J. Physiol. (1971), **219**, *pp.* 587–610
With 6 text-figures
Printed in Great Britain

THE LOCATIONS AND ACTIVITIES OF MEDULLARY NEURONES ASSOCIATED WITH RUMINANT FORESTOMACH MOTILITY

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(*Received 4 June 1971*)

SUMMARY

1. Neuronal activity bearing a temporal relationship with spontaneous reticulo-ruminal movements was recorded with micro-electrodes from the medulla oblongata in halothane-anaesthetized sheep. Recording sites were located histologically after causing electro-coagulation at the micro-electrode tip.

2. One hundred and forty-four gastric units were recorded from the dorsal vagal nucleus and up to 1 mm dorsal and lateral to the nucleus between transverse planes 1 mm caudal, and 4 mm rostral, to the obex. It is considered that records were obtained from the regions of cell bodies.

3. The discharges of thirty-two vagal preganglionic motoneurones were identified by an antidromic collision technique. Conduction velocities ranged from 10–26 m/sec. They were located in the dorsal vagal nucleus and up to 0·5 mm dorsal and lateral to the nucleus. The majority of motoneurones innervated either the reticulum or the rumen. One ruminal unit discharged during both primary and secondary cycle movements.

4. One hundred and twelve units which were not orthodromically or antidromically activated by stimulating the vagus nerves were considered to be interneurones. Four types were distinguishable on the basis of their patterns of discharge during primary cycle movements.

5. The discharges of Type A interneurones resembled those of gastric motoneurones, having no resting discharge between contraction cycles. Their discharges were temporally related to either reticular contractions or rumen contractions during primary and secondary cycle movements.

6. Types B and C interneurones have resting discharges which, respectively, increased and either decreased or stopped during each primary cycle movement.

7. Discharges of only three units identified as interneurones resembled the discharges of gastric vagal afferent units.

INTRODUCTION

Ruminant forestomach movements are dependent upon the integrity of the vagal nerves (Duncan, 1953). Iggo (1951, 1956) concluded that reflex centres exist at the level of the hind-brain, because forestomach movements persisted after sectioning the brainstem through the mid-brain and through the spinal cord. The regions and structures of the hind-brain functioning as reflex centres have been investigated (i) by stimulation with implanted electrodes (Bell & Lawn, 1955; Andersson, Kitchell & Persson, 1959; Dussardier, 1960), (ii) by making lesions (Beghelli, Borgatti, Mavrulis & Parmeggiani, 1964), (iii) by observing retrograde degeneration after sectioning gastric nerve fibres (Bell, 1960; Dussardier, 1960) and after ablation of the reticulum and rumen (Szabo & Dussardier, 1964), and (iv) by using micro-electrodes to record neuronal activity temporally related to the forestomach movements (Beghelli, Borgatti & Parmeggiani, 1963; Howard, 1968; Leek, 1968). These investigations show general agreement on the regions of the hind-brain involved, but controversy exists on the precise structures involved within these regions. The disparities arise because hitherto there has been a failure to differentiate between interneurones, motoneurone cell bodies and axones, and vagal sensory fibres involved in activating forestomach movements.

In this investigation neuronal activity in the gastric centres has been recorded with microelectrodes. Motoneurone activity has been identified by an antidromic spike collision technique and the recording sites have been marked by causing electro-coagulation at the tip of the micro-electrode. Tip positions have been located afterwards by histological staining of serial transverse sections of the medulla oblongata.

This work is an extension of that briefly described by Leek (1968, 1969*a*) and by Harding & Leek (1970, 1971).

METHODS

Experimental animals. Seventy-eight adult Cheviot, Blackface and cross-bred sheep were used. They were maintained on a diet of hay and water *ad lib* for at least 2 weeks before the experiment.

Anaesthesia. Anaesthesia was induced with 4 % halothane in O_2 administered by a face mask. After tracheal intubation, anaesthesia was maintained with 1–3 % halothane in O_2 delivered by a closed-circuit system. A Harvard respiration pump was used to ventilate sheep which failed to respire adequately. Concentration of CO_2 in the respired gases was monitored with a Beckman CO_2 analyser (model LB–1).

Preparatory surgery. Following a median laparotomy, a 1 l. rubber balloon was inserted and tied into the reticulum at its ventral pole. A small balloon or an open tipped catheter was inserted into the contents of the dorsal sac of the rumen through an acutely implanted cannula. After the insertion of recording balloons, the sheep was placed on a canvas sling in a position of brisket recumbency. Ear bars were

inserted, and the head held in a ventroflexed position by a jaw clamp. The occipital bones and the cerebellum were removed. Haemorrhage was controlled with Surgicel (Ethicon). The cavity thus formed exposed the dorsal surface of the hind brain from 1 cm caudal to the obex to 2 cm rostral to it, and it was filled with warm liquid paraffin.

Both vagi were exposed in the neck, and a pair of Ag/AgCl stimulating electrodes was placed around each nerve.

Recording movements of the reticulo-rumen. Primary cycle contractions of the reticulo-rumen were evoked reflexly by inflating the reticular balloon with 200–800 ml air. Contractions of the reticulum were recorded manometrically under isometric conditions. Movements of the rumen were recorded as small pressure fluctuations by the small rubber balloon or the open-tipped catheter in the dorsal sac. Pressures within the reticulum and rumen were monitored with strain-gauge transducers (Consolidated Electrodynamics, Type 4/326/L212).

The presence of continuous cycles of forestomach motility was essential to this study. The problems involved in maintaining these in halothane-anaesthetized sheep have been discussed by Iggo & Leek (1967*a*). In the present study, an additional factor which prejudiced movements of the reticulo-rumen was the extensive haemorrhage which sometimes resulted from the removal of the cerebellum.

Recording neuronal activity from the medulla. Unitary activity was recorded with electrolytically sharpened tungsten micro-electrodes, which were insulated with a single coat of Insl-X and had tip impedences of 5–12 MΩ. The recorded activity was amplified and displayed using a cathode follower, preamplifier and oscilloscope. Recording conditions were often unstable due to pulsation of the medulla oblongata with a cardiac or a respiratory rhythm, and records of unitary activity were frequently lost after only a few minutes. Attempts to limit the pulsation with paraffin wax or agar gel were usually unsuccessful. Records of activity from the units reported in this study were obtained during at least three successive primary cycle contractions of the forestomach, and, for the most part, for periods of more than 15 min. A few units were held for more than an hour.

Localization of micro-electrode recording sites. The recording sites of unitary activity were localized by passing a current of 20–40 μA through the tip of the micro-electrode for 5–10 sec. Subsequently the medulla was fixed in formol-saline, embedded in wax, and serially sectioned at 25 μ. The sections were stained for Nissl substance with Toluidine blue. The coagulated tissue appeared in the sections as a deeply stained spot 0·1–0·2 mm in diameter.

RESULTS

Definition of gastric centres. In the context of the present investigation the term 'gastric centre' refers to one or other of the bilaterally paired reflex centres in the medulla oblongata which appear to be involved with ruminant forestomach motility, insofar as the discharges in gastric centre neurones bear a temporal relationship to movements of the reticulum and rumen. This is comparable to the criteria given by Iggo & Leek (1967*a*) for the identification of efferent gastric vagal units during 'single fibre' studies on the cervical vagus of sheep. They presumed that efferent vagal activity was 'gastric' if (*a*) a discharge of impulses appeared, or an existing discharge was modified, at the same period during each gastric contraction, (*b*) if this discharge did not occur during the inactive phase of the cycle of

contractions, and (*c*) if the discharge was appropriately changed with both spontaneous and reflexly induced variations in the amplitude and frequency of gastric movement. This third criterion of Iggo & Leek (1967*a*) is not entirely applicable to the present study, as the discharges of many gastric centre neurones were not altered by spontaneous and reflexly induced variations in the amplitude of the forestomach movements, although the temporal relation between the discharges and the contractions was constant.

In defining 'gastric centres' in this way, we are, nevertheless, aware that other parts of the brain stem may play some part in the complex reflex integration and co-ordination processes involved in forestomach motility, even though the neuronal activity in these regions may not fulfil the above criteria and has failed to be identified.

The topographical location of the gastric centres. The first experiments were designed to determine the topography of the gastric centres as a prerequisite to recording sample activity of its component units (Leek, 1968). Micro-electrodes with tip impedences of about 5 MΩ were used, as these were found to record multi-unit gastric activity from a wider area than that recorded by the micro-electrodes of higher impedence used in later experiments. The obex was used as a reference point and the positions of micro-electrode penetrations were expressed by the co-ordinates of transverse and sagittal planes relative to the obex. The medulla was explored systematically on a grid plan with intervals of 0·5 mm between each penetration. The micro-electrode was advanced vertically downwards in steps of 100–200 μ. By this means, neuronal discharges related to movements of the reticulorumen were detected and a three-dimensional map of the gastric centres, as defined above, was obtained.

The gastric centres are bilaterally paired. Each centre is spindle-shaped and diverges from a sagittal plane in a rostro-lateral direction. The rostrocaudal limits are approximately 1 mm caudal to the obex and 6 mm rostral to the obex. The locations of micro-electrode penetrations recording gastric neuronal activity are shown in Fig. 1.

In six sheep, twenty micro-electrode penetrations were made between transverse planes 1 mm caudal and 3 mm rostral to the obex, and during the withdrawal of the micro-electrode, the dorsal and ventral limits of the region from which gastric centre activity had been recorded were marked by micro-coagulation at the electrode tip. This procedure rendered the micro-electrode unusable for further recording, but it allowed the zone of gastric centre activity to be identified during subsequent histological examination. This zone involved the dorsal vagal nucleus and the grey reticular formation up to 1 mm dorsal and lateral to the nucleus (Fig. 2*A*). Gastric centre activity was recorded from the grey matter ventral to the nucleus in only two penetrations and medial to the nucleus in no cases.

In several experiments an attempt was made to trace gastric neuronal activity laterally from the centres by making penetrations at 0·25–0·5 mm intervals. In no instance could activity be recorded at more than 1 mm lateral to the dorsal vagal nucleus even though, on subsequent histological examination, micro-electrode tracks were found to pass through tracts of vagal fibres. In the discussion (p. 604) it is concluded from this and other

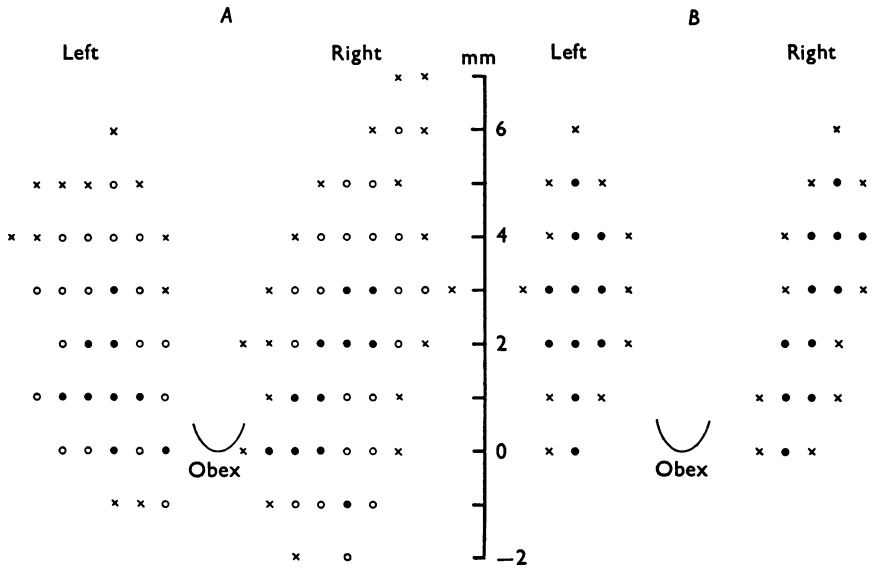

Fig. 1. Plan view of dorsal surface of the medulla *in vivo* showing the locations of micro-electrode penetrations at which gastric neuronal activity was recorded.

A shows the results of 563 penetrations in seventy-eight sheep; at points marked × no gastric activity was found, at ○ gastric activity was found in less than 50 % of the penetrations and at ● gastric activity was found in more than 50 % of the penetrations.

B shows the results of forty-four penetrations in one sheep. At × no gastric activity was found and at ● gastric activity was recorded.

findings, that the records of gastric neuronal activity obtained under the conditions of these experiments were most probably from the vicinity of cell bodies rather than from axones.

Differentiation between types of gastric centre neurones by electrical stimulation of the cervical vagi. In later experiments the objects were to record unitary activity from gastric centre neurones and to identify the type of neurone from which the unitary activity was being recorded (Harding & Leek, 1970). Unitary records were most easily obtained using micro-electrodes with a slightly higher impedance (8–12 MΩ) than those used in the earlier experiments.

As an aid to differentiating between the different kinds of gastric centre neurones, their responses to electrical stimulation of the cervical vagi were examined. Stimulating electrodes were placed on each intact vagus nerve in its mid-cervical region. The stimuli (10–15 V, 0·5–1 msec, 1–3 mA) were supramaximal for exciting efferent gastric vagal fibres, judged by the

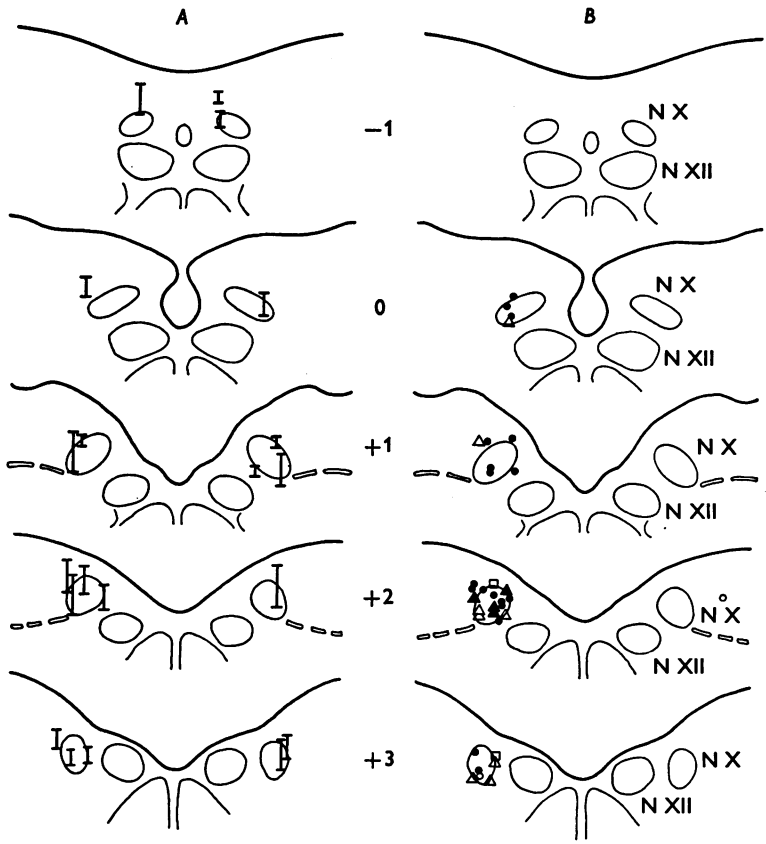

Fig. 2. Transverse sections of the dorsal region of the medulla showing the locations at which gastric neuronal activity was recorded. Plane 0 is at the level of the obex, +1, *etc*; is 1 mm, *etc*. rostral to the obex and −1 is 1 mm caudal to the obex.

A shows the regions of twenty penetrations in six sheep from which gastric activity was recorded. On withdrawing the micro-electrode the lower and upper limits of the regions were marked by point electro-coagulation and were identified by subsequent histological examination.

B shows points in sheep at which activity was recorded from units which had been identified as vagal preganglionic motoneurones (●), gastric centre interneurones (Type A △; Type B □; Type C ▲) and gastric afferent-type interneurones (○). After each recording a micro-coagulation point was made. Details in text.

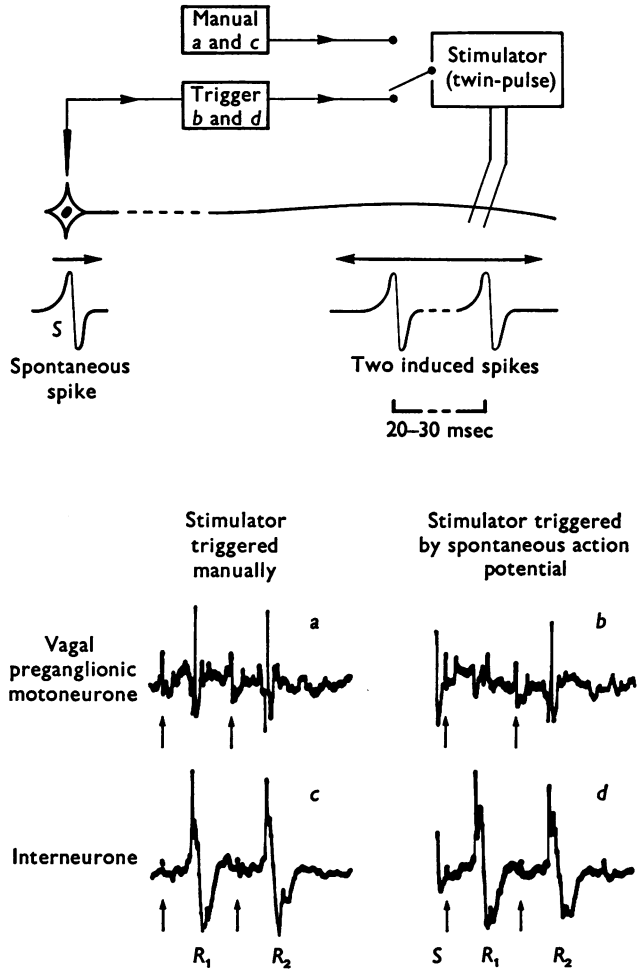

Fig. 3. Cervical vagal stimulation used as an aid to differentiating between the different kinds of gastric centre neurones.

In the upper part the stimulator was set to give, after a delay of less than 4 msec, twin pulses with an interval of 20–30 msec between them, when it was triggered either manually or by a recorded action potential.

In the lower part, the responses evoked at the recording sites of two gastric centre neurones by twin pulse vagal stimulation are shown when the stimulator was triggered manually (*a* and *c*) and by action potentials spontaneously discharged by the units (*b* and *d*). The stimuli (artifacts marked with arrows) evoke responses R_1 and R_2. In both cases, when the stimulator is triggered manually (*a* and *c*) the responses evoked by both stimuli are similar. When an action potential from a vagal preganglionic motoneurone triggers the stimulator (*b*), the response (R_1) following the first stimulus lacks a component which corresponds to the triggering spike (*S*). When an action potential from an interneurone triggers the stimulator, the responses evoked by both stimuli are identical (*d*).

effectiveness of a short train of stimuli of low frequency to produce a maximal directly evoked contraction of the reticulo-rumen. The stimulator was set to give, after a delay of less than 4 msec, twin pulses with an interval of 20–30 msec between them. The stimulator could be triggered either manually or by a spike recorded from a gastric centre neurone (Fig. 3).

A response was evoked at the recording site of each of 144 gastric centre units recorded from forty sheep when the cervical vagus, ipsilateral to the recording site, was stimulated with single pulses. In each case, there was no response when the contralateral cervical vagus was stimulated.

The responses evoked at the recording sites of the gastric centre units fell into four classes: (1) those in which there was a field potential, with no recognizable spikes (twenty-three units); (2) those in which the evoked response consisted of a field potential upon which were superimposed 1 or more spikes which were either of variable latency or too low in amplitude to have been antidromic or orthodromic impulses from the unit under study (sixty-two units); (3) those in which the response included one or more spikes of constant form and latency which resembled in form and amplitude the spikes spontaneously discharged by the unit under study, but which were not cancelled when the stimulator was triggered with action potentials spontaneously discharged from the unit (twenty-seven units, Fig. 3); (4) those which included a spike of constant form and latency which could be cancelled through collision with spontaneously discharging spikes from the unit (thirty-two units, Fig. 3).

The eighty-five units at which the responses evoked by vagal stimulation included either no spike, or spikes which could not be regarded as antidromic or orthodromic because of their varying latency or insufficient amplitude, were considered to be gastric centre interneurones.

The thirty-two units at which the evoked response included a spike which could be cancelled by a spontaneously occurring spike from the unit were considered to be vagal preganglionic motoneurones. The possibility of these units being neurones which were activated by recurrent collaterals of motoneurone axones is discussed later (p. 604).

The remaining twenty-seven units at which the response evoked by vagal stimulation included spikes of constant latency and amplitude could, theoretically, be either vagal sensory units or interneurones synaptically activated by vagal stimulation. It is considered that these units were interneurones and not vagal afferent units (1) because the pattern of discharge of each unit, during contractions of the reticulo-rumen, differed from those of gastric afferent units recorded from the cervical vagus by a 'single-fibre' technique (Iggo, 1955; Leek, 1969*b*); (2) because the responses of these units to induced changes in the tension of the walls of the reticulo-rumen differed from those of the vagal afferent units recorded by Iggo (1955) and

Leek (1969*b*) and (3) because units with similar patterns of discharge to these twenty-seven units have been recorded from the gastric centres after both vagus nerves had been sectioned in the neck and the spinal cord sectioned at a cervical level (Beghelli, Harding & Leek 1971).

Gastric vagal preganglionic motoneurones

Twenty-nine motor units were recorded as single units. In each of three other cases, discharges from other gastric units were present, but the discharges from the motor units could be recognized by their ability to cancel repeatedly a component of the response evoked by vagal stimulation.

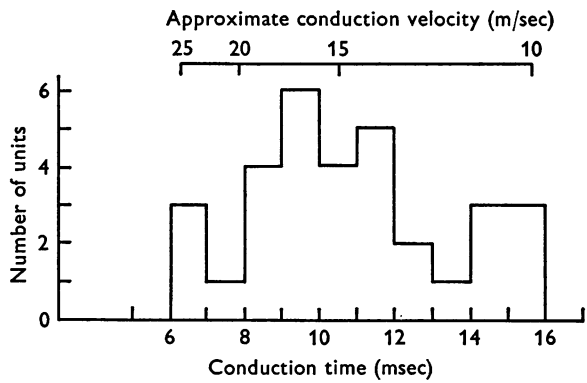

Fig. 4. Histogram of conduction times between the gastric centre and the cervical vagus in identified vagal gastric preganglionic motoneurones. Assuming a mean conduction distance of 15·7 cm, the approximate conduction velocities are shown on the scale above the histogram. See text for details.

Conduction velocities

The conduction time in the efferent axon, between the point of stimulation of the vagus and the medullary recording site was taken as the interval between the stimulus artifact and the antidromic impulse evoked in the medulla. In three experiments, the conduction distances were measured as 14, 16 and 17 cm (mean 15·7 cm), after dissection *in situ* of the vagus nerve. These values assume a direct path for the motor axons through the medulla from the vagal rootlets to the recording site. The conduction velocities of five gastric motor units located in these three sheep were 14·3, 16·0, 16·5, 18·0 and 23·4 m/sec. Conduction times for the other twenty-eight gastric motor units ranged from 6·0–15·5 msec, but conduction distances were not measured. Assuming a conduction distance of 15·7 cm in these experiments, the range of approximate conduction velocities is 10–26 m/sec (Fig. 4).

TABLE 1*a* and *b*. The discharges in 138 identified gastric centre neurones and their temporal relationship to reticular contractions. They were recorded from thirty-nine sheep. Intervals referring to periods before or after the peak of the reticular contraction are tabulated, respectively, as negative or positive. Ranges of values are given in brackets.

The time of maximum discharge frequency of units which discharge at a uniform frequency was taken as the mid-point of the discharge. Values marked† refer to the period of increased discharge; those marked * refer to the period of reduced discharge; and those marked × refer to the minimum discharge frequency

			Unitary discharge				Intervals to peak of reticular contraction		
	Type of unit	No. of units	Resting discharge (sec^{-1})	Total no. of spikes	Maximum frequency (sec^{-1})	Duration of discharge (sec)	Start of discharge (sec)	Maximum frequency (sec)	End of discharge (sec)
Vagal motoneurones	'Early' biphasic	8	—	48·0 (12–95)	3·7 and 16·1 (1·5–7) and (4–40)	10·1 (7–17)	−6·9 (−4 to −16)	−5·7 and −1·9 (−14 to −4) and (−5 to −1)	+3·2 (+1 to +5)
	'Early' monophasic	12	—	22·4 (6–55)	6·8 (2–22)	6·0 (2–10)	−3·7 (−7 to −1)	−1·9 (−5 to −0·5)	+2·2 (+1 to +5)
	'Late'	6	—	33·0 (8–102)	5·7 (2–12)	10·4 (4–20)	−0·1 (−2 to +2)	+4·0 (−1 to +9·0)	+9·1 (+3 to +18)
Type A interneurones	'Early' biphasic	9	—	39·2 (13–94)	4·3 and 9·7 (2–8) and (6–25)	6·6 (4·5–11)	−6·1 (−8 to −4·5)	−5·0 and −1·4 (−7 to −3·5) and (−2 to −0·5)	+0·4 (−0·5 to +3)
	'Early' monophasic	27	—	18·9 (±3·3 S.E.)	4·3 (±0·4 S.E.)	8·0 (±0·9 S.E.)	−5·1 (±0·5 S.E.)	−2·1 (±0·5 S.E.)	+2·8 (±0·6 S.E.)
	'Late'	22	—	28·0 (±5·4 S.E.)	4·5 (±0·5 S.E.)	10·0 (±1·0 S.E.)	−0·9 (±0·7 S.E.)	+2·5 (±0·6 S.E.)	+9·1 (±1·0 S.E.)
Type B interneurones		19	1·8 (±0·2 S.E.)	—	11·6 (±2·5 S.E.)	12·3† (±1·1 S.E.)	−7·8† (±0·4 S.E.)	−2·7 (±0·2 S.E.)	+4·2† (±0·8 S.E.)
Type C interneurones		32	2·1 (±0·3 S.E.)	— —	0·23 × (±0·1 S.E.)	16·6* (±2·4 S.E.)	−6·5* (±0·8 S.E.)	−4 ± 0·6 S.E. × to +4·5 ± 1·4 S.E.	+10·2* (±2·0 S.E.)
Afferent-like interneurones		3	0·3 (0·2–0·5)	—	5·1 and 11·0 (0·2–8) and and (6–20)	6·7† (5–10)	−2·7† (−2 to −3)	−0·8 and +1·0 (−2·5 to −0·5) and (0 to +2·5)	+4·0† (+2 to +8)

TABLE 1*b*

		Reticular contractions				
		Intervals to peak of reticular contraction				
		Start to peak (sec)	Peak to end (sec)	Amplitude (mm Hg)	Reticular volume (ml.)	Endoreticular pressure (mm Hg)
Vagal moto-neurones	'Early' biphasic	5·3 (3–13)	2·4 (1·5–5)	17·0 (3–31)	650 (400–800)	12·6 (7–22)
	'Early' monophasic	3·6 (2–4·5)	2·9 (2–8)	11·4 (2–31)	617 (450–850)	11·8 (8–16)
	'Late'	4·5 (3·5–6·0)	4·0 (2–7)	20·2 (11–33)	517 (200–800)	9·8 (7–14)
Type A inter-neurones	'Early' biphasic	5·0 (1·5–5·2)	4·7 (1·7–7·0)	13·8 (9–20)	511 (200–800)	10·3 (5–15)
	'Early' monophasic	3·2 (± 0·8 S.E.)	3·4 (± 0·3 S.E.)	11·1 (± 1·4 S.E.)	615 (± 38 S.E.)	11·3 (± 0·9 S.E.)
	'Late'	3·8 (± 0·3 S.E.)	3·5 (± 0·6 S.E.)	11·3 (± 1·5 S.E.)	686 (± 41 S.E.)	12·5 (± 0·9 S.E.)
Type B interneurones		4·0 (± 0·3 S.E.)	3·0 (± 0·3 S.E.)	9·1 (± 1·9 S.E.)	731 (± 17 S.E.)	14·1 (± 1·3 S.E.)
Type C interneurones		3·6 (± 0·3 S.E.)	3·3 (± 0·5 S.E.)	12·0 (± 1·2 S.E.)	630 (± 39 S.E.)	11·6 (± 0·9 S.E.)
Afferent-like interneurones		4·1 (3·2–5·5)	3·9 (1·8–8)	24 (14–33)	633 (500–700)	11·3 (8–14)

Patterns of discharge

Discharges from twenty-nine motor units occurred only during contractions of the reticulo-rumen; no discharges were recorded from these units in the interval between successive forestomach movements (Table 1 and Fig. 5*a*, *b*). Twenty-six of these twenty-nine units were classified as being 'early' or 'late' according to whether the discharges predominantly preceded or predominantly followed the peak of the reticular contraction. Discharges from three units were not enumerated as discharges from other gastric units were present in the same records.

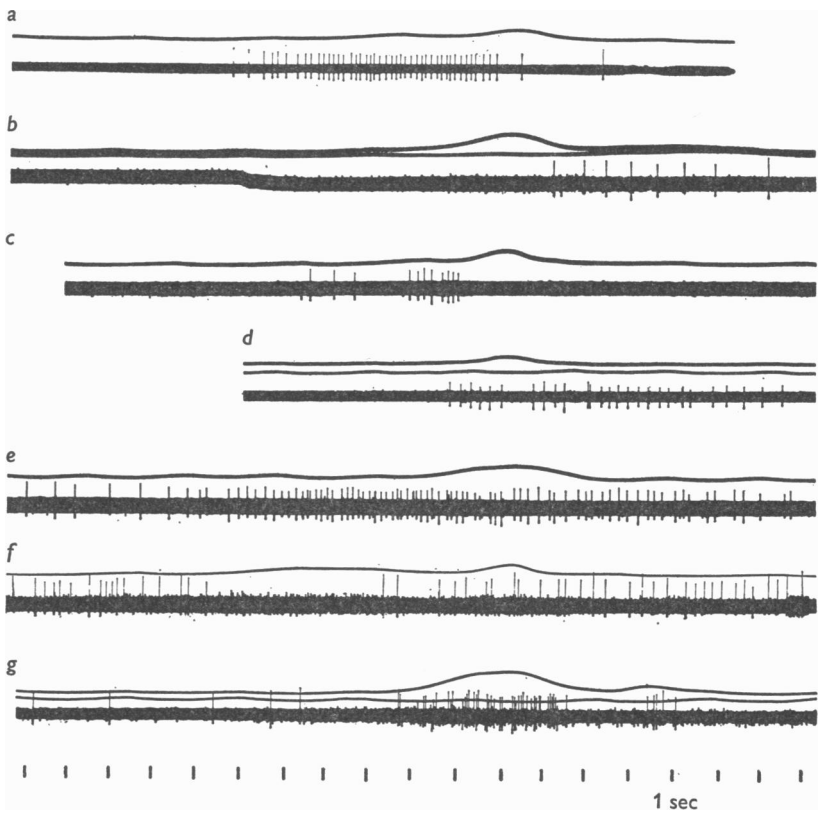

Fig. 5. The discharges of identified gastric centre neurones during primary cycle movements. The upper trace in each record is the pressure recorded from the reticular balloon. In *b*, *d* and *g* the middle trace is the pressure recorded from the mid-dorsal sac of the rumen. The peaks of the reticular contractions are aligned. The discharges are from gastric preganglionic motoneurones with 'early' (*a*) and 'late' (*b*) activities, from Type A interneurones with 'early' (*c*) and 'late' (*d*) activities, from a Type B interneurone (*e*), a Type C interneurone (*f*) and from an interneurone with afferent-like activity (*g*).

'Early' units

Eight of the units with 'early' discharges had a biphasic pattern of discharge, each phase preceding a phase of the biphasic reticular contraction. The discharges commenced 1–4 sec before the intra-reticular pressure began to increase, and the maximum discharge frequency (4–40/sec) occurred 1–5 sec before the peak of the second phase of the reticular-contraction. In each case the maximum frequency of the second phase exceded that of the first. The discharges stopped 1–5 sec after the peak of the reticular contraction.

The discharge patterns of another twelve 'early' units were monophasic. The discharge of seven of these units started 1–6 sec before the pressure began to rise within the reticulum. The discharge from four units showed no clearly defined peak frequency, but in each of the seven units, the maximum discharge frequency (2–22/sec) occurred from 0·5 to 5 sec before the peak of the reticular contraction. The discharge of five 'early' units started after the pressure within the reticulum began to increase. The maximum discharge frequency of these units (4–8/sec) occurred 1–2 sec before the peak of the second phase of the reticular contraction.

'Late' units

The discharges from six units occurred predominantly after the peak of the reticular contraction. Discharges from all but one unit commenced after the start of the reticular contraction, and the discharges of four units did not start until after the peak of the reticular contraction. The maximum discharge frequency (2–12/sec) of five units occurred 1–9 sec after, and in the other 1 sec before, the peak of the reticular contraction.

Discharges from one 'late' unit were recorded during secondary cycle contractions as well as during primary cycle contractions (Fig. 6). The total number of spikes was 2–3 times greater during secondary cycle movements and the pressure increment recorded from the dorsal ruminal sac was twice as great as that recorded during primary cycle movements. During primary cycles, the discharge occurred during or after the peak of the dorsal sac contraction whereas, during secondary cycle contractions, the discharge preceded the peak of the contraction of the dorsal ruminal sac. It is probable that this unit innervated a caudal region of the dorsal sac, as this part of the rumen contracts earlier during secondary cycles, and later during primary cycles, than the mid-region of the dorsal sac from which the pressure record was obtained.

Other motor units

Three motor units discharged at a frequency of 1–2/sec during the period between forestomach movements. The discharges from one unit ceased 5–6 sec before the peak of the reticular contraction, and reappeared at a frequency of 4–8/sec during the reticular contraction, after which the discharges stopped for a period of 4–10 sec. Discharges from the other two units ceased, or became less frequent, for 1–2 sec during the reticular contraction.

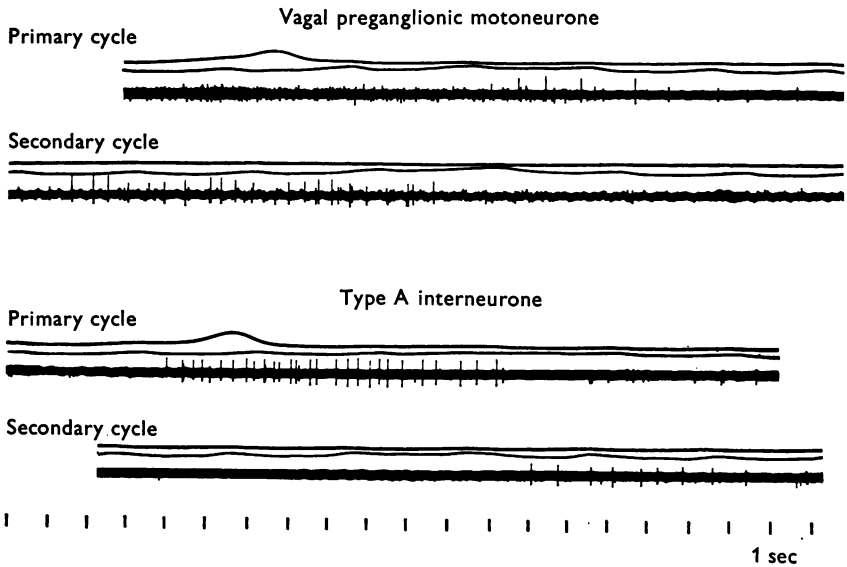

Fig. 6. The discharges of a vagal gastric motoneurone with 'late' activity and of a Type A interneurone with 'late' activity during primary and secondary cycle movements of the forestomach. The upper and middle traces are, respectively, pressure recorded from the reticulum and from the mid-region of the dorsal sac of the rumen. The pressure peaks from the rumen are aligned. Details in text.

Locations of motoneurones

The recording sites of eighteen motor units were located histologically between the level of the obex and 3 mm rostral to this (Fig. 2*B*). Eleven units were localized by coagulating the tissue at the micro-electrode tip at the recording site. The recording site of each of the other seven units was estimated from the location of a coagulation at another point in the same micro-electrode track, after allowing for shrinkage of the tissue during histological preparation. The recording sites of eleven motor units lay within the dorsal vagal nucleus, and seven were located up to 0·5 mm

dorsal and lateral to the dorsal vagal nucleus. During the course of five micro-electrode penetrations, an 'early' and a 'late' unit were located in the same track. In each case, the 'late' unit was recorded at a more ventral level than the 'early' unit.

Gastric centre interneurones

Four distinct types of discharge pattern were recognized among the 112 gastric units identified as interneurones (Table 1*a* and *b*, Fig. 5*c–g*).

Type A interneurones

The discharges of fifty-eight interneurones resembled those of the majority of the above motoneurones. The discharges occurred during contractions of the reticulo-rumen and there was no resting discharge (Fig. 5*c*, *d*). These units have been grouped, as were the motor units, on the basis of the temporal relation between their discharges and the peaks of the reticular contractions as either 'early' or 'late' units.

'Early' units

Discharges from nine 'early' units occurred in two phases each of which preceded a phase of the reticular contraction. The greatest discharge frequency from each unit occurred during the second phase of the discharge. The discharge from each unit commenced before the beginning of the pressure increment within the reticulum. The maximum frequency during the first phase of the discharge was 2–8/sec, and occurred 3·5–7·0 sec before the peak of the second phase of the reticular contraction. The maximum frequency of the second phase of the discharge (6–25/sec) occurred 0·5–2 sec before the peak of the reticular contraction.

The discharges of twenty-seven 'early' units occurred as a single phase and commenced, in twenty-one cases, before the start of the reticular contraction and after this in six cases. In those units from which the discharge reached a peak frequency, this occurred 0–5 sec before the peak of the reticular contraction. The maximum discharge frequency ranged from 1–10/sec and, in each unit, occurred before the peak of the reticular contraction.

'Late' units

Discharge from twenty-two Type A interneurones occurred predominantly after the peak of the reticular contraction. The discharges from six units commenced after the peak of the reticular contraction, and from 1–10 sec before it in the other sixteen units. The maximum discharge frequency ranged from 1–10/sec and occurred from 1 sec before, to 10 sec after, the peak of the reticular contraction.

Discharges from five 'late' units were recorded during secondary cycle movements as well as during primary cycles (Fig. 6). The discharge from two units preceded the peak of the dorsal sac contraction during secondary cycles, whereas during primary cycle movements, the discharge was coincident with the dorsal sac contraction. The discharges from three units occurred predominantly after the peak of the dorsal sac contraction during secondary cycles, but during primary cycle contractions, the discharge from two of these units was coincident with the dorsal sac contraction, and occurred only after it in one unit. The maximum discharge frequency during secondary cycle contractions was 1–3/sec.

Type B interneurones

Nineteen units identified as interneurones discharged continuously during the period between successive movements of the forestomach, and discharged with increased frequency in association with each cycle of contraction (Fig. 5*e*). The discharges during the quiescent phase of the forestomach cycle occurred at 0·2–5/sec, and showed little fluctuation. The discharge frequency began to increase 2–8 sec before the start of the pressure increment within the reticulum, and 5–11 sec before the peak of the reticular contraction. The peak frequency of the discharges (3–42/sec) occurred 1–5 sec before the peak of the reticular contraction. The discharge frequency returned to the basal level from 2 sec before, to 13 sec after, the peak of the reticular contraction.

Type C interneurones

The discharges from thirty-two units occurred continuously during the period between the movements of the reticulo-rumen and ceased, or slowed, during each movement (Fig. 5*f*). The discharge frequency between successive contraction cycles was constant, and ranged from 0·5–5/sec. The discharges from twenty-nine units started to decline in frequency before the start of the reticular contraction. In the three other units, the discharge began to slow before the peak of the reticular contraction.

The minimum discharge frequency ranged from 0–3/sec. In only six units the longest interval between impulses was less than 1 sec, and in the other twenty-six units, the quiescent period during each forestomach movement ranged from 1–40 sec. The period during which minimum discharge occurred started from 16 sec before, to 2 sec after, the peak of the reticular contraction, and ended from 4 sec before, to 32 sec after, the peak of the reticular contraction.

The discharge frequency returned to its resting value from 2 sec before, to 50 sec after, the peak of the reticular contraction.

The discharge patterns of three units were biphasic. In each case there

were two periods of reduced discharge separated by a period of discharge of 1–3 sec duration, when the discharge frequency returned to the resting value.

Afferent-like interneuronal discharges

The discharges from three units identified as interneurones occurred between forestomach movements and their frequency could be increased or decreased at short latency by, respectively, inflating or deflating the balloon in the reticulum. The discharge frequency increased during each primary cycle movement (Fig. 5*g*). The discharge frequency began to increase coincident with, or later than, the start of the pressure increment within the reticulum. The peak discharge frequency (6–20/sec) coincided with, or occurred after, the second peak of the reticular contraction, and was directly related to the amplitude of the reticular contraction. The discharge patterns of these units are distinct from those of Type B interneurones in that the frequency of the discharge from the latter interneurones began to increase before the start of the reticular contraction, and reached a maximum level before the peak of the reticular contraction.

The discharges from these units resemble those recorded by a 'single-fibre' technique from afferent gastric vagal fibres innervating 'in-series' tension receptors in the reticulo-rumen (Leek, 1969*b*). It is probable that the 3 units described here were synaptically activated by sensory fibres of the vagus from 'in-series' tension receptors in the wall of the reticulum.

Locations of interneurones

The recording sites of six Type A interneurones were marked by micro-coagulations between transverse planes 1 and 3 mm rostral to the obex (Fig. 2*B*). Five units were located in the dorsal vagal nucleus and one was 0·5 mm lateral to the nucleus. The recording site of each of a further two Type A units was estimated from the location of a coagulation at another site in the micro-electrode track. Both units were located within the dorsal vagal nucleus.

The sites of two Type B units were marked by micro-coagulation at the recording site, and they were located within the dorsal vagal nucleus. The recording sites of four Type C units were localized by micro-coagulation; three were located within the dorsal vagal nucleus and one occurred 0·3 mm dorso-lateral to the nucleus. One interneurone with 'afferent-like' activity was located 1 mm lateral to the dorsal vagal nucleus and one was located within the nucleus. The micro-electrode track from which another interneurone with afferent-like activity was recorded was 0·5 mm lateral to the nucleus, but the recording site of the unit was not identified.

DISCUSSION

The first question which arises when neuronal activity is recorded extracellularly with micro-electrodes is whether the electrical activity is being recorded from the region of cell bodies, of axones or of both cell bodies and axones. With the micro-electrodes used in the present investigation, it seems most likely that activity was recorded from the vicinity of cell bodies, (1) because no records of efferent gastric vagal motoneurone activity were obtained from the fibre tracts between the dorsal vagal nucleus and the vagal rootlets, (2) because the usual form of recorded action potentials was biphasic rather than the triphasic form of action potentials recorded from axones in a volume conductor (Cooper, Robson & Waldron, 1969), (3) because few afferent-like discharges were recorded, despite the large numbers of reticulo-ruminal tension receptors which are known to be active under these experimental conditions (Leek, 1969*b*), and (4) because the discharges of a single unit could be recorded over a vertical distance of up to 300 μ.

The validity of the conclusions based on the tests used to differentiate between motoneurones, interneurones and afferent-like neurones depends not only on the results of the antidromic spike collision technique but on a knowledge of the discharge characteristics of the various neurones both under standard experimental conditions and when subjected to reflex changes (Iggo & Leek, 1967*b*; Leek, 1969*b*; R. Harding & B. F. Leek, in preparation). Considered alone, the results of the antidromic spike collision technique are open to certain criticisms. Units that have been labelled 'interneurones' may include, theoretically, sympathetic motoneurones and vagal motoneurones with such small diameter axones that they would not be excited by electrical stimulation having the foregoing parameters. At present, it seems unlikely that sympathetic (splanchnic) motoneurones, would arise in the region of the dorsal vagal nucleus or that they would be tonically active either in conscious sheep (Duncan, 1953) or in sheep under the present experimental conditions (Iggo & Leek, 1967*b*). Likewise, the possibility of failing to stimulate all the gastric fibres in a vagal nerve is remote, because stimuli with the parameters used have been shown to excite vagal C fibres in a different investigation (R. Harding & B. F. Leek, unpublished observations). A criticism of labelling some units as 'vagal motoneurones' arises because the antidromic spike collision test would not distinguish between motoneurones and neurones, such as Renshaw cells, activated by recurrent collateral branches of the motoneurone. If the Renshaw cell activity has a 1 : 1 relationship with the motoneurone activity, records from Renshaw cells would reflect motoneuronal discharges with a synaptic delay which would be an insignificant fraction of the conduction

time along these slow efferent gastric vagal fibres. Renshaw cells typically do not have 1:1 discharge ratio with a motoneurone (Renshaw, 1946) and would therefore not reflect the activity of a single motoneurone. Therefore, each electrical stimulus applied to an entire vagus might be analogous to stimulation of ventral roots, which produces high frequency discharges. Multiple identical spikes evoked by a single vagal shock were not observed in the present investigation and it is therefore considered that units in which antidromic spike collisions occur exhibit the discharge patterns of motoneurones, irrespective of whether the discharge is recorded from near a motoneurone itself or whether, as seems unlikely, it is recorded from a recurrent collateral system.

The spike discharge patterns of the motoneurones resembled those recorded electromyographically from the diaphragm reinnervated by the vagus (Dussardier, 1960) and those recorded by a 'single unit' technique from efferent gastric vagal fibres (Iggo & Leek, 1967*a*). Units with early discharges, presumably in motoneurones innervating the reticulum, resembled the Type I, II and III units described by Iggo & Leek (1967*a*) although in the present work Type II units were less frequent and their discharges were shorter. Units with late discharges, presumably in motoneurones innervating the rumen, resembled the Type IV activity of Iggo & Leek (1967*a*) and some of the 'attivita postuma' described by Beghelli & Mavrulis (1966). These units also discharged during secondary cycle movements, which involve only the rumen, and it is concluded that Type IV motoneurones provide a common path to the rumen for both primary and secondary cycle movements. The region innervated by one of these motoneurones was considered to be in the caudo-dorsal blind sac because of the different temporal relationship between its discharge and the dorsal sac contraction recorded in the mid-dorsal region. During the primary cycle, in which the dorsal sac movement starts cranially and ends caudally, the discharge occurred after the contraction peak, whereas, during the secondary cycle, in which the movement starts caudally and ends cranially, the discharge preceded the contraction peak.

Estimates of the conduction velocity in efferent gastric vagal fibres based on the measurements of conduction times of antidromic impulses range from 10–26 m/sec. These values are higher than 1–16 m/sec given by Iggo (1956) based on a compound action potential technique. This discrepancy may have arisen, in part, because Iggo's measurements were made over a length of vagus in the cervical region whereas the measurements in this investigation were proximal to this region and it has been shown (Iggo, 1958) that small nerve fibres taper and have slower conduction velocities in the more distal regions.

The results of this investigation provide some more and some new

evidence that various zones of the reticulo-rumen receive independent vagal motor innervation, for which the activity is co-ordinated in the central nervous system. The evidence in favour of this view is (i) that electrical stimulation of the peripheral end of a cut ventral thoracic vagal trunk evokes a contraction of the reticulum without a contraction of the rumen (e.g. Habel, 1956), (ii) that differential cooling of a cervical vagus may reduce reticular contractions without affecting ruminal contractions (Leek, 1967, Fig. E1), (iii) that the patterns, temporal relationships and reflexly induced changes of spike discharges in motoneurones innervating the reticulum are clearly different from those innervating the rumen (Leek, 1967; Iggo & Leek, 1967*a*, *b*; this investigation), (iv) that small regions of the reticulo-rumen appear histologically as independent motor units (Comline & Message, 1965) and (v) that co-ordination in the central nervous system allows the same ruminal motoneurone (one unit) and late Type A (i.e. ruminal) interneurones (five units) to be involved in both primary and secondary cycle movements (this investigation) even though the direction and amplitude of the contraction sequence in the dorsal sac of the rumen differs in each cycle (e.g. review by Sellers & Stevens, 1966).

Four main types of gastric centre interneurones were found. The discharges of Type A interneurones bore some resemblance to the above motoneurone discharges and to the discharges of efferent gastric vagal fibres (Iggo & Leek, 1967*a*). An investigation which fails to differentiate between Type A interneurones and vagal motoneurones may therefore obtain confusing results. A greater proportion of Type A interneurones than motoneurones had a 'late' discharge, presumably associated with ruminal movements. In halothane-anaesthetized sheep, rumen contractions are depressed to a greater extent than reticular contractions and the above results point to the possibility that the anaesthetic may be exerting its depressant effect at sites between the Type A interneurones and the ruminal motoneurones.

The Types B and C interneurones have discharges which are readily distinguishable from those of Type A interneurones and of motoneurones, as the former possess resting discharges during the quiescent period between primary cycle forestomach movements. In association with the movements, the Type B discharges increase whereas the Type C discharges are reduced or absent. Type C interneurones have an activity which appears to be complementary to the Type A interneurones. The probable roles of the various interneurones are contained in a later investigation (R. Harding & B. F. Leek, in preparation). We do not consider that the short high-frequency discharges recorded by Howard (1968) during the course of only one primary cycle represent the activity of gastric centre interneurones.

The limitation of the technique used in the present investigation lay in

our ability to recognize that a particular form of spike discharge was related to forestomach movements. This limitation is acknowledged in our definition of 'gastric centres' (p. 589). The gastric centres are bilaterally paired, and are located in the rostral part of the dorsal vagal nucleus and the grey reticular formation up to 1 mm dorsal and lateral to the nucleus between transverse planes 6 mm rostral and 2 mm caudal to the obex. These rostrocaudal limits accord with those of Beghelli *et al.* (1963), although these authors thought that neuronal activity was confined to the dorsal vagal nucleus itself. Dussardier (1960) and Szabo & Dussardier (1964) demonstrated retrograde chromatolysis throughout the entire length of the dorsal vagal nucleus but the reason for the discrepancy may be that their vagal sections also cut efferent vagal fibres which were not concerned with forestomach motility. Results obtained by making brain stem lesions are difficult to compare with our results because the lesions, to be effective, must be large and bilateral (e.g. Beghelli *et al.* 1964). Thus it is impossible to define precisely those regions included in the lesions responsible for any observed dysfunction and furthermore to decide whether the lesion had affected neuronal networks or whether it had interrupted tracts of axones carrying information to or from the gastric centre. Similar problems arise from the interpretation of results obtained by point stimulation of the brain stem. What is the extent of the spread of the stimulus? Are the effects of stimulation the result (i) of stimulating motor tracts as Bell & Lawn (1955) seem to have done, (ii) of disrupting co-ordination systems in the gastric centres, so that there is forestomach stasis, as seems likely in the experiments of Andersson *et al.* (1959), Dussardier (1960), Howard (1970) or (iii) of excitation of specific afferent pathways and non-specific parts of the reticular formation, causing movements to be enhanced (Dussardier, 1960)?

In the present study there was no evidence of an asymmetry between the two gastric centres. Lewis, Scott & Navaratnam (1970) found that the cells affected by section of the gastric vagal branches in the rat lay mainly in the dorsal vagal nucleus on the left side, indicating that the majority of gastric motor fibres are in the left cervical vagus nerve. In the sheep, however, forestomach motility is affected equally when the left or right cervical vagus nerves are sectioned. The axones of each of the motoneurones located on both sides of the medulla in the present study travelled in the cervical vagus ipsilateral to the recording site. This finding supports the observation of Bell & Lawn (1955) and Bell (1960).

Recently, Kerr (1969) has stated that, in the cat, the dorsal vagal nucleus does not give rise to the motor fibres of the visceral smooth muscle. This was based on a comparison of the effects of stimulating the vagus nerves on smooth muscle responses of the duodenum and bronchioles, before and

after unilateral destruction of the dorsal vagal nucleus. The gastric response was not examined. The majority of gastric motoneurones located in the present study lay in the dorsal vagal nucleus, but some were located in the grey matter up to 0·5 mm dorsal and lateral to the nucleus. Three possibilities exists to account for the conflict between our findings and those of Kerr (1969): (i) a species difference may exist, but this is unlikely in view of the large body of evidence that vagal preganglionic fibres to the viscera arise from the dorsal vagal nucleus in non-ruminant species (see review by Mitchell & Warwick, 1955); (ii) it is possible that vagal motoneurones lying close to the dorsal vagal nucleus survived electrocoagulation of the nucleus by Kerr (1969) and allowed sufficient numbers of motor axones to remain intact to produce a response similar to the control response; (iii) since Kerr (1969) did not observe the response of the gastric smooth muscle, it is possible that the response of this muscle may be affected by destruction of the dorsal vagal nucleus.

It is concluded from the present study that neurones with discharges temporally related to movements of the reticulo-rumen are located within the dorsal vagal nucleus and up to 1 mm dorsal and lateral to the nucleus between transverse planes 1 mm caudal and 5–6 mm rostral to the obex. Records of neuronal activity were obtained from the regions of cell bodies. No records of vagal gastric afferent activity were obtained, presumably because the micro-electrodes used in this study did not record activity from axones. This is the possible reason that no records of gastric activity were obtained from the region medial to the dorsal vagal nuclei even though cross connexions exist between the two gastric centres.

The discharge patterns of many interneurones resemble those of gastric vagal motoneurones, and this emphasizes the need to distinguish between the two types of unit. Motoneurones and Type A interneurones with 'late', ruminal activity are part of a common pathway for rumen movements during both primary and secondary cycle movements. This suggests a complex organization within the gastric centres as the rumen movements during each type of contraction cycle progress in opposite directions.

We are indebted to the Agricultural Research Council for financial support of this investigation and to Miss Sheila Valentine and Mr Alyn Parry for invaluable technical assistance.

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