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THE ROLE OF VAGAL AND INTRAMURAL INHIBITORY REFLEXES IN THE REGULATION OF INTRAGASTRIC PRESSURE IN THE FERRET

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SUMMARY

1. The step inflation technique combined with nerve section and pharmacological receptor blockade was used to determine the gastric distribution of the vagal inhibitory fibres and their role in the regulation of intragastric pressure in the anaesthetized ferret.

2. Under the conditions described the predominant effect of the vagus was inhibitory. The dorsal abdominal vagus mediated more inhibition than the ventral vagal trunk. There was partial overlap in the inhibitory effects of the two trunks but, in contrast to excitatory effects, this overlap was less than complete.

3. No evidence was found to indicate that the intramural inhibitory neurones (demonstrated pharmacologically) could be activated by a local distensive stimulus or that local inhibitory reflexes played an independent role in the regulation of intragastric pressure. The vago–vagal inhibitory reflexes play the major (possibly the only) part in the over-all regulation of intragastric pressure.

4. After a step gastric inflation (50 ml. in < 3 sec) the time taken for the intragastric pressure to fall to $1/e$ of the peak pressure was a function of the number of inflations; the time decreased with increased number of inflations. It was apparently unaffected by nerve lesions.

5. The intragastric pressure in response to an inflation, besides being a function of the neural reflexes evoked, was also related to the gastric weight and hence the size of the animal.

6. The sum of the prevailing intragastric pressure and the response to vagal stimulation (10 Hz, 10 sec) was similar, irrespective of the intragastric fluid volume (10–80 ml.).

7. The volume of fluid in the corpus was shown to be a major determinant of the contraction produced in response to vagal stimulation: as the corpus volume increased the corpus contraction decreased but the relaxation that follows the contraction increased. A similar effect was observed with intra-arterial ACh which implies that the response to stimulation was modified directly by the degree of stretch of the smooth muscle cells. Inhibitory effects were not apparent unless the stomach was moderately inflated.

8. The relative roles of the vagal reflexes, intramural reflexes and the smooth

muscle in the regulation of intragastric pressure are discussed in the light of the above observations.

INTRODUCTION

In a recent study electrical stimulation and selective section of vagal branches were used to assess the role of each branch in the regulation of intragastric pressure (Andrews, Lawes & Bower, 1980*c*). Total functional overlap was demonstrated between the dorsal and ventral abdominal trunks of the vagus in their effect on intragastric pressure. Stimulation of the dorsal trunk produced a greater increase in intragastric pressure than stimulation of the ventral trunk. A possible explanation for the differential effect of dorsal and ventral vagal stimulation was that the ventral trunk had a greater influence than the dorsal trunk on the non-adrenergic intrinsic inhibitory nerves. If so, electrical stimulation at intensities supramaximal for C fibres would activate simultaneously both excitatory and inhibitory axons and evoke more inhibition when applied to the ventral trunk. The gastric response to ventral stimulation would therefore be smaller than the response to dorsal stimulation.

In the present study the vagal-inhibitory reflexes were reflexly activated by gastric distension to determine their distribution (Andrews, Grundy & Lawes, 1980*a*). We attempted to eliminate other known gastro–gastric reflexes and leave only the vago–vagal inhibitory ones; vago–vagal excitatory reflexes can be blocked by atropine (Andrews *et al.* 1980*a*), vago–splanchnic (Cragg & Evans, 1960) and splanchno–splanchnic (Abrahamsson, 1974) reflexes by bilateral splanchnic nerve section. The role of local reflexes dependent on nicotinic cholinergic receptors was studied by examining the effects of hexamethonium on intragastric pressure.

The principal aim of the present study was to determine whether the organization of vagally mediated gastric relaxation was similar to that of vagally mediated gastric excitation. The results of the principal study were extended by administering drugs or stimulating cervical vagi in ferrets with intact or divided stomachs containing different volumes of fluid.

METHODS

Reflex studies

Animals and surgery

Thirty-six male and female ferrets weighing 510–1270 g were used. They were anaesthetized with Urethane (1·5 g kg^{-1}; 50 % solution in 0·9 % saline I.P.). A tracheostomy was performed and the right external jugular vein was cannulated for intravenous injections. An oesophageal tube (firm plastic, 0·5 cm diameter) was secured in the stomach by an external ligature at mid-cervical level. It led from the stomach to a pressure transducer (Bioscience pressure monitor) and amplifier, the output of which was displayed on a chart recorder (Bryans 28000). A second tube inserted into the stomach from a duodenostomy was secured by an external ligature above the entry of the bile duct into the duodenum. This tube was used for inflation and drainage of the stomach. All animals were bilaterally splanchnectomized via an abdominal exposure. The abdomen was then closed with Michelle clips. Rectal temperature was maintained at 37–39 °C by radiant heat and a homeothermic blanket.

After surgery an interval of 20 min elapsed to allow stabilization of gastric activity. In all animals 50 ml. saline at 38 °C was then injected into the stomach, within 3 sec. Five minutes later the stomach was deflated and care was taken to recover all 50 ml. of saline.

The animals were then divided into six groups, each of six ferrets. One minute after the first inflation, atropine sulphate (1 mg kg^{-1} I.V.) at a dose sufficient to block vagally evoked gastric

contractions (Andrews & Scratcherd, 1980) was administered to five groups of six animals. The thirty atropinized animals and six unatropinized animals had a second gastric inflation 10 min later.

One group of six animals had a dorsal vagotomy, another group a ventral vagotomy and two more groups both a dorsal and a ventral vagotomy. Ten minutes later the stomach was inflated for the third time. Subsequently in animals with one vagotomy the converse vagotomy was performed. One of the two groups which had both trunks sectioned was given a low dose of hexamethonium (1 mg kg^{-1} I.V.). The other group was given a high dose (20 mg kg^{-1} I.V.). All six groups of animals then received a fourth inflation. The neuroectomies were confirmed by extensive dissection at post-mortem.

Analysis of results

Previous studies (Andrews *et al.* 1980*a*) had shown that after inflation intragastric pressure rises to an immediate peak and then drops to a plateau level in under 5 min. In these experiments the peak increase in intragastric pressure and the plateau amplitude 5 min later were measured after each procedure. Contractions superimposed upon the static pressure level were not included in the measurement of pressure. The volume of fluid (50 ml.) used for the inflations is within the physiological range for the ferret although the rate of inflation is unphysiological (Andrews, Grundy & Scratcherd, 1980*b*). The peak pressure during a step inflation was about twice that for a physiological ramp inflation of the same volume. However the plateau pressure levels were the same for both types of inflation, and therefore the plateau levels of pressure from the present study correspond to physiological levels. The main reason for using a step inflation was to study whether vagotomy, atropine or hexamethonium affected the time course of the gastric pressure adaptation. The time taken for the pressure to fall by 0·632 (i.e. $1/e$ of the amplitude from peak to plateau) was used as an index of the rate of adaptation of the gastric musculature. It was also expected that the higher pressures reached after step inflation would favour activation of inhibitory reflexes.

The results from each group of animals was subjected to a two-way analysis of variance, and the results from different groups were subjected to a one-way analysis of variance. Responses from within a group of animals were then compared using a paired-sample t test, and responses from different groups of animals were compared using an unpaired-sample t test. Corrections for equal variance were employed where necessary.

The incidence of rhythmic contractions before and after inflation was determined. These results were subjected to the McNemar test for the significance of changes in related samples.

Vagal stimulation studies

Animals and surgery

Fifteen male animals weighing 700–1400 g were used. They were anaesthetized with either Urethane (1·5 g kg^{-1}; 50 % solution in 0·9 % saline I.P.) or Inactin (120 mg kg^{-1} I.P.). An endotracheal tube was inserted via the mouth and the right external jugular vein was cannulated for intravenous injections. The stomach was intubated via the mouth and the tube secured by a ligature around the cervical oesophagus. The abdominal viscera were exposed via a mid-line incision and the greater splanchnic nerves were sectioned bilaterally. The stomach was either left intact and the pylorus occluded by a ligature or it was divided into corpus and antrum as previously described (Andrews *et al.* 1980*b*). Pressure in the antrum was monitored via a fluid-filled tube inserted into the antrum from the duodenum and connected to a pressure transducer (Bioscience, pressure monitor). The pressure transducer outputs were displayed on either a Bryans 28000 recorder or a four-channel Gould recorder (2400). Systemic arterial blood pressure was measured from a cannula sited in either the right common carotid artery or the right femoral artery. Heart rate was monitored from the e.c.g.

The vagi were mobilized in the neck over a distance of 5 mm and were initially left intact. A paraffin pool was made in the neck and the temperature was monitored and maintained at 39 °C. After the vagi were sectioned they were placed over bipolar silver stimulating electrodes to which pulses were delivered from a constant-current stimulator (Neurolog NL800) driven by a DS2 stimulator (Devices). The pulses were timed by a Digitimer (D4030). Pulses of up to 20 V of 0·5 msec duration (maximum current 1·5 mA) and variable frequency were used. The nerve was not desheathed prior to stimulation.

Drugs were administered either into the jugular vein (atropine 0·5–1 mg kg^{-1}; hexamethonium 1 or 20 mg kg^{-1}) or directly into the gastric blood supply via a cannula inserted retrogradally into

the splenic artery. Drugs injected into the gastric circulation were acetylcholine (ACh) chloride (10 μg in 0·1 ml.), veratrine (100–500 μg in 0·5 ml.), phenyldiguanide (100–300 μg in 0·3 ml.), nicotine (5–20 μg in 0·1 ml.).

Results from this part of the study are expressed as mean $\pm$ S.E. of mean and statistical significance was tested using a t test.

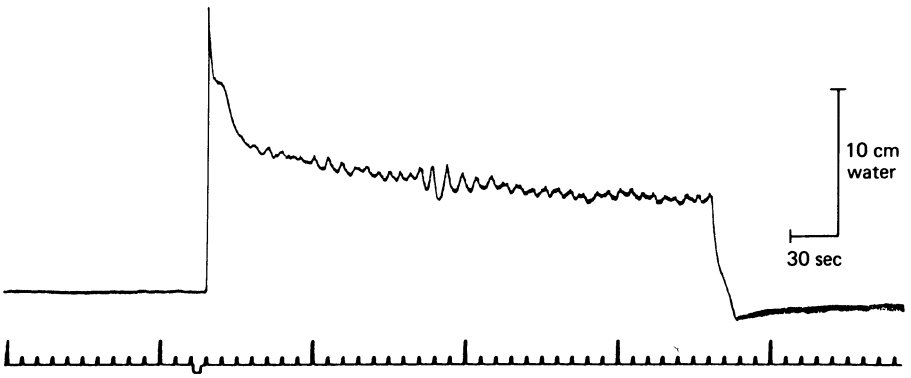

Fig. 1. The rise in intragastric pressure in response to a step inflation of warm saline (50 ml.). Pressure was measured at the peak and 5 min later. Rhythmic contractions were evident when the stomach was inflated in this animal.

RESULTS

Inflation studies

Validity of intergroup comparisons

All thirty-six animals had a bilateral splanchnectomy followed by a step inflation of 50 ml. The intragastric pressure rose to a peak of 22·1 $\pm$ 1·0 cm H_2O and then declined rapidly to a plateau of 6·6 $\pm$ 0·3 cm H_2O 5 min after the peak. An example of a typical response from one animal is shown in Fig. 1. The animals were divided into six groups and a one-way analysis of variance was carried out on each of the three measures (peak and plateau pressures and the time taken to fall to 0·368 of the difference between the two). None of the variance ratios was significant ($F = 0{\cdot}35$ for peaks; $F = 0{\cdot}44$ for plateaus; $F = 0{\cdot}80$ for the time course, $\text{d.f.}_1 = 5$, $\text{d.f.}_2 = 30$). This confirms that the animals were assigned to different groups without bias, so comparisons between them could be made with confidence.

The effects of repeated inflation

The control group was given a bilateral splanchnectomy followed by four inflations at the same intervals as for the experimental groups. A two-way analysis of variance was carried out on each of the three measures, and showed that while repeated inflation had no effect on peak ($F = 0{\cdot}87$, $\text{d.f.}_1 = 3$, $\text{d.f.}_2 = 15$) or plateau ($F = 0{\cdot}57$, $\text{d.f.}_1 = 3$, $\text{d.f.}_2 = 15$) pressures, the time course of adaptation was significantly affected ($F = 6{\cdot}63$, $\text{d.f.}_1 = 3$, $\text{d.f.}_2 = 15$, $P < 0{\cdot}01$). This means that changes in peak or plateau pressures in the experimental groups may be attributed to the experimental treatment. Conversely, without independent evidence, changes in the time course of adaptation may not be attributed to the experimental treatment.

The effects of atropine

A second control group was given a bilateral splanchnectomy and a first inflation, then atropine (1·0 mg kg^{-1}). After atropine this group was given three additional inflations. A two-way analysis of variance on each of the three measures for the three post-atropine inflations was performed (i.e. excluding the pre-atropine inflation). It showed that neither peak ($F = 0{\cdot}13$, $d.f._1 = 2$, $d.f._2 = 10$) nor plateau ($F = 1{\cdot}23$,

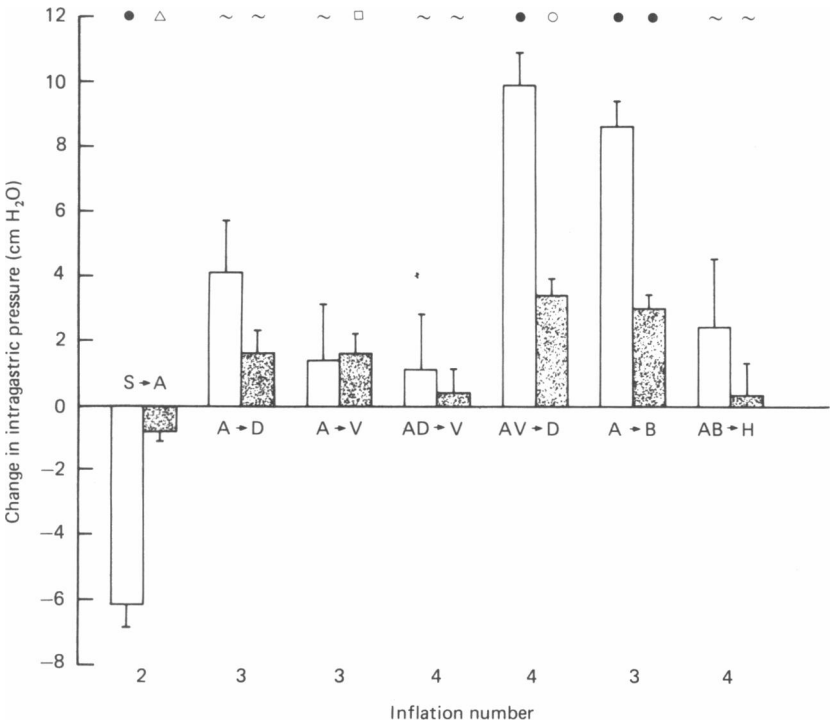

Fig. 2. Changes in intragastric pressure responses to step inflation. The first bar of each pair represents the change in peak pressure (increases shown as positive) and the second bar represents the change in plateau pressure. Responses decrease when atropine follows splanchnectomy (S → A). Responses increase when dorsal vagotomy (A → D), ventral vagotomy (A → V) or both dorsal and ventral vagotomy (A → B) follow atropine. Responses increase when ventral vagotomy follows dorsal vagotomy and atropine (AD → V), when dorsal vagotomy follows ventral vagotomy and atropine (AV → D) and when hexamethonium (20 mg kg^{-1}) follows both vagotomies and atropine (AB → H). Standard errors and the levels of significance are also shown. ●: $P < 0{\cdot}001$. ○: $P < 0{\cdot}002$. △: $P < 0{\cdot}01$. □: $P < 0{\cdot}05$. ~: not significant.

$d.f._1 = 2$, $d.f._2 = 10$) pressures were affected by repeated inflation, but the time course of adaptation was significantly affected ($F = 5{\cdot}32$, $d.f._1 = 2$, $d.f._2 = 10$, $P < 0{\cdot}05$). Consequently any change in peak or plateau pressures after vagotomy or hexamethonium may be attributed to the treatment and not to any preceding dose of atropine. Changes in time course will be dealt with in a separate section.

All remaining animals (four groups) were given a bilateral splanchnectomy, a first inflation, atropine (1 mg kg^{-1} I.V.), then a second inflation. The reduction in peak

and plateau pressures after atropine for these four groups combined with the atropine control group are shown in Fig. 2.

A paired-sample t test was performed on the results and showed that a mean decrease of $6{\cdot}1 \pm 0{\cdot}7$ cm H_2O in the peak pressure was significant ($P < 0{\cdot}001$); a mean decrease of $0{\cdot}8 \pm 0{\cdot}26$ cm H_2O in the plateau pressure was also significant ($P < 0{\cdot}01$). From closer inspection of the data it appeared that atropine had a greater effect if the response to the control inflation was high. If the response to the control inflation was low the effect of atropine was barely distinguishable from the effect of a second control inflation. This suggests that high control pressures may be due to cholinergic excitation whereas low control pressures may be due, at least in part, to the absence of cholinergic excitation.

Effects of vagotomy on pressure

Twelve of the ferrets discussed in the preceding section then had either a dorsal or a ventral abdominal vagotomy followed by a third inflation. Subsequently they received whichever vagotomy they had not yet received, and then had a fourth inflation. The changes in amplitude of responses to the third and fourth inflations are plotted in Fig. 2.

Two-way analysis of variance showed that significant changes in pressure occurred in both groups. Paired-sample t tests showed that the increase in plateau pressure of $1{\cdot}6 \pm 0{\cdot}6$ cm H_2O following ventral vagotomy was significant ($P < 0{\cdot}05$). Apart from this the effects of single truncal vagotomy were not statistically significant. The effect of ventral vagotomy was, if anything, smaller than the effect of dorsal vagotomy, and statistically there was no significant difference between them.

After receiving a second truncal vagotomy, however, peak pressure rose by $8{\cdot}2 \pm 1{\cdot}2$ cm H_2O ($P < 0{\cdot}001$) over the level following atropine alone. Plateau pressures increased by $3{\cdot}5 \pm 0{\cdot}6$ cm H_2O ($P < 0{\cdot}001$). This implies that either one of the vagal abdominal trunks is capable of maintaining gastric relaxation in the absence of the other trunk, and that in the absence of both trunks the stomach shows a significant increase in its pressure response to inflation.

In the case of plateau pressures this increase is to a level significantly above the control level before atropine ($2{\cdot}7 \pm 0{\cdot}7$ cm H_2O, $P < 0{\cdot}01$). This suggests that the degree of inhibition exerted by the vagus was greater than the degree of excitation under the prevailing conditions.

That these increases in pressure after vagotomy were genuine and not the consequence of repeated inflation was shown by comparing them to the responses to a fourth inflation in the atropine control group. The peak pressure after vagotomy was $7{\cdot}7 \pm 3{\cdot}4$ cm H_2O higher than in the atropine control group ($P < 0{\cdot}05$), and the plateau pressure was $2{\cdot}7 \pm 1{\cdot}1$ cm H_2O higher ($P < 0{\cdot}05$).

The increase when dorsal vagotomy followed ventral vagotomy ($9{\cdot}9 \pm 1{\cdot}0$ cm H_2O for peaks, $3{\cdot}4 \pm 0{\cdot}5$ cm H_2O for plateaus) were significantly greater ($P < 0{\cdot}01$) than the increases when ventral vagotomy followed dorsal vagotomy ($1{\cdot}1 \pm 1{\cdot}7$ cm H_2O peaks, $0{\cdot}4 \pm 0{\cdot}7$ cm H_2O for plateaus (Fig. 2). This suggests that despite the fact that either can inhibit the stomach without the other, the dorsal trunk exerts a greater effect.

The combined effects of hexamethonium and vagotomy

Two groups of six animals with a bilateral splanchnectomy and atropine then had both a dorsal and ventral vagotomy simultaneously. After a third inflation they were then given hexamethonium, either 1·0 mg kg^{-1} or 20·0 mg kg^{-1} I.V. (A pilot study showed that 20 mg kg^{-1} of hexamethonium was necessary to block the gastric inhibitory effects of electrical stimulation of the vagi). The changes in pressures are shown in Fig. 2. Two-way analysis of variance of all the results showed that statistically significant changes took place. Paired-sample *t* tests were performed to determine whether the significant changes were attributable to the atropine, vagotomy or hexamethonium, and the results of these are detailed below.

The *t* tests showed that the increase in peak pressure after vagotomy (8·6 ± 0·8 cm H_2O) was significant ($P < 0·001$) and so was the increase in plateau pressure (3·0 ± 0·4 cm H_2O, $P < 0·001$). Once again, when compared to the responses to a third inflation in the atropine control group, the increase in peak pressure relative to the control group pressure remained significant (8·5 ± 4·0 cm H_2O, $P < 0·05$) although the increase in plateau pressure just failed to reach significance (2·4 ± 1·3 cm H_2O, $P < 0·10$). This lends further weight to the assumption that the effect of vagotomy is real and not due to atropine wearing off or to repeated inflation. Furthermore, pressures after a third inflation in the two simultaneous vagotomy groups were indistinguishable from pressures after a fourth inflation in the two sequential vagotomy groups ($F = 1·74$ for peaks; $F = 2·94$ for plateaus, $d.f._1 = 1$, $d.f._2 = 22$, using a one-way analysis of variance).

Following hexamethonium, small increases occurred for both peak and plateau pressures but these increases were not statistically significant whether hexamethonium was used at a dose of 1 mg kg^{-1} or 20 mg kg^{-1}. All the significant changes occurring in this group were therefore attributable to vagotomy.

Effect of nicotine, veratrine and phenyldiguanide

It was unexpected that we were unable to demonstrate any intramural pressure regulating reflex involving activation of the intrinsic inhibitory neurones by a cholinergic pathway sensitive to blockade by hexamethonium. To test whether the intrinsic inhibitory neurones could be driven by activation of nicotinic cholino-receptors, injections of nicotine were given into the gastric circulation after the animals had been vagotomized and atropinized. As the corpus is the area of stomach which exhibits the greatest ability to relax in response to activation of the vagal inhibitory fibres the stomach was divided and the pressure monitored in the corpus to increase the likelihood of observing relaxation. Nicotine (20 μg) produced a prompt long-lasting fall in intracorpus pressure (Fig. 3) which was abolished by prior administration of hexamethonium (20 mg kg^{-1} I.V.). Veratrine and phenyldiguanide have previously been shown to activate mechano- and chemoreceptors (Paintal, 1954; Paton & Vane, 1963). A fall in intracorpus pressure was produced by veratrine (Fig. 4) or phenyldiguanide injected into the gastric circulation of atropinized, vagotimized animals. The responses to these substances was reduced 50% by pre-treatment with hexamethonium.

Time course of pressure adaptation after step inflation

After the first inflation the pressure took 15·6 ± 1·4 sec to fall by 62·2 % (1/*e*) of the difference between peak and plateau amplitudes. There was a significant reduction in the time course of adaptation in the control group with successive inflations ($F = 6{\cdot}63$, d.f.$_1 = 3$, d.f.$_2 = 15$, $P < 0{\cdot}01$). In order to determine whether any of the time course changes in the other groups were attributable to their experimental

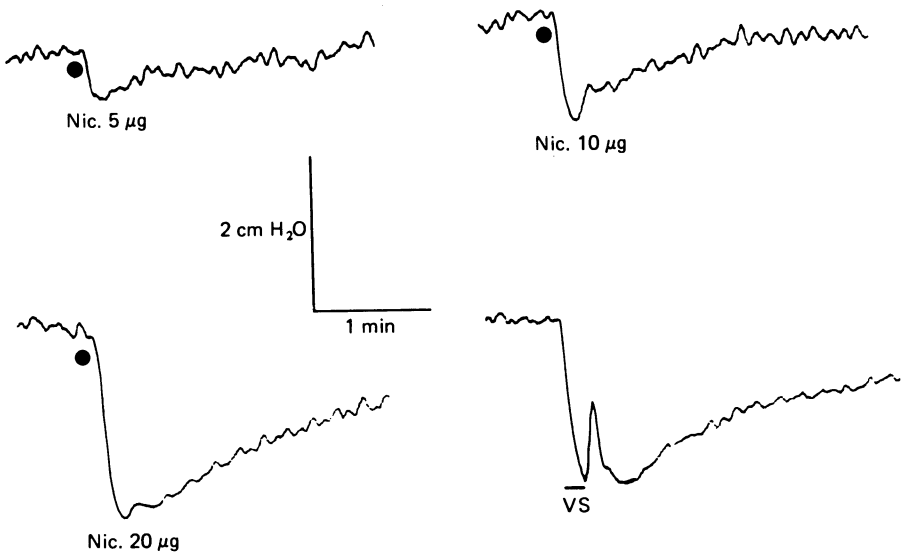

Fig. 3. The effect of intra-arterial nicotine (Nic.) on the intracorpus pressure in an atropinized animal (1 mg kg^{-1}). The response to vagal stimulation (VS) is shown for comparison.

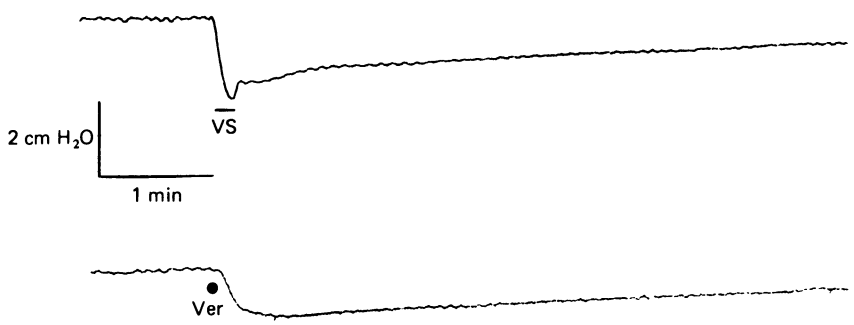

Fig. 4. The effect of vagal stimulation (VS) (20 V, 10 Hz, 0·5 msec, 10 sec) and veratrine (Ver. 100 μg) on the intracorpus pressure in an atropinized (1 mg kg^{-1}) animal.

treatment rather than to repeated inflation, factor analysis was performed. It was found that the differences between groups were not significant ($F = 2{\cdot}23$, d.f.$_1 = 5$, d.f.$_2 = 120$) whereas the effect of repeated inflation was significant ($F = 35{\cdot}74$, d.f.$_1 = 3$, d.f.$_2 = 120$, $P < 0{\cdot}01$). There were no significant interactions between groups and inflations ($F = 0{\cdot}76$, d.f.$_1 = 15$, d.f.$_2 = 120$). In other words the change in time

course with repeated inflation was not influenced by atropine, vagotomy or hexamethonium. The time course of adaptation for all thirty-six animals is plotted in Fig. 5. The greatest fall occurred between the first and second inflations.

Rhythmic contractions

The 5 min periods preceding and following each inflation were examined for the presence of rhythmic contractions. Any discernable periodic deflexion in the trace was accepted as the occurrence of motility.

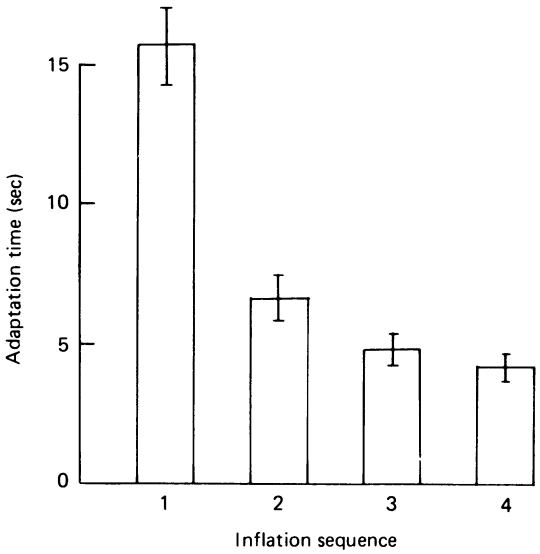

Fig. 5. The time for the pressure to fall 62·2 % ($1/e$) of the difference between peak and plateau pressures, plotted against the number of inflations.

Before the first inflation there were rhythmic contractions in 36 % of the animals. After the first inflation this proportion rose to 89 % ($P < 0{\cdot}025$).

Following atropine, the incidence of spontaneous rhythmic contractions fell to 11 % ($P < 0{\cdot}025$). The incidence of rhythmic contractions evoked by inflation fell to 13 % ($P < 0{\cdot}001$).

Neither dorsal nor ventral vagotomy on its own significantly increased the incidence of rhythmic contractions relative to the incidence after atropine. When both trunks were cut, however, rhythmic contractions were seen in 63 % of records prior to inflation ($P < 0{\cdot}025$) and in 83 % of records after inflation ($P < 0{\cdot}025$).

Hexamethonium had no significant effect on the incidence of rhythmic contractions.

Cervical vagi and resting tone

The effects of cervical vagotomy on the prevailing intracorpus pressure at a volume of 20 ml. were examined. Section of any one cervical nerve produced a small but significant rise in pressure ($P < 0{\cdot}05$). Section of the second cervical nerve resulted in a much larger rise in pressure ($P < 0{\cdot}01$). The results were similar irrespective of

which nerve was cut first. The increase in pressure after the second vagotomy was significantly greater than the increase after the first vagotomy ($P < 0.05$). This complements the finding of partial overlap between dorsal and ventral abdominal trunks by showing that such overlap also applies to cervical vagi. Left and right cervical vagi are therefore equivalent in their inhibitory effects, as has been shown

TABLE 1. Linear regression of intragastric pressure (y) against wet gastric weight (x) after sympathectomy or sympathectomy plus atropine

		r	P
Post-sympathectomy peak	$y = 38{\cdot}25 - 2{\cdot}45x$	$-0{\cdot}40$	$< 0{\cdot}02$
Post-sympathectomy plateau	$y = 15{\cdot}51 - 1{\cdot}34x$	$-0{\cdot}65$	$< 0{\cdot}001$
Atropine peak	$y = 37{\cdot}83 - 3{\cdot}27x$	$-0{\cdot}64$	$< 0{\cdot}001$
Atropine plateau	$y = 12{\cdot}83 - 1{\cdot}06x$	$-0{\cdot}61$	$< 0{\cdot}001$

previously for their excitatory effects (Andrews *et al.* 1980*c*). Since intracorpus pressure increased after cervical vagotomy the predominant effect of the vagus prior to vagotomy was presumably inhibitory. The increase caused by vagotomy occurred even in animals pre-treated with atropine so it represents a loss of inhibition and not a change in excitation.

The effect of gastric weight and volume

In a previous paper (Andrews *et al.* 1980*a*) it was observed that 'smaller animals showed larger intragastric pressure rises associated with 50 ml. gastric inflations than did larger animals'. This observation has been extended in the present study to define the relationship between intragastric pressure and gastric weight. Peak and plateau pressures after sympathectomy and after atropine were found to be negative linear functions of gastric weight (Table 1). Non-linear regression was not attempted because scatter diagrams of pressure against gastric weight did not warrant it. As the Table shows, the correlations between gastric weight and intragastric pressure were low but nevertheless significant.

Closer inspection of the data suggested that in heavier stomachs the balance between excitation and inhibition shifted towards greater inhibition. If a constant volume of fluid in stomachs of different sizes altered the balance of excitation and inhibition then different volumes in a stomach of constant size might have the same effect. This was tested explicity by stimulation of the distal ends of cut cervical vagi with a standard stimulus (10 S, 10 Hz, 0·5 msec, 20 V) while gastric volume was varied. The results are plotted in Figs. 6 and 7. As can be seen from Fig. 6 increasing the volume of fluid in the stomach reduces the amplitude of vagally evoked response. At the largest volume used (80 ml.) the amplitude of vagally evoked response was reduced to 40 % of the maximal. The difference in amplitude between 20 and 40 ml., and between 40 and 60 ml., was significant ($P < 0.05$ in both cases). Furthermore, the degree of relaxation following vagally evoked contraction also appeared to increase (Fig. 7).

The shift in balance of excitation and inhibition caused by altering intragastric volume might have been uniform throughout the stomach. On the other hand it might have represented an alteration in the preponderance of activity between corpus and

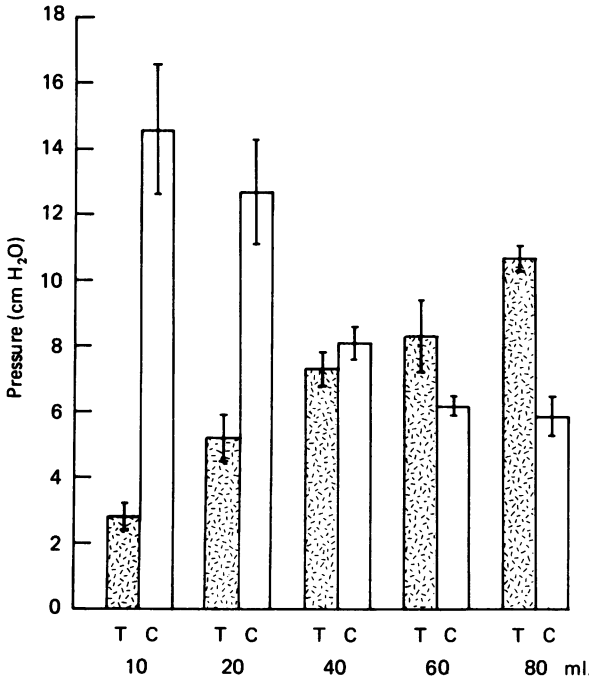

Fig. 6. The effect of changes in the volume of fluid in the stomach (10–80 ml.) on the intragastric pressure (T) and the response to cervical vagal stimulation (C) (10 Hz, 10 sec, 20 V, 0·5 msec). $n = 5$ animals, body wt. 1214 ± 97 g.

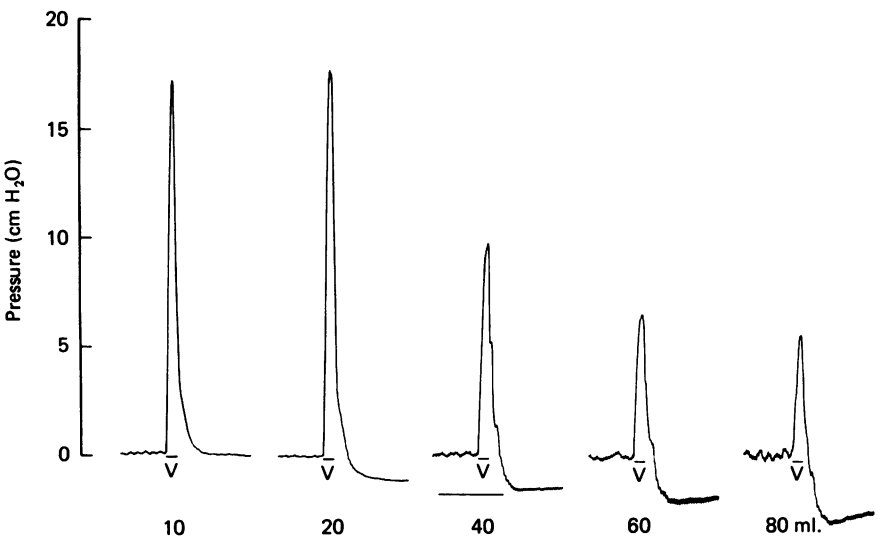

Fig. 7. The effect of changes in the volume of fluid in the stomach (10–80 ml.) on the response to cervical vagal stimulation (10 Hz, 10 sec, 20 V, 0·5 msec). Zero pressure in this Figure refers to the prevailing pressure at each volume (see Fig. 6 for values). Note not only the decrease in the evoked contraction but also the increase in the magnitude of the relaxation that follows the contraction.

antrum. This hypothesis was tested by repeating the experiment using a divided stomach and monitoring the pressure in the corpus and antrum. Increasing the distension of the antrum from a volume of 5 to 15 ml. reduced the response to vagal stimulation, but decreasing the distension did not restore the amplitude of response to control levels. The antrum is clearly less able to accommodate increases in volume and can be readily over-distended (Andrews *et al.* 1980*b*). In view of this no further comment will be made on antral pressures. Distension of the corpus with 50 ml. saline produced a similar percentage reduction in vagally evoked responses as did 80 ml. in the intact stomach. After atropinization the relaxations produced by vagal stimulation were increased by an increase in corpus volume from 20 to 30 ml. The relaxation returned towards pre-stimulus levels more quickly at higher volumes. It can be seen therefore that the corpus behaves in much the same way as the intact stomach. The shift in the balance of excitation and inhibition is therefore occurring locally rather than being mediated by a shift from corpus to antrum or vice versa.

If increasing gastric volume in the vagal-stimulation study is analogous to using stomachs of smaller size in the inflation study, then these results appear to contradict each other. This apparent contradiction may be resolved by taking the resting gastric pressure into account in the vagal stimulation study. Fig. 6 shows that as the intragastric volume increases so too does the resting pressure. If the amplitude of vagally evoked response is added to the resting pressure, the total pressure is found to be $16{\cdot}4 \pm 5{\cdot}4$ cm of water ($n = 25$). There were no significant differences between the total pressures at different volumes. This has considerable bearing on the interpretation of vagal stimulation studies (see Discussion).

The constancy of total pressure suggests that the reduction in amplitude of vagally evoked responses by increasing volumes is less likely to be a modulation of vagal effects by intramural reflexes and more likely to be a limitation of the pressure which the contracting stomach wall can exert. This hypothesis was investigated by administering ACh through a cannula inserted into the splenic artery in a retrograde direction. The effects of ACh on corpus pressure were measured after inflating the divided stomach with varying volumes. The corpus was used because it is known that the corpus and antrum behave differently and we wished to avoid obscuring alterations in the balance of activity within one region of the stomach with changes between regions. Fig. 8 shows that the response to intra-arterial ACh was significantly reduced ($P < 0{\cdot}01$) by increasing the volume in the stomach from 20 to 50 ml. The response to ACh was abolished by atropine (1 mg kg^{-1}). It therefore appears that the modulation of evoked gastric contractions by changes in gastric volume is mediated directly by the smooth muscle of the stomach and not by activation of a local reflex.

Time course of relaxation after vagal excitatory fibre stimulation

Previously it was demonstrated that the time course of pressure adaptation after step inflation was a function of the number of inflations (see Fig. 5). Stimulation of the vagus nerve evokes a contraction followed by a long-lasting period of relaxation when the stimulus is removed (Andrews & Scratcherd, 1980). In this part of the study it was found that the time taken for the gastric pressure to return towards pre-stimulation levels after vagal stimulation was a function of the gastric volume. Fig. 9 plots the magnitude of the relaxation just after vagal stimulation and 2 min

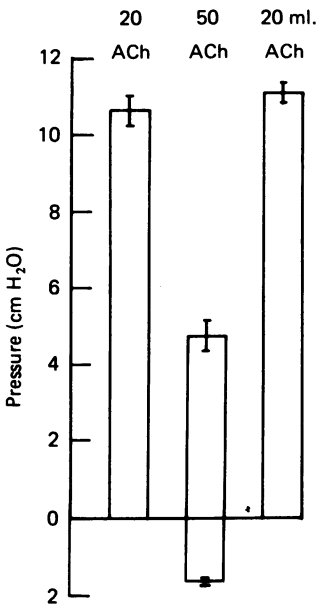

Fig. 8. The amplitude of corpus contractions evoked by I.A. ACh (10 μg, three injections). As the corpus volume was increased from 20 to 50 ml. the response was reduced and was followed by a relaxation (plotted downwards). The responses returned to the control level on deflation.

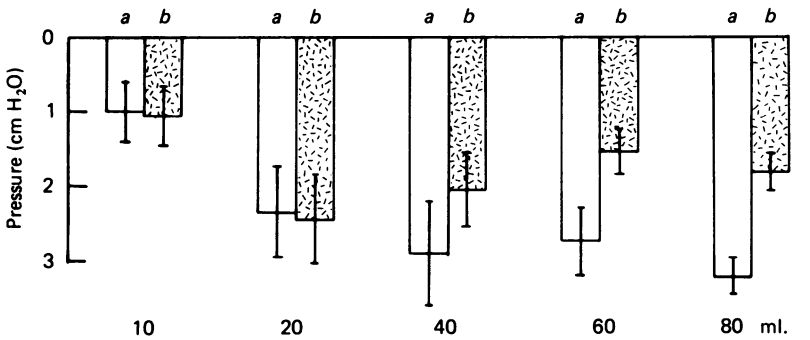

Fig. 9. The effect of changes in the volume of fluid in the stomach on the magnitude of the gastric relaxation that follows the vagally evoked contraction. Open bars (*a*) pressure 30 sec after the stimulus stopped. Shaded bars (*b*) pressure 2 min 30 sec after the stimulus stopped. 10 ml. volume and 20 ml. volume (*a vs. b*) not significant; 40 ml. volume (*a vs. b*) $P < 0{\cdot}05$; 60 ml. and 80 ml. volume (*a vs. b*) $P < 0{\cdot}01$. $n = 5$ animals, body wt. 1214 ± 97 g. Zero cm H_2O refers to the pressure immediately before nerve stimulation.

later. At volumes of 10 and 20 ml. the pressure after 2 min was the same as the pressure immediately following stimulation. As the volume was increased, however, the pressure returned to pre-stimulus levels more rapidly. With 40 ml. in the stomach the pressure 2 min after stimulation was significantly greater than the pressure immediately following stimulation ($P < 0{\cdot}05$), indicating that post-stimulus relaxation was wearing off. At 60 ml. the post-stimulus relaxation had fallen to 58% by 2 min

($P < 0{\cdot}01$). Thus although the post-stimulus relaxation increased with increasing volume, it was of progressively shorter duration. The effect of changes in corpus volume on the relaxation produced by activation of vagal inhibitory fibres (in atropinized animals) is plotted in Fig. 10. It was observed that whereas the amplitude and time course of the vagally induced relaxation were affected by intracorpus volume, the amplitude of the rebound contractions which occur on cessation of vagal

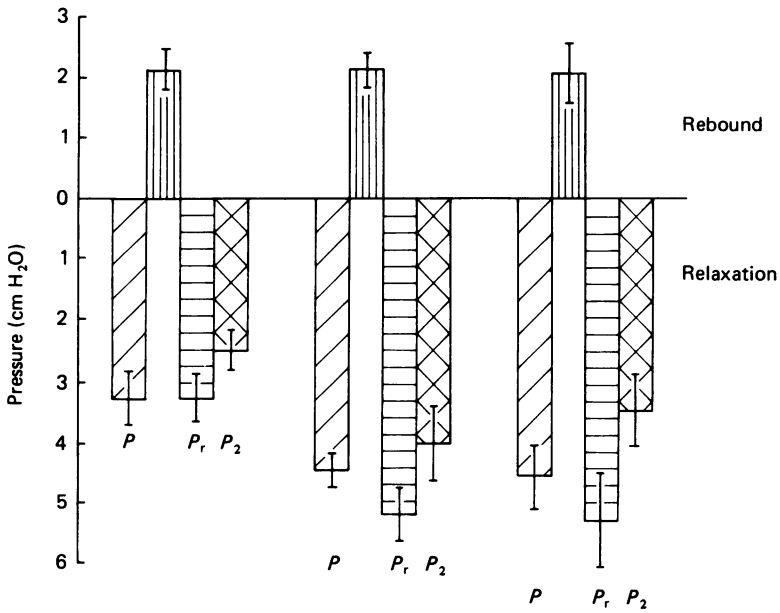

Fig. 10. The effect of changes in corpus volume on several parameters of the relaxation evoked by activation (10 Hz, 10 sec, 20 V, 0·4 msec) of the vagal inhibitory fibres in the atropinized animal. P: the pressure at the end of the 10 sec stimulation period; P_r: the pressure after the rebound contraction; P_2: the pressure 2 min after P_r. 20 ml. volume P_r *vs.* P_2; $P < 0{\cdot}05$; 30 ml. volume P_r *vs.* P_2: $P < 0{\cdot}1$; 50 ml. volume P_r *vs.* P_2: $P < 0{\cdot}01$. For the corpus relaxation 0 cm H_2O refers to the pressure immediately before vagal stimulation and for the rebound contraction it refers to the pressure at point P.

stimulation remained constant (Fig. 10). The mean amplitude of the rebound contractions was $1{\cdot}0 \pm 0{\cdot}09$ cm H_2O and there were no significant differences at the different intracorpus volumes.

DISCUSSION

Although the first objective of this study was the determination of the pattern of distribution within the vagus of its inhibitory effects on gastric smooth muscle, a number of other observations were made which are perhaps of greater general significance. These concern the effects of gastric weight and gastric volume on intragastric pressure, and the effect of repeated inflation on the time course of adaptation.

The amplitude of the responses to step inflations of the stomach was shown to be

functions of gastric weight. Although the correlation coefficients were low, they were significant. If control and experimental groups are not matched for gastric weight a spurious difference may emerge, or conversely a real difference may be obscured by the effect of gastric weight. This is particularly important if control and experimental groups are studied at different times of year since the mean body weight of ferrets available from suppliers shows an annual variation, and gastric weight is a function of body weight (P. L. R. Andrews & I. N. C. Lawes, unpublished observations).

As a corollary to the effect of gastric weight, the relative volume of fluid within the stomach determines the amplitudes of contraction and relaxation following vagal stimulation. In particular, at low volumes relaxation will not be apparent and at high volumes the amplitude of excitatory responses will be obscured by a high resting tone. Since intragastric pressure is a direct physical function of intramural tension (as opposed to a physiological function) these caveats apply even when intramural tension is measured. Experiments aimed at discovering the inhibitory transmitter in the stomach will have little chance of success when empty stomachs are studied (e.g. Gustafsson, 1980). The limitation in maximum pressure achieved appears to be imposed by the stomach wall itself since it occurred even after intra-arterial ACh, the response to which was entirely blocked by atropine.

It should be remembered that the gastric volumes used are within those that the ferret usually drinks and it is therefore possible that the modulation of the vagal responses described here occur in the conscious animal, particularly after a very large meal.

The number of inflations administered to an animal is an overriding factor in the time course of pressure adaptation. The initial peak and the plateau reached after 5 min were apparently uninfluenced by the number of inflations, but the time taken to reach an intervening pressure 63 % lower than the initial peak–plateau difference was overwhelmingly determined by the number of preceding inflations. This factor entirely obscured any influence of drugs or nerve lesions, which suggests that the response of the stomach wall itself was changed by inflation. The importance of the influence of repeated inflation to studies of mechanoreceptors cannot be over-emphasized. A tension receptor found early in the course of an experiment might appear to be slowly adapting because intragastric pressure (and therefore intramural tension) will be decreasing slowly. Later on, the same mechanoreceptor will appear to be much more rapidly adapting because intragastric pressure will be decreasing much more rapidly. Unless the simultaneous pressure (or intramural tension) are known the proper classification of mechanoreceptors will be impossible.

The original objective of this study was the determination of the functional organization of gastric inhibitory fibres in the vagus. It was found that either one of the abdominal trunks could exert marked inhibition on the stomach, but neither trunk exerted as much inhibition as both trunks acting simultaneously. In contrast it was shown previously that the dorsal trunk was capable of as much excitation as both trunks acting together (Andrews *et al.* 1980*a*). If dorsal vagotomy followed ventral vagotomy the subsequent release from inhibition was greater than when ventral vagotomy came second. The dorsal trunk is therefore responsible for more of both excitation and inhibition than the ventral trunk. This is in accord with the

observation that in the ferret the dorsal trunk is usually larger in diameter than the ventral trunk.

Successive section of cervical vagi indicated that there was a significant loss of inhibition when one nerve was cut, but this was not as great as when the second nerve was cut. This applied whichever cervical nerve was cut first. The two cervical vagi are therefore responsible for as much gastric inhibition as each other, and there is some overlap between them. Unlike the case of their excitatory effects (Andrews *et al.* 1980*c*), overlap of inhibition is incomplete since both nerves together have a greater inhibitory effect on the stomach than either nerve alone.

As far as local (intramural) inhibitory reflexes are concerned, we found no evidence that they operate independently from the vagus. After atropine and vagotomy the response to step inflations was unaltered by hexamethonium in either a high or a low dose. On the other hand inhibition was obtained by administration of nicotine or chemical activation of afferents even after vagotomy and atropine. In both cases pre-treatment with hexamethonium greatly reduced or even abolished the inhibition. Why inflation was unable to activate these local inhibitory nerves despite the crude pharmacological demonstration of their existence is unclear. One possibility is that a background of vagal activity is required to facilitate the local reflexes; however, such activity is not required for the local reflexes occurring in the small intestine (Costa & Furness, 1976). A second possibility is that the intramural reflexes are not mediated by cholinergic synapses. In the guinea-pig stomach Bülbring & Gershon (1967) demonstrated that the vagal intrinsic inhibitory neurones possess receptors for both 5-hydroxytryptamine (5-HT) and ACh. The involvement of 5-HT in the activation of these neurones via local reflexes cannot be excluded. However, preliminary studies indicate that methysergide maleate (1 mg injected into the gastric blood supply) is without effect on the spontaneous motility or the response to vagal stimulation.

In conclusion, the gastric response to step inflations and vagal stimulation is influenced by gastric weight, intragastric volume and the number of preceding inflations. Most of these effects operate directly on the stomach wall and appear to be independent of gastric innervation. The distribution of inhibitory functions in the vagus resembles the distribution of excitatory functions in that the two cervical vagi are equivalent and the dorsal abdominal trunk is responsible for more of both functions than the ventral abdominal trunk. Inhibitory functions differ from excitatory functions in the extent to which vagal branches overlap with each other: the overlap is complete for excitation and less than complete for inhibition. Intrinsic inhibitory nerves, although pharmacologically demonstrable, are not apparently activated by gastric inflation unless the vagus is intact.

Finally, the precise role of the gastric myenteric plexus in the regulation of intragastric pressure remains to be determined. From our results it is apparent that vago–vagal reflexes make a significant contribution to the over-all regulation of intragastric pressure mainly by controlling the activity of the muscle in the corpus region but it is also clear that the gastric smooth muscle has an intrinsic capacity to respond to changes in gastric volume. There was no indication that the myenteric plexus had any independent role to play in the regulation of the over-all intragastric pressure, and it therefore appears that its function is to disseminate the effect of

activation of the small number of vagal efferent fibres over the entire stomach and to act as a co-ordinator for the rhythmic activity of the smooth muscle.

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