

Gastric Motility Patterns for Digestion and Vomiting Evoked by Sympathetic Nerve Stimulation and 5-hydroxytryptamine in the Dogfish *Scyliorhinus canicula*

P. L. R. Andrews and J. Z. Young

Phil. Trans. R. Soc. Lond. B 1993 **342**, 363-380
doi: 10.1098/rstb.1993.0165

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: <http://rstb.royalsocietypublishing.org/subscriptions>

Gastric motility patterns for digestion and vomiting evoked by sympathetic nerve stimulation and 5-hydroxytryptamine in the dogfish *Scyliorhinus canicula*

P. L. R. ANDREWS¹ AND J. Z. YOUNG²

¹*Department of Physiology, St George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, U.K.*

²*Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, U.K.*

SUMMARY

The sympathetic control of motor activity in the stomach of the dogfish *Scyliorhinus canicula* and its pharmacology was studied in freshly killed animals with the abdominal viscera superfused *in situ* with elasmobranch Ringer by recording gastric pressure and by quantitative analysis of video-tape recordings of the activity.

Little spontaneous activity was seen in the stomach, although in two animals retrograde contractions occurred in the spiral intestine. The effects of electrical stimulation of the splanchnic (sympathetic) nerves varied markedly with frequency. At 4 Hz after a latency of about 1 min there was a slight increase in the overall level of contractile activity in both cardiac and pyloric regions of the stomach, which persisted throughout the stimulation period (5–7 min). At 16 Hz a contraction was visible in the pyloric region within 10 s of the start of stimulation. Over the next 30 s this contraction proceeded cranially becoming more powerful as it swept into the cardiac region. This contraction returned the contents of the pyloric stomach to the cardiac stomach past the valve-like junction between them. This contraction continued into the cardiac stomach and in some animals propelled the contents into the oesophagus.

About 2 min after the start of stimulation there followed a series of contractions in both gastric regions, predominantly in the circular muscle. In the cardiac region these contractions occurred alternately in the proximal and distal regions. Occasionally a large contraction passed a bolus of material from the cardiac to the pyloric region. No movements or pressure changes were observed in the stomach after stimulation of the vagus, although contractions were readily induced in the oesophagus.

It is proposed that the initial large retrograde contraction provides a mechanism by which the animal can vomit indigestible or accidentally ingested material. In contrast the later motor patterns suggest that this type of activity is involved in more normal digestive functions of mixing food with gastric secretions, trituration and gastric emptying.

Evidence is presented which implicates 5-hydroxytryptamine as a principal neurotransmitter involved in the genesis of the retrograde contraction by the splanchnic nerve.

1. INTRODUCTION

Studies of the influence of the sympathetic nerves on the motility of the dogfish and skate stomach by using recordings of muscle tension *in vitro* have provided an insight into the nature of the autonomic control of gastric motility in elasmobranchs (Young 1980a, 1983), and shed light on the evolution of autonomic control of visceral function.

In *Scyliorhinus* the cardiac region of the stomach is a large sac which receives the prey (small fish or crustaceans) intact and stores it during the digestive process (figure 1). In contrast the pyloric region is a narrow elongated muscular tube along which the liquified products of digestion are conveyed to the pylorus and spiral intestine. This organization has certain similarities to that in carnivorous mammals.

Electrical stimulation of the sympathetic nerves *in*

vitro induces motor changes in both cardiac and pyloric regions of the stomach (Young 1980a). The responses recorded included inhibition of contractions and relaxation of the cardiac region, followed by 'rebound contractions' when the stimulus stopped. In the pyloric stomach there was inhibition of contractions, increased amplitude and 'rebound contractions'. Pharmacological studies showed that adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT), and adrenaline are involved in these responses (Young 1983). Studies in the skate (*Raja*), showed that substance P and pentagastrin (a synthetic gastrin analogue) were excitatory to the cardiac and pyloric gastric muscle (Andrews & Young 1988). Immunocytochemical studies have demonstrated the presence of gastrin, cholecystokinin (CCK), bombesin, somatostatin, vasoactive intestinal peptide (VIP), substance P and 5-HT immunoreactive nerves in the stomach of

the dogfish *Scyliorhinus stellaris* (Cimini *et al.* 1985). In another type of dogfish, *Squalus acanthias*, Holmgren & Nilsson (1983, 1984) have described gastrin-/CCK, somatostatin, bombesin, VIP and 5-HT-like immunoreactivity in the myenteric plexus of the stomach and in some cases in nerve fibres entering the muscle layers. These observations suggest that several neurotransmitters including peptides may be involved in mediating the sympathetic motor responses.

The studies outlined above have in general been done *in vitro* and have shed little light on the effects of the sympathetic nerves on the movements of gastric contents as part of digestion. Because of the complex effects of sympathetic nerve stimulation on motility in the two gastric regions it was decided that the simplest means of identifying the overall effect of nerve stimulation on the stomach was to make video-tape recordings of the stomach *in situ* and subject them to quantitative analysis. By using this technique it has been possible to propose functional motor correlates of sympathetic stimulation and to provide evidence that 5-HT is involved in mediating some of the effects of sympathetic nerve stimulation.

A preliminary account of aspects of this work has been presented to the Physiological Society (Andrews & Young 1989).

2. METHODS

(a) *Animals*

Dogfish (*Scyliorhinus canicula*) of either sex weighing 1–2 kg were housed in a large sea water aquarium and fed regularly on pieces of fresh squid or small, whole, dead fish. Animals were kept for about 7 d after capture before experimentation, were removed from the aquarium in a large net, manually restrained and killed by destruction of the brain and spinal cord. The entire procedure took less than 30 s.

(b) *Preparation*

The animal was placed on its back and the abdominal cavity opened by a midline incision. The liver and ovaries (if present) were removed. When food was present in the stomach it was pushed into the oesophagus by gentle manipulation and removed using forceps inserted via the mouth. In some studies food was left in the stomach, or after it had been emptied the stomach was inflated with 10–30 ml of sea water.

In some experiments markers were placed by inserting black suture thread (3/0 Ethicon) into the gastric serosa to provide sites for measurement from the videotapes. The markers (usually 8–10) were so placed that measurements of activity in both circular and longitudinal muscle axes of both the cardiac and pyloric gastric regions could be made. They were placed with reference to easily identified anatomical features (e.g. oesophageal–cardiac stomach junction, cardiac–pyloric boundary) in the middle of the ventral surface of the cardiac and pyloric regions, at the extreme edges of the mid-point of the pyloric stomach,

and at the extreme edges of the proximal cardiac stomach about 1 cm from the oesophago-gastric junction, and the distal cardiac stomach about 1 cm proximal to the junction with the pyloric stomach.

Throughout the experiment the abdominal contents were intermittently superfused with chilled elasmobranch Ringer prepared as previously described (Young 1980a).

(c) *Pressure recording*

In animals prepared as described above, a cannula was inserted via the mouth into the stomach so that its tip was in the cardiac portion. It was secured in place with a ligature placed around the abdominal portion of the oesophagus taking care to exclude the vagus and sympathetic nerves. A cannula was also inserted into the pyloric stomach via an incision in the pylorus–spiral intestine junction and secured with a ligature. The stomach was inflated with 10 ml of sea water, the cannulae connected to pressure transducers (Palmer Bioscience) and the output displayed on a chart recorder (Bryans 2800). During the course of the experiment the junction between the cardiac and pyloric regions was sometimes occluded by placing two ligatures around the stomach at this point.

(d) *Nerve stimulation*

The splanchnic sympathetic nerve was identified on the right-hand side of the animal either at the level of the first sympathetic ganglion, where it is free of vagal contamination, or running with the coeliac artery where some vagal fibres may be included in the nerve bundle (Young 1933). For stimulation the nerve was ligated and the peripheral end placed over bipolar stimulating electrodes to which square wave pulses (20 V, 0.5 ms) were delivered from a Grass stimulator. Based on previous studies two stimulus frequencies were selected to represent 'low' (4 Hz) and 'high' (16 Hz) frequency activation. Considerable differences related to frequency have been reported in the motor responses of the two gastric regions (Young 1983). In most studies, stimulation was continued for 5–7 min as there was some indication that motor responses may be delayed, particularly in the cardiac region (Young 1980a). With contractions occurring perhaps as infrequently as every 2 min, short periods of stimulation might not have revealed the full spectrum of sympathetic effects.

The right visceral vagus nerve was identified in the abdominal cavity as it coursed over the oesophagus (figure 1). It was mobilized, ligated and the peripheral end placed over bipolar stimulating electrodes (parameters as above). In some animals the right vagus was identified and stimulated at the level of the last branchial arch to control for the possibility that effects seen were due to contamination by sympathetic fibres, which may occur when the vagus is stimulated at more caudal sites.

At least 30 min elapsed between stimulation periods and no more than three periods of stimulation were performed in any one animal.

(e) Drugs

Atropine methyl nitrate (1 mg kg^{-1} , Sigma), BRL43694 (5 mg kg^{-1} , SKB), guanethidine (5 mg kg^{-1} , Roche), hexamethonium bromide (30 mg kg^{-1} , Sigma), ketanserin (10 mg kg^{-1} , Janssen) methysergide maleate (10 mg kg^{-1} , Sandoz), reserpine ($5 \text{ mg kg}^{-1} \text{ d}^{-1}$ 2 days before experimentation, Sigma), were all given by intraperitoneal injection dissolved in elasmobranch Ringer ($< 2 \text{ ml}$). With the exception of reserpine, drugs were administered 60–90 min before testing the effects of nerve stimulation.

5-hydroxytryptamine creatinine sulphate (Sigma) was dissolved in elasmobranch Ringer and applied topically to the entire ventral surface of the stomach at a concentration of 10^{-5} M . A total volume of 1 ml was applied over a period of 2 s.

(f) Video-tape recording

A portable video-camera (Panasonic F2 CCP) was mounted on a tripod above the animal and the field of view adjusted to encompass the entire abdominal cavity so that activity in the intra-abdominal oesophagus, stomach, spiral intestine and rectum could all be recorded. Illumination was provided by normal room lighting and a 'cold' fibre optic light source (Nache). The output from the camera was recorded in real time on a portable video-cassette recorder (Panasonic NV-180B) and monitored on a 21" colour monitor (Amstrad). A ruler displaying 1 mm and 1 cm calibration marks was included in the picture and stimulation periods indicated by inserting a marker into the picture at one corner.

Comments made from direct observation of the stomach during stimulation were recorded using the microphone attachment to the video-camera and the video-tape sound track. This was particularly useful in facilitating description of the motor patterns.

(g) Analysis of motility patterns**(i) Qualitative analysis**

The descriptions of the motor patterns reported in the results were assembled from observations of spontaneous and sympathetically evoked motility recorded on 18 h of video-tape from 25 animals. These observations are supplemented by direct observations of a further five animals. In general we describe the most consistent and striking motor changes but also include observations of rare motor events (retrograde contractions of the spiral intestine) because they illustrate the motor capabilities of a particular region.

During the course of the study we discovered serendipitously that by replaying the videotapes at up to five times normal speed the relatively slow motor patterns of the stomach were emphasized and a better impression of the overall change in activity could be gained. This fast replay technique was therefore used routinely in the analysis.

(ii) Quantitative analysis

In animals in which markers were inserted into the

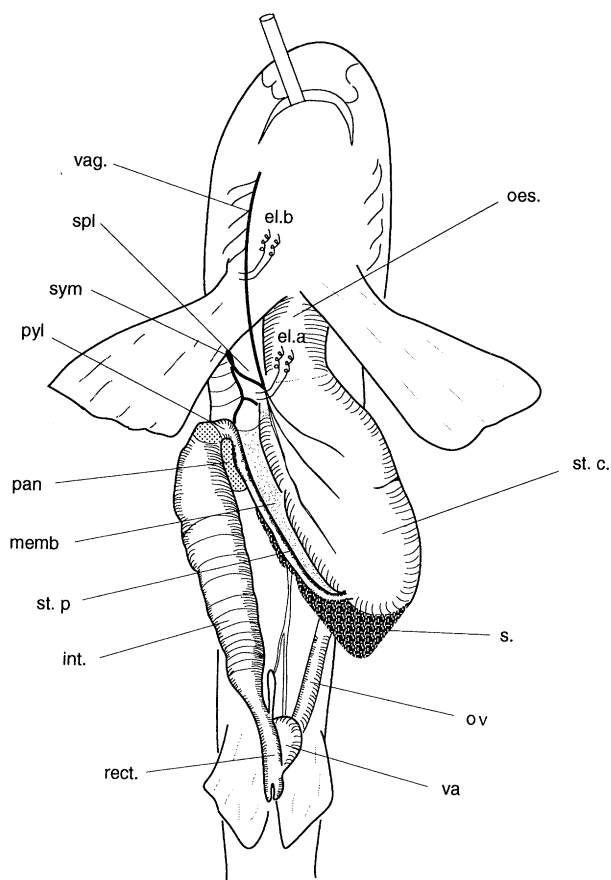


Figure 1. Diagram of the gastrointestinal tract in a female dogfish *in situ*. Note the acute angle between the cardiac and pyloric regions of the stomach. The membrane (memb) between the cardiac and pyloric regions of the stomach is shown stretched. Abbreviations: can, cannula inserted into the stomach via the mouth; el.a., stimulating electrode on the mixed vago-splanchnic nerve trunk; el.b., stimulating electrode on the vagus in the region of the last branchial arch; int, spiral intestine; memb., membrane between cardiac and pyloric regions of the stomach; oes, oesophagus; ov, oviduct; pan, pancreas; pyl, pylorus; rect, rectum; s, spleen; spl, splanchnic sympathetic nerve; st. c., cardiac region of the stomach; st. p., pyloric region of the stomach; sym, sympathetic nerve chain; va, vagina; vag, vagus nerve.

gastric wall measurements were made of the relationships between each pair of the markers every 10 s during a pre-stimulus period (1–5 min) the stimulation period (5–7 min) and the post-stimulus period (5 min or longer). The measurements were made from $12 \times 18 \text{ cm}$ black and white photographs taken directly from the television monitor.

From the qualitative observations and the quantitative studies using markers it became apparent that the most striking quantifiable changes in activity occurred in the circular orientation of the lower part of the cardiac stomach during the first 90 s of stimulation. Measurements of this activity were used for the quantitative assessment of the activity of drugs on the response to sympathetic stimulation. The longitudinal axis of the cardiac stomach was divided into thirds and the apparent diameter of the stomach measured at right angles to this line at each point.

(h) Statistics

Results are expressed as mean \pm s.e.m. (number of animals) and statistical significance tested between 'control' and drug treated groups of animals using an unpaired sample Student *t*-test.

3. RESULTS**(a) Anatomy of the stomach**

The major anatomical features of the stomach will be outlined because as the study progressed it became clear that nerve stimulation induced considerable transient changes in the morphology of the stomach and some of the anatomical features appeared to play a role in determining the nature of the motor responses (figure 1).

On opening the abdominal cavity the stomach was the most prominent feature, particularly if the animal had been fed. The cardiac stomach was distended and elongated so that the caudal extremity often lay at the level of the uro-genital opening. The cardiac stomach often contained large quantities of food: for example in four animals with a mean body mass of 1 kg the mean solid gastric contents (fish) weighed 72 ± 21 g. In animals recently fed with whole fish it was noted that they were intact and orientated along the major axis of the cardiac region. Solid undigested food was never seen in the pyloric region but on one occasion the distal portion of a crab claw was found in this region. In the empty state the cardiac stomach accounts for $92 \pm 0.5\%$ of the weight and the pyloric region only $7.6 \pm 0.5\%$ ($n = 13$ animals). In freshly killed animals the only material found in the pyloric stomach was a crab claw in one animal or parasitic nematode worms, suggesting that the pyloric region does not act as a long-term reservoir for food.

The pyloric region lies parallel to the cardiac region and is attached to it by a transparent membrane (figure 1). The pyloric stomach makes a very acute angle with the cardiac stomach. Observations on distending empty stomachs with fluid revealed that during filling the angle between the cardiac and pyloric regions of the stomach becomes more acute. The effect of this may be to occlude the lumen between the two regions reducing the possibility of undigested food entering the pyloric stomach. The membrane linking the two gastric regions may serve to ensure that the pyloric and gastric regions remain in the correct anatomical relationship for this 'valve' effect to occur.

As the abdominal incision was being made the stomach often herniated, indicating that the abdominal wall normally constrains the stomach in this sleek elongated fish.

(b) Spontaneous motor activity in the gut

In general, no obvious activity was observable in the gut either before or after surgical manipulation. However, 'fast replay' and quantitative analysis revealed that some activity was occasionally present

particularly in the distal cardiac and/or pyloric regions. The activity was of a low frequency (1–2 contractions per minute) and did not appear to move the gastric contents. Contractions were not visible on the pressure records in the four animals studied with this technique.

In contrast, striking motor activity was seen in the spiral intestine on two occasions (figure 2). A ring of contraction originated in the terminal portion of the spiral intestine at the junction with the rectum. The ring of contraction then extended orally (retrograde) and after about 90 s included the entire intestine. The net effect of this tonic contraction was to narrow the spiral intestine and increase its length by 28%. As the contraction reached the most rostral part of the spiral some liquid material was seen to be returned to the pyloric region of the stomach. Following contraction, the spiral became quiescent and failed to repeat this type of activity for the rest of the experiment either spontaneously or during sympathetic stimulation.

No spontaneous activity was observed in the rectum.

(c) Gastric responses to sympathetic stimulation

To simplify the description of the motor activity evoked by nerve stimulation the motor patterns occurring during the first few minutes of stimulation will be described separately from those in the later phase as the two types of activity appear to be quite different in their effect on the movement of gastric contents.

(i) Initial responses

This section will describe the gastric motor changes occurring during approximately the first 2 min of the response to sympathetic nerve stimulation with 4 or 16 Hz.

Stimulation with a frequency of 4 Hz did not produce any immediately visible change in the activity of the stomach (cf. 16 Hz below). After about one minute a low level of contractile activity was seen to be present in the pyloric stomach and distal part of the cardiac region. The visual observation was confirmed in two animals by recording gastric pressure during nerve stimulation at 4 Hz. The activity involved mainly irregular rhythmic contractions of the muscle in the circular orientation in the two gastric regions, with activity in the cardiac stomach being the more pronounced (figure 3).

The responses to 16 Hz stimulation were markedly different both qualitatively and quantitatively. Within 10 s of the start of stimulation, enhanced contractile activity was apparent in the pyloric stomach and in some animals a wave of contraction was clearly seen to progress retrogradally over about 15 s towards the cardiac–pyloric junction. Pressure recordings from the pyloric region showed an almost immediate increase (figure 4). In all animals a contraction was clearly visible in the region of the cardiac–pyloric junction within 30 s of the onset of stimulation. Recordings from the cardiac region showed that the rise in pressure occurred after that recorded in the pyloric region.

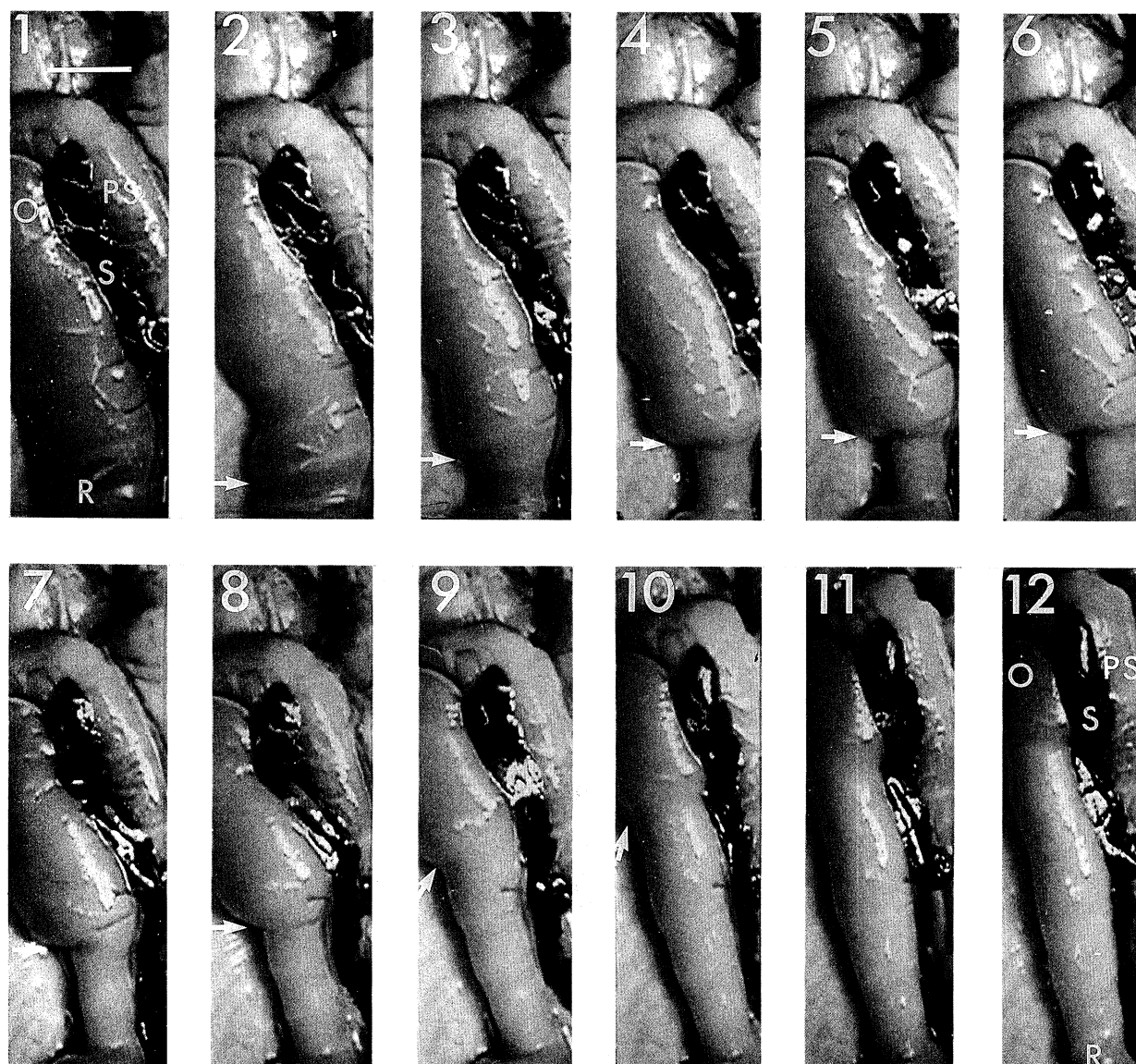


Figure 2. Consecutive series of photographs taken every 10 s showing the retrograde propagation (from the rectal region towards the stomach) of a spontaneous contraction along the entire length of the spiral intestine. O, oral end; R, rectal end of spiral intestine. The arrow indicates the location of the wave-front where it is clearly visible. PS, pyloric stomach; S, spleen. Scale bar in panel 1 = 10 mm.

These recordings support the impression gained from the visual observations that the contractile activity originated in the pyloric region and propagated to the cardiac region (figure 4). The contraction continued sweeping orally into the cardiac region stopping about half way up the cardiac stomach 90–120 s after the start of stimulation (figure 5). The lower part of the cardiac stomach usually then remained in a tonically contracted state for 20–30 s before the beginning of the motor patterns described below.

When food was left in the stomach the retrograde contraction propelled it into the proximal part of the cardiac stomach and in some animals into the oesophagus. In three experiments sea water stained with dye was placed in the stomach and a window made in the oesophagus in its pharyngeal course. In these animals expulsion of coloured fluid into the oesophagus coincided with the contraction of the lower part of the cardiac stomach. In three animals a plastic sphere 10 mm in diameter was inserted into the stomach via

the oesophagus. Stimulation of the sympathetic nerve at 16 Hz lead to the expulsion of this sphere into the oesophagus within 60 s of the onset of stimulation.

In two animals frequency of stimulation was incremented in 4 Hz steps from 4 to 16 Hz. This showed that this response requires a threshold frequency of 12 Hz or greater for its initiation. This type of response was never seen using 4 Hz stimulation but was reliably induced at 16 Hz.

In an attempt to characterize this retrograde contraction further, the stomach was transected in the region of the cardio-pyloric junction in animals in which semi-solid food residues were present in the stomach. Care was taken to exclude the macroscopically visible extrinsic nerves from the transection. Following the transection there was no leakage of gastric contents from the cut ends of the pyloric or cardiac regions. Stimulation of the sympathetic nerve at 16 Hz initiated activity in the distal pyloric stomach and the contents were ejected when the wave of contrac-

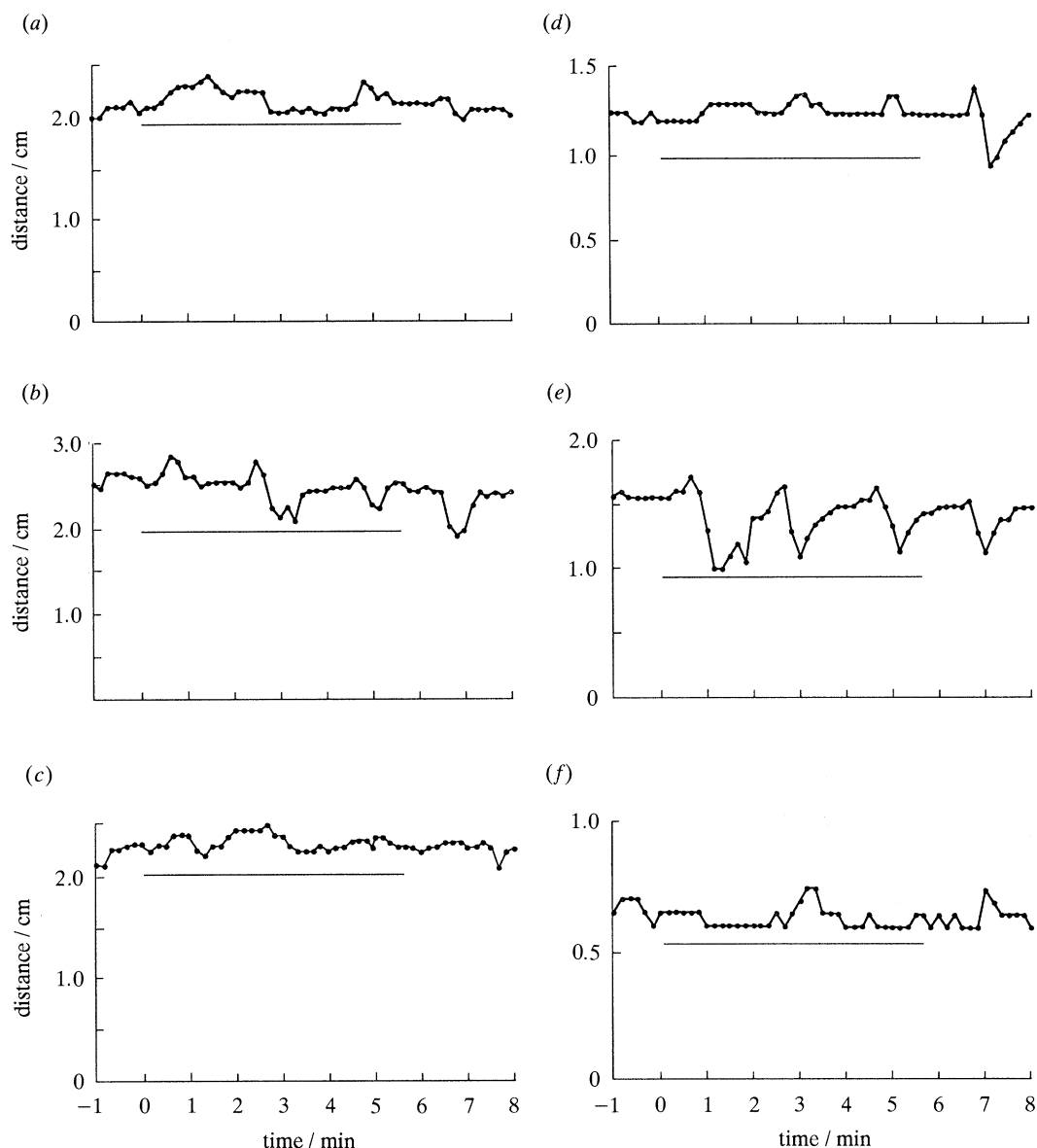


Figure 3. Analysis of the motor patterns in various stomach regions in an individual animal during sympathetic nerve stimulation at 4 Hz (horizontal bars). (a) Cardiac region lesser curve, longitudinal muscle; (b) cardiac region greater curve, longitudinal muscle; (c) pyloric region, longitudinal muscle; (d) cardiac region proximal, circular muscle; (e) cardiac region distal, circular muscle; (f) pyloric region, circular muscle. The level of activity evoked is low when compared to 16 Hz (see figure 8). The Y-axis shows the distances in cm between markers located on the serosal surface of the stomach in the regions indicated to measure the changes in dimension in the muscular orientations indicated. Contraction of the muscle results in a decrease in the dimension and relaxation in an increase.

tion reached the opening made in the proximal part. Measurements in one animal revealed the expulsion of pyloric contents 16 and 60 s after the onset of stimulation with ejection of material lasting about 5 s in each case.

Measurements of activity in the cardiac region of this animal showed that material was ejected from the pyloric stomach before the onset of activity in the cardiac stomach. The retrograde contraction was still present in the cardiac region beginning between the first and second ejections of pyloric material (figure 6).

In intact stomachs as the wave of contraction spread into the cardiac region the membrane linking the pyloric and cardiac regions was considerably stretched (figure 5).

In addition to the retroperistaltic activity of the

pyloric and distal cardiac stomach, early activity was also induced in the spiral intestine but this was only convincingly observed in two animals. About 30 s after the start of stimulation, at the time the retrograde contraction had spread to the lower cardiac stomach, a contraction became apparent just distal to the pylorus and extended over the following 40 s to involve the first third of the spiral intestine. The net effect of this activity was to narrow and elongate the upper part of the spiral intestine. This region receives the pancreatic and biliary ducts and hence may be compared to the mammalian duodenum. Some luminal contents were propelled by the contraction of this proximal region into the caudal spiral, which was seen to become distended with dark material. No activity was seen to be evoked in the rectum by splanchnic

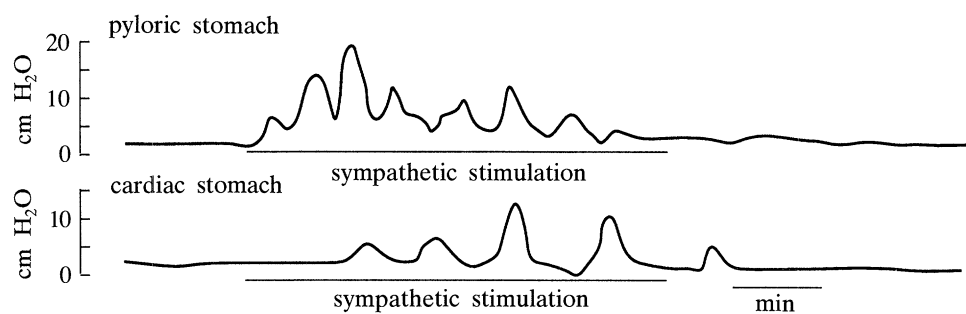


Figure 4. Recording of the pressure in the cardiac and pyloric regions of the stomach during splanchnic nerve stimulation at 16 Hz. Note the rapid onset of a response in the pyloric region in comparison to the cardiac region.

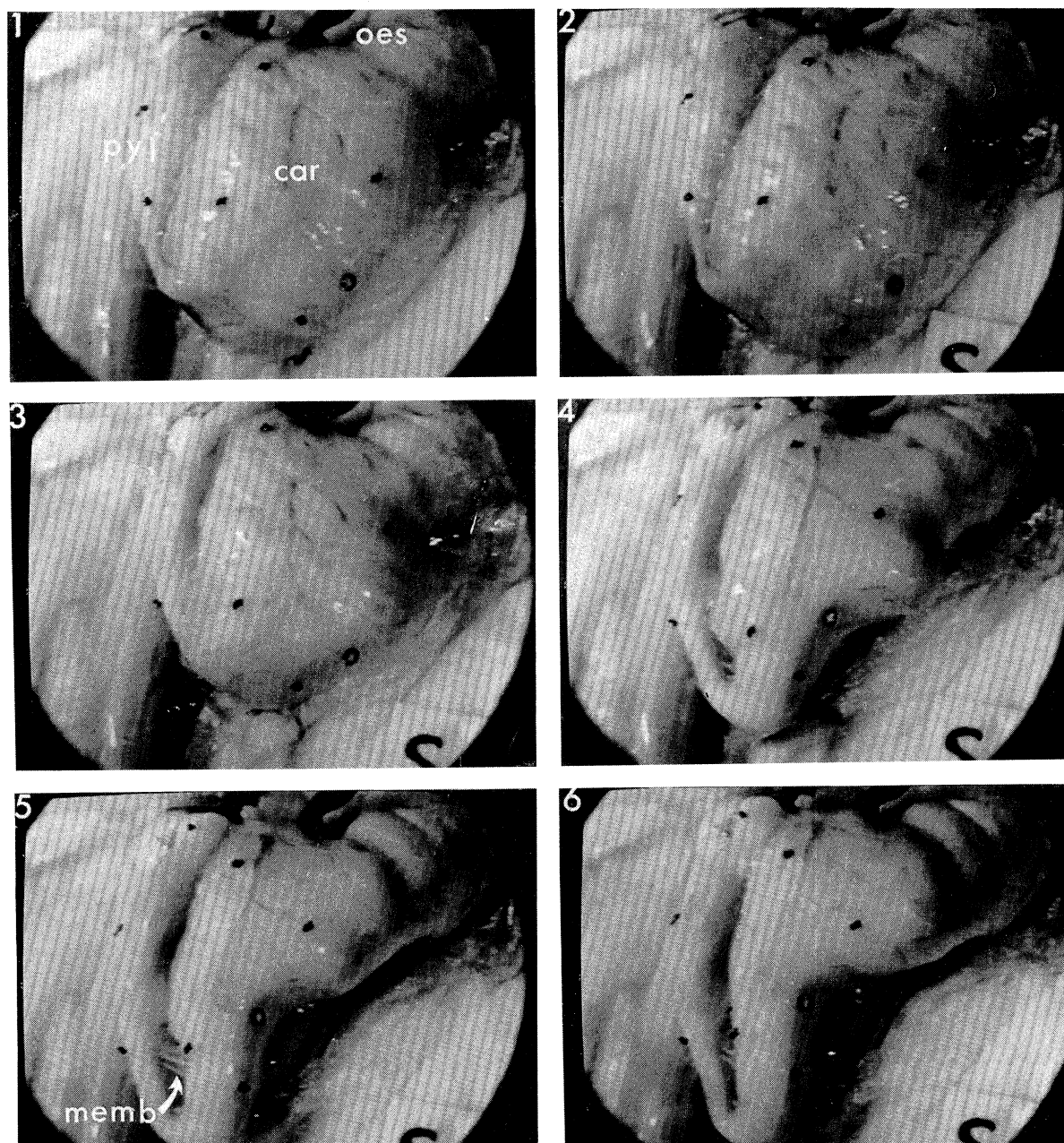


Figure 5. Consecutive photographs of the stomach taken every 10 s during sympathetic stimulation at 16 Hz indicated by S in the lower right of each panel. Note the origin of the contraction in pyloric stomach and the progression of the contraction into the cardiac region propelling gastric contents into the proximal cardiac stomach. The membrane (memb) linking the cardiac and pyloric gastric regions can be seen in panel 5.

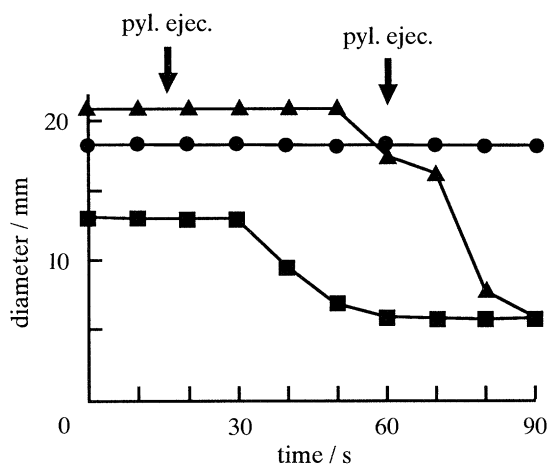


Figure 6. A plot of the diameter of the upper (circles), middle (triangles) and lower (squares) regions of the cardiac stomach during the first 90 s of sympathetic stimulation at 16 Hz in a stomach in which the pyloric and cardiac regions have been surgically separated and the pyloric–cardiac junctions left patent. Ejection of material from the cardiac end of the pyloric region (pyl.ejec.) occurs before the onset of the retrograde contraction in the cardiac regions indicated by the decrease in dimensions of the lower and middle cardiac regions. A second episode of pyloric ejection occurs when the retrograde contraction is in the cardiac region.

sympathetic stimulation but it is activated by fibres in the posterior splanchnic nerve (Young 1983).

(ii) *Later responses*

This section describes the motor responses occurring after the first two minutes of stimulation. In contrast to the initial responses these later responses were variable and complex and do not lend themselves so readily to quantification. The description below outlines the most common overall changes observed and illustrates them with measurements from representative animals.

Stimulation at 4 Hz produced a general increase in the level of activity of the stomach, most clearly visible in the circular muscle of the distal cardiac stomach when the video-tape was replayed at a fast speed. The activity was of a low level and persisted throughout the stimulation period (figure 3). Because of its low level it was difficult to measure reliably from photographs and hence little further comment can be made.

The responses to 4 Hz again contrast with those to 16 Hz which will now be described. After the invasion of the cardiac stomach by the large retrograde contraction there was a gradual increase in rhythmic contractile activity. This was principally reflected in the transverse dimensions with little change in the longitudinal dimension (figure 7). The contractions occurred at a frequency of about one per minute. The circular muscle of the pyloric region also contracted rhythmically at a frequency of about two per minute. In both the cardiac and pyloric regions the rhythmic contractions were superimposed upon an overall sustained increase in the tone (figure 7). Unlike the initial response the activity of the cardiac and pyloric regions in the later phase did not appear to be temporally coupled. Observation of the cardiac stomach showed

that the contractions mixed the contents in the following way: the lower cardiac stomach circular muscle contracts pushing the contents orally; the circular muscle in the upper region then contracts displacing the contents caudally. This alternation of contraction can be seen in the plot of activity in figure 8 and presumably serves to mix and mechanically disrupt the contents. Occasionally this pattern of activity was interrupted by a contraction originating in the middle of the cardiac region and dividing it into two compartments with a ‘dumbbell’ in shape (figure 9). This contraction then passed away and the rhythmic activity resumed.

This type of activity continued with varying degrees of intensity throughout the stimulation period and often persisted for several minutes after cessation of the stimulus (figure 7). In contrast to the skate stomach (P. L. R. Andrews & J. Z. Young, unpublished observations) post-stimulus rebound contractions were never observed in the dogfish stomach *in vivo*.

In addition to the above pattern of activity another clearly identified pattern occurred in which waves of contraction passed from the cardiac to the pyloric region propelling a bolus of gastric contents into the pyloric region and occasionally beyond (figure 9). During such activity the junction region between the cardiac and pyloric divisions underwent a remarkable transformation shown in figure 9. The bolus of gastric contents moved into the pyloric region was often then returned to the cardiac stomach by a retrograde contraction of the pyloric stomach.

Pressure recordings showed that rhythmic contractile activity was present in the cardiac and pyloric region throughout the stimulus period (figure 4).

(d) *Absence of effect of vagal nerve stimulation*

The possible effect of stimulation (1–20 Hz) of the peripheral cut end of the vagus at the level of the terminal branchial arch was investigated in three animals. Low frequency stimulation (1–15 Hz) induced twitch-like contractions in the terminal oesophagus (composed of striated type muscle) which fused into sustained contractions at higher frequencies (above 15 Hz). Although the stimulus was clearly adequate for activation of vagal fibres supplying the oesophagus no effect was detected on the stomach as assessed either by direct observation or pressure measurement. This contrasts with the observations of Campbell (1975). In addition, simultaneous vagal stimulation appeared to have no effect on the gastric response to sympathetic stimulation but this must be treated as a preliminary observation until quantitative studies have been undertaken. Vagal stimulation was without apparent effect on the spiral intestine or the rectum.

(e) *The effect of drugs on the gastric responses to sympathetic stimulation*

The nature of the neurotransmitter responsible for the mediation of the responses to sympathetic nerve stimulation was investigated by examining the effect

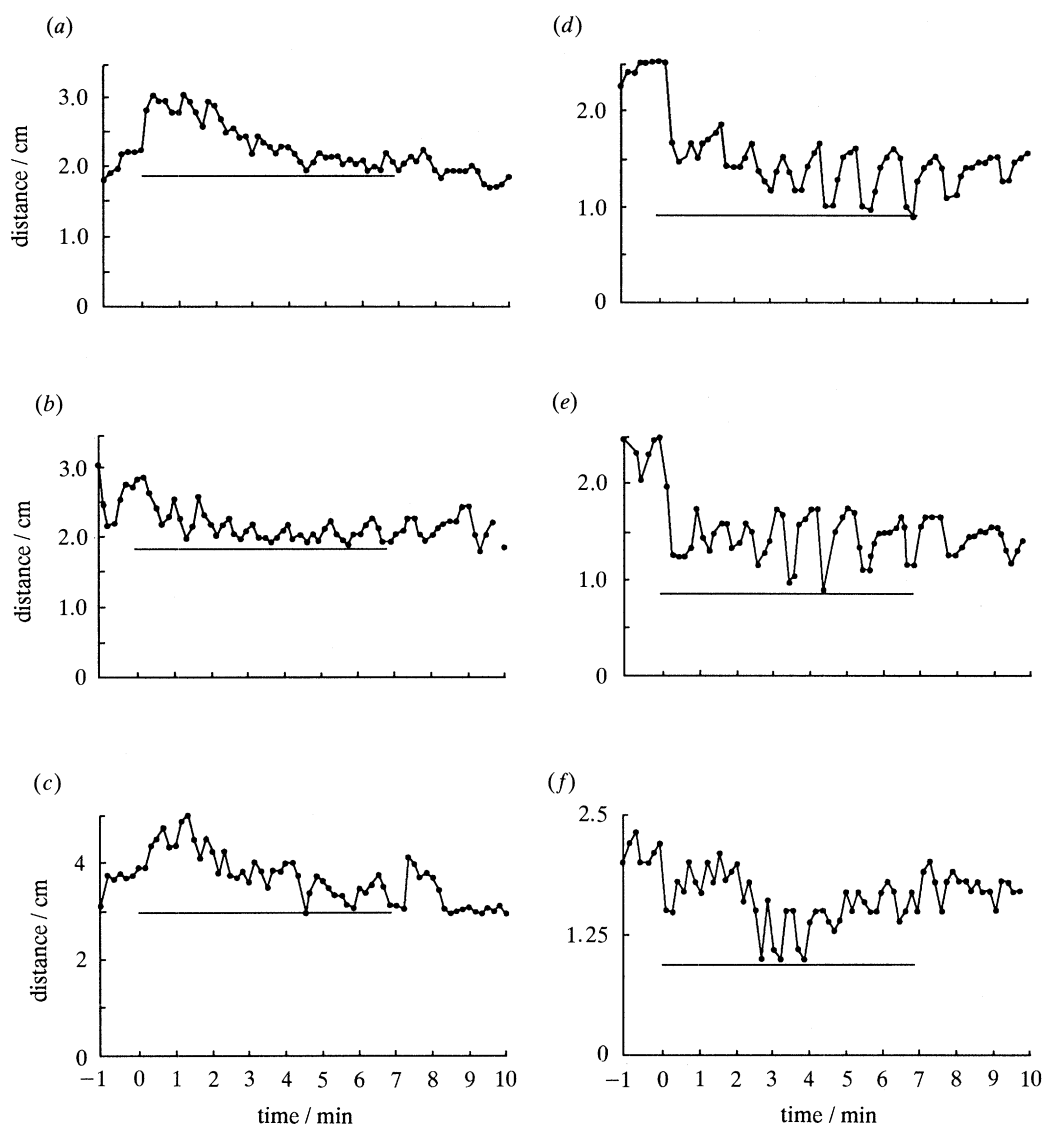


Figure 7. Changes in several gastric dimensions measured simultaneously during a prolonged period of sympathetic stimulation (7 min, 16 Hz indicated by horizontal bar) in an individual animal. (a) Cardiac region lesser curve, longitudinal muscle; (b) cardiac region greater curve, longitudinal muscle; (c) pyloric region, longitudinal muscle; (d) cardiac region proximal, circular muscle; (e) cardiac region distal, circular muscle; (f) pyloric region, circular muscle. The Y-axis shows the distances in cm between markers located on the serosal surface of the stomach in the regions indicated to measure the changes in dimension in the muscular orientations indicated. Contraction of the muscle results in a decrease in the dimension and relaxation in an increase. Note that after the initial intense tonic contraction of the proximal and distal cardiac region circular muscle (d,e), this region settles into a pattern of regular rhythmic contractions which continue throughout the stimulus period.

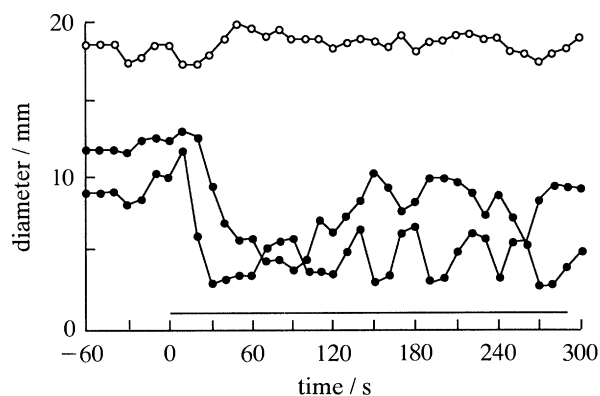


Figure 8. Changes in the diameter of the cardiac stomach in two circular (upper and lower) and one longitudinal dimension during the first 5 min of sympathetic stimulation at 16 Hz. Open circles, longitudinal; filled circles (upper trace), circular upper; filled circles (lower trace), circular lower. This animal was selected as it illustrates the alternating pattern of cardiac contractile activity. This is shown by the 180° phase lag between the dimension changes of the lower and upper regions clearly seen after the first 60 s of stimulation.

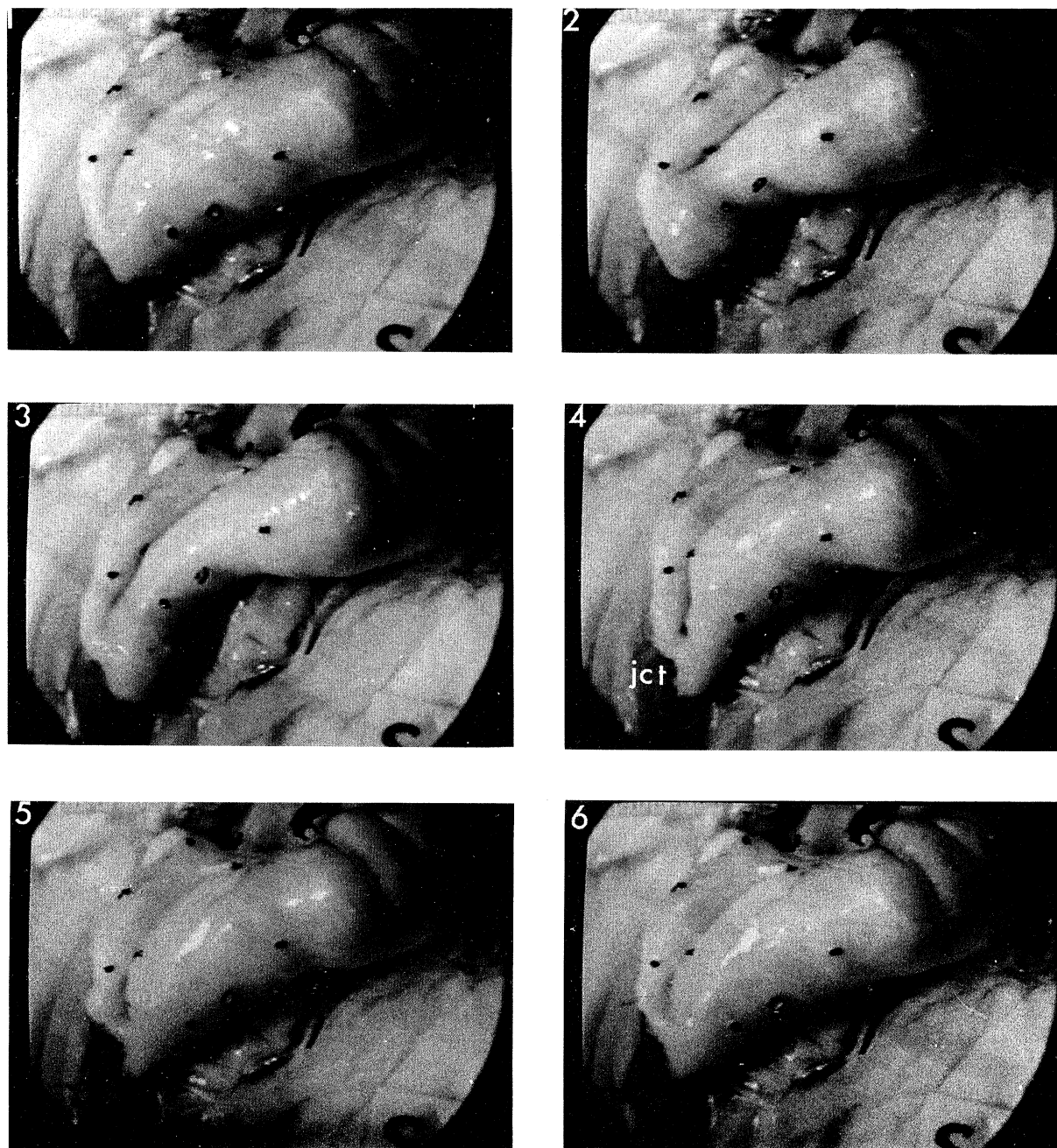


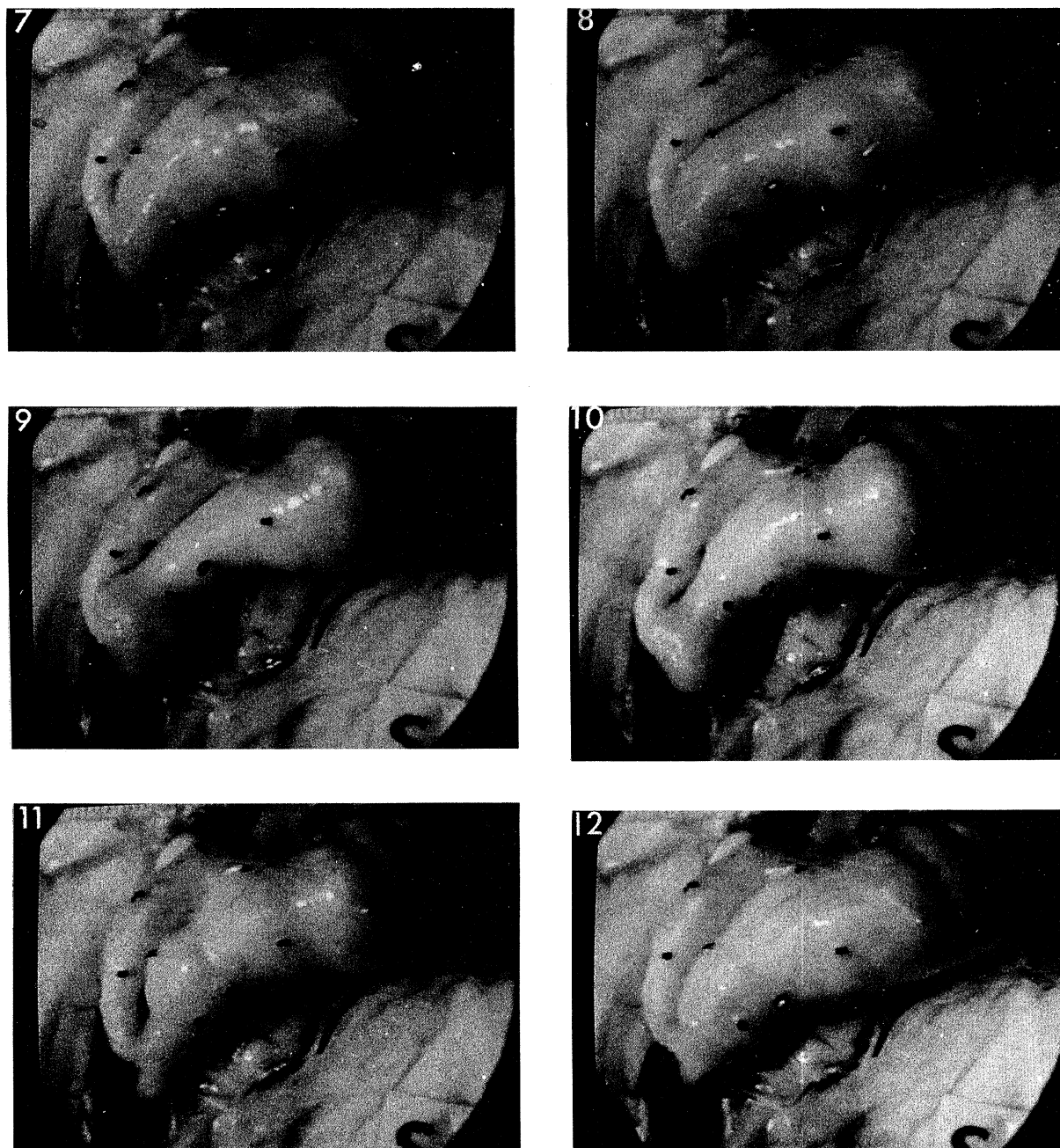
Figure 9. Consecutive photographs taken every 10 s, 3 min into a 7 min period of sympathetic stimulation at 16 Hz. Note the contraction moving contents from the cardiac to pyloric region and the morphological changes associated with this in the junction (jct) between the cardiac and pyloric region. The complete activity cycle is seen in panels 1-6 and repeated in panels 7-12. Measurements from these pictures were used in the construction of figure 8.

of neurotransmitter receptor antagonists or depleting agents. As the initial retroperistaltic response to sympathetic stimulation at 16 Hz was highly reproducible this component alone was subjected to quantitative analysis to investigate the effect of the pharmacological interventions. The effect of the same treatments on the motor response to topically applied 10^{-5} M 5-HT was quantified in the same animals to give some indication of the selectivity of any effects of the treatments against nerve stimulation. Two types of study were performed. Firstly, a pilot study in which measurements of gastric activity were made from video-tape recordings and secondly a more extensive study using

recordings of intra-gastric pressure. The results are reported separately.

(i) *Video-tape study*

The apparent diameter of the cardiac stomach was measured at two points (33% and 66% along the major axis) before stimulation and at the same points 30, 60 and 90 s after the onset of stimulation. Reserpine treatment did not block the response and if anything enhanced it, whereas methysergide totally blocked the response (figure 10). Ketanserin was studied in two animals and also totally abolished the response to sympathetic nerve stimulation at 16 Hz.

Figure 9. *Continued*(ii) *Pressure recording study*

Splanchnic sympathetic nerve stimulation.

Stimulation at 16 Hz for 5 min was used as a standard stimulus against which to test the effect of drug treatments. These stimulus parameters evoked a series of contractions beginning 24 ± 7 s ($n=9$) after the start of stimulation. The first contraction peaked 68 ± 8.5 s, the second 145 ± 15 s, the third 190 ± 16 s and the fourth 243 ± 17 s after the start of stimulation. The amplitude of the response declined progressively after the first contraction (figures 11 and 13). The effects of drugs on all contractions was measured but for simplicity we only present results for the first contraction, which is the pressure correlate of the retrograde contraction recorded in the video-tape part of this study (figure 12).

Methysergide (5 mg kg^{-1} , i.p., $n=3$) produced a dramatic decrease in the amplitude of the response and ketanserin (10 mg kg^{-1} , i.p., $n=3$) abolished the response. As there were only three animals in each group statistical analysis was not possible. BRL43694 (5 mg kg^{-1} , i.p.) abolished the response in all five animals tested ($p=0.0001$). Guanethidine (5 mg kg^{-1} , i.p., $n=5$) produced a large and significant ($p=0.001$) reduction in the response. Hexamethonium (30 mg kg^{-1} , i.p., $n=4$) reduced the amplitude of all contractions by about 50% but this just failed to achieve statistical significance. In contrast atropine methyl nitrate (1 mg kg^{-1} , i.p., $n=4$) was without effect on the first contraction but increased the amplitude of subsequent contractions, although not to a statistically significant level.

None of the effects on the evoked responses were

considered to be due to changes in the prevailing basal tone produced by the drugs as drug treatment appeared to have little effect on this parameter. Tone values were: control 4.2 ± 0.23 cm H₂O; methysergide 4.4 ± 0.4 cm H₂O; ketanserin 5.2 ± 0.5 cm H₂O; BRL43694 3.9 ± 0.67 cm H₂O; guanethidine 4.1 ± 0.3 cm H₂O; hexamethonium 4.6 ± 0.32 cm H₂O; atropine methyl nitrate 4.5 ± 0.68 cm H₂O.

Topical application of 5-HT

Application of 10^{-5} M 5-HT evoked a series of contractions most clearly visible in the cardiac stomach. In all eight animals studied, two contractions occurred, with the second contraction always being larger than the first (figures 11 and 13). In six of the eight animals three contractions occurred and in three animals a series of four contractions was evoked. The latency to the beginning of the first contraction was 18 ± 4 s after application with the peaks of the first three contractions being at 55 ± 9 s ($n=8$), 149 ± 19 s ($n=8$) and 254 ± 34 s ($n=6$) after 5-HT application.

As the second contraction was the largest and occurred in all control animals for simplicity the effects of the various drug treatments is described mainly with reference to their effect on this component (figure 12). Methysergide (5 mg kg⁻¹, i.p.) and ketanserin (10 mg kg⁻¹, i.p.) both produced a dramatic decrease in the response to 5-HT (figure 12) but as only three animals were studied for each statistical analysis was not possible. BRL43694 (5 mg kg⁻¹, i.p., $n=5$) produced a significant reduction ($p=0.038$, $n=5$) in the response as did guanethidine (5 mg kg⁻¹, i.p., $p=0.017$, $n=5$). Hexamethonium (30 mg kg⁻¹, i.p., $n=4$) and atropine methyl nitrate (1 mg kg⁻¹, i.p., $n=4$) both increased the amplitude of the first three contractions, although this failed to achieve statistical significance.

4. DISCUSSION

This study is the first to report a detailed and quantitative description of the patterns of gastric motility evoked by sympathetic splanchnic nerve stimulation of the intact gut *in situ* in *Scyliorhinus canicula* or any other elasmobranch fish. For convenience, the various experiments will be discussed separately before drawing an overall conclusion.

(a) Spontaneous motility

Small, irregular contractions were present in the distal cardiac and pyloric regions of the stomach. Spontaneous activity is present *in vitro* in most longitudinal muscle strips from both gastric regions but the level of activity is very variable (Young 1983; Andrews & Young 1987). The spontaneous activity *in vitro* was not abolished by tetrodotoxin suggesting that it is to some degree of purely myogenic origin. In the anaesthetized dogfish (*Mustelus canis*) Alvarez (1927) described some spontaneous activity in the stomach with contractions in the cardiac region rarely passing to the pyloric region.

Although little activity was seen in the stomach

some remarkable examples of spontaneous activity were seen in the spiral intestine in the form of a retrograde contraction associated with elongation, which has also been described by Alvarez (1927) and Young (1983). Such retrograde activity could serve to retain the semi-solid contents in the spiral intestine during digestion and absorption.

(b) Sympathetically evoked activity

In adult mammals the gastric motor response to sympathetic nerve stimulation is inhibition of ongoing contractions and reduction in tone (e.g. Jansson & Martinson 1966; Andrews & Lawes 1984). In marked contrast, as this study shows, in *Scyliorhinus canicula* the gastric response is excitatory as is the case in *Squalus acanthias* (Holmgren & Nilsson 1984). The present observations in this elasmobranch confirm the *in vitro* studies by Young (1980a, 1983) and *in vivo* and *in vitro* studies of other elasmobranchs such as the skate (*Raja diaphanes*, *Raja stabuliformis*, Babkin *et al.* 1935; Nicholls 1933), and ray *Torpedo*, (Bottazzi 1902). In teleost fish also the predominant response in the stomach to sympathetic stimulation is excitation (e.g. brown trout, Burnstock 1958; eel, pike and perch, Muller & Liljestrand 1918) but Young (1980b) also showed evidence for the presence of inhibitory effects in the angler fish *Lophius*.

All the previous studies have examined the motor responses of the stomach primarily from the aspect of identifying whether the sympathetic responses were excitatory or inhibitory and characterizing the nature of the transmitter involved. In the present study the emphasis has been on the motor patterns and their possible functional significance and therefore the two main components of the response will be discussed separately.

(i) Initial response

The character of the initial response was frequency dependent. At 4 Hz there was a stimulation of activity in both gastric regions at a low level, which began slowly but continued throughout and often beyond the stimulus period of many minutes. This delayed onset of activity in the cardiac stomach confirms the observations of Young (1980) of a close relationship between the onset of contraction and frequency of sympathetic stimulation between 2 and 16 Hz. An increase in gastric motility by frequencies of stimulation as low as 1 Hz has been reported in the isolated perfused stomach of *Squalus acanthias* (Nilsson & Holmgren 1983; Holmgren & Nilsson 1984). We cannot be certain whether these contractions in the dogfish have a function but the impression gained was that they lead to some mixing and redistribution of the contents of the cardiac stomach. There was no evidence at this frequency of net transfer of material from the cardiac to the pyloric region or of gastric emptying into the proximal spiral intestine.

Activity induced by 16 Hz originated early in the terminal portion of the pyloric stomach and swept rapidly orally into the cardiac stomach. This retrograde peristaltic contraction lead to the retropulsion

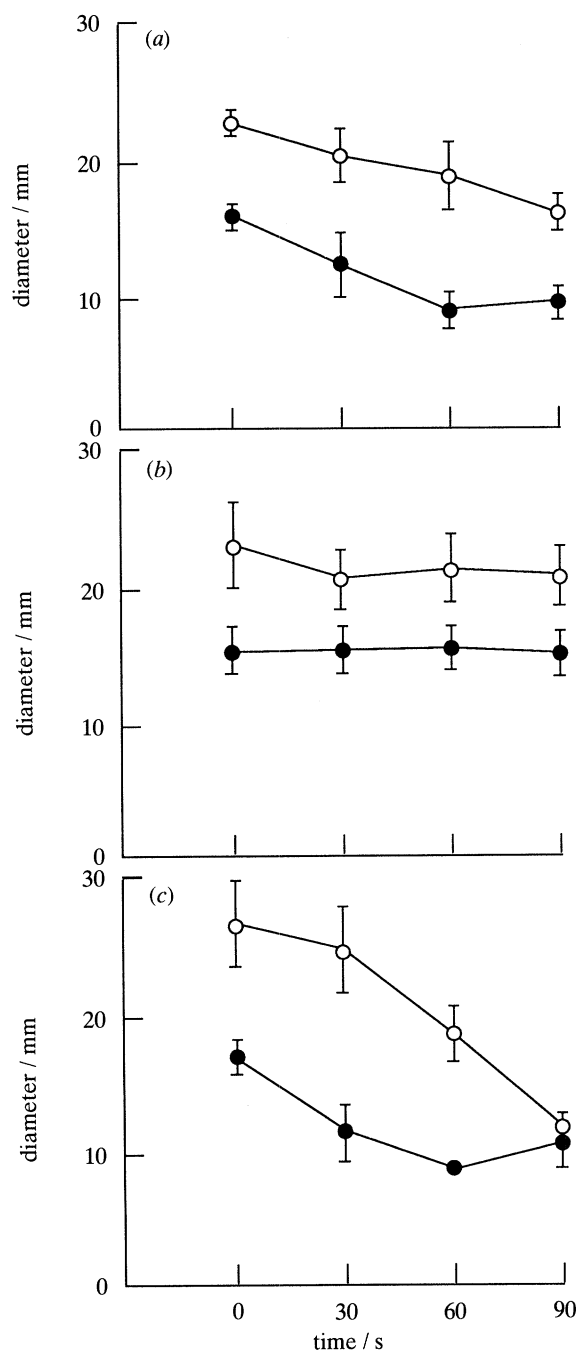


Figure 10. The diameter (mean \pm s.e.m.) of the rostral (open circles) and caudal (filled circles) regions of the cardiac stomach during the first 90 s of sympathetic nerve stimulation at 16 Hz, showing: (a) control; (b) the abolition of the contractile response by pretreatment with methysergide (5 mg kg^{-1} , i.p., $n=4$); and (c) the lack of effect of reserpine (5 mg kg^{-1} , $n=4$). Contraction of the muscle results in a decrease in the diameter of the stomach.

of gastric contents (fluid, partly digested fish or plastic balls) into the proximal cardiac stomach and on some occasions into the oesophagus. Two possible functions are proposed for this activity.

Regulation of gastric emptying

Formal measurements of gastric emptying rates have not been made in elasmobranchs but several observations indicate that it is slow. In the dogfish the

food, usually small dead fish (up to 10 cm long in our study), is swallowed whole without chewing. Thus digestion relies solely on gastric acid and enzyme secretions from the stomach, intestine and pancreas (Barrington 1942) and mechanical breakdown by gastric motility.

The process of digestion appears to be slow as we have observed virtually intact fish in the cardiac stomach of animals killed at least 24 h after feeding. Only when solubilized are the gastric contents transferred to the spiral intestine. One function of the retroperistaltic contractions may be to facilitate the retention of undigested food in the stomach for further digestion. It is likely that food is retained in the stomach for many days. In the dogfish (species not stated) van Slyke and White (1911) estimated that protein digestion takes six times longer than in a mammal and in a study of several elasmobranchs (*Raja*, *Torpedo*, *Scyllium*) food was found in the stomach up to 18 days after feeding (Weinland 1901, quoted in Barrington 1942). Studies of gastric secretion suggest that this may not reach a maximum level until 2–3 days after feeding (Soulima 1919, quoted in Barrington 1942). Transit through the entire gut also appears to be prolonged as defaecation was first reported 5 days after a meal in the dogfish *Scyllium canicula* (Dobreff 1927, quoted in Barrington 1942).

In view of these observations we propose that one function of the retroperistaltic contraction is to facilitate the retention of food in the cardiac stomach during digestion. This may also be achieved by the elongation and tonic contraction of the circular muscle of the pyloric stomach occluding the lumen, particularly as pyloric contractions can occur with stimulation of the splanchnic sympathetic nerve at frequencies below 16 Hz (Young 1980a).

Vomiting

With stimulation at 16 Hz it was possible in some animals to demonstrate ejection of the gastric contents into the oesophagus. We propose that this motor pattern provides a major mechanism by which fish vomit. In mammals the expulsive component of the vomiting reflex is brought about by compression of the stomach by synchronous tonic contraction of the costal diaphragm and anterior abdominal muscles under the influence of spinal somatic motor nerves and coordinated from the brainstem (for reviews, see Andrews & Hawthorn 1988; Grundy *et al.* 1991). As fish do not have a diaphragm and contraction of the 'abdominal' muscles would dramatically interfere with swimming, ejection of gastric contents in fish must be by a different mechanism from mammals. We propose that ejection is achieved by this large retroperistaltic gastric contraction delivering the gastric contents into the short (2.6 ± 0.2 cm long in dogfish with a nose-to-tail length of 54.8 ± 2.4 cm, $n=8$ animals) striated muscle oesophagus and pharynx. From there they are ejected to the outside world by the 'cough' reflex described in another elasmobranch *Heterodontus Portus Jacksoni* (Satchell & Maddalena 1972). The expulsion may be facilitated by contraction of the

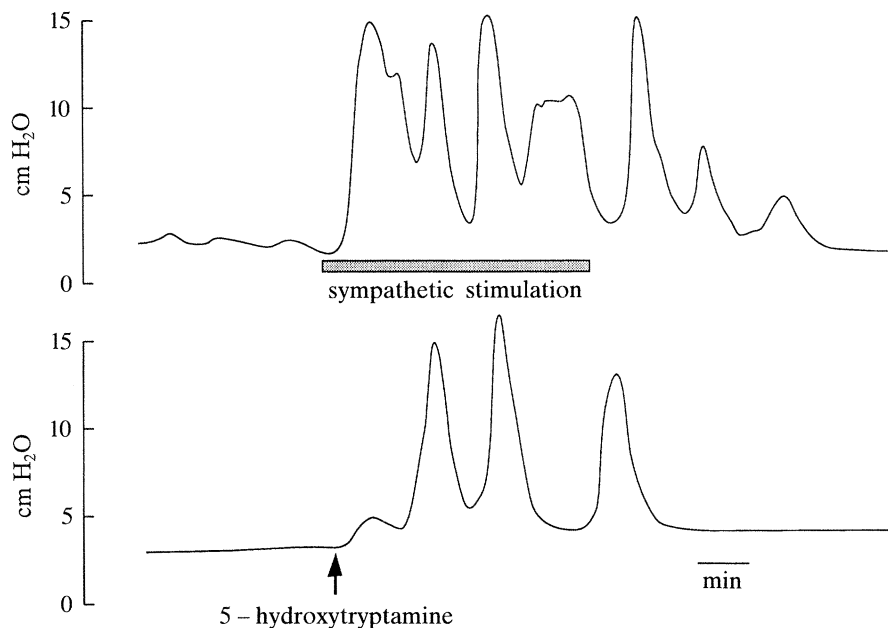


Figure 11. Examples of the gastric pressure responses induced by splanchnic sympathetic nerve stimulation (16 Hz, 5 min, 20 V, 0.5 ms) and topical 5-hydroxytryptamine (10^{-5} M).

oesophagus under the control of the vagus (see § 3*d*) and such activity has been reported to be involved in the emetic response in the dog (Lang 1992). Vomiting has been reported in a number of teleost fish (e.g. brown trout, pike, perch, killifish, walleye, largemouth bass, bullhead catfish, cod, yellowhead wrasse, pinfish and striped bass) in response to stimuli such as apomorphine and other emetic drugs (Gerhart 1991; Tiersch & Griffith 1988; Foster 1977; Jernejcik 1969; Puerta 1959; Webster 1942), and even motion (cod, McKenzie 1935). Vomiting and retching were described in the decerebrate dogfish *Mustelus canis* by Alvarez (1927) in response to pharyngeal stimulation and traction of the stomach or oesophagus. Rectal obstruction also results in food regurgitation in the dogfish (van Slyke & White 1911). Alvarez (1927) also described the gastric retroperistaltic wave.

In mammals and other vertebrates vomiting is viewed as a protective reflex (Davis *et al.* 1986) comparable to coughing, which is a reflex also present in fish including elasmobranchs (Alvarez 1927; Satchell & Maddalena 1972). Although vomiting probably has a protective role in the dogfish, we believe that it may also play a part in the normal course of digestion. The diet of many fish, including elasmobranchs contains chitinous and other relatively indigestible material (e.g. squid beaks and seaweed; Clarke & Stevens 1974). Such material will gradually accumulate in the stomach particularly in elasmobranchs as no solid matter enters the spiral intestine. We propose that this indigestible material is periodically cleared from the gut by the retroperistaltic gastric motility described here probably supplemented by contraction of the oesophagus. Although we are unaware of reports of periodic purging of the gut in elasmobranchs, such behaviour has been reported in a teleost, the tuna, in the open ocean (Carey *et al.* 1984).

In mammals the somatic component of the vomiting reflex is preceded immediately by a large retrograde

contraction originating in the mid-small intestine progressing to the gastric antrum (Lang *et al.* 1986) under vagal control. This contraction has been proposed to return toxin contaminated gut contents to the stomach prior to ejection. It is tempting to suggest that this retrograde contraction represents a conserved motor pattern.

(ii) *Later response*

The motor patterns evoked by sympathetic nerve stimulation after the first few minutes of stimulation were quite different from the initial responses. The main difference was that the activity appeared to lead to a mixing of the contents of the cardiac stomach and the oscillation of contents between the cardiac and pyloric regions. This is the type of activity which might be expected to occur during the long process of digestion of prey swallowed whole.

One of the most striking observations was the changes undergone by the cardiac-pyloric junction. In the resting state the oblique angle between the cardiac and pyloric regions and its maintenance by the linking membrane and the elongated nature of the abdomen must impede the passage of material between the two regions. This junction zone may therefore act as a functional sphincter and contribute to ensuring that material passing towards the spiral intestine is sufficiently digested and fluid.

A puzzling feature of the present study is that only rarely were peristaltic contractions seen which passed over the whole stomach to the spiral intestine. Only occasionally were contractions seen to travel over the entire stomach in Alvarez's (1927) study of *Mustelus canis* and 'at no time were deep forwarding contractions seen in the pyloric region'. The nature of the motor activity leading to the progressive emptying of the stomach and the way in which it is controlled remain to be elucidated. At the time when the gastric contents are sufficiently digested to pass to the duode-

num they are likely to be very liquid in nature and hence large macroscopically visible contractions may not be necessary. In addition, the movement of material from the stomach to the spiral intestine will depend upon the relative level of motor activity of the stomach, the proximal part of the spiral intestine and any sphincter located between the two. It is therefore conceivable that the reason why gastric contents were not transferred from the stomach to the spiral intestine in spite of enhanced gastric motility is that the sympathetic stimulation induced contraction of a sphincter located between the two regions. Alvarez (1927) reported the presence of 'a definite pylorus' at the lower end of the stomach where it joined the spiral intestine and histology of this region revealed that the muscle fibres broke up 'into small islands surrounded by connective tissue', an arrangement which he comments is similar to that seen in the region of the pyloric sphincter in man.

Another aspect requiring some discussion is the relationship of the results obtained in the present study to those from previous *in situ* (Young 1980) and *in vitro* (Young 1983; Nilsson & Holmgren 1983; Holmgren & Nilsson 1984) studies of the stomach in two species of dogfish. The main difference between the present and previous studies is that no 'rebound' contractions were observed in the present study either on the pressure record or the video-recording, although this requires some explanation. In the present study contractile activity continued at an enhanced level after cessation of sympathetic stimulation but we have not termed these contractions 'rebound' contractions because they had neither a fixed temporal relationship to the cessation of the stimulus nor were they larger than the preceding contractions occurring during stimulation. In the studies of whole stomach motility it was reported that 'rebound' contractions began 1–2 min after the end of stimulation (Nilsson & Holmgren 1983; Holmgren & Nilsson 1984) whereas in the *in situ* study by Young (1980) recording the contractions of the stomach at single points on the cardiac and pyloric regions, contractions began within 20–30 s. At present there is no clear explanation for the differences between the various studies, although two points should be mentioned. Firstly, it is possible that any 'rebound' contraction may not have been large enough to be visible, although using an identical technique, 'rebound' contractions are readily visible in the stomach of the skate (P. L. R. Andrews & J. Z. Young, unpublished observations). The experiments recording pressure in the stomach would have been expected to detect even very weak contractions but it should be remembered that the pressure recorded is the result of activity at all points in the stomach. Thus it is possible that an increase in pressure caused a 'rebound' contraction in one gastric region which could be obscured by a relaxation in another region. Secondly, the stimulus duration in the present study was much longer than used previously and a study in the anaesthetized ferret revealed that the magnitude of the 'rebound' contraction in the gastric corpus reduced as the duration of stimulation increased (Andrews & Grundy 1981).

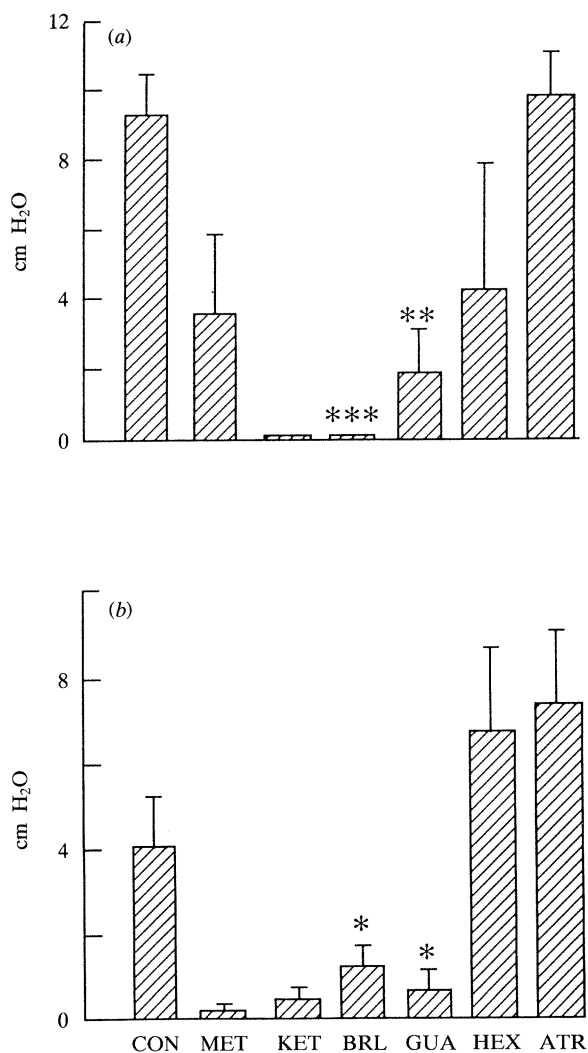


Figure 12. Histogram showing the effect of drug treatment on the pressure response to: (a) sympathetic nerve stimulation (16 Hz, 5 min, 20 V, 0.5 ms); and (b) topical 5-hydroxytryptamine (10^{-5} M). For sympathetic stimulation the amplitude of the first contraction is shown and for 5-HT the second contraction (see text for details and number of animals per group). Results are expressed as mean \pm s.e.m. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$. ATR, atropine; BRL, BRL43694 (granisetron); CON, control response; GUA, guanethidine; HEX, hexamethonium; KET, ketanserin; MET, methysergide. See text for doses.

A further difference between the present study and those of Young (1980, 1983) is that no evidence was obtained for inhibitory effects of sympathetic nerve stimulation manifest as a reduction in the overall level of muscle tone and an inhibition of ongoing contractile activity. Unless the relaxation was profound, it is unlikely that it would have been detected using the video-taping technique. While pressure recording has been used in mammalian studies to record gastric relaxation (e.g. Andrews & Lawes 1985a), the magnitude of the effect recorded depends upon the prevailing gastric pressure and volume (Andrews & Lawes 1985b). As the prevailing gastric pressure (tone) and level of spontaneous contractile activity were both relatively low in the present study, it may not have been possible to record clear inhibitory effects. In

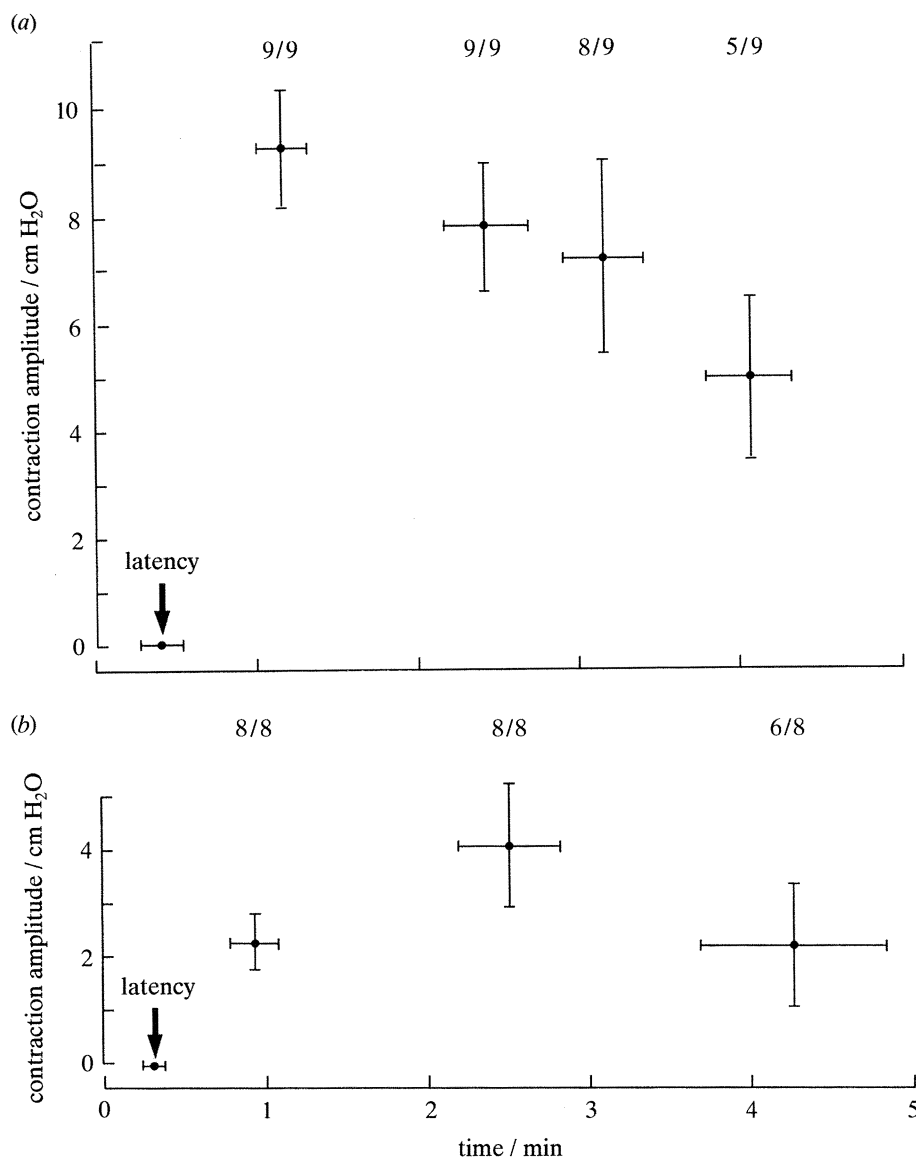


Figure 13. Analysis of the gastric pressure responses to: (a) splanchnic sympathetic nerve stimulation (16 Hz, 5 min, 20 V and 0.5 ms); and (b) 5-hydroxytryptamine (10^{-5} M). The values (mean \pm s.e.m.) were obtained from measurements of the original pressure records and plot the time of occurrence of each contraction peak and its amplitude. The latency of onset of the first contraction after application of the stimulus ($t=0$) is shown on the X-axis. The numbers above each point refer to the number of animals with a contraction/number of animals tested.

addition, inhibitory effects in one gastric region may have been obscured by excitatory effects in an adjacent region. In view of the clear demonstration of inhibitory effects of sympathetic stimulation by Young (1980, 1983) this aspect requires further study in the intact stomach using a variety of techniques for recording gastric motility under better experimental conditions.

(c) *The pharmacology of the motor responses*

In vitro studies in elasmobranchs (Young 1980a; Nilsson & Holmgren 1983; Holmgren & Nilsson 1984) and teleosts (Holmgren *et al.* 1985; Nilsson & Holmgren 1989) have implicated 5-HT in mediating the motility effects of nerve stimulation and therefore our preliminary *in vivo* pharmacological studies investigated the possible role of 5-HT in the retroperistaltic response.

The involvement of 5-HT in the response to sympathetic nerve stimulation is supported by the studies showing a reduction or abolition of contractions by methysergide, ketanserin or BRL43694. The effect of these agents on the response to topical 5-HT provides further evidence for 5-HT as a neurotransmitter in the elasmobranch stomach.

The antagonists used act on the three major subtypes of 5-HT receptor identified from mammalian studies: 5-HT-1-like (methysergide), 5-HT-2 (ketanserin), 5-HT-3 (BRL43694) (Bradley *et al.* 1986). It is not known whether these subtypes of 5-HT receptor are present in elasmobranchs. The results indicate that either all three sub-types are involved in mediating the response to sympathetic stimulation and 5-HT or that one type of 5-HT receptor is present which can be influenced by all three antagonists. Until more quantitative pharmacological dose-response studies are undertaken in elasmobranchs to characterize the

selectivity of a range of 5-HT receptor antagonists and agonists, these possibilities cannot be distinguished. However, in spite of these limitations the present study has clearly implicated 5-HT in mediating the effects of sympathetic stimulation in *Scyliorhinus canicula*, although it is uncertain at which point 5-HT is involved. For example, in the rainbow trout stomach, evidence has been presented that part of the contractile effect of substance P is via a release of 5-HT from the enteric neurons (Holmgren *et al.* 1985). Substance P is excitatory in the skate (Andrews & Young 1988) and dogfish (P. L. R. Andrews & J. Z. Young, unpublished observations) stomachs *in vitro* but the effects of selective 5-HT receptor antagonists on the response have not been studied.

The studies with the cholinergic receptor antagonists hexamethonium and atropine give a further insight into the transmitter systems involved in the control of motility. Hexamethonium reduced the response to nerve stimulation by about 50% indicating that at least part of the response was mediated by a pathway containing a nicotinic receptor. In *Squalus acanthias*, acetylcholine induced gastric contractions but the receptor type was not investigated (Holmgren & Nilsson 1984). The lack of effect of hexamethonium and atropine on the response to topical 5-HT indicated that in the stomach of *Scyliorhinus canicula*, unlike the mammalian gut, the response to 5-HT is not mediated indirectly by activation of cholinergic neurons (Sanger & Wardle 1990). No evidence was found for an involvement of muscarinic cholinergic receptors in mediating the sympathetic response and this is consistent with the previous studies in *Squalus acanthias* (Nilsson & Holmgren 1983).

The reduction in the response to sympathetic nerve stimulation by guanethidine also implicated adrenergic transmission in the response and this is consistent with the previous study by Young (1980) demonstrating a stimulation of motility by adrenaline. Guanethidine also reduced the response to 5-HT suggesting that 5-HT may act by activation of an adrenergic neuron. 5-HT_{1B} and 5-HT₃ receptors have been located on sympathetic neurons in mammals (Costall & Naylor 1990).

The present study has focused on the involvement of 5-HT in mediating the responses to sympathetic stimulation. However, the possible involvement of peptides should not be neglected in view of the wealth of immunocytochemical evidence (see §1) and the demonstration of stimulatory effects of bombesin, CCK and substance P on gastrointestinal tract motility in elasmobranchs (Andrews & Young 1988; Bjenning *et al.* 1990; Jensen & Holmgren 1991). Indeed, in view of the complexity of the motor effects induced by sympathetic stimulation it is highly unlikely that only a single neurotransmitter is involved. The peptides could be involved in the response in a number of ways acting as co-transmitters (Nilsson & Holmgren 1998) as an intermediate transmitter with the final effect being due to 5-HT for example (Holmgren *et al.* 1985) or by potentiating the effect of a non-peptide transmitter as demonstrated for bombe-

sin and acetylcholine in the cod and trout stomachs (Thorndyke & Holmgren 1990). Although the neurotransmitter role of peptides is the one of most relevance to the present study where the circulation is arrested, the endocrine role of peptides such as gastrin and cholecystokinin in the regulation of motility and in potentiating the effect of the nerves should not be overlooked.

These results provide a body of evidence for an involvement of 5-HT and possibly adrenaline in mediating the gastric motor responses to sympathetic nerve stimulation in elasmobranchs. However, the results must be treated with some caution because as relatively few pharmacological studies have been undertaken in elasmobranchs we have little idea of the receptor selectivity of the drugs at the doses used *in vivo*.

(d) *The control of the motor responses*

The intramural mechanisms involved in the genesis of the motor responses to stimulation are not known. In the dog intestine the giant retrograde contraction preceding retching is proposed to be generated by sequential activation of vagal efferents supplying muscle at progressively more oral locations (Lang & Sarna 1989). In the dogfish stomach a different mechanism appears to operate as the entire motor sequence occurs in response to continual stimulation of the entire nerve trunk. This requires detailed neurophysiological investigation of the intramural mechanisms.

One difficulty with the present study is that the motor patterns have all been evoked using electrical stimulation of nerves and, although they demonstrate some features of the nervous control, they must be treated with caution until they can be demonstrated to be evoked by natural stimuli (e.g. gastric distension) in intact animals.

We thank the Director and staff of the Plymouth Laboratory of the Marine Biological Association for providing the facilities for this research. John Mavin gave valuable technical assistance. The study was supported by grants from The Royal Society Brown Fund and The Wellcome Trust.

REFERENCES

- Alvarez, W.C. 1927 Peristalsis in the dogfish and ray. *Am. J. Physiol.* **80**, 493–501.
- Andrews, P.L.R. & Grundy, D. 1981 The effect of stimulus characteristics on the rebound contraction in the gastric corpus. *J. Physiol.* **313**, 24–25P.
- Andrews, P.L.R. & Hawthorn, J. 1988 The neurophysiology of vomiting. *Bailliere's clin. Gastroenterol.* **2**, 141–168.
- Andrews, P.L.R. & Lawes, I.N.C. 1984 Interactions between splanchnic and vagus nerves in the control of mean intragastric pressure in the ferret. *J. Physiol.* **351**, 473–490.
- Andrews, P.L.R. & Lawes, I.N.C. 1985a Characteristics of the vagally driven non-adrenergic, non-cholinergic inhibitory innervation of ferret gastric corpus. *J. Physiol.* **363**, 1–20.
- Andrews, P.L.R. & Lawes, I.N.C. 1985b Gastric tone modifies responses to extrinsic neural stimuli in the anaesthetized ferret. *J. Physiol.* **366**, 1–16.

- Andrews, P.L.R. & Young, J.Z. 1988 The effects of peptides on the motility of the stomach, intestine and rectum in the skate. *Comp. Biochem. Physiol.* **89C**, 343–348.
- Andrews, P.L.R. & Young, J.Z. 1989 Videotape recording of elasmobranch stomach activity patterns *in vivo*. *J. Physiol.* **407**, 8P.
- Babkin, B.P., Friedman, M.H.F. & MacKay-Sawyer, M.E. 1935 Vagal and sympathetic innervation of the stomach in the skate. *J. biol. Board Can.* **1**, 239–250.
- Barrington, E.J.W. 1942 Gastric digestion in the lower vertebrates. *Biol. Rev.* **17**, 1–27.
- Bjønning, C., Jonsson, A.-C. & Holmgren, S. 1990 Bombesin-like immunoreactive material in the gut, and the effect of bombesin on the stomach circulatory system of an elasmobranch fish, *Squalus acanthias*. *Reg. Peptides* **28**, 57–69.
- Bottazi, F. 1902 Untersuchungen über das viscerale Nervensystem der Selachier. *Z. Biol.* **43**, 3–72.
- Bradley, P.B., Engel, G., Feniuk, W., Fozard, J., Humphrey, P.P.A., Middlemiss, D.N., Myelcharane, E.W., Richardson, B.P. & Saxena, P.R. 1986 Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* **25**, 563–576.
- Burnstock, G. 1958 The effect of drugs on spontaneous motility and on responses to stimulation of the extrinsic nerves of the gut of a teleostean fish. *Br. J. Pharmacol. Chemotherapy* **13**, 216–226.
- Campbell 1975 Inhibitory vagal innervation of the stomach in fish. *Comp. Biochem. Physiol.* **50C**, 169–170.
- Carey, F.C., Kanwisher, J.W. & Stevens, E.D. 1984 Bluefin tuna warm their viscera during digestion. *J. exp. Biol.* **109**, 1–20.
- Cimini, V., Van Noorden, S., Giordano-Lanza, G., Nardini, V., McGregor, G.P., Bloom, S.R. & Polak, J.M. 1985 Neuropeptides and 5-HT immunoreactivity in the gastric nerves of the dogfish (*Scyliorhinus stellaris*). *Peptides* **6**, 373–377.
- Clarke, M.R. & Stevens, J.D. 1974 Cephalopods, blue sharks and migration. *J. mar. biol. Ass. U.K.* **54**, 949–957.
- Costall, B. & Naylor, R.J. 1990 5-hydroxytryptamine: new receptors and novel drugs for gastrointestinal motor disorders. *Scand. J. Gastroenterol.* **25**, 769–787.
- Davis, C.J., Harding, R.K., Leslie, R.A. & Andrews, P.L.R. 1986 The organization of vomiting as a protective reflex. In *Nausea and vomiting: mechanisms and treatment* (ed. by C. J. Davis, G. V. Lake-Bakaar & D. G. Grahame-Smith), pp.65–75. Berlin: Springer-Verlag.
- Foster, J. 1977 Pulsed gastric lavage: an efficient method of removing the stomach contents of live fish. *Prog. Fish Culture* **39**, 166–169.
- Gerhart, D.J. 1991 Emesis, learned aversion and chemical defense in octocorals: a central role for prostaglandins. *Am. J. Physiol.* **260**, R839–843.
- Grundy, D., Blackshaw, A. & Andrews, P.L.R. 1991 Neural correlates of the gastrointestinal motor changes in emesis. In *Brain gut interactions* (ed. Y. Tache & D. Wingate), pp. 325–338. Boca Raton: CRC Press.
- Holmgren, S., Grove, D.J. & Nilsson, S. 1985 Substance P acts by releasing 5-hydroxytryptamine from enteric neurons in the stomach of the rainbow trout, *Salmo gairdneri*. *Neuroscience* **14**, 683–693.
- Holmgren, S. & Nilsson, S. 1983 Bombesin-, gastrin/CCK-, 5-hydroxytryptamine-, neurotensin-, somatostatin- and VIP-like immunoreactivity and catecholamine fluorescence in the gut of the elasmobranch, *Squalus acanthias*. *Cell Tiss. Res.* **234**, 595–618.
- Holmgren, S. & Nilsson, S. 1984 The innervation of the stomach wall in the dogfish *Squalus acanthias*. *Acta physiol. scand.* **120**, 1–8A.
- Jansson, G. & Martinson, J. 1966 Studies on the ganglionic site of action of sympathetic outflow to the stomach. *Acta physiol. scand.* **68**, 184–192.
- Jensen, J. & Holmgren, S. 1991 Tachykinins and intestinal motility in different fish groups. *Gen. comp. Endocrinol.* **83**, 388–396.
- Jernejcik, F. 1969 Use of emetics to collect stomach contents of walleye and large mouth bass. *Trans. Am. Fish Soc.* **98**, 698–702.
- Lang, I.M. 1992 New perspectives on the mechanisms controlling vomitus expulsion. In *Mechanisms and control of emesis* (ed. A. L. Bianchi, L. Grelot, A. D. Miller & G. L. King), pp. 71–81. Colloque Inserm. John Libbey, Eurotext, 2–23.
- Lang, I.M. & Sarna, S.K. 1989 Neural control of initiation and propagation of the retrograde giant contraction associated with vomiting. *Falck Symp.* **50**, 726–731.
- Lang, I.M., Sarna, S.K. & Condon, R.E. 1986 Gastrointestinal motor correlates of vomiting in the dog: quantification and characterization as an independent phenomenon. *Gastroenterology* **90**, 40–47.
- Müller, E. & Liljestrand, G. 1918 Anatomische u. experimentelle Untersuchungen über das autonome Nervensystem der Elasmobranchen nebst Bemerkungen über die Darmnerven bei den Amphibien und Säugetieren. *Arch. Anat. Physiol.* **1918**, 137–172.
- Nicholls, J.V.V. 1933 The effect of temperature variations and of certain drugs upon the gastric motility of elasmobranch fishes. *Contr. Can. Biol. Fish.* **7**, 449–463.
- Nilsson, S. & Holmgren, S. 1983 Splanchnic nervous control of the stomach of the spiny dogfish, *Squalus acanthias*. *Comp. Biochem. Physiol.* **76C**, 271–276.
- Nilsson, S. & Holmgren, S. 1988 Novel neurotransmitters in the autonomic nervous systems of nonmammalian vertebrates. *Pharmacol. Therapy* **41**, 257–287.
- Puerta, G.C. 1959 the effects of tranquilizing drugs on tropical fish. *Archs Int. Pharmacodyn.* **CXXI**, 404–414.
- Sanger G.J. & Wardle, K. 1990 5-hydroxytryptamine and cholinergic function in the gastrointestinal tract. In *Serotonin: from cell biology to pharmacology and therapeutics* (ed. R. Paoletti, P. M. Vanhoutte, N. Brunello & F. M. Maggi), pp. 251–255. Kluwer Academic Publishers.
- Satchell, G.H. & Maddalena, D.J. 1972 The cough or expulsion reflex in the Port Jackson shark, *Heterodontus Portus Jacksoni*. *Comp. Biochem. Physiol.* **41A**, 49–62.
- Thorndyke, M. & Holmgren, S. 1990 Bombesin potentiates the effect of acetylcholine on isolated strips of fish stomach. *Reg. Peptides* **30**, 125–135.
- Tiersch, T.R. & Griffith, J.S. 1988 Apomorphine induced vomiting in rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **91A**, 721–725.
- Van Slyke, D.D. & White, G.F. 1911 Digestion of protein in the stomach and intestine of the dogfish. *J. biol. Chem.* **9**, 209–217.
- Webster, D.A. 1942 Food progression in young white perch *Morone americana* (Gmelin) from Bantam Lake Connecticut. *Trans. Am. Fish Soc.* **72**, 136–144.
- Young, J.Z. 1933 The autonomic nervous system of selachians. *Q. J. microsc. Sci.* **75**, 571–624.
- Young, J.Z. 1980a Nervous control of stomach movements in dogfishes and rays. *J. mar. biol. Ass. U.K.* **60**, 1–17.
- Young, J.Z. 1980b Nervous control of gut movements in *Lophius*. *J. mar. biol. Ass. U.K.* **60**, 19–30.
- Young, J.Z. 1983 Control of movements of the stomach and spiral intestine of *Raja* and *Scyliorhinus*. *J. mar. biol. Ass. U.K.* **63**, 557–574.

Received 20 July 1992; revised 2 April 1993; accepted 15 April 1993

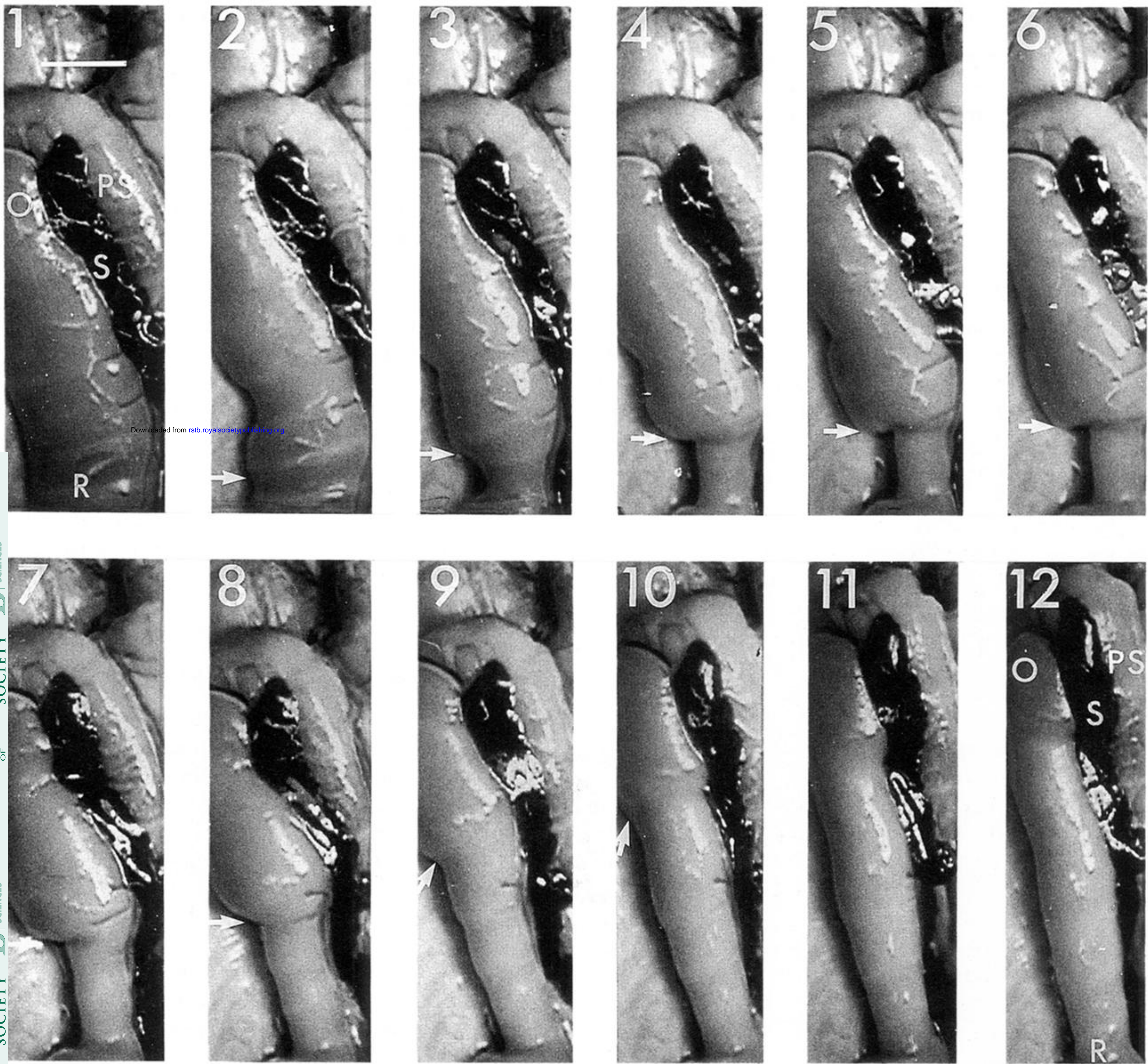


Figure 2. Consecutive series of photographs taken every 10 s showing the retrograde propagation (from the rectal region towards the stomach) of a spontaneous contraction along the entire length of the spiral intestine. O, oral end; R, rectal end of spiral intestine. The arrow indicates the location of the wave-front where it is clearly visible. PS, pyloric stomach; S, spleen. Scale bar in panel 1 = 10 mm.

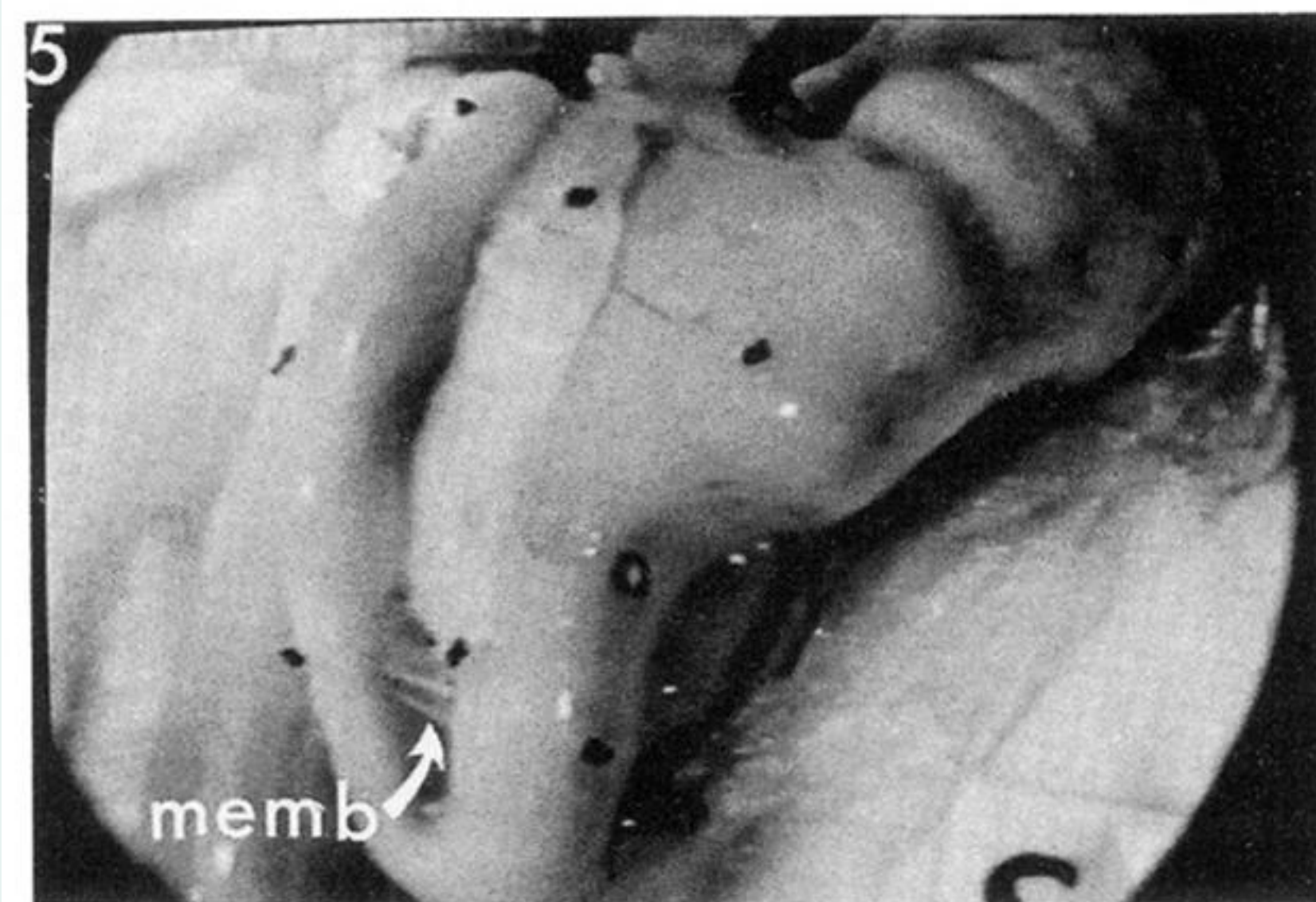
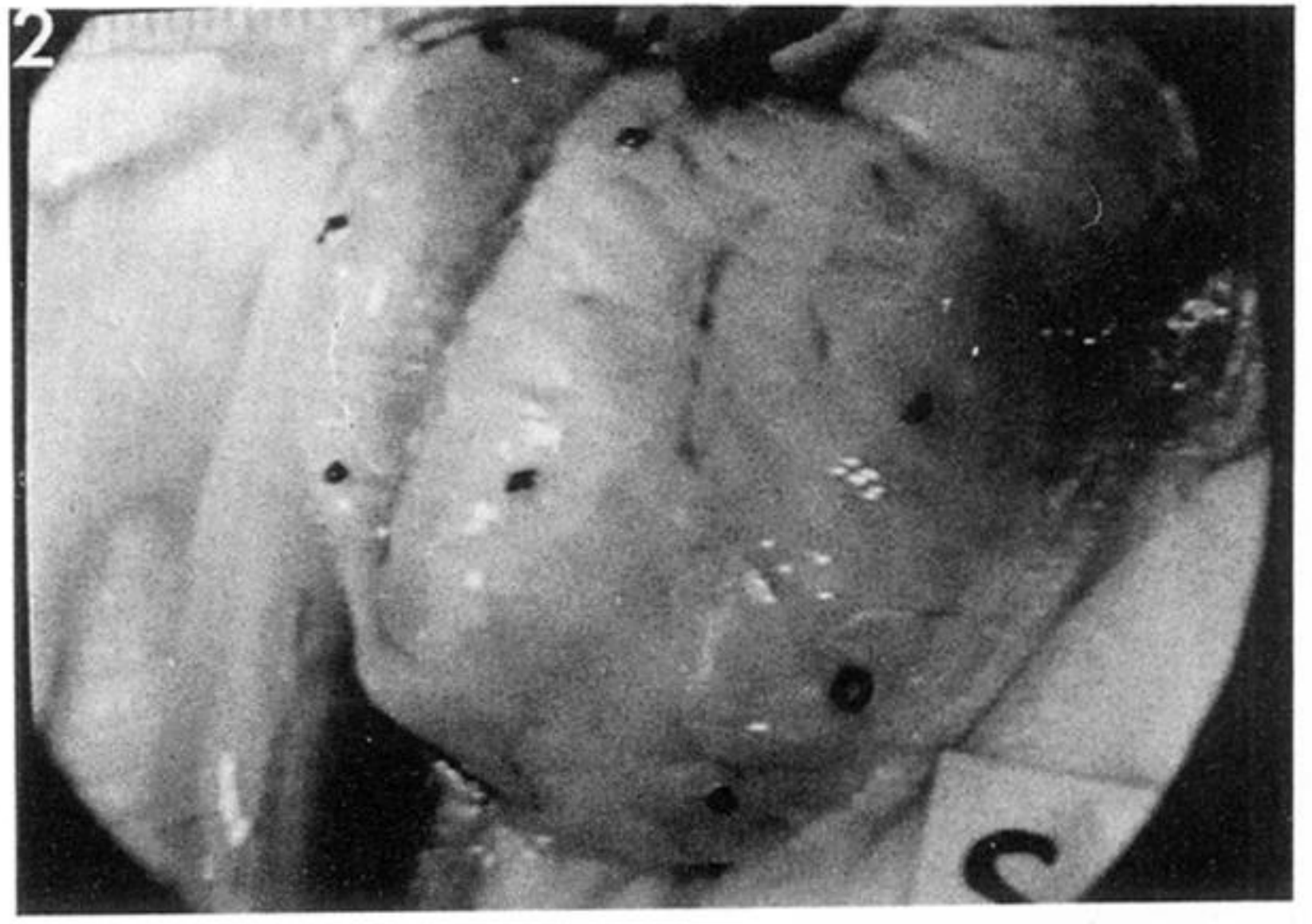
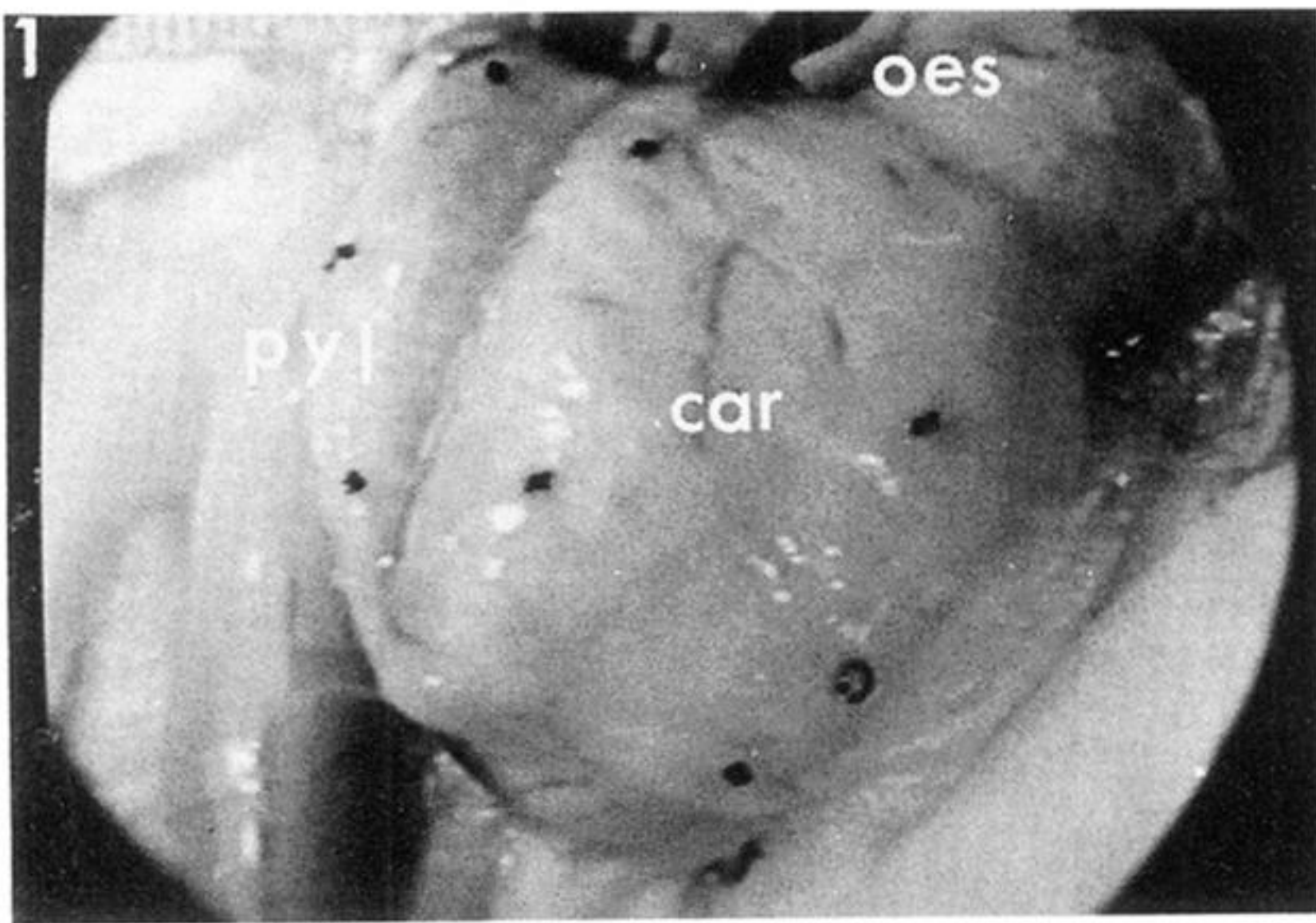


Figure 5. Consecutive photographs of the stomach taken every 10 s during sympathetic stimulation at 16 Hz indicated by S in the lower right of each panel. Note the origin of the contraction in pyloric stomach and the progression of the contraction into the cardiac region propelling gastric contents into the proximal cardiac stomach. The membrane (memb) linking the cardiac and pyloric gastric regions can be seen in panel 5.

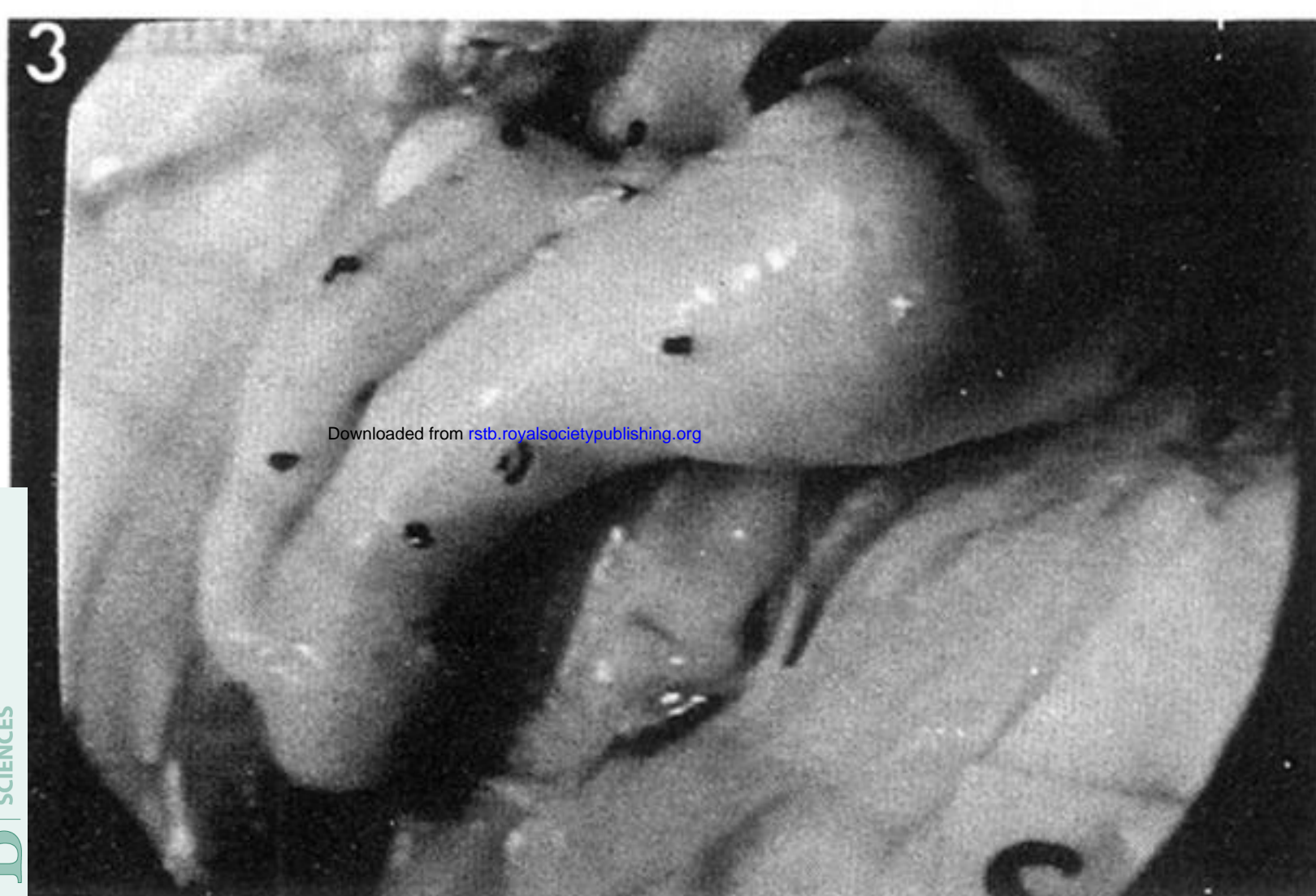
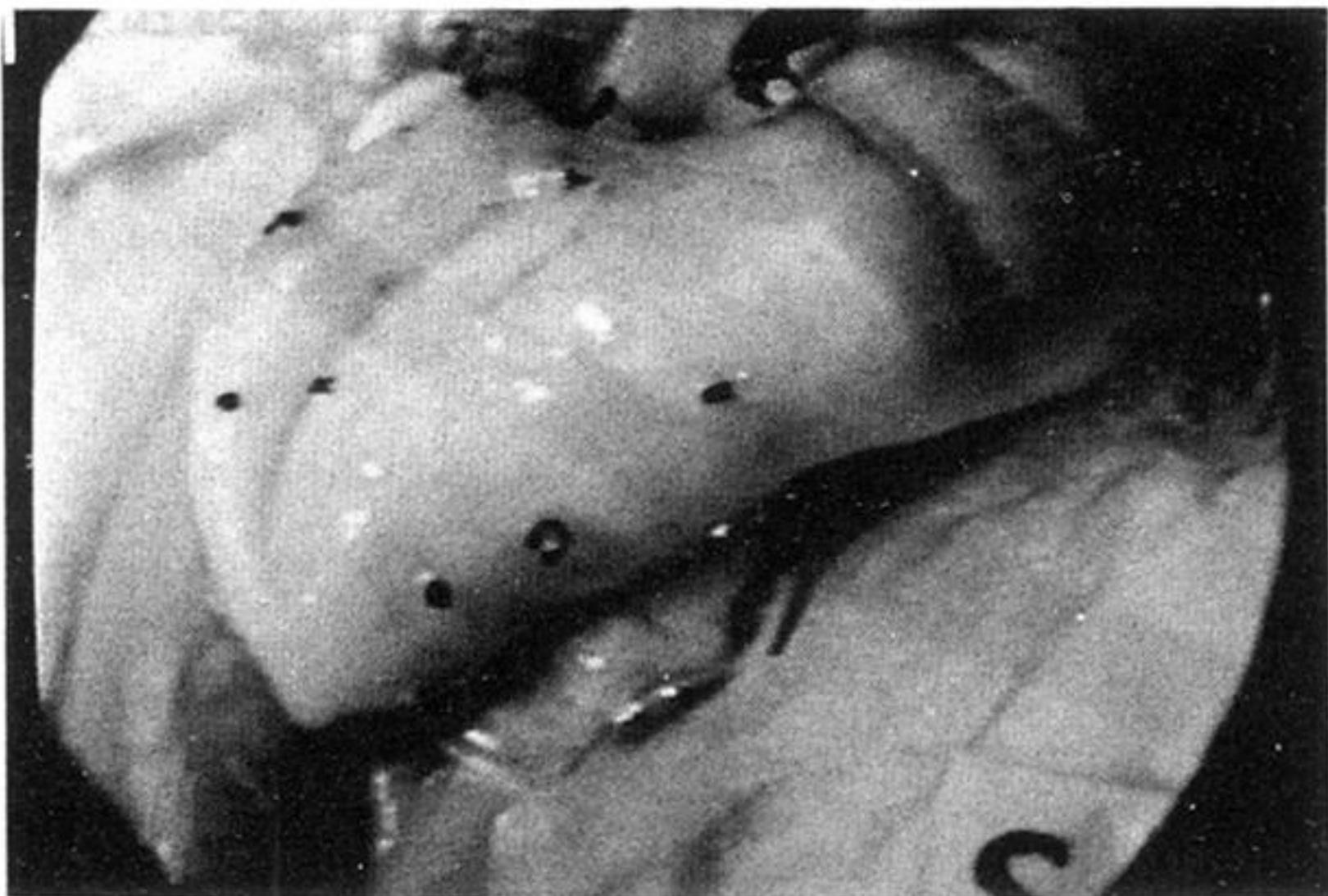


Figure 9. Consecutive photographs taken every 10 s, 3 min into a 7 min period of sympathetic stimulation at 16 Hz. Note the contraction moving contents from the cardiac to pyloric region and the morphological changes associated with this in the junction (jct) between the cardiac and pyloric region. The complete activity cycle is seen in panels 1–6 and repeated in panels 7–12. Measurements from these pictures were used in the construction of figure 8.

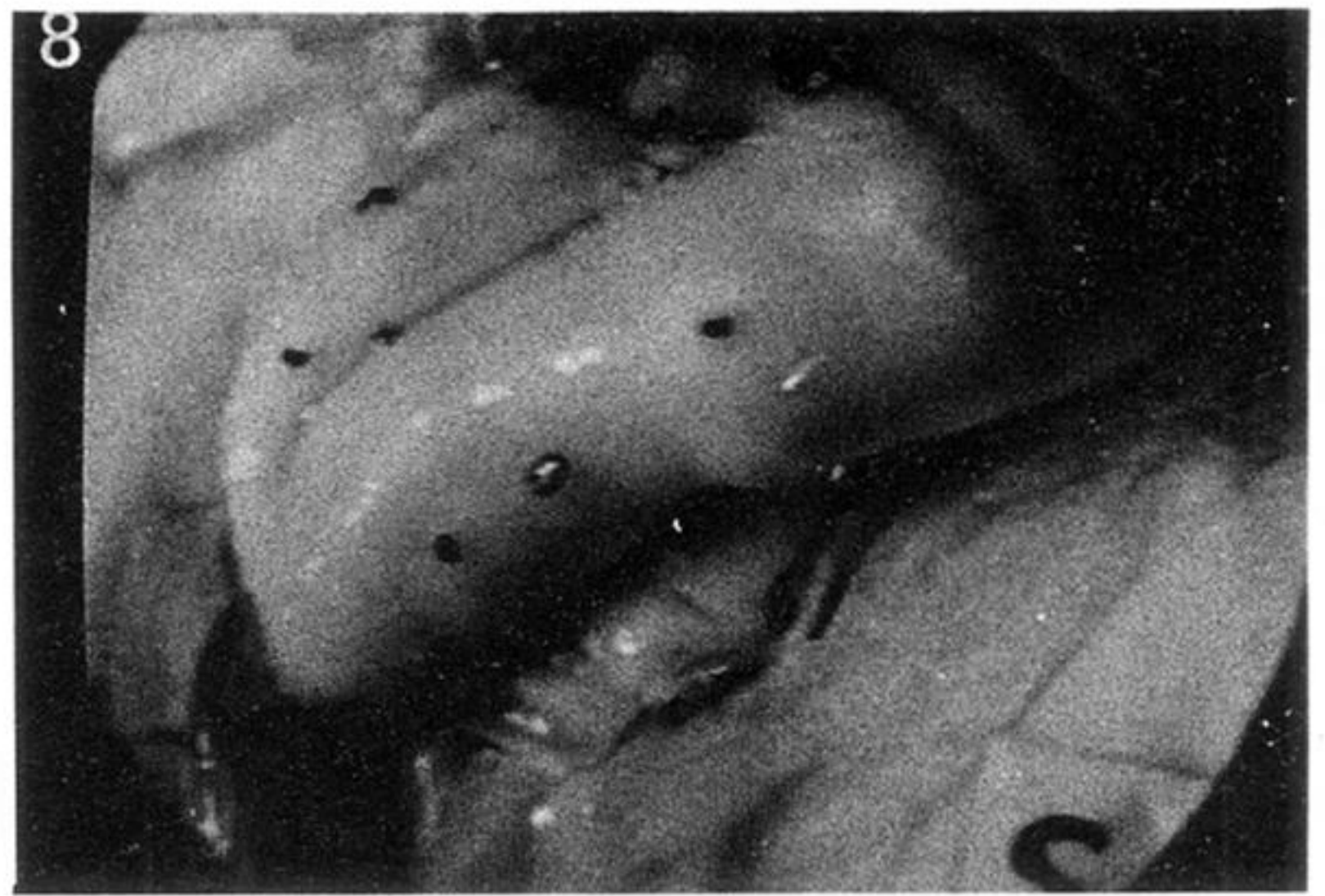
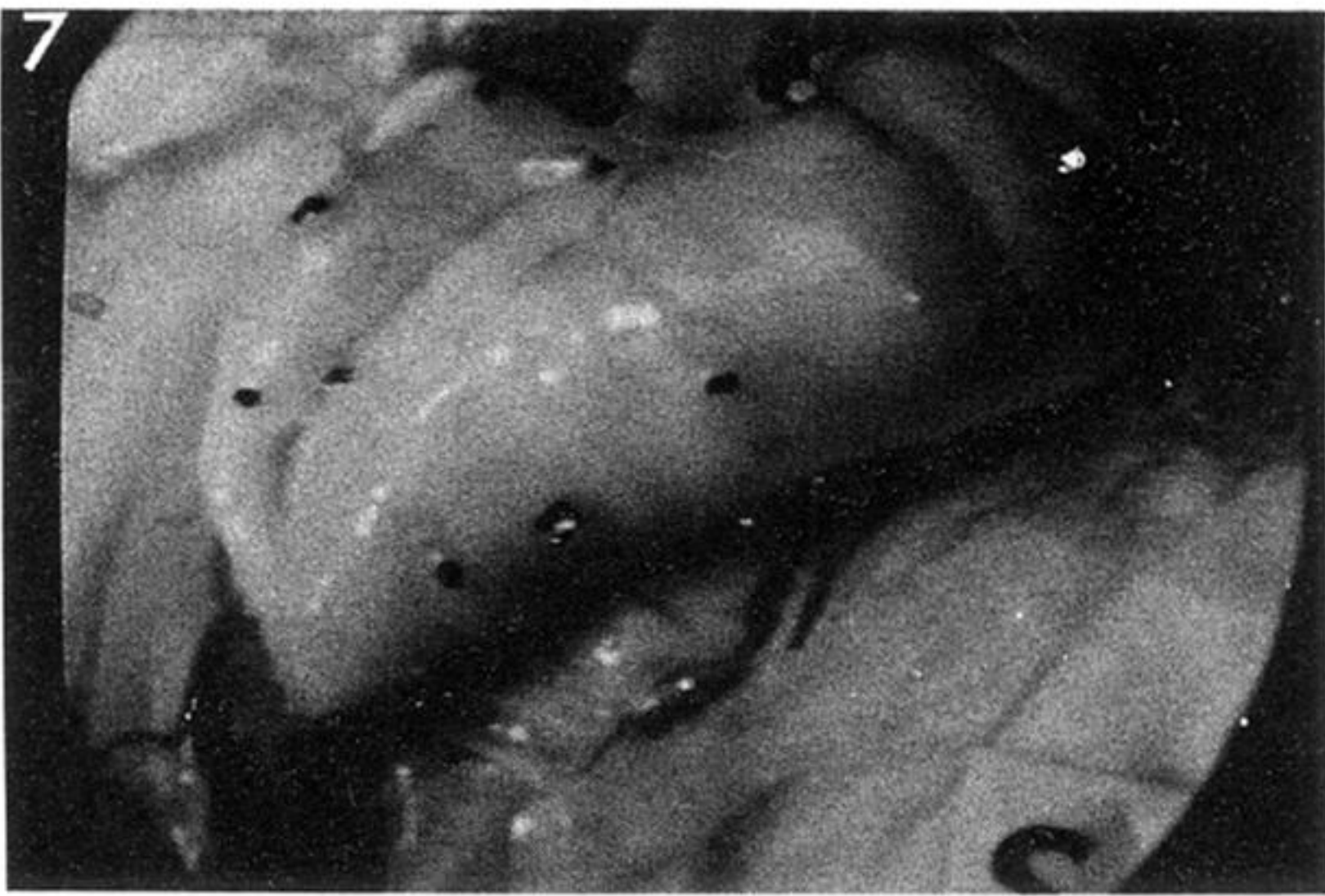


Figure 9. *Continued*