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BREATHING IN FETAL LAMBS: THE EFFECT OF BRAIN STEM SECTION

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SUMMARY

1. The effects of section of the brain stem caudally (through the upper pons or mid-collicular) or rostrally (through the caudal hypothalamus or anterior commissure–suprachiasmatic nucleus) were studied in fetal lambs from 118 days gestation, after recovery *in utero*.

2. In lambs sectioned *caudally*, breathing movements and electrocortical activity were dissociated. After some days recovery breathing tended to become continuous, with an abnormal prolongation of inspiratory time. Isocapnic hypoxia caused an increase in the rate and amplitude of breathing. After carotid denervation hypoxia still caused an increase in the amplitude of breathing.

3. In lambs sectioned *rostrally*, there was also dissociation between breathing movements and electrocortical activity. Breathing remained episodic, with an incidence similar to that of intact fetal lambs. In two lambs after 10 days of recovery the breathing and electrocortical rhythms returned, from time to time, to their normal phasic relationship. Isocapnic hypoxia caused a diminution or arrest of breathing, as in intact lambs.

4. The cardiovascular effects of transection were examined. Baroreflex sensitivity was normal in those lambs sectioned caudally and enhanced in those sectioned rostrally.

5. It is concluded first that as a result of rostral section, independent episodic rhythms of fetal breathing and electrocortical activity can be dissociated. Secondly, moderate isocapnic hypoxia causes arrest of fetal breathing indirectly, requiring the integrity of a suprapontine mechanism. And thirdly, after caudal section of the brain stem, hypoxia causes enhancement of fetal breathing efforts, independently of the carotid chemoreceptors. Possible mechanisms are discussed.

INTRODUCTION

In fetal lambs the electrocorticogram begins to differentiate into episodes of low- and high-voltage activity at about 115 days gestation. Before this time breathing movements are almost continuous. Thereafter they become increasingly episodic,

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with longer intervening periods of apnoea (Dawes, Gardner, Johnston & Walker, 1980*a*; Bowes, Adamson, Ritchie, Dowling, Wilkinson & Maloney, 1981). Near term breathing normally occurs only during episodes of low-voltage electrocortical activity, characteristic of rapid-eye-movement sleep (Dawes, Fox, Leduc, Liggins & Richards, 1972). Hypercapnia increases the amplitude of breathing, but it is still only present during low-voltage electrocortical activity (Boddy, Dawes, Fisher, Pinter & Robinson, 1974; Dawes, Gardner, Johnston & Walker, 1982).

Two hypotheses were considered for explaining these phenomena: first, that breathing is abolished in high-voltage electrocortical activity by descending inhibition, or secondly that breathing is enabled in low-voltage activity by descending facilitation of respiratory control in the medulla. Since breathing is almost continuous before differentiation of electrocortical activity, the first hypothesis was preferred. Section of the brain stem, to separate the lower pons and medulla from the source of high-voltage electrocortical activity, was undertaken to discriminate between the two hypotheses.

There was some evidence which suggested that it might be possible to relieve the episodic respiratory inhibition by operative interference. Though Barcroft & Barron (1937) devoted more attention to lambs before 100 days gestation, Barcroft (1947, p. 265) described one lamb 'sectioned anterior to the upper border of the pons and...observed as late as the 132nd [day]' with 'almost continuous respiratory movement and that without any specific stimuli'. The unoperated twin 'exhibited the properties of a normal foetus, its potential movement being profoundly inhibited'. And he added that 'the seat of inhibition is definitely in front of the pons'. Since he believed that breathing was totally inhibited from 60 days gestation, the observation has been difficult to interpret; intact fetal lambs near term may have episodes of breathing lasting for more than an hour and also long periods of apnoea.

We were also aware of experiments, published after this work had begun, by Robinson, Kingston & Thorburn (1980), who observed prolonged episodes of breathing in fetal lambs hypophysectomized by transcerebral passage of a cautery to the sella turcica. Electrocortical activity and the anatomical distribution of the lesions were not recorded. These observations suggested a more rostral location for the site responsible for episodic respiratory inhibition in the fetus.

Our first experiments showed that section of the brain stem through the upper pons or inferior colliculus caused dissociation of electrocortical activity and fetal breathing, and also abolished the arrest of breathing caused by mild or moderate hypoxia in intact fetal lambs (Dawes, Gardner, Johnston & Walker, 1980*b*). The results have been extended by rostral transection of the brain stem.

METHODS

In sixteen ewes at 118–123 days of pregnancy catheters were implanted in a carotid artery and jugular vein under halothane anaesthesia. Catheters were also implanted into a fetal carotid artery, jugular vein, trachea and the amniotic cavity, together with pairs of stainless steel electrodes on the parietal dura, around one orbit and into the diaphragm, intercostal and nuchal muscles (Dawes *et al.* 1972, 1982).

The fetal brain stem was sectioned, at levels which varied from the upper pons to the superior colliculus, by inserting a spatula through a small trephine hole 4–5 mm from the mid-line and half-way between the occipital and parieto-occipital sutures; the spatula was swept from side to

side against the base of the skull (lambs 1–5, 10, 11: Table 1). In two other fetuses the brain was transected in a similar manner at the level of the optic chiasma, using a trephine hole 5 mm rostral to the coronal suture (lambs 6, 7: Table 1). In two others a blunt knife, formed from stainless steel wire 0·75 mm in diameter shaped into a 12 mm diameter quadrant at its lower end, was introduced through a parasagittal slit in the temporal and parietal bones, and swept against the base of the skull 120° to either side of the mid-line to separate the cortex from the diencephalon (lambs 8, 9: Table 1).

TABLE 1. Lesions of the brain stem in operated fetal lambs

	Fetus no.	Position of lesion	Survival (days)	Gestational age at birth or termination	Birth wt. (kg)
Caudal transection	1	Upper pons	7	131[a]	4·4
	2	Upper pons	14	135[a]	4·1
	3	Upper pons	20	139[b]	3·4
	4	Pons–mid-brain	22	145[bt]	4·2
	5	Mid-brain–caudal hypothalamus	14	134[bt]	3·0
Rostral transection	6	Mid-hypothalamus	14	134[e]	4·4
	7	Optic chiasma–anterior hypothalamus	13	136[a]	4·2
	8	Anterior commissure–optic chiasma	21	138[bt]	3·5
	9	Anterior commissure–suprachiasmatic nucleus	18	137[e]	4·1
Caudal transection and carotid denervation	10	Upper pons	12	131[e]	—
	11	Upper pons	13	132[e]	—
Degenerate brain	12, 13, 14	Brain degenerate above cervical cord	6–19	122–132[a or e]	3·3–4·0

[a] Asphyxial death. [b] Spontaneous birth. [e] Elective section. [t] Twin.

Fetal arterial pressure (minus amniotic fluid pressure subtracted electronically), heart rate, electrocortical and electro-ocular activity, tracheal pressure (minus amniotic fluid pressure) and the electromyograms (e.m.g.s: direct or/and integrated) of the diaphragm, intercostal and nuchal muscles were recorded on Schwarzer polygraphs continuously, night and day. After the fourth post-operative day experiments were undertaken to measure the response to hypoxia as described by Boddy *et al.* (1974); the ewe was given either air to breathe in control periods, or 8·5 % O_2 with 2·5 % CO_2 in N_2 to maintain isocapnia. The sensitivity of fetal breathing to P_{a,CO_2} was estimated by raising the maternal inspired P_{CO_2} stepwise to 2·5, 5, 7·5 and 10 % for 6 min at a time. The details of data collection and analysis on a PDP 11/34 computer have been described elsewhere (Dawes *et al.* 1982). As a result of those experiments we chose to measure inspiratory time (T_I) from the diaphragmatic e.m.g., and CO_2 sensitivity from the change in tracheal pressure amplitude. Baroreflex sensitivity was measured after injection of phenylephrine (Dawes, Johnston & Walker, 1980). The effect of administration of pentobarbitone to the ewe was compared with the results in intact fetuses as described by Boddy, Dawes, Fischer, Pinter & Robinson (1976).

Blood samples were taken every day, and more often before, during and after hypoxia or hypercapnia, for analysis of blood gases and pH (Radiometer). For measurement of catecholamine content, samples were taken less frequently, and the frozen plasma stored for subsequent analysis.

The fetal brains were fixed *in situ* by arterial perfusion with formal saline (10 % w/v), removed when hardened and stored in formal saline for 4 weeks. They were examined to identify the site of the transection on the dorsal and ventral surfaces, and each was then divided longitudinally in the mid-line. One half was embedded and sectioned at 15 μm intervals. Three of every hundred

sections were stained with Haematoxylin and Eosin, Weil's or Luxolfast blue (or in one instance Cresyl Violet).

All tabulated results are expressed as means ± S.E.

RESULTS

Two lambs failed to survive more than 24 h after section of the brain stem, as a result of massive intracranial haemorrhage. Of the other fourteen, at autopsy three had total degeneration of the central nervous system above the spinal cord. There remained eleven in which the site and extent of the lesion were identified by post mortem examination and histological section in the parasagittal plane (Table 1). All the lambs were well grown.

The lesions varied from a narrow cut with little surrounding necrosis (1: Fig. 1), to a much larger loss of brain tissue in the immediate neighbourhood (3: Fig. 1). The positions of the cuts were identified and are shown diagrammatically in Fig. 2. In two brains (2 and 3: Fig. 2*B*) there was extensive loss of tissue in the upper pons and caudal mid-brain. In one brain (6: Fig. 2*C*) the section, centred approximately on the anterior hypothalamus and optic chiasma, had removed the hypothalamus and overlying thalamic pathways bilaterally.

Electrocortical activity

The pattern of low- or high-voltage electrocortical activity was not sufficiently clear to be measured until 4·0 ± 0·5 (S.E.) days post-operatively in fetuses sectioned caudal to the mid-brain (at or caudal to the root of the oculomotor nerve), or 6·3 ± 1·8 days for those fetuses sectioned rostrally.

Thereafter the ratio of the time spent in low activity to that spent in high activity was calculated day by day for each fetus, averaged, and the means measured for each group. Table 2 shows that data loss from all causes was greater for lambs transected rostrally; in the latter the electrocortical activity was more often of an indeterminate character. In one lamb (7: Table 1) the pattern never became clear; this lamb died after 13 days. In intact fetuses the ratio of low- to high-voltage electrocortical activity was 1·29 ± 0·6 (from Dawes & Robinson, 1976) as in the remaining three lambs with more rostral transections. In those sectioned more caudally the ratio was almost doubled.

There was no evidence of decerebrate rigidity from such e.m.g. records as were made *in utero*, or in those lambs which were delivered alive. One such lamb was allowed to survive for a short while, was in good condition as judged by its blood gas values and developed rigidity after several hours. One singleton lamb (3) was delivered spontaneously at 139 days gestation, which suggests that transection in the upper pons may not have impaired the fetal control of parturition.

Breathing after caudal section of the brain stem

After caudal section of the brain stem the incidence of breathing gradually increased (Fig. 3, interrupted line) so that after the tenth post-operative day the lambs were breathing almost continuously. Breathing was dissociated from electrocortical activity (Fig. 4).

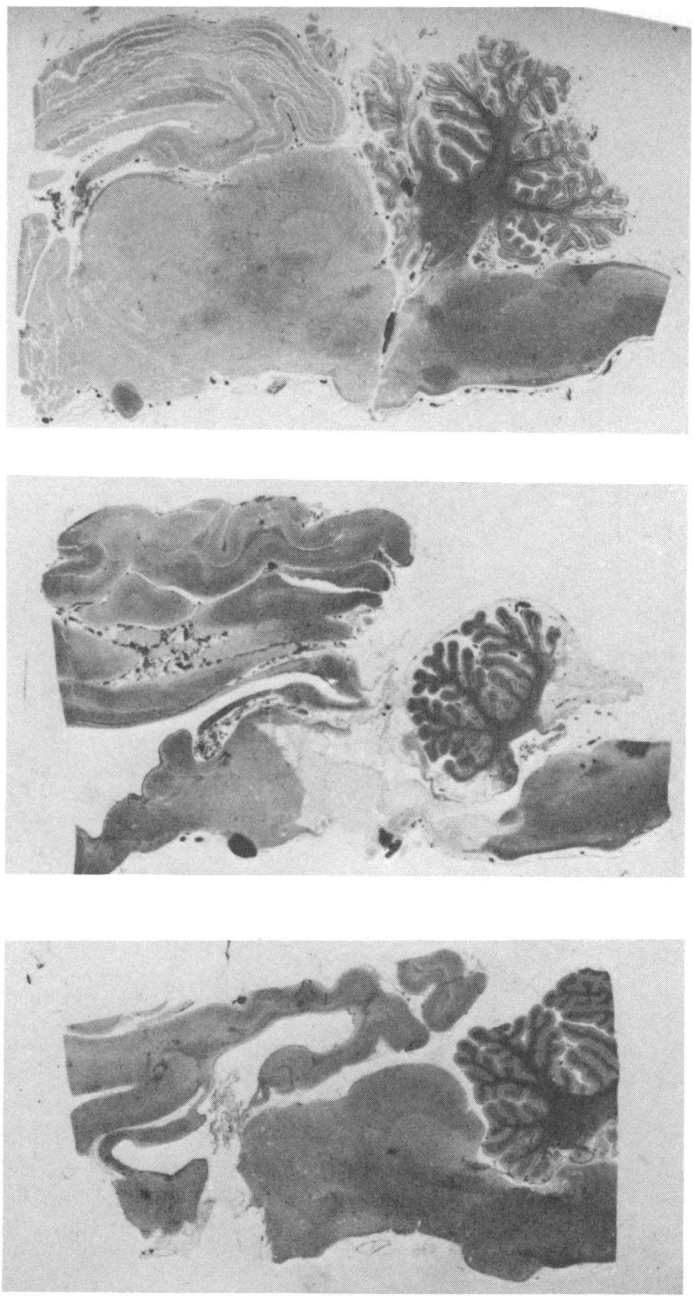

Fig. 1. Sections of brains, cut in the parasagittal plane 180 μm from the mid-line and stained with Weil's stain, from fetuses transected either caudally (1, 3) or rostrally (6) and enlarged twofold. The numbers refer to Table 1.

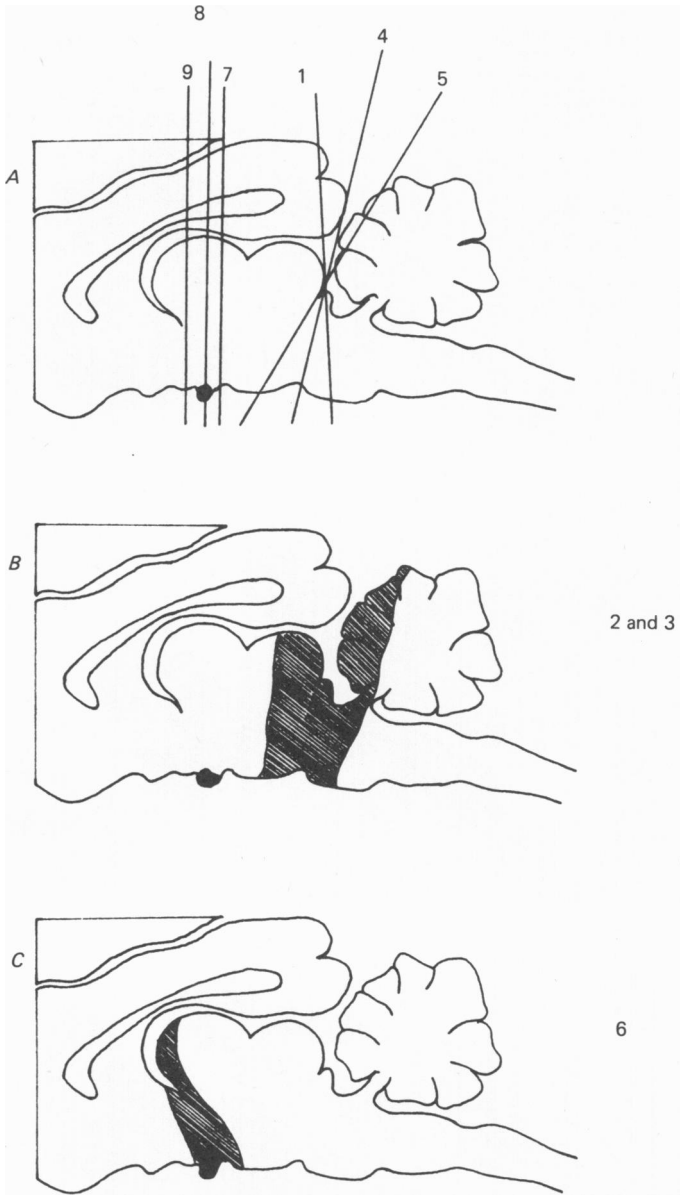

Fig. 2. Schematic diagram of the planes of section in fetuses with smaller (*A*) or greater (*B*, *C*) regions of brain necrosis. The numbers refer to Table 1.

Table 3 is designed to compare the character of fetal breathing in operated and normal lambs at the same gestational ages. Records were made continuously on all lambs. The data selected for computer processing were from at least an hour on at least 3 days, and comprised many thousands of breaths. The values for normal lambs are summarized from a previous paper (Dawes *et al.* 1982). When breathing was present in lambs 1–4, where the section entered the pons, inspiratory time was

prolonged ($T_I > 1$ s) compared with that in intact lambs (0·44 s). There was little difference in the amplitude of breathing measured from the tracheal pressure. As in intact lambs the frequency distribution of breath intervals was rather flat. In some lambs the modal breath interval was above that of intact lambs (Table 3), but the

TABLE 2. Ratio of low- to high-voltage electrocortical activity in fetal lambs whose brains were transected at different levels

	Period of observation (days)	Data loss* (%)	Ratio of low to high electrocortical activity
Caudal transections	40	14·9 ± 3·5	2·54 ± 0·13
Rostral transections	27	24·6 ± 7·5	1·36 ± 0·17
Five intact fetuses†	35	—	1·29 ± 0·06

* Proportion of time excluded because of experimental interventions, or (rarely) through instrumental failure, or because the record was of indeterminate character.

† Calculated from the data of Dawes & Robinson. (1976).

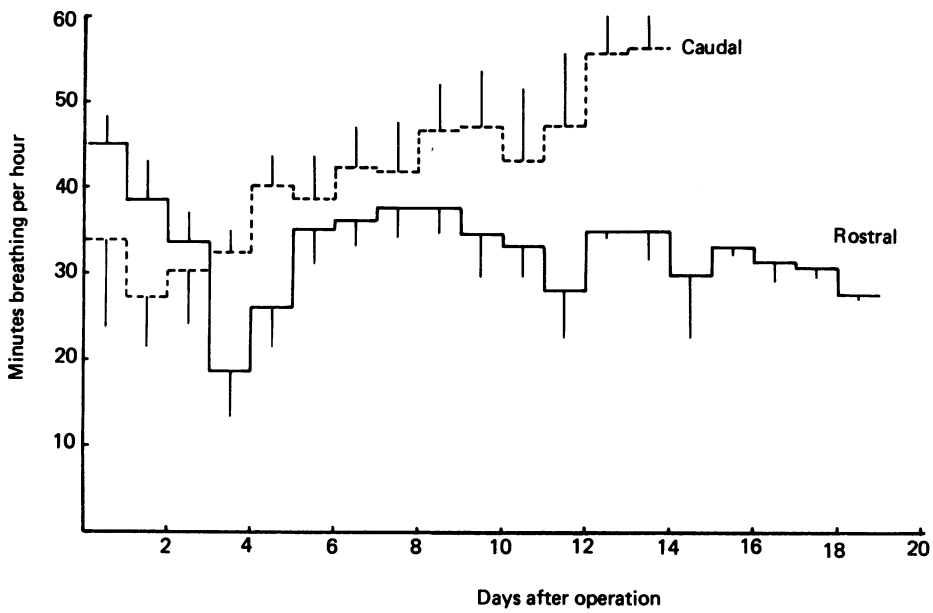

Fig. 3. Changes in the incidence of breathing (means ± S.E.) with time from operation in five lambs transected caudally (interrupted line) and in four transected rostrally (continuous line).

flatness of the frequency distribution made it impossible to conclude that the rate of breathing was significantly different.

The '*deep inspiratory efforts*' or '*gasping*' which occur from time to time during high-voltage electrocortical activity in intact fetuses (Dawes *et al.* 1972; Harding, 1980) were not identified in the breathing records of fetuses transected caudally. In one of these (5) breathing was often of such amplitude (> 10 mmHg tracheal pressure) as to make such discrimination difficult.

Table 4 (normoxia) shows that the blood gas values in lambs sectioned caudally were within the normal range for chronic unanaesthetized preparations *in utero*. The sensitivity of fetal breathing to changes in P_{a,CO_2} was tested by stepwise increases of maternal P_{CO_2}, while the inspired P_{O_2} was reduced to maintain the fetal P_{a,O_2} as nearly constant as possible. Table 3 shows that the CO_2 sensitivity (as judged by the increase in tracheal pressure change) was at least as great as that in intact fetuses. As in intact fetuses there was no significant change in mean or modal breath interval when the P_{a,CO_2} was increased. However, as in intact fetuses, the frequency

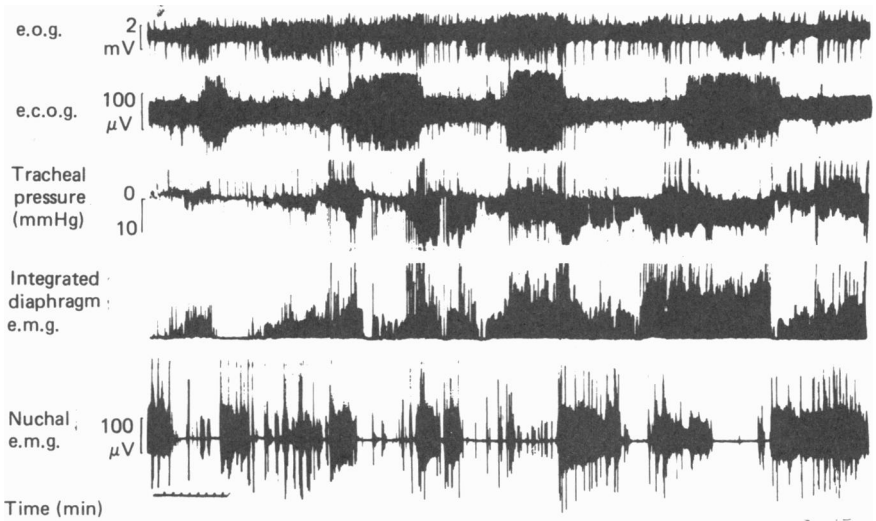

Fig. 4. Observations on the seventh post-operative day on lamb no. 5, sectioned in the mid-brain, in which breathing was almost continuous and dissociated from electrocortical (e.c.o.g.) activity. The eye movement record (e.o.g.) often showed episodic activity dissociated from both electrocortical and breathing.

distribution was reduced (i.e. breathing became more regular) during hypercapnia. Hypercapnia caused no significant change in inspiratory time.

In intact fetal lambs isocapnic hypoxia causes an arrest or gross diminution of fetal breathing movements when the P_{a,O_2} is reduced from its normal value (about 23 mmHg) by 5–7 mmHg (Boddy, *et al.* 1974). In fetal lambs with caudal section of the brain stem breathing was almost continuous before hypoxia was induced. The P_{a,O_2} fell from 22 to 13 mmHg with no significant change in P_{a,CO_2} or pH (Table 4). Fetal breathing was not merely well maintained, but increased in rate and, on average, more than doubled in depth over 10 min in twenty-eight trials in the lambs transected caudally (Fig. 5*A*). There was no consistent change in inspiratory time.

The possibility was considered that the enhancement of fetal breathing during hypoxia might have been due to an increase in the activity of the carotid chemoreceptors. In two fetal lambs, therefore, the carotid nerves were cut and the bifurcations were thoroughly stripped at the time of caudal brain transection (lambs 10 & 11 of Tables 1 and 3). Their blood gas values were in the normal range; the amplitude of

TABLE 3. Breathing in brain-transected lambs at 125–132 days, and in intact lambs

Lesion	Fetus no.	Tracheal pressure amplitude (mmHg)	T_I*(s)	Modal breath interval*(s)	CO_2 sensitivity† (mmHg t.p./ mmHg P_{a,CO_2})	Response to hypoxia
Caudal transection	1	4·4 ± 0·5	1·8 ± 0·2	0·78	0·30 ± 0·04 (1)	+
	2	—	1·6 ± 0·4	1·60	—	+
	3	3·0 ± 0·15	1·1 ± 0·1	0·72	0·23 ± 0·06 (3)	+
	4‡	7·2	1·2	—	—	+
	5	4·1 ± 0·9	0·6 ± 0·07	1·1	0·53 ± 0·09 (1)	+
Rostral transection	6	3·8 ± 0·5	0·46 ± 0·04	0·60	0·43 ± 0·12 (1)	−
	7	4·9 ± 0·4	0·26 ± 0·03	0·60	0·59 ± 0·12 (3)	−
	8	5·5 ± 1·4	0·31 ± 0·3	0·61	0·98 ± 0·12 (1)	−
	9	3·8 ± 0·4	0·24 ± 0·02	0·51	0·42 ± 0·15 (3)	−
Caudal transection and carotid denervation	10	6·2 ± 0·8	1·3 ± 0·1	1·10	—	+
	11	7·2 ± 0·7	1·6 ± 0·1	0·86	—	+
None§		4·0 ± 0·3	0·44 ± 0·02	0·69	0·23 ± 0·08 (8)	−

The number of experiments is given in parentheses. +, maintenance of breathing and increase in amplitude; −, decrease or arrest of breathing.

* Measured from diaphragm e.m.g.

† Computed from tracheal pressure (t.p.).

‡ No computer records; data estimated from polygraph records.

§ Five lambs at 122–130 days gestation (Dawes *et al.* 1982).

TABLE 4. Changes in carotid blood gas values in fetal lambs subjected to hypoxia for 15 min, arranged according to the site of brain section

	Number of experiments		P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH
Caudal transection*	28	Normoxia	22·4±0·56	48·4±0·68	7·343±0·006
		Hypoxia	12·9±0·28	48·5±0·69	7·327±0·007
Rostral transections	10	Normoxia	21·7±0·68	51·2±1·5	7·376±0·013
		Hypoxia	13·5±0·47	46·7±0·3	7·379±0·013
Degenerate	6	Normoxia	25·2±0·50†	49·4±0·67	7·369±0·007
		Hypoxia	13·1±1·4	61·0±5·0	7·232±0·027

* Including two lambs with carotid denervation.

† $P < 0·05$, compared with normoxic values of lambs with successful caudal or rostral transections.

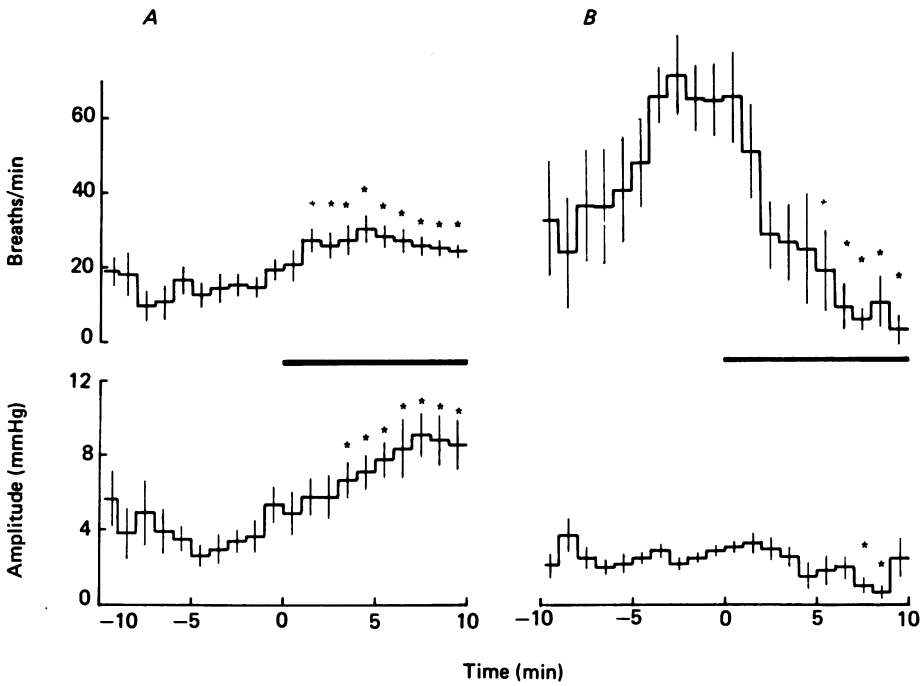

Fig. 5. Changes in breathing on exposure to hypoxia, from time zero (heavy line) in lambs transected caudally (*A*, twenty-eight experiments) and in four transected rostrally (*B*, ten experiments). The values shown are the means±S.E. (*$P < 0·05$ from the mean of control values in the 10 min before hypoxia).

breathing was high and inspiration was prolonged (Table 3; the plane of section was through the upper pons). The modal breath interval was within the normal range for lambs sectioned at this level. Judged by these criteria, breathing in normoxia was not altered by carotid denervation. The sensitivity to CO_2 was not tested. Isocapnic hypoxia was induced to the same degree as in the previous experiments. During hypoxia, breathing movements were increased in amplitude ($P < 0·05$ in six trials); the rate did not change significantly. We conclude that the maintenance or

enhancement of fetal breathing during hypoxia in the caudally sectioned fetuses was not mediated by the carotid chemoreceptors.

The possibility was also considered that the enhancement of fetal breathing during hypoxia in lambs with caudal section of the brain stem might be due to progressive development of metabolic acidosis in the medulla, whose blood supply might have been impaired as a result of the operation. If this were so, then more prolonged exposure to hypoxia, in place of the 10–15 min trials normally used, might be expected to produced a different outcome. In experiments on three fetuses sectioned caudally (with carotid nerves intact) the greater depth of breathing was well maintained during isocapnic hypoxaemia for 1–1·5 h (e.g. Fig. 6).

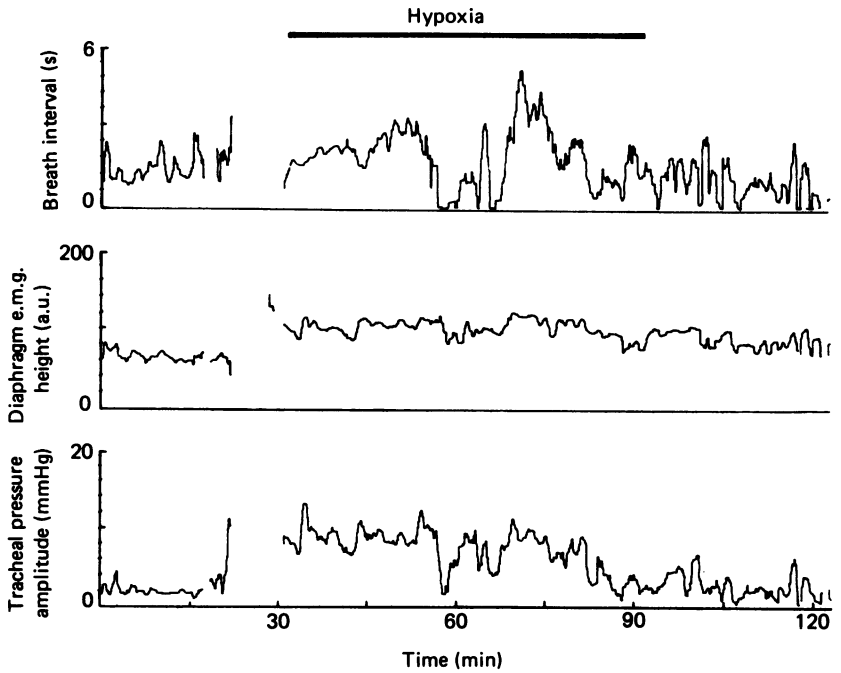

Fig. 6. Re-plot from the computer analysis of data from fetus 3, transected caudally, to show the persistence of vigorous breathing during an hour's hypoxia. The P_{a,O_2} fell from 21·7 to 12·8 mmHg with no significant change in P_{a,CO_2} or pH.

Breathing after rostral section of the brain stem

Rostral section of the brain stem gave different results in many respects. After rostral section the fetuses exhibited a pattern of episodic breathing which occupied about half the time after the tenth post-operative day (Fig. 3, continuous line), not very different from intact fetuses. But initially there was a brief increase in the incidence of breathing, lasting on average not more than a day, during which breathing often was continuous for some hours.

In three fetuses with rostral sections, in which episodic low or high electrocortical activity eventually appeared, although the breathing also was episodic there was no consistent relation between these episodes and low-voltage electrocortical activity

(e.g. Fig. 7) based on analysis of 630 hours of data. However, in two of these lambs (8, 9) after 10 days post-operative recovery an association did appear, discontinuously, between episodic breathing and low-voltage electrocortical activity, which persisted from time to time until termination by birth or elective Caesarean section 8–11 days later.

The mean tracheal pressure amplitude, inspiratory time and the modal breath interval of fetuses with rostral sections were similar to those in intact fetal lambs (Table 3). Trains of regular 'deep inspiratory efforts' also were present between episodes of rapid irregular breathing. Their blood gas values in normoxia were within the normal range (Table 4). The response to hypercapnia was qualitatively normal but, on average, more than doubled in amplitude (Table 3).

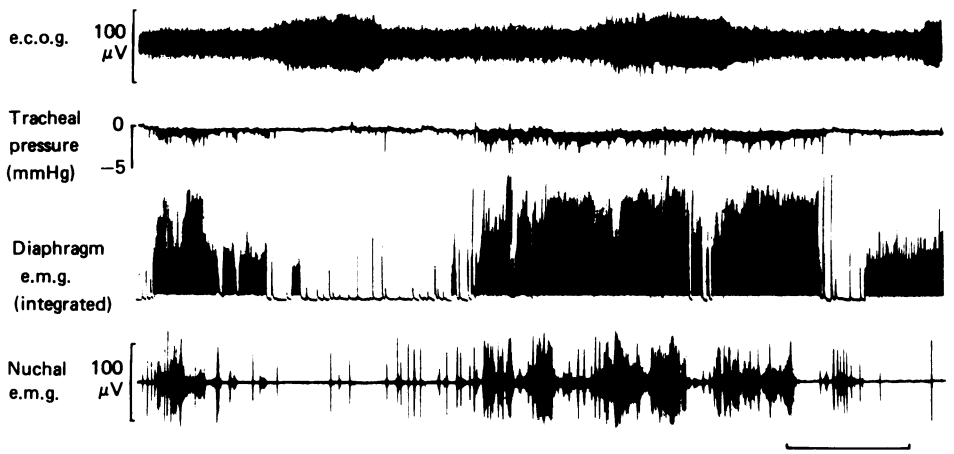

Fig. 7. Observations on the thirteenth post-operative day on lamb no. 8, sectioned rostrally, in which both electrocortical activity (e.c.o.g.) and breathing were episodic but dissociated. Nuchal activity was closely associated with breathing movements.

Isocapnic hypoxia was induced to the same extent as already described, with a mean fall in P_{a,O_2} from 22 to 13 mmHg (Table 4), 12–15 min after the start of an episode, i.e. when the mean rate had risen to about 60/min (Fig. 5*B*). These fetuses behaved like intact lambs; the incidence of breathing fell rapidly to approach zero after a few minutes. Breathing was grossly diminished or arrested.

Cardiovascular status

The resting arterial blood pressure and heart rates of the brain-sectioned fetuses (excluding those with degenerate brains) were within the normal range for their gestational ages (Table 5). The baroreflex, tested by injection of 50–100 μg phenylephrine into the fetal jugular vein to cause a rise in arterial pressure to 65 mmHg or more, was present in all of them. It was somewhat greater in the lambs sectioned rostrally than in intact lambs. Heart rate variability appeared unaltered.

In seventeen experiments on six lambs hypoxia caused a small rise in arterial pressure and fall in heart rate, followed by a transient post-hypoxic tachycardia,

irrespective of the level of section. In one lamb (3) there was no bradycardia in four such experiments. There was a similar rise in arterial pressure and post-hypoxic tachycardia in the lambs whose carotid bifurcations were denervated. There was no systematic change with age (121–137 days gestation) in normoxic plasma noradrenaline, sampled every other day. Hypoxia caused a rise in plasma adrenaline and noradrenaline of the same size as in intact lambs, irrespective of the level of section (H. Lagercrantz, personal communication).

TABLE 5. Cardiovascular data in fetal lambs with caudal or rostral transections, or with degenerative brains

Lesion	Fetus no.	Arterial* pressure (mmHg)	Heart rate* (/min)	Baroreflex sensitivity† (ms/mmHg)	
Caudal transection	1	43·8 ± 1·0	170 ± 3·0	3·0 (1)	5·5 ± 0·8
	2	45·6 ± 1·2	168 ± 3·5	7·0 ± 1·3 (6)	
	3	45·9 ± 0·6	169 ± 2·6	5·9 ± 1·3 (5)	
	4	41·6 ± 1·0	166 ± 3·0	4·5 ± 0·6 (7)	
	5	42·0 ± 1·3	178 ± 2·4	7·2 ± 2·2 (4)	
Rostral transection	6	47·1 ± 2·0	150 ± 3·1	5·1 ± 1·1 (4)	9·5 ± 1·6
	7	44·2 ± 1·7	150 ± 3·0	12·1 (2)	
	8	44·9 ± 1·2	168 ± 3·2	8·8 ± 2·6 (6)	
	9	49·7 ± 1·0	186 ± 2·9	11·9 ± 1·8 (6)	
Degenerate brain	12	39·8 ± 0·7	134 ± 1·3	0 (3)	
	13	27·7 ± 1·5	155 ± 3·0	0 (2)	
	14	40·0 ± 0·7	—	—	
None‡		44·0 ± 1·8	156 ± 1·5	5·4 ± 0·5	

* Mean of the average daily arterial pressure and heart rate from day 3 post-operatively to 3 days before death or delivery (range 125–140 days).

† Slope (pulse interval/arterial pressure): number of phenylephrine injections in parentheses.

‡ Mean values in four normal lambs (Dawes, Johnston & Walker, 1980).

Nuchal and oculomotor activity. In intact lambs the activity of the neck muscles recorded from e.m.g. electrodes was greater during high-voltage electrocortical activity. In lambs whose brains were transected caudally there was more nuchal activity, not related to episodes of electrocortical activity (Fig. 4). In lambs with rostral transections there was some evidence of episodic activity, in association with high-voltage activity or with breathing (e.g. Fig. 7).

It was to be expected that the more caudal transections would have damaged the oculomotor nuclei and outflow tracts, and indeed the records from the eye often were bizarre (e.g. Fig. 4). Neither hypercapnia nor hypoxia caused a consistent change in nuchal or oculomotor activity.

Pentobarbitone. Intravenous injection of 240–300 mg pentobarbitone to the ewe (calculated as 4–5 mg/kg) caused a minor degree of maternal sedation and no significant change in fetal blood gas values. This result was similar to that observed previously in ewes with intact fetuses (Boddy *et al.* 1976), in which, however, there was arrest of normal rapid fetal breathing movements for 30–40 min accompanied by prolonged high-voltage electrocortical activity. In contrast, in six trials in three ewes with fetuses whose brains were sectioned at the upper pons or hypothalamus

(2, 3, 6) fetal breathing movements persisted after pentobarbitone injection. There was no systematic change in fetal electrocortical activity. In one trial on fetus 9, with the most rostral section, breathing ceased.

Lambs with degenerate brains. In three lambs the brains were found at autopsy to be degenerate above the upper end of the cervical cord. The P_{a,CO_2} was within the normal range, but the P_{a,O_2} was greater (Table 4). No electrocortical activity or breathing movements were observed, in eucapnia, hypercapnia or hypoxia. The heart rate record was flat, showing no evidence of normal short-term variation. Hypoxia, induced in the standard way by giving the ewe 8·5% O_2 with 2·5% CO_2 in N_2 to breathe, caused the normal changes in the maternal blood gases, and reduced the fetal P_{a,O_2} to 13 mmHg as in the other groups; however, the P_{a,CO_2} rose to 61 mmHg on average and pH fell (Table 4). This was a consequence of progressive deterioration in one lamb. There was usually a small rise in arterial pressure (4–9 mmHg) in six trials. There was no fall in heart rate; after several minutes there was a gradual rise in heart rate to > 200 beats/min. There was no baroreflex as judged by the heart rate response to phenylephrine injection.

DISCUSSION

It was interesting to find that the lambs fell so clearly into two groups, according to the level of section caudally or rostrally, in terms of the effect of section on electrocortical activity, on breathing and on the response of breathing to hypoxia. The results were similar whether the section led to little necrosis or to a relatively large loss of brain tissue (Fig. 1). We can therefore be reasonably confident that the longer term results, with passage of days or weeks from operation, were not attributable to reconnexion of pathways. On the other hand there were more rapid changes, such as the brief increase in the incidence of fetal breathing in lambs sectioned rostrally (Fig. 3, continuous line) during the two days after operation, which might have been due to local haemorrhage or oedema, with subsequent recovery.

Electrocortical activity. In normal mature fetal lambs *in utero* there is a diurnal variation in electrocortical activity and in fetal breathing (Boddy, Dawes & Robinson, 1973; Dawes & Robinson, 1976). We did not attempt to measure this in the present experiments. When experimental interventions were excluded the ratio of low- to high-voltage electrocortical activity was similar in intact fetuses and in those with rostral sections. However, in fetuses with caudal sections the ratio on average was nearly doubled; the incidence of low-voltage activity was thus increased from about 56% to 71% (ignoring sections of record which could not be analysed or were otherwise excluded). The figure of 56% for the normal incidence of low-voltage activity agrees well with that reported in other experiments (55·4%: Boddy *et al.* 1974). The results show that, in the fetal lamb as in adult animals (Villablanca, 1962; Sprague, 1967; Moruzzi, 1972), episodic variations in electrocortical activity return after gross sections of the brain stem, whether or not there is extensive loss of tissue in the upper pons. The fact that the incidence was changed also agrees with observations in adult cats, which recover rapidly from mid-pontine transections, when the proportion of low-voltage fast electroencephalogram activity is increased compared with the intact cats (Batini, Moruzzi, Palestini, del Rossi & Zanchetti, 1969).

The absence of electrocortical activity in the lambs with degenerate brains shows that its presence in other lambs is not an artifact.

The results also have some bearing on the site of origin of the episodic element in electrocortical activity. In two lambs (8, 9) the cortex was separated from the diencephalon by a wide (240°) cut, leaving only a slim connexion to the base of the brain caudally. Yet in these two fetuses episodic variations in electrocortical activity eventually returned, at a normal low- to high-voltage ratio. This would be consistent with the conclusion that, as in adult cats, gross transverse sections to divide the cortex and mid-brain do not abolish episodic variations in electrocortical activity. We cannot be sure that the activity we have recorded arises from the cortex; it could arise from deeper structures.

Breathing. One of the unexpected results is illustrated in Fig. 7, which shows that, as a result of rostral section of the brain stem, for many days breathing and electrocortical activity were both episodic yet dissociated. The spontaneous episodes of apnoea were independent of high-voltage electrocortical activity. In these circumstances both activities had independent natural episodic rhythms, which came into phase from time to time after 10 days recovery in the two lambs with the most rostral transections. The operation of rostral transection might be regarded as a perturbation which had temporarily thrown the two systems out of phase, but had not permanently disconnected them.

It could still be that under natural circumstances, with an intact brain, breathing movements are inhibited, for progressively increasing periods of time with increasing age, by signals from the structures responsible for high-voltage electrocortical activity, and that these are permanently disconnected from the medulla by caudal transection. Even in these lambs, in which breathing movements ultimately became present all the time, there were intervening days in which breathing was episodic and dissociated from electrocortical activity. Whether that could be attributed to episodic failure of the hypothetical rhythm-generator to reach threshold as a result of the operation, and of the anatomical and physiological adjustments during recovery, is uncertain.

Hypercapnia. The experiments with hypercapnia showed that, in spite of the apneustic breathing (i.e. with prolonged inspiration) of lambs in which the section passed through the upper pons, the response to CO_2 was similar to that in lambs with intact brains. Judged by this criterion the main respiratory centre was intact. And the results were the same whether the section was more caudal (1–3: with prolonged inspiration), or with more normal breathing (5: Table 3). The sensitivity to CO_2 was greater than normal in lambs transected rostrally (Table 3), suggesting that there was normally a degree of control by higher centres.

Hypoxia. The effect of a mild degree of isocapnic hypoxia in intact fetal lambs is to cause depression or arrest of breathing movements (Dawes, 1973; Boddy *et al.* 1974). This response of the fetus differs remarkably from the hyperventilation induced by moderate hypoxia post-natally, which is largely due to carotid body stimulation. The fetal response is appropriate to energy conservation in the face of a reduction in O_2 supply. When this hypoxic depression of fetal breathing was first observed we assumed that it could be attributed to a direct action on the medullary centres, as is commonly supposed in adult animals subjected to severe hypoxia. The

present evidence demonstrates that, in the fetal lamb near term, the effect is indirect and requires the presence of a region between the caudal hypothalamus and the inferior colliculus. It does not follow that this region should of itself be especially sensitive to hypoxia, only that it should be intact and connected to the medulla.

Reference has already been made to the experiments of Robinson *et al.* (1980) who observed abnormal prolongation of episodes of fetal breathing after transcerebral cauterization of the fetal pituitary. The present observations suggest the possibility that this procedure may entail damage more caudally, a conclusion supported by further experience with this method of hypophysectomy (M. Parkes & D. Walker, unpublished observations), with occasional extensive degeneration of the cortex and mid-brain, and persistence or enhancement of fetal breathing during hypoxia.

The possibility was considered that the increase in breathing on exposure to hypoxia of lambs with caudal section of the brain stem might have been due to the rapid development of local respiratory acidosis in the medullary centres. While the observations made do not exclude this possibility, it is unlikely for two reasons. First, the same result was obtained in all seven fetuses (five with caudal section alone, two with carotid denervation as well), independent of the level of section from the pons to mid-brain (Table 1), and with a wide variation in tissue loss (compare the upper two sections of Fig. 1). And secondly the enhancement of breathing was well maintained but not progressive, even when hypoxia was continued for an hour (Fig. 6).

Analogies with experiments in animals after birth are not simple, since the range of P_{a,O_2} (24–13 mmHg on average in the present experiments) is below that usually investigated post-natally and since the fetal carotid chemoreceptors are not readily excited to induce breathing, even by local injection of cyanide (Dawes *et al.* 1969); the doses were higher than those required postnatally, This lack of responsiveness may be due to relative insensitivity of the fetal peripheral transducer mechanism or of the respiratory centre to carotid afferent signals. The present experiments show that if the latter explanation is correct, it cannot be due to descending inhibition from above the pons.

Tenney, Scotto, Ou, Bartlett & Remmers (1971) produced a schematic diagram of the principal excitatory and inhibitory pathways they considered important in hypoxic ventilatory control, based on their extensive work in adult cats. Our data do not fit their paradigm in three respects. First , we found an *inhibition* of breathing from a suprapontine region below the cortex. Secondly, there was excitation of breathing from centres below the mid-brain other than via the carotid chemoreceptors. And thirdly, in contrast to what has generally been believed to be true in adults, hypoxia (13 mmHg P_{a,O_2}) did not depress the respiratory centres by a direct action in the fetal lamb.

We come to visualize the control of breathing in the fetal lamb near term as dependent on a wide region of the brain stem, from the medulla to the colliculi. The fact that breathing movements continue, though with prolonged inspiration, after section of the upper pons, shows that the executive neurones lie more caudally. But properly co-ordinated movements, with full range of normal responsiveness to changes in the blood gases, require the integrity of the upper pons and brain stem as far forward as the caudal hypothalamus. Whether any part of the region deserves

the description of central chemoreceptor (to O_2 lack), as the term has been used elsewhere, is uncertain.

We may also consider, in the same context, the mechanism by which the ratio of low to high electrocortical activity is varied upwards or downwards by a rise in P_{a,CO_2} or fall in P_{a,O_2} respectively (Boddy *et al.* 1974). Yamasaki & Lico (1981) have shown that electrocortical activity can be modulated by micro-injection of 5-hydroxytryptamine into the nucleus of the solitary tract or by lesions of the area postrema. So it may be economical to suppose that modulation of the electrocorticogram by changes in the blood gases is effected through the same transducer mechanisms which control breathing, rather than by a direct action.

Finally we may consider the cortex and brain stem in their conjoint role of co-ordinating the patterns of fetal breathing, electrocortical activity and muscular movements. The pattern that is finally established in late gestation begins to be laid down at about 110–115 days of gestation. In our present series of experiments conducted between 125 and 140 days of gestation we have seen, in lambs sectioned rostrally, a reversion to immature patterns (Dawes *et al.* 1980*a*); Fig. 7 shows an example. So we conclude that though the mid-brain may generate the episodic rhythm which controls breathing and the activity of other fetal muscles, for full co-ordination connexion with an intact cortex also is required.

The experiments with low doses of pentobarbitone show that, like hypoxia, it normally arrests fetal breathing by an indirect suprapontine mechanism. In normal lambs it caused a prolonged episode of high-voltage electrocortical activity during which breathing ceased (Boddy *et al.* 1976). In the present experiments with section of the brain stem, the same dose had no effect on fetal breathing except in one fetus sectioned most rostrally. The consequences of hypoxia or pentobarbitone in intact lambs are not identical, since hypoxia causes only an increased incidence of high-voltage electrocortical activity. Low-voltage activity does occur for a reduced proportion of time, but breathing movements are nevertheless arrested or much reduced in amplitude. Therefore, though it seems likely that the same region of the mid-brain is required to be intact and connected via the pons to the medulla for the normal exhibition of the effects of hypoxia and pentobarbitone, the sites of action may not be identical.

Lambs with degenerate brains. The lambs with degenerate brains survived for many days after operation. They were characterized by the absence of breathing movements in normoxia, hypercapnia or hypoxia, together with a flat heart rate trace and the absence of a baroreflex. The fact that short-term variations in heart rate were absent in these lambs, though apparently of normal size in lambs with caudal transection of the brain stem, shows that the variations have a medullary origin independent of the cerebral cortex.

Because of the arrangement of the fetal circulation, a reduction in O_2 uptake results in a rise in P_{a,O_2}, up to a maximum of 45–50 mmHg (limited by O_2 consumption in the placenta: Dawes, 1968). Hence the fact that the P_{a,O_2} of this group of lambs was above normal (Table 4) could be due to reduced O_2 uptake, through loss of their brain and less muscular movements including breathing. The mature intact fetus is well able to respond to hypoxia by redistributing its cardiac output in such a way as to maintain or increase umbilical flow and maintain O_2 supply to the heart and the central nervous

system; acidaemia is of slow onset (e.g. Boddy *et al.* 1974). The lambs with more caudal brain stem transections had a slightly greater fall in pH over 15 min than those cut rostrally (Table 4), suggesting that the mid-brain makes a contribution to the normal response. The observations on lambs whose brains were degenerate above the cervical cord suggests that the response in normal lambs depends also on the integrity of the medullary centres.

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