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THE ROLE OF NON-MYELINATED VAGAL AFFERENT FIBRES FROM THE LUNGS IN THE GENESIS OF TACHYPNOEA IN THE RABBIT

BY A. GUZ AND DIANA W. TRENCHARD
From the Department of Medicine, Charing Cross Hospital Medical School, Fulham Hospital, London. W. 6

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SUMMARY

1. The use of a direct current (d.c.) to produce a differential block of conduction in the cervical vagus nerves of rabbits is described; the myelinated fibres are blocked, while the non-myelinated 'C' fibres conduct normally. The method produces reproducible and reversible results.

2. The block is equally effective for low and high frequencies of discharge (1–100 Hz). During recovery or development of the block, lower frequencies of discharge can pass but higher frequencies cannot.

3. Block of conduction in myelinated fibres is associated with slower, deeper breathing, confirming previous work with cooling.

4. A further slowing and deepening of breathing may occur when a differentially blocked ('non-myelinated') nerve is sectioned, and this is mainly apparent when there are pathological conditions in the lungs.

5. The respiratory response to the right atrial injection of phenyl diguanide is mediated by non-myelinated thoracic vagal afferent fibres.

6. The tachypnoeic response to lung deflation is not mediated by non-myelinated fibres.

7. Head's Paradoxical reflex has been demonstrated during partial recovery of conduction in myelinated fibres when only lower frequencies of afferent discharge can pass the area of block.

8. A standard technique for providing a reproducible vagally mediated, tachypnoeic response to pulmonary micro-embolism is described using inert carbon-coated microspheres of 50 μm diameter. This tachypnoeic response was unchanged during a differential block indicating that the response was mediated by non-myelinated 'C' fibres.

9. Pathological changes such as haemorrhage, oedema, infarction and collapse were absent after micro-embolism, and there were no systematic

changes in lung resistance and compliance. The walls of arterioles and adjacent alveoli are distorted by the emboli and these areas are the probable sites of afferent stimulation.

INTRODUCTION

Over 50% of the vagal afferent supply to the mammalian lung consists of non-myelinated fibres with a diameter of 1 μm or less (Agostini, Chinnnock, Daly & Murray, 1957). The function of these fibres is largely unknown, although Paintal (1955, 1969) has recorded impulse activity in them in response to pulmonary embolism with starch, intravenous phenyldiguanide and pulmonary congestion. In addition Frankstein & Sergeeva (1966) found increased activity in these fibres when the lungs were inflamed. There is no evidence, however, that the ventilatory changes seen in all these experimental conditions are produced as a result of the discharge in these fibres, since myelinated fibres could also be involved.

The present studies were designed to block conduction in the myelinated fibres of the cervical vagus nerve leaving the non-myelinated fibres functioning. Observations could then be made in rabbits with normal and with damaged lungs, to determine any separate role of these fibre groups with respect to the control of the pattern of breathing and the mediation of pulmonary vagal reflexes.

The most appropriate method for producing an adequate differential block of the type required appeared to be the use of a direct current applied to the nerve (Mendell & Wall, 1964). Most of this current will flow along the surface of the nerve where there is least resistance. However, some of the current will enter the nerve at the anode, travel along individual fibres and leave at the site of the cathode. Since fibre resistance is inversely proportional to the square of the fibre diameter, the larger fibres will have a smaller resistance to the flow of current, which therefore travels preferentially along the fibres of larger diameter. In addition, for myelinated fibres, current entry may be concentrated at the nodes of Ranvier, thus creating areas of higher current densities for lower total current flows when compared with fibres without a myelin sheath. The block is produced at the anode, since the fibre membranes at this point become hyperpolarized thereby preventing the normal passage of impulses. By adjusting the strength of the d.c. it should be possible to produce the block of conduction in myelinated fibres only.

METHODS

General experimental procedure

Rabbits (3–5 kg) were used for these studies. The surgical procedures were performed under 0·5–1·5 % halothane in 50 % N_2O in O_2, and this was replaced by light chloralose anaesthesia (Merck, 40 μkg/kg) before recordings were made. Intravenous atropine (1 μkg/kg) was usually administered before any of the studies were made, to eliminate any cardiac effects of efferent vagal discharge evoked by the manipulation of the nerves or by the experimental procedures. A tracheal cannula was inserted and O_2 was added to the inspired air to achieve a $P_{a,\,O_2} > 200$ mm Hg and thus minimize chemoreceptor stimulation.

The right cervical vagus nerve was separated from the carotid artery and surrounding tissue, leaving the nerve sheath intact. The left vagus nerve was always sectioned since it is very difficult to perform this differential block on both nerves simultaneously.

Differential block techniques

The saline wick electrodes, through which the d.c. was passed to produce the differential block, were placed on the right vagus nerve with the anode central. The d.c. was supplied from a 20 V dry battery source with variable resistors in the circuit to adjust the strength of the current, this being measured by a micro-ammeter, also in the circuit. It was necessary to monitor the conduction of the constituent waves in the cervical vagal electroneurogram, through the area of block. The 'A' wave (conduction velocity 5–60 m/sec) is composed of potentials from the larger myelinated fibres, the 'B' wave (conduction velocity 2–25 m/sec) from the smaller myelinated fibres, while the 'C' wave is from the non-myelinated fibres with a conduction velocity of less than 2 m/sec (Paintal, 1963; Widdicombe, 1964). The desired degree of block was the abolition of the 'A' and 'B' waves leaving the 'C' wave intact. Silver–silver chloride electrodes were used for recording and stimulating the electroneurograms, and for earthing. The stimulating electrodes were placed on the cervical vagus nerve as low in the neck as possible. Liquid paraffin warmed to 40° C was applied to the vagus nerve at the sites of stimulating and recording electrodes to prevent drying and to maintain the nerve at or near body temperature and hence prevent slowing of conduction in the fibres. Similarly, 0·9 % saline, also warmed to 40° C, was added at the site of contact of the wick electrodes supplying the d.c. The evoked electroneurograms were recorded biphasically by the recording electrodes placed on the nerve as high in the neck as possible. The tips of the electrodes were 5–10 mm apart. The earthing electrode was usually on the nerve between the d.c. electrodes and the stimulating electrodes, but occasionally on adjacent tissue. Simultaneous continuous monitoring of 'A + B' and 'C' waves was necessary during the differential blocking procedure, but since the large artifact produced by the stimulus eliciting the 'C' wave would swamp the 'A + B' wave, two stimulators (Devices, Mark IV) were used with approximately 5 msec delay between them. The stimulators and the sweep of the oscilloscope were triggered by a Digitimer (Devices) at a frequency of 1 Hz. The one stimulator evoked the 'A' and 'B' waves using stimulation parameters of 0·5–1·5 V and 50 μsec duration, and the other stimulator evoked the 'C' wave with stimulation parameters of 5–15 V and 200–1000 μsec duration. The stimulating voltages were gradually increased until they were supramaximal for the recorded 'A + B' and 'C' waves respectively. The electroneurograms were recorded through an a.c.-coupled differential preamplifier (Tektronix, Type 122) and displayed on an oscilloscope. Two oscilloscopes were used for display of the different waves (Tektronix, Type 564; Devices, Type 3120). The arrangement of the

electrodes and recording system (Fig. 1) was such that the initial negativity of excitation at the first recording electrode gave an upward deflexion on the oscilloscope. In the later stages of the study a first-stage differential amplifier was added to provide a perfect balance of the recorded signals with high common-mode rejection. This amplifier contained a dual field-effect transistor with a 20-turn potentiometer giving a fine adjustment of the relative gains of the two sides. This permitted reduction in the amplitude and spread of the artifact and eliminated any artifactual initial positive wave (Trenchard, 1970). The entire recording system had an available pass-band from 1 Hz to 10 kHz (3 db down from 0·2 to 40 Hz). To ensure that the actual frequency band width used was the widest compatible with a minimum noise level, maximum zero stability and minimal distortion, the qualitative appearance of the

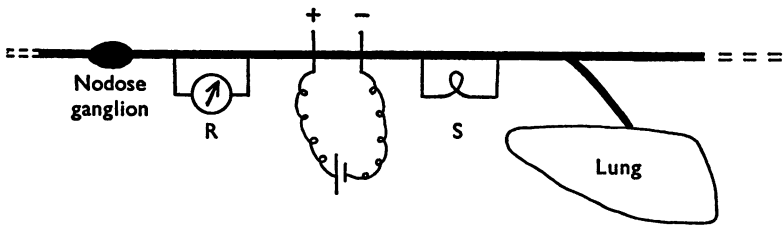

Fig.1. Arrangement of electrodes on cervical vagus nerve for technique of differential block by d.c. S, stimulating electrodes; R, recording electrodes.

waves, i.e. their size and shape, were compared with themselves before and after being submitted to improved band width settings of the differential preamplifier. There was no difference between the appearance of the 'A' and 'B' waves at the two upper band-width settings (3 db down at 40 kHz and 10 kHz respectively) but on occasion the waves were distorted at the next upper band width setting (3 db down at 1 kHz). There was no difference in the form of the 'C' wave at any of the high frequency settings, so all measurements were therefore made at the 10 kHz 3 db point. There was no change in the 'A' or 'B' waves when the lower level of the pass-band was increased to 120 Hz (3 db down at 8 Hz), while the 'C' wave was unchanged up to 15 Hz (3 db down at 8 Hz), but showed a gross reduction on increasing to 120 Hz. All recordings were therefore made at either 15 Hz (3 db down at 8 Hz) or 1·5 Hz (3 db down at 0·8 Hz) low frequency band widths.

Cardiopulmonary methods

The respiratory and cardiovascular variables were recorded on a 12-channel pen recorder writing on heat-sensitive paper (Cardiac Recorders Ltd.). The deflexions of the pens were linear and their frequency response was flat to 40 Hz. Air flow was continuously monitored by a pneumotachograph (Fleisch 0 or 00) and a differential pressure transducer (Statham, PM 97). Airway pressure was measured with a strain gauge (Statham, P 23 Db). P_{a,O_2} and P_{a,CO_2} were measured with a Severinghaus–Clarke electrode assembly and Vibron electrometer (E.I.L.). Cardiovascular pressures were recorded by strain gauges (Statham, P 23 Db) through polyvinyl catheters inserted via the femoral artery and vein. The femoral venous catheter was floated centrally in an attempt to reach the pulmonary artery but usually only the right ventricle or atrium were reached. The exact sites were determined from the pressures monitored during the studies and confirmed at post-mortem examination.

Intrapleural pressure was measured through a single Malecot catheter inserted through the right chest wall. Transpulmonary pressure was then recorded as the

difference between airway and intrapleural pressure using a differential strain gauge (UP-1, Langham Thomson). Total lung resistance was measured by subtracting a voltage proportional to lung volume (compliance pressure) from the pressure axis of a pressure–flow (X–Y) trace recorded on an oscilloscope. The angle between the 'subtracted' pressure and flow was the total resistance (Mead & Whittenberger, 1953; Nadel & Widdicombe, 1962) and could be read to an accuracy of 0·5 cm H_2O/l. sec. (one degree). The resistance values so obtained included the resistance of the tracheal cannula and pneumotachograph, which together represented 2·2 cm H_2O/l. sec. Static compliance measurements were made during the apnoeic responses to inflation, as the volume change produced by the corresponding transpulmonary pressure change. These volume changes were of the order of 1–1½ times tidal volume and the measurement therefore represented the static compliance at this degree of inflation above functional residual capacity.

Studies

Pulmonary reflexes. Inflations were achieved by switching the airways during inspiration into a weighted bag filled with 100 % O_2. By varying the weight, different inflations at constant pressures could be achieved at or above end-inspiratory level. The strength of the response was expressed as an inhibitory ratio, which is the duration of the apnoeic response to an inflation compared with the duration of the preceding respiratory cycle. The ratio was related to the inflation volume or transpulmonary pressure (Widdicombe, 1961). A Malecot catheter was inserted into the right chest cavity and normally attached to a source of negative pressure of -10 cm H_2O. The right lung could then be deflated by removing the negative pressure and injecting known quantities of air (usually 30 ml.) through the Malecot catheter, to produce a deflation reflex. Phenyl diguanide was injected through the femoral venous catheter that had been directed centrally to the proximity of the right atrium. In addition, wires were inserted into the abdominal side of the central diaphragm in two rabbits to record the electromyogram with the same equipment used for recording the electroneurogram. These studies were repeated in three rabbits with the abdominal vagi cut by transection of the oesophagus at the level of the diaphragm.

Pulmonary micro-embolism. Pulmonary micro-embolism was produced by the intravenous injection of plastic microspheres of 50 ± 10 μm diameter (3 M Co. Ltd.). These carbon-coated microspheres were nearly spherical and made of a biologically inert, plastic matrix with specific gravity between 1·1 and 1·6. The emboli (20,000 spheres/μkg) were suspended in saline in a concentration of 20 μkg/ml. to which was added a drop of Tween 20 to prevent aggregation (Kaihara, van Heerden, Migita & Wagner, 1968). The suspension was then placed in 5 ml. syringes containing magnetic 'fleas' and attached to a tapering polyethylene catheter previously inserted into a femoral vein. The syringe rested on a magnetic stirrer to keep the emboli continuously suspended in the saline. The emboli were smoothly injected at the rate of 40 μkg/min until the monitored arterial pressure began to fall. The injections were then immediately stopped. In one rabbit, in which the microspheres injected included a proportion of ^{85}Sr labelled spheres, a 99·4 % recovery was demonstrated within the lungs.

Four control groups of rabbits were studied to establish the nature and reproducibility of the response to the embolism. The first group had both vagi intact; the second group had received a prior administration of atropine; the third group was atropinized and had the left cervical vagus sectioned, and the fourth group was atropinized and had both cervical vagi sectioned. The experimental group of rabbits was atropinized, had the left vagus sectioned and was given the emboli during a period of differential block of conduction in the right vagus nerve. The adequacy

of the block was assessed by continually monitoring the 'A', 'B' and 'C' waves of the electroneurogram once every 5 sec, before, during and after the embolism.

Within 30 min of completion of the embolism the lungs were removed and fixed by the intratracheal infusion of 10% formal saline. This solution displaced air and was given until it reached a vertical height of 20 cm above the lung hila, with the lungs held vertically by the trachea. Routine histology with light microscopy was then performed.

Statistical evaluation of results

The significance of changes in respiratory rate, tidal volume and minute ventilation as a result of an experimental procedure has been assessed by a paired t test. The level of significance has been taken as $P < 0{\cdot}05$ in a two tail t table.

The significance of differences between small groups of animals has been examined using the non-parametric Mann–Whitney U test. The level of significance has been taken as $P < 0{\cdot}05$ in a one-tailed test. The respiratory rate data were submitted to the Mann–Whitney U test in the following four forms: change in respiratory rate, % change in respiratory rate, change in duration of breaths and % change in duration of breaths.

RESULTS

Adequacy of differential blocks

When the strength of the d.c. was slowly raised, the 'A' and 'B' waves were progressively slowed and temporally dispersed until they were finally abolished; the waves could not be seen even though the gain of the C.R.O. was increased tenfold. There was no preferential block of faster or slower wave components. The amount of current necessary to ensure complete abolition of the 'A' wave also blocked the 'B' wave, but left the 'C' wave intact or minimally slowed. Any further increase in the strength of the d.c. resulted in abolition of the 'C' wave. The degree of block shown in Fig. 2 was achieved in each experiment by careful adjustment of the current in the range 20–250 μA.

The 'A' and 'B' waves could not be elicited after they had been abolished during the block, either by changing the frequency of stimulation over the range 1–100 Hz (three rabbits) or increasing the duration or voltage of the stimulus (two rabbits). The 'C' wave occasionally showed minimal slowing during the block, but never more than that illustrated in Fig. 2.

When the d.c. was removed, the 'A' and 'B' waves reappeared first as slower, flattened waves, which with time gradually re-assumed the forms of the control waves. The length of time needed for complete recovery depended on the duration of the application of the d.c. The current could be reapplied up to six times to produce the same degree of block without any permanent changes in the electroneurogram. However, on occasion there was no return of the 'A' and 'B' waves after repeated and prolonged application of the current indicating permanent damage to large fibres.

As the block developed or recovered, with slowing and temporal dispersion of the waves, low-frequency impulses passed the area of block

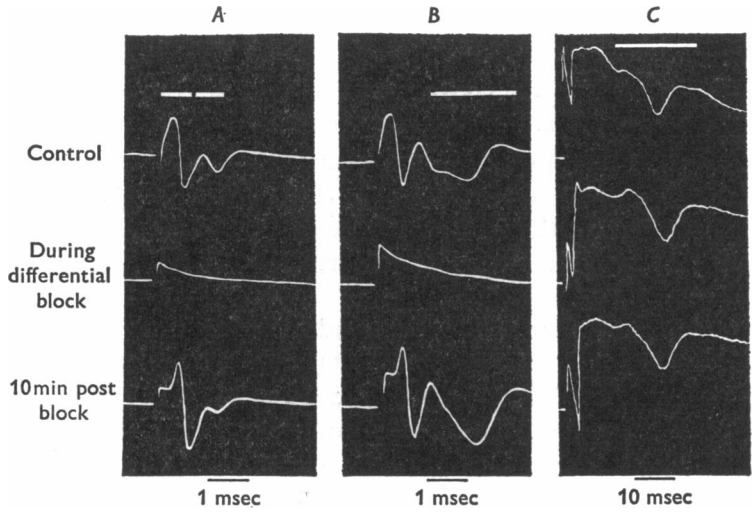

Fig. 2. Effect of a differential block of conduction by d.c. on the 'A', 'B' and 'C' waves of the cervical vagal electroneurogram, and their subsequent recovery. The horizontal bars above the records indicate respectively the 'A' and 'Aδ' waves (left-hand column), the 'B' wave (middle column) and the 'C' wave (right-hand column). In each trace the stimulus is shown first as a brief downwards deflexion (break in base line) followed by a slowly declining upwards deflexion. The artifact became successively larger with the 'B' and 'C' waves because of the increase in stimulating voltage necessary to elicit these waves. The 'C' wave is therefore superimposed on a large declining artifact.

Note that during the block the 'A' and 'B' waves are eliminated leaving only the stimulus artifacts, while the 'C' wave is almost unchanged.

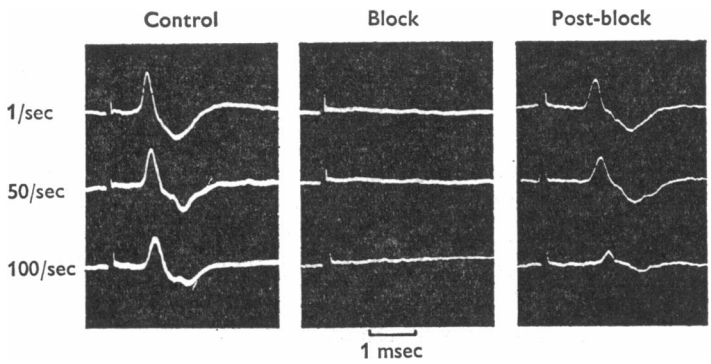

Fig. 3. Effect of frequency of stimulation during recovery from block. Note the reduced passage of high frequency discharge (100/sec) compared with the low frequency (1/sec).

whereas those of high frequency did not. Thus in Fig. 3, with incomplete recovery from the block, the 'A' wave at 100/sec was delayed and more temporally dispersed than at 1/sec. The wave was completely absent at even higher frequencies.

Ventilatory effects of differential block

The initial application of the d.c. was sometimes accompanied by an alteration in breathing which often took the form of an expiratory apnoea lasting 10–30 sec. As the strength of the d.c. was raised until complete abolition of the 'A' and 'B' waves, breathing became regular and of constant amplitude.

This stable ventilatory state was studied during sixty-nine blocks (of 3–10 min duration) in twenty-two rabbits, and all the results are shown in Fig. 4. In forty-six of the blocks performed in seventeen of the rabbits, the 'C' wave was shown to be present. In the remainder it was either not tested or the presence of a large stimulus artifact made it difficult to observe. The respiratory rate decreased on sixty-five occasions, was unchanged in two and increased in the remaining two. Breathing thus became significantly slower ($P < 0{\cdot}001$) and deeper ($P < 0{\cdot}001$). The changes in respiratory rate were significantly correlated with the changes in tidal volume (linear regression $r = 0{\cdot}52$; $P < 0{\cdot}001$ t test for r). There was a reduction in minute ventilation significant at the level $0{\cdot}05 > P > 0{\cdot}025$. When the d.c. was removed breathing remained unchanged, but reverted to the control state when the 'A' and 'B' waves reappeared.

The application of the d.c. to a vagus nerve that had previously been sectioned below the stimulating electrodes only produced the initial ventilatory disturbance described above. After this period, while the current was still being applied, the pattern of breathing remained as in the control state.

Ventilatory effects mediated by non-myelinated vagal afferent fibres

The differentially blocked nerve was sectioned below the region of block through an area to which local anaesthetic (2% lignocaine) had been applied a few seconds previously, to prevent irritative stimuli arising from the cut end of the nerve. The effects on breathing first appeared to be variable, a few rabbits showing negligible or no change, while others showed a marked slowing and deepening. The lungs of many of these rabbits were fixed in 10% formal saline and were examined by histopathologists who had no knowledge of the physiological data. The histopathological report was that in general the rabbits who had shown no obvious change in breathing had apparently normal lungs. On the other hand, those rabbits who had shown a further slowing and deepening of breathing had pathological changes in their lungs.

The results were therefore divided into two groups. The first group comprised seven rabbits whose lungs were macroscopically normal, and where the duration of the experiment had been less than 1 hr and the lungs had not been subjected to manoeuvres such as repeated inflations and deflations; in two of this group, histological examination was performed and

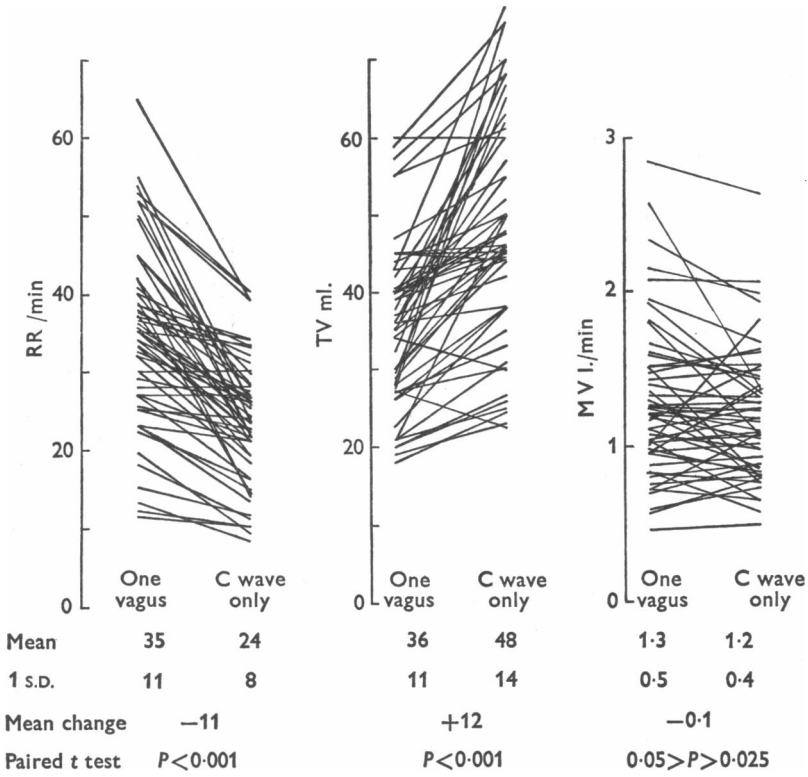

Fig. 4. Effect on respiratory rate (RR), tidal volume (TV) and minute ventilation (MV) of a d.c. block of conduction in myelinated fibres.

confirmed the normality of the lungs. The second group consisted of seventeen rabbits where the experiment was prolonged over 2 hr (usually because the lungs had been repeatedly inflated and deflated) or where macroscopic examination at post-mortem showed obvious pathology such as patchy areas of collapse; in ten of these rabbits, histological examination was performed and confirmed that the lungs had patchy areas of collapse, haemorrhage or oedema. The results in these two groups are shown in Fig. 5. There was no significant fall in respiratory rate or increase in tidal volume in the 'normal' group, whereas there was a significant slowing ($P < 0{\cdot}001$) and deepening ($0{\cdot}005 > P > 0{\cdot}001$) of breathing in the

'pathological' group. The changes in minute ventilation were not significant in either group.

Vagal stimulation during the differential block

High frequency stimulation (50–100 Hz) of the cervical vagus nerve sufficient only to elicit the 'A' wave always produced an expiratory apnoea. After abolition of this wave by d.c. the stimulation produced no ventilatory effect.

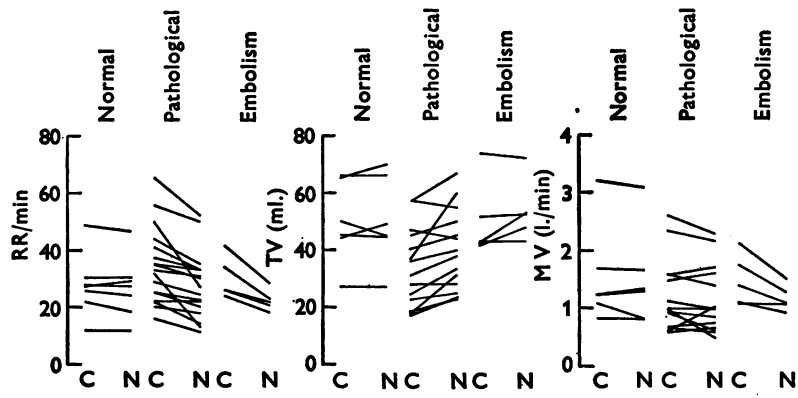

Fig. 5. Effect on respiratory rate (RR), tidal volume (TV) and minute ventilation (MV) of section of a cervical vagus nerve with only the non-myelinated fibres functioning. The other nerve had been sectioned previously.

The 'normal' group comprised rabbits whose lungs were macroscopically normal, and where the duration of the experiment was less than 1 hr, with no studies involving inflations or deflations. The 'pathological' group comprised rabbits with obvious lung pathology such as patchy areas of collapse. In two of this group the abdominal vagi were previously sectioned. The 'embolism' group were rabbits in which the 50 μm microspheres had been given after a differential block had been established. C, control value with only non-myelinated fibres functioning; N, after vagal section.

The higher voltage and longer duration of stimulus necessary to elicit the 'C' wave (without d.c. block) increased both the functional residual capacity and the respiratory frequency, and reduced tidal volume. These effects became more marked with an increasing frequency of stimulation until they merged into an inspiratory apnoea (Fig. 6). These patterns of response at the various frequencies were not affected by a differential block (Fig. 7) but were absent when the d.c. was increased to block the 'C' wave.

Pulmonary reflexes

Inflation reflex. The reflex apnoeic response to an inflation invariably disappeared with the establishment of the differential block and reappeared with recovery (Fig. 8). This result has been obtained on sixty-one occasions during twenty-seven differential blocks in thirteen rabbits. The

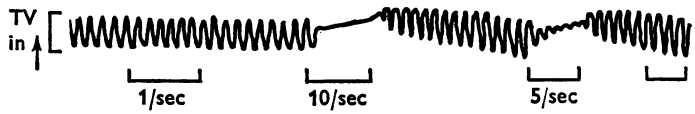

Fig. 6. The effect on breathing produced by the stimulation of the cervical vagus nerve necessary to elicit the 'C' wave. TV, tidal volume. Note the increasing effect as the frequency of stimulation is increased. Stimulation parameters 13·3 V and 500 μsec. Calibration lines: vertical = 30 ml., horizontal = 5 sec.

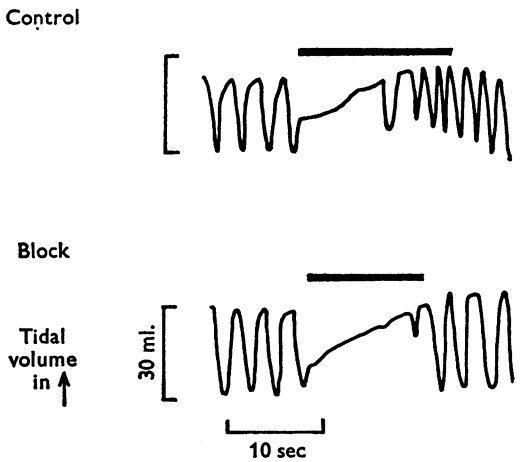

Fig. 7. Comparison of effects on ventilation of stimulation of the intact right vagus nerve before and during a differential block of conduction. Left vagus previously sectioned. The period of stimulation is indicated by the horizontal line above each record. The stimulus in both cases was 15 V and 500 μsec duration at a frequency of 10 Hz. The response is similar in both records and was abolished either by section of the nerve or increasing the strength of the d.c. block until the 'C' wave disappeared.

inflation reflex was abolished both while the d.c. was on and also after the current had been removed, but before any return of the 'A' wave.

Head's paradoxical reflex. The presence or absence of this reflex, i.e. a maintained inspiratory response to an inflation, can only be demonstrated by recording phrenic or diaphragmatic activity, or intrathoracic or transpulmonary pressures. In the two rabbits in which the diaphragmatic

electromyogram was recorded, the reflex was observed during recovery from differential blocks at a time when slowed and temporally dispersed 'A'+'B' waves were present (Fig. 9). It was never seen with complete abolition of the 'A' and 'B' waves. This stage only lasted a few minutes and was followed by the return of these waves to their control forms, when the apnoeic response to an inflation reappeared.

Deflation reflex. The tachypnoeic response to a deflation by a unilateral pneumothorax of up to 30 ml. was abolished during eleven differential

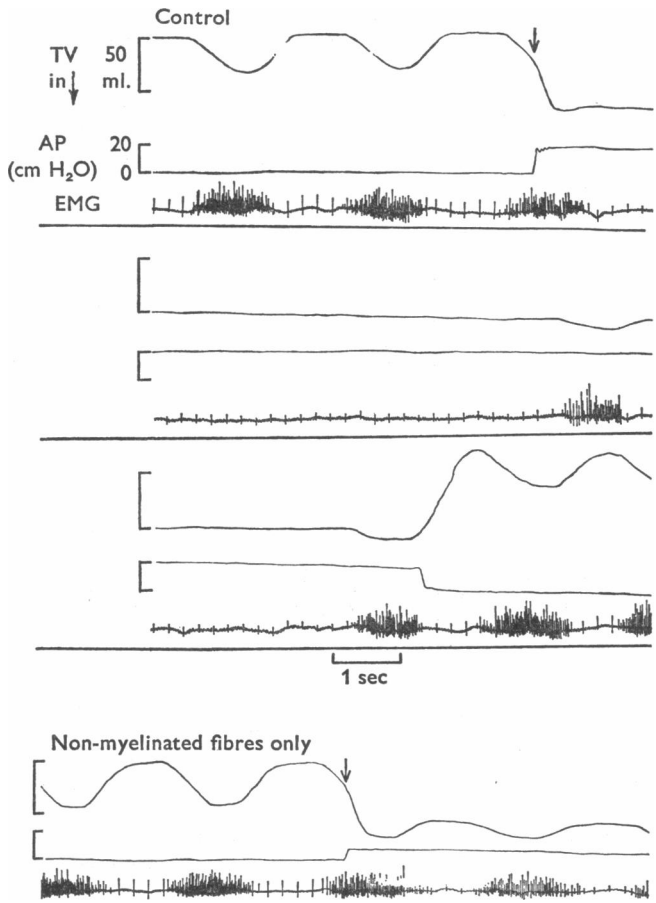

Fig. 8. Reflex responses to inflations with right vagus nerve intact, before and during a differential block of conduction. TV, tidal volume; AP, airway pressure; EMG, electromyographic recording from electrode wires inserted into the undersurface of the diaphragm. The upper three records are continuous. Note the absence of any effect on respiratory frequency in the lowest record, although there is a decrease in magnitude of the EMG which remains after the second vagus nerve has been sectioned.

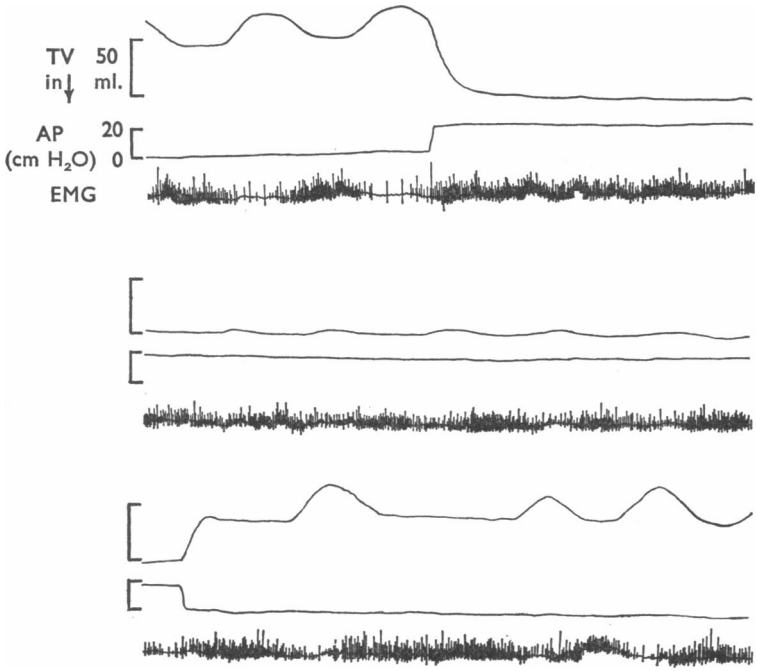

Fig. 9. Head's Paradoxical reflex response to an inflation during partial recovery from a d.c. block of conduction in myelinated fibres. The three records are continuous. TV, tidal volume; AP, airway pressure; EMG, electromyographic recording from diaphragm. Note prolonged inspiratory activity during response to the inflation compared with the usual expiratory apnoea (Fig. 8).

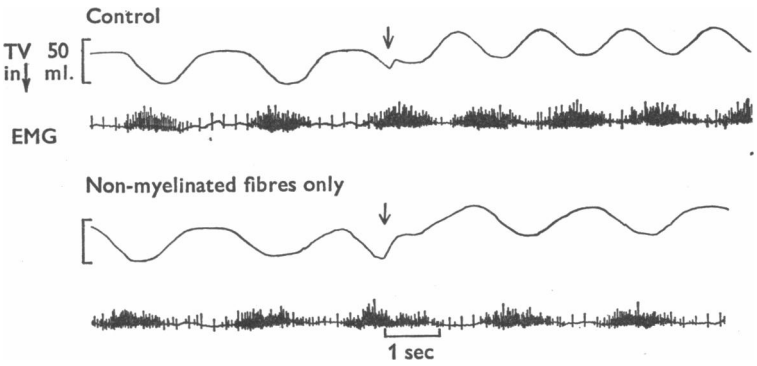

Fig. 10. Comparison of respiratory response to a pneumothorax of 30 ml. (at arrows) in a rabbit with right vagus intact and during d.c. block of conduction in myelinated fibres. The left vagus nerve was sectioned previously. TV, tidal volume; EMG, electromyographic recording from the diaphragm. Note abolition of response in lower record.

blocks in five rabbits (Fig. 10). The deflation reflex was only present when the inflation reflex was present and it was impossible to separate the reflexes at any time.

Response to intravenous phenyl diguanide. The ventilatory response to the right atrial injection of phenyl diguanide (10–50 μkg/kg) consisted of a tachypnoea, a decrease in tidal volume and usually an increase in functional residual capacity; these changes all commenced within 1–2 sec of injection. As the dose was increased the tachypnoea became more rapid and merged into a maintained inspiration. The dose chosen in any individual rabbit was that which gave a minimal, well defined response. The blood pressure fell by 20–40 mm Hg at the same time, but there was no bradycardia since atropine had been administered.

During a differential block the response to a right atrial injection of phenyl diguanide was always present, and usually stronger (Fig. 11). This has been seen on twenty-seven occasions in eleven rabbits. The response with only the non-myelinated fibres functioning was comparable to that obtained in the control with a stronger dose of the drug. This ventilatory response disappeared when conduction in the non-myelinated fibres was abolished by section. Occasionally the weak and delayed (4–6 sec) increase in tidal volume in response to phenyl diguanide, mediated by receptors at the carotid bifurcation, was seen after vagal section (Dawes, Mott & Widdicombe, 1951). The degree of fall in blood pressure in response to the drug was unchanged by differential block.

The results of the pulmonary reflex studies in the rabbits with abdominal vagi cut did not differ from the other results.

Pulmonary micro-embolism

The results are given in Table 1.

Control. In all the control rabbits with at least one intact vagus there was an increase in respiratory frequency (t test, $P < 0{\cdot}001$) and a decrease in tidal volume (t test, $P < 0{\cdot}001$). There was no significant difference whether or not atropine was administered beforehand (groups A and B: U test gave respiratory rate $P = 0{\cdot}43$, tidal volume $P = 0{\cdot}43$). There was also no difference between the control animals with both vagi intact and those with the left vagus sectioned (groups A and B compared with group D: U test gave respiratory rate $P = 0{\cdot}33$, tidal volume $P = 0{\cdot}13$). There was almost complete absence of any change in breathing when both vagus nerves had been previously sectioned (group C). In all cases with a vagus nerve functioning, over the next 30 min following the embolism tidal volume returned to or approached the control values, while the respiratory rate remained at its increased level. The results in one rabbit (R. 115) are shown in Fig. 12. The P_{a,O_2} always remained above 180 mm Hg, but

any changes with embolism, either an increase or more usually a decrease had no significance since the regulation of the amount of O_2 added to the inspired air could only be approximately controlled with the flowmeter available. There were variable small changes in P_{a,CO_2}. The control measure-

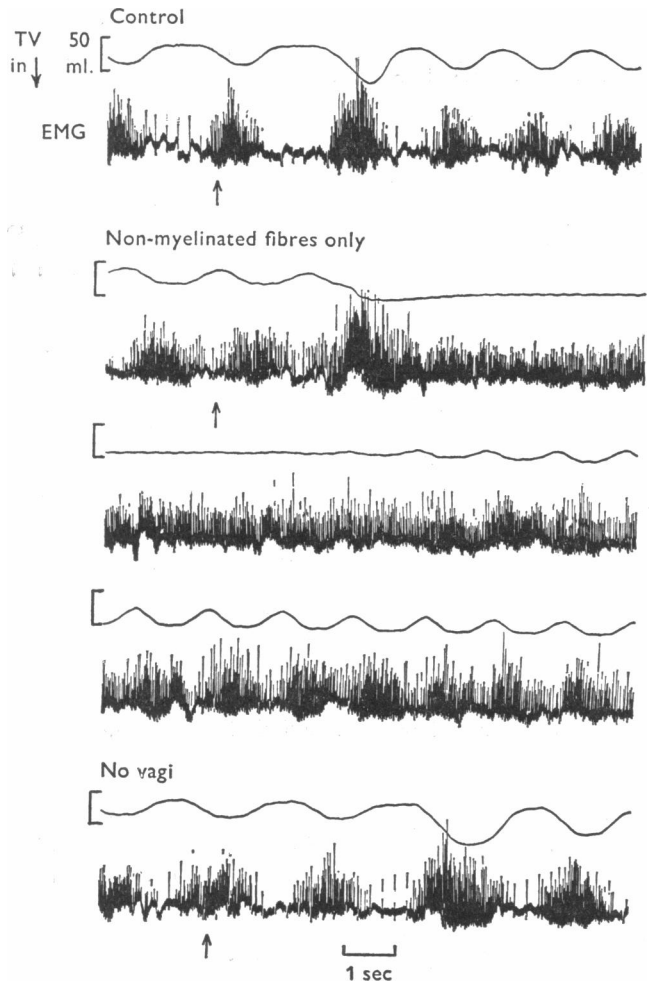

Fig. 11. Responses to right atrial injections of phenyl diguanide (at arrows) with the right vagus nerve intact (control), during a differential block (non-myelinated fibres only) and after the right vagus nerve had been sectioned (no vagi). There is a gap of 5 sec between the successive traces in the middle records. TV, tidal volume; EMG, electromyographic recording from the diaphragm. Note the enhancement of the response when only the non-myelinated fibres are conducting. With no vagal conduction the delayed response that occurs after 4 sec consists of an increase in tidal volume without increase in respiratory frequency (see text).

TABLE 1. Effect of pulmonary micro-embolism in rabbits

Rabbit no.	Weight (kg)	Left vagus	Right vagus	Atropine (1 μkg/kg)	No. of emboli × 10^6	Respiratory rate (min^{-1})		Tidal volume (ml.)		Minute ventilation (l./min)		P_{a,O_2} mm Hg (P_{I,O_2} > 400 mm Hg)	
						Control	Change (%)	Control	Change (%)	Control	Change (%)	Control	Change (%)
A 114	4·0	Intact	Intact	−	3·8	26	+35	36	−25	0·94	+4	220	−14
115	3·5	Intact	Intact	−	4·5	23	+24	45	−22	1·01	+3	260	−13
143	3·9	Intact	Intact	−	4·0	65	+14	19·5	−5	1·27	+13	410	−46
Mean	3·8				4·1	38	+24	33·5	−17	1·07	+7	296	−24
B 108	3·8	Intact	Intact	+	3·6	24	+44	35	−14	0·84	+3·5	—	—
110	4·0	Intact	Intact	+	3·4	28	+11	33	−30	0·72	−0·5	310	+6
111	3·1	Intact	Intact	+	3·2	30	+20	34	−24	0·96	−2	255	−2
117	4·0	Intact	Intact	+	3·4	25	+48	37	−24	0·98	+5	—	—
Mean	3·7				3·4	25	+31	35	−23	0·88	+1·5	282	+2
C 109	4·0	Cut	Cut	+	4·0	17	0	52	−4	0·89	−4·5	210	+24
139	3·5	Cut	Cut	+	2·0	18	+11	39	+7·5	0·70	+26	255	−29
140	3·2	Cut	Cut	+	1·6	25	+2	28	+3·5	0·70	+5·5	460	−18
141	3·7	Cut	Cut	+	3·2	11	0	101	0	1·11	0	—	—
142	3·2	Cut	Cut	+	3·2	9	0	57	+5	0·51	+6	—	—
Mean	3·5				2·8	16	+2·5	55	+2·5	0·78	+6·5	308	−8
D 118	4·3	Cut	Intact	+	3·4	30	+23	39	−15	1·17	+4·5	280	−5
119	4·2	Cut	Intact	+	3·6	27·5	+15	34	−19	0·94	−21	230	−17
138	3·9	Cut	Intact	+	2·6	24	+29	31·5	−11	0·76	+14·5	330	+6
Mean	4·1				3·2	27	+22	35	−15	0·96	−1	280	−5
E 131	3·6	Cut	D.C.	+	1·8	22	+32	48	−6	1·06	+13	380	−16
132	3·5	Cut	D.C.	+	5·0	20	+23	60	−2·5	1·21	+18	450	−20
133	5·0	Cut	D.C.	+	7·2	21	+29	62	−8	1·30	+18·5	300	+17
135		Cut	D.C.	+	5·6	24	+42	52	0	1·25	+28	415	−4
136	4·0	Cut	D.C.	+	4·6	22	+27	34	+38	0·89	+2	320	+16
137	4·0	Cut	D.C.	+	4·6	20	+30	37	−5	0·79	+2·5	390	−6
Mean	4·0				4·8	21·5	+31	49	+2·5	1·10	+14	376	−2

TABLE 1 (*cont.*)

Rabbit no.	P_{a,CO_2} (mm Hg) Control	P_{a,CO_2} (mm Hg) Change (%)	Total lung resistance (cm H_2O/l. sec) Control	Total lung resistance (cm H_2O/l. sec) Change (%)	Static compliance (ml./cm H_2O) Control	Static compliance (ml./cm H_2O) Change (%)	P, pulmonary artery pressure V, right ventricular pressure A, right atrial pressure C, central venous pressure (cm H_2O)	Control	Δ	(mm Hg) Arterial pressure (mm Hg) Control	Δ
A 114	37	+12	21	+14	10·8	−13	V	17/0	+5/12	118/91	+12/11
115	42	−7	15	0	7·8	+85	V	9·5/0	+9·5/1	92/68	−15/16
143	34·5	+7	12·8	+12	—	—	V	31/2·5	+29/4	80/79	−10/18
Mean	38	+4	16	+9	9·3	+25				96/79	−11/8
B 108	—	—	—	—	—	—	C	0·8	+3·2	88/68	−2/2
110	40·5	−1	—	—	3·5	+5·5	C	0	+3	116/86	−8/10
111	42	+2	25	−12	10·2	+16	V	20/2	+5/8	88/72	−20/20
117	41·5	−7	15·4	−7	14·0	−16·5	C	—	—	84/64	−8/8
Mean	41	−2	20	−9·5	9·0	+2				94/72	−9·5/10
C 109	37·5	+28	—	—	—	—	C	—	—	84/70	−28/26
139	41·5	+7	—	—	—	—	V	23·5/0	+19·5/0	85/73	−8/8
140	40	+5	—	—	—	—	C	0	+3	71/62	−11/20
141	44	0	—	—	—	—	V	35/0	+5/0	92/82	−12/10
142	41	+2	—	—	—	—	P	33/0	+5/7	106/89	−14/18
Mean	41	+8								88/55	−15/16
D 118	38	+1	21·5	+7	9·1	−11	C	—	—	118/93	−6/9
119	41	+2	23·5	0	4·9	−14	C	0·5	+5	132/92	−19/18
138	25·5	+8	—	—	—	—	C	0	+7	125/107	−10/11
Mean	35	+4	22	+3·5	7·0	−12				125/97	−12/13
E 131	41	—	—	—	—	—	V	19/0	+7/0	78/55	−4/5
132	39	—	—	—	—	—	V	15/2	+3/3	120/80	−3/5
133	42·5	−14	—	—	—	—	V	27/1·5	+5/1·5	160/112	−7/7
135	37	—	—	—	—	—	A	2·5	+2	113/78	−14/12
136	37	+9	—	—	—	—	A	0	+2·5	120/100	−8/8
137	42·5	—	—	—	—	—	A	0·5	+2	165/124	−23/19
Mean	40	−2·5								126/91	−10/9

ments of total lung resistance were in the range described by others in the rabbit (15·3–42·0 cm H_2O/l. sec; Karczewski & Widdicombe, 1969). The administration of atropine reduced the resistance by values ranging from 1 to 7 cm H_2O/l. sec (mean 4·0). Embolization of the lungs was associated with small, variable changes in resistance whether or not atropine was

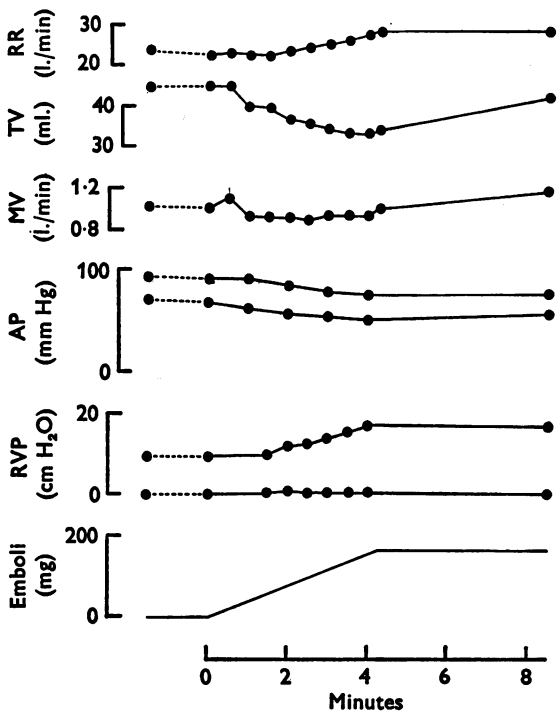

Fig. 12. Respiratory and cardiovascular responses in rabbit (R. 115) to intravenous injection of 50 μm emboli (20,000 spheres/μkg). RR, respiratory rate; TV, tidal volume; MV, minute ventilation; AP, systemic arterial pressure; RVP, right ventricular pressure. The control values represent stable measurements over a period of at least 15 min before the start of embolization.

present. The control values for static compliance were also close to the normal range of 3·5–10·8 ml./cm H_2O established by others (Karczewski & Widdicombe, 1969). The variable changes in the values of static compliance after embolization do not appear to be correlated with the magnitude of the ventilatory response. In all studies except one, the systemic arterial pressure fell. Tnis was usually mimimal since any fall seen was used as an index to stop further injection of emboli. Right-sided pressures (right atrial, right ventricular or central venous) always rose.

Differential block. The response to pulmonary micro-embolism in rabbits

with a differential block of conduction in the vagus nerves are shown in group E in Table 1, and the response in one rabbit (R. 137) is illustrated in Fig. 13. The increase in respiratory frequency with the embolism is not significantly different from the response in the control rabbits (group E

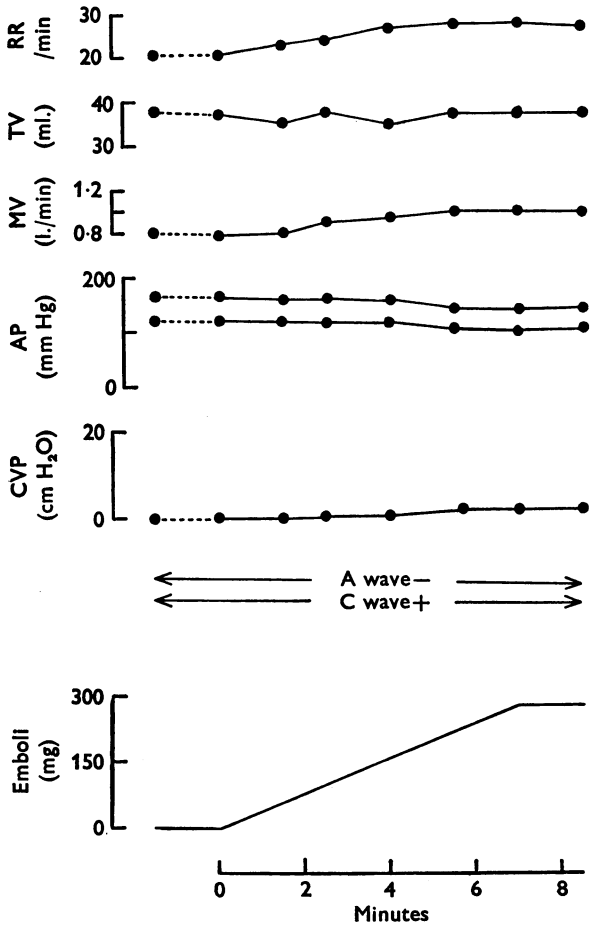

Fig. 13. Response of a rabbit (R. 137) to intravenous injection of 50 μm emboli (20,000 spheres/μkg). RR, respiratory rate; TV, tidal volume; MV, minute ventilation; AP, systemic arterial pressure; CVP, central venous pressure. The 'A' and 'C' waves of the electroneurogram were monitored throughout, the former being absent and the latter present and not slowed.

compared with groups A, B and D: U test, $P > 0{\cdot}4$). There were usually minimal changes in tidal volume but one rabbit (R. 136) showed a large (+38 %) increase. The small changes in tidal volume are similar to those occurring in the control group when both vagi had been sectioned prior to the embolism (group E compared with group C: U test, $0{\cdot}01 > P > 0{\cdot}001$).

The changes in all cardiovascular pressures were comparable to the controls. Measurements of total lung resistance and static compliance were not made since these variables had not changed systematically in response to embolism in the control groups.

Section of the differentially blocked nerve in five of these rabbits after embolism eliminated the increase in respiratory frequency (paired t test, $0{\cdot}010 > P > 0{\cdot}005$). There was no significant effect on tidal volume

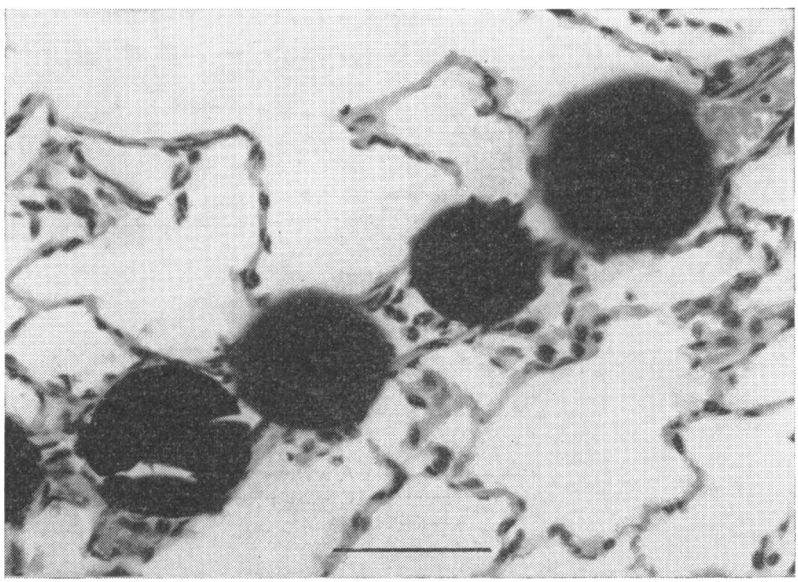

Fig. 14. Histological section of lung parenchyma of a rabbit showing a column of 50 μm microspheres in a pulmonary blood vessel. The emboli are distorting the walls of the vessels and the adjacent alveoli. Calibration line 50 μm.

(Fig. 8), but this variable had not changed in response to embolism in this group of animals.

Pathological changes with embolism. Macroscopic examination of the lungs at post-mortem showed no obvious areas of collapse or haemorrhage, but a diffuse greyness was present presumably due to the presence of the black emboli. Columns of emboli could be seen in vessels on the surface of the lung with $\times 10$ magnification. Microscopic examination of the lungs confirmed the absence of collapse, oedema, haemorrhage or infarction, and showed the 50 μm emboli scattered throughout the lung apparently within blood vessels of smaller size than their own diameter (Fig. 14). The walls of these arterioles and the adjacent alveolar walls were distorted by the presence of the emboli.

DISCUSSION

Adequacy of differential block

The use of d.c. to block differentially a mixed nerve so that only the non-myelinated fibres conduct was established by Mendell & Wall (1964). They found that a simple polarization of the sural nerve of the cat produced a block at the anode dependent on the strength of d.c. used. By grading the strength of current and monitoring the degree of block with electroneurograms they showed that conduction in small non-myelinated fibres (conduction velocity of 1 m/sec and less) could be preserved while larger fibres were blocked. They also showed that the method was reversible and repeatable.

This technique has now been applied to the cervical vagus nerve in order to produce a block of conduction in the myelinated fibres (both 'A' and 'B' groups) leaving the non-myelinated fibres functioning normally. In the present studies no detailed investigations have been made of the ability to separate the different groups of myelinated fibres by d.c., but observations have indicated that the entire 'A–B' complex was abolished simultaneously, with the same current strength.

The interpretation of these results depends on the adequacy of the differential block, that is, whether conduction in fibres of the 'A' and 'B' groups were abolished in the absence of change in conduction in fibres of the 'C' group. The electroneurograms (magnified $\times 10$ on the C.R.O.) provided the principal evidence of completeness of the block, by demonstrating the absence of 'A' and 'B' waves with any voltage, duration and frequency of stimulus used. By contrast during an incomplete block, low impulse frequencies could pass more readily than higher ones. There are serious problems of interpretation with this type of 'frequency dependent' block, and this has been discussed by Paintal (1966) in relation to the use of cooling as a blocking agent. The present results have therefore only been obtained when the block was complete, and not partial and hence 'frequency dependent'.

Although the 'A' and 'B' waves of the electroneurogram were abolished, there can be no certainty that conduction in all the fibres contributing to these waves were blocked. In a study on the reliability of using a d.c. to produce a differential block of conduction in the sural nerve of cats, Casey & Blick (1969) recorded from single fibres as well as the electroneurograms. They showed that the 'Aδ' wave was abolished before the rest of the 'A' wave as the strength of the d.c. was increased, and that some (four out of nine) 'Aδ' fibres were still conducting when the simultaneously monitored 'Aδ' wave in the compound action potential had disappeared and the rest of the 'A' wave was still present. This type of

study has not yet been repeated in the vagus nerve, but the results of Casey & Blick are of sufficient interest to suggest that caution is needed before any claim is made that all fibres have ceased to conduct when the relevant waves in the electroneurogram have been eliminated.

The abolition of the inflation reflex and the production of slow, deep breathing with our degree of differential block clearly resembles the effect of cooling the vagus nerves to 8–12° C, studied by Head (1889), Hammouda & Wilson (1935), Dawes *et al.* (1951) and Karczewski & Widdicombe (1969). In addition our results are similar to those of Douglas & Malcolm (1955) in cats; these authors correlated the effects of cooling the vagus nerve with changes in the compound action potential. One of their Figures (Fig. 12) shows that at 9·5° C the 'A–B' complex has disappeared, but the 'C' wave is still present although reduced in amplitude. It was therefore important in the present studies to establish that the non-myelinated fibres were still functioning normally during the differential block. The principal evidence was again based on the form of the electroneurogram, and studies were therefore made only if the 'C' wave was either unchanged or slowed by less than 10%. The effect on the 'C' wave of frequency of stimulation was also examined over the range 1–10 Hz and this confirmed that no 'frequency dependence' of impulse conduction was present. Additional evidence that the non-myelinated fibres were conducting during the block was supplied by the effects on ventilation of stimulating the vagus nerve to elicit the 'C' wave. The similarity in the response with and without functioning myelinated fibres and its disappearance when the 'C' wave was blocked indicated that the response was being mediated by non-myelinated fibres.

The initial ventilatory disturbances produced by application of the d.c. are similar whether the vagus nerve is intact or cut peripherally. This suggests that as the current is increased a stimulating effect occurs at the cathode, presumably on pulmonary stretch fibres. This effect is not blocked at the anode until the membrane is adequately hyperpolarized. The fact that removal of the d.c. causes no immediate change in the ventilatory pattern supports the view that this stimulating effect is only present initially. All our studies avoided this period.

Ventilatory response to phenyl diguanide

Paintal (1957) using single fibre recordings of non-myelinated fibres from pulmonary receptors showed that there was a large discharge in response to right atrial injections of phenyl diguanide. The present studies are in keeping with this and provide proof that the respiratory response to phenyl diguanide is mediated by non-myelinated vagal afferent fibres. A possible explanation of the increase in strength of the response when the myelinated

fibres were blocked is that the control response was being modified by pulmonary stretch receptor activity. It is suggested that with the first inspiration resulting from the phenyl diguanide response a pulmonary stretch receptor discharge ensues, consequent upon increase in lung size and this inhibits inspiratory drive. Lung size then decreases, stretch receptor discharge falls off, permitting the phenyl diguanide-activated fibres to produce an inspiratory drive again. This oscillation results in tachypnoea. In the absence of stretch receptor discharge (differential block) the inspiratory-inhibiting effect is lost, and a maintained inspiration results. The possibility that stretch receptor discharge may modify the ventilatory response to phenyl diguanide was suggested by Dawes *et al.* (1951).

Pulmonary micro-embolism

The results obtained with a standardized form of pulmonary micro-embolism (50 μm inert spheres) clearly show that the resultant tachypnoeic response was mediated by non-myelinated vagal afferent fibres. The absence of typical secondary pathological changes, e.g. haemorrhage, oedema, infarction or collapse, such as is usually found with starch embolism (Trenchard, 1970), as well as the absence of any consistent mechanical changes in the lungs ensured that only the response to a specific stimulus was studied. It is possible that the haemodynamic changes acted as the stimulus, but Whitteridge (1950) and Knisely, Wallace, Mahaley & Satterwhite (1957) have presented evidence that this is not so in view of the fact that the nature of the respiratory response depends on the size of the emboli even though there are comparable haemodynamic changes. It seems more likely that the sites of stimulation were the areas of the stretched arterioles and distorted inter-alveolar walls. Paintal (1969, 1970) has recently produced physiological evidence that the receptive endings ('J' receptors) of the non-myelinated pulmonary vagal afferent fibres lie in the interstitial spaces between capillaries and alveoli, where they would be ideally situated for stimulation by these emboli.

Mills, Sellick & Widdicombe (1969, 1970) have demonstrated a discharge from lung irritant receptors in response to pulmonary embolism with starch and barium sulphate, and postulated that the tachypnoeic response is at least part due to this discharge. These authors did not report on whether any secondary pathology existed in the lung. Stimulation of irritant receptors is thought to be associated with an increase in bronchomotor tone (Karczewski & Widdicombe, 1969). It is therefore of interest that the tachypnoea in our studies with embolism was not accompanied by any consistent increase in airway resistance, and furthermore was not diminished in the presence of atropine. The wide range of conduction velocities of these irritant fibres (3·6–25·8 m/sec) suggests that most, if

not all, would be blocked by our technique, while the similarity of the tachypnoeic response with the differential block also suggests that these irritant fibres were not primarily involved in our studies.

The over-all ventilatory response to micro-embolism during differential block differed from the control response in that tidal volume did not decrease and this presents a problem in interpretation. It may be postulated that the reduction in tidal volume in the controls was due to an alteration in the pattern of discharge from pulmonary stretch receptors consequent upon the increase in respiratory frequency and inspiratory flow rates (Davis, Fowler & Lambert, 1956). In the absence of functioning pulmonary stretch receptors this would not occur.

Activity in non-myelinated fibres and breathing pattern

It has been known for many years (Hering, 1868; Breuer, 1868) that section of the vagus nerves results in slow, deep breathing. It has since been assumed that this effect results from the abolition of the pulmonary stretch receptor discharge described by Adrian (1933). In studies on vagal block by cooling in the rabbit, doubt was thrown on this view since the slowing and deepening of breathing associated with block of stretch receptor activity was often followed by a further slowing and deepening of breathing when the temperature of the block was lowered (Karczewski & Widdicombe, 1969). These authors concluded that lowering the temperature had blocked fibres of smaller diameter than those conducting the stretch receptor discharge, and that the activity in these smaller fibres was also modulating the pattern of breathing. Our results with section of the 'non-myelinated' nerve show that the influence of activity in 'C' fibres, probably of pulmonary origin, can be as large as the influence of the pulmonary stretch receptors, but the effect is mainly apparent in the presence of pathological conditions of the lung such as patchy collapse and micro-embolism. This evidence supports the view that the non-myelinated fibres originating from 'J' receptors in the lung may possibly constitute a nociceptive system.

Deflation reflex

Breuer (1868) and Head (1889) demonstrated that the tachypnoeic response to a lung deflation depended on the presence of intact vagus nerves. Hammouda & Wilson (1935) with the cat and Troelstra (1960) with the rabbit showed that the inflation reflex could be abolished by cooling the vagus nerves to approximately 5–8° C while the deflation reflex was still present. They proposed separate sets of receptors and fibres for the inflation and deflation reflexes, with the fibres mediating the latter reflex being of much smaller diameter. However, it is possible that the separation of the inflation and deflation reflexes by cooling results from the frequency

dependent nature of this block, and the fact that higher frequencies of discharge are probably involved in the inflation reflex. Paintal (1953) recorded a transient discharge in a few of the non-myelinated vagal afferent fibres from pulmonary receptors which responded to a passive deflation of the lungs; these receptors were called 'deflation' receptors and it became generally accepted that this group of fibres were mediating the deflation reflex although Paintal himself never claimed this. More recently these receptors have been renamed 'J' receptors as mentioned above (Paintal, 1969). Our results supply conclusive direct evidence that the deflation reflex is not mediated by non-myelinated fibres. The inability to separate the deflation and inflation reflexes by our differential block does not imply that these reflexes are mediated by the same group of myelinated fibres since the entire 'A–B' complex in the electroneurogram is abolished simultaneously. These results are compatible with the evidence of Sellick & Widdicombe (1969) that the tachypnoeic response to lung deflation may be mediated by a discharge in fibres from irritant receptors.

Head's Paradoxical Reflex

The results demonstrate that Head's Paradoxical Reflex occurs during recovery of the 'A–B' complex from a block produced by a technique other than cooling. At this time there is certainly a 'frequency dependence' of impulse conduction but the experiments do not clarify whether it is this situation that results in this reflex (Paintal, 1966), or whether other groups of myelinated fibres are involved, such as those from irritant receptors (Sellick & Widdicombe, 1970).

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