PRESSURE-FLOW RELATIONSHIPS IN THE PULMONARY VASCULATURE OF FETAL LAMBS DURING *IN UTERO* VENTILATION

by

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A DISSERTATION

Presented to the Department of Physiology
and the Oregon Health Sciences University
School of Medicine
In partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

May 1987

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(Dr. John A. Resko, Chairman, Graduate Council)

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ACKNOWLEDGEMENTS

Many members of the Department of Physiology and the "fetal research group" have generously lent support and encouragement these past five years; those friendships will not be forgotten.

As always, there are those without whose help this project would not have been possible. Mr. Nick Schiller sacrificed many evenings and weekends to perform the blood gas analyses critical to this study. Mr. Tom Green wrote essential computer programs and solved too many problems to mention. Ms. Pat Renwick provided cheerfully efficient surgical assistance and good companionship. Mr. Bob Webber and Ms. Karen Lentfer expertly prepared the sheep for surgery; Ms. Karen Lentfer also helped to monitor post-surgical progress of the sheep.

Special thanks is extended to Ms. Jackie Niemi whose professional integrity has been privotal in completion of this manuscript. Ms. Pat McDaniel has played a critical role in the preparation of the figures for this thesis; for her assistance I am also grateful.

Dr. J. Job Faber has put much energy into many facets of my education. His willingness to discuss scientific concepts has always been appreciated and is now gratefully acknowledged. Dr. Debra Anderson has also made cherished contributions to my scientific development; her influence has been an important aspect of my training.

Several people have helped to hone the ideas presented in this thesis. Dr. Debra Anderson, Dr. Job Faber, Dr. Roger Hohimer, Dr. William Holden and Dr. Mark Morton all contributed much good advice in their official capacity as my thesis advisory committee. Dr. George Giraud and Dr. Mark Reller have also made valuable contributions to this project.

It has been my particular good fortune to have had Dr. Kent Thornburg as mentor. With rare talent, he combined a challenge to achieve goals beyond my own vision with constant support and friendship. It has been a priveledge to have worked with him; I look forward to our continued association.

Finally, my heartfelt thanks to my husband, Dallas A. Carter, who has shared the joy and withstood the pain of the "transition period."

ABSTRACT

Pulmonary vascular resistance is high in the fetus. At birth, resistance falls sharply, about ten-fold. The factors which stimulate this fall are not well defined. This study was designed to test the hypothesis that mechanical ventilation, increased arterial PO_2 and decreased PCO_2 all play important roles in the fall in pulmonary vascular resistance at birth.

Eleven mature lamb fetuses were equipped with tracheal catheters for positive pressure in utero ventilation. Catheters were placed in the pulmonary artery, left atrium, carotid artery, right atrium, and the pericardial and amniotic fluid spaces for pressure measurement. An electromagnetic flow sensor was placed around the left pulmonary artery for flow measurement. Inflatable balloon occluders were placed on the ductus arteriosus and the postductal main pulmonary artery so that driving pressure through the pulmonary vascular bed could be raised and lowered to generate pressure-flow curves.

Blood flow to the left lung, heart rate and pressures were measured during six treatment conditions to determine the pressure, heart rate and flow at which the fetus operates under each given condition. A pressure-flow curve was also generated during each given condition. Treatment conditions were: 1) control (non-ventilated resting state); 2) ventilation with 3% O_2-7% CO_2 in N_2 (mechanical ventilation); 3) ventilation with 3% O_2 in N_2; 4) ventilation with room air; 5) ventilation with 95% O_2-5% CO_2; and 6) ventilation with 100% O_2.

Mechanical ventilation with no change in fetal arterial gases decreased pulmonary vascular resistance from 1.3 $\pm$ 1.3 (SD) during

control to 0.4 ± 0.4 (SD) mm Hg·min·ml^{-1}. Increased pulmonary arterial PO_2 and decreased pulmonary arterial PCO_2 were significantly associated with further decreases in pulmonary vascular resistance. In summary, mechanical ventilation, increased pulmonary arterial PO_2, and decreased pulmonary arterial PCO_2 were all important factors which decreased pulmonary vascular resistance in mature lamb fetuses during positive pressure *in utero* ventilation.

INTRODUCTION

At birth, the site of gas exchange moves from the placenta of the fetus to the expanded lungs of the neonate. A marked decrease in the resistance of the pulmonary vessels accommodates the sudden increase in pulmonary blood flow which is necessary for proper ventilation-perfusion relationships in the neonate. The factors which mediate the decline in pulmonary vascular resistance at birth are of great significance, but are poorly understood. Before the determinants of pulmonary vascular resistance can be addressed, relevant differences between the fetal and adult circulations will be reviewed.

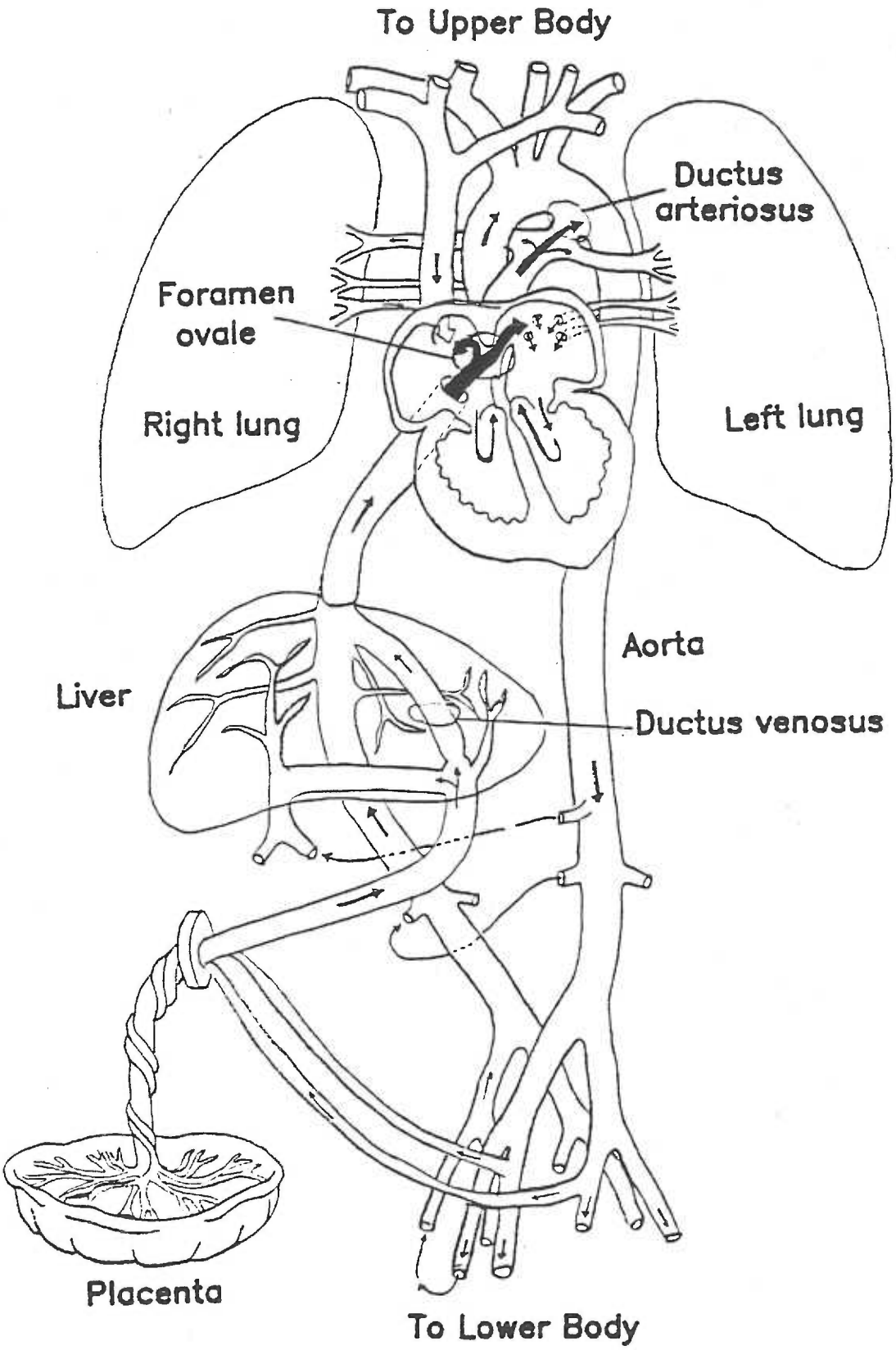
HEMODYNAMIC AND ANATOMIC CHARACTERISTICS OF CIRCULATION

FETAL CIRCULATION

The fetal circulatory system is uniquely arranged to permit the survival of the fetus in the intrauterine environment. Four special vascular structures allow the fetus to circulate blood to the placenta for oxygenation rather than to the lungs (Figure 1). These structures include: 1) the ductus venosus, a conduit between the umbilical vein and the inferior vena cava; 2) the foramen ovale, a valve-like communication between the right and left atria of the fetus; 3) the ductus arteriosus, a vessel between the pulmonary artery and aorta; and 4) the umbilical vessels, a vascular circuit which permits placental exchange of nutrients, wastes and respiratory gases between fetal and maternal organisms.

Another characteristic which differentiates the fetal from the adult circulation is the high vascular resistance of the fetal pulmonary bed. The mechanisms which maintain this high pulmonary

Figure 1: Diagrammatic representation of the human fetal circulation. Four specialized fetal structures are indicated: placenta, ductus venosus, foramen ovale and ductus arteriosus. Direction of blood flow is indicated by arrows. Obvious differences in the lamb circulation include: 1) a single major arterial branch, the brachiocephalic artery, which serves the fetal upper body , 2) in the sheep there are multiple placentas called cotyledons, and 3) the lamb fetus has two umbilical veins instead of one as depicted here for the human. (Modified from Moore, 1982, p 334).


To Upper Body
Ductus arteriosus
Foramen ovale
Right lung
Left lung
Aorta
Liver
Ductus venosus
Placenta
To Lower Body

vascular tone in the fetus are not understood. However, the high resistance to blood flow serves to divert blood away from the inactive fetal lungs, which are not needed for respiration in fetal life, to the placenta which is the fetal organ of gas exchange.

As a result of the fetal circulatory arrangement, the distribution of blood flow from the fetal heart is particularly suited to the intrauterine "low oxygen" environment. Of the total combined output of the fetal right and left ventricles, 40% perfuses the placenta (Rudolph and Heymann, 1970; Mott, 1982). The hemoglobin in blood flowing through the placenta acquires oxygen by diffusion (Faber and Thornburg, 1983) and returns to the fetus through the umbilical veins 80% saturated with oxygen (Dawes, 1968). In the liver, the umbilical vein bifurcates to form two veins, the ductus venosus and the portal sinus. Some of the umbilical blood perfuses both the right and left lobes of the liver before it joins the inferior vena cava in the liver (Edelstone et al., 1978). The ductus venosus joins the inferior vena cava where umbilical blood mixes with oxygen-depleted blood returning from the fetal hindlimbs; here the hemoglobin is 67% oxygen saturated (Dawes, 1968). Both the inferior and the superior vena caval bloods flow toward the right atrium. Superior vena caval blood drains the fetal upper body and is relatively desaturated (about 31% saturated, Dawes, 1968). Due to the anatomical arrangement of the inflow vessels, the blood stream entering the right atrium from the superior vena cava does not mix completely with the stream from the inferior vena cava.

Most of the oxygenated blood returning to the heart via the inferior vena cava bypasses the right atrium. The kinetic energy of the stream in the inferior vena cava propels it through the channel of

the foramen ovale and into the left atrium (Anderson et al., 1981; 1985). This flow through the foramen ovale represents a flow shunt, from the right side of the circulation to the left, which permits oxygenated blood to bypass the pulmonary circulation. Most of the blood returning from the upper body via the superior vena cava enters the right atrium, crosses the tricuspid valve and flows into the right ventricle. Blood leaves the right ventricle via the pulmonary artery, 52% saturated with oxygen (Dawes, 1968). The pulmonary artery bifurcates at the ductus arteriosus providing a route by which blood leaving the right ventricle can flow either through the ductus arteriosus or into the pulmonary vascular bed. Due to the high vascular resistance of the pulmonary circuit, compared to the systemic circuit, most of the pulmonary artery blood flow is diverted through the ductus arteriosus to the systemic circulation. This creates a second fetal right-to-left shunt. These two shunts permit the fetal lungs to receive a small fraction of the combined output of both ventricles, rather than the entire cardiac output as in the adult circulation (Dawes, 1968).

Blood returning from the lungs flows through the four pulmonary veins which, in sheep, empty individually into the left atrium. This blood mixes in the left atrium with the well-oxygenated blood entering through the foramen ovale from the right atrium and is ejected from the left ventricle into the aorta. The coronary arteries branch immediately from the aorta and supply the active fetal myocardium with well oxygenated blood. A single branch of the aorta, the brachiocephalic artery, supplies the fetal upper body; it forms the carotid and brachial arteries. The carotid arteries supply blood to a major portion of the fetal brain. This anatomic arrangement provides

to the fetal brain blood which is about 62% saturated with oxygen (Dawes, 1968). Thus the liver, the myocardium and the fetal upper body, including the brain, receive the most highly oxygenated blood supply in the fetus. At the ductus arteriosus the less oxygenated blood (52% saturated, Dawes, 1968) is shunted from the pulmonary artery and joins blood in the aorta that perfuses the rest of the body with blood which is about 58% saturated with oxygen. Much of this blood flows to the placenta where oxygen and nutrients are replenished and wastes are removed.

As one can gather from the above description, the fetal circulation is different from the adult circulation, where blood is pumped through the systemic and pulmonary circuits in series; the fetal circulations, on the other hand, are arranged in parallel. Therefore, instead of referring to a cardiac output as that from a single ventricle as in the adult, fetal cardiac output is expressed as biventricular output, the sum of the outputs of the left and right ventricles. The ovine fetal right ventricle contributes slightly more to the combined output of the heart than does the left. Estimates of right ventricular output range from 60 to 67% biventricular output (Heymann et al., 1973; Anderson et al., 1981; Thornburg et al., 1986). In spite of initial reports to the contrary (St. John Sutton et al., 1984; Wladimiroff et al., 1982), it now appears that these percentages may be applicable to the human fetus as well as to the lamb (Kleinman and Donnerstein, 1985).

TRANSITIONAL CIRCULATION

The period during which mammalian circulation changes from the

fetal pattern to the adult-like pattern of neonatal life is termed the transition period. As parturition begins, several changes in the fetal circulation become characteristic of the "transitional circulation." The most readily observable of these is the loss of the placental circulation as the umbilical cord is tied or severed. Collapse and functional closure of the umbilical vessels and of the ductus venosus closely follows the events of birth (Dawes, 1968). In the fetus, the placenta has a low vascular resistance; this helps to maintain a relatively low systemic vascular resistance. And, as one would expect, when the neonate is separated from the placenta, systemic vascular resistance rises.

The most obvious feature of birth is the onset of ventilation. Since 1952, it has been known that the first breaths of air cause pulmonary vascular resistance to fall dramatically (Adran et al., 1952). The drop in resistance is associated temporally with increased pulmonary blood flow (Dawes et al., 1953), blood volume (Walker et al., 1975) and left atrial pressure (Dawes, 1968). As left atrial pressure increases, the pressure gradient across the foramen ovale reverses. This acts to reverse the pressure gradient through the foramen ovale and in some species, closes the septum secundum, a flap valve on the left side of the atrial septum, and inhibits left-to-right flow through the foramen ovale (Dawes, 1968). This functionally closes the foramen ovale.

The combination of decreased pulmonary vascular resistance and increased systemic resistance causes a reversal in the direction of flow through the ductus arteriosus (Assali et al., 1962). Under these conditions, pulmonary blood flow is probably more than 50% of biventricular cardiac output. The blood flowing through the pulmonary

vessels exchanges gases with those filling the neonatal lung. Thus, blood leaving the lungs of the neonate has a higher oxygen content and tension and lower carbon dioxide tension than ever before encountered by the fetus. Human neonates attain carotid arterial PO_2 values slightly greater than 60 mm Hg and PCO_2 values slightly below 40 mm Hg within one hour of birth (Oliver et al., 1961). The increased oxygen tension has several actions on the circulation. It appears to stimulate constriction of the systemic vessels, increasing systemic vascular resistance beyond that expected by the loss of the placental circulation (Dawes, 1968). Second, it causes constriction of the ductus arteriosus. Thus, two major shunts of the fetal circulation close when the neonate begins to breathe and, over a short period of time, the series circulations of the adult are established.

Closure of the ductus arteriosus in response to increased oxygen concentration has been studied in detail and is the subject of a recent review article (Clyman, 1987). Upon closure of the ductus arteriosus, pulmonary and systemic arterial pressures change in opposite directions. Pulmonary arterial pressure declines with decreasing pulmonary vascular resistance to a value still greater than normal adult pressure, but substantially lower than fetal pulmonary artery pressures (Rudolph and Heymann, 1974). In contrast, aortic pressures increase several mm Hg when the ductus arteriosus closes (Dawes, 1968). It should be noted that the closure of the ductus arteriosus appears to be due to active constriction and that the constricted condition can be reversed, during the first few hours of life, by a decline in arterial oxygen tension (Clyman, 1987).

A third major circulatory change which accompanies birth is an increase in cardiac output. Changes in heart rate and stroke volume both contribute to increased output at birth, but stroke volume is the primary factor. Stroke volumes increase from 1.1 and 1.4 $ml \cdot kg^{-1}$ for the left and right fetal ventricles, respectively, to approximately 2.0 $ml \cdot kg^{-1}$ with right and left ventricular output equal within 24 hours of birth (Morton et al., 1987; Erath et al., 1981). Some of the changes of birth can also be mimicked by *in utero* ventilation with oxygen (Morton et al., 1987).

In the adult, the four well recognized mechanisms which can mediate increases in ventricular stroke volume include increased sarcomere length (preload), increased strength of contraction (contractility), decreased tension on the sarcomeres during contraction (afterload) and increased chamber size (Braunwald, 1980). Mean atrial pressure is one of the more commonly used indices of preload. The relationship between mean atrial pressure and stroke volume has been studied extensively in the fetal lamb (Kirkpatrick et al., 1976; Gilbert, 1980; Thornburg and Morton, 1983). The fetus operates at a filling pressure which produces near maximal stroke volume so that it has little preload reserve. Therefore, increases in atrial pressure above the operation point cause only very small increases in stroke volume. One must conclude then, that increased atrial pressure is not the mechanism which augments stroke volume at birth.

Other mechanisms that may be responsible for birth-related increases in stroke volume have been studied by ventilating fetuses *in utero*. During *in utero* ventilation and at birth, the ventricular function curve which relates mean atrial pressure to stroke volume is shifted upward (Morton et al., 1987), so that stroke volume is larger

for the same filling pressure. An increase in contractility could cause such a shift. To shed light on the contractility issue, the shift in dimension of the left ventricular anterior-posterior minor axis has been examined in the fetal and neonatal states (Kirkpatrick et al., 1973; Anderson et al., 1982). Although percentage fractional shortening ([end diastolic dimension - end systolic dimension] ÷ end diastolic dimension · 100%) is greater in neonates than in fetuses (27.7 vs. 24.7%, Anderson et al., 1982), this dimension change may not be due entirely to changes in contractility alone and can not account for the magnitude of change in stroke volume at birth. The effect of arterial pressure on stroke volume has been examined in the fetus as a method to estimate afterload sensitivity (Thornburg et al., 1983), although it should be noted that the relation between arterial pressure and systolic wall tension has not been adequately studied in the fetus. This study showed that the right ventricle is very sensitive to decreases in arterial pressure. However, the left ventricle is not very sensitive to changes in arterial pressure over the physiologic range (Morton et al., 1987). Even if the left ventricle were sensitive to arterial pressure, systemic arterial pressure generally increases at birth and thus would not be a mechanism for increasing stroke volume. The final mechanism available to the fetus to augment stroke volume at birth is an acute increase in ventricular chamber size. Although this possibility has not yet been adequately studied, evidence from fetal and neonatal humans using ultrasonic imaging suggests that left ventricular dimension increases and right ventricular dimension decreases slightly after birth (Wladimiroff et al., 1982). This may

come about as the result of ventricular interaction with the formation of a left-right ventricular pressure gradient.

ADULT CIRCULATORY PATTERNS

As mentioned previously, the adult circulation is arranged as two circuits in series, whereas the two circulations are in parallel in the fetus. The structures unique to the parallel circulation of the fetus are the ductus arteriosus, ductus venosus and foramen ovale; structures which are no longer patent in the adult circulatory system. However, their remnants are recognized in adults as the ligamentum arteriosum, ligamentum venosum and the fossa ovalis. Likewise the vessels of the umbilical circulation can be identified in the adult. The umbilical vein is termed the ligamentum teres while the umbilical arteries are referred to as the lateral umbilical ligaments (Arey, 1966).

Following birth, the pulmonary vascular resistance continues to decline with development so that it is even lower in adulthood than in the neonatal period. This is due primarily to anatomic development; that is, the cross-sectional area of the vasculature increases as new capillary growth occurs in the alveolar region (Reid, 1968). The decrease in pulmonary vascular resistance with maturity is also accompanied by regression of the medial muscle layer in small arteries and by increased diameter of larger arteries (Hislop and Reid, 1973). Recent evidence also suggests that the fall in resistance from neonatal to adult life may be accounted for in part by the physiologic effect of oxygen on the pulmonary vessels (Custer et al., 1985).

PULMONARY VASCULAR ANATOMY

The lungs are supplied by two arterial systems. The bronchial

circulation arises from the thoracic aorta and provides blood to the airways and large vessels. That portion of the bronchial flow which is returned via pulmonary veins comprises less than 5% of fetal pulmonary blood flow (Campbell et al., 1967). The pulmonary circulation supplies blood to the respiratory tissues of the lungs and serves the alveoli for the purpose of gas exchange. Anatomically, the pulmonary arteries accompany the airways. However, the arteries have a more extensive branching pattern. This results in two classes of arteries: preacinar pulmonary arteries accompany airways, intraacinar arteries lie within the respiratory tissue (Reid, 1968).

In the adult, the structure of the pulmonary arteries change in a predictable way with diameter. (McFadden and Braunwald, 1980). Arteries in excess of 2000 μm diameter are classified as "elastic"; "muscular" arteries comprise the 30 to 2000 μm diameter range. Arteries smaller than 250 μm range from muscular to nonmuscular. As the vessel diameter of muscular arteries becomes smaller, the solid muscular coat which completely surrounds the small arteries becomes progressively thinner. Eventually only a spiral of muscle can be found and even that muscle disappears so that no muscle exists in the walls of the smallest vessels (Reid, 1977).

Reid also studied the wall composition of fetal vessels (Hislop and Reid, 1972). In the fetus, elastic arteries can be found at much lower diameters. In fact, elastic arteries exist at the same airway level in both fetal and adult lungs. However, the structure of nonelastic fetal arteries is again dependent on diameter. Hislop and Reid (1972) reported considerable overlap in vessel structure at any given vessel diameter. In a 140 day human fetus, the largest non-

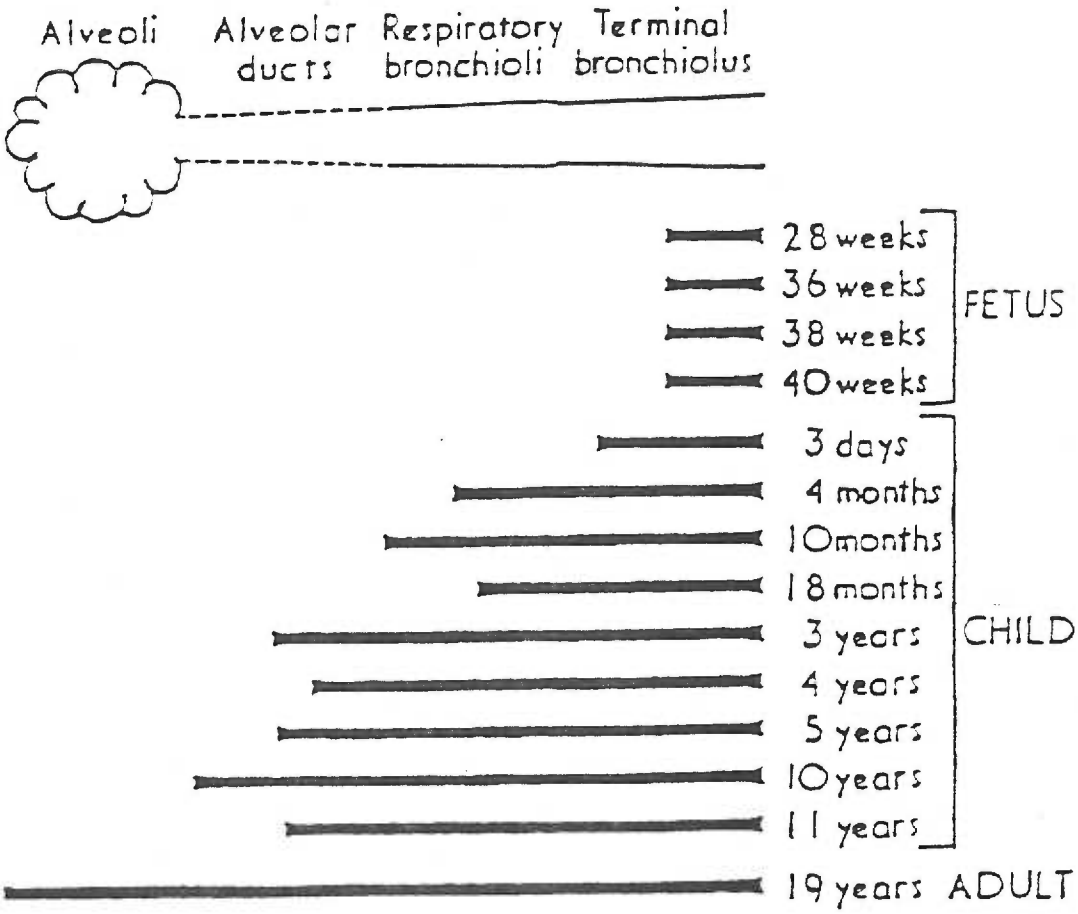
muscular artery was found to be 107 μm in diameter. Partially muscular arteries ranged from 37 to 160 μm and the smallest muscular artery was 95 μm diameter. This relationship is close to that described earlier in the adult.

In terms of anatomic location Hislop and Reid's data indicate that in the adult, muscular arteries are found to the alveolar level, whereas in the fetus no muscular arteries are found distal to the terminal bronchioli. However, partially muscular arteries may be found at the acinar level in the fetus (Hislop and Reid, 1972). The change in the location of muscular pulmonary arteries with increasing maturity is demonstrated in Figure 2. At all arterial levels, The fetal lung vasculature is more muscular than the adult, i.e. the percentage of the vessel wall composed of muscle is greater in the fetus. In fact, the percentage wall thickness for a vessel of a given diameter is twice as great for fetal arteries as for adult arteries (Hislop and Reid, 1972).

Dilation of the muscular arteries of less than 200 μm in diameter accompanies the drop in pulmonary vascular resistance at birth (Hislop and Reid, 1973; Rabinovitch, 1985). The decrease in resistance from fetal to adult levels is accompanied by the medial regression and dilation of larger arteries (Hislop and Reid, 1973).

However, the changes which occur in the pulmonary vessels in the perinatal period can be influenced by both in utero and postnatal conditions. The lung vasculature is extremely plastic in nature. That is, the vessel walls are able to remodel in reaction to the conditions to which they are subjected. In particular, hypoxia and pulmonary hypertension have been shown to elicit a restructuring of the pulmonary vascular wall muscle composition (Reid, 1977; Levin et al., 1978).

Figure 2: Presence of smooth muscle in smallest arteries of the pulmonary vasculature as a function of maturity and airway level. Bar represents level to which muscle is found. Muscular arteries do not extend beyond the terminal bronchioles in the fetus but can be found to the alveolar level in adults. (Adapted from Hislop and Reid, 1973, p 133).


Alveoli
Alveolar ducts
Respiratory bronchioli
Terminal bronchiolus
28 weeks
36 weeks
38 weeks
40 weeks
FETUS
3 days
4 months
10 months
18 months
3 years
4 years
5 years
10 years
11 years
CHILD
19 years
ADULT

REGULATION OF ADULT PULMONARY VASCULAR RESISTANCE

In hydraulic systems which operate in a fashion analagous to Ohm's law, resistance, R, in mm Hg $min \cdot min^{-1}$ is defined as the ratio of the driving pressure and flow or:

$$R = P/\dot{Q} \qquad (1)$$

where $\dot{Q}$ is flow in ml/min, and ΔP is the difference in mm Hg between inflow pressure and outflow pressure. Pouiseuille's law describes the relationship between driving pressure and steady laminar flow of a Newtonian fluid through a rigid tube. In this case:

$$\dot{Q} = \frac{\pi \cdot \Delta P \cdot r^4}{8 \eta \ell} \qquad (2)$$

where η is the viscosity of the fluid, in mm $Hg \cdot min \cdot cm^{-3}$, ℓ is the length in cm of the tube and r equals the radius in cm of the tube. Rearrangement of equations (1) and (2) yields an important relationship for resistance in these tubes:

$$R = \frac{8\eta}{\pi} \cdot \frac{\ell}{r^4} \qquad (3)$$

Thus, when the laws of Ohm and Poulseuille are applicable, resistance is dependent upon the viscous characteristics of the fluid and upon the dimensions of the tube.

One might ask, do these relationships hold within the hemodynamic constraints of the pulmonary vasculature? It is immediately clear that several of the basic assumptions of Poulseuille's law are not strictly met. Blood, a non-Newtonian fluid, flows through short branched distensible vessels of the lung under the influence of a pulsatile driving pressure.

In vascular systems, inflow pressure is usually arterial blood pressure and the outflow pressure is venous or mean atrial pressure. However, in some instances, a pressure exceeding the outflow pressure surrounds the vessel distal to the arteries. This pressure is often referred to as a "surrounding pressure." When surrounding pressure is elevated above the normal outflow pressure, flow through the vessel is determined by the driving pressure which is now arterial pressure minus surrounding pressure. This condition, where driving pressure is independent of outflow pressure, is commonly referred to as the waterfall phenomenon, since the height of a waterfall (the outflow pressure) does not determine the flow of water over a falls. When surrounding pressure exceeds outflow pressure the relationship between flow and apparent driving pressure does not pass through the origin. Instead it intersects the zero flow abcissa at a positive pressure indicating the magnitude by which surrounding pressure exceeds outflow pressure.

It has long been known that pulmonary vascular resistance is not constant under varying conditions. Factors which change pulmonary vascular resistance can be classified as either passive or active. Active changes are those which are mediated by a change in the vascular tone of smooth muscle in the vessel walls. All other factors which change pulmonary vascular resistance are termed passive.

PASSIVE DETERMINANTS OF PULMONARY VASCULAR RESISTANCE

The ratio of driving pressure to blood flow in the lung is sensitive to passive alterations in the dimensions of resistance vessels with changes in vascular pressures and lung volume. The

fraction ℓ/r^4 in equation (3) represents the influence of vascular dimension (vessel length, ℓ, and radius, r) on the resistance and will be referred to as the geometrical factor. This quotient, which is constant in rigid tubes, changes dramatically in the lung under the influence of passive forces.

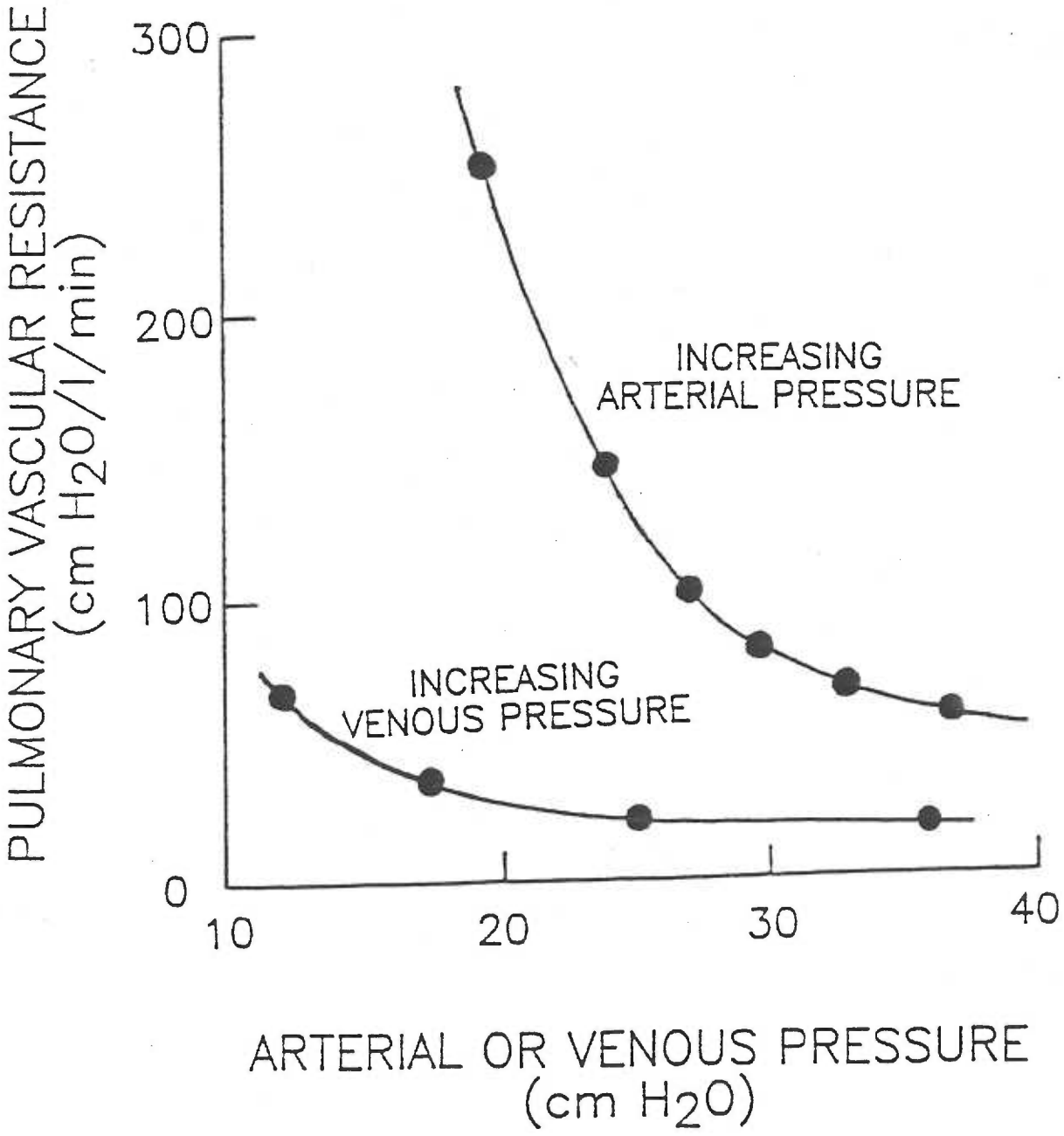
Vascular Pressures

In the vessels of the adult lung (Fishman, 1985), changes in transmural pressure, the hydrostatic pressure difference across the wall of the vessel, have a remarkable influence on the geometrical factor through vessel distension (increase in vessel radius) or recuitment (opening of new vessels). Increased arterial pressure increases transmural pressure and distends pulmonary vessels. Even small increases in vessel radius greatly decrease the geometrical factor and decrease resistance. If outflow pressure is decreased (at constant arterial pressure), transmural pressure of the "resistance vessels" will decrease, the vessel radius will decrease and pulmonary vascular resistance will increase. Therefore, increases in either pulmonary arterial pressure or left atrial pressure will decrease pulmonary vascular resistance (Figure 3).

Lung Gas Volume

Pulmonary vascular resistance can also be passively altered by changes in lung gas volume. The effect of a change in lung volume is dependent upon the anatomic location of the vessel being affected and on the posture of the animal. A change in lung gas volume can alternately narrow and lengthen a vessel or shorten and distend it, depending upon its orientation and location in the lung. Pulmonary vascular resistance is increased by both high and low lung volumes.

Figure 3: Diagrammatic representation of the independent effects of increasing pulmonary arterial and venous pressures on pulmonary vascular resistance on the isolated adult dog lung. The "venous" outflow pressure was kept constant while arterial pressure was altered and vice versa. Increase in either pulmonary arterial or venous pressure is associated with decreased pulmonary vascular resistance; the relationship is more powerful for arterial than venous pressure. Note pressures are in cm H_2O and not mm Hg. (Adapted from West, 1985, p 36).


PULMONARY VASCULAR RESISTANCE
(cm H2O/l/min)
300
200
100
0
10
20
30
40
INCREASING
ARTERIAL PRESSURE
INCREASING
VENOUS PRESSURE
ARTERIAL OR VENOUS PRESSURE
(cm H2O)

ACTIVE DETERMINANTS OF PULMONARY VASCULAR RESISTANCE

The Autonomic Nervous System

Although stimulation of autonomic nerves can elicit small changes in pulmonary vascular resistance, the autonomic nervous system is not considered an important regulator of pulmonary vascular resistance. This topic has recently been reviewed by Fishman (1980). It is clear, however, that the pulmonary vasculature is sensitive to many circulating substances and to local chemical stimuli.

Humoral Substances

Circulating agents which cause vasoconstriction in the lung include serotonin, angiotensin, catecholamines, phenylephrine, leukotrienes, histamine, endoperoxidases, thromboxane, prostaglandin $F_{2\alpha}$ (Fishman, 1985). Acidosis also leads to pulmonary vasoconstriction; and potentiates the vasoconstrictive actions of hypoxia on pulmonary vessels (Enson et al., 1964). Acetylcholine, bradykinin, isoproterenol, theophylline, and prostaglandin I_2 all have pulmonary vasodilating properties (Fishman, 1985).

Oxygen

The effect of decreased oxygen tension on pulmonary vascular resistance is opposite its effect on systemic vascular resistance. Systemic vessels generally dilate in response to decreased plasma oxygen tension and constrict when oxygen tensions rise. This is believed to be the mechanism behind autoregulation (Johnson, 1986). On the other hand, pulmonary resistance vessels dilate in response to increasing oxygen tension and constrict when oxygen tension decreases.

This phenomenon is termed pulmonary hypoxic vasoconstriction. In the adult, hypoxic vasoconstriction serves to divert blood away from poorly ventilated areas of lung and therefore enhances efficient respiratory exchange by the lung.

The location of the site that senses oxygen tension is unknown, but in the adult it has been postulated to exist closer to the alveolar than to the arterial side of the alveolar gas diffusion barrier (Hauge, 1969; Hyman et al., 1975; Bjertnaes et al., 1980; Hyman et al., 1981; Marshall et al., 1983).

The relative importance of oxygen in the alveoli versus the pulmonary arteries has been a question of considerable interest for several decades. Most evidence suggests that alveolar oxygen tension is the primary determinant of pulmonary vascular resistance during hypoxia. However, there are data suggesting that pulmonary arterial oxygen levels may influence pulmonary vascular resistance (Bergofsky et al., 1963; Hauge, 1969 Benumof et al., 1981; Pease et al., 1982; Marshall et al., 1983). The mechanism by which oxygen exerts its influence on pulmonary vascular resistance is the subject of intense investigation and has been recently reviewed (Fishman, 1976; Voelkel, 1986).

CHANGES IN FETAL PULMONARY VASCULAR RESISTANCE AT BIRTH

It has been recognized for at least 35 years that pulmonary vascular resistance decreases at birth. But as knowledge of transition physiology has grown, so has the level of understanding of the regulation of pulmonary vascular resistance. Ardran and colleagues (1952) first used artificial positive pressure ventilation in

anesthetized fetuses at surgery. They reported an immediate fall in pulmonary arterial pressure after ventilation and a large increase in the velocity of blood moving through the lungs (as measured by pulmonary circulation times). This was the first published evidence of changes in the pulmonary vasculature at the onset of ventilation. Decreased pulmonary vascular resistance was also demonstrated in several early experiments by Dawes and coworkers (1952; Dawes et al., 1953). Reynolds, a member of the research team of Ardran et al., conducted a histologic investigation of the fetal vasculature and concluded that the high resistance of the unventilated lung was a morphological consequence of the deflated state of the fetal lung (Reynolds, 1956). His theory was opposed by investigators whose infusions of acetylcholine and histamine caused vasodilation. It was believed by these latter researchers that high fetal pulmonary vascular resistance was due to tonic vasoconstriction (Dawes and Mott, 1962). It is now known that anesthesia, surgical trauma and exteriorization of the fetus each change the fetal condition enough to influence pulmonary vascular resistance (Heymann and Rudolph, 1967). Furthermore, it is now believed that fetal pulmonary vessels are neither actively constricted nor dilated in the normal resting fetus (Lewis et al., 1976; Fishman, 1985).

STUDIES ON ANESTHETIZED EXTERIORIZED FETUSES

The bulk of the information in the literature regarding fetal pulmonary vasculature has been gathered from experiments performed on anesthetized, exteriorized, open-chest fetal lambs which had just undergone rigorous surgical procedures. Data from these experiments probably do not reflect the normal physiological regulation of fetal

pulmonary vascular tone; nevertheless, much useful information has been gleaned from these experiments.

Fetal Lungs Without Ventilation

Effect of nerve and pharmacologic intervention on pulmonary vascular resistance. The effects of the autonomic nervous system on fetal ovine pulmonary vascular resistance have been studied in several ways. Stimulation of the parasympathetic system by electrical excitation of the vagus nerves caused large increases in pulmonary blood flow. Increased flow was accompanied by a decreased perfusion pressure (Dawes and Mott, 1962; Cassin et al., 1964a; Colebatch et al., 1965). The vasodilatory effect of stimulation of the parasympathetic vagus nerve was blocked by administration of the competitive muscarinic antagonist, atropine (Colebatch et al., 1965). In addition, bolus injection of the parasympathomimetic drug, acetylcholine, into the pulmonary arteries produced substantial inceases in fetal pulmonary blood flow (Dawes and Mott, 1962). It is now generally believed that parasympathetic action in the pulmonary vascular bed is vasodilatory.

Pulmonary vasoconstriction can be brought about by electrical stimulation of thoracic sympathetic nerves, by the administration of adrenaline and by noradrenaline (Colebatch et al., 1965). Adrenalectomy has been shown to decrease pulmonary vascular resistance in immature ovine fetuses, suggesting a tonic release of epinephrine from the adrenal glands in control animals (Cassin et al., 1964a). However, since bilateral thoracic sympathectomy had no effect on pulmonary vascular resistance, it appears that the pulmonary vascular resistance of these fetuses is not under the influence of chronic sympathetic tone (Colebatch et al., 1965). Interestingly, Dawes (1966)

later cites Colebatch's work (1965) as evidence that there is sympathetic tone in the fetal lung. Infusions of the β-agonists isoprenaline and isoproterenol have been shown to cause pulmonary vasodilation (Colebatch et al., 1965; Barrett et al., 1972), while infusion of methoxamine, an α-agonist, causes increased flow and increased arterial pressure (Barrett et al., 1972).

The eicosanoids, a family of naturally occurring vasoactive substances are derivatives of arachadonic acid. This family includes leukotrienes, thromboxanes and prostaglandins. The prostaglandins are very active in the fetal pulmonary vascular bed and act as either constrictive or dilatory agents, depending on chemical class. Specific actions have been described for each prostaglandin series. Prostaglandins of the D, E and I series are all pulmonary vasodilators (Cassin et al., 1981; Leffler and Hessler, 1979; Phillips and Lyrene, 1983; Cassin et al., 1979). Notably, PGI_2 release appears to be under the influence of distention forces in the lung (Leffler et al., 1984). Prostaglandins from the F series, however, appear to exert vasoconstrictive actions in fetal goats (Kadowitz et al., 1974). Thromboxane A_2 and Leukotriene D_4 both appear to act as pulmonary vasoconstrictors in fetal sheep (Solfer et al., 1984; Cassin, 1987). Investigation of these actions is currently a very active area of research.

Unfortunately, little is known of the roles of other autocoids in regulating fetal pulmonary vascular resistance, but two autocoids, histamine and bradykinin, cause pulmonary vasodilation in fetal lambs and goats (Campbell et al., 1968; Gilbert et al., 1973; Dawes and Mott, 1962).

Effects of oxygen on pulmonary vascular resistance. Studies on the effects of oxygen tension in the pulmonary vasculature have been primarily concerned with acute hypoxic episodes. In the first study of its kind, Dawes and Mott (1962) reported that the administration of 7 to 10% oxygen to the ewe induced fetal hypoxia and caused vasconstriction. Fetal asphyxiation by umbilical cord compression also induced an increased pulmonary vascular resistance (Dawes and Mott, 1962; Campbell et al., 1967).

Campbell and associates (1967) studied the effects of oxygen and sympathetic tone in regulating pulmonary vascular resistance in both mature and immature lambs. In a cross-circulation experiment, fetuses were made hypoxic by cord compression while the pulmonary blood vessels were perfused with normoxic blood from a donor fetus after a large increase in pulmonary vascular resistance (Campbell et al., 1965; Campbell et al., 1967b). When normoxic blood was infused into the pulmonary vessels of distinctly immature lambs, pulmonary vascular resistance returned to control levels. However, mature fetuses behaved differently. They still exhibited a slightly elevated pulmonary vascular resistance, even after infusion of normoxic blood, which could only be brought completely to normal by sympathectomy. Pulmonary vascular resistance of fetuses with normal peripheral arterial oxygen tensions increased when pulmonary vasculature was perfused with blood from an asphyxiated donor animal (Campbell et al., 1967a). Thus, from these acute experiments, one could conclude that decreased pulmonary arterial oxygen tension causes elevated pulmonary vascular resistance and that the sympathetic nervous system plays only a minor role in regulation of pulmonary vascular resistance.

Cassin et al. (1964a) quantified the effects of arterial oxygen tension in the unexpanded lung in fetal lambs. Using data collected during spontaneous changes in pulmonary vascular resistance, they found a significant negative correlation between pulmonary vascular resistance and arterial oxygen tension.

Assali and coworkers (1968) first studied the effect of oxygen on pulmonary vascular resistance in unanesthetized exteriorized fetal lambs using a hyperbaric chamber. At three atmospheres absolute pressure, the ewe was ventilated with 100% oxygen. Fetal pulmonary arterial PO_2 rose from 16 to 47 mm Hg, and pulmonary blood flow increased three-fold on average while mean pressure in the pulmonary artery dropped 5 mm Hg. Flow through the ductus arteriosus was also studied. In every animal, right-to-left flow decreased, and in one-half the fetal lambs the direction of ductal flow reversed forming a left-to-right shunt. In another study with hyperbaric oxygen, Heymann and colleagues (1969) demonstrated that the pulmonary vasodilator, bradykinin, was produced by the fetal lungs in response to increased oxygen tension.

Ventilated Fetal Lungs

Effects of nerve and pharmacologic intervention on pulmonary vascular resistance. Most of what is known about regulation of pulmonary vascular resistance by the autonomic system and by autocoids has been studied in the unventilated fetus. Only two autocoids, histamine and acetylcholine, have been studied in the ventilated exteriorized fetus. The effects of acetylcholine and histamine are not as great in the ventilated fetus as in the non-ventilated fetus (Dawes and Mott, 1962; Dawes, 1966). Even dosages 5-10 times the dosage

effective in the unexpanded fetal lung did not increase flow further in the ventilated pulmonary bed (Dawes and Mott, 1962).

Effects of mechanical ventilation on pulmonary vascular resistance. The effects of mechanical ventilation have been studied in exteriorized fetal sheep. In 1953, Dawes and his colleagues reported that during mechanical ventilation, pulmonary blood flow began to rise within 2-3 breaths, rapidly increased over the next minute and increased slowly for an additional 5-10 minutes until it reached a new steady level. This response was observed regardless of the composition of the inspiratory gas mixture, which ranged from 0 to 100% oxygen. In the same study these investigators reported that distension of the lungs with saline increased vascular resistance.

Since these early studies, several researchers have sought to critically evaluate the contribution of ventilation to the decline in pulmonary vascular resistance. Cook et al. (1963) and Enhorning et al. (1966) found that slow inflations of fetal lungs with nitrogen failed to increase pulmonary blood flow and that flow was greater during deflation than inflation. Thus, both groups concluded that simple distension of the lung was not the mechanism which causes decreased vascular resistance at birth.

Cook's group (1963) found that during ventilation with air, a decrease in either tidal volume or frequency caused reversible falls in pulmonary blood flow. Maximal tidal volume was 40-50 ml per 3-4 kg with 30 breaths per minute. In the same study, flow was measured during rhythmic ventilation with nitrogen and with room air while pulmonary arterial pressure was maintained at constant levels. During N_2 ventilation, maximal blood flow through the pulmonary vascular circuit varied between animals depending on fetal maturity. The

smallest fetuses (about 2.2 kg) had little or no response, while the largest (about 3.3 kg) had up to a seven-fold increase in flow. It was noted that addition of 10% CO_2 to the inspired gas mixture caused flow to drop to one-half its original value. Therefore, the authors concluded that the effect of initial ventilation with N_2 was due to a fall in PCO_2 and that the effect of mechanical ventilation alone was minimal because flow did not change during slow inflation with N_2. These issues still need to be investigated.

The importance of mechanical ventilation in the regulation of pulmonary vascular resistance was also examined by Cassin and his associates in 1964 (a). Single five-second expansions of the lungs of fetal lambs with 3% O_2 and 7% CO_2 in N_2 were reported to increase lung blood flow. Rhythmic rebreathing produced no more effect than the single brief inflation. These investigators also inflated the lungs with 16-20 ml/kg warm amniotic fluid or normal saline which had oxygen and carbon dioxide tensions similar to fetal arterial blood. These inflations produced small transient falls in pulmonary vascular resistance. These data contradicted previous reports that distension of the lungs with saline increased pulmonary vascular resistance (Dawes et al., 1953). This controversy has not been settled.

Several other studies (Cassin et al., 1964b; Colebatch et al., 1965) have been conducted on the effects of rhythmic lung expansion, some with a "fetal" gas mixture designed to leave fetal arterial PO_2 and PCO_2 unaltered (3% O_2, 7% CO_2, 90% N_2), and some with saline. Conductance (pulmonary blood flow/mm Hg driving pressure) was nearly doubled when the lungs were ventilated with a gas mixture that did not change systemic carbon dioxide tension or pH (with a slight but

significant decline in arterial oxygen tension). When Lauer and colleagues (1965) ventilated fetal lambs with 5.6% O_2 and 6.3% CO_2 in N_2, they observed no change in arterial oxygen tension or pH, but pulmonary vascular resistance fell to approximately one-fourth the value they recorded in unventilated fetuses.

In general, the data from these acute experiments suggest that mechanical ventilation alone contributes to the decline in pulmonary vascular resistance at birth. Interestingly, mechanical ventilation has been associated with release of the vasodilator PGI_2 by the lung (Leffler et al., 1980; 1981).

Mechanical ventilation may affect more than the resistance of the pulmonary vascular bed. Studies in acutely prepared fetal goats suggest that the pressure which surrounds the pulmonary blood vessels also falls in response to mechanical ventilation (Gilbert et al., 1972).

Effect of pH and PCO_2 on pulmonary vascular resistance. Few studies have addressed the importance of carbon dioxide tension and pH as mediators of the changes which occur during the onset of ventilation. The work of Cook et al. (1963) has already been mentioned. These authors found that the addition of carbon dioxide to the inspiratory air mixture resulted in vasoconstriction. In their studies, pulmonary blood flow fell to 50% of the value measured during ventilation with air. This level is comparable to that seen in the same study during 100% nitrogen ventilation. Cassin et al. (1964b) studied the role of carbon dioxide in regulating pulmonary vascular resistance in seven fetal lambs. During ventilation with 6.0-8.4% CO_2 in N_2 carbon dioxide tension was maintained at about 36 mm Hg and oxygen tension dropped slightly from 20 $\pm$ 1.4 (SEM) to 17.4 $\pm$ 1.0 mm

Hg; pulmonary vascular conductance rose from 1.0 ± 0.2 to 1.5 ± 0.2 ml/min· mm Hg. When the ventilatory gas was switched to nitrogen, carbon dioxide fell to 26.3 ± 1.4 mm Hg and pulmonary vascular conductance rose to 2.6 ± 0.3 ml/min·mm Hg. Ventilation with 6.7 to 7.6% CO_2 in air raised arterial oxygen tension to 33.4 ± 4.9 mm Hg and carbon dioxide tension to 42.0 ± 1.7 mm Hg; vascular conductance was not different from the conductance measured during nitrogen ventilation. With a drop in carbon dioxide tension to 24.8 ± 1.3 during ventilation with air, conductance rose to 3.6 ± 0.6 ml/min·mm Hg. Thus, PCO_2 appears to have an important effect on pulmonary vascular resistance, though the point has not yet been adequately addressed.

Effect of oxygen on pulmonary vascular resistance. The role of oxygen in the changes in pulmonary vascular resistance during the initiation of ventilation has also been examined. An early investigation by Dawes and co-workers (1953) found no difference in the effects of oxygen, air or nitrogen ventilation upon the pulmonary vascular resistance. Having observed that oxygen can change pulmonary vascular resistance in the unventilated fetus, Dawes and Mott (1962) re-evaluated the role of oxygen during fetal ventilation in mediating changes in pulmonary vascular resistance. Four of the six fetal lambs they studied responded with pulmonary vasodilation when the inspiratory gas was switched from nitrogen to air. Blood gas data were not made available. Two subsequent studies yielded similar results, still without blood gas data. In one study by Cook et al. (1963) pulmonary blood flow rose two- to four-fold and in a second study by Cassin et

al. (1964b) it rose by not quite two-fold when air was substituted for nitrogen as a ventilatory mixture.

Summary

Early studies in exteriorized anesthetized fetal lambs indicate that the tone of fetal pulmonary vasculature is sensitive to several chemical substances. Acetylcholine, bradykinin and histamine have all been shown to dilate the pulmonary vasculature in the acute fetal preparation. Noradrenaline and adrenaline appear to vasoconstrict the pulmonary vasculature in the acute fetal preparation. In the acutely prepared fetus, ventilation with 3% O_2, 7% CO_2 in N_2 drops pulmonary vascular resistance about two-fold. Removal of carbon dioxide from the ventilation gas mixture provides further vasodilation; the addition of oxygen to the mixture dilates the pulmonary vasculature even more.

Aside from the obvious difficulties which arose from studying acutely prepared animals, many of the fetuses in the earlier studies mentioned above were also of low weight, indicating immaturity. Recent evidence indicates that gestational age may be a critical factor in the preparation of the lungs for proper gas exchange (Jobe et al., 1983); all data on acutely prepared immature fetuses must be interpreted with caution. This and the effects of anesthesia and trauma may be important explanations for some of the inconsistencies between studies.

STUDIES ON UNANESTHETIZED FETUSES

Fetal Lungs Without Ventilation

In 1972, Rudolph and Heymann published in abstract form, the results of the first chronic study of pulmonary blood flow in fetal lambs. Three fetuses, ranging in gestational age from 115 to 135 days,

were studied 4-5 days after surgery. The wave form of postductal main pulmonary artery blood flow was first described as a flow pattern with a distinctive "prominent reversal of flow in early diastole" (Rudolph and Heymann, 1972).

Effects of neural and pharmalogic intervention on pulmonary vascular resistance. The direct effects of the autonomic nervous system on pulmonary vascular resistance have not been studied in the chronically instrumented fetus. However, Rudolph and Heymann (1972) reported that infusion of the parasympathomimetic agent, acetylcholine, decreased pulmonary vascular resistance and diminished the negative phase of the pulmonary artery flow wave form. It was later reported that the response to acetylcholine was age-dependent, with older fetuses (from 100-140 days) more sensitive to the vasodilatory effects of acetylcholine than immature fetuses (Lewis et al., 1976; Heymann et al., 1977).

Effects of oxygen on pulmonary vascular resistance. Several investigators have studied the effect of oxygen on pulmonary vascular resistance in the non-ventilated fetus. Investigators in Rudolph's laboratory reported that the fetus responds to hypoxic insult by increasing pulmonary vascular resistance but the response appears to be dependent upon gestational age and appears after gestational day 120 in the fetal sheep (Rudolph and Heymann, 1972; Lewis et al., 1976; Heymann et al., 1977). In a study by Lewis et al. (1976), α- and β-adrenergic blockade with phentolamine and propranalol did not alter the vasoconstrictive effects of hypoxia.

The temporal aspects of pulmonary vasoconstriction were studied by Abman and colleagues (1986). Pulmonary vascular resistance immediately returned to control value upon return to normoxia if the hypoxic

incident was 30 minutes or less; an hypoxic episode of 120 minutes caused pulmonary vascular resistance to remain elevated for at least one hour after oxygen tension and pH had returned to control levels (Abman et al., 1986a). The sustained pulmonary vasoconstriction was attenuated but not corrected by α-adrenergic blockade with phentolamine (Abman et al., 1986).

The effects of increased arterial oxygen tension on pulmonary vascular resistance were studied by Goetzman et al. (1984) by the microsphere method. One hundred per cent oxygen was administered to five ewes causing fetal lung blood flow to increase two-fold, while ascending aortic oxygen tension increased from about 20 to 26 mm Hg.

Accurso and associates (1986) recently studied the temporal response of pulmonary blood flow to increased pulmonary arterial oxygen tension. An electromagnetic flow sensor was placed about the left pulmonary artery of 13 fetal lambs (Accurso et al., 1986). After a four day recovery period, 100% oxygen was administered to the ewe; fetal pulmonary artery oxygen tension rose from 18.7 ± 0.7 to 23.8 ± 0.7 mm Hg. While pulmonary arterial and left atrial blood pressures remained constant, fetal left pulmonary artery flow blood rose to 2.7 times control value within one hour. Over the next hour of the study, flow declined to approximatley 1.1 times original baseline flow. These investigators concluded that the fetus has mechanisms which resist the vasodilatory stimulus of increased oxygen tension.

Ventilated Fetal Lungs

Effect of mechanical ventilation on pulmonary vascular resistance. Investigators in Rudolph's lab (1986) have published, in abstract form, the only study to date which claims to have addressed the effects of

mechanical ventilation on pulmonary vascular resistance in chronically instrumented fetuses. In this study, 8 fetal lambs of 132-135 days gestation were examined 2-3 days after surgery. Distribution of cardiac output was assessed using the microsphere method. Pulmonary vascular resistance was calculated from pulmonary blood flow and pulmonary artery pressure; resistance fell 30-fold during ventilation with 3% oxygen in nitrogen. The authors conclude from this work that rhythmic lung expansion is the dominant factor in the reduction of pulmonary vascular resistance at birth. However, as mentioned, the "mechanical effects of ventilation" were assessed using a gas mixture of 3% O_2 in N_2 to ventilate the fetuses. Other investigators have reported decreased arterial carbon dioxide tensions during ventilation of conscious fetuses with oxygen and nitrogen gas mixtures (Willis et al., 1986; Morton et al., 1983). Thus, it is likely that the "mechanical" effect reported by the authors was a combination of changes in arterial or alveolar carbon dioxide tension, pH, and mechanical ventilation (Rudolph, 1986).

Effect of oxygen on pulmonary vascular resistance. Rudolph et al. (1986) also conducted the only published study on the role of increased oxygen tension in affecting pulmonary vascular resistance in chronically catheterized ventilated fetal sheep. During ventilation with 100% oxygen, carotid arterial oxygen tension rose from 18 $\pm$ 2.9 to 205 $\pm$ 154.8 mm Hg. Corresponding to the rise in oxygen tension was a three-fold drop in pulmonary vascular resistance from the resistance values obtained during ventilation with 3% oxygen. This fall represented a 90-fold drop from the control, non-ventilated fetal

state. Pulmonary arterial oxygen tension was not reported, nor were carbon dioxide tensions.

Effect of pH and PCO_2 on pulmonary vascular resistance. The effects of PCO_2 and pH on pulmonary vascular resistance have not been studied in the chronically catheterized mature fetus.

Summary

Increased fetal oxygen tension causes pulmonary vascular resistance to fall in chronically instrumented fetal lambs. There is evidence which suggests that increased oxygen tension in the absence of ventilation is not an adequate stimulus for sustained decreases in pulmonary vascular resistance. One initial report suggests a potent effect of mechanical ventilation on pulmonary vascular resistance. Neither the effects of carbon dioxide tension and pH, nor the effect of mechanical ventilation alone have been studied in chronically prepared fetuses.

REGULATION OF NEONATAL PULMONARY VASCULAR RESISTANCE

The regulation of pulmonary vascular resistance in neonates is peripheral to the subject matter of this thesis and therefore will not be covered in detail. Instead, pertinent physiological differences between newborns and adults or newborns and fetuses will be highlighted with respect to the regulation of blood flow through the lung.

In adults, prostaglandins D_2 and E_2 are pulmonary vasoconstrictors (Kadowitz et al., 1980; Wendling et al., 1981). In contrast, anesthetized neonatal and fetal lambs react to infusions of both prostaglandin D_2 and E_2 with pulmonary vasodilation (Phillips et al.,

1983; Leffler and Hessler, 1979; Cassin et al., 1979). Infusion of prostaglandin D_2 also prevents the normal vasoconstrictive effects of a one-minute hypoxic incident (Phillips et al., 1983). The vasodilator effects of prostaglandin D_2 are dose-dependent (Cassin et al., 1981).

Contrary to its vasodilatory effects on the lungs of anesthetized fetal lambs (Dawes and Mott, 1962), histamine is a pulmonary vasoconstrictive agent in both normoxic and hypoxic neonates (Lock et al., 1980).

Custer and Hales (1985) studied the pulmonary vascular response to the fraction of inspired oxygen in both neonatal and adult sheep. They compared regional pulmonary vasoconstriction in newborn (3-21 days) and adult sheep at graded levels of alveolar oxygen tension to determine whether the pulmonary response to oxygen was age related. In adults and neonates, one lung of each animal was ventilated with nitrogen while the other was ventilated with oxygen. Pulmonary vascular resistance was assessed by the changes in blood flow to the test lung. The vasoconstrictor response to hypoxia was more powerful in the neonates than in adults. In the neonates, blood flow was reduced by alveolar oxygen tensions below 360 mm Hg. Thus, neonates exhibited some "hypoxic" pulmonary vasoconstriction even during ventilation with room air; the adult sheep exhibited no such response.

The effects of pH and carbon dioxide tension have been studied in the neonate but not in the fetus; data from neonatal studies may be useful. Rudolph and Yuan (1966) studied the effect of metabolic alkalosis on the pulmonary vascular resistance of anesthetized newborn calves. During normoxia, alkalosis decreased pulmonary vascular resistance and acidemia increased vascular resistance. Rudolph's study also demonstrated that pH greater than 7.3 opposed the vasoconstrictive

effects of hypoxia. Lyrene and associates (1984) investigated the roles of metabolic and respiratory alkalosis (hyperventilation) in newborn lambs and found that increased pH attenuated the response to hypoxia, regardless of the classification of alkalosis. Thus, it has been suggested by these investigators that increased hydrogen ion concentration is more important than decreased PCO_2 in regulating pulmonary vascular resistance in neonates (Lyrene et al., 1984; Lyrene et al., 1985; Schreiber et al., 1984).

PERSISTENT PULMONARY HYPERTENSION SYNDROME OF THE NEONATE

The failure of the pulmonary vessels to vasodilate normally at birth results in a syndrome termed persistent pulmonary hypertension syndrome of the neonate. Its incidence is thought to be about 1 in 1500 live births and associated mortality rates range from 20 to 50% (Brown, 1974; Heymann, 1985).

Drummond (1983) and Heymann (1985) have recently reviewed the characteristics of persistent pulmonary hypertension. The predominant feature of this devastating syndrome is the inappropriate maintenance of pulmonary vascular resistance near normal fetal levels. This syndrome is associated with low pulmonary blood flow, elevated pulmonary arterial pressure, hypoxemia, and continued right-to-left shunts through the foramen ovale and ductus arteriosus (Drummond et al., 1977). Unlike infant respiratory distress syndrome, this disease commonly occurs in term and post-term infants. The etiology of this syndrome is not understood, but chronic intrauterine stress has been cited as one possible cause (Heymann, 1985).

The usual therapeutic approach to treatment of this syndrome is merely supportive in nature (Levin et al., 1976) and is directed toward the alleviation of troublesome symptoms. Oxygen demands are minimized by careful maintenance of thermal-neutral ambient air temperatures and care is taken to stabilize systemic blood pressure (Heymann, 1985). The aim of the ventilatory regimen is to maintain systemic arterial PO_2 above 50-60 mm Hg and to prevent acidemia; even with the administration of 100% oxygen in the ventilator, this is often a formidable task (Heymann, 1985). One current therapeutic modality intentionally creates respiratory alkalosis by hyperventilation with 100% oxygen, using respiratory rates set in excess of 100 breaths per minute concurrent with controlled paralysis of the infant. The mechanism for the relative success of the treatment is unknown (Drummond, 1983). Vasodilator therapy has also been attempted clinically for many years, but thus far, no effective treatment program has been established (Drummond, 1981), since no specific pulmonary vasodilator has been identified.

In summary, persistent pulmonary hypertension syndrome of the neonate remains an unexplained, relatively untreatable affliction of a significant number of mature newborns. Little is known about the pathophysiology of this syndrome; indeed, little is known about the normal changes which occur in pulmonary vascular resistance at birth. Until the normal physiology is thoroughly understood in normal fetuses and newborns, it is unlikely that a successful approach to therapy for persistent pulmonary hypertension will develop.

INTRODUCTION TO THE PROBLEM

In almost every review written on the subject, oxygen has been

credited as the primary chemical stimulus which dilates the pulmonary vasculature at birth (Dawes, 1966; Heymann, 1985; Rudolph, 1974; Fishman, 1985). The effects of mechanical ventilation, PCO_2 and pH have not been investigated to any great extent. That oxygen is considered the predominant influence on pulmonary vascular resistance appears to be inconsistent with the fact that oxygen therapy is ineffective in the treatment of many infants with persistent pulmonary hypertension syndrome (Heymann, 1985), although one could argue that the response to oxygen is abnormal in these infants. Evidently, something other than oxygen must also be important in the regulation of pulmonary vascular resistance of these newborns.

These facts lead to the conclusion that the roles of oxygen, pH and mechanical ventilation in altering pulmonary vascular resistance at birth are not well understood and that these roles need to be carefully re-evaluated in healthy, conscious animals. The objective of this project was to study pulmonary vascular resistance as it is affected by mechanical ventilation, increased arterial oxygen tension pH and decreased carbon dioxide tension in the perinatal period.

METHODS AND MATERIALS

In utero ventilation was selected as the method of study because fetal gases can easily be manipulated by use of different inspiratory mixtures (Willis et al., 1986). The *in utero* ventilation preparation was developed in the laboratory of Dr. J. Faber (Faber et al., 1979) and was therefore a familiar technique at this institution. Fetal lambs were chosen as the experimental model because the intricacy of

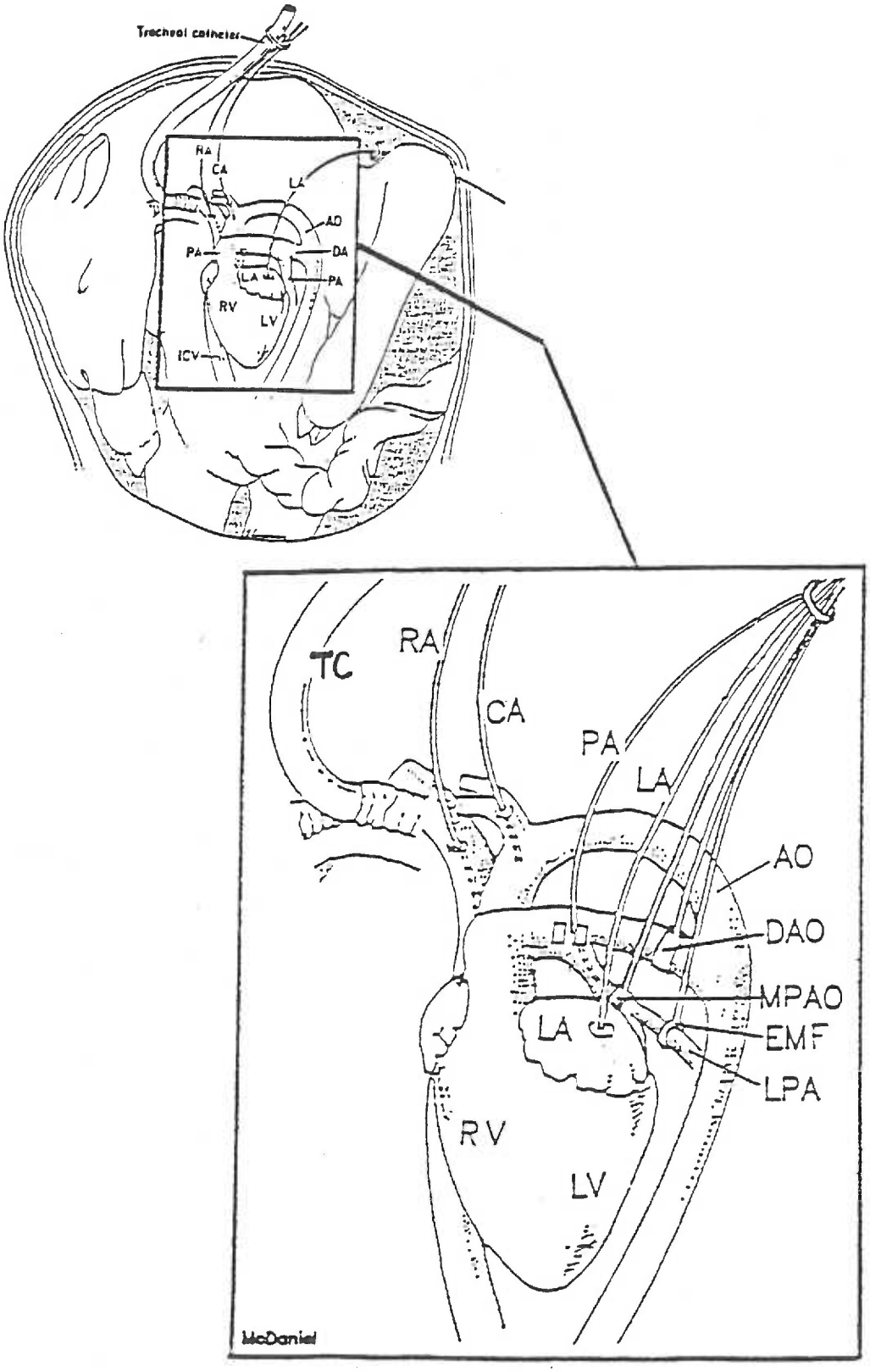
the surgical procedure required use of a large animal and because their relative lung maturation rate is similar to that of humans. In addition, most research conducted on animals during the perinatal period has been done on fetal and neonatal lambs. Positive pressure ventilation was used because ventilatory parameters, such as frequency, minute volume and airway pressures, could be controlled.

SURGICAL PROCEDURES

Fetuses from 22 domestic sheep of mixed western breeds of 130 ± 2 (SD) days gestation were surgically instrumented with catheters, a breathing tube, two inflatable balloon occluders and a flow sensor. The instrumented fetus is illustrated in Figure 4. Pregnant ewes were purchased from the Department of Animal Care at OHSU; breeding dates were provided for all animals and pregnancy was confirmed by X-ray examination. The ewes were brought to the laboratory animal quarters approximately one week before the scheduled surgery day. They were given food and water ad libidum and were allowed to adjust to their new surroundings. Food was withheld for 24 hours and water for two hours before surgery. On the scheduled surgery day, the ewe was brought to a surgical scrub room.

To induce anesthesia, an intravenous catheter was inserted into a maternal jugular vein through a small incision in the neck. The catheter was attached to an elevated one liter bag of sterile lactated Ringer's solution. After approximately 250 milliliters saline had been administered to the ewe, a solution of 10 mg/ml methohexitol sodium (Lilly) was administered to the ewe until she was unconscious. An endotracheal tube was inserted into the trachea, the endotracheal cuff inflated, and the animal was lifted to an adjustable surgical table and

Figure 4: Illustration of surgical preparation in fetal lamb. Enlarged portions show placement of the tracheal catheter (TC), right atrial (RA), carotid arterial (CA), pulmonary arterial (PA) and left atrial (LA) catheters. Inflatable balloon occluders are indicated on the ductus arteriosus (DAO) and main postductal pulmonary artery (MPAO). The electromagnetic flow sensor (EMF) is shown on the left pulmonary artery (LPA). Anatomic structures indicated are: aorta (AO), right ventricle (RV), left ventricle (LV) and the left atrium (LA).


Tracheal catheter
RA
CA
LA
AO
PA
DA
PA
LA
RV
LV
ICV
TC
RA
CA
PA
LA
AO
DAO
MPAO
LA
EMF
LPA
RV
LV
McDaniel

placed in the supine position. The endotracheal tube was then attached to a ventilator/anesthesia machine. Anesthesia was maintained with about one percent halothane in a 1:1 mixture of oxygen and nitrous oxide. Halothane was chosen as the anesthetic agent because it anesthetized both ewe and fetus. In preparation for sterile surgery, the maternal abdomen, thighs and flanks were shaved, cleansed and sterilized with an iodine solution. The animal was transported to a surgical suite and draped for surgery.

During surgery, the fetal upper body was delivered through a midline laparotomy extending from the umbilicus to the udder and a ten centimeter long uterine incision through a purse-string suture. The amnion and chorion were stitched to the uterus to keep them from slipping around the fetus. To prevent umbilical cord occlusion, the ventral and dorsal sides of the fetus were sutured to the uterus with a single stitch on each side.

After the fetus was satisfactorily positioned, a short midline incision was made in the fetal neck overlying the trachea, using an electrocautery (Valley Lab). The distal end of the right jugular vein was ligated with a 2-0 silk suture and a loose ligature was placed around the proximal end of the segment. The tip of a saline-filled 1.7 mm outside diameter (O.D.) polyvinyl (Bolab) catheter was introduced into the lumen of the vein and advanced to a position estimated to be just cephalad to the right atrium in the ascending vena cava. The procedure was repeated to cannulate the right carotid artery and the tip was advanced to the aortic arch. Both catheters were anchored to the subcutaneous tissue. Next, the trachea was isolated and ligatures of number one silk were passed around the ends of the exposed segment.

An incision was made in the trachea with a scalpel and the beveled end of a number 10 french dual lumen nasogastric feeding tube was inserted into the tracheal lumen and advanced to a position just short of the carina. The catheter was tightly anchored with the ligature. The opposite end of the tracheal catheter was connected to a number 18 french dual lumen nasogastric feeding tube. The small end of this catheter system was then attached to the fetal skin to provide continuous communication between tracheal and amniotic fluid. The skin incision was closed using a continuous stitch with 2-0 silk suture. Catheters were anchored to the skin outside the suture. Both catheters were previously attached to 12 ml syringes filled with sterile normal saline and the catheters were flushed to prevent clot formation in the catheter tip. An additional 40 ml sterile normal saline were administered to prevent blood volume depletion.

Next, the fetus was repositioned to permit access to the left thorax. A left thoracotomy was performed using the electrocautery. The initial incision overlying the fourth rib was made first through the skin, then through the underlying muscle layers. The pleura was incised along the periosteum of the rib and the opening was extended from the sternum to the left scapula. The ribs were separated by insertion of a rib spreader which was slowly opened and positioned for the most favorable exposure of the heart and great vessels. Small gauze sponges were inserted to keep lung lobes out of the surgical field. Epipericardial adipose tissue and thymus were carefully dissected from the pericardium. Perpendicular cuts were made in the pericardium. A 3 cm lateral incision followed the line of the thoracotomy. A second incision extended from the middle of the lateral incision to the lower margin of the left atrium. The edges of the

pericardial sac were sutured to the skin to cradle the heart above the thoracic cavity and improve surgical exposure.

With adequate exposure it was possible to see the left atrium, pulmonary artery, ductus arteriosus and left pulmonary artery branch. The left pulmonary artery, main postductal pulmonary artery and ductus arteriosus were carefully dissected from the surrounding connective tissue using low current electrocautery. A 12 cm length of umbilical tape was placed around each isolated portion to permit "atraumatic" handling of the vessels and to mark the areas where instruments would be placed later in surgery.

A polyvinyl (Bolab) catheter of 1.3 mm outside diameter with a one centimeter long, 1.7 mm outside diameter cuff on the tip was constructed prior to the surgery for placement in the left atrium. The left atrial appendage was gently held with two curved atraumatic forceps. A 16 gauge needle was used to make a hole in the atrium between the two forceps. The hole was dilated with a small pair of forceps and the catheter tip was carefully inserted into the left atrium. A ligature was tied around the catheter at the hole and the appendage was released from the forceps. The catheter was then pulled back until the cuff was against the ligated atrial wall. Blood was withdrawn into the catheter to confirm that the catheter tip was properly positioned. Fluid was then flushed into the catheter to prevent clot formation in the catheter during surgery. Through this catheter the fetus was given an additional 30 ml sterile normal saline to offset fluid loss during the surgery.

The next step was to equip the fetus with a catheter in the postductual main pulmonary artery. This procedure is illustrated in

Figure 5: Schematic diagram of pulmonary artery catheter placement. The catheter tip is shown in the postductal main pulmonary artery, beyond the inflatable balloon occluder. The electromagnetic flow sensor is on the left branch of the pulmonary artery; the artery serves the entire left lung.

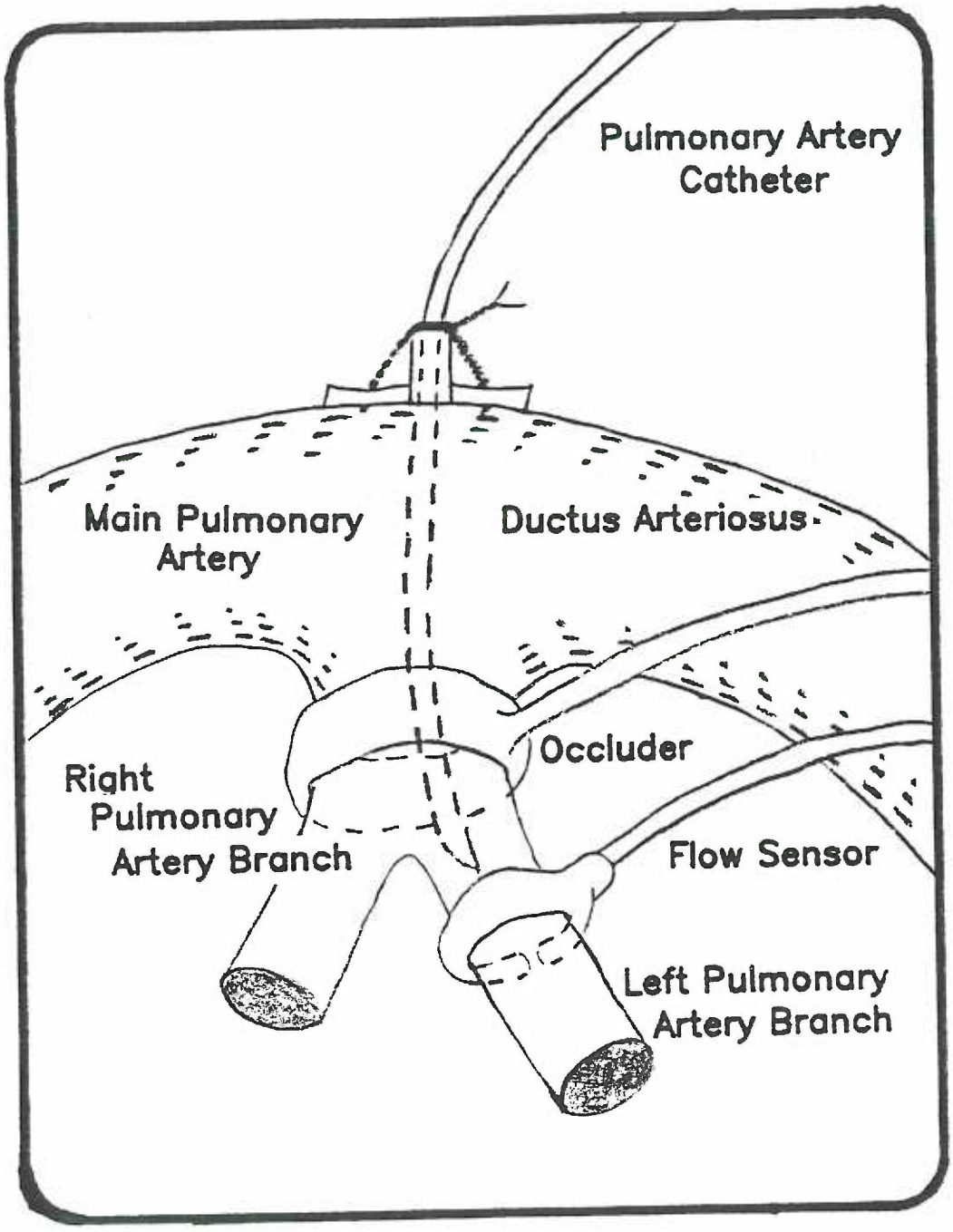

Pulmonary Artery Catheter
Main Pulmonary Artery
Ductus Arteriosus
Occluder
Right Pulmonary Artery Branch
Flow Sensor
Left Pulmonary Artery Branch

Figure 5. A 3 x 7 mm piece of teflon felt was threaded on a 5-0 prolene double-arm suture. Each arm was then used to make a 6 mm stitch through the adventitia and muscle layers of the pulmonary artery. The needles were pulled through their respective ends of a second 3 x 7 mm teflon felt piece. A specially constructed catheter was made to access the left pulmonary artery from the entry site. A 1 cm long, 1.7 mm outside diameter polyvinyl (Bolab) cuff was positioned 1.5 cm from the beveled end of a 1.3 mm outside diameter polyvinyl (Bolab) length of catheter tubing. A curved atraumatic forcep was placed deep around the area of the teflon pads. A 19 gauge needle was used to make a puncture wound between the teflon pads; the site was carefully cleared of blood and the tip of the cannula was inserted into the hole and directed into the left pulmonary artery branch, with the catheter cuff remaining on the outside of the vessel wall (Figure 5). The suture, still attached to the teflon pads, was then used to secure the catheter to the pads.

With the two cardiac catheters securely anchored, inflatable balloon occluders were placed around both the main postductal pulmonary artery and the ductus arteriosus and an electromagnetic flow probe was placed around the left branch of the pulmonary artery. The vessels, which had previously been dissected free, were measured to estimate the size needed for the two occluders and the flow sensor. The occluders were filled with saline and the contacts on the electromagnetic flow probe were polished. These items were placed in the following order: ductus arteriosus occluder, main postductal pulmonary artery occluder, and finally, the left pulmonary artery flow sensor.

A right angle hemostat was inserted under the ductus arteriosus while the vessel was lifted with the umbilical tape placed around it.

The upper lip of the occluder was carefully positioned in the space between the ductus arteriosus and the back wall of the fetal chest. The occluder was carefully pushed through the space. The two edges of inflatable balloon occluder were then tied together with a length of silk suture.

The second occluder was placed around the postductal main pulmonary artery in a manner similar to that used to implant the ductus arteriosus occluder. A right angle hemostat was inserted beneath the vessel while traction was gently applied using the umbilical tape previously placed around it. The lip of the occluder was centered in the opened space and pushed under the vessel. It was then tied and placed in the desired position.

The umbilical tape around the main pulmonary artery was left on to be used in conjunction with the umbilical tape around the left branch of the pulmonary artery to provide gentle traction during placement of the electromagnetic flow sensor. The opening in the flow sensor was positioned perpendicular to the longitudinal plane of the vessel, gently slipped onto the vessel and rotated. The cable of the flow sensor was anchored to the pericardium to prevent the head of the sensor from coming off the vessel.

Lastly, a 1.3 mm outside diameter polyvinyl catheter connected to a 3 cm long silastic tip with side holes was slipped into the pericardial space along the ventricular septal groove, pointing toward the apex of the heart. The catheter was then anchored to the pericardial sac. Normal saline was infused through this catheter to flush any remaining blood from the pericardial cavity and the fluid was removed by suction.

The pericardial sac was left open. The rib spreaders were removed from the chest cavity. Two 2-0 silk sutures were passed through the second and fourth intercostal spaces and the ribs were brought back to their normal positions as the sutures were tied. The muscles of the chest wall were anatomically repaired in two layers, allowing the catheters to exit the chest one at a time with at least one stitch between them. This improved the seal so that healing would occur more rapidly and no leaks would occur when the fetus was ventilated. Finally, the skin layer was closed with a continuous stitch of 2-0 silk suture material.

The catheters and probe cable were carefully looped under the foreleg and anchored to the skin. A 1.3 mm outside diameter catheter with side holes was tightly secured for measuring amniotic fluid pressure. The catheters were anchored in three places along the fetal chest and neck until the jugular venous, carotid arterial and tracheal catheters joined the thoracic catheters where one last anchoring stitch was made.

All the catheters were flushed with heparinized saline and were then tied off. The fetus was returned to the uterus. Amniotic fluid was replenished with sterile normal saline and the amniotic and allantoic membranes were released. The uterus was sewn together with close continuous stitches with a catheter exiting every second or third stitch. The uterus was then oversewn along the same plane. One million units of penicillin G were then flushed into the amniotic fluid sac to minimize risk of infection.

Next, holes on each side of the maternal abdominal incision were made by pushing a small hemostat through the tissue from the inside. A large Peon clamp was inserted through the hole from the outside and a

purse-string suture was sewn around the clamp on the outside. The tracheal catheter loop was pushed through the hole on the left side; the wound was sealed with the purse string. The flow sensor head, catheters and occluders were brought out on the right side in a similar manner. The peritoneum was then closed with number one silk using an interrupted stitch.

The flow probe head was anchored into the end of a 0.5 m long metal rod and the catheters were pulled through a loop attached to the rod. The catheters and probe cable were then exteriorized, using the metal rod, through a small opening in the right flank of the ewe. This same procedure was repeated to exteriorize the tracheal catheter loop to the left flank of the ewe. The subcutaneous layer of the abdominal incision was closed from the proximal and distal ends of the incision with vicryl suture. The skin was closed with wound clips and the external incision site was swabbed with iodine solution.

The catheter exit wounds were repaired and sealed and a cloth pouch was sewn on each flank. Catheters and cables were wrapped in gauze and placed in the pouches. The tracheal catheter was carefully placed to prevent kinking and fetal lung damage.

The sheep was finally transported to the recovery pen. Within 10-15 minutes, most sheep were able to stand up to drink water and eat.

The recovery of each sheep was carefully monitored and assessed daily for the following seven to ten days. The ewe was transported to the laboratory where the catheter tracts were cleansed and fresh Betadine-soaked bandages were wrapped around the catheters. Fetal heart rate was occasionally monitored to assess the condition of the fetus. These procedures also helped to familiarize the ewe with the

transport procedure and to acclimate her to the laboratory surroundings.

EXPERIMENTAL PROCEDURES

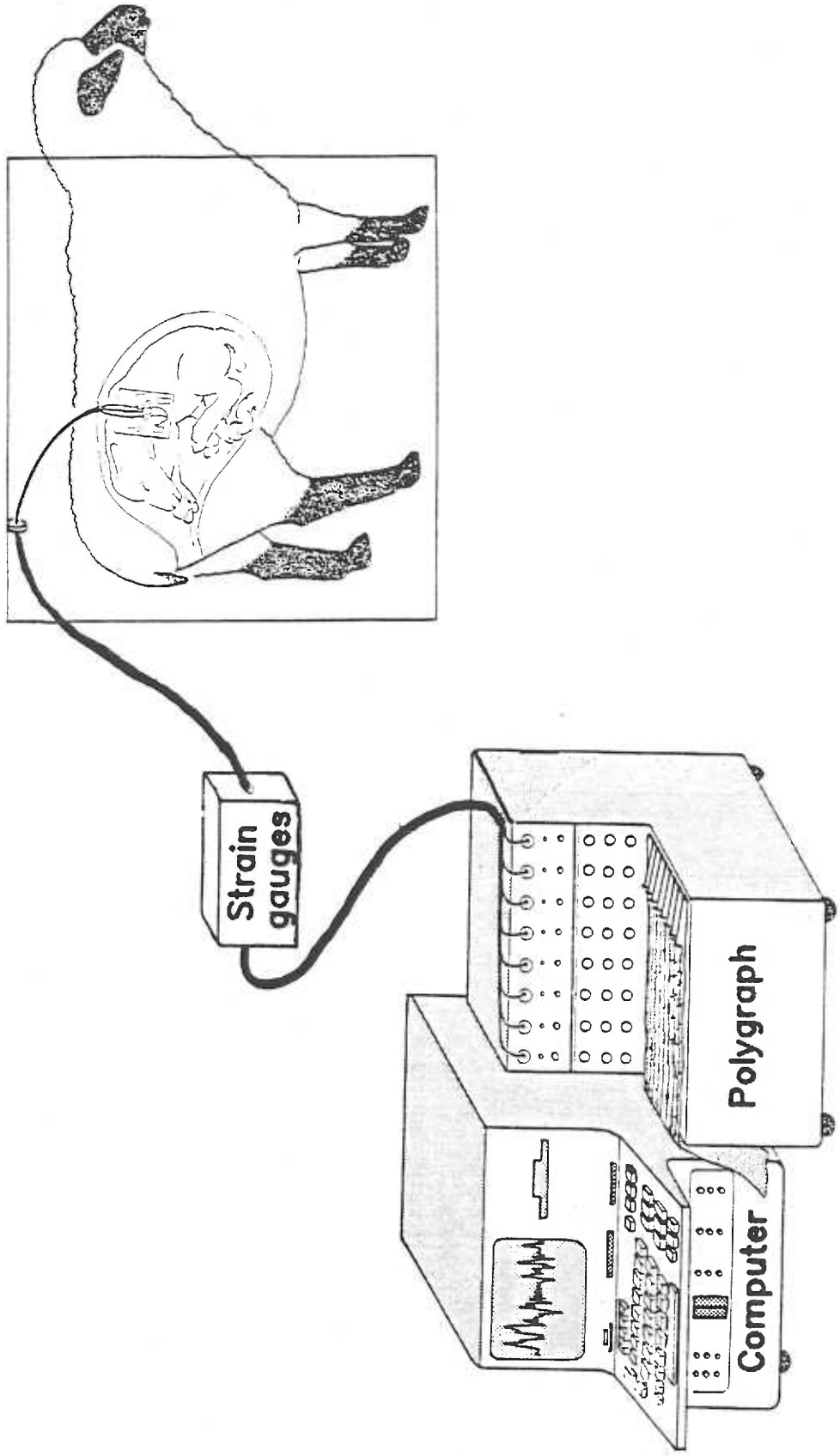
On the morning of the scheduled experiment, the sheep was transported to the laboratory in a mobile metal stanchion cart. Food and water were supplied and the ewe generally stood quietly and ate for the duration of the experiment. At times a ewe would not placidly accept the laboratory setting. When this dissatisfaction was vocally communicated to the investigators, a companion sheep was brought into the room. This invariably comforted both species.

Six channels of an 8-channel Gould 2800S recorder were carefully calibrated to Gould-Statham P23 ID strain gauges for full scale deflection at 50 or 100 mm Hg with a mercury manometer. The seventh channel of the polygraph was calibrated to record flow from a Gould-Statham SP2202 flowmeter for full scale deflection. The eighth channel housed a Gould cardiotachometer coupler which was calibrated for full scale deflection at 500 beats per minute.

A Hewlett-Packard 9826-S computer was used to acquire data directly from the polygraph. Analogue data were digitized and stored on floppy disk. Acquisition programs were written by Thomas J. Green.

Next, strain gauges were cleansed and sterilized with a 95% ethanol solution; gauge height was adjusted to the estimated mid-uterine level. The catheters were removed from the left flank pouch on the ewe, cleaned and opened; each was attached to a 35 ml saline-filled syringe through two stopcocks and a 19 gauge needle. The catheters were attached to the strain gauges which were filled with sterile

Figure 6: Experimental layout. The catheters were connected to strain gauges whose voltage output is proportional to hydrostatic pressure changes. The voltage outputs were recorded on the polygraph and were also digitized by the computer on-line and stored on floppy disk.


Strain gauges
Polygraph
Computer

saline. Special attention was paid to insure that no air was left in the catheters or the domes. All pressures measured by strain gauges were referred to pericardial pressure. A 3 ml blood sample was taken from the carotid artery catheter into a heparinized syringe. Blood samples were analyzed for PO_2, PCO_2 and pH by a Radiometer BMS3 Mk2 blood microsystem and for oxygen content with a Lex-O_2-Con-K (Cavitron) apparatus. Blood gas data were used to assess the initial condition of the fetus.

The flow signal was displayed on a Tektronix 2230 oscilloscope cathode ray tube. Zero flow was set equal to zero on the polygraph by two methods. First, the occluder on the postductal main pulmonary artery was inflated until no fluctuation was visible; the polygraph pen was adjusted to zero. Second, the electrical zero of the Gould flowmeter was recorded. These two values were usually identical. When they were not, the manual occlusion was assumed to be the more reliable zero.

The experimental setup is illustrated in Figure 6. Catheters, strain gauges, flowmeter and polygraph were connected to the polygraph:

Channel 1: Amniotic fluid or airway pressure

2: Pericardial fluid pressure

3: Right atrial pressure

4: Left atrial pressure

5: Carotid artery pressure

6: Left pulmonary artery pressure

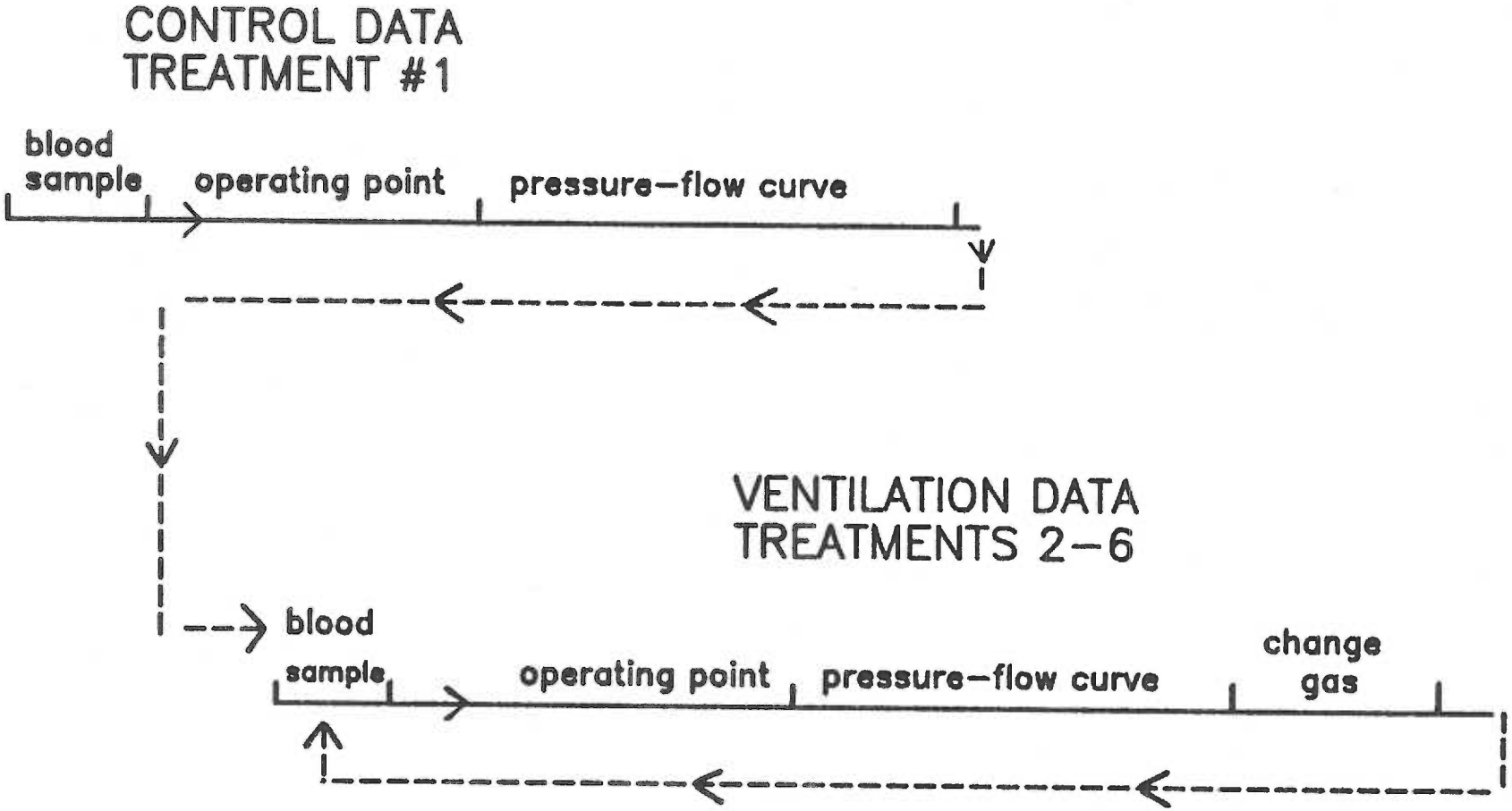
7: Left pulmonary artery flow

8: Heart rate

The experimental protocol is summarized in Figure 7. The experiment was divided into six treatment groups as follows.

Figure 7: Experimental protocol. Two data sections were recorded for each treatment. In the first section, pressure, flow and heart rate data were recorded to determine the fetal "operating point." In the second section, a pressure-flow curve was generated by inflating one of the balloon occluders. The process was repeated for each treatment. Treatments were: 1) control, 2) ventilation with 3% O_2-7% CO_2 in N_2, 3) ventilation with 3% O_2 in N_2, 4) ventilation with room air, 5) ventilation with 95% O_2-5% CO_2, and 6) ventilation with 100% O_2.

EXPERIMENTAL PROTOCOL



Treatment 1: Control. Measurements were taken in the non-ventilated fetus to determine normal resting pressures, flows, heart rate and the pressure-flow relationship.

Treatment 2: Mechanical ventilation with 3% O_2 and 7% CO_2 in N_2. This inspiratory gas mixture has been shown to produce no change in fetal arterial oxygen and carbon dioxide tensions and pH in other laboratories (Cassin et al., 1964a). This gas mixture was used to examine the effects of mechanical ventilation on fetal resting hemodynamic parameters and on pulmonary vascular resistance.

Treatment 3: Mechanical ventilation with 3% O_2. Ventilation with 3% O_2 was expected to maintain arterial PO_2 at normal fetal levels while decreasing arterial PCO_2 and increasing pH. This protocol was designed to determine the role of changes in arterial PCO_2 or pH, at normal fetal arterial PO_2 levels, in affecting the pressure-flow relationships of the pulmonary vascular bed.

Treatment 4: Mechanical ventilation with air. Fetuses were ventilated with air to test the effect of "neonatal level" arterial oxygen tensions on pulmonary vascular resistance.

Treatment 5: Mechanical ventilation with 95% O_2 and 5% CO_2. This gas mixture was used to increase arterial PO_2 as much as possible while maintaining normal fetal arterial PCO_2 and pH. The protocol was included to examine the effect of increased oxygen tension on pulmonary vascular resistance at normal fetal arterial PCO_2 levels.

Treatment 6: Mechanical ventilation with 100% O_2. Ventilation with this gas mixture was expected to maximally increase oxygen tension and pH while decreasing PCO_2 levels. Based on acute studies in fetal

lambs, this mixture was expected to bring about a maximal decrease in pulmonary vascular resistance (Cassin et al., 1964a; Dawes, 1966).

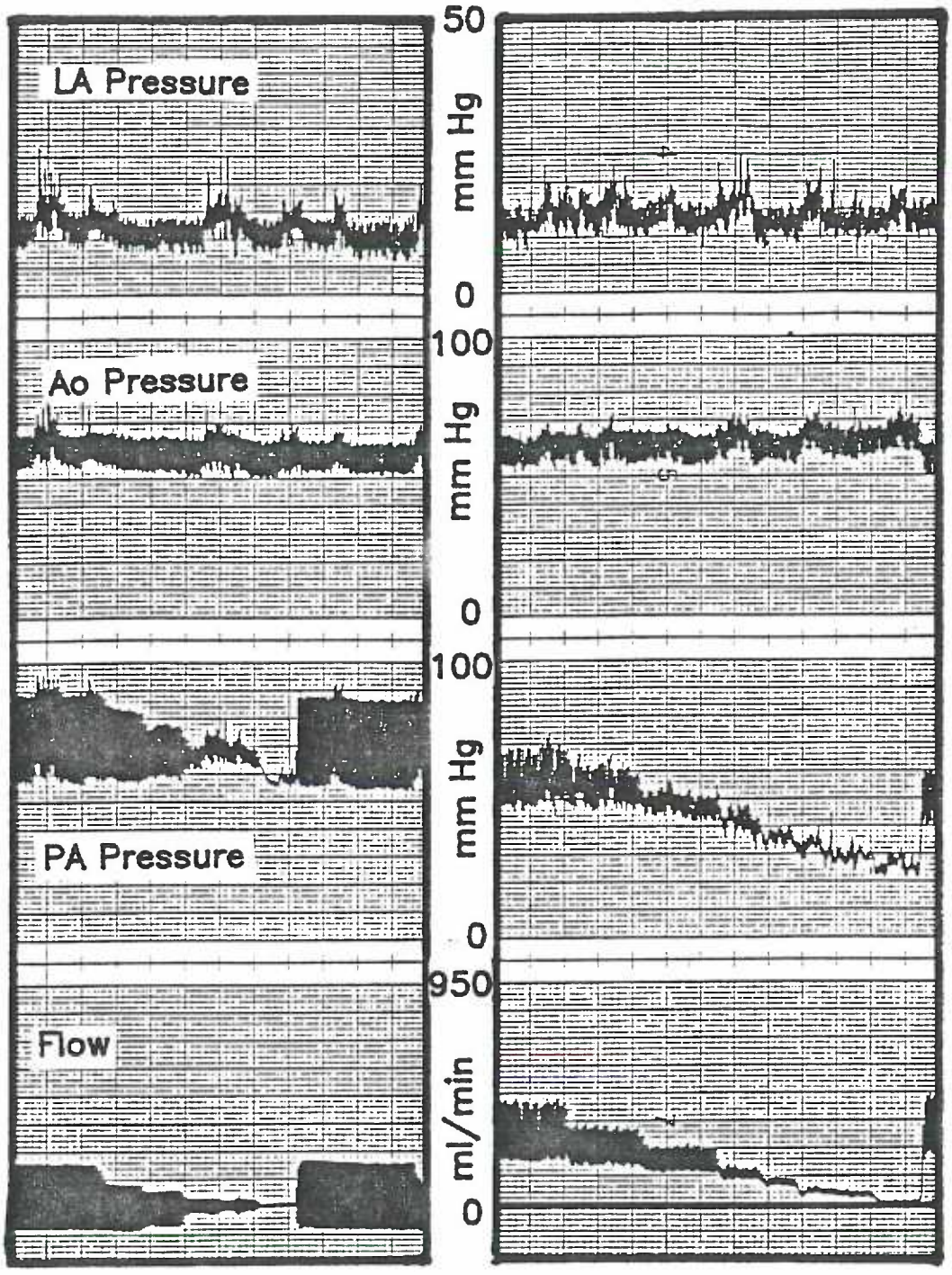
Before data were recorded for each protocol, pulmonary and carotid arterial blood samples were taken. Both were analyzed for oxygen and carbon dioxide tensions, pH and hematocrit. In addition, the pulmonary arterial blood was analyzed for oxygen content when possible. Three ml and 1 ml samples of blood were removed from the pulmonary and carotid arterial catheters, respectively. An equal or greater volume of lactated Ringer's solution was reinfused into the fetus after the blood sample was removed. Samples were capped and placed in an ice bath for later analysis. Samples were usually analyzed within 30 min.

One of three drug regimens was administered to fetuses before control data were taken. Initial experimental studies were conducted with no drug therapy. However, after several fetuses died suddenly during the experiment from apparent bradycardiac episodes, cardiac blockade therapy was initiated. The parasympathetic antagonist, atropine (1.5 mg), was selected to prevent vagally-induced episodes of bradycardia. The β-adrenergic antagonist, propranolol (3.0 mg), was also administered to prevent the remaining tachycardia. To determine the role of propranolol treatment on pulmonary vascular resistance, a third drug regimen which used atropine only was included.

For each treatment, two types of data were recorded. The first section (Section A) consisted of recording normal pressures and flows. Data was collected for a one-minute sampling period; each channel was sampled at 100 times/sec and averaged over a five-second interval. A similar section was recorded before any autonomic antagonists were administered at the beginning of the experiment, and again after autonomic blockage was administered.

Figure 8: Polygraph record during inflation of balloon occluder around the main post-ductal pulmonary artery. Left atrial (LA), carotid artery (AO), pulmonary artery (PA) and left pulmonary artery flow are shown during control and during ventilation with room air (Treatment 4) in one animal. Note that pulmonary artery pressure during zero flow exceeds left atrial pressure during both treatments. Note that pulmonary artery pressure is quite high when flow is zero. This figure is not representative in this regard but is included to emphasize the fact that P^{O} often changed with treatment groups (see Table 7). Pericardial pressure was 9.2 mm Hg throughout.

PRESSURE–FLOW CURVE GENERATION



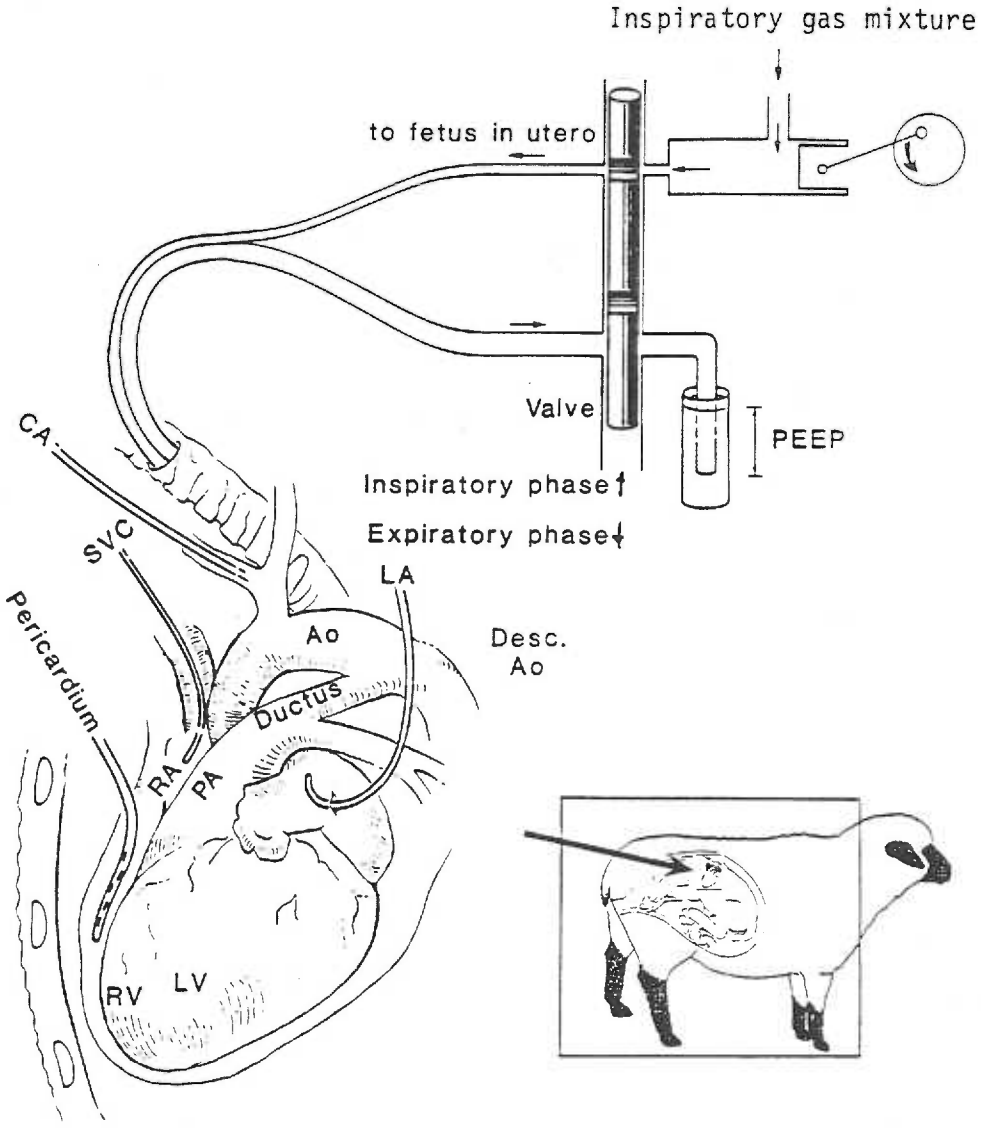
In the second section (Section B), data were collected while one (or both) of the occluders was inflated in stepwise manner to generate a pressure-flow curve (illustrated in Figure 8). The computer cathode ray tube displayed points as they were collected. During most experiments, the main pulmonary occluder was fully inflated for a five-second period to record the pressures at which blood flow through the left pulmonary artery ceased. In addition to data stored on disk, inspiratory gas mixtures, pulmonary arterial blood gases, ventilation frequency and inspiratory stroke volume (tidal volume) were all manually recorded for every section.

After control data were recorded, the fetus was ventilated. The *in utero* ventilation setup is illustrated in Figure 9. The tracheal catheter was removed from the left flank pouch of the ewe, cleaned and drained. The expiratory tubing was connected to a mechanical ventilator valve (Harvard) which was open only during expiration. The end of the expiratory tube was immersed in water to adjust fetal positive end expiratory pressure (PEEP) during ventilation.

The inspiratory tube was attached to the ventilator. A pressure line was attached between the inspiratory tube and strain gauge number one. This allowed a continuous record of airway pressure during ventilation. The desired premixed inspiratory gas was slowly released from a high pressure gas tank (Airco) into a low pressure gas reservoir bag attached to the respirator intake valve.

The Harvard volume respirator can be adjusted to deliver a desired volume, up to 125 ml at a pre-set frequency up to 50/min. Once the tubing was connected and the reservoir was filled with a desired gas mixture, the pump was switched on. A single stroke of 25 ml was given

Figure 9: *In utero* ventilation preparation. Gas mixtures are supplied to the piston of the respirator. The gas is pumped into the fetus through the inspiratory tubing. Stroke volume and frequency are adjustable. Expired gas travels through the tubing to a water bottle which maintains a positive end expiratory pressure. (Adapted from Morton et al., 1986).


Inspiratory gas mixture
to fetus in utero
Valve
PEEP
Inspiratory phase
Expiratory phase
CA
SVC
Pericardium
LA
Ao
Desc.
Ao
Ductus
RA
PA
RV
LV

and the tracheal pressure was examined to monitor the resultant tracheal pressure. A peak end expiratory pressure of 1-2 mm Hg above pericardial pressure and a peak inspiratory pressure of approximately 30 mm Hg above the pericardial pressure were targeted. Frequency was gradually increased up to 40 min^{-1} at each volume setting. As tracheal fluid was washed out of the lungs and they gradually became more compliant, larger tidal volumes were administered. This process continued until the fetal airways stabilized at the targeted peak pressures. Stable state was estimated to be a time period during which a constant tidal volume and frequency produced consistent tracheal pressure fluctuations.

At least ten minutes were allowed between ventilation treatments for blood flow and pressure to stabilize at a new level. Atropine was readministered before data were recorded for each ventilation treatment and blocking doses of propranolol were readministered every hour. Data were then taken for Sections A and B. This process was repeated for each individual inspiratory gas mixture administered on a given day.

In the first several initial experiments, data were collected using a predetermined sequence of inspiratory gas mixtures. The order was as follows: 1) control, 2) 3% oxygen and 7% carbon dioxide in nitrogen, 3) 3% oxygen in nitrogen, 4) air, 5) 95% oxygen and 5% carbon dioxide in nitrogen, and 6) 100% oxygen. As the project progressed, possible deterioration of the experimental preparation over time became a concern when in some animals ventilation seemed to be less efficacious with time. In order to avoid biasing the data in these events, two changes were made. 1) The order in which inspiratory mixtures were given was varied. It was reasoned that even without a true "random" design the data would at least be <u>evenly</u> biased by changing the order

of treatments if time were really a factor. 2) After data had been taken for all 5 protocols, the protocol was repeated with the 100% oxygen inspiratory gas. In most cases, the results were reassuring and similar.

At the completion of the experiment, the ewe and fetus were killed with an overdose of barbiturate (Euthanol) administered through the maternal jugular vein. Necropsy was then performed to retrieve the occluders and flow probe, confirm the positions of the catheter tips, assess the gross appearance of the fetal lungs, and to measure fetal weight.

Blood gas values, notable events during the experiment and the necropsy report were all recorded in a laboratory notebook.

DATA ANALYSIS

Mean pressures, flow and heart rate were determined for the normal fetal operating point from the first data section recorded during each treatment protocol. Peak inspiratory pressure, positive end expiratory pressure, minute volume (V_I) and pulmonary pressure at zero flow were all read from the polygraph charts and recorded.

The flow-pressure relationship was assessed in two ways. The "intercept method" used the difference between the mean pulmonary arterial pressure at the fetal operating point obtained from Section A and the pulmonary artery pressure at zero flow to determine driving pressure. The slope of the flow-pressure relationship was calculated by dividing flow by the calculated driving pressure. This slope is an estimate of pulmonary vascular resistance.

The second technique used the sum of the least squares regression

method which minimizes the error in flow at a given pulmonary artery pressure to estimte the slope of the flow-pressure relationship from individual points collected as pressure was reduced during pulmonary artery occlusion. Slopes and abscissa intercepts were calculated by this "regression method."

To determine the effect of drug treatment on the pressure-flow relationship, two-way analysis of variance was performed, testing the pressure-flow relationship between drug regimens and treatment groups. Paired t-test was performed to determine whether the two techniques for assessing pulmonary vascular resistance and conductance differed. The Bonferroni correction (Wallenstein, 1980) was applied to account for multiple testing. Student's t-test was performed on pre- and post-blockade hemodynamic data to determine the effect of the administered cholinergic and β-adrenergic blocking drugs on fetal hemodynamics. One-way analysis of variance was performed on all hemodynamic, blood and ventilatory parameters. When differences across treatment groups were found by analysis of variance, Duncan's multiple comparisons test was used to determine separation of groups at the 0.05 level. Multiple linear regression was performed to determine whether pulmonary vascular resistance was correlated with carotid arterial PO_2 and PCO_2 or PO_2 and pH, pulmonary arterial PO_2 and PCO_2 and pH, as well as with fractional inspired O_2 and CO_2. It was assumed that PO_2 and PCO_2 were independent variables.

RESULTS

Of 22 fetuses surgically prepared for experimentation, 15 survived the recovery period. Three of the 15 fetuses could not be ventilated and were therefore not included in the study. A fourth fetus died during the experiment before ventilation data could be recorded. Table

TABLE 1. INITIAL FETAL VALUES

Animal Number	Days Gestation	Fetal Weight (kg)	Recovery Period (days)	Carotid Artery				
				PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	Hct (%)	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})
86-108	136	6.8	6	14.4	50.6	7.34	42	4.4
86-109	137	3.4	5	17.6	45.3	7.39	42	---
87-6	136	5.0	9	20.1	48.1	7.32	42	---
97-19-2	141	5.8	8	21.2	50.5	7.37	38	7.2
87-27-2	139	5.0	9	19.5	44.7	7.37	--	---
87-33	140	4.2	12	19.0	51.5	7.32	37	6.2
87-45-2	141	4.6	10	21.3	45.6	7.40	35	---
87-47-1	143	3.8	13	15.4	46.7	7.37	47	---
87-54	139	5.1	7	17.4	53.1	7.35	35	7.3
87-58-2	139	5.5	8	19.6	44.4	7.36	34	---
87-63-2	140	5.4	7	21.7	47.7	7.31	38	8.3
$\bar{X}$	139	5.0	8	18.8	48.0	7.35	39	6.7
S.D.	2	0.9	2	2.3	2.9	0.03	4	1.3
n	11	11	11	11	11	11	10	5

TABLE 2. NUMBERS OF ANIMALS IN TREATMENT GROUPS

Drug Regimen	Treatment Groups					
	Control	3% O_2-7% CO_2 in N_2	3% O_2 in N_2	Room air	95% O_2-5% CO_2	100% O_2
No drugs	4	1	3	2	1	3
Propranolol & atropine	8	6	7	3	4	8
Atropine	4	4	4	1	3	4
Total*	11	6	10	5	4	10

*After the elimination of duplicate animals

1 shows that the 11 fetuses included in the study were allowed 8 ± 2 (SD) days to recover from surgery before experimentation, were 139 ± 2 (SD) days gestational age at experiment, and weighed 5.0 ± 0.9 (SD) kg at autopsy. Initial carotid arterial PO_2 was 18.8 ± 2.3 (SD) mm Hg, PCO_2 was 48.0 ± 2.9 (SD) mm Hg and pH was 7.35 ± 0.03 (SD); oxygen content was 6.7 ± 1.3 (SD) ml O_2/100 ml blood and hematocrit was 39 ± 4 (SD)%.

For reasons mentioned in the Methods section, some animals were given no drugs, some were treated with the muscarinic cholinergic antagonist, atropine, and some with both atropine and the β-adrenergic antagonist, propranolol. The number of animals studied for each of three drug regimens for each treatment group is reported in Table 2. Note that all six treatments were not necessarily administered to each fetus. It was important to know whether these animals represented three different populations or whether they could be treated as one group. Two-way analysis of variance on the slope of the pressure-flow relationship was used to test for differences between the drug groups. Drug regimen could not be shown to affect the slope of the pressure-flow relationship or the pulmonary artery pressure at the zero flow intercept ($p < 0.8$). Therefore, all drug regimens in a single treatment group were combined. If an animal was used in more than one drug regimen, duplicate experiments were eliminated from the study. For these experiments, the experimental day chosen for inclusion in the study was based on the number of treatment groups completed on the day in question, without regard to outcome.

Table 3 shows hemodynamic data before and after blocking doses of atropine (1.5 mg) alone or atropine and propranolol (3.0 mg) were

TABLE 3. EFFECT OF CHOLINERGIC AND β-ADRENERGIC BLOCKADE ON HEMODYNAMIC VALUES ($\bar{X}$ ± SD)

	Pre-blockade	Cholinergic blockade
Pulmonary artery pressure (mm Hg)	52.5 ± 4.3	53.5 ± 12.5
Carotid artery pressure (mm Hg)	46.9 ± 2.8	49.0 ± 8.6
Left atrial pressure (mm Hg)	3.8 ± 1.2	3.6 ± 2.1
Right atrial pressure (mm Hg)	4.1 ± 1.2	4.6 ± 3.0
Left pulmonary artery flow (ml/min)	41.8 ± 24.4	40.8 ± 19.0
Heart rate (beats·min^{-1})	130 ± 14	195 ± 23**
n	4	4

	Pre-blockade	β-adrenergic and cholinergic blockade
Pulmonary artery pressure (mm Hg)	55.8 ± 9.1	61.4 ± 9.8
Carotid artery pressure (mm Hg)	46.5 ± 5.5	52.4 ± 7.5
Left atrial pressure (mm Hg)	2.5 ± 1.5*	2.7 ± 1.4*
Right atrial pressure (mm Hg)	3.2 ± 1.8	3.9 ± 1.9
Left pulmonary artery flow (ml/min)	30.5 ± 19.7	39.9 ± 28.4
Heart rate (beats.min^{-1})	159 ± 15	187 ± 18**
n	8	8

*n=7

**different from pre-blockade ($p < 0.05$), Student's t-test

$\bar{X}$ = mean

administered to the fetus. These doses have been shown in previous studies to give adequate blockade (Thornburg and Morton, 1983). Heart rate rose from 130 ± 14 (SD) to 195 ± 23 (SD) ($p<0.05$) beats per minute after blockade with atropine; combined administration of atropine and propranolol resulted in increased heart rate from 159 ± 15 (SD) to 187 ± 18 (SD) beats per minute ($p<0.05$), an unexpected result. The reader is directed to Appendix 1 where hemodynamic data from individual animals are tabulated by treatment group.

Blood gas data for each experimental treatment are listed in Table 4. During ventilation with 3% oxygen-7% carbon dioxide in nitrogen, fetal arterial blood gas tensions did not change. During ventilation with 3% O_2 in N_2, carbon dioxide tensions fell from control 51.8 ± 2.6 (SD) to 41.4 ± 5.8 (SD) mm Hg in the pulmonary artery and from 48.1 ± 2.5 (SD) to 35.5 ± 7.2 (SD) mm Hg in the carotid artery. pH in the pulmonary artery did not show a statistical change from control when ventilated either with 3% O_2-7% CO_2 in N_2 or with 3% O_2 in N_2. However, arterial pH increased significantly from 7.31 ± 0.03 (SD) during ventilation with 3% O_2-7% CO_2 in N_2 to 7.40 ± 0.07 with 3% O_2 in N_2.

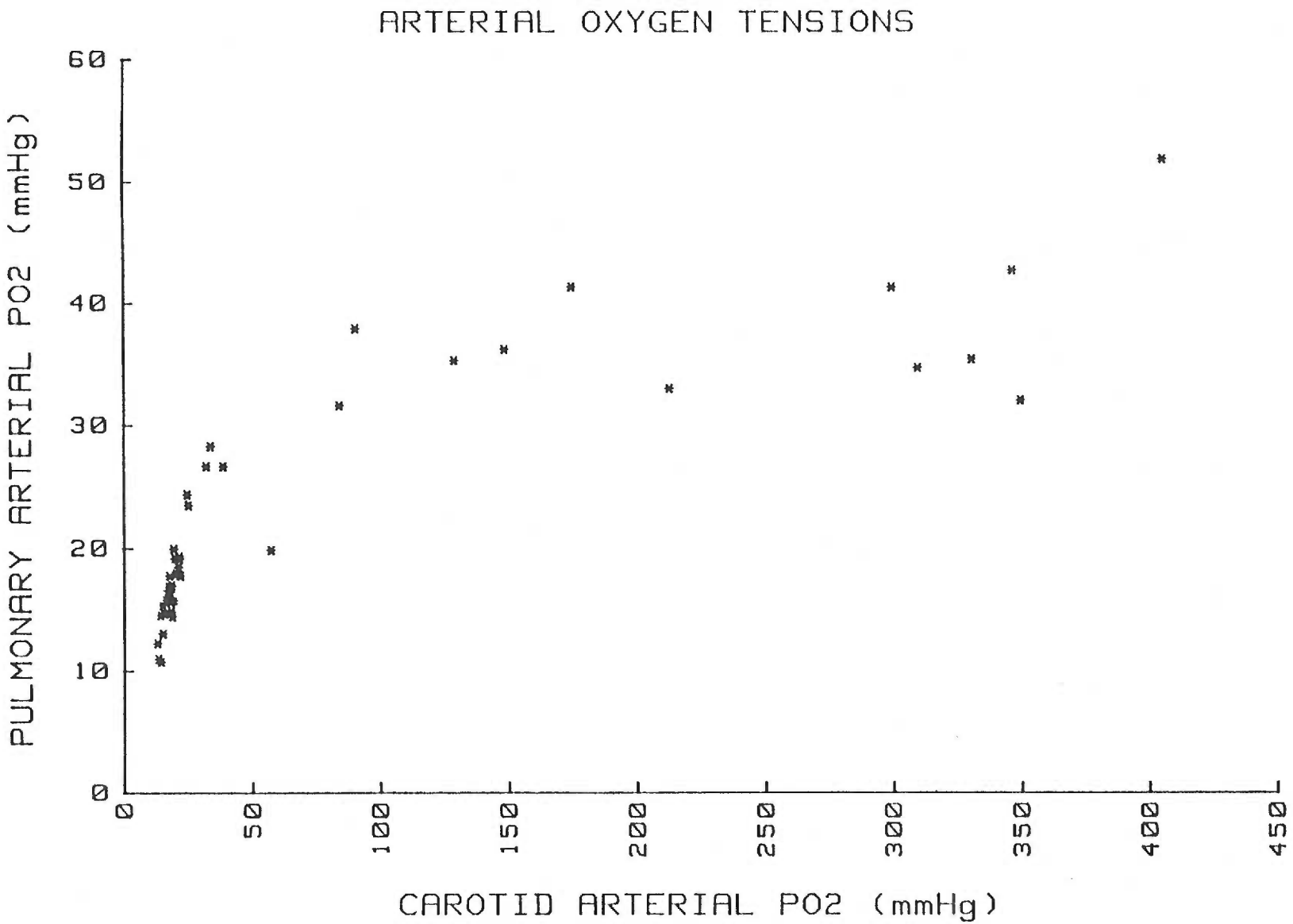
As expected, oxygen tensions rose when fetuses were ventilated with O_2 rich gas mixtures; PO_2 in the pulmonary artery increased from 15.8 ± 1.9 (SD) mm Hg during control to 24.3 ± 5.1 (SD) mm Hg during ventilation with room air; oxygen content also increased significantly. Carotid arterial PO_2 increased from a control value of 18.4 ± 2.4 (SD) to 45.4 ± 26.0 (SD) mm Hg with air ventilation. During ventilation with 95% oxygen-5% carbon dioxide, pH and carbon dioxide tensions differed neither from control nor from treatment with 3% O_2-7% CO_2 in N_2. Oxygen tensions in the pulmonary artery rose from 15.8 ± 1.9 (SD)

TABLE 4. PULMONARY AND CAROTID ARTERIAL BLOOD VALUES IN DIFFERENT TREATMENT GROUPS ($\bar{X}$ ± SD)

	Pulmonary artery					Carotid artery				
	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	Hct (%)	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	Hct (%)	n
1. Control	15.8±1.9	51.8±2.6	7.33±0.03	40±5 (n=10)	5.6±1.5 (n=7)	18.4±2.4	48.1±2.5	7.35±0.03	39±4	11
2. 3% O_2-7% CO_2 in N_2	16.7±2.9	49.4±4.8	7.31±0.06	39±4	6.7±2.6 (n=5)	19.1±3.1	46.8±3.9	7.32±0.06	38±4	6
3. 3% O_2 in N_2	16.0±2.8	41.4±5.8	7.40±0.07	40±4 (n=9)	6.5±2.1 (n=8)	16.9±2.2 (n=9)	35.5±7.2	7.45±0.10	39±4 (n=8)	10
4. Room air	24.3±5.1	39.2±6.1	7.42±0.07	40±4	10.9±1.3 (n=4)	45.4±26.0	33.7±8.5	7.46±0.10	37±1 (n=4)	5
5. 95% O_2-5% CO_2	34.5±5.4	49.2±3.3	7.31±0.05	38±4	12.6±1.3	188.3±122.4	45.0±2.2	7.32±0.05	38±2	4
6. 100% O_2	36.2±8.1	38.9±4.6	7.42±0.06	38±3 (n=8)	12.4±0.9 (n=7)	213.9±143.4	34.9±6.4	7.45±0.08	39±4	10
Group Differences ($p<0.05$)	4>1,2,3 5,6>1,2,3,4	1,2,5> 3,4,6	4,6>2,5 3>2,5	NSD*	4,5,6> 1,2,3	5,6>1,2,3,4	1,2> 3,4,5,6	3,4,6> 1,2,5	NSD	

*NSD = no significant differences found between treatment groups; $\bar{X}$ = mean
Analysis of variance, Duncan's multiple comparisons test

Figure 10: Relationships between carotid and pulmonary arterial PO_2 for all treatment groups. In general, pulmonary artery PO_2 increased linearly with increases in carotid arterial PO_2 up to about 30 mm Hg. Above 30 mm Hg, pulmonary arterial PO_2 changes very little with increases in carotid arterial PO_2.


ARTERIAL OXYGEN TENSIONS
PULMONARY ARTERIAL PO2 (mmHg)
0
10
20
30
40
50
60
0
50
100
150
200
250
300
350
400
450
CAROTID ARTERIAL PO2 (mmHg)

to 34.5 ± 5.4 (SD) mm Hg and in the carotid artery from 18.4 ± 2.4 (SD) to 188.3 ± 122.4 (SD) mm Hg during ventilation with 95% O_2-5% CO_2. During ventilation with 100% oxygen, all blood gas values were different from both control and ventilation with 3% O_2-7% CO_2 in N_2. Oxygen tension rose in the pulmonary artery from 15.8 ± 1.9 (SD) to 36.2 ± 8.1 (SD) mm Hg, and in the carotid artery from 18.4 ± 2.4 (SD) to 213.9 ± 143.4 (SD) mm Hg. Carbon dioxide tensions decreased in both arteries and pH rose significantly to 7.42 ± 0.06 (SD) mm Hg in the pulmonary artery and 7.45 ± 0.08 (SD) mm Hg in the carotid artery. The relationship between PO_2 in the pulmonary artery and the carotid artery was not linear and is shown in Figure 10. Interestingly, the relationship is apparently linear up to about 30 mm Hg; at carotid artery PO_2 values above 30 mm Hg, pulmonary artery PO_2 changed very little.

Hemodynamic data for each treatment are reported in Table 5. Significant differences were seen between three variables: left pulmonary artery flow, pulmonary artery pressure, and pulmonary artery pressure at zero flow. During ventilation with 3% oxygen in nitrogen, flow increased significantly in comparison to control, from 37.5 ± 27.6 (SD) to 255.5 ± 137.7 (SD) ml/min.

Pulmonary arterial pressure was analyzed as a function of treatment groups. Pressure ranged from about 56 to 42 mm Hg in the control and 100% O_2 groups, respectively. Statistical analysis showed that pulmonary arterial pressure fetuses in ventilated with 100% O_2 and with air were less than control, but no other combination was different. The general trend for pulmonary arterial pressure to drop with ventilation is not entirely unexpected if pulmonary vascular resistance drops considerably. It is interesting that the pulmonary

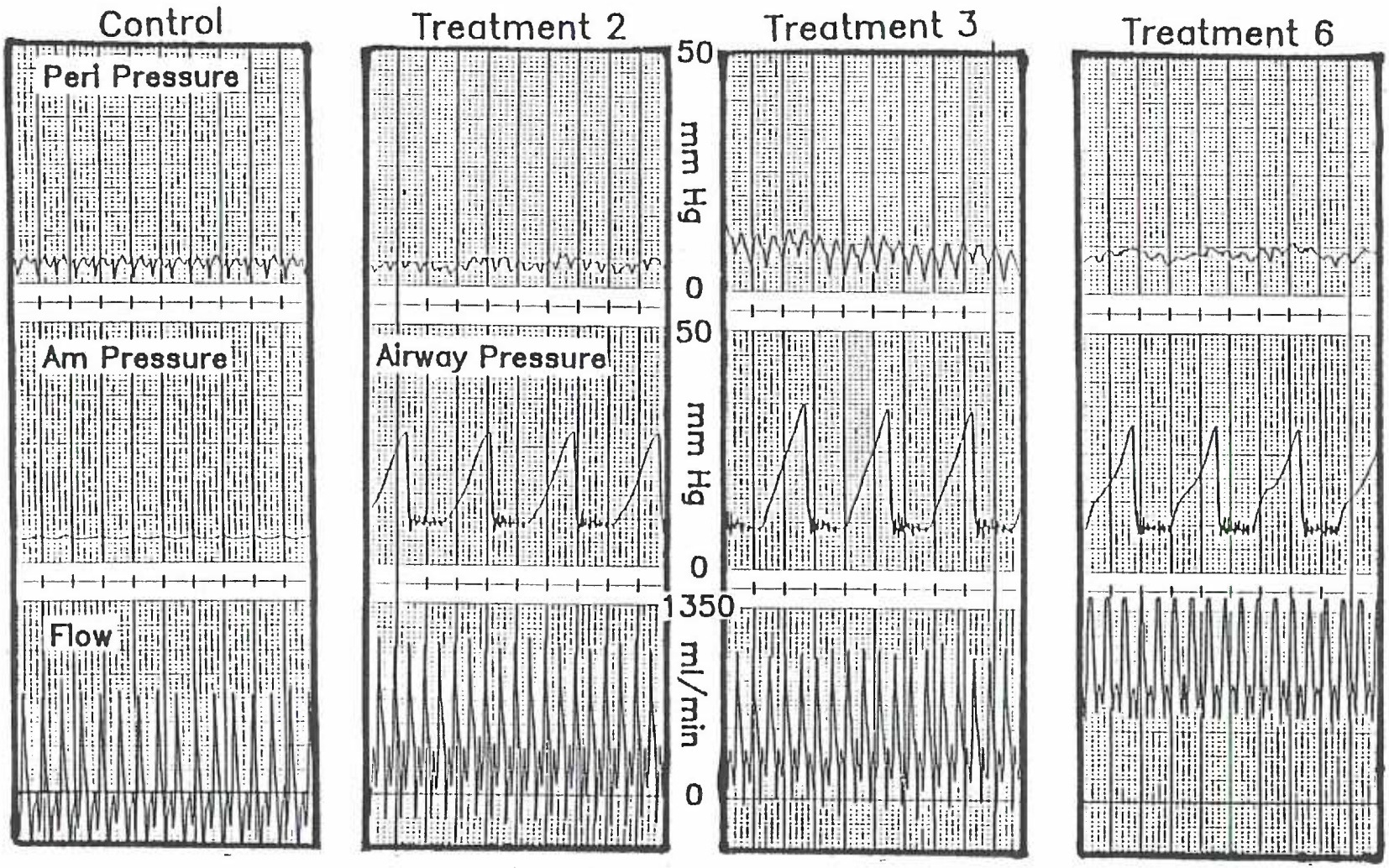
TABLE 5. HEMODYNAMIC VALUES WITH DIFFERENT TREATMENT GROUPS ($\bar{X} \pm SD$)*

Treatment Number	p_{PA} (mm Hg)	p_{CA} (mm Hg)	p_{LA} (mm Hg)	p_{RA} (mm Hg)	p_{Peri} (mm Hg)	p_{O} (mm Hg)	$\dot{Q}_{LPA}$ (ml/min^{-1})	HR ($beat \cdot min^{-1}$)	n
1. Control	55.9±11.7	50.1±8.1	2.7±2.6 (n=9)	3.7±2.7	8.0±1.9 (n=10)	24.9±11.6 (n=9)	37.5±27.6	175±26	11
2. 3% O_2-7% CO_2 in N_2	53.1±10.6	47.9±4.4	3.5±1.8	3.9±2.1	9.0±1.5	19.6±16.2 (n=5)	117.7±78.3	200±30	6
3. 3% O_2 in N_2	48.5±9.3	48.7±4.5	3.4±2.0 (n=8)	2.9±1.8	9.8±2.2 (n=8)	10.8±4.8 (n=8)	255.5±137.7	193±40	10
4. Room air	44.3±8.4	46.8±7.8	3.4±1.8 (n=4)	4.1±1.4	11.8±1.9	6.0±5.7	271.8±87.5	163±20	5
5. 95% O_2-5% CO_2	45.8±5.6	46.7±2.4	4.9±1.5	3.9±1.4	10.6±0.9	9.5±5.2	281.5±61.2	193±22	4
6. 100% O_2	42.3±7.3	49.6±4.8	4.9±2.0 (n=8)	3.8±2.5	9.7±2.4 (n=9)	8.5±4.5 (n=8)	374.0±201.0	177±28	10
Group Differences ($p<0.05$)	1>4,6	N.S.	N.S.	N.S.	4>1,2	1>3,4,5,6 2>4	4,5,6>1,2 3>1	N.S.	

*p_{PA} = pulmonary artery pressure; p_{CA} = carotid artery pressure; p_{LA} = left atrial pressure; p_{RA} = right atrial pressure; p_{O} = pulmonary artery pressure at which flow ceases; $\dot{Q}_{LPA}$ = left pulmonary artery flow; HR = heart rate; n = number of animals; N.S. = no significant difference between groups; $\bar{X}$ = mean
Analysis of variance, Duncan's multiple comparisons test

Figure 11: Pulmonary artery flow waveforms. Shown are representative examples of pericardial (peri) pressure, amniotic (Am) pressure and airway pressure during control ventilation with 3% O_2-7% CO_2 in N_2 (Treatment 2), ventilation with 3% O_2 in N_2 (Treatment 3) and ventilation with 100% O_2 (Treatment 6) in a single animal. During the control period, a large component of the pulsatile flow was negative. During Treatment 2 much of the negative portion had diminished. Diastolic flow in Treatment 3 approximates zero and in Treatment 6 there is a continuous positive flow through the left pulmonary artery, possibly due to left-to-right shunt through the patent ductus arteriosus.

REPRESENTATIVE PULMONARY ARTERY FLOW WAVEFORMS



artery pressure at zero flow also decreased from 24.9 ± 11.6 (SD) to 10.8 ± 4.8 (SD) mm Hg during ventilation with 3% O_2 in N_2.

In Figure 11, changes in the pulsatile flow wave form in the pulmonary artery are shown for an individual animal for several different treatments. The initial flow signal has a negative flow component as originally described by Rudolph and Heymann (1972). Flow signals are shown for control and three ventilation conditions. The flow signal becomes progressively more positive with each of the particular gas mixtures that were used for ventilation.

Table 6 lists the various pressures that were used in ventilating fetuses. Mean airway pressure (MAP) was about 6 mm Hg on the average, peak inspiratory pressure was about 24 mm Hg, and the average positive end-expiratory pressure was 0.1 mm Hg, each referred to pericardial pressure. A small positive end expiratory pressure (PEEP) was used to keep airways open between breaths (Willis et al., 1986). The average minute volume was 3.5 L/min.

Two features of the flow-pressure relation in the fetal lung were particularly important in helping to understand the effect of ventilation on pulmonary blood flow, the slope of the pressure-flow relationship and the pressure where flow was zero (Table 7). The slope of the relation between lung blood flow and pulmonary arterial pressure indicates the increase in blood flow for each mm Hg increase in pressure. As explained in the Methods sections, two methods were used to evaluate the relationship. First, the relationship was estimated by a least squares fit of the flow-pressure data in each condition and second, the linear relationship between the pressure-flow point where the animal operated and the pressure point where flow was zero during occlusion of the pulmonary artery was determined. Figure 12 shows

TABLE 6. VENTILATION VALUES IN DIFFERENT TREATMENT GROUPS ($\bar{X} \pm SD$)

	MAP (mm Hg)	PIP (mm Hg)	PEEP (mm Hg)	$\dot{V}$ (ml/min)
Control	--	--	--	--
3% O_2-7% CO_2 in N_2	6.7 ± 1.7	29.5 ± 10.1	0.4 ± 2.0	3800 ± 632
3% O_2 in N_2	6.6 ± 2.3	23.3 ± 4.8	1.0 ± 2.3	3334 ± 1062
Room air	4.5 ± 1.1	21.2 ± 3.2	-1.6 ± 1.0	3560 ± 542
95% O_2-5% CO_2	5.8 ± 2.0	22.7 ± 2.5	-0.4 ± 1.5	3500 ± 500
100% O_2	6.7 ± 2.3	23.3 ± 3.0	0.3 ± 2.0	3600 ± 710
Group differences ($p<0.05$)	N.S.	N.S.	N.S.	N.S.

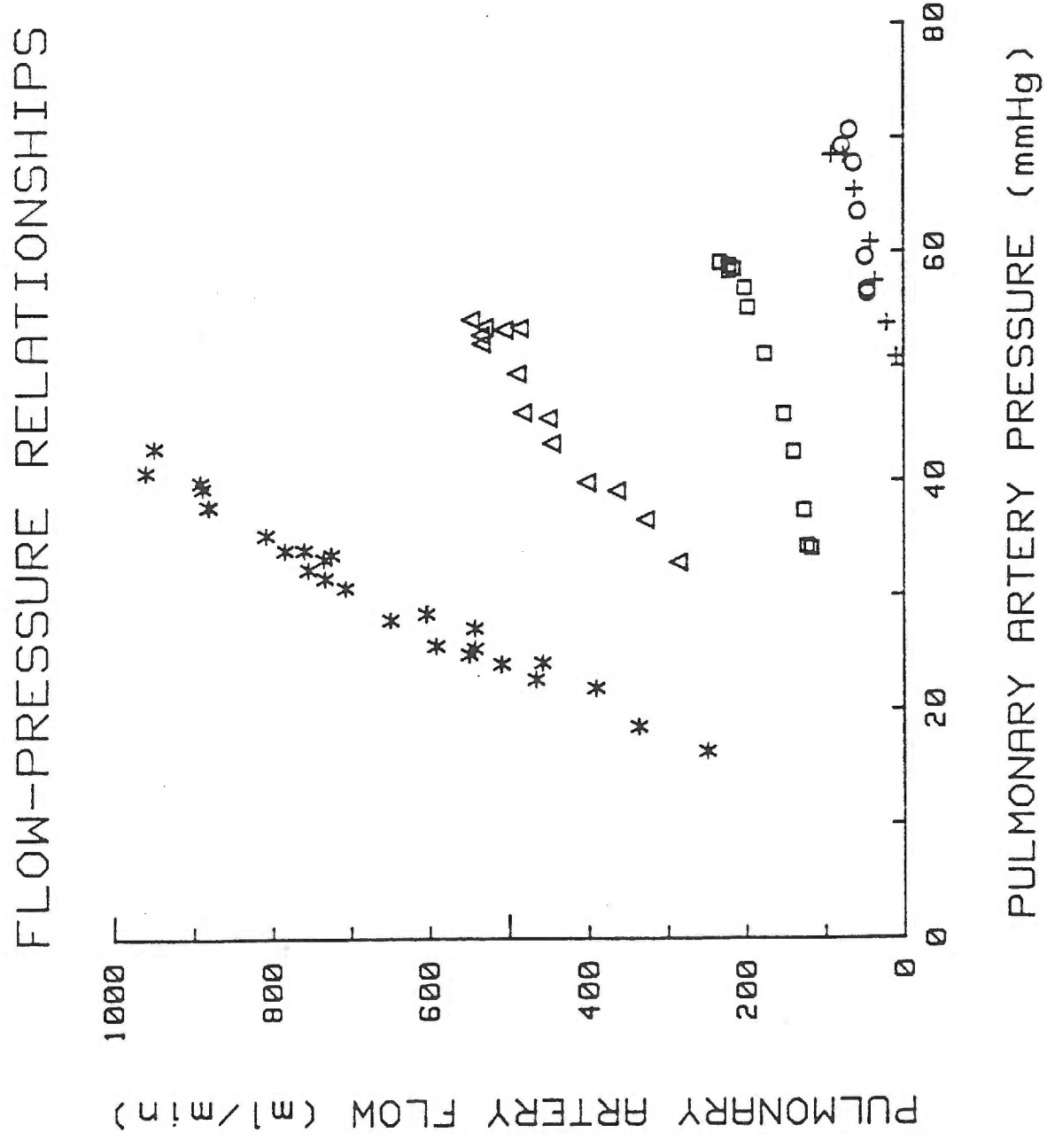
MAP = mean airway pressure; PIP = peak inspiratory pressure; PEEP = positive end expiratory pressure; $\dot{V}$ = minute volume; NS = no significant differences exist between groups; $\bar{X}$ = mean
Analysis of variance

TABLE 7. PRESSURE-FLOW RELATIONSHIP IN DIFFERENT TREATMENT GROUPS ($\bar{X}$ ± SD)*

	$P/\dot{Q}$ (mm Hg·min·ml^{-1})		$\dot{Q}/P$ (ml·min^{-1}·mm Hg^{-1})		P^O (mm Hg)	P^R (mm Hg)
	intercept method	regression method	intercept method	regression method	intercept method	regression method
Control	1.3 ± 1.3 (n=9)	0.6 ± 0.2	1.5 ± 0.9	1.8 ± 0.6	25 ± 12 (n=9)	27.8 ± 25.4
3% O_2-7% CO_2 in N_2	0.4 ± 0.4 (n=5)	0.4 ± 0.3	3.5 ± 1.7	3.5 ± 1.9	20 ± 16 (n=5)	20.1 ± 17.4
3% O_2 in N_2	0.2 ± 0.1 (n=8)	0.2 ± 0.1	8.4 ± 7.8	7.7 ± 5.0	11 ± 5 (n=8)	15.1 ± 8.1
Room air	0.2 ± 0.1	0.1 ± 0.1	7.1 ± 2.1	8.2 ± 2.6	6 ± 6	12.4 ± 7.2
95% O_2-5% CO_2	0.1 ± 0.02	0.1 ± 0.02	7.8 ± 1.2	8.5 ± 1.6	10 ± 5	12.8 ± 4.4
100% O_2	0.1 ± 0.1 (n=8)	0.1 ± 0.04	10.7 ± 6.8	13.8 ± 7.8	9 ± 5 (n=8)	10.8 ± 6.7
Group differences ($p<0.05$)	1>2,3,4,5,6	1>2,3,4,5,6 2>3,4,5,6	6>1,2 3>1	6>1,2,3,4,5 2,3,4>1	1>3,4,5,6 2>4	N.S.

*P^O = positive pulmonary artery pressure at zero flow; P^R = pressure intercept of regression relationship; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship; $\bar{X}$ = mean
Analysis of variance, Duncan's multiple comparisons test

Figure 12: Relationships between left pulmonary artery flow and pulmonary artery pressure in a single animal. This figure illustrates curves during control (+), ventilation with 3% O_2-7% CO_2 in N_2 (□), ventilation with 3% O_2 in N_2 (△), ventilation with 100% O_2 (*) and 45 minutes after ventilation (o).


FLOW-PRESSURE RELATIONSHIPS
PULMONARY ARTERY FLOW (ml/min)
1000
800
600
400
200
0
0
20
40
60
80
PULMONARY ARTERY PRESSURE (mmHg)

flow-pressure relationships during different treatments in an individual animal. Note that the steepness of the slope depends upon the gas mixture administered and that the slope returns to its initial position after ventilation. Data are summarized for all animals in Figure 13. The slope of each line, an estimate of conductance, is the average flow-pressure relationship for each treatment; the x-intercept represents the average positive pulmonary artery pressure at zero flow. The mean slope of the flow-pressure relationship during each treatment is also shown in a composite histogram in Figure 14. The slope of the flow-pressure relatioship shows a trend toward increasing values with changes in experimental condition. During ventilation with 3% O_2 in N_2, 95% O_2-5% CO_2, and with 100% O_2, the slopes are different from control ($p<0.05$). It is likely that the slope during ventilation with air would also reach statistical significance if more than five animals had been studied for this treatment. The other feature of the flow-pressure relationship which could have physiological significance is the pulmonary artery pressure at which flow becomes zero. This pressure was determined in two ways, as described in the Methods section. The intercept pressure generally exceeded the left atrial pressure and when the intercepts derived by the two methods were compared by paired t test (with Bonferroni correction), the values could not be shown to be different. The inverse of the flow-pressure relationship describes the association of driving pressure and blood flow in the lung, and is a likely approximation of pulmonary vascular resistance. The slope of this pressure-flow relationship was significantly higher during control conditions than during any ventilation treatment regardless of the method by which the slope was

Figure 13: Composite flow-pressure curves for all animals. Curves are shown for control (Treatment 1, n=9), ventilation with 3% O_2-7% CO_2 in N_2 (Treatment 2, n=5), ventiation with 3% O_2 in N_2 (Treatment 3, n=8), ventilation with room air (Treatment 4, n=5), ventilation with 95% O_2-5% CO_2 (Treatment 5, n=4), and ventilation with 100% O_2 (Treatment 6, n=8). Data for construction of curves were based on slope calculated by the intercept technique and actual pressure intercepts were taken from polygraph records during full occlusion of postductal pulmonary artery.

COMPOSITE FLOW–PRESSURE CURVES

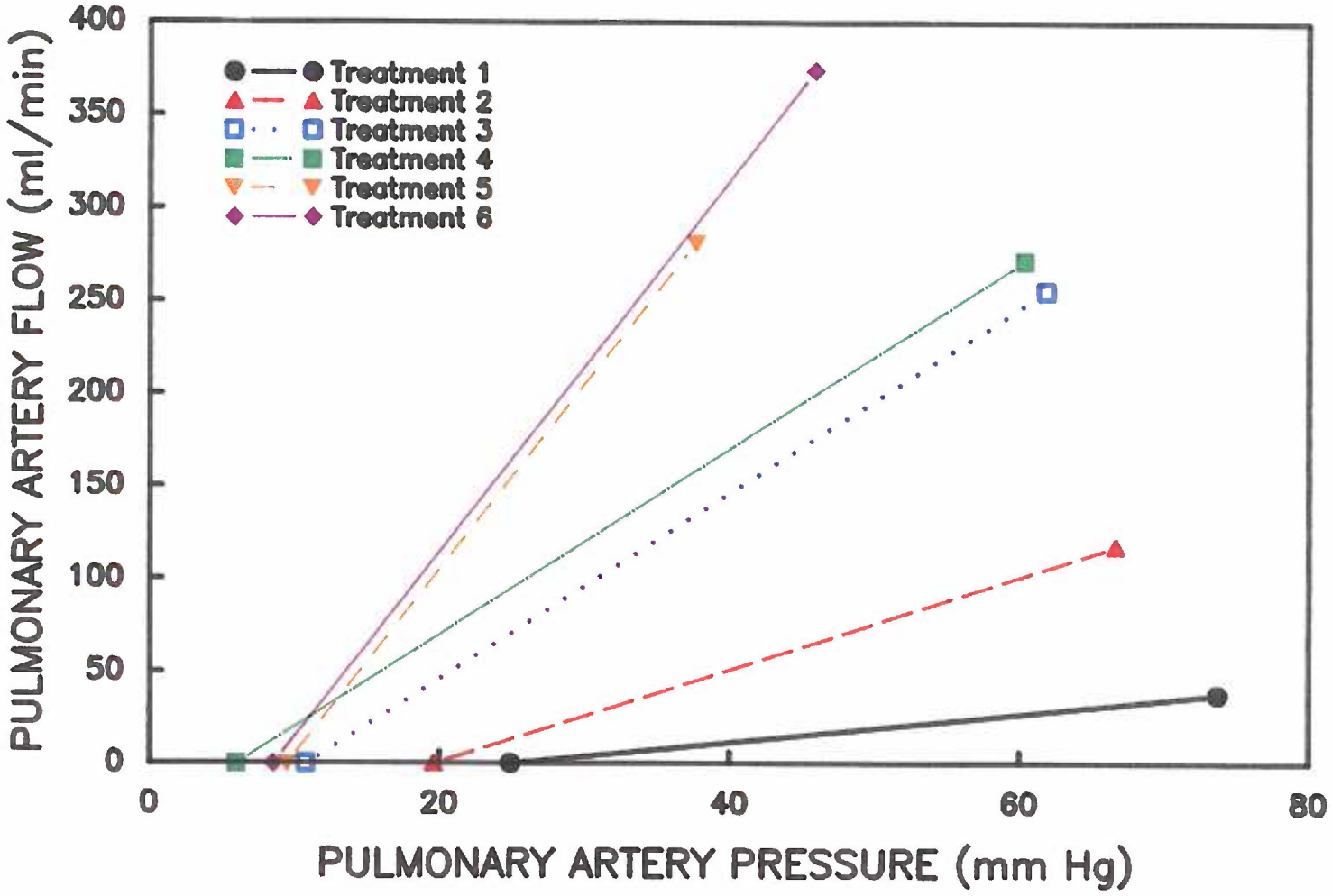
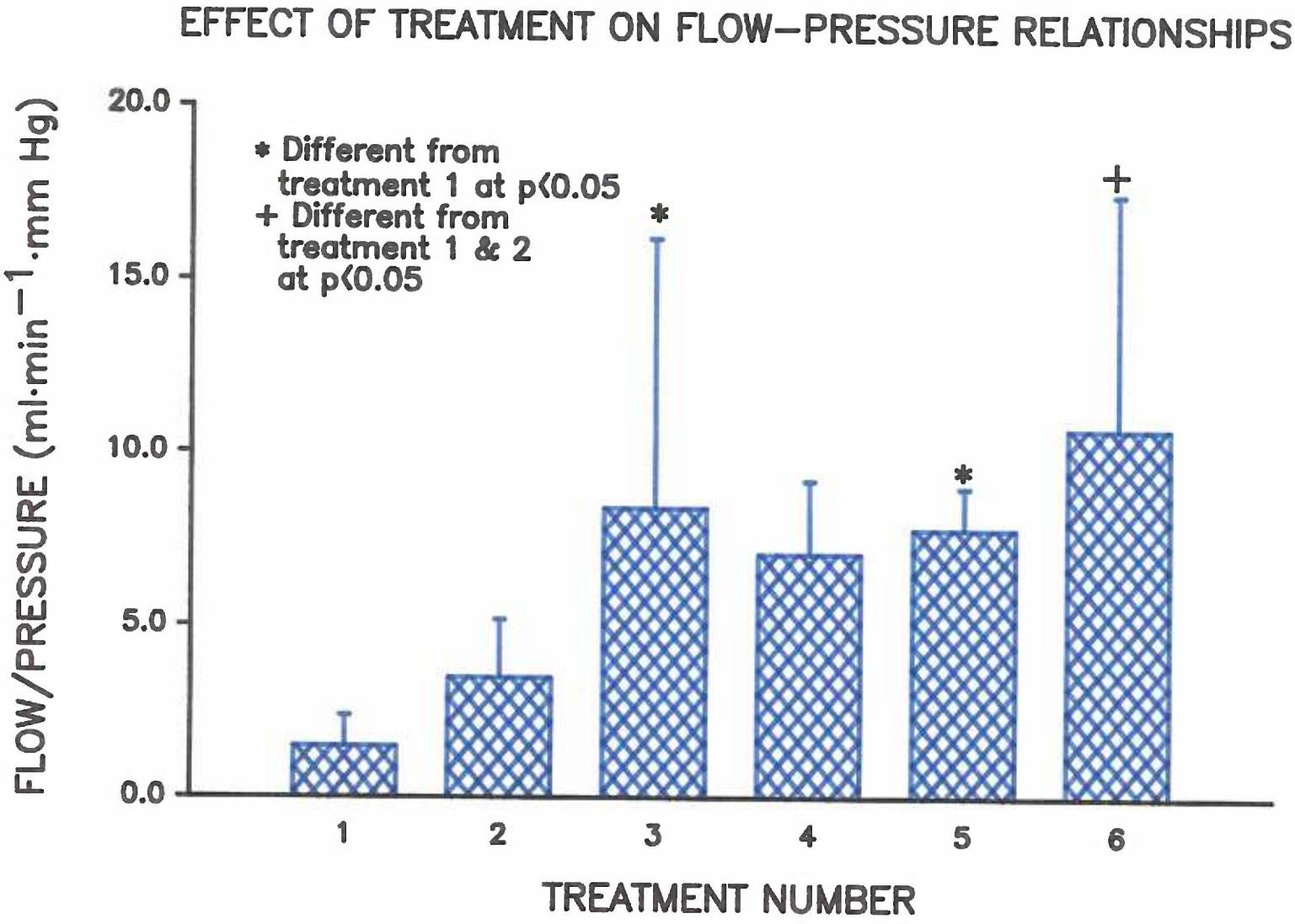


Figure 14: Composite histogram of flow-pressure relationship. Pulmonary flow-pressure relationships calculated by the intercept method are shown for control (Treatment 1, n=9), ventilation with 3% O_2-7% CO_2 in N_2 (Treatment 2, n=5), ventilation with 3% O_2 in N_2 (Treatment 3, n=8), ventilation with room air (Treatment 4, n=5), ventilation with 95% O_2-5% CO_2 (Treatment 5, n=4), and ventilation with 100% O_2 (Treatment 6, n=8). Treatments 2, 3, 4, 5 and 6 are significantly lower than Treatment 1. In general, pressure/flow value decreased as treatments progressed from left to right.


EFFECT OF TREATMENT ON FLOW–PRESSURE RELATIONSHIPS
* Different from treatment 1 at p<0.05
+ Different from treatment 1 & 2 at p<0.05
FLOW/PRESSURE (ml·min⁻¹·mm Hg)
20.0
15.0
10.0
5.0
0.0
*
*
+
1
2
3
4
5
6
TREATMENT NUMBER

calculated. During ventilation with 3% O_2-7% CO_2 in N_2, the slope calculated by regression method was also statistically different from all other treatments. Changes in the slopes of the pressure-flow relationship are shown in the composite histogram in Figure 15. The slope of the pressure-flow relation changed as expected from Figures 12, 13, and 14, with all ventilation treatments being different from that of control.

Multiple regression analysis was used to describe the effect of pulmonary and carotid arterial PO_2, PCO_2, pH and inspired O_2 and CO_2 fraction on the slope of the pressure-flow relationship (PVR). The independent variables were analyzed in pairs, i.e., PVR versus PO_2 and PCO_2, PO_2 and pH, and inspired O_2 and CO_2 fraction. Table 8 shows that PVR was related to pulmonary arterial PO_2, PCO_2 and pH and also by inspired O_2 and CO_2 fraction, as indicated by their significant regression coefficients. Thus, the relationship between PVR and pulmonary arterial PO_2 and PCO_2 has been quantitatively described in the chronically prepared ventilated fetus.

DISCUSSION

Pulmonary arterial pressure-flow relationships were studied in healthy unanesthetized mature fetal lambs. Mean carotid arterial PO_2 was 18.8 mm Hg, a value within the normal range even though no animals were discarded because of low PO_2. As shown in Table 1, two animals had a carotid arterial PO_2 less than 17 mm Hg. One of these (#86-108) did not respond in the same fashion as other fetuses to ventilation with 3% O_2 in N_2, the only inspiratory mixture used in that fetus. It is not apparent whether this unusual response is a function of hypoxia

Figure 15: Composite histogram of average pulmonary pressure-flow relationships calculated by the intercept method. Control (Treatment 1, n=9), ventilation with 3% O_2-7% CO_2 in N_2 (Treatment 2, n=5), ventilation with 3% O_2 in N_2 (Treatment 3, n=8), ventilation with room air (Treatment 4, n=5), ventilation with 95% O_2-5% CO_2 (Treatment 5, n=4), and ventilation with 100% O_2 (Treatment 6, n=8).

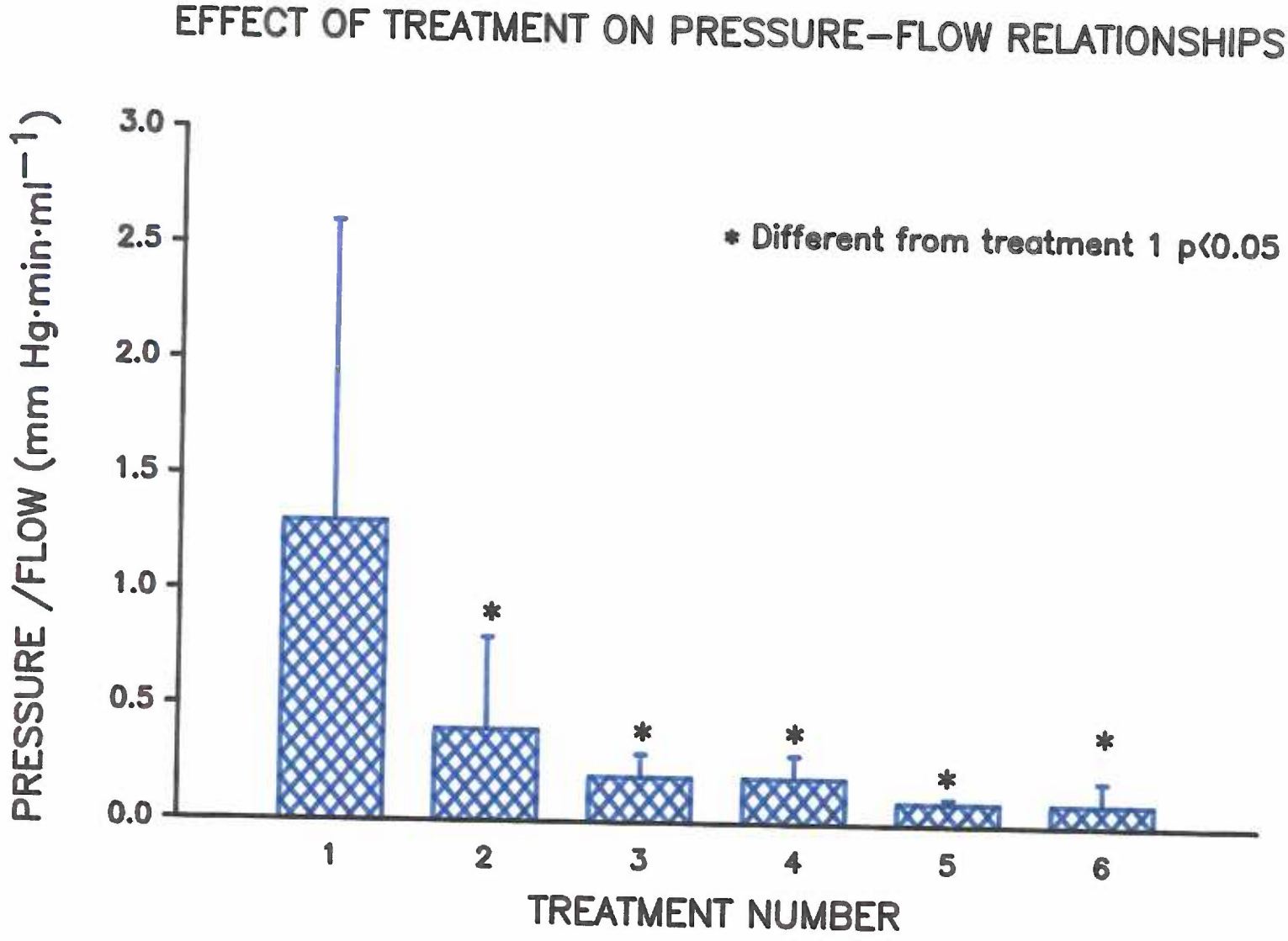

EFFECT OF TREATMENT ON PRESSURE–FLOW RELATIONSHIPS
PRESSURE /FLOW (mm Hg·min·ml⁻¹)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
* Different from treatment 1 p<0.05
*
*
*
*
*
1
2
3
4
5
6
TREATMENT NUMBER

TABLE 8. MULTIPLE REGRESSION VALUES FOR SLOPE OF PRESSURE-FLOW RELATIONSHIP OF THE FORMULA $y = a + bx + cz$*

Equation #	y	x	z	a	t	p	b	t	p	c	t	p	r^2	n
1	PVR	PPO_2	PpH	6.5	2.83	<0.01	-0.006	-2.64	<0.025	-0.8	-2.67	<0.025	0.37	35
2	PVR	PPO_2	$PPCO_2$	-0.1	-0.66	N.S.	-0.006	-2.48	<0.025	0.01	2.83	<0.01	0.38	35
3	PVR	CPO_2	CpH	4.8	2.33	<0.05	-0.0002	-1.08	N.S.	-0.6	-2.22	<0.05	0.22	34
4	PVR	CPO_2	$CPCO_2$	-0.06	-0.41	N.S.	-0.0003	-1.08	N.S.	0.007	1.88	<0.1	0.18	34
5	PVR	FIO_2	$FICO_2$	0.2	5.23	<0.001	-0.1	-2.54	<0.025	2.5	3.12	<0.005	0.37	35

*PVR = slope of pressure-flow relationship; PPO_2, PpH, $PPCO_2$ = pulmonary arterial PO_2, pH and PCO_2, respectively; CPO_2, CpH, $CPCO_2$ = carotid arterial PO_2, pH and PCO_2, respectively; FIO_2 = fractional inspired O_2; $FICO_2$ = fractional inspired CO_2; N.S. = not significant

or of animal variability. Even though the accepted standard recovery period is 2-3 postoperative days (Anderson et al., 1981), a longer period was allowed in this study to assure recovery from the extensive open-chest surgery.

Fetuses were ventilated with or without changes in PO_2 and PCO_2 to study the separate influences of these gas mixtures on the slope and relative position of the pressure-flow relationship. Since not all suspected regulators of fetal pulmonary vascular tone could be investigated, several specific factors were chosen for study: the effect of positive pressure ventilation in utero, arterial carbon dioxide tension and arterial oxygen tension. As discussed in the introduction, evidence suggests that each of these might be important in the fall in pulmonary vascular resistance at birth. No effort was made to differentiate the separate roles of arterial and alveolar gas tensions or arterial CO_2 gas tension from pH, although these should be investigated in the future.

Neither cholinergic blockade alone nor the combined effects of cholinergic and β-adrenergic blockade by administration of atropine and propranolol, had an effect on the slope of the pressure-flow relationship. This finding is interesting in light of the fact that Cassin and associates (1964a) reported decreased pulmonary vascular resistance when the adrenal glands were removed from anesthetized exteriorized fetal lambs. Little else is known about adrenergic tone of the fetal pulmonary vessels, but results from the present study suggest that in normal fetal lambs as in the adult (Fishman, 1985), the autonomic nervous system is not an important determinant of resting tone in the pulmonary vascular bed. Barrett et al. (1972) studied α-agonist action

in anesthetized fetuses by infusing agonist doses of methoxamine. Their experiments yielded ambiguous results since both pulmonary blood flow and arterial pressure increased. Because the Barrett study was not designed to generate pressure-flow curves, it was not possible to determine whether their data represented hypertension without vasoconstriction, or changes in the slope of the pressure-flow curve. Left unanswered then, is the question, what is the normal resting tone of the α- and β-adrenergic nerves in the mature fetal lung?

A previous study by Thornburg and Morton (1983) could not demonstrate an effect of cholinergic and β-adrenergic blockade on hemodynamic parameters in conscious sheep fetuses. In the present study, the only effect shown by cholinergic blockade with atropine alone and in combination with β-adrenergic blockade with propranolol, was an increased heart rate. The increase in heart rate associated with β-adrenergic and cholinergic blockade was unexpected. This effect has not been seen in other studies (Thornburg and Morton, 1983) and cannot presently be explained.

In this study, most hemodynamic variables were constant throughout the experiments, regardless of ventilation treatment. Only left pulmonary artery flow and pulmonary arterial pressure exhibited significant differences in response to ventilation treatment. In addition, the pulmonary arterial pressure at which flow became zero (P^0) was affected by ventilation. Blood flow to the left lung was significantly elevated from control for all ventilation treatments except ventilation with 3% O_2-7% CO_2 in N_2. In addition, flow was significantly greater than ventilation with 3% O_2-7% CO_2 in N_2 in the three ventilation treatments which raised fractional inspired oxygen and arterial PO_2 (room air, 95% O_2-5% CO_2 and 100% O_2). Pulmonary

artery pressure was significantly lower than control both during ventilation with air and with 100% oxygen. No other significant differences in pulmonary artery pressures between ventilation treatments could be shown. The pulmonary artery pressure at which flow became zero (P^0) was significantly lower than control during ventilation with 3% O_2 in N_2, room air, 95% O_2-5% CO_2, and with 100% O_2; the P^0 was also lower than ventilation with 3% O_2-7% CO_2 in N_2 during ventilation treatments with room air and 100% O_2. These data support the previously published findings of Gilbert and colleagues (1972).

As expected, the composition of the inspiratory gas mixture had striking effects on arterial gas tensions (Willis et al., 1986). Ventilation with gases devoid of carbon dioxide caused arterial carbon dioxide tensions to fall to neonatal levels or lower. Addition of oxygen to the inspiratory mixture was associated with increased arterial oxygen tension. As is evident in Figure 10 the relationship between carotid arterial and pulmonary arterial oxygen tensions was not linear. Pulmonary artery PO_2 did not increase to the same levels as in the carotid artery when systemic PO_2 was above 30 mm Hg. This is due to the complexities of the transitional circulatory system which include variable shunt flow patterns and an intact umbilical circulation.

Vascular resistance is defined as the driving pressure per unit flow through the vascular bed. In an individual organ, driving pressure is generally considered to be arterial minus venous pressure. However, in some cases a pressure surrounding the vasculature exceeds venous pressure. In such cases, driving pressure is equivalent to

arterial minus surrounding pressure (Green, 1982). In this study, the occluder around the pulmonary artery was used to reduce pulmonary arterial pressure until flow through the pulmonary bed stopped. The pressure at which flow became zero (P^0) was always greater than or equal to left atrial pressure. The source of this "intercept" pressure (P^0) is unknown; it may include both an interstitial pressure component and a critical closing pressure (active smooth muscle) component. Regardless, the pressure intercept estimates surrounding pressure, since it is the intercept of a fairly linear relationship between flow and pressure in the pulmonary vascular bed. For purposes of calculation, individual pulmonary arterial pressure values at zero flow were used as the downstream component of the driving pressure for flow through the pulmonary vascular bed. It appears that the slope of the pressure-flow relationship is therefore a fair estimate of the vascular resistance of the pulmonary bed under these experimental conditions. It is interesting that the slope of the relationship between pressure and flow using the least squares regression method is about the same value as the vascular resistance calculated using the intercept method (Table 7).

In utero ventilation is an effective way to select for certain birth related changes while holding other factors such as temperature and muscular activity constant. However, positive pressure ventilation does not simulate normal birth. In adults, positive pressure ventilation causes some increase in pulmonary vascular resistance (Green, 1982). This may also be true for positive pressure ventilation in the fetus. Positive pressure ventilation may have elevated pulmonary vascular resistance during all ventilation treatments to a level higher than that which would have been attained by a neonate

breathing the same gas mixtures with normal negative-pressure ventilation. In spite of such an increase, positive-pressure ventilation with 3% O_2-7% CO_2 in N_2 caused a substantial decline in pulmonary vascular resistance in the absence of changes in arterial gas tensions. This change in pulmonary vascular resistance is related to the mechanical effects of positive pressure ventilation alone since arterial blood gases did not change. These experiments, using positive pressure ventilation, may actually underestimate decreases in vascular resistance in response to mechanical ventilation. This aspect of the study supports the preliminary conclusion reported by Rudolph et al. (1986) that mechanical ventilation is an important stimulus for decreased pulmonary vascular resistance at birth. Data in anesthetized fetuses suggest that the mechanical effect of ventilation may be mediated by prostaglandin I_2 (Leffler et al., 1980; 1981). Much more work must be done to examine how this mechanism works and how to manipulate the mechanism in a clinical setting.

Five gas mixtures were included in this experimental design. These were chosen to study the contribution of changes in PO_2 and PCO_2 to the changes seen in pulmonary vascular resistance at birth. The gas mixtures were selected to produce interesting combinations of high and low alveolar and arterial gas tension of oxygen and carbon dioxide. Analyses by multiple linear regression of the data collected during ventilation with these gas mixtures show significant correlations between pulmonary vascular resistance, inspired gases and blood gases: pulmonary vascular resistance correlated with arterial PCO_2 and PO_2, and pH and also with FIO_2 and $FICO_2$. Pulmonary vascular resistance is inversely related to PO_2, pH and FIO_2. Likewise, pulmonary vascular

resistance correlated with arterial PCO_2 and $FICO_2$. This supports data from anesthetized fetuses which indicate that PO_2 and PCO_2 both have important effects on pulmonary vascular resistance.

The regression coefficients for these data present an irresistible opportunity to predict the theoretical contributions of pulmonary arterial PO_2, PCO_2 and pH to the drop in pulmonary vascular resistance at birth. Under the conditions from which the data were derived, pulmonary vascular resistance changed by 0.006 resistance units per mm Hg PO_2 and 0.114 resistance units per mm Hg PCO_2. One might be tempted to assume from these data that arterial PCO_2 is more powerful than PO_2, but caution is advised since the average change in pulmonary arterial PCO_2 at birth is only a few mm Hg, whereas the change in pulmonary arterial O_2 tension is on the order of 20 mm Hg.

In individual animals, the separate effects of increased PO_2 and decreased PCO_2 did not appear to be additive. For example, the effect of decreasing carbon dioxide tension may be seen when ventilation with 3% O_2-7% CO_2 in N_2 is compared with 3% O_2 in N_2 when PO_2 does not change. On the average, the decrease in PCO_2 which accompanied the change in ventilation mixture, was associated with a decrease in pulmonary vascular resistance from 0.44 to 0.19 mm Hg·min·ml^{-1}. The effect of increased PO_2 can be seen by comparing the vascular resistance during ventilation with 3% O_2-7% CO_2 in N_2 to resistance during ventilation with 95% O_2-5% CO_2 when CO_2 tension does not change. Here, vascular resistance drops from 0.44 to 0.13 mm Hg·min·ml^{-1}. This represents a fall of 0.25 and 0.31 mm Hg·min·ml^{-1} for decreased PCO_2 and increased PO_2, respectively. If the effects of CO_2 and O_2 were additive, ventilation with 100% oxygen could be expected to drop pulmonary vascular resistance by about 0.56 mm Hg·min·ml^{-1}. Instead,

ventilation with 100% oxygen dropped pulmonary vascular resistance about 0.32 mm Hg·min·ml^{-1}, a fall not very different from the change predicted with either PCO_2 or PO_2 individually. This result would be expected if the vasculature was maximally vasodilated with relatively small increases in PO_2 or decreases in PCO_2, or if both PO_2 and PCO_2 share a common pathway in the steps which lead to vasodilation. If one particular component of the pathway were maximally stimulated so that an additional stimulus would be ineffective, then the two effects would not be additive.

It is also interesting that the biochemical mechanism(s) that underlie the role of carbon dioxide tension in regulating pulmonary vascular resistance have not been studied in the fetus; neither are they clearly understood in the adult lung. Research in the adult lung indicates that hydrogen ion concentration and not necessarily carbon dioxide tension itself mediates changes in pulmonary vascular tone. However, this issue has not been examined in the fetus.

There is evidence that suggests that one of the mediators of the "oxygen effect" in the fetal lung is bradykinin (Heymann et al., 1969), but this should be explored further. In the adult, the mechanism of action by which oxygen exerts an effect on pulmonary vascular tone is controversial (Fishman, 1976). There are two distinct schools of thought. One is based on a direct action of oxygen on vascular smooth muscle. The other school is based on the belief that the oxygen effect is mediated by a chemical substance.

The present study suggests that while there may be no single regulator of pulmonary vascular resistance during the first few hours of breathing, mechanical ventilation alone, increased pulmonary

arterial PO_2, decreased PCO_2 (and/or increased pH) as well as increased FIO_2 and decreased $FICO_2$ all contribute to the decrease in pulmonary vascular resistance during *in utero* positive-pressure ventilation in fetal lambs.

An understanding of the bases for the normal fall in pulmonary vascular resistance at birth is necessary before the pathological condition in which pulmonary vascular resistance remains elevated can be appropriately treated. Persistent pulmonary hypertension syndrome of the neonate occurs in approximately 1 in 1500 newborns; the associated mortality rate is 20 to 50% (Brown, 1974; Heymann, 1985). Drummond (1983) has suggested that persistent pulmonary hypertension of the neonate has a multi-faceted etiology. This study supports the idea that there is more than one possible cause of persistent pulmonary hypertension of the neonate.

As expected, this study has raised new questions regarding changes in the fetal pulmonary circulation at birth. For example, what factors contribute to P^0? Why does P^0 drop during positive-pressure ventilation? Can the fetus distribute its blood flow preferentially to ventilated areas of the lung? In what way does PCO_2 affect pulmonary vascular resistance; is the "CO_2 effect" really the effect of pH? Lastly, and perhaps most interestingly, are the pulmonary vasodilatory effects of PO_2 and PCO_2 mediated by alveolar or arterial gas tensions? This study shows that it is possible to critically assess pulmonary vascular resistance in the fetus by generating pressure-flow "curves" over a wide range of pressures and flow. This study also provides evidence that increased PO_2, decreased PCO_2 and mechanical ventilation are all important features in the regulation of pulmonary vascular resistance in the transitional period.

SUMMARY AND CONCLUSIONS

Pressure-flow relationships were successfully studied in 11 fetal lambs under several experimental conditions. The following conclusions were made:

1. Blood flow through the pulmonary vascular bed ceases at a pulmonary arterial pressure which exceeds left atrial pressure. Therefore, left atrial pressure probably does not represent the true downstream component of driving pressure through the pulmonary vascular bed. The actual downstream pressure can best be estimated by measurement of pulmonary arterial pressure during manual occlusion of the main postductal pulmonary artery.
2. Positive pressure *in utero* ventilation with an inspiratory gas mixture of 3% O_2-7% CO_2 in N_2 did not alter fetal arterial blood gas values. Even so, the mechanical effects of ventilation produced, on average, a 3-fold fall in pulmonary vascular resistance.
3. Increased pulmonary arterial PO_2 during ventilation with oxygen-rich mixtures was associated with a fall in pulmonary vascular resistance beyond that measured during ventilation with 3% O_2-7% CO_2 in N_2.
4. Lowering pulmonary arterial PCO_2 decreased pulmonary vascular resistance in comparison to ventilation with 3% O_2-7% CO_2 in N_2.

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APPENDIX 1

"RAW DATA FOR ALL TREATMENT GROUPS"

TABLE A. HEMODYNAMIC RAW DATA DURING CONTROL*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})	$\dot{Q}$ (ml $\cdot$ min^{-1})	P^{PA} (mm Hg)	P^0 (mm Hg)	$P/\dot{Q}$ (mm Hg $\cdot$ min $\cdot$ ml^{-1})		$\dot{Q}/P$ (mm Hg $\cdot$ min $\cdot$ ml^{-1})	
								intercept method	regression method	intercept method	regression method
86-108	14.5	53.1	7.33	4.6	63.7	53.5	28	0.39	0.33	2.57	3.06
86-109	15.8	51.5	7.38	--	24.9	45.1	34	0.45	0.59	2.24	1.71
87-6	17.8	51.9	7.31	--	20.1	49.2	--	--	0.59	--	1.70
87-19-2	14.8	52.8	7.35	4.5	105.0	56.9	15	0.40	0.88	2.51	1.14
87-27-1	15.6	54.0	7.34	5.9	19.8	51.0	39	0.61	0.56	1.65	1.80
87-33	14.4	53.9	7.30	4.1	53.3	63.4	--	--	0.75	--	1.33
87-45-2	18.4	46.8	7.38	--	17.7	61.2	34	1.54	0.39	0.65	2.57
87-47-1	13.0	50.7	7.36	--	43.6	35.2	13	0.51	0.64	1.97	1.56
87-54	14.6	55.9	7.32	4.9	14.3	79.2	16	4.92	1.00	0.23	1.00
87-58-2	15.7	49.0	7.34	6.6	18.0	67.1	36	1.73	0.50	0.58	2.01
87-63-2	19.1	50.2	7.30	8.3	32.2	53.5	9	1.38	0.74	0.72	1.35
$\bar{X}$	15.8	51.8	7.33	5.6	37.5	55.9	25	1.27	0.63	1.45	1.75
SD	1.9	2.6	0.03	1.5	27.6	11.7	12	1.30	0.20	0.92	0.61
n	11	11	11	7	11	11	9	9	11	9	11

*PO_2, PCO_2, pH and O_2 content represents values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^0 = pulmonary artery pressure at which flow becomes zero; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship

TABLE B. HEMODYNAMIC RAW DATA DURING VENTILATION WITH 3% O_2-7% CO_2 IN N_2*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})	$\dot{Q}$ (ml·min^{-1})	pPA (mm Hg)	pO (mm Hg)	P/$\dot{Q}$ (mm Hg·min·ml^{-1})		$\dot{Q}$/P (mm Hg·min·ml^{-1})	
								intercept method	regression method	intercept method	regression method
87-33	16.9	49.6	7.33	5.7	109.0	42.5	--	--	0.36	--	2.82
87-45-2	17.7	44.3	7.38	--	32.1	55.1	45	0.31	0.44	3.19	2.28
87-47-1	17.7	47.0	7.35	9.1	120.0	43.4	19	0.20	0.18	4.92	5.61
87-54	10.9	58.2	7.20	2.7	41.1	71.3	22	1.20	0.96	0.83	1.04
87-58-2	17.9	47.0	7.32	7.8	162.0	56.1	10	0.29	0.27	3.51	3.69
87-63-2	19.1	50.2	7.30	8.3	242.0	50.1	2	0.20	0.18	5.03	5.73
$\bar{X}$	16.7	49.4	7.31	6.7	117.7	53.1	20	0.44	0.40	3.50	3.53
SD	2.9	4.8	0.06	2.6	78.3	10.6	16	0.43	0.29	1.70	1.87
n	6	6	6	5	6	6	5	5	6	5	6

*PO_2, PCO_2, pH and O_2 content represent values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^{O} = pulmonary artery pressure at which flow becomes zero; P/$\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}$/P = slope of flow-pressure relationship

TABLE C. HEMODYNAMIC RAW DATA DURING VENTILATION WITH 3% O_2 IN N_2*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})	$\dot{Q}$ (ml·min^{-1})	P^{PA} (mm Hg)	P^0 (mm Hg)	$P/\dot{Q}$ (mm Hg·min·ml^{-1})		$\dot{Q}/P$ (mm Hg·min·ml^{-1})	
								intercept method	regression method	intercept method	regression method
86-108	17.6	49.6	7.37	6.8	54.1	55.7	--	--	0.34	--	2.94
87-6	19.9	48.5	7.32	--	443.0	42.6	--	--	0.07	--	14.19
87-19-2	10.7	36.8	7.48	3.2	144.0	55.8	15	0.28	0.22	3.53	4.47
87-27-1	16.8	43.5	7.39	8.3	477.0	32.9	15	0.04	0.06	26.65	18.07
87-33	16.5	37.7	7.43	5.5	184.0	41.0	13	0.15	0.17	6.47	5.88
87-45-2	16.4	32.7	7.51	--	377.0	49.5	14	0.09	0.11	10.62	9.34
87-47-1	15.3	36.9	7.45	8.5	283.0	40.2	2	0.14	0.11	7.41	9.16
87-54	12.2	45.9	7.31	3.9	171.0	64.0	11	0.37	0.26	2.67	3.82
87-58-2	15.7	37.4	7.43	7.4	182.0	53.1	11	0.23	0.25	4.32	4.05
87-63-2	19.1	46.1	7.32	8.5	240.0	50.3	5	0.19	0.20	5.30	5.10
$\bar{X}$	16.0	41.4	7.40	6.5	255.5	48.5	11	0.19	0.18	8.37	7.70
SD	2.8	5.8	0.07	2.1	137.7	9.3	5	0.11	0.09	7.80	5.01
n	10	10	10	8	10	10	7	7	10	7	10

*PO_2, PCO_2, pH and O_2 content represent values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^0 = pulmonary artery pressure at which flow becomes zero; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship

TABLE D. HEMODYNAMIC RAW DATA DURING VENTILATION WITH ROOM AIR*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml $O_2 \cdot 100$ ml blood^{-1})	$\dot{Q}$ (ml$\cdot$min^{-1})	P^{PA} (mm Hg)	P^0 (mm Hg)	$P/\dot{Q}$ (mm Hg$\cdot$min$\cdot$ml^{-1})		$\dot{Q}/P$ (mm Hg$\cdot$min$\cdot$ml^{-1})	
								intercept method	regression method	intercept method	regression method
86-109	19.3	45.1	7.40	--	324.0	36.5	0	0.11	0.10	8.88	9.85
87-19-2	31.6	35.2	7.49	11.1	383.0	56.7	11	0.12	0.09	8.38	10.91
87-54	19.8	31.8	7.49	9.9	167.0	36.5	3	0.21	0.17	4.80	6.06
87-58	26.6	38.3	7.41	12.6	280.0	45.6	13	0.12	0.11	8.59	9.07
87-63-2	24.3	45.7	7.31	10.0	205.0	46.1	3	0.21	0.21	4.76	4.87
$\bar{X}$	24.3	39.2	7.42	10.9	271.8	44.3	6	0.15	0.13	7.08	8.15
SD	5.1	6.1	0.07	1.3	87.5	8.4	6	0.05	0.05	2.11	2.57
n	5	5	5	4	5	5	5	5	5	5	5

*PO_2, PCO_2, pH and O_2 content represent values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^0 = pulmonary artery pressure at which flow becomes zero; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship

TABLE E. HEMODYNAMIC RAW DATA DURING VENTILATION WITH 95% O_2-5% CO_2*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml O_2·100 ml blood^{-1})	$\dot{Q}$ (ml·min^{-1})	P^{PA} (mm Hg)	P^0 (mm Hg)	$P/\dot{Q}$ (mm Hg·min·ml^{-1})		$\dot{Q}/P$ (mm Hg·min·ml^{-1})	
								intercept method	regression method	intercept method	regression method
87-47-1	35.4	45.6	7.36	13.5	205.0	39.1	13	0.13	0.12	7.85	8.67
87-54-1	33.0	53.7	7.24	11.4	265.0	52.7	13	0.15	0.15	6.68	6.75
87-58-2	41.3	48.8	7.32	13.9	348.0	46.9	10	0.11	0.10	9.43	10.58
87-63-2	28.2	48.8	7.30	11.7	308.0	44.5	2	0.14	0.13	7.25	8.00
$\bar{X}$	34.5	49.2	7.31	12.6	281.5	45.8	10	0.13	0.12	7.80	8.50
SD	5.4	3.3	0.05	1.3	61.2	5.6	5	0.02	0.02	1.19	1.60
n	4	4	4	4	4	4	4	4	4	4	4

*PO_2, PCO_2, pH and O_2 content represents values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^0 = pulmonary artery pressure at which flow becomes zero; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship

TABLE F. HEMODYNAMIC RAW DATA DURING VENTILATION WITH 100% O_2*

Animal #	PO_2 (mm Hg)	PCO_2 (mm Hg)	pH	O_2 Content (ml O_2·100 ml blood^{-1})	$\dot{Q}$ (ml·min^{-1})	P^{PA} (mm Hg)	P^0 (mm Hg)	$P/\dot{Q}$ (mm Hg·min·ml^{-1})		$\dot{Q}/P$ (mm Hg·min·ml^{-1})	
								intercept method	regression method	intercept method	regression method
86-109	37.9	38.2	7.45	--	367.0	35.9	4	0.09	0.08	11.51	13.21
87-6	23.4	47.0	7.32	--	345.0	46.6	--	--	0.11	--	9.47
87-19-2	42.7	34.9	7.49	12.9	319.0	50.7	11	0.12	0.09	8.04	11.64
87-27-1	36.2	39.5	7.40	11.6	467.0	34.9	--	--	0.05	--	19.11
87-33	51.8	35.4	7.44	12.1	85.2	35.3	14	0.25	0.17	4.00	6.07
87-45-2	41.3	37.2	7.47	--	865.0	42.7	10	0.04	0.03	26.45	31.91
87-47-1	34.7	35.2	7.45	13.9	247.0	34.1	5	0.12	0.13	8.49	7.84
87-54	32.0	33.7	7.45	12.1	290.0	39.4	8	0.11	0.05	9.24	19.37
87-58-2	35.3	42.7	7.39	12.9	419.0	53.1	14	0.09	0.09	10.72	11.46
87-63-2	26.6	44.9	7.32	11.5	336.0	49.9	2	0.14	0.12	7.02	8.29
$\bar{X}$	36.2	38.9	7.42	12.4	374.0	42.3	8.5	0.12	0.09	10.68	13.84
SD	8.1	4.6	0.06	0.9	201.0	7.3	4.5	0.06	0.04	6.78	7.76
n	10	10	10	7	10	10	8	8	10	8	10

*PO_2, PCO_2, pH and O_2 content represents values from the pulmonary artery; $\dot{Q}$ = left pulmonary artery flow; P^{PA} = pulmonary arterial pressure; P^0 = pulmonary artery pressure at which flow becomes zero; $P/\dot{Q}$ = slope of pressure-flow relationship; $\dot{Q}/P$ = slope of flow-pressure relationship