INVESTIGATIONS ON RESPIRATORY MOTONEURONES.

by

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Thesis submitted for the Degree of Doctor of Philosophy in the Australian National University 1963
The investigations described in this thesis are essentially my own original work.

The initial experiments using the technique of intracellular recording were made under the guidance of Dr. Rosamond Eccles with Dr. C.N. Shealy as collaborator. The investigations described in Section IV were made with the collaboration of Dr. P. Andersen.

With these exceptions I hereby declare that the investigations were entirely my own work.

T. A. Sears
As a result of the investigations described in this thesis the following publications have appeared.


ERRATA

p.31 line 8: Should read "internal intercostal nerves, not muscles."

p.34 line 3: Q_{10} not T_{10}.

p.45 lines 15 and 16: Should read "between the external and internal intercostal muscles,"

p.75 line 13: Should read "filaments innervating the external and internal intercostal muscles".

p. 86 line 1: Should read "It follows that membrane potentials lower than the 'resting' potential are probably due to synaptically induced depolarization.

p. 89 line 18: Should read "comparatively few low-gain records".

p.117 line 21: Delete "The threshold of the motoneurone axons."

p. 130 line 14: Should read "raise the threshold."
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VII. GENERAL DISCUSSION.
Due undoubtedly to the early influence exerted by the work of Sherrington, our understanding of the physiology of limb movements is now firmly based on a detailed knowledge of the properties of spinal motoneurones and of the segmental reflexes controlling them. In contrast, a review of the literature reveals that our understanding of the physiology of respiratory movements has no similar, firm basis. The cause for this may be traced to the profound influence exerted on the experimental investigation of respiratory movements by the concept of a 'respiratory centre' introduced early in the last century. One consequence of the subsequent pre-occupation with the organisation and localisation of the 'centres', for example, is a dearth of information concerning the segmental proprioceptive reflexes of respiratory muscles. In fact, with few exceptions, the effects of afferent discharges from somatic proprioceptors has been considered only in relation to their influence on the centres. Conversely, the various concepts of the 'centres' ignore the problem of how the co-ordination of segmental proprioceptive reflexes is achieved.

The author is of the opinion that the term 'respiratory centre' is not particularly meaningful and he believes, in agreement with a previous reviewer Liljestrand (1953), that preferably it should not be used. In order to justify this statement and also to indicate the need for new experimental approaches in this field, a critical account is given of the origin of present views concerning the various concepts of the respiratory centres. This is followed by an account
of the proprioceptive reflex regulation of respiratory movements and the introduction closed with a general summary.

A. THE RESPIRATORY CENTRES.

It is convenient to review the evidence for the localisation and organisation of the centres in terms of the three principal methods of investigation: 1. Serial ablation of, and the placement of, localised lesions within the brain stem; 2. Electrical and chemical stimulation of the brain stem; 3. Electrical recording from the brain stem.

1. Ablation Studies.

Without doubt, Legallois (1812) originated the concept of a medullary centre which is vital to the genesis of respiratory movements. Writing in "Experiences sur le principe de la vie" he states "Ce n'est pas du cerveau tout entier que depend la respiration mais bien d'un endroit assez circonscrit de la moelle allongee, lequel est situe a une petite distance du trou occipital et vers l'origine des nerfs de la huiteme paire (ou pneumo-gastriques). Car, si l'on ouvre le crane d'un jeune lapin, et que l'on fasse l'extraction du cerveau, par portions successives d'avant en arriere en le coupant par tranches, on peut enlever de cette maniere tout le cerveau proprement dit, et ensuite tout le cervelet et une partie de la moelle allongee sans que la respiration s'arrete. Mais elle cesse subitement lorsque on arrive a comprendre dans une tranche l'origine des nerfs de la huiteme paire". More specifically he emphasises that the region of the medulla "dans
"lequel reside le premier mobile de la respiration" must be left in continuity with the spinal cord for respiration to continue. Thus was initiated the concept of a medullary centre of first-order importance to the production of respiratory movements.

Flourens (1842) was responsible for the description 'noeud vitale' which was the site of a bilateral lesion which when placed in the dorsal reticular formation just rostral to the level of the calamus scriptorius immediately abolished respiration. However he later modified this opinion (1858) asserting that a 5 mm wide spatula had to be inserted at the level of the obex for respiration to be abolished. Gierke (1873) believed that the alleged function of the 'noeud vitale' was due to the tractus solitarius. Previously however, Longet (1847) had shown that section of the pyramids and restiform bodies was without effect, while lesions of the reticular formation at that level instantly abolished respiration. Essentially the same results were obtained by Mislawsky (1885) and Gad and Marinesco (1892).

Entirely new observations were made by Langendorff and Nitschmann (1880), Langendorff, Nitschmann and Witzack (1881). When young rabbits were made spinal just below the level of the calamus scriptorius and maintained an artificial respiration, the diaphragm gave periodic contractions some time after stopping artificial respiration, the movement of air being sufficient to operate a Marey
tambour. Although such contractions might develop spontaneously, it was usually necessary also to apply some forms of sensory stimulation, for example stroking or lightly blowing on the fur over the thorax, or faradic stimulation of the sciatic nerve. Older animals manifested the same phenomena if given small doses of strychnine. Although he did not deny the importance of brain-stem centres Langendorff invoked the notion of spinal shock to explain the depression of spinal respiratory centres following spinal transection. This conclusion was however considered invalid by Porter (1894) and by Deason and Robb (1911) in their work on the descending respiratory pathways.

Lumsden, (1923a,b) proposed the existence of four centres located at different levels in the medulla. The most caudal of these was a 'gasperg' centre and just rostral to it the 'apneustic' centre. Activity of the latter was manifest as a series of maintained inspirations lasting many seconds. The 'apneustic' centre seems to be co-extensive with the region from which in the rabbit Markwald (1887) had obtained respiratory movements in response to electrical stimulation and which he had thought to be responsible for the 'inspiratory cramps' he observed in ablation experiments. Both workers claimed that the inspiratory (apneustic) centre was inhibited by a more rostrally located centre. Markwald placed this in the region of the posterior colliculus, whereas Lumsden placed it in the rostral two thirds of the pons and called it the 'pneumotaxic centre'. If this centre was intact, bilateral vagotomy was not followed by apneusis. The alternative proposition of 'inherent
rhythmicity of medullary centres was put forward earlier by Rosenthal (1865) who found that contractions of the diaphragm persisted when transections were made of the cervical cord below C5 and of the medulla above the striae acusticae, and in addition the vagi and the cervical dorsal roots were sectioned.

Stella (1938a,b; 1939) confirmed Lumsden and showed also that CO$_2$ excess and O$_2$ lack, acting through peripheral chemoreflex mechanisms, could intensify the depth of inspiratory apneusis caused by temporary blocking of the vagi in low-decerebrate animals. Stella also showed that cutting all the cervical and thoracic spinal roots did not affect apneusis thus discrediting the suggestion made earlier by Henderson and Sweet (1929) that apneusis was a decerebrate rigidity of the respiratory muscles. However, as Stella's preparations showed no intercostal respiratory movements, and as it is now known that decerebrate rigidity may arise in two different ways (Granit, Holmgren and Merton, 1955), his results are difficult to assess.

The entire problem concerning the relation between the various centres was reassessed by Hoff and Breckenbridge (1949, 1950). They defined apneusis as a steadily maintained inspiratory tone on which phasic respiratory movements may, or may not, be superimposed and showed that it was not the inevitable consequence of low-decerebration combined with vagotomv. In confirmation of an earlier report by Nicholson and Hong (1942), Hoff and Breckenbridge found that transection of the brainstem still more caudally was followed
by a resumption of normal breathing (cf. Lumsden's 'gasping' centre). They interpreted apneusis as a 'sort of decerebrate rigidity of the inspiratory system' adopting for this purpose the newly established concepts of the brain stem facilitatory and inhibitory systems of Magoun and Rhines (1946a,b). Their interpretation thus opposed the conclusion drawn by Pitts (1946) in his important review. This was that apneusis represents the unrestrained activity of the medullary inspiratory centre freed from all inhibitory influences, or, as Pitts states, "the rhythm of breathing is impressed upon the respiratory centre by inhibitory mechanisms operating from without".

A different localisation altogether was found by Hukuhara, Nakayama, Baba and Odanaka (1951), and Hukuhara, Sumi and Okada (1953), in the lateral reticular formation at the level of the striae acusticae. At the level of the obex, the bilateral lesion had to be placed more medially to be effective and so they presumed there is convergence of the descending respiratory tracts. Hukuhara, Sumi and Okada (1953) found that lesions placed medially, 2 mm rostral to the obex, 1mm lateral to the mid-line and 3mm deep, caused no appreciable change in respiration. This is particularly significant since these lesions lie in the heart of the inspiratory area defined by Pitts, Magoun and Ranson (1939a) using electrical stimulation.

Wang, Ngai and Frumin (1957) obtained similar results to those of Lumsden (1924a), Pitts et al. (1939a) and Tang (1953), with regard to the lowest level of medullary transection compatible with
normal or 'gapping' breathing and to the localisation of the
'pneumotaxic centre' in the rostral pons, but they offered a
new interpretation. They believe that the primary respiratory
act is a sustained inspiration to which rhythmicity is imparted
via pneumotaxic or vagal afferent mechanisms. But whereas Pitts
et al. attribute apneusis to the unrestrained activity of a
medullary inspiratory centre, Wang et al. attribute it to the un­
restrained activity of the 'pontile apneustic centre' (cf. Lumsden,
1924a), which in turn is inhibited by a pneumotaxic centre in
the rostral pons at the lateral portion of the isthmus, in agreement
with the localisation shown by Tang (1953). Wang et al. (1957)
suggest that the inherent rhythmicity of the medullary centres
(at times manifest as gasping, at others as normal, respiratory
movements) is suppressed under normal conditions by the modulated
activity of the apneustic centre and that the medullary centres
"probably function only as a sub-ordinate component, and as the
efferent relay for the central respiratory control" (the 'apneustic-
pneumotaxic centre' complex).

The belief that the 'pneumotaxic' centre plays a vital
role in the neural mechanism of respiration has been seriously
challenged by Hukuhara and Nakayama (1959) who showed that the
rostral pons in vagotomised cats and dogs could be entirely removed
without apneusis ensuing, provided that the vagi were not sectioned
immediately prior to the transection of the pons.
A final complication in assessing the effects of lesions, is the claim by Salmoiraghi & Burns (1959b) that a longitudinal incision through the mid-line abolished respiratory movements. In contradistinction Lewandowski (1878), Langendorff, Nitschmann and Witzack (1881) and Hukuhara et al. (1953) claimed that respiratory movements persisted following such a lesion and their findings are consistent with the preponderantly unilateral projection of the descending efferent pathways described by Pitts (1940).

From the foregoing account it is clear that the studies employing local destructions or ablations of the medulla and pons have revealed that these areas play an important, and in some instances essential, role in the production of respiratory movements. However, the function, and therefore the importance, accorded to various areas differs considerably and depends principally on whether the investigator arbitrarily regards the residual respiratory movements as 'normal' or unrelated to normal (such as the gasp).

Not only are the facts therefore in dispute but so also are the definitions on which the localisation and organisation of the centres depend. It seems doubtful whether any further advances can be made using ablation techniques unless more precise methods are used to measure the changes in activity of the inspiratory and expiratory motoneurones, the activity of the latter having been ignored in most investigations.
2. **Stimulation Studies**

   a. Electrical stimulation. In 1883 Markwald showed that electrical stimulation of the brain stem caused 'inspiratory cramps' similar to those which followed bilateral vagotomy in an animal in which the upper medulla had been transected. Earlier, Markwald and Kronecker (1880) had shown that electrical stimulation of the central end of the vagus gave inspiratory and expiratory reactions which they attributed to corresponding centres in the medulla. Markwald concluded from his results that inspiratory and expiratory centres were located just caudal to the striae medullares and had an inherent rhythmicity expressed in the alternating inspiratory cramps.

   This form of investigation was not used again until Monnier (1938a,b; 1944) repeated the experiments with more refined techniques and full histological control of the electrode placements. He grouped the various types of response and emphasised the differential effects on the rhythmic and static components of normal respiration. Thus he obtained inspiratory displacements of the thorax and diaphragm without any effect on the phasic inspiratory and expiratory movements, although intense inspiratory displacements could inhibit periodic respiration. Monnier introduced the terms 'postural inspiratory' and 'postural expiratory hypertonia', to distinguish these effects from those exerted on body posture as a whole. Thus he took into account the extra-respiratory effects of stimulation in this region, as described later by Magoun and Rhines (1946a,b), Bach (1952), and Alexander (1946).
Pitts, Magoun and Ranson (1939a,b) placed an entirely
different interpretation on essentially the same data. They defined
the respiratory centres operationally as the anatomical distribution
of the stimulating points which gave maximal, uninterrupted
inspiratory and expiratory 'apnoeas'; these responses were clearly
the same as Monnier's hypertonias. The centres so defined are
in the medulla, and comprise an inspiratory centre in the ventral
reticular formation overlying the cephalic four fifths of the
inferior olive; superior to it, and slightly more rostral, is the
expiratory centre.

Brookhart (1940) and Amorosa, Bell and Rosenberg (1951)
agreed that the neurones comprising the centre were mostly in the
reticular formation, but would not concede the existence of two
anatomically distinct zones. Dirken and Woldring (1951), on the
other hand, agreed with the localisation described by Pitts et al.
and provided supplementary evidence from electrical recording
studies. Rijlant (1942) also was unable to find a sharp delimitation
into inspiratory and expiratory zones. He thought that the
medullary region giving respiratory responses (his 'moderator
centre') exerted a tonic facilitatory action on the motoneurones
and that this activity summated at the level of the spinal
motoneurones with activity from a supra-bulbar 'phasic centre'.
Furthermore he claims to have demonstrated two distinct descending
pathways to the motoneurones, a lateral pathway which makes mono-
synaptic connections with the phrenic motoneurones, and a paramedian
pathway which exerts its effects with longer latencies (Rijlant,

Chatfield and Purpura (1953) studied the effects of vagal stimulation on the response to electrical stimulation of the inspiratory centre of Pitts et al. (1939a). They found that the phasic respiratory movements interrupting apneusis (which movements Pitts et al. had ignored for the purpose of their definition) were due to phasic relaxations of the diaphragm and that these were inhibited by concomitant stimulation of the vagus. They concluded that the tonic component of apneusis is due principally to the intercostal musculature. Their results suggest the independence of the mechanisms responsible for phasic respiration and apneusis, and give support to the views held by Monnier (1938) and Rijlant (1950).

Liljestrand (1952) was unable to confirm the existence of anatomically separate inspiratory and expiratory areas in the medial reticular formation although the tonic effects he obtained did show an inspiratory dominance ventrally and an expiratory dominance dorsally.

Although it is clear that electrical stimulation experiments have revealed the presence in the medulla of important descending systems of fibres, their role in the production of normal respiratory movements is far from clear. The delineation of the centres by Pitts et al. (1939a,b) is based on highly selected data, namely, the sites giving maximal inspiratory and expiratory apneusis. Yet, as an examination of the diverse responses produced by brain stem stimulation shows, the same sites may produce
merely shifts in the inspiratory or expiratory position of the chest without interrupting the periodic respiratory movements, hence the use of the terms 'inspiratory' and 'expiratory hypertonia' by Monnier (1938) and of the 'moderator centre' by Rijlant (1942).

The significance of the results obtained with electrical stimulation rests entirely on the validity of the definitions employed. This is especially important in relation to the work of Pitts, Magoun & Ranson (1938a,b) which appears generally to be accepted as the standard work. The fundamental question arises, what is the relation of inspiratory and expiratory apneusis to normal breathing? As discussed above, and earlier in connection with the ablation studies, there is no agreement on this so the validity of the delineation rests entirely upon the point of view adopted by each investigator. Brodal (1957) comments pertinently on this problem: "Nature does not necessarily work in those terms of function which we have set up as working tools in our attempt to understand biological problems".

Finally, as Wyss (1954) points out, complete destruction of the regions giving apneusis on electrical stimulation should be followed by irreversible loss of respiration. He thought that such experiments had not been performed. However, as described in the previous section, lesions so placed by Hukuhara et al. (1953) were without appreciable effect on respiration. Also, earlier Henderson and Craigie (1936) described a preparation (cat 'h') in which respiratory movements persisted following a transverse lesion
which histologically was shown to extend laterally at least to the fasciculus solitarius on either side, ventrally to the dorsal surface of the inferior olive, and approximately 1.4 mm rostral to the obex. If these important, but overlooked results are confirmed, it is clear that an entirely different significance must be placed on the responses evoked by electrical stimulation of the medial reticular formation.

b. Chemical Stimulation. The question of whether the neurones comprising the respiratory centres are themselves sensitive to CO₂ has been given scant attention. Both Gesell (1940) and Pitts (1946) are clearly of the opinion that they are. Pitts believes that under the combined influence of chemical and synaptic stimulation the neurones of the respiratory (inspiratory) centres discharge repetitively. In accordance with this view, Comroe (1943) found that local injections of buffered carbon-dioxide sodium bicarbonate solutions into the medulla produced hyperpnoea, the distribution of the active areas corresponding approximately to the inspiratory and expiratory centres delineated by electrical stimulation. However, Liljestrand (1953) found that the effective areas were located in the lateral reticular formation at the level of the inferior olive and thus did not correspond to the respiratory centres defined by electrical stimulation.

Euler and Soderberg (1952a,b) found that certain respiratory reflexes which they considered to be mediated via the
respiratory centres (they used the term 'respiratory reflex and coordination centre') were unaffected by concentrations of chloralose which abolished the stimulating action of CO₂. They concluded that the central respiratory effect of CO₂ is due to special chemoreceptors and in support of this claim they find in the area of the respiratory centres 'slow' potentials which occur specifically in response to CO₂ but not in response to respiratory reflexes.

3. **Electrical Recording**

Experiments utilising electrical recording from the brain stem and spinal cord were initiated by the work of Adrian and Butendijk (1931) on the goldfish, although these workers were concerned more with the general problem of autorhythmicity of neurones than with the localisation and organisation of respiratory centres. The first systematic study in the mammal was made by Gesell, Bricker and Magee (1936) who made a comprehensive survey of the timing, configuration and distribution of potentials with a respiratory rhythm in the medulla and spinal cord. On the basis that no other structure yielded respiratory potentials in such abundance, they concluded that the reticular formation is of paramount importance as an integrating centre for the orderly recruitment of respiratory muscles. They found no evidence of separate inspiratory and expiratory regions. Nor later, did Amoroso, Bainbridge, Bell, Lawn and Rosenberg (1951). The latter authors, noting the relative scarcity of respiratory potentials
in the medulla, concluded that the neuronal aggregates vital to the genesis of respiration lie in the spinal cord (cf. Brown-Sequard, 1860; Langendorff, 1880; Cordier and Heymans, 1955). Dirken & Woldring (1951) did find, however, that neurones firing with an inspiratory and expiratory rhythm had different anatomical distribution.

In confirmation of their previous findings using local destructions, Hukuhara, Nakayama and Okada (1954) found a lateral distribution in the reticular formation of cells firing with a respiratory rhythm. Haber, Kohn, Ngai, Holaday and Wang (1957) also found respiratory discharges in the lateral reticular formation between 3 mm rostral and 3 mm caudal to the obex. The expiratory discharges were concentrated caudally (corresponding to the areas from which Ngai and Wang (1957) obtained expiratory spasms on electrical stimulation) whereas the inspiratory discharges were located rostrally. This location of inspiratory and expiratory neurones does not correspond to that described by Pitts et al. using electrical stimulation.

Baumgarten (1956), Baumgarten, Baumgarten and Schaefer (1957) used a more precise definition of respiratory neurones than had been accepted hitherto and employed succinylcholine neuromuscular blockade and diffusion respiration to exclude the possibility of recording periodic discharges evoked by periodically activated proprioceptors. A region exclusively
comprised of units discharging during inspiration was found laterally in the reticular formation, rostral to the obex and ventral to the tractus solitarius (cf. Hukuhara et al. 1953). Expiratory neurones were concentrated in the region caudal to the inspiratory region. Baumgarten et al. (1957) emphasised that the localisation of centres defined by electrical stimulation shows no correspondence at all to the areas where the activity of respiratory neurones may be recorded. As stated in the previous section, there is a similar lack of correspondence between the results of electrical stimulation and the placement of localised lesions. In a later study Baumgarten & Kanzow (1958) describe two types of respiratory (inspiratory) neurone. One type, their Rα, is believed to drive the inspiratory motoneurone. The other type, Rβ, is believed to be inhibitory to the Rα type and they suggested that it may mediate either the reflex inhibitory, or intra-central inhibitory actions associated with vagal afferents discharge and the intrinsic inspiratory-inhibitory mechanisms (e.g. the pneumotoxic centre) respectively.

Salmoiraghi and Burns (1960) confirmed the findings of Amoroso et al. (1951), and Hukuhara et al. (1954) both with regard to the location, and the lack of segregation of inspiratory and expiratory neurones. Furthermore, Salmoiraghi & Burns (1960a) found that progressive isolation of the brain stem by caudal, rostral and mid-line transection, markedly reduced the number of
units active with a respiratory rhythm (cf. Euler & Soderberg, 1952). They concluded that there was no evidence for pacemaker cells capable of spontaneous discharge in the absence of neighbouring neural systems. The presence of such cells would be expected according to the theory proposed initially by Rosenthal (1875) and adopted by later workers such as Lumsden (1924), Pitts, Magoun, and Ranson (1939), Wang et al. (1957). Burns and Salmoiraghi (1960b) interpreted their results as supporting the hypothesis that the maintained discharge of respiratory neurons is due to a process of self-excitation within the networks of inspiratory and expiratory neurones. The reciprocal inhibitory connections between these two networks could ensure that the activity of one system increases as the activity of the other diminishes, and that cumulative inhibition or fatigue, would terminate the process (Burns and Salmoiraghi, 1960c). This postulate of self-excitation in closed networks of neurones was earlier suggested as a possible basis of the sustained augmenting activity of the respiratory centres (e.g. Pitts et al. 1939b).

B. THE PROPRIOCEPTIVE REGULATION OF RESPIRATORY MOVEMENTS.

1. Proprioceptive reflexes of pulmonary origin.

Following Rosenthal's (1865) experiments using electrical stimulation of the vagus nerve, Hering and Breuer (1868) used alterations in lung volume to study the effects on respiration of natural stimulation of the pulmonary end-organs of the vagus nerve. They demonstrated an inspiratory-inhibitory, expiratory-
activating reflex produced by lung inflation and an expiratory-inhibiting, inspiratory-exciting reflex elicited by lung deflation. These two reflex actions constitute the self-regulating mechanism ("Selbststeuerung") of respiration, whereby each expansion of the lungs in inspiration inhibits the action which caused it and so initiates the following expiration. Conversely, the expiratory collapse of the lungs is excitatory for the subsequent inspiration. Hering and Breuer explicitly stated that both the force and the duration of inspiration and expiration are modified. Recently, measurements made only of the duration of the reflex, (i.e. of the apnoea) led Widdicombe (1961) to conclude possibly erroneously, that in man the Hering-Breuer reflex is weak. Confirmation of Hering and Breuer's findings was made on the diaphragm-slip preparation of the rabbit by Head (1889) and of the cat by Barry (1913). Using partial blocking of the vagus by cold, Head disclosed a third reflex (Head's paradoxical response) which is a strong, sustained inspiratory-exciting reflex produced by lung inflation. There is a possibility that Head's paradoxical response may be associated with the gasp produced by lung inflation in the new-born (Cross, Klaus, Tooley and Weisser, 1960). Other authors (Worzniak and Gesell, 1939; Knowlton and Larrabee, 1946; and Widdicombe, 1954) have studied a transient inspiratory effort evoked by lung inflation in the cat.

Electrophysiological investigation by Adrian (1933) showed that lung inflation excites slowly adapting pulmonary
receptors which, according to Knowlton and Larrabee (1946) and Paintal (1953), travel in fast conducting fibres of the vagus. The existence of specific 'deflation' receptors, as initially described by Adrian, appears still to be equivocal, as also is their role in eupneic respiration. Knowlton and Larrabee found deflation receptors that were not excited also by inflation. Paintal described two deflation receptors that did not respond to inflation, but contrary to Adrian, found them to be discharging at normal expiratory lung volumes. In 400 individual vagal afferents examined by Widdicombe (1954) only 7 did not respond to lung inflation. This question is of importance in relation to the role and mechanism of the Hering Breuer reflexes in normal respiration. According to Wyss (1954) specific deflation receptors play no role in normal breathing. He believes that the Hering Breuer reflexes are to be explained solely on the basis of a frequency conditioned reversal of the central effects of the pulmonary inflation receptor activity when their low frequency discharge during inspiration increases to a high frequency discharge during inspiration. He bases this interpretation on the differential effects of low and high frequency electrical stimulation of the vagus.

The central pathway through which these reflexes are mediated, the 'inspiratory' and 'expiratory reflex centres' have been localised by Anderregen, Oberholzer and Wyss (1946a, y) in the
tractus solitarius and nearby reticular formation (the nucleus tractus solitarius). Interestingly, this is the region which Baumgarten, Balthasar and Koepchen (1960) have localised as the site of inspiratory neurones capable of autochthonous activity. However, Wyss (1954) believes that the vagal reflexes are mediated through the ventral medial reticular formation in the region designated by Pitts et al. (1939a) as the inspiratory centre.

Further detailed consideration of the mechanism of the vagal reflexes would seem unwarranted in view of the uncertainty concerning the localisation and organisation of the respiratory centres through which the reflexes are presumed ultimately to operate.

2. **Proprioceptive reflexes of somatic origin.**

Comparatively little information is available concerning the role of somatic proprioceptive reflexes in respiration. What evidence there is relates almost exclusively to the role played by proprioceptive afferents in controlling the activity of the centres without serious consideration being given to possible reflex actions at the segmental or motoneuronal levels. Surprisingly, in this latter connection, no use has been made of the diaphragm-slip preparation, the diaphragm being one of the few respiratory muscles which has been successfully isolated for the recording of contractions. Cleland and Tait (1927) did however describe a reflex contraction in response to stretch
of the isolated muscles of the abdominal wall, accompanied by an inhibition of the contralateral muscles. They also described an inhibition of tone in these muscles with each inspiration.

The effects of cutting the dorsal roots of the spinal nerves have contributed some information concerning the importance of proprioceptive afferent discharge. Coombs (1918) found that this greatly diminished intercostal respiration in the cat, and even abolished it in kittens (Coombs and Pike, 1930). Stella (1938a) claimed that dorsal root section is without effect on respiration, but it must be pointed out that his conclusion cannot be regarded as refuting the findings of Coombs (1918) since Stella's preparation showed no costal respiration before dorsal root section.

In contrast to the widespread de-afferentation employed by previous workers, Sears (1958) restricted the section of the dorsal roots to one or two segments and used electromyography of the muscles of that segment to assess the effects. He found that the recruitment of units and the increased frequency of discharge of already active units, evoked by closing the trachea at the peak of inspiration (the inspiratory-inhibiting, expiratory activating reflex of Hering and Breuer), were greatly diminished by dorsal root section. His results strongly suggested that there was an effective proprioceptive reflex of myotatic nature in the intercostal muscles, which would be consistent with their abundance of muscle spindles (Huber, 1901). Ramos and Mendoza (1959) described reflex contractions of the intercostal muscles in response
to stretch. These contractions were also abolished by section of the dorsal roots. Nathan and Sears (1960) reported that in man section of the cervical dorsal roots leads to a temporary paralysis of the hemi-diaphragm, similar effects being observed in the intercostal muscles. However, by the technique of cutting the dorsal roots Sant 'Ambrogio, Wilson and Frazier (1962) could find no evidence for a proprioceptive reflex in the diaphragm of the cat; nor, on electrical stimulation of the cervical dorsal roots was there a monosynaptic reflex discharge of phrenic motoneurones. They did however obtain polysynaptic reflex discharges, thus refuting, in common with Dowman (1955), the claim by Calma (1952) that the discharge of phrenic motoneurones is controlled exclusively through the descending pathways from the respiratory centres. Dowman also described intercostal to intercostal nerve reflexes whose central latency and discharge characteristics conformed with those of the polysynaptic limb reflexes.

Fleisch (1928, 1929, 1930), using his pneumotachograph, measured the changes in air flow and tracheal pressure consequent on sudden partial obstructions of the air passages. As the changes he observed persisted after vagotomy (in his animal experiments), he attributed them to a series of compensatory proprioceptive reflexes. Fleisch's interpretation of the facts was seriously challenged by Niekerk and Ter Braak (1935) who were unable to find the afferent pathway for the alleged reflexes and hence attributed the responses to the viscous-elastic properties of the
respiratory muscles. Their conclusion was later given further support by Riedstra and Dirken (1953), who obtained similar responses in simulated experiments on cadavers. Dolivo (1946) also obtained similar responses to those described by Fleisch, but, as he also could not entirely abolish them by total deafferentation, he supposed that the afferents must pass in the ventral roots, a view which he also holds in connection with the mechanism of the 'crossed phrenic phenomenon'.

Other evidence pointing to the presence of a somatic proprioceptive control of respiratory movements derives from the recording of afferent discharges from respiratory muscles and associated structures. Although earlier workers had recorded afferent discharges arising in respiratory muscles from the dorsal columns in the cervical region, from the relay nuclei (Gesell, Bricker and Magee, 1936; Gesell, Magee and Bricker, 1960; Yamamoto, Miyajima and Urabe, 1960), and from the phrenic nerve (Cardin, 1944; Cuenod, 1961) the first report of recording from the thoracic dorsal roots is that of Siebens and Puletti (1961). Unitary discharges with a respiratory periodicity were found throughout the respiratory cycle. Of the 65 units studied 31 were inspiratory and 19 expiratory when classified according to the relation of their peak discharge frequency to the respiratory cycle. Critchlow and Euler (1962) also recorded the activity of single units from thoracic dorsal roots, but following Matthews (1933), they further classified them as belonging to muscle spindles or Golgi tendon organs. Furthermore, they interpreted their results from using
procaine blocking of the small fibres in the intercostal nerves as indicating that the fusimotor innervation of the intercostal muscles is subject to a spinal reflex control initiated by the respiratory movements. This latter report appeared after experiments to be described in this thesis had shown that the intercostal fusimotoneurones are in fact under a central control resembling that which governs the discharge of alpha respiratory motoneurones.

C. SUMMARY

The concept of a respiratory centre has been deemed necessary to account for the co-ordinated action of diverse, widely scattered groups of motoneurones innervating the respiratory muscles. The origin of this point of view may be traced to the earliest of the ablation studies which demonstrated the abrupt cessation of respiratory movements consequent to high-spinal transection. Although the wealth of evidence suggests that the medullary reticular formation plays an indispensable role in the neural mechanism of respiration, what this role is, is far from clear. The interpretation of data has been greatly influenced by a desire to find, if not a 'noeud vitale' in the sense implied by Fluorens, at least a circumscribed group of neurones from which arise axons passing to the spinal motoneurones. These axons are supposed to carry impulses, the temporal patterns of which determines the periodic and alternate excitation of inspiratory and expiratory motoneurones. To the best of my knowledge no advocate of a
'respiratory centre' has proposed that inhibition of the respiratory motoneuron plays any role in the nervous mechanism of respiration. Nothing is known of the essential features of the efferent discharge which is cut off by high-spinal transection. There is suggestive but not conclusive evidence for a segmental proprioceptive regulation of respiratory motoneuron discharge, but no concept appears to have developed to indicate how these spinal reflexes are integrated with the activities of the 'centres'.

In recent years, the delineation and mode of operation of the respiratory centres as suggested by Pitts et al. (1939a) has proved most popular with teachers and authors of textbooks, due presumably to the clarity of their concept. However, the position now is that the relationship between electrically induced apneusis and normal respiration must be regarded as highly equivocal (cf. Baumgarten et al. 1957). Unless future work can show a precise correlation between the results from using different methods, it is clearly preferable that the term 'respiratory centre' should not be used unless specifically defined for the problem under consideration.

D. SCOPE OF INVESTIGATIONS DESCRIBED IN THIS THESIS.

As indicated at the commencement of the introduction, the investigation of the mechanism of respiratory movements has proceeded quite differently from that of the limbs where the study of the motoneurone and of its segmental connections has been pre-
- eminent. To study the respiratory motoneurone from this point of view appeared to offer a fresh approach towards a better understanding of the neural mechanism of respiration. Furthermore, the technique of intracellular recording from motoneurones (Brock, Coombs and Eccles, 1952; Woodbury and Patton, 1952) lends itself admirably to this end.

The starting point of the investigation was to use intracellular recording to define the synaptic events leading to the discharge of respiratory motoneurones and to investigate some of the reflex pathways acting on them. The intercostal and abdominal motoneurones of the thoracic spinal cord were chosen for investigation, as the evidence in the literature suggested that the proprioceptive reflexes acting on them might be better developed than those of the phrenic motoneurones. Also, the presence of motoneurones innervating the internal and external intercostal muscles allowed the possibility of recording both from inspiratory and expiratory motoneurones in the same segment.

In the course of this work it was found necessary to broaden the scope of the investigations since it was discovered that the fusimotor neurones innervating the external and internal intercostal muscle spindles are controlled in a very similar manner to the control of inspiratory and expiratory alpha motoneurones. These investigations are described in Section V, and others which they led to, concerning the innervation and mechanical properties of intercostal muscles, are described in sections IV.
Section III deals with histology, Section VI with intracellular recording and Section VII contains a general discussion.
II. ANATOMY, DISSECTION AND METHODS.

A. ANATOMY OF INTERCOSTAL NERVES.

As it appears that there are no previous detailed descriptions of the innervation of the cat intercostal muscles, the anatomy and dissection is described in detail and a nomenclature is introduced.

Fig. 1 illustrates the principle anatomical features of a lower thoracic segment (T.6 - T.11) including the innervation of the abdominal wall. The innervation of the intercostal muscles of the upper thoracic segments is similar. The ventral spinal ramus divides into two branches, the larger of which (Fig. Ia), here referred to as the 'internal intercostal nerve' (known generally as 'the intercostal nerve'), gives off numerous filaments which innervate the internal intercostal muscle (Fig. Ib) and the intra-costalis muscle (not shown in Fig. I, see instead Fig. 3).

A lateral branch of the internal intercostal nerve (Fig. Ic) innervates through one division the abdominal oblique muscle (Fig. Id) and through two others, the lateral and ventro-lateral skin (Fig. Ie). Ventrally, the internal intercostal nerve provides branches which innervate the rectus abdominus muscle (Fig. If) and the ventral skin (Fig. Ig). Because the internal intercostal, the abdominal oblique and rectus abdominis muscles are expiratory muscles, the internal intercostal nerve thus carries the axons of all the expiratory motoneurones whose outflow is in the ventral spinal ramus.
After its origin, the smaller branch of the ventral ramus (Fig. Ih), described here as the 'external intercostal nerve', penetrates the internal intercostal muscle and passes laterally in the intercostal space in a position immediately caudal to the rib giving off filaments which innervate the external intercostal muscle (Fig. Ii). Distal to the angle of the rib the external intercostal nerve crosses the lateral intercostal nerve and completes its distal course in the middle of the intercostal space to terminate just short of the ventral limit of the external intercostal muscle. As the latter is the inspiratory muscle of the thoracic cage, the external intercostal nerve at its origin carries the axons of all the inspiratory motoneurones whose outflow is in the ventral spinal ramus.

B. DISSECTIONS AND PREPARATIONS.

1. General.

Cats were anaesthetised with sodium pentobarbitone (55 mg/kg I.V.,) or with Urethane - Chloralose mixture (5.0 ml/kg I.P., of a solution containing 10% Urethane and 1% Chloralose). After venous cannulation and tracheostomy the cat was placed prone and a mid-line incision made from T.4-L.1. The latissimus dorsi muscle was cut through bilaterally close to the mid-line and sewn back to the skin edges. The spinalis, longissimus dorsi and iliocostalis muscles were removed bilaterally from T.7-T.11 and a laminectomy made if required. These procedures gave a clear exposure of the external intercostal muscles extending from the vertebrae to beyond the angle of the rib.
2. Dissection of intercostal nerves and their filaments.

In section V it has been found convenient to use the terms 'inspiratory nerve filament' and 'expiratory nerve filament' respectively, for filaments dissected from the external and internal intercostal muscles. Their dissection is described below.

a. 'Inspiratory nerve filaments'. The dissection is illustrated in Fig. 2A and B. An incision was made through the caudal border of the external intercostal muscle (heavy dotted line in A) which was reflected forwards so exposing the external intercostal nerve applied to the rostral, inner surface of the external intercostal muscle (B). After arising from the parent nerve, the filaments are across the interspace where caudally they form 2 or 3 branches which are distributed T-fashion before taking up their intra-muscular course. A cotton loop was tied to the central end of the filament which was cut at the level of the branching and the filament was then freed from the parent nerve as far proximally as possible by opening the loose sheath which united them. The filaments were never sub-divided so as to avoid damage to the fibres and thus maintain the proportionality between spike height and fibre diameter which obtains for myelinated fibres in intact nerve (Blair and Erlanger, 1933; Gasser and Grundfest, 1939).

b. 'Expiratory nerve filaments'. The dissection is illustrated in Fig. 2C. A cut was made through the internal intercostal muscle immediately caudal to the rib to expose the internal intercostal nerve lying between the internal intercostal muscle and a deeper, extremely thin and often discontinuous layer
of muscle which I believe to be homologous with the human intra-costalis muscle described by Walmsley (1915). By stripping the internal intercostal muscle fibres back towards their caudal insertions the filaments were exposed across the full width of the intercostal space; they were prepared in a similar manner to the inspiratory nerve filaments.

3. Dissection and general arrangements for intracellular recording.

The external and internal intercostal muscles were freed for stimulation close to the vertebral column as indicated by the white areas on these nerves (Fig. 1). This made it possible to activate antidromically either the inspiratory or expiratory motoneurones independently, or to assess the reflex effects of stimulation of afferents in the intercostal nerves. Other nerves prepared were the medial and lateral divisions of the internal intercostal nerve, muscular (to the spinalis dorsi and multifidus spinae muscles (Fig. Ij) and cutaneous divisions (Fig. Ik) of the posterior ramus, and the contralateral nerves corresponding to those mentioned.

The greatest obstacle to intracellular recording from thoracic motoneurones in the spontaneously breathing animal was the periodic displacement of the spinal cord caused by movements of the underlying vertebrae; those movements were prevented as follows. The laminectomy was limited to 3 segments, usually between T.8 and T.10, although other combinations were possible. The vertebral column was stretched tautly between flat-jawed clamps gripping the spinous processes of T.6 and T.7 and pincer clamps gripping the mammillary processes of T.II. The vertebrae of T.8 and T.9 were immobilized
by pressing against their lateral processes with screw-operated side clamps after opening the capsules of the tubercle articulations. Movements of the spinal cord due to venous pulsation were reduced by supporting the pelvis so that the abdomen was pendant and not compressed. Transection of the spinal cord below the level investigated was not necessarily advantageous as this often exaggerated the movements of the spinal cord caused by traction from intercostal nerves from the upper thoracic segments. The dura was opened widely and sometimes it was found advantageous to pass threads through the cut edges to allow the spinal cord to be lifted away from the vertebral column. In general, each preparation was treated independently to obtain good conditions for satisfactory recording.

The cat was heated by electric heaters placed beneath the abdomen and the exposed tissues were covered with mineral oil maintained at 37°C by electric immersion heaters.

4. **Dissection and arrangements for mechanical recording.**

As shown in Fig. 3, the rostral border of a rib (T.7-T10) was cleared of periosteum at two places approximately 1.5 cm apart so that the rib could be firmly clamped by two pairs of artery forceps mounted on bars fixed to the animal frame. At two places on the rib caudal to the fixed rib, corresponding to the position of the clamps, a curved, chisel-edged instrument was passed completely under the rib severing the periosteum from the bone. At these two places, the ribs were cut across. One end of the severed length of rib was drawn outwards, and after identifying the plane separating the external from the internal intercostal muscle the caudal insertions
of the latter were cut through across the width of the sector, thus isolating a sector of external intercostal muscle with its innervation and blood supply intact.

The internal intercostal muscle was prepared in a similar fashion after removal of the external intercostal muscle. This dissection was considerably more difficult and its success depended mainly on the intra-costalis muscle being well-developed so that it could be left intact, together with the pleura, without a pneumothorax resulting. When the latter occurred, unless the hole could be closed, the experiment was done with the animal on artificial respiration.

Attachment of the freed rib to the recording strain gauge (Statham GI - 1.5 - 300, or GI - 8 - 350) was made through thread as indicated in Fig. 3, care being taken to align the strain gauge with the direction of the muscle pull. As the muscle was deep in a pool of mineral oil and as the initial tension (50 - 100 gms) was 10-20 times greater than the weight of the sector, there was negligible sagging of the muscle even though it was not vertical.

For stimulation in continuity, the appropriate intercostal nerve was freed from surrounding tissues immediately proximal to the sector and all of its proximal filaments divided. Contractions of the distal musculature were eliminated by dividing the same nerve immediately distal to the sector; and in order to prevent contraction of other muscles when the ventral roots were stimulated, the other intercostal nerve was divided at its origin from the ventral ramus. Free access to the extremely short ventral roots was secured by removing two or three segments of spinal cord. All tissues were
covered with mineral oil maintained at 37-38°C. This temperature regulation was essential and was checked repeatedly as the speed of muscle contraction has a high temperature coefficient \( T_{10} = 1.53 \) according to Gordon and Phillips (1953).

C. ELECTRICAL RECORDING TECHNIQUES.

1. Intracellular recording.

Intracellular recording was done with glass micro-electrodes pulled to a fine tip by the instrument described by Winsbury (1956). They were filled either with 3M KCl, 3M KCl containing 1% agar, or 2M K citrate, and after inspection of their tips with a water immersion microscope the resistances of those selected for use were usually between 5 and 10 M\( \Omega \). The micro-electrodes were connected through a cathode follower stage to a low-gain D.C. amplifier for the measurement of membrane potentials (registered on a meter or on a pen-recorder), and to a high-gain D.C. amplifier with condenser coupling available as required for the measurement of synaptic potentials etc. Other methods which were used will be described in the relevant sections.

2. Recording of nerve and muscle action potentials.

Nerve action potentials were recorded monophasically through fine platinum electrodes. Great caution was necessary when recording fusimotor discharges from the fine intercostal nerve filaments because at the high amplification required, attenuated action currents arising in immediately adjacent active muscle fibres could be recorded even though differential recording was used through
an amplifier with a high rejection ratio greater than 10,000).
These 'false' leading conditions were overcome by placing an
additional earthed electrode proximal to the proximal electrode
on the filament. Similar pairs of platinum electrodes were placed
1-2 mm apart on the exposed surface of the intercostal muscles to
record their electromyograms. For recording the electromyogram of
the diaphragm the insulation was removed for 1 mm from adjacent
spirals about 10 cm from the end of a pair of varnished copper wires
(gauge 36) twisted together. A curved sewing needle was used to
pass the wires through the abdominal wall below the 13th rib,
through the costal margin of the diaphragm and out through the 11th
or 12th intercostal space, until, as judged by the electromyogram,
the bared portion of the wires came to lie in the diaphragm.

Nerve and muscle action potentials were led through a
differential, low noise level pre-amplifier (Tektronix 122) with
a high input-impedance, further amplified, and displayed on a
cathode ray oscillograph.

3. Stimulation

Stimulation was by condenser discharges passed through an
isolating transformer and fed to the nerves through platinum electrodes.
Three stimulators were available, one of which could be made to provide
continuous or pulsed trains of tetanic stimuli. In order to stimulate
the vagus nerve an electrode was devised which consisted of two inter-
locking perspex half cylinders one of which carried a pair of platinum
electrodes. The vagus nerve on the left side was freed and the two halves
of the electrode were placed around the nerve and secured with thread;
this arrangement allowed the nerve to be stimulated in continuity if
required.
III. HISTOLOGY OF THE INTERCOSTAL NERVES

A. INTRODUCTION AND METHODS.

Apart from data concerning the human lateral cutaneous intercostal nerve given by Ranson, Droegemueller, Davenport and Fisher (1934), there appear to be no published fibre-calibre spectra for the intercostal nerves of the cat or of other species to provide a correlation with the physiological investigations described in this thesis. The calibre spectra of thoracic ventral roots of the cat (Duncan, 1934) of the macaque (Haggqvist, 1937) and of man (Swensson, 1938) are unsuitable for this purpose due to the presence of the small myelinated fibres of the pre-ganglionic sympathetic outflow which could not be distinguished from the fusimotor fibres assumed to be therein. Accordingly, histological experiments were undertaken to determine the fibre-calibre spectrum of sensory and motor fibres of those intercostal nerves which had been used most commonly in the electrophysiological studies. Operations were performed aseptically on 4 cats, a total of 8 dorsal roots and 3 ventral roots being divided. Distal to the ganglion, the dorsal root fibres interdigitate immediately with the fibres of the ventral root making it impossible to remove the ganglion without damaging the ventral root fibres. However, such probably uniform destruction of ventral root fibres of different sizes was preferred to leaving ganglion cells intact and the difficulty of interpretation arising therefrom. The technique employed was first to divide the dorsal roots 2-3 mm central to the ganglion. The latter was gripped with forceps, its dural sheath opened, and a fine glass hook used...
to tease the dorsal roots distal to the ganglion away from the ventral roots before the ganglion was removed. The ventral roots were divided between ligatures to avoid the troublesome bleeding that otherwise occurred. In order to minimise complications due to regeneration into the intercostal nerves of motor fibres injured in the ventral root, the survival time was 28 days in contrast to the 45 to 58 days employed by Eccles & Sherrington (1930) and Boyd and Davey (1961) on limb muscle nerves, and the intercostal nerves were removed for sectioning as far distal as possible compatible with the information required.

The cats were anaesthetised and the nerves removed and attached under light tension to frames prepared from card. The nerves were stored in freshly prepared Flemming's fluid for 24 hours and stained by the modified Weigert's stain (the Flemming-Wolter technique) according to the detailed method described by Williams and Wendell-Smith (1960). To check if de-afferentation was complete, the ventral spinal ramus, the ventral root and distal stump of dorsal root were removed and serial, 20 μ paraffin sections prepared for staining with Cresyl Violet. Every section was examined and counts made of the number of ganglion cell bodies present. In one segment only of each of two animals was de-afferentation found to be complete. The nerves were well stained and undistorted and one set of them (T.8), provide the photographs and histograms shown in Figs. 5, 6, 7 and 8. Unfortunately, the sections of the contralateral nerves, which were to be used as controls, proved unsuitable for the construction of fibre-calibre spectra, although sufficient evidence was obtained from them.
to show that there was nothing atypical in the fibre complement of the nerves of these animals. The control nerves were therefore taken from the same segment in another animal. All measurements were made from enlargements at 500 diameters from photographs taken with a Leitz Panphot apparatus. All the photographs and prints together with enlargements of a micrometer scale were made in one batch. Measurements made from the photographs of the diameters of the largest fibres were checked against their direct visual image in the microscope with an occular micrometer. The external diameters of the fibres were measured in groups of 2 \( \mu \) by means of a thin perspex sheet on which were engraved a series of circles of diameter 500 x 2 \( \mu \), 500 x 4 \( \mu \), etc.

B. RESULTS.

A few explanatory comments seem necessary.

Due to cutaneous fibres entering in its lateral and ventral branches, (Fig. 1e and g), the internal intercostal nerve is mixed throughout its entire length. The lateral branch (Fig. 1c) is also mixed until it divides to provide the branch innervating the abdominal oblique muscle (Fig. 4, AO) and usually two cutaneous branches (Fig. 4c). No cutaneous fibres are present in the filaments of the internal intercostal nerve which innervate the internal intercostal muscle, or in the external intercostal nerve and its filaments which innervate the external intercostal muscle.

I. Normal Nerves.

The normal internal and external intercostal nerves contain myelinated fibres ranging from 2-20 \( \mu \) in diameter with
usually a few still larger fibres being present (Fig. 4A, B and D; photographs of nerves in Fig. 7). These values cover the entire range of diameters described for motor: (Eccles and Sherrington, 1930; Rexed and Therman, 1948; Boyd and Davey, 1961) and sensory fibres (Lloyd and Chang, 1948; Rexed and Therman, 1948) of various muscle nerves of the hind-limb and of limb cutaneous fibres (Gasser and Grundfest, 1939; see also the diameters predicted from conduction velocity measurements on cutaneous fibres of the cat by Hunt and McIntyre, 1960).

The distribution is bi-modal but less markedly than that of the limb muscle nerves due principally to the presence of the cutaneous fibres. When the internal intercostal nerve divides, the lateral branch (Fig. 4, B2, and Fig. 7 B2) takes the greater share of the small, and the internal intercostal nerve (Fig. 4 B1), takes the greater share of the large diameter fibres. The external intercostal nerve is also bi-modal but the large fibre peak occurs at 10-12 µ compared with the 12-14 µ peak of the internal intercostal nerve.

2. De-afferented nerves.

The distribution of motor fibres in the internal intercostal nerve is markedly bimodal, (Fig. 5A). The large diameter fibres range from 10-20 µ (2 fibres are greater than 20 µ) of which 21% are over 16 µ and in which the 16-18 µ group are as numerous as any others. The other, completely de-afferented internal intercostal nerve (T.7), referred to in the methods, similarly showed fibres ranging from 10-20 µ and there were a substantial number in the range 17.5-20 µ. The size and distribution of these large diameter fibres is equalled in limb nerves only by the semitendonousus nerve (Eccles
and Sherrington, 1930) the popliteus nerve (Boyd and Davey, 1961) and the distributions of fibres in the ventral root shown by both authors, especially those of the latter.

Already apparent in the photograph of Fig. 7A (de-afferented) is the striking segregation of the motor fibres which occurs in the internal intercostal nerve prior to the formation of the lateral intercostal nerve. This lateral intercostal nerve (Fig. 7 B2) contains approximately 70 fibres, also segregated, which eventually form a separate nerve which innervates the abdominal oblique muscle (Fig. 7 C1). The two cutaneous divisions of the lateral intercostal nerve (Fig. 7 C2 and 3) show a complete absence of fibres thus providing further proof of the completeness of the de-afferentation. The few, darkly stained rings are blood vessels which are more numerous in these de-afferented nerves.

The large diameter fibres of the external intercostal nerve also show a wide range of diameters with a well-defined peak at 10-12 \( \mu \) and a subsidiary peak at 14-16 \( \mu \) (Fig. 5D). These values may be contrasted to the slightly different distribution of the internal intercostal nerve which shows peaks at 12-14 \( \mu \) and 16-18 \( \mu \) in both its lateral and medial divisions.

Both the external and internal intercostal nerves contain small diameter fibres with a well-defined peak at 4-6 \( \mu \). However, the presence of fibres of intermediate size, especially in the external intercostal nerve, makes the division between the groups of large and small diameter fibres less prominent than in the limb muscle nerves.

In confirmation of Boyd and Davey (1961) the group of
small diameter fibres is comprised of thinly and thickly myelinated fibres, the diameter of the latter tending to be greater. However, no distinct grouping may be recognised in the fibre-calibre spectrum, which could be due to the coarser grouping of the measurements employed here or could equally be the true situation since not all of the hind-limb nerves showed two distinct groups of small diameter fibres (Boyd and Davey, 1961).

3. **De-efferented nerves.**

The histograms of Fig. 6A and D, are from T8 of the same animal, and that of Fig. 6C is from T9 of a different animal.

Proximally, the afferent fibres of the internal intercostal nerve show a prominent peak of 4-6 μ and a broad distribution of the larger fibres ranging from 8-20 μ (Fig. 6A). There is no tri-modal distribution comparable to that of the limb muscle nerves (Lloyd and Chang, 1948) although in part, this difference might be due to the presence of the cutaneous fibres. Fig. 6C shows the distribution of afferent fibres in the cutaneous divisions (C2 and C3) and the muscular division (Cl; note the different ordinate scale), of the internal intercostal nerve. Whereas the cutaneous divisions contain comparatively few fibres over 14 μ, the muscular division has a prominent 14-16 μ peak, and of its fibres greater than 12 μ in diameter, 25% are over 16 μ.

The external intercostal nerve shows many afferent fibres of intermediate size with a peak of 8-10 μ (Fig. 6D). However, the largest fibres extend into the same upper range of diameters shown by the muscular division of the lateral intercostal nerve.
4. **Intercostal nerve filaments.**

Examples of the filaments which innervate the intercostal muscles may be seen in Fig. 7B (normal) and Fig. 7B, C and D (de-afferented) and Fig. 7D (de-efferented). Fig. 8 shows some of these filaments at a higher magnification, together with a normal (Fig. 8A) and a de-afferented filament (Fig. 8D) from the other de-afferented preparation (T.7) referred to above. The normal filaments (Fig. 8A, B and C), contain 30 to 60 myelinated fibres whose diameters show the full range from 2-20 μ characteristic of the parent nerves. Fig. 8A is of interest since this filament contains an unusually large number of fibres whose diameters are 20 μ or above, a feature which is shared by the parent nerve as may be seen in the upper part of the photograph. The diameter of the motor fibres in the de-afferented filament from the same animal, also attain a high value (20 μ) but none are quite so large as the largest fibres (afferent fibres) present in the normal filament.

The de-afferented filaments like their parent nerves, also show small diameter fibres. These are best seen in Fig. 8F where they are particularly numerous and where the staining is heaviest.

**C. DISCUSSION**

The main points of comparison between the fibre-calibre spectra of intercostal and limb nerves have already been stated, so that only a few comments are made below.

Any proximal transverse section of the external or internal intercostal nerves must include fibres about to leave via a filament to innervate the adjacent sector of intercostal muscle,
and fibres destined to pass to the ventral part of the intercostal space, up to 5 cm or more distally. This proximal versus distal distribution of the fibres may be associated with a tendency for the distally directed fibres to be of smaller diameter, as in limb muscles (Fernand and Young, 1951). Also, fibres about to leave the parent trunk may undergo splitting prior to their taking an intramuscular course not more than 2 cm away (cf. Eccles and Sherrington, 1930). Both these factors may contribute to the broad range of fibre diameters present in the intercostal nerves but the anatomical complexities may preclude in the future a precise quantitative estimation of their relative importances.

A comparison of the total number of motor and sensory fibres in the operated nerves to the total number of fibres in the normal nerve shows, bearing in mind that these figures derive from different animals, that approximately 30% of the fibres are motor and 44.5% are sensory, the deficiency of 25% being almost certainly in the motor fibres due to injury to many of them at the time of operation. This proportion of sensory to motor fibres should be contrasted to the 10% of afferent fibres, of which in turn only 10% are above 12 μ, reported to be present in the phrenic nerve of the cat by Hinsey, Hare and Phillips (1939).

Histologically, the filaments were uniform in that they always contained large and small diameter fibres, although the numbers of each varied considerably. This variation is probably due to the variation in the number and types of muscle spindle present in the sector of intercostal muscle served by each filament. Huber (1901)
reported that the upper 6 intercostal spaces of the cat contained 60-100 muscle spindles and although his figure for 'sensory endings' probably included the multiple secondary endings of complex spindles, I have confirmed that muscle spindles are indeed numerous in the intercostal muscles. In serial, transverse sections of the internal and external intercostal muscles (taken separately from the 4th intercostal space), I have found that each block of tissue, approximately 1 cm wide, contains at least one, or as many as four independent (i.e., not tandem arranged) muscle spindles.
IV. INTERCOSTAL MUSCLES; THEIR CONTRACTIONS AND THEIR INNERVATION.

A. MECHANICAL PROPERTIES OF INTERCOSTAL MUSCLES

1. Introduction.

Contractions of intercostal muscles were first recorded by Martin and Hartwell (1879) and later by Scharpey-Schafer and MacDonald (1925). However, these preparations, like the diaphragm slip preparation, were not then used, or subsequently, to obtain information concerning either the innervation or the mechanical properties of the intercostal muscles. Because such information is necessary to provide corroborative evidence for the conclusions reached in section V, a preparation was devised whereby it was possible to record the contractions of the intercostal muscles in response to stimulation of the ventral roots or of the intercostal nerves. Furthermore, as the results of the histological and electrophysiological investigations were suggestive of differences between the external and intercostal muscles, advantage was taken of their separate innervation (see Section II) to record the contractions of these muscles independently. Briefly, pairs of stout artery forceps about 2 cm apart and firmly fixed to the frame were used to immobilise a sector of a selected rib. The muscle fibres inserted in this sector were traced caudally to delineate their origin and the corresponding sector of the caudal rib freed so as to permit attachment of the isolated sheet of intercostal muscle to the myograph. The muscle was stimulated indirectly either through electrodes on the appropriate intercostal nerve or on the ventral roots. Contractions of muscles extraneous to the isolated sector were prevented by denervation.
During the course of these experiments the work of Glebovskii (1961a,b) and Biscoe (1961; 1962) became available. Their results will be compared to the present results in the discussion at the end of this section.

2. **Single isometric contractions.**

In agreement with the findings of Banus and Zetlin (1958), and Buller, Eccles and Eccles (1960) on limb muscles of the cat, it was found that as the intercostal muscle was progressively lengthened the twitch tension also increased and reached a maximal value at a muscle length approximating to its physiological length. If the muscle was extended beyond this optimal length the twitch tension declined. Around the optimal length, the contraction time (time from onset to summit) and the half relaxation time (time from summit to half twitch tension) increased with muscle lengthening. To allow comparison of these times in different preparations all measurements were made with the muscle at its optimal length, the initial tension usually being between 50-100 gms. Table I shows the mean values for the contraction and half relaxation times of 13 sectors of intercostal muscle (segments T7-T10) prepared in 10 animals. There were 9 sectors of the external and 4 of the internal intercostal muscle.
TABLE I.

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<tbody>
<tr>
<td>Contraction time</td>
<td>msec</td>
<td>26.3 (S.D. ± 4.3; n = 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-relaxation time</td>
<td>msec</td>
<td>24.2 (S.D. ± 5.3; n = 13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus interval at half maximal tetanus: twitch tension ratio</td>
<td>msec</td>
<td>30.4 (S.D. ± 7.2; n = 9)</td>
<td></td>
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<tr>
<td>Tetanus: twitch tension ratio</td>
<td></td>
<td>4.8 (S.D. ± 1.7; n = 9)</td>
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The absolute tensions developed in response to indirect stimulation varied considerably (10-60 gm) from one preparation to another. In the regions worked on, the internal intercostal muscle is approximately twice the thickness of the external intercostal muscle so that the values for the former tended to be higher. The low values were usually due to inadvertent damage to the nerve filaments innervating the sector so that relatively few units could be activated by indirect stimulation.

The mean contraction time of 26.3 msec shown in Table I may be compared to the lowest value of 23.4 msec described by Cooper and Eccles (1930) and 19.2 msec by Gordon & Phillips (1953) for extensor digitorum longus, and the mean of 27 msec for the pooled values for several fast muscles (lateral and medial gastrocnemius, flexor digitorum longus and flexor hallucis longus, the plantaris and gracilis muscles) given by Buller et al. (1960).

3. Fast and slow muscles

Early in the course of these experiments, the records illustrated in Fig. 9 were obtained from two preparations in which only a few motor units could be activated in each sector. The records
of Fig. 9 A-D show that, as the stimulus intensity was increased, the twitch tension increased in steps instead of continuously as was more usual, and reached a maximum at 1.7 times the threshold of the most excitable fibre. At 2.2 times threshold a late hump appeared (Fig. 9 E) the occurrence of which made, however, little difference to the time of half relaxation, or to the maximal twitch tension. Subtraction of curve Fig. 9 D from E, gave a curve with a time to peak of approximately 60 msec, as shown in Fig. 9 G. This result gave evidence for an admixture of slow and fast muscles in the intercostal muscles. Attempts to record in this preparation the activity of the slow unit in isolation by stimulating filaments of the ventral roots were unsuccessful. However, in a different sector of the same animal, a slow unit was isolated (Fig. 9 I) with a contraction time of 45 msec, in contrast to the contraction time of 28.0 msec for a single fast unit (Fig. 9 H) similarly isolated.

Records A-D and H, of Fig. 9 show that the form of the fast muscle response resembles an isosceles triangle (c.f. records of flexor hallucis longus of an adult cat in Fig. 2D of Buller et al. 1960) whereas the form of the slow muscle twitch is asymmetrical about its apex, the relaxation time being relatively prolonged. Although the records J, K and L of Fig. 9 comprise but a few units, they are completely typical of the most commonly encountered forms of the intercostal isometric twitch. The record shown in Fig. 9E, although not unique, is of interest in that the fast units present were as fast, and the slow unit as slow, as any which subsequently were isolated. This presumably fortuitous conjunction was important, for it showed that the half-relaxation time of the intercostal muscles
was not in itself a clear index of the presence of slow muscles in the intercostal muscles.

The composite nature of the intercostal twitch is clearly demonstrated in Fig. 10 in which records obtained by stimulating different combinations of ventral root filaments at different intensities allowed the activity of two fast units and 3 slow units to be recognised. Fig. 10 A shows a compound twitch of slow muscle fibres evoked either in isolation (a) or summating with a compound twitch of fast muscles as in the superimposed trace (b). Further sub-division of the ventral roots isolated a compound fast muscle twitch (B), which, by an adjustment of stimulus intensity, was further resolved into the unit shown in C. Record D, evoked by stimulating a different filament, is a compound slow muscle twitch identical to that seen in (a) which could be resolved by stimulus threshold into a single unit (E) and a response of intermediate size (F). An enlarger was used to make tracings on graph paper of magnified records of A and B, and photographs of each were taken at the same magnification as shown in G and H respectively. The difference obtained graphically between the magnified tracings of (a) and (b) was transferred to the photographic enlargements as shown by the dotted lines in G and H. It may be seen that the compound curve b is satisfactorily accounted for by summation of the compound twitch of the fast muscles in B with the slow muscles of D.

These records, together with those of Fig. 9H and I and Fig. 9B and C, also demonstrate that the twitch tension of individual fast units is greater than that of the slow units (usually by a factor of 2 or more) a difference which was observed repeatedly when a fast
and slow unit was isolated from the same sector.

Table II shows the contraction times of 14 units which were activated in isolation and showed all or nothing contractions in response to stimulating ventral root filaments. The mean contraction time for the pooled values was 35 msec with a broad distribution as indicated by the large standard deviation. As this distribution was distinctly bimodal, with no values lying between 30 and 40 sec, the values were placed into two groups as shown in Table II.

**TABLE II.**

<table>
<thead>
<tr>
<th>Contraction time</th>
<th>msec.</th>
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<tbody>
<tr>
<td>Pooled values</td>
<td>35.7 (S.D. ± 12.4; n = 14)</td>
</tr>
<tr>
<td>Fast units (contraction times 30 msec or less).</td>
<td>24.6 (S.D. ± 4.0; n = 7)</td>
</tr>
<tr>
<td>Slow units (contraction time 40 msec or more)</td>
<td>47.0 (S.D. ± 5.1; n = 7)</td>
</tr>
</tbody>
</table>

The units belonging to the group with fast contraction times were also characterised uniformly by an isosceles form, and a half relaxation time less than the contraction time. In contrast, the units with a slow contraction time were asymmetrical about their apices and their half-relaxation times were longer than their contraction times. They also differed in their responses to tetanic stimulation as later described.

4. **Nerve thresholds of fast and slow muscle units**

By analogy with fast and slow limb muscles, as exemplified by the gastrocnemius and soleus muscles, it was anticipated that the fast and slow intercostal muscle units would be innervated by fibres of
different conduction velocity and therefore, it was assumed, of
different electrical threshold. This did not prove to be the case.
Thus, a just threshold stimulus to the nerve or to the ventral root
filaments might excite either a slow or a fast motor unit. Stimulus
intensity alone, without division of the ventral roots into filaments,
was usually not very effective in isolating one or other type of unit.
The curve relating stimulus intensity to the growth of the peak twitch
tension (the peak being dependent on the fast units) did not differ
significantly from the curve relating the tension measured 45 msec
(the mean contraction time of the slow units) after the onset of
contraction. These facts all indicate comparative homogeneity of the
electrical thresholds of the fast and slow muscle fibres when considered
as a group although individual units might indeed show the expected
difference in threshold (cf. Fig. 9). The short conduction distances
available precluded the accurate determination of the conduction
velocity of the fibres.

5. **Isometric tetanic contractions.**

In 9 compound preparations, the effects of repetitive
electrical stimulation were examined over a standard range of frequencies.
To permit comparison of results from different preparations, the relation
between stimulus interval and the ratio of the tetanic tension to the
twitch tension was plotted for each muscle. The points fell on S-shaped
curves (cf. Cooper and Eccles, 1930; Buller et al. 1960) from which the
stimulus interval giving the half-maximal tetanic to twitch tension ratio
could be obtained. The mean value so obtained is given in Table I.

The maximal tension produced in the tetanus reached a plateau
at about the fusion frequency, which was usually between 50-64 c/s, at times higher. At other times, a slowly or even steeply climbing plateau resulted, which was thought possibly to be due to the slow muscle present. This expectation was confirmed by recording the effects of repetitive indirect stimulation on single fast and single slow units as illustrated in Fig. II. It may be seen how the horizontal tension plateau of the slow muscle (Fig. II A) contrasts with the rising plateau of the slow muscle (Fig. II B). These differences were confirmed in 3 other slow and 2 other fast units investigated. The fusion frequency of the slow muscles was between 30 and 40 c/s, whereas that of the fast muscles was 60 c/s or above. Although the tetanus:twitch tension ratio of the slow muscles tends to be higher than that of the fast muscles, the dominant influence of the greater tension produced by the fast muscle is probably responsible for the horizontal rather than the climbing plateau of the responses of the compound preparations.

B. INNERVATION OF INTERCOSTAL MUSCLES.

The original purpose of these experiments was to compare the electrical thresholds and conduction velocities of the alpha and fusimotor fibres (only the activity of the former is manifest as external tension in the muscle according to Kuffler, Hunt and Quilliam, 1951) and to correlate them with the stimulus thresholds evoking the different components of the ventral root compound action potential. Because of the short conduction distances and the consequent short conduction times of less than 0.4 msec, of which a significant fraction is occupied by utilization time (0.1 msec, Blair and Erlanger, 1936), the conduction velocity measurements proved to be unreliable
so they are not included. The procedure adopted was to place the stimulating electrodes 8-10 mm apart, if possible on a branch-free portion of the relevant intercostal nerve, with the cathode distally, and then to determine the relation between the stimulus intensity and the twitch tension as partially illustrated and plotted for an internal intercostal muscle in Fig. 12 and for an external intercostal muscle in Fig. 13. Recordings were then made of the growth of the ventral root compound action potential with increasing stimulus intensity as shown in Fig. 12L, and in Fig. 13 B and C, which partially illustrate the full series of points plotted in Fig. 13D. It was found essential to reverse the polarity of the stimulating electrodes to take these series, because when the cathode was distal, interfering effects arose at the anode with the higher stimulus intensities.

Successful experiments were completed on 5 external and 2 internal intercostal muscles. A maximal twitch was obtained with a mean value of stimulus intensity for the 7 preparations of 2.01 times the threshold of the lowest threshold motor fibre present. In both muscles, 80-90% of the peak twitch tension was produced at about 1.4 times threshold but the mean value for the maximal twitch of the internal intercostal muscle was 1.71 compared to the mean value of 2.13 times threshold for the external intercostal muscle.

In most preparations, the ventral root compound action potential showed two components, here to be described as $a_1$ and $a_2$, which could be partially differentiated according to their electrical thresholds. The slower, higher threshold component ($a_2$) is seen as
the notch on the descending limb of the \( \alpha I \) component in the low-gain record of Fig. 12L; in other preparations, the \( \alpha 2 \) component might attain a greater amplitude than the \( \alpha I \) component, as in Fig. 13 B.

In Fig. 12, the \( \alpha I \) component was maximal at 1.4 times, and the \( \alpha 2 \) component maximal at 2.2 times, \( \alpha I \) threshold, the low-gain record shown in Fig. 12L being supra-maximal for both, as well as being greatly supra-maximal for the maximal twitch. Fig. 12L shows that a stimulus 3.4 times \( \alpha I \) threshold evoked in the wake of the \( \alpha 2 \) component a small deflection which reached a maximum at 4.4 times \( \alpha I \) threshold. Two other late waves were maximally evoked with stimuli 6.1 and 10.0 times \( \alpha I \) threshold respectively.

In Fig. 13, a plateau of tension was reached at 1.4 times \( \alpha I \) threshold; a further small increment of tension occurred between 2.15 and 2.27 times \( \alpha I \) threshold but thereafter the twitch tension remained constant even though the stimulus intensity was increased to 30 times \( \alpha I \) threshold. Recordings from the ventral root showed that the \( \alpha I \), \( \alpha 2 \) components were maximal at 2.15 times \( \alpha I \) threshold, whereas the threshold of the late wave was 3.0, and it was maximal at 4.2 times \( \alpha I \) threshold. A second component was maximal at 6.05 times, and additional minor elevations appeared at 15 times \( \alpha I \) threshold. Pooled values are shown in Table III.
TABLE III

<table>
<thead>
<tr>
<th>Stimulus intensity as multiples of $\alpha I$ thresholds (mean values).</th>
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<tbody>
<tr>
<td>Maximal twitch tension</td>
</tr>
<tr>
<td>Maximal $\alpha I$, $\alpha 2$ wave.</td>
</tr>
<tr>
<td>Threshold of late wave.</td>
</tr>
</tbody>
</table>

C. DISCUSSION.

The presence of fast and slow muscles in the intercostal muscles seems proved beyond reasonable doubt, a result comparable to that described for the tibialis anterior muscle by Gordon and Phillips (1953). The lower twitch tension of the slow units means that the number of them which contribute to the compound response is greater than suggested by a superficial inspection of the records. Thus in Fig. 10, the compound response was in fact due to the activity of 3 slow and 2 fast units.

No clear correlation was established between the contraction times of the fast and slow muscle fibres and the relative electrical thresholds of their axons as might be expected from the work on limb muscles by Eccles, Eccles and Lundberg (1958). It should be noted, however, that whereas the contraction times of slow and fast muscles (soleus and gastrocnemius) differ in the ratio of 2.8, the same ratio for the intercostal muscle is 1.9. This smaller ratio may be associated with a smaller difference between their electrical thresholds, a difference which our technique has failed to disclose except in one isolated case where significantly, the contraction time was also extremely slow (Fig. 9).
According to Glebovskii (1961), the properties of intercostal muscles are intermediate between those of fast and slow limb muscles and he gives values for their contraction times of 46 msec. He also describes inflections on the rising phase of the tension curve which he attributes to fast muscles with a contraction time of 20-30 msec. The major discrepancy between the two investigations is that in his records, the response of the slow units determines the crest time, in ours, it is determined by the fast units as shown by the correspondence between the contraction times of the fast units and that of the compound twitches. Biscoe (1961) also finds that the intercostal muscles are fast, but his contraction times of 30-35 msec are slower than reported here.

It is difficult to account for the discrepancies in the three investigations. It is highly probable that Biscoe and Glebovski did not stimulate the external intercostal muscle in their combined external and internal intercostal muscle preparations, since, as judged from their descriptions the 'intercostal nerve' they stimulated was actually the 'internal intercostal nerve' which innervates solely the internal intercostal muscle. Thus, the presence in their preparations of two diagonally running layers of muscle fibres, one layer of which was not contracting, introduces a mechanical complexity the effects of which are difficult to assess. Biscoe's values did however include measurements from slips of internal intercostal muscle alone, taken parasternally, and these values did not differ significantly from the remainder, so that the discrepancies must be regarded as unresolved.

In general there was a good correlation between the stimulus
intensity giving a maximal twitch and that evoking a maximal α1, α2 wave in the ventral roots. The values for the latter were usually greater due probably to the presence of fibres other than those innervating the sector, such as those from the abdominal oblique and rectus abdominis muscles, or from distal parts of the rib. The mean value of 2.01 times threshold for the stimulus giving a maximal twitch, reflects the wide range of motor fibre diameters present in the intercostal nerves. These values may be compared to the lower value of 1.2 to 1.55 times threshold given for the nerve to soleus by Harvey and Matthews (1961) and as may be inferred from the mean value of 1.72 of the values they give for the maximal alpha wave in the ventral roots arising from the mesial gastrocnemius nerve. Neither of these limb muscle nerves shows such a wide range of diameters as here disclosed in the intercostal nerves.

The late waves in the ventral root compound action potential, evoked by a stimulus intensity which was supra-maximal for the α1, α2 waves, and which did not cause tension to be produced in the muscle, are clearly to be associated with the excitation of fusimotor fibres in the intercostal nerves. The thresholds and forms of the late components showed considerable variation from one preparation to another and a much larger number of experiments is required, with several experiments on each segment, before any classification into groups could be justified.
V. Efferent Discharges in Alpha and Fusimotor Fibres of the
Intercostal Nerves.

A. Introduction.

The recording of efferent discharges from intercostal nerves was first described by Bronk and Ferguson (1934). By microdissection, they sub-divided the nerve filaments innervating the external and internal intercostal muscles until preparations of single active units were obtained. From the discharge patterns of single units thus obtained Bronk and Ferguson concluded that the external intercostal muscles are active in inspiration and that the internal intercostal muscles are active during expiration, a finding which settled a long standing dispute concerning the precise respiratory function of these muscles.

As the function of the small motor fibres had not then been elucidated (Leksell, 1945; Kuffler, Hunt & Quilliam, 1951), Bronk and Ferguson naturally assumed that they had recorded efferent impulses that directly caused contraction of the extrafusal muscle fibres of the intercostal muscles. However, as shown in Section III B, the intercostal nerves contain numerous small as well as large diameter motor fibres, as would be expected from the abundance of muscle spindles in the intercostal muscles (Huber, 1901). The possibility therefore arose that Bronk and Ferguson may have recorded from fusimotor as well as alpha motor fibres.

Since the de-afferented intercostal nerve filaments contain relatively few motor fibres (15–30 according to the size of the filament) the attempt was made to record efferent discharges
present in them during normal and stimulated respiration. It was anticipated that, under conditions of asynchronous, physiological activation, the activity of single units could be detected, as in fact proved to be the case. It was of advantage to record from whole, naturally occurring filaments, in contrast to micro-dissected nerve strands, because by so doing the proportional relationship between spike height and fibre diameter (Blair and Erlanger, 1933; Gasser and Grundfest, 1939) is preserved. This method has been used by several workers to investigate the activity of alpha and fusimotor fibres in limb muscle nerves (Hunt, 1951; Eldred and Hagbarth, 1954; Pascoe, 195%; Diette-Spiff and Pascoe, 1959; Laporte, Bessou and Voorhoeve, 1958; and Voorhoeve, 1960).

The dissection of the filaments and the recording methods are described in detail in Section II. As emphasised by Hunt (1951) great care must be taken when using potential size as an index of fibre diameter, and the precautions he described were strictly observed.

B. RESULTS

I. Patterns of activity in normal respiration.

a. General features. Many patterns of activity may be observed when recordings are made from inspiratory or expiratory nerve filaments (see Section 2B 2 for definition) in the quietly breathing animal. Nevertheless, this activity showed several characteristic features which were observed repeatedly and consistently. Recordings from expiratory filaments of three different animals are shown in Fig. 14 A, B and C, and from inspiratory filaments of two
other animals in Fig. 14 D and Fig. 16. In all records from both types of filaments, the activity consisted of spikes of two distinct sizes, and the frequency and pattern of these two types of discharge showed characteristic differences. In Fig. 14A, the repetitive firing of the small spikes occurred periodically with, and throughout each expiratory pause. The small spike of Fig. 14B fired more or less continuously throughout the respiratory cycle but the discharge frequency showed a striking periodicity. This frequency was lowest at the height of inspiration (about 6 c/sec), it increased abruptly the instant inspiration ceased and then showed a progressive increase throughout the expiratory pause and eventually attained a maximal frequency 3-4 times greater than at the height of inspiration. Prior to the onset of inspiration, the discharge frequency of the small spike decreased and throughout inspiration it slowed still further, to reach its lowest value at the height of inspiration. Although the discharge pattern of the small spike in Fig. 14 C is essentially the same as in B, the discharge frequency is much higher, ranging from approximately 50 c/sec during inspiration to almost 100 c/sec during expiration, additional units also being recruited into activity. In contrast, the large spikes in the expiratory filaments of Fig. 14 A, B and C fired at much lower frequencies (not usually greater than 9 c/sec in quiet respiration), they did not commence firing until much later, commonly not until the latter half of the expiratory pause, and they ceased firing earlier than the small spikes.

Small and large spikes are also present in the recordings
from the inspiratory filaments of Fig. 14 D and Fig. 16, but their periodic discharge, or their periodic acceleration in frequency, occur during inspiration. Unless the small spikes were firing throughout the respiratory cycle, as in Fig. 16 C and D, the difference in discharge frequency between the small and large spikes in quiet respiration was usually not so great as in the expiratory nerve filaments, nor was the maximum discharge frequency of the small spikes usually as high. The activities of the small spikes in Fig. 16 C and D are of interest in that their early onset of discharge, their early acceleration in frequency and their progressive increase and decrease in frequency during inspiration and expiration respectively, closely parallel, although in the opposite half cycle of respiration, the small spike activity of the expiratory nerve filament shown in Fig. 14 B.

In recordings made from 31 filaments innervating the external intercostal muscles and 63 filaments innervating the internal intercostal muscles, taken from the mid-line dorsally to just beyond the level of emergence of the lateral intercostal nerve in the 4th-11th thoracic segments, there was not one exception to the rule that the former filaments could be classified as inspiratory and the latter as expiratory nerve filaments according to the phasing of the spike activity recorded from them. In the few instances where the maximal activity did not occur in the expected phase of the respiratory cycle, it was found that 'false' leading was occurring from active muscle fibres closely adjacent to the root of the filament. "False leading" was usually recognised
because the longer duration and diphasicity of the muscle action potentials gave them a characteristic sound over the loudspeaker monitor. Such spikes were invariably abolished by placing an earthed electrode on the filament between the proximal recording electrode and the volume conductor; conversely, a crush proximal to the proximal recording electrode failed to abolish them.

The spikes recorded from the intercostal nerve filaments were abolished by sectioning the ventral roots of the same segment thus proving they were neither injury discharges nor impulses propagating antidromically in branches of afferent fibres, the so-called 'axon reflex' discharges described by Adrian, Cattell and Hoagland (1931). Large and small spikes were present in filaments of intercostal nerves which had been deafferented by dorsal root section 30 days previously. Finally the extremely remote possibility that they arose in sympathetic post-ganglionic fibres was also excluded. A retro-pleural approach was used to expose the sympathetic grey ramus communicantes from the sympathetic ganglion in the same segments; cutting the grey ramus was without effect on the discharges.

b. Effects of changing levels of anaesthesia. The single factor which most influenced the pattern of spike activity in quiet respiration, was the level of anaesthesia. Animals which showed large and small spikes in expiratory or inspiratory nerve filaments always displayed some evidence of active costal respiration. At this level of anaesthesia, the animal was likely still to have a fairly brisk pupillary light reflex, a conjunctival or corneal reflex, and a brief, non-sustained flexor withdrawal of the fore but not usually of the hind-limb (cf. Petersen, 1952). If the depth of
anaesthesia was increased by further anaesthetic, the costal respiratory movements diminished and in the filaments there was a concomitant reduction in the number of small and large spikes (Fig. 15). In the expiratory filaments this depression tended to fall most heavily on the large spikes, so that the recordings might then show small spike activity alone (Fig. 15 D). Conversely, it was extremely common to record initially the periodic activity of only small spikes, a subsequent lightening of anaesthesia causing large spikes to appear. In inspiratory nerve filaments, the effects of anaesthesia tended to fall more equally on the large and small spikes. With experience it was possible to judge accurately which type of spike was present.

c. Effects of passive limb movements. If the animal was left undisturbed for some time, in the absence of extraneous stimulation, there was frequently a complete disappearance of small and large spike activity, or the large spikes might be present alone as shown for an inspiratory filament in Fig. 16 A. Initially, it was thought that this was due to the anaesthesia deepening to a level beyond that at which the effects of increasing anaesthesia described above were obtained. But strong pinching of the claws, the skin, or the joint capsules, or twisting of the pinna were usually ineffective in evoking activity. However, if the limbs, especially the fore-limbs, were repeatedly flexed and extended, these passive movements within a few seconds induced a remarkable activation of the small spikes which, as soon as they appeared, resumed the periodic respiratory activation described above, and as is seen sequentially in the recordings of Fig. 16 B, C and D.
Exactly similar changes were observed when recording from expiratory nerve filaments. Once aroused, the small spike activity could persist for hours without abating; but if it did abate, it could be re-evoked by passive limb movements.

2. Function of fibres giving rise to large and small spikes.

Kuffler, Hunt and Quilliam (1951) and Hunt and Kuffler (1951a) established that the fusimotor innervations of muscle spindles was exclusively through small diameter fibres conducting at 15-50 m/sec. Previously, Blair and Erlanger (1933) had found a proportional relationship between spike height and conduction velocity and this linear relationship was shown by Gasser and Grundfest (1939) to give the best fit for their reconstructed and recorded action potentials. It followed that the fusimotor fibres should give rise to small spikes and subsequently, Hunt (1951) provided proof that potential size was an adequate criterion for identifying the activity of alpha and fusimotor spikes.

In the present investigation large and small spikes were so well differentiated that they may be assigned with assurance to the groups of large and small diameter motor fibres respectively, whose presence was disclosed histologically (Section III) and whose different functions were established by mechanical recording (Section IV). Supporting evidence for this conclusion is given below under five subheadings. (a) At any instant in the respiratory cycle, if both small and large spikes were present, the frequency of the former was always greater than the frequency of the latter, usually by a factor of between 2 and 10 according to the type of small spike discharge present. (b) The absolute peak
discharge frequency of the large spikes never exceeded 15 c/s in quiet respiration and was usually below 11 c/s, whereas the small spike frequency ranged from 20-100 c/s according to the type of discharge present. (c) In quiet respiration, the peak discharge frequency of alpha intercostal motoneurones recorded as single units in the electromyogram was always low, a mean value of 11.0 c/s (S.D. ± 2.7; n = 30) being obtained. (d) The large spike activity only occurred during inspiration or expiration, according to the filament, but not continuously throughout the respiratory cycle, which correlated with the observation that at the levels of anaesthesia employed, it was rare to obtain electromyographic evidence for continuous alpha motoneurone activity throughout the cycle. (e) Small spike activity alone was often recorded from filaments which, before they had been isolated for recording, innervated sectors of muscle in which there was no electromyographic evidence of alpha motoneurone activity; nor could any be detected in adjacent parts of the intercostal space or in other segments. Collectively therefore, the evidence provides strong justification for attributing the small spikes to activity of fusimotor fibres and the large spikes to activity of alpha motor fibres. Throughout the thesis the terms alpha and fusimotor will be used accordingly.

The inspiratory or expiratory phasing of the activity of fusimotor neurones closely parallels the activity of the alpha motor fibres present in their respective filaments. This fact suggests that the activity of alpha and fusimotor neurones is
under the control of the same central mechanism and the extent to which this appears to be the case will now be described.

3. **Effects of hyperventilation.**

Rosenthal (1865) found that hyperventilation caused 'apnoea', that is to say, it abolished spontaneous respiration. Hering and Breuer (1868) showed that the rhythmic excitation of pulmonary receptors of the vagus nerve decisively influenced the time course of the apnoea, an action which Head (1889) described as the 'negative after action' of the vagus. Later, however, it was established that the principal cause of 'apnoea' is the hypocapnia induced by hyperventilation (see Haldane and Priestley, 1920 for review).

In the present experiments hyperventilation was effected by artificial respiration under positive pressure as a simple means of abolishing spontaneous respiration. An apnoea of 10-20 seconds duration was induced by 2-3 mins of artificial respiration at a normal tidal volume but at 30 strokes per min., i.e. about twice the normal rate. Providing the pump was stopped, it was found that during the apnoea all periodic alpha spike activity of both inspiratory and expiratory nerve filaments was abolished, as illustrated for an expiratory filament in Fig. 17 D. Furthermore, the tonic fusimotor discharge which persisted (Fig. 17 B, C and D) showed no respiratory periodicity. Prolonged hyperventilation often abolished the tonic fusimotor discharge, but the tonic discharge could then be re-evoked by making passive movements of the
limbs. As shown in Fig. 17 E, at the very first sign of renewed inspiratory activity (diaphragmatic, or of the external intercostal muscle), the fusimotor discharges of the expiratory filament were inhibited, immediately to become active again at the onset of the first expiratory pause, and subsequently, with each expiration. Fusimotor spikes always resumed a periodic respiratory discharge earlier than the alpha fibres. In inspiratory nerve filaments the first sign of renewed respiratory activity was an acceleration in frequency of the fusimotor discharges and the onset of alpha motoneurone activity might not occur until the second or third cycle.

When the apnoeic state is reached, the animal no longer breathes against the pump and the fusimotor discharges are aperiodic. However, as seen in Fig. 17 B and C, there now occurs a periodic burst of repetitive firing with each inflation of the lungs and chest, the faster rhythm of the pump being clearly evident. This periodic activity of the alpha expiratory motoneurones is a reflex arising from proprioceptors in the chest wall. Thus, section of the dorsal roots in the same and immediately adjacent segments abolished the response. If the tidal volume was increased, the frequency of discharge increased and the activity was present for a greater fraction of the inspiratory phase of the cycle. Conversely, if the stroke volume was reduced below a critical value, the periodic alpha fibre activity was abolished. In confirmation of previous findings (Sears, 1958), the same observation was made electromyographically on the internal intercostal muscles.
In the experiment illustrated, it may be noted that the alpha spike firing during spontaneous respiration was the one which showed the reflex activation and with the loudspeaker it was possible to follow continuously the transition from one mode of activation to the other.

A second observation of great interest, made on several occasions during artificial respiration, was that if fusimotor fibre activity had subsided, then reflex alpha motoneurone activity in response to inflation did not occur. It could be made to do so however, if the fusimotor fibre activity was re-established by moving the fore-limbs passively.

4. Effects of hypercapnia and anoxia.

When examining the effects of anoxia and of hypercapnia it was essential to ensure that air-flow resistance to and from the lungs was not increased otherwise vagal and proprioceptive reflexes complicated the result. A narrow, open-ended tube with the tip turned backwards was sealed into the straight limb of the tracheal cannula with the tip just beyond the level of the side arm which was left open. A steady flow of gases (5% CO₂ in air; or 8% or 12% O₂ in N₂ contained in pressure cylinders), was passed under low pressure through the tube so displacing the air in the dead space from which the animal breathed. Control experiments using compressed air produced no stimulation of respiration. Under these conditions, hypercapnia and anoxia exerted similar actions: an increase in respiratory rate; a stimulation of inspiratory alpha and fusimotor neurones; and a striking inhibition of fusimotor fibre activity in
expiratory nerve filaments during inspiration. These three phenomena are illustrated in Fig. 18 which shows the effect of 12% O₂ in N₂. The resting respiratory rate was 18/min, and in the control recordings from the inspiratory nerve filament (Fig. 18 A and B) there were two alpha spikes firing at about 8 c/sec and a single fusimotor discharge at approximately 22 c/s at the peak of inspiration. The expiratory filament showed intense fusimotor fibre activity and at the height of expiration, when the activity was maximal, the spikes of 6 fusimotor fibres were identified. No alpha fibres were discharging spontaneously but they could be evoked by the inspiratory-inhibitory, expiratory-activating Hering Breuer reflex (see sub-section 4) as seen in the centre of Fig. 18 A. Fig. 18 D shows that 30 seconds after causing the animal to breathe 12% O₂ in N₂, the respiratory rate had increased to 22 c/s, the peak discharge frequency of the alpha spikes had increased to 10 c/s, fresh alpha units were recruited into activity and they showed synchronisation. The peak frequency of the inspiratory fusimotor neurone discharge had increased to about 40 impulses/sec. A slight increase also occurred in the expiratory fusimotor neurone activity during expiration, but the most striking effect exerted on them was a virtual total inhibition of their activity during inspiration, in contrast to their former sustained activity. When air breathing was resumed, the tonic fusimotor discharge during inspiration was restored (Fig. 18E). A comparison of Fig. 18 C and D shows that the degree of inhibition was greater, the greater the intensity of the inspiratory discharge.
This relationship between increased inspiratory activity and increased inhibition of expiratory fusimotor neurone activity during inspiration may also be recognised in the moment to moment variations seen during eupnoea.

Fig. 19 A, B and C illustrates the reduced number and Fig. 19 D, E and F, the reduced frequency of discharge, of individual expiratory fusimotor neurones during inspiration when respiration was stimulated by 5% CO₂ in air.

Due to the shortening of the expiratory pause at the faster respiratory rate, the stimulating action of carbon dioxide excess and oxygen lack on expiratory fusimotor neurones is complex and has not been examined in any detail. Since their discharge is inhibited during inspiration the expiratory fusimotor neurones then usually show a faster acceleration in frequency at the onset of expiration, but the peak frequency to which they rise may be lower as illustrated in Fig. 19 F.

5. **Effect of vagal afferent discharge on the activity of alpha and fusimotor discharges.**

a. Stimulation of vagal pulmonary afferents by natural stimulation. Hering and Breuer (1868) showed in the rabbit that closure of the trachea at the height of inspiration, before the expiratory collapse of the lungs occurs, prolongs the subsequent expiratory pause and evokes a powerful contraction of the expiratory muscles, the 'inspiratory inhibitory, expiratory activating reflex'. Conversely, closure of the trachea prolongs the duration and increases the intensity of the following inspiration and inhibits
the activity of expiratory muscles, the 'inspiratory-activating, expiratory inhibitory' reflex. Subsequently, the use of the diaphragm-slip preparation (Head, 1890) has caused the expiratory component of the former reflex to be overlooked. Furthermore, in the rabbit, the 'inspiratory inhibitory' reflex is so powerful that the animal may die of asphyxia and this has led to a greater emphasis being placed on the control of respiratory rhythm by the reflex, than on the control of the force of expiratory contraction. Again, the diaphragm slip preparation fails to reveal that in fact the animal may die in expiratory spasm.

In a previous investigation it was shown electromyographically that the internal intercostal and abdominal oblique muscles are active during the prolonged expiratory pause of the 'inspiratory-inhibitory' reflex (Sears, 1958). In the present study, tracheal closure at the peak of inspiration increased the frequency and duration of discharge of alpha spikes previously active in expiration and recruited fresh units into activity (Figs. 18A, 20B, 21B, and Fig. 25 B and D. However, as shown in Fig. 20 B there is also a concomitant activation of the expiratory fusimotor neurones. The filled circles in Fig. 20 C show the periodic fluctuation throughout the respiratory cycle of the number of fusimotor spikes which fired per 100 msec, counted irrespective of their sizes. At least 3 individual units fired at the height of expiration. When the trachea was closed at the peak of inspiration, the fusimotor discharges persisted throughout the prolonged expiratory pause and the number of discharges per 100 msec reached a higher level than in the control period (open circles). Individual fusimotor fibres
showed an increased frequency of discharge as illustrated in Fig. 21. In the control record (Fig. 21A) two alpha spikes and one fusimotor spike fired with each expiration, the lower trace being the electromyogram of the internal intercostal muscle. Below, plotted on the same time scale, are the variations in the mean spike frequency of the 3 units measured over 200 msec periods. The higher peak frequency and the more rapid acceleration of the fusimotor spikes (filled circles) with each expiration is well shown. During the prolonged expiratory pause caused by closing the trachea at the peak of inspiration the fusimotor spike reached a higher peak frequency and the duration of its discharge was prolonged (open circles). The activity of the small and large alpha spikes (triangles and squares respectively) was also increased during the pause, but this effect fell most heavily on the small alpha spikes. This latter effect was a regular finding; so also was the fact that the first alpha units to become active, were almost invariably smaller than those eventually recruited.

The influence of the 'inspiratory activating' reflex on inspiratory alpha and fusimotor neurones is illustrated in Fig. 22. Closing the trachea at the onset of inspiration caused the alpha fusimotor neurones to discharge for a longer period and at a higher frequency than in the control recording.

b. Electrical stimulation of the vagus nerve. The lowest threshold fibres in the vagus nerve are those mediating the Hering-Breuer reflexes (Knowlton and Larrabee, 1946; Paintal, 1953). Low intensity, high frequency stimulation of the central end of the cut vagus nerve, or of the nerve left in continuity, inhibits
inspiration (see Wyss, 1954a for references) and extensive use was made of this procedure to provide a simple means of modifying respiration during intra-cellular recording, as described in Section VI.

The immediate effect of stimulating the vagus nerve at 200-300 c/s was either the abrupt termination of any coincident inspiratory alpha motoneurone activity, or as shown in Fig. 23B, the next inspiration did not occur at the expected time. That this was not due simply to the next inspiration being delayed may be seen by the 'escape' from vagal restraint, of a single (small) alpha unit which fired one impulse (open squares Fig. 23C), and the slight but definite 'escape' of the inspiratory fusimotor neurone from inhibition as shown by its acceleration in frequency preceding the alpha motoneurone discharge (open circles Fig. 23C). In the second 'escape', which was more powerful, the small alpha spike fired several impulses at a higher frequency and an additional alpha unit was recruited. The increase in fusimotor discharge again preceded the alpha discharge and the change in frequency was greater. Fig. 24 illustrates the effect of vagal stimulation on a single inspiratory fusimotor spike which in the control recording showed a remarkable modulation of the discharge frequency ranging from 0-70 c/s (Fig. 24C, closed circles). Vagal stimulation prolonged the expiratory pause during which time the fusimotor spike frequency rose to a slightly higher level than in the control recording, fell abruptly during 'vagal escape' and rapidly increased once more to an even higher level, this cycle repeating itself.
(Fig. 24C open circles). Note that although the fusimotor discharge was inhibited during a second 'escape' there was no concomitant 'escape' of alpha inspiratory motoneurones.


According to Sears (1958) section of the dorsal roots of the same segment reduces or abolishes the effect that tracheal closure at the peak of inspiration has in increasing the frequency of already active units and in recruiting fresh motor units. It was assumed, although not stated in that brief report, that dorsal root section interrupted the afferent limb of a proprioceptive stretch reflex of the intercostal muscles; this possibility was considered in more detail elsewhere (Nathan and Sears, 1960).

In the present study, section of the dorsal roots has always resulted in a diminution in the number of alpha spikes evoked by tracheal closure. However, because the investigations with intracellular recording demonstrated that expiratory intercostal motoneurones receive monosynaptic excitation from afferents in the same and adjacent intercostal nerves (Section VI) all three dorsal roots were cut in the present experiments. In the control recording from an expiratory filament (T.8) shown in Fig. 25A, a single alpha fibre fired 3-5 impulses at 8-9 c/s and at least two fusimotor fibres fired at a peak frequency of about 50 c/s with each expiration. Fig. 25E shows the fusimotor fibre activity recorded at a faster film speed for clarity. During the subsequent prolonged expiratory pause, tracheal closure at the peak of inspiration evoked a powerful augmentation of alpha and fusimotor fibre activity (Fig. 25B)
After cutting the dorsal roots of T7, 8 and 9 no alpha spikes fired repeatedly with each expiration but the periodicity of the fusimotor fibre activity was unaffected (Fig. 25C). Tracheal closure now evoked the discharge of only a single alpha unit for a shorter period of time and at a lower frequency (Fig. 25D) than in the control response. It is stressed that this loss of alpha motoneurone activity following dorsal root section is a threshold phenomenon. Thus if respiration was stimulated, for example by the inhalation of 5% CO₂ in air, alpha motoneurone activity could then be evoked.

C. DISCUSSION

The phasing of the periodic activity recorded from nerve filaments innervating the intercostal muscles is in complete accord with the findings of Bronk and Ferguson (1934) who concluded that the former muscle is inspiratory and the latter expiratory in function. Moreover, the present work demonstrates for the first time that the activity of fusimotor neurones innervating the inspiratory and expiratory muscle spindles is also phased during inspiration and expiration respectively. In fact, these fusimotor neurones are subjected to a central control closely related to, if not the same as, that which controls the activity of alpha respiratory motoneurones. As far as the intercostal muscles are concerned it seems logical therefore to introduce the terms 'inspiratory' and 'expiratory fusimotor neurones'.

It should be mentioned that a different conclusion was
reached recently by Critchlow and von Euler (1962). By recording the afferent discharges in the dorsal roots, they obtained indirect evidence of a periodic modulation of intercostal fusimotor fibre activity, which they attributed to a spinal reflex initiated by movements of the thoracic wall. Their interpretation is invalidated by the data here obtained by direct recording of intercostal fusimotor fibre activity.

The mechanism of the efferent control over the muscle spindle afferent discharge appears to be extremely complex. Thus recent physiological studies show that the static and dynamic responses of primary endings may be controlled independently by fusimotor fibre activity (Jansen and Matthews, 1962), possibly through the two histologically identifiable types of intrafusal muscle fibre which receive independent motor innervations (Boyd 1961, 1962). In contrast, the small dynamic response of the secondary endings (Cooper, 1959, 1961; Bessou and Laporte, 1962) is little effected by fusimotor fibre activity, although their static response is increased (Jansen and Matthews, 1962).

From the earlier work on the reflex effects of fusimotor fibre activity, in particular from the work of Hunt (1951), Hunt and Kuffler (1951, 1952), Granit and Kaada (1952) and Eldred, Granit and Merton (1953), two divergent views have arisen as to the role of fusimotor neurone activity in the nervous mechanism of movement. Hunt and Kuffler conceived the fusimotor fibre system as a mechanism for keeping the muscle spindle correctly biased to the most sensitive part of its range. On the other hand, Eldred et al. working with
the decerebrate animal, regarded the spindle with its efferent control as a motor instrument or 'error detector' which, through reflex action via the monosynaptic pathway, causes the extrafusal muscle fibre length to 'follow' the length of the intrafusal muscle fibres, their so-called 'indirect' route (the 'gamma loop') of muscle activation. Because Hunt and Paintal (1958) found that in the spinal animal comparatively few fusimotor fibres showed a lower threshold for reflex activation than alpha motoneurones, Hunt and Perl (1960), in their review, concluded that the pathway via fusimotor neurones is not likely to be the primary route for the initiation of motoneurone discharge. This comparison between the results from spinal and decerebrate animals cannot however be used as direct evidence against the hypothesis of Eldred et al. This hypothesis was based on the acceleration in muscle spindle discharge during active contraction, and the persistence of this acceleration when dorsal root section had abolished alpha motoneurone activity. As they expressed it "In the intact preparation activity, by biasing the spindles sets up an afferent discharge which contributes decisively to the excitation of the alpha cells" (Eldred et al. 1953, p.520). Eldred et al. did not, however, put their hypothesis to a crucial test by measuring the magnitude of possible sub-liminal changes produced in the motoneurones by the 'direct' pathway. Thus it is equally possible that the contribution made by the 'gamma loop' to the alpha motoneurone excitability was not in fact 'decisive' for excitation, but by summation with the sub-liminal effects of the 'direct' pathway it could cause alpha moto-
neurones to discharge.

In the present study on the intact, anaesthetised cat, the lower threshold for the periodic (respiratory) activation of fusimotor neurones was consistently a conspicuous feature. Thus periodic fusimotor discharges were often present when electromyography, or recording from several filaments in the same segment disclosed that there were no alpha motoneurones discharging. The onset of fusimotor fibre firing was earlier, its duration of discharge longer and its discharge frequency higher than alpha motoneurones. Finally, periodic fusimotor discharge was not dependent on the dorsal roots being intact. In contrast, for some intercostal alpha motoneurones, their periodic discharge was dependent on afferent impulses in the dorsal roots.

The intercostal fusimotor neurone discharge always 'leads' the alpha motoneurone discharge, so that increased activity of the former would cause an increased afferent discharge from the muscle spindles. Because the lowest threshold muscle afferents in the intercostal nerves exert a monosynaptic excitatory action on intercostal motoneurones (Section VI), periodic fusimotor neurone activity will cause a periodic depolarisation of the alpha motoneurones which would either cause their discharge or at least facilitate their excitation through other pathways.

As emphasised earlier, the central mechanism determining periodic fusimotor neurone discharge is closely related to the central mechanism causing alpha respiratory motoneurone discharge. Indeed the simplest hypothesis would be that they have a common mechanism,
differences in the properties of the two types of motoneurone giving rise to their threshold differences. It follows that any periodic fusimotor-driven output from the spindles is most likely to occur concomitantly with the periodic depolarisations (the 'central respiratory drive potential') of at least some of the alpha motoneurones due to activity in a 'direct' pathway. Concomitant activation of both the 'indirect' and the 'direct' pathways has the advantage that for the respiratory motoneurone the time course of the depolarisation produced by either pathway would be similar. These findings thus give support for the 'follow-up servo hypothesis' of Eldred et al. (1953). The inspiratory and expiratory phasing of the fusimotor neurone discharge to inspiratory and expiratory muscle spindles ensures that the 'error' signal is fed back from the appropriate muscle according to the phase of the respiratory cycle.

The significance of this central control of intercostal fusimotor fibre activity is seen as follows. Failure of the extrafusal fibres to shorten at the same rate as the intrafusal fibres, due to increased resistance to air flow to and from the lungs, would lead to an increased discharge from the muscle spindles. Under such conditions the muscle spindle acts as an 'error detector', the output from which, via the monosynaptic pathway, would exercise an increased excitatory drive on the intercostal motoneurones. There would be summation with the 'central respiratory drive potential' resulting in an increase in the discharge frequency of active motoneurones and the recruitment of others into activity. Such discharge of intercostal motoneurones arising from summation of the effects of activity in the 'direct' and
'indirect' pathway, has been demonstrated (Section VI).

The extreme example of increased resistance to air-flow is seen when the trachea is closed at different phases of the respiratory cycle to evoke the Hering Breuer reflexes. But the mechanism considered above could clearly operate at all levels of resistance down to the condition of normal unimpeded air flow. Thus from these results there emerges a new concept of the role of the Hering Breuer reflexes. This is that pulmonary afferent discharges not only determine the rhythm of breathing, but, in conjunction with the central mechanism governing intercostal fusimotor neurone activity, serve to control the muscle spindles of the intercostal muscles. These muscle spindles would then serve as 'error detectors'. At least part of the increased force of contraction of respiratory muscles during the Hering Breuer reflexes is due therefore to afferent discharges from the concommitantly activated muscle spindles acting at the motoneuronal level. This mechanism was indicated previously by the partial or complete loss of expiratory alpha motoneurone recruitment, caused by tracheal closure, from deafferented intercostal segments (Sears, 1958).

That dorsal root section leads to loss of some alpha motoneurone activity in eupnoea, emphasises the importance of afferent discharge in maintaining alpha motoneurone excitability. After bilateral vagotomy, some recruitment of units persists in the internal intercostal muscles when the trachea is closed at the peak of inspiration (Sears, 1958). In the diaphragm Cuenod (1961) made a similar observation on the effects of vagotomy on the 'inspiratory activating reflex'. These
procedures reveal that the segmental reflex operating alone is reduced in effectiveness when its muscle spindles are deprived of the vagally initiated increase in fusimotor activity. Since bilateral vagotomy abolishes the rhythm controlling component of the Hering Breuer reflex, which is the classical basis for attributing these reflexes only to pulmonary afferents, some modification in activity of the 'direct' route is also to be expected.

It now seems highly probable, that the measurement of the effectiveness of the Hering Breuer reflexes in various animals and in man, by the duration of the apnoea produced by tracheal closure (Widdicombe, 1961) measures the least significant component of the reflex. And in view of the inter-relationship between vagal and segmental reflexes indicated above, the dismissal of the vagal reflexes as being responsible for the effects of respiratory loading in man (Campbell, Dinnick and Howell, 1961) needs to be re-appraised.

The recordings from nerve filaments in animals in which spontaneous respiration was abolished by hyperventilation showed that the internal intercostal muscles are activated by a segmental reflex during lung inflation. These findings are in agreement with previous electromyographic observations (Sears, 1958). This reflex is presumably activated by stretch of the expiratory muscles and must normally be inhibited during inspiration since these muscles are then either inactive or least active. Two mechanisms are available for this inhibition. Firstly, the expiratory fusimotor neurone activity is least during inspiration and the subsequent reduction in spindle bias would reduce muscle spindle afferent discharge during inspiration. Further-
more, the degree of this inhibition was found to be greater, the
greater the intensity of inspiratory motoneurone discharge, as
was seen most clearly when respiration was stimulated in various
ways. The second mechanism, which is described and discussed in
Section VI, is the active inhibition of expiratory motoneurones
during inspiration by hyperpolarisation.
VI. INTRACELLULAR RECORDING FROM RESPIRATORY MOTONEURONES OF THE THORACIC SPINAL CORD.

A. INTRODUCTION AND GENERAL ACCOUNT OF EXPERIMENTAL PROCEDURES.

I. Introduction and aims.

Respiratory movements are caused by the alternating contractions of inspiratory and expiratory muscles. These contractions in turn result from the alternating, periodic, repetitive discharge of inspiratory and expiratory motoneurones. The need is clearly indicated for a study of the mechanism of these activities on the motoneurone itself. For this purpose the technique of intracellular recording from neurones in the central nervous system (Brock, Coombs, and Eccles, 1952a, 1953; Woodbury and Patton, 1952) lends itself admirably. The aims of the experiments were two-fold. Firstly to obtain information of the processes underlying the periodic, repetitive and alternating discharge of inspiratory and expiratory motoneurones. Secondly, to define some of the characteristics of the segmental reflex actions which intercostal nerve afferents exert on thoracic motoneurones.

2. Design of experiments.

The general design of the experiments was based on previous investigations from this laboratory (Brock, Coombs and Eccles, 1952a, 1953; Coombs, Eccles and Pott, 1955a, b and c; Eccles, Eccles and Lundberg, 1957a, b and c; Coombs, Curtis and Eccles, 1957a and b; Eccles, Eccles and Lundberg, 1958; Curtis and Eccles, 1960).

The microelectrodes were inclined laterally 5 - 10° and inserted into the spinal cord immediately medial to the dorsal
root entry zone through a hole made in the pia. Motoneurones were located by searching for their soma-dendritic (SD spike) and synaptic field potentials evoked by stimulating various intercostal nerves. Often their location required extensive tracking since in the thoracic region the ventral horn is a thin, nearly vertical column not more than 0.5 mm wide.

By stimulating the external and internal intercostal nerve separately, it was possible to evoke the field potentials of inspiratory or of expiratory motoneurones. These field potentials were made as large as possible, by stimulating the two nerves close to their origin from the ventral spinal ramus so exciting all the available axons.

The field potentials of inspiratory and expiratory motoneurones, which were usually located at a depth of 2.75-3.5 mm from the surface, were not mutually co-extensive. Whereas the expiratory motoneurones were encountered across the full width of the ventral horn, the inspiratory motoneurones showed a definite medial location to a degree which gave a valuable guide as to the position of the tip of the microelectrode.

When a motoneurone was impaled it was possible by means of a rotary switch to stimulate quickly each of the nerves and so to establish by antidromic invasion if the cell was an inspiratory or expiratory motoneurone, and also to assess the reflex actions exerted on them by intercostal nerve afferents. D.C. recordings were also made on slowly moving film of the spontaneous electrical activity on which the experimentally induced activity was superimposed.
Other experimental procedures used will be described later.

B. SPONTANEOUS ACTIVITY OF THORACIC MOTONEURONES.

1. Membrane potential.

In general, it was difficult with thoracic motoneurones to obtain such stable recording conditions as with lumbar motoneurones. Apart from the greater liability of movement dislodging the microelectrode, the small size and mostly non-spherical, fusimotor form of the motoneurones (unpublished observations; cf. the description of the phrenic motoneurones by Keswani, Groat and Hollinshead, 1954; Keswani and Hollinshead, 1956), made them more difficult to impale than most lumbar motoneurones.

There were wide variations in the membrane potentials of different cells. Such variation is common to all intracellular investigations, and is due in part to the chance of obtaining a satisfactory impalement, presumably of the soma, and the subsequent adequate self-sealing of the membrane (Brock, Coombs and Eccles, 1952a). Although membrane potentials of -70 to -80 mV were obtained in a few cells the values most commonly recorded were in the range -40 to -65 mV with a mean of about -55 mV compared to the 'resting' potential of -70 mV of lumbo-sacral motoneurones, given by Brock et al. (1952).

However, these differences almost certainly in part depend on a physiological factor. Unlike limb motoneurones, respiratory motoneurones are spontaneously active in the anaesthetised animal and others may readily be evoked into activity by segmental
reflex action (Section V). It follows lower than the 'resting' potential due to synaptically induced depolarisation. Therefore it is not known whether the lower values of the membrane potentials of thoracic motoneurones constitute a genuine difference from lumbo-sacral motoneurones, due to the different functional states of the two types of motoneurones, or whether they indicate a greater liability to damage by impalement.

2. **Rhythmic fluctuations in membrane potential having a respiratory periodicity.**

The membrane potentials of different cells, to a varying degree, were subjected to slow, rhythmic fluctuations having a respiratory periodicity as exemplified by the collection of records, which are all from different cells, illustrated in Fig. 26 (see also Figs. 27-32). These slow potentials showed certain constant characteristics which are enumerated under sub-headings below.

(I). In motoneurones identified by antidromic invasion from the external intercostal nerve, the depolarising phase of the slow potential was in phase with inspiration as denoted by the electromyogram of the diaphragm (Fig. 26 A, B and C; see also Figs. 27, 29, 30 and 32). Conversely, the depolarising phase of the slow potentials recorded from motoneurones antidromically invaded from the internal intercostal nerve occurred during the expiratory pause (Figs. 26 D, E and F, 28, 30A and 31D. Thus in cells which according to independent criteria were identified as inspiratory or expiratory motoneurones (Section V), the phase of depolarisation occurred in the phase of the respiratory cycle when such a depolarisation would be predicted. This relationship was observed regularly in antidromically identified
inspiratory and expiratory motoneurones of the same (as in Fig. 30),
and of different animals. (2). When periodic, repetitive firing
of motoneurones occurred, the period of the discharge coincided with
the depolarising phase of the slow potentials (as seen in Figs. 27 and
29). (3). In eupnoea, the maximum amplitude of the slow potentials
of the inspiratory motoneurones (range 5 to 12 mV) was greater than
that of the expiratory motoneurones (range 0.5 - 4.0 mV). This dif­
ference in amplitude of the slow potentials was associated with a
Corollary, inspiratory motoneurones were invariably first identified
over the loud-speaker before the test of antidromic invasion was applied
or d.c. recording made, by the intensity of this synaptic noise.

The above cited evidence supports the view that the slow
potentials are normal occurrences in respiratory motoneurones of the
spontaneously breathing cat. Although movements of the microelectrode
with respect to the spinal cord would be expected to be phase-locked
to the respiratory movements (the actual movement taking on any form
in different preparations) such movements could not consistently have
given rise to potentials which were specifically phased in identifiable,
functionally distinct cells. Movement artefacts could of course occur,
especially when there were visible movements of the spinal cord. Such
artefacts were usually recognised by their sharply contoured profile,
the absence of related, periodic synaptic noise, and their non-system­
atic occurrence in expiratory and inspiratory motoneurones. Inconclusive
but supporting evidence for the physiological basis for these
potentials was obtained in records such as that of Fig. 27 where it may be seen that the onset of the depolarisation preceded the onset of activity in the diaphragm, to which any movement artefacts would be expected to be linked.

Slow potentials of the type illustrated were recorded from thoracic motoneurones subjected to a bilateral de-afferentation (acute) extending to three segments on either side of the segment from which recording was made. Since it has been established in the present investigation that thoracic motoneurones receive monosynaptic connexions principally from the same segment, with only a small contribution from the adjacent segments (Section VI.D), it is highly improbable that the slow potentials were dependent on proprioceptive reflexes initiated from the thoracic wall. The further conclusion is therefore drawn that the slow potentials are 'central' in origin. In view of the obvious causal relationship between the slow potentials and periodic respiratory motoneurone discharge, it has been suggested (Eccles, Sears and Shealy, 1962) that they be called 'central respiratory drive potentials' (abbreviated to CRDP).

3. The excitatory phase of the central respiratory drive potential.

As already indicated, the repetitive discharges of respiratory motoneurones arise from the depolarising phase of their CRDP's. This is best seen in the inspiratory motoneurones illustrated in Figs. 27 and 29 C, D and E, where the decrease in membrane potential and the progressive augmentation in amplitude of the synaptic noise well precede the onset of spike discharge. Spontaneous repetitive firing of an expiratory motoneurone is illustrated in Fig.
Expiratory motoneurones were impaled with a higher incidence than inspiratory motoneurones (approximately 6 - 1 in 430 motoneurones), as would be expected from their greater numbers (see Section III), but few showed sustained, repetitive firing even though in independent experiments the nerve recordings showed alpha expiratory motoneurone activity. It has since been realised that this was probably due to the loss of alpha motoneurone excitability attendant on the virtually complete de-afferentation which resulted from dividing the internal and external intercostal nerves for stimulation. This interpretation is supported by the subsequent finding that repetitive stimulation of the low-threshold intercostal nerve afferents, by virtue of the summed depolarisation which it causes, may evoke repetitive firing of the expiratory motoneurones (Section VI.C). The greater probability of recording repetitive discharges of inspiratory motoneurones in eupnoea, also correlates with the greater amplitudes of their CRDP's.

As attention was focussed on the recording of the slow potentials, comparatively few long-gain records were taken of the spontaneously occurring spikes. The spike potentials were usually either of the same order of magnitude as the membrane potentials, or lower, i.e. between 50 and 60 mV. even with the use of compensation for the input capacity of the recording system, so that the overshoot potentials were negligible. When the membrane potential of lumbo-sacral motoneurones with initially a high resting potential were depolarised by an extrinsic current to between -50 and -60 mV, the spike then showed no overshoot potential (Eccles, 1957, Fig. 25). In some motoneurones, such as the inspiratory motoneurone illustrated
in Fig. 28, the summit of the spike potential fell progressively by as much as 3 mV throughout the period of discharge.

Under these conditions of natural, orthodromic activation, the intense synaptic noise and presence of multiple local responses made it difficult to determine the firing threshold of the motoneurones. Nevertheless, it was usually quite evident, as in Fig. 27 and 28, that there was a definite, progressive increase in threshold of the spike during periodic firing, especially in motoneurones showing a prominent augmenting pattern of depolarisation. This increase in threshold was also manifested by the fact that in the wake of the last spike, the membrane potential was more depolarised than at the onset of the first spike in the periodic train of discharges.

4. The inhibitory phase of the central respiratory drive potential.

Although the various concepts of the mode of operation of the 'respiratory centres' differ, they have in common the view that inspiratory and expiratory motoneurones are subjected alternately to a periodic barrage of excitatory impulses from their respective centres. As may be seen in Figs. 26 - 32, with respect to its maximal level of polarisation the CRDP may be regarded as a monophasic wave of potential. From this it could be concluded that the CRDP is due simply to the waxing and waning of the postulated excitatory synaptic drive. However, the following evidence shows that this is not the case.

Some minutes after impalement of respiratory motoneurones with a KCl-filled microelectrode, it was often observed that the CRDP changed from a single to a double phase of depolarisation with each cycle of respiration. This change occurred without any appreciable
displacement of the membrane potential, and evoked activities, such as the antidromic SD spike or the monosynaptic EPSP were unaffected, thus indicating that no essential change had resulted in the recording conditions.

According to the work of Coombs, Eccles and Fatt (1955b) on the ionic mechanism of inhibition, this reversal of a repolarising (hyperpolarising) potential strongly suggested the presence of a phase of active inhibition of expiratory and inspiratory motoneurones during the repolarising phase of their respective CRDP's. The explanation of the reversal of inhibition given by Coombs et al. (1955b) is that the increase in Cl⁻ ion concentration in the cell, due to diffusion from the microelectrode, lowers the equilibrium potential for Cl⁻ ions; hence during the specific increase in Cl⁻ ion conductance caused by the inhibitory transmitter, the net flow of Cl⁻ ions is directed outwards so depolarising the membrane.

By passing hyperpolarising currents through KCl-filled electrodes Cl⁻ ions were injected into the respiratory motoneurones in order to accelerate the otherwise slow and at times uncertain process of Cl⁻ ion diffusion. These experiments were difficult and frustrating. In addition to the difficult problem of obtaining stable recording of the CRDP's, the prolonged passage of the ion-injecting current often led to the withdrawal of the electrode from the cell. Ion injections were attempted in approximately 50 cells. Examples of the reversal of inhibition caused by Cl⁻ ion injections into expiratory and inhibitory motoneurones are illustrated in Figs. 29 and 32.
When the expiratory motoneurone of Fig. 28 was first impaled, the membrane potential was approximately -50 mV, but within a minute it had fallen to the level shown in the control record (A). With each inspiration the membrane potential showed a pronounced, augmenting repolarisation. Two successive injections of Cl\textsuperscript{-} ions are illustrated. The injections were made by passing hyperpolarising currents of 20 and 40 x 10\textsuperscript{-9}A for periods of 30 sec through the micro-electrode. By using the diaphragm electromyogram as the reference phase, it may be seen that whereas the augmenting repolarisation during inspiration was abolished, the depolarisation during expiration was unaffected. When, after the second injection, the membrane potential had increased to a higher level than before the injection, there was a slight but definite tendency for the motoneurone to show a wave of depolarisation during inspiration. Thereafter, the membrane potential steadily increased and there was a concomitant increase in the amplitude of the depolarisation which occurred during inspiration. Accompanying these changes there was a striking increase in the synchronous synaptic noise (cf. Araki, Ito and Oscarsson, 1961).

The record shown in Fig. 28G was made immediately after removing a depolarising current of 50 x 10\textsuperscript{-9}A which was passed for 50 sec. The effect of this current was to abolish almost completely the depolarisation during inspiration without affecting significantly the depolarisation during expiration. About one minute later, the record was restored to the form it had before the depolarising current was passed (H). This passage of a depolarising current was
tried three times in this experiment with exactly the same result. A depolarising current through a KCl-filled electrode suppresses the anionic diffusion from the microelectrode with the consequence that the diffusional exchange across the membrane causes the Cl\(^-\) ion concentration in the cell to approach its normal level (Coombs, Eccles and Fatt, 1955b).

Whereas spontaneous reversal of the repolarising potentials occurred with KCl-filled electrodes it never occurred with electrodes filled with 2M K citrate in recordings made sequentially in the same animal with the two types of electrode, or in different animals. The citrate ion is one of a series which, unlike Cl\(^-\) ions do not cause reversal of the IPSP to a depolarising potential (Coombs, Eccles and Fatt, 1955b; Araki, Ito and Oscarrson, 1961a and b; and Ito, Kostyuk and Oshima, 1962). On the other hand, passage of a depolarising current into a cell through a K citrate-filled electrode did cause a temporary reversal which recovered with a time constant of approximately 25 sec. Such a reversal obtained by passing a depolarising current through a citrate-filled electrode has been attributed to the increased inward flux of Cl\(^-\) ions carrying the depolarising current across the motoneuronal membrane (Coombs, Eccles and Fatt, 1955b).

From these results it is concluded that the depolarising phase of the CRDP is a phase of hyperpolarisation and therefore, a phase of inhibition. This hyperpolarisation occurs as the result of a phased, specific increase in ion conductance which is assumed to be due to the release of an inhibitory chemical transmitter.
The further conclusion is drawn that the alternating, periodic discharge of inspiratory and expiratory motoneurones may no longer be assumed to be due simply to their exposure to a periodic barrage of excitatory impulses.

5. **Spontaneous changes in the activity of respiratory motoneurones.**

While recording intracellularly from respiratory motoneurones, slow, periodic changes were often observed to occur in the average level of the membrane potential on which were superimposed the CRDP's. When the average level of the membrane potential was close to the firing threshold, the effect of these changes was very dramatic. For example, Fig. 29 shows a sequence of records made from an inspiratory motoneurone which was discharging periodically with each inspiration. In A, the frequency of the near continuous discharge was modulated with a respiratory rhythm, the peak discharge frequency reaching 12 - 14 c/sec at the height of inspiration. Over the following 6 min the membrane potential steadily increased, the discharge became entirely periodic and the peak frequency during inspiration was 10 c/sec. It may also be seen in B that the periodic discharge arises during the depolarising phase of the CRDP which in A is masked by the abrupt onset of the first spike. Further slight increases in the membrane potential resulted in still lower peak discharge frequencies (C and D; note change of gain between C and D) and the duration of this discharge was curtailed to the effect that in one cycle of inspiration shown, only a single discharge occurred. The record shown in E was made 15 min. later when the membrane potential had decreased again and a regular, well-sustained discharge occurred throughout most of inspiration.
This slowly occurring change in the pattern of discharge occurred repeatedly throughout the entire period of 110 min. during which intracellular recording was made. Further cause for believing that these records demonstrate a physiological regulation, is that precisely similar patterns of discharge were recorded from intercostal nerve filaments. Furthermore, the frequencies of discharge thus encountered (Section V) fell within the same range as described above. These intracellular recordings of spontaneous activity may therefore be distinguished from those made from Betz cells of the motor cortex where the regular discharge obtained intracellularly, could not be correlated with the pattern of activity of the cell before its impalement by the microelectrode (Phillips, 1959).

6. Effects of electrical stimulation of the vagus nerve on the central respiratory drive potentials.

As described in Section V, repetitive (250 - 300 c/sec) stimulation of the vagus nerve inhibits inspiration and prolongs the expiratory pause, during which time there may be an active discharge of expiratory motoneurones. During the stimulation, the activity of the inspiratory motoneurones may 'escape' the vagal restraint. And, as judged from the electromyogram of the diaphragm or of the external intercostal muscles, each successive 'escape' increases in intensity. On the other hand, if the expiratory motoneurones become active during the expiratory pause, their discharge is inhibited with each 'escape'.

Intracellular recordings from inspiratory and expiratory motoneurones during vagal stimulation are illustrated in Figs. 29-32. In Fig. 30 the recordings from an expiratory motoneurone (A) and an
inspiratory motoneurone (B) and (C), were made within 15 min. of each other. As the rhythm of breathing was unaltered during that time, the records may be compared as if they had been recorded simultaneously. The controls show that, concomitant with the depolarising phase of the CRDP of the inspiratory motoneurone there is an augmenting hyperpolarisation of the expiratory motoneurone of closely similar time course. Conversely, the depolarising phase of the expiratory motoneurone augmented little throughout the expiratory pause and the hyperpolarisation tended towards a plateau for approximately half of the expiratory pause.

In the expiratory motoneurone, the onset of vagal stimulation fell during the expiratory pause with the result that the membrane depolarised to a lower level than occurred during any of the preceding expirations. In the inspiratory motoneurone, the onset of vagal stimulation fell during inspiration and this caused the abrupt termination of the depolarising phase of the concurrent CRDP. The membrane potential also increased to a level slightly higher than that reached in the preceding expiratory pauses, and remained there throughout the prolonged expiratory pause. Fig. 30C shows a second trial of the effects of vagal stimulation when the membrane potential had slightly increased. On this occasion, there was virtually a total inhibition of the depolarising phase of the CRDP. These records also illustrate the slow depolarisation, and the increase in amplitude and duration of the CRDP's, which were seen regularly, and reversibly to occur in the post-vagal stimulation period in both inspiratory and expiratory motoneurones.
While recording during vagal stimulation from the inspiratory motoneurone illustrated in Fig. 29, vagal 'escape' occurred. In D, where the average level of the membrane potential was highest, it is seen that with each 'escape' there was a brief depolarisation of the membrane, and with each successive 'escape' this depolarisation increased in amplitude. When, as in C, the membrane potential was slightly lower, the second and third 'escapes' evoked one and four spikes respectively. At a still lower membrane potential, as in B, even the first 'escape' was adequate to evoke spikes, and the successive 'escapes' readily evoked them. These intracellular records of the effects of vagal stimulation should be compared to the recordings from inspiratory nerve filaments illustrated in Fig. 23.

During vagal stimulation, when each 'escape' occurs, there is a corresponding repolarisation of the expiratory motoneurones. That this is in fact an inhibitory hyperpolarisation is shown by the reversal of the inhibition which occurs when KCl-filled electrodes are used. In the control record of Fig. 31A, the expiratory motoneurone showed a well-marked depolarisation during vagal stimulation, and when the 'escape' occurred, the motoneurone abruptly repolarised. Fig. 31B records the reversal of the inhibitory hyperpolarisation taking place. After this reversal had occurred, the cell showed two phases of depolarisation with each respiratory cycle, the reversed inhibition being of relatively greater amplitude than the expiratory depolarisation as was usually the case with expiratory motoneurones. That this interpretation is correct was shown by the fact that in C, vagal stimulation abruptly shortened the concurrent, reversed inhib-
iation although the expiratory motoneurone showed the expected increasing depolarisation throughout the prolonged expiratory pause (note different time scale for C).

Fig. 32 illustrates the effects of the vagal stimulation on the reversed inhibition of an inspiratory motoneurone. In the control recording of A, taken immediately after impalement, the motoneurone showed a normal CRDP. During vagal stimulation the cell hyperpolarised and with each 'escape', there occurred a depolarisation the membrane potential between each 'escape' tending progressively to increase as indicated by the dotted line. 14 min. after impalement of the motoneurone, the membrane potential had increased and there were some indications that reversal had occurred. After the passage of a hyperpolarising current of 30x 10⁻⁹ A the synaptic noise showed a striking increase and when subsequently the membrane potential increased to beyond its former level, the motoneurone then showed two phases of depolarisation with each respiratory cycle. This reversal of the inhibition was itself temporarily reversed by the application of a depolarising current through the micro-electrode (Fig. 32C), and two minutes later, the reversed inhibition was re-established (Fig. 32D).

Stimulation of the vagus then caused a slight, progressive depolarisation throughout the expiratory pause (Fig. 32E; dotted line) on which were superimposed the depolarisations due to each vagal escape. When the stimulation of the vagus was stopped, the membrane potential increased 2-3 mV due firstly to the completion of the concurrent inspiratory depolarisation and secondly, to the loss of the synaptic drive causing the reversed inhibition.
C. DISCUSSION OF SUB-SECTION A AND B.

The evidence for believing that the CRDP's are directly responsible for the periodic, repetitive discharge of respiratory motoneurones has already been discussed. This mechanism of respiratory motoneurone excitation and inhibition must be distinguished from the overall nervous mechanism of respiration whose activity gives rise to the inhibitory and excitatory synaptic drives producing the CRDP in individual motoneurones.

The evidence shows that hyperpolarisation and depolarisation of inspiratory and expiratory motoneurones are concurrent activities which have considerable interdependence (but see final discussion). Thus an increase in the intensity of the inspiratory discharge is associated with an increase in the concomitant hyperpolarisation of expiratory motoneurones, and vice versa.

No information was obtained to indicate the nervous mechanism of the inhibition. One possibility was considered that the inhibition might be due to Renshaw cells driven by the activity of respiratory motoneurones of the opposite kind. However, CRDP's of normal form were present in respiratory motoneurones where electromyography of the muscles of the same segment, or recordings from the intercostal nerve filaments, showed no evidence of alpha motoneurone discharge.

The periodic inhibition of respiratory motoneurones by hyperpolarisation is as much a normal feature of their activity
as their periodic excitation by depolarisation. Therefore any future theory concerning the overall nervous mechanism of respiration, in order to be complete, must be stated in terms of the synaptic drives giving rise to the periodic inhibition, as well as to the periodic excitation, or respiratory motoneurones. The recordings of repetitive firing of respiratory motoneurones are of some interest in relation to the mechanism of repetitive firing of motoneurones. The CRDP clearly controls the phasing of respiratory motoneurone discharge. If the motoneurone is discharging repetitively throughout the cycle, the periodic modulation in the frequency of discharge denotes the presence of the underlying CRDP which may be unmasked by an increase in the average level of the membrane potential, or, as often occurred, by suppression of spike generation by catelectrotonic depression. The order of magnitude of this concealed depolarisation could be estimated since it has been established (Section VI.D) that over a 5-fold change of frequency (8 - 40 c/sec) there is an approximately linear relation between frequency and depolarisation of the motoneurone (cf. similar relation between the muscle spindle receptor potential and the spindle discharge frequency; Katz, 1950).

During orthodromic, repetitive firing there is an increase in the threshold of the spike. Such an increase was observed by Kolmodin and Skoglund (1958), who compared their findings to results obtained on the crayfish stretch receptor (Eyzaguirre and Kuffler, 1955). Eyzaguirre and Kuffler did not however observe such an increase in threshold in fresh preparations.
Since the IS spike (or the A spike of Fuortes Frank and Becker, 1957) is the penultimate step in the initiation of axonal spikes, although occasionally the latter may arise first (Coombs, Curtis and Eccles, 1957b), the apparent accommodation of the IS segment is clearly of physiological significance. According to Eccles (1957), accommodation of the initial segment is probably indicated by the fact that the terminal, slowly rising phase of the monosynaptic EPSP is ineffective in evoking spike discharge. Furthermore, the direct application of steady depolarising currents through a microelectrode evokes a repetitive discharge of a few hundred spikes per second which declines within a few seconds (Brock and McIntyre, 1953; Coombs, Eccles and Fatt, 1955a). Also, Coombs, Curtis and Eccles (1957b) found an increase in threshold of the IS spike in motoneurones depolarised for 12 msec by a steady, extrinsic current but there was uncertainty in the allowance made for the coupling resistance between the current passing and recording microelectrodes. According to more recent investigations, during a steady depolarising current, the change in membrane threshold is correlated accurately with the change in membrane potential and is therefore not strictly an example of accommodation of the membrane as had previously been assumed (Araki, Ito and Oshima, 1961). Measurements made by Frank and Fuortes (1960) and Bradley and Somjen (1961) by passing linearly rising currents through microelectrodes inserted into motoneurones, showed, in partial agreement with Sasaki and Otani (1961), that accommodation is slow and negligible, as agreed by Eccles (1961). As pointed out by Bradley and Somjen (1961) the proper experimental approach to obtain the true accommodation curve is
to measure the displacement of the membrane potential at the
instant of excitation, but this has not yet been done with com­
plete control over the stimulus artefact.

In order to explain the steady discharge which results
from uniform synaptic bombardment, it has also been suggested
(Eccles, 1957, p.66; 1961, p.359) that possibly accommodation is
less effective under such conditions because of the unsteadiness
in the depolarisation due to the random synaptic bombardment.
Previously, however, it has been asserted that under conditions
of physiological activation synaptic depolarisation is continuous
and uniform (Fuortes, 1954; see also Fuortes and Mantegazzini, 1962)
and that this would result from the "combined effect upon a cell
of repetitive impingement arriving asynchronously from different
fibres" (Frank and Fuortes, 1961). As exemplified by the respir­
atory motoneurone, depolarisation as a consequence of physiological
activation is far from being continuous and uniform. The component
waves of the synaptic noise of inspiratory motoneurones may easily
achieve a peak to peak amplitude of 2 - 3 mV even during the relat­
ively weak synaptic drives associated with eupnoea. Another import­
ant factor is the postulated role of the after - hyperpolarisation
of motoneurones in the generation of rhythmic discharges (Eccles, 1936;
Eccles, 1953, p.174; Eccles, Eccles and Lundberg, 1958; Eccles, 1961,
p.355). Interaction experiments between the IS spike and the monosyn­
aptic EPSP show that the IS spike is generated on a segment of the
motoneurone on which there are few, if any, excitatory synapses such
as on the axon hillock Brock, Coombs and Eccles, 1953; Fatt, 1956b;
Coombs, Curtis and Eccles, 1957a). The excitatory synapses lie on a
membrane which is actively discharged by the SD spike since the latter erases completely the depolarisation caused by a concurrent EPSP. Furthermore, the excitatory synapses do not completely recover their ability to depolarise the membrane for as long as 50 msec after the spike, this recovery occurring in two phases (Coombs, Eccles and Fatt, 1955c).

As seen in Figs. 27 and 29, the after-hyperpolarisation following each spike increases the membrane potential to well above the firing level of the first and subsequent spikes. Thereafter, the rebuilding of the synaptically induced depolarisation occurs at a rate complexly determined by the recovery from the EPSP depression, as discussed above, and the intensity and degree of modulation of the excitatory synaptic drive. This progressive depolarisation which follows in the wake of the spike provides the outward current on which the depolarisation of the IS segment is dependent. The time-course of this depolarisation and the local responses and possible accommodation which it engenders, is presumably the most important factor determining the firing of the next impulse in the train. The SD after-hyperpolarisation, the mechanism of which has been previously described (Coombs, Eccles and Fatt, 1955a; Ito and Oshima, 1962), may be looked upon as temporarily isolating the IS segment (the trigger zone for initiating axonal impulses) from the synaptically induced depolarisation.

Repetitive firing of the IS segment in response to extrinsic depolarising currents does indeed occur but the negligible after-hyperpolarisation of the IS spike (Brook, Coombs and Eccles, 1953) exerts little limitation on the discharge frequency until much higher
frequencies than normally allowed by the SD after-hyperpolarisation in orthodromic activation. This difference is also manifested by the ability of the IS spike to follow high frequencies of antidromic invasion (Brock, Coombs and Eccles, 1953).

In conclusion, the physiologically distinct properties of the IS and SD membranes, especially with regard to the common locus for EPSP depolarisation and the SD after-hyperpolarisation, are clearly of great importance in determining the rhythm of motoneurone discharge. In contrast, in neurones with small after-hyperpolarisations, the rhythm of discharge and the timing of individual discharges, may well be governed, to a greater extent than in the motoneurone, by the temporal and spatial pattern of the synaptic bombardment.
D. ANTIDROMIC INVASION OF RESPIRATORY MOTONEURONES OF THE
THORACIC SPINAL CORD.

1. **Periodic antidromic invasion.**

As the microelectrode penetrated the spinal cord the field responses evoked by stimulating the external and internal intercostal nerves at 10 shocks per second were displayed continuously on the cathode ray oscillograph. The stimuli were approximately five times the nerve threshold so as to excite all the alpha motor axons. With the microelectrode close to a motoneurone it was often observed that the SD spikes due to antidromic invasion occurred in an "all or nothing" fashion: during inspiration in motoneurones activated by stimulating the external intercostal nerve; and during expiration for motoneurones excited by stimulating the internal intercostal nerve.

In the inspiratory motoneurone of Fig. 33 antidromic invasion occurred only occasionally during inspiration and never during expiration, even with the use of paired shocks to the external intercostal nerve, as in A, or with an antecedent shock to the internal intercostal nerve as in D. When however paired shocks were applied to the external intercostal nerve, antidromic SD invasion occurred in some sweeps when the stimuli were applied early during inspiration (B) and in all sweeps when the stimuli fell during the height of inspiration (C). Similarly, an antecedent shock to the internal intercostal nerve as in F facilitated antidromic SD invasion during inspiration.

Since antidromic SD invasion is aided by depolarisation and opposed by hyperpolarisation of the motoneuronal membrane (Brock,
Coombs and Eccles, 1953) it may be assumed that this inspiratory motoneurone displayed a CRDP, and that the synaptic actions evoked by the antecedent stimuli (see next section) summed with the depolarising phase of the CRDP and so facilitated antidromic invasion. Periodic antidromic invasion occurred in cells which according to extracellular recording showed no evidence of orthodromic firing, as for example in Fig. 33. Such findings provided evidence that periodic antidromic firing was not dependent on the collision of orthodromic and antidromic impulses. It also gave indirect confirmation of the existence of CRDP's.

2. The antidromic spike.

The amplitudes of the antidromic SD spikes were of the same order of magnitude as the membrane potentials, with little or no overshoot potential even in well-impaled cells as judged by the negative after-potential immediately in the wake of the spike (cf. Brock, Coombs and Eccles, 1953). The rise time of the SD spike, as measured from the inflection on the rising phase to the summit of the spike was 0.41 msec (S.D. ± 0.12) in a group of 20 motoneurones with a membrane potential of over-55 mV. The duration of the declining phase of the spike was not measured because of the considerable uncertainty in deciding where the spike terminated.

3. Repetitive antidromic invasion.

By the use of paired shocks, as in Fig. 34 B, the antidromic spike was shown to have the typical double composition described for lumbo-sacral motoneurones by previous workers (Brock, Coombs and Eccles, 1951, 1952, 1953; Woodbury and Patton, 1952; Araki, Otani
and Furukawa, 1953; Araki and Otani, 1955; Coombs, Eccles and Fatt, 1955a; Frank and Fuortes, 1955a; Fuortes, Frank and Becker, 1957; Fatt, 1957a). When the stimulus interval was reduced, the interval between the IS and SD spikes (the A and B spikes of Fuortes et al., 1957) increased, there was intermittent failure of the SD spike, and at the shortest intervals, intermittent and finally complete failure of the IS spike to leave a small M spike as the only response to the second stimulus. In this motoneurone, the least interval between stimuli which gave a second SD spike was 3.7 msec, which was a typical value for thoracic motoneurones and corresponded to the shortest values given for lumbo-sacral motoneurones by Brock et al. (1953).

The dual composition of the motoneurone spike was also revealed during antidromic invasion evoked at increasing frequencies of stimulation, by the failure firstly of the SD spike then secondly, by the failure of the IS spike. This differential failure of the spike generating mechanism of the SD and IS membranes is illustrated in Fig. 34 A. At frequencies of 50 c/sec and below, both the SD and the IS spikes followed each stimulus without intermission. At higher frequencies there was failure of the SD spike but characteristically for these motoneurones the SD spike was able to follow quite high frequencies for three or four impulses without intermission, the discharge later becoming intermittent. Table IV shows the results obtained on a group of 30 cells in which the effects of repetitive antidromic invasion were examined over a wide range of frequencies. In 21 of the cells the spike amplitudes and after-hyperpolarisations were also recorded. As may be seen at
250 c/sec, 66% of the cells were able to give 3 SD spikes and 44% of them 4 SD spikes.

**TABLE IV.**

Repetitive antidromic activation of respiratory motoneurones.

30 cells examined with frequencies ranging from 80-400 c/s.

- Resting potential: 55.0 mV S.D. ± 8.8 (30)
- Amplitude of spike: 50.5 mV S.D. ± 9.8 (21)
- Amplitude of after-hyperpolarisation (AHP): 2.5 mV S.D. ± 1.5 (21)
- AHP/Spike: 4.9%

Duration of AHP; range 65-110 msec

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<th>Tetanus frequency</th>
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E. SEGMENTAL REFLEX ACTIONS EXERTED ON RESPIRATORY MOTONEURONES OF THE THORACIC SPINAL CORD.

1. Monosynaptic excitation of respiratory motoneurones.

a. General features. Stimulation of either the internal or external intercostal nerves below the threshold of the motor axons, at an intensity adequate to excite only the lowest threshold afferent fibres, evoked a brief depolarisation of simple time course (Figs. 35 C, 36 and 37). The peak amplitude of this depolarisation was graded according to stimulus intensity but within limits to be described later, the time course was relatively unaffected by the stimulus intensity. The latency of onset of the depolarisation, was measured from the summit of the initial positivity of the triphasic action potential of the afferent volley recorded from the cord dorsum (Fig. 35 A and B), and was usually between 0.6 to 0.8 msec. In 23 motoneurones with a mean membrane potential of 55 mV (S.D. ± 10.0), the rise time of the depolarisation was 1.4 msec (± 0.43). The time for decay to half - maximum ranged from 3.0 to 6.0 msec; occasionally it was considerably longer even when precautions were taken to minimise contamination by later polysynaptically induced activity. No essential differences were observed between inspiratory and expiratory motoneurones.

These general features closely resemble those described for the monosynaptic EPSP of lumbosacral motoneurones. Direct comparison is hampered by the presence of cutaneous fibres in the internal intercostal nerve (see Section II, Fig. I). However, a comparison of the histograms of the afferent fibres of the muscular branch (C I)
and of the cutaneous branches (C2 and C3) of the lateral intercostal nerve shows that the cutaneous fibres are of smaller diameter (Fig. 6C, "de-efferented"). It is reasonable to assume that the cutaneous fibres innervating the ventral skin are similarly smaller than the afferent fibres of the internal intercostal and the rectus abdominus muscles, all of which pass in the internal intercostal nerve. Stimulation of the lowest threshold afferents in either the internal or external intercostal nerves, most probably excites only the large diameter fibres (primary afferents) coming from the muscle spindles which these muscles are known to contain (Section V). These facts, and the assumptions, lead to the conclusion that the brief depolarisation is a monosynaptically evoked EPSP.

b. The cord dorsum potential evoked by stimulating the internal intercostal nerve and its relationship to monosynaptic excitatory action. The records in Fig. 35 A illustrate the action potential recorded from the dorsal root entry zone evoked by stimulating the internal intercostal nerve. The compound action potential so recorded consisted invariably of two components with clearly distinct electrical thresholds. The lowest threshold component will here be described as the "Ia" component because of its obvious association with the Ia component of limb muscle nerves the inverted commas signifying that it arises from a nerve which is not wholly muscular in distribution to which nerves, the description "Ia" strictly pertains. The "Ia" component is shown at a stimulus strength 2.0 times threshold which was in fact slightly supra-maximal. The higher threshold component is shown at a sub-maximal (3.06 times threshold) and a supra-maximal (5.56 times
threshold) intensity. The clear separation of these components was further shown by the use of paired, closely spaced shocks to the same nerve, so timed that the second or test shock fell in the relatively refractory period of the first or conditioning shock. Thus by the action potential that it evoked, the test stimulus revealed the fibre population not excited by the conditioning stimulus. Such a series of records obtained by altering the intensity of the conditioning stimulus, is partially illustrated in Fig. 35B, and is plotted in Fig. 35C. When the intensity of the test shock was increased, the amplitude of the "Ia" component decreased and it was no longer detectable when the test shock was more than 1.8 times threshold. At this intensity of the conditioning stimulus, the "b" wave was seen in isolation. At lower intensities of the conditioning stimulus, the "b" wave was actually smaller due to overlap with the "Ia" component. When the intensity of the conditioning stimulus was further increased, the "b" wave decreased in amplitude and was abolished completely at about 3.5 times threshold to unmask a still higher threshold component labelled as "c".

Collected results from 16 experiments in which paired stimuli were used to measure the thresholds and maxima for the two components are shown in Table V.
TABLE V

Stimulus intensity to give a maximal "Ia" response 1.6T. (± 0.37)
Threshold stimulus intensity for "b" response 1.87T. (± 0.30)
Stimulus intensity to give maximum "b" response 3.0 T. (± 0.78)

These values conceal the fact that a high stimulus intensity for a maximal "Ia" response in an individual animal, was correspondingly associated with a high threshold for the "b" component.

The records of the monosynaptic EPSP's shown in Fig. 35C, were recorded from an expiratory motoneurone in the same segment a few minutes after taking the records of A and B. They partially illustrate the plotting in Fig. 35E, of the relationship between stimulus intensity and the amplitude of the monosynaptic EPSP. The growth in amplitude of the latter is seen to correlate closely with the stimulus intensities which evoke the "Ia" component. This was a regular finding and applied equally to the growth in amplitude of the monosynaptic EPSP evoked in inspiratory motoneurones by stimulation either of external or internal intercostal nerves.

As judged by the formation of a plateau, the amplitude of the EPSP was already maximal, or within about 10% of maximum, by the time the stimulus intensity was adequate to excite the axon of the impaled motoneurone. Records such as those of Fig. 35C, taken at 3.89 times threshold, provided confirmatory evidence of the justness of this conclusion. Spontaneous failure of the IS, SD spike caused only the M spike to appear with the result that it made possible the measurement of the EPSP at higher stimulus intensities. The slight dip in the curve is due to inhibition as will be described later.
2. The convergence of "Ia" monosynaptic excitatory action on inspiratory and expiratory motoneurones.

a. Expiratory motoneurones. The convergence on respiratory motoneurones of monosynaptic excitatory action (Eccles, Eccles and Lundberg, 1957) was determined by measuring the peak amplitude of the EPSP's evoked by stimulating the internal intercostal nerves one, two or three segments rostral and caudal to the segment in which recording was made. The ipsi-segmental external intercostal nerve was also stimulated and in some experiments the contra-lateral intercostal nerves. The experiment was subsequently simplified when it was ascertained that it was comparatively rare for the expiratory motoneurones, and never for the inspiratory motoneurones, to receive monosynaptic activation other than from the same and immediately adjacent segments. Monosynaptic activation from the contralateral nerves was never observed.

In all segments examined (T5 to T11), the ipsi-segmental internal intercostal nerves evoked the largest EPSP's in expiratory motoneurones. The mean amplitude of the EPSP in 202 cells, measured without regard for the absolute value of the membrane potential, was 1.1 mV, of which 10% of the cells had EPSP's of over 3 mV, the largest individual EPSP being 5.0 mV. In this group of 202 cells, 2.5% of them received a small monosynaptic EPSP from the ipsi-segmental external intercostal nerve, but the amplitude was never more than 40% of the EPSP evoked from the ipsi-segmental internal intercostal nerve.

Individual expiratory motoneurones received monosynaptic EPSP's from the internal intercostal nerve of the rostral and caudal segments, often, from only one or the other nerve. In the group of 202
expiratory motoneurones, the amplitude of the EPSP due to the caudal segment was 30%, and that due to the rostral segment was 22%, of the amplitude of the ipsi-segmental EPSP. The aggregate EPSP in 10% of the cells was over 3.0 mV. All these values would have been higher had each motoneurone been tested from all sources, and had the low membrane potentials of many of the cells been taken into account.

The impression was gained that the EPSP's were of greater amplitude in the motoneurones with axons of highest electrical threshold. Accordingly, the data was analysed to select a group of motoneurones in which it was known for certain that the axon threshold had been accurately measured with respect to the nerve threshold, and in which the EPSP evoked by stimulating the ipsi-segmental, the rostral and caudal internal intercostal nerves, had been recorded for every motoneurone. The group of 95 cells so selected, were divided into groups according to their axon thresholds and the mean amplitudes of their respective EPSP's were plotted on the graph shown in Fig. 36. This plotting revealed a slight but definite trend for the motoneurones of highest electrical threshold to have the highest aggregate EPSP. This trend appears largely to be due to the greater contribution of monosynaptic activation from the adjacent segments.

b. Inspiratory motoneurones. The distribution of monosynaptic excitation to inspiratory motoneurones was greatly more restricted than to expiratory motoneurones. Approximately 30% of inspiratory motoneurones did not receive any significant monosynaptic activation from the ipsi-segmental external intercostal nerves.
This was not due to an axonal rather than soma-dendritic location for the microelectrode because the same cell could receive monosynaptic excitation from the internal intercostal nerve. In fact, about 70% of the inspiratory motoneurones received monosynaptic excitation from the ipsi-segmental internal intercostal nerve but only 6 received it from the adjacent internal intercostal nerves out of 40 motoneurones examined.

3. **Monosynaptic reflex discharge of respiratory motoneurones.**

   The monosynaptic EPSP evoked by stimulating the "Ia" afferents could lead to the reflex discharge of motoneurones as illustrated in Fig. 37 and 38. Such discharges could occur regularly in response to each stimulus, especially in cells with low membrane potentials, or was phased either during inspiration or expiration according to the type of motoneurone. This phasing was due to the depolarisation of the EPSP summing with the depolarising phase of a concurrent CRDP, as shown in Fig. 37. The arrows in A and B indicate the intracellularly recorded monosynaptic reflex discharges of an expiratory motoneurone recorded on sweeps which were superimposed precisely due to the use of a short time constant (0.01 sec) in the a.c. amplifier. The remaining sweeps in A and B show the same responses recorded simultaneously with d.c. amplification. In A, the stimuli at 10 shocks per second were applied during expiration and the shifting level of the onsets of the sweeps in the d.c. recordings indicate the shifting level of the depolarising phase of the concurrent CRDP. In five of the seven sweeps, as most easily distinguished in the a.c.
recording, the EPSP summed with the CRDP and evoked reflex discharges the timing of which was clearly determined by the monosynaptic EPSP. In B, the sweeps occurred during the transition from expiration to inspiration as the membrane repolarised, at first rapidly, then more slowly. The three stimuli which occurred when the membrane was most hyperpolarised failed to evoke reflex discharges.

Since phased reflex discharges were also recorded from intercostal nerve filaments in response to stimulation of the "Ia" afferents in the internal intercostal nerve, it is concluded that monosynaptic reflex activation of respiratory motoneurones is not necessarily an abnormal feature of the motoneurone due to their impalement by the microelectrode.

4. Segmental reflex inhibition of respiratory motoneurones

In B of Fig. 38, the amplitudes of the monosynaptic EPSP's of an expiratory motoneurone illustrated in A, are plotted as ordinates against the respective stimulus intensities which evoked them measured relative to threshold. With stimuli of intensity 1.3 to 1.6 times threshold, the EPSP showed no significant increase in amplitude. Because of summation with the depolarising phase of the CRDP, orthodromic reflex firing occurred during each expiration as shown for a stimulus intensity of 1.43 times threshold. This firing during expiration occurred for the entire range of stimulus intensities indicated between the two arrows in B. With stimulus intensities greater than 1.6 of threshold, the reflex firing ceased, due to a reduction which occurred in the peak amplitude of the EPSP. In C,
the tracings a, b, c and d were made from the records of the EPSP's evoked by the stimulus intensities indicated. Tracings e, f and g, show the difference obtained graphically by subtracting curves b, c and d from the curve a, the assumption being that the curves so obtained represent the time course of the inhibition. The inhibitory curve shows two phases; an early phase which prevents the EPSP from reaching its peak amplitude, and is due to relatively low threshold fibres (1.66 times threshold); and a later phase, which is responsible for the late concavity of the EPSP which becomes more prominent at the higher stimulus intensities. The onset of the inhibition occurs within 0.6 to 0.8 msec of the onset of the EPSP. Early inhibition cutting off orthodromic spikes which occurred at lower stimulus intensities, was observed in five other cells. In one of them, the orthodromic discharge was also evoked by stimulating the adjacent internal intercostal nerve and this discharge was similarly abolished when the stimulus intensity was increased. A further twelve expiratory motoneurones which showed no reflex discharges, nevertheless showed a diminution in amplitude of the EPSP with increasing stimulus intensity. This inhibition appeared to develop at stimulus intensities below those which excited a significant fraction of the motoneurone axons. The threshold of the motoneurone axons. The threshold of the motor axon of the cell illustrated in Fig. 38 was itself comparatively high (2.6 times threshold), but the mean thresholds of eleven other motoneurones impaled in the same segment was 2.0 times, and none was below 1.8 times threshold.

In six expiratory motoneurones, a hyperpolarising synaptic potential occurred with stimuli applied to the adjacent
internal intercostal nerves. The shortest latency of such inhibitory potentials was 0.8 msec longer than the latency of the EPSP which the same stimulus had evoked in other expiratory motoneurones. In not one instance was inhibition observed to occur with the stimulus intensities below that which evoked a maximal "$Ia$" component in the cord dorsum potential, or a maximal EPSP in the motoneurone.

5. **Repetitive activation of monosynaptic excitatory pathway; potentiation of the monosynaptic EPSP.**

While examining the monosynaptic reflex discharges of expiratory motoneurones it was noted that increasing the stimulus repetition rate from 1 c/sec to 25 c/sec notably augmented the reflex discharge. Since in general the monosynaptic reflex of limb motoneurones is depressed by increasing the frequency of repetition (Jefferson and Schlapp, 1953; Lloyd and Wilson, 1957; Lloyd, 1957) it was considered worthwhile examining the effects of repetition on the amplitude of the monosynaptic EPSP.

Two procedures were adopted to measure the size of the EPSP at different frequencies. The first method was applicable at low frequencies (below 50 c/sec) and consisted simply of superimposing the EPSP's at the required frequency, as illustrated in Fig. 39C. When the sizes of the superimposed EPSP's expressed as a fraction of their control size at 1 c/sec were plotted against frequency, there was no appreciable change in the size of the EPSP until about 8 c/sec. Thereafter, the EPSP amplitude increased as the frequency was raised until, at 43 c/sec, the EPSP amplitude was
twice its control value.

The second method is illustrated in Fig. 40. The end of each repetitive response shown in Fig. 40 A was expanded on a fast sweep as seen in Fig. 40 B. Following Eccles and Curtis (1960) the assumption was made that the decline of the penultimate EPSP followed the same time course as the last EPSP; hence by subtraction of this assumed curve the size of the last EPSP was determined. Both methods gave substantially the same results and showed conclusively that the EPSP is strongly potentiated by increasing frequency of repetition; the second method indicated that this potentiation continued until frequencies of approximately 200 c/sec.

The records of Fig. 40 clearly demonstrate the progressive nature of the potentiation until a steady state was reached. Absolute potentiation of the EPSP amounting to an average increase of about 100% was obtained in 14 out of 18 motoneurones on which this test was applied. In one of the cells there was an increase of 200% at 150 c/s as measured by the amplitude of the terminal EPSP.


Respiratory motoneurones may show repetitive firing in response to repetitive activation of "Ia" afferent fibres. The records from the expiratory motoneurone illustrated in Fig. 41 are of interest in that stable intracellular recording conditions existed for approximately 25 min during which time it was possible to examine repeatedly the effects of a wide range of stimulation frequencies on a motoneurone which showed no significant CRDP and was not itself
discharging spontaneously. The sample records B, C, D, E and F were all taken when the membrane potential at the onset of the tetanus was -58 mV. The frequencies of stimulation in c/sec are shown above the records. Stimulation frequencies of 64 c/sec and below, failed to evoke any discharges. At 80 c/sec three discharges were evoked (note the progressively augmenting amplitude of the EPSP's occurring in the wake of the second discharge). As the stimulus frequency was increased, the discharge frequency increased and the firing was more regular. The relationship between the discharge frequency and the stimulus frequency is plotted in Fig. 41 G. Each ordinate value was the mean of several observations in which the membrane potential at the onset of the sweep was within 0.5 mV of -58 mV. This value was the mean membrane potential over the period during which all the records were taken. The relation between the discharge frequency and the stimulation frequency is approximately linear.

Had firing not occurred in this motoneurone in response to the tetanus of EPSP's, each burst of stimulation would have evoked a sustained depolarisation the amplitude of which would have been related to the stimulating frequency as shown in Fig. 40. This relationship was not examined because of the firing. However, the inset H shows the relationship between stimulus frequency and the plateau height of the depolarisation evoked in the motoneurone whose records are shown in Fig. 40. Each value is the mean of at least two trials at the same frequency. The relation between stimulus frequency and the depolarisation it produces is seen to be linear.
The absolute discharge frequency resulting from a given frequency of stimulation depended on the steady level of the membrane potential on which the evoked, sustained depolarisation was superimposed. Each pair of records shown in Fig. 42 A was evoked at the same stimulus frequency but at times when the membrane potential at the onset of each sweep was different due to slow, spontaneous changes in the membrane potential. The difference in the membrane potential is indicated by the values given between each pair of records. The discharge pattern of each record is shown schematically in Fig. 42 B, the record taken at the lower membrane potential being placed above. The numbers in small figures to the right of each sweep show the mean discharge frequency measured as the reciprocal of the mean of the spike intervals. The numbers in heavy type shown to the right in B show the frequency of discharge produced per mV change in membrane potential calculated from the values obtained from these records. The constancy of the slope of the relationship between the discharge frequency and membrane potential, indicates the simple manner in which a spontaneous change in membrane potential is equivalent to an evoked change in membrane potential with regard to their effects on discharge frequency. The assumption made here is that there were no spontaneous changes in the membrane potential throughout the period of the testing tetanus.

7. Interaction between the central respiratory drive potential and the effects of repetitive activation of the monosynaptic excitatory pathway.

The effects of repetitive activation of the monosynaptic
excitatory pathway on an expiratory motoneurone showing a CRDP are illustrated in Fig. 43. This motoneurone showed no spontaneous discharges and repetitive stimulation at 50 and 60 c/sec also failed to evoke them. At these frequencies temporal summation of the EPSP's was still slight. However, at 100 and 125 c/sec the evoked, sustained depolarisation thereby added to the CRDP caused rhythmical discharging at 13.9 c/sec and 16.6 c/sec respectively. This evoked firing only occurred during the depolarising phase of the CRDP.

If a motoneurone was already discharging periodically with each depolarising phase of its CRDP, then repetitive activation of the "Ia" afferent fibres could convert this periodic discharge to a continuous discharge, though frequency-modulated, throughout the respiratory cycle. Such an effect is illustrated in the records from an expiratory motoneurone presented in Fig. 44. Measurements of the instantaneous discharge frequency (reciprocal of the spike intervals) of this motoneurone are shown in Fig. 45. With each expiration the motoneurone discharged repetitively at 8 to 12 c/sec (filled circles). When the "Ia" afferents were stimulated at 80 c/sec, the discharge frequency, as seen in Fig. 44 B, increased abruptly to reach a peak at about 20 c/sec (filled triangles, Fig. 45). At the onset of inspiration, the frequency immediately decreased, remained at a lower level throughout inspiration and then increased again to about 20 c/sec during the next expiratory pause. The stimulus was removed in the middle of the expiratory pause with the result that the discharge frequency fell abruptly to the same level that it had in the control records (open triangles for clarity). The records of Fig. 44 C and D show the effect of a more prolonged period of stim-
ulation at a higher frequency (125 c/sec). The discharge frequency of the control record in Fig. 44A is plotted as the open circles in Fig. 45 B. The single filled circle in Fig. 45 B represents the fact that the first interspike interval of the record shown in Fig. 44 C corresponded to the same discharge frequency as in the previous control record. Stimulation of the "Ia" afferents at 125 c/sec caused an abrupt increase in the discharge frequency (filled squares) which reached a peak value of 25 c/sec at the height of the expiratory pause then fell again during inspiration; this sequence was repeated for three cycles of respiration. The stimulus was removed in the middle of the expiratory pause and it may be seen that the discharge frequency fell instantaneously back to the level it had in the control recordings. No discharge occurred in the subsequent inspiration and during the following expiration the discharge pattern was exactly similar to the previous control records. These records obtained by adding a constant depolarisation to the motoneurone should be compared to those of the inspiratory motoneurone illustrated in Fig. 29 where conversely, an increase in the average level of the membrane potential caused continuous firing to be converted to periodic firing.

F. DISCUSSION OF SUB-SECTIONS D AND E.

In general, the respiratory motoneurones followed higher frequencies of antidromic invasion than described for lumbo-sacral motoneurones by Brock, Coombs and Eccles (1953). This difference is probably not due to the low membrane potentials because a decline in the membrane potential was found usually to impair anti-
dromic invasion. Nor is this difference attributable to a shorter duration of the after-hyperpolarisation, because the range of values obtained was identical to the relatively brief after-hyperpolarisation of flexor motoneurones given by Eccles, Eccles and Lundberg (1958), Kuno (1959). Furthermore, it is doubtful whether the concomitant synaptically induced depolarisation aided antidromic invasion because the summation of the after-hyperpolarisations (Brock, Coombs and Eccles, 1953; Ito and Oshima, 1962) usually dominated the recordings. It may be that a morphological factor is involved related to the fact that many of the thoracic motoneurones have a fusimotor, nonspherical form. The geometry of the motoneurone is of significance in the interpretation of the motoneuronal spike potentials (Brock, Coombs and Eccles, 1953).

The presence of the monosynaptic pathways to the respiratory motoneurones is compatible with the presence of muscle spindles in the intercostal and abdominal muscles. Long ago, Huber (1901) specifically drew attention to the abundance of muscle spindles in the intercostal muscles and he postulated, with great insight, their important participation in the nervous mechanism of respiration.

The relatively small amplitudes of the EPSP's in response to single shock stimulation means that monosynaptically determined reflex discharges occurred only in those motoneurones with membrane potentials poised close to threshold. As demonstrated in the records of Section V, comparatively few inspiratory or expiratory alpha motoneurones were discharging in eupnoea. The detection
of their monosynaptic reflex discharge was entirely dependent on using the same favourable conditions provided by the recording from the filaments which allowed the recording of single units during eupnoea. The failure previously to detect these monosynaptic pathways by monosynaptic reflex discharge is therefore not surprising. Thus the intercostal to intercostal, and the ipsi-segmental intercostal reflexes, described by Downman (1953, 1955), had long central delays indicative of multisynaptic pathways.

Expiratory motoneurones of the lower thoracic segments are the site of convergence of monosynaptic excitation from the same and immediately adjacent segments, and occasionally, from two segments away. This distribution reflects not only the synergism to be expected between the internal intercostal muscles of different intercostal spaces, but their synergism with the abdominal oblique and rectus abdominis muscles, all three of which are expiratory in function; it also reflects the synergism to be expected between different parts of a muscle whose innervation is pluri-segmental, such as that of the abdominal oblique muscle.

Inspiratory motoneurones were found to receive monosynaptic connexions from the external intercostal muscles via the external intercostal nerve. It was completely unexpected, however to find that they also received them from their direct antagonists, the expiratory muscles. Similarly, expiratory motoneurones are in receipt of a small amount of excitation from the external intercostal muscles. An explanation of this mutual
facilitatory action between antagonistic muscles is advanced in the final discussion.

The two to three-fold potentiation of the monosynaptic EPSP in respiratory motoneurones may be contrasted to the depression to below control values generally observed in lumbosacral motoneurones during repetitive activation of synapses (Curtis and Eccles, 1960). These authors did observe absolute potentiation in three of the fourteen motoneurones which were studied. The phase of relative potentiation between 10 and 50 c/sec that they found corresponds to the phase of absolute potentiation observed here; but whereas in the former potentiation had reached a maximum at between 80 and 100 c/sec, the maximum potentiation was not reached in the latter until about 200 c/sec. Potentiation of the EPSP occurs also at the synapses made by Ib afferents on the cells of the ventral spinocerebellar tract (Eccles, Hubbard and Oscarrson, 1961), at synapses of the corticospinal tract on fore-limb motoneurones of the baboon (Landgren, Phillips and Porter, 1952), and at the synapses on frog motoneurones made by fibres of the lateral tract (Fadiga and Brookhart, 1962).

Recently, 'mobilisation' and 'depletion' of transmitter have been implicated as two opposing processes which underly the potentiation and depression of synaptic transmission which occur during repetitive activation. The magnitude of the potentiation and the limited depression which occurs at these "Ia" synapses on respiratory motoneurones suggests a special role for 'mobilisation' at these sites. Such a mechanism provides a reliable
safeguard that any sudden demand for an increase in the force of respiratory movements, such as in respiratory obstruction, may be met by the throwing into full activity of the drives of segmental origin converging on the respiratory motoneurones. It is not known whether the nervous pathways responsible for the central respiratory drive potentials employ a similar mechanism.

Little may be said at present concerning the origin of the fibres giving rise to the segmental inhibition. Although it is tempting to suppose that the inhibition subserves a proprioceptive function, such as autogenetic inhibition, particularly in view of the inhibition of spike discharge which was observed, a cutaneous source cannot be excluded. In fact, a cutaneous source is equally indicated on account of the work of Kugelberg and Hagbarth (1958), who have described in man a series of abdominal skin reflexes in which inhibition is as prominent as excitation.

The sustained reflex discharge of respiratory motoneurones in response to repetitive activation of "Ia" afferents in the anaesthetized cat resembles the similarly evoked rhythmic discharges of extensor motoneurones of the decerebrate cat described by Alvord and Fuortes (1953). Both results may be contrasted to the poorly sustained discharge of extensor motoneurones of the anaesthetized cat as reported by Eccles and Rall (1951). These differences depend presumably on differences in excitability since when this was high Eccles and Rall did observe repetitive firing. On the other hand, Eccles and Rall concluded that spatial summation is much more important than temporal summation in generating
repetitive discharges that characterise natural movements. However, this does not obtain for respiratory motoneurones since temporal summation is here shown to play a decisive role in the evocation of their rhythmic discharge.
VII. GENERAL DISCUSSION.

The results of the experiments described in this thesis show that the respiratory motoneurone is converged on by inhibitory and excitatory pathways from both central and segmental sources. More particularly, inspiratory and expiratory motoneurones are shown to be excited and inhibited in alternating sequence. This periodic inhibition is an active process dependent on hyperpolarisation of the motoneuronal membrane caused by the periodic release of an inhibitory transmitter. Furthermore, in spontaneous respiration the inhibition of inspiratory motoneurones is an inevitable concomitant of excitation, liminal or sub-liminal, of the expiratory motoneurones, and vice versa. This interdependence reveals the inadequacy of existing concepts of the 'respiratory centres' which conceive the inspiratory and expiratory motoneurones as being exposed alternately to a barrage solely of excitatory impulses.

In the historical introduction criticism has already been fully made on other accounts, of the concept of a respiratory centre and it will not here be re-iterated. Nor will any attempt be made to formulate a new concept since, as stated in the introduction, the author is of the opinion that the term respiratory centre should not be used. Instead, the results will be discussed briefly in relation to some of the problems mentioned in the introduction, particularly those related to the proprioceptive regulation of respiration. An unexpected finding was the mutual synergism displayed in the distribution to inspiratory and expiratory motoneurones of the "Ia" excitation arising from their respective
muscles. This paradoxical distribution to antagonistic muscles may satisfactorily be accounted for by implicating two separate mechanisms which have been disclosed in the present investigation. Firstly, there is the factor of the phased discharge of inspiratory and expiratory fusimotor neurones as described fully in the discussion of Section V. Increased fusimotor activity, whether the spindle acts as an 'error detector' or as a proprioceptor, will probably lower the threshold (cf. Kuffler, Hunt and Quilliam, 1951; Eldred, Granit and Merton, 1953) and increase the sensitivity (cf. Whitteridge, 1959; Harvey and Matthews, 1951b; Jansen and Matthews, 1962) of the muscle spindles in response to stretch. Conversely, the phased inhibition of fusimotor activity will lower the threshold and diminish the sensitivity of the spindle. This inhibition ensures that during inspiration and expiration respectively, the passive stretching of the antagonistic muscles is less likely to excite the muscle spindles so that reflex contraction of the muscle is prevented or minimised. Any afferent discharge is further rendered ineffective by the hyperpolarising phase of the CRDP concurrently proceeding in that motoneurone.

The need to inhibit the proprioceptive reflexes of the respiratory muscles is best recognised in connection with the 'inflation reflex' of the expiratory muscles described by Sears (1958). He found that during artificial respiration in the animal rendered apnoeic by hyperventilation the expiratory muscles show a reflex contraction with each inflation of the lungs and chest. As he pointed out, this inflation reflex must be inhibited during normal
inspiration because the expiratory muscles are then least active, or inactive. The mechanism of this inhibition is thus explained. On the other hand, when the animal is apnoeic, the CRDP is abolished and with it the periodic inhibition so that the proprioceptive inflation reflex is dis-inhibited.

The question now arises, under what circumstances are the proprioceptive reflexes normally brought into operation? As already outlined in Section V, the monosynaptic pathway provides the afferent limb of a segmental servo-loop the efferent limb of which is controlled through the phased activity of the respiratory fusimotor neurones. Thus controlled, the muscle spindle, acting as an 'error detector', provides under conditions of hindered breathing an increased output which, through the monosynaptic pathway, facilitates or evokes the reflex discharge of the appropriate motoneurones. The effectiveness of these monosynaptic pathways must not, and cannot, be judged by the feebleness of their monosynaptic reflexes. The full effectiveness of these pathways is revealed when, under normal physiological circumstances they are activated repetitively. The ensuing depolarisation, derived as it is from pluri-segmental sources and sustained by potentiation, is of sufficient magnitude to evoke repetitive firing over a wide range of frequencies. This mechanism is likely to be thrown into activity under all circumstances when extra force of contraction of respiratory muscles is required, either subtly in the moment to moment adjustments of normal breathing which occur unconsciously, during the volitional control of the respiratory apparatus as in speech,
and during the maximum respiratory efforts as in straining. The suggestion therefore that the 'gamma loop' is involved in the neural mechanism of respiration, based on the effects of dorsal root section in man (Nathan & Sears, 1960), is given adequate substantiation from these animal experiments.
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Schematic diagram of the anatomy of the intercostal nerves.

a. Internal intercostal nerve.
b. Internal intercostal muscle.
c. Lateral intercostal nerve.
d. Abdominal oblique muscle.
e. Cutaneous branches of lateral intercostal nerve.
f. Rectus abdominis muscle.
g. Cutaneous branches of lateral intercostal nerve.
h. External intercostal nerve.
i. External intercostal muscle.
j. Muscular branch of posterior spinal ramus.
k. Cutaneous branches of posterior spinal ramus.

White areas on a and h indicate site of stimulation for experiments described in section VI.
FIG. 2. Schematic diagram of the dissection of the external and internal intercostal nerves and their filaments.
FIG. 3. Schematic diagram of dissection and mounting for mechanical recording.
FIG. 4. Fibre-size histograms of the external diameter of fibres in normal intercostal nerves (T.8); Flemming-Wolter method. A, B, C and D indicate the approximate levels of the transverse sections from which the measurements were made; the corresponding photomicrographs are shown in Fig. 7. Bl, internal intercostal nerve; B2, lateral intercostal nerve. Ordinates, external diameter of myelinated fibres in 2 μ groupings.
FIG. 5. Same as in Fig. 4, nerves de-afferented 28 days previously. C1, muscular branch to the abdominal oblique muscle.
FIG. 6. Same as in Fig. 4, nerves de-efferented 28 days previously. C1, muscular division, C2 and C3 cutaneous divisions of lateral intercostal nerve.
FIG. 7. Photomicrographs of normal, de-afferented and de-efferented intercostal nerves stained by Flemming-Wolter method. A, B, C and D refer to the levels indicated in Fig. 4. B1, C1 etc., same as in Figs. 5 and 6. Scale 10 μ.
FIG. 6. Photomicrographs of intercostal nerve filaments stained by Flemming-Wolter method. Normal filaments, A, B and C; de-afferented filaments, D, E and F.
**FIG. 9.** Isometric twitch contractions of sectors of external intercostal muscle prepared in T.10 (A to E) and T.8 (H to L). In F, the isometric twitch tension (filled circles, T.10 and open circles, T.8), is plotted against stimulus intensity measured as a multiple of the threshold of the most excitable motor fibre. The inset G, shows tracings of records D and E and the difference between them, obtained graphically, is shown by the dotted line.
FIG. 10. Isometric twitch of the external intercostal muscle (T.9) evoked by stimulating filaments of the ventral roots. By stimulating independently two sub-divisions of the filaments the response of the fast muscles (B) and the slow muscles (D) were obtained alone and in each case an adjustment of stimulus intensity gave a further separation in C, of a single fast component and in E and F, two slow components. Records A and B are shown at greater magnification in G and H respectively. The graphically obtained difference between the curves a and b, is shown by the dotted lines.
FIG. 11. Isometric contraction of individual slow (A) and fast (B) motor units in response to repetitive stimulation at the above indicated frequencies per second. Calibration bar for isometric twitch responses, 5 gms in A, 10 gms in B. In C, the tetanus : twitch tension ratios for each unit are plotted as ordinates and the stimulus intervals of the tetani (i.e. the reciprocal of the frequencies), as abscissae.
FIG. 12. Relation between isometric twitch tension of the intercostal muscle and the ventral root compound action potential with stimuli of increasing intensity applied to the internal intercostal nerve. A-J isometric twitches at stimulus intensities of 1.0, 1.14, 1.28, 1.36, 1.43, 1.52, 1.71, 2.14, 3.57, 14.0 times threshold. These records partially illustrate the full series of responses plotted in K where the twitch tensions are plotted as ordinates and the stimulus intensities, measured relative to the threshold, as abscissae. L shows the ventral root compound action potential evoked by stimuli at the indicated intensities. Note the two components of the alpha wave (α1 and α2).
FIG. 13. Relation between isometric twitch tension of the external intercostal muscle and the ventral root compound action potential, with stimuli of increasing intensity applied to the external intercostal nerve. A, isometric twitches and B, compound action potentials recorded (low-gain) from the ventral root at the indicated stimulus intensities measured relative to the threshold for the twitch. C, ventral root compound action potential recorded at high gain at the indicated stimulus intensities. The records in A and B partially illustrate the full series of responses plotted in D, where the twitch tensions are plotted as ordinates and the relative stimulus intensities as abscissae.
FIG. 14. Patterns of alpha and fusimotor neurone discharge in eupnoea (sodium-pentobarbital anaesthesia) recorded from filaments of the intercostal nerves. Upper traces of A, B and C 'expiratory nerve filaments', and of D, an 'inspiratory nerve filament'; lower traces electromyogram of diaphragm. Time scale 1 sec, vertical calibration for nerve recordings 100 µV (alpha spikes retouched).
FIG. 15. Effects of increasing the depth of anaesthesia on alpha and fusimotor neurone discharges. Upper traces, recordings from an expiratory nerve filament (T.10); lower traces, electromyogram of diaphragm. A, control. Note high frequency, periodic discharge of fusimotor spikes; at least 3 units may be distinguished in original records. B, C and D, 60, 80 and 100 sec after giving 10 mg/Kgm sodium pentobarbital (I.V.). E, recorded at faster film speed 4 min after injection.
FIG. 16. Effects of passive limb movements on alpha and fusimotor neurone discharge. Upper traces, recording from an inspiratory nerve filament (T.8); lower traces, electromyogram external intercostal muscle (T.8). A, control; C and D are recordings made continuously following several, repeated flexions and extensions of the right fore-limb made during the recording of B.
FIG. 17. Effects of hyperventilation on activity of expiratory alpha and fusimotor neurones. Upper traces, recordings from expiratory nerve filament (T.9); lower traces, electromyogram of diaphragm. A, control. B, recorded during artificial respiration when the animal was apnoeic. The periodic alpha spike activity occurred with each inflation of the lungs; the frequency of the respiratory pump was about twice the frequency of the spontaneous respiratory movements. C, same as B, recorded at higher gain. D, recorded during apnoea but with the respiratory pump stopped. E, recorded when spontaneous respiration first resumed; note earlier onset of periodic fusimotor discharge.
FIG. 18. Effects of breathing 12% O₂ in N₂ on the activity of alpha and fusimotor neurones. Upper traces, recordings from an inspiratory nerve filament (T.8). Lower traces, recordings from an expiratory nerve filament; only fusimotor discharges were present during eupnoea. Note the alpha spike evoked by closing the trachea at the height of inspiration (T.C.); trachea opened at T.O. C and D, 10 and 30 sec after onset of breathing 12% O₂ in N₂. E, 2 min after resuming air breathing.
FIG. 19. Effects of breathing 5% CO₂ in air on fusimotor neurone activity. A and B, and upper traces of D and E, recordings from and 'expiratory nerve filament' (T.8); lower traces in D and E, electromyogram of external intercostal muscle. A and D, controls; B and E, 1 min. after onset of breathing 5% CO₂ in air. C, shows the variation throughout the cycle in the number of fusimotor spikes per unit time, and F, the variation in frequency of an individual fusimotor spike, during air breathing (open circles) and breathing 5% CO₂ in air (filled circles). Note that no alpha spikes are present.
FIG. 20. Effect of tracheal closure at the peak of inspiration (the 'inspiratory inhibitory, expiratory activating' reflex of Hering and Breuer) on expiratory fusimotor neurones. Upper traces of A and B, recordings from an 'expiratory nerve filament' (T.9); lower traces, electromyogram of diaphragm. A, control; B, trachea closed at the peak of inspiration (T.C.) and not opened again (T.O.) until the onset of activity in the diaphragm. C, number of fusimotor spikes per 100 msec (counted irrespective of size) as ordinates, plotted against the times in the respiratory cycle at which the measurements were made as abscissae; the points were plotted in the middle of the corresponding 100 msec period.
FIG. 21. Effect of closing the trachea at the peak of inspiration on the activity of expiratory alpha and fusimotor neurones. Upper traces of A and B, recordings from an expiratory nerve filament (T.9); lower traces, electromyogram of internal intercostal muscle. A, control; B, the trachea was closed at the peak of inspiration. C, mean spike frequency measured over 200 msec periods as ordinates, plotted against times in the respiratory cycle at which measurements were made as abscissae. The points were plotted in the middle of the corresponding 200 msec period. The fusimotor spike is shown by circles, the small alpha spike by triangles and the large alpha spike by squares. Measurements from A, plotted as filled symbols, measurements from B, as open symbols.
FIG. 22. Effect of closing the trachea at the onset of inspiration on the activity of inspiratory alpha and fusimotor neurones. Upper traces of A and B, recordings from an 'inspiratory nerve filament' (T.S); lower traces, electromyogram of diaphragm. A, control. B, the trachea was closed (T.C.) at the onset of inspiration and not opened again (T.O) until all inspiratory activity had ceased. In C, the instantaneous frequencies (i.e. the reciprocal of spike intervals) of the single fusimotor spike (C) and the small alpha spike (D) are plotted against time. Filled symbols control, open symbols during tracheal closure. The values obtained from the first inspiration in B, provide the points plotted as the controls in C and D.
FIG. 23. Effects of electrical stimulation of the vagus nerve on the activity of inspiratory alpha and fusimotor neurones. Upper traces of A and B, recording from inspiratory nerve filament (T.8); lower traces, electromyogram of diaphragm. A, control; B, stimulation of left vagus nerve at 250 c/s. Note vagal escape of alpha and fusimotor neurones. In C, the numbers of spikes per 250 msec. are plotted as ordinates, against the times at which the measurements were made as abscissae; the ordinates were plotted in the middle of the corresponding periods. Filled symbols, measured from A, open circles measured from B. The fusimotor spike, is shown by circles and the small alpha spike which showed 'vagal escape' by squares.
FIG. 24. Effects of vagal stimulation on an expiratory fusimotor neurone. Upper traces, recording from an expiratory nerve filament (T.8); lower traces, electromyogram of external intercostal muscle. A, control. B, at arrow, onset of stimulation of central end of right vagus at 250 c/sec. Note in the centre of the record the 'vagal escape' of the inspiratory alpha motoneurones and the concomitant inhibition of the expiratory fusimotor neurone. C, plot of mean discharge frequency of fusimotor neurone measured over 200 msec intervals. Filled circles, control; open circles, during vagal stimulation; points plotted in the middle of the interval over which measurements were made.
FIG. 25. Effects of dorsal root section on the activity of expiratory alpha and fusimotor neurones. Upper traces, recordings from expiratory nerve filament (T.8); lower traces, electromyogram of diaphragm. A, control. B, the trachea was closed at the height of inspiration (T.C.) and was not opened again until the prolonged expiratory pause terminated spontaneously (T.O.). C, control record after section of the dorsal roots of T.7, 8 and 9. D, repeat tracheal closure at the peak of inspiration. E, control recording made at faster film speed to show fusimotor spikes more clearly.
Fig. 26. Central respiratory drive potentials (CRDP's) of inspiratory and expiratory motoneurones. Upper traces, intracellular d.c. recordings from inspiratory and expiratory motoneurones; lower traces, electromyogram of diaphragm. Note the difference in amplification for the records from the two types of motoneurone.
FIG. 27. Intracellular recording from an inspiratory motoneurone during normal respiration. A and C, intracellular d.c. recording at high gain. D shows the spike recorded at low gain (a.c. recording, TC 0.03 sec). B, the electromyogram of the diaphragm.
FIG. 28. Effects of passing hyperpolarising and depolarising currents through a KCl-filled microelectrode on the CHDP of an expiratory motoneurone. Upper traces, intracellular d.c. recording from an expiratory motoneurone (T.9). A and B partially illustrate the effects of passing successively hyperpolarising currents of 20 nA ad 40 nA for 30 sec. C, D, E and F illustrate, at the times indicated, the increase in membrane potential and synaptic noise which occurred spontaneously. Note change of gain in E, and the two phases of depolarisation with each cycle of respiration due to the 'reversal' of inhibition. G, immediately after, and H approximately 1 min after a depolarising current of 50 nA for 30 sec.
FIG. 29. Spontaneous changes in the discharge of an inspiratory motoneuron (T.6). Upper traces, intracellular d.c. recording; note changes in amplification. Beginning of lower trace in A shows spike at low gain (a.c. recording). Effects of electrical stimulation of the left vagus nerve at 300 c/sec for the periods indicated. Lower traces, electromyogram of the diaphragm. Time in min indicates the period elapsed following penetration of the cell.
FIG. 30. Effects of vagal stimulation on the CRDP's of respiratory motoneurones. Upper traces, intracellular d.c. recordings from an expiratory motoneurone in A, and an inspiratory motoneurone in B and C; lower traces, electromyogram of diaphragm. A and B were recorded within 15 min of each other. C was recorded 10 min after B when the membrane potential had increased.
FIG. 31. Effect of vagal stimulation on the CRDP of an expiratory motoneurone before and after 'reversal' of inhibition. Upper traces, intracellular d.c. recording; lower traces, electromyogram of diaphragm. A, control; effects of stimulating central end of left vagus at 300 c/s. B, spontaneous 'reversal'. C, effects of vagal stimulation after 'reversal'; note different time scales for A and B.
FIG. 32. Effects of passing hyperpolarising and depolarising currents through a KCl-filled electrode on the CRDP of an inspiratory motoneurone. Upper traces, intracellular d.c. recording (T.8); lower traces, electromyogram of diaphragm. The control record A also shows the effect of stimulating the vagus nerve at 300 c/sec for the period indicated by the bar line. B, effects of a hyperpolarising current of 30 nA passed for 30 sec. C, effects of a depolarising current of 10 nA passed for 45 sec. E, effects of vagal stimulation after 'reversal' of inhibition.
FIG. 33. Influence of CRDP on antidromic invasion. Upper traces, extracellular recording of SD field potentials evoked by stimulating external intercostal nerve; lower traces, electromyogram of diaphragm. A, B and C, paired stimuli to external intercostal nerve. D, E and F, stimulus to external intercostal nerve preceded by stimulus to internal intercostal nerve. The mV calibration refers to the upper traces.
FIG. 34. Repetitive antidromic invasion of a respiratory motoneurone. Intracellular a.c. recording from an inspiratory motoneurone. A, stimulation of external intercostal nerve at the indicated frequencies in c/sec. B, stimulation of external intercostal nerve with closely paired shocks at different intervals.
**FIG. 35.** Correlation of monosynaptic EPSP to "Ia" component of afferent volley. A, action potential recorded at dorsal root entry zone evoked by stimulation of the internal intercostal nerve (T8) at the indicated stimulus intensities measured relative to threshold. B, same as in A, but two stimuli (indicated by arrows) 0.6 msec apart. The intensity of the first stimulus was varied as indicated; the second stimulus was kept constant at 5.56 times threshold. The ordinates in D show the amplitudes of the three components "Ia", "b" and "c" as indicated in B, plotted against the intensity of the first shock measured relative to threshold. C, Intracellular recording from expiratory motoneurone (T8) in same animal as for A and B. Monosynaptic EPSP's evoked by stimulation of the internal intercostal nerve at the indicated intensities. In E, the amplitudes of the EPSP's are plotted against stimulus intensities relative to threshold.
FIG. 36. Relation between group "Ia" receptiveness of expiratory motoneurones and the thresholds of their axons. Ordinate shows the mean amplitude of the EPSP's evoked by stimulating the ipsi-segmental, rostral and caudal internal intercostal nerves (squares, crosses and triangles respectively; filled circles, aggregate EPSP). Each point is the mean of at least 5 cells; each cell was tested from all three sources.
**FIG. 37.** Summation of monosynaptic EPSP with CRDP. The arrows in A and B indicate the intracellularly recorded monosynaptic reflex discharge of an inspiratory motoneurone, recorded with an a.c. amplifier and short time constant (0.01 sec) in order to attenuate the CRDP. The remaining sweeps were recorded simultaneously with a d.c. amplifier. The stimuli were applied at 10/sec during expiration in A, and during inspiration in B; note different levels of the onsets of the sweeps due to the CRDP.
FIG. 38. Segmental reflex inhibition of a respiratory motoneurone. The upper traces in A show intracellular a.c. recordings from an expiratory motoneurone (T.9) evoked by stimulation of the internal intercostal nerve at the indicated stimulus intensities measured relative to threshold. Note at 1.43 times threshold reflex discharge in some sweeps. B, plotting of peak amplitude of the EPSP against stimulus intensity measured relative to nerve threshold. Horizontal line indicates range of stimulus intensities at which reflex discharge occurred. C, a, b, c and d tracings of the records at the indicated stimulus intensities; e, f and g obtained by graphical subtraction of the curves b, c and d respectively, from curve a.
FIG. 39. Effect of repetition rate on EPSP. Intracellular recording (a.c. amplification) from an expiratory motoneurone (T.9); spike potential and EPSP shown in inset. A, plot of EPSP amplitude against stimulus intensity measured relative to the threshold of the nerve. B, superimposed EPSP's evoked at the repetition rates indicated in c/sec; the stimulus intensity was 2.0 times threshold. The ordinates in C show the amplitudes of the EPSP's expressed as a fraction of the control amplitude at a repetition rate of once per second; the abscissae show the repetition rates in c/sec.
FIG. 40. Potentiation of EPSP. A and B, d.c. intracellular recordings from an expiratory motoneurone of the EPSP evoked at the indicated stimulation frequencies in c/sec; membrane potential -62 mV. B, the last part of each record in A recorded on a fast sweep. In C, the open circles indicate the size of the terminal EPSP of the series partially illustrated in B, measured relative to the mean value of the EPSP after rest periods of several seconds; filled circles, amplitude of superimposed EPSP's measured with respect to same control as used for plotting amplitudes of terminal EPSP's.
FIG. 41. Repetitive monosynaptic activation of an expiratory motoneurone. A, antidromic spike and EPSP recorded intracellularly with d.c. amplification. B to F, orthodromic discharges evoked by stimulating "Ia" afferents in the internal intercostal nerve at the frequencies indicated in c/sec. The ordinates plotted in G show the discharge frequencies, and the abscissae the stimulation frequencies in c/sec. The ordinates of the inset H show in mV the amplitudes of the depolarisation plateau evoked at the stimulation frequencies plotted as abscissae; measurements made from records of the motoneurone illustrated in Fig. 40.
FIG. 42. Repetitive monosynaptic activation of an expiratory motoneurone as in Fig. 41. A, recordings made at the same frequencies, but at different membrane potentials as indicated in mV. between each pair of records. The discharge pattern of each record is shown schematically in B, with the record taken at the lower membrane potential placed above. The numbers in small figures show the mean discharge frequency (reciprocal of the mean of the spike intervals); the numbers in large figures show the change in discharge frequency produced per mV change in membrane potential.
FIG. 43. Repetitive monosynaptic activation of expiratory motoneurone. Intracellular d.c. recording. A, antidromic spike. B, superimposed monosynaptic EPSP's evoked by stimulation of "Ia" afferents at the indicated frequencies in c/sec. The upper traces of C, D, E and F show d.c. recordings of the CRDP. Arrows indicate the onset of repetitive stimulation of the internal intercostal nerve at the indicated frequencies; offset of stimulation not shown. Numbers immediately following the spikes show the mean discharge frequency (reciprocal of mean spike interval).
FIG. 44. Repetitive monosynaptic activation of expiratory motoneurone. Upper traces, intracellular d.c. recording from expiratory motoneurone (T.9), spike summit cut off; lower traces electromyogram of diaphragm. A, control. B, between arrows, stimulation of "Ia" afferents at 80 c/s. C and D are continuous, and show the effects of stimulation of "Ia" fibres at 125 c/sec.
FIG. 45. Plotting of discharge frequency against time for records illustrated in Fig. 44. Ordinates in A and B show the instantaneous discharge frequency measured as the reciprocal of the spike interval. A. Filled circles, control; filled triangles, during stimulation at 80 c/sec. B. Open circles, same control as in A; filled squares, during stimulation at 125 c/sec.