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THE RESPONSE OF FOETAL SHEEP AND LAMBS TO PULMONARY INFLATION

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SUMMARY

1. Pulmonary inflation through a tracheostomy consistently produces an inspiratory response or gasp in the foetal lamb. This response is reversibly removed by vagal cooling or xylocaine local anaesthesia to the air passages. It is abolished by bilateral vagotomy. It is therefore suggested that this gasp response involves a reflex whose afferent pathway is in the vagus nerve.

2. This gasp reflex is also found in lambs aged 1 hr to 30 days.

3. Lambs and foetuses possess a cough reflex.

4. A Hering–Breuer inflation reflex can be demonstrated in lambs.

5. The gasp reflex is distinct from either of these last two.

6. The significance of the gasp reflex in pre- and post-natal life is discussed.

INTRODUCTION

Inspiratory responses to pulmonary inflation have been described in the rabbit (Head, 1889), the cat (Larrabee & Knowlton, 1946; Widdicombe, 1954*a*, *b*, *c*; Reynolds, 1962) and the new-born infant (Cross, Klaus, Tooley & Weisser, 1960). Although Barcroft (1946) studied respiratory movements in foetal lambs, he did not mention such a response. In the course of experiments to measure foetal arterial oxygen tensions under different conditions a Malecot catheter was inserted into the intrapleural space and intrapleural pressures measured as a gauge of respiratory movement. The trachea was cannulated and it was noted that inflation through this cannula produced marked negative pressure deflexions, i.e. gasps. We therefore compared the responses of foetal and new-born lambs and adult sheep to inflation of the lungs.

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METHODS

Foetuses

Experiments were carried out on foetuses obtained by breeding 2-year-old Rambouillet cross ewes to Suffolk rams.

Twenty-five foetuses between 90 days gestational age and term (147 days) were studied. In these experiments ewes were given a spinal anaesthetic of 1 or 2% xylocaine administered intrathecally by a subdural polyethylene catheter. They were laid on their right side, and the foetuses delivered by Caesarian section on to a heated table level with the ewe. A saline filled surgical glove was placed over the head of the foetus and once the preparation was complete the foetus was covered to prevent cooling. Care was taken not to twist or stretch the umbilical cord. We observed no placental separation or cord spasm, and could continue experiments for several hours with the foetus in good physiological condition as judged by the following criteria: *Rectal temperature* was $40 \pm 1°$ C on delivery, and never fell below 38° C; The *blood pressure* and *pulse rate* were within the ranges given by Barcroft (1946), whilst the *carotid arterial blood oxygen tensions* (18–25 mm Hg) corresponded to his values of saturation and did not fall during the course of experiments.

Local anaesthetic (1% xylocaine) was infiltrated over an area of the right chest wall and neck. A rubber Malecot catheter (Bardex No. 10 or 12) was inserted through the sixth or seventh rib space into the right pleural cavity and connected to a Statham strain gauge to record intrapleural pressure. Tracheostomy was performed and a glass T-tube cannula inserted. One arm of this was connected to another Statham gauge to record intratracheal pressure whilst the other arm was used for inflation. As we felt that care was needed with such inflations of foetal lung, they were carried out by means of a 50 ml. tight fitting syringe or by direct oral inflation through a short length of rubber tubing. The trachea was found to be fluid-filled on incision. The fluid was allowed to drain but was not deliberately aspirated.

Types of inflation. Three types of inflation were used. Type A were brief inflations of 1 sec or less with a rapid rise of intratracheal pressure to 20–50 mm Hg. Type B were prolonged inflations of about 10 sec producing an approximately 'square wave' intratracheal pressure change. Type C were also prolonged inflations of about 10 sec, but taking 6–8 sec to reach a plateau. Types B and C inflation were usually carried out at intratracheal pressures of 20–30 mm Hg. When a syringe was used in the foetus the volume of air introduced to produce the necessary pressure rise was 10–15 ml. Figure 1 is a diagram of the types of inflation and the actual changes in intratracheal and intrapleural pressures that occurred with the three types of inflation in a foetus aged 124 days.

Local anaesthetic. 1% xylocaine (between 1·5 and 2·5 ml./kg body weight) was instilled through the tracheal cannula.

One or both vagus nerves were cut high in the neck in some experiments. The vagal trunks were cooled to 4° C in four foetuses by placing them on hollow brass thermodes (1·2 cm long) through which ice water was circulated.

A carotid artery was cannulated to record pulse rate and blood pressure with another Statham gauge: samples of carotid blood (1–2 ml.) were withdrawn and their oxygen tensions measured using a modified Clarke oxygen electrode and a Beckman 160 read-out meter. Simultaneous tracings from the strain gauges were recorded on a multi-channel Grass polygraph.

Lambs

Fifteen lambs between the ages of 1 hr and 30 days were studied. Those less than 24 hr old (three animals) were delivered by Caesarian section and the remainder were born spontaneously. Anaesthesia was as follows:

1. Six animals received intravenous thiopentone, using 10–15 mg/kg body weight for

induction and additional doses as required. These lambs were deeply anaesthetized during the initial surgical procedures, but were lightly anaesthetized during the inflations.

2. Nine animals received pentobarbitone sodium (Diabutal) given intraperitoneally or by slow intravenous injection (25–30 mg/kg body weight). This produced deep anaesthesia throughout the experiment.

Otherwise the same procedures as those employed for foetuses were used.

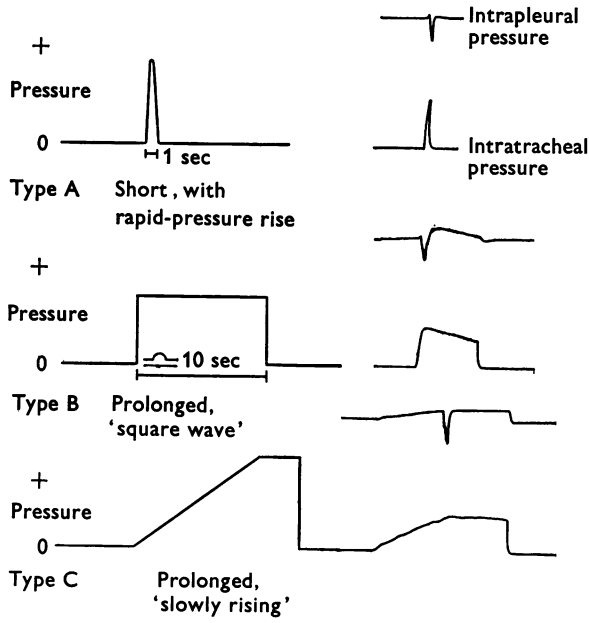

Fig. 1. The three types of inflation and results obtained in a 124-day old foetus.

RESULTS

In the foetus

After the preparation was complete the foetuses lay still on their left side. Any generalized body or limb movements if disturbed were associated with positive intrapleural pressure changes. Few animals made any spontaneous inspiratory efforts associated with negative intrapleural pressure changes. Such changes were usually only 2–4 mm Hg which were much less than those that could be produced by inflation (*see below*). These small inspiratory efforts often appeared in bursts of three or four within 10 sec, the bursts being separated by at least half a minute. Such spontaneous inspiratory efforts were commoner in the younger foetuses and did not become more frequent as the experiments proceeded. The number of such efforts was counted for a 5 min period after the completion of the preparation but before any inflations were begun. Their incidence per minute is shown in Fig. 2. From this it will be seen that sixteen animals

made *no* spontaneous inspiratory efforts, and that only one of the nine which did was over 127 days old. This exception was a 146-day-old foetus (from ewe no. 228) who was making marked respiratory efforts on delivery and continued to do so.

In all twenty-five foetuses an inspiratory response or gasp was produced by all three types of inflation. In every instance all three types were tried, but in the foetus type A was the one most extensively studied. These inflations produced an immediate gasp with a negative pressure swing of between 4 and 40 mm Hg. The resting slightly negative intrapleural pressure (usually 1 mm Hg) often showed a positive phase before the negative swing occurred. Response times in terms of milliseconds were not measured, but scrutiny of the records, including those made at faster paper speeds,

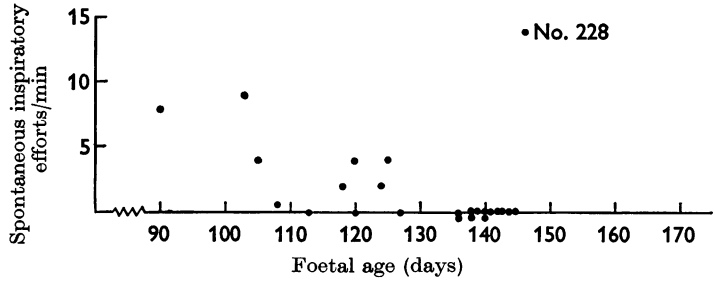

Fig. 2. Incidence of spontaneous inspiratory efforts in twenty-five lamb foetuses of different gestational ages.

shows that the maximum negative intrapleural pressure was reached never more than 0·2 sec after the maximum inflating pressure. Inflations were usually carried out at intervals of 5 sec or more. In one foetus aged 139 days successive inflations sufficient to produce gasps were carried out as rapidly as possible allowing for the intrapleural pressure to return to its resting value. Inflations following as close as 2·5 sec apart continued to evoke a response.

Figure 3 shows a series of inflations carried out in a 127-day-old foetus to determine if there was a threshold pressure above which a gasp was always obtained. The results of 208 inflations carried out over a period of 35 min were analysed as shown in Table 1 from which it will be seen that at pressures above 40 mm Hg a gasp always occurred. A second experiment in a 140-day-old foetus gave a similar result. When a gasp was evoked the response as measured by the negative intrapleural pressure increased with increasing inflating pressure (see bottom line of Table 1).

With type B inflations the response was again immediate. Sometimes there were two or three negative pressure swings during these prolonged inflations and on three occasions a negative swing occurred when the

inflating pressure was released. No attempt to determine a threshold was made.

Type C inflations often produced multiple gasps which were sometimes delayed (Fig. 4). The thresholds were studied in the two foetuses used for the determination of the threshold for type A responses and were 20 and 25 mm Hg respectively, rather lower than that for the rapid inflations.

Brief or prolonged negative intratracheal pressures (deflations) corresponding to types A and B inflation were performed on forty-one occasions in seven foetuses aged 118–144 days. Though pressures of up to 60 mm Hg were employed no gasps were obtained.

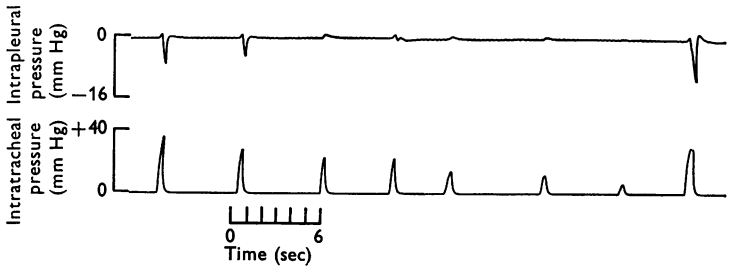

Fig. 3. Series of type A inflations in 127-day-old foetus.

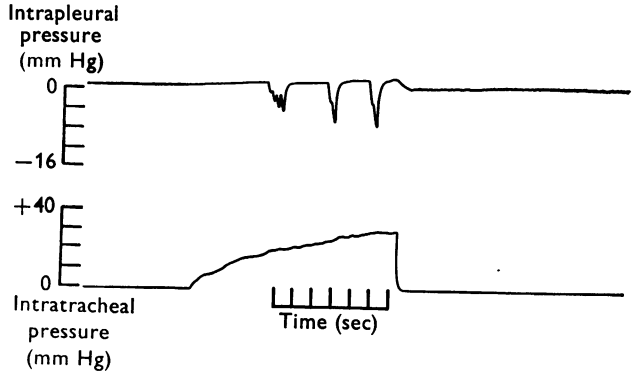

Fig. 4. Multiple and delayed inspiratory responses to type C inflation in a foetus aged 136 days.

TABLE 1. Response of 127-day-old foetus to 208 inflations

Inflating pressure (mm Hg)	0–9	10–19	20–29	30–39	>40
No. of inflations	25	30	63	49	41
No. of gasps	0	0	34	46	41
Percentage response	0	0	54	94	100
Mean negative intrapleural pressures (mm Hg) when response obtained	—	—	7·3	9·3	11·1

Insertion of a catheter as far as the carina produced a coughing response with multiple positive pressure swings in two foetuses aged 120 and 140 days.

Effect of local anaesthetic. In three foetuses in whom there was a consistent inspiratory response to type A inflation 1% xylocaine solution (1·5 ml./kg body weight) was instilled through the tracheal cannula Further inflations were then made at the same or greater pressures than before. The gasp response was lost within 15 sec of the instillation but reappeared 40–45 min later in every case.

Experiments with the vagus nerve. Bilateral cervical vagotomy abolished the inspiratory response to inflation in seven foetuses. Right vagotomy alone abolished the response in one out of five foetuses. The response persisted in two foetuses after left vagotomy alone.

Vagal cooling was carried out in four foetuses. Cooling both vagi always abolished the response, though cooling neither alone did so. The response returned when the cooling had been discontinued.

In the lamb

Inspiratory responses. The response to pulmonary inflation depended on the anaesthetic employed. An inspiratory response to inflation was obtained in all six lambs under thiopentone but only one out of nine under pentobarbitone anaesthesia. In the responsive animals gasps could be elicited by all three types of inflation. The most effective was the slowly rising type C at moderate pressures (15–25 mm Hg) and most studies were made with this type. The threshold for these type C inflations in a 3-day-old lamb was found to be 25 mm Hg (Table 2). An inspiratory response alone was never found as any inflation producing a gasp also led to apnoea (see below). Usually the gasp occurred after some seconds of apnoea (see Fig. 5), though occasionally it preceded a period of apnoea.

The gasp in the lamb produced negative intrapleural pressure changes of the same order as in the foetus. The changes were at least twice as great as those produced during normal respiration as shown in Fig. 5. Here the gasp was associated with a negative pressure change of 18 mm Hg compared to the usual change of 5 mm Hg during normal breathing.

Even in responsive animals the inspiratory response would sometimes disappear, though the apnoeic one persisted. Inflations were usually performed in series of up to ten at a time at least 10 sec apart within a period of 2–3 min. An inspiratory response was more often seen with the first few inflations of any series. Further if the response disappeared it would often reappear when the animal was allowed to breathe quietly for 5–10 min before retesting. In the six lambs under thiopentone thirty-three series of type C inflations with 3–10 inflations in each were performed. In these,

gasps occurred in response to the first inflation in twenty-nine (88 %), to the second in twenty-three (79 %) and to the third in twenty (60 %). In all 190 type C inflations were carried out in these six animals 158 of which produced gasps (83 % positive response).

Apnoeic response. Apnoea occurred in response to all three types of inflation whichever anaesthetic was used and whether a gasp occurred or not. The threshold inflating pressure also seemed lower, around 15 mm Hg (see Table 2). Apnoea was often prolonged for up to 30 sec after the end of an inflation.

TABLE 2. Responses of a 3-day-old lamb under thiopentone to forty-four type C inflations

Maximum intratracheal pressure (mm Hg)	< 10	10–14	15–19	20–24	25–29	> 30
No. of inflations	8	6	7	8	8	7
Apnoea	2	5	7	8	8	7
Gasp	0	2	3	6	8	6

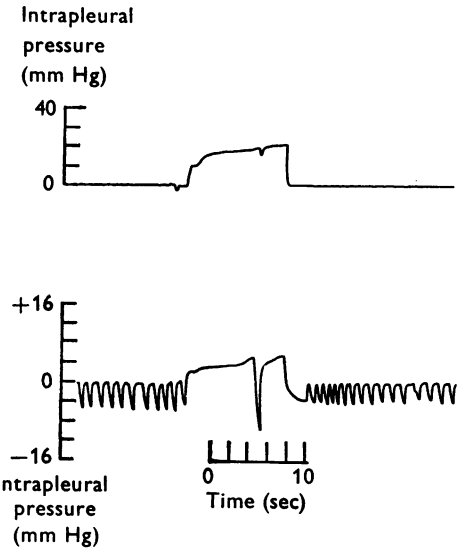

Fig. 5. Gasp response in a 2-day-old lamb under pentobarbitone anaesthesia.

Other stimuli. Negative intratracheal pressures of up to 60 mm Hg did not produce gasps or apnoea. A catheter inserted into the trachea consistently produced a coughing response. This consisted of a positive intrapleural pressure change or else a positive pressure swing followed by a negative one. Inflating a balloon in the lower cervical trachea above the carina produced gasps. However, if the balloon was placed in the upper trachea just below the larynx, inflation produced prolonged apnoea without a gasp.

This lasted over a minute on four occasions, the longest period before breathing recommenced being 152 sec.

Effect of xylocaine. 1% xylocaine solution (0·5 ml./kg body weight) instilled into the trachea produced a cough and then subsequently removed the cough reflex for 30 min. The gasp and apnoeic responses persisted. This was a lower dosage than that used in the foetus.

Experiments with the vagus nerve. (1) Bilateral cervical vagotomy in four lambs showing a gasp reflex abolished the inspiratory response, though cutting either nerve alone did not do so. The cough and apnoeic responses were also abolished by bilateral vagotomy. Other effects of bilateral vagotomy were to abolish the spontaneous gasps that occur fairly frequently in new-born lambs and to increase the depth and decrease rate of respiration.

(2) Cooling the vagus nerves in two animals removed the gasp and apnoeic responses which returned with warming.

DISCUSSION

The results reported in this paper indicate that inflation of the lungs of foetal sheep and lambs through a tracheal cannula leads to marked inspiratory efforts as measured by negative intrapleural pressure changes. Such changes do not occur in response to stimuli other than inflation and are abolished by bilateral vagotomy. Such a response depending on the integrity of the nervous system suggests a reflex action. Such a reflex has not previously been described in this species. It is present in foetal sheep and lambs. It is most consistently found and more easily studied in the foetus which does not have regular spontaneous respiratory movements and therefore does not have the Hering–Breuer inflation reflex to complicate the pattern. Though circulatory reflexes in the sheep foetus have been described by Barcroft (1946) and Reynolds (1960) we can find no description of respiratory reflexes apart from the rhythmical movements that follow tapping the face of the young foetus (Barcroft, 1946). It is also interesting that this inspiratory reflex was present in a foetus as young as 90 days at an age when the foetal lung can be rather more difficult to inflate (Born, Dawes & Mott, 1955).

These results in the foetus were particularly striking as relatively large negative intrapleural pressure changes associated with the gasps could be obtained so consistently. These were easily distinguished from the much smaller spontaneous efforts and from the generalized writhing movements the foetuses sometimes made in response to external stimuli and which were associated with positive intrapleural pressure changes. That the inflatory pressures needed to produce these inspiratory responses are

rather high may be related to the relatively low compliance of foetal lung.

It is difficult to see the purpose of the reflex in the lamb foetus which is not normally breathing in the latter part of pregnancy. It is known that reflex pathways can exist before they are brought into use (Downing, 1960) and it may be that it has a part to play in the initiation of the first deep breath after delivery; in addition this reflex might be elicited during attempts to resuscitate new-born animals or babies when artificial inflations could produce quite large active gasps by the neonate.

During the course of natural respirations most animals, including man, take periodic deeper breaths or sighs. Benedixen, Smith & Mede (1964) have studied this in man and defined such sighs as 'breaths larger than 3 times the average tidal volume'. There is also evidence (McCutcheon, 1953) that such periodic or spontaneous deeper breaths increase in frequency with decreasing body size. Lambs make such periodic sighs and it may be that the gasp reflex described here plays some part in their production. Such a role for an excitatory inspiratory reflex in cats was, in fact, suggested by Knowlton & Larabee (1946). The lungs of new-born lambs are relatively unstable and their compliance decreases rapidly with time (Williams, Tierney & Parker, 1966). Periodic deeper breaths would be expected to increase the compliance and this has, in fact, been demonstrated. It may be that a naturally occurring or spontaneous gasp reflex is responsible for maintaining the patency of the terminal air units and normal lung compliance. Though we have not made simultaneous studies of changes in compliance and in threshold of the gasp reflex in lambs, there is some indirect evidence and it is more easily elicited under conditions of low compliance. Gasps were obtained in response to inflation most commonly with the first inflation of any series. Further, when the animal was apparently refractory, gasps could be obtained by letting the animal breathe quietly for 5–10 min (during which time a fall in compliance might be expected to occur) and then retesting. These results are similar to those of Reynolds (1962); with adult cats he found that inflation-induced and spontaneous gasping was related to a reduced end-expiratory lung volume and compliance, although, unlike our results, there was also a long refractory period (97–130 sec).

Increasing inspiratory activity in response to inflation of the lungs has been described by several workers. This has been measured as a shortening of expiration (Head, 1889), an accelerated respiratory rate (Hammouda & Wilson, 1934, 1935; Steffensen, Brookhart & Gesell, 1937) and an increase in phrenic motor neurone activity (Larrabee & Knowlton, 1946; Widdicombe, 1954*b*). Head referred to his effect as a 'paradoxical reflex' to distinguish it from the usual inhibition produced by inflation. Subsequent

authors have kept the title 'Head's Paradoxical Reflex' to describe such effects. Several authors have employed differential vagal cooling to distinguish it from the other respiratory reflexes (see Widdicombe, 1954*a*, *b*, *c*). The receptors for such a reflex have not yet been identified with certainty however (Widdicombe, 1963).

The gasp reflex described here in the sheep resembles the paradoxical reflex in that it involves an inspiratory response to inflation. The afferent pathway is in the vagus nerves as shown by the experiments in which the vagi were cut or cooled. The receptors appear to be in the lower trachea, bronchi, bronchioles, or further down the respiratory tract. In the present study gasps were produced by moderate pressure changes. In the new-born lambs we had to use pressures (10–20 mm Hg) rather higher than those used by Cross *et al.* (1960) who found that babies would gasp after the application of pressures as low as 2·2 cm water (1·7 mm Hg). It is interesting that the reflex is present both in the fluid-filled foetal lung and the air-filled lamb's lung. It appeared to be distinct from the cough reflex in that the latter, though present in lambs and foetuses, was elicited by a mechanical stimulus and usually consisted of one or more expiratory movements associated with positive intrapleural pressure swings. In contrast to the findings of Widdicombe (1954*a*) in the cat, an inspiratory component to the cough reflex was encountered rather infrequently in the lamb. The level of anaesthesia and type of anaesthetic agent used were important in the production of the gasp reflex.

The new-born lamb also has an active Hering–Breuer inhibitory reflex resulting in apnoea which is often prolonged. This reflex is more easily elicited than the gasp reflex and is present under thiopentone or pentobarbitone anaesthesia. This response is also abolished by bilateral vagotomy. The Hering–Breuer reflex has been shown to be present in the new-born of other species such as the rabbit (Dawes & Mott, 1959) and human baby (Cross *et al.* 1960) but has not previously been described in the lamb.

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