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TYPES OF AFFERENT NERVOUS ACTIVITY WHICH MAY BE MEASURED IN THE VAGUS NERVE OF THE SHEEP FOETUS

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SUMMARY

1. Afferent activity was measured in the vagus nerves in sixteen foetuses within 2–3 days of natural term and in two foetuses 130 and 135 days gestational age, exteriorized by hysterotomy from seventeen ewes given either a spinal anaesthetic (ten) or pentobarbitone sodium (seven).

2. Activity from aortic chemoreceptors was observed which increased in rate with foetal hypoxia, NaCN or nicotine and temporary occlusion of the umbilical cord or veins. Activity was abolished or reduced with saline equilibrated with air.

3. Activity from baroreceptors was observed which was synchronous with the arterial pulse and which followed changes in arterial pressure.

4. Activity designated as arising from stretch receptors in the lungs or lower airways was observed which consisted of a regular discharge at 15–45 impulses sec^{-1}, which varied with intra-tracheal pressure, which increased with negative intrathoracic pressure generated by spontaneous respiratory movements, which, when the umbilical cord was occluded, at first increased in rate and then fell to zero at or about the occasion of the first breath. After breathing had been established, activity assumed the rhythmical pattern and showed evidence of slow adaptation of receptors seen in the adult. This activity was frequently modulated by the heart beat and after breathing had started it was reduced when carbon dioxide was raised.

5. Respiratory movements with a frequency of 10–20/min occurred spontaneously in foetuses from ewes given a spinal anaesthetic and were associated with a modulation of the stretch receptor discharge. They could be induced by withdrawing fluid from the trachea and so lowering both intra-tracheal pressure and receptor discharge and were abolished by raising intra-tracheal pressure. These changes were abolished by sectioning the vagi just caudal to the nodose ganglion.

6. These results indicate that there are excitatory and inhibitory pathways in the vagus nerves which are active in the foetus and which may well be concerned with the limitation of respiratory movements of the foetus and the onset of maintained respiration at birth.

INTRODUCTION

It is known that when the umbilical cord is occluded and breathing starts in the foetus, there is a large increase in both cerebral blood flow and oxygen uptake and that this increase is abolished if the vagi have previously been cut (Purves & James, 1969). This observation suggests that there must be a pathway in the vagus which is excitatory to the central nervous system and of whose activation the rise in oxygen uptake and blood flow are indices. Alternatively, the vagal activity could be inhibitory and itself be inhibited at the moment of birth.

In this paper, we give the results of a study of afferent activity in the vagus nerves of foetal sheep and of additional experiments which indicate that this activity can affect respiratory movements in the mature foetus. Some of the results have been communicated to the Physiological Society (Ponte & Purves, 1972).

METHODS

Eighteen foetuses from seventeen Clun-Hampshire ewes were studied. They were all within 2 or 3 days of natural term (147–150 days) except two whose gestational age was 130 and 135 days. Ten of the ewes were anaesthetized with a spinal anaesthetic (10 ml. 2% lignocaine, Astra Chemicals Ltd) given through a needle and supplemented at approximately half hourly intervals with lignocaine given through an indwelling subarachnoid catheter. Seven ewes were given pentobarbitone sodium (25 mg/kg) I.V. and this was supplemented with pentobarbitone 4 mg/kg given hourly through a brachial vein catheter. The foetuses were exteriorized and lay on the table beside the mother. In the first five experiments, the sheath of the umbilical cord was removed by dissection for a length of 8 cm and the umbilical vessels painted with formaldehyde (40%, w/v) so that the umbilical veins could be separated from the arteries and compressed individually without fear of spasm. In these and in the other experiments, the umbilical cord was continuously irrigated with warm saline which effectively prevented the spasm of vessels which occurs with time and with repeated occlusion of the cord between finger and thumb.

Immediately each foetus had been exteriorized, it was wrapped in cotton wool and radiant heat was used to prevent shivering and to maintain the rectal temperature at 40 ± 0·5° C. The snout was enclosed with a condom holding 75–100 ml. saline which ensured that no air was inhaled with respiratory movements but which allowed free passage of fluid from upper airways and trachea. In each foetus, the trachea was opened and a T-piece inserted (Fig. 1). The sidearm of the T-piece connected with a length of silicone rubber tubing and was closed with a tap (1) which was opened only after the umbilical cord had been clamped to allow either spontaneous or assisted breathing. From the sidearms of the two interposed T-pieces in this segment of tubing, tracheal fluid could be withdrawn through tap (2) into a syringe, or saline, warmed to 40° C, injected, and from the second sidearm the pressure in this segment was continuously measured with a Statham 23 AC transducer, the reference point being taken as the level of the foetal atrium. In eight foetuses, a silicone rubber catheter, saline filled and open ended, was inserted into the oesophagus, the tip being placed in the lower third of the thoracic oesophagus. Intra-oesophageal pressure was measured with a Statham PM131TC transducer and the difference between intra-tracheal and intra-oesophageal pressures used as an index of transpulmonary pressure.

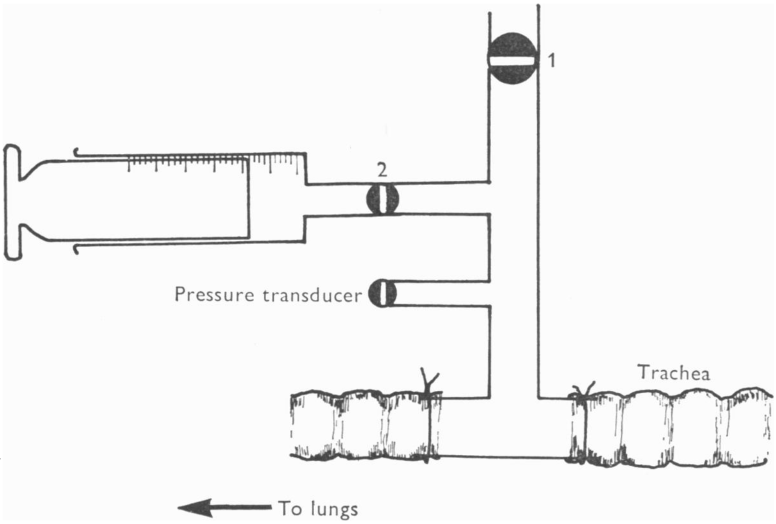

Fig. 1. The arrangement in the foetal and new-born lamb for measuring intra-tracheal pressure and for injecting air or saline into the trachea or withdrawing fluid. Pressure was measured via the sidearm and tap as indicated. Tap 1 was closed while fluid or air was injected or withdrawn through tap 2, and during these manoeuvres, the trachea was clamped on the laryngeal side of the T-piece. After birth, the lungs were ventilated with a pump through tap 1.

A catheter was inserted retrogradely into the femoral artery for the measurement of pressure in the lower aorta and for the taking of blood samples. Arterial blood gas tensions and pH were measured immediately from 0·25 ml. blood samples using appropriate Radiometer electrodes at 39° C. A second catheter was inserted into the femoral vein and advanced until the pressure record showed the tip to lie in the right ventricle. A third catheter, used for the injection of drugs, was inserted via the left brachial artery until the tip was in the left ventricle and then was withdrawn so that the tip lay at the root of the aorta. The pressure trace obtained from this catheter indicated that systolic pressure was 10–15 mm Hg lower than that seen in the femoral venous catheter which provided further evidence that the tip of the latter was in fact in the right ventricle. The position of the tips of each catheter was always checked post-mortem. The frequency response of the catheter–strain-gauge–recorder system was flat to between 15 and 25 Hz, the lower response being obtained from the relatively long (35 cm) catheters. The outputs of the pressure transducers and other physiological variables were displayed on a Grass polygraph recorder.

After the initial dissection, recordings were made from single or few fibre preparations of the vagus nerves as described below. A series of tests was then carried out to identify the receptors whose afferent discharge was being measured. The response of aortic chemoreceptors was elicited to changes in the arterial oxygen and carbon dioxide tensions, the local injection into the root of the aorta of warm saline equilibrated with air and of between 5 and 25 μg nicotine or NaCN in 0·5 ml. saline. The response of pulmonary stretch receptors was obtained to changes in intra-tracheal pressure, to the administration of 5 % carbon dioxide in air to the ewe and to the injection of between 10 and 20 μg nicotine or 20–35 μg veratridine in 0·5 ml. saline into the right ventricle. The responses of both types of receptors were obtained to temporary occlusion ($<$ 1 min) of the umbilical cord. When these tests had been completed in the foetus, the umbilical cord was tied and respiration started spontaneously or was assisted mechanically. In some lambs, the relation between neural discharge and intra-tracheal pressure was determined after the lamb had been paralysed with gallamine triethiodide (10 mg/kg) and was being artificially ventilated. The pump volume was set so that end tidal carbon dioxide, monitored with a Beckman LB1 infra red gas analyser, was approximately 5·5 %. At intervals, the pump was stopped, tap (1) (Fig. 1) was closed and various increments of air injected into the airway so that the desired range of intra-tracheal pressure was obtained. After each volume of air had been injected and intra-tracheal pressure and neural discharge had become steady, the pump was restarted, tap (1) opened and after a further 30–40 sec, a further measurement was made.

Nerve recording. The left vagus was usually dissected. It was freed from the carotid sheath for a distance of about 2 cm in the mid-cervical region and also separated from the sympathetic nerve which lay medial to it. The nerve was found to consist of between twenty-five and forty bundles separated from each other by highly vascular connective tissue. There was considerable passage of fibres from one bundle to another so that it was possible to divide one of these bundles and peel it away from the rest of the nerve for a distance of not more than 7–8 mm. Great care was taken to preserve as far as possible an intact vascular supply to the nerve so that there should be normal conduction in the rest of the nerve.

One or more of these bundles was dissected in a pool of liquid paraffin until a single- or few-fibre preparation had been obtained. Activity from these fibres was recorded unipolarly and conventionally amplified and monitored and then either photographed on to moving film or stored on tape. The action potentials were also used to trigger standard pulses from a pulse generator whose output could be matched with the original potentials and which was counted with a rate-meter (J. & P. Engineering, Reading) and displayed on one channel of the polygraph recorder.

RESULTS

Effects of anaesthesia. The type of anaesthesia given to the mother did not appear to affect the ease or otherwise of finding fibres in the vagus with particular patterns of activity nor did the anaesthetic affect the responses of the receptors to the various stimuli used. The type of anaesthetic did affect the time to onset and types of respiratory activity observed. Thus, in the foetuses from ewes given pentobarbitone, there was no evidence of spontaneous respiratory activity and it was virtually impossible to induce such movements by the withdrawal of tracheal fluid and reduction of intra-tracheal pressure, a response which was easily elicited as described in a later section in foetuses from ewes given a spinal anaesthetic. Secondly, it was observed that when the umbilical cord was temporarily occluded, the time to onset of regular respiration was delayed in the foetuses from ewes given pentobarbitone compared to the other group. In the former, the delay was in the range 25–55 sec, in the latter 10–25 sec. Further, in four of the foetuses from ewes given pentobarbitone breathing was irregular and poorly maintained and their ventilation had to be assisted mechanically.

Types of afferent activity observed in the foetal vagus

Aortic chemoreceptor. Activity designated as arising from aortic chemoreceptors was found in only five out of eighteen foetuses. There was no evidence of a separate aortic nerve nor of localization of these fibres in a specific bundle in the vagus. Satisfactory single-fibre preparations were obtained in two foetuses but both were lost with the writhing movements associated with occlusion of the umbilical cord: the other three were few-fibre preparations.

With femoral arterial blood values in the range P_{a,O_2} 22–25 mm Hg, P_{a,CO_2} 39–45 mm Hg and pH 7·38–7·41 units, the rate of activity in the single fibres or from identifiable single units in the few-fibre preparations varied between 0·9 and 4·5 impulses/sec. The discharge was quite irregular and had no relation to the heart beat. When the mother was paralysed with gallamine and ventilated with 5% oxygen in nitrogen instead of air, P_{A,CO_2} being maintained constant in the range 35–40 mm Hg, foetal P_{a,O_2} fell to the range 13–17 mm Hg (ten tests). Identifiable discharge in single- or few-fibre preparations increased in rate by between 37 and 45% of control and there was also evidence of recruitment of activity from other receptors (Fig. 2). Activity from these receptors at least doubled when between 5 and 25 μg NaCN or nicotine in 0·5 ml. saline was injected into the root of the aorta through the left brachial artery catheter or, as is illustrated in Fig. 3, through the femoral venous catheter whose tip lay in the right ventricle. The delay between injection via the brachial catheter and the rise in discharge was 1–2 sec; between injection into the right ventricle and rise in discharge, 5–6 sec.

The effect of temporary occlusion of the umbilical veins or of the whole cord for up to 1 min was tested on nine occasions and a typical response is illustrated in Fig. 4. After a delay of between 4 and 7 sec, the discharge increased to approximately double the resting rate and there was a similar delay between release of the cord and the return of the discharge toward resting levels. Activity in these fibres was invariably (ten tests) abolished or reduced if 0·5–1·0 ml. 0·9% saline equilibrated with air

(P_{O_2} 150 mm Hg) and warmed to 40° C was injected at the root of the aorta.

Aortic baroreceptors. Activity was observed in six fibres in six foetuses which most probably was derived from the aortic group of baroreceptors. As with activity designated as coming from aortic chemoreceptors, there was no evidence of localization within the vagus nerve and fibres were found purely by chance. The activity was in all respects similar to that described by Biscoe, Purves & Sampson (1969) in the sinus nerve of the foetal sheep: that is, it was synchronous with the aortic pulse wave, the inter-spike intervals varying so that the shortest coincided with the upstroke of the pressure wave, and the rate of discharge increased and there was evidence of recruitment when aortic pressure was raised either

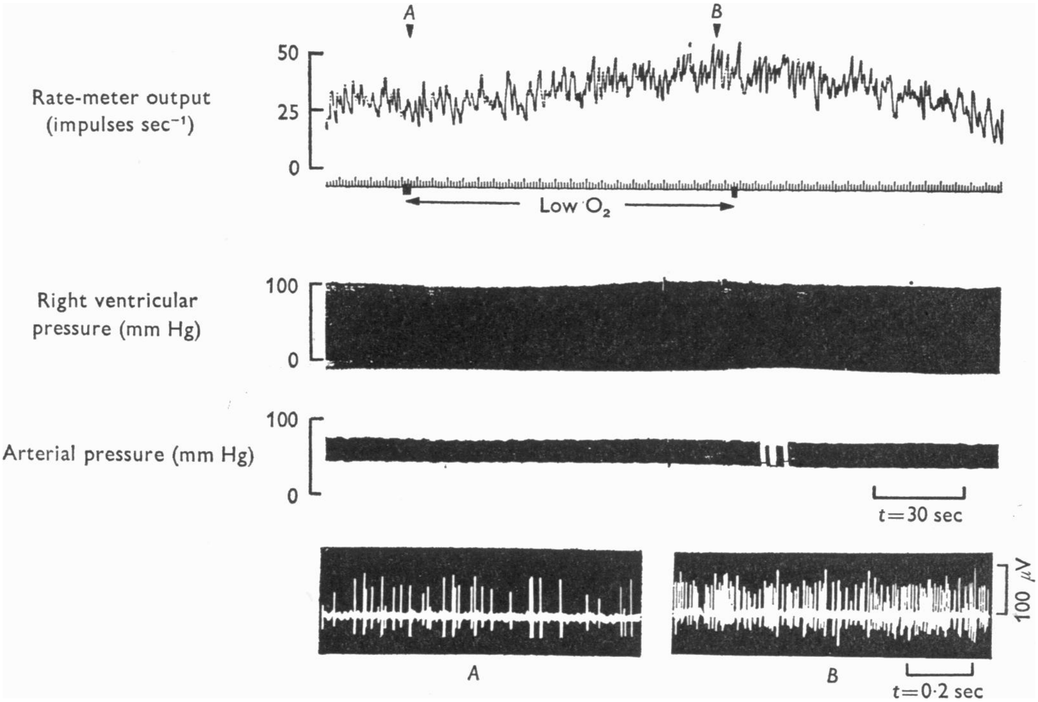

Fig. 2. The changes which occurred in a few-fibre preparation of the right vagus in a foetus 147 days gestational age when the ewe was given 5% oxygen in nitrogen to inhale for the period shown by the mark. Traces from above down: rate-meter output, right ventricular pressure and arterial pressure. The neurograms *A* and *B* were recorded at the points indicated on the rate-meter record. P_{a,O_2} in the control period was 23 mm Hg and in a sample of blood taken at the break in the arterial record was 16 mm Hg.

by clamping the foetal abdominal aorta or when 5 μg noradrenaline in 0·5 ml. saline was injected I.V. This type of activity also followed the changes in pressure associated with occlusion of the umbilical cord. The irregularity of the inter-spike interval within each pulse cycle served to distinguish it from the pulse-modulated discharge from pulmonary stretch receptors (Fig. 9, neurogram *D*).

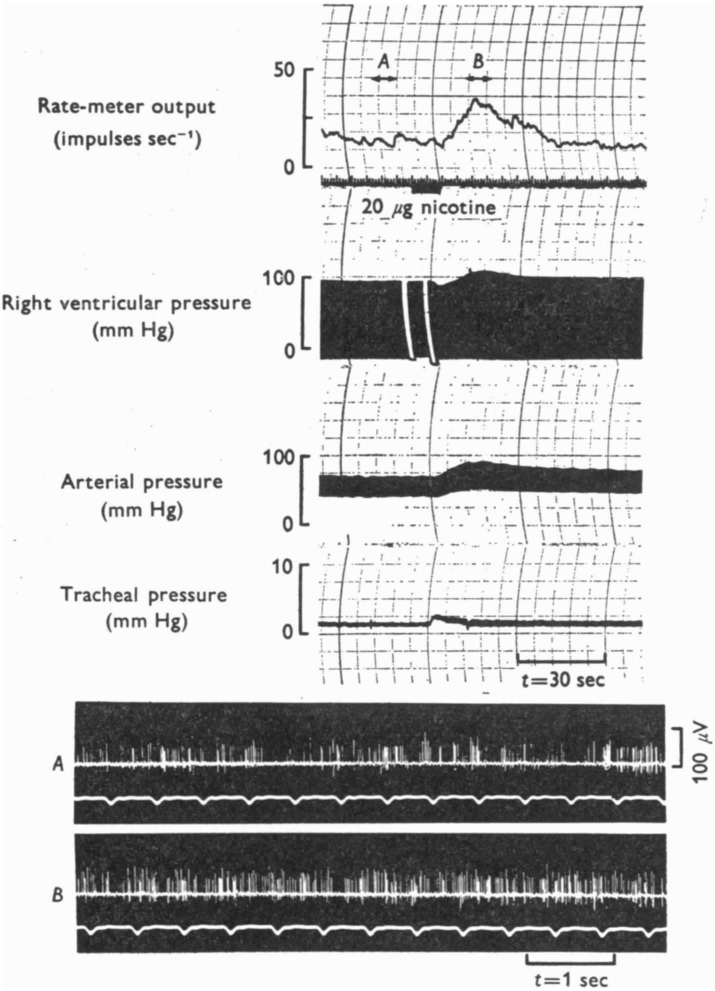

Fig. 3. The effect of injecting 20 μg nicotine into the right ventricle, as shown by the bar on the time marker, upon aortic chemoreceptor activity in a few-fibre preparation in the right vagus in a sheep foetus 147 days gestational age. Traces from above down: rate-meter output and time marker (t = 30 sec), right ventricular, arterial and tracheal pressure. The neurograms *A* and *B* were recorded as indicated on the rate-meter record.

Pulmonary stretch receptors. By far the commonest type of activity was that designated as arising from pulmonary or lower airway stretch receptors. Such activity was seen on the oscilloscope or heard on the audio-monitor in virtually every multi-fibre preparation from the vagus laid on the electrodes: satisfactory single-fibre preparations were obtained on thirty-seven occasions in eighteen foetuses. There was no difference in the pattern of discharge or of the response to various stimuli between the

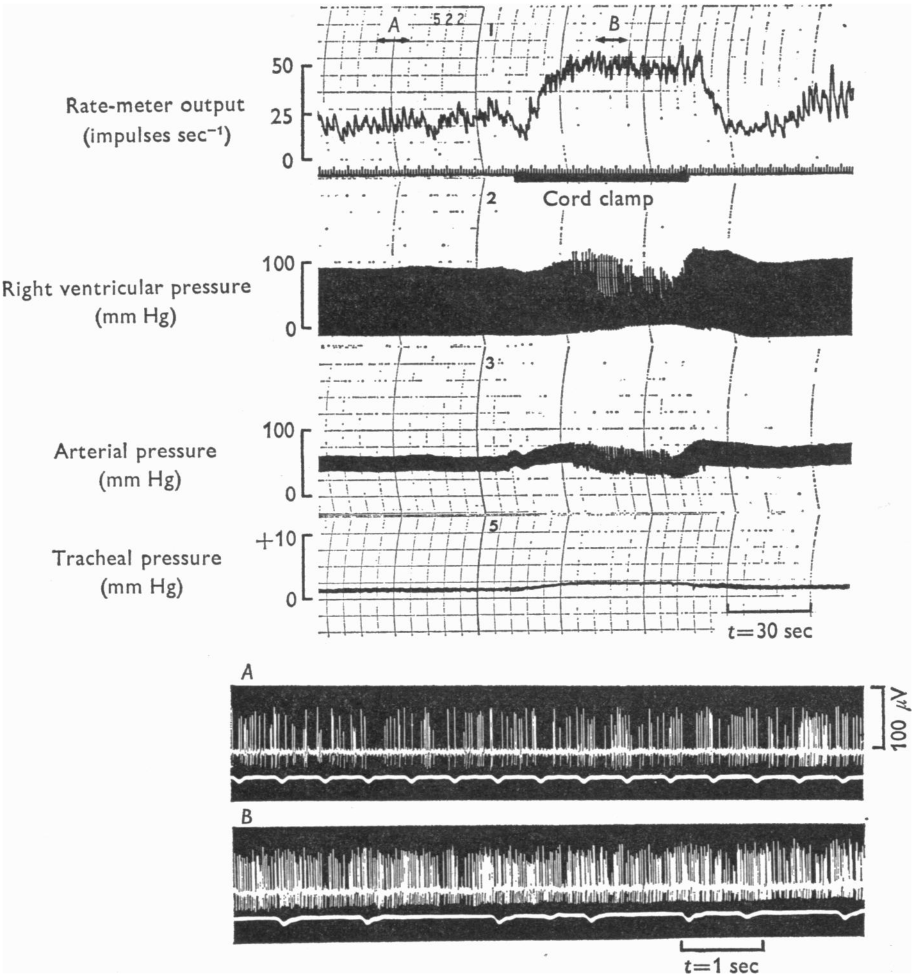

Fig. 4. The effect of occluding the whole umbilical cord for the period indicated by the bar on the time marker upon aortic chemoreceptor activity, same foetal preparation as in Fig. 3. Traces from above down: rate-meter output, right ventricular, arterial and tracheal pressures. Neurograms *A* and *B* recorded as indicated on the rate-meter trace.

two younger and the mature foetuses. Under resting conditions in the exteriorized foetuses, intra-tracheal pressure was found to be in the range -1 to $+3$ mm Hg with respect to atmosphere at the mid-thoracic level in the foetus. Over periods of 1–2 hr, this pressure varied spontaneously; in some foetuses, pressure gradually increased by up to 3 mm Hg and then, associated with a spontaneous foetal movement, intra-tracheal pressure fell to control levels ± 1 mm Hg. This cycle of a gradual rise in pressure and return to normal levels was repeated with an irregular period of between 5 and 10 min. In other foetuses, intra-tracheal pressure slowly fell over the period of observation without foetal movements or readjustment of pressure. In one of the younger foetuses and in nine out of the sixteen mature foetuses, there were regular spontaneous movements of the thorax which produced changes in intra-tracheal pressure similar to that shown in Figs. 7 and 11. These changes were clearly to be distinguished from artifacts in the intra-tracheal or intra-oesophageal pressure records due to the heart beat or which were synchronous with fasciculation of intercostal or scalene muscles giving rise to a noisy signal, i.e. with a frequency of up to 5 Hz and with an amplitude equivalent to ± 4 mm Hg. This latter type of pressure signal appeared and disappeared spontaneously and could last for up to 15 min at a time and in these respects was similar to the type of record described by Merlet, Hoerter, Devilleneuve & Tchobroutsky (1970) and by Dawes, Fox, Leduc, Liggins & Richards (1970, 1972) and interpreted by them as being respiratory in origin.

Under resting conditions with respect to intra-tracheal pressure, activity designated as arising from pulmonary stretch receptors consisted of a very regular discharge of between 15 and 45 impulses sec^{-1}. The regularity of the discharge is illustrated by interval histograms from three fibres over 3 min runs (Fig. 5). This confirms that the bulk of intervals fell within a range of the mean ± 5 msec. The conduction velocity was measured in three fibres and found to be 38, 43 and 46 msec^{-1} respectively. Because of the size of the whole nerve and possibly because of the highly vascular tissue connecting the individual bundles of fibres, it proved difficult at first to cool the nerves sufficiently, as indicated by a locally placed thermocouple, to affect conduction. However, using the method devised by Franz & Iggo (1968) of placing agar round the nerve on the thermode, local temperature fell to 8° C and at this level, conduction in these fibres was effectively blocked.

Discharge in this type of fibre was affected by altering intra-tracheal pressure by withdrawing fluid or injecting saline warmed to 40° C. Because of the free flow of fluid between trachea and condom, in these studies the trachea was clamped above the T-piece. The relation between the level of discharge and intra-tracheal pressure is shown for three fibres from two foetuses in Fig. 6. Over the range tested, the relation was linear and it was clear that there was still a substantial level of discharge at sub-atmospheric pressures. The range of the slopes of this relation, obtained in seven tests, varied between 4 and 10 impulses $\sec^{-1}$ mm Hg^{-1} intra-tracheal pressure.

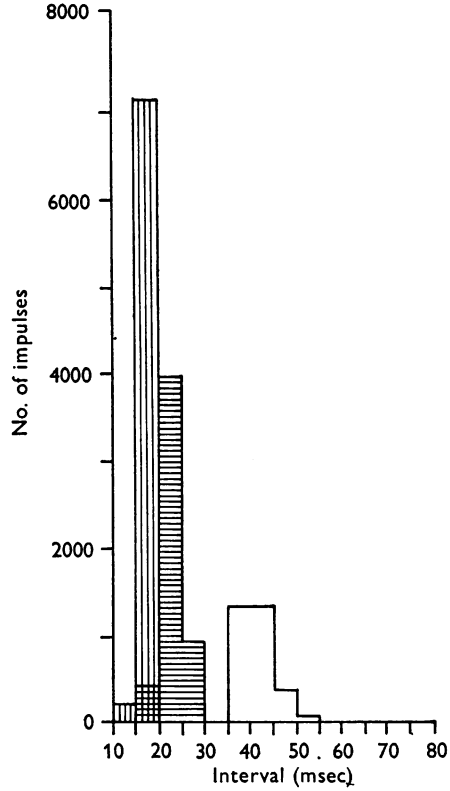

Fig. 5. Histograms of the distribution of interspike intervals taken from three fibres from pulmonary stretch receptors in the vagus of a sheep foetus of 147 days gestational age. Action potentials were accumulated over 3 min periods.

It was not possible to measure the rate of adaptation of these receptors in the fluid filled lungs to changes in intra-tracheal pressure because immediately following withdrawal or reinjection of fluid, there was a change in pressure and subsequent adjustment over 15–20 sec. In Fig. 6, the responses of two of these fibres to changes in intra-tracheal pressure after birth are shown. The rate of adaptation, an index of which is described by $(R_1 - R_2)\ 100/R_1$ where R_1 and R_2 are the rates of discharge at the end of 1 and 2 sec respectively following a step change of intra-tracheal pressure, was determined in nine fibres. In all tests, the rate of adaptation was less than 30 % indicating that the receptors involved were slowly adapting (Davis, Fowler & Lambert, 1956). The relationships between discharge and intra-tracheal pressure after birth were tested in five fibres in addition to the two shown in Fig. 6. The slope of this relation after birth was between

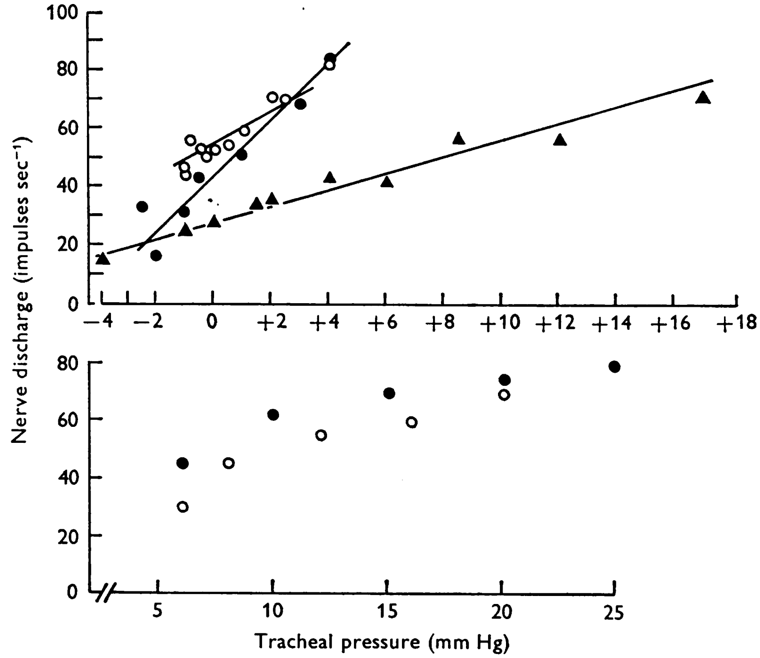

Fig. 6. The relation between the discharge from single fibre afferents from pulmonary stretch receptors (impulses sec^{-1}) and above, in the foetus, changes in intra-tracheal pressure (mm Hg) brought about by injection or withdrawal of fluid, and below, in the lamb shortly after birth, by the injection into the trachea of various volumes of air. The foetal records above are from three separate fibres in three foetuses (open and filled circles, filled triangles), those below are from two fibres in two lambs artificially ventilated. All points are taken from steady rates of discharge 10–15 sec after the step change in pressure was made.

0·35 and 0·55 impulses sec^{-1} mm Hg^{-1} intra-tracheal pressure and, as is shown in Fig. 6, it was clear that in the range of intra-tracheal pressure 0–10 mm Hg, the curve ceased to be linear. However, in those fibres where discharge was measured over comparable ranges of intra-tracheal pressure before and after birth, it was equally clear that the slope of the relation between discharge and intra-tracheal pressure was substantially reduced after birth.

Spontaneous respiratory movements were seen in ten foetuses, all from mothers given spinal anaesthetic. Such movements could also be readily induced by withdrawing fluid from the trachea and lowering the pressure as is shown in Fig. 7. These movements were regular, with an interval of between 2 and 5 sec and were associated with the generation of sub-atmospheric pressures in oesophagus or trachea of up to 20 mm Hg. As is shown from the accompanying rate-meter record, these movements were associated with modulation of the discharge pattern from the stretch receptors and under these circumstances, the distribution of inter-spike intervals was bi-modal. It is noteworthy that in this as in other records, despite comparatively large fluctuations in intra-tracheal pressure, the amplitude of modulation of discharge was small and, in contrast to the comparable situation in the air filled lung (Fig. 11), discharge remained high at end-expiration.

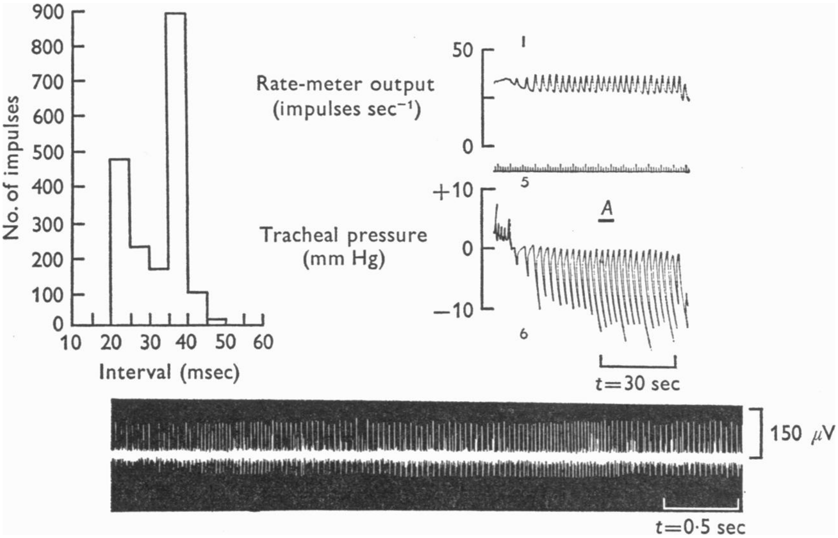

Fig. 7. The effect of reducing intra-tracheal pressure in a foetus 147 days gestational age. On the right, above, the rate-meter output from a single afferent fibre from a pulmonary stretch receptor and below, the corresponding changes in intra-tracheal pressure. The neurogram recorded at the mark *A* covers approximately 1·5 respiratory cycles. During the control period, the intra-tracheal pressure had been + 2 mm Hg and at the left of the tracheal pressure record are small artifactual movements associated with preparing to remove fluid from the trachea. Shortly after pressure was reduced to zero, respiratory movements started.

On the left, an interval histogram obtained from activity in the same fibre measured over a 1·5 min period. The modulation of the discharge to give interval peaks at 20–25 and 35–40 msec is to be compared with the histograms obtained under steady condition (Fig. 5).

The injection of nicotine (10–20 μg) or veratridine (20–35 μg) into the right ventricle invariably caused an increase in stretch receptor activity and an example of the response is shown in Fig. 8. Activity in nine such tests increased by between 25 and 45 % of control when the drugs were given in these quantities. If these drugs were given into the root of the aorta, they were ineffective. The receptors were unaffected by NaCN 10–20 μg given into the right ventricle, by the reduction of foetal P_{a, O_2} to the range 14–17 mm Hg (three tests) or by raising foetal P_{a, CO_2} to 48–51 mm Hg (four tests) despite the fact that with each of these manoeuvres there was a rise of both right ventricular and aortic pressure of up to 15 mm Hg.

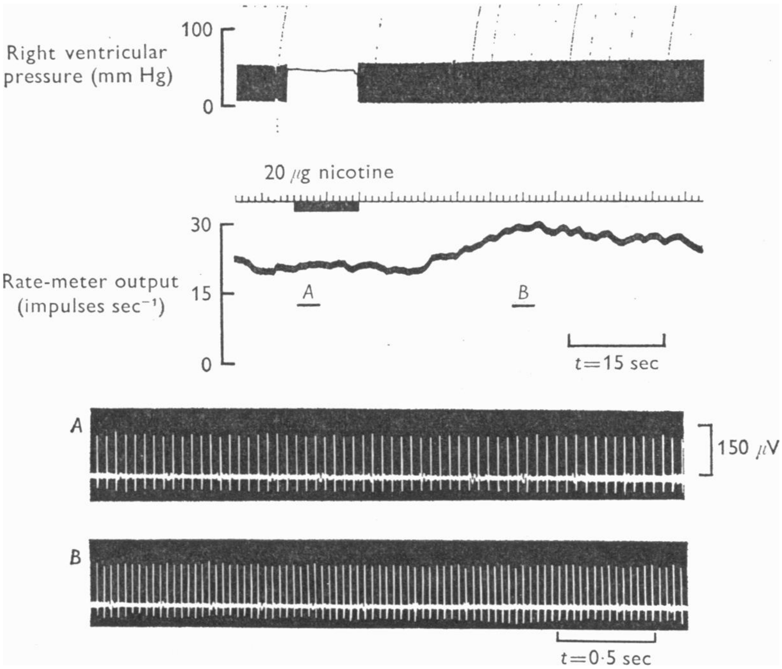

Fig. 8. The effect of injecting 20 μg nicotine in 0·5 ml. saline into the right ventricle for the period shown by the bar on the time marker upon activity from a single stretch receptor in the lung of a foetus, 147 days gestational age. From above down: right ventricular pressure, time marker, rate-meter output, neurograms A and B recorded as indicated in the rate-meter trace.

The effect of temporary (< 90 sec) occlusion of the umbilical cord upon the discharge was tested in thirteen foetuses including the two younger foetuses. The results were similar and will be described together. In all foetuses, shortly after the cord was occluded, the discharge from the receptors increased and then fell abruptly to zero. After a further 15–20 sec and if the umbilical cord had been released, the discharge returned usually with a rhythm modulated by the heart beat towards control levels. If the cord was permanently occluded, regular breathing started and the pattern of discharge was similar to that observed in the adult animal. A representative example of the relation between the discharge from a receptor and the vascular changes seen with occlusion of the cord is shown in Fig. 9. At first sight the changes in the level of discharge follow those in right ventricular pressure, but on closer inspection in this and in the other experiments, there is clear dissociation. The delay between occlusion of the cord and the rise in the rate of discharge varied between 10 and 60 sec. The peak rate of discharge (neurogram *B*) was in this as in the other tests approximately twice control and this phase of increased rate also varied in duration such that the subsequent fall of discharge to zero (neurogram *C*) occurred between 20 and 110 sec after the cord was first occluded.

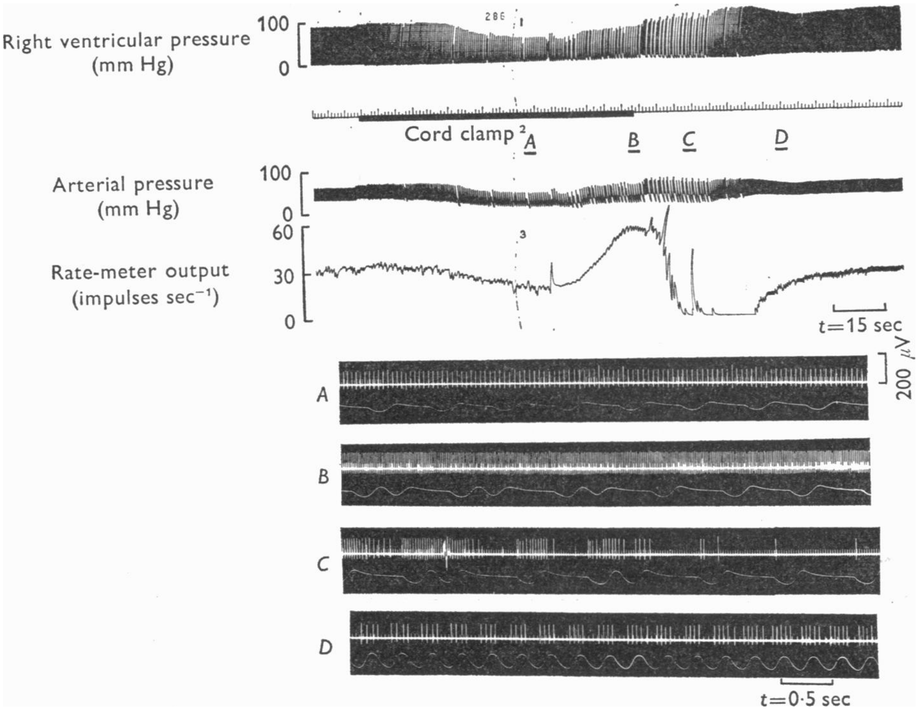

Fig. 9. The effect of occluding the umbilical cord for the period shown by the bar on the time marker upon the activity from a stretch receptor in the vagus in a foetus 147 days gestational age. From above: right ventricular pressure, time marker, arterial pressure and rate-meter output. Neurograms *A–D* recorded as indicated above the arterial pressure trace. In each neurogram, above, discharge from vagal fibre, below, right ventricular pressure.

Coinciding with this abrupt reduction in discharge from pulmonary stretch receptors was the first breath. In the example shown in Fig. 9, the first respiratory movement which caused a significant (i.e. > 5 mm Hg) reduction in intra-tracheal pressure was signalled by the recording artifact which occurred some 5 sec after the discharge fell to zero. The earlier artifacts seen in the ratemeter record were due to writhing or jerking movements of the foetus which commonly heralded the first breath. The course of events shown in Fig. 9 was similar to that seen in six other foetuses. In four others, the first breath was delayed for 10–15 sec after the discharge had fallen to zero and, in the remaining two foetuses, the first breath occurred as the discharge was falling to zero.

In none of the foetuses was there any change in intra-tracheal or intra-oesophageal pressure or the difference between the two which could have accounted either for the increase in receptor discharge which was first observed after cord occlusion or for the subsequent fall in discharge to zero. A possibility which was considered was that the receptors responded to changes in pressure in the lung tissue outside the wall of the lower airways and which, in the absence of any change in intra-tracheal pressure would cause a change in trans-mural pressure. Attempts were made to increase the volume of pulmonary interstitial fluid by injecting large (> 200 ml.) quantities either of maternal blood or of normal saline into the foetal circulation. Neither of these manoeuvres had any effect upon the discharge from pulmonary stretch receptors.

The effect of altering intra-tracheal pressure upon respiratory movements. During the course of the tests in which the rate of discharge from stretch receptors was related to intra-tracheal pressure, it was observed on many occasions that if fluid was removed from the trachea and in particular when this was sudden, a series of respiratory movements was provoked and an example is given in Fig. 7. This was observed in sixteen out of nineteen tests in ten foetuses from ewes given a spinal anaesthetic. In two foetuses from ewes given pentobarbitone, this stimulus was ineffective. There did not appear to be a critical level of intra-tracheal pressure below which respiratory movements could be elicited.

If these movements had been elicited or if they occurred spontaneously, they could be rapidly abolished by injecting warm saline into the trachea and so raising the intra-tracheal pressure. An example of this is given in Fig. 10 in which, with increments of added saline, respiratory movements at first became less frequent and then ceased. This type of response was elicited in the same ten foetuses on seventeen occasions and the increase in intra-tracheal pressure necessary to abolish respiratory movements varied between 2 and 8 mm Hg. In three foetuses, the vagi were cut in the neck 2 cm caudal to the nodose ganglia and these tests were repeated. A reduction in intra-tracheal pressure no longer provoked respiratory movements and in the one foetus in which spontaneous movements were still present, an increase of intra-tracheal pressure sufficient to embarrass the venous return, + 14 mm Hg, was without effect upon the movements.

Pulmonary stretch receptor activity after birth. Nine lambs were studied after the umbilical cord had been tied, and either spontaneous respiration had developed (five lambs) or respiration had to be assisted (four lambs). In the spontaneously breathing lambs, by 45 sec after birth the pattern of stretch receptor activity well known in the adult had been established. The only difference was the variable level of discharge at end-expiration: and this may simply reflect an incompletely established functional residual capacity.

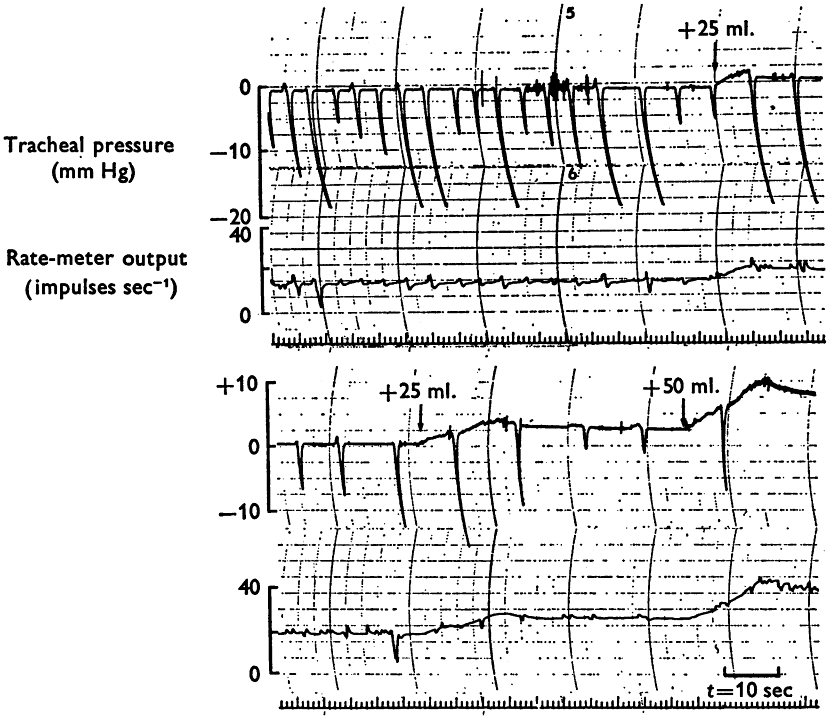

Fig. 10. The effect of increasing intra-tracheal pressure by injecting increments of saline as indicated into the trachea upon the frequency and depth of spontaneous respiratory movements in a sheep foetus 147 days gestational age. The upper and lower traces are continuous in each, above, tracheal pressure and below, the rate-meter output from a single afferent fibre in the vagus from a pulmonary stretch receptor. t = 10 sec.

In the lambs with assisted ventilation or in the two which were paralysed with gallamine and mechanically ventilated, the pattern of discharge was also similar to that observed in the adult. Of particular interest was the observation that if 5 % carbon dioxide was added to the air at the inlet of the pump, this was followed after a delay of between 1·5–2·0 min by a reduction in the level of discharge at peak inflation and an increase in the level of discharge at the end of the period of elastic recoil. The reduction in discharge at peak inflation with carbon dioxide has been reported in the adult rabbit (Mustafa & Purves, 1972) but in that series if any activity occurred at end-expiration, it too was abolished or reduced with carbon dioxide. We considered that the rise in discharge at end-expiration might be related to the rise in vascular pressure, seen particularly clearly in the right ventricle in Fig. 11 shortly after carbon dioxide was given. We repeated the test, but on this occasion substituting a mixture carbon dioxide/oxygen/nitrogen for air so that end-tidal carbon dioxide remained

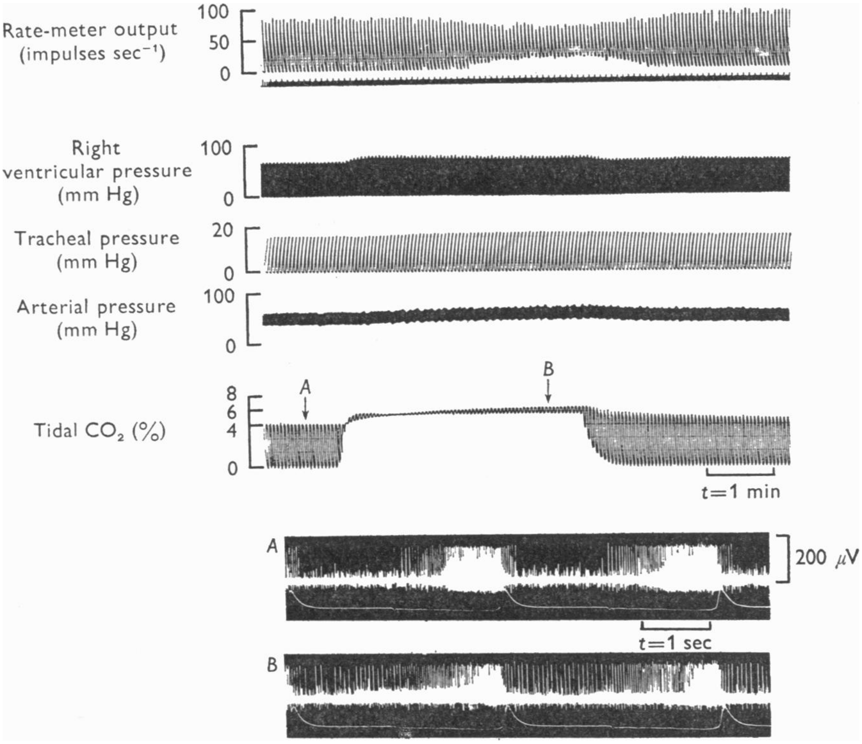

Fig. 11. The effect of administering 5 % carbon dioxide in air upon the discharge from a single pulmonary stretch receptor. Traces from above down: rate-meter output, right ventricular, tracheal and arterial pressures, tidal carbon dioxide. Neurograms *A* and *B*, receptor discharge above and tracheal pressure below, recorded as indicated in the tidal carbon dioxide trace. Lamb, 10 min after cord occlusion, artificially ventilated.

constant and P_{a,O_2} was reduced to 33 mm Hg from the control level of 69 mm Hg. The increase in right ventricular pressure was even greater than that observed when carbon dioxide was given but the pattern of stretch receptor discharge was completely unaffected.

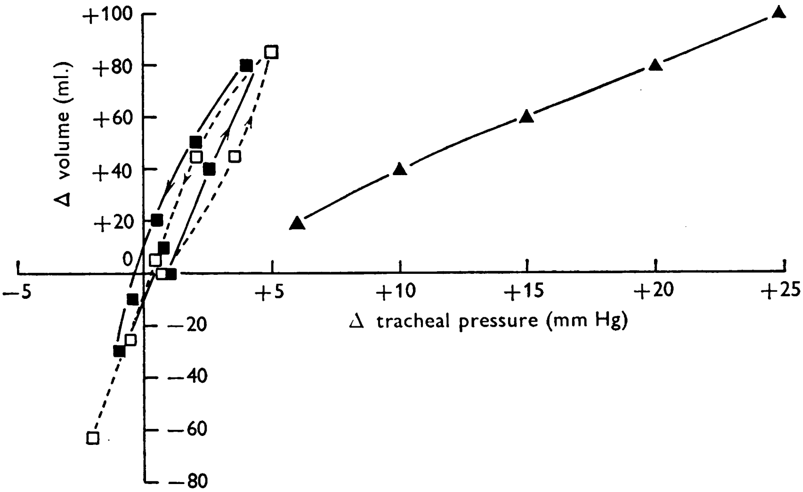

Fig. 12. The pressure/volume curves obtained from steady measurements of intra-tracheal pressure with the withdrawal or injection of various volumes of fluid in two foetuses (symbols, open and filled squares). Values for intra-tracheal pressure relate to that obtained just before the test started: those for volume to the amount of fluid added or withdrawn. The lines joining the points indicate the order of measurement. The curve represented by filled triangles is from the foetus represented by filled squares and relates changes in intra-tracheal pressure with addition of various volumes of air, 15 min after the umbilical cord was occluded, the lamb paralysed with gallamine and mechanically ventilated.

Pressure/volume curve before and after birth. In two foetuses, 147 days gestational age, the relation between intrathoracic volume and trans-thoracic pressure was determined and in one of the foetuses, this relation was obtained after the umbilical cord had been tied and the lamb paralysed with gallamine and mechanically ventilated. The results, shown in Fig. 12, are expressed as the volume of fluid or air added or withdrawn and the corresponding intra-tracheal pressure, the values being related to those obtaining 15–20 min after the trachea was cannulated in the foetuses or 30 min after the umbilical cord had been tied and ventilation started in the lamb. In the foetuses, where more numerous measurements were possible, the pressure/volume plots showed the expected hysteresis and total thoracic compliance over the range tested was 20 and 17 ml. mm Hg^{-1} intra-tracheal pressure. In the first of these foetuses, the corresponding value after birth was 4·5 ml. mm Hg^{-1}.

DISCUSSION

The present study provides the first direct evidence for the existence of active chemoreceptors in the aortic region of the sheep foetus. The resting level of discharge and responses to various stimuli were not obviously different at 130 days from 147 days gestation. Activity in these receptors has hitherto been inferred from the reflex responses to NaCN (Dawes, Lewis, Milligan, Roach & Talner, 1968) or reflex changes in peripheral vascular beds in response to hypoxia (Dawes, Duncan, Lewis, Merlet, Owen-Thomas & Reeves, 1969*a*, *b*). The precise location of the receptors is not known for certain but the fact that there was a small but noticeable increase in the delay before activity increased when NaCN was injected into the right side of the heart compared with the effect when the drug was injected into the root of the aorta suggests (*a*) that the receptor involved did not obtain its blood supply from the pulmonary artery (cf. Boyd, 1934) and (*b*) that the receptors lie somewhere between the root of the aorta and ductus arteriosus.

The finding that the aortic group of chemoreceptors is active in the foetus is in contrast to the observation by Biscoe *et al.* (1969) that the carotid body chemoreceptors are virtually completely insensitive to changes in the chemical composition of the carotid arterial blood or to drugs in the mature sheep foetus. The carotid receptors are activated by occlusion of the umbilical cord and by stimulation of the sympathetic nerves and activation of the chemoreceptors is most probably mediated by the sympathetic nerves since this activity also rises with umbilical cord occlusion. It is quite possible that there is a similar reflex component in the response of the aortic chemoreceptors to hypoxia or to occlusion of the umbilical cord since it is known that in the adult the responses of aortic chemoreceptors are affected by sympathetic efferent fibres (Mills, 1968). This point was not tested in the present study so the importance of this component is unknown. However, there is some indication that over birth, there is a change in the sensitivity of aortic receptors to chemical stimuli. In the present study, the level of discharge of the receptors with $P_{\mathrm{a,O_2}}$ 22–25 mm Hg was similar to that described by Lee, Mayou & Torrance (1964) in the adult cat with $P_{\mathrm{a,O_2}}$ 90–100 mm Hg and it is therefore possible that the difference in sensitivity to chemical stimuli between carotid and aortic chemoreceptors in the foetus is simply one of degree. The importance of discovering precisely how this change of sensitivity comes about in both sets of receptors is enhanced.

With respect to the activity in vagal fibres designated as arising from pulmonary stretch receptors, three questions, at least, require to be answered. First, how good is the evidence that this activity is from such receptors; secondly, to what stimuli do these receptors naturally respond in the foetus and thirdly, what physiological function, if any, does this activity have in the control of respiration in the foetus and at the moment of birth. The principal argument which can be brought to bear in connexion with the first question is that the properties of the receptors and the pattern of discharge from them compares closely with those which have been described in the adult and extensively reviewed (e.g. Widdicombe, 1964); that is, there is a linear relation between the level of discharge and intra-tracheal pressure, the receptors are affected by nicotine and veratridine but not by NaCN, conduction velocity falls in the range 38–45 msec^{-1} and conduction is blocked when the nerve fibres are cooled to 8° C, which are both features of $A\alpha$ and $A\beta$ fibres in the adult (Wyss, 1954; Paintal, 1953; Douglas & Malcolm, 1955). Further, after the cord had been clamped and breathing started, the discharge from these receptors assumed the adult pattern of discharge with modulation during the respiratory cycle and it was possible to confirm that the receptors adapted slowly to changes in pressure. Whereas these points indicate that the receptors from which afferent discharge was being recorded in the vagus were responsive to stretch rather than to, for example, chemical or mechanical irritation, it is by no means certain where in the lungs or airways the receptors were located. From the rather different slopes of the relation obtained between discharge and static pressure, it is probable that the receptors involved were variously located with respect to the bronchial lumen so that the same range of intra-tracheal pressure affected them quite differently. But we do not know whether the receptors were subepithelial in trachea or bronchioles (Elftman, 1943), or in bronchial smooth muscle (Larsell & Dow, 1933), or whether they were the encapsulated or unencapsulated endings described by these authors in the smaller bronchioles, atria, alveolar ducts or alveoli. Uncertainty on this point has made it difficult to interpret the results which might shed light on the stimulus to which these receptors respond in the foetus.

The results illustrated in Figs. 6 and 12 indicate clearly that between the foetal and new-born states there is a reduction both in the relation between stretch receptor discharge and intra-tracheal pressure and in pulmonary compliance. At the same time, as is shown in Figs. 7 and 11, either with respiratory movements in the foetus or with assisted respiration in the newborn, the pattern of discharge from the stretch receptors is exactly the opposite of what would be predicted from Fig. 6: that is, with changes in intra-thoracic pressure of the order of 10–20 mm Hg, modulation of discharge with respiration in the newly born is not less than twice as great as that seen in the foetus. In order to resolve this discrepancy, two questions require to be answered. First, why does pulmonary compliance fall so markedly at birth and, secondly, why is the apparently greater sensitivity of stretch receptors to changes in intra-tracheal pressure under static conditions in the foetus not confirmed with the occurrence of spontaneous or elicited respiratory movements?

The fall in pulmonary compliance over birth cannot simply be due to the difference between fluid- and air-filled lungs since in the foetus, but not in the paralysed new-born lamb, it is probable that part of the response to alterations in intra-tracheal pressure was contributed by chest wall muscles and diaphragm. However, it is probable that the major part of the reduction in compliance was due to the establishment of an air liquid interface in alveoli and lower air passages since the reduction in slope of the pressure/volume curve is similar to that seen by Neergaard & Wirz (1927) and by Neergaard (1929) in the fluid- and air-filled lungs of dogs. The corresponding reduction in the slope relating stretch receptor discharge and pressure suggests either that the receptors are located in the areas where it is generally thought that the surface tension properties of the liquid lining film are most effective, i.e. in alveoli or alveolar ducts: alternatively, that the receptors are located in smooth muscle of small bronchioles and that the performance of the smooth muscle and therefore of the receptors is affected by the lining film. The facts necessary to resolve this question completely are not yet available.

With regard to the second question, that of the difference in sensitivity of the stretch receptors to changes in intra-tracheal pressure before and after birth, two points may be made immediately. First, although comparison of Fig. 7 with Fig. 11 suggests that whereas the increase in discharge before birth was approximately 1 impulse sec^{-1} mm Hg^{-1} intra-tracheal pressure and after birth, 3 impulses sec^{-1} mm Hg^{-1} intra-tracheal pressure, it is by no means certain that these figures are strictly comparable since in the foetus, intra-thoracic pressure was negative and in the lamb it was above atmospheric since respiration was mechanically assisted. Further, it was by no means certain in the foetus as in the new-born lamb that the relation between discharge and pressure was linear over the range of pressure -10 to zero and we were unable to be certain of this point because our attempts to study the relation between discharge and pressure in this range were frustrated since withdrawal of fluid invariably caused the onset of respiratory movements. The second point to be emphasized is that the measurements shown in Fig. 6 were made under static conditions with respect to pressure while those shown in Figs. 7 and 11 were recorded under dynamic conditions. It is most probable that the effective stimuli to the stretch receptors in the lung were quite different under the two sets of conditions although the range of pressure generated was similar. Thus, since the dynamic viscosity of water is many times greater than that of air, a correspondingly smaller volume of fluid would be displaced in the airways than air for a given change in pressure per unit time. This point has been confirmed experimentally by Howatt, Humphreys, Normand & Strang (1965) who showed under comparable conditions to those of the present experiments that with the generation of up to -10 mm Hg pressure in the thorax, only 3–5 ml. fluid were displaced. If, as is generally believed, the stretch receptors of the lung and lower airways respond to the degree of distortion of their surrounding tissue, it follows that the smaller the change in airway volume, the less will the distortion and the smaller the change in discharge. Further, if as the present experiments indicate, activity from these receptors is inhibitory to respiration, the presence of fluid in the airways will tend to maintain the level of inhibition in two ways. First, the respiratory modulation of the discharge will be slight and secondly, because of the increased inertia of the fluid filled lung, the receptor discharge will not fall to zero or close to it at end-expiration as it does in the air filled lung. This high rate of discharge during expiration is well demonstrated in Fig. 7.

The other outstanding question raised by these experiments and which it has not been possible to answer is the mechanism whereby stretch receptor discharge at first rises and then falls to zero following occlusion of the umbilical cord. A number of possible explanations have been entertained. The receptors, assuming them to be in smooth muscle of small bronchioles, could have been affected by changes in compliance of smooth muscle. In the context of umbilical cord occlusion, this could have been due to the rise in carbon dioxide or fall in oxygen; but in separate experiments, neither of these stimuli separately was found to affect the discharge although in the new-born, carbon dioxide was found to reduce discharge as it does in the adult rabbit (Mustafa & Purves, 1972). Similarly, smooth muscle tone could have been changed reflexly. Vagally mediated efferent activity did not appear to be responsible since the changes in discharge were observed after vagotomy higher in the neck. The possibility that sympathetic activity relayed from the stellate ganglion was responsible was not excluded. Although it is known that sympathetic activity in the cervical sympathetic nerve increases with umbilical cord occlusion (Biscoe *et al.* 1969), it is difficult to see how such a rise in sympathetic efferent activity to bronchial smooth muscle can have accounted for both the early rise in discharge from pulmonary stretch receptors and the later fall. Another factor could have been the release of catecholamines in response to the asphyxia associated with cord occlusion (Comline, Silver & Silver, 1965). However, this mechanism would be unlikely to explain the early changes in discharge, i.e. after 10–15 sec of cord occlusion and in any case, intravenous injection of noradrenaline and adrenaline was found to be without effect upon receptor discharge.

An alternative possibility was that the receptors responded to changes in pressure in the vascular or interstitial compartments outside the bronchioles. Some evidence for this was the frequently observed modulation of the discharge by the pulse. However, following cord occlusion there was clear dissociation of the receptor discharge from changes in either aortic or pulmonary arterial pressure. The receptors could have responded to changes in interstitial pressure, for in the absence of any significant changes in intra-tracheal pressure these would effectively be changes in transmural pressure. It is known that shortly after birth by Caesarean section, there is a reduction in the over-all fluid content of the lungs (Adams, Yanagisawa, Kuzela & Martinek, 1971) and that over the same period there is evidence of peri-arterial distension in lung interstitial tissue and opening up of lymphatics (Aherne & Dawkins, 1964). This is consistent with evidence of very considerable increase in the rate of lymphatic flow from the lungs of the foetus (Humphreys, Normand, Reynolds & Strang, 1967). Although in these studies, the measurements have been made over minutes or hours rather than seconds, it is clear from studies in the adult that the transfer of water from pulmonary capillaries to the interstitial spaces can be accomplished very quickly (Freinkel, Schreiner & Athens, 1952; Chinard & Enns, 1954; Lillienfield, Freis, Partenope & Morowitz, 1955) and this may be accentuated during cord occlusion since it is known that the rate of capillary filtration is enhanced with transient episodes of hypoxia in the foetus (Seller & Spector, 1964). Substantial changes in the volume and pressure of fluid in the pulmonary interstitial space are therefore to be expected in association with occlusion of the umbilical cord. Further studies will be required to show whether in fact these changes do occur.

The importance of answering this question is enhanced by the observation in the present study and made previously by Dawes (1968) that respiratory movements can be elicited by a reduction in intra-tracheal pressure and abolished by a rise in pressure and that these changes can be abolished after vagotomy. In this connexion, it is of interest that we were unable to confirm that an increase in intra-tracheal pressure, however sudden, gives rise to a gasp in the foetus (Hughes, Parker & Williams, 1967). We are uncertain how to reconcile this difference in results: it may be that since these workers used air at room temperature with which to inflate the lungs of the foetus, they may have excited receptors sensitive, for example, to changes in temperature.

The question as to what physiological function the aortic chemoreceptors and pulmonary stretch receptors have in the foetus and at the moment of birth is, as always, difficult to answer and inevitably comparisons have to be made with the known function of these receptors in adult life. The aortic chemoreceptors are known to be excitatory to respiration (Comroe, 1939; Daly, Hazzledine & Howe, 1965) and their stimulation also causes vasoconstriction in various vascular beds in the adult (Daly *et al.* 1965) and in the foetus (Dawes *et al.* 1968). It is also possible that the aortic group of chemoreceptors are involved in the regulation of cerebral blood flow since it has been shown recently that stimulation of the carotid body chemoreceptors causes large increases in cerebral blood flow (Purves & Ponte, 1972). If it can be shown that the aortic chemoreceptors have a similar action, then this would go some way towards explaining why the large increases in cerebral blood flow which occur at the moment of birth and with the onset of respiration are abolished if the vagi have previously been cut (Purves & James, 1969).

With respect to the pulmonary stretch receptors, the evidence from the present study indicates that their afferent discharge is inhibitory to respiration, and in the foetus they may share this action with other receptors in the naso-pharynx which are also sensitive to the presence or pressure of fluid. This would suggest that the Hering Breuer reflex is important in the foetus and possibly as powerful as it has been shown to be in the newborn infant (Cross, Klaus, Tooley & Weisser, 1960). However, the importance of this reflex in the foetus and at birth has been put in question by the observation that there is a second type of movement in the foetus which is irregular and rapid, up to 4 Hz, and whose incidence is unaffected by block of both vagi and sympathetic nerves in the neck with local anaesthetic (Merlet *et al.* 1970; Dawes *et al.* 1970, 1972). On this basis, these movements are clearly to be distinguished from those observed in the present study and in numerous others (e.g. Howatt *et al.* 1964; Dawes, 1968) and which correspond much more closely to the pattern of respiratory activity seen immediately after birth. The question as to the origin and significance of these two types of foetal movement and the associated question as to the physiological importance of the Hering Breuer reflex in the foetus will remain unresolved until parallel records are made of phrenic nerve, intercostal muscle or diaphragmatic activity.

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