STIMULATION OF OESOPHAGEAL MOTILITY IN THE CAT

A thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Science in Physiology at Massey University of Manawatu in the Department of Veterinary Biology.

JOHN SPENCER WHEELER

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TO MY PARENTS

A C K N O W L E D G E M E N T S

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S U M M A R Y

Literature concerned with reports of stimuli which cause or modify oesophageal activity have been reviewed and has been associated with the evidence obtained in the experiments undertaken.

The preparations of cats which have been used in these experiments were:-

Anaesthetised cats. Chloralose 70 mgm/kgm injected as a solution in saline (70°C), or an organic solvent (dimethyl sulphoxide).

Decerebrated cats.

Decerebrated cats with the spinal cord sectioned at the sixth cervical vertebra.

Decerebrated cats with the spinal cord sectioned at the third cervical vertebra.

Cats with the central nervous system totally destroyed by pithing.

Oesophageal motility has been recorded by isometric and isotonic systems. In all cases oesophageal contractions were detected by balloons 3-5 cc volume.

Contractions of the terminal oesophagus in response to distension of this region have been obtained in the absence of an extrinsic

innervation. These results, and those obtained in decerebrate preparations, indicated the existence of both local and inter-oesophageal reactions. These intrinsic reactions were found to be reflexly modified by stimuli arising in other parts of the preparations, particularly the alimentary tract. The reflexes appeared to be mediated certainly through vagal and possibly through sympathetic afferents to centres in the medulla oblongata or pons. Vagal efferent pathways have been shown to affect the oesophagus, and indications of a sympathetic efferent innervation of the oesophagus have been obtained.

Stimuli which facilitated the local responses of the terminal oesophagus to distension were:-

Greater degrees of distension of this region of the oesophagus. (Very high levels of distension tended to be inhibitory.)

Acidification of the mucosa of the terminal regions of the oesophagus.

Acidification of the isolated stomach to a pH of about 2.0.

Stimuli which inhibited the response of the terminal oesophagus to distension were:-

Distension of a more cranial region of the oesophagus.

High degrees of distension of the oesophagus at the point from which the recording was taken.

Distension of the stomach.

Noxious stimuli arising from the operative procedures.

These facilitatory and inhibitory stimuli were found to summate. The oesophageal response observed was a reflection of the nett afferent discharge.

Reactions of the oesophagus to cholinergic and adrenergic drugs and blocking agents such as atropine, hexamethonium and nicotine have been studied.

The results are discussed in relation to the function of the oesophagus in the intact animal and in relation to how co-ordinated responses are obtained in swallowing and other circumstances.

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Chapter 1

THE OESOPHAGUS - HIPPOCRATES TO 1965

It is reported that Hippocrates, after administering a coloured beverage to pigs and simultaneously slitting their throats, concluded: "When liquid is swallowed, a portion goes to the lung and from there is filtered into the pericardial sac, where it lubricates and cools the feverish heart. The rest of the liquid is exhaled as vapour." According to Eykman (1747), this notion was refuted by the publication of Fridericus Bernardus Albinus's "De Deglutione", which established that, normally, swallowed liquids never entered the respiratory tract (cited by Sanchez, Kramer and Ingelfinger, 1953).

The accessibility, for motility studies, of the oesophagus was realised in the 1880's by Kronecker and Meltzer. They published their findings in 1883 and at that time recognised that a contraction wave followed a buccopharyngeal movement of swallowing, and also that distension of the oesophagus by balloons led to a contraction wave. (A reproduction of one of their records is contained in a paper published by Sanchez et al., 1953.)

Cannon made his first contribution to the study of oesophageal motility in 1896 by the use of the fluoroscopic technique. Three years after this, Meltzer postulated the existence of two forms of oesophageal motility:-

> Primary peristalsis - the contraction wave initiated by a buccopharyngeal movement of swallowing.

Secondary peristalsis - the contractions following distension of the oesophagus.

Meltzer sought to separate these two phenomena on the grounds of their control. He proposed that primary peristalsis was controlled entirely by a central nervous system mechanism. He did not think discharge from receptors in the oesophagus was essential for its progression (see Ingelfinger, 1958). This concept has been re-stated by Davenport (1962). In contrast, Meltzer considered that the secondary peristaltic wave was dependent upon the successive activation of receptors as the bolus moved caudally down the oesophagus. Evidence to justify the above postulates was:-

Oesophageal transection did not stop the progression of primary peristalsis;

secondary peristalsis was less sensitive to central nervous system depressants;

and section of one vagus nerve in the neck had little effect on primary peristalsis, although it either abolished secondary peristalsis or increased the threshold for its stimulation.

These proposals of Meltzer's stimulated others to study oesophageal motility, and work done at this time generally supported his postulates.

In a study he made of the effects of section of the vagus nerves, Cannon (1907) reported that, after bilateral section of the vagi, the striated muscle of the upper oesophagus of cats was paralysed while

the lower (unstriated muscle) showed a peristaltic contraction to a local distending stimulus. Cannon also studied the effects of partial sympathectomy at different levels of the oesophagus and various combinations of denervation procedures. These studies of the extrinsic nerves of the oesophagus were continued by a group of workers under A.J. Carlson in the early 1920's (Carlson, Boyd and Pearcy, 1922). These, and later experiments aimed at the definition of the extrinsic innervation of the oesophagus, were concentrated on the reactions of the terminal oesophagus. Carlson concluded that the splanchnic nerves and vagus nerves contained both excitatory and inhibitory fibres. Since this report, nerve stimulation studies, pharmacological studies, and *in vitro* investigations have been reported which support, in various degrees, this original observation.

Hanzlik and Butt (1928) separated the circular and longitudinal components of oesophageal motility in the pigeon crop, but attempts to follow up this work in other species have not been reported in the literature.

Oesophageal motor responses to distension of the stomach by water were studied by Payne and Poulton (1928). These workers used a technique and approach which had not been used up to this time. This approach was the deliberate distension of the oesophagus to provide an excitatory background on which other stimuli (such as distension) may be superimposed and assessed. Payne and Poulton's results were supported by Goldberg (1931), who found that reflux of stomach contents into the oesophagus occurred at a lower threshold if the pyloric antrum was distended. The motility of the oesophagus was not recorded in this

study. Pennington (1946) noted that distension of the jejunum lowered the tonicity of the cardia in unanaesthetised dogs. The more recent work of Schenk and Fredrickson (1959) refers to a reflex inhibition of the resting tone of the terminal oesophagus with distension of the pylorus.

Knight (1934a) reported a series of investigations on the motor innervation of the cat's oesophagus; earlier (1933) he had postulated that the anti-reflux mechanism at the caudal end of the oesophagus was an intrinsic smooth muscle sphincter rather than a mechanism involving the cruciate fibres of the diaphragm or the presence of a mucosal cuff. Knight's conclusions were supported by the results of an investigation of this anti-reflux mechanism by Zeller and Burget (1937).

These investigations on the anti-reflux mechanism were taken up again in the mid 1950's by various groups, whose conclusions supported one, but usually several, of five postulated mechanisms:-

An intrinsic smooth muscle sphincter: Fyke, Code and Schlegel (1956); Fleshler, Hendrix, Kramer and Ingelfinger (1958).

A mucosal cuff or rosette: Dornhurst (1955-56); Nauta (1956); Wolf (1960); Hayward (1961); Milstein, Edwards and Berridge (1961).

An obliquity of the gastro-oesophageal junction: Hoag (1954); Marchant (1955); Cohn, Close and Weston (1956).

An involvement of the cruciate fibres of the diaphragm: Peters (1955); Nauta (1956); Schenk and Fredrickson (1959).

An involvement of the abdominal segment of the oesophagus: Creamer, Harrison and Pearce (1959); Wolf (1960); Nagler and Spiro (1961); MacLaurin (1963).

In general, studies of the cervical oesophagus had not received the attention that was given to the terminal regions of the oesophagus. Hwang, Grossman and Ivy (1947) postulated that the cervical oesophagus was not innervated by the recurrent laryngeal nerves. It was proposed that a small branch of the superior laryngeal nerve, called the pharyngo-oesophageal nerve, carried the sensory and motor fibres responsible for motility of the cervical oesophagus. Chauveau (1862) is reported (by Hwang, Grossman and Ivy, 1948) to have considered the contribution of this nerve in the horse, cow, lamb, cat, dog and rabbit. Hwang, in collaboration with various other workers, continued to work on the innervation of the oesophagus until he published a paper in 1954 entitled "The Mechanism of Transportation of Contents in the Oesophagus." In this paper he expresses these findings as a total integrative statement of motility of the oesophagus.

During this period, 1948-49, two workers who were to become pre-eminent in oesophageal motility studies began their contributions. Ingelfinger, in collaboration with Kramer and Sanchez (1953), studied oesophageal motility, at all levels, in response to swallowing or oesophageal distension in healthy and diseased human patients. He proved, in these early stages, powerful in influencing thoughts about oesophageal motility. For example, when intraluminal recording of oesophageal motility was introduced in 1953 by Butin, Olsen, Moersch

and Code, he was able to offer, and have accepted, some very pertinent criticisms of this method (Ingelfinger, Kramer and Sanchez, 1954). However, he adopted this method of recording himself in later years and used it to extend his studies in human patients. His critical review articles in 1956, 1958, 1961 and 1963 indicate his knowledge and contribution to the study of oesophageal motility.

The introduction of intraluminal recording apparatus in Code's laboratory at the Mayo Clinic at the time appeared to provide the solution to the gastroenterologist's problem of preventing stimulation by a bulky recording device. Code and his associates (in particular, Schlegel) adopted a relatively simple and repeatable method of serial motility studies, and its application by various groups of workers has provided large volumes of data on motility patterns in man. The early oesophageal studies were repeated, using these small pressure recording units, and this emphasised the differences between primary and secondary peristalsis. It was presumed that the small recording units allowed the detection of motility following a buccopharyngeal movement of swallowing or a distension of the oesophagus without modification of the response by its presence. Specific studies on this problem (Hwang, 1954; Fleshler, Hendrix, Kramer and Ingelfinger, 1959; Siegel and Hendrix, 1961) led the workers concerned to conclude that the oesophageal motor response was the same, regardless of the initiating stimulus. In 1956 Andrew carried out the first and, until very recently, the only electrical investigation of motor and sensory activity of the oesophageal innervation and musculature. His study was concerned with the cervical oesophagus of rats.

The majority of the work on oesophageal motility since 1953 has been related to clinical investigations. Motility patterns have been studied in normal patients and patients with disorders such as cardiospasm, hiatal herniation, scleroderma, oesophagitis, heartburn and other less frequently reported disorders. Records have been obtained in conscious human subjects with the same type of pressure recording units and all have yielded essentially the same record. In 1958 Code and Schlegel published data which suggested that the records of the lower oesophageal sphincter activity obtained by this system were suspect, and modified their recording system. Other investigators altered their systems and the motility studies continued. Some workers, however, have not altered their methods, and the type of pressure sensing device must be carefully considered in each instance. The procedure used by most workers depends upon the respiratory reversal point to position the recording units relative to the oesophageal hiatus of the diaphragm. The value of this method as a reliable indication of position has been questioned in the report of Geisel, Aria, Jefferson and Necheles (1965). They suggest that this point is variable and may occur in the fundus of the stomach or the thoracic regions of the oesophagus. This report, if substantiated, may result in doubt being cast on the value of records obtained and the methods employed in the analysis and interpretation in many studies of oesophageal motility.

In the light of observations on the effects of section of the vagus nerves on oesophageal motility, presented by Dagradi, Stempien, Seifer and Weinberg (1962, 1963), and the original statement of

automaticity by Cannon (1907), experimental procedures using dogs in which the vagus nerves were cut were designed at the Mayo Clinic Physiology Unit. These experiments were designed to study the dependence or independence of the motility pattern (measured by intraluminal pressure changes) on the extrinsic nerve supply. Carveth, Schlegel, Code and Ellis (1962), and Greenwood, Schlegel, Code and Ellis (1962) reported that the relaxation, in response to a swallow, of the area of high tonus at the terminal end of the oesophagus continued after partial sympathectomy, vagotomy, phrenicotomy and oesophageal interruption. This study, plus other work in the same laboratory (Helm, Schlegel, Code and Summerskill (1965), on the transmucosal junction potentials at the gastro-oesophageal junction; and the very recent work of Arimori, Schlegel and Code (1965) on the electromyography of the oesophagus), places this group at the Mayo Clinic as the most active in this branch of gastrointestinal physiology.

In recent years the attention of various groups has been concentrated on specific clinical problems involving the oesophagus. The studies have been undertaken in hospitalised patients suffering from the specific complaints mentioned above and compared with results obtained from control subjects. In 1961 two groups published the results of studies on heartburn (Nagler and Spiro, 1961; Tuttle, Rufin and Bettarello, 1961). This work was continued by Siegel and Hendrix (1963). Siegel (1964), reviewing the work done on heartburn, indicated the way in which these specific problems had been investigated and introduced into the literature the idea that the oesophagus exhibits different levels of excitability. The oesophageal response to acid

infusion was thought to be dependent upon the background level of excitability.

A further specific study which appears to have just been started is a study of belching. McNally, Kelley and Ingelfinger (1965) published the results of a study of this problem, and it might be foreseen that this investigation may be developed in a manner similar to that of the investigations on heartburn which followed the original observation by Lazer, Pulletti, Douglass, Danovitch and Texter (1959), that abnormal motility patterns followed acid infusion of the oesophagus.

An important aspect of oesophageal motility which has not been studied in detail is the sensory innervation of the oesophagus. This is probably due to the idea that the motility response is largely central in origin. However, both direct and indirect evidence does exist for a sensory innervation.

The use of oesophageal motility studies in clinical investigation is established, and its continued application will provide data on motility patterns in various clinical conditions. Winship, Poindexter, Thayer and Spiro (1965) have reported that the oesophagus of the monkey is similar in its motility patterns to man. This may allow a bolder approach than used at present. Further progress in the understanding of oesophageal motility will be gained from stimulating it in a variable manner by a stimuli other than swallowing and by recording its motility by a method more direct in its measurement than intra-luminal pressure recording, for example, by recording the electrical

activity of the musculature or separation of its walls.

The experimental investigations reported in this thesis have been concerned with the following:-

The nature of the oesophageal response to distension of itself.

Interrelationships between different levels of the oesophagus and how this might lead to an integrated response of the oesophagus as a whole to a stimulus.

The effects stimulation or changes in conditions in different parts of the alimentary tract have on oesophageal motility.

The sensory and motor innervation of the oesophagus.

The action of selected pharmacological agents on oesophageal motility.

The dependence of oesophageal motility on an extrinsic innervation.

This has provided a start towards an integrated approach to a subject which has been treated in a particulate fashion by most workers because of preoccupation with the solution of various clinical problems. Ingelfinger (1963) made the comment that: "... but acute experiments performed under anaesthesia and applying electrical and classical pharmacologic stimuli to severely manipulated gastro-oesophageal segments have probably been exploited to the maximum and have little more to offer." It is hoped that the experimental observations

presented in this thesis, coupled with clinical investigations, chronic experimental investigations, histological studies, or taken alone, may make some contribution to the understanding of oesophageal motility.

Chapter 2

STIMULI WHICH CAUSE OR ALTER OESOPHAGEAL MOTILITY IN THE CAT

INTRODUCTION

Evidence for a sensory innervation of the oesophagus has been derived from four sources:-

Subjective sensations arising from the oesophagus.

Reflex effects arising from stimulation of the oesophagus.

Recording from afferent nerve fibres from the oesophagus.

Histological evidence of sensory nerve endings in the oesophagus.

Subjectively oesophageal sensation may be recognised. Pain and gross distension can be noted and referred to the oesophagus. Recent work on heartburn would indicate that the pain associated with this condition is due to abnormal motility caused by the acid stimulation (Siegel, 1964).

Reflex effects due to oesophageal stimulation have been reported. Kay (1958) has shown that tactile stimulation of the thoracic oesophagus of the sheep will cause reflex salivation. Phillipson and Reid (1958) have demonstrated an oesophageal distension-salivatory reflex in the calf; Sellers and Titchen (1959) have also shown this in the sheep, and that distension may cause an inhibition of rumen-

reticulum movements. This effect was elicited at a lower threshold at two regions - the anterior thoracic and terminal oesophagus. The sensory nerve fibres of these reflexes appear to be carried in the vagus nerves. Oesophageal responses to distension of the oesophagus have been reported by various workers (Andrew, 1956b; Creamer and Schlegel, 1957). Hazarika, Coote and Downman (1964) report that the abdominal segment of the cat's oesophagus has a sensory nerve supply carried via the dorsal roots T_3-T_{10}. They employed a technique introduced by McDowall (1925). An afferent discharge (in this case stimulated electrically or by pinching) causes an inhibition of the oculo-motor constrictor tone. This causes dilatation of the pupil of a cat under chloralose anaesthesia.

Neurophysiological recording from the oesophagus and afferent nerve fibres arising from the oesophagus has provided definitive evidence for the presence of receptors in the oesophagus. Andrew (1956b, c) has recorded discharges from receptors sensitive to distension and tactile stimulation. It also appeared that these receptors were activated by a contraction of the oesophagus. Iggo (1957a) distinguished an oesophageal receptor activated by contraction. These reports are confirmed by Mei (1965), who has suggested that these receptors which are activated by distension or a contraction are concentrated in two regions - the anterior thoracic and the terminal oesophagus (see Sellers and Titchen, 1959).

Histological evidence for receptors is scanty. DeWitt (1900 - cited by Dougherty, Habel and Bond, 1958) described fine sensory nerve

endings of the subepithelial cells. Also, dense "end balls" of interdigitating aborisations were noted. These sensory nerve endings were located at the pharyngo-oesophageal junction. Comline and Hessage (1964) have identified nerve fibres in the oesophagus of the sheep which have low cholinesterase levels. They suggest that these are sensory fibres.

The evidence cited would indicate that there does exist a sensory innervation of the oesophagus. The fibres of this innervation are, in all probability, carried via the vagus nerves and sympathetic nerve trunks. The receptors present appear to be sensitive to both fixed and moving distension stimuli and also the passage of a contraction. Tactile and acid receptors are also indicated by the observations cited above. The evidence is not unequivocal, and a characterisation of receptors, such as Iggo and Paintal have carried out for gastro-intestinal receptors (see Paintal, 1963), is required.

The reactions of the oesophagus to distension of itself have long since been recognised. Two forms of distension have been employed, namely, with a fixed balloon distension (Carlson et al., 1922; Zeller and Burget, 1937; Kramer and Ingelfinger, 1949; Baylis, Kauntze and Trounce, 1955; Creamer and Schlegel, 1957), and with a fluid or moving balloon distension (Hwang, Essex and Mann, 1947; Hwang, 1954; Creamer and Schlegel, 1957). Definitive observations on the nature of these responses is lacking. The frequency and threshold of these responses depended on the region of the oesophagus distended, and an inverse relationship between amplitude and frequency appeared to exist

(Ingelfinger, 1958).

Oesophageal distension has been reported to have an inhibitory effect on oesophageal activity caudal to the point of distension and an excitatory effect on oesophageal activity cranial to the applied distension (Hwang, 1954; Creamer and Schlegel, 1957). These results were not supported by the experimental observations made by Dornhurst, Harrison and Pierce (1954).

A fall in tone of the terminal regions of the oesophagus in response to the buccopharyngeal movement of swallowing, a distension of the oesophagus, or contractions of the oesophagus cranial to this region, has attracted the attention of many workers (Zeller and Burget, 1937; Butin, Olsen, Moersch and Code, 1953; Fyke, Code and Schlegel, 1956; Code, Creamer and Schlegel, 1958; Kelley, Wilbur, Schlegel and Code, 1960). The records obtained have shown a fall in tone 1.5 - 2.5 seconds after the buccopharyngeal movement of swallowing which continues for 5-12 seconds. The fall in tone was followed by a contraction. If a second swallow was stimulated when the tone had been inhibited, the contraction was further delayed. Whether this inhibitory phase of swallowing is present throughout the oesophagus and whether it is caused by the buccopharyngeal movement of swallowing (Kronecker and Meltzer, 1883; Sanches et al., 1953; Dornhurst et al., 1954; Ingelfinger, 1958), or contraction or distension of the cervical oesophagus (Zeller and Burget, 1937; Hwang, 1954; Creamer and Schlegel, 1957), has not been determined.

Kronecker and Meltzer (1883) recognised that a contraction wave of

the oesophagus followed the buccopharyngeal movement of swallowing. The form of this wave has been investigated by numerous workers with many different forms of recording methods (Burget and Zeller, 1936; Kramer and Ingelfinger, 1949; Butin et al., 1953; Code et al., 1958). Originally this contraction wave was differentiated from the contraction wave initiated by distension of the oesophagus in both its motility characteristics and the nervous elements involved. However, the reports of Hwang (1954); Fleshler et al. (1959); and Siegel and Hendrix (1961) indicate that the "primary" and "secondary" contraction waves of the oesophagus are similar in form and mechanism. These reports simply suggest the manner of their initiation is different.

The effect of gross distension of the lower oesophagus appears to have an inhibitory effect on the tonus of this area (Nagler and Spiro, 1963; Vinnik and Kern, 1964). These workers noted, with prolonged gastric intubation, that material was refluxed into the oesophagus. They suggested the resting tone was lost, and that this was due to the gross distension of the terminal oesophagus. This reflux could also have been interpreted as arising from the straightening of the obliquity of the gastro-oesophageal junction.

Siegel (1964) suggested that acid applied to the lower oesophagus would cause abnormal motility of the distal oesophagus. This editorial comment, related to a consideration of heartburn, was based on the observation of Lazer et al. (1959), that abnormal motility patterns followed acid infusion of the oesophagus. Siegel and Hendrix (1960, 1963) published their findings following both positive and negative evidence from other workers (Tuttle, Bettarello and Grossman, 1961;

Tuttle, Rufin and Bettarello, 1961). They reported that abnormal motility patterns were observed following acid infusion into the terminal oesophagus. In some cases acid infusion into the terminal oesophagus was associated with the substernal pain characteristic of heartburn. It was suggested by Siegel that failure to obtain these effects may have been due to their subjects being in a supine position which did not allow concomitant distension of the oesophagus with the acid infusion.

Cineradiographic studies reported by Dougherty and Habel (1955), working with conscious sheep, indicated that the terminal oesophagus may be sensitive to tactile or chemical stimuli. They noted the reactivity and motility of this area increased markedly when material to be ruminated entered the terminal oesophagus.

The responses of the oesophagus to changes in conditions in the stomach have received little systematic consideration. Most of the observations made have been incidental to a study of reflux into the oesophagus of stomach contents. However, Payne and Poulton (1928) did show that distension of the stomach caused a wave of contraction to pass over the entire length of the oesophagus. No mention of the direction of this wave was made. The oesophagus was, in their experiments, subjected to distension by the large recording balloons employed. They also observed that a similar distension of the stomach caused an inhibition of contractions which had been stimulated by distension of the oesophagus. Goldberg (1931) found that when distension of a pyloric pouch caused vomiting there was a concomitant

reduction in the tonus of the terminal oesophagus. Results obtained by Schenk and Fredrickson (1959) supported this observation. An inhibition of the terminal oesophageal tone, as measured by the resistance to reflux of stomach contents to the oesophagus, was obtained with distension of the entire stomach. Distension of a fundic pouch of the stomach did not cause this inhibition. The distensions of the stomach in experimental animals which are reported as being inhibitory are only likely to occur in intact animals during vomiting or during strong contractions of the abdominal walls. In this connection, McNally et al. (1965) report that 1000-2000 cc of air may be introduced to the stomach with a rise in pressure of only 4-7 mm Hg.

Pennington, Haney and Youmans (1946) reported that distension of the jejunum caused a fall in tone of the terminal oesophagus. Published observations of the effects of other stimuli applied to other parts of the gastrointestinal tract are not available. This is in contrast to what one might have expected to have found after Ingelfinger made the statement in his 1958 review: "Presumably, however, the wave is susceptible to afferent stimuli originating from various sources, and this susceptibility may account for the inconstant features of all types of oesophageal peristalsis with respect to incidence, point of origin, force and course."

The experiments reported in this chapter have been carried out to determine the effect which various stimuli may have on the activity of the resting or active terminal oesophagus. The nature of the responses has been investigated in terms of their latency, duration and direction.

The sensory pathways involved in these responses have also been studied.

A preliminary communication of the results cited in this chapter has been presented (Fitchen and Wheeler, 1966).

METHODS AND MATERIALS

Decerebrated, anaesthetised, isolated medullary, and pithed preparations of cats have been used in a study of oesophageal motility of the cat. In all preparations anaesthesia was induced with ethyl chloride and continued with ether-oxygen inhalation. Tracheal, femoral venous, and femoral arterial cannulae were inserted in the usual manner (Liddell and Sherrington, 1929). In some experiments an endotracheal tube was inserted instead of a tracheal cannula.

Unless specified to the contrary, all dissection procedures referred to were undertaken during the period of ether anaesthesia.

The method and sequence of decerebration was as described by Comline and Titchen (1951a). Following decerebration, the preparations were placed on their right side and the right femoral artery was cannulated. The terminal oesophageal balloon and balloon used to record respiration were connected to the recording apparatus. The femoral arterial cannula was connected to a mercury manometer, and recording commenced. The carotid clamps used during decerebration were removed after these procedures had been completed.

The anaesthetised preparations referred to were prepared in the following manner: Anaesthesia was induced using ethyl chloride inhalation followed by an ether-oxygen mixture. Venous and tracheal cannulae were inserted. Chloralose made up in saline (70°C), or in an organic solvent (di-methyl sulphoxide), was injected intravenously. The dose usually given was 70 mgm/kgm body weight. All other operative procedures were carried out while the animal was under the chloralose

anaesthesia.

For the investigations of vago-vagal reflexes involving the oesophagus, isolated medullary preparations were made. The procedure was similar to that for decerebrate preparations. Just prior to decerebration the spinal cord (contained within the dura) was crushed with mosquito forceps through an exposure at the atlanto-occipital junction made immediately before.

Procedures which involved spinal cord section were carried out. The laminectomy required was made during the ether anaesthesia and the spinal cord was sectioned during the experiment.

Cats with their central nervous system totally destroyed were prepared. These preparations are hereinafter referred to as totally pithed preparations. Anaesthesia was induced with ethyl chloride and maintained with ether. The trachea was cannulated and a venous tube inserted into the femoral vein. A laminectomy was then made cranial to the sacrum, and the spinal cord was exposed. The exposure was several segments long and the spinal cord was cut at this point. The carotid arteries were tied and the vago-sympathetic nerve trunks were sectioned in the neck. The tracheal vein was tied and both jugular veins were isolated. One of these was tied while the other allowed venous drainage from the head. A large packing needle was passed under the spinal column, behind the wings of the atlas, to carry a cord which was tied tightly to occlude the vertebral arteries. The second jugular vein was then tied. The administration of the anaesthetic agent then ceased and the subject respired from a positive

pressure respiratory pump timed to approximately match the previous respiratory rate. The cord was exposed at the atlanto-occipital articulation and the artificial ventilation adjusted to create a sufficiently high intrathoracic pressure to cause haemorrhage from the cervical exposure. The brain was then pithed and swabs were forced forward to ensure total destruction. A plastic covered wire was then passed down the spinal column and out the sacral laminectomy wound. This wire was then used to pull a rubber tube down the column to remove the cord entirely and fill the evacuated space. This procedure was devised after attempts to completely remove the spinal cord failed because of a coronary thrombosis, due to either air or fat embolisms. This was overcome by creating the continuous (even on expiration) positive pressure within the thorax by manipulation of the respiratory pump output and amount of expiration possible. Thus the venous channels from the spinal cord did not accept air or fat emboli during the negative thorax pressure of inspiration. Iorio and McIsaac (1965), in a short note, made mention of a similar procedure, but a consideration of the difficulties was not undertaken.

To study the effects of changes in the conditions of the stomach on oesophageal motility the stomach was isolated from the oesophagus by a special cannulation technique. Under ether anaesthesia a ventral abdominal incision about 10 cm long was made. The pylorus was located and was tied lightly with a double ligature. An incision 2-3 cm long in the stomach was made 5-6 cm from the cardia on the greater curvature of the stomach. A flexible tube was passed down the oesophagus to facilitate identification of the point of connection of the oesophagus

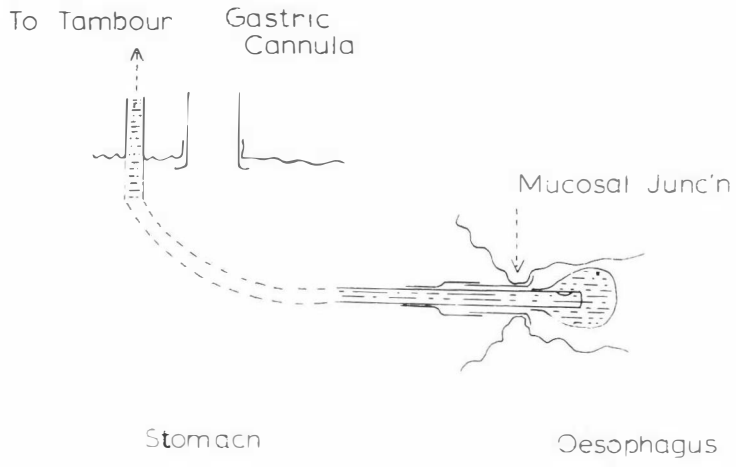
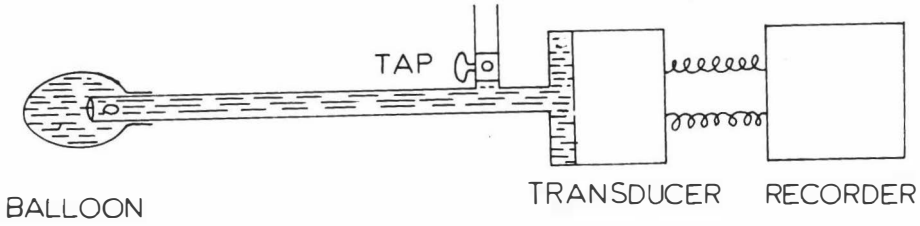


Fig. 2.1. Diagram of cannulation technique used to separate the stomach and the oesophagus at the gastro-oesophageal mucosal junction. The oesophageal cannula also provides a means of balloon placement at the terminal site. The stomach cannula was designed to allow infusion of fluids through the stomach and also fluid distension of the stomach.

with the stomach. The gastro-oesophageal mucosal junction was located and a purse-string suture, which passed into the muscularis of the stomach, was placed to tie a cannula in at this region. Attached to this cannula was an oesophageal balloon; the tube to which this was attached passed out of the stomach to the recording apparatus (see fig. 2.1). A gastric cannula (8-9 mm diameter) was tied into the stomach incision with a purse-string suture. The rest of the stomach incision and the skin incision was closed. The form of the oesophageal cannula altered, depending on whether drainage of the oesophagus was required. In this case a large bore tube was attached to the outside of the oesophageal cannula and passed out of the stomach. The cannulation of the oesophagus in this manner allowed a balloon, or balloons, to be introduced into the oesophagus without pharyngeal stimulation. It also permitted saliva or fluid which entered the oesophagus to drain from the caudal end and thus avoided a fluid distension of the oesophagus. The gastric cannula was inserted in order to allow the introduction of fluids into the stomach, provide quick drainage of the stomach contents, and allow the distension of the stomach by a column of fluid.

A study of the effect of various stimuli on the terminal oesophagus was carried out while this region of the oesophagus was distended by water-filled balloons. These balloons were 1.2 - 1.7 cm long and 1-2 cm diameter at 20 cm H_2O pressure. Since they were readily distensible, they permitted varying degrees of distension to be delivered in the same preparation under different conditions as well as in different preparations.



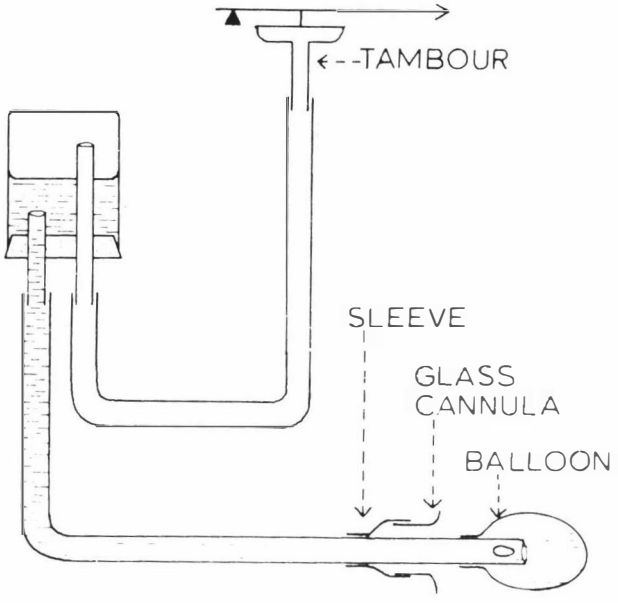


Fig. 2.3. Diagram of isometric form of balloon recording. Oesophageal balloon connected to transducer (AIDL) external to animal. The transducer output is translated by multi-pen recorder (Sanborn).

Fig. 2.2. Diagram of isotonic form of balloon recording. Oesophageal balloon is connected to a variable head pressure dome. The fluid movement is changed to air movement in the closed system. The air movement is measured by tambour writing on kymograph.

The methods used to record the motility measured by these oesophageal balloons were two:-

The tube to which the balloons were attached (both tube and balloons were water-filled) was connected to a dome in which the displacement of water was converted to a displacement of air. This displacement was translated into a kymographic record with the use of a tambour-lever system. The pressure in the recording part of the system was atmospheric and the pressure at the balloon was that column of water which it supported in the system. The resistance a contraction experienced was approximately the same throughout its course, the recording system measuring only a displacement. This system will hereinafter be referred to as the Isotonic recording system (see fig. 2.2).

The availability of a Sanborn electro-physiograph allowed precise recording of pressure changes within the oesophagus. Minute recording balloons were not used because these did not provide a concomitant distension with recording. Large air-filled balloons (1.5 cm long, variable diameter) were attached, via tubing, to transducers (AIDL). The technique of enlarging these balloons to a degree sufficient to accord distension and establish excitability was continued. The displacement of air from the balloon to the transducer in this system was very small. The contraction of the oesophagus was therefore close to being isometric, as opposed to an isotonic contraction record

in the kymograph-balloon-tambour system. This system will hereinafter be referred to as the Isometric system (see fig. 2.3).

These two forms of recording are similar to those used by Setekleiv (1964a).

Respiration was recorded in all experiments. This indicated what portion of the oesophageal record was due to respiratory movements. The record of respiratory movements was obtained by attaching a balloon (1-2 cm long and 1.5 - 2 cm diameter) to the external thorax by means of an inflexible cuff, thus measuring expansion of the thoracic cavity. In all records inspiration is indicated by an upward movement of the writing lever.

Blood pressure was recorded from a femoral artery. In kymograph recording a mercury manometer was used. In Sanborn records a transducer and arterial tube were filled with heparinised saline and calibrated by reference to a mercury manometer attached in parallel.

Stimulation of the vagus nerves required their isolation and section. This was done as soon before stimulation as possible, to minimise nerve damage. For electrical stimulation, bipolar shielded electrodes or saline-glass bead electrodes were used (Porter and Allamon, 1936 - cited by Comline and Titchen, 1951a). The glass bead electrodes gave repeatable conditions for stimulation. A Palmer student's stimulator was used to deliver the stimuli (at frequencies up to 100 impulses/sec and intensity 0-25 volts).

Saline used was 0.9% w/v and kept in a thermostatically controlled water bath at 37°C.

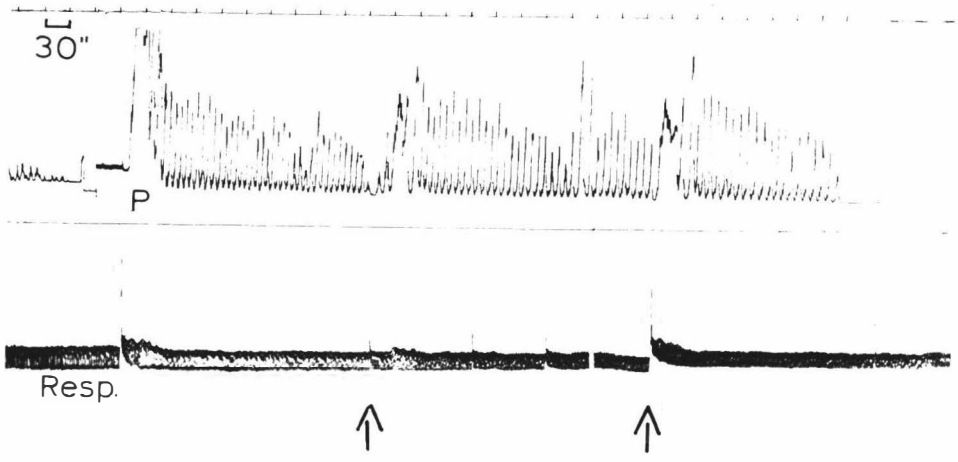


Fig. 2.4. Decerebrate cat. Response of the terminal oesophagus to distension by a balloon in this region. Coupled with this record is shown the excitatory effect of deep inspiratory movements. Records from above downwards: 30 sec time-marker, terminal oesophagus, respiration. The balloon was distended at P, and the arrows signal the deep inspiratory movements.

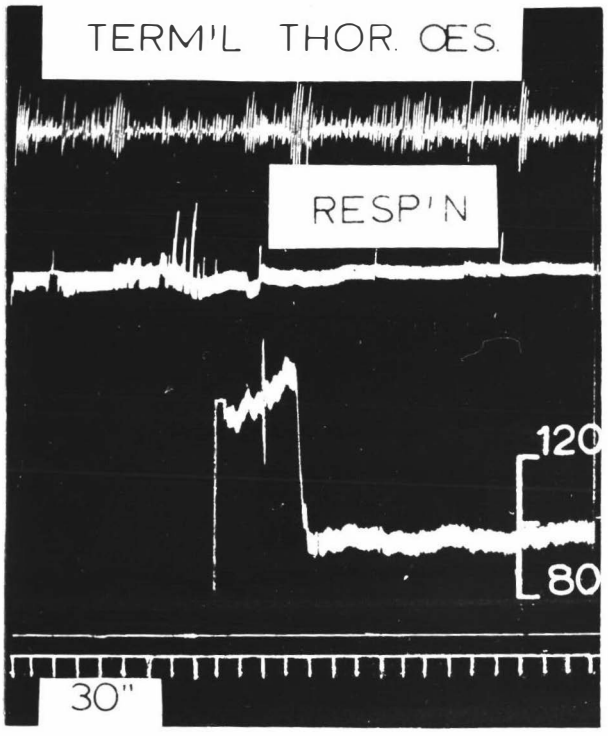


Fig. 2.5. Decerebrate cat. Blood pressure response to removal of the clamps attached to the carotid arteries in the neck during decerebration. The terminal oesophageal activity present within 10 minutes of decerebration is unaffected by the hypotension. Records from above downwards: terminal oesophagus, respiration, blood pressure (mm Hg), signal, 30 sec time-marker.

RESULTS

Contractions of the terminal thoracic or abdominal oesophagus were stimulated in decerebrate preparations of cats by distension of a balloon in this region of the oesophagus. This response was recorded as soon as 10 minutes after decerebration (the earliest opportunity of observation). Inflation with 2-4 ml of H_2O of balloons 1.2 - 1.7 cm long to a diameter of 1-2 cm diameter with a head of 20 cm H_2O stimulated a series of contractions which continued for four to 20 minutes. The contractions occurred at a frequency of about six/minute; both frequency and intensity of the contractions gradually declined after distension (fig. 2.4).

The duration of the response and the magnitude of the individual contractions varied in different preparations. Decerebrate preparations which did not display corneal and pinna reflexes, a mean arterial blood pressure of 60 mm Hg or more, regular respiration, and moderate rigidity within 30-40 minutes of decerebration, were discarded.

Oesophageal contractions stimulated by distension were observed in decerebrate preparations before removal of the clamps applied to the carotid arteries during decerebration. The responses appeared to be unaffected by the fall in blood pressure which occurred when the clamps were removed (Pagano-Hering reflex). This fall in blood pressure itself provided an indication of the condition of the preparation (fig. 2.5).

Stimuli other than distension modified the oesophageal responses. Gentle abduction of the hind limb in which the venous cannula was inserted lessened the intensity of the oesophageal contractions (fig.

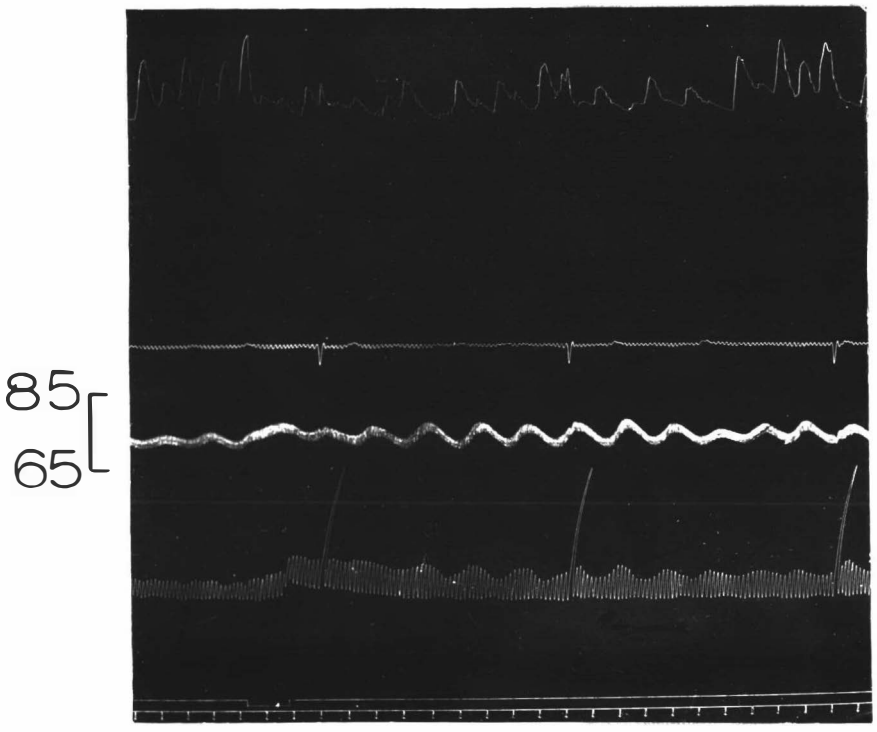


Fig. 2.6. Decerebrate cat. Inhibition of terminal oesophageal motility stimulated by gentle abduction of the left hind limb in which a femoral venous cannula had been inserted. Records from above downwards: terminal oesophagus, anterior thoracic oesophagus, blood pressure (mm Hg), signal, 10 sec time-marker. The signal marks six gentle abductions of the hind limb.

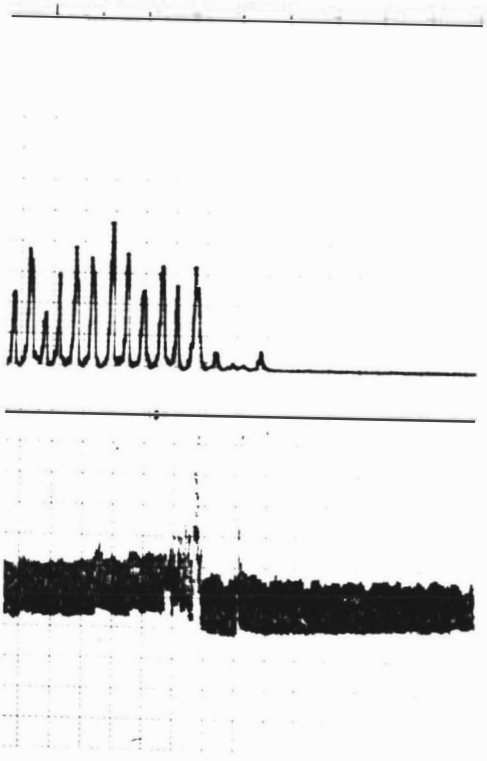


Fig. 2.7. Decerebrate cat. Inhibition of terminal oesophageal activity following manipulation of the stomach cannula. Records from above downwards: 30 sec time-marker, terminal oesophagus signal, respiration.

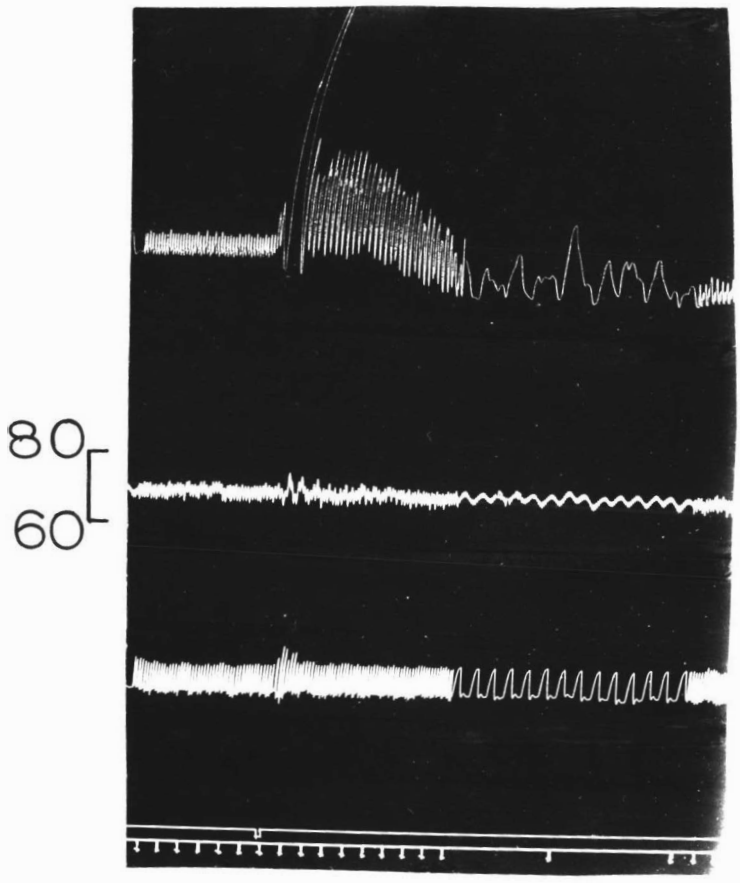


Fig. 2.8. Decerebrate cat. Response of the terminal oesophagus to increased balloon distension in this region. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker. Signal indicates an increase of the balloon head from 15 cm to 35 cm H_2O.

2.6) with little change in their frequency. The inhibition persisted for two minutes. Similarly, a reduction in oesophageal contractions was observed with manipulation of the gastric cannula (fig. 2.7). In contrast, it was observed that a deep inspiratory effort increased the oesophageal contractions (see fig. 2.4) or, when these had disappeared, led to their re-appearance. These observations influenced the conduct of the experiments. Care was taken to avoid manipulations or interferences which might change the oesophageal response. When such interferences were unavoidable, they were succeeded by a period during which no stimuli were delivered. This allowed the resting activity of the oesophagus to be established before any observations were undertaken on the responses of the oesophagus to a change or stimulus.

Once the response of the terminal oesophagus to its initial distension had wholly or largely ceased, the activity returned if the distension was increased. The first of the series of contractions stimulated by a further distension was observed 20-30 seconds after the application of this further distension and resetting of the recording apparatus; thus the response exhibited a latency of up to or less than 30 seconds (fig. 2.8). The duration of the response to increased distension varied in different preparations and according to the level of distension.

The terminal thoracic oesophagus responded to the flow over its mucosa of $^{N}/_{10}$ HCl with a series of contractions. The oesophageal cannula used allowed fluid to be washed over the oesophageal mucosa between the recording balloon and the cannula with a minimal amount of

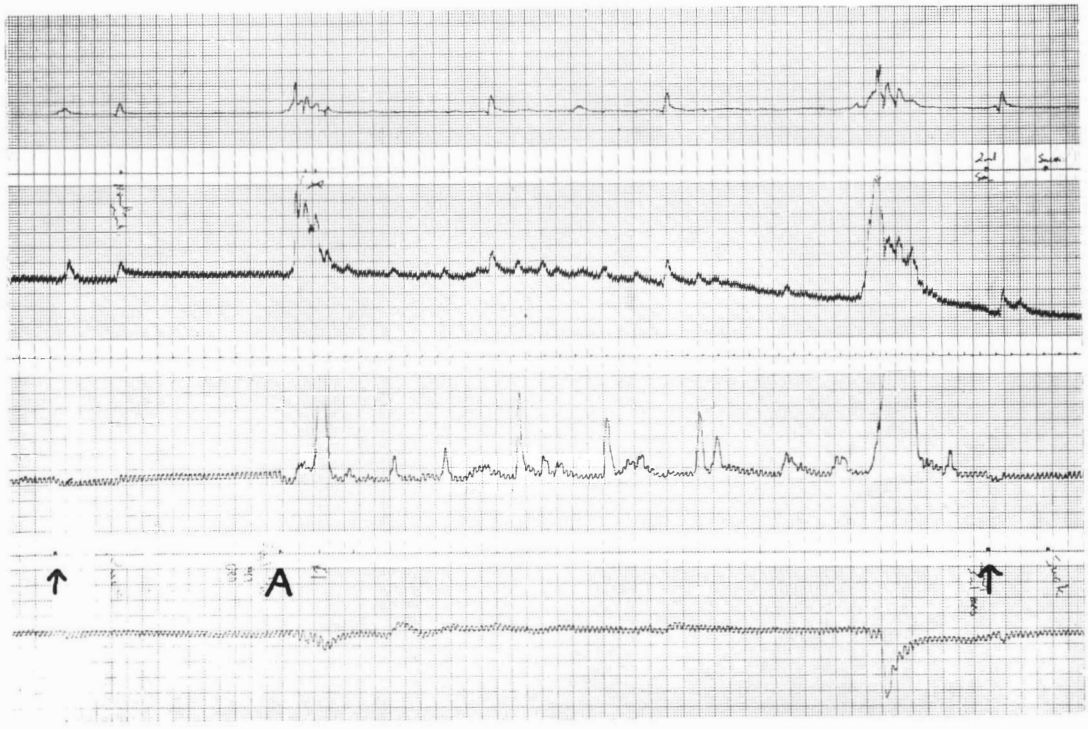


Fig. 2.9. Decerebrate cat. The response of the oesophagus to the application of 2 ml $^{N}/_{10}$ HCl. Complete involvement of the oesophagus is recorded with rhythmic activity in the mid-thoracic and terminal oesophagus following the application of the acid. The response is terminated by the application of 2 ml saline to the terminal oesophagus. Fluid in the oesophageal cannula was sucked out at various intervals as signalled. Records from above downwards: cervical oesophagus, mid-thoracic oesophagus, 10 sec time-marker, terminal oesophagus, signal, respiration. The arrowed signals mark the introduction of saline to the terminal oesophagus. The signal marked "A" signals the application of 2 ml $^{N}/_{10}$ acid to the terminal oesophagus. The other signals indicate sucking of the oesophageal cannula.

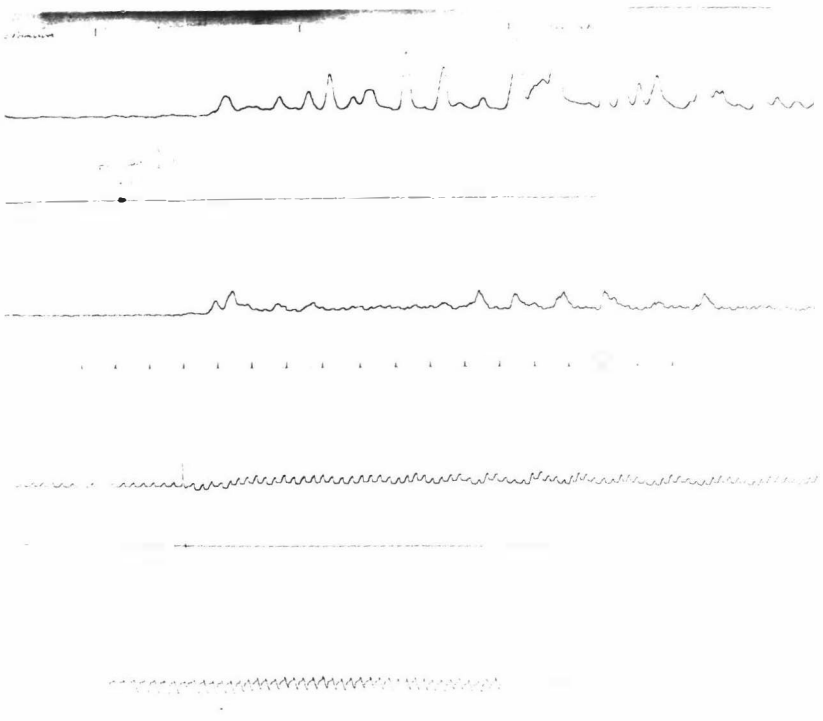


Fig. 2.10. Decerebrate cat. Contractions of the terminal and mid-thoracic regions of the oesophagus stimulated by 0.2 ml $^{N}/_{5}$ HCl. Twenty seconds elapsed before the response was noted in the mid-thoracic oesophagus. Records from above downwards: terminal oesophagus, signal, mid-thoracic oesophagus, 10 sec time-marker, cervical oesophagus, respiration. The signal marks the application of 0.2 ml $^{N}/_{5}$ HCl to the terminal region of the oesophagus.

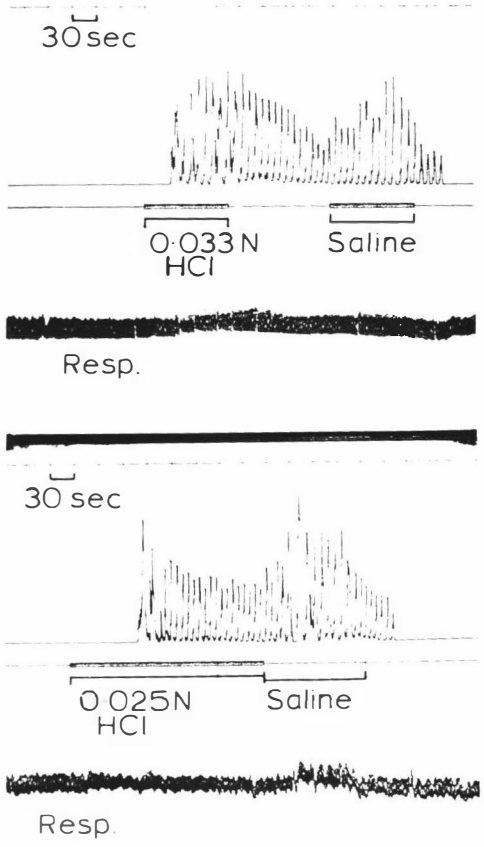


Fig. 2.11. Decerebrate cat. Response of the terminal oesophagus to adification of the stomach contents. Records from above downwards: 30 sec time-marker, terminal oesophagus, respiration. The lower half of the figure shows the same records. The signal marks the time during which an infusion of the stomach with acid or saline was made.

distension. In some cases continual sucking off of the contents of the cannula was undertaken in order to avoid, as far as possible, distension by the fluid introduced. Within 5-60 seconds of the introduction of the $^{N}/_{10}$ HCl the first of a series of contractions of the terminal oesophagus occurred. The duration of the response to acid was 10-20 minutes and it ceased within 10 seconds of a control infusion of saline. Shorter enduring responses of more cranial regions of the oesophagus were observed with this stimulus (fig. 2.9). The latencies and duration of the responses of the terminal and more cranial regions of the oesophagus varied (see figs. 2.10 and 2.9). In general, the latency of this response was 5-10 seconds.

Changes in conditions in the stomach affected the terminal oesophagus. Acidification of the contents of the stomach stimulated oesophageal contractions; distension of the stomach inhibited contractions of the terminal oesophagus.

Hydrochloric acid in strengths between $^{N}/_{40}$ and $^{N}/_{10}$ infused through the stomach increased both the activity of the terminal oesophagus and its responsiveness to other stimuli (such as distension of itself). The latency of the responses to the gastric infusions with acid varied according to the strengths of acid used. This can be seen in fig. 2.11, in which responses to $^{N}/_{30}$ and $^{N}/_{40}$ HCl infused into the stomach are compared in the same experiment. With $^{N}/_{30}$ HCl, 40 seconds elapsed before the first oesophageal contraction occurred, whereas it was 105 seconds after $^{N}/_{40}$ HCl infusion before a response of the terminal oesophagus was recorded. Care was taken in all experiments

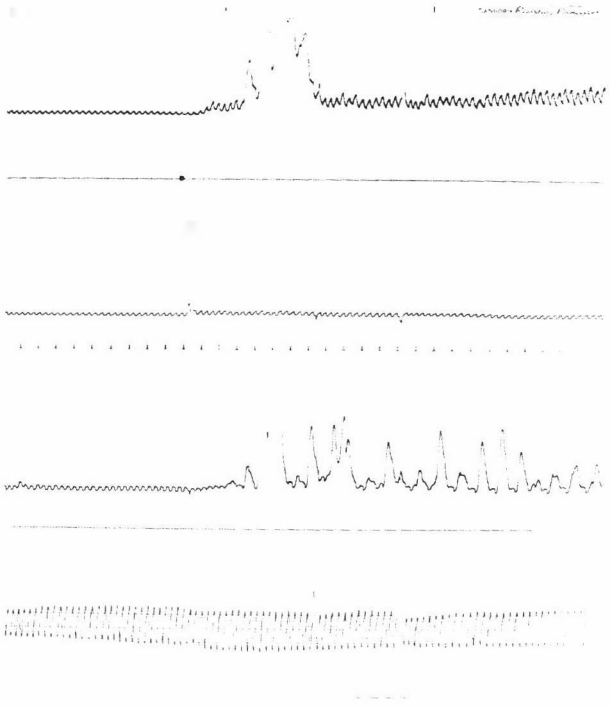


Fig. 2.12. Decerebrate cat. The response of the oesophagus, distended at the terminal and mid-thoracic levels, to the removal of a 100 ml $^{N}/_{10}$ HCl distension of the stomach. Records from above downwards: terminal oesophagus, signal, pharynx, 10 sec time-marker, mid-thoracic oesophagus, respiration. Note the degree of responsiveness of the mid-thoracic oesophagus which is determined by the relative placement and degree of distension. The signal marks the removal of 100 ml $^{N}/_{10}$ HCl from the stomach.

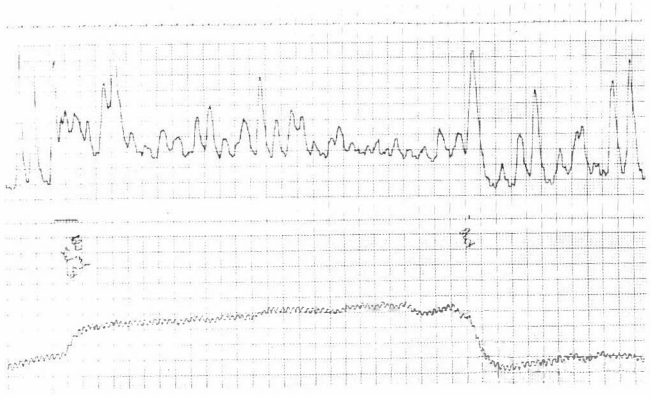


Fig. 2.13. Decerebrate cat. Inhibition of terminal oesophageal distension due to distension of the stomach. Records from above downwards: 30 sec time-marker, terminal oesophagus, signal, respiration.

on the infusion of acid into the stomach to avoid distension of the stomach. The cannulation procedure described above (Methods and Materials, Chapter 2) prevented the entry into the oesophagus of solutions introduced into the stomach in these experiments. It is interesting to note the differences in the latency of the responses to the introduction of acid into the oesophagus and the stomach (figs. 2.9, 2.10, 2.11). Control infusions of 0.9% saline were without effect provided no gastric distension was present.

Distension of the stomach with 100 ml of saline (37°C) reduced the activity of the terminal oesophagus. The responsiveness to other stimuli was also reduced. Fig. 2.12 shows the form of record which led to this finding. The distension of the stomach with acid was ineffective. However, on removal of the distension, the terminal oesophagus contracted strongly. The latency of the terminal oesophageal response was, in this instance, only 10–15 seconds. Inhibition of terminal oesophageal motility was regularly observed with distension of the stomach. The effect of gastric distension (50–100 ml) depended both on its extent and the level of terminal oesophageal distension. A low level of terminal oesophageal distension was inhibited by a 50 ml saline distension of the stomach. Higher oesophageal distensions, such as that accorded by a head of 30 cm H_2O, required higher levels of gastric distension to cause an inhibition.

Terminal oesophageal contractions were inhibited within 10–20 seconds of gastric distension, depending upon the intensity of the activity present in the terminal oesophagus (fig. 2.13). The inhibition generally lasted for the duration of the gastric distension. The

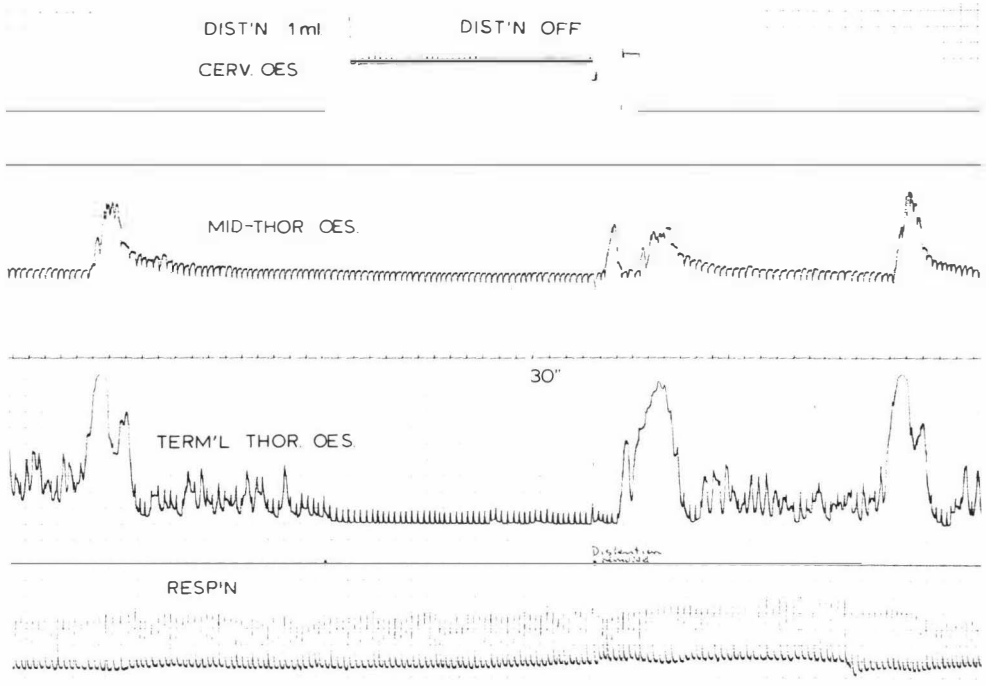


Fig. 2.14. Decerebrate cat. Response of the terminal oesophagus to distension by a balloon of the cervical oesophagus. Records from above downwards: cervical oesophagus, mid-thoracic oesophagus, 30 sec time-marker, terminal oesophagus, signal, respiration.

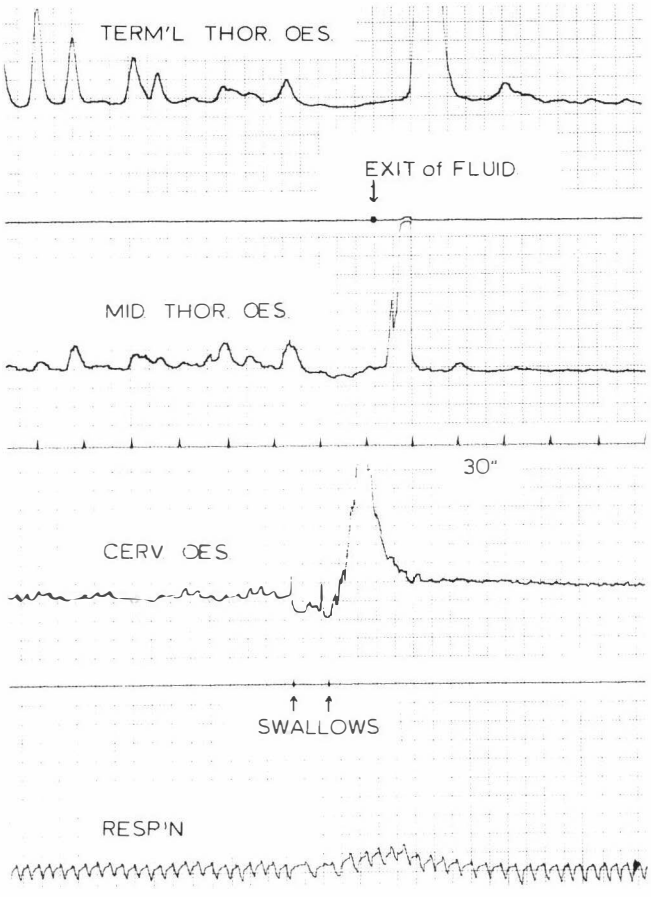


Fig. 2.15. Decerebrate cat. Record of two buccopharyngeal movements followed by a contraction wave sequentially involving the whole oesophagus. Note the inhibitory effect of the buccopharyngeal movements on activity of the terminal and mid-thoracic oesophagus. Records from above downwards: terminal oesophagus, signal marking exit of fluid from the terminal oesophageal cannula, mid-thoracic oesophagus, 30 sec time-marker, cervical oesophagus, signal marking swallows, respiration.

inhibition of strong terminal oesophageal activity by a low level of gastric distension was, in some cases, not effective throughout the gastric distension. Higher levels of gastric distension caused an inhibition which continued after removal of the distension.

Distension, by a balloon, of the cervical oesophagus caused an inhibition of terminal oesophageal contractions in response to a distension (fig. 2.14). This inhibition invariably occurred within five seconds, and normally continued for the duration of the distension of the cervical oesophagus. Release of the distension was, in most cases, followed almost immediately by increased activity of the terminal oesophagus. The inhibitory effect of a more cranial oesophageal distension on the activity of the terminal oesophagus could be elicited at all levels of the oesophagus.

Contraction of the terminal oesophagus usually followed, with variable latencies, the buccopharyngeal movement of a swallow. The occurrence of a contraction wave of the oesophagus following a buccopharyngeal movement stimulated by fluid introduced to the pharynx appeared to depend on the presence of fluid or a distension in the cervical oesophagus in addition to the buccopharyngeal movement of swallowing (fig. 2.15).

Electrical stimulation of the central end of the superior laryngeal nerve can be used reliably to initiate the buccopharyngeal movement normally associated with swallowing and an oesophageal contraction wave. Fig. 2.16 is a record obtained from a decerebrate cat by stimulating the central end of the superior laryngeal nerve at

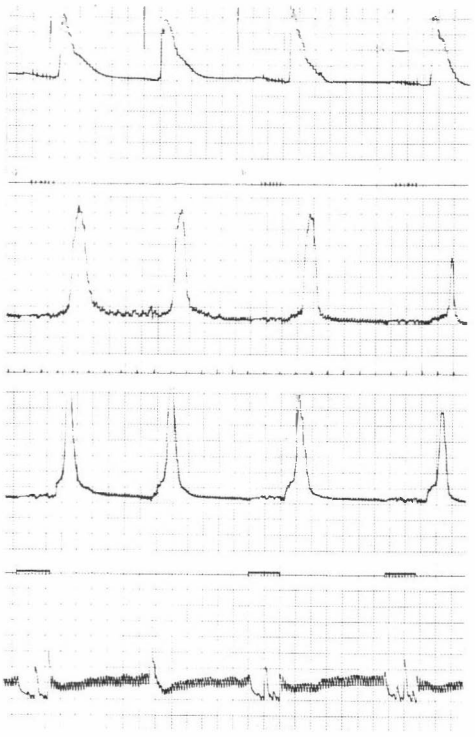


Fig. 2.16. Decerebrate cat. Oesophageal response to stimulation of the central end of the superior laryngeal nerve. Records from above downwards: caudal cervical oesophagus, signal marking buccopharyngeal movements, terminal oesophagus, 10 sec time-marker, mid-thoracic oesophagus, signal marking nerve stimulation, respiration. Note the contraction wave following the deep inspiration.

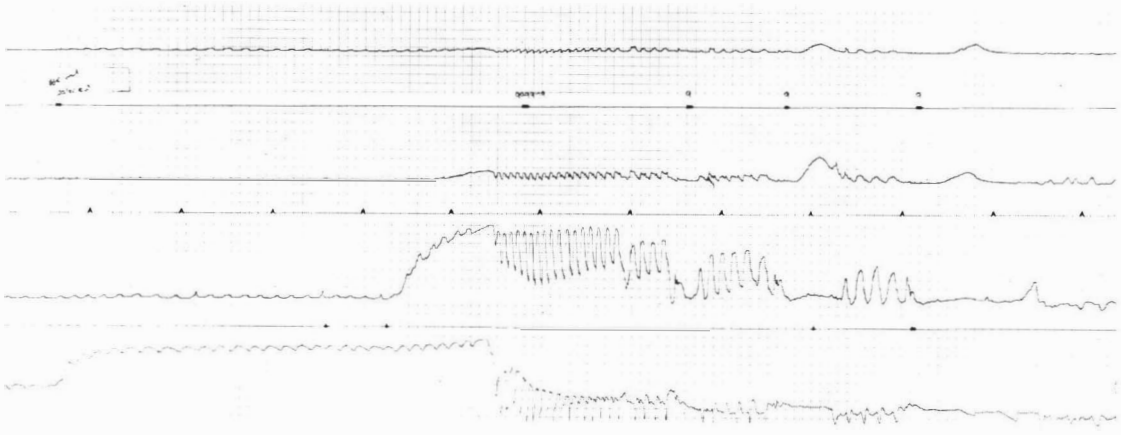


Fig. 2.17. Decerebrate cat. Vomiting in response to 3.25 mgm apomorphine injected intravenously. A 100 ml saline distension of the stomach was removed 30 sec prior to the act of vomiting. Records from above downwards: terminal oesophagus, events signal, mid-thoracic oesophagus, 10 sec time-marker, mid-cervical oesophagus, signal marking buccopharyngeal movements, respiration.

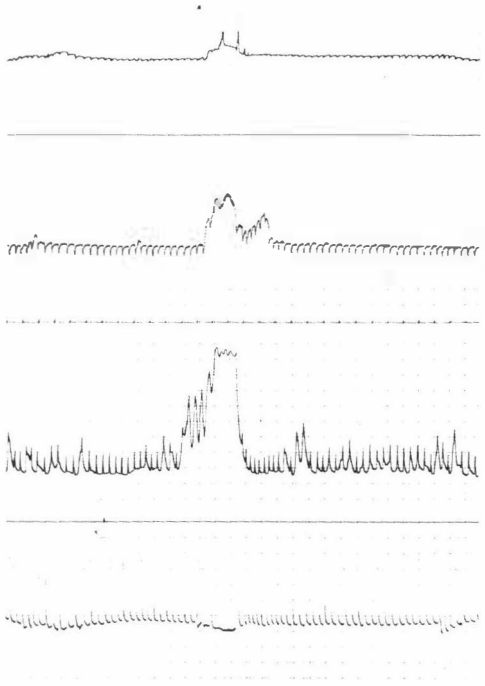


Fig. 2.18. Decerebrate cat. Contraction of the terminal oesophagus associated with an inhibition of respiration which occurs at the height of the terminal oesophageal contraction. Records from above downwards: mid-cervical oesophagus, mid-thoracic oesophagus, 10 sec time-marker, terminal oesophagus, respiration.

a frequency of five shocks/second for 20 seconds at a strength of 0.6V. Six buccopharyngeal movements were stimulated and the inhibition of respiration which occurs as a part of this reflex was apparent with each such movement. After the stimulus ceased, there was a very rapid caudally moving wave of contraction. The responses to three separate periods of stimulation are shown to indicate their repeatability. The terminal oesophageal contraction which occurred at the end of the swallowing act occurred 3-40 seconds after the buccopharyngeal movement of swallowing.

An inhibitory phase of the buccopharyngeal movement of swallowing was identified. Terminal oesophageal contractions in response to distension were inhibited 2-3 seconds after the buccopharyngeal movement was noted. This inhibition lasted until the contraction of this region occurred (see fig. 2.15).

Vomiting, whether elicited by distension of the stomach with saline, or by the intravenous injection of apomorphine, was not followed or associated with any detectable reaction of the terminal oesophagus (fig. 2.17).

Distension of the terminal oesophagus was noted in some decerebrate preparations to cause an inhibition of respiration. The latency of this inhibition was 2-3 seconds. An inhibition of respiration has also been noted with contraction of the terminal oesophagus (fig. 2.18). This inhibition occurred at the height of the contraction of the terminal oesophagus or within 20-25 seconds after commencement of the contraction and it was also observed after the intravenous injection of atropine,

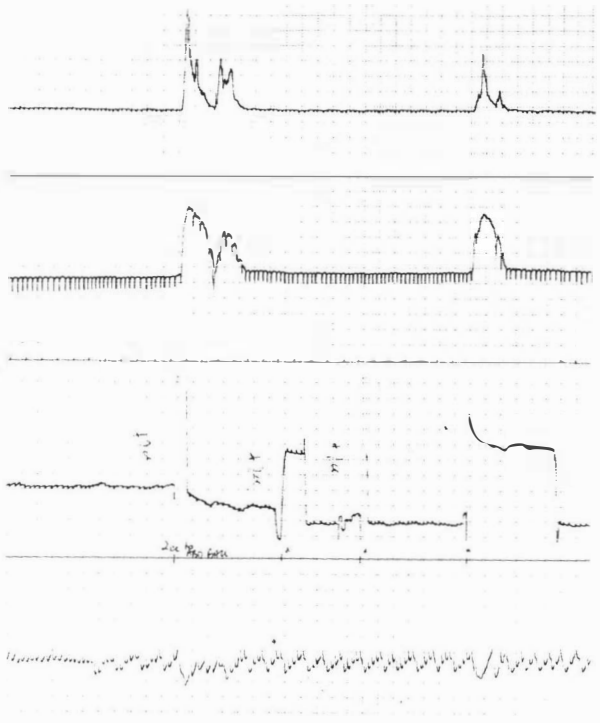


Fig. 2.19. Decerebrate cat. Response of oesophagus to terminal oesophageal distension following injection of 0.65 mgm atropine intravenously. Note the inhibition of respiration following the distension. Records from above downwards: mid-cervical oesophagus, mid-thoracic oesophagus, 10 sec time-marker, terminal oesophagus, signal, respiration. Each signal marks a 2 ml distension of the terminal oesophageal balloon.

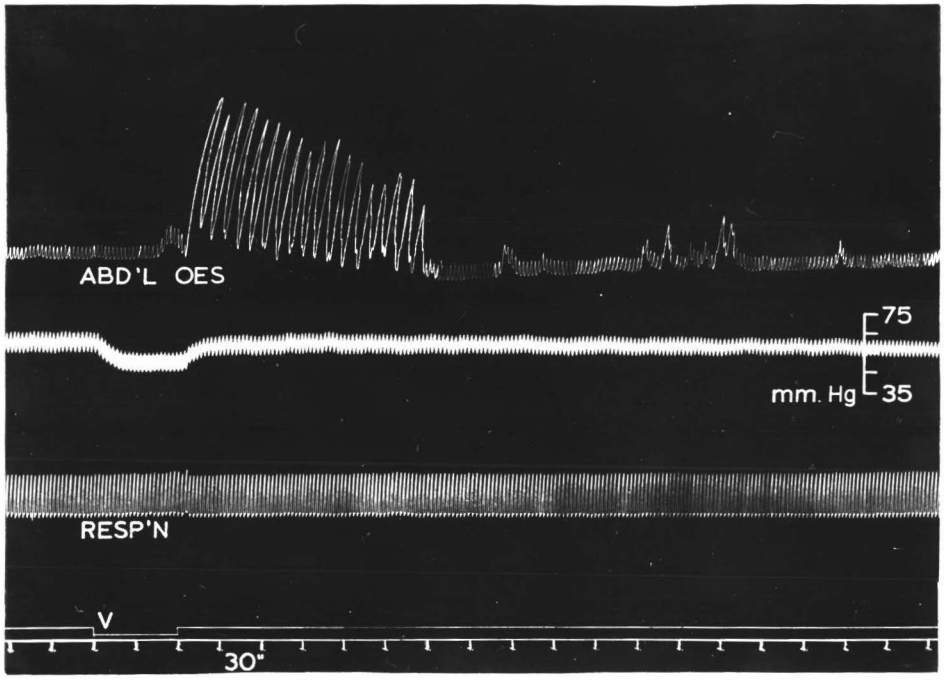


Fig. 2.20. Isolated medullary preparation of a cat. Response of the terminal oesophagus to low frequency stimulation of a vagus nerve cut in the neck in an afferent sense. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker. The arrowed signal marks the application of the stimulus: five shocks/sec, 12.5V, for 60 sec.

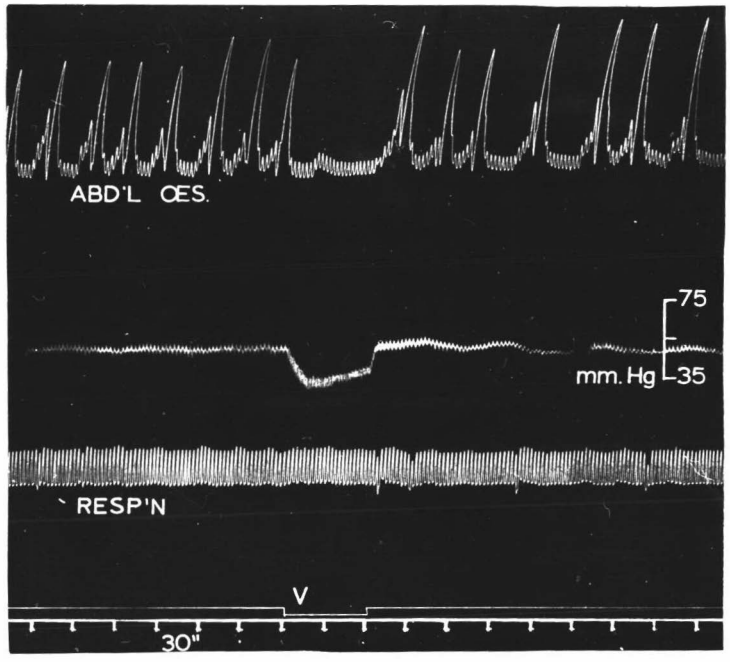


Fig. 2.21. Isolated medullary preparation of a cat. Inhibition of terminal oesophageal motility by high frequency stimulation of the central end of a vagus nerve cut in the neck. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker. Signal (arrowed) marks stimulus: 30 shocks/sec, 12.5V, for 60 sec.

which blocked all activity of the terminal oesophagus (fig. 2.19). In contrast to this, it was observed in decerebrate preparations that a deep inspiratory movement caused increased activity of the terminal oesophagus or, where there was no activity, initiated it (see fig. 2.4).

Because of this terminal oesophageal-respiratory interaction, studies on the afferent vagus stimulation, particularly the stimulation of a vagus nerve cut in the neck, were undertaken in partially isolated medullary preparations to avoid Hering-Breuer reflex modification of respiration.

Electrical stimulation of the vagus nerve in an afferent sense caused both inhibition or excitation of the activity of the terminal oesophagus, depending on the frequency of the stimulation. In decerebrate and partially isolated medullary preparations of cats, stimulation at frequencies of five/second initiated activity of the terminal oesophagus usually after the stimulus was ceased. Tonus changes were sometimes noted during the period of stimulation. The duration of the activity after the withdrawal of the stimulus was 5-8 minutes (fig. 2.20). At frequencies of stimulation of, or above, 30/second, an inhibition of terminal oesophageal contractions was produced (fig. 2.21). The latency of this response was 5-10 seconds and the duration depended largely upon the degree of oesophageal distension. Normally, terminal oesophageal contraction commenced within 10-20 seconds of withdrawal of the stimulus.

Experiments were designed to investigate the pathways of the sensory fibres involved in the gastric distension inhibitory effect on

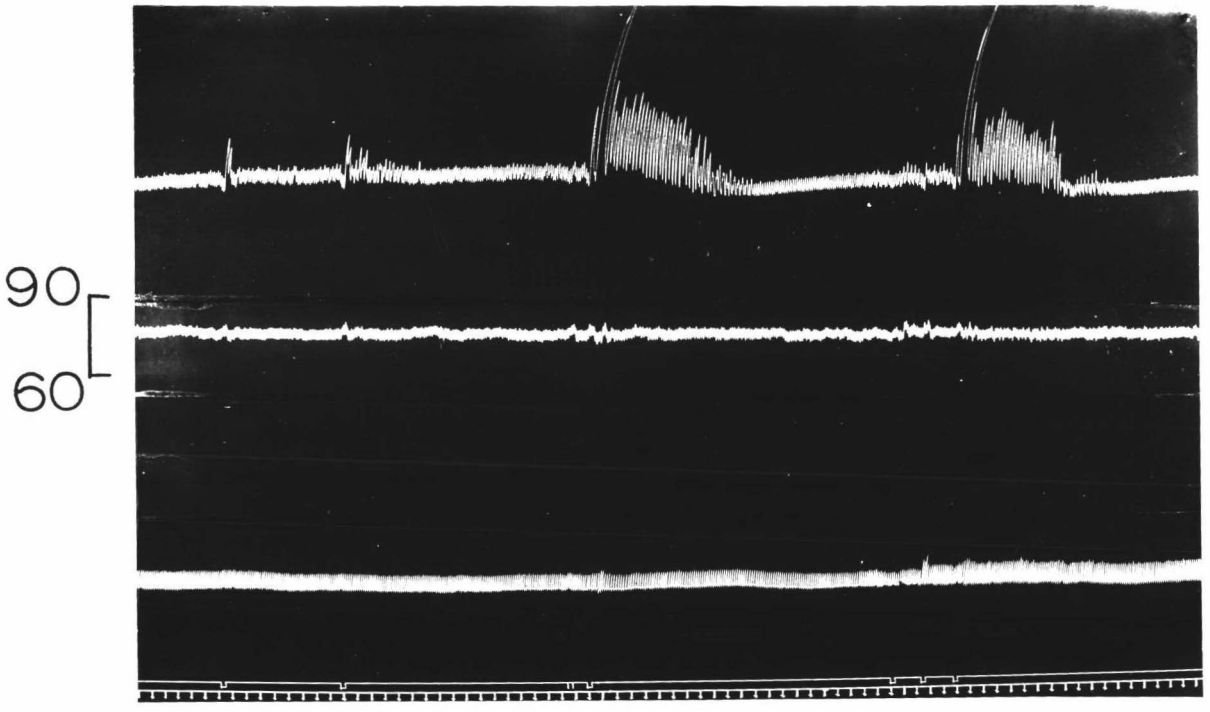


Fig. 2.22. Decerebrate cat. The response of the terminal oesophagus to stimulation of the central end of a vagus nerve cut in the abdomen. The response is determined by the separation of the stimuli. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker. Each signal indicates the application of an electrical stimulus: 20/sec, 12.5V for 10 sec.

the terminal oesophagus. Procedures involving spinal cord section at the thoraco-lumbar junction were carried out in three preparations. Two forms of record were obtained. Moderate or gross distension had no effect on terminal oesophageal activity. Moderate distension caused increased activity, while higher degrees of gastric distension did not cause an inhibition. In contrast, it was recorded in partially isolated medullary preparations that distension of the stomach caused an inhibition of terminal oesophageal activity.

The ventral branch of the abdominal vagus nerve of decerebrate cats was stimulated by means of a unipolar glass bead electrode. Stimulation of this nerve at a frequency of 20/second, at intensity 12.5V for 10 seconds, caused a series of contractions of the terminal oesophagus which lasted for 3.5 - 5 minutes. It was usually necessary to establish an initial excitation by a preliminary stimulus before an effective stimulus was noted. The time separation of successive stimuli determined the efficacy of the succeeding stimuli. Fig. 2.22 compares the effect of two stimuli separated by 30 seconds with three stimuli separated by 60 seconds. Within limits, distension of the terminal oesophagus increased the response to nerve stimulation. With a moderate (23 cm) distension from the balloon there was a greater response to afferent vagus nerve stimulation than there was with the distension, due to a head of 15 cm H_2O. The response when the terminal oesophagus was subjected to a larger distension (accorded by a head of 35 cm) was only slightly greater than the response noted with the distension with a head of 23 cm. Stimulation during a distension accorded by a head of 55 cm H_2O was less effective than with the

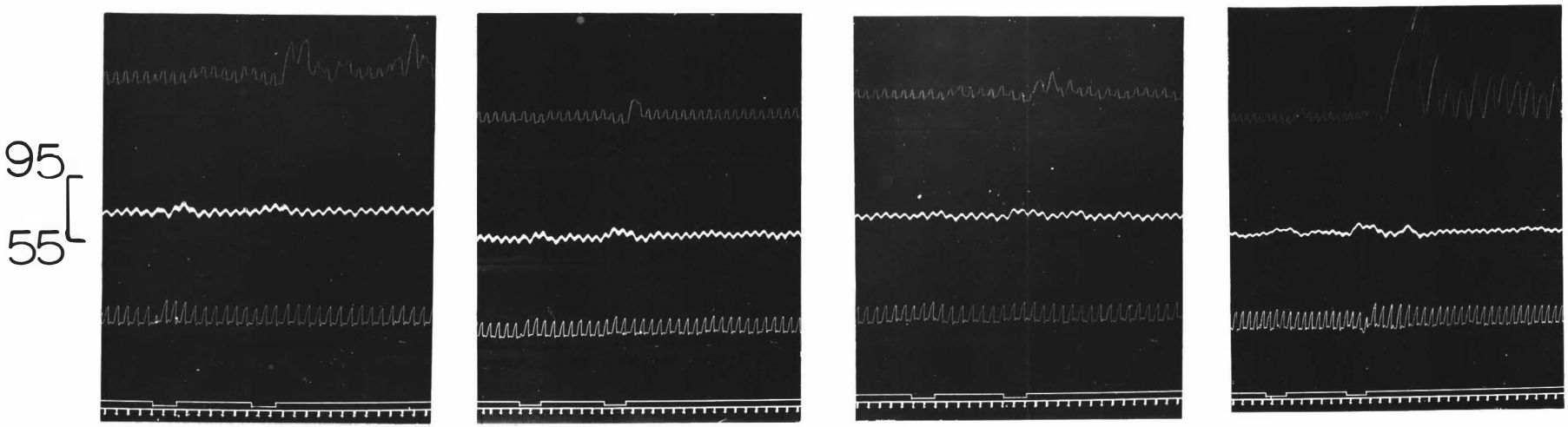


Fig. 2.23. Decerebrate cat. Effects of electrical stimulation of the central end of the ventral branch of the vagus nerve in the abdomen when the terminal oesophagus was subjected to varying degrees of balloon distension. Records from above downwards in each figure: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 5 sec time-marker. Figures left to right - effects of stimulation during the oesophageal distension accorded by a head of: 25 cm; 15 cm; 35 cm; 15 cm three minutes after the removal of the distension due to the 55 cm head. The signal in each figure marks the application of the stimulus: 20/sec, strength 12.5V, for 10 sec.

distension at heads 23 and 35 cm H_2O. The response of the terminal oesophagus after the head was reduced from 55 cm to 15 cm was recorded and was greater than all levels previously noted (fig. 2.23).

DISCUSSION

Rhythmic contractions of the terminal oesophagus occur when this region of the oesophagus is subjected to distension by a balloon. This response has been recorded in totally pithed preparations and after large doses of nicotine had been administered, indicating that this response is independent of an extrinsic innervation and may be a direct oesophageal response. In the decerebrate cat this activity could be modified by chemical and physical stimuli applied to the oesophagus or more distant regions. Evidence suggests that the modification of this basic activity is reflex in nature. Stimulation, in an afferent sense, of a vagus nerve in the neck and of a branch of the vagus nerve in the abdomen has facilitated and inhibited terminal oesophageal motility. In partially isolated medullary preparations, section of the other vagus nerve in the neck, or the administration of atropine, has abolished these responses. Further indications of the reflex nature of the responses described above comes from the character of the responses and some of their properties, namely, latency, inhibition, after-discharge, and summation of stimuli. The responses to stimulation of the central end of a vagus nerve cut in the neck never occurred less than four seconds after the commencement of stimulation.

The reflex centre, or centres, is probably located in the medulla oblongata. The responses were recorded in partially isolated medullary preparations. The centres are unlikely to be situated in the pons which may suffer damage during decerebration by mechanical transection.

The medullary site of the reflex centres is supported by the report of Lawn (1964), who recorded contractions of the oesophagus of the sheep following stimulation through electrodes inserted into the medulla oblongata.

Stimuli which facilitate contractions of the terminal oesophagus are: distension of this region of the oesophagus, acidification of the terminal oesophagus, and acidification of the contents of the stomach. The response to distension of the terminal oesophagus is present in the totally pithed preparation. This would indicate that the response is either direct or mediated by a short intrinsic ganglionated or non-ganglionated neural pathway. These possibilities have not been investigated. The facilitation of terminal oesophageal activity by acidification of the oesophageal mucosa in this region generally had a shorter latency than the facilitation accorded by acidification of the gastric mucosa. The latter response was also more easily inhibited than the response to acidification of the terminal oesophagus. Whether this response is present in the totally pithed preparation has not been investigated, but the dependence of this response on extrinsic nerves may be less than the response to acidification of the contents of the stomach.

The acid sensitive receptors in the stomach which have been identified by Iggo (1957b) are presumably responsible for the activation of the afferent limb of this reflex. Receptors which are responsive to acid in the oesophagus have not been identified, but the oesophageal response to acidification of its mucosa has been previously recognised in conscious humans and unanaesthetised dogs (Siegel and Hendrix, 1963).

Stimuli which inhibit contractions of the terminal oesophagus are: a more cranial oesophageal distension, gross terminal oesophageal distension, and distension of the stomach. The latter effect resembles the inhibition of reticulum movements by distension of the abomasum reported by Phillipson (1939), Dussardier (1955), and Titchen (1958a) in intact anaesthetised and decerebrate sheep. The afferents involved in this reflex inhibition of reticulum movements were found to occur in both the splanchnic and vagus nerves (Dussardier, 1955: Titchen, 1958a). Facilitation of reticulum movements on distension of the stomach was found to be due to vagal afferent fibres (Titchen, 1958a). The presence of these fibres was only appreciated after section of both splanchnic nerves. The evidence obtained in these experiments indicates that the passage of the inhibitory afferents may course in the splanchnic nerves. The inhibition of terminal oesophageal activity evoked by stimulation of the ventral abdominal branch of the vagus nerve and the presence of this reflex in partially isolated medullary preparations provides strong evidence for the presence of vagal inhibitory afferents being stimulated by distension of the stomach. The facilitation of this abdominal vagal branch suggests that careful investigation of the pathway of sensory pathways may show that distension of the stomach at lower degrees of distension facilitates terminal oesophageal activity. A complete investigation of the paths of sensory fibres in the splanchnic, spinal and vagal nerves is suggested by the above. The inhibitory effects of oesophageal distension are still present in totally pithed preparations and are probably mediated by intrinsic ganglionated or non-ganglionated

pathways. When it has been ascertained that a particular response may be obtained when all extrinsic nervous pathways have been removed, the difficulty which then arises is to determine whether extrinsic nervous pathways are normally involved in the intact animal. The problems associated with such an investigation resemble the difficulties involved in deciding between nervous and hormonal motor pathways in other gastrointestinal reflexes (see Gregory, 1962, p. 103). An investigation of the electrical discharge in extrinsic sensory nerves following the application of one of the above stimuli might indicate whether extrinsic pathways were involved. Assessing their relative contribution still remains an apparently insuperable problem.

Two prominent features of these reflex modifications of terminal oesophageal motility are the long latencies and the long duration of the effects after stimulation had ceased. This property may account for the ability of several inadequate stimuli, separated by some minutes, to summate and cause a response (see fig. 2.23). Salivatory responses and a facilitation or inhibition of rumen-reticulum movements obtained with an oesophageal distension accorded by a moving balloon were found to be greater than the response to a fixed balloon distension (Sellers and Titchen, 1959). This may be explained by this after-discharge and summation phenomenon. The successive stimuli being delivered will be more effective as the site of summation is conditioned by the preceding afferent discharge. The summation of stimuli and the conditioning of one response by another stimuli has been reported in connection with the reflex stimulation of rumen-reticulum movements in decerebrate sheep (Comline and Titchen, 1961;

Reid and Titchen, 1965). From the results presented above, it would appear that similar mechanisms are operating in the control and stimulation of terminal oesophageal activity. The site of this conditioning and summation may be either at the medullary reflex centres or at the site of activity - the terminal oesophagus. The method of investigation of this problem would be extremely difficult, if at all possible, to undertake.

The receptors in the oesophagus which are sensitive to stretch or distension by a fixed or moving balloon have been identified but not fully characterised. Andrew (1956b), who studied the electrical discharge of vagal afferents in anaesthetised rats, recorded an afferent discharge with both a distension and with the passage of a contraction in the cervical oesophagus. Mei (1965) has confirmed this report in both the skeletal and smooth muscle regions of the oesophagus of the cat. The facilitation of terminal oesophageal activity during the negative thoracic pressure encountered during a deep inspiratory movement, coupled with these results, might provide an indication that the oesophageal stretch receptors may be responsive to both a passive displacement of the walls of the oesophagus and the displacement associated with the passage of a contraction. This conclusion is supported by the identification of receptors in the stomach which are activated by both stretch and the passage of a contraction (Paintal, 1953d; Iggo, 1957a). The susceptibility of the contractions of the striated muscle of the sheep oesophagus to D-tubocurarine has been reported (Sellers and Titchen, 1959), and Ingelfinger (1958) suggests that these regions of the oesophagus are essentially similar to skeletal

muscle elsewhere. These reports, coupled with the results of Andrew (1956b) and Mei (1965), may suggest that the slowly adapting stretch receptors in skeletal muscle may be present in the oesophageal striated muscle and are responsible for afferent discharge in the vagal fibres. The form and characteristics of the smooth muscle receptors responsive to both active and passive stretch cannot, at the moment, be known. An analysis of vagal afferent fibres activated by stimuli applied to the oesophagus and a histological examination of the receptive endings of nerve fibres are required for a full understanding of the sensory innervation of the oesophagus.

The investigation of segmental spinal reflexes has not been studied, but an indication that a dorsal root innervation of the oesophagus is present has been obtained (Kirk and Wheeler, unpublished observations). The influence of the cerebellum on visceral activity has been reviewed by Moruzzi (1950). Although Titchen (1958a) has indicated that the cerebellum is not necessary for the occurrence of some visceral reflexes, it is probable that the cerebellum exerts some influence on the reflex centres in the medulla. A report of hypothalamic stimulation causing contractions of the oesophagus (Kazawa, 1964) indicates that higher regions of the central nervous system play a part in the control of oesophageal motility. This control is probably exerted by a regulation of the medullary reflex centres.

Chapter 3

THE MOTOR INNERVATION OF THE OESOPHAGUS OF THE CAT

INTRODUCTION

Branches of the vagus nerve innervate the cervical oesophagus of all mammalian species studied. The anterior cervical regions of various species is innervated by an oesophageal branch of the superior laryngeal rami of the vagus nerve. This was first noted by Chauveau (1886) in his studies on horses, cows, lambs, dogs, cats, and rabbits. His work is cited by Hwang *et al*. (1948), who called this branch the pharyngo-oesophageal nerve. Bilateral section of this nerve causes a loss of contractile activity in the anterior cervical oesophagus. However, a return of activity occurs within a few weeks in dogs (Hwang, 1953). This functional recovery is attributed to the recurrent laryngeal nerve, which is normally predominantly sensory, assuming the motor requirements of this region of the oesophagus. The number of large diameter sensory fibres in the recurrent laryngeal nerve of the cat has been found to decrease in the more cephalad regions (Murray, 1957). This provides an indication that the recurrent laryngeal nerve contributes to the motor innervation of the more caudal regions of the oesophagus. The electrophysiological studies of Andrew (1956*b*, *c*) with the cervical oesophagus of the rat provide evidence that efferent fibres in the vagus are concerned in both primary and secondary peristalsis of the cervical oesophagus.

The thoracic oesophagus receives a motor innervation from fibres carried in oesophageal branches of the vagus nerves (Mitchell, 1953). Unilateral section of one vagus nerve in the neck does not qualitatively affect the oesophageal response in swallowing (Hwang, Essex and Mann, 1947). Section of both vagus nerves causes paralysis of the skeletal muscle portions and a loss of purposeful motility of the smooth muscle regions of the more caudal oesophagus. A spasm or rise in tone has been noted in the terminal oesophageal regions after both vagus nerves were cut (Hwang, Essex and Mann, 1947). These workers also report that small irregular and strong tetanic contractions of the skeletal muscle regions, even after bilateral vagotomy, may be evoked by distension.

The pathological condition of cardiospasm or mega-oesophagus indicates that the myenteric plexus of Auerbach is necessary for the orderly progression of oesophageal contractions and the normal functioning of the terminal oesophagus (Ingelfinger, 1953).

The neural pathways responsible for the inhibition of the tone of the terminal oesophagus have been investigated by many workers. Zeller and Burget (1937) reported that the inhibition of the tonus of the distal oesophagus in response to vagal stimulation was not abolished by bilateral section of the vagi below the hilus of the lung. Grondahl and Haney (1940) repeated this work and showed that the inhibitory vagal impulses were abolished by dividing the oesophageal musculature 4 cm above the oesophageal hiatus. This was confirmed by Lehmann (1945). In 1947, Hwang, Essex and Mann showed that high bilateral

vagotomy caused a narrowing of the distal oesophagus, but vagotomy below the hilus of the lung did not produce this response. Essentially the same observation was made by Alnor and Ohnesorge (1958). It appears from the above reports that the vagal fibres responsible for the contraction which occurs in this region were carried in the main thoracic vagal trunks. The recent work of Carveth et al. (1962), and Greenwood et al. (1962) shows that the relaxation or inhibition which was presumed to be due to vagal impulses carried in the walls of the oesophagus is not dependent upon any extrinsic innervation. This report apparently removes the necessity for the inhibitory fibres which were supposedly carried within the walls of the oesophagus as opposed to the thoracic vagal trunks. Further, it would explain the difficulty in locating these fibres.

Whether the resting discharge of the vagal fibres is excitatory or inhibitory is an important question which must be asked if a complete understanding of the vagal efferent contribution to the resting oesophagus is to be obtained. Dagradi et al. (1962) reported observations on 14 cases of high bilateral vagotomy in human patients. They consistently observed an obstruction to swallowed food, and radiologically noted a narrowing of the distal oesophagus. This report, coupled with the achalasia which follows degeneration of the myenteric ganglion cells (Ingelfinger, 1956; Scherb and Arias, 1962), would indicate that the predominant vagal influence is inhibitory (see Butin et al., 1953; and Manzano, Torres, Hall and Cobo, 1964). These reports are supported by the report of Higgs and Ellis (1965),

that bilateral supra-nodose vagotomy causes an achalasic condition in dogs.

The role of the sympathetic nerves is uncertain. Knight (1934b) reported that cervical sympathetic nerve stimulation potentiated vagal stimulation of the skeletal muscle portions of the cervical oesophagus. Sympathetic denervation does not affect cervical oesophageal motility. Early workers suggested that the sympathetic innervation is excitatory to motility in the distal thoracic oesophagus, which was considered a sphincter, and inhibitory in the body of the oesophagus. Part of the evidence cited in favour of this is the greater tonus developed after vagotomy. However, this implies that it is only a sympathetic excitatory drive which is subservant to the vagal innervation. Carlson et al. (1922) showed that both section of the splanchnic nerves, and stimulation of them, had excitatory effects. They concluded that the splanchnics carried excitatory and inhibitory efferents to the distal oesophagus. Knight (1934a, b) reported that stimulation of the stellate ganglia potentiated vagally stimulated contractions of the upper thoracic, but diminished those of the lower thoracic oesophagus. These variable results would possibly indicate a relation between the response obtained and the resting tonus, the form and parameters of stimulation, the method of recording, and possibly the conditions under which the experiment was undertaken. One aspect of this problem which has been well confirmed is that orderly progression of a contraction wave occurs after substantial sympathectomy (Greenwood et al., 1962; Carveth et al., 1962; Manzano et al., 1964).

A final point to consider is the dependence of oesophageal motility upon the extrinsic motor innervation. It would appear from the report of Hwang, Essex and Mann (1947) that the bilateral sectioning of the pharyngo-oesophageal nerves and the vagi in the neck of the dog and cat causes the loss of all contractile activity in the cervical and thoracic regions of the oesophagus. A spasm, or hypertonicity, of the terminal oesophagus was noted. Dagradi et al. (1962) reported that high bilateral vagotomy in humans caused essentially the same effects. The presence of an innervation similar to the pharyngo-oesophageal nerve has not been established in humans (Hwang, 1953).

Sympathectomy has been shown not to have an effect on either the resting tone or the progression of a contraction wave (Greenwood et al., 1962; Carveth et al., 1962; Manzano et al., 1964). It would thus appear that the contractile activity of the proximal portions of the oesophagus is dependent upon an intact vagal innervation while the distal oesophagus possesses some motor function after denervation. O'Mallane (1954) made the observation that there was no sphincter at the terminal oesophagus, as a ganglion blocking agent did not affect the threshold pressure for reflux of stomach contents to the oesophagus. He assumed, in making such a comment, that an innervation was necessary for closure of this region in response to a challenging pressure. Greenwood et al. (1962) showed that the relaxation which follows deglutition continued after denervation (including oesophageal interruption). These workers do not commit themselves to a mode of control, but one assumes they would postulate hormones. One of the

difficulties associated with denervation studies which are undertaken with a view to allowing observations to be made on recovery from the operative procedures is that there may be recurrent or unrecognised courses taken by nerve fibres. For example, Harper, McSwiney and Suffolk (1935) obtained evidence that afferent nerve fibres passed from the vagus nerves into intercostal nerves without entering sympathetic nerve trunks. Further evidence for the presence of unmapped nervous pathways involved in visceral reflexes has been cited by Titchen (1954a). A gastro-colic reflex elicited by electrical stimulation of an abdominal branch of the vagus nerve continued after both vagus nerves had been cut in the neck. The afferent fibres stimulated were presumed to pass into the spinal cord with the sympathetic nerve trunks.

The oesophageal response to distension, or stretch of its walls, has not been studied to a great extent, but it was shown by Cannon (1907) that the smooth muscle portion of the oesophagus responded to stretch after complete denervation. A confirmation of this comes from Hwang (1949). Sympathectomy does not reduce the hypertonicity, or hypersensitivity, to tactile or distending stimuli associated with achalasia. If the observed effects of vagal denervation in this condition were due to an autonomic imbalance, sympathectomy would be effective, but there must be some other driving force which is subservient to the vagal innervation. The electromyographic work of Minzuno (1964) would indicate that action potentials in the lower oesophagus increase after unilateral vagotomy.

The results and discussion presented in this chapter are based on experiments on variously modified cats. The ablation of areas of the central nervous system, coupled with the results of pharmacological blocking presented in Chapter 4, have provided evidence for the path of motor fibres. The resting discharge and effects of stimulation have been considered and discussed. The use of totally pithed preparations has allowed the centrally denervated oesophagus to be studied. This has provided evidence for the control of motility exerted by its extrinsic innervation and by the intrinsic nervous elements.

METHODS AND MATERIALS

The preparation of the animals used to study the motor innervation of the oesophagus have been described in the Methods and Materials section, Chapter 2. Both the isotonic and isometric systems of recording from oesophageal balloons were employed. Respiration was recorded in all cases from an external thoracic balloon and blood pressure was recorded from the femoral artery by a mercury manometer or calibrated transducer.

Various autonomic blocking and stimulating drugs have been used in conjunction with nerve stimulation experiments. The dosages are expressed as mgm of the salt, injections and dilutions being made in 0.9% w/v saline. The stimulation of the various nerves was derived from a Palmer student's stimulator, using fluid (unipolar) electrodes or silver bipolar electrodes.

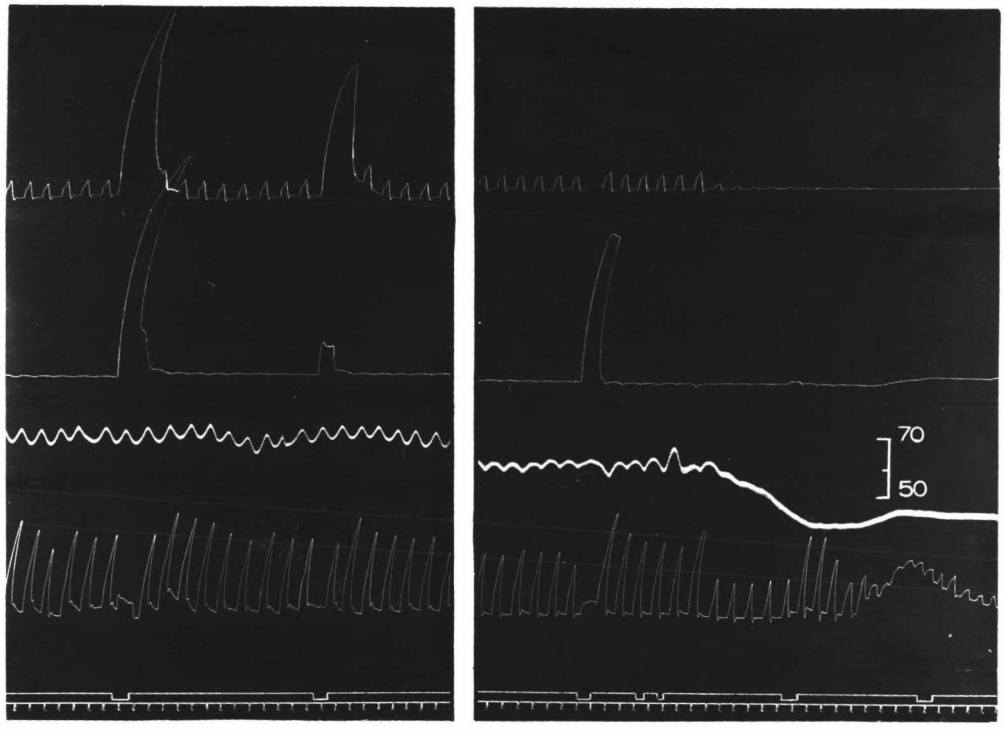


Fig. 3.1. Decerebrate cat. Response of the anterior and terminal regions of the thoracic oesophagus to stimulation of the peripheral end of a vagus nerve cut in the neck. The blocking action of atropine and D-tubocurarine is recorded. Records from above downwards: anterior thoracic oesophagus, terminal oesophagus, blood pressure (mm Hg), respiration, signal, 5 sec time-marker. "a" marks the application of the stimulus: 30/sec, 12.5V for 5 sec. "b" marks the application of the stimulus: 5/sec, 12.5V for 5 sec. Between the two records 1 mgm/kgm atropine was injected intravenously. The arrowed signal marks the injection of 0.1 mgm/kgm D-tubocurarine; 2 ml saline followed this injection (signalled). Note the slight effect atropine has on the form of the contraction of the anterior thoracic oesophagus (see fig. 3.2).

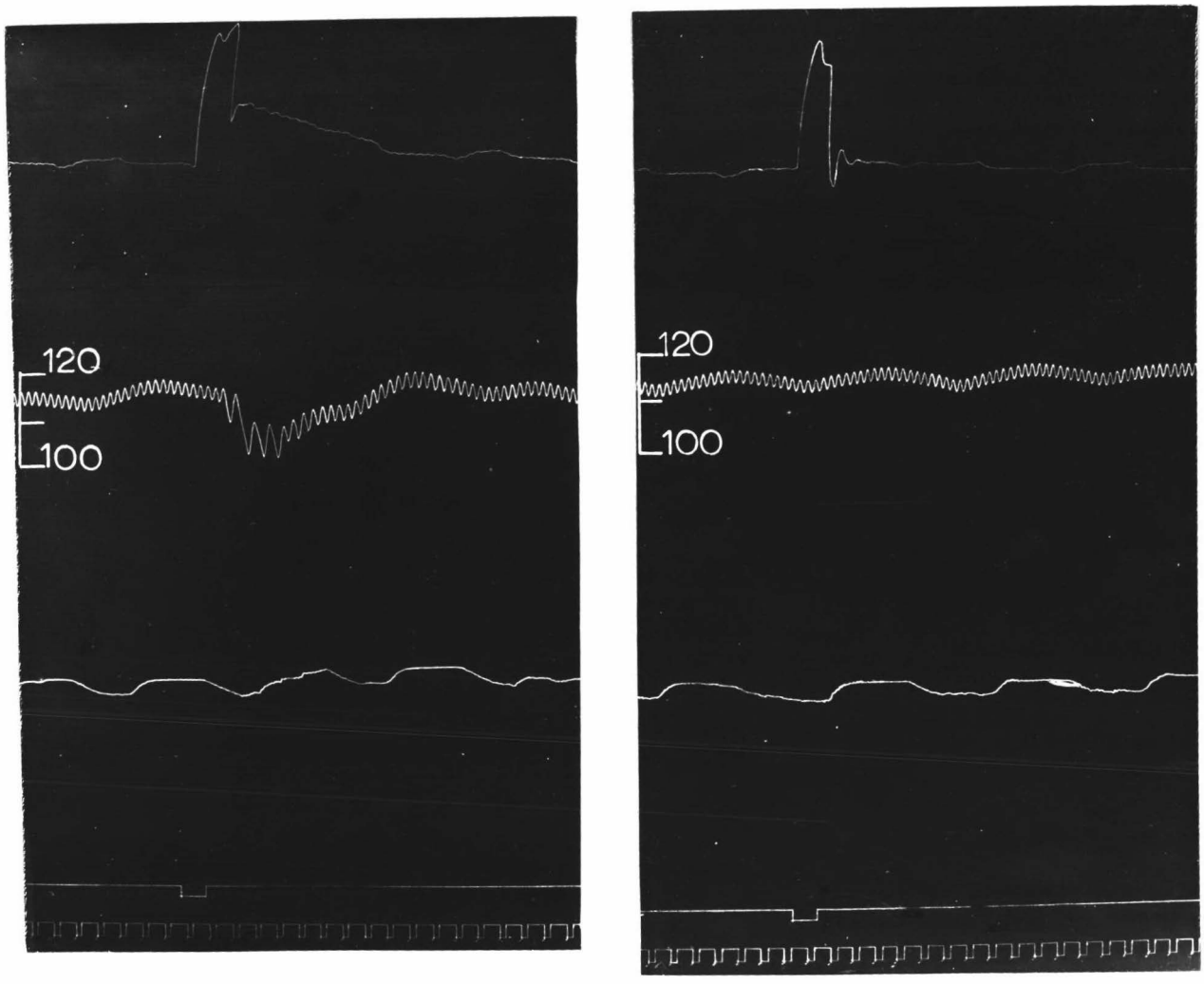


Fig. 3.2. Anaesthetised cat. Chloralose 70 mgm/kgm in saline solution (70°C). Contraction of the mid-thoracic oesophagus in response to stimulation of the peripheral end of the left vagus nerve cut in the neck before and after the injection of 1 mgm/kgm atropine. The final slow portion of the contraction and the bradycardia are abolished by the atropine leaving the initial fast portion of the oesophageal contraction. Records from above downwards: mid-thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 1 sec time-marker. The signal in each record marks the application of the stimulus: 25/sec, 15.0V for 1 sec. Between the records shown 1 mgm/kgm atropine was injected intravenously.

RESULTS

Electrical stimulation of the vagus nerves, their section, spinal cord section, and other simplifications of the central nervous system have been undertaken in these studies on the contribution of the extrinsic innervation of the oesophagus. The effects of autonomic blocking agents have been used to provide a further definition of the form of the motor pathways involved.

Contractions of the anterior, mid, and terminal regions of the oesophagus have been evoked by electrical stimulation of the peripheral end of either vagus nerve cut in the neck. These responses were independent of the hypotension which resulted when the stimulus applied was sufficiently intense to cause bradycardia. The stimuli used were chosen to minimise these effects on blood pressure. In fig. 3.1 a contraction of the anterior thoracic and terminal oesophagus is shown. These regions are skeletal and smooth muscle respectively. Also shown in fig. 3.1 is the oesophageal response to peripheral vagus stimulation following the administration of atropine and D-tubocurarine. The contraction of the smooth muscle region is blocked by atropine and the skeletal muscle region is slightly affected. Curare injected intravenously abolishes this contraction in response to peripheral vagus stimulation before the contractions of the diaphragm are blocked (see fig. 3.1). The junctional region of the two types of muscle appears to be in the mid-thoracic region of the oesophagus. A balloon placed at this point recorded a response to stimulation of the peripheral end of a vagus nerve which was made up of two portions (see

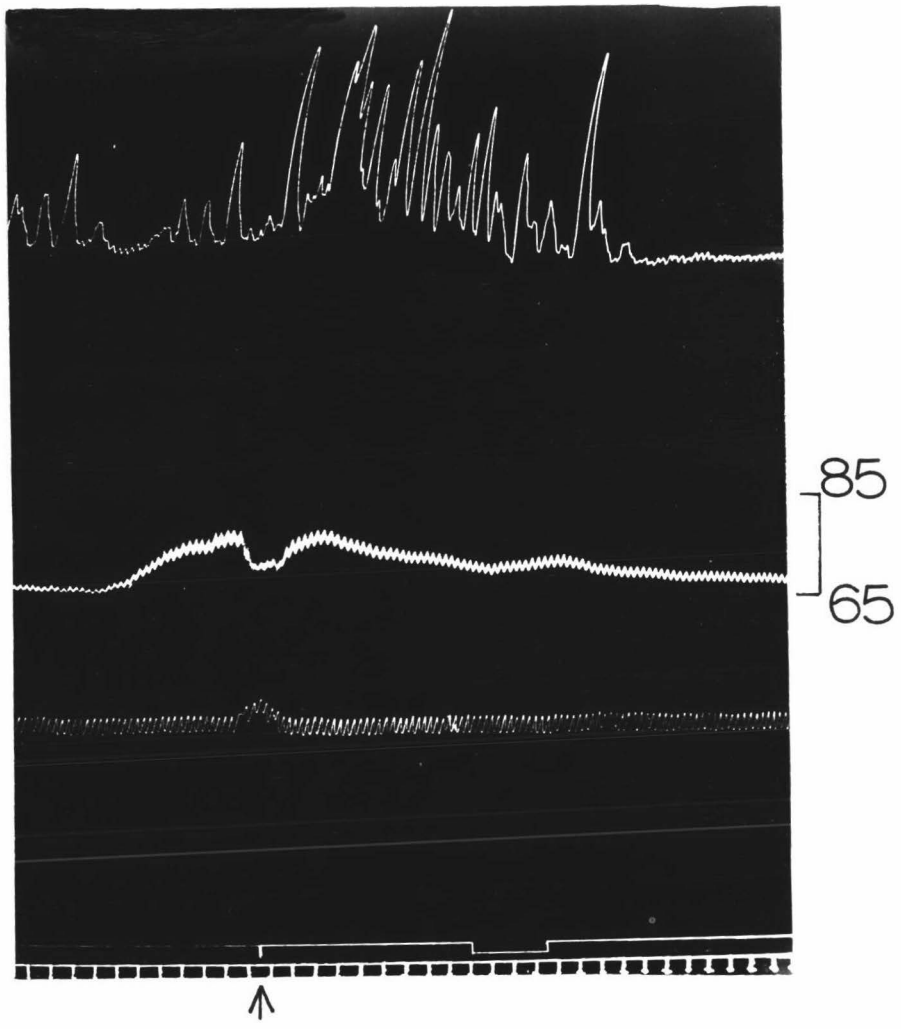


Fig. 3.3. Decerebrate cat. Response of the terminal oesophagus to section of the right vagus nerve in the neck. The left vagus nerve had been cut previously. The application of a stimulus (50/sec, 17.5V for 35 sec) to the peripheral end of the left vagus nerve cut in the neck caused an inhibition of the activity. Removal of the stimulus was followed by a strong contraction which ended the oesophageal response. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 10 sec time-marker. The signal (arrowed) marks the section of the right vagus nerve. The other signal marks the stimulation of the left vagus nerve in an efferent sense.

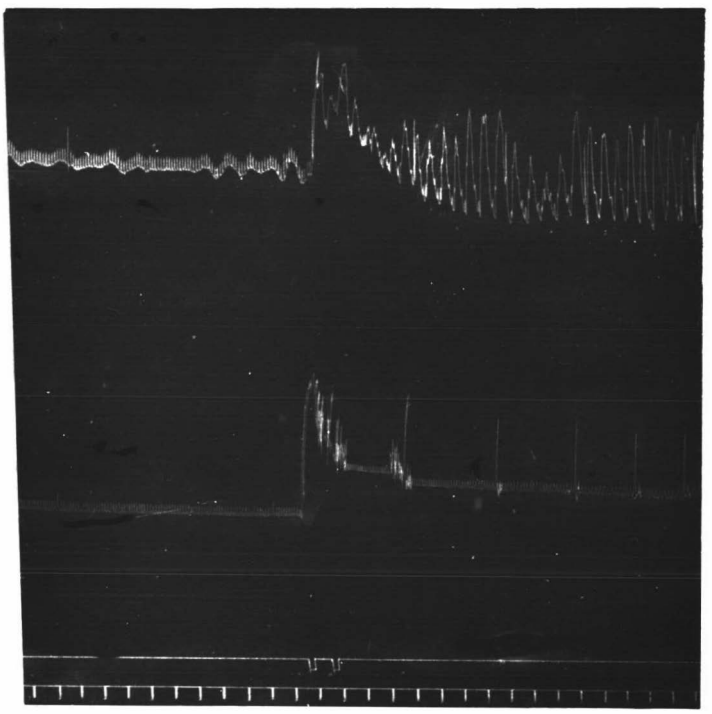


Fig. 3.4. Decerebrate cat. Response of the terminal oesophagus to section of the spinal cord at the sixth cervical segment. The increased activity of the terminal oesophagus continued for longer than 10 minutes. Records from above downwards: terminal oesophagus, respiration, signal, 30 sec time-marker. The signals mark first the spinal cord section and secondly the introduction of a cotton swab between the cut ends of the spinal cord.

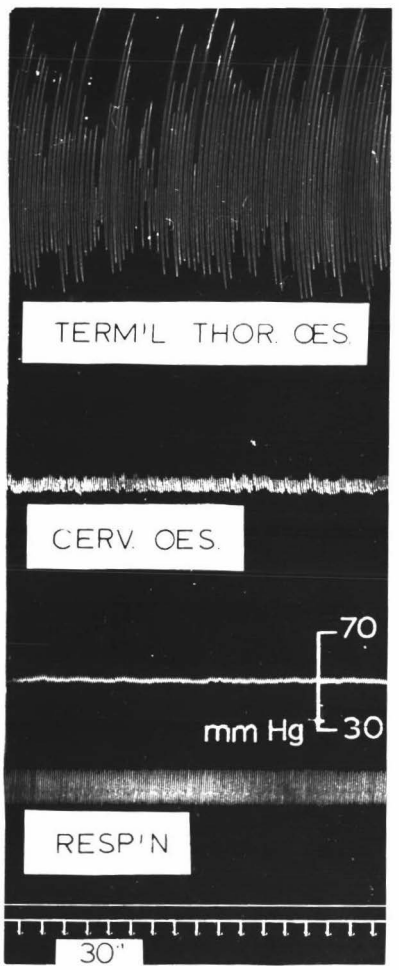


Fig. 3.5. Totally pithed preparation of a cat. Activity of the terminal oesophagus in response to a balloon distension of this region of the oesophagus. The frequency of the contractions is similar to that observed in decerebrate preparations. It appeared that the amplitude of the contractions was greater than those observed in decerebrate preparations. Records from above downwards: terminal oesophagus, cervical oesophagus, blood pressure (mm Hg), respiration, 30 sec time-marker.

fig. 3.2). A rapidly developing and short duration contraction was followed by a more slowly decaying contraction. The latter portion of the contraction was blocked by atropine which did not affect the rapid, initial portion of the contraction.

Unilateral or bilateral section of the vagus nerves in the neck of decerebrate preparations was found to increase the activity of the distended terminal oesophagus (fig. 3.3). This response was generally initiated by a contraction at all regions of the oesophagus which were being recorded from in the particular experiment. This initial response may be attributed to the mechanical stimulation during section of the nerve. The response of the terminal regions of the oesophagus continued for 2-10 minutes and resembled the original response to distension.

Section of the spinal cord at the fifth or sixth cervical segment was carried out in three preparations. In all these preparations an increased response of the distended terminal oesophagus was noted (fig. 3.4).

From the above results it appeared that the response of the terminal oesophagus to distension of itself was not dependent upon the extrinsic innervation of the oesophagus. To investigate the influence of the extrinsic nervous pathways, totally pithed preparations of cats were made. The response of the terminal oesophagus to distension of itself in such a preparation did not qualitatively differ from that obtained in the decerebrate cat (fig. 3.5). The frequency (six/minute) was approximately the same, and the amplitude of the

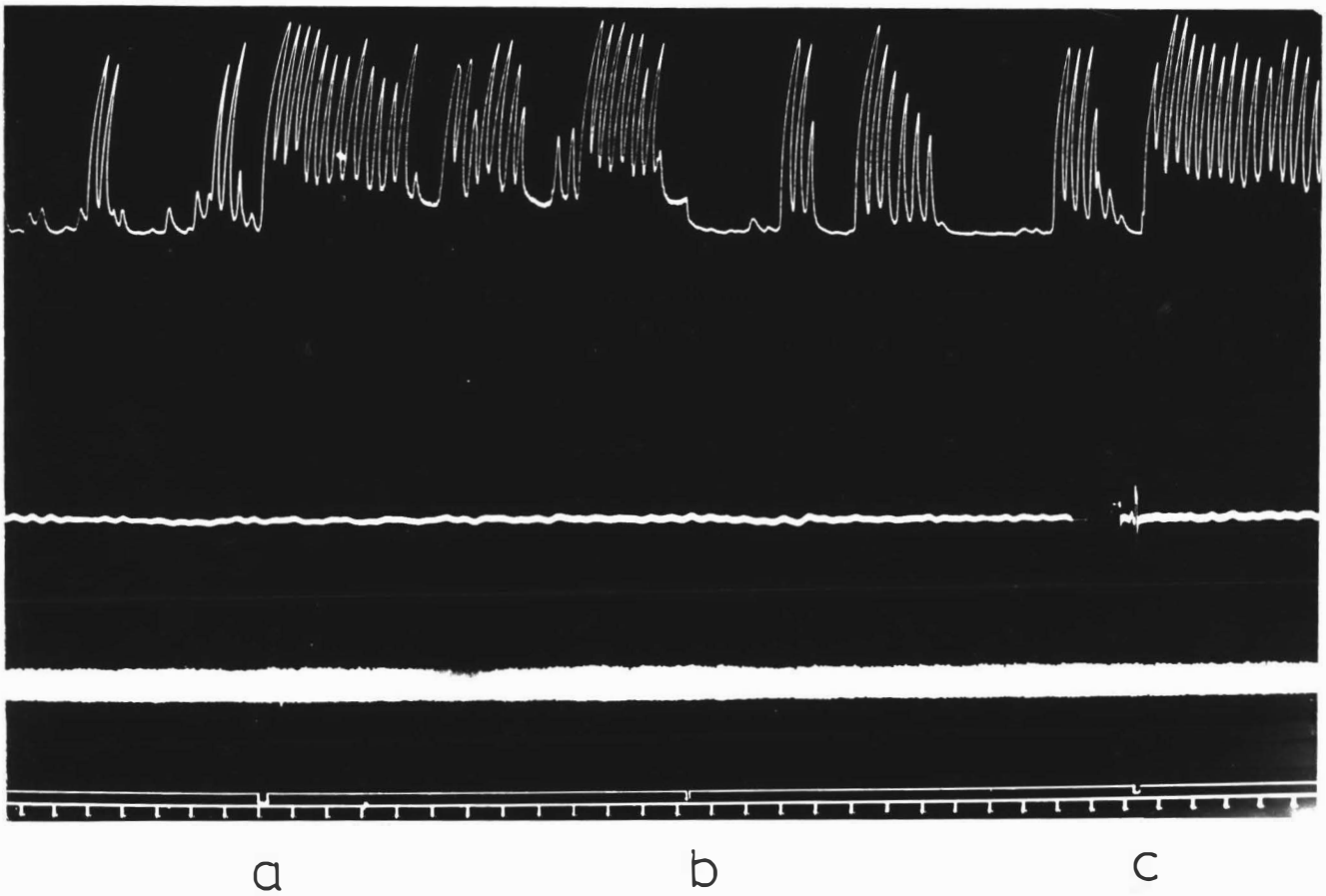


Fig. 3.6. Totally pithed preparation of a cat. The response of the terminal oesophagus to different degrees of balloon distension of this region. Increased distension continues to cause contractions, but over similar periods of time less contractions were recorded. Reduction of the distension resulted in an increased oesophageal response. Records from above downwards: terminal oesophagus, blood pressure (stabilised at 60 mm Hg by a reservoir), respiration, signal, 30 sec time-marker. Between "a" and "b" and the period following "c" the terminal oesophagus was subjected to a distension accorded by a head of 25 cm H_2O. Before "a", and between "b" and "c", the distending head was 40 cm H_2O. Base-line shifts are due to the closed nature of the recording system.

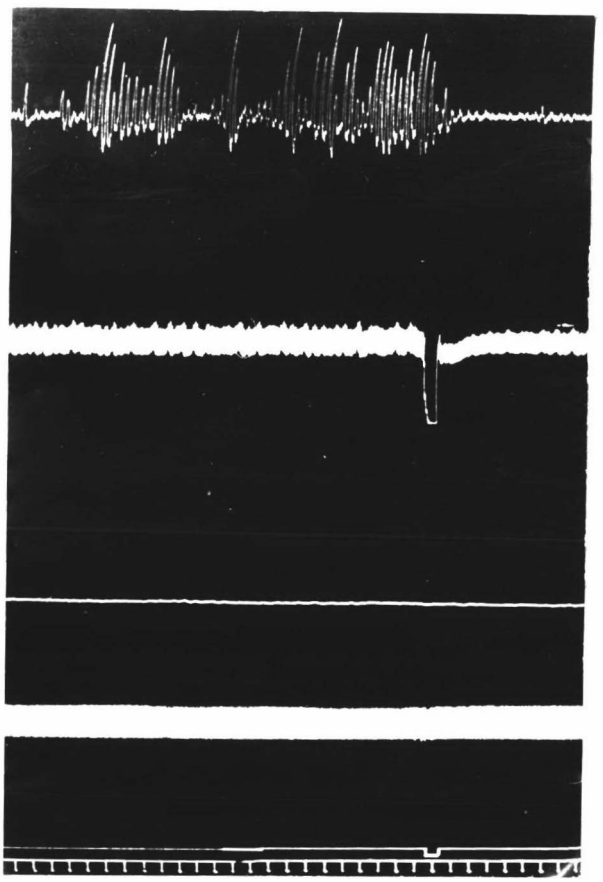


Fig. 3.7. Totally pithed preparation of a cat. Inhibition of terminal oesophageal motility in response to distension of the anterior thoracic recording balloon. Records from above downwards: terminal oesophagus, anterior thoracic oesophagus, blood pressure (stabilised at 50 mm Hg by a reservoir), respiration, signal, 30 sec time-marker. The signal marks the distension of the anterior thoracic oesophagus. The distension is maintained, but the base-line has been reset.

contractions, although difficult to measure with the recording technique used, appeared to be greater than that observed in decerebrate preparations.

The degree of balloon distension of the terminal oesophagus in totally pithed preparations was observed to alter the intensity of the contractions in this region. The greater degree of distension, although it continued to cause contractions, produced a shorter series of contractions than the lesser degree of oesophageal distension (fig. 3.6).

The inhibitory effect on terminal oesophageal motility exerted by a more cranial oesophageal distension was observed in the totally pithed preparation (fig. 3.7). The inhibition occurred 30-40 seconds after the more cranial distension was applied, and persisted until the distension was removed. The form of this response was essentially the same as that observed in decerebrate preparations (see fig. 2.14).

DISCUSSION

The reflexes described in the previous chapter which modify the terminal oesophageal response to distension appear to have their motor fibres in the vagus nerves. The facilitation of contractions of the terminal, and other more cranial, regions of the oesophagus in response to peripheral vagus stimulation, and the presence of these responses in partially isolated medullary preparations, support this conclusion.

The nature of the efferent vagal pathway to the terminal oesophagus is suggested by the form of the response and the action of pharmacological blocking agents. The smooth muscle portions of the oesophagus appear to be innervated by cholinergic muscarinic fibres. In the anterior thoracic oesophagus (striated muscle) some portions appear to be sensitive to atropine (see fig. 3.1). Whether the portion affected was smooth muscle cells or some other atropine sensitive mechanism of the striated muscle contraction was not indicated. The basic mechanism of the terminal oesophageal contractions appears to be independent of ganglionated neural pathways, yet remains susceptible to atropine (see figs. 4.4 and 4.7). This may indicate that the mechanism involved in the rhythmic contractions of the smooth muscle in response to distension has, as an integral part, a neuro-effector site, the transmitter for which is acetyl choline. The form of the extrinsic motor pathways is not shown by the evidence.

In the decerebrate preparations used, it appears that the resting discharge in motor pathways to the oesophagus is predominantly

inhibitory. This is probably a reflection of the acute nature of the preparations and the surgical, and other, modifications employed in these investigations. Removal of the extrinsic pathways by surgical or pharmacological means appeared to remove this inhibitory discharge suppressing the more basic activity which is not dependent on these extrinsic neural pathways. The independence of the terminal oesophageal contractions of extrinsic nerves is supported by the studies of achalasia (Hwang, Essex and Mann, 1947; Ingelfinger, 1956). The increased tone of this region and the associated resistance to the passage of material would indicate that the denervation which is reported to cause this condition (Ingelfinger, 1961) removes inhibitory influences from this region. This basic activity, which is subservant to the extrinsic innervation, provides an alternative drive to the sympathetic innervation. The sympathetic drive was reported to cause the increased activity with the autonomic balance due to removal of the parasympathetic effects (Manzano et al., 1964). The mechanical transection of the motor pathways is both irreversible and drastic. A more precise indication of their contribution would be obtained with a reversible and unstimulating section such as that possible with cooling of the nerve trunks.

The effects of stimulation of the peripheral end of the splanchnic nerves has not been investigated. The results of Carlson et al. (1922) and Knight (1934a) indicate that the efferent sympathetic fibres may be both excitatory and inhibitory to terminal oesophageal motility. This conclusion is supported by the facilitation and inhibition of the oesophageal contractions, stimulated by distension, in response to

adrenergic drugs (see figs. 4.9, 4.11, 4.12 and 4.13).

In totally pithed preparations, the presence of a modification of the basic oesophageal response by stimuli applied to positions other than the site of action indicates that intrinsic nervous pathways are involved. The form of these pathways may, in their most elaborate form, be ganglionated neural pathways. The pithed preparation, being essentially a preganglionic preparation, allows the following forms of neural pathway to exist: two neuron parasympathetic pathway; two neuron sympathetic pathway; axon reflex pathway; and direct intramural conduction. The investigation of these possibilities would be difficult, but is essential to the understanding of the manner in which the extrinsic nervous pathways affect this basic activity. The site and form of the conjunction of the extrinsic nerves and intrinsic mechanism of the basic activity is also of great interest.

The discussion above raises the question of the level to which the oesophageal innervation must be simplified to, to study the form and degree of extrinsic control. The use of <u>in vitro</u> experiments to study these basic mechanisms may be profitable, provided the oesophageal muscle can be isolated without excessive damage to the intramural connections which may be involved. The presence of the sympathetic post-ganglionic pathways in the totally pithed preparations must be considered as contributing to the motility and the modification of this in these preparations. A further analysis by the use of specific adrenergic blocking drugs, coupled with the ablation of the

sympathetic ganglia in totally pithed preparations, may give an indication of the contribution of the sympathetic efferents to this basic activity.

Chapter 4

THE RESPONSES OF THE OESOPHAGUS OF THE CAT TO SELECTED PHARMACOLOGICAL AGENTS

INTRODUCTION

Pharmacologic investigations on the oesophagus have been reported in a number of species and under a variety of conditions. In man, observations have been made in normal conscious subjects, patients suffering from a variety of oesophageal disorders, and in subjects who have undergone varying degrees of sympathectomy and section of the vagus nerves. Isolated strips of the oesophagus have been obtained from adult humans during thoracotomy and post mortem from human foetuses. The responses of the oesophagus in anaesthetised and conscious experimental animals have also been studied.

In both man and experimental animals most work has been directed towards an investigation of the action of cholinergic and adrenergic drugs, drugs which block the action of these agents, and of drugs which affect ganglionic or neuromuscular transmission. In all reports of drug action on the oesophagus a number of factors such as the following have to be borne in mind:-

Route of administration. A pure effect of a given substance may be difficult enough to obtain in an anaesthetised subject when given by the intravenous route; it may be impossible to demonstrate when given by the intramuscular or subcutaneous

route. In these latter instances there is ample time for compensatory mechanisms or reactions to develop as the agent is slowly absorbed and presented to the reacting tissue.

Anaesthetic agents may interfere with the responses due to a particular substance.

Few of the so-called blocking agents are specific in their actions.

Even the intravenous injection of a substance may lead to confusing results because of: effects on distant structures (e.g., central nervous system), effects such as a facilitation of receptors, stimulation of reflex compensatory reactions masking the pure reactions to the agent, concomitant changes in blood supply and therefore oxygenation. (This is of particular importance in smooth muscle which exhibits strong contractions when anoxia develops.)

These comments, which are both cursory and perhaps better fitted for a discussion, are thought to be desirable to preface a survey of observations such as those that follow in this introduction to the pharmacological investigations reported later in this chapter.

Cholinergic drugs have been reported to exert only a mild action on the oesophagus. Sleisenger, Steinberg and Almy (1953 - cited by Ingelfinger, 1958) reported that these agents exert no marked effects on motility in response to distension or oesophageal mouth to stomach transit time in anaesthetised or conscious human patients. However,

cholinergic drugs administered by various routes in a variety of subjects have been reported to: cause a spasm of the terminal segment of the oesophagus (Bettarello, Tuttle and Grossman, 1960; Schenk and Fredrickson, 1961); increase the reflex activity of the oesophagus (Bühler-Vieira, 1963); and cause a shortening of the oesophagus (Schenk and Fredrickson, 1961). Physostigmine and neostigmine have been reported to be excitatory to oesophageal activity, especially in the terminal regions (Robins and Jankelson, 1926; Ellis et al., 1960).

Ellis et al. (1960), with human oesophageal muscle strips, recorded contractions of both circular and longitudinal muscle preparations from the distal and thoracic regions of the oesophagus in response to acetyl choline. This report is supported by Schenk and Fredrickson (1961), with the exception that they report acetyl choline as having no effect on the circular muscle of the distal oesophagus.

Oesophageal motility can be easily altered by cholinergic blocking agents. Carlson et al. (1922) showed that the frequency of contractions in the lower oesophagus in the cat, dog, and rabbit, in response to distension, was reduced with the administration of atropine. The contractile response to peripheral vagus or splanchnic stimulation was also blocked by atropine. In man, cholinergic blocking agents (banthine, atropine and murel) disrupt the propulsive nature of oesophageal contractions before the contractions are blocked (Ingelfinger et al., 1954; Flood and Fink, 1960). The reports on the responses of the terminal oesophagus are varied. Increased strength of contracture (Ingelfinger et al., 1954) and increased relaxation associated with

reflux of stomach contents into the oesophagus (Robins and Jankelson, 1926; Bettarello *et al.*, 1960) have both been noted after the intravenous injection of atropine. The contractions of the thoracic oesophagus, in response to a distension, of the sheep are susceptible to the blocking action of D-tubocurarine (Sellers and Titchen, 1959).

In vitro experiments with human (Ellis *et al.*, 1960), guinea pig (Mann, Schlegel, Ellis and Code, 1962), and chick (Bowman and Everett, 1964) muscle strips from the terminal oesophagus indicate that atropine blocks the responses to nerve stimulation. The contraction in response to nicotine was also abolished by atropine (Ellis *et al.*, 1960).

Curare has been reported to block the effects of vagus stimulation on the isolated strips of rat oesophagus (Hughes and McDowall, 1954). Succinyl choline does not block the effect of acetyl choline on muscle strips from the lower human oesophagus.

The effects of ganglion blocking drugs on the oesophagus have not received much attention from workers in this field. O'Mallane (1954 - cited by Ingelfinger, 1958) reported that a ganglion blocking agent had no effect on the threshold of reflux of stomach contents to the oesophagus. This led him to discount the existence of an active sphincter in the lower oesophagus. A report by Flood and Fink (1960) indicates that various ganglion blocking drugs may cause the disarrangement of an ordered contraction wave. Bowman and Everett (1964), using an isolated preparation of the chick oesophagus, found that nerve stimulation was not effectively blocked by ganglion blocking

agents. They suggest that ephaptic transmission between the pre- and post-ganglionic neurons is responsible for this result (see Martin and Pilar, 1963a, b).

At this point it is opportune to mention the phenomena of cardiospasm, or achalasia. This disease condition appears to be due to a degeneration or destruction (in the case of the endemic South American mega-oesophagus - Bühler-Vieira, 1963; Ingelfinger, 1963) of the ganglion cells of Auerbach's plexus. Patients with this condition have been found to be hypersensitive to cholinergic drugs. The reason proposed for this phenomena is the sensitisation of the post-ganglionically, cholinergically, denervated smooth muscle (Kramer and Ingelfinger, 1951). The symptoms of this condition are: retention of fluid in the oesophagus, lack of tone in the body of the oesophagus, and hyperactivity to cholinergic drugs. The condition of achalasia is reported in many places in the literature. The accounts of Kramer and Ingelfinger (1949), Ingelfinger et al. (1954), Hightower, Olsen and Moersch (1954), and Bühler-Vieira (1963), cover the main points of the pharmacology of achalasia of the oesophagus of humans.

The reports of the effects of adrenergic drugs on motility of the oesophagus are variable. Carlson's dogs, when unanaesthetised, responded to adrenaline by a contraction of the terminal oesophagus. When the same dogs were anaesthetised, the terminal oesophagus relaxed. Excitatory effects of adrenergic drugs have been reported in the pigeon (Hanzlik and Butt, 1928), cat (Schenk and Fredrickson, 1961),

guinea pig (Bailey, 1965), and human (Ellis *et al.*, 1960). The above workers found the excitatory effects in both circular and longitudinal layers of the terminal oesophagus.

Inhibitory effects of adrenergic drugs have been reported. The hyperactivity of the thoracic oesophagus associated with Chaga's disease is depressed by adrenaline (Bühler-Vieira, 1963). Hughes (1957), investigating the responses of the human foetal oesophagus to nerve stimulation, showed that sympathetic stimulation inhibited spontaneous activity. Similar sympathetic stimulation relaxed the contraction of the oesophagus induced by parasympathetic stimulation. Nor-adrenaline has been reported to have the same effects as adrenaline on isolated strips of the human terminal oesophagus (Ellis *et al.*, 1960) and the guinea pig terminal oesophagus (Bailey, 1965). The report of Bailey (1965) on the action of isoprenaline on the smooth muscle of the guinea pig oesophagus is alone and suggests that this amine acts on beta receptors to cause a relaxation.

The action of adrenergic blocking agents on the oesophagus has not been extensively studied. The *in vitro* work of Ellis *et al.* (1960), using muscle strips from the human distal oesophagus, showed that phentolamine blocked the contraction but did not affect the relaxation caused by adrenaline. On the basis of this observation, they postulated a population of receptors to adrenaline which was greater in the area of functional relaxation, the terminal oesophagus, and less at higher levels of the oesophagus. This report, coupled with the observation of Hanzlik and Butt (1928), that the effects of

sympathetic nerve stimulation were not affected by ergotoxine, represents the studies made on the effects of sympathetic blocking drugs on oesophageal motility.

The evidence cited from the literature, viewed in the light of the comments made at the start of this section, indicated that a systematic investigation of the responses of the oesophagus to autonomic stimulating and blocking drugs was needed. By means of close intra-arterial injections of drugs and a consideration of the latencies of the responses obtained, the oesophageal reactions presented are considered to be attributable to the agents injected. The responses to drugs injected into anaesthetised, decerebrate and totally pithed preparations have permitted the effects of the anaesthetic and autonomic drugs on the central nervous system to be assessed.

METHODS AND MATERIALS

Decerebrate, anaesthetised and totally pithed preparations of cats have been used to study the responses of the oesophagus to selected pharmacological agents. (See Methods and Materials, Chapter 2, for details of animal preparation.)

To ensure a close association of the administered drug and the oesophagus, injections were made by a cannula placed in the thoracic aorta. A polyethylene tube was passed from the femoral artery to lie in the thoracic aorta. In some experiments a cannula was passed down the carotid artery to lie at the junction of the common carotid arteries. This enabled small volumes of low concentrations of drugs to be administered almost directly to the oesophagus. The effects of the injected drugs on structures other than the oesophagus, such as the heart and central nervous system, were thus minimised.

Three different recording systems have been used:-

Water-filled balloons of approximately 3 cc volume. This system, as described in the Methods and Materials section, Chapter 2, (isotonic system), allowed a distension to be applied to the oesophagus at various levels.

Large air-filled balloons which recorded motility on the Sanborn electro-physiograph (isometric system). This system also allowed the oesophagus to be distended to note the effects of drugs which blocked the motility induced by distension. The effects of drugs which potentiated motility, or enhanced receptors, were also

studied on this background of excitatory oesophageal distension.

Very small air-filled balloons, dimensions 10 mm long and diameter 5 mm. This method allowed the effects of drugs on the quiescent, unstimulated oesophagus to be observed. These balloons recorded motility by activating transducers which recorded by way of the Sanborn electro-physiograph.

The doses of drugs injected are expressed as a weight of the salt. All injections and dilutions were made in 0.9% w/v saline, unless otherwise stated.

The drugs used were:-

Cholinergic drugs:

Acetyl choline hydrochloride (Merche). Injected as a 5% Na_2HPO_4 solution of the powder.

Physostigmine salicylate, B.P. (MacFarlane Smith Ltd.). Injected as a 5% Na_2HPO_4 solution of the powder.

Nicotine (40% aqueous solution of the alkaloid). Injected as a 0.9% saline dilution of the aqueous alkaloid.

Cholinergic blocking agents:

Atropine sulphate, B.P. (British Drug Houses Ltd.). Injected as a 0.9% saline solution of the tablet.

D-tubocurarine chloride, B.P. ("Tubarine", Burroughs Wellcome and Co., London.). Injected as the manufacturer's solution diluted in 0.9% saline.

Adrenergic drugs:

Adrenaline tartrate, B.P. (May and Baker Ltd., Dagenham.). Injected as the manufacturer's solution diluted in 0.9% saline.

L-Nor-adrenaline (Koch-Light Lab.). Injected as a 5% Na_2HPO_4 solution of the powder.

Isoprenaline sulphate, B.P. (Burroughs Wellcome and Co. (N.Z.) Ltd.). Injected as a 5% Na_2HPO_4 solution of the powder.

Adrenergic blocking agents:

Tolazoline, 2-benzyl-imidazoline-hydrochloride, B.P. ("Priscol", CIBA Laboratories Ltd.). Injected as the manufacturer's solution diluted in 0.9% saline.

Ganglionic blocking agents:

Hexamethonium bromide, B.P.C. ("Vegolyson", May and Baker Ltd., Dagenham.). Injected as the manufacturer's solution diluted in 0.9% saline.

Nicotine (40% aqueous solution of the alkaloid). Injected as a 0.9% saline dilution of the aqueous alkaloid.

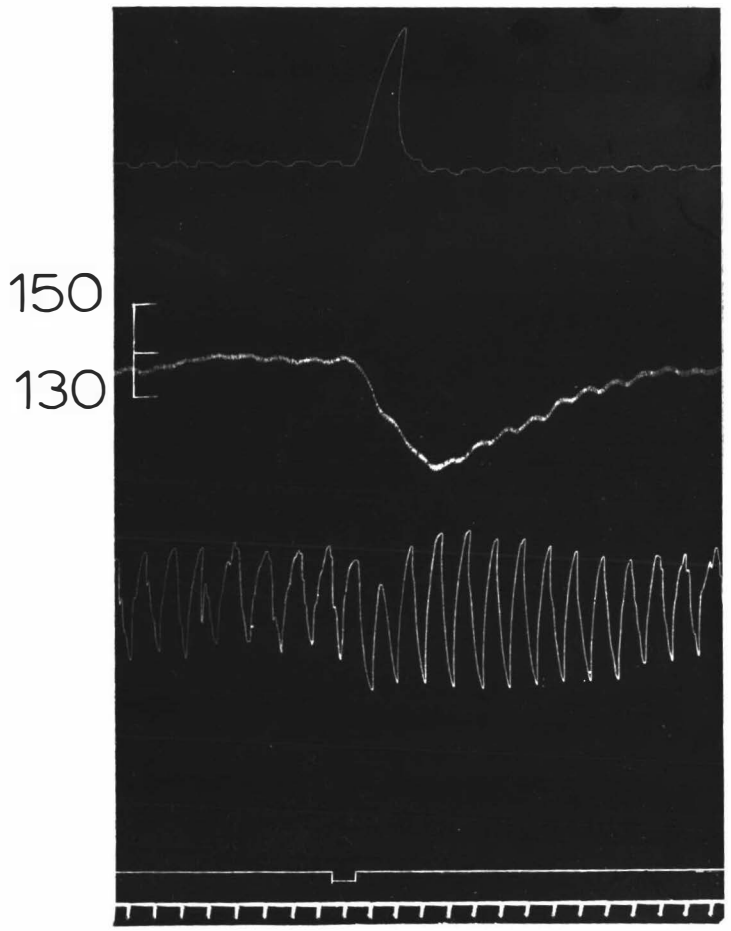


Fig. 4.1. Anaesthetised cat. Chloralose 70 mgm/kgm. Response of the mid-thoracic oesophagus to an intra-arterial injection (thoracic aorta) of 0.1 μgm acetyl choline. Records from above downwards: mid-thoracic oesophagus, femoral blood pressure, respiration, signal, 5 sec time-marker.

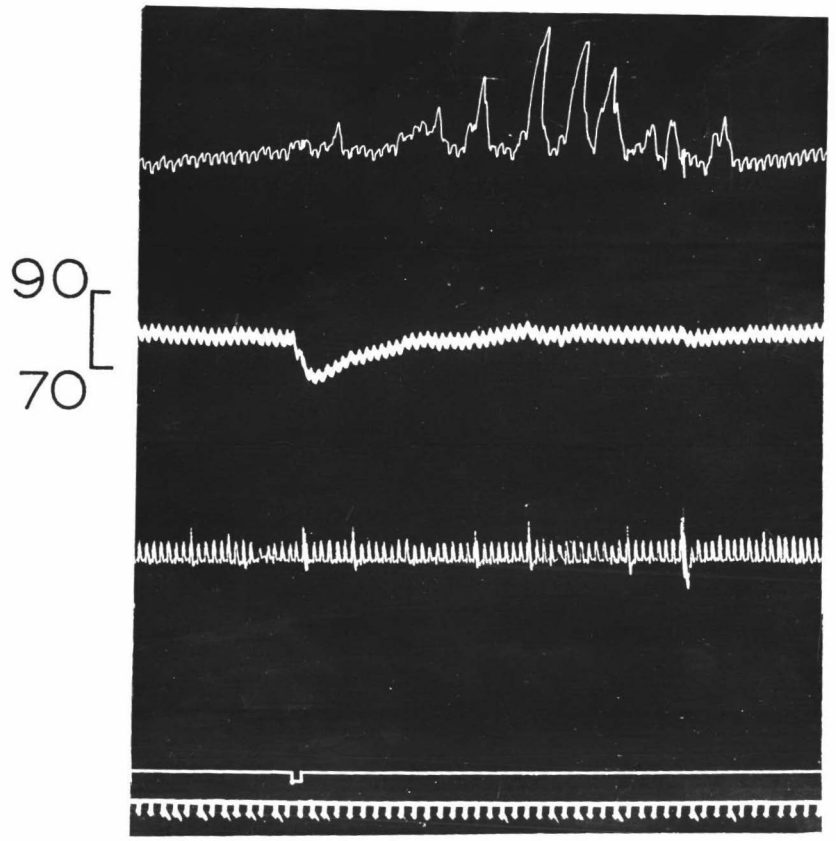


Fig. 4.2. Anaesthetised cat. Chloralose 70 mgm/kgm. Response of the distended mid-thoracic oesophagus to the intra-arterial injection (thoracic aorta) of 0.5 μgm/kgm acetyl choline. Records from above downwards: mid-thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 10 sec time-marker.

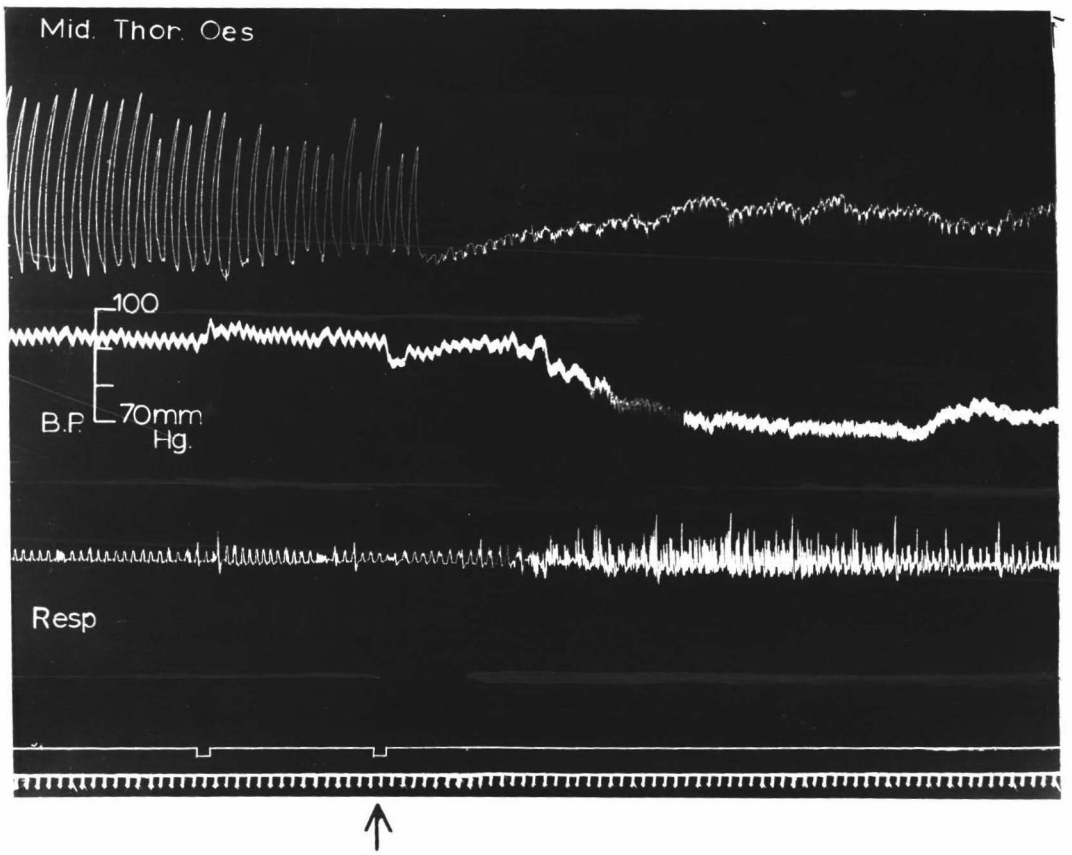


Fig. 4.3. Anaesthetised cat. Chloralose 70 mgm/kgm. Inhibition of mid-thoracic oesophageal contractions stimulated by distension of this region following the intravenous intra-arterial injection of 0.23 mgm/kgm eserine. Records from above downwards: mid-thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 10 sec time-marker.

RESULTS

The responses of the oesophagus to some pharmacological agents have been investigated in anaesthetised, decerebrate, partially isolated medullary, and totally pithed preparations of cats. Routes of administration of the drugs have been intravenous and close intra-arterial. In all instances attempts have been made to reduce the vascular effects of the drugs to a minimum.

The close intra-arterial injection of acetyl choline caused a single contraction of the mid-thoracic oesophagus. The contraction occurred 3-5 seconds after the commencement of the injection. The oesophageal response occurred at the same time as both bradycardia and hypotension. The amplitude of the contraction varied according to the dose injected, and responses were obtained with 5.0 - 0.005 µgm/kgm doses. Fig. 4.1 shows the contraction recorded after the injection of 0.1 µgm acetyl choline into the thoracic aorta. A series of contractions could also be evoked by acetyl choline. The response started 5-10 seconds after the commencement of the injection and was of variable duration (fig. 4.2). Such a response was generally noted after the oesophagus had been subjected to an inhibition or was subjected to a greater degree of distension. The initial contraction due to acetyl choline appeared to trigger the response to distension.

Eserine injected intra-arterially was found to have an initial excitatory effect which was followed by an inhibition of the mid-thoracic oesophageal contractions in response to distension (fig. 4.3).

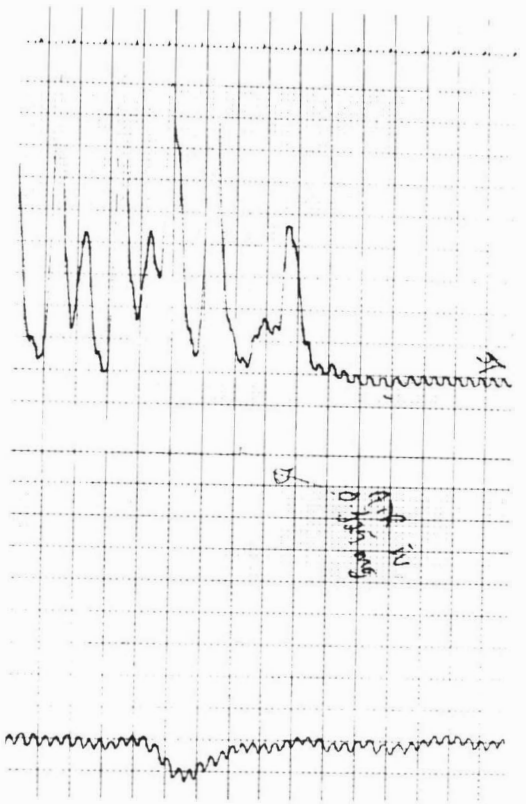


Fig. 4.4. Decerebrate cat. Terminal oesophageal response to the intravenous injection of 0.1 mgm/kgm atropine. Records from above downwards: 10 sec time-marker, terminal oesophagus, signal, respiration.

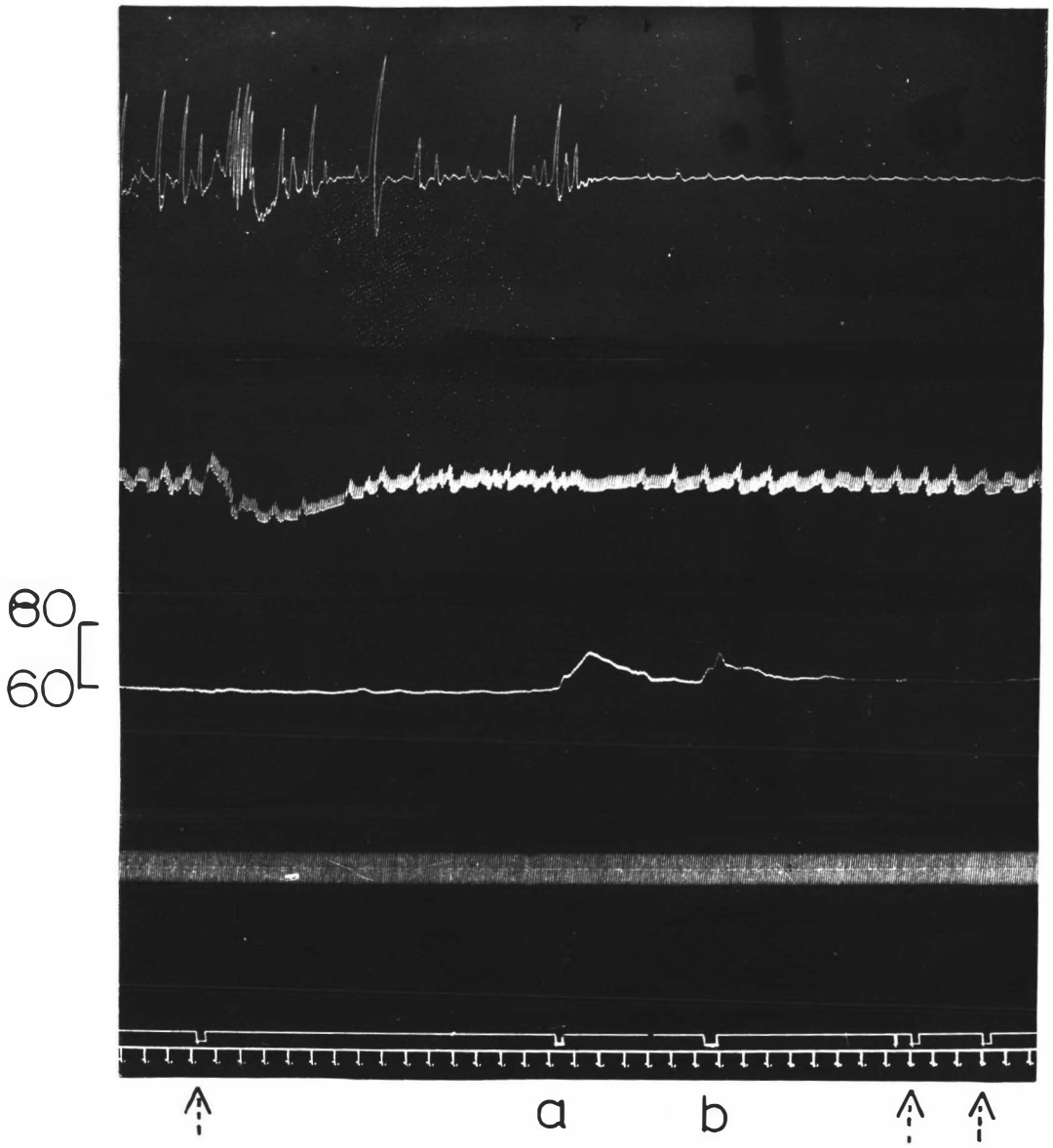


Fig. 4.5. Totally pithed preparation of a cat. Block of terminal oesophageal contractions following the intravenous injection of 1 mgm/kgm atropine. The peripheral end of the left vagus nerve was stimulated before and after administration of atropine. Records from above downwards: terminal oesophagus, anterior thoracic oesophagus, blood pressure (mm Hg stabilised by a reservoir), respiration, signal, 30 sec time-marker. The arrowed signals mark the application of the peripheral vagus stimulation: 30/sec, 12.5V for 10 sec. "a" marks the injection of 1 mgm/kgm atropine, "b" marks the injection of a further 2 mgm/kgm atropine.

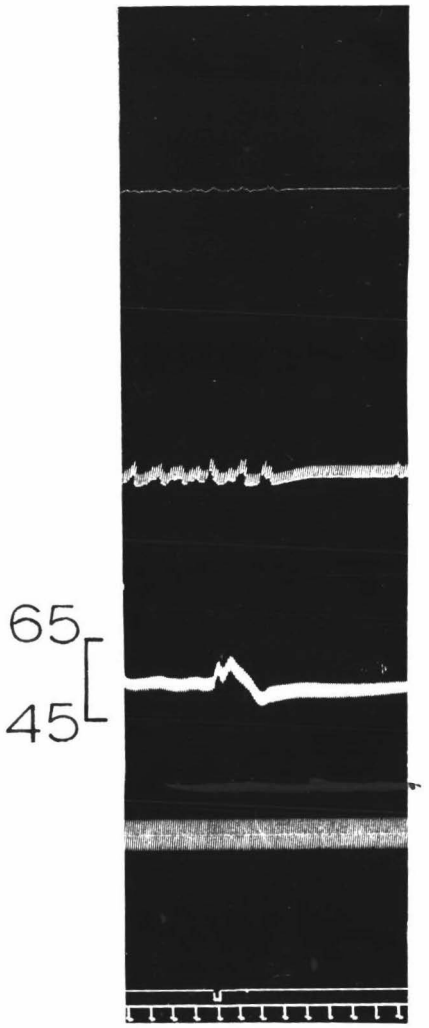


Fig. 4.6. Totally pithed preparation. The response of the distended anterior thoracic oesophagus to the injection of 1 mgm/kgm D-tubocurarine. 3 mgm/kgm atropine had been injected intravenously 10 minutes previously. Nicotine (1.1 ml 40% solution) had also been administered. Records from above downwards: terminal oesophagus, anterior thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker.

The inhibition of contractions occurred 30-40 seconds after commencement of the injection and continued for greater than ten minutes. Eserine was administered in doses of 0.1 - 0.5 mgm/kgm. The excitatory effect of eserine was more evident after injection of a small dose.

Atropine caused the disappearance of both the contractions of the smooth muscle portions of the oesophagus evoked by distension of itself and those produced by stimulation of the peripheral end of a vagus nerve cut in the neck. Injected intravenously in doses (0.2 - 1 mgm/kgm) to anaesthetised, decerebrate, medullary and totally pithed preparations, a complete cessation of contractions was seen within 30-60 seconds (figs. 4.4 and 4.5). The contractile response of the skeletal muscle regions of the oesophagus to distension and stimulation of the peripheral end of a vagus nerve was largely unaltered (see figs. 3.1 and 3.2).

The intravenous injection of D-tubocurarine blocked the contractile response of the skeletal muscle portions of the oesophagus. Following the injection of 1 mgm/kgm atropine intravenously, a contractile response of the anterior thoracic oesophagus to stimulation of the peripheral end of a vagus nerve remained. This was blocked by 0.1 mgm/kgm D-tubocurarine injected intravenously (fig. 3.1). Distension of the cranial thoracic oesophagus in a totally pithed preparation caused a rhythmic tonus change of this region. This response persisted after the intravenous injection of a 1 mgm/kgm and a 2 mgm/kgm dose of atropine. A dose of nicotine (0.37 mgm/kgm of a 40% solution) had also been administered. Stimulation of the peripheral end of a vagus

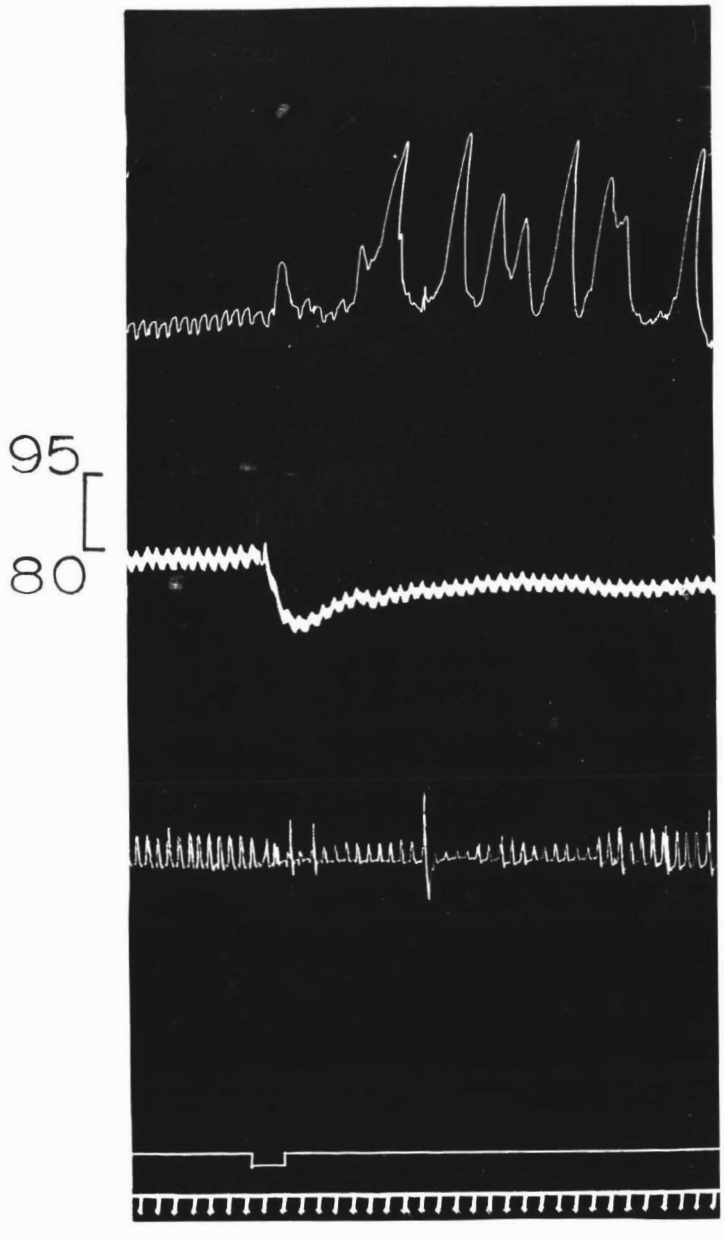


Fig. 4.7. Anaesthetised cat. Chloralose 70 mgm/kgm. Response of the mid-thoracic oesophagus to the intravenous injection of 1 mgm/kgm hexamethonium. The ganglion block produced may be interpreted as an unstimulating release of the oesophageal muscle from the pre-ganglionic innervation. Records from above downwards: mid-thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 10 sec time-marker.

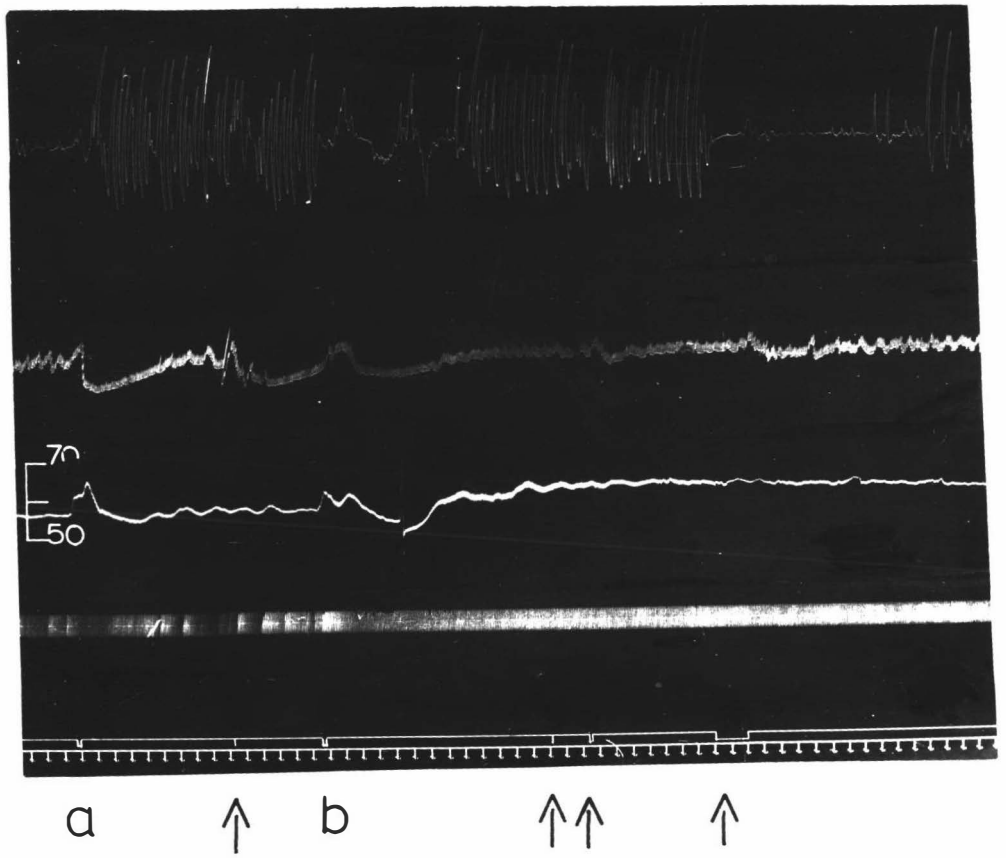


Fig. 4.8. Totally pithed preparation of a cat. Comparison of the effects of a low and high dose of nicotine on the terminal and anterior thoracic regions of the oesophagus. The initial dose (0.1 ml 40% solution) stimulated a series of contractions of the terminal oesophagus; the tone of the anterior thoracic oesophagus was inhibited. A further dose (1 ml 40% solution) of nicotine caused an inhibition of terminal oesophageal contractions. Stimulation of the peripheral end of the vagus nerve cut in the neck continued to cause an inhibition of terminal oesophageal contractions after the administration of nicotine. Records from above downwards: terminal oesophagus, anterior thoracic oesophagus, blood pressure (mm Hg stabilised by a reservoir), respiration, signal, 30 sec time-marker. "a" marks the intravenous injection of 0.1 ml 40% solution of nicotine, "b" marks the intravenous injection of 1 ml 40% nicotine solution. The arrowed signals mark the peripheral vagus stimulation: 30/sec, 12.5V for the periods indicated.

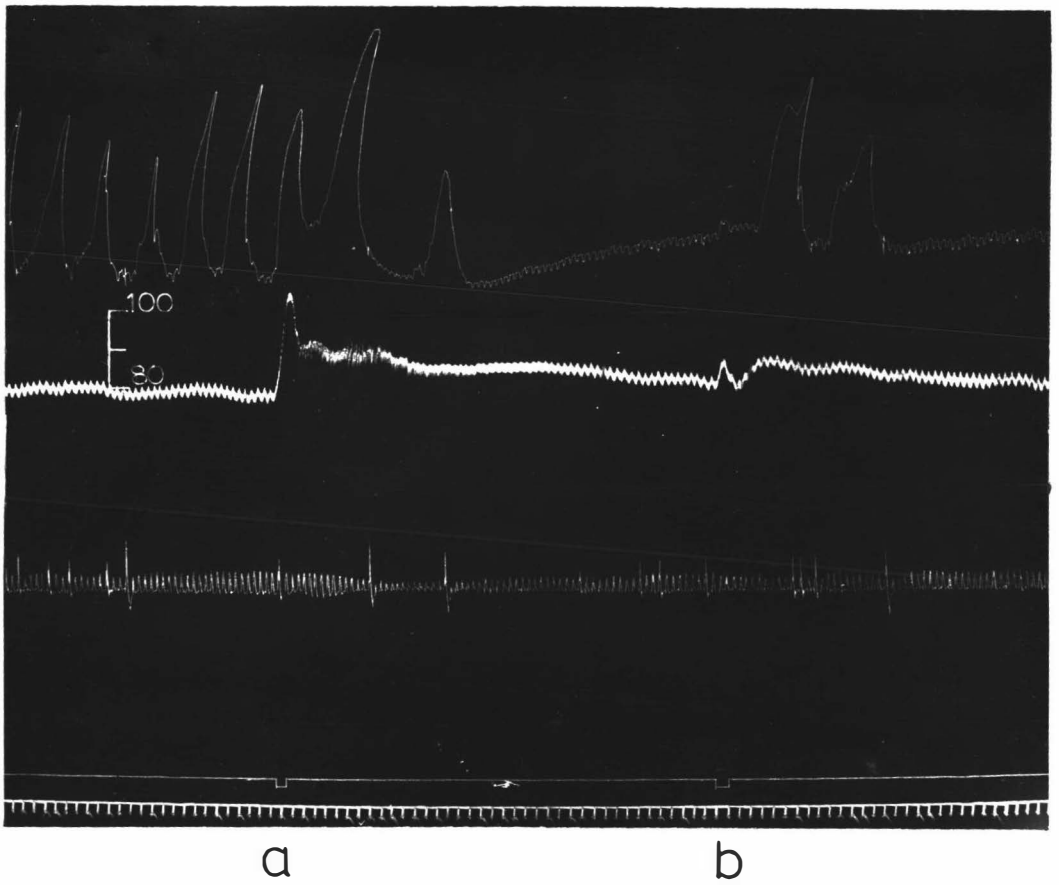


Fig. 4.9. Anaesthetised cat. Chloralose 70 mgm/kgm. Record of mid-thoracic oesophagus showing both the excitatory and inhibitory effects of adrenaline on this region of the oesophagus. Records from above downwards: mid-thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 10 sec time-marker. "a" marks the injection of 5 μgm/kgm adrenaline (intra-arterial, thoracic aorta), "b" marks the injection of 0.5 μgm/kgm adrenaline.

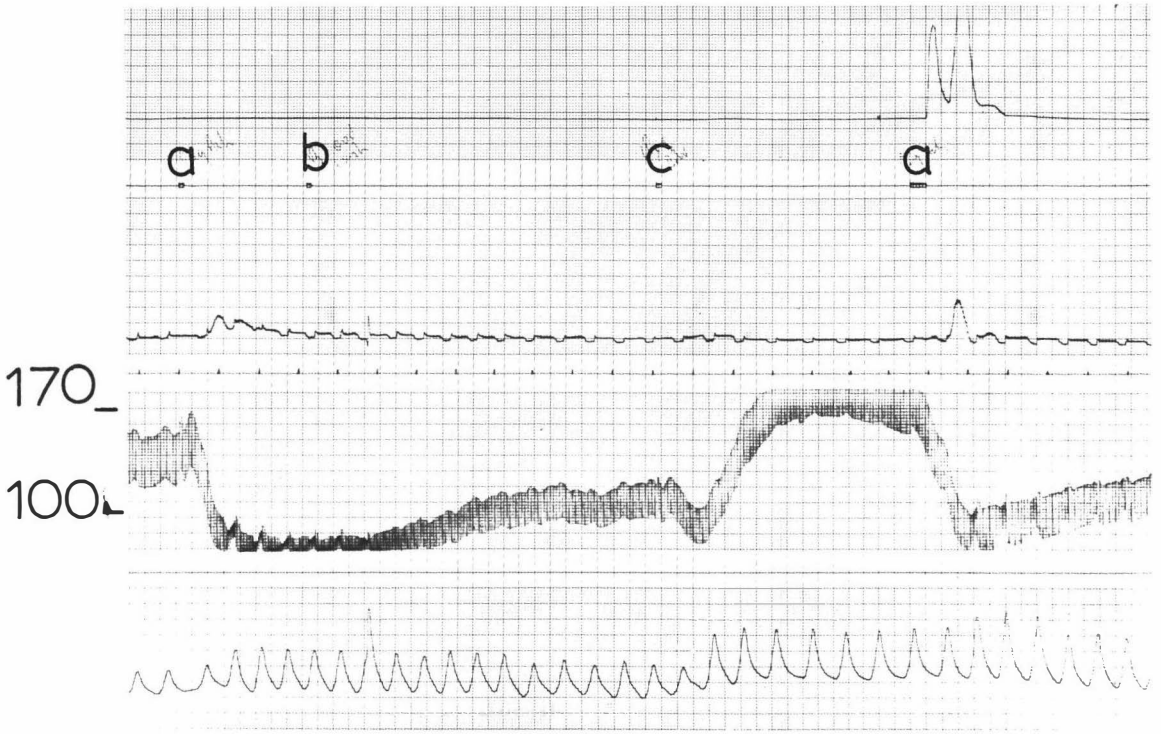


Fig. 4.10. Anaesthetised cat. A comparison of the effects of injecting 10 µgm acetyl choline intra-arterially alone and following the injection of 10 µgm adrenaline intra-arterially (thoracic aorta). Records from above downwards: caudal cervical oesophagus, signal, mid-thoracic oesophagus, 5 sec time-marker, blood pressure, respiration. Signal "a" marks 10 µgm acetyl choline (I.A.), "b" marks saline wash of intra-arterial cannula, "c" marks 10 µgm adrenaline (I.A.), "a" marks 10 µgm acetyl choline (I.A.)

nerve was without effect. This rhythmic activity was blocked by the intravenous injection of 1 mgm/kgm D-tubocurarine (fig. 4.6).

In the decerebrate cat, hexamethonium bromide administered intravenously, in doses of 0.2 - 2 mgm/kgm, had an excitatory action on the distended smooth muscle portions of the oesophagus. Fig. 4.7 shows the effect of 1 mgm/kgm hexamethonium on the terminal thoracic oesophagus. The hypotension developed within five seconds and the increased activity within 60 seconds.

Nicotine, injected in doses of 0.01 - 0.03 mgm/kgm, was excitatory to the activity of the terminal oesophagus in both totally pithed and decerebrate preparations. Higher doses (0.5 mgm/kgm) were found to cause an inhibition of contractions which lasted for up to five minutes. Contractions of this region of the oesophagus returned after this period (fig. 4.8). Stimulation of the peripheral end of a vagus nerve cut in the neck continued to exert an inhibitory effect on the contractions of the terminal oesophagus (see fig. 4.8).

Adrenaline administered as a close intra-arterial injection had both an excitatory and inhibitory effect on the smooth muscle portions of the oesophagus. The intra-arterial injection of adrenaline in doses of 1-5 μgm, when the oesophagus was contracting rhythmically in response to a distension, usually caused an initial excitation which was followed by an inhibition (fig. 4.9). The excitation was apparent 30-50 seconds after the injection and the inhibition appeared 100-130 seconds after the injection. A similar injection made when the oesophagus was not contracting was generally excitatory. The

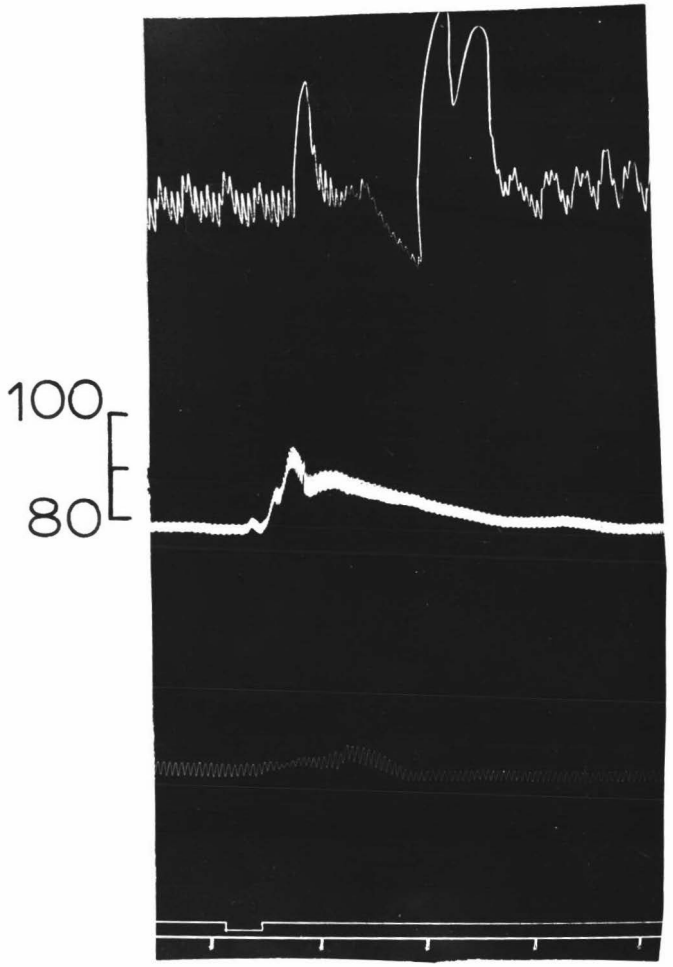


Fig. 4.11. Decerebrate cat. Response of the terminal oesophagus to 5 µgm/kgm nor-adrenaline. The response appears to comprise both excitatory and inhibitory effects. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker.

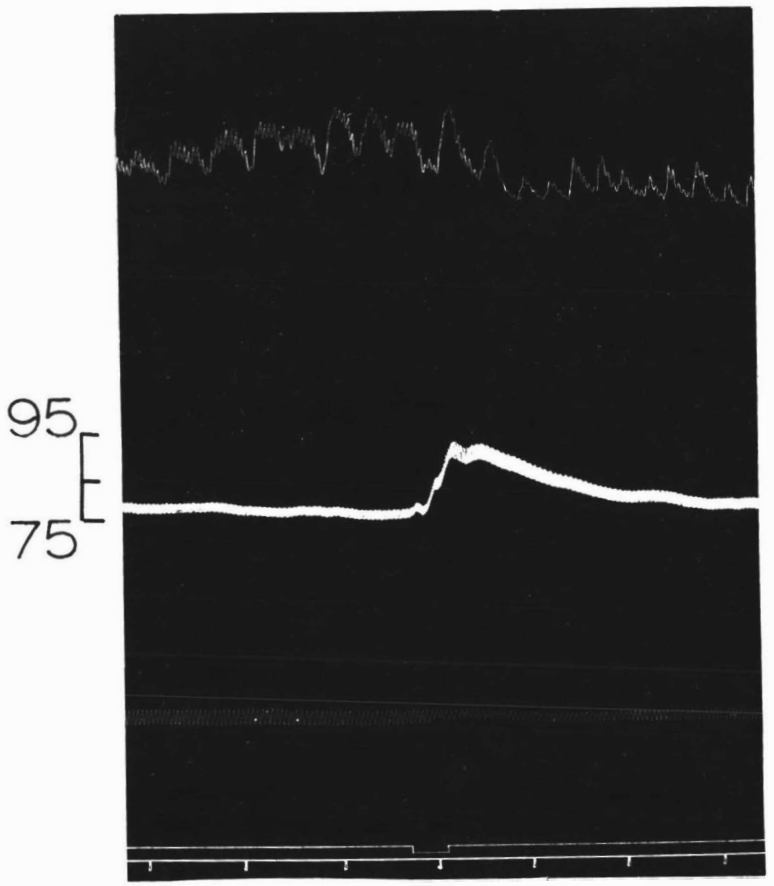


Fig. 4.12. Decerebrate cat. Inhibition of terminal oesophageal tone and strength of contraction following the intravenous injection of 5 µgm/kgm nor-adrenaline. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker.

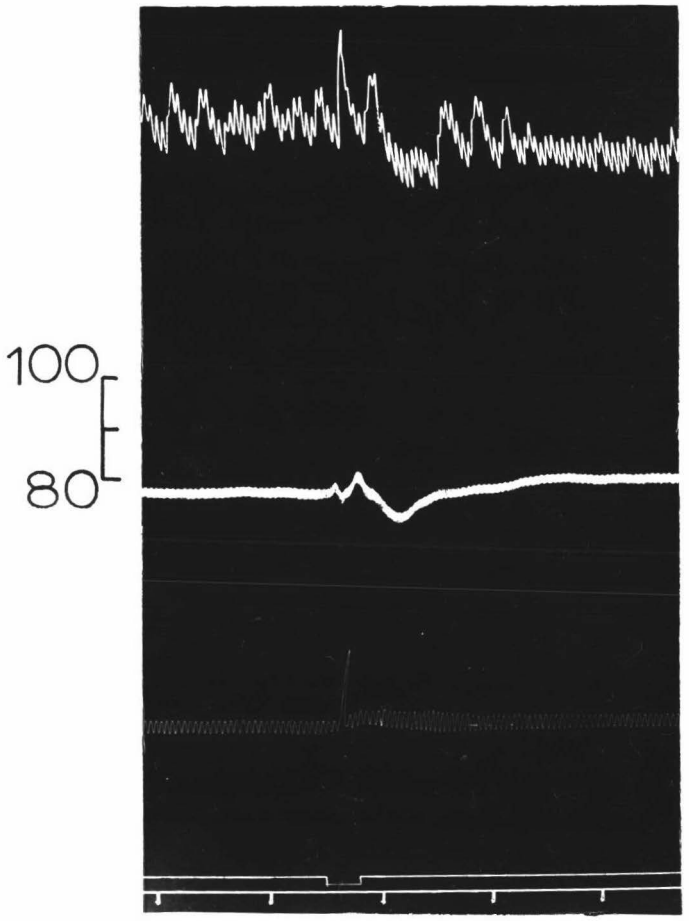


Fig. 4.13. Decerebrate cat. Inhibition of terminal oesophageal contractions following the intravenous injection of 5 μgm/kgm isoprenaline. Records from above downwards: terminal thoracic oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker.

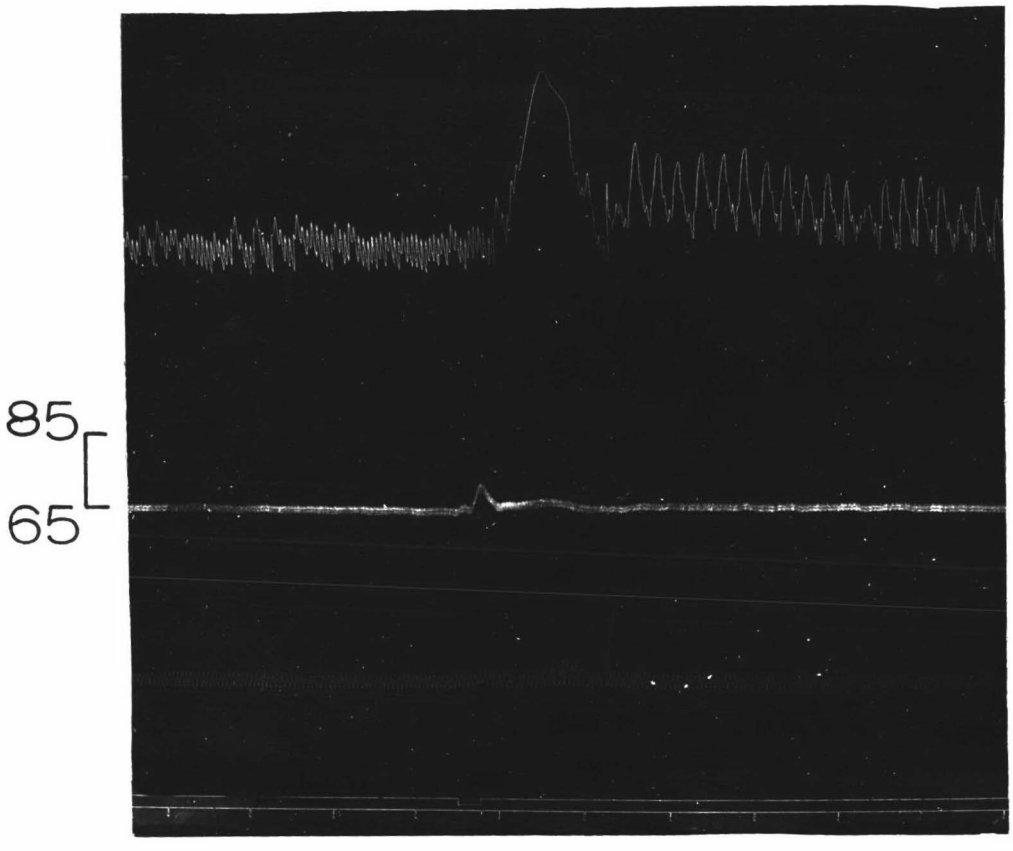


Fig. 4.14. Decerebrate cat. Effect of an intravenous injection of 0.25 mgm priscol. Records from above downwards: terminal oesophagus, blood pressure (mm Hg), respiration, signal, 30 sec time-marker. The onset of the activity might be compared with the unstimulating nerve block by hexamethonium (see fig. 4.7).

contractions evoked (usually one or two) occurred 30-50 seconds after the time of injection (fig. 4.9). Adrenaline was also found to sensitise the caudal regions of the oesophagus to acetyl choline. Fig. 4.10 shows the effect of 10 µgm acetyl choline alone and a repeat injection 160 seconds after the injection of 10 µgm adrenaline. The contraction occurred simultaneously with the hypotension produced by acetyl choline. In this experiment the oesophageal motility was recorded by balloons (10 mm long and 5 mm diameter). The oesophagus was therefore under little or no distension. This response was seen in only one anaesthetised preparation.

The oesophageal response to nor-adrenaline and isoprenaline has been observed in one decerebrate preparation. Nor-adrenaline (5 µgm/kgm) caused both facilitation and inhibition of terminal oesophageal activity (figs. 4.11 and 4.12). Isoprenaline (5 µgm/kgm) had a consistent inhibitory effect on both the tone and contractions of the terminal oesophagus (fig. 4.13).

The intravenous injection of priscol in 0.1 - 1 mgm/kgm doses consistently caused increased activity of the distended terminal oesophagus. The response to priscol was observed 10-20 seconds after the completion of the injection and the response continued for greater than ten minutes. Large contractions of the terminal oesophagus were generally seen at the commencement of the oesophageal response to priscol (fig. 4.14).

DISCUSSION

The pharmacological investigations reported in this chapter indicate that the smooth muscle portions of the oesophagus possess a muscarinic cholinergic innervation. The contractions of the oesophageal striated muscle display a resistance to the blocking action of atropine but are susceptible to curare. Although similar to skeletal muscle in this respect, further studies are required to ascertain the form of the innervation of these more cranial regions of the oesophagus of the cat.

The studies of Ellis *et al*. (1960) with strips of human distal oesophagus showed that contractions could be obtained with acetyl choline and these responses could be blocked by atropine. The effects of nerve stimulation on the isolated chick oesophagus preparation could be, in part, blocked by ganglion blocking agents, and cholinergic drugs were effective in producing contraction (Bowman and Everett (1964). These reports support the conclusions presented above.

The junctional region of the skeletal and smooth muscle in the mid-thoracic regions of the oesophagus of the cat is a region of great interest. The degree to which the different muscle cells, and the characteristics of each muscle type, overlap is uncertain. Studies involving histological, pharmacological, and *in vitro* examination are necessary for a systematic investigation of this region of the oesophagus. The presence of ganglion cells in the skeletal muscle regions has been observed by various workers (see Ingelfinger, 1961).

Comline and Message (1964) suggest these ganglion cells may be involved in vascular control. These workers have also identified, in the terminal regions of the oesophagus of the sheep, motor end plates similar to those found in skeletal muscle but which are approximately one-fourth to one-sixth the size. Some striated muscle cells were also seen to have a series of short linear nerve terminations along the fibre. This form of motor nerve fibre was previously thought to occur only in the slow striated fibres in the postural muscles of amphibia and some birds. This evidence might suggest a mechanism by which the striated muscle of the oesophagus can respond slowly and discreetly to various stimuli rather than rapidly and completely as in swallowing, regurgitation and eructation. The ability of atropine to separate the contraction of the oesophagus at this junctional region (see fig. 3.2), and the effect atropine has on the contraction of a region 5-7 cm craniad to this junctional region (fig. 3.1), would suggest that there is both an overlap of cells and of muscle characteristics. The problem of assessing whether the smooth muscle regions are innervated only by post-ganglionic fibres is more difficult, as pharmacological blocking agents which block the pre-ganglionic fibres have effects such as ganglion stimulation or a blocking action at the neuro-effector site.

Rhythmic contractions of the anterior thoracic oesophagus in response to distension of this region (see fig. 4.6) persisted after the administration of nicotine and atropine. This response appears to suggest: that the innervation is a direct one and does not involve a nicotine-sensitive ganglion; that the response which occurred simply

involved a post-ganglionic neuron; or the reaction was a direct response of the oesophageal muscle. The last possibility appears to have been dismissed by the blocking action of curare which does not affect the muscle itself but blocks transmission from the neurone. It produces a neuro-effector block and not a direct muscular paralysis. The ability of the thoracic oesophagus to respond to a distension appears to be part of a protective mechanism which is largely independent of extrinsic nervous pathways. Where portions of the thoracic oesophageal muscle are striated in character, such as in the sheep or cat, mechanisms may have been developed or retained to allow these local responses to occur. The slow-fibre innervation described by Somline and Message (1964) and the response of striated muscle after atropine and nicotine described above both may be taken as indications that primitive local mechanisms exist in the striated muscle regions. The characterisation of the innervation of striated oesophageal muscle, a description of the local responses, a comparison with the local responses of the smooth regions of the oesophagus, and the degree to which these local responses are present in other species, would be a probable study.

The effects of stimulating and blocking drugs on the vasculature of a smooth muscle organ, such as the caudal oesophagus, makes the interpretation of the results difficult. The well known sensitivity of smooth muscle to anoxia, which may occur following a vaso-constriction, can be cited as a possible effect of vascular changes on the thoracic oesophagus of the cat.

In the experiments reported here a systematic study of the action of ganglionic blocking agents on the thoracic oesophagus has not been undertaken. Any attempt to do so must involve experiments with controlled blood pressure conditions and a comparison of the effects of ganglion blocking agents which either act by producing a persistent depolarization of the post-synaptic membrane or by producing a competitive block at the post-synaptic membrane. It must also involve an investigation of the effects of the above ganglion blocking agents after the administration of sympatholytic agents. This would exclude the possibility of the effects being attributable to a block of vasomotor fibres or even possibly a block of a direct sympathetic innervation of the oesophageal skeletal muscle. Goodman and Gilman (1955, p. 619) report: "Hexamethonium (C6) also stabilises the postsynaptic membrane; however, under certain experimental conditions C6 can stimulate ganglia, but there is no evidence that any of its close congeners normally causes depolarisation." The observation that terminal and mid-thoracic oesophageal activity increases after administration of hexamethonium becomes of particular moment as it may be interpreted as an unstimulated release of oesophageal muscle from post-ganglionic, parasympathetic and sympathetic innervations. No response of the terminal oesophagus to peripheral vagus stimulation following the administration of hexamethonium has been observed. This would confirm the suggestion (from the use of atropine) that the innervation of this region of the oesophagus is post-ganglionic.

Nicotine was both excitatory and inhibitory to terminal oesophageal activity. This may be due to the stimulation or the block of both

parasympathetic or sympathetic post-ganglionic nerves. The persistence of an inhibitory effect of peripheral vagus stimulation following the administration of 0.6 mgm/kgm of nicotine might suggest two possibilities: the presence of the synapse above the site of stimulation of the vagus nerve in the neck; or ephaptic transmission between the pre- and post-ganglionic neurones. Bowman and Everett (1964) have reported a similar phenomenon in the isolated, innervated chick oesophagus and offer the latter as an explanation of their results.

Acetyl choline has been found to only cause a contraction of the oesophagus. The inhibitory effect of eserine on terminal oesophageal contractions indicates that acetyl choline released or administered in large doses may act as an inhibitory transmitter in the thoracic oesophagus. In the experiments reported, only an indication has been obtained that the oesophagus may be inhibited by cholinergic nerve fibres. In contrast to this, the administration of sympathomimetic drugs (adrenaline, nor-adrenaline and isoprenaline) cause both facilitation and inhibition of oesophageal motility. The presence of excitatory and inhibitory adrenergic receptors has been deduced from experiments in the human terminal oesophagus (Ellis et al., 1960) and in the chick (Bowman and Everett, 1964). The effects of these drugs on a background of oesophageal activity have been recorded, but the reports of Hanzlik and Butt (1928) and Knight (1934b) indicate that adrenaline may facilitate the effects of stimulation of the vagal efferents. In one experiment an interaction between acetyl choline and adrenaline was noted. This may indicate that a study of the

interactions of the two efferent pathways and their transmitters would be profitable.

Hanzlik and Butt (1928) and Ellis *et al*. (1960) have reported that the longitudinal and circular elements of the oesophageal musculature are innervated separately and differently. These conclusions have been based on the responses of the oesophagus and strips of oesophageal muscle to drugs. The interactions and innervation of these two portions of the oesophageal walls have not been studied in the present experiments. A clear-cut way of doing so under *in vivo* conditions may be with the use of electrophysiological techniques with simultaneous recording of mechanical and electrical activity.

The removal of sympathetic effects from the oesophagus may be done partially by surgical ablation of the sympathetic pathways. Complete removal, however, requires the use of pharmacological blocking agents. The drugs available for this are both non-specific in their actions and may also act directly on the effector organ. The effect of priscol (tolazoline) reported above may be due to a removal of a sympathetic inhibitory discharge or a direct action on the terminal oesophageal musculature. Goodman and Gilman (1955, p. 583) refer, for example, to the "labile" block of sympathetic neurons produced by it, its direct actions on smooth and cardiac muscle, and its histamine-like effects. The time course of the oesophageal response to priscol was generally faster than the non-stimulating block of the post-ganglionic innervation by hexamethonium. This might indicate the

presence of a direct effect of priscol (see fig. 4.14). A further clarification of the sympathetic innervation might be obtained by the use of more specific blocking agents, which might selectively block the excitatory and inhibitory adrenergic receptors. Stimulation of the peripheral end of a splanchnic nerve at different frequencies, under different conditions and after the administration of various drugs, is essential to obtain evidence on this problem.

The portion of the oesophageal responses which are due to the facilitation or stimulation of receptors by pharmacological agents is of importance. Paintal's report (1954) that adrenaline might facilitate the discharge of stretch receptors in the cat's stomach indicates that this possibility must be considered. The difficulty of assessing the contribution of such a sensory discharge might be overcome by using preparations with a de-afferentated oesophagus.

These preliminary observations suggest the need for an extended systematic and comparative observation of the reactions of both smooth muscle and skeletal muscle parts of the oesophagus to selected pharmacological agents. A more complete understanding may be obtained by using a variety of animal preparations, namely:

The isolated oesophagus in <u>in vitro</u> studies, with a para-sympathetic, post-ganglionic innervation.

Isolated strips of oesophageal muscle independent of the myenteric ganglia. Obtained by dissection, or from embryos before the parasympathetic innervation is established.

Totally pithed preparations with both a parasympathetic and a sympathetic post-ganglionic innervation.

Totally pithed preparations with the sympathetic elements removed as much as is possible to allow a comparison of the influences of the parasympathetic and sympathetic nerves.

Totally pithed preparations with the oesophagus divided to remove the possibility of inter-oesophageal reactions.

However, the use of such animal preparations will not overcome the limitations of the pharmacological agents. The problems which arise from this have been briefly mentioned in relation to the use of priscol, but they can perhaps be overcome by the use of a variety of agents with similar effects but different side-effects.

Chapter 5

GENERAL DISCUSSION

Rhythmic contractions of localised regions of the smooth and skeletal muscle portions of the thoracic oesophagus have been evoked by local distension of these regions by balloons (3-5 cc volume). These contractions do not appear to be dependent on an extrinsic innervation as they have been recorded in preparations with their central nervous systems totally destroyed by pithing and also after the administration of ganglion blocking agents.

In decerebrate preparations the response of the terminal thoracic oesophagus to distension of itself was either facilitated or inhibited by stimuli applied to the oesophagus (at this or other regions); other regions of the alimentary tract; and more distant parts of the preparation. Effects of a change in one part of the oesophagus on the activity of another part of the oesophagus (inter-oesophageal responses) may be mediated over intrinsic pathways within the oesophagus itself. The importance of extrinsic nervous pathways in these reactions has not been defined. The effects of extra-oesophageal stimuli are mediated by extrinsic nerves and the various afferent nerves appear to converge on the medulla oblongata. The effects of these stimuli reflected back to the oesophagus appeared to be mediated certainly by vagal and possibly also sympathetic efferent fibres.

The recurring contractions of both the caudal and higher regions of the oesophagus which may be evoked by distension, and the inter-

oesophageal reactions described above, may represent an oesophageal protective mechanism. The existence of such mechanisms does not prevent the oesophagus in these regions from participating in other more complete activities of the oesophagus as a whole. However, it does appear that the protective function can be demonstrated in the absence of an extrinsic innervation and is therefore a more basic or elementary form of response.

More complete actions of the oesophagus are superimposed and at times submerge the more direct oesophageal reactions. Complete involvement of the oesophagus which may follow the buccopharyngeal movements of swallowing also involve that part of the oesophagus which displays the most obvious local responses - the terminal thoracic oesophagus. In contrast to the variable form of the local responses of the thoracic oesophagus, its complete involvement in an event such as swallowing was found to be, in any one preparation, remarkably consistent. This might be expected if the more local reactions can be regarded as due to intrinsic mechanisms modified by a variable degree of activity of the extrinsic innervation. As mentioned previously, the activity of the oesophagus stimulated by distension of itself may be affected by a variety of stimuli which are either facilitatory or inhibitory. The nett or final effect of the total afferent input from the various sensory areas will determine the effect the extrinsic innervation has on the intrinsic activity of the thoracic oesophagus. During swallowing there appears to be a dominating influence exerted on the oesophagus to produce a co-ordinated response. To what degree the central nervous system alone

orders or predetermines the sequence and form of the oesophageal response is not known. There exists an orderliness of oesophageal response without the extrinsic nervous system and it might be suggested that the central nervous system causes a facilitation or inhibition of this intrinsic oesophageal orderliness.

Separation of the oesophageal response from the buccopharyngeal movement of swallowing, and the observation that the oesophageal contraction wave of swallowing may not traverse the whole oesophagus (Hwang, 1954), indicates that the sequential contraction of the oesophagus in swallowing requires the presence of a stimulating "bolus" in the oesophagus. Creamer and Schlegel (1957) and Siegel and Hendrix (1961) concluded from the similarities of the oesophageal response following buccopharyngeal movements of swallowing and those evoked by distension of the oesophagus that a central mechanism which ordered the form of oesophageal response existed and was stimulated by either of these stimuli. The presence of one mechanism for ordering a sequential contraction may preclude the need for duplication; however, for adequate functioning there should surely be a means of adjusting the degree of response according to conditions or requirements. The presence of a central mechanism which may adjust the degree but not the form of oesophageal response in swallowing would explain the recording of greatly different rates of passage of the contraction wave without much variation in the form of the contraction wave. (Ingelfinger, 1958, has reported the wide range of velocity of oesophageal contraction observed in swallowing even in the same species.) Evidence has been obtained that conditions in the oesophagus

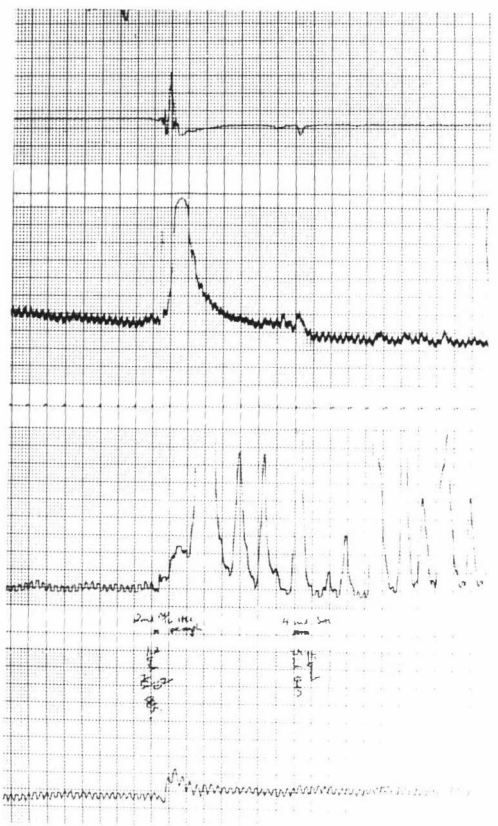


Fig. 5.1. Decerebrate cat. Stimulation of an oesophageal contraction wave moving in a cranial direction by the application of 2 ml $^{N}/_{10}$ HCl to the terminal regions of the oesophagus. The cervical oesophageal contraction is associated with, or stimulated by, a buccopharyngeal movement similar to that observed during swallowing. Records from above downwards: anterior cervical oesophagus, anterior thoracic oesophagus, 10 sec time-marker, terminal oesophagus, signal, respiration.

and other parts of the alimentary tract affect the degree of central facilitation of the oesophageal sequential response to a distension and could thus lead to changes in the speed of the contraction wave. This interpretation is contrary to the contention that the oesophageal component in swallowing is not dependent on an afferent discharge of oesophageal receptors. The use of preparations with a de-afferentated oesophagus might provide an indication of the validity of this contention.

For a contraction wave to pass over the oesophagus in a craniad direction, the facilitation of the oesophagus which occurs as a local response (inter-oesophageal) above the point of contraction or distension must be inhibited. If this facilitatory discharge either ceased or changed to an inhibitory discharge, a distension or stimulation by other means of the caudal regions of the oesophagus could be expected to lead to either a contraction which progressed cranially over the oesophagus or to an overall inhibition of the oesophagus. Contraction waves moving cranially have been recorded in the decerebrate cat (figs. 2.18 and 5.1) and occur in ruminants during regurgitation. In ruminants, in eructation, there may be simply a passive movement of gas up the oesophagus, although this point requires more investigation.

Buccopharyngeal movements of swallowing have also been observed following the passage of a wave of contraction cranially from the terminal to the cervical regions of the oesophagus (fig. 5.1). This "swallow" could arise from stimulation of the cervical oesophagus in

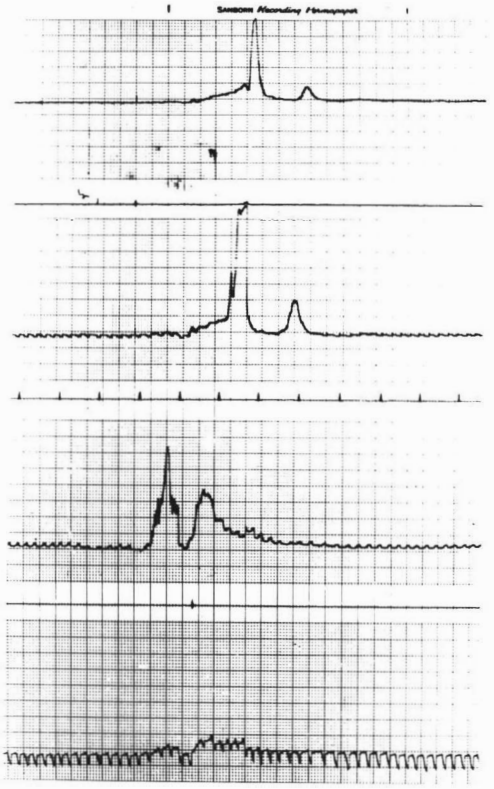


Fig. 5.2. Decerebrate cat. Fluid distension of the cervical oesophagus caused a contraction of this region of the oesophagus. This appeared to stimulate a buccopharyngeal movement similar to that observed during swallowing. A contraction wave of the oesophagus followed the buccopharyngeal movement. Records from above downwards: terminal oesophagus, signal marking fluid distension of cervical oesophagus, mid-thoracic oesophagus, 10 sec time-marker, cervical oesophagus, signal marking buccopharyngeal movement, respiration.

the course of the passage of the contraction. Hwang (1954) reported that swallowing could be stimulated by distension of the cervical oesophagus. This type of response has also been obtained in the present studies (see fig. 5.2).

The reflex modification of the basic contractile activity of the terminal oesophagus described might indicate the way in which this region will function in controlling the passage of stomach contents into the oesophagus. Rather than presenting a barrier of resting high tone, as suggested by many workers, it would provide a region which would be stimulated by refluxing material. The ease with which this region of the oesophagus would be stimulated might depend not only on the nature of the material but also on the conditions existing elsewhere in the alimentary tract. Material passing from these terminal regions, as far craniad as the anterior thoracic oesophagus, would encounter a similar mechanism stimulated in essentially the same manner. Greater stimulation, or the stimulation of more cranial regions, would then lead to a more complete oesophageal clearing mechanism being brought into play.

The oesophagus appears to be capable, in its more caudal regions, of intrinsic responses to applied stimuli: those that can be modified by conditions which exist elsewhere in the oesophagus and in more distant areas, particularly of the alimentary tract. The modification may be an intrinsic reaction, as inter-oesophageal reactions, or mediated by extrinsic pathways as reflex effects. Reflex centres appear to exist either in the medulla or pontine regions of the

central nervous system. Higher regions of the central nervous system may also affect the efficacy of the reflex modification of oesophageal activity if an analogy can be made with the medullary control of respiration, blood pressure, and other activities.

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