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ON THE TRANSMISSION OF THE STIMULATING EFFECTS OF CARBON DIOXIDE TO THE MUSCLES OF RESPIRATION

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SUMMARY

1. Electromyography was used to measure the response of the diaphragm and intercostal muscles to CO_2 in artificially ventilated decerebrate cats.

2. Hypocapnia produced tonic activity in either inspiratory or expiratory muscles or both, according to the preparation.

3. A graded effect of CO_2 on both rhythmic and tonic activity was observed and for the latter this could be seen at as low as 10 torr P_{A,CO_2}.

4. In one human subject tonic firing of expiratory motoneurones was also induced by hypocapnia and this activity showed a graded increase with increasing CO_2.

5. A saggital incision of the medulla aimed at interrupting inspiratory bulbospinal axons abolished activity in inspiratory muscles and at eupnoeic levels of CO_2 converted the activity of expiratory muscles from a periodic to a tonic firing pattern.

6. Following such lesions the threshold for rhythmic excitation of expiratory muscles was elevated and this revealed that the graded effect of CO_2 on tonic expiratory activity extends to as high as 60 torr.

7. The tonic activation of respiratory muscles in response to CO_2 ceased after cervical cord transection or when the saggital incision in the medulla was extended caudally to the first cervical segment.

8. It is concluded that the CO_2 dependent activation of spinal respiratory motoneurones is conveyed by bulbospinal axons which decussate in the vicinity of the obex and that this activation can be rhythmic or tonic.

9. It is suggested that the rhythmic excitation of expiratory muscles derives from a periodic inhibition of expiratory bulbospinal neurones which are subjected to a tonic CO_2 dependent excitation which is continuously variable over the physiological range.

INTRODUCTION

Carbon dioxide is the most important chemical stimulus to breathing. Although CO_2 clearly stimulates ventilation via peripheral chemoreceptors (for review see Biscoe, 1971) and via structures on the ventral surface of the medulla (Mitchell, Loeschcke, Severinghaus, Richardson & Massion, 1963; Schläfke, See & Loeschcke, 1970) the neuronal pathways between these receptors and the respiratory motoneurones are unknown. Furthermore the minimum level of CO_2 which is an effective

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respiratory stimulus is not known (cf. Bainton & Mitchell, 1966) nor is it known which elements in the neural network are under CO_2 control.

CO_2 responsiveness is usually described for the rhythmic state but it is also possible that CO_2 exerts a tonic control over respiratory muscles. The purpose of the experiments described here was to compare the effects of CO_2 on motoneurones innervating both diaphragm and thorax in tonic and rhythmic states in an attempt to answer these questions.

Evidence for phasic and tonic inputs to these neurones is as follows. Thoracic motoneurones are characterized by periodic bursts of activity produced by alternating synaptic excitation and inhibition (Sears, 1964*c*). Increased discharges during the excitatory phases may be produced either by increasing the phasic excitation, i.e. by increasing the amplitude of the central respiratory drive potential (CRDP, Sears, 1964*c*) or else by increasing the mean level of depolarization upon which this is superimposed, i.e. by altering the 'operating point' (Sears, 1964*c*, 1966*b*). The latter concept derives from the slow variations in membrane potential described by Sears (1964*c*, *d*) in intracellular recordings from respiratory motoneurones during spontaneous breathing.

Evidence for a tonic component in the control of respiratory muscles comes from a variety of sources including Head's work on the diaphragm slip (1889), from studies on apneusis (Lumsden, 1923; Stella, 1938*a*, *b*; Euler, Marttila, Remmers & Trippenbach, 1967), through investigations of vagal mechanisms (Wyss, 1954) and from observations on intercostal alpha motoneurones (Massion, Meulders & Colle 1960; Duron, 1966) and fusimotor neurones (Sears, 1963, 1964*a*; Eklund, von Euler & Rutkowski, 1964). Similarly the tonic activation of medullary respiratory neurones can be seen for both inspiratory and expiratory groups in hypocapnic apnoea (Batsel, 1967; Nesland & Plum, 1965; Cohen, 1968; Fallert, Böhmer & Dinse, 1977).

Although Duron (1966, 1973) has emphasized a purely 'postural' role for the tonic activation of the intercostal motoneurones, such activation can equally well be understood as having an important respiratory role by stabilizing the configuration of the rib cage and hence optimizing the action of the diaphragm (see Da Silva, Sayers, Sears & Stagg, 1977).

Experiments in the decerebrate cat described in this paper investigate whether or not tonic activity of respiratory muscles is directly controlled by $P_{A,\,CO_2}$ and how it is related to the control of respiratory rhythm. To do this we investigated muscle electrical activity (e.m.g.) in two particular conditions: (1) below the normal CO_2 threshold for respiratory rhythm; (2) at higher levels of CO_2 but with the threshold for rhythm raised by means of a saggital brain-stem lesion.

We found that we could demonstrate a graded effect of CO_2 on tonic muscle activity to as low as $P_{A,\,CO_2}$ 10 torr. The level of tonic activation increased smoothly and continuously with transitions to rhythmic breathing suggesting that the two states, tonic or rhythmic, are not independent but rather represent two aspects of control through the same bulbospinal pathway. Preliminary accounts of this work have already appeared (Bainton, Kirkwood & Sears, 1974; Sears, Kirkwood & Bainton, 1975).

METHODS

These experiments were carried out on twenty decerebrate cats, one cat under pentobarbitone anaesthesia, and a conscious human volunteer, one of the experimenters (T.A.S.). The cats were initially anaesthetized with halothane-oxygen mixture, tracheostomized and the common carotid arteries tied, one being cannulated for the measurement of blood pressure using a Statham pressure transducer. The cats were supported in the prone position with the thorax and abdomen pendant by means of ear bars and clamps at T1, T11 and on the iliac crests. The animal was then artificially ventilated with the oxygen–halothane mixture and end-tidal CO_2 (P_{A,CO_2}) held at its eupnoeic level (25–35 torr) by the addition of CO_2 to the inspired air. A craniotomy was made, the saggital sinus divided between ligatures and suction used to decerebrate the animal at the mid-collicular level. Anaesthesia was further maintained while the medulla was widely exposed by occipital craniotomy and removal of the dorsal part of the Atlas. The posterior part of the vermis was removed by gentle suction to give free access to the medulla in the mid line extending some 5–8 mm rostral to the obex. On completion of these procedures the halothane was withdrawn and the preparation left undisturbed for about 30 min. Prepared in this way, under artificial ventilation with O_2 throughout, the cats had systolic blood pressures of 100 to 160 torr (mean 120 torr) and brisk ventilatory responses to inhaled CO_2.

Electromyography. The e.m.g.s were recorded through pairs of fine, diamyl insulated stainless-steel wires (diameter 0·16 mm) inserted into the relevant muscles through hypodermic needles which were subsequently withdrawn leaving the wires, which carried hooks, held in position. Electrodes were placed in the costal parts of the diaphragm, in several intercostal spaces, usually between T3 and T9 and in some experiments also in the rectus abdominus muscle. Commonly up to eight pairs of wires were thus positioned, and in each case repeated sampling was done to locate the electrodes in the most active sector ('low threshold' regions) of the muscles. For the intercostal muscles these are in the dorso-medial aspects of the segments, in the regions lying beneath the longissimus dorsi and iliocostalis muscles, inspiratory activity being more prominent in the rostral segments, expiratory activity caudally. The signals were amplified, full-wave rectified and filtered with a time constant of 200 msec, this 'integrated' e.m.g. together with the original signal, being recorded on magnetic tape (Ampex, F.R. 1200) usually at $3\frac{3}{4}$"/sec and displayed on a U-V galvanometer recorder (Aviation Instruments Ltd.). For analysis it was often found convenient to replay the magnetic tapes at the fast rate of 30"/sec which gave time scale compression and thus facilitated the recognition of slow, time-dependent changes (e.g.Fig. 8).

Artificial ventilation. Throughout the experiment the preparations were artificially ventilated under positive pressure from a Palmer Ideal Pump, with the expired gas returned to the pump and exhausted through its expiratory valve. The O_2, and CO_2 composition of the inspired gas could be controlled by flowmeters which were fed from storage cylinders through two-stage reducing valves. P_{A,CO_2} was measured with a Capnograph infra-red gas analyser (Godart) which was calibrated during each experimental run using test gases switched into the circuit. All measurements were available at the outputs of the individual amplifiers and at any time up to seven of them could be recorded on magnetic tape.

General experimental procedure. When the above procedures were completed (commonly about one hour after the discontinuance of anaesthesia and when decerebrate rigidity was fully established) the animal was allowed to breathe spontaneously and its eupnoeic P_{A,CO_2} measured. Note was made of the general pattern of breathing, in particular of the degree of expiratory muscle activation, which nearly always occurred in the 'low threshold' regions with the cat suspended prone, as is also the case for the lightly anaesthetized Nembutal animal (cf. Da Silva *et al.* 1977). The cat was then artificially ventilated at a fast rate (52/min) and small stroke volume such that without the addition of CO_2 to the inspired air, the P_{A,CO_2} fell to about 10–15 torr. When this level had been held constant for several minutes recordings were made, following which the P_{A,CO_2} was either increased in steps while recordings of 20–30 sec duration were made at each steady level, or was slowly increased as a continuous record was made. The pump rate was deliberately set high in relation to the respiratory periodicity in order to facilitate the distinction between central rhythm generation and the segmental reflex activation of the expiratory muscles, the so-called 'inflation' reflex described by Sears (1958, 1964*a*, *d*). In this paper we refer to this as the chest wall inflation reflex to distinguish it from the Breuer-Hering inflation reflex. We mainly relied on using the loudspeaker to check that the phasic nerve activity of the

expiratory muscles was in phase with the inflation stroke of the respiratory pump, and on two occasions checked such observations by recording the airway pressure (not illustrated).

Lesions. In most cats, following the titration of e.m.g. activity against P_{A,CO_2} a saggital section of the medulla of varying extent was made. The aim was to study separately the effects of sectioning the descending axons of the bulbospinal inspiratory and expiratory neurones of the ventral respiratory group which, according to Merrill (1974), mostly decussate respectively rostral or caudal to the obex. The medulla was first probed in the mid line with a micrometer mounted needle to determine the depth of the bone ventrally and hence the ventral surface of the medulla so that the medulla could be as near as possible completely sectioned (within chosen limits) without severing the basilar artery. A thin sliver of razor blade mounted on a micromanipulator so as to move in the saggital plane was used for making the cut. This was usually done in two stages, either first rostral to obex, then further caudally, or vice versa, with measurements of the responses to CO_2 after each stage. Often the sections were then further extended to check on the completeness of the effects observed. In two cats the spinal cord was also sectioned transversely at C1.

Post mortem the brain stem was removed and preserved in formol-saline. The material was embedded in paraffin wax and transverse sections (10 μm) were cut, mounted and stained with haematoxalin and eosin so as to establish the extent of the lesions.

Human subject. The e.m.g.s of inspiratory (external) and expiratory (internal) intercostal muscles were recorded through wire electrodes following the procedures and precautions described by Newsom Davis & Sears (1970), with the subject seated. Positive pressure artificial ventilation was applied through a mouthpiece from a respirator, which, as in the cat experiments, was set at a fast rate (30/min) and the stroke volume adjusted such that in the absence of additional CO_2 the P_{A,CO_2} fell to about 15 torr. The experimental protocol of switching from one gas mixture to another was devised entirely by the other authors and all sensory clues as to what was being done were carefully eliminated so that the subject had no idea of the nature of the prevailing experimental run. Indeed, it was his experience that after becoming accustomed to the procedure and other experimental modifications he had no idea whether his respiratory muscles were active or not either at eupnoeic or subeupnoeic levels of CO_2 as the different gas mixtures were changed.

RESULTS

Pattern of breathing in eupnoea

In the present investigation the mean P_{A,CO_2} during eupnoea of the decerebrate cats was initially 28 torr (range 25–35 torr). These values, which tended slowly to increase over the course of the experiment, occurred in the face of quite wide variations in the actual pattern of breathing between different animals, as well as during the experiment in individual animals.

In the inspiratory phase, the external intercostal muscles were invariably active in the 2nd to 7th spaces, especially dorsally in the sectors of muscle proximal to the angle of the rib underlying the iliocostalis and longissimus dorsi muscles. This meant that there was no overt paradoxical movement of the rib cage due to the action of the diaphragm, which was always active. In the expiratory phase, there was nearly always some activity of the internal intercostal muscles especially proximally in the lower intercostal spaces (T5–T11) again notably in the sectors underlying the dorsal axial muscles. This activity, together with that of the deep layer of the parasternal region (cf. Da Silva *et al.* 1977 for the anaesthetized cat and Taylor, 1960, for man) caused compression of the rib cage and, because the rectus abdominus muscle was often also active, there was elevation of the ventral abdominal wall as recently described by Da Silva *et al.* (1977) in their stereophotographic analysis of breathing movements. Most of these e.m.g. features can be seen in the control recordings of Figs. 1, 4, 5, 9 and 10.

Effects of hyperventilation in intact preparations

When the cats were ventilated without the addition of CO_2, so that the P_{A,CO_2} fell to between 15 and 20 torr a variety of patterns of motoneurone activity occurred which, as we came to recognize, represented for each preparation its particular inspiratory or expiratory bias. We were never able to identify the origin of this variability by changes in lung volume, for example, or by vagal section, and in this particular respect our experience with decerebrate preparations closely resembles that of previous workers (Stella, 1938*b*; Cohen, 1964).

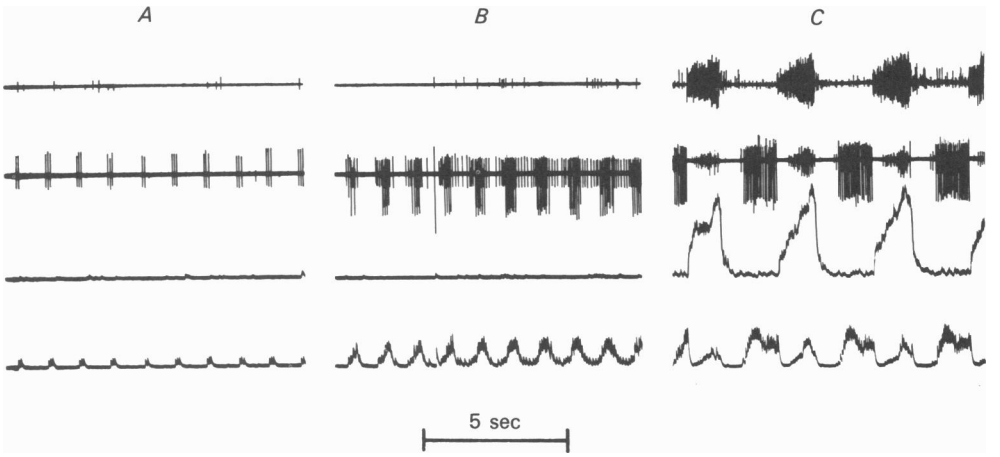

Fig. 1. E.m.g. recordings from an 'expiratory biased' preparation at three different levels of CO_2. Upper two traces: diaphragm and expiratory intercostal respectively (some inspiratory contamination of expiratory trace at high CO_2); lower two traces: integrated versions of upper two. P_{A,CO_2} 28, 32 and 42 torr for *A*, *B* and *C* respectively.

Preparations with an expiratory bias

Animals with an expiratory bias were those which were dominated by tonic expiratory activity when P_{A,CO_2} was reduced below the threshold for rhythmic breathing. Fig. 1 illustrates typical recordings from a preparation showing an expiratory bias. The upper traces show the e.m.g.s from the diaphragm and the intercostal muscles at T4, with their respective integrated records below. In the control recordings shown on the right, both the internal and external muscles were strongly active at the elevated CO_2 level of 42 torr and a central respiratory rhythm occurred at 20/min. When the CO_2 level was reduced to 32 torr the diaphragm activity was completely abolished and the intercostal recording showed some units firing in phasic bursts with each inflation phase of the pump (52/min) together with others that fired tonically, although also rate modulated with the pump cycle (Fig. 1*B*). The tonic component of the activity was dependent on the prevailing CO_2 level, as was shown by the abolition of this component when the CO_2 was further reduced to 28 torr leaving only a single unit firing at the height of each inflation (Fig. 1*A*). This phasic activation represents the chest wall inflation reflex of the internal intercostal muscles which is reduced, or abolished by section of the dorsal roots in the same segments (Sears, 1958, 1964*d*; Ramos & Mendoza, 1959) and is thought to be subserved by

afferents from the muscle spindle primary and secondary endings which monosynaptically excite the internal intercostal motoneurones (Sears, 1964*b*; Kirkwood & Sears, 1974).

As shown in Fig. 2, the reflex nature of the response to inflation of the chest wall is readily demonstrated by increasing the stroke volume of the pump. This record shows the summation of the reflex and the central respiratory drive. At the lowest stroke volume (75 ml.) the discharge was only achieved by a summation of these two inputs, the central drive being entrained at every third pump stroke. When the pump stroke was increased to 100 ml. the inflation-induced input was strong enough

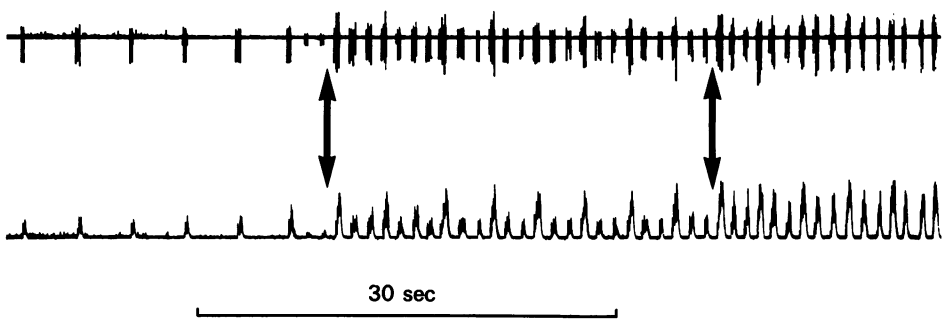

Fig. 2. Demonstration of the inflation reflex in an expiratory intercostal e.m.g. recording. The record is continuous, the stroke volume being increased quickly at the arrows from 75 to 100 ml. and from 100 to 125 ml. Upper trace is the e.m.g. recording, lower trace is the integrated version of the upper trace.

to exceed threshold at every stroke, but the record nevertheless clearly shows the summation. At 125 ml. the chest wall inflation reflex dominates; its magnitude could also be increased by causing a greater share of the stroke volume to inflate the rib cage. This was done simply by using a sling to compress the abdomen thus decreasing the compliance of the diaphragm–abdominal pathway (cf. Konno & Mead, 1968; D'Angelo and Saint'Ambrogio, 1974; Da Silva, *et al.* 1977) and hence causing a greater inflation of the rib cage.

The extent to which we were able to study the relation between P_{A,CO_2} and tonic firing of expiratory motoneurones in individual animals depended entirely on the threshold for central rhythm generation. The most effective way to study this relationship in the intact animal was to expose the animal first to a low level of CO_2 (10–15 torr) which abolished rhythm generation and then progressively to increase the CO_2 concentration in small steps as described in Methods. The reverse procedure of slowly lowering the CO_2 in decrements from a high level invariably led to rhythm generation persisting at an apparently lower level of CO_2, although eventually the central rhythm was abolished. Thus the system showed hysteresis probably due to the wash-out time of CO_2 from the brain.

The behaviour of the chest wall inflation reflex at levels of CO_2 just at threshold for rhythm generation is illustrated by records from a different preparation shown in Fig. 3. In *A* there was no diaphragmatic activity at a P_{A,CO_2} of 19·5 torr, whereas the sampled expiratory intercostal muscle showed continuous activity which increased in intensity with each inflation of the lungs (best seen in the integrated

record below). A small increase of CO_2 to a new steady-state level of 20 torr caused weak and brief bursts of diaphragm activity at 26/min, i.e. at half the pump rate, and with each burst the chest wall inflation reflex was partly inhibited. We presume that this inhibition was due to the post-synaptic inhibition of expiratory motoneurones which, at segmental level, is linked reciprocally to the intensity of the

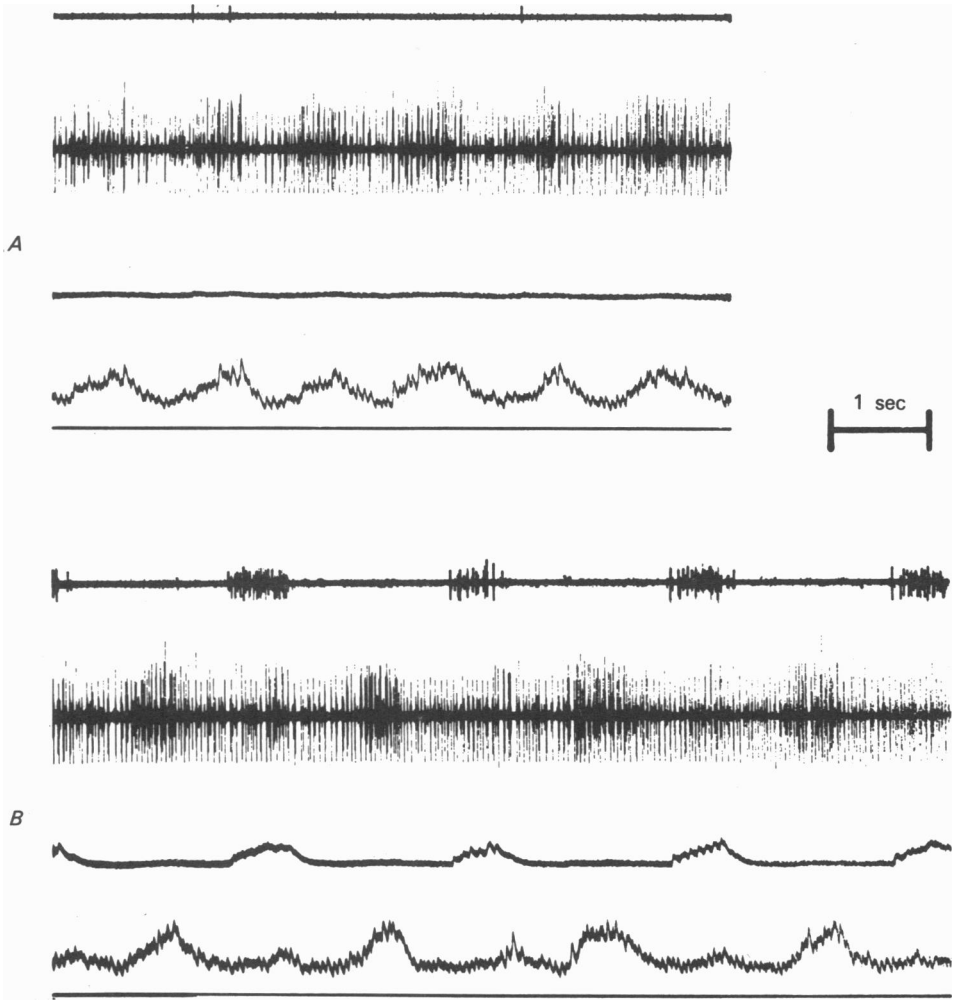

Fig. 3. Interaction between the inflation reflex in an expiratory biased preparation and a near threshold central respiratory drive. Upper two traces: diaphragm and expiratory intercostal e.m.g.s respectively; lower two traces: integrated versions of the upper two. Note base line of integrator for expiratory trace. P_{A,CO_2} (*A*) 19·5 torr and (*B*) 20 torr.

central inspiratory drive (Sears, 1964*c*). The fact that such inhibition did not occur at the slightly lower level of CO_2 suggests that no appreciable subliminal *phasic* drive occurred to the relevant intercostal inspiratory motoneurones at that lower level of CO_2.

A more complete titration of the effects of CO_2 in the same preparation is shown

in Fig. 4. These records illustrate the full sequence of events as the tonic activity of expiratory motoneurones at low CO_2 levels evolved into the periodic discharge characteristic of eupnoea or of stimulated breathing. The records show a progressive increase in intensity of diaphragm burst activity associated with a progressive decrease in frequency of the bursts as the coupling between the central rhythm and pump-induced rhythms changed. As the burst activity of the diaphragm increased in intensity, there was an associated deepening of the reciprocal inhibition of the expiratory motoneurone activity (note zero level of integrator), this being complete

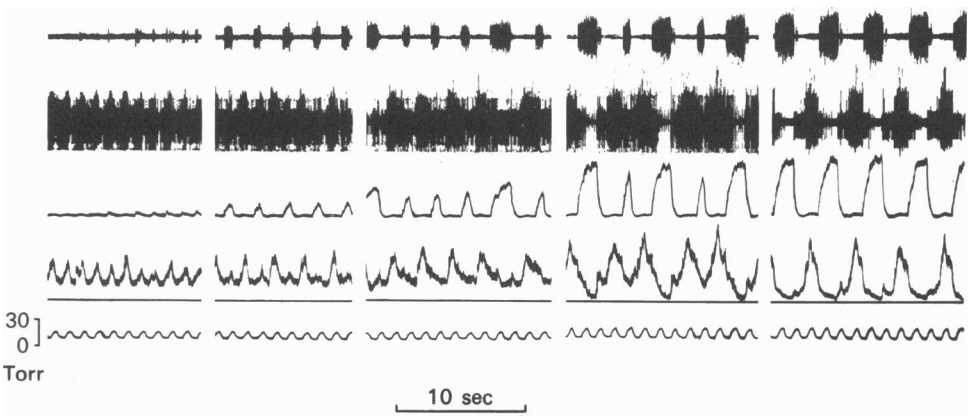

Fig. 4. Recordings as Fig. 3. (same preparation) but more compressed time scale and wider range of P_{A,CO_2}. Lowest trace is capnograph output.

at the eupnoeic level of CO_2 save for the small chest wall inflation reflex coincident with the rapid offset of each inspiratory burst. With the increased diaphragmatic activity at higher levels of CO_2 there was a concomitant increase in the peak intensity of expiratory activity. Thus, the response of the expiratory motoneurones to CO_2 can be seen as a continuum, extending from their graded tonic discharge at the lower levels of CO_2, with facilitation of the segmental reflexes, through to the progressive increase in the peak intensity of their periodic discharge as the respiratory rhythm develops (cf. Fig. 8).

Preparations with an inspiratory bias

Animals with an inspiratory bias were those that were dominated by tonic inspiratory activity or showed a persistent fast rhythm at low P_{A,CO_2} (cf. Cohen, 1964). They were characterized by greater variability during the experiment and greater complexity, as revealed by the changes consequent on stopping the pump after a period of hyperventilation at low P_{A,CO_2} (e.g. 10 torr). Fig. 5*B* shows control recordings with the animal breathing spontaneously at a P_{A,CO_2} of 35 torr. These control recordings correspond to the period between arrows of the time-compressed recording shown in *A*. The tracings at the left in *A* were taken during artificial ventilation without the addition of CO_2 so that P_{A,CO_2} was 10 torr. The inspiratory intercostal muscles were inactive while the diaphragm showed tonic firing (note mean level of integrator) modulated at the pump rate of 52/min. On stopping the pump the diaphragm discharged tonically for approximately 25 sec, disturbed only by minor

phasic perturbations visible in the integrated records, following which a clear cut rhythm developed consisting of progressive increases in peak intensity of activation alternating with deepening inhibitions, the pattern of firing eventually assuming the wholly periodic character of eupnoeic (or stimulated) breathing, as in *B*.

Later in the same experiments, as shown in Fig. 6, a rhythm at 24/min persisted when the pump was stopped, causing approximately a 50 % modulation of the tonic activity. As with the tonic diaphragm activity in Fig. 5, the amplitude and frequency of the 24/min rhythm remained essentially unaltered for nearly 1 min even though

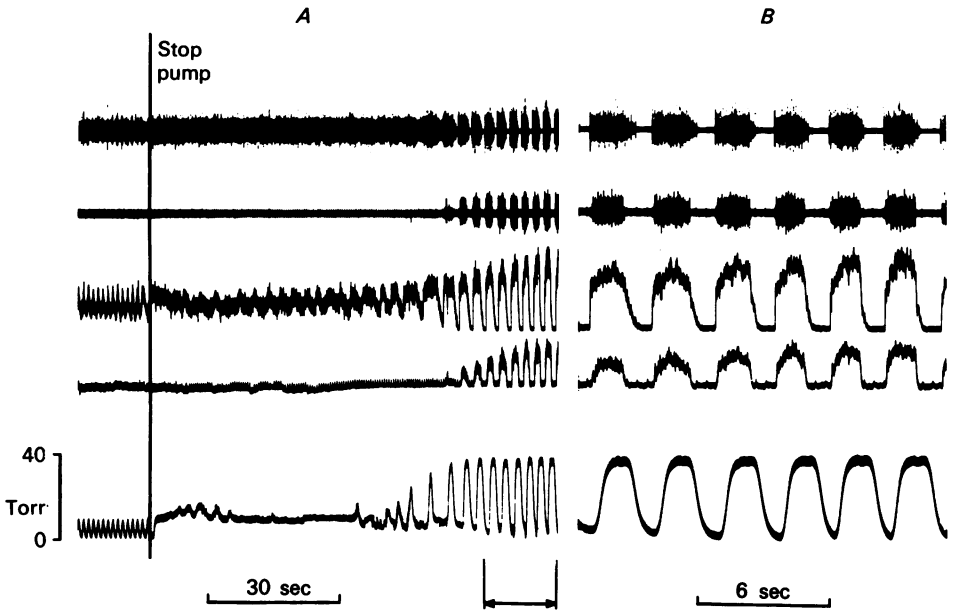

Fig. 5. Behaviour of an 'inspiratory biased' preparation in hypocapnia. *A*, upper two traces: diaphragm and inspiratory intercostal e.m.g.s respectively; next two traces: integrations of the upper two. Lowest trace, capnograph output; this trace is only interpretable when there is lung ventilation, i.e. at the start and end of the record. *B*, the same recordings, from the period indicated by the double arrow, but on a more expanded time scale.

CO_2 must have been accumulating during this time. Subsequently this rhythm progressively increased in amplitude both by increasing inhibition of the tonic component and by increases in the peak level of activation. A small dose of Brietal (sodium methohexitone) was then given (2 mg) which both suppressed the fast rhythm (including the initial pump-locked activity) and elevated the threshold of the rhythm responsive to CO_2, thus revealing the tonic stimulating effect of CO_2 on inspiratory motoneurones in the absence of rhythm generation, as was the case for the expiratory motoneurones in the expiratory biased animals.

In addition, some inspiratory biased animals showing pump-locked activation of the diaphragm at low CO_2 revealed, surprisingly, that the increase in activity occurred with each *inflation* stroke of the pump. This is best seen in Fig. 7 at a faster time scale where the bursts of diaphragm activity at 52/min with each inflation

alternate with the activity of the expiratory intercostal muscle. Such a pattern may be compared with the pump-locked activities of Fig. 3*B* in which the pump entrained central rhythm gave activation of the diaphragm at approximately half the pump rate and thus caused partial inhibition of the chest wall inflation reflex only on alternate cycles of the pump. This unexpected excitation of the diaphragm with the

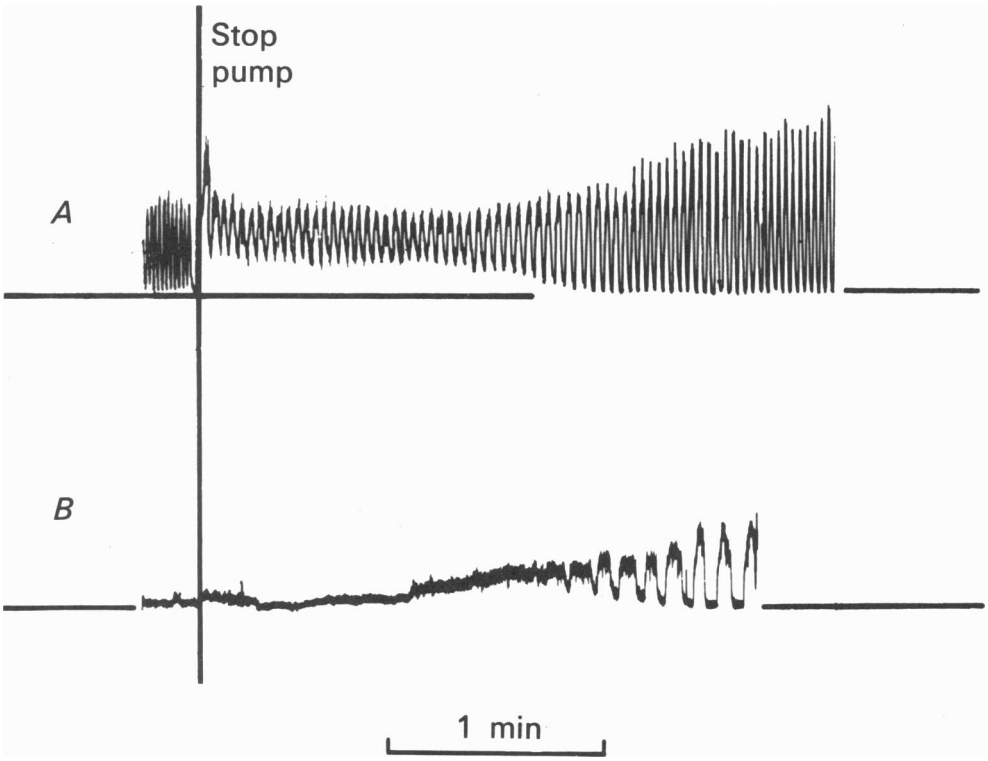

Fig. 6. Variants of the response shown in Fig. 5. Same preparation as Fig. 5, but later in the experiment. Only the integrated diaphragm record is shown (horizontal lines give the zero level of integrator). Between *A* and *B*, 2 mg Brietal was given I.V.

inflation phase of the pump (see however Ramos & Mendoza, 1959) is to be distinguished from the Breuer-Hering reflex, as was confirmed by its persistence after vagotomy in one preparation in which this was done.

Reciprocal tonic activation of inspiratory and expiratory motoneurones in hypocapnia

It is known from several investigations that the central respiratory drives to the inspiratory and expiratory motoneurones give reciprocal inhibition to their respective antagonist motoneurones (Sears, 1964*c*, 1966; Aminoff & Sears, 1971; Merrill, 1974). It might be expected, therefore, that these tonic effects of CO_2 in hypocapnia would similarly manifest themselves in a reciprocal manner. This indeed proved to be the case as illustrated in Fig. 8. At low CO_2, the expiratory and inspiratory intercostal activity showed reciprocal pump-locked activities superimposed upon a tonic dis-

charge. As the P_{A,CO_2} was then increased from about 15 torr upwards, both the mean level of activity and the amplitude of the chest wall inflation reflex in the expiratory intercostal e.m.g. increased, paralleled by a progressive reciprocal decline in the tonic activity of the inspiratory intercostal muscles. At 28 torr a central rhythm at about half the pump rate developed and was characterized by a more rapidly accelerating growth in the amplitude of the inspiratory compared to the expiratory bursts and by a progressively deepening inhibition of the expiratory motoneurones during

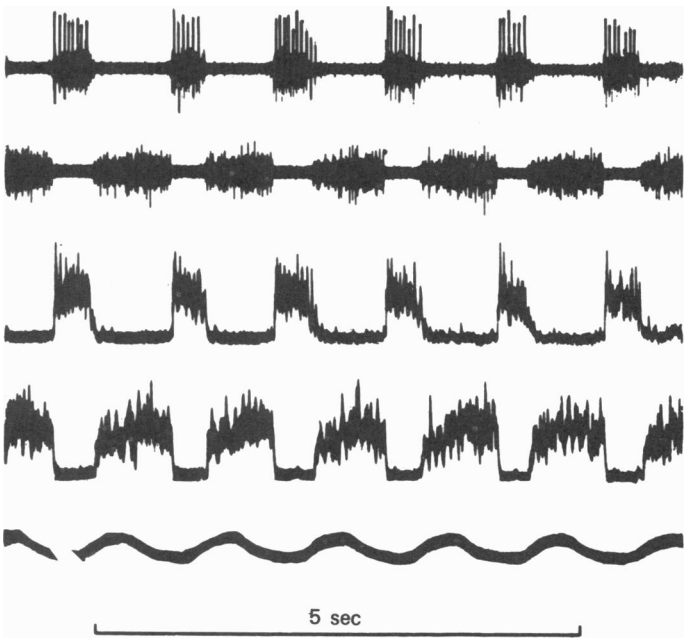

Fig. 7. Pump-locked rhythmic activity in an inspiratory-biased animal. First two traces: diaphragm and expiratory intercostal e.m.g.s respectively; next two traces: integrated versions of first two. Lowest trace: capnograph output to show pump rate. The phasing of the pump cannot be deduced from this record because of an unknown delay in the sampling tube. $P_{A,CO_2} = 20$ torr.

inspiration. A similar sequence from the same preparation later in the experiment, when the threshold for central rhythm generation was at a higher level of CO_2, is illustrated in Fig. 8*B*, and clearly reveals the manner by which the central rhythm at 15/min evolved in a reciprocal fashion from the underlying reciprocally distributed tonic activities. One interesting feature of the records was the sporadic occurrence of brief bursts of inspiratory e.m.g. activity which possibly were 'augmented' breaths, although their occurrence frequency seemed too high for this. At low CO_2 these bursts were accompanied by reciprocal inhibition of expiratory activity, but at higher levels of CO_2, reciprocal inhibition was an inconstant feature unlike that associated with the regular rhythm of breathing which occurred at these higher levels. These differences raise the possibility that the nature and origin of these bursts of inspiratory activity was different from that constituting regular breathing.

The above illustrations (Fig. 8*A*, *B*) were derived from an animal showing an

expiratory bias. On the other hand Fig. 8*C* was taken from an animal showing inspiratory bias. Note that the reciprocal relationship of tonic activity in expiratory and inspiratory intercostal muscle was also present but that as P_{A,CO_2} was raised, inspiratory activity increased while expiratory activity decreased.

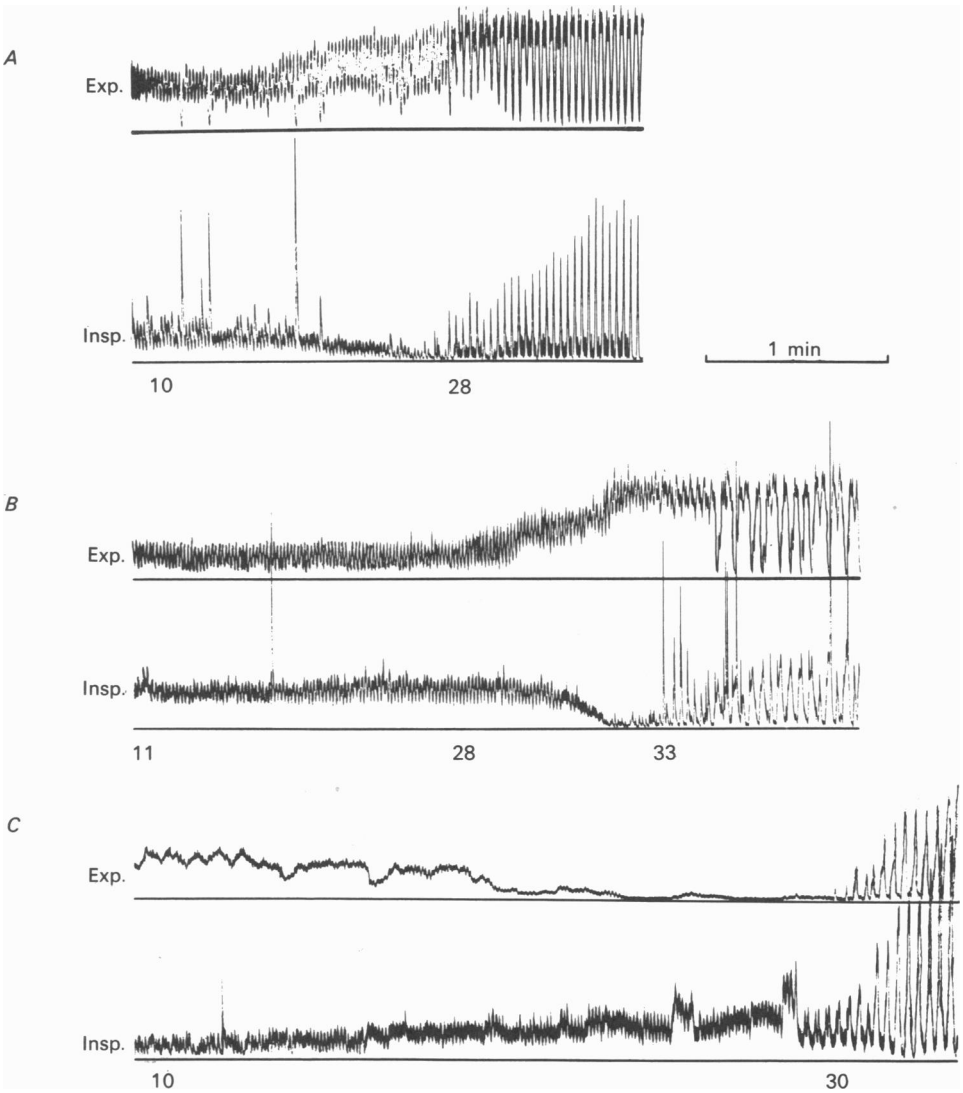

Fig. 8. Continuous titrations of e.m.g. activity with P_{A,CO_2}. In each sequence (*A*, *B*, *C*) the level of CO_2 was slowly raised, the numbers under each record giving the appropriate values of P_{A,CO_2}. In each case the upper trace is the integrated e.m.g. signal from an expiratory intercostal site and the lower trace from an inspiratory site. *A* and *B* are two sequences from the same (expiratory biased) animal and *C* is from an inspiratory biased animal. The fast periodic components that do not reach the base line (up to 28, 33 and 30 torr P_{A,CO_2} for *A*, *B* and *C* respectively) were in phase with the pump and constitute the inflation-reflex (cf. Figs. 1–3) for the expiratory or a corresponding deflation reflex for the inspiratory recordings. At higher levels of CO_2 in each case a respiratory rhythm with a lower frequency ensued.

The experiments described above reveal the existence during hypocapnia of CO_2 dependent tonic excitation of respiratory motoneurones. However, interpretation of the results is complicated by the fact that the central excitatory synaptic drives to the inspiratory and expiratory motoneurones reciprocally inhibit their respective antagonistic motoneurones (Sears, 1963, 1964*c*), while the onset of central rhythm generation at relatively low levels of CO_2 hinders study of these mechanisms at eupnoeic levels of CO_2 and above. In the hope of dissociating these phenomena we tried the effects of saggital incisions in the vicinity of the obex since lesions there abolish spontaneous breathing movements while leaving intact the apneustic response to electrical stimulation (Sears, 1966*a*). As described in the next section, these experiments led to the discovery that saggital incisions rostral to the obex elevate the CO_2 threshold for rhythm generation so enabling the study of the tonic excitatory effects of CO_2 in the eupnoeic range and above.

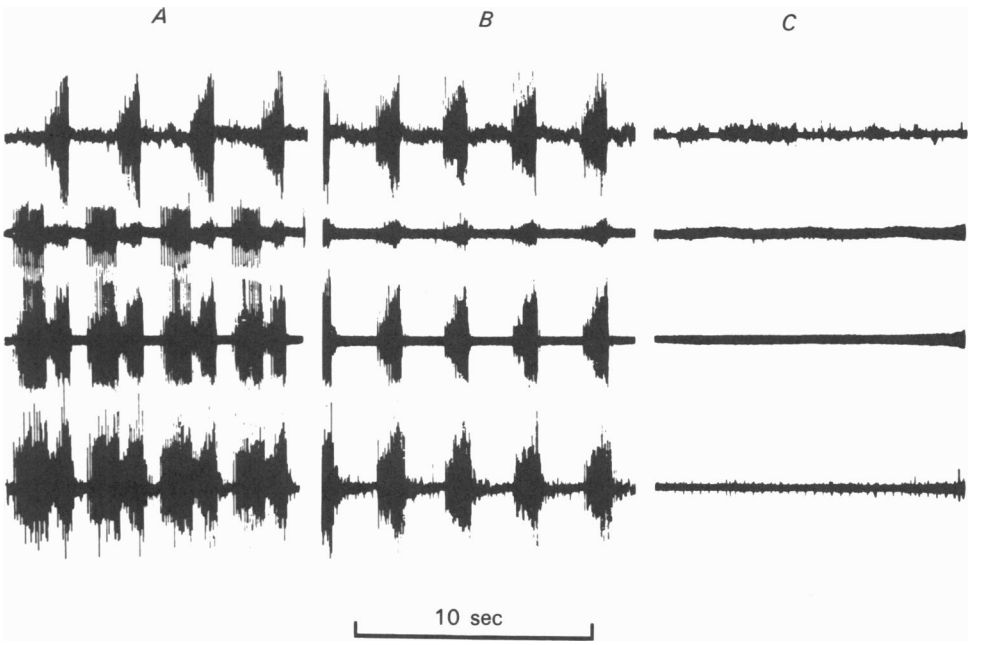

Fig. 9. Effect of a saggital section of the medulla, starting caudal to obex. E.m.g. recordings, first trace diaphragm, other traces mixed intercostal T6, T7, T3 respectively. *A* is a control recording, *B* is after sectioning below obex and *C* is after extending the section 4 mm more rostrally. *A* and *B*, spontaneous breathing; *C*, artificial respiration at approximately the same P_{A,CO_2} as *A* and *B*. Nembutal anaesthetized cat.

Selective interruption of bulbospinal respiratory pathways

From the lesioning experiments of Salmoiraghi & Burns (1960) and Sears (1966*a*) it is known that the breathing movements of the diaphragm and rib cage depend on decussating pathways extending a few millimetres rostral and caudal to the obex. Merrill (1970) and Bianchi (1971) have shown by antidromic invasion that the axons of bulbo-spinal respiratory neurones are substantially crossed at the level of the obex, those from expiratory neurones lying caudal, and those from inspiratory

neurones rostral to the obex. In the experiment of Fig. 9 the recording wires sampled activity from the internal and external intercostal muscles so that in the control recording (Fig. 9*A*), two bursts of activity occurred in each respiratory cycle as can be seen by reference to the diaphragm e.m.g. A saggital incision extending from the obex to the rootlets of the first cervical segment, selectively abolished the expiratory phased activity while leaving the inspiratory phased activity

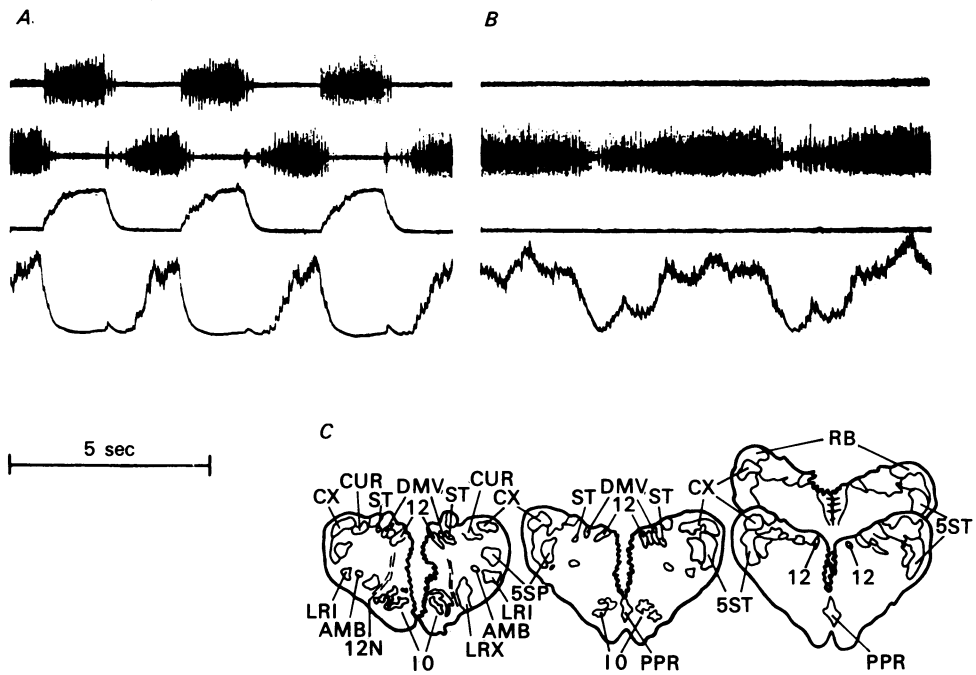

Fig. 10. Effect of a saggital section of the medulla starting rostral to obex. Upper two traces: diaphragm and expiratory intercostal e.m.g.s respectively; lower two traces: integrated versions of the upper two. *A* is the control recording and *B* is after the section was made from 3 mm rostral to 2 mm caudal to obex. Extending the lesion more caudally abolished all activity. Artificial respiration, P_{A,CO_2} constant at 31·5 torr. *C*, transverse sections of the medulla from this cat to show rostral extent of lesion (1·5, 1·0 and 0·5 mm between sections caudal to rostral). Notation following Berman (1968): 5SP, spinal trigeminal nucleus; 5ST, spinal trigeminal tract; 12, hypoglossal nucleus; 12N, hypoglossal nerve; AMB, nucleus ambiguus; CUR, cuneate nucleus, rostral division; CX, external cuneate nucleus; DMV, dorsal motor nucleus of vagus; IO, inferior olive; LRI, lateral reticular nucleus, internal division; LRX, lateral reticular nucleus, external division; PPR, post-pyramidal nucleus of the raphe; RB, restiform body; ST, solitary tract.

of the external intercostal muscles and of the diaphragm wholly intact (Fig. 9*B*). This confirms the previous reports that with such a lesion inspiratory movements persist (Sears, 1966*a*) and that expiratory motoneurone activity is selectively abolished (Merrill, 1972). With extension of the lesion 4 mm rostral to the obex all inspiratory activity was abolished (Fig. 9*C*).

We next tried to abolish selectively the inspiratory bulbospinal drive by making discrete lesions in the vicinity of the obex as illustrated in Fig. 10. In such experi-

ments the P_{A,CO_2} was held constant throughout the experiment, in this case at 31·5 torr. In the control recording the diaphragm and internal intercostal muscles showed typical alternating activity, the latter, in addition, being modulated by the segmental chest wall inflation reflex during the expiratory phase. The records in Fig. 10*B* were taken approximately 5 min after a saggital incision extending 3 mm rostral to the obex and 2 mm caudal to it. They show a total loss of diaphragmatic activity while the expiratory activity now persists throughout the slightly slowed respiratory cycle (16–12c/min), save for a very brief period of total loss of activity. However, the peak intensity of the expiratory activity was unaltered. Evidently the lesion had not interrupted a significant fraction of the expiratory drive to the spinal cord. The apparent prolongation of the expiratory phase could be due either to the absence at *segmental* level of the reciprocal inhibition of the expiratory motoneurones consequent on the loss of the inspiratory drive, or, to the loss of inspiratory inhibition of the expiratory bulbospinal neurones (Merrill, 1974).

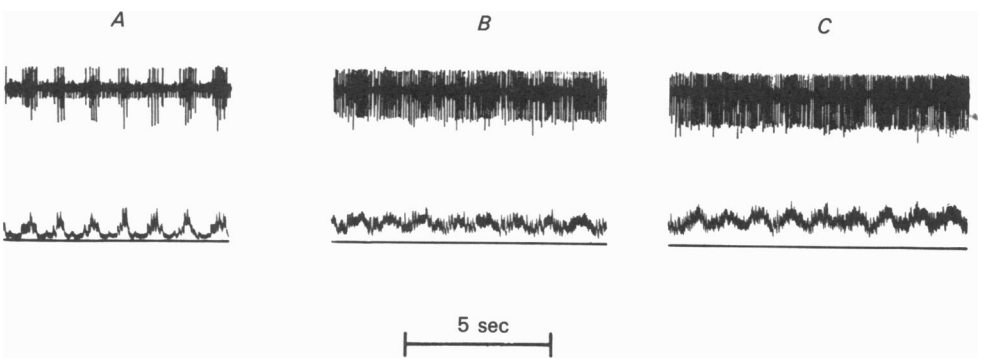

Fig. 11. Titration of expiratory e.m.g. activity (upper trace) following an extensive saggital lesion rostral to obex. Lower trace is the integrated version of the upper trace (note base line for the lower trace which is the zero level for the integrator). P_{A,CO_2} 28, 32 and 42 torr respectively for *A*, *B* and *C*. The diaphragm was silent for all three levels of CO_2. Same animal as Fig. 1.

When such lesioned animals were removed from the respirator, vigorous rhythmic expiratory movements occurred but this purely expiratory mode of ventilation, occurring at low lung volume, resulted in a P_{A,CO_2} of between 60 and 70 torr in the two animals examined in this way.

While investigating the effects of such lesions, we found that with some of them, especially those extending more rostrally from the obex (5·0 mm), the CO_2 threshold for rhythm generation was elevated above eupnoeic levels and could be as high as 50–60 torr. In these instances, the persistence of central rhythm generation had to be inferred from the periodic inhibition of the expiratory activity. Such lesions enabled us to examine the response of expiratory motoneurones to a wider range of CO_2 levels than was possible in the intact animal. An example of this is illustrated in Fig. 11 from an animal in which the eupnoeic level of CO_2 was 28 torr before the lesion (same animal as Fig. 1). Following the lesion all inspiratory activity ceased and central rhythm generation was blocked. At low CO_2 (28 torr) the chest wall

inflation reflex of the expiratory motoneurones was clearly in evidence against a background of only a modest level of tonic activity. At 32 torr there was about a two-fold increase in the tonic activity and a further marked augmentation occurred on increasing the CO_2 level to 42 torr still without the intervention of central rhythm generation. Another example of mid line lesioning above the obex and the titration of CO_2 on the resulting tonic activity is seen in Fig. 12, this time from the same preparation as illustrated in Fig. 8*C*. Before lesioning, the threshold for rhythm generation was approximately 30 torr and below this level a reciprocal relationship existed between the tonic inspiratory and expiratory activities, the former dominating as CO_2 was progressively raised. After lesioning, the inspiratory activity was abolished and the threshold for rhythm generation elevated to above 60 torr, thus enabling the entire response to CO_2 to be recorded over the range of 17–69 torr.

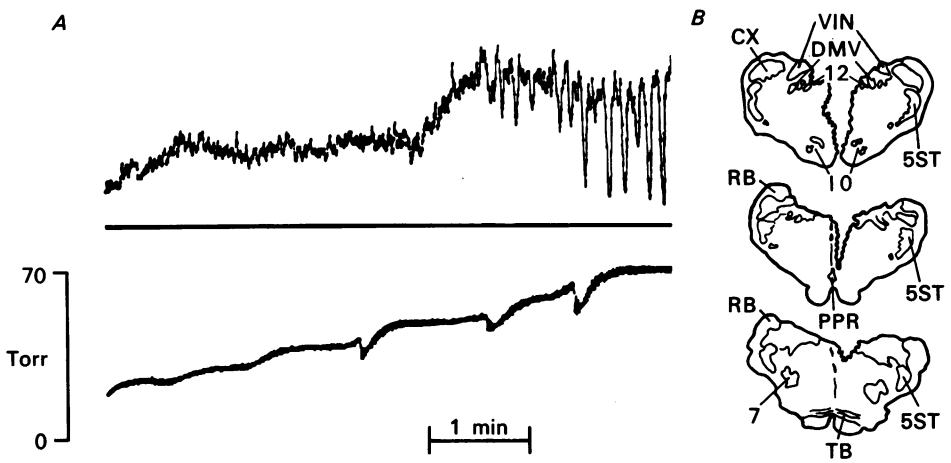

Fig. 12. *A*, continuous titration of integrated e.m.g. recording (upper trace) from expiratory intercostal muscle after a saggital lesion of the medulla rostral to obex. Lower trace: capnograph output. Same animal as Fig. 8*C*. *B*, transverse sections of the medulla from this cat (1·0 mm between sections) to show rostral extent of lesion. Notation as Fig. 10, plus: 7, facial nucleus; TB, trapezoid body; VIN, inferior vestibular nucleus.

All CO_2-related responses were abolished when the saggital incision was extended caudally to the level of the first cervical segment indicating that these effects were dependent on fibres decussating at these levels. It also disappeared after cervical cord transection in two cats.

Because the lesions were frequently extended after the CO_2 titrations were made, it was not possible to make an exact correlation between the physiological results and the anatomical structures involved in the lesions. However, two examples are shown in Figs. 10 and 12. Fig. 10 is a case where the threshold for rhythm was not appreciably elevated by the rostral lesion and Fig. 12 is one where a clear effect was present. In both cases, however, the majority of the inspiratory bulbospinal axons were cut as shown by the loss of all inspiratory discharges in diaphragm and intercostal muscle up to a P_{A,CO_2} of 70 torr. The rostral extents of the lesions correspond to the states represented by the responses to CO_2 but the caudal extents are unknown since in both cases the lesion was later extended caudally.

Effects of hyperventilation on expiratory motoneurones in man

The response of human expiratory motoneurones to hyperventilation induced hypocapnia was remarkably similar to that of the decerebrate cat, as illustrated in Fig. 13. The recording is completely typical of the response to several similar sequences of exposing the subject to 10 min of artificial ventilation with oxygen which reduced P_{A,CO_2} to 25 torr. Inspired CO_2 was then added gradually, while

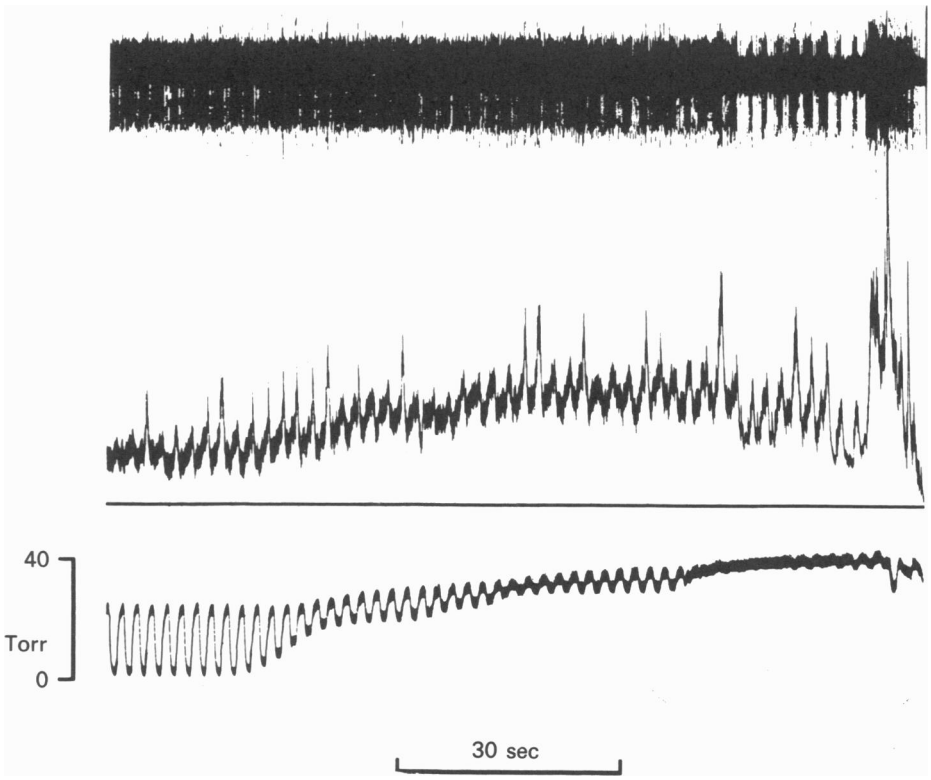

Fig. 13. Titration of expiratory intercostal e.m.g. activity (top trace) from a human subject against P_{A,CO_2} (bottom trace), starting from a hypocapnic level. Middle trace as the integrated version of the e.m.g. record (base line drawn in).

maintaining the same ventilation, returning the P_{A,CO_2} to the subject's eupnoeic level. At 25 torr the sampled internal intercostal muscle showed tonic activity modulated by a chest wall inflation reflex synchronous with the pump. As the CO_2 level was progressively elevated, the mean level of tonic activity progressively increased with spasmodic increases in the peak amplitude of the chest wall inflation reflex. At the end of the record, central rhythm generation ensued, and this evolved by a periodic and progressively deepening inhibition of the tonic activity accompanied by a progressive increase in the peak level of activity in the expiratory phase, just as we have described for the decerebrate cat. The presence of the chest wall inflation reflex under conditions of CO_2 dependent, but tonic firing of expiratory moto-

neurones, provides independent evidence of a stretch reflex in the human intercostal muscles and thus confirms the conclusions reached by Newsom Davis & Sears (1970) on the basis of loading experiments.

DISCUSSION

Bulbospinal neurones. In setting out to examine if the brain stem mediated, CO_2 dependent, excitation of motoneurones is separable into tonic and periodic components, our motivating idea was that two antomically distinct systems of fibres might be involved. This was because previously it had been shown (Sears, 1966*a*) that saggital incisions above and below the obex abolish spontaneous breathing but leave intact the inspiratory apneusis evoked by electrical stimulation in the region of the medial reticular formation which Pitts, Magoun & Ranson (1939; see also Pitts, 1946) had designated as their 'inspiratory centre'. However, the present experiments strongly indicate that for expiratory motoneurones a single anatomical system can give rise to the tonic and periodic excitation of motoneurones. Thus, both the periodic excitation of expiratory motoneurones during spontaneous breathing and the CO_2 dependent tonic excitation of expiratory motoneurones in animals with lesions rostral to the obex, are dependent on fibres which decussate *below* the obex, since a lesion there abolished both phenomena. These fibres are likely to be the axons of expiratory bulbospinal neurones in the nucleus retroambigualis (Merrill, 1970) or ventral respiratory nucleus of Bianchi (1971), which decussate caudal to the obex (Merrill, 1974) and which we presume to be responsible for the strong expiratory apneusis evoked by electrical stimulation just below the obex in the mid line (Sears, 1966*a*).

Central and peripheral sensitivity to CO_2. The central stimulating action of CO_2 on respiratory movement is probably mediated by its effects on H^+ concentration. However, little can be said at present concerning the manner in which the central CO_2 chemo-receptive regions of the ventral medulla described by Mitchell, Loeschcke, Severinghaus, Richardson & Massion (1963) and Loeschcke, Lattre, Schläfke & Trouth (1970) might project to the bulbospinal or other neurones belonging to the respiratory complex. Similarly, there is no direct evidence available for the manner in which the stimulating action of CO_2 on chemoreceptors is conveyed to the expiratory bulbospinal neurones, although the work of Davies & Edwards (1975) and of Eldridge (1976) indicates the possibility of fairly direct pathways (see Sears, 1977 for a more detailed discussion).

Central rhythm generation and apneusis. Because pontine (pneumotaxic) mechanisms are generally held to play an essential role in the generation of central respiratory rhythm we were surprised to find that relatively discrete saggital incisions of the lower medulla elevated the CO_2 threshold for rhythm generation in the expiratory intercostal muscles, indeed abolishing rhythm altogether over the normal physiological range (e.g. see Fig. 12). We know nothing specifically of the origins and destinations of the relevant decussating axons, but in this region, the decussating axons of the 'early burst' inspiratory neurones, elegantly demonstrated by Merrill (1974) using microstimulation techniques, are obvious candidates (Fig. 14*B*).

We, of course, have no direct information about the rhythmic state of inspiratory

neurones in the medulla after the mid line lesion even if we can infer the state of the expiratory bulbospinal population from the activity of the expiratory motoneurones. In particular, we do not know whether the lesion actually inhibits rhythm generation altogether, whether rhythm persists but the lesion disconnects the medullary respiratory neurones from its influence, or whether the lesion merely removes the rhythmic drive from the expiratory group perhaps by means of severing the axons of the 'early burst' cells mentioned above.

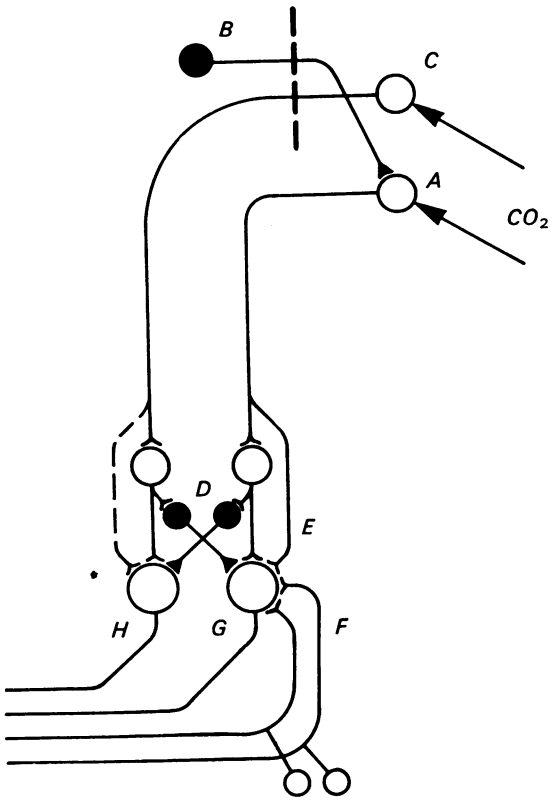

Fig. 14. Neural connexions assumed in the Discussion. *A*, bulbospinal expiratory neurones; *B*, 'early burst' inspiratory neurones (Merrill, 1974); *C*, inspiratory bulbospinal neurones; *D*, reciprocal inhibitory segmental internuncial network (Aminoff & Sears, 1971); *E*, monosynaptic excitation of expiratory motoneurones from the medulla (Kirkwood & Sears, 1973); *F*, monosynaptic excitation from primary and secondary endings of muscle spindles (Kirkwood & Sears, 1974); *G*, expiratory motoneurone; *H*, inspiratory motoneurone. Interrupted line represents lesion rostral to obex.

These lesions produced a state of tonic firing of expiratory motoneurones, in effect expiratory apneusis. This behaviour would appear to correspond to what Lumsden (1923) observed, but in fractionated form, as the brief but powerful expiratory efforts which intervene between the periods of prolonged inspiratory apneusis. Such expiratory efforts would be expected to be strong because of the high level of CO_2 which must have prevailed in his experiments.

In that we have demonstrated tonic activity dependent on CO_2, our experimental result on expiratory motoneurones is strikingly similar to (i) the results obtained by

Stella (1938*b*) for the effects of CO_2 on inspiratory apneusis evoked by cold blocking of the vagi in low decerebrate cats, (ii) to the effects of raised CO_2 on both inspiratory apneusis and the intervening expiratory spasms noted by Lumsden (1923) and (iii) the recent measurements of apneusis by Euler *et al.* (1975). Stella demonstrated that inspiratory apneusis was abolished by deep hypocapnia induced by hyperventilation, but when the respirator was removed there was a progressive increase in the intensity (depth) of inspiratory apneusis as the CO_2 accumulated. Our own measurements of the CO_2 sensitivity of tonic activity in both diaphragm and intercostals extend these findings of Stella (1938*b*).

For Lumsden and for Stella the dependency of apneusis on CO_2 was an expression, in static terms, of the normal stimulating action of CO_2 on spontaneous respiratory movements. The notion of the CO_2 sensitivity of the pontine apneustic centre (the 'inspiratory centre' for Stella) was incorporated in Pitt's (1946) theory that the cells comprising his 'inspiratory' centre (then located by electrical stimulation to the *medial* reticular formation of the medulla in the nucleus reticularis gigantocellularis) are directly sensitive to CO_2. But the medial reticular formation appears to be devoid of the rhythmic respiratory neuronal activity that his theory demanded, since from Hukuhara, Nakayama & Okada (1954) and Achard & Bucher (1954) onwards, most authors have found that such neurones have an abundant distribution in the lateral medulla (e.g. Merrill, 1970; Bianchi, 1971) or dorsolaterally in the region of the nucleus of the solitary tract (e.g. Baumgarten, Balthasar & Koepchen, 1960; Bianchi, 1971) although an exception to this has recently been reported for unidentified neurones in the medial reticular formation (Vibert, Bertrand, Denavit-Saubié & Hugelin, 1976). However, the hypothesis of medial reticular formation neurones having a tonic CO_2 sensitivity is still tenable.

Our discovery that the CO_2 threshold for rhythm generation is elevated following saggital incisions rostral to the obex was most fortunate. It allowed us to extend the observations we had made on hypocapnic states in the intact animal, to show that the responses of expiratory motoneurones to the brain stem mediated effects of CO_2 is a graded continuum, extending from hypocapnic through eupnoeic to hypercapnic levels of CO_2. The unmasking of this relationship between CO_2 level and the tonic firing of expiratory motoneurones was undoubtedly simplified by the fact that the saggital incisions of the caudal medulla also interrupted the inspiratory bulbospinal axons (cf. Merrill, 1974), thus relieving the expiratory motoneurones from reciprocal inhibition (Sears, 1964*c*; Aminoff & Sears, 1971) as represented in Fig. 14*C*, *D*.

These lesions obviously left the expiratory motoneurones exposed to fewer synaptic inputs so that under the experimental conditions of central rhythm suppression, their discharge patterns were simplified and can now be explained in terms of their known bulbospinal and proprioceptive monosynaptic inputs, notwithstanding the possible contribution of tonic inputs from segmental interneurones. This assertion is justified because by means of spike triggered averaging Kirkwood & Sears (1973) have proved that the expiratory bulbospinal neurones of the ventral respiratory nucleus monosynaptically excite the thoracic expiratory motoneurones (Fig. 14*E*). The other known sources of monosynaptic excitation are the Group Ia fibres from intercostal muscle spindle primary endings (Sears, 1964*b*), which Kirk-

wood & Sears (1974) have confirmed by spike triggered averaging, and afferents from spindle secondary endings whose monosynaptic connexions Kirkwood & Sears (1974) have also demonstrated with the same technique (Fig. 14*F*). These stretch activated receptors are responsible for the chest wall inflation reflex of expiratory muscles since this is abolished by section of dorsal roots of the same and immediately adjacent segments innervating the heteronymous muscles (Sears, 1964*d*). The ease with which the chest wall inflation reflex can be demonstrated in hypocapnic apnoea, as well as during the expiratory phase of spontaneous breathing, can thus now be understood in terms of the CO_2 dependent tonic facilitation or excitation of the relevant expiratory motoneurones which still occurs in the absence of central rhythm generation.

Entrainment of central rhythm generation. The pump-induced entrainment of central rhythm generation during hypocapnia is a new observation and is dependent on a reflex mechanism which is opposite in sign to that of the Breuer-Hering inflation reflex in which lung inflation is inspiratory inhibitory. We have not further analysed this somewhat fickle phenomenon which occurred in inspiratory biased animals which had the tendency to show fast spontaneous rhythm at low CO_2 (cf. Figs. 5 and 6). Two possibilities can, however, be usefully discussed. This behaviour is unlikely to be an expression of the response of the intercostal-to-phrenic reflex (Decima, Euler & Thoden, 1968; Decima & Euler, 1969) to artificial ventilation. In the present work, inspiratory intercostal as well as phrenic motoneurones were concomitantly excited by lung inflation, and when this behaviour occurred there was no chest wall inflation reflex of the expiratory motoneurones. This suggests that the responses were mediated supraspinally, via inspiratory bulbospinal pathways giving reciprocal excitation and inhibition to the inspiratory and expiratory motoneurones. The intercostal-to-phrenic reflex as described by Decima *et al.* is a purely spinal reflex affecting only the phrenic motoneurones and does not in itself have the requisite properties to account for what we have observed. According to Remmers & Tsiaras (1973), compression of the mid and upper rib cage leads to a reflex inhibition of phrenic and laryngeal motoneurones, which is mediated via bulbar mechanisms, this response being abolished by bilateral lesions of ascending fibres running superficially (1·0 mm deep) in the lateral columns of the upper cervical spinal cord. The relevant spindle afferent fibres (Group II) are in the nerves to the external intercostal muscle, whose spindles should be unloaded by lung inflation so that the entrainment we have observed could represent disinhibition via the proprioceptive supraspinal reflex described by Remmers & Tsiaras (1973). It is also possible that pump-entrained rhythms seen at higher levels of CO_2, where the phasing of the diaphragm activity coincided with inflation but where the respiratory rhythm was only at a sub-multiple of the pump rate (e.g. Fig. 3*B*), may arise by the same mechanism, but vagal effects may also be involved (cf. Cohen, 1960).

Expiratory motoneurone control and functional significance. In the intact animal the pattern of expiratory motoneurone discharge is determined by three principal inputs; the excitatory and inhibitory inputs associated with the CO_2 dependent bulbospinal activities and proprioceptive inputs reflecting the local configuration of the rib cage and abdomen. During inspiration there is excitation of inspiratory bulbospinal neurones and, according to Merrill (1974) and Mitchell & Herbert (1974), an associated reciprocal inhibition of expiratory bulbospinal neurones

(Fig. 14*B*). Correspondingly, at segmental levels, there is excitation of inspiratory motoneurones and reciprocal post-synaptic inhibition of expiratory motoneurones, (Fig. 14*D*) which serves to inhibit the reflex excitation which would otherwise occur due to the inflation-induced stretch reflexes (Sears, 1964*c*, *d*) (Fig. 14*F*). During the expiratory phase the expiratory bulbospinal neurones are disinhibited and with an appropriate time course assume a discharge rate proportional to the prevailing synaptic drive from peripheral and central chemoreceptors. This expiratory bulbospinal drive monosynaptically excites the expiratory motoneurones, now released from inspiratory bulbospinal inhibition, and in summation with segmental and other non-specified inputs (e.g. propriospinal) cause the depolarizing phase of the CRDP which may, or may not reach firing threshold. This mechanism would be responsible for actively determining the control of end expiratory volume. At eupnoeic levels of CO_2 the expiratory motoneurone discharge may be restricted only to the most proximal regions of the intercostal muscles, nevertheless the expiratory half of the central mechanism is clearly in widespread operation as evidenced by the occurrence even in deep anaesthesia of expiratory bulbospinal neurone activity (Merrill, 1974) and of augmenting depolarizing phases of the CRDPs (Sears, 1964*c*). Thus the normal periodic pattern of expiratory motoneurone discharge is sculptured by inspiratory-linked inhibition from an underlying tonic process of CO_2 dependent excitation. The graded intensity of inspiratory motoneurone discharge with CO_2 described so clearly by Stella for apneusis (1938*b*), and which here we have demonstrated in hypocapnia, probably also depends on an equally simple circuit. However nothing is known of the mechanism which transforms such a drive into the normal augmenting pattern of inspiratory (and expiratory) bulbospinal discharge.

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