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EFFECTS OF HYPERCAPNIA ON TRACHEAL PRESSURE, DIAPHRAGM AND INTERCOSTAL ELECTROMYOGRAMS IN UNANAESTHETIZED FETAL LAMBS

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SUMMARY

1. The electromyographic (e.m.g.) activity of the diaphragm and intercostal muscles has been recorded during breathing movements of unanaesthetized lambs *in utero* (109–135 days), and compared with the changes of tracheal pressure.

2. The diaphragm e.m.g. was irregular in size, shape and timing, with a variable rate of rise during inspiration, often with a flattening of integrated activity before the end of a breath and with little or no post-inspiratory activity.

3. The diaphragm e.m.g. gave the most reliable measurements of breath interval and incidence: in eucapnia mean T_I was $0{\cdot}45 \pm 0{\cdot}02$ (S.E. of mean) and T_E was $0{\cdot}74 \pm 0{\cdot}05$ sec, and 58–100 % of the diaphragm bursts were associated with identifiable and appropriate changes of tracheal pressure.

4. During fetal hypercapnia, produced by increasing the maternal inspired CO_2 in a single change or series of step changes, tracheal pressure amplitude and its rate of change during inspiration increased progressively over the P_{a,CO_2} range of 37–87 mmHg.

5. In eucapnia the area, amplitude and inspiratory slope of the integrated diaphragm e.m.g. were not always correlated with tracheal pressure amplitude, and in hypercapnia they increased only in the lower part of the P_{a,CO_2} range. Inspiratory intercostal activity increased progressively as the P_{a,CO_2} was raised.

6. The frequency histograms of variables derived from the tracheal pressure, diaphragm and intercostal e.m.g.s were skewed to the left in eucapnia but became normalized during hypercapnia. The rate and depth of breathing became regular.

7. The response to mild asphyxia was a combination of the responses to hypercapnia and hypoxia.

8. The interpretation of the tracheal pressure and diaphragm e.m.g. as measures of the 'depth' of breathing and respiratory 'drive' in the fetal lamb is discussed.

INTRODUCTION

Rapid irregular breathing in unanaesthetized fetal sheep normally occurs in episodes associated with low voltage electrocortical activity and rapid eye movements (Dawes, Fox, Leduc, Liggins & Richards, 1972). High-voltage electrocortical activity

is accompanied by apnoea or occasional deep gasps or sighs possibly associated with swallowing (Harding, 1980). Previous studies have shown that an increase in P_{a,CO_2} causes an increase in the depth and regularity of breathing but has comparatively little effect on its episodic appearance (Boddy, Dawes, Fisher, Pinter & Robinson, 1974; Chapman, Dawes, Rurak & Wilds, 1980).

Tracheal pressure and the diaphragm electromyogram (e.m.g.) have been used to measure the depth and timing of breathing in fetal lambs in eucapnia and hypercapnia (Maloney, Adamson, Brodecky, Cranage, Lambert & Ritchie, 1975; Chapman *et al.* 1980; Bowes, Wilkinson, Dowling, Ritchie, Brodecky & Maloney, 1981), but the relationship between the two measurements has not been thoroughly examined. In anaesthetized and conscious adult animals, and in conscious man, the relationship between the electrical activity of the diaphragm and ventilation is such that change of tidal volume may be estimated from the diaphragm e.m.g. at various levels of chemical drive (Katz, Fink & Ngai, 1962; Lourenco, Cherniak, Mann & Fishman, 1966; Lopata, Evanich & Lourenco, 1977; Lopata, Onal, Evanich & Lourenco, 1980). The rates of rise of integrated electrical activity of the diaphragm or phrenic nerve have been regarded as measures of the 'central drive' to the inspiratory muscles (Bradley, von Euler, Martilla & Roos, 1975) and are predictably increased by hypercapnia or stimulation of the carotid sinus nerves (von Euler, Martilla, Remmers & Trippenbach, 1976; Eldridge & Gill-Kumar, 1980; Lopata *et al.* 1980).

In the present work we have examined the electrical activities of the diaphragm and intercostal muscles to establish their relationships with the changes of tracheal pressure during breathing in unanaesthetized fetal lambs *in utero*. In these, breathing movements are associated with only small variable movements of liquid in the trachea (Dawes *et al.* 1972) and with irregular movements of the chest wall (Poore & Walker, 1980).

METHODS

Operations were performed on eleven fetuses at 100–115 days gestation (term is 147 days). Under aseptic conditions and Halothane anaesthesia catheters were implanted in the amniotic cavity, a carotid or brachial artery, jugular vein and trachea of the fetus, and into the maternal carotid artery and jugular vein. One or two pairs of stainless steel wires (Cooner Wire Co., California), insulated to the tip, were implanted in the fetal diaphragm about 6 mm apart through a right thoracotomy between the seventh and eighth ribs. Similar pairs of electrodes were implanted in the intercostal muscles in the mid-axillary line between the fourth and eighth intercostal space, usually encompassing both the internal and external muscles. About 4 mm of bare wire was implanted in a loose loop, anchored at the ends by 4-0 silk thread, so as to avoid crushing the muscle.

Carotid and tracheal pressure measurements were corrected for changes of amniotic pressure by an electronic subraction circuit and, after suitable amplification, all signals were displayed on a polygraph (Schwarzer). Recordings were begun 12–24 hr after surgery and were continued until fetal death or delivery (6–21 days). During an experiment signals were either recorded on an FM tape recorder, by a computer on-line, or both.

The fetal arterial P_{O_2} and P_{CO_2} were altered by allowing the ewe to breathe air containing various concentrations of O_2, CO_2 and N_2 from a large plastic bag tied over her head with the outflow around the neck. The bag was flushed with air at 40 l./min for 1–2 hr as a control before the onset of hypercapnia. Hypercapnia was started at any point in the electrocortical cycle, but always encompassed at least one complete low voltage period. The inspired CO_2 was increased either as a single step (5 % CO_2) lasting 30–90 min, or as a series of steps (2·5, 5·0, 7·5 and 10 % CO_2) each lasting 6 min. In some experiments hypercapnia was associated with hypoxia to produce a mild

degree of fetal asphyxia. The ewe was given air to breathe from the head bag for 30–60 min after the end of the hypercapnia or asphyxia.

Data collection and analysis were performed on a PDP 11/34 computer. The tracheal pressure signal was amplified, inverted and band-pass filtered to remove high-frequency base-line noise and slow drift. The diaphragm and intercostal e.m.g.s were filtered (30–1000 Hz) and passed through a 'leaky' integrator to provide an envelope representative of frequency and amplitude (e.g. Fig. 1); time constants of 15–30 msec usually proved satisfactory. All signals were digitized at 10 msec intervals.

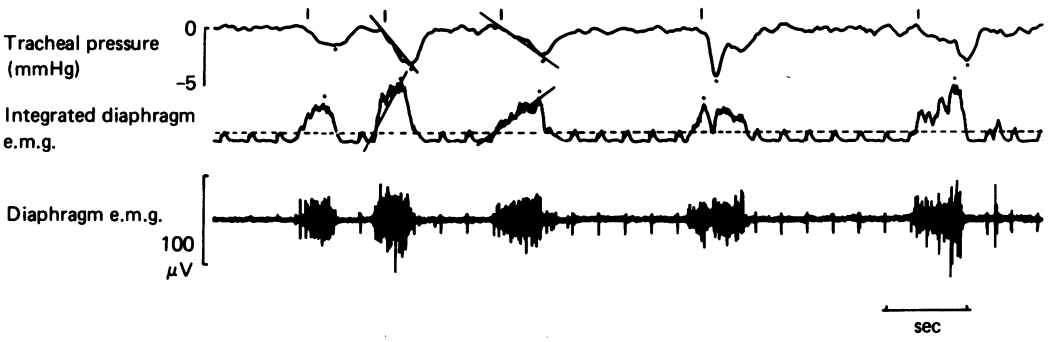

Fig. 1. Tracheal pressure, integrated and raw diaphragm e.m.g. records to illustrate the computer analysis. The interrupted line through the integrated diaphragm trace indicates the threshold, above which the timing and calculation of the breath was begun. The dash above each tracheal pressure deflexion indicates the point identified by the computer as the start of the breath for calculation of breath variables from tracheal pressure. The dots below each tracheal pressure deflexion and above each integrated diaphragm record indicate the peak activity identified by the computer. The lines drawn through the second and third breaths indicate the inspiratory slope that would be calculated for these breaths.

The programme determined, and updated every 6 sec, a running minimum value between bursts of activity in the diaphragm and intercostal muscles. A threshold, adjusted for individual experiments to exclude base line noise, was set to locate the start and finish of each breath. Three independent criteria were used for validation of the presence of a breath, namely the height above the base line, the total area above the threshold, and time below the threshold at the end of a breath. This procedure excluded e.c.g. artifacts and distinguished between the discrete e.m.g. burst of a breath and longer periods of complex diaphragm activity which were clearly non-respiratory. No attempt was made to remove artifacts within an e.m.g. burst or tracheal pressure deflexion due to the e.c.g. or cardiac pulse respectively. The diaphragm e.m.g. was used to determine the initiation of a breath; the tracheal pressure and intercostal signals were then examined simultaneously, and for up to 100 msec after the termination of diaphragm activity, to identify the minimum and maximum values associated with the breath. The criteria were reset for each fetus and before the start of each experiment to minimize artifacts as judged by eye.

Up to twelve variables were measured for each breath. Inspiratory time was calculated as the width of the diaphragm and intercostal bursts of activity and as the time from threshold crossing to maximum tracheal pressure change. Expiratory time was calculated as the time from the end of one diaphragm burst to the start of the next. The amplitude of tracheal pressure changes, height of the integrated e.m.g. activity, and total area of activity under each integrated deflexion were determined. The slopes of the inspiratory phase of tracheal and diaphragm signals were determined by calculating the slope of a line connecting the start of each breath and the peak value (Fig. 1). All derived variables were stored in the computer buffer; every 1000 breaths the programme was interrupted for 10 sec to allow transfer to disk. Subsequent analysis was performed off-line. All variables over the whole or part of a run were averaged and correlation and regression matrices formed from the individual breaths within each run. Frequency histograms were recorded on an X–Y plotter.

RESULTS

Observations in normal conditions

Primary measurements were made of breathing movements, tracheal pressure, and diaphragm and intercostal muscle e.m.g. activity. From these were derived peak height, area, inspiratory slope and time, expiratory time and breath-to-breath interval. The blood gas values in seventeen control runs in eleven fetuses are shown in Table 1.

TABLE 1. Fetal carotid blood gas values (mean ±S.E. of mean) before and after administration of 5% CO_2 in air to ewes in seventeen experiments

	Control	Hypercapnia
P_{CO_2} (mmHg)	47·2±0·9	55·5±1·3
P_{O_2} (mmHg)	19·9±0·7	22·3±2·9
pH	7·35±0·01	7·29±0·01

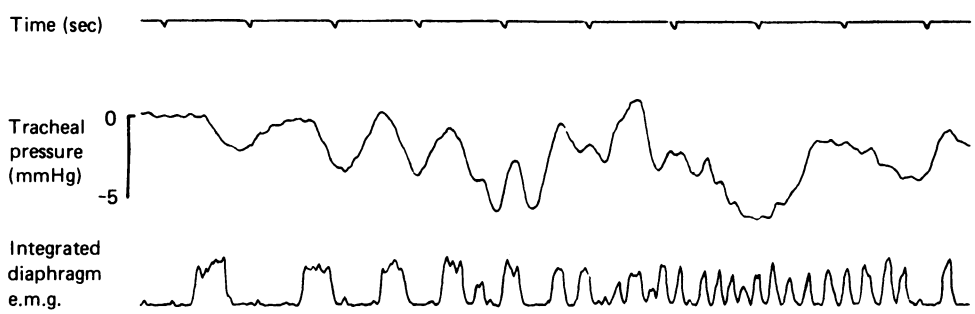

Fig. 2. Records of tracheal pressure (above) and integrated diaphragm e.m.g. (below) from a eucapnic fetal lamb at 116 days gestation in low-voltage electrocortical activity. The tracheal pressure record fails to follow the more rapid changes in diaphragm activity.

Almost all diaphragmatic activity, verified by the computer as breathing, occurred during low-voltage electrocortical activity. Longer lasting activity which was clearly non-respiratory, and the occasional deep inspiratory efforts usually present during high-voltage activity, have not been considered in the following analyses; it was removed by the computer editing.

Individual bursts of diaphragmatic activity usually began and ended abruptly, so that after integration the envelope of activity was approximately rectangular (Fig. 2). Otherwise inspiration was characterized by a progressive increase in activity, comparable to the inspiratory slope observed in adult anaesthetized animals. A gradual decrease in activity at the end of a breath ('post-inspiratory activity' in the adult) was rarely seen (Figs. 1 and 2).

The bursts of e.m.g. activity were irregular in timing, height and duration, often showed several components, and, especially in the younger fetuses, sometimes followed one another in rapid succession. The tracheal pressure changes were then complex, and it was difficult to distinguish successive breaths from these alone (Fig. 2). Discrete bursts of diaphragm activity were usually, but not always, associated

with clear tracheal pressure changes. In the seventeen control runs from six fetuses (36,360 breaths) on average 82·5% (range 58–100) of diaphragm e.m.g. bursts validated by the computer were associated with validated tracheal pressure deflexions.

Fig. 3*A* shows a complete episode of breathing which lasted 23 min and contained 1493 bursts of diaphragm activity identified by the computer as breathing; 1093

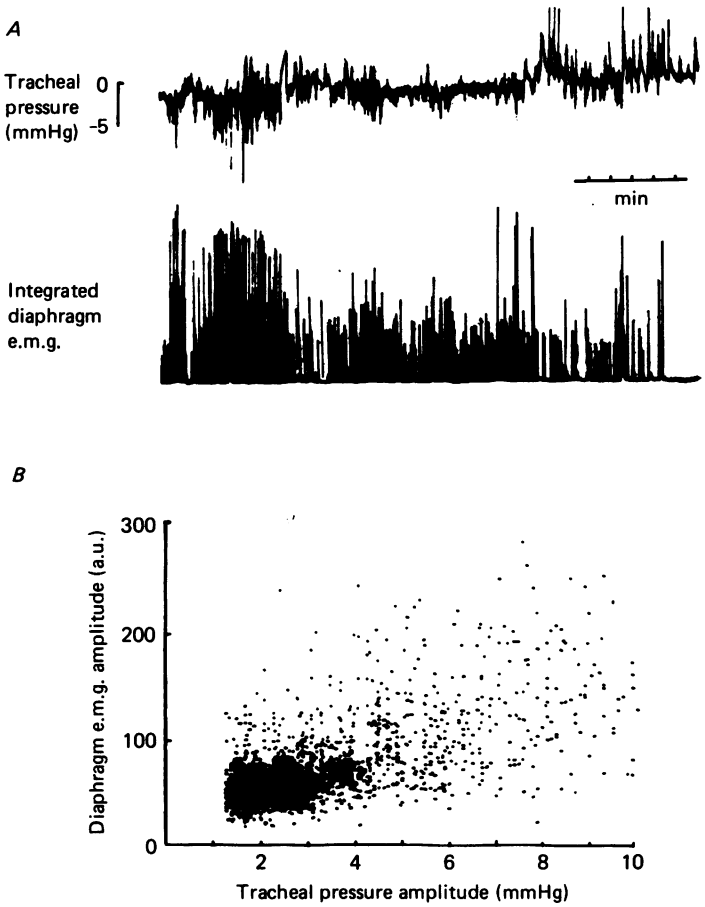

Fig. 3. *A*, 20 min record of tracheal pressure (above) and integrated diaphragm e.m.g. (below) from a eucapnic fetal lamb at 124 days gestation during low-voltage electrocortical activity (not shown); 1493 breaths occurred during this episode. *B*, scattergram of tracheal pressure amplitude (mmHg) and height of the diaphragm e.m.g. (arbitrary units; a.u.) for the breathing episode shown in (*A*). Correlation coefficient = 0·66.

(73%) of these were associated with identifiable and appropriate changes of tracheal pressure. While a relationship between the height of the integrated diaphragm e.m.g. and tracheal pressure amplitude is apparent, there was considerable scatter (Fig. 3*B*); the correlation coefficient was 0·66. Other aspects of the diaphragm e.m.g. (e.g. area of the integrated e.m.g. and inspiratory slope) which might have related to the total activation of the diaphragm, were not related to tracheal pressure amplitude.

A linear correlation was derived between each of the variables in seventeen control runs (eleven fetuses) while the ewe was breathing room air. The highest correlations were between the amplitudes of tracheal pressure and the diaphragmatic and intercostal e.m.g.s (Table 2). This Table only includes those pairs of variables with correlations in excess of 0·3. Others were statistically significant (the numbers of breaths were large) but biologically less important. And indeed even for the variables in Table 2 the correlation was not high.

TABLE 2. Average (±S.D.) of nineteen to twenty-six separate correlation coefficients each based on > 100 breaths while the ewes breathed room air

Fetal variables*	Mean correlation coefficient
Tracheal pressure amplitude: diaphragm e.m.g. amplitude	0·67±0·29
Tracheal pressure amplitude: intercostal e.m.g. amplitude	0·52±0·17
Tracheal pressure amplitude: diaphragm e.m.g. area	0·47±0·18
Tracheal pressure slope: diaphragm e.m.g. amplitude	0·36±0·21
Tracheal pressure slope: diaphragm e.m.g. slope	0·53±0·25

* Only variables with a mean correlation coefficient in excess of 0·3 are shown; all are significant ($P < 0·001$).

Several measures of inspiratory and expiratory times were examined, based on tracheal pressure, and diaphragmatic or intercostal activity (Table 3). Although the fall in tracheal pressure started after the onset of diaphragmatic activity and reached its peak shortly after diaphragmatic activity ceased, inspiratory times measured from the two signals were similar and highly correlated ($r > 0·75$). Intercostal activity usually began after the start of diaphragmatic activity and finished at about the same time, and therefore the intercostal time was less than that of either of the tracheal pressure or diaphragm signals.

In eucapnia, the frequency distributions of all the variables were skewed to the left (e.g. Fig. 4, thin lines).

Hypercapnia

The measurement of diaphragmatic and intercostal activity, as well as tracheal pressure, provided an opportunity of testing which variable, among the many derived measurements, correlated best with the change in fetal arterial P_{CO_2}.

The responses of the diaphragm e.m.g to an increase in maternal inspired P_{CO_2} to 5% were studied on eleven fetuses in twenty-six experiments in twenty of which tracheal pressure and in thirteen of which intercostal e.m.g.s also were measured. The length of the control and hypercapnia periods varied, but each contained at least one complete low-voltage electrocortical episode. The blood gas values in hypercapnia are shown in Table 1.

Table 3 shows the mean and modal values before and during hypercapnia. The first eight of the thirteen variables may be regarded as measures of the depth of breathing, and all these showed a significant increase in mean values in response to CO_2. There was a large increase in intercostal e.m.g. activity; the proportion of breaths

TABLE 3. Mean (±S.E. of mean) and the average modal values for the respiratory variables during normocapnia (P_{a,CO_2}, 47·2±0·9 mmHg) and hypercapnia (P_{a,CO_2}, 55·5±1·3 mmHg) from ten fetuses 109–135 days gestation. Height and area of the integrated e.m.g.s are expressed as arbitrary units (a.u.) after analogue-to-digital conversion

		Normocapnia		5% CO_2	
	n†	Mean±S.E. of mean	Mode±S.E. of mode	Mean±S.E. of mean	Mode±S.E. of mode
Tracheal pressure amplitude (mmHg)	20	3·12±0·19	1·95±0·17	4·20±0·32**	3·52±0·35
Diaphragm e.m.g. height (a.u.)	26	100·0‡	78·8±7·98	137·4±9·10**	118·0±8·08*
Diaphragm e.m.g. area (a.u.)	26	100·0	51·4±16·4	129·9±6·14**	107·0±9·83**
Intercostal e.m.g. height (a.u.)	14	100·0	46·6±10·5	140·3±16·9*	118·9±26·1
Intercostal e.m.g. area (a.u.)	14	100·0	34·2±9·5	170·2±30·5*	156·1±35·6
Diaphragm and intercostal e.m.g. area (a.u.)	14	100·0	51·7±10·3	152·0±13·9**	146·8±14·6*
Inspiratory slope					
Tracheal pressure (mmHg/sec)	20	9·79±0·54	5·41±0·54	13·95±1·17**	9·72±0·97**
Diaphragm e.m.g. (a.u./sec)	26	100·0	58·9±7·49	122·8±9·83*	85·1±8·23**
T_I (sec)					
Tracheal pressure	20	0·40±0·018	0·34±0·026	0·34±0·017	0·33±0·019
Diaphragm	26	0·45±0·022	0·32±0·024	0·43±0·021	0·40±0·026
Intercostal	14	0·26±0·017	0·20±0·030	0·31±0·026	0·31±0·034*
T_E (sec)					
Diaphragm	26	0·74±0·053	0·36±0·039	0·55±0·038**	0·41±0·030
T_T (sec)					
Diaphragm	26	1·12±0·059	0·72±0·056	0·93±0·047*	0·83±0·042

* $P < 0·05$, compared to normocapnia. ** $P < 0·01$, compared to normocapnia. † Number of experiments. ‡ Mean values for normocapnia scaled to equal 100; corresponding values during hypercapnia scaled proportionately.

(estimated from diaphragmatic activity) which contained intercostal activity rose from 28·5 to 92%.

There was no significant correlation over all the experiments between the mean value of each of the e.m.g. variables and mean fetal arterial P_{CO_2}, whereas there was a correlation between mean tracheal pressure amplitude or slope and P_{CO_2}. There was no significant change in inspiratory time (T_I) as measured from the diaphragm, but there was a small decrease in mean expiratory time.

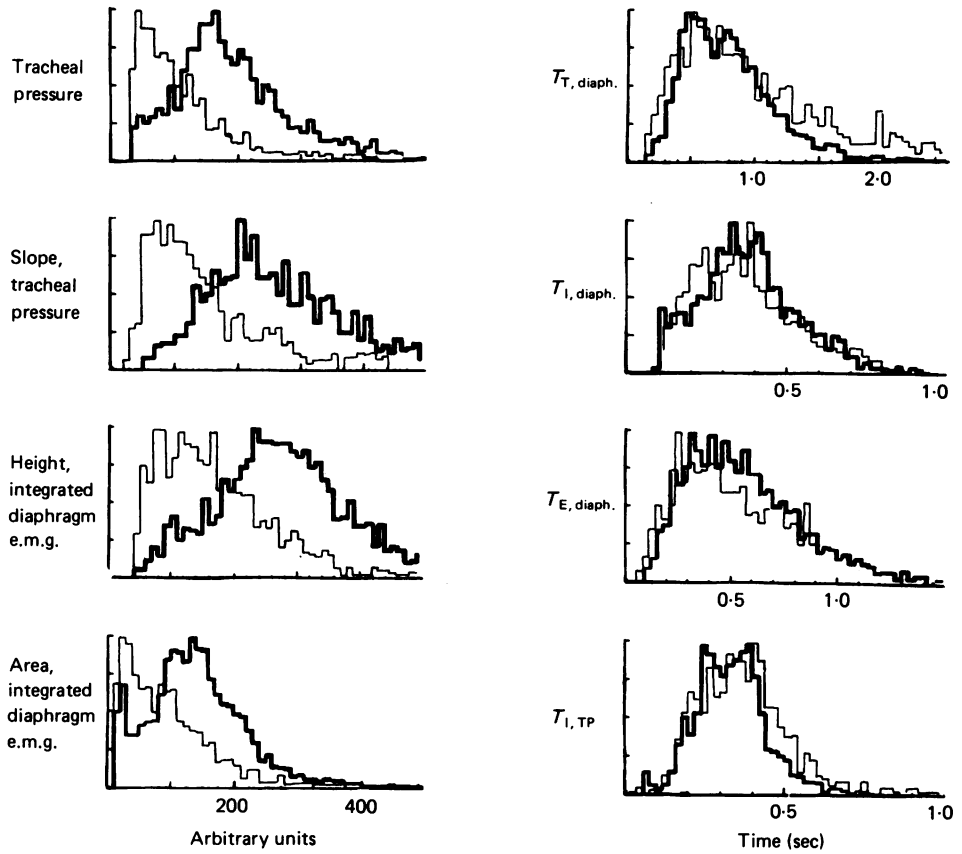

Fig. 4. Frequency histograms during eucapnia (thin lines) and hypercapnia (heavy lines; ewe breathing 5% CO_2), of tracheal pressure amplitude and inspiratory slope (tracheal pressure $\div T_{I,TP}$), height and area of the integrated diaphragm e.m.g., inspiratory times from the diaphragm e.m.g. ($T_{I,\ diaph}$) and tracheal pressure ($T_{I,TP}$), expiratory time from the diaphragm e.m.g. ($T_{E,\ diaph}$), and breath-to-breath interval from the diaphragm e.m.g. ($T_{T,\ diaph.}$). Fetus E72/79, 109 days gestation. In each instance peak frequency was scaled to the ordinate maximum.

The frequency distributions of many variables became more normal during hypercapnia; e.g. tracheal pressure amplitude and inspiratory slope, diaphragm e.m.g amplitude and area (Fig. 4, left-hand side), even at 109 days of gestation, before electrocortical differentiation. The frequency distributions of variables related to the timing of fetal breathing (T_I and T_E) were not greatly altered (e.g. Fig. 4, right-hand

side) in the experiment illustrated. In older fetal lambs breath intervals became more regular, confirming the findings of Chapman *et al.* (1980).

In 8 experiments on four fetuses the arterial P_{O_2} was lowered by a mean 3·9 mmHg and the P_{CO_2} was raised by 6·5 mmHg for up to 65 min; there was a mean fall in pH of 0·07. The changes of the tracheal pressure and diaphragm e.m.g. variables were not different from those observed during hypercapnia alone, but there was a reduction in the over-all amount of breathing with lower P_{O_2} values.

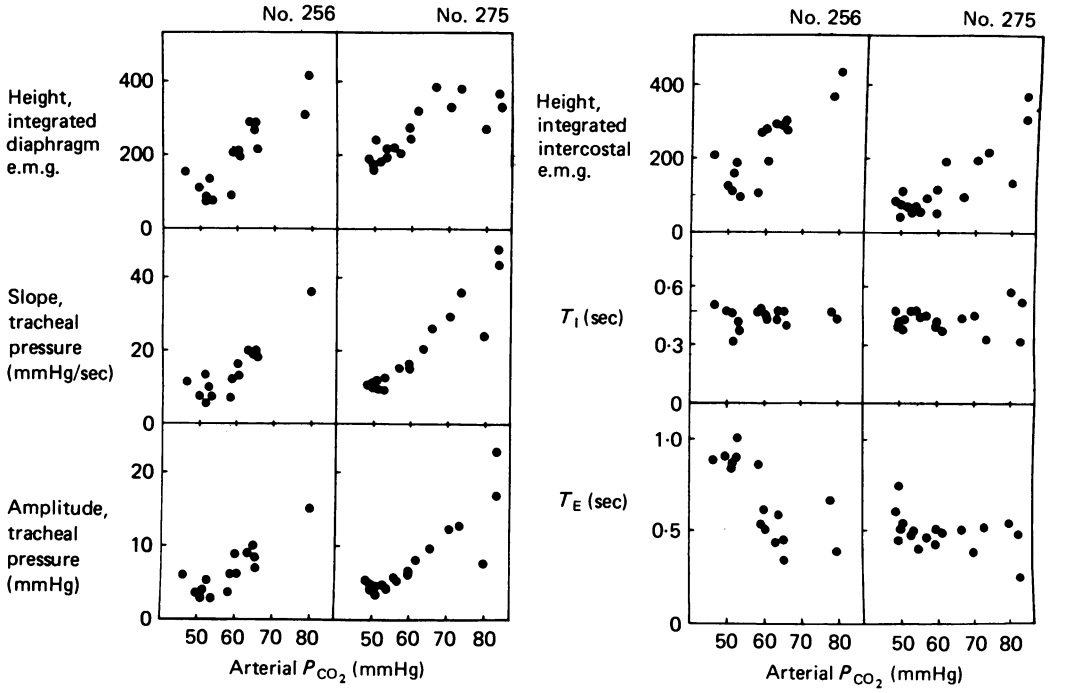

Fig. 5. Fetal arterial P_{CO_2} plotted against breathing variables for fetus 256 (three experiments) and 275 (four experiments). The ewe breathed 2·5, 5, 7·5 or 10% CO_2 in air for 6 min. Each symbol is the mean of 70–260 breaths from the last 3 min of each run.

Responses to progressive stepwise increments in CO_2 during fetal low-voltage electrocortical activity

Either air or 2·5, 5·0, 7·5 or 10% of CO_2 (in air with added N_2) was administered for periods of 6 min to the ewe. The O_2 content of the gas mixture was adjusted so as to minimize changes in fetal arterial P_{O_2}. Where possible the effects of all five gas mixtures were examined sequentially, rising progressively, with return to air at the end. Occasionally an experiment was interrupted by a return to high-voltage electrocortical activity and consequent cessation of fetal breathing movements; the ewe was then given room air to breathe until low-voltage activity recurred. Eleven sets of observations were completed satisfactorily in four fetuses.

Tracheal pressure and inspiratory slope increased linearly over the whole range of P_{a,CO_2} (Fig. 5), with mean slopes of 0·23 ± 0·08 mmHg . mmHg CO_2^{-1} and 0·75 ± 0·19 mmHg . sec^{-1} . mmHg CO_2^{-1} respectively.

The amplitude of the diaphragm e.m.g. increased progressively in two fetuses (e.g. fetus no. 256, Fig. 5); in the other two it reached a plateau (e.g. fetus no. 275, Fig. 5) or was variable. The amplitude of the intercostal e.m.g. also increased progressively, in one instance to reach a plateau. There was clear evidence of recruitment (Fig. 6). In general mean T_I remained constant whereas mean T_E shortened as P_{a,CO_2} increased (Fig. 5). At lower fetal P_{a,CO_2}s the modal value of T_E was much less than the mean, reflecting the skew distribution of breath intervals. At higher P_{a,CO_2}s the modal and mean values of T_E became closer.

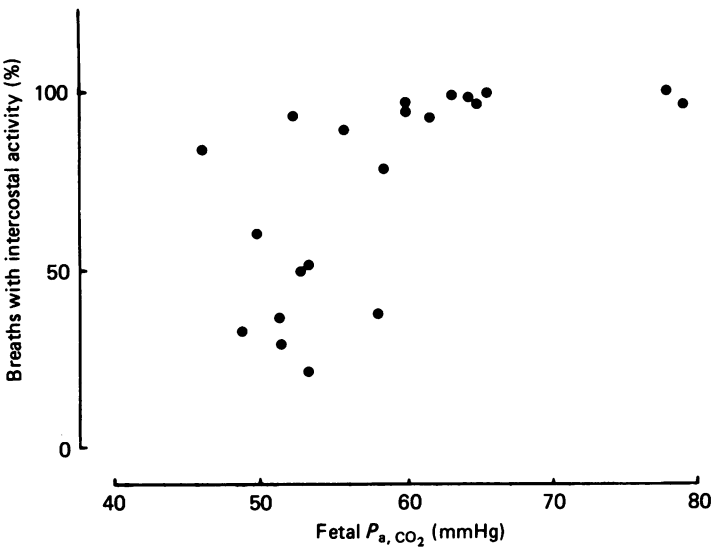

Fig. 6. The percentage of breaths with intercostal activity during inspiration, plotted against the arterial P_{CO_2} for a fetus of 123 days gestation. The P_{CO_2} was raised by progressively increasing the maternal inspired CO_2 in 6 min steps as discussed in Methods. The data from four runs are shown. Each symbol is the mean of the breathing which occurred over periods of between 3 min (hypercapnia) and up to 30 min (eucapnia).

DISCUSSION

Most previous studies of breathing in the chronic unanaesthetized fetal lamb *in utero* have been qualitative or only semi-quantitative. Chapman *et al.* (1980) were limited by computer size to the study of short records. Maloney *et al.* (1975) and Bowes *et al.* (1981) used the diaphragm e.m.g to estimate inspiratory and expiratory times only, and did not take account of electrocortical activity.

In the anaesthetized adult cat and dog breathing is normally regular and there is a progressive increase in firing and recruitment of inspiratory neurones as inspiration proceeds, with a consequent rise of activity in the phrenic nerve and diaphragm (Adrian & Bronk, 1928; Pitts, 1942; Lourenco *et al.* 1966; Eldridge, 1975; Iscoe, Dankoff, Migicovsky & Polosa, 1976). Especially in the younger fetuses this orderly recruitment was usually not seen and bursts were sometimes so fragmented that it

was difficult to distinguish discrete breaths. Clearly, this irregularity reflects irregular respiratory activity at the level of the brain stem. Whether this is of special significance is difficult to ascertain, since breathing in the new-born and adult during active (rapid eye movement) sleep is also irregular in depth and rate (Henderson-Smart & Read, 1978; Philipson, 1978). Immaturity and absence of normal afferent feed-back from the lungs and chest wall may also contribute to instability.

There are problems in interpreting the diaphragm e.m.g. In the unanaesthetized animal activity in different parts of the diaphragm is not uniform (Grassino, Whitelaw & Milic Emili, 1976; Henderson-Smart, Johnson & McClelland, 1979). Some of the differences we observed between fetuses may arise from this. E.m.g. amplitude is sensitive to electrode movements, to changes in the tissue covering the electrodes, to changes in initial length of the muscle fibres, to volume changes of the lung and to body posture (Grassino *et al.* 1976). In the fluid-filled fetal lung with variable and unpredictable efflux and influx of fluid, the initial lung volume and thus the initial muscle stretch are likely to be more variable than in the adult. Contractions are likely to be more nearly isometric. The relationship between electrical activity and contraction of the diaphragm is unlikely to be simple and it is perhaps not surprising that the relationship between the diaphragm e.m.g. and tracheal pressure amplitude in the fetal lamb is not as clear as that found in the adult when the e.m.g. and tidal volumes are compared (Katz *et al.* 1962; Lopata *et al.* 1980).

The first of the two types of hypercapnia experiments gives an estimate, based on many hours of observation, of the direction and degree of change of the major fetal respiratory variables in response to a single stepwise change in CO_2. The second set of experiments is concerned with smaller CO_2 changes over a wide range. The two studies are complementary and the results consistent.

The first study shows that both the diaphragm e.m.g.s and tracheal pressure are necessary to describe fetal breathing. Both the amplitude and inspiratory slope of tracheal pressure were closely correlated with P_{a,CO_2}; they might therefore be regarded as measures of breath depth, or central respiratory drive analogous to the use of tidal volume in the adult.

In contrast to the changes of tracheal pressure, the diaphragm e.m.g. was not consistently changed when the P_{a,CO_2} was raised. When single or sequential changes of P_{CO_2} were made the height, area and slope of the integrated diaphragm e.m.g. did not always increase progressively or proportionately as is usually found in conscious adult man (Lopata *et al.* 1980) and anaesthetized cats (Katz *et al.* 1962) and dogs (Lourenco *et al.* 1966).

Unlike the height, slope and area of the integrated e.m.g. the measurements of breath timing from the diaphragm allow of little ambiguity and are preferable to measurements derived from the tracheal pressure. This study shows that fetal breathing responds to changes in P_{a,CO_2} at an early age, before the differentiation of electrocortical activity into predominantly low or high voltage.

The second study with sequential changes of P_{CO_2} up to high (65–80 mmHg) levels shows that the response to P_{a,CO_2} is not all-or-none, but proportional over a wide range above the normal fetal values. Central chemoreceptors almost certainly mediate this response and appear to be mature as early as 110 days gestation.

At high levels of CO_2 both diaphragm and intercostal e.m.g.s showed evidence of

a plateau. This is consistent with findings in the adult; in conscious man, the CO_2 response curve eventually flattens (Lambertson, 1974), as also in the anaesthetized cat (von Euler, Herrero & Wexler, 1970; Richardson & Widdicombe, 1969). This plateau could explain the variable responses of the diaphragm e.m.g. in the first series of experiments, the change of diaphragm e.m.g. height depending on the initial position on the CO_2 response curve. The plateau could be due to saturation of either neuronal firing or of muscle fibre response.

Once again, tracheal pressure amplitude and inspiratory slope showed the most consistent and linear increase with increasing P_{CO_2}. The slope of the relationships provides a form of quasi-steady-state CO_2 response curve. Moss & Scarpelli (1979) have studied fetal lambs partially removed from the uterus with the ewe under spinal anaesthesia but their results are not directly comparable with ours. They have described CO_2 response curves obtained during maternal CO_2 re-breathing. There was no evidence in the present study to support a meaningful concept of CO_2 threshold; breathing failed to respond to CO_2 only when there was a switch from low- to high-voltage electrocortical activity. Moreover, steady-state responses in which equilibration of blood gases between brain stem and arterial blood is allowed to occur cannot be quantitatively compared with the re-breathing situation where there is a continually and unpredictably changing gradient for blood gases throughout the body.

Considering both sets of results, it is not unreasonable to conclude that T_I is not changed by CO_2 and that T_E either remains constant or shortens, especially at and just above the eucapnic range. However, changes in shape of their distributions with CO_2 makes interpretation difficult but suggest that there is a different preponderance of mechanisms working in hypercapnia than in eucapnia. In conscious man, in whom the pulmonary stretch reflex is weak (Guz, Noble, Eisele & Trenchard, 1970; Gardner, 1977), and in the conscious cat (Gautier, Remmers & Bartlett, 1973) an increase of CO_2 shortens T_E with little change of T_I over the eucapnic range. In the anaesthetized cat with a strong pulmonary stretch reflex both T_I and T_E shorten (Clark & von Euler, 1972). The present results resemble those in the conscious adult with the implication of weak feed-back from pulmonary afferents. Perhaps this is hardly surprising since the tidal volume of the fetal lungs is so small, and indeed, local block of the vagus nerve did not cause a gross change in fetal breathing (Dawes *et al.* 1972).

The breath-by-breath pattern *within* one level of chemical drive for both conscious man and the anaesthetized cat shows a positive correlation of tidal volume with both T_I and T_E (Priban, 1963; Clark & von Euler, 1972). Again, since tidal volume is normally so small, this association was not expected, or found, in the fetal lamb. The low correlation ($\sim 0{\cdot}01$) between tracheal pressure amplitude and expiratory time (measured between bursts of diaphragmatic activity) indicates that each breath has little influence on the occurrence or characteristics of the next.

In the new-born human hypoxia first increases and then reduces minute volume (Cross & Warner, 1951). Although hypoxia alone only decreases fetal breathing *in utero* (Boddy *et al.* 1974) it was thought that hypoxia might have a different effect in mild hypercapnia. However, the results suggest a simple addition between the depressant effect of hypoxia (Boddy *et al.* 1974) and the stimulant effect of CO_2. 'Picket fence' breathing as described by Chapman, Dawes, Rurak & Wilds (1978) in fetal lambs before death was never seen and must be attributed to a mechanism

other than asphyxia alone. There was no direct evidence from these experiments that the peripheral chemoreceptors, either aortic or carotid, actively influence respiratory activity *in utero*.

The variability between fetuses in the incidence of intercostal activity in eucapnia, and the recruitment during hypercapnia, is interesting and warrants further investigation. Intercostal activity is diminished or absent during rapid eye movement sleep after birth in lambs (Henderson-Smart & Read, 1978), and probably decreases during gestation, so that little can be recorded after 135 days (Harding, 1980). The role of reflex inhibition from intercostal muscle spindles and joint afferents in the fetus is unknown; nor is it known whether CO_2 provides an equivalent drive to diaphragm and intercostal spinal motoneurones. The fact that CO_2 caused an increase of pre-existing intercostal activity or the appearance of activity at sites previously silent suggests that their threshold for activity is at, or just below, the eucapnic range. It is possible that this threshold is raised progressively with advancing gestational age, and that the incidence of intercostal activity consequently decreases.

In conclusion, both the diaphragm e.m.g and the tracheal pressure are necessary to obtain a complete measure of breathing in the fetal lamb. The response to CO_2 is so similar to known responses in conscious man and the anaesthetized cat that it is almost certain that the central chemoreceptors are involved and functioning normally in the fetus at least after 109 days of gestation.

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