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THE NERVOUS CONTROL OF THE CIRCULATION IN THE FOETAL AND NEWLY EXPANDED LUNGS OF THE LAMB

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Previous experiments on foetal lambs have shown that the high vascular tone usually observed in the unexpanded lung is very labile and can be altered by changes in arterial P_{O_2} and P_{CO_2}, and in other ways (Dawes & Mott, 1962; Cassin, Dawes, Mott, Ross & Strang, 1964). For instance rhythmical ventilation with gas mixtures which produced little or no alteration in arterial P_{O_2} and P_{CO_2} caused pulmonary vasodilatation. The present experiments were undertaken to find out whether these changes in pulmonary vascular tone depended on the nerve supplies to the lung, and whether a single brief expansion of the lung would cause the same vasodilatation as that seen on rhythmic ventilation. A brief account has been published elsewhere (Colebatch, Dawes, Goodwin & Nadeau, 1965).

METHODS

The term resistance, used to denote changes in vascular tone, has been applied in a number of different ways. In order to avoid misinterpretation the term vascular conductance is used to define Δ flow/Δ pressure, measured from the steep part of the pressure–flow curve, where Δ pressure is calculated as the difference in pressure at two given flows corrected for the pressures in the part of the apparatus outside the animal (Cassin, Dawes, Mott, Ross & Strang, 1964). The intercept of this part of the pressure–flow curve on the pressure axis (the pressure intercept) is obtained by extrapolation. Knowledge of the vascular conductance and pressure intercept permits reconstruction of the relevant part of the pressure–flow curve as

$$y = a + x/b,$$

where y is pressure, x is flow, b is vascular conductance and a pressure intercept. Changes in vascular tone may be indicated by a statistically significant alteration in either vascular conductance or pressure intercept or in both.

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Thirty-four foetal lambs of 127–143 days gestation age, weighing 2·1–5·3 kg, were delivered by Caesarean section under light chloralose anaesthesia (30 mg/kg I.V. initially). Further doses of chloralose 10 mg/kg I.V. at intervals of 1–2 hr caused no discernible change in pulmonary vascular tone. The lambs were placed on a warmed table alongside the mother, still attached by the umbilical cord. A cannula filled with saline was introduced into the foetal trachea and the chest was opened on the left side to give access to the origin of the left pulmonary artery within the pericardium. Heparin (10 mg/kg I.V.) was injected and the peripheral end of the left pulmonary artery was connected to the central end of the left carotid artery. The connecting tubes (vol ~ 15 ml.) were filled with freshly drawn heparinized maternal blood. A vertical polyethylene tube 100 cm long and 6 mm internal diameter was attached to one connecting tube. When filled with blood the hydrostatic pressure in this system (~ 80 mm Hg) was greater than mean arterial pressure. Pulmonary arterial pressure was recorded with an Elema inductance manometer from the connecting tube, and left atrial pressure was recorded from a catheter passed into a femoral vein and through the foramen ovale. The left atrial pressure was subtracted from pulmonary arterial pressure by an electronic method (Puckle, 1952) and the difference was used to produce the pressure deflexion for pressure–flow curves on an $X-Y$ recorder, from which the vascular conductance (Δ flow/Δ pressure in the steep part of the curve) and the intercept on the pressure axis were measured as described previously (Cassin, Dawes, Mott, Ross & Strang, 1964). Pulmonary arterial flow was measured with an electromagnetic flowmeter (Wyatt, 1961). In three lambs blood flow was measured in the abdominal umbilical vein with a cuff electromagnetic flowmeter (Wyatt, 1964). In both these instruments the difference between the zero reading when the magnet was switched off and that observed on occlusion of the blood vessel was less than 1 % of scale. The maximum error observed on *in vivo* trials of the cannulated flowmeter was ± 2 %, and of the cuff flowmeter was ± 7 %; the average error was considerably less.

In ten lambs the thoracic sympathetic ganglia were removed. The lamb was laid on its left side after delivery and portions of three to four ribs were taken out. After the right sympathetic chain had been removed to well below the root of the lung (T 1–8 or 9) the chest was closed and the lamb was laid on its right side. The left side of the chest was then opened and, after a similar operation, the left pulmonary artery was exposed and joined to the left carotid as described above. This preparation took about $1\frac{1}{2}$ hr from delivery as compared with about an hour without bilateral sympathectomy.

Shortly before the lungs were to be expanded the T-shaped tracheal cannula was drained of fluid and a gas mixture containing 3 % O_2 and 7 % CO_2 in N_2 was sucked through until the effluent O_2 content recorded on a paramagnetic O_2 analyser (Cambridge Instrument Co.) was 3 %. The trachea and cannula remained full of fluid to within 1 cm of the T-junction. A syringe, which had been repeatedly flushed with the gas mixture, was then attached to the T-junction and the lungs were expanded by injection of a known volume of gas. Later, the lungs were rhythmically ventilated with a constant-volume Starling Ideal pump which had been modified to prevent leaks. The pump was usually stopped while pressure–flow curves were produced; this brief period of arrest did not modify the result. The composition of the inspired gas mixtures used was checked by analysis in the Lloyd–Haldane apparatus (Lloyd, 1958).

Arterial blood samples (0·7 ml.) were taken from the carotid-pulmonary loop into 1 ml. syringes, in which the dead space was filled with a solution of heparin 0·4 % and NaF 8 g/100 ml. They were analysed at once for pH with a Metrohm capillary glass electrode at 39° C and an E.I.L. pH meter model 23A, and for P_{CO_2} and P_{O_2} with electrodes (Severinghaus & Bradley, 1958) using a Beckman 160 gas analyser. Duplicate pH measurements agreed within $\pm 0{\cdot}01$ unit. The gas tension electrodes were calibrated with analysed gas mixtures, and duplicate blood samples agreed within ± 1 mm Hg over the range of P_{O_2} and P_{CO_2} used. The observations were corrected for the difference in temperature between

the electrodes and the foetus, measured from a thermometer in its oesophagus (Bradley, Stupfel & Severinghaus, 1956; Severinghaus, Stupfel & Bradley, 1956).

The peripheral ends of the left cervical vagus and of sympathetic nerves to the left lung were stimulated with rectangular constant current pulses of 5–10 msec duration at frequencies of 10–30/sec using bipolar electrodes.

RESULTS

Vasomotor responses to pressure changes

Pulmonary arterial pressure–flow curves were produced by interrupting the connexion with the carotid artery and allowing blood to flow in from a vertical tube (between the arrows in Fig. 1). The blood supply from the left carotid was restored after 10–15 sec and pulmonary arterial pressure gradually rose to its initial level. During this time there was usually a large increase in pulmonary blood flow, lasting 20–40 sec, which rose above the initial level and often exceeded the maximum observed

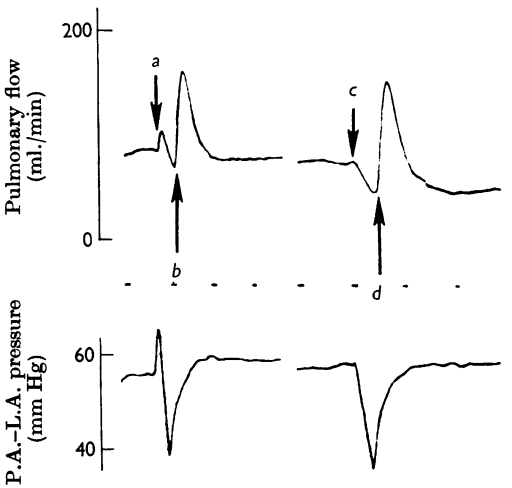

Fig. 1. Foetal lamb, 134 days gestation, 3·4 kg. Records of left pulmonary arterial flow (above), time in 30 sec intervals, and transpulmonary pressure (arterial–left atrial, below). Pressure–flow curves were generated by flow from a vertical tube between *a* and *b* and *c* and *d*. At *a* the arterial pressure was raised above the initial level, at *c* it was allowed to fall from the initial level. In both instances this was followed by vasodilatation.

during the pressure–flow curve; this was attributed to pulmonary vasodilatation. This phenomenon was observed repeatedly in every lamb. As Fig. 1 shows, the vasodilatation was seen whether the pressure in the vertical tube was raised above the initial mean arterial pressure (*a*) or was allowed to fall from this level (*c*). The only occasions on which pressure–flow curves were not followed by vasodilatation in unexpanded foetal lungs

were when the pressure was raised above the initial level and allowed to fall down to but not below it.

When the connexion with the vertical tube was shut off, and the blood supply from the left carotid artery was interrupted, pulmonary arterial pressure fell rapidly to a value 3–4 mm Hg greater than that in the left atrium. Restoration of the blood supply from the left carotid was followed by brief pulmonary vasodilatation even when blood flow was interrupted for only a few sec. Infusion of up to 15 ml. foetal blood previously removed from the lamb (the maximum volume returned during the production of a pressure–flow curve) over 10–15 sec, while the blood supply from the carotid was stopped, and at a rate so that the height of the blood in the vertical tube did not rise by more than 1 cm above the initial value,

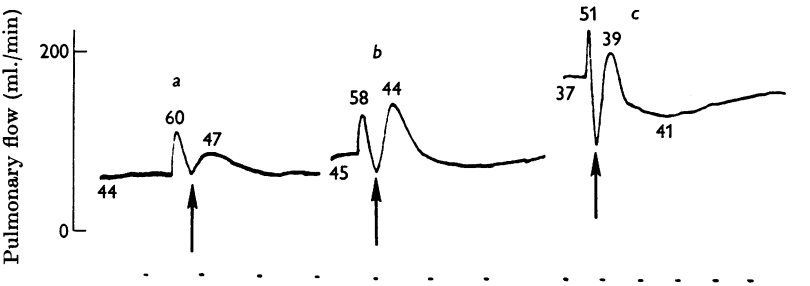

Fig. 2. Foetal lamb, 133 days gestation, 3·8 kg. Records of left pulmonary arterial flow before, during and after pressure–flow curves generated by flow from a vertical tube. The end of each procedure is indicated by an arrow. The numbers on the record indicate the mean transpulmonary (pulmonary arterial-left atrial) pressure in mm Hg at selected times. The three records (*a*, *b*, *c*) were taken during a period of spontaneous vasodilatation over half an hour. Time marks indicate 30 sec intervals.

did not cause vasodilatation. Therefore the vasodilatation was due neither to the removal of the normal pressure pulse nor to the return of blood which had been allowed to cool in contact with glass.* It was concluded that it was probably due to the fall in arterial pressure.

The vasomotor responses depended on the initial tone of the pulmonary blood vessels. In foetal lambs in which they were very constricted and pulmonary flow was low, the increase in flow above the initial value after a pressure–flow curve was small (Fig. 2*a*). It became larger as the vessels dilated (Fig. 2*b*), and when resting pulmonary flow was still greater, pressure-flow curves were followed by a complex response in which flow rapidly increased to or above the initial level and then fell below it again (Fig. 2*c*). Analysis of simultaneous pressure and flow records (on the *X*–*Y* recorder) showed that the latter changes corresponded to a brief initial vasodilatation followed by a longer period of vasoconstriction.

* See note added in proof.

Ventilation with 3% O_2 and 7% CO_2 in N_2 only caused a small increase in pulmonary blood flow and no change in these vasomotor responses. But on ventilation with air pulmonary blood flow always increased greatly and there was little or no vasomotor response after pressure–flow curves (in eight lambs). When the lungs were then ventilated with 7% CO_2 in air, or 10% O_2 in N_2, or when they were filled with O_2 and allowed to collapse, pulmonary blood flow decreased and the vasomotor responses returned.

In seven lambs the cervical vago-sympathetic nerves were cut on both sides. This did not alter these vasomotor responses, which were also observed after bilateral thoracic sympathectomy and vagotomy in eight lambs.

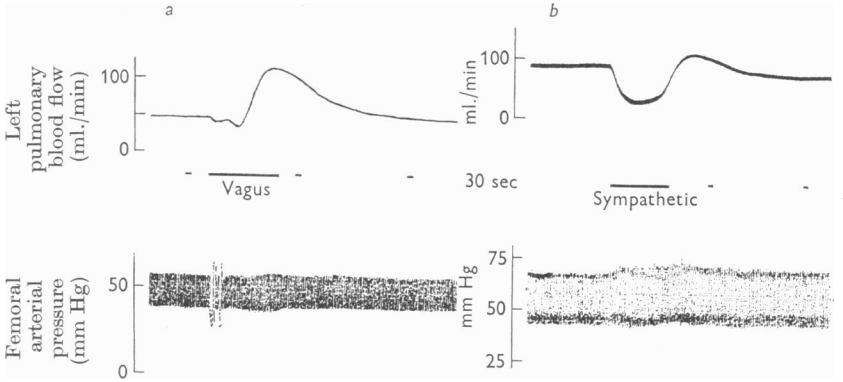

Fig. 3. Records of mean left pulmonary arterial flow (above) and of femoral arterial pressure (below) in foetal lambs, (*a*) 142 days gestation, 4·9 kg, during stimulation of the peripheral end of the left vagus during the signal mark with 5 msec, 2 mA pulses at 15/sec; and (*b*) 143 days gestation, 3·9 kg, during stimulation of sympathetic nerves to the left lung with 5 msec, 0·4 mA pulses at 30/sec. Time marks indicate 30 sec intervals.

The effects of autonomic nerve stimulation and section on pulmonary vascular tone

Electrical stimulation of the peripheral end of the cut left cervical vagus nerve caused bradycardia and an immediate fall of arterial pressure in nine mature foetal lambs. There was a brief decrease in left pulmonary arterial blood flow followed by an increase which was often very large, usually after a delay of 10–15 sec (Fig. 3*a*). This increase in flow was associated with a small fall in left pulmonary arterial pressure and little change in left atrial pressure. It was observed on left vagal stimulation after cutting the left cardiac branches, which abolished the bradycardia and the large instantaneous fall in arterial pressure and also reduced the delay. The main vagal trunk which runs over the ductus arteriosus was

left intact, but all the branches running medially towards the heart between the ductus arteriosus and the aortic arch were cut. Figure 4 illustrates the changes in pressure–flow relation during left vagal stimulation after dividing the cardiac branches. Pressure–flow curve *a* was obtained before vagal stimulation, and curve *b* during stimulation when the vasodilatation had almost reached its maximum. The arrow indicates the direction of movement of the pen of the *X–Y* recorder as a result of vagal stimulation, almost at right angles to curve *a*, with a delay of only 2–3 sec. It was concluded that vagal stimulation caused vasodilatation by a direct action on the pulmonary blood vessels, irrespective of the immediate fall of arterial pressure due to bradycardia.

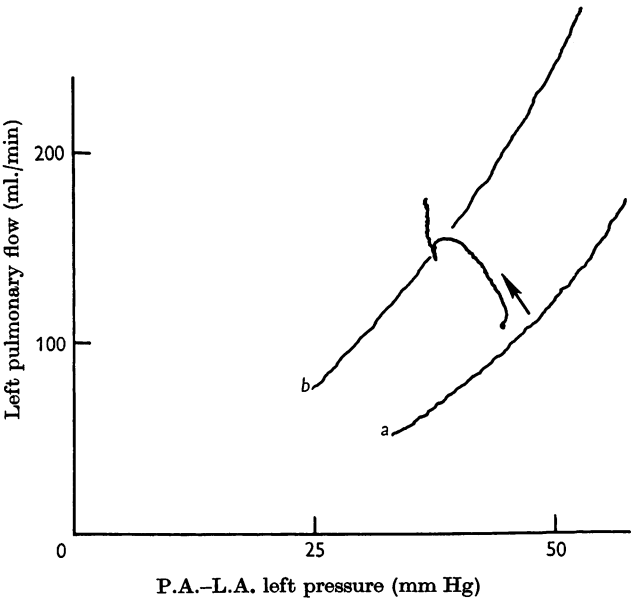

Fig. 4. Foetal lamb, 140 days gestation, 4·6 kg, cardiac branches of left vagus cut. Pulmonary arterial pressure–flow curve *a* was obtained before vagal stimulation. The arrow indicates the direction of movement of the pen of the *X–Y* recorder on stimulation of the peripheral end of the left vagus. Curve *b* was obtained during stimulation with 10 msec, 2 mA pulses at 15/sec.

Pulmonary vasodilatation was also observed on vagal stimulation after cutting both vago-sympathetic trunks in the neck, together with bilateral thoracic sympathectomy. The vasodilatation was abolished by intravenous injection of atropine (1 mg/kg) in three lambs.

The branches of the thoracic sympathetic chain arising from the third, fourth and fifth ganglia were cut as they ran across the posterior thoracic wall towards the left hemiazygos vein in four lambs. Electrical stimulation of the peripheral ends caused a reduction in left pulmonary blood flow

due to vasoconstriction (Fig. 3*b*). When observed on a pressure–flow diagram the effect of sympathetic stimulation was to cause the pen to move downwards and to the right, at right angles to the pressure–flow curve, and in the opposite direction to that shown in Fig. 4. There was a small rise in left pulmonary arterial pressure and no change in left atrial pressure. There was also an increase in heart rate, confirming the observations of Waites (1957).

Bilateral section of the cervical vago-sympathetic trunks did not alter pulmonary vascular tone in foetal lambs. After bilateral thoracic sympathectomy (T 1–8), the heart rate (100–150 beats/min) was less than in other lambs (170–220), the arterial pressure (45–60 mm Hg) was no different and pulmonary vascular tone was less. Pulmonary vascular conductance and pressure intercept were calculated from pressure–flow curves shortly

TABLE 1. The effect of bilateral thoracic sympathectomy on pulmonary vascular resistance in foetal lambs

	Normal	Sympathectomized
Number	21	9
Arterial P_{O_2} (mm Hg)	25·6 ±0·9	*23·7 ± 1·1
Arterial P_{CO_2} (mm Hg)	38·7 ±0·8	†43·0 ± 1·8
Vascular conductance/kg (ml./min.mm Hg)	0·91±0·13	*1·23±0·21
Pressure intercept (mm Hg)	31·7 ±1·4	‡22·2±2·6

* Not significantly different; † $P < 0{\cdot}05$; ‡ $P < 0{\cdot}002$.
In all tables the figures indicate the means ± S.E.

before and after the withdrawal of arterial blood samples in normal and sympathectomized lambs. As Table 1 shows, the arterial P_{O_2} was not significantly different in the two groups, but the P_{CO_2} was significantly greater in the sympathectomized lambs. Nevertheless, the pressure intercept was significantly less in the latter, and mean pulmonary vascular conductance was also increased, but not significantly.

In four foetal lambs with bilateral vagotomy and thoracic sympathectomy, in which the lungs were not expanded, occlusion of the umbilical cord to cause acute asphyxia for 1–1·5 min caused intense pulmonary vasoconstriction (Fig. 5) and bradycardia. When the umbilical cord was released the left pulmonary arterial pressure–flow curve returned to its original position over a period of about 10 min, and there was an increase in heart rate above the initial value lasting about the same time.

In five lambs the lungs were rhythmically ventilated, after bilateral cervical vagotomy and thoracic sympathectomy, with 3% O_2 and 7% CO_2 in N_2. Subsequent ventilation with air raised the arterial P_{O_2} and lowered the P_{CO_2}, and always caused a large pulmonary vasodilatation (Fig. 5 and Table 2), and also a fall in heart rate. In two of the five lambs

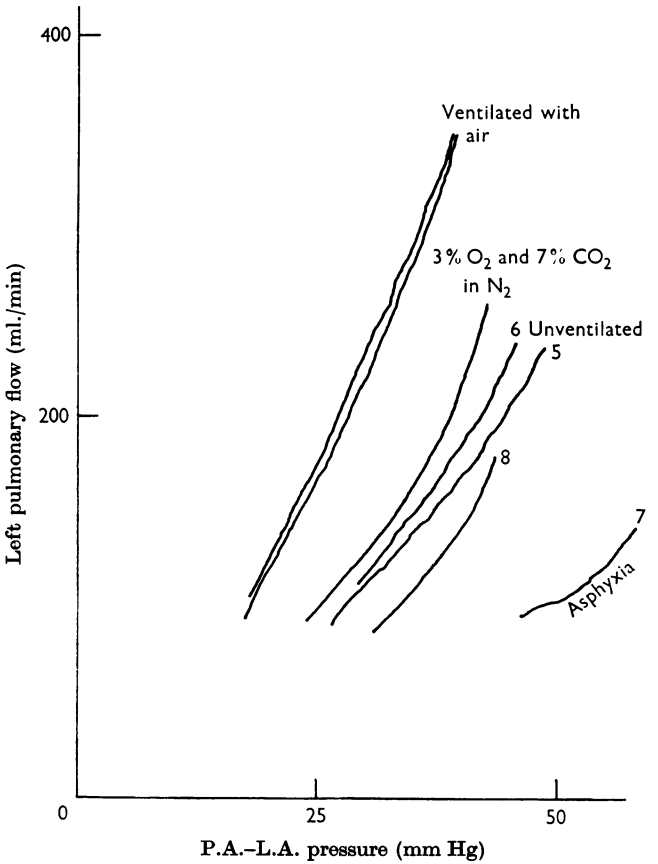

Fig. 5. Foetal lamb, 138 days gestation, 3·8 kg; both cervical vago-sympathetic nerves cut and bilateral thoracic sympathectomy. Pulmonary arterial pressure–flow curves were obtained before (5, 6), during (7) and after (8) acute asphyxia caused by occluding the umbilical cord for one minute; and also during rhythmic ventilation of the lungs with 3 % O_2 and 7 % CO_2 in N_2, and with air.

TABLE 2. Effect of gaseous ventilation in newly delivered lambs after bilateral vagotomy and sympathectomy

	Ventilated with	
	7 % CO_2 and 3 % O_2 in N_2	Air
Number of observations/lambs	5/5	11/5
Arterial P_{O_2} (mm Hg)	19·6 ± 1·8	30·6 ± 1·6
Arterial P_{CO_2} (mm Hg)	54 ± 3·9	36·7 ± 1·7
Vascular conductance per kg (ml./min.mm Hg)	1·49 ± 0·02	3·39 ± 0·39
Pressure intercept (mm Hg)	16·6 ± 2·1	14·1 ± 1·3

gas mixtures containing 10% O_2 in N_2 and 7% CO_2 and 21% O_2 in N_2 were substituted for air as the ventilating gas. These caused a small vasoconstriction and a rise in heart rate, both of which were abolished when ventilation with air was restored.

Expansion of the lungs

Previous experiments have shown that rhythmic ventilation of the lungs of foetal lambs with 3% O_2 and 7% CO_2 in N_2 causes little change in arterial P_{O_2} or P_{CO_2} (Cassin, Dawes, Mott, Ross & Strang, 1964). The effect of a brief initial expansion of the lungs with this gas mixture was examined in twelve foetal lambs which had a mean arterial P_{O_2} of $25{\cdot}3 \pm 1{\cdot}1$ (S.E.) mm Hg, a P_{CO_2} of $41{\cdot}5 \pm 1{\cdot}4$ mm Hg and (in seven) a pH of $7{\cdot}51 \pm 0{\cdot}024$. Both lungs were expanded by a single injection into the tracheal cannula of 25 ml./kg (mean; range 13–38) made as rapidly as possible from a syringe (see Methods). This caused a rise of intratracheal pressure of 34–51 mm Hg, which subsided over the next 5 sec to 21–32 mm Hg, after which the trachea was opened to the atmosphere.

This brief expansion of the lungs caused an immediate rise in left pulmonary blood flow accompanied by a fall in pulmonary arterial pressure and in the pressure difference between the left pulmonary artery and the left atrium (Fig. 6) and usually a drop in heart rate lasting a few seconds only. After a short interval of time in eleven of the twelve lambs there was a further gradual increase of pulmonary blood flow which usually did not reach a peak until 5–10 min after the lungs had been expanded, and which also was accompanied by a decrease in the pressure difference between the pulmonary artery and left atrium. Figure 6 shows a good example of this sequence of events. The initial small increase in pulmonary flow was accompanied by a large fall in arterial pressure, while the later larger increase in flow was associated with a smaller fall in pressure. Figure 7 shows two pressure–flow curves before expansion of the lungs (*a*), the immediate effect of expansion (indicated by the arrow) and two pressure–flow curves taken 10–15 min later when the secondary vasodilatation had reached its maximum (*b*).

Pressure–flow curves were taken just before inflation and when the secondary increase in flow had reached its peak, 10–12 min later, in seven lambs. The measurements on these curves (Table 3) show that there had been a considerable pulmonary vasodilatation as judged both by the increase in vascular conductance and the decrease in pressure intercept ($P < 0{\cdot}001$). During this period there was a small decrease in arterial P_{O_2} and rise in P_{CO_2}, each of 3 mm Hg on the average. These changes in blood gas tensions can be attributed, first, to the fact that the lungs had been expanded with a gas containing O_2 at a lower and CO_2 at a higher partial pressure than that in the blood, and secondly to a small decrease

in umbilical blood flow. Umbilical flow was measured in three lambs, and decreased abruptly when the arterial blood pressure fell on expansion of the lungs; it then rose gradually to reach a value somewhat less than that observed initially.

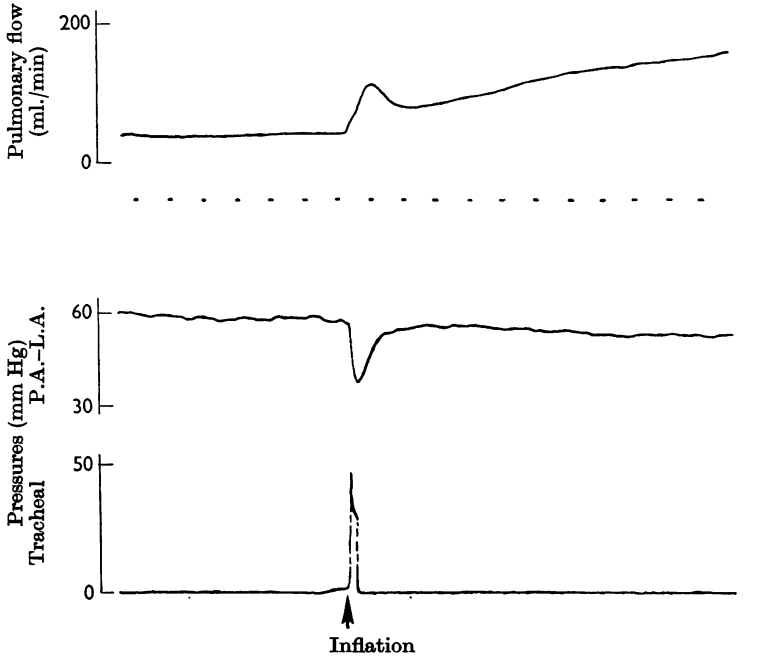

Fig. 6. As Fig. 1. Records from above downwards of left pulmonary arterial flow, time in 30 sec intervals, transpulmonary (arterial–left atrial) pressure, and intratracheal pressure. The lungs were expanded with 100 ml. 3 % O_2 and 7 % CO_2 in N_2 for 5 sec at the arrow.

In seven of the twelve lambs, 10–15 min after the initial expansion of both lungs, the same volume of the same gas mixture was again injected into the trachea (which had been thoroughly flushed with it previously). This caused a brief drop in pulmonary arterial pressure and flow, usually followed by a small increase in flow lasting less than a minute. This response was obtained repeatedly, but was much smaller than the effects observed on first expansion of the lungs. If the lungs were not re-expanded, they began to collapse after about ½ hr, and as they collapsed pulmonary vascular tone increased. In seven lambs whose lungs had recently been expanded by one or more brief inflations with 3 % O_2 and 7 % CO_2 in N_2, rhythmic ventilation with rebreathing from a bag filled with the same gas mixture caused no further change in pulmonary vascular tone. Subsequent ventilation with air always caused a large vasodilatation.

Bilateral section of the cervical vago-sympathetic trunks abolished the

bradycardia usually seen on expansion of both lungs, but did not abolish the vasodilatation, nor (in one lamb) did previous administration of atropine (1 mg/kg I.V.). Bilateral section of the thoracic sympathetic nerves also did not abolish the pulmonary vasodilatation in three lambs. Six lambs were subjected to both bilateral vagotomy and thoracic sympathectomy. The pulmonary vascular tone was considerably less than that in normal lambs; subsequent brief expansion of both lungs caused a vasodilatation which was less than that in normal lambs (Table 3).

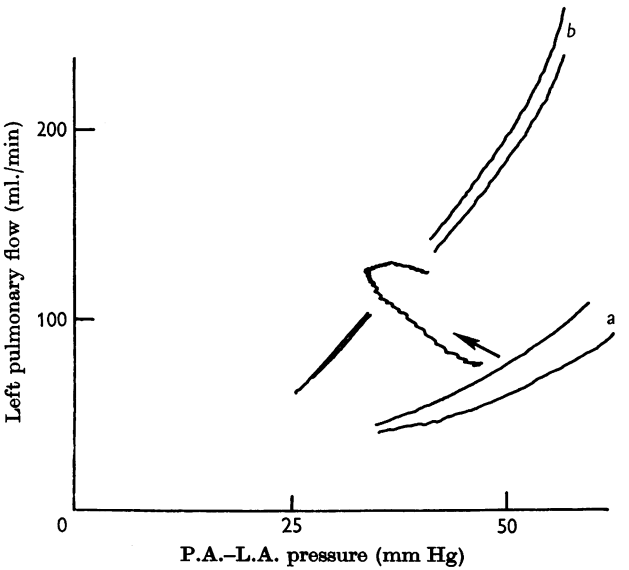

Fig. 7. Foetal lamb, 137 days gestation, 4·4 kg. Left pulmonary arterial pressure–flow curves were obtained 10 and 4 min before (*a*) and 5 and 16 min after (*b*) expansion of the lungs with 100 ml. 3 % O_2 and 7 % CO_2 in N_2 for 5 sec. The arrow indicates the direction of movement of the pen of the *X–Y* recorder on inflation.

TABLE 3. The effect of a brief expansion of the lungs with 3 % O_2 and 7 % CO_2 in N_2 on pulmonary vascular resistance in foetal lambs

	Normal (7)	Bilateral vagotomy and thoracic sympathectomy (6)
Pulmonary vascular conductance/ kg (ml./min.mm Hg)		
Before lung expansion	0·70 ± 0·12	1·21 ± 0·25
After lung expansion	*1·28 ± 0·13	‡1·61 ± 0·20
Pressure intercept (mm Hg)		
Before lung expansion	32·0 ± 2·6	23·0 ± 3·1
After lung expansion	*21·9 ± 1·8	†17·8 ± 1·4

The figures in brackets indicate the number of lambs.
Significantly different: * $P < 0·001$; † $P < 0·02$; ‡ $P < 0·05$.

In three lambs the left bronchus was obstructed by inflating a small balloon passed down the trachea. A subsequent brief inflation of the *right lung only* with 12–15 ml./kg of 3% O_2 and 7% CO_2 in N_2 caused an immediate fall in arterial pressure followed, after a brief delay, by an increase in *left* pulmonary blood flow (Fig. 8). In one of these lambs the cervical vagi were cut and this abolished both the fall of pressure and the increase in pulmonary flow.

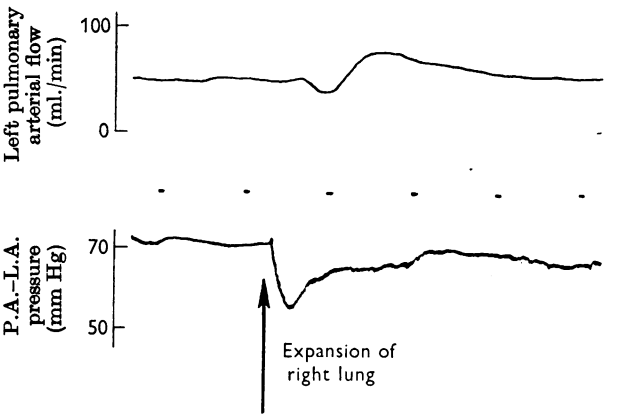

Fig. 8. Foetal lamb, 140 days gestation, 4·2 kg. Records of left pulmonary arterial flow (above), time intervals in 30 sec, and transpulmonary pressure (arterial–left atrial; below) during expansion of the right lung only with 60 ml. 3% O_2 and 7% CO_2 in N_2 (at the arrow).

In three lambs the lungs were expanded by injection of 16–20 ml./kg warm amniotic fluid or 0·9% sodium chloride solution, which had been equilibrated with 3% O_2 and 7% CO_2 in N_2 for an hour or more, and the intratracheal pressure rose to 6–16 mm Hg. The trachea was opened to atmospheric pressure after 30–50 sec and the fluid drained out; almost all was recovered within 1 min. The injection always caused an immediate fall in arterial pressure. This was followed within 5–10 sec by a small increase in flow (while the pressure was still reduced) which lasted up to a minute only.

Other procedures which usually caused an abrupt transient fall in arterial pressure, such as compression of the foetal head, extension of the head, or insertion of an object into the mouth to induce sucking movements, also led to a small brief increase in pulmonary blood flow in seven lambs. Even extrasystoles sometimes had the same effect. It is evident that such small changes should be interpreted with caution. None of these measures ever led to the prolonged vasodilatation observed on gaseous expansion of both lungs (Fig. 6).

Unexplained pulmonary vasodilatation in unexpanded lungs

In most foetal lambs (whose lungs are not yet expanded) pulmonary blood flow reaches a steady level soon after the preparation has been completed, but in some there is an unexplained large prolonged pulmonary vasodilatation within the next half hour (Cassin, Dawes, Mott, Ross & Strang, 1964). The arterial blood gas tensions may remain unaltered, or the P_{O_2} may fall and the P_{CO_2} rise. The incidence of this phenomenon observed in the previous and present experiments combined has been thirteen of 65 lambs (i.e. 20%). These thirteen include two which

TABLE 4. Measurements on seven foetal lambs at the peak of unexplained pulmonary vasodilatation

Arterial P_{O_2} (mm Hg)	$22{\cdot}4 \pm 1{\cdot}3$
Arterial P_{CO_2} (mm Hg)	$37{\cdot}7 \pm 3{\cdot}1$
Vascular conductance per kg (ml./min.mm Hg)	$2{\cdot}34 \pm 0{\cdot}30$
Pressure intercept (mm Hg)	$18{\cdot}7 \pm 2{\cdot}9$

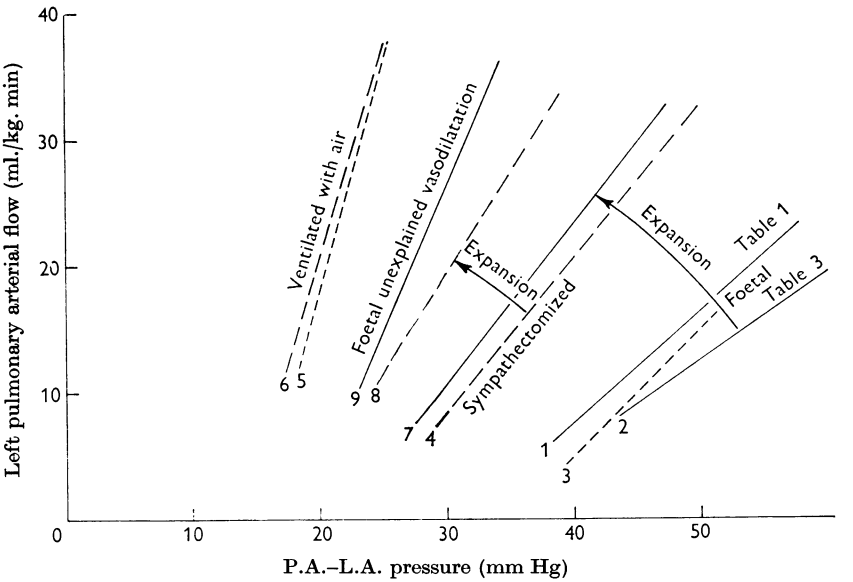

Fig. 9. The mean left pulmonary vascular conductances per kg and pressure intercepts were used to construct mean pressure–flow diagrams for normal foetal lambs (Table 1 (1), and Table 3 (2), and from Cassin, Dawes, Mott, Ross & Strang (1964; 3)). For comparison are shown the effects of bilateral thoracic sympathectomy from Table 1 (4); of ventilation with air from Table 2 (5) and from Cassin, Dawes, Mott, Ross & Strang (1964; 6); of brief expansion with 3% O_2 and 7% CO_2 in N_2 in normal foetal lungs (7) and in foetal lungs after bilateral vagotomy and thoracic sympathectomy (8); and of unexplained spontaneous vasodilatation (Table 4, 9).

had had bilateral vagotomy and thoracic sympathectomy and one which had thoracic sympathectomy alone. In seven lambs left pulmonary blood flow exceeded 50 ml./kg. min (range 51–78) at the peak of the vasodilatation. The measurements of left pulmonary vascular conductance and pressure intercept at this peak are shown in Table 4. It is evident that the vasodilatation was substantially greater than that observed on bilateral thoracic sympathectomy (compare Fig. 9, pressure–flow diagrams 4 and 9). In one lamb the left vagus was cut at the peak of the vasodilatation, which continued. In two lambs pulmonary blood flow was stopped for 5 min at the peak of the vasodilatation; on release, pulmonary flow rose at once to near the original value. The peak vasodilatation was less than that observed on ventilation with air (Fig. 9), but in one lamb it approached this value closely.

DISCUSSION

Previous observations showed that arrest of pulmonary arterial blood flow in unexpanded foetal lungs for 2 min was followed by vasodilatation when flow began again (Dawes & Mott, 1962). This phenomenon was described as reactive hyperaemia by analogy with similar observations in the systemic circulation of adult animals. The present results demonstrated a brief vasodilatation after total arrest of pulmonary flow for a much shorter period of time, or when pulmonary arterial pressure and flow were allowed to decrease for 10–15 sec to about two-thirds of their original value (Fig. 1). The fact that this vasodilatation was seen after bilateral section of the autonomic nerve supply to the lung suggests that it might be due to a direct effect on vascular smooth muscle, analogous to that described in the systemic circulation by Bayliss (1902), Hilton (1959) and Folkow (1962). Although the usual response was vasodilatation, when the vasomotor tone was low and pulmonary flow was high the response was biphasic and appeared superficially like a damped oscillation. There are therefore several possibilities which require further exploration.

In adult lungs hypoxia causes vasoconstriction, and this is probably due to a local action as it is seen in isolated perfused preparations (Nisell, 1948; Duke, 1951). It is not a necessary consequence of systemic hypoxaemia, either in human congenital cardiac anomalies or in new-born calves in which a right-left shunt has been produced surgically (Reeves, Leathers, Eiseman & Spencer, 1962). Conversely substitution of air for N_2 as the ventilating gas in isolated perfused newly ventilated lamb's lungs caused vasodilatation (Born, Dawes & Mott, 1955). Yet arrest of pulmonary blood flow for up to 5 min in unexpanded foetal lungs did not cause subsequent vasoconstriction. Therefore either the fall in arterial pressure was predominant in causing vasodilatation, or no asphyxia was produced. But

there is good reason to suppose that the foetal lung consumes oxygen *in vivo* (Dawes, Mott & Widdicombe, 1954) as it does *in vitro*. When the pulmonary circulation was arrested pulmonary arterial pressure fell to within a few mm Hg of left atrial pressure, suggesting that bronchopulmonary anastomoses are not very large or plentiful in the foetus. More detailed investigation of the bronchial blood supply to the foetal lungs might provide an answer to the apparent paradox that local hypoxia produces vasoconstriction after ventilation, but ischaemia does not so do before ventilation.

Electrical stimulation of the parasympathetic and sympathetic nerves to the foetal lung can cause large changes in pulmonary vascular tone (Figs. 3 and 4), and these appear to be relatively larger than those seen in the adult. This is probably because pulmonary vascular tone is usually higher in the unexpanded foetal lung. A further rise in pulmonary vascular tone will divert blood flow from the lungs to the parallel circuits through the other foetal tissues and the placenta and this could serve a useful purpose during partial asphyxia. There was sympathetic tone in the pulmonary vascular bed in our preparations, but no parasympathetic tone. This is similar to the situation in the normal systemic circulation in adults, but further experience is required with a variety of different physiological conditions before we can be sure that the nervous mechanisms play a substantial part in the regulation of pulmonary blood flow during foetal life.

In foetal lambs after bilateral thoracic sympathectomy and vagotomy, acute asphyxia still caused pulmonary vasoconstriction, and ventilation of the lungs with air caused vasodilatation, both of about the same magnitude as that seen when the lungs were innervated (Dawes & Mott, 1962; Cassin, Dawes, Mott, Ross & Strang, 1964). Consequently the nerve supply to the lung is not essential for regulation of its vascular tone, as was indeed apparent from experiments on isolated perfused foetal lungs (Born *et al.* 1955). Figure 9 summarizes some of the observations. Mean left pulmonary pressure–flow diagrams have been constructed for the foetal condition from the data in Table 1 (1) and Table 2 (2) and also for previous observations (3, ----, Cassin, Dawes, Mott, Ross & Strang, 1964). Bilateral thoracic sympathectomy (4, — — —) caused vasodilatation. On rhythmic ventilation with air pulmonary vascular tone was reduced to the same level whether the lungs were innervated (5) or denervated (6).

In the present experiments there were large changes of heart rate during and after asphyxia, and on substituting air for 7% CO_2 in air or in N_2 as the ventilating gas, in lambs in which the autonomic nerve supply to the heart and lungs had been removed. In every instance a rise in heart rate was accompanied by pulmonary vasoconstriction, and a fall by vaso-

dilatation, and it is likely that both were in part due to changes in the concentration of circulating sympathomimetic amines as a result of alterations in blood gas tensions. For instance Comline & Silver (1961) showed that acute asphyxia released noradrenaline and adrenaline from the foetal adrenals, and both can cause pulmonary vasoconstriction in the lamb (Cassin, Dawes & Ross, 1964). The pulmonary vasoconstriction caused by asphyxia in immature foetal lambs was not abolished by adrenalectomy, but was abolished by injection of dibenamine, which blocked the vasoconstrictor effects of adrenaline and noradrenaline. Asphyxia might release these catecholamines from organs other than the adrenals, and possibly in the lung itself, as Barer (1963) has proposed from experiments on collapsed lungs in adult cats.

Expansion of the lungs

Previous experiments (Cassin, Dawes, Mott, Ross & Strang, 1964) showed that rhythmic ventilation of the lungs with a gas mixture which caused little or no change in blood gas tensions produced a considerable persistent pulmonary vasodilatation. The present results showed that a single brief expansion of the lungs with a similar gas mixture had an identical action (Fig. 9, 2 → 7), and that when this vasodilatation reached its peak, rhythmic ventilation with this gas mixture had no further effect on vascular tone. From the evidence presented (Fig. 6) it might be thought that this vasodilatation was due to two components, an initial rapid and a later slow response. There is another possibility, that the immediate vasodilatation is interrupted after an interval of 5–10 sec by vasoconstriction due to another mechanism and lasting 3 min or more. Just such a vasoconstriction has been described in the lungs of lambs 2–6 weeks of age after a single large inflation, and has been attributed to a local effect (Colebatch & Nadeau, 1964). We have also seen a rise in pulmonary arterial pressure on deep inflation of the lungs of a lamb breathing spontaneously and less than 12 hr old. We did not see this phenomenon in the present experiments when, 10–15 min after the initial expansion of the lungs, the same volume of the same gas mixture was injected into the trachea. But this may have been due to the fact that the lungs had already been distended, and so recently. The vasodilatation on first expansion of the lungs was not abolished by cutting the cervical vago-sympathetic trunks nor by bilateral thoracic sympathectomy (Fig. 9, 4 → 8). The effect of sympathectomy was more difficult to interpret than that of vagotomy, because the former decreased vascular tone while the latter did not alter it. The pulmonary vasodilatation which followed a single inflation in the intact foetus may have been partly due to a reflex inhibition of sympathetic tone, yet after sympathectomy expansion of the lungs caused a small

vasodilatation. Therefore there was a local effect of expansion upon the lungs themselves.

Various other possibilities were considered. Brief expansion of the right lung alone caused a fall of arterial pressure, and a small transient increase of blood flow through the unexpanded left lung (Fig. 8). The increase in blood flow could, however, have been a secondary consequence of the fall in arterial pressure rather than a reflex response. Expansion of the lungs with amniotic fluid or saline also caused a transient fall of arterial pressure and increase in pulmonary flow. It is hardly surprising that this small brief increase in flow was not noticed in previous experiments with a slower, more extensive dissection and a cruder type of flowmeter (Dawes, Mott, Widdicombe & Wyatt, 1953). This increase also could have been partly a secondary consequence of the fall in arterial pressure and a result of an increased capacity of the pulmonary vascular bed. It was impossible to maintain expansion of the lungs with a fluid without applying a positive pressure to the trachea, whereas after gaseous expansion the alveoli remained distended when the trachea was opened to the atmosphere.

Cook, Drinker, Jacobson, Levison & Strang (1963) described experiments in which the left lung was slowly inflated and deflated with either air or N_2 from a syringe. They obtained erratic results; in some lambs (on deflation) there was an increase in pulmonary flow at constant arterial pressure, but in others there was no change. They divided the left hemiazygos vein, freed the pulmonary artery from the pericardium and exposed and cannulated the left bronchus. It is possible that the sympathetic nerves to the left lung were injured in some of these preparations, as they run along the left hemiazygos vein and then dive in to the root of the lung. Their observations are not inconsistent with the present results, taking into consideration the differences in anaesthetic (pentobarbitone) and experimental procedures.

The present results add further evidence to support the view that vascular tone in the foetal lung is very labile. It may readily be altered by stimulation of the autonomic nerves, by injection of small doses of sympathomimetic amines, acetylcholine or histamine, by small changes in arterial P_{O_2} or P_{CO_2}, by a brief fall in arterial pressure, by gaseous expansion of the lung and by some as yet unexplained mechanism. Similar changes have been observed in the foetal lung after bilateral thoracic sympathectomy and vagotomy. Some of the changes in vascular tone associated with variations in blood gas tensions could be due to blood-borne hormones. But we must also, in the future, give serious consideration to the possibility of local regulation.

SUMMARY

1. In mature foetal lambs a fall in pulmonary arterial pressure usually caused pulmonary vasodilatation, both before and after bilateral vagotomy and thoracic sympathectomy.

2. Electrical stimulation of the peripheral end of the ipsilateral vagus caused a large vasodilatation, and stimulation of the sympathetic nerves caused vasoconstriction in the foetal lung.

3. Bilateral section of the cervical vago-sympathetic trunks did not alter pulmonary vascular tone in the foetus, but bilateral thoracic sympathectomy caused vasodilatation.

4. After denervation of the foetal lung, acute asphyxia still caused pulmonary vasoconstriction and positive pressure ventilation with air still caused vasodilatation.

5. A single brief expansion of the foetal lungs with 3% O_2 and 7% CO_2 in N_2 caused prolonged pulmonary vasodilatation with little or no change in arterial P_{O_2} or P_{CO_2}. This vasodilatation was also observed after bilateral vagotomy and thoracic sympathectomy.

6. It was concluded that the pulmonary circulation in mature foetal lambs was under nervous control as well as being susceptible to both blood-borne hormones and local influences.

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Note added in proof. Subsequent experiments in lambs have shown that contact of glass with foetal blood can, under favourable circumstances, rapidly lead to the formation of a substance which, like bradykinin, causes a transient pulmonary vasodilatation in this preparation (Campbell, Dawes, Fishman & Hyman, unpublished). Although the vertical tube used for generating pressure–flow curves in the present experiments was made of plastic, it was attached to the connexion between the carotid and left pulmonary artery by a T-piece. Any procedure which caused a fall of arterial pressure allowed blood to re-enter the circuit from the vertical tube through this T-piece; this could explain the consequent brief pulmonary vasodilatation. The same mechanism may also account for the 'spontaneous' vasodilatation.