NORMAL GROWTH OF THE PULMONARY CIRCULATION IN THE PIG AND THE EFFECT OF AORTO-PULMONARY SHUNTS IN IMMATURE ANIMALS: A STRUCTURAL AND FUNCTIONAL STUDY

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This Thesis is dedicated to

MY SON

ABSTRACT

In congenital heart disease, lung structure can be altered directly by the abnormal haemodynamic state but this may also interfere with further development of the pulmonary circulation. Although it has been suggested that immature animal models should be used for the study of these diseases, pulmonary vascular development has not yet been compared in human and animal.

In the present work the structure of the pulmonary vasculature in the pig was investigated, using light microscopy and quantitative morphometric techniques. The study included, in animals from birth to adult life, assessment of the alveolar zone, the arterial and venous circulation and the ventricular weights.

Studies of lung function and pulmonary haemodynamics were also performed in normal swine, from the second week to the fourth month of life.

During postnatal life, the porcine lung followed a similar pattern of growth to the human, but both the structural and functional changes were telescoped into a much shorter period of time.

Experimental aorto-pulmonary shunts were performed in growing swine aged between one and three months. The follow-up period lasted between one and three months, during which time studies of cardio-pulmonary function were performed. After sacrifice, the lungs and

hearts were studied by the same techniques applied to the normal.

The main effects of an increase in pulmonary blood flow and pressure on the immature pig lung were: structurally, a decrease in arterial size, together with an increase in muscularity within the acinar zone shown by increase in the thickness of the arterial muscle coat and of muscle extension abnormally far along the peripheral arterial pathway; functionally, dynamic compliance was decreased and pulmonary arterial pressure and resistance elevated. These changes were more striking in the animals operated early in life.

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INTRODUCTION

THE INVESTIGATION OF THE PULMONARY CIRCULATION

Early Studies

Since the time of Galen (131-201), experiments in animals have helped man to understand the pulmonary circulation. Galen studied the circulation of blood in living animals and was the first to postulate the existence of two types of blood, venous (containing the natural spirits and the waste products of the body) and spiritual (containing the vital spirits). He also assumed that the liver was the site of origin of the venous blood and that the left ventricle was the site of origin of the spiritual blood. According to his theories there were invisible pores in the interventricular septum which allowed the passage of blood from the right to the left ventricle where it was "revitalized" by receiving air coming from the lungs. Galen also admitted the movement of blood from the pulmonary artery to the lungs but his general theory of blood circulation was based on the concept of "ebb and flow" through the various organs of the body. The influence of Galen was considerable, and because of the dogmatic atmosphere of the Middle Age which did not encourage deductive and experimental work, his theories were accepted until the Renaissance.

Leonardo da Vinci (1452-1519) reassumed experiments in animals. He studied the contractility of the heart in the living pig and demonstrated the active contraction of the cardiac musculature as opposed to Galen's theory of passive dilatation.

Although Ibn an-Nafis (1210-1288) admitted that blood circulated from the pulmonary artery to the lungs and returned to the heart, the work of Servetus (1509-1553) regarding the pulmonary circulation, stood as the first movement against the galenic concepts of blood circulation in a Europe dominated by the Inquisition and religious wars. Servetus was essentially a theologian and there is no evidence that he performed experimental work; nevertheless, he wrote that the blood moved from the right ventricle to the lungs and from there to the heart. These and other ideas published in his book "Christianismi Restitutio" led him to be burned for heresy in Geneva in 1553.

The dissection of the human body by Vesalius (1514-1564) and by Columbus (1516-1599) finally established that the interventricular septum was intact and confirmed Servetus's theory.

William Harvey (1578-1657) described the circulation of blood in man and showed the importance of the heart as responsible for the forward movement of blood in arteries and for the venous return. Harvey studied in Italy, namely in Padua, for two years; there he gained the anatomical knowledge of the cardiovascular system which, together with his animal experiments on arterial and venous circulation enabled him to establish the concept of unidirectional blood flow in the body.

Marcello Malpighi (1628-1694), the first experimental physiologist to use the microscope, observed the movement of blood

cells in the systemic capillaries of living frogs and also described pulmonary capillaries in tortoises and frogs after drying the lungs.

In the same century, Boyle (1627-1691) and Hooke (1635-1703) demonstrated in dogs that the inspired air was essential to life. In the late eighteenth century Priestley (1733-1804) isolated oxygen as an element and Lavoisier (1743-1794) stated that during the passage of blood through the lungs a combustion occurred, with oxygen being consumed and carbon dioxide released. This phenomenon produced heat in the lungs which was distributed to the whole body by the veins.

Although this was false, Lavoisier's work was very important for two reasons: (i) it pointed out the relationship between the respiration and the pulmonary circulation; (ii) it stimulated other investigators to test the validity of his theories. In order to do so, Gustav Magnus (1802-1870), Gay-Lussac (1778-1804) and Magendie (1783-1885) had to develop techniques for measuring blood temperatures and the relative concentrations of oxygen and carbon dioxide in arteries and veins. Their work on the other hand, stimulated Claude Bernard (1813-1878) to perform the first cardiac catheterization using intact horses and dogs to measure the temperature and pressure of the blood in each ventricular cavity. During the same period Carl Ludwig (1816-1895) measured the pulmonary arterial pressure in a dog with the chest opened.

Stephen Hales (1677-1761) was the first to measure arterial and venous pressures in intact horses using peripheral vessels and

not cardiac catheterization. In 1861, Chaveau and Marey assessed intra-ventricular pressures in intact dogs and horses and correlated these measurements with the cardiac apex beat. The first measurements of cardiac output using the method described by Adolph Fick (1829-1901) were performed in dogs, sixteen years later, by Grehant and Quinquaud (1886). The simultaneous measurement of both pulmonary artery and aortic pressures, together with the assessment of the cardiac output and of the composition of both arterial and venous blood gases was reported two years later, by Nathan Zuntz (1847-1920) in horses.

Not until the twentieth century were attempts made to develop similar studies in the human. Forssman (1929) performed the first catheterization of the right atrium in himself, while pulmonary angiography was introduced by the Portuguese Egas Moniz, Lopo de Carvalho and Almeida Lima (1931), by catheterizing the right atrium and injecting solutions of sodium iodide.

Between 1939 and 1945 Andre Cournand, Dickinson Richards, Robert Darling and others, working at Columbia University, systematically developed cardiac catheterization in the human, both in health and disease and introduced the new diagnostic procedure to clinical practice. Cournand wrote recently about cardiac catheterization (1975): "these lines of progress opened new fields of investigation of cardiac function and systemic and pulmonary circulation in health and disease. In the entire development the clinical physiologist did not always play second fiddle to the

experimental physiologist. Our original plan was indeed fulfilled, but with far more ramifications than we had dreamed of at the time of its conception". (sic).

Structure of the Pulmonary Vascular Bed in the Adult Lung

Human Studies

The first overall study of the pulmonary vascular bed was performed by Brenner in 1935 using uninflated and uninjected lungs. He assessed the vascular structure from the main pulmonary arteries and veins to the capillaries and measured the thickness of the media at various levels and expressed this as a percentage of the "total vascular diameter", but gave no information on the actual limits of the media wall. By comparing the aorta with the pulmonary artery, he showed that the pulmonary artery had a more irregular structure with the elastic laminae scanty and widely spaced than the aorta in the media. Inside the lung and until the vessels reached 1 mm in diameter the arterial structure was similar to that of the main vessels with the number of elastic laminae ranging between 16-20 in arteries 5-6 mm in diameter and 3-4 in those of 1 mm. The smaller vessels, between 0.1-1 mm had a muscular media bounded by two elastic laminae. Arterioles and venules were similar in structure, with a thin muscular wall, whereas in the pre-capillary vessels a single elastic lamina was found, which seemed to be continuous with the external elastic lamina. The veins were thinner than arteries over any size range, particularly

in vessels smaller than 1 mm in diameter. In the larger veins the media was irregularly organized and contained elastic and connective tissue.

The structure of the pulmonary vascular bed in man was further described by Wagenvoort, Heath and Edwards (1964). Again using lungs with uninjected vasculature, they confirmed Brenner's finding (1935) that all the arteries with an external diameter exceeding 1 mm were elastic and stated that vessels between 1 mm and 500 µm were transitional with a progressive loss of the elastic tissue in the media until finally the media became solely muscular, with the external and internal elastic laminae as boundaries. The muscular arteries became arterioles by a slow loss of the muscular cells, a complete muscular coat being found in arteries 70 µm in external diameter. The veins also acquired a muscular layer when their external diameter was 80 µm. Over any size range the venous wall contained more elastic and connective tissue than the arterial one, particularly in the larger vessels.

Elliott (1964) injected the pulmonary arterial bed using a barium sulphate and gelatin mixture at a constant pressure of 100 cm of water. He studied not only the arterial wall structure, represented by the medial thickness expressed as a percentage of the external diameter, but from serial sections he also related the structure of the pulmonary arteries to their position within the lung. He described three structural types of pulmonary arteries: (i) elastic, with more than seven continuous elastic laminae, running around the media; (ii) muscular, with a defined internal and external

elastic laminae and a predominant muscular media; (iii) transitional, with an intermediate structure between the two above mentioned types. In this study it was also shown that the muscular arteries ranged between 2000 and 30 μm in diameter, suggesting a larger muscular segment than previously reported. Along any arterial pathway there was a point where the completely muscular coat gave way to a spiral of muscle (partially muscular) before it disappeared (non-muscular). The arterial size at which these changes occurred varied, so that at any diameter below 150 μm the arterial population was mixed. The study of the arterial branching pattern showed that the number of arterial branches outnumbered that of the bronchial generations; these extra-arteries were named "supernumerary".

Singhal, Henderson, Horsfield, Harding and Cumming (1973) performed measurements of arterial length and diameter which were related to the arterial branching order. Their study was based on a vascular cast from one human lung injected with a synthetic resin mixture at a constant pressure of 25 cm of water. All the arteries down to 0.8 mm in diameter were measured whereas the values for branches less than 0.1 mm were taken from the literature. These data were used to deduce cross-sectional and total vascular area and volume for each branching order of arteries.

Animal Studies

McLaughlin, Tyler and Canada (1961) studied the broncho-vascular branching pattern in various mammalian species using corrosion casts. From their work three types of branching patterns

were described: (i) Type I, in the cow, sheep and pig, in which the bronchial pathway and the arterial and venous beds were closely related throughout their intrapulmonary course from the hilum to the periphery; (ii) Type II, in the monkey, dog and cat, in which the pulmonary veins were not related to the broncho-arterial bundle neither at the hilum nor at the lung periphery; (iii) Type III, in the horse, in which the venous branching pattern was similar to the Type II at the hilum and to the Type I at the periphery.

Best and Heath (1961) studied arterial and venous wall structure in animals by measuring the thickness of the media in vessels smaller than 300 μm in external diameter. Several species were included: monkey, dog, fox, goat, mouse, rabbit, rat, sheep, seal, pig, cow, cat, civet and squirrel. Their findings suggested that there was more arterial muscle in animals than in the human, particularly in the smaller vessels down to a diameter of 20 μm. The veins of the rat, squirrel and mouse were described as having striated cardiac muscle in their larger branches; on the other hand, the pig and the cow showed thick walled veins with irregular fibromuscular prominences.

Alexander (1962) studied the structure of the pulmonary vascular bed in the bovine lung and confirmed the existence of a distinct muscular media in arteries down to a diameter of 20 μm. This was also described in the rat by Kay and Heath (1969), who assessed arterial wall structure in vessels ranging between 20-350 μm in external diameter.

Studies of the pulmonary vasculature in primates have recently been performed by Jones (1969) in the vervet monkey, by Heath, Jones and Housley (1970) in the patas monkey and by Smith, Heath, Wright and McKendrick (1973) in the baboon. Their findings, based on the thickness of the pulmonary trunk and of the muscular arteries showed that the vervet monkey had higher values for the arterial wall thickness than the other two species and man.

All the above mentioned studies used uninjected lungs and in most the airways were not inflated.

The pulmonary arterial bed of the rat lung has recently been studied in detail, using both light and electron microscopy (Hislop and Reid, 1976), (Meyrick, 1976), (Meyrick, Hislop and Reid, 1976), (Meyrick and Reid, 1976). The arteries were injected using the method described by Elliott (1964). According to this work the arterial branching pattern of the rat lung showed similarities to the human, particularly in the existence of supernumerary arteries. In the rat the main pulmonary artery was muscular with 1-3 elastic laminae in the media; a similar structure was maintained along two-thirds of the pathway until the vessels were around 1000 μm in external diameter. Smaller vessels showed a muscular media but at the end of the pathway, and like the human (Elliott, 1964), a mixed population of muscular, partially muscular and non-muscular arteries was also found. The percentage of arterial wall thickness also was similar to that described in the human (Reid, 1967) if only the arteries containing circular muscle fibers were included.

This finding was emphasized by Meyrick, Hislop and Reid (1976), who showed that the rat lung contains within the arterial muscular region of any pathway, an arterial segment of thick muscle orientated obliquely. They suggested that measurements of wall thickness from the above mentioned segment would have caused the initial reports on the higher wall thickness of the arterial bed in the rat when compared to the human.

STRUCTURAL DEVELOPMENT OF THE LUNG

Antenatal

Airways and Alveoli

The structure of the fetal lung was not studied until the nineteenth century and for a long period was mainly concerned with airway development.

According to Flint (1906-1907) the first description of the fetal lung was that of von Baer (1828) in the chick embryo. Fifty one years later, Kölliker (1879) using the rabbit admitted that the lung developed by a process of centrifugal budding during fetal life, the total number of terminal airways units being achieved before birth.

The first reports on the development of the human lung were by His (1887) who described the right and left stem bronchus as well

as their further division by dichotomy to form the distal airways. He also stated that the bronchial tree was asymmetric from early in its development.

Flint (1906-1907) studied the development of the lung of the pig and showed that the extra-lobular airways and vessels were present before birth when the embryo was 200-230 mm long (length at term -290 mm).

In the human embryo the presence of lobar bronchi was noted by Heiss (1919) and confirmed by Boyden (1955) who demonstrated that in the human, subsegmental bronchi were present at 40 days.

Bucher and Reid (1961) showed that the number of bronchial generations in the human was established by the sixteenth week of fetal life and that it was similar to the adult number of bronchial generations (Hayward and Reid, 1952).

The development of the terminal airways during fetal life was studied by Palmer in the human (1936), and, in the pig, by Flint (1906-1907) and by Clements (1937-1938). Although all these workers mentioned that the areas where respiration (gas exchange) would take place were not present during fetal life, their description of the epithelial lining of the lung was conflicting, because Flint admitted the existence of "non-nucleated plated" and Clements and Palmer suggested that the respiratory epithelium was progressively disrupted due to the capillary growth. This last hypothesis was further developed by Policard (1926) who

admitted that the alveolar epithelium was never present and by Short (1950) who proposed that there was a period of epithelial desquamation during fetal lung development. The use of the electron microscope solved this controversy by showing a continuous epithelial layer throughout the rat adult lung (Low, 1952). Similar studies during fetal life, both in the human (Campiche, Gautier, Hernandez and Raymond, 1963), in the mouse (Buckingham and Avery, 1962), in the sheep (Kikkawa, Motoyama and Cook, 1965) and in the rabbit (Kikkawa, Motoyama and Gluck, 1969), (Reid and Meyrick, 1969) showed that the various lung structures including the epithelial cells were present before birth.

Dubreuil, Lacoste and Raymond (1936) divided the development of the fetal lung into several periods: a glandular phase until seven months, during which the airways developed and branched; a canalicular period, from seven months until birth, when capillaries appeared and an alveolar phase occurring after birth, when definitive alveoli were formed.

Loosli and Potter (1951) also studied the antenatal development of the lung and their observations were in accordance with those of Dubreuil, Lacoste and Raymond (1936), although they suggested a different time sequence: the glandular period until the fourth month of intra-uterine life; the canalicular period between four and six months of fetal age, and the alveolar period after the sixth month of intra-uterine life.

The Commission on Embryological Terminology (1970) has recently suggested the following definitions to describe the various stages of fetal lung development: 1. embryonic period - the first five weeks after ovulation; 2. pseudoglandular period - from five to seventeen weeks; 3. canalicular period - from thirteen to twenty five weeks (Boyden) or from sixteen to twenty four weeks (Loosli and Potter); 4. terminal sac period - until term.

Hislop and Reid (1974) described the development of the acinus in the human lung reviewing their own material and Boyden's waxplate reconstructions of the human acinus. They showed that the intra-acinar airways were present before birth, the respiratory bronchioli by the nineteenth week of intra-uterine life; the alveolar ducts and saccules by the twentieth week; and that alveoli described as such did not appear until after birth.

Arteries

Although Flint (1906-1907) had mentioned the similarities between bronchial and arterial antenatal development in the pig, the human pulmonary arterial development was first described by Congdon (1922) who, using the Born wax plate method of reconstruction, showed that the adult branching pattern was already established by the fifth day of intra-uterine life. Heath, Wood, DuShane and Edwards (1959) studied the pulmonary trunk in normal human fetus throughout gestation and showed that it was similar to the aorta, both in structure and wall thickness.

The structure of the muscular pulmonary arteries in the fetal lung was assessed by several workers. Civin and Edwards (1951) using uninjected material noted the extremely narrow lumen of the pulmonary arteries in the human fetus. O'Neal, Ahlvin, Bauer and Thomas (1957), also from uninjected material, reported a progressive increase in smooth muscle within the arterial wall during the last months of fetal life. A similar finding was reported by Naeye (1961), who used planimetry to compare in a growing artery the area of its muscle with that of its intima. Wagenvoort, Neufeld and Edwards (1961) still in uninjected material, showed that this increase in wall muscle during late fetal life was due to lengthening of the muscular coat along the peripheral arteries because mean medial thickness when expressed as a percentage of the external diameter did not change during that period.

In 1972, Hislop and Reid published their work on the pulmonary arterial development of the human fetus, using lungs injected at a constant pressure and applying precise quantitative morphometric techniques. They showed that the pre-acinar arterial branching pattern was established half-way through fetal life and followed that of the bronchi. The structure of the pre-acinar arteries was also established at the same time and did not differ from that of the adult. However, as in the adult lung (Elliott and Reid, 1965), there were "extra" or "supernumerary arteries" along the pathway which, unlike the "conventional arteries", did not accompany airways; the ratio of "conventional" to "supernumerary" arteries did not change during fetal life. They also demonstrated that arterial wall thickness was greater in the fetus than in the adult

or child but after twelve weeks found no change in the wall thickness between the various gestational ages. Inside the acinar zone they showed that throughout fetal life, the arteries lacked a muscular coat.

Levin, Rudolph, Heymann and Phibbs (1976) studied the pulmonary vascular development of the fetal lamb using injected specimens and quantitative morphometric techniques. They were able to demonstrate an increase in the total number of small arteries per unit volume of lung tissue with increasing gestational age. These results, which suggested an increase in the capacity of the arterial bed, helped to explain why pulmonary arterial pressure and resistance decreased during late fetal life in the lamb, at a time when pulmonary blood flow was known to increase (Rudolph, 1974).

Veins

Ader (1948), Butler (1952) and Neil (1956) described the origin of the pulmonary veins from the splanchnic plexus and their drainage directly to the heart. Patten (1958) emphasized the independence of the pulmonary veins from other systemic venous channels and attributed this to the fact that the lungs were phylogenetically relatively new structures.

Wagenvoort, Heath and Edwards (1964) considered that human pulmonary venous structure did not change during fetal life and was similar to that seen in the adult lung.

In 1973, Hislop and Reid described the fetal development of the pulmonary veins in the human. Not only did they confirm that the vein wall structure did not change throughout fetal life, but also they were able to show that like the arteries and the airways, the pre-acinar venous branching pattern was completed long before birth.

Postnatal

Airways and Alveoli

Broman (1923) reported that in the middle lobe of the human infant the number of airways, including those which contained alveoli, was similar to that counted in the corresponding lobe of an adult lung before reaching the alveolized pathways; this observation suggested that the pre-acinar airways increased in number during postnatal life.

Bucher and Reid (1961) studied the pre-acinar airways in most segments of the human lung during fetal life, using bronchial axial pathways which are those directed to the distal pleural surface, and showed that the adult number of bronchial generations as determined by Hayward and Reid (1952) was already established by the sixteenth week of intra-uterine age. Cudmore, Emery and Mithal (1962) confirmed this finding using casts of the right upper lobe; they also suggested that the increase in size was faster in the proximal than in the distal airways.

Hislop, Muir, Jacobsen, Simon and Reid (1972) studied the dimensions of selected pre-acinar airways during growth and concluded that the non-respiratory zone of the newborn lung was similar to the adult and that the increase in length and diameter of the axial pathways was symmetrical, each individual branch bearing a constant relationship with the whole throughout life.

Willson (1928) working on both mice and human lungs suggested that alveolar multiplication occurred during postnatal life but he also described an increase in the number of the non-respiratory airways between birth and adult life. Similarly, Bremer (1935) suggested that in the rabbit lung the respiratory airways were converted into non-respiratory airways during postnatal growth.

Engel in 1947 suggested that the infant lung was different from the adult lung and that new acini were formed after birth. Boyden and Tompsett (1961)(1965) studied the development of the respiratory airways in the dog and in the human lung, and contrary to Bremer, described, particularly in the dog, a substantial reduction in the number of the non-respiratory generation during postnatal life. They admitted that this was due to the transformation of the terminal bronchioli in respiratory bronchioli which in turn, also decreased, becoming alveolar ducts.

The work by Hislop and Reid (1974) on the development of the human acinus, in which they reviewed both their own material and Boyden's results showed that between birth and seven years of life

the number of airway generation transformed to respiratory bronchioli was about two and that during childhood alveolar ducts were formed from the alveolar saccules and from one generation of respiratory bronchioli either by division or by alveolization.

Alveolar multiplication has been suggested to occur mainly during postnatal life since the work of Willson in 1928. Emery and Mithal (1960) using the alveolar radial counts in a large series of children's lungs showed that the number of alveoli increased rapidly during the first year of life and more steadly after that period. Dunnill (1962) using the quantitative morphometric techniques described by Weibel and Gomez (1962), found a five fold increase on the total number of alveoli during infancy and stated that alveolar multiplication ceased by the eight year of life. Similar results were obtained by Davies and Reid (1970) and by Hislop and Reid (1972) although their absolute numbers were slightly higher (10%) than those reported by Dunnill. Angus and Thurlbeck (1972) and Thurlbeck and Angus (1975) showed that in both the adult human and during postnatal life there was a wider range of variation in the total number of alveoli per lung than had been previously reported. This was attributed both to individual variation and to the complexity of the measuring technique. While they confirmed that alveolar multiplication occurred in the human lung throughout childhood, they considered it might continue until somatic growth stopped.

In the rat, Burri, Dbaly and Weibel (1974) showed that

between the fourth and the thirteenth day of postnatal life, new alveoli were formed causing a 66% increase of the alveolar surface area.

Arteries

Human studies

The important role played by the pulmonary circulation in congenital heart disease was the stimulus to various workers to study the normal pulmonary arterial bed during postnatal life (Edwards, 1950). Heath, DuShane, Wood and Edwards (1959) compared the structure and the thickness of the main pulmonary trunk with that of the ascending aorta during postnatal life. They described a fetal period until the sixth month after birth during which the pulmonary artery wall thickness and structure did not change considerably although some fragmentation of the medial elastic laminae occurred. This phase was followed by the transitional period, between six months and two years of age, during which there was a relative decrease in wall thickness of the pulmonary artery trunk when compared to the aorta. During this period there was also considerable fragmentation of the elastic laminae in the media of the pulmonary artery, the adult pattern being reached by the end of the second year of life.

Saldaña and Arias-Stella (1936) performed similar studies to the ones mentioned above and considered that although the adult structure of the pulmonary artery was reached around the first

year of life in most humans, some had a slower process of change with the adult pattern being only established around nine years of age.

Civin and Edwards (1951) described a considerable decrease in arterial wall thickness of the intra-pulmonary vessels during early infancy, the adult structure being established by the age of six months. Damman and Ferencz (1956) quantitated the structural changes in the pulmonary arteries by determining the ratio of lumen size to wall thickness, obtained by dividing the diameter of the vessel lumen by twice the thickness of the media and intima added together; they showed a decrease in wall thickness between birth and four weeks of age with further thinning during the first two years of life. Rosen, Bowden and Uchida (1957) studied the structure of the pulmonary arteries in infants, using the ratio of internal diameter (lumen and intima) to external diameter (lumen, intima and media) and found the lumen significantly larger in the cases older than one month of age, when compared to the younger ones.

Naeye (1961) used planimetry to determine the cross-sectional area of the media and compared it with that of the intima. This technique allowed him to demonstrate a decrease in the amount of muscle in individual arteries during the first two weeks of life. Wagenvoort, Neufeld and Edwards (1961) measured the "relative medial wall thickness" expressed as the percentage of the shortest diameter. They described a rapid decrease in wall thickness between birth and the second week of life and a slower reduction until eighteen months,

when the adult values were reached. All the studies that we have mentioned so far used uninjected specimens and while concerned with the structure of pulmonary arteries did not relate this to the position within the lung.

Libi-Sylora, Greco and Ferencz (1968) assessed pulmonary arterial growth in the human lung at different ages. Post-mortem pulmonary arteriograms were obtained after distension of the lungs with air and injection of the arterial tree by Lipiodol at a low pressure, just sufficient to fill the vessels. No further information was given regarding the injection procedure but a good filling of the peripheral arteries was obtained. For the histological study fixation was achieved by injecting 10% formalin solution into the arterial and bronchial trees. From the assessment of arterial length, size and structure they distinguished two vascular segments in the lung, the "capacitance" containing the elastic arteries and the "resistance" containing the muscular arteries. It was also suggested that the postnatal increase in arterial length and diameter occurred mainly in the "capacitance" segment, the "resistance" one maintaining similar dimensions throughout growth.

Davies and Reid (1970) studied arterial development in the human lung using detailed quantitative techniques after previous injection of the vascular bed with barium sulphate and gelatin mixture at a constant pressure (Elliott, 1964), (Elliott and Reid, 1965). They showed that the arterial wall thickness decreased with age, the adult values being reached at four months. The number of

the small intra-acinar arteries, less than 200 μm, increased significantly between four months and three years during the period of alveolar multiplication. During childhood and adolescence muscle extended gradually along the intra-acinar arteries, the adult pattern being established by the nineteenth year of life. The weight of the right ventricle was also measured throughout growth and compared with that of the left ventricle plus septum. These measurements showed that the relative "dominance" in weight of the right ventricle at birth, decreased during postnatal life and that the adult pattern of "left ventricular dominance" was found at 10 months of age. Hislop and Reid (1973) extended this work using similar techniques and described the arterial changes occurring mainly at intralobular and intra-acinar level. In this lung zone, arterial branching pattern and dimensions increased with age and, contrary to what was seen at pre-acinar level, the intra-acinar "conventional" and "supernumerary" arteries increased in number until the age of eight years, particularly the "supernumerary" ones. By relating arterial wall thickness to external diameter they showed that, when related to size, the drop in wall thickness was not uniform during growth. Immediately after birth the wall thickness of the arteries less than 200 μm in external diameter dropped so that the adult levels were demonstrated at three days, while similar values were reached only by four months of age in the larger vessels.

Animal studies

The earliest report on the structure of the pulmonary arteries in immature animals was by Ferguson, Berkas and Varco (1953) who used

the lumen to wall ratio, measured from internal to external lamina, to assess the structure of small arteries in puppies three to four months old. They found an arterial wall ratio of 3.5-9.0. A few years later, Campbell (1959) reported that in the puppy there was an abrupt decrease in medial wall thickness apparent by microscopy, after the fifth day of life. Philips, DeWeese, Manning and Mahoney (1960) studied the arterial lumen to wall ratio in puppies aged between one day and thirteen weeks. They found a gradual decrease in the arterial ratio until the end of the first month, when the adult values were reached. Wagenvoort and Wagenvoort (1969) also studied the pulmonary vasculature in cattle at different ages, from fetal to adult life. They described an immediate decrease in medial wall thickness, expressed as a percentage of the external diameter, by the first day of life; this was followed by a more gradual decrease during the first year. Friedli, Kent and Kidd (1975) assessed medial arterial wall thickness (Wagenvoort, Heath and Edwards, 1964) in growing pigs aged one to eleven months and reported no changes during that period. Although their series did not include newborns, they claimed that there was no postnatal drop in arterial wall thickness in the pig lung. All the studies that are mentioned in this paragraph used uninjected lungs.

Reeves and Leathers (1968) injected the pulmonary arterial bed in calves aged one day, two weeks, two and five months using a micropaque and gelatin mixture. The lungs were examined combining macro- and micro-angiographic techniques with a non-quantitative histological assessment. They reported a better filling of the small

arteries in the two weeks old animals, when compared to the newborns, and suggested that this was due to postnatal arterial multiplication.

Ferencz (1969) used the method described in the human by Libi-Sylora, Greco and Ferencz (1968), to study the postnatal changes in the pulmonary vasculature in various mammalian species. In each case she compared a newborn and an adult lung. Two types of "arterial design" were described: (i) Type A - deer, monkey, dog, cat and mouse, in which a correlation was found between structure and size, the muscular arteries having a similar external diameter in all species; (ii) Type B - rabbit, guinea-pig and rat, in which no correlation was found between arterial size and structure due to the wide variation in external diameter over any structural type. As in the human it was possible to distinguish the "capacitance" and the "resistance" segments, but only in Type A was the pattern of growth similar to the human.

A different approach to postnatal lung development was used by Weibel in the rat (1967). Based on a physical model of the gas exchange area and using morphometric techniques he showed that during growth, the pulmonary gas exchange area increases in proportion to body weight. This work was recently completed by Burri, Dbaly and Weibel (1974) and by Burri (1975) who described three phases of development in the rat lung: (i) lung expansion (between birth and four days); (ii) tissue proliferation (between four and thirteen days) during which there was a relative increase in alveolar

surface area and capillary volume; (iii) equilibrated growth (from two weeks till adult life).

Veins

Damman and Ferencz (1956) described the pulmonary veins in the newborn as being thin walled but did not measure vein wall thickness. Wagenvoort, Heath and Edwards (1964) mentioned that the structure of the venous wall did not change during postnatal life.

Hislop and Reid (1973 b) studied the branching pattern and structure of the pulmonary venous bed in the human lung during childhood. They found that vein branches, like the arteries, could be divided into "conventional" and "supernumerary", when considered in relation to the bronchial generations. Throughout growth, there were more supernumerary veins than arteries both in the pre- and intra-acinar regions. Inside the acinus, there was new growth of veins during childhood. On the other hand, vein wall thickness did not change between birth and the tenth year of life.

Laws of Human Lung Development

The pattern of growth of the human lung was summarized in The Laws of Human Lung Development presented by Reid (1967 a) and modified by Hislop and Reid (1973 c)(1974 b):

1. The bronchial tree is developed by the sixteenth week of

intra-uterine life;

2. Alveoli develop after birth, increasing in number until the age of eight years and in size until growth of the chest wall finishes with adulthood;

3. The pre-acinar vessels, arteries and veins, follow the pre-acinar development of the airways; the intra-acinar that of the alveoli. Muscularization of the intra-acinar arteries does not keep pace with the arrival of new arteries.

POSTNATAL PULMONARY PHYSIOLOGY

During the first postnatal ~~weeks~~ days respiration and circulation are rapidly adapted to extra-uterine life (Dawes, 1968). The onset of breathing, the loss of the placenta and later, the closure of the ductus arteriosus and of the foramen ovale, all call for rapid modification of the circulation and respiration. Thus, during the neonatal period, it is difficult to separate the changes due to new environmental conditions from those that represent the beginning of postnatal growth and development.

Respiratory Physiology

Human Studies

Cook, Helliesen and Agathon (1958) have demonstrated that from birth till adulthood, dynamic compliance is relatively constant

when related to functional residual capacity. Thus the ratio between these two variables, termed as the specific compliance, remained unchanged throughout growth. This was confirmed by Godfrey (1974) who reviewed the literature on pulmonary physiology during infancy, childhood and adolescence. According to Polgar (1974) functional residual capacity gave the best functional assessment of overall lung growth. Because of this we shall concentrate here on studies of both dynamic compliance and functional residual capacity throughout growth.

The first measurements of lung function in infants were performed by Cross (1949) and by Cross and Oppé (1952), who measured respiratory frequency, tidal volume and minute ventilation at birth.

McIlroy and Tomlinson (1955) reported the first measurements of lung mechanics in infancy using an esophageal balloon. However, the first large series was published by Cook, Sutherland, Segal, Cherry, Mead, McIlroy and Smith (1957). The value they reported for dynamic compliance during the first week of life, 5.2 ml/cmH_2O, was later confirmed by other workers (Chu, Dawson, Klaus and Sweet, 1964), (Polgar and String, 1966), (Howlett, 1972); from the above mentioned studies the dynamic compliance during the neonatal period is found to range between 5.2 and 8.8 ml/cmH_2O.

Auld, Nelson, Cherry, Rudolph and Smith (1963) reported the first measurements of thoracic gas volume in neonates and their value of 85 ml was later confirmed by Chu, Dawson, Klaus and Sweet

(1964), Howlett (1972) and Radford (1974). According to all these studies thoracic gas volume in neonates ranged between 85-140 ml.

Studies of dynamic compliance during the first year of life were reported by Krieger (1963), Phelan and Williams (1969), Doershuk, Downs, Mattews and Lough (1970) and by Ahlström and Jonson (1974 a and b). According to this last study, the most detailed and complete of the series, dynamic compliance in the first year of life ranged between 4.2 ml/cmH_2O (body height, 40 cm) and 9.3 ml/cmH_2O (body height, 75 cm) a significant linear correlation being found between the two variables. Phelan and Williams (1969) reported higher values for dynamic compliance in infancy, up to .13 ml/cmH_2O, but their series included longer infants.

Measurements of functional residual capacity during infancy were performed by Phelan and Williams (1969), Howlett (1972) and by Radford (1974). They also found that there was a significant correlation between changes in functional residual capacity and age throughout infancy. When related to body weight, functional residual capacity ranged between 30 ml/kg and 35.5 ml/kg.

Throughout infancy and childhood there was an increase in both dynamic compliance and functional residual capacity. Height has been accepted as the best of the body measurements to which to relate the changes in lung function with growth. These relationships are represented either by linear (Polgar and Promadhat, 1971) or exponential functions (Godfrey, 1974).

Polgar and Promadhat (1971) have further shown, by reviewing the literature from 1958 to 1969, that the measurements of dynamic compliance (x) and functional residual capacity (y) in boys and girls from four to nineteen years of age, could be related by a linear equation $y = 17.0945x + 0.0459$.

Animal Studies

Avery and Cook (1961) measured static compliance in goats, from birth to adulthood. They reported a significant correlation between static compliance and both age and body weight throughout the growing period. In rats, Joanson and Pierce (1973) also found a significant correlation between functional residual capacity and either age or body weight between the fourth and eighteenth month of life.

Pulmonary Haemodynamics

Patten (1930) measured the weight and volume of the ventricles and the size of the various vascular orifices in fetal and neonatal hearts. According to his findings "...the idea that the pulmonary blood flow is negligible before birth and rises abruptly to full power with the beginning of respiration is, as far as a diligent search of the literature reveals, pure dogma". (sic). This statement has been confirmed by structural and functional studies both in the human and the lamb, which showed that the transition from the fetal

regime of "low flow - high pressure" to the adult regime of "high flow - low pressure" was a slow process, initiated before birth, with rapid changes in the neonatal period, and continuing throughout infancy and childhood (Davies and Reid, 1970), (Rudolph, 1970), (Hislop and Reid, 1972, 1973 a, b and c), (Rudolph and Heymann, 1974), (Levin, Rudolph, Heymann and Phibbs, 1976).

Human Studies

The early studies of the human neonatal circulation were mainly concerned with the closure of the ductus arteriosus. Keith and Forsyth (1950) and Lind and Wegelius (1954) performed angiograms in neonates, as early as 12 hours after birth, and reported that no contrast entered the ductus from the aorta. Eldridge and Hultgren (1955) however, measured hand-foot oxygen saturations in a series of sixty infants and found a considerable veno-arterial shunt at ductal level until 72 hours of life. These findings were confirmed by Adams and Lind (1957) who reported the first cardiac catheterization studies in a series of eight normal newborn infants ranging in age between seven hours and fourteen days. Not only were they able to show a considerable left-to-right shunt through the ductus after the third day of life, but they also demonstrated that the pressure adjustments occurred gradually after birth, a moderate degree of pulmonary hypertension being found in the first days of life.

James and Rowe (1957) published the first report on pulmonary arterial pressure measurements in normal humans during the first year

of life. They stated that the adult levels of pressure were reached within the first two weeks.

Serial measurements of pulmonary arterial pressure and resistance were reported by Lucas, St. Geme, Anderson, Adams and Ferguson (1961) in infants and children aged between twenty one days and sixteen years. This group suggested a much longer period of maturation for the pulmonary circulation because they found that total pulmonary vascular resistance did not fall to the normal adult level until four years of age. This fall in resistance with increasing age correlated with their histological assessment of the arterial wall thickness/lumen diameter which also reached the adult value at the same age.

Krovetz and Goldbloom (1972 a and b) reviewed the data from the literature on cardiovascular function in normal humans aged between one month and twenty years. They concluded that no significant change in pulmonary arterial pressure occurred during that period. Total pulmonary vascular resistance decreased with age and the relationship between both variables could be expressed by an exponential curve. However, they draw attention to the fact that this change depended solely on cardiac output which increased with body growth (Cayler, Rudolph and Nadas, 1963), while pulmonary pressure remained constant. This finding made the assessment of "functional" pulmonary vascular maturation difficult to define, especially if based only on the changes in pulmonary vascular resistance with age.

Rudolph (1974) has recently suggested that the gradual decrease in pulmonary vascular resistance seen in the human during the first 2-3 years of life was due to structural changes occurring in the intra-acinar zone after birth with an increase in number of both alveoli and arteries (Davies and Reid, 1970), (Hislop and Reid, 1973).

Animal Studies

The first functional studies on neonatal circulation were performed in lambs. Using angiographic techniques Barcklay, Franklin and Prichard (1944) analysed the patterns of blood flow inside the heart cavities immediately after birth. This work was extended by Dawes and his group (1953-1968) using lambs and rhesus monkeys. The haemodynamic studies showed that, immediately after birth, there was an abrupt decrease in pulmonary arterial pressure and resistance accompanied by a great increase in pulmonary blood flow. The ductus arteriosus did not close immediately after birth however, and the adult pulmonary arterial pressure values did not occur until several days after birth.

By taking direct pulmonary artery pressure in thoracotomized puppies, aged between one day and thirteen weeks, Phillips, DeWeese, Manning and Mahoney (1960) demonstrated that the adult values were reached by the end of the first week of life. However, the adult ratio of mean pulmonary to mean systemic arterial pressure was only reached by the first month. This finding was related to the lumen

diameter to arterial wall thickness ratio which also reached the adult value by the first month of life.

Rudolph, Auld, Golinko and Paul (1961) performed cardiac catheterizations in puppies and goats aged between one and thirty six days. In puppies, the systolic pulmonary pressure decreased rapidly during the first five to ten days of life, whereas in the goat it took until the end of the second week.

Reeves and Leathers (1964) performed cardiac catheterizations in calves between birth and eight weeks of age and measured pulmonary and systemic pressures together with pulmonary flows, using the Fick method. They found a rapid decrease in pulmonary arterial pressure between two and twelve hours of life, followed by a slower reduction up to the age of two weeks when the adult value was reached. From their data it was possible to show that total pulmonary vascular resistance fell progressively during the first eight weeks of life in the calf.

Friedli, Kent and Kidd (1975) studied pulmonary haemodynamics in pigs aged between one and nine months. According to their work neither pulmonary arterial pressure nor resistance changed significantly in the pig during that period of life.

THE HEART DURING POSTNATAL LIFE - VENTRICULAR CHANGES

The first report on heart changes during growth was by Müller in 1883 who assessed ventricular weights in human hearts at various ages and showed that the rate of increase in left ventricular weight dominated that of the right ventricle during postnatal life.

Patten (1930) performed similar measurements and related the right ventricular preponderance in weight at birth to the pattern of right ventricular dominance found in the electrocardiogram during the neonatal period. He also showed that during the first five months of life, left ventricular weight increased to equalise that of the right ventricle, the adult left ventricular preponderance being established by the second year of life. According to his work these relative changes in the ventricular musculature were due to the establishment of the postnatal circulation with closure of the ductus and foramen ovale and an increased loading of the left ventricle during growth.

Keen (1955) reviewed Müller's cases and compared them with his own findings. He assessed free wall ventricular weights and also the ratio between left ventricular plus septal weight and right ventricular free wall weight. He found that both ventricles had a similar weight at birth, although in some cases the right ventricle was heavier than the left. The postnatal changes occurred mostly during the first month of life and the left ventricular preponderance in weight was apparent soon after birth. The adult proportions of ventricular weights were established around the age of six months.

Recavarren and Arias-Stella (1964) used the method described by Herrmann and Wilson (1922). They studied human hearts from birth to maturity by comparing the weights of ventricular slices. According to their findings the adult ratio of left to right ventricular weight was established by the fourth month of life.

Recently, Davies and Reid (1970) assessed ventricular weights in infancy and childhood using the method described by Fulton, Hutchinson and Jones (1952), who had suggested that the septum should be included with the left ventricle. They found the postnatal decrease in relative right ventricular mass continued until the tenth month of life, when the adult ratio between the left ventricle plus septum weights and the right ventricle weight was also reached. These right ventricular changes were related to the gradual decrease in wall thickness of the pulmonary arteries, which also reach the adult values at about the same time (Hislop and Reid, 1972).

A similar decrease in the right ventricular dominance after birth was described in the rabbit (Latimer and Sawin, 1960) and in the dog (Averill, Wagner and Vogel, 1963). More recently Lee, Taylor and Downing (1975) assessed ventricular weights in seven species - sheep, swine, dogs, cats, rabbits, guinea pigs and rats. They reported that within the same species the ratio between the mass of right ventricular free wall and that of the left ventricle, was always significantly higher in the newborn than in the adult. They also found considerable differences between species regarding right and left ventricular weights at birth. These values ranged

from 86-100% (right ventricular weight expressed as a percentage of the left ventricle) in dogs, rabbits and rats, to 55-77% in sheep, swine, cats and guinea pigs.

Ultrastructurally Zak (1973) has shown that cardiac development occurred after birth, with enlargment of the muscle cells which became progressively organized in a parallel fashion and an increase in the number of the transverse intercalated discs. The work by Boerthe (1972) in the cat and of Hopkins, McCutcheon and Wekstein (1973) in the rat using isolated hearts, suggested that the adult pattern of left ventricular function was not reached in both species until the fourth week of life. Romero, Covell and Friedman (1972) compared pressure-volume curves from both ventricles during growth in the sheep. They showed that the right ventricle was always more compliant than the left at any age and that the adult values of compliance were reached earlier in life on the left side than on the right one.

THE PULMONARY CIRCULATION IN CONGENITAL HEART DISEASE WITH LEFT-TO-RIGHT SHUNTS

Pulmonary Hypertension - Structural and Functional Studies

In 1927 Moschcowitz noticed that in cases of systemic arteriosclerosis the pulmonary circulation was not affected. Because he

accepted that systemic hypertension played an important role in the genesis of the vascular lesions, he postulated that pulmonary hypertension would produce structural changes in the pulmonary vasculature. Although the lesions were described as arteriosclerosis he mentioned that not only the main pulmonary vessels were affected but also the capillaries, with thicker walls and hyaline degeneration. The clinical conditions considered by Moschcowitz as liable to develop "pulmonary arteriosclerosis" were: mitral valve disease, chronic lung disease, cases with "marked diminution of lung volume", Kyphoscoliosis, patent ductus arteriosus and congenital intra-cardiac shunts.

Stewart and Crawford in 1933 described the pulmonary vascular lesions in a condition similar to that studied by Eisenmenger in 1897 and consisting of a high ventricular septal defect, a dextroposition of the aorta and a dilated pulmonary artery. Although they admitted a syphilitic origin for the lesions, the small pulmonary arteries were considered fewer in number and with thicker walls than normal.

With the advent of cardiac catheterization in man (Cournand and Ranges, 1941) a better understanding of the relations between structure and function in congenital heart disease was achieved. The initial catheter studies were reported by Brannon, Ween and Warren (1945) in atrial septal defects, by Baldwin, Moore and Noble (1946) in ventricular septal defects, by Dexter, Haynes, Burwell, Eppinger, Gosman and Evans (1947) in patent ductus arteriosus and other congenital cardiac defects.

Welch and Kinney (1948) studied the pulmonary vasculature in cases of patent ductus arteriosus, interauricular and interventricular congenital heart disease. They found vascular lesions - intimal proliferation or hialinization and medial changes - only in occasional cases and more frequently if two of the defects were simultaneously present. Increased pulmonary blood flow was considered as the common cause for the structural changes.

Edwards in 1950 reviewed the previous pathological studies of the pulmonary vascular bed in congenital heart disease and related them to the early catheterization data. Regarding the left-to-right shunts, a variety of lesions were described from few changes in the uncomplicated cases with normal or slightly elevated pulmonary arterial pressure, to intimal thickening in patients with atrial septal defects and associated mitral stenosis (Lutembacher's syndrome) or medial hypertrophy and intimal fibrosis of the peripheral muscular arteries as in cases of "atypical" ductus arteriosus. Edwards related the nature and progression of the lesions to the immediate postnatal changes of the pulmonary circulation and admitted that in some cases of ventricular septal defects the normal decrease in arterial wall thickness associated with a fall in pulmonary arterial pressure and resistance occurring soon after birth, caused death, due to an excessive pulmonary blood flow and a diminished systemic blood flow. In more complex cases such as the Eisenmenger complex,medial hypertrophy was evident but intimal fibrosis was not seen in the younger patients suggesting that it developed only after pulmonary hypertension was established

and as a consequence of it. In the Eisenmenger complex and in some cases of ductus arteriosus it was suggested that the high resistance fetal pulmonary circulation persisted during postnatal life.

The initial clinical haemodynamic studies by Wood (1950 a, 1950 b) and Dexter (1950) and Shepard (1954) showed that congenital heart disease with left-to-right shunts had a different evolution according to the type and size of the defect. Pulmonary hypertension was not seen in uncomplicated atrial septal defects until adult life, whereas ventricular septal defects tended to develop higher pulmonary arterial pressures than patent ductus arteriosus during adolescence. The effects of increased blood flow and pressure on the pulmonary vascular bed were further studied by Damman and Ferencz (1956), who were able to show in cases of large ventricular septal defects, a progressive decrease in pulmonary blood flow and increase in pressure and resistance with age. These haemodynamic changes were related to the simultaneous increase in wall thickness and decrease in lumen diameter of the small pulmonary arteries. Similar lesions were also described by Whitaker, Heath and Brown (1955) and by Heath and Whitaker (1957) in cases of patent ductus arteriosus and in atrial septal defects and related to the increase in pulmonary arterial pressure found at cardiac catheterization.

The importance of the increased muscularity of the pulmonary arterial bed in cases of left-to-right shunts was further discussed by Edwards (1957), who suggested that the increase in pulmonary arterial pressure and resistance could be due in part to the

vasoconstriction of the peripheral arteries. The idea of a reactive pulmonary circulation in cases of congenital heart disease associated with left-to-right shunts was not new; Wood, Magidson and Wilson (1954) had suggested a similar mechanism to explain the pulmonary hypertension found in cases of large ventricular septal defects, more than 1 cm in diameter. Chapman and Robbins (1944) reported a patient with patent ductus arteriosus and cyanosis, who became acynotic with the inhalation of 100% of oxygen for thirty minutes suggesting a decrease in the pulmonary arterial pressure. Although no catheterization was performed, pulmonary vascular lesions were found at the post-mortem study. The lesions were mainly on the pulmonary arterial bed, the capillaries and the veins showing little change. There was generalized thickening of the arterial wall from the large to the small branches with a considerable increase in elastic tissue. Intimal proliferation was also present causing a considerable reduction in the arterial lumen diameter. The smallest arteries and arterioles were considered normal and the alveolar wall showed only a minimal degree of edema and fibrosis.

The catheter studies by Harris (1955, 1957) finally demonstrated that there was a decrease in pulmonary arterial pressure in certain cases of congenital heart disease when acetycholine was injected in the pulmonary artery. The cases with muscular hypertrophy and elevated pulmonary arterial pressure showed a greater decrease than those with a similar degree of hypertension, but with intimal fibrosis.

The importance of vasoconstriction in pulmonary hypertension was further discussed by Wood (1958 a and b), who considered

that the initial increase in pulmonary pressure was hyperkinetic, the changes in the muscular arteries being reversible during that period. Doyle, Goodwin, Harrison and Steiner (1957) studied pulmonary haemodynamics in cases of congenital heart disease with left-to-right shunts and showed that, when pulmonary hypertension was associated with a decrease in pulmonary flow there was no vascular response to the injection of tolazoline in the pulmonary artery. Their structural study which included post-mortem arteriograms showed a significant dilatation of the main arteries and a considerable degree of medial hypertrophy and intimal proliferation, particularly in cases with a fixed pulmonary hypertension. Similar studies were performed by Evans and Short (1958) who showed that there was a poor filling of the peripheral pulmonary arterial bed in the post-mortem arteriograms, whereas the histological study demonstrated muscular contracture of the arteries less than 2 mm in diameter, together with a variable degree of intimal proliferation.

At the Mayo Clinic, clinicians, surgeons, physiologists and pathologists worked together to study the problems of pulmonary hypertension before and after correction of congenital heart disease. Their work is a landmark in the field. In 1958 Heath and Edwards classified the structural changes in the pulmonary vasculature in cases of congenital heart disease with left-to-right shunts. They divided the lesions into six grades according to the structural changes in the media and intima of the peripheral pulmonary arteries:

Grade 1 - Retention of fetal type pulmonary arteries. This definition based on Edwards concept (1950) of a postnatal fetal

pulmonary circulation in certain cases associated with increased flow and pressure, created a lot of controversy. According to a more recent review - by Wagenvoort, Heath and Edwards (1964) - it meant that in these conditions the abrupt thinning of the arterial wall occurring immediately after birth was not followed by the normal gradual decrease in arterial wall thickness. In fact, these cases showed a gradual thickening of the small pulmonary arteries during postnatal life.

Grade 2 - Medial hypertrophy with cellular intimal reaction.

Grade 3 - Progressive fibrous vascular occlusion.

Grade 4 - Progressive generalized arterial dilatation with formation of complex dilatation lesions.

Grade 5 - Chronic dilatation with numerous dilated vessels through the lung with pulmonary hemosiderosis.

Grade 6 - Fibrinoid necrosis of the media.

By correlating the above mentioned changes with the haemodynamic findings, Heath, Helmholz, Burchell, DuShane and Edwards (1958) showed that grades 1-3 were present in cases with an elevated pulmonary arterial pressure which was likely to fall on inhalation of 100% oxygen, suggesting a functional vasoconstrictive component in the vascular changes. Grades 5-6 were associated with a pulmonary vascular bed which did not respond to the inhalation of 100% oxygen. When these patients were operated upon it was shown that immediately after surgery, cases with grades 1-3 had a reduction in p ulmonary arterial pressure, whereas cases with grades 5-6 remained hypertensive (Heath, Helmholz, Burchell, DuShane, Kirklin and Edwards, 1958).

The initial reviews on the haemodynamics of the pulmonary circulation in patients with congenital heart disease and left-to-right shunts did not consider that a progression of the vascular lesions was likely during infancy and childhood, with the probable exception of patent ductus arteriosus (Nadas, Rudolph, Gross, 1960). However, Wagenvoort, Neufeld, DuShane and Edwards (1961) showed that in patients dying of ventricular septal defects in infancy and childhood, although the medial thickness and the medial surface area of the small pulmonary arteries decreased during the first five weeks of life as in normal infants, by eight weeks after birth an increase in both parameters was already noticeable. Hislop, Haworth, Shinebourne and Reid (1975) have recently studied a similar group of patients and suggested that in some cases of ventricular septal defects the normal postnatal decrease in arterial wall thickness does not occur.

The studies on the evolution of the pulmonary vascular lesions during life and the reversibility of the changes after surgical correction were one of the reasons for the development of open heart surgery in infancy. This produced new patients and urged a reassessment of the various concepts of the normal maturation of the pulmonary circulation and of its changes in cases of hypertension associated with congenital heart disease.

Pulmonary Hypertension in the Immature Lung

The initial structural studies on the normal postnatal changes of the pulmonary arteries performed by Civin and Edwards (1951) and by Damman and Ferencz (1956 a) showed a significant decrease in arterial wall thickness together with an increase in lumen diameter during the first months of life. Damman and Muller (1953) and Damman (1959) also noticed that the relationship between blood pressure and flow was quite different in the newborn and in the adult lung. In the newborn, a much smaller increase in flow was required to produce a simultaneous increase in pressure, whereas in the adult, pulmonary blood flow had to increase about three times before an increase in pressure was obtained (Cournand, 1947).

The importance of structural changes in lung maturation and their effects on pulmonary haemodynamics were further discussed by Rudolph (1974). He pointed out that normal infants and children born at high altitude had more "muscularized" pulmonary arteries than those born at sea level (Naeye and Letts, 1962) and showed a slower decrease in pulmonary vascular resistance with age (Peñaloza, Arias-Stella, Sime, Recavarren and Marticorena, 1964). He related these findings to the lower incidence of heart failure in cases of ventricular septal defects at high altitude when compared to the sea level. Vogel, McNamara and Blount (1967) had also shown a greater response to tolazoline in children younger than two years with ventricular septal defects, born and living at high altitude,

when compared with similar children born at sea level. In cases of atrial septal defects it was also demonstrated that pulmonary hypertension and changes in the pulmonary vasculature did not occur before the age of twenty, unless the patient lived at high altitude (Dalen, Bruce and Cobb, 1962).

In premature babies, with the respiratory distress syndrome and a patent ductus arteriosus, impairement of lung development and congenital left-to-right shunts were also associated. It was recently shown that in these cases, when hypoxia and acidosis were corrected as part of the management, the simultaneous decrease in pulmonary vascular resistance caused a large increase in pulmonary blood flow and precipitated left ventricular failure. It was speculated that because of prematurity, the pulmonary vascular bed had not developed enough muscle at arterial level to respond to the increase in flow with an increase in pulmonary vascular resistance (Neal, Barringer, Hunt and Lucas, 1975), (Lees, 1975).

The work on normal vascular development of the human lung by Davies and Reid (1970) and by Hislop and Reid (1972, 1973) showed not only that arterial wall thickness decreased soon after birth but also that during postnatal life muscle extended along the small pulmonary arteries. Intra-acinar arteries increased in number and size during the period of alveolar multiplication suggesting an increased capacity of the pulmonary vascular bed with age. Using the same precise morphometric techniques, Hislop, Haworth, Shinebourne and Reid (1975) have recently demonstrated that in

cases of ventricular septal defects in early infancy and childhood there was a decrease in both arterial multiplication and size; this suggested that the effects of increased pulmonary blood flow and pressure on the lung of infants and children with congenital heart disease could not be estimated only by the changes in arterial wall size and structure and that an impairement in lung maturation had occurred.

Reversibility of the Vascular Lesion after Surgical Correction

The main purpose of the initial studies on pulmonary hypertension associated with congenital heart disease was to predict whether the pulmonary vascular pressure and resistance decreased and if possible would return to the normal levels after surgical correction. When Heath and Edwards together with Wagenvoort (1964) reassessed their previous work on the regression of the pulmonary hypertension based on structural and haemodynamic data (Heath and Edwards, 1958) they emphasized that their initial study was related to the immediate intra-operative changes and that "...it is not known with certainty that pulmonary arterial pressure in patients with grades 1-3 vascular disease which falls to normal levels after closure of a septal defect will remain so under the influence of exercise and activity of everyday life". (sic).

Very little is known about the behaviour of the pulmonary circulation during exercise or other stress conditions, after the

surgical correction of congenital left-to-right shunts. Hallidie-Smith, Hollman, Cleland, Bental and Goodwin (1968) described a considerable increase in pulmonary arterial wedge pressure during mild exercise in patients with moderate residual post-operative pulmonary hypertension after correction of ventricular septal defects.

Epstein, Beiser, Goldstein, Rosing, Redwood and Morrow (1973) found that the haemodynamic response to intense upright exercise was impaired in patients with a repaired atrial septal defect and with no haemodynamic changes either at rest or mild exercise. Maron, Redwood, Hirshfeld, Goldstein, Morrow and Epstein (1973) described similar findings in cases of ventricular septal defects and found a direct relation between the age at surgery and the abnormal response of the pulmonary arterial pressure to intense exercise.

Studies of Lung Mechanics

In 1953, Mead and Whittenberger described methods for studying lung mechanics during spontaneous breathing in man. Using these techniques a significant reduction in compliance was described in cases of mitral valve disease (Mead, Frank, Lindgren, Gaensler and Whittenberger, 1953). Such a decrease was confirmed by other workers, but no consistent relationship was found between the decrease in compliance and either pulmonary vascular pressures or resistances (Saxton, Rabinowitz, Dexter and Haynes, 1956) The studies in

experimental animals by Borst, Berglund, Whittenberger, Mead, McGregor and Collier (1957) and by Cook, Mead, Schreiner, Frank and Craig (1959) and in normal man by Bondurant, Mead and Cook (1960) further showed that acute pulmonary congestion was not associated with a decrease in compliance.

Studies of lung mechanics in congenital heart disease were reported by Ayres, Kozam and Lukas (1960) who found that patients with increased pulmonary blood flow and normal pressures had a decreased compliance, whereas it was normal in one patient with increased pulmonary vascular resistance and a normal pulmonary blood flow. Studies by Wallgren, Guebelle and Koch (1960) by Woolf (1963) and by Howlett (1972) suggested the importance of an increased pulmonary arterial pressure as a major cause for the decrease in compliance. However, they were not able to find a significant correlation between the changes in compliance and the degree of pulmonary hypertension. Such a correlation was described by Griffin, Ferrara, Lax and Cassels (1972) between peak pulmonary arterial pressure and compliance and by Ahlström (1974) between mean pulmonary arterial pressure and compliance.

Davies, Williams and Wood (1962), Gazetopoulos, Olivier and Deuchar (1966), Gazetopoulos and Davies (1966) and Davies and Gazetopoulos (1967) confirmed the previous studies and related the changes in compliance both to increased pulmonary blood flow and pressure. They commented on the structural changes of the pulmonary vessels mentioning the fact that a rigid, highly muscularized

vascular tree would affect the elastic properties of the lung. Linde, Siegel, Martelle and Simmons (1964) also emphasized on the fact that the vascular lesions would decrease lung distensibility, particularly in cases of congenital left-to-right shunts with pulmonary hypertension. They also showed that in such cases vital capacity and total lung capacity were reduced. Lees, Way and Ross (1967) assessed pulmonary ventilation and the distribution of oxygen, carbon dioxide and nitrogen in arterial blood and alveolar gas in infants with increased p ulmonary blood flow and pressure due to congenital heart disease.

Dollery, West, Wilcken, Goodwin and Hugh-Jones (1961) had already analysed regional pulmonary blood flows in cases of circulatory shunts using radioactive isotope techniques. According to their work, an increase in pulmonary blood flow with normal pulmonary artery pressure was associated with a evener perfusion pattern than normal, whereas when pulmonary hypertension was present, there were generalized changes in perfusion. However, the inversion of the normal perfusion pattern, as seen in mitral stenosis, was never found in cases of circulatory shunts.

Griffin, Ferrara, Lax and Cassels (1972) showed a significant improvement in compliance after the surgical correction of congenital left-to-right shunts in infancy. They suggested that the analysis of this physiological feature should be used as a reliable non-invasive method to assess the reversibility of the pulmonary vascular lesions. However, work done by Ahlström (1974) did not confirm that suggestion.

Although he showed improvement in most of the operated cases, he did not find in all patients a significant correlation between clinical improvement and changes in lung compliance.

THE EXPERIMENTAL CONTRIBUTION TO THE STUDY OF THE PULMONARY CIRCULATION IN CONGENITAL HEART DISEASE WITH LEFT-TO-RIGHT SHUNTS

Systemic-to-Pulmonary Shunts

In 1939 Levy and Blalock published their work "on the effects of connecting by suture the left main pulmonary artery to the systemic circulation". (sic). They used large mature dogs and performed an end-to-end anastomosis between the left pulmonary artery and the left subclavian artery. The animals were followed for several months and haemodynamic studies were performed which included measurements of pulmonary blood flow using the Fick method and of systemic and pulmonary arterial pressures by needle puncture. After sacrifice, the pulmonary arteries were injected with a radiopaque material and biopsy tissue of the lung was analysed to detect any change in the pulmonary vasculature. No structural lesions were found in the lungs and the pressure in the pulmonary artery distal to the anastomosis was approximately half of that in the aorta. The authors suggested that the pressure in the peripheral pulmonary vessels was normal. Six years later, in 1945, Alfred Blalock and Helen Taussig published the first report on palliative surgery in children with a decreased pulmonary blood flow, using a similar type of shunt.

Another important landmark in cardiac surgery was the report by Gross and Hubbard in 1939 on the first successful ligation of the ductus arteriosus in the human. This operation stimulated interest in the physiology of the patent ductus arteriosus and attempts were made to create a similar condition in the experimental animal. Eppinger, Burwell and Gross (1941) performed end-to-side anastomosis between the left subclavian artery and the main or left pulmonary artery. Leeds (1943) anastomosed laterally the aorta to the left pulmonary artery in some of his cases, while in others he performed end-to-end anastomosis between the left subclavian and the left pulmonary artery. These experiments were all performed in adult dogs and in every case the pulmonary arterial pressure remained within the normal range, even several months after surgery. These findings were explained on the basis of a low peripheral resistance in the pulmonary circulation as confirmed later by studies of human pulmonary haemodynamics both in normal cases (Cournand, 1947) and after pneumonectomy (Cournand, Riley, Himmelstein and Austrian, 1950).

It was only in 1951 that Ekström, Ekman and Müller described the first case of pulmonary hypertension following an experimental systemic-to-pulmonary shunt. Using a three month old mixed harrier breed dog, weighing 12 kg, a side-to-side anastomosis was performed between the aorta and the left pulmonary artery. Two months after surgery, at cardiac catheterization, the pulmonary arterial pressure had increased to four times its original value and the animal died of cardiac failure, ten days after the study. This experiment was undertaken mainly to reproduce certain

"atypical" forms of ductus arteriosus, which developed p ulmonary hypertension early in life, contrary to what was usually seen in cases of patent ductus arteriosus. Unfortunately although an immature animal was used with considerable success, no mention was made to the influence of age and no further studies were reported.

Potts and Riker (1952) performed side-to-side aorto-pulmonary shunts in piglets weighing 17-22 pounds, the animals being killed nine months after surgery. Because a similar type of shunt had been described in the human (Potts, Smith and Gibson, 1952), the main purpose of this experimental work was to study the changes in the anastomosis during growth. The lungs were described as normal on routine histological assessment although one animal had to be killed two weeks after surgery, due to "respiratory difficulty". (sic).

The first systematic approach to the problem of experimental pulmonary hypertension due to an increase in blood flow and pressure was made in 1953 by both Muller, Damman and Read and by Ferguson, Berkas and Varco. The first group, anastomosed the aorta to the left pulmonary artery (side-to-end) in adult dogs and performed lung biopsies eight months and one year after surgery. They reported a high mortality but the survivors showed medial hypertrophy and intimal thickening of the small pulmonary arteries. The second group of workers used puppies aged between two and four months; they performed various types of systemic-to-pulmonary shunts, the more significant increase in pulmonary arterial pressure being found in

the cases with an end-to-end anastomosis between the left subclavian artery and the left pulmonary artery together with a left upper lobectomy. The histological study showed medial hypertrophy of the peripheral pulmonary arteries (50-200 μm in diameter) as early as two weeks after surgery, but intimal proliferation was seen only several months after.

Damman, Baker and Muller (1957) performed end-to-end anastomosis between the left subclavian artery and the left upper lobar pulmonary artery in adult dogs and described the sequential haemodynamic and structural changes in the pulmonary vascular bed. The pulmonary arterial pressure was assessed by needle puncture immediately after surgery and during the first twelve post-operative weeks showed a five fold increase when measured distal to the shunt. Lung biopsies taken simultaneously demonstrated that two weeks after surgery there was widespread parenchymal hemorrhage together with fragmentation of the internal elastic lamina and in some cases of the media. By the third week, medial hypertrophy was already apparent whereas intimal proliferation could only be seen by the eleventh post-operative week.

The reversibility of vascular lesions was also studied experimentally. Ferguson and Varco (1955) and Ferguson, Berkas and Varco (1959) performed end-to-end anastomosis between the left subclavian and the left lower lobar artery in young adult dogs. The shunt was closed after being patent for period between one and eight months and, whereas the pulmonary arterial pressure returned

to its normal pre-operative values, the structural changes failed to regress, during a time interval similar to that required for them to develop, even when only medial hypertrophy was observed. Blank, Muller and Damman (1959) also used the adult dog to assess the regression of the vascular lesions after aorto-pulmonary shunt. Their results showed that there was an increase in arterial diameter of the peripheral pulmonary vessels as early as eleven days after the occlusion of the shunt. However the structural changes, such as fragmentation of the elastic laminae and intimal fibrosis did not improve with the re-establishment of a normal pulmonary pressure.

Heath, Donald and Edwards (1959) studied the peripheral pulmonary arteries of an adult dog with an aorto-pulmonary shunt, 5 mm in diameter, for a period of four years. Although no haemodynamic measurements were performed the histological study showed vascular lesions grades 4-6 according to the Heath and Edwards classification of pulmonary vascular disease (1958). This case showed lesions similar to those described in humans with congenital heart disease and irreversible pulmonary hypertension and their production in an experimental animal suggested that time, together with shunt size were important factors in the development and severity of the lesions.

Ellison, Hall, Yeh, Mobarhan, Rossi and Ellison (1961) also using adult dogs, studied cardio-respiratory function in cases with increased pulmonary blood flow and with or without increased pulmonary artery pressure. Lung biopsies were also performed to assess the changes in the peripheral pulmonary arteries. This work showed that

both functional and structural changes were only apparent when systolic pulmonary arterial pressure exceeded 40 mm Hg. Those findings supported the initial suggestion by Ferguson and Varco (1955) that pulmonary vascular changes were more easily induced by an end-to-end than by a side-to-side systemic-pulmonary anastomosis.

Work done by Downing, Pursel, Vidone, Brandt and Liebow (1962) in adult dogs suggested that pulmonary hypertension secondary to experimental aorto-pulmonary shunts could be caused by disseminated embolization of the vascular bed; however, this suggestion was not confirmed by other workers. Geer, Glass and Albert (1964) performed shunts between the aorta and the left lower lobar pulmonary artery in adult dogs. The histological study showed no evidence of clot embolism and just the usual lesions with medial hypertrophy and various degrees of intimal lesions. In the cases with a high pulmonary arterial pressure (systolic higher than 50 mm Hg) severe intimal thickening and plexiform lesions were present and had not resolved thirty seven weeks after the occlusion of the shunt. Esterly, Glagov and Ferguson (1968) used the electron microscope to assess the changes in the pulmonary vascular bed after a systemic to pulmonary shunt. Using adult dogs, anastomosis were performed between the left pulmonary artery and the left subclavian artery with ligation of the left lower lobar artery. The pulmonary arterial pressure distal to the shunt ranged from 30-90 mm Hg. No evidence of thrombus was found and the initial ultrastructural changes, assessed from lung biopsies, consisted of hyperplastic reaction in the endothelium and media followed by the appearance of extensive projections of the medial smooth-muscle

cells into the sub-endothelial space until in some cases the lumen was occluded.

Recently Hawe, Tsakiris, Rastelli, Titus and McGoon (1972) performed side-to-side anastomosis between the ascending aorta and the main pulmonary artery in adult dogs and reported that they were not able to produce pulmonary hypertension. This was attributed to the low resistance of the pulmonary vascular bed, which tolerated large shunts, sufficient to cause left ventricular failure, without a significant increase in pulmonary arterial pressure. Similar findings were reported by Fixler, Saunders and Sugg (1974), who used adult lungs in an acute aorto-pulmonary anastomosis preparation and reported that with large shunt flows the decrease in aortic pressure was associated with a decrease in coronary artery pressure and with myocardial ischemia particularly in the sub-endocardial region.

Rudolph, Scarpelli, Golinko and Gootman (1964) also studied the acute haemodynamic effects of an aorto-pulmonary shunt. Adult dogs were used in the experiment and a silicone rubber prosthesis with a variable diameter was anastomosed between the thoracic descending aorta and the main pulmonary artery. A continuous high pulmonary flow was observed throughout the diastolic phase of the cardiac cycle and it was suggested that this continuous distention of the pulmonary arterial bed played an important role in the development of the pulmonary vascular changes. It was also noted that the older animals developed cardiac failure earlier than the

younger ones probably due to a more efficient and balanced performance of both ventricles in the more immature animals.

The importance of the ventricular function in cases of aorto-pulmonary anastomosis had already been emphasized by Evans, Bresler, Lancaster, Stewart, Harrison and Moulder (1964), who showed that adult dogs with biventricular hypertrophy (caused by banding both the aorta and the pulmonary artery) were able to sustain much larger experimental left-to-right shunts, than under normal conditions of heart function. Similar findings were also reported by Dart, Montgomery and Peters (1968), who produced the biventricular hypertrophy by creating a femoral arteriovenous fistula.

It is important to emphasize that apart from the initial study by Levy and Blalock (1939) in which the pulmonary arterial bed was injected and post-mortem radiographs taken, all the other studies in which a structural analysis of the pulmonary vascular bed was performed used uninjected and uninflated lungs and that in the great majority of the cases only biopsy material was studied.

Intra-Cardiac Shunts

Ventricular Septal Defects

In 1928, Holman and Beck created experimental ventricular septal defects in dogs by introducing a knife in the left ventricle and piercing the septum. Four animals survived out of ten cases,

and were followed for periods ranging between seven weeks and eight months during which the systemic arterial pressure was noted to decrease initially with a return to its normal values a few months after surgery. Similar defects were also created in adult dogs by Eppinger and Gross (1941), who showed that after surgery there was an increase in cardiac output assessed by the Fick method. Studies of pulmonary haemodynamics after the experimental creation of a ventricular septal defect also in adult dogs were reported by Griffin and Essex (1951), who showed a fifty per cent increase in pulmonary artery pressure after surgery.

In 1961, Siegel studied the haemodynamic mechanisms involved in the adaptation to an acute ventricular septal defect in adult dogs. He suggested that the basic compensatory mechanisms were both an increase in central blood volume and in pulmonary vascular resistance, and tried to explain both by a reflex affecting simultaneously the systemic venous bed and the lungs.

Atrial Septal Defects

Blalock and Hanlon in 1948 described a method of creating atrial septal defects in adult dogs which was later employed in the human. Experimental studies by Dow and Maloney (1951) in adult dogs showed that in cases of large defects the pressures in both atria were similar and suggested the importance of the ventricular distensibility as a major cause for the amount of blood shunted through the defect. Similar haemodynamic studies were performed

later both in acute (Weldon, 1966) and chronic experiments (Douglas, Rembert, Sealy and Greenfield, 1969).

Unlike the cases of systemic-to-pulmonary anastomosis the pulmonary vascular bed has not been studied after the creation of intra-cardiac shunts.

The Use of Growing Animals

In 1961, Richardson, Philips, DeWeese, Manning and Mahoney performed end-to-end left subclavian to left pulmonary artery anastomosis in puppies aged between three and eleven weeks. The animals were followed for a period ranging between one hour and fourteen weeks and vascular pressure studies and lung biopsies were performed in an attempt to correlate the structural with the functional changes. The pulmonary arterial pressure was significantly increased in all animals but only the older animals showed arteries with thicker walls and smaller lumens at biopsy.

Rudolph, Neuhauser, Golinko and Auld (1961) performed right pneumonectomies in puppies aged between one and two months and in adult dogs. They showed a gradual rise in pulmonary arterial pressure only in the younger animals. The arteriograms, performed in vivo, showed dilatation of the main pulmonary arteries and tortuosity of the peripheral branches, particularly in the animals operated during adult life. The histological assessment of the pulmonary vascular

bed showed medial thickening and intimal fibrosis in the small arteries and dilatation of the arterioles. There were no age related differences in the structural study.

Mansion and Schilling (1964) performed lung resections in puppies aged between one and seven days and found that pulmonary arterial hypertension was present when the animals reached maturity. Similar results were not found by Kato, Kidd and Olley (1971), who performed left pneumonectomies in older puppies, aged between four and eight weeks, and found no significant increase in pulmonary arterial pressure and no structural change in the vascular bed by fourty weeks after surgery.

Vogel, Averil, Pool and Blount (1963) and Vogel, McNamara, Hallman, Rosenberg, Jamiesen and McCrady (1967) ligated either of the pulmonary arteries in calves. In their first study, reported at an high altitude of 5000 feet, they were able to produce pulmonary hypertension if either of the vessels was ligated between one and three months of life. In the following study they showed that the ligation of the left pulmonary artery in the newborn calf at sea level, was not associated with an increase in pulmonary arterial pressure; on the other hand, some degree of hypertension was found after the ligation of the right pulmonary artery. From this work it was suggested the importance of the increase in pulmonary blood flow as a direct cause for the pulmonary hypertension.

Left pneumonectomies were also performed in minipigs (Kato, Kidd and Olley, 1971) and in piglets (Friedli, Kent and Kidd, 1975).

Pulmonary arterial pressure was increased in all cases, as early as three weeks after surgery. The histological study revealed that whereas the minipig rapidly developed medial hypertrophy of the small pulmonary arteries, the piglet showed an initial relative decrease in wall thickness due to the dilatation of the arterial lumen. After the first six post-operative months, an increase in the medial wall thickness then occurred.

As in the experimental systemic to pulmonary anastomosis, the structural study of the pulmonary vascular bed was performed using uninflated and uninjected lungs.

THE PIG AS AN EXPERIMENTAL ANIMAL

The domestic pig (sus scrofa) is one of the five species included in the genus "Sus" belonging to mammalian order Arteriodactyla. There are various breeds of domestic pigs, the most common in Britain being the Large White (Mount, 1968).

The pig was domesticated in China nearly 5000 years ago. Leonardo da Vinci (1452-1519) introduced the species in research performing experiments in living animals to demonstrate the active contraction of the heart. John Hunter (1728-1793) considered the pig as a useful animal for laboratory experiments (Gloyne, 1950), but Pavlov (1849-1936) could not use swine for his experiments on the autonomic nervous activity and considered them "hysterical" (Marcuse and Moore, 1944).

The fetal piglet was used for early studies on the embryology of the respiratory system both by Flint (1906) and Clements (1937).

During this century pigs have been increasingly used not only in cardio-respiratory research but also in the fields of immunology, to study the effects of radiation and the various aspects of growth and nutrition, renal function and organ transplantation (Mount and Ingram, 1971).

Anatomy of the Respiratory and Cardiovascular Systems

Respiratory

The lungs of the pig are asymetrical, the right one having four lobes and the left only three. In both lungs the lobes are termed apical, cardiac and diaphragmatic whereas the fourth one, called intermediate, is located in the middleline towards the diaphragmatic surface and has a pyramid-shape with the apex looking the hilum. The right apical bronchus originates directly from the trachea a few centimeters above the carina (Talanti, 1959).

McLaughlin, Canada and Tyler (1961) described the vascular and bronchial branching pattern of the pig lung and reported on a close relationship between the bronchial pathway and the arterial and venous beds along all their intrapulmonary course from the hilum to the periphery. They also showed that the lung in swine has a thick pleura and well developed interlobular septa.

Cardiovascular

Engelhardt in 1966 demonstrated that the relative heart weight in swine, expressed as a percentage of the body weight, decreased with age. He attributed this to selective breeding which caused the rate of growth in swine to double since 1910 and suggested that because heart growth had not followed body growth, this phenomenon might cause a permanent strain on the heart of this species. However, the study of the heart weights in various mammalian species showed that larger and more active animals, like horses and dogs, had larger hearts than smaller and less active ones like pigs, sheep and rabbits (Lee, Taylor and Downing, 1975).

The anatomy of the coronary arteries in the pig is similar to the human, both in the branching pattern of the main vessels (Howe, Fehn and Pensinger, 1968), (Fehn, Howe and Pensinger, 1968) and at intra-mural ventricular level (Jönsson, 1975). The ascending aorta has a relatively short course and its first branch is the brachio-cephalic artery, which gives origin to the right subclavian artery and to the cephalic artery, which in turn, bifurcates into the two common carotid arteries. The left subclavian artery originates as a separate branch from the aortic arch (Becker, Lord and Dobell, 1972).

Physiology of the Respiratory and Cardiovascular Systems

Respiratory

Respiratory frequency and tidal volume in the awake pig were measured by Ingram (1964) and Ingram and Legge (1970). Studies of

lung function in the anaesthetized animal were performed by Attinger and Cahill (1960) who measured not only respiratory frequency and tidal volume, but also airways flow and resistance, static compliance and functional residual capacity.

Brown, Woolcock, Vincent and Macklem (1969) and Woolcock and Macklem (1971) studied the mechanics of the airways in the pig lung. They showed that in this species, compliance was dependent on respiratory frequency even at normal resting values, a phenomenon not seen in either man or dog and suggested that this could be due to the well defined lobulation of the pig lung.

Cardiovascular

In 1966 Engelhardt reviewed cardiovascular physiology in swine. He related systemic arterial blood pressure and heart rate to body weight and showed that the various measurements of cardiac output in swine varied considerably, the highest values obtained by using the Fick method and the lowest by using dye-dilution curves.

Right ventricular and pulmonary arterial pressures in the pig have been considered higher than in other species (Attinger and Cahill, 1960), (Engelhardt, 1966) but no simultaneous measurement of arterial blood gas tensions and pH were reported. More recent work has shown that when these variables were monitored both in the anaesthetized (Evans, Rowe, Downie and Roswell, 1963), (Friedli, Kent and Kidd, 1975) and in the conscious animal (Boothe, Maaske and Nielsen, 1966) the pulmonary arterial pressure was similar to that found in other species.

Experiments in Growing Pigs

The changes occurring immediately after birth during the first weeks of life in the pig are well documented in the studies by McCance and Widdowson (1957), Glauser (1966) and Book and Bustad (1974), who considered this species as a suitable experimental animal model for paediatric research, because of its similarities to the human.

Evans, Rowe, Downie and Roswell (1963) studied the cardiovascular changes occurring immediately after bith in the pig and showed that as in other species, the pulmonary arterial pressure decreased soon after birth. Using phonocardiograms they were able to demonstrate that the ductus arteriosus was functionally closed between the second and third week of life in the pig. This finding confirmed the early structural study by Schaeffer in 1941, who showed that complete obliteration of the ductus was achieved by the second week of life in the pig. Ductus flow as well as the response of the pulmonary circulation to acute hypoxia was studied by Rowe, Sinclair, Kerr and Gage (1964) in newborn animals using an "open-chest" preparation. Acute hypoxia induced a significant increase in the right-to-left shunting of blood through the ductus due to a significant increase in pulmonary arterial pressure which reached systemic arterial levels.

Glauser, McCance and Widdowson (1962) and Glauser (1966) studied the functional and structural changes in the lungs of newborn pigs living for a period of 24 hours in an atmosphere consisting of

7-8% oxygen and 10% carbon dioxide for twenty four hours. The animals hyperventilated and arterial blood gas studies showed a lower pH, PO_2 and oxygen saturation together with an elevated PCO_2. Structurally the lungs looked hyperinflated and there was rupture and thinning of the alveolar walls. The effects of an increase in environmental temperature and humidity on the respiratory frequency of young pigs were studied by Ingram (1964, 1965). He showed that because swine do not sweat and are dependent on changes in the respiratory frequency to increase the heat loss, under high temperatures conditions their respiratory frequency increased up to 400 breaths/min.

Tawes, Aberdeen and Berry (1968) studied the growth of an aortic anastomosis in piglets. They considered the species suitable for cardiovascular experiments during growth not only because the structure of the aorta in the pig is similar to the human, but also because its diameter triples between six weeks and six months of life, a much faster rate than the human in whom a comparable change in diameter occurs from birth to maturity.

PREPARATION OF LUNG AND HEART TISSUES - QUANTITATIVE ASSESSMENT OF THE PULMONARY VASCULATURE

Fixation of the Lung

Formaldehyde

The gas formic aldehyde was discovered in 1863 by August Wilhelm von Hoffmann first director of the Royal College of Chemistry

in London (Tompsett, 1970). Thirty years later, Cohn (1828-1898) experimented with its solution, formalin, as a fixative agent for plants.

In the lungs, formalin has been used as a fixative introduced in the airways both in liquid and vapour phases.

Vapour formalin

The early attempts by Blumenthal and Boren (1959) to fix the lungs using air saturated with cold formalin were not very successful. Pratt and Klugh (1961) used warm concentrated formalin at a temperature of 60° C and inflated the lungs by placing them in a perspex box and applying a negative pressure. This also gave poor results histologically, particularly in the terminal airways and at alveolar level, due to poor fixation. Recently Wright, Slavin, Kreel, Callan and Sandin (1974) used a similar technique, but recommended that further fixation of pulmonary tissues was needed in a 10% formalin solution, prior to histological processing.

Weibel and Vidone (1961) also distended the lungs by placing a negative pressure, but used formalin steam as a fixative. This method was usefully applied to the adult human lung, free of pulmonary disease but was not successful when applied to the diseased lung (Reid - personal communication; Weibel - personal communication, in Hislop, 1971). The Panel on Pathology of the Medical Research Council Committee on Research into Chronic Bronchitis (1972)(1975) also considered it inadequate for the study of the pulmonary vasculature.

A further criticism to this method was made by Wright, Slavin, Kreel, Callan and Sandin (1974), who suggested that fixation was not uniform because condensation of steam inside the lungs caused accumulation of water with dissolved formaldehyde in the air spaces, preferentially at the bases.

Liquid formalin

Hartrof and Macklin (1943) were the first to attempt fixation of the lungs at a constant infusion pressure followed by floating the lungs in the fixing solution. Similar method was used by Gough and Wentworth in 1949. Heard (1958) described a method of maintaining a constant pressure throughout fixation and showed that less shrinkage of pulmonary tissues occurred, when compared to the cases where the lungs were simply floated in formalin.

The Committee on Emphysema, set up by the Aspen Conference (1959) recommended the use of a fixing solution containing 10% neutral formalin and 5% sodium acetate injected into the trachea at a normal pressure of 20.3-30.5 cm (8-12 inches) of water until the lung surface showed normal contours. The lungs were then submerged in a fixative for a period of at least three days.

The Panel on Pathology of the Medical Research Council Committee on Research into Chronic Bronchitis (1972)(1975) has recently reviewed the various methods on whole lung fixation with formalin. The report emphasised the fact that any technique required distention of the lung to a certain volume, which for most of the

current methods is total lung capacity, and argued that structural measurements at this point of inflation were difficult to correlate with functional studies.

In our department the lungs are fixed by injecting liquid formalin through the airways. The method is similar to that recommended by the Committee on Emphysema of the Aspen Conference (1959), except that the inflation pressure is somewhat higher - 45.7 cm (18 inches) of water rather than up to 12 inches. Using this technique good fixation was achieved for both young and adult lungs, with and without pathological lesions (Elliott, 1964)(Davies, 1969)(Hislop, 1971) (Haworth, 1975).

Rapid Freezing Techniques

These techniques have been used to correlate more closely the functional and structural changes occurring in the lung, particularly in the alveolar capillary zone.

Staub and Storey (1962) were the first to use liquid propane to freeze the pulmonary parenchyma of cats; the animals had the chest opened and were artificially ventilated. Good histological sections were obtained from lung specimens 2-3 cm long, frozen to a depth of 0.2 cm in two seconds. The freezing methods were further developed by Glazier, Hughes, Maloney, Pain and West (1966) to study alveolar morphology. They totally froze greyhounds, using solid carbon dioxide for a period ranging from twenty four to forty eight hours. The morphology of the capillary bed in the lungs was further studied

by Glazier, Hughes, Maloney and West (1969), who used liquid freon to rapidly freeze pieces of tissue from an isolated lung preparation.

Fixation of the Heart - Ventricular Weights

The early attempts by Müller (1883) to divide the heart in the fresh state were not very successful. Since then all the work in the assessment of ventricular weights has been performed after fixation of the heart in formalin. Herrmann and Wilson (1922) suggested that prolonged immersion in the fixative could cause a considerable decrease from the fresh weight and that care should be taken to keep the hearts in formalin only for a period of four days prior to the weighing. However, further studies by Millard (1965) showed that the heart weight decreased about five per cent during the first four days of fixation and that it remained constant thereafter.

The division of the ventricular cavities has been performed in various ways. Müller divided the weight of the septum between the two ventricles in proportion to the weight of the free walls. Lewis (1914) separated each ventricle as a completely closed cavity and considered as septum a thin layer of muscle that remained between. Herrmann and Wilson (1922) cut the ventricles in horizontal sections and divided each one into right and left by dissecting through a white line in the septum.

Fulton, Hutchinson and Jones (1952) studied cases of ventricular

hypertrophy and showed that in cases of hypertrophy the increase in septal weight always followed that of the left ventricle. Based on this finding, they suggested that the ventricular weights should be expressed as a ratio between the weight of the left ventricle plus the septum to that of the right ventricular free wall plus any papillary muscle on the right side of the septum.

This method has been used in our department to assess changes in human hearts during fetal life (Hislop and Reid, 1972) and post-natal development (Davies and Reid, 1970) and also in cases of chronic obstructive pulmonary disease (Millard, 1965).

Injection of the Pulmonary Vascular Bed

Vascular Casts

Leonardo da Vinci made the first wax cast to study the cerebral ventricles. This material was also used for vascular injections later being replaced by rose's metal, a low melting point mixture of metal, with which bronchial casts were made (Aeby, 1880) (Ewart, 1889). Further progress was achieved with the introduction of celloidin which allowed good filling of any cavity or vascular bed; Narath (1901) used this method to study the branching of the bronchial tree.

In 1936, Narat, Loef and Narat described a vinyl resin, known as vinylite, which has since then been widely used in corrosion casts.

It was using this material that Boyden (1955) described the segmental broncho-vascular anatomy of the lung. Patel and Burton (1957) also employed vinylite casts to demonstrate for the first time, the capacity of the small pulmonary arteries of the rabbit to actively constrict after the injection of norepinephine. The casts of the small vessels in the experimental group of animals were markedly grooved and contorted when compared to the control ones, which appeared smooth and straight.

Various types of latex also have been used to fill the bronchial tree and the pulmonary vascular bed as in the cases of Pump (1961) (1962) (1964), who used Vultex moulage to study the intra-acinar vasculature, and of Oderr, Dauzat and Montamel (1963), who used latex of different colours to fill the pulmonary arterial and venous bed.

Radiopaque Materials

Gull and Sutton (1872) were the first to use an aqueous barium sulphate mixture to study a vascular bed, in their case, the kidney. Vascular injections were also performed in the same organ by Hinman and Morison (1924), who proposed that gelatin should be added to the aqueous mixture to allow the cutting of the specimen and the identification of the injected vessels under the microscope. Harrison and Wood (1949) used a barium gelatin mass to inject the coronary arteries at the known diastolic pressure of the patient or, if that was unknown, at a standard pressure of 90 mm Hg.

In 1951, Evans emphasized the importance of the post-mortem

pulmonary arteriogram as an adjunct to histology in the study of pulmonary hypertension. The method of vascular injection was described by Short (1956), who used a radiopaque medium (a bismuth oxychloride gelatin suspension) heated at 80° C at a pressure of 50 mm Hg (100 mm Hg in cases of pulmonary hypertension). By this method vessels down to a 30 um in diameter were filled.

Silver (1951) used an aqueous barium sulphate solution to fill the pulmonary arteries, the injection being made with a hand syringe. He tried different concentrations and reported that the 50% solution leaked from the vessels during the cutting of the lung, whereas the 85% solution remained in the vessels. He also noted that the suspension did not penetrate into the capillary bed. An aqueous barium sulphate gelatin mixture was also used by Collister, Dankmeijer, Snellen and van der Wel (1953) in a post-mortem angiographic study of the pulmonary vascular bed in congenital heart disease. According to their report no histological study was performed due to the leaking of the contrast, when the lungs were cut. Reid (1955) recommended that gelatin should be added to the barium sulphate mixture for the injection of both airways and vessels in the lung; she commented that this method allowed better cutting for the histological processing.

Doyle, Goodwin, Harrison and Steiner (1957) applied the method described by Harrison and Wood (1949) for the study of the coronary arteries to the pulmonary arterial bed. They used a warm barium sulphate gelatin mixture (37° C) at a pressure of 80 mm Hg or at the value of the pulmonary arterial systolic pressure during life, if it was known to have been higher than 80 mm Hg. Dunnill (1961) also

used a barium sulphate gelatin mixture at a pressure ranging between 50-100 mm Hg to perform post-mortem pulmonary arteriograms.

The use of a hypertensive pressure for the injection was criticized by Wagenvoort (1960) and by Wagenvoort, Heath and Edwards (1964), who recommended the use of an injection pressure not exceeding the pulmonary arterial pressure during life. They stated that overdistension of the vascular bed would create artefacts and that spilling of contrast medium into the surrounding tissues could occur.

Elliott in 1964 used a barium sulphate gelatin mixture injected at a constant pressure of 100 cm of water, to study the pulmonary arterial bed in the normal adult human lung. Using this technique distension of all vessels was obtained as judged by the flattening of the elastic laminae in the media, but no damage was caused to the arterial wall. The mixture filled vessels down to a 15 μm external diameter and produced no leakage into the parenchyma. This method has been used since then in the study of the normal human pulmonary circulation during fetal (Hislop, 1971) and postnatal (Davies, 1969) life, in the elderly age group (Semmens, 1970) and also in the assessment of the changes caused in the pulmonary vascular bed by congenital heart disease (Hislop, 1971)(Haworth, 1975), cystic fibrosis (Ryland and Reid, 1975), scoliosis (Davies, 1969), primary and thrombo-embolic hypertension (Anderson, Simon and Reid, 1969), chronic obstructive pulmonary disease (Shelton, Keal and Reid, 1976) and acute hepatic failure (Williams, Reid, Trewby and Williams, 1976). The

same technique has also been used to study the normal structure of the pulmonary arterial bed in the rat (Hislop and Reid, 1976) and the vascular changes caused either by feeding with the seeds of Crotolaria spectabilis (Hislop and Reid, 1974 c) or by chronic hypoxia (Meyrick, 1976)

Quantitative Assessment of the Pulmonary Vasculature

Measurements from Casts and Angiograms

Using a corrosion cast from a normal adult human lung, Singhal, Henderson, Horsfield, Harding and Cumming (1973) measured the length and diameter of the proximal arteries and peripherally down to a diameter of 0.8 mm and attempted to relate these morphological values to the predicted blood flow in the lung.

The importance of correlating the histological study with the post-mortem arteriogram was emphasized by Evans (1951), Silver (1951), Short (1956) and Doyle, Goodwin, Harrison and Steiner (1957), but their assessment was only qualitative.

Millard (1965) performed measurements directly from the arteriograms and related the changes on lumen diameter along the pathway to its total length. This method has further been used in the study of normal and diseased human lungs (Davies, 1969), (Hislop, 1971), (Haworth, 1975).

The Capillary Bed

Attempts to study the capillary network in the lungs have been made in vivo using a thoracic window by Wearn, Ernstene, Bromer, Barr, German and Zschesche (1934), Irving, Burrage, Aimar and Chestnut (1954), Knisely (1960) and Krahl (1963). Post-mortem studies were reported by Staub (1961) and by Reid and Heard (1962). These observations were only qualitative and although in some cases, measurements of capillary diameter were performed no attempt was made to assess the overall capillary network of the lungs. This was first attempted by Weibel and Gomez (1962) and by Weibel (1963) using morphometric techniques. The proposed model is becoming progressively more sophisticated and recently Weibel (1970) (1971) has proposed a morphometric method to estimate the area available for gas diffusion across the alveolar surface.

The morphology of the pulmonary capillaries was also studied by Glazier, Hughes, Maloney and West (1969) using isolated, perfused dog lungs and a quick-freezing technique. By changing the vascular pressures in the gravity dependent preparation, they were able to show that the capillary width and the density of red blood cells inside the capillaries also changed accordingly. These variations, however, were not uniform throughout the lung. Based on the nomenclature proposed by West, Dollery and Naimark (1964) and West (1966), they admitted that, in zone II (in the mid lung) where the pulmonary arterial pressure (P_a) is higher than alveolar pressure (P_A), which in turn is higher than pulmonary venous pressure (P_v) - there was recruitment of new vessels whereas in Zone III (in the bases) $P_a > P_v > P_A$ the main change was dilatation of already open capillaries.

The alveolar microcirculation in the cat has recently been studied using a low viscosity silicone elastomer which filled the capillary bed completely (Fung and Sobin, 1969) (Sobin, Tremer and Fung, 1970) (Sobin, Fung, Tremer and Rosenquist, 1972) (Fung and Sobin, 1972 a, b) (Rosenquist, Bernick, Sobin and Fung, 1973). The complex and detailed analysis of the vasculature led to the presentation of a "sheet flow" model for the pulmonary circulation, the importance of which is still being debated.

Microscopic Study of the Arterial and Venous Beds

Microscopic measurements of wall thickness and lumen diameter were first performed in systemic arteries by Gull and Sutton (1872) and by Kernohan, Anderson and Keith (1929).

Brenner in 1935 was the first to attempt a complete quantitative analysis of the pulmonary vasculature. He used uninjected specimens and expressed the thickness of the media of both arteries and veins as a percentage of the external diameter excluding the adventia. O'Neale, Thomas and Hartcroft (1955) also using uninjected lungs, introduced the measurement of medial area, which was obtained by subtracting lumen area from total area. They also attempted to landmark the arteries within the lung, by relating these with the accompanying airways. Heath and Best (1958) used measurements of both thickness of the media, expressed as a percentage of the external diameter, and cross-sectional area of the muscle of the media, to study various forms of pulmonary hypertension. Other arterial measurements have been used such as the mean Lumen-to-wall ratio

(diameter of the lumen divided by twice the thickness of the media and adventitia) by Damman and Ferencz (1956 a and b), the internal (lumen and intima) to external diameter (lumen, intima and media) ratio by Rosen, Bowden and Uchida (1957).

Naeye (1961) using planimetry calculated lumen, medial and intimal areas and used the third of these measurements, as a base line value, not affected by either vasoconstriction or vasodilatation. Wagenvoort (1960) injected the pulmonary arterial bed and measured the actual amount of arterial muscle tissue per unit area of lung parenchyma. This method also avoided errors arising from differences in degree of vascular dilatation.

Elliott (1964) injected the arterial bed using a constant pressure of 100 cm water to achieve distension of the vessels. This allowed a more accurate comparison between the various cases. He expressed twice the medial wall thickness (between the internal and external elastic lamina) as a percentage of the total diameter (between the external elastic lamina). He also related the structure and size of the pulmonary arteries to their position inside the lung by performing serial sections along an arterial pathway, both in the pre- and intra-acinar areas and landmarking the vessels by their accompanying airway. These techniques, together with the counting of alveoli and arteries between 25 and 200 μm in external diameter per unit area of lung tissue have been used in our department for the study of pulmonary vasculature during growth, both in health and in disease (Davies, 1969) (Davies and Reid, 1970) (Hislop, 1971) (Hislop and Reid, 1972) (Haworth, 1975).

THE PRESENT STUDY

The structural development of the human lung has been studied in detail using precise quantitative techniques with particular emphasis on the pulmonary vasculature.

Experimental attempts to produce changes in the pulmonary circulation by systemic-to-pulmonary anastomosis have given variable and conflicting results. Although the main purpose of that work was to reproduce similar conditions to those found in human congenital heart disease with left-to-right shunts, few experiments were performed on immature animals. Similarly, studies on the normal pulmonary circulation in the laboratory animals are fragmented and no complete comparison has been made between human and animal pulmonary vascular growth and development.

In the first part of the present work the normal postnatal development of the pig lung is described. The features of lung structure, respiratory physiology and pulmonary haemodynamics during growth will be compared with those of the human in order to assess the suitability of the pig as a model for the study of the pulmonary circulation during postnatal life.

The results obtained from aorto-pulmonary anastomosis performed in swine at four, eight and twelve weeks of age and followed for one to three months are reported in the second part of the present work. Studies of lung structure and function similar to those reported

for the normal animals are also described in an attempt to assess the effects of increased pulmonary blood flow and pressure in the immature lung.

MATERIAL

Sixty seven Large White pigs were used in the present study. They were all obtained from litters free from enzootic pneumonia. While in the laboratory, the animals received pelleted food (Spillers) and water, ad libitum.

The structural analysis of the normal pulmonary vasculature was performed in 22 cases, from birth to adult life; details of age, sex and body weight are given in Table II 1. Cardiopulmonary function was assessed in 37 normal animals, aged between two and sixteen weeks (Table II 2). Eighteen aorto-pulmonary anastomosis were performed in swine aged between four and twelve weeks of life (Table II 3). Some animals were included in the three series. When surgery followed a normal functional study, a period of one week always separated the two procedures.

In the pig, if sexual maturity is taken as a criterion of adulthood, the female is mature at about seven months of age and the male between eleven and twelve months (Mount and Ingram, 1971).

METHODS

The Experimental Procedure

Surgical Creation of the Aorto-Pulmonary Shunt

All animals were deprived of food and water twelve hours before

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TABLE II 1

STRUCTURAL STUDIES. NORMAL ANIMALS (BIRTH TO 3½ YEARS)

Case No.	Age	Sex	Body Weight (kg)
A.1	Newborn	M	1.5
A.2	"	F	1.2
A.3	"	M	1.0
A.4	"	F	1.5
A.5	3 days	M	2.0
A.6	3 "	F	2.0
A.7	1 week	M	2.5
A.8	2 weeks	F	3.0
A.9	2 "	M	3.5
A.10	2 "	M	4.5
A.11	5 "	M	10.0
A.12	5 "	M	9.5
A.13	8 "	F	19.5
A.14	8 "	M	21.5
A.15	8 "	M	18.0
A.16	12 "	M	38.0
A.17	16 "	F	58.5
A.18	16 "	M	60.5
A.19	20 "	M	77.0
A.20	20 "	F	87.5
A.21	Adult (1 year)	F	180.0
A.22	" (3½ years)	M	-

TABLE II 2

FUNCTIONAL STUDIES. NORMAL ANIMALS (2 TO 16 WEEKS)

Case No.	Age (weeks)	Sex	Body Weight (kg)
B.1	2	F	3.0
B.2	2	M	3.5
B.3	3	M	7.0
B.4	4	F	9.0
B.5	4	M	10.0
B.6	4	M	10.5
B.7	5	M	10.5
B.8	5	F	12.0
B.9	5	M	10.0
B.10*	5	M	10.0
B.11	5	M	17.5
B.12	6	F	11.0
B.13	6	F	12.0
B.14	6	M	11.0
B.15	7	F	16.0
B.16	7	M	14.0
B.17	7	F	15.0
B.18*	8	F	19.5
B.19	8	M	17.0
B.20	8	M	24.0
B.21	8	M	25.0
B.22	9	M	14.0
B.23	9	F	20.0
B.24	10	M	26.0
B.25	10	M	22.0
B.26	10	F	23.0
B.27	10	F	22.0
B.28	11	M	25.5
B.29	11	M	25.0
B.30	12	M	23.0
B.31	12	F	22.0
B.32	12	M	30.0
B.33	12	M	24.0
B.34	14	F	42.0
B.35	16	M	38.0
B.36	16	F	50.0
B.37	16	M	30.0

*used also for structural studies

TABLE II 3

AORTO-PULMONARY SHUNTS. SEX, AGE AND BODY WEIGHT OF PIGS AT THE TIME OF SURGERY (4 TO 12 WEEKS)

Case No.	Sex	Age (weeks) At the time of surgery	Body Weight (kg)
C.1*	M	4	11.0
C.2	M	4	12.0
C.3	F	4	11.5
C.4	M	4	10.5
C.5	M	4	10.0
C.6*	M	5	10.5
C.7*	M	5	12.0
C.8	F	6	11.5
C.9*	F	6	15.0
C.10*	M	8	17.0
C.11*	F	8	15.0
C.12	M	8	25.0
C.13	M	8	19.0
C.14	M	8	19.5
C.15	M	8	25.0
C.16	F	10	24.0
C.17*	M	12	22.0
C.18*	F	12	28.0

*used for normal function studies (one week before surgery)

surgery. Pre-medication was given intra-muscularly and consisted of fentanyl and droperidol (29 µg fentanyl and 1.4 mg droperidol/Kg) together with atropine (50 µg/Kg), (Piermattei and Swan, 1970). Ten minutes later, anaesthesia was induced using halothane, 1.5-2% nitrous oxide in oxygen. Endo-tracheal intubation with a cuffed oral tube was carried out under spontaneous breathing. The animals were then placed on a left lateral recumbent position and d-tubocurarine chloride, 0.3 mg/Kg was administered via an ear vein. Respiration was controlled using a Brompton-Manley ventilator, delivering 50% nitrous oxide in oxygen; the gas flow ranged between 4 and 10 liters/minute according to the size of the animal and the tidal volume was adjusted to 20-25 cc/Kg. Anaesthesia was maintained using an intra-venous infusion of fentanyl and droperidol (0.5 mg fentanyl and 12.5 mg droperidol in 100 ml saline). This was administered via an ear vein at a rate of 1-2 ml/minute (Piermattei and Swan, 1970). Atropine, 12.5 µg/Kg was given intra-muscularly every 30 minutes. Halothane 0.5-1% was also used intermittently to maintain anaesthesia during surgery, but was discontinued immediately after the aorto-pulmonary anastomosis was established. Arterial blood samples were collected by needle puncture before and after the completion of the shunt. The Pa CO_2 was found to be always below 49 mm Hg (for details on blood analysis see Functional Studies). The ECG was monitored during surgery.

Under aseptic conditions, a left thoracotomy was performed through the third intercostal space and the aorta and the main pulmonary artery were exposed by downward retraction of the left upper and middle pulmonary lobes. The aorta was mobilized along the

first three to four centimeters of its thoracic descending segment and nylon tapes were placed around the vessel at the top and bottom of the dissected zone; care was taken not to damage the adventitia during this procedure. The pericardium covering the main pulmonary artery was opened and the vessel was also mobilized; in some cases, two nylon tapes were also placed around the main pulmonary artery. The aorta and the pulmonary artery were partially constricted along the main axis, using vascular serrated clamps; longitudinal arteriotomies were made in each vessel and the shunt performed using a silicone-rubber prosthesis* (Ashton, Lightwood and Hardman, 1967) - 6 mm, i.d., in animals aged between 4 and 6 weeks, and 8 mm, i.d., in the older cases (Fig. II 1).

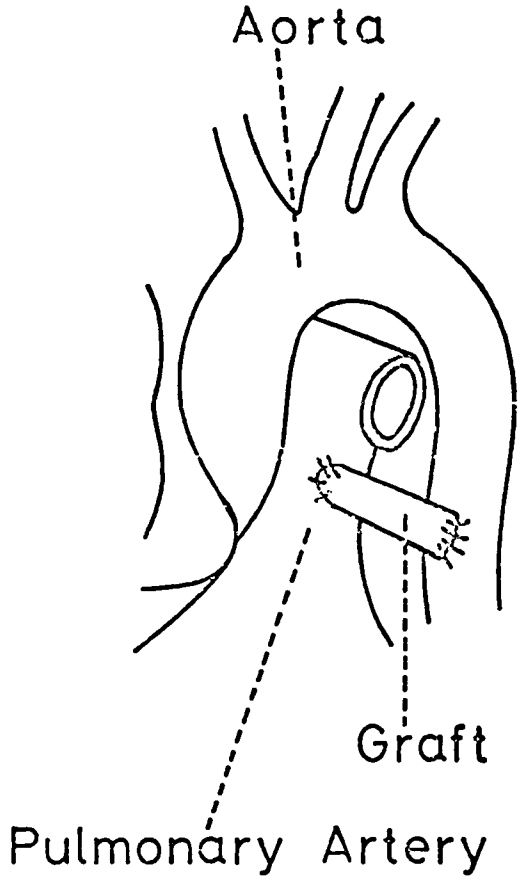
All the grafts were preclotted before insertion. Running sutures of 5.0 Prolene (Ethilon, Ltd.) were used on each side and care was taken to avoid intimal injury during suturing. Any bleeding was controlled by intermittent pressure and additional matress sutures, if required, were also used.

No antibiotics were administered during the follow-up period. A description of the methods used in the intra-operative assessment of the shunt is given below (see Functional Studies). Twenty eight animals were operated upon. Two (7%), died at surgery due to excessive bleeding from the aortic anastomoses, eight (29%), died of pulmonary edema within the first 24 postoperative hours. The present study is concerned with the remaining 18 cases (64%).

* This material was supplied to us by Professor Ashton, Department of Surgery, University of Birmingham.

Fig. II 1

Diagram illustrating the experimental aorto-pulmonary shunt. The vascular prothesis is inserted between the thoracic descending aorta and the main pulmonary artery


Aorta
Graft
Pulmonary Artery

Sequence of the Follow-up Studies

All the animals were given a period of fifteen days to recover. In each case, the follow-up period ranged between one and three months during which serial measurements of lung function were performed. The animals were also catheterized at the end of the follow-up. Immediately before sacrifice, some cases had a thoracotomy and the pulmonary haemodynamic state was investigated.

A scheme of the follow-up protocol is given in Fig. II 2. Details of all these procedures are given below (see Functional Studies).

Structural Studies

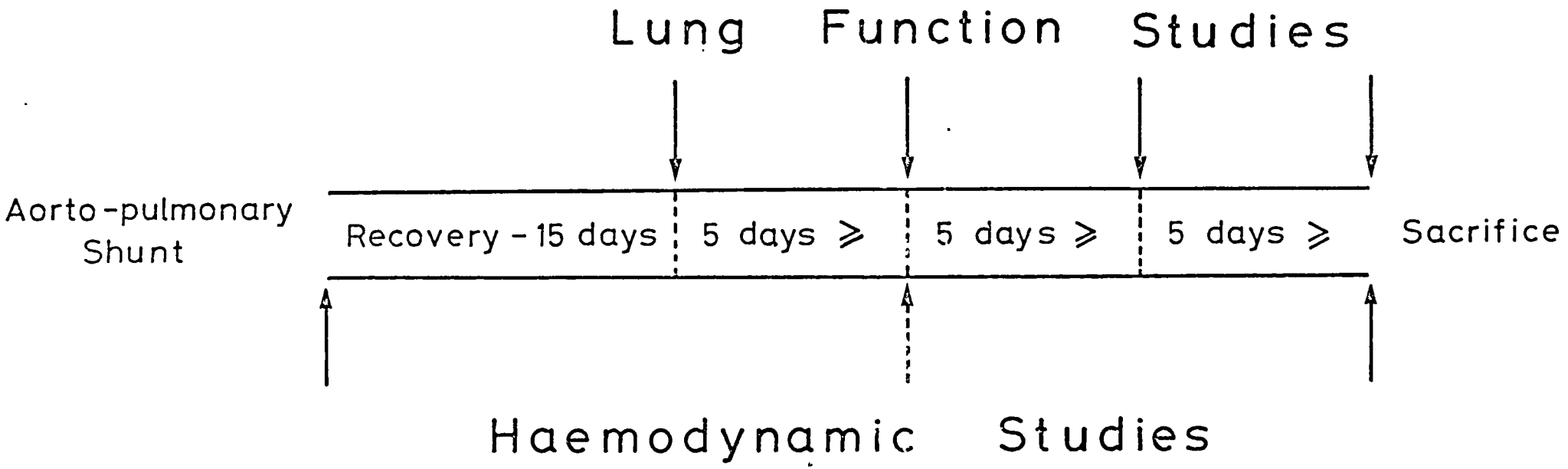
Similar methods were used for the normal and experimental cases.

Collection and Storage of the Specimens

All the animals were sacrificed with an intra-cardiac injection of pentobarbitone sodium (Euthatal - dose 200 mg/ml). Immediately after death the cardiopulmonary block was removed and stored at a temperature of -20° C.

Fig. II 2

Diagram showing the sequence of the follow-up studies. Each animal had several measurements of lung function. Pulmonary haemodynamic studies were performed at the time of the initial surgery and immediately before sacrifice; the dotted arrow represents cardiac catheterization studies performed in some cases, during the follow-up period, which were not included in the present study


Lung Function Studies
Aorto-pulmonary Shunt
Recovery - 15 days
5 days ≥
5 days ≥
5 days ≥
Sacrifice
Haemodynamic Studies

Preparation of Lung Tissue

Post-mortem pulmonary vascular injection

Before injection, the lungs were kept at room temperature for a period of sixteen hours. The main pulmonary artery was cannulated in six normal specimens, from animals younger than two weeks; the ductus arteriosus was patent in these cases and had to be ligated before the vascular injection. In the other specimens, the arterial cannula was placed in the left pulmonary artery. The right pulmonary veins were cannulated separately in all cases, except in the newborn where the venogram was performed using a cannula placed in the left atrium.

Two hours prior to the injection, the lungs were placed in an incubator at a temperature of 37° C.

A similar radiopaque medium was used for arteries and veins. This medium was prepared by mixing 500 cc of Micropaque Liquid Dispersion* (Damancy and Company), with 50 g of gelatine powder dissolved in 200 cc of distilled water; a few phenol crystals were added to inhibit bacteral growth.

The radiopaque mixture was injected into the vessels at a temperature of 60° C and at a pressure of 100 cm H_2O for five minutes. A liquid reservoir was used to maintain a constant injection pressure measured in a water-column manometer (Hislop, 1971).

*A fluid dispersion containing 100% weight in volume of mucophilic, electronegatively charged barium sulphate.

In all cases except in the newborn, in which it was slower, filling of the peripheral vessels occurred within the first minute of the injection and could be seen through the pleural surface.

At the end of the injection the vessel or vessels were clamped to maintain a constant intravascular pressure during the setting of the injection mixture.

This technique has been previously used in our department. Elliott, in 1964, showed that the density of the mixture only allows vessels larger than 15 µm in diameter to be injected with consequently no filling of the capillary bed. By this method a complete distension of the vascular bed was obtained in all cases, both normal and abnormal, and this allowed for accurate comparison between specimens.

Fixation

The lungs were fixed by running a 10% buffered formol saline solution into the airways at a pressure of 45.5 cm until the pleural surface was tense. The trachea was cannulated in the seven cases younger than two weeks; in all the other specimens the cannula was placed in the main bronchi. After inflation, the trachea or the bronchi were clamped and the lungs floated in formol saline for a period of fifteen days.

Radiography

After injection, inflation and fixation, the lungs were

radiographed using a Watron MKII portable x-ray unit and Kodirex Auto-process film. The tube distance was 50 cm and the values for kilovoltage and milliamperage were respectively 50 and 15; the time of exposure varied between 0.5 and 1.5 seconds depending upon the size of the lung. The films were developed using an "X-Omat" automatic processing machine.

Evaluation of the Angiogram

The general assessment of the angiograms gave information on the vascular branching pattern and of the size and length of its various segments. The length of the axial pathway of the left lower lobe artery and of the contralateral vein were measured from the hilum to the lung periphery; the lumen diameter was also measured at the hilum and at 25% intervals to the periphery (Hislop, 1971).

Using this angiographic method, Elliott in 1964 has shown that vessels down to 160 µm can be seen as separate lines. The peripheral filling on the arteriogram is given by the small arteries that cannot be individually seen. According to Elliott (1964), Davies (1969), Hislop (1971) and Haworth (1975) these vessels make up the so called "background haze", which can be related to the histological assessment of arterial number (vessels smaller than 200 µm in external diameter).

Measurement of Lung Volume and Lung Length

After fixation, lung volumes were measured by displacement of

water (Dunnill, 1962 a). The length of the left lower lobe was measured on the arteriograms from the apex to the diaphragmatic surface.

Macroscopic Point Counting. Selection of Blocks

The lungs were cut in slices 0.5 cm thick. The volume proportions of the various compartments in the lung were assessed macroscopically using the point counting method described by Dunnill (1962). The following structural components were included: parenchyma, arteries, veins, bronchi and connective tissue, the proportion of each being expressed as a percentage of the total number. Three series each of one thousand points were counted per lung and the mean value of the three estimated.

Ten blocks were taken from each lung using a stratified sampling method (Dunnill, 1962). Standardized size blocks, 1x1 cm, were always taken, using a transparent grid. Additional blocks were taken from the main pulmonary artery immediately above the pulmonary valve in the normal cases, and from the left main artery at hilar level in both normal and experimental animals.

In three normal cases (newborn, two and eight weeks of age), blocks were also taken from the followign sites along the left lower arterial pathway during macroscopic dissection (see below): (i) at the origin, (ii) halfway along the vessel length and (iii) from the various side-branches at their origin.

Processing. Sectioning. Staining

All specimens were processed on a Histokinette automatic processor. The blocks were cut using a Leitz base sledge microtome and the sections were 5 µm thick.

All sections were stained with Verhoeff's elastic and counterstained with Van Gieson's stain (Hislop, 1971). Alternate sections were stained with haematoxylin and eosin (Culling, 1957). Shrinkage due to processing and sectioning was assessed by comparing the initial standardized 1x1 cm block with the size of the section after staining. According to Hislop (1971) there is a negligible amount of shrinkage of the artery lumen after processing and no correction factor is needed in the measurement of arteries or veins.

Macroscopic Dissection of the Pre-Acinar Arterial Pathway

In three normal cases, aged one hour, two and eight weeks, the left lower lobe artery and its accompanying airway was dissected using a Zeiss dissecting microscope. This was done to determine whether the pig, like the human, had more arteries than airways branches along an axial pathway (Elliott and Reid, 1965).

No reconstruction of the pre-acinar branching pattern was made.

Microscopy Study of the Lung

All slides were examined using a Zeiss EA 38 microscope.

General assessment

The overall structure of the lung was studied. Particular attention was given to the distribution of connective tissue, because previous workers (McLaughlin, Tyler and Canada, 1961) have suggested that the pig lung has well developed interlobular septa. In the experimental cases, changes in the alveolar wall thickness and evidence of pulmonary edema were also noted. Arteries and veins were classified according to their medial structure (definitions are given at the end of this chapter). Any intimal change was also recorded.

Microscopic point counting

At microscopic level, the volume proportions of the various components of the lung were assessed using the point-counting method (Weibel and Gomes, 1962) (Dunnill, 1962 a). An integrating eye-piece (Zeiss I) was used and a 1000 point per slide were counted on fields selected at random (x25 objective). The structures counted were: alveolar air, alveolar duct air, alveolar wall, arteries and parenchymal tissue. The problems of differentiating alveolar duct space from alveolar space in a two dimensional section have been emphasized by Davies (1969) and recently by Hansen and Ampaya (1974).

Pre-acinar arteries. Structure and medial wall thickness

The wall thickness of the media was measured using a calibrated eye-piece graticule and factors were determined for each objective to convert scale units to μm for greater accuracy. All measurements

were performed at the highest magnification possible. At least three measurements were made at sites where the media was compact and the mean value calculated. The number of elastic fibers contained within the two elastic laminae was also counted.

Particular attention was given to the degree of fragmentation of the elastic fibers, as well as to the amount and distribution of muscle cells and connective tissue in the media.

Peripheral arterial bed. Quantitative studies

Measurements were made using a calibrated graticule in the eye-piece and factors were determined for each objective to convert scale units to μm.

External diameter All the measurements were performed using the highest magnification that included the whole vascular structure in the field.

External diameter was measured as the distance between the outside of the external elastic lamina taken along the diameter of the vessel. When arteries were cut in true cross-section, two measurements were made at right angles and the mean value recorded. When vessels were cut obliquely, the mean diameter was taken as the narrowest diameter of the section (Davies, 1969).

Wall thickness. Percentage arterial wall thickness The wall thickness was always measured using a x100 objective. It was taken as the distance from the inside of the internal elastic lamina to the outside of the external elastic lamina. When the artery was cut in true cross-section four measurements were made along two diameters at right angles and the mean value recorded. If the artery was sectioned obliquely only two measurements were made along the smallest diameter. The wall thickness was expressed as a percentage of the external diameter using the formula:

$$\% \text{ wall thickness} = \frac{2 \times \text{wall thickness}}{\text{external diameter}} \times 100$$

Population studies The study of the arterial "populations" allowed comparison between size and structure at the periphery of the arterial pathway. The work done by Elliott (1964) and by Hislop (1971) has shown that in the human lung the arteries below 150 µm in diameter could be either muscular, partially muscular or non-muscular.

In the present study the same structural pattern was also found in the pig and so, population counts were performed in the normal and in the experimental cases. Six to eight sections per lung were examined. The external diameter and the structure of all arteries below 200 µm was recorded, the vessels later being grouped according to size. The range of each group was 10 µm up to 100 µm and 50 µm above that diameter. The percentage of each structural type within each size range was assessed and the overall results were shown as "population curves".

Arteries accompanying airways The size and the structure of arteries accompanying terminal bronchioli, respiratory bronchioli and alveolar ducts were noted. Identification of the intra-acinar airways was essential for this method, because they "landmarked" the arteries. This permitted comparison between arterial size, structure and position at the periphery of a vascular pathway not only within the same lung but between different specimens. Injected vessels found in the alveolar walls were also noted and their size and structure recorded.

Number of arteries and alveoli per unit area of lung tissue The counting of alveoli per unit area has been previously used by Dunnill (1962 a), Davies (1969) and Hislop (1971), among others.

This method assumes that all the lungs are similarly fully inflated and that shrinkage due to the preparation of the tissues is similar between cases.

Davies (1969) and Hislop (1971) have related the number per unit area of lung tissue of alveoli to that of small arteries below 200 μm external diameter.

In the present study, a modification of the Davies and Hislop methods was used; that was introduced by Haworth (1975). Using an integrating eye-piece (Zeiss I) of a known area, and a x25 objective alveoli and arteries were counted in twenty five fields, chosen at

random from six to eight lung sections per case. The alveoli and arteries were related to square centimeter and the alveolar/arterial ratio calculated. When there was significant variation in number of the arteries they were recounted in fifty additional fields; if these results differed from the initial counting, another twenty five fields were used to count both alveoli and arteries.

The peripheral venous bed. Quantitative studies

Measurements of external diameter and wall thickness were also performed using the technique described for the arteries except that due to the absence of an internal elastic lamina in porcine pulmonary veins, wall thickness was measured from the external elastic lamina to the internal surface of the innermost muscle cell. Percentage vein wall thickness was then calculated as described for arteries.

Ventricular Weights

The hearts were fixed in 10% buffered formol saline solution. Millard (1965) has shown that the heart loses about five per cent of its weight in the first four days of fixation in formalin; after that period, the weight remains constant. Therefore all the hearts used in the present study, from both normal and experimental cases, remained in formalin solution for at least seven days and no correction factor was used for the initial shrinkage.

Each heart was dissected according to the method described

by Fulton, Hutchinson and Jones (1952). The atria and the great vessels as well as the valve leaflets were separated from the ventricles and all fat removed. The ventricles were then separated by cutting along the angle between the right ventricle and septum, and the right ventricular wall removed from the left ventricle and septum. Any papillary muscles on the right side of the septum were removed and included with the right ventricle. The left ventricle plus septum (LV+S) was weighed separately from the right ventricle (RV); the ratio LV+S was calculated.

Functional Studies

A 6-channel system (S.E. Laboratories, "EMMA") was used for amplification and display of the signals, and data was recorded on an ultraviolet oscillograph (S.E. Laboratories, 3006/DL).

In some animals, studies of lung mechanics and pulmonary haemodynamics were performed on separate occasions; in other animals both studies were performed at the same session, the respiratory measurements being followed by cardiac catheterization.

Lung Function

The same methods were used for both the normal and the experimental animals.

The pre-medication, induction and intubation protocol was similar to the one already described for the surgical procedure. After endo-tracheal intubation the animals were placed in a dorsally recumbent position and anaesthesia was continued with 0,5-1% halothane in 50% nitrous oxide in oxygen, delivered to the spontaneously breathing animal through the Jackson-Rees modification of an Ayres' "T" piece.

The overall procedure from endo-tracheal intubation until the end of the study lasted an average of 30 minutes, and respiratory function remained stable during this period as judged by serial measurements of arterial blood gas tensions taken at the 1st, 15th and 30th minute.

Dynamic compliance

Intra-pleural pressure was measured according to the techniques described in man by Milic-Emili, Mead, Turner and Glauser (1964) and in the dog by Gillespie, Lay and Hyatt (1973). An air-filled latex balloon, 5.0 cm long, 3.5 cm in circumference was fused to the distal end of a length of polyethylene tubing (100 cm x 0.15 cm i.d.); there were multiple perforations in the tubing covered by the balloon. This assembly was placed in the stomach of the animal and the proximal end of the tubing was connected to a pressure transducer (S.E. Laboratories, 1150/D5964). An air volume of 0.5 cc was injected into the balloon, which was slowly pulled up into the esophagus, until it lay in the lower third of this organ, at the level where the end-expiratory pressure was most negative and the cardiac artefact least evident (Macklem, 1974).

Gas flow was measured using a heated Fleisch pneumotachograph (no. 1), which was connected directly to the endo-tracheal tube, and a pressure transducer (Elema-Schönander, EMT 32). Tidal volume was derived by electronic integration of the flow signal.

Three series of ten breaths were recorded during each study for the determination of dynamic compliance, and each series was preceeded by three passive breaths of approximately twice the resting tidal volume in an attempt to eliminate alveolar collapse (Mead and Collier, 1958). Care was taken to avoid any phase difference between the flow and the pressure signals (Macklem, 1974).

Dynamic compliance was calculated from each breath by dividing the tidal volume by the change in esophageal pressure between points of zero flow. The mean of fifteen breaths was taken in each case, and expressed in ml/cm H_2O. Tidal volume (ml) and respiratory frequency (breaths/min) were also calculated.

Thoracic gas volume

Thoracic gas volume was determined by placing the intubated air-breathing animal in a pressure type whole body plethysmograph and proceeding according to the method described by DuBois, Botelho, Bedell, Marshall and Comroe (1956). Because the measurements of thoracic gas volume were made during short intervals, the wooden plethysmographic box was not absolutely leak-free and a temperature control system was not used (Leith and Mead, 1974).

Mouth pressure and plethysmograph pressure calibrated in terms of a volume change, were measured by means of pressure transducers (J. Langham Thompson, Ltd - Type UP/1355 and 1051). Tidal volume was displayed as described for the measurements of dynamic compliance and this allowed the airways to be occluded precisely at the end of a normal expiration.

Three series of five breaths were recorded during each study. Thoracic gas volume was calculated from each breath using the following formula, derived from Boyle's Law:

Thoracic gas volume = (atmospheric pressure - water vapour pressure)/(change in mouth pressure per unit change of box volume)

The mean value of fifteen breaths was calculated and expressed in ml.

Specific compliance

The ratio between dynamic compliance and thoracic gas volume, termed specific compliance, was also calculated in ml/cm H_2O per ml.

Pulmonary Haemodynamics

Intra-operative measurements

The animals were under automatic ventilation during these procedures (see Surgical Creation of the Aorto-Pulmonary Shunt) and all the measurements were taken with the ventilator stopped, for

for short periods at end-expiration and were referred to zero at the mid-chest. Arterial blood samples were collected immediately before and after each study and stored in ice to be later analysed for blood gas tensions, pH and haematocrit (see Cardiac Catheterization).

At the time of the creation of the shunt After the opening of the chest, both the aortic and the pulmonary artery pressures were assessed by direct needle puncture. In some cases, the main pulmonary blood flow was also measured using a sine wave electromagnetic flowmeter (SEM 275, Laboratories, Engineering Ltd.) and a cuff-type head transducer (series SEM 230, S.E. Laboratories, Engineering Ltd.). After the shunt had been completed, both pressure and flow measurements were repeated with the additional assessment of the shunt flow, using a second sine-wave flowmeter (SEM 275, S.E. Laboratories, Engineering Ltd.).

At the end of the follow-up period A left thoracotomy was performed through the third intercostal space, as described in the surgical protocol, and the lung freed from any adhesions. It was originally intended to measure shunt flow and pulmonary flow during this procedure. However, the silicone-rubber prosthesis was always surrounded by a thick fibrous capsule which did not allow for cuff-head transducer to be properly placed. Thus, the measurements were restricted to the assessment of the flow in the main pulmonary artery, distal to the shunt, using the sine-wave electromagnetic

flowmeter and a cuff-head transducer (S.E. Laboratories, Engineering Ltd.). Aortic and pulmonary artery pressures were also assessed by direct needle puncture.

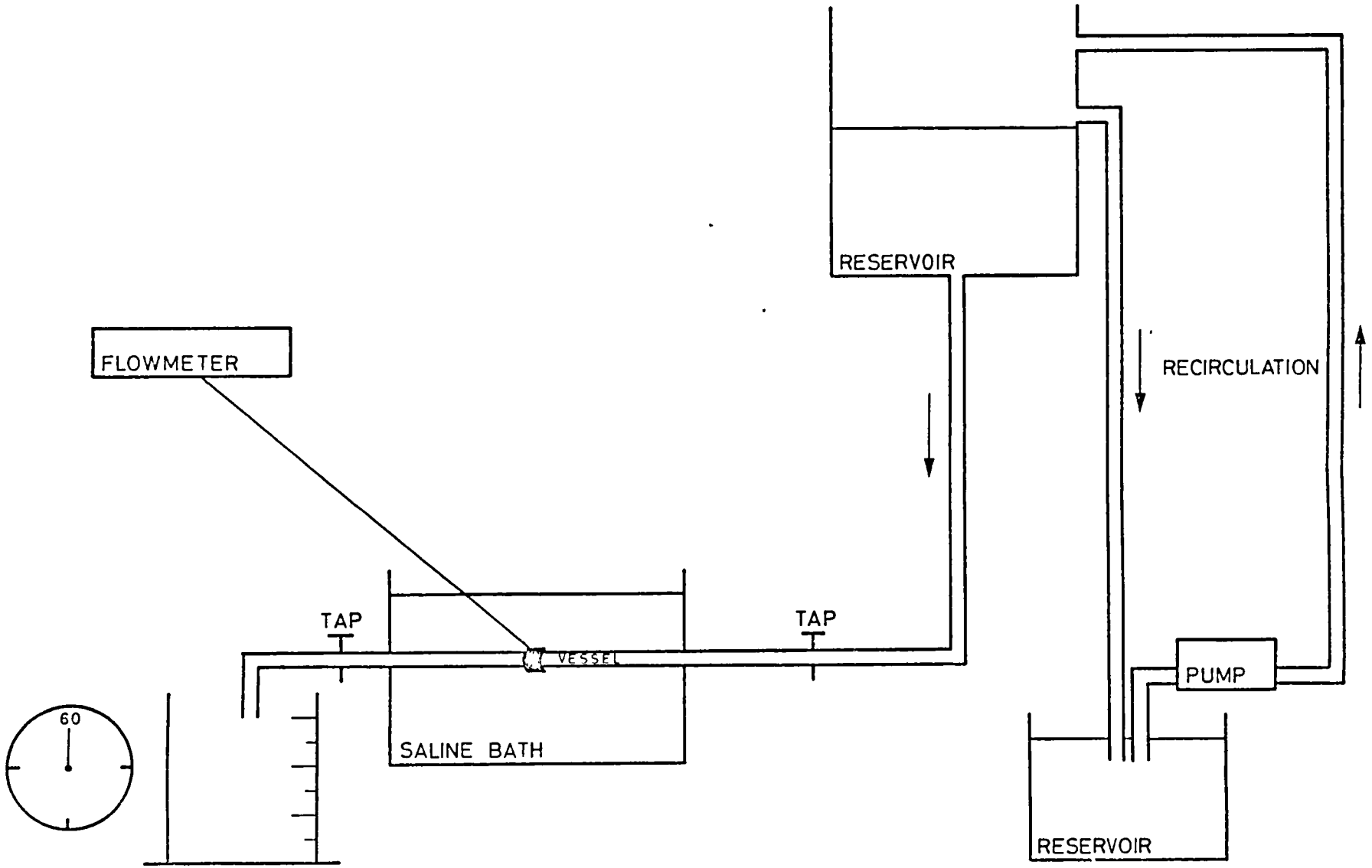
Calibration of the flow transducers All the cuff-head transducers were calibrated in vitro, to check the pre-calibration factors supplied by the manufacturer. A system was designed in the laboratory to test the flowmeter and transducer performance (Fig. II 3). It consisted of a large perspex reservoir, various length of polyethylene tubing and a specially earthed perspex chamber (Carolina Medical Instruments Ltd.), in which the vessel either human or porcine, together with the cuff-head transducer, was submerged in saline. A Sarns roller pump was used to maintain the reservoir level. In all the studies the calculated flow was related to the volume collected in a measuring cylinder during a timed interval.

Each transducer was tested under a range of gravity flow rates from 100-1000 ml/min, using both physiological saline (0.9 % Na Cl) and porcine blood at room temperature (18-20^{o} C). Both during in vitro calibration and in the experiments, care was taken in the selection of the transducer size. According to Spencer and Denison (1963) the cuff-head should reduce the cross-sectional area of the vessel by approximately five to ten per cent to assure a firm contact between the electrodes and the vascular wall.

Mean flow was obtained by electrical damping of the signal. The electronic zero from the sine-wave flowmeter was checked in vitro by

Fig. II 3

Diagram of the system designed to test the flowmeter and transducer performance in vivo. The vessel together with the cuff-head transducer is submerged in the saline bath and submitted to a range of gravity flow rates, using either saline or blood


RESERVOIR
FLOWMETER
RECIRCULATION
TAP
TAP
VESSEL
PUMP
60
SALINE BATH
RESERVOIR

mechanical occlusion of the vessel and a ± 5% error was found, between the mechanical and the electronic zero; this is in accordance with previous reports (Spencer and Denison, 1963)(Hickman, Croisier, Smith, Immelman and Terblanche, 1975).

A linear and significant response was obtained with all the probes ($P<0.001$) both with saline and blood. The variation between reading for the same probe made on different days, never exceeded 10%.

No tests were performed in vitro on the effects of pulsatile blood flow, changes in blood haematocrit or temperature.

Cardiac catheterization

The same techniques were used for both the normal and the experimental animals. The shunted cases were catheterized at the end of the follow-up period.

The pre-medication, induction and intubation protocol was similar to the one already described. When cardiac catheterization was the sole procedure, anaesthesia was maintained using an intravenous infusion of fentanyl and droperidol (0.5 mg fentanyl and 12.5 mg droperidol in 100 ml saline), (Piermattei and Swan, 1970). This was administered via an ear vein at a rate of 1-2 ml/min; halothane was discontinued shortly after endo-tracheal intubation, and atropine (12.5 µg/Kg) was given intra-muscularly every 30 minutes. The same drugs were also given during the haemodynamics performed following

lung mechanics, halothane being discontinued immediately before the start of cardiac catheterization. Manual ventilatory assistance was performed throughout the procedure.

The femoral artery was cannulated with a 20 gauge, 2" Argyle Medicut (Sherwood Medical Instruments Inc.) to permit blood sampling and continuous monitoring of systemic arterial blood pressure.

Under aseptic conditions, the external jugular vein was approached through a longitudinal incision in the neck and exposed by blunt dissection. Right heart catheterization was performed by this route using a thermal dilution floating catheter (Type 3753, size 3F, 110 cm long. Cardio Vascular Instruments Ltd.). Pressures were recorded with Consolidated Electrodynamics transducers (Type 4-327, L 221, Bell and Howell, Ltd.). All measurements were made during the expiratory phase of the respiratory cycle and referred to zero at the mid-chest.

Cardiac output was measured by the thermal dilution method (Branthwaite and Bradley, 1968). three successive injections of normal saline at room temperature being delivered through a short catheter terminating in the right atrium via the external jugular vein - 2 ml were used for animals weighing less than 15 Kg and 5 ml for animals weighing more than this. Cardiac output was derived automatically using a prototype of the Cardio Vascular Instruments Ltd. thermal dilution cardiac output system; this assumes an injectate volume of 10 ml and the values displayed were therefore corrected according to the actual volume of injectate used (Wyse, Pfitzer, Rees,

Lincoln and Branthwaite, 1975).

Immediately after the cardiac output measurements had been completed, the thermal dilution catheter was replaced by a Swan-Ganz double lumen catheter (Type 93-110, 5F, 110 cm long, Edwards Laboratories), and the pulmonary arterial wedge pressure was determined.

The electrocardiogram was monitored throughout the procedure. The duration of cardiac catheterization averaged 60 minutes. Arterial blood was sampled, on four occasions, at the beginning of the experiment, immediately after the insertion of the arterial cannula, before the cardiac output was determined and after the wedge pressure had been measured. Mixed venous blood was withdrawn on the second and third occasions at the same time as the arterial samples. These samples were drawn into heparinised all glass syringes and analysed for gas tensions and pH using a BMS-3 Electrode System (Radiometer) calibrated with known gas mixtures. All the samples were stored in ice between collection and analysis.

Body surface area was calculated according to the formula:

$$\text{Surface area } (m^2) = K \times \text{Weight}^{2/3}$$

K for swine = 0.090 (Guyton, Jones and Coleman, 1973).

Standard equations were used to calculate stroke volume, cardiac index and both pulmonary vascular and total pulmonary resistance (Yang, Bentivoglio, Maranhão and Goldberg, 1972). Conversion from Traditional to SI units: 1 mm Hg $\approx$ 0.133 kPa.

Assessment of the shunt at catheterization In the animals with an aorto-pulmonary anastomosis, the presence of the left-to-right shunt was detected during cardiac catheterization by the shape of the thermal dilution curve. When compared to the normal cases, there was a rapid change in the downslope component of the curve caused by recirculation (Fig. II 4, 5).

Further assessment of the shunt was made by calculating the pulmonary-to-systemic flow ratio using the formula:

$$\frac{PBF}{SBF} = \frac{\text{Content } O_2 \text{ SA} - \text{Content } O_2 \text{ MV}}{\text{Content } O_2 \text{ SA} - \text{Content } O_2 \text{ Pa}}$$

(Yang, Bentivoglio, Maranhão and Goldberg, 1972) (Rudolph, 1974)

PBF - pulmonary blood flow

SBF - systemic blood flow

Content O_2 SA - oxygen content of blood, systemic artery

Content O_2 MV - oxygen content of blood, mixed venous return. In the present case, the right ventricle, as the chamber proximal to the shunt.

Content O_2 PA - oxygen content of blood, main pulmonary artery. The pulmonary-to-systemic resistance ratio was also calculated by dividing the flow-ratio by the ratio between the mean pulmonary and systemic arterial pressures.

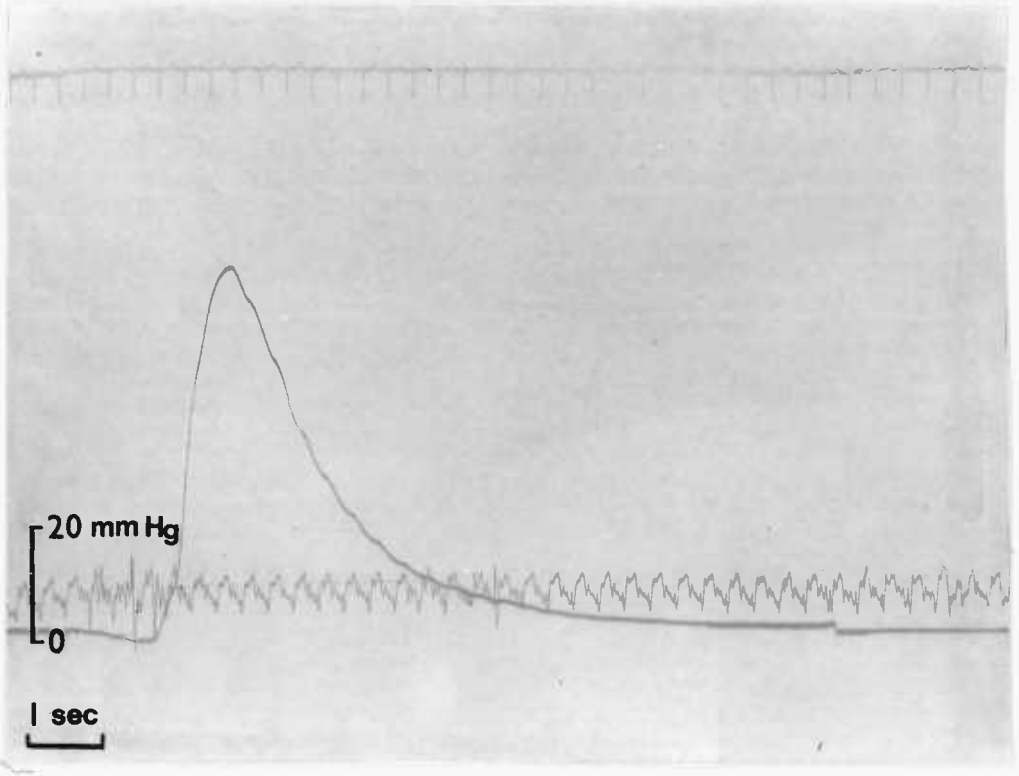
Before the cardiac output curves were obtained, blood was withdrawn from the three above mentioned locations in the following sequence: (i) simultaneously, from the systemic and pulmonary artery; (ii) immediately afterwards, from the right ventricle.

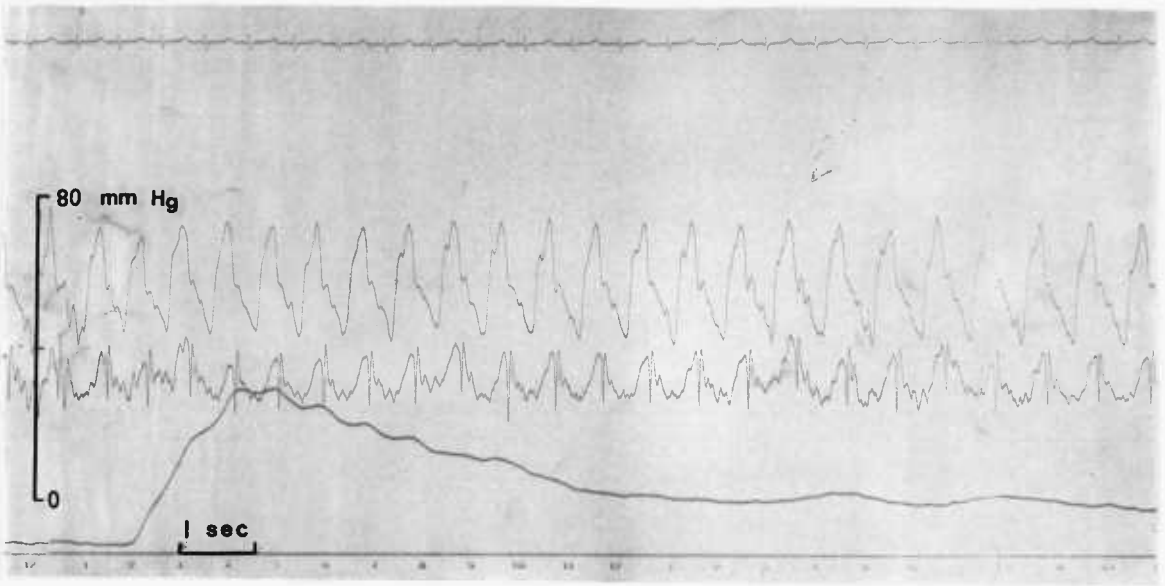
Fig. II 4

Thermal dilution curve. Normal case

Fig. II 5

Thermal dilution curve, case with an aorto-pulmonary shunt. There is evidence of recirculation as shown by a rapid change in the downslope part of the curve which remains above the base line. These features are not seen in the normal case (Fig. II 4)


20 mm Hg
0
1 sec


80 mm Hg
0
1 sec

The same blood sample was not used for measurements of both oxygen content and tension, because the first requires complete mixing, which is performed by agitation with a small bead of mercury, this in turn, causes disruption of the red blood cells and leads to errors in the assessment of the oxygen tension (Branthwaite, 1974).

Oxygen content was determined using a Lexington Oxygen Content Analyser (Lex-02-Con, Albury Instruments, Ltd.), which had been validated by comparison with the electrode method (Branthwaite, 1974).

The haematocrit was also measured in each arterial sample using a Hawkesley microhaematocrit centrifuge.

STRUCTURAL STUDIES - DEFINITIONS

(Reid and Simon, 1958) (Elliott, 1964) (Reid, 1967 b) (Reid, 1968)

Airways

Axial Pathway

An axial pathway runs from the hilum to the distal surface of a segment.

Generations

A generation lies between two successive branches of bronchus or artery.

Bronchi

Bronchi are those airways running proximal to the last plate of cartilage.

Bronchioli

Bronchioli are those airways lying distal to the last plate of cartilage and proximal to the alveolar region.

Terminal Bronchiolus

A terminal bronchiolus lies proximal to the respiratory bronchiolus.

Respiratory Bronchiolus

A respiratory bronchiolus is an airway that has alveoli opening into its lumen.

Acinus

An acinus includes all lung tissue distal to a terminal bronchiolus; it comprises respiratory bronchioli, alveolar ducts and alveoli and is therefore the respiratory part of the lung.

Arteries

Conventional Arteries

Conventional arteries are those accompanying airways.

Supernumerary Arteries

Supernumerary arteries arise as side branches from the main artery, but do not have a corresponding airway. They eventually join some type of respiratory structure and perfuse respiratory tissue. They increase the number of branches from the present artery relative to the parent bronchus.

Elastic Artery

The media of an elastic artery is bound by an internal and external elastic lamina and contains seven or more concentric elastic laminae.

Transitional Artery

A transitional artery is similar to an elastic artery but contains only five or six elastic laminae within the media. This type of artery is found between the elastic and muscular vessels along any pathway.

Muscular Artery

The media of a muscular artery consists of a continuous coat of muscle limited by an internal and external elastic lamina. In larger muscular arteries there are up to four elastic laminae in the media which are usually fragmented.

Partially Muscular Artery

In this type of vessel, the muscular layer is not continuous and therefore appears as a crescent when the vessel is cut in cross-section.

Non-Muscular Artery

A non-muscular artery is larger than a capillary but has no muscle in its wall, which consists of collagen with or without a single elastic lamina.

Arteriole

The term arteriole has not been used because it is open to different interpretations, both functional and structural; it seems better to use the descriptive terms of muscular, partially muscular and non-muscular arteries.

CHAPTER III

STRUCTURAL DEVELOPMENT OF THE PULMONARY VASCULATURE

This study was performed in a series of twenty two animals from birth to three and a half years of age (for details of age, sex and body weight see Table II 1).

The porcine left lung has three lobes, apical, cardiac and diaphragmatic. The right lung has these and in addition the so-called intermediate lobe, that resembles a triangular pyramid, with apex directed to the right hilum and base to the diaphragm. The right apical bronchus originates directly from the trachea, several centimeters above the carina.

Lung Volume and Lung Length

During the first year of life, lung volume (cc) and lung length (cm) increased (Table III 1).

The changes in both lung volume and length were related to age (weeks) and body weight (Kg) using linear, power and exponential equations (Table III 2). All the correlation coefficients were highly significant ($P<0.001$), but the rate of change of the two variables was different during growth; this could be demonstrated by the fact that there was, on average, an eighty fold increase in lung volume during the first year of life, whereas lung length increased only five times during the same period (Fig. III 1).

Half of these changes - a forty fold increase in volume and a

TABLE III 1

LUNG VOLUME AND RADIOGRAPHIC LUNG LENGTH OF THE ANIMALS INCLUDED IN THE PRESENT STUDY (FROM BIRTH TO 3½ YEARS)

Case No.	Pulmonary Vessels Injected	LV (cc)	LV/BW (cc/100g)	LL (cm)	LL/BW (cm/100g)
A.1	LL+RL-A	60	4.0	6.2	0.41
A.2	LL+RL-A	55	4.6	5.4	0.45
A.3	LL+RL-V	40	4.0	-	-
A.4	LL+RL-A	70	4.7	-	-
A.5	LL+RL-A	100	5.0	6.3	0.31
A.6	LL+RL-A	105	5.2	6.7	0.33
A.7	LL+RL-A	155	6.2	7.8	0.31
A.8	LL-A	175	5.8	-	-
A.9	LL-A	150	5.0	9.0	0.26
A.10	LL-A;RL-V	180	4.0	9.5	0.21
A.11	LL-A	220	2.2	14.2	0.14
A.12	LL-A;RL-V	235	2.5	13.6	0.14
A.13	LL-A;RL-V	540	2.8	-	-
A.14	LL-A	435	2.0	18.7	0.09
A.15	LL-A	520	2.9	18.2	0.10
A.16	LL-A;RL-V	1280	3.4	19.8	0.05
A.17	LL-A	1835	3.1	20.0	0.03
A.18	LL-A;RL-V	1795	3.0	23.3	0.04
A.19	LL-A	2045	2.7	22.0	0.03
A.20	LL-A;RL-V	2120	2.4	24.3	0.03
A.21	LL-A;RL-V	4950	2.7	32.7	0.02
A.22	LL-A	4600	-	30.1	-

Mean value for each case
LL - left lung
RL - right lung
A - arteries
V - veins

LV - lung volume
LL - lung length
BW - body weight

TABLE III 2

LUNG VOLUME AND LUNG LENGTH DURING GROWTH. RELATION WITH AGE AND BODY WEIGHT (FROM BIRTH TO THE FIRST YEAR OF LIFE)

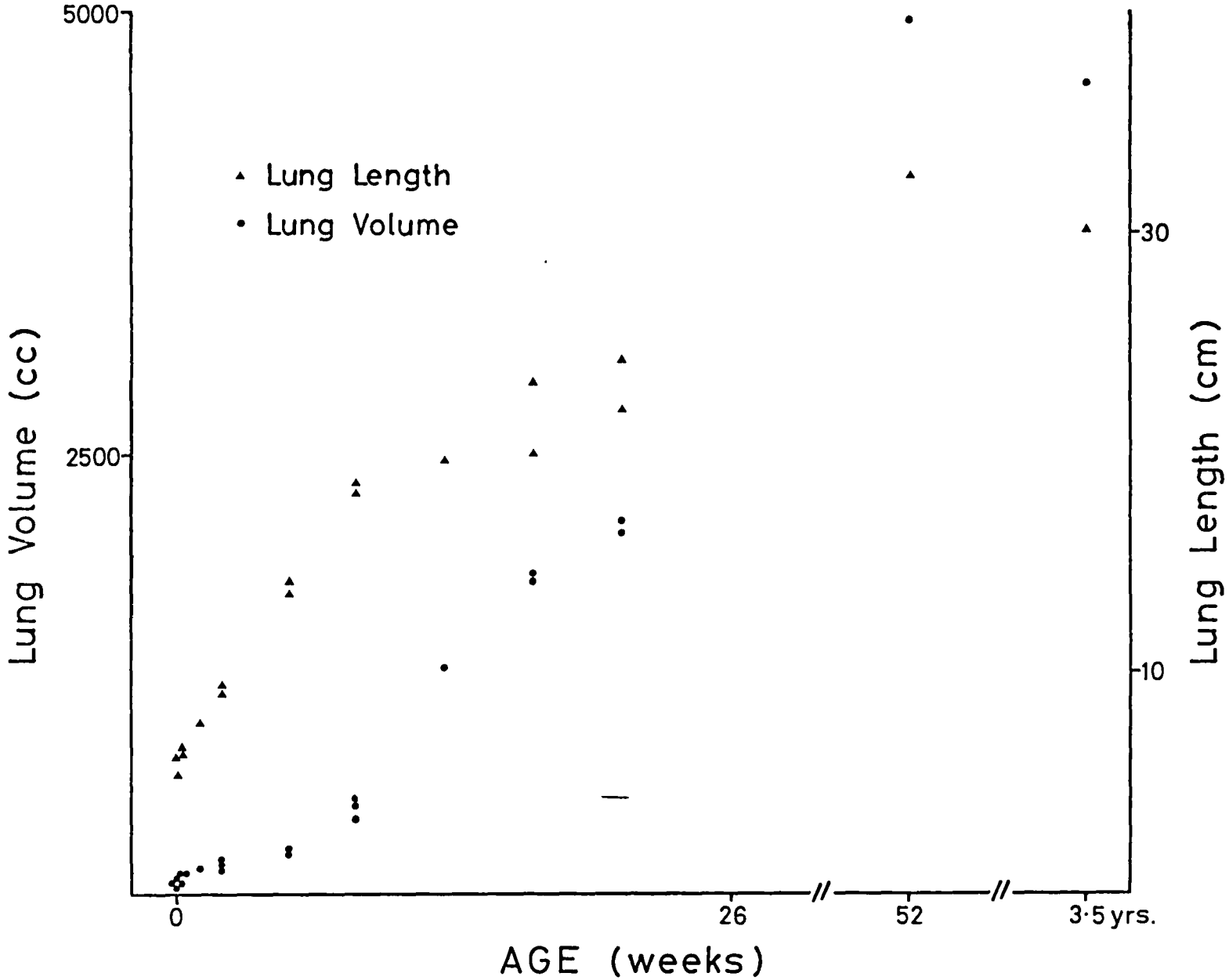
		Age (weeks)			Body Weight (Kg)			Mean	SD	SEM
		linear	power	exponential	linear	power	exponential			
Age	r	-	-	-	0.991	0.864	0.835	-	-	-
n=19	P				<0.001	<0.001	<0.001			
LV	r	0.989	0.866	0.854	0.996	0.987	0.848			
(cc)	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	-
LV/BW	r	0.520	0.512	0.509						
(cc/100 g)	P	<0.05	<0.05	<0.05	-	-	-	3.7	0.23	0.03
LL	r	0.902	0.890	0.796	0.894	0.985	0.781	-	-	-
(cm)	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			
n=15										
LL/BW	r	0.697	0.842	0.835						
(cm/100 g)	P	<0.001	<0.001	<0.001	-	-	-	0.17	0.145	0.035
n=15										

see Table III 1 for symbols

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
SD - standard deviation
SEM - standard error of the mean

Fig. III 1

Diagram showing the relation between lung volume and lung length with age (from birth to 3 1/2 years). Notice that whereas the changes in volume are evenly distributed throughout growth, the increase in lung length is faster during the first months of life slowing down afterwards


5000
2500
Lung Volume (cc)
Lung Length
Lung Volume
30
10
Lung Length (cm)
0
26
52
3·5 yrs.
AGE (weeks)

two and a half increase in length - had occurred respectively at twenty weeks and at eight weeks of age, suggesting that whereas the rate of change in lung volume was evenly distributed throughout growth, the increase in lung length was faster during the first months of life.

The ratio between lung volume and body weight, and between lung length and body weight throughout growth confirmed this suggestion (Fig. III 2). Whereas the rate of increase in length was always slower than that of body weight and decreased significantly during growth, lung volume increased faster than body weight during the first two weeks of life and was slower after the fifth week.

GENERAL STRUCTURAL ASSESSMENT. POINT COUNTING TECHNIQUES

Each lobe was divided by thick septa that passed deep into the lung and could be seen in the central regions. The macroscopic assessment of the volume proportions occupied by the various structures within the lung (Table III 3) showed that their relationship remained fairly constant throughout growth. This was illustrated by the fact that the volume proportions of the non-parenchymatous structures - arteries, veins, bronchi and connective tissue - ranged between 16.1% and 9.7% in all the animals from birth to adult life.

Microscopic point counting (Table III 4) showed that between

Fig. III 2

Diagram showing the ratios between lung volume and body weight and between lung length and body weight throughout growth. Notice that lung volume increases faster than body weight during the first two weeks of life; the increase in lung length is always slower than that of body weight

Lung Volume/Body Weight (cc/100g)
3
6
0
AGE (weeks)
25
52
0·1
0·3
Lung Length (cm/100g)
Lung Length
Lung Volume

TABLE III 3

VOLUME PROPORTIONS (%) OCCUPIED BY THE VARIOUS COMPONENTS OF LUNG TISSUE DURING GROWTH

(MACROSCOPIC POINT COUNTING)

Age	Parenchyma	Arteries	Veins	Bronchi	Connective Tissue
Newborn*	89.7	1.3	1.5	2.4	5.1
3 days*	86.1	2.8	0.9	4.4	5.8
1 week**	83.9	4.8	2.0	3.4	5.9
2 weeks*	86.0	4.1	2.4	3.8	3.7
5 " *	87.2	3.6	1.5	4.3	3.4
8 " *	86.5	4.7	2.8	2.9	4.1
12 " **	84.2	3.1	2.9	4.5	5.3
16 " *	90.3	2.5	2.6	1.5	3.1
20 " *	89.7	2.9	2.0	3.0	2.4
Adult*	88.9	2.6	2.3	3.7	2.5

** 1 case

* 2 cases

TABLE III 4

VOLUME PROPORTIONS (%) OCCUPIED BY THE VARIOUS COMPONENTS OF LUNG TISSUE DURING GROWTH

(MICROSCOPIC POINT COUNTING)

Age	Alveolar Air	Alveolar Wall	Arteries	Alveolar Duct Air	Connective Tissue
Newborn*	57.1	12.8	2.2	15.8	12.1
3 days*	65.1	9.5	3.5	13.9	8.0
1 week**	66.8	9.6	3.0	12.3	8.3
2 weeks*	62.6	10.9	2.4	17.1	7.0
5 " *	68.3	8.4	4.1	14.7	4.5
8 " *	68.5	7.8	3.4	15.0	5.3
12 " *	69.7	9.3	2.3	15.6	3.1
16 " **	73.7	7.6	3.1	12.0	3.6
20 " *	70.1	9.2	2.5	13.8	4.4
Adult*	75.8	6.1	2.3	14.3	3.5

** 1 case

* 2 cases

birth and the third day of life the proportion of lung volume occupied by alveoli increased from 57.1% to 65.1%, a value similar to that found throughout growth, which ranged between 66.8% and 75.8%. Alveolar wall thinning was apparent on microscopic examination, particularly during the first weeks of life and could be demonstrated by the reduction in the volume proportion of alveolar wall, from 12.8% at birth to 9.5% at three days of life and to 8.3% at eight weeks, a similar value to that found in the adult animal.

There was also a reduction in the volume proportion of lung tissue occupied by the connective tissue septa. This change could be traced by macroscopic point counting (Table III 3), but was more evident at microscopic level (Table III 4), where a fall could be demonstrated between birth and the fifth week of life, from 12.8% to 4.5%, the adult values being reached at that age.

THE PULMONARY ARTERIAL BED

The arterial bed was investigated along the whole pathway and the study included an assessment of arterial branching pattern, size, length, number and structure throughout growth and in the adult animals.

Structure of the Main and Left Pulmonary Arteries

The main and the left pulmonary arteries maintained an elastic

structure from birth to adult life - internal and external elastic laminae demarcating the media, which contains seven or more elastic laminae - (Elliott and Reid, 1965).

There was a considerable decrease in medial wall thickness between birth and the second week of life and the number of elastic fibers also decreased during that period (Table III 5). There was a slight disruption of the elastic fibers, apparent only in the inner third of the vascular wall (Fig. III 3).

Muscle cells were apparent in the main and left pulmonary arteries by the eighth week of age (Fig. III 4)(Fig. III 5).

Fragmentation of the elastic fibers was not striking until the twelfth week of life and even at that age, the fibers were still numerous and parallel to the vessel wall particularly in the outer third of the media.

A considerable thickness of muscle cells was seen in the medial coat of the main pulmonary artery by the twentieth week of life (Fig. III 6), together with disruption of the elastic fibers. This pattern was more evident in the left pulmonary artery at the same age (Fig. III 7). These features were very similar to those found in the adult animals (Fig. III 8).

The arterial intima consisted of a single layer of endothelial

TABLE III 5

STRUCTURE OF THE MAIN AND LEFT PULMONARY ARTERIES DURING GROWTH

Age	Main Pulmonary Artery		Left Pulmonary Artery	
	Tickness of the Media (μm)	Number of Elastic Laminae	Tickness of the Media (μm)	Number of Elastic Laminae
Newborn	950.0 56.3	33.0 4.6	535.0 13.9	19.0 1.7
2 weeks	512.0 68.7	22.0 5.7	380.0 7.8	11.0 0.5
5 "	490.0 47.8	14.0 1.1	228.0 23.7	10.0 0.4
8 "	379.0 22.4	13.0 1.2	196.0 14.7	8.0 0.7
12 "	392.0 18.8	15.0 0.7	188.0 23.5	7.0 0.3
16 "	343.0 18.9	11.0 1.3	165.0 12.1	7.0 0.4
20 "	336.0 12.2	12.0 0.9	194.0 4.4	8.0 0.3
Adult	488.0 24.4	11.0 0.8	230.0 13.3	7.0 0.5

Mean and standard error of the mean

Fig. III 3

Photomicrograph of the main pulmonary artery from a normal case aged two weeks. Notice the orderly arrangement of the elastic fibers in the media (x170)

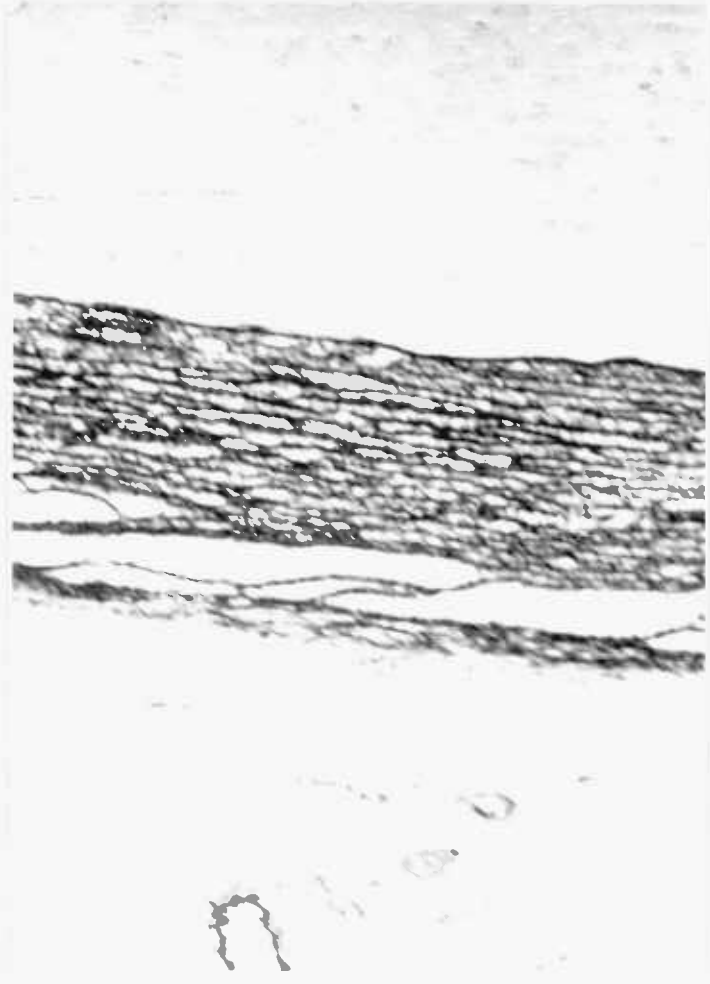
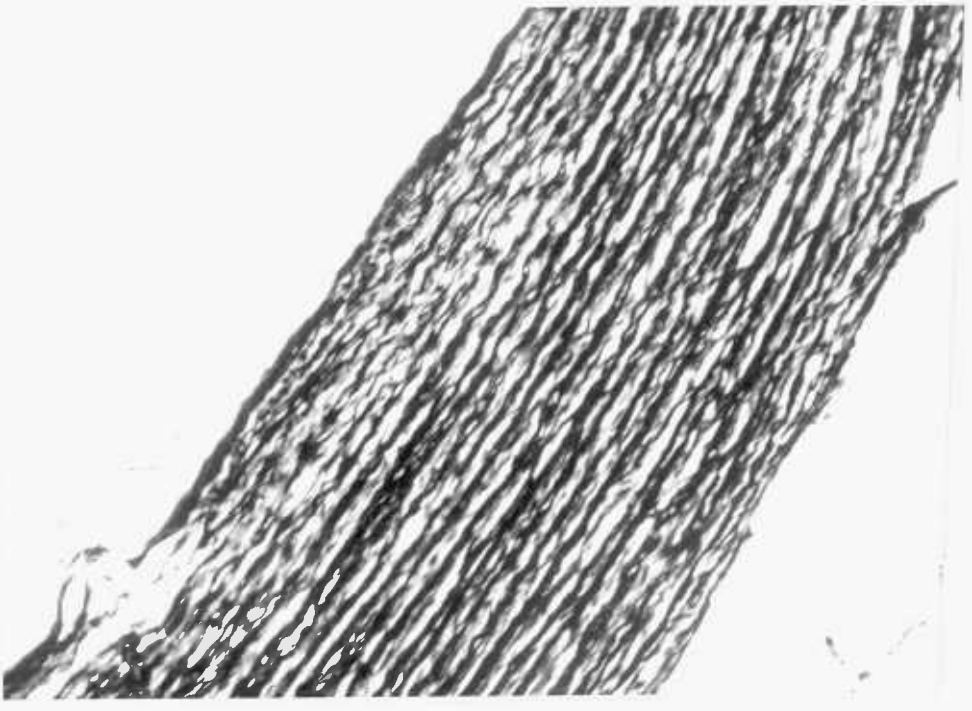


Fig. III 4

Photomicrograph of the main pulmonary artery from a normal case aged eight weeks. Notice the appearance of muscle cells between the organized elastic fibers whose fragmentation is only apparent in the inner third of the medial wall (x420)
(lumen to the left of the figure)

Fig. III 5

Photomicrograph of the left pulmonary artery from a normal case aged eight weeks. The disruption of the elastic fibers is more apparent than in the main vessel (Fig. III 4) (x420)
(lumen to the right of the figure)



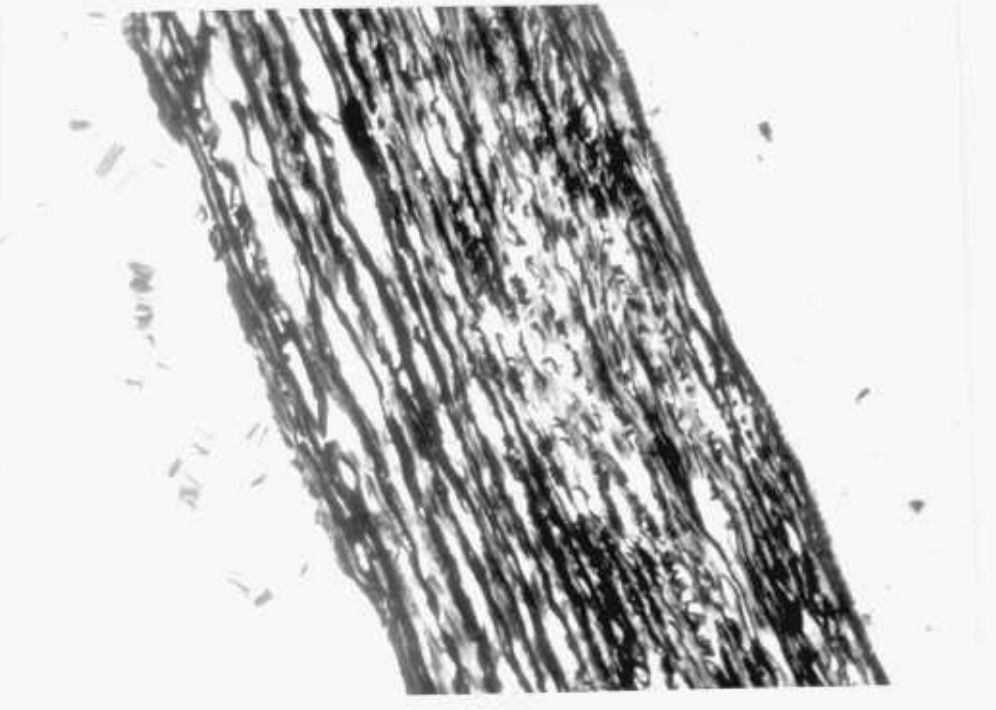


Fig. III 6

Photomicrograph of the main pulmonary artery from a normal case aged twenty weeks. Notice the disruption of the elastic fibers in the medial wall (x170)
(lumen to the upper part of the figure)

Fig. III 7

Photomicrograph of the left pulmonary artery from a normal case aged twenty weeks. Notice the increase in muscle cells between the fragmented elastic fibers (x420)
(lumen to the left of the figure)

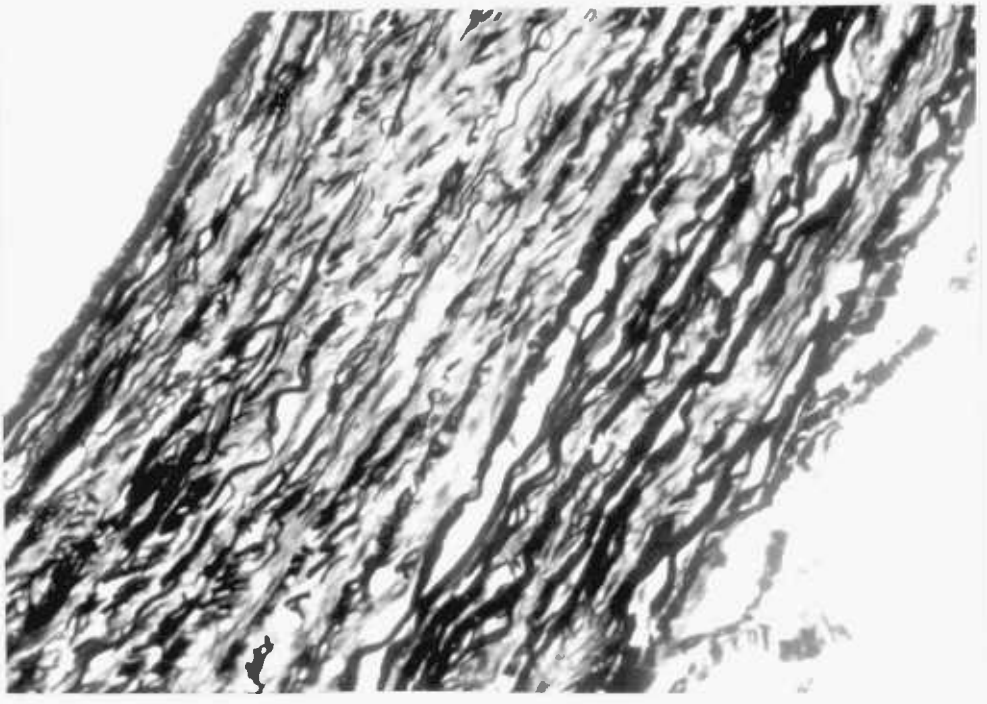
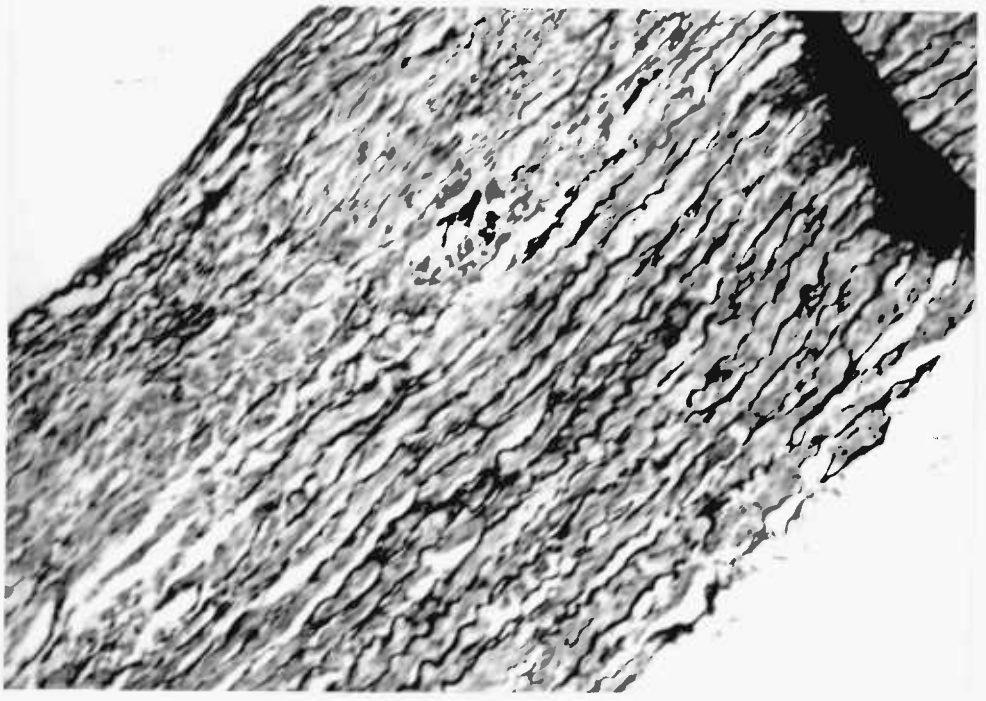


Fig. III 8

Photomicrograph of the left pulmonary artery from a normal adult case. Notice the thinning and fragmentation of the elastic fibers; muscle cells are increased (x420)



cells and was thin in all specimens except those from the adult animals in which there was slight intimal proliferation.

Pre-Acinar Arteries

At pre-acinar level, the arterial branching pattern was assessed on the arteriograms and by macroscopic dissection. The arterial wall structure was studied along the axial pathway length and at the point of origin of the conventional branches.

The arteriogram

A good filling of the pulmonary vascular bed was obtained in all cases. The arterial branching pattern was similar throughout growth (Fig. III 9-17).

Between birth and the third day of life, a considerable increase in the background haze had already occurred. This feature was also described in the human over a longer time, by Davies and Reid (1971), who showed that appearance of background haze on the arteriograms reflected the increase in intra-acinar arteries which occurred during growth. These changes will be further discussed with the histological studies of the intra-acinar arteries.

The vascular injection did not fill any pulmonary veins or bronchial vessels.

Fig. III 9

Pulmonary arteriogram of a newborn pig (x1.1)

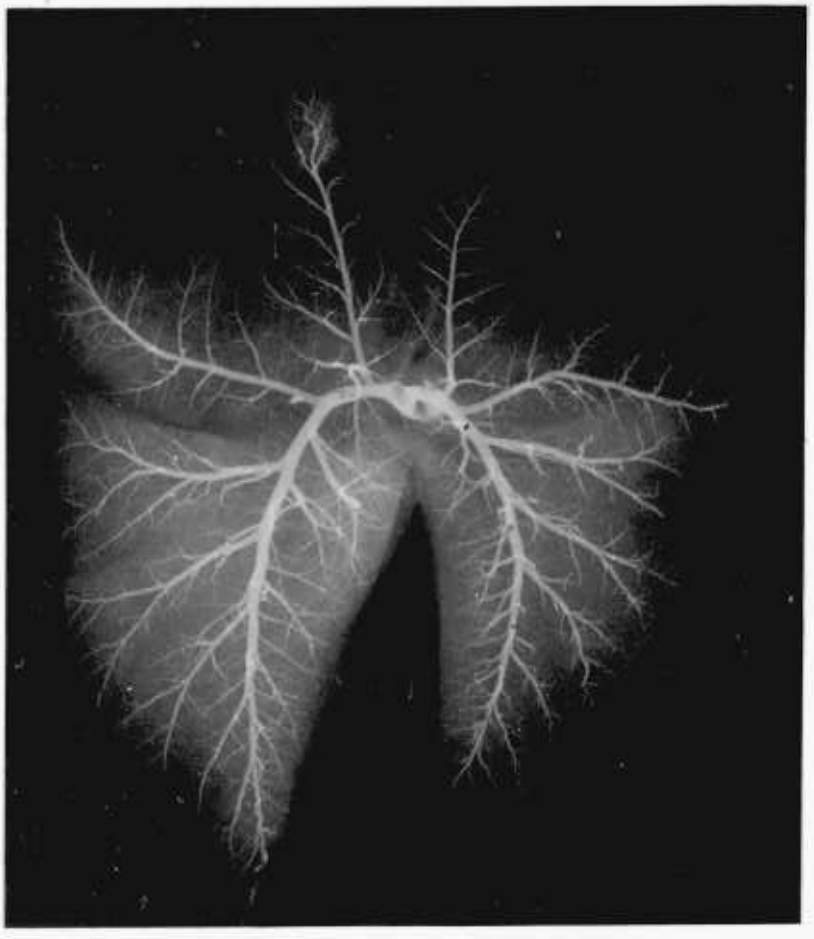


Fig. III 10

Pulmonary arteriogram of a pig aged three days
(xl.l)

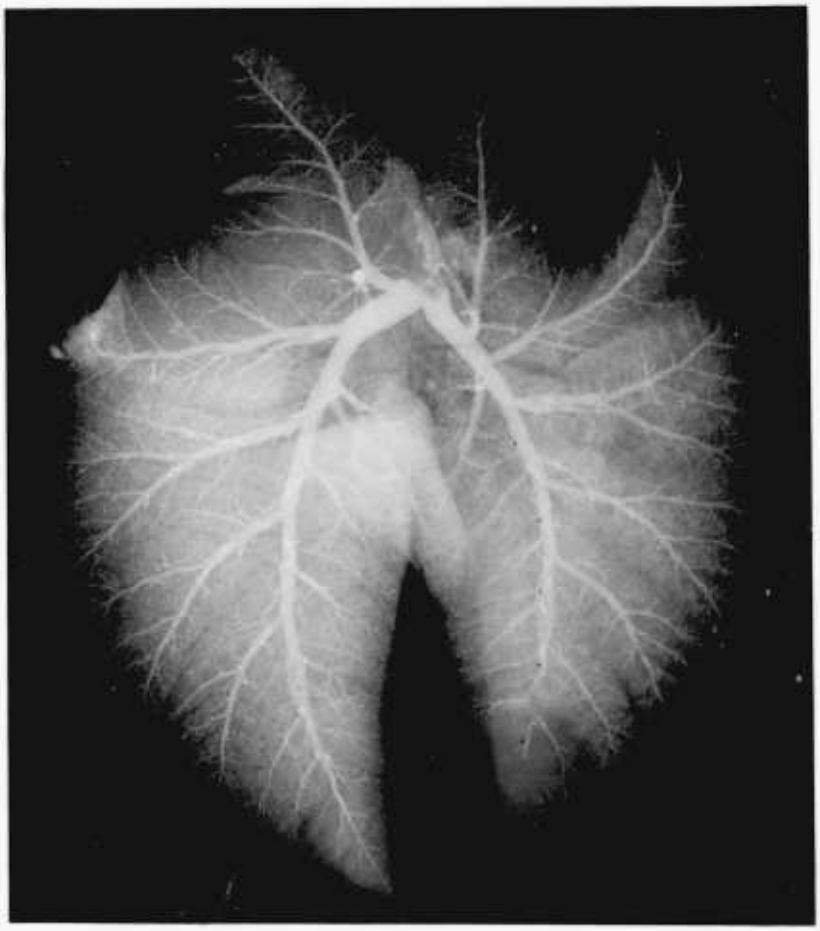


Fig. III 11

Pulmonary arteriogram of a pig aged one week
(x0.7)

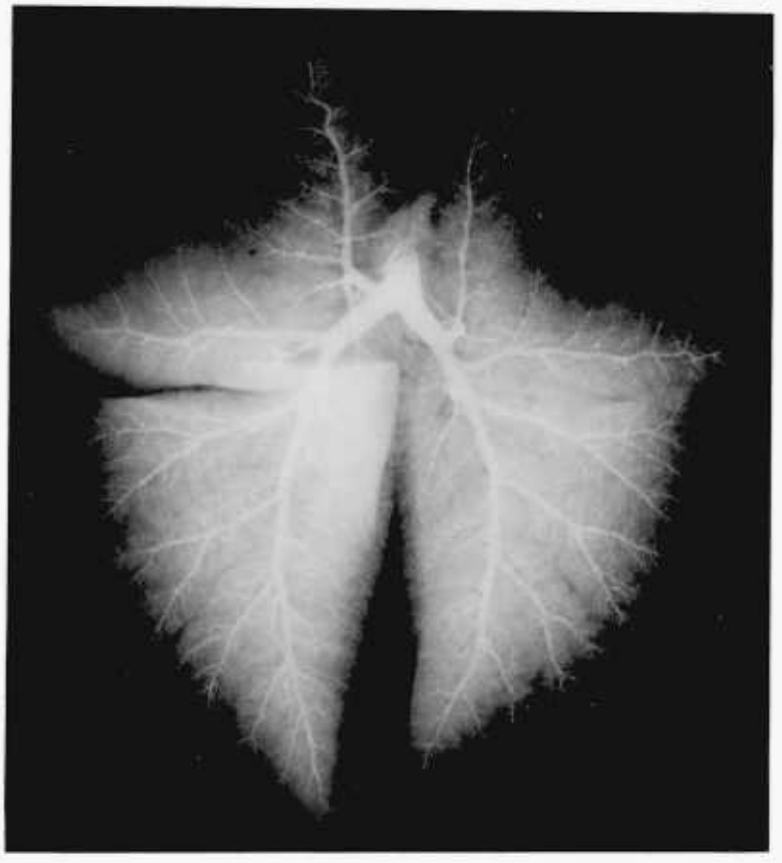


Fig. III 12

Pulmonary arteriogram of a pig aged two weeks (left lung)
(x1.1)

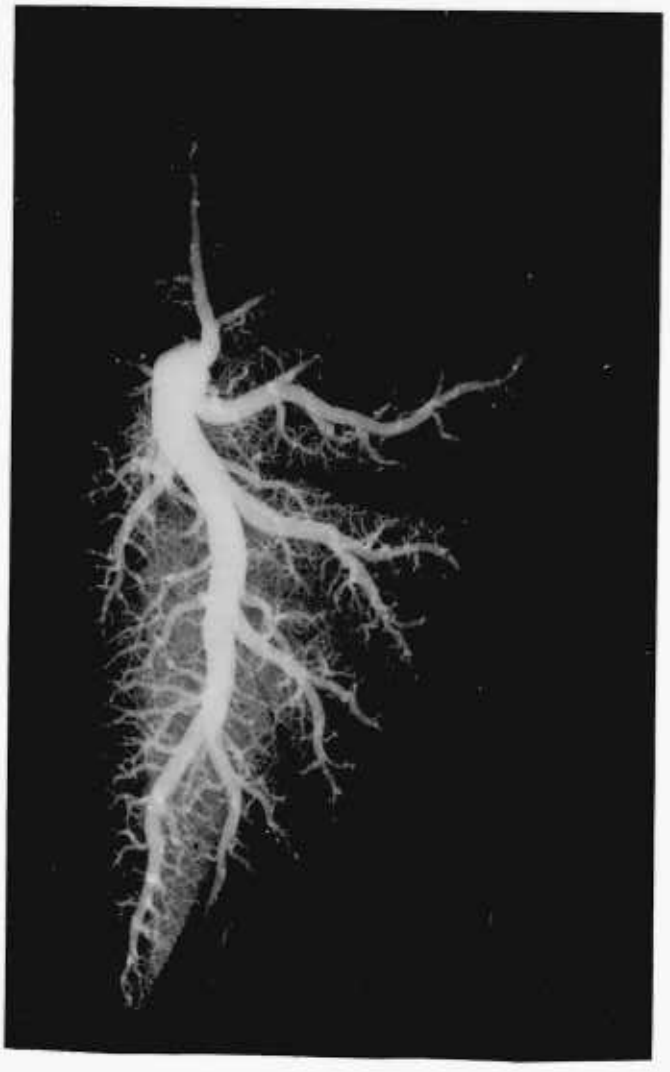


Fig. III 13

Pulmonary arteriogram of a pig aged five weeks (left lung)
(x0.8)

Fig. III 14

Pulmonary arteriogram of a pig aged eight weeks (left lung)
(x0.7)

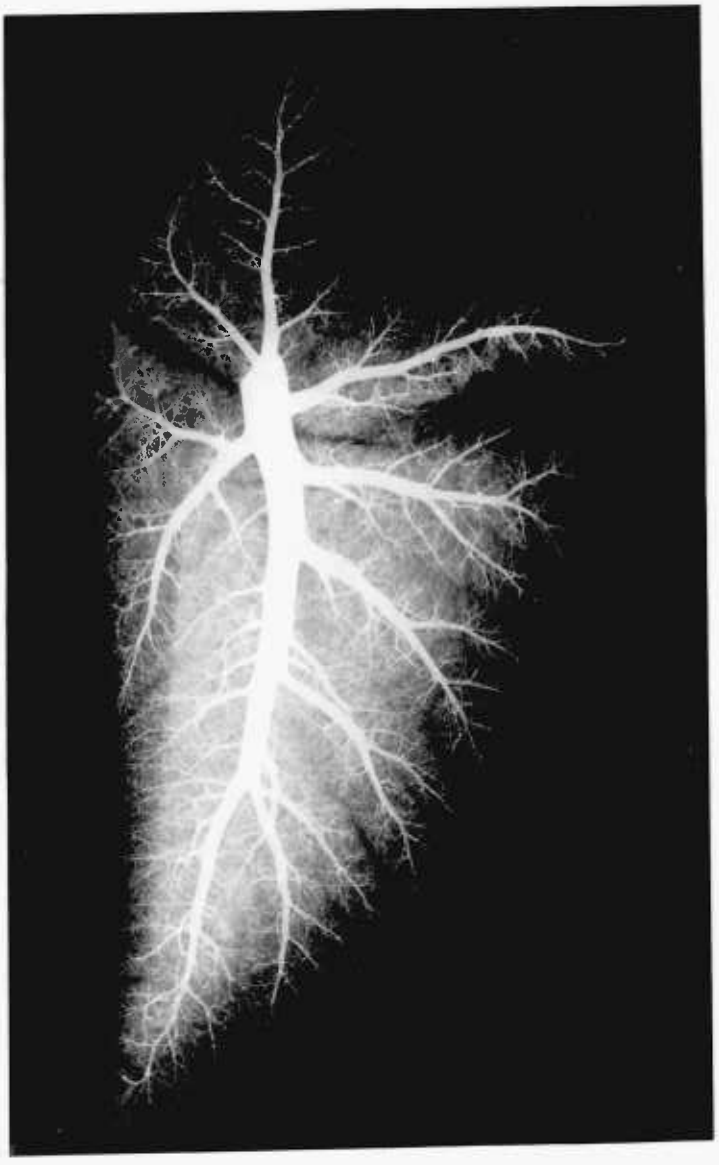


Fig. III 15

Pulmonary arteriogram of a pig aged twelve weeks
(left lung) (x0.6)

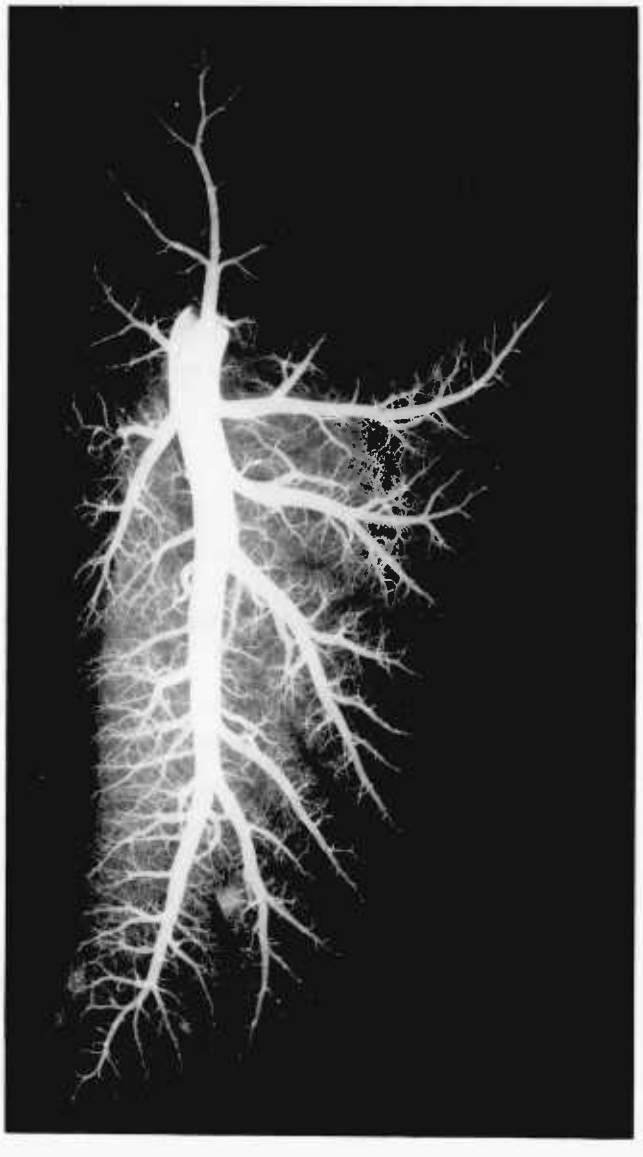


Fig. III 16

Pulmonary arteriogram of a pig aged twenty weeks (left lung)
(x0.4)

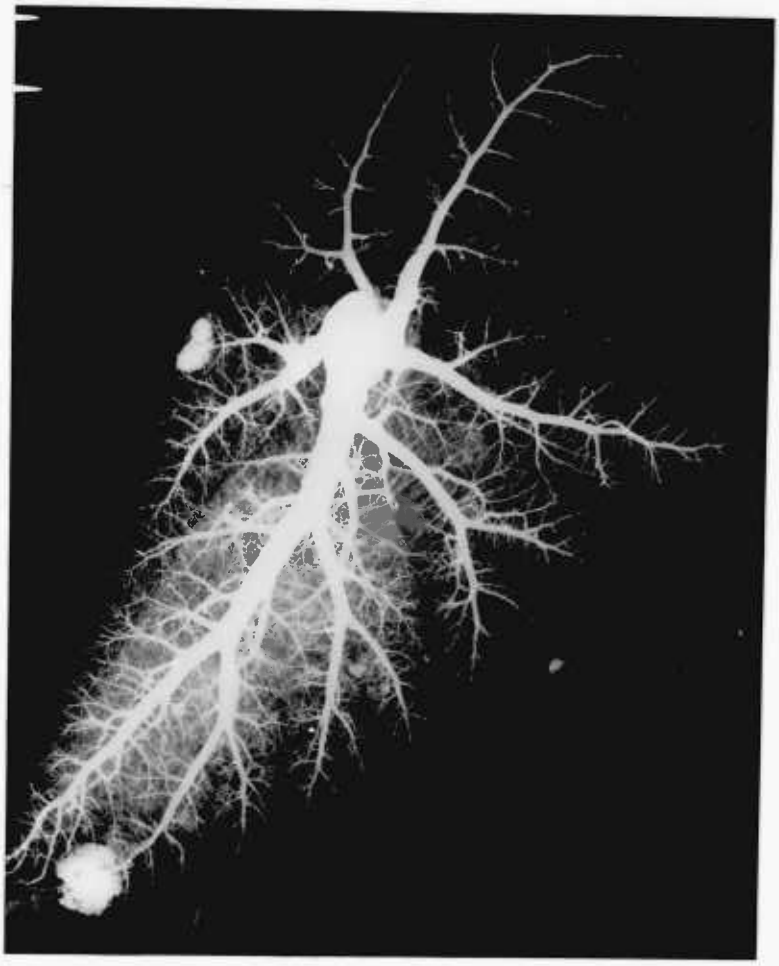
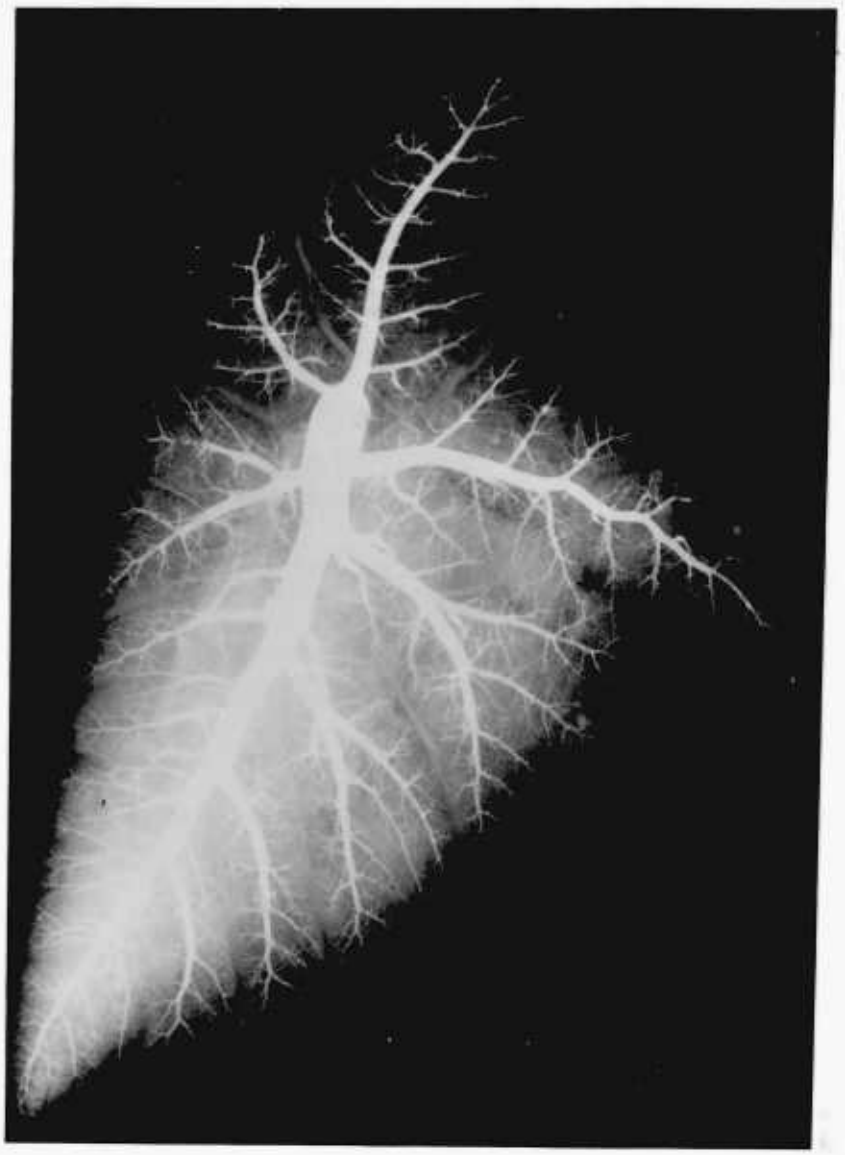


Fig. III 17

Pulmonary arteriogram of an adult pig (one year)
(x0.3)



Measurements of axial length and diameter The length and diameter of the pre-acinar arteries increased during growth (Table III 6).

There was five fold increase in axial length between birth and the first year of life, and half of that change had already occurred in the first two months, suggesting that the rate of increase in axial length was faster in early life.

The rate of increase in lumen diameter along the axial pathway was also faster during the first two months of life (Table III 6). When the changes in diameter at the hilum were compared with those at the periphery (75% of the axial length) throughout growth (Fig. III 18), it was noticed that the hilar vessels increase an average of 9.4 fold in diameter, whereas the peripheral ones increased a 7.5 fold between birth and adult life.

Number of generations from the axial pathway

Macroscopic dissection of the lower left lobar arterial and bronchial pathways in three specimens (cases A.1 - newborn, A.9 - two weeks, A.13 - eight weeks) revealed nineteen generations of bronchi in each case, whereas 16, 18 and 17 branches, respectively, were counted accompanying the airways - the "conventional arteries" (Elliott and Reid, 1965).

As in the human, extra or "supernumerary" arteries were also found in the pig (Hislop and Reid, 1973). These increased in number from 21 at birth to 31 at five weeks, increasing the ratio of

TABLE III 6

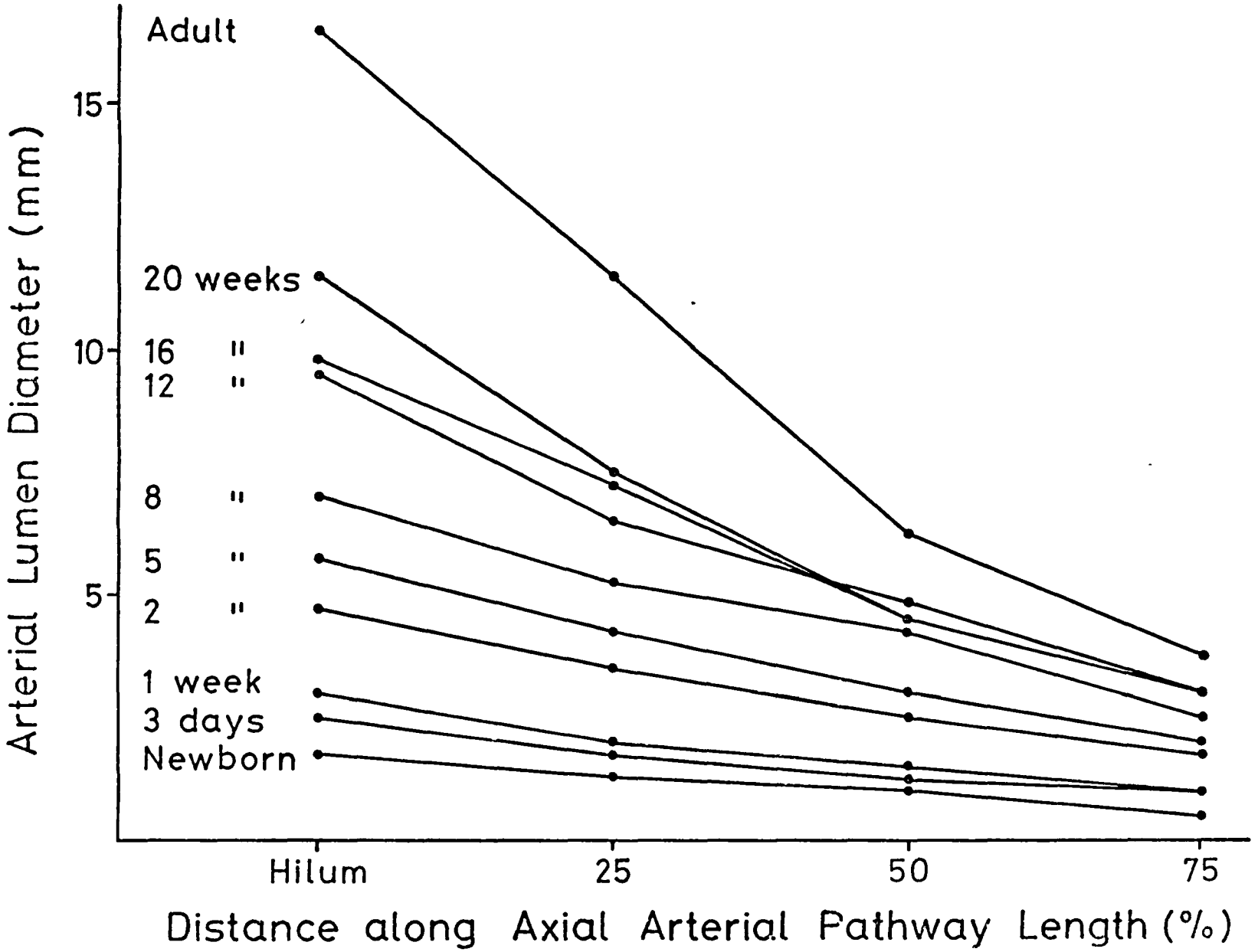
MEASUREMENTS FROM THE ARTERIOGRAMS IN NORMAL ANIMALS. LEFT LOWER LOBE ARTERY - TOTAL AXIAL LENGTH, DIAMETER AT THE HILUM AND AT 25% INTERVALS ALONG THE PATHWAY

Age	Length (mm)	Diameter (mm) Hilum	25	50	75
Newborn	57	2.0	1.5	1.0	0.5
"	49	1.5	1.0	1.0	0.5
3 days	51	2.0	1.5	1.0	1.0
3 "	59	3.0	2.0	1.5	1.0
1 week	71	3.0	2.0	1.5	1.0
2 weeks	70	4.5	3.0	2.5	1.5
2 "	78	5.0	4.0	2.5	2.0
5 "	97	6.0	4.5	3.0	2.5
5 "	107	5.5	4.0	3.0	1.5
8 "	129	7.0	5.0	4.0	2.0
8 "	138	7.0	5.5	4.5	3.0
12 "	159	9.5	6.5	4.5	3.0
16 "	167	9.5	7.5	5.0	3.5
16 "	173	10.0	7.0	4.5	2.5
20 "	194	12.0	8.5	4.0	3.0
20 "	214	11.0	6.5	5.0	4.0
Adult (1 year)	290	18.0	13.0	7.0	4.0
" (3½ years)	271	15.0	10.0	5.5	3.5

Mean value for each case

Fig. III 18

Diagram illustrating the changes in lumen diameter of the pre-acinar arteries with age. Measurements from the arteriograms; mean left lower lobe artery, diameter at the hilum and at 25% intervals along the axial pathway


Adult
20 weeks
16 =
12 =
8 =
5 =
2 =
1 week
3 days
Newborn
15
10
5
Arterial Lumen Diameter (mm)
Hilum
25
50
75
Distance along Axial Arterial Pathway Length (%)

"supernumerary" to "conventional" arteries, from 1.3 to 1.7.

In the three cases studied, aged two hours, two and eight weeks, a similar structural pattern was found along the left lower lobe artery. The vessel was elastic from its origin to the 7th or 9th generation, approximately halfway along the axial pathway. It was transitional until the 10th generation and muscular afterwards as far as the lung periphery.

The structure of the conventional branches, at their origin from the left lower lobe artery, was also similar in the three cases. The elastic segment of the axial length gave conventional branches which either were elastic, transitional or muscular (internal and external laminae and less than four laminae in the predominantly muscular media, Elliott and Reid, 1965). The branches originating from the transitional and muscular segments were always muscular at their site of origin from the axial pathway (Table III 7).

The structure of the "supernumerary" arteries was not studied.

In all cases the macroscopic dissection was possible until approximately 5 mm from the pleural surface. This could account for some error in the assessment of the total number of generations, mainly because the pre-acinar pathway might extend further to the lung periphery. In the human, using serial reconstructions, Hislop (1971) has shown that the length of the intra-acinar axial pathway increased from 1330 to 1520 μm between birth and four months of age and from 2520 to 5180 μm between eighteen months and eleven years.

STRUCTURE OF THE LEFT LOWER LOBE ARTERY AND ITS CONVENTIONAL BRANCHES AT THE SITE OF ORIGIN

Generation No.	Arterial Structure					
	Newborn		2 weeks		8 weeks	
	Axial Path.	Convent. Branch	Axial Path.	Convent. Branch	Axial Path.	Convent. Brach
1		E		E		E
2		T		E		E
3	E	E		T		T
4		E		T	E	M
5		T	E	M		T
6		M		M		M
7		M		M		M
8	T	M		M		M
9		M		M		M
10		M	T	M	T	M
11		M		M		M
12	M	M		M		M
13		M		M		M
14		M		M	M	M
15		M	M	M		M
16		M		M		M
17				M		M
18				M		

E - elastic
T - transitional
M - muscular

Path. - pathway
Convent. - conventional

The Peripheral Arterial Bed

The media of the muscular pulmonary arteries in the pig was bounded by two distinct elastic laminae (Fig. III 19). Like the human and the rat, at the periphery of any arterial pathway, the completely "muscular" coat is replaced by a spiral of muscle to give a "partially muscular" artery, until the vessel finally became "non-muscular" (Reid, 1968)(Hislop and Reid, 1976)(Meyrick, 1976).

The arteries found within the lung periphery could be grouped by size and by structural type, and the proportion of each structural type within any size group determined, this analysis being described as a "population study". Where a small artery accompanied a small airway, it was also characterized by reference to the type of airway it accompanied. Thus, the structure of an artery could be related to its size and airway level - in this way, extension of muscle along the peripheral arterial pathway during growth was established.

Percentage medial wall thickness

Peripheral arteries less than 1000 μm in external diameter were extremely thick walled at birth (Table III 8). From the diagram relating the percentage wall thickness to the external diameter at this age, two separate compartments could be identified (Fig. III 20). One included the smaller vessels (< 200 μm in external diameter), in which the values for the mean percentage wall thickness were between 5.4 and 11.4, the mean value being 8.0. The other included the larger arteries (200-1000 μm in external diameter), in which the mean percentage

Fig. III 19

Photomicrograph of a muscular pulmonary artery from a normal adult pig showing the media bounded by two distinct elastic laminae (x420)

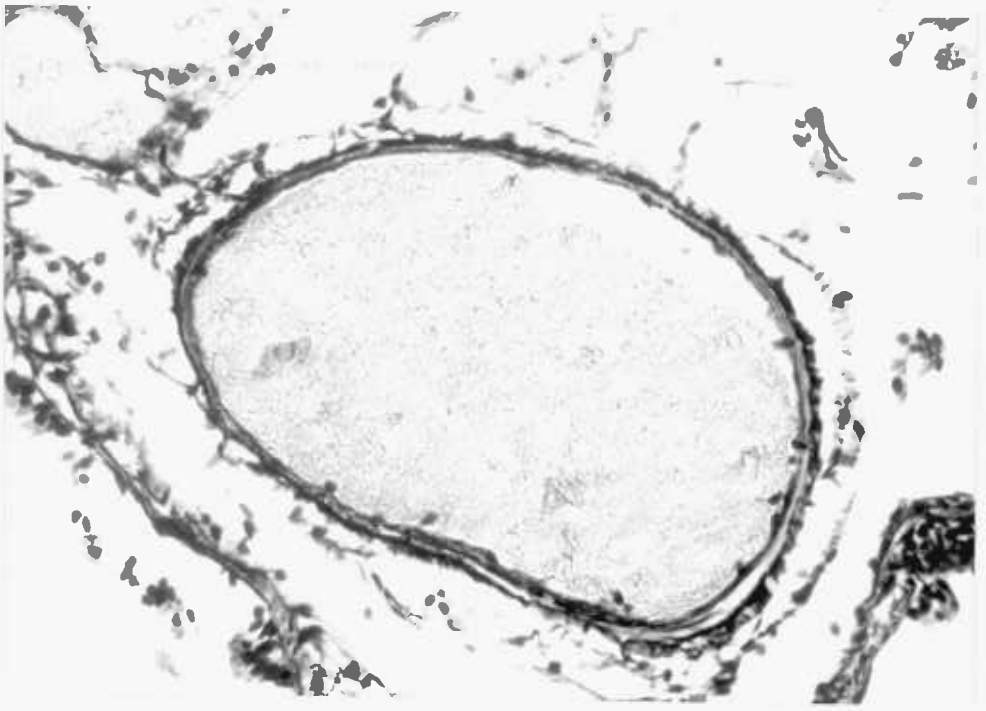


Fig. III 20

Diagram relating percentage arterial wall thickness to external diameter (µm) in the newborn pig. Mean and standard deviation values are represented for every size range (arteries smaller than 1000 µm). Notice the extreme wall thickness of the peripheral muscular arteries at birth

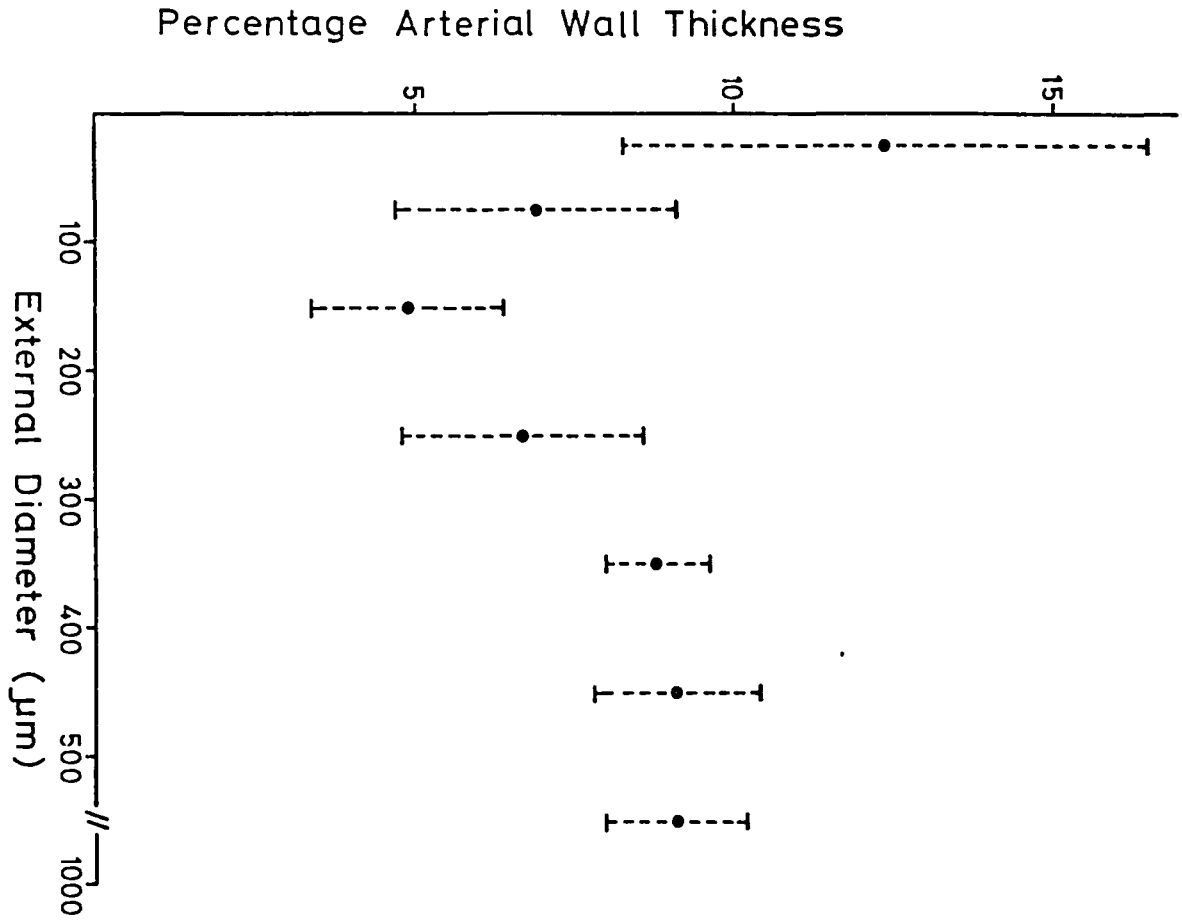

Percentage Arterial Wall Thickness
5
10
15
External Diameter (μm)
100
200
300
400
500
1000

TABLE III 8

PERCENTAGE ARTERIAL WALL THICKNESS DURING GROWTH

Age	External Diameter (μm) 0-50	50-100	100-200	200-300	300-400	400-500	500-1000
Newborn	12.4	6.9	4.9	6.7	8.8	9.1	9.1
	0.96	0.44	0.38	0.66	0.60	0.95	0.58
	111	26	15	3	2	2	4
"	10.4	7.6	6.0	6.9	8.4	7.8	10.9
	0.27	0.32	0.42	0.26	0.75	0.60	1.23
	113	34	13	5	5	3	4
3 days	7.5	4.9	4.0	4.7	4.3	4.4	4.7
	0.46	0.26	0.16	0.48	0.25	0.25	0.29
	38	51	19	4	3	3	3
3 "	7.1	3.9	3.4	3.8	4.5	4.9	4.2
	0.34	0.16	0.25	0.47	0.50	0.12	0.23
	46	53	15	5	3	3	3
1 week	6.8	3.8	3.4	4.3	4.0	5.8	5.6
	0.36	0.14	0.23	0.36	0.60	0.95	0.56
	21	50	35	10	5	2	5
2 weeks	7.0	4.1	3.9	4.5	4.5	3.8	4.9
	0.24	0.11	0.27	0.49	0.5	0.55	0.53
	54	95	17	10	6	2	9
2 "	6.2	3.8	2.3	4.0	2.9	4.0	3.1
	0.40	0.11	0.15	0.63	0.26	0.53	0.29
	112	47	26	12	7	4	9
5 "	8.0	4.3	3.9	3.1	3.5	3.5	3.6
	0.34	0.21	0.20	0.25	0.25	0.19	0.20
	57	59	28	3	3	3	3
5 "	7.3	4.1	3.1	3.5	3.2	3.8	3.4
	0.17	0.09	0.20	0.25	0.22	0.32	0.20
	162	149	33	5	3	5	3
8 "	7.5	3.4	2.4	2.1	1.9	1.9	1.5
	0.26	0.07	0.09	0.66	0.15	0.11	0.07
	72	94	63	12	8	4	6
8 "	8.7	3.6	2.6	2.6	3.5	3.2	2.1
	0.71	0.04	0.09	0.20	0.55	0.56	0.63
	253	250	79	18	5	3	3
12 "	8.3	3.8	3.2	3.0	3.5	3.2	2.5
	0.32	0.14	0.16	0.63	0.34	0.63	0.32
	73	49	41	7	6	3	3
16 "	7.2	3.6	2.1	1.7	1.4	1.7	1.6
	0.32	0.09	0.09	0.14	0.04	0.20	0.31
	47	81	48	6	5	3	3
16 "	7.4	3.8	2.7	2.6	2.8	2.0	2.0
	0.39	0.10	0.12	0.23	0.36	0.27	0.14
	55	100	48	6	5	3	2
20 "	8.5	4.4	2.6	3.1	2.5	2.9	2.2
	0.41	0.52	0.12	0.23	0.25	0.44	0.45
	192	84	54	9	9	5	6
20 "	8.3	4.0	2.8	3.1	2.9	2.9	3.0
	0.33	0.12	0.14	0.33	0.49	0.44	0.34
	74	75	35	10	3	5	4
Adult (1 year)	7.3	3.6	2.8	2.3	2.5	2.9	2.5
	0.20	0.11	0.13	0.20	0.42	0.55	0.48
	89	55	37	10	4	2	4
Adult	6.6	3.7	2.1	2.8	2.1	1.9	1.8
(3½ years)	0.22	0.09	0.10	0.15	0.34	0.15	0.40
	71	75	63	18	6	3	4

Mean, standard error and no. of vessels measured in each case

wall thickness was between 6.8 (for arteries 200-300 μm) and 10.0 (for arteries 500-1000 μm), the mean value being 8.4.

By the third day of life, there was a considerable decrease in wall thickness in arteries of all sizes (Fig. III 21), particularly in those smaller than 200 μm, since the mean percentage wall thickness had already dropped to 5.1, a value similar to adult range of 4.1-4.6 (Table III 9).

In the larger arteries, it was not until eight weeks of age (Fig. III 22) that the mean arterial wall thickness reached 2.3, a value within the mean adult range of 2.1-2.5 (Table III 9)(Fig. III 23-24).

Population studies

In the newborn pig lung all the arteries over 100 μm had a complete muscular coat (Fig. III 25), while the smallest muscular artery was 25 μm in external diameter (Table III 10). The "population curves" (Fig. III 26) at this age showed that muscle was found at the very distal end of the vascular bed. At ~~three days~~ one week of life, the shape of the "muscular curve" changed considerably (Fig. III 27), the smallest muscular artery being 42 μm in external diameter: at ~~one and~~ two weeks of age, similar muscular populations were found (Table III 10).

These findings suggested that the immediate postnatal increase in arterial size was not accompanied by a proportional increase in

Fig. III 21

Diagram relating percentage arterial wall thickness to external diameter in swine at three days of age. Mean and standard deviation are represented for every size range (arteries smaller than 1000 μm). Notice the considerable drop in wall thickness in vessels of all sizes when compared with the newborn (Fig. III 20). Vessels smaller than 200 μm in external diameter reach the adult wall thickness at this age

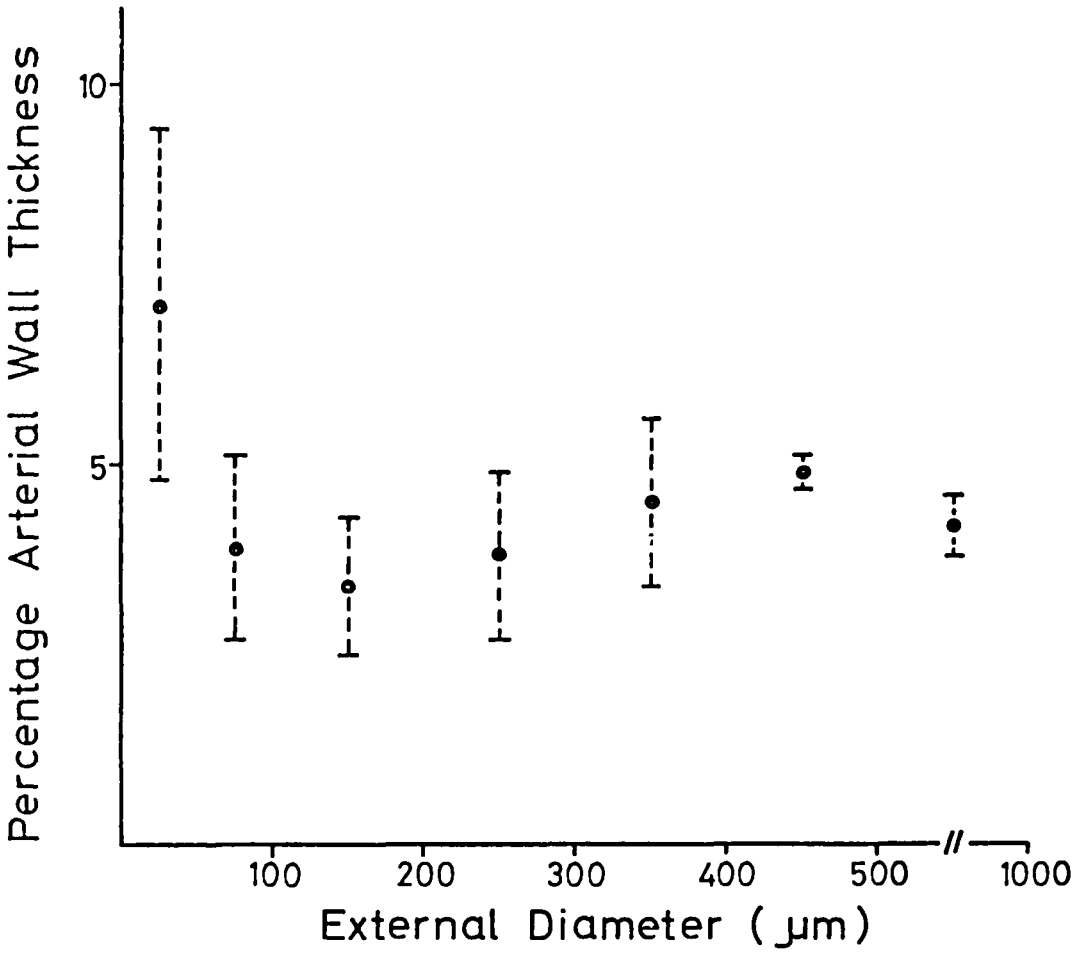

Percentage Arterial Wall Thickness
10
5
100
200
300
400
500
1000
External Diameter (µm)

TABLE III 9

ARTERIES - PERCENTAGE WALL THICKNESS DURING GROWTH

Age	External Diameter (µm)									
	0-50	50-100	100-200	200-300	300-400	400-500	500-1000		0-200	200-1000
Newborn *	11.4	7.2	5.4	6.8	8.6	8.4	10.0	***	8.0	8.4
	1.0	0.35	0.55	0.10	0.20	0.65	0.90		1.78	0.66
	224	60	28	8	7	5	8			
3 days *	7.3	4.4	3.7	4.2	4.4	4.6	4.4	***	5.1	4.4
	0.20	2.50	0.30	0.45	0.10	0.24	0.24		1.11	0.09
	84	104	34	9	6	6	6			
1 week **	6.8	3.8	3.4	4.3	4.1	5.8	5.6	***	4.7	4.9
	0.36	0.14	0.23	0.36	0.60	0.95	0.56		1.07	0.17
	21	50	35	10	5	2	5			
2 weeks*	6.6	3.9	3.1	4.2	3.7	3.9	4.0	***	4.5	3.9
	0.40	0.14	0.80	0.24	0.80	0.10	0.90		1.06	0.10
	166	142	43	22	13	6	18			
5 " *	7.6	4.2	3.5	3.3	3.5	3.6	3.5	***	5.1	3.5
	0.35	0.10	0.40	0.20	0.14	0.14	0.10			
	219	208	61	8	6	8	6			
8 " *	8.1	3.5	2.5	2.3	2.7	2.5	1.8	***	4.7	2.3
	0.60	0.10	0.10	0.24	0.60	0.65	0.30		1.72	0.18
	325	344	142	30	13	7	9			
12 " **	8.3	3.8	3.2	3.0	3.5	3.2	2.5	***	5.1	3.0
	0.32	0.14	0.16	0.63	0.34	0.63	0.32		1.61	0.22
	73	49	41	7	6	3	3			
16 " *	7.3	3.7	2.4	2.1	2.1	1.8	1.8	***	4.5	1.9
	0.10	0.10	0.30	0.45	0.70	0.14	0.20		1.46	0.09
	102	181	96	12	10	6	5			

20 "	8.4 0.10 266	4.2 0.20 159	2.7 0.10 35	3.1 0.05 19	2.7 0.20 12	2.9 0.05 10	2.6 0.40 10	***	5.1 1.71	2.8 0.09
Adult (1 year) **	7.3 0.20 89	3.5 0.11 55	2.8 0.13 37	2.3 0.20 10	2.5 0.42 4	2.9 0.55 2	2.5 0.48 4	***	4.5 1.40	2.5 0.12
Adult (3½ years)**	6.6 0.22 71	3.7 0.09 75	2.1 0.10 63	2.8 0.15 18	2.1 0.34 6	1.9 0.15 3	1.8 0.4 4	***	4.1 1.32	2.1 0.22

* 2 cases - mean, standard error of the mean and total number of vessels measured (from the original means - Table III 8)

** 1 case - mean, standard error of the mean and total number of vessels measured

*** Mean and standard error of the mean (from the means shown in this Table)

Fig. III 22

Diagram relating percentage arterial wall thickness to external diameter in swine at eight weeks of age. Mean and standard deviation are represented for every size range (arteries smaller than 1000 µm). Vessels larger than 200 µm decrease in wall thickness until the eighth week of life when the adult values are reached

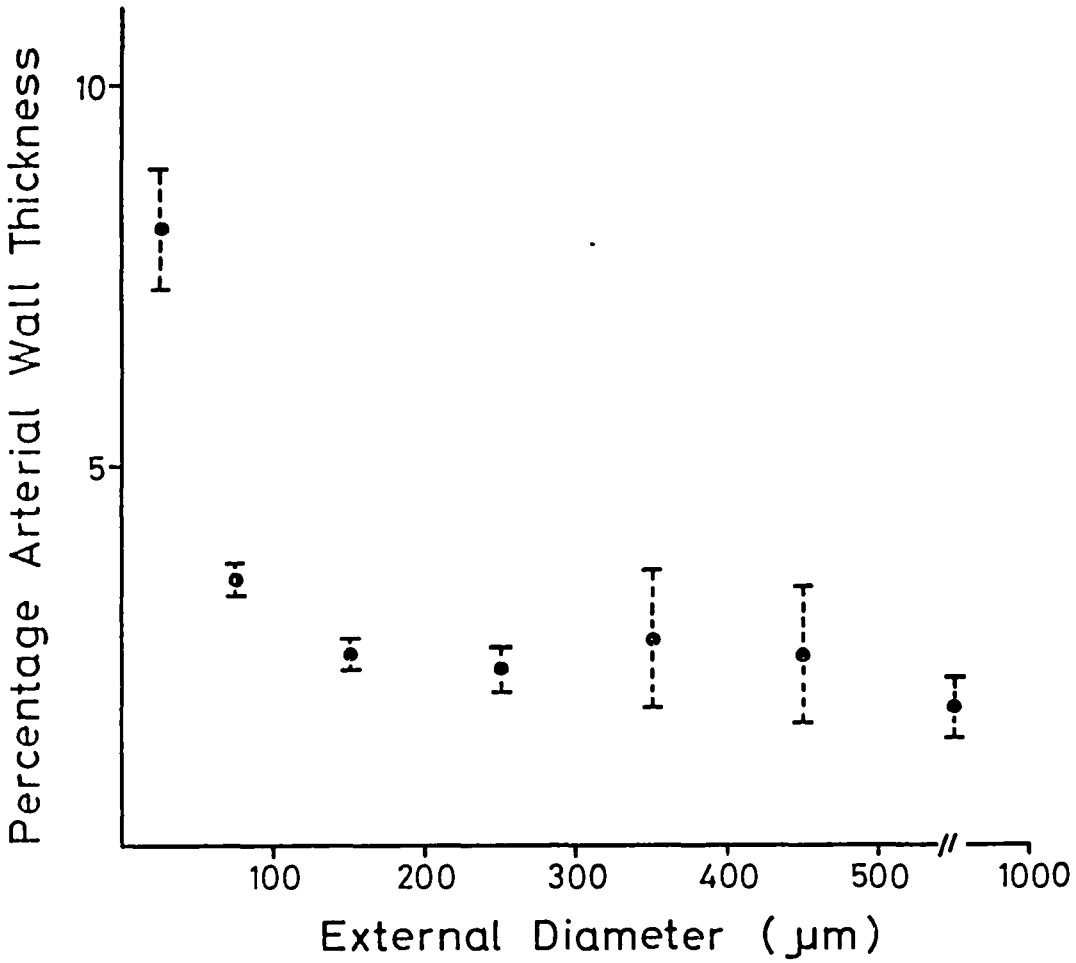

Percentage Arterial Wall Thickness
10
5
100
200
300
400
500
1000
External Diameter (μm)

Fig. III 23

Diagram relating percentage arterial wall thickness to external diameter in adult swine. Mean and standard deviation are represented for every size range (arteries smaller than 1000 µm)

Percentage Arterial Wall Thickness
10
5
100
200
300
400
500
1000
External Diameter (µm)

Fig. III 24

Diagram relating mean percentage arterial wall thickness to external diameter (μm) in normal swine throughout growth. Newborn (2 cases), 3-14 days (5 cases), 5 weeks (2 cases) and 2 months-adult (9 cases)

Mean value for every size range

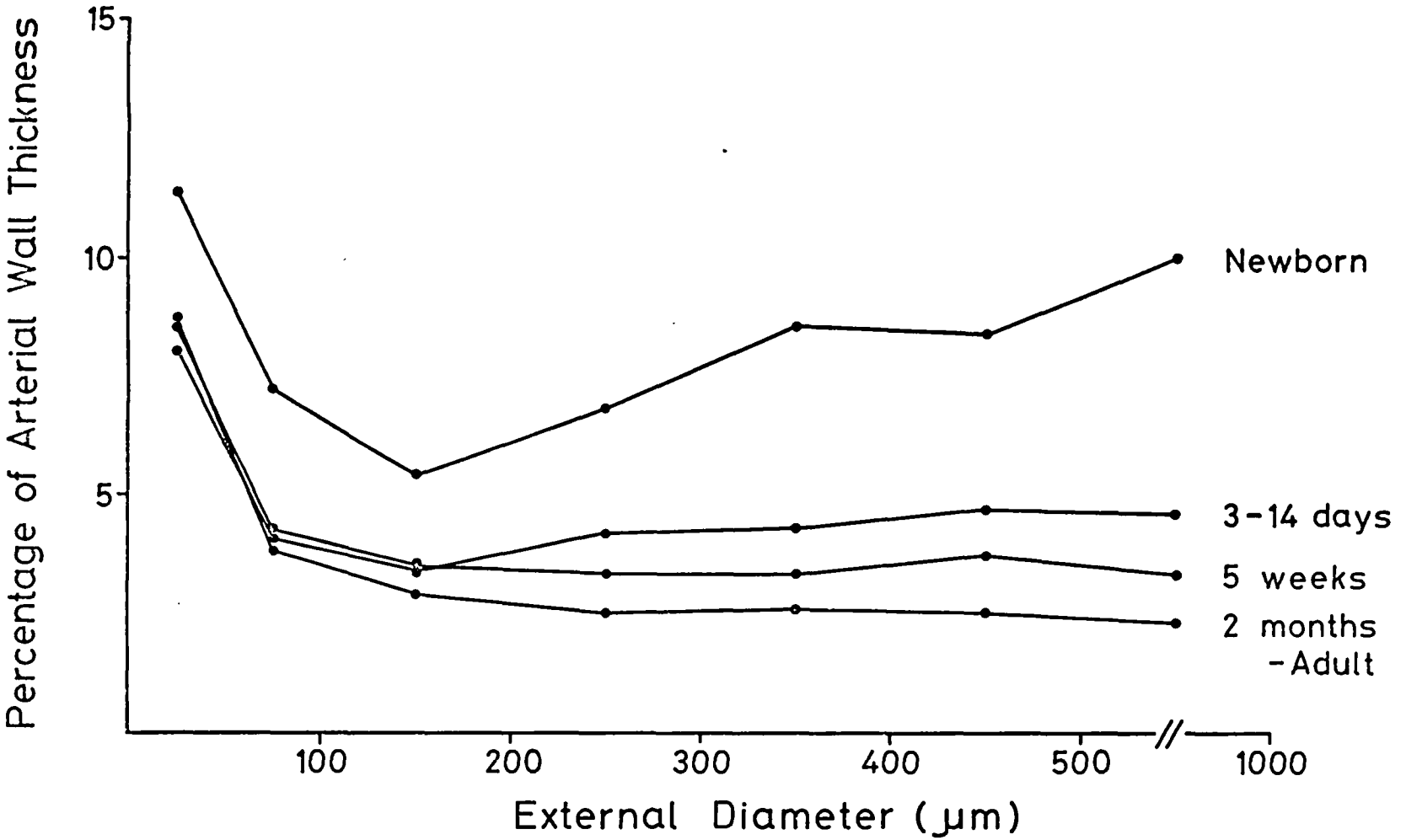

Percentage of Arterial Wall Thickness
15
10
5
100
200
300
400
500
1000
External Diameter (µm)
Newborn
3-14 days
5 weeks
2 months
-Adult

Fig. III 25

Photomicrograph of a peripheral pulmonary artery from a newborn swine. Notice the continuous muscular media (x680)

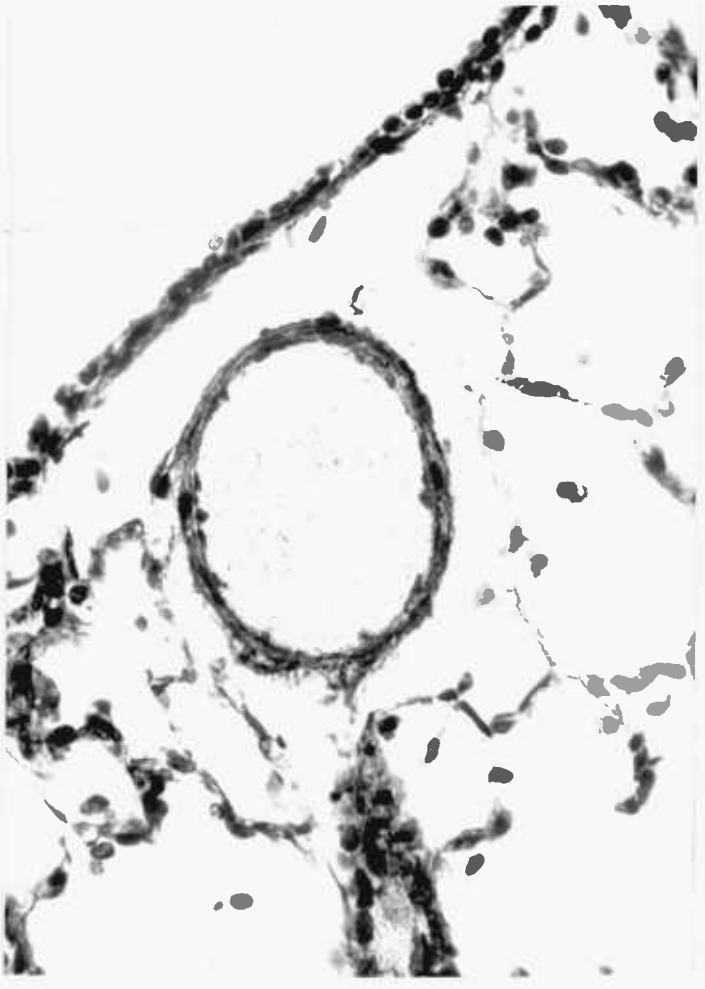
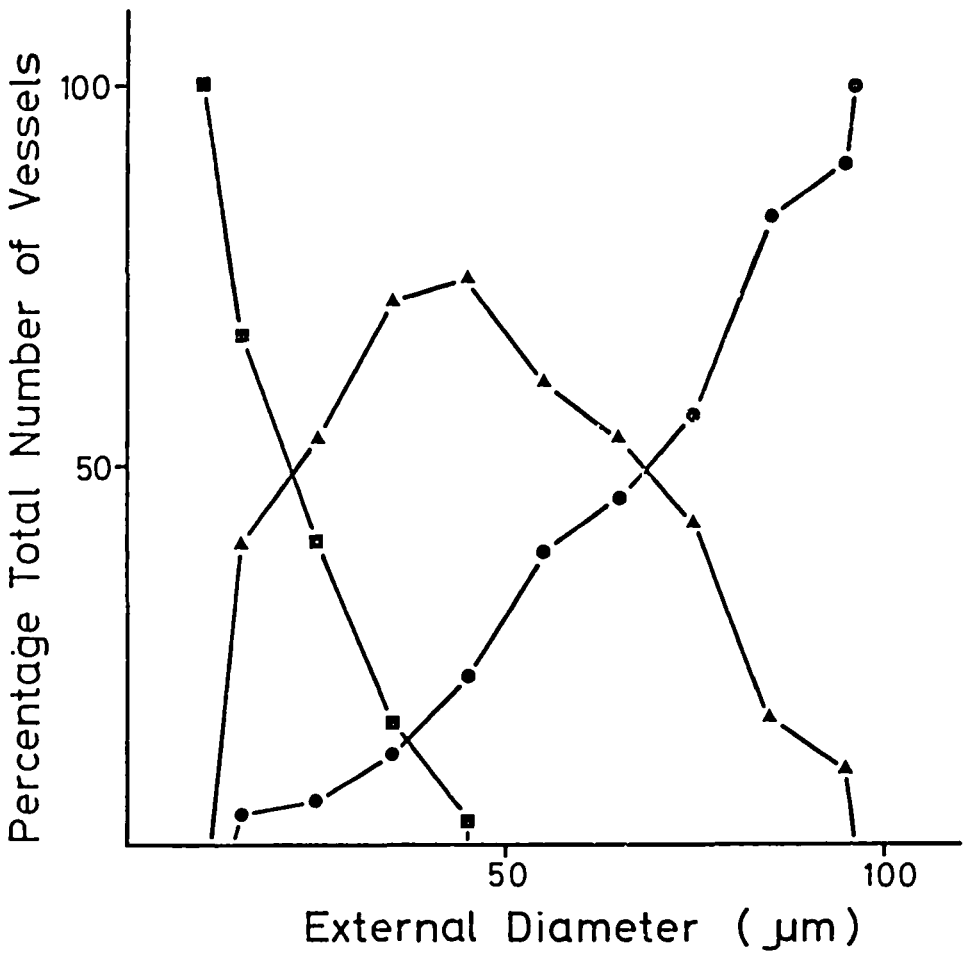


Fig. III 26

Diagram showing the arterial population curves. The percentage of each type of vessel in each size range is shown. Notice that muscle is already present in very small arteries at birth

● - muscular arteries
▲ - partially muscular arteries
■ - non-muscular arteries


Percentage Total Number of Vessels
100
50
50
100
External Diameter (µm)

TABLE

POPULATIONS STUDIES

Age	External 10-20	20-30	30-40	40-50	50-60	60-70
Newborn	59	42	30	0	0	0
	41	55	58	51	33	27
	0	3	12	49	67	73
"	67	42	40	15	5	0
	33	56	58	75	63	41
	0	2	2	10	32	59
3 days	82	81	58	42	34	10
	18	19	38	44	53	62
	0	0	4	14	13	28
3 "	93	65	60	44	36	22
	7	35	40	56	54	59
	0	0	0	0	10	19
1 week	100	84	75	63	48	30
	0	16	25	37	52	70
	0	0	0	0	0	0
2 weeks	100	77	48	40	15	11
	0	23	52	60	75	81
	0	0	0	0	0	8
2 "	100	93	82	78	70	57
	0	7	18	22	30	26
	0	0	0	0	0	17
5 "	95	88	53	47	27	20
	5	12	47	50	65	61
	0	0	0	3	8	19
5 "	85	64	58	47	20	10
	15	36	42	53	70	80
	0	0	0	0	10	10
8 "	70	65	60	47	30	20
	30	35	40	43	55	57
	0	0	0	10	15	23
8 "	77	54	36	13	10	0
	23	46	64	75	71	74
	0	0	0	12	19	26
12 "	86	85	46	18	0	0
	14	15	50	67	65	58
	0	0	4	15	35	42
16 "	70	60	33	21	13	0
	30	40	67	79	72	74
	0	0	0	0	15	26
16 "	80	64	56	45	22	17
	20	36	44	52	68	57
	0	0	0	3	10	16
20 "	53	33	20	4	0	0
	47	67	77	73	71	62
	0	0	3	23	29	38
20 "	60	25	23	13	0	0
	40	75	73	67	61	57
	0	0	4	20	39	43
Adult (1 year)	55	23	10	0	0	0
	45	77	80	76	66	55
	0	0	10	24	34	45
" (3½ years)	65	45	25	0	0	0
	35	55	73	70	57	53
	0	0	2	30	43	47

For each case, % of non-muscular, partially muscular and

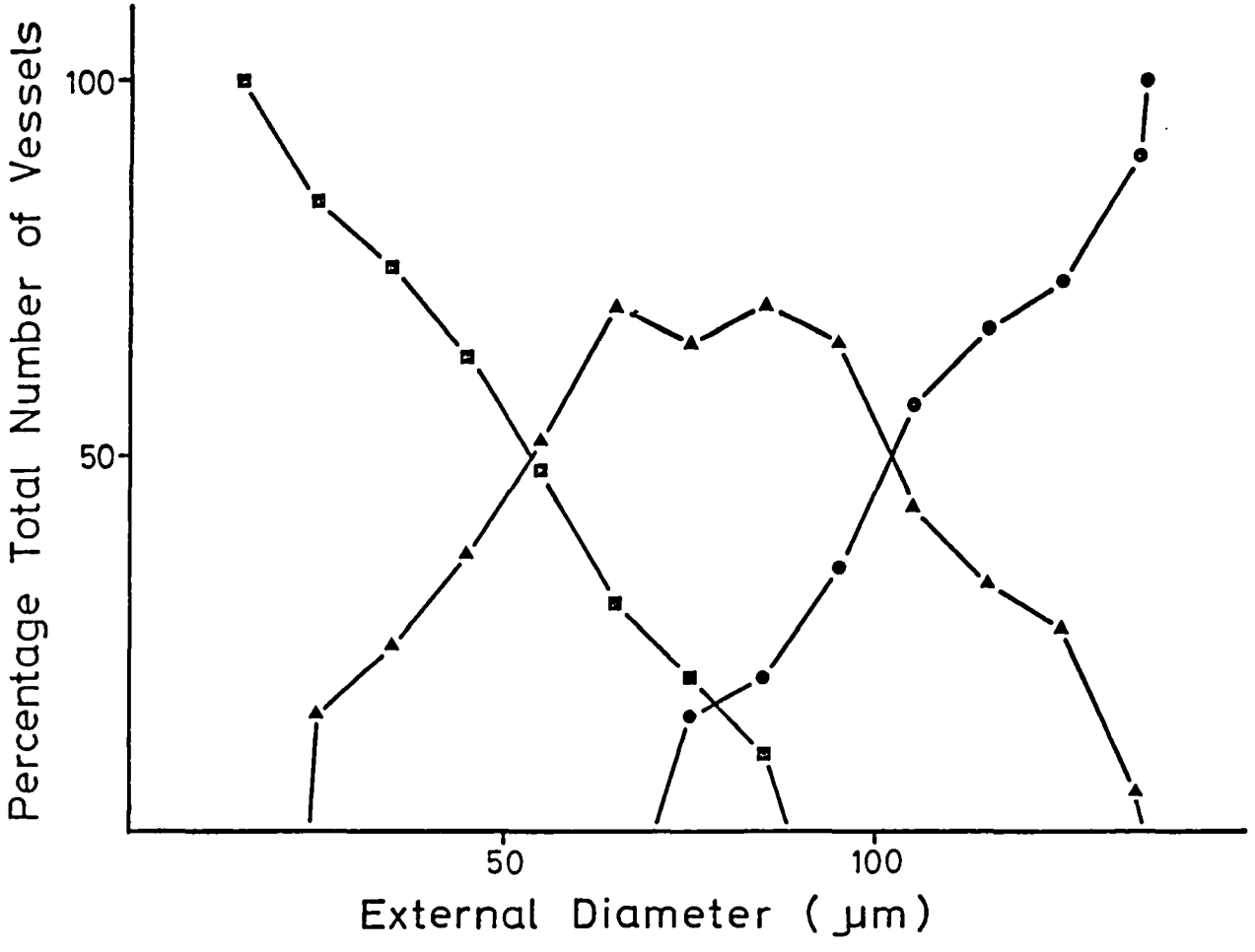
Diameter (μm)							
70-80	80-90	90-100	100-110	110-120	120-130	130-140	140-15(
0	0	0	0				
20	17	7	0				
80	83	93	100				
0	0	0	0				
25	12	11	0				
75	88	19	100				
5	0	0	0	0	0		
59	34	30	23	15	0		
36	66	70	77	85	100		
10	5	0	0	0	0	0	
63	58	41	35	27	10	0	
27	37	49	65	73	90	100	
20	10	0	0	0	0	0	
65	70	65	43	33	27	0	
15	20	35	57	67	73	100	
9	0	0	0	0	0	0	
74	80	69	40	27	13	0	
17	20	31	60	73	97	100	
50	20	5	0	0	0	0	0
30	45	85	59	32	30	20	
20	35	35	41	68	70	100	100
0	0	0	0	0			
74	60	35	20	10			
26	40	55	80	100			
4	5	0	0	0			
61	55	73	20	5			
25	45	27	80	95			
10	0	0	0	0	0		
66	55	30	15	10	0		
24	45	70	85	90	100		
0	0	0	0	0			
67	35	20	5	0			
33	65	80	95	100			
0	0	0	0	0	0	0	
43	41	35	20	10	10	0	
57	59	65	80	90	90	100	
0	0	0	0	0	0		
63	53	38	21	13	0		
37	47	62	79	87	100		
0	0	0	0	0	0	0	
62	60	43	40	18	10	0	
38	40	57	60	82	90	100	
0	0	0	0	0	0		
45	30	14	10	5	0		
55	70	86	90	95	100		
0	0	0	0	0			
56	47	35	20	0			
44	53	65	80	100			
0	0	0	0	0	0		
40	12	10	5	5	0		
60	88	80	95	95	100		
0	0	0	0	0	0	0	0
47	40	45	35	20	10	5	0
53	60	55	65	80	90	95	100

muscular arteries in every size range

Fig. III 27

Diagram showing the arterial population curves at three days of age. The percentage of each type of vessel in each size range is shown. Notice the decrease in the percentage of small muscular arteries when compared with the newborn (Fig. III 26)

● - muscular arteries
▲ - partially muscular arteries
■ - non-muscular arteries


Percentage Total Number of Vessels
100
50
50
100
External Diameter (µm)

muscle and that development of new muscle cells in the arterial wall lagged during the first weeks of life.

Later, as the animals grew, muscle was present in progressively smaller arteries and by the twelfth week of life (Fig. III 28) the adult distribution of muscular, partially muscular and non-muscular arteries was already present (Fig. III 29).

The partially muscular arteries increased in number and in size, relative to the other type, from birth until the second week of life. At this age, the total length of the peripheral pathway where partially muscular vessels could be identified, was 112 µm, the maximum for any age, and 30% of all the arteries between 80-90 µm in external diameter were partially muscular. At twelve weeks, the partially muscular vessels ranged between 31 and 119 µm in size and only 41% of the arteries between 80-90 µm were partially muscular, suggesting that as muscle extended along the arterial pathway, there was a shift to the left of the partially muscular population curve.

Arteries accompanying airways

During growth, the structure and the size of the intra-acinar arteries accompanying the various airway types also changed.

Wall structure In the newborn, very few partially muscular arteries were found accompanying alveolar ducts and muscular arteries were absent at that airway level (Table III 11), although there was

Fig. III 28

Diagram showing the arterial population curves at twelve weeks of age. The percentage of each type of vessel in each size range is shown. Notice that muscle is again found in small pulmonary arteries making the curves similar to those found at birth

● - muscular arteries
▲ - partially muscular arteries
■ - non-muscular arteries

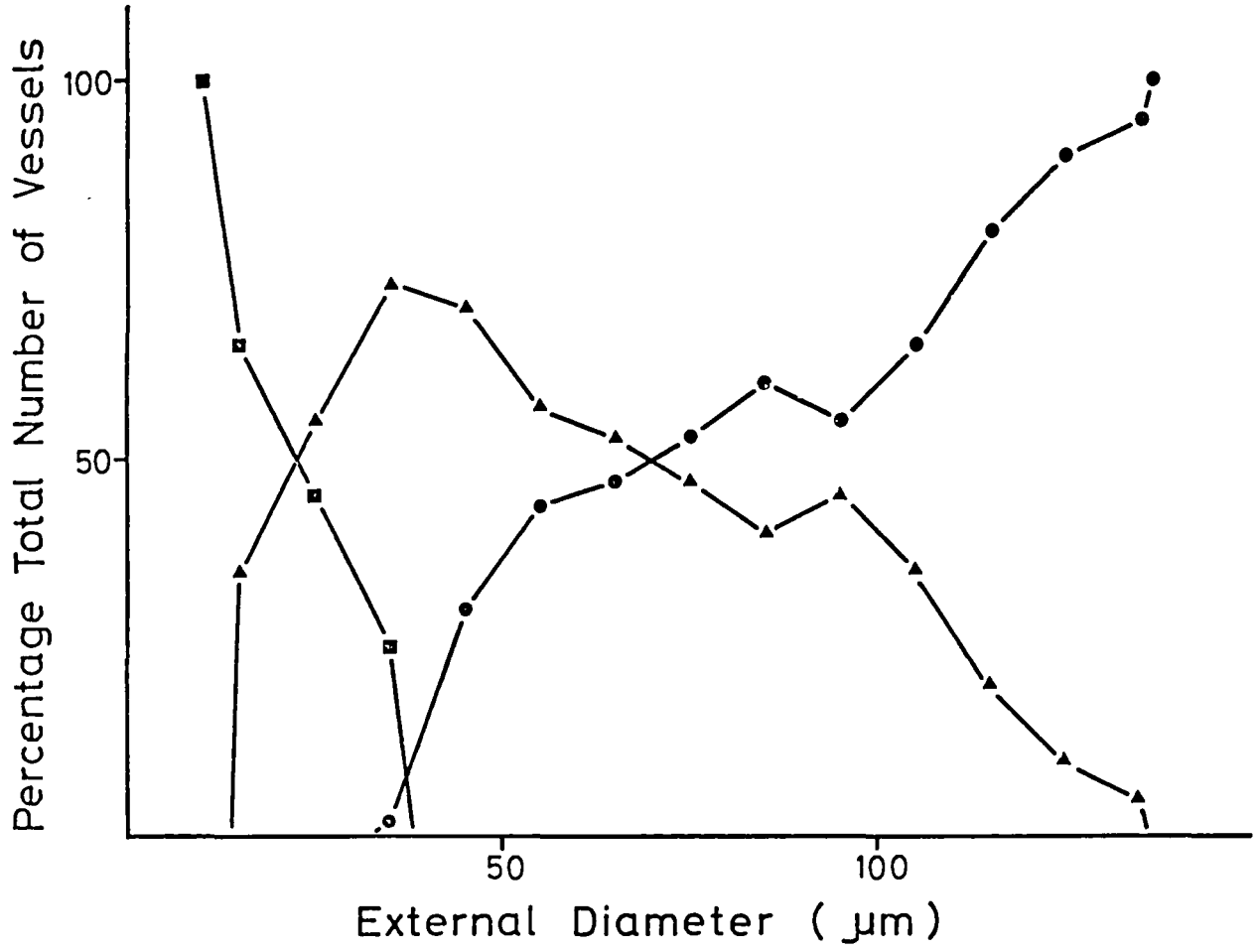

Percentage Total Number of Vessels
100
50
50
100
External Diameter (µm)

Fig. III 29

Diagram showing the arterial population curves in the adult pig. The percentage of each type of vessel in each size range is shown

● - muscular arteries

▲ - partially muscular arteries

■ - non-muscular arteries

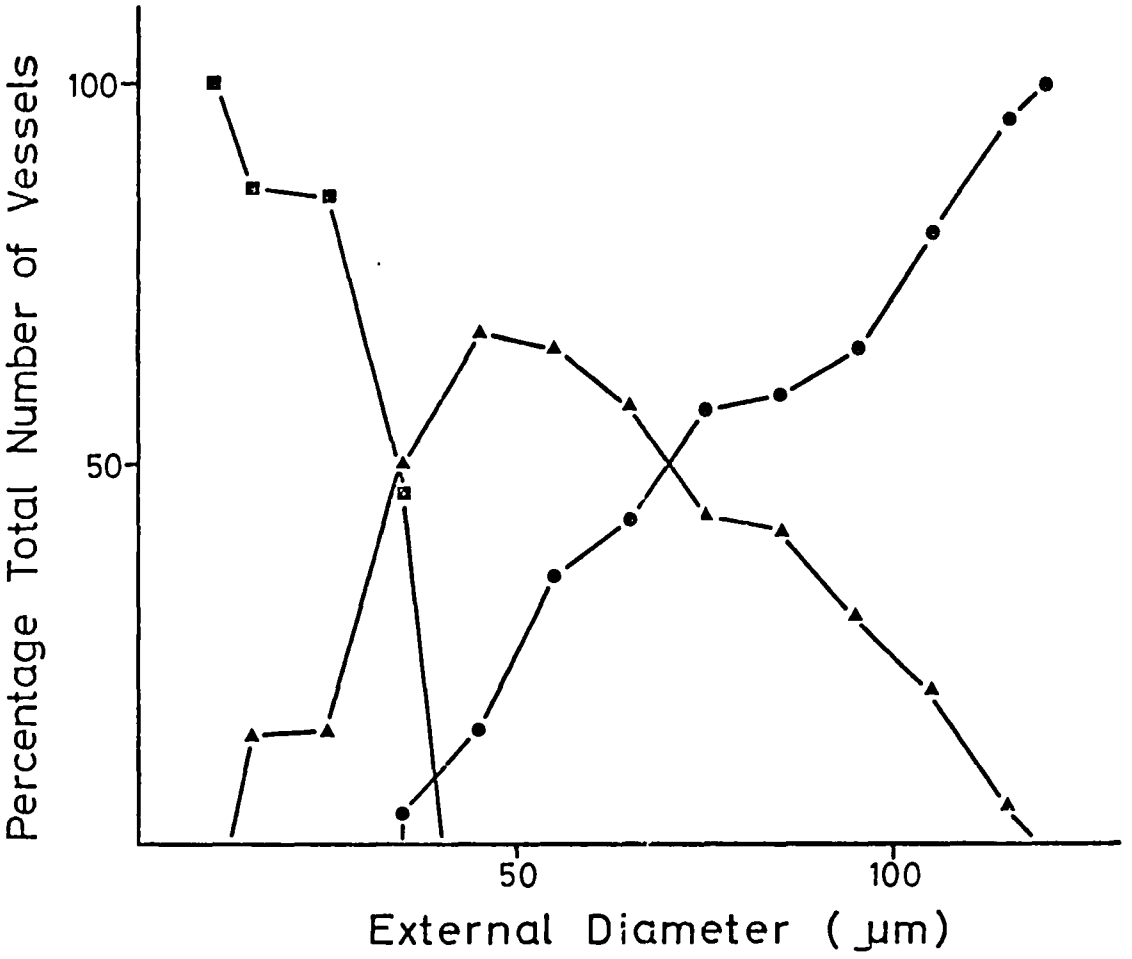

Percentage Total Number of Vessels
100
50
50
100
External Diameter (µm)

TABLE III 11

STRUCTURE OF ARTERIES ACCOMPANYING INTRA-ACINAR AIRWAYS

Age	Term. Bronch.			Resp. Bronch.			Alveolar Duct		
	nm	pm	m	nm	pm	m	nm	pm	m
Newborn	0	71	29	20	60	20	94	6	0
"	0	82	18	14	74	12	93	7	0
3 days	0	79	21	23	77	0	67	33	0
3 "	0	88	12	42	58	0	76	24	0
1 week	0	83	17	38	62	0	84	16	0
2 weeks	0	74	26	36	64	0	84	16	0
2 "	0	86	14	39	61	0	55	45	0
5 "	0	12	88	0	38	62	19	67	14
5 "	0	6	94	0	75	25	52	48	0
8 "	0	0	100	0	35	65	12	76	12
8 "	0	0	100	0	46	54	24	67	9
12 "	0	0	100	0	48	52	0	80	20
16 "	0	0	100	0	45	55	0	88	12
16 "	0	0	100	0	65	35	0	90	10
20 "	0	0	100	0	13	87	0	56	44
20 "	0	0	100	0	30	70	0	67	33
Adult (1 year)	0	0	100	0	9	91	0	66	34
" (3½ years)	0	0	100	0	15	85	0	41	59

Term. Bronch. - terminal bronchiolus
Resp. Bronch. - respiratory bronchiolus

nm - non-muscular arteries
pm - partially muscular arteries
m - muscular arteries

already a considerable proportion of muscular vessels accompanying both terminal and respiratory bronchioli.

Muscle "extended" along the peripheral pathway during growth (Fig. III 30), so that by the fifth week of life, half the arteries at alveolar duct level were partially muscular and muscular vessels were already present. The muscular population continued to increase with age and at twenty weeks, half of the arteries at alveolar duct level were muscular, the other half being partially muscular - a similar distribution to that found in the adult animal (Fig. III 31-32).

External diameter

The external diameter of the intra-acinar arteries increased progressively with age (Table III 12). Between birth and the third day of life, there was an approximate two fold increase in vascular size at the three airway levels. After that age, the more proximal arteries accompanying terminal bronchioli, doubled their external diameter at eight weeks of age, whereas a similar change occurred only at sixteen weeks in the more distal vessels accompanying respiratory bronchioli and alveolar ducts (Fig. III 33). After that age, the rate of increase in external diameter was again similar at the three airway levels until adult life.

Number of arteries and alveoli per unit area of lung tissue

The number of alveoli and intra-acinar arteries per unit

Fig. III 30

Diagram illustrating the level to which peripheral muscular arteries extend, landmarked by their accompanying airways. Throughout growth muscle extends along the intra-acinar arterial pathway, despite the fact that a considerable amount of muscular arteries is already present at birth

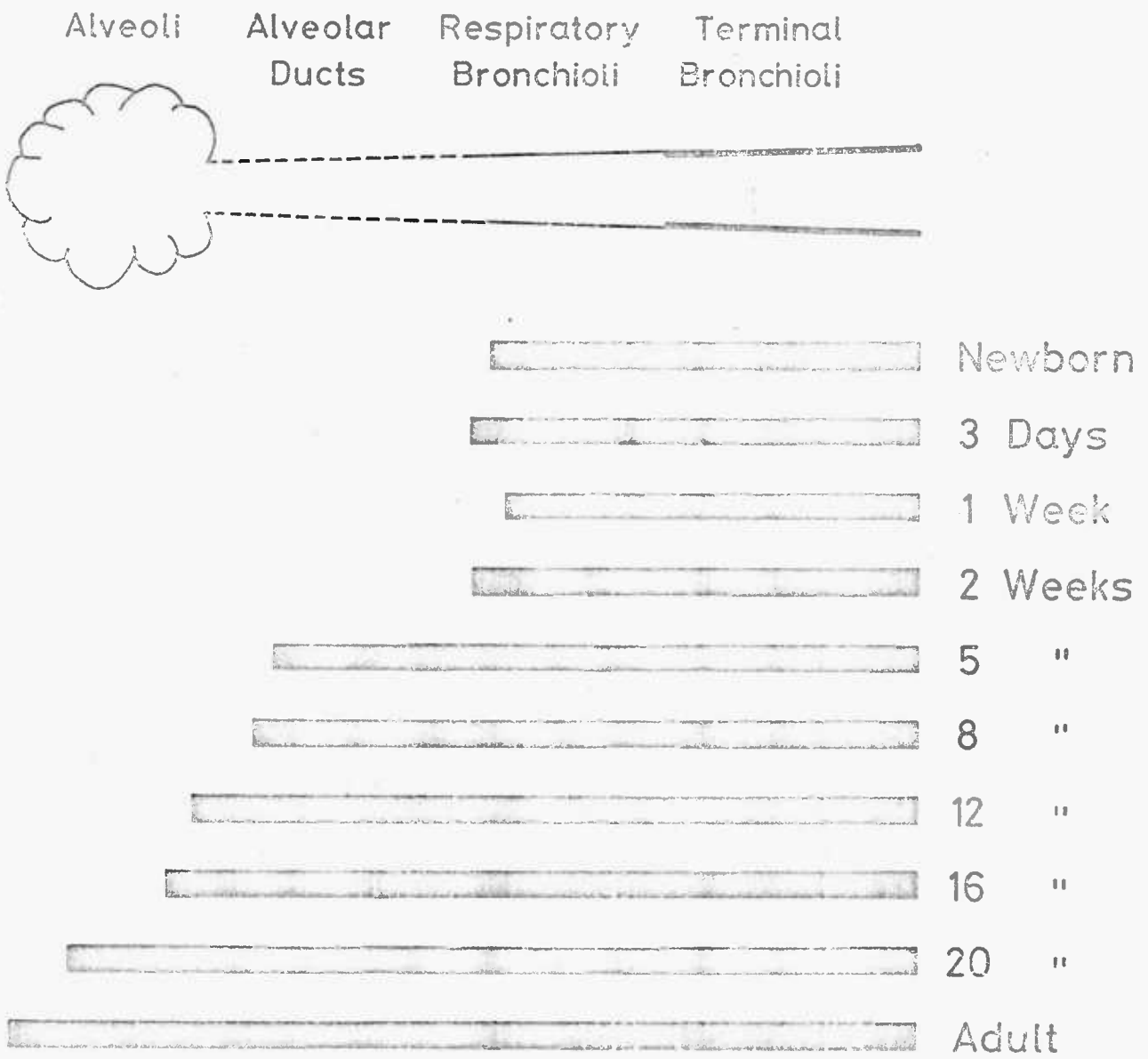

Alveoli
Alveolar Ducts
Respiratory Bronchioli
Terminal Bronchioli
Newborn
3 Days
1 Week
2 Weeks
5 "
8 "
12 "
16 "
20 "
Adult

Fig. III 31

Photomicrograph of a small muscular artery from a normal swine at twenty weeks of age. The vessel is situated in the junction between a respiratory bronchiolus and an alveolar duct (x420)

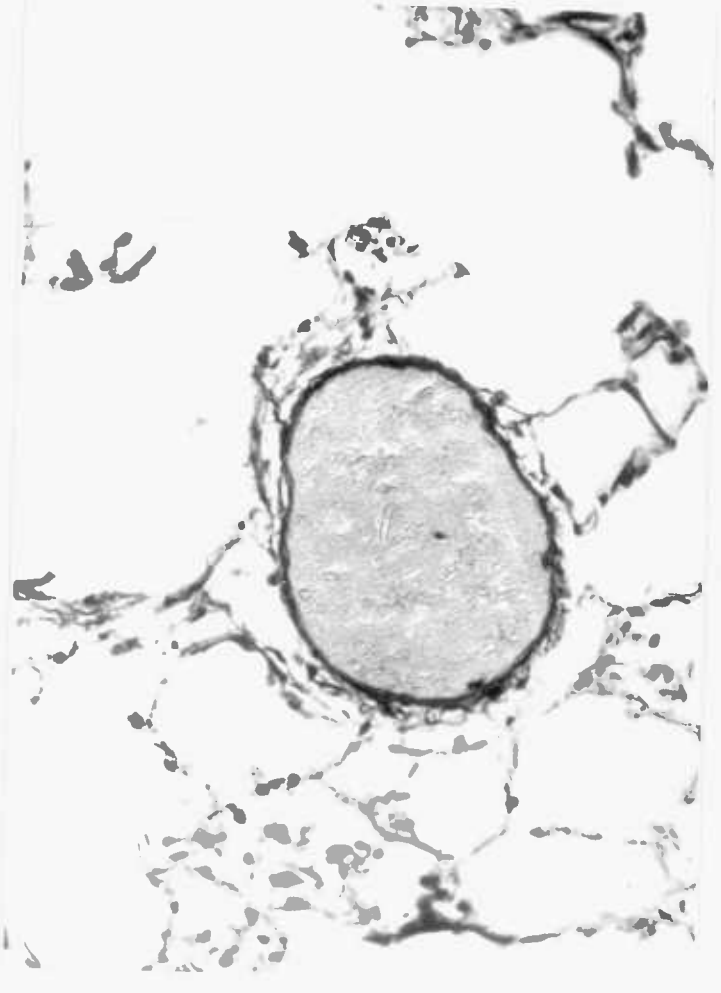


Fig. III 32

Photomicrograph of a partially muscular artery from a normal adult swine. Notice the non-muscular vessel arising as a branch (x680)

TABLE III 12

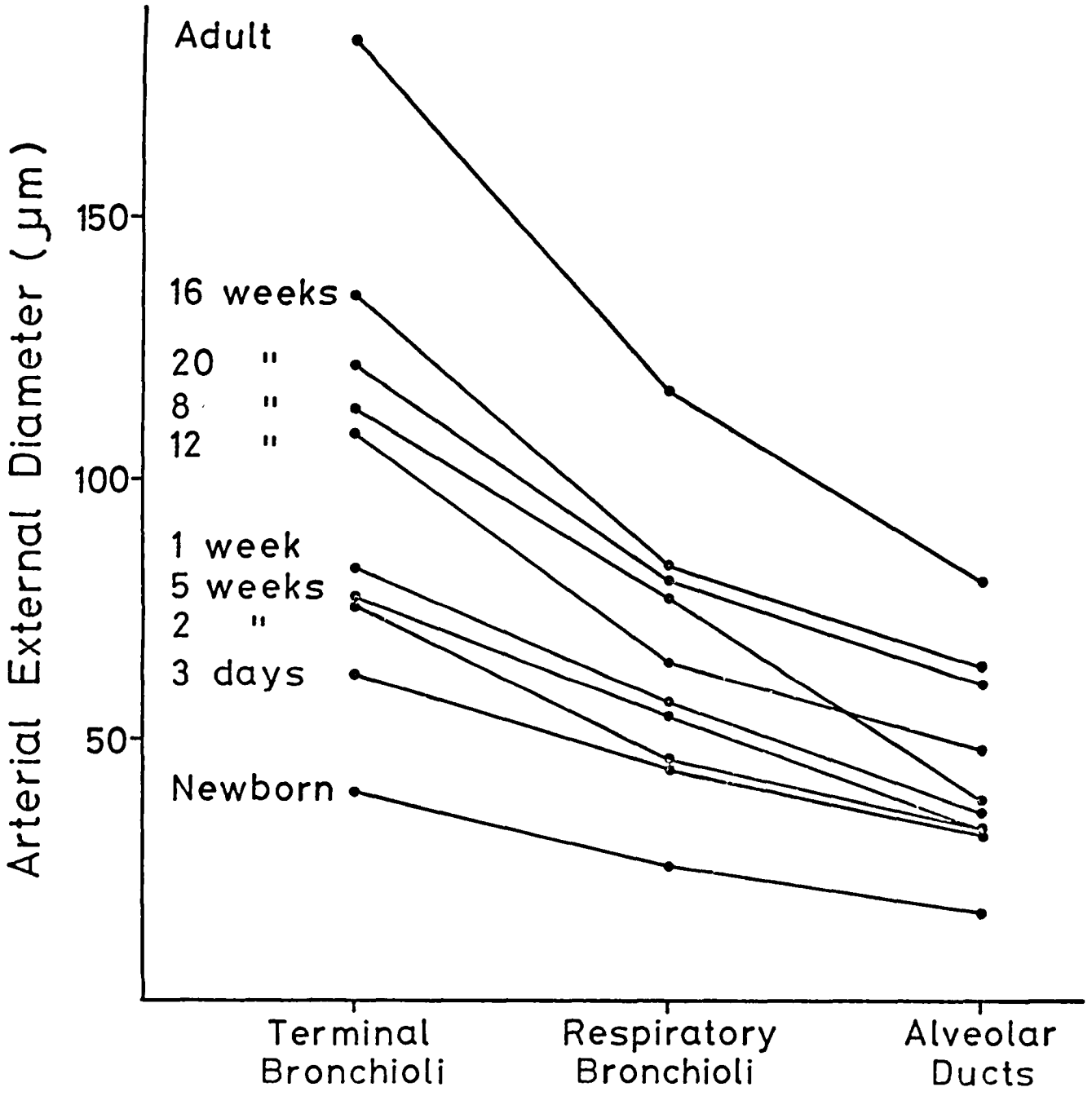
SIZE OF ARTERIES ACCOMPANYING INTRA-ACINAR AIRWAYS

Age	External Diameter (μm)		
	Term. Bronch.	Resp. Bronch.	Alveolar Duct
Newborn	38	24	15
	2.7	1.7	0.9
	24	20	16
"	42	28	18
	1.6	0.8	0.8
	84	65	30
3 days	61	47	34
	3.3	2.1	1.7
	34	30	30
3 "	64	42	30
	3.9	2.5	1.5
	33	24	29
1 week	83	57	36
	4.7	2.3	1.3
	36	37	37
2 weeks	66	39	27
	2.5	1.6	1.4
	38	31	19
2 "	86	53	40
	5.7	2.1	2.3
	19	28	19
5 "	83	55	32
	6.2	2.6	2.5
	26	42	21
5 "	72	54	34
	3.6	2.2	2.2
	37	40	27
8 "	133	88	42
	9.9	4.5	3.3
	12	29	20
8 "	109	66	35
	5.5	2.9	1.9
	32	54	33
12 "	94	65	48
	5.2	3.4	6.8
	26	27	15
16 "	147	93	67
	12.8	7.0	2.9
	22	33	24
16 "	123	83	61
	6.9	3.7	2.4
	22	37	30
20 "	114	74	62
	7.2	4.2	2.6
	11	32	32
20 "	130	79	59
	9.6	3.5	4.0
	16	30	18
Adult (1 year)	170	108	73
	13.4	9.7	2.1
	27	23	33
Adult (3½ years)	197	126	88
	10.5	5.5	4.1
	26	36	44

Mean, standard error of the mean and total no. vessels measured

Fig. III 33

Diagram illustrating the changes in mean external diameter (μm) of the intra-acinar arteries throughout growth, landmarked by their accompanying airways. Notice the considerable increase in size occurring between birth and the third day of life


Adult
16 weeks
20 "
8 "
12 "
1 week
5 weeks
2 "
3 days
Newborn
150
100
50
Arterial External Diameter (µm)
Terminal Bronchioli
Respiratory Bronchioli
Alveolar Ducts

area of lung tissue changed with age (Table III 13). These numbers represent a balance between multiplication of the structures and distension of the lung from thoracic growth. Since both processes occur at the same time, interpretation of changes in alveolar and intra-acinar arterial number is complex. An increase in alveolar or arterial number was considered as evidence that multiplication predominated whereas a reduction in number was taken as an evidence of lung distension secondary to thoracic and lung increase in volume. The alveolar/arterial ratio was used to assess changes in the relative proportions of alveoli and arteries during growth (Fig. III 34).

Between birth and the second week of life, alveolar number per unit area decreased, probably as the result of alveolar expansion that occurred after birth. Arterial number increased during that period, implying that arterial multiplication was already occurring. This conclusion was supported by the decrease in the alveolar/arterial ratio during that period (Fig. III 34). Between two and five weeks of age the number of both alveoli and arteries increased, together with an increase in the alveolar/arterial ratio, suggesting that the alveolar multiplication was relatively greater than the arterial one. By eight weeks of age both numbers had decreased, indicating that now multiplication was not keeping pace with increase in volume of the thoracic cage; the lowering of the alveolar/arterial ratio suggested that arterial multiplication was greater than alveolar.

At twelve weeks, the number of both structures per unit area fell, but the ratio was similar to that found at eight weeks, suggesting that the increase in both alveolar and arterial number kept pace with

TABLE III 13

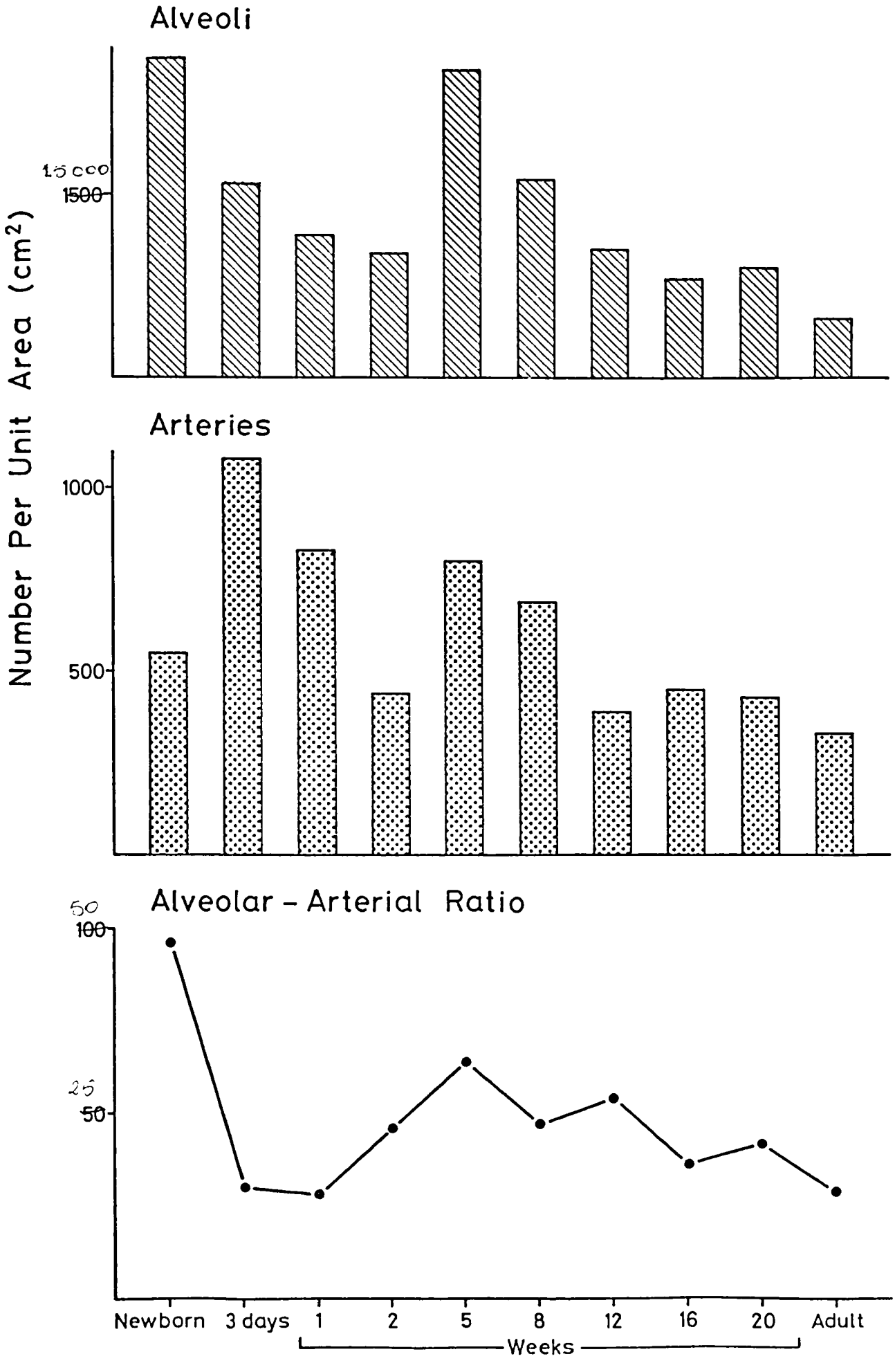
ALVEOLAR AND INTRA-ACINAR ARTERIAL NUMBER PER UNIT AREA OF LUNG TISSUE DURING POSTNATAL GROWTH

Age	Number per Unit Area (cm^2)		
	Alveoli	Arteries	Ratio
Newborn	26208 384	548 29	47.8
3 days	16051 329	1079 52	14.9
1 week	11755 190	833 69	14.1
2 weeks	10183 164	439 37	23.2
5 "	25547 307	804 37	31.8
8 "	16336 238	695 33	23.5
12 "	10622 175	392 29	27.1
16 "	8164 230	450 66	18.1
20 "	9111 168	429 37	21.2
Adult (1 year)	4676 110	328 26	14.3

Mean
Standard error of the mean

Fig. III 34

Diagram illustrating the changes in alveolar and intra-acinar arterial number from birth to adult life. Notice the changes in the alveolar/arterial ratio occurring between birth and the eighth week of life


Alveoli
1500
Arteries
1000
500
Number Per Unit Area (cm²)
Alveolar – Arterial Ratio
50
100
25
50
Newborn
3 days
1
2
5
8
12
16
20
Adult
Weeks

the growth of the thoracic cage. The same pattern was seen until twenty weeks of life; after that age and until adult life (1 year), alveolar number fell, whereas the arterial number remained fairly constant, causing a reduction in the ratio and suggesting that arteries, even if not alveoli, were still increasing until somatic growth was complete.

In the pig lung, as in the human, the appearance of background haze on the arteriograms reflected the increase in intra-acinar arteries during growth (Davies and Reid, 1970). In the newborn animal, only the pre-acinar vessels were visible in the arteriogram (Fig. III 9), whereas as early as three days of life, a considerable degree of background haze was already present (Fig. III 10). This was confirmed by the fact that arterial number, assessed microscopically, also increased considerably during that period (Fig. III 34).

THE PULMONARY VENOUS BED

Pre-Acinar Veins

The venous branching pattern was assessed from the venograms. The structure of the pre-acinar veins was not studied.

The venogram

A good filling of the pulmonary venous bed was obtained in all cases. The branching pattern of the venograms was similar to that

of the arteriograms and did not change throughout growth (Fig. III 35-38).

A considerable degree of background haze was already present at birth and did not change until adult life. This better filling of the peripheral veins, when compared to the arteries in the newborn was also found in the human by Hislop and Reid (1973), who suggested that this reflected the greater number of intra-acinar veins than arteries at birth and confirmed it, by assessing the number of vessels per unit area of lung tissue.

The venous injection did not fill any pulmonary arteries or bronchial vessels.

Measurements of axial length and diameter There was a five fold increase in length of the venous pathway between birth and the first year of life and also, like the arteries, half of that change had already occurred in the first two months (Table III 14).

The rate of increase in lumen diameter was also faster during the first two months of life (Fig. III 39), but this change was uniform along the pathway throughout growth, as shown by the fact that between birth and adult life, the hilar vessels increased, on average, 8.2 fold in diameter, whereas the peripheral ones (75% of the axial length) increased a 8.0 fold during the same period.

Fig. III 35

Pulmonary venogram of a newborn pig (right lung)
(x1.4)

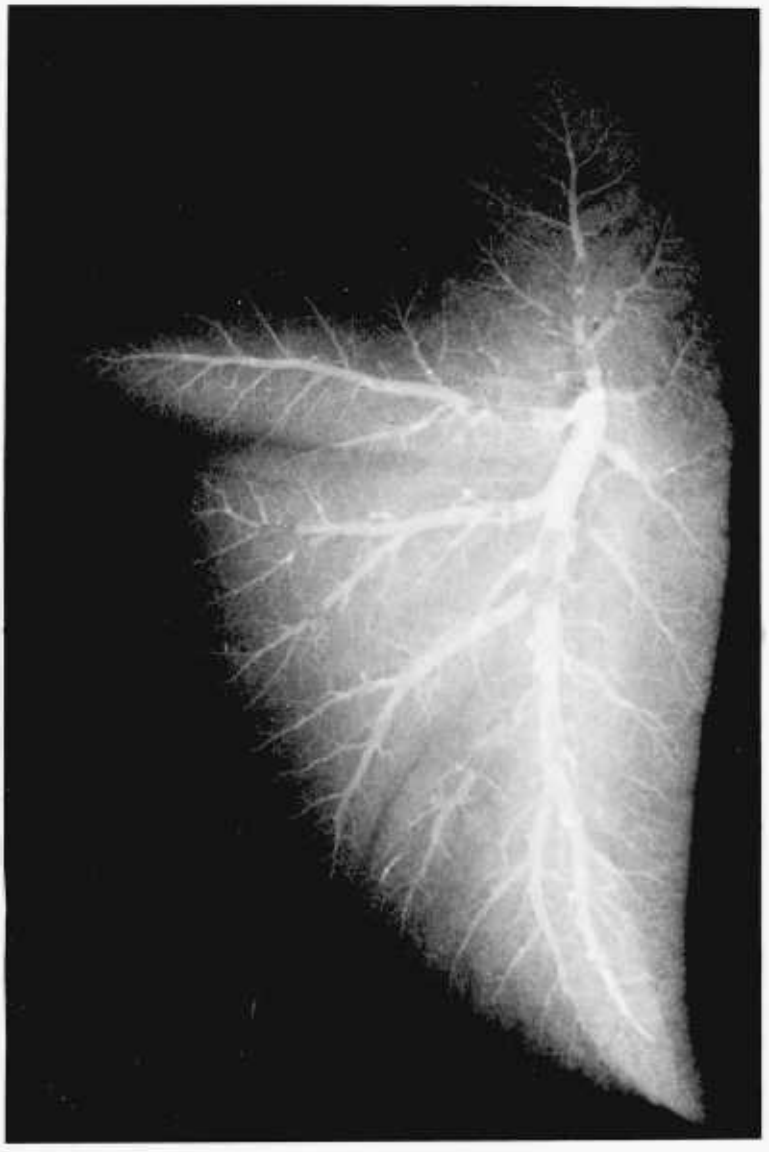


Fig. III 36

Pulmonary venogram of a pig aged eight weeks (right lung)
(x0.6)

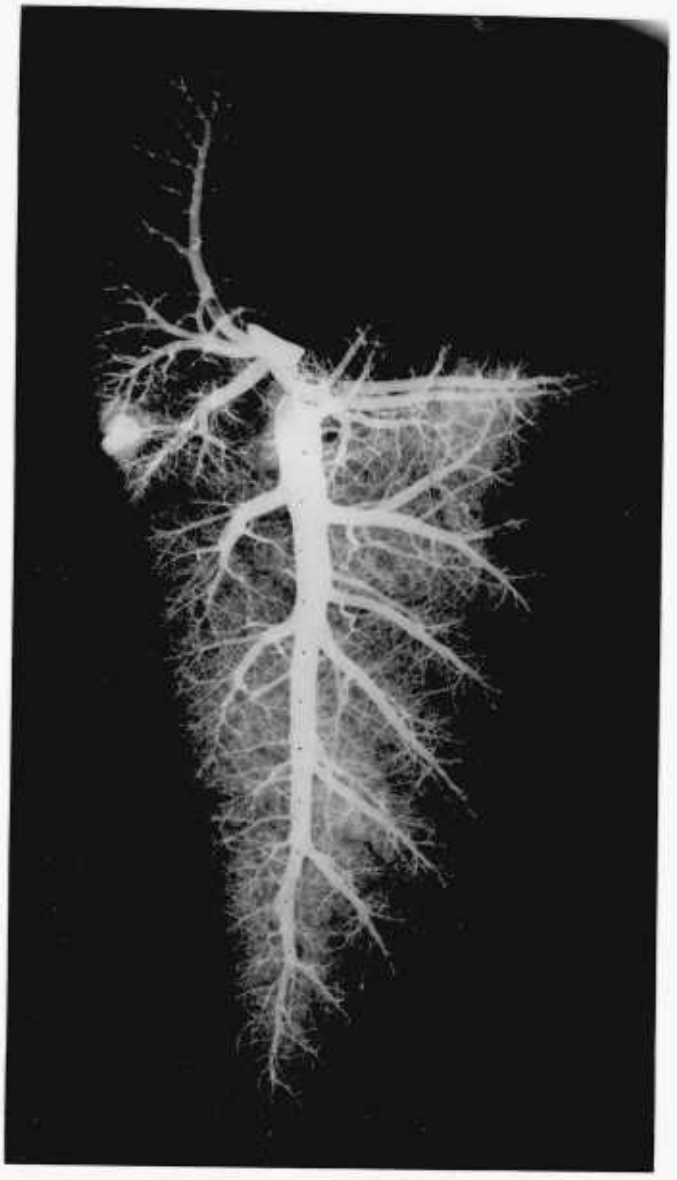


Fig. III 37

Pulmonary venogram of a pig aged twenty weeks (right lung)
(x0.4)

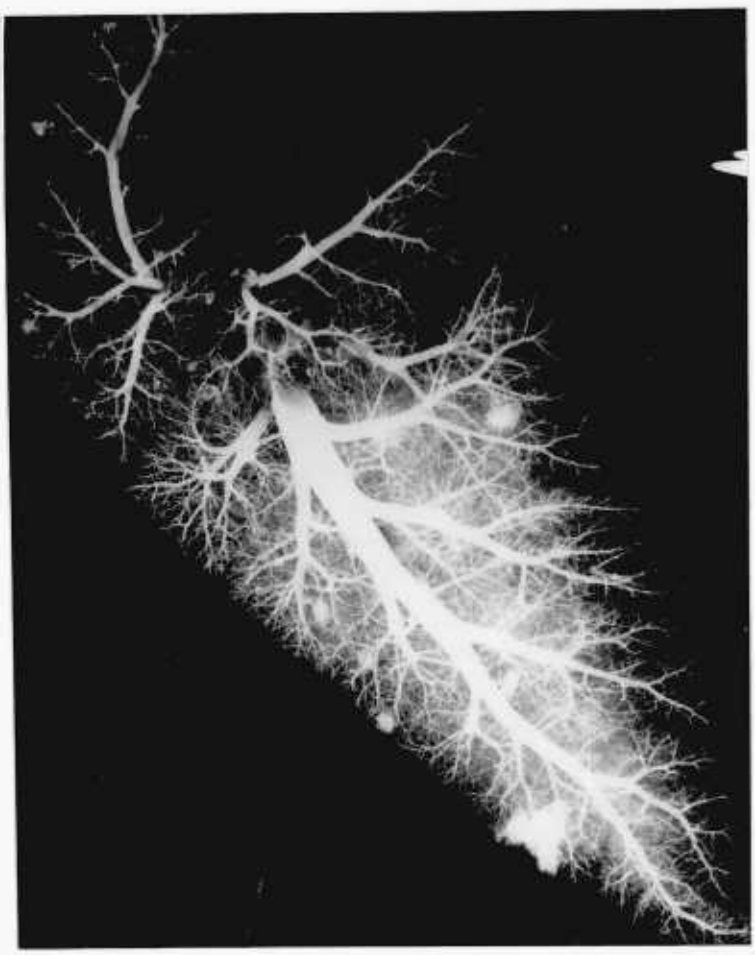


Fig. III 38

Pulmonary venogram of an adult pig (one year) (right lung)
(x0.4)

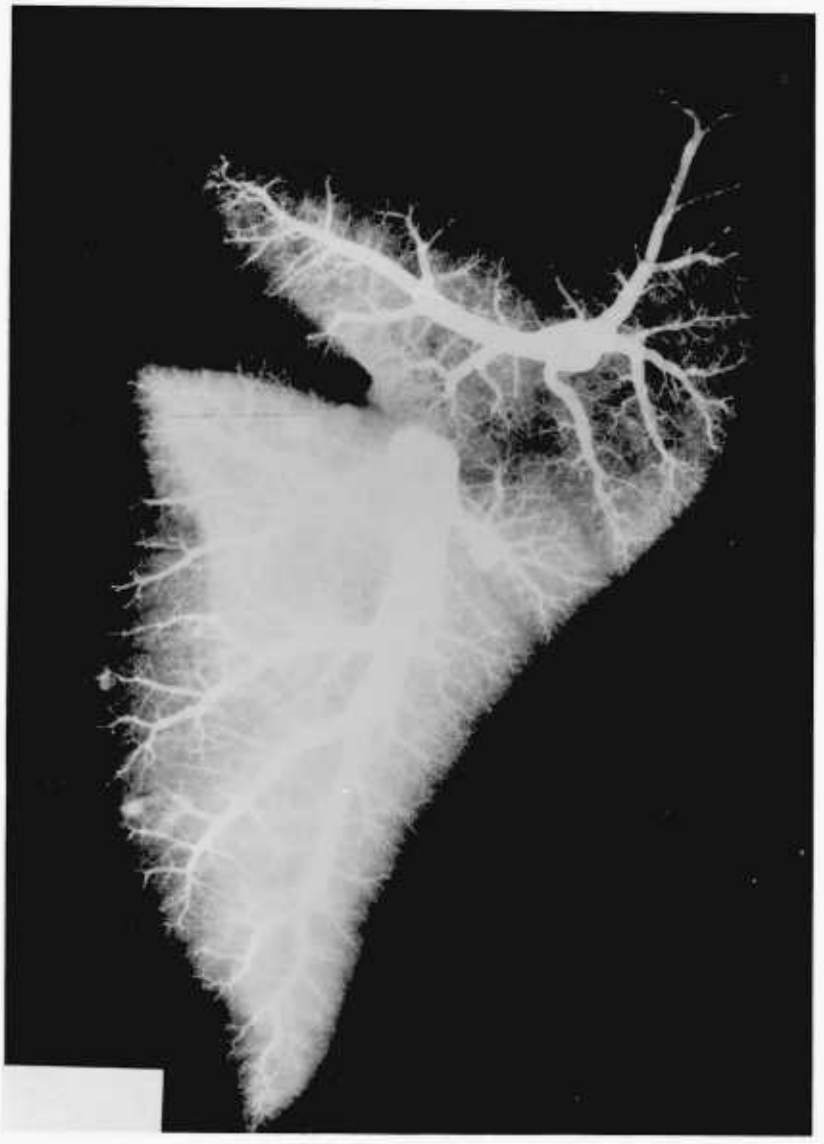


TABLE III 14

MEASUREMENTS FROM THE VENOGRAMS IN NORMAL ANIMALS.

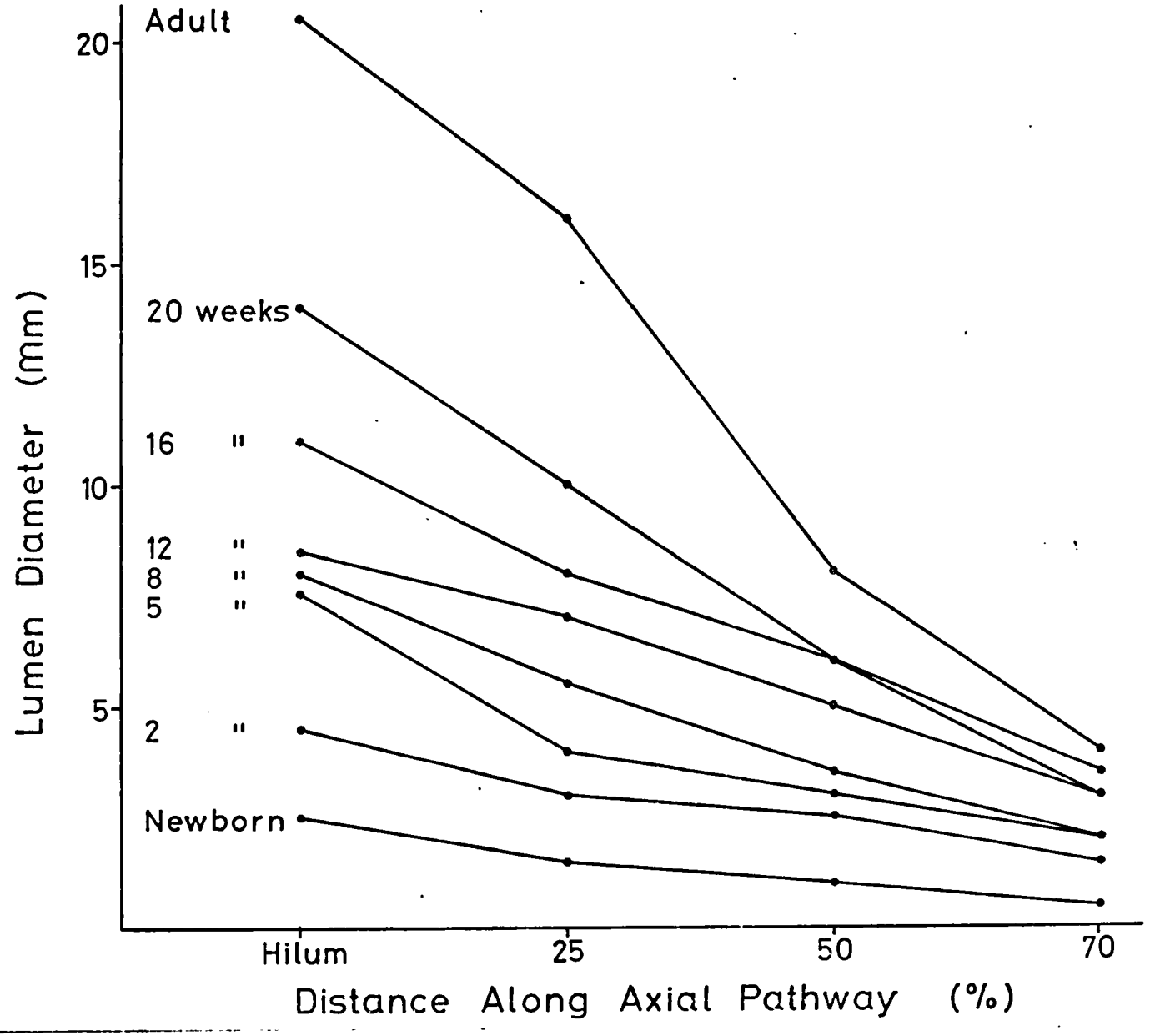
LEFT LOWER LOBE VEIN - TOTAL AXIAL LENGTH, DIAMETER AT THE HILUM AND AT 25% INTERVALS ALONG THE PATHWAY

Age	Axial Length (mm)	Diameter (mm)			
		Hilum	25	50	75
Newborn	53	2.5	1.5	1.0	0.5
2 weeks	75	4.5	3.0	2.5	1.5
5 "	114	7.5	4.0	3.0	2.0
8 "	125	8.0	5.5	3.5	2.0
12 "	163	8.5	7.0	5.0	3.0
16 "	171	11.0	8.0	6.0	3.0
20 "	216	14.0	10.0	6.0	3.5
Adult (1 year)	267	20.5	16.0	8.0	4.0

Mean value for each case

Fig. III 39

Diagram illustrating the changes in lumen diameter of the pre-acinar veins with age. Left lower lobe vein. Mean Diameter at the hilum and at 25% intervals along the axial pathway


Adult
20 weeks
16 "
12 "
8 "
5 "
2 "
Newborn
20
15
10
5
Lumen Diameter (mm)
Hilum
25
50
70
Distance Along Axial Pathway (%)

The Peripheral Venous Bed

The structure of the venous wall in the pig did not change significantly throughout growth. The media contained collagen, elastic fibers and smooth muscle cells which increased slightly in number with age. The internal elastic lamina was always absent whereas the external elastic lamina was thick, particularly in the older animals (Fig. III 40). The intima consisted of a single layer of endothelial cells.

Percentage medial wall thickness

At birth (Fig. III 41), veins of any size had a thin wall and this feature did not change during growth (Fig. III 42).

Mean percentage wall thickness of veins smaller than 200 µm in external diameter was 4.8 throughout growth, the mean values for all age groups included in the present study ranging between 4.5 and 5.7. For larger vessels, the mean value was 2.0 with a range between 1.3 and 2.8 (Table III 15)(Fig. III 43).

VENTRICULAR WEIGHTS

There was an average 49 fold increase in total ventricular weight from birth to adulthood, i.e. 52 weeks (Table III 16). During the same period, the right ventricular weight (RVW) increased on average 32 fold, whereas the left ventricle and septum (LVW+SW) increased on

Fig. III 40

Photomicrograph of a peripheral vein from a normal swine at twenty weeks of age. Notice the muscular media and the absence of an internal elastic lamina

(x680)

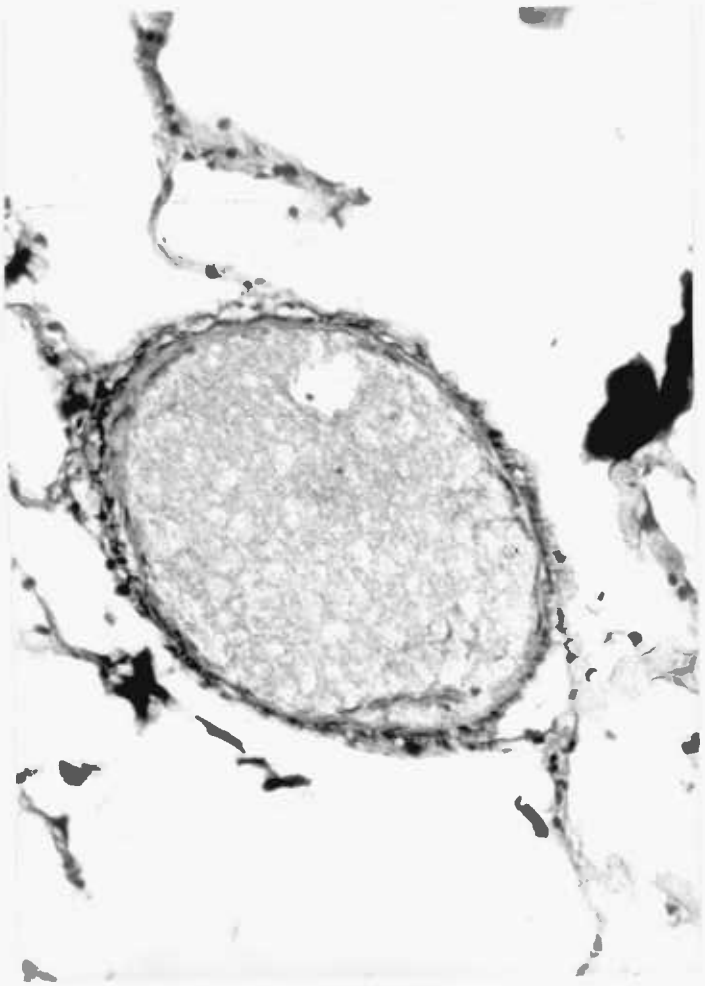


Fig. III 41

Diagram relating venous wall thickness to external diameter (µm) in the newborn pig. Mean and standard deviation are represented for every size range (veins smaller than 1000 µm)

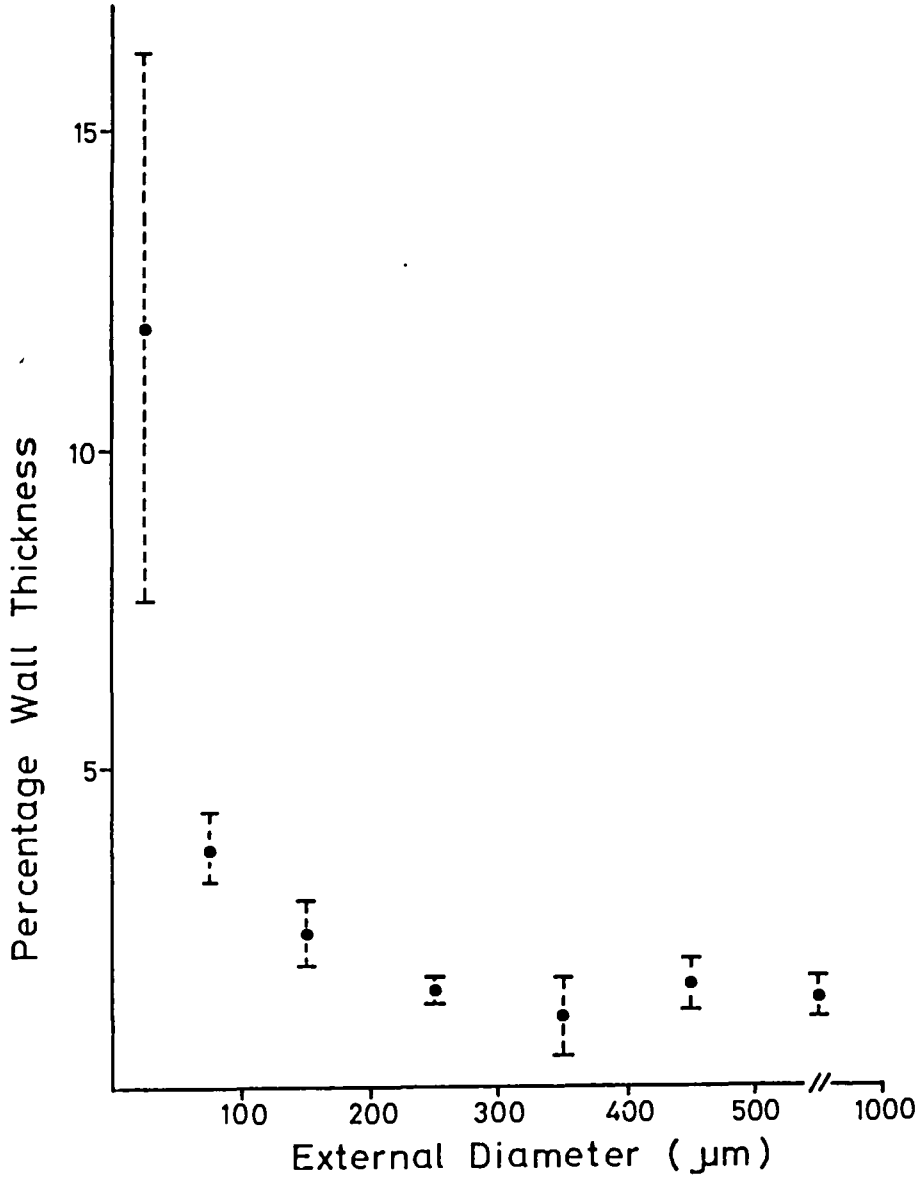

Percentage Wall Thickness
15
10
5
100
200
300
400
500
1000
External Diameter (µm)

Fig. III 42

Diagram relating percentage venous wall thickness to external diameter (µm) in the adult pig. Mean and standard deviation are represented for every size range (veins smaller than 1000 µm)

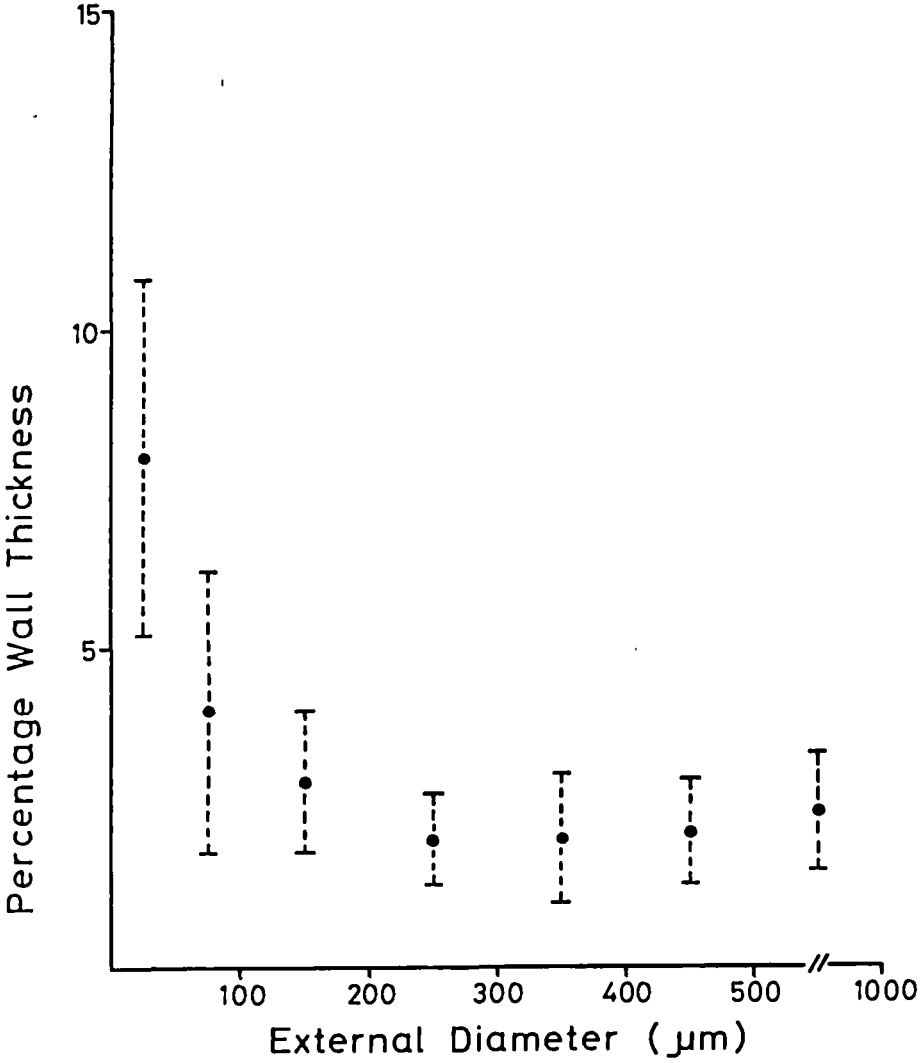

15
10
5
Percentage Wall Thickness
100
200
300
400
500
1000
External Diameter (μm)

TABLE III 15

VEINS - PERCENTAGE WALL THICKNESS DURING GROWTH

Age	External Diameter (µm)								
	0-50	50-100	100-200	200-300	300-400	400-500	500-1000	0-200	200-1000
Newborn *	10.9	3.7	2.4	1.5	1.1	1.6	1.4	** 5.7	1.4
	0.67	0.16	0.14	0.10	0.33	0.21	0.15	4.58	0.22
	88	15	12	3	3	3	3		
2 weeks *	6.8	3.8	3.0	2.5	3.1	2.9	2.7	** 4.5	2.8
	0.20	0.13	0.22	0.40	0.06	0.19	0.12	2.00	0.26
	77	73	19	10	3	3	3		
5 " *	8.2	3.6	2.3	2.0	1.5	1.3	1.1	** 4.7	1.5
	0.53	0.11	0.20	0.14	0.09	0.13	0.20	3.10	0.39
	40	34	21	6	10	4	3		
8 " *	8.1	3.7	1.8	1.4	1.6	1.2	1.0	** 4.5	1.3
	0.45	0.17	0.07	0.15	0.17	0.05	0.30	3.23	0.26
	47	53	37	13	3	4	3		
12 " *	7.6	3.5	2.7	2.6	2.7	2.6	2.2	** 4.6	2.5
	0.34	0.09	0.14	0.15	0.15	0.21	0.06	2.63	0.22
	49	52	30	14	9	6	3		
16 " *	8.4	3.8	2.5	1.9	2.3	2.0	1.7	** 4.9	2.0
	0.56	0.23	0.13	0.25	0.38	0.17	0.38	3.10	0.25
	56	33	18	10	5	3	2		
20 " *	8.0	3.4	3.4	2.9	2.5	2.2	1.8	** 4.9	2.3
	0.35	0.34	0.22	0.32	0.22	0.47	0.37	2.66	0.46
	54	38	14	13	6	3	4		
Adult (1 year) *	8.0	4.0	2.9	2.0	2.0	2.1	2.4	** 5.0	2.1
	0.41	0.93	0.23	0.29	0.36	0.45	0.44	2.68	0.19
	48	45	24	7	3	3	4		

* 1 case - mean, standard error of the mean and total number of vessels measured
** Mean, standard error of the mean (from the means shown in this table)

Fig. III 43

Diagram relating percentage venous wall thickness to external diameter (μm) throughout growth. Mean and standard error of the mean are represented for every size range (veins smaller than 1000 μm). Nine cases from birth to adult life

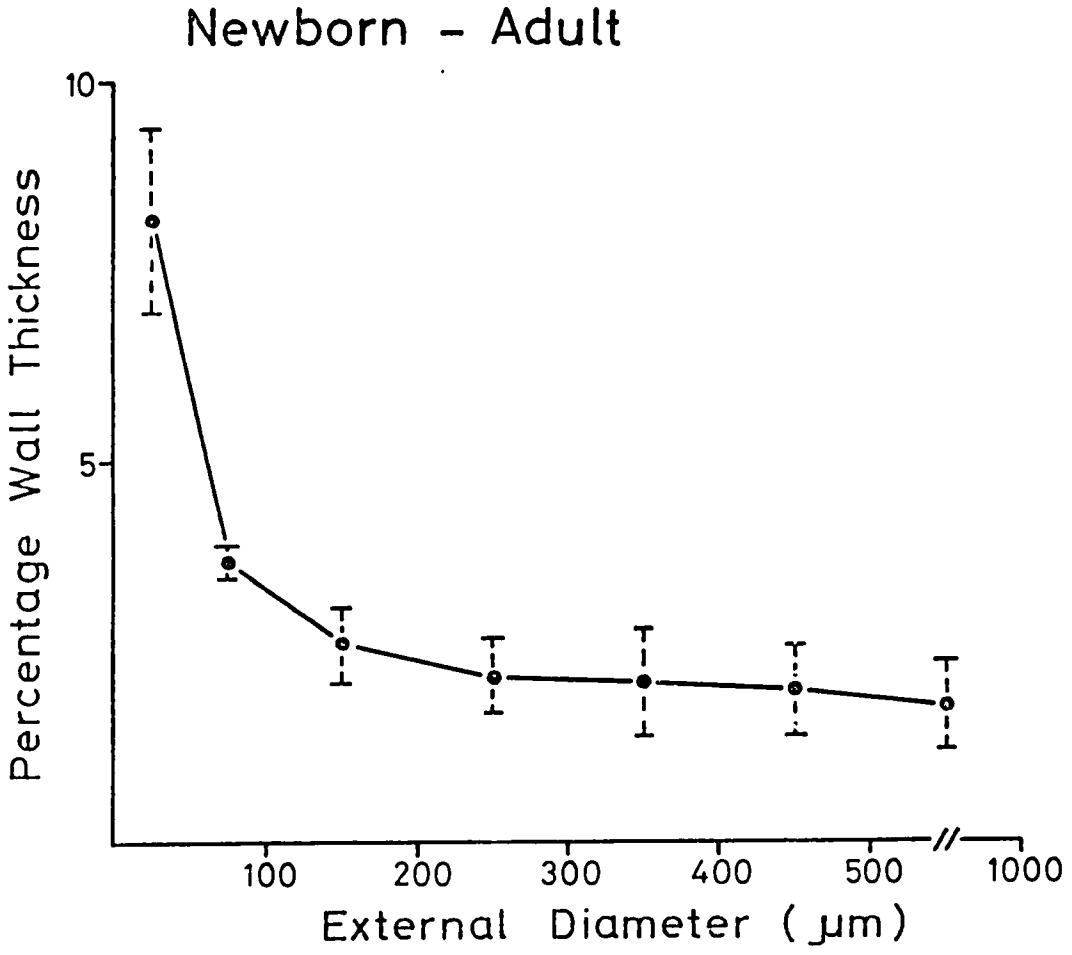

Newborn - Adult
Percentage Wall Thickness
10
5
100
200
300
400
500
1000
External Diameter (µm)

TABLE III 16

VENTRICULAR WEIGHTS DURING GROWTH

Age	RV+LV+S (g)	RV+LV+S/BW (g/100g)	RV (g)	LV+S (g)	$\frac{LV+S}{RV}$ (%)
Newborn	7.657	0.51	2.708	4.949	1.827
"	6.076	0.51	2.472	3.604	1.458
3 days	7.744	0.39	2.358	5.386	2.223
3 "	6.741	0.34	2.054	4.687	2.622
1 week	10.629	0.42	3.098	7.531	2.431
2 weeks	18.625	0.62	5.888	12.377	2.102
2 "	15.382	0.44	4.390	10.922	2.504
5 "	41.621	0.42	9.127	32.494	3.561
5 "	35.581	0.37	8.129	27.452	3.377
8 "	50.692	0.26	10.841	39.851	3.676
8 "	58.458	0.27	12.033	46.425	3.858
12 "	64.390	0.17	15.550	48.840	3.141
16 "	85.818	0.15	19.760	66.058	3.343
16 "	92.512	0.15	21.730	70.782	3.257
20 "	106.106	0.14	23.844	82.262	3.450
20 "	199.678	0.23	46.752	152.926	3.271
Adult (1 year)	336.788	0.19	83.460	253.328	3.035
" (3½ years)	405.879	-	98.011	307.868	3.141

Mean value for each case

RV - right ventricle
LV - left ventricle
S - septum
BW - body weight

average 59 fold. This relative difference occurred only during the first five weeks of life where the increase in weight of RVW and of LVW+SW was 3.3 and 7 fold, respectively. After that age, the rates of increase in weight with age were similar in both ventricles.

These changes can also be assessed by calculating the ratio between LVW+SW and RVW during growth. At birth, the RVW was relatively greater than that of LVW+SW, as compared with the adult animal, giving $\frac{LVW+SW}{RVW}$ of 1.642. This ratio increased during the first month of life (Table III 16) and by five weeks of age, it reached a mean value of 3.469, not different from the value (3.088) for the mature animals. Thus, the adult pattern of left ventricular dominance was virtually established during the first month of life.

SUMMARY OF RESULTS

1. The structure of the main and left pulmonary arteries changed considerably between birth and adult life, but both vessels remained elastic throughout growth and in the adult animals. There was a decrease in wall thickness during the first five weeks of life, but fragmentation of the elastic fibers within the media was only striking at 12 weeks of age. The adult pattern with disruption of the fibers and a considerable thickness of muscle cells in the media was only apparent by the twentieth week.

2. The pre-acinar arterial bronchial pathways did not change between birth and eight weeks of life. This suggested that during the period of remodeling of the intra-acinar zone, the arterial pre-acinar branching pattern and structure was already established.

3. The rate of increase in lumen diameter of the pre-acinar arteries, measured on the arteriograms, was faster during the first two months of life and similar at any point along the axial length.

4. The media of the muscular arteries in the pig was bounded by two distinct elastic laminae. As in the human and the rat, a mixed population of "muscular", "partially muscular" and "non-muscular" arteries could be identified at the periphery of any arterial pathway.

5. In the pig, muscle was already present in small vessels, 25 μm, at birth. Postnatally, arterial wall thickness rapidly decreased in vessels smaller than 200 μm in external diameter, reaching the adult value by the third day of life. Larger muscular arteries dropped to the adult wall thickness by eight weeks of age.

6. During the first two weeks of life, there was an apparent lag in muscle development in the arterial wall, probably because the changes in arterial diameter occurred very quickly during that period. Later, muscle was again present in very small arteries, 20 μm, as in the newborn, the adult pattern being reached by the twelfth week of life.

7. The arterial population studies reflected this change. The partially muscular arteries increased in number and size during the first two weeks of life and their maximum number, expressed as a percentage of the total number of vessels in a given size range, was found in the vessels between 80-90 μm in external diameter at two weeks of age contrasting with a range of 30-40 μm at birth. This early postnatal shift to the right of the partially muscular population was followed by a longer period, until twenty weeks of life, during which this population again moved to the left, as muscle extended along the peripheral pathway.

8. This "extension" of muscle along the peripheral arterial

bed, could also be demonstrated by studying the peripheral arteries "landmarked" by their accompanying airways. The newborn pig already showed a considerable number of muscular vessels accompanying both terminal and respiratory bronchioli, but no muscular arteries were found at alveolar duct level. In contrast, by the twentieth week of life, half of the vessels accompanying the alveolar ducts were muscular and this type of artery could also be found in the alveolar walls. A similar distribution was also seen in the adult animals.

9. The intra-acinar arteries increased in size throughout growth. Between birth and the third day of life, there was a two fold increase in external diameter at any level along the pathway. After that age, the more proximal arteries showed a faster rate of increase than the peripheral ones, until the fourth month of age, the rate of increase being again similar at all levels after that period and until adult life.

10. After birth, alveoli and intra-acinar arteries increased in number particularly during the first eight weeks of life and in size until adult life. This change in arterial number could be traced on the arteriogram by the appearance of background haze, particularly between birth and three days of life.

The Pulmonary Venous Bed

11. The rate of increase in lumen diameter of the pre-acinar veins, measured on the venogram, was faster during the first two months

of life and similar at any point along the pathway.

12. The appearance of background haze in the newborn venogram suggested a greater number of intra-acinar veins than arteries at birth.

13. The peripheral veins in the pig lacked an internal elastic lamina, but the external one was thick, particularly in the older animals. There was no change in venous wall thickness throughout growth.

Ventricular Weights

14. The adult pattern of left ventricular dominance, as assessed by weight, was established at five weeks of age.

CHAPTER IV

POSTNATAL PULMONARY PHYSIOLOGY

A total of 37 animals were included in the present study, details of age, sex and body weight are given in Table II 2.

Between two and sixteen weeks of life, there was a highly significant correlation ($P<0.001$) between age (weeks) and body weight (Kg) and the changes could be equally represented by linear, power and exponential equations (Table IV 1). The best fit was given by the power regression, the standard error of the regression coefficient being $\pm$ 5.34%.

LUNG FUNCTION

A total of twenty one animals were investigated (Table IV 2). During each experiment the carbon dioxide tension of the arterial blood (Pa CO_2) was always below 50 mm Hg. The mean Pa CO_2 being 44.5 $\pm$ 2.0 (standard deviation).

Respiratory Frequency

Respiratory frequency ranged between 11 and 54 breaths/min in all cases, with a mean value of 31 (Table IV 3). When related to age and body weight using linear and power equations the correlation coefficients were highly significant ($P<0.001$) the best fit being given by the power regression with body weight (standard error of the regression coefficient - 21.43%) (Table IV 4). The exponential

TABLE IV 1

PULMONARY PHYSIOLOGY. RELATION BETWEEN AGE AND BODY WEIGHT OF THE ANIMALS INCLUDED IN THE PRESENT STUDY FROM TWO TO SIXTEEN WEEKS OF LIFE

n = 35		Age (weeks) Linear	Power	Exponential
Body Weight (Kg)	r	0.926	0.953	0.905
	P	<0.001	<0.001	<0.001
	B	2.510	1.086	0.143
	SEB	0.175	0.058	0.011

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
B - regression coefficient
SEB - standard error of the regression coefficient

TABLE IV 2

PULMONARY PHYSIOLOGY. NATURE OF PROCEDURE

Procedure Case No.		
Lung Function	Pulm. Haem.	Pulm. Haem. and Lung Function
B.5	B.1	B.2
B.6	B.4	B.3
B.11	B.7	B.10
B.21	B.8	B.13
B.26	B.9	B.14
B.27	B.12	B.16
B.32	B.15	B.17
B.33	B.18	B.20
B.37	B.19	B.29
	B.22	B.31
	B.23	B.34
	B.24	B.36
	B.25	
	B.28	
	B.30	
	B.35	

Pulm. Haem. - pulmonary haemodynamics

TABLE IV 3

NORMAL CASES. LUNG FUNCTION MEASUREMENTS BETWEEN TWO AND SIXTEEN WEEKS OF LIFE

Case No.	f breaths/min	VT ml	VT/BW ml per Kg	CDyn ml/cm H_2O	CDyn/BW ml/cm H_2O per kg	TGV ml	TGV/BW ml per Kg	CSpecif ml/ cmH_2O per ml
B.2	48	24	6.9	8.8	2.5	189	54.0	0.047
B.3	54	23	3.3	10.6	1.5	194	27.7	0.079
B.5	45	90	9.0	18.7	1.9	281	28.1	0.067
B.6	39	56	5.6	18.2	1.7	244	23.2	0.074
B.10	40	110	6.3	15.3	0.9	299	17.1	0.051
B.11	27	110	11.0	17.1	1.7	286	28.6	0.062
B.13	37	75	6.8	23.4	2.1	301	27.4	0.078
B.14	36	94	7.8	29.5	2.4	456	38.0	0.065
B.16	24	96	6.9	34.0	2.4	451	32.2	0.075
B.17	28	95	6.3	48.2	3.2	537	35.8	0.090
B.20	24	123	4.9	39.8	1.6	419	16.8	0.095
B.21	25	90	3.7	42.3	1.8	455	18.9	0.093
B.26	24	108	4.9	44.6	2.0	442	20.1	0.101
B.27	26	163	7.1	63.1	2.7	485	21.1	0.130
B.29	25	168	6.7	41.6	1.7	525	21.0	0.079
B.31	33	94	4.3	53.4	2.4	618	28.1	0.086
B.32	20	192	6.4	46.1	1.5	661	22.0	0.070
B.33	11	171	7.1	49.9	2.1	739	30.8	0.054
B.34	24	192	4.6	68.2	1.6	814	19.4	0.084
B.36	23	236	4.7	96.7	1.9	1358	27.2	0.071
B.37	30	167	5.6	118.3	3.9	1135	37.8	0.104
Mean	31	118	6.2	40.3	2.1	518	27.4	0.079
SD	12.3	55.7	1.79	28.46	0.65	298.0	8.86	0.019
SEM	2.7	12.2	0.39	6.07	0.14	65.0	1.93	0.004

f - respiratory frequency
VT - tidal volume
BW - body weight
CDyn - dynamic compliance
TGV - thoracic gas volume
CSpecif - specific compliance

Mean value for each case; mean, standard deviation (SD) and standard error of the mean (SEM) for each variable

TABLE IV 4

LUNG FUNCTION STUDIES DURING GROWTH. RELATION WITH AGE AND BODY WEIGHT

n=19		Age (weeks) linear	power	exponential	Body Weight (Kg) linear	power	exponential
f	r	-0.666	-0.696	-0.630	0.673	-0.729	-0.623
	P	<0.001	<0.001	<0.01	<0.001	<0.001	<0.01
	B	-1.976	-0.451	-0.085	0.641	0.420	0.020
	SEB	0.508	0.107	0.024	0.162	0.090	0.006
VT	r	0.872	0.882	0.808	0.895	0.892	0.793
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	11.698	0.951	0.118	4.348	0.862	0.042
	SEB	1.508	0.116	0.020	0.497	0.100	0.007
VT/BW	r	-0.326	-0.209	-0.262	-	-	-
	P	>0.1	>0.1	>0.1			
	B	-0.139	-0.107	-0.018			
	SEB	0.093	0.115	0.015			
CDyn	r	0.916	0.953	0.933	0.812	0.889	0.826
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	6.143	1.166	0.154	1.961	0.978	0.050
	SEB	0.617	0.084	0.014	0.323	0.115	0.008
CDyn/BW	r	0.267	0.201	0.246	-	-	-
	P	>0.1	>0.1	>0.1			
	B	0.042	0.110	0.018			
	SEB	0.035	0.123	0.016			
TGV	r	0.916	0.936	0.958	0.874	0.880	0.380
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	65.702	0.879	0.122	22.749	0.743	0.041
	SEB	6.598	0.075	0.008	2.901	0.092	0.005
TGV/BW	r	-0.230	-0.326	0.191	-	-	-
	P	>0.1	>0.1	>0.1			
	B	-0.491	-0.173	-0.014			
	SEB	0.476	0.115	0.016			
CSpecif	r	0.367	0.485	0.403	0.292	0.451	0.315
	P	>0.1	<0.05	>0.1	>0.1	<0.05	>0.1
	B	0.002	0.213	0.024	0.005	0.178	0.007
	SEB	0.001	0.088	0.012	0.004	0.081	0.005

See table IV 3 for symbols

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
B - regression coefficient
SEB - standard error of the regression coefficient

equations were less significant ($P<0.01$).

Tidal Volume

Between two and sixteen weeks of life the increase in tidal volume (ml) was an average ten fold (Table IV 3). These changes were all highly significant ($P<0.001$) when related to age and body weight, the best fit being given by the linear regression with body weight (standard error of the regression coefficient - 11.43%) (Table IV 4).

Throughout growth the ratio between tidal volume and body weight (ml/Kg) remained unchanged ($P>0.1$) (Table IV 4), the mean value being 6.2 with a range between 3.3 and 11.0 (Table IV 3).

Dynamic Compliance

Dynamic compliance (ml/cm H_2O) also increased during the period of life presently investigated (Table IV 3). The changes were also highly significant ($P<0.001$) when related to both age and body weight, the best fit being given by the power regression with age (standard error of the regression coefficient - 7.20%) (Table IV 4).

The ratio between dynamic compliance and body weight (ml/cm H_2O

per Kg) with age ranged between 0.9 and 3.9 with a mean value of 2.1 (Table IV 3). These changes were not significant ($P>0.1$) throughout growth (Table IV 4).

Thoracic Gas Volume

Thoracic gas volume (ml) followed a similar pattern to dynamic compliance, the best of the highly significant fits being given by the exponential regression with age (standard error of the regression coefficient - 6.56%) (Tables IV 3-4).

The ratio between thoracic gas volume and body weight (ml/Kg) with age did not change significantly ($P>0.1$) (Table IV 4), the mean value being 27.4 with a range between 16.8 and 54.0 (Table IV 3).

Specific Compliance

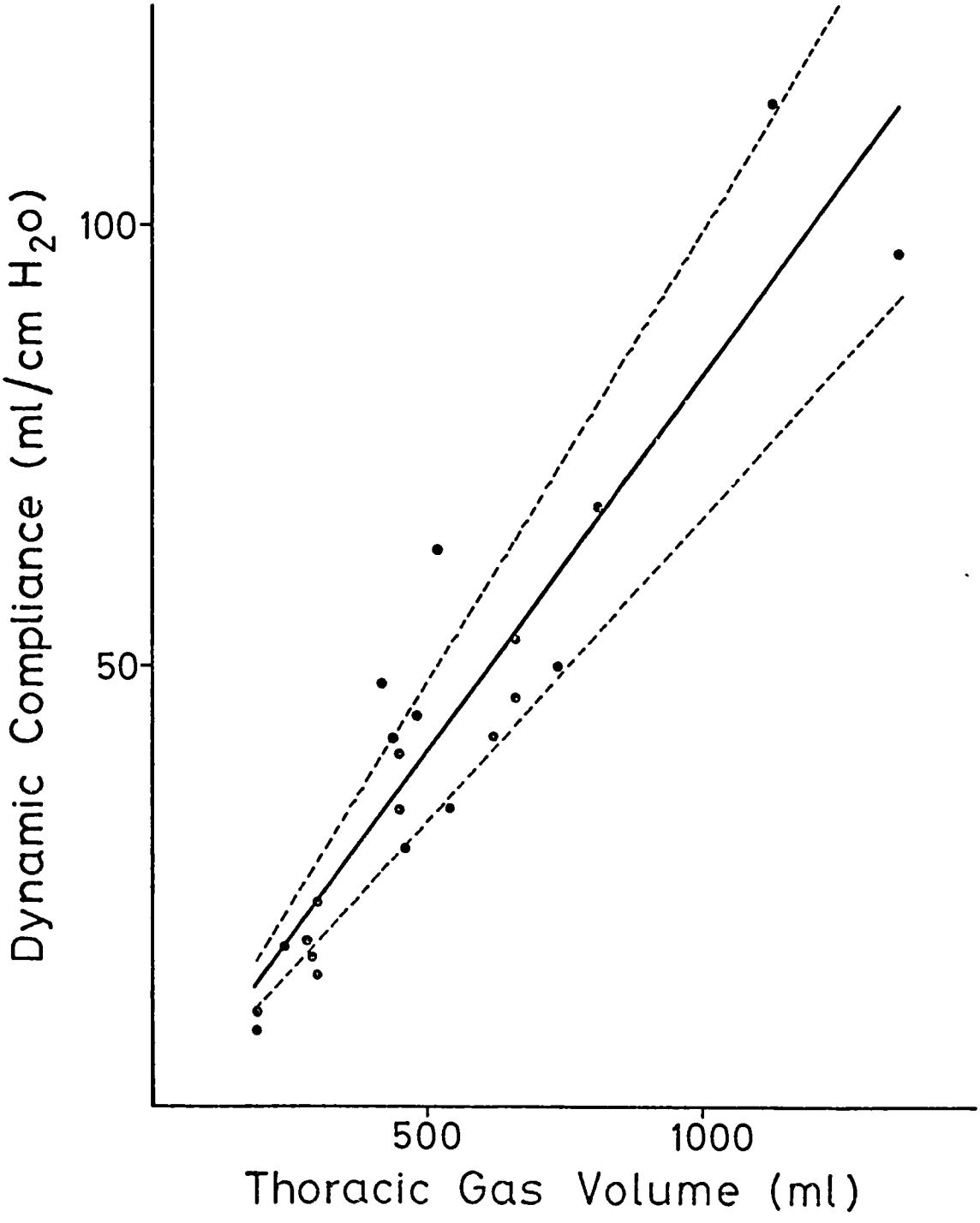
Dynamic compliance and thoracic gas volume increased at a similar rate during the period of development studied, and the relationship between these two variables could be represented by a linear, power or exponential equation (Fig. IV 1).

The ratio between dynamic compliance and thoracic gas volume termed specific compliance (ml/cm H_2O per ml) remained unchanged

Fig. IV 1

Diagram showing the correlation between thoracic gas volume and dynamic compliance throughout growth. The continuous line represents the ~~regression coefficient~~ linear regression and the dotted lines represent 2 standard error ~~of the regression coefficient~~ (P<0.001)

$Y = 0.077 x + 1.104$


Dynamic Compliance (ml/cm H_2O)
100
50
500
1000
Thoracic Gas Volume (ml)

throughout growth (Fig. IV 2). The best of the fits was given by the power regression, but even that relation was not highly significant either with age or body weight ($0.05 > P > 0.01$) (Table IV 4).

PULMONARY HAEMODYNAMICS

A total of twenty eight animals were investigated (Table IV 2). For details of age, sex and body weight see Table I 2.

There were no significant changes in the carbon dioxide tension and pH of either arterial or venous blood during the cardiac catheterization studies. The Pa O_2 was always above 90 mm Hg (Table IV 5).

Blood Pressures

Systemic Arterial

The changes in systemic arterial blood pressure (mm Hg) with either age or body weight were only highly significant for the power regression with age ($0.01 > P > 0.001$) (Tables IV 6-7). The mean systemic arterial pressure was 59 mm Hg between two and sixteen weeks of life (Fig. IV 3).

Fig. IV 2

Specific compliance measurements throughout growth. Correlation with age. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 3)

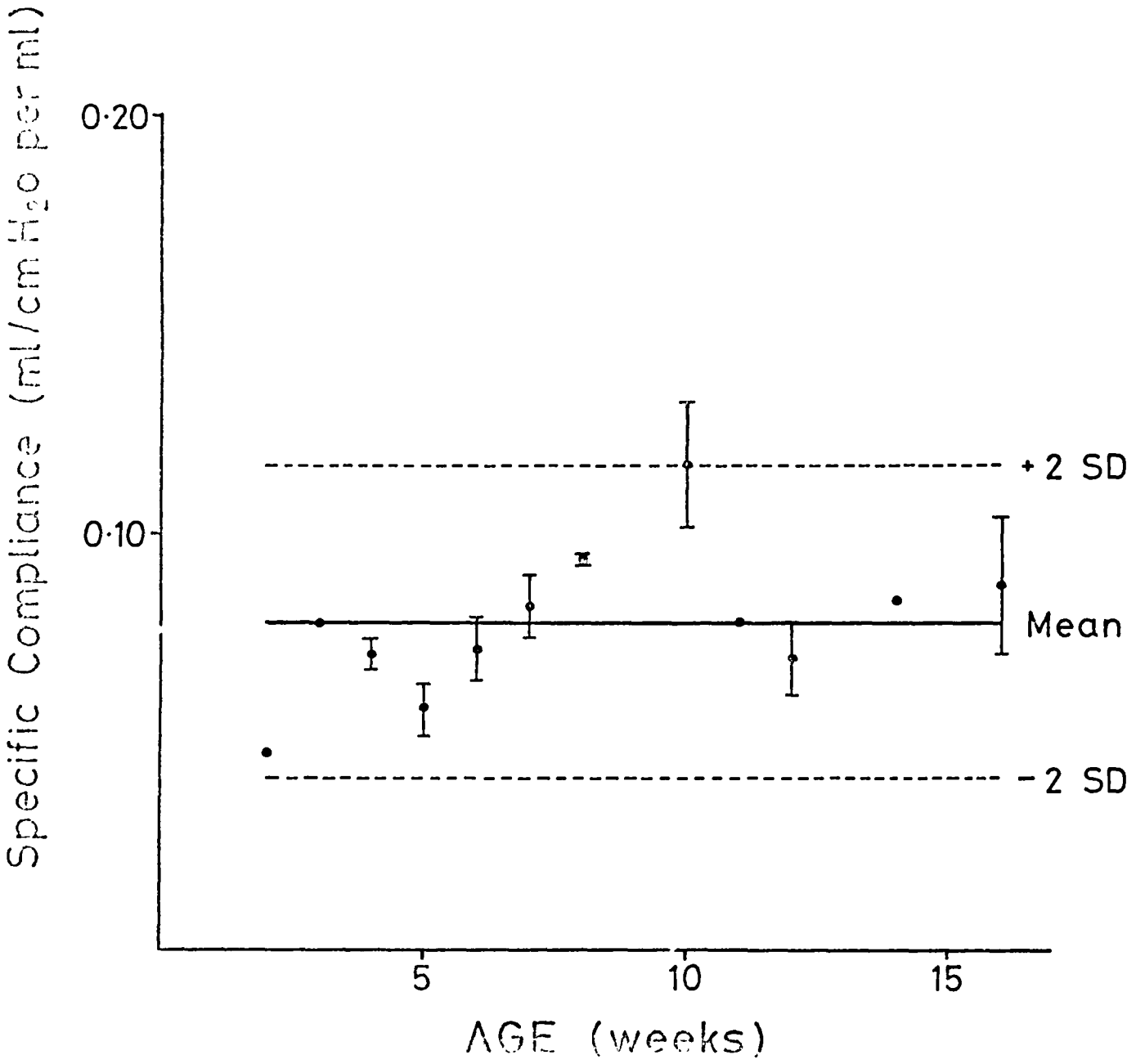

Specific Compliance (ml/cm H_2O per ml)
0·20
0·10
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

TABLE IV 5

ARTERIAL (a) AND MIXED VENOUS (V) BLOOD GAS TENSIONS AND pH MEASUREMENTS DURING CARDIAC CATHETERIZATION. 28 ANIMALS FROM TWO TO SIXTEEN WEEKS OF LIFE

Blood Gases	Mean	Standard Deviation	Standard Error
Pa O_2	168	26.9	6.3
Pa CO_2	41	4.1	1.0
pH	7.41	0.07	0.02
PV O_2	43	7.8	1.8
PV CO_2	49	8.4	2.0
pH	7.34	0.07	0.02

TABLE IV 6

NORMAL CASES. BLOOD PRESSURE MEASUREMENTS BETWEEN TWO AND SIXTEEN WEEKS OF LIFE

Case No. (28)	Blood Pressures (mm Hg)										
	SA			RV		RA	PA			PAW	mean PA
	syst	diast	mean	syst	end-diast	mean	syst	diast	mean	mean	mean SA
B.1	52	23	33	18	1	2	17	7	10	-	0.30
B.2	82	49	60	21	1	2	21	6	11	4	0.18
B.3	88	20	43	22	2	1	20	7	11	5	0.26
B.4	70	37	48	19	1	2	17	7	10	4	0.21
B.7	71	34	46	14	0	3	14	5	8	-	0.17
B.8	86	28	47	23	0	2	22	9	13	6	0.28
B.9	89	51	64	25	1	1	16	5	9	-	0.14
B.10	65	44	51	19	0	3	19	9	12	-	0.23
B.12	75	46	56	24	3	3	24	12	16	-	0.29
B.13	103	59	74	18	1	2	18	7	11	4	0.15
B.14	113	56	75	14	2	1	14	5	8	-	0.11
B.15	105	58	74	24	0	3	24	8	12	7	0.16
B.16	105	60	75	21	1	3	21	7	12	7	0.16
B.17	67	43	51	17	0	1	14	5	8	4	0.16
B.18	91	35	54	21	1	2	21	7	12	5	0.22
B.19	86	39	55	21	1	2	20	6	11	4	0.20
B.20	73	45	54	14	1	1	16	7	10	4	0.18
B.22	107	55	72	23	0	3	21	9	13	10	0.18
B.23	69	41	50	19	2	1	19	9	12	6	0.24
B.24	65	36	51	23	1	2	23	13	16	9	0.31
B.25	65	45	52	15	3	1	12	5	7	5	0.13
B.28	95	59	71	20	0	1	20	4	9	3	0.13
B.29	84	60	68	13	1	2	13	5	8	4	0.12
B.30	86	41	56	23	2	3	23	13	16	7	0.29
B.31	76	52	60	12	0	1	14	5	8	3	0.13
B.34	81	46	58	15	1	1	15	4	8	3	0.14
B.35	125	62	83	25	0	3	25	8	14	-	0.17
B.36	80	49	59	16	1	1	16	5	9	4	0.15
Mean	84	45	59	19	1	2	18	7	11	5	0.19
SD	17.0	11.3	11.7	3.9	0.2	0.8	3.7	2.5	2.6	2.0	0.061
SEM	3.2	2.1	2.2	0.7	0.04	0.2	0.7	0.5	0.5	0.4	0.011

SA - systemic artery
RV - right ventricle
RA - right atrium
PA - pulmonary artery
PAW - pulmonary artery wedge

syst - systolic
diast - diastolic
SD - standard deviation
SEM - standard error of the mean

Mean value for each case; mean, SD and SEM for each variable

TABLE IV 7

BLOOD PRESSURE MEASUREMENTS DURING GROWTH. RELATION WITH AGE AND BODY WEIGHT

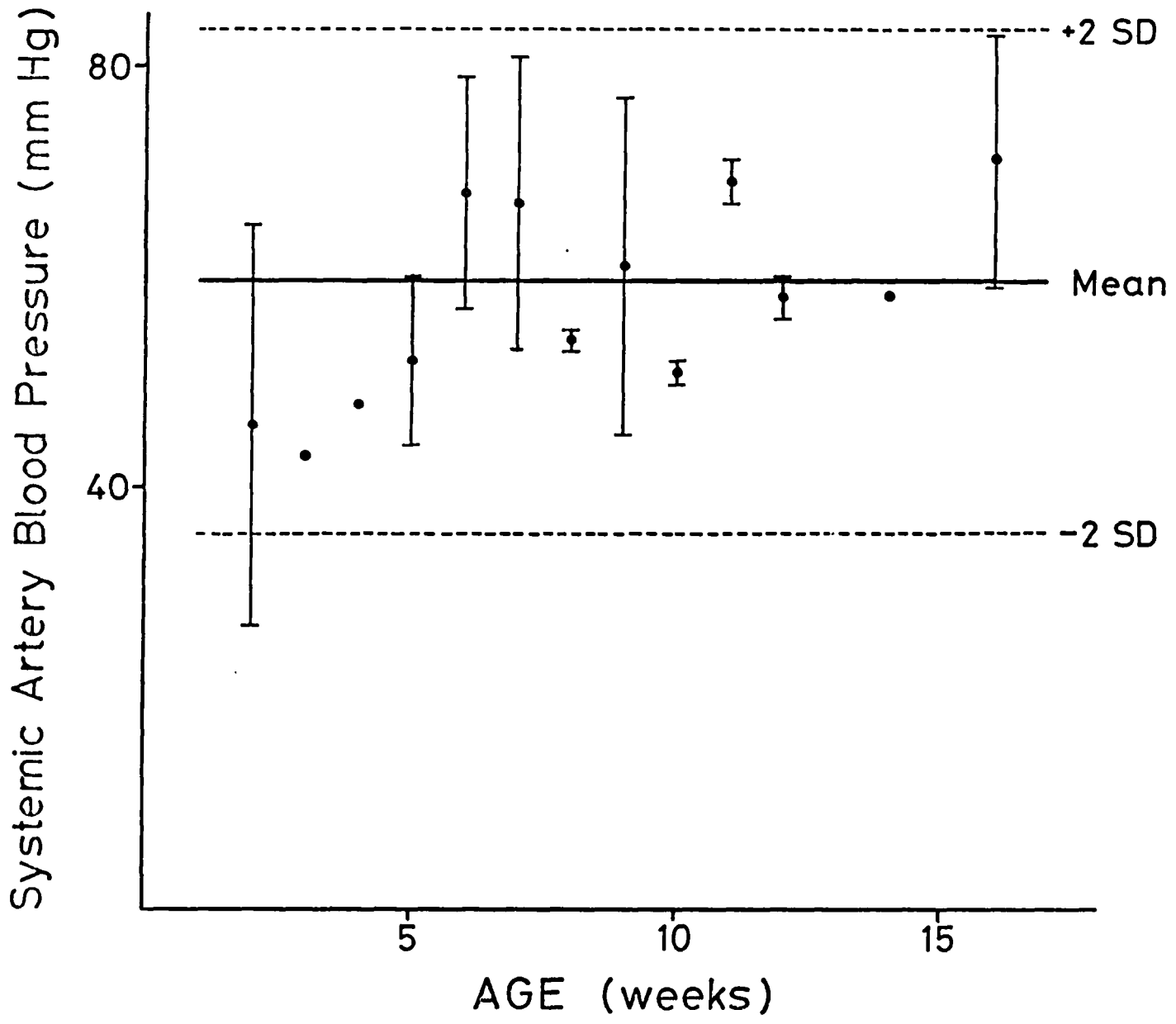
		Age (weeks)			Body Weight (Kg)		
n=26		linear	power	exponential	linear	power	exponential
SAP	r	0.411	0.495	0.438	0.371	0.459	0.390
mean	P	<0.05	<0.01	<0.05	<0.1	<0.05	<0.05
	B	1.273	0.187	0.024	0.396	0.145	0.007
	SEB	0.554	0.064	0.010	0.194	0.055	0.003
RV	r	-0.154	-0.179	-0.181	-0.078	-0.139	-0.106
syst	P	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	B	-0.160	-0.071	-0.010	-0.028	-0.046	-0.002
	SEB	0.201	0.077	0.011	0.068	0.065	0.004
RAP	r	-0.117	-0.142	-0.163	-0.128	-0.182	0.158
mean	P	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	B	-0.026	-0.122	-0.020	-0.010	-0.131	-0.007
	SEB	0.043	0.167	0.024	0.015	0.139	0.008
RAP	r	0.004	-0.027	-0.043	-0.013	-0.050	-0.034
mean	P	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
	B	0.003	-0.012	-0.003	-0.003	-0.018	-0.001
	SEB	0.134	0.085	0.012	0.046	0.071	0.004
PAWP	r	-0.100	0.006	-0.188	-0.214	-0.156	-0.271
mean	P	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
(n=19)	B	-0.057	0.006	-0.020	-0.045	-0.104	-0.011
	SEB	0.130	0.226	0.025	0.048	0.152	0.009
mean PAP	r	0.325	0.356	0.328	0.284	0.349	0.295
mean SAP	P	<0.1	<0.1	<0.1	>0.1	<0.1	>0.1
	B	0.144	0.200	0.026	0.043	0.165	0.008
	SEB	0.082	0.103	0.015	0.029	0.087	0.005

see Table IV 6 for symbols

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
B - regression coefficient
SEB - standard error of the regression coefficient

Fig. IV 3

Systemic artery blood pressure measurements throughout growth. Correlation with age. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 6)


Systemic Artery Blood Pressure (mm Hg)
80
40
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

Right-Side Heart

Right ventricular and atrial pressures (mm Hg) did not change during this growth period ($P>0.1$) (Tables IV 6-7). The mean right ventricular systolic pressure being 19 mm Hg whereas the mean right atrial pressure was 2 mm Hg (Fig. IV 4).

Pulmonary Arterial

Both pulmonary arterial and wedge pressures (mm Hg) remained unchanged during the period of development ($P>0.1$) (Tables IV 6-7). The mean pulmonary arterial pressure ranged between 8 and 16 mm Hg, with a mean value of 11 mm Hg (Fig. IV 5). The mean pulmonary arterial wedge pressure ranged between 3 and 10 mm Hg with a mean value of 5 mm Hg (Fig. IV 6).

The ratio between mean pulmonary and mean systemic arterial pressures also remained unchanged between two and sixteen weeks of life ($0.1>P>0.05$) (Tables 6-7).

Heart Rate, Cardiac Output and Stroke Volume

Heart Rate

The changes in heart rate (beats/min) were highly significant, when related to either age or body weight ($0.01>P>0.001$) (Tables IV 8-9). The mean value for all the cases was 111 beats/min with a range between 82 and 159.

Fig. IV 4

Right ventricle systolic blood pressure measurements throughout growth. Correlation with age. Mean and standard deviation values for each age group. The continuous and the dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 6)

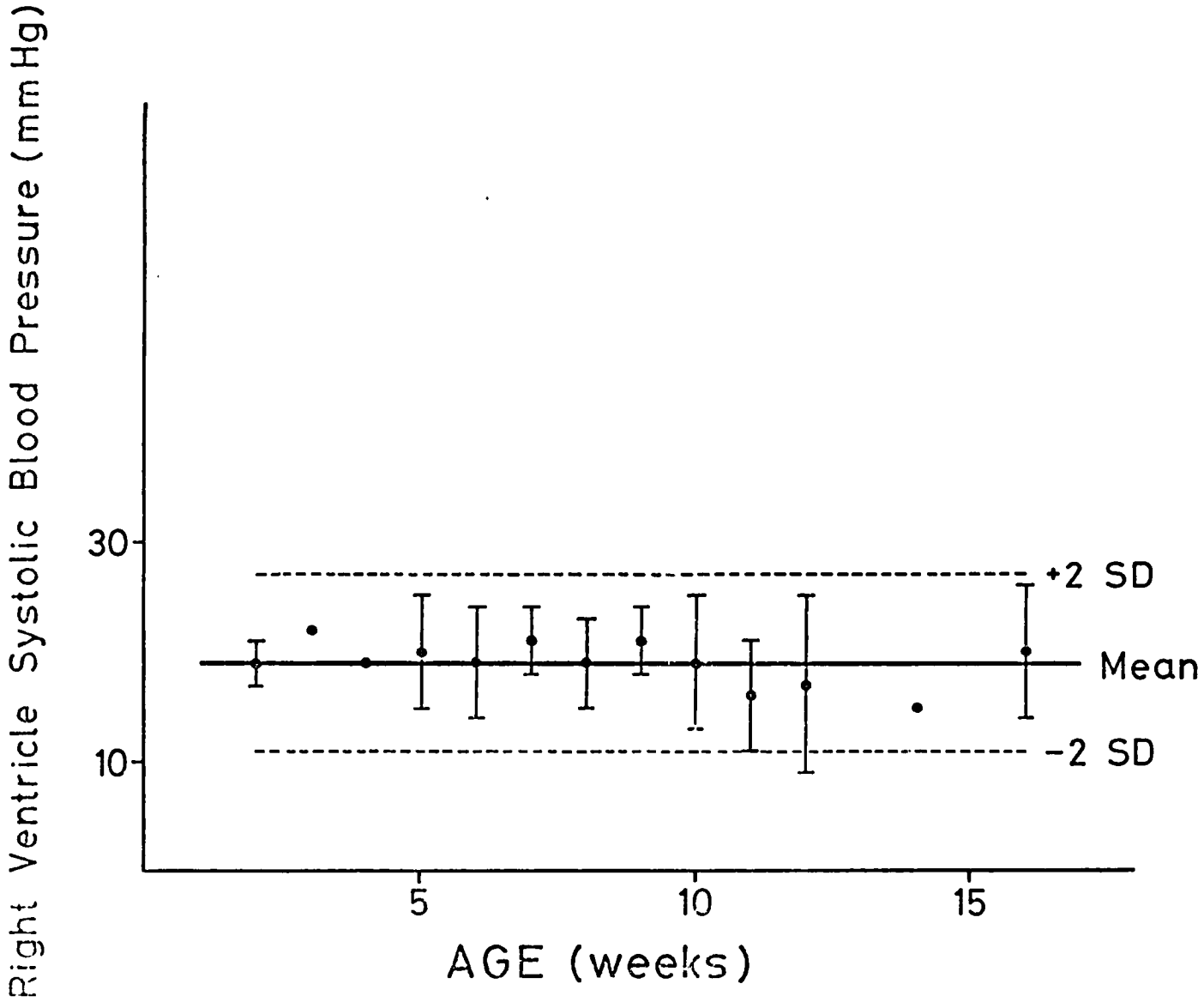

Right Ventricle Systolic Blood Pressure (mm Hg)
30
10
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

Fig. IV 5

Mean pulmonary artery pressure measurements throughout growth. Correlation with age. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 6)

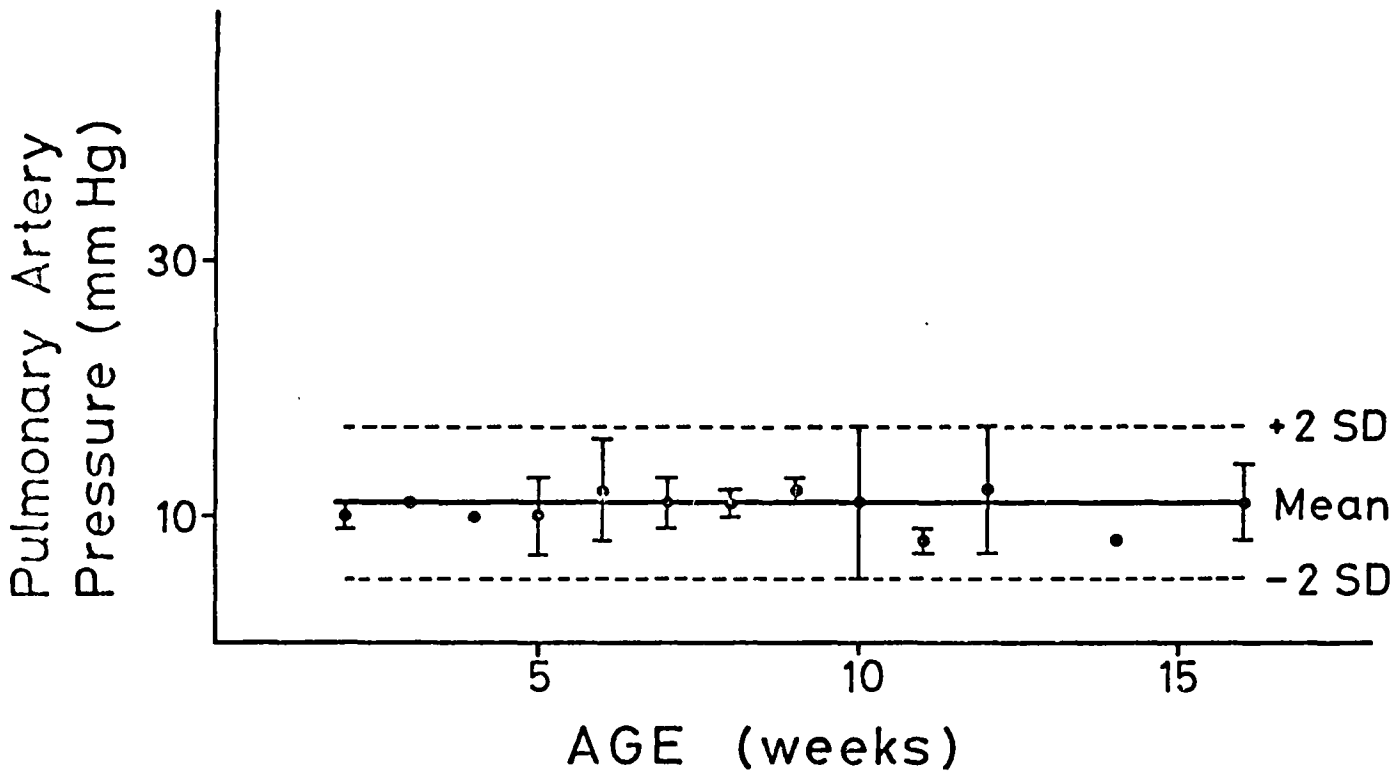

Pulmonary Artery
Pressure (mm Hg)
30
10
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

Fig. IV 6

Mean pulmonary artery wedge pressure measurements throughout growth. Correlation with age. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 6)

Pulmonary Artery Wedge Pressure (mm Hg)
10
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

TABLE IV 8

NORMAL CASES. HEART RATE, CARDIAC OUTPUT AND STROKE VOLUME MEASUREMENTS BETWEEN TWO AND SIXTEEN WEEKS OF LIFE

Case No.(28)	HR beats/min	CO ml/min	CO/BW ml/min per Kg	CI l/min per m^2	SV ml/beat
B.1	113	390	130.0	2.4	3.4
B.2	136	550	157.1	3.2	4.0
B.3	159	750	107.1	3.1	4.7
B.4	113	600	66.7	2.2	5.3
B.7	98	850	80.9	2.7	8.7
B.8	117	760	76.0	2.7	6.5
B.9	152	980	81.7	3.1	6.4
B.10	109	885	88.5	3.2	8.1
B.12	114	975	88.6	3.2	8.5
B.13	130	950	79.2	3.1	7.3
B.14	101	1010	91.8	3.4	10.0
B.15	104	1750	125.0	5.1	16.8
B.16	122	1250	83.3	3.6	10.2
B.17	125	1335	83.4	3.7	10.7
B.18	82	1100	59.5	2.8	13.4
B.19	86	1500	62.5	3.4	17.4
B.20	106	1175	69.1	3.1	11.1
B.22	134	2375	169.6	7.0	17.7
B.23	101	1985	99.2	5.0	19.6
B.24	85	2850	109.6	6.2	33.5
B.25	103	1925	87.5	4.6	18.7
B.28	106	2700	108.0	6.1	25.5
B.29	106	2050	80.4	4.6	19.3
B.30	105	2800	127.3	6.7	26.7
B.31	93	2175	94.6	5.1	23.4
B.34	86	3500	83.3	6.0	40.7
B.35	109	3750	75.0	5.9	34.4
B.36	104	3450	90.8	7.0	33.2
Mean	111	1656	94.8	4.2	15.8
SD	18.9	970.6	26.60	1.51	10.65
SEM	3.6	58.6	5.03	0.29	2.05

HR - heart rate
CO - cardiac output
BW - body weight
CI - cardiac index
SV - stroke volume
SD - standard deviation
SEM - standard error of the mean

Mean value for each case; mean, SD and SEM for each variable

TABLE IV 9

HEART RATE, CARDIAC OUTPUT AND STROKE VOLUME MEASUREMENTS DURING GROWTH. RELATION WITH AGE AND BODY WEIGHT

		Age (weeks)			Body Weight (Kg)		
n=26		linear	power	exponential	linear	power	exponential
HR	r	-0.488	-0.526	-0.486	-0.482	-0.533	-0.488
	P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	B	-2.428	-0.159	-0.021	-0.823	-0.135	-0.007
	SEB	0.852	0.051	0.007	0.293	0.042	0.002
CO	r	0.949	0.942	0.944	0.914	0.919	0.871
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	242.887	1.056	0.152	80.552	0.862	0.048
	SEB	15.285	0.074	0.010	7.024	0.073	0.005
CO/BW	r	-0.138	0.223	-0.113	-	-	-
	P	>0.1	>0.1	>0.1			
	B	-0.967	-0.105	-0.008			
	SEB	1.363	0.090	0.013			
CI	r	0.823	0.785	0.823	-	-	-
	P	<0.001	<0.001	<0.001			
	B	0.327	0.506	0.076			
	SEB	0.044	0.078	0.010			
SV	r	0.929	0.950	0.942	0.936	0.931	0.878
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	2.562	1.220	0.173	0.869	1.001	0.055
	SEB	0.200	0.079	0.012	0.060	0.077	0.006

see Table IV 8 for symbols

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
B - regression coefficient
SEB - standard error of the regression coefficient

Cardiac Output

There was a considerable increase in cardiac output (ml) throughout growth (Table IV 8). These changes were all highly significant ($P<0.001$), when related to both age and body weight, the best fit being given by linear regression with age (standard error of the regression coefficient - 6.29%) (Table IV 9).

The ratio between cardiac output (ml/min) and body weight (Kg) remained unchanged between two and sixteen weeks of life ($P>0.1$) (Table IV 9). The mean value for this variable was 94.8 ml/min per Kg with a range between 59.5 and 169.6 (Fig. IV 7).

Cardiac index (l/min per m^2) increased with age (Fig. IV 8); this change was highly significant ($P<0.001$) (Table IV 9).

Stroke Volume

Stroke volume (ml/beat) increased throughout growth (Table IV 8). The best of the all highly significant fits ($P<0.001$) was given by the power regression with age (standard error of the regression coefficient - 6.47%) (Table IV 9).

Pulmonary Vascular Resistances

Total Pulmonary Resistance

Total pulmonary resistance - TPR (Units - mm Hg per l/min) -

Fig. IV 7

Cardiac output measurements throughout growth. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and 2 standard deviation values for the whole series (see Table IV 8)

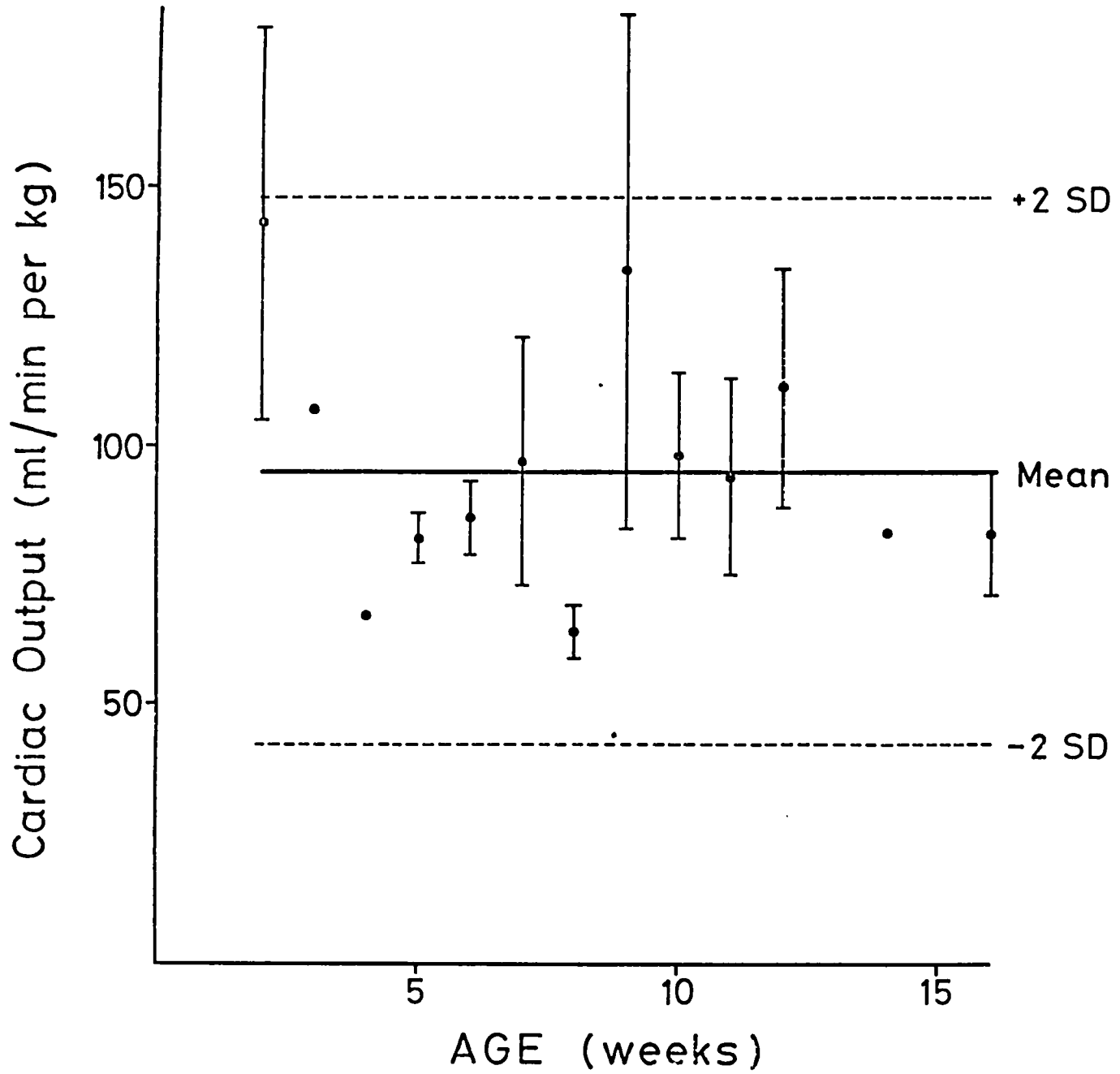
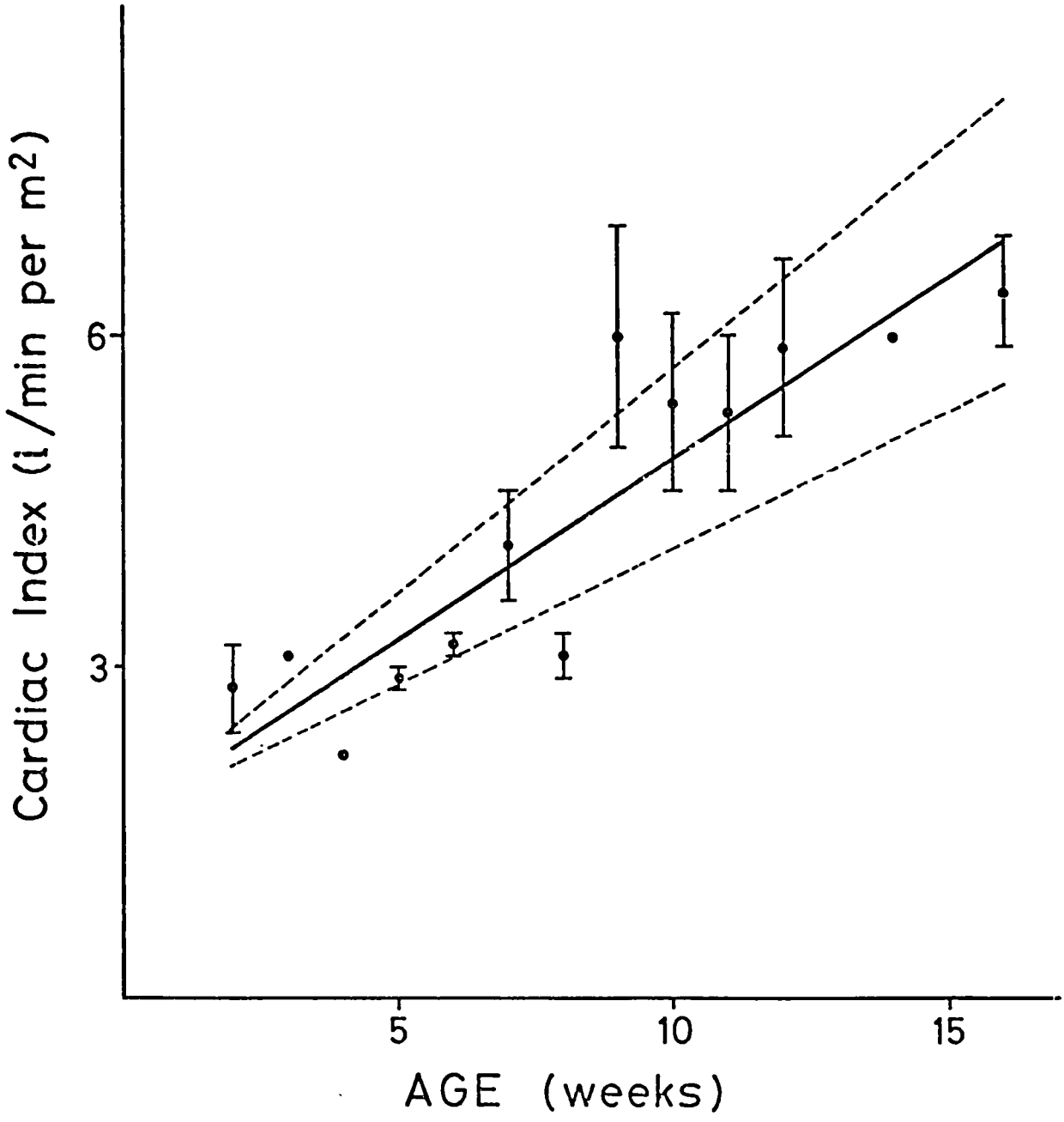

Cardiac Output (ml/min per kg)
150
100
50
+2 SD
Mean
-2 SD
5
10
15
AGE (weeks)

Fig. IV 8

Correlation between age and cardiac index throughout growth. Mean and standard error values for each age group. The continuous line represents the regression coefficient and the dotted lines represent 2 standard error values of the regression coefficient


Cardiac Index (l/min per m2)
6
3
5
10
15
AGE (weeks)

decreased throughout growth, all the values being below 6 Units after the eighth week of life (Fig. IV 9). In the period of development studied, these changes were highly significant, when related to both age and body weight ($P<0.001$), the best fit was given by the exponential regression with age (standard error of the regression coefficient - 9.03%) (Tables IV 10-11).

Total pulmonary resistance index - TPRI (mm Hg per l/min per m^2 - decreased significantly with age ($P<0.001$) (Table IV 11).

Pulmonary Arterial Resistance

The changes in pulmonary arterial resistance - PAR (Units - mm Hg per l/min) - were similar to those described for the total pulmonary resistance (Tables IV 10-11). After the age of eight weeks all the values for PAR were below 3.2 Units (Fig. IV 9).

Pulmonary arterial resistance index - TPRI (mm Hg per l/min per m^2) - also decreased significantly during growth ($0.01>P>0.001$) (Table IV 10-11).

Fig. IV 9

Total pulmonary resistance (TPR) and pulmonary arterial resistance (PAR) measurements throughout growth. Mean values for each case. The continuous and dotted lines represent respectively the regression coefficients for TPR and PAR (see Table IV 11)

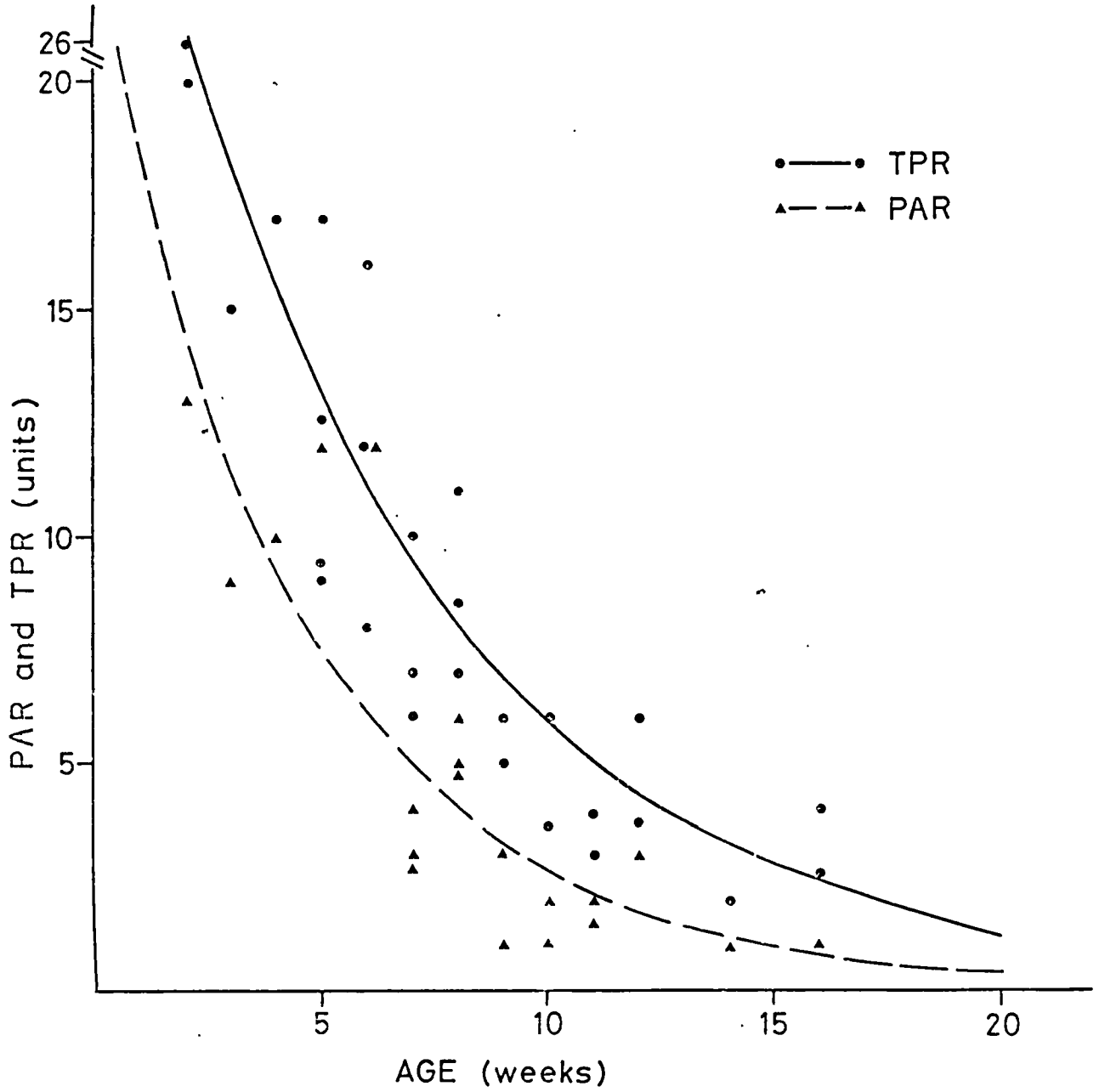

26
20
15
10
5
PAR and TPR (units)
TPR
PAR
5
10
15
20
AGE (weeks)

TABLE IV 10

NORMAL CASES. PULMONARY VASCULAR RESISTANCE MEASUREMENTS BETWEEN TWO AND SIXTEEN WEEKS OF LIFE

Case No. (28)	TPR mm Hg per l/min	TPRI mm Hg per l/min per m^2	PAR mm Hg per l/min	PARI mm Hg per l/min per m^2
B.1	25.6	4.1	-	-
B.2	20.0	3.4	12.7	2.2
B.3	14.7	3.5	9.3	1.9
B.4	16.7	4.5	10.0	2.7
B.7	9.4	3.0	-	-
B.8	17.1	4.8	9.0	1.9
B.9	9.2	2.9	-	-
B.10	13.6	3.8	-	-
B.12	16.4	4.9	-	-
B.13	11.6	3.6	7.4	2.3
B.14	7.9	2.4	-	-
B.15	6.9	2.3	2.9	1.0
B.16	9.6	3.4	4.0	1.4
B.17	6.0	2.2	3.0	1.1
B.18	10.9	4.2	6.4	2.5
B.19	7.3	3.2	4.7	2.0
B.20	8.5	3.1	5.1	1.9
B.22	5.5	1.9	1.3	0.4
B.23	6.0	2.4	3.0	1.2
B.24	5.6	2.6	2.5	1.1
B.25	3.6	1.5	1.0	0.4
B.28	3.3	1.5	1.5	1.0
B.29	3.9	1.7	1.9	0.9
B.30	5.7	2.4	3.2	1.3
B.31	3.7	1.6	2.8	1.2
B.34	2.3	1.3	1.4	0.8
B.35	3.7	2.4	-	-
B.36	2.6	1.3	1.4	0.7
Mean	9.2	2.9	4.5	1.4
SD	5.87	1.07	3.36	0.62
SEM	1.11	0.20	0.73	0.15

TPR - total pulmonary resistance
TPRI - total pulmonary resistance index
PAR - pulmonary arterial resistance
PARI - pulmonary arterial resistance index
SD - standard deviation
SEM - standard error of the mean

Mean value for each case; mean, standard deviation (SD) and standard error of the mean (SEM) for each variable

TABLE IV 11

PULMONARY VASCULAR RESISTANCE MEASUREMENTS DURING GROWTH. RELATION WITH AGE AND BODY WEIGHT

		Age (weeks)			Body Weight (Kg)		
		linear	power	exponential	linear	power	exponential
TRP	r	-0.825	-0.901	-0.909	-0.763	-0.887	-0.841
n=26	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	-1.280	-1.069	-0.155	-0.395	-0.882	-0.049
	SEB	0.172	0.101	0.014	0.066	0.090	0.006
TRPI	r	-0.710	-0.700	-0.752	-	-	-
n=26	P	<0.001	<0.001	<0.001			
	B	-0.206	-0.511	-0.079			
	SEB	0.040	0.102	0.014			
PAR	r	-0.840	-0.830	-0.823	-0.747	-0.793	-0.738
n=19	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	B	-0.804	-1.234	-0.176	-0.267	-1.023	-0.059
	SEB	0.119	0.190	0.028	0.055	0.185	0.012
PARI	r	-0.675	-0.602	-0.616	-	-	-
n=19	P	<0.01	<0.01	<0.001			
	B	-0.130	-0.645	-0.095			
	SEB	0.033	0.196	0.028			

see Table IV 10 for symbols

n - degrees of freedom
r - correlation coefficient
P - level of significance (Student's t-test)
B - regression coefficient
SEB - standard error of the regression coefficient

SUMMARY OF RESULTS

1. Studies of lung function and pulmonary haemodynamics have been performed in growing swine from the second week to the fourth month of life.

Lung Funtion

2. The respiratory frequency ranged between 11 and 54 breaths/minute in all cases with a mean value of 31.

3. Tidal volume, dynamic compliance and thoracic gas volume increased significantly between two weeks and four months of life, and these changes were highly significant when related to both age and body weight ($P<0.001$).

4. Specific compliance remained unchanged throughout growth, ranging between 0.047 and 0.130 with a mean value of 0.079 ml/cm H_2O per ml.

Pulmonary Haemodynamics

5. In the absence of any abnormality of gas exchange, the systemic, right ventricular and pulmonary arterial pressures remained unchanged throughout growth.

6. The mean value for heart rate in all cases was 111 beats/minute with a range between 82 and 159.

7. Both the cardiac output and stroke volume increased throughout growth and these changes were all highly significant ($P<0.001$), when related to both age and body weight. The ratio between cardiac output and body weight remained unchanged until the sixteenth week of life and the mean value for this variable was 94.8 ml/min/Kg. The cardiac index (l/min per m^2) increased with age and this change was highly significant ($P<0.001$).

8. Total pulmonary and pulmonary arterial resistances decreased throughout growth, and after the eighth week of life all values for these variables were below 6.0 and 3.2 Units respectively.

CHAPTER V

AORTO-PULMONARY SHUNTS IN GROWING SWINE - THEIR FUNCTIONAL AND STRUCTURAL EFFECT ON THE PULMONARY VASCULATURE

Eighteen animals were studied. The age at surgery varied between four and twelve weeks of life and the duration of the follow-up period ranged between four and eleven weeks (Table V 1).

Four cases (C. 1, 5, 8 and 16) were found to have no signs of recirculation during the cardiac catheterization studies (see Functional Studies), and the post-mortem examination confirmed that in these animals the grafts were occluded. They were studied both functionally and structurally in the same way as were the abnormal cases and used as controls.

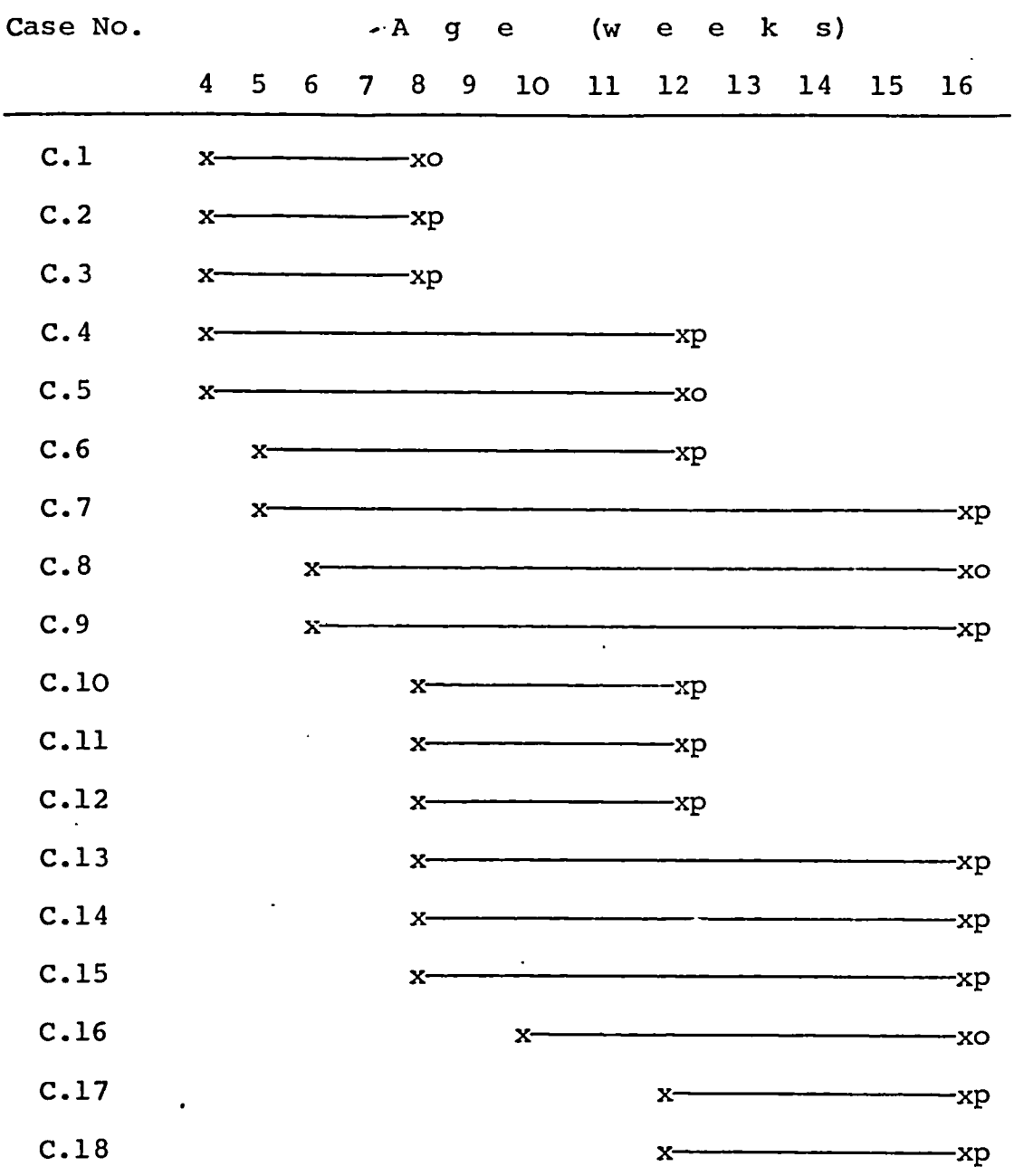
The changes in body weight during the follow-up period (Table V 2) were similar in both shunted and control cases ($P>0.1$); they did not differ significantly from those found in the normal animals throughout growth ($P>0.1$).

FUNCTIONAL STUDIES

Each case was studied sequentially as follows: (i) at surgery, pulmonary haemodynamics were investigated before and after the creation of the aorto-pulmonary anastomosis; (ii) during the follow-up period, serial lung function studies were performed; and (iii) immediately before the sacrifice, pulmonary haemodynamics were assessed by cardiac catheterization and in some cases in the thoracotomized animal.

TABLE V 1

AORTO-PULMONARY SHUNTS. DURATION OF THE FOLLOW-UP PERIOD



o - shunt occluded

p - shunt patent

TABLE V 2

AORTO-PULMONARY SHUNTS. BODY WEIGHT (Kg) DURING THE FOLLOW-UP PERIOD

Case No.	Age (weeks)												
	4	5	6	7	8	9	10	11	12	13	14	15	16
C.1	11.0	-	15.0	18.0	19.0								
C.2	12.0	-	19.0	20.0	22.0								
C.3	11.5	-	15.0	21.0	24.0								
C.4	10.5	-	14.0	16.0	17.0	-	-	-	26.0				
C.5	10.0	-	15.0	17.5	21.0	24.0	-	-	36.0				
C.6		10.5	-	14.0	21.0	24.0	-	-	32.0				
C.7		12.0	-	26.0	-	-	-	-	-	35.0	36.0	-	45.0
C.8			11.5	-	-	24.0	-	-	-	35.5	38.0	-	39.5
C.9			15.0	-	-	25.0	30.0	-	-	-	-	-	45.0
C.10					17.0	-	28.0	33.0	36.0				
C.11					15.0	-	20.0	23.0	25.0				
C.12					25.0	-	30.0	33.0	36.0				
C.13					19.0	-	23.5	-	-	36.0	36.0	-	54.0
C.14					19.5	-	-	-	-	36.0	40.0	-	52.0
C.15					25.0	-	-	36.0	-	-	42.0	-	45.0
C.16							24.0	-	30.0	37.0	-	-	52.0
C.17									22.0	-	33.0	35.0	40.0
C.18									28.0	-	30.0	36.0	38.0
Normal*	9.0	9.5	11.0	14.0	17.0	14.0	22.0	25.0	22.0				30.0
range	10.5	17.5	12.0	16.0	25.0	20.0	26.0	25.5	38.0	-	42.0	-	60.5

*from Tables II 1 and 2

Pulmonary Haemodynamics

Intra-Operative Measurements

In the experimental cases, aortic and pulmonary artery pressures were measured immediately after opening the chest by direct needle puncture (Table V 3). A sample of arterial blood was taken to be analysed later for gas tensions, pH and haematocrit. Main pulmonary artery blood flow was also assessed in some cases, using an electromagnetic flowmeter and a cuff-head transducer.

After the completion of the aorto-pulmonary anastomosis, these measurements were repeated with the additional assessment of the shunt flow and in some cases, of main pulmonary artery flow (Tables V 4-5). A second sample of arterial blood was taken at the time of these measurements.

Before establishing the shunt

Heart rate The mean value for the heart rate in all cases was 120 beats/min, a figure not significantly different ($P>0.1$) from that found in the normal animals at cardiac catheterization (Table V 6)

Systemic arterial pressure Systemic arterial pressure was also similar in both the open-chest animals and at cardiac catheterization ($P>0.1$) (Table V 7).

TABLE

INTRA-OPERATIVE MEASUREMENTS. HEART RATE AND BLOOD

Case No.	HR (beats/min)		SAP (mm Hg)					
	B	A	B			A		
			s	d	m	s	d	m
C.1	62	106	84	42	56	45	22	30
C.2	133	183	85	48	60	75	33	47
C.3	132	147	78	47	57	46	24	31
C.4	123	135	61	37	45	56	26	36
C.5	125	125	73	43	53	62	40	47
C.6	176	186	76	43	54	61	26	38
C.7	116	157	70	51	57	63	38	46
C.8	131	145	102	65	77	65	35	45
C.9	148	153	-	-	-	-	-	-
C.10	120	120	-	-	-	-	-	-
C.11	140	109	68	43	51	65	41	49
C.12	101	118	74	52	59	47	28	34
C.13	98	99	80	54	63	59	31	40
C.14	108	121	89	50	63	70	24	41
C.15	139	147	73	52	59	59	32	41
C.16	114	127	103	50	68	73	40	51
C.17	100	107	88	46	60	68	30	43
C.18	92	120	94	38	54	73	27	42
Mean	120	134	81	47	59	62	31	41
SD	25.1	25.2	11.9	7.1	7.2	9.5	6.2	6.2

HR - heart rate
SAP - systemic arterial pressure
PAP - pulmonary arterial pressure
B - before shunt
A - after shunt
s - systolic pressure
d - diastolic pressure
m - mean pressure
SD - standard deviation

Mean value for each case; mean and SD for each variable

V 3

PRESSURES BEFORE AND AFTER CREATION OF THE SHUNT

PAP (mm Hg)						m/m	PAP/SAP
	B			A		B	A
s	d	m	s	d	m		
3	4	7	14	6	9	0.17	0.30
2	6	8	15	10	12	0.13	0.25
3	5	8	16	12	13	0.14	0.42
5	6	9	17	14	15	0.20	0.42
4	4	7	19	14	16	0.13	0.34
6	4	7	18	14	15	0.13	0.39
17	3	8	20	15	17	0.16	0.37
19	5	10	21	12	14	0.13	0.31
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
20	8	12	23	10	14	0.23	0.29
15	4	8	18	5	9	0.14	0.26
19	5	10	21	7	12	0.16	0.30
17	6	10	20	11	14	0.16	0.34
14	6	9	16	8	11	0.15	0.27
16	5	9	17	10	12	0.13	0.23
13	5	8	15	10	12	0.13	0.28
12	4	7	14	8	10	0.11	0.24
15	5	9	18	10	13	0.15	0.31
2.6	1.2	1.4	2.7	3.0	2.4	0.03	0.06

TABLE V 4

INTRA-OPERATIVE MEASUREMENTS. SHUNT FLOW IN SIXTEEN CASES

Case No.	Shunt Flow (mean) ml/min	ml/min per Kg	% CO* (ml/m per Kg)
C.1	258	23.4	(24.7)
C.2	257	29.7	(31.3)
C.3	476	41.4	(43.7)
C.4	498	47.4	(50.0)
C.5	295	29.5	(31.2)
C.6	462	44.0	(46.4)
C.7	553	46.0	(48.5)
C.8	555	48.3	(50.9)
C.9	-	-	-
C.10	-	-	-
C.11	531	35.4	(37.3)
C.12	500	20.0	(21.1)
C.13	812	42.7	(45.0)
C.14	713	37.5	(39.6)
C.15	987	39.5	(41.2)
C.16	575	24.0	(25.3)
C.17	885	40.2	(42.4)
C.18	978	34.9	(36.8)

Mean value for each case

* Predicted from the normal studies. Thermaldilution (Table IV 8)

TABLE V 5

INTRA-OPERATIVE MEASUREMENTS. BLOOD FLOW MEASUREMENTS BEFORE AND AFTER THE CREATION OF THE SHUNT

Case No.	MPBF		MSF				
	B	A	From MPBF A-B	Actual Flow	%MPBF B	From MPBF A-B %B	%CO
C.1	703	-	-	258	36.7	-	24.7
C.2	746	-	-	357	47.8	-	31.3
C.3	838	-	-	476	56.8	-	43.7
C.12	1840	2245	405	500	27.2	22.7	21.1
C.14	1250	1905	655	731	58.5	52.4	39.6
C.15	1331	2120	789	987	64.5	59.3	41.2
C.17	2654	3197	543	885	33.5	20.5	32.8

Mean value for each case

MPBF - mean pulmonary blood flow
MSF - mean shunt blood flow
B - before shunt
A - after shunt
CO - cardiac output (thermaldilution)

TABLE V 6

INTRA-OPERATIVE MEASUREMENTS. HEART RATE; COMPARISON WITH THE NORMAL CASES (CARDIAC CATHETERIZATION)

Heart Rate		Before Shunt (18)	After Shunt		
			Total (18)	4-6 w (9)	8-12 w (9)
	Mean	120	134	149	119
	SD	25.1	25.2	25.6	13.7
normals (28)	111	NS	P<0.01	P<0.001	NS
	18.9				
before shunt			NS	P<0.01	NS
after shunt (operated 4-6 w)					P<0.01

( ) - total number of measurements
SD - standard deviation
NS - P>0.1

TABLE V 7

INTRA-OPERATIVE MEASUREMENTS. SYSTEMIC ARTERIAL PRESSURE; COMPARISON WITH THE NORMAL CASES (CARDIAC CATHETERIZATION)

Systemic Arterial Pressure		Mean / SD	Before Shunt (16) s	Before Shunt (16) d	Before Shunt (16) m	After Shunt (18) s	After Shunt (18) d	After Shunt (18) m
		Mean	81	47	58	62	31	41
		SD	11.9	7.1	7.2	9.5	6.2	6.2
normals (28)	s	84 17.0	NS			P<0.001		
	d	45 11.3		NS			P<0.001	
	m	59			NS			P<0.001
before shunt	s					P<0.001		
	d						P<0.001	
	m							P<0.001

( ) - total number of measurements
SD - standard deviation
s - systolic pressure
d - diastolic pressure
m - mean pressure
NS - P >0.1

Pulmonary arterial pressure Both systolic and diastolic pressures were lower in the thoracotomized animals (15-5 mm Hg with a mean of 9), than in the normal cases, at cardiac catheterization (18-7 mm Hg with a mean of 11) and these differences were highly significant ($0.01 > P > 0.001$) (Table V 8).

The ratio between the mean pulmonary and the mean systemic arterial pressures was also highly significantly different ($0.01 > P > 0.001$) from that found in the catheter studies (Table V 9).

Pulmonary blood flow The pulmonary flow wave contour was constant in all cases (Fig. V 1); it increased sharply to a systolic peak, decreased rapidly to zero and showed a reverse pattern coincident with the end of systole. A similar wave pattern has been recently described by Guntheroth, Gould, Butler and Kinnen (1974) in the main pulmonary artery of the dog.

Main pulmonary blood flow (ml/min) was measured in seven normal cases, three aged four weeks (C. 1, 2 and 3), three aged eight weeks (C. 12, 14 and 15) and one aged twelve weeks (C. 17). Additionally, this measurement was also performed in a case from the normal series (B. 35), aged sixteen weeks (Table V 10).

The main purpose of this procedure was to obtain control values for the pulmonary blood flow in thoracotomized animals. These results were later compared with those obtained from the shunted animals, both at the time of surgery and at the end of the follow-up period.

TABLE V 8

INTRA-OPERATIVE MEASUREMENTS. PULMONARY ARTERIAL PRESSURE; COMPARISON WITH THE NORMAL CASES (CARDIAC CATHETERIZATION)

Pulmonary Arterial Pressure			Before Shunt s	Before Shunt d	Before Shunt m	After Shunt s	After Shunt d	After Shunt m
		Mean	15	5	9	18	10	13
		SD	2.6	1.2	1.4	2.7	3.0	2.4
normals (28)	s	18	P<0.01			=		
		3.7						
	d	7						
		2.5		P<0.01			P<0.001	
	m	11			P<0.01			NS
		2.6						
before shunt	s					P<0.01		
	d						P<0.001	
	m							P<0.001

( ) - total number of measurements
SD - standard deviation
s - systolic
d - diastolic
m - mean pressure
NS - P>0.1

TABLE V 9

INTRA-OPERATIVE MEASUREMENTS. RATIO BETWEEN MEAN PULMONARY AND SYSTEMIC ARTERIAL PRESSURES; COMPARISON WITH NORMAL CASES (CARDIAC CATHETERIZATION)

Mean PAP / Mean SAP		Before Shunt	After Shunt		
			Total (16)	4-6 w (8)	8-12 w (8)
	Mean	0.15	0.31	0.35	0.28
	SD	0.031	0.061	0.061	0.035
normals (28)	0.19 0.061	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$
before surgery			$P < 0.001$	$P < 0.001$	$P < 0.001$
after shunt (operated 4-6w)					$P < 0.01$

( ) - total number of measurements
SD - standard deviation
PAP - pulmonary arterial pressure
SAP - systemic arterial pressure

Fig. V 1

Normal pulmonary arterial blood flow (main pulmonary artery). Measurement using the electromagnetic flowmeter. Notice the negative component at the end of systole. ECG recorded on the top (DII)

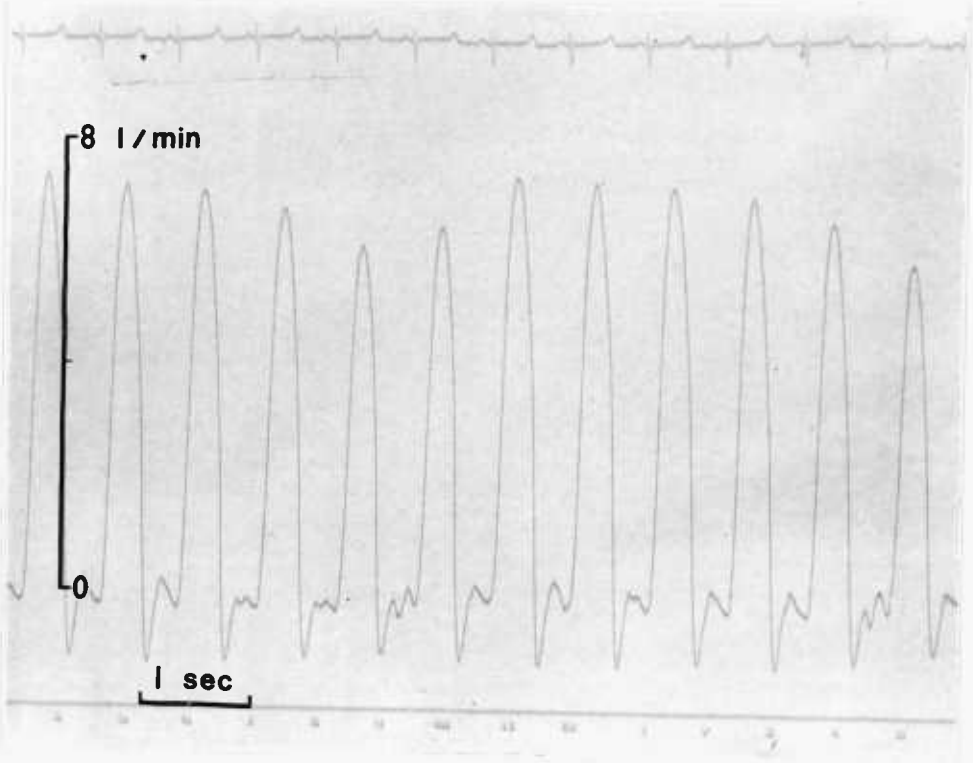

8 l / min
0
1 sec

TABLE V 10

INTRA-OPERATIVE MEASUREMENTS. COMPARISON BETWEEN THE FLOWMETER AND THE THERMALDILUTION METHODS IN A SERIES OF FIFTEEN ANIMALS

Age (weeks)	Case (no.)	MPBF (ml/min)	*	CO (ml/min)
4	C.1	703		
	C.2	746		
	C.3	836		
	B.4			600
8	C.12	1840		
	C.14	1250		
	C.15	1431		
	B.18			1100
	B.19			1500
	B.20			1175
12	C.17	2654		
	B.30			2800
	B.31			2175
16	B.35	3540		3750
	B.36			3450

Mean value for each case

MPBF - mean pulmonary blood flow (flowmeter-thoracotomy)

CO - cardiac output (thermaldilution-catheterization)

* significance of the linear correlation coefficient between the two variables ($P<0.001$)

After establishing the shunt

Heart rate In most cases, there was an immediate increase in heart rate, after the shunt had been completed (Table V 3). In the whole series this change was from a mean of 120 to one of 134 beats/min ($P > 0.1$). This increase occurred mainly in the younger animals, operated between four and six weeks of life (C. 1-9); in this group, the mean heart rate increased highly significantly to 149 beats/min ($P < 0.001$ or $0.01 > P > 0.001$) (Table V 6). In the older animals, the changes in heart rate were not significant (Table V 6).

Systemic arterial pressure The systemic arterial pressure decreased immediately after surgery, the mean value for the whole series being 41 mm Hg (Table V 3). This value was highly significantly lower ($P < 0.001$) than those found during the pre-shunt measurements and at cardiac catheterization (Table V 7). There were no age related differences in the systemic arterial response to the shunt, the mean value being 40 and 43 mm Hg respectively, for the younger (c. 1-9) and older animals (C. 11-18).

Pulmonary arterial pressure The pulmonary arterial pressure increased immediately after the completion of the shunt (Table V 3). The mean pressure value for the whole series was 13 mm Hg, although this difference was highly significant ($P < 0.001$), when compared with the pre-shunt measurements, it did not differ from the cardiac catheterization findings ($P > 0.1$) (Table V 8). Diastolic pulmonary pressure, however,

was significantly increased ($P<0.001$) when compared both with the cardiac catheterization and the pre-shunt measurements (Table V 8).

Similar changes occurred in both age groups, with a mean pressure of 14 mm Hg for the younger animals (C. 1-8) and 12 mm Hg for the older ones (C. 10-18).

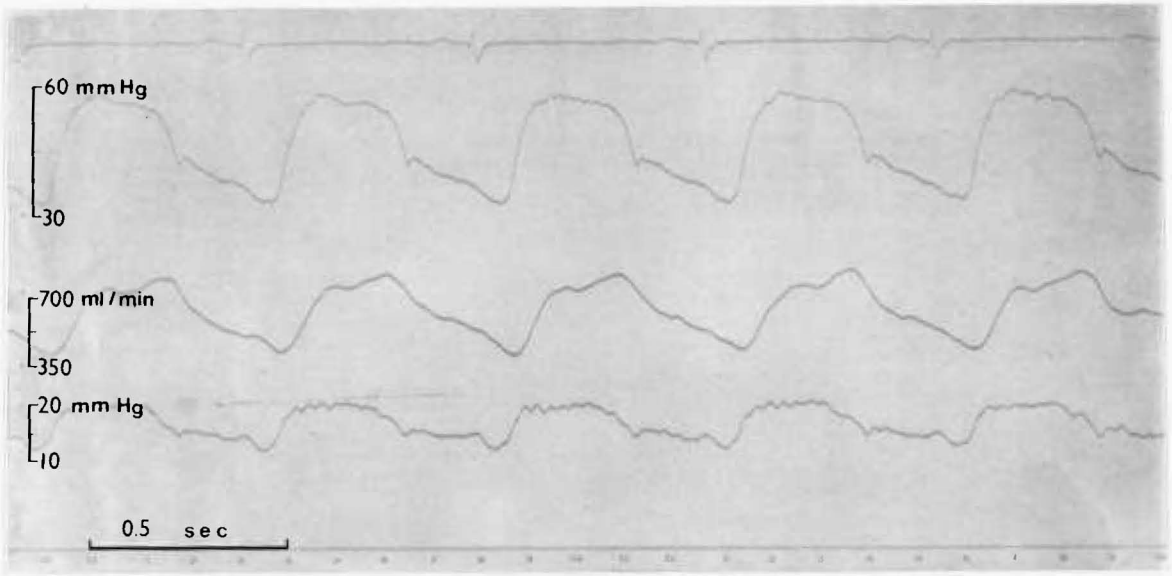
The ratio between mean pulmonary and mean systemic pressures increased from 0.15 to 0.31 (Table V 3). This difference was highly significant ($P<0.001$) when compared both with the cardiac catheterization and pre-shunt measurements (Table V 9). The cases operated earlier in life showed an higher ratio between the mean pulmonary and systemic arterial pressures (0.35), than the older ones (0.28) and this difference was highly significant ($0.01>P>0.001$) (Table V 9).

Blood flow The blood flow through the shunt was continuous during the cardiac cycle. The wave contour was pulsatile with a peak coinciding with the systolic components of both aortic and pulmonary pressure curves (Fig. V 2-3).

Mean shunt flow ranged between 257 and 987 ml/min (20.0-48.3 ml/min per Kg) in the whole series (Fig. V 4). These figures represented between 21.1% and 50.9% of the predicted cardiac output (ml/min per Kg), assessed using the thermal dilution method in the normal cases. When the series was divided in two groups, according to the age at surgery, the mean shunt produced in the younger animals (1-8) was 40.8% of the

Fig. V 2 and 3

Inter-operative assessment of shunt blood flow. Fig. V 2 (case C. 5) and Fig. V 3 (case C. 7). Simultaneous recording of systemic arterial blood pressure (top), shunt flow (middle) and pulmonary arterial pressure (bottom). Notice that the contour of the shunt flow wave is pulsatile with a peak coinciding with the systolic components of both aortic and pulmonary pressure curves. ECG recorded on the top (DII)


60 mm Hg
30
700 ml / min
350
20 mm Hg
10
0.5 sec

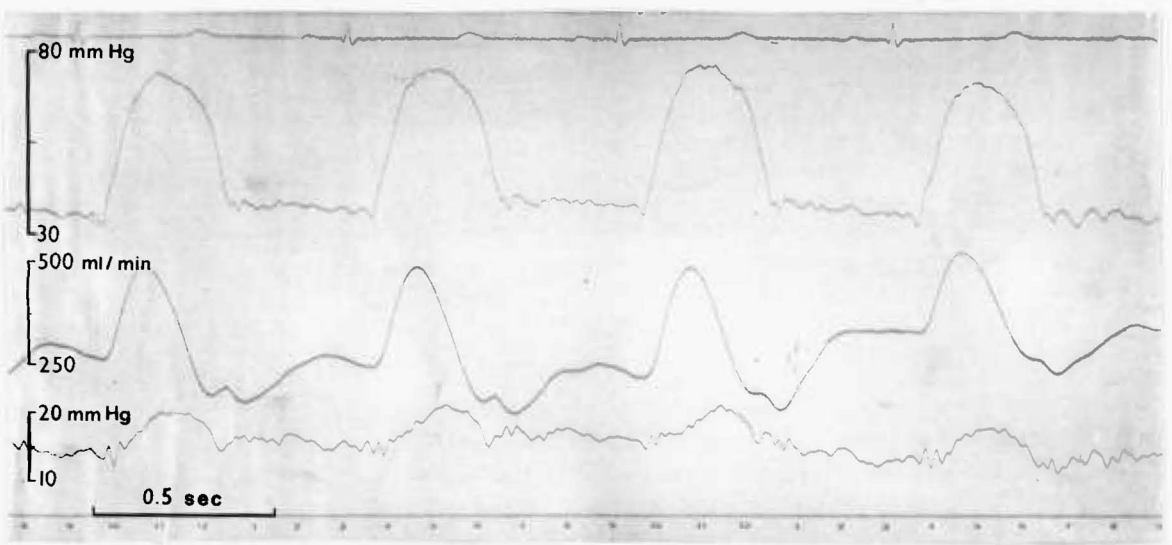
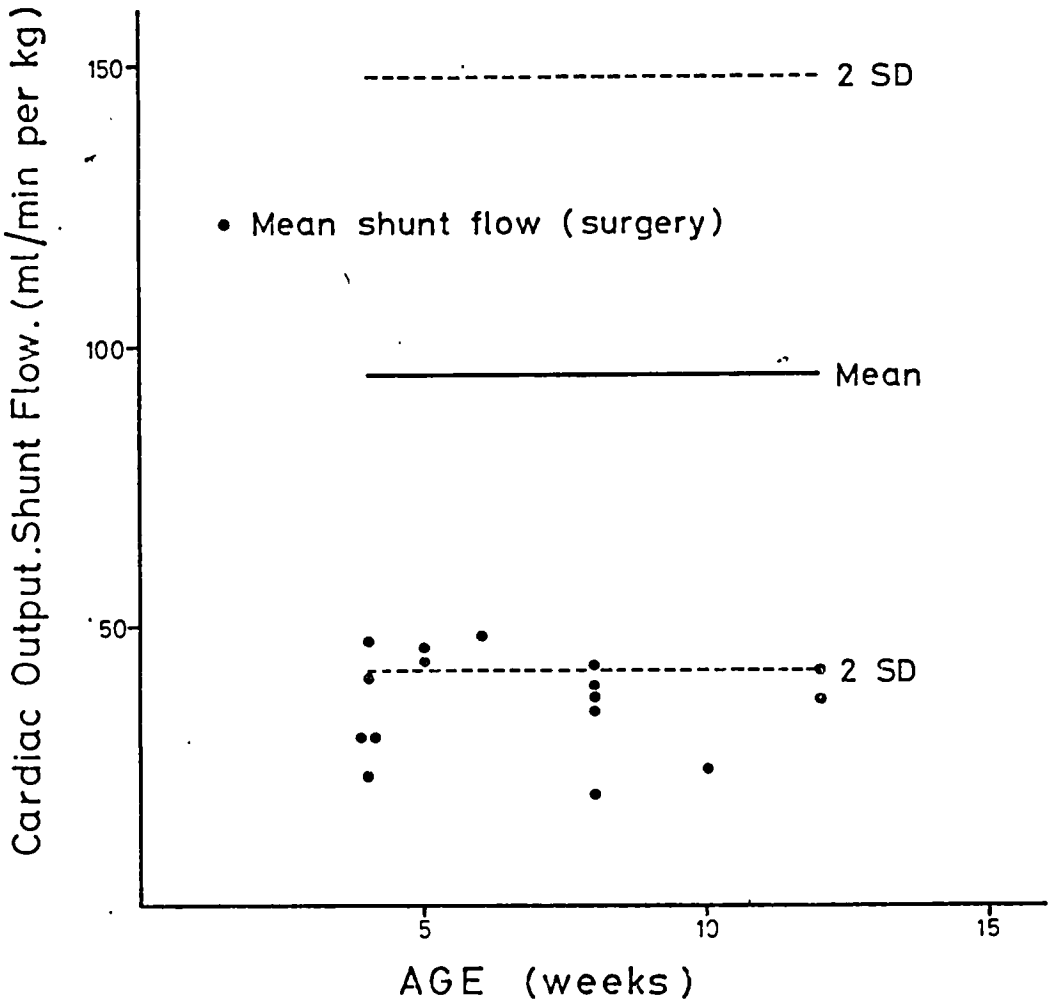

80 mm Hg
30
500 ml / min
250
20 mm Hg
10
0.5 sec

Fig. V 4

Diagram illustrating the different values of shunt blood flow measured at surgery. Mean value for each case expressed in ml/min per kg. The continuous and dotted lines rpresent respectively the mean and 2 standard deviation values for the cardiac output obtained at cardiac catheterization


Cardiac Output.Shunt Flow. (ml/min per kg)
150
100
50
2 SD
• Mean shunt flow (surgery)
Mean
2 SD
5
10
15
AGE (weeks)

cardiac output (range 24.7-50.9%), whereas in the older cases, the mean shunt flow was 39.4% (range 21.1-45%). These differences were not significant (P 0.4) and allowed comparison between the different age groups (Table V 4).

In seven cases (C. 1, 2, 3, 12, 14, 15 and 17), it was possible to relate the mean shunt flow to the actual pulmonary blood flow, measured at surgery, before the creation of the shunt (Table V 5). The percentage increase in blood flow ranged between 27.2 and 64.5 of the initial value and although these results were higher than those predicted from the cardiac output measurements, the difference between the two groups was not statistically significant ($0.1 > P > 0.05$).

In four of the above mentioned seven cases (C. 12, 14, 15 and 17), the main pulmonary blood flow was also measured, after the completion of the shunt. Most of the attempts to assess this flow did not meet with success mainly because the presence of the graft with two recent vascular anastomosis did not allow a full manipulation and the cuff-head transducer could not be correctly placed on the vessel. Another important reason for the fewer measurements was that the left-to-right shunt created obvious turbulence in the flow pattern of the main pulmonary artery at that level, thus introducing a considerable error in the measurements (Spencer and Denison, 1963). These artefacts were found because the flow wave was continuously traced on the oscilloscope and the abnormal patterns discarded. In the four present cases, the differences between the final (post-shunt) and the initial (pre-shunt) mean flow values were similar to the actual mean shunt flow ($0.1 > P > 0.05$) (Table V 5).

There was no close relationship between the amount of blood shunted (Table V 4) and the immediate changes in pulmonary arterial pressure (Table V 3). Nevertheless, the cases with a larger shunt flow tended to have a greater increase in pulmonary pressure, particularly in the younger age group.

The pulmonary flow wave contour changed considerably after the completion of the shunt in that the zero flow level was not reached with consequent disappearance of the reversed flow pattern at the end of the systole (Fig. V 5). These features could be rapidly reversed by occluding the shunt for short periods of time (5-15 s) (Fig. V 6).

Blood gas tensions, pH and haematocrit (Table V 11) During these measurements, the mean Pa CO_2 was 42 mm Hg, with a range between 35-49 mm Hg in all cases. The mean $P_a O_2$ was always above 98 mm Hg. The mean pH was 7.39.

The mean value for the haematocrit was 33 mm.

Cardiac Catheterization

All the eighteen animals were catheterized at the end of the follow-up period (Table V 12).

Heart rate

Comparison between the values of the heart rate in the normal,

Fig. V 5

Pulmonary arterial blood flow after the completion of the aorto-pulmonary shunt. Both the phasic and the mean flows are recorded (top). Systemic (middle) and pulmonary (bottom) arterial blood pressures are also recorded. Notice the disappearance of the negative component of the pulmonary flow wave

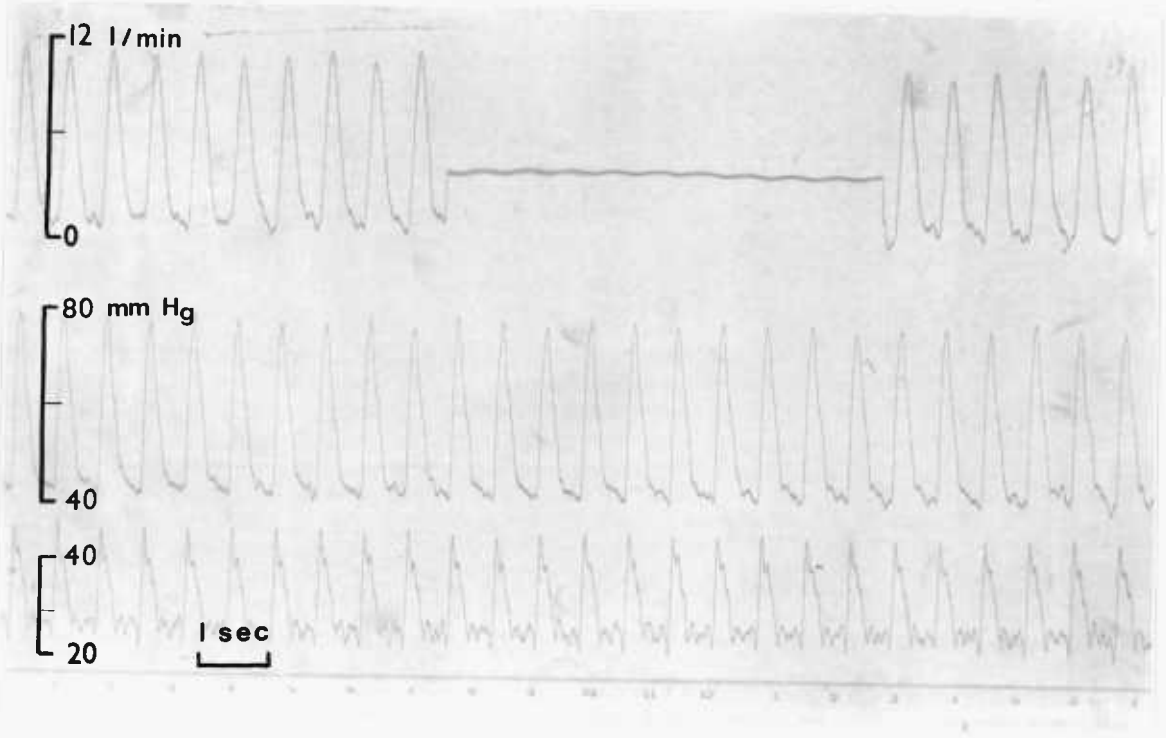

12 l/min
0
80 mm Hg
40
40
20
1 sec

Fig. V 6

Recording illustrating the effect of the temporary occlusion of the shunt on both systemic (top) and pulmonary (bottom) arterial pressures. ECG recorded on the top (DII)

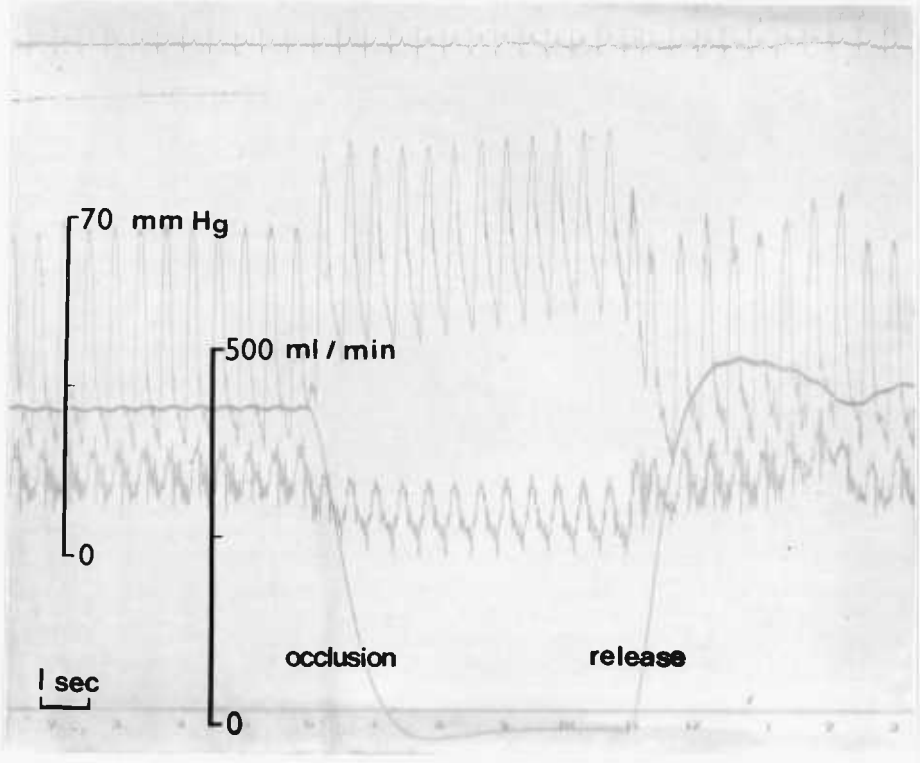

70 mm Hg
0
500 ml / min
0
occlusion
release
1 sec

TABLE V 11

ARTERIAL BLOOD GASES AND HAEMATOCRIT DURING SURGERY

AT THE TIME OF THE HAEMODYNAMIC STUDIES

Blood Gases	Mean	Standard Deviation	Standard Error
Pa O_2	137	24.6	7.9
Pa CO_2	42	3.9	1.0
pH	7.39	0.06	0.01
Htc	33	2.9	0.7

TABLE

FOLLOW-UP STUDIES. CARDIAC CATHETERIZATION,

Case No.	HR beats/min	SAP mm Hg s	SAP mm Hg d	SAP mm Hg m	RVP mm Hg s	RVP mm Hg d	RAP mm Hg m
C.2	154	87	46	60	34	2	2
C.3	120	71	40	51	39	1	3
C.4	139	75	42	53	41	2	2
C.6	150	81	40	54	35	1	1
C.7	136	74	40	51	36	2	2
C.9	121	120	54	76	31	0	3
C.10	94	114	57	76	34	0	3
C.11	84	98	60	73	33	1	1
C.12	118	119	83	95	38	0	1
C.13	131	77	51	60	26	1	3
C.14	85	73	44	54	30	0	1
C.15	83	113	44	67	40	3	3
C.17	85	79	36	50	27	2	2
C.18	116	106	41	63	36	1	3
Controls							
C.1	120	113	57	76	18	0	3
C.5	121	87	61	70	19	1	1
C.8	92	81	51	61	18	0	1
C.16	94	100	56	71	15	0	2
Normals*							
Mean	111	84	45	59	19	1	2
SD	18.9	17.0	11.3	11.7	3.9	0.2	0.8

HR - heart rate
SAP - systemic arterial pressure
RVP - right ventricular pressure
RAP - right atrial pressure
PAP - pulmonary arterial pressure
PAWP - pulmonary arterial wedge pressure
$\frac{QP}{QS}$ - pulmonary to systemic flow ratio (blood oxygen contents)
$\frac{RP}{PS}$ - pulmonary to systemic resistance ratio
s - systolic pressure
d - diastolic pressure
m - mean
SD - standard deviation
* - Tables IV 8, 10

Mean value for each case

COMPARISON BETWEEN SHUNTED, CONTROL AND NORMAL CASES

PAP mm Hg s	d	m	PAWP mm Hg m	m PAP / m SAP	QPA / QSA	RPA / RSA
26	22	23	7	0.38	1.5	0.25
41	29	33	7	0.65	2.2	0.29
54	34	40	8	0.75	2.4	0.31
39	28	32	5	0.59	1.7	0.35
43	33	36	6	0.71	2.0	0.35
34	26	29	-	0.38	1.5	0.25
38	20	26	-	0.34	1.9	0.18
33	20	24	6	0.33	1.7	0.19
41	17	25	5	0.26	1.7	0.15
30	22	25	6	0.42	1.8	0.23
34	26	29	6	0.54	2.1	0.26
49	25	33	6	0.49	2.4	0.20
29	21	24	6	0.48	2.0	0.24
36	20	25	7	0.40	2.3	0.17
20	8	12	4	0.16	1.0	0.16
16	4	8	4	0.11	1.0	0.11
15	10	12	3	0.20	1.0	0.20
17	4	8	7	0.11	1.0	0.11
18	7	11	5	0.19		
3.7	2.5	2.6	2.0	0.06		

control and shunted animals (complete series) showed that the differences were not significant ($P > 0.5$ in all cases). The animals operated earlier in life, had a faster heart rate (137 beats/min) than those operated at or after eight weeks of age (99 beats/min); this difference was found to be highly significant ($0.01 > P > 0.001$) (Table V 12).

Systemic arterial pressure

The changes in arterial pressure were not highly significant in either age group ($P > 0.01$ in all cases) although the control animals tended to have higher pressures than the normal ones, and the younger cases (C. 2, 3, 4, 6, 7 and 9) showed the lowest values of the four groups (Table V 12).

Right-side heart pressures

The right ventricular systolic pressure was highly significantly increased in the two shunted series, when compared to the control and normal animals ($P < 0.001$). There were no age related differences, the mean values being respectively 36 mm Hg for the younger animals, and 33 mm Hg for the older ones (Table V 12).

The right ventricular end-diastolic and the atrial pressures were not increased in the animals with an aorto-pulmonary shunt (Table V 12).

Pulmonary arterial pressures

The mean pulmonary arterial pressure was highly significantly increased ($P<0.001$) in the experimental series (mean value of 29 mm Hg, opposed to 11 mm Hg and 10 mm Hg respectively in the normal and control series).

Comparison between the two age groups showed that the animals operated earlier in life had a higher mean pulmonary arterial pressure, 32 mm Hg, when compared to the older ones, 26 mm Hg. It was also found that hwereas the pulmonary systolic pressures were similar in both series,39 and 36 mm Hg respectively, the difference between the diastolic pressures, 29 and 21 mm Hg respectively, was more noticeable ($0.01>P>0.001$) (Table V 13).

The pulmonary arterial wedge pressure did not change in the experimental cases, remaining within the normal and control range (Table V 12), (Fig. V 7).

The ratio between the mean pulmonary and systemic pressures was highly significantly increased in the experimental cases ($P<0.001$). The age related differences were also significant ($0.05>P>0.01$) (Table V 12).

Pulmonary-to-systemic flow ratio

The assessment of the pulmonary-to-systemic flow ratio by measuring the blood oxygen contents showed that the degree of shunting was similar in the two experimental series (Table V 12), as had

TABLE V 13

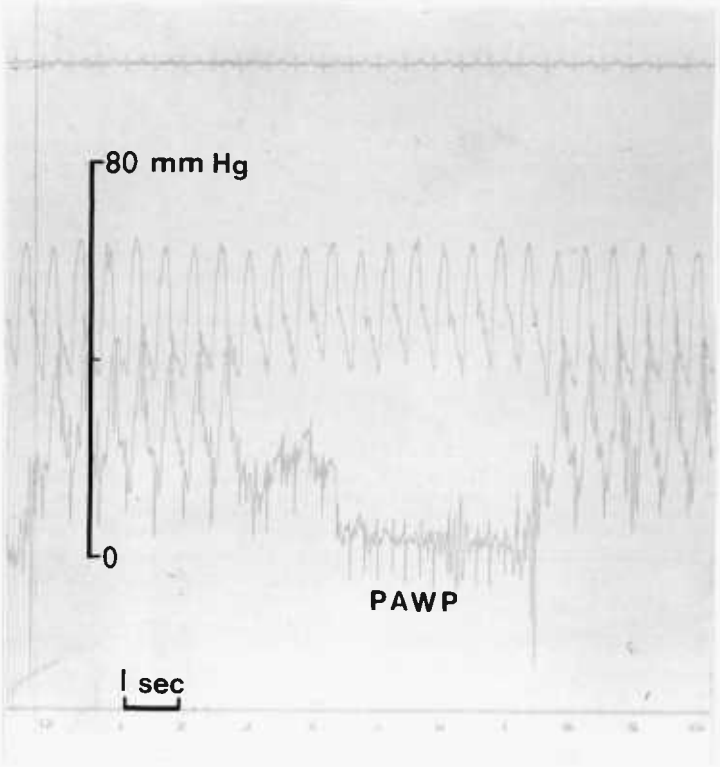
FOLLOW-UP STUDIES. CARDIAC CATHETERIZATION - PULMONARY ARTERIAL PRESSURE; COMPARISON BETWEEN THE EXPERIMENTAL, CONTROL AND NORMAL SERIES

Pulmonary Arterial Pressure		Controls (4)			Shunt 4-6 w (6)			Shunt 8-12 w (8)		
		s	d	m	s	d	m	s	d	m
	Mean	17	6	10	39	29	32	36	21	26
	SD	2.2	3.0	2.3	9.0	4.5	5.8	6.5	2.9	3.1
normals (28)	s	18 3.7	NS		P ⟨0.001			P ⟨0.001		
	d	7 2.5		NS		P ⟨0.001			P ⟨0.001	
	m	11		NS			P ⟨0.001			P ⟨0.001
controls	s				P ⟨0.001			P ⟨0.001		
	d					P ⟨0.001			P ⟨0.001	
	m						P ⟨0.001			P ⟨0.001
shunt 4-6 w	s							NS		
	d								P ⟨0.01	
	m									P ⟨0.05

(see Table V 7 for symbols)

Fig. V 7

Recording illustrating the measurement of pulmonary artery wedge pressure (PAWP) during cardiac catheterization in a shunted case (Swan-Ganz catheter). Notice the low value of the pulmonary arterial wedge pressure when compared to the pulmonary artery pressure (same recording, each side of PAWP). ECG (DII) (top) and systemic artery blood pressure (middle) are also recorded


80 mm Hg
0
PAWP
1 sec

already been predicted from the intra-operative blood flow measurements (Table V 5). It also demonstrated that the pulmonary pressure response to the left-to-right shunt was different in the two experimental groups. This can be exemplified by comparing C. 4 with C. 15, both cases having a similar QP/QS (2.4). The younger animal (C. 4) showed the highest $\frac{\text{m PAP}}{\text{m SAP}}$ of the whole series, 0.75, and a pulmonary arterial diastolic pressure of 34 mm Hg, two months after surgery, the older case, catheterized after a similar follow-up period, showed a $\frac{\text{m PAP}}{\text{m SAP}}$ of 0.49 and pulmonary arterial diastolic pressure of 25 mm Hg.

Pulmonary-to-systemic resistance ratio

The use of the resistance ratio (Rp/Rs), which correlated the flow assessment with both mean pulmonary and systemic arterial pressures showed that the animals operated late in life, had a Rp/Rs very similar to the one found in the control series ($P > 0.1$), whereas the pigs operated between four and six weeks of life showed a highly significantly increase in the Rp/Rs, 0.30 ($P < 0.001$), when compared with both the older age group, 0.20, and the control series, 0.14.

Blood gas tensions and pH

The mean arterial PCO_2 was 40 mm Hg in all cases with a mean pH value of 3.79 and a PO_2 above 82 mm Hg (Table V 14).

The mixed venous blood, collected from the right ventricle, because of the shunt at the pulmonary artery level, had a mean PCO_2

TABLE V 14

FOLLOW-UP STUDIES. ARTERIAL (a) AND MIXED VENOUS (V) BLOOD GAS TENSIONS AND pH DURING CATHETERIZATION

Blood Gases	Mean	Standard Deviation	Standard Error
Pa O_2	159	42.9	26.9
Pa CO_2	40	5.7	1.3
pH	7.39	0.06	0.01
PV O_2	36	9.1	2.2
PV CO_2	48	6.9	1.7
pH	7.30	0.04	0.01

of 48 mm Hg with a mean pH of 7.30 (Table V. 14).

Final Study. Open-Chest Measurements

Immediately before sacrifice, most of the animals were reoperated in an attempt to reassess the pulmonary blood flow. This procedure was not always successful mainly because of the adhesions surrounding the main vessels, which made the complete dissection of the pulmonary artery difficult and did not allow for a good contact between the cuff-head transducer and the vessel.

In four cases (C. 3, 4, 14 and 15) it was possible to measure the pulmonary blood flow distal to the shunt together with the systemic and pulmonary arterial pressures (Table V 15).

Heart rate

At the end of the follow-up period, the heart rate remained faster in the animals shunted early in life (C. 3-4), 140 beats/min, compared to those operated later (C. 14-15), 99 beats/min. These results were similar to those obtained at cardiac catheterization at the end of the follow-up period (Table V 12).

Systemic arterial pressure

The changes in systemic arterial pressure at the end of the follow-up period were not significantly different from the cardiac catheterization findings ($P > 0.1$), the mean value for the whole series being 60 mm Hg (Table V 15).

TABLE V 15

HAEMODYNAMIC STUDIES AT THE END OF THE FOLLOW-UP PERIOD. MEASUREMENTS OF PULMONARY PRESSURE AND FLOW IN FOUR CASES WITH AN AORTO-PULMONARY SHUNT

Case No. Age	HR beats/min	SAP mm Hg			PAP mm Hg			MPBF ml/min	PVR units
		s	d	m	s	d	m	*	*
C.3	119	77	49	58	43	25	31	2740	11.3
8 w								(1474)	(6.5)
C.4	161	78	42	54	48	34	39	3860	10.1
12 w								(2654)	(3.0)
C.14	91	89	50	63	30	22	25	6340	3.9
16 w								(3850)	(2.3)
C.15	108	108	47	67	41	26	31	6510	4.8
16 w								(3850)	(2.3)

Mean value for each case

*( ) normal cases - open-chest measurements (Tables V 3,5 and 10)

HR - heart rate
SAP - systemic arterial pressure
PAP - pulmonary arterial pressure
MPBF - mean pulmonary blood flow (flowmeter)
PVR - pulmonary vascular resistance

Pulmonary arterial pressure

The mean pulmonary arterial pressure was 31 mm Hg and again the animals operated earlier in life had a higher value, 35 mm Hg, when compared to the older ones, 28 mm Hg, thus confirming the cardiac catheterization findings (Table V 12).

Pulmonary blood flow and vascular resistance

The flow was increased in all cases, confirming the existence of the left-to-right shunt (Table V 15). The contour of the pulmonary wave remained similar to that described immediately after the creation of the anastomosis with a diastolic flow above zero and no reverse wave at the end of systole.

The assessment of the pulmonary vascular resistance in these cases further confirmed the increase above the normal values, suggested at cardiac catheterization by the changes in the Rp/Rs (Table V 12). The highest value for the pulmonary vascular resistance was found in case C. 4, which had a value of 10.1 Units (3.0 Units in the normal animals at the same age - Table IV 10). The increase in pulmonary resistance was smaller in the animals operated later in life (Table V 15).

Blood gas tensions, pH and haematocrit

The mean Pa CO_2 was 34 mm Hg with mean value pH of 7.42. The Pa O_2 was above 190 mm Hg in all cases (Table V 16).

The haematocrit ranged between 30 and 35 mm.

TABLE V 16

ARTERIAL (a) BLOOD GAS TENSIONS AND HAEMATOCRIT DURING THE FINAL HAEMODYNAMIC STUDY (4 CASES)

Blood Gases	Mean	Standard Deviation	Standard Error
Pa O_2	224	40.8	20.2
Pa CO_2	34	2.4	1.2
pH	7.42	0.013	0.006
Htc	31	1.4	0.07

Lung Function

The eighteen experimental cases were followed for periods that ranged between four and eleven weeks, during which they had at least three sequential measurements of lung function.

The mean arterial PCO_2 was 47 mm Hg (2.5 SD) in all cases and the pH ranged between 7.30 and 7.37; the PO_2 was always above 75 mm Hg.

Respiratory Frequency

Respiratory frequency did not change significantly during the follow-up period. It ranged between 15 and 39 breaths/min in all experimental cases, compared with 14 and 40 in the controls and 10 and 36 in the normal series (Table V 17). The mean differences between these groups were not statistically significant ($P>0.1$ in all cases).

Tidal Volume

Tidal volume increased with age in all the experimental cases (Table V 18). In the animals operated between four and six weeks of age, the increase in tidal volume was slower than normal, during the first month of the follow-up period (Fig. V 8); however, a similar pattern was found in the control case operated at the same age (Fig. V 8), thus suggesting that the thoracotomy might cause some reduction in the tidal volume in younger animals, particularly in the early

TABLE

FOLLOW-UP STUDIES. LUNG FUNCTION -

Case No.	f	W e e k s		o f	
	4	5	6	7	8	9
C.2	s	-	30	27	26	
C.3	s	-	39	31	21	
C.4	s	-	36	31	27	-
C.6		s	-	25	38	25
C.7		s	-	29	-	-
C.9			s	-	-	20
C.10					s	-
C.11					s	-
C.12					s	-
C.13					s	-
C.14					s	-
C.15					s	-
C.17						
C.18						
Controls						
C.1	s	-	28	22	23	
C.5	s	-	40	34	33	37
C.8			s	-	-	17
C.10						
Normals*						
f (mean)			37	28	25	-
			36	24	24	

f - respiratory frequency (breaths/min)

s - shunt

* - Table IV 3

Mean value for each case

MEASUREMENTS OF RESPIRATORY FREQUENCY

		Life				
10	11	12	13	14	15	16
–	–	28				
–	–	36				
–	–	–	19	29	–	20
23	–	–	–	–	–	16
28	25	30				
21	26	27				
23	18	17				
29	–	–	28	18	–	28
–	–	–	22	19	–	26
–	15	–	..	21	–	20
		s	–	27	19	21
		s	–	24	21	17
–	–	29				
–	–	–	24	28	–	14
s	–	29	24	–	–	23
26	25	33	–	24	–	30
24		11				23

TABLE

FOLLOW-UP STUDIES. LUNG FUNCTION -

Case No.	V T and V T / B W					
	4	5	6	7	8	9	10
C.2	s	-	83 4.4	95 4.7	141 6.4		
C.3	s	-	61 4.1	75 3.6	93 3.9		
C.4	s	-	57 4.1	61 3.8	86 5.1	-	-
C.6		s	-	57 4.1	61 2.9	86 3.6	-
C.7		s	-	53 2.0	-	-	-
C.9			s	-	-	70 2.8	90 3.0
C.10					s	-	117 4.2
C.11					s	-	139 2.8
C.12					s	-	147 4.9
C.13					s	-	65 2.8
C.14					s	-	-
C.15					s	-	-
C.17							
C.18							
Controls							
C.1	s	-	72 4.8	112 6.2	97 5.1		
C.5	s	-	71 4.7	53 3.0	80 3.8	78 3.2	-
C.8			s	-	-	112 4.7	-
C.16							s
Normals*							
VT			110 75	96 95	123 90	-	163 108
VT/BW			11.0 6.8	6.9 6.3	4.9 3.7	-	7.1 4.9

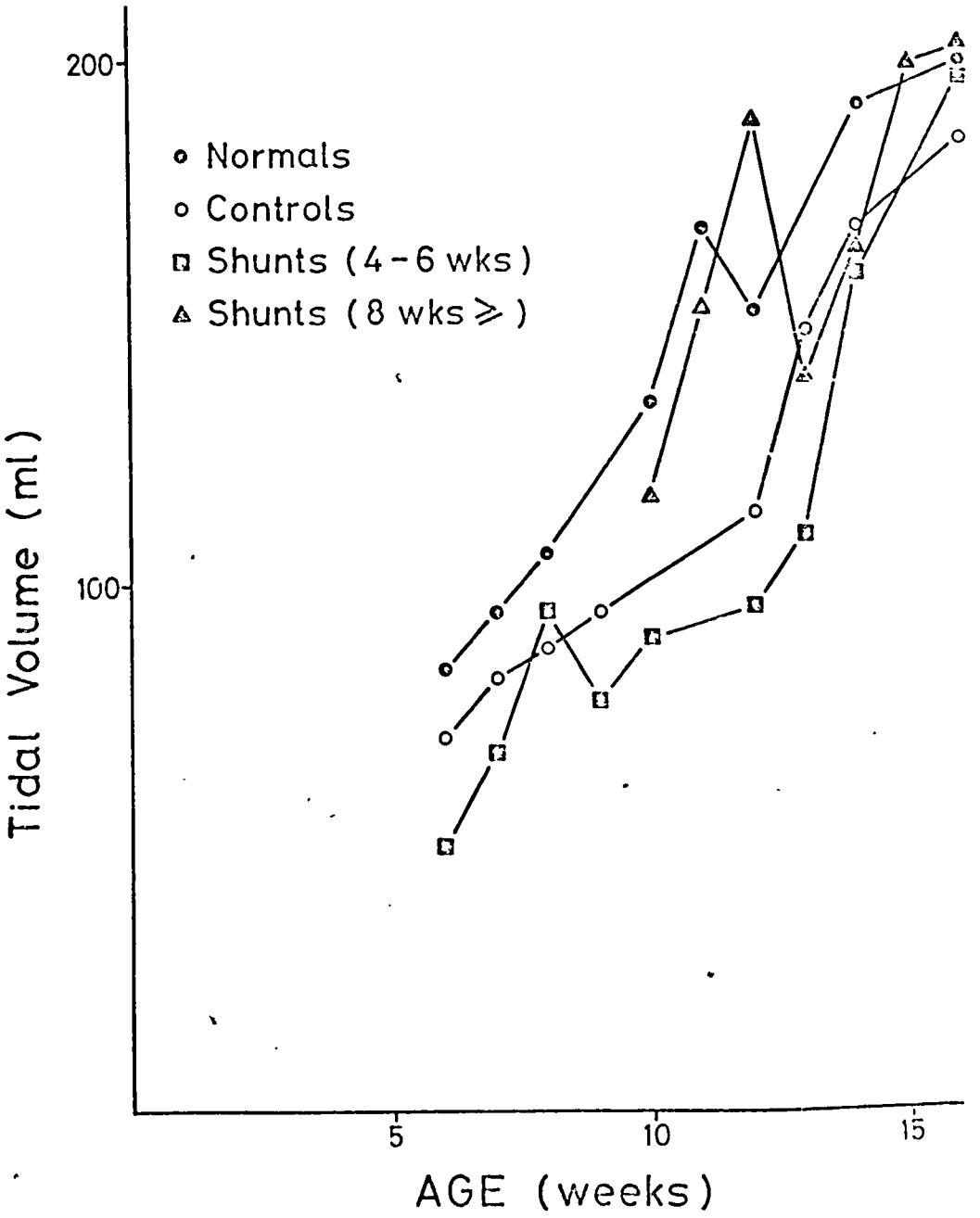
VT - tidal volume (ml)
BW - body weight (Kg)
s - shunt
* - Table IV 3

Mean value for each case

Weeks of Life					
11	12	13	14	15	16
-	96 3.7				
-	96 3.0				
-	-	110 3.1	160 4.4	-	274 6.1
-	-	-	-	-	122 2.7
121 3.7	163 4.5				
156 6.8	191 7.6				
212 6.4	213 5.9				
-	-	156 4.3	129 3.6	-	291 5.4
-	-	105 2.9	178 4.4		208 4.0
122 3.4	-	-	139 3.3	-	169 3.7
	s	-	169 5.1	174 5.0	159 4.0
	s	-	162 5.4	228 6.3	192 5.0
-	114 4.4				
-	-	171 4.8	169 4.4	-	186 4.7
-	114 3.8	127 3.4	-	-	251 4.8
-	168	192 94		192	236 167
	6.7	6.4 4.3	-	4.6	5.6 4.6

Fig. V 8

Diagram illustrating the increase in tidal volume with age both in the experimental, normal and control series. Mean value for each age group


200
100
Normals
Controls
Shunts (4-6 wks)
Shunts (8 wks ≥)
Tidal Volume (ml)
5
10
15
AGE (weeks)

post-operative period. The animals operated at eight weeks or later in life showed a normal increase in tidal volume during growth (Fig. V 8).

These measurements were also related to body weight and the mean value for each series compared with the normal findings (these had already shown that the ratio between tidal volume and body weight - VT/BW - did not change throughout growth - Tables III 3-4). VT/BW was highly significantly reduced in the controls and shunted cases (total series), when compared to the normals ($P < 0.001$). But the difference between controls and shunted animals was not significant ($P > 0.1$ in all cases). When the two experimental groups were compared with one another (Table V 19), a highly significant difference ($P < 0.001$) was found, suggesting that apart from the effect of the thoracotomy there could be an additional decrease in VT/BW caused by the aorto-pulmonary shunt in the animals operated earlier in life that persisted throughout growth.

Dynamic Compliance

The normal increase in dynamic compliance with age did not occur in the experimental cases (Table V 20); the abnormality could be defined in both age groups as a maintenance of the values found at the first post-operative study in contrast to the steady increase seen in the normal animals throughout growth (Fig. V 9). The control cases showed normal pattern during the follow-up period (Table V 20).

The ratio between dynamic compliance and body weight (CDyn/BW)

TABLE V 19

FOLLOW-UP STUDIES. LUNG FUNCTION - TIDAL VOLUME RELATED TO BODY WEIGHT. COMPARISON BETWEEN CASES SHUNTED AT VARIOUS AGES, CONTROLS AND NORMALS

Tidal Volume Body Weight (ml per kg)		Controls (14)	Total Shunted (46)	Shunted 4-6 w (21)	Shunted 8-12 w (25)
	Mean	4.3	4.2	3.8	4.7
	SD	0.43	0.82	0.53	0.92
normals (21)*	6.2 1.79	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.01$
controls			NS	NS	NS
shunted 4-6 w					$P < 0.001$

( ) - total number of measurements
SD - standard deviation
NS - $P > 0.1$
* Table IV 3

TABLE

FOLLOW-UP STUDIES. LUNG FUNCTION -

Case No.	CDyn and CDyn/BW 4	5	6	7	8	Weeks 9	10
C.2	s	-	41.8 2.2	48.0 2.4	44.9 2.0		
C.3	s	-	18.3 1.2	37.3 1.8	25.5 1.1		
C.4	s	-	18.9 1.3	20.3 1.3	18.1 1.1	-	-
C.6		s	-	30.1 2.1	39.4 1.9	24.2 1.0	-
C.7		s	-	26.0 1.0	-	-	-
C.9			s	-	-	35.0 1.4	33.6 1.1
C.10					s	-	49.6 1.8
C.11					s	-	39.6 2.0
C.12					s	-	58.9 2.0
C.13					s	-	24.8 1.0
C.14					s	-	-
C.15					s	-	-
C.17							
C.18							
Controls							
C.1	s	-	52.2 4.8	55.5 3.1	71.8 3.8		
C.5	s	-	25.4 1.7	29.4 1.7	33.0 1.6	63.5 2.6	-
C.8			s	-	-	24.4 1.0	-
C.16							s
Normals*							
CDyn			29.5 23.4	48.2 34.0	42.3 38.9	-	63.1 44.6
CDyn/BW			2.4 2.1	3.2 2.4	1.8 1.6	-	2.7 2.0

CDyn - dynamic compliance (ml/cm H_2O)
BW - body weight
s - shunt
* - Table IV 3

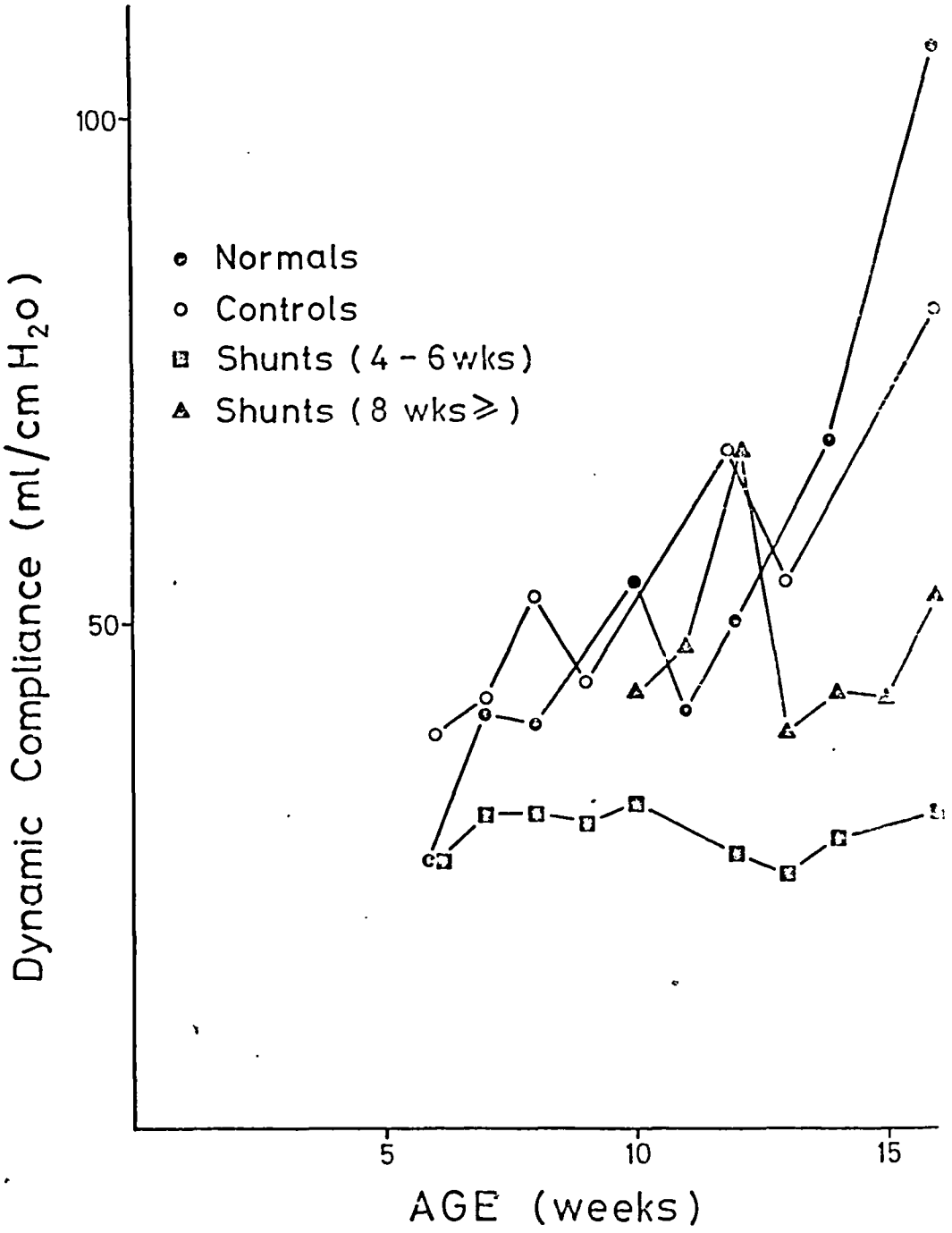
Mean value for each case

MEASUREMENTS OF DYNAMIC COMPLIANCE

		o f		L i f e	
11	12	13	14	15	16
-	23.2 0.9				
-	24.6 0.8				
-	-	20.5 0.6	26.8 0.7	-	31.6 0.7
-	-	-	-	-	46.4 1.0
49.4 1.5	73.3 2.0				
62.2 2.7	60.7 2.4				
54.1 1.6	68.0 1.9				
-	-	37.7 1.0	37.2 1.0	-	41.1 0.8
-	-	40.8 1.1	41.1 1.1	-	67.5 1.5
25.3 0.7	-	-	38.8 0.9	-	46.5 1.0
	s	-	54.2 1.6	61.2 1.7	71.5 1.8
	s	-	49.6 1.6	53.6 1.5	65.6 1.7
-	73.5 2.0				
-	-	32.2 0.9	26.8 1.8	-	66.2 1.7
-	60.9 2.7	75.3 2.0	-	-	96.1 1.8
41.6	53.4 46.1	-	68.2	-	118.3 96.7
1.7	2.4 1.5	-	1.6	-	3.9 1.9

Fig. V 9

Diagram illustrating the changes in dynamic compliance with age in the experimental cases. Comparison with the normal and control series. Mean value for each age group. Notice that the animals operated between 4 and 6 weeks of age failed to show a normal increase in dynamic compliance during the follow-up period. These changes were less striking in the animals operated at or after the eighth week of life


Dynamic Compliance (ml/cm H_2O)
100
50
Normals
Controls
Shunts (4 - 6 wks)
Shunts (8 wks ≥)
5
10
15
AGE (weeks)

did not change during growth (Table IV 4). This ratio was decreased in all shunted cases ($P<0.001$) and the difference between the two mean values, in the younger and older age groups, was also highly significant ($P<0.001$) (Table V 21). When the ratio CDyn/BW was related to age, it was found that most of the fall occurred only after the fourth post-operative week in both experimental series (Fig. V 10), thus suggesting a progressive decrease in lung compliance as a response to the aorto-pulmonary anastomosis.

Thoracic Gas Volume

There was increase in thoracic gas volume throughout growth in both shunted and control cases (Fig. V 11). The figures for the animals operated earlier in life, with a patent and occluded shunt, were slightly higher than the normal cases, suggesting a certain degree of air trapping after surgery (Table V 22). When related to body weight however, the changes in thoracic gas volume throughout growth were not highly significant between any of the various groups, i.e., (i) normals; (ii) controls; (iii) shunted, complete series; (iv) shunted 4-6 weeks and (v) shunted 8 weeks or more ($0.05>P>0.01$ in all cases).

Specific Compliance

There was a decrease in specific compliance in all the shunted cases (Table V 23). This difference was highly significant ($P<0.001$) when compared to the control cases, which maintained constant and normal values throughout growth (Table V 23). The abnormal pattern

TABLE V 21

FOLLOW-UP STUDIES. LUNG FUNCTION - DYNAMIC COMPLIANCE RELATED TO BODY WEIGHT. COMPARISON BETWEEN CASES SHUNTED AT VARIOUS AGES, CONTROLS AND NORMALS

Dynamic Compliance Body Weight $\left(\frac{ml/cm\ H_2O}{Kg}\right)$		Controls (14)	Total Shunted (46)	Shunted 4-6 w (21)	Shunted 8-12 w (19)
	Mean	2.2	1.3	1.1	1.5
	SD	0.60	0.42	0.4	0.33
normals (28)*	2.1	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
	0.65				
controls			$P < 0.001$	$P < 0.001$	$P < 0.001$
shunted 4-6 w					$P < 0.001$

( ) - total number of measurements
SD - standard deviation
NS - $P > 0.1$
* Table IV 3

Fig. V 10

Diagram illustrating the changes in dynamic compliance related to body weight in the experimental cases during growth. Mean and standard deviation values for each age group. The continuous and dotted lines represent respectively the mean and the 2 standard deviation values for dynamic compliance related to body weight obtained from the normal series. The changes are less striking in the older age group

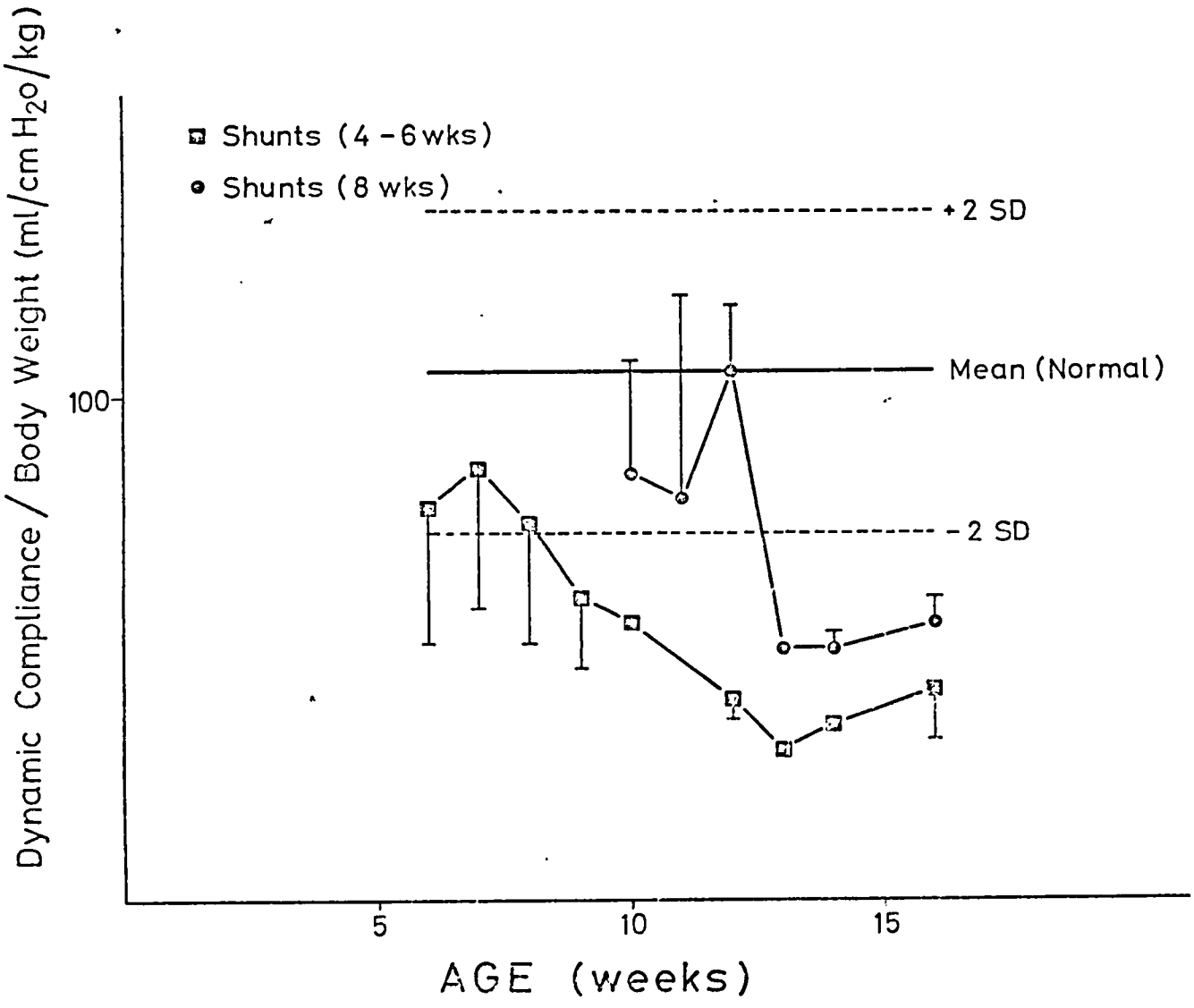
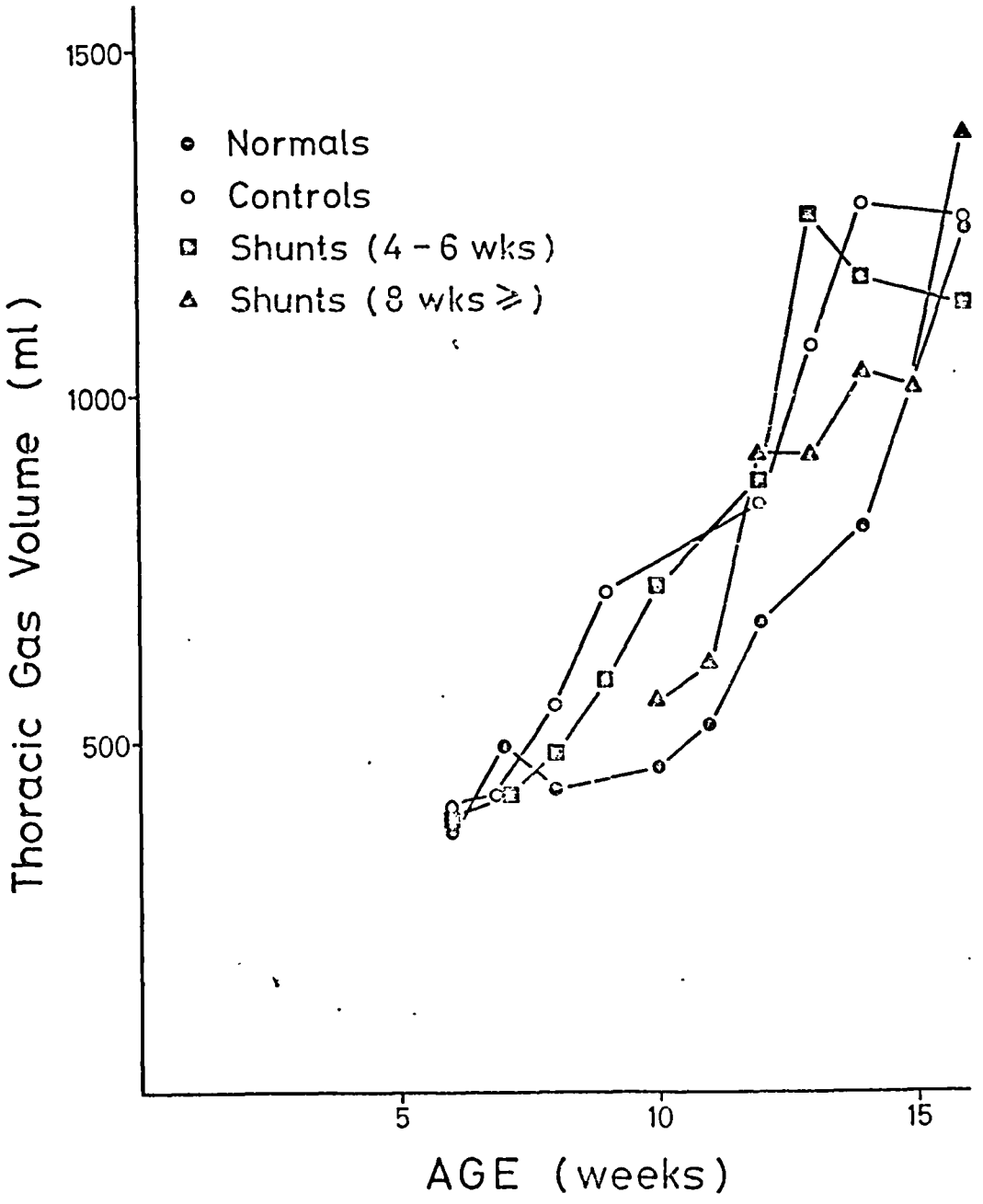

Shunts (4 - 6 wks)
Shunts (8 wks)
+2 SD
Mean (Normal)
-2 SD
100
Dynamic Compliance / Body Weight (ml/cm H2O/kg)
5
10
15
AGE (weeks)

Fig. V 11

Diagram illustrating the increase in thoracic gas volume with age in both the experimental, normal and control series. Mean value for each age group


1500
1000
500
Thoracic Gas Volume (ml)
Normals
Controls
Shunts (4 - 6 wks)
Shunts (8 wks ≥)
5
10
15
AGE (weeks)

TABLE

FOLLOW-UP STUDIES. LUNG FUNCTION - T G V and T G V / B W

Case No.	4	5	6	7	8	9
C.2	s	-	427 22.5	456 22.8	524 23.8	
C.3	s	-	374 24.9	380 18.9	513 21.4	
C.4	s	-	388 27.7	398 22.7	409 24.1	-
C.6		s	-	529 37.8	504 24.0	637 26.5
C.7		s	-	645 24.8	-	-
C.9			s	-		552 22.1
C.10					s	-
C.11					s	-
C.12					s	-
C.13					s	-
C.14					s	-
C.15					s	-
C.17						
C.18						
Controls						
C.1	s	-	420 28.0	424 23.5	543 28.6	
C.5	s	-	392 26.1	426 24.3	564 26.9	654 27.2
C.8			s	-	-	784 32.6
C.16						
Normals*						
TGV			456 301	537 451	455 419	-
TGV/BW			38.0 27.4	35.8 32.2	18.9 16.9	

TGV - thoracic gas volume (ml)
s - shunt
BW - body weight (Kg)
* - Table IV 3

Mean value for each case

Weeks of Life						
10	11	12	13	14	15	16
-	-	698 26.8				
-	-	1065 33.3				
-	-	-	1061 30.3	1168 32.4	-	1273 28.3
731 24.4	-	-	-	-	-	998 22.2
420 15.0	424 12.8	643 17.9				
644 32.2	679 29.5	1002 40.1				
631 21.0	863 26.1	1107 30.7				
557 23.7	-	-	856 23.8	1288 35.8	-	1541 28.5
-	-	-	980 27.2	1350 33.7	-	1646 31.6
-	640 17.8	-	-	872 20.8	-	1260 28.0
		s	-	817 24.8	976 27.9	1073 26.8
		s	-	832 27.7	1044 34.8	1374 36.1
-	-	770 21.4				
-	-	-	1154 32.5	1274 33.5	-	1352 34.2
s	-	931 31.0	997 26.9	-	-	1153 22.2
485 442	525	739 618	-	814	-	1358 1135
21.1 21.0	1.0	30.8 21.0	-	19.4		37.8 27.2

TABLE

FOLLOW-UP STUDIES. LUNG FUNCTION -

Case No.	CSpecif		Weeks		of	
	4	5	6	7	8	9
C.2	s	-	0.098	0.105	0.086	
C.3	s	-	0.049	0.098	0.050	
C.4	s	-	0.049	0.051	0.044	-
C.6		s	-	0.057	0.078	0.038
C.7		s	-	0.040	-	-
C.9			s	-	-	0.063
C.10					s	-
C.11					s	-
C.12					s	-
C.13					s	-
C.14					s	-
C.15					s	-
C.17						
C.18						
Controls						
C.1	s	-	0.124	0.131	0.132	
C.5	s	-	0.065	0.069	0.058	0.097
C.8			s	-	-	0.031
C.16						
Normals*						
CSpecif			0.078	0.090	0.095	-
			0.065	0.075	0.093	

CSpecif - specific compliance (ml/cm H_2O per ml)

BW - body weight (Kg)

s - shunt

* - Table IV 3

Mean value for each case

MEASUREMENTS OF SPECIFIC COMPLIANCE

		Life				
10	11	12	13	14	15	16
-	-	0.033				
-	-	0.023				
-	-	-	0.019	0.023	-	0.025
0.046	-	-	-	-	-	0.046
0.118	0.116	0.114				
0.061	0.092	0.061				
0.093	0.063	0.061				
0.044	-	-	0.044	0.029	-	0.027
-	-	-	0.042	0.030	-	0.039
-	0.039	-	-	0.044	-	0.037
		s	-	0.066	0.063	0.067
		s	-	0.060	0.051	0.480
-	-	0.095				
-	-	-	0.028	0.053	-	0.049
s	-	0.074	0.075	-	-	0.083
0.130	0.079	0.086	-	0.084	-	0.104
0.101		0.054				0.071

was only evident however, after the first post-operative month in both experimental groups suggesting a progressive change throughout growth, as already predicted from the dynamic compliance measurements (Table V 20-21). Contrary to this variable, specific compliance was not highly significantly different ($0.05 > P > 0.01$) between both experimental groups (Table V 24).

STRUCTURAL STUDIES

Lung Volume and Lung Length

The lung volume (cc) in the shunted cases, at the end of the follow-up period, did not differ significantly from that found in the normal and control animals at the same age ($0.1 > P > 0.05$) (Table V 25).

The lung length (cm) was smaller in the shunted cases when compared with the normal values (Table V 25), particularly in the animals operated earlier in life and followed for two months. But the same also occurred in the younger controls thus suggesting that the thoracotomy early in life might have had some effects on lung length throughout growth, at least during the first post-operative months.

TABLE V 24

FOLLOW-UP STUDIES. LUNG FUNCTION - SPECIFIC COMPLIANCE, COMPARISON BETWEEN CASES SHUNTED AT VARIOUS AGES, CONTROLS AND NORMALS

Specific Compliance (ml/min per Kg)		Controls (14)	Total Shunted (46)	Shunted 4-6 w (21)	Shunted 8-12 w (19)
	Mean	0.076	0.052	0.044	0.061
	SD	0.020	0.020	0.019	0.020
normals (28)*	0.079 0.019	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
controls			$P < 0.001$	$P < 0.001$	$P < 0.01$
shunted 4-6 w					$P < 0.05$

( ) - total number of measurements
SD - standard deviation
NS - $P > 0.1$
* Table IV 3

TABLE V 25

LUNG VOLUME AND LUNG LENGTH IN THE EXPERIMENTAL CASES

Case No.	LV (cc)	LV/BW (cc per 100g)	LL (cm)	LL/BW (cm per 100g)
C.2	365	1.9	13.3	0.07
C.3	405	1.8	12.5	0.06
C.4	910	3.5	15.0	0.06
C.6	925	2.6	16.0	0.04
C.7	1870	4.1	18.7	0.04
C.9	1020	2.3	17.5	0.04
C.10	1085	3.0	18.5	0.05
C.11	995	4.0	14.0	0.06
C.12	950	2.6	15.0	0.04
C.13	1785	3.3	16.8	0.03
C.14	1645	3.2	14.6	0.03
C.15	2020	4.5	16.8	0.04
C.17	1890	4.7	17.5	0.04
C.18	2000	5.3	16.0	0.04
Controls				
C.1	430	2.3	15.5	0.08
C.5	1095	3.0	18.5	0.05
C.8	1480	3.7	16.8	0.04
C.16	1895	3.6	16.5	0.03
Normals (Table III 1)				
8 weeks	435-540	2.0-2.9	18.2-18.7	0.09-0.10
12 "	1280	3.4	19.8	0.05
16 "	1795-1835	3.0-3.1	20.0-23.3	0.03-0.04

LV - lung volume

BW - body weight

LL - lung length

General Structural Assessment. Point Counting Techniques

Macroscopic Appearance

The lungs did not show evidence of atelectasis, and the pleura was normal and thin, except around the left upper lobe, and in some cases around the middle one, where it was thicker than normal due to adhesions to the chest wall at the level of the thoracotomy scar. The lower lobe which constitutes the greater part of the left lung was not affected by the adhesions.

Point counting

Macroscopically, the volume proportions of lung tissue occupied by the arteries was slightly lower than normal in the animals operated earlier in life and followed for one month (C. 2-3); cases from the same age group but studied for longer periods tended to show an increase in the volume proportion of arterial tissue, 3.3-3.7% at twelve weeks of age and 3.1-3.5% at sixteen weeks, compared with 3.1-4.1% at twelve weeks (normals and controls) and 2.0-4.4% at sixteen weeks (normals and controls) (Table V 26). This increase above normal was apparent also in the older age group, particularly in the animals followed until sixteen weeks of age, 3.9-4.7%. The volume proportions of the other components of lung tissue did not change after the shunt was made.

Microscopic Appearance

The general architecture of the lung was not affected by the

TABLE V 26

VOLUME PROPORTIONS (%) OF THE VARIOUS COMPONENTS OF LUNG TISSUE (MACROSCOPIC POINT COUNTING) IN THE EXPERIMENTAL CASES

Case No.	Parenchyma	Arteries	Veins	Bronchi	Connective Tissue
C.2	91.0	3.9	1.0	2.0	2.1
C.3	89.4	3.1	2.2	3.3	2.0
C.4	85.3	3.3	1.8	4.5	5.1
C.6	86.4	3.7	2.4	4.1	3.4
C.7	85.4	3.1	2.2	3.8	5.5
C.9	90.0	3.5	1.9	2.1	2.5
C.10	85.9	2.6	2.1	3.4	6.0
C.11	84.0	3.4	2.7	2.7	4.5
C.12	88.0	3.9	3.0	2.0	3.1
C.13	87.1	4.1	3.4	2.5	2.9
C.14	84.4	4.0	2.2	4.8	4.6
C.15	87.9	3.8	3.3	2.9	4.2
C.17	85.6	4.7	2.5	3.8	3.4
C.18	89.0	3.9	2.5	4.9	2.7
Controls					
C.1	81.5	4.7	2.6	7.1	4.1
C.5	84.7	4.1	3.1	4.2	3.9
C.8	82.3	3.6	4.4	4.5	3.7
C.16	88.0	2.9	2.0	2.2	4.9
Normals (Table III 3)					
8 weeks	86.5	4.7	2.8	2.9	4.1
12 "	84.2	3.1	2.9	4.5	5.3
16 "	90.3	2.5	2.6	1.5	3.1

aorto-pulmonary anastomosis. The connective tissue septa looked normal in structure and thickness and the alveolar walls were normally thin. There was no evidence of perivascular or peribronchiolar edema.

Point counting

The animals that were submitted to a thoracotomy showed the highest volume proportion of alveolar air when compared with the normal cases (Table V 27), but the differences between the two groups, shunted and controls, was not highly significant ($0.02 > P > 0.01$ in all cases). Microscopic arterial volume was smaller in cases of all ages with the longer follow-up, but, as in the macroscopic studies, although a trend was shown, the differences were not highly significant from the normal and control cases ($0.02 > P > 0.01$ in all cases). The other components of the lung tissue were not changed in the shunted animals.

The Pulmonary Arterial Bed

Structure of the Left Pulmonary Artery

In the shunted cases, there was an increase in medial wall thickness of the left pulmonary artery. This change was already apparent in the animals studied for only one month in the various age groups but became more significant the longer the animals were followed. Cases C. 7 and 9 had the highest values, 276 and 312 µm,

TABLE V 27

PROPORTIONS OF LUNG VOLUME (%) OCCUPIED BY THE VARIOUS COMPONENTS OF LUNG TISSUE (MICROSCOPIC POINT COUNTING) IN THE EXPERIMENTAL CASES

Case No.	AA	AD	AW	AR	P
C.2	74.0	9.1	9.3	2.7	4.9
C.3	73.9	10.1	11.7	2.9	3.4
C.4	75.6	9.4	9.4	2.4	3.2
C.6	74.1	10.2	10.6	2.5	2.6
C.7	75.7	9.7	12.6	2.1	1.9
C.9	73.0	12.0	11.0	2.0	2.0
C.10	75.0	11.0	9.0	3.0	2.0
C.11	69.1	10.7	15.0	2.8	2.4
C.12	77.0	8.9	10.2	2.2	1.7
C.13	75.4	7.1	13.4	1.7	2.4
C.14	76.1	10.1	11.9	1.8	2.0
C.15	82.0	6.0	6.0	2.0	4.0
C.17	77.6	5.4	11.9	2.6	2.5
C.18	76.5	4.0	14.3	2.5	2.7
Controls					
C.1	74.8	6.7	10.6	2.8	5.1
C.5	79.4	3.1	11.6	2.9	3.0
C.8	73.7	8.8	12.9	2.5	2.1
C.16	76.7	6.1	11.8	3.7	1.7
Normals (Table III 4)					
8 weeks	68.5	15.0	7.8	3.4	5.3
12 "	69.7	15.6	9.3	2.3	3.1
16 "	-3.7	12.0	7.6	3.1	3.6

AA - alveolar air
AD - alveolar duct air
AW - alveolar wall
AR - arteries
P - parenchyma

compared with a range of 165-228 μm in the age-matched normal and control series. Cases C. 13, 14 and 15 operated later in life (eight weeks) and followed until the sixteenth week of life also showed a similar increase, the range in wall thickness being 247-296 μm, although the absolute change was smaller than in the younger series (Table V 28).

The number of the elastic fibers contained within the media did not change considerably, although the animal with the highest medial wall thickness showed the highest number of elastic fibers in the media, 11, compared with 7-8 in the age-matched control normal series.

Intimal proliferation was seen in some regions of the arterial wall but no defined pattern of intimal thickening could be found in any of the experimental cases at the level of the left pulmonary artery.

The Arteriogram

A good filling of the pulmonary arterial bed was obtained in all the experimental cases. In no experimental case were abnormal communications found either with the pulmonary venous circulation or with the bronchial vessels.

At the end of the first post-operative month, only the animals that were shunted at twelve weeks of age, C. 17 and 18, showed dilatation of the pre-acinar arteries (Fig. V 12); the other cases

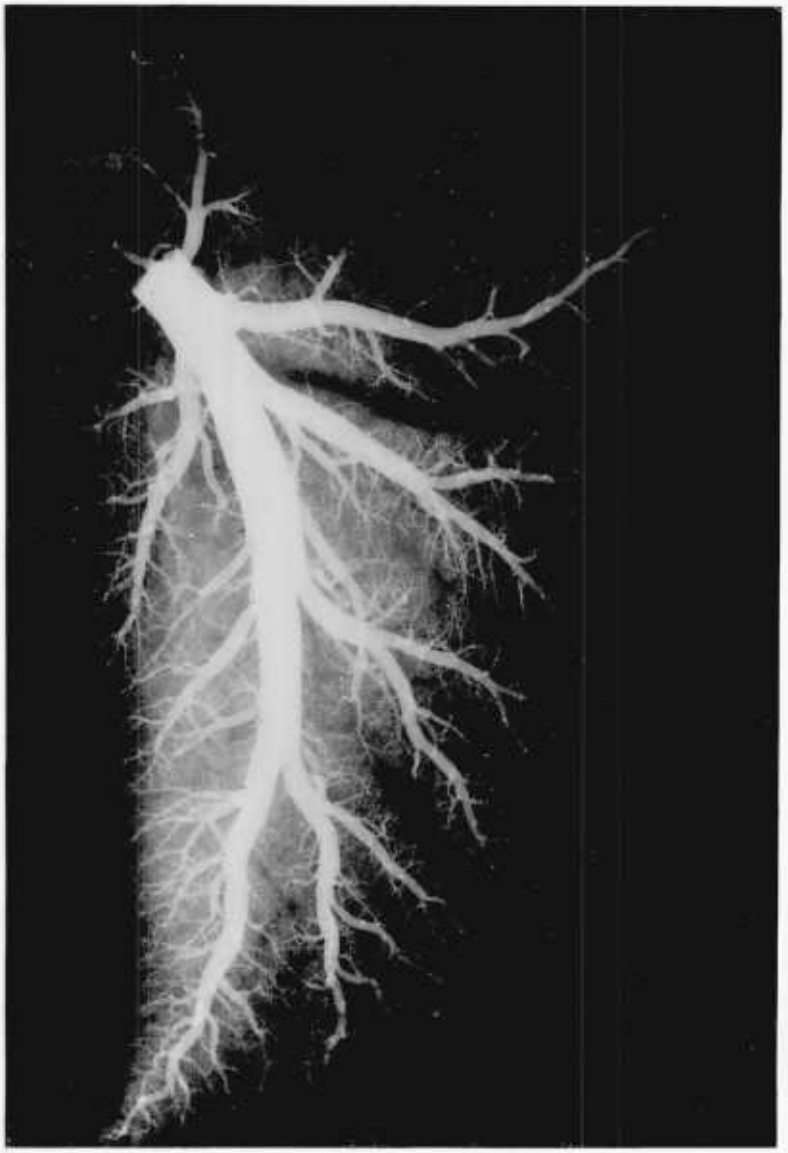
TABLE V 28

EXPERIMENTAL CASES. STRUCTURE OF THE LEFT PULMONARY ARTERY

Case No.	Left Pulmonary Artery	
	Thickness of the Media (μm)	Number of Elastic Laminae
C.2	250 36.4	9 0.9
C.3	219 9.5	8 0.3
C.4	254 13.6	9 0.4
C.6	231 14.2	8 0.6
C.7	276 14.5	10 0.5
C.9	312 25.2	11 0.6
C.10	216 35.3	8 0.7
C.11	147 10.6	8 0.3
C.12	233 30.5	10 0.9
C.13	247 19.2	8 0.3
C.14	296 7.4	11 0.8
C.15	250 8.7	10 0.8
C.17	238 38.8	10 1.0
C.18	202 23.4	10 0.7
Controls		
C.1	228 14.8	7 0.5
C.5	177 9.1	9 0.4
C.8	139 7.3	8 0.6
C.16	182 10.5	7 0.3
Normals (Table III 5)		
8 weeks	196 14.7	8 0.7
12 "	188 23.5	7 0.3
16 "	165 12.1	7 0.4

Fig. V 12

Pulmonary arteriogram of an experimental case shunted at twelve weeks of age and sacrificed at sixteen weeks (x0.8)
Left lung



were similar to the control and normal animals at the same age (Fig. V 13). By the end of the second post-operative month, dilatation of the pre-acinar arteries was also apparent in the animals that were operated at eight weeks of life (Fig. V 14).

The animals shunted at four weeks of age showed less dilatation of the pre-acinar arteries when compared with the older ones (Fig. V 15). An increase in the tortuosity of the peripheral vessels was found in all the series, particularly after the first post-operative month.

The background haze was not reduced in any of the experimental cases, thus suggesting that the number of the small intra-acinar arteries was not significantly reduced.

Measurements of axial length and diameter

The radiographic measurements showed that the length of the left lower lobe artery was slightly reduced in the younger age group particularly in the animals with the smallest follow-up period; by the end of the third post-operative month, the arterial axial length was again within the normal and control range (Table V 29). The same pattern was seen in animals operated at eight weeks of life, the decrease in length being apparent only at the end of the first post-operative month. The animals operated at twelve weeks did not show any decrease in arterial axial length by the end of the first post-operative month.

Fig. V 13

Pulmonary arteriogram of an experimental case shunted at four weeks and sacrificed at eight weeks of age (x0.9)

Left lung

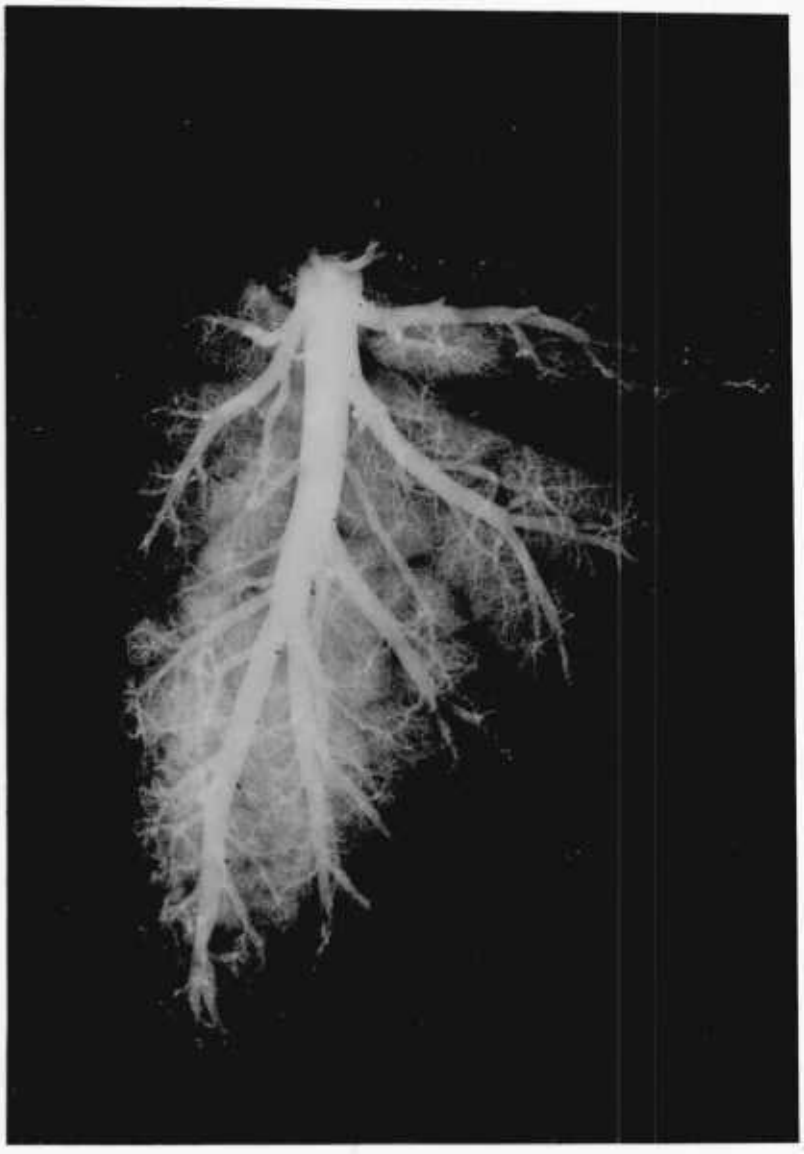


Fig. V 14

Pulmonary arteriogram of a case shunted at four weeks and sacrificed at sixteen weeks of age. Notice the dilatation of the proximal arteries and the increased tortuosity at the lung periphery (x0.7)

Left lung

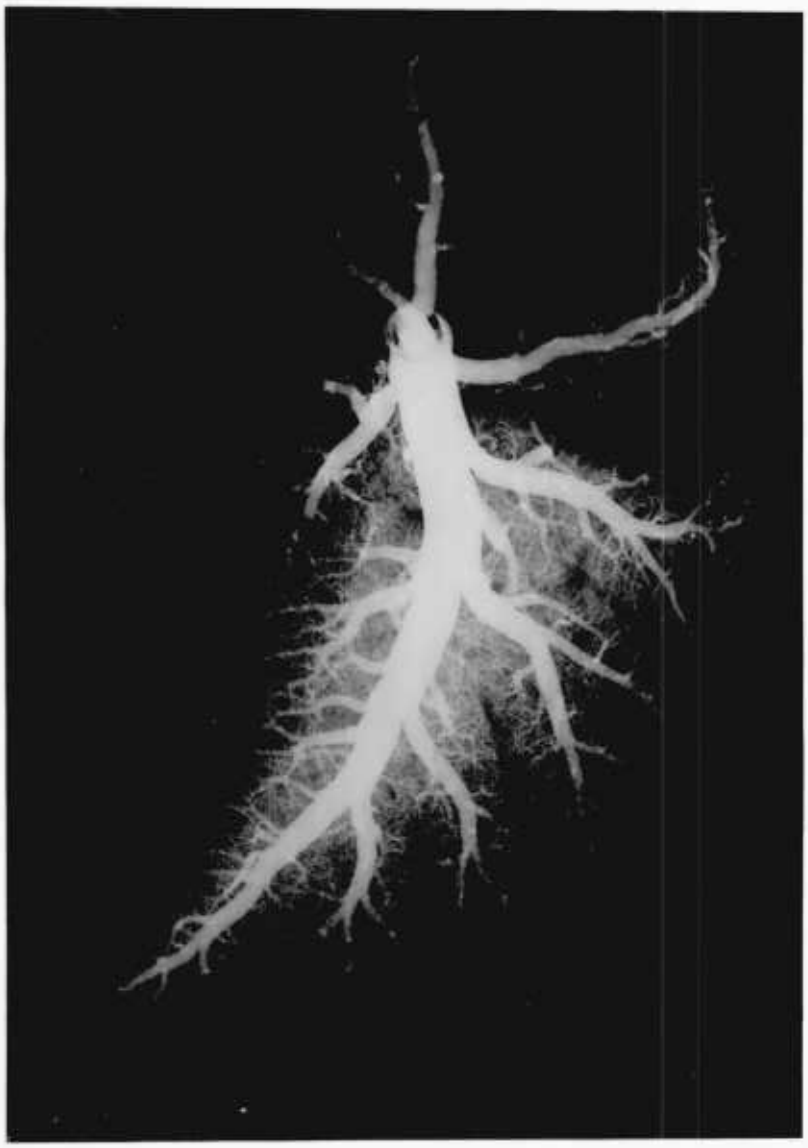


Fig. V 15

Pulmonary arteriogram of a case shunted at eight weeks and sacrificed at sixteen weeks (x0.6). Notice the dilatation of the proximal arteries and the increased tortuosity at the lung periphery. The pre-acinar changes are more striking in the animals operated late in life than in the younger series

Left lung

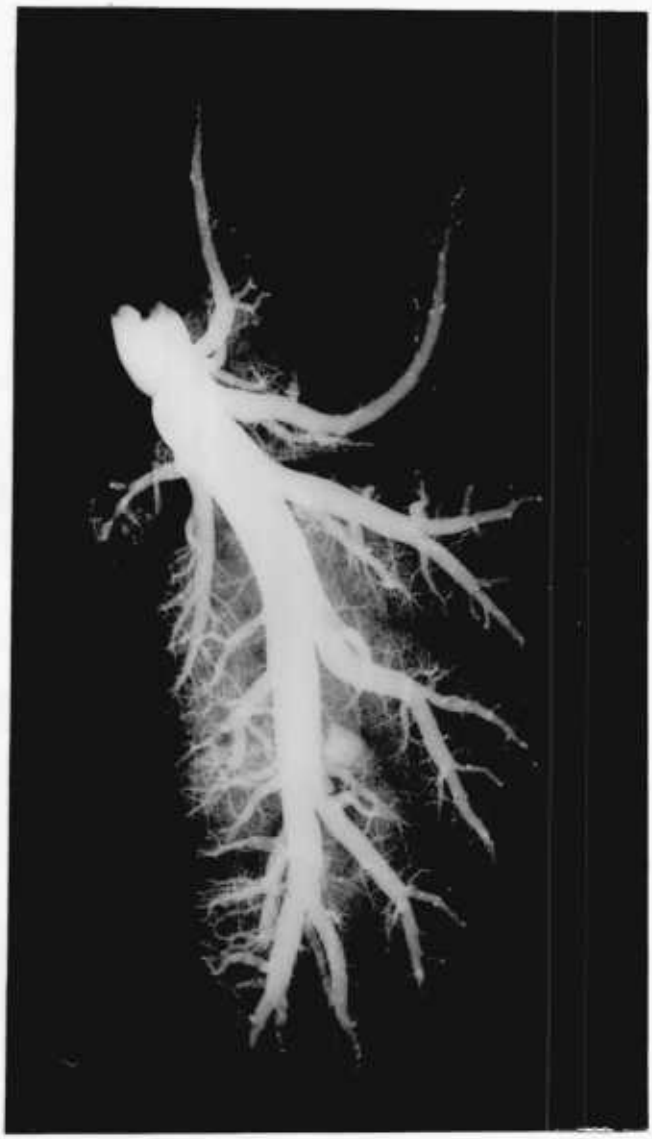


TABLE V 29

MEASUREMENTS FROM THE ARTERIOGRAMS IN THE EXPERIMENTAL CASES. LEFT LOWER LOBE ARTERY - TOTAL AXIAL LENGTH, DIAMETER AT THE HILUM AND AT 25% INTERVALS ALONG THE PATHWAY

Case No.	Length (mm)	Diameter (mm) Hilum	25%	50%	75%
C.2	115	8.0	5.7	4.7	2.0
C.3	110	8.0	6.2	3.7	2.5
C.4	130	9.0	7.5	5.5	3.0
C.6	140	10.0	7.0	5.0	3.5
C.7	168	10.0	6.0	3.7	2.0
C.9	165	10.5	6.2	5.0	2.5
C.10	173	8.5	7.0	4.5	2.5
C.11	110	9.0	5.7	3.7	2.0
C.12	120	9.5	6.0	4.5	2.5
C.13	155	11.0	8.2	5.0	3.0
C.14	130	9.0	7.0	4.0	2.5
C.15	151	13.0	11.5	8.7	5.7
C.17	165	11.5	10.0	8.0	4.7
C.18	150	10.0	8.7	6.5	4.0
Controls					
C.1	140	7.5	6.0	4.5	2.2
C.5	160	9.0	6.0	4.0	2.0
C.8	143	10.5	6.5	5.5	2.5
C.16	155	10.0	7.5	5.5	2.7
Normals (Table III 6)					
8 weeks	129	7.0	5.5	4.5	3.0
8 "	138	6.5	5.0	4.0	2.0
12 "	159	9.5	6.5	4.5	3.0
16 "	167	10.0	7.5	5.0	3.5
16 "	173	9.5	7.0	4.5	2.5

Mean value for each case

The changes in arterial lumen diameter along the pre-acinar pathway (Table V 29) were more apparent in the older age group, particularly in cases C. 17 and 18 and in cases C. 13, 14 and 15 where a considerable dilatation of the arterial bed was seen (Fig. V 16). Although some increase in arterial size had also occurred in the younger age group, that is animals operated early in life, these changes were less striking than those found in the older cases (Fig. V 16).

The Peripheral Arterial Bed

The structure of the muscular pulmonary arteries was not changed in the experimental cases when compared with the normal animals; there was no evidence of intimal thickening in any of the cases included in the present study.

Percentage medial wall thickness

The changes in wall thickness of the muscular arteries were not striking in the three different groups by the end of the first post-operative month (Table V 30). There was some increase in percentage wall thickness in the larger arteries, those between 200 and 1000 μm in external diameter, the range in all experimental cases being 2.3-3.4 with a mean of 3.0 (Fig. V 17), compared with a range of 2.2-3.0, mean 2.6, in the control and normal cases. The percentage wall thickness for the smaller arteries, 0-200 μm, in the experimental series, was within the normal and control range (Table V 30).

Fig. V 16

Diagram illustrating the changes in arterial lumen diameter (measured from the arteriogram) in the experimental cases. The changes are ony striking in the animals operated late in life

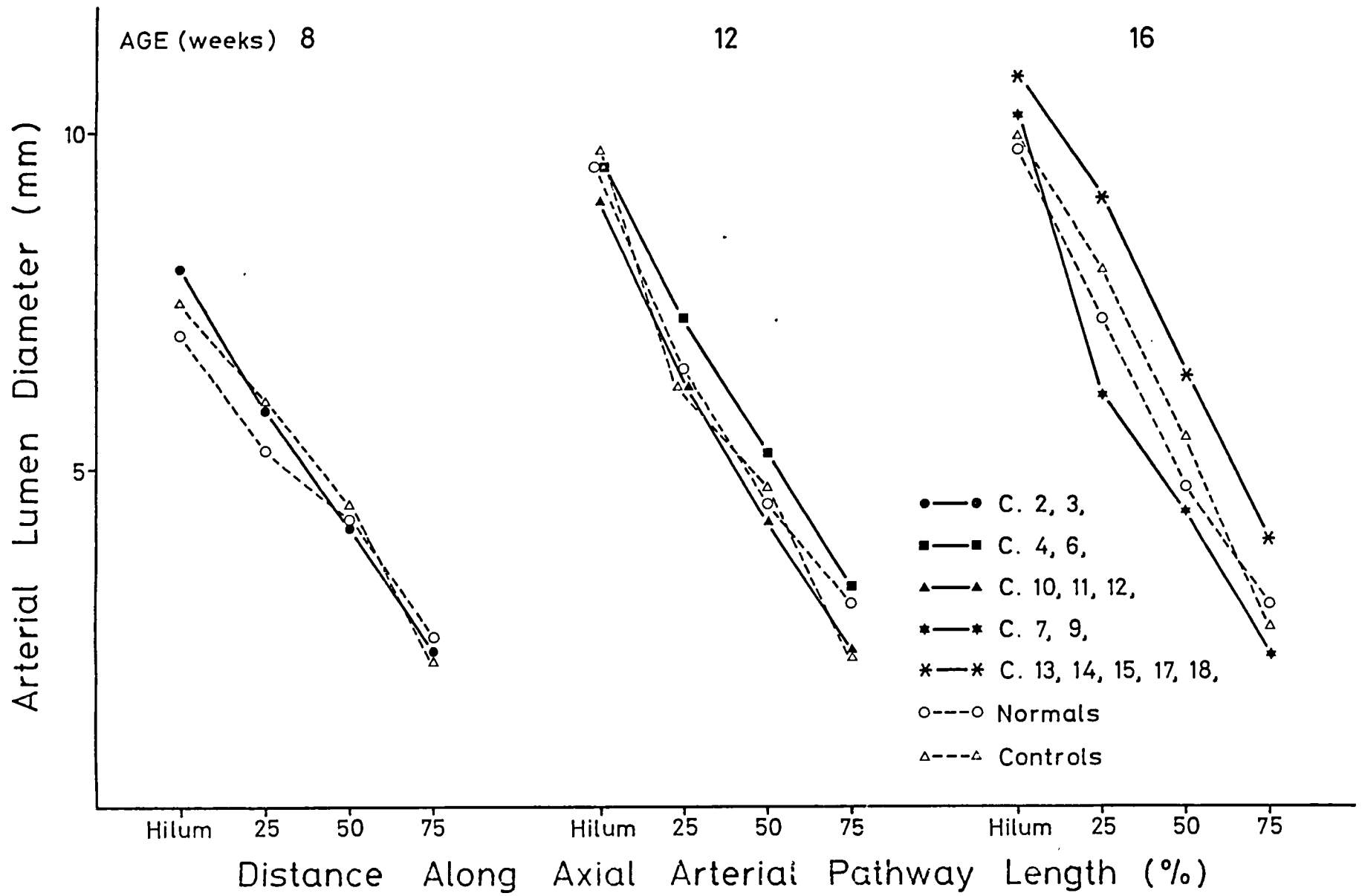

PRE-ACINAR ARTERIES - SIZE (mm)
AGE (weeks) 8
12
16
Arterial Lumen Diameter (mm)
10
5
C. 2, 3,
C. 4, 6,
C. 10, 11, 12,
C. 7, 9,
C. 13, 14, 15, 17, 18,
Normals
Controls
Hilum
25
50
75
Distance Along Axial Arterial Pathway Length (%)

TABLE V 30

PERCENTAGE ARTERIAL WALL THICKNESS. EXPERIMENTAL CASES

Case No.	External Diameter (μm)								
	0-50	50-100	100-200	200-300	300-400	400-500	500-1000	0-200	200-1000
C.2	6.1	4.4	2.7	3.2	2.4	2.4	2.7	4.4	2.7
	0.34	0.66	0.17	0.17	0.23	0.43	0.19	0.35	0.12
	30	76	37	13	10	5	6	143	34
C.3	6.2	3.9	3.3	3.8	4.1	2.8	2.8	4.5	3.4
	0.30	0.10	0.15	0.16	0.29	0.29	0.14	0.14	0.13
	44	90	53	19	12	5	2	187	38
C.4	8.2	6.2	6.0	4.9	5.8	5.5	4.8	6.8	5.2
	0.25	0.23	0.22	0.58	0.85	0.27	0.80	0.16	0.34
	108	79	32	8	2	3	2	219	15
C.6	8.3	6.5	5.4	4.7	4.2	4.5	4.3	6.7	4.4
	0.37	0.44	0.16	0.38	0.46	0.51	0.70	0.31	0.28
	188	186	84	25	3	6	3	458	37
C.7	9.3	6.9	5.9	4.8	5.8	5.4	5.6	7.4	5.4
	0.31	0.21	0.33	0.55	0.91	0.62	1.40	0.18	0.35
	56	134	30	11	4	8	3	220	26
C.9	10.1	6.8	6.3	6.7	6.9	5.3	5.5	7.7	6.1
	0.20	0.21	0.28	0.73	1.09	0.79	0.71	0.15	0.43
	94	96	46	9	5	4	2	236	21
C.10	6.7	3.8	3.8	3.9	4.0	2.9	2.2	4.8	3.2
	0.25	0.10	0.17	0.91	1.60	0.30	0.23	0.11	0.56
	56	139	64	8	4	2	3	259	17
C.11	7.3	3.8	3.0	2.5	2.3	2.7	1.9	4.7	2.3
	0.20	0.10	0.15	0.10	0.31	0.25	0.20	0.16	0.10
	69	114	54	11	5	2	2	237	20
C.12	6.6	3.9	2.7	2.6	2.5	3.0	4.7	4.4	3.2
	0.27	0.40	0.10	0.13	0.39	0.12	1.90	0.13	0.18
	36	95	55	19	4	3	2	186	28

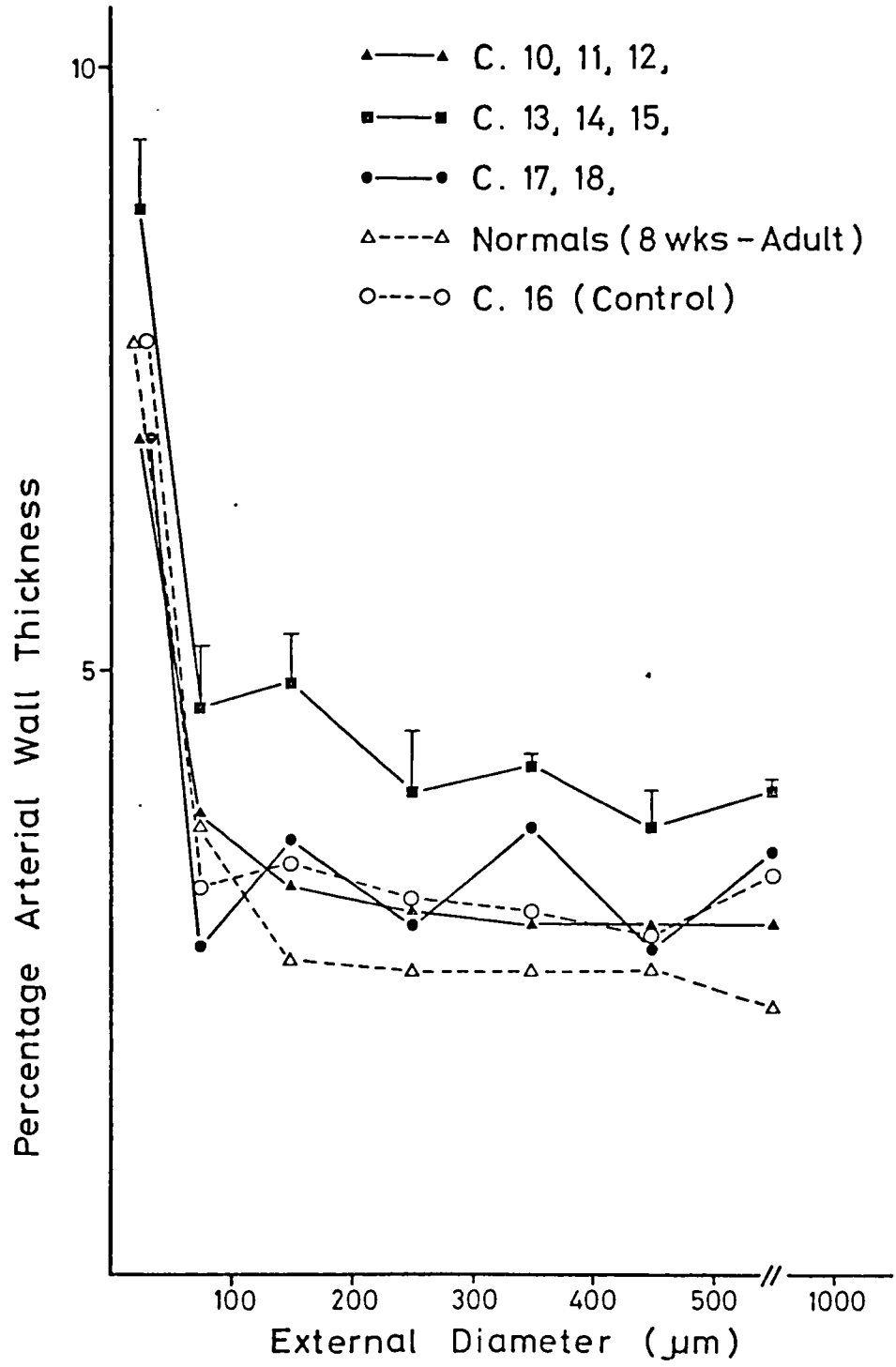
C.13	8.1	4.7	5.3	3.0	3.9	3.9	4.0	6.0	3.7
	0.33	0.11	0.18	0.35	0.35	0.69	0.60	0.12	0.23
	36	72	44	19	6	4	5	152	34
C.14	8.3	3.8	4.0	4.5	4.3	3.1	4.1	5.4	4.0
	1.04	0.10	0.14	0.44	0.64	0.90	0.53	0.19	0.28
	74	74	44	7	3	3	7	192	20
C.15	9.9	5.5	5.4	4.4	4.3	4.2	4.0	6.9	4.2
	0.54	0.22	1.70	0.22	0.59	0.33	0.25	0.32	0.14
	135	103	48	14	3	4	6	286	27
C.17	7.4	4.5	3.8	3.0	3.6	3.1	3.4	5.2	3.3
	0.81	0.21	0.51	0.30	0.46	0.70	0.31	0.16	0.25
	35	78	70	12	7	3	12	183	34
C.18	6.3	3.7	3.4	2.6	3.8	2.1	3.4	4.5	3.0
	0.22	0.09	0.51	0.22	1.38	0.39	0.55	0.12	0.27
	35	80	71	20	6	5	9	186	40
Controls									
C.1	7.9	3.6	2.8	2.2	2.8	1.7	2.2	4.8	2.2
	1.12	0.08	0.10	0.13	0.27	0.29	1.12	0.18	0.11
	59	107	68	11	6	3	8	234	28
C.5	7.7	3.5	3.9	3.0	2.8	2.1	1.8	5.0	2.4
	0.89	0.16	0.47	0.15	0.58	0.51	0.41	0.13	0.27
	58	119	55	13	4	5	7	232	29
C.8	6.4	4.3	2.9	3.8	2.7	2.9	2.0	4.5	2.8
	0.20	0.44	0.19	1.19	0.53	0.27	0.27	0.13	0.38
	70	104	48	13	9	8	5	222	35
C.16	7.7	3.1	4.4	3.1	3.0	2.8	3.2	5.1	3.0
	0.95	0.16	0.17	0.23	0.23	0.21	0.23	0.38	0.16
	65	76	65	24	12	5	3	206	44

Normals (see Table III 9)

Mean, standard error of the mean and total number of vessels measured

Fig. V 17

Diagram illustrating the measurements of percentage arterial wall thickness in the experimental cases (animals operated at or after the eighth week of life). Notice that the changes are only apparent after the second month of the follow-up period (C. 13-15)


C. 10, 11, 12,
C. 13, 14, 15,
C. 17, 18,
Normals (8 wks – Adult)
C. 16 (Control)
10
5
Percentage Arterial Wall Thickness
100
200
300
400
500
1000
External Diameter (μm)

Two months after surgery both the younger animals, C. 4 and 6, operated at four weeks of age, and the older ones, C. 13, 14 and 15 shunted at eight weeks, showed a considerable increase in arterial wall thickness in muscular vessels of all sizes. Cases C. 4 and 6 (Fig. V 18) had the highest values, 6.7-6.8 for the smaller arteries, 0-200 μm, and 4.4-5.2 for the larger ones, 200-1000 μm in external diameter. The older cases (Fig. V 17), showed a similar change although the wall thickness of the larger vessels, 3.7-4.2, was less significantly increased.

The two cases that were followed for three months, C. 7 and 9, showed the highest values of arterial wall thickness (7.4-7.7 for arteries 0-200 μm in diameter and 5.4-6.1 for those 200-1000 in diameter). These findings suggested that at least in the younger animals there was a direct relationship between the increase in arterial wall thickness and the duration of the follow-up period.

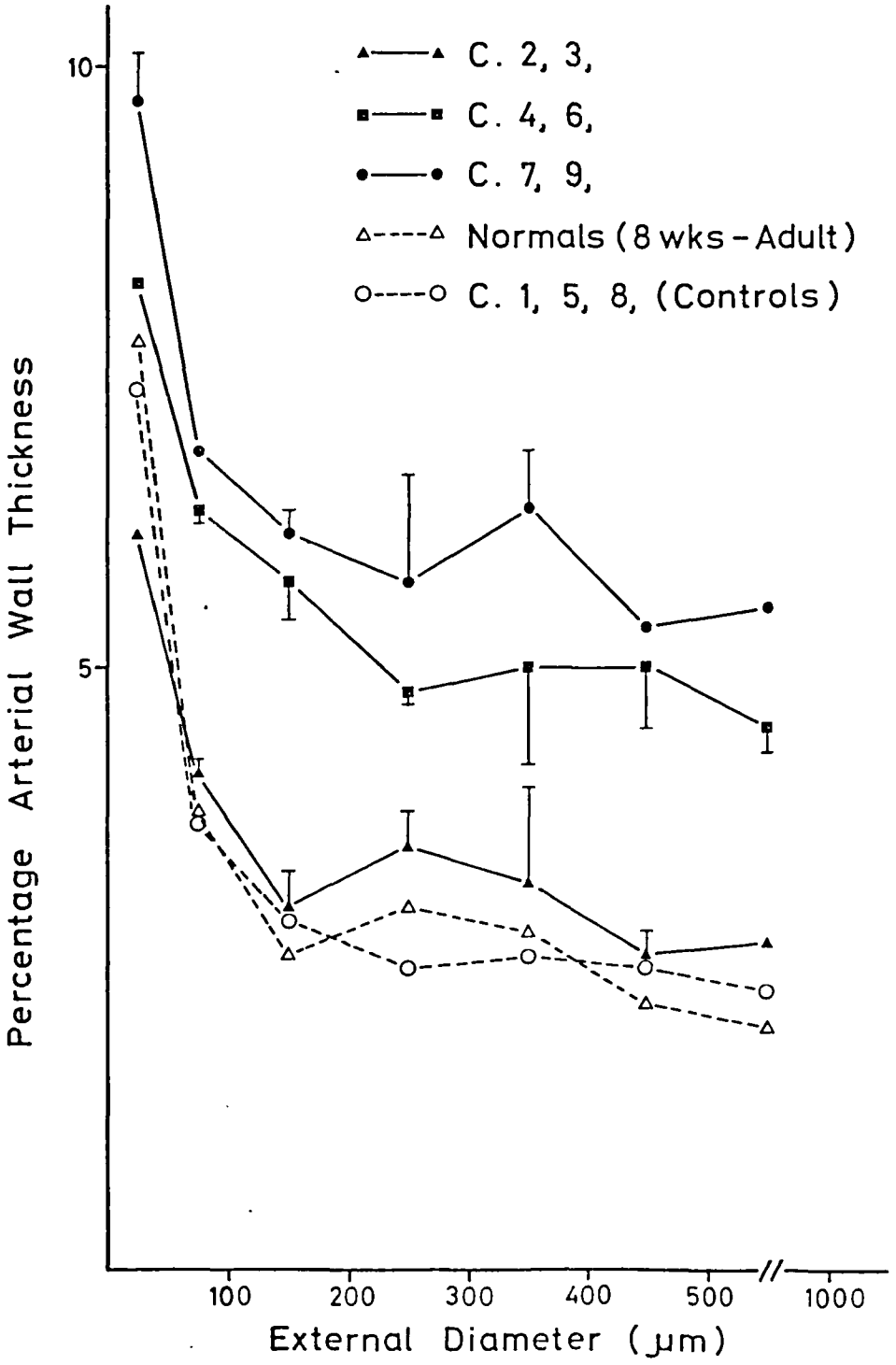
Population studies

The normal pig showed a considerable number of muscular vessels at the periphery of the arterial pathway during normal postnatal development. This feature, which is different from the human, made the assessment of the arterial population changes in the experimental cases difficult, because all the variation occurred within the last 100 μm in external diameter of the peripheral arterial bed, where the muscular, partially muscular and non-muscular vessels mixed.

There was a definite increase in the number of muscular and

Fig. V 18

Diagram illustrating the measurements of percentage arterial wall thickness in the experimental cases (animals operated between the fourth and the sixth week of life). Notice the increase in wall thickness between the cases followed for three months (C. 7-9) and those followed for two months (C. 4-6)


C. 2, 3,
C. 4, 6,
C. 7, 9,
Normals (8 wks - Adult)
C. 1, 5, 8, (Controls)
Percentage Arterial Wall Thickness
10
5
100
200
300
400
500
1000
External Diameter (μm)

partially muscular vessels at the periphery of the arterial bed (Table V 31) in the shunted cases, and this was accompanied by a progressive decrease in the number of the non-muscular ones (Fig. V 19-21).

These changes were already apparent in the younger cases (C. 2 and 3) by the end of the first post-operative month, with muscular arteries being found within the 20-30 μm external diameter range, where as normally, a complete muscular coat was only found in arteries between 40-50 μm in external diameter for the normal and control animals at the same age (Table V 31). This difference was less evident in the older animals at the end of the first post-operative month.

Two months after surgery, muscular arteries were found within the 10-20 μm range in both series, C. 4 and 6 operated at four weeks and C. 13, 14 and 15 shunted at eight weeks. The partially muscular and the non-muscular populations were also similar in both series (Table V 31) and even after the third month of follow-up, cases C. 7 and 9, the younger cases with the longer follow-up, were not different from the older ones (Fig. V 20).

Arteries accompanying airways

Wall structure As with the population studies, changes in the structure of the intra-acinar arteries reflected extension of muscle

TABLE V 31

POPULATION STUDIES IN THE EXPERIMENTAL CASES

Case No.	External Diameter (μm)												
	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	100-110	110-120	120-130	130-140
C.2	59	46	26	0	0	0	0	0	0	0	0		
	41	39	71	86	81	64	67	55	45	30	0		
	0	15	23	14	19	36	33	45	55	70	100		
C.3	90	71	38	8	0	0	0	0	0	0	0	0	
	10	29	55	90	90	72	71	61	60	40	33	0	
	0	0	7	2	10	28	29	39	40	60	67	100	
C.4	57	27	0	0	0	0	0	0	0				
	36	66	78	56	54	42	12	8	0				
	7	7	22	44	46	58	88	92	100				
C.6	64	25	2	0	0	0	0	0	0	0			
	36	66	75	65	52	36	13	9	4	0			
	0	9	23	35	48	64	87	91	96	100			
C.7	33	8	0	0	0	0	0	0	0	0			
	67	77	90	80	52	60	23	34	20	0			
	0	15	10	20	48	40	77	66	80	100			
C.9	71	18	0	0	0	0	0	0	0	0			
	20	73	89	84	70	50	28	19	8	0			
	9	9	11	16	30	50	72	81	92	100			
C.10	80	59	41	17	6	0	0	0	0	0	0		
	20	41	59	78	84	70	73	59	35	25	0		
	0	0	0	5	10	30	27	41	65	75	100		
C.11	67	33	5	5	3	0	0	0	0	0	0	0	0
	33	63	75	82	91	84	57	38	33	31	33	21	0
	0	4	25	13	6	16	43	62	67	69	67	79	100
C.12	70	33	10	0	0	0	0	0	0	0	0	0	
	30	67	82	79	94	81	63	71	73	60	20	0	
	0	0	8	21	6	19	37	29	27	40	80	100	

C.13	80	39	0	0	0	0	0	0	0	0	0	0
	20	52	100	94	79	80	53	45	45	25	20	0
	0	9	0	6	21	20	47	55	55	75	80	100
C.14	40	28	0	0	0	0	0	0	0			
	50	69	96	83	75	64	36	26	0			
	10	3	4	17	25	36	64	74	100			
C.15	23	26	0	0	0	0	0	0				
	69	66	62	57	43	37	20	0				
	8	8	38	43	57	63	80	100				
C.17	67	27	6	0	0	0	0	0	0	0	0	
	33	73	82	78	75	51	50	39	14	26	0	
	0	0	12	22	25	49	50	61	86	74	100	
C.18	90	80	30	0	0	0	0	0	0	0	0	0
	10	20	70	100	94	83	60	54	64	60	30	0
	0	0	0	0	6	17	40	46	36	40	70	100
Controls												
C.1	60	43	31	9	0	0	0	0	0	0	0	0
	40	57	69	91	93	86	81	80	79	55	20	0
	0	0	0	0	7	14	19	20	21	45	80	100
C.5	64	34	25	27	0	0	0	0	0	0	0	0
	36	66	75	63	62	64	77	87	50	30	9	0
	0	0	0	10	38	36	23	13	50	70	91	100
C.8	50	60	50	36	0	0	0	0	0	0	0	0
	50	40	50	60	94	90	87	79	62	47	33	14
	0	0	0	4	6	10	13	21	38	53	67	86
C.16	75	56	6	0	0	0	0	0	0	0	0	0
	25	44	90	94	74	81	62	50	40	17	0	100
	0	0	4	6	26	19	38	50	60	83	100	

Normals (see Table III 10)

For each case, % of non-muscular, partially muscular and muscular arteries in every size range

Fig. V 19

Diagram illustrating the changes in the arterial population curve in the experimental cases. Notice that both C. 7 (operated at four weeks and sacrificed at sixteen weeks) and C. 14 (operated at eight weeks and sacrificed at sixteen weeks of age) show an extension of muscle into smaller arteries than nromal at that age

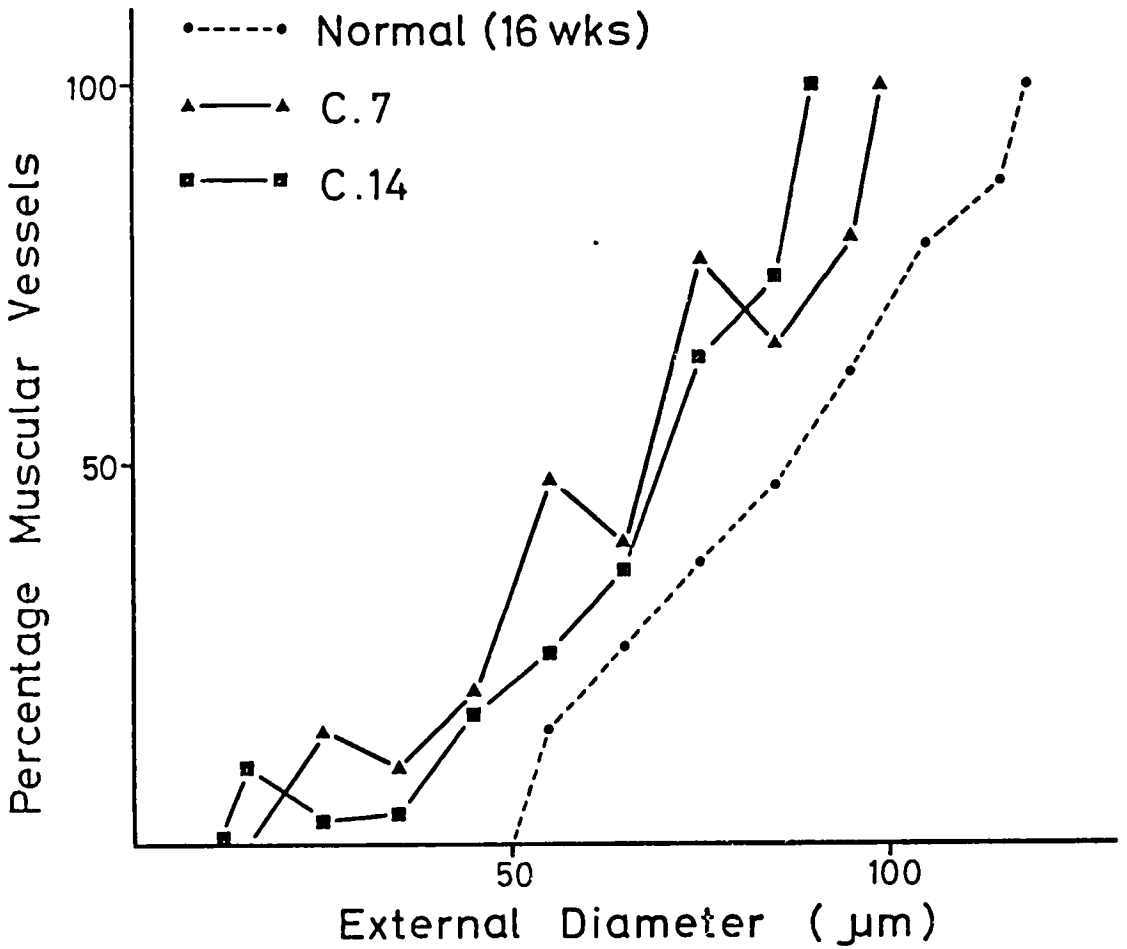

Normal (16 wks)
C.7
C.14
100
50
Percentage Muscular Vessels
50
100
External Diameter (µm)

Fig. V 20

Diagram illustrating the changes in the partially muscular arterial population curve in the experimental cases .

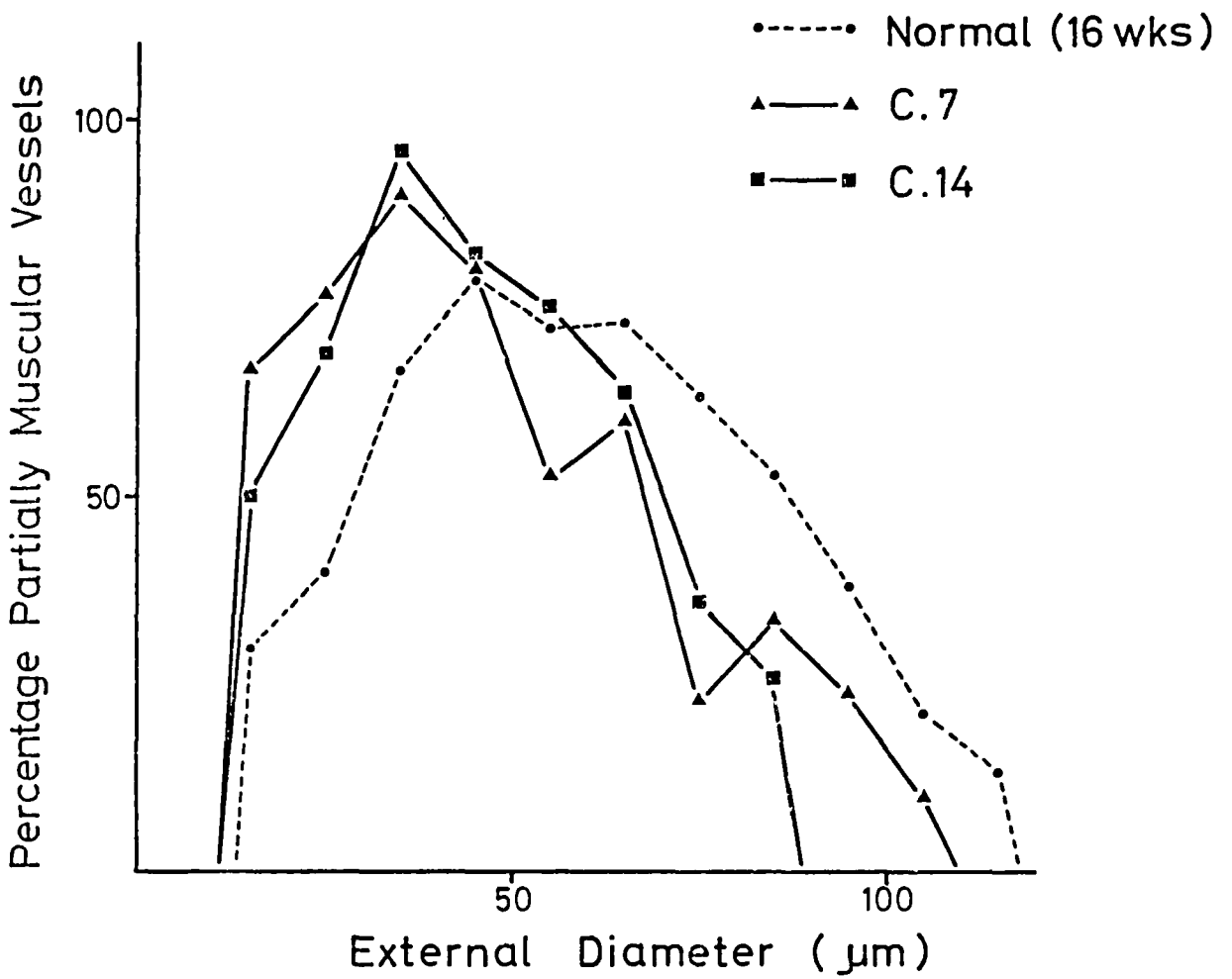
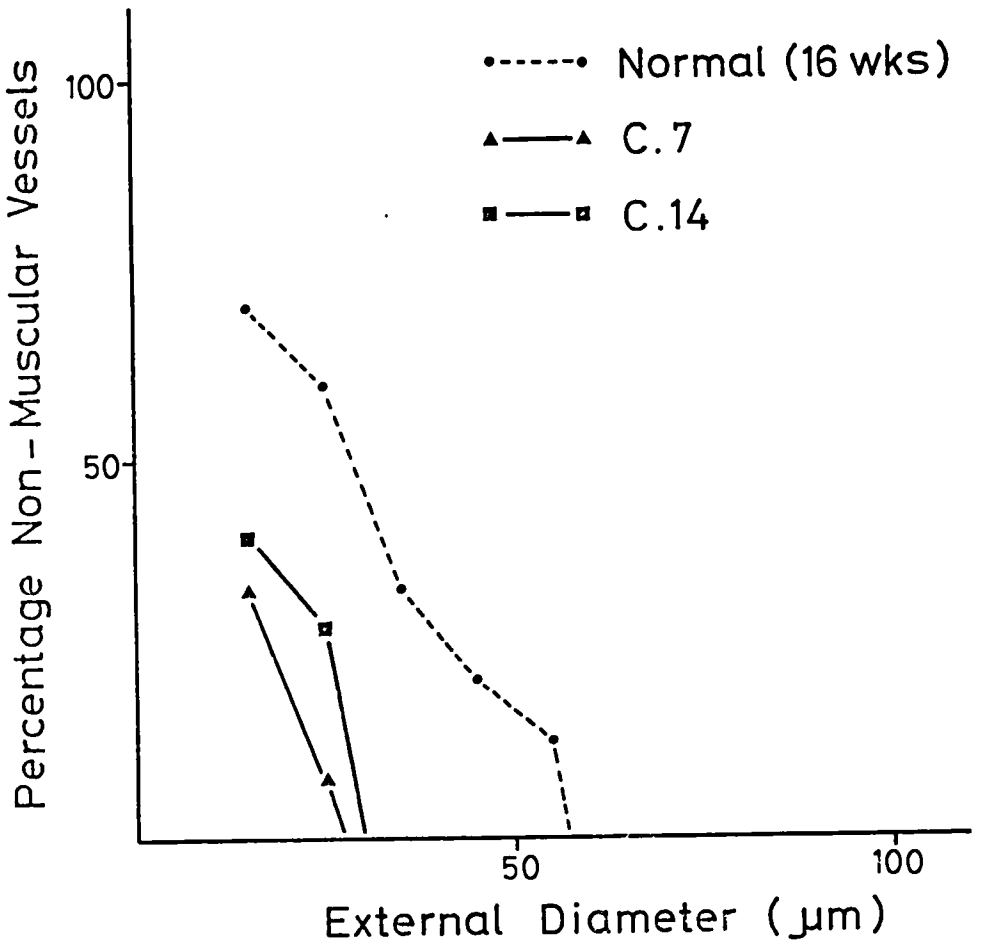

Normal (16 wks)
C.7
C.14
Percentage Partially Muscular Vessels
100
50
50
100
External Diameter (μm)

Fig. V 21

Diagram illustrating the changes in the non-muscular arterial population curve in the experimental cases. Notice a decrease in the percentage of the small non-muscular vessels when compared with the normal series


Normal (16 wks)
C.7
C.14
Percentage Non-Muscular Vessels
100
50
50
100
External Diameter (µm)

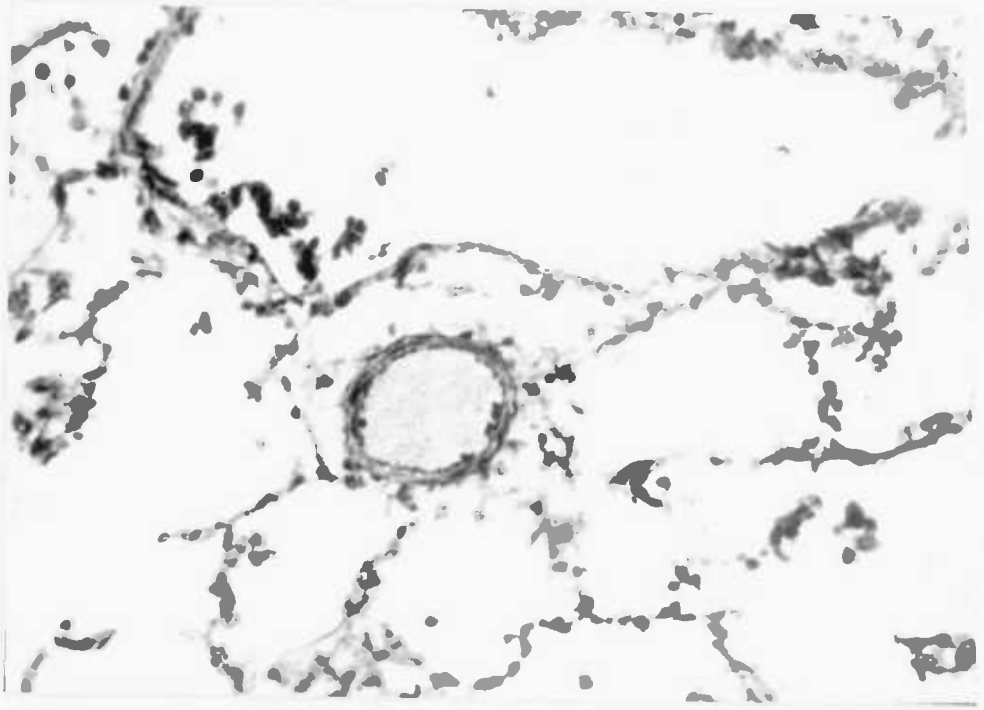
along the arterial pathway. Because the normal pig already had a considerable amount of muscle at alveolar duct level at the ages included in the present experimental series, it was difficult to find striking changes in any age group. The relevant finding was an increase in the percentage of muscular arteries both at alveolar ductal and respiratory bronchiolar level particularly after the first post-operative month (Fig. 22-23). The difference between the various age groups was not significant (Table V 32).

External diameter At the end of the first month of the follow-up period, the cases operated at four weeks, C. 2 and 3, showed a slight decrease in external diameter, when compared with age-matched normals and controls, where similar measurements in older experimental cases were within the normal and control range (Table V 33).

The age-related difference persisted and increased during the follow-up period as demonstrated by comparing cases C. 4 and 6 with C. 13, 14 and 15 followed for a similar period of time (Fig. V 24). Although there was a decrease in external diameter in both series at any level along the intra-acinar airway, the changes were always greater in the younger cases. No comparison was possible beyond the two months period, but the animals operated early in life showed a persistent abnormality in arterial size, particularly at alveolar duct level, where the vessels remained with the same external diameter, 41 µm ($\pm$ 1.4, SEM), at the eighth post-operative week, and 42 µm

Fig. V 22 and 23

Photomicrographs showing the existence of intra-acinar arteries with a complete muscular coat (x420 - Fig. V 22 and x680 - Fig. V 23)



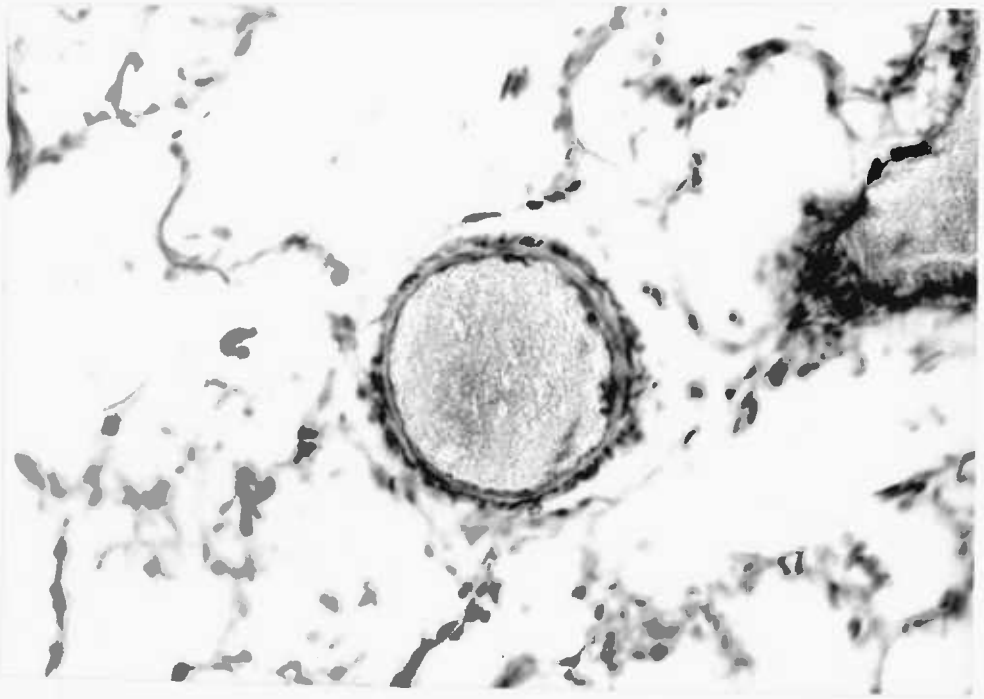


TABLE V 32

STRUCTURE OF ARTERIES ACCOMPANYING INTRA-ACINAR AIRWAYS IN THE EXPERIMENTAL CASES

Case No.	Term. Bronch.			Resp. Bronch.			Alveolar Duct		
	nm	pm	m	nm	pm	m	nm	pm	m
C.2	0	0	100	0	26	74	0	79	21
C.3	0	0	100	0	35	65	0	65	35
C.4	0	0	100	0	45	55	0	57	43
C.7	0	0	100	0	31	69	0	20	80
C.9	0	0	100	0	30	70	0	34	66
C.10	0	0	100	0	28	72	10	76	14
C.11	0	0	100	0	35	65	0	74	26
C.12	0	0	100	0	17	83	0	64	36
C.13	0	0	100	0	32	68	0	30	70
C.14	0	0	100	0	26	74	0	36	64
C.15	0	0	100	0	23	77	0	32	68
C.17	0	0	100	0	36	64	0	100	0
C.18	0	0	100	0	25	75	0	60	40
Controls									
C.1	0	0	100	0	91	9	30	70	0
C.5	0	0	100	0	79	21	0	89	11
C.8	0	0	100	0	50	50	0	87	19
C.16	0	0	100	0	51	49	0	87	13
Normals (Table III 11)									
8 weeks	0	0	100	0	35	65	12	76	12
8 "	0	0	100	0	46	54	24	67	9
12 "	0	0	100	0	48	52	0	80	20
16 "	0	0	100	0	45	55	0	88	12
16 "	0	0	100	0	65	35	0	90	10

nm - nom-muscular
pm - partially muscular
m - muscular

Term. Bronch. - terminal bronchiolus
Resp. Bronch. - respiratory bronchiolus

TABLE V 33

SIZE OF ARTERIES ACCOMPANYING INTRA-ACINAR AIRWAYS IN THE EXPERIMENTAL CASES

Case No.	External Diameter (μm)		
	Term. Bronch.	Resp. Bronch.	Alveolar Duct
C.2	91	54	39
	5.1	2.3	2.3
	29	33	24
C.3	100	68	44
	5.7	5.6	2.1
	100	33	29
C.4	87	54	40
	5.0	2.4	1.6
	44	51	40
C.6	88	52	42
	5.8	2.2	1.9
	35	42	40
C.7	87	65	40
	10.5	4.6	3.0
	14	22	18
C.9	104	64	45
	5.9	3.1	2.8
	34	36	29
C.10	107	73	54
	3.6	2.6	2.4
	43	50	48
C.11	112	75	45
	6.4	2.6	2.7
	27	43	31
C.12	106	70	50
	5.6	3.0	2.6
	30	30	32
C.13	114	79	61
	7.6	3.5	2.8
	20	22	20
C.14	105	72	53
	5.1	2.9	3.0
	23	34	31
C.15	126	69	57
	12.2	4.7	3.4
	17	30	22
C.17	122	87	60
	10.0	5.4	3.8
	20	25	20
C.18	123	83	57
	6.1	3.7	2.9
	26	35	26

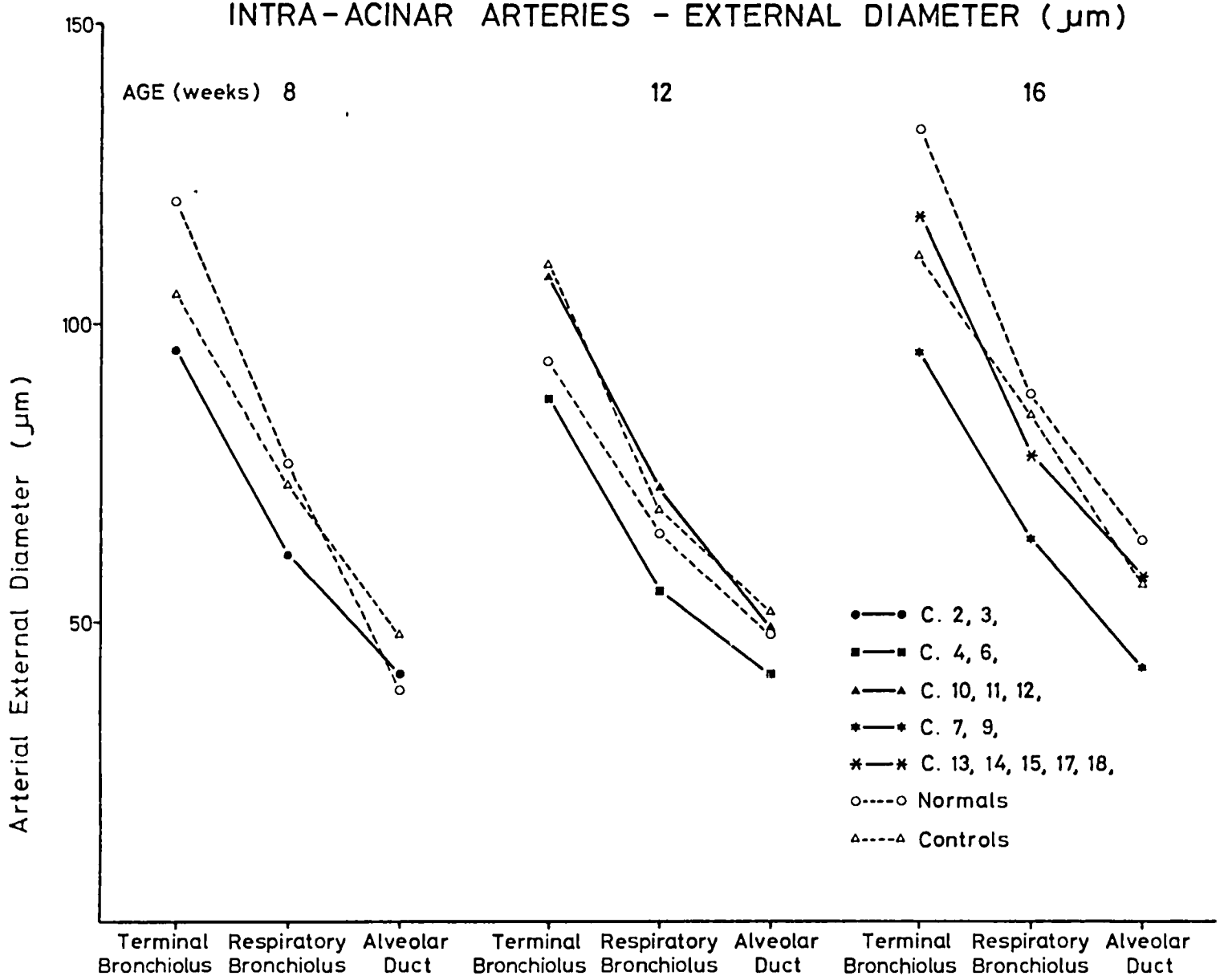
(Table to be continued in the next page)

Controls			
C.1	105	73	48
	4.3	2.4	2.0
	45	54	44
C.5	110	69	52
	6.8	2.5	2.0
	41	47	44
C.8	117	84	62
	5.6	2.8	2.8
	27	34	26
C.16	112	85	57
	5.0	2.9	1.9
	40	41	37
Normals (Table III 12)			
8 weeks	133	88	42
	9.9	4.5	3.3
	12	29	20
8 "	109	66	35
	5.5	2.9	1.9
	32	54	33
12 "	94	65	48
	5.2	3.4	6.8
	26	27	15
16 "	147	93	67
	12.8	7.0	2.9
	22	33	24
16 "	123	83	61
	6.9	3.7	2.4
	22	31	30

Mean, standard error of the mean and total number of vessels measured

Fig. V 24

Diagram illustrating the changes in size of the intra-acinar arteries in the experimental cases. Notice the decrease in size apparent by the second post-operative month, particularly in the animals operated early in life (C. 7 and C. 9)


INTRA-ACINAR ARTERIES - EXTERNAL DIAMETER (µm)
AGE (weeks) 8
12
16
150
100
50
Arterial External Diameter (µm)
C. 2, 3,
C. 4, 6,
C. 10, 11, 12,
C. 7, 9,
C. 13, 14, 15, 17, 18,
Normals
Controls
Terminal Bronchiolus
Respiratory Bronchiolus
Alveolar Duct
Terminal Bronchiolus
Respiratory Bronchiolus
Alveolar Duct
Terminal Bronchiolus
Respiratory Bronchiolus
Alveolar Duct

($\pm$ 3.5, SEM), at the twelfth week, compared with 50 µm ($\pm$ 2.83, SEM) and 62 µm ($\pm$ 4.1, SEM) respectively, in the age-matched normal and control series (Table V 33).

Number of arteries and alveoli per unit area of lung tissue

At the end of the first post-operative month, the alveolar number per unit area was considerably reduced particularly in the younger series, C. 2 and 3, in which it had a value of 7770-9885, compared with the normal value of 16336 (Table V 34). The control case C.1, also showed a lower alveolar number, 10484, when compared with the normal value and this suggested that the thoracotomy had some effect on the alveolar number particularly when performed early in life. It is also important to consider that alveolar volume, assessed by microscopic point counting, was slightly increased in the experimental cases, thus suggesting an increase in alveolar size during the early post-operative period.

These changes were also apparent in the older cases, although to a smaller degree; they also tended to be less striking with an increase in the duration of the follow-up period (Table V 34).

There was no definite pattern of response of intra-acinar arteries to the experimental aorto-pulmonary shunt, but the changes were small when compared with the normal and control values, thus suggesting no developmental impairment. The lowest number, 271, was found in case C. 15 operated at eight weeks of age and followed for two months, whereas the highest number, 540, occurred in case C. 2

TABLE V 34

ALVEOLAR AND INTRA-ACINAR ARTERIAL NUMBER PER UNIT AREA OF LUNG TISSUE IN THE EXPERIMENTAL CASES

Case No.	Number per Unit Area (cm^2)		
	Alveoli	Arteries	Ratio
C.2	7770 249	540 51	14.4
C.3	9885 124	539 33	18.3
C.4	7532 128	314 36	24.0
C.6	7934 150	475 51	16.7
C.7	6991 99	335 37	20.9
C.9	9723 164	331 87	29.4
C.10	7250 222	539 48	13.4
C.11	7655 120	380 37	20.1
C.12	7554 190	439 66	17.2
C.13	6355 186	347 44	18.3
C.14	5899 183	281 51	21.0
C.15	8901 235	278 30	23.5
C.17	6751 110	318 29	21.2
C.18	5558 95	328 37	16.9
Controls			
C.1	10484 135	598 95	17.5
C.5	8483 121	424 40	20.0
C.8	5916 230	376 44	15.7
C.16	6961 210	485 75	14.3
Normals (Table III 13)			
8 weeks	16336 238	695 33	23.5
12 "	10622 175	392 29	27.1
16 "	8164 230	450 66	18.1

Mean and standard error of the mean

operated at four weeks and sacrificed at the end of the first post-operative month (Table V 34).

The Pulmonary Venous Bed

The Venogram

A good filling of the pulmonary veins was obtained in all the experimental cases. No vascular communications were apparent either with the pulmonary arterial bed or the bronchial circulation.

The pre-acinar branching pattern and degree of background haze were similar to those found in the normal animals and did not change during the follow-up period (Fig. V 25-26).

Measurements of axial length and diameter

The length of the right lower lobe vein did not change significantly during the follow-up period when compared with the normal and control series (Table V 35).

Similarly, the size of the pre-acinar veins followed the normal developmental pattern and no significant change was found during the post-operative period (Table V 35).

The Peripheral Venous Bed

The wall structure of the peripheral veins did not differ from

Fig. V 25

Pulmonary venogram (right lung) of a case shunted at four weeks and sacrificed at twelve weeks of age (x0.8)

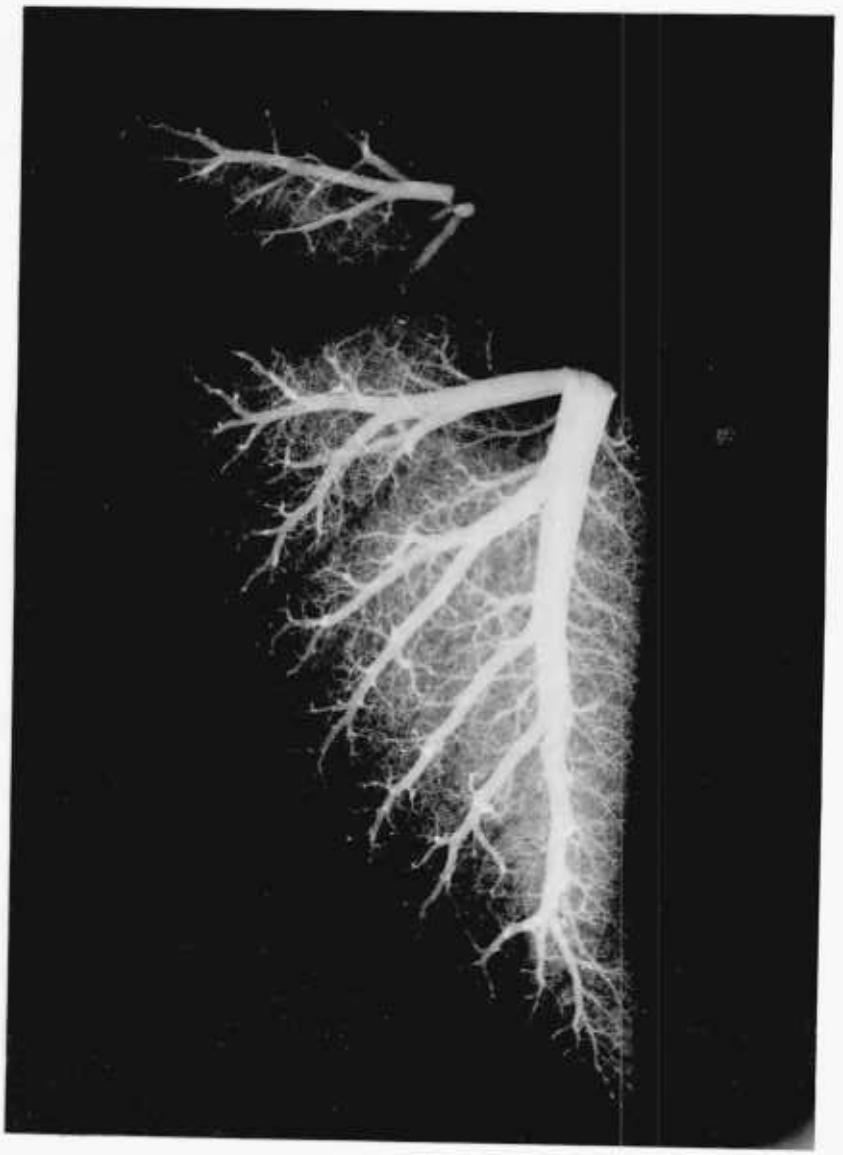
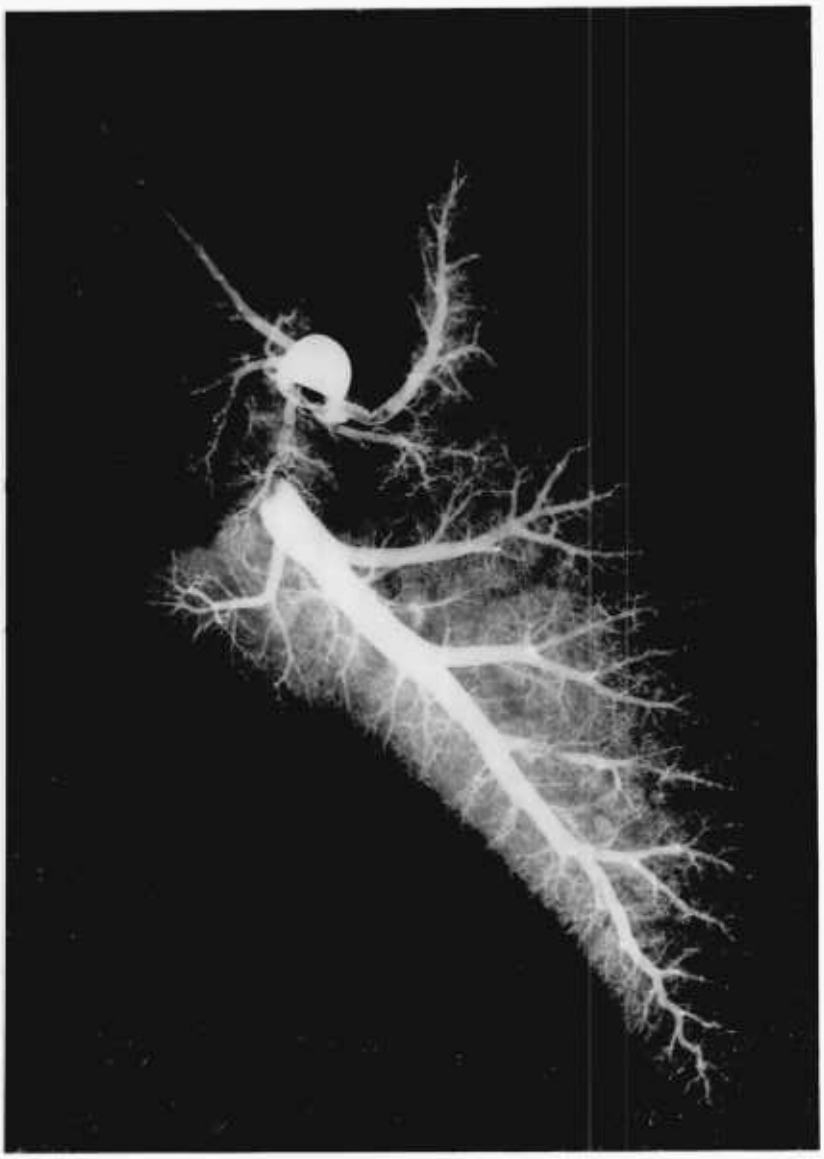


Fig. V 26

Pulmonary venogram (right lung) of a case shunted at eight weeks and sacrificed at sixteen weeks of age (x0.5)



MEASUREMENTS FROM THE VENOGRAMS IN THE EXPERIMENTAL CASES. LEFT LOWER LOBE VEIN - TOTAL AXIAL LENGTH, DIAMETER AT THE HILUM AND AT 25% INTERVALS ALONG THE PATHWAY

Case No.	Length (mm)	Diameter (mm) Hilum	25%	50%	75%
C.2	115	7.5	5.0	3.5	2.0
C.3	123	8.5	5.0	3.0	2.5
C.4	158	9.5	6.5	4.0	2.0
C.6	169	9.0	7.0	5.0	3.0
C.7	181	10.5	7.0	5.5	3.5
C.9	176	11.0	8.0	6.0	3.0
C.10	149	8.0	6.0	3.0	2.0
C.11	138	9.0	5.5	5.0	3.0
C.12	150	9.5	6.5	5.0	2.5
C.13	187	10.0	8.5	6.0	3.5
C.14	190	11.5	9.5	6.5	3.0
C.15	161	11.0	7.5	5.5	2.0
C.17	175	12.0	9.0	7.0	3.0
C.18	188	11.5	8.5	6.0	3.0
Controls					
C.1	120	8.0	5.0	3.0	2.0
C.5	169	9.5	6.0	5.0	3.0
C.8	183	11.0	5.5	4.5	2.5
C.16	194	11.5	7.0	5.5	2.5
Normals (Table III 14)					
8 weeks	125	8.0	5.5	3.5	2.0
12 "	163	8.5	7.0	5.0	3.0
16 "	171	11.0	8.0	6.0	3.0

Mean value for each case

that described for the normal cases. Like the arteries no intimal changes were found in any experimental case.

Percentage medial wall thickness

The medial wall thickness of the pulmonary veins was not affected by the aorto-pulmonary shunt. At any time of the follow-up period, the measurements were similar to the normal and control series. The range of percentage medial wall thickness in the experimental cases was 3.9-5.3 for normal veins up to 200 μm, compared with a range of 4.1-4.9 for the same size vessels in the normal and control series. Larger veins, between 200-1000 μm in external diameter, had a range between 0.9 and 2.4 in the experimental cases and between 1.0 and 2.5 in the normal and control series (Table V 36).

Ventricular Weights

Total ventricular weight was always increased in the experimental cases (Table V 37). Even the four animals with an occluded shunt showed a certain degree of increase in ventricular weight particularly at left ventricular level. This finding suggested that in the control series the shunts could have been patent for a certain period of time, long enough for the cardiac changes to occur. This was not the case, because catheter studies performed in the four controls and in some shunted cases, two weeks after surgery and not included in the present work, showed that

TABLE V 36

VEINS - PERCENTAGE WALL THICKNESS IN THE EXPERIMENTAL CASES

Case. No.	External Diameter (μm)								
	0-50	50-100	100-200	200-300	300-400	400-500	500-1000	0-200	200-1000
C.2	6.4 0.21 42	3.7 0.10 62	1.7 0.05 34	1.1 0.09 11	1.7 0.03 3	1.7 0.24 2	1.9 0.36 2	3.9 1.37	1.6 0.17
C.3	6.4 0.18 55	3.6 0.11 60	3.0 0.15 37	2.9 0.38 3	2.0 - 1	1.4 - 1	1.7 - 1	4.3 1.05	2.0 0.32
C.4	6.8 0.21 49	3.7 0.09 62	2.0 0.65 25	1.5 0.30 4	0.7 - 2	0.8 0.35 2	1.4 0.12 9	4.2 1.40	1.1 0.21
C.6	6.5 0.23 64	3.9 0.51 19	2.3 0.33 8	2.2 0.44 5	2.9 0.93 4	2.7 0.44 3	2.4 0.29 3	4.2 1.23	2.5 0.16
C.7	7.5 0.29 38	3.6 0.12 37	2.2 0.19 20	1.7 0.13 10	1.1 0.13 8	- - -	1.3 0.24 6	4.4 1.59	1.4 0.17
C.9	6.6 1.47 39	4.3 1.56 59	2.7 0.25 20	2.3 0.22 11	2.2 0.43 3	1.7 0.20 2	1.7 0.03 3	4.5 1.14	2.0 0.15
C.10	7.5 0.42 34	3.9 0.13 75	2.3 0.14 30	2.1 0.29 5	2.0 0.15 3	1.8 0.12 3	2.2 0.05 2	4.6 1.54	2.0 0.07
C.11	7.0 0.35 29	3.9 0.12 52	3.0 0.17 22	1.7 0.03 4	2.3 0.18 4	2.4 0.78 4	2.4 0.22 3	4.6 1.22	2.2 0.17
C.12	7.7 0.36 50	3.7 0.12 48	2.1 0.11 24	1.4 0.18 8	1.3 0.10 2	1.1 - 1	0.8 0.06	4.5 1.67	1.1 0.13

C.13	7.7	4.5	2.1	1.2	1.0	0.7	0.6	4.8	0.9
	0.30	0.68	0.14	0.10	0.10	0.21	0.24	1.62	0.13
	56	60	40	10	14	3	3		
C.14	7.2	3.6	2.4	1.3	1.3	-	1.1	4.4	1.2
	0.30	0.10	0.14	0.17	0.14	-	0.34	1.44	0.08
	49	45	26	14	2	-	4		
C.15	6.7	4.5	3.2	2.2	-	-	1.3	4.8	1.7
	0.20	0.26	0.27	0.33	-	-	0.07	1.02	0.45
	51	39	19	6	-	-	5		
C.17	7.7	4.5	3.6	2.4	2.1	2.8	1.8	5.3	2.3
	0.47	0.95	0.44	0.16	0.23	0.25	0.61	1.24	0.21
	35	41	52	9	3	4	4		
C.18	6.9	3.8	2.6	1.4	2.5	3.5	1.1	4.4	2.4
	0.19	0.11	0.18	0.19	0.50	-	0.24	1.28	0.55
	55	64	25	7	2	1	3		
Controls									
C.1	6.7	3.8	2.0	2.0	1.3	1.1	1.1	4.2	1.4
	0.28	0.09	0.09	0.12	0.07	0.12	0.09	1.37	0.21
	38	53	29	3	4	3	3		
C.5	7.3	4.2	2.4	2.5	-	1.8	1.6	4.6	2.0
	0.26	0.21	0.15	0.43	-	0.35	0.24	1.43	0.27
	54	40	21	6	-	2	2		
C.8	6.6	3.8	1.9	1.8	1.0	0.7	0.7	4.1	1.0
	0.19	0.10	0.08	0.72	0.22	0.13	0.14	1.37	0.26
	40	59	42	11	3	10	6		
C.16	7.1	4.1	2.3	3.2	1.5	2.2	1.1	4.5	2.0
	0.27	0.24	0.14	1.08	0.12	-	0.17	1.40	0.46
	42	31	35	15	4	1	3		
Normals (see Table III 15)									

Mean, standard error of the mean and total number of vessels measured

occlusion or patency of the graft was already apparent at early stage of the follow-up period.

Comparison between the two age groups, that were followed for four months, C. 2, 3, 4 and 6 and C. 10, 11, 12, 13, 14 and 15 showed that, initially, there was a rapid increase in left ventricular and septal weight (LVW+SW/RVW) not accompanied by a similar increase in right ventricular weight (RVW) probably reflecting the primary response of the systemic ventricle to the left-to-right shunt. This was demonstrated by the fact that the LVW+SW/RVW showed higher values than normal particularly in the older animals (Table V 37).

The decrease in the LVW+SW/RVW found in the two cases operated early in life and followed for three months, C. 7 and 9, suggested that a certain degree of right ventricular hypertrophy occurred, as the pulmonary arterial bed became structurally affected, particularly with an increase in wall thickness and a decrease in vascular size at intra-acinar level.

TABLE V 37

VENTRICULAR WEIGHTS IN THE EXPERIMENTAL CASES

Case No.	Ventricular Weights				
	RV+LV+S (g)	RV+LV+S/BW (g/100g)	RV (g)	LV+S (g)	LV+S/RV (g)
C.2	92588	0.42	20705	71883	3472
C.3	80600	0.34	16460	64140	3897
C.4	124913	0.48	36080	108832	3016
C.6	126298	0.39	29611	96687	3265
C.7	161120	0.36	37816	113304	2996
C.9	162546	0.36	41688	120858	2899
C.10	133209	0.37	22763	110446	4852
C.11	81530	0.33	14707	66823	4544
C.12	130844	0.36	31684	99160	3130
C.13	140801	0.26	34801	106000	3046
C.14	149965	0.29	33155	116810	3523
C.15	174869	0.39	30026	144843	4824
C.17	132324	0.33	30724	101600	3307
C.18	125555	0.33	27578	97977	3553
Controls					
C.1	67588	0.36	15705	51883	3303
C.5	98640	0.30	20687	77953	3768
C.8	115720	0.29	21916	83804	3824
C.16	106519	0.20	20068	86451	4308
Normals (Table III 16)					
8 weeks	50692	0.26	10841	39851	3676
"	58458	0.27	12033	46425	3858
12 "	64390	0.17	15550	48840	3141
16 "	85818	0.15	19760	66058	3343

RV - right ventricle
LV - left ventricle
S - septum
BW - body weight

SUMMARY OF RESULTS

1. Eighteen animals were included in the present study. They were operated between four and twelve weeks of life and the duration of the follow-up period ranged between four and eleven weeks. Each case was studied functionally as follows: (i) at surgery, pulmonary haemodynamics were investigated before and after the creation of the aorto-pulmonary shunt; (ii) serial lung function studies were performed during the follow-up period; and (iii) immediately before the sacrifice, pulmonary haemodynamics were assessed by cardiac catheterization and in some cases in animals with the chest open; the structural studies were similar to those described for the normal cases.

2. Four of the eighteen cases were found to have an occluded graft. They were used as controls.

FUNCTIONAL STUDIES

Pulmonary Haemodynamics

Immediately after establishing the shunt

3. The heart rate was increased particularly in the animals operated early in life. There was a decrease in the systemic arterial pressure in the shunted animals and the value was significantly lower compared with the cardiac catheterization

and the pre-shunt measurements ($P<0.001$). The diastolic pulmonary arterial pressure was significantly increased when compared both with the cardiac catheterization and the pre-shunt measurements ($P<0.001$).

4. The blood flow throught the shunt was continuous during the cardiac cycle and the wave contour was pulsatile with the peak coinciding with the systolic components of both aortic and pulmonary pressures. The flow in the main pulmonary artery was increased after the creation of the shunt and the wave contour changed considerably because zero flow was not reached in diastole and so the usual normal end-systolic pattern of reversed flow was not present after the creation of the shunt.

Cardiac catheterization

5. The heart rate in the experimental series was not significantly different when compared with the normal value at cardiac catheterization. The younger animals however, tended to show a faster heart rate than the animals operated later in life. The systemic arterial pressure was similar in both experimental, normal and control series. The right ventricular systolic pressure was significantly increased in the shunted cases when compared to the control and normal animals ($P<0.001$). The mean pulmonary arterial pressure was also significantly increased in the experimental animals ($P<0.001$). Comparison between the two

age groups showed that the younger cases had higher mean and diastolic pressures and that the differences were significant ($0.05 > P > 0.001$). The pulmonary wedge pressure was not changed after the creation of the aorto-pulmonary shunt. The pulmonary-to-systemic resistance ratio was increased in the shunted animals, when compared with the normal and control series and this change was highly significant ($P > 0.001$); the age-related differences in the experimental cases were also significant ($0.05 > P > 0.01$).

Final study. Open chest measurements

6. The final assessment of the pulmonary blood flow showed that the wave contour remained similar to that described immediately after the creation of the anastomosis with a diastolic flow above zero and no reverse wave at the end of the systole.

Lung Function

7. Respiratory frequency did not change significantly during the follow-up period in any of the experimental cases.

8. Tidal volume increased during growth in the shunted animals, although the cases operated early in life tended to show lower values particularly during the first two months of the follow-up period; this difference was highly significant ($P > 0.001$). This feature was also found in the control cases operated early in life.

9. Dynamic compliance was decreased in the experimental animals of all ages particularly after the first post-operative month. The changes were highly significant in the animals operated early in life ($P<0.001$).

10. The changes in thoracic gas volume during growth in the experimental cases were not significantly different from those found in the normal and control series.

11. Specific compliance was reduced in the experimental cases after the first post-operative month, but unlike dynamic compliance, there were no highly significant age-related differences in the experimental cases ($P<0.05$).

STRUCTURAL STUDIES

12. Lung volume was similar in the shunted, control and normal series.

13. Lung length was highly significantly reduced in the animals operated early in life at the end of the first post-operative month ($0.01>P>0.001$). After that age and in the older cases the differences were not highly significant when compared to the normal and control series.

14. The volume proportions of the various components of lung tissue, assessed both by macroscopic and microscopic point counting did not change considerably at any stage of the follow-up period.

The Pulmonary Arterial Bed

15. The wall thickness of the left pulmonary artery was increased as early as the end of the first post-operative month, the highest values from the whole experimental series were found in cases C. 7 and 9 operated early in life and with the longest follow-up period. These cases also showed the highest number of elastic fibers within the medial wall. Although the older cases also showed an increase in wall thickness of the left pulmonary artery, the changes were less striking than in the younger animals. Intimal proliferation was seen in some regions of the arterial wall, but no defined pattern of intimal thickening could be found in the experimental cases.

16. In the arteriogram a considerable dilatation of the pre-acinar arteries was already apparent at the end of the first operative month in animals operated at twelve weeks of age, whereas in this respect the younger cases did not differ from the normal and control series. Although a certain degree of dilatation of the pre-acinar arteries was apparent in the animals with the longer follow-up period, the changes were only evident in the animals that were operated late in life. In all the experimental cases, there was an increase in the tortuosity of the peripheral pulmonary vessels particularly in animals with the longest follow-up period. The degree of background haze in the shunted animals was not different from that found in the normal and control series, thus suggesting that there was no impairement in the multiplication of the intra-acinar arteries.

17. The percentage medial wall thickness was not significantly increased in any of the experimental cases at the end of the first post-operative month. Two months after surgery, both the younger and the older cases showed a considerable increase in arterial wall thickness in muscular vessels of all sizes. The highest values being found in the animals operated early in life, when compared with the normal and control series ($P<0.001$). The older cases showed a similar and highly significant increase in the vessels between 0-200 μm in external diameter whereas the larger vessels between 200-1000 μm tended to have smaller change when compared with the normal and control series. The two cases that were followed for three months showed the highest values in all the experimental cases (7.4-7.7 for arteries up to 200 μm in diameter and 5.4-6.1 for arteries between 200-1000 μm). There was no evidence of intimal thickening in the shunted animals.

18. Throughout growth the normal pig already showed a considerable number of muscular arteries at the periphery of the pathway. This made the comparison between the population studies in both experimental, normal and control series difficult because the extension of muscle along the peripheral arterial pathway could only be assessed in vessels smaller than 100 μm in external diameter, where the three populations of muscular, partially muscular and non-muscular vessels are mixed. There was an extension of muscle along the peripheral arterial pathway that could be found at the end of the first post-

operative month particularly in the animals operated early in life. When the various age groups were compared with one another the changes were not significant, particularly after the first post-operative month and in all cases muscle was found in the small peripheral arteries in the range between 10-20 μm in external diameter.

19. The extension of muscle along the arterial pathway was assessed also by studying the structure of the intra-acinar arteries landmarked by the accompanying airways. The shunted cases showed an increase in the percentage of muscular arteries both at alveolar duct and at respiratory bronchiolar level and this change was striking after the first post-operative month. As in the population studies, there were no age-related differences.

20. The size of the intra-acinar arteries was smaller than normal in the animals operated early in life, and this change became more evident the longer the duration of the follow-up period. Animals shunted at eight weeks of life also showed some decrease in arterial size at intra-acinar level by the end of the second post-operative month, but the changes were less evident than in the younger series.

21. The number of alveoli per unit area of lung tissue was reduced in most of the experimental cases, when compared with the normal series; these changes were more evident in the animals operated early in life. No reduction in the arterial number

was evident in any of the experimental cases, thus suggesting as it already had been predicted from the assessment of the degree of background haze from the arteriogram, that there was no decrease in the number of intra-acinar arteries in the experimental animals.

The Pulmonary Venous Bed

22. There were no changes in pulmonary venous branching pattern, length and pre-acinar size in the experimental cases.

23. The structure and the wall thickness of the pulmonary veins were not affected by the creation of the aorto-pulmonary anastomosis and the changes from both the normal and control series were not significant even in the animals with the longest follow-up period.

Ventricular Weights

24. At the end of the first month of the follow-up period, the shunted cases showed an increase in the weight of both ventricles. This increase was greater in the left ventricle than in the right one, particularly in the animals operated late in life, as shown by the increase in the ratio LVW+SW/RVW, which was higher than that found in normal and control animals of the same age. As the follow-up period increased, the ratio decreased particularly in the animals operated early in life, suggesting that some degree of right ventricular hypertrophy was occurring

as the pulmonary arterial bed became more structurally affected, with an increase in wall thickness in vessels of all sizes and a decrease in size of the intra-acinar arteries.

CHAPTER VI

DISCUSSION

The present study is particularly concerned with the postnatal development of the lung.

Knowledge on the pulmonary circulation has greatly benefited from the structural assessment of the vascular bed using quantitative techniques. The early work by Elliott (1964) on the branching pattern and structure of the normal human lung, was applied to the child lung by Davies (1969) and further developed by Hislop (1971), who studied the branching pattern and structure of the pulmonary vascular bed in both fetus and children throughout development. She and others also used the same quantitative techniques to assess the changes in the pulmonary circulation in cases of Tetralogy of Fallot early in childhood and in cases of ventricular septal defects in infancy (Hislop, 1971) (Hislop, Haworth, Shinebourne and Reid, 1975). The structural studies of the pulmonary circulation in cases of congenital heart disease were extended by Haworth in 1975, who studied cases of hypoplastic left heart syndrome, pulmonary atresia and total abnormalous pulmonary venous return in early infancy.

The studies on the normal human lung during pre and postnatal life were summarized in the Laws of Human Lung Development (Hislop and Reid, 1974 b), which related the prenatal development of the airways with that of the pre-acinar arteries and demonstrated that the development of the intra-acinar vessels occurred mainly after birth, accompanying the development of the intra-acinar air spaces.

Studies of the pulmonary circulation in congenital heart

disease have further shown that lung growth and development can be impaired by changes in blood flow and pressure and that the use of the present quantitative techniques, which assess not only vascular structure, but also branching pattern, size and number of branches from the pathway, allow for a better comparison between the structural and the functional findings (Hislop, 1971)(Haworth, 1975)(Hislop, Haworth, Shinebourne and Reid, 1975).

Although it has been suggested that animal studies of the pulmonary circulation relevant to congenital heart disease (Hawe, Tsakiris, Rastelli, Titus and McGoon, 1972) should use growing rather than adult animals, no attempt has been made to compare human and animal pulmonary vascular development.

The purpose of the present work was to describe the significant features of the structural development of the pig pulmonary circulation and to compare it with the human, using similar techniques. Some aspects of postnatal pulmonary physiology were also assessed during growth, in an attempt to relate the normal structural and functional findings.

Furthermore, aorto-pulmonary shunts were performed in growing swine, in an attempt to study the effects of an increase in pulmonary blood flow and pressure on the immature lung.

EVALUATION OF THE TECHNIQUES USED IN THE PRESENT WORK

Structural Studies

Vascular Injection

In our department a standardized pressure of 100 cm of water has been used in the injection of both pulmonary arterial and venous beds. The method has been continously used in the study of the human lung, in the normal adult (Elliott, 1964) in the fetus and throughout childhood (Davies, 1969)(Hislop, 1971) and in the old age group (Semmens, 1970)(Semmens, 1971). It has also been used in the study of the pulmonary circulation in congenital heart disease (Hislop, 1971)(Hislop, Haworth, Shinebourne and Reid, 1975) and (Haworth, 1975). The technique has been applied to the study of the pulmonary vasculature in the normal adult rat (Hislop and Reid, 1976).

Post-mortem pulmonary injection pressures higher than the normal physiological values, had already been advocated by Short (1956), who used pressures ranging between 50-100 mm Hg, by Doyle, Goodwin, Harrison and Steiner (1957), who used a pressure of 80 mm Hg or the patients pulmonary arterial pressure if it was known to be higher than that value. Wagenvoort, Heath and Edwards (1964) submitted that the use of hypertensive pressures to inject the pulmonary arterial bed would cause damage to the arterial wall and bursting of the contrast media to the surrounding parenchyma.

This suggestion was not confirmed in our department, where the continuous use of this injection technique confirmed the early

findings by Elliott (1964) that a high injection pressure caused the full distension of the vascular bed, assessed by the fact that elastic laminae were smooth in every case, and that vessels down to 15 μm in external diameter were filled with contrast. The technique is reproduceable and even in cases of pulmonary atresia, where the vessels were abnormally thin (Haworth, 1975), no rupture of the arterial wall was found. The use of a standardized technique, as in the present case, also allowed for a more accurate comparison between various cases, either normal or hypertensive.

The two important draw-backs found during the use of this method were: (i) the fine histological detail was lost due to the use of a high temperature (60°C) (Sobin and Rosenquist, 1973); and (ii) because the contrast media does not fill the capillary bed, that vascular area was not assessed quantitatively. Some of these problems were recently dealt by Meyrick (1976), who studied the pulmonary vascular bed in the normal adult rat using both light and electron microscope techniques. She used a method of simultaneous injection with glutaraldehyde by way of the trachea and of the pulmonary artery, tying the pulmonary veins prior to the fixation; using this method it was found that the glutaraldehyde did not penetrate beyond the capillaries and that the pre-capillaries veins could be distinguished from their corresponding arteries, because they were found to have red blood cells within their lumen; the technique used also an injection pressure of 100 cm of water. Although it is doubtful whether this technique could have been applied to larger lungs for electron microscopic studies, it should be

considered as a correct method to study the three vascular compartments within the lung using vascular injection.

Fixation of the Lung

In our department, the lungs have always been fixed using a standardized pressure of 45.5 cm of water to inflate the airways. This value is higher than that recommended by the Committee on Emphysema set up by the Aspen Conference (1951) and by the Panel on Pathology of the Medical Research Council Committee on Research into Chronic Bronchitis (April, 1972,1975), who have suggested a pressure ranging between 20-25 cm H_2O, but in any of the previous cases, as in fact during the present study, no alveolar or pleural rupture was seen to occur.

Post-Mortem Pulmonary Angiography

Both in the human and in the animal lung, the use of post-mortem pulmonary angiography can give important information for the study of the vascular bed either in the isolated lung or compared with studies performed in vivo. It was possible to assess the pre-acinar branching pattern and also to quantify some of these features by measuring the arterial axial length and diameter from the hilum to the periphery. The degree of background haze seen at the lung periphery on the arteriograms has been used as an useful means of assessing the number of the small intra-acinar arteries, which may be reduced in cases of pulmonary hypertension both in the adult human lung (Anderson, Simon and Reid, 1973), in cases of

congenital heart disease (Hislop, 1971) and in experimental animals (Hislop and Reid, 1974 c).

Histological Assessment of the Pulmonary Vasculature

Wall structure

Parenchymal collapse has been shown to affect measurements of arterial wall thickness (Best and Heath, 1961). On the other hand, Meyrick (1976) reported that the arterial injection with gluteraldehyde, at a pressure of 100 cm H_2O, caused some distension of the vascular bed, but because the medium is more fluid than the Micropaque - gelatin mixtue - the vessels showed a percentage arterial wall thickness twice that of those injected with Micropaque.

This finding reinforced the idea, that the quantitative evaluation of medial wall thickness, particularly in cases associated with pulmonary hypertension, where various degrees of increased muscularity were present, should be made using a standardized injection technique, which by assuring a complete distension of the vascular wall allows a better comparison between the specimens.

The finding that both in the human and in the rat lung, at the periphery of any arterial pathway, there was a mixed population of muscular, partially muscular and non-muscular arteries (Elliott, 1964) (Davies, 1969) (Hislop, 1971) (Meyrick, 1976) was also apparent in the pig. This was confirmed in the present study, not only by the assessment of the arterial structure in each individual vessel, but

also by the use of serial sections in two cases, one newborn and one aged eight weeks, which were not included in the results section.

Size

The fact that the intra-acinar arteries in the pig lung could be landmarked by their accompanying airways, as already shown in the human and in the rat (Elliott, 1964) (Davies, 1969) (Hislop, 1971) (Meyrick, 1976), allowed for a better study of arterial wall structure and size along the intra-acinar arterial pathway particularly because changes in structure have been shown to occur during normal growth in the human lung and also to be considerably affected in cases of congenital heart disease (Haworth, 1975).

Number

The assessment of the number of intra-acinar arteries per unit area of lung tissue has been performed in the human lung and shown that arterial multiplication occurred throughout childhood (Davies, 1969) (Hislop, 1971). The human work has further shown that in certain clinical conditions such as emphysema of childhood (Hislop and Reid, 1970) (Hislop and Reid, 1971) (Henderson, Hislop and Reid, 1971) and as congenital diaphragmatic hernia (Kitagawa, Hislop, Boyden and Reid, 1971), arterial branching pattern and multiplication could be considerably impaired, whereas in congenital heart disease arterial development could either be affected or progress normally (Hislop, Haworth, Shinebourne and Reid, 1975) (Haworth, 1975).

Functional Studies

Anaesthetic Protocol

It is becoming more evident, that animal studies of cardio-respiratory function, relevant to human disease, should be performed in animals awake and unsedated (Vatner and Braunwald, 1975). When this is not possible, as in the present case, care should be taken to use an anaesthetic protocol that causes the smallest degree of cardio-respiratory depression, and that at the same time allows easy manipulation of the animal throughout the experiment.

The pig is a difficult animal to handle and restrain in the laboratory. This became more important, when as in the present study, attempts were made to use a common protocol to animals ranging in weight between 10 and 50 Kg, throughout the closed and open chest studies and during surgery.

The initial technique which included induction by means of halothane, using a close fitting mask, and endo-tracheal intubation after the intra-venous injection of thiopentonesodium and maintenance using the same drug, proved unsucessful. Endo-tracheal intubation was only performed under respiratory arrest, and in some cases even under cardio-respiratory arrest; when the animals were properly ressuscitated and the experiment was continued, a permanent abnormality of gas exchange was present, as assessed by an increase in P CO_2 and a decrease in the pH in the arterial blood.

The use of neuroleptanalgesia in swine was advocated by

Piermattei and Swan in 1960. This technique proved to be successful in experimental cardiac surgery on swine. Although more recently reports by Becker, Lord and Dobell (1972) and by Ragan and Gillis (1975) suggested that barbiturates particularly from the ultra-short acting group, could be used successfully for various surgical procedures in swine, it was decided to attempt the use of neuroleptanalgesia in the present work.

Neuroleptanalgesia is based on the use of a tranquilizer and a narcotic drug administered intravenously (Fox, Fox and Crandell, 1967). Droperidol, a butyrophenone is the neuroleptic component; it produces an α-adrenegic blockade which causes peripheral vaso-dilatation and mild hypotension. Fentanyl citrate, a piperidine derivative, is an extremely potent analgesic with minimum side effects. The mixture is known to produce minimal effects on myocardial contractility and a certain degree of respiratory depression (Prys-Roberts and Kelman, 1967) (Dixon, Nolan, Stewart and Morrow, 1970) (Graves, Downs and Browne, 1975).

In the present study, it was found that the intramuscular administration of fentanyl and droperidol as premedication allowed for a successful induction of anaesthesia using low doses of halothane and an easy endo-tracheal intubation under spontaneous breathing. The lung function measurements, the quickest of all the procedures, were performed under halothane anaesthesia, whereas the cardio-catheterization studies and the surgical procedures, were performed using intravenous administration of fentanyl and droperidol in normal saline. Using this method, no abnormalities in gas exchange

occurred throughout the experiments, as assessed by the measurements of arterial blood gas tensions and pH. Furthermore, and in the case of the surgical experiments, the fact that the myocardial contractility was preserved must have played an important role in the capacity of the left ventricle to respond to the acute increase in blood volume, loading occurring immediately after the left-to-right shunt was established.

Lung Function Studies

Measurements of dynamic compliance have been performed in normal humans of all ages, in cases of cardio-respiratory diseases and in various experimental conditions.

The technique has recently standardized in a publication by the Division of Lung Diseases, National Heart and Lung Institute (Macklem, 1974). Similar measurements have also been performed in several laboratory animals (Crosfill and Widdicombe, 1961) (Spell, 1969) particularly in the dog (Gillespie, Lai and Hyatt, 1973).

The problems related with the use of esophageal balloons in healthy subjects are well known: compression by the mediastinum and diaphragmatic structures, intrinsic motility of the oesophagus, changes in the surface acting forces in the alveoli and a reduction in the number of open lung units caused by atelectasis or air trapping (Mead and Gaensler, 1952). Additional errors caused by disease can occur particularly in situations associated with an increasing thoracic blood volume causing an engorgment of the

vessels in and around the esophagus, which makes it less compliant and affect the measurements of oesophageal pressure. These artefacts cannot be ruled out and must be added in the case of the present work to the effects caused by anaesthesia, although the animals were lightly sedated, maintained under spontaneous breathing and submitted to artificial sighs (Mean and Collier, 1958). Nevertheless, when all these factors are taken into account, dynamic compliance must still be considered as a simple and reproduceable technique to assess the overall mechanical behaviour of the lungs (Gibson and Pride, 1976).

The measurement of thoracic gas volume using a body plethysmograph is also well documented in the literature and recent attempts have also been made to standardize the procedure (Leith and Mead, 1974). It is important to emphasize that the present technique measures the volume of gas inside the thorax, whether in free communication with the airways or not, contrary to functional residual capacity, which is the volume of gas that is in communication with the airways at the end of the normal expiration (Auld, 1975). While this difference is not relevant in normal cases, it can be significant in cases of air trapping in which the thoracic gas volume values are higher than those of the functional residual capacity.

Haemodynamic Studies

Right sided heart catheterization was uneventful both with the micro-floating catheter (Cardiovascular Instruments, Inc.) and with the flow-directed balloon-tip catheter, Swan Ganz (Edwards Laboratories).

In some cases, ventricular tachycardia occurred while the catheter passed through the pulmonary valve but it reverted to sinus rythm when the catheter was positioned in the main pulmonary artery never requiring the administration of drugs.

The measurement of the cardiac output using the thermaldilution technique, introduced by Fegler(1954) in the anaesthetized animal, and validated in the human by Branthwaite and Bradley (1968) is a well established technique, both on clinical and experimental grounds. The use of the thermaldilution curves to study circulatory shunts had already been suggested by Paul, Rudolph and Rappaport (1958) and by Cooper, Braunwald, Riggle and Morrow (1960), who validated the method by comparing it with simultaneous dye-dilution curves. In the present study it was possible to compare the thermaldilution curves with the quantitative assessment of the shunt using the blood oxygen contents.

According to Mills (1972), the sine wave flowmeter is at present the best device used in blood flow studies. It proved effective in the present work, particularly when the pulsatile pressure and flow waves were recorded simultaneously thus keeping a better overall picture of the vascular bed. Rudolph, Scarpelli, Golinko and Gootman (1964) performed aorto-pulmonary shunts in dogs and measured both the shunt flow and the pulmonary blood flow using a sine wave electromagnetic flowmeter with satisfactory results.

The only problem found in the present work regarding the blood flow measurements was the zero offset, which is defined as the difference

between the output from the flowmeter, when the magnet is not energized and that when the magnet is energized, but the flow through the probe is zero (Mills, 1972). The proper choice of the cuff size as related to vessel size and in some cases the use of a swab soaked in saline surrounding the probe, while the measurements were performed, assured a better contact between the electrodes and the conducting vessel wall and gave a satisfactory zero flow stability throughout the measurements.

POSTNATAL DEVELOPMENT OF THE PULMONARY VASCULAR BED

Structural Studies

In the pig, during the first weeks of life, the rate of increase in lung volume is faster than body weight; later it slows down. Throughout growth, the rate of increase in lung length is slower than that of body weight. The changes in body weight with age, reported in the present study, are in agreement with those described by other workers in the pig during the same period of life (Mount, 1968) (Mount and Ingram, 1971).

Both the main and the left pulmonary arteries remain elastic throughout growth and in the adult animal. Although the thickness of the media decreases during the first weeks of life, fragmentation of the elastic fibers is only evident at twelve weeks of age, whereas the adult pattern, with a considerable thickness of muscle cells in

the media is apparent only by the twentieth week of age.

The number of pre-acinar conventional arteries did not change during growth. This finding confirms the studies performed by Flint in 1906, who suggested, based on bronchial vascular casts of fetal pig lungs at various ages, that the adult macroscopic bronchial and arterial branching patterns were already established between the fourteenth and the fifteenth week of gestation, that is about two weeks before term, when the fetus is around 220 mm in length (Book and Bustad, 1974).

The rate of increase in length and lumen diameter of the pre-acinar arteries is faster during the first two weeks of life and similar at any point along the axial length. The rate of increase in external diameter of the intra-acinar arteries, particularly in the more proximal ones, (e.g. those accompanying terminal bronchioli), is also faster during the early postnatal weeks. During this period arteries increase in number within the acinus and it is possible that this non-uniform change in lumen diameter along the vascular bed reflects the fact that whereas pre-acinar arteries can only increase their capacity to accomodate progressively larger volumes of blood by increasing in size, at intra-acinar level, this is achieved not only by an increasing size, but also by an increase in arterial number.

The structure of the pre-acinar arteries, whether elastic or muscular, is already established at birth, not only along the axial

pathway, but also along the lateral conventional branches.

At the lung periphery, immediately after birth, the wall thickness of the muscular pulmonary arteries falls considerably. By the third day of life, the vessels smaller than 200 μm in external dimater have already reached the adult values. In larger muscular arteries, between 200-1000 μm in external diameter, this occurs later in life, around the eighth week. Our results contradict the recent suggestion that in the pig, there is no immediate postnatal decrease in wall thickness (Friedli, Kent and Kidd, 1975), but their work does not include any animals younger than one month. Between three days and five weeks of life, there is an apparent lag in the development of muscle at the periphery of the arterial pathway. This coincides both with the period of rapid multiplication and that of faster increase in arterial size. During this phase, muscle is not seen in the small peripheral arteries, probably because muscular development does not keep pace with the increase in arterial size and number.

The pig has a considerable number of muscular intra-acinar arteries at birth. During postnatal growth, muscle extends along the intra-acinar arterial pathway and by the fourth month of life, muscular arteries are already found in the alveolar walls.

The venous branching pattern follows closely the arterial one. Venous wall thickness does not change significantly throughout growth, the values being similar to those found in the adult animal.

The adult pattern of left ventricular dominance is established

in the pig by the first month of life. Our results are very similar to those reported by Lee, Taylor and Downing (1975), their LVW+SW/RVW ratio being 2.646 for pigs aged between two and fourteen days (our ratio - 2.167) and 3.188 for adult animals (our ratio - 3.088).

Comparison with the Human and other Species

The pig is characterized by a uniform and well marked pattern of lung septation, apparent at any age. In this respect, it differs from the human lung, in which at any age, the connective tissue septa are irregularly distributed, being more numerous at the sharp edges of the lung and along the costal-vertebral margins (Reid and Rubino, 1959)(Reid, 1959).

In the human lung, the rate of increase both in lung volume and length with age is much slower than in the pig (Hislop and Reid, 1973 c)(Hislop and Reid, 1974 b), although the growing rat shows a pattern of change in lung volume with age similar to the one we have described in the pig (Burri, Dbaly and Weibel, 1974)(Thurlbeck, 1975).

The structure of the pulmonary artery and its main branches is well documented in the human throughout growth. Heath, DuShane, Wood and Edwards (1959) suggested that the adult structure is reached by the end of the second year of life with fragmentation of the elastic fibers and disappearance of their orderly circular arrangment in the media. Saldaña and Arias-Stella (1963) described a longer transitional period and admitted that although the adult pattern could be present in some cases by the end of the first year of life,

it could take up to nine years before the adult pattern was reached in some normal specimens. Farrar, Blomfield and Reye (1965) analysed the human main pulmonary arteries both histologically and chemically paying particular attention to the amount and distribution of elastin and collagen, and demonstrated that both premature and infant had a high proportion of soluble elastin which decreased with age. After the first year of life, there was a rapid decrease in elastin accompanied by a slight increase in collagen throughout growth.

In the pig, at any postnatal age, the main and left pulmonary arteries are elastic and retain more organised structural pattern that the human; the elastic fibers are more regularly arranged and although fragmentation occurs at twelve weeks of age, even in the mature animals, well defined elastic fibers, running in the media, are parallel to the vessels lumen.

In the pig as in the human (Hislop, 1971), the branching pattern of the pre-acinar arteries is already established at birth as is the distribution of muscular and elastic arteries along this region of the arterial pathway.

The pattern of increase in pre-acinar arterial size is also similar in both species, but the pig shows a faster rate than the human, particularly during the first weeks of life.

During postnatal life, intra-acinar arteries increase in size and number in both species. In the pig, however, the phase of rapid arterial multiplication is condensed into a period of five weeks,

shorter than the eighteen months of the human (Hislop and Reid, 1973 a).

The arteries of the newborn pig have a considerably higher percentage wall thickness than the human ones, particularly in vessels between 200 and 1000 μm in external diameter. The shape of the "wall thickness/external diameter diagram" is similar in both species with a "dip", due to a reduced wall thickness in arteries 100-200 μm in external diameter. Like the human, the wall thickness falls considerably between birth and three days of life, particularly in arteries smaller than 200 μm in external diameter, in which the adult values of wall thickness are found at this age in both species. In larger vessels, the low adult values are reached between five and eight weeks of age (at four months in the human - Hislop and Reid, 1973 a).

The adult values of arterial wall thickness are similar in both species, contrary to what has been suggested by Best and Heath (1961), who reported that in the pig, uninjected arteries smaller than 300 μm in external diameter, had a thicker muscle coat than the human ones. Our methods of distending the pulmonary vessels with injected material allow for more precise measurements of arterial wall thickness and possibly for a more accurate comparison between species.

Previous studies done on arterial wall thickness in immature animals have used only uninjected lungs. In both the cow (Wagenvoort and Wagenvoort , 1969) and the dog (Phillips, DeWeese, Manning and

Mahoney, 1960), a rapid postnatal fall in wall thickness has been described, in the cow the adult values being reached by one year of life in arteries of all sizes and in the dog by the end of the first month in vessels smaller than 200 μm.

The study of the distribution of muscular, partially muscular and non-muscular arteries during growth shows that in the pig "population curves" are similar to the human, save that the lag in muscular development is apparent earlier in life in the pig. This is due to the fact that a period of rapid increase in arterial size and number occurs earlier in life in swine than man.

Muscle extends along the peripheral arterial pathway during postnatal life in both species. In the newborn pig lung, many arteries within the acinus are already muscular, contrary to what is seen in the human infant. The rate of muscular extension is faster in the pig, muscular arteries being found accompanying alveolar ducts at five weeks of life and in the alveolar walls by twenty weeks. In the human lung, similar findings were reported only by ten months and eleven years of age respectively (Hislop and Reid, 1973 a).

During postnatal growth, venous branching pattern closely follows the arterial one. Since we have not carried out macroscopic dissections on the pulmonary venous bed, we cannot compare its branching pattern with the human, but in the pig, as in the human, vein wall thickness does not change significantly from birth to adult life (Hislop and Reid, 1973 b). As in the arteries, our results show a significant lower percentage wall thickness than those reported by other authors

(Best and Heath, 1961), but their measurements were performed in uninjected lungs. Vein wall thickness in the pig is slightly higher, however, than that found in the human lung studied with similar techniques.

In the pig, the adult pattern of left ventricular dominance is established between the first and the second month of life and in the human between the fourth and tenth month. In both cases, this corresponds to the period when arterial wall thickness falls to the adult level, suggesting a close relationship between right ventricular and pulmonary vascular maturation.

Structurally, the pig lung "telescopes" the human postnatal pulmonary development into a much shorter period of time (Table VI 1), but the pattern of lung development is similar in both species, particularly regarding the pulmonary vascular bed, which makes the pig lung a suitable species for studying the pulmonary circulation during growth.

The different rates of lung growth merely reflect the different rates in overall body growth. The newborn pig weighs around 1 Kg and is similar in size to the 3 Kg human infant; but whereas the piglet doubles its birth weight in one week, the baby takes six months to achieve the same relative increase. Related to mature size, the pig at birth is only 1% of the mature adult size, whereas the human infant is about 5% (Mount, 1968). The growth pattern similar to the pig, is also found in the sheep and in the cow and the slow pre-pubertal gain in weight in man has no counterpart in most of the mammals (Brody, 1945).

TABLE VI 1

POSTNATAL LUNG DEVELOPMENT - COMPARISON BETWEEN HUMAN AND SWINE. AGE AT WHICH VARIOUS ADULT VALUES ARE REACHED IN THE TWO SPECIES

	SWINE	HUMAN*
Lung Length	1 year	19 years
Alveolar and Arterial Numbers	1 year	5 years
Arterial Wall Thickness		
200 um diameter	3 days	3 days
200 um diameter	2 months	4 months
Peripheral Muscle Extension	3 months	19 years
Vein Wall Thickness	birth	birth

* Hislop and Reid, 1973 c

In a review of the prenatal development of the mammalian lung, it was suggested that a common fundamental architecture is present at birth (Alcorn, Alexander, Mahoney, Ritchie and Walker, 1974), if allowance is made for intra-species differences in the rate of growth. That study did not include the fetal pig but a recent work by Baskerville (1976) showed that the pig follows a similar pattern.

The present study has demonstrated that postnatal lung development is also similar in the pig and in the human; this suggests that the "Laws of Human Lung Development" can be applied generally to the mammalian lung.

Functional Studies

Lung Function

The growing pig

Attinger and Cahill (1960) measured tidal volume, dynamic compliance and functional residual capacity in a series of pigs weighing between 9.1 and 45.5 Kg, but their study did not explore the changes occurring during growth. The findings in the present study are similar to those reported by Attinger and Cahill, when the variables are related to body weight in Kg.

Work done by Brown, Woolcock, Vincent and Macklem (1969) and

by Woolcock and Macklem (1971) has suggested that lung compliance in the pig lung may be normally dependent on respiratory frequency, particularly when this variable had higher values than 40 breaths/min. In the present series, the respiratory frequency ranged between 11 and 58 breaths/min, mean 31 $\pm$12 and was only above 40 in three cases aged two, three and four weeks respectively.

Comparison with the human and other species

The findings that the respiratory variables are related to body weight during growth in the pig, is in accordance with the results reported in other species. Avery and Cook (1961) reported a similar finding for static compliance in goats from birth to maturity, and Johanson and Pierce (1973) found the same for functional residual capacity in rats aged between four and eighteen months.

Between birth and sixteen years of age, the dynamic compliance of the human lung increases from 6.5 to 125 ml/cm H_2O and the functional residual capacity increases from 123 to 2.600 ml (Godfrey, 1974). Specific compliance is unchanged throughout growth and averages 0.05 to 0.06 ml/cm H_2O per ml. This compares with a value for adult human lung of 0.08 ml/cm H_2O per ml and a mean value in the growing swine in the present study of 0.07 ml/cm H_2O/ml.

According to Polgar and Promadhat (1971), dynamic compliance in children aged between four and nineteen years can be related to functional residual capacity by the formula y=0.0459x+17.094, r=1.00,

where y=dynamic compliance and x=functional residual capacity. In the pig a similar relationship between the two variables has been demonstrated - y=0.077x+1.104; r=0.930.

Pulmonary Haemodynamics

The growing pig

The values for right ventricular and pulmonary arterial pressure in the present study are similar to those reported for conscious pigs (Booth, Maaske and Nielsen, 1966)(Friedli, Kent and Kidd, 1975), in which arterial blood gas tensions and pH have been controlled. These results contradict the initial report by Engelhardt (1966), who reviewed cardiovascular physiology in swine and reported that both the right ventricular and the pulmonary arterial pressure could be significantly higher than the values reported for other laboratory animals, most of the work that he reviewed however, was without control of blood gas tensions and pH.

The ductus arteriosus was completely closed in all animals studies, as judged by the absence of recirculation on the termaldilution (cardiac output) curves together with the anatomical evidence of closure in the two cases sacirficed at two weeks of life. These findings are in agreement with earlier studies in swine (Evans, Rowe, Downie and Rowse, 1963)(Rowe, Sinclair, Kerr and Gage, 1964) and (Schaeffer, 1941), who also showed that in swine the ductus arteriosus was completely obliterated by the beginning of the second week of life.

When compared with other series (Engelhardt, 1966), the absolute values for cardiac output measured with the thermaldilution method lie between those previously reported with the dye-dilution technique

which are lower (Rowe, Sinclair, Kerr and Gage, 1964), and those with the Fick technique (Friedli, Kent and Kidd, 1975), which are higher. In the pig, cardiac output increases linearly with age whereas the ratio of cardiac output to body weight decreases throughout growth.

Comparison with the human and other species

The blood pressures found in the present study do not differ significantly from those found in the human (Rowe and James, 1957) (Krovetz and Goldbloom, 1972 b) (Rodbard, Brown and Katz, 1949) studied during similar periods of life. Similarly, the time of closure of the ductus arteriosus in swine did not differ from that occurring in other species (Rudolph, Auld, Colinko and Paul, 1961) and in man (Rowe and James, 1957).

The decrease in the ratio of cardiac output to body weight with age, found in the pig, has also been described in the lamb (Cross, Dawes and Mott, 1959).

Krovetz and Goldbloom (1972 a) reviewed data on cardiac output in normal human subjects aged between one month and twenty years. During that period, there is a highly significant correlation between cardiac output, age, weight, height and surface area. A similar correlation between cardiac output and both age and body weight has been shown for the pig in the present study. In the human, the mean cardiac index increases during early life and decreases afterwards. The time sequence of these changes is not clearly defined: Krovetz and Goldbloom (1972 b) reported a decrease in the cardiac index after the third year of life, whereas according to Guyton, Jones and Coleman (1973) the period of increase continues until late adolescence.

The mean cardiac index for swine in the present study was 4.2, $\pm$ 1.51 l/min per m^2. This figure is not different from the mean cardiac index in man, between one month and twenty years of life (4.04 $\pm$ 1.02 l/min per m^2) (Krovetz and Goldbloom, 1972 a).

Both pulmonary resistances decrease with age in the human (Krovetz and Goldbloom, 1972 b) and the results of the present study show that the same occurs with the pig. In both species, these changes are best represented by exponential curves.

Postnatal Development of the Lung - Correlation between Structural and Functional Studies

The present work included a structural analysis of the normal lung from birth to adult life. The functional studies were only performed in animals between two and sixteen weeks, thus missing the perinatal and the pre adult stages of development.

Respiratory studies

Structural studies of the mammalian adult lung in various species (Tenney and Remmers, 1963) have shown that lung volume, measured by water displacement, can be related to body weight, the work being done in 26 species including the extremes of body size. On the other hand, lung diffusion assessed as the internal alveolar surface area correlates with the metabolic activity or better still with the resting oxygen comsumption; this applies to the entire range of the mammalian size. Also according to this work, alveolar size is not always related to body size, although most of the small

animals possess alveoli which are smaller than those of the larger animals. Similar reports by Bartels (1976) admit that lung volume and heart weights are related to body weight in mammals of all sizes, whereas ventilation and cardiac output are more closely related to the metabolic rate than to the body weight. Similar attempts to correlate lung structure with function have not been made in any animal species throughout growth.

At birth, the liquid lung becomes aerated. Some of the requirements for birth, as far as cardiopulmonary physiology is concerned, have been recently summarized by Scarpelli (1975): (i) development and maintenance of the proper static and dynamic pulmonary mechanics to allow easy ventilation and the atteinment of a normal functional residual capacity; (ii) appropriate circulatory adjustements where the fetal circulation in parallel, becomes circulation in series; (iii) establishment of appropriate ventilation/perfusion relationship and gas diffusion capability. All these dramatic changes occur during the first three days of life and after that period it is probable that the adult patterns of ventilation/perfusion and diffusion are already established.

Once the perinatal transitional stage is finished both the structural and the functional changes throughout growth are much smoother.

The present work describes in the neonatal pig lung considerable structural changes, particularly an increase in the proportion of lung

volume occupied by the alveoli, from 57.1% at birth to 65.1% by the third day of life, a value similar to that found throughout growth, which ranges between 66.8% and 75.8%. This change is also accompanied by a decrease in alveolar wall thickness, that can play an important role in increasing the capability of the respiratory gases to diffuse. The changes in perfusion are equally dramatic, not only because the percentage arterial wall thickness of the vessels smaller than 200 μm in external diameter - those situated inside the lung zone where gas exchange can occur (Staub, 1961) (Staub and Storey, 1962) (Meyrick and Reid, 1975) - decrease to the adult value by the third day of life, but also because these vessels double their size during the same period. Studies of the number of intra-acinar arteries per unit area of lung tissue have also shown that, in the pig, as in the human, some increase in arterial number is already occurring between birth and the third day of life.

Cook, Helliesen and Agathon (1958) stated that compliance of the infant lung is about half the compliance of older individuals, when this variable was divided by lung weight as a basis of comparison; these results suggest that the elastic properties of the newborn lung are different from those of the adult. Further studies by Stigol, Vawter and Mead (1972) found that static compliance at a given per cent of total lung capacity was the same throughout growth. The structural basis for these findings is conflicting. Whereas Reid and Rubino (1959) and Reid (1959) stated that the distribution of connective tissue septa is unchanged

throughout fetal and postnatal development and similar to that found in the adult human lung, Stiegel, Vawter and Mead thought that newborns and infants might have a larger number of connective tissue septa per unit area of lung than the older children and that the fiber content of connective tissue septa changed during the first year of life. In the pig, there is a considerable reduction in the microscopic volume proportion occupied by the connective tissue septa between birth (12.1%) and the third day of life (8.0%), and this decrease continues until the fifth week of age where values similar to adult ones are reached (4.5%). This reduction is not apparent in the macroscopic study.

The fact that most of the respiratory parameters included in the present study are unchanged when related to a standard reference such as body weight reflects the fact that respiration and gas exchange are set to meet the metabolic needs of the body and that, if the perinatal period is excluded, these are practically related to the size of the individual whether as an adult or during postnatal development (Doershuk, Fisher and Matthews, 1975).

Circulatory studies

It has already been mentioned that soon after birth an almost ideal ventilation/perfusion ratio is achieved throughout the lung, both by an increase in the alveolar surface area and by a significant increase in the pulmonary vascular area, which has suddenly to accomodate the total right ventricular output. Furthermore, it is admitted that this is achieved not only by a

considerable increase in size, but also by an increase in intra-acinar arterial number and a decrease in the wall thickness of the small arteries occurring between birth and the third day of life. After this period the changes in the respiratory parameters are much smoother throughout growth and the same can be said about the circulatory changes.

The present study raises several questions connected with pulmonary vascular structure and function during normal development, but most of the present knowledge on the subject is based on studies performed on adult human or animals and only recently on the fetus.

Using fetal lambs, Rudolph and Heymann (1970)(1974) have shown that during the second half of gestation there is a marked increase in pulmonary blood flow through the lungs, not accompanied by a corresponding increase in pulmonary arterial pressure. This suggests a marked reduction in pulmonary vascular resistance, which shows a ten fold decrease, during this period of intra-uterine life. Recently, the same group of investigators assessed the morphological development of the pulmonary vasculature in the fetal lamb (Levin, Rudolph, Heymann and Phibbs, 1976) and suggested that this increase in pulmonary blood flow and consequent decrease in pulmonary vascular resistance was accompanied by the addition of new small peripheral arteries which would increase the total vascular area and enable the lung to cope with the increase in pulmonary blood flow.

In the human lung, the original studies done by Hislop (1971)

suggested that the intra-acinar arterial multiplication occurred only after birth, although it was inferred that a considerable increase in the size of the intra-acinar arteries occurred between the twenty eighth week of gestation and birth.

The morphometric method used by Rudolph and his group needs to be validated with further quantitative studies and although the differences between the species cannot be ruled out, it is evident that more studies both of pulmonary vascular structure and function during fetal life in the laboratory animals are needed.

The present work can only point out the relations between the changes in size, number and structure of the intra-acinar arteries and the changes in pulmonary arterial pressure and resistance occurring in the pig during the first months of postnatal life, thus confirming the work done by Hislop (1971) in the human.

In the pig, the ratio between the volume of lung tissue occupied by the small intra-acinar arteries and the alveolar volume is more or less constant throughout postnatal life. Similar findings have already been reported in the human using the same technique (Hislop, 1971)(Hislop and Reid, 1973 c); and in the rat by measuring alveolar and capillary volumes (Weibel, 1967)(Burri, Dbaly and Weibel, 1974). This seems to confirm the suggestion that the appropriate ventilation/perfusion relationships and corresponding gas diffusion capabilities are achieved soon after birth and remain unchanged throughout growth and in adult life.

The structural and functional changes in pulmonary vascular mechanics, defined as the relationships between blood pressure, flow and resistance in the lung, occur more slowly, probably to allow for gas exchange conditions as ideal as possible at any developmental stage. Nevertheless, it is important to realize that the progressive postnatal decrease in pulmonary vascular resistance described in the human (Lucas, St. Geme, Anderson, Adams and Ferguson, 1961) and also found in the present study in the pig, cannot be necessarily related to "functional maturation of the pulmonary vasculature" because pulmonary vascular resistance measurements depend upon the absolute values of the cardiac output and this changes with age. This is true despite the fact that "structural maturation of the pulmonary vasculature" can be traced both in the human and in the pig by a considerable increase in diameter of the pre-acinar arteries throughout growth, together with intra-acinar arterial multiplication, which facilitates the accomodation of progressively higher cardiac outputs.

The changes in pulmonary vascular pressure, flow and resistance have been studied in normal adult lungs, both in the human and in animal preparations. It is now certain that the lung can be classified as a low pressure and low resistance vascular circuit and that the major fraction of the pulmonary vascular resistance is related to the gas exchange zone (McDonald and Butler, 1967)(Staub and Storey, 1971). Nevertheless, because no haemodynamic analyses have been performed during normal growth, it remains controversial how these findings apply to a developing pulmonary vasculature, where structural changes have been shown to occur, both in the human and, as shown in the present study, in swine.

The effects of gravity on the pulmonary vascular pressures have been demonstrated by West and co-workers in the upright adult and in the excised lung (West, 1971), by showing that the blood flow is least at the apices than at the bases.

These regional variations in perfusion have been shown to play an important role in the adaptability of the pulmonary vascular bed. Indeed it is known than an increase in pulmonary blood flow and pressure would cause better perfusion to the apex and a more even perfusion throughout the lung (West and Dollery, 1960)(West, Dollery and Naimark, 1964)(West and Dollery, 1965). This has been confirmed in experimental cases by Glazier, Hughes, Maloney and West (1969), who have shown that recruitment of new capillary units occurs mainly in the upper zones of the lung, whereas capillary distensibility takes place in the lower zones.

All these changes have been considered as part of a normal adaptive mechanism of the pulmonary circulation, which has been shown to able to cope with a three five fold increase in pulmonary arterial flow without a corresponding increase in pulmonary arterial pressure (Robin, 1970)(Lee, 1971).

When these findings are applied to the child lung at any stage of development, it is tempting to admit that during growth vascular perfusion is more homogenous than in the adult. Although these measurements have not been performed in the normal child, studies of pulmonary blood flow using radio isotopes have described a more even perfusion in certain cases of congenital heart disease associated

with an increase in pulmonary blood flow and pressure (Dollery, West, Wilcken, Goodwin and Hugh-Jones, 1961)(Friedman, Braunwald and Morrow, 1968).

It remains to be investigated how both recruitment and distensibility act in the small lung of the normal child. The present evidence seems to suggest that these adaptive mechanisms are less developed in the child making him more vulnerable to increases in pulmonary blood flow and pressure. Hoffman (1975) has recently admitted that distensibility only would play a vital role in the vascular reverse capacity of the child lung; he also suggested that this would be possible because of the decreased muscularity of the small arteries throughout childhood. This might permit a greater distensibility than in similar size vessels at an older age.

The role played by the intra-acinar arterial multiplication, which has been demonstrated in the normal human (Davies and Reid, 1969)(Hislop and Reid, 1971) and in the pig lung in supplying newly formed channels both for vascular recruitment and distensibility remains to be fully investigated.

In the present study we have shown that the three most important differences between the pulmonary vascular bed in the pig and in the human throughout postnatal development are:

1. a more organized pattern in the main pulmonary artery and its branches in the pig than in the human, characterized by the presence of more continuous and parallel elastic fibers in the media;

2. extension of muscle down to very small arteries even at birth;

3. slight increase in medial wall thickness of the peripheral pulmonary veins with muscle found in very small vessels.

The functional importance of these changes is not apparent under normal steady conditions because, as we have shown, with no abnormality of gas exchange, both the pulmonary pressure and resistances follow a pattern of change similar to that found in the human throughout growth. Nevertheless, these inter-species structural differences must play an important tole in the response of the pulmonary circulation to various haemodynamic stimuli.

A recent confirmation of this hypothesis has come from the work done by McMurtry, Frith and Will (1973) who showed that swine at an altitude of 5400 meters developed marked pulmonary hypertension and right ventricular hypertrophy, which was proportional to the increase in pulmonary arterial pressure. Comparing their findings with those obtained from other species submitted to high altitude, including the human, the cow and the sheep, they postulated that whereas cattle represented the hyperreactors, and sheep represented the hyporreactors, because they failed to develop pulmonary hypertension, the pig is situated in an intermediate group with man, but probably rather more reactive than the human.

Although both neurological and humoral inter-species differences cannot be ruled out in explaining the various animal responses to chronic hypoxia (Fishman, 1976), inter-species differences in the

structure of the pulmonary arterial bed also play an important role (Tucker, McMurtry, Reeves, Alexander, Will and Grover, 1975). Based on our findings, it is not difficult to admit that the distensibility of the small intra-acinar arteries might be less in the pig than in the human, thus reducing one of the important normal "buffer" mechanisms of the pulmonary circulation and allowing for the rapid development of pulmonary hypertension.

The role played by the larger arteries in the transmission of both the pressure and the flow waves has been analysed in the adult human (Patel, Schilder and Mallos, 1960) (Karatzas and Lee, 1969) and in the dog (Attinger, 1963) (Milnor, Bergel and Bargainer, 1966). This work has shown that the main pulmonary vessels are highly compliant in both species. Our structural studies indicate that there is a more organized elastic pattern in the wall of the main pulmonary artery and its branches in swine than in man at any stage throughout growth. This difference had also been suggested by Attinger and Cahill (1967) based on catheterization findings in swine and might explain why we have found an increase in the number of pre-acinar supernumerary arteries between birth and the second week of life in the pig, contrary to what has been described in the human; it is possible that the existence of these additional vascular channels might improve perfusion in a less compliant pulmonary circulation.

The above mentioned differences between man and swine must be take into account in the analysis of the present animal model, but the faster "vascular reactivity" in swine makes this species

a suitable experimental animal in assessing the time-related responses of the pulmonary circulation to various hypertensive stimuli.

STRUCTURAL AND FUNCTIONAL EFFECTS OF AN INCREASED PULMONARY BLOOD FLOW AND PRESSURE ON THE IMMATURE LUNG

Chronic Experiments

Most of the work has been performed in adult animals, mainly in dogs, and since the early experiments by Levy and Blalock (1939) it became apparent that one intact lung was able to sustain considerable increase in blood flow, produced by direct anastomosis of the left pulmonary artery to the left subclavian artery, without any apparent structural or functional damage.

To summarize the present knowledge on the effects of an experimental aorto-pulmonary shunt on the adult animal lung, it can be said that structural changes are produced only when a significant increase in pulmonary arterial pressure (systolic) between 30-40 mm Hg, is applied either to a reduced volume of lung (lobe) or to a reduced vascular bed (ligation of lobar arteries) (Ferguson, Berkas and Varco, 1959). The most striking morphological change is an increase in wall thickness of the peripheral arteries, between 50-200 μm in external diameter, apparent only four to six weeks after surgery. When the progression of the changes is assessed,

using weekly lung biopsies, it is demonstrated that a certain degree of alveolar hemorrhage and a considerable vascular damage, such as wall disruption and necrosis, occur before the final increase in wall thickness is established (Ferguson and Varco, 1955) (Damman, Baker and Muller, 1957) (Esterly, Glagov and Ferguson, 1968).

Significant changes in the pulmonary arterial bed after an increase in pulmonary blood flow and pressure, were also described by Heath, Donald and Edwards (1959), in one adult dog, four years after the creation of an aorto-pulmonary anastomosis (Pott's type - side-to-side anastomosis between the left pulmonary artery and the thoracic descending aorta. No haemodynamic studies were performed. This case is the only one reported in the literature, in which severe structural changes in the peripheral arterial bed were described, that are characterized by generalized and complex forms of vascular dilatation, considered, according to the Heath and Edwards' classification (1958), as pathognomonic of severe forms of pulmonary hypertension. This work suggests the importance of the time factor as a major cause in the development of the vascular lesions.

On the other hand very little is known about the structural and functional behaviour of the remaining of the arterial pathway, nor indeed of the pulmonary veins and capillaries, after the creation of an aorto-pulmonary shunt. The work done by Elkins, Peyton and Greenfield (1974) on chronic pulmonary hypertension after pulmonary embolism in dogs suggested that a mean pressure ranging between 34-52 mm Hg produced considerable arterial stiffening, probably due to a

change in the elastic properties of the main arteries caused by the hypertension.

The physiological studies performed by Ellison, Hall, Yeh, Mobarhan, Rossi and Ellison (1961) showed that no significant impairement of lung function occurred five to thirty six months after the establishment of an aorto-pulmonary shunt in mature dogs. The only changes were some decrease in the arterial PO_2 (in cases with a pulmonary arterial pressure above 40 mm Hg), together with a certain impairement in lung diffusion probably due to an increase in the arterial to venous shunting and not to any change in the normal distribution of ventilation/perfusion.

Very few studies have been performed on the effects of increased pulmonary blood flow and pressure on the immature vascular bed. Rudolph, Neuhauser, Golinko and Auld (1961) performed pneumonectomies on puppies, aged between one and two months, and on mature dogs and although they were not able to find any significant structural changes in the peripheral muscular pulmonary arteries, they demonstrated, by angiographic techniques performed in vivo, that a considerable dilatation of the proximal arteries occurred, but only in the adult animals; this change was not apparent in the cases operated early in life.

Unilateral ligation of the pulmonary artery in newborn calves, produced a significant increase in pulmonary arterial pressure and resistance, together with medial hypertrophy of the peripheral muscular arteries (Vogel, Averill, Pool and Blount, 1963). On the other hand, Kato, Kidd and Olley (1971) attempted to perform

pneumonectomies in dogs between birth and four weeks of life, but failed to report any survivors; whereas in pigs, Friedli, Kent and Kidd (1975) were able to produce a moderate increase in pulmonary arterial pressure and structural lesions of increased muscularization of the peripheral arteries, if the pneumonectomy was performed at four weeks of age.

The work done by Richardson, Phillips, DeWeese, Manning and Mahoney (1961) remains, as far as we were able to find, the sole attempt to study the pulmonary vascular bed in immature animals after a chronic systemic-to-pulmonary shunt. They used puppies aged between three and eleven weeks and performed end-to-end anastomosis between the left subclavian and the left pulmonary artery. Although they failed to report in detail most of their haemodynamic findings, their structural changes showed that the animals with a more immature pulmonary vasculature at the time of the shunt had arteries with thinner walls and larger lumens, whereas the animals operated later in life showed arteries with thicker walls and smaller lumens.

To summarize the already reported findings on the effects of an increasing pulmonary blood flow and pressure in immature animals, it is apparent that although both the structural and the functional changes can be more easily produced than in the adult lung, there are considerable inter-species differences, probably due to changes in the pulmonary vascular structure and to some variation in the pattern of the pre and postnatal lung development in the various experimental animals.

Acute Experiments

Nearly all the present knowledge on pulmonary haemodynamics is based on acute measurements in animals performed either in vivo, at thoracotomy or with the chest closed, or in vitro on the isolated lung.

To assess the acute haemodynamic changes caused by an aorto-pulmonary shunt in dogs with a closed chest, Rudolph, Scarpelli, Golinko and Gootman (1964) inserted between the aorta and the pulmonary artery, a prosthesis whose internal diameter could be controled. Using both blood flow and pressure measurements, they described a considerable increase in the volume load of the heart including left atrial hypertension and an increase in left ventricular-end diastolic pressure after the systemic-to-pulmonary shunt was established. The pulmonary blood flow was also significantly increased, particularly during diastole thus suggesting a continuous expansion of the pulmonary vessels during the whole cardiac cycle. They were also able to report that the older adult animals developed failure more rapidly than the young adult animals; the latter tolerated the shunt for longer periods, which, in any case, were not more than a few hours. These findings suggest that the ventricles of the young animals are more efficient in handling a sudden increase in volume load than those of the older ones.

The role played by the left ventricle in cases of an acute aorto-pulmonary shunt was further investigated by Fixler, Saunders and Sugg (1974), who demonstrated that there was not only an increase in the volume load of the left side of the heart, but also a decrease in diastolic aortic pressure, resulting in a reduction of the coronary

blood flow to the sub-endocardial region of the left ventricle. This could induce ischemia and precipitate left ventricular failure.

The effect of distension without occlusion of the main pulmonary artery has been recently described by Laks, Juratsch, Garner, Beazell and Criley (1975) in conscious dogs. This group of investigators were able to produce a significant increase in pulmonary arterial pressure after inflating a non-occlusive balloon in the main pulmonary artery. The only other haemodynamic abnormality observed was a significant increase in right ventricular systolic pressure, whereas both left atrial and pulmonary artery wedge pressures and cardiac output remained unchanged. They suggested that distension of the main pulmonary artery in the conscious dog would produce reflexely some constriction of the distal pulmonary arteries and probably veins. Similar findings had already been reported by Hyman (1968) in the anaesthetized dog.

Maloney, Rooholamini and Wexler (1970) studied the mechanical properties of the pulmonary vessels in the dog by measuring their static pressure-diameter relations in an isolated perfused preparation using a radiographic technique. Their work demonstrated that under the range of pressure studied, varying from 10 to 45 cm H_2O in relation to alveolar pressures at the bottom of the lung, the smaller arteries (800-1200 μm) showed the greatest increase in diameter, followed by the smaller veins (800-1200 μm), the larger arteries (3200-3600 μm) and the larger veins (2400-2800 μm). Similar findings had already been reported by Caro (1965) in the isolated rabbit lung.

The importance of these acute haemodynamic studies was to further broaden the problem of assessing the effects of increasing pulmonary blood flow and pressure on the lung, by pointing to the fact that both the structural and the functional changes must be evaluated on the whole of the pulmonary vasculature and on the heart, if they are to be considered as valid models in the study of the human pulmonary circulation.

Studying these features in the immature lung, adds the additional factor of growth to the complexity of the problem.

The Present Study

Contrary to what had been previously reported, the present work suggests that it is possible to produce experimental hypertension in the intact pulmonary vascular bed, by increasing both pressure and flow to the lungs using an aorto-pulmonary shunt. This success is due to several factors, among which, the use of an immature vascular bed is a major one.

In the light of the earlier discussion, it is submitted that an immature pulmonary circulation is less able to sustain probably at all vascular levels and compartments, an increase in blood flow and pressure, without developing both structural and functional changes.

By looking at the previous studies, it is doubtful, whether

findings obtained from experiments in which only part of the pulmonary vascular bed is submitted to the increase in blood flow and pressure can be safely applied to the overall behaviour of the complete pulmonary vasculature. Furthermore, there is doubtless a considerable inter-species and even intra-species or individual variability in the pulmonary vascular reactivity and this in the development of pulmonary hypertension (Grover, Vogel, Averill and Blond, 1966).

The fact that the pig already has a considerable number of muscular arteries at the lung periphery at birth and that muscle is also present in the small pulmonary veins, probably has an important role in explaining why most of the animals included in the present study did not develop left ventricular failure and pulmonary edema. The exact role played by a more muscular and less distensible pulmonary vasculature in triggering the development of pulmonary hypertension is unknown, but it is likely that this increased muscularity allows the pig lung to produce a more effective and sustained vascular response when blood flow and pressure are increased. On the other hand, it is doubtful whether immature animals with a more distensible pulmonary arterial tree would be able to sustain a significant increase in blood flow and pressure early in life without developing left ventricular failure and pulmonary edema.

There is no report on the structural changes produced by an increasing pulmonary blood flow and pressure to the intact pulmonary vasculature in immature animals, but work done in dogs: pneumonectomies

(Rudolph, Neuhauser, Golinko and Gootman, 1961)(Kato, Kidd and Olley, 1971) and subclavian to pulmonary anastomosis (Philips, DeWeese, Manning and Mahoney, 1960) early in life, suggests that this species, probably with less muscle at the periphery (Fishman, 1962) either reaches rapidly a stage of passive pulmonary vascular dilatation and cardiac failure, or develops minor structural and functional vascular changes.

The present work shows that in the pig, with an aorto-pulmonary shunt, the larger pulmonary arteries become thicker and fail to distend, particularly in the animals operated early in life, confirming the initial work by Rudolph, Neuhauser, Golinko and Gootman (1961). It also demonstrates that the peripheral muscular arteries develop a considerable increase in arterial wall thickness after the first post-operative month. The decrease in arterial size at the lung periphery, more evident on the animals operated earlier in life, must play an important role in the increase in pulmonary vascular resistance, particularly because it reduces considerably the vascular area at that level. It is interesting to notice that the intra-acinar arterial multiplication, normally occurring throughout growth, was not impaired, even three months after surgery, thus suggesting that recruitment of additional vessels, rather than distensibility, plays a major role in the "defense" to an increase in pulmonary blood flow and pressure.

Another important structural finding from the present work is the extension of muscle into very small peripheral arteries, where it is not present either in control or normal animals of the same age.

This finding had already been reported after the creation of experimental aorto-pulmonary shunts in adult dogs (Damman, Baker and Muller, 1957).

Meyrick (1976) described a similar feature in the adult rat, exposed to chronic alveolar hypoxia, and studied it in detail using the electron microscope. According to her findings, new muscle cells are formed in the normally non-muscular region of the pulmonary artery; these cells appear to differentiate from the pericyte and from what she has called an "intermediate" cell, which is located in the normal non-muscular arterial segment and appears to be a cell midway between the pericyte and the smooth muscle cell, representing a transitional stage between the two types. These new muscle cells develop inside the single elastic lamina of this region, since this is the site of the precursor cells. After twenty days exposure to hypoxia, they ressemble ultrastructurally to mature smooth muscle cells containing both microfilaments and dense bodies.

If the same occurs in the immature pig lung, submitted to an increase in pulmonary blood pressure and flow, both studies are in accordance with the recent hypothesis developed by Rodbard (1970) (1975), who tried to relate the structural changes of the vessels in the overall circulation to changes in vascular tension, caused by variations in both blood pressure and flow. Previous studies by Thoma (1893) had already suggested that vascular structure could be significantly changed by the pattern of blood flow within them.

According to Rodbard the development of muscular hypertrophy

and thus increased wall thickness is induced by an acceleration of tension caused by a chronic increase in intra-vascular pressure. Furthermore, he suggested that a persistent and continuous increase of the cardiac cycle, prevents relaxation of the contractile elements in the arterial wall and so contributes to elongation and extension of muscle. The early experiments by Rudolph, Scarpelli, Golinko and Gootman emphasizing the increased diastolic pulmonary blood flow following an acute aorto-pulmonary shunt in dogs, give some haemodynamic support to this concept.

In the present work, a significant relationship is found between the increase in pulmonary diastolic pressure and flow and the development of the vascular changes, particularly in the animals operated early in life. Based on these findings, it seems likely that the persistence of an increased vascular tension during diastole plays a significant role in the development of the initial pulmonary arterial changes and particularly in the extension of muscle along the vascular pathway.

It is not surprising that significant intimal changes failed to occur in the present study; nor are such changes apparent in cases of congenital heart disease, with left-to-right shunt, studied in infancy (Rudolph and Nadas, 1962 a) even in cases associated with a significant increase in pulmonary arterial pressure and resistance (Hislop, Haworth, Shinebourne and Reid, 1975).

The fact that pulmonary venous changes are not present, together with the initial development of left ventricular hypertrophy, correlates

with the finding of a normal pulmonary arterial wedge pressure at the end of the follow-up period and points to the important role played by the heart in the adaptative mechanisms developed in response to an aorto-pulmonary shunt in the immature pig, as in the human infant (Rudolph and Nadas, 1962 b).

The Present Work in Relation to the Study of Congenital Heart Disease Associated with Left-to-Right Shunts in Infancy and Childhood

A great deal of clinical research has been carried out in paediatric cardiology in recent years. The natural history of congenital heart disease is now well established and it is possible to diagnose and in most cases to medically treat or surgically correct, if necessary, cases liable to be at risk during infancy or early childhood or those prone to develop irreversible pulmonary vascular lesions later in life.

The fact that all the steps in the clinical management, from diagnosis to prevention, seem covered, may at first sight suggest that there is little room for further experimental research in the field. But this is not the case. Very little is known about the progressive adaptability of the growing human lung to an increase in pulmonary blood flow and pressure, although clinical experience has separated atrial septal defects from ventricular septal defects and patent ductus arteriosus, the former seldom producing pulmonary hypertension and only late in adult life, the later being sometimes

associated with severe pulmonary vascular changes in early childhood (Rudolph and Nadas, 1962 a).

Furthermore, there are still doubts as to what structural features correspond to what is haemodynamically considered as the reliable index for operative indication in patients with pulmonary hypertension due to left-to-right shunts and diagnosed in infancy and early childhood (Rudolph, 1974)(Rowe, Kidd, Fowler, Olley, Izukawa, Rose and Trusler, 1974; particularly because the series based in which Heath and Edwards proposed their classification of pulmonary vascular disease (1958) does not include infants.

On the other hand, cardiac surgery performed early in life, is creating a growing population of children, who it is hoped will only be functionally studied (Krovetz, Rowe, Haller and Gott, 1973) (Ho, Krovetz, Strife, Brawley and Rowe, 1973)(Pieroni, Strife, Donahoo and Krovetz, 1973) and for whom a structural picture of the pulmonary vasculature is lacking.

Quantitative morphological techniques have already been applied to the infant lung with ventricular septal defect by Hislop, Haworth, Shinebourne and Reid (1975), who demonstrated that not only there was an increase in arterial wall thickness at the lung periphery, as already mentioned by previous authors, but also that there was a decrease in size of the intra-acinar arteries,together with extension of muscle into smaller peripheral arteries than normal, and with a reduction in the number of intra-acinar arteries, probably representing a failure of the normal postnatal multiplication.

Muscular venous hypertrophy was also found in most cases particularly in those associated with a higher pulmonary vascular resistance.

In the experimental cases, the initial structural changes are left ventricular hypertrophy, and an increase in wall thickness of the vessels larger than 200 μm in external diameter associated with extension of muscle into more peripheral arteries than normal. After the first post-operative month, there is an evident increase in wall thickness of the arteries smaller than 200 μm in external diameter together with a decrease in size at intra-acinar level. This suggests that the growing lung responds sequentially and progressively to an increase in pulmonary blood flow and pressure and that vascular size, structure and number play a determinant role in that response.

In the present work, it was also demonstrated a relation between the elevated pulmonary arterial pressure and the decrease in lung compliance. This finding agrees with previous studies performed in infants with left-to-right shunts and pulmonary hypertension (Howlett, 1972)(Griffin, Ferrara, Lax and Cassels, 1972)(Ahlström, 1974). The fact that dynamic compliance was only significantly decreased when the arteries smaller than 200 μm in external diameter were thicker than normal, confirms close relationship between vessels and airways at the lung periphery (Mead and Whittenberger, 1964) (Maloney, Cannata and Ritchie, 1976), and suggests they influence the elastic properties of the lung.

It is important to notice that studies performed in both the

normal human (Bondurant, Mead and Cook, 1960) and animal (Borst, Berglund, Whittenberger, Mead, McGregor and Collier, 1957) suggested that acute changes in pulmonary arterial pressure and flow when leading to vascular congestion were not associated with a significant decrease in lung compliance. These findings led most investigators to attribute the changes in pulmonary mechanics found in cases of heart disease, such as mitral stenosis (Saxton, Rabinowitz, Dexter and Haynes, 1956) to chronic changes in the lung parenchyma and not directly to the abnormal haemodynamic condition. Although these factors cannot be also ruled out in the cases of congenital heart disease, the present work, by demonstrating a close relationship between increased arterial muscularity and decreased lung compliance, confirms the suggestion (Davies, Williams and Wood, 1962) that a more rigid pulmonary vasculature would affect the elastic behaviour of the lung.

The use of growing animals will provide further knowledge of the normal structural and functional development of the pulmonary circulation particularly during the perinatal period. It is important that further haemodynamic studies will be performed, together with an analysis of the distribution of ventilation and perfusion and an assessment of gas diffusion in the lung throughout development. These results must be compared with structural findings obtained using quantitative techniques.

In relation to congenital heart disease, such further studies

are needed, involving animals with lesions made early in life, as close as possible to birth, and followed for longer periods of time. Whenever possible the defects should also be corrected in an attempt to assess the reversibility of the pulmonary vascular lesions and of any modification of lung growth.

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