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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 fore-stomachs 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 ½ 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 (P 23) 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 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

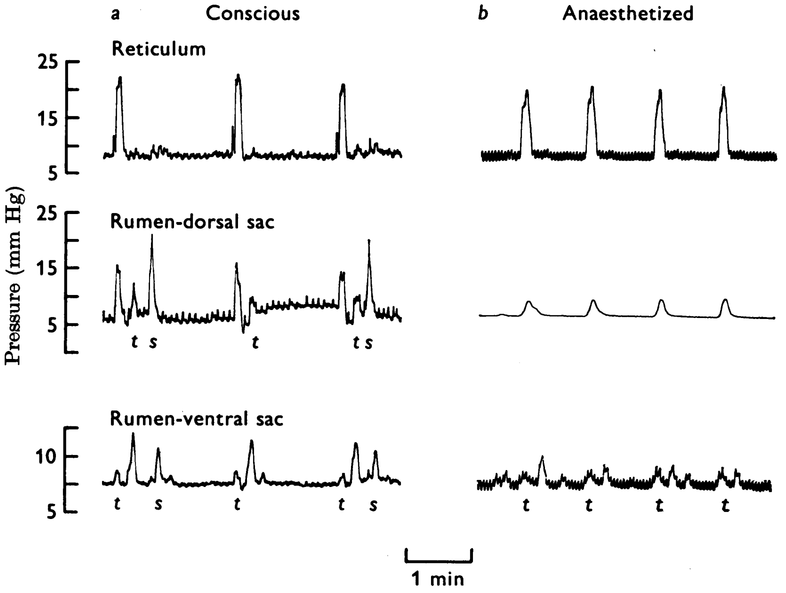

Fig. 1. Movements of the reticulo-rumen in (*a*) a standing conscious sheep fitted with a rumen cannula and (*b*) a halothane-anaesthetized sheep lying on its right side. The records were made under identical conditions with air-filled balloons connected by polythene tubing to strain-gauge manometers. 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 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

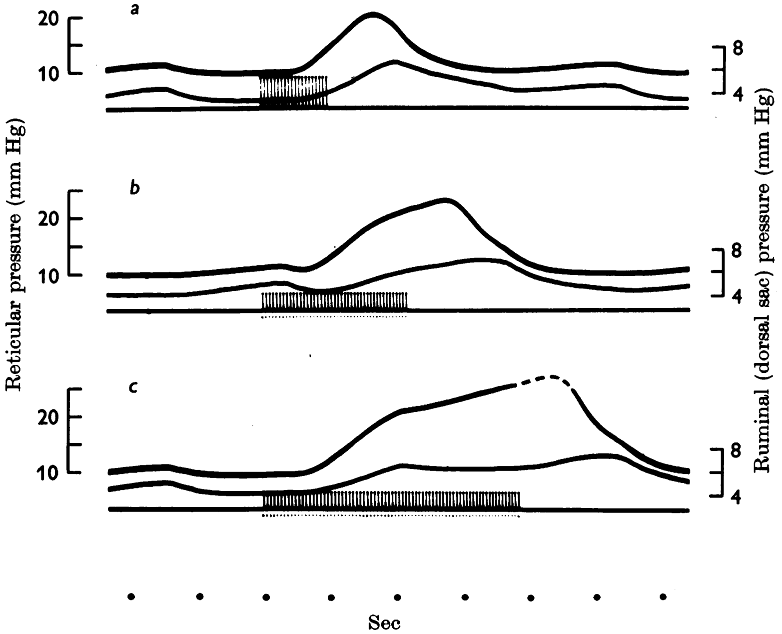

Fig. 2. Contractions of the reticulum (*upper trace*) and dorsal sac of the rumen (*middle trace*) caused by electrical stimulation at maximal intensity (*lower trace*) of efferent fibres in the intact left cervical vagus in a halothane-anaesthetized sheep. Maximal contractions were obtained only when 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*).

reticular contraction was about 1·3 sec (1·2–1·5 sec). This interval is slightly shorter that 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 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-

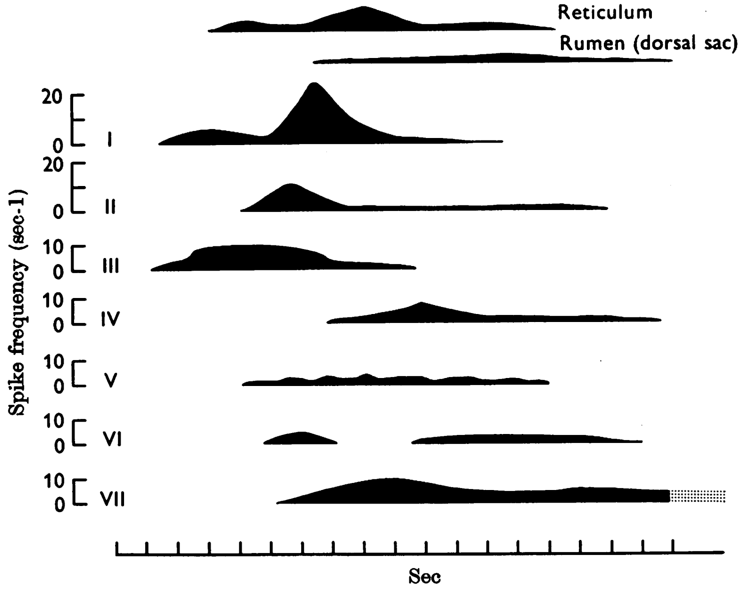

Fig. 3. The temporal relationship between gastric (reticulo-ruminal) contractions constituting the 'primary cycle' and the discharge patterns in the various types of efferent units. The (second) peak of the reticular contraction has been used as the ordinate for alignment of the frequency curves constructed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

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, 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 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.

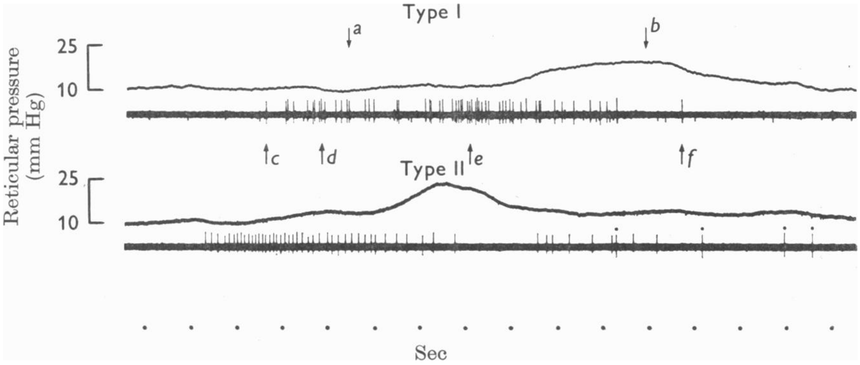

Fig. 4. The discharges of Types I and II efferent unit (*lower traces in each record*) and the corresponding reticular contractions (*upper traces*). A Type IV unit fires with a low frequency in the later part of the lower record (*spikes marked with a dot*).

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*.

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*.

		Reticular contraction		Efferent unitary discharge							
					Peak frequencies			Intervals			
Unit no.	Vol. in balloon (ml.)	Amplitude at *b* (mm Hg)	Duration *a–b* (sec)	Total no. spikes	1st at *d*	2nd at *e*	Duration *c–f* (sec)	*c–e* (sec)	*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	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	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

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

			Reticular contraction		Efferent unitary discharge					
								Intervals		
Type	Unit no.	Vol. in balloon (ml.)	Amplitude at *b* (mm Hg)	Duration *a–b* (sec)	Total no. spikes	Peak frequency (sec^{-1})	Duration *c–f* (sec)	*c–e* (sec)	*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	**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	9·0	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.

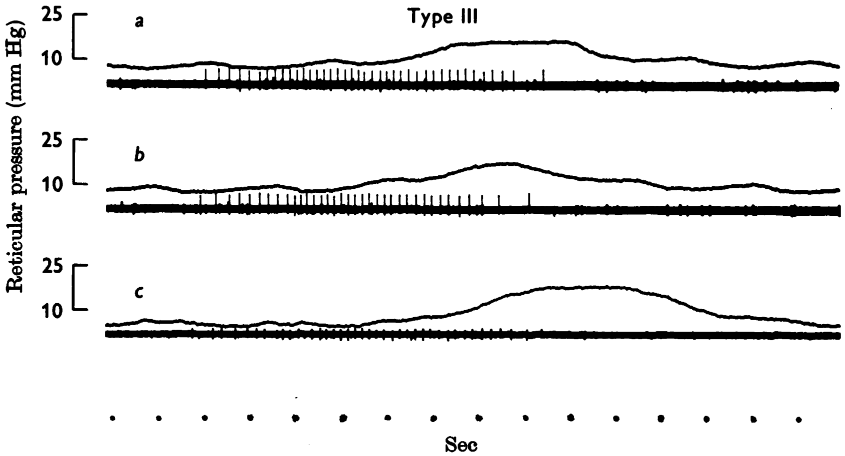

Fig. 5. The discharges of a Type III unit (*lower traces*) and the corresponding reticular contractions (*upper traces*). *a* and *b* are consecutive reticular contractions recorded under 'standard' conditions: the efferent discharges are similar but the contractions appear to be slightly different because they are superimposed upon pressure fluctuations due to respiratory movements. *c* is from the same unit 5½ 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 phase of the primary cycle of gastric contractions. For these reasons the Type V units are regarded as a distinctive group.

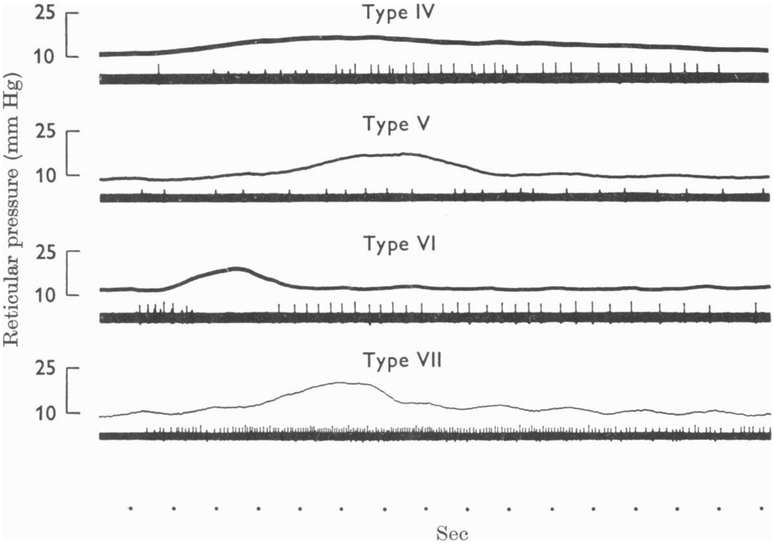

Fig. 6. Examples of gastric efferent discharges (*lower traces*) in units innervating structures other than the reticulum. In each record the *upper trace* shows the reticular contraction. *a* shows both a Type IV unit (*large spikes*) and a Type V unit (*small spikes*). The main part of the Type IV 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 up to 10 sec before the start of the reticular contraction.

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 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

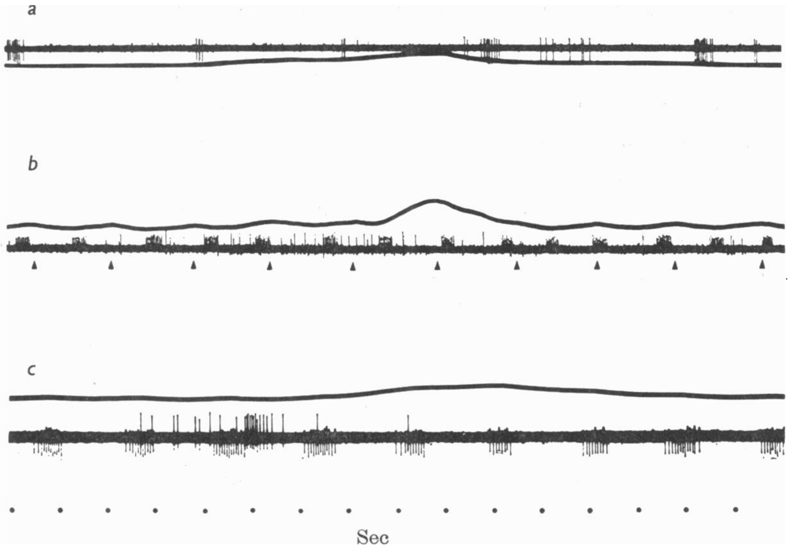

Fig. 7. Examples of single unit discharges not associated with gastric movements. *a* shows the discharges produced during swallowing in a unit innervating the oesophagus. *b* shows bursts of activity which were in phase with the arterial pulse. They are clearly not related to respiration (end of inspiration 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).

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 function 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 interneurones.

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 and would 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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