bilateral vagotomy was performed in two groups of rats at the time of pylorus ligation in order to assess what happens in a preparation in which gastric secretion is mainly

vagal dependent (Alphin and Lin 1959). A further group was prepared in which unilateral vagotomy was carried out one week prior to pyloric ligation to exclude the effects of trauma.

In another set of studies, the dog was used as the experimental animal. The effect of unilateral vagotomy was assessed by observing the responses to sham feeding and to gastrin infusions and comparing these with control studies in the same animals before nerve section.

EXPERIMENTS IN THE RAT

METHOD

The Material.

Wistar rats, weighing from 150 to 230 g, were used in this study. The rat is a particularly suitable animal for experimental procedures as it is a hardy animal with great and rapid powers of recovery. This means that the effect of 'invalidism' on results is kept to a minimum. The 'interdigestive secretion' of the rat is continuous (Friedman 1943; Hirschovitz, O'Leary, and Marks 1960) and in this respect it is like man (Hirschovitz, Streeter, London and Pollard 1957; Van Goidsenhoven, Wilkoff and Kirsner 1958). The rat has the further advantage in that, although it possesses both cephalic (Lin and Alphin 1958) and humoral (Komarov, Shay, Rayport and Fels 1944) phases of gastric secretion, its spontaneous secretion is mainly cephalic (i. e. vagal dependent), in origin (Alphin and Lin 1959).

Gastric Juice Collection and Operative Technique.

The method used for the collection of gastric juice was a modification of a technique first described by Shay, Komarov, Fels, Meranze and Siplet in 1945 for the experimental production of gastric ulceration in the rat and later revised by Shay, Sun and Gruenstein (1954). The animals were starved for 48 hours before operation although full access to water was allowed. During this time and between operations the animals were housed in cages

with raised wire mesh bottoms and this precaution was especially necessary as they are coprophagous by nature and more avidly so when starving. They were kept throughout in one room at a constant temperature and no form of restriction or muzzling was used to overcome the coprophagous tendency as it has been shown (Bonfils and Lambling 1963) that this alters the normal gastric physiology in the rat and leads to 'restraint induced ulcer'. The rats were randomly allocated to four groups:

(i) a control group,

(ii) an acute abdominal unilateral vagotomy group,

(iii) a group where unilateral cervical vagotomy was carried out one week prior to pyloric ligation.

(iv) an acute bilateral abdominal vagotomy group.

THE CONTROL GROUP.

After starvation for 48 hours the animals were lightly anaesthetized with ether in a Kilner jar. They were then weighed and placed on their backs on a small operating table while the anaesthetic was continued via a 'Schimmelbusch-type' mask.

The abdomen was opened by a mid-line incision extending from the xiphoid for three to four cms. The abdomen was not shaved as it was found that post-operatively the animals cleaned themselves and swallowed any loose pieces of fur and this added to the amount of solid in the stomach (see later).

The duodenum was identified without handling the stomach and the pylorus tied with a thread ligature. The vagi were identified, if necessary by traction in the greater omentum or the pyloric ligature. No dissection of the nerves was carried out as a sham operation since none was necessary in the vagotomy groups as the vagi were easily visible. Furthermore the danger of neuropraxia to such fine nerves precluded the sham handling of them. A size 8 F.G. Jaques soft rubber catheter was passed into the stomach via the oral route till the tip was visible against the stomach wall (Figure 1) and the tongue was gently pulled forward with non-toothed forceps to prevent respiratory obstruction. The stomach was emptied of any contents and then four mls of physiologic saline were injected via the catheter and immediately aspirated. At least four mls had to be recovered to ensure that no fluid was left in the stomach. The catheter was then withdrawn and passed a second time to confirm that the stomach was empty. The abdomen was closed in layers with continuous thread sutures (Figure 2).

The animal was replaced in its cage and it usually recovered within a few seconds. Nothing more was allowed orally and four hours later it was re-anaesthetised and the abdomen re-opened. A thread ligature was placed around the oesophago-gastric junction and the stomach removed from the animal (Figure 3). The anterior surface of the stomach was carefully dried of blood and a small incision was made along

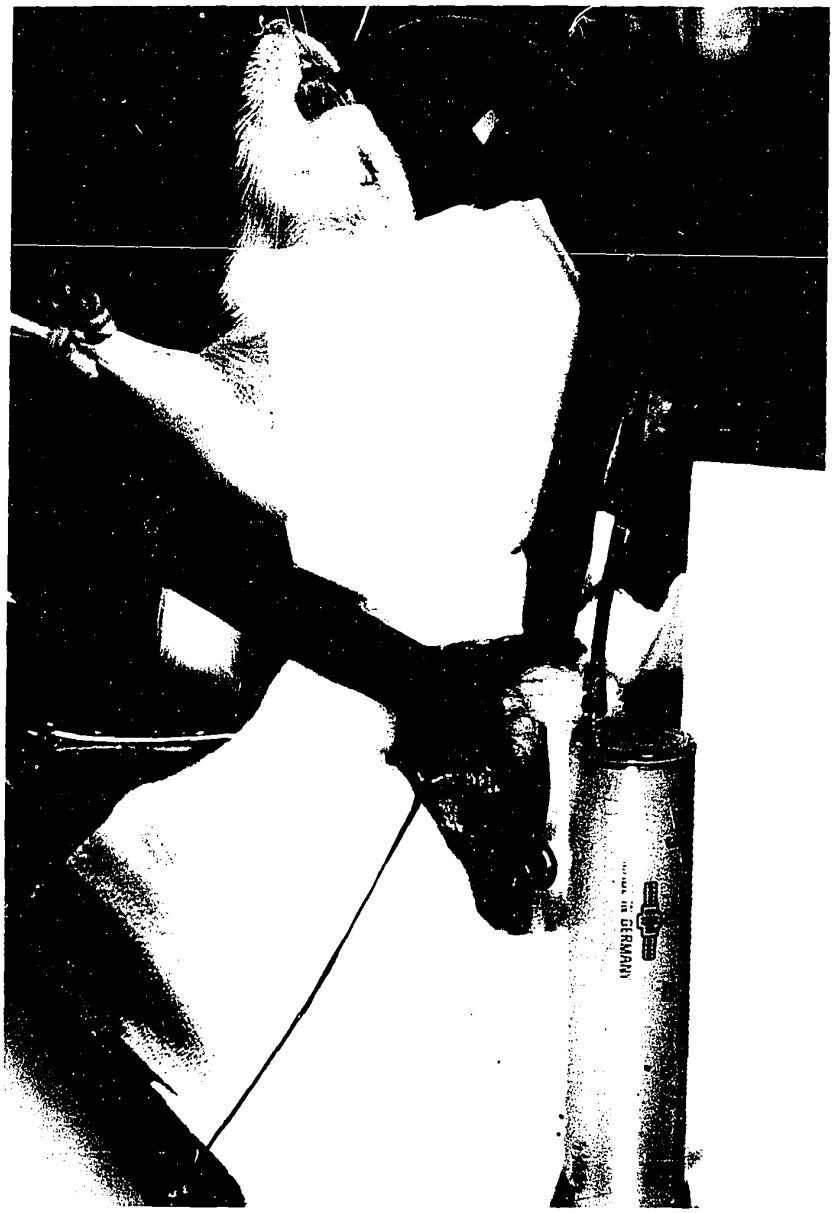

FIGURE I

Shows the abdominal operation. The pyloric ligature is in place and the soft rubber catheter has been passed into the stomach. The arrow shows where the tip of the catheter is in contact with the stomach wall.

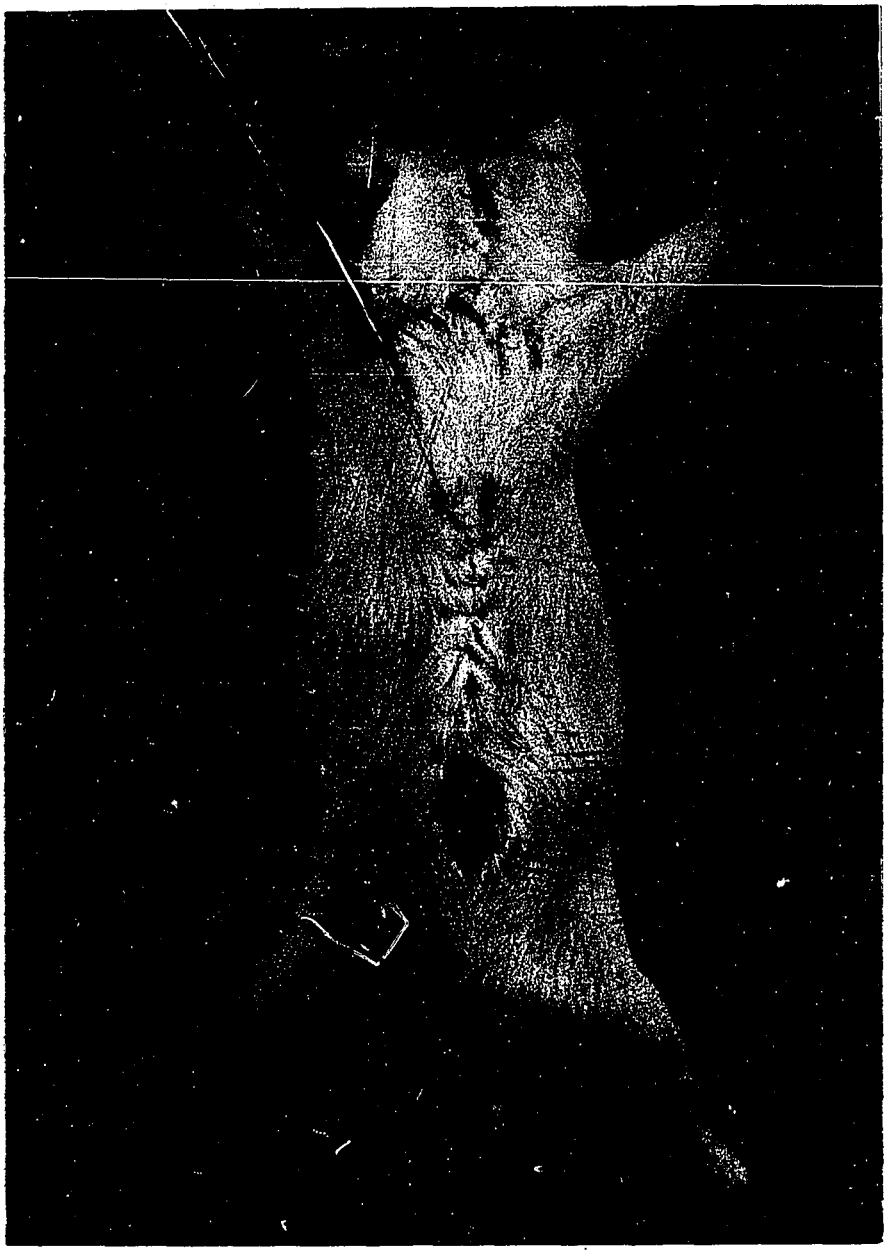

FIGURE 2

Shows the abdominal incision being closed in two layers. Note the 'Schimmelbusch - type' mask.

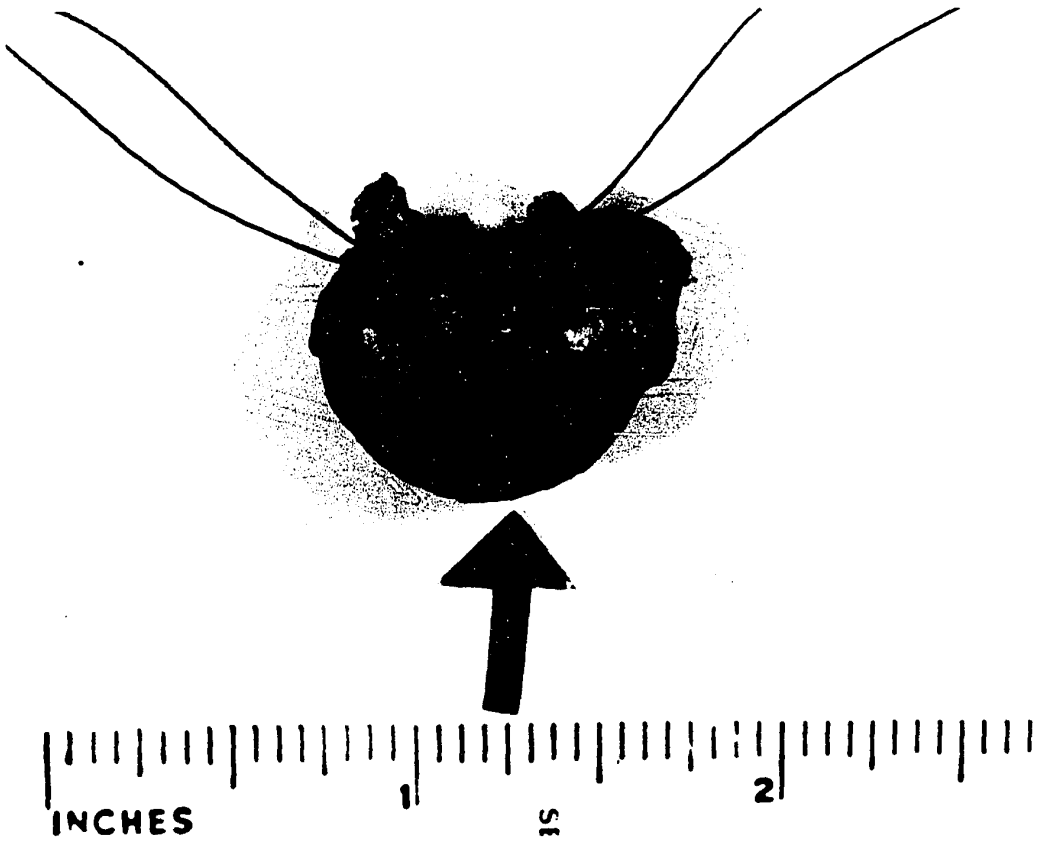

FIGURE 3

Shows the stomach with pyloric and cardiac ligature after its removal from the rat. It is distended with gastric juice and the arrow shows the line of demarcation between the glandular part of the stomach to the left and the squamous part to the right.

the greater curve. The stomach contents were drained into a graduated centrifuge tube, and centrifuged at 3000 rpm for 10 minutes. Any specimen containing more than 0.6 ml of solids after centrifuging was discarded, as suggested by Shay et al (1954).

Each group consisted initially of 18 rats but in the control group (with intact vagal innervation) five samples were discarded as the solid content was in excess of 0.6 ml leaving 13 specimens for analysis. In each of the other three groups, samples were collected until 13 acceptable specimens were available; any remaining animals were discarded without analysis of the gastric contents.

THE ACUTE ABDOMINAL UNILATERAL VAGOTOMY GROUP.

This group was treated pre-operatively in the same way as the control group. At the time of the pyloric ligation, however, either the anterior or posterior vagus nerve was divided in the upper abdomen. The anterior nerve divides into a 'lesser curve nerve' and a 'fundic branch' and care was taken to divide both of these completely (Figure 4). The posterior nerve enters the abdomen and joins the left gastric artery at the oesophago-gastric junction. The posterior nerve was cut above this junction in order to avoid the gastric ulcers that may occur if this artery is damaged in the rat. Great care was taken again not to handle the stomach and any traction needed, was obtained by pulling on the greater omentum or the pyloric ligature. This was done to prevent the

FIGURE 4

Shows the anterior abdominal vagus. A card has been placed between the nerve and oesophagus to demonsirate the constant Y division of the nerve, the branch in the left of the picture being distributed along the lesser curve and the branch on the right being distributed to the fundic area of the stomach. The arrow points to the oesophag-gastric junction and the pyloric tie is in place.

presence of blood in the gastric juice which may result from handling of the stomach. Blood in the gastric juice lowers the pH and any specimen so contaminated was discarded.

THE PRIOR CERVICAL UNILATERAL VAGOTOMY GROUP.

This group had unilateral vagotomy carried out one week prior to pyloric ligation in order to prevent the trauma of the vagotomy influencing the results.

Some initial hesitation was experienced about carrying out a prior unilateral vagotomy in the neck rather than in the abdomen. Nevertheless it was considered advisable that no previous operative procedures in the abdomen should mar the pyloric ligation. In addition Evans and Murray (1954) and Angostini, Murray etal. (1957) have shown in their studies in the rabbit and the cat that each of the cervical vagi contains approximately the same number of fibres as each other, as do each of the abdominal vagi. It could reasonably be assumed that unilateral vagotomy in the neck would produce a 50% vagal nerve fibre interruption to the stomach in the same way as a unilateral vagotomy in the abdomen. Furthermore seven of the unilateral cervical vagotomies were carried out on the right side and six on the left side and seven of the unilateral abdominal vagotomies were carried out posteriorly and six anteriorly in order to minimise any possible differences.

Due to the decussation of the vagi in the oesophageal plexus it was appreciated that a unilateral neck vagotomy would probably denervate a

a different territory from a unilateral abdominal vagotomy.

This was not considered a contra-indication as the design of the experiment was to interrupt 50% of the vagal fibres to the stomach irrespective of whether this denervated the anterior wall of the stomach, the posterior wall or part of each.

A possible criticism is that unilateral cervical vagotomy may influence cardiac and pulmonary function sufficiently to alter the animals' internal environment and lead to altered gastric function.

However the author had previously (Rivilis 1965) carried out nearly 200 cervical vagotomies in Wistar rats varying in age from a few weeks to adult life and had observed no immediate or long term (up to four months) effects on activity, ability to thrive and put on weight, or on respiratory function. Weight gain and respiratory function were considered good parameters of the health of the Wistar rat, especially the latter. The Wistar rat is very prone to respiratory infections and any adverse factors usually lead to loss of the animal. That this did not occur in the author's previous or present experiments make any marked cardio-pulmonary changes unlikely and that these could effect gastric secretion, remote.

The cervical vagotomy was performed either on the right or left vagus by opening the neck through a small (2 cm) incision. The neck incision was increased by gently extending the neck using a tape to pull on the large upper incisor teeth of the rat. The deep cervical fascia was

incised and the sternomastoid muscle retracted laterally (Figure 5). This exposed the carotid sheath from which the vagus was gently freed and 2-4 mm of the vagus was excised. The neck was then closed with a continuous thread suture to the skin. Pyloric ligation was carried out one week later as in the control group, with visualization of the abdominal vagi.

THE ACUTE BILATERAL ABDOMINAL VAGOTOMY GROUP.

This group had bilateral vagotomy performed in the upper abdomen at the time of pyloric ligation.

Gastric Juice Measurements and Statistical Analysis.

Gastric acid concentration was measured by titration with 0.1 N NaOH using phenolphthalein indicator. Shay (1954) has shown that the volume of gastric juice secretion bears a direct linear relationship to the weight of the animal (i. e. a 200 g rat secretes twice as much gastric juice as a 100 g rat in a fixed period of time) and that the most reliable index for comparison between groups is the mean acid output, which also takes into consideration the mean acid concentration. The sex of the animal does not influence this relationship.

The results are given as the mean rate of secretion per 100 g body weight per four hours and as the mean acid output in μEq per 100 g body weight per four hours. Duncan's (1955) multiple range test was calculated for individual mean comparisons.

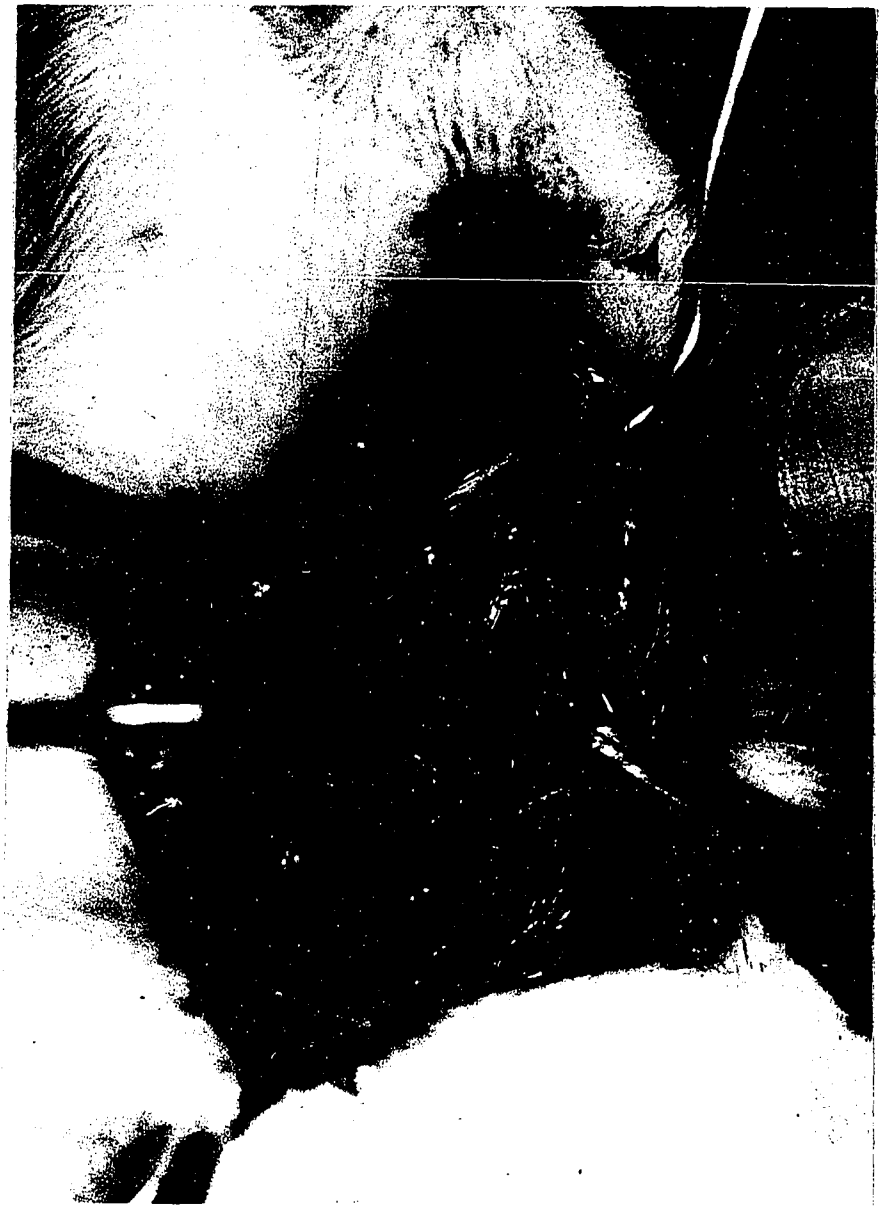

FIGURE 5

Shows the neck dissection. The right cervical vagus is seen lying next to the right internal jugular vein. A good exposure has been obtained by retracting the right sternomastoid muscle laterally (to the left in the picture).

RESULTS

The individual results for each rat and each group are given in Tables I, II, III, and IV. Acute bilateral vagotomy caused a marked reduction in the rate of secretion of gastric juice, the acid concentration, and the acid output in four hours in the pylorus-ligated rat (Table IV). The acid output after unilateral vagotomy was significantly smaller than the acid output of the control rats and significantly greater than the acid output of the group subjected to bilateral vagotomy. These differences were apparent whether the unilateral vagotomy was performed in the neck prior to pyloric ligation, or in the abdomen at the time of pyloric ligation. There was no significant difference between the mean acid outputs of both unilateral vagotomy groups.

In the groups subjected to unilateral cervical vagotomy, six rats had the left vagus divided, and the mean acid output of this subgroup was 178 μEq/100 g/4 hrs; seven had the right vagus divided and their mean acid output was 210 μEq/100 g/4 hrs. In the groups in which unilateral vagotomy was performed in the abdomen, seven rats had the posterior vagal trunk divided and the mean acid output in this subgroup was 130 μEq/100 g/4 hrs; six had the anterior vagal trunk divided and their mean acid output was 175 μEq/100 g/4 hrs.

TABLE I

THE CONTROL GROUP

(13 animals)

Animal No.	61	62	63	64	66	67	68	69	70	71	72	73	98	
Sex	f	f	f	m	m	m	m	f	f	f	f	m	f	
Wt. in grams	170	165	175	195	210	210	240	160	160	190	205	170	180	Av. wt = 186
Rate/100g/ 4 hrs in mls	2.18	2.91	2.73	2.74	1.81	2.19	2.17	4.25	2.88	1.15	3.02	2.65	2.44	Mean ± S. E. 2.55 ± 0.19
Mean acid conc. in µEq/ml	54	112	106	122	122	96	98	98	112	86	104	86	96	99 ± 8
Mean acid output in µEq/100 g/ 4 hrs.	120	330	290	300	220	210	210	420	320	100	310	280	230	257 ± 24

1: f = female; m = male.

TABLE II

THE ACUTE UNILATERAL VAGOTOMY GROUP
(13 animals)

Animal No.	84	85	86	88	90	91	92	93	94	95	99	108	109	
Sex.[1]	f	m	m	f	m	m	m	f	f	f	m	f	m	
Position of vagotomy[2]	P	A	A	P	P	A	P	A	P	A	A	P	P	
Wt. ingrams	200	210	220	195	185	190	195	200	180	180	200	145	145	Av. wt = 196
Rate/100g/ 4 hrs in mls	1.60	3.50	0.82	1.85	1.19	1.79	1.59	1.20	2.11	1.40	2.50	1.24	1.65	Mean±S. E. 1.73± 0.19
Mean acid conc. in µEq/ml	78	114	72	70	100	90	104	60	54	52	114	50	116	83 ± 7
Mean acid output in µEq/100 g/ 4 hrs.	130	400	60	130	120	160	170	70	110	70	290	60	190	151 ± 27[3]

1: f = female; m = male. 2: A = anterior abdominal vagotomy; P = posterior abdominal vagotomy.
3: mean acid output after anterior abdominal vagotomy was 175 µEq/100 g/4 hrs. and after posterior abdominal vagotomy was 130 µEq/100 g/ 4 hrs.

TABLE III

THE PRIOR CERVICAL VAGOTOMY GROUP
(13 animals)

Animal No.	111	112	113	114	116	118	119	120	121	124	126	128	129	
Sex [1]	f	m	f	m	m	m	f	m	m	f	f	f	f	
Position of vagotomy [2].	L	R	L	L	R	R	R	R	L	L	L	R	R	
Wt. in grams.	160	155	155	180	190	175	175	185	165	150	160	160	140	Mean = 165
Rate/100g/ 4 hrs in mls.	1.63	2.00	3.23	2.11	2.95	2.40	2.63	2.05	1.70	2.00	2.31	3.13	1.58	Mean ± S. E. 2.29 ± 0.15
Mean acid conc. in µEq/ml	84	74	96	90	96	86	98	42	78	78	62	114	76	83 ± 5
Mean acid output in µEq/100 g/ 4 hrs.	140	150	310	190	280	210	260	90	130	160	140	360	120	195 ± 23 [3]

1: f = female; m = male. 2. R = right cervical vagotomy; L = left cervical vagotomy.
3: mean acid output after right cervical vagotomy was 210 µEq/100 g/4 hrs and after left cervical vagotomy, 178 µEq/100 g/ 4 hrs.

TABLE IV

THE ACUTE BILATERAL ABDOMINAL VAGOTOMY GROUP
(13 animals)

Animal No.	75	76	77	78	79	80	81	82	83	87	97	100	101	
Sex [1]	f	f	m	m	f	m	m	m	f	f	m	m	m	
Wt. in grams.	190	195	215	225	200	215	230	195	195	195	205	175	190	Mean = 202
Rate /100g/ 4 hrs in mls.	0.53	1.03	0.84	0.44	1.25	1.20	1.00	0.70	0.31	1.44	0.98	1.43	1.58	Mean±S.E. 0.98±0.11
Mean acid conc. in µEq/ml	30	25	25	20	35	35	15	35	25	35	30	25	20	27 ± 2
Mean acid output in µEq/100 g/ 4 hrs.	20	30	20	10	50	40	20	20	10	50	30	30	30	28 ± 7

1: f = female; m = male.

DISCUSSION

Unilateral vagotomy has been shown to reduce significantly the mean acid output in pylorus-ligated rats (Table V). Shay, Komarov, and Gruenstein (1949) reported that unilateral vagotomy reduced the rate of secretion of gastric juice in the pylorus-ligated rat to 78% of control values; in these experiments, the mean rate of secretion of gastric juice in both unilateral groups (taken together) was 79% of the control values. Shay et al. found no difference in the acid concentration after unilateral vagotomy, but unilateral vagotomy did reduce the acid concentration in this study to 84% of the control values. The mean rate of secretion of gastric juice and the mean acid concentration after bilateral vagotomy were similar in the two studies.

It is possible that the slight reduction in the rate of secretion of gastric juice described by Shay et al. after acute unilateral vagotomy may have been due to excessive handling of the glandular stomach and other abdominal structures at the time of nerve section. However, unilateral vagotomy in the neck seven days prior to pyloric ligation also caused a significant reduction in acid output (Table V); this excludes the possibility that the difference in the acute experiments was due to trauma. Cervical unilateral vagotomy was chosen in order to leave the abdomen free of adhesions for pyloric ligation one week later.

TABLE V

GASTRIC SECRETION IN THE PYLORUS LIGATED RAT AFTER UNILATERAL OR BILATERAL VAGOTOMY

Group	Number of animals.	Mean rate of gastric secretion in ml/100 g/ 4 hrs. ± S.E.	Mean acid concentration in μEq/ml. ± S.E.	Mean acid output in μEq/100 g/ 4 hrs.[1] ± S.E.
Control (vagus intact)	13	2.55 ± 0.19	99 ± 8	257 ± 24
Acute abdominal unilateral vagotomy	13	1.73 ± 0.19	83 ± 7	151 ± 27
Prior cervical unilateral vagotomy	13	2.29 ± 0.15	83 ± 5	195 ± 23
Acute bilateral abdominal vagotomy	13	0.98 ± 0.11	27 ± 2	28 ± 7

1. Comparisons between acid output means (multiple range test).
   Control v. acute abdominal unilateral vagotomy: difference 106 μEq, $p < 0.01$.
   Control v. prior cervical unilateral vagotomy: difference 62 μEq, $p < 0.05$.
   Control v. acute bilateral vagotomy: difference 229 μEq, $p < 0.01$.
   Acute abdominal unilateral vagotomy v. prior cervical unilateral vagotomy: difference 44 μEq, $p > 0.05$.
   Acute abdominal unilateral vagotomy v. acute bilateral vagotomy: difference 123 μEq, $p < 0.01$.
   Prior cervical unilateral vagotomy v. acute bilateral vagotomy: difference 167 μEq, $p < 0.01$.

Canon and Rosenblueth (1937) postulated that autonomic nerves might supply only certain "key" cells, and that diffusion of the transmitter substances from this region was able to influence more distant effectors. This theory implies that partial division of the nerve supply of a gland would have little, if any, influence on the amount of secretion produced by a standard nervous stimulus. In the salivary glands electrical stimulation of the chorda appears to cause maximal rates of secretion (Emmelin 1955); it seems probable that this form of excitation stimulates all the secretory units of the gland.

Hillarp (1949) found that stimulation of the chorda after destruction of some of the parasympathetic fibres caused cytological signs of secretory activity in some acini whilst neighbouring acini seemed to be at rest. This was taken was evidence that diffusion of a chemical mediator from its site of liberation does not play a major role in the excitation of more remote glandular secretory units.

It has been suggested that the spontaneous secretion in the unanaesthetized pylorus-ligated rat is almost entirely due to vagal influences (Shay et al. 1949; Lin and Alphin 1958).

Brodie and Knapp (1966) have demonstrated that the output of gastric juice in the pylorus-ligated rat is due to a vago-vagal reflex as a result of gastric distension. This is produced by the combined volume of swallowed saliva and the spontaneous gastric secretions,

and not to the stimulation of gastric secretion in the rat by a specific substance in the rat saliva (Levine 1965).

The finding that unilateral vagotomy significantly reduces this spontaneous secretion argues against the diffusion theory of Canon and Rosenblueth, and favours the view that individual vagal fibres supply separate "secretory units".

As far as can be ascertained from the literature this is the only experimental evidence that partial destruction of the parasympathetic nerve supply to a glandular organ will cause a significant reduction in its external secretion.

In the dog, Sebus and Charbon (1963) claimed that the gastric secretory response to histamine and to insulin hypoglycemia was not altered by cutting 75% of the vagal fibres in the thorax. This may represent a species differences and so the experiments to be described in the second part of this section were carried out.

SUMMARY

Unilateral vagotomy reduced the spontaneous secretion of gastric juice in the pylorus-ligated rat. The mean acid output after unilateral vagotomy was significantly smaller than the acid output in control rats, and significantly greater than the mean acid output after bilateral vagotomy. Similar differences were observed whether unilateral vagotomy was performed in the abdomen at the time of pyloric ligation, or in the neck one week prior to pyloric ligation.

EXPERIMENTS IN THE DOG

The Material.

Three mongrel dogs weighing 16, 17 and 27 kilograms were chosen for their good behaviour in standing on tables in harness for periods of eight to nine hours and for their willingness to sham-feed for up to 32 minutes after a period of fasting. Dogs were also chosen for having a long neck with a loose skin (see operative techniques later). Unlike the rat and man, the dog's interdigestive secretion is intermittent (Babkin 1950). However both parameters of gastric secretion to be tested in these experiments were of stimulated secretion. Furthermore, the advantages of repeated estimations on a large animal with easily cannulated veins more than offset any slight dissimilarities compared with gastric secretion in man. Like man, the dog has a cephalic (Pavlov and Mme Schumova-Simanowskaya 1895) and a humoral (Uvnas 1942) phase of a gastric secretion. Between experiments the animals were kept in separate cages on a balanced soft minced diet.

Operative Techniques.

An oesophagostomy was prepared in two stages by the method of Olbe (1959). For the first operation the dog was anaesthetized with nembutal and a wide bore polythene tube was passed down its oesophagus as a guide. Two vertical incisions were made, one on each side of the

tube and parallel to it, after the position of the tube had been checked by palpation through the extended neck. The incisions were about four cms apart and were extended in a gentle curve laterally at the upper and lower ends.

The skin incisions were undermined extensively so that the two lateral flaps could be brought together under the central skin bridge, which was completely separated from the underlying tissues.

Following this the deep cervical fascia was incised in the mid-line and the long and short strap muscles separated also in the mid-line. The oesophagus was identified behind the trachea usually to the left side, but occasionally it was found to the right side.

The oesophagus was gently mobilized, care being taken not to damage the nearby vessels and nerves, especially the large veins lying in close proximity. The oesophagus was brought out anterior to the strap muscles which were then sutured behind it. All fascial bridges which might later cause obstruction were divided at the upper and lower ends of the wound. The central bar of skin was wrapped around the oesophagus and sutured to itself posteriorly. The lateral skin flaps were then brought together with interrupted sutures in the mid-line behind the newly formed oesophageal tube. The dogs were given Fortimycin post-operatively and allowed only fluids for the first few days.

There was a 50% mortality with this stage of the operation mainly due to a breakdown of the skin flaps, but in one case due to ischaemic necrosis of the oesophagus in the skin tube with a resultant fatal mediastinitis.

A successful surgical outcome after stage one is illustrated in Figure 6. When the dog was well enough to be fed, his food had to be made up in the form of a stew or 'mush' as any large pieces of meat or biscuit could cause obstruction in the oesophageal tube. The skin tube is usually anaesthetic and at a later date a ligature can be placed very tightly around the lower end so as to completely obstruct the oesophagus without complaint from the dog.

After allowing the animal at least three weeks to recover from the first operation for sound healing of the skin flaps, the animal was re-anaesthetized and laparotomy performed. Through a mid-line incision a wide bore (20 mm) metal cannula was inserted into the gastric lumen and brought out through the most dependent part of the stomach on the greater curve in the pyloric antral region so as to assure free drainage of gastric juice. The cannula was inserted through one gastrostomy incision which was closed, and brought out through another to ensure a tight fit.

At the same time as the gastrostomy was made an oesophagostomy was fashioned by making a small circular opening in the ventral wall of the 'oesophageal tube' and the mucosa was sutured to the skin with

FIGURE 6

Shows the oesophagostomy after the first stage of the operation. Note the anaesthesia of the tube on firm pressure and the ample space behind the tube for placing of the ligature.

interrupted catgut sutures. The following day a plastic oesophageal cannula was inserted via the mouth in the conscious dog, usually with some difficulty.

A minimum of three weeks elapsed between surgery and secretory studies. After the control studies had been carried out a unilateral vagotomy was performed. This was done through a thoracotomy incision in the bed of the right or left fifth rib, cutting the right vagus in two dogs and the left vagus in one. The vagi were divided from below the lung roots almost down to the diaphragm. The cut ends were ligated and the chest closed in layers without drainage.

The Preparation of Gastrin.

The method used was modified from the procedure of Gregory and Tracy (1964). The fresh pyloric end of the stomach of hogs was collected from the abattoir of Canada Packers in bags of polyethylene film packed in ice. Excessive washing of the stomachs with water was avoided. At the laboratory all fundic and duodenal mucosa was trimmed off and discarded. The pyloric gland mucosa was dissected off the muscle and cut into cubes about 1 cm square.

Materials: diethylaminoethylcellulose 'floc' (Whatman DE 50).

cellulose powder (Whatman CF 2, coarse grade).

filter paper circles (15 cm Whatman No. 4).

glacial acetic acid (reagent grade).

Materials contd/....

dipotassium hydrogen phosphate (K_2HPO_4) (reagent grade).

isopropanol (2-propanol (Spectrophotometric grade).

diethyl ether (anhydrous, peroxide free, reagent grade, freshly opened).

Hyflo supercel (John Mansville).

(Floc, cellulose powder, and Hyflo were washed with O.1 N HCl and then with water before use).

Procedure: -

In a 13 quart stainless steel bucket, six liters of distilled water was brought to the boil using a 2000 watt electric hot plate. One kilogram of frozen antral mucosa was added and the mixture stirred continuously with a motor-driven stirrer while the solution was boiled for 30 minutes.

After cooling to 15° C in an ice bath with continuous stirring the fat was skimmed off the surface with a tea strainer. The mixture was poured through a 12 inch diameter coarse mesh metal kitchen strainer into another 13 quart bucket and the meat in the strainer discarded.

Fifty grams of cellulose powder (washed with distilled water to rid it of small dust particles and drained through a nylon cloth) were added to the mixture. After stirring for one minute this was then filtered through

a square of nylon tricot cloth laid over the large kitchen strainer. The residue was washed through the nylon cloth two times with two liters of water and the residue on the cloth then discarded.

To the filtrate, 25 g of floc was added and the solution stirred vigorously with a motor-driven stirrer for three hours.

Two batches were made by the above method and were combined and filtered through nylon cloth. After washing the floc four times with two liters of water all the fluid was discarded and only the floc retained. This floc was transferred to a 15 cm Buchner funnel with a Whatman #4 filter paper, overlayed with a thin cake of acid-washed Hyflo, and the water removed by suction.

Two hundred and fifty ml O.1 N NaOH was added to the floc over a clear flask. The floc was allowed to stand without suction for 10 minutes, then suction was applied until no further came through. Four more 250 ml extractions with NaOH were carried out, and all extraction fluid was pooled in the same flask.

These NaOH washings were transferred to a two liter beaker and the pH adjusted to 7 with glacial acetic acid. The fluid was cooled to 15°C, stirring continuously, and the pH then adjusted to 4 with glacial acetic acid with continuous stirring.

The solution was placed in the refrigerator overnight and the next day spun at 9000 rpm for 10 minutes in 250 ml plastic bottles in

a Sorvall centrifuge. The supernatant was poured off.

The precipitate was put into a one liter beaker using about 160 ml distilled water in the transfer for rinsing of centrifuge bottles. After stirring with a magnetic stirring bar until the mixture was homogeneous, concentrated NH_4OH was added until the pH was 9.8. The volume was then made up to 200 ml with water and the solution cooled to 20°C in an ice bath. One hundred grams of K_2HPO_4 and 160 ml isopropanol were added, with continuous stirring for a further 30 minutes at 20°C. The fluid was poured into two 250 ml glass centrifuge bottles and spun at 3000 rpm for 10 minutes. Three layers appeared. The lower layer was clear and yellow, the middle was a cake of solids, and the upper was clear. The upper phase (isopropanol) was removed with a pipette.

This alcohol phase was divided between two 250 ml glass centrifuge bottles and 40 ml distilled water added to each. They were topped up with anhydrous ethyl ether, mixed well, and centrifuged one minute. Aspiration removed the ether phase which was discarded. Ether washings were repeated twice.

The residual solution was transferred to an evaporating dish and stirred while heating (magnetic stirrer, electric hot plate combination) to 45° C in a hood. Stirring for 30 minutes at room temperature removed all traces of ether. The pH of the solution was adjusted to 8 with glacial acetic acid. The final volume was increased with water to allow one ml

of fluid for each 10 g of extracted mucosa (200 ml for 2 kilograms).

The crude gastrin was placed in a plastic bottle and stored in the deep freeze.

One batch was used for all experiments and the doses are expressed as the equivalent weights in grams of wet hog antral mucosa extracted.

Sham-feeding Techniques.

The dogs were starved for 48 hours to ensure a good appetite although water was allowed ad libitum. They were then brought down to the laboratory and stood in a harness in the same way that they had been trained to stand in many preliminary trials. The neck and abdominal cannulae were opened and a tight ligature was placed around the oesophagus below the cervical cannula. Gastric juice was collected for 1/2 hour to ensure that the dogs were secreting under basal conditions; if not, the dog concerned was returned to its cage and the experiment repeated at a later date. For the sham feed the dogs were given a gruel made from two tins (approximately 900 g) of commercial dog food (Ballards Animal Foods Ltd., Montreal) mixed with 600 mls of warm water. They were sham fed for 2, 8, and 32 minutes on separate occasions and each experiment was repeated. The metal bowels from which the dogs ate were placed in such a way that the masticated food fell out of the oesophagostomy into the same dish and was continually

reconsumed. Collections of gastric juice were made every 15 minutes from the beginning of sham-feeding and continued for three hours.

The Gastrin Infusion Experiments.

After fasting for 24 hours, with water ad libitum, the dogs were brought down to the laboratory and an intravenous infusion of normal saline was set up at a constant rate of 100 ml/hr using a Harvard peristaltic pump (Harvard Apparatus Co., Dover, Mass.) and the previously prepared solutions of normal saline and gastrin were infused, beginning with gastrin solution containing 0.5 g of wet hog antral mucosa per kilogram of body weight per hour. Each dose was infused for one hour, due time having been allowed for passage through the tubing and the doses were increased by doubling up for seven consecutive hours until the animals were receiving a solution containing 32 g of wet hog antral mucosa per kilogram body weight per hour. Gastric juice was collected at 15 minute intervals after a 1/2 hour basal collection during which normal saline alone was infused. The experiments were terminated after 7 1/2 hours as the dogs became restive. Each experiment was repeated twice. No animal received more than two infusions in any one week, as these experiments made severe electrolyte demands on the dogs.

Gastric Juice Measurements and Statistical Analysis.

Gastric juice volumes were recorded and the gastric acid concentrations measured by titration of the samples with 0.1 N NaOH using

phenolphthalein indicator.

In the sham-feeding experiments the peak 15 minute mean acid output and the three hour mean acid output were calculated and expressed in mEq/15 minutes and in mEq/three hours respectively. The results were plotted against the duration of the sham-feeding times (Figures 7 and 8).

In the gastrin infusion experiments only the last two 15 minute collections in each hour were used in calculating the average 15 minute response in each hour. This was done to allow for equilibration of the gastrin in the circulation. The average 15 minute mean acid output was expressed in mEq/15 minutes and plotted against the dose of gastrin in grams of wet antral mucosa (Figure 9).

The statistical significance was determined by the t test for paired values.

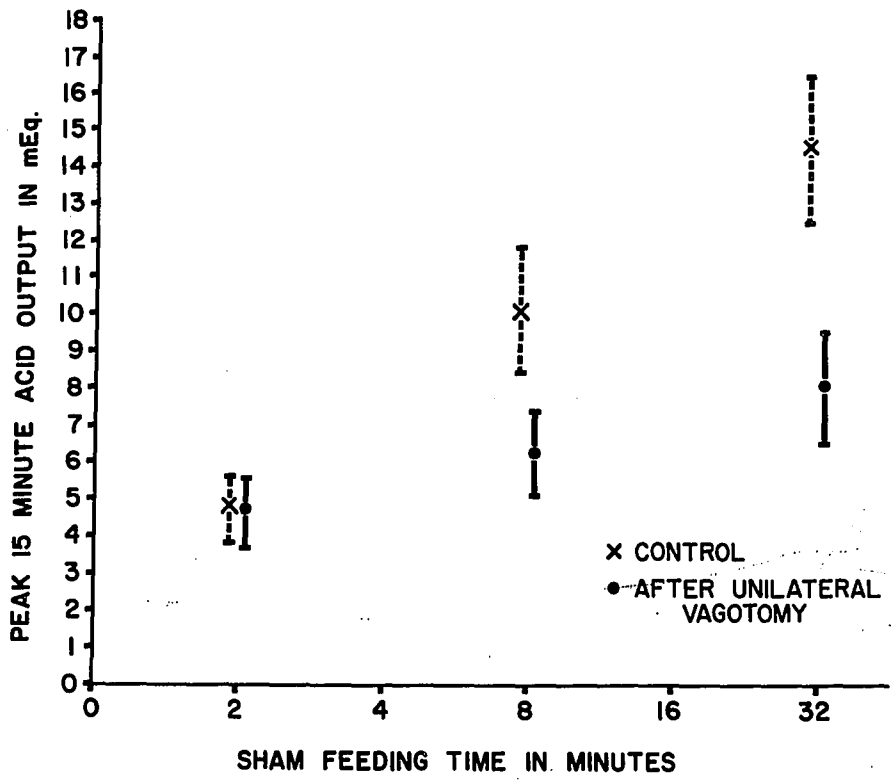

FIGURE 7

The peak 15 minute mean acid output response to sham-feeding.

Note: On all graphs each point represents the mean of six experiments in three dogs. The vertical line represents the standard error of the mean.

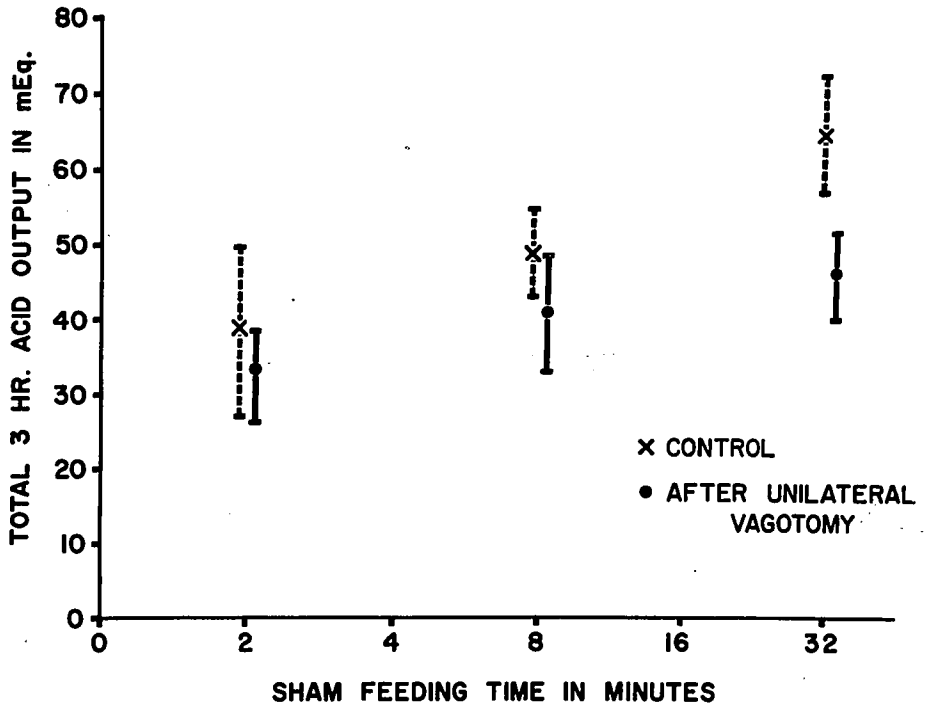

FIGURE 8

The total three hour mean acid output response to sham-feeding.

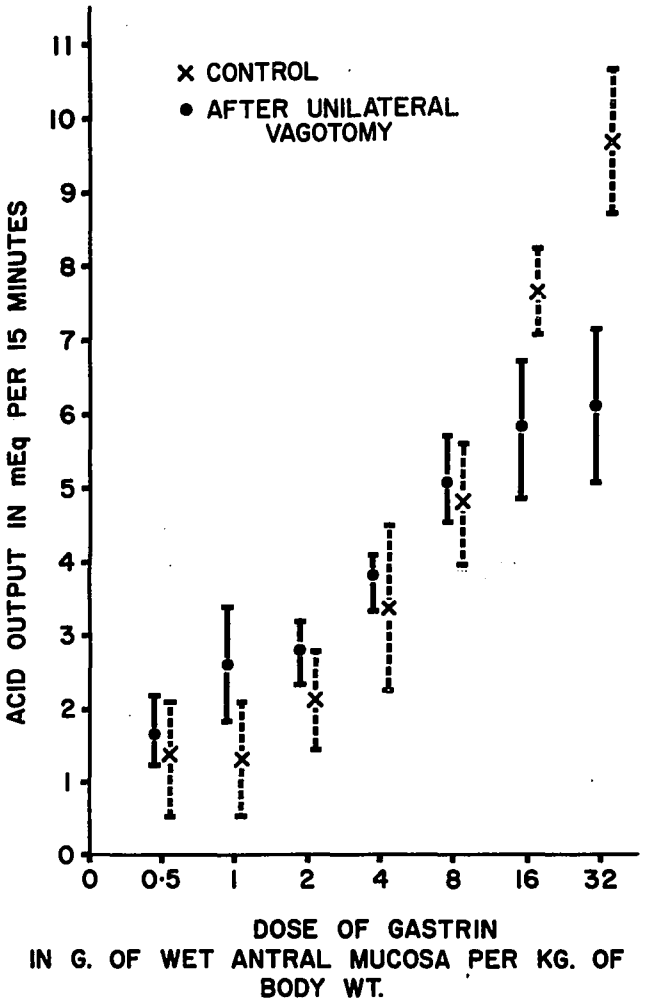

FIGURE 9

The average fifteen minute mean acid output response to graded doses of gastrin.

RESULTS.

The Sham-feeding Experiments.

The individual results are shown in Tables VI, VII, VIII and IX. There was no difference in the peak 15 minute and the total three hour acid outputs after two and eight minutes of sham-feeding after unilateral vagotomy compared with the control values (Tables X and XI).

After 32 minutes of sham-feeding there was a significant reduction in the peak 15 minute ($p < .05$), but not in three hour total mean acid outputs ($p < .01$) after unilateral vagotomy compared with the control outputs.

The Gastrin Infusion Experiments.

The individual results are shown in Tables XII and XIII. The gastrin dose response curves in the control and unilateral vagotomy groups (Figure 9) overlapped at the lower ranges but gradually separated as the dose of gastrin was increased. In the seventh hour of collection, when the dose of gastrin was equivalent to 32 g of wet antral mucosa per kilogram of body weight, there was a significant difference (Table XIV) between the results in the control and unilateral vagotomy experiments ($p < .05$).

TABLE VI

The peak 15' response to sham feeding in mEq before unilateral vagotomy.

	2'		8'		32'	
DOG A	2.60 5.40	) 4.0	6.85 8.16	) 7.50	14.31 13.95	) 14.13
DOG C	2.07 8.66	) 5.37	14.60 16.24	)15.92	18.94 20.98	) 19.96
DOG E	4.96 4.90)	) 4.93	6.06 8.58	) 7.32	9.62 7.78	) 8.70

TABLE VII

The peak 15' response to sham feeding in mEq after unilateral vagotomy.

	2'		8'		32'	
DOG A	3.81 2.97	) 3.39	3.90 4.73	) 4.32	6.30 5.04	) 5.67
DOG C	5.42 9.10	) 7.26	8.60 11.25	) 9.93	12.15 13.16	)12.66
DOG E	2.44 4.66	) 3.55	4.90 4.06	) 4.48	4.52 6.19	) 5.36

TABLE VIII

The total three-hour response to sham feeding in mEq before unilateral vagotomy.

	2'		8'		32'	
DOG A	11.46 29.15	20.31	32.73 38.66	35.70	47.27 63.30	55.29
DOG C	8.94 88.25	48.60	72.25 65.76	69.01	87.35 100.65	94.00
DOG E	44.20 49.55	46.88	32.91 50.75	41.83	50.05 44.68	47.37

TABLE IX

The total three-hour response to sham feeding in mEq after unilateral vagotomy.

	2'		8'		32'	
DOG A	30.11 22.10	) 26.11	28.61 24.83	) 26.72	50.81 39.08	) 44.95
DOG C	29.31 60.43	) 44.87	40.63 79.04	) 59.84	59.21 62.40	) 60.81
DOG E	17.61 40.14	) 28.88	43.22 28.83	) 36.03	28.05 42.19	) 35.12

TABLE X

The peak 15' response to sham feeding before and after unilateral vagotomy.

Sham feeding time in minutes.	15' peak mean acid output before vagotomy in mEq. ± S. E.	15' peak mean acid output after unilateral vagotomy in mEq. ± S. E.	p. Value.
2	4.77 ± 0.96	4.73 ± 0.98	NS
8	10.08 ± 1.74	6.24 ± 1.22	NS $p < .10$
32	14.26 ± 2.09	7.89 ± 1.53	S $p < .05$

TABLE XI

The total three-hour acid response to sham feeding before and after unilateral vagotomy.

Sham feeding time in minutes.	Total 3 hr. mean acid output before vagotomy in mEq. ± S. E.	Total 3 hr. mean acid output after unilateral vagotomy in mEq. ± S. E.	p. Value.
2	38.59±12.00	33.28±6.27	NS
8	48.84±6.36	40.86±7.48	NS
32	65.55±8.69	46.96±4.84	NS $p < .10$

TABLE XII

Gastrin dose response results before unilateral vagotomy

Dose in grams of wet antral mucosa per hr.	15' acid output in mEq during two experiments. A	C	E	The mean acid output in mEq. A	C	E
0.5	.08	1.85	.45			
	.01	5.20	.36	.05	3.53	.41
1.0	.07	1.10	.31			
	.36	5.56	.28	.22	3.32	.30
2.0	.35	3.68	1.97			
	.03	3.85	2.40	.19	3.77	2.19
4.0	.96	4.72	3.97			
	.41	7.62	2.20	.69	6.17	3.09
8.0	4.28	4.29	6.92			
	4.42	7.05	1.66	4.35	5.67	4.29
16.0	5.40	6.79	8.72			
	8.91	8.87	6.76	7.16	7.83	7.74
32.0	6.74	12.38	9.75			
	9.90	11.94	8.28	8.32	12.16	8.28

TABLE XIII

Gastrin dose response results after unilateral vagotomy.

Dose in grams of wet antral mucosa per hr.	15' acid output in mEq during two experiments. A	C	E	The mean acid output in mEq. A	C	E
0.5	.42	2.90	2.17			
	.07	2.08	2.18	.25	2.49	2.18
1.0	.83	5.53	2.77			
	.08	3.58	2.86	.46	4.56	2.82
2.0	1.46	3.28	4.05			
	.63	4.00	3.29	1.05	3.64	3.67
4.0	4.05	3.89	4.01			
	1.72	4.82	3.52	2.89	4.36	3.77
8.0	5.60	2.90	5.81			
	3.89	7.14	4.99	4.75	5.02	5.38
16.0	8.50	1.07	6.96			
	4.84	7.63	5.76	6.67	4.35	6.36
32.0	7.68	1.10	7.50			
	5.61	8.19	6.33	6.65	4.65	6.92

TABLE XIV

Gastrin dose response results before and after unilateral vagotomy.

Dose in grams of wet antral mucosa per hr.	Mean 15' acid output in mEq in control animals. ± S.E.	Mean 15' acid output in mEq after unilateral vagotomy ± S.E.	p value.
0.5	1.33 ± 0.82	1.64 ± 0.46	NS
1.0	1.28 ± 0.87	2.61 ± 0.80	NS
2.0	2.05 ± 0.66	2.79 ± 0.58	NS
4.0	3.31 ± 1.10	3.67 ± 0.42	NS
8.0	4.77 ± 0.82	5.05 ± 0.61	NS
16.0	7.58 ± 0.60	5.79 ± 1.09	NS
32.0	9.59 ± 0.99	6.07 ± 1.07	S $p < .05$

DISCUSSION.

In the presence of an intact antrum (Olbe 1963), sham-feeding is undoubtedly one of the best methods of testing vagal function (Preshaw and Webster 1967). Following the original work of Uvnas (1942) there has been a great deal of evidence (Pe Thein and Schofield 1959; Nyhus et al. 1960) to show that not only does the vagus have a direct effect on the acid secreting cells of the stomach but that it regulates both the release and action of humoral stimuli (Olbe 1963). The present experimental results show that after unilateral vagotomy, no difference in acid secretion is detectable after a relatively small stimulus, such as a two or eight minute sham feed, but suggests that after increasing the stimulus to thirty-two minutes of sham feeding the remaining vagal fibres may not be able to transmit all the impulses rapidly enough to the animal's stomach and so the 15 minute peak response is reduced. Yet over a period of three hours the vagus seems to be able to compensate by a more prolonged period of secretory activity than in the controls.

It seems improbable that the drop of nearly 50% in the mean 15 minute peak acid output after a 32 minute sham feed in an animal that has had a 50% vagotomy could be due to partial loss of vagal tone alone, although this probably does contribute.

An alternative explanation is that the remaining vagal fibres are

carrying their normal impulses and that the defect is at the periphery. There is experimental evidence that in the rat anterior abdominal vagotomy produces Wallerian degeneration in all the vagal fibres proximal to Auerbach's plexus (Rivilis 1965). Recent work by LeGross (1968) also supports this. Spontaneous gastric secretion in the unanaesthetized pylorus-ligated rat is said to be almost entirely due to vagal influences (Shay et al. 1949; Lin and Alphin 1958) and the finding that unilateral vagotomy significantly reduces the spontaneous secretion favours the view that individual vagal fibres supply separate 'secretory units'.

Furthermore in the dog Pritchard, Griffith and Harkins (1968) using the neutral red technique, have clearly shown that the anterior and posterior abdominal vagi innervate their respective gastric walls and also that each vagus divides into branches that supply the stomach wall in a segmental fashion.

The gastrin dose response curve after unilateral vagotomy shows a significant difference at the highest dose of gastrin used and therefore agrees with previous observations on the interaction between vagal impulses and gastrin in the control of gastric secretion (Uvnas et al. 1966). In these experiments the pitfall that acid in the antrum or duodenum can inhibit gastric acid secretion, based on the findings of Andersson (1960) and Wormsley and Grossman (1964) was avoided by

having a large open cannula in the most dependent part of the stomach.

The present experiments in the dog support the previous findings in the rat and tend to confirm at a secretory level a segmental vagal innervation in the dog.

No comparable studies of the effect of unilateral vagotomy on gastric secretion in the dog have been found in the literature except for the study of Sebus and Charbon (1963). They reported no difference after histamine and after insulin hypoglycaemia, even after a 75% vagotomy. However careful examination of their results shows that, in addition to the author's admitted doubts about the adequacy of their collections through an oesophageal tube, one out of their three dogs did have a reduction in histamine response after partial vagotomy.

It is interesting to compare the results of unilateral vagotomy and gastrin infusion in this experiment with those of Emas and Grossman (1967) after bilateral truncal vagotomy. They showed that the gastrin dose response curve in their dogs was shifted to the right, suggesting that the bilateral vagotomy had eliminated the tonic vagal impulses which normal sensitize the glands to the stimulus; it should be noted that they produced maximal responses to gastrin whereas in this study, this was not done.

They found the percentage reduction decreased with increasing secretory rates after bilateral vagotomy whilst decreases were only seen

in the responses to the highest dose of gastrin in this study of unilateral vagotomy.

SUMMARY

Unilateral vagotomy was found to decrease the acid output in gastric fistula dogs after sham-feeding and gastrin infusions. Differences between the pre- and post-vagotomy results only became apparent at the upper levels of stimulation. Explanations have been advanced for the results but the gross reduction may represent the sum of a number of different effects. It is suggested that only 'maximal stimuli' may be useful experimentally and clinically for differentiating varying degrees of partial denervation of the stomach.

SECTION II

INTRODUCTION.

Anatomy of the Sympathetic Supply to the Stomach.

The stomach receives its nerve supply from the autonomic nervous system, from both its sympathetic and parasympathetic components (Gray 1962).

The efferent sympathetic supply comes from the thoracic sympathetic outflow via the splanchnic nerve, which in man contains fibres from T 5-12 inclusive. These fibres are pre-ganglionic and most of them are said to synapse in the coeliac ganglion and travel as a plexus along the branches of the coeliac axis to reach the stomach (McCrea 1924, McSwiney 1931). A few fibres, however, synapse in small masses of ganglionic tissue that lie outside the main coeliac ganglion (Langley 1896).

The afferent fibres pursue the same route in a reverse direction but instead of synapsing in the coeliac ganglion they continue on into the spinal cord via the posterior spinal routes. The cell bodies are located in the posterior spinal route ganglia (Mitchell 1959).

The experimental data concerning the influence of the sympathetic nervous system on the secretory activity of the stomach are scarce and controversal (Baxter 1934; Forrest 1956).

A Brief History of Immuno-sympathectomy.

Recently a new method of destroying the sympathetic nervous system of animals by immuno-sympathectomy has been discovered. This development began when Bueker (1948) reported that following the implantation of fragments of mouse sarcoma 180 into the body wall of a two day chick embryo the adjacent sensory ganglia underwent an increase in size. Nearly fifteen years elapsed before a re-investigation of the phenomenon by Levi-Montalcini and Hamburger (1951) showed that not only had the sensory ganglia supplied nerve fibres to the transplanted tumor to an extent 20 to 40% in excess of that evoked by the transplanting of a limb bud of similar volume but that the adjacent sympathetic ganglia became five or six times greater than those in comparable controls. Furthermore they found that the sympathetic innervation in the embryo appeared at the end of the first week instead of as usual at the end of the third week and that the outgrowth of fibres was so prolific that they invaded all organs even forcing the tubules of the mesonephros apart.

The next step was to find out the nature of the causal agent and so the fragments of the sarcoma were grafted on to the chorio-alantoic membrane of four to six day embryos thus sharing the circulation of the embryo without direct physical contact. A similar growth response was evoked and thus evidence was produced for the existence of a "Nerve

Growth Factor" (NGF) substance. Instead of continuing with experiments using chick embryos, a similar in vitro method using tissue culture was developed and it was found that ganglia incubated with fragments of sarcoma responded by an enormous "halo" of outgrowing nerve fibres (Levi-Montalcini, Meyer and Hamburger, 1954).

This paved the way for the isolation of a growth producing nucleo-protein by Cohen in 1954, who then tried to find whether the factor lay in the intact nucleo-protein or in the nucleic acid or proteinic fractions. To do this Cohen used phosphodiesterase obtained from snake venom in order to degrade the nucleic acid component and to his surprise he found that this evoked an even greater response than the sarcoma extract itself. Brilliantly arguing that NGF had been originally discovered in a mouse tumor and that it was in the salivary gland of the snake that an even more potent NGF was present, Cohen made extracts from adult male mouse salivary glands which proved to be ten thousand times more potent than the tumor NGF and ten times more potent than snake venom NGF. This male mouse salivary gland NGF was shown to be a heat labile protein of molecular weight in the order of 44,000 (Cohen 1956).

The same worker then prepared an anti-serum on the NGF by injecting purified NGF protein into rabbits and when this anti-serum was injected into newborn mice, rats, rabbits, and kittens, and these animals sacrificed some weeks later, the sympathetic chain ganglia were virtually

absent. Further work (Zaimis, Berk and Callingam 1965) has revealed that para-vertebral ganglia are affected more than the pre-vertebral ganglia (Figure 10), and whereas up to 97% of the superior cervical ganglia cells might be absent only up to about 75% of the cells in the coeliac ganglion are absent. Moreover the cell density in the coeliac ganglia of the treated animals was about 20% lower than that of the controls (Figures II and 12).

The varying extent to which the ganglia are affected is possibly due to the fact that some ganglia are more developed at birth and so are less susceptible to the anti-serum; probably only injection in utero would overcome this. The immuno-sympathectomized animals lead a normal life under sheltered laboratory conditions. They are more sensitive to circulating catechol-amines and this is easy to demonstrate using an isolated heart preparation from these animals. It is difficult to carry out a blind trial using control and immuno-sympathectomized rats as they usually have a fairly obvious ptosis (Figure 13).

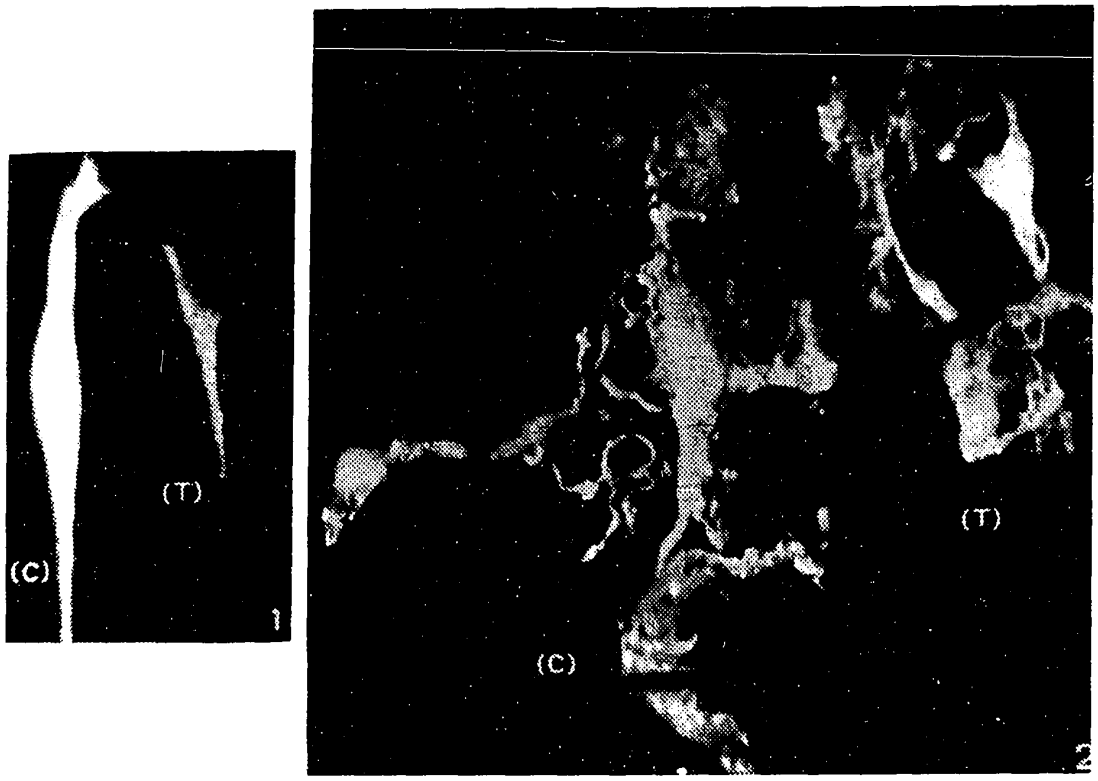

FIGURE 10

(1) on the left shows a normal rat superior cervical ganglion labelled (C) compared with a treated ganglion (T).

(2) on the right shows a normal (C) and a treated (T) rat coeliac ganglion.

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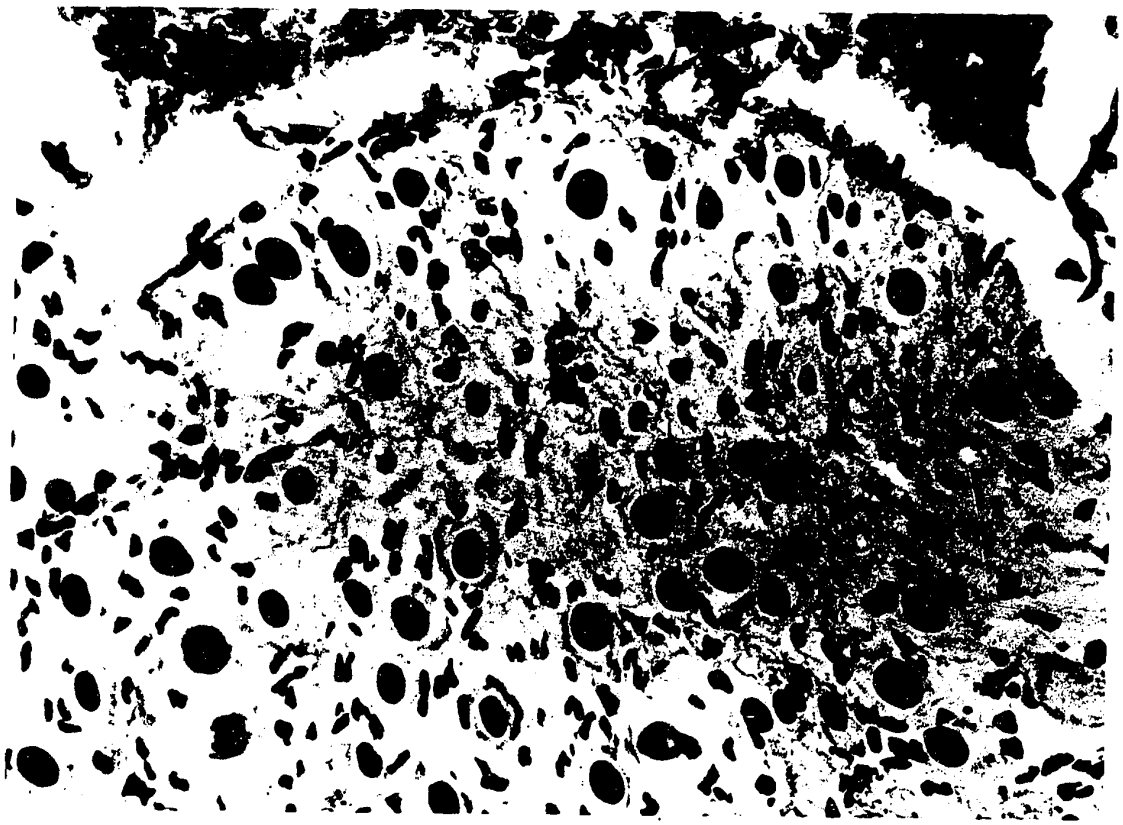

FIGURE 11

Shows a section through a normal rat coeliac ganglion stained with silver. Note the ganglion cells with their large well staining nuclei and compare this with Figure 12.

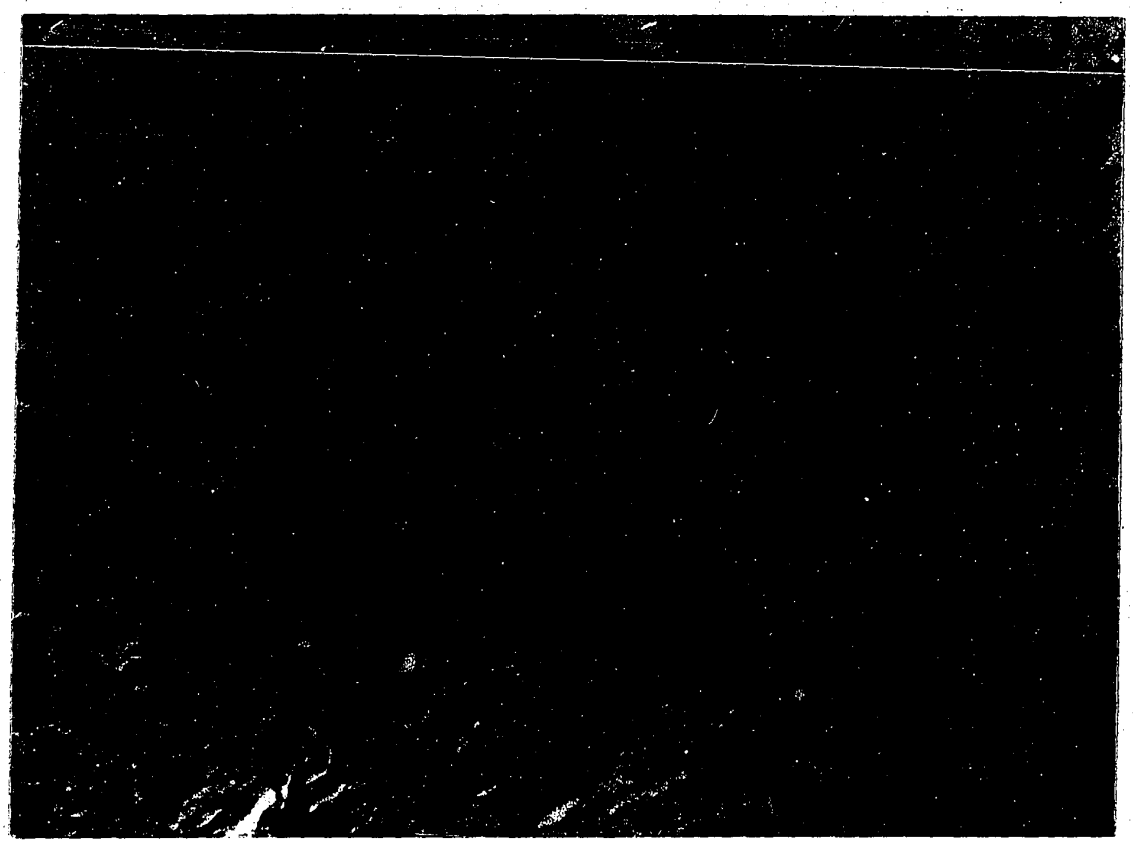

FIGURE 12

Shows a section through the coeliac ganglion of an immuno-sympathectomized rat taken through silver staining simultaneouslt with the section in Figure 11. Note the relative paucity of ganglion cells and the abnormal staining characteristics of the nuclei that are present. Many abnormal nuclei are visible and there is replacement of part of the ganglion by fibrous tissue.

FIGURE 13

Shows an immuno-sympathectomized rat on the left and a normal rat on the right. These animals are litter-mates yet note the marked ptosis of the treated rat on the left. The appearance of the fur in the treated animal is an artefact, as the animal required washing before photograph.

Results of a preliminary study.

In a preliminary study carried out under Professor A. J. Harding Rains in the Department of Surgery, Charing Cross Hospital, London, and under Professor E. Zaimis in the Department of Pharmacology, Royal Free Hospital, London, the author observed the effects of immuno-sympathectomy on gastric secretion. The relevant results are briefly summarized as follows and the histological findings are presented.

After only two hours of pyloric ligature, the stomachs of the five control animals were macroscopically and microscopically normal (Figure 14), whereas all five immuno-sympathectomized (IS) animals showed mucosal changes. Four out of the five IS rats had ulcers and some hyperaemia in the glandular portion of the stomach, and the fifth animal had marked hyperaemia but no ulcer.

Microscopically, the ulcers were shallow, and certainly acute (Figure 15) as there was virtually no "cellular response"; around the ulcers were numerous red cells confirming the hyperaemia seen macroscopically. When stained for mucus with PAS and Alcian Blue, the mucus in the surface cells stained red whilst that deeper in the gastric glands stained a light blue. The staining in the control animals is illustrated in Figure 16; Figure 17 shows how the staining in both sites in the IS group is diminished.

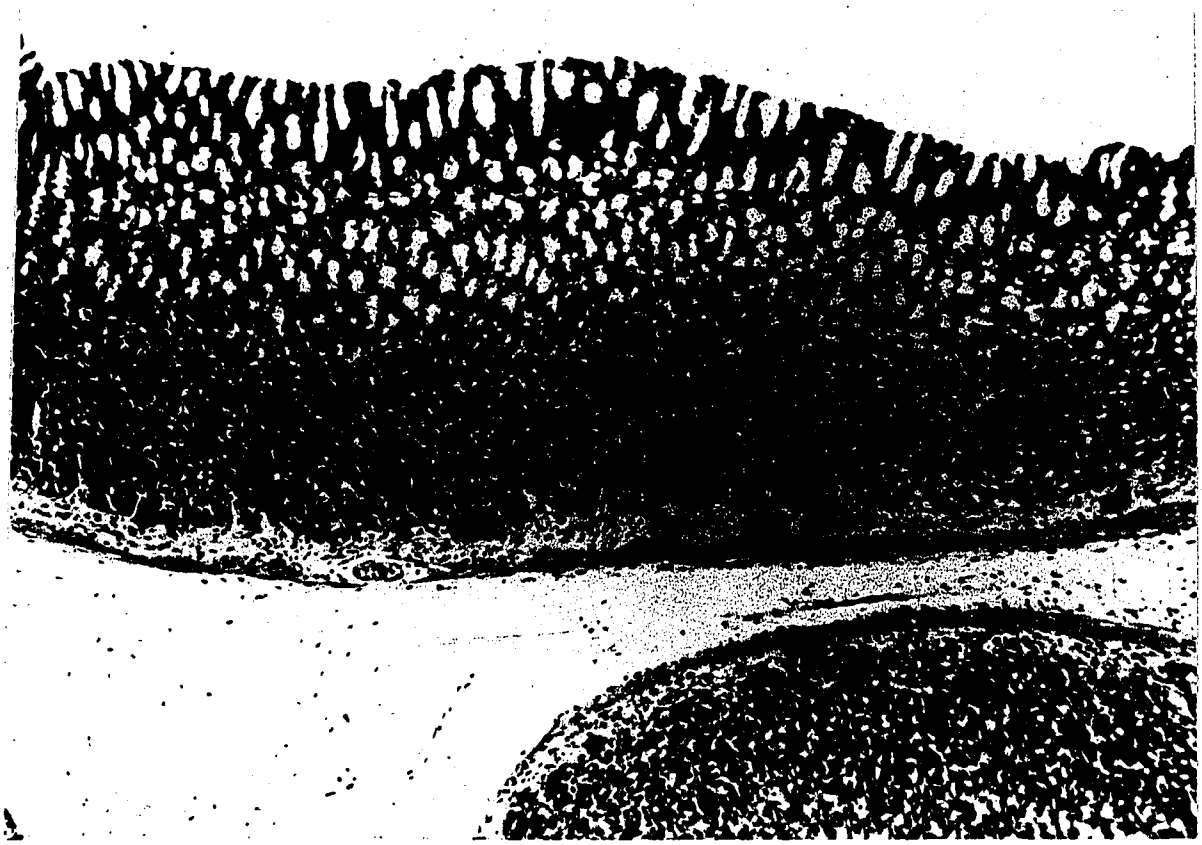

FIGURE 14

Shows the gastric mucosa from a control animal.

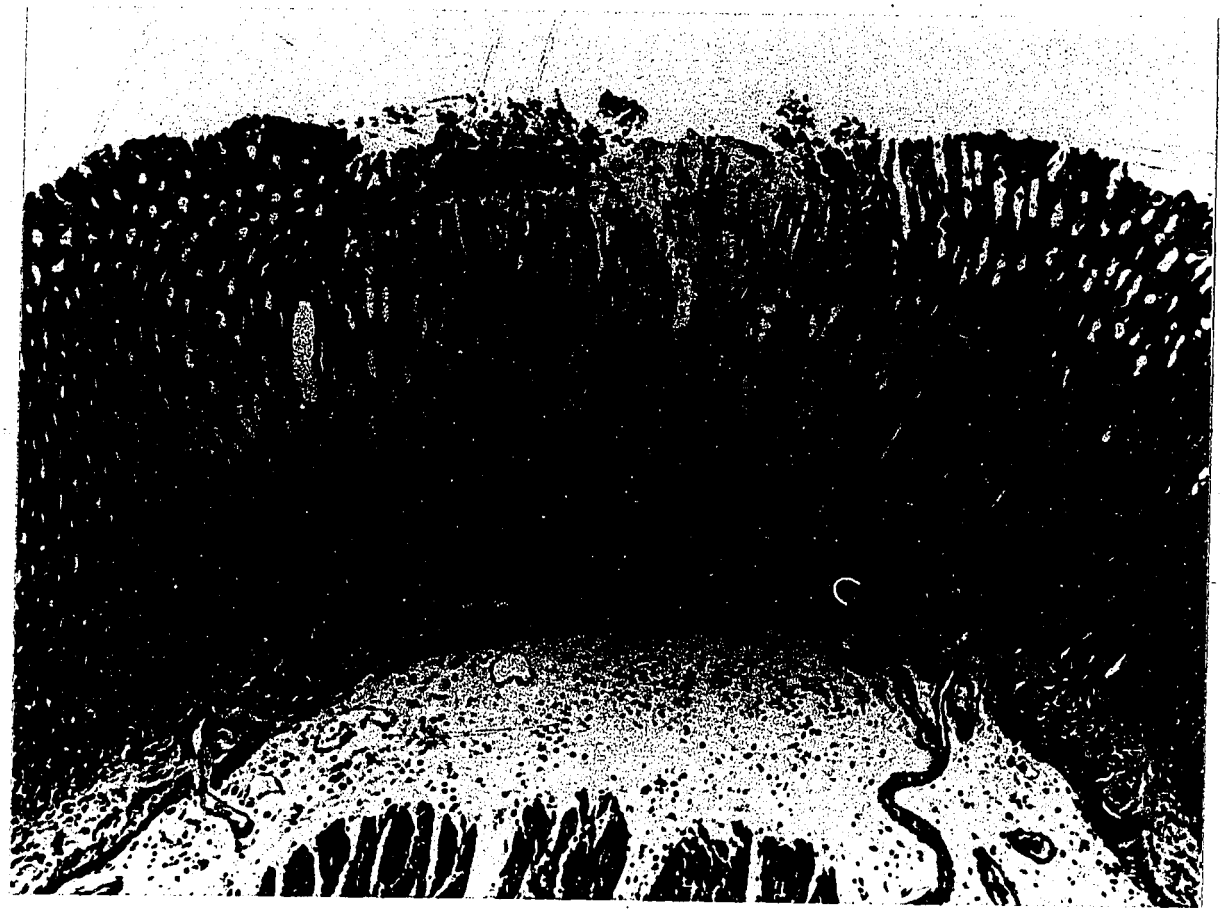

FIGURE 15

Shows the stomach from an I.S. animal after two hours pyloric tie.

Note the shallow "acute" ulcer.

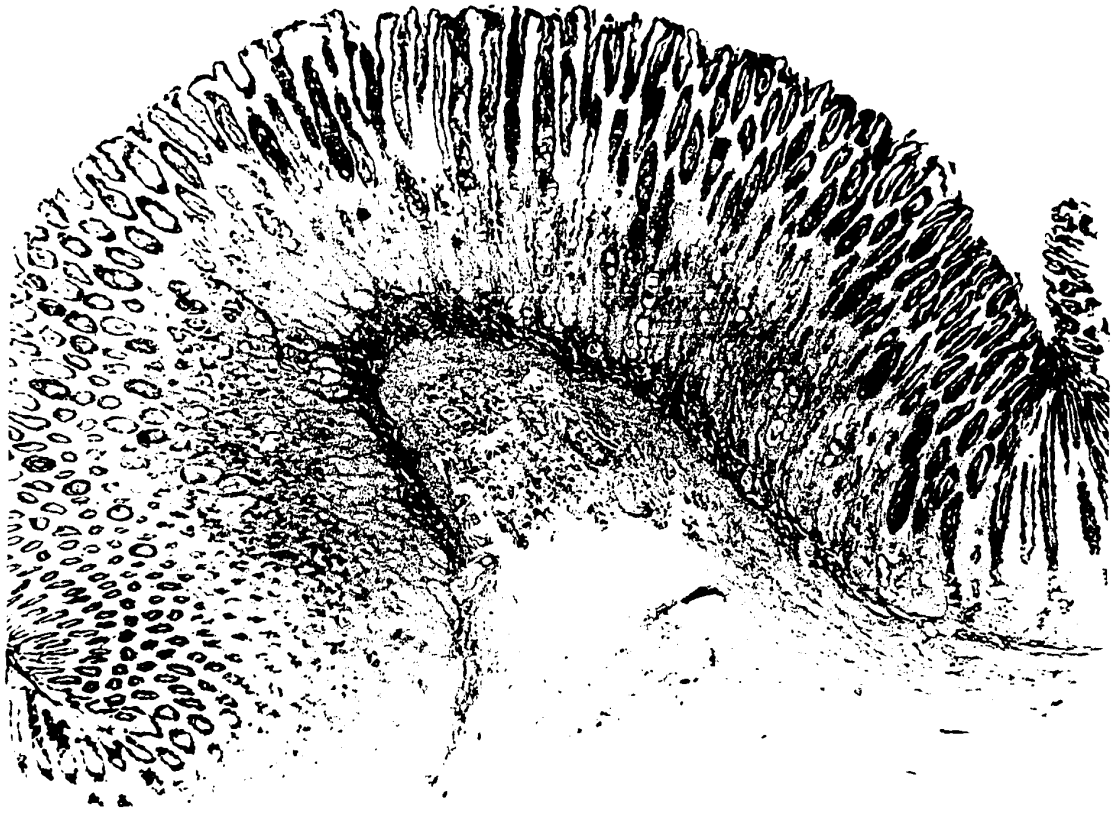

FIGURE 16

Shows the mucus in the epithelium of a control animal (stained with P. A. S. and Alcium Blue).

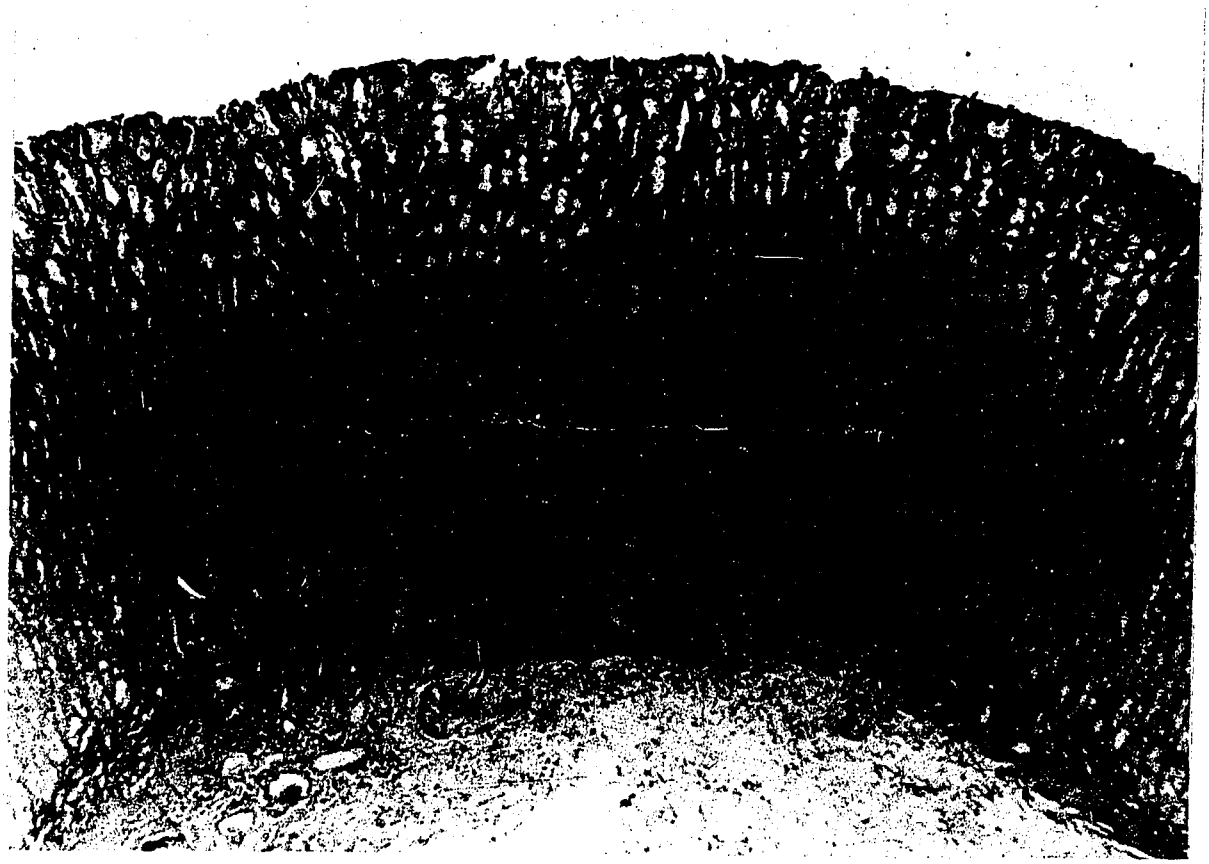

FIGURE 17

Shows the diminution of mucus in the gastric epithelium of an I.S. animal, (stained with P.A.S. and Alcium Blue).

No change was found in acid or pepsin production between these two groups of snimals that would account for the changes seen. Indeed the acid and pepsin outputs were similar in each group. However, the number of animals in each group (five) was small and the ulcer and mucus findings were qualitative rather than quantitative. Therefore the present study was carried out in order to assess the effects of immuno-sympathectomy on mucus secretion, gastric ulceration, and gastric secretion in larger groups of animals. The possible action of adrenaline in reversing these effects was also investigated.

METHOD

Material.

The stomach of the rat is divided into two completely different regions by a firm raised ridge (Berg 1942); the proximal part, or rumen, is a pale pinkish colour and it is lined by stratified squamous epithelium. It is continuous with the oesophagus which opens into it just proximal to the dividing ridge on the lesser curve (Figure 18).

The distal part of the stomach is said to be divided into a functional, or pre-pyloric, part mainly located on the lesser curvature and a corpus which comprises the rest of the glandular part of the stomach (Sun and Chen 1963). The corpus is lined by columnar cells which are continuous into the gastric pits; the gastric glands contain mucus neck cells and parietal cells and chief cells. The glands of the antral region are shorter and consist mainly of mucus-type cells. The muscularis mucosa and the muscular layers are continuous with the same layers in the rumen (Figure 19).

The Production of Immuno-sympathectomy.

Litters of Wistar rats were divided at birth into two groups. Immuno-sympathectomy was produced in one group by giving O.25 ml. of equine antiserum to Nerve Growth Factor for the first five days after birth. The antiserum had a potency of 9,600 anti-units per ml. (Wellcome Research Laboratories, Beckenham, Kent, England - -

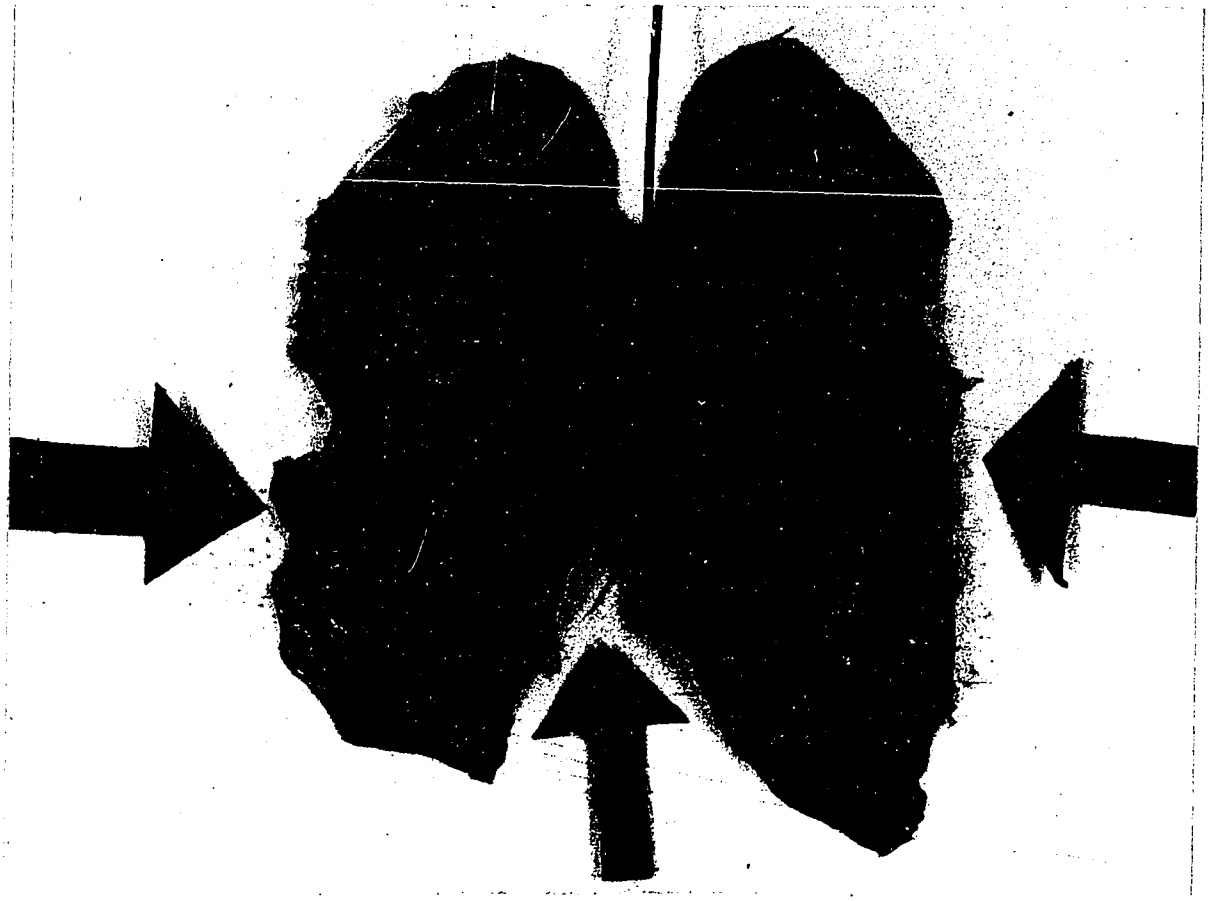

FIGURE 18

Shows the stomach of the rat opened out. The lowest arrow points to the pylorus; the other two arrows indicate the junction between the squamous part of the stomach in the upper half of the picture and the glandular part in the lower half. There is a bristle in the oesophageal opening into the squamous part.

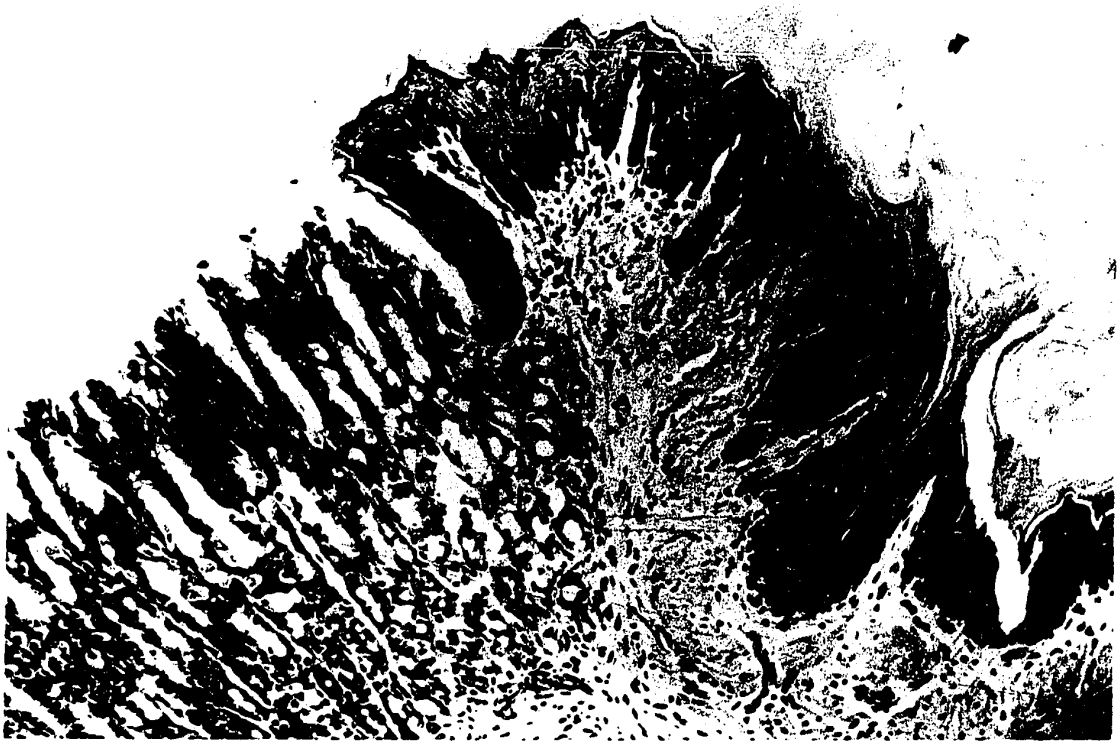

FIGURE 19

Shows the dividing ridge with glandular epithelium on the left and squamous epithelium on the right. Note the intact layer of keratin on the squamous part - not digested by the action of the gastric juice and therefore suggesting that the ridge is competent against reflux of gastric juice, from the glandular part of the stomach.

Batch Nos. 4812/3/4). The other group was injected with a similar amount of normal horse serum and used as controls.

All animals were kept on a normal diet (Purina Checkers) from weaning until they weighed approximately 225 grms. Four days prior to gastric collection, the IS group was separated into two sub-groups, one receiving an empirical dose of adrenaline in peanut oil subcutaneously as follows, 1 mg on the first day, O.5 mg on the second and third days, and 1 mg on the morning of operation.

Collection of Gastric Juice.

This will be described briefly as the method has been given in detail in Section I of this thesis. Gastric secretion was collected by the method of Shay, Sun, and Gruenstein (1954). After fasting for forty-eight hours in individual cages with free access to water, pyloric ligation was carried out under light ether anaesthesia and the stomach washed out with O.15 M NaCl through a soft rubber catheter passed orally. Four hours later, the animals were re-anaesthetized with ether and a ligature placed at the cardia and the stomach removed. Gastric contents were drained into graduated tubes and centrifuged at 3,OOO rpm for ten minutes. Any specimen containing more than O.6 ml solids after centrifuging was discarded, as suggested by Shay et al. (1954).

Animals, selected at random, were operated on until ten

acceptable specimens were obtained from the control and IS groups. In the adrenalin treated immuno-sympathectomy (AIS) group, only nine acceptable specimens were obtained.

Gastric Juice Measurements and Statistical Analysis.

Gastric acid concentration was measured by titration with O.1 N NaOH using phenolphthalein as indicator. The pepsin concentration was estimated by the author's modification of the method of Anson and Mirsky (1932) using casein as the substrate.

Method for determination of Pepsin.

Introduction.

Methods for the estimation of pepsin in gastric juice have been described by many authors and these methods have usually employed different substrates, such as haemoglobin by Anson and Mirsky (1932), or dried plasma or serum by Hunt (1948). The preparation of the haemoglobin substrate (Anson and Mirsky 1932) was found to be too laborious and time consuming. Furthermore, the solution did not keep well and gave high reagent blank readings. Similar high reagent blank readings occur when dried serum or dried citrated plasma as recommended by Hunt (1948) was used and although other substrates such as egg albumin or gelatin were tried, these also gave solutions containing trichloroacetic acid soluble chromogens. The technique to be described used a pure casein substrate (Hammarsten) and has been found to give consistent results

for the estimation of peptic activity in gastric juice.

Principle.

A substrate consisting of casin (Hammersten) was incubated at pH 1.8 with gastric juice and the end products in the protein - free filtrate were determined by means of the blue colour produced by the Folin and Ciocalteu reagent after alkaline copper treatment.

Reagents.

Casein substrate.

One point six g of casein was dissolved in 100 ml of distilled water adjusting the pH to 10 by the addition of NaOH. Before use the solution was filtered through a Whatman No.1 paper. Four batches of casein, obtained from two manufacturers (Hopkin & Williams, and B.D.H.) gave similar results. The stability of the substrate was tested by taking three aliquots from the same preparation, one being stored at room temperature, one at +4°C and one at -10°C. The sample left at room temperature showed marked increase in the extinction of the reagent blank after 48 hours; after storage at +4°C the substrate only began to deteriorate at the end of one week. However, when the solution was stored at -10°C no deterioration was found after one month.

Alkaline copper reagent.

This was made up freshly before use from stock solutions in the following manner; 0.1 ml. of 5% (w/v) $CuSO_4$, $5H_2O$; 0.9 ml. of 1.1%

(w/v) sodium potassium tartrate; 50 ml. of 2% (w/v) sodium carbonate in 0.2 N-NaOH. The addition of alkaline copper reagent renders not only the tyrosine and tryptophan end products of peptic digestion suitable for estimation by the Folin and Ciocalteu reagent, but also soluble peptides (Lowry, Rosebrough, Farr and Randall, 1951).

Stock standard tyrosine solution.

One hundred and forty-five mg. dl-tyrosine was dissolved in 100 ml. of distilled water.

Standard tyrosine solution.

The stock solution was diluted ten fold with distilled water. (0.8 mM solution).

Other reagents.

Folin and Ciocalteu (1927) phenol reagent, diluted with an equal volume of water.

0.12 N-HCl.

0.06 N-HCl.

20% (w/v) trichloracetic acid.

METHOD.

Gastric juice was diluted 100 fold with 0.06 N HCl. As a control. An aliquot was boiled to inactivate pepsin. Casein substrate (1.25 ml.) and 0.12 N HCl (1.25 ml) were introduced to each of four tubes and placed in a water bath at 37°C for 5 minutes. Aliquots (0.1 ml.) of diluted

gastric juice and boiled diluted gastric juice, were added to each of two tubes labelled T (Test) and C (Control) whilst dilute acid (O.1 ml. O.O6 HCl) was added to the other two tubes labelled S (Standard) and B (Blank). Each tube was then incubated for thirty minutes. The mixtures were deproteinized with 1 ml. 20% w/v trichlow acetic acid. After being shaken gently they were left to stand for five minutes, before being centrifuged at 3000 rpm for three minutes. One ml. of each supernatant was pipetted into another four tubes. O.2 ml. of standard tyrosine solution was added to tube S; distilled water (O.2 ml.) was added to the other tubes. Finally, alkaline copper reagent (2.5 ml.) was added to all the tubes and after standing for ten minutes, dilute Folin and Ciocalteu (O.25 ml) was pipetted into each one. They were left for thirty minutes and read at 680 mu. A Beckman D.B. Spectrophotometer was used throughout, employing a 10 mm cell.

Calculations.

$$\text{Peptic activity per ml of gastric juice per minute} = \frac{\text{test - control}}{\text{standard - blank}} \times \text{concentration of tyrosine} \times \frac{1}{\text{incubation time in minutes.}}$$

$$\times \text{ dilution of gastric juice} \times \text{amount of gastric juice in the supernatant taken to a unit of 1 ml. of gastric juice.}$$

$$= \frac{T - C}{S - B} \times O.16 \times \frac{1}{30} \times 100 \times \frac{1}{\left(\frac{O.2}{3.7}\right) \times 1}$$

$$= \frac{T - C}{S - B} \times 9.8 \ \mu \text{ mole tyrosine per minute per ml.}$$

Reproduceability.

Fifteen assays were carried out on the same specimen of gastric juice and gave the following results using the macromethod: - 7.1, 7.3, 6.6, 6.4, 6.9, 7.0, 7.4, 6.9, 7.2, 6.5, 6.7, 6.4, 6.6, 6.9, 6.8. This gave a mean of 6.8, the standard deviation being 0.3. The acceptable range is 2 x 5D (0.6) i.e. 6.2 to 7.4 and all results came within this range.

Linearity.

The linearity of the techniques was investigated using various concentrations of enzyme and of tyrosine. The calibration curve followed Beer's Law in each case up to a concentration of 14 units. Gastric juice giving a result higher than this range should be repeated using a greater dilution of the gastric juice (e.g. 1 in 200).

The mucus concentration was determined by measuring the sialic acid content of the clear gastric juice by the method of Warren (1959).

Sialic Acid Method.

Reagents.

A - Sodium Metaperiodate: 0.2 molar in 9M. H_3PO_4

B - Sodium Arsenite: 10% solution in 0.5M Sodium Sulphate and 0.1 N H_2SO_4.

C - Thiobarbituric Acid: 0.6% in 0.5 M Sodium Sulphate.

D - Cyclohexanone.

Procedure.

Gastric juice (40λ) was diluted to O.2 ml. with water and O.1 ml. of reagent A added. After mixing, it was allowed to stand for twenty minutes. One ml. reagent B was introduced and mixed until the yellow colour disappeared. Three ml. of reagent C was then added and the solution mixed and heated in a boiling water bath for fifteen minutes while covered with a glass bubble. It was cooled in water for five minutes. After the addition of 4.3 ml. of cyclohexanone, the solution was shaken to extract the colour, centrifuged and read in the Beckman Spectrophotometer at 5490 A using a reagent blank. The colour developed is linear up to O.O6 μM sialic acid.

Calculations.

Mucus output per ml. G. J.
Expressed in μ grams.
N-acetyl neuraminic acid = Optical density x Conversion factor.

x Dilution factor.

= O. D. x 25 x O.O75

In the calculations all the sialic acid has been assumed to be N-acetyl neuraminic acid (MW = 309), the conversion factor being O.O75.

The results of the gastric analyses are given as the mean rate of secretion per 100 gm body weight per four hours and as the mean acid output in μEq per 100 gm per four hours. The mean pepsin output was expressed in μ mols tyrosine per ml/min per 100 gm body weight per

four hours, and the mean mucus output in μ gms. N-acetyl neuraminic acid per ml. per 100 gms body weight per four hours.

The results of the IS and the AIS groups were compared with the controls using the Student t test.

Assessment of Gastric Ulceration.

The presence or absence was recorded at the time of sacrifice by the author and his assistant (M. Yaffe). The severity of each lesion was also jointly assessed and then each stomach was photographed twice in colour. These photographs were projected later in a random distribution and two independent assessors (Dr. D.R. Webster and Dr. R.M. Preshaw) ranged each of the slides, giving O for no ulcer, 1 for a small ulcer, 2 for moderate ulceration, and 3 for severe ulceration. Each stomach was thus graded five times and the total score ranked by the Kruskall-Wallis (1956) one way analysis of variance by ranks.

RESULTS

No difference was found in the rate of secretion of gastric juice between the control (individual results - Table XV; composite results - Table XVIII) and IS groups (Table XVI). Nor was any difference observed in the concentration and output of acid and pepsin between these groups. Some reduction in the acid pepsin values were noted in the AIS group (individual results - Table XVII; composite results - Table XVIII).

There was a very marked and statistically significant drop in the mucus concentration of the gastric juice in the IS group compared with the control group, but the mucus concentration was at normal or above normal levels in the AIS group (individual results - Table XV, XVI, and XVII; composite results - Table XIX).

At the time of sacrifice four ulcers were found in the ten control animals. All ten IS animals had ulcers whereas in the nine AIS animals there were only three ulcers and one doubtful one. Applying the Kruskall-Wallis test the ranking totals for the three groups (Table XIX) were 122, 218, and 95 respectively.

TABLE XV
THE CONTROL (HORSE-SERUM) GROUP
(10 animals)

Animal No.	10	12	14	16	18	20	22	24	28	34
Sex.	F	F	F	M	M	M	F	F	F	M
Mean wt. in grams.	180	175	210	285	300	230	210	210	195	250
Mean rate in mls/ 4 hrs/100 gms.	2.61	1.65	0.90	0.42	0.73	0.73	2.76	0.76	0.92	0.88
Mean acid conc in μEq/ml	120	112	114	128	104	118	114	94	108	62
Mean acid output 4 hrs/100 gms in μEq	310	180	100	50	80	90	310	70	100	50
Mean pepsin conc in μ mols tyrosine/ml/min.	32	3.21	5.12	9.91	3.29	3.62	1.39	4.21	1.24	0.27
Mean pepsin output/4 hrs/ 100 gms in μ mols tyrosine/ml/min.	0.84	5.30	4.61	4.16	2.40	2.64	3.84	3.20	1.14	0.24
Presence of ulcer at sacrifice.	-	+	-	+	-	+	-	-	-	+
Ulcer score Mean	0.2	1.6	0.6	1.0	0.8	0.8	0.0	0.6	0.2	1.8
Rank	6	21	10.5	16	13	13	2.5	10.5	6	23.5
Mean mucus conc as μ gms N-acetyl neuraminic acid /ml of G. J.	0.47	0.43	0.61	1.06	0.59	0.77	0.28	0.58	0.42	0.40
Mean mucus output/ 4 hrs/100 gms as μ gms N-acetylneuraminic acid /ml of G. J.	1.22	0.71	0.54	0.44	0.43	0.56	0.77	0.44	0.39	0.35

TABLE XVI

IMMUNOSYMPATHECTOMY GROUP

(10 animals)

Animal No.		3	4	11	13	15	17	19	21	23	25
Sex		M	M	F	F	F	M	M	M	F	F
Mean wt. in gms.		240	220	185	175	200	280	310	255	185	200
Mean rate in mls/ 4 hrs/100 gms.		1.25	1.36	0.49	2.74	0.45	1.64	1.35	0.78	1.08	1.30
Mean acid conc in µEq/ml		106	126	94	118	130	126	96	108	88	100
Mean acid output/ 4 hrs/100 gms in µEq		130	170	50	320	60	210	130	80	100	130
Mean pepsin conc in µ mols tyrosine/ml/min.		2.41	1.34	2.39	5.02	0.85	2.63	0.90	2.78	3.50	0.92
Mean pepsin output/4 hrs/ 100 gms in µ mols tyrosine /ml/min.		3.01	1.82	1.17	13.75	0.35	4.31	1.22	2.19	3.78	1.20
Presence of ulcer at sacrifice		+	+	+	+	+	+	+	+	+	+
Ulcer score	Mean	1.8	1.8	0.8	2.0	1.2	1.8	1.0	2.4	1.4	2.0
	Rank	23.5	23.5	13	26.5	18	23.5	16	28	19.5	26.5
Mean mucus conc as µ gms N-acetyl neuraminic acid/ml of G. J.		0.59	0.38	0.48	0.09	0.57	0.22	0.24	0.27	0.42	0.27
Mean mucus output/ 4 hrs/100 gms as µ gms N-acetyl- neuraminic acid/ ml of G. J.		0.73	0.52	0.24	0.25	0.26	0.36	0.33	0.21	0.46	0.35

TABLE XVII

THE ADRENALIN TREATED IMMUNOSYMPATHECTOMY GROUP
(9 animals)

Animal No.	41	42	43	44	47	51	58	59	60
Sex.	M	M	F	F	M	M	F	F	F
Mean wt. in gms.	270	290	185	215	305	320	160	200	190
Mean rate in mls/ 4 hrs/100 gms.	0.78	0.48	3.68	0.93	1.74	0.51	2.00	1.60	1.16
Mean acid conc. in µEq/ml	80	110	86	78	40	58	84	38	54
Mean acid output 4 hrs/100 gms in µEq	60	50	320	70	60	30	170	60	60
Mean pepsin conc in µ mols tyrosine /ml/min.	0.09	0.97	1.46	1.15	1.36	2.07	1.38	1.25	1.09
Mean pepsin output/ 4 hrs/100 gms in µ mols tyrosine./ml/min.	0.15	0.65	5.37	1.06	2.37	1.06	2.76	2.00	1.26
Presence of ulcer at sacrifice.	-	+	+	-	?	+	-	-	-
Ulcer score Mean	0.0	1.4	3.0	0.2	0.4	1.0	0.0	0.0	0.4
Rank	2.5	19.5	29	6	8.5	16.0	2.5	2.5	8.5
Mean mucus conc as µ gms N-acetyl neuraminic acid /ml of G. J.	0.57	0.79	0.43	0.55	0.75	0.64	0.43	0.49	1.24
Mean mucus output/ 4 hrs/100 gms as µ gms N-acetylneuraminic acid / ml of G. J.	0.44	0.38	1.49	0.51	1.31	0.33	0.86	0.78	1.43

TABLE XVIII

Groups	Control Group	I.S. Group	A.I.S. Group
Nos in each group	10	10	9
Sex	6F — 4M	5F — 5M	5F — 4M
Mean wt. in gms.	225	225	237
Mean rate in mls/ 4 hrs/100 gms.	1.24 ± 0.26[1]	1.24 ± 0.21[2]	1.43 ± 0.33[7]
Mean acid conc in μEq/ml	107 ± 6	109 ± 7[3]	70 ± 8[8]
Mean acid output/ 4 hrs/100 gms in μEq	130 ± 30	140 ± 30[4]	98 ± 30[9]
Mean pepsin conc in μ mols tyrosine per ml/min.	3.26 ± 0.91	2.27 ± 0.42[5]	1.20 ± 0.17[10]
Mean pepsin output/ 4 hrs/100 gms in μ mols tyrosine per ml/min.	2.84 ± 0.54	3.28 ± 1.23[6]	1.85 ± 0.52[11]

1 = standard error of the mean

2 = control vs IS = NS
3 = control vs IS = NS
4 = control vs IS = NS
5 = control vs IS = NS
6 = control vs IS = NS

7 = control vs AIS = NS
8 = control vs AIS = S $p < .005$
9 = control vs AIS = NS
10 = control vs AIS = S $p < .05$
11 = control vs AIS = NS

S = significant
NS = not significant

TABLE XIX

Group	Control Group	I.S. Group	A.I.S. Group
Number in group	10	10	9
Mean mucus conc as μ gms N-acetyl neuraminic acid per ml of G.J.	0.56±0.07[1]	0.35±0.05[2]	0.65 ± 0.08[3]
Mean mucus output 4 hrs/100 gms as μ gms N-acet. neuram. acid per ml of G.J.	0.59±0.08	0.37±0.07[4]	0.84 ± 0.16[5]
Numbers of animals with ulcers at time of sacrifice.	4	10	3-4[6]
Scores of ulcers in each group	122	218	95[7]

1 = standard error of the mean
2 = IS group vs control $p < .005$, significant.
3 = AIS group vs control $p < .025$, significant.
4 = IS group vs control $p < .005$, significant.
5 = AIS group vs control, $p < .005$, significant.
6 = Three definite and one doubtful ulcer.
7 = The null hypothesis that there is no difference between the groups can be rejected with $0.02 > p < 0.05$

DISCUSSION

It is often a difficult procedure to section the sympathetic nerve supply to the stomach and the operative trauma may interfere with gastric function studies, especially if they are performed at the time of nerve section. Yet if they are performed after some time, the results may be invalidated by ' collateral nerve sprouting'. a process which has been shown to occur in the autonomic nervous system (Murray and Thompson 1957; Rivilis 1965) and lead to functional recovery.

Drugs such as reserpine have the disadvantage that they possess central or other side effects which may give false results. Immunosympathectomy therefore is a useful research tool for studying the effects of sympathetic denervation, although it could be observed histologically that not all ganglion cells are destroyed.

Experimental ulcer production in the pylorus ligated rat was first described by Shay, Fels, Merance, Gruenstein, and Siplet in 1945. These ulcers occurred almost solely in the squamous lined part of the stomach and increased in number and severity (i. e. perforation and haemorrhage) in direct proportion to the length of time of the pyloric tie. In the present experiments, all the ulcers occurred in the glandular part of the stomach and there was a pronounced increase in the number of ulcers produced in the IS animals (4/10 → 10/10). However, no corresponding increase in acid or pepsin production was observed;

the output of each component was the same in each group suggesting that the sympathetic nervous system plays little or no part in acid or pepsin secretion whilst having a marked influence on mucosal integrity. Adrenaline effected a reverse on the rate of ulceration in IS rats (10/10→3-4/9), accompanied by some drop in acid and pepsin output.

As no change in acid or pepsin accompanied the high incidence of ulcer in the IS group, it seems unlikely that the reduction in acid and pepsin in the AIS group was responsible for protecting the gastric mucosa against ulceration provoked by immuno-sympathectomy. Changes in sialic acid containing mucus mirrored the number of ulcers. The IS animals showed a large drop in the mucus content of their gastric juice compared with controls and following a large dose of adrenaline the AIS group showed a mucus production within normal or above normal limits.

The loss of mucus in the gastric lumen corresponds with the previous findings (Rivilis 1966 a, 1966 b) in which mucus was greatly diminished in sections of IS animals' stomachs stained histochemically.

Sialic acid containing mucus in the gastric lumen can be derived from salivary secretions as well as from the gastric mucosal epithelium. In these experiments it was assumed that equivalent amounts of saliva would reach the stomach in all animals; the reduced mucus content of the IS group may be partly due to lowered sialic acid containing mucus from the salivary glands as well as from the gastric source.

The sympathetic nervous system may have an as yet unelucidated "trophic influence" on the gastric epithelium and a breakdown of this control, further aggravated by exposure to unbuffered gastric juice in the fasting pylorus ligated rat may be the explanation for the ulceration.

Sympathetic ablation increases visceral blood flow and could be expected to lead to improved nutrition of the mucosa. The results do not show the beneficial effects of an increased visceral blood flow, in fact the reverse is true.

The sympathetic nervous system probably plays an important and direct role in mucus secretion as suggested by Baxter (1934) who found that vagally denervated whole stomach preparations in the cat and the dog produced a steady secretion of mucus on splanchnic stimulation and on repeated injections of adrenaline. Immuno-sympathectomy would thus lead to loss of the first line of defense of the gastric mucosa, according to Hollander's hypothesis (1954).

Another possible explanation of the findings stems from the work on intestinal renewal by Dupont, Biggers, and Sprinz (1965), who discovered that jejunal villus epithelial cell renewal was markedly decreased in both reserpine treated and IS animals compared with a bovine serum treated control group. By injecting tritiated thymidline followed by radio-autography, they showed that after forty-eight hours there was

epithelial cell spillover from the tips of the villi of the control group, whereas in the two experimental groups the active cells had progressed only halfway up the villus.

In the present experiments the cell turnover rate may be lower, resulting in poor cellular function and a concomitant drop in mucus production. It is not yet clear which factors are the most critical and probably more than one factor is responsible for the final outcome of gastric glandular ulceration.

SUMMARY

The immunologically suppressed sympathetic nervous system gives rise to an increased incidence of gastric glandular ulceration in pylorus ligated rats. This is accompanied by a decreased mucus content in the gastric juice with no appreciable change in acid or pepsin output. Adrenaline treatment of these animals seems to reverse the ulcerogenic effects of sympathetic ablation and restores mucus production to normal and above normal levels.

CONCLUSIONS

SECTION I

Incomplete vagotomy in surgical patients is not a rare occurrence. The commonest fault is the failure to divide a complete vagal nerve trunk. It is therefore important to know what the effect of this may be in man.

The only three references in the literature disagree as to the effects of unilateral vagotomy. One of the studies was done on the rat and another on the dog. The third author did not stipulate his experimental animal.

In Section I of this thesis it has been shown that unilateral vagotomy does significantly reduce the mean acid output in the rat and the dog. It favours the view that the vagus supplies the stomach in a segmental fashion and that individual vagal fibres supply separate secretory units. The gross reduction, however, probably represents the sum of a number of different effects.

It is suggested that only maximal stimuli may be useful in clinical practice for differentiating the varying degrees of partial denervation of the stomach.

SECTION II

The gastric barrier has always proved difficult to assess. The problem has been to damage the gastric barrier with trauma to the mucosa and with the continued production of normal amounts of acid and pepsin.

This has been achieved in rats by immunologically suppressing the sympathetic nervous system.

These animals show an increased incidence of gastric glandular ulceration and a concommitant decreased mucus production with no appreciable change in acid or pepsin production.

Adrenaline pre-treatment of immuno-sympathectomized animals reverses the ulcerogenic effects and returns the mucus values to normal or above normal.

It is suggested that the sympathetic nervous system may play an important role in "the gastric barrier".

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