

# Intrinsic mechanisms controlling the mammalian gastro-oesophageal sphincter deprived of extrinsic nerve supply

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The oesophagus and gastro-oesophageal junction of 13 guinea-pigs and 14 3-week-old kittens were removed and placed in oxygenated Tyrode's solution at 36 to 37° C. Sphincteric pressures were recorded before and after oesophageal distension. In these preparations changes in the tone of the gastro-oesophageal sphincter were observed in response to lower oesophageal distension. The sphincter of the guinea-pig responded by relaxation, whereas the sphincter of the kitten responded more often by contraction. The relaxation/contraction responses of the sphincter to oesophageal distension were independent of any extrinsic nerve supply and could be abolished by removal of a circular cuff of oesophageal muscle between the source of the stimulus and the gastro-oesophageal sphincter. The intramural mechanisms affecting gastro-oesophageal sphincteric tone probably are present in all mammals, including man.

It is now generally acknowledged that a physiological sphincter is present in man between the lower oesophagus and the stomach (Atkinson, Edwards, Honour, and Rowlands, 1957; Fyke, Code, and Schlegel, 1956), but the mechanisms of its control are not fully understood. The extrinsic nerve supply to the lower oesophagus and gastro-oesophageal sphincter consists of the vagus nerves and sympathetic fibres (Gray, 1967; Ingelfinger, 1958; Mitchell, 1953), and much is known of their action on the cardia (Cannon, 1907; Carlson, 1922; Carveth, Schlegel, Code, and Ellis, 1962; Greenwood, Schlegel, Code, and Ellis, 1962; Ingelfinger, 1958; Knight, 1934). However, although high bilateral division of the vagus nerves in experimental animals often produces an achalasia-like picture (Alnor, 1958; Cannon, 1907; Carveth *et al.*, 1962; Ferguson, 1936; Friedberg, 1950; Ingelfinger, 1958; Knight, 1934; Long, Nice, Thal, and Truex, 1959), low vagal division in man (Friedberg, 1950; Ingelfinger, 1958; Jefferson, Phillips, Proffitt, and Necheles, 1951; Knight, 1934) and animals (Greenwood *et al.*, 1962; Grondahl and Haney, 1940; Ingelfinger, 1958; Jefferson *et al.*, 1951; Lehmann, 1945) does not cause such severe motility disturbances in the oesophagus, and sphincteric function, although affected, continues. This difference in

effect on oesophageal emptying between high suprahiatal and lower infrahiatal vagus division is so pronounced that it indicates that mechanisms present in the wall of the lower oesophagus must help to control the actions of the lower oesophagus and the sphincter. There have been many suggestions that such mechanisms exist within the oesophageal wall (Cannon, 1907; Ferguson, 1936; Grondahl and Haney, 1940; Lehmann, 1945; Meltzer and Auer, 1906) and that their absence as a result of damage to nerve cells in the wall can produce achalasia or a similar picture.

This investigation was undertaken to determine whether mechanisms exist in the oesophageal wall that can influence the gastro-oesophageal sphincter independent of the extrinsic nerve supply.

## METHOD

Complete removal of all vagal and sympathetic nerves to the region of the gastro-oesophageal sphincter is virtually impossible in surviving animals without at the same time dividing the blood vessels. Thus, an 'in-vitro' method was used for the investigation. By this technique, complete severance of all extrinsic nerves is assured, and, although the blood supply is

divided, oxygen, glucose, and electrolytes are provided by the perfusion fluid. Because survival of the tissue is dependent on diffusion processes, only very thin-walled organs were suitable for the experiments. The oesophagus of the guinea-pig and of the 3-week-old kitten was found to be sufficiently thin to remain viable for hours in the perfusion arrangement, and at the same time it was sufficiently large to enable pressure measurements to be made.

All the animals were anaesthetized with ether, and the entire oesophagus and the upper part of the stomach were rapidly removed and transferred to an organ bath containing oxygenated Tyrode's solution at 36 to 37° C. (Fig. 1). The composition of the

a second small water-filled 'recording' balloon was placed in the gastro-oesophageal junction via the stomach (Fig. 1). In the guinea-pig the stimulating balloon was 3 × 3 mm. and in the kitten 5 × 5 mm.; the recording balloon used in the guinea-pig was 2 mm. wide and 5 mm. long, and that used in the kitten was 5 mm. wide and 10 mm. long (Fig. 2). All balloons were attached to water-filled no. 50 polyethylene tubing, except the tiny recording balloon used in the guinea-pig, which was mounted on the end of a differential transformer-transducer of the type devised by Gauer and Gienapp (1950). The water-filled tubes were attached individually to strain gauges (Statham, Model P-23 De). The outputs of the strain-gauge pressure transducers were connected to d'Arsonval type galvanometers (Honeywell, Model 40-1000). A mirror attached to the coil suspension of the galvanometers reflected light beams to photographic paper moving at a constant rate of 15 cm./minute. The circuit was arranged so that an increase in pressure within the balloon produced an upward deflection on the records. The arrangement for the differential-transformer-pressure transducer

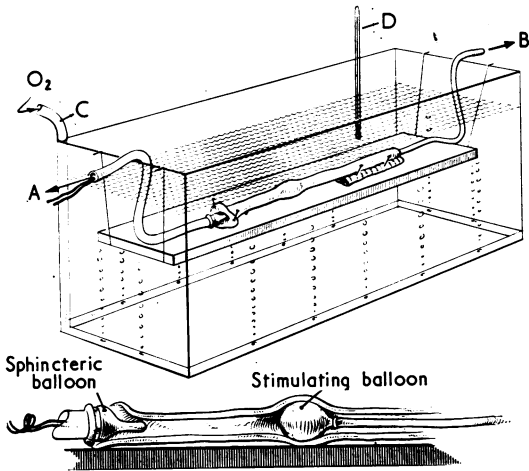


FIG. 1. *Isolated preparation. Oesophagus and attached cuff of stomach are shown pinned out in organ bath. Enlarged diagram below organ bath shows positioning of recording (sphincteric) and stimulating balloons. A, lead from differential transformer transducer mounting the recording balloon; B, water-filled tube connecting stimulating balloon to syringe and strain gauge; C, oxygen feed line; and D, thermometer. The water-heater is not shown.*

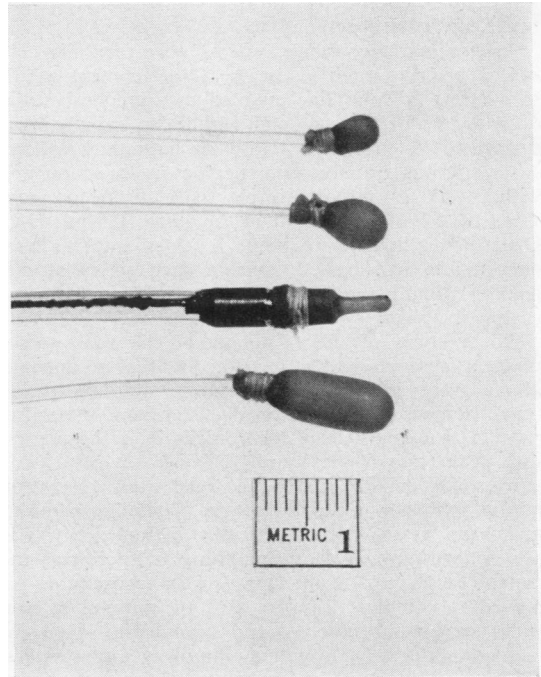


FIG. 2. *Recording and stimulating balloons. From above downward, guinea-pig stimulating balloon (3 × 3 mm.), kitten stimulating balloon (5 × 5 mm.), guinea-pig recording balloon (2 × 5 mm.), and kitten recording balloon (5 × 10 mm.). All balloons were water-filled and mounted on No. 50 polyethylene tubing, except the guinea-pig recording balloon, which was mounted directly to a miniature differential transformer-transducer.*

Tyrode's solution we used has been published previously (Code and McIntire, 1956). With practice, the time to remove and transfer the tissue was less than 3 minutes. The oesophagus was pinned out in the organ bath to the same length as it had been before removal by placing the pins in the cuff of stomach in a small piece of trachea left attached to the upper oesophagus for this purpose. All adventitious tissues, including nerve fibres, were then carefully dissected off the surface of the oesophagus and gastro-oesophageal junction.

When the specimen had been firmly pinned, a small 'stimulating' balloon, which could be inflated with water, was threaded caudad into the oesophagus, and

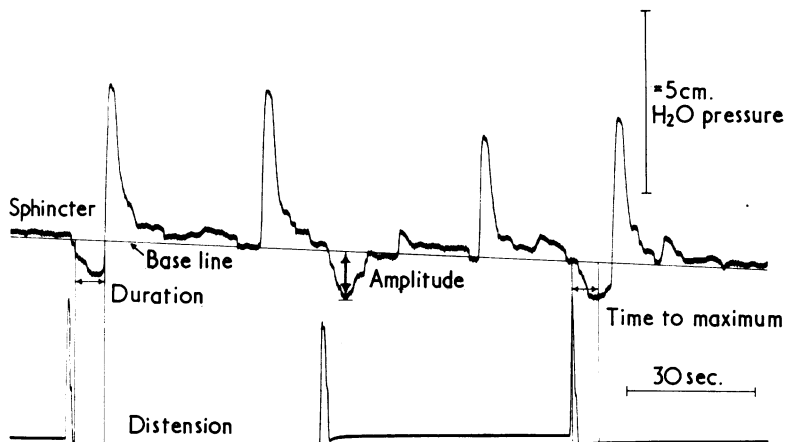


FIG. 3. Method of measurement of relaxations; identical methods were used for contractions. Each sphincteric response to oesophageal distension was measured for duration, amplitude, and time to maximal response.

was similar, except that an amplifier was inserted between the transducer and the galvanometer.

The pressure recording systems were carefully calibrated prior to each study. A 'T' attachment to the stimulating balloon line enabled measured quantities of water to be injected and withdrawn rapidly by a syringe to distend and collapse the balloon. Less than 1 second was usually required for the injection and withdrawal. In the guinea-pig each distension consisted of 0.2 ml. H<sub>2</sub>O, and in the kitten 0.5 ml. H<sub>2</sub>O. Distension stimuli were never repeated until at least one minute had passed. During each series of distending stimuli, the distance of the balloon from the gastro-oesophageal junction was noted.

The records of the responses of the gastro-oesophageal sphincter to distension of the oesophagus above were analysed for duration, amplitude, and time to maximal response (Fig. 3). Each distension stimulus was marked on the records by a sharp pressure peak (Figs 3, 4, 5, and 7).

In some of the guinea-pig tests, after relaxation of the sphincter in response to a distending stimulus had been established, a circular myomectomy was performed between the stimulating balloon and the gastro-oesophageal sphincter. The procedure removed a complete circular cuff of muscle, 5 mm. wide, leaving the mucosal and submucosal layers intact. A series of distending stimuli were reapplied above, and in some cases below, the myomectomy site.

#### RESULTS

**GUINEA-PIG** After the technique had been established, observations were made on the oesophagus of 13 animals. An increase of pressure over that in the gastric remnant or in the oesophagus was detected in all the isolated gastro-oesophageal

sphincters. The mean pressure of the gastro-oesophageal sphincter of the 13 preparations was 7.4 cm. H<sub>2</sub>O (range 2.25 to 20.4 cm. H<sub>2</sub>O). In all but one of the sphincters spontaneous alterations of sphincteric tone occurred in a definite pattern. The pressure fluctuations were significant in 10 of the 13 specimens (Fig. 4). In three sphincters, occasional exceptionally large, spontaneous elevations of pressure, exceeding 5 cm. H<sub>2</sub>O, were observed (Fig. 4). The frequency of the spontaneous activity was usually 10 or 12 pressure peaks per minute, but varied between 6 and 14 per minute. Spontaneous relaxation of the sphincter was never observed.

Distension was applied at various levels in the 13 preparations. The sphincter never responded to distension of the upper half of the oesophagus. By contrast sphincteric responses to distension in the lower half of the oesophagus were obtained in all preparations.

A total of 165 distension stimuli were applied to the lower half of the oesophagus of the 13 preparations: on 134 occasions delayed relaxation of the sphincter occurred (Figs 3 and 4); on four additional occasions a definite interruption of spontaneous activity occurred. No response was obtained to 27 distensions. Sphincteric contraction did not occur in response to oesophageal distension in the guinea-pig. The means and standard errors of the means of the duration and the amplitude of relaxation were  $8.6 \pm 0.6$  seconds and  $1.0 \pm 0.1$  cm. H<sub>2</sub>O. The mean and standard error of the mean of the time from distension to maximal relaxation (Fig. 3) was  $6.2 \pm 0.3$  seconds.

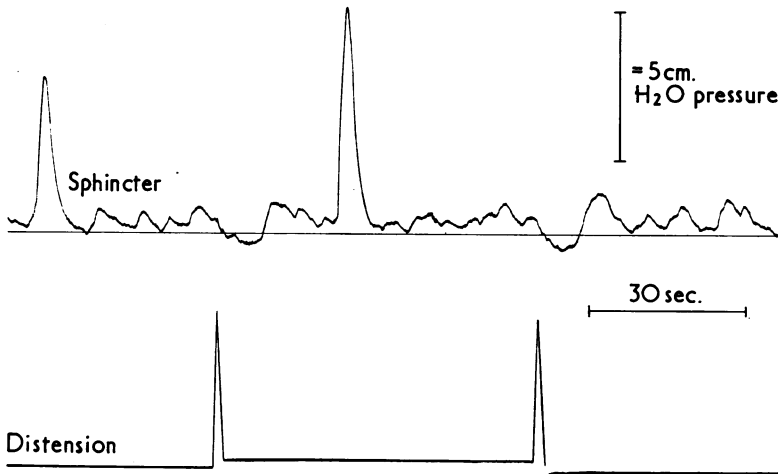


FIG. 4. *Spontaneous activity and responses to oesophageal distension of isolated guinea-pig gastro-oesophageal sphincter. Typical pattern of spontaneous variations in sphincteric tone, with two large spontaneous sphincteric contractions. Two sphincteric relaxations in response to oesophageal distensions occur, similar to but smaller than those of Figure 3.*

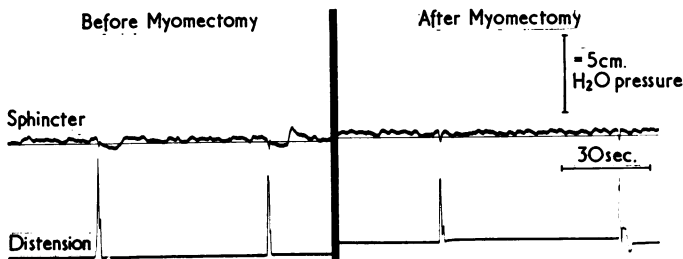


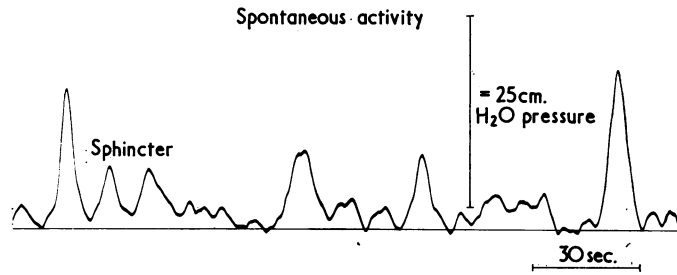
FIG. 5. *Effect of oesophageal myomectomy on isolated gastro-oesophageal sphincter of the guinea-pig. Left panel: Two sphincteric responses to oesophageal distension. Right panel: Same preparation after myomectomy. No sphincteric response was obtained after oesophageal distensions above the myomectomy site.*

In five preparations, after relaxation in response to oesophageal distension had been recorded in the sphincter, a myomectomy was done. After the myomectomy, the pressures and spontaneous activity of the resting sphincter were unchanged, but sphincteric relaxation in response to a distension orad to the myomectomy site was abolished (Fig. 5). In two of the five experiments, the stimulating balloon was repositioned caudad to the myomectomy site; distension at this site produced relaxation of the sphincter.

**KITTEN** Tests were made on the oesophagus of 14 kittens. The mean pressure of the resting gastro-oesophageal sphincter in the kitten was

less than in the guinea-pig, being 3.7 cm. H<sub>2</sub>O (range 0.8 to 5.0 cm. H<sub>2</sub>O). As with the guinea-pig, spontaneous activity of the sphincter was observed in all preparations, with occasional elevations of pressure to 25 cm. H<sub>2</sub>O (Fig. 6). The mean rate of spontaneous activity was 6.5 pressure peaks per minute, with a range of 4 to 10 per minute. Occasionally spontaneous relaxation of the sphincter was seen (Fig. 6).

Like that of the guinea-pig, the sphincter of the kitten did not respond to distension of the upper half of the oesophagus. Unlike that seen in the guinea-pig, however, the usual response of the sphincter of the kitten to lower oesophageal distension was contraction (Fig. 7). All the



### Distension

FIG. 6. Typical pattern of spontaneous activity, with two large spontaneous sphincteric contractions in the isolated gastro-oesophageal sphincter of a 3-week-old kitten.

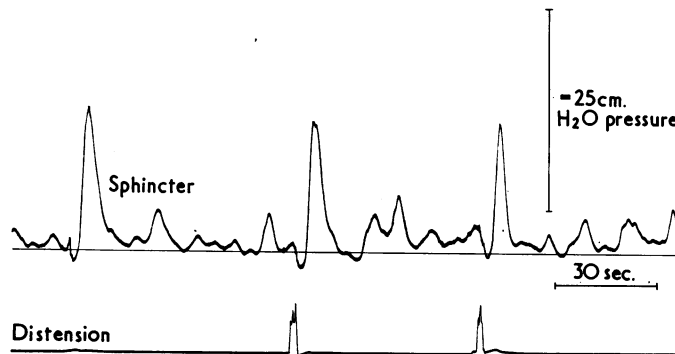


FIG. 7. Pressures from isolated gastro-oesophageal sphincter of a 3-week-old kitten. Spontaneous sphincteric contraction before distension (left) and the typical contraction observed after two oesophageal distensions.

sphincters responded to distension of the lower half of the oesophagus.

Of the 193 lower oesophageal distensions delivered to the 14 preparations, 71 produced a delayed sphincteric contraction (Fig. 7). Twenty-one caused relaxation of the sphincter; in 12 instances the relaxation was followed by sphincteric contraction. The mean duration, amplitude, and the temporal sequence of relaxation and contraction in response to distension are given in the Table.

Myomectomy was not done on the oesophagus of the kitten.

TABLE

RESPONSES OF THE ISOLATED KITTEN GASTRO-OESOPHAGEAL SPHINCTER TO LOWER OESOPHAGEAL DISTENSION<sup>1</sup>

Response	No.	Duration (sec.)		Amplitude (cm. H <sub>2</sub> O)		Time to Maximal Response (sec.)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
Relaxation	21 <sup>2</sup>	3.9	0.2	1.0	0.2	2.7	0.2
Contraction	59 <sup>3</sup>	5.5	0.2	6.5	1.0	7.1	0.2

<sup>1</sup>Total number of distensions=193; in 113 there was no response.

<sup>2</sup>Of the 21, 9 were relaxation alone and 12 were relaxation followed by contraction.

<sup>3</sup>In addition to these 59 contractions, there were 12 in which the contraction was preceded by relaxation.

## DISCUSSION

These experiments demonstrate that the gastro-oesophageal sphincter of the guinea-pig and the kitten will respond to lower oesophageal distension by relaxing and contracting when all external nerves have been severed. These responses are lost when the muscular coat of the oesophagus of the guinea-pig is divided between the site of the distension and the sphincter. Although Cannon (1907) postulated that the property of independent motor action of the oesophagus would be found only in those parts composed of smooth muscle, it is clear from these experiments that impulses which produce relaxation and contraction of the sphincter are transmitted equally well by structures in the striated muscle wall in the oesophagus of the guinea-pig (Muller Botha, 1958), and in the unstriated muscle wall of the kitten. Because this transmission is indifferent to muscle type, it may reside in the nerve tissue that is common to both types of wall. But this is not proved by our experiments.

Although sphincteric relaxation to oesophageal distension was seen in both guinea-pig and kitten, the usual response of the sphincter of the kitten was contraction. Carlson (1922) and others (Cannon, 1907; Lehmann, 1945; Meltzer and Auer, 1906; Veach, 1925) found that sphincteric reaction to stimulation depended not only on the strength and duration of the stimulus but also on the muscle tone prevailing at the moment of stimulation. Our results are in accord with this concept; the sphincter of the guinea-pig, with relatively high resting pressure, relaxed in response to oesophageal distension, whereas the flaccid low-pressure sphincter of the kitten more often contracted. The kitten preparation may not have been as well preserved in our tests as the guinea-pig preparation. Preparations of both animals were dying. We had no measure of neural function as opposed to muscular function. Our experiments simply establish that the lower oesophagus and its sphincter can function in a relatively normal fashion when completely isolated and that distension of the lower oesophagus produces responses in the sphincter that are transmitted to the sphincter via the wall of the oesophagus, completely independently of the

external nerve supply. Our observations do not define whether the conduction in the oesophageal wall occurs by way of neural or muscular channels. The results offer the prospect that disordered function of the gastro-oesophageal sphincter could result from interruption of conduction mechanisms in the wall of the oesophagus.

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