SUMMARY Pulmonary arterial (PA) blood flow patterns, changes in pulmonary blood flow, and pulmonary vascular responses to graded hypoxemia and intravenous acetylcholine (ACh) were studied in 15 fetal lambs in utero 3–12 days after surgical implantation of an electromagnetic flow transducer and PA catheter. Phasic PA flow in the fetus was forward only during the first third of systole, almost zero during mid-systole, and backward during late systole and early diastole. In contrast, neonatal lambs showed forward PA flow throughout systole. The constriction of the fetal pulmonary vasculature in response to progressive hypoxemia varied with gestational age. At 103 days there was no significant drop in PA flow and only a small increase in pulmonary vascular resistance (Rp). With hypoxemia, the greatest increase in Rp was seen in fetuses after 121 days of gestation. This response was unaffected by α- and β-sympathetic and parasympathetic blockade. Similarly, the pulmonary vascular response to ACh injected into the fetal jugular vein depended on gestational age. Little or no increase in pulmonary flow was noted in the youngest fetus, whereas ACh produced a marked increase in pulmonary flow in fetuses over 120 days of gestation. These data suggest that the mechanisms by which hypoxemia constricts and ACh relaxes the pulmonary vascular smooth muscle are not fully developed in fetal lambs at 100 days of gestation and furthermore, that these mechanisms progressively develop during the last third of gestation.

THE PULMONARY CIRCULATION of the fetus has been shown to be more responsive to hypoxemia and to vasoactive agents than is that of the adult.** However, previous observations on these responses were made on fetal lambs exteriorized from the uterus, usually with the ewe under general anesthesia. Since these procedures alter the distribution of blood flow in the fetus' and may modify responses of the pulmonary circulation, pulmonary vascular pressures of the undisturbed fetus in utero could not be defined. Furthermore, in previous studies little attempt was made to delineate the effects of graded hypoxemia, nor was there a detailed consideration of possible gestational differences in pulmonary vascular responses.

We designed a preparation to study changes in pulmonary blood flow and pulmonary vascular responses of fetal lambs in utero. After recovery from surgery, pulmonary blood flow was monitored continuously with an electromagnetic flow transducer, and pulmonary arterial pressures were measured. Constriction of the pulmonary vascular bed by graded hypoxemia and dilation of the pulmonary vascular bed by the infusion of acetylcholine (ACh) were studied during advancing gestation. The role of the sympathetic nervous system in the hypoxic response was assessed.

Methods

Twenty-six time-dated pregnant sheep with gestational periods ranging from 0.66 to 0.93 (100–140 days) were studied. Low spinal analgesia was produced with 2–3 ml of 1% tetracaine hydrochloride. The abdomen was opened in the midline, a small incision was made in the uterine wall to expose a fetal hindlimb, and polyvinyl catheters were inserted into a hindlimb artery and vein.** Through a separate incision catheters were inserted into the fetal carotid artery and jugular vein and, through a purse-string suture, the trachea. A thoracotomy then was performed in the 3rd or 4th left intercostal space, the pericardium was opened, and the main pulmonary trunk was isolated.

In the fetal lamb the right ventricle ejects into the pulmonary trunk, which continues into the ductus arteriosus. The blood vessels to the lung arise as a common main pulmonary artery segment which divides into the left and right branch pulmonary arteries (Fig. 1). The main pulmonary artery is of variable length; in nine fetuses a precalibrated electromagnetic flow transducer was placed around it...
but in the other 17 the vessel was too short, therefore the flow transducer was placed around the left pulmonary artery just beyond its origin (Fig. 1). The flow transducer was selected to provide a snug fit around the vessel. The lumen diameters were 4-6 mm.

A polyvinyl catheter was inserted directly into the main pulmonary artery through a purse-string suture in the pulmonary trunk, adjacent to the origin of the main pulmonary artery. The chest was sutured, the fetus was returned to the uterus, and the uterus was closed and returned to the abdomen. The catheters and flow transducer cable were led to a pouch sewn to the skin of the ewe's flank. The abdomen then was closed and the fetus and ewe were allowed to recover from surgery. All ewes received 1 million U of penicillin and 400 mg of kanamycin daily as prophylaxis against infection. Eighteen fetuses survived the surgical procedure for more than 2 days; however, in three, satisfactory recordings of pulmonary flow could not be obtained. The remaining 15 fetuses were studied 3-4 days after surgery, while the ewe stood quietly in a cage. In several fetuses repeat observations were performed on separate days for up to 12 days. Studies were not continued beyond this time because we felt that growth of the fetus would result in the development of constriction of the pulmonary artery (PA) by the flow transducer, which had a fixed diameter. Total pulmonary blood flow or left pulmonary arterial blood flow was measured with a Statham SP2202 electromagnetic flowmeter (frequency response flat to 100 Hz; ±5% error; ±2% reproducibility). The validity and accuracy of these measurements have been published previously.10 Systemic, PA, and tracheal pressures were measured with Statham P23Db pressure transducers, and pressures and flow were recorded continuously on a Beckman Dynograph recorder. We used the tracheal pressure as zero reference for the intravascular pressures because we have shown that over the age range studied tracheal pressure is only 1-2 mm Hg above intraamniotic pressure. Pulmonary vascular resistance ($R_p$) was calculated by dividing the mean transpulmonary pressure difference (PA - LA) by the pulmonary blood flow ($Q_p$). The equation used was: $R_p = (PA - LA)/Q_p$. Left atrial (LA) pressure was assumed to be 3 mm Hg above intratracheal pressure. This assumption was based on studies in other fetal lambs of similar gestational ages in which left atrial catheters were implanted chronically. In these, changes in left atrial pressure during periods of hypoxemia were insignificant. In those fetuses in which left PA flow was measured, it was assumed that this represented 40% of total pulmonary blood flow. This assumption is based on the results of studies using radionuclide-labeled microspheres.8,9 The figures for total $R_p$ were used for comparing fetuses. Variations in the proportion of flow to each lung may have altered the calculation of total $R_p$ in some fetuses, but did not influence the measurement of the percentage changes in $R_p$ related to hypoxemia.

A loose-fitting plastic bag was placed over the ewe's head and connected to a gas mixing chamber by a flexible hose.11 Control measurements were obtained while the ewe received compressed air at a flow rate of at least 20 liters/min. Progressive fetal hypoxemia without alteration of pH and PCO$_2$ was slowly-produced over 30-40 minutes by giving the ewe compressed air with increasing proportions of nitrogen and 5% CO$_2$ to breathe, thereby decreasing frac-
tional inspired oxygen concentration (FIO₂) from 21% to 6%. Fetal PA blood gas measurements were made serially, using a Radiometer blood gas meter and appropriate electrodes. After fetal PA blood PO₂ had been lowered to 10–13 torr, the ewe was returned to room air and allowed to recover. The hypoxemia studies were not performed during periods of spontaneous fetal respiratory movements, since considerable increases in pulmonary flow were produced by these movements.

Twenty-six hypoxemia studies were performed in the 15 fetuses. Two studies were performed in one fetus at 103 and 104 days of gestation; four in two fetuses between 112 and 119 days; 17 in nine fetuses between 121 and 130 days; and three studies in three fetuses between 132 and 138 days of gestation. Serial observations were made in 10 fetuses. The effects of α- and β-adrenergic blockade on the hypoxemic response were studied in four fetuses of 120–130 days' gestation. α-Adrenergic blockade was produced with (iv) phentolamine (1 mg/kg of estimated fetal weight), and β-adrenergic blockade with propranolol (1 mg/kg, iv). The effectiveness of the blockade was tested by administering methoxamine and isoproterenol. The effect of parasympathetic blockade on the hypoxemic response was examined in four fetuses after administering atropine in doses of 0.1–0.2 mg/kg. Fetal arterial blood PO₂ was increased slightly in four fetuses by administering 100% oxygen to the ewe to breathe.

The effect of ACh on the pulmonary circulation was examined in nine fetuses. In five, increasing amounts of ACh (0.4–2.7 μg/kg) were injected as a bolus into the fetal superior vena cava while systemic and PA blood pressures and pulmonary blood flow were monitored continuously. In the other four, ACh (7 μg/kg of estimated fetal wt/min) was infused continuously into the fetal jugular vein while monitoring pressures and flow.

The actual weight of each fetus was determined at termination of the study. Statistical analyses were performed by Student's paired and unpaired t-tests.

**Results**

During the control period, prior to each study, PA blood pH was 7.37 ± 0.03 (SEM); PO₂, 19 ± 2 (SEM) torr; and PCO₂, 42 ± 4 (SEM) torr. PA mean blood pressures ranged from 30 to 57 mm Hg, with the higher pressures in the older fetuses (Fig. 2). With advancing gestation, total PA blood flow increased from 90 ml/min in the immature to 195 ml/min in the oldest fetuses. However, pulmonary blood flow per kilogram of fetal body weight did not change significantly (Fig. 2). Although actual R p decreased with advancing gestation, the resistance calculated in relation to fetal body weight did not change significantly (Fig. 2).

The contour of the phasic PA flow tracing in the fetal lamb was strikingly different from that in the postnatal lamb (Fig. 3). Postnatally, pulmonary flow persists throughout systole with only a small amount of backflow coincident with the dicrotic notch of the PA pressure. During diastole little or no forward flow occurs. In the fetus, forward flow occurs only during the first third of systole, there is almost no flow during midsystole, and backward flow occurs during late systole and early diastole; throughout the remainder of diastole there is no significant forward or backward flow. The dicrotic notch of the PA pressure pulse is associated with the negative flow phase of the flow tracing.

**RESPONSES TO PO₂ CHANGE**

Reduction of the inspired oxygen fraction of the ewe resulted in a progressive fall in fetal PA blood PO₂. The lowest level reached varied from 10 to 13 torr. Pulmonary arterial pH (7.33 ± 0.04) and PCO₂ (45 ± 5 torr) were not significantly altered. Pulmonary and systemic arterial mean pressures increased by 1–5 mm Hg, but the change also was not significant (Fig. 2).

At PA blood PO₂ levels of 10–13 torr, pulmonary blood flow fell in most fetuses (Fig. 2). In the youngest fetus (103–104 days), there was no significant drop in flow; the reduction in flow in four studies in the two fetuses of less than 120 days of gestation averaged 31%, but this too was not significant. The pulmonary flow fell 57% in the fetuses...
beyond 121 days of gestation and this change was significant \((P < 0.01)\). There was no difference between the responses at 121–130 days and at 132–138 days of gestation. \(R_p\) increased in all fetuses. In the fetus of 103–104 days’ gestation there was an average rise of 19\%, and in the fetuses of 112–119 days a rise of 33\% above control levels. In the fetuses of 121–138 days’ gestation \(R_p\) increased by 141\% \((P < 0.05)\). This increase was significantly greater \((P < 0.05)\) than that in fetuses of under 120 days’ gestation. No significant difference was noted between the increase in \(R_p\) in the fetuses of 121–130 and 132–138 days’ gestation. In individual fetuses studied serially, a similar progressive increase in the hypoxicemic response occurred.

The inspired oxygen fraction was increased in four ewes with gestations above 120 days; this resulted in an increase in fetal PA blood \(P_{O_2}\) to 24 ± 2 (SEM) torr. PA blood pressure did not change, but pulmonary blood flow increased from 40 ± 17 ml/kg per min to 60 ± 35 ml/kg per min; however, this change was not significant. \(R_p\) fell by 27\% of control, and this too was not significant.

The pattern of response of \(R_p\) to hypoxemia was clearly related to gestational age of the fetus (Fig. 4). In a 103-day fetus a progressive decrease of PA blood \(P_{O_2}\) produced a small increase in \(R_p\), only at a \(P_{O_2}\) below 12 torr. With advancing gestation not only was the maximal response greater, but the \(R_p\) increased with progressively smaller reductions in \(P_{O_2}\). In the older fetuses there was a curvilinear relationship between PA blood \(P_{O_2}\) and \(R_p\).

Associated with the increase in \(R_p\), there was a change in the configuration of the PA flow tracing. The duration of forward flow in early systole decreased, whereas the duration of backflow during diastole increased.

**AUTONOMIC BLOCKADE**

Administration of phentolamine, propranolol, or atropine to the fetus prior to induction of hypoxemia did not alter the vascular responses to \(P_{O_2}\) alterations.

**ACETYLCHOLINE RESPONSES**

Administration of ACh either by bolus injection or continuous infusion into the fetal jugular vein catheter produced a marked increase in pulmonary blood flow. This did not appear to result from an increase in heart rate, since pulmonary blood flow in fetuses above 120 days’ gestation increased by 200–300\%, while heart rate response varied but did not increase or decrease by more than 25\%. PA blood pressure fell 2–5 mm Hg; calculated \(R_p\) decreased markedly. The pulmonary vasculature of the younger fetuses was much less sensitive to ACh than that of the older fetuses. Whereas ACh in doses of 0.4 \(\mu\)g/kg of fetal body weight produced a marked increase in pulmonary blood flow in the fetuses above 120 days’ gestation, no response was noted with doses of up to 1.3 \(\mu\)g/kg in the fetus of 103–104 days gestation. Doses of 2.7–4.0 \(\mu\)g/kg produced an increase of pulmonary blood flow of only about 25\% in this fetus, while 0.4–0.7 \(\mu\)g/kg consistently resulted in a pulmonary flow increase of at least 200\% in the older fetuses.

Continuous administration of ACh in amounts of 7 \(\mu\)g/kg per min into the jugular vein catheter produced a sustained increase in pulmonary blood flow over the duration of the infusion. Associated with the increased pulmonary flow was a persistence of forward flow throughout most of systole, a decrease or disappearance of backflow, and a small but consistent continuous forward flow throughout diastole (Fig. 5).

**Discussion**

Most previous studies of the fetal pulmonary circulation have been performed acutely in exteriorized fetal lambs with open chest. In most instances the ewes received general anesthesia, and the pulmonary artery to the left lung or left lower lobe was cannulated. These studies provided important information regarding the capability of the pulmonary vasculature to respond to pharmacological agents, and to changes in blood gases and pH. However, they could not define the responses of the normal pulmonary circulation, because general anesthesia may have affected vascular responses. Furthermore, passage of blood through rubber or plastic tubes may result in release of vasoactive substances which may drastically affect vascular resistance. In most of the previous reports, pulmonary blood flow was quite low as compared to measurements made in fetal lambs in utero using the radionuclide-labeled microsphere technique. This suggests that pulmonary vasoconstriction was present even in the control periods.
In the present series, the total pulmonary blood flows were slightly higher than those measured in two different groups of fetuses using the microsphere method, or an electromagnetic flow transducer. Flow in this study averaged 55 ml/kg of fetal body weight, as compared to 40 ml/kg of body weight in our two previous studies. In the majority of fetuses in the earlier studies the flow measurements were made on the same day that surgery had been performed on the ewe and fetus, and full recovery may not have occurred. In this study we assumed, on the basis of the microsphere studies, that left lung flow was 40% of total pulmonary blood flow. If this assumed proportion was low, then total pulmonary blood flow would not have been as high as estimated.

Even though there is a slight discrepancy between the current and previous studies, we confirmed that pulmonary blood flow represents only 10–11% of combined ventricular output in the fetus, and that Rp is very high; this high resistance helps to explain the unusual flow patterns observed. Only about 10–15% of blood ejected by the right ventricle passes through the lungs, the remaining 85–90% passing through the ductus arteriosus to the descending aorta. Forward flow into the pulmonary arterioles occurred only during the first third of systole to the peak of the blood velocity through the pulmonary trunk. During the latter part of systole, flow continued through the pulmonary trunk and ductus arteriosus, but PA flow ceased. During diastole, when there was no flow through the pulmonary valve, a significant backflow was recorded by the flowmeter. This probably represents flow of blood from the large pulmonary arteries which preferentially passed to the ductus and relatively low resistance lower body and placental circulations, rather than through the high resistance pulmonary circulation. When Rp was further increased by hypoxemia, the magnitude and duration of the forward flow was reduced. Acetylcholine, a potent pulmonary vasodilator, produced a marked increase in the magnitude and duration of systolic flow and reduced or eliminated backflow.

Studies on the pulmonary vascular response to hypoxemia in newborn calves showed a curvilinear response with a progressive increase in Rp with decreasing PO2 to levels of about 20 torr. It was not possible to achieve lower PO2 levels as circulatory failure occurred. Our studies in fetal lambs have demonstrated that there is a progressive curvilinear increase in Rp with reduction of PO2 to 10–13 torr. The potentiation of the hypoxic vasconstrictor response by acidaemia, noted in the newborn calves, was not examined in these fetuses.

In our studies pharmacological blockade of parasympathetic and α- and β-adrenergic activity did not influence the hypoxic response, indicating that neither the sympathetic nor parasympathetic nervous system mediates the hypoxic response directly. This is at variance with the observations of Cassin et al. and Campbell et al., who noted that α-adrenergic blockade consistently decreased the response to hypoxemia. The differences can probably be explained by the more intense degree of hypoxemia and also the production of concomitant acidemia in their studies, since PO2 was reduced by compression of the umbilical cord. The severe hypoxemia, hypercarbia, and acidemia which occurred has been shown to stimulate norepinephrine release. Norepinephrine produces mild pulmonary vasoconstriction and thus α-adrenergic blockade could have abolished the norepinephrine-induced constriction, while not directly affecting the hypoxic response.

The increasing responsiveness of the pulmonary circulation to both hypoxemia and Ach infusion with advancing gestational age is of considerable interest. It had been suggested that the thickness of the medial muscular layer in pulmonary arterioles increases during the latter third of gestation. This could explain our findings, since the greater the quantity of smooth muscle in each vessel, the more vasoconstriction or dilation could occur. However, recently we have shown that the thickness of the medial muscular layer is constant throughout the latter half of gestation in fetal lambs, but that the total number of vessels increases. Although the total Rp could change to a greater extent with hypoxemia or Ach in the more mature animals, the percentage change should not differ. Our data therefore suggest that the mechanism by which hypoxemia constricts the pulmonary vascular smooth muscle is not fully developed in the fetal lamb at 100 days (0.66) gestation, and that it progressively develops toward term. Similarly, the smooth muscle relaxation with Ach is poorly developed early in gestation and this suggests either that receptors are not developed, are not responsive, or that there is an immaturity of the intrinsic mechanism for the Ach response. A detailed study of the development of pulmonary vascular response to hypoxemia and to pharmacological agents may help to explain the basic mechanisms responsible for vasoconstriction and dilation.

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References

Vasomotor Control of Capillary Transit Time
Heterogeneity in the Canine Coronary Circulation

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SUMMARY A set of indicator-dilution studies of the coronary circulation of the intact functioning heart was carried out in the dog to provide a data base for defining the effect of changes in vasomotor control on the exchange of materials across the myocardial capillaries. The reference substance used was \(^{14}C\)-labeled albumin; the diffusible substance used was \(^{14}C\)-labeled sucrose. Model analyses of the data were carried out. In previous models of the capillary exchange in the coronary circulation, it had been assumed that a single capillary transit time is representative of the whole. The data acquired here indicate that there is a very large heterogeneity of capillary transit times in the intact heart, and that the single transit time model is approximately true only when the resistance vessels are maximally dilated. The present pattern of findings is explained best by a model of the coronary microcirculation based on capillary-large vessel units with a variable heterogeneity of flow or capillary lengths, hence of transit times. We conclude that a major determinant of the extraction of each diffusible substance, on a microscopic level, is the distribution of capillary transit times.

Although the construction of mathematical models of the single capillary is relatively advanced, \(^{14}C\) it rarely has been possible to isolate a single capillary and its surrounding tissue. The transport data available have been those from whole organs and are a summation of effects at the level of millions of capillaries, which may differ in their lengths, flow rates, and diffusional interactions. In order to extract information on the intrinsic characteristics of transport by the capillary wall and tissue cells, the organ model must be extended to include this heterogeneity.

Goresky et al. \(^1\) previously have considered two models representing the extreme cases, i.e., no heterogeneity, and maximum heterogeneity in capillary transit times. Multiple indicator-dilution data from the liver fit the latter model very well. \(^2\) The data from the heart, on the other hand, have always been assumed to exhibit very little or no heterogeneity of capillary transit times and to represent the other extreme, \(^3\) although the matter really has not previously been tested.

In the present study we found, in the course of analyzing many multiple indicator-dilution experiments on the heart, that this assumption did not provide an adequate description of the data, that it approximated the data only when the coronary circulation was maximally dilated, and that in general there was evidence of a large degree of heterogeneity, which varied with the degree of coronary vascular resistance. To account for this, we developed a mathematical model of the coronary circulation based on capillary-large vessel units in which there is a heterogeneity of flow, hence of capillary transit times, and, with this, we then were

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