J. Physiol. (1964), **171**, *pp.* 80–89
With 5 text-figures
Printed in Great Britain

PULMONARY BLOOD FLOW AND VASCULAR RESISTANCE IN IMMATURE FOETAL LAMBS

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(*Received* 18 *July* 1963)

The first object of the present experiments was to measure pulmonary vascular resistance in the foetal lamb at an early gestational age (75–90 days), at a time when the lungs cannot be expanded by gaseous ventilation, for comparison with observations on mature foetal lambs near term (147 days; Cassin, Dawes, Mott, Ross & Strang, 1964). The second object was to determine whether the tone of the pulmonary vascular bed was variable and under physiological control at the earlier age, as compared with mature lambs (Dawes & Mott, 1962).

METHODS

Nineteen Clun-Hampshire foetal lambs of 84–90 days gestation age, weighing 504–816 g, and 3 lambs of 75–81 days gestation age weighing 260–391 g, were delivered by Caesarean section under light chloralose anaesthesia (40 mg/kg I.V. initially). The umbilical cord remained intact. The preparation and methods for measuring pulmonary vascular resistance were similar to those described by Cassin *et al.* (1964), except that the vertical tube which was filled with blood in order to produce pressure–flow curves, was smaller in diameter (3·5 mm) than that used previously. The output from the electromagnetic flowmeter (Wyatt, 1961) was increased by a further stage of amplification (5–10 times) to produce full-scale output for a flow of 40 ml./min. Frequent zero adjustments were made to compensate for temporary changes in base line. Otherwise the recording equipment was the same as that described previously (Dawes & Mott, 1962). Doses of drugs are given in terms of base.

RESULTS

Pulmonary vascular conductance has been calculated as Δ flow/Δ arterial pressure in the steep part of the pressure–flow curve, and the intercept of the conductance line on the pressure axis has been measured as described in the preceding paper (Cassin *et al.* 1964). Left atrial pressure showed little variation and was low when compared with pulmonary and systemic arterial pressures. No spontaneous variations in

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conductance comparable to those in lambs near term, were observed in these immature lambs.

There was an insignificant difference in mean pressure intercept be-

TABLE 1. Effect of age on left pulmonary vascular conductance and pressure intercept in foetal lambs

Age (days)	85·3 (range 75–90)	138 (range 135–143)
Number	14	23
Weight (kg)	0·55 (range 0·26–0·76)	3·92 (range 2·38–5·18)
Conductance (ml./min.mm Hg)	1·48 ± 0·19	3·44 ± 1·42
Conductance per kilogram	2·69 ± 0·31	0·89 ± 0·17
Pressure intercept (mm Hg)	33 ± 1·3	36 ± 1·7

The figures indicate means ± S.E. unless otherwise stated.

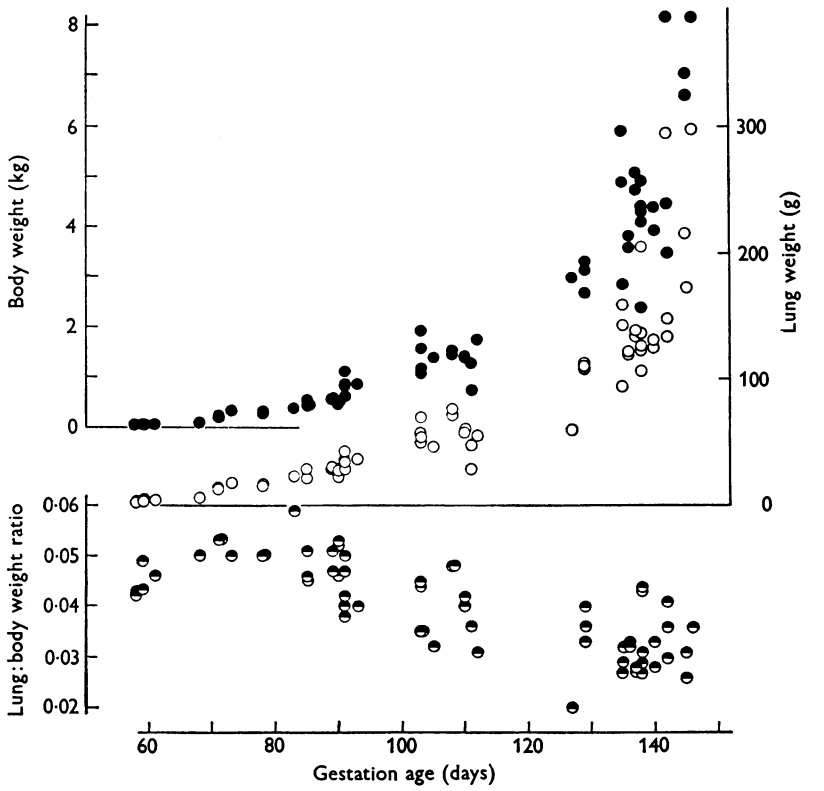

Fig. 1. Body weight (●), lung weight (○) and lung:body-weight ratio (◓) in sixty-two Clun-Hampshire foetal lambs during the latter part of gestation.

tween lambs of 75–90 and of 135–143 days gestation age (Table 1). Left pulmonary vascular conductance was less in the younger lambs. Foetal lambs increase in weight more than sevenfold during this period of gestation. When the results were expressed in terms of body weight, left

pulmonary vascular conductance was on the average 3 times greater in younger than in older foetal lambs. The weight of the lungs does not increase proportionately as much in foetal lambs as does total body weight during the last half of gestation (Fauré-Fremiet & Dragoiu, 1923; Carlyle, 1945). Figure 1 shows measurements of body weight (●), lung weight (○) and of lung:body-weight ratio (◓) in the variety of sheep used in the present experiments (but not in the same animals). The lung:body-weight ratio decreased from a mean of $0{\cdot}048 \pm 0{\cdot}0013$ (S.E.) in sixteen lambs of 78–91 days gestation to $0{\cdot}032 \pm 0{\cdot}0012$ (S.E.) in twenty lambs of 135–145 days gestation. The difference between the means is highly significant ($P < 0{\cdot}001$). Left pulmonary vascular conductance was 5·6 ml./min.mm Hg per 100 g total lung (wet weight) in the younger lambs, as compared with 2·8 in older lambs.

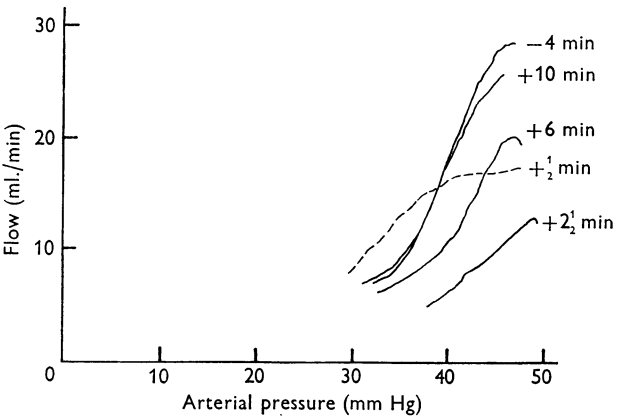

Fig. 2. Foetal lamb, 90 days gestation, 670 g. Left pulmonary arterial pressure–flow curves traced from oscilloscope photographs before and after injection of 0·1 μg noradrenaline into the blood supply to the left pulmonary artery at time zero.

Noradrenaline and adrenaline

Single injections of 0·05–0·25 μg noradrenaline into the blood supply to the left pulmonary arteries of eleven immature lambs caused vasoconstriction and a small rise of arterial pressure which persisted for several minutes. The magnitude and duration of the vasoconstriction depended on the rate of blood flow at the time of injection. In the experiment illustrated in Fig. 2 this was 10·5 ml./kg.min, and it was 10 min before the pressure–flow curve had returned to its original position. In another lamb with a larger left pulmonary flow (15·2 ml./kg.min) the same dose of noradrenaline caused a relatively smaller vasoconstriction, which lasted less than 6 min. Its twin had a flow of 5·3 ml./kg. min, and the effect

was greater and lasted more than 20 min. It is therefore not possible to give a simple quantitative measure of the vasoconstrictor effect of noradrenaline injected directly into the pulmonary blood stream. When noradrenaline was infused into the external jugular vein of two foetal lambs at 0·5 μg/kg.min there was little change in the pressure intercept, but vascular conductance decreased from 1·76 and 1·74 ml./min.mm Hg. kg to 0·68 and 0·94 respectively within 4–5 min.

In four of ten lambs noradrenaline also caused transient vasodilatation after injection into the blood supply to the left pulmonary artery. There was a small rise in pulmonary flow 10–15 sec after the injection, at a time when arterial pressure was beginning to increase. Pressure–flow curves taken at this time showed changing characteristics (Fig. 2, ---) and lay partly to the left of the initial observations made before administration of noradrenaline. Administration of dibenamine (3 mg/kg) always abolished the vasoconstrictor action of noradrenaline; when given to one of the four lambs in which vasodilatation had been seen, a small vasodilator effect remained and there was no rise in arterial pressure. As Fig. 2 shows the vasoconstrictor action is normally much greater and longer lasting.

Single injections of 0·05–0·2 μg adrenaline into the blood supply to the left pulmonary artery in seven lambs caused vasoconstriction and a small rise of blood pressure of about the same magnitude and duration as that observed on injection of equal doses of noradrenaline. Transient vasodilatation was also seen in five of these lambs. The vasoconstrictor effect of adrenaline was abolished by the administration of dibenamine.

Isoprenaline, acetylcholine and histamine

Injection of isoprenaline (0·1–0·3 μg to six lambs), acetylcholine (1–10 μg to eight lambs) or histamine (1–4 μg to ten lambs) into the blood supply to the left pulmonary artery all caused a very considerable increase in pulmonary blood flow and a small fall in arterial pressure. Figure 3 illustrates the effects of acetylcholine and isoprenaline on the pressure–flow curves. In three lambs the pressure intercept was reduced (to as low as 10 mm Hg) with little change in conductance (i.e. in the maximum slope of the pressure–flow curve). The increase in pulmonary blood flow was accompanied by a fall in femoral arterial pressure and a rise in heart rate.

Ischaemia and asphyxia

When the left pulmonary artery was divided, and the peripheral end was first connected to the cardiac end of the left carotid artery, flow increased considerably and then returned to a lower value, in lambs of

75–90 days gestational age as in mature foetal lambs. In three premature lambs flow was deliberately arrested for 2 min, and a similar transient increase in flow was observed after this period of ischaemia.

The mean arterial pressure (measured in a side arm of the external circuit which joined the left carotid to the left pulmonary artery) was 28 (range 25–33) mm Hg in eight lambs. When the umbilical cord was occluded to cause asphyxia there was an instantaneous rise in pressure of 5–7 mm Hg, which was usually succeeded by a small fall during the next

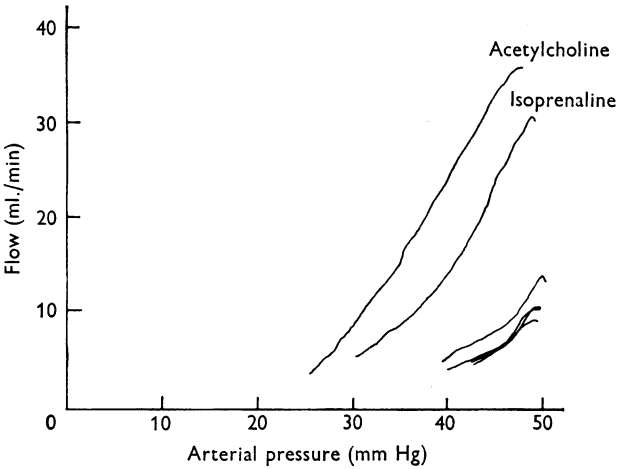

Fig. 3. Foetal lamb, 86 days gestation, 545 g. Left pulmonary arterial pressure–flow curves traced from oscilloscope photographs before, during and after the vasodilatation caused by injection of 2 μg acetylcholine and 0·2 μg isoprenaline into the blood supply to the left pulmonary artery.

10–15 sec. Then the pressure gradually rose to reach a mean value of 44 (range 38–56) mm Hg after 2 min (Fig. 4*a*). Pulmonary flow rose for the first 30 sec, and then gradually decreased to a mean value rather less than that before asphyxia. Pressure–flow curves taken after 2 min asphyxia showed severe vasoconstriction (Fig. 5). The loop of string round the umbilical cord was loosened after 2·5–3·0 min asphyxia; recovery required at least 10 min and usually 15 min. Asphyxia for 2 min caused the pressure intercept to rise in all but one of eight lambs, though the increase was small (Table 2). There was a decrease in vascular conductance in all eight lambs which averaged 65 %.

In each of seven lambs injection of 1–2 mg dibenamine into the blood supply to the left pulmonary artery caused a fall in pulmonary vascular resistance, which was slow in onset and prolonged. After administration of dibenamine, asphyxia for 2 min caused neither a rise in blood pressure

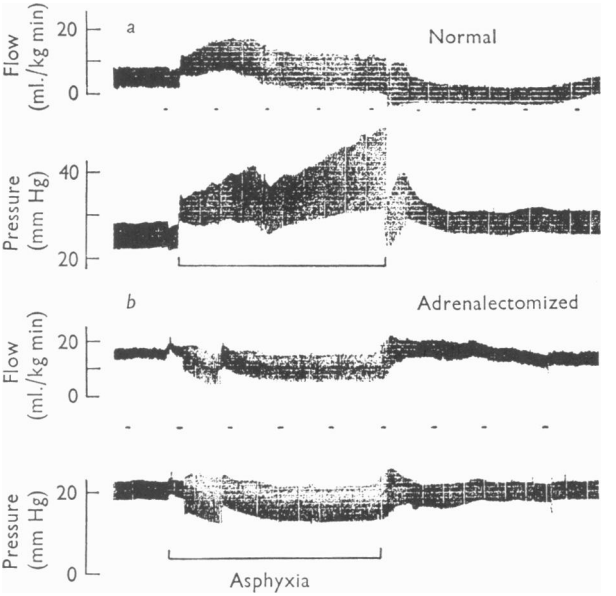

Fig. 4. Records of left pulmonary arterial flow (above), time marker, 0·5 min, left pulmonary arterial pressure (below) and signal mark in 2 twin lambs of 89 days gestation, (*a*) 525 g normal and (*b*) 605 g, adrenalectomized, during asphyxia caused by occlusion of the umbilical cord for 2 min.

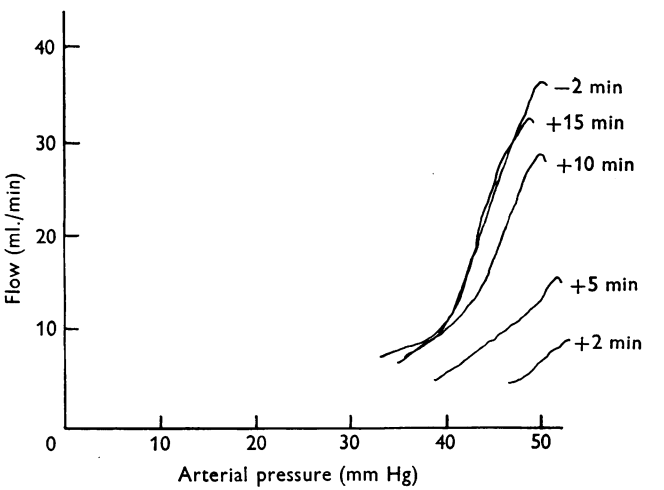

Fig. 5. Foetal lamb, 86 days gestation, 545 g. Left pulmonary arterial pressure–flow curves traced from oscilloscope photographs before, during and after asphyxia caused by occlusion of the umbilical cord at time zero and lasting 2·5 min.

nor pulmonary vasoconstriction in four lambs (Table 2); in one lamb there was transient vasodilatation.

Both adrenal glands were removed from four lambs, each of them twins to those in which the effects of asphyxia have been described. After adrenalectomy the mean pulmonary pressure intercept was significantly less ($P < 0.05$) and the vascular conductance per kilogram was significantly greater ($P < 0.01$) than in normal lambs. Asphyxia for 2 min caused only a small rise of blood pressure (Fig. 4*b*), the mean being 2 mm Hg. Examination of the pressure–flow curves showed that there was a small increase in the pressure intercept and that pulmonary vascular

TABLE 2. Effect of asphyxia by occlusion of the umbilical cord for 2 min on pulmonary vascular resistance in lambs of 84–90 days gestation

		Before cord occlusion		2 min after cord occlusion	
	No.	Pressure intercept (mm Hg)	Conductance per kilogram (ml./min.mm Hg)	Pressure intercept (mm Hg)	Conductance per kilogram (ml./min.mm Hg)
Normal	8	34·7 ± 1·8	2·77 ± 0·39	38·5 ± 1·9	0·96 ± 0·10
After dibenamine	4	29·8 ± 1·8	2·40 ± 0·33	26·5 ± 2·7	2·20 ± 0·08
After adrenalectomy	4	27·5 ± 1·0	6·66 ± 2·71	29·2 ± 1·9	2·50 ± 0·45

The figures indicate the means ± S.E.

conductance decreased considerably (Table 2). The proportionate reduction in pulmonary vascular conductance per kilogram during asphyxia was the same after adrenalectomy as before.

Lambs of 75–81 days gestation

In the three lambs of 75–81 days gestation the effects of injection of adrenaline, noradrenaline, isoprenaline, histamine and dibenamine, and of asphyxia for 2 min, were both qualitatively similar to those observed in lambs of 84–90 days gestation, and quantitatively similar when account was taken of the difference in weight.

DISCUSSION

A previous attempt to measure pulmonary blood flow and vascular resistance *in vivo* in foetal lambs of 90 days gestation age or less, i.e. at an age when the lungs cannot yet be expanded by gaseous ventilation, was unsuccessful (Born, Dawes & Mott, 1955). The smaller size and greater friability of the pulmonary arteries in younger lambs made it impossible to use the methods which were successful in lambs near term. Since that time two changes in technique have made the preparation easier: first, connexion of the peripheral end of the left pulmonary artery

to the cardiac end of the left carotid, so as to avoid double cannulation of a short pulmonary artery (Dawes & Mott, 1962), and secondly, substitution of an electromagnetic flowmeter (Wyatt, 1961) for the clumsier density flowmeter (Dawes, Mott & Vane, 1953). The application of these methods was successful, but the first experiments showed that measurements of pressure and flow separately were a poor guide to changes in vascular resistance in very young lambs. For instance, injection of acetylcholine often caused only a small increase in pulmonary blood flow, accompanied by a decrease in perfusion pressure. It was suspected, and this suspicion proved correct, that the small magnitude of the increase in flow was due to the fall in arterial pressure combined with the shape of the pressure–flow curves before and after administration of acetylcholine. When the pressure–flow curves were obtained by displaying flow on the Y axis and pressure on the X axis of an oscilloscope or X-Y recorder (while blood ran into the left pulmonary artery from a vertical tube) the change in vascular resistance on injecting acetylcholine was obvious and large. Subsequently this method was applied to lambs near term and proved valuable in giving an immediate accurate measure of vascular resistance, from which decisions could be taken at once about the conduct of an experiment.

In the last three centuries ideas as to the rate of blood flow through the foetal lungs at different stages of gestation have varied from those of Harvey (1628), who considered that there was almost no pulmonary flow in the foetus, to those of Kilian (1826) who thought it was very large. Others expressed the opinion that it might be small at first, increasing towards term (Bichat, 1801; Patten, 1930). The present experiments do not answer the question directly, as they have been concerned more with the measurement of pulmonary vascular conductance and its physiological control than with measurement of flow under natural conditions *in utero*. They show that pulmonary vascular conductance is greater earlier in gestation, whether expressed in terms of total body weight or of lung weight, under the conditions of our experiments. The variation in calculated conductances was large, particularly in the mature lambs. Apart from experimental error some of this variation may have been due to anaesthesia, delivery of the foetus from the uterus and to dissection. We cannot exclude the possibility that these factors had a greater effect in the mature than in the premature lambs. The experiments suggest the possibility, which from previous observations might have been regarded as implausible, that under natural conditions *in utero* pulmonary blood flow could be relatively high throughout the latter half of gestation.

In lambs of 75–90 days gestation age, moderate doses of noradrenaline (or adrenaline) caused a considerable decrease in pulmonary vascular con-

ductance (Fig. 2), but acetylcholine and isoprenaline (or histamine) caused a large increase (Fig. 3). Hence even at this early age, when gaseous expansion is impossible, the lungs have vasomotor tone which might be altered in either direction by variations in the internal environment. The small transient vasodilatation caused by adrenaline (which was also seen after administration of dibenamine) and the profound vasodilatation caused by histamine are more like that observed in the systemic circulation than in the pulmonary circulation of the normal adult sheep (J. Colebatch, personal communication).

In previous observations on immature foetal lambs under pentobarbitone anaesthesia, hypoxaemia (induced by giving the ewe 10, 7·5 or 5% O_2 in N_2 to breathe) caused a small increase in heart rate but little rise in blood pressure (Born, Dawes & Mott, 1956). The responses were much less than in foetal lambs near term. The present experiments have demonstrated that immature lambs under chloralose anaesthesia react vigorously to brief but profound asphyxia. There was extreme pulmonary vasoconstriction which was abolished (in one instance reversed) by dibenamine, which also abolished the vasoconstriction caused by adrenaline and noradrenaline. Comline & Silver (1961) showed that the quantity of catecholamines released by asphyxia from the adrenals was considerably less at this age than in mature lambs near term. In our experiments asphyxia still caused proportionately the same fall in pulmonary vascular conductance after adrenalectomy; this may have been due to release of catecholamines into the circulating blood from the organs of Zuckerkandl (West, 1955) and chromaffin tissue elsewhere. There are other possibilities, such as by an effect on the arterial chemoreceptors and reflex nervous control of the pulmonary vascular bed. It is possible that a number of different mechanisms may be involved simultaneously.

SUMMARY

1. Immature foetal lambs of 75–90 days gestation age were delivered by Caesarean section, and left pulmonary arterial pressure–flow curves were produced by running blood in from a vertical tube.

2. Mean pulmonary vascular conductance was greater, when compared with that of mature foetal lambs at term, both in terms of body weight or of lung weight.

3. Injection of noradrenaline or adrenaline caused vasoconstriction, often preceded by brief vasodilatation. Injection of isoprenaline, acetylcholine or histamine caused profound vasodilatation.

4. Asphyxia for 2 min caused a rise of arterial pressure and pulmonary vasoconstriction. These effects were abolished by dibenamine.

5. It was concluded that at this age, when the lungs cannot be ventilated, they have vasomotor tone which can be greatly changed by alterations in the internal environment.

We thank Dr J. C. Mott, Dr L. B. Strang, A. Ryder and A. Stevens for help with the experiments. We are also grateful to Mr H. B. Parry and Dr H. Shelley for some of the lung and body weights.

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