

An electrophysiological analysis of the reflex regulation of  
reticulo-ruminal movements

by

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SUMMARY

The reflex regulation of reticulo-ruminal movements was analysed in the present experiments using an electrophysiological 'single fibre' recording technique. The afferent input to and the efferent output from the 'gastric (reticulo-ruminal) centres' was repeatedly sampled by recording from single gastric units dissected from the cervical region of the left vagus in 8 sheep anaesthetized with chloralose and 70 with halothane. Primary cycle movements of the reticulo-rumen were usually evoked by distending a reticular balloon with 400-600 ml air.

By recording from afferent gastric units, it was found that the majority of gastric mechanoreceptors are slowly-adapting 'in series' tension receptors situated in the muscle layers of, principally, the reticulum, the reticulo-ruminal fold and the cranial sac (dorsal rumen). The afferent discharge from these receptors increases during passive distension and during isometrically-recorded contractions. Receptors in the lips and the floor of the reticular groove and omasal canal respond both during a contraction and, particularly, to pressure. The 'resting discharge' generated by tension receptors is largely determined by the intrinsic motility of smooth muscle cells. The mean conduction velocity in afferent gastric fibres is 12.4 m/sec.

By recording from efferent gastric units, at least 7 distinctive types were discernible and, by relating their discharges temporally to movements of the reticulum and of the rumen, it was concluded that Types I, II and III occur in fibres innervating the reticulum or associated structures, Type IV the rumen and Types V, VI and VII other gastric structures not yet identified. In only Type/

Type VII units is there a 'resting discharge' during the quiescent part of the gastric cycle. By cold blocking vagal nerves, it was demonstrated that separate efferent fibres innervate the reticulum and the rumen and, that the dorsal vagal trunk carries predominantly excitatory afferent fibres to the gastric centres whereas the ventral vagal trunk carries predominantly either excitatory or inhibitory fibres depending on the experimental conditions.

By recording from afferent and from efferent gastric units at the same time as altering conditions in the reticulum either physically or with certain drugs, the changes in the afferent input to and the efferent output from the gastric centres evoked by these manoeuvres were recorded and estimates of the total and the central reflex time for gastric reflexes were made.

It is concluded from the present investigation that:

- (a) the co-ordination of the complex sequence of primary cycle movements is a function of the 'gastric centres', through their ability to determine the parameters and temporal interrelationships of efferent nervous discharges in the various types of gastric units innervating different regions of the forestomach.
- (b) the tonic afferent input from 'in series' reticular tension receptors during the quiescent period of the primary cycle provides a reflex 'drive' to the gastric centres and largely determines the rate, the duration and the amplitude of reticular and ruminal contractions.
- (c) the enhanced afferent input to the 'gastric centres', occurring during a reticular contraction recorded under isometric conditions, modifies the form, the amplitude, the duration and the delay in onset of the later parts of the contraction sequence of the reticulum and the rumen.

## FOREWORD

The present investigation arose as a sequel to the demonstration by Iggo (1951, 1956) that 'centres' in the hind-brain are responsible for the reflex regulation of reticulo-ruminal movements and that the necessary afferent 'drive' may be provided through the activity of tension receptors located near the reticular groove (Iggo, 1955). The extent and the nature of reflex regulation was analysed in the present experiments by the use of an electrophysiological 'single fibre' recording technique. By repeatedly sampling the afferent input to and the efferent output from the 'gastric (reticulo-ruminal) centres' under a variety of experimental conditions, one hoped to elucidate the manner in which nervous integration was performed by the centres in the regulation of gastric movements.

For ease of description and discussion, the present investigations are divided into six sections. Section A is concerned with the means of obtaining gastric (primary cycle) movements in sheep during acute experiments. Section B deals with the distribution and physiological properties of mechanoreceptors in the reticulo-rumen and of the afferent vagal fibres by which they are innervated. The various types of nervous activity present in efferent gastric units are described and classified in Section C and the manner in which this activity may be reflexly modified is considered in Section D. The reflex effects of interfering with nervous transmission in afferent and efferent vagal pathways are dealt with in Section E and, finally, the reflex consequences of administering drugs which affect neurohumoral transmission and gastric smooth muscle motility are described in Section F.

Parts of the investigation comprising this thesis have already been published/

published or have been accepted for publication, i.e. Leek (1963, 1966, 1967) and Iggo & Leek (1966, 1967a, 1967b). A copy of each of these, except for the last one, has been bound as part of the appendix.

INTRODUCTION

The ruminant stomach consists of a non-secretory forestomach divided into three compartments, viz. the reticulum, the rumen and the omasum, and a peptic acid-secreting compartment, viz. the abomasum. The anatomy of these structures is given by Sisson & Grossman (1961) and, throughout this thesis, the nomenclature recommended by the International Committee on Veterinary Anatomical Nomenclature (Habel, 1965) has been used as far as possible.

Ruminant gastric movements are of two physiologically different types, i.e., intrinsic and extrinsic. Intrinsic movements consist of localised contractions of the reticulo-ruminal wall, appearing as 'ripples' when observed visually or radiographically (Ash & Kay, 1959; Phillipson, 1939) and on kymographic recordings (Brunaud & Dussardier, 1953; Dussardier, 1960, Fig. 4). They are still present after the extrinsic nerves to the ruminant stomach have been cut (Duncan, 1953). Intrinsic movements in the reticulo-rumen are functionally ineffective per se for the purposes of propelling or of mixing the voluminous contents of these compartments, although results obtained during the present experiments suggest that intrinsic movements may affect extrinsic movements of the reticulo-rumen reflexly. Intrinsic movements may therefore be of greater significance than has hitherto been considered.

Extrinsic movements of the reticulo-rumen are responsible for mixing and propelling the fluid contents and for eliminating rumen gases. These movements are forceful and constitute co-ordinated sequences, involving in turn various regions of the forestomach compartments. It is generally agreed that two different sequences exist, although various names have been given/

given to them, e.g. 'primary and secondary cycles' (Schalk & Amadon, 1928), 'backward and forward moving contractions' (Weiss, 1953), 'mixing and belching cycles' (Reid & Cornwall, 1959) and 'A and B sequences' (Reid, 1963). Extrinsic movements are dependent upon the presence of at least one intact vagal nerve or one thoracic/abdominal vagal trunk (Mangold & Klein, 1927; Popow, Kudrjavcev & Krasausky, 1933; Höflund, 1940; Duncan, 1953; Weiss, 1953; Clark, 1953; Habel, 1956; Howard, 1966). In the present experiments, using anaesthetized sheep, only the sequence comprising the primary cycle has been studied and the details of this sequence are of moment for two reasons. First, records of nervous activity in afferent gastric units (Section B) innervating mechanoreceptors in various discrete regions of the reticulo-rumen have enabled the onset and the duration of the movement in each of these regions to be determined precisely and without many of the draw-backs of the classical recording methods discussed below. Secondly, since the forestomach region innervated by a particular efferent unit whose nervous activity was recorded (Section C) could not be determined directly, (because the 'single fibre' technique used necessitated sectioning the unit peripheral to the recording site), it was deduced from a knowledge of the time relationships which exist between the sequential movements in the various forestomach regions comprising the primary cycle.

One of the classical methods for detecting reticulo-ruminal movements involves chronically fistulated animals. This preparation was first used by Flourens (1833) and movements may be observed by visual examination, by intra-ruminal/

ruminal palpation or by recording the associated pressure changes using either balloons or, as recently, open-tipped catheters (Wester, 1926; Schalk & Amadon, 1921, 1928; Phillipson, 1939; Dziuk & McCauley, 1965). The disadvantages of the first two methods are that permanent records are not obtained and that only one region at a time may be inspected or palpated. The principal drawbacks of recording manometrically are that certain regions are unsuitable for applying this technique, e.g. the folds and pillars, that (when using balloons) the record obtained will depend on whether the balloon is located in the lumen of the compartment or is pressed against the wall, that manometric devices record gross movements (including some from adjacent compartments) and that movements which lead to a propulsion of contents with only a slight pressure rise will be less readily detected than those in which the movement is relatively isometric, i.e., is associated with a pressure rise accompanied by only a slight reduction in the volume of the contents of that particular region.

Another classical method makes use of x-rays. One advantage is that the animal is both conscious and intact but the disadvantages are that it can only be applied to the small ruminants, that some structures cannot be delineated readily without the use of contrast medium or implanted radio-opaque 'tags' and that serial fluorography or cinefluorography can only be used over a very limited period. This technique has been employed by Czepa & Stigler, 1929; Magee, 1932; Phillipson, 1939; Benzie & Phillipson, 1957; Dougherty & Meredith, 1955. Another method applicable to intact ruminant animals involves simultaneously/

simultaneously auscultating the reticulum and palpating the dorsal ruminal sac but no permanent records are obtained (Williams, 1955).

The newer recording techniques have been developed to overcome the above problems, particularly with the object of recording the movements of discrete regions and of establishing the temporal inter-relationships of the various parts of the contraction sequence. Reid & Titchen (1959) and Reid (1963) have examined, in conscious surgically-prepared sheep, the motility of localized regions of the reticulum and rumen which have been partially exteriorized (herniated). Movements of the ventral pole of the reticulum, the cranial ruminal sac and the ruminal pillars have been recorded, using a vertical displacement technique, by Reid & Cornwall (1959). Movements of the ruminal pillars have been recorded with an electromagnetic induction technique (Lucas & Dougherty, 1964). Chiesa, Vacirca & Colombo (1965a,b) have attached a series of small transducers to specific sites on the external surfaces of the reticulum and the rumen and have recorded movements from discrete areas after the sheep had regained consciousness. For the same purpose, Leek & Ullah (1967) have implanted the sealed pressure-sensitive endings of polythene micro-cannulae between the muscle layers at discrete sites in the reticulo-rumen and, during the subsequent fortnight, have recorded manometrically movements localized to these regions, using transducers connected to a hot-wire pen recorder. In anaesthetized sheep, the motility of discrete regions has been obtained by recording the afferent discharge from units innervating mechanoreceptors at sites subsequently located by manipulation of the reticulo-ruminal/

ruminal wall (Section B).

The results obtained by the various techniques are in general agreement. The original terms, 'primary and secondary cycles' (Schalk & Amadon, 1928) will be used to describe the two sequences. 'Primary cycle' will refer to a sequence which starts with contraction of the reticulum and is immediately followed by a contraction of the dorsal ruminal sac et seq and 'secondary cycle' will refer to a cycle in which the dorsal ruminal sac contraction et seq is not immediately preceded by a reticular contraction. The frequency, the form and the duration of each cycle depends largely upon the ruminant species, the state of the animal, i.e., whether it is eating, ruminating or doing neither, and on other factors, such as posture, type of food, interval since previous feed and the absence of disease. Comparative studies of reticulo-ruminal movements in sheep, ox and goats are the most numerous (e.g., Phillipson, 1939; Dziuk & McCauley, 1965) but records have also been obtained from the white-tailed deer (Dziuk, Fashingbauer & Idetrom, 1963), the American bison (Dziuk, 1965) and the alpaca (Vallenas, 1965). The basic sequences described below occur when the ruminant is neither ruminating nor feeding unless there is a statement to the contrary and are therefore likely to correspond most closely to the state in the anaesthetized sheep used in the present investigations.

The primary cycle commences with the reticulum contracting twice during a period of about 5 sec. In cattle the reticulum relaxes completely between each contraction (Webster, 1926; Schalk & Amadon, 1928; Dziuk & McCauley, 1965), whereas in sheep and goats, there is usually only a partial relaxation  
or/

or a pause with no relaxation between the first and second contractions (Czepa & Stigler, 1926, 1929; Magee, 1932; Phillipson, 1939; Dziuk & McCauley, 1965). Czepa & Stigler, (1929), using a radiographic technique, concluded that the reticular contraction was total but Wester (1926) claimed that a wave of peristalsis swept across the reticulum, after which followed a wave of anti-peristalsis. Using small transducers applied to specific sites on the parietal surface of the sheep's reticulum in order to record localized movements at their sites of application, Chiesa et al (1965a) have shown that the reticular contraction starts on the (left) dorso-lateral region and subsequently involves, in turn, the (left) cranio-lateral, the ventro-lateral, the (right) ventro-medial and the dorso-medial regions. The duration of the contraction diminishes in the same order. From their illustrations it appears that the left (lateral) wall undergoes a double (biphasic) contraction and the right (medial) wall undergoes a single (monophasic) contraction. During rumination there is an extra reticular contraction which precedes the contractions described above (Schalk & Amadon, 1928). At its peak the cardia opens and the cud 'bolus' is drawn into the oesophagus (Downie, 1954; Bell, 1958). This extra contraction has a shorter duration in the cow (1.0 - 1.5 sec) than in the sheep and goat (2.0 - 4.0 sec), and consequently, in the latter, the next contraction is of low amplitude or absent (Dziuk & McCauley, 1965). Henceforth, these one, two or three movements of the reticulum will be referred to as 'the reticular contraction' and each of its component contractions as a 'phase'.

Associated/

Associated with the reticular contractions are movements of the reticulo-ruminal fold and the reticulo-omasal sphincter. Using induction coils clipped on to the reticulo-ruminal fold of fistulated cows, Lucas & Dougherty (1964) showed that the fold underwent a biphasic contraction at the same time as the (biphasic) reticular contraction. From their records it appears, however, that relaxation after the second phase is much slower for the fold than for the reticulum and another contraction of the fold may occur at the time of the dorsal ruminal sac contraction. Reid & Cornwall (1959) using a vertical displacement recording device in fistulated cows, also found that the fold underwent a biphasic contraction coincident with that of the reticulum. Variations in the aperture of the reticulo-omasal orifice are related to the phase of the reticular contraction. Wester (1926) observed that the orifice was partially open during most of the cycle in cows, that it was widely dilated during the reticular contraction (at which time there was a rush of reticular contents through it) and that it was closed immediately afterwards. Schalk & Amadon (1928) concluded that the wide dilation was related to the second phase of the reticular contraction. Balch, Kelly & Heim (1951), by recording pressure changes in balloons inserted into the orifice, demonstrated that the orifice was loosely open to 60-70% of the cycle but closed during the first phase of the reticular contraction, widely dilated during the second phase and then closed again for several seconds ( $\sim$ 15 sec) afterwards. This sequence has been confirmed by Brunaud & Dussardier, (1953), Stevens et al (1960), Borgatti & Matscher (1958) and Ohga et al (1965).

In the primary cycle, a contraction of the rumen follows that of the reticulum./

reticulum. The early literature on this subject is controversial. Czepa & Stigler (1926, 1929) concluded that rumen contractions in sheep and goats were total not peristaltic and independent of reticular contractions, whereas Wester (1926) and Schalk & Amadon (1928) considered the rumen contraction in cows to be linked to the reticular contraction and to consist of a peristaltic wave which originated near the reticular groove and moved caudally across the dorsal sac. Wester (1926) described this as a spiral wave affecting, in turn, the cranial sac, the cranial pillar, the longitudinal pillars and dorsal sac, the caudal and dorsal coronary pillars and caudo-dorsal blind sac, the ventral and caudo-ventral blind sacs. The description given by Schalk & Amadon (1928) is similar to Wester's except that (a) the caudal, longitudinal and dorsal coronary pillars contract simultaneously with the cranial pillar, (b) the caudo-ventral blind sac contracts after the ventral coronary pillars but before the ventral sac and (c) the caudo-dorsal blind sac contracts after the ventral sac. Phillipson (1939), using both balloon and radiographic methods in sheep, as well as by palpation through a fistula in a cow, concludes that structures contract in the following sequence:-

- (a) the reticulum (first phase)
- (b) the reticulum (second phase) and the cranial, caudal and longitudinal pillars,
- (c) the cranial sac, dorsal and caudo-dorsal blind sac and
- (d) the caudo-ventral blind sac and ventral coronary pillars. Dorsal and ventral sac contractions were regarded as total and not as peristaltic.

The idea that the dorsal ruminal sac (primary cycle) contraction was not

a total contraction but occurred as a wave, spreading across the wall from the cranial to the caudal regions was revived by Weiss (1953), although it was not implied that this wave was 'peristaltic', as defined by Bayliss & Starling, (1899), or involved an intrinsic reflex, e.g., the 'myenteric reflex'. Weiss (1953) called it a 'backward (i.e. caudally) moving contraction' and most observations since then have confirmed this. Reid & Cornwall (1959) give the following sequence of contractions for cattle:-

- (a) first phase in the reticulum and reticulo-ruminal fold,
- (b) second phase in above,
- (c) the cranial sac
- (d) the dorsal sac and the cranial, caudal and dorsal coronary pillars,
- (e) ventral displacement of the cranial pillar together with contractions of the caudal and ventral coronary pillars, the ventral sac and the caudo-dorsal blind sac.

Dziuk & McCauley (1965) describe a sequence similar to the above for cattle, sheep and goats except that the caudo-dorsal blind sac contraction precedes the ventral sac movements. In decerebrate sheep with evoked rumen contractions, Reid & Titchen (1965) have clearly demonstrated a wave of contraction moving caudally across the dorsal sac. Lucas & Dougherty (1964) claim that, when the wave of muscular activity reaches the anterior pillar, the posterior pillar and also the musculature of the dorsal ruminal sac contract simultaneously but Chiesa et al (1965b), using small transducers applied to the parietal/

parietal surface of the sheep's rumen, observed that the contraction arises in the left cranial region and spreads rapidly along the left longitudinal pillar to the caudal and then the right longitudinal pillars and slowly outwards from the pillars through the rumen walls both dorsally and ventrally. From the values given in their tables, it appears that dorsal sac activity arises mainly from the left pillar and therefore, precedes the ventral sac activity which arises mainly from the right pillar.

The ruminal contraction comprising the secondary cycle are not preceded by a reticular contraction. Wester (1926) described this cycle in cows as an 'antiperistaltic wave' passing cranially over the caudal and dorsal coronary pillars, the longitudinal pillars, the cranial pillar and finally the cranial sac, whereupon the cardia opened and rumen gases were eructated. Schalk & Amadon (1928), however, concluded that the secondary cycle contraction of the rumen was identical with that of the primary cycle except that the ventral sac contraction was more pronounced. Magee (1932) did not observe secondary cycles and suggested that those described above might have been artefacts! Weiss (1953) describes the secondary cycle in sheep as a 'forward (i.e. cranially) moving contraction' of the dorsal sac of the rumen, followed by a contraction of the ventral sac. This has been confirmed by Reid & Titchen (1965) using decerebrate sheep. According to Dziuk & McCauley (1965) the caudo-dorsal blind sac contraction had an amplitude and duration which were greater in cattle and less in sheep and goats in the secondary cycle than in the primary. In cattle the caudo-dorsal blind sac contraction started before the dorsal sac contraction but both sacs reached their peaks at the same/

same time, whereas in sheep and goats the starts and peaks occurred simultaneously although the amplitudes were different. The cranial sac did not contract during the secondary cycle. Dougherty & Habel (1955), during radiographic studies on sheep, found that the reticulo-ruminal fold contracted during the secondary cycle and formed a barrier which effectively kept the cardia clear of ingesta and assisted the expulsion of gas. Lucas & Dougherty (1964) observed no contraction of this fold in cattle: instead there was a strong contraction of the cranial pillar. This species difference may be the functional correlate of the structural differences, namely, that the cranial pillar is more prominent than the reticulo-ruminal fold in cattle and vice versa in sheep. It is claimed that the secondary cycle in sheep usually starts with a contraction of the caudo-ventral blind sac 1-7 sec before the dorsal sac contraction (Reid, 1960), using sheep with specific regions of the rumen partially exteriorised. This has not been confirmed by Leek & Ullah (1967), using sheep with implanted micro-cannulae, who found that secondary cycles usually start without a prior contraction of the caudo-ventral blind sac. When this contraction was present, it was more closely related to the preceding primary cycle than to the subsequent secondary cycle.

Sellers & Stevens (1966) have reviewed the motor function of the ruminant forestomach and conclude that there is general agreement on certain aspects of reticulo-ruminal motility, viz., 'After the reticular contraction(s) the primary cycle wave of contraction passes (caudally) over the rumen, resulting in a lifting of the cranial sac, contraction of the cranial, caudal and dorsal coronary pillars and compression of the dorsal sac of the rumen. The/

The wave continues over the caudo-dorsal blind sac, ventral coronary pillars, ventral sac and caudo-ventral blind sac, and the cranial pillar may be ventrally displaced at this time. ... (For the secondary cycle) in the cow the dorsal coronary pillar or caudo-dorsal blind sac can contract before the cranial pillar and dorsal sac. Then follows contraction of the ventral sac(s). In sheep the caudo-dorsal blind sac and dorsal sac contract simultaneously.' Despite this 'general agreement', the precise movements of certain regions and their time relationship to movements in other regions remain either controversial or unsolved. To some extent the experiments described later (in Sections B and C), in which the activity in afferent and efferent gastric vagal units has been recorded, have helped to elucidate these issues.

The basic sequence of movements described above may show modifications which either arise for reasons not as yet known or are induced by factors such as feeding and rumination. The most common modification is the absence of a primary cycle ventral sac contraction. This is more prevalent (a) when the animal is not feeding, (b) when the animal is ruminating, (c) when the primary cycle is followed by a secondary cycle and (d) in cattle than in sheep and goats (Phillipson & Reid, 1960; Dziuk & McCauley, 1965; Phillipson, 1966). A detailed description of the changes in the sequence which occur in sheep during fasting, feeding and immediately after feeding is given by Reid (1963). The changes were more pronounced when hay was fed than when fresh cut lucerne was given. The principal effects of feeding are (a) an increase in the amplitude and duration of the contractions, (b) a decrease in the duration/

duration of each cycle and (c) a decrease in the rate at which the wave of contraction spreads across the dorsal and ventral ruminal sacs.

The primary and secondary cycle sequences also involve contractile activity in certain regions of the omasum. Movements of the omasum have been described by Wester (1926), Schalk & Amadon (1928), Phillipson (1939), Balch et al (1951), Brunaud & Dussardier (1953), Stevens, Sellers & Spurrell (1960) and Ohga et al (1965). There is general agreement on the movements involving the reticulo-omasal orifice, described earlier (p. I 7). These consist of closure during the first phase of the reticular contraction, wide dilatation during the second phase, then closure for several seconds and partial dilatation for the remainder of the primary cycle. The confusion that exists between the results obtained for other regions of the omasum can largely be resolved by realising firstly, that the movements in different regions of the omasum are not the same and secondly, that some of the movements are temporally related to reticulo-ruminal movements whilst others are not. According to Stevens et al, (1960) the omasal canal gave a large prolonged pressure wave coincident with the dorsal ruminal sac contraction of both primary and secondary cycles. In contrast the omasal body gave protracted contractions of great amplitude (i.e., up to 40 mm Hg for 1-2 min) with a frequency which was less than half that of the primary (reticulo-ruminal) cycles. Superimposed on these tonic contractions, which were independent of the reticulo-rumen, were marked relaxations coincident with the dorsal ruminal sac and omasal canal contractions of the primary cycle only. The secondary cycle had no effect on the omasal body/

body movements.

Movements of the abomasum seem to be largely independent of movements in any compartments of the forestomach, despite a contrary view by Schalk & Amadon (1928). Using radiographic techniques, it appears that the fundus is more or less inert but strong peristaltic contractions sweep across the pyloric region (Czepa & Stigler, 1929; Krzywaneck & Quast, 1937; Phillipson, 1939; Benzie & Phillipson, 1957). This has been confirmed by observations made visually and by recording with balloons (Dukes & Sampson, 1937; Magee, 1932; Brunaud & Dussardier, 1953). The last authors also describe tonus waves with superimposed peristaltic contractions in the prepyloric regions. Both movements were present after bilateral vagotomy, although the tonus waves then had a longer duration.

Part of the present investigation is concerned with recording the nervous discharge in efferent gastric vagal units arising from 'gastric centres' in the medulla. Unlike most movements of the non-ruminant stomach and of the abomasum, the extrinsic movements of the ruminant forestomach compartments (i.e. primary and secondary cycle movements) are dependent upon the presence of at least one intact vagal nerve or one thoracic/abdominal vagal trunk (Mangold & Klein, 1927; Popow, Kudrjavcev & Krasausky, 1933; Hoflund, 1940; Duncan, 1953; Weiss, 1953; Clark, 1953; Habel, 1956; Howard, 1966). If both vagi are sectioned, reticulo-ruminal stasis occurs. Eructation and rumination are abolished and there is no transfer of digesta from the reticulum, via the omasum, to the abomasum. The last structure retains a reduced capacity/

capacity for propulsive motility and this results in delayed emptying and dilatation (Duncan, 1953). Although the rumen is innervated almost solely by branches from the dorsal vagal trunk, normal ruminal motility is re-established within 3 weeks from the time it is sectioned (Weiss, 1953; Duncan, 1953; Habel, 1956). Whilst there is no apparent permanent impairment of motility after sectioning the left or right cervical vagal or the dorsal or ventral thoracic/abdominal vagal trunk, section of more than one nerve or trunk, exclusive of total vagotomy, causes a persistent dysfunction (Duncan, 1953; Habel, 1956).

Excitatory motor nerves to the ruminant stomach are present in the vagi. Stimulation of the vagi causes contraction of the stomach compartments (e.g. Mangold & Klein, 1927; Habel, 1956). The gastric efferent fibres are parasympathetic and cholinergic in type (Brunaud, Dussardier & Labouche, 1950) and have a conduction velocity in the B range characteristic of preganglionic fibres, i.e., 1-16 m/sec (Iggo, 1956). Inhibitory gastric motor fibres are present in the splanchnic nerves. Splanchnic nerve stimulation reduced the amplitude of contraction of the reticulum, rumen and abomasum and the tonus of the rumen and abomasum after these had been elicited in anaesthetized sheep by vagal stimulation (Brunaud & Navarro, 1953). Bilateral splanchnotomy had no apparent effect on reticulo-ruminal motility (Duncan, 1953). This division of motor fibres into excitatory fibres in the vagi and inhibitory fibres in the splanchnic nerves may not, however, be absolute, since Comline & Titchen (1951, 1961) have demonstrated the presence of some inhibitory fibres in the vagus/

vagus and some excitatory fibres in the splanchnic nerves of atropinized, acute spinal goats. Using a histochemical technique for the localization of cholinesterases, Comline & Massage (1965) have shown that (a) preganglionic and postganglionic cholinergic fibres are present in all compartments of the sheep's stomach, (b) the principal ganglia lie between the inner and outer layers of smooth muscle and (c) each fasciculus of smooth muscle appears to be innervated by its own set of cholinergic nerve fibres. In similar studies but using a silver impregnation technique, Morrison & Habel (1964) concluded that few preganglionic fibres directly innervated ruminal structures distant from the reticular groove region. They considered that this region served as a peripheral co-ordinating centre for intrinsic and extrinsic nervous activity and caused contractions in the rumen musculature through extensive multisynaptic connexions by postganglionic cells in the intramural plexuses. The concept of 'peripheral' co-ordination is contrary to the conclusions drawn from the results of the experiments described later, in Sections C, D and E.

The central nervous control of reticulo-ruminal movements is carried out by centres in the medulla oblongata. Iggo (1951) obtained spontaneous normal gastric movements in sheep which had been decerebrated at the intercollicular level and in one decerebrate sheep in which the spinal cord also had been sectioned at the level of the second thoracic vertebra. These contractions were absent when nerve impulse conduction was abolished by blocking/

blocking the vagi with local anaesthetic agents, cooling or pressure. In preparations where contractions were not present spontaneously, they could often be evoked by tetanic stimulation of the central end of a sectioned vagus or abomasal nerve. It was postulated therefore, that the hind-brain contains 'reticulo-ruminal motor centres' which are 'triggered' by vagal afferent impulses and give rise to the vagal gastric efferent discharge responsible for co-ordinated activity in the reticulum and rumen. Clark (1953) was unable to confirm this observation, since his decerebrate sheep showed no spontaneous reticulo-ruminal movements nor could these be evoked by rubbing the mucosa with roughage. Since then, numerous investigators have recorded reticulo-ruminal movements in decerebrate sheep and Clark's inability to evoke them probably resulted from his use of an inadequate stimulus on sheep devoid of spontaneous movements (Titchen, 1953, 1958, 1960; Dussardier & Albe-Fessard, 1954).

The location and behaviour of the 'reticulo-ruminal motor centres' (henceforth called 'gastric centres') has been studied by stereotaxic electrical stimulation in decerebrate sheep and goats (Bell & Lawn, 1955; Dussardier, 1960; Howard, 1966) and in conscious goats with implanted electrodes (Andersson, Kitchell & Persson, 1958). Electrophysiological recordings of nervous activity in the medulla associated with reticulo-ruminal movements in anaesthetized and decerebrate sheep have been made with glass micro-electrodes (Beghelli, Borgatti & Parmeggiani, 1963; Howard, 1966). Bell & Lawn (1955) found that the reactive loci lay mainly in the dorsal part of the lateral/

lateral reticular formation at the level of the obex. On stimulation there was a strong contraction of the reticulum followed by a contraction wave in the rumen which started in the cranial sac and spread caudally across the dorsal sac only. The contraction lasted for as long as the duration of the stimulus. There appeared to be no intramedullary decussation of motor fibres. Andersson et al (1958) have evoked opposing effects from two different sites, firstly, an acceleration of the movements (through stimulation of white reticular formation) and secondly, a slowing of the movements (through stimulation of grey reticular formation). The latter effect was followed by an acceleration of movements when stimulation ceased and they concluded that this was a rebound phenomenon. Dussardier (1960) has observed three possible effects arising out of stimulation, viz., direct motor, acceleratory and inhibitory. He regards the direct motor effects as equivalent to those induced by Bell & Lawn (1955) and attributes them to direct stimulation of the motoneurons or their processes. The acceleratory effects are presumed to be due to the stimulation of areas which act as 'pacemaker' and 'reticulo-ruminal co-ordinating' networks. The inhibitory effects are discussed in the light of three possibilities, namely, that they might be due to electrical stimulation of splanchnic (inhibitory) afferent pathways, fibres from higher (inhibitory) centres or inhibitory nerve nets responsible for the quiescent phase of the primary cycle. Howard (1966) has demonstrated by electrical stimulation and by micro-electrode recordings, that certain areas in the dorsal vagal nucleus rostral to the obex are concerned with excitatory and with inhibitory effects.

One/

One of the objects of recording from single efferent gastric vagal units in the present investigation is to examine the character of the efferent discharge by a direct method, in order to determine the extent to which the integration and co-ordination responsible for the complex sequence of primary and secondary cycle movements occurs in 'gastric centres' in the medulla or in 'peripheral co-ordinating centres'. Only three previous investigations have been concerned with recording efferent gastric activity and two of these recorded nervous activity by means of micro-electrodes inserted into the medulla. With this technique it seems difficult to decide whether nervous activity, preceding the reticular contraction or the ruminal contraction by an appropriate interval, represents the discharge in the motoneurone or merely a discharge in an interneurone which will be subjected to further integration. Using micro-electrodes inserted into the medulla of chloralose-anaesthetized lambs, Beghelli et al (1963) have recorded nervous activity in the dorsal vagal nucleus associated with evoked reticular contractions. They have recorded (a) bimodal activity which precedes the biphasic reticular contraction and therefore is presumed to be directly associated with the reticulum, i.e., impulses in a motoneurone or its processes and (b) nervous activity which occurs after the reticular contraction and, since there were no rumen contractions in these lambs, is assumed to represent the discharge in interneurons in reverberatory circuits. Similar results were obtained by Howard (1966), using halothane-anaesthetized adult sheep with spontaneous reticulo-ruminal movements. Eleven types of discharge were recorded, two of which were presumed to be closely related to efferent gastric vagal activity.

The/

The other investigation of efferent gastric vagal activity is that of Dussardier (1958, 1960), who used an indirect but most ingenious method. He cross-sutured the central end of a cut thoracic vagus to the peripheral end of a cut phrenic nerve and allowed the cut vagal preganglionic fibres to regenerate in the phrenic nerve stump and to reactivate the diaphragmatic muscle. He was then able to record the effects of vagal efferent activity electromyographically from muscle fibres in a partially exteriorised diaphragm. Thirteen different patterns of activity are given diagrammatically (Dussardier, 1960 Fig. 20). Three of these precede and are likely to be connected with the reticular contraction and the remainder occur after it and presumably are related to activity in the rumen. Except for a few units (Fig. 22) there was no discharge during the quiescent part of the primary cycle. In the present investigation (Section C), a direct method of recording efferent activity in gastric vagal units is employed.

The rate, form and amplitude of reticular and ruminal contractions and the ratio of primary:secondary cycles are variable. Under normal conditions, these variations are brought about by factors such as feeding and rumination (Schalk & Amadon, 1928; Balch, 1952; Reid & Cornwall, 1959; Phillipson & Reid, 1960; Reid, 1963; Dziuk & McCauley, 1965; Ohga et al, 1965; Phillipson, 1966), 'apparent sleep' (Balch, 1955; Dziuk & McCauley, 1965), the consistency of the diet (Schalk & Amadon, 1928; Weiss, 1953; Freer, Campling & Balch, 1962; Reid, 1963), posture (Balch, 1952) and intercurrent disease (e.g., Blood & Henderson, 1960). Under experimental conditions, variations/

variations may be induced by stimulating either reflexogenic zones, afferent nerve pathways or higher centres in the central nervous system itself.

In the present investigation, the reflex effects upon efferent gastric vagal discharges of increasing and of decreasing the 'afferent input' to the gastric centres (by a variety of means) is examined, in order to determine the efficacy of a particular stimulus, the reflexogenic zones, the afferent pathways and the reflex effects of applying or removing the stimulus. Two facets of this study which receive considerable emphasis are the dependence of the 'gastric centres' upon a tonic afferent drive from the periphery and the extent of 'feed-back' (particularly from the reticulum), whereby movements occurring during the early part of the primary cycle reflexly modify those occurring during the later parts (Section D).

There are numerous accounts of gastric movements being affected by stimuli applied to the reticulo-rumen. Wester (1926) describes the enhanced frequency of movements due to increasing the rumen contents. Distension of the reticulum has been shown to evoke or increase the frequency and amplitude of gastric movements in decerebrate sheep (Iggo, 1951; Titchen, 1953) in chloralose-anaesthetized sheep (Dussardier, 1955, 1960) and in conscious fistulated cows (Stevens & Sellers, 1959). In decerebrate sheep, Titchen (1960) found that stretching the reticulo-ruminal fold was a more potent stimulus than distending the reticulum, to evoke or enhance ruminal contractions. Mechanical stimulation of the reticular groove region, reticulum, reticulo-ruminal fold and cranial pillar were particularly efficacious in reflexly exciting reticulo-ruminal movements, rumination and parotid secretion in conscious sheep with large bore cannulae, although more forceful stretching and squeezing/

squeezing of the mucosa inhibited these activities (Ash & Kay, 1959). In the present investigation, the behaviour of mechanoreceptors (Section B) and their reflex effects upon efferent gastric vagal discharges (Section D) receive particular emphasis.

Gaseous insufflation of the rumen increases the rates of eructation and secondary cycle contractions (Dougherty, 1940; Cole, et al 1942; Weiss, 1953; Dougherty & Meredith, 1955; Stevens & Sellers, 1959). In an analysis using fistulated cows, Stevens & Sellers (1959) demonstrated that primary and secondary cycles were independently excitable. After removing the contents of the reticulo-rumen, primary and secondary cycles still occurred. Both cycles increased in rate and amplitude when either normal or simulated ruminal contents were replaced. In the case of the secondary cycles, it was concluded that this increase was due to increased pressure or tension rather than to chemical to tactile stimuli. The enhancement of primary cycles resulting from ruminal insufflation was not affected by local anaesthesia of the reticulum (excluding the reticular groove region), of the dorsal ruminal mucosa or of the ruminal nerves. In contrast, secondary cycles were abolished by anaesthesia of the dorsal ruminal mucosa in the caudal region and of the ruminal nerves, whereas they were enhanced by local pressure applied to the caudal dorsal sac and by stimulating either the ruminal nerves or the dorsal abdominal vagal trunk, using implanted electrodes. It was inferred that a reflexogenic area for secondary cycles exists in the caudal regions of the dorsal ruminal sac which responds to pressure or tension and receives its afferent innervation through the ruminal nerve branches of the dorsal vagal trunk./

trunk. One of the objectives of the afferent gastric unit studies described in Section B was to test this conclusion.

Reticulo-ruminal motility is reflexly affected by physical and chemical conditions in the abomasum. Phillipson (1939) showed in conscious cannulated sheep, that distension of the abomasum with a balloon or with 500-600 ml solutions of either normal saline, warm water, 1% sodium bicarbonate or 0.1 N HCl caused primary contractions to cease completely for 8-12 minutes and secondary ruminal movements to be reduced in amplitude and frequency. A similar effect was recorded by Weiss (1953) and in decerebrate sheep by Titchen (1958). Sectioning the splanchnic nerves abolished the inhibition and instead, distension produced an excitatory effect. Titchen (1958) also observed that acidifying the abomasal contents with 0.2 N HCl to attain a pH of not more than 1.0 evoked or enhanced reticulo-ruminal movements. Ash (1959) showed that 100 ml of unbuffered 150-200 mM V.F.As. solutions introduced into the abomasum of conscious cannulated sheep enhanced reticulo-ruminal movements after a latency of 2 min for a period of up to 50 min. Under these conditions the abomasal pH was usually 2.0-3.0 and never below 1.3. Titchen (1958) concludes that 'acid receptors' with a threshold of about pH 1.0 exist in the walls of the fundic glands and are stimulated during the secretion of abomasal acid and are therefore more or less independent of the pH of the contents found in the lumen of the abomasum. These receptors are considered to provide a tonic afferent drive to the 'gastric centres' for the reflex excitation of reticulo-ruminal movements. This hypothesis has been tested to some extent during the present experiments (Sections A, D and F).

Numerous/

Numerous attempts to analyse the reflex regulation of gastric movements have involved studies of visceral afferent pathways; three general approaches having been employed. In the first category are those investigations in which the existence of specific and non-specific afferent pathways have been demonstrated by stimulating the central end of a cut or blocked nerve. This approach has produced some profitable results. The idea that the gastric centres are dependent upon a tonic afferent drive stems from Iggo's demonstration (1951) that tetanic stimulation of the central end of a cut cervical nerve showed no motility. Using anaesthetized sheep, Dussardier (1955, 1960) showed that continuous stimulation (more than 10 stimuli/sec) of the central end of a cut cervical vagus reflexly evoked contractions for as long as the stimulation lasted. The frequency of the contractions increased as the rate of stimuli increased but there was no change in their amplitude. When spontaneous contractions were present, stimulation often enhanced them but occasionally inhibited them. Similar results were obtained with stimulation of the central end of the cut abomasal nerve. The latter has been confirmed by Matscher & Beghelli (1957, 1958). Titchen (1958), by stimulating the central ends of the ventral thoracic vagal trunk and the abomasal nerve in decerebrate sheep, observed an enhancement of motility with low stimulus frequencies (20-60/sec) and an inhibition with frequencies greater than 60/sec. Stimulation of the dorsal thoracic vagus produced no reflex effects on motility, whereas stimulation of the central end of a sectioned splanchnic nerve resulted in reflex inhibition.

The/

The second category of investigations consist of those in which test stimuli have been applied before and after blocking the afferent nerves, in order to identify the effective stimulus, the reflexogenic zone and the reflex response to stimulation. Reversible block of vagal pathways has been employed in decerebrate sheep (Iggo, 1951), conscious sheep (Dussardier, 1960; Popow, et al, 1933) and conscious cows (Dziuk & Sellers, 1955; Stevens & Sellers, 1956). In decerebrate sheep with spontaneous reticulo-ruminal contractions, Iggo (1951) blocked one vagus with local anaesthesia, cooling or pressure. This resulted in reversible, graded depression or complete abolition of the contractions. Block of motor fibres may have accounted for the former result but the latter must be due to block of a tonic afferent input. Using conscious sheep, Popow et al (1933) cooled both vagi after they had been exteriorized in a skin loop and this abolished movements. Dussardier (1960) combined unilateral cervical vagotomy with cooling the other vagus by means of a chronically implanted thermode and, in accordance with Popow et al (1933), movements ceased: these experiments did not differentiate between afferent and efferent nerve block. Dziuk & Sellers (1955) and Stevens & Sellers (1956, 1959) implanted assemblies on the ventral and dorsal abdominal vagal trunks and the ruminal nerves, which allowed the nerve to be stimulated electrically before and after procaine anaesthetization of the nerve peripheral to the point of stimulation. They showed that stimulation of the dorsal abdominal trunk and ruminal nerves increased the ruminal contraction rates (i.e. secondary cycles?) and eructation frequency. Block at these sites reduced the amplitude of ruminal contractions and the frequency of eructation but this was reversed by electrical stimulation central to the blocked region.

Stimulation/

Stimulation through the assembly on the ventral abdominal trunk increased the rate of secondary ruminal contractions and initiated rumination but had no effect on eructation. Block resulted in an atonic reticulum. It was concluded that the principal afferent pathway for the eructation reflex is through the dorsal vagal trunk. The reflexogenic zone for eructation would therefore, lie within the rumen and, for rumination, it would not lie in the rumen. An extension of these investigations is used on some of the present experiments (Section E), in which selected vagal branches are reversibly blocked by cooling and electrical stimuli given above and below the blocked region. The reflex effects of these manoeuvres are observed as a result of the changes which take place in efferent gastric vagal discharges recorded from single efferent units.

The third category of investigations into afferent gastric activity employs the electrophysiological technique of recording from 'single afferent gastric units'. Apart from present experiments detailed in Section B, the only previous electrophysiological investigations are those of Iggo (1954, 1955, 1956), who recorded the activity in 22 'single afferent units' innervating the reticulum. He describes 19 afferent units which produced a slowly adapting or non-adapting response to reticular distension (Iggo, 1955) and three units which gave a rapidly-adapting response (Iggo, 1954). Three of the 19 slowly-adapting units had a resting discharge even when the stomach was empty and 8 others developed a sustained regular discharge, when the reticulum was inflated. The resting discharge frequency of afferent impulses increased./

increased, when reticular tension was increased either passively due to inflation of the reticular balloon or actively during a reticular contraction. A further 8 slowly-adapting units had an intermittent resting discharge which appeared in bursts lasting 1-5 sec and recurred at intervals of up to 30 sec. The discharge in these units was also increased by passive and active rises in reticular tension. It was concluded, therefore, that all of the 19 units innervated 'in series' tension receptors. By manipulation inside the reticulum, the receptors of 5 units which had given an intermittent discharge beforehand were located to the lips of the reticular groove and on pulling and stretching the lips these units produced a sustained discharge. One unit which had had a regular sustained discharge before manipulation was thought to have a receptive area in the cranial wall of the reticulum. The conduction velocities of four afferent units was measured and those were 12, 6, 5 and 2 m/sec (Iggo, 1955). Using a compound action potential method, Iggo (1956) showed that tetanic stimulation of afferent fibres with conduction velocities estimated at 10-13 m/sec reflexly evoked reticulo-ruminal movements in decerebrate sheep and stimulation of units with conduction velocities of less than 10 m/sec reduced the interval between contractions.

In view of the many investigations on reticulo-ruminal movements, eructation, rumination and salivation, which have implicated receptors in the reticulum reticular groove, reticulo-ruminal fold, cranial pillar and dorsal ruminal sac, the results obtained by Iggo, (1954, 1955) are clearly inadequate. This has led to further investigations of afferent activity arising from the reticulo-rumen and the results are detailed in Section B.

### The objects of the present experiments

The present experiments were designed to study the reflex regulation of reticulo-ruminal movements, using an electrophysiological technique. The afferent input to and the efferent output from the gastric (reticulo-ruminal) centres were sampled repeatedly by recording the nervous activity in 'single fibres' dissected from the left cervical vagus. This necessitated, at first, a method of preparing sheep for acute experiments such that reticulo-ruminal movements would be present under defined standard conditions, so that results obtained at different times in the same sheep or in different sheep might be satisfactorily compared.

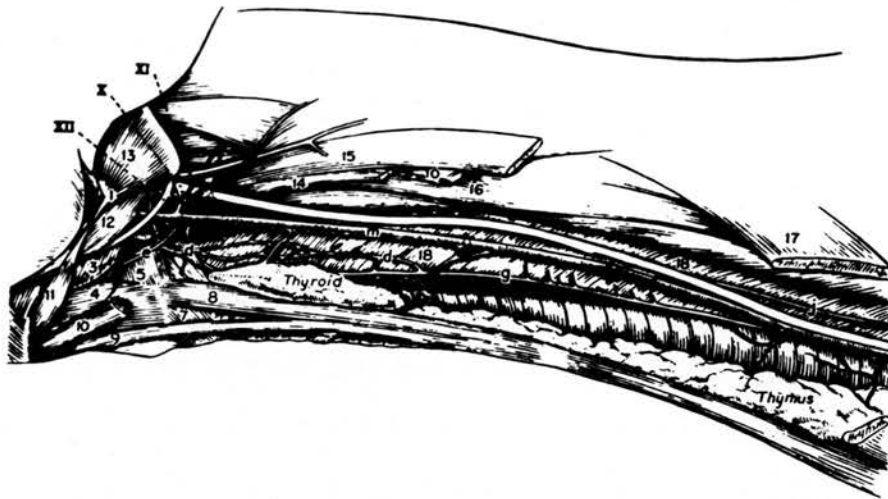
The objects of recording from afferent gastric units were to determine:

- (a) the types of receptors present in the reticulo-rumen.
  - (b) the distribution of receptors in the reticulo-rumen.
  - (c) the level in the wall at which receptors are located.
  - (d) the effective stimulus for each type of receptor.
  - (e) the physiological properties of receptors in terms of their responses to stimulation.
  - (f) the pathway taken by afferent fibres innervating gastric receptors.
  - (g) the conduction velocity of afferent gastric fibres.
  - (h) the afferent input to the gastric centres when reticulo-ruminal movements are present. The input was recorded during both the active and the quiescent parts of the primary cycle at different initial levels of reticular distension and also with the reticulum contracting under both isometric and isotonic recording conditions.
- (i)/

- (i) the pattern of movements which discrete regions of the reticulo-rumen undergo.
- (j) the temporal relationship which exists between the sequential contractions of the various regions of the reticulo-rumen.
- (k) the action of certain drugs on the reticulo-rumen.

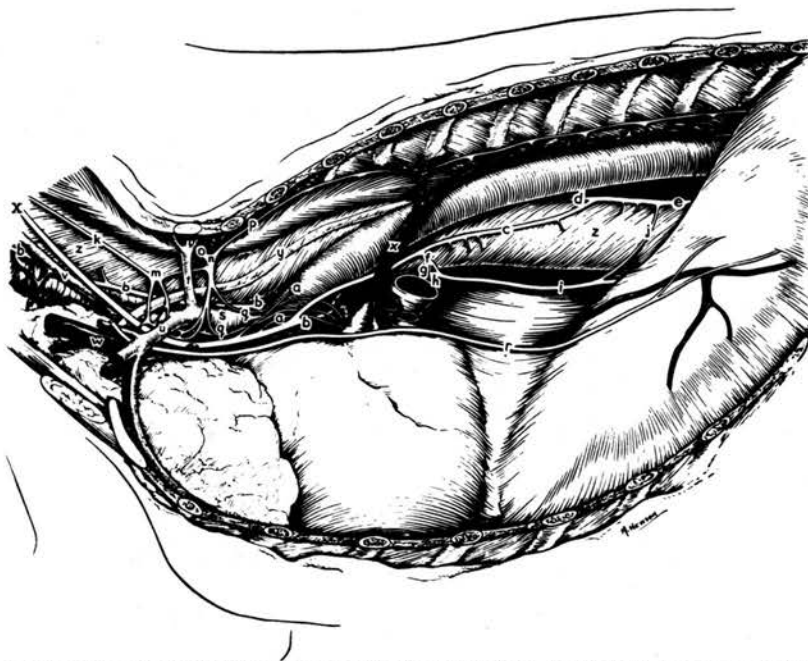
The objects of recording from efferent gastric units were to determine:

- (a) the efferent output from the gastric centres during both the quiescent and the active parts of the primary cycle.
- (b) the different types of efferent discharge patterns.
- (c) the relationship between the efferent discharges and the form of contractions in various regions of the reticulo-rumen.
- (d) the reflex effects upon the gastric centres, and hence upon the efferent gastric discharges, of altering physical conditions within the reticulo-rumen and chemical conditions within the abomasum.
- (e) the dependence of the gastric centres upon a 'drive' provided by afferent vagal inputs.
- (f) the extent of central/reflex and peripheral actions of certain drugs on the reticulo-rumen.
- (g) the total and the central reflex times for gastric reflexes.



(d) Pharyngoesophageal nerve; (e) cranial laryngeal nerve; (f) external laryngeal branch; (g) left recurrent laryngeal nerve; (j) cervical sympathetic trunk; (m) common carotid artery; (1) muscular angle of stylohyoid bone; (3) hyopharyngeus muscle; (4) thyrohyoid muscle; (5) thyropharyngeus muscle; (7) cricothyroid muscle; (8) sternothyroid muscle; (9) sternohyoid muscle; (10) omohyoid muscle; (11) stylohyoid muscle; (12) digastric muscle; (13) jugulothyroid muscle; (14) sternomastoid muscle; (15) cleidomastoid muscle; (16) longus capitis muscle; (17) scalenus muscle; (18) esophagus; (X, XI, XII) cranial nerves.

Fig. 2—The left side of the neck of the sheep.



(a) Cardiovascular nerves; (b) left recurrent laryngeal nerve; (c) dorsal branch of left vagus nerve; (d) dorsal branch of right vagus nerve; (e) dorsal vagal trunk; (f) ventral branch of left vagus nerve; (g) ventral branch of right vagus nerve; (h) bronchial branches; (i) ventral vagal trunk; (j) anastomosis (variable); (k) cervical sympathetic trunk; (m) middle cervical sympathetic ganglion; (n) cervicothoracic sympathetic ganglion; (o) vertebral nerve; (p) thoracic sympathetic trunk; (q) cardio-sympathetic nerves; (r) phrenic nerve (roots removed); (s) common brachiocephalic trunk; (t) costocervical trunk; (u) left subclavian artery; (v) common carotid artery; (w) jugular vein; (x) azygos vein; (y) thoracic duct; (z) esophagus; (X) cervical vagus.

Fig. 4—Nerves in the thorax of the sheep.

EXPERIMENTAL METHODSExperimental animals

Seventy-eight adult Scotch Blackface sheep were used. They weighed 20-40 kg and were 8-18 months old except for a few aged ewes. The sheep were held indoors, usually for at least a fortnight, before use and they received  $\frac{1}{2}$  lb (0.27 kg) oats/day and an unrestricted amount of hay. This practice was found to result in more satisfactory experiments than those in which the sheep had been starved for 24 hr before the experiment or had undergone a change of diet and environment during the previous 2 weeks.

Surgical procedures

Anaesthesia was induced with a 4% halothane B.P./oxygen mixture by a semi-closed method employing a facemask. Then an endotracheal tube (McGill No. 10) was inserted. For the first 8 experiments, anaesthesia was maintained with warm 1% chloralose solution, given intravenously in an initial dose of 4 ml/kg body weight followed by maintenance doses of 1 ml/kg body weight approximately every hour. In subsequent experiments, anaesthesia was maintained with a controlled mixture of halothane and oxygen using a 'Fluotec' halothane vaporizer and a circle type, closed-circuit method incorporating a respiration pump. In the early experiments this was a Starling pump and in the later ones a Harvard 'variable phase large animal respirator' (Model 613). Because swallowing and reflex limb movements seriously interfered with recording from single units, the level of anaesthesia had to be comparatively deep. The corneal reflex was either absent or sluggish and the/

the limbs and tail were flaccid.

The horns were cut off with a hacksaw and bleeding stopped with bone wax and gauze pads. An intravenous cannula was inserted into the left lateral tarsal vein. A large rubber balloon (a 1 litre anaesthetic bag) was inserted into the reticulum either through the reticulo-ruminal orifice (reached by way of a rumenotomy incision in the left sublumbar triangle) or, more usually, through the ventral pole of the reticulum (exposed by a median laparotomy). Sometimes small balloons were inserted into the dorsal and ventral ruminal sacs and a small cannula was put in the abomasum. The animal was then transferred to the experimental table where the sheep's rectal temperature was maintained at  $38^{\circ}\text{C}$  by means of a thermostatically controlled electric blanket. The sheep was laid on its right side with a block of wood under its neck and a saliva tray below its mouth. Further surgery was delayed until reticulo-ruminal movements were recorded. When spontaneous contractions were not already present, they could usually be evoked by distending the reticular balloon with 200-600 ml of air.

The left cervical vagus was exposed by incising the skin for 15 cm along the line of the jugular groove and excising the left sternocephalic muscle. The edges of the skin wound were sutured to a horizontal ring of solder to form a pool for liquid paraffin B.P. A silver earth electrode was embedded and sutured in the longus colli muscle. About 1.5 cm of vagus was freed from underlying connective tissue and a rigidly held black perspex dissecting plate was placed under this region. When required, Ag/AgCl stimulating electrodes or the thermode were inserted beneath the nerve on either side of the plate.

When

When the surfaces of the reticulum and rumen were manipulated in order to locate the sites of receptors, the hand was inserted usually through a large fistula made through the sublumbar triangle into the dorsal sac but occasionally into the reticulum directly (see below). Frequently, ribs 7-9 and the left lung were removed for a variety of reasons, e.g.,

- (a) the removal of the left lung largely eliminated afferent pulmonary discharges, whose presence made the recognition of gastric afferent discharges much more difficult,
- (b) the dorsal branch of the left thoracic vagus and the dorsal and ventral thoracic vagal trunks were accessible for the application of stimulating electrodes or thermodes and
- (c) by incising the diaphragm, the left (lateral) wall of the reticulum could be observed directly or could be opened to allow direct access to the reticular surface for the purpose of locating mechanoreceptors.

In three experiments the greater splanchnic nerves were cut at the level of the diaphragm and both adrenal glands were removed.

Anaesthesia and gastric contractions were maintained for up to 19 hr.

#### Nerve recording and stimulating techniques

Extracellular recordings were made from fine nerve strands dissected from the left cervical vagus and placed across a pair of fine Ag/AgCl wire recording electrodes carried on a micromanipulator, as described by Iggo & Vogt (1960) for recording from preganglionic cervical sympathetic fibres. The dissecting instruments consisted of fine watchmaker's forceps, a beading needle held in a pinchuck and a piece of razor blade sharpened to a point on  
Arkansas/

Arkansas stone and also held in a pinchuck. With the aid of a Zeiss microscope, the nerve/<sup>sheath</sup> was incised for about 1 cm of its length to expose underlying fasciculi. The sheath of one fasciculus was opened as above and then a fine strand was parred away from its parent fasciculus by cutting centrally in the case of afferent fibres and distally in the case of efferent fibres. The strand was subdivided on the dissecting plate using either forceps alone or combined with the beading needle (Fig. M 1). The vagus and the fine strand dissected from it were at all times immersed in a pool of warm liquid paraffin. The action potentials ('spikes') were pre-amplified 1000X by a Tektronic (Type 122) low-level pre-amplifier, set with a high-frequency response of 1 KC and a low-frequency response of 80 c/sec, and further amplified by and displayed on either a Tektronic 502 dual beam oscilloscope or in later experiments a Tektronic 565 dual beam oscilloscope. With the spots stationary on the X axis, movements along the Y axis were recorded photographically on moving bromide paper using a Cossar camera mounted in front of the oscilloscope screen. The amplified spikes were also fed into an audio-amplifier and loudspeaker and, when desired, into a Thermionic Products 8 channel tape recorder and into a frequency meter connected to one channel of a Devices 8 channel pen recorder.

Electrical stimulation was made through Ag/AgCl electrodes connected via a stimulus isolation unit to a Grass S4 stimulator. When intermittent trains of stimuli were required a second stimulator was connected to the first to act as a modulator.

Recording forestomach movements/

Recording forestomach movements

Movements of the various compartments of the reticulo-rumen were recorded manometrically using air filled balloons (Fig. M 2). Both a narrow polythene tube, with a tap through which air could be introduced or removed rapidly by means of a 200 ml hypodermic syringe, and a wide polythene tube were passed through the neck of the 1 litre balloon in the reticulum. The wide tube had a side-arm which branched and was connected to a Marey tambour writing on a smoked kymograph paper and to either a C.E.C. or a Statham (P23) strain-gauge manometer or, in the early experiments, a glass diaphragm which activated the grid pin of a R.C.A. 5734 valve. The other end of the wide tube was connected to the top of a 10 litre aspirator flask and the latter was joined to a similar flask by a very short length of wide tubing between the openings near their bases. The top of the second flask was connected to a 600 ml float recorder, which recorded the volume changes, or to a Greer micro-manometer, which recorded the very low pressure changes in the aspirator bottle caused by an (imperfectly isotonic) reticular contraction. The flasks were half-filled with water, so that a head of pressure could be established in the reticular balloon and, due to the size of the tubing and the flasks, this pressure changed by not more than 2 mm Hg during the course of a reticular contraction. The recording conditions were, therefore, approximately isotonic, since the contraction resulted in a change in the volume but not in the pressure of the reticular contents. When the wide tube was clamped between the first aspirator flask and the side-arm leading to the Marey tambour and the transducers, isometric recording conditions were obtained/

obtained, since the reticular contraction then resulted in a rise in pressure without a reduction in the volume of the contents. The small balloons in the dorsal and ventral ruminal sacs were connected by polythene tubing to C.E.C. strain-gauge manometers. Each manometer was connected to a separate channel on the Devices pen recorder and one or two were also connected to the oscilloscope, so that pressure changes were displayed simultaneously with the spike discharge. The pre-amplifier outputs from the Devices pen recorder were tapped and recorded on magnetic tape along with the spike discharge described above. A Venner Electronics Frequency Source (T.S.A. 602/A) was used as a time base for recordings made on bromide paper and magnetic tape.

#### pH recording

An E.I.L. Vibret pH meter and glass electrode were used to measure the pH of abomasal and ruminal contents in vitro and, by inserting the glass electrode through a cannula, ruminal contents in vivo. A continuous record of pH was made on a channel of the pen recorder in a few experiments.

#### Nerve cooling

Nerve transmission could be reversibly blocked by applying a thermode to a length of the nerve. The thermode consisted of (a) a curved metallic shield with a groove, the shield being placed under the nerve so that the nerve lay in the groove, and (b) a drum which fitted into the shield and through which fluid was cooled and rapidly circulated by means of a Colora (KT 10K) low temperature bath with a built-in pump. For total block of nerve/

nerve transmission, the fluid circulating to the thermode was usually cooled to less than 4°C.

### Drugs

Drugs were administered intravenously through the cannula inserted into the left lateral tarsal vein. The doses and manufacturers of the drugs are given in the relevant Sections (D and F).

Fig. M1. The dissection of 'single fibres' from the left cervical vagus in sheep.

A shows the vagus lying on top of a black perspex dissecting plate. The nerve sheath has been slit longitudinally to expose the underlying fasciculi. The exposed tissues are immersed in a pool of warm liquid paraffin.

B shows a multifibre strand which has been dissected from one of the fasciculi after slitting its sheath. The strand is laid on the dissecting plate, where it is subdivided. The dissecting instruments shown are (a) fine watchmakers forceps, (b) a scalpel made from a piece of razorblade, held in a pinchuck and (c) a beading needle held in a pinchuck.

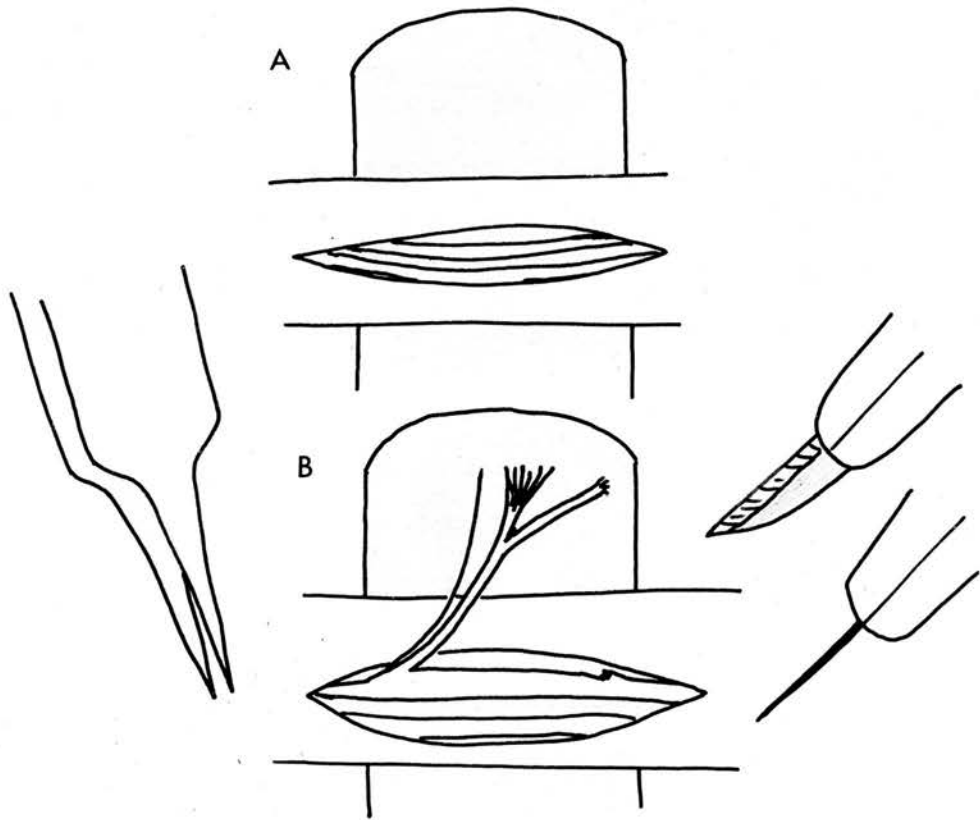


Fig. M2. The experimental design used in the first experiments.

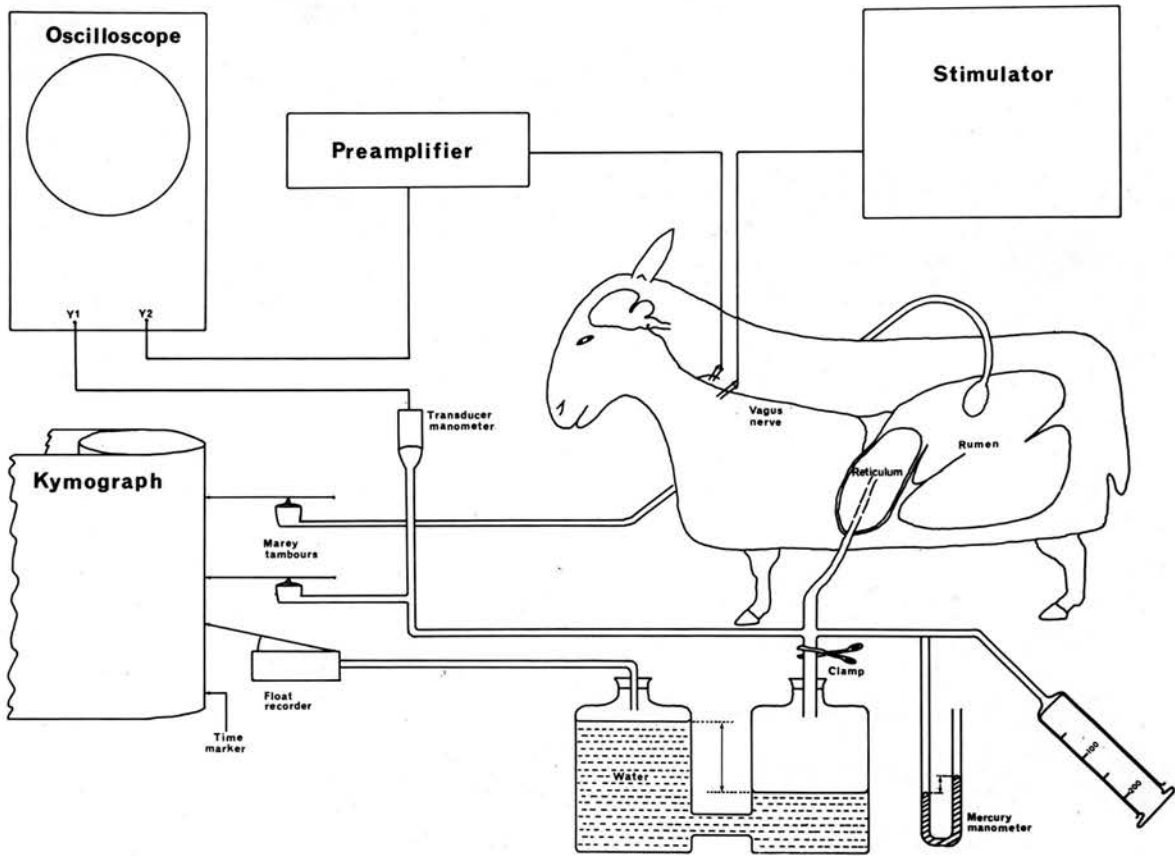
'Single fibres' were isolated from the cervical region of the left vagus and their nervous activity was recorded extracellularly with a pair of Ag/AgCl electrodes, amplified and displayed on an oscilloscope. A stimulator and electrodes were available for conduction velocity measurements, etc., (see Sections A, B and E).

A large balloon was inserted into the reticulum and reticular contractions were recorded either isometrically (i.e. when the clamp was closed) or isotonically (i.e. when the clamp was removed). Air could be suddenly introduced or removed by means of the large syringe. Rumen movements were recorded with small balloons inserted into the dorsal and ventral sacs.

Not shown are (a) the closed 'circle type' anaesthetic circuit incorporating a Beaumont anaesthetic machine, a respiration pump and a McGill intratracheal tube, (b) the cannula in the tarsal vein, (c) the thermode with its refrigerator and pump and (d) the glass electrode and pH meter.

Later modifications were the replacement of the kymograph with a 6 or 8 channel 'hot wire' pen-recorder, the Marey tambours with strain gauge transducers and the single connection to the reticular balloon with three separate connections, i.e. for the isometric system, the isotonic system and the syringe connection. Signals from both the pen-recorder and the oscilloscope could be transferred to and stored on 7 channel magnetic tape.

# EXPERIMENTAL DESIGN



SECTION AGastric movements in anaesthetized sheepINTRODUCTION

One of the main problems in the study of gastric motility in sheep is that the reticulo-ruminal movements are abolished by many anaesthetic agents (Iggo, 1956). The movements may be present in decerebrate preparations of sheep (Iggo, 1951; Titchen, 1953) but they are not always present and cannot always be evoked. When present, they may be different in form and frequency from those in the intact animal and may persist for a relatively short time. These features of decerebrate preparations make them unsuitable for the kind of work to be described in this thesis, in which it is necessary to obtain regularly recurring contractions of more or less normal shape and size, particularly since reflex modification of these movements is also studied. A further disadvantage of decerebrate preparations is that they may exhibit reflex somatic movements, elicited by cutaneous and other stimulation arising from the experimental manipulations. These movements interfere both electrically and mechanically with single unit recording and so an attempt was made to find a preparation with recurrent reticulo-ruminal movements as near as possible to those occurring in the conscious animal.

This section with only slight modification has already been published (Iggo & Leek, 1967a).

RESULTS

Brunaud & Dussardier (1951) described active gastric movements in sheep anaesthetized with chloralose and a modification of this method was tried.

Particular/

Particular difficulty was found in maintaining an optimum anaesthetic level and because of the long interval between the injection of chloralose and its effective action, estimation of maintenance doses was not easy. Eight sheep were prepared in this way. Two of these had been fed out-of-doors on turnips until two days before use; in one no gastric movements were evoked, and in the other they persisted for only 6 hours. The remaining 6 all produced gastric contractions which were intermittent, because of the variations in anaesthetic level.

During the preparation of sheep for decerebration, halothane was used to induce anaesthesia and it was discovered that gastric movements were often present or could easily be elicited in sheep maintained under a halothane/oxygen mixture. The use of this anaesthetic offers several advantages over both decerebrate and chloralose-anaesthetized preparations. These are (a) the absence of swallowing and reflex limb movements, (b) the persistence of gastric movements for very long periods, as long as 19 hours and (c) the ease of rapidly adjusting the anaesthetic level while retaining the ability to maintain a fairly constant level of anaesthesia for a long time. A respiration pump was incorporated into the anaesthetic circuit, since this permitted the anaesthetic level to be kept more constant and caused gastric movements to be larger than those present during spontaneous breathing.

The halothane/oxygen method of anaesthetizing sheep was used in 39 animals included in the present study. In 16 of these animals gastric movements were present for longer than 12 hours and in some they were still present/

present after 19 hours. Twenty-three animals produced gastric contractions that persisted for less than 12 hours but these included 6 animals with extensive surgery and 6 which were starved for 24 hours prior to the experiment. Only 2 failed to produce any gastric movements and these included 1 which was only 5 months old.

Gastric movements were present in only 8 of the sheep after induction of anaesthesia at the start of an experiment, but in a further 36 animals it was possible to elicit them reflexly. A simple and effective way to do this was to inflate the reticular balloon with 400-600 ml air, causing an intraluminal pressure of about 10 mm Hg. These conditions were adopted as the 'standard' procedure and the comparisons of single unit activity made later are based, as far as possible, on recordings under these conditions. Other procedures that were known to evoke reticular contractions in decerebrate sheep were also tried. Electrical stimulation of the central end of a cut cervical vagus or of an intact cervical vagus or acidification of abomasal contents were ineffective in sheep in which reticular contractions could not be evoked by reticular distension. These procedures did elicit reticular contractions for a short time from some of those animals in which, either previously or subsequently, reticular contractions were produced by reticular distension. The addition of 50 ml of 0.2 N hydrochloric acid to the abomasum sometimes produced a temporary enhancement of the amplitude or frequency of reticular contractions that had been evoked by reticular distension. Gastric contractions were reduced in amplitude or frequency when the abomasum or the rumen became markedly distended, for example, when the ruminal pressures exceeded/

exceeded 20 mm Hg. During the early stages of an experiment, bacterial fermentation in the rumen caused the rapid accumulation of gas and resulted in high intraruminal pressures. If reticular contractions were absent in these conditions, they re-appeared 1-4 minutes after the gas was released by inserting a large bore needle through the flank of the animal into the rumen. An optimal intraruminal pressure appeared to be necessary, since the reticular contractions did not re-appear if too much gas was removed.

The movements of the different parts of the stomach were affected to varying degrees by anaesthesia. The pressure changes associated with reticular contractions were approximately the same as those recorded in conscious animals, as regards their frequency, wave-form, duration and amplitude (Fig. A 1 & B 9). The typical isometric reticular contraction was biphasic. Initially the pressure rose sharply at a rate of 4 mm/sec for 1.5-2.0 sec to reach a low first peak of 6 mm Hg. During the next 1.5 sec there was usually a slight drop in pressure (4 mm Hg) but there might be no fall or even a slight rise at this time. Then followed an even sharper rise in pressure at the rate of 4.5 mm/sec lasting 1.5-2.0 sec, so that a high second peak of pressure was reached, 15 mm Hg above the resting pressure. After this the pressure fell quickly at first, at the rate of 4 mm/sec for 2-3 sec to reach 4-7 mm Hg, and then followed a slow terminal fall back to the resting pressure during the next 4-6 sec. When large ruminal contractions were present, this terminal phase usually had superimposed upon it a slight rise and fall of pressure coincident with the dorsal sac contractions.

The/

The reticular pressure changes were compared, on a few occasions, with movements of the (left) lateral wall of the reticulum, that had been exposed by resecting ribs 9-11, removing the left lung and incising the diaphragm. The reticular movement was seen to start as a weak contraction involving simultaneously all parts of the lateral wall of the reticulum. Then followed, in sequence, a slight incomplete relaxation, a sharp strong contraction and a complete relaxation which was rapid at first but terminated slowly. There were no signs of waves of contraction or of regions which were inactive for a part, or whole, of the sequence, as was suggested by some of the earliest investigators (Wester, 1926; Schalk & Amadon, 1928).

Ruminal contractions in the anaesthetized animals were usually much weaker than in the conscious animal, especially in the more caudal and ventral parts of the rumen. The largest recorded dorsal ruminal sac contractions lasted 10-12 sec and reached a peak pressure of 10 mm Hg about 4 sec after the second peak of the reticular contraction. Dorsal sac contractions were more often absent or weak. They were largest when the level of anaesthesia was light or when the reticular distension was large (600-1000 ml). In the present experiments the reticular balloon scarcely projected over or stretched the reticulo-ruminal fold. Contractions of the caudo-dorsal blind sac and the ventral ruminal sac, were sometimes present, though always of very low amplitude. The position of the sheep affects the ruminal movements, e.g., secondary cycles of contraction were found to be absent when the animal was on its side.

Reticulo-ruminal contractions were most often present or most easily evoked/

evoked when there was only a short interval between the induction of anaesthesia and the insertion of the reticular balloon, and its inflation. For this reason the reticular balloon was always put in place as soon as possible after anaesthetizing the sheep and before completion of the other surgical procedures that were required for the electrical recording. The reticular movements were always reduced in amplitude and rate by even relatively minor surgical procedures, e.g., incising the skin, and they were completely abolished for 5-10 minutes by more extensive surgery, e.g., exposure of the cervical vagus, although they regained their former amplitude and rate over the course of a further 5-10 min. This effect was seen during surgery at any site on the body, either superficial or deep, and also, when exposed viscera and the edges of unsutured wounds were manipulated. The effect appeared to be independent of anaesthetic level and was also present in adrenalectomised animals in which both major splanchnic nerves had been cut. Blood loss during surgery was quite small and was unlikely to have contributed to the effect.

For comparison with the gastric movements elicited reflexly by distending the reticulum, a brief study was made of movements evoked directly, by stimulating electrically the peripheral end of a cut cervical vagus at intensities that were sufficient to cause maximal reticular contractions when continued for 5 sec at 20/sec. Using a train of stimuli at this rate, which is the average peak frequency of discharge recorded from the Type I units described later, a stimulation period of approximately 1 sec was necessary to produce a reticular contraction similar in amplitude to those occurring/

occurring spontaneously under the standard conditions described on p. A 3. The reticular contraction was monophasic, began 0.7 to 1.0 sec after the start of stimulation and reached a maximum amplitude if the stimulus was continued for 5 sec. For submaximal contractions the phase of contraction lasted for as long as the period of stimulation (Fig. A 2). The interval between the middle of a 1 sec train of stimuli and the peak of the reticular contraction was about 1.3 sec (1.2 to 1.5 sec). This interval is slightly shorter than the average interval (1.8 sec) between the peak of the impulse discharge in Type I units (described later) and the second peak of the associated reticular contraction. Electrical stimulation at frequencies of less than 20/sec caused the rate of pressure rise in the reticulum to be slower. Electrical stimulation of a vagus produced similar effects in the rumen, except that the latency in the mid-dorsal sac was 0.8 sec longer than the reticular latency.

#### DISCUSSION

One of the principal difficulties encountered in analysing the reflex basis of reticulo-ruminal motility has been that of maintaining gastric movements in suitable experimental conditions. In the present experiments halothane anaesthesia has allowed the movements to be investigated in the anaesthetized animal for up to 19 hours. This has avoided the use of decerebrate preparations, in which gastric movements are often difficult to evoke and maintain for long periods (Iggo, 1956; Titchen, 1958). It was also more convenient and reliable than the chloralose anaesthesia method used by Brunaud & Dussardier (1951) and also in the first 8 of the present experiments.

A number of other conditions were also found to result in more reliable preparations. Gastric movements were most easily elicited and maintained in anaesthetized sheep which had not been starved before the beginning of the experiment, or which had not recently had a change of diet. This usually leads to a reduction in appetite for a few days. Active ruminal fermentation prior to an experiment seems, therefore, to be associated with more lively reflex preparations.

Reticulo-ruminal movements evoked under halothane anaesthesia showed some differences from those recorded in the conscious animal. Reticular movements were similar so far as the frequency, form, duration and amplitude of the biphasic contractions were concerned and it is concluded that the observations made on reticular function during these experiments would also hold for conscious animals. This was not so for the rumen, because, although the dorsal sac contractions had a similar form and duration in conscious and anaesthetized animals, they were of smaller amplitude in the latter. Ventral sac contractions were either very small or absent under anaesthesia. It is likely that the reduction in ruminal motility was due to reflex and central factors rather than to a transmission block in the motor pathway, even though halothane in high concentration blocks peripheral nervous transmission and ruminal movements are known to be more susceptible than reticular movements to the action of ganglion blocking agents (Brunaud & Navarro, (1954). This conclusion is based on a comparison of our results with those of Dussardier (1960), who used the cross-sutured nerve technique in conscious animals, and recorded many more units with a ruminal or late discharge (equivalent to the Type IV units described in Section C) than with a reticular or early discharge (equivalent to the units of Types I-III). Dussardier's ruminal/

ruminal units also had many more spikes per discharge and higher peak frequencies.

Further experiments are required, in order to determine the extent to which halothane was depressing the reflex centres for ruminal motility and the extent to which the experimental conditions diminished reflex excitatory effects and introduced or enhanced reflex inhibitory actions. It is likely that halothane had a stronger central depressant action on the 'ruminal centres' than on the 'reticular centres' because lightening the anaesthetic level alone often led to the appearance or increase in amplitude of ruminal movements without a change in those of the reticulum, i.e. in conditions in which there is unlikely to be any modification of gastric afferent input. In the present experiments the reticular balloon scarcely projected through the reticular ruminal orifice and hence did not stretch the reticulo-ruminal fold appreciably. The sheep were, therefore, deprived of a stimulus which Titchen (1960) found to be very effective in evoking reflex ruminal contractions in decerebrate sheep. Other peripheral factors might include the abnormal position of lateral recumbency, a posture which is known to influence rumen movements (Balch, 1952; Reid & Titchen, 1965) and also physico-chemical changes in the rumen contents resulting from ruminal stasis and the lack of an inflow of saliva.

In some experiments reticular movements were present before the reticular balloon was inflated but usually it was necessary to add 300-600 ml air to evoke reticular contractions which were comparable in rate, form, duration and amplitude to those in the unanaesthetized sheep. The results obtained from studies of gastric afferent units might be significant in this connection. Reticular distension of about 400 ml was required to change the discharge pattern/

pattern in a gastric tension receptor during the inactive phase of the gastric cycle from one of irregular bursts of activity to one in which the discharge was continuous and regular (Iggo, 1955). Results described in Section B confirm that mechanoreceptors in the reticulum and cranial sac (dorsal rumen) produce either no discharge or an intermittent discharge during the inactive part of the gastric cycle when the reticulum contains less than about 400 ml air, whereas these receptors tend to produce an uninterrupted resting discharge at greater volumes of distension.

The suppression of reticulo-ruminal movements which occurred (even at the deepest planes of anaesthesia), when surgery was performed or contact made with exposed tissues, was most striking and was evident within a few seconds of applying noxious stimuli. The full effect took about 30 sec to develop. This suppression was present even in sheep which had been adrenalectomised and had had their splanchnic nerves cut, although in these animals the effect seemed to persist for a shorter time. Titchen (1958, 1960), using decerebrate sheep, had observed similar reflex suppression of reticular contractions through manipulation of the viscera (particularly the pylorus) and distension of the abomasum. He concluded that the splanchnic nerves were providing an afferent pathway for these effects, except for the ones from the pylorus. In the present experiments the suppression took the form of an absence or suppression of a discharge in the efferent fibres, so that the inhibition was a central phenomenon. The inhibitory mechanisms require further study.

Although reticulo-ruminal movements in the halothane-anaesthetized sheep are,/

are, to a slight extent, subnormal, the preparation has several merits:

(a) by standardising the experimental conditions, it becomes possible to compare units recorded from a large number of animals, (b) recordings are made directly from efferent gastric fibres and the afferent and efferent pathways in both vagi remain intact, apart from the fascicule from which the fibres have been dissected, (c) the anaesthetized sheep, unlike decerebrate animals, are free from reflex limb and neck movements which can seriously interfere with single unit recordings, and the gastric movements persist for much longer.

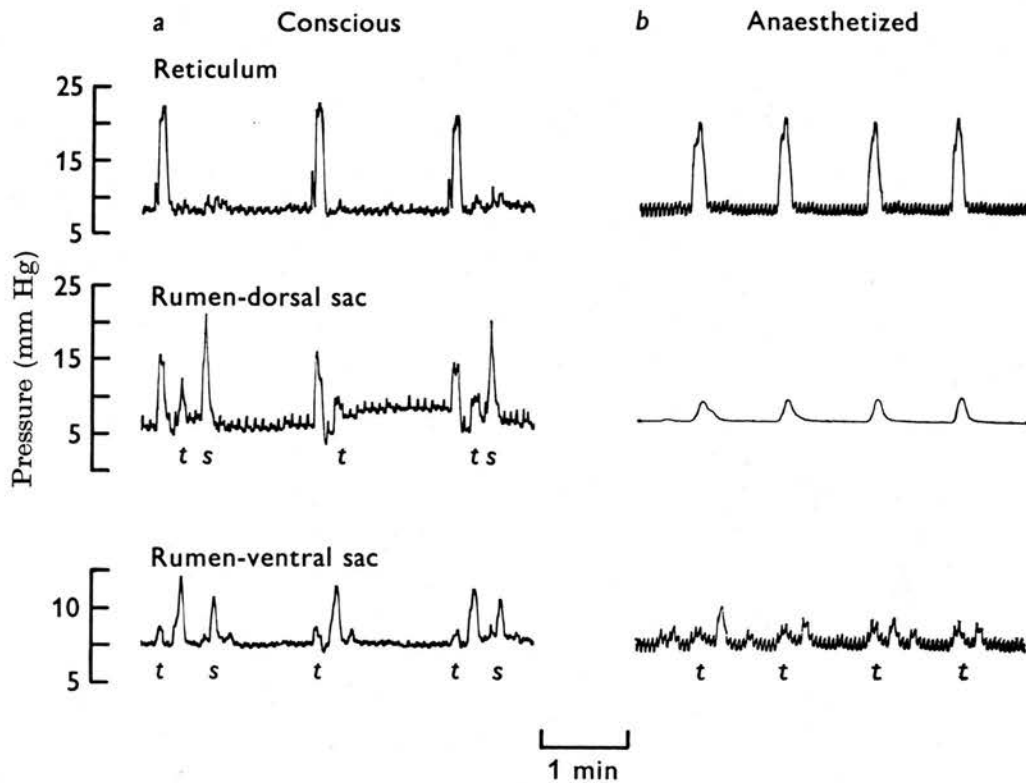


Fig. A1. Movements of the reticulo-rumen in (a) a standing conscious sheep fitted with a rumen cannula and (b) a halothane-anaesthetized sheep lying on its right side. The records were made under identical conditions with air-filled balloons connected by polythene tubing to strain-gauge manometers. The first phase of the reticular contraction is small and followed by a relaxation in the conscious animal but large, with only a slight relaxation in *b*. The 'primary' ruminal contractions are smaller in the anaesthetized animal. 'Secondary' ruminal cycles (*s*) are present in the conscious sheep but absent from *b*. Certain pressure changes (*t*) in the rumen are passively transmitted from the dorsal to the ventral sac and vice versa.

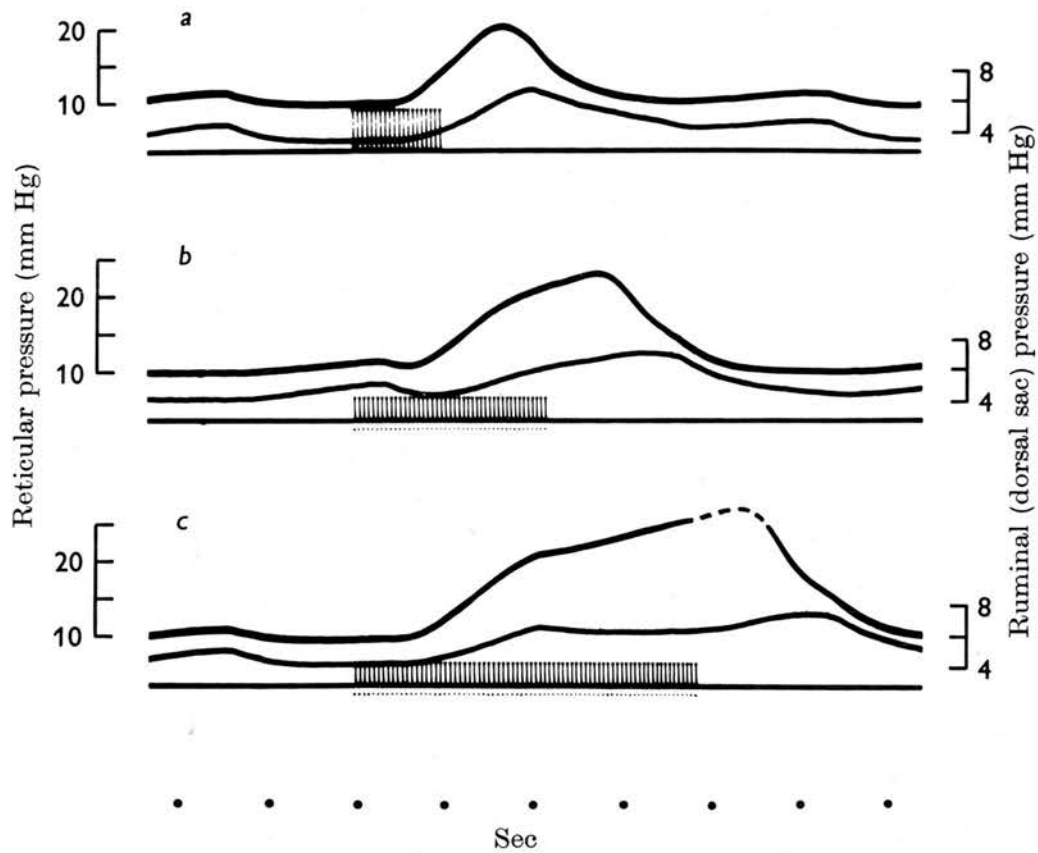


Fig. A2. Contractions of the reticulum (*upper trace*) and dorsal sac of the rumen (*middle trace*) caused by electrical stimulation at maximal intensity (*lower trace*) of efferent fibres in the intact left cervical vagus in a halothane-anaesthetized sheep. Maximal contractions were obtained only when the stimulus lasted for at least 5 sec. Submaximal contractions are illustrated and in all of them the phase of contraction has approximately the same duration as the period of stimulation but starts after a latency of 0.7 sec. The contractions are superimposed upon pressure fluctuations due to respiratory movements. The stimulus artifacts in *a* are larger than in *b* and *c* because of a change in recording conditions.

Trains of stimuli (30 V) at a frequency of 20/sec were given at intervals of 1 min for durations of 1 sec (*a*), 2 sec, (*b*) and 4 sec (*c*).

SECTION BThe afferent discharges arising from mechanoreceptors in the reticulo-rumenINTRODUCTION

Forestomach movements and the production of saliva are reflexly modified and the processes involved in eructation and rumination are reflexly triggered by applying stimuli to the surface of the reticulo-rumen (p. I 39 et seq). Apart from the reflex effects produced by altering the pH of the rumen contents described by Ash (1959), most effects have been induced by mechanical stimuli, e.g., in decerebrate sheep, it appears that some degree of reticular distension, either alone (Iggo, 1951; 1955) or combined with stretching of the reticulo-ruminal fold (Titchen, 1958), is necessary to provide the 'gastric centres' with a tonic afferent drive.

Investigations of sensory mechanisms which depend on observing a reflex effect after applying a test stimulus usually suffer from three important drawbacks:

- (a) the stimulus is ill-defined e.g., 'stroking', 'scratching', 'stretch', 'distension'. By 'stretch' and 'distension', is it meant that a region is being lengthened, tensed in a tangential direction (in accordance with the concepts of 'surface tension' physics) or compressed between the inner and outer walls of the viscus?
- (b) the receptive area is often vaguely located
- (c) it is usually technically difficult and undesirable to stimulate and record activity from the same region, since the recording device may interfere/

interfere with the application of the stimulus or may itself provide an additional stimulus.

These problems have been overcome by Iggo (1954, 1956) using a 'single fibre technique' for recording afferent gastric vagal unitary discharges in decerebrate sheep. Unfortunately, only a few units were isolated and, in most of his sheep, gastric movements were not occurring spontaneously but were induced by tetanic stimulation of the central end of a cut cervical nerve.

In the present experiments the same 'single afferent fibre technique' has been used to investigate the locations, types and physiological properties of mechano-receptors in the reticulo-rumen. This technique for examining the sensory input to the 'gastric centres' is complementary to those methods in which reflex effects are studied, since the 'single fibre technique' per se provides no indication of the reflex consequences of the afferent discharges which are recorded. Although Titchen (1958) has showed, in decerebrate sheep, that very low abomasal acidity provides an important tonic excitatory drive to the 'gastric centres', acid receptors in the abomasum have not been examined in the present experiments. There appear to be no a priori reasons why abomasal acid receptors should differ substantially from those described for the cat's stomach (Iggo, 1956).

Brief reports of this work have been published (Leek, 1966, 1967).

#### RESULTS

Sixty-six afferent units were isolated from 15 sheep. In 41 units the activity was recorded under conditions when (spontaneous) reticulo-ruminal movements/

movements were present. The sites of the receptors were located for 13 of the 41 units and the conduction velocities were measured for 4 of the units. The afferent activity in a further 25 units was examined in sheep with no spontaneous reticulo-ruminal movements, principally to determine the location of their receptors, the conduction velocity of the afferent fibres and, for 11 units, the thoracic vagal branch in which the fibres were to be found. Due to the inhibitory effect upon gastric movements (discussed on p. A 10) caused by surgical procedures and the manipulation of wound edges and viscera, the manoeuvres necessary for locating receptors often resulted in the abolition of spontaneous movements.

#### The location of receptors

The locations of the forestomach mechanoreceptors responsible for the discharge in 38 single afferent units, were determined by manual exploration of the mucosal surface of the reticulo-rumen. Access to the interior of the reticulo-rumen was gained through fistulae made either in the dorsal ruminal sac, using a sub-lumbar approach, or in the reticulum, using a transthoracic approach (p. M 3). It was usually necessary to remove some of the rumen contents and to partially or completely deflate the reticular balloon, if present. Using fingers or a glass rod, the mucosal surface of the forestomach was stimulated mechanically by gentle stroking, pinching, pressing with one finger, pulling on strips of mucosa and stretching the wall by opening two fingers applied to adjacent areas of the wall. In this way the location of mechanoreceptor sites and their distribution were found (Table B 1) and the afferent discharges elicited by manipulation are illustrated in Fig. B 2 (A & B).

The/

The majority of receptors (15 units) were found in the walls of the reticulum, predominantly in the medial wall adjacent to the lips of the reticular groove. Few receptor sites were found in the caudal wall and even less in the cranial wall. No receptors were located to the lateral (left) wall of the reticulum in these experiments. In only one of the 41 units was it possible to elicit an afferent discharge when the mucosa was gently stroked. The receptive field of this unit was located in the cranial wall of the reticulum; stretching the wall also caused a short burst of afferent impulses but a sustained discharge could not be produced. In the remaining 14 reticular units there was no response to pinching or gentle pressing. Harder pressing, pulling on the mucosa or stretching (in the manner described above) caused a sustained discharge. The frequency of the discharge increased as the stretch increased. It was established that the receptive field for one of the units was in the cranial wall of the reticulum, which had been exposed by the transthoracic approach. While recording the afferent discharge in this unit, the receptive area was compressed between the finger (on the mucosal surface) and the thumb (on the peritoneal surface). No discharge was evoked by transmural compression, although a discharge was readily elicited by tangential stretch of the same region. Similarly, with 600-1,000 ml air in the reticular balloon, it was not possible to evoke afferent discharges (in any of the several units tested) by exploring the peritoneal surface of the reticulum manually and pressing on the outer side of receptor area.

Six units had receptive fields in or near the reticular groove. One of these was situated very close to the cardiac sphincter, another in the floor of/  
of/

of the omasal canal, about 1 cm distal to the reticulo-omasal orifice, and two others in the floor of the reticular groove. All of these units gave a high frequency sustained discharge when the site was pressed upon with a finger. The remaining two units had receptive fields located in the cranial and caudal lips of the reticular groove respectively. An afferent discharge appeared in these two units when the lips were stretched, and more particularly when they were pressed and pinched. By their response to pressing and pinching, the receptors in the lips of the reticular groove clearly differed from those in the reticulum.

Four units with receptors situated in the reticulo-ruminal fold were isolated. A high frequency (up to 100 spikes/sec), sustained, regular afferent discharge was produced by stretching the fold, the frequency being directly related to the degree of stretch. The discharge in these units was not evoked by lightly pressing or stroking the fold, nor by compressing the fold between the finger and thumb. When a forceful stretch was maintained for several seconds, it often caused a local contraction of the fold, which could be readily felt beneath the fingers and which gave rise to a short burst of afferent activity with an even higher discharge frequency than that which could be evoked by stretch alone.

Thirteen units had receptors in the rumen and 9 of these were in the cranial sac (dorsal rumen). Seven of the 9 cranial sac units had receptors located in the medial wall, one in the ventral wall and one in the dorsal wall. Stretching, but not pinching or stroking, stimulated the receptors and caused a sustained discharge in the afferent unit. Usually the discharge was not/

not regular, however, because the stretch stimulus induced local contractions of the muscle in the area being stretched. As in the case of the reticulo-ruminal fold described above, these contractions were easily detected by the fingers and caused an extra burst of afferent impulses. After a series of these local contractions ('ripples') the afferent discharge sometimes diminished or ceased even though the stretch was maintained. During the preliminary exploration of the mucosal surface and before the actual receptive field was located and stretched, the resting discharge often increased in frequency. Moreover, after a series of applied 'stretches', the resting discharge often took the form of frequent bursts of activity, each of which was associated with a local contraction of that region. Once this pattern of resting discharge became established it usually lasted for at least 5-10 minutes (Fig. B 2).

Only four single units were isolated from other parts of the rumen (Table B 1). In two experiments a deliberate attempt was made to isolate only ruminal afferent units and, during the process, afferent activity in multiunit strands was frequently detected, when stretch was applied to the reticulum, reticular groove and cranial sac regions, whereas afferent activity arising from receptors in other parts of the rumen was observed very infrequently. Stretch was the effective stimulus for the 4 units which were eventually isolated. As for the cranial sac and the reticulo-ruminal fold, stretch evoked local contractions, which could be felt and, which enhanced the afferent discharge. In the case of the unit with receptors in the cranial wall of the ventral sac, the discharge during stretch was not sustained but fluctuated/

fluctuated in time with these local contractions.

The paths and conduction velocities of gastric afferent fibres

For 28 of the 34 units whose receptor sites had been located, the conduction velocities were measured by the techniques of Paintal (1953, 1963) and Iggo (1958). Stimulating electrodes were placed either on the left cervical vagus (peripheral to the recording electrodes), on the left thoracic dorsal vagal branch or on the ventral thoracic trunk. By stimulating the last two nerves, it was possible to decide whether a particular unit had its pathway in the dorsal or ventral vagal trunk. Excision of the left lung, besides largely abolishing respiratory afferent activity in the left vagus, facilitated access to the thoracic vagal branches, which were kept moist under a pool of warm liquid paraffin. If the nerve strand on the recording electrodes contained more than one live fibre, electrical stimulation evoked a compound action potential. The recognition of the contribution to the compound action potential by the gastric unit, whose conduction velocity was being determined, was based on the demonstration of a refractory period in the gastric unit. With the stimulating electrodes close to the recording electrodes on the cervical vagus, the 'natural' spike was made to trigger the stimulator after an appropriate delay. When the delay was less than the absolute refractory period, the evoked compound action potential was devoid of the spike component attributable to the gastric unit. Alternatively, the nerve could be stimulated repetitively and on occasions the gastric unit component would be absent from the evoked compound action potential, because, on these occasions, electrical stimulation would have occurred during the refractory period/

period resulting from the natural spike. The latter method alone was used to identify gastric units when thoracic vagal branches were stimulated.

The path taken by 8 of the afferent fibres was determined during the course of those conduction velocity measurements which involved stimulation of thoracic branches of the left vagus. The path of one other unit was determined by blocking nervous transmission in turn in the left dorsal and the left ventral thoracic branches by cooling, using the thermode described on p. M 6. This last unit had a receptive field on the medial wall of the reticulum about 2 cm caudal to and level with the reticulo-omasal orifice and the afferent discharge was abolished by cooling the left dorsal thoracic vagal branch. By the method of electrical stimulation, the dorsal vagal trunk was found to provide a path for afferent fibres with receptors in the medial wall of the reticulum (1 unit), the caudal (right) lip of the reticular groove (1 unit), the lateral region of the reticulo-ruminal fold (1 unit), the floor of the omasal canal (1 unit) and the medial wall of the cranial sac of the dorsal rumen (2 units). The ventral vagal trunk contained afferent fibres innervating the medial wall of the reticulum (1 unit) and the medial aspect of the reticulo-ruminal fold (1 unit).

The mean value for conduction velocity in gastric vagal afferent fibres is  $12.4 \text{ m/sec} \pm 1.0 \text{ m/sec}$  (standard error of mean) and the distribution of the conduction velocities is shown in Fig. B 1. The individual values for conduction velocity are given in relation to the receptor sites in Table B 1. These values have been arbitrarily divided into two groups according to whether the unit is known or is likely to have a pathway either in the dorsal or in the ventral/

ventral vagal trunk, on the basis of the nerve distribution given by Habel (1956). In the case of units in which the path was not determined experimentally, it was presumed that units had a pathway in either the ventral vagal trunk, if the receptors were located in the cranial and cranio-medial wall of the reticulum, the cranial lip and floor of the reticular groove and the floor of the omasum, or in the dorsal vagal trunk, if the receptors were located in the cardia, caudal lip of the groove, caudal and caudo-medial wall of the reticulum the reticulo-ruminal fold, cranial sac and other parts of the rumen (Fig. B 1). On this basis, the mean conduction velocity for gastric afferent fibres with pathways in the ventral vagal trunk is  $6.6 \text{ m/sec} \pm 0.5 \text{ m/sec}$  (standard error of mean) and in the dorsal vagal trunk is  $14.5 \text{ m/sec} \pm 1.0 \text{ m/sec}$ . These values are statistically significant at the 0.1% level ( $P = \text{less than } 0.001$ ).

#### The nervous discharge in single gastric afferent units

The nervous discharge will be described as it occurs firstly during the quiescent part of the 'primary cycle' and secondly during the contraction or active phase of the cycle, recorded under isometric conditions.

Forty-one units were examined with reticulo-ruminal movements present (Table B 2) and only 4 of these had no discharge during the quiescent phase of the cycle. The remaining 37 units had one of the following 'resting' discharges patterns:

- (a) An occasional spike, e.g., 1 spike every 11 sec, or a 'doublet' of spikes e.g., 1 'doublet' every 7 sec (Fig. B 3A).
- (b) Rhythmic bursts of spikes lasting 1-2 sec, recurring every 4 sec and related to inspiration (positive pressure ventilation). When the left lung was removed, as was the case in most experiments, the intra-reticular/

reticular pressure was scarcely affected by respiratory excursions and this type of resting afferent discharge was not seen. When both lungs were present, the resting discharge frequently had a respiratory rhythm superimposed on it.

- (c) Intermittent bursts of spikes with a non-respiratory rhythm. The bursts lasted 2-5 sec and recurred every 4-10 sec (Fig. B 3 B&C).
- (d) A sustained discharge with frequencies ranging from 1/sec to 28/sec (Fig. B 3 D&E).

A particular afferent unit often possessed either one or more of the types of resting discharge outlined above depending principally on the recording conditions. In general there was a gradation from either no discharge or an occasional spike to an intermittent discharge and then to a sustained, regular discharge as the reticular balloon was progressively distended (Fig. B 3). In some units it was possible to relate the intermittent bursts which had a non-respiratory rhythm to local contractions, which were felt as 'ripples' during manual exploration. If the reticular balloon was suddenly emptied, the resting discharge was usually abolished for 1-10 minutes and thereafter re-appeared but at a much lower frequency. A resting discharge was still present in many of the units examined either when there were no reticulo-ruminal movements or after the contents of the reticulum and rumen had been more or less removed. This discharge took the form of a low frequency steady discharge or of infrequent bursts of activity, the latter being particularly prevalent in units innervating the cranial sac of the dorsal rumen.

The pattern of the afferent discharges associated with the active phase of/

of the primary cycles may be divided into two groups. The first group (Table B 3) contains 27 units (henceforth termed Type A units) whose discharge either starts or increases above the resting level 3-5 sec prior to the second peak of the reticular contraction, reaches its maximum discharge frequency at about the same time as this second peak and ceases 1-2 sec afterwards. Between the start and the maximum of the discharge, there is often a raised level of activity corresponding to the first phase of the reticular contraction, when this is clearly present (Fig. B 8B). The receptive fields of 8 Type A units (Fig. B 5) were localised to sites in or near the reticulum, namely, the medial wall of reticulum (3 units), the caudal wall of reticulum (2 units) and the reticular groove (3 units:- one in each lip and one in the floor).

The second group comprised of 10 units (henceforth termed Type B units) in which the afferent discharge consisted of a submaximal burst of spikes (occasionally absent) at the time of the (second) peak of the reticular contraction and a maximal burst 2-9 sec afterwards. Two of these units were found to innervate receptors in the medial wall of the cranial sac, one of which was active 6-9 sec after the reticular contraction peak (Fig. B 6B), whereas the other (from a different sheep) showed submaximal activity at the time of this peak and maximal activity 3 sec after it (Fig. B 6C). A third unit was found to innervate receptors in the lateral part of the reticulo-ruminal fold and although slight activity occurred coincident with the peak of the reticular contraction, the greatest activity occurred 5-8 sec afterwards (Fig. B 6A). The receptive fields of the other 7 units were not located but the pattern of the afferent discharge in 6 of them (Table B 4), resembled that in the unit with receptors located in the reticulo-ruminal fold described above./

above. The seventh unit had a steady discharge of 4 spikes/sec lasting from 1 sec before to 7 sec after the (second) peak of the reticular contraction.

The remaining four units had afferent discharge patterns which were substantially different from either of the previous groups. In two of these units the discharge occurred during only the active phase of the cycle but had a variable time relationship to the reticular contraction. The discharge consisted of a burst of activity lasting 3-4 sec and started, for example, 11, 9, 6, 2 and -3 sec before the second peak of the reticular contraction. Another unit had no resting discharge but was active from 5 sec before to 3 sec after the peak of the reticular contraction and two maxima occurred, which were 3 sec before and 1-2 sec after this peak. The fourth unit was active from 1 sec before to 3 sec after the reticular peak.

#### The afferent discharge recorded under isotonic conditions

The afferent discharges in 7 units were examined under both isometric and isotonic recording conditions. The change from the former to the latter was effected by removing the clamp from the airline above the aspirator bottle (see p. M 5) and vice versa by replacing the clamp. The duration of the afferent discharge was the same in 4 units and shorter in 3 units, when recording under isotonic conditions. During the course of the isotonic reticular contraction, despite an increase in pressure of not more than 2 mm Hg, the spike frequency of the afferent discharge associated with the early or first phase of the reticular contraction was similar to that occurring under isometric recording conditions, but that related to the second phase of the reticular contraction/

contraction was much lower in all the units. It was, however, always greater than the 'resting' discharge. Two Type A units and 1 Type B unit had a phase of activity during the period 2-9 sec after the (second) peak of the reticular contraction and the number of spikes and the frequency of the afferent discharges during this phase were reduced in Type A units and enhanced in Type B units, when recordings were made under isotonic conditions compared with those made under isometric conditions (Fig. B 7). The receptors for the 2 Type A units were not localised. The Type B unit had a receptive field in the medial wall of the cranial sac. The remaining 4 units, which did not have a third phase of activity, innervated receptors in the floor of the reticular groove (1 unit), the caudo-medial wall of the reticulum (1 unit) and unlocated regions, presumably in the reticulum (2 units). In all the units, the 'resting discharge' was the same under both isometric and isotonic recording conditions, provided that the pressure head in the aspirator bottles had been adjusted so that it was equal to the existing intrareticular pressure prior to removing the clamp.

The effect on the afferent discharge of suddenly inflating or deflating the reticular balloon

The effects of progressively inflating and deflating the reticular balloon in steps of 200 ml were examined in 16 afferent units. Intrareticular volumes of not more than 1,200 ml were used, because, at high levels of distension the frequency of afferent impulses became too great to photograph except on fast film speeds and, because reticulo-ruminal movements were less readily maintained on subsequently deflating the reticular balloon (p. ). The effects of inflation will be described below. The effects of deflation were the/

the converse of these and will not be described.

As detailed earlier, most units were inactive or had a low 'resting' discharge frequency when the reticular balloon was empty. When the balloon was inflated the 'resting' discharge frequency increased, resulting in a low frequency sustained and regular discharge or in intermittent bursts of activity unrelated to respiratory or cardiac movements. Further inflation caused an increase in the resting discharge and took one of several forms:-

- (a) low frequency regular discharges increased in frequency.
- (b) intermittent discharges became sustained discharges with intermittent fluctuations in frequency and the interval between these periods of enhanced activity was reduced.
- (c) intermittent discharges became sustained regular discharges.

These changes are illustrated in Fig. B 3.

If the left lung had not been removed, a rhythm due to respiratory movements was often superimposed on the above patterns of resting discharge. When 200 ml was suddenly added or removed from the reticular balloon during the course of  $\frac{1}{2}$ - $\frac{1}{2}$  sec, a change in the frequency of the afferent discharge was observed about 100 msec after the start of the inflation or deflation. After inflation a high discharge frequency was reached  $\frac{1}{2}$ - $\frac{1}{2}$  sec later and persisted for about 2 sec before falling to its new value during the course of a further 3 sec. This is illustrated in Fig. B 4.

Inflating the reticular balloon caused the interval between primary cycle contractions to be reduced (i.e. the frequency of movements to be increased) and the amplitude and the duration of the reticular and the ruminal contractions to be increased. If ruminal contractions were absent at low levels of distension, inflation usually evoked them (Fig. B 8). Associated with these changes/

changes in the form of the contractions were increases in all parameters of the afferent discharges related to the first and second phases of the reticular contraction and, in some Type A and B units, an enhancement or an appearance of a third phase of activity 2-9 sec after the (second) peak of the reticular contraction. In Fig. B 8 C&D is shown the discharge recorded from a Type B unit at low and at moderate levels of reticular distension. In the former, a burst of activity occurs only in association with the second peak of the reticular contraction and there is no ruminal contraction, whereas in the latter, in addition to this activity there is a pronounced burst of spikes occurring at the time of the third phase of the reticular contraction shown on the record, which itself coincided with a prominent dorsal ruminal sac contraction (not shown). Despite the reticular contraction being much greater in C than in D (Fig. B 8), the burst of activity associated with the second peak of the reticular contraction is only slightly enhanced.

#### DISCUSSION

The distribution of mechanoreceptor sites identified by the procedures described on p. B 3 shows that the majority are in the reticulum (45%) and the cranial sac of the rumen (27%) and that fewer are in the reticulo-ruminal fold (12%), the reticular groove, including the cardia and omasal canal, (18%) and other parts of the rumen (12%). This finding is in agreement with the conclusions of Ash & Kay (1959), who found that a gradient of sensitivity radiated caudally from the cardia and reticulum, as judged by the reflex effect of mechanical stimulation on parotid gland secretion, reticular motility and the/

the onset of rumination. The five receptors located by Iggo (1955) to the region of the reticular groove were probably not a representative sample. Further investigations seemed necessary to determine whether the rumen, excluding the cranial sac, is really as insensitive as the present experiments and those of Ash & Kay (1959) suggest, despite various hypotheses which implicate reflexogenic zones in the rumen, to account for reflex phenomena related particularly to secondary cycle movements and eructation, (e.g., Weiss, 1953; Stevens & Sellers, 1959; see p. 139). Perhaps these reflexogenic zones are situated more cranially in the rumen (i.e. in the cranial sac) than is generally suggested by the literature, firstly, because the present experiments have demonstrated that the cranial sac has a good afferent innervation and secondly, because Dougherty et al (1958) were able to evoke eructation even after ablating most of the dorsal and ventral sacs of the rumen.

Thirty of the 34 receptive fields examined manually responded to stretch but not to pressing, compression (either transmurally or by pinching) or to stroking. They were therefore neither tactile nor pressure receptors, but were a form of stretch receptor, because they responded to passive lengthening of the walls of the viscus. The response to stretch was 'non-adapting' for 13 of these units and also for 28 units whose receptive fields were not located. The afferent discharge was found to increase during reticulo-ruminal contractions recorded under isometric conditions and, to a lesser extent, under isotonic conditions. Using the same criteria as Iggo (1955) for the classification of receptors, these receptors, like his, are non-adapting gastric tension receptors 'in series' with the muscle cells, because the afferent/

afferent discharge increased with tension developed both passively, by inflation of the reticular balloon, and actively, during a contraction involving the receptor zone (Matthews, 1933). The latter response distinguishes them from volume receptors 'in parallel' with muscle cells. Under isotonic recording conditions, the discharge from volume receptors would be expected to diminish and that from tension receptors to be unaltered. In fact the discharge increased slightly, partly because the isotonic conditions were not perfect, but this is inadequate to account for all of the increased discharge, however, and an additional factor may be that the connective tissue 'in series' with both the muscle cells and the tension receptors is less elastic than the tension receptors themselves.

Of the remaining four units, one situated in the omasal canal near to the reticulo-omasal orifice responded to light pressure, one innervated 'touch receptors' situated in the cranial wall of the reticulum (light stroking evoked a discharge which was 'rapidly-adapting'), and two units, with receptors in the lips of the reticular groove, responded to stretch but primarily to pinching. In the latter respect they differ from the tension receptors described above, since they appear to be sensitive to a deformation (produced by pinching, stretching, or actively developed tension) rather than to tangential lengthening (produced passively by stretching or actively by contraction of 'in series' muscle cells). Iggo (1955) failed to make this differentiation, because all the receptors which he located were found to be in the lips of the reticular groove and like the two units above, responded passively to stretch and to pinching as well as actively, during a contraction.

In/

Ash & Kay (1959) have shown that gentle stroking (more so than stretch) was a particularly effective stimulus for enhancing parotid secretions and reticular movements and for inducing rumination. They have suggested that this stimulus may excite tactile receptors situated superficially in the mucosa. Methylene blue stained structures have been observed by Hill (1959) in new-born lambs and kids, although, in the absence of physiological evidence, there seems to be no justification for regarding them as receptors particularly as the forestomach in a new-born ruminant animal is both structurally and functionally underdeveloped and as these structures have not been demonstrated in the adult. In the present experiments, only one afferent unit, which gave a rapidly-adapting response, was excited by gently stroking the mucosa. To account for the disparity between the last observation and the results of Ash & Kay (1959), several possibilities exist, i.e. either gentle stroking excites receptors in deeper locations than those assumed by Ash & Kay (1959) or the receptors are not innervated by afferent fibres in the left vagus or records from the particular afferent fibres have not been obtained for technical reasons. In the present experiments, it has been shown that gentle stroking does not excite directly the tension receptors described above, although there is often a subsequent increase in their resting discharge frequencies (Fig. B2), from which it is inferred that the intrinsic motility of the 'in series' muscle cells has been increased. On the basis of this, one hypothesis which may be tentatively proposed is that mucosal tactile receptors are not innervated by extrinsic vagal afferent fibres but are innervated by intrinsic sensory neurones/

neurones thus forming part of an intrinsic nerve network capable of eliciting intrinsic movements akin to peristaltic movements, as described for the guinea-pig ileum by Bulbring, Lin & Schofield (1958). In this way, gentle tactile stimulation of the mucosa would enhance intrinsic motility, through an intrinsic reflex, and this, in turn, would enhance the resting discharge in 'in series' tension receptors which are extrinsically innervated by afferent vagal fibres. There remains the possibility that mucosal tactile receptors may have been overlooked in the present experiments for technical reasons, e.g. the difficulty of isolating units of very small diameter (C fibres). This possibility is discussed more fully later (p. G8).

Localized muscular activity in vivo in the reticulo-rumen has been described in sheep before (Brunaud & Dussardier, 1953; Ash & Kay, 1959) and after section of both vagi (Duncan, 1953). Since bilateral vagotomy abolishes the primary and secondary cycle movements, these localized movements are intrinsic and either originate myogenically or from intrinsic reflexes such as that discussed above. Iggo (1955) suggested that the intermittent bursts of afferent activity comprising the 'resting' discharge in some units might be due to localized gastric movements but he was unable to confirm this idea. In the present experiments the relationship between bursts of afferent activity and localized movements of the wall was firmly established and most easily demonstrated for the wall of the cranial sac and the reticulo-ruminal fold/

fold and less readily for the reticulum. The existence of intrinsic movements in strips taken from the reticulo-ruminal wall has been demonstrated in vitro by Dussardier & Navarro (1953) and Duncan (1954). Although intrinsic movements are not directly responsible for primary and secondary cycle contractions in the manner implied by the earliest investigators (e.g., Wester, 1926), it does appear, from the present experiments, that they may influence primary and secondary cycles in two possible ways, firstly, by raising the level of excitability in the muscle cells, so that extrinsic nerve impulses from efferent vagal fibres are more effective, and secondly, by enhancing the 'resting' discharge in 'in series' tension receptors, thereby increasing the afferent input to the 'gastric centres' and leading to extrinsic reflex effects. Physical, chemical and pharmacological factors which affect intrinsic movements may thereby influence extrinsic reflex movements both directly and indirectly.

Besides ascertaining the character of the gastric afferent input and the properties and types of reticulo-ruminal mechanoreceptors, recording from afferent units has thrown some light on the temporal relationship existing between the sequential movements of different regions of the reticulo-rumen during the course of primary cycles. The limitations of the present experiments are

- (a) that the afferent discharge in only one gastric unit is recorded at any one time,
- (b) that comparisons have to be made of activity in units isolated from different sheep, in which experimental conditions and the rate and form of the contractions are not the same and
- (c) that gastric movements were being recorded manometrically from a limited number of sites.

The results/

The results supplement those obtained for gross movements by the classical manometric and radiographic methods (discussed on p. I 17) and for discrete regions by techniques involving partial herniation (Reid & Titchen, 1959), implanted transducers (Chiesa et al., 1965a) implanted microcannulae (Leek & Ullah, 1967) and others discussed earlier (p. I 18). One of the major problems of all manometric methods is in determining which of the recorded pressure changes are due, firstly, to movements of the compartment in which the sensing device is located and secondly, to pressures transmitted from adjacent compartments during the course of their movements. This problem existed also in the present experiments to some extent. From an examination of the afferent discharge it was usually not difficult to decide which was the principal contraction in a particular region but it was often less easy to differentiate between an afferent discharge due to a partial contraction and one due to a transmitted pressure or a passive stretch of the wall. The premise underlying the identification of the principal contractions at a particular receptor site is based on an observation made by Iggo (1955) and confirmed during the present experiments, namely, that 'the rate of (the afferent) discharge was often greater during isometric contractions than the maximal rate during gastric distension'. Periods of high frequency afferent discharges were, therefore, assumed to indicate contractions in the region containing the receptors innervated by the unit.

In the first group of 27 units described on p. B 11 the highest impulse frequency in the afferent discharge occurred at the time of the main or second peak of the reticular contraction recorded by the reticular balloon. For 8 units/

units (with receptors located to sites in the reticulum or reticular groove) it was established that their receptor sites were undergoing contraction at this time. Conversely, for the other 19 Type A units, their receptors, although not located manually, were presumed to be in the reticulum, because their afferent discharges resembled those of the previous 8 units. When the reticular contraction was clearly biphasic a phase of increased afferent activity with a submaximal frequency was associated with the first phase of the reticular contraction (Fig. B 8B). Because the spike frequency associated with the first phase of the reticular contraction exceeded that, which would have resulted from passively distending the intrareticular balloon to establish a similar tension, it is concluded that the first phase of the afferent discharge in these units is due actively to a contraction phase at the site of the receptors and is not due passively to pressures developed by and transmitted from a contraction in an adjacent site. As the receptive fields for some of these units were located in the medial wall of the reticulum, it is concluded that this region may undergo a biphasic contraction. The same argument holds for the reticular groove region and this is clearly shown in Fig. B 5H, where the reticular contraction is, in this instance monophasic, yet the afferent discharge in a unit innervating receptors in the floor of the groove is biphasic, as presumably is the contraction in the reticular groove region.

The main part of the afferent discharge in 10 units occurred 2-9 sec (mainly 5-7 sec) after the (second) peak of the reticular contraction, coincident with or sometimes 1-2 sec later than the pressure change recorded in the dorsal ruminal sac. The receptors for two of these units were located to the cranial sac/

sac (Fig. B 6 B&C) and, for one other, to the reticulo-ruminal fold (Fig. B 6A). It is concluded that units with this pattern of afferent discharge innervate units in the reticulo-ruminal fold, cranial sac or other regions in the dorsal sac. Most descriptions of reticulo-ruminal fold movements indicate that it contracts biphasically coincident with the biphasic reticular contraction but Lucas & Dougherty (1964, Fig. 4), using cows, have shown that it may contract a third time and sometimes with greater force, coincident with the dorsal sac contraction. The latter observation is supported by the results of the present experiments. In addition to this late activity, most of the units in this group showed some afferent activity at the time of the second and occasionally the first phases of the reticular contraction. I cannot decide whether this afferent activity was developed actively by contractions in the receptor region or passively by stretch or distension, transmitted from contractions in the reticulum or possibly the reticulo-ruminal fold.

Under conditions of moderate reticular distension (800-1,100 ml) a third pressure rise is recorded by the reticular balloon (recording under isometric conditions) 2-9 sec after the (second) peak of the reticular contraction and this coincides approximately with the dorsal ruminal sac contraction. One of the problems was deciding whether this pressure rise was due to a third contraction phase in the reticulum itself or whether it was due to pressures developed by and transmitted from the dorsal sac contraction. An examination of pressure recordings made from the reticulum and dorsal sac favours the former possibility, because the temporal relationships and the amplitudes of the third phase did not always show consistent changes with variations in the dorsal/

dorsal ruminal sac contraction (Fig. B 8). Associated with this third phase of contractile activity, 7 of the 26 Type A units showed a third phase of afferent activity having spike frequencies in excess of those likely to have been caused by tensions developed passively. Two of these units innervated receptors located in the cranial part of the reticulum and therefore would probably have been less susceptible to transmitted tensions. This evidence suggests that a third phase of reticular contraction does occur under certain conditions. Conclusive evidence for this idea comes from a comparison of spike discharge patterns recorded under isometric and under isotonic recording conditions for Types A and B afferent units and Type I efferent units (p. D 3). After changing to isotonic recording conditions the third phase of a reticular contraction is not observed, whereas often the dorsal ruminal sac contraction increases in amplitude (Fig. F I). As a corollary to this the third phase of activity is reduced or abolished in Type A (i.e. reticular) afferent units (Fig. B 7 C&D), is enhanced in Type B (i.e. ruminal) afferent units (Fig. B 7 E&F) and is reduced or abolished in Type I (i.e. reticular) efferent units (Fig. D 1).

The mean conduction velocity for gastric afferent nerve fibres (12.4 m/sec) is greater than that obtained by Iggo (1955) from 4 units, i.e. 6 m/sec. It seems likely that this difference is due to the latter being a small and unrepresentative sample. Conduction velocities measured by the compound action potential method gave values of 10-13 m/sec for the most excitable gastric afferent fibres (Iggo, 1956) and these are similar to the values obtained/

obtained from 'single fibre' measurements during the present experiments. The observed paths of 8 afferent fibres in dorsal and ventral vagal trunks were found to be in accordance with those expected, on the basis of Habel's (1956) anatomical observations. The conduction velocity of units known or presumed to have a pathway in the dorsal vagal trunk was significantly higher ( $P = \text{less than } 0.001$ ) than for those in the ventral vagal trunk.

From the present experiments it is concluded that:-

- (a) The majority of reticulo-ruminal mechanoreceptors are 'in series' tension receptors located in the medial walls of the reticulum and cranial sac.
- (b) The 'resting discharge' from mechanoreceptors is largely determined by the intrinsic motility of the muscle cells. Reticular distension is one of the factors which excites this intrinsic motility.
- (c) The afferent discharge developed during a contraction in Type A (i.e. reticular) units is greater under isometric recording conditions than under isotonic recording conditions.
- (d) The afferent discharge developed during a contraction in Type A units recorded under isometric conditions exceeds that which may be evoked passively by distension alone.
- (e) In halothane-anaesthetized sheep the primary cycle contraction may be monophasic, biphasic or triphasic in the reticulum and reticular groove. The main contraction of the cranial sac and the reticulo-ruminal fold occurs 2-9 sec after the (second) peak of the reticular contraction.
- (f)/

- (f) Afferent fibres from reticulo-ruminal receptors have pathways in both the dorsal and the ventral vagal trunk, the mean conduction velocities being 14.5 m/sec and 6.6 m/sec in each trunk respectively.

Table B1. The locations of receptors innervated by 38 gastric afferent vagal units and the conduction velocities of 28 of these units.

Site	No. of units	Conduction velocities (m/sec)	Mean C.V. (m/sec)
cranial wall	2	6, 8	
Reticulum medial wall	8	5, 7, 8, 10, 11	11
caudal wall	5	14, 21, 24	
Cardia	1	19	19
Omasal canal	1	7	7
Reticular groove	4	5, 15	10
Reticulo-ruminal fold	4	12, 16	14
Cranial ruminal sac	9	9, 10, 11, 11, 13, 16, 10	11
Caudodorsal blind sac (dorsal wall)	1	15	15
Mid-dorsal sac (medial wall)	1	16	16
Right longitudinal pillar	1	23	23
Ventral sac - cranial surface	1	11	11
	—		—
	Total = 38	Overall mean =	12.4
	—		—

Table B2. The form of the 'resting discharge' at the start (a-e) and after inflating the reticular balloon (f-h) in 41 units innervating reticuloruminal tension receptors. This includes 27 Type A units and 10 Type B units. In most sheep the left lung had been removed.

Form of 'resting discharge'	No. of units
(a) no spikes	4
(b) occasional spikes (< 1/sec)	7
(c) respiratory rhythm	3
(d) intermittent non-respiratory bursts	16
(e) continuous discharge (> 1/sec)	6
(f) a + b + c + e	1
(g) c + e	2
(h) d + e	2
	—
	41
	—

Table B3. The afferent discharge in 27 Type A (i.e. 'reticular') units. The spike frequency represents the number of spikes occurring in 1 sec. Several values for the interval between the bursts of activity of the 'resting discharge' have been given to illustrate their variability. The right-hand column shows the interval between the main peak of the discharge and of the reticular contraction (i.e. the second peak).

Unit & site	Resting discharge		Interval between bursts (sec)	Contraction discharge			Pk->Pk (sec)
	Spike frequencies (sec-1)	min max		Spike frequencies (sec-1)	1st phase	2nd phase	
12/8/65							
C ret-cd	5	5	continuous	17	45	28	12
D	2	2	continuous	11	11	7	9
F ret-cd/md	0	5	1, 3	12	19	5	12
15/3/66							
A	9	10	continuous	24	36	0	5
C	6	11	4, 6	17	60	0	6
2/6/66							
D	0	0	-	47	40	0	3
F (100 ml)	0	2	4, 6	2	42	-	14
(300 ml)	16	28	3, 6	51	>100	38	3
13/6/66							
A	0	8	13	8	18	0	4
B	0	21	3	54	>70	0	7
C	0	20	5, 7	26	44	20	10
E-ret-cd	0	1	continuous (0.2/sec)	18	12	0	5
F-groove-cr	11	35	4, 6	35	50	28	10
G-groove-cd	1	7	2, 4	14	27	0	4
28/7/66							
A	0	8	5	10	11	0	4
B-ret-cr/md	0	10	2, 4	17	28	16	9

2/8/66

A	3	6	3 (resp)	12	22	22	11	-0.2
B	0	1	10	0	16	8	6	0.8
C ret-cr/md	1	2	reg.	6	8	4	9	0.7

9/3/67

A	0	6	4, 6	36	45	12	10	-0.3
B	0	3	3	0	8	0	4	0.2
C	0	4	4 (resp)	9	16	6	10	0.7
E	0	7	6, 8	0	11	8	13	0.4
F	0	4	6	4	11	4	12	-0.3
G ret-cd/md	0	20	3, 8	33	53	0	9	0.0

22/3/67

C	0	9	3, 6	7	19	0	7	0.0
D ret-cr/md	0	37	10, 20	0	58	0	8	-0.7

27/3/67

A groove-floor	0	10	3, 6	25	40	0	6	-0.2
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Mean  $\pm$  S.E.    1.9 $\pm$ 0.8    10.4 $\pm$ 1.9    5.7 $\pm$ 0.6    17.7 $\pm$ 3.0    33.2 $\pm$ 4.1    7.4 $\pm$ 2.0    8.1 $\pm$ 0.6    0.4 $\pm$ 0.1

Ret = reticulum    cr = cranial    cd = caudal    md = medial    resp = respiratory rhythm  
reg. = regular

Table B4. The afferent discharge in 11 Type B (i.e. 'ruminal') units. The spike frequency is the number of spikes occurring in 1 sec. Several values for the interval between the peaks of bursts of activity in the 'resting discharge' have been given to illustrate their variability. The interval given in the right-hand column (Pk->Pk) is measured from the peak of the afferent discharge and the second peak of the reticular contraction.

Unit & site	Resting discharge		Contraction discharge			Pk->Pk (sec)
	Spike frequencies (sec-1)	Interval between bursts (sec)	Spike frequencies (sec-1)	Duration (sec)		
	min	max	1st phase	2nd phase	3rd phase	
12/8/65						
D	0	2	4	8	28	7
E (small)	0	1	2, 6	14	13 & 16	14
E (large)	0	0	0	0	7	2
2/6/66						
A	0	5	4 (resp)	26	41	14
B	0	6	4 (resp)	21	31 & 31	12
C	0	1	3	0	32 & 50	12
7/6/66						
B-c.s.-md	0	3	3, 6	0	15	13
13/6/66						
D-(2 units)	8	27	4 (resp)	65	82	8
1/9/66						
A-fold-lat	0	8	4 (resp)	19	54	15
22/3/67						
B	1	3	reg	0	11	11
27/3/67						
B-c.s.-md	0	8	4, 6	32	35	7
				18	27 & 24	9
Mean $\pm$ S.E.	0.8 $\pm$ 0.7	5.8 $\pm$ 2.3	4.3 $\pm$ 0.6	15.3 $\pm$ 5.5	33.2 $\pm$ 6.2	10.3 $\pm$ 1.1
			16.9 $\pm$ 5.4			

c.s. = cranial sac fold = reticulo-ruminal fold md = medial lat = lateral reg = regular

Fig. B1. The conduction velocities of 28 gastric afferent units.

The overall mean is  $12.4 \text{ m/sec} \pm 1.0 \text{ m/sec S.E.}$  (standard error of mean). The mean for units either known or presumed (see text for details) to have pathways in the ventral thoracic vagal trunk (hatched area on histogram) is  $6.6 \text{ m/sec} \pm 0.5 \text{ m/sec S.E.}$  and in the dorsal thoracic vagal trunk (clear area on histogram) is  $14.5 \text{ m/sec} \pm 1.0 \text{ m/sec S.E.}$

The location of the receptors innervated by these units is given in Table B1.

The conduction velocity in gastric afferent units.

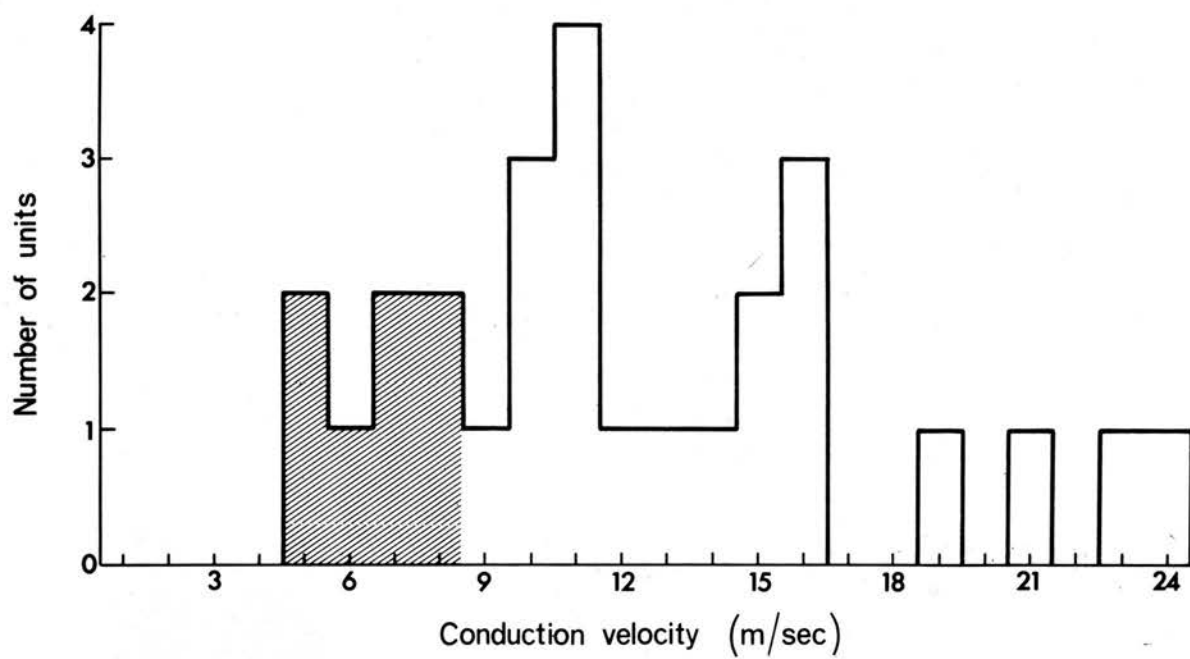


Fig. B2. The effect upon the afferent discharge, recorded from gastric units innervating 'in series' tension receptors in the reticulum, of manipulating the receptive field per manus.

A and B were recorded from the same unit. The receptors were stimulated by splaying open two fingers applied to the reticular wall containing the receptive field, thereby lengthening the reticular wall. The period of stimulation is indicated by the bars beneath the tracings: the narrow part representing a period of slight splay and the thick part representing a wide splay. Between the two periods of stimulation in A, a burst of spikes appeared spontaneously and was associated with a local contraction of the reticular wall, which was discernible as a 'ripple' beneath the finger tips. After the stimulation in B, spontaneous bursts of activity were present.

C and D were recorded from the same unit under conditions when cyclical reticulo-ruminal movements were present and the reticular balloon contained the same volume of air and was at the same pressure. Between C and D, the balloon was temporarily deflated and the reticulum manipulated, to locate the receptive field innervated by this afferent unit. Although recording conditions were returned to their former state before D, the 'resting discharge' was much greater than in C.

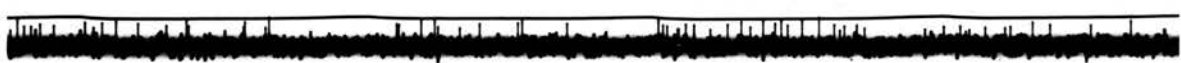
A



B



C



D



1 sec

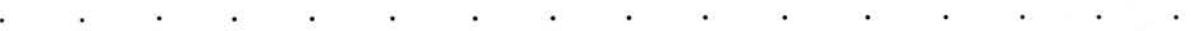
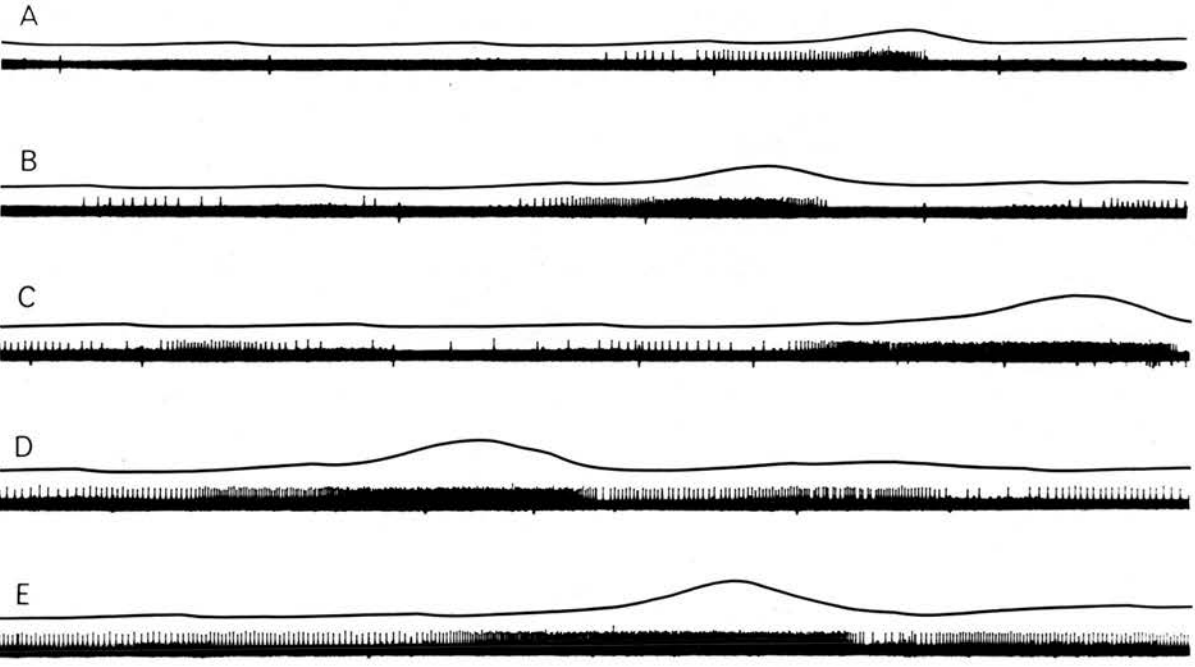
Fig. B3. The delayed effects upon the afferent discharges, recorded from a unit innervating reticular tension receptors, of increasing the volume of air in the reticular balloon.

In A, B, C, D and E, the reticular balloon contains 100 ml, 200 ml, 300 ml, 400 ml and 600 ml air respectively. All the records were obtained from the same units.

In A there is negligible 'resting discharge': a doublet of spikes (not shown) occur approximately every 7 sec. The reticular contraction has a small amplitude and the afferent discharge is correspondingly of relatively low frequency. As the level of distension is increased the 'resting discharge' is also increased. At first, intermittent bursts of spikes appear (B) and, upon further inflation, the interval between bursts is reduced and the peak discharge frequency reached during each burst is increased (C). Finally, the 'resting discharge' becomes continuous, although it fluctuates at a rhythm unrelated to respiration (D and E).

At the higher levels of distension the durations and amplitudes of the reticular contractions are greater and, associated with these, the afferent discharges are enhanced. In E, the peak frequency is greater than 100 spikes/sec.

A small non-gastric 'contaminant' spike, which extends below the base line, is also present but should be disregarded.



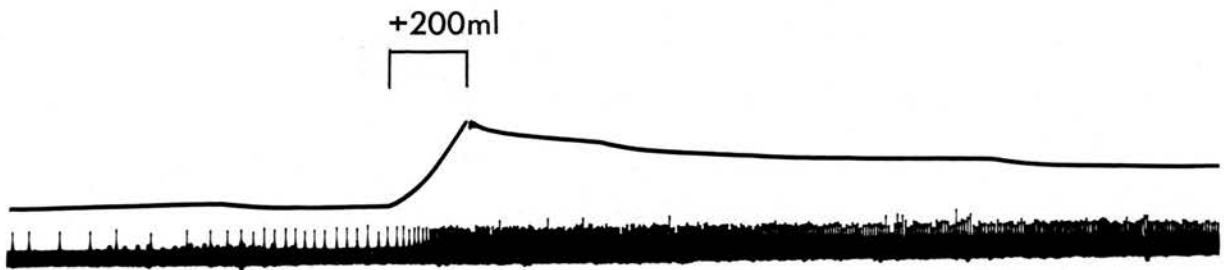
1 sec

Fig. B4. The immediate effect upon the afferent discharge, recorded from a unit innervating reticular tension receptors, of suddenly injecting 200 ml air into the reticular balloon.

This unit is the same as that shown in the previous figure (Fig. B3). It demonstrates that tension receptors are excited both during the active part of the primary cycle in the reticulum, and during passive distension of the reticulum. This characterizes the receptors as being 'in series' with the smooth muscle cells.

The latency between the rise in pressure (upper trace) and the increase in afferent discharge (middle trace) is approximately 100 msec.

The high frequency discharge occurring at the end of inflation falls steadily over the course of the next 10 sec to a new rate, which is greater than that prior to inflation.



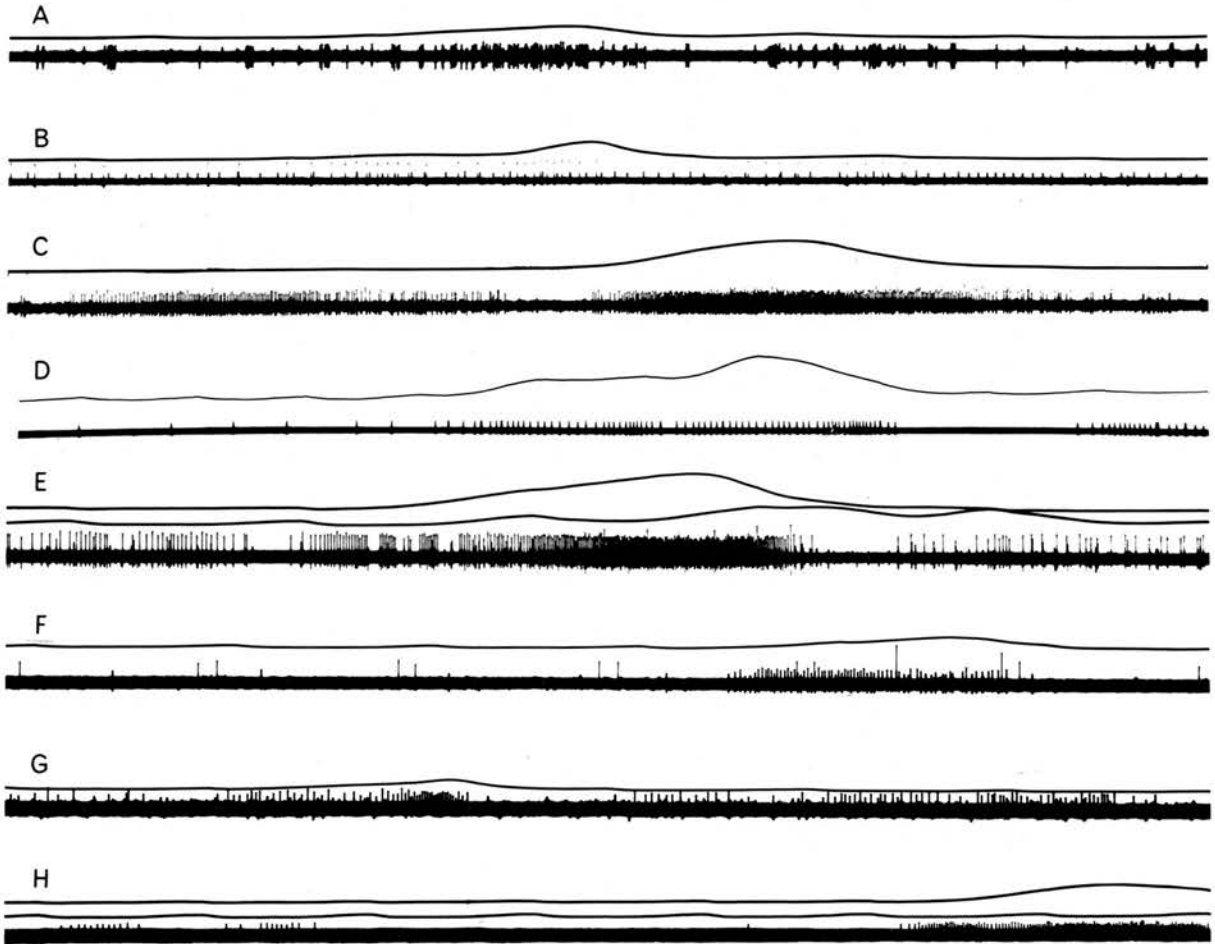
1 sec

Fig. B5. The afferent discharge in 8 Type A units, for which the discharge during spontaneous reticular contractions was recorded and the location of the receptors innervated by these units was established by manipulation.

The same amplification was not used for each reticular pressure tracing (upper trace in each record). In E and H, the upper trace is the reticular pressure and the middle trace is the dorsal ruminal sac pressure.

The locations of the receptive fields are:

- A. Reticulum - cranio medial wall, about 2 cm cranial to the reticulo-omasal orifice.
- B. Reticulum - (tall, thin spikes) - cranio-medial wall, as A.
- C. Reticulum - cranio-medial wall, as A.
- D. Reticulum - medial wall, between the reticulo-omasal orifice and the reticulo-ruminal fold.
- E. Reticulum - medial wall, about 1 cm caudal to the reticulo-omasal orifice.
- F. Reticulum - (small spikes) - caudal wall, about 1 cm below the reticulo-ruminal fold.
- G. Reticular groove - ventral end of caudal (right) lip. Responded to pinching > stretching.
- H. Reticular groove - middle of floor. This unit responded to light pressure as well as during a contraction. Note that the discharge in this record is biphasic, although the reticular contraction is monophasic.



.....  
1 sec

Fig. B6. The afferent discharge in 3 Type B units, for which the discharge during spontaneous gastric contractions was recorded and the location of the receptors innervated by these units was established by manipulation.

The reticular pressure is shown in the upper trace. The middle trace in C is incidental and should be disregarded.

The locations of the receptive fields are:

- A. Reticulo-ruminal fold - (left) lateral border.
- B. Rumen - cranial sac, medial wall.
- C. Rumen - cranial sac, medial wall.

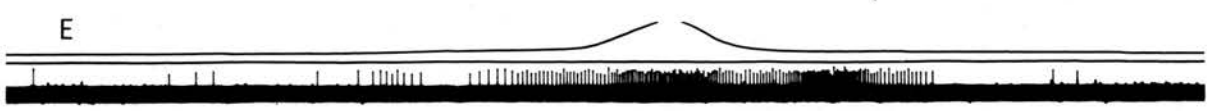
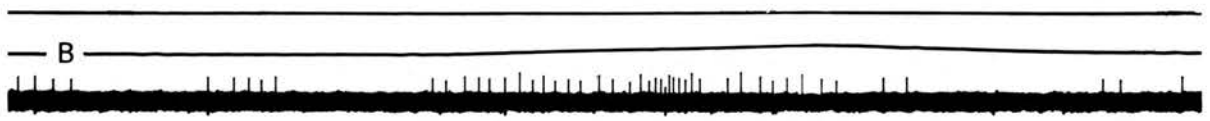
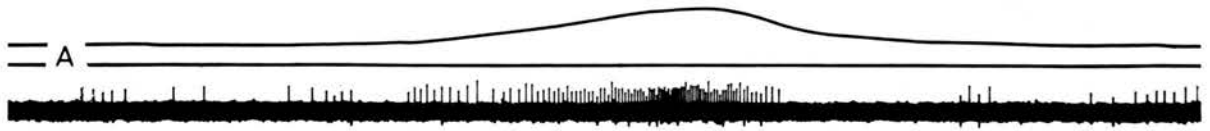


Fig. B7. Comparisons of the afferent discharges, recorded from units innervating tension receptors, when the reticular contractions are recorded under isometric conditions (A, D and E) and under isotonic conditions (B, C and F).

A and B show sequential contractions of the reticulum whilst recording from the same unit: a tension receptor in the reticulum. In A and B, the upper trace records reticular pressure and the middle trace records the volume of air displaced by the reticular contraction. The afferent discharge, when the reticulum contracts under isotonic recording conditions (B), is less than, when it contracts under isometric recording conditions (A).

C and D show sequential contractions in the reticulum, whilst recording from a unit innervating tension receptors in the reticulum. The upper trace shows pressure changes in the reticulum. The discharge frequency was so great relative to the paper speed that it is not possible to make the same comparisons as in A and B. Note that under isotonic conditions (C), there is no enhancement of the afferent discharge at the time of the third phase of the reticular contraction shown in D.

E and F show the afferent discharges in a unit innervating the cranial sac of the rumen (as Fig. B6C), when the reticulum contracts under isometric recording conditions in E (shown as a pressure rise in the upper trace) and under isotonic recording conditions in F (shown as a volume change in the middle trace). Under isotonic conditions in the reticulum, the ruminal contraction (not shown) had a greater amplitude and, correspondingly, the afferent discharge occurring after the reticular contraction and associated with the ruminal contraction was enhanced.



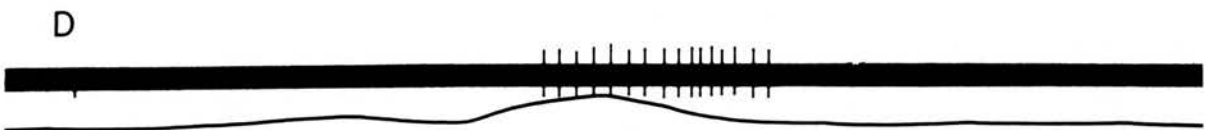
..... 1 sec

Fig. B8. The influence of the initial level of reticular distension upon the third phase of a reticular contraction and upon the ruminal contraction.

A and B are recordings of the afferent discharges in the same Type A unit, innervating tension receptors in the reticulum. The spikes have a different size in A and in B, because of a change in electrical recording conditions. For A, the initial level of reticular distension was low (400 ml) and there is neither a third phase in the reticular contraction nor a corresponding enhancement in the discharge. For B, the initial level of reticular distension was moderate (800 ml) and there is both a third phase in the reticular contraction and a corresponding enhancement of the afferent discharge.

C and D (thick spikes only) are records of afferent discharges in the same Type B unit, innervating tension receptors in the cranial sac (dorsal rumen) at a moderate level, i.e. 900 ml (C) and a low level i.e. 300 ml (D) of reticular distension. At the low level, the ruminal contraction (not shown) and the corresponding enhancement of the afferent discharge are absent, whereas with a moderate level of reticular distension, ruminal contractions and the corresponding enhancement of the afferent discharge are present (C).

In each record, the lower trace represents the reticular pressure.

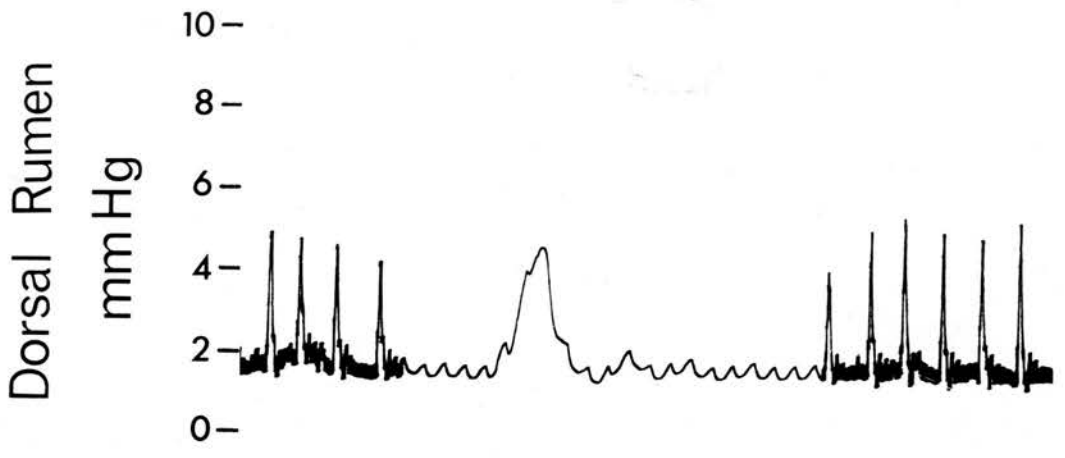
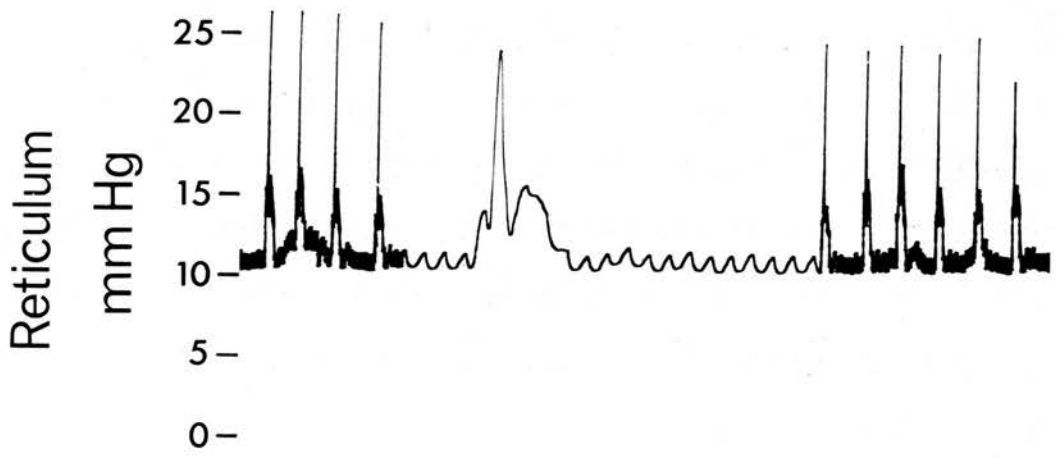


• • • • • • • • • •

1 sec

Fig. B9. The form and temporal relationship of reticular and dorsal ruminal sac contractions in a halothane-anaesthetized sheep.

In the middle part of the record, the paper speed was increased. On the dorsal rumen trace, the first and second notches correspond to the second and third peaks of the reticular contraction respectively. The peak of the dorsal ruminal sac contraction occurs about 5 sec and about 2 sec after the second and the third peaks of the reticular contraction respectively. The ventral sac contraction (not shown) reached a peak about 11 sec after the peak of the dorsal sac contraction and was responsible for the slight rise in dorsal sac pressure shown on the lower trace.



1 min

10 sec

SECTION CThe nervous discharge in efferent gastric vagal fibresINTRODUCTION

Forestomach movements comprising the primary and secondary cycles are activated by nervous discharges which arise in the 'gastric centres' (Iggo, 1951; Bell & Lawn, 1955; Andersson et al., 1959; Howard, 1966) and pass to the stomach along efferent pathways in the vagi (Mangold & Klein, 1927; Hoflund, 1940; Popow et al., 1933; Iggo, 1951, 1956; Duncan, 1953; Dussardier, 1960; Titchen, 1953, 1958, 1960; Reid & Titchen, 1965). Experimental methods which depend upon the presence or absence of reticulo-ruminal movements as evidence for the existence of efferent gastric vagal pathways provide no information on the patterns and durations of the nervous discharges using these pathways. The only previous attempts to record efferent vagal activity associated with reticulo-ruminal movements have been by Dussardier (1958, 1960), Beghelli et al., (1963) and Howard (1966). Dussardier cross-sutured the vagus and phrenic nerves and recorded electromyographically the activity in the reinnervated diaphragm initiated by vagal efferent fibres. He established, on the basis of differing discharge patterns, that there are a variety of vagal efferent fibres, and this result has been confirmed by Beghelli et al., (1963) and Howard (1966) who recorded electrical activity, presumably from gastric motoneurons or their processes, using micro-electrodes inserted into the dorsal vagal nucleus in the medulla oblongata. In the experiments described below, efferent gastric vagal activity/

activity was repeatedly sampled in halothane-anaesthetized sheep with spontaneous gastric movements (described in Section A) by recording from single efferent units dissected from the left vagus, using the 'single fibre technique' described on p. M 3. This method allowed direct sampling of nervous activity emerging from the gastric centres without appreciably interfering with either the afferent or efferent pathways. The objects of this analysis were to determine (a) the extent to which the gastric centres emitted a 'tonic' efferent discharge during the quiescent part of the primary cycles, (b) whether co-ordination for the complex sequence of forestomach movements was attributable to activity in the 'gastric centres' or in peripheral co-ordinating plexuses in the forestomach itself and (c) the relationship between the pattern of nervous discharges in efferent gastric vagal units and the forestomach movements with which the discharges were presumed to be associated.

The results and discussion given in this section have been published briefly (Leek, 1963, 1966; Iggo & Leek, 1966) and wholly, except for minor alterations, by Iggo & Leek, 1967a.

### RESULTS

The results detailed in this section were obtained by recording from strands dissected from fasciculi in the left vagus. The strand was transected at the peripheral end of the exposed fasciculus and parred away centrally from the underlying nerve fibres. Various patterns of nervous discharge were obtained. In some units, the discharge had a clearly recognisable relationship to cardiac, respiratory or swallowing movements. A brief description of/

of these units is given later in this section. Principal interest centred on efferent units having a nervous discharge pattern related in time to contractile events in the reticulo-rumen.

#### Gastric (reticulo-ruminal) units

Single unit discharge patterns associated with reticulo-ruminal movements were readily distinguished from other patterns since the gastric movements had a characteristic and regular cycle which was unrelated to the movements of other thoracic or abdominal viscera. Two criteria had to be satisfied for a unit to be classed as a gastric unit, namely:-

- (a) a discharge of impulses must appear, or an existing discharge must be modified, at the same period during each gastric contraction and
- (b) the discharge should change appropriately with both spontaneous and reflexly induced variations in the amplitude and frequency of gastric movements.

Sixty-four single units satisfying these criteria were isolated, as well as more than fifty strands containing 2-4 active gastric units some of which could be used for the purposes of analysis and classification (Fig. C 1). In addition there were many more strands in which gastric units were present together with active non-gastric units, but these strands were much less useful for purposes of analysis. Prolonged recording was possible from many of the single units and recordings were made from twenty-seven of them for longer than one hour and, in two instances, for as long as  $5\frac{1}{2}$  hours. The single units were usually lost either as a result of physical damage to the very fine nerve strands resting across the recording electrodes, caused by slight movement of the/

the neck or oesophagus, or due to further dissection of a strand in an attempt to improve the recording conditions.

For any individual unit, the pattern of discharge was very similar during successive gastric cycles throughout the recording period lasting several hours, provided that the experimental conditions remained the same or, if altered, were subsequently returned to the original condition. This is illustrated in Fig. C 4. The discharge was, on some occasions, so regular that the units could be mistaken for afferent fibres. The following tests were used to ensure that the gastric units were efferent:

- (a) the reticulum was suddenly distended with 200 ml air. Iggo (1956) showed that this procedure either initiates or enhances a resting discharge in the afferent gastric units. No resting discharges were observed in the gastric efferent units, with the exception of those classed as Type VII.
- (b) Drugs which block impulse transmission distal to the recording electrodes were administered (tetraethylammonium chloride, 1 mg/kg body weight, probanthine hydrochloride, 0.02 mg/kg body weight). These drugs caused both gastric contractions and the corresponding phase of an afferent discharge to be abolished but an efferent discharge was still present (see pages D 6, F 5).

All the gastric units described below satisfied one, or both, of the above criteria.

#### Classification of gastric efferent units

Sixty-four gastric efferent single units were classified into seven types  
on/

on the basis of their discharge patterns, and the time relationship of this discharge to the gastric contraction. Fig. C 2 summarises the results in the form of frequency curves. Detailed results are given in Tables C 1 and C 2. Each class of gastric unit was distinctive and quite separate from the others for the following reasons

- (a) No discharge pattern changed in type during recording sessions lasting as long as  $5\frac{1}{2}$  hours, either spontaneously or as a result of deliberately altering gastric conditions in a way which reflexly modified the activity of the unit (Fig. C 4).
- (b) In some multi-unit records (Fig. C 1), when each unit had a distinguishable spike waveform and amplitude, it was possible to identify several active units which could be of different types e.g., Types I, II and III were present in all combinations.
- (c) During an experiment, units of several types could be isolated, so the presence of one type or another did not depend only on the experimental conditions.

In the classification which follows, a functional grouping has been used; Types I-III are believed to represent units that innervate the reticulum or neighbouring structures, Type IV is thought to innervate the rumen, and Types VI and VII probably innervate special regions of the reticulo-rumen, e.g., sphincters, and/or pillars.

#### Type I gastric efferent units

Twenty-five single units of this type were examined (Fig. C 3, Table C 1). The standard discharge was bimodal, the frequency of the first peak (6/sec) being/

being much lower than of the second (20/sec). These units were active only during the contraction phase of a cycle and were silent during the quiescent part in between the contractions. Most of the action potentials, including the peak frequency of the discharge, preceded the peak of the reticular contraction. The peak frequency of discharge under 'standard conditions' was never greater than 45/sec. It preceded the peak of second reticular contraction by an average of 1.6 sec for all the units, and varied within the range of 0.5 to 2.5 sec for individual units. In addition to this consistent temporal relation between the Type I discharge and the reticular contractions, there were the following similarities between the pattern of the discharge and the form of the contraction.

- (a) Both the discharges and the contractions were biphasic and the intervals between the peaks were similar (3.5 sec and 3.0 - 3.5 sec respectively), although it was not easy to identify consistently the peak of the first reticular contraction.
- (b) The mean ratio of spike frequencies of the first and second peaks of the efferent discharge was 1:4 and the mean ratio of the amplitudes of the first and second peaks of the reticular contraction was 1:4 (Table C 1).
- (c) The interval between the second peak and the end of the spike discharge (3.5 sec) was similar to the phase of the reticular relaxation (6 - 8 sec). There was, however, considerable variation in the various time relations.
- (d) The average interval from the start of the discharge in the unit to its/

its peak (5.0 sec) was similar to the average duration of the phase of reticular contraction (4.9 sec).

#### Type II gastric efferent units

Seven single units of this type were isolated (Fig. C 3, Table C 2). The Type II discharge was always unimodal, consisting of an early peak with a long tail. The peak discharge was less than for the Type I units and rarely exceeded 18/sec. Occasionally, up to 3 spikes preceded the main part of the discharge in 3 of the units. The presence, number and position of these early impulses were erratic, even for successive contraction cycles, and they were, therefore, disregarded when measuring the intervals detailed in Table C 2. The start-to-peak interval for the discharge was very short (1.7 sec), whereas the peak-to-end interval was very long (9sec). The overall duration of the discharge (11 sec) was the same as for Type I units (10.8 sec), although the number of spikes (41) and the peak frequency (12/sec) were less. The interval between the peak frequency of the discharge and the second peak of the reticular contraction (2.1 sec) was longer than for the Type I units. Because of the similarity of these units to those of Type I, particular care was taken to ensure that they were, indeed a separate group. For example, Types I and II discharges were, on at least one occasion, recorded simultaneously from a multi-unit strand, so the experimental conditions were not important in determining whether one or the other type of discharge was present in the unit. Both types of unit were isolated from animals at different times and were not in any particular sequence. Furthermore, the Type II discharge always started after the beginning of a reticular contraction and could be present/

present even when the reticular contractions were clearly biphasic. For these reasons, the Type II units have been assigned to a separate category.

#### Type III gastric efferent units

Eight single units of this class were isolated (Fig. C 4, Table C 2). The discharge of Type III units began at about the same time as in Type I units but, unlike the latter, the discharge was in the form of a fairly even, extended plateau and did not exhibit sharp peaks. The peak frequency was also lower with an average value of 9.4/sec. The period during which a fairly steady frequency of discharge was present in the Type III units was at least twice as long as the period for the peak discharge in either the Type I or II units: 4.4 sec, compared with about 2 sec. An interesting feature of the discharge of a Type III unit was that the discharge appeared at the same time as that of Type I units, reached a plateau almost coincident with the peak of the first discharge in Type I units, and fell fairly abruptly just after the peak of the second discharge of Type I units. Although, therefore, the discharge patterns for these Types I, II and III unit were quite different, the principal part of the discharge in each case occurred at about the same time. For each type of unit the discharges appeared before or during the earlier part of reticular contractions and for this reason it is likely that all 3 types were in some way associated either with these contractions or with the contraction of other structures closely associated with the reticulum.

#### Type IV gastric efferent units

Fifteen units of this type were examined, 10 as single units (Fig. C 5, Table C 2)/

Table C 2) and 5 that were clearly distinguishable in multi-unit records. The Type IV discharge began after the second peak of the Type I discharge, i.e. during the second phase of the reticular contraction, reaching its peak shortly after the second reticular contraction peak. The discharge then continued on for several seconds at a lower frequency. Both the peak frequency (7/sec) and the total number of spikes in any one cycle of contraction were less than for the Types I, II or III unit. A discharge of impulses began 1.2 seconds before, and reached its peak frequency 1.8 seconds after the second peak of reticular contraction. This was the most striking difference between Type IV units and those of Types I, II and III, as is illustrated in Fig. C 2.

Activity in Type IV units was present under those conditions which also led to the appearance of large dorsal ruminal sac contractions. The effective conditions were a preparation in which reticular contractions could be readily evoked, a relatively light plane of anaesthesia and a moderately high reticular distension (600-1,000 ml). There were several occasions, when a Type IV discharge and ruminal contractions suddenly appeared whilst recording from a strand which initially had no Type IV discharge in it. The discharge and the contractions were, in these circumstances, elicited by an increase in the reticular distension.

The time relationship of the Type IV discharge to reticular contractions also supports the identification of these units as ruminal efferent units. During primary gastric cycles, the peak of a dorsal ruminal sac contraction occurred/

occurred about 4.5 (2-9) sec after the peak of a reticular contraction (Figs. A 1 & B 9). The peak of the Type IV efferent discharge also occurred, on an average, 3.5 sec after the peak of the Type I discharge (Fig. C 2). The Type IV discharge preceded dorsal ruminal sac contractions: the peak discharge being on an average 2.4 sec earlier than the peak of the dorsal sac contractions. The latter was difficult to assess accurately since the ruminal sac contractions tended to be slow and of low amplitude. The interval is similar to the latency of ruminal contractions (Fig. A 2) elicited by direct electrical stimulation of the peripheral cut end of the vagus (2.2 sec).

#### Type V gastric efferent units

Six single units of this type were found (Fig. C 5). The common feature of this group was a very low frequency of discharge with no obvious or consistent peak. It lasted about 10 seconds and began 4 seconds before the peak of the reticular contraction. The Type V discharge, therefore, began at about the same time as the Type II and earlier than the Type IV discharge but had ceased before the end of either. The frequency of discharge was very irregular and was scarcely affected by experimental procedures that caused pronounced reflex effects in the Types I, II, III and IV units. There was no discharge during the inactive phase of the primary cycle of gastric contractions. For these reasons the Type V units are regarded as a distinctive group.

#### Type VI gastric efferent units

Five units of this type were found (Fig. C 5). The discharge appeared in two separate bursts with a silent interval of 2.5 sec coincident with the peak/

peak of the reticular contraction. The peak frequency of the discharge was low (4.4/sec), and occurred during the first burst of impulses, which lasted only 2.5 sec. The second burst was much longer (7.6 sec) but had a lower frequency of discharge (2/sec). This pattern of discharge is, therefore, quite dissimilar from any of the preceding types. Like Types I, II, III and IV, it could be modified reflexly. It is suggested in the discussion that Type VI units may innervate special regions, such as gastric sphincters or pillars.

#### Type VII gastric efferent units

Only three units of this type were found (Fig. C 5), all of which survived for less than 10 minutes. The discharge began just after the start of the first reticular contraction and reached a peak frequency of 17/sec 1 second after the peak of the reticular contraction, at a time during which the Type VI units were silent. This peak discharge, therefore, occurred after the peaks of activity in Types I, II and III but before the peak of activity in Type VI units. The unique and distinctive feature of this type was the presence of a discharge at a low frequency (about 1/sec) that persisted throughout the greater part of the inactive phase of a primary gastric cycle. This persistent activity disappeared for at least 10 seconds prior to a reticular contraction.

#### Miscellaneous units

Recordings were made from only one unit whose discharge was related to gastric contractions but in which the discharge was intermittent, i.e. it appeared during only 2 out of 3 gastric cycles. The discharge reached a peak about 2 seconds before the peak of the reticular contraction. No other gastric unit/

unit was observed in any of the single or multi-unit recordings, made under the 'standard' conditions, which was not active during every primary cycle.

Several units were found which had a tonic or resting discharge with a respiratory rhythm, superimposed on which was an additional discharge during a reticular contraction. The spike amplitudes and regularity of the response of these units to pulmonary inflation indicated that they were pulmonary inflation afferent units. It was inferred that the superimposed gastric discharge arose because the receptors were in a lobe or part of the lungs adjacent to the diaphragm and reticulum and were excited by pressure changes or mechanical displacement caused by reticular contractions.

#### Oesophageal units

Swallowing movements were often present when the anaesthetic level was light. Normally, anaesthesia was adjusted to prevent these movements, since they interfered mechanically with the recording from the fine nerve strands in the neck. On a number of occasions unitary activity was recorded which bore a temporal relation to the contractions of the cervical oesophagus. The discharge consisted of 8 - 14 impulses at a frequency of about 8/sec (Fig. C 6). A similar discharge associated with swallowing in conscious sheep was observed by Dussardier (1960, Fig. 17). Although the conduction velocities of these oesophageal units were not measured, their spike amplitudes were much greater than those of any of the gastric units, from which it might be inferred that their axonal diameters were greater.

#### Cardiac units

Single/

Single units with a cardiac rhythm were isolated occasionally. An example is shown in Fig. C 6, in which a burst of 18-20 action potentials accompanied each pulsation in the carotid artery, observed through the paraffin pool. Although the nerve strand lying across the recording electrodes was cut distally, the active unit was not necessarily efferent since, as Holmes (1954) and Jewett (1965) have demonstrated, the existence of an afferent discharge arising from the carotid sinus, may be recorded in fibres dissected from the central end of a cut aortic nerve. This phenomenon has been attributed to a bifurcation of the afferent fibre at some point central to the recording site; a situation comparable to that described for frog tactile receptors by Adrian, Cattell and Hoagland (1931). The afferent discharge in these cardiac units was very similar to the discharge in carotid sinus baroreceptors, and it is concluded that they were afferent fibres.

#### Respiratory units

Single units with a respiratory rhythm were encountered more frequently than those with a cardiac rhythm. In the example shown in Fig. C 6 the discharge was related in time to the small pressure waves recorded by the reticular balloon. These waves are respiratory in origin and inspiration is recorded as a rise in pressure. Some of these respiratory units may have been afferent and to test for this point the endo-tracheal tube was clamped or a graded distension was applied to the lungs. When this was done it was possible to differentiate between afferent and efferent fibres. The discharge in an afferent fibre became steady after clamping the endo-tracheal/

tracheal tube and increased in frequency as lung distension was increased, whereas the discharge rate and rhythm in efferent fibres were not substantially altered by these procedures. The recordings obtained were comparable to those described for pulmonary inflation receptors by Paintal (1963), for pulmonary efferents by Widdicombe (1961, 1966) and laryngeal efferents by Andrew (1955).

#### DISCUSSION

The results obtained by recording from single vagal units provide information not previously available and allow a start to be made on the analysis of the underlying reflex mechanisms. There was no difficulty in establishing that the gastric vagal discharge was efferent for the reason given on p. C 4. The fact that the discharge still appeared at the expected times when gastric contractions had been abolished by the action of drugs that are known to block both pre- and post-ganglionic transmission demonstrates, incidentally, that the gastric efferent discharge is being transmitted in pre-ganglionic fibres at the cervical vagal level and that the post-ganglionic fibres are cholinergic, since probanthine hydrochloride exerts an action similar to atropine (Goodman & Gilman, 1956). These results are also consistent with the observation of Iggo (1956), who measured the conduction velocities of gastric efferent fibres in cervical and thoracic vagi by a compound action potential method and showed that they had conduction velocities in the range (1-16 m/sec) that would be expected for parasympathetic, preganglionic axons.

The efferent discharge could be classified into several distinct types and/

and it is reasonable to conclude that different structures were innervated by the various classes. One possibility to be considered, however, is that the various patterns resulted from an inability to standardise experimental conditions and not from the existence of several different categories of unit. The evidence for rejecting this hypothesis is that dissimilar types of discharge pattern were often seen in successive units during the course of an experiment on the same sheep, that units with different types of pattern could be recorded simultaneously in multi-unit records (Fig. C 1), and that each pattern was distinctive and for any individual unit remained basically constant for several hours, despite reflex and incidental changes in experimental conditions (Fig. C 4).

The evidence for the hypothesis that each type of gastric unit innervated a functionally and anatomically distinct region of the reticulo-rumen is strongest for Types I and IV. The Type I units are considered to innervate the reticulum, because (a) vagal denervation abolishes reticular contractions, (b) the biphasic contraction peculiar to the reticulum was matched by a biphasic efferent discharge pattern in the Type I units, (c) the interval between the first and second peaks of impulse discharge was equal to the interval between the peaks of the first and second reticular contractions, (d) the ratio of the first to second peak spikes frequencies was similar to the ratio of the amplitudes of the first and second reticular contractions. Further support for this identification was that the interval between the peak of the second discharge preceded the peak of the second contraction by an interval of 1.8 sec, only slightly longer than the latency of reticular contractions elicited/

elicited by electrical stimulation of the cervical vagus at 20/sec. There was not always an exact match between the discharge pattern of the unit and the ensuing reticular contraction but this is what would be expected since the reticular contraction would be the resultant of the activity in a large number of these Type I units. There may be significant variations in the form and in the times of the start, peak and end of the contraction in different parts of the reticulum, as suggested by Chiesa et al (1965 b).

The Type IV gastric efferent units, for reasons similar to those detailed above for the Type I units, are associated with, and considered to give rise to, contractions of the dorsal ruminal sac. The discharge pattern matched the rate, form, duration and amplitude of the dorsal ruminal sac contractions, the peak frequencies occurred at appropriate intervals before ruminal contraction and, in particular, a discharge in Type IV unit was present only when dorsal ruminal sac movements also occurred.

The functions of Type II and Type III units are not so clear. The main part of the discharge preceded the peak of reticular contraction and it is likely, therefore, that these units are involved in movements either of the reticulum, or of adjacent structures that contract at the same time, e.g., the reticulo-ruminal fold, or the oesophageal groove. Although visual examination of the left side of the reticulum confirmed that the whole wall contracted in the biphasic manner expected from manometric records, it was not possible to observe directly the medial wall, which may contract monophasically (Chiesa et al, 1965b), and the structurally specialised region around the reticular groove.

The function of the Type V units is not known, except that the discharge was clearly related to the presence of gastric contractions. The fact that these/

these low frequency units could be picked up from multi-unit strands prior to subdivision makes one confident that no gastric units were being overlooked due to this particular technical factor of low frequency, and perhaps small amplitude, in multi-unit recordings.

The Type VI and VII gastric efferent units, although few in number had very distinctive patterns and they may be associated with the movements of certain specialized structures, including sphincters or pillars. The bimodal discharge of Type VI units is unlikely to bear the right time relationship to the contraction of the reticulo-ruminal fold described by Lucas & Dougherty (1964), as the second part of the discharge starts too late. It seems more likely that the discharge is related to the movements of the reticulo-orifice described on p. I 21. The Type VII discharge may be associated either with a contraction of the omasal canal (see p. I 29) or of the cranial pillar, which may remain in a partially contracted state throughout most of what is regarded as the quiescent phase of the cycle for other parts of the reticulo-rumen (Reid & Cornwall, 1959).

There are only three other published investigations of gastric efferent vagal activity; Dussardier (1960) Beghelli et al (1963) Howard (1966). Beghelli et al (1963) recorded electrical activity from the medulla oblongata that had the same rhythm as gastric motility. They used curarised lambs (20-25 days old) anaesthetized with chloralose. Spontaneous reticular contractions, as would be expected, were absent, since reticulo-ruminal structure and function in lambs of this age, according to Wardrop and Coombe (1961), would still have been in a very primitive stage of development.

Reticular/

Reticular contractions were evoked by distending the reticulum or stimulating the central end of a cut abomasal nerve. The records obtained from the dorsal motor nucleus of the vagus showed multi-unit activity, and it is possible to identify several different types of discharge in their illustrations on the bases of spike amplitude and frequency and the temporal relationship of the discharge to the reticular contraction. The interval between the peak of the discharge (in those units having an early, high frequency discharge) and the peak of the reticular contraction was 1.2-2.0 sec, similar to the Types I, II and III units. In a study involving mature sheep in which reflex reticular movements were present, Howard (1966) has also recorded several kinds of unitary discharge in the dorsal motor nucleus of the vagus. Some of his units correspond to the Types I and IV units but, in addition, there were several others, which were probably interneurons.

Dussardier (1960, Fig. 20) illustrates 13 examples of efferent activity recorded in his cross-sutured animals. With two exceptions they could be incorporated in my classification. The principle differences were that the number of spikes per discharge, and the peak spike frequencies were generally less than those recorded during the present experiments and there was also a preponderance of units with a late discharge, which I would have grouped together as Type IV units. In addition there were two examples with a very late low frequency discharge, which I did not find. Dussardier does not say how common the various examples were, except that units with an early discharge were relatively uncommon. He recorded, very infrequently, units having a tonic/

tonic discharge equivalent to the Type VII units and others with a very early brief discharge similar to one of the Type I units (no. 25). The prevalence of units having a late discharge in Dussardier's experiments on conscious animals is probably due to the higher level of ruminal activity in his preparations.

From my results, together with those of Dussardier and Beghelli et al., it is now clear that the total efferent discharge passing from the gastric centres to the reticulum and rumen consists of several distinct and independent types of unitary activity. Each of these has patterns of activity which, in the course of time, will probably be related to the form, duration and amplitude of movements of some particular part of the stomach. Activity in the various types of efferent unit occurs in a sequence that could produce a co-ordinated series of movement in the reticulum and rumen. It is my view that they actually cause the movement. The orderly sequence of events that constitutes the primary gastric cycle can, therefore, be attributed to this co-ordinated efferent output which arises in the gastric centres rather than in the periphery. This view is contrary to that of Morrison and Habel (1964) who argued that the existence of multi-synaptic pathways in the myenteric plexus of the ruminant stomach implied that 'co-ordination' could and would be largely a peripheral phenomenon. It seems much more likely that the complexity of these myenteric pathways is related to the large area of the ruminal walls rather than to the need for a peripheral co-ordinating mechanism. The internal organisation of the gastric centres is probably very complex; e.g., Dussardier (1960).

Dussardier (1960) and Howard (1966) have established that there are powerful inhibitory interactions within the dorsal motor nucleus of the vagus itself.

Several firm conclusions can be drawn from the investigation detailed in this section.

- (a) Halothane-anaesthetized sheep are suitable for acute experiments on the reflex mechanisms underlying reticular motility but may be less suitable for studies of ruminal motility.
- (b) There are at least seven different types of gastric efferent fibres with characteristic patterns of discharge. Except for one of these groups, there is no resting discharge in efferent fibres during the quiescent phase of the gastric cycle. The form, duration and peak frequency of certain types of units can be related to the form, duration and amplitude of the movements of particular regions on the reticulum or rumen.
- (c) The co-ordination of the complex sequence of movements comprising the primary cycle of gastric contraction in ruminant animals is a function of the 'gastric centres' in the hind-brain, through their ability to determine the forms, durations, and spike frequencies and temporal inter-relationships of efferent discharges in nerve fibres innervating different parts of the stomach.

Table C1. The efferent discharges in 25 Type I single units and their time relationship to reticular contractions. Each set of values is representative of the unit and was obtained during one contraction cycle under 'standard' recording conditions. 23 sheep were used.

Refer to Fig. C3 for the positions in the reticular contraction and efferent discharge indicated by a-f.

Unit No.	Vol. in balloon (ml)	Reticular contraction		Efferent unitary discharge							
		Amplitude at b (mm Hg)	Duration a-b (sec)	Total No. spikes	Peak frequencies 1st at d, 2nd at e	Duration c-f (sec)	Intervals d-e (sec), e-f (sec), e-b (sec)				
1	100	-	5.2	72	9	18	12	6	4	5	2.0
2	100	11	6.7	56	2	24	14	5	4	8	3.7
3	300	4	3.3	40	8	32	6	2	1	4	1.2
4	300	9	5.5	33	5	7	13	6	5	7	1.5
5	300	12	5.5	45	6	11	9	6	4	4	2.0
6	300	14	6.0	27	1	8	7	5	4	2	1.0
7	300	17	6.5	41	5	13	10	5	4	5	1.5
8	300	18	6.5	99	11	17	18	5	5	12	1.4
9	400	3	2.5	69	15	25	12	6	3	7	1.8
10	400	4	5.7	28	2	13	6	4	3	2	1.7
11	500	9	3.0	88	5	29	13	7	4	8	2.0
12	600	-	6.0	70	10	18	13	6	5	7	1.7
13	600	6	3.2	32	5	10	9	4	3	5	1.0
14	600	10	5.0	56	9	14	10	4	3	5	4.0
15	700	9	4.5	33	2	20	9	5	3	5	2.3
16	700	17	5.0	74	3	14	15	4	4	10	2.5
17	800	2	5.5	105	2	35	18	8	4	10	0.8
18	800	6	1.7	93	20	45	7	5	4	2	1.5
19	800	9	4.5	46	2	18	12	7	5	5	0.5
20	800	9	4.0	34	4	15	5	4	2	1	1.5
21	800	20	4.8	52	10	30	6	2	2	4	0.5
22	900	7	6.2	74	2	29	14	-	-	13	1.7
23	1000	8	4.0	86	9	17	16	7	4	9	1.0
24	1100	16	4.2	78	3	25	10	4	3	6	1.2
25	-	26	4.2	20	4	14	7	3	2	4	5.0
Mean	563	10.6	4.8	58.0	6.2	20.0	10.8	5.1	3.5	6.0	1.8
±S.E.	57	1.3	0.2	6.9	0.9	1.9	0.8	0.3	0.2	0.6	0.2

Table C2. The efferent discharges in Type II, III and IV single units and their time relationships to reticular contractions. Each set of values is representative of the unit and was obtained during one contraction cycle under 'standard' recording conditions. 23 sheep were used.

Refer to Fig. C3 for the positions in the reticular contraction and the efferent discharge indicated by a-f. e represents the peak frequency in Type II and IV and the mid-point of the 'plateau' in Type III.

Type	Unit No.	Vol. in balloon (ml)	Reticular contraction			Efferent unitary discharge					
			Amplitude at a-b (mm Hg)	Duration a-b (sec)	Total No. spikes	Peak frequency (/sec)	Duration c-f (sec)	c-e (sec)	Intervals e-f (sec)	e-b (sec)	
II	26	300	20	4.0	46	10	17	2	15	2.7	
	27	400	4	3.7	40	12	13	1	12	2.0	
	28	500	8	6.8	30	18	6	1	5	1.0	
	29	600	4	4.5	32	16	10	3	7	1.5	
	30	600	9	4.5	44	11	12	2	10	4.0	
	31	900	17	3.5	27	8	9	1	8	0.7	
	32	1400	23	4.7	72	12	9	2	7	2.5	
	Mean±S.E.		<u>671±141</u>	<u>12±2.9</u>	<u>4.5±0.41</u>	<u>42±6</u>	<u>12±1.3</u>	<u>11±1.3</u>	<u>1.7±0.29</u>	<u>2±1.3</u>	<u>2.1±0.43</u>
	III	33	150	4	6.0	51	13	7	3.4	3.6	4.2
		34	200	11	5.5	81	10	13	5.2	7.8	2.0
		35	300	3	5.5	55	10	10	4.3	5.7	4.0
		36	400	5	4.0	40	7	8	3.4	4.5	3.0
37		700	17	7.2	35	7	7	3.7	3.5	4.0	
38		900	19	5.0	14	5	5	1.9	3.1	3.0	
39		1100	6	4.5	59	14	12	3.0	9.0	3.5	
40		1200	1	-	32	9	6	2.5	3.5	-	
Mean±S.E.			<u>619±146</u>	<u>8.3±2.3</u>	<u>5.4±0.40</u>	<u>45±7</u>	<u>9.4±1.1</u>	<u>8.5±1.0</u>	<u>3.4±0.36</u>	<u>5.1±0.8</u>	<u>3.4±0.30</u>
IV		41	300	4	-	55	10	15	4.0	11	-2.5
		42	400	1	-	15	7	4	2.0	2	-2.0
		43	450	14	-	27	5	11	2.0	9	-2.0
	44	700	11	-	30	3	18	1.5	17	-3.0	
	45	800	5	-	46	17	7	4.0	3	-1.0	
	46	800	5	-	14	4	10	1.0	9	0.0	
	47	800	10	-	55	8	11	5.5	5	-2.0	
	48	800	20	-	27	4	11	3.0	8	-2.0	
	49	1000	10	-	31	6	7	3.0	4	-2.0	
	50	-	-	-	45	6	11	4.0	7	-1.5	
	Mean±S.E.		<u>672±78</u>	<u>9±1.9</u>	<u>35±5</u>	<u>7.0±1.3</u>	<u>10.5±1.3</u>	<u>3.0±0.44</u>	<u>7.5±1.4</u>	<u>1.8±0.26</u>	

Fig. C4. An example of efferent gastric vagal activity recorded from a fine multiunit strand.

The record contains one non-gastric unit and at least four efferent gastric units, of which two (? Type III) show activity related to the reticular contraction and two (Type IV) show activity which follows the reticular contraction and is presumably associated with the ruminal contraction (not shown).

This record demonstrates (a) that multiunit recordings are unsatisfactory for the type of analysis required by the present investigation and (b) that the reticulum and the rumen appear to be independently activated by discharges in separate efferent nerve fibres.



1sec

Fig. C2. The temporal relationship between gastric (reticulo-ruminal) contractions constituting the 'primary cycle' and the discharge patterns in the various types of efferent units. The second peak of the reticular contraction has been used as the ordinate for alignment of the frequency curves constructed from the mean values given in Tables C1 and C2.

Types I, II and III are considered to innervate the reticulum or adjacent structures, Type IV the rumen and Types V, VI and VII special regions, e.g. pillars and/or sphincters, which have not yet been identified.

# GASTRIC EFFERENT DISCHARGES

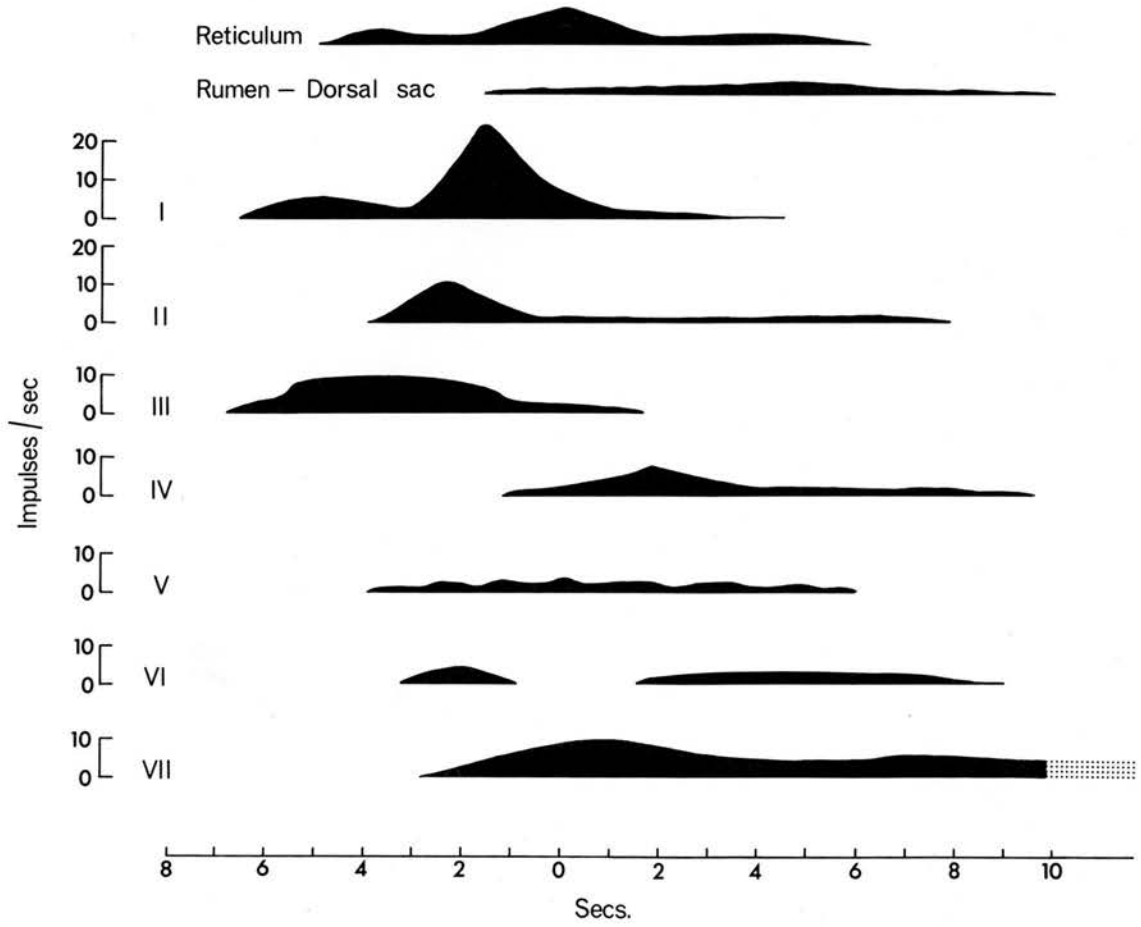


Fig. C3. The discharges of Types I and II efferent units (lower traces in each record) and the corresponding reticular contractions (upper traces). A Type IV unit fires with a low frequency in the later part of the lower record (spikes marked with a dot).

The points labelled a-f provide the key for Tables C1 and C2: a indicates the start of the reticular contraction and b its second peak. The efferent discharge commences at c, reaches its first peak at d, its second peak at e and ends at f.

The scale on the left of each record represents a reticular pressure of 10 mm Hg at its lower end and of 25 mm Hg at its upper end. This convention has also been used for Figs. C4-5.

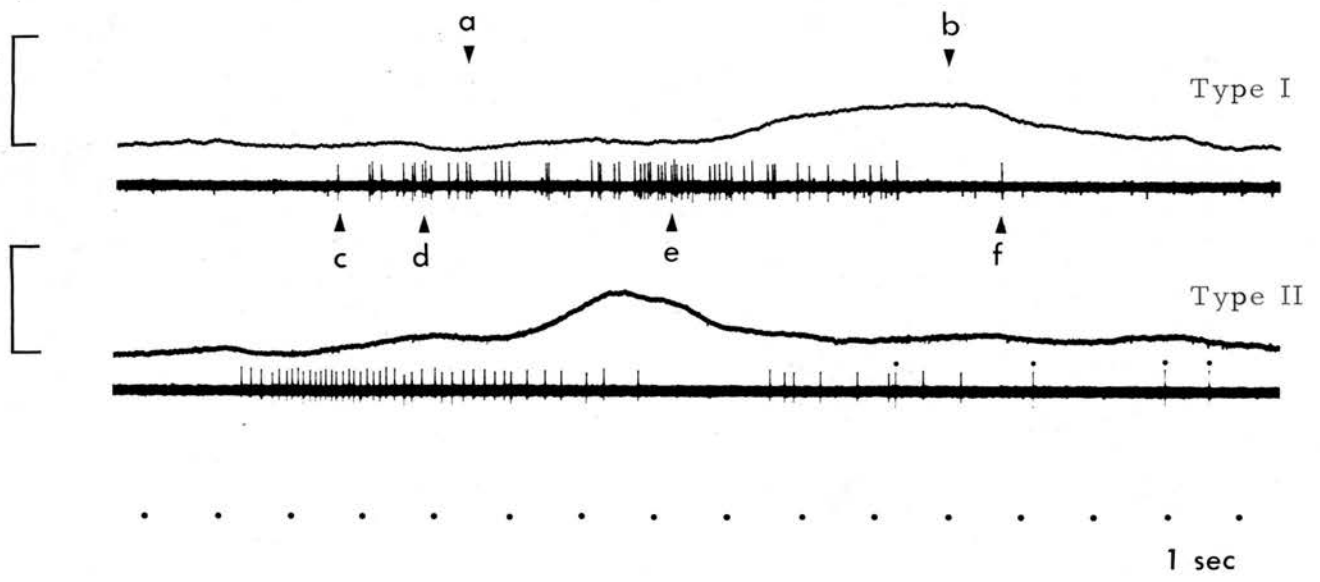
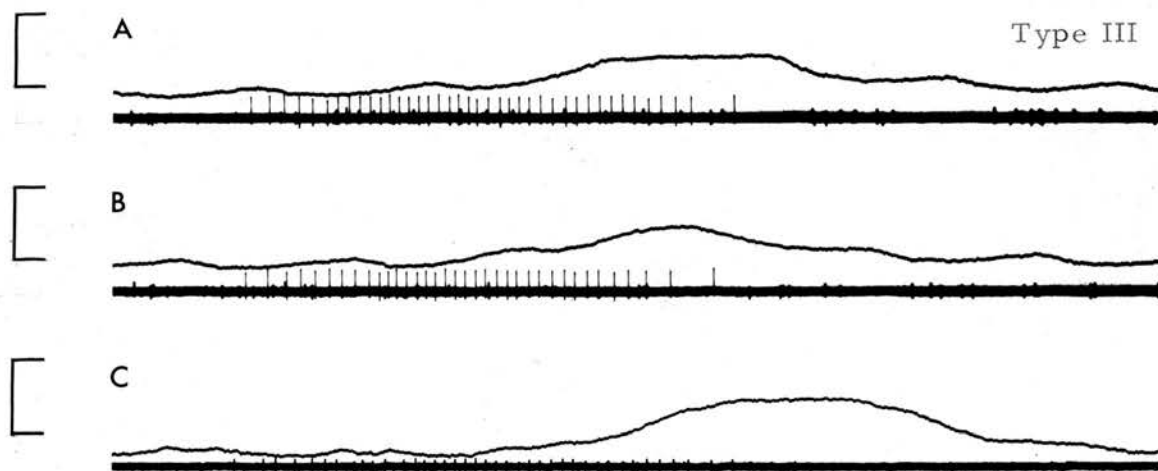
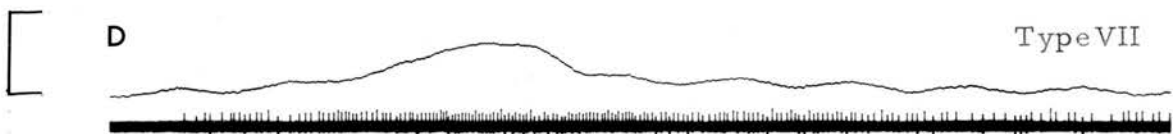


Fig. C4. The discharges of a Type III unit (lower traces) and the corresponding reticular contractions (upper traces). A and B are consecutive reticular contractions recorded under 'standard' conditions: the efferent discharges are similar but the contractions appear to be slightly different because they are superimposed upon pressure fluctuations due to respiratory movements. C is from the same unit  $5\frac{1}{2}$  hr later when, after a variety of experimental procedures, the recording conditions were once more 'standard'. The discharges are similar in A, B and C but the spike amplitude is smaller in C due to an alteration in electrical recording conditions.



1 sec

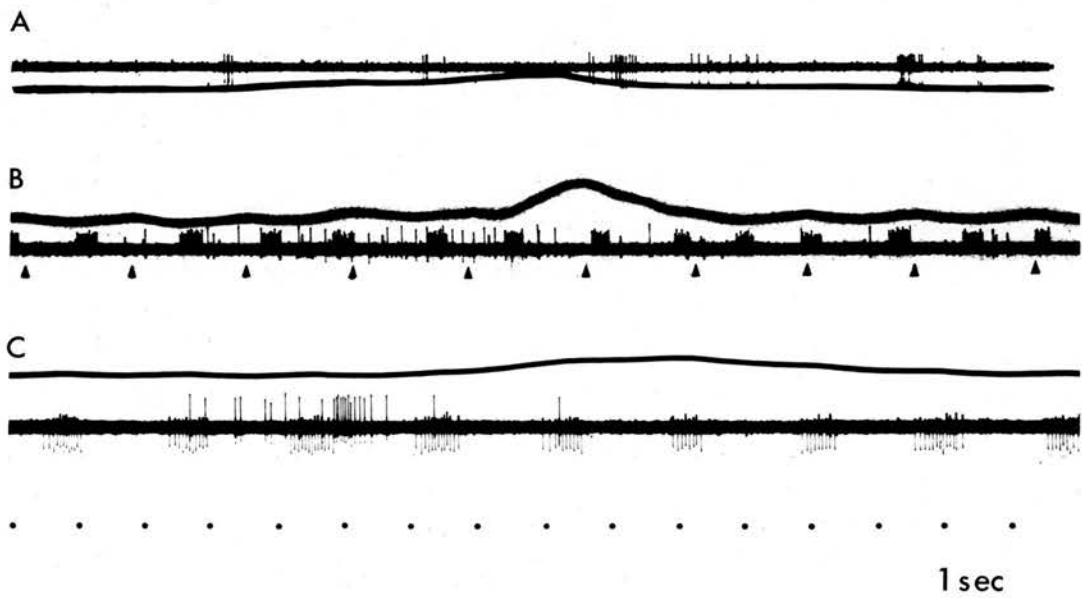
Fig. C5. Examples of gastric efferent discharges (lower traces) in units innervating structures other than the reticulum. In each record the upper trace shows the reticular contraction. A shows both a Type IV unit (large spikes) and a Type V unit (small spikes). The main part of the Type IV discharge occurs during the phase of reticular relaxation and is associated with the ruminal contraction. B shows a Type V unit which has the typical irregular discharge of low frequency. C shows a Type VI discharge which is characterized by the pause during reticular contraction and the long, low frequency discharge afterwards. D shows a Type VII discharge. This is maximal near the peak of the reticular contraction and is followed by a low frequency discharge which persists until upto 10 sec before the start of the reticular contraction.



1 sec

Fig. C6. Examples of single unit discharges not associated with gastric movements. A shows the discharges produced during swallowing in a unit innervating the oesophagus. B shows bursts of activity which were in phase with the arterial pulse. They are clearly not related to respiration (end of inspiration marked with triangles). C shows bursts of activity in a pulmonary afferent fibre (spikes below the line) and an unusually early reticular efferent unit (spikes above the line). In all cases the strand from which the recordings were made had been cut distal to the recording electrodes.

Spikes are recorded in the upper trace in A and the lower traces in B and C. The pressure line (lower trace in A and upper traces in B and C) shows the reticular contraction superimposed on respiratory movements (inspiration giving an upward deflection).



SECTION DReflexly induced changes in the efferent gastric vagal unitary dischargesINTRODUCTION

The forestomachs of ruminant animals undergo complex cycles of contraction which are dependent upon extrinsic reflexes integrated by 'gastric centres' in the hind brain. Reticulo-ruminal motility may be altered by a variety of procedures; e.g., by distending the reticulum (Iggo, 1956; Titchen, 1958; Dussardier, 1960), stretching the reticulo-ruminal fold (Titchen, 1958) and by altering the pH of the ruminal (Ash & Kay, 1959) or the abomasal contents (Phillipson, 1939; Titchen, 1958). The vagi provide afferent and efferent pathways for these reflexes. Electrophysiological studies on some of the vagal afferent fibres involved has been reported previously (Iggo, 1956). Gastric efferent vagal activity has been recorded indirectly from the diaphragm using electromyography after vago-phrenic nerve anastomosis (Dussardier, 1960) and directly using a single vagal fibre technique (Section C; Leek, 1963; Iggo & Leek, 1967).

Two limitations of previous experimental work on reticulo-reticular reflexes were, firstly, that the reflex responses of the reticulum were often initiated by changes in the recording conditions, e.g., the use of the same balloon to provide the stimulus (distension) and to record the evoked contraction, and, secondly, that the extent to which the observed changes were due to a local mechanism or to an extrinsic reflex could not be determined. These technical limitations have been circumvented in the present experiments by/

by recording the efferent discharges in gastric vagal units and determining the reflex changes elicited in these, as a result of distending the reticulum and acidifying the abomasal contents. This technique has also provided information on the dependence of the gastric centres upon a tonic excitatory input from receptors in the stomach, the extent of reflex feedback whereby the early stages of the contraction cycle influence the later ones and, finally, on the time taken for the central nervous integration of gastric reflexes. A further problem was to see to what extent the further excitation of gastric 'in series' receptors during the reflexly elicited contractions modified the behaviour of the gastric centres (Iggo, 1955, 1956).

Brief reports of these experiments have already been published (Iggo & Leek, 1966; Leek, 1966). This Section has been published with only minor alterations by Iggo & Leek (1967b).

### RESULTS

The results described below were obtained during the course of the experiments reported in Section C. The 53 experimental animals, the surgical procedures and the technique for recording from single efferent units were therefore the same.

Simultaneous recordings of the nervous activity in single vagal gastric efferent units and of reticulo-ruminal movements were made before, during and after the administration of drugs and alterations in reticular tension and abomasal pH. Reflex changes in the various types of discharge pattern elicited by these procedures are described below. A detailed account of seven vagal gastric efferent discharge patterns is given in Section C. Briefly,  
Types/

Types I, II and III are presumed to activate the reticulum or adjacent structures. In Type I the discharge pattern is bimodal, preceding and corresponding to the typical biphasic reticular contraction. Type II is unimodal and consists of a sharp peak, which precedes the second peak of the reticular contraction, and a long tail. The Type III discharge has a flattened peak or 'plateau' which precedes the reticular contraction, although it has approximately the same duration. Type IV units are considered to innervate the rumen. Their discharges are unimodal. They commence during and reach a peak after the reticular contraction, but before the ruminal one. Units having discharge patterns of Types V, VI and VII are presumed to innervate specialized structures e.g., pillars and sphincters, which have not as yet been identified. Type V units have a discharge with a low, irregular frequency, occurring during the reticular contraction and relaxation. The Type VI discharge pattern consists of a brief burst of impulses then a pause of 2.5 sec, during which there are no impulses, and this coincides with the second peak of the reticular contraction. Then follows a low frequency discharge lasting 7.6 sec. The discharge in Type VII units begins after the start of the reticular contraction and reaches its peak frequency during the phase of reticular relaxation. A low frequency discharge persists throughout most of the quiescent part of the gastric cycle in Type VII units only.

Comparisons of efferent discharges recorded under isometric and isotonic conditions in the reticulum

The reflex effect of the enhanced gastric afferent input which occurs during the course of an isometric reticular contraction (Iggo, 1955) were tested/

tested by comparing the patterns of the various types of gastric efferent discharge under isometric and under isotonic recording conditions. Reticular movements were usually recorded isometrically, but by removing the clamp from the airline between the intrareticular balloon and the first aspirator bottle, isotonic conditions were immediately obtained and vice versa.

The reflex effects of changing from isometric to isotonic recording conditions in the reticulum and vice versa were examined repeatedly in 20 units isolated from 10 sheep. The change was made during a quiescent part of the gastric cycle. Usually 4-6 primary cycles were recorded under one set of conditions before switching to the other. This procedure did not affect the interval between the reticulo-ruminal contractions constituting the primary cycles. No changes in the pattern of the gastric efferent discharge were observed in 3 units. All three units had an early discharge which had finished before the reticulum contracted. Two of these units were of Type I and one of Type II. The remaining 17 units were all affected and the results are summarized in Table D 1 and an example is shown in Fig. D.1. The overall effects in Type I units were a marked increase in the maximal frequency of discharge together with a reduction in the discharge frequency at other times, so that the total number of spikes was usually reduced. Although the duration of the efferent discharge was either unchanged or reduced, the interval between the start of the discharge and its peak was often increased, whereas that between the peak and the end of the discharge was always reduced. The effect on 3 Type II units tested was a reduction in the total number of spikes, the duration of the discharge and the interval between its peak and the end. The peak discharge occurred/

occurred at a very early stage relative to the reticular contraction in two of these units and there was no apparent effect upon the start-to-peak interval of the discharge or on the peak frequency. The peak frequency in the third unit was higher under isotonic conditions, as with the Type I units. In this unit the peak frequency of discharge occurred later during the phase of reticular contraction than in the case of the other two units. Four Type III units were tested. Apart from one unit with a very early discharge which showed no changes in the discharge pattern, they showed a reduction in the duration of the discharge, particularly its later phase. Most of the 'plateau' or flat peak occurred early in the phase of reticular contraction and it was unaffected by a change in recording conditions. Consequently, the peak frequency and the total number of spikes in the Type III units showed variable changes.

Two Type IV (i.e. ruminal) units were tested. Due to oscillations in the anaesthetic level which caused changes in the reticular contraction rate and amplitude the results obtained from one of these units was discarded. The other unit showed an overall increase in both the duration and the total number of spikes. On a few occasions, whilst recording from Type I, II and III units, a low frequency Type IV unitary discharge also appeared when recording conditions became isotonic and disappeared on reverting to the isometric state (Fig. D 1). Of the four Type V units examined, three showed no changes in the discharge pattern and one had fewer spikes and a shorter duration. The one Type VI unit showed an increase in the total number of spikes, the peak frequency and the duration of discharge. Several spikes occurred/

occurred during the 'pause' in the discharge, which coincided with the second peak of the reticular contraction and characterized this type of unit under isometric conditions.

In all units the converse effects were observed when conditions reverted to the isometric state.

The reflex effect of preventing the reticular contraction by the use of drugs

An isotonic reticular contraction differed from an isometric one firstly, by the absence of a rise in pressure during the contraction and secondly, by a reduction in reticular volume during the contraction. As the reflex effects described above might have been a consequence of either or both of these factors, the analysis was carried a step further by examining the effects in the absence of both a pressure increment and a reduction in reticular volume. This was achieved by administering drugs which block neurohumoral transmission in the autonomic nervous system. Tetraethylammonium chloride (T.E.A.) was used to block transmission at preganglionic nerve endings and probanthine hydrochloride at postganglionic cholinergic nerve endings, predominantly those of the alimentary tract (Goodman & Gilman, 1956). The doses were adjusted so that a total block of gastric contractions developed after  $\frac{1}{2}$ -2 minutes and lasted 5-10 minutes. Recovery took place progressively during the next 10-20 minutes.

Ten units were tested with T.E.A. alone, three with probanthine alone and three others with both drugs. These drugs act at different sites and, although they produce opposite effects on the cardiovascular system, both blocked the efferent gastric discharge distal to the recording site. This abolished/

abolished reticulo-ruminal contractions and resulted in identical reflex changes in the pattern of the efferent vagal discharge (Fig. D 2). The interval between the bursts of efferent activity responsible for the primary cycle movements was unaltered by these drugs.

Six Type I units were tested with T.E.A. alone and two with both drugs. The first contraction after the administration of the drugs often showed only partial block. The next 4 or 5 contractions were completely blocked. The features common to these cycles in all the units were a reduction in the total number of spikes, a sharp increase in the peak frequency of the discharge, an increased interval between the start of the discharge and its peak and, finally, a decreased interval between the peak and the end of the discharge. These effects are summarized in Table D 2 and resemble those seen when recording conditions were changed from the isometric to the isotonic and similarly were most pronounced when the preceding ('control') reticular contractions had a large amplitude. After about the fifth cycle and until about half way through the recovery period there was an overall reduction in the efferent discharge, so that not only were there fewer spikes but also the peak frequency and the duration of the discharge were diminished.

One Type II unit was tested with T.E.A. alone and one with probanthine alone. In both cases the peak frequency of the discharge occurred before the reticulum contracted and remained unaltered during the second to fifth cycles after administering the drugs. The number of spikes occurring after the peak and also the interval between the peak and the end of the discharge was reduced. As with Type I units, there was a general reduction in the efferent/

efferent discharge from about the fifth cycle until the recovery period. This was evident as a reduction in the peak frequency and duration of the discharge.

Two Type III units were tested with T.E.A. alone, one with probanthine alone and two with both drugs. In all cases the total number of spikes and the duration was reduced, due to a shortening of the final part of the discharge. Once again there was a general reduction in the discharge after about the fifth cycle.

Like Type II units, most of the discharge in Type III units occurs either before or during the early part of the reticular contraction. The reflex effects of changing to isotonic conditions and (for the second to fifth cycles) of administering blocking agents are, therefore, restricted to the tail of the discharge.

One Type IV unit was tested with T.E.A. alone. During the first two cycles after giving the drug, the peak frequency was higher than before but, by the third cycle the peak frequency, total number of spikes and duration had reached very low values.

One Type V unit was tested with T.E.A. alone. The only noticeable effect was a slight reduction in the total number of spikes and the duration of the discharge. One Type VI unit was tested with probanthine. There was a general reduction in the efferent discharge and like the isotonic effect described above in a different Type VI unit, some spikes were present during what had previously been the silent period or 'pause'.

The immediate reflex effect of suddenly deflating the reticulum

The above experimental procedures had little reflex effect on the main part/

part of the gastric efferent discharge in units of Types II and III. Most of the discharge in these units occurred before or during the earliest phase of the reticular contraction: it was probable that the absence of an effect was due to the lack of any change in the afferent input. Clearly a different technique was required to test whether such discharges could be altered reflexly. The procedure adopted was that of suddenly deflating or inflating the reticular balloon by removing or injecting 200 ml air at the beginning of the reticular contraction or the efferent discharge, in contrast to the first set of experiments in which the change was made during a quiescent part of the cycle. This procedure also allowed the reflex time to be assessed. The effects of the manoeuvre were examined at both high and low levels of reticular distension. Although reticular deflation and inflation were carried out in turn on all the units described below, the first description will be confined to the effects of suddenly deflating the reticulum, as these largely resembled the effects of changing from isometric to isotonic recording conditions and of administering blocking agents.

The reflex effect upon the efferent discharge of suddenly removing 200 ml air by means of a large syringe connected to the reticular balloon was examined in 30 units. The most pronounced changes were seen when this was done either at the start of the efferent discharge or of the reticular contraction, whichever was the earlier. Moreover, the alterations which this procedure induced depended upon whether the reticulum was previously distended at a low level (400-700 ml air in balloon), a moderate level (600-1,100 ml) or a high level (more than 1,000 ml). The divisions can be seen to slightly overlap/

overlap, presumably because the units were isolated from different sheep. The significance of these divisions will become clearer later, when the effects of inflating the reticular balloon are considered. The efferent discharge associated with the contraction cycle in which the reticulum was deflated, was markedly different from those of both the preceding cycles and the third and subsequent cycles. The efferent discharge associated with the second cycle following deflation was transitional between the first, which showed the immediate effects of deflation, and the third and subsequent contractions, which showed the delayed or final effects of deflation.

Eleven Type I units were repeatedly tested and all were affected. The responses depended on the initial volume of the reticulum. Eight were tested under conditions of moderate (600-1,100 ml) reticular distension (Fig. D 3). The immediate effects of deflation on the 8 units were a marked increase in the peak frequency of the discharge and, except for one unit, a reduction in the total number of spikes, the duration of the discharge, the frequency of the discharge between the first and second peaks and the interval between the second peak and the end of the discharge. The exceptional unit was tested at a reticular distension of 1,100 ml, which was on the borderline between the moderate and high levels of distension. In the one other unit examined at high reticular distension (1,300 ml) the immediate effects of deflation were an increase in the total number of spikes, the frequency of the discharge between the first and second peaks and, on most occasions, the duration of the discharge and the interval between the peak and the end of the discharge, but there was no change in peak frequency. Two units were also tested with the reticulum/

reticulum at a low level of distension (600 and 700 ml). The immediate effects of deflation were a reduction in the total numbers of spikes, the duration and the interval between the peak and the end of the discharge but either no increase or a reduction in the peak frequency of the discharge. Thus the effect of sudden deflation on these units was to convert the 'isometric' type response to the 'isotonic' type. The initial level of inflation had an important modifying influence.

Five Type II units were tested with the reticulum moderately distended. The effects of deflation in all these units were similar. The peak frequency of the discharge was increased but the total number of spikes, the duration of the discharge and the interval between the peak and the end of the discharge were reduced. The effects were, therefore, similar to those found for the Type I units at moderate distension.

Seven Type III units were tested with the reticulum moderately distended. Unlike the Types I and II units there was no consistent change in peak frequency of the discharge; three units showing a reduction, one an increase, two no change and one both an increase and a reduction at consecutive tests. Like the Types I and II units, however, all the Type III units showed, as a result of reticular deflation, a reduction in the total number of spikes, the duration of the discharge and the interval between the peak or central point of the 'plateau' and the end of the discharge.

Three Type IV units were tested. In all cases there was a reduction in the total number of spikes, the duration and the peak frequency of the discharge. Two Type V units were tested. With the reticulum moderately distended, /

distended, deflation caused little change in the first half of the discharge but reduced the frequency and the duration of the second part of the discharge. With the reticulum at high distension, deflation resulted in an increase in the spike frequency, total number of spikes and the duration of the discharge. One Type VI unit was tested. This unit was from a different sheep than were those Type VI units subjected to isotonic conditions and to probanthine described above. However, the immediate effect of deflation upon the efferent discharge was similar to the effects caused by the isotonic conditions and probanthine. The 'pause' between the two bursts of spikes which typified these units under normal isometric conditions was absent from the discharge associated with the first contraction after the reticulum had been deflated. The overall duration of the discharge was reduced.

The immediate reflex effect of suddenly inflating the reticulum

Repeated observations of the immediate effects of inflating the reticulum at the start of the reticular contraction were made on the units described in the preceding section. For the most part, the effects were the converse of those produced by deflation but the effect of the level of reticular distension prior to inflation was more pronounced. For Type I units at low levels of distension, sudden inflation caused an increase in the total number of spikes, the duration and the peak frequency of the discharge (Fig. D 4). At moderate reticular distension, however, sudden inflation resulted in an increase in the total number of spikes and the duration of the discharge but a marked reduction in its peak frequency. When the reticulum was subjected to high levels of distension, /

distension, further inflation caused the efferent discharge in three units tested to show a variable but marked reduction in the total number of spikes, the duration and the peak frequency of the discharge (Fig.D 5 ). In two of these units the total number of spikes in the discharge was less than 10 per cent of its previous value with an overall duration of only 2-4 seconds.

In the Type II units, inflation superimposed on a low reticular distension caused an increase in the total number of spikes and the duration of the discharge with no change in its peak frequency. At moderate levels of reticular distension, inflation resulted in an increase in the number of spikes and the duration of the discharge but a reduction in its peak frequency. At high levels of reticular distension, sudden inflation caused in two Type II units an abbreviation of the discharge similar to that observed in the Type I units described above, after inflation.

Seven Type III units were tested at moderate levels of reticular distension. Inflation caused an increase in the total number of spikes in all the units, an increase in the duration of the discharge in five units and an increase in the peak frequency in three units, but no change in two and a reduction in one. The terminal part of the discharge, in particular, was enhanced in those units in which the duration was increased.

Following reticular inflation, the Type IV units showed an increase in the total number of spikes and the duration of the discharge but no marked change in the peak frequency. One Type V unit was tested with the reticulum moderately distended and inflation caused a discharge of longer duration with more spikes whereas, in another Type V unit with the reticulum greatly distended/

distended, it produced either a reduced discharge or, in one test, no discharge at all. No Type VI units were tested with inflation.

#### The delayed effect of inflating the reticulum

The results described as 'immediate effects' in the preceding two sections refer to those discharges associated with the actual contraction cycles in which the reticulum was inflated or deflated. The delayed effects of inflation were observed in the subsequent contraction cycles in 25 units over a narrow range (not more than 3 x 200 ml air added) with initial reticular distensions of 200-1,300 ml and in two units over a fuller range from 400-1,600 ml. Few units were examined at extreme reticular distensions, because, on subsequent deflation, a greater degree of reticular distension was then necessary, to evoke reticular contractions of the same rate and amplitude as those occurring before the extreme distension. Furthermore these very large reticular distensions often induced reflex swallowing and limb movements which were, in part, passively transmitted to the recording site and, thereby, often caused the nerve strand to be broken between the electrodes and the nerve trunk.

The results obtained from one of the latter two units (Type III) are shown in Figs. D 6 and D 7. At the start of approximately every fifth contraction, 200 ml air was suddenly added to the reticular balloon. The delayed effects of this procedure were incremental changes in

- (a) the reticular pressure, the greatest increments being from 600-800 ml and 800-1,000 ml;
- (b) the amplitude of the isometric reticular contractions up to a maximum reached/

reached with a resting pressure of 15 mm Hg and 600 ml air in the reticulum. At higher volumes and pressures the amplitude progressively decreased;

- (c) the frequency of reticular contractions up to a maximum of 1.72/min at a reticular volume of 1,200 ml;
- (d) the number of spikes/discharge up to a maximum of 90 at 800 ml and
- (e) the peak frequency of the discharge up to a maximum of 21 impulses/sec with a reticular volume of 1000 ml.

At values of distension greater than those producing maximal effects, further inflation caused a reduction in each of these parameters.

The effect on the gastric efferent discharge in this unit of incrementally distending the reticulum up to very high levels may be examined in more detail by making three comparisons: firstly, of the first discharge occurring after inflation with that preceding it (i.e. the immediate effects), secondly, of the fourth or fifth discharge after inflation with that preceding inflation (i.e. the delayed effects) and thirdly, of the fourth or fifth discharge with the first discharge after inflation (i.e. the transition from the immediate to the delayed effects). After sudden inflation, the intrareticular pressure is very high momentarily but falls exponentially during the next 1-2 min to a new steady level, which is higher than before inflation. This is presumably due to the 'viscous' properties of the gastric and abdominal walls. The immediate effects of inflation on this unit, with initial reticular distensions of not more than 1,000 ml, were an increase in the total number of impulses, the duration of the discharge, particularly the interval between the peak ('plateau' mid/

mid point) and the end of the discharge, and the peak frequency. With initial reticular distensions of 1,000 ml to 1,200 ml, further inflation caused no change in the total number of impulses and the duration of the discharge, although the peak frequency was slightly less. With initial distensions greater than 1,200 ml, further inflation resulted in decremental changes in the total number of spikes and the peak frequency but the duration of the discharge remained the same.

The delayed effects of inflation of the reticular balloon can be seen by comparing the discharge in the cycle prior to inflation with that associated with the fourth or fifth contraction afterwards, by which time the resting reticular pressure had reached a stable value, and successive contractions and efferent discharges were identical. With initial reticular distensions of not more than 600 ml, further inflation resulted in an increase in the total number of impulses, the duration of the discharge (particularly the interval between the 'peak' and the end) and the peak frequency. At greater reticular distensions, inflation resulted in a reduction in the number of impulses, the duration of the discharge (particularly the peak-to-end interval) and the peak frequency. The 'plateau' duration was either the same or less.

Comparison of the discharges associated with the first and either the fourth or fifth contractions after inflation demonstrates the transition occurring between the immediate and the delayed effects seen 2-4 min later. The latter have fewer spikes, a shorter duration of discharge (particularly of the above interval) and a lower peak frequency.

The effects described above for a Type III unit were seen also in the other/

other types of unit tested over a more limited range of distension. Two additional features were: (a) that in Type I units there was no immediate effect on the interval between the start and the peak of the discharge, whereas the delayed effect was a lengthening of this interval, (b) in those instances at high levels of reticular distension, when further inflation resulted in an immediate reduced discharge in some Type I and II units, the discharge associated with the second contraction after inflation showed the features described above as 'immediate effects'. (Fig. D 5).

The converse of the changes described above relating to inflation was seen when the reticulum was progressively deflated by suddenly removing 200 ml air at the beginning of every fourth or fifth contraction. Once again the 'viscosity' effects were seen (this time in reverse). Sudden deflation caused an immediate reduction in intrareticular pressure with an exponential rise over the course of 1-2 minutes to a new stable level which was lower than before deflation.

#### Reflex time for reticulo-reticular reflexes

For each unit, the efferent discharges associated with successive cycles of contraction were very similar, provided that recording conditions had been stabilised. It was, therefore, possible to determine a reflex time for reticulo-reticular reflexes by comparing the efferent discharge which was recorded, when 200 ml air was suddenly added to or removed from the reticulum just after the beginning of the contraction, with that discharge, which might have been expected to occur had this procedure not intervened. The discharge recorded during the previous cycle was used as the expected or control pattern.

The/

The addition or withdrawal of air took  $\frac{1}{2}$ - $\frac{1}{2}$  sec. The time between the start of the addition or withdrawal of air and the first noticeable difference between the two efferent discharges was measured on 89 occasions in 20 units. A skewed distribution of values was obtained, having a mean of  $1,020 \pm 48$  msec (mean  $\pm$  standard error) and a mode of 800 msec (Fig. D 8). There was no significant difference between the values obtained for the different types of unit, although the values obtained for some of the Type I units were probably too high, due to the difficulty of comparing the low frequency and rather irregular discharges occurring between their first and second phases, which often was the time when the reflex effects ought to have been first noticeable. It is likely, therefore, that the mode rather than the mean value is more truly indicative of reflex time measured from the start of inflation or deflation to the first noticeable change in the efferent discharge. This interval represents the time taken for the reflex by the receptors, the afferent nerves, central integration processes in the gastric centres and the efferent pathway from the medulla to the recording site on the left vagus at the mid-cervical level.

#### Acidification of the abomasum

Titchen (1958) has described the potent excitatory reflex effects of introducing 0.2 N HCl into the abomasum in eliciting reticular contractions in decerebrated sheep. This procedure was employed in 10 sheep under halothane anaesthesia in the present experiments. In all except 3 of these, acidification of the abomasal contents, in order to lower their pH to less than 1.0, did/

did not affect the prevailing reticulo-ruminal movements, which often had a large amplitude even when the abomasal pH was as high as 4.2. The acidity of the abomasal contents does not appear to be so critical therefore, for reflex excitation of the movements in anaesthetized sheep. In the 3 sheep which did respond to acidification of the abomasal contents, an increase in the amplitude of the reticular contractions appeared after 1-2 min and lasted about 10 min. The record obtained from a single efferent unit before and again 90 sec after abomasal acidification is shown in Fig. D 9. There is an increase in the total number of spikes, the peak frequency of the discharge and its overall duration.

#### DISCUSSION

The 'gastric centres' in the hind brain give rise to several types of efferent discharge, which are presumed to account for the complex but co-ordinated sequence of movements in the various regions of the reticulo-rumen comprising the 'primary cycle' of contractions (Section C; Iggo & Leek, 1967a). In halothane-anaesthetized sheep the patterns of the efferent discharges, the reticulo-ruminal contractions for which they are responsible and the duration of the quiescent period between contractions are modified by the degree of reticular distension and the acidity of the abomasal contents. The effects of both reticular distension and the increased tension developed during an isometric reticular contraction differ according to the type of gastric unit from which the efferent discharge is being recorded (see Table D 2), although within any one type the reflex responses of the units are similar. This finding supports the classification of gastric unitary efferent discharge patterns/

patterns into functionally distinctive types not only on the basis of the parameters of the discharges and their time relationships to the reticular and ruminal contractions (Section C; Iggo & Leek, 1967a) but also on the basis of their reflex responses.

Titchen (1958) demonstrated, in decerebrate sheep, that reticulo-ruminal movements were reflexly enhanced when abomasal acid secretion was stimulated or when the abomasal pH was reduced to less than 1.0. He concluded that mucosal 'acid receptors' were stimulated by these procedures and that their afferent activity excited the 'gastric centres'. Under normal conditions in the conscious sheep on 'ad lib' feeding, the secretion of abomasal acid is continuous (Hill, 1960) and, therefore, 'acid receptor' activity is likely to provide a tonic excitatory afferent drive to the 'gastric centres'. Several indirect pieces of evidence arise from the present experiments which suggest that abomasal acid secretion is present in halothane-anaesthetized sheep and may reflexly excite reticulo-ruminal movements, viz. (a) movements were present in some sheep even before the reticular balloon was inflated, (b) movements were usually not enhanced by acidifying the abomasal contents, (c) difficulty was experienced in evoking movements in starved sheep and others which had undergone a dietary change just before the start of the experiment (Section A) - procedures which Hill (1960, 1965) has shown to cause a reduction in abomasal acid secretion and (d) movements were abolished when pentagastrin was given in high doses which were likely to inhibit abomasal acid secretion (Section F). Although this evidence is consistent with the view that 'acid receptor' activity is stimulated by the secretion of abomasal acid and provides a tonic excitatory afferent/

afferent drive to the 'gastric centres' for the elicitation of reticulo-ruminal movements, further experimental work would be required to confirm this.

Besides the above mechanism, the 'gastric centres' appear to receive a tonic afferent drive from tension receptors in the reticulum. In most sheep with no air in the intrareticular balloon, there were no gastric efferent discharges or reticulo-ruminal (gastric) contractions, whereas with 400-600 ml air in the balloon, contractions of normal amplitude and frequency were usually present. A pressure of about 10 mm Hg was created by this volume and is similar to that found in the conscious sheep, in which it seems likely, therefore, that reticular tension receptors would also provide a tonic afferent drive. As the intrareticular volume is increased up to about 1,000 ml, the reflex excitatory drive also increases as judged by the frequency and amplitude of the reticular contractions, which are increased. At greater volumes and tensions, the frequency and amplitude of the reticular contractions are reduced. High levels of reticular distension, therefore, elicit reflex inhibitory effects. Similar phenomena have been observed for reticular movements by Dussardier (1960, p. 93) and for salivary secretion by Ash & Kay (1959) and Kay & Phillipson (1959); moderate levels of reticular distension causing/

causing reflex excitation and high levels causing reflex inhibition. Reid & Titchen (1965) reported that quite low levels of reticular distension (i.e. above 8-12 cm H<sub>2</sub>O pressure) inhibited secondary cycle contractions of the rumen and eructation. The afferent drive to the 'gastric centres' provided by mechanoreceptors in the reticulum thus appears to consist of an excitation at low tensions and a combination of excitation and inhibition at higher tensions, the latter becoming progressively more dominant as the tension is raised.

The changes in the pattern of the gastric efferent discharge which are produced, either when there is a switch from isometric to isotonic recording conditions or when blocking agents are administered, are identical. Therefore, in so far as these reflex effects are concerned, the reflex drive i.e. the afferent input, present during a contraction involving a reduction in volume against a constant pressure head, is the same as during no contraction. This eliminates the possible involvement of 'in parallel' volume receptors, which would be off-loaded during the isotonic contraction, and tactile receptors, which presumably would be excited during the isotonic contraction, due to the movements of the reticular wall against the balloon. Iggo (1956), during single vagal afferent fibre studies, could find no evidence for volume receptors in the reticulum, but only 'in series' tension receptors.

The increased afferent activity occurring during an isometric contraction, whilst modifying the later phases of the efferent discharge associated with that contraction cycle, does not have a residual effect upon succeeding contractions. The first isotonic contraction after isometric ones is no different from the next isotonic contractions and there is no difference in the/

the duration of the quiescent periods under isometric or isotonic conditions or after administering the blocking agents, providing that the resting intrarecticular pressure remains unaltered. If the resting pressure alters under either of these conditions, however, there is a corresponding change in the frequency of the contractions and the parameters of the efferent discharge. The rate and amplitude of the gastric contractions are thus determined by the afferent activity arising from 'in series' reticular tension receptors during the quiescent phase of the primary cycles; a conclusion that supports the previous work that implicated these receptors in an excitatory reticulo-ruminal drive (Iggo, 1956).

Tension in the wall of the reticulum is due to two components, namely; the passive tension of the elastic elements and the active tensions developed by the muscle cells through their intrinsic activity. Normally, in the quiescent stomach, the latter are insignificant in comparison to the passive tensions and the reticular pressure/volume relationship appears to be the same in both the living and the dead animal, except when the volume is suddenly increased. This causes the pressure to be very high momentarily before falling exponentially to its new value. The rate of fall is faster in the dead than in the living animal, from which it is inferred that in the latter an active factor is also involved; presumably, stretching the wall excites intrinsic smooth muscle contractions. This conclusion is supported by evidence from (single) afferent gastric unit responses (described in Section B), in which stretching the reticular wall manually, in the region of the receptor whose afferent discharge was being recorded, often elicited a localized contraction of/

of that region. This was felt by the fingertips and also recorded as an extra afferent discharge following that provoked directly by stretching the wall. These intrinsic smooth muscle contractions are probably of consequence in relation to excitation of tension receptors 'in series' with them only when the reticulum is suddenly distended, particularly at the higher levels.

The effect upon the efferent gastric discharge of suddenly inflating the reticulum when it has a low initial level of distension, is an overall excitation which is seen as an increase in all its parameters, i.e. peak frequency, duration, number of impulses. In contrast, the effect when the reticulum has an initial high level of distension is an inhibition, which overrides the reflex excitation and produces a general decrease in all its parameters. Inhibition is most pronounced immediately after inflation, when the efferent discharge may be reduced, but does not persist except at the very high initial levels of reticular distension. These observations support the hypothesis that reticular distension causes reflex excitation at low thresholds and inhibition at high thresholds. High thresholds are only likely to be reached

- (a) during the contraction phase of an isometric contraction,
- (b) at maintained very high levels of reticular distension and
- (c) immediately after a sudden inflation when the reticular tension is exceptionally high for a short time, due to passive 'viscosity' and active intrinsic contractile properties.

This hypothesis will also account for the effects upon the efferent discharge observed when the reticulum is deflated or inflated at moderate levels of distension, /

distension, when changing from isometric to isotonic recording conditions or after administering blocking agents. Under these circumstances, inflation and the increased tension developed by the isometric contraction, compared with the isotonic and 'blocked' contractions, evoke simultaneously both excitatory and inhibitory effects. These are seen as an increase in the duration of the discharge and the total number of spikes but a decrease in the peak frequency of the discharge. Thus the tensions developed by the early phases of the reticular contraction affect its later phases through a reflex 'feed-back' loop. The pathway of this loop is through the vagi and the 'gastric centres', since the same effects are observed both before and after section of the splanchnic nerves.

The reflex effects described above are most likely to be due to the afferent activity arising from 'in series' tension receptors and not from 'in parallel' volume receptors or tactile receptors. These investigations have not, however, differentiated between two possibilities to account for the low threshold excitatory effects and the high threshold inhibitory effects, namely; whether the inhibition is due to a separate set of high threshold receptors which inhibit the 'gastric centres' or whether the high frequency afferent discharge arising from low threshold receptors at high levels of reticular tension causes inhibitory connexions in the 'gastric centres' to be brought into play.

The mode value for the time between inflating or deflating the reticulum and observing a reflex effect upon the efferent discharge recorded at the mid-cervical level in the vagus is 800 msec. Afferent discharges from reticular/

reticular receptors recorded at a similar site give values of about 100 msec between the start of inflation or deflation and a change in the rate of the afferent input (see Section B). Assuming a distance of 15 cms between the recording site and the 'gastric centres' and the conduction velocity of 10 m/sec for both afferent and efferent fibres, the central reflex time =  $800 - 100 - 15 - 15 = 670$  msec or, if the minimum value for reflex time (500 msec) had been taken, 370 msec.

The latent period between stimulating the cervical vagus distally and recording (a) a reticular contraction is 800 msec and (b) a dorsal ruminal sac contraction is 1,600 msec. The time between applying a stimulus to the reticulum and recording the start of an effect would be, therefore, about 1,600 msec and certainly not less than 1,300 msec for the reticulum and about 2,400 msec (not less than 2,100 msec) for the rumen. Thus, during the course of an isometric reticular contraction the tensions developed during its early phases are likely to reflexly affect that phase of the reticular contraction occurring not less than  $1\frac{1}{2}$  sec later and of the ruminal contraction occurring not less than 2 sec later. This supports the conclusions of Leek (1966) that the first phase of the biphasic reticular contraction reflexly modifies the form and amplitude of its second phase (occurring about 2-3 sec later) and the latter, in turn, modifies the dorsal ruminal sac contraction. This normally starts during the second phase of the reticular contraction and reaches its peak several seconds after the (second) peak of the reticular contraction depending on the region. Using microcannulae implanted between the muscle layers of the ruminal wall, S. Ullah (personal communication) has recorded contractions/

contractions occurring in discrete regions of the rumen and has established the time relationships which exist between their phases of contraction. For primary cycles in conscious sheep, the average interval between the (second) peak of the reticular contraction and the peak of the ruminal contraction is 2.5 sec for the cranial dorsal sac, 3.5 sec for the mid-dorsal sac and 4.2 sec for the caudal dorsal blind sac. Similar values have been obtained for the halothane-anaesthetized sheep.

Titchen (1960) has evoked ruminal contractions in decerebrate sheep by distending the reticulum and, especially, by stretching the reticulo-ruminal fold. This observation is confirmed by the present experiments in which the discharge in Type IV (i.e. ruminal) units was found to increase in all its parameters as the reticular balloon was inflated, although the extent to which this was due to distending the reticulum and which to stretching the reticulo-ruminal fold was not ascertained. In addition to this reflex excitatory effect of the reticulum upon the rumen, there appears also to be reflex inhibition. The Type IV discharge was enhanced under isotonic recording conditions and also after the administration of the blocking drugs from which it is inferred that reflex inhibition resulted from the increased afferent activity arising from reticular tension receptors during the course of isometric contractions. Although this conclusion is based on only a few observations, there is other evidence to support it; both the afferent discharge from receptors located in the ruminal wall and the amplitude of the ruminal contraction are greater when the reticulum contracts isotonically than when it does so isometrically (see Section B). This evidence opposes an idea of Iggo (1951), that the afferent/

afferent discharge increment arising from tension receptors in the reticulum during its contraction activates the 'ruminal centres' and leads to the ruminal movements.

From this investigation it is concluded that

- (a) the 'gastric centres' are dependent upon 'low threshold' excitatory drive provided by the tonic afferent input of 'in series' tension receptors during the quiescent period of the primary cycle and this, modified at high levels of distension by an inhibitory effect, largely determines the rate and amplitude of both reticular and ruminal contractions,
- (b) the earlier phases of the increased afferent input arising during the course of an isometric reticular contraction reflexly modify both the later phases of the reticular contraction, after a total reflex time of more than 1,300 msec, and of the dorsal ruminal sac contraction, after a total reflex time of more than 2,100 msec. In the case of reticulo-reticular reflexes the reflex modification is a combination of excitation and inhibition, with the latter predominating at high initial levels of reticular distension, whereas for reticulo-ruminal reflexes, reflex inhibition predominates even at moderate initial levels of reticular distension.

Table D1. Summary of the comparisons made between the efferent gastric discharge in single vagal units recorded under isotonic recording conditions and those recorded under control isometric conditions. The change from isometric to isotonic recording conditions was made during the quiescent phase of the primary (reticulo-ruminal) cycle.

+ = increase, - = decrease, o = no change.

Type	No. of units	Total No. spikes	Peak frequency	Parameters Duration	Intervals	
					start -> peak	peak -> end
I	4	o/-	+	o/-	+/o	-
II	3	-	+/o	-	o	-
III	3	-	+/o/-	-	o	-
IV	1	+	+	+	-	+
V	3	o/-	o	o/-	o	o/-
VI	1	+	+	+	-	+

Two Type I units and one Type III unit have been excluded from this table. Their discharges, which occurred before the start of the reticular contraction, were identical under both isometric and isotonic recording conditions.

Table D2. Summary of the results of comparisons made between the efferent gastric discharge in single vagal units recorded under the conditions specified and the discharge recorded under control isometric conditions.

+ = increase, ++ = large increase, - = decrease,

-- = large decrease, o = no change.

The initial level of reticular distension is indicated by l for low (400-700 ml), m for moderate (600-1100 ml), and h for high (> 1000 ml) volumes.

Type of unit	Parameters	Isotonic	Blocking drugs *	Sudden deflation			Sudden inflation		
				<u>l</u>	<u>m</u>	<u>h</u>	<u>l</u>	<u>m</u>	<u>h</u>
I	No. of spikes	-/(o)	-	-	-	+	+	--	
	peak frequency	++	++	o/-	++	+	--	--	
	duration	o/-	o/-	-	-	+	+	--	
	start -> peak	+	+	-	-	+	+	-	
	peak -> end	--	+	-	-	+	+	-	
II	No. of spikes	-	-	-	-	+	+	-	
	peak frequency	o	o	+	+	o	-	-	
	duration	-	-	-	-	+	+	-	
	start -> peak	o	o	-	-	-	-	-	
	peak -> end	--	-	-	-	-	-	-	
III	No. of spikes	-/(o)	-	-	-	+	-	-	
	peak frequency	o/(-)	o	o	o	+	-	-	
	duration	--	-	-	-	+/ (o)	-	-	
	start -> peak	o	o	-	-	-	-	-	
	peak -> end	--	-	-	-	++	-	-	
IV	No. of spikes	+	-	-	-	+	-	-	
	peak frequency	+	+	-	-	o	-	-	
	duration	+	-	-	-	-	-	-	
	start -> peak	-	-	-	-	-	-	-	
	peak -> end	-	-	-	-	-	-	-	
V	No. of spikes	o/(-)	(-)	-	+	+	-	-	
	peak frequency	o	(-)	-	+	+	-	-	
	duration	o/(-)	(-)	-	+	-	-	-	
	start -> peak	o	-	-	+	-	-	-	
	peak -> end	o	-	-	-	-	-	-	
VI	No. of spikes	+	(-)	(-)	(-)	-	-	-	
	peak frequency	+	(-)	(-)	(-)	-	-	-	
	duration	+	(-)	(-)	(-)	-	-	-	
	start -> peak	-	-	-	-	-	-	-	
	peak -> end	-	-	-	-	-	-	-	
	'pause'	a	a	a	a	-	-	-	

\* = early stage of total block

a = absence of pause

Types I and III include only those units in which the discharge was affected. The other three units are discussed in the text.

Fig. D1. Records showing the discharge patterns in 2 efferent gastric vagal units (lower trace in each record), when the reticulum contracted (A) under isotonic recording conditions and (B) under isometric recording conditions. Most of the spikes are in a Type I unit and activity in the other unit (Type IV) is marked with dots. Under isotonic recording conditions, the reticular contraction caused a reduction in intrareticular volume (not shown on this record) but no rise in intrareticular pressure (upper trace). The small pressure oscillations were due to respiratory excursions of the diaphragm.

The reflex effects on the Type I unit of the pressure increment in B are an increase in the total number of spikes, in the interval between the peak frequency and the end of the discharge and in the discharge frequencies both before and after the peak. The peak frequency itself and the interval between the start and the peak of the discharge are reduced. The reflex effect on the Type IV discharge is a reduction in the total number of spikes and, in particular, a suppression of the early part of the discharge.

In Figs. D2, 3, 4, 5 and 8 the records are presented in the same way.

A



B

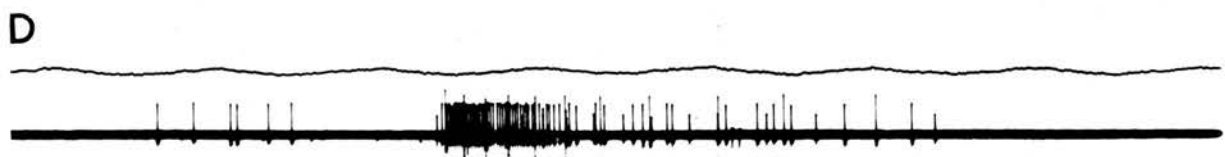


• • • • • • • • • • • • 1 sec

Fig. D2. The reflex effect on a Type I unit (with a prominent early discharge) of administering drugs which block the transmission of efferent activity and thereby abolish reticular contractions. A and C are control records obtained (A) immediately before injecting 35 mg tetraethylammonium chloride i/v and (C) 25 min after A and immediately before injecting 1 mg probanthine hydrochloride. The immediate reflex effects of these drugs are shown in B and D recorded 3 and 5 min respectively, after A and C.

Reticular contractions were abolished by both drugs and there was a marked increase in the peak frequencies of the discharge, but all other parameters of the discharge show a reduction (B and D). These responses are, in general, similar to those obtained when recording under isotonic conditions, as in Fig. D1A.

The strand contains, in addition to the Type I unit, at least two other units, which are more prominent in C and D because of a movement of the nerve strand on the recording electrodes.



. . . . .

1 sec

Fig. D3. The immediate (B) and delayed effects (C) of suddenly deflating the reticular balloon. Record A shows the efferent discharge in a single Type I vagal gastric unit (lower trace) in the cycle preceding that in which 200 ml air was suddenly removed from the intrareticular balloon. This was done at the start of the efferent discharge associated with the reticular contraction of the cycle shown in B. Record C was obtained 5 min after B, and is four contraction cycles later.

Compared with A the efferent discharge in B has lower frequencies before and after the peak which itself is greatly increased. The total number of spikes is less.

In C the number of spikes, the peak frequency and the duration of the discharge are less than in A and B. The frequencies of the discharge before and after the peak are however greater than in B but lower than in A.

A Type IV unit (tall, thin spikes) is also present. Following deflation its duration is reduced but several early spikes appear during the contraction phase of the reticulum.



Fig. D4. The immediate reflex effects of suddenly inflating the reticular balloon with the reticulum initially distended at a low level (B) and a moderate level (D). Records A and C were obtained during the contraction preceding B and D respectively.

In A the resting reticular pressure was 5 mm Hg and the reticular contraction (upper trace) was very small. In B, the sudden addition of 200 ml air to the reticular balloon at the start of the efferent discharge of the Type III gastric unit caused, reflexly, an increase in the total number of spikes, duration and peak frequency of the efferent discharge and in the amplitude and duration of the contraction.

In C the resting reticular pressure was 10 mm Hg. In D, sudden inflation of the reticulum, by adding 200 ml at the start of the Type I gastric efferent discharge, reflexly enhanced the total number of spikes and the frequencies of the discharge before and after the peak and caused an increase in the duration and amplitude of the reticular contraction. The peak frequency in D, where there is a moderate level of distension is much lower than in C; whereas in B, where there is a low initial level of distension, the peak frequency is higher than in A.

A



B

+200ml



C



D

+200ml



1 sec

Fig. D5. The reflex effects on a Type I discharge of suddenly inflating the reticular balloon with the reticulum distended at a high level (22 mm Hg). A, B and C are consecutive contractions and 200 ml air was added to the reticular balloon at the start of the discharge in B.

The immediate reflex effect (B) of inflating the highly distended reticulum is a reduction in all the parameters of the efferent discharge. In the next contraction (C), however, there is a great increase in all the parameters.

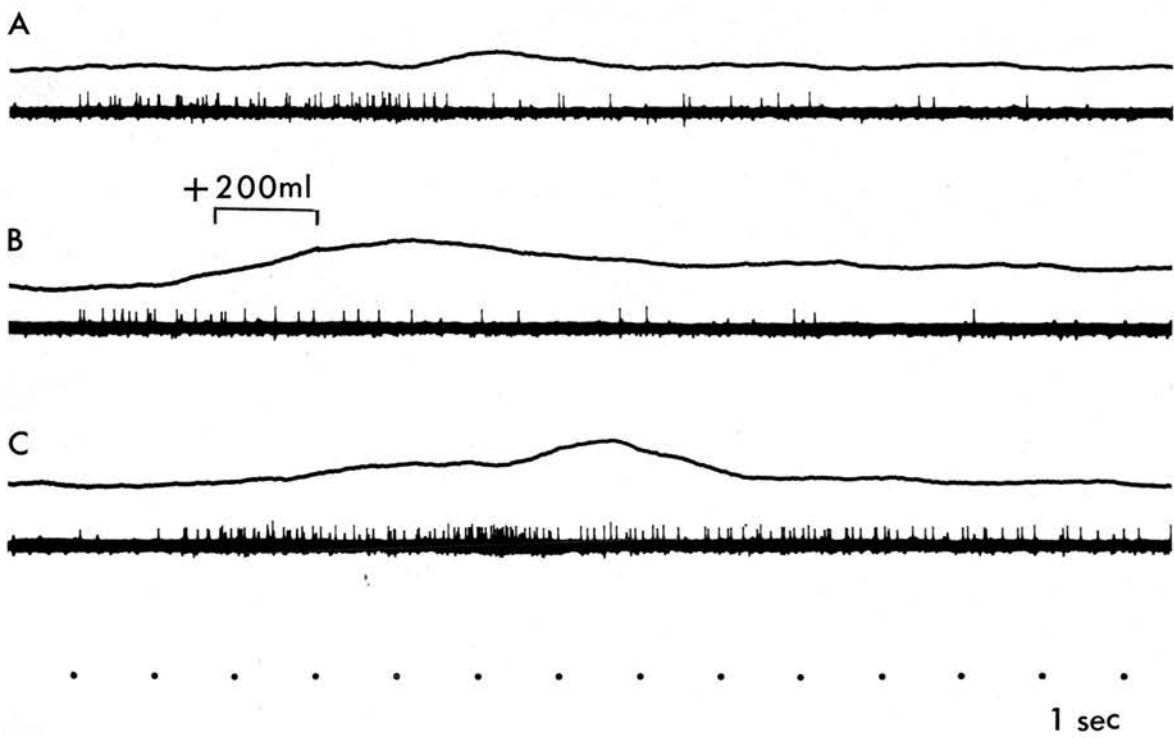


Fig. D6. The spike discharge frequency curves of a Type III gastric vagal efferent fibre at low, moderate and high levels of reticular distension (400, 800 and 1400 ml air respectively). Each record was taken not less than 5 min after inflation. The discharge is enhanced as the initial level of distension is increased from a low to a moderate level but it is depressed as the distension is further raised to a high level.

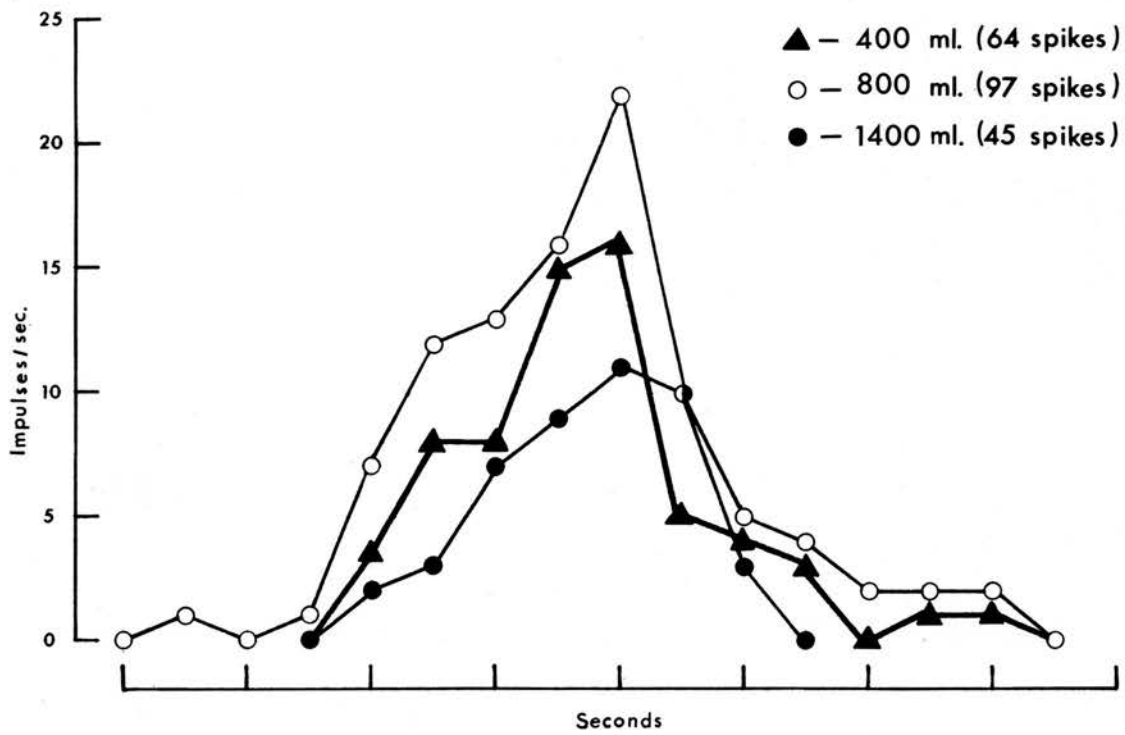


Fig. D7. The effect of incremental inflation of the reticular balloon by adding 200 ml air immediately before the contractions marked with an arrow. In A O—O indicates this maximal reticular pressure during a contraction and ●—● the reticular pressure during the quiescent part of the gastric cycle. B is the pressure rise developed during each contraction (i.e. the difference between O—O and ●—● in A), C is the total number of spikes during each discharge cycle. D is the frequency of contraction, i.e. the reciprocal of the duration of individual gastric cycles.

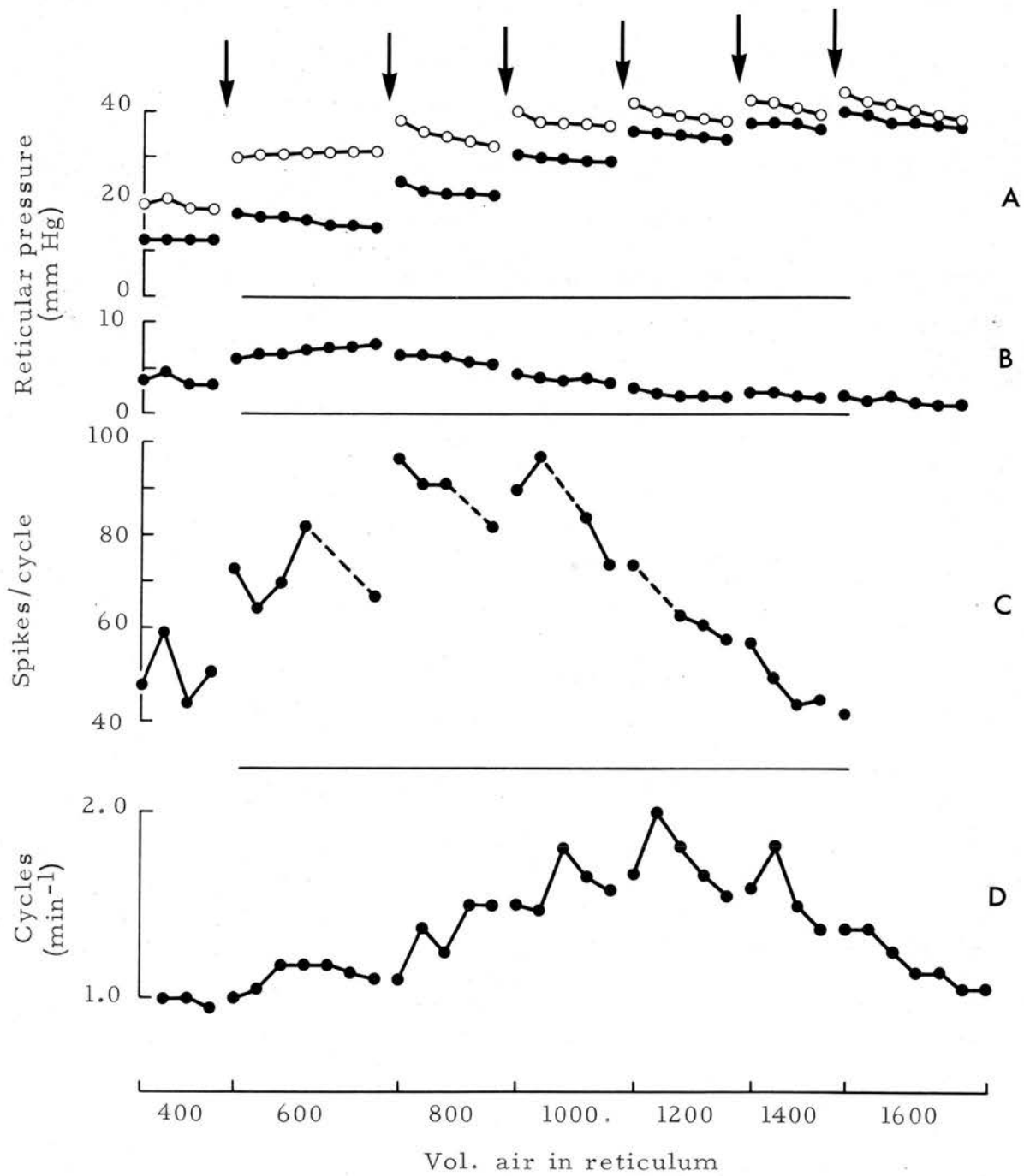


Fig. D8. Histograms showing the distribution of 89 values obtained for the interval between applying a stimulus (measured from the start of either a sudden deflation or inflation of the reticulum) and the appearance of a reflex response in gastric efferent discharges recorded from Type I, II and III units dissected from the mid-cervical region of the left vagus (see text for further details). The 54 values obtained from Type I units are shown as the hatched portion of the histogram.

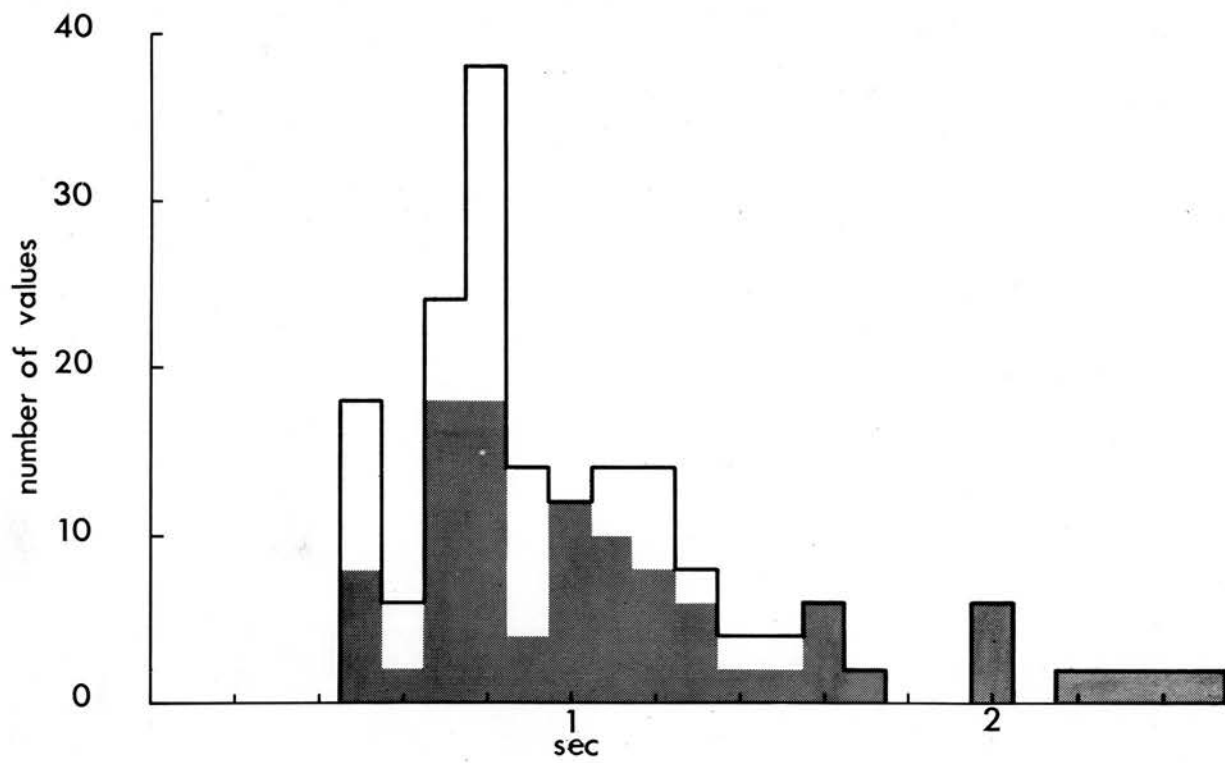
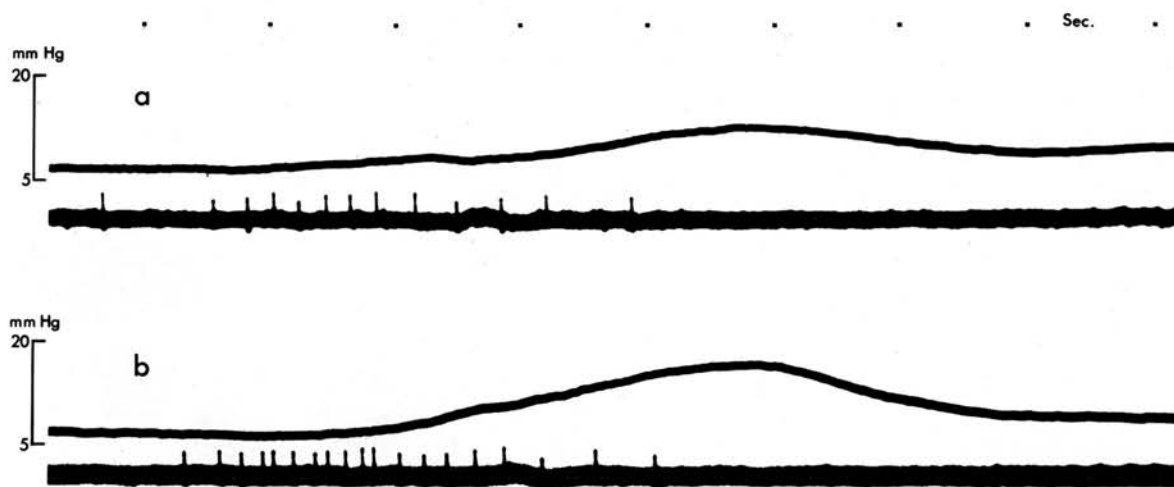


Fig. D9. The effect of acidifying abomasal contents on the discharge in a Type I unit. A is the control, obtained immediately before adding 50 ml 0.2 N HCl to the abomasum. B was recorded 90 sec afterwards. In B, the total number of spikes, the peak frequency of the discharge and the size of the reticular contraction are enhanced.

Effect of abomasal acid on the  
discharge of gastric motorneurons during reflex gastric contraction (sheep, halothane)



SECTION EThe effects of blocking transmission and stimulating the vagus nerve branchesINTRODUCTION

The dependence of reticulo-ruminal movements upon the presence of the vagi has been demonstrated by experiments involving chronic vagal denervation (p. I 31; Mangold & Klein, 1927; etc.), blockage of vagal nerve transmission by cooling (p. I 46; Popow et al, 1933; Iggo, 1951; Dussardier, 1960) and by local anaesthetics (Dziuk & Sellers, 1955; Stevens & Sellers, 1956, 1959). The limitations of chronic denervation experiments are firstly, that denervation is not reversible and secondly, that only the delayed effects and not the immediate ones are observed. These drawbacks do not apply to the method of blocking nerve transmission by cooling, which was therefore used in the present experiments in conjunction with single afferent and efferent unit recordings. The temperature that will produce cold block of transmission in vagal fibres depends upon their diameters and an effective blocking temperature of not more than 4°C was anticipated by comparison with that required to block Type B atrial receptors, having a conduction velocity range of 8-29 m/sec (Whitteridge, 1948; Torrance & Whitteridge, 1948). This is similar to the range for reticulo-ruminal afferent fibres, i.e. 5-23 m/sec (p. B 8), and reticulo-ruminal efferent fibres, i.e. 11-16 m/sec for the most excitable fibres and 1-11 m/sec for the less excitable ones (Iggo, 1956). The dependence of the cold block temperature upon the fibre diameter in the case of myelinated fibres is opposed, however, by Paintal (1965). He concludes that the two factors are not consistently related.

The/

The objects of the present experiments were to determine

- (a) whether the various branches of the vagi serve predominantly as afferent pathways or as efferent pathways for reflexes involving reticulo-ruminal movements.
- (b) whether a vagal branch contains afferent units having an overall reflex inhibitory effect or an overall reflex excitatory effect on reticulo-ruminal movements.
- (c) the reflex effect of reducing the afferent input during both the quiescent period and the active period of the primary cycle.
- (d) the reflex effect of electrically stimulating, in turn, vagal branches central and peripheral to the blocked region.

The effects of cold block were usually confirmed towards the end of the experiment by sectioning the vagal nerve branches.

### RESULTS

Nerve transmission in vagal nerves and their branches was blocked by cooling a region of the nerve with a thermode (p. M 6) in 14 sheep. During cold block, recordings of nervous activity were made from four gastric efferent vagal strands and one afferent strand. Before satisfactory unitary records were obtained, numerous units were lost due to reflex swallowing, coughing and limb movements evoked when the thermode was placed in position.

The temperature in the cooled nerve was not measured. The temperature of the refrigerant liquid entering and leaving the thermode was recorded. The flow rate through the thermode in situ was sufficiently high that the influx-efflux temperature difference was less than  $0.1^{\circ}\text{C}$ . The effectiveness of the cold/

cold block was tested either by recording from the afferent unit and observing a cessation of afferent activity or by electrically stimulating the nerve central to the thermode (25 stimuli/sec) and observing the abolition of evoked reticulo-ruminal contractions (Fig. E 1). With an influx temperature of  $4^{\circ}\text{C}$  and above, no block was observed in gastric efferent fibres. With influx temperatures of  $1^{\circ}\text{C}$  and below, a block of gastric efferent fibres was usually established in less than 3 min. After the removal of the thermode, nerve conduction was re-established within 10-60 sec. The blocking temperature for the afferent unit was not determined but block was established with an influx temperature of  $1^{\circ}\text{C}$ . After cooling the nerve, in this unit there was neither a resting discharge nor a discharge during each reticular contraction even though the reticular contractions were only slightly reduced in amplitude. The thermode had been applied to the left dorsal thoracic branch of the vagus (henceforth abbreviated to L.D.Th.V.).

The effect of cooling the left cervical vagus (L.C.V.) in 3 sheep was a reduction in the amplitudes of the spontaneous reticular contractions but no change in their frequencies. When this nerve was subsequently sectioned in two of the sheep, the same result was obtained. In one sheep, cooling with influx temperatures of  $1^{\circ}\text{C}$ ,  $0.5^{\circ}\text{C}$  and  $-0.7^{\circ}\text{C}$  completely abolished the evoked reticular contraction ~~of the reticulum~~ but left the evoked ruminal contraction unaffected (Fig. E 1). In 3 sheep, electrical stimulation central to the blocked region induced swallowing, coughing and even limb movements, which interfered with recording, whereas electrical stimulation peripheral to the blocked region caused the subsequent 3-10 contractions to have a greater frequency/

frequency and amplitude. The stimulus consisted of a train of biphasic pulses (each lasting 2 msec) given at the rate of 30/sec for 5, 10, or 20 sec.

Cooling the (common) dorsal thoracic vagal trunk (D.Th.V.) in 4 sheep caused the amplitude and the rate of reticular contractions to fall on all occasions. Cooling the ventral thoracic vagal trunk (V.Th.V.) caused a reduction in the amplitude of the (spontaneous) reticular contractions in 4 sheep, an increase in one sheep (Fig. E 2) and no change in another. The frequency of contractions was reduced in two sheep, increased in one, showed no change in two and had a variable effect in another. Even when cooling caused an enhancement or no change in the amplitude of the first and second phases of the reticular contraction, the third phase (discussed earlier: p. B 23, Fig. B 9) was invariably reduced or abolished.

Electrical stimulation (parameters as above) of the V.Th.V. and L.D.Th.V. nerves peripheral to the thermode caused an increase in the rate and the amplitude of the subsequent reticular contractions. Electrical stimulation of the same nerve central to the thermode usually caused the spontaneous contractions to be inhibited for about 2-3 min, following which there was a much reduced contraction and then 5-10 contractions which had often a lower amplitude but usually a greater frequency than those before stimulation (Fig. E 3).

The effects of sectioning the L.D.Th.V., L.C.V. and V.Th.V. successively were examined in 3 sheep. In one sheep the reticulum was distended with 1,000 ml air, so that the spontaneous contractions had initially a high frequency and amplitude. After sectioning the L.D.Th.V. these parameters were/

were reduced but after then cutting the L.C.V., they were enhanced slightly and finally after sectioning the V.Th.V., so that only the right dorsal thoracic vagal branch (R.D.Th.V.) remained intact, they were reduced again. The amplitude of the contraction was very low, the pressure rise being less than 2 mm Hg, but the frequency was 1 contraction per 5 min. A similar effect was observed in another sheep after section of the L.D.Th.V. and the V.Th.V., the amplitude of contractions being very low but the frequency was only reduced to 0.5 cycles/min. In a third sheep after sectioning the L.D.Th.V. and V.Th.V., only one more spontaneous contraction occurred.

Due to technical difficulties, gastric efferent activity was successfully examined in only four strands under conditions of cold block of thoracic vagal branches. In one sheep, cooling the V.Th.V. caused the amplitude of the reticular contraction to be reduced but the frequency of the contractions, the total number of spikes and the peak frequency in a Type III efferent gastric unitary discharge to be enhanced (Fig. E 2). In another sheep, a strand, containing 2-3 gastric efferent units (not separately identifiable) but no non-gastric units, was isolated. Cooling the L.D.Th.V. in this sheep caused a slight increase on one occasion and no change on another occasion in the amplitude of the second peak of the reticular contraction but, as usual, an abolition of its third phase. On both occasions in this sheep, the frequency of reticular contractions was increased and the number of spikes associated with that part of the efferent discharge occurring before the second peak of the reticular contraction was increased, whereas the number of spikes associated with the remainder of the efferent discharge was much reduced.

From/

From one sheep two separate Type IV (i.e. ruminal) efferent units were isolated. In the first unit, cooling the V.Th.V. caused the frequency and amplitude of the reticular contractions to be reduced, the third phase of the contraction to be abolished and the efferent discharge to be much reduced, particularly that part previously associated with the third phase. In the same unit, cooling the L.D.Th.V. caused no change in the frequency of reticular contractions and the amplitude of the second peak of the contractions but the third phase was abolished and the efferent discharge was much reduced (Fig. E 4). In the second Type IV unit, cooling the V.Th.V. caused the amplitude and the frequency of the reticular contractions to diminish and the duration and total number of spikes of the efferent discharge to be reduced. The peak frequency was raised but made its appearance before, instead of after, the (second) peak of the reticular contraction. This phenomenon was peculiar to this unit but occurred consistently during each period of cooling the V.Th.V. In contrast, cooling the L.D.Th.V. caused a marked diminution in the amplitude of the reticular contractions with abolition of the third phase but only a slight reduction in the frequency of the contractions. The total number of spikes and the duration of the efferent discharge was diminished but its peak frequency and the temporal relationship of the discharge peak to the second peak of the reticular contraction was unchanged.

Whilst continuing to record from the above unit the V.Th.V. was sectioned and its peripheral end stimulated electrically. This procedure resulted in increases in the amplitude and the frequency of the reticular contractions and the duration and peak frequency of the efferent discharges. Before sectioning the V.Th.V. it was stimulated electrically, as above, on four separate occasions/

occasions and each of these resulted after a quiescent period lasting 3 min in an increase in the frequency and the amplitude of second peak of the reticular contractions. On three occasions there was also an enhancement of the third phase and the duration and peak frequency of the efferent discharges. On the remaining occasion, the third phase was unaltered and similarly there was no change in the duration or peak frequency of the efferent discharges. The 3 min 'quiescent period' immediately following stimulation was identical with that observed earlier after stimulating the V.Th.V. central to a cooled region. During this 'quiescent period' the efferent unit gave a regular low-frequency discharge which terminated in a subnormal burst of activity corresponding to the much reduced reticular contraction which marked the end of the quiescent period. Thereafter, no 'resting' efferent discharge was recorded and the next contraction and its corresponding efferent discharge were greater than normal.

#### DISCUSSION

The temperature of the fluid entering the thermode needed to be not more than 1°C, in order to block all gastric afferent and efferent vagal activity. Although the temperature in the cooled region of the nerve itself was not measured, it was surprising that circulating fluid of such a low temperature was required in view of that required to block Type B atrial afferent fibres (Whitteridge, 1948; Torrance & Whitteridge, 1948) and others, reviewed by Paintal (1963, 1965). In fibres dissected from the saphenous nerve of the cat, Franz & Iggo (1967) found that cold block was established/

established for myelinated, afferent fibres (conducting at 5-100 m/sec) when the nerve itself was cooled to  $7.2^{\circ}\text{C}$  and for non-myelinated fibres when the nerve itself was cooled to  $2.6^{\circ}\text{C}$ , although occasionally temperatures as low as  $0.3^{\circ}\text{C}$  were necessary. It seems likely that many of the gastric efferent vagal fibres have conduction velocities at the lower end of the range (1-16 m/sec) given by Iggo (1956).

By examining the reflex effects of blocking ~~the~~ various vagal nerve branches in anaesthetized sheep with spontaneous gastric movements and by making certain assumptions, it is possible to decide whether a particular branch provides an afferent and/or an efferent pathway for gastric reflexes and whether the overall afferent activity in that branch has a reflex excitatory or a reflex inhibitory action. A vagal branch is presumed to be providing a pathway for efferent gastric fibres if, during the cold block, there is either

- (a) an enhancement of the efferent discharge (i.e. increases in the total number of spikes, the peak spike frequency and the duration of the discharge) but either no increase or a reduction in the amplitude of the gastric contractions,
- (b) no change in the efferent discharge but a reduction in the amplitude of the gastric contractions or
- (c) a directly-evoked gastric contraction when the nerve branch is electrically stimulated peripheral to the cooled region.

A vagal branch is presumed to be providing a pathway for afferent fibres involved in the reflex regulation of gastric movements if, during the cold block, there is either

- (a)/

- (a) a reduction in the efferent discharge together with a reduction in the rate and the amplitude of the gastric contractions or
- (b) an enhancement of the efferent discharge accompanied by an increase in the rate of the gastric contractions. The amplitude of the gastric contractions, under these circumstances, may either increase, remain the same or decrease, depending on the extent to which the blocked vagal branch was serving as an efferent pathway prior to cooling.

Using these criteria, it is concluded from the results described above that

- (a) the dorsal thoracic vagal trunk, to a greater extent, and the ventral thoracic vagal trunk, to a lesser extent, provide paths for efferent fibres innervating the reticulum.
- (b) the dorsal thoracic vagal trunk provides the only pathway for efferent fibres (in the left cervical vagus) innervating the rumen.
- (c) the dorsal thoracic vagal trunk provides a pathway for afferent vagal fibres (innervating unspecified structures) which have an overall potent excitatory reflex action on gastric movements.
- (d) the ventral thoracic vagal trunk provides a pathway for afferent vagal fibres (innervating unspecified structures) which reflexly affect gastric movements as a result of either an overall excitatory action or an overall inhibitory action, depending on the experimental conditions.
- (e) the left cervical vagus has properties with a greater resemblance to those of the ventral thoracic vagus than those of the dorsal thoracic vagus.

These/

These conclusions support those of Habel (1956), that the dorsal vagal trunk is the predominant pathway for efferent fibres innervating the reticulum and the only pathway for those to the rumen, and of Titchen (1958), that the overall reflex effect of stimulating the central end of the ventral trunk could be either excitation or inhibition, depending on the parameters of the stimuli.

Stimulation of the ventral thoracic trunk or of the left cervical vagus at a site peripheral to the blocked or sectioned region resulted in an excitation of gastric movements starting 1-2½ min afterwards and lasting 5-10 min. The mechanism for this phenomenon depends upon an excitatory afferent pathway forming a positive feed-back loop from structures, activated by the peripherally-<sup>-ed</sup> direct stimuli, to the 'gastric centres' in the hind-brain. The region activated by the stimulation and responsible for the reflex excitation was not determined. One possibility is that the intrinsic motility of the gastric musculature was enhanced, which would have led to an increased afferent input to the 'gastric centres' from 'in series' tension receptors, as described in Section B. Another possibility is that the secretion of acid in the abomasum might have been increased and this would have reflexly excited gastric movements, in the manner described by Titchen (1958).

Stimulation of intact nerve branches or of regions situated central to blocked or sectioned parts usually resulted in a 'quiescent period' lasting 2½-3 min during which there were no contractions. During this period the efferent discharge exhibited a 'resting discharge' which terminated eventually in a subnormal burst of activity in association with a much reduced reticular contraction. Similar efferent activity was encountered on several occasions, as a sequel to 200 ml air being suddenly introduced into the reticular balloon during/

during the course of a contraction and, when adrenaline was administered intravenously (p. F. 6 ). In these situations presumably, the 'gastric centres' are suddenly swamped with afferent impulses, the effect of which appears to be a disorganisation of their integrative activities, with the result the efferent discharge is no longer confined to periodic (i.e. primary cycle) bursts of activity.

In previous Sections (A and C), the requirement of the 'gastric centres' for an afferent drive from gastric tension receptors has been established. Provided that the level of afferent drive is great enough, it appears that a minimum of one intact vagal nerve branch (R.D.Th.V.) is necessary to provide an afferent pathway, for the 'centres' to be driven at a rate within the normal range.

In one experiment, reticular and ruminal efferent fibres in the left cervical vagus were blocked differentially. In response to electrical stimulation of the region central to the thermode, there were evoked contractions of both the reticulum and the rumen prior to cooling and of only the rumen during cooling (Fig. E 1). This demonstrates unequivocally that the reticular and the ruminal musculature is innervated independently and supports the conclusion reached in Section C, that the co-ordination for the sequence of events constituting primary cycle (reticulo-ruminal) movements occurs in the central nervous system and not in a peripherally-sited co-ordinating centre of the kind suggested by Morrison & Habel (1964).

The 'third phase' in the pressure changes recorded by the reticular balloon is due to a third phase of the reticular contraction proper (p.

B 23), superimposed on which is the passively transmitted pressure change resulting from the ruminal contraction. Cooling a vagal nerve branch usually reduced or abolished this 'third phase'. It also reduced the afferent input to the gastric centres and the reflex effect of this, on the limited number of 'reticular' (Types I-III) efferent units examined, was an enhancement of the peak frequency of that part of the efferent discharge associated with the (second) peak of the reticular contraction and a reduction of that part associated with the third phase proper of the reticular contraction. In 'ruminal' (Type IV) units the efferent discharge was markedly reduced. In the case of 'reticular' efferent (Types I-III) units but not of 'ruminal' (Type IV) units the above effects resemble those observed after changing from isometric to isotonic recording conditions (p. D 3). Under isotonic recording conditions, the afferent discharge arising from 'in series' gastric tension receptors during the course of a reticular contraction is less than that arising under isometric recording conditions. The reflex effect of this is an increased spike frequency associated with the second peak of the reticular contraction and a reduction in the later part of the discharge, i.e. that associated with the third phase. The reduction in the efferent discharge in Type IV units resembles that observed when the reticular balloon is deflated, the diminished afferent input thereby reducing the drive to that part of the 'gastric centres' concerned with rumen movements.

The main conclusions arising from the experiments described in this Section are:

(a)/

- (a) the rumen and the reticulum are innervated by separate groups of efferent gastric fibres.
- (b) the dorsal vagal trunk contains afferent fibres, which normally exert an overall potent excitatory action on the gastric centres, and efferent gastric fibres which independently innervate the reticulum and the rumen.
- (c) the ventral vagal trunk contains afferent fibres, which may exert either an overall excitatory or an overall inhibitory action on the gastric centres, and efferent gastric fibres, which innervate the reticulum but not the rumen.

Fig. E1. An example of differentially blocking nerve impulse conduction in gastric efferent fibres.

Stimulating electrodes were placed on the intact left cervical vagus and a train of stimuli (25/sec) given for 5 sec at 1 min intervals. Each train evoked a reticular and a ruminal contraction, through stimulating gastric efferent fibres. A cooling thermode was placed on the nerve peripheral to the stimulating electrodes and refrigerant fluid at  $1^{\circ}\text{C}$  was circulated through the thermode during the period marked by a horizontal bar. The reticular contractions show a progressive reduction in amplitude, whereas there is no change in the amplitude of the ruminal contractions.

The reticular balloon contained 300 ml air and, under these conditions in this sheep, no spontaneous reticulo-ruminal movements were present. Besides evoking the above contractions, the trains of stimuli reflexly evoked reticular contractions (marked with dots) but not ruminal contractions after every second train of stimuli under the control conditions but only after every third or fourth train of stimuli when the nerve was cooled peripheral to the stimulating electrodes. Similar effects were observed on three subsequent occasions.

When the cold block was applied central to the stimulating electrodes there was no change in the amplitude of the reticulo-ruminal contractions resulting from direct stimulation of the efferent fibres or in the frequency of the reflexly-evoked reticular contractions, although the amplitudes of the latter were reduced to the same extent as above.

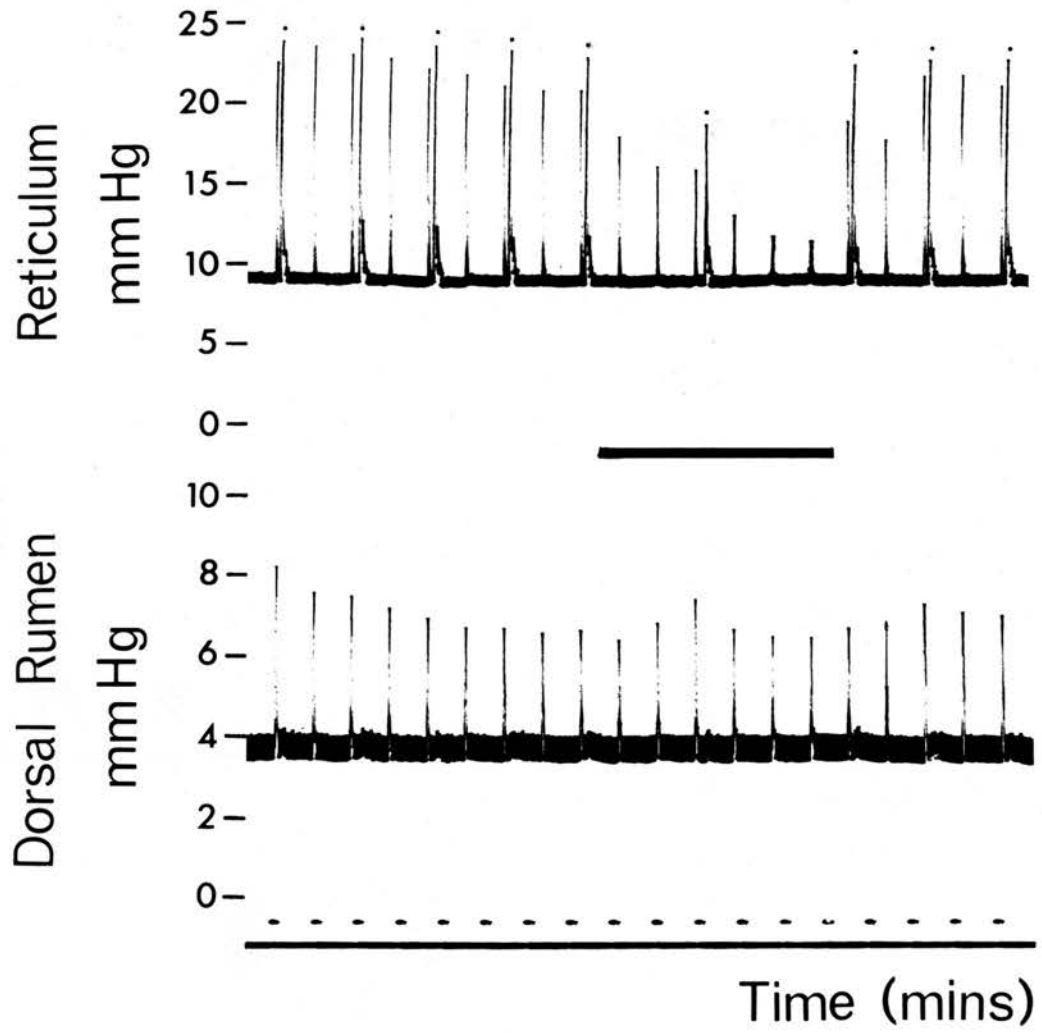


Fig. E2. The effect of applying a cold block to the ventral thoracic vagal trunk.

During the period of cold block, the amplitude of the reticular contractions increased (A) and the number of spikes occurring in a Type III gastric efferent unit during the course of a contraction was greater during the period of the block (C) than prior to it (B).

Contrast this result with that shown in the next figure (Fig. E3).

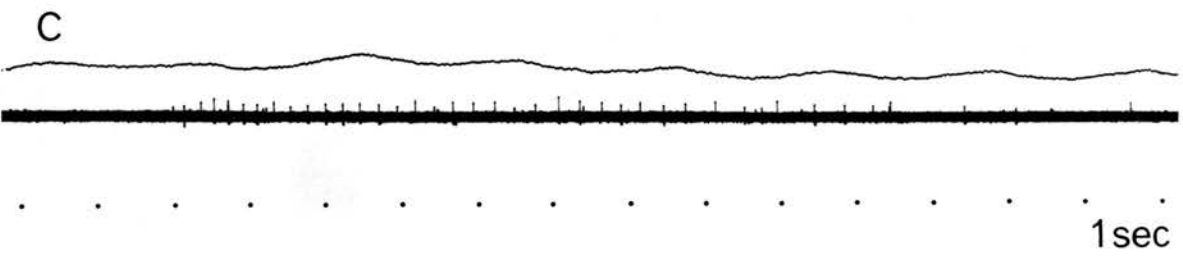
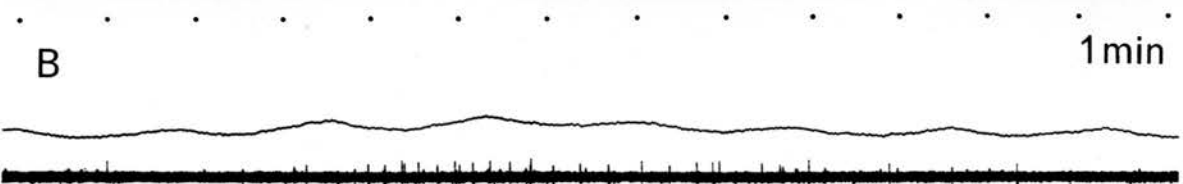
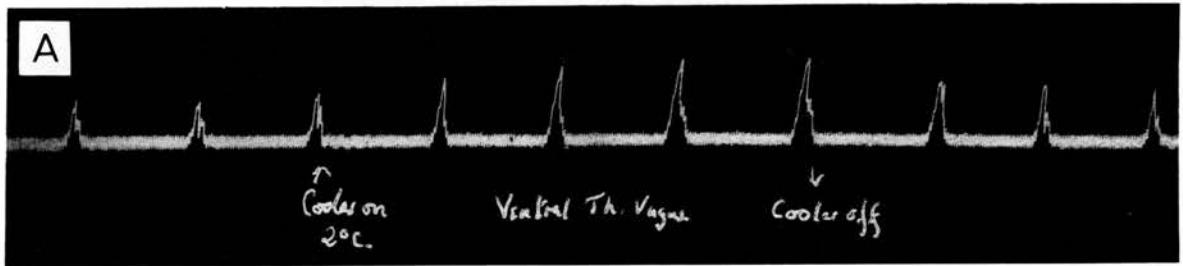


Fig. E3. The effects upon reticular contractions of cold blocking, sectioning and electrically stimulating thoracic vagal trunks.

A shows the effect upon reticular contractions of applying a cold block, firstly, to the left dorsal thoracic vagal branch (L.D.Th.V.) and, secondly, to the ventral thoracic vagal trunk (V.Th.V.). In both cases the amplitudes of the reticular contractions are reduced and the interval between contractions is prolonged by cooling the V.Th.V. Contrast this result with that shown in Fig. E2.

B shows that the effects of sectioning the V.Th.V. resemble the effects of cold block. It also shows that, whereas a 10 sec train of electrical stimuli (25/sec) applied to the peripheral stump of the sectioned V.Th.V. reflexly enhance the following cyclical contractions, a similar train of stimuli applied to the central stump of the sectioned V.Th.V. completely inhibit the contractions for 2-3 minutes, although much abbreviated efferent discharges were recorded from a gastric unit at the times marked with a white dot. The first 4 discharges appeared with a subnormal interval between them but were ineffective in causing a recordable reticular contraction.

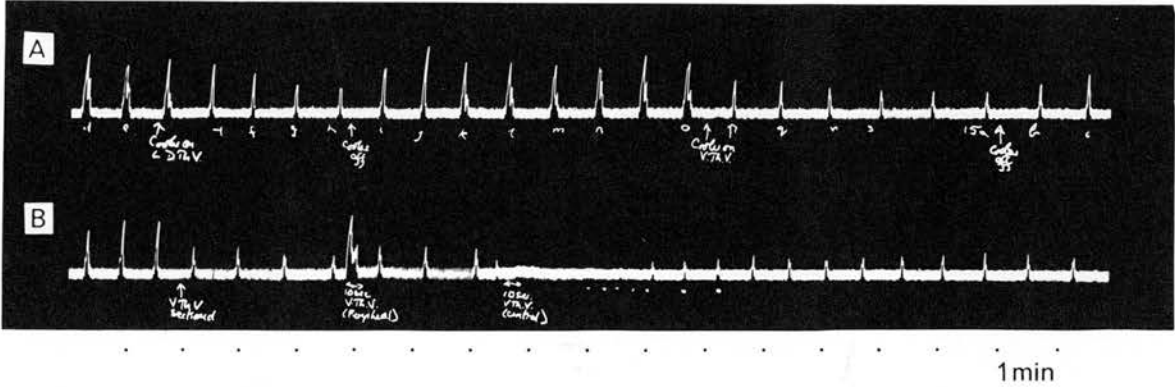
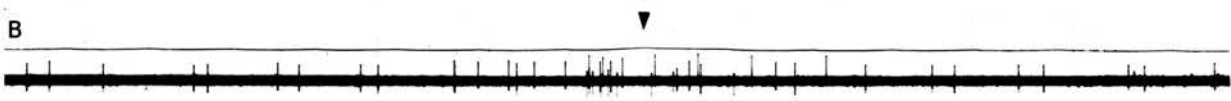


Fig. 24. The effects upon the efferent discharges in a multiunit cervical vagal strand of cold blocking the left dorsal thoracic vagal branch.

The strand contains at least four active units, viz, a respiratory efferent unit (most clearly observed at the start of B), a Type I, a Type III and a Type IV efferent gastric unit. The amplifier gain for the reticular pressure trace was set too low but the peaks of the reticular contractions (12 and 3 mm Hg in A and B respectively) have been marked with an arrowhead.

As a result of the block, the efferent discharges in all the gastric units is markedly reduced and, from a kymograph record (not shown), one observes that the reticular amplitude is reduced by 75% and the frequency of primary cycle contractions is reduced by 50%.



..... 1sec

SECTION FThe effect upon afferent and efferent gastric discharges of modifying  
reticulo-ruminal contractions by the use of drugsINTRODUCTION

Two conclusions reached in earlier Sections were that

- (a) intrinsic movements of the reticulum occurring during the quiescent part of the primary cycle influence the rate and amplitude of reticulo-ruminal contractions through a 'feed-back' to the 'gastric centres' of activity generated by 'in series' tension receptors.
- (b) intrareticular tensions, developed during the course of a reticular contraction recorded isometrically, reflexly affect the amplitude and duration of the later parts of the contraction.

The experiments described below were designed to test these conclusions, by using drugs either to modify the intrinsic movements or to modify the effectiveness of the efferent gastric discharge and the tensions developed during the ensuing extrinsic reticulo-ruminal contraction. At the same time, the techniques of recording the discharges in afferent and efferent gastric units has provided some new evidence on the mode of action of certain drugs on the ruminant forestomach.

The efferent vagal innervation of the reticulo-rumen is predominantly cholinergic. Acetylcholine, acting locally, causes a contraction of reticular and ruminal muscle, as shown in vitro with strips of muscle suspended in Tyrode's solution (Duncan, 1954; Dussardier & Navarro, 1953) and in vivo by injection into the musculature directly (Duncan, 1954) or into the coeliac artery/

artery (Dussardier, 1954). Acetylcholine, acting systemically after intravenous injection, usually inhibits reticulo-ruminal contractions (Quin, van der Wath & Myburgh, 1938; Dougherty, 1942; Brunaud, Dussardier & Labouche, 1950). This systemic effect has been attributed to an indirect nicotinic effect upon the adrenal medulla (Duncan, 1954) and to an inhibitory effect upon the gastric centres (Dussardier, 1954). Other choline esters, e.g., carbachol, have been shown to enhance reticulo-ruminal contractions in small doses and inhibit them in large doses (Quin et al, 1938; Dougherty, 1942; Duncan, 1954). Carbachol stimulates intrinsic movements in the reticulum and particularly in the rumen (Brunaud & Navarro, 1955).

The action of acetylcholine is potentiated by anti-cholinesterases, e.g., physostigmine, neostigmine. These enhance the frequency and amplitude of reticular (Duncan, 1954) and ruminal contractions in conscious animals (Dougherty, 1942; Clark & Weiss, 1954; Duncan, 1954) and in chloralose-anaesthetized sheep (Brunaud & Navarro, 1954). The actions of acetylcholine at post-ganglionic nerve endings are blocked by atropine and atropine-like drugs, e.g., probanthine hydrochloride, and in ganglia by blocking agents such as tetraethylammonium chloride (p. D 6). The last two drugs abolish extrinsic gastric contractions by blocking transmission of the efferent discharge.

When administered to conscious animals (Magee, 1932; Dougherty, 1942; Weiss, 1953; Duncan, 1954) or to chloralose-anaesthetized sheep with spontaneous reticulo-ruminal movements present (Dussardier, 1954), adrenaline inhibits these movements. When administered to anaesthetized, decerebrate or vagotomized animals in which no spontaneous (extrinsic) movements are present, adrenaline/

adrenaline usually causes a contraction (Brunaud & Dussardier, 1951), which is not inhibited by atropine but is blocked by 933F (Comline & Titchen, 1951; Duncan, 1954). Duncan, (1954) showed that a strip of reticulum in vitro contracted in response to adrenaline. Close arterial injection of adrenaline causes an increase in reticular tonus (Dussardier, 1954).

In the experiments described below, observations have been restricted to the effects of neostigmine, tetraethylammonium chloride, adrenaline, carbachol and pentagastrin, using halothane-anaesthetized sheep and recording unitary discharges from afferent and efferent gastric vagal units.

### RESULTS

#### Neostigmine

Neostigmine methylsulphate B.P. ('Prostigmin', Roche Products Ltd.) was injected into the tarsal veins of 7 sheep in single doses.

The effects of neostigmine upon reticulo-ruminal contractions and the afferent discharge in a unit with receptors in the reticular wall 1 cm caudal to the reticulo-omasal orifice are shown in Figs. F 1 and F 2. The maximal effects of injecting 0.5 mg neostigmine i/v were observed after an interval of 5 min. There was no increase in the frequency of reticulo-ruminal contractions but the amplitude of the reticular contraction was increased by 40% and the amplitude of the ruminal contraction was increased by 150%, when the reticulum contracted under isometric recording conditions, and 200%, when the reticulum contracted under isotonic recording conditions. The resting discharge in the afferent unit increased, primarily as a consequence of a reduction in the interval between bursts of activity from 16 sec to 8 sec. Whilst recording from/

from this unit, there was no third phase of reticular contraction and similarly there was no third phase in the afferent discharge, nor was there a discharge at the time of the rumen contractions even though these were very prominent, particularly under isotonic reticular recording conditions.

In 6 other sheep, there was an enhancement of the amplitude but no change in the frequency of reticulo-ruminal contractions. The enhancement took the form of an increase in the amplitude of the second phase of the reticular contraction in all cases and, in most cases, either an increase in or the elicitation of the first and third phases of the reticular contraction. The reflex effect upon a Type I gastric efferent discharge is shown in Fig. F 3. The early part of the discharge is enhanced, the part associated with the second peak of the reticular contraction is either unchanged or slightly reduced and the later part of the discharge is very much enhanced. The duration of the discharge is prolonged and the total number of spikes increased, in this case from 89 spikes (Fig. F 3A) to 130 spikes (Fig. F 3B). Similar effects were observed in four other units of Types II and III. The effects of neostigmine were first observed about 1 min after the injection, reached a maximal level 3-7 min later and lasted about 20 min.

In one sheep, on two occasions, 2.5 mg neostigmine was given i/v after spontaneous movements had been abolished by partially deflating the reticular balloon. On each occasion the drug evoked one burst of efferent activity which gave rise to a reticular contraction, 24-30 sec after injection, following which there was a prolonged tonic contraction of the reticulum, lasting 30 and 60 min. During the course of this tonic contraction, the efferent discharges were much reduced in all their parameters but the interval between discharges was much shorter. The efferent discharges did not cause a 'phasic' or extrinsic/

extrinsic contraction to be superimposed on the tonic contraction.

#### Tetraethylammonium chloride

As discussed in Section D, tetraethylammonium chloride abolishes reticulo-ruminal contractions and the immediate reflex effects of this upon efferent gastric discharges resemble the effects of changing from an isometric to an isotonic recording system for the reticular contractions, i.e. the efferent discharge is reduced in all its parameters, except for the peak frequency which is greatly increased (Figs. D 1 and D 2). One of the delayed effects is a reduction in the peak frequency of the efferent discharge but the interval between the discharges is unchanged.

The activity in one afferent vagal unit was recorded after administering 35 mg tetraethylammonium chloride. In this sheep the reticular contractions were not completely abolished but were reduced by 65%. For the afferent discharge, the resting discharge frequency was reduced by not more than 15% whereas the number of spikes associated with the contraction was reduced by 90%.

In another sheep the reticulo-ruminal contractions were completely abolished by T.E.A. and there was no increase in the discharge in a Type A afferent unit at the times when a reticular contraction would otherwise have occurred. The resting discharge was unaltered, however. It consisted of a continuous discharge with intermittent fluctuations, presumably related to intrinsic muscle contractions. Both the mean discharge frequency and the interval between the fluctuations were unaffected by the drug.

#### Adrenaline/

Adrenaline

When 0.1-0.5 mg adrenaline tartrate solution B.P. (1/1,000 'Evans Medical Co.') was injected intravenously in halothane-anaesthetized sheep with spontaneous gastric movements present, a sustained intrinsic contraction of the reticulum and the rumen was usually produced after a latency of 10-30 sec (Fig. F 4). This contraction developed during the course of about 3 sec and resulted in a reticular pressure increment of up to 10 mm Hg. It decayed slowly during the next 2-3 min. Then followed a period of 5-15 min, during which no reticular or ruminal movements were present, and a further period of 10-20 min, during which reticulo-ruminal contractions with a subnormal amplitude and a supranormal frequency were present. Contractions of normal rate and amplitude were present again 15-35 min after the injection.

The discharge in two Type A afferent units was recorded before, during and after the injection of 0.5 mg adrenaline i/v which produced effects on the reticulum as described above. In one unit (Fig. F 5) there was no change in its resting discharge until the start of the sustained contraction, whereupon a very high frequency discharge (about 80/sec) developed and lasted for 17 sec. The frequency then fell to about 40/sec and persisted for 75 sec. Suddenly, at the end of the sustained contraction, the continuous discharge changed to one in which a  $\frac{1}{2}$  sec burst of 3 spikes occurred approximately every four seconds. After a lapse of 10 min, a persistent discharge developed and lasted 44 sec. The frequency at the start was 26 spikes/sec and this fell to 8/sec at the end. No pressure rise was recorded by/

by the reticular balloon during this activity. Then followed a period of 45 sec, during which there were no spikes, and thereafter bursts of 2-3 spikes recurring every 2 sec. The first signs of a small reticular contraction were evident 15 min after the injection. After this the resting discharge markedly increased, appearing as bursts of about 42 spikes lasting 2 sec and recurring at intervals of 1-2 sec.

In the other afferent unit a high discharge frequency occurred at the time of the sustained contraction 30-126 sec after injecting 0.2 mg adrenaline. Then followed a fluctuating resting discharge which was approximately normal throughout the period prior to the return of small reticular contractions 7 min later. There was, however, no increase in the afferent discharge at the time of the reticular contractions until these had regained their previous amplitude (20 min after the injection). This unit innervated receptors located to the medial wall of the reticulum, 2 cm caudal to the reticulo-omasal orifice.

The discharges in one Type I and two Type III efferent units were recorded before, during and after adrenaline was injected. In the Type I unit, 25 sec after injecting 0.1 mg adrenaline, an abnormal discharge appeared during the decay of the sustained intrinsic contraction (Fig. F 6B). Then a 'resting efferent discharge' consisting of 1 spike every 4 sec occurred until the next burst of efferent activity occurred 3 min after the injection. This burst (Fig. F 6C) resembled the ones obtained under isotonic reticular recording conditions (Fig. D 1A). Very small reticular contractions (less than 2 mm Hg pressure rise) were recorded at this time and the interval between the/

the discharges was slightly less than previously. Subsequently (after 10 min) slightly larger reticular contractions appeared and, as these increased in size, so the efferent discharge changed from that resembling the one recorded under isotonic conditions to that recorded under isometric conditions (Figs. F 6D and D 1), and the interval between contractions reverted to its previous value. The overall effect of the drug lasted 30 min.

In two different sheep when 0.5 mg adrenaline was injected a sustained contraction started 20 sec after the injection, reached an amplitude of 10 mm Hg and lasted 30 sec. The first very small reticular contraction appeared 1 min after the start of the sustained contraction and these recurred at twice the rate of the contractions prior to the injection. Five minutes later the amplitude started to increase steadily and normal movements were present 35 min after the injection. The discharge in two Type III efferent units recorded during this procedure showed many of the features of the efferent unit described above. There was a much reduced discharge of abnormal pattern during the decay of the contracture. Thereafter, associated with each small reticular contraction there was a subnormal discharge, which increased in step with the progressive increases in the amplitude of the reticular contractions.

#### Carbachol (carbaminoylecholine chloride)

0.5 mg carbachol ('Carbachol injection B. Vet. C.', May & Baker, Ltd.) was injected intravenously into three halothane-anaesthetized sheep with spontaneous reticulo-ruminal movements present, causing similar effects in each. After a latency of 10 sec a sustained contraction of large amplitude (15 mm Hg) developed./

developed, which lasted 15 sec and was followed by a slightly raised intrareticular pressure ( $\sim 2$  mm Hg) for 30 min, due to increased tonus in the reticulum (Fig. F 7). The first signs of a phasic reticular contraction appeared 6 min after the contracture and normal reticular contractions superimposed on the remaining tonus were present 15 min after the injection.

The efferent discharge in a Type I unit was recorded before, during and after the injection. No efferent activity was present during the sustained contraction. The first burst of activity occurred  $4\frac{1}{2}$  min after this contraction (Fig. F 8B). It had more spikes and a longer duration than the control discharge obtained before the injection (Fig. F 8A). No sign of an extrinsic reticular contraction resulted from neither this efferent discharge nor the next one but the subsequent discharge was associated with the first very small reticular contraction noted above. The interval between the discharges was 30% less than before the injection. Thereafter the amplitude of the reticular contractions and the interval between the discharges became longer. Although, after 15 min, the amplitude of the reticular contraction was the same as the control, the efferent discharge was much enhanced, e.g., there were nearly twice as many spikes (Fig. F 8C). Throughout the period from the appearance of the first burst of activity  $4\frac{1}{2}$  min after the injection to the disappearance of reticular 'tonus' 30 min later, there were occasional spikes, at intervals of 5-10 sec, between the bursts of activity.

#### Pentagastrin

25  $\mu$ g pentagastrin was injected intravenously on four occasions in two halothane-anaesthetized sheep and each time this caused spontaneous reticular movements/

movements to stop for up to 20 min. On one occasion recordings were made of the activity in an afferent gastric unit (Type A). After the injection the fluctuations in the discharge frequency became more prominent. The peak frequencies developed during the fluctuation were higher and the interval between these peaks was reduced.

#### DISCUSSION

By comparing the changes evoked in the extrinsic reticular contractions and the afferent and efferent gastric discharges before and after administering a drug, it has been possible to decide whether the drug exerts

- (a) an action at a site peripheral to <sup>the</sup> 'single fibre' recording site either directly or indirectly through a humoral mechanism, or
- (b) an action involving reflex pathways through the 'gastric centres'.

Neostigmine is a potent anticholinesterase but one of its subsidiary actions is thought to be direct stimulation of autonomic ganglia (Goodman & Gilman, 1956). The results described above are compatible with both of these actions. The intrinsic motility of the reticulum was enhanced by neostigmine. With a low dose (0.5 mg) there was no obvious increase in reticular tension recorded by the balloon but the resting discharge in an afferent unit was increased. With higher doses (2.5 mg) a prolonged raised tonus was recorded by the balloon. No 'resting discharge' was recorded from efferent units, so it is concluded that neostigmine was capable of exerting an action on the ganglia or the smooth muscle itself. This conclusion may also account for the apparent refractoriness of the reticulum to efferent gastric activity during the period of high tonus. Efferent gastric activity is enhanced or evoked by/

by neostigmine and results either directly from an action on the gastric centres or indirectly through increased afferent activity having a reflex excitatory effect on the centres. In part, the latter is likely to be due to the increased afferent activity from reticular tension receptors but also to the activity from other regions, e.g., acid secretion by the abomasum may be potentiated by neostigmine and lead to excitation of the gastric centres in the manner described by Titchen (1958). 'Feed-back' from tension receptors is also suggested, however, by the form of the efferent discharges. With low doses (0.5 mg) of neostigmine the predominant effect is an increase in the amplitude of the reticular contraction and the efferent discharge has a form which is reminiscent of that observed after suddenly inflating the reticulum (at moderate initial levels of distension) or after changing from an isotonic to an isometric reticular recording system, viz. there are increases in the total number of spikes, the duration of the discharge, the intervals between the start to the peak and the peak to the end of the discharge, but either no increase or a reduction in the peak frequency. With high doses of neostigmine (2.5 mg) the form of the efferent discharges resembles that described for the reticulum at high levels of distension (p. D 16), viz. abbreviated discharges with only short intervals between them.

The immediate reflex effects upon efferent gastric discharges of administering drugs, such as T.E.A. and probanthine which block neural transmission peripheral to the single fibre recording site have been discussed in Section D. It is concluded that the effects result from the abolition of reticular contractions and of the increased afferent activity arising from 'in series' tension receptors during the course of isometrically recorded reticular/

reticular contractions. This reflexly modifies the form of the later parts of the efferent discharges. The delayed effect of both drugs is a general reduction in the various parameters of efferent gastric discharges. From an examination of records obtained from afferent gastric units after the administration of T.E.A., it does not appear that there is a substantial reduction in intrinsic reticular motility, since the frequency and rhythmic fluctuations in the resting afferent discharge are unaltered. It seems more likely that T.E.A. (and probanthine) block autonomic nerve transmission to structures other than gastric muscle, the activity of such structures being normally under some degree of resting or tonic neural activation and exerting a reflex excitatory action on extrinsic reticular movements through afferent 'feed-back' connexions with the 'gastric centres'. The fact that the resting discharge in afferent gastric units is scarcely unaltered by T.E.A. suggests also that intrinsic reticular motility is essentially myogenic rather than neurogenic in origin.

The effects upon the reticulum of injecting adrenaline intravenously will be discussed in three stages. The first stage consists of the sustained contraction during which the intrareticular tension and the activity in afferent units from reticular tension receptors is greatly enhanced. This contraction is not associated with an efferent discharge but is due to adrenaline acting peripherally, probably on the muscle cells directly. Only during the decay of the contracture is there a discharge in efferent gastric units and, due to peripheral inhibition, this discharge is ineffective in evoking a phasic (extrinsic) reticular contraction. This result confirms earlier/

earlier results obtained both in vitro and in vivo under certain conditions, viz. that adrenaline may cause a contraction in reticular muscle (Comline & Titchen, 1951; Brunaud & Dussardier, 1951; Duncan, 1954). The efferent discharge appearing during the decay of the sustained contraction is presumably evoked reflexly as consequence of the tension rise due to this contraction. During the next stage the muscle cells are relaxed, since the activity in afferent tension receptor units is absent. Adrenaline therefore inhibits intrinsic motility during this second stage. When the afferent activity returns, efferent gastric discharges also appear but are ineffective in causing the reticulum to contract, i.e. there is peripheral inhibition of the efferent discharge. The discharge shows an overall reduction and its form resembles that recorded under isotonic reticular conditions more than under isometric reticular conditions (p. D 3). This accords with the observation that the afferent 'resting discharge' is also much reduced and that there is no intrareticular tension rise associated with the efferent discharges. The third stage is the period, during which the afferent 'resting discharge' regains its previous rate and the peripheral inhibition of the efferent discharge is progressively reduced. In consequence, the reticular contractions increase in amplitude, the parameters of the efferent discharge return to their previous values, and, in form, the discharge changes progressively from the 'isotonic' to the 'isometric' type. The peripheral inhibitory action following the sustained contraction is presumably analogous to the inhibition of gastric movements observed in conscious ruminant animals (p. F 2; Magee, 1932; etc).

The effects of carbachol, like acetylcholine (outlined on p. F 1) appear to be due to a combination of direct and indirect actions. Duncan (1954) has/

has suggested that acetylcholine exerts its indirect action through a nicotinic effect on the adrenal medulla, since this action was less evident in adrenalectomized sheep. The effects of injecting carbachol will be discussed in three stages, as for adrenaline. The first stage, consisting of the sustained contraction, is even more pronounced than for adrenaline. This may have resulted from carbachol acting synergistically both directly and indirectly through an adrenaline mechanism. The intrareticular pressure increment was greater and so was the discharge in an afferent gastric unit. This sustained contraction did not evoke an efferent discharge (apart from two spikes). Applying the 'two thresholds' hypothesis advanced in Section D, that reticular tension receptors give rise to reflex gastric excitation under conditions of moderate reticular distension and reflex gastric inhibition under conditions of high reticular distension, the large sustained contraction caused by carbachol may have resulted in a situation equivalent to high reticular distension, which would account for the appearance of only two spikes, in contrast to the more pronounced burst of efferent activity evoked during the more moderate contraction caused by neostigmine and adrenaline. Following the sustained contraction, the next stage consisted of a prolonged period of raised tonus, as observed by Brunaud & Navarro (1955). This contrasted with the similar stage after adrenaline, because after carbachol the intrinsic reticular motility and the efferent discharges were enhanced. The increased intrinsic motility is presumably a direct effect of carbachol and may account, in part at least, for the reflex gastric excitation made evident by the much enhanced efferent discharges. During this stage, as with adrenaline, /

adrenaline, the efferent discharges were ineffective in eliciting an extrinsic reticular contraction and this may have been due to either a refractoriness at the level of the ganglia or of the muscle or to an indirect effect of carbachol, resulting perhaps from the release of adrenaline and the production of peripheral inhibition. The next (i.e. the third) stage was protracted. Small (extrinsic) reticular contractions were recorded accompanied by efferent discharges which were much enhanced in comparison with those recorded prior to injecting carbachol. It therefore appears, that carbachol probably exerts a direct excitatory effect on the peripheral ganglia and/or smooth muscle cells accompanied by a refractoriness or an indirect peripheral inhibition, possibly through an adrenaline mechanism as suggested by Duncan (1954) for acetylcholine. The present results oppose the view of Dussardier (1954) that acetylcholine (and hence presumably carbachol) exerts its inhibitory effects through an action on the central nervous system, because the effect of this would have been a suppression of efferent gastric activity whereas there was an enhancement even during the period when no (extrinsic) reticular contractions were recorded!

Gastrin has been extracted from the abomasum and shown to stimulate gastric secretion in sheep (Anderson et al 1962). Titchen (1958) has demonstrated the importance of abomasal pH for the reflex regulation of reticulo-ruminal movements in decerebrate and, therefore, pentagastrin (a potent synthetic analogue of gastrin) was administered, in order to promote abomasal acid secretion. Because a high dose was administered, however, the inhibitory effect described by Tracy & Gregory (1964) was probably evoked. The ensuing reduction in the secretion of abomasal acid could account for the observed abolition of reticulo-ruminal movements on the basis of the reflex mechanism discussed by Titchen (1958). It is interesting/

interesting that the 'resting discharge' in afferent gastric units increased, as pentagastrin has been observed to enhance intrinsic motility in the non-ruminant stomach (R. A. Gregory, personal communication). No other records of nervous activity in afferent gastric units following pentagastrin administration are known to exist at the moment.

Under certain conditions the reticular pressure record shows a third pressure rise at about the time of the dorsal ruminal sac contraction. In an earlier discussion (p. B 23) it was concluded that this third rise was due to a third phase of contraction in the reticulum and not to passively transmitted pressures produced by the dorsal sac contraction. This conclusion is confirmed by the observation of activity in a Type A afferent unit innervating receptors located 2 cm caudal to the reticulo-omasal orifice after the administration of neostigmine, which caused the ruminal contraction to develop a very large amplitude. Although the receptive field was located in a region which was most likely to be stretched passively by large ruminal contractions, no third phase of afferent activity was recorded and this emphasises the independence of reticular and ruminal tensions under the conditions of the present experiments.

The present experiments with drugs must be regarded only as a preliminary investigation. The techniques employed have, however, allowed one to differentiate between various categories of drug action, viz:-

- (a) reflex and central excitation
- (b) reflex and central inhibition
- (c) peripheral excitation
- (d)/

(d) peripheral facilitation

(e) peripheral inhibition and/or refractoriness

Without further refinements the techniques do not differentiate between the direct actions of the drug and the indirect actions operating through, for example, endocrine glands and changes in the blood supply.

In general, observations on the reflex effects of drug actions accord with those anticipated from the results of earlier Sections, viz, (a) moderate increases in intrinsic reticular motility result in reflex excitation of the 'gastric centres' and larger increases result in reflex inhibition and (b) the tensions developed during the earlier parts of the reticular contraction reflexly modify the later parts of the efferent discharge and of the reticular and the ruminal contractions.

Fig. M. The effect upon the reticular and the ruminal contractions of administering 0.5 mg neostigmine methylsulphate i/v (at the point marked with an arrow).

For the first four contraction cycles after the injection, reticular contractions were recorded under an isometric system; for the next three cycles, under an isotonic system and, for the last four cycles, under an isometric system.

Neostigmine causes the amplitude of both the reticular and the ruminal contractions to increase. As at other times, the ruminal contractions are greater when the reticulum contracts under an isotonic system. The frequency of the cycles remains the same.

The cycles marked a, b and c are the ones during which records A, B and C in Fig. F2 were obtained.

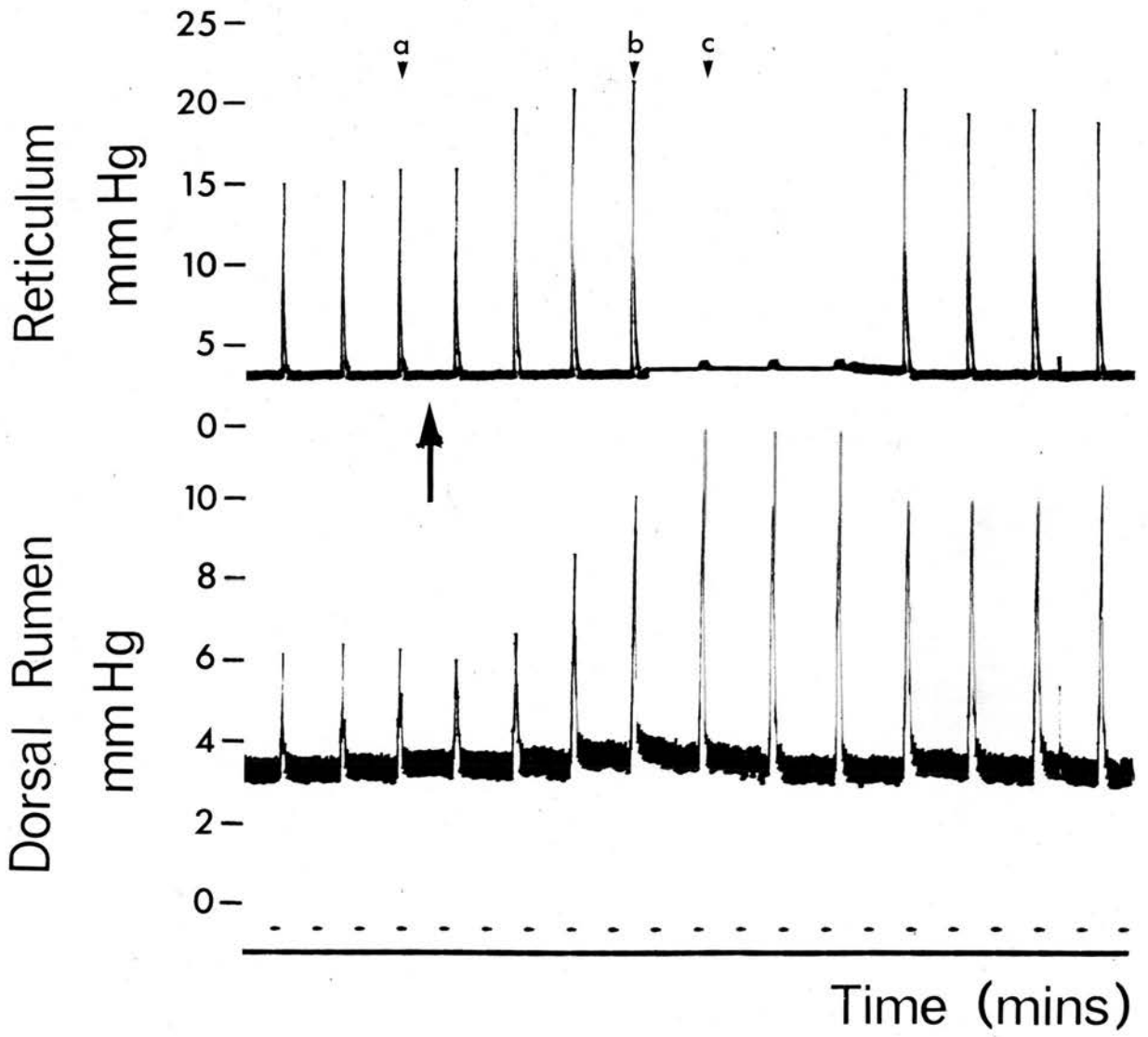


Fig. F2. The effect of neostigmine upon the reticular contraction (upper trace in each record), the ruminal contraction (middle trace) and the afferent discharge in a unit innervating tension receptors situated 1 cm caudal to the reticulo-omasal orifice (lower trace).

A is the control record taken during the cycle preceding the i/v injection of 0.5 mg neostigmine methylsulphate (a in Fig. F1).

B was obtained 5 min after A, with the reticulum contracting under isometric recording conditions. The amplitudes and the durations of both the reticular and the ruminal contractions are enhanced and so is the afferent discharge. The ruminal contraction reaches its peak later than in A.

C was obtained during the cycle following B, with the reticulum contracted under isotonic recording conditions. There is no pressure rise in the reticulum and the afferent discharge is much less than in B. The ruminal contraction is greater than in B.

Although the field innervated by the afferent unit is close to the rumen, it appears to be unaffected by contractile events in the rumen, even when these have an unusually large amplitude.

These records were obtained during the cycles shown in Fig. F1.

A



B



C



..... 1sec

Fig. F3. The effect of neostigmine upon the reticular contraction (upper trace in each record) and the discharges in a Type I (unmarked spikes) and a Type IV efferent gastric vagal unit (spikes marked with a dot).

A is the control record, taken during the cycle preceding the i/v injection of 0.5 mg neostigmine methylsulphate. B was taken 4 min later.

In B the amplitude of the reticular contraction is greater and in the Type I unit, the discharge has nearly 50% more spikes. Its duration is slightly prolonged and the numbers of spikes in the early and late parts of the discharge are enhanced, although the peak frequency is reduced.

In B, the discharge in the Type IV unit has more spikes and a longer duration. This would correspond to an enhanced ruminal contraction due to the action of neostigmine, as seen in Figs. F1 and 2.

A



B



..... 1sec

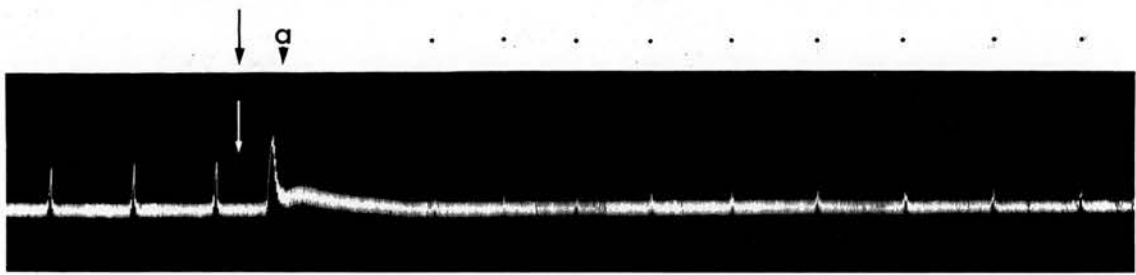
Fig. F4. The effect upon the reticular contraction of administering 0.1 mg adrenaline i/v at the point marked with an arrow.

After a latency of about 30 sec, there is a large contraction of the reticulum (pressure increment = 18 mm Hg) which is followed by a period of raised tonus, which lasts about 2 min in this case, and then the cyclical reticular movements reappear with a very small amplitude at first. Normal contractions were present again 30 min later.

The large contraction was not associated with a discharge in efferent gastric units. After the peak of this contraction (at a) an abnormal discharge was recorded (Fig. F6B) and further discharges were recorded at the points marked with dots. The amplitudes of the latter contractions were lower than those likely to have occurred with comparable discharges in the absence of the drug.

With larger doses of adrenaline, there was a prolongation of the intervals between the initial large contraction and the reappearance of efferent discharges and, later still, of cyclical movements.

0.1mg  
adrenaline



1min

Fig. F5. The effect upon the afferent discharge recorded from a gastric unit innervating reticular tension receptors of injecting 0.5 mg adrenaline i/v.

A is the control record and shows the afferent discharge during the reticular contraction prior to injecting adrenaline. There is a continuous but fluctuating 'resting discharge' and an 'active discharge' associated with the first, second and third phases of the reticular contractions.

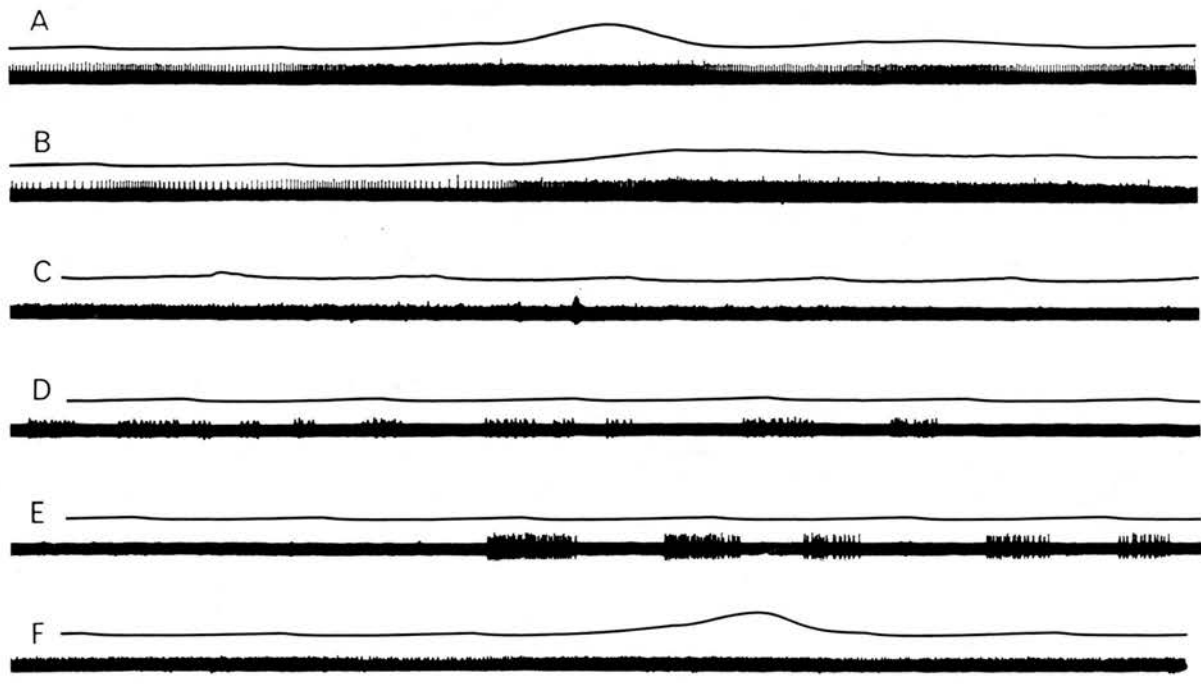
B shows the development of a high frequency discharge at the start of the large contraction evoked directly by adrenaline.

C shows the sudden termination of this high frequency discharge (about two-thirds along the trace) 92 sec after the injection.

D shows the end of a 44 sec irregular discharge of spikes having a moderately high frequency. This burst was not accompanied by a detectable rise in reticular pressure. It was preceded by  $\frac{1}{2}$  sec bursts (each with only three spikes) occurring at 4 sec intervals and was followed by no spikes for 45 sec.

E shows the 'resting discharge' after the first obvious but small reticular contraction 15 min after the injection. Bursts of about 42 spikes/2 sec were recorded at intervals of 1-2 sec.

F shows the afferent discharge 30 min after the injection. The resting and active discharges are similar to the control (A) but recording conditions at the electrode have deteriorated, so that the spikes are small and do not reproduce well.



. . . . . 1sec

Fig. F6. The effect upon the efferent discharge in a Type I efferent gastric vagal unit of administering 0.1 mg adrenaline i/v.

These records were obtained during the events shown in Fig. F4.

A is the control and shows the reticular pressure (upper trace in each record) and the efferent discharge (lower trace) associated with the primary cycle contraction preceding the injection of adrenaline.

B shows the abnormal efferent discharge which occurred (at the point marked a in Fig. F4), during the decay of the contraction directly-evoked by adrenaline.

C shows the abnormal presence of a 'resting discharge' which occurred for 2 min between B and the first incipient reticular contraction (marked by the first dot in Fig. F4).

D shows the return of an approximately normal efferent discharge, although the amplitude of the reticular contraction is only 70% of that in A. This record was obtained 15 min after the injection.

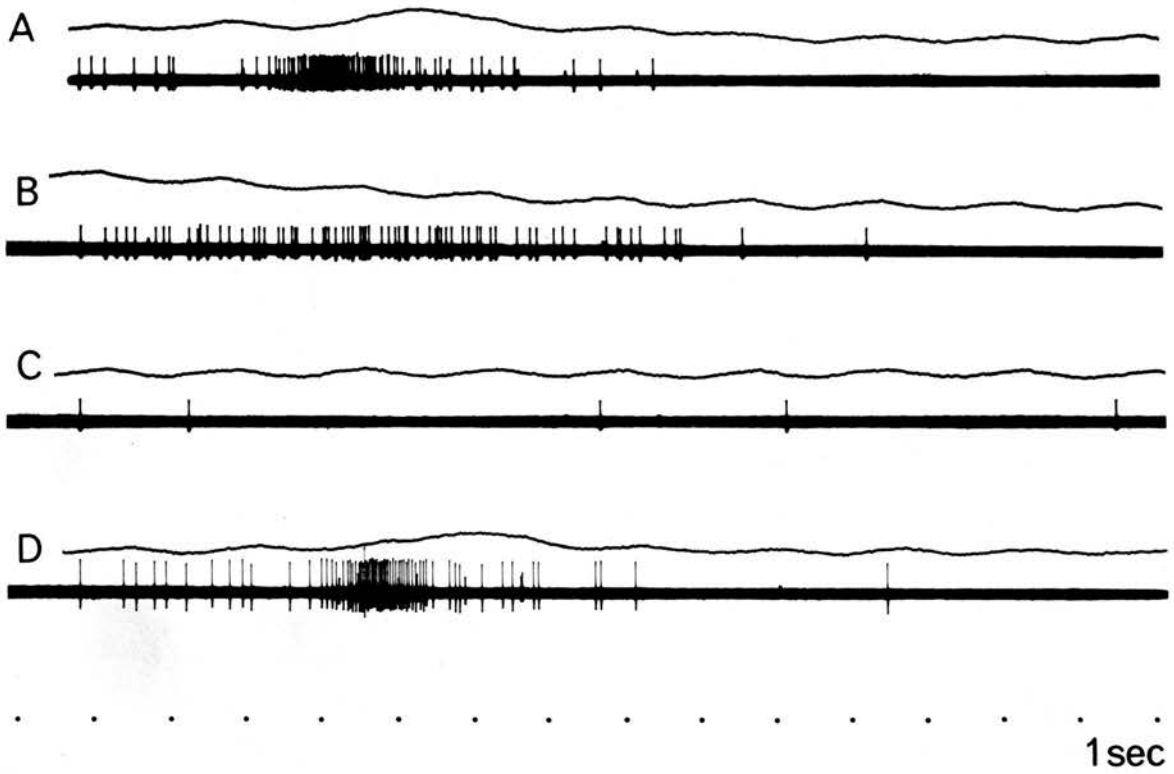


Fig. F7. The effect upon reticular contractions of administering 0.5 mg carbachol i/v (at the point marked with an arrow).

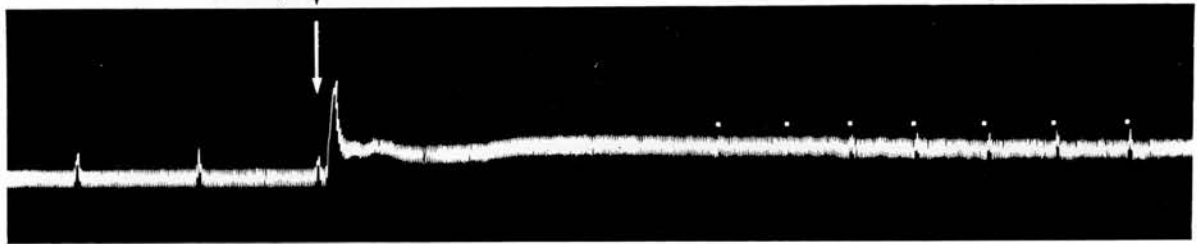
Prior to injection the reticular contractions had a small amplitude (4 mm Hg). 10 sec after injecting carbachol a large reticular contraction ensued and lasted for 15 sec. Then followed a period of raised tonus. The times at which an efferent discharge was recorded from a Type I unit after giving the injection are marked with a white dot. The first discharge appeared  $4\frac{1}{2}$  min after the injection and the first detectable contraction appeared 6 min after the injection. The effects of the carbachol lasted about 30 min.

Efferent discharges recorded from a Type I unit at the points marked a and b on this kymograph tracing are shown in Fig. F8. The interval between discharges is less than before giving carbachol.

0.5mg  
carbachol

a

b



1 min

Fig. F8. The effect upon the discharges in a Type I efferent gastric vagal unit of administering 0.5 mg carbachol i/v.

These records were obtained during the events shown in Fig. F7.

A is the control record and shows the efferent discharge associated with the reticular contraction marked a in Fig. F7.

B shows the discharge in the same unit at the point marked b in Fig. F7, i.e. it is the first discharge which occurred,  $4\frac{1}{2}$  min after the injection. Although the discharge has as many spikes as the control (A), there is no detectable reticular contraction.

C shows the discharge 15 min after the injection, by which time the amplitude of the reticular contractions was nearly the same as in A but the efferent discharge in C (102 spikes) was much greater than in A (59 spikes).



..... 1sec

GENERAL DISCUSSION AND CONCLUSIONS

The electrophysiological investigation reported in this thesis necessitated the use of acutely prepared sheep in which cyclical movements of the reticulo-rumen were present. The form of primary cycle movements present in chloralose-anaesthetized and in halothane-anaesthetized sheep is described in Section A. One of the advantages of these preparations is that standard conditions can be readily established and the results obtained at different times and from different animals may be compared more satisfactorily. In comparison with gastric movements in conscious sheep, those occurring in anaesthetized sheep under the standard conditions are similar for the reticulum but are different for the rumen because the amplitude is subnormal. This difference probably accounts for the relative infrequency of Type IV (i.e. ruminal) efferent units found in the present experiments compared with the high incidence of similar units recorded by an indirect method in conscious sheep by Dussardier (1960).

Several earlier investigations have demonstrated the dependence of reticulo-ruminal movements upon efferent vagal activity (p. I 31 et seq) which originates from 'gastric (reticulo-ruminal) centres' in the hind-brain (p. I 33). The gastric centres integrate afferent nervous activity arising in peripheral structures and in higher centres of the central nervous system. From the results of the present experiments it is concluded that the gastric centres determine (a) the frequency of primary cycle contractions, (b) the amplitude and the duration of the contractions and (c) the temporal relationships existing between the sequential contractions of the various forestomach regions./

regions. To some extent (b) and (c) are influenced by the 'feed-back' of afferent activity from 'in series' gastric tension receptors during the active part of the contraction cycle. As discussed in Section D, the tensions developed during the early parts of the reticular contractions reflexly modify the gastric efferent discharges to and hence the tensions developed by the later parts of the reticular contraction and by the ruminal contraction. These reflex effects are attributable to the change in afferent activity generated by reticular tension receptors as a result of increased reticular distension (see Sections B, D and F) and resemble the change in motility seen immediately after the end of a meal compared with that immediately before a meal (Reid, 1963). It seems likely, therefore, that such a change in motility may be largely explicable on the basis of changes in reticular tension receptor activity induced by newly ingested foodstuffs.

The activity in the gastric centres is influenced not only by afferent activity fed back during the active part of the contraction cycle but also by a continuous afferent input throughout the entire cycle. As demonstrated by cold block, nerve section and centripetal stimulation in Section E, the afferent input to the gastric centres is a combination of inputs having reflex excitatory effects and of inputs having reflex inhibitory effects, although it must be emphasised once again, that, whilst afferent activity in certain pathways will always exert either a reflex excitatory or a reflex inhibitory effect, there probably exist other pathways in which the afferent activity may exert reflexly either an excitatory effect or an inhibitory effect, depending on the frequency of the afferent discharge.

Whether/

Whether the afferent input merely modifies inherent rhythmical activity in the gastric centres or whether the centres are actually 'driven' by the afferent input is worth considering further. The requirement of decerebrate sheep and goats for a continuous excitatory afferent input to activate or 'drive' the gastric centres has been established by the experiments of Iggo (1951, 1955) and Titchen (1953, 1958). In decerebrate preparations devoid of cyclical movements in the reticulo-rumen, such movements could frequently be evoked by reticular distension, stretching the reticulo-ruminal fold, acidifying the abomasal contents or centripetal electrical stimulation of a vagal nerve branch. Likewise, in the present experiments on halothane-anaesthetized sheep, the level of the excitatory afferent input (from reticular tension receptors in particular) was shown to be a limiting factor in the activation of the gastric centres. Gastric movements (Section A) and efferent gastric vagal activity (Section D) were abolished, if

- (a) intrareticular tension was very low (Sections A and D),
- (b) excitatory afferent pathways were blocked (Section E) or
- (c) intrinsic movements of the gastric musculature were reduced, and hence the afferent activity from 'in series' tension receptors was reduced, by the use of drugs, e.g. adrenaline (Section F).

Conversely, in these preparations, cyclical movements of the reticulo-rumen and the associated discharges in gastric efferent units could often be evoked by

- (a) distending the reticulum (Sections A and D),
- (b) acidifying the abomasal contents (Sections A and D),
- (c)/

- (c) restoring vagal nervous transmission by removing a cold block  
(Section E)
- (d) increasing activity at those peripheral sites which have excitatory 'feed-back' connexions with the gastric centres, e.g. by using certain drugs (Section F) and by electrically stimulating vagal nerve branches peripheral to a sectioned or blocked region (Section E)
- (e) by increasing the afferent input through electrical stimulation of vagal nerve branches central to a sectioned or blocked region (Section E).

It is quite clear that a minimum level of excitatory afferent input is required to activate the gastric centres in both decerebrate and anaesthetized preparations. It seems unlikely that this input is required in these preparations merely to compensate for the depression caused by decerebration or by anaesthesia. The standard conditions (Section A), under which cyclical movements are usually present in the halothane-anaesthetized sheep (i.e. an intrareticular volume of about 500 ml and a pressure of about 10 mm Hg), are similar to those found under normal conditions in conscious sheep and, which presumably provide a similar drive, because, even in some conscious sheep, total removal of reticulo-ruminal contents abolishes cyclical movements (Ash & Kay, 1959).

Two other hypotheses to account for the mechanism by which the complex sequence of movements is co-ordinated are worth considering in the light of the present investigation. One hypothesis assumes that the afferent discharge increment arising from tension receptors during the active part of the contraction cycle in the reticulum may trigger the emission from the gastric centres  
of/

of the efferent discharges to the rumen. Several pieces of evidence oppose this idea, that the sequence of contractions is based on serial feed-back triggering-pulses, e.g. (a) ruminal contractions still occur even though reticular contractions have been abolished by local anaesthetization of either the reticular mucosa (Ash & Kay, 1959) or the ventral abdominal vagal trunk (Stevens & Sellers, 1959) and (b) Type IV (i.e. ruminal) efferent gastric vagal discharges were still recorded in the present experiments both after the gastric contractions had been abolished by blocking agents (T.E.A. or probanthine HCl) and after the afferent discharge increment had been largely prevented by causing the reticulum to contract under isotonic conditions. Rather than acting as a trigger for the ruminal contraction, the afferent discharge increment elicited by an isometrically-recorded reticular contraction was observed to cause a delay in the onset of the Type IV (i.e. ruminal) discharge and a reduction in its parameters.

Another hypothesis has been postulated by Morrison & Habel (1964), who concluded that co-ordination is a peripheral function attributable to 'co-ordinating centres' in the region of the reticular groove, from which multisynaptic post-ganglionic pathways spread through the rumen wall. This conclusion is based, firstly on histological evidence, that there is a dearth of preganglionic fibres in the rumen, and, secondly, on pharmacological evidence, that ganglionic blocking agents are more effective at low concentrations on the rumen than on the reticulum. From this hypothesis of 'peripheral co-ordination' one might deduce, that ruminal movements in particular would have a stereotyped sequence, whereas, in fact, this is not the case. The sequence/

sequence is quite different for primary cycles (i.e. caudally directed movements) and for secondary cycles (i.e. cranially-directed movements with no involvement of the cranial sac). Furthermore, the pattern, the time relationships and the contraction durations for different parts of the sequence varies according to certain conditions, e.g. whether the animal is feeding, ruminating or resting and whether these occur immediately after a meal or after a period of starvation (p. I 36 et seq). Three more pieces of evidence which oppose the hypothesis of a peripheral co-ordinating centre are provided by the present experiments:

- (a) by cooling the cervical vagus it was possible to block differentially the afferent activity in fibres innervating the reticulum, whilst leaving those to the rumen largely unaffected (Fig. E 1). This means that the reticulum and rumen are both innervated and activated independently,
- (b) from recordings of efferent gastric activity described in Section C, at least 7 different types of efferent units were found, including 2 types (Types I and IV) in which the discharges were clearly related to the form, amplitude and duration of the reticular and dorsal ruminal sac contractions respectively, whereas, if a peripheral co-ordinating centre were to exist, it might be expected that the efferent gastric vagal discharge would take the form of a relatively simple and undifferentiated triggering pulse,
- (c) the reticular groove, which normally undergoes a biphasic contraction coincident with that of the reticulum, is shown in Fig. B 5H to undergo  
a/

a biphasic contraction at a time when the reticulum undergoes a monophasic contraction: a situation which seems extremely unlikely, if a single peripheral co-ordinating centre were to exist.

In halothane-anaesthetized sheep, the predominant afferent drive to the gastric centres has its origin in tension receptors, especially those of the reticulum. Tension receptors are 'in series' with smooth muscle cells and, together, these are 'in parallel' with fibrous elements of connective tissue in the wall of the forestomach. The tension developed in the reticulum by distending a balloon is passive and is due to the elasticity of fibrous elements. Because the tension receptors are 'in parallel' with these elements, receptor activity is largely independent of overall reticular tension, except in so far as distension affects the intrinsic motility of muscle cells 'in series' with the receptors. The activity of tension receptors is determined by the tension developed locally by the smooth muscle cells and the results of experiments described in Sections B and F emphasise the relationship between tension receptor activity and intrinsic contractions of smooth muscle cells. The contractions appear to be mainly myogenic, although they may be influenced by other factors, such as local or intrinsic neurogenic activity and humoral substances, e.g. hormones, metabolites, electrolytes and drugs. Due to the absence of any resting discharges in recordings made from efferent gastric vagal units (Section C), intrinsic motility is probably independent of tonic extrinsic nervous activation and this view is consistent with the further observations, that intrinsic movements were unaffected by block of vagal nervous transmission, using drugs (Section/

(Section F), or by nerve cooling or section (Section E). The lack of dependence of tension receptor activity upon passively developed reticular tension is shown most clearly by a comparison of the afferent discharges arising from tension receptors after the administration of carbachol and of adrenaline (Section F). In both cases the reticular pressure returns to its previous level after an initial period of contraction; thereafter, receptor activity is much increased after carbachol, whereas it is absent after adrenaline. The inferences are that intrinsic movements are increased by carbachol and inhibited by adrenaline, and that these movements, under most conditions, make no contribution to the tensions recorded by the intrareticular balloon. The effect upon afferent gastric activity observed when the reticulum is distended probably arises indirectly as a result of distension causing the smooth muscle cells to be lengthened and to increase their intrinsic motility.

Besides the tension receptors discussed above, there exist receptors in the lips and the floor of the reticular groove and in the floor of the omasal canal, whose properties are a combination of those described for tension receptors and for slowly-adapting pressor receptors. These receptors may be involved in reflexes which are quite different from those subserved by the tension receptors described above.

In the present experiments, no mechanoreceptors were located to the superficial regions of the reticular mucosa and few receptors were detected in the caudal and ventral regions of the rumen. Technical reasons may account for this, e.g., if the afferent fibres had small diameters (i.e. C fibres).

Iggo/

Iggo (1958) has shown that vagal nerve trunks at the level of the diaphragm contain many non-myelinated fibres, although the proportion of these (in relation to small myelinated fibres) is less in ruminant animals than in non-ruminant animals. Moreover, some of the fibres which are non-myelinated at the level of the diaphragm are myelinated in the upper thoracic and cervical regions. C fibres would have been difficult to isolate for purposes of measuring their conduction velocities and this may account for the absence of such fibres from conduction velocity measurements given in Table B1 and Fig. B1. I feel, however, that it ought to have been possible to detect C fibre activity even in fine multi-unit strands and that this technical difficulty alone does not account for the absence of units ascribable to the innervation of mucosal receptors and receptors in the caudal and ventral regions of the rumen. The failure to demonstrate superficially-located receptors may have been because such receptors either do not exist or are not innervated by vagal nerve fibres. The former possibility is not ruled out either by the histological evidence of Hill (1959) that methylene blue stained structures were observed in the mucosal layer of the reticulo-rumen, since he used newborn lambs and kids and only assumed a receptor role for the structures, or by the physiological evidence of Ash & Kay (1959) that light stroking of the reticulo-ruminal mucosa in its cranial regions excited gastric and salivary reflexes since this stimulus may have activated receptors at a level deeper than they assumed. Alternatively, superficially-located receptors may form part of an intrinsic reflex, so that light tactile stimuli excite intrinsic movements/

movements of the reticulo-ruminal musculature and these movements, in turn, evoke or enhance activity in 'in series' tension receptors innervated by vagal afferent fibres.

The relative scarcity of afferent units innervating mechanoreceptors located in the more caudal and ventral regions of the rumen may be attributed either to technical factors (e.g., the use of an inadequate stimulus or the difficulty of recording from non-myelinated fibres) or to a low density of mechanoreceptors in these regions or to a combination of both possibilities. Assuming that technical factors alone do not account for this, the rarity of mechanoreceptors in the caudal and ventral regions is consistent with the observations of Ash & Kay (1959) that reflex effects on gastric movements and salivation were less readily evoked by mechanical stimulation of these regions than of the cranial regions. The above observation conflicts with the view of Stevens & Sellars (1959) that the reflexogenic area for the elicitation of secondary cycles and eructation is located in the caudo-dorsal blind sac. From the results of the present experiments, it seems likely that distension-sensitive receptors involved in secondary cycle movements and eructation may be situated more cranially in the rumen than was previously supposed. A reappraisal of existing literature also supports this conclusion, e.g., Weiss (1953) observed that secondary cycles were increased by raising the hind-quarters of sheep, Dougherty et al (1958) evoked eructation after ablating the caudal and ventral regions of the rumen and Stevens & Sellars (1959) observed that procaine block of the ruminal nerves was more effective in abolishing eructation than surface anaesthetization of the caudo-dorsal blind sac alone.

The/

The conclusions reached as a result of the present investigations are

that:-

- (1) halothane-anaesthetized sheep are suitable acute preparations in which to analyse electrophysiologically the reflex regulation of reticular and, to a lesser extent, ruminal movements
- (2) in these sheep, movements are usually present for up to 19 hr: reticular contractions are approximately normal but ruminal contractions are often subnormal
- (3)/

- (3) standard conditions may be readily defined, so that results obtained at different times and from different sheep may be compared satisfactorily
- (4) noxious stimuli elicited by surgical and other manipulations inhibit gastric movements reflexly via the gastric centres
- (5) the majority of reticulo-ruminal mechanoreceptors are 'in series' tension receptors located in the muscle layers of principally the reticulum, the reticulo-ruminal fold and the cranial sac (dorsal rumen). Their responses are slowly adapting.
- (6) receptors in the lips and floor of the reticular groove and in the omasal canal respond to pressure as well as during an isometric contraction. Their responses are also slowly adapting.
- (7) the 'resting discharge' from 'in series' tension receptors is intermittent or fluctuating and is largely determined by the intrinsic motility of smooth muscle cells. Reticular distension is one of the factors which enhances this intrinsic motility.
- (8) the afferent discharge developed during a contraction in Type A (i.e. reticular) units is greater under isometric recording conditions than under isotonic recording conditions.
- (9) the afferent discharge developed during a contraction in Type A units recorded under isometric conditions exceeds that which may be evoked passively by distension alone.
- (10) the afferent discharge developed during a contraction in Type B (i.e. ruminal) units is greater when recording conditions in the reticulum are isotonic than when they are isometric.
- (11)/

- (11) in halothane-anaesthetized sheep, the primary cycle contractions may be monophasic, biphasic or triphasic in the reticulum and reticular groove. The main contraction of the cranial sac and reticulo-ruminal fold reaches its peak 2-9 sec after the second peak of the reticular contraction.
- (12) the mean conduction velocity of afferent gastric fibres is 12.4 m/sec. The mean conduction velocities for afferent gastric fibres in the dorsal and in the ventral thoracic vagal trunks are 14.5 m/sec and 6.6 m/sec respectively.
- (13) efferent gastric fibres in the cervical vagi are preganglionic.
- (14) at least 7 different types of efferent gastric units exist. By relating their discharges temporally to movements of the reticulum and of the rumen, it was concluded that Types I,II and III occurred in fibres innervating the reticulum or associated structures, Type IV the rumen and Types V,VI and VII other gastric structures not yet identified. Apart from Type VII units, there was no 'resting discharge' in efferent fibres during the quiescent part of the gastric cycle.
- (15) the co-ordination of the complex sequence of primary cycle movements is a function of the 'gastric centres', through their ability to determine the parameters and the temporal interrelationships of efferent discharges in the various types of gastric units innervating different regions of the forestomach.
- (16) the total reflex time is not less than 1.3 sec for reticulo-reticular reflexes and not less than 2.1 sec for reticulo-ruminal reflexes.

The/

The central reflex time is not less than 370 msec.

- (17) afferent activity arising from 'acid receptors' in the abomasum may provide a tonic excitatory drive to the 'gastric centres' for the elicitation of reticulo-ruminal movements in halothane-anaesthetized sheep.
- (18) in halothane-anaesthetized sheep fed 'ad lib' up to the time of experiment, abomasal acid secretion appears to be adequate for reflexly evoking reticulo-ruminal movements. The addition of more acid to the abomasum is usually ineffective.
- (19) efferent fibres innervating the reticulum are distinct from those innervating the rumen, as shown by differential cold block.
- (20) electrical stimulation of vagal nerve branches peripheral to a region that has been sectioned or cold blocked causes reticulo-ruminal movements to be enhanced as a result of a reflex 'feed-back' mechanism.
- (21) electrical stimulation of vagal nerve branches peripheral to a region that has been sectioned or cold blocked usually causes reticulo-ruminal movements to be abolished for about 5 min and then to reappear with an increased rate and amplitude.
- (22) a minimum of one intact thoracic vagal nerve branch is necessary for excitatory afferent pathways carrying the input which 'drives' the gastric centres.
- (23) the dorsal thoracic vagal trunk carries efferent fibres, which innervate the reticulum and the rumen, and afferent fibres, whose overall effect on the gastric centres is excitatory.
- (24) the ventral thoracic vagal trunk carries efferent fibres, which innervate the reticulum but not the rumen, and afferent fibres, whose overall effect on the gastric centres may be either excitatory or inhibitory.
- (25)/

- (25) the effects of cold block applied to the left cervical vagus resemble those resulting from cold block of the ventral thoracic vagal trunk more than of the dorsal thoracic vagal trunk.
- (26) neostigmine exerts its effects at low concentrations by potentiating acetylcholine released by the efferent gastric discharges and at higher concentrations by increasing intrinsic smooth muscle motility probably through a direct stimulatory effect on ganglia.
- (27) T.E.A. and probanthine block efferent nervous transmission and abolish cyclical movements of the reticulo-rumen without appreciably affecting intrinsic motility, which is therefore myogenic rather than neurogenic in origin. The delayed reflex depression of efferent gastric vagal activity is probably due to block of tonic efferent activity to sites other than the reticulo-rumen which normally exert an excitatory 'feed-back' drive on the gastric centres.
- (28) Adrenaline evokes a local contraction of the gastric musculature and this<sup>is</sup> followed by a relaxation together with either peripheral inhibition of or refractoriness to efferent vagal discharges.
- (29) Carbachol induces a prolonged contraction of the gastric musculature, during which time there is either peripheral inhibition of or refractoriness to efferent vagal discharges.
- (30) high doses of pentagastrin (0.7 ug/kg) enhance intrinsic movements and reflexly abolish extrinsic movements of the reticulo-rumen.
- (31) in general, the effects observed after the administration of drugs are consistent with the conclusions given in (7), (32) and (33).
- (32)/

- (32) the tonic afferent input from 'in series' reticular tension receptors during the quiescent period of the primary cycle provides a reflex drive to the gastric centres and largely determines the rate, the duration and the amplitude of reticular and ruminal contractions.
- (33) the enhanced afferent input to the gastric centres, occurring during a reticular contraction recorded under isometric conditions, modifies the form, the amplitude, the duration and the delay in onset of the later parts of the contraction sequence of the reticulum and the rumen. The reflex effects upon the reticulum are predominantly excitatory at low initial levels of reticular tension, inhibitory at high levels and a combination of excitatory and inhibitory at moderate levels. The reflex effects upon the rumen are a reduction in the amplitude and in the duration of the contraction and a delay in its onset.

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APPENDIXData Processing

The data in Sections B and C subjected to statistical analysis was processed by the Edinburgh Regional Computing Centre KDF9 Computer using one or other of the following programs, written in 'Atlas Autocode' with data on punched tape.

Program A

This program is designed to calculate and print out in tabular form the mean, the standard deviation, the standard error of the mean and the number of values for an unlimited number of sets of data. Each set is terminated with a label '-1' and the final set with an additional label '-2'. Data containing the values '-1' or '-2' cannot therefore be processed by this program. Most lines carry an explanatory comment:

```

begin
integer n; comment n = number of values
real x,q,s,t; comment x = value q = sum of squares, s = sum of numbers
comment t = standard deviation
777: n = 0; q = 0; s = 0; t = 0; comment zeroing of counters
888: read (x); comment read the next value in the data
if x = -2 then stop; comment end of data reached
if x = -1 then → 999; comment end of set reached
n = n+1; comment count of number of values
q = q+(x * x); comment summing squares of data
s = s+x; comment sum of data
→ 888; comment jump to line labelled 888
999: if n < 30 then t = sq rt ((q - ((s * s)/n))/(n - 1))
if n > 30 then t = sq rt ((q - ((s * s)/n))/(n))
comment S.D. is calculated = t
comment below are print out instructions for each set of computed data
caption # MEAN
print (s/n,3,3)
caption # S.D.
print (t,3,3)
caption # S.E.
print (t/sq rt (n),3,3)
caption No. OF VALUES
print (n,3,0)
→ 777; comment prepare for next set of data
end of program

```

Program B

This program is designed to calculate and print out in tabular form the mean, the minimum value, the maximum value, the standard deviation, the standard error and the number of values together with the title of each set of results. In addition a 't test' can be requested at any stage and for any two sets of data.

The program is written in the form of 'routines', so that writing subsequent programs may be facilitated by employing these tested routines.

The end of the title is denoted by an asterisk 'x', then follows a number between 1-30 used to label that set of data, the data and finally '-1' to terminate the set. A 't test' may be requested at any stage by punching a title ending with 'x', followed by '-3' (which calls in the 't test' routine) and the labelling numbers of the two sets of data. The data is terminated with the label '-2'.

```

begin
real x, min, max, q,p,s,t
integer n, h, a, b, c, i
integer array title (1:30)
integer array na (1:30)
array pa (1:30)
array va (1:30)
routine spec TL
routine spec TP
routine spec TST
routine spec VP
routine spec VR
caption n MEAN ssssss MIN sssss
caption MAX ssss S.D. ssss S.E. sssss NO.
15: n = 0; p = 0; q = 0; min = 10@6; max = 0
TL
read (a)
if a = -3 then -> 44
if a ≠ -3 then -> 55

```

```

44: TST
    → 15
55: VR
    → 15
    routine TL; comment storage of title
    h = 1
13: read symbol (i)
    if i = 'x' then → 13
    if i = -2 then stop
    TITLE (h) = i
    h = h+1
    → 13 unless i = 'x'
    end
    routine TP; comment print out of title
    h = 1
33: print symbol (TITLE (h))
    h = h+1
    → 33 unless TITLE (h) = 'x'
    end
    routine TST; comment routine for 't test'
    read (b)
    read (c)
    s = sq rt ((va(b) + va(c))/(na(b) + na(c)-2))
    t = ((pa(b)/na(b)) - (pa(c)/na(c)))/(s + sq rt ((1/na(b)) + (1/na(c))))
    newline
    spaces (24)
    caption SERIES  $\hat{s}$ 
    print (b,2,0)
    space
    print (c,2,0)
    spaces (3)
    TP
    caption  $\hat{T} = \hat{s}$ 
    print (T,3,3)
    space
    caption DEGREES OF FREEDOM
    space
    print (na(b) + na (c)-2,3,0)
    end
    routine VP; comment print out sequel to VR
    va (a) = q - ((p * p)/n)
    na (a) = n
    pa (a) = p
    newline
    print (p/n, 3, 2)
    space
    print (min,3,2)
    space
    print (max,3,2)
    space

```

```

print (sq rt (va(a)/(n-1)),2,3)
space
print ((sq rt (va(a)/(n-1)))/(sq rt (n)),2,3)
space
print (n,3,0)
spaces (3)
TP
print (A,2,0)
end
routine VR; comment preparation of variance calculation
20: read (x)
if x = -2 then stop
if x = -1 then → 22
n = n+1
q = q+(x * x)
p = p+x
if x min then min = x
if x max then max = x
→ 20
22: VP
end
end of program

```

#### Publications

- (1) Leek (1963)
- (2) do (1966)
- (3) do (1967)
- (4) Iggo & Leek (1966)
- (5) do (1967a)
- (6) do (1967b) - in the press

### Single unit activity in cervical vagal efferent axons associated with reticulo-ruminal movements

By B. F. LEEK. *Department of Veterinary Physiology, University of Edinburgh*

Anaesthesia of the sheep is induced with halothane, and after endotracheal intubation this is maintained with a controlled mixture of halothane and oxygen by a closed-circuit method. Reticular distension is provided by a 1 l. rubber bag inserted into the reticulum through its ventral pole, exposed by laparotomy. The same balloon is used for recording reticular pressure, kymographically by a Marey tambour and photographically by a transducer manometer and oscilloscope. A small balloon placed in the dorsal sac of the rumen records ruminal activity, which is displayed kymographically. Electrical activity in preganglionic vagal axons is recorded from small strands of the left cervical vagus, which is undisturbed except for the fascicle from which the bundles of axons are dissected. The right vagus is intact. Electrical stimulation of the left vagus to elicit reflex or direct contractions of the stomach is obtained by a pair of electrodes placed proximal or distal to the recording electrodes. The electrophysiological techniques are based on methods described by Iggo (1955, 1956).

At the operative level of anaesthesia (surgical stage, plane two) reticulo-ruminal contractions are usually absent. Apparently normal reflex diphasic reticular contractions can be elicited by reticular distension, by acidification of the abomasal contents with N/5 HCl or by a combination of both procedures. The reflex excitability of the preparation is greater, and can be maintained more readily for long periods, than with the decerebrate preparations previously preferred for this kind of work.

Several distinctive patterns of unitary efferent vagal activity associated with gastric contractions have been observed. There are two principal types. (1) Activity which appears before any mechanical change is detectable in the reticulum. This discharge increases during about 2 sec to reach a peak, falls off slightly and then increases again to a second higher maximum frequency. The contraction has a similar shape, with each pressure peak occurring about 2 sec after the efferent action potentials' maxima. This efferent discharge, for various reasons, is considered to cause the reticular contraction. (2) The second pattern typically begins in other efferent fibres about the peak of the reticular contraction and persists for several seconds after it. It probably causes the ruminal contractions. The patterns of discharge can be modified reflexly, as will be demonstrated. A dissociation of vagal efferent activity and gastric movements

can be obtained by increasing the depth of halothane anaesthesia—the efferent activity continues but the movements disappear. This effect is caused by the peripheral autonomic block caused by halothane, and it is interesting that the gastric centres are still actively discharging under these conditions.

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## THE REFLEX REGULATION OF RETICULO-RUMINAL MOVEMENTS

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The stomachs of ruminant animal undergo two distinctive cycles of motility termed *primary and secondary cycles* (SCHALK and AMADON, 1928). The primary cycle consists of an active phase lasting 15-25 secs recurring regularly approximately every minute, and is composed of a biphasic contraction of the reticulum, followed by a contraction of the anterior and posterior ruminal pillars, then of the dorsal ruminal sac and finally of the ventral ruminal sac. Interspersed between some of the *primary cycles* are *secondary cycles* (WEISS, 1963; REID, 1963).

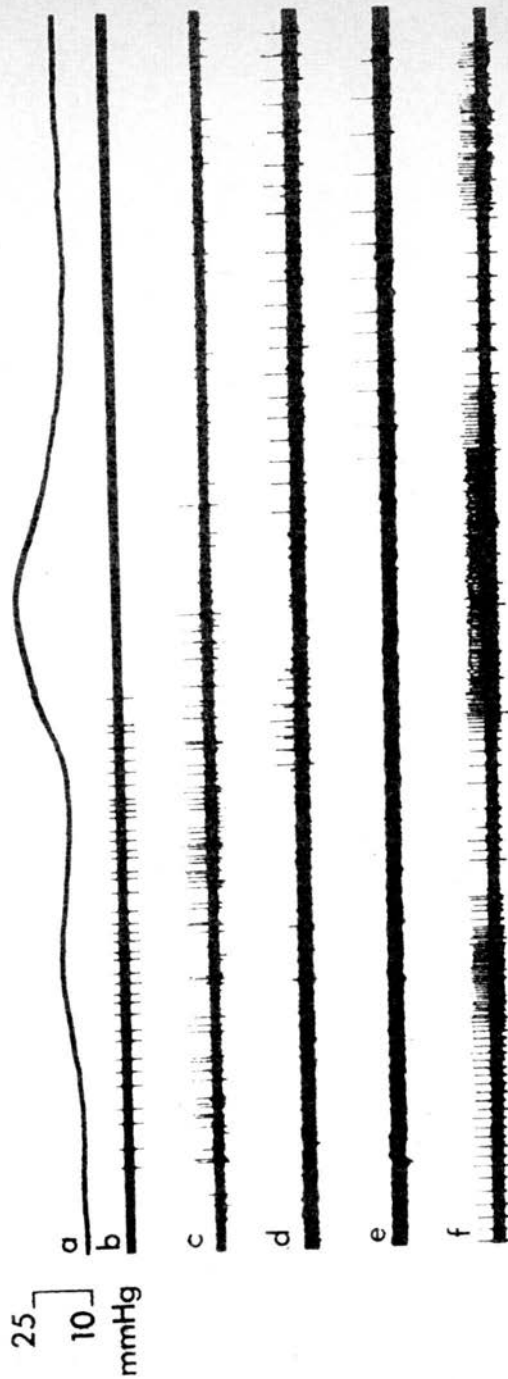
The reticulo-rumen is innervated by both vagal and splanchnic nerves but DUNCAN (1953) has demonstrated that apparently normal motility persisted after section of the splanchnic nerves whereas section of the vagi resulted in complete stasis. Therefore, the complex gastric motility cycles were dependant upon vagal activity and IGGO (1951, 1956), TITCHEN (1953, 1958 and 1960) and TITCHEN and REID (1965) have shown that they resulted from extrinsic reflexes some of which possessed both afferent and efferent pathways in the vagi. These reflexes were integrated in the central nervous system at the level of the hind-brain, as shown by brain-stem and spinal cord section (IGGO, 1951) and this has been confirmed by ANDERSSON *et al.* (1958).

The present investigations were concerned with determining the extent to which and the mechanism by which physical conditions within the reticulum reflexly influenced the movements of the reticulum and rumen constituting the *primary cycle*. The nervous activity in sensory vagal nerve fibres passing from tension receptors in the reticulum to the gastric centres in the medulla was repeatedly sampled by recording electrophysiologically from single afferent fibres dissected from the left vagus in the cervical region. Similarly the nervous activity in efferent (parasympathetic) vagal fibres passing from the gastric centres to the different regions of the reticulo-rumen was recorded and it was, therefore, possible to determine the relationship which existed between reticular tension and the nervous activity in both afferent and efferent nerves and to make certain inferences regarding reflex integration within the gastric centres.

The techniques involved the use of sheep maintained under closed-circuit anaesthesia with Halothane B. P. Whereas most anaesthetics abolished reticulo-ruminal movements, these movements were present or could be readily evoked in 95 % of the halothane anaesthetized sheep by moderately distending the reticulum by introducing 400-600 ml. of air into a large recording balloon inserted into the reticulum through an incision in its ventral pole. Balloons were also placed in the rumen, so that movements of the various compartements could be recorded manometrically using strain gauge pressure transducers. Reticular movements in the halothane anaesthetized sheep (fig. 1) were of a similar rate, form and amplitude to

FIG 1

1 sec



Examples of the nervous discharge in efferent and afferent gastric fibres in relation to the reticular contraction. Trace a) the biphasic pressure changes due to reticulum contracting isometrically; b) and c) efferent discharges in fibres presumed to be innervating the reticulum; d) an efferent discharge which pauses during the second contraction; e) an afferent discharge in a fibre presumed to be innervating the rumen; f) an afferent discharge due to a *slowly adapting, in series* tension receptor situated in the medial wall of the reticulum.

those in the conscious sheep but the amplitude of ruminal contractions was depressed. The method of recording from single vagal nerve fibres resembled that used by ICGO and VOCT (1960) to record from preganglionic cervical sympathetic fibres in the cat. With the sheep lying on its right side, the left cervical vagus was exposed by incising the skin for 10 cm. along the jugular groove and excising the left sternocephalic muscle. The edges of the skin incision were sutured to a large ring of solder to form a pool which was then filled with warm liquid paraffin B. P., to protect the exposed tissues. A narrow, rigidly held perspex dissecting plate was inserted under the nerve. Fine strands were dissected from the left cervical vagus and placed across a pair of fine Ag/AgCl wire recording electrodes carried on a micromanipulator. When recording afferent activity the fine strands were cut at the central end, and when recording efferent activity they were cut at the peripheral end. The electrical activity in the strands was amplified, displayed on an oscilloscope together with the manometric recording of reticulo-ruminal movements and photographed onto moving bromide paper. The basis for identifying afferent fibres innervating reticular mechanoreceptors was an alteration in their electrical activity when the reticulum was passively distended and or at the time of a reticular contraction. Similarly, efferent fibres innervating the reticulo-rumen were identified as such if the changes in their electrical activity bore a fixed temporal relationship to the active phase of each *primary cycle*. Usually the fine strands also contained electrical activity due to non-gastric fibres. It was necessary, therefore, to repeatedly sub-divide the strand and eliminate the latter until, ultimately, only a single active gastric fibre remained.

Afferent fibres innervating mechanoreceptors in the wall of the reticulum were found to have conduction velocities of 6-14 m/sec which was equivalent to a fibre diameter of 1.2-3 $\mu$  (assuming a conversion factor of 1/6). All the receptors examined so far have given a *slowly-adapting* discharge linearly proportional to the tension in the reticulum during the inactive phase of the *primary cycles* and a discharge which increased in frequency when the reticulum contracted spontaneously (isometrically) and when it was passively distended by insufflation. Therefore, these receptors were *in series* with the contractile elements in the reticular wall, as was also observed by ICGO (1955, 1956). Often it was possible to locate the position of the receptive field by inserting the hand through a rumenotomy incision and manually exploring the surface of the reticulum, where upon the afferent discharge recorded in a single fibre was increased by stretching or pinching the appropriate region of the wall. The receptive fields were 1-2 sq. cms. in area and were located mainly around the ventral part of the oesophageal groove, and in the medial and caudal walls of the reticulum.

The afferent discharge of a typical reticular tension receptor is shown in fig. 1 f. There was no discharge when the reticular balloon contained only 300 ml. air at a pressure of 7.5 mm. Hg. When the balloon was further distended, the discharge appeared and increased linearly at a rate of 0.7 impulses/sec/mm. Hg. rise in intrareticular pressure. When the recording system was arranged so that a reticular contraction caused rises in pressure with little change in volume (i. e., an isometric contraction) the peak frequencies of the afferent discharge were proportional to the first and second peak pressure developed by the biphasic reticular contraction. Conversely, when the recording system was arranged so that a reticular contraction caused a reduction in the volume of its contents with little rise in pressure i. e., an isotonic contraction the afferent discharge showed only a slight increase. Similarly, when the contraction were abolished by administering drugs which block ganglionic (Tetraethylammonium chloride) and post-ganglionic transmission (Probanthine hydrochloride), the afferent discharge remained unaltered.

The efferent discharge in gastric motoneurons has been recorded by LEEK (1963) using the *single fibre* technique, BECHELLI *et al.* (1963) using micro-electrodes inserted into the gastric centres and by DUSSARDIER (1957) using an indirect method. The efferent fibres have conduction velocities of 1-6 m/sec (ICCO, 1956). The *single fibre* technique entailed cutting the nerve strand distal to the recording electrodes. It was, therefore, impossible to subsequently locate the region innervated by efferent fibres whose electrical activity was being recorded. Eighty-five single units showing eight distinctive efferent discharge patterns have been recorded and, by comparing their temporal relationship with the sequential contractions of various regions of the reticulo-rumant, it was possible to deduce their probable sites of innervation (fig. 1 b-e). Efferent fibres innervating the reticulum and rumen possessed no discharge during the inactive phase of the *primary cycle*, nor when the intrareticular pressure fell below the threshold value (about 8 mm. Hg. necessary to reflexly evoke contractions).

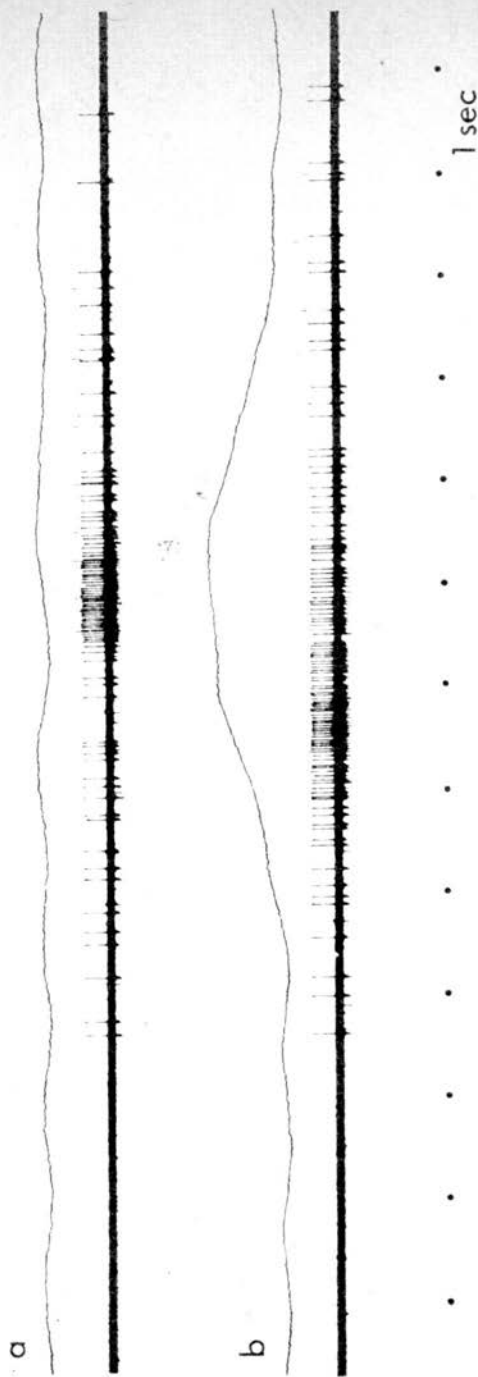
The reflex effect of withdrawing air from the reticular balloon during the inactive phase was to reduce the rate and amplitude of the reticular contractions and to reduce the number of impulses, peak frequency and duration of the efferent discharge. Conversely, insufflation of the reticular balloon up to about 1,000 ml. reflexly increased the rate and amplitude of the reticular contractions and also the number of impulses, peak frequency and duration of the efferent discharge. Further insufflation caused initially a reduction in the amplitude of the reticular contractions and in the efferent discharge, followed later by a reduction also in the rate of reticular contractions.

Efferent discharges recorded during isometric and isotonic conditions were compared (fig. 2). In contrast to the isotonic record the *isometric one* had more nerve impulses and a peak frequency which was earlier, lower and longer lasting. Similar effects were observed before and after reticular contractions had been abolished by Probanthine hydrochloride or Tetraethylammonium chloride. Therefore the afferent discharge elicited by the isometric reticular contraction ad a reflex excitatory effect on the overall number of impulses and the rate of attainment of the peak frequency but a reflex depression of its magnitude. The reflex time occupied 3-4 seconds. Comparable effects were recorded when 200 ml. air was suddenly removed from or added to the reticular balloon at the commencement of a contraction. In the former case the number of impulses was much reduced although the peak frequency was higher but of shorter duration. Conversely in the latter case, the number of impulses was much increased and the peak frequency of longer duration. At low initial volumes the sudden insufflation caused an increased peak frequency, at moderate initial volumes (600-1,600 ml.) it caused a reduced peak frequency and at more extreme initial volumes it practically abolished the entire discharge. Therefore, it appeared that in addition to the low threshold of reticular tension (300 ml. 8 mm. Hg.) above which there was reflex excitation there existed a high threshold (1,000 ml. 20 mm. Hg.) above which reflex inhibition occurred. The latter was reached by extreme distension of the intrareticular balloon and also during isometric contractions.

From the results obtained so far it seems justifiable to propose tentatively the following hypothesis regarding central nervous integration:

a) the gastric centres are dependant upon the drive provided by a continuous or *tonic* afferent input from reticular tension receptors which thereby determines the rate and amplitude of reticulo-ruminal contractions,

FIG 2



Reflex modification of the efferent discharge by tension developed during the reticular contraction. Trace a) shows an isotonic contraction with no rise in intrareticular tension (upper trace) and the corresponding efferent discharge (lower trace). Trace b) shows the same efferent unit discharging (lower trace) when the reticulum contracts isometrically (upper trace) so that intrareticular tension rises. In b) the peak frequency is reached earlier, has a longer duration but a lower magnitude, due to reflex effects caused by the initial rise in tension.

b) the afferent discharge developed by the first part of a biphasic isometric reticular contraction reflexly modifies the amplitude, form and duration of the second part.

c) the afferent discharge developed by the second part of the biphasic reticular contraction probably reflexly influences the contraction of the rumen, which reaches a peak about 5 seconds after the former, as this is equal to the reflex time for reticulo-ruminal reflexes.

## SUMMARY

A *single fibre* method of recording afferent and efferent impulses in nerves innervating the reticulo-rumen is described using Halothane anaesthetized sheep. The location and properties of tension receptors in the reticular wall is discussed and also their effect on the gastric centres and reflexly on the efferent discharges responsible for reticulo-ruminal movements.

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## Reflex Regulation of Gastric Activity

By

A. Iggo and B. F. Leek

With 5 Figures

The reflex regulation of gastric motility is very highly developed in ruminant animals, and the principles of the central nervous regulation which are revealed by experimental investigation of such species are probably, with appropriate modifications, of general application. The complex sequences of gastric (reticular and ruminal) contractions that occur in the normal mixing and propulsive movements of the fore-stomach, in the removal of gas by eructation and in the regurgitation of ingesta for remastication are entirely dependent on the vagi. If these nerves are blocked or cut in conscious animals the normal propulsive reticular and ruminal movements cease (*Popow, Kudrjavcev and Krasausky, 1933; Duncan, 1953*). This disappearance arises directly from the interruption of the efferent vagal axons that provide the motor innervation of the stomach. The axons arise in motoneurons in the gastric motor centres in the medulla which can function in the decerebrate animal to give an apparently normal cycle of gastric movements (*Iggo, 1951, 1956; Titchen, 1958*). Reflex discharge of the gastric motoneurons can be elicited by appropriate and effective natural stimuli such as distension of the stomach and acidification of the abomasal gastric contents (*Titchen, 1958*).

Electrical records made from single gastric vagal afferent fibres show that in the sheep and goat a discharge of impulses is elicited by distension of the reticulum (*Iggo, 1955*) and that as the distension is increased the rate of firing in the afferent fibres is enhanced. During a gastric contraction the discharge increases still further (Fig. 1). Electrophysiological and histological work has established that reflex excitation of the medullary gastric centres is caused by these "in series" stretch receptor afferent units. There is no published work on acid-sensitive gastric receptors in ruminant animals but they have been described in the cat (*Iggo, 1957*) and it is likely that similar slowly-adapting afferent units underly the reflex response to acidification of gastric contents in ruminant animals.

The excitatory afferent fibres travel in the vagi and electrical stimulation of the central cut end of one cervical or ventral thoracic vagus or of an abdominal branch of the vagus will elicit a reflex contraction of the reti-

culum and/or rumen, so long as the contralateral vagus is intact to provide an efferent pathway. The exact role of the afferent fibres in determining the reflex discharge of the gastric vagal motoneurons cannot, for technical reasons, be established by de-afferentation of the stomach or by interfering differentially with the conduction of impulses in the vagus because both the afferent and efferent fibres of the gastric reflexes travel in the vagus and the axons are of similar diameters (Iggo, 1956) and there is no separation of the axons into afferent and efferent roots on entry into the medulla. It follows that any interference with one set of fibres will also alter the activity of the others. Thus in acute experiments, block of one cervical vagus causes a reduction in the amplitude of the contractions. This is due partly to a reduction in the number of motor impulses reaching the stomach because

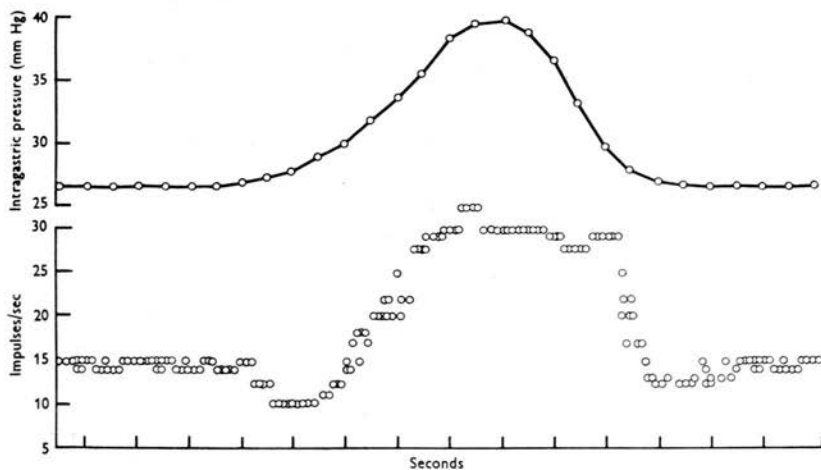


Fig. 1. The discharge of impulses in a slowly-adapting gastric vagal afferent fibre (o o o: lower graph) during sustained distension of the reticulum in a goat. The frequency of this discharge was about 15 imp./sec. This distension elicited a reflex reticular contraction during which the reticular pressure (o—o) rose, and caused a further increase (to 30 imp./sec.) in the frequency of discharge in the afferent fibre (modified from Iggo, 1955). Pentobarbital anaesthesia.

some of the efferent fibres are blocked, and partly to a weaker discharge of the intact gastric vagal motoneurons because the excitatory afferent inflow from the stomach is reduced since the afferent axons in one cervical vagus are blocked.

A solution to this problem is to record from the efferent vagal fibres. One interesting attempt was made by *Dussardier* (1958) who anastomosed the central end of one cut thoracic vagus with the peripheral cut end of one phrenic nerve and, after allowing time for nerve regeneration, recorded electro-myographically from the diaphragm. In this way he was able to record muscle action potentials coincident with gastric contractions, and showed that there was repetitive discharge in muscle units. In some work in progress in Edinburgh (*Leek*, 1963) we are recording directly from pre-ganglionic vagal motor fibres in the cervical vagus. In these experiments the

sheep are under continuous halothane anaesthesia and regular reflex contractions of the reticulum, and sometimes of the rumen, can be elicited by distension of the reticulum and/or acidification of the abomasal contents. Both the intensity of the contractions and their frequency can be modified reflexly, as described by Iggo (1956) and Titchen (1958).

*Discharge of single gastric vagal motoneurons.*

Single unit recording from vagal motor nerve fibre has established that some vagal motoneurons have a distinctive pattern of discharge in relation to the gastric contraction. Fig. 2 illustrates characteristic and stable types of discharge in several units examined under comparable standardised isometric pressure conditions. They probably represent different kinds of moto-

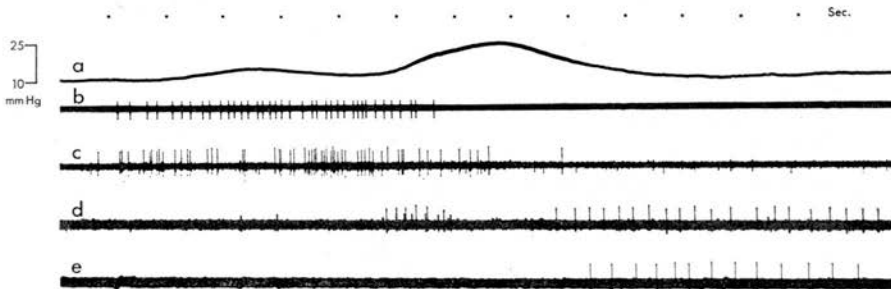


Fig. 2. (Sheep. Halothane anaesthesia.) The discharge of impulses in medullary preganglionic motoneurons. The upper trace a) shows the intra-reticular pressure changes during a reflex contraction, recorded under isometric conditions. The other traces b, c, d and e are records of the discharge of impulses in fine strands dissected from the cervical vagus. b) and c) are motor units that discharge early in the reticular contraction, and are presumed to cause the contraction. e) is a unit with a low frequency late discharge and is presumed to be a ruminal motoneurone. d) is particularly interesting since it shows several units. The very small action potentials are from a unit similar to c) with an early discharge in two parts and clearly related to the reticular contraction. The largest action potentials in d) have a late discharge similar to e) associated with ruminal contractions. The time marks are at 1 sec. intervals at the top.

neurons that innervate either different parts of the stomach or go to different elements in the same part of the stomach. There are several common features; the absence of background discharge, a variation in frequency of discharge to a peak of up to 60 impulses/sec. in some units and, in some units, two or more periods of high frequency activity. These features are all seen most clearly in Fig. 2 b and c. There is no background discharge, the discharge first appears at low frequency and rises rapidly to 25 impulses/sec. and finally falls quickly and eventually disappears. The reticular contraction is clearly related to this discharge and presumably similar units are causing the contractions. The interval between the main burst of action potentials and the maximal contraction pressure has varied in different units from 2 to 3 sec. Part of this delay will be conduction time in the motor axon but the greater part is intramural, probably in the muscle coats. In addition to the kinds of unit in which the discharge is clearly related to the reticular contractions (e. g., Fig. 2 b, c and Fig. 3) there are several other patterns,

examples of which are shown in Fig. 2 d and e. These types of discharge were found less often and are probably recorded from ruminal motoneurons. In general the frequency of discharge in this latter kind of unit has been less than 3 impulses/sec., there has been less tendency for the discharge to reach a high frequency peak and there has been a longer period of discharge. These features are typical of ruminal contractions, which in the normal primary gastric contraction cycle a) follow the double reticular contraction after a few seconds and b) are slower to develop and last longer than the reticular contractions. These results imply that the various parts of the stomach are innervated by different motoneurons.

*Reflex modification of gastric vagal motoneuronal discharge.*

In the experiments reported here the sheep were anaesthetized and there were no gastric contractions in the absence of an appropriate stimulus.

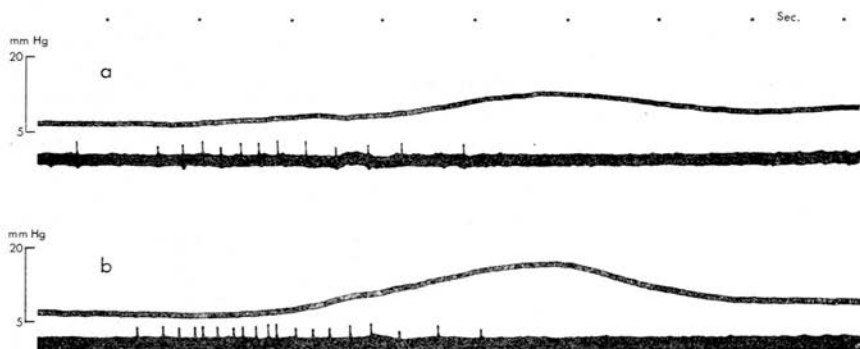


Fig. 3. The effect of acidification of the gastric contents on the discharge of a reticular vagal motor unit. After the upper a) control reflex reticular contraction was recorded, the abomasal contents were acidified by the addition of 50 ml 0.2 N HCl and 90 sec. later the lower record b) was taken. A distinct enhancement of the discharge in the motor unit is evident, but the pattern of discharge is the same, and the reticular contraction is stronger. The time marks, at the top, are at 1 sec. intervals.

Distension of the reticulum with 200—400 ml. air in a large balloon usually elicited a regular series of contractions, the amplitude and frequency of which were greater at the larger volumes of inflation. The contraction illustrated in Fig. 2 a was obtained in this way. Another method of eliciting the contractions, or of accelerating them, is to increase the acidity of the gastric contents (Titchen, 1958). When this is done (Fig. 3) the contractions are more frequent and larger, but of the same general form. The motoneuronal discharge, as might be expected, shows a more vigorous discharge but the general pattern of discharge in an individual unit was the same (Fig. 3 b). These kinds of gastric stimuli are excitatory for the gastric centres in a general way, leading to a greater or lesser degree of activity, but not modifying the general pattern of motoneuronal discharge.

Both the course of the gastric contractions and conditions within the stomach may modify the discharge of the gastric centres. One reason for

expecting "feed-back" during the contraction is that the distension-sensitive receptors that form the afferent limb of the reflex arc are excited still further by the gastric contractions they evoke (Fig. 1). We have examined some features of the reflex mechanism by altering the pressure/volume conditions in the stomach during the course of a contraction. The standard situation was to use *isometric* recording conditions, so that the volume of the gastric balloon did not change during a contraction and there was a rise in pressure of about 25 mm. Hg. In these conditions a substantial increase in the afferent inflow from the "in series" stretch receptors would occur (Fig. 1). A typical gastric motoneuronal discharge in these conditions is shown in Fig. 4 a. The recording conditions were then made *isotonic*, so that there was a large decrease in volume during a contraction and only a slight rise in pressure (Fig. 4 b). The distension which initiated the contraction was the same in both conditions. The reflex motoneuronal discharge was much

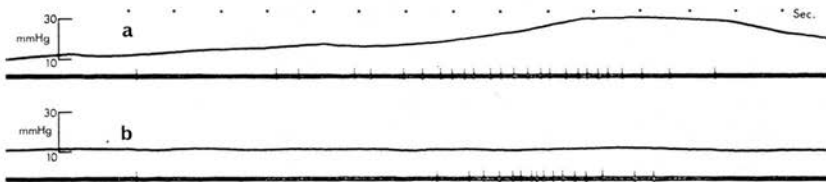


Fig. 4. The effect of "feed-back" on the discharge of the medullary gastric centres. The upper record a) illustrates a reflex reticular contraction recorded under the standard 'isometric' conditions. During the contraction on the incompressible balloon containing 300 ml. the pressure rose to a peak of 28 mm. Hg. The reticular recording conditions were then changed from "isometric" to "isotonic", arranged so that the basal reticular volume and pressure were identical. The next reticular contraction is recorded in b). This time the motor unit discharge shows a higher frequency discharge, and lacks the pronounced early discharge seen in a). During the contraction in b) about 200 ml. was expelled from the reticulum, but the reticular pressure did not rise more than a few mm. Hg. In a) there would be an increase in afferent inflow from the receptors in and about the reticulum (see Fig. 1) whereas in b) such a marked acceleration of the discharge would not occur. Conditions within the reticulum during a gastric discharge very clearly modify the form and pattern of the gastric motoneuronal discharge. Time in 1 sec. intervals at top of figure.

less in the isotonic conditions. It is clear that the gastric conditions during the contraction can modify the actual excitability of the gastric centre during a discharge. In the experiments just described the effect appears to be to adjust the discharge so as to prolong the contraction if there is an obstruction and accelerate the contraction if there is free movement of the contents.

Another method used to alter the gastric conditions was to inflate or deflate the reticular balloon after a normal gastric centre discharge had begun. A typical result is shown in Fig. 5. The sudden withdrawal of 100 ml. of air from the reticular balloon about 1 sec. after the first sign of a contraction abbreviated the motoneuronal discharge and brought the peak discharge earlier. The effect is to make the contraction shorter and sharper. The converse happens if the isometric distension is increased after a contraction has started.

These results establish that the gastric motoneurons in the medulla can be activated reflexly by general excitatory stimuli and that the discharge of the individual neurones can be modified reflexly by the events in the stomach during the course of their discharge. The detailed interrelations of the gastric motoneurons and the dependence of their orderly sequential discharge on feedback of information from the stomach are the subject of current work.

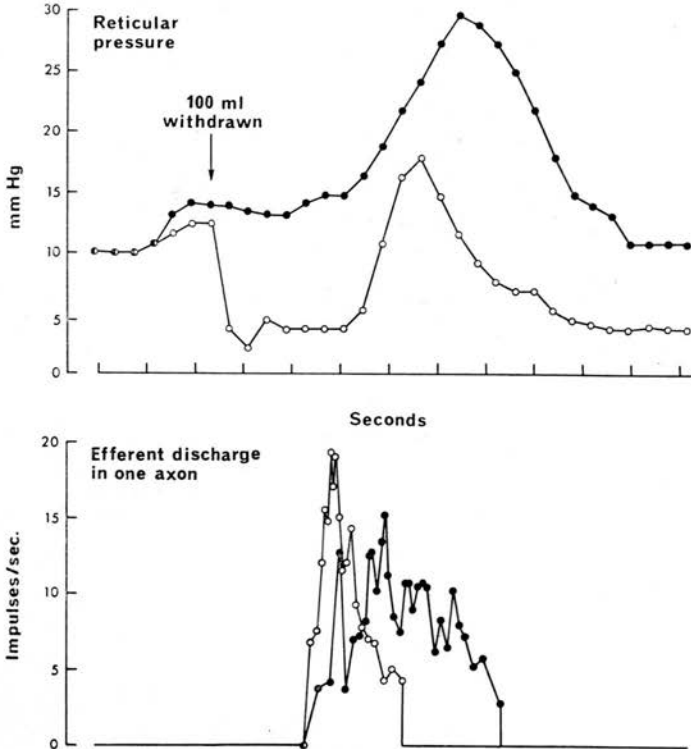


Fig. 5. "Feed-back" from the reticulum during a reflex reticular contraction. In this experiment a gastric motor unit which discharged before the major reticular contraction was recorded in the standard isometric conditions (reticular balloon containing 300 ml.) ●-●. During the next reticular contraction (○-○) 100 ml. of air were withdrawn from the balloon, as indicated in the upper graph. The gastric motor unit discharge started at the same time as in the previous cycle but was both accelerated and abbreviated.

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#### Summary

Impulses have been recorded from single vagal gastric motor axons in sheep during reflex gastric contractions. Several different patterns of activity associated with contractions of different parts of the stomach were found. Each unit had a stable and characteristic pattern of discharge. The discharge of a unit could be

modified by a general excitatory stimulus e. g., acidification of the gastric contents or by afferent "feed-back" from the part of the stomach that was contracting.

### Zusammenfassung

Impulsentladungen einzelner Axone des motorischen Vagusnerven wurden beim Schaf während gastrischer Reflexkontraktionen registriert. Verschiedene unterschiedliche Aktivitätsformen ließen sich als Begleiterscheinungen der Kontraktion einzelner Teile des Magens feststellen. Jede Einheit hatte eine gleichmäßige und charakteristische Entladungsform. Die Entladung einer Einheit konnte durch eine allgemeine Reizung modifiziert werden, d. h. durch Ansäuerung des Mageninhalts oder durch einen afferenten "feed-back" aus dem Anteil des Magens, welcher eine Kontraktion aufwies.

### Résumé

Les auteurs ont enregistré les impulsions déchargées par des axones isolés du nerf vague moteur gastrique pendant les contractions réflexes gastriques chez le mouton. Ils ont pu identifier des types de réactions électrographiques différents suivant les différentes parties de l'estomac en activité. La réaction électrographique de chaque unité est caractéristique et stable. La décharge de l'unité peut être modifiée par une excitation générale, par exemple par acidification du contenu de l'estomac ou par «feed-back» provenant de la partie de l'estomac en contraction.

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AN ELECTROPHYSIOLOGICAL  
STUDY OF SINGLE VAGAL EFFERENT UNITS ASSOCIATED  
WITH GASTRIC MOVEMENTS IN SHEEP

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SUMMARY

1. A method for obtaining reticulo-ruminal movements for up to 19 hr in halothane-anaesthetized sheep is described. The duration, wave form and frequency of the movements resembles those in the conscious animal except that ruminal movements have a lower amplitude.

2. A method of recording for up to 5½ hr single efferent unit discharges from fibres presumed to innervate the reticulo-rumen is described.

3. At least seven distinguishable types of discharge pattern were recorded. By relating these temporally to movements of the reticulum and rumen it was concluded that Types I, II and III occurred in fibres innervating the reticulum or associated structures, Type IV the rumen and Types V, VI and VII other gastric structures not yet identified. Apart from Type VII units there was no 'resting discharge' in efferent fibres during the quiescent phase of the gastric cycle.

4. We conclude that the co-ordination of the complex sequence of gastric movements in ruminant animals is a function of the 'gastric centres' in the hind-brain through their ability to determine the pattern, durations, spike frequencies and temporal interrelationships of discharges in gastric motoneurons innervating the different parts of the forestomach.

INTRODUCTION

Two distinct cycles of movements are now recognized in the forestomachs of ruminant animals and have been termed 'primary and secondary cycles' (Schalk & Amadon, 1928), 'backward-moving and forward-moving ruminal contractions' (Weiss, 1953), 'mixing and belching cycles' (Reid & Cornwall, 1959) and 'A and B sequences' (Reid, 1963). Most attention has been given to the reticulum and rumen, although simultaneous movements of the omasum and abomasum also occur

(Brunaud & Dussardier, 1953; Ohga, Ota & Nakazato, 1965). The primary cycle consists of a biphasic reticular contraction followed by contraction of the cranial and caudal ruminal pillars, then of the dorsal ruminal sac and, finally, of the ventral ruminal sac. The contraction phase of the cycle lasts 15–25 sec and recurs regularly after an inactive period of 45–75 sec. The duration of these phases is influenced by a number of factors such as the nature of the diet, interval since feeding, and whether the animal is ruminating or eating (Schalk & Amadon, 1928; Phillipson, 1939; Balch, 1952; Reid, 1963). Secondary cycles of contraction are interspersed among the primary cycles and may be accompanied by eructation. The secondary cycle consists of a contraction of the caudo-ventral ruminal sac (Reid, 1960, 1963), followed by a 'forward-moving contraction' of the dorsal ruminal sac and then a contraction of the ventral ruminal sac (Weiss, 1953; Reid & Titchen, 1965).

The complex gastric movements are dependent upon reflexes with afferent and efferent pathways in the vagi (Iggo, 1951, 1956; Duncan, 1953; Titchen, 1953, 1958, 1960; Reid & Titchen, 1965). The reflex centre is in the hind-brain (Iggo, 1951; Andersson, Kitchell & Persson, 1959). Attempts have been made recently to record electrically the vagal efferent activity associated with reticulo-ruminal contractions in order to analyse the nervous control mechanisms that underlie the movements. Dussardier (1957, 1960) cross-sutured the vagus and phrenic nerves and recorded electromyographically the activity in the diaphragm initiated by vagal efferent fibres. He established that there is a variety of different kinds of vagal efferent fibres associated in some way with gastric contraction, a result which is confirmed by Beghelli, Borgatti & Parmeggiani (1963), who recorded with micro-electrodes from the dorsal motor-nucleus of the vagus.

The present investigation was started as the first step in an electrophysiological analysis of the reflex mechanisms that underlie reticulo-ruminal movements. In particular, a search was made for an experimental preparation which would allow the simultaneous recording of efferent vagal discharge and the application of a variety of different kinds of stimuli that are known reflexly to alter the activity of gastric centres, in order to examine their effects on the central discharge. A technique is described for eliciting gastric movements in anaesthetized sheep together with the simultaneous recording of nerve impulses in single gastric efferent units dissected from the left cervical vagus. The method used allowed direct sampling of the activity emerging from the gastric centres along the efferent fibres without appreciably interfering with either the afferent or efferent pathways.

Brief reports of the present work have already been published (Leek, 1963, 1966; Iggo & Leek, 1966).

## METHODS

*Experimental animals.* Forty-seven adult Scotch Blackface sheep were used. They weighed 20–40 kg and were mostly 8–18 months old, although a few aged ewes were also used. The sheep were held indoors before use, usually for a fortnight, during which time they received  $\frac{1}{2}$  lb (0.27 kg) oats/day and an unrestricted amount of hay. This practice was found to result in more satisfactory experiments than those in which the sheep had been starved for 24 hr before the experiment or had undergone a change of diet and environment during the previous 2 weeks.

*Surgical procedures.* Anaesthesia was induced with a 4% halothane B.P./oxygen mixture by a semi-closed method employing a face-mask. It was maintained, after endotracheal intubation (McGill, No. 10), either with warm 1% chloralose solution, given intravenously in an initial dose of 4 ml./kg body weight followed by maintenance doses of 1 ml./kg body weight approximately every hour, or with a controlled mixture of halothane and oxygen administered by a circle type, closed-circuit method incorporating a Starling respiration pump. Because swallowing and reflex limb movements seriously interfered with recording from single units, the level of anaesthesia needed to be comparatively deep, the corneal reflex being either absent or sluggish.

An intravenous cannula was inserted into the left lateral tarsal vein. A 1 l. rubber balloon was inserted into the reticulum either through the reticulo-ruminal orifice reached by way of a rumenotomy incision in the left sublumbar triangle or, more usually, through the ventral pole of the reticulum exposed by a median laparotomy. Sometimes a small balloon was placed in the dorsal sac of the rumen.

With the sheep lying on its right side recordings of reticulo-ruminal movements were started and then the left cervical vagus was exposed by incising the skin for 15 cm along the jugular groove and excising the left sternocephalic muscle. The edges of the skin wound were sutured to a horizontal ring of solder to form a pool for liquid paraffin B.P. A silver earth electrode was embedded and sutured in the longus colli muscle. About 1.5 cm of vagus was freed from underlying connective tissue and a rigidly held black Perspex dissecting plate was placed under this region. When required, Ag/AgCl stimulating electrodes were inserted beneath the nerve on either side of this plate.

Throughout the experiment the sheep's intra-abdominal temperature was held at 38° C by means of a thermostatically controlled electric blanket. Anaesthesia and gastric contractions were maintained for up to 19 hr.

*Recording technique.* Fine strands were dissected from the left cervical vagus and placed across a pair of fine Ag/AgCl wire recording electrodes carried on a micromanipulator, as described by Iggo & Vogt (1960) for recording from preganglionic cervical sympathetic fibres. The vagus and the fine strand dissected from it were at all times immersed in a pool of liquid paraffin. The action potentials ('spikes') were amplified, displayed on an oscilloscope and recorded photographically on moving bromide paper (Iggo, 1955, 1956).

Movements of the reticulum and rumen were recorded manometrically. The respective balloons were connected by polythene tubing to Marey tambours writing on smoked kymograph paper. In addition the pressure line from the reticulum was taken to a transducer where a glass diaphragm actuated the grid pin of a R.C.A. 5734 valve. Thus pressure changes in the reticulum could also be displayed on the oscilloscope at the same time as the nervous discharge. In the most recent experiments a Statham strain-gauge manometer (P23) has been used.

## RESULTS

## A. Gastric movements in anaesthetized sheep

One of the main problems in the study of gastric motility in sheep is that the reticulo-ruminal movements are abolished by many anaesthetic agents (Iggo, 1956). The movements may be present in decerebrate preparations of sheep (Iggo, 1951; Titchen, 1953) but they are not always present and cannot always be evoked. When present, they may be different in form and frequency from those in the intact animal and may persist for a relatively short time. These features of decerebrate preparations make them unsuitable for the kind of work to be described in the present paper, in which it is necessary to obtain regularly recurring contractions of more or less normal shape and size, particularly since in subsequent work reflex modification of these movements is studied. A further disadvantage of decerebrate preparations is that they may exhibit reflex somatic movements, elicited by cutaneous and other stimulation arising from the experimental manipulations. These movements interfere both electrically and mechanically with single unit recording and so an attempt was made to find a preparation with recurrent reticulo-ruminal movements as near normal as possible.

Brunaud & Dussardier (1951) described active gastric movements in sheep anaesthetized with chloralose and a modification of this method was tried. Particular difficulty was found in maintaining an optimum anaesthetic level and, because of the long interval between the injection of chloralose and its effective action, estimation of maintenance doses was not easy. Eight sheep were prepared in this way. Two of these had been fed out-of-doors on turnips until 2 days before use; in one no gastric movements were evoked, and in the other they persisted for only 6 hr. The remaining six all produced gastric contractions which were intermittent because of the variations in anaesthetic level.

During the preparation of sheep for decerebration, halothane was used to induce anaesthesia and it was discovered that gastric movements were often present or could easily be elicited in sheep maintained under a halothane/oxygen mixture. The use of this anaesthetic offers several advantages over both decerebrated and chloralose-anaesthetized preparations. These are (a) the absence of swallowing and reflex limb movements, (b) the persistence of gastric movements for very long periods, as long as 19 hr and (c) the ease of rapidly adjusting the anaesthetic level while retaining the ability to maintain a fairly constant level of anaesthesia for a long time. A respiration pump was incorporated into the anaesthetic circuit, since this permitted the anaesthetic level to be kept more constant

and caused gastric movements to be larger than those present during spontaneous breathing.

The halothane/oxygen method of anaesthetizing sheep was used in thirty-nine animals included in the present study. In sixteen of these animals gastric movements were present for longer than 12 hr and in some they were still present after 19 hr. Twenty-three animals produced gastric contractions that persisted for less than 12 hr but these included six animals with extensive surgery and six which were starved for 24 hr before the experiment. Only two failed to produce any gastric movements and these included one which was only 5 months old.

Gastric movements were present in only eight of the sheep after induction of anaesthesia at the start of an experiment, but in a further thirty-six animals it was possible to elicit them reflexly. A simple and effective way to do this was to inflate the reticular balloon with 400–600 ml. air, causing an intraluminal pressure of about 10 mm Hg. These conditions were adopted as the 'standard' procedure and the comparisons of single unit activity made in this paper were, as far as possible, based on recordings under these conditions. Other procedures that were known to evoke reticular contractions in decerebrate sheep were also tried. Electrical stimulation of the central end of a cut cervical vagus or of an intact cervical vagus or acidification of abomasal contents were ineffective in sheep in which reticular contractions could not be evoked by reticular distension. These procedures did elicit reticular contractions for a short time from some of those animals in which, either previously or subsequently, reticular contractions were produced by reticular distension. The addition of 50 ml. of 0.2 N-HCl to the abomasum sometimes produced a temporary enhancement of the amplitude or frequency of reticular contractions that had been evoked by reticular distension. Gastric contractions were reduced in amplitude or frequency when the abomasum or the rumen became markedly distended, for example, when the ruminal pressures exceeded 20 mm Hg. During the early stages of an experiment, bacterial fermentation in the rumen caused the rapid accumulation of gas and resulted in high intraruminal pressures. If reticular contractions were absent in these conditions they re-appeared 1–4 min after the gas was released by inserting a large bore needle through the flank of the animal into the rumen. An optimal intraruminal pressure appeared to be necessary, since the reticular contractions did not re-appear if too much gas was removed.

The movements of the different parts of the stomach were affected to varying degrees by anaesthesia. The pressure changes associated with reticular contractions were approximately the same as those recorded in conscious animals, as regards their frequency, wave form, duration and amplitude (Fig. 1). The typical isometric reticular contraction was bi-

phasic. Initially the pressure rose sharply at a rate of 4 mm/sec for 1.5–2.0 sec to reach a low first peak of 6 mm Hg. During the next 1.5 sec there was usually a slight drop in pressure (4 mm Hg) but there might be no fall or even a slight rise at this time. Then followed an even sharper rise in pressure at the rate of 4.5 mm/sec lasting 1.5–2.0 sec, so that a high second peak of pressure was reached, 15 mm Hg above the resting pressure. After this the pressure fell quickly at first, at the rate of 4 mm/sec for 2–3 sec to reach 4–7 mm Hg, and then followed a slow terminal fall back

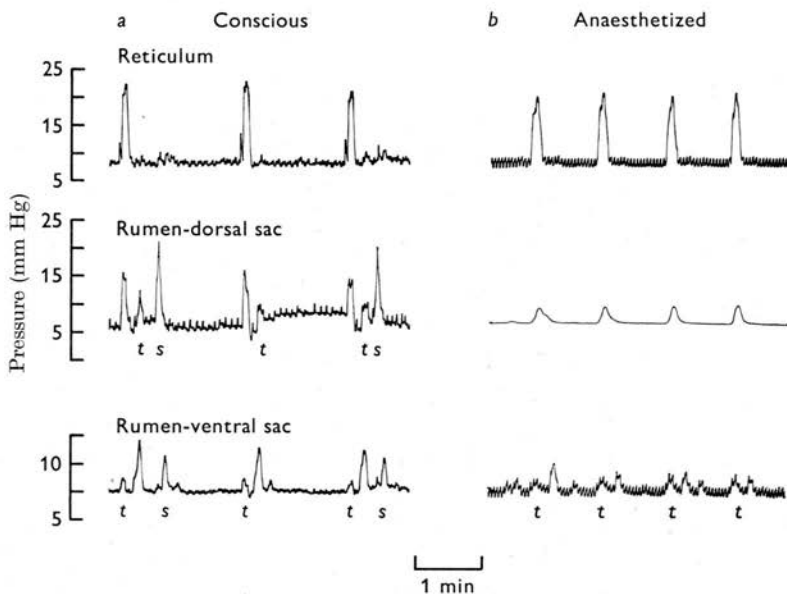


Fig. 1. Movements of the reticulo-rumen in (a) a standing conscious sheep fitted with a rumen cannula and (b) a halothane-anaesthetized sheep lying on its right side. The records were made under identical conditions with air-filled balloons connected by polythene tubing to strain-gauge manometers. The first phase of the reticular contraction is small and followed by a relaxation in the conscious animal but large, with only a slight relaxation in *b*. The 'primary' ruminal contractions are smaller in the anaesthetized animal. 'Secondary' ruminal cycles (*s*) are present in the conscious sheep but absent from *b*. Certain pressure changes (*t*) in the rumen are passively transmitted from the dorsal to the ventral sac and vice versa.

to the resting pressure during the next 4–6 sec. When large ruminal contractions were present, this terminal phase usually had superimposed upon it a slight rise and fall of pressure coincident with the dorsal sac contractions.

The reticular pressure changes were compared, on a few occasions, with movements of the (left) lateral wall of the reticulum, that had been exposed by resecting the ribs 9–11, removing the left lung and incising the

diaphragm. The reticular movement was seen to start as a weak contraction involving simultaneously all parts of the lateral wall of the reticulum. Then followed, in sequence, a slight incomplete relaxation, a sharp strong contraction and a complete relaxation which was rapid at first but terminated slowly. There were no signs of waves of contraction or of regions which were inactive for a part, or whole, of the sequence, as was suggested by some of the earliest investigators (Wester, 1926; Schalk & Amadon, 1928).

The ruminal contractions in the anaesthetized animals were usually much weaker than in the conscious animal, especially in the more caudal and ventral parts of the rumen. The largest dorsal ruminal sac contractions recorded lasted 10–12 sec and reached a peak pressure of 10 mm Hg about 4 sec after the second peak of the reticular contraction. Dorsal ruminal contractions were more often absent or weak. They were largest when the level of anaesthesia was light or when the reticular distension was large (600–1000 ml.). In the present experiments the reticular balloon did not project over and, therefore, stretch the reticulo-ruminal fold, a stimulus that was found by Titchen (1960) to be very effective in eliciting ruminal contractions in decerebrated sheep. Contractions of the caudo-dorsal blind sac and the ventral ruminal sac, were sometimes present, though always of very low amplitude. The position of the sheep affects the ruminal movements. Secondary cycles of contraction were absent when the animal was on its side.

Reflex reticular and reticulo-ruminal contractions were most easily evoked when there was only a short interval between the induction of anaesthesia and the insertion of the reticular balloon, and its inflation. For this reason the reticular balloon was always put in place as soon as possible and before completion of the other surgical procedures that were required for the electrical recording. The reticular movements were always reduced in amplitude and rate by even relatively minor surgical procedures, e.g. incising the skin, and they were completely abolished for 5–10 min by more extensive surgery; for example, exposure of the cervical vagus, although they regained their former amplitude and rate over the course of a further 5–10 min. This effect was seen during surgery at any site on the body, superficial or deep, and also when exposed viscera and the edges of unsutured wounds were manipulated. The effect appeared to be independent of anaesthetic level and was also present in adrenalectomized animals in which both major splanchnic nerves had been cut. Blood loss during surgery was quite small and was unlikely to have contributed to the effect.

For comparison with the gastric movements elicited reflexly by distending the reticulum, a brief study was made of movements evoked

directly by stimulating electrically the peripheral end of a cut cervical vagus at intensities that were sufficient to cause maximal reticular contractions when continued for 5 sec at 20/sec. Using a train of stimuli at this rate, which is the average peak frequency of discharge recorded from the Type I units described later, a stimulation period of approximately 1 sec was necessary to produce a reticular contraction similar in amplitude to those occurring spontaneously under the standard conditions described

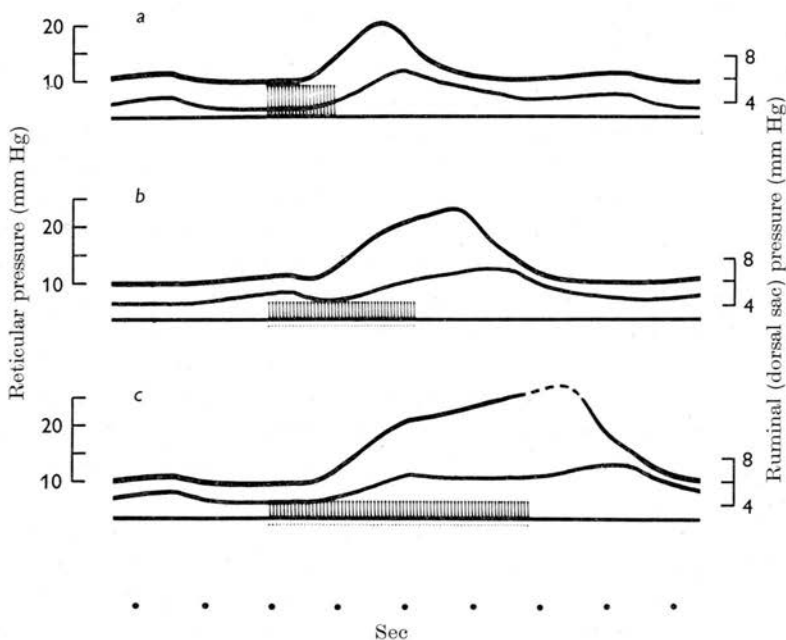


Fig. 2. Contractions of the reticulum (*upper trace*) and dorsal sac of the rumen (*middle trace*) caused by electrical stimulation at maximal intensity (*lower trace*) of efferent fibres in the intact left cervical vagus in a halothane-anaesthetized sheep. Maximal contractions were obtained only when the stimulus lasted for at least 5 sec. Submaximal contractions are illustrated and in all of them the phase of contraction has approximately the same duration as the period of stimulation but starts after a latency of 0.7 sec. The contractions are superimposed upon pressure fluctuations due to respiratory movements. The stimulus artifacts in *a* are larger than in *b* and *c* because of a change in recording conditions.

Trains of stimuli (30 V) at a frequency of 20/sec were given at intervals of 1 min for durations of 1 sec (*a*), 2 sec, (*b*) and 4 sec (*c*).

on p. 182. The reticular contraction was monophasic, began 0.7–1.0 sec after the start of stimulation and reached a maximum amplitude if the stimulus was continued for 5 sec. For submaximal contractions the phase of contraction lasted for as long as the period of stimulation (Fig. 2). The interval between the middle of a 1 sec train of stimuli and the peak of the

reticular contraction was about 1.3 sec (1.2–1.5 sec). This interval is slightly shorter than the average interval (1.8 sec) between the peak of the impulse discharge in Type I units (described later) and the (second) peak of the associated reticular contraction. Electrical stimulation at frequencies of less than 20/sec caused the rate of pressure rise in the reticulum to be slower. Electrical stimulation of a vagus produced similar effects in the rumen, except that the latency in the mid-dorsal sac was 0.8 sec longer than the reticular latency.

### B. Discharge in efferent vagal fibres

The results detailed in the present paper were obtained by recording from the left cervical vagus of adult sheep and units with various patterns of discharge were obtained. In some units the discharge had a clearly recognizable cardiac or respiratory pattern and a brief description of these units is given later in the paper. Principal interest centred on efferent units in which the discharge bore a temporal relation to contractile events in the reticulum and rumen.

#### *Gastric (reticulo-ruminal) units*

Single unit discharge patterns associated with reticulo-ruminal movements were readily distinguished from other patterns since the gastric movements had a characteristic and regular cycle which was unrelated to the movements of other thoracic or abdominal viscera. Several criteria had to be satisfied for a unit to be classed as a gastric unit; (a) a discharge of impulses must appear, or an existing discharge must be modified, at the same period during each gastric contraction, (b) this discharge should not occur during the inactive phase of the cycle of contractions and (c) the discharge should change appropriately with both spontaneous and reflexly induced variations in the amplitude and frequency of gastric movements.

Sixty-four single units satisfying these criteria were isolated, as well as more than fifty strands containing 2–4 active gastric units some of which could be used for the purposes of analysis and classification. In addition there were many more strands in which gastric units were present together with active non-gastric units, but these strands were much less useful for purposes of analysis. Prolonged recording was possible from many of the single units and recordings were made from twenty-seven of them for longer than 1 hr and, in two instances, for as long as 5½ hr. The single units were usually lost either as a result of physical damage to the very fine nerve strands resting across the recording electrodes, caused by slight movement of the neck or oesophagus, or owing to further dissection of a strand in an attempt to improve the recording conditions.

For any individual unit the pattern of discharge was very similar during successive gastric cycles throughout the recording period lasting several hours, provided that the experimental conditions remained the same or, if altered, were subsequently returned to the original condition. This is illustrated in Fig. 5. The discharge was, on some occasions, so regular that the units could be mistaken for afferent fibres. The following tests were used to ensure that the gastric units were efferent: (a) the reticulum was suddenly distended with 200 ml. air. Iggo (1956) showed that this procedure either initiates or enhances a resting discharge in the afferent fibre. No resting discharges were observed in the gastric efferent units, with the

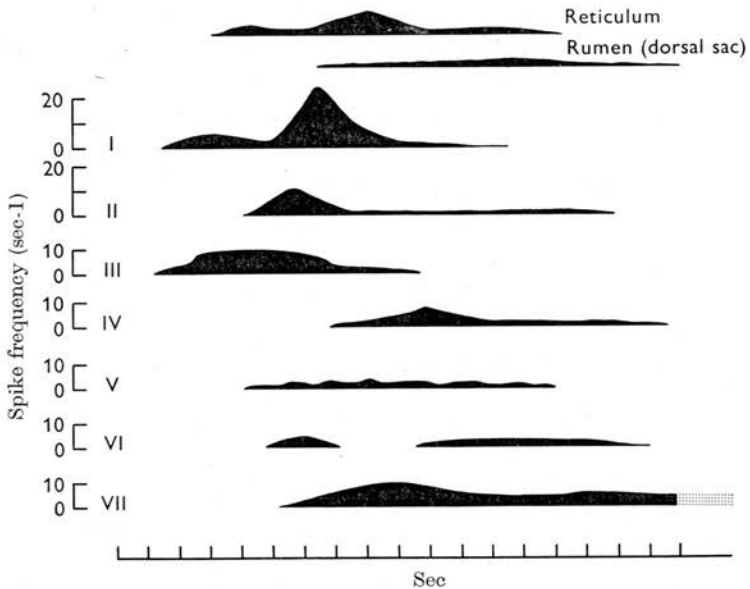


Fig. 3. The temporal relationship between gastric (reticulo-ruminal) contractions constituting the 'primary cycle' and the discharge patterns in the various types of efferent units. The (second) peak of the reticular contraction has been used as the ordinate for alignment of the frequency curves constructed from the mean values given in the tables.

Types I, II and III are considered to innervate the reticulum or adjacent structures, Type IV the rumen and Types V, VI and VII special regions, e.g. pillars and/or sphincters, which have not yet been identified.

exception of those classed as Type VII. (b) Drugs which block impulse transmission distal to the recording electrodes were administered (tetra ethylammonium chloride, 1 mg/kg body weight, probanthine hydrochloride, 0.02 mg/kg body weight). These drugs caused both gastric contractions and the corresponding phase of an afferent discharge to be abolished but an efferent discharge was still present (B. F. Leek, un-

published observations). All the gastric units described below satisfied one, or both, of the above criteria.

#### Classification of gastric efferent units

Sixty-four gastric efferent single units were classified into seven types on the basis of their discharge patterns, and the time relationship of this discharge to the gastric contraction. Figure 3 summarizes the results in the form of frequency curves. Detailed results are given in Tables 1 and 2. Each class of gastric unit was distinctive and quite separate from the others for the following reasons: (a) No discharge pattern changed in type during recording sessions lasting as long as 5½ hr, either spontaneously or as a result of deliberately altering gastric conditions in a way which reflexly modified the activity of the unit. (b) In some multi-unit records,

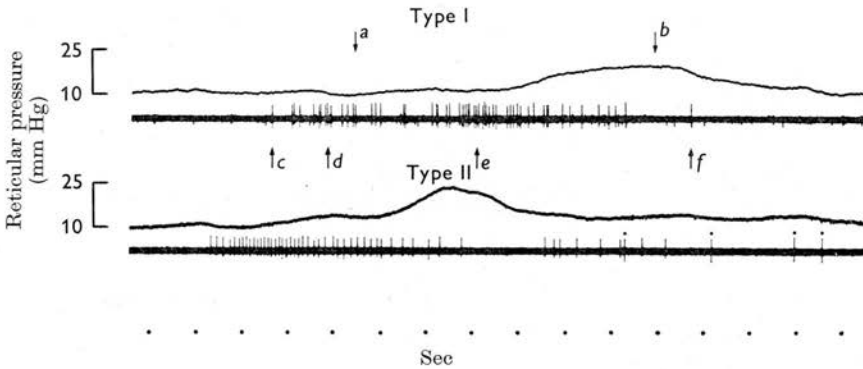


Fig. 4. The discharges of Types I and II efferent unit (*lower traces in each record*) and the corresponding reticular contractions (*upper traces*). A Type IV unit fires with a low frequency in the later part of the lower record (*spikes marked with a dot*).

The points labelled *a-f* provide the key for Tables 1 and 2: *a* indicates the start of the reticular contraction and *b* its second peak. The efferent discharge commences at *c*, reaches its first peak at *d*, its second peak at *e* and ends at *f*.

when each unit had a distinguishable spike wave form and amplitude, it was possible to identify several active units which could be of different types, e.g. Types I, II and III were present in all combinations. (c) During an experiment, units of several types could be isolated so that the presence of one or another did not depend only on the experimental conditions. In the classification which follows, a functional grouping has been used; Types I-III are believed to innervate the reticulum or neighbouring structures, Type IV is thought to innervate the rumen, and Types VI and VII probably innervate special regions of the reticulo-rumen, e.g. sphincters, and/or pillars.

TABLE 1. The efferent discharges in Type I single units and their time relationship to reticular contractions. Each set of values is representative of the unit and was obtained during one contraction cycle under 'standard' recording conditions. Twenty-one sheep were used

Refer to Fig. 4 for the positions in the reticular contraction and efferent discharge indicated by *a-f*.

Unit no.	Vol. in balloon (ml.)	Reticular contraction		Efferent unitary discharge			Intervals				
		Amplitude at <i>b</i> (mm Hg)	Duration <i>a-b</i> (sec)	Total no. spikes	Peak frequencies 1st at <i>d</i> 2nd at <i>e</i>	Duration <i>c-f</i> (sec)	<i>c-e</i> (sec)	<i>d-e</i> (sec)	<i>e-f</i> (sec)	<i>e-b</i> (sec)	
1	100	—	5.2	72	9	18	12	6	4	5	2.0
2	100	11	6.7	56	2	24	14	5	4	8	3.7
3	300	4	3.3	40	8	32	6	2	1	4	1.2
4	300	9	5.5	33	5	7	13	6	5	7	1.5
5	300	12	5.5	45	6	11	9	6	4	4	2.0
6	300	14	6.0	27	1	8	7	5	4	2	1.0
7	300	17	6.5	41	5	13	10	5	4	5	1.5
8	300	18	6.5	99	11	17	18	6	5	12	1.4
9	400	3	2.5	69	15	25	12	6	3	7	1.8
10	400	4	5.7	28	2	13	6	4	3	2	1.7
11	500	9	3.0	88	5	29	13	7	4	8	2.0
12	600	—	6.0	70	10	18	13	6	5	7	1.7
13	600	6	3.2	32	5	10	9	4	3	5	1.0
14	600	10	5.0	56	9	14	10	5	3	5	4.0
15	700	9	4.5	33	2	20	9	4	3	5	2.3
16	700	17	5.0	74	3	14	15	5	4	10	2.5
17	800	2	5.5	105	2	35	18	8	4	10	0.8
18	800	6	1.7	93	20	45	7	5	4	2	1.5
19	800	9	4.5	46	2	18	12	7	5	5	0.5
20	800	9	4.0	34	4	15	5	4	2	4	1.5
21	800	20	4.8	52	10	30	6	2	2	4	0.5
22	900	7	6.2	74	2	29	14	—	—	13	1.7
23	1000	8	4.0	86	9	17	16	7	4	9	1.0
24	1100	16	4.2	78	3	25	10	4	3	6	1.2
25	—	26	4.2	20	4	14	7	3	2	4	5.0
Mean	563	10.6	4.8	58.0	6.2	20.0	10.8	5.1	3.5	6.0	1.8
± S.E.	57	1.3	0.2	6.9	0.9	1.9	0.8	0.3	0.2	0.6	0.2

*Type I gastric efferent units.* Twenty-five single units of this type were examined (Fig. 4, Table 1). The standard discharge was bimodal, the frequency of the first peak (6/sec) being much lower than of the second (20/sec). These units were active only during a cycle of contraction and were silent in between the contractions. Most of the action potentials, including the peak frequency of the discharge, preceded the peak of the reticular contraction. The peak frequency of discharge was never greater than 45/sec. It preceded the peak of second reticular contraction by an average of 1.6 sec for all the units, and varied within the range of 0.5–2.5 sec for individual units. In addition to this consistent temporal relation between the Type I discharge and the reticular contractions, there were the following similarities between the pattern of the discharge and the form of the contraction. (1) Both the discharges and the contractions were biphasic and the intervals between the peaks were similar (3.5 sec and 3.0–3.5 sec respectively), although it was not easy to identify consistently the peak of the first reticular contraction. (2) The mean ratio of spike frequencies of the first and second peaks of the efferent discharge was 1:4 and the mean ratio of the amplitudes of the first and second peaks of the reticular contraction was 1:4 (Table 1). (3) The interval between the second peak and the end of the spike discharge (3.5 sec) was similar to the phase of the reticular relaxation (6–8 sec). There was, however, considerable variation in the various time relations. (4) The average interval from the start of the discharge in the unit to its peak (5.0 sec) was similar to the average duration of the phase of reticular contraction (4.9 sec).

*Type II gastric efferent units.* Seven single units of this type were isolated (Fig. 4, Table 2). The Type II discharge was always unimodal, consisting of an early peak with a long tail. The peak discharge was less than for the Type I units and rarely exceeded 18/sec. Occasionally, up to three action potentials preceded the main part of the discharge for three of the units. The presence, number and position of these early impulses was erratic, even for successive contraction cycles, and they were, therefore, disregarded when measuring the intervals detailed in Table 2. The start-to-peak interval for the discharge was very short (1.7 sec), whereas the peak-to-end interval was very long (9 sec). The over-all duration of the discharge (11 sec) was the same as for Type I units (10.8 sec), although the number of spikes (41) and the peak frequency (12/sec) were less. The interval between the peak frequency of the discharge and the second peak of the reticular contraction (2.1 sec) was longer than for the Type I units. Because of the similarity of these units to Type I, particular care was taken to make sure that they were, indeed, a separate group. For example, Types I and II discharges were, on at least one occasion, recorded simultaneously from a multi-unit strand, so that the experimental

TABLE 2. The efferent discharges in Types II, III and IV single units and their time relationship to reticular contractions. Each set of values is representative of the unit and was obtained during one contraction cycle under 'standard' recording conditions. Twenty-three sheep were used.

Refer to Fig. 4 for the positions in the reticular contraction and the efferent discharge indicated by *a-f*. *e* represents the peak frequency in Types II and IV and the mid-point of the 'plateau' in Type III

Type	Unit no.	Reticular contraction			Efferent unitary discharge						
		Vol. in balloon (ml.)	Amplitude at <i>a-b</i> (mm Hg)	Duration <i>a-b</i> (sec)	Total no. spikes	Peak frequency (sec <sup>-1</sup> )	Duration <i>c-f</i> (sec)	Intervals			
								<i>c-e</i> (sec)	<i>e-f</i> (sec)	<i>e-b</i> (sec)	
II	26	300	20	4.0	46	10	17	2	15	2.7	
	27	400	4	3.7	40	12	13	1	12	2.0	
	28	500	8	6.8	30	18	6	1	5	1.0	
	29	600	4	4.5	32	16	10	3	7	1.5	
	30	600	9	4.5	44	11	12	2	10	4.0	
	31	900	17	3.5	27	8	9	1	8	0.7	
	32	1400	23	4.7	72	12	9	2	7	2.5	
	Mean	671	12	4.5	41	12	11	1.7	9	2.1	
	III	33	150	4	6.0	51	13	7	3.4	3.6	4.2
		34	200	11	5.5	81	10	13	5.2	7.8	2.0
35		300	3	5.5	55	10	10	4.3	5.7	4.0	
36		400	5	4.0	40	7	8	3.4	4.5	3.0	
37		700	17	7.2	35	7	7	3.7	3.5	4.0	
38		900	19	5.0	14	5	5	1.9	3.1	3.0	
39		1100	6	4.5	59	14	12	3.0	3.0	3.5	
40		1200	1	—	32	9	6	2.5	3.5	—	
Mean		619	8.3	5.4	41	9.4	8.5	3.6	5.1	3.4	
IV		41	300	4	—	55	10	15	4.0	11	—2.5
	42	400	1	—	15	7	4	2.0	2	—2.0	
	43	450	14	—	27	5	11	2.0	9	—2.0	
	44	700	11	—	30	3	18	1.5	17	—3.0	
	45	800	5	—	46	17	7	4.0	3	—1.0	
	46	800	5	—	14	4	10	1.0	9	0.0	
	47	800	10	—	55	8	11	5.5	5	—2.0	
	48	800	20	—	27	4	11	3.0	8	—2.0	
	49	1000	10	—	31	6	7	3.0	4	—2.0	
	50	—	—	—	45	6	11	4.0	7	—1.5	
Mean	672	9	—	35	7.0	10.5	3.0	7.5	—1.8		

Conditions were not important in determining whether one or the other type of discharge was present in the unit. Both types of unit have been isolated from an animal at different times and not in any particular sequence. Furthermore, the Type II discharge always started after the beginning of a reticular contraction and could be present even when the reticular contractions were clearly biphasic. For these reasons, the Type II units have been assigned to a separate category.

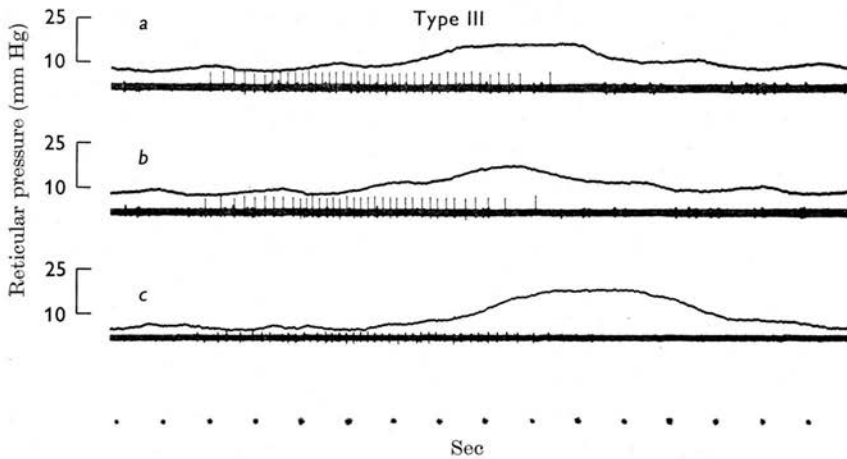


Fig. 5. The discharges of a Type III unit (*lower traces*) and the corresponding reticular contractions (*upper traces*). *a* and *b* are consecutive reticular contractions recorded under 'standard' conditions: the efferent discharges are similar but the contractions appear to be slightly different because they are superimposed upon pressure fluctuations due to respiratory movements. *c* is from the same unit  $5\frac{1}{2}$  hr later when, after a variety of experimental procedures, the recording conditions were once more 'standard'. The discharges are similar in *a*, *b* and *c* but the spike amplitude is smaller in *c* owing to an alteration in recording conditions.

*Type III gastric efferent units.* Eight single units of this class were isolated (Fig. 5, Table 2). The discharge of Type III units began at about the same time as in Type I units but, unlike these latter, the discharge was in the form of a fairly even, extended plateau and did not exhibit sharp peaks. The peak frequency was also lower and the average value was 9.4/sec. The period during which a fairly steady frequency of discharge was present in the Type III units was at least twice as long as the period for the peak discharge in either the Type I or II units: 4.4 sec, compared with about 2 sec. An interesting feature of the discharge of a Type III unit was that the discharge appeared at the same time as the Type I and reached a plateau about coincident with the peak of the first discharge for the Type I units, and that the discharge fell fairly abruptly

just after the peak of the second discharge of the Type I units. Although, therefore, the discharge patterns for these Types I, II, and III unit were quite different the principle part of the discharge in each case occurred at about the same time. For each class of unit the discharge appeared before or during the earlier part of reticular contractions and for this reason it is likely that all three classes were in some way associated either with this contraction or with the contraction of other structures closely associated with the reticulum.

*Type IV gastric efferent units.* Fifteen units of this type were examined, ten as single units (Fig. 6, Table 2) and five that were clearly distinguishable in multi-unit records. The discharge began after the peak of the second discharge of the Type I units, i.e. during the second part of the reticular contraction, and reached a peak of activity shortly after the reticular contraction peak. The discharge then continued on for several seconds at a lower frequency. Both the peak frequency (7/sec) and the total number of spikes in any one cycle of contraction were less than for the Types I, II or III unit. A discharge of impulses began 1.2 sec before, and reached its peak frequency 1.8 sec after, the second peak of reticular contraction. This was the most striking difference from the Types I, II and III units, as is illustrated in Fig. 3.

Activity in Type IV units was present in those conditions which also led to the appearance of large dorsal ruminal sac contractions. The effective conditions were a preparation in which reticular contractions could be readily evoked, a relatively light plane of anaesthesia and a moderately high reticular distension (600–1000 ml.). There were several occasions when a Type IV discharge and ruminal contractions suddenly appeared whilst recording from a strand which initially had no Type IV discharge in it. The discharge and the contractions were, in these circumstances, elicited by an increase in the reticular distension.

The time relationship of the Type IV discharge to reticular contractions also supports the identification of these units as ruminal efferent units. During primary gastric cycles, the peak of a dorsal ruminal sac contraction occurred about 4.5 sec after the peak of a reticular contraction (Fig. 1). The peak of the Type IV efferent discharge also occurred about 3.5 sec after the peak of the Type I discharge (Fig. 3). The Type IV discharge preceded dorsal ruminal sac contractions and the peak discharge was 2.4 sec earlier than the peak of the dorsal sac contractions. This maximum was difficult to assess accurately since the ruminal sac contractions tended to be slow and of low amplitude. The interval is similar to the latency of ruminal contractions elicited by direct electrical stimulation of the peripheral cut end of the vagus (2.2 sec).

*Type V gastric efferent units.* Six single units of this type were found

(Fig. 6). The common feature of this group was a very low frequency of discharge with no obvious consistent peak. It lasted about 10 sec and began 4 sec before the peak of the reticular contraction. The Type V discharge, therefore, began at about the same time as the Type II and earlier than the Type IV discharge but had ceased before the end of either. The frequency of discharge was very irregular and was scarcely affected by experimental procedures that caused pronounced reflex effects in the Types I, II, III and IV units. There was no discharge during the inactive

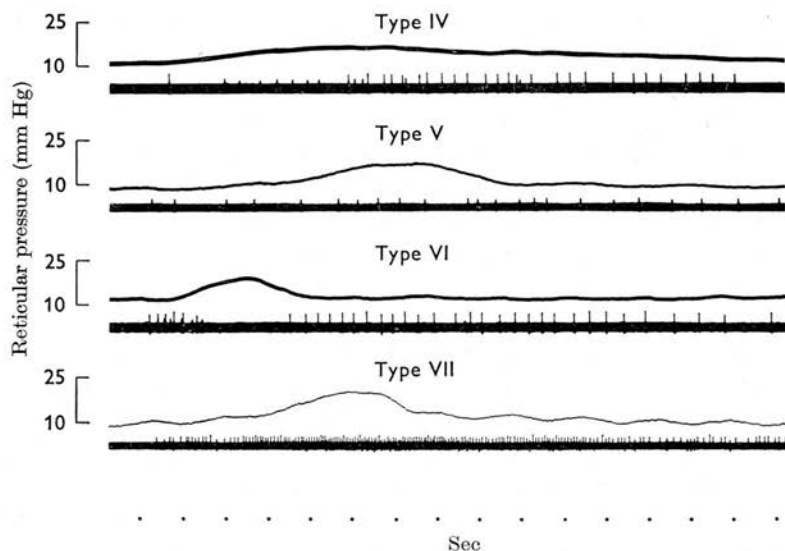


Fig. 6. Examples of gastric efferent discharges (*lower traces*)<sup>2</sup> in units innervating structures other than the reticulum. In each record the *upper trace* shows the reticular contraction. *a* shows both a Type IV unit (*large spikes*) and a Type V unit (*small spikes*). The main part of the Type IV discharge occurs during the phase of reticular relaxation and is associated with the ruminal contraction. *b* shows a Type V unit which has the typical irregular discharge of low frequency. *c* shows a Type VI discharge which is characterized by the pause during reticular contraction and the long, low frequency discharge afterwards. *d* shows a Type VII discharge. This is maximal near the peak of the reticular contraction and is followed by a low frequency discharge which persists until up to 10 sec before the start of the reticular contraction.

phase of the primary cycle of gastric contractions. For these reasons the Type V units are regarded as a distinctive group.

*Type VI gastric efferent units.* Five units of this type were found (Fig. 6). The discharge appeared in two separate bursts with a silent interval of 2.5 sec coincident with the peak of the reticular contraction. The peak frequency of the discharge was low (4.4/sec), and occurred during the first

burst of impulses, which lasted only 2.5 sec. The second burst was much longer (7.6 sec) but had a lower frequency of discharge (2/sec). This pattern of discharge is, therefore, quite dissimilar from any of the preceding types. Like Types I, II, III and IV, it could be modified reflexly. It is suggested in the discussion that Type VI units may innervate gastric sphincters or pillars.

*Type VII gastric efferent units.* Only three units of this type were found (Fig. 6), all of which survived for less than 10 min. The discharge began just after the start of the first reticular contraction and reached a peak frequency of 17/sec 1 sec after the peak of the reticular contraction, at a time during which the Type VI units were silent. This peak discharge, therefore, occurred after the peaks of activity in Types I, II and III but before the peak of activity in Type VI units. The unique and distinctive feature of this type was the presence of a discharge at a low frequency (about 1/sec) that persisted throughout the greater part of the inactive phase of a primary gastric cycle. This persistent activity disappeared for at least 10 sec before a reticular contraction.

*Miscellaneous units.* Recordings were made from only one unit whose discharge was related to gastric contractions but in which the discharge was intermittent, i.e. it appeared during only two out of three gastric cycles. The discharge reached a peak about 2 sec before the peak of the reticular contraction. No other gastric unit was observed in any of the single or multi-unit recordings, made under the 'standard' conditions, which was not active during every primary cycle.

Several units were found which had a tonic or resting discharge with a respiratory rhythm, superimposed on which was an additional discharge during a reticular contraction. The spike amplitudes and regularity of the response of these units to pulmonary inflation indicated that they were pulmonary inflation afferent units. It was inferred that the superimposed gastric discharge arose because the receptors were in a lobe or part of the lungs adjacent to the diaphragm and reticulum and were excited by pressure changes or mechanical displacement caused by reticular contractions.

*Oesophageal units.* Swallowing movements were often present when the anaesthetic level was light. Normally, anaesthesia was adjusted to prevent these movements, since they interfered mechanically with the recording from the fine nerve strands in the neck. On a number of occasions unitary activity was recorded which bore a temporal relation to the contractions of the cervical oesophagus. The discharge consisted of 8-14 impulses at a frequency of about 8/sec (Fig. 7). A similar discharge associated with swallowing in conscious sheep was observed by Dussardier (1960, Fig. 17). Although the conduction velocities of these oesophageal units were not measured, their spike amplitudes were much greater than those of any of

the gastric units, from which it might be inferred that their axonal diameters were greater.

*Cardiac units.* Single units with a cardiac rhythm were isolated occasionally. An example is shown in Fig. 7, in which a burst of 18–20 action potentials accompanied each pulsation in the carotid artery, observed in the paraffin pool. Although the nerve strand lying across the recording electrodes was cut distally, the active unit was not necessarily efferent

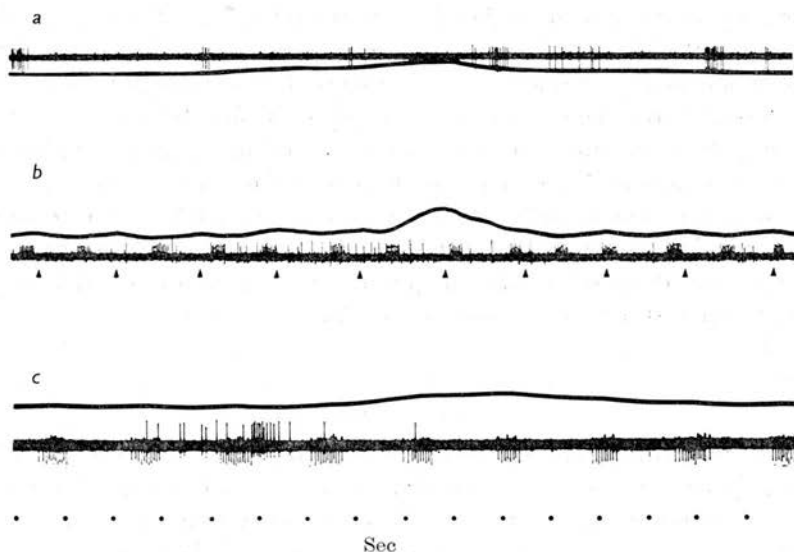


Fig. 7. Examples of single unit discharges not associated with gastric movements. *a* shows the discharges produced during swallowing in a unit innervating the oesophagus. *b* shows bursts of activity which were in phase with the arterial pulse. They are clearly not related to respiration (end of inspiration marked with triangles). *c* shows bursts of activity in a pulmonary afferent fibre (spikes below the line) and an unusually early reticular efferent unit (spikes above the line). In all cases the strand from which the recordings were made had been cut distal to the recording electrodes.

Spikes are recorded in the *upper trace* in *a* and the *lower traces* in *b* and *c*. The pressure line (*lower line* in *a* and *upper traces* in *b* and *c*) shows the reticular contraction superimposed on respiratory movements (inspiration giving an upward deflexion).

since, as Holmes (1954) and Jewett (1964) have demonstrated, the existence of an afferent discharge arising from the carotid sinus may be recorded in fibres dissected from the central end of a cut aortic nerve. This phenomenon has been attributed to a bifurcation of the afferent fibre at some point central to the recording site; a situation comparable to that described for frog tactile receptors by Adrian *et al.* (1931). The afferent

discharge in these cardiac units was very similar to the discharge in carotid sinus baroreceptors, and it is concluded that they were afferent fibres.

*Respiratory units.* Single units with a respiratory rhythm were encountered more frequently than those with a cardiac rhythm. In the example shown in Fig. 7 the discharge was related in time to the small pressure waves recorded by the reticular balloon. These waves are respiratory in origin and inspiration is recorded as a rise in pressure. Some of these respiratory units may have been afferent and to test for this point the endo-tracheal tube was clamped or a graded distension was applied to the lungs. When this was done it was possible to differentiate between afferent and efferent fibres. The discharge in an afferent fibre became steady after clamping the endo-tracheal tube and increased in frequency as lung distension was increased, whereas the discharge rate and rhythm in efferent fibres was not substantially altered by these procedures. The recordings obtained were comparable to those described for pulmonary inflation receptors by Paintal (1963), for pulmonary efferents by Widdicombe (1961, 1966) and laryngeal efferents by Andrew (1955).

#### DISCUSSION

One of the principal difficulties encountered in analysing the reflex basis of reticulo-ruminal motility has been that of maintaining gastric movements in suitable experimental conditions. In the present experiments halothane anaesthesia has allowed the movements to be investigated in the anaesthetized animal for up to 19 hr. This has avoided the use of decerebrate preparations, in which gastric movements are often difficult to evoke and maintain for long periods (Iggo, 1956; Titchen, 1958). It was also more convenient and reliable than the chloralose anaesthesia method used by Brunaud & Dussardier (1951), and by ourselves for the first eight experiments. A number of other conditions were also found to result in more reliable preparations. Gastric movements were most easily elicited and maintained in anaesthetized sheep which had not been starved before the beginning of the experiment, or which had not recently had a change of diet. This usually leads to a reduction in appetite for a few days. Active ruminal fermentation before an experiment seems, therefore, to be associated with more lively reflex preparations.

Reticulo-ruminal movements evoked under halothane anaesthesia showed some differences from those recorded in the conscious animal. Reticular movements were similar so far as the frequency, form, duration and amplitude of the biphasic contractions were concerned and it is concluded that the observations made on reticular function during these experiments would

also hold for conscious animals. This was not so for the rumen, because, although the dorsal sac contractions had a similar form and duration in conscious and anaesthetized animals they were of smaller amplitude in the latter. Ventral sac contractions were either very small or absent under anaesthesia. It is likely that the reduction in ruminal motility was due to reflex and central factors rather than to a transmission block in the motor pathway, even though halothane in high concentration blocks peripheral nervous transmission and ruminal movements are more susceptible than reticular movements to the action of ganglion blocking agents (Brunaud & Navarro, 1954). This conclusion is based on a comparison of our results with those of Dussardier (1960), who used the cross-sutured nerve technique in conscious animals, and recorded many more units with a ruminal or late discharge (equivalent to our Type IV units) than with a reticular or early discharge (equivalent to our Types I-III). Dussardier's ruminal units also had many more spikes per discharge and higher peak frequencies.

Further experiments are required to determine the extent to which halothane was depressing the reflex centres for ruminal motility and the extent to which the experimental conditions diminished reflex excitatory effects and introduced or enhanced reflex inhibitory actions. It is likely that halothane had a stronger central depressant action on the 'ruminal centres' than on the 'reticular centres' because lightening the anaesthetic level alone often led to the appearance or increase in amplitude of ruminal movements without a change in those of the reticulum, i.e. in conditions in which there is unlikely to be any modification of gastric afferent input. In the present experiments the reticular balloon did not project through the reticular ruminal orifice and hence did not stretch the reticulo-ruminal fold. The sheep were, therefore, deprived of a stimulus which Titchen (1960) found to be very effective in evoking reflex ruminal contractions in decerebrate sheep. Other peripheral factors might include the abnormal position of lateral recumbency, a posture which is known to influence rumen movements (Balch, 1952; Reid & Titchen, 1965) and also physico-chemical changes in the rumen contents resulting from ruminal stasis and the lack of an inflow of saliva.

In some experiments reticular movements were present before the reticular balloon was inflated but usually it was necessary to add 300-600 ml. air to evoke reticular contractions which were comparable in rate, form, duration and amplitude to those in the unanaesthetized sheep. The results obtained from studies of gastric afferent units might be significant in this connexion. Reticular distension of about 400 ml. was required to change the discharge pattern in a gastric tension receptor during the inactive phase of the gastric cycle from one of irregular bursts of activity to one in which the discharge was continuous and regular (Iggo, 1955). More

recently, it has been observed that similar receptors, principally in the region of the oesophageal groove, were silent during the inactive phase of the gastric cycle when the reticulum contained less than about 400 ml. air, whereas these receptors produced a steady resting discharge at greater volumes of distension (B. F. Leek, unpublished observations).

The suppression of reticulo-ruminal movements which occurred (even at the deepest planes of anaesthesia), when surgery was performed or contact made with exposed tissues, was most striking and was evident within a few seconds of applying noxious stimuli. The full effect took about 30 sec to develop. This suppression was present even in sheep which had been adrenalectomized and had had their splanchnic nerves cut, although in these animals the effect seemed to persist for a shorter time. Titchen (1958, 1960), using decerebrate sheep, had observed similar reflex suppression of reticular contractions through manipulation of the viscera (particularly the pylorus) and distension of the abomasum. He concluded that the splanchnic nerves were providing an afferent pathway for these effects, except from the pylorus. In the present experiments the suppression took the form of an absence or suppression of a discharge in the efferent fibres, so that the inhibition was a central phenomenon. The inhibitory mechanisms require further study.

Although reticulo-ruminal movements in the halothane-anaesthetized sheep were, to some extent, subnormal, the preparation has several merits: (a) By standardizing the experimental conditions it becomes possible to compare units recorded from a large number of animals. (b) Recordings are made directly from efferent gastric fibres and the afferent and efferent pathways in both vagi remain intact, apart from the fascicule from which the fibres have been dissected. (c) The anaesthetized sheep, unlike decerebrate animals, are free from reflex limb and neck movements which can seriously interfere with single unit recordings, and the gastric movements persist for much longer.

The results obtained by recording from single vagal units provide information not previously available and allow a start to be made on the analysis of the underlying reflex mechanisms. There was no difficulty in establishing that the gastric vagal discharge was efferent for the reasons given on page 186. The fact that the discharge still appeared at the expected times when gastric contractions had been abolished by the action of drugs that are known to block both pre- and post-ganglionic transmission demonstrates, incidentally, that the gastric efferent discharge is being transmitted at the cervical level in preganglionic fibres and that the post-ganglionic fibres are cholinergic, since probanthine hydrochloride exerts an action similar to atropine (Goodman & Gilman, 1956). These results are also consistent with the observation of Iggo (1956), who

measured the conduction velocities of gastric efferent fibres in cervical and thoracic vagi by a compound action potential method and showed that they had conduction velocities in the range (1–16 m/sec) that would be expected for parasympathetic preganglionic axons.

The efferent discharge could be classified into several distinct types and it is reasonable to conclude that different structures were innervated by the various classes. One possibility to be considered, however, is that the various patterns resulted from an inability to standardize experimental conditions and not from the existence of several different categories of unit. The evidence for rejecting this hypothesis is that dissimilar types of discharge pattern were often seen in successive units during the course of an experiment on the same sheep, that units with different types of pattern could be recorded simultaneously in multi-unit records, and that each pattern was distinctive and for any individual unit remained basically constant for several hours, despite reflex and incidental changes in experimental conditions.

The evidence for the hypothesis that each type of gastric unit innervated a functionally and anatomically distinct region of the reticulo-rumen is strongest for Types I and IV. The Type I units are considered to innervate the reticulum, because (*a*) vagal denervation abolishes reticular contractions, (*b*) the biphasic contraction peculiar to the reticulum was matched by a biphasic efferent discharge pattern in the Type I units, (*c*) the interval between the first and second peaks of impulse discharge was equal to the interval between the peaks of the first and second reticular contractions, (*d*) the ratio of the first to second peak spikes frequencies was similar to the ratio of the amplitudes of the first and second reticular contractions. Further support for this identification was that the interval between the peak of the second discharge preceded the peak of the second contraction by an interval of 1.8 sec, only slightly longer than the latency of reticular contractions elicited by electrical stimulation of the cervical vagus at 20/sec. There was not always an exact match between the discharge pattern of the unit and the ensuing reticular contraction but this is what would be expected, since the reticular contraction would be the resultant of the activity in a larger number of these Type I units.

The Type IV gastric efferent units, for reasons similar to those detailed above for the Type I units, are associated with, and considered to give rise to, contractions of the dorsal ruminal sac. The discharge pattern matched the rate, form, duration and amplitude of the dorsal ruminal sac contractions, the peak frequencies occurred at appropriate intervals before ruminal contraction and, in particular, a discharge in Type IV units was present only when dorsal ruminal sac movements also occurred.

The functions of Type II and Type III units are not so clear. The main

part of the discharge preceded the peak of reticular contraction and it is likely, therefore, that these units are involved in movements either of the reticulum, or of adjacent structures that contract at the same time, e.g. the reticulo-ruminal fold, or the oesophageal groove. Although visual examination of the left side of the reticulum confirmed that the whole wall contracted in the biphasic manner expected from manometric records, it was not possible to observe directly the medial wall and the structurally specialized region around the oesophageal groove. Another possibility is that the Type II units might innervate the reticulo-ruminal fold, which has been shown by Lucas & Dougherty (1964) to contract monophasically at the same time as the biphasic contraction of the reticulum.

The function of the Type V units was not at all clear, except that the discharge was clearly related to the presence of gastric contractions. The fact that these low frequency units could be picked up from multi-unit strands before subdivision makes us confident that no gastric units were being overlooked owing to this particularly technical factor of low frequency, and perhaps small amplitude, in multi-unit recordings.

The Type VI and VII gastric efferent units, although few in number, had very distinctive discharge patterns and they might be associated with the movement of certain structures such as sphincters or pillars. Published data is available only for movements of the reticulo-ruminal orifice or sphincter, omasal canal, omasum and abomasum (Balch, Kelly & Heim, 1951; Borgatti & Matscher, 1958; Stevens & Sellars, 1960; Ohga *et al.* 1965). The results indicate that the movements are not identical in sheep and cattle. Ohga *et al.* (1965) report a weak tonic contraction of the omasal canal in sheep during the quiescent phase of the gastric cycle, a relaxation during the reticular contraction, followed by a powerful contraction of the canal (lasting about 8 sec) just after the second reticular contraction. This contraction was succeeded by a relaxation and then a further slowly developing contraction. Omasal movements were not examined in the present experiments, so that we cannot test the hypothesis that Type VII units could have accounted for omasal canal activity, although the behaviour of these units in our experiments does match the omasal canal activity reported by Ohga *et al.* (1965). No muscular activity corresponding to the Type VI units has come to our attention, and we are unable to suggest a functional role for these units.

There are only two other published investigations of gastric efferent vagal activity; Dussardier (1960) and Beghelli *et al.* (1963). The latter recorded electrical activity from the medulla oblongata that had the same rhythm as gastric motility. They used curarized lambs (20–25 days old) anaesthetized with chloralose. Spontaneous reticular contractions, as would be expected, were absent, since reticulo-ruminal structure and

action in lambs of this age, according to Wardrop & Coombe (1961), would still have been in a very primitive stage of development. Reticular contractions were evoked by distending the reticulum or stimulating the central end of a cut abomasal nerve. The records obtained from the dorsal motor nucleus of the vagus showed multi-unit activity, and it was possible to identify several different types of discharge on the basis of spike, amplitude and frequency and the temporal relationship of the discharge to the reticular contraction. The interval between the peak of the discharge (in those units having an early, high frequency discharge) and the peak of the reticular contraction was 1.2–2.0 sec, similar to our Types I, II and III units. It is likely therefore, that these and our units are identical. In a study involving mature sheep in which reflex reticular movements were present, Howard (1966) has also recorded several kinds of unitary discharge in the dorsal motor nucleus of the vagus. Some of his units correspond to our Types I and IV, but in addition there were several others, including interneurons.

Dussardier (1960, Fig. 20) illustrates thirteen examples of efferent activity recorded in his cross-sutured animals. With two exceptions they could be incorporated in our classification. The principle differences were that the number of spikes per discharge, and the peak spike frequencies were generally less than those we recorded and there was also a preponderance of units with a late discharge, which we would have grouped together as Type V units. In addition there were two examples with a very late low frequency discharge, which we did not find. Dussardier does not say how common the various examples were, except that units with an early discharge were relatively uncommon. He recorded very infrequently from units having a tonic discharge equivalent to Type VII and others with a very early brief discharge similar to one of our Type I units (no. 25). The prevalence of units having a late discharge in Dussardier's experiments on conscious animals is probably due to the higher level of ruminal activity in his preparations.

From our results, together with those of Dussardier (1960) and Beghelli *et al.* (1963) it is now clear that the total efferent discharge passing from the gastric centres to the reticulum and rumen consists of several distinct and independent types of unitary activity. Each of these has patterns related to the form, duration, and amplitude of movements of some particular part of the stomach and occurs in a sequence that could produce a co-ordinated series of movement in the reticulum and rumen. It is our view that they actually cause the movement. The orderly sequence of events that constitutes the primary gastric cycle can, therefore, be attributed to this co-ordination of efferent output and consequently it arises in the gastric centres. This view is contrary to that of Morrison & Habel (1964)

who argued that the existence of multi-synaptic pathways in the myenteric plexus of the ruminant stomach implied that 'co-ordination' could be largely a peripheral phenomenon. It seems to us much more likely that the complexity of these myenteric pathways is related to the large size of the ruminal walls rather than to the need for a peripheral co-ordinating mechanism. The internal organisation of the gastric centres is probably very complex; e.g. Howard (1966) has established that there are powerful inhibitory interactions within the dorsal motor nucleus of the vagus itself.

Several firm conclusions can be drawn from the present investigation. First, halothane-anaesthetized sheep are suitable for acute experiments on the reflex mechanisms underlying reticular motility but may be less suitable for studies of ruminal motility. Secondly, there are at least seven different types of gastric efferent fibres with characteristic patterns of discharge. Except for one of these groups, there is no resting discharge in efferent fibres during the quiescent phase of the gastric cycle. The form, duration and peak frequency of certain types of units can be related to the form, duration and amplitude of the movements of particular regions on the reticulum or rumen. Finally, the co-ordination of the complex sequence of movements comprising the primary cycle of gastric contraction in ruminant animals is a function of the 'gastric centres' in the hind-brain, through their ability to determine the forms, durations, and spike frequencies and temporal interrelationships of efferent discharges in nerve fibres innervating different parts of the stomach.

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An Electrophysiological Investigation into the Sensory Innervation  
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The frequency and form of reticulo-ruminal (gastric) contractions and the secretion of saliva are reflexly altered by changing the physical conditions inside the fore-stomachs. These changes are sensed by gastric mechanoreceptors and the location, type and properties of those in the reticulum and cranial regions of the rumen were examined in halothane-anaesthetised sheep. Spontaneous gastric movements were recorded manometrically. Afferent nerve impulses were recorded by a 'single fibre' technique. Manual explorations to locate receptors were made through a large ruminal fistula. Receptors were most numerous in the medial walls of the reticulum, reticulo-ruminal fold and cranial dorsal ruminal sac and less numerous in other parts of these structures and in the ventral rumen. They responded to stretch but not to pinching or transmural compression. With reticular volumes greater than 400 ml a resting discharge was present and was increased both by passive distension and during a spontaneous contraction. The receptors were therefore 'in series' with the contractile tissues and the afferent discharge was 'slowly adapting' in response to a change of conditions. Mechanoreceptors in the lips and floor of the oesophageal groove responded to both stretch and pinching and appeared to adapt more quickly. The conduction velocity in afferent fibres was 4-18 m/sec. The abundance of mechanoreceptors in the locations described could account for the reflexogenic potential of these zones in relation to gastric motility and salivary secretion.

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