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THE EFFECTS OF END-TIDAL CO_2 ON THE DISCHARGE OF INDIVIDUAL PHRENIC MOTONEURONES

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The activity of single phrenic motoneurones was first studied by Adrian & Bronk (1928). They noted that impulse frequency in individual units increased during asphyxia, and suggested that the increase in ventilation during asphyxia was due to an increase in discharge frequency of units already active in normal respiration, rather than to recruitment of additional units. Subsequently, Bronk & Ferguson (1935), recording from intercostal motoneurones, found that asphyxia not only increased impulse frequency in units already discharging, but also caused recruitment of new units. Later, Gesell, Atkinson & Brown (1941), and Pitts (1942) found evidence of recruitment during asphyxia in phrenic motoneurones as well.

In the above studies the responses observed could have resulted from the interplay of several factors. For example, the preparations generally had afferent connexions intact, so that bulbar respiratory neurones were subject to sensory input from carotid and aortic chemoreceptors, from receptors in the lungs, diaphragm and chest wall, as well as from other sources. Also, since asphyxia was generally employed, both hypoxia and hypercapnia might have been responsible for the responses observed. There has been no quantitative study on the relation between CO_2 levels acting on the respiratory centres and the resulting discharge in phrenic motoneurones.

The present study was designed to analyse the relation between end-tidal CO_2 levels and the discharge in single phrenic motoneurones in a preparation in which the respiratory centres were deprived of their usual sensory input. The results obtained show the manner in which the activity of phrenic motoneurones is altered by the action of CO_2 on the respiratory centres in the absence of most secondary effects. The quantitative description of the relation between unit phrenic motoneurone activity and CO_2 levels indicates the relative roles of recruitment and increase in the discharge of individual motoneurones in increasing the discharge in the phrenic nerves as end-tidal CO_2 is increased.

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METHODS

Adult cats were decerebrated at the intercollicular level under ether anaesthesia. Cranial nerves IX, X, XI, and XII were cut close to the jugular foramen and the spinal cord was transected at the level of the 7th or 8th cervical segment. The upper roots of the phrenic nerves on the two sides were dissected free in the neck and cut, the lower roots also being cut when they could be conveniently reached. The trachea was cannulated and artificial respiration applied through a demand valve respirator (Ensco). The pressure of gas inflating the lungs and the duration of inspiration and expiration could be adjusted in order to vary ventilation. The inspired gas mixture could be changed by a valve system. The following gas mixtures were used, their composition being determined by the use of a Scholander gas-analysis apparatus: 2·01 % CO_2 in O_2; 3·93 % CO_2 in O_2; 5·81 % CO_2 in O_2 and 100 % O_2.

An infra-red CO_2 analyser (Liston-Becker; microcatheter detector unit) was employed to analyse continuously the CO_2 percentage of gas in the trachea. The detecting unit was connected to the tracheal cannula through a fine polyethylene catheter. A small volume of tracheal gas was drawn continuously through the system by a suction pump. As the concentrations of CO_2 in different inspired gas mixtures were accurately known, these CO_2 levels were used for calibration. The output of the CO_2 analyser was recorded on one beam of a dual trace cathode-ray oscilloscope (Tektronix 532).

Thin filaments were separated from the cut central end of the upper root of the phrenic nerve on one side, subdivision usually being carried out until only a single responding motor unit was present. However, if a filament contained 2–3 units it was not subdivided if the impulses from individual units could be distinguished. Platinum electrodes were used for recording. These were connected to the other beam of the oscilloscope through an a.c. pre-amplifier and records were taken on moving film.

Exposed tissues were covered by a pool of paraffin oil. The body temperature was maintained within 1° C, in the range 36–38° C. To prevent muscular movement, Flaxedil (gallamine hydrochloride, American Cyanamid Company) was given intravenously in doses of 1–2 mg/kg when required.

Experimental procedure

End-tidal CO_2 was altered in two ways. A level of ventilation with 100 % O_2 was selected which produced an end-tidal CO_2 in the range of 5–6 % except when high-threshold units were studied. Increase in end-tidal CO_2 was produced by switching the inspired gas mixture to 2, 3·9, or 5·8 % CO_2 in O_2. Under these conditions arterial O_2 saturation was assumed to have been complete. Decrease in end-tidal CO_2 was produced by increasing the level of ventilation in a stepwise manner with the animal inspiring 100 % O_2. These changes in inspired gas mixtures or in the level of ventilation were maintained for at least 5 min periods, during which time a sample of the unit discharge was recorded at intervals of 1–2 min. The end-tidal CO_2 reached a plateau within 2–3 min of a change of inspired gas mixture or of ventilation, and the unit activity usually reached a steady value within 2 min of the establishment of the end-tidal CO_2 plateau. It was therefore assumed that conditions at the end of the 5 min period were approximately steady and that P_{CO_2} in blood and tissues was not changing significantly. Accordingly, unit activity recorded at the end of such periods was used for analysis. Barometric pressure was about 640 mm Hg.

RESULTS

A total of 29 units were studied in decerebrate preparations. A number of other units were analysed in preliminary experiments with anaesthetized preparations. While the results were generally similar, only the data from decerebrate cats are reported. Filaments of phrenic nerve were examined

for discharge during a period when the end-tidal CO_2 was raised. This permitted detection of units with varying thresholds. When discharge occurred, it usually consisted of recurrent trains of impulses separated by silent periods. These cycles of respiratory discharge appeared to be independent of the cycles of artificial ventilation. The number of impulses per cycle, the average frequency of impulses during the discharge phase, the durations of the discharge phase and silent period and the number of discharge cycles per minute were determined for each unit. In addition, in a number of units, the reciprocals of the intervals between individual impulses during the discharge phase were plotted as a function of time during the discharge period.

The responses of individual units

Figure 1 shows a number of characteristic features of the response of a phrenic motoneurone to variations in end-tidal CO_2. Unit activity is recorded together with CO_2 level. In *A*, the animal was inspiring 3·9 % CO_2 in O_2, the end-tidal CO_2 being about 6·7 % ($P_{CO_2} \simeq 40$ mm Hg). The unit discharge consisted of trains of 18–20 impulses recurring approximately every 2·7 sec. Record *B* was taken at the same level of ventilation when the animal had been breathing 100 % O_2, the end-tidal CO_2 being 5·5 % ($P_{CO_2} \simeq 33$ mm Hg). The number of impulses in each discharge period was reduced to 11–12 and the interval between the inspiratory cycles was reduced. Record *C* was taken 5 min after a stepwise increase in ventilation, the animal still inspiring 100 % O_2. The end-tidal CO_2 had fallen to 4·7 % and the discharge phase consisted of 8–9 impulses. The duration of the silent period was further reduced. Records *D*, *E* and *F* were taken in succession after a further stepwise increase in ventilation causing the end-tidal CO_2 to fall to a value of 4·4 %. Accompanying the fall in end-tidal CO_2, the unit discharge showed a progressive decrease in the number of impulses in each cycle, the average frequency of impulses in each cycle was reduced together with a reduction in the duration of the discharge phase, and the silent period became shorter. As a result of the shortening of the durations of the active and silent phases, the number of cycles per minute increased as end-tidal CO_2 fell (see below). The unit ceased to discharge at an end-tidal CO_2 of 4·4 % ($P_{CO_2} \simeq 26$ mm Hg).

Increase in end-tidal CO_2 above the threshold for initiation of discharge in a phrenic motoneurone usually resulted in a rise of discharge to some maximum, following which further increments of CO_2 failed to increase the unit discharge. An example is shown in Fig. 2. Records *A–D* show the discharge of this unit at four different levels of CO_2. In each case a complete discharge phase is shown on the left. In *A* (end-tidal CO_2 10·2 %, $P_{CO_2} \simeq 61$ mm Hg) the discharge consisted of 31 impulses at

an average frequency of 26/sec. The cycles recurred at a frequency of about 15/min. In *B* (end-tidal CO_2 9 %), and *C* (end-tidal CO_2 7·3 %) the number and frequency of impulses, and the frequency of bursts was the same as in *A*. In *D* the end-tidal CO_2 was 4·9 %, and unit discharge then consisted of 7–8 impulses per cycle at an average frequency of about

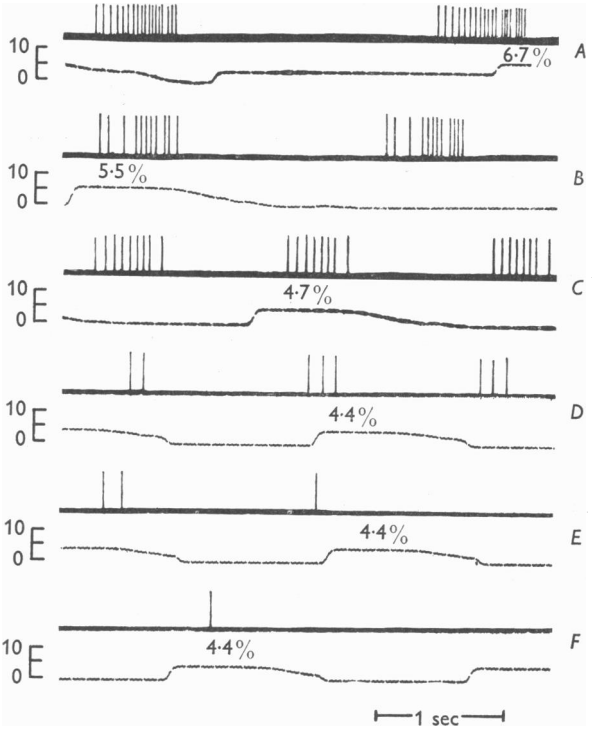

Fig. 1. Response of a phrenic motoneurone at various levels of end-tidal CO_2. Upper trace, unit discharge; lower trace, tracheal CO_2 (%); the value of end-tidal CO_2 given numerically; calibration on left, % CO_2. *A*: inspired gas 3·9 % CO_2 in O_2; downward deflexion in CO_2 record below level in inspired gas was due to room air being drawn into sampling tube from open expiratory valve. *B*: inspired gas 100 % O_2. *C*: after ventilation was increased stepwise from level in *B*. *D*, *E* and *F*: in succession after a further stepwise increase in ventilation with 100 % O_2. Some spikes retouched.

11/sec. The frequency of bursts was increased to 25/min. It is clear that above 7·3 % further increments of CO_2 failed to have significant effects on the discharge of this unit. Similar effects were noted in most of the units studied.

During the discharge phase the impulse frequency tended to show a gradual increase followed by a more abrupt fall. This was most evident when the discharge was near maximum value (see Figs. 1 and 2). When

the number of impulses in the discharge phase was low, this tendency for acceleration of impulse frequency during the inspiratory cycle was usually less. At a given level of end-tidal CO_2, the frequency pattern of successive discharge cycles was generally fairly constant. Figure 3 shows plots of the impulse frequency, measured as the reciprocal of the interval between successive impulses, as a function of time for four successive discharge cycles in two units. While there are minor variations from one cycle to another, the discharge pattern for each unit remains essentially constant at a single level of end-tidal CO_2.

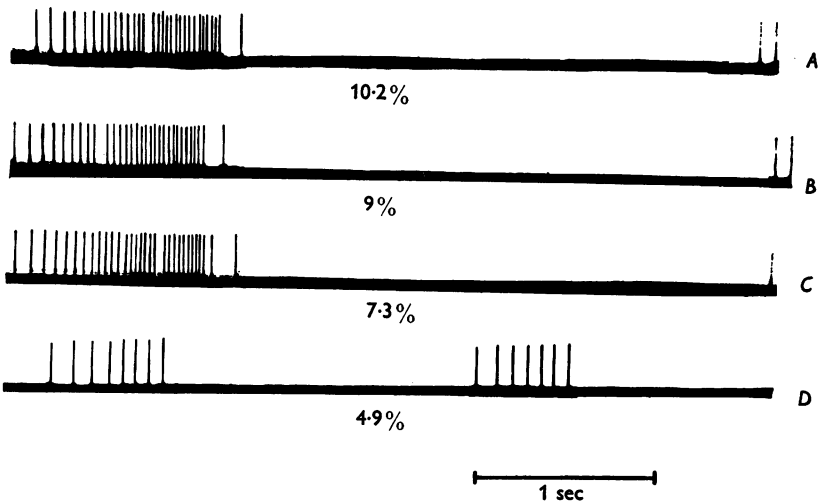

Fig. 2. Response of a phrenic motoneurone at several levels of end-tidal CO_2. The figures below each trace give the end-tidal CO_2 level (%). *A*: animal inspiring 5·8 % CO_2 in O_2. *B*: animal inspiring 3·4 % CO_2 in O_2. *C*: inspired gas 100 % O_2, level of ventilation same as in *A* and *B*. *D*: after a stepwise increase in ventilation. Spikes retouched.

All the units showed essentially the same type of response to changes in end-tidal CO_2 as is illustrated in Figs. 1 and 2. In many of them, while the activity continued to show clear discharge and silent phases as end-tidal CO_2 was reduced, the number of impulses and the pattern of intervals during the discharge phase became irregular near threshold levels of CO_2. Two units differed in that the discharge was not interrupted by a silent period near the threshold level of CO_2. Hukuhara & Okada (1956), and Burns & Salmoiraghi (1960) have observed similar behaviour in the inspiratory neurones of the respiratory centre. Figure 4 illustrates the response of one of these units. At an end-tidal CO_2 of 9·4 % (*A* and *B* in the figure) the unit showed a typical periodic discharge. A complete discharge phase is shown in *A*, and the later part of the cycle shown in *A* and

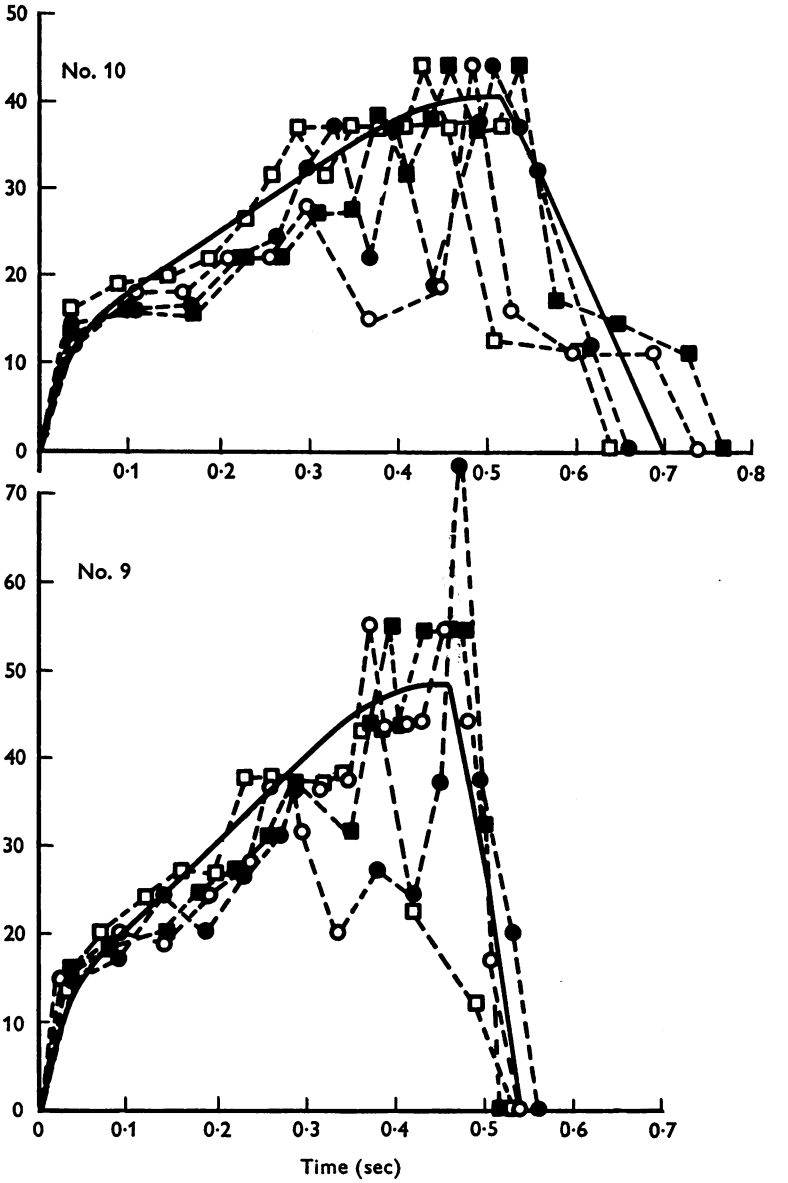

Fig. 3. Plot of impulse frequency (measured as the reciprocal of interval between impulses) of two units in four successive cycles against the time after the onset of the burst (●, ○, ■, □). Solid line indicates approximate average curve.

the beginning of the next cycle are shown in *B*. In *C*, at an end-tidal CO_2 of 5·4 %, the discharge was still periodic. When the end-tidal CO_2 was reduced to 4·2 % (*D* and *E* in the figure) the discharge at first became irregular (*D*) and then became continuous without any distinct silent periods, although periodic modulation of impulse frequency remained (*E*).

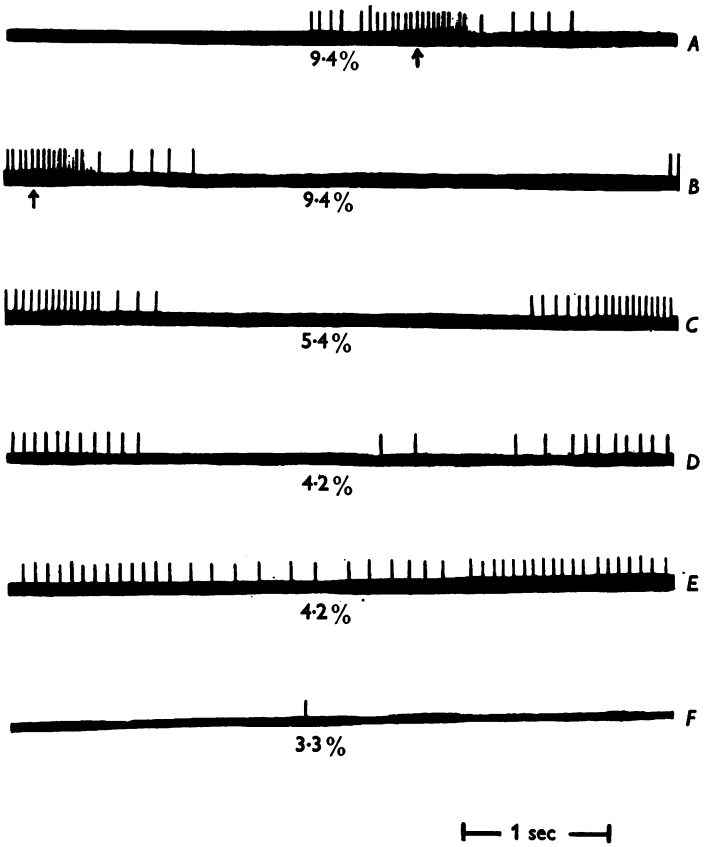

Fig. 4. Discharge recorded from a phrenic nerve filament at different levels of end-tidal CO_2. The figures below each trace give the end-tidal CO_2 (%). *A* and *B*: animal inspiring 5·8 % CO_2 in O_2; arrows indicate corresponding points in the discharge phase (see text). *C–F*: inspired gas 100 % O_2, with stepwise increases in ventilation between *C* and *D*, and between *E* and *F*. Spikes retouched.

In *F* the end-tidal CO_2 had fallen further to 3·3 % and the discharge had nearly ceased.

Measurements of several parameters of the discharge of a single phrenic motoneurone in response to changing end-tidal CO_2 are plotted in Fig. 5. All values are averages of five or more cycles. Initially the end-tidal CO_2

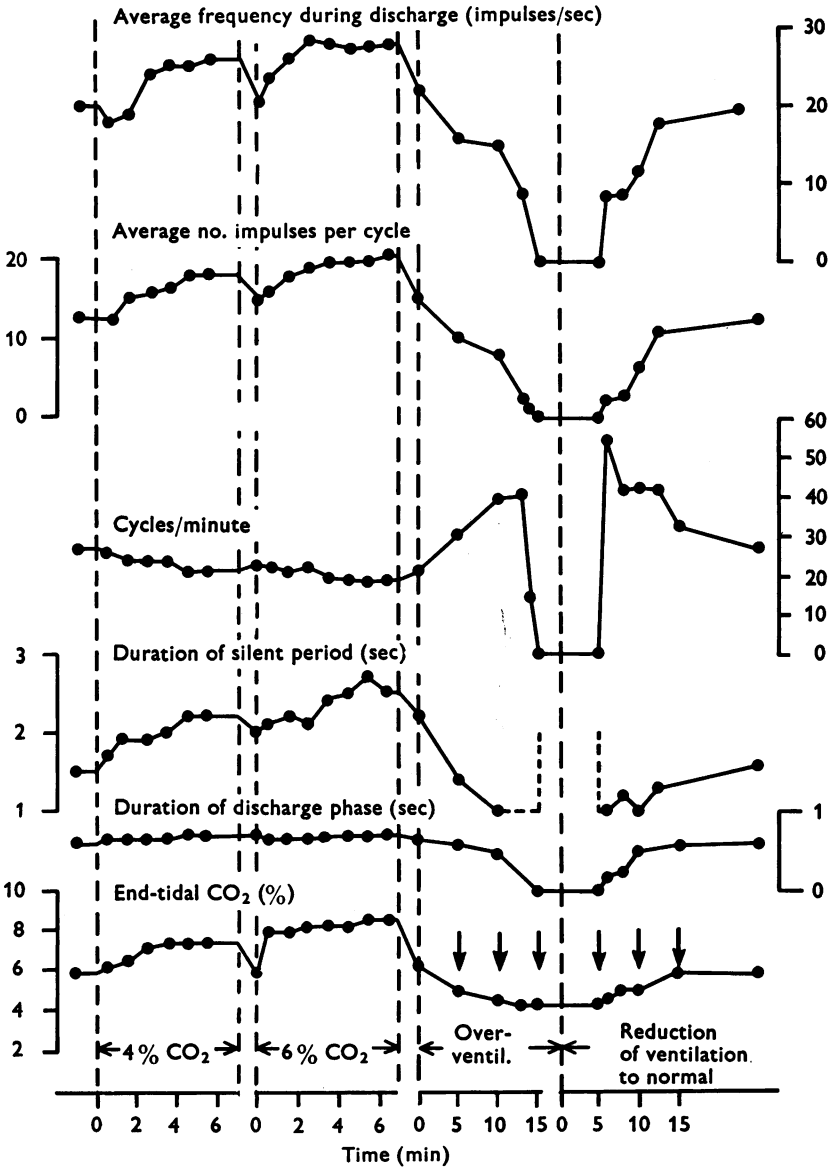

Fig. 5. Plot of various parameters of discharge in a single phrenic motoneurone, when end-tidal CO_2 was varied by altering inspired gas mixtures or levels of ventilation. Arrows indicate onset of stepwise changes in ventilation. For details see text.

level was 5·8 %, and the unit discharge consisted of 13 impulses per cycle, the discharge phase lasted about 0·7 sec, and the average impulse frequency was 20/sec. The silent period had a duration of 1·5 sec and cycles recurred 27 times per minute. The inspired gas was then changed. The end-tidal CO_2 rose gradually and became steady at 7·3 %. Accompanying this rise the number of impulses per cycle increased to 16 with little change in the duration of the discharge phase. The average frequency of impulses during the discharge phase rose to 25/sec, and the number of cycles/min fell to about 20 as a result of prolongation of the silent period. Following a period of CO_2-free O_2 and further change of gas mixture the end-tidal CO_2 rose to 8·25 %. The number of impulses per cycle increased further to 21, and with no significant change in the duration of the discharge phase the average impulse frequency during the discharge phase reached about 26/sec. The duration of the silent period increased still further, resulting in a further decrease in the number of cycles/min. After a period of CO_2-free O_2 inhalation ventilation was increased stepwise for 5 min periods and then decreased in a similar manner to control levels. As end-tidal CO_2 fell during over-ventilation the number of impulses per cycle and their average frequency fell, discharge ceasing when end-tidal CO_2 reached 4·1 %. Accompanying the reduction in discharge during the active phase was a decrease in the duration of the silent period and to a less extent of the discharge period. The number of cycles per minute therefore increased. When ventilation was decreased, end-tidal CO_2 rose again and discharge was re-initiated. The number of cycles per minute showed an initial overshoot to a value higher than that observed at comparable levels of CO_2 during the over-ventilation periods. This was a common finding. The various parameters of the unit discharge and the end-tidal CO_2 then returned towards their control values.

The relation between the parameters of the discharges and the end-tidal CO_2

Figure 6 illustrates the various parameters of the activity of the same unit, plotted as a function of end-tidal CO_2. These measurements were all made after increasing end-tidal CO_2 (see below). The number of cycles per minute rose to a peak soon after the end-tidal CO_2 exceeded the threshold value, and then fell as end-tidal CO_2 rose further. The average duration of the active phase increased initially to reach a plateau as end-tidal CO_2 increased further. The number of impulses per cycle, their average frequency, and the duration of the silent phase all increased in roughly the same manner as end-tidal CO_2 rose. The number of impulses per cycle and their frequency might be considered proportional to the degree of activity of the inspiratory neurones in the bulbar respiratory centres. The parallelism between the number and frequency of impulses during the

discharge phase and the duration of the silent phase suggests that the latter may depend on the degree of activity of the inspiratory neurones of the respiratory centres.

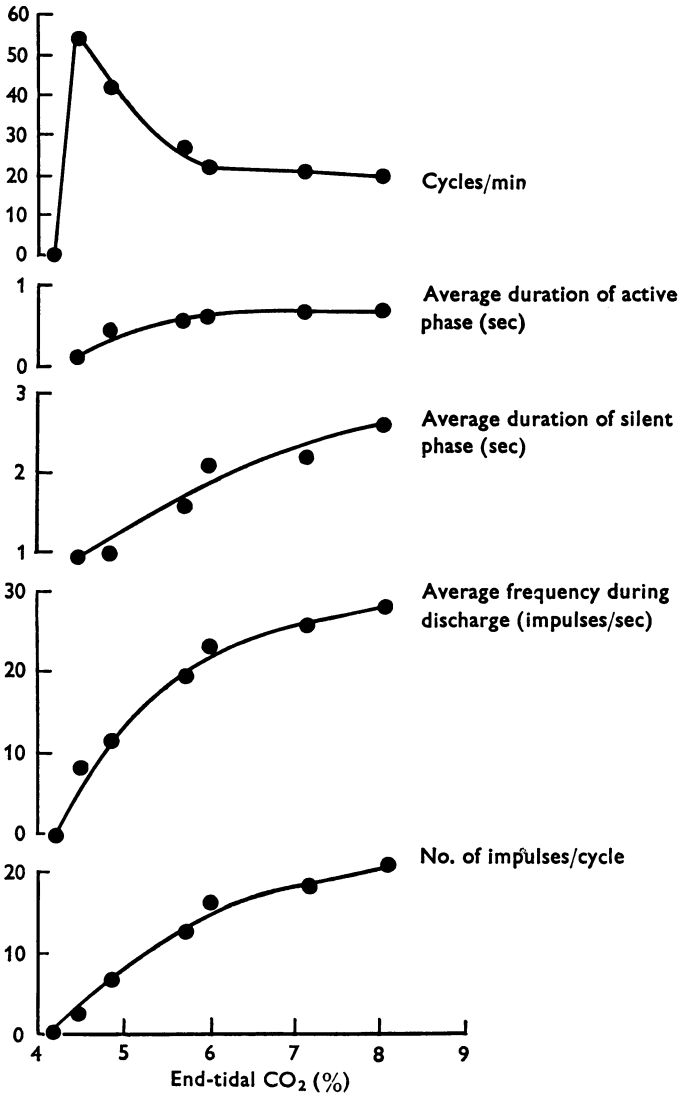

Fig. 6. Parameters of phrenic unit discharge as a function of end-tidal CO_2 from the data of Fig. 5.

Intensity of discharge and end-tidal CO_2 in several units

The relation between the number of impulses per cycle and the end-tidal CO_2 level for five units from a single preparation is compared in Fig. 7. Unit *A* showed the lowest threshold and began to discharge at an end-tidal CO_2 of 4·6 %. The number of impulses per cycle showed a plateau of 28–29 as the CO_2 level increased above 6·5 %. Units *B* and *C* were similar,

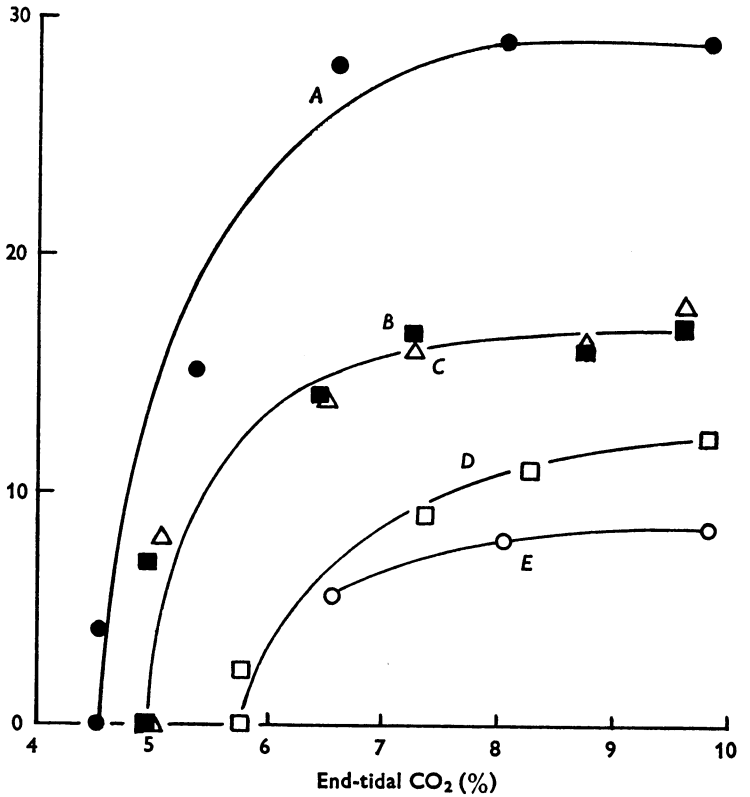

Fig. 7. Relation between number of impulses per cycle to end-tidal CO_2 in 5 units from a single preparation. Measurements taken as CO_2 levels increased.

both beginning to discharge at an end-tidal CO_2 of slightly over 5 %, and reaching a maximum in number of impulses per cycle at CO_2 levels above 7 %. This maximum of about 17 impulses/cycle was much lower than the maximum in unit *A*. Unit *D* had a still higher threshold and reached a lower maximum value. A similar plot of 23 units from 5 different preparations is shown in Fig. 8. It may be noted that as CO_2 levels were raised above threshold, unit discharge generally tended to increase at first rapidly and then more slowly, often but not always reaching a plateau.

The differences in threshold and in the maximal number of impulses per cycle between different units are considerable. There is some tendency for higher threshold units to attain a lower maximal level of discharge, but there are many exceptions to this trend.

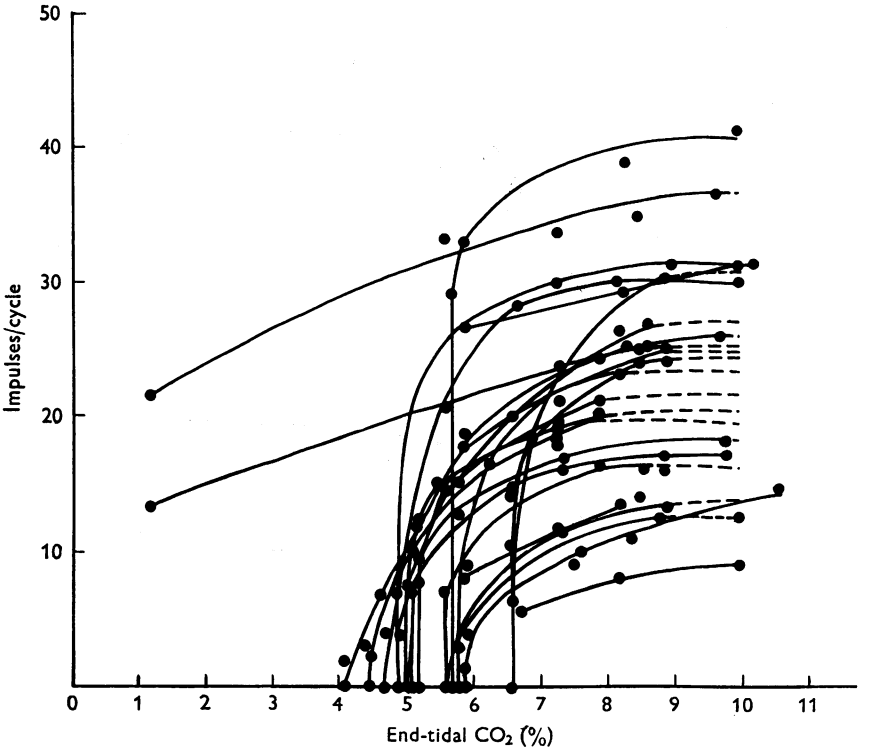

Fig. 8. Relation between number of impulses per cycle to end-tidal CO_2 in 23 phrenic units; interrupted lines indicate extrapolations of curves.

The cumulative discharge that would result from 20 of the units shown in Fig. 8 as end-tidal CO_2 was raised, was estimated by summing the number of impulses per cycle of all the units discharging, at intervals of 0·5 % CO_2. Since the curves relating the number of impulses/cycle to CO_2 level did not all cover the same range these were extrapolated (interrupted lines in Fig. 8) when required. The resultant curve relating the cumulative number of impulses per cycle to end-tidal CO_2 is shown in Fig. 9. This curve is approximately sigmoid in shape, the increase in total discharge being greatest and nearly linear between end-tidal CO_2 levels of 4·5 and 7 % (P_{CO_2}, 27–42 mm Hg).

Variations in the thresholds of single units

An interesting feature of most of the units studied was a difference between the unit threshold to CO_2 for initiation of discharge when end-tidal CO_2 was raised and its threshold for cessation of discharge when end-tidal CO_2 was reduced. The level of rising CO_2 required to initiate discharge was higher than that at which discharge ceased when CO_2 was lowered. Thus, curves relating the number of impulses per cycle to end-tidal CO_2 showed a hysteresis effect as CO_2 was first decreased and then increased.

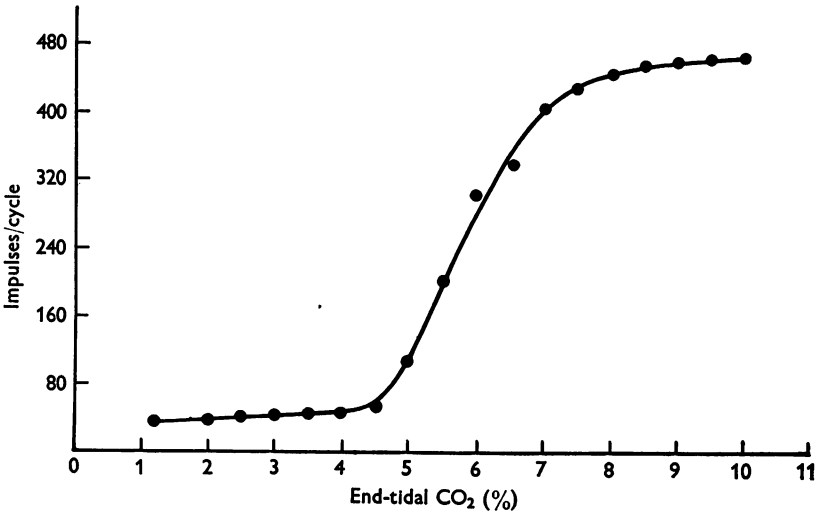

Fig. 9. Cumulative discharge of 20 of the units shown in Fig. 8 as a function of end-tidal CO_2; see text.

For this reason, the measurements of the relation between number of impulses per cycle and end-tidal CO_2 were taken during increasing CO_2 levels. The magnitude of the threshold difference varied in different units. Some examples of the hysteresis effect are shown in Fig. 10, the effect being especially pronounced in unit 6.

DISCUSSION

In the preparations employed in this study afferent input to the bulbar respiratory centres from the diaphragm, pulmonary receptors, carotid and aortic chemo- and baroreceptors, other receptors with fibres in the IX–XII cranial nerves, and all segments of the body below C7 or C8, was interrupted. There remained the input from the cervical segments and the trigeminal and other intact cranial nerves. However, since muscular contraction was prevented by administration of Flaxedil and since there

was no air flow through nasal passages, which might influence inspiratory discharge (Rijlant, 1942), the afferent input from these sources may be considered to have been essentially constant. Decerebration permitted elimination of anaesthesia and also eliminated influences from structures rostral to the mid-brain. The preparation thus consisted of a largely de-afferented brain stem containing neurones of the respiratory centres connected to phrenic motoneurones. The effects of CO_2 on single phrenic

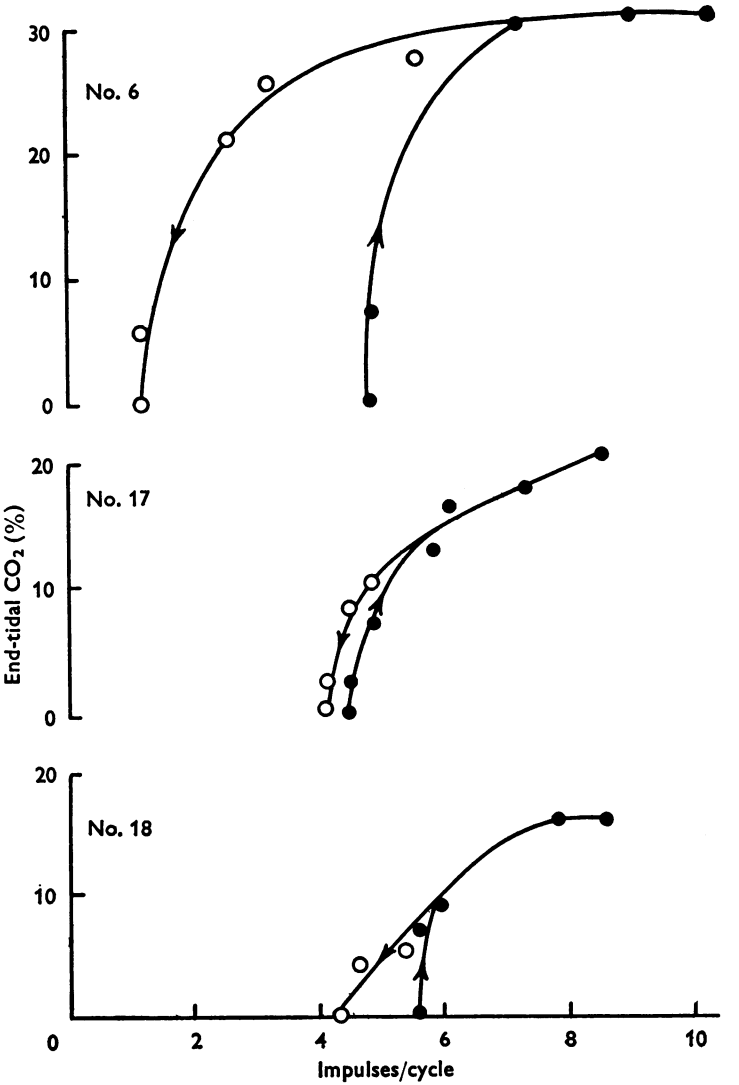

Fig. 10. Relation between number of impulses per cycle and end-tidal CO_2 when CO_2 level was falling (○) or rising (●).

units reported in this study thus represent the direct effects of the gas on the respiratory centres uncomplicated by any interaction between central and peripheral effects. However, it is not possible from the present study to exclude any effects of CO_2 on spinal interneurones in the respiratory pathways or directly on phrenic motoneurones.

The phrenic units differed chiefly in their threshold to CO_2 and in the maximum number of impulses per cycle. It may be assumed that each motoneurone receives connexions, directly or indirectly, from a number of descending fibres of bulbar inspiratory neurones. Activity in descending groups of fibres that are excitatory to phrenic motoneurones may be expected to vary both as to the number of fibres that are active and their frequency of discharge. The discharge of a given motoneurone at any level of CO_2 would be expected to depend upon its excitability, the number of excitatory fibres impinging on it, and on the activity of these fibres. The threshold to end-tidal CO_2 could vary among different phrenic units because of variations in the distribution of connexions from the population of excitatory fibres descending from the respiratory centres as well as in the excitability of the motoneurones themselves. The plateau in the relation between discharge and end-tidal CO_2 observed in phrenic units could result from the following causes: (1) At plateau the motoneurone might be discharging at its maximum rate, so that further increase in synaptic excitation would be ineffective in increasing the discharge. However, phrenic motoneurones can discharge at frequencies above 400/sec (Purpura & Chatfield, 1953). (2) All the excitatory fibres impinging on the cell might be discharging at their maximum frequency. (3) CO_2 may not be able to excite neurones of the respiratory centre beyond a certain point. The actual cause of the plateau can only be determined by studying the behaviour of the bulbar inspiratory neurones.

The discharge pattern in phrenic motoneurones observed in the present and in previous studies is similar to the pattern in bulbar respiratory neurones that discharge during inspiration (Gesell, Magee & Bricker, 1940; Pitts, 1942; Hukuhara, Nakayama & Okada, 1954; Nelson, 1959). Knowledge of the details of coupling between bulbar respiratory neurones and phrenic motoneurones must await more detailed study. Gesell, Atkinson & Brown (1940) found that when respiration was stopped in curarized animals the discharge pattern in single phrenic units was still of the slowly augmenting type. Since under these conditions there was no phasic variation of the sensory input to the respiratory centres, they concluded that the discharge pattern was determined by central factors. The present results support their conclusion.

The present results relating the activity of phrenic units to end-tidal CO_2 level may be compared to the responses of inspiratory intercostal muscle

units in dogs to rebreathing, described by Gesell *et al.* (1941). These authors noted that maximum frequency of discharge, with the animal rebreathing into a reservoir of O_2, was related to the number of breaths rebreathed. The relation showed a plateau such that beyond a certain number of breaths the discharge frequency did not increase.

In the de-afferented preparations used in the present study increasing end-tidal CO_2 caused an increase in the number and frequency of impulses per cycle but a decrease in the frequency of bursts (Fig. 6). This is in contrast to preparations with intact afferent pathways, where both the respiratory frequency and the discharge per cycle increase as blood P_{CO_2} rises (Adrian, 1933; Pitts, 1942; Burns & Salmoiraghi, 1960). It is thus clear that the increase in respiratory frequency in response to CO_2 is not a direct effect of the gas on the respiratory centres. The difference in the behaviour of these two types of preparations may be attributed to the effect of pulmonary stretch receptors, possibly proprioceptors in the respiratory muscles, and any pulmonary receptors stimulated during expiration. Adrian (1933), Pitts (1942) and Tang (1962) have made some observations on the effect of CO_2 on the respiration of decerebrate cats after double vagotomy. Adrian reported that the increase in respiratory frequency after vagotomy was less. Pitts found that in some animals there was no increase in respiratory frequency after vagotomy, whereas in others the respiratory frequency still showed an increase. Tang, however, reports a 10% reduction in respiratory frequency in response to administration of CO_2 after vagotomy. The precise role of the different receptors in altering the respiratory frequency response to CO_2 therefore still remains to be ascertained.

The results presented in Fig. 8 indicate that while an increase in the number of impulses in individual units must play a significant role in the increase in total phrenic discharge in response to increasing levels of end-tidal CO_2, recruitment of new units is also a major factor. Even though differences in threshold of respiratory centre neurones to CO_2 may influence the results drawn from a number of preparations, similar threshold difference between units from the same preparation (Fig. 7) support the suggestion that recruitment plays an important role.

Comparison of Fig. 9 with the integrated response recorded from the whole phrenic nerve at similar end-tidal CO_2 levels would permit an estimate of the adequacy of sampling in the present study. Unfortunately no such whole-population response is available. Nielsen & Smith (1951) and Lambertsen, Kough, Cooper, Emmel, Loeschcke & Schmidt (1953) have studied the relation between arterial P_{CO_2} and minute ventilation in man, over P_{CO_2} levels ranging from resting values to about 50 mm Hg. Over the whole of this range in the figure given by Nielsen & Smith, and

over a large part of the range in the figure given by Lambertsen *et al.*, the relation is linear. In ventilation studies, however, steady-state observations cannot be extended to CO_2 levels lower than resting values, for obvious reasons. Nielsen & Smith, besides demonstrating a CO_2-threshold effect during hypoxia, extrapolated the ventilation–alveolar P_{CO_2} line at normal and raised alveolar P_{CO_2} in the direction of hypocapnia and regarded the point of intersection of the curve with the abscissa (alveolar P_{CO_2}) as the threshold of the respiratory centre for CO_2. It is clear from the results of the present study that such an extrapolation is not justified, as the lower part of the curve may not be linear (Fig. 9). As a matter of fact the threshold of the respiratory centre cannot be determined by ventilation studies, as air movement cannot occur till the force developed by the inspiratory muscles exceeds the various resistive forces such as airway resistance and resistance of the pulmonary tissues and thoracic cage. Moreover, some qualitative observations made on the discharge of the whole phrenic nerve have shown that as the animal is over-ventilated the discharge loses its phasic character and the activity becomes continuous before disappearing finally. This type of activity cannot cause ventilatory excursions. These observations have been made on animals anaesthetized with pentobarbitone and with the sensory input to the respiratory centre intact.

The detailed analysis of unitary activity has shown that the activity of most of the units became irregular as CO_2 approached its threshold level and in some units the discharge became continuous before the units became silent (see Results). Units that discharge during the expiratory phase of respiration when near threshold could produce a small maintained contraction or tone of the diaphragm during expiration. As their discharge becomes more regularly phasic and confined to the inspiratory period, this tone would decrease. A decrease in diaphragmatic tone following administration of CO_2 was noted by Scott (1908).

More work needs to be done to determine the cause of the differences in recruitment and de-recruitment thresholds of phrenic units observed in the present study.

SUMMARY

1. Discharge of 29 individual phrenic motoneurones in response to different levels of end-tidal CO_2 was examined in decerebrate cats in which the brain-stem respiratory centres were essentially de-afferented.

2. As end-tidal CO_2 was raised, phrenic units began to discharge at particular levels of CO_2. The threshold varied considerably among different units. Near threshold the discharge usually became irregular although retaining its phasic character. Occasionally units showed a continuous

discharge near threshold which became typically rhythmic when CO_2 tension was raised.

3. As end-tidal CO_2 was raised above the threshold level for a unit the number of impulses per cycle and the average frequency during the discharge phase increased to some maximum, following which further increase in CO_2 caused no change in the discharge. In the de-afferented preparations employed the increase in number of impulses per cycle was accompanied by a decrease in the frequency of bursts.

4. Both recruitment of additional units and increase in discharge of units already active play a role in increasing the phrenic discharge when end-tidal CO_2 increases.

5. The end-tidal CO_2 level associated with initiation of discharge in a phrenic unit, as CO_2 was increased, was often higher than the level associated with cessation of discharge when CO_2 was reduced.

6. The relation of these findings to the functional organization of respiratory neurones is discussed.

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REFERENCES

ADRIAN, E. D. (1933). Afferent impulses in the vagus and their effect on respiration. *J. Physiol.* **79**, 332–358.

ADRIAN, E. D. & BRONK, D. W. (1928). The discharge of impulses in motor nerve fibres. Part 1. Impulses in single fibres of the phrenic nerve. *J. Physiol.* **66**, 81–101.

BRONK, D. W. & FERGUSON, L. K. (1935). The nervous control of intercostal respiration. *Amer. J. Physiol.* **110**, 700–707.

BURNS, B. D. & SALMOIRAGHI, G. C. (1960). Repetitive firing of respiratory neurones during their burst activity. *J. Neurophysiol.* **23**, 27–46.

GESELL, R., ATKINSON, A. K. & BROWN, R. C. (1940). The origin of respiratory activity patterns. *Amer. J. Physiol.* **128**, 629–634.

GESELL, R., ATKINSON, A. K. & BROWN, R. C. (1941). The gradation of intensity of inspiratory contractions. *Amer. J. Physiol.* **131**, 659–673.

GESELL, R., MAGEE, C. & BRICKER, J. (1940). Activity patterns of the respiratory neurones and muscles. *Amer. J. Physiol.* **128**, 615–628.

HUKUHARA, T., NAKAYAMA, S. & OKADA, H. (1954). Action potentials in the normal respiratory centres and its centrifugal pathways in the medulla oblongata and spinal cord. *Jap. J. Physiol.* **4**, 145–153.

HUKUHARA, T. & OKADA, H. (1956). On the excitation and inhibition of the inspiratory neurones in the respiratory centres. *Acta med. Okayama*, **10**, 151.

LAMBERTSEN, C. J., KOUGH, R. H., COOPER, D. Y., EMMEL, G. L., LOESCHCKE, H. H. & SCHMIDT, C. F. (1953). Comparison of relationship of respiratory minute volume to pCO_2 and pH of arterial and internal jugular blood in normal man during hyperventilation produced by low concentrations of CO_2 at 1 atmosphere and by O_2 at 3 atmospheres. *J. appl. Physiol.* **5**, 803–813.

NELSON, J. R. (1959). Single unit activity in medullary respiratory centres of the cat. *J. Neurophysiol.* **22**, 590–598.

NIELSEN, M. & SMITH, H. (1951). Studies on the regulation of respiration in acute hypoxia. *Acta physiol. scand.* **24**, 293–313.

PITTS, R. F. (1942). The function of components of the respiratory complex. *J. Neurophysiol.* **5**, 403–413.

PURPURA, D. P. & CHATFIELD, P. O. (1953). Changes in action potentials of single phrenic motor neurones during activity. *J. Neurophysiol.* **16**, 85–92.

RIJLANT, P. (1942). Contribution à l'étude du contrôle reflexe de la respiration. *Bull. Acad. Méd. Belg.* ser. 6, **7**, 58–107.

SCOTT, F. H. (1908). On the relative parts played by nervous and chemical factors in the regulation of respiration. *J. Physiol.* **37**, 301–326.

TANG, P. C. (1962). Brain-stem control of respiration in the cat. *Abstr. XXII int. physiol. Cong.* No. 314.