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VAGAL CONTROL OF LOWER OESOPHAGEAL SPHINCTER MOTILITY IN THE CAT

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SUMMARY

1. The effects of vagal efferent fibre stimulation on the smooth muscle of the lower oesophageal sphincter have been studied on the anaesthetized animal and on the isolated and perfused organ.

2. In both muscle layers (longitudinal and circular) vagal stimulation elicits two types of electromyographic (e.m.g.) potentials: (*a*) excitatory junction potentials (e.j.p.s) where there is a depolarization of the smooth muscle fibres. E.j.p.s can give rise to spike potentials inducing a contraction of the sphincter; (*b*) inhibitory junction potentials (i.j.p.s) where there is hyperpolarization of the smooth muscle fibres, often followed by a transient depolarization which may initiate spikes (post-inhibitory rebound).

3. Pure i.j.p.s are observed after atropine treatment which suppresses e.j.p.s. Under these conditions, a long lasting vagal stimulation induces a long duration hyperpolarization concomitant with an opening of the lower oesophageal sphincter followed after the cessation of stimulation by a powerful rebound leading to a strong contraction which closes the sphincter.

4. Several arguments, pharmacological (action of acetylcholine (ACh), atropine and hexamethonium) and physiological (threshold and latency of responses) lead to the following conclusions.

Preganglionic vagal fibres are cholinergic and they activate (*a*) intramural excitatory cholinergic neurones; (*b*) intramural non-adrenergic

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inhibitory neurones (purinergic neurones). Preganglionic fibres leading to inhibition have a higher threshold than those leading to excitation.

Both excitatory and inhibitory pathways are interconnected inside the intramural network. In particular, activation of intramural inhibitory neurones, by relaxing the oesophagus orally to the lower oesophageal sphincter, inhibits intramural excitatory neurones and subsequently blocks vagal excitatory responses.

5. Two functions may be attributed to the vagal extrinsic innervation: (*a*) closure of the lower oesophageal sphincter by maintaining the basal tone of the sphincter; this would imply that at rest the inhibitory control is supplanted by the excitatory one; (*b*) sphincter opening during swallowing by suppressing the excitatory stimulus and reinforcing the inhibitory one (it may be recalled that after bilateral vagotomy, swallowing is no longer followed by a relaxation of the sphincter).

INTRODUCTION

The lower oesophageal sphincter is a constricted area with an intraluminal pressure, higher than that in the stomach or other parts of oesophagus. During deglutition, this pressure drops soon after the onset of the bucco-pharyngeal stage (opening of the sphincter); then, at the end of the oesophageal stage, it gradually rises until it exceeds the resting level before it slowly drops to the resting level (closing of the sphincter).

In the cat, as in man and primates, the distal part of the thoracic oesophagus and the lower oesophageal sphincter consists exclusively of smooth muscle. Following bilateral vagotomy this region is not paralysed and still exhibits peristalsis, the sphincter seeming to behave normally (Cannon, 1907; Jurica, 1926; Hwang, 1948; Roman & Tieffenbach, 1971). Thus, a distension of the oesophagus, by inflating a balloon, initiates a 'tertiary' or 'autonomic' peristalsis which propels the balloon down into the stomach; the lower oesophageal sphincter, previously closed, opens as soon as the onset of distension and closes again when the balloon has passed (Roman & Tieffenbach, 1971). Even *in vitro*, the sphincter still exhibits some tone (Thomas & Earlam, 1972); a distension of the oesophagus orally to the sphincter elicits an opening, and then, a phasic closing at the end of distension (Mann, Code & Schlegel, 1968). These observations are similar to those of Bayliss & Starling (1899) on the intestine, leading them to state their famous 'law of the intestine'. Subsequently, it was shown that the phenomena described by Bayliss & Starling were due to the activity of intramural plexuses (see Wood, 1975).

Data from section or stimulation of nerves indicate that vagal innervation controls the lower oesophageal sphincter. Intrathoracic section

has little effect on its motility (Zeller & Burget, 1937); section in the neck, even though some results are controversial, seems to have a marked effect on the sphincteric function. A permanent spasm (Jourdan, 1957), a transient spasm (Zeller & Burget, 1937) or a simple atony of the sphincter (Hwang, Essex & Mann, 1947) have been described in dogs. According to Langley (1898), vagal stimulation in rabbits closed the sphincter in curarized animals, while it opened it after curare plus atropine. In the same species Meltzer & Auer (1906) described either a contraction or a relaxation according to the stimulation intensity.

In the present work, the problem of vagal control of the lower oesophageal sphincter was examined using electromyographic techniques. Fundamentally, our experiments consisted in stimulating vagus nerves and recording both electromyogram (e.m.g.) of sphincter smooth muscle and intraluminal pressure modifications. Experiments were performed *in vivo* on acute animals, or *in vitro* on the isolated and perfused organ. In addition, the sucrose gap method was used to test circular muscle strips responses of the lower oesophageal sphincter to ACh.

METHODS

In vivo experiments

Adult cats of either sex were used. The trachea was cannulated and a polyethylene catheter was inserted into the great saphenous vein under ether anaesthesia. Anaesthesia was then continued with I.V. injections of a 10% sodium thiopentone (Nesdonal) solution. The first injection of Nesdonal was of 50 mg/kg, the depth of anaesthesia being verified by the absence of palpebral reflex. It was then established in test experiments that repeated injections of Nesdonal (20 mg/kg) every hour allowed to maintain a convenient level of anaesthesia. Moreover, all surgical wounds were regularly infiltrated with a 2% (0·02 g/ml.) procaine solution; cardiac rhythm and blood pressure were systematically recorded throughout all the experiments in order to detect any variation of the anaesthesia level and to maintain it as constant as possible. During recordings, animals were curarized by I.V. injections of gallamine (Flaxedil, 5 mg/kg) to prevent movements due to contractions of the striated muscle of the proximal part of the oesophagus. Both vagi were dissected in the neck and transected, the peripheral ends being placed on electrodes connected to a stimulator. The approach to the lower oesophageal sphincter was carried out on the left side after 8th, 9th and 10th rib resection. The animal was artificially ventilated; the diaphragmatic lobe of the left lung was removed. After dissection of the visceral pleura, the sphincter and lower oesophagus were immersed in paraffin oil maintained at 37 °C. Stimulation pulses were delivered to the vagus through an insulation unit, by means of two silver wires inserted in a Plexiglas chamber which received the nerve.

The intraluminal pressure was recorded by means of one or two rubber balloons connected by a polyvinyl catheter to a pressure transducer.

The e.m.g. recording was made with extracellular pressure electrodes. They consisted of 270 μm diameter copper wire, insulated except at the tip, and in the shape of a helical spring of about 1 cm length to allow the electrode to follow the movements

of the organ. A photograph of this type of electrode has been previously published (Gonella, 1972). Recording was monopolar with AC or DC amplifiers. The focal electrode was on the lower oesophageal sphincter area, the indifferent electrode being on the diaphragm as near as possible to the active electrode to avoid recording the electrocardiogram. The active electrode was connected to the recording device in such a way that its positive changes would give an upward deflexion.

E.m.g., pressure variations and stimulating pulses were recorded using an electromagnetic writer with ink projection galvanometers (Mingograf 81, Elema Shönander, band pass: 0–700 Hz).

In vitro experiments

The surgical phase was identical to that previously described, except that the thoracic window was larger. All the small arteries of the dorsal part of the aorta were ligated at 5 mm from their origin. The oesophagus was excised, from the level of heart to the stomach, with aorta and vagi. The oesophagus was then placed horizontally in an organ bath (Gonella, 1972) filled up with a saline solution containing NaCl 133 mM, KCl 4·7 mM, $CaCl_2$ 1·6 mM, $MgCl_2$ 0·49 mM, $NaHCO_3$ 16·3 mM, glucose 7·8 mM, maintained at 36 °C and bubbled with 95 % O_2 and 5 % CO_2.

The oral part of the oesophagus was fixed and a tension of 3–5 g was applied to the gastric side. The peripheral end of the aorta was ligated and a catheter introduced into the cardiac end to allow the physiological solution to perfuse the local circulatory system; veins from the oesophagus were cut. Before beginning this perfusion, diluted (1 part of blood, 3 parts of physiological solution), oxygenated and heparinized blood was injected into the aorta, to test the quality of the perfusion. Direct examination under a surgical microscope showed muscle colouring and the possible leakages from aorta to have stopped. 1 or 2 cm of the distal part of a thoracic vagus nerve were cleaned from connective tissue and connected to a stimulation device (see Jule & Gonella, 1972).

The e.m.g. recording was as described for the experiments *in vivo* with the recording electrodes placed either on serosa or directly on the circular layer through a small window made through the longitudinal muscle. The indifferent electrode was immersed in the bath.

Sucrose gap experiments

The surgical phase was identical to that described in the preceding section. Helical strips of circular muscle of 0·8–1 mm width and 40 mm long were used. In most of the experiments, the longitudinal muscle remained attached to the circular muscle. Strips were mounted in an apparatus similar to that described by Bülbring & Tomita (1969). Tension was applied to the strip in order to give it approximately its length *in situ*. Silver-silver chloride pick-up electrodes were connected by means of a cathode follower (Clottes, 1969) to the DC amplifier of a cathode ray (Tektronix 502 oscilloscope).

RESULTS

I. *Localization of the lower oesophageal sphincter*

The existence of a physiological sphincter in the distal part of oesophagus is demonstrated by the presence of a high intraluminal pressure zone; it can be observed by pulling an open tip catheter, connected to a pressure transducer, from stomach up to oesophagus (Fig. 1). The pressure, which is nearly constant as long as the catheter remains in the stomach,

increases in the sphincteric zone and decreases again to a lower value when the catheter is in the oesophagus. The location of the lower oesophageal sphincter is determined by measuring, from the incisors, the catheter length necessary to reach the high pressure zone. Negative pressure in the pleural cavity is probably responsible for a lower pressure in the oesophagus than in the stomach (Fig. 1). In the cat, the sphincter is

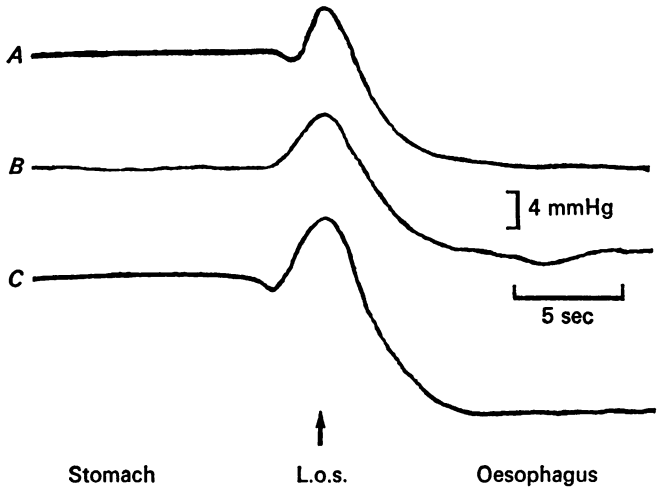

Fig. 1. Pressure variations, observed in anaesthetized cats, by withdrawing an open tip catheter from stomach to oesophagus. Each tracing comes from a different animal. The peak of pressure corresponds to the high intraluminal pressure zone of the lower oesophageal sphincter (l.o.s.). Pressure is higher in stomach than in oesophagus.

located at the most distal part of oesophagus, just at the level of the diaphragm, where visual examination shows a constricted zone joining the oesophagus to the stomach (in the cat, there is no subdiaphragmatic oesophagus). The length of the sphincteric high pressure zone is shorter in cat than in man or dog (Schlegel & Code, 1958; Waldeck, 1972). Moreover, in dog the physiological sphincter is located 2–3 cm above the oesophagogastric junction (Waldeck, 1972; Miolan & Roman, 1973).

II. *Effects of vagal stimulation in vivo*

Stimulation by a single pulse (0·5 msec, 6–8 V) elicits a slow depolarization corresponding to an e.j.p. (Fig. 2); no change in intraluminal pressure is observed. More complex functional events are sometimes observed (Fig. 2); they consist of biphasic potentials with an initial depolarization followed by a hyperpolarization. The biphasicity becomes obvious by increasing stimulation intensity. Spike potentials, and

consequently contraction, are rarely observed by using single pulse stimulation. With a brief train (3 pulses at 15 Hz, 8–12 V) a short latency excitatory response occurs consisting of an e.j.p. with superimposed spike potentials; at the same time an increase in intraluminal pressure, due to a phasic contraction of the sphincter, is recorded (Fig. 3). E.j.p. latencies are similar to those in the body of oesophagus in the cat (Tieffenbach & Roman, 1972).

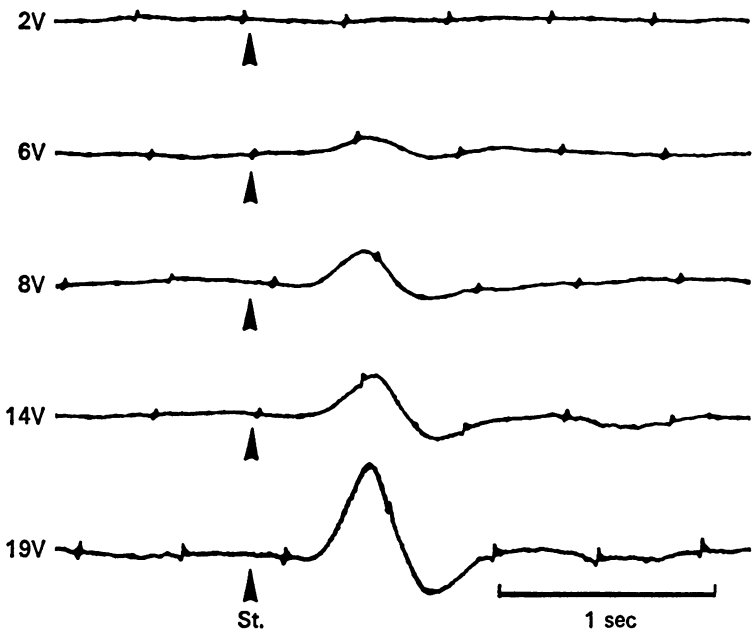

Fig. 2. Effects of increasing the intensity of stimulation *in vivo*, under Flaxedil. Stimulus (St.): single pulses, 0·5 msec in duration. Monopolar record on the lower oesophageal sphincter; DC amplification. The response to a weak stimulation is a depolarization (e.j.p.). With 8 V a biphasic potential with initial depolarization is observed. Biphasicity of the responses increases with increasing strength of stimuli.

(1) *Action of atropine.* With single or double stimuli immediately after I.V. injection of atropine (0·1 mg/kg) the vagal excitatory effect decreases and then disappears. If the intensity of the stimulus is increased, a slow hyperpolarization corresponding to an i.j.p. is observed. I.j.p.s can be elicited by a single stimulus. Nevertheless they can be more easily obtained with a double pulse (Fig. 4*A*). In some cases, i.j.p.s are followed by a transient depolarization (Fig. 4*B*).

The repetition of short trains of stimuli (5–6 pulses at 20 Hz) elicits a succession of i.j.p.s and a concomitant fall in intraluminal pressure

indicating the opening of the sphincter (Fig. 4*C*). A train of longer duration (20 Hz during several seconds) induces a sustained hyperpolarization of smooth muscle and an opening of the sphincter (Fig. 4*D*). In the same conditions of stimulation, about one second after stimulation was stopped, a transient depolarization initiating a burst of spike potential was observed (Fig. 4*E*). At the same time, a powerful contraction closing the sphincter occurred. This post-inhibitory excitation represents a rebound phenomenon.

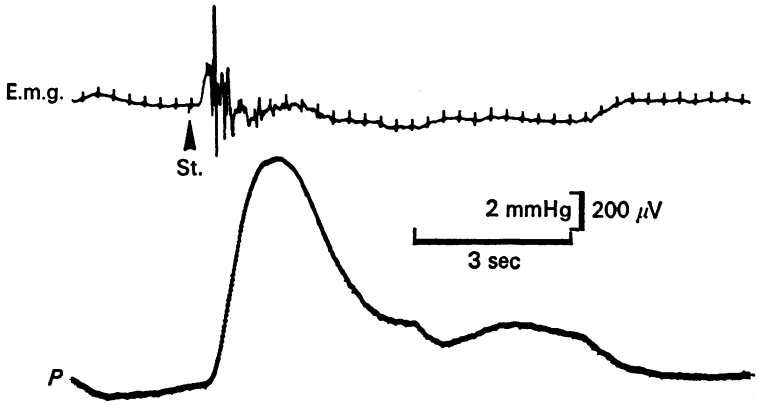

Fig. 3. Effects of a vagal stimulation, with a brief train *in vivo* and under Flaxedil. Stimulus (St.): 3 pulses, 0·5 msec, 12 V, 15 Hz. Upper record: sphincter e.m.g.; monopolar reception; 2·5 sec time constant. Lower record: sphincter intraluminal pressure (*P*); DC record. Vagal stimulation evokes an e.j.p. with superimposed spike potentials triggering a contraction of the lower oesophageal sphincter with the consequent rise of intraluminal pressure.

(2) *Compared latencies of e.j.p.s and i.j.p.s.* This comparison was made on the same animal and for the same position of the focal electrode on the sphincter. For fifteen e.j.p.s and i.j.p.s, mean latencies were respectively 330 ± 17 and 425 ± 19 msec. These mean values are significantly different at the threshold of 0·001. The conduction length from neck to the recording point was 12–15 cm.

III. *Effects of vagal stimulation in vitro on the isolated organ*

(1) *Various kinds of responses.* Single pulses elicit e.j.p.s from the longitudinal muscle with superimposed spike potentials (Fig. 5*A*). The lower latencies obtained *in vitro* (about 250 msec) with respect to those obtained *in vivo* (330 ± 17 msec) may be very likely related to a shorter distance of conduction in the former case. This finding fits well with conduction velocities measured by Jule (1975) in the rabbit vagus nerve. E.j.p.s are blocked by atropine ($5 \cdot 10^{-8}$ g/ml.) or by hexamethonium

(10^{-8} g/ml.). In some cases i.j.p.s may occur (Fig. 7*A*); but they generally appear only after atropine treatment (Fig. 6). I.j.p.s are often followed by a transient depolarization initiating spike potentials (post-inhibitory rebound). I.j.p.s are blocked by hexamethonium (10^{-8} g/ml.).

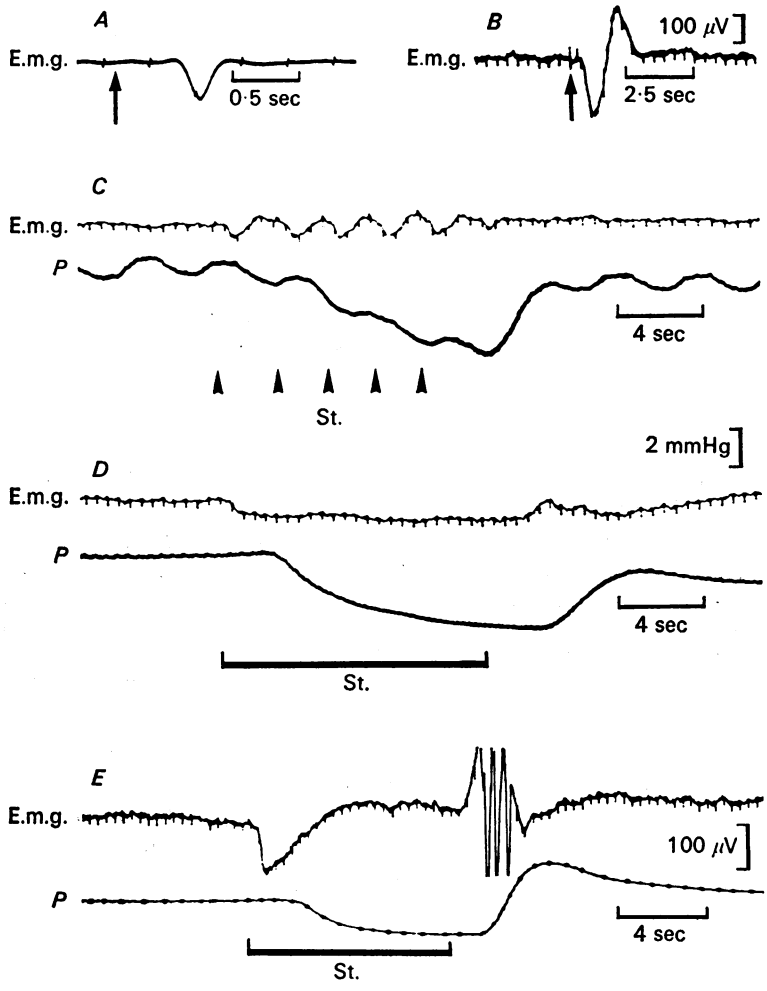

Fig. 4. For legend see facing page.

When an electrode is placed directly on circular muscle (window through longitudinal muscle), e.j.p.s and i.j.p.s are also recorded (Figs. 5*B* and 6*B*); the last ones are generally well marked after atropine. Moreover, the threshold of excitatory response (e.j.p.s + spike potentials) seems higher for circular muscle than for longitudinal. But only spike potentials from

circular muscle elicit an increase in intraluminal pressure. A prominent feature of electrical activity recorded from circular muscle is an amplitude several times greater than corresponding potentials recorded on

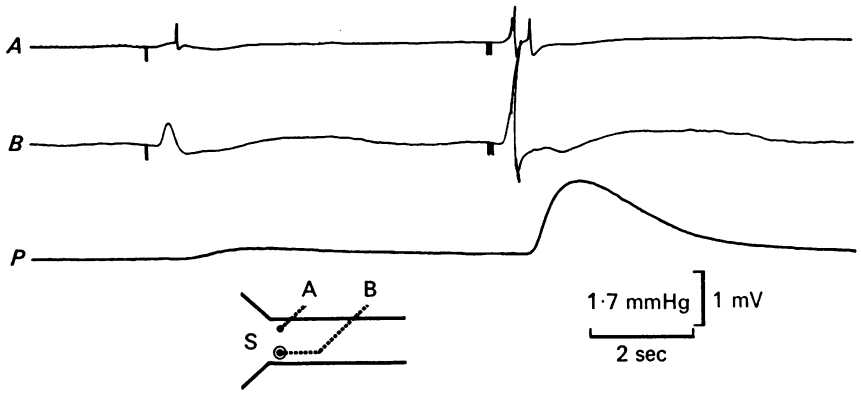

Fig. 5. Excitatory vagal effects recorded *in vitro* on longitudinal and circular muscle of the sphincter. Monopolar record; RC amplification (time constant 0·1 sec). Recording: *A*, on longitudinal muscle; *B*, on circular muscle (window through the longitudinal layer). The first stimulation (single pulse, 1 msec; 15 V) evokes an e.j.p. which initiates a spike on the longitudinal muscle and only an e.j.p. of high amplitude on the circular muscle. The second stimulation (2 pulses, 30 Hz, 1 msec, 15 V) evokes e.j.p.s initiating two small spikes on longitudinal muscle and one large spike on circular muscle. Circular muscle contraction leads to a high intra-luminal pressure increase.

Fig. 4. Effects of vagal stimulation *in vivo* under Flaxedil plus atropine. Monopolar records on the lower oesophageal sphincter; *A* and *B*: DC amplification. Hyperpolarization downwards. Stimulus indicated by arrows (0·5 msec, 19 V). In *A*, stimulation evokes a pure hyperpolarization; in *B* the i.j.p. is followed by a transient depolarization: the post-inhibitory rebound. *C*, *D*, *E*: upper record e.m.g. (monopolar record); lower record: intraluminal sphincter pressure. DC amplification for *C*, *D*, *E* pressure and *C* and *D* e.m.g. In *E*, AC amplification for e.m.g. (time constant 2·5 sec). In *C*, brief trains of stimuli (5 pulses, 20 Hz, 0·5 msec, 19 V) indicated by arrows, evoke a series of i.j.p.s and an opening of the lower oesophageal sphincter. In *D*, a long lasting train (straight line under the tracings: pulses 0·5 msec, 20 Hz, 19 V) induces a sustained hyperpolarization and an opening of the sphincter (decrease of intraluminal pressure). In *E*, the long lasting stimulus (straight line under the tracings: pulses 0·5 msec, 20 Hz, 19 V) induces a hyperpolarization and an opening of the sphincter (the hyperpolarization seems to be transient because of the RC amplification). 1 sec after the end of stimulation, appears a post-inhibitory rebound characterized by a depolarization with spike potentials superimposed, and an exaggerated closing of the sphincter. The top of spikes are cut off because of the high amplification; small vertical accidents of e.m.g. tracings are the electrocardiogram.

longitudinal muscle (Fig. 5). It has been suggested (Gonella, Condamin & Roman, 1975) that the greater amplitude of spikes from circular layer might be related to a better electrical coupling between circular fibres due to a greater richness in nexuses in this layer.

With an electrode placed on the serosa a complex response is sometimes observed, namely e.j.p.s and eventually spikes of low and high amplitude; only the latter are accompanied by an increase in intraluminal pressure,

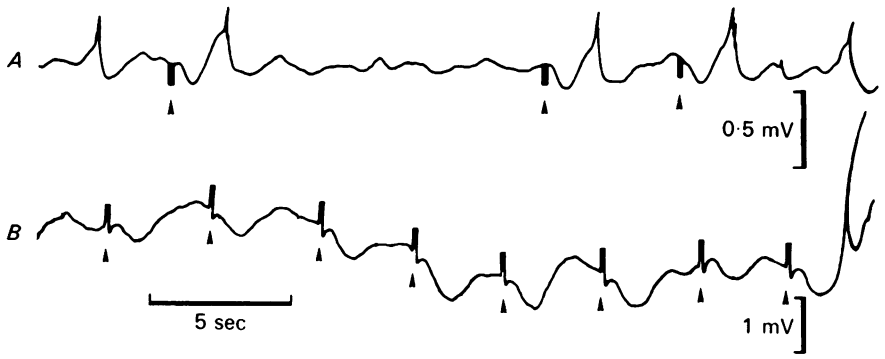

Fig. 6. Inhibitory vagal effects recorded *in vitro* in the presence of atropine on longitudinal and circular muscle of the sphincter. In *A* and *B*, e.m.g. recorded from two different animals: monopolar records; RC amplification. *A*: electrode on longitudinal muscle (RC = 0·1 sec). *B*: electrode on circular muscle (window through longitudinal muscle; RC = 2·5 sec). Stimulation of the vagus with brief trains (5 pulses at 30 Hz; 1 msec; 18 V). I.j.p.s are observed on both muscle layers. In *A* each i.j.p. is followed by a rebound depolarization with one or several spikes superimposed. In *B*, the rebound appears at the end of the series of stimuli.

suggesting that they originate in the circular layer. This interpretation is strongly supported by results of simultaneous records with an electrode on serosa and a second one directly on circular layer with e.j.p.s and spikes being recorded from both muscle layers.

E.j.p.s amplitude is higher *in vitro* than *in vivo* and spikes are more easily elicited on the former preparation. This may be related to a depressant effect of anaesthesia; it is also possible that some blocking effect of circulating catecholamines should be taken into account since we have demonstrated (Niel, 1975) that sympathetic stimulation decreased the size of e.j.p.s evoked by vagal stimulation.

In vitro as well as *in vivo* i.j.p.s, either from longitudinal or circular muscle, were unaffected by guanethidine (10^{-5} g/ml.). In view of this resistance to guanethidine, i.j.p.s might be attributed to purinergic nerves activity (Burnstock, Campbell, Bennett & Holman, 1964; Burnstock, 1975). Such i.j.p.s in response to extrinsic parasympathetic nerve

stimulation have been found in various parts of the digestive tract (Jule & Gonella, 1972; Diamant, 1973; Gonella & Gardette, 1974; Jule, 1975).

(2) *Interactions between excitatory and inhibitory vagal effects.* When biphasic junction potentials (like those of Fig. 2) occur, repetitive vagal stimulations produced a regular increase of the e.j.p. component until it

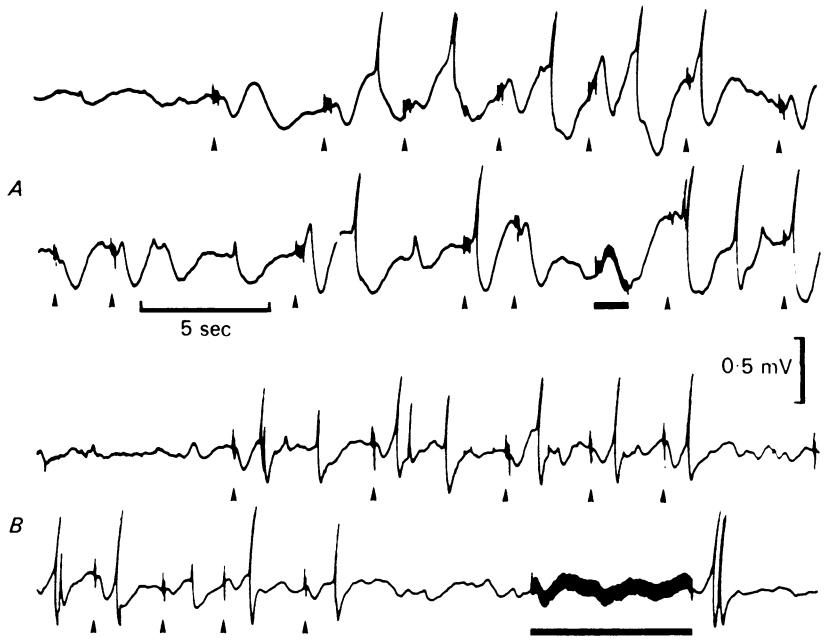

Fig. 7. Interaction between the excitatory and inhibitory effects of vagal stimulation. Monopolar record of the e.m.g. at the same point, on the longitudinal layer of the lower oesophageal sphincter (RC = 1 sec for *A* and 0·1 sec for *B*). In *A*, before the administration of atropine; repeated brief trains (5 pulses, 30 Hz, 1 msec, 5 V) evoke biphasic slow responses: a depolarization (e.j.p.) followed by a hyperpolarization (i.j.p.). The respective amplitude of both components fluctuates e.j.p. component increases periodically until it initiates a spike, after that, responses with a high i.j.p. component are recorded, then the e.j.p. component increases again and so on. In *B*, after atropine (10^{-5} g/ml.) administration only i.j.p.s followed by a rebound are observed.

initiated a spike. Afterwards, responses with pure or high i.j.p. component were recorded, the e.j.p. component increased again, and so on (Fig. 7*A*).

(3) *Effects of oesophagus distension on the sphincteric response to vagal stimulation.* In the absence of distension, the response was an e.j.p., usually with superimposed spikes. A distension of the oesophagus 3–4 cm

orally to the sphincter caused disappearence of spikes, whereas e.j.p.s were markedly decreased in amplitude or completely obliterated (Fig. 8).

IV. *A comparison of the effects of vagal stimulation on the lower oesophageal sphincter and the body of the oesophagus*

There appears to be no qualitative or quantitative difference between the responses of the lower oesophageal sphincter and the body of oesophagus to vagal stimulation. E.j.p.s and i.j.p.s have been recorded on both muscle layers in both regions.

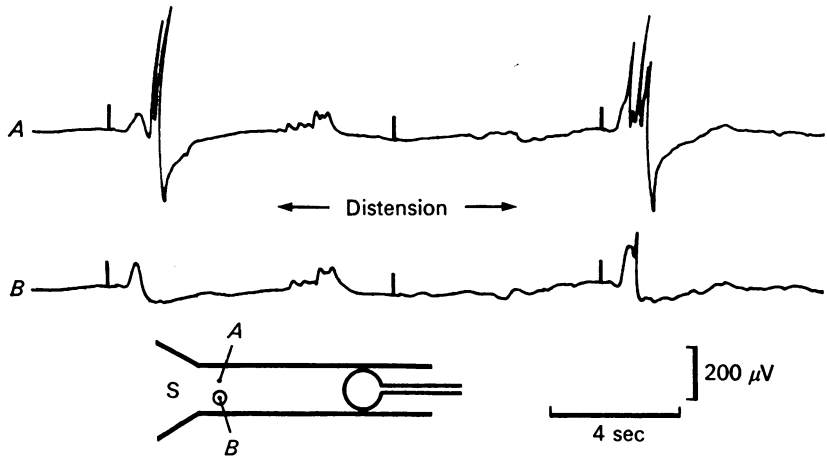

Fig. 8. Effects of distension of oesophagus on the sphincteric response to vagal stimulation. *A* and *B*: e.m.g. monopolar records and RC amplification (time constant = 0·1 sec). *A*, electrode on the longitudinal muscle; *B*: electrode on the circular layer (window through longitudinal muscle). Stimulation of the vagus with brief trains (3 pulses at 40 Hz, 1 msec, 5 V). In the absence of distension, vagal stimulation induces on both muscular layers excitatory responses, i.e. e.j.p.s with or without superimposed spikes. During a distension of the oesophageal body 3–4 cm orally to the sphincter the vagal excitatory response is completely abolished.

V. *Action of acetylcholine on circular muscle strips from the lower oesophageal sphincter and the body of the oesophagus*

ACh (10^{-5} g/ml.) depolarized oesophageal and sphincteric muscle as well. The results are summarized in Fig. 9. On strips taken from oesophagus 4–5 cm orally to the lower oesophageal sphincter, ACh caused a depolarization (about 10 mV) generating spikes. Whereas in strips from the sphincter, the depolarization usually did not elicit spikes. In both cases the depolarization was blocked by atropine (10^{-5} g/ml.).

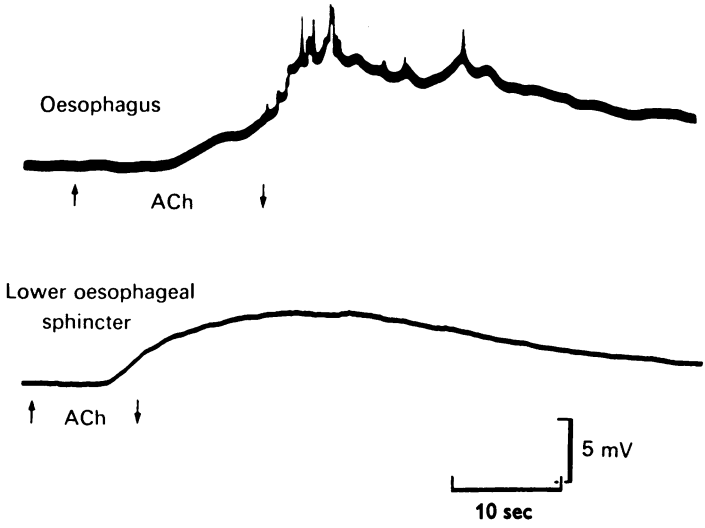

Fig. 9. Action of acetylcholine on circular muscle strips from the sphincter and the body of the oesophagus (4–5 cm orally to the lower oesophageal sphincter). Sucrose gap experiment. Notice that acetylcholine (10^{-5} g/ml.) induces on both strips a depolarization, but spiking is observed only on the oesophageal strip.

DISCUSSION

A surprising feature in view of the tonic closure of the sphincter was the absence, *in vitro* as well as *in vivo*, of permanent electrical activity in the sphincteric region. This is consistent with observations of Arimori, Code, Schlegel & Sturm (1970) and Miolan & Roman (1973) who observed on dogs, with chronically implanted electrodes, only a very slight tonic activity; they suggested that this discrete tonic activity could be the consequence of an asynchrony in smooth muscle bundles discharge, and a correlated difficulty or impossibility to record such an electrical activity with external electrodes. An alternative explanation could be that the tonic closing of the lower oesophageal sphincter would be achieved by a contraction caused by a permanent depolarization without spike potentials, something like a 'physiological contracture' similar to that described by Johanson (1971) and Bohr (1973) in vascular smooth muscle. This view is consistent with the finding of E. E. Daniel (personal communication) that in the opossum the membrane potential is significantly slower in sphincter than in oesophageal smooth muscle, and with our observation that generally ACh depolarized sphincter smooth muscle without initiating spikes.

In the sphincter as in the body of oesophagus stimulation threshold, at least for spike activity, is higher for circular than for longitudinal

muscle (Tieffenbach & Roman, 1972). Two explanations stated for other parts of the digestive tract and which do not exclude each other may be proposed. First, a greater inhibitory innervation of circular muscle (Hirst, 1975; Wood, 1975; E. E. Daniel, personal communication), although this does not seem to be a general feature of the digestive tract (see Suzuki & Kuriyama, 1975). Secondly, a particular property of circular muscle (Kuriyama, Mishima & Suzuki, 1975; Suzuki & Kuriyama, 1975).

The blockage of e.j.p.s by hexamethonium and atropine indicates that the excitatory pathway is entirely cholinergic, that is, that cholinergic excitatory preganglionic fibres excite cholinergic intramural excitatory neurones which in turn activate smooth muscle. However, although some characteristics of intramural excitatory network organization have been described for the cat oesophagus (Roman & Tieffenbach, 1971) and more recently for the guinea-pig small intestine by Hirst, Holman & McKirdy (1975), it is not yet possible to determine on what points of intramural network preganglionic fibres do establish their connexions.

The longer latencies for i.j.p.s than for e.j.p.s confirm earlier findings (Bennett, 1972; Gonella & Gardette, 1974; Jule, 1975). Moreover, i.j.p.s have also a higher threshold than e.j.p.s. Both of these properties of i.j.p.s suggest that inhibitory parasympathetic fibres are smaller in diameter than excitatory fibres. This hypothesis is consistent with recent findings of Jule (1975) that the rabbit vagus extrinsic inhibitory fibres to the proximal colon have a conduction velocity of 0·5 m/sec compared with 1 m/sec for excitatory fibres. If the results of Jule are extrapolated to our preparation, we find for lengths of nerve ranging between 12 and 15 cm differences in latencies from 120 to 150 msec. These values are fairly similar to our values. These characteristics of extrinsic inhibitory fibres (smaller diameter) explain the difficulties in obtaining pure i.j.p.s without atropine.

The origin of i.j.p.s recorded with an electrode directly on circular layer is certain; as for those obtained by an electrode on serosa, the presence of i.j.p.s from circular layer recorded through the longitudinal one cannot be excluded. It can be noted that E. E. Daniel (personal communication) did not find any i.j.p. in longitudinal muscle strips by transmural stimulation.

According to recent findings of Burnstock, Cocks, Paddle & Staszwewska-Barczak (1975) the post-inhibitory rebound following hyperpolarization is due to prostaglandin liberation resulting from purinergic nerve stimulation.

Fluctuations in respective amplitude of e.j.p.s and i.j.p.s, according to the moment of vagal stimulation, suggest the existence of connexions between excitatory and inhibitory intramural pathways. The existence

of such connexions is also strongly supported by the decrease in size of sphincteric e.j.p. or even its suppression by a distension of the thoracic oesophagus. Thus, intramural inhibitory neurones excited by distension elicit in an aboral direction not only a muscular hyperpolarization, as stated by Diamant (1973), but also an inhibition of intramural excitatory neurons of the same region. Such an interconnexion between excitatory and inhibitory intramural systems was postulated by Furness (1969) in the colon and by Hirst (1975) in the small intestine of the guinea-pig.

As a conclusion, vagal excitatory and inhibitory effects are, at least qualitatively, identical in the lower oesophageal sphincter and in the body of oesophagus. The vagal control of the sphincter is not a particular one; it includes first, extrinsic preganglionic cholinergic neurones exciting intramural cholinergic neurones, which in turn excite smooth muscle fibres, leading to contraction of the body of oesophagus and closing of the sphincter and secondly, extrinsic cholinergic preganglionic neurones, connected with intramural non-adrenergic inhibitory neurones. When called into play, they relax the body of oesophagus and open the lower oesophageal sphincter. Thus, the vagal control of the sphincter is not purely inhibitory as claimed by Chauveau (1862) and Jourdan, Hutet, Sagols & Faucon (1955). Our results are in agreement with the finding of Langley (1898) who described for the first time the dual control of the sphincter by the vagi of the rabbit. This duality has been established now for many parts of the digestive tract: in the stomach (Martinson & Muren, 1963; Miolan & Roman, 1971), the proximal colon of the rabbit (Jule, 1975). Dual control has also been described in the distal colon of the rabbit (Jule & Gonella, 1972) and in the colon of the cat (Gonella & Gardette, 1974).

Concerning the physiological significance of this dual control of the lower oesophageal sphincter, it could be supposed that excitatory command is permanent and participates in the basal tone of the sphincter. If indeed the sphincter deprived of its extrinsic innervation remains closed, the intrasphincteric pressure is lower than in normal animals (Hwang, Essex & Mann, 1947; Motta, 1955; Higgs & Ellis, 1965; Jennewein, Siewert, Koch & Waldeck, 1975). As for the inhibitory command, its function should be essentially to open the sphincter just after the beginning of swallowing; this phenomenon seems to be programmed in the central nervous system. This opening is likely to invoke an active inhibition of smooth muscle, for suppression of excitatory command alone would leave intact the 'intrinsic' tone of the sphincter, the tone observed in the absence of extrinsic innervation. The hypothesis we present here is in accord with findings of Miolan & Roman (1973) and J. P. Miolan (personal communication) on conscious dogs.

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REFERENCES

ARIMORI, M., CODE, C. F., SCHLEGEL, J. F. & STURM, R. E. (1970). Electrical activity of the canine esophagus and gastroesophageal sphincter. *Am. J. dig. Dis.* **15**, 191–208.

BAYLISS, W. B. & STARLING, E. H. (1899). The movements and innervation of the small intestine. *J. Physiol.* **24**, 99–143.

BENNETT, M. R. (1972). *Autonomic Neuromuscular Transmission.* Monographs of the Physiological Society. Cambridge: University Press.

BOHR, D. F. (1973). Vascular smooth muscle updated. *Circulation Res.* **32**, 665–678.

BÜLBRING, E. & TOMITA, T. (1969). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc.* B **172**, 89–102.

BURNSTOCK, G. (1975). Purinergic transmission. In *Handbook of Psychopharmacology*, vol. 5, Synaptic Modulators, ed. IVERSON, L. L., IVERSON, S. D. and SNYDER, S. H., pp. 131–194. London: Plenum.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M. R. & HOLMAN, M. E. (1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? *Int. J. Neuropharmacol.* **3**, 163–166.

BURNSTOCK, G., COCKS, T., PADDLE, B. & STASZWEWSKA-BARCZAK, J. (1975). Evidence that prostaglandin is responsible for the rebound contraction following stimulation of non-adrenergic, non-cholinergic (purinergic) inhibitory nerves. *Eur. J. Pharma.* **31**, 360–362.

CANNON, W. B. (1907). Esophageal peristaltis after bilateral vagotomy. *Am. J. Physiol.* **19**, 436–444.

CHAUVEAU, A. (1862). Quoted after JOURDAN, F., HUTET, G., SAGOLS, L. and FAUCON, G. (1955). *J. Physiol., Paris* **5**, 337.

CLOTTES, A. (1969). Abaisseur d'impédance pour enregistrement des potentiels physiologiques par microélectrodes neutres ou polarisées. *Eléctronique méd.* **53**, 93–95.

DIAMANT, N. E. (1973). Electrical activity of the cat smooth muscle esophagus: a study of hyperpolarizing responses. *Proc. IVth international Symposium on Gastrointestinal Motility* (Banff, Canada), pp. 593–605. Vancouver: Mitchell.

FURNESS, J. B. (1969). An electrophysiological study of the smooth muscle of the colon. *J. Physiol.* **205**, 549–562.

GONELLA, J. (1972). Modifications of electrical activity of the rabbit duodenum longitudinal muscle after contractions of the circular muscle. *Am. J. dig. Dis.* **17**, 327–332.

GONELLA, J., CONDAMIN, M. & ROMAN, C. (1975). Relation between the amount of nexuses in digestive smooth muscle and the amplitude of spikes recorded by extracellular electrodes. *Proc. Vth international Symposium on Gastrointestinal motility* (Leuwen). Herentals, Belgium: Typoff.

GONELLA, J. & GARDETTE, B. (1974). Etude électromyographique in vivo de la commande nerveuse extrinsèque parasympathique du colon. *J. Physiol., Paris* **68**, 395–413.

HIGGS, B. & ELLIS, F. M. (1965). The effect of bilateral supranodosal vagotomy on canine oesophageal function. *Surgery* **58**, 828–834.

HIRST, G. D. S. (1975). The function of the myenteric plexus. Guest lecture, *Proc. Vth international Symposium on Gastrointestinal motility* (Leuwen). Herentals, Belgium: Typoff.

HIRST, G. D. S., HOLMAN, M. E. & MCKIRDY, H. C. (1975). Two descending nerve pathways activated by distension of guinea-pig small intestine. *J. Physiol.* **244**, 113–127.

HWANG, K. (1948). On tertiary peristaltis of esophagus of the cat. *Am. J. Physiol.* **159**, 574.

HWANG, K., ESSEX, H. E. & MANN, F. C. (1947). A study of certain problems resulting from vagotomy in dogs with special reference to emesis. *Am. J. Physiol.* **149**, 429–448.

JENNEWEIN, H. M., SIEWERT, R., KOCH, A. & WALDECK, F. (1975). The effect of vagotomy on the resting pressure and reactivity of the lower esophageal sphincter (LES) in man and dog. *Proc. Vth international Symposium on Gastrointestinal motility* (Leuwen). Herentals, Belgium: Typoff.

JOHANSSON, B. (1971). Electromechanical and mechanoelectrical coupling in vascular smooth muscle. *Angiologica* **8**, 129–143.

JOURDAN, F. (1957). L'innervation du sphincter cardiaque et sa mise en jeu. *J. Physiol., Paris* **49**, 227.

JOURDAN, F., HUTET, G., SAGOLS, L. & FAUCON, G. (1955). L'innervation du cardia. *C. r. Séanc. Soc. Biol.* **149**, 1571–1573.

JULE, Y. (1975). Modifications de l'activité électrique du côlon proximal de Lapin in vivo, par stimulation des nerfs vagues et splanchniques. *J. Physiol., Paris* **70**, 5–26.

JULE, Y. & GONELLA, J. (1972). Modifications de l'activité électrique du côlon terminal de Lapin par stimulation des fibres nerveuses pelviennes et sympathiques. *J. Physiol., Paris* **64**, 599–621.

JURICA, E. J. (1926). Studies on the motility of the denervated mammalian esophagus. *Am. J. Physiol.* **77**, 371–384.

KURIYAMA, H., MISHIMA, K. & SUZUKI, H. (1975). Some differences in contractile responses of isolated longitudinal and circular muscle from the guinea-pig stomach. *J. Physiol.* **251**, 317–331.

LANGLEY, J. N. (1898). On inhibitory fibres in the vagus for the end of the oesophagus and the stomach. *J. Physiol.* **23**, 407–414.

MANN, C. V., CODE, C. F. & SCHLEGEL, J. F. (1968). Intrinsic mechanisms controlling the mammalian gastroesophageal sphincter deprived of extrinsic nerve supply. *Thorax* **23**, 634–639.

MARTINSON, J. & MUREN, A. (1963). Excitatory and inhibitory effects of vagus stimulation on gastric motility in the cat. *Acta. physiol. scand.* **57**, 309–316.

MELTZER, S. J. & AUER, J. (1906). Vagus reflexes upon oesophagus and cardia. *Br. med. J.* **2**, 1906–1907.

MIOLAN, J. P. & ROMAN, C. (1971). Modification of the electromyogram from the canine stomach after stimulation of extrinsic nerves. *J. Physiol., Paris* **63**, 561–576.

MIOLAN, J. P. & Roman, C. (1973). Activity of vagal efferent fibres innervating the dog's cardia. *J. Physiol., Paris* **66**, 171–198.

MOTTA, G. (1955). Contributo sperimentale allo studio del tono muscolare dell' esofago et del cardias nel cane. *Arch. Fisiol.* **55**, 1–38.

NIEL, J. P. (1975). La commande extrinsèque de la musculeuse lisse du sphincter oesophagien inférieur. Thèse de spécialité en Neurophysiologie, Marseille.

ROMAN, C. & TIEFFENBACH, L. (1971). Motricité de l'oesophage à musculeuse lisse après bivagotomie; étude électromyographique (EMG). *J. Physiol., Paris* **63**, 733–762.

SCHLEGEL, J. F. & CODE, C. F. (1958). Pressure characteristics of the oesophagus and its sphincters in dogs. *Am. J. Physiol.* **193**, 9–14.

SUZUKI, H. & KURIYAMA, H. (1975). Electrical and mechanical properties of longitudinal and circular muscles of the guinea pig ileum. *Jap. J. Physiol.* **25**, 759–773.

THOMAS, P. A. & EARLAM, R. J. (1972). Electrical activity of the isolated perfused canine gastroesophageal junction. *Arch. fr. Mal. app. Dig.* **61**, 22.

TIEFFENBACH, L. & ROMAN, C. (1972). Rôle de l'innervation extrinsèque vagale dans la motricité de l'oesophage à musculeuse lisse; étude électromyographique chez le Chat et le Babouin. *J. Physiol., Paris* **64**, 193–226.

WALDECK, F. (1972). A new procedure for functional analysis of the lower oesophageal sphincter (LES). *Pflügers Arch. ges. Physiol.* **335**, 74–84.

WOOD, J. D. (1975). Neurophysiology of Auerbach's plexus and control of intestinal motility. *Physiol. Rev.* **55**, 1307–1324.

ZELLER, W. & BURGET, G. E. (1937). A study of the cardia. *Am. J. dig. Dis.* **4**, 113–120.