THE FETAL AND CHILDHOOD DEVELOPMENT OF THE PULMONARY CIRCULATION AND ITS DISTURBANCE IN CERTAIN TYPES OF CONGENITAL HEART DISEASE

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ABSTRACT

This study was designed to establish the developmental patterns of the pulmonary arterial and venous systems during fetal life and childhood, considering both vessel wall structure and branching pattern.

In 25 fetal and 18 normal childhood lungs the vessels were injected with a radio-opaque material under pressure and the lungs inflated with liquid formalin.

The pre-acinar arteries and veins were present by mid-gestation and the change in their size and structure during fetal life has been determined. Both the arterial and venous systems developed in similar manner with conventional and supernumerary vessels being found in both systems in the same proportion. After birth, only within the acinus, vessels continued to branch, following alveolar multiplication.

During fetal life the arterial media was relatively thick: a postnatal lag in muscle cell development reduced wall thickness. The most striking structural finding was that even large intra-acinar arteries remained non-muscular during childhood. Venous medial muscle increased during gestation but changed little during childhood.

To elucidate further the development of the pulmonary vessels, the effect on their growth of changes in blood flow and pressure were studied in lungs from six cases with tetralogy of Fallot (potentially decreased flow) and six with ventricular

septal defect (potentially increased flow).

The application of precise quantitative methods to normal lungs has provided a sound basis for assessment of developmental abnormalities and revealed new facts.

In tetralogy of Fallot similar changes were found at all ages - a reduction in size of arteries and veins and an increase in their number in the acinar region.

In ventricular septal defect the changes varied but seemed to fall into two groups: increased flow associated with increased arterial and venous size with or without muscle hypertrophy; and in other cases, muscle hypertrophy was associated with decreased lumen diameter and reduced flow.

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At birth there are rapid and dramatic changes in the functioning of the lung. These changes will ultimately cause modification to its structure but for these time is necessary. The blood vessels of the lungs will be particularly affected by the alteration in blood flow that follows closure of the foramen ovale and ductus arteriosus in the heart and by change in blood gas tensions.

This thesis is concerned with the development of the blood vessels during fetal+ life and soon after birth but the vascular findings must be related to the general embryonic development of the lung as well as to concepts of circulatory function. To quantitatively describe the vessels measuring techniques must be used and these call for precise methods of preparation.

This work on normal lungs offers a basis for assessing changes that occur in disease, particularly in congenital heart disease and the effect of two lesions has been traced: ventricular septal defect, high flow and tetralogy of Fallot, low flow.

EMBRYOLOGY OF THE LUNG

Development of Airways

Though Malpighi described the adult lung in 1661 it was not until 1882 that Kölliker commented on the neonatal lung. He stated that it possessed the full complement of acini and that any

+ This spelling is recommended by the Oxford English Dictionary, see B.H.Bass, Lancet, 2, 1194 (1969)

subsequent growth was due to an increase in size of acini and alveoli. Short in 1950, working on rabbit, supported this opinion.

His, in 1887, published the first substantial account of the development of the human lungs. He described at an early stage a right and left stem bronchus passing into a primary lung sac. From these, three branches passed to the right and two to the left, after which there was dichotomous branching to form the lung branching system. Heiss (1919) realised that there was not even development throughout the lung, but that each lobe showed an independent pattern of branching. He made Born wax-plate reconstructions of the developing lungs in the human fetus and showed the primary lung sacs in an embryo of 5 - 7 mm (28 - 30 days). The lobar bronchi were present by 33 days.

Boyden (1955) verified the work of Heiss and made models of the bronchial tree up to the age of 40 days when all subsegmental bronchi are present and the subsegments can be identified on the outside of the lung.

The intra-pulmonary airways continue to divide and according to Broman (1923) in the middle lobe there are 18 generations at birth. Palmer (1936) counted 17 generations between trachea and terminal bronchiolus in the right upper lobe of a 21 week fetus. Bucher and Reid (1961) found that the total number of bronchial generations found at birth was already present by the sixteenth week of intra-uterine life

and that, as in the adult (Hayward and Reid, 1952), the number varied in different segments from 16 - 22. They described a burst of activity between 10 and 14 weeks. There was a slight drop in the mean number between 24 and 40 weeks, caused by the development of the alveoli from the last airways. At birth there were slightly fewer generations than had been found in the adult which was either individual variation or might have suggested that new branches formed after birth. The latter would have agreed with Engel (1947) who stated that new acini were formed up to four years of age.

Willson (1928) working on mice suggested that there was an increase in both respiratory and non-respiratory branches from birth to adulthood. However, Boyden and Tompsett (1962) showed by reconstruction that there was no increase in the number of non-respiratory airways in the child lung. Earlier (1961) the same authors had shown be reconstruction of the dog lung that in this species there is a reduction in number of generations between the newborn and the adult. This is due to alveolisation of non-respiratory bronchioli. A similar but less striking change was found in the human due to alveolisation of the terminal branches (Boyden and Tompsett, 1965). Emery and Wilcock (1966) using a single lung slice and counting airways demonstrated this conversion of non-respiratory to respiratory branches as well as growth of new respiratory bronchioli during fetal life. The differences found between authors on the number of branches before and after birth are probably due to variability in the lungs of the various segments and between individuals.

Development of Alveoli

Palmer (1936) stated that in the fetus there was a continuous epithelial lining until 20 weeks. After this time capillary loops bulged into the lumen, some covered by thinned epithelium but the majority having no cover. Using the electron microscope Campiche, Gautier, Hernandez and Raymond (1963) showed that there was a continuous epithelium over all the capillaries, too thin to see with the light microscope. There is a direct transition from cuboidal epithelium to the alveolar lining cells or pneumonocytes.

In 1936 Dubreuil, Lacoste and Raymond outlined three stages of differentiation in the developing lung:- 1. a glandular period while bronchial divisions are formed, 2. a canalicular stage, from the seventh month to birth, while the respiratory portion is delineated and becomes vascularised and 3. an alveolar period after birth while the definitive alveoli are produced. Loosli and Potter (1951) agreed with these stages but stated that the glandular period was only up to four months, the canalicular from four to six months, while the alveolar began at six months of intra-uterine life; thus some alveoli develop before birth. Parmentier (1962) confirmed their findings and showed that the alveoli had developed before birth whether the lung was filled with air, as after a normal birth, or liquid, as in a stillborn infant, and even if there were no fetal respiratory movements to aspirate amniotic fluid. This he investigated by decapitating fetal rats which were then allowed to develop to term in utero. Mithal and Emery (1961a)

using their method of radial alveolar counting, found that premature babies had fewer alveoli at the equivalent age of post natal maturity than stillborn cases at term. Alveolar multiplication was therefore more rapid in utero and birth did not stimulate a rapid phase of growth.

Short (1950), studying the rabbit, believed that the alveoli were formed not by budding but by the development of septa dividing further the air spaces. These septa arose as a result of tension on the elastic fibres in the alveolar wall due to lung expansion at birth. Later development was only an increase in alveolar size. In the human, Loosli and Potter (1959) and Loosli and Baker (1962) expressed a similar opinion describing 'evagination' of alveoli before birth, the respiratory bronchioli, alveolar ducts and alveoli being smaller at birth than in the adult. Willson (1928) stated that the alveoli in children under seven years were larger than in the adult. Between seven and thirteen years of age he considered invagination of the alveolar walls occurred to give an increase in alveolar number and at thirteen the lung was as complex as in the adult. Engel (1947) realised that there were large immature alveoli in the fetus, after birth they differentiated and increased in number up to six years and then in size up to adulthood. Boyden (1965) using reconstruction methods showed that the alveoli at birth are shallow indentations in the walls of saccules which he regards as the physiological units of gas exchange at this age. Increase in number of alveoli is, at first, by centripetal

alveolisation of existing airways and later deepening of the shallow alveoli.

Dunnill (1962a and b) and Davies and Reid (1970) have demonstrated the increase in number of alveoli during childhood by quantitative analysis and shown that the 20 million alveoli present at birth increase to the adult number of 300 million by eight years.

During this time there is also some increase in size. Dunnill showed by direct measurement of the alveoli that there was little increase in size until eight years, but Davies and Reid, who estimated size from the number of alveoli per cubic centimetre of lung tissue showed an increase in size from three years, which continued as the chest cavity grew to adult dimensions. Emery and Mithal (1960) using their radial alveolar count estimated a rather lower, a nine fold, increase in the number of alveoli per terminal unit from birth to twelve years. Emery and Wilcock (1966) showed an increase in number up to 20 years but showed an increase in size only after ten years. Until ten growth was by multiplication.

In summary the alveoli develop mainly after birth by multiplication within each acinus to the age of eight years. Growth in size continues until adulthood.

Development of Pulmonary Arteries

Congdon (1922) used a wax plate reconstruction to describe the early embryonic development of the pulmonary artery. At 31 days, as the lung bud is growing from the

oesophagus, there is a net of capillaries and angioblastic cords from the aortic sac to the lungs. These will later form the pulmonary arteries. The pulmonary arches grow separately from the paired dorsal aortae to the aortic sac and are complete at 36 days. By the fiftieth day the adult pattern is complete. The right arch disappears save for a small part which becomes part of the right pulmonary artery. The left arch gives rise to the main pulmonary artery and the left and right pulmonary arteries. There is now no connection to the dorsal aorta except via the patent ductus arteriosus. Only one pair of systemic arteries from the dorsal aorta to the lungs are left, these form the bronchial arteries.

Later development of the pulmonary arteries has not been described, though Loosli and Baker (1962) commented that since the pulmonary arteries follow the system of the airways it is likely that all arterial branching to the level of the terminal bronchiolus is complete by the end of the fourth month, and capillaries in the respiratory portion grow adjacent to the epithelium from the fourth month to the seventh month.

Development of Pulmonary Veins

Three authors have described the early embryonic development of the pulmonary veins using serial reconstruction. Aller (1948) described the developing vein in the human embryos of 2 - 7 mm. He described caudal and cranial evaginations of the sinu-atrial region. The cranial type gives rise to the stem of the pulmonary vein which makes contact with the

capillaries of the splanchnic plexus surrounding the oesophagus and trachea. Butler (1952) noted that the vein stem from the left atrium connects with the pulmonary part of the splanchnic plexus near the bifurcation of the lung bud at the eight millimetre stage (31 days). At this time it is canalised and contains blood (Neill, 1956).

All three authors noted that the pulmonary and systemic veins develop from the same splanchnic plexus and at first all drain to the systemic veins; only later (28 - 30 days), does the pulmonary section make connection with the heart via the pulmonary veins. In the human adult there are still a few connections between the two systems in the form of bronchial veins near the lung roots.

In 1967a Reid summarised the work on the development of the lung in the following three Laws of Lung Development:

1. The bronchial tree is developed by the sixteenth week of intra-uterine life.
2. Alveoli, as commonly understood, develop after birth, increasing in number until the age of eight years and in size until the growth of the chest wall is finished.
3. Blood vessels are remodelled and increase certainly while new alveoli are forming and probably until the growth of the chest is complete.

The first two laws were based on many observations but little work has been done on the development of the blood vessels. One of the aims of the present study was to provide the quantitative basis for the third law and to extend it.

PULMONARY ARTERY CIRCULATION

Early Theories

'All the blood in the body is under the control of the heart..... The blood current flows continuously in a circle and never stops.' So wrote the Chinese Emperor, Hwang Ti, in his book Nei Ching (2650 B.C.). This was possibly the first and only correct description of the blood flow for 4000 years. However it was a theory and not the result of dissection and the information did not reach Europe until after Harvey's description had been published.

For the Egyptians (Guthrie, 1945) in the second and first millennia before Christ, medicine was mainly mystical although they did note that the pulse could be found in many parts of the body and that it was synchronous with the heart beat. They described three vessels in each arm and leg and four others, one in each of the head, liver and each lung. They thought that respiration took place through the ears 'the breath of life enters by the right ear and the breath of death by the left ear'. Even at this time some attempt was being made to understand the mechanism of the blood flow.

The Greeks advanced the knowledge of medicine in that they made it observational rather than philosophical or magical. Empedocles (504 - 443 B.C.) described the heart as an organ which took 'pneuma' or spirit through the body. He thought that breathing took place through the lungs and also the skin.

Aristotle (384 - 322 B.C.) furthered his knowledge by dissection of animals. In studying the development of a chick he noted that the heart was present within three days and thought

therefore that it must be the seat of intelligence.

Erasistratus (300 - 250 B.C.) of the Alexandrian School, dissected the human body and described two separate circulations separated in the heart by a septum (Cournand, 1959). Air he thought was taken from the lungs via the venous artery to the left ventricle of the heart where it was changed to vital spirit and sent round the body in the arteries. Some reached the brain and here became animal spirit which was conveyed to the body by the nerves which were thought to be hollow. The veins carried blood from the heart to the body by an ebb and flow movement.

Galen (129 - 199 A.D.) who also was trained at Alexandria, made further observations by dissection of apes and pigs, since human dissection was not then allowed. He was a great experimental physiologist and proved that there was blood in the arteries, in this taking a contrary view to that of Erasistratus. This he demonstrated by showing blood in the artery after cutting it between two ligatures. Unfortunately his theory of the circulation was not as accurate as many others that he put forward. He stated that 'pneuma' entered the lung in breathing and mingled with the blood. This Vital Spirit passed from the lungs through the arterial vein to the left ventricle and then, to provide power, went in the arteries to the body. Venous blood with natural spirit he thought was formed in the liver and passed to the right ventricle, and thence to the organs of the body, including the lungs, to provide nourishment. The veins supplied the so called heavy organs and the arteries the light organs. He thought that pores between the two ventricles carried with vital blood, some 'spirit' from

the left to the right ventricle, so re-vitalising the venous blood. Galen's views were accepted by the church without question and his theory was taught as religious dogma for 1500 years, which greatly impeded progress in the discovery of the circulation. The main false premises were that of the existence of pores in the interventricular septum and of the ebb and flow movement of the blood.

After Galen, Europe entered the dark ages when experiment was considered dangerous; but experiment was still carried on in the East and later this habit filtered back to Western Europe.

This reawakening or Rennaissance started in Europe in the 13th century. Leonardo da Vinci (1452 - 1519) was the first to make accurate drawings of the human heart showing valves and vessels, (Morgan-Jones, 1957). He demonstrated contraction of the heart in the living pig and disproved the idea of passive movement formulated by Galen. He still thought that there was an ebb and flow in the circulation of the blood, however he did try to calculate the outflow of blood.

Michael Servetus (1509 - 53) revolted against the dogma of the church and was eventually burnt at the stake charged with heresy. In 1553 in 'Christianismi Restitutio' he denied the presence of pores in the intraventricular septum and described the true route of the blood through the lungs; 'However this communication is made not through the middle wall of the heart, as is commonly believed, but by a very ingenious arrangement the subtle blood is urged forward by a long course

through the lungs, becomes reddish-yellow and is poured from the pulmonary artery into the pulmonary vein. Then in the pulmonary vein it is mixed with inspired air and through expiration it is cleansed of its sooty vapours. Thus finally the whole mixture.....is drawn onward from the left ventricle of the heart by diastole'.

At the same time in Padua in 1543 Vesalius (1514-64) in his book 'De Humani Corporis Fabrica' denied the presence of pores in the septum. It was his pupil Colombo (1510-99) who independently of Servetus described the course of the blood through the lungs in 'De re Anatomica' (1559). He understood the mechanism of the valves in the heart and that the heart had its own blood supply through the coronary arteries. He argued that if vital spirit was in fact made in the heart it would not need its own blood supply. Concerning the blood circulation he writes: 'But they are in great error for the blood is carried through the pulmonary artery to the lung and is there attenuated, then it is carried along with air, through the pulmonary vein to the left ventricle of the heart. Hitherto no one has noticed this or left it in writing.' It seems from this last sentence that Colombo was not aware of the work of Servetus nor of that of Ibn an-Nafis (1210-88). The latter studied in Damascus and was Dean of the hospital in Cairo. He also realised that there were not pores in the intraventricular septum and speculated in 1245 in his 'Commentary on Anatomy of Avicenna's Canon' that blood must pass through the lungs where it seeps into the alveoli and mixes with air. His writings were not rediscovered in Europe until 1924 and it seems unlikely that they had any influence on the work of Colombo or Servetus.

Harvey (1578-1657) in 'De Moru Cordis' (1628) correlated his many observations on the valves of the heart and veins, with the pulsation of auricles and ventricles. He noted that more blood left the left ventricle in one hour than filled the whole body. He deduced (1) that blood must circulate round the body and only passes from the veins to the arteries in the heart, (2) that circulation is in one direction and (3) that the sole reason for the heart pulsating is to circulate the blood. He did many experiments on animals and man to prove his points. He realised that blood passed through the lungs via 'porosites' before being passed back to the body and that there must be an anatomical structure responsible for this.

Malpighi (1628-94) used a simple lens microscope to study the lung and described it (1661) as fleshy, made up of lobules of cavities like 'honeycomb alveoli of bees'. He also saw the movement of blood from arteries to veins in a live frog lung. Using a dried frog lung he saw the network of tiny vessels between the artery and vein and so concluded that the blood always runs through these tubules and does not 'gape' into the common substance of the lung.

Both Harvey and Malpighi realised that breathing supplied something necessary to the blood. It was the work of three seventeenth century Oxford scientists which established what. Boyle (1660) and Hooke (1667) proved that air was needed for life and Lower (1669) showed that breathing caused blood to change colour in the lungs.

Prior to the nineteenth century the approach to the study of lungs and their blood vessels was functional and any anatomy was gross rather than microscopical. With the improvement of microscopes there was a swing to observation of fine structure of the vessels; this was related to function and the changes in disease.

Arterial Muscle Hypertrophy

Thoma (1893) stated that blood vessels reflect in their structure the stresses to which they are subjected and that hyperplasia of muscle is a response to increased tension. Weibel (1958) showed this experimentally in vivo, in the rat. By rhythmic stretching of the mesenteric arteries by attaching them to the diaphragm, longitudinal muscle appeared in the artery.

Harvey (1628) commented that the pulmonary artery had a thinner wall than the systemic artery because there was less thrust. He noted that the right ventricle was thinner than the left since it did not need to be so strong because the lungs were spongy. He also realised that the arteries nearer the heart were stronger because of the force of the heart while the veins were thin walled.

In his account of experiments made on the blood vessels of animals, Hales (1733) showed that there was less pressure needed to force blood through the lungs than through the body, and that the pressure in the lungs must be lower because of the thin walled right ventricle.

The first microscopical observations of muscle hypertrophy in the arterial wall were made over a hundred years ago. Johnson (1850) while studying the kidneys of patients with Bright's Disease, noted that there was an increase in both circular and longitudinal muscle in the small arteries of the Malpighian Body, but the veins were not affected. He believed, at that time, that the blocking of the kidney tubules caused back pressure in the capillaries and that it was this impediment to flow which caused the hypertrophy, the muscle being to assist in driving the blood through the capillaries. In 1868 he rejected this theory as 'physiologically untenable'. He now believed that the increase in muscle was due to the small arteries stopping the blood going to the kidneys because of the reduced work of the kidneys. He emphasised the fact that there was true hypertrophy of muscle since the muscle layers were thicker and the vessels had become long and tortuous. By now he had found muscle hypertrophy in the vessels of the cerebrum, small intestine and skin of the hand in these patients, having used Prussian blue fluid injected into the arteries to identify them. He noted that the greater the hypertrophy of the heart the more were the arteries hypertrophied. He concluded that in Bright's Disease 'hypertrophy is due to overaction in opposition to the heart'.

Gull and Sutton (1872) also studied the arteries in Bright's Disease. They examined the walls after injection with Beale's blue translucent fluid. They found no evidence of muscle hypertrophy, in fact they believed that the muscle may have been atrophied. They explained that the increase in wall thickness

was due to hyalinisation of the intima and some hyalin-fibroid deposits external to the muscle layer. Much argument on these rival views followed in the next fifty years, some authors believing that increase in wall thickness was due to hypertrophy, others to hyalinisation associated with muscle atrophy.

It was only when Kernohan, Anderson and Kieth (1929) used careful and controlled measurements that muscle hypertrophy was definitely demonstrated in the small arteries in cases of systemic hypertension. They believed that the hypertension was due to the changes in the artery wall increasing resistance and the first change was vasoconstriction eventually leading to hypertrophy. Short (1962) later believed in contracture eventually becoming irreversible.

There has been much interest in the presence of muscle hypertrophy in the pulmonary circulation as a result of primary lung disease and heart diseases such as mitral stenosis and congenital heart lesions. The question of whether the hypertrophy causes hypertension or whether the histological changes are a result of changes in pressure and flow has still to be answered to everyone's satisfaction.

TECHNIQUES OF PREPARATION

Before the lungs and their vessels can be studied they have to be prepared and numerous methods have been used since the lung presents special problems because of its consistency. It is also essential to have methods for describing the normal structure precisely and thus for estimating change in the abnormal.

Inflation and Fixation

In a letter to Borrellius in 1661, Marcellus Malpighius described how he prepared lungs for examination. He passed water through the arteries and then inflated the lung with air through the trachea, leaving the lung to dry. This is the earliest report of a method for preserving the lung prior to demonstrating by dissection the alveoli and branching pattern of airways and vessels. In 1823 this method was still being used by Picard who preferred to cut the lung in slices rather than to dissect it. Later Laennec (1826) commented that this 'insufflation and dessication always causes contraction or crisping which diminishes regularity'. The air drying method was used again by Tobin in 1952a when he used compressed air to maintain full distension. The final result was the texture of a 'wasp's nest' which he found easy to handle for radiological studies and dissection of the bronchial tree.

It was not until the nineteenth century that formaldehyde was introduced as a fixative by Blum (1893) who simply immersed all organs in it, the lungs being no exception. In 1935 Moolten combined the methods and both inflated the lungs with air and then fixed them with Kaiserling I, a fluid that includes formalin. He inflated the lungs by applying a negative pressure of 10 - 20 cm of water. The fixative was then run into the inflated lungs and the suction maintained for 12 to 18 hours. The resulting specimen was well preserved and the alveoli very distinct.

Methods of fixation, currently being advocated, involve inflation by a fixative, in either the vapour or liquid phase.

Vapour fixation

Blumenthal and Boren (1959) inflated a lung for three to five days with air that had been bubbled through cold formalin. The lungs were then dried for a further seven days by a continuous current of air. The result was a rigid dry lung that could be cut into slices. This method is fine for stereoscopic study but the authors themselves admit that another lung is needed for histological study. Jones (1960) inflated similarly with a mixture of 50% formalin and 50% ethyl alcohol using various pressures. He had disappointing results and felt that the method was impractical for routine work especially since any obstruction in the bronchus inhibited the fumes. To inflate the lung, Cureton and Trapnell (1961), used the vapour in the space above 40% formaldehyde stored in a Carboy. The lungs were then floated in 20% formalin. This method gave very good results on radiography and the histology was adequate.

The main problems with these methods is the length of time needed for fixation, and that they have given unsatisfactory fixation of diseased lungs. By warming the formalin vapour the time can be reduced. Pratt and Klugh (1961) used warm vapour at 60°C and the vapour in the lung was changed by making the lung 'breathe', the fumes being forced in and out by a cylinder and piston at a fixed pressure calculated to take into account the fresh lung volume. The lung fixes in 12 - 18 hours and it is then dried by inflating with air for five to eight days. This led to good stereoscopic results but poor histology.

It seems that drying the lungs distorts them so Weibel and Vidone (1961) devised a method of inflation under negative

pressure and then fixation by bubbling in hot formalin steam. This method only takes two hours and the lungs are then fixed overnight in Zenkers' fluid which hardens the outside of the lung so that it can be easily sliced. The slices are then fixed in Zenkers' fluid. This method gives very good histology because it is so rapid and the lung is easily handled. Silverton in his review article of 1965 commented that it was not necessary to double fix and that floating the lung in formalin was just as effective. This method was in fact used by Greenberg, O'Neal and Jenkins in 1964 with good results. It is now recognised that even this technique is not efficient on diseased lungs (Reid, personal communication) and Weibel, now, does not use this method (personal communication).

Constant Pressure Liquid Inflation

Fixing with a liquid would seem to be the method of choice since it is less time consuming and more reliable for diseased lungs. Hartcroft and Macklin in 1943 wanted to measure the size of alveoli as in life. To try to avoid artefact they inflated all lungs with 3% formalin run into the trachea at a constant pressure of 30 inches of water. They inflated to 15% more than the measurements of the thorax, to allow for shrinkage. Gough and Wentworth (1949) used a mixture of formol-acetate and injected the bronchus from a height of four feet until the lung was fully distended. They then floated it in a large container of fixative. This overcame one of the disadvantages of liquid inflation, that is, distortion from pressure on the pleura and the effect of gravity on the fluid in the lung.

Heard (1958) devised a method whereby the pressure in the lung could be kept constant throughout fixation. The fresh lung was floated in water and distended gently at a pressure of 10 - 12 inches of 20% formol saline. When it was fully distended it could be attached to an apparatus that pumped formalin through the lung at a constant pressure for several days. He found that this gave less shrinkage than when lungs were floated in formalin and he also proved that the volume remained constant over several months. Hartung (1962) criticised this method for over-inflation and preferred a method of 'inhaling' liquid formalin under controlled negative pressure until the lung reached inspiratory volume, which was estimated from weight and volume measurements.

A Committee set up by the Aspen Conference on Emphysema (1959) reported that the best preparation for lungs was to run 10% neutral formalin and 5% sodium acetate into the trachea at a pressure of 8 - 10 inches until the lung borders showed the normal contours. The lung should then be submerged in the fixative for at least three days. Elliott (1964), Reid (1967b) and Davies (1969) all used this method but increased the pressure head of formalin to 30 inches.

Injection Techniques

As early as 1661 it was realised that identification of different structures is made easier if they are differentially injected with chemicals. Malpighi emptied the pulmonary artery of blood, inflated it with air and tied the end so that the branches stood out like a tree. He also commented that

if the arteries were injected with mercury they were more obvious. In 1704 Jahre used an injection technique to demonstrate the coronary arteries. An illustration of this is seen in Schoenmacker's exhaustive review article on vascular injection (1960).

Reisseisen in 1822 also used mercury to help identify the arteries, veins and bronchi and their branching pattern in the lung. Laennec (1826) in his treatise criticised this method saying that the mercury filled all structures leaving a confused mass.

Further developments in injection techniques were directed to one of three aims, that is, identifying structures macroscopically, radiologically or microscopically.

By using differently dyed masses combined with dissection, Rossignol (1846) described the branching pattern of the airways in the lung and its division into lobules each attached to a terminal branch of the bronchial tree. Methods have been refined and in 1963 Oderr, Dauzat and Montamal used thin latex of different colours injected into the arteries and veins under pressure. To set the latex 4% acetic acid was injected into the bronchi. It was then possible to dissect the veins and arteries and to define where they met at alveolar level.

Elegant demonstrations of the pattern of the vascular and bronchial system and their changes in disease have been produced by making casts using synthetic resins such as vinylite (Liebow, Hales, Lindskog and Bloomer, 1947; Marchand, Gilroy and Wilson, 1950; Tompsett, 1952) and Vultex moulage (Pump, 1964). The disadvantage of these methods is that the lung

tissue is no longer available for macroscopic or microscopic study.

The advantages of injecting material into one system so that it can be easily identified microscopically have been recognised by many authors. It is even better if the substance used is radio-opaque and therefore suitable for X-ray analysis. Collister, Dankmeijer, Snellen and van der Wel (1953) and Harrison and Asling (1955) used an aqueous barium sulphate mixture for radiological studied but unfortunately this runs out when the specimens are cut and is not a help in microscopy. Hinman and Morrison (1924) used a method which they ascribed to Gross (1917), of mixing gelatine with the warmed barium sulphate. Since this sets solid at room temperature it remains in the vessels while they are prepared for microscopy. They applied this method to the radiographic and microscopic analysis of kidney lesions.

Methods of combining X-ray analysis and histological study have been applied to many organs but particularly to the kidney, heart and lung. Several types of injection mass have been used, usually depending on the size of vessels to be reached. Schlesinger (1938) used agar mixed with lead phosphate which penetrated to a diameter of 40 μm. Since they pass through the capillary bed, but do not diffuse through the cell membranes (Barclay, 1947), colloids such as thorotrast can be used to study capillaries. Gelatine with India ink will also pass through the capillaries but it is not radio-opaque (Reid and Heard, 1963). The most commonly used substances are the heavy metals in the form of red lead oxide and barium sulphate

which will not pass through the capillary bed because of particle size. Reid (1955) found that lead oxide as used by Miyata (1939) was too hard to be used for histology and therefore recommended barium sulphate which is radio-opaque and can be handled and cut easily.

Schlesinger (1957) used gelatine kept liquid by potassium iodide, and barium sulphate as the contrast medium. By adding formalin of varying concentrations just prior to injection there was solidification at a pre-determined time. On this depended the level to which the injection mass extended. Cicero and Celis in 1955 injected the vessels under fluoroscopic control until all the fine branches had been filled.

The pressure at which the material is injected also effects penetration. It has been varied considerably and caused much discussion. Ljungquist (1963) used hypertensive systemic pressure while injecting kidneys with aqueous Micropaque (a barium suspension), since he was dealing with diseased as well as normal kidneys. Similarly Short (1956) and Dunnill (1961) used 50 - 100 mm of mercury when injecting normal and abnormal pulmonary arteries with Micropaque and gelatine. This pressure insured that all arteries were fully distended and that arteries down to 30 μm were filled. Doyle, Goodwin, Harrison and Steiner (1957) followed this example and injected arteries at 80 mm of mercury pressure. However Wagenvoort (1960) and Reeves, Tweeddale, Noonan, Leathers and Quigley (1966) used a pressure equal to the pulmonary systolic measured in life; this varied from case to case. Wagenvoort, Heath and Edwards (1964) have warned that higher pressures will cause artefacts due to splitting of the artery wall and that, in any case, they are unnecessary since the extent of filling depends on viscosity rather than pressure.

Elliott (1964) took the view that it was essential that all vessels should be fully distended in normal and diseased lungs. He also argued that all lungs should be injected under the same conditions so that this at least was comparable between specimens. He therefore used the hypertensive pressure of 100 cm of water. He found that this high pressure did not cause damage to the arterial wall and that all vessels were fully distended with the elastic laminae smooth and free of wrinkling. The barium sulphate-gelatine solution which he used filled arteries down to 15 μm in diameter. Davies (1969) using the same method found that, even in the child lung, there was no bursting of the medium into the surrounding parenchyma.

More detailed work on the angiograms, produced after injection with radio-opaque medium can be done using the technique of microangiography which involves secondary magnification of the angiogram onto fine grain photographic film. It is possible to trace the course of small vessels and capillaries. This technique has been developed by Barclay (1947) working on the kidney. Turner-Warwick (1963) and Robertson (1968) have used it extensively in studying bronchial arteries and broncho-pulmonary pre-capillary anastomoses.

Arterial Measurement

A subjective impression of the structure of artery walls and the changes in disease is not sufficient; one cannot rely on the 'ideal section' of the earlier anatomist. It is essential to have quantitative assessment by making measurements of the blood vessels in order that hypertrophy in disease can be

recorded accurately.

As early as 1872 Gull and Sutton measured wall thickness and lumen diameter to demonstrate hyalinisation causing thickening of the renal arteries in chronic Bright's Disease. They identified the arteries by injection with Beale's blue translucent fluid.

Kernohan, Anderson and Keith (1929) studied the arterioles in cases of systemic hypertension using a similar method to Gull and Sutton. They used the ratio of lumen to wall thickness, measuring the arteries in cross-section and taking the average of four wall thicknesses and two lumen diameters. Unfortunately they did not state exactly what constituted the wall of the artery and their work is difficult to repeat. Their results are valid since they did use control material in the study.

In 1935 Brenner wrote a monograph which has become the basis for most later work on the pathology of the pulmonary vessels. He measured the thickness of the media and expressed it as a percentage of the total diameter. Again he did not define the limits of the wall except to state that the external diameter excluded the adventitia. His own criticism of this method is that the figures are higher than would be found during life since the lumen is not distended. Injection would have overcome this difficulty. Similar methods were used by Goodale and Thomas (1954) who produced a ratio of media to external diameter, and Dammann and Ferencz (1956a) who related lumen to twice the media plus intima. This was essentially the same as the method of Larrabee, Parker and Edwards (1949).

Unfortunately measurements of lumen and wall thickness do not take into account contracture of the wall so that a contracted large artery cannot be distinguished from a smaller artery with a thicker wall. Short (1957) showed that injection of the vessels would overcome rigor mortis but not hypertrophy.

O'Neal, Thomas and Hartcroft (1955) were aware that the wall thickness would alter with contraction and therefore used measurement of medial area. Muscle volume does not alter in contraction and area is a function of volume. Using arteries cut only in true cross section they estimated medial area by subtracting luminal area from total area. In order to further eliminate inaccuracies they selected arteries running with the proximal and peripheral respiratory bronohioli, assuming that they would be similar in size and type. These could then be compared with arteries from the same position in diseased lungs. This method assumed that the vessels running with certain airways would have a standard dimension. Elliott (1964) showed that in fact there can be great variation.

By using projection and planimetry Naeye (1961a) extended and refined the methods of medial area measurement. He estimated medial and lumen areas and related these to the area of the intima which remains constant in disease and ageing. This method though laborious has led to reproducible and significant results. It is not necessary to define the limits of the media since only the muscle layer is measured. This method leads to results on the relative medial area and dilatation of the individual arteries but does not show overall changes in the total arterial population. This problem was helped by Wagenvoort (1960) who measured the total muscle mass per unit

area of lung. He injected the arteries with barium sulphate and gelatine solution which overcame contraction. He then measured the external diameter and width of media from which he estimated the medial area and ratio of media to external diameter of each vessel. To find the muscle mass per unit area of lung he multiplied the average area by the number of arteries per square cm of tissue. Taken together, these two methods of measurement gave an indication of the amount of muscle in the arteries of the lung. If the medial wall thickness increased it could be the result of contracture or hypertrophy, but if there was also an increase in medial area per unit lung area there must be a true increase in muscle tissue. Wagenvoort found that his results varied only within 20% whether the arteries were injected or not and his later work was done on uninjected material only.

Elliott (1964) used an injection technique to fully distend all arteries. He maintained that measurements made of external diameter and wall thickness would be comparable between cases if all arteries were fully distended. This was demonstrated microscopically by flattened laminae in the media. He expressed twice the medial wall thickness as a percentage of the total diameter. His results were consistent for normal lungs and gave very little variation in any size range. Differences found in diseased lungs were statistically significant. This method was later used by Davies (1969) and Davies and Reid (1970) who found that it was possible to repeat Elliott's results accurately. This method is the most informative and precise of those previously used and has been used in the present study.

These methods of measurement do not show the overall

structure of the lung. It is essential to have a three dimensional picture of the lung; for this there must be reconstruction of the branching patterns of airways, arteries and veins, in addition to information on their structure.

Measurements from Arteriograms

By injecting arteries with a radio-opaque medium it is possible, with the use of radiographs, to produce an overall picture of the branching pattern both in normal and abnormal lungs. This method has been used several times but rarely with quantitative assessment. Miyata (1939) injected with red lead oxide. He noted the treelike appearance of the arterial bed and the fact that there is more rapid branching to the periphery. In mitral stenosis he described the increase in size of the hilar vessels.

Silver (1952) used injection of barium sulphate to describe the normal branching pattern of the bronchial and pulmonary arteries. Short (1956) was the first to study arteriograms and relate his findings to the microscopic appearance. He described the branching pattern with progressive decrease in calibre and noted the variation between specimens. He commented on changes in embolism, mitral stenosis, emphysema and pulmonary hypertension which he described later in more detail (1957).

Doyle et al (1957) described the dilatation of proximal vessels in cases with increased pulmonary flow due to septal defects of the heart. These cases were further analysed by Harrison (1958) who related the arteriograms to histological

and dissection studies. In the arteriogram only the lumen diameter can be measured, the external diameter may be quite different if there has been muscle hypertrophy or thinning. A dilated artery may appear normal if the walls are hypertrophied. It is therefore necessary to use microscopy of comparable arteries. Harrison therefore stressed the need to use the two methods in parallel. Dunnill (1961) used similar combined methods to study the relationship of emphysema and cor pulmonale.

More accurate results can be obtained by direct measurement of the arteriograms. Millard (1965) measured the lumen diameter in adults at the branching points and related this to the percentage distance along the pathway. In this way he could compare pathways of different lengths. He showed that the measurements made in this way were very similar to those obtained microscopically by measuring the same artery at the same point.

Davies (1969) modified this method and only took measurements at 10% intervals along the pathway. He demonstrated the changes with age in the normal and the effect of cystic fibrosis and scoliosis.

Methods of Reconstruction

A three dimensional picture can also be obtained by microscopic reconstruction from serial sections of both the bronchial and the circulatory system. Though not using serial reconstruction Brenner (1935) described the structure of the artery at various levels in the lung and commented on the branching pattern.

Measuring is essential in reconstruction and in 1964 Pump dissected and measured Vultex moulage casts of both systems. His careful measurements were only made on intra-acinar structures. Careful measurements of the pre-acinar region of both bronchi and arteries have been made by Cumming and his co-workers using resin casts (Horsfield, Cumming and Hicken, 1966; Cumming, Henderson, Horsfield and Singhal, 1969). They described dichotomous branching in the bronchial tree and monopodial branching of the artery; they also related diameter to generation number.

When a cast has been made it is impossible to see the microscopic structure. Reconstruction using the Born wax-plate method has been widely used and Boyden (1965, 1969) has demonstrated the three dimensional pattern and the histological structure of the growing acinus by tracings from the sections and wax models made from them.

The importance of a model of branching used in conjunction with the results obtained by random measurement was stressed by Elliott (1964). He made serial sections from the hilum to periphery of an axial pathway and examined each slide measuring the main artery and bronchus and noting the position, size and structure of any branch. This painstaking work on a few cases led to an accurate description in the normal adult of the branching pattern related to structure.

STRUCTURE OF THE PULMONARY ARTERY SYSTEM

The Pulmonary Artery in the Normal Adult

Two types of study have been made on the pulmonary artery. The first type involves the pattern of branching and the second the structure of the arterial wall. It is essential that both should be studied.

Branching Pattern - Pre-Acinar

It is generally assumed that the pulmonary arteries run adjacent to the bronchi and branch with them.

Boyden (1955) has described the hilar arrangement of the vessels in great detail and stressed the variability between cases. This variability was also commented on by Harrison (1958) while studying arteriograms.

Very little study had been made until recently on the branching pattern of the intra-pulmonary arteries of the pre-acinar region. Brenner (1935) described the structure of arteries from hilum to periphery, however he did not relate structure to the position in the lung and only commented briefly on the branching pattern: 'within the lungs a branch follows each of the larger subdivisions of the bronchi, but in the remote subdivisions the arteries divide more frequently than the bronchi'. Miller (1937) made note of no extra branches until the level of the ductulus alveolaris where there were branches to each saccule. von Hayek (1953) observed extra branches leaving the pulmonary artery at right angles in addition to those running with bronchi. These he traced to the bronchi and termed 'Rami Pulmobronchiales'.

In 1964 Elliott (Elliott and Reid, 1965) undertook the extensive task of reconstructing the pulmonary artery, from hilum to acinus. His intention was to relate structure and dimensions of the artery to the position in the branching system. He found, as generally assumed, that the pulmonary artery follows closely the branching pattern of the bronchial tree. But there were in addition extra arterial branches which he termed 'supernumerary arteries'. They occurred throughout the length of the artery from the hilum to the level of the capillaries, but were more numerous towards the periphery. At all levels they outnumbered the branches accompanying airways, 'conventional arteries'. They accounted for 40% of the total cross-sectional area of the pulmonary artery branches. It is of interest to know whether these branches are present from the start of development or whether they appear with the increasing blood supply to the lung at birth. A suggestion that at least some are present early was given by Robertson (1967a) who found 'abrupt' extra branches near the hilum when studying infant lungs.

Branching Pattern - Intra-Acinar

According to Pump (1961), who used injection of Vultex moulage, proximal to the primary lobule the arteries ran with the bronchial tree. At the level of the alveolar duct the artery broke up into a network of pre-capillaries and capillaries in the alveolar wall. This network was continuous from one alvolus to another. Alveoli adjacent to pre-alveolar duct vessels were fed directly from these vessels. In 1962 he showed that each acinus was supplied by a single central artery which progressively branched to supply the bronchioli. The peripheral zone could be supplied by arteries from outside the acinus.

Knisely (1960), Krahl (1962) and Reid and Heard (1963) using open chest illumination, chest window observation and India ink and gelatine injection, respectively, described how the arterioles supplying the alveolar region gave off daughter branches at acute angles that showed a gradual diminution in size as well as right angled small branches direct to alveoli. The latter could possibly be compared to supernumerary branches. Reid and Heard also noted that the arteries accompanying the respiratory bronchioli and alveolar ducts gave off many side branches. Boyden found small 'adventitious' branches between the main branches within the acinus in a 37 day old infant (1965) and in a 30 week fetus (1969).

Pulmonary Artery Structure

Most authors while studying the arteries in disease have described the structure in the normal adult, since this must be used as a control. Of the early work the most important was that of Brenner (1935). His classification of artery size and structure has been used by most authors. Working on uninjected material he divided arteries into three types according to wall structure and external diameter. He also measured the percentage wall thickness. His classification is as follows: Elastic arteries, with layers of elastic and some muscle in the media and a thin endothelium; diameter over one millimetre with a wall thickness of 5 - 15%: Muscular arteries, diameter 0.1 mm to 1 mm with media made up of circular muscle between an internal and external elastic lamina and a wall thickness of 6 - 32%: Arterioles, below 100 μm

in diameter with a wall of endothelium and a spirally wound elastic fibril continuous with the external elastic lamina, which occasionally splits enclosing a single muscle cell with a wall thickness of 4 - 9%. These arterioles were very different from systemic ones that have a very thick wall. Brenner could not distinguish the arterioles of the pulmonary circulation from the venules since they had the same wall structure.

In 1939 Miyata was the first to measure injected material. He injected lungs with Berlin Blue and gelatine and then microscopically measured the lumen diameter and related this to wall structure. He also recorded the number of arteries in each size range. He found that above 150 μm all arteries were surrounded by a muscular coat with an internal and external elastic lamina. At 80 μm all continuous muscle was finished and at this point there were isolated muscle fibres and an external elastic lamina; arteries of this type were most common at 40 μm and he termed them arterioles. Under 40 μm there was no muscle and these vessels had the structure of the capillaries but with a larger lumen; he called these pre-capillaries. These results compare well with those of Elliott which is probably due to the injection. Miyata pointed out that his results could only be compared with others using the same methods.

von Hayek (1953) accepted similar definitions of arterial type and related arterioles to the airway branching pattern, saying that they were found with the first generation of respiratory bronchioli.

Some careful studies on injected and uninjected material were made by Wagenvoort (1960), who showed a percentage wall thickness of 2.9 - 4.8% in the adult. He found that the wall thickness was higher in the small vessels than the large. Later Wagenvoort and Wagenvoort (1965a) showed that there was no change in the amount of muscle with old age but that the media became irregular, perhaps because of change in elasticity.

Elliott (1964) made a careful study of the structure of the pulmonary artery from hilum to periphery and related the structure to diameter and position in the lung with relation to generation number and length. He compared his findings with those of Brenner and reclassified wall structure into five histological types which allowed each artery to be characterised by its structure rather than diameter. He defined elastic, transitional, muscular, partially muscular and non-muscular arteries. The first three were artificially divided depending upon the number of elastic laminae between the internal and external laminae. Elastic arteries had more than seven, transitional four to seven and muscular less than four. Partially muscular arteries had at least part of their wall without muscle.

He found that, above a diameter of 150 μm, structure was related to diameter. Below this it was impossible to predict structure from diameter because there was a 'mixed population'. Elastic and transitional arteries were found down to 2,000 μm. Muscular arteries then extended as far down as 30 μm, a much larger extent of muscle than Brenner had described. Below 150 μm partially and non-muscular arteries appeared and were found in varying proportions.

By fully distending the arteries it was possible to get a repeatable range of wall thicknesses. He found that in the larger arteries the average percentage wall thickness was 1.5% but that it rose to 12% in the smaller arteries within the acinus. It is possible to compare results from diseased lungs with these findings if the same methods are used.

The methods, definitions and results of Elliott have been referred to throughout this study.

The Structure of the Pulmonary Artery in Fetal Life and Childhood

It is only in recent years that measurements have been made on the pulmonary artery during fetal life. Civin and Edwards (1951) measured the wall thickness including the adventitia and arterial lumen in uninjected material and found that during fetal life the lumen was less than the wall thickness. During the first month of life there was an increase in lumen diameter and by the sixth month there was the adult appearance of large lumen and thin wall.

Much interest is centred around the changes occurring at birth and the time at which the adult pattern is reached. As a method of assessing muscularity Dammann and Ferencz (1956a) in uninjected material, used ratio of lumen diameter to twice the width of media plus intima. They showed that the newborn had pulmonary arteries with thick walls like the systemic arteries. In the first two or three months of life there was a rapid thinning of the artery walls and thereafter a more gradual thinning.

Granston (1958) found a percentage wall thickness of 15 - 23% in the uninjected fetus while in the adult it was 3 - 8%, and Rosen, Bowden and Uchida (1957) using a ratio of internal diameter to external diameter found a significant thinning of the media in children over one month.

Using the same measurements on premature and newborn infants Larroche, Nodot and Minkowski (1959) found that it took ten days for the lumen to open and that it was the length of time after birth and not the gestational age which caused the change.

O'Neal, Ahlvin, Bauer and Thomas (1957) reported that in fetal life not only were the walls thicker but that muscle was found in smaller vessels as fetal life progressed. This extra muscle was lost in the first six months of life. Using planimetry in arteries under 50 um in diameter, Naeye (1961a) found that from 20 weeks of gestation to birth there was an increase in medial area related to intima in both pulmonary and systemic arteries. After birth the systemic medial area continued to rise but the pulmonary decreased rapidly, 30% being lost in the first week. In 1966(a) he showed that muscle mass decreased by a drop in the number of muscle fibres in the media, the individual cells did not lose mass.

A careful study on a series of 33 fetuses and newborn infants was reported by Wagenvoort, Neufeld and Edwards (1961) and Wavenvoort and Wagenvoort(1965b). They used the methods of measuring wall thickness and estimating medial area of Wagenvoort (1960) and showed that the percentage wall thickness was high in the fetus, 17%. There was a sudden drop in the first two weeks of life and then a gradual drop up to 18 months.

The medial area per unit area of lung had increased from 21 weeks gestation to birth and since the wall thickness remained the same it seemed that there was extension of muscle as described by O'Neal et al (1957). After birth the medial area dropped, probably due to expansion of the lung. The decrease in wall thickness at first reflected dilatation and then possibly atrophy since this would take place more slowly. They felt that it was the smaller pulmonary arteries which dilated since in fetal life the small arteries had a higher wall thickness than the larger; during the first few weeks this was reversed, the larger arteries having the greater wall thickness. Later this changed again and returned to the smaller arteries having the relatively thicker wall. This dilatation was demonstrated by Parmentier (1962) who showed using an injection technique that even high pressures would not fill capillaries at birth, but gradual relaxation of the 'arteriolar fetal sphincter' allowed easier filling after birth.

Kaufman (1965) described histological changes in the artery wall during fetal life but did not use measurement. He described four types of embryonic wall structure during development quite apart from elastic, mixed and muscular. He maintained that all vessels passed through this undifferentiated state and it was at this time that the lumen was at its narrowest.

Sylora, Greco and Ferencz (1968) using injection and measurement showed that structure was related to diameter in children as in adults, but that a larger proportion of smaller vessels contained muscle in the newborn infant. They did not discuss the thickness of the wall. Davies (1969) and Davies and Reid (1970) using the precise methods of Elliott (1964) showed that

the percentage wall thickness was high at birth and dropped to within the adult range by four months of age. They also found that muscle did not extend into such small arteries in childhood as it did both at birth and in the adult.

In this study it is hoped to use precise methods to describe the changes in fetal life and to analyse more extensively the apparent 'loss' of muscle in the intra-acinar region of the child lung.

FUNCTIONAL CHANGES AT BIRTH

In the circulation structure and function are intimately related. The changes described of the structure of the pulmonary artery have already been considered. Much work has also been reported in the functional changes at birth and the haemodynamics of the fetal circulation. These are important in relation to the abnormal circulation found in association with congenital heart disease and in the correlation of these changes to structure. It is therefore necessary to refer briefly to the more important work on the ante-natal circulation and the changes at birth.

Harvey described the difference in the heart structure in the fetus and the changes which occur after birth and he suggested the correct path of blood flow during fetal life. It was not until 1939 that the fetal circulation was visualised in vivo. Barclay, Barcroft, Barron and Franklin (1939) made radiographic demonstration of the blood flow in fetal sheep near term using a radio-opaque medium in lambs prematurely delivered.

Lind Stern and Wegelius (1964) used a similar method to

study the circulation in 12 - 24 week human fetuses and showed a similar circulation. Oxygenated blood passes from the umbilicus through the liver and the ductus venosus to the inferior vena cava and right atrium. From here it passes through the patent foramen ovale to the left atrium and thence through the left ventricle and aorta to the head and the rest of the body. De-oxygenated blood returns via the superior vena cava to the heart into the right atrium and then the right ventricle. Some of this blood - 12% according to Lind (1960) - passes to the lungs but most of it passes through the ductus arteriosus to the aorta and back to the umbilicus. Due to the high resistance in the pulmonary circulation there is very little blood in the fetal lungs at any time.

At birth there is an increase in flow to the lungs. The placenta is removed from the circuit, and the ductus venosus, foramen ovale and ductus arteriosus all close. The heart ventricles are now working in series rather than in parallel.

Much work has been done on the causes of these changes. It seems likely that the onset of breathing is the primary cause. Barron (1944) believed that the respiratory centre is stimulated by carbon dioxide excess when the placenta stops supplying the baby. Ardran, Dawes, Pritchard, Reynolds and Wyatt (1952), studying the lamb, showed that ventilation in its turn causes a decrease in pulmonary resistance and an increase in flow. This decrease in resistance was noted when the lungs were inflated with air, oxygen or nitrogen but not when they were expanded with saline. The gas inflation must play a part. They noted that after a few minutes there was a reversal of shunt through the patent ductus arteriosus indicating a rapid decrease in resistance. This shunt helps with the oxygenation by recircling the blood, as at this stage the lung

is rather inefficient (Dawes, 1959). The patent ductus also allows reversal to the fetal state if there is underventilation of the lung. It closes completely from obliteration by two months (Lind et al 1964).

Cook, Drinker, Jacobson, Levison and Strang (1963) analysed further the effect of ventilation on blood flow in fetal lambs. They found that slow inflation with oxygen and then deflation gave no great change in flow. However rhythmic ventilation caused a six-fold increase after between 8 and 30 minutes. Unlike Ardran et al they found that nitrogen inflation caused no increase in flow and they felt that the change in tone was related to oxygen and carbon dioxide tension which acted directly on the blood vessels and that the high vascular resistance was under neurohumoral control. Adams (1966) concluded that in the fetus there was neurohumoral control since epinephrine and norepinephrine cause vasoconstriction and acetyl choline and isoprenaline cause vasodilatation.

The decrease in resistance in the lungs causes an increase in flow. Everett and Simmons (1954) used isotopic tracer techniques to quantify the increase in flow in guinea pigs. They found that after five minutes of breathing the pulmonary blood volume had increased by 25% and after 16 hours by 100%. After this there was no further increase. Assali, Sehgal and Marable (1962) recorded a four to six fold increase in pulmonary flow in the lamb.

Reynolds (1956) showed that in the unexpanded lung of guinea pig the capillaries are convoluted. When the lung expands they are stretched and separated and he believed that it was this that reduced the resistance to flow. Dawes and Mott (1962) felt that

the resistance in the lamb was due to muscular tone rather than contortion of the blood vessels since resistance could be altered in utero by introduction of histamine or acetylcholine to the blood stream or by increasing or decreasing oxygen tensions. Another opinion was put forward by Lind et al (1964) who suggested that the resistance to flow in fetal life is due to the fluid in the alveoli exerting pressure laterally so compressing the pulmonary capillaries. At the first breath the formation of the gas-fluid interphase caused an inward pull on the alveolar walls, so opening up the blood vessels.

With the fall in resistance from 8,000 dynes during fetal life to 600 dynes after birth (Zeigler, 1965), the pressure falls in the pulmonary artery. Rowe and James (1957) showed that by 11 days it had fallen to normal adult values. A drop in pressure occurs within one hour of birth due to drop in resistance, but further decrease is a result of decrease in wall thickness (Lind et al, 1964).

OTHER ASPECTS OF THE PULMONARY CIRCULATION

Pulmonary Veins

Wall Structure

The veins contain relatively little muscle since they are in the connective tissue septa and hence do not have to resist respiratory movements; also the pressure within them is low (Hyman, 1966). The first accurate description of the structure of the pulmonary veins was made by Brenner in 1935. The pulmonary venules arise from the capillaries and according to Brenner their wall consisted of endothelium and an elastic membrane. Up to a

diameter of 0.15 - 0.25 mm he could not differentiate them from arterioles. From this diameter to 1 mm he described irregular elastic fibres with between them connective tissue and a few muscle cells. Wagenvoort et al (1964) stated that muscle appeared first in venules as small as 80 μm in diameter. Takino and Miyake (1936) showed these muscle fibres in the form of ridges, which they called a 'string of beads' appearance, in venules of 50 - 80 μm. As the veins increased in size the media developed a connective tissue with fine irregular elastic and smooth muscle cells; there was no demarcation between the media and adventitia (Brenner, 1935; von Hayek, 1953). According to Harris and Heath (1962) there is much less muscle in the veins than in the arteries; it is irregular in thickness with bundles of obliquely and circularly arranged muscle fibres.

Brenner also noted that the extrapulmonary part of the veins was characterised by a layer of cardiac muscle fibres in the adventitia which was an extension from the heart. These myocardial sleeves extended for varying distances with the superior veins having more cardiac muscle than the inferior. These sleeves were made up of one or more layers of muscle fibres arranged circularly, longitudinally, obliquely or in a spiral (Nathan and Eliahim, 1966). Using electron microscopy in the rat (Ludatscher (1968) has shown that the cardiac muscle is first seen in veins of 25 μm diameter.

Very little notice has been taken of vein structure during fetal life. Dammann and Ferencz (1956) noticed that in the newborn the vein walls were thinner than the adult. Wagenvoort et al (1964) and Wagenvoort (1970) believed there to be no basic difference in structure in the perinatal period. Detailed study using accurate measuring techniques is still to be made on the veins during growth

in intra-uterine life and childhood.

Though they are thin walled, there is some resistance in the veins; Rudolph (1962) and Hyman (1969) showed it to be active since pressure could be affected by vasoconstrictive drugs. They act as a reservoir since they can respond to increase in blood volume by changes in tone so that there is not necessarily any change in pressure (Hyman, Burch, DePasquale, 1964; DePasquale, Hyman and Burch, 1965). These active changes are abolished if there is bilateral vagotomy (Hyman and Sanchez, 1969).

Branching Pattern

According to Miller (1937), the pulmonary veins, unlike the arteries, 'are situated as far removed from the bronchus and its subdivisions as possible'. They run at first in the connective tissue septa between the lobules and continue centipetally to the hilum where they approach the bronchial system. Boyden (1955) has described in detail the segmental arrangement of the pulmonary veins.

The veins could be traced to four sources of origin within the lung (Miller, 1937). The first was the capillary network of the pleura, second the capillary network of the alveolar duct which gives rise to two venous radicles, the third the 'broncho-pulmonary veins' from the bronchial branching point and fourth the capillary bed of the alveolar region.

Knisely (1960), using an in vivo technique, showed that the capillaries could enter a vein at right angles, usually causing no increase in its size, as well as merging with it gradually when an increase in the size of the venule was usually apparent. Using

corrosion casts (Krahl (1962) saw no angular tributaries to the veins of the acinus while this pattern was common in artery branches. His casts also demonstrated that the veins lay in septa between lobules. Spencer (1968) states that the tributaries from the lobule join the interlobular veins opposite points where the respiratory passages divide. Apart from these no study has been made on the intra-pulmonary, pre-acinar branching pattern of the veins. Lauweryns (1968) showed an angiogram of the venous system but did not describe it in detail since he was using it to determine pressures for filling vessels.

Bronchial Arteries

The bronchial arteries are the systemic nutrient vessels of the lung. They originate from the descending aorta and supply the hilar region of the lung and the walls of bronchi and large vessels. Using an injection technique Verloop (1948) demonstrated that they supply the bronchi as far as the respiratory bronchioli and a large part of the pleura, via four main branches.

In the human the bronchial arteries appear at the ninth week of intra-uterine life as segmental branches from the paired dorsal aortae (Tobin, 1952b; Boyden, 1970). They tap the capillaries of the plexus surrounding the trachea, oesophagus and bronchi by induction. Up until this time the lung has been supplied only by the pulmonary artery. Boyden (1970) has described in a 12 week fetus three bronchial arteries to the left and one to the right lung. Along the posterior basal bronchus they could be traced as far as the cartilage had developed. Clarke (1966) showed that the vasa vasorum of the pulmonary trunk which arise

from the bronchial arteries are present from the sixteenth week and that the pattern, as at birth, is present by 34 weeks gestation. In the adult the vasa vasorum reach arteries of one millimetre diameter (Brenner, 1935).

Changes in Disease

There are two types of situation when the bronchial arteries enlarge, one when the pulmonary arteries are absent or occluded and the other when new tissue is formed in the lung (Liebow, 1967). These changes can occur rapidly. In a case with tetralogy of Fallot who died at 17 days Ferencz (1960a) described an increase in size of bronchial vessels assuming them to be normal at birth. If the bronchial arteries connect to the pulmonary artery branching pattern the blood supply can be sufficient to avoid cyanosis (Lintermans, Guntheroth and Figley, 1966). Liebow (1962) ligated the pulmonary artery of young rats inducing thereby a marked collateral circulation. He showed that there was hormonal control since cortisone would impair its development. In the same year Averill, Wagner and Vogel measured flow in bronchial arteries and found a marked increase in a case of tetralogy of Fallot of six years, where 25% of the left ventricular output passed through the collateral circulation to the lung and into the left atrium.

Broncho-Pulmonary Anastomoses

There has been much confusion and contradiction as to the presence or absence of anastomoses between the two circulations of the lung. Using injection of Berlin Blue in gelatine Miller (1937) proved conclusively that there were no anastomoses between the

bronchial artery and pulmonary artery at pre-capillary level. Surprisingly in 1948 Verloop, using gelatine and chalk, found numerous anastomoses in the human, confirmed by sectioning. He commented that there were more in the adult than the child.

Anastomoses have been demonstrated in the normal adult and child lung. Marchand, Gilroy and Wilson achieved this using vinylite casts (1950). Tobin (1952) described two types, one short (1 - 2 mm) with a 50 - 100 μm diameter and the second 10 - 40 mm long, running a spiral course between the bronchial arteries with segmental bronchi and the pulmonary artery branches. Weibel (1959), von Hayek (1960) and Wagenvoort and Wagenvoort (1967) found fewer in the adult than the fetus; Robertson (1967a and b; 1969) demonstrated the gradual appearance of anastomoses in late fetal life and early infancy when they reached 300 μm in diameter. This was followed by a gradual obliteration by fibrin and then, after ten weeks, by muscle. The last two authors also demonstrated bronchopulmonary (that is, bronchial vessels supplying alveolar walls) and pulmobronchial arteries (that is, pulmonary vessels supplying bronchial walls) which followed the same pattern of development.

Silver (1952) and Turner-Warwick (1963) both using angiographic techniques combined with histological examination, found no anastomoses in normal lungs but demonstrated them in diseases where the bronchial artery supply was increased.

Arterio-Venous Anastomoses

von Hayek (1953) in his monograph, claims to have seen arterior-venous anastomoses as had Tobin (1952b). The latter author (1966), using casts and injection of glass spheres, showed them to be

at the apex of the acinus and in the form of loops. Verloop (1948) was not able to demonstrate these in the adult nor could Wagenvoort et al (1964) although they used many specimens.

Groniowski (1960), using an injection technique, demonstrated a pre-capillary arterio-venous shunt during human fetal life which was lost as soon as the infant had breathed. There appeared to be a short cut during fetal life in addition to the normal pathway through the capillary bed.

'Sperrarterien'

'Sperrarterien' or blockade arteries were first described by von Hayek (1940) in the human lung. They are bronchial arteries with a thin media and the lumen almost obstructed by longitudinal bundles of muscle running in steep helices. He believed that they were linking vessels between the bronchial and pulmonary circulation and provided a regulatory mechanism for blood flow.

Wagenvoort (1962) commented that he had seen these vessels in normal fetuses and newborns, and Robertson (1969) believed that the anastomoses of fetuses went through a stage when they looked like 'Sperrarterien' before they were completely obliterated. They may therefore be a transitory form. Yoshino (1966) studied a large group of human newborn lungs to find these vessels. He found in the pulmonary arteries intimal cushions bulging into the lumen and almost obliterating it. He called these the 'Sperrarterien' since he found no bronchopulmonary connections. Since these cushions had disappeared by eight days, he felt that they regulated flow during fetal life in the same way as von Hayek's blockade arteries.

Heart - Ventricular Weights

Galen (Prendergast, 1928) described the differences in the heart during fetal life and the changes that took place after birth. He noted that within a few days the valve closed the foramen ovale and that the ductus arteriosus did not grow like the rest of the vessels but became thinner and eventually disappeared. Harvey (1628) described the fetal heart and commented that because of the structure of the valves the blood flows from right to left through the foramen ovale and ductus arteriosus. He states 'thus in the embryo, while the lungs are idle..... Nature uses the two ventricles of the heart as one for the transmission of the blood'. He also commented on the ventricles 'It is noteworthy that this is otherwise in the embryo, where there is no such difference between the ventricles. As in the double kernels of a nut, they about equal each other and the tip of the right reaches the apex of the left, so that the heart appears as a double pointed cone'.

It is possible to discern differences in the right and left ventricles and changes in the total size of the heart with age purely by measurement of the thickness of the wall. This method is not very accurate particularly when applied to small hearts. A more accurate method is by weighing and several methods have been described. Muller (1883) cleared the heart of all but the ventricles and then divided these into right and left walls and septum. The septum was then divided by weight giving 30% to the right and 70% to the left.

Herrman and Wilson (1922) cut the heart transversely in sections one and a half centimetres thick and cut along a line

in the middle of the septum which they believed separated the two ventricles. They then weighed the sections and derived a ratio of left to right. Fulton, Hutchinson and Jones (1952) showed that the septum behaved functionally as part of the left ventricle, they therefore weighed left ventricle plus septum together and the right ventricle and expressed them as a ratio. Their method has been used in this study.

Heart Weights in Fetal Life and Childhood

Muller (1883) published heart weights in the fetus and child. In fetuses up to 28 weeks the left ventricle was larger than the right; while up to birth the right ventricle was the larger.

Taking the weight of a whole fresh heart, Smith (1928) found that at birth it was about 20 grm, 0.98% of the total body weight. In the adult it weighed 250-294 grm and constituted 0,42% of total body weight. He found that the heart weight related to body weight not age. Later workers reverted to weighing only the ventricles. Patten (1930) stated that by four months after birth the left ventricular muscle had increased to equal the right and by two years the left ventricle was greater than the right.

In 237 fixed hearts, by separating the left and right ventricles from the septum, Keen (1955) found that at birth the right free wall equalled the left or was sometimes greater. By one month the left was greater than the right and by six months the proportions were the same as in the adult. He found that the right ventricle actually lost weight after birth and the child was one year before the birth weight was regained. He agreed with Fulton et al., that the septum grows with the left ventricle.

de la Cruz, Anselmi, Romero and Monroy (1961) measured the thickness of the ventricle walls in a series of unfixed hearts from six months gestation to 12 years of age. They found that the thickness of the left and right ventricle increased with age but the former more than the latter. The right ventricle was always thinner than the left even in premature babies.

Emery and Macdonald (1960) found, using the method of Herrman and Wilson, that under 32 weeks of gestation the left and right ventricles were almost equal. After this time until birth the right ventricle was greater than the left. This seems to correlate with the results of Muller. Emery and Mithal (1961) showed that by four weeks the left ventricle was again larger than the right, though there was a gain in the weight of the right ventricle. They did not demonstrate the atrophy of Keen. Mithal and Emery (1961b) demonstrated that this comparative increase in the left ventricle occurred only after birth. Using the same methods Recavarren and Arias-Stella (1964) showed that there was in fact atrophy of the right ventricle after birth for the first four months, when the adult ratio was reached.

Fulton et al gave the normal values for the adult as having a ratio of left ventricle plus septum to right ventricle of 2.3-3.3:1. Using this same method Davies and Reid (1970) showed that this ratio was reached by ten months but that at birth the ratio was 1.2:1 indicating a relatively large right ventricle.

THE PULMONARY CIRCULATION IN DISEASE

As commented upon earlier most quantitative work on the normal has been incidental to the study of changes that occur in the pulmonary circulation as a result of lung and heart disease, often using uninjected material. Congenital heart lesions change the haemodynamics of blood flow to the lungs and thus indirectly affect the structure of the pulmonary vessels, while lung diseases may also do this by inducing vasoconstriction and hypertrophy as a result of hypoxia.

The most common change in the circulation is the development of pulmonary hypertension which leads to right ventricular hypertrophy.

Wood (1959) defined hypertension, which normally refers to arterial hypertension, as a pulmonary pressure over 50/24 mm Hg and he stated that in severe cases it could reach systemic level but was rarely over 150 mm Hg. Resistance is a more sensitive measure of the energy necessary to produce forward blood flow through the lung; this is often used to describe the degree of hypertension. Resistance is estimated by relating pressure (P) to flow (F) as $R = \frac{P}{F}$: if they both increase as in exercise there may be little change in resistance although pulmonary artery pressure may be raised. The normal value of resistance is of the order of 2.5 units derived as follows:

$$\frac{12.5 \text{ mm Hg}}{5 \text{ litres/min}} = 2.5 \text{ units (Wood, 1952).}$$

Wood recognised three basic types of hypertension:

1. Passive (due to venous pressure) as in mitral stenosis.

2. Hyperkinetic (due to increased blood flow). If flow increases more than three times there is an increase in pulmonary vascular resistance, e.g. Ventricular Septal Defect.

3. Vaso-occlusive (due to obstruction). This may may due to obstruction from outside agents such as emboli, or to obliteration of vessel lumen as a result of structural disease or of vasoconstriction of the arteries. The latter includes the effect of hypoxaemia resulting from high altitude, obesity or obstructive lung disease.

The following report is concerned only with the work on the structure of the pulmonary arteries in mitral stenosis, an example of passive hypertension, hypoxaemia which is a vasoconstrictive disease and congenital heart disease with changing flow.

Mitral Stenosis

Parker and Weiss (1936) found that the changes in arteries in cases of mitral stenosis were intimal thickening and proliferation of cells into the lumen rather than muscle hypertrophy; these arterial changes were associated with thickening of the alveolar walls. Larrabee, Parker and Edwards (1949) found medial hypertrophy in 18 out of 20 cases. This was confirmed by Henry (1952) who showed a correlation between the amount of thickening and severity of the stenosis. Similarly Enticknap (1953) related the muscle hypertrophy of arterioles to an increase in pulmonary artery pressure. This was supported by the results of Heath and Whitaker (1955a) who also reported extension of muscle. On the other hand Goodale and

Thomas (1954) and O'Neal, Thomas and Hartcroft (1955) denied the presence of medial thickening but demonstrated intimal proliferation and fibrosis. Ferencz and Dammann (1957) using lumen to wall ratio found medial hypertrophy and intimal thickening to be of greater severity in mitral stenosis with a rise in venous pressure, than in cases with increase of flow due to congenital heart disease. This was the only condition in which they found changes in the capillaries and veins which were dilated, engorged and with intimal proliferation. Evans and Short (1957) described muscularisation of the arterioles and pruning of small arteries which, in its turn, was associated with right ventricular hypertrophy. Harrison (1958) working on injected material from cases of mitral stenosis showed medial hypertrophy and intimal thickening, both contributing to narrowing of the arterial lumen. Changes were also found in the veins.

Overall opinion is that mitral stenosis causes medial hypertrophy of varying severity probably related to the pressure that develops in the pulmonary artery. It may be that there are two reactions to the same lesion.

Hypoxaemia

Naeye and Bickerman (1959) and Naeye (1961b) noted that adults with hyperventilation due to obesity or who lived at high altitude showed arterial medial hypertrophy and dilatation of the arterial and capillary beds. The veins and bronchial vessels were normal. They thought that these changes were due to hypoxaemia and, in 1959, proved this by producing similar vascular changes in rats kept in an oxygen deficient atmosphere, or in one at a reduced pressure (equivalent to 16,000 ft).

In 1961(c) Naeye also demonstrated muscle hypertrophy with no intimal changes in cases of kyphoscoliosis who had exhibited hypertension and hypoxaemia during life. He maintained that the hypoxaemia caused vasoconstriction which later became permanent and led to an increase in vascular resistance. In 1962 Naeye and Letts and Naeye described the effect on the pulmonary vascular bed of prolonged neonatal hypoxia as in respiratory distress syndrome of the newborn. They found that the normal, infant, reduction in muscle mass did not occur and that hypoxaemia maintained the fetal structure. This increase in muscle led to hypertension with further right ventricular hypertrophy. Later Naeye (1965) proved experimentally that there was both hyperplasia and hypertrophy of smooth muscle as a direct result of hypoxia. He used dogs with one ligated bronchus so that the pulmonary artery would be normal to one airless lung. The arterial vasoconstriction in the collapsed hypoxic lung shunts blood away from it and leads to arterial hypertrophy in it. Muscle atrophied if the hypoxia was relieved. Wagenvoort, Wagenvoort and Vogel (1969) showed similar results on cattle and James and Thomas (1968) on mice who also developed right ventricular hypertrophy.

In an elegant set of experiments on the guinea pig, Tanaka, Tzen, Asai, Kai, Tanae, Nakagawa and Gagawa (1966) demonstrated that it is hypoxia and not hypoxaemia which cause constrictive changes of the arteries, and that the changes are found in the arteries running with respiratory bronchioli. They suggested that it was the direct action of alveolar gas on the vascular wall which caused this constriction.

Arias-Stella and Saldana (1962, 1963) were able to confirm

Naeye's results on people native to high altitudes. They used methods of landmarking the arteries by reference to terminal bronchioli and alveolar ducts and estimated their wall thickness, medial area and extension of muscle. When compared with people living at sea level there was an extension of muscle in all ages over one month in people at high altitude. There was also an increase in medial area giving a reduced luminal area and increased resistance which led to hypertension. Later work by Arias-Stella and Castillo (1966) showed that there was no difference at birth between babies born at high or low altitudes so that the changes reported at one month must have been acquired but might have been maintenance of the fetal state. These changes were reflected in the heart and Arias-Stella and Recavarren (1962) showed by dissection that the right ventricle was hypertrophied in all children at high altitude from immediately after birth since no drop in weight was seen, as in the controls. Two years later Penaloza, Arias-Stella, Sime, Recavarren and Marticorena (1964) demonstrated electrocardiographically that there was persistence of the right ventricular preponderance of fetal life, suggesting it was due to maintenance of hypertension in children born at high altitude.

Hasleton, Heath and Brewer (1968) put forward the interesting idea that the hypertrophy due to hypoxia was different to that found in congenital heart lesions. Studying cases from high altitude and those with kyphoscoliosis, obesity and emphysema they found no increase in wall thickness but the appearance of a muscle coat in the small arterioles, that is an extension of muscle. There was also appearance of longitudinal muscle in the intima. They offered evidence that hypoxia hypertension is reversible, if high altitude residents move to sea level or if acetyl choline is administered to such residents.

Contrary to this Semmens (personal communication) showed that in lungs whose arterial tree had been injected hypoxia due to chronic bronchitis and emphysema caused hypertrophy of the media and Davies (1969) found hypertrophy in cases of cystic fibrosis and of kyphoscoliosis.

Congenital Heart Disease

Pulmonary hypertension can develop in those anomalies where there is an increase in flow to the lungs as a result of arterio-venous communications at any level; these allow the lung to be subjected to systemic pressures. In other anomalies where the blood flow is reduced, there is a different type of pathological reaction in the blood vessels. In the work reported in this thesis the lungs have been examined in cases representative of the two groups. Past work on the lungs of cases with ventricular septal defect and tetralogy of Fallot will be considered separately. The general principles and changes related to increase in flow are first considered.

Edwards (1950) reported that arterial hypertrophy was present only when there was hypertension and it was not flow which led to arterial lesions, since most cases of ventricular septal defect and patent ductus arteriosus had no medial thickening. He believed that hypertrophy started as maintenance of the fetal state as a protective mechanism and only later was there intimal fibrosis which led to high resistance and therefore hypertension as seen in Eisenmenger's syndrome.

Dammann and Muller (1953) stressed that in any discussion of congenital heart disease associated with a communication between the systemic and pulmonary system the state of the pulmonary artery

bed must be considered. According to these authors changes in the pulmonary circulation after birth may follow one of the three following courses:

1. The fetal type of arterial wall is kept for some months after which the media thins and there is a left to right shunt with little blood reaching the body. There is a high output cardiac failure.

2. Thick walls persist and there is a balance of the blood flowing to the lungs and body. This is the optimum and is hardly ever seen.

3. Persistence of thick walls but intimal changes leading to increase in resistance and a right to left shunt.

Valenzuela, Toriello and Thomas (1954) demonstrated how thick walls could be produced when the lungs were subjected to high pressures in a case with tetralogy of Fallot and a patent ductus arteriosus. Brewer (1955) and Whitaker, Heath and Brown (1955) showed that hypertension was related to intimal changes in the arteries. Heath and Whitaker (1955b) reported cases of patent ductus arteriosus with degrees of hypertension and concluded that the higher the pressure the more severe were the changes in the pulmonary arteries. In 1957 working on cases with atrial septal defect they again found hypertrophy and fibrosis in cases with increased pressure but normal arteries where there was increase flow and no change in pressure. Wagenvoort, Neufeld, Dushane and Edwards (1961a) confirmed that an increase in flow in this anomaly gave only a slight degree of medial hypertrophy though the main change was dilatation of the arteries. Besterman (1961) showed

that only 16% of these cases get hypertension and that this was possibly due to congenital persistence of the fetal state but more likely acquired due to increase in flow. Heath, Swan, Dushane and Edwards (1958) reported an example of death due to premature thinning of walls causing heart failure, in two cases of patent ductus arteriosus. Other patients with this lesion lived longer when they maintained the fetal wall thickness.

In the same year Heath and Edwards put forward a classification of the structural changes that occur in the pulmonary arteries as a result of increase in flow and even more of increase in pressure in the pulmonary artery.

Grade 1. Medial hypertrophy of arteries and arterioles, no intimal changes.

Grade 2. Medial hypertrophy with cellular intimal proliferation.

Grade 3. Medial hypertrophy, intimal fibrosis and early generalised vascular dilatation.

Grade 4. Generalised dilatation and occlusion by intimal fibrosis. Appearance of plexiform lesions.

Grade 5. Appearance of vein-like branches of hypertrophied muscular arteries, angiomatoid lesions making a highly vascular lung.

Grade 6. Necrotising arteritis.

Heath, Helmhlz, Burchell, Lushane and Edwards (1958) reported that the grades related to increasing levels of resistance. High pressures with high flow and low resistance usually had reversible lesions (grades 1 - 3). Very high resistance with a low flow and right to left shunt had irreversible lesions (grades 5 - 6).

Heath, Helmotz, Burchell, Dushane, Kirklin and Edwards (1958) demonstrated reversibility in cases with grades 1 to 3 after closure of defects. In the same year Evans and Short looked at cases of congenital heart disease with hypertension, for the first time using vessels distended by injection. Arteriograms showed dilatation of proximal arteries and restriction of the peripheral bed. Histological examination showed intimal thickening in arteries less than two millimetres and in these vessels there was muscle hypoplasia. In the small arterioles there was medial contracture and extension of the muscle to the periphery.

After studying 106 cases of transposition with atrial septal defect, Ferencz (1966) believed that flow produced hypertrophy and therefore hypertension. Wagenvoort, Nauta, van de Schaar, Weeda and Wagenvoort (1967a) on a series of 1000 cases of septal defect correlated increase in muscle with pressure and not flow and in 1968 the same group showed that increase in flow with low pressure showed dilatation of the arteries and thinning of the media.

There is still no clear answer as to the cause of hypertrophy of muscle in the wall of pulmonary arteries - an increase in flow or pressure.

Ventricular Septal Defect

This is one of the commonest heart lesions, there are two per thousand live births and 20% of cases of congenital heart disease have a solitary ventricular septal defect (Nadas and Fyler, 1968). Much work has been published on its effect on the pulmonary circulation.

During fetal life the pulmonary and systemic circulation are in parallel and communicating at the foramen ovale and patent ductus arteriosus. The high resistance in the fetal lung ensures that little blood enters the lung but bypasses it by way of these connections. A ventricular septal defect simply forms another bypass. After birth the two circulations become separated but if there is a septal defect a connection remains and blood can flow either way depending upon pressure gradients. Normally, after birth, there is a higher pressure in the systemic system so that there is a flow from left to right with increasing flow to the lungs. The pulmonary arteries react to this increase in flow and it is with this response that we are concerned.

The first histological description of the changes in Eisenmenger's syndrome (ventricular septal defect with dextroposition of the aorta) was made by Stewart and Crawford (1933). They described, in a man of 60, arteriosclerosis of the pulmonary arteries, veins and bronchial arteries with severe thrombosis. The next case of Eisenmenger's complex was described by Old and Russell (1950). They found necrotising arteritis limited to the pulmonary arteries and suggested that this was the anatomic evidence of pulmonary hypertension and that the hypertension was the result of the heart lesion.

Civin and Edwards (1950) explained the findings as a compensation for the increase flow to the lungs because of the ventricular septal defect. The lungs maintained the fetal state to keep a high resistance to the flow. There was later medial hypertrophy and intimal thickening leading to a high resistance and reversal of flow. Goldberg, Silber, Gordon and Katz (1951) demonstrated by catheter studies that there was an increase in

pressure in the pulmonary artery in Eisenmenger's complex associated with increase in resistance.

Kroop and Grishman (1950) described three cases of simple ventricular septal defect and found dilatation of the pulmonary arteries and medial thickening. They thought that the dilatation was congenital and may have led to the medial thickening. Selzer and Laqueur (1951) were the first authors to state that there was no dividing line between the commonly seen ventricular septal defect and a child with Eisenmenger's syndrome. The latter is a more severe form with an increase in resistance in the pulmonary artery causing a right to left shunt and high pulmonary artery pressure. Wood (1958) extended this and defined Eisenmenger's syndrome as pulmonary hypertension with reversed shunt at any level due to high resistance.

Using pathological measurements of wall thickness to demonstrate their point and a series of normals as controls, Dammann and Ferencz (1956b) showed that in cases with a 'common ejectile force' there is a changing pattern depending on the flow, which is at first excessive, then diminishes as medial hypertrophy develops until the resistance to flow is higher than in the systemic system and then there is reversal of flow. In 1960 Dammann, Thompson, Sosa and Christlieb related the type of change to the size of the lesion. Adams, Lucas, Ferguson and Lillelei (1957) graded biopsies from cases according to the degree of intimal thickening and found that if it was great there was little chance of survival. Neither pressure, flow nor resistance could be related to survival and thus to the intimal changes.

A quantitative analysis of medial thickness and medial area per unit area of lung tissue on a series of cases from 26 weeks of fetal life to 12 years was made by Wagenvoort, Neufeld, Dushane and Edwards (1961b) and Wagenvoort (1962). These were compared with a control group. They found that the normals were the same as the abnormal group after birth until five weeks. At this time there was a marked increase in the medial muscle in the abnormals. Intimal proliferation was not common until one year. They believed the change to be a result of increased pressure. Edwards (1962) also believed that intimal thickening came later and that this is irreversible and causes the increase in resistance.

Using planimetry Naeye (1966b) also demonstrated increase in muscle mass in small pulmonary arteries in cases over the age of two months. This increase was in the size of individual muscle cells. Over six months he demonstrated thrombi and sclerosis.

Though much work has been done on the pathological and radiographic changes as well as by catheter studies (Deuchar and Knebel, 1952; Weidman, Dushane and Kincaid, 1963; Iverson, Linde and Kegel, 1966; Park, Nicodemus, Downes, Miller and Waldhausen, 1969; Ritter, Feldt, Weidman and Dushane, 1965) few authors have correlated results from histological and pathological examination with radiographs and catheter results. Dammann, Thompson, Sosa and Christlieb (1960) demonstrated four different reactions of the lung to ventricular septal defect using results from clinical and biopsy studies. They described two reactions to a marked increase in flow; the first when there is a low resistance leading to dilatation of the arteries and later high output cardiac failure; the second when the high flow leads to high vasoconstrictive tone and hypertrophy and eventually pulmonary resistance exceeds

systemic and there is a right to left shunt. If there is a medium sized defect the flow is still great but there is some resistance to flow leading to a pressure drop between systemic and pulmonary system so that the changes in the pulmonary circulation are not so pronounced. There is often early dilatation followed by medial hypertrophy which increases the pressure. Often when there is only a small defect the increase in flow can be handled by the pulmonary artery without any increase in pressure. The authors stressed the need for correlation and particularly the serial assessment of changes. Anderson, Levy, Naeye and Tabakin (1967) realised that the changes can be rapid in cases with ventricular septal defect and that serial studies must be made so that operation can be at the right time.

In the present study of congenital heart disease arteriograms have been used, as did Harrison (1958), and histological sections which have been assessed quantitatively, using the methods of Elliott (1964). These results have been interpreted with relation to radiographs, angiograms and catheter results.

Tetralogy of Fallot

Fallot's tetralogy (ventricular septal defect, over-riding aorta, right ventricular hypertrophy and pulmonary atresia) of the heart causes a reduction in flow of blood to the lungs. Bing, Vandam and Gray (1947) studying the clinical features of 120 cases showed that the blood flow through the pulmonary artery is reduced according to the degree of stenosis. The systemic flow in the body ranges from values just above to those below normal with the variations related to the size of shunt which is from right to left. They found that the pulmonary capillary blood flow exceeded pulmonary

artery flow, therefore there was collateral circulation to the lung. The general metabolic rate was below normal, which may help to compensate for anoxaemia.

In 1948 Rich studied the arteries of 21 patients who died at operation. He found widespread new and recanalised thrombi in both arteries and veins. He suggested that these were due to increase in viscosity due to polycythaemia and also to the slow flow and low pressure in the pulmonary arteries. Dammann and Ferencz (1956a) studied the arteries in pulmonary stenosis and found that the lumen to wall ratio was the same as in the normal: in this condition there is a low or normal flow and low or normal pulmonary artery pressure. Bronchial collateral circulation had developed in these cases. In a later study Ferencz (1960a) showed that in patients with tetralogy of Fallot aged between 17 days and 26 years the wall thickness of pulmonary arteries was normal at birth but that in older patients their lumen was larger and their wall thinner. The capillaries were congested probably due to increase flow from the bronchials, a change observed by 17 days. She found thrombi in both arteries and veins due presumably to slow flow and polycythaemia. At the same time (1960b) she found that if the blood supply was increased by a systemic to pulmonary arterial anastomosis and too large a flow produced there was medial muscle hypertrophy and intimal thickening. This was also found by Daoud, Kaplan and Helmsworth (1966). Ferencz in 1964 showed that the thrombotic lesions would disappear if an anastomosis with a good flow was made.

Heath, Dushane, Wood and Edwards (1958) agreed that thromboses were only found in cases of tetralogy of Fallot when there was a reduced flow and polycythaemia. If there was normal or high flow

in the pulmonary artery, structural changes characteristic of hypertension were also found. Granston (1958) measuring arteries found that the ratio of media to external diameter was the same in this disease as in the normal adult. On the other hand, Best and Heath (1958) showed a reduction in percentage wall thickness in uninjected vessels over 100 μm diameter from 5% in the normal to 1.75% in tetralogy of Fallot. They characterised the changes as arterial hypoplasia, widespread pulmonary artery and vein thrombosis with the development of bronchial collateral circulation.

Naeye (1961d) using planimetry, also found that even before birth there was a reduction in medial area. The systemic arteries were normal. He found no structural abnormalities in pulmonary or bronchial arteries or veins. These were all young babies however and thromboses had probably not had time to develop.

Wagenvoort and Edwards (1961) demonstrated a drop in percentage wall thickness and medial tissue per unit area of lung in two cases. This shows a definite drop in muscle not a dilatation of the arteries. The changes were present at two days and probably, therefore, before birth. They found thrombotic lesions in cases over six months and believed that muscle had atrophied due to very low pressure. A later study (Wagenvoort, Nauta, van de Schaar, Weeda and Wagenvoort (1967b) on a larger series of 117 cases, showed a thin media and increased lumen size, with many organised thrombi. The width of the lumen and thinness of the wall increased, as did the thrombi, with a rise in haematocrit value. Increase in the haematocrit reading was usually related to reduction in flow.

Fragoyannis and Kardalinos (1962) noted that in early infancy children with ischaemic lung disease had arteries of the normal external diameter with a reduced wall thickness and no

extension of muscle into smaller vessels than normal. A systemic to pulmonary anastomosis made the arteries return to normal and they suggested that it was the flow of blood which maintained the correct muscle mass.

THE PRESENT STUDY

The purpose of the present study was to extend the precise quantitative methods used by Elliott (1964) to the study of the developing circulation during fetal life. As well as the heart all aspects of the three systems of pulmonary circulation have been studied - the pulmonary arteries and veins and the bronchial arteries.

In view of the interesting findings of Davies (1969) on the relative lack of muscle in the intra-acinar pulmonary arteries during childhood an additional and detailed study was made of this region in a series of children.

Changes in the dynamics of blood flow due to congenital heart disease cause changes in the blood supply to the lungs. The precise methods that have been developed are here used to assess these changes and to compare them with the normal for the same age. Two types of heart lesion have been chosen for study, one showing an increase in blood flow to the lungs - ventricular septal defect - and the other a decrease in flow - tetralogy of Fallot. The pathological changes found can be related to the changes in the haemodynamics as assessed by the clinical studies made before death.

CHAPTER II

MATERIAL AND METHODS

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MATERIAL

Normal Lungs

To establish the normal pattern of development of the circulatory system it was essential to obtain a series of lungs and hearts from normal fetuses and children representing a range of ages.

For the early stages of intra-uterine development fetal lungs were obtained at abortion; lungs at the later gestational ages were from stillborn infants and neonatal deaths. The series covered gestational ages from 12 to 40 weeks. Successful arterial injections were made on 19 fetal lungs and successful venous injections on six. Details of the age, sex, cause of death and source of these cases are shown in Table II 1.

The lungs were considered to be normal in structure since abortion was usually carried out for psychosocial reasons and in the older cases the cause of death was unconnected with the lungs. There was no reason to believe that the lungs from babies dying with hyaline membrane disease were anatomically abnormal and this has been confirmed by this study. Gestational age was assessed using crown-rump length and weight (Arey, 1954). If there was any inconsistency between them the mean of the two ages was used.

Normal child lungs are difficult to obtain since it is essential that the cause of death is not because of lung disease. Specimens from children of four months, five years and 11 years previously studied by Davies (1969) have been used again and a further 16 specimens of normal lungs obtained from Coroner's post mortems were used for study of arteries and veins. The age, sex, cause of death and

TABLE II 1

Normal Lungs Examined (Ante-Natal)

Age in weeks	Sex	Cause of Death	Source	Vessels Injected
12	M	Psychiatric Abortion	R.M.H.	A
14	F	Maternal Diabetes	R.M.H.	A
16	M	Psychiatric Abortion	R.M.H.	A
17	F	Psychiatric Abortion	R.M.H.	A
18	M	Psychiatric Abortion	R.M.H.	A
19	F	Psychiatric Abortion	R.M.H.	A
20	M	Psychiatric Abortion	R.M.H.	A
20	M	Psychiatric Abortion	R.M.H.	A
20	F	Psychiatric Abortion	R.M.H.	V
20	M	Maternal Mumps	R.M.H.	V
23	F	Psychiatric Abortion	R.M.H.	A
26	M	Maternal Appendicitis	H.H.	A
28	M	Subarachnoid and intra ventricular haemorrhage	H.H.	A
28	M	-	H.H.	A
28	-	Atelectasis	H.H.	V
28	M	Atelectasis	H.H.	V
32	M	Intra-partum Asphyxia	H.H.	A
33	M	Respiratory Distress	R.P.H.	A
36	M	Respiratory Distress	H.H.	A
34	M	Ante-partum haemorrhage	H.H.	V
37	F	Atelectasis	H.H.	V
38	F	Ante-partum haemorrhage	R.P.H.	A
39	-	-	H.H.	A
40	F	Asphyxia	R.P.H.	A
40	M	Asphyxia	R.P.H.	A

R.M.H. = Royal Marsden Hospital
H.H. = Hillingdon Hospital
R.P.H. = Royal Postgraduate Medical School, Hammersmith
A = Arteries
V = Veins

source of these cases are given in Table II 2. This series is incomplete in that there are no specimens between five and ten years but over a period of four years no specimen from this age group has become available.

Lungs from Children with Congenital Heart Disease

Over a period of several years the lungs and hearts have been collected from a series of cases with congenital heart disease. For detailed study lungs were selected that showed either an increase or decrease in blood flow.

In the first group were six cases showing an abnormal communication between the left and right side of the heart at least at one level. Five of these cases had a ventricular septal defect, in only one case was this the only lesion. In one case there was in addition an atrial septal defect and in three an atrial septal defect and a patent ductus arteriosus. The sixth case had only a patent ductus arteriosus.

In the second group were six cases all with a diagnosis of tetralogy of Fallot. Details of age, sex, diagnosis and source of these cases are listed in Table II 3. Clinical details are presented in detail in Appendix B.

TABLE II 2

Normal Lungs Examined (Children)

Age	Sex	Cause of Death	Source	Injection
3 days	M	Meconium Ileus	Q.E.H.	A
5 days	F	Meconium Ileus	Q.E.H.	A
4 months	M	Meconium Ileus	Q.E.H.	A
10 months	M	Fire Asphyxia	West.C.	A
11 months	F	Cleft Palate	West.C.	A
18 months	M	Fire Asphyxia	West.C.	A
22 months	F	Brain Tumour	R.M.H.	A
23 months	M	Fire Asphyxia	West.C.	A
2½ years	M	Fire Asphyxia	West.C.	A
2½ years	M	Fire Asphyxia	West.C.	A
3 years	F	Fire Asphyxia	West.C.	A
3 years	M	Fire Asphyxia	West.C.	A
3 years	F	Carbon Monoxide	West.C.	A & V
4 years	M	Fire Asphyxia	West.C.	A
4 years	M	Fire Asphyxia	West.C.	A
5 years	M	Road Accident	G.H.	A
10 years	M	-	S.C.	A & V
11 years	M	Electrocution	Batt.C.	A

Q.E.H. = Queen Elizabeth Hospital
West.C. = Westminster Coroner
R.M.H. = Royal Marsden Hospital
G.H. = Guys Hospital
S.C. = Southwark Coroner
Batt.C. = Battersea Coroner
A = Arteries
V = Veins

TABLE II 3

Cases of Congenital Heart Disease

Case	Age	Sex	Diagnosis	Source
V.S.1	3 week	M	V.S.D.	B.H.
V.S.2	2 week	M	A.S.D. V.S.D. P.D.A.	B.H.
V.S.3	2 month	F	A.S.D. V.S.D.	B.H.
V.S.4	3 month	M	A.S.D. V.S.D. P.D.A.	B.H.
V.S.5	3 month	F	A.S.D. V.S.D. P.D.A.	B.H.
V.S.6	3 month	M	P.D.A.	B.H.
T.F.1	13 month	F	Tetralogy of Fallot	B.H.
T.F.2	$3\frac{3}{4}$ year	F	Tetralogy of Fallot	S.G.H.
T.F.3	$4\frac{1}{4}$ year	F	Tetralogy of Fallot	B.H.
T.F.4	$6\frac{1}{4}$ year	M	Tetralogy of Fallot	B.H.
T.F.5	8 year	M	Tetralogy of Fallot	B.H.
T.F.6	$4\frac{1}{2}$ year	M	Tetralogy of Fallot	B.H.

B.H. = Brompton Hospital
S.G.H. = St George's Hospital
V.S.D. = Ventricular Septal Defect
A.S.D. = Atrial Septal Defect
P.D.A. = Patent Ductus Arteriosus

METHODS

The methods used in this study were the same for both normal and diseased lungs except where otherwise stated.

Preparation of Specimens

Collection and Storage

The lungs were collected, fresh, as soon as possible after post mortem. They were then stored at -20°C until required for study.

Injection Technique

A similar injection technique was used for arteries and veins.

Before injection all lungs were thawed at room temperature. The fetal lungs only required two to three hours, while the child lungs were left overnight. Two hours before injection the lungs were warmed to 37°C in an incubator. They were not removed from the incubator until they were required for injection and care was taken to keep the lungs warm during the injection procedure.

A cannula was tied into the vessel selected for injection. For arterial injection in a fetal lung, the main pulmonary artery was injected through the right ventricle, the ductus arteriosus having first been identified and ligated. When only one lung was available for injection the main artery to the lung was cannulated. In the normal children either the main pulmonary artery was cannulated or each pulmonary artery separately. In the

cases of heart disease the lungs had always been separated from the heart and so where possible the main pulmonary artery was cannulated. If this had been dissected at post mortem as many as possible of the lobar branches were injected separately.

For injection of veins a cannula was tied into each pulmonary vein at the hilum, except in the fetus of 20 weeks gestation when injection was made from the left atrium.

All lungs except those of the fetuses that had not breathed were inflated with air before proceeding with the injection. This ensured that there was no obstruction to flow from kinking of the arteries.

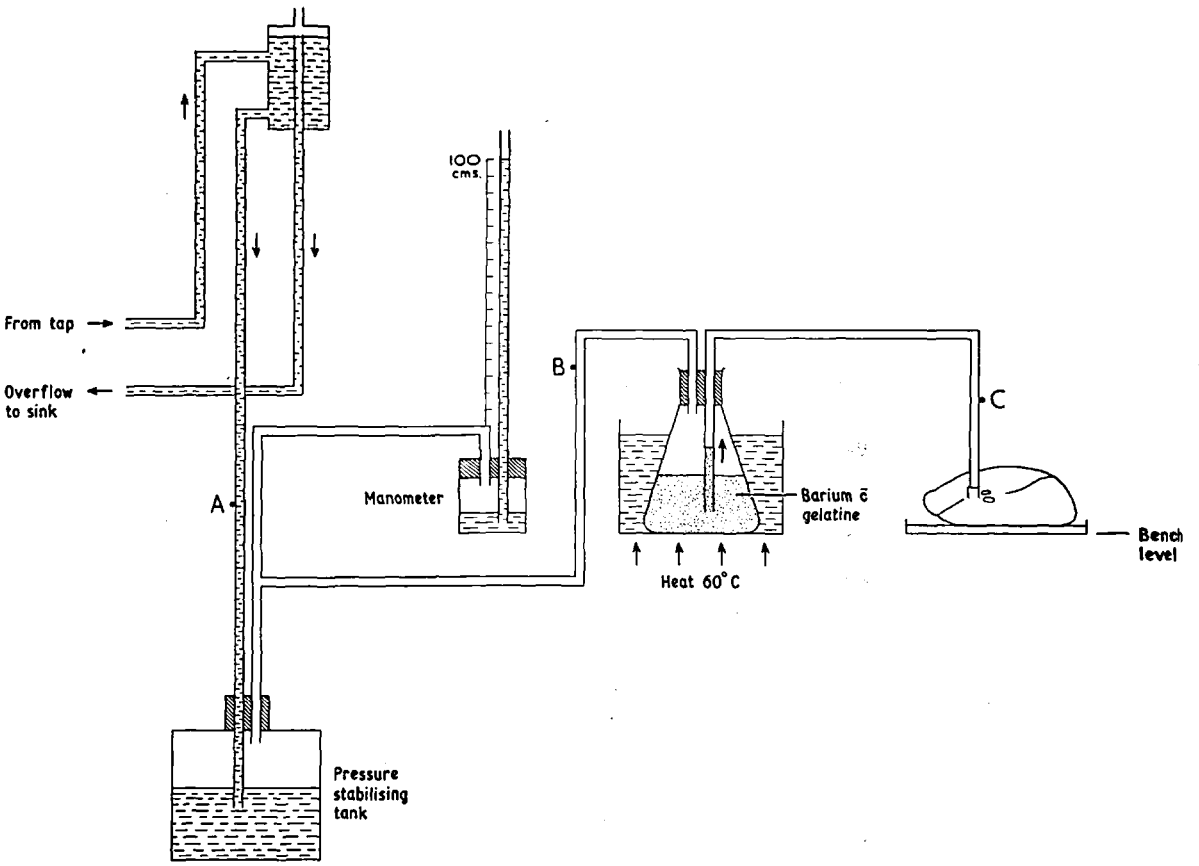
The arteries and veins were injected with a mixture of 'Micropaque' and gelatine. The medium was made up from 500 cc Micropaque (Damancy and Company Ltd), 50 gm of Gelatine dissolved in 200 cc of distilled water, and a few crystals of phenol to inhibit bacterial growth. This medium can be stored in this form for long periods of time. The mixture is solid at room temperature but at 60°C it has the consistency of thin cream.

The Micropaque is injected into the arteries and veins at 60°C and at a pressure of 100 cm of water for five to seven minutes. The pressure is maintained by a liquid reservoir and recorded by a manometer. A diagram of the apparatus is shown in Figure II 1. Any small leaks which occur during injection are clamped at the time they occur. At the end of the injection time the cannula is clamped to maintain the pressure within the lung and to prevent any leakage. The lung is allowed to cool at room temperature, so setting the micropaque, before the clamp is removed.

Figure II 1

Diagram of apparatus for injecting vessels under pressure. In the present series a barium sulphate - gelatine solution was used.

A. B. and C. are control clips.


From tap
Overflow to sink
100 cms.
A
Manometer
B
C
Barium ē gelatine
Heat 60°C
Bench level
Pressure stabilising tank

It has been found (Elliott, 1964; Davies, 1969) that using this technique vessels to a diameter of 15 μm are filled. Due to the particle size of the barium sulphate no medium passes through the capillary bed. Any reflux through veins, arteries or bronchial arteries must be due to pre-capillary anastomoses.

The high pressure used ensures that all vessels are fully distended both in normal and abnormal lungs and that contracture has been eliminated. Microscopically the elastic laminae of the media are flattened. All lungs injected by this technique can be regarded as comparable. Even in very small lungs the high pressure rarely led to any rupture of vessel walls. The same high pressure was found to be necessary for the veins despite the reduced pressure in them during life. Attempts made to inject veins at 20, 40, 60 and 80 cm of water pressure were all unsuccessful. The injection medium did not pass into the small vessels and the veins filled were not fully distended.

Fixation

Steam inflation and fixation were not used in this study. Since this method has been found to be inadequate for lungs with airways obstruction it is not used generally in this Department. Since the present study, though not on diseased lungs, was made at least in part to serve as the basis for the study of disease and also to be compared with the work of Elliott and Davies, liquid formol saline was used.

The lungs were inflated through the bronchi with 10% buffered formol saline at pH 7 (see Appendix A). The fixative was run into the lungs with a pressure of 18 inches of formalin. The lungs were suspended freely and were inflated until the pleural

surface was tense and formol saline was running out of the bronchus (see Figure II 2). This was then clamped and the lung floated in the formol saline for at least one week, when the clamp could be removed. The small fetal lungs were not inflated but fixed by immersion in formol saline.

Measurement of Lung Volume

Lung volume was estimated immediately after inflation with formol saline by water displacement. After fixation the total volume was again estimated. Before the lungs were cut each was measured separately. It was possible to estimate the amount of shrinkage in the lung due to fixation.

Radiography

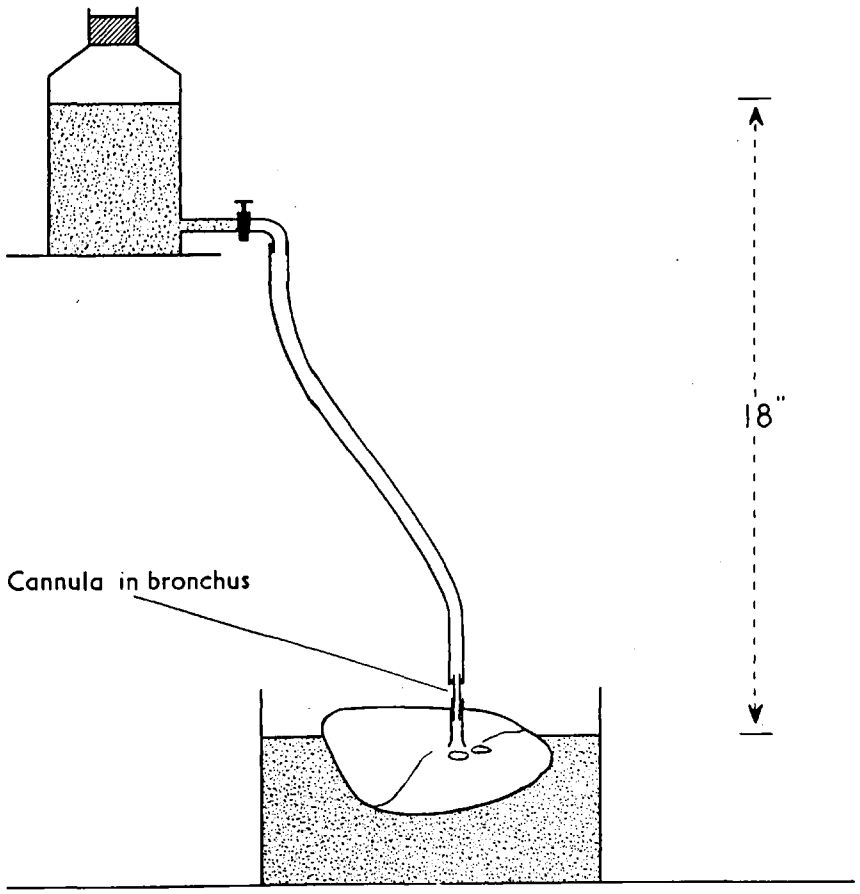
The injection medium is radiopaque and therefore the fixed and inflated lungs were radiographed using a Watson MKII portable X-ray unit. Kodirex Auto-Process film was used at a tube distance of 50 cm using 50 Kilovolts and 15 Milliamperes for 0.5 to 1.5 seconds, depending upon the size of the lung. This film resolves arteries to a diameter of 160 μm as separate lines (Millard, 1965). The films were developed by the 'X-omat' automatic process.

Some specimens were radiographed using Kodak Crystallex film at 59 Kilovolts and 100 Milliamperes. This film is of finer density and it was hoped to achieve better resolution. Results were disappointing in that although the contrast was better it was not possible to see any more vessels than before, even after magnification.

Figure II 2

Diagram of apparatus for inflation of lungs, under pressure, with formol saline.

LUNGS FIXED AFTER INJECTION BY PERFUSION WITH 10% FORMALIN UNDER 18" PRESSURE



Ilford N5.50 Line film was also used in some specimens. Exposures of 24 seconds were made with a tube length of 11 inches at 35 Kilovolts and 15 Milliamperes. The film was developed for eight minutes with five minutes in fixer. The radiographs obtained were very clear but did not appear to show up any more arteries. Turner-Warwick (1961) stated that arteries of 30 μm diameter could be discerned under magnification. The results from the line film though good could not be relied upon for tracing and counting of arterial or venous branches especially since the vessels overlay each other on the radiograph. To investigate the possibility of using the arteriograms to count branches 500 μm sections were cut and radiographed. Branches could be identified but it was felt that the results were not as reliable as those obtained by serial sectioning.

Selection of Specimens for Study

It is not always possible in the young lungs to see from external examination if filling is complete at the time of injection. In adults and normal children if the lung is satisfactorily filled, the filling medium can be seen through the pleura. In fetal lungs and some of the abnormal lungs very often there is no filling visible when the radiograph shows that it is quite adequate. The arteriograms and venograms are studied and only lungs which are well filled are selected for further study.

Evaluation of Arteriograms and Venograms

Arteriograms and venograms were appraised before measurements were made. It was possible to see the changing pattern with age and also to detect regions of poor filling.

The radiographs of the lungs from cases with congenital heart disease were examined and the general pattern compared with a normal of the same age. It was possible to detect increase or decrease in lumen diameter, changes in the segmental branching pattern, changes in the relative sizes of side branches and axial pathways, tortuosity or pruning of the blood vessels and changes in the amount of background filling.

Measurements on Arteriograms and Venograms

Measurements were made on the lungs of all cases studied. In each upper and lower lobe measurements were made of the arterial or venous lumen at the hilum and at 25% intervals along the axial pathway to the periphery of the lung and in addition the length of the pathway. In the small lungs measurements were made using a graticule eyepiece in a dissecting microscope. The larger vessels were measured using a ruler. Measurements were also made in all injected lobes one centimetre from the pleural edge. In the lungs from the cases with congenital heart disease the main pulmonary artery and the lobar arteries were also measured.

The results from the normal lungs gave an idea of the changing size and shape of the axial pathway with age. These could then be used for comparison with abnormal lungs. This method is simpler than that used by Millard (1965) and Davies (1969) on arteriograms. They estimated the lumen diameters of upper and lower axial pathways by making measurements at ten per cent intervals along the pathway.

Selection of Blocks

Block selection varied with the feature under study.

Selection of Arteries in Fetal Lungs In the small fetal lungs the whole of the left lower lobe was cut into centimetre thick blocks at right angles to the posterior basal artery. This pathway is one of the largest axial arterial pathways, is easily identified and was the one chosen for special study. In the older fetal lungs the course of the posterior basal artery was established by examination of the arteriogram together with the lung. A core of tissue around this artery was then cut from the lobe and sliced into centimetre thick blocks at right angles to the artery. In some cases blocks were taken from the left upper lobe and the right lung for comparison with the left lower lobe.

Selection of Veins in Fetal Lungs In two cases blocks were taken from the posterior basal segment of the left lung, the cut surface being at right angles to the main vein as it passed to the hilum. These centimetre thick blocks were used for serial reconstruction. Random blocks to include central and peripheral veins were taken from other lobes in these cases and throughout the lungs in the other cases examined.

Selection of Arteries in Child Lungs (either normal or abnormal) In all cases blocks were taken from each lung to include proximal and peripheral vessels. In addition in a series of normal child lungs and one case of ventricular septal defect and two of tetralogy of Fallot, a block was taken for serial reconstruction. In these cases a block was taken from the diaphragmatic surface of the

posterior basal segment of the lower lobe approximately one centimetre deep. It was ensured that part of an axial pathway was included in each block.

Selection of Veins in Child Lungs (either normal or abnormal)

Blocks were taken from all regions of the lung to include veins in all diameter ranges. In addition blocks were taken from the periphery of the posterior basal segment one centimetre from the origin of a main vein, for reconstruction of the peripheral drainage pattern.

Preparation of Tissue

Processing All tissues were processed on a Histokinette automatic processor. A 24 hour schedule was used and the tissue was dehydrated through graded alcohols, cleared in chloroform and impregnated with wax.

Sectioning All blocks were cut using a Leitz base sledge microtome. Sections were cut of a thickness of 7 μm.

The blocks of the posterior basal artery or vein in the fetus were cut serially and every section mounted, usually with four being included on each slide. In the older fetuses, it was possible to use step sections through the hilar blocks. One section was mounted every 50 or 25 μm depending upon the size of the lung, the rest of the sections then being discarded. The centimetre blocks from the periphery of the child lungs were cut and mounted as complete serials. Four sections were cut from each random block and each mounted on a separate slide.

Shrinkage Due to Processing During processing and cutting there is shrinkage of the block. This can be estimated by measuring the block before processing and the section of the slide after staining. The shrinkage is mainly in the alveolar region.

Frozen sections were cut from several blocks containing injected arteries. The blocks were then processed, cut and mounted in the usual manner. The arteries of both sets of sections were measured. It was found that though the sections were smaller after processing, there had been a negligible amount of shrinkage of the injection medium and hence of artery lumen. No correction factor was used in the study of the arteries or veins.

In this series, in many cases lung volumes were estimated only after fixation. All volumes therefore, refer to fixed volume and no allowance has been made for loss due to fixation. It is not possible to estimate how much vessels shrink during fixation.

Staining All sections taken from random blocks and in the series of step sections were stained with Verhoeff's elastic stain (Culling, 1957) and counter-stained with Van Gieson's stain (Department modification, see Appendix A). Where a complete serial had been cut alternate slides were stained with elastic Van Geison (E.V.G.) and Ehrlich's Haematoxylin and Eosin (Culling, 1957).

Choice of Stain

Fetal lung tissue differs from adult lung tissue in that it has a much higher water and glycogen content.

It was found that a standard Verhoeff's E.V.G. stain did not stain the fetal material as effectively as it did in the adult. It was therefore necessary to attempt either to alter the E.V.G. or to try to find another stain which would show muscle and elastic in the fetus. Any new stain had to give results which would be comparable with the adult.

A series of tests with different stains was made using fetal and adult material.

The Verhoeff's elastic Van Gieson stain gave poor results even with adjustments to the staining procedure such as altering the length of time of staining both for the elastic and muscle stain and the amount of differentiation used for each.

The main difficulty was that the muscle could not be discerned between the elastic fibres. It was felt that the Verhoeff's elastic stain was masking the Van Gieson in these small arteries. Elastic stains which give finer definition were therefore tried. Both Hart's (Culling, 1957) and French's (Drury and Wallington, 1947) modification of Weigert's elastic stain were investigated. The results were no better than with Verhoeff's stain and these were not improved by variation of time and concentration of stain used. Since these stains were not as effective on the adult tissue it seemed better to carry on using Verhoeff's elastic stain and attempt to find a more efficient counterstain for muscle.

Martius Yellow, Brilliant Crystal Scarlet and Aniline Blue (Lendrum, Fraser, Slidders and Henderson, 1962) were used, with or without an elastic stain, but still no muscle could be discerned.

Verhoeff's elastic counterstained with Masson's trichrome (Culling, 1957) gave a very good red muscle stain on adult tissue. However, when it was used on fetal material it was not effective, particularly after counterstaining with Light Green. Post-fixing with 2.5 acidic potassium dichromate (Zenker's fixative) for two to three hours improved the staining of the muscle but the stain became less specific.

Lendrum's Acid Picro-Mallory stain (Culling, 1957) which stains fibrin red and collagen blue was effective on this material, but is difficult to use on large numbers of sections and only by inference is muscle identified.

In older fetuses muscle was more easily identified. It seemed that in young fetuses muscle was present but immature. It was decided, since the new stains gave no better results, to stain the sections with Verhoeff's elastic Van Gieson and Haematoxylin and Eosin.

Microscopic Studies

Reconstruction of Arterial and Bronchial Branching Pattern

A Gillett and Sibert Conference Microscope was used for examination of all slides.

For the reconstruction of the arterial pathway all sections of the series were examined. The main axial pathway was plotted schematically from hilum to periphery on graph paper, one millimetre square being the equivalent of one 7 μm section. In the fetus the arterial filling medium reached but rarely went beyond the terminal bronchiolus and therefore reconstruction of

of the filled arteries could be made from the hilum of the lobe to the terminal bronchiolus. In the child lung reconstruction was made to the end of the filling medium at the level of alveoli; the whole acinar branching pattern was traced.

Branches were identified and recorded as they left the main arterial pathway and an accurate plan of the bronchial branching pattern was built up at the same time. It was possible to relate the branching pattern of the artery and airway and therefore distinguish conventional from supernumerary arteries. These types are defined on page 107. The original diagrams were later scaled down and the airways drawn alongside their related conventional artery (see Figure III 21). Lateral branches could be traced in a similar manner to the axial pathway.

While tracing the pathways the structure and size of the airways and arteries was noted. The arteries were measured using a calibrated graticule scale in a Zeiss focussing eyepiece. This method is described in detail below.

The centimetre blocks from the older children were analysed in a similar manner. An axial pathway with its accompanying artery was traced distally, each section being represented on the chart. It was possible to identify the type of bronchiolus and therefore delineate the lobule and the acinus, and relate the arteries to these structures.

In two cases the axial pathway was entirely reconstructed for a second time after a three year interval. It was found that the results of the two reconstructions were the same, showing the method to be reliable.

Reconstruction of the Venous System

A similar tracing to that described above was made of a main vein and its tributaries from periphery to hilum in two fetal lungs of different ages, and of the peripheral centimetre in two children. As before, the size and structure of each vessel was noted and the region drained by each vein.

Structure of Walls of Arteries and Veins

While tracing the pathways of arteries and veins the structure of the walls was noted, both the main and side branches. It was possible to relate structure to the size of a given vessel and also to its position in the lung, and in the case of arteries to the position and type of its accompanying airway.

The basic structure of the media of arteries was noted, whether elastic, transitional, muscular, partially muscular or non-muscular. (Definitions of these terms are given at the end of this chapter.) The intima was examined in normal and diseased lungs and any changes noted.

The structure of the vein wall was described, commenting upon intima, elastic and muscle configuration in the media and adventitia with special reference to changes of age and of disease.

Measurement of Wall Thickness and External Diameter - Arteries

Measurements of the total diameter and thickness of the media of a vessel were made along the main pathways and on vessels picked at random through the rest of the lung. It was possible to compare vessels of the main pathway with other vessels in the same lobe and to compare vessels from different lobes and lungs.

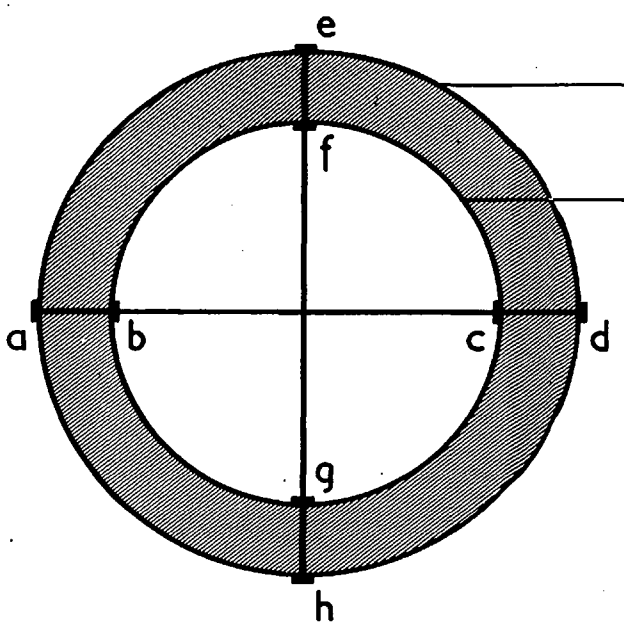
Measurements were made using a calibrated graticule eyepiece. A factor was determined for each magnification to convert units of the scale to um. The measurements were made using the highest magnification with which it was possible to view the whole of the structure being measured; this leads to greater accuracy. There is slight variation in measurements using different objectives, therefore the same one was used as far as possible so that the effect would be minimal. The wall thickness was always measured using an x40 or x100 objective.

External Diameter The external diameter was taken as the distance between the outside of the two external elastic laminae intersected by the diameter. When the arteries are fully distended the elastic laminae are flattened and these measurements can be made accurately. If the vessels are not completely injected the laminae are not smooth and the point of measurement could not be standard. When the vessel is cut in true cross-section it is possible to make two measurements of external diameter at right angles. Many of the vessels to be measured were cut obliquely however, so that they appear oval in cross-section. When a tube is cut obliquely the narrowest dimension of the oval is the true diameter (Davies, 1969). It was therefore possible to measure diameter on all vessels whether cut obliquely or at right angles to the axis.

Wall Thickness This is measured from the inside of the internal elastic lamina to the outside of the external elastic lamina. When a vessel was cut in true cross-section four measurements were made where the diameters for measurement of external diameter crossed the media; these could then be averaged (Figure II 3).

Figure II 3

Diagram to illustrate the method of measurement of external diameter and wall thickness in arteries cut in cross-section (Elliott, 1964).



External elastic lamina

Internal elastic lamina

ad, eh, external diameter

ab, cd, ef, gh, wall thickness

When the vessel was cut obliquely two measurements were made at the point of intersection of the smallest diameter.

Percentage Wall Thickness Using the average figures for diameter and wall thickness it is possible to calculate the percentage wall thickness. This is the total wall thickness expressed as a percentage of the external diameter. This value is derived from the equation:

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

Medial Area Using the same figures it is possible to calculate the area of the media. This is equal to the total area of the artery minus the area of the lumen. The calculations for this method are long and arduous. Medial areas were estimated along the main artery in those fetal lungs where a serial reconstruction was made. Since the results showed essentially the same features as the percentage wall thickness further calculations on arteries from random sections and from other cases were not made.

Measurement of Wall Thickness and External Diameter - Veins

There is no definite external elastic lamina delineating the outside of the media in the veins. Collagen is mixed with muscle and increases towards the external edge. In the veins measured the last muscle fibre was taken as the outer limit of the media. Measurements of external diameter were made between the extreme limits of the muscle on each side of the vein. Wall thickness was measured from the inside of the internal elastic lamina to the outer limit of the muscle cells. Percentage wall thickness was then estimated in the same manner as for the arteries.

Reliability of Method of Measuring Wall Thickness

Repeatability of Measurements All the arteries from one section were measured and their structure noted. Measurements on the same section were repeated six months and two years later. The results showed little variation; although there was a slight difference in absolute measurement doubtless due to variations in the part of the arterial wall measured, the percentage wall thickness was in the same range. The identification of structure of the artery wall was always the same.

Adequacy of Sampling It was necessary to make sure that the arteries measured were representative of the whole lung. Several sections were taken from the same block and all arteries measured. It was found that all sections gave similar results and fell within the same narrow range. When different blocks from the same lobe were compared, it was again found that all figures fell within the same range. Blocks from different lobes showed no statistical difference. This confirms work by Simons and Reid (1969) on the adult. Although in a normal lung any section was representative of the whole, several sections were used in all cases to give a larger number of arteries to measure. Ranges in the normal were narrow and abnormal lungs usually showed results well outside these ranges.

Population Studies

In the adult, in larger arteries the wall structure is related to the external diameter. It is possible to predict the wall structure if the diameter is known. However below a certain level, 150 μm in the adult (Elliott, 1964), there is a

mixed population in each size range.

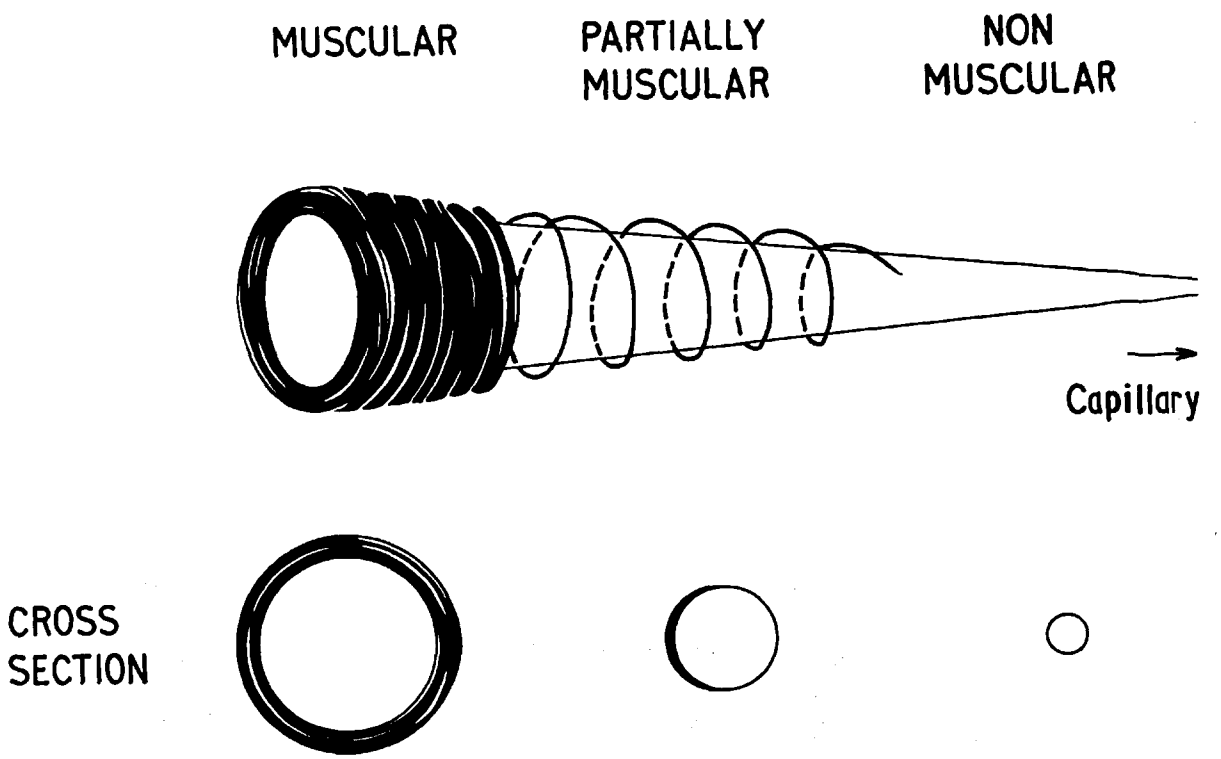
Along any arterial pathway the wholly muscular coat gives way to a spiral of muscle before it finally disappears and the artery becomes 'non-muscular', though still pre-capillary. The arteries with a spiral of muscle are called 'partially muscular' since in cross-section the muscle appears as a crescent in part of the wall (Figure II 4). The diameter at which the changes in wall structure occur varies from pathway to pathway. It is possible to estimate the proportions of each type of vessel in any one size range by examining a large number of arteries. The result of this method is termed a 'Population Count' (Elliott, 1964). These counts showed that the extent of muscle along the arterial pathway varied with age and disease.

A population count entails scanning the whole of a section and measuring all arteries below 400 μm. The external diameter is measured and the type of wall ascertained. Up to 150 μm the arteries were grouped by size range at 25 μm intervals and above this at 50 μm intervals. The percentage of each type of artery in each size range could be calculated and the results plotted graphically (see Figure IV 10). At least 200 arteries were examined in each case. Where possible a larger number, up to 500, were measured to give smoother curves to the distribution graph.

When making a population count sections from throughout the lung were included to ensure that a representative population was being studied. In the young fetuses arteries in this size range were found accompanying bronchioli and small bronchi, while in the lungs of children they were all within the acinus. It was

Figure II 4

Diagram to illustrate the changing structure of the pulmonary artery wall at the distal end of any pathway. A muscular wall structure gives way to a spiral of muscle before finally disappearing. The spiral of muscle appears as a crescent in cross-section. The non-muscular arteries are still pre-capillary.


MUSCULAR
PARTIALLY MUSCULAR
NON MUSCULAR
Capillary
CROSS SECTION

possible to estimate the total percentage of each type of artery in the population studied as well as that for each size range.

Arteries with Airways

Arteries can be identified by their position in relation to recognisable airways. This can be done both by serial reconstruction and on random sections. By these methods it is possible to determine structure and size of comparable arteries and these can be compared between cases. Landmarking of arteries in random sections is particularly useful when comparing normal and diseased lungs since serial reconstruction is time consuming and this can be done using a few microscope sections. This method was used by Elliott (1964) on the adult and in part by Davies (1969) on children.

Using random sections it was possible in the present study to measure arteries accompanying terminal bronchioli, respiratory bronchioli, alveolar ducts and alveoli. It was possible to recognise in cross-section the last three by their structure. Terminal bronchioli could only be identified when they ran into respiratory bronchioli and both were cut in longitudinal section. Results from random sections were compared with those from serial reconstruction and in the normal it was found that results were similar.

Number of Arteries and Alveoli per Unit Area

Wagenvoort (1960) estimated arterial muscularity in a lung section by measuring the medial area of all arteries within a given section area. This method was adapted by Davies (1969) who grouped

the arteries into size ranges and estimated the medial area of an average artery in each size range. This was then multiplied by the number of arteries in each size range and an index of medial area estimated.

This method has been further simplified here and used only as an estimation of the number of arteries of each size range in an area of lung. In each case an area of one square centimetre was marked out on a microscope section. In some cases only one section was used and in others several. Hilar areas were avoided. The total number of arteries below 200 μm in diameter was recorded. The arteries were divided into groups by size of 25 - 50 μm, 50 - 75 μm, 75 - 100 μm, 100 - 150 μm and 150 - 200 μm external diameter. The percentage falling into each size range was estimated. Over the same area the number of alveoli per square centimetre was also estimated. The total number in a microscope field was counted, the average of ten such fields was taken and the number per square centimetre calculated. It was then possible to determine a ratio of the number of alveoli to small arteries in a unit area. This was estimated for the normal at different ages for comparison with the abnormal cases. It was assumed, when counting alveoli in a given area, that there was the same shrinkage in each case as a result of fixation and processing and that all had been fully inflated.

Number of Veins per Unit Area

The number of veins per unit area was estimated in the same way as the number of arteries.

Estimation of Ventricular Weights

The method of Fulton, Hutchinson and Jones (1952) has been used for measuring ventricular weights. Millard (1965) showed that the heart lost about five per cent of its weight in the first four days of fixation in formalin and that thereafter the weight remained constant. All hearts were therefore left at least one week in formol saline before being dissected. No correction was made for initial shrinkage since all hearts were treated in the same way and comparisons were based on the ratio of ventricular weights.

The atria and vessels were dissected from the ventricles. The valve leaflets were removed and any fat stripped off. The ventricles were then separated; a cut was made in the angle between the right ventricular wall and the septum at each side. The free right ventricle wall was removed leaving a complete left ventricle. Any papillary muscles on the right side of the septum wall were removed and grouped with the right ventricle. The ventricular muscle was weighed in two separate portions, the left ventricle plus septum and the right ventricle. The ratio of the left ventricle plus septum to the right ventricle was calculated.

DEFINITIONS

The definitions of some terms employed frequently in this study are given below.

Airways (Reid, 1950)

Axial Pathways These are the pathways which run from hilum to the distal surface of a segment. They include the longest pathways in each segment. Some segments have longer pathways than others. Lateral pathways supply regions between the hilum and the distal pleural surface but are not as long as axial pathways.

Generations A generation lies between two successive branches of a bronchus or artery. Any airway or artery is given a number which refers to the number of generations or branches that separate it from the hilum. The first part of the pathway is the first generation; the first branch and the continuation of the parent structure are known as generation two and so on. The convention for intersegmental comparison is to count the segmental structure as one.

Bronchi Bronchi are those airways lying proximal to the last plate of cartilage along an airway.

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

Terminal Bronchiolus The terminal bronchiolus is that immediately 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 the lung distal to a terminal bronchiolus. It includes all airways with alveoli opening into them, alveolar ducts and alveoli and is therefore the respiratory part of the lung.

Lobule A lobule is made up of a group of acini, and their terminal bronchioli which cluster at the end of any pathway.

Arteries (Elliott, 1964)

Conventional Arteries Conventional arteries are those which accompany an airway.

Supernumerary Arteries Supernumerary arteries are those arteries which arise as side branches from the main artery but do not have a corresponding airway. They eventually join some type of respiratory structure either airway or alveolar. They increase the number of branches from the parent artery relative to the parent bronchus.

Elastic Artery Elastic arteries are the largest found in any lung. The media is made up of seven or more concentric elastic laminae with muscle cells and collagen between. The media is delineated by an internal and external elastic lamina (see Figure II 5). It may be that these vessels are not very distensible since an elastic fibre stretches only one third the amount of a muscle fibre and has a high tensile strength. It maintains the tension in the blood vessel wall to keep it open (Burton, 1954). The main pulmonary arteries, the hilar vessels and the proximal airways have an elastic wall structure.

Transitional Artery A transitional artery is similar to an elastic artery but contains only five or six elastic laminae between the internal and external elastic lamina (see Figure II 6). This type of artery is found between the elastic and muscular vessels along any pathway.

Figure II 5

Photomicrograph of the wall of an elastic artery from a normal five year old child. There are numerous thick elastic laminae with muscle cells in between (for further description see text). (x300).

Figure II 6

Photomicrograph of the wall of a transitional artery from a three month old infant. Six elastic laminae are seen between the internal and external elastic laminae (for further description see text). (x590).

Figure II 5

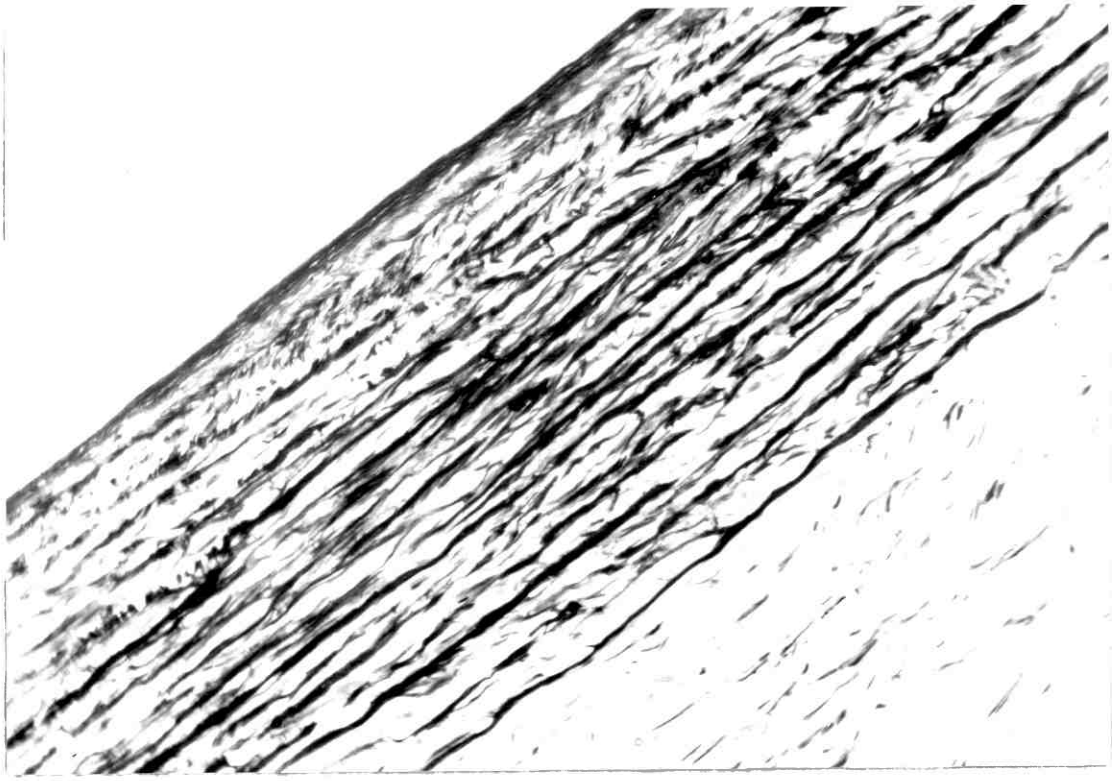
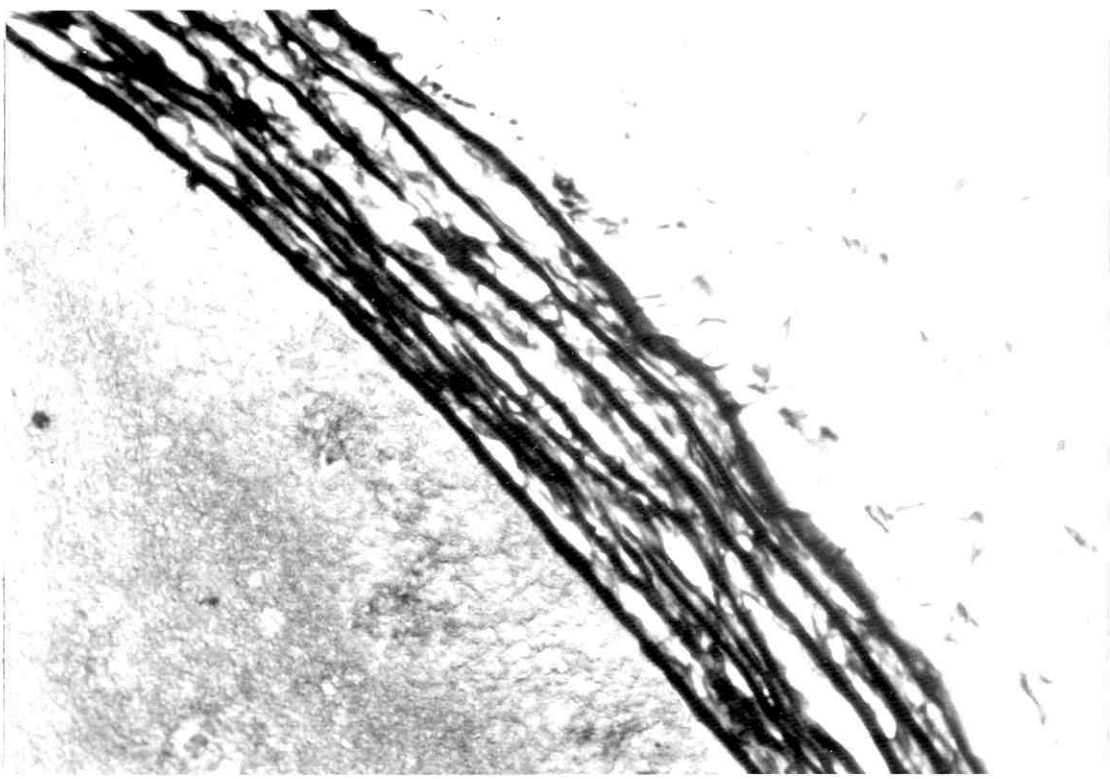


Figure II 6



Muscular Artery The media of a muscular artery consists of a continuous muscle coat limited by an internal and external elastic lamina. In larger muscular vessels there are up to four additional but fragmentary elastic laminae between these two (Figure II 7). They form the majority of the vessels in the lung.

Partially Muscular Artery A partially muscular artery is one that in cross section has a crescent of muscle between two elastic laminae. This muscle is not continuous round the whole of the vessel and the rest of the wall is either a single elastic lamina or collagen. This crescent of muscle is due to the muscle layer at this point consisting of a spiral of muscle not a continuous sheet (Figure II 8).

Non-muscular Artery A non-muscular artery is one that is larger than a capillary but has no muscle in its wall. The wall is made up of collagen with or without a single elastic lamina. This single elastic lamina may represent the previously external (Brenner, 1935) or internal (von Hayek, 1960).

Arterioles The term arteriole has not been used in this thesis. Anatomists have given many different definitions of this small artery, some in terms of diameter and some by structure. The term usually refers to the small pre-capillary arteries. Since diameter varies with the state of the lung it would seem better to define by structure. Because of the confusion over the term arteriole it seems better to use the descriptive terms of muscular, partially muscular and non-muscular arteries.

Veins

The veins are described and defined in Chapter V.

Figure II 7

Photomicrograph of the wall of a muscular artery from a normal ten year old child. There is a clear internal and external elastic lamina with a muscular media between, containing fragments of elastic fibrils. Nuclei of the muscle cells are also visible (for further description see text). (x590)

Figure II 8

Photomicrograph of a partially muscular artery from a four month old child. The muscle of the media is discontinuous at the point marked by an arrow (for further description see text). (x675)

Figure II 7

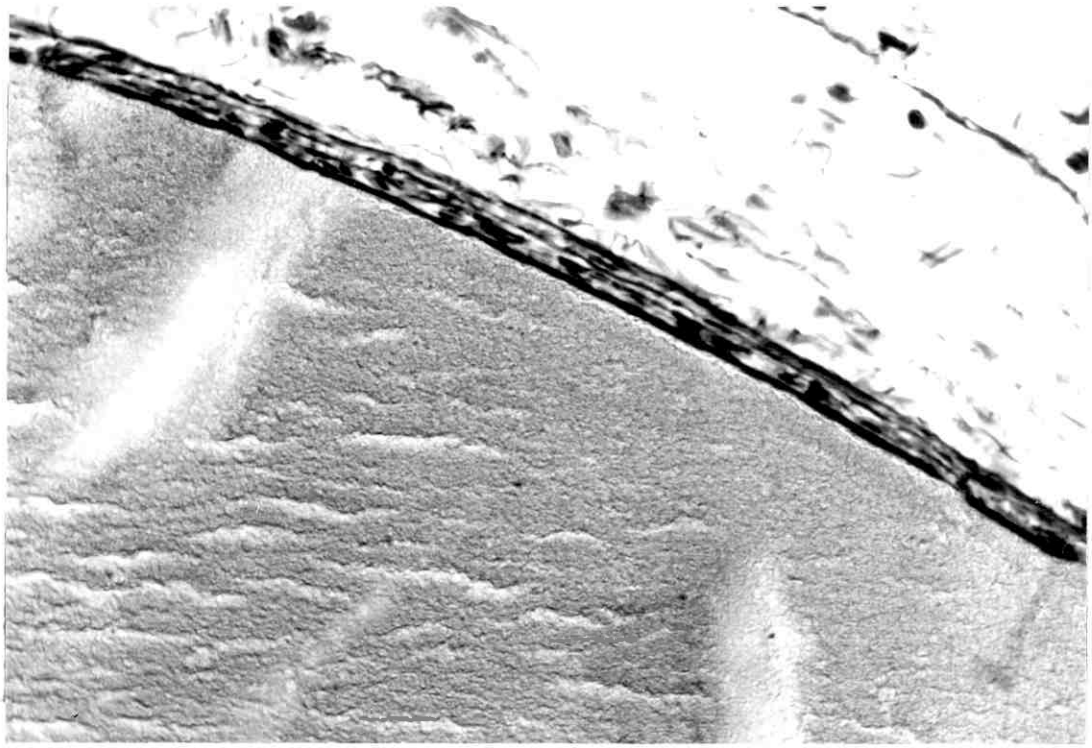
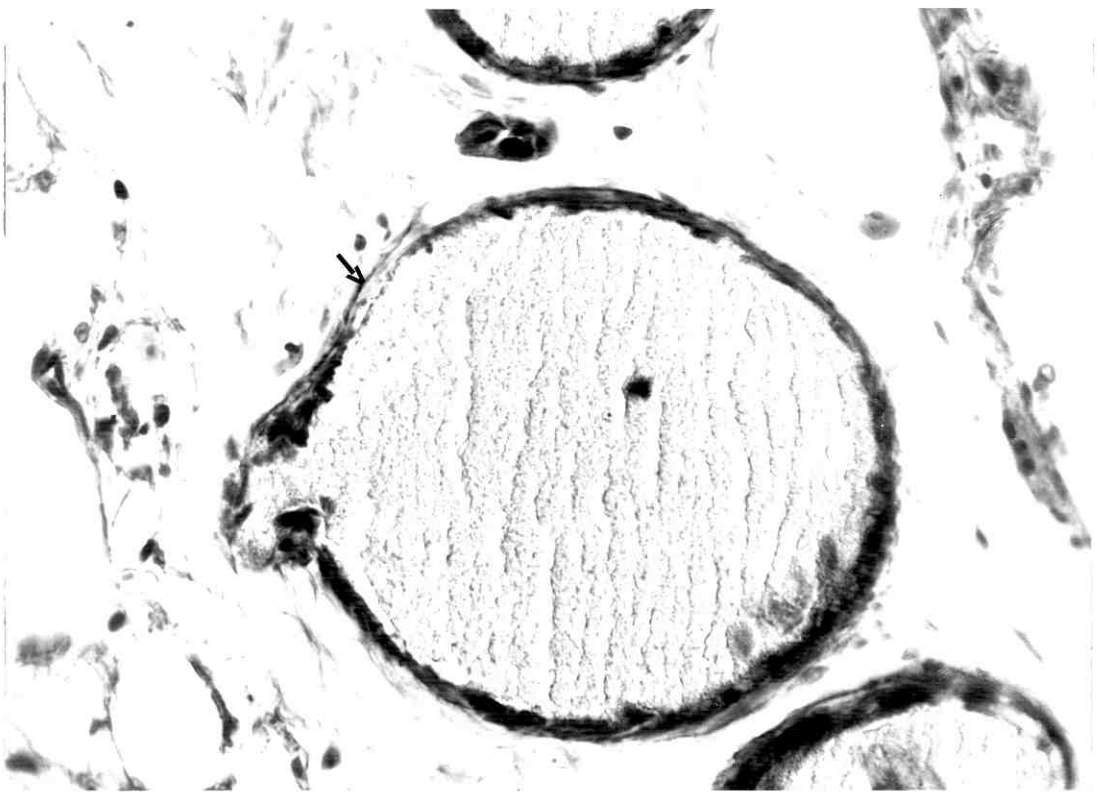


Figure II 8



CHAPTER III

DEVELOPMENT OF THE PULMONARY ARTERY BRANCHING PATTERN

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Introduction

The development of the pulmonary arterial system has been studied by two different but related methods. The first method is to reconstruct the branching pattern of the arteries thus creating a three dimensional picture of shape and size of the arterial tree at different ages: the second is to relate arterial structure to size by studying random sections.

Material and Methods

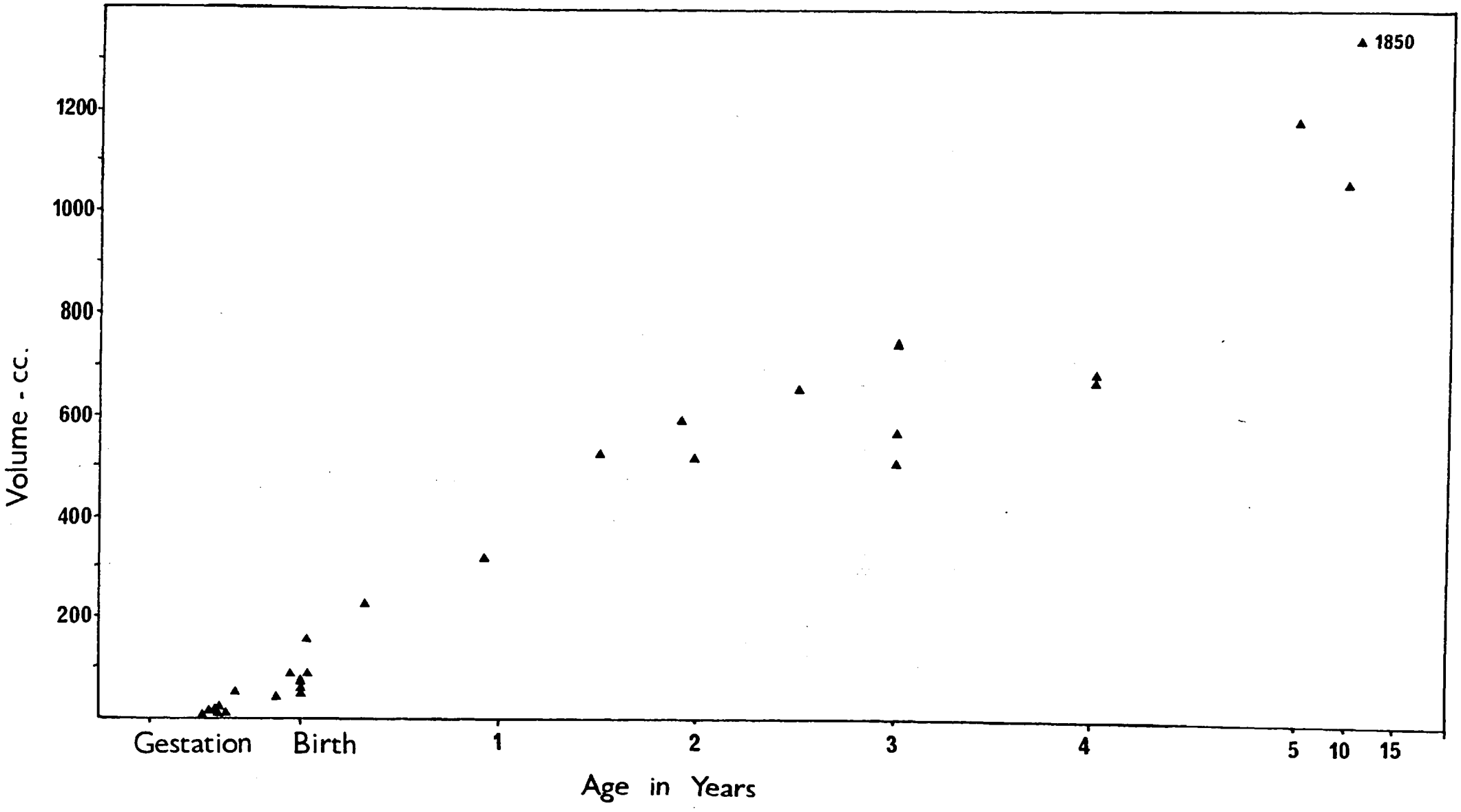
A series of lungs from fetuses of 12 to 40 weeks gestation and from children of three days to 11 years have been studied. Details of the material are given in Tables II 1 and 2. The specimens used for each study will be listed with the results. Three of the specimens - from children of four months, five years and eleven years - had been used previously by Davies (1969). Since normal material is extremely difficult to obtain, new material was taken from these lungs for the present study.

LUNG VOLUME

Vessel development was related to the increase in total lung size. The total fixed lung volume was measured by water displacement, the younger fetal lungs being uninflated and the older fetal and child lungs having first been inflated with formalin. Figure III 1 shows the total lung volume plotted against age. The volume increases steadily with age. The figures for the three children used by Davies (1969) are taken from his work since the lungs were cut and not available for further measurement.

Figure III 1

The total lung volume measured by water displacement plotted against age. The scale changes at five years of age.


1850
1200
1000
800
600
400
200
Volume - cc.
Gestation
Birth
1
2
3
4
5
10
15
Age in Years

The volume increased from 4 cc at 14 weeks gestation to 64 cc at birth, a 16 fold increase in 26 weeks: by ten months there was a further increase to 320 cc, a five fold increase. There is a drop in the rate of increase after birth. By 22 months there had only been a two fold increase, a further drop in the rate. By 11 years the volume was 1850 cc, a three fold increase in nine years. Though there is a steady increase in volume the rate of increase is slower during childhood than in utero.

ARTERIOGRAMS

Successful arteriograms were made of all cases; only a few are illustrated here and are representative of the whole series (Figures 2 - 10). The smallest lungs that it was possible to inject successfully using the present method were those of a 12 week old fetus. In this arteriogram the main branches of the pulmonary artery to the level of the subsegmental branches can be seen, though at this time they are very fine. Smaller branches, that is those below 160 um (Millard, 1965), cannot be discerned on the arteriogram because of the grain size of the film. By 14 weeks there has been an increase both in size and number of vessels at the periphery. This increase continues so that in the arteriograms of a 20, 23 and 32 week fetus the adult pattern of branching can be seen, in miniature, with the arteries tapering gradually towards the periphery. By 39 weeks the number of small arteries at the distal end of the pathway has evidently increased enough to give rise to a slight background haze. Five days after birth the pattern is the same but by ten months peripheral filling is profuse and the background haze obscures the clear lines of

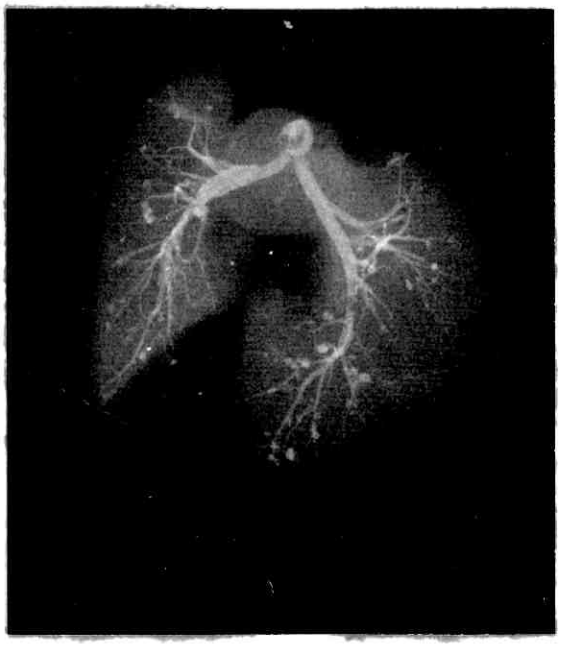


Figure III 2

Arteriogram of a

12 week fetus (x 3)

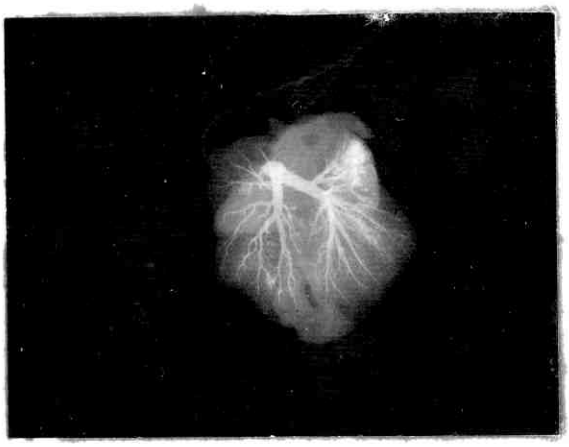


Figure III 3

Arteriogram of a

14 week fetus (x 1)

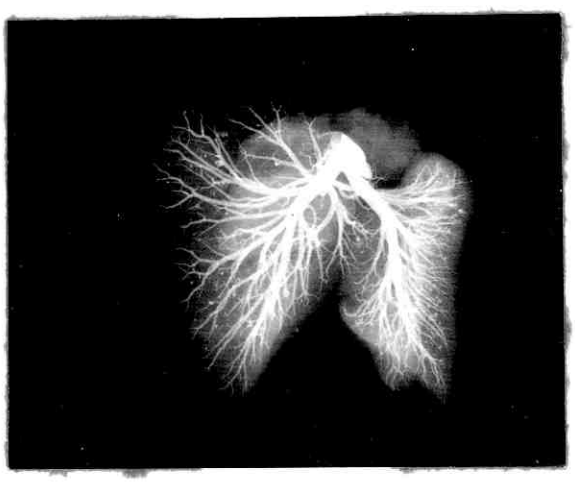


Figure III 4

Arteriogram of a

20 week fetus (x 1)

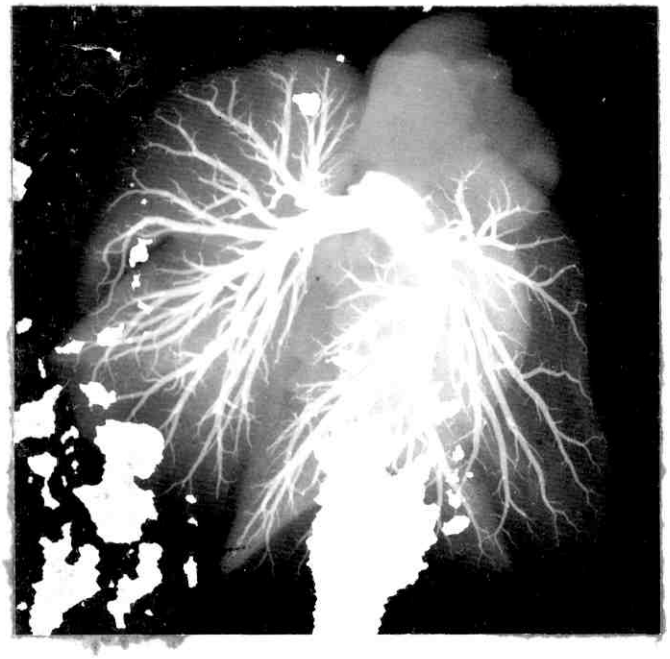


Figure III 5

Arteriogram of a

23 week fetus (x 1.4)

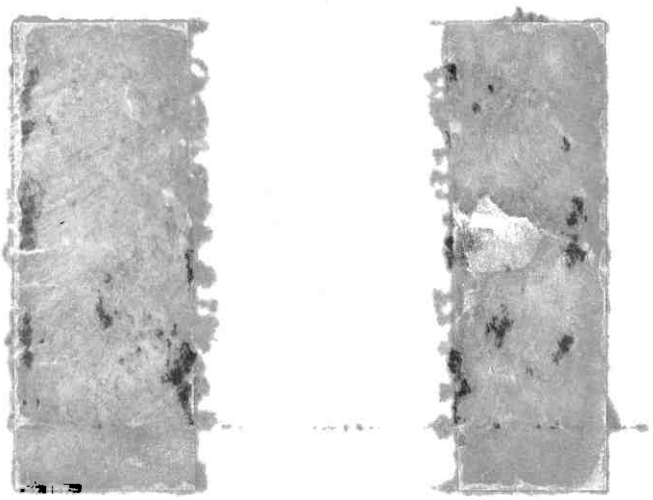


Figure III 6

Arteriogram of a

32 week fetus

(left lung x 1)

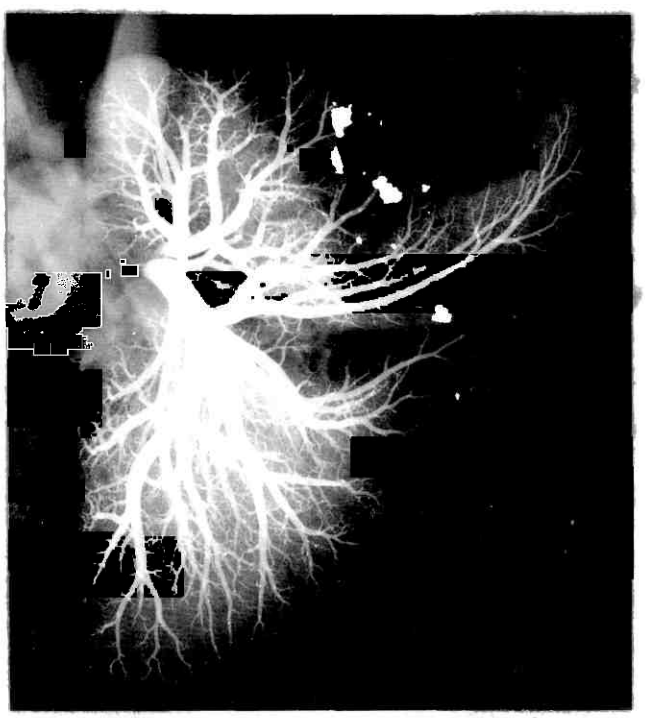


Figure III 7

Arteriogram of a

39 week fetus

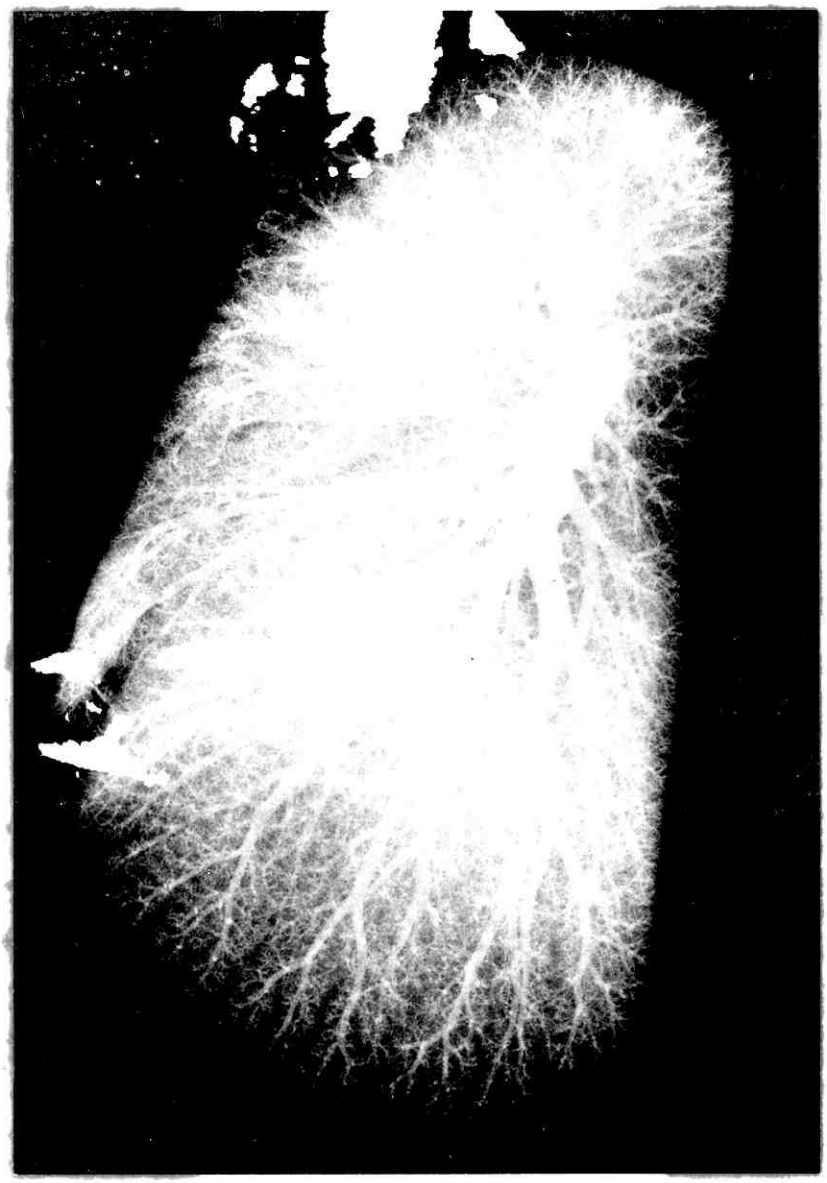
(left lung x 1)

Figure III 8



Anteriogram of both lungs of a normal infant aged five days (x 1).

Figure III 9



Arteriogram of the left lung of a normal child aged ten months (x 1.1)

Figure III 10



Arteriogram of the right lung of a normal child aged ten months (x 1.2)

Figure III 11

Arteriogram of the left lung of a normal child aged 18 months, sibling of the case shown in Figure III 12 (x 0.75)

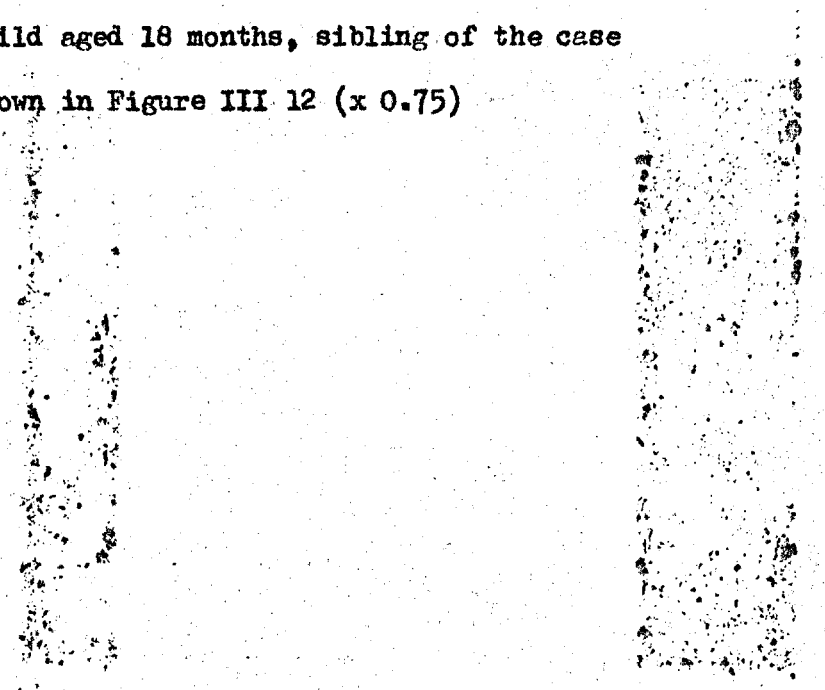
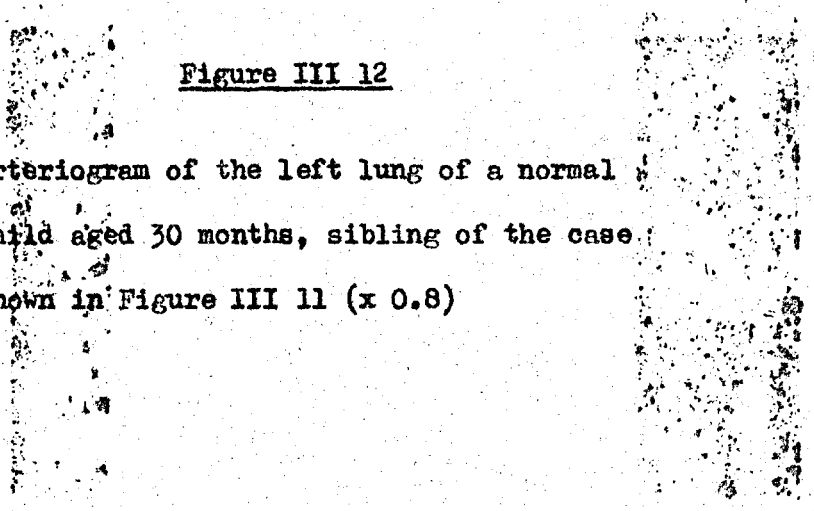
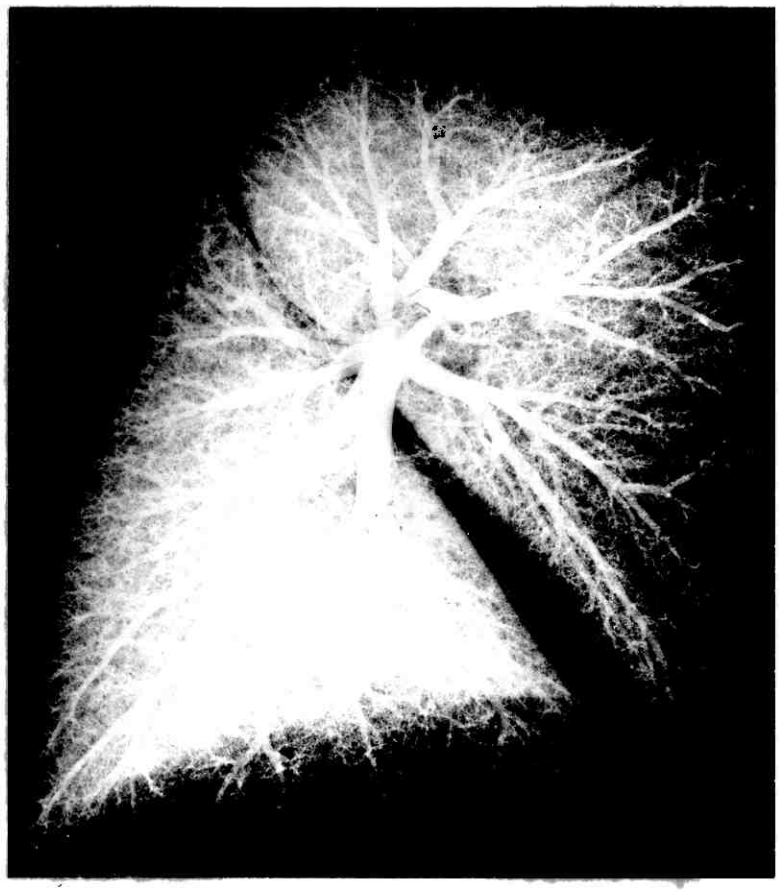
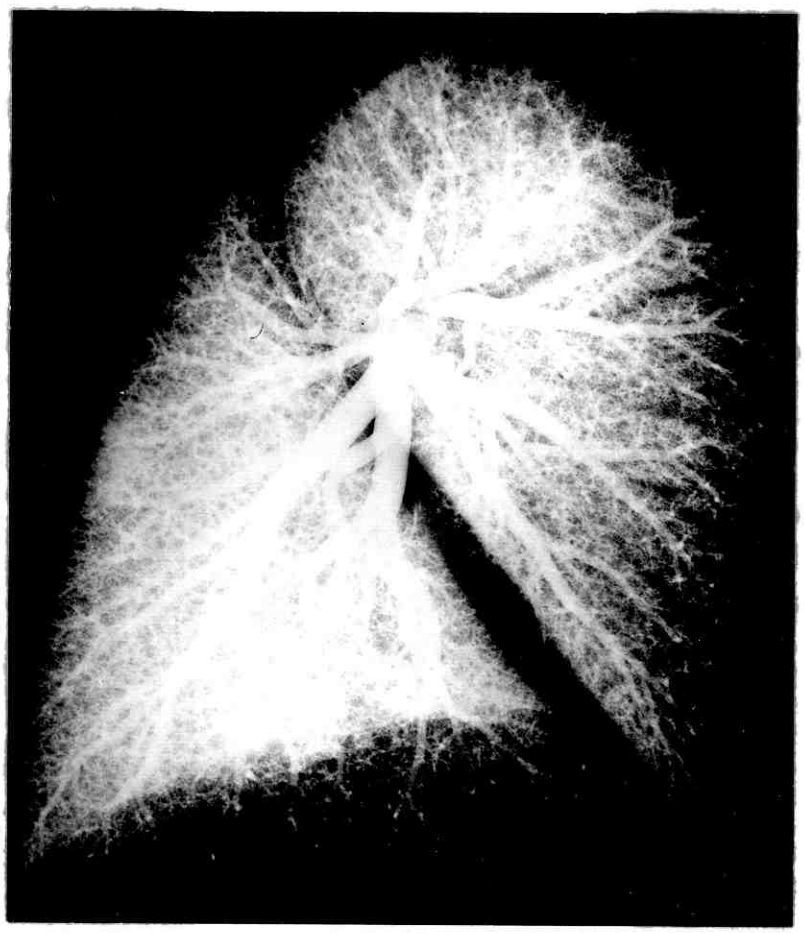


Figure III 12

Arteriogram of the left lung of a normal child aged 30 months, sibling of the case shown in Figure III 11 (x 0.8)







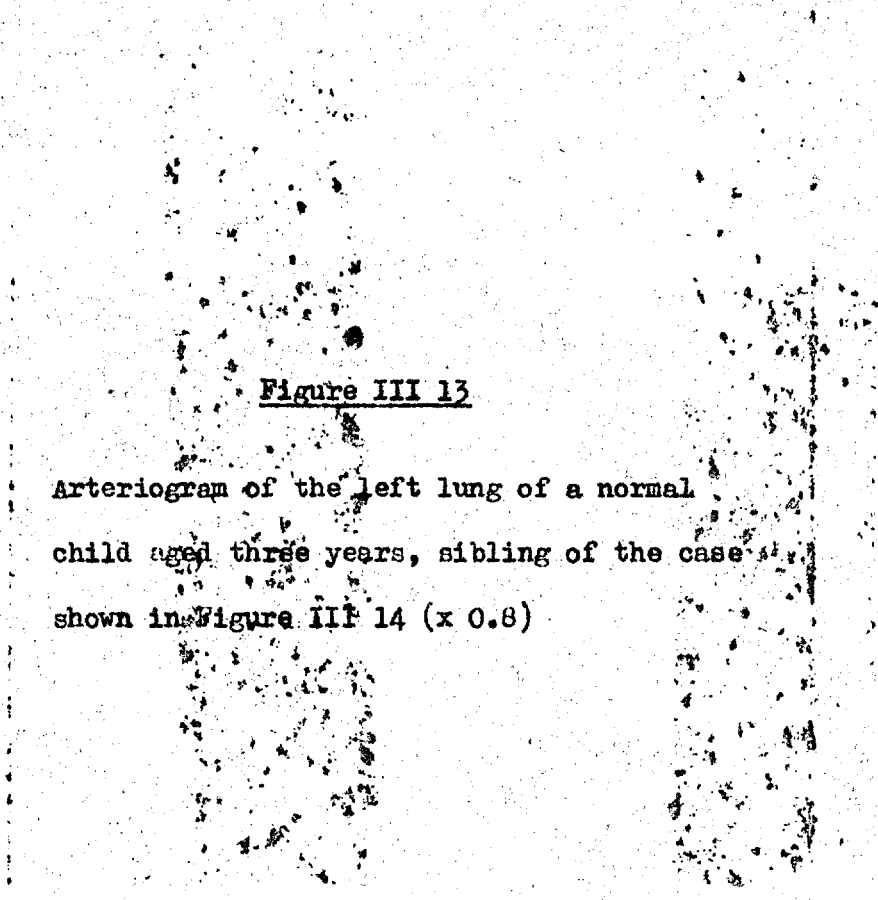


Figure III 13

Arteriogram of the left lung of a normal child aged three years, sibling of the case shown in Figure III 14 (x 0.8)

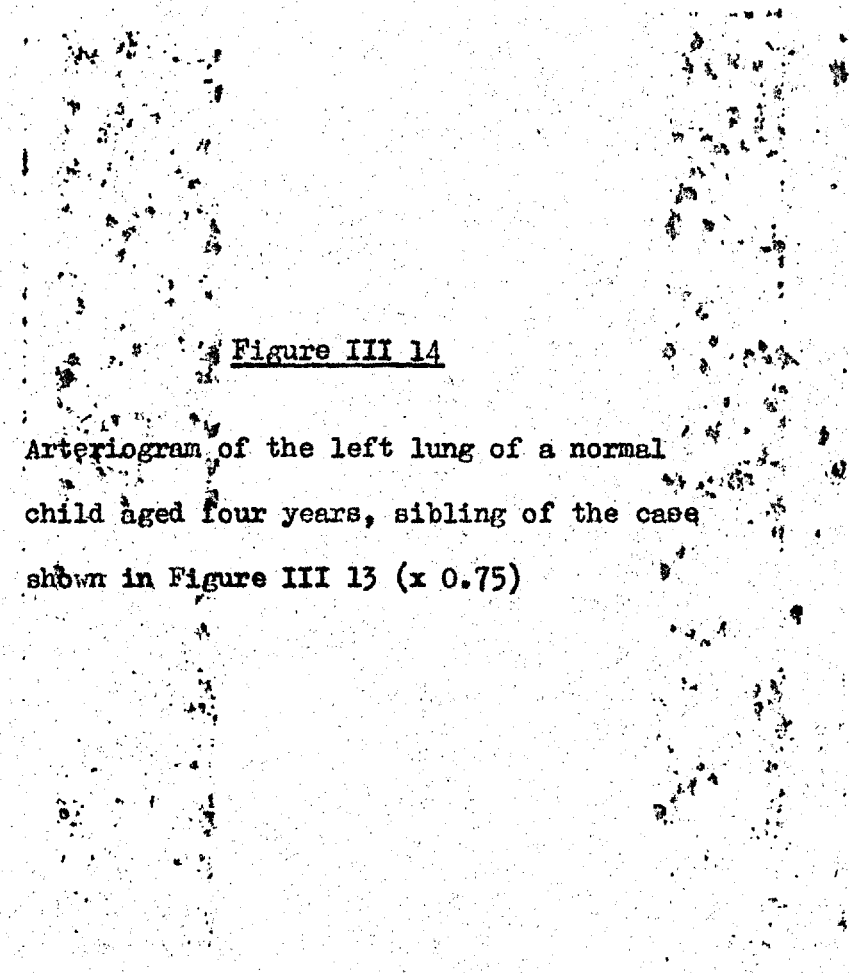
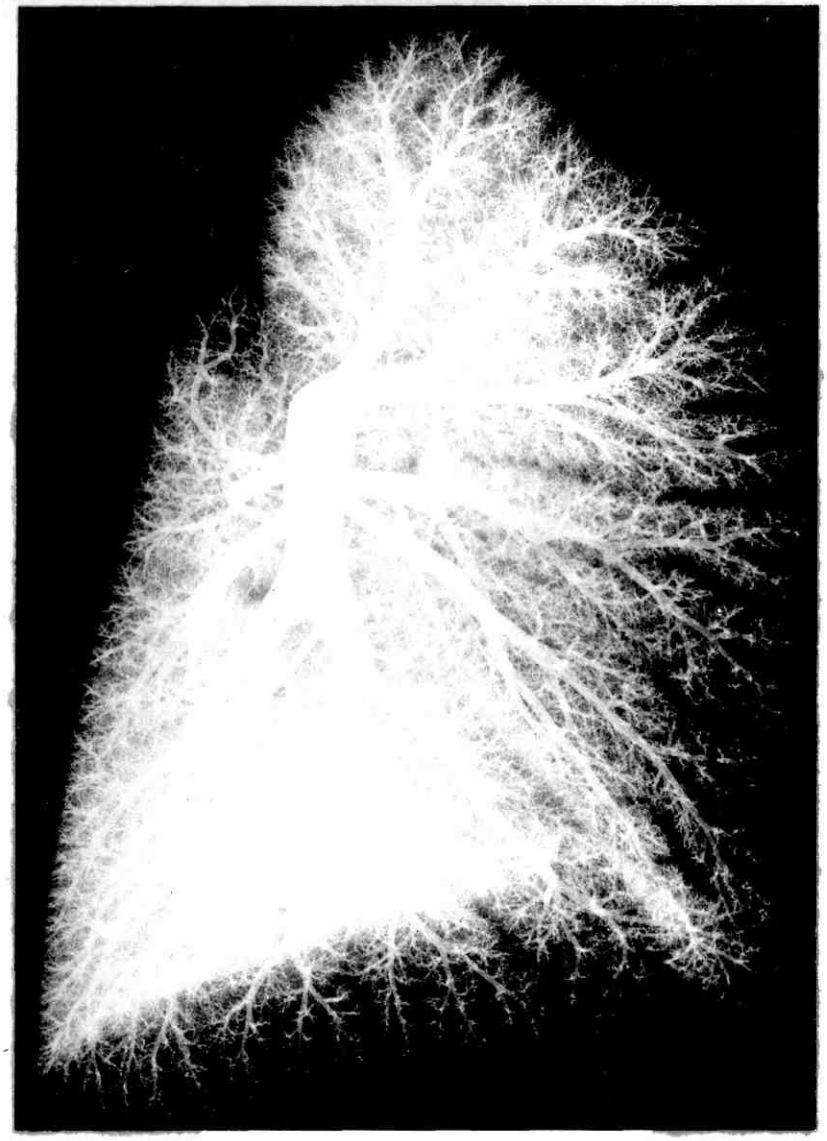
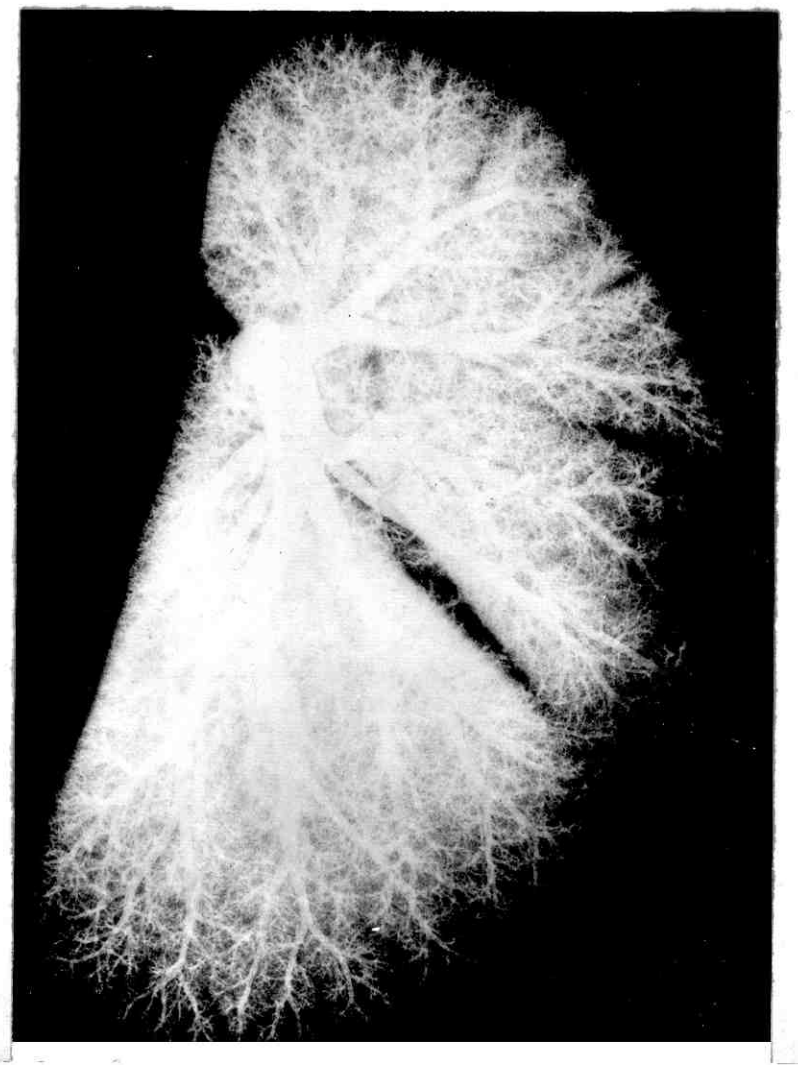


Figure III 14

Arteriogram of the left lung of a normal child aged four years, sibling of the case shown in Figure III 13 (x 0.75)





the main pathways, as it does in the adult. Between birth and ten months there has been a marked proliferation of peripheral vessels.

The arterial branching pattern seen in the arteriograms though broadly similar, varies from case to case. During the present study the lungs from three families were examined, two from each of two families and four from the third. A marked family resemblance was noted in each series of arteriograms. Figures III 11 and 12 show the left lung of two brothers aged 18 and 30 months. A resemblance is also seen in Figures III 13 and 14 - the arteriograms from another pair of siblings, but the difference between the pairs is considerable.

In none of the cases was filling of the pulmonary veins or bronchial arteries apparent on the arteriograms.

Measurements from Arteriograms

Measurements were made of the total length of the longest axial pathway in upper and lower lobes of each case. This was the artery to the posterior basal segment in the lower lobe and the apical segment in the upper lobe. The diameter of the lumen of these pathways was measured at the hilum and at 25% intervals along the pathway. These results are given in Tables III 1 and 2 and the diameters only are represented diagrammatically in Figure III 15.

Length of Axial Pathway

At all ages the upper lobe artery is shorter than that of the lower lobe. With age both show a gradual increase in length, more rapid during fetal life than during childhood, particularly in the lower lobe. There is a 13 fold increase in length of the lower lobe artery between 12 weeks gestation and adulthood and an 11 fold increase in the upper lobe. In the upper and lower lobe the length of the pathway increases as the volume increases, the relationship being more nearly linear if the cube of artery length is plotted against lobar volume.

Diameter of Lumen

Figure III 15 shows the reduction in size, seen on the arteriograms, of the lumen diameter towards the periphery for the upper and lower lobes. Usually the left lung is represented but occasionally the right when the left was not available. The diameter at all levels increases with age and the lower lobe arteries are smaller than those of the upper lobe. At all ages the diameter is greater at the hilum than the periphery with different rates of increase for the upper and lower lobes.

The arterial diameter at the hilum increases with age and volume of the lung and is directly related to the length of pathway which it is supplying both in the upper and lower lobe (Figure III 16). There is an apparent flattening of the curve for the upper lobe in the older cases where the diameter increases with little increase in length. This will lead to an increase in gradient.

The increase with age in the diameter of the hilar vessel

TABLE III 1

Measurements from Arteriograms of Fetal Lungs

Fetal age weeks	Total length (cm) Upper Lobe Artery	Total length (cm) Lower Lobe Artery	Diameter (mm) Percentage distance from hilum — Upper Lobe 0	25	50	75	Lower Lobe 0	25	50	75
12	0.8	1.2	0.3	0.2	0.15	0.1	0.4	0.3	0.25	0.1
14	0.6	1.8	0.5	0.3	0.2	0.1	1.2	0.6	0.5	0.3
17	1.0	1.8	0.5	0.4	0.25	0.1	1.3	0.8	0.4	0.2
18	1.2	2.0	0.9	0.6	0.4	0.2	1.6	0.9	0.5	0.3
19	1.2	2.0	0.9	0.5	0.3	0.1	1.2	0.8	0.6	0.3
20	1.3	2.3	1.5	0.7	0.4	0.2	1.5	1.0	0.7	0.5
20	1.5	2.8	0.9	0.5	0.3	0.1	1.3	0.8	0.6	0.5
23	1.5	3.2	1.2	0.9	0.6	0.4	2.8	1.5	1.0	0.4
26	2.2	4.1	2.0	1.2	0.8	0.4	2.5	1.6	1.1	0.7
28	2.2	3.7	2.5	1.8	0.6	0.3	2.9	2.0	1.1	0.5
32	2.4	3.9	2.0	1.0	0.6	0.3	3.0	2.0	1.2	0.8
33	3.0	4.8	1.6	1.0	0.8	0.4	3.2	1.7	1.3	0.7
36	2.2	5.5	3.0	1.3	1.1	1.0	4.1	2.4	1.2	0.9
38	3.1	4.8	1.7	1.0	0.9	0.6	3.8	2.3	1.4	0.7
39	3.1	4.8	2.0	1.7	1.1	0.6	3.8	2.5	1.6	0.8
40	3.1	5.4	2.0	1.2	0.8	0.4	4.1	2.7	1.5	0.7
40	3.0	6.0	3.0	2.0	1.4	0.5	4.0	2.5	1.7	0.6

Figure III 15a

Measurements from arteriograms. Diameter is shown (mm) at 25% intervals along axial pathway from hilum to periphery for the upper and lower lobes.

Fetal age in weeks is shown in the top right hand corner of each square.

Upper lobe - open circles.

Lower lobe - closed circles.

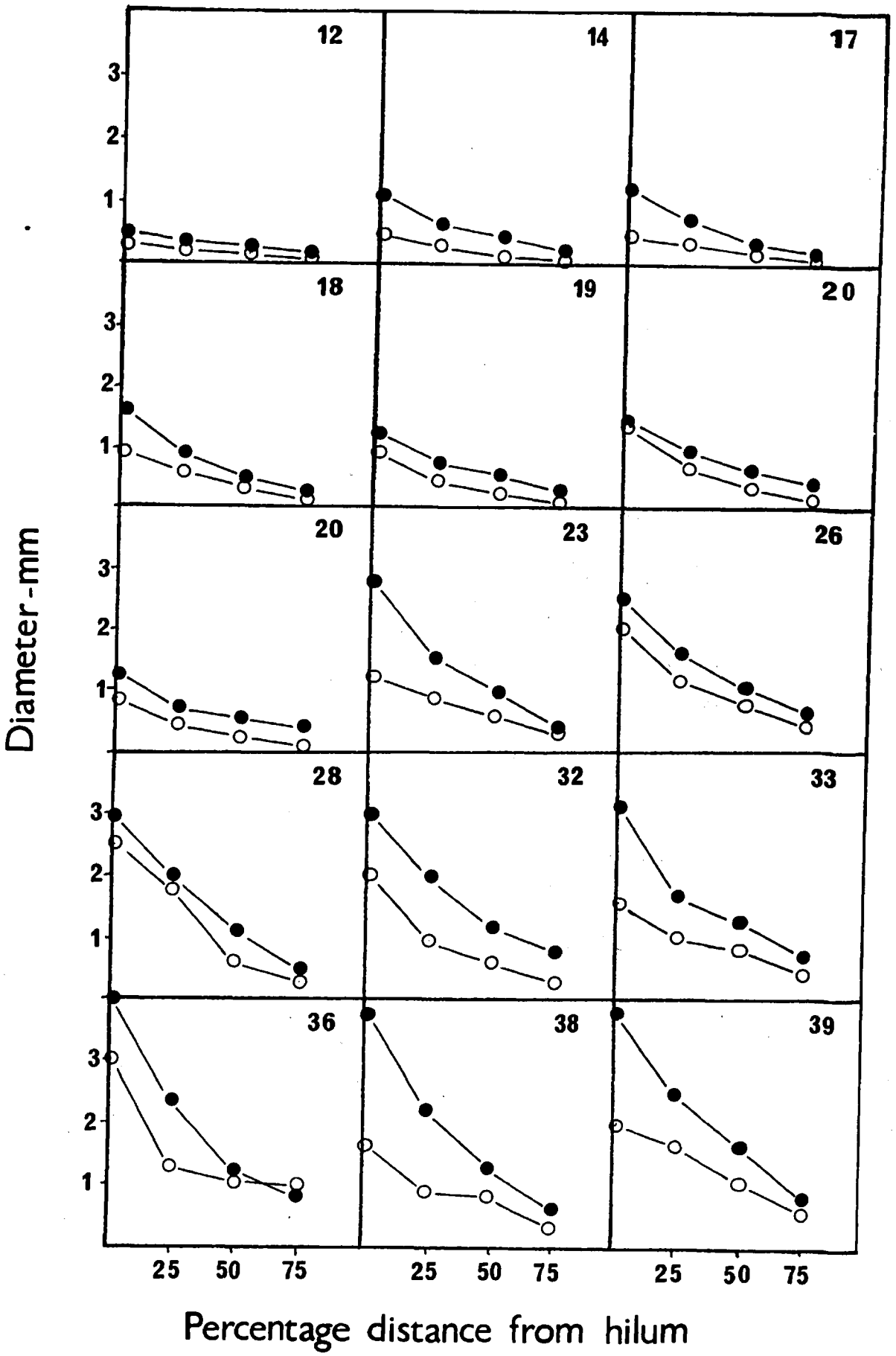

12
14
17
18
19
20
20
23
26
28
32
33
36
38
39
3
2
1
25
50
75
Diameter-mm
Percentage distance from hilum

Figure III 15b

Measurements from arteriograms. Diameter is shown (mm) at 25% intervals along the axial pathway from hilum to periphery for the upper and lower lobes.

The age is shown in the top right hand corner of each square in weeks, days or months.

Upper lobe - open circles.

Lower lobe - closed circles.

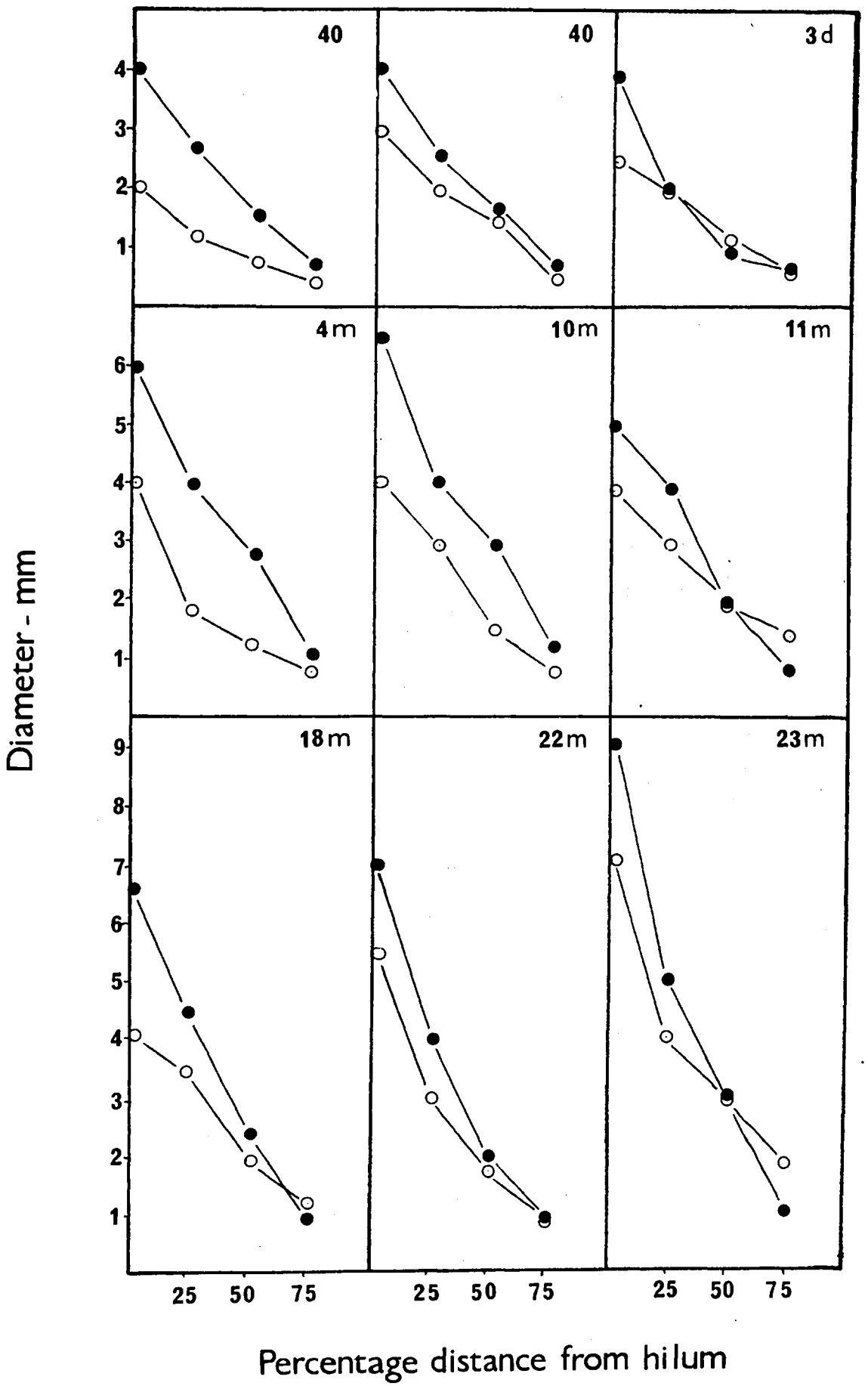

40
40
3d
4m
10m
11m
18m
22m
23m
Diameter - mm
Percentage distance from hilum
25
50
75

Figure III 15c

Measurements from arteriograms. Diameter is shown (mm) at 25% intervals along the axial pathway from hilum to periphery for the upper and lower lobes. The age is shown in the top right hand corner of each square in months or years.

Upper lobe - open circles.

Lower lobe - closed circles.

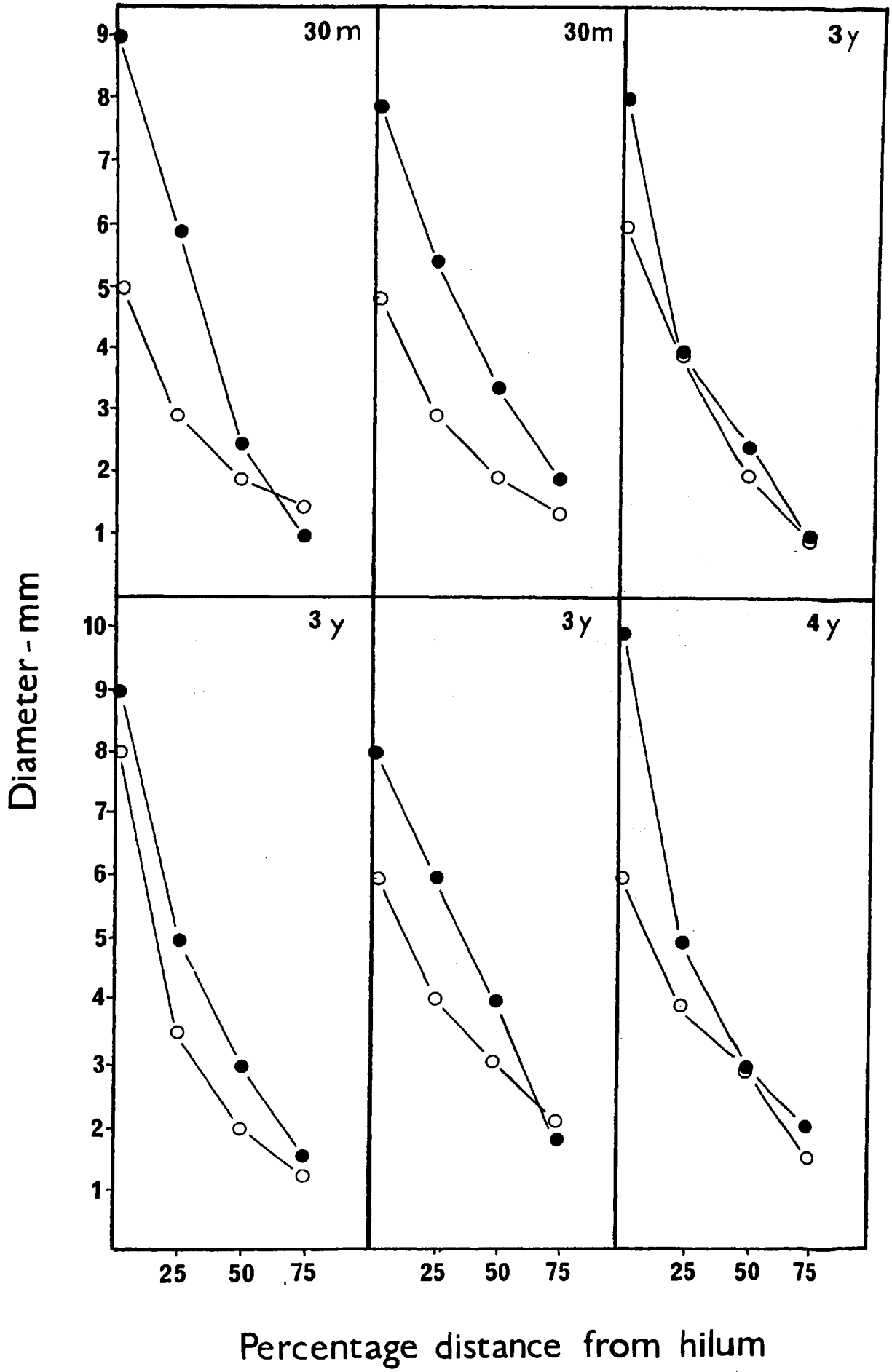

30 m
30m
3y
3 y
3y
4 y
Diameter - mm
Percentage distance from hilum
25
50
75

Figure III 15d

Measurements from arteriograms. Diameter is shown (mm) at 25% intervals along the axial pathway from hilum to periphery for the upper and lower lobes.

The age is shown in the right hand corner of each square in years.

Upper lobe - open circles.

Lower lobe - closed circles.

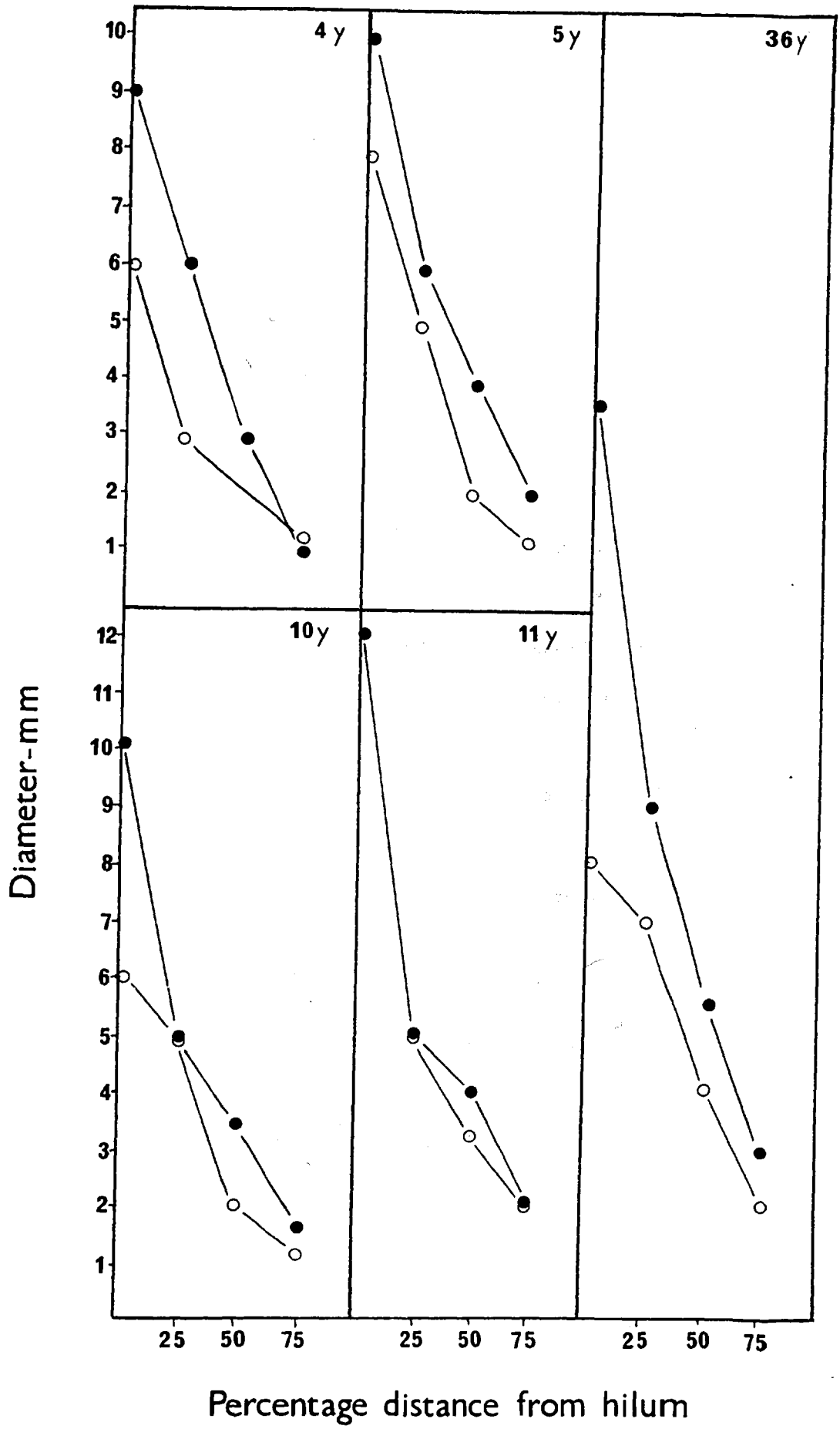

4 y
5 y
36 y
10 y
11 y
Diameter-mm
Percentage distance from hilum
25
50
75

TABLE III 2

Measurements from Arteriograms of Children and one Adult

Age	Total length (cm) Upper Lobe Artery	Total length (cm) Lower Lobe Artery	Diameter (mm) Percentage distance from hilum — Upper Lobe 0	25	50	75	Lower Lobe 0	25	50	75
3 day	4.0	5.9	2.5	2.0	1.2	0.7	4.0	2.0	1.0	0.7
5 day	3.0	5.0	2.5	1.5	1.0	0.5	4.0	2.0	1.5	0.8
4 mnth	5.0	6.6	4.0	1.8	1.2	0.8	6.0	4.0	2.8	1.0
10 mnth	4.2	8.4	4.0	3.0	1.5	0.8	6.5	4.0	3.0	1.2
11 mnth	6.0	8.0	4.0	3.0	2.0	1.4	5.0	4.0	2.0	0.9
18 mnth	5.9	9.6	4.2	3.5	2.0	1.2	7.0	4.5	2.5	1.0
22 mnth	6.9	8.6	5.5	3.0	1.8	1.0	7.0	4.0	2.0	1.0
23 mnth	5.2	9.0	7.0	4.0	3.0	1.8	9.0	5.0	3.0	1.0
30 mnth	7.0	11.4	5.0	3.0	2.0	1.5	9.0	6.0	2.5	1.1
30 mnth	6.5	10.1	5.0	3.0	2.0	1.5	8.0	5.5	3.5	2.0
3 yrs	5.2	9.2	6.0	4.0	2.0	1.0	8.0	4.0	2.5	1.0
3 yr	5.5	10.5	8.0	3.5	2.0	1.2	9.0	5.0	3.0	1.5
3 yr	4.6	9.5	6.0	4.0	3.0	2.0	8.0	6.0	4.0	1.8
4 yr	6.2	10.0	6.0	4.0	3.0	1.5	10.0	5.0	3.0	2.0
4 yr	5.7	10.0	6.0	3.0	-	1.2	9.0	6.0	3.0	1.0
5 yr	6.2	11.2	8.0	5.0	2.0	1.2	10.0	6.0	4.0	2.0
10 yr	5.4	11.6	6.0	5.0	2.0	1.2	10.0	5.0	3.5	1.6
11 yr	7.0	11.0	-	5.0	3.2	2.0	12.0	5.0	4.0	2.0
36 yr	8.6	16.0	8.0	7.0	4.0	2.0	16.0	9.0	5.5	3.0

to the lower lobe is shown in Figure III 17. The increase is more rapid during fetal life than in childhood and a quarter of the total increase to adulthood has occurred by birth. The diameter 25% from the periphery of the lung also increased with age and therefore volume (Figure III 17) but the changes were not of the same magnitude. Here again the increase was more rapid during fetal life.

The length of the axial vessels both in upper and lower lobe showed a similar change with age, the increase being far more rapid during fetal life. The change in rate of increase, both in diameter and length, occurred at between four and ten months after birth.

Gradient along the Axial Pathway

It can be seen that the gradient along the axial pathways increases with age since the hilar diameter increases by a greater percentage than the peripheral diameter in both upper and lower lobes. During early fetal life the hilum and periphery increase at the same rate but during the perinatal period and the first few days of life the peripheral arteries do not increase as rapidly so the gradient steepens.

Between 12 weeks gestation and birth the hilum of the lower and upper lobe each increases ten fold and the periphery only six and five fold respectively. After birth there is, in the lower lobe, a four fold increase at the hilum and at the periphery, while in the upper lobe there is a three fold increase at both levels. The total increase from 12 weeks gestation to adulthood in the lower lobe at the hilum is 48 fold for the diameter, an area increase of 6436, while the peripheral increases are only 30 and 883 fold

Figure III 16

Length of arterial axial pathway plotted against its diameter at the hilum, for upper and lower lobes, measured on arteriograms from fetal and childhood lungs.

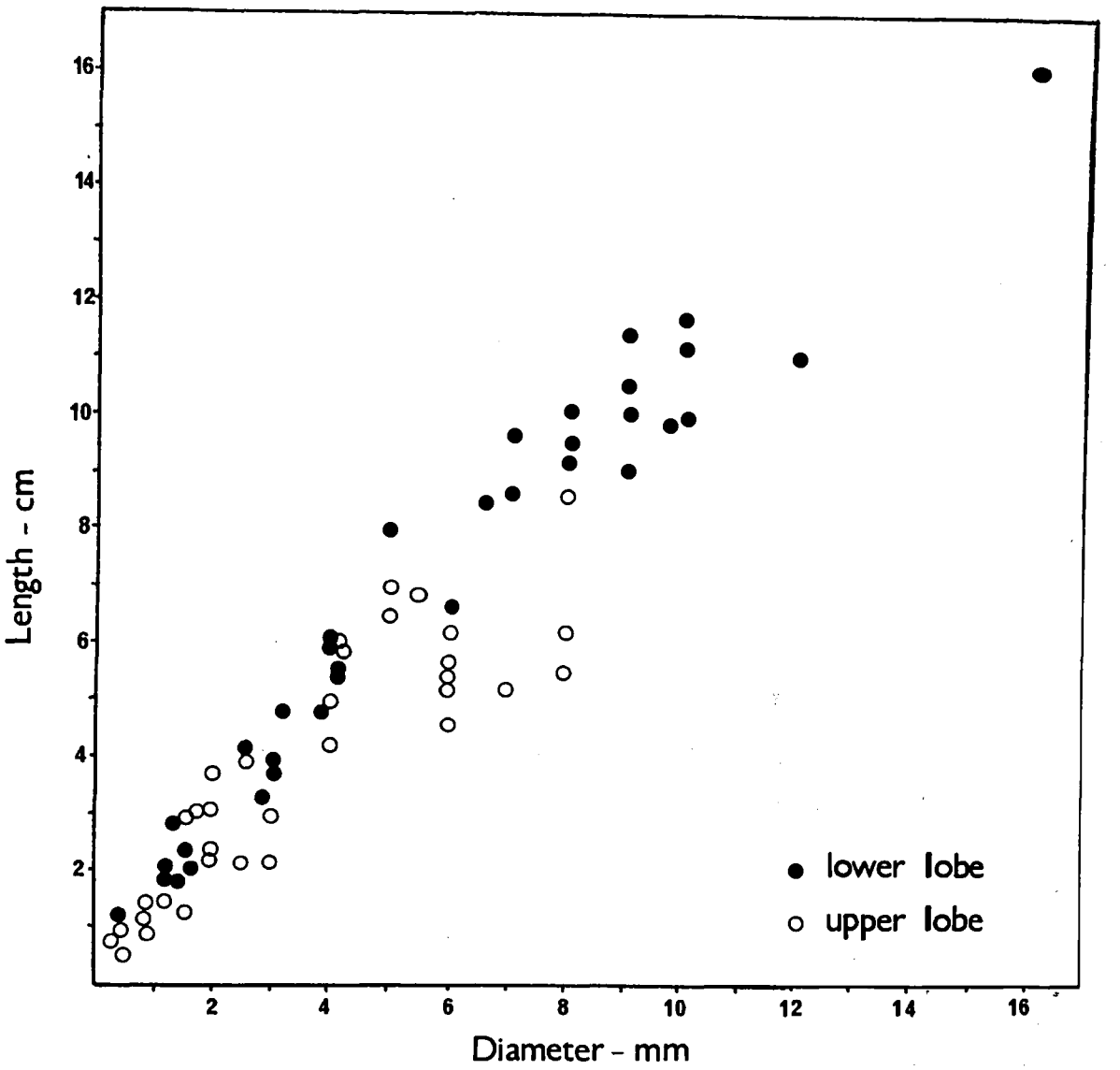
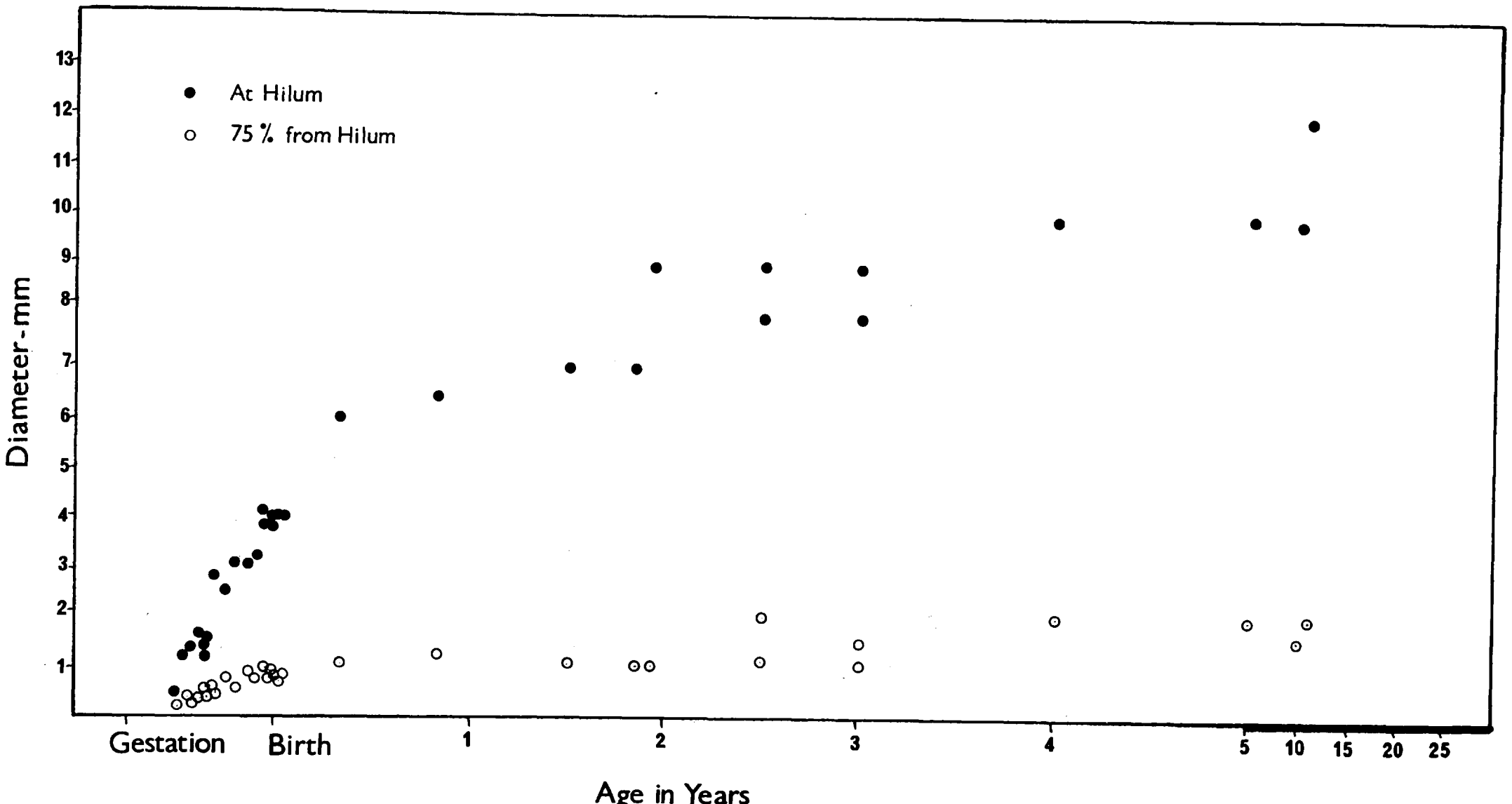

Length - cm
16
14
12
10
8
6
4
2
2
4
6
8
10
12
14
16
Diameter - mm
lower lobe
upper lobe

Figure III 17

Lumen diameter, measured on the arteriogram for the lower lobe, both at the hilum and at the point 75% from the hilum plotted against age.

Note the change in age scale at five years.


At Hilum
75 % from Hilum
Diameter-mm
13
12
11
10
9
8
7
6
5
4
3
2
1
Gestation
Birth
1
2
3
4
5
10
15
20
25
Age in Years

respectively. Although the number of lobules or acini does not change significantly over this time the numbers of arteries within the acinus increases. This relatively greater increase in cross-sectional area at the hilum allows for the increase in the number of branches at the peripheral level.

In Figure III 18 the gradient is plotted as estimated from the diameter and the length of each pathway represented by one hundred units so that all lengths are reduced to the same absolute value. As the hilar diameter increases relatively more than the periphery the gradient increases.

If the diameter is related to the actual length (Figure III 19) the gradient remains much the same throughout growth but there is a slight increase during fetal life. The gradient in the upper lobe was not as steep as that of the lower lobe when both were considered on a 100 unit scale. This was because the diameter in the upper lobe was less.

When the diameter is related to actual length the gradient is steeper in the upper lobe. The lower lobe is known to have a greater flow and the decreased gradient in this lobe would favour this flow. It is essential to consider the true length of the artery when comparing diseased lungs with the normal since the gradient is of haemodynamic significance.

Diameter One Centimetre from the Periphery

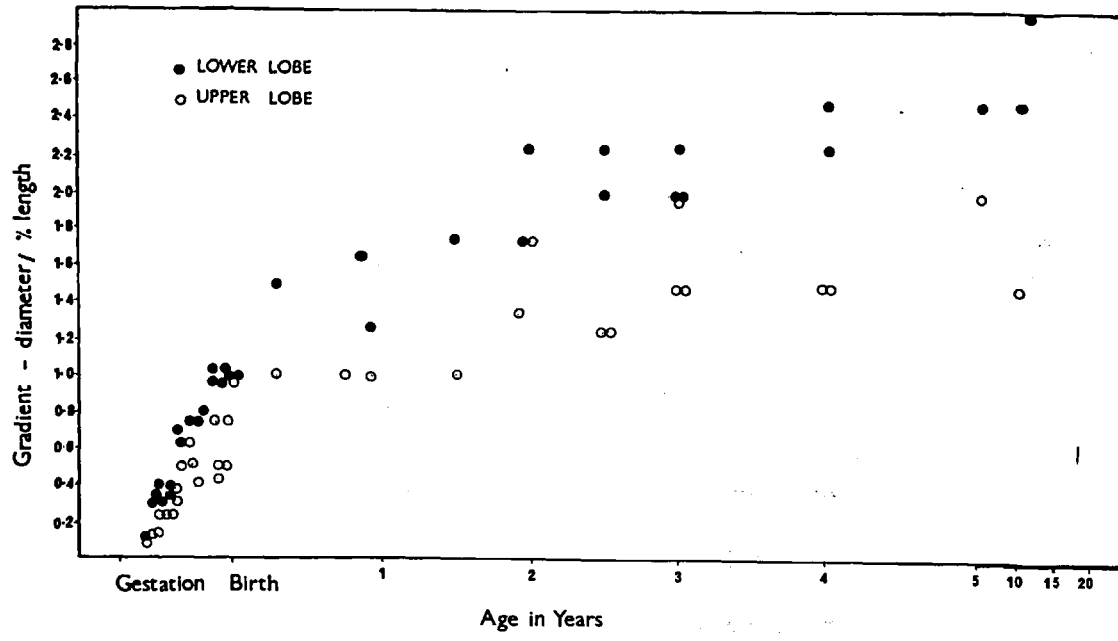
It seems that the diameter of the artery is related to the volume of lung which the artery is supplying, as exemplified by

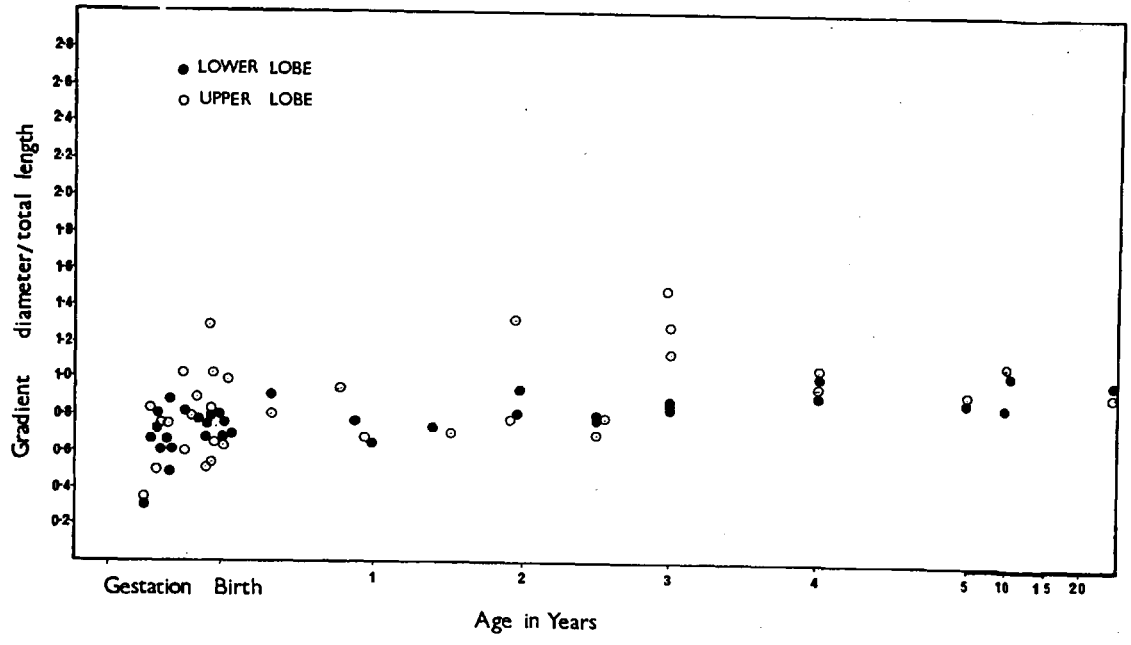
Figure III 18

Gradient along axial pathway to upper and lower lobe estimated from diameter and length reduced to an absolute value plotted against age. Note the change in scale at five years.

Figure III 19

Gradient along axial pathway to upper and lower lobe estimated from diameter and total measured length plotted against age. Note the change in scale at five years.


LOWER LOBE
UPPER LOBE
Gradient - diameter/ % length
Gestation Birth
1
2
3
4
5
10
15
20
Age in Years


LOWER LOBE
UPPER LOBE
Gradient diameter/total length
Gestation Birth
1
2
3
4
5
10
15
20
Age in Years

the hilar arteries. To test this hypothesis measurements were made on all cases, in the upper and lower lobes, one centimetre from the periphery. The results are shown in Table III 3 and Figure III 20. There was a slight increase in diameter of an artery one centimetre from the pleura between 12 weeks gestation and two years, however within this age group there was a wide range from 0.4 to 1.0 mm. Over two years of age all fell within the same smaller range of 0.8 to 1.0 mm diameter with the same range being found in each age group.

Surprisingly 11 out of 18 fetal lungs and 20 out of 27 child lungs had larger arteries in the upper lobes. It may be that these are dilated to favour flow which is less than in the lower lobe due to the gradient.

RECONSTRUCTION OF THE PRE-ACINAR BRANCHING PATTERN OF THE ARTERIAL AXIAL PATHWAY

The pre-acinar (see Definitions p. 106) arterial branching pattern in the adult has been described by Elliott (1964) and Elliott and Reid (1965). They described the conventional arteries running with airways and in addition supernumerary branches supplying the alveolar region of the lung by a more direct route. By using a method of serial reconstruction it has been possible to study the developing arterial system during fetal life and childhood.

Studies of a total pathway before the acinus was reached were made on 12 cases of fetal ages 12, 14, 16, 17, 18, 19, 20, 23, 28, 36, 38 and 40 weeks. The peripheral part of the pre-acinar region was reconstructed in children aged three days to 11 years. The

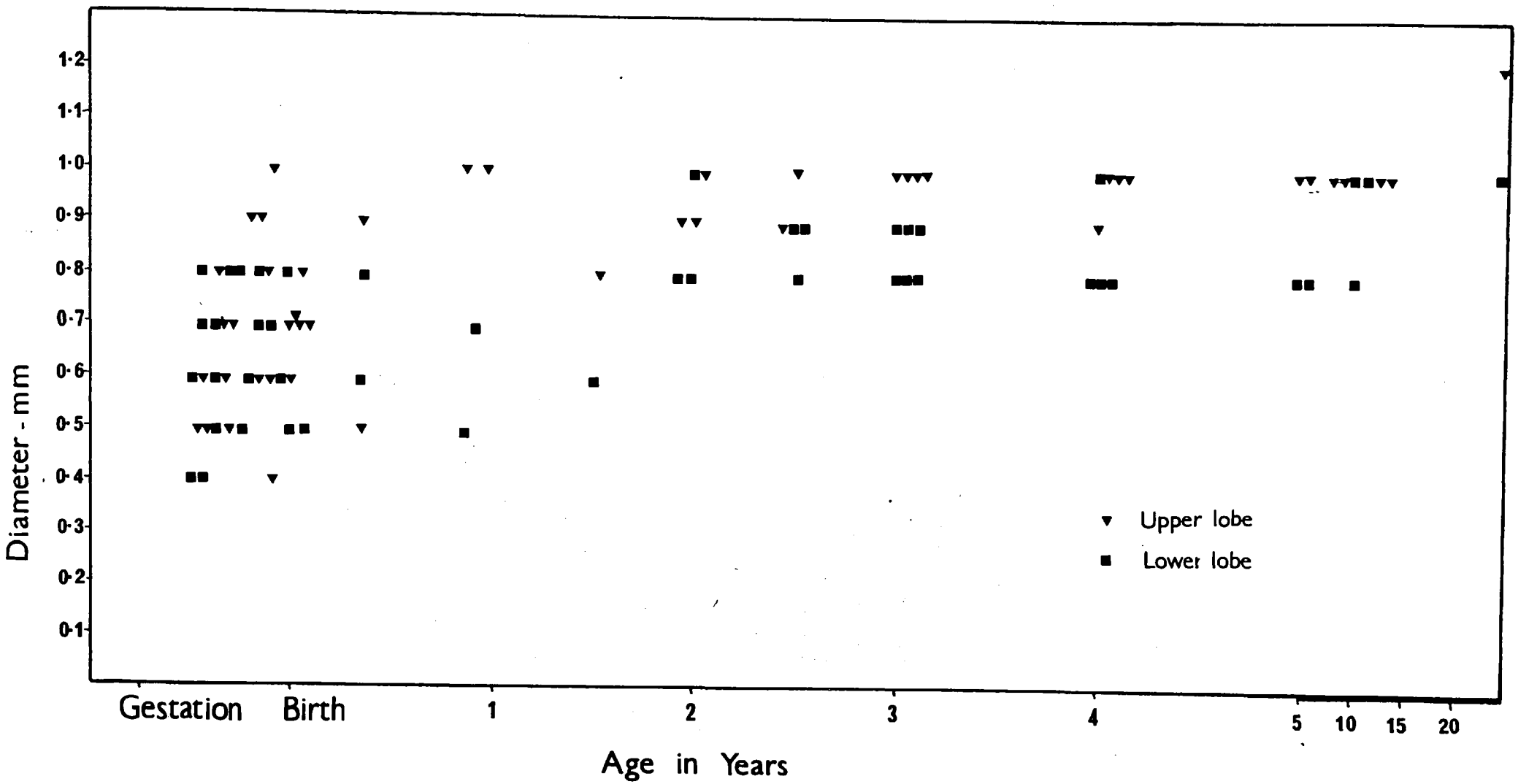
TABLE III 3

Diameter of Arteries 1 cm from the Periphery

Fetal Age (weeks)	Upper Lobe (mm)	Lower Lobe (mm)	Post Natal Age	Upper Lobe (mm)	Lower Lobe (mm)
12	0.6	0.4	3 day	0.7,0.7	0.7,0.7
14	0.5	0.6	5 day	0.8,0.9	0.8,0.9
17	0.5	0.4	4 mnth	0.5,0.9	0.6,0.8
18	0.7,0.8	0.8,0.7	10 mnth	1.0	0.5
19	0.5	0.7	11 mnth	1.0	0.7
20	0.6	0.5	18 mnth	0.8	0.6
20	0.7	0.6	22 mnth	0.9	0.8
23	0.9,0.9	0.8,0.8	23 mnth	0.9,1.0	0.8,1.0
26	0.6	0.5	30 mnth	1.0	0.9
28	0.8	0.7	30 mnth	0.9	0.8
32	0.4	0.6	3 yr	1.0,1.0	0.9,0.8
33	0.6	0.8	3 yr	1.0	0.9
36	1.0	0.7	3 yr	1.0,0.9	0.8,0.8
38	0.6	0.6	4 yr	1.0,0.9	1.0,0.8
39	0.7	0.8	5 yr	1.0,1.0	0.8,0.8
40	0.7	0.5	10 yr	1.0,1.0	0.8,1.0
40	0.8	0.5	11 yr	1.0	1.0
			36 yr	1.2	1.0

Figure III 20

Diameter of arteries one centimetre from the periphery of the lung, measured on arteriograms plotted against age. The upper lobe measurements are on the whole larger than those of the lower lobe. Note change in scale at five years.


Diameter - mm
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
Upper lobe
Lower lobe
Gestation
Birth
1
2
3
4
5
10
15
20
Age in Years

posterior basal artery and airway were studied since they form the longest pathway and are easily identified. On the arteriograms this is the obvious main pathway to the lower lobe. The bronchus to this segment however is only of the same order as the other segmental bronchi and does not stand out.

Pattern at Birth

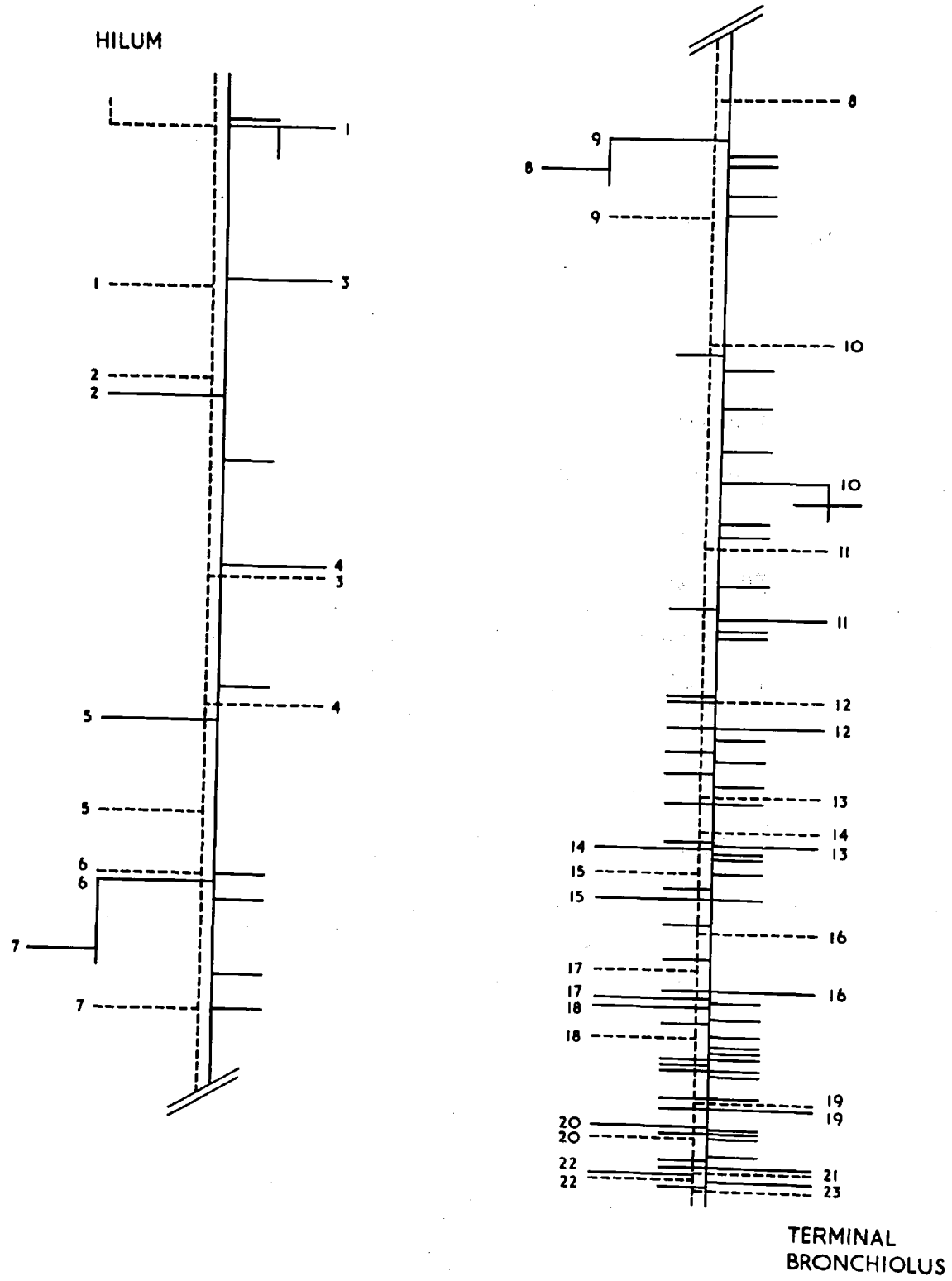
The pattern of branching at birth is described first since it is the most complex of those studied and, as will be seen, is the adult pattern in miniature. For this reason it became unnecessary to reconstruct the total pre-acinar branching pattern after birth.

The lung at birth is in the 'alveolar' stage of growth as described by Loosli and Potter (1952). Serial sections of the posterior basal segment were made and a reconstruction of the branching pattern of the axial airway and artery was produced in a stillborn child of 38 weeks gestation. Figure III 21 shows a graphic representation of this pathway.

The total length of the pathway from the hilum of the lower lobe to the terminal bronchiolus was 30.1 mm. On the arteriogram this pathway measured 41.0 mm. This reduction is due to shrinkage and compression during fixation, processing and cutting. Over this distance there were 23 bronchial branches of which 21 were intra-segmental (that is in this reckoning the segmental airway is the third generation). This number is within the normal range for the adult (Hayward and Reid, 1952) and newborn (Bucher and Reid, 1961). The first 18 generations contained cartilage, again within the normal range.

Figure III 21

Reconstruction of the branching pattern of the airway and artery to the posterior basal segment of the left lower lobe in a fetus of 38 weeks gestation. The airways and their accompanying arteries are numbered from hilum to terminal bronchiolus. The supernumerary arteries are plotted but not numbered. The length is estimated from the number of sections cut.


HILUM
1
1
3
2
2
4
3
5
4
5
6
6
7
7
8
9
8
9
10
10
11
11
12
12
13
14
14
13
15
15
16
17
17
16
18
18
19
19
20
20
22
22
21
23
TERMINAL
BRONCHIOLUS
Actual length
30·1 mm.
Artery
Airway

Accompanying the 23 bronchial branches were 21 conventional arteries branching from the main pathway. Two bronchial branches, numbers seven and eight, were supplied by branches of the conventional arteries accompanying bronchial branches six and nine. As can be seen in the diagram the artery and its bronchus often branched from the main pathway some distance apart, but they eventually came to be found in the same connective tissue sheath. The arteries have been designated the same generation number as the bronchus which they accompany. The first branch of the airway is generation one.

In addition to the conventional artery branches there were 65 supernumerary branches which did not accompany branches from the main bronchus. It seems then that these arteries first described by Elliott (1964) are present at birth. The ratio of supernumerary to conventional arteries in this case was 3.1:1. In the anterior basal segment of the adult Elliott reported a ratio of 3:1. The ratio of supernumerary arteries to bronchial branches in this case was 2.82:1.

Proximally the bronchi and conventional arteries branched at widely spaced intervals of approximately the same length (see Table III 4). The first seven bronchial generations (between conventional arteries 6 and 9) comprised half the total length. Towards the periphery, particularly after the 12th generation, the distance between branches decreased and then again further, within the lobule at the 21st generation.

The supernumerary arteries also arose more frequently

TABLE III 4

Diameter and Length of Arterial Generations along Axial Pathway at 38 weeks Gestation

Airway Generation	Diameter of Arterial Branch (μm)	Diameter of Axial Artery (μm)	Distance from hilum (mm)
1	1150	4000	0.7
2	2500	2500	2.8
3	1500	2700	4.4
4	1550	2700	6.8
5	1800	2200	8.9
6	1600	1800	11.0
9	1400	1100	15.4
10	860	830	20.3
11	490	800	21.7
12	340	800	23.1
13	350	500	25.03
14	470	500	25.07
15	260	520	25.84
16	350	-	27.0
17	147	-	27.07
18	250	380	27.21
19	220	310	28.75
20	100	270	29.17
21	90	250	29.45
22	75	250	26.66
23	77	250	29.73

towards the periphery. Over the first eight bronchial generations there were only seven supernumeraries (ratio 0.875:1). In the next eight there were 35 (ratio 3.375:1), from generation 16 to 23 there were 23 (ratio 3.28:1). Surprisingly within the lobule and prior to the acinus where there were three conventional generations (21 - 23) there were only two supernumerary arteries. In the adult it is within the lobule that the greatest relative number of supernumeraries are found. It is probable that there is growth of intra-lobular pre-acinar supernumeraries during childhood.

Diameter of Arteries

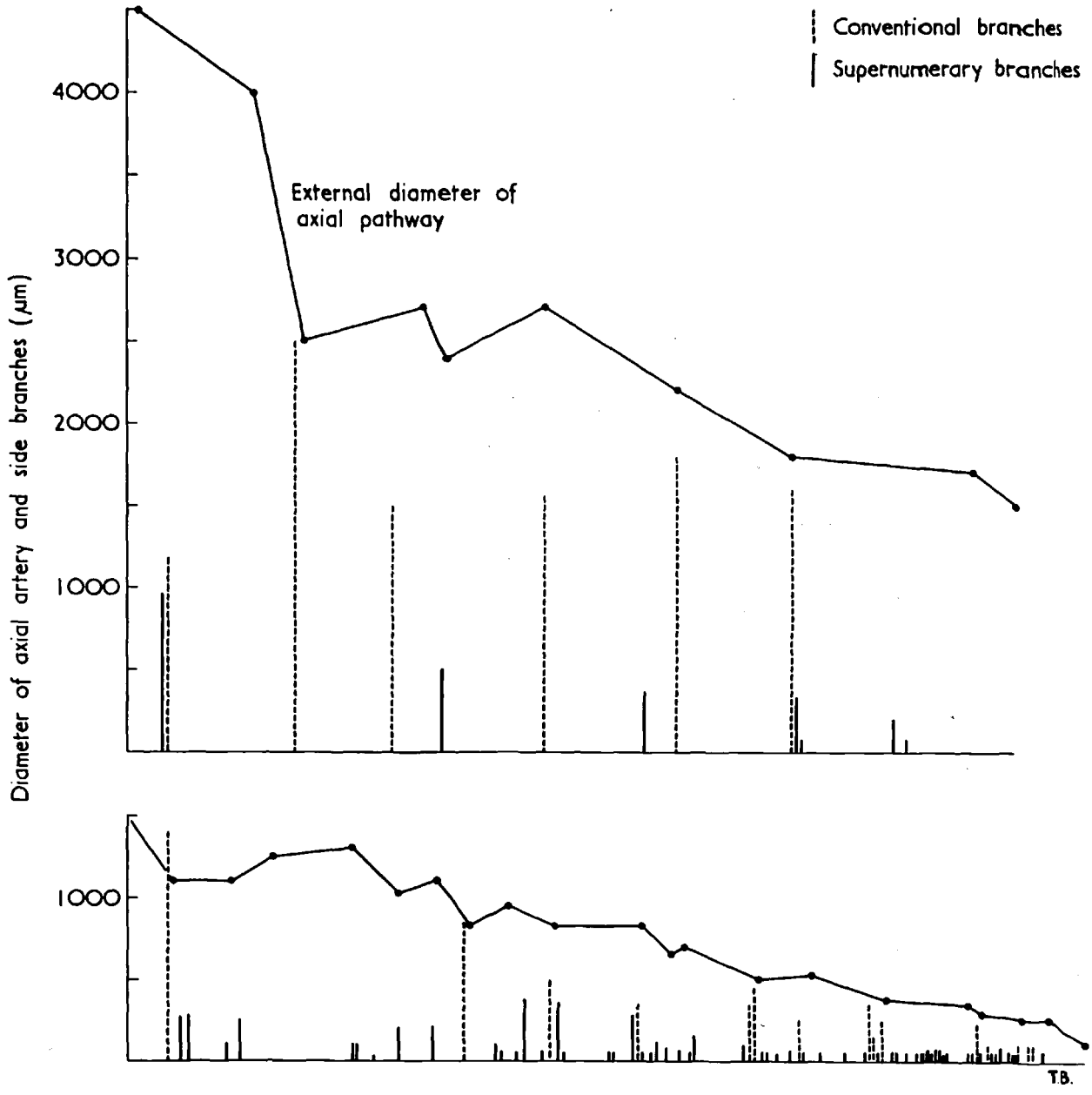
The diameter of the axial pathway is included in Figure III 22 and Table III 4. At the hilum its external diameter was 4.5 mm similar to measurements made on the arteriogram. The diameter decreased steadily from hilum to periphery with more sudden drops at some branching points of conventional arteries. The conventional arteries were usually formed by dichotomous branching so that it was at these levels that the main artery dropped considerably in diameter. The supernumeraries arose in a monopodial fashion from the main pathway and had relatively little effect on the axial diameter.

The last bronchus, that is an airway with cartilage in its wall, was generation 18. At this point the artery had a diameter of 380 μm. In the bronchiolar region the diameter fell further so that the artery at the level of the terminal bronchiolus was 250 μm in diameter.

The conventional and supernumerary branches are also represented in Figure III 22. The diameters of these branches and

Figure III 22

The diameter of the axial pathway of 38 weeks traced in Figure III 21 with the diameter of the conventional and supernumerary arteries, from the hilum to terminal bronchiolus (TB).


Conventional branches
Supernumerary branches
External diameter of axial pathway
Diameter of axial artery and side branches (μm)
4000
3000
2000
1000
1000
T.B.

the axial pathway are tabulated in Tables III 4 and 5. The diameter and frequency of each type of vessel are clearly seen from the diagram. The conventional branches are always larger than adjacent supernumerary branches although proximal supernumeraries are larger than peripheral conventionals. The conventional arteries are similar in diameter to their parent artery at their point of origin, though usually smaller. In this case there was only one exception, conventional branch number nine, which was larger than the axial pathway at its origin.

The conventional branches decreased in diameter towards the periphery. The largest was 2500 μm in diameter and ran with the bronchus to the anterior basal segment. The smallest conventional artery was 75 μm in diameter and ran with a terminal bronchiolus. The mean size of the conventional branches was 741.85 μm but because of the wide range the standard deviation (S.D.) was 714 and the standard error of the mean (S.E.), 156.

The diagram demonstrates clearly the increase in number of supernumeraries towards the periphery and their gradual diminution in size. The largest was 950 μm and very quickly came to accompany a bronchus. No other supernumerary was over 500 μm in diameter and most were below 250 μm. The mean diameter was 110 μm with a range of 15 - 950 μm (S.D. 149; S.E. 18.62). The mean of the supernumerary arteries was considerably smaller than that of the conventional branches.

It seems then that, at birth, the pattern of arterial branching is very similar to that seen in the adult in the pre-lobular region: it is in the lobular region and particularly

TABLE III 5

Diameter, Generation and Distance from Hilum of Supernumerary Arteries along the Posterior Basal Artery at 38 Weeks

Airway Generation	External Diameter (μm)	Distance from hilum (mm)	Airway Generation	External Diameter (μm)	Distance from hilum (mm)
0	950	0.7	12	60	23.9
3	500	5.25	12	45	24.11
4	370	8.82	12	148	24.18
5	330	11.41	12	80	25.02
6	85	11.48	14	40	25.21
6	75	12.46	14	40	25.28
6	÷	13.23	14	30	25.49
9	260	15.61	14	50	25.7
9	280	15.75	15	60	25.91
9	112	16.38	15	40	26.19
9	260	16.59	15	57	26.61
9	100	18.48	15	40	26.96
9	112	18.55	17	35	27.14
9	15	18.83	18	50	27.42
9	205	19.25	18	37	27.49
9	200	19.81	18	30	27.63
10	90	20.79	18	35	27.84
10	57	20.86	18	35	27.91
10	70	21.24	18	50	27.98
10	80	21.38	18	40	28.05
10	48	21.66	18	50	28.05
11	350	21.8	18	50	28.12
11	50	21.87	18	30	28.19
11	65	22.71	18	50	28.25
11	40	22.78	18	47	28.6
11	260	23.13	18	33	28.67
12	50	23.27	20	55	28.82
12	50	23.41	20	97	29.03
12	100	23.55	20	62	29.1
12	70	23.69	20	40	29.1
			20	55	29.17
			20	33	29.38
			20	23	29.45
			21	50	29.45
			23	65	29.87

within the acinus that new branches form after birth.

Pattern at 12 weeks Gestation

The youngest lung studied was that of a 12 week fetus. At this age the full complement of bronchial branches is not yet present (Bucher and Reid, 1961), and it might be expected that the arterial pattern in this case would also be incomplete.

At this age there is no identifiable terminal bronchiolus since the lung is still at the glandular stage. Serial reconstruction was therefore made of the main artery of the posterior basal segment, between the hilum of the left lower lobe and the end of an axial bronchus, where it becomes a blind-ended tubule in the mesenchyme near the pleura.

The total length of this pathway was 7 mm, less than a quarter of its length at birth. The total number of bronchial generations was 15 and running with them were only 11 conventional arteries. Bronchial branch number two received an artery from an adjacent main arterial pathway and branches three, seven and eight were accompanied by early and large branches of conventional arteries, number five and six. This may be evidence of remodelling at a later stage when the bronchi will all be supplied by conventional arteries. The branching pattern is shown in Figure III 23. It was more irregular than at birth with the bronchus and its accompanying artery often being some distance apart at their origin from the axial pathways. Occasionally a second airway arose from the main pathway and ran in a different direction before the first has received its accompanying arterial

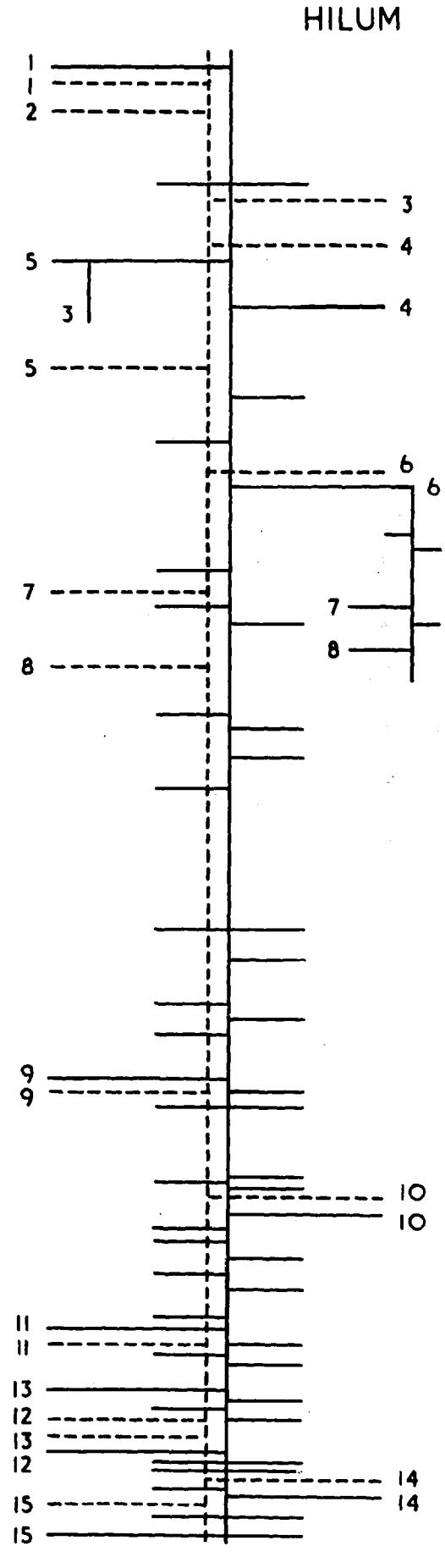
branch, for example, generations 12 and 13. Artery or bronchus may branch first. Elliott (1964) noted that the artery often took a shorter route than the bronchus by branching earlier, these he called short-cut arteries. Irregular branching is more marked in the present study than that described in his.

Supernumerary arteries are present even at this early stage of development. Along this main pathway there were 43, giving a ratio of supernumerary to conventional vessels of 3.9:1. As at birth the supernumeraries were more frequent at the periphery. In the first four arterial generations there were only four (ratio 1:1), in the next two 19 (ratio 8.5:1) and in the last five 20 (ratio 4:1). This increase in the fourth to sixth generations was partly due to the long gap between generations as it was in this part of the pathway that there are two bronchial branches without accompanying arteries. There was a true increase to the periphery since in the first 3.5 mm of the pathway there were 11 supernumerary arteries and in the second half 32. The lack of conventional arteries also accounts for the raised ratio of supernumerary to conventional arteries. If there were 15 conventional arteries, the number expected by airways, the ratio would be 2.9:1. In the first 15 generations of the full term infant there were 37 supernumerary vessels. There may have been loss in number in the proximal region between 12 weeks (when there were 43) and birth but it is more likely that the difference is only due to individual variation.

The arteries develop by growing from the hilum, the distal end being the actively growing region. As the arteries appear they are arranged in the ultimate adult pattern. The number of supernumeraries is related to the number of conventional branches and not to the

Figure III 23

Reconstruction of posterior basal artery and airway to the left lower lobe in a 12 week fetus. The airway generations are numbered with their accompanying conventional arteries. The 43 supernumerary vessels are also shown. Length is estimated from the number of sections cut.



Actual length
7mm.

Artery
Air

length of the pathway since there is no increase in the number as the pathway increases in length. At 12 weeks 43 supernumeraries are found in only 7 mm and at 38 weeks 37 are found in 26 mm.

The conventional branches are more frequent at the periphery as in the case of the 38 week lung. In the older lungs these branches will come to be further apart and more central as new branches appear at the periphery. There is increase in length along the whole pathway as well as an extension at the periphery. Since the last 7 mm of the 38 week fetus shows a similar pattern to the whole length of the 12 week fetus it may be that the interval between branches is related to distance from periphery and capillary bed.

If the pathway of the 38 week fetus and 12 week fetus are each represented as 100 units, it is found that generations one to seven form a greater proportion of the whole length in the older lung. In the 38 week fetus it is half the length and in the 12 week only just over one third. Generations 11 to 15 cover the same proportion at both ages which means that there is a difference in the rate of lengthening in different regions.

Diameter of Arteries

The diameter of the axial pathway is shown in Figure III 24. The diameter at the hilum was only 470 μm and there was a gradual fall to the periphery. This fall was at the same rate as that in the axial pathway of the 38 week fetus beyond the point where its diameter was 470 μm. The 38 week fetus has a steeper gradient near the hilum than the 12 week fetus, a feature also seen on the arteriograms. It seems that the gradient beyond a given point relates to the diameter of the artery at that point not to its

Figure III 24

Diagram of the external diameter of the axial pathway of 12 weeks shown in Figure III 23 and the diameters of the conventional and supernumerary arteries along this pathway. It was not possible to measure the last four conventional arteries and their intervening supernumeraries and these are therefore represented as the same diameter.

Diameter of axial artery and side branches (μm)
500
400
300
200
100
Conventional branches
Supernumerary branches
External diameter of
axial pathway

position in the lung. The total gradient at 12 weeks was 1 in 14 and at 38 weeks 1 in 7.

Airways containing cartilage were found only as far as the seventh generation where the arterial diameter was 250 μm. Filling ended in the artery at 65 μm diameter at the level of the 12th bronchial generation, measurements were not made on the uninjected artery since they would not be comparable.

As in the 38 week fetus the conventional arteries shown also in Figure III 24 are larger than the supernumerary arteries but smaller than the axial pathway at the same level. The size and frequency of branching are shown in Tables III 6 and 7. The mean diameter for the seven measured conventional arteries was 158.5 μm with a range of 55 - 330 μm (S.D. 107.9; S.E. 40.87). The mean for the supernumerary arteries was 36.7 μm with a range of 16 - 75 μm (S.D. 17.4; S.E. 3.13).

Pattern at 19 weeks Gestation

By 16 weeks gestation all bronchial branching is complete (Bucher and Reid, 1961) and the lung enters the 'canalicular' stage (Loosli and Potter, 1952). In fact at this time the epithelium at the distal end of the tubules is converted to alveolar type epithelium - no layer being visible with the light microscope. Capillaries are found in close relationship with the epithelium (Campiche, Gautier, Hernandez and Reymond, 1963). The pattern at 19 weeks of intra-uterine life is described here as an example of this stage.

TABLE III 6

Diameter and Spatial Arrangement of Conventional Arteries and Axial Pathway in Case of 12 Weeks Gestation

Airway Generation	External Diameter of Conventional Artery (μm)	Diameter of Axial Pathway (μm)	Distance from Hilum (mm)
1	230	420	0.28
5	330	370	1.19
4	115	330	1.54
6	240	260	2.38
9	55	125	5.04
10	85	82	5.74
11	55	75	6.3
12	-	-	6.58
13	-	-	6.79
14	-	-	7.07
15	-	-	7.21

TABLE III 7

Diameter and Spatial Arrangement of Supernumerary Arteries in Case of 12 Weeks Gestation

Airway Generation	External Diameter of Supernumerary Artery (µm)	Distance from Hilum (mm)
1	40	0.63
1	75	0.63
4	55	1.61
4	55	1.82
6	75	2.7
6	32	2.91
6	53	2.98
6	37	3.4
6	37	3.47
6	30	3.54
6	17	3.61
6	25	4.38
6	63	4.45
6	21	4.52
6	27	4.73
6	27	4.8
6	40	4.87
9	65	5.15
9	20	5.22
9	27	5.29
9	35	5.5
9	35	5.57
9	57	5.64
10	20	5.81
10	16	5.88
10	16	5.95
10	18	6.02
10	40	6.09
10	25	6.23
11	20	6.37
11	-	6.44
11	35	6.51
12	-	6.65
12	-	6.65
12	-	6.72
13	-	6.86
13	-	6.86
13	-	6.93
13	-	6.93
13	-	7.0
14	-	7.07
14	-	7.14
14	-	7.21

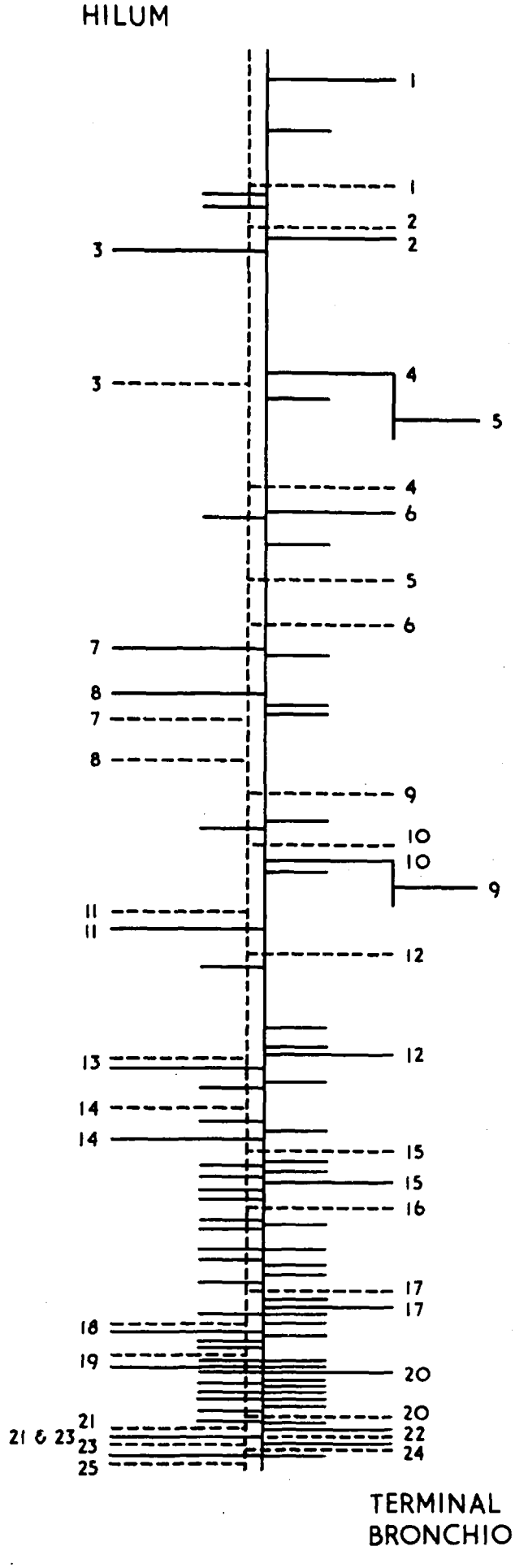
The total length from the lobar hilum to the end of the posterior basal artery of the left lower lobe was 16 mm. The artery terminated in mesenchyme accompanying a multi-branched tubule at the end of the bronchial pathway. This pathway is diagrammatically represented in Figures III 25 and 26. There were 25 bronchial generations to this level, probably the terminal bronchiolus. This falls within the normal range for this segment (Bucher and Reid, 1961). There were 17 generations of bronchi and eight bronchioli, again within the normal range. The increase in length from 12 weeks was partly due to new branches at the periphery and partly to an increase in length of existing arteries and bronchioli since now the first six generations were the same length as the total pathway at 12 weeks gestation. By this time the differential growth of the proximal airways must have occurred since the first seven generations already comprised the first half of the pathway, as at birth.

Running with the 25 bronchial generations were 21 conventional arteries. Airway generations 5, 9, 16 and 23 were supplied by branches of arterial generations 4, 10, 15 and 21 respectively.

There were at this time 58 supernumerary arteries giving a ratio of supernumerary to conventional arteries of 2.76:1 and a ratio of supernumerary arteries to airway generations of 2.32:1. This is slightly lower than the ratio at birth but is likely to be due to individual variation. There may have been a loss in the number of supernumerary branches in the proximal regions since in the first 15 generations there were only 23 while at 12 weeks there were 43. This may have little significance since in the case of 38 weeks there were 37 in this length.

Figure III 25

Reconstruction of the posterior basal artery and airway of the left lower lobe in a 19 week old fetus. The airways and their accompanying conventional arteries are numbered, the supernumerary arteries are also shown.
Length is estimated from the number of sections cut.



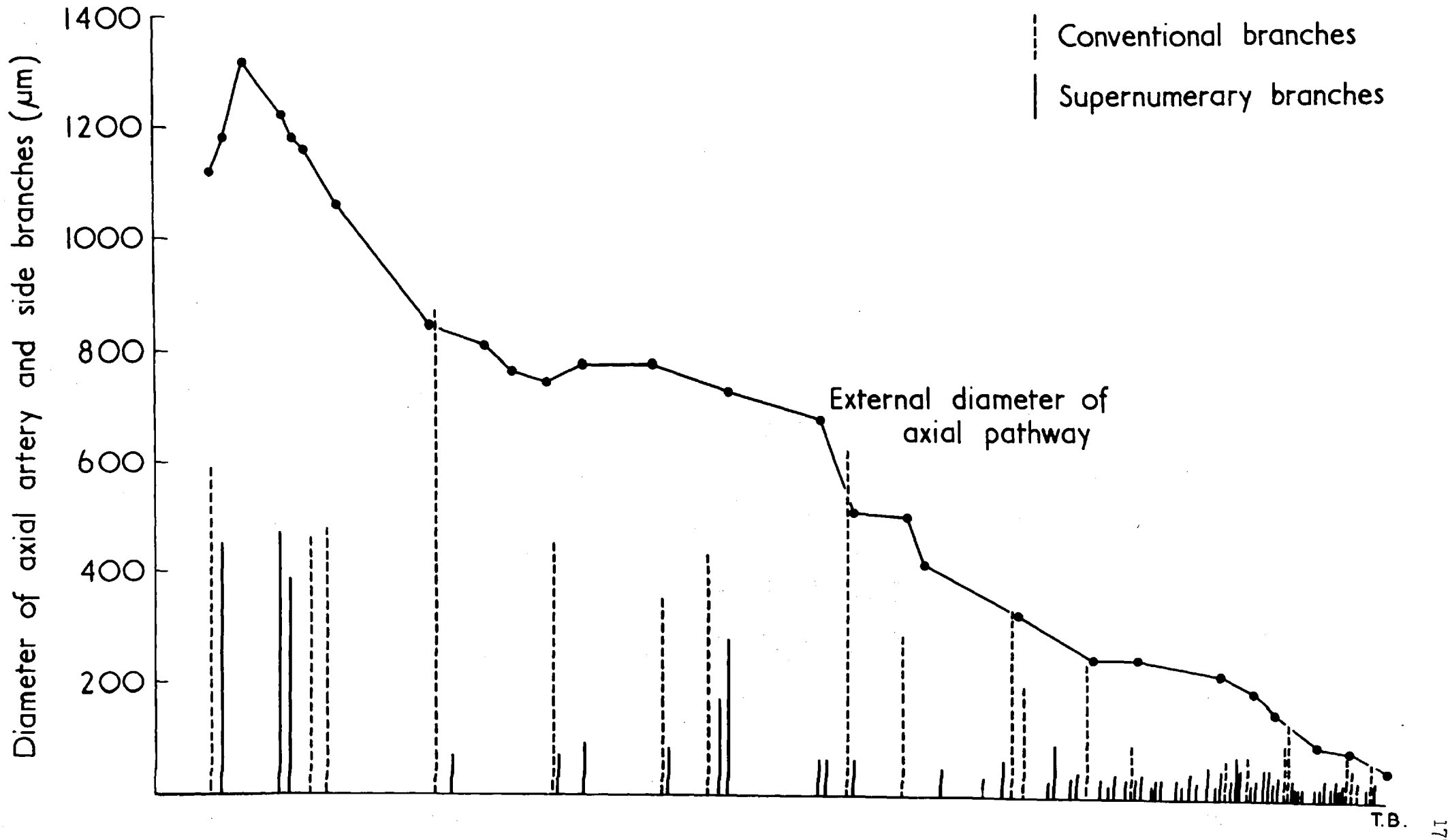
Actual length
16 mm.

—— Artery
------ Airway

Figure III 26

Diagram to show the diameter of the arterial pathway of 19 weeks traced in Figure III 25 and the diameter of conventional and supernumerary arteries.

(T.B.) - Terminal bronchiolus.


Diameter of axial artery and side branches (μm)
1400
1200
1000
800
600
400
200
Conventional branches
Supernumerary branches
External diameter of axial pathway
T.B.

As in the previous two cases the branching of both the conventional and supernumerary arteries was more frequent at the periphery than the hilum. The conventional arteries were particularly close together distal to the 20th generation and, as just stated, the first seven generations comprised half the pathway. In the first 14 bronchial generations there were only 17 supernumerary arteries (ratio 1.2:1) while between generation 15 and 21 there were 39 (ratio 6.5:1). In the last four generations there were only two supernumeraries, a ratio of only 0.5:1. This drop in the last few generations was also seen in the 38 week fetus at the level of the lobule.

Diameter of Arteries

Figure III 26 shows the diameter of the axial pathway and the branches from it. The drop in external diameter at the hilum is probably due to the angle of cutting during preparation. The diameter fell gradually from 1300 μm to 45 μm over the lenth of 16 mm. Similarly the diameter 16 mm from the periphery in the 38 week old fetus was 1300 μm. The distal 7 mm of the 19 week pathway was similar to the total length (7 mm) at 12 weeks. It seems that like the lumen diameter, as measured on the arteriogram, the external diameter relates directly to the subsequent length of the artery. The diameter of the main artery at the level of the last plate of cartilage (17th generation) was 210 μm and at the level of the prospective terminal bronchiolus was 45 μm.

At points along the main pathway there were branches of the artery of the same size as the main vessel. These are points of true dichotomy, for example generations 4 and 8. More often the conventional arteries were smaller than the axial pathway. The conventional arteries had a mean diameter of 282.7 μm with a range

of 32 - 590 μm and a standard error of the mean of 57.08 (Table III 8). This average is greater than that of the 12 week fetus. The conventional arteries were larger on average than the supernumeraries though as before there were supernumeraries of larger diameter than conventional arteries. The average diameter was 62.6 μm with a range of 12.5 - 470 μm and a standard error of the mean of 12.77 (Table III 9).

Pattern of Branching Throughout Fetal Life

Thus the pattern at 19 weeks gestation was very similar to that seen at birth, and it is unnecessary to describe in detail further pathways at other intervals up to birth. The results of eight other cases are included here in a summary of this section, together with the three already described. In these eleven cases the posterior basal artery and bronchus of the left lower lobe were traced by serial section from hilum to periphery: their ages were 12, 14, 16, 17, 18, 19, 20, 23, 28, 36 and 38 weeks of intra-uterine life. The three cases described in detail in this section are underlined.

Total Length of Posterior Basal Artery

The total length of the artery from hilum to periphery increased with age (see Table III 10). This increase was at approximately the same rate throughout fetal life. The pulmonary arteries appear in the fourth week of gestation and by 20 weeks the axial pathway is 17 mm long. By 38 weeks of gestation it has reached 34 mm.

TABLE III 8

Diameter and Spatial Arrangement of the Conventional Arteries and Axial Pathway in the 19 Week Fetus

Airway Generation	External Diameter of Conventional Artery Branch (μm)	Axial Artery Diameter (μm)	Distance from Hilum (mm)
1	590	1120	0.7
2	460	1100	1.96
3	480	1060	2.17
4	870	850	3.57
6	450	750	5.11
7	350	780	6.51
8	430	730	7.07
10	620	510	8.89
11	280	500	9.59
12	330	320	10.99
13	190	300	11.13
14	230	240	11.97
15	87	240	12.53
17	63	210	13.72
18	67	180	14.0
19	90	140	14.49
20	130	130	14.56
21	70	80	15.26
22	58	70	15.33
24	32	60	15.4
25	60	45	15.54

TABLE III 9

Diameter and Distance from Hilum of Supernumerary Arteries in 19 Week Fetus - Grouped by Airway Generation

Airway Generation	Diameter (μm)	Distance from Hilum (mm)	Airway Generation	Diameter (μm)	Distance from Hilum (mm)
1	450	0.84	15	25	13.37
1	470	1.61	15	48	13.51
1	390	1.75	15	22	13.65
4	75	3.78	15	37	13.65
6	70	5.18	17	25	13.79
6	90	5.53	17	68	13.86
7	70	6.58	17	45	13.93
8	170	7.21	18	17	14.07
8	275	7.35	18	25	14.14
8	60	8.47	18	50	14.21
8	60	8.61	18	50	14.28
10	60	8.95	18	20	14.28
11	45	10.08	18	30	14.35
11	30	10.5	20	12.5	14.56
11	55	10.71	20	12.5	14.56
13	25	11.48	20	20	14.63
13	90	11.55	20	20	14.69
13	30	11.76	20	20	14.83
13	40	11.83	20	12.5	14.9
14	30	12.18	20	25	14.97
14	20	12.25	20	30	15.04
14	35	12.32	20	25	15.11
14	30	12.46	20	30	15.18
15	25	12.6	20	20	15.18
15	32	12.67	20	17	15.26
15	17	12.81	24	17	15.47
15	25	12.81	25	25	15.61
15	25	12.88			
15	25	13.16			
15	30	13.23			
15	35	13.3			

TABLE III 10

Summary of Findings During Fetal Life

Age in Weeks	12	14	16	17	18	19	20	23	28	36	38
Total Length (mm)	7	8	7	11.9	11.9	16	16.8	21	28	34	31.01
Bronchial Generations	15	18	18	18	24	25	20	25	28	26	23
Conventional Arteries	11	15	18	16	23	21	19	23	26	24	21
Supernumerary Arteries	43	40	46	40	45	58	43	57	75	76	64
Ratio Sup. : Con. Arteries	3.9	2.6	2.5	2.5	1.9	2.8	2.3	2.5	2.8	3.1	3.1
Ratio Sup. : Bronchial Branches	2.8	2.2	2.5	2.2	1.8	2.3	2.1	2.3	2.7	2.9	2.8

Number of Bronchial Generations

The total number of bronchial generations increased with age. After the 19th week the terminal bronchiolus could be accepted as the end of the pathway (Table III 10). In the pre-acinar region the full complement of bronchial branches was present in the 18 week fetus. Until this age bronchial branching at the periphery was still taking place. Between 18 and 38 weeks gestation there was little variation in the number of pre-acinar generations (23 - 28) except in the case of 20 weeks. It is possible that in this lung one of the shorter pathways of the posterior basal segment had been traced. It is still within the normal values described by Bucher and Reid (1961).

Number of Conventional Arteries

For the pre-acinar region the total adult number of conventional arteries was present by 18 weeks gestation (Table III 10). After this time the number varied, the range being between 20 and 27 branches. In nine out of the eleven cases studied there were fewer conventional arteries than bronchi. These bronchi without their own conventional artery were accompanied by branches from another axial pathway or from a branch of a nearby conventional artery. Making the artery rather than the airway the reference structure these bronchi could be termed aberrant bronchi since they run a shorter course by number of branches than their accompanying artery and they always end up with an accompanying artery. This type of bronchus was not always present, for example in the 16 and 20 week fetus.

Number of Supernumerary Arteries

At all ages there were supernumerary arteries present. The number varied between 40 and 76 (Table III 10). Over 28 weeks of gestation there were over 60 in each case and below 19 weeks there were no more than 46. In the transitional period between 18 and 28 weeks there were 58, 43 and 57. There was an overall increase in number as the number of conventional arteries increased. It seems that over the age of 18 weeks there is considerable individual variation between cases and along different pathways.

Ratio of Supernumerary to Conventional Arteries

Over the whole of the ante-natal development the ratio of supernumerary to conventional arteries varied from 1.9:1 to 3.9:1 with a mean of 2.73:1 (S.D. - 0.52; S.E. - 0.156). The highest ratio was seen in the 12 week fetus, partly due to the absence of four conventional arteries. The variation in the other cases is due to individual variation and pathway variation within a lung. The ratio of supernumerary arteries to airways shows less variation with a range of 1.8 to 2.9, and a mean of 2.42 (S.D. - 0.349; S.E. - 0.105). The number of supernumeraries may be 'controlled' by the number of bronchial branches.

Pattern of Branching

The number of supernumerary arteries between each conventional branch, that is over each arterial generation, is shown in Table III 11, as well as the average for each generation in each case. The average per generation per case has been described above. The number per generation varies from case to case but generally in each the number

TABLE III 11

Number of Supernumerary Branches in Each Arterial Generation

Artery Gen.	Age in Weeks 12	14	16	17	18	19	20	23	28	36	38	Mean No.
1	2	1	0	0	1	3	2	0	0	1	0	.909
2	0	0	0	1	0	0	1	1	3	1	0	.636
3	2	1	0	1	1	0	1	0	3	0	1	.909
4	13+	2	1	2	1	1	2	7	1	4	1	3.18
5	6	4	6	0	0	2	1	2	0	0	1	2
6	6	6+	1	2	4	1	8+	2	3	3	3	3.5
7	3	4	2	2	3	4	4	1	4	5	9	3.5
8	3	1	2	5	1	1	1	1	1	0	5	1.9
9	5	4	9+	9+	1	3	6	4	0	3	5	4.5
10	2	5	1	3	0	0	1	0	4	7	8	2.8
11		2	4	3	2	4	2	3	10	4	0	3.4
12		1	8	1	2	4	4	2	4	6	4	3.6
13		1	0	0	2	12+	0	1	8	0	4	2.8
14			1	5	1	3	3	2	0	0	0	1.66
15			1	1	0	6	4	5	5	9+	1	3.4
16			4		2	0	0	1	0	2	13+	2.75
17			3		6+	12	1	5	3	4	0	4.25
18			3		0	0	1	1	1	5	7	2.25
19					0	0		3	1	2	1	1.16
20					1	1		2	0	6	0	1.66
21					1	1		3	13+	2	1	3.5
22								8+	0	1		3
23								0	5	7		4
24									4	2		3.0
25									2			2.0

increases in the more peripheral generations. The generation in each case with the greatest number is marked with an asterisk. This varies in position but is more often at the periphery. In the case of 12 weeks the large number at generation 4 is due to the lack of conventional vessels.

The average figures for each generation show that there are few supernumerary arteries in the first three generations but in later generations no trend is apparent. This is because larger numbers at the periphery are reached earlier in the younger cases because branching is incomplete. In some generations the standard deviation is very high so that the average figures have little value. By totalling the averages the number of supernumeraries in 25 generations is 63.57, an average per generation of 2.54. This is almost the same as the average ratio of supernumeraries to conventionals and shows that all cases show the same basic features.

In all the cases where the lobule could be defined, there was a smaller number of supernumeraries in the intralobular pre-acinar region than found in the adult, suggesting that there is growth of new vessels in this region during childhood.

The distance between the conventional branches, that is the generation length, for all the cases studied is shown in Table III 12, with the mean, standard deviation and standard error of the mean for each generation and each case.

There is a general trend to a decreasing generation length towards the periphery. There are variations so that in some cases there are short generations near the hilum so that there is an apparent trichotomy, for example the eighth generation of the 20 week

TABLE

Length of Generations

Artery Generation	Age in Weeks 12	14	16	17	18	19	20
1	0.56	0.42	0.35	0.35	0.28	1.61	3.08
2	0.35	0.98	0.56	2.73	0.28	1.61	1.75
3	1.05	0.35	0.21	0.28	2.03	1.54	0.42
4	2.8	0.42	0.77	0.63	0.42	1.4	0.49
5	0.63	1.84	1.4	0.03	0.14	0.56	1.75
6	0.56	1.3	0.07	1.07	3.01	1.82	4.06
7	0.28	0.56	0.28	1.2	1.75	0.7	1.54
8	0.21	0.24	0.35	2.24	0.35	1.61	0.07
9	0.17	0.38	0.84	1.36	0.42	0.28	1.47
10		0.28	0.14	0.35	0.7	1.68	0.14
11		0.28	0.42	0.56	0.84	1.19	0.35
12		0.21	0.63	0.28	0.28	2.1	0.35
13		0.10	0.07	0.07	1.05	0.77	0.07
14		0.14	0.14	0.21	0.14	0.28	0.35
15			0.07	0.28	0.42	0.35	0.35
16			0.21		0.21	0.35	0.05
17			0.21		0.42	0.28	0.07
18					0.14	0.14	0.04
19					0.07	0.14	
20					0.07	0.7	
21					0.14	0.7	
22					0.07		
23							
24							
25							
Mean	0.73	0.53	0.39	0.76	0.6	0.88	0.91
S.D.	0.82	0.49	0.85	0,79	0.74	0.69	1.15
S.E.	0.27	0.13	0.079	0.206	0.16	0.15	0.27

III 12

during Fetal Life (mm)

23	26	36	38	Mean	S.D.	S.E.
2.3	2.59	1.26	2.1	1.35	1.03	0.31
1.47	4.06	3.78	1.61	1.74	1.29	0.39
1.54	1.68	0.35	2.45	1.08	0.8	0.24
3.71	2.38	5.95	2.1	1.91	1.73	0.52
1.47	0.07	0.63	2.17	0.97	0.77	0.23
1.05	2.8	4.41	4.41	2.23	1.58	0.48
1.05	2.66	6.22	4.76	1.9	1.9	0.57
0.7	0.98	0.14	1.96	0.8	0.8	0.24
1.82	0.7	2.1	1.47	0.99	0.66	0.2
0.14	1.33	1.75	1.68	0.82	0.7	0.22
1.33	2.52	1.05	0.7	0.92	0.66	0.21
0.63	0.98	0.63	1.26	0.73	0.58	0.18
0.35	1.54	0.7	0.1	0.48	0.51	0.16
0.49	0.14	0.77	0.1	0.28	0.21	0.06
0.84	1.4	1.26	1.4	0.71	0.52	0.17
0.14	0.03	0.56	0.28	0.23	0.17	0.06
0.49	0.45	0.49	0.63	0.38	0.18	0.06
0.21	0.14	0.98	0.07	0.24	0.32	0.12
0.21	0.28	0.35	0.07	0.18	0.11	0.05
0.21	0.14	0.7	0.07	0.21	0.24	0.09
0.14	0.84	0.14		0.26	0.32	0.14
0.49	0.05	0.14		0.19	0.2	0.1
	0.28	0.63		0.45	0.24	0.17
	0.28			0.28		
	0.14			0.14		
0.94	1.13	1.5	1.4	0.77		
0.87	1.12	1.8	1.34	0.62		
0.18	0.22	0.37	0.3	0.12		

fetus and the fifth generation of the 28 week fetus. The mean figure for each generation shows a gradual diminution towards the periphery.

With age, there is an increase in the average length of a generation in any one case, though here again there is a wide range and hence a large standard error. The increase in generation length is related to the total increase in length and in all the cases over 12 weeks gestation the first seven generations constitute half the total length. It seems that before a pathway, arterial or bronchial, is complete the proximal half grows faster but once complete there is equal growth along its whole length. The lung arteries and bronchi are both growing symmetrically.

Diameter of the Axial Pathway

In all ante-natal lungs the arterial diameter decreased towards the periphery. Table III 13 shows the diameter of the axial artery at various levels - at the hilum, at the level of the last plate of cartilage and at the level of the terminal bronchiolus. The diameter at the hilum increased with age. The 16 week fetus had an exceptionally small pulmonary artery system throughout the whole lung and the main pathway was rather short for age, however the branching pattern was normal. There was a ten fold increase in hilar diameter between 12 and 38 weeks which is the same as that measured on the arteriograms.

The last plate of cartilage was seen in the seventh generation at 12 weeks gestation, by the 14th week cartilage had reached the 14th generation. In the other cases the generation to which it reached, varied and did not relate to the total number of generations.

TABLE III 13

Diameter of Axial Artery During Fetal Life

(μm)

Age (wks)	12	14	16	17	18	19	20	23	28	36	38
At hilum	470	1050	650	1200	980	1300	2100	2500	3300	3900	4500
At last plate of cartilage	240 (7)+	180 (14)	137 (11)	200 (11)	260 (11)	210 (17)	290 (12)	212 (17)	500 (15)	600 (16)	380 (18)
At terminal bronchiolus	-	-	-	-	-	45	-	60	70	112	250
7 mm from periphery	470 (1)+	800 (2)	650 (1)	640 (5)	640 (5)	480 (9)	730 (7)	530 (11)	680 (14)	600 (14)	780 (11)

+ Generation at this level

Since the position varied so did the diameter at this level but even so it was still possible to see an increase in size with age.

Where a terminal bronchiolus could be identified the diameter of the accompanying artery increased with age.

The diameter was also measured 7 mm proximal to the terminal bronchiolus. Here the diameter varied between 470 μm and 800 μm. This was similar to measurements made on the arteriograms one centimetre from the pleura. The generation at this point varied depending on the age of the fetus, being in the later generations in the older cases. This confirms that the diameter is related to the distance the pathway subsequently runs and not to the pattern of arterial branching.

Diameter of Conventional Arteries

Table III 14 shows the diameter of the conventional branches for the 11 cases. In each case the arteries became smaller towards the periphery. There was some variation so there was not necessarily a steady reduction in size. In each case the arteries dropped to about the same size but in the older cases this occurred after more branches and over a longer pathway. In the proximal generations the arterial size increased with age if allowance is made for variation between pathways. A small branch may be found between two large ones in one case but after both of them in another. The mean figures for each generation show a reduction in size to the distal end. The standard deviation is large because of the great increase in size with age particularly at the hilum.

When the average of all the branches in each case was estimated there was an increase with age from 158 μm at 12 weeks to 742 μm at 38 weeks. The average for the 16 week fetus was low because of the

TABLE

Size of Conventional Artery Branches

Artery Generation	Age in Weeks 12	14	16	17	18	19	20
1	230	800	250	530	540	590	610
2	330	380	410	1000	760	460	1020
3	115	240	420	345	980	480	430
4	240	450	212	600	920	870	770
5	55	250	195	250	520	450	710
6	85	310	260	320	310	350	710
7	55	112	140	340	112	430	390
8	-	80	55	250	350	620	460
9	-	57	120	160	160	280	250
10	-	80	45	65	230	330	200
11	-	65	40	128	60	190	240
12		60	40	62	115	230	112
13		48	30	50	65	87	80
14		37	-	48	37	63	155
15		-	-	30	-	67	65
16		-	-	30	-	90	50
17			-		-	130	65
18					-	70	50
19					-	58	110
20					-	32	
21					-	60	
22					-		
23					-		
24							
25							
26							
Mean	158	212	170	263	368	282	340
S.D.	107	217	136	264	325	233	296
S.E.	40.5	58	37.7	66	86.9	50.9	68

III 14

During Fetal Life (μm)

23	28	36	38	Mean	S.D.	S.E.
1500	1350	2650	1150	927	707	213
1550	2720	2000	2500	1193	873	263
800	1100	880	1500	667	418	144
1050	1200	1600	1550	860	472	142
620	1040	1250	1800	649	526	158
400	1100	820	1600	569	450	135
550	800	540	1400	442	392	118
125	855	540	860	419	300	95
540	620	520	490	320	203	64
255	245	430	340	222	128	40.5
100	95	330	350	160	112	35.4
100	470	680	470	233	224	70.8
95	270	380	260	136	121	38
100	270	100	350	129	111	35
305	100	250	147	137	103	39
73	187	87	250	109	79	30
73	137	90	220	119	57	23
70	115	95	100	83	24	10
93	112	175	90	106	39	15.9
75	95	70	75	69	23	10.3
50	85	85	77	71	16	7.17
62	87	150		99	45	26
62	75	55		64	10	5.78
	40	37		38	5	3.54
	48					
	25					
376	509	575	742			
454	623	677	714			
94.7	122	138	156			

small size of the lung. That of the 18 week was high because the distal nine generations which are the smaller could not be measured since injection was not complete. The standard deviation was high in all cases because of the marked change in size from hilum to periphery.

Diameter of Supernumerary Arteries

In each of the generations the diameter of all the supernumeraries has been averaged. The results are shown in Table III 15: at all ages this average is smaller than for the conventional arteries. This method has the disadvantage that there are a different number of vessels in each generation. The particularly high average seen in some generations near the hilum is due to one very large artery which would fall into the category of an 'aberrant' artery since it does not supply the acinus directly; towards the periphery these larger vessels are not seen. In each case the supernumerary arteries decreased in size towards the periphery, the average over each bronchial generation of all the cases also decreased towards the periphery. After the ninth generation the arteries remained much the same size with a smaller standard deviation. This was due to the lack of 'aberrant' arteries (see page 160).

The average size of the supernumeraries in each case increased with age. It is interesting that the mean derived by this method for the 12, 19 and 38 week fetus was higher than the actual mean of all the supernumerary arteries shown earlier. This is probably because the larger aberrant arteries will have a greater effect on the mean when the number of small vessels has been decreased by averaging the number in each generation.

TABLE

Size of Supernumerary Arteries during

Age in Weeks

Artery Generation	12	14	16	17	18	19	20
1	357.5	260	-	-	375	436	740
2	-	-	-	45	135	-	85
3	55	87	75	62	75	-	-
4	37.3	60	115	76	-	75	337
5	39.9	75	35	42.3	79	80	205
6	22.5	54.3	36	210	-	70	89.2
7	27.5	45.6	25	36.8	60	141.2	77
8		-	34.2	39.2	-	60	215
9		66.6	50	27.4	-	43.3	45
10		25	31.3	-	25	-	65
11		35	23.1	33.5	-	46.2	30
12		15	-	-	26	28.8	36.3
13		40	12	25	43.5	28.8	-
14				15	55	46	49
15				17	-	32	31.6
16				25	19	-	-
17				-	25	20.4	-
18					35.4	-	
19					37	-	
20					15	17	
21					20	25	
22					-		
23					-		
24							
25							
Mean	40	69	44	50	46	76	154
S.D.	14	66	30	51	32	104	198
S.E.	5.7	19.9	9.5	14.2	8.26	26.9	55

III 15

Fetal Life (μm) (Mean size over each artery generation)

23	28	36	38	Mean	S.D.	S.E.
-	-	450	950	482	322	122
250	716	137	-	228	248	102
-	310	240	500	175	161	57
209	710	-	370	221	220	73
73.5	-	107	-	81	51	17
101.5	194	97.8	163	103	65	21
150	106	-	171.5	84	54	17
250	175	-	129	128	87	33
55.5	-	79	153	64	38	13
-	41.3	53.5	75.3	45	20	7.6
53.3	37.4	48	-	39	11	3.9
32	100.4	59.8	40	42	27	9.6
25	46	-	49.3	33.6	13	4.6
26.5	-	47.5	-	39	15.5	6.4
21	37.4	30	35	29	7.4	2.8
73	-	52.8	38.6	42	22	9.9
23	40	31.25	61.8	33.5	15.5	6.4
-	-	55.6	35.3	42	12	6.9
23.5	35	51.1	-	37	11	5.5
15	-	27.5	-	19	6	3.0
26.6	37.8	-	47.3	31	11	4.9
20.87	-	31.7		26	8	5.7
	19	27.5		23	6	4.3
	19	25		22	4	2.8
	29	20		25	6	4.3
79	156	84	187			
80	223	100	250			
18.9	54.1	22.4	64.5			

- No supernumerary arteries present

Branching Pattern of a Lateral Branch

A study was made of a lateral pathway in the 19 week old fetus to ascertain whether a pattern similar to that of the axial pathway was seen throughout the lung.

A serial reconstruction was made of the seventh bronchial bronchial branch and its accompanying artery from the axial pathway described previously (page 170). Figure III 27 shows the branching pattern of this pathway and Table III 16 the tabulated results. The total length of this pathway from its origin at the axial pathway to the terminal bronchiolus was 4.9 mm. Over this length there were 11 bronchial branches with 11 accompanying conventional arteries. There were in addition 40 supernumerary arteries giving a ratio of supernumerary to conventional arteries of 3.63:1. This ratio is higher than that for the total axial pathway since it does not include the proximal generations where there are fewer supernumeraries. It is similar to the last 11 generations of the axial pathway which had 44 supernumeraries.

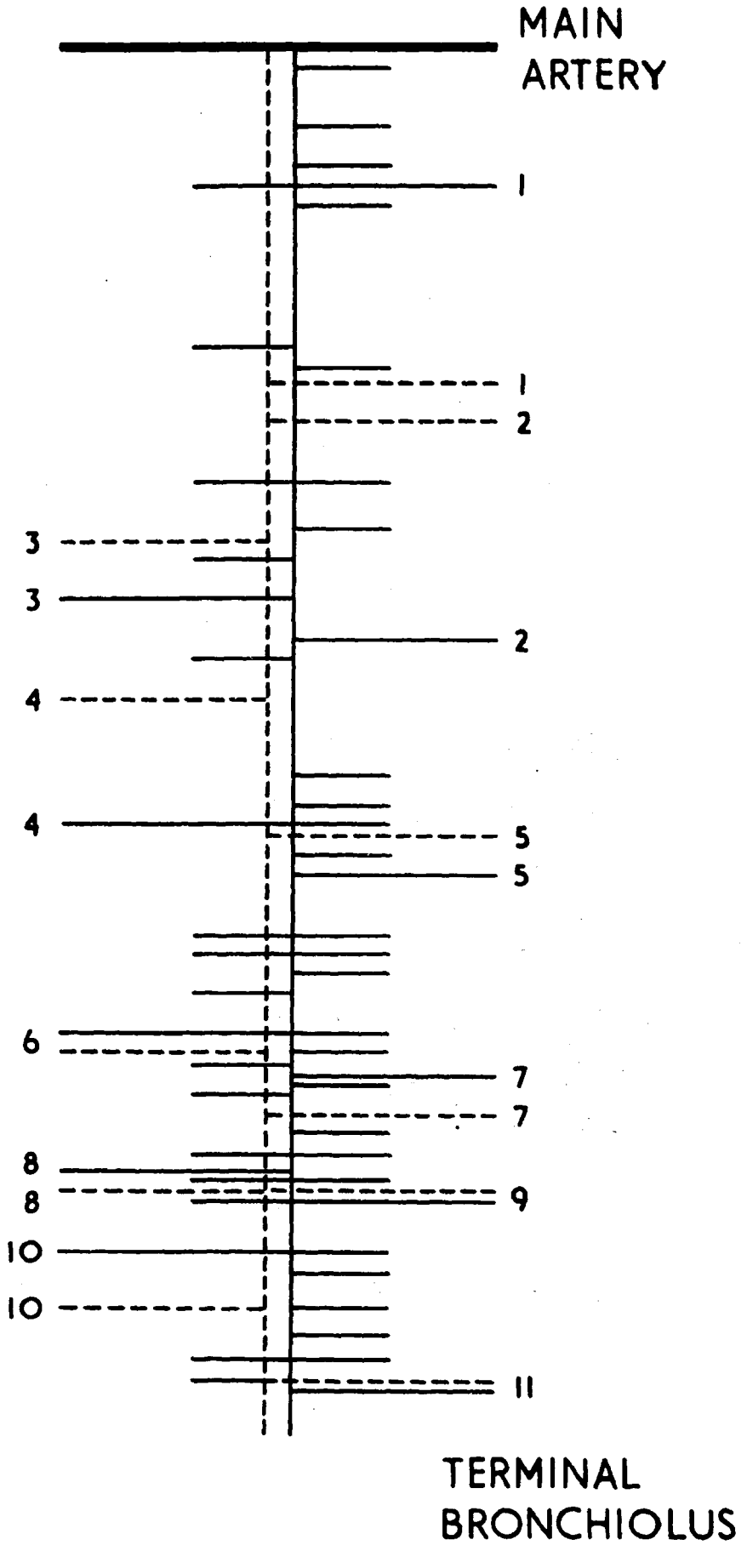
Cartilage extended to the fifth generation of this lateral pathway; this is the 12th from the hilum. This was not as far along as the axial pathway where it reached the 17th generation. However, this was a shorter pathway and there were only six generations of bronchioli in comparison with eight along the axial pathway.

The bronchial branching and conventional artery branching became more frequent towards the periphery as in the axial pathway. The supernumerary arteries also arose at more frequent

Figure III 27

Reconstruction of the branching pattern of conventional branch number 7 in the 19 week old fetus. The airway branches and their accompanying conventional arteries are numbered, the main artery being regarded as generation 0.

The length is derived from the number of sections cut.



Actual length
4·9 mm.

——— Artery
----- Airway

intervals but the number between generations did not increase. This was also true of the last eleven generations of the main pathway.

This lateral artery when it left the main pathway was 360 μm in diameter and it reduced steadily in size towards the periphery. At the terminal bronchiolus it was 37 μm and the diameter at the end of the cartilage was 170 μm. These figures are slightly lower than those of the axial pathway, where they are 45 and 210 μm respectively.

The conventional arteries decreased in size towards the periphery though as before there was no set pattern of reduction. The average size of the conventional arteries was 107 μm with a range of 35 - 240 μm (S.D. - 112; S.E. - 33.7). The average of the last 11 generations of the axial pathway was similar at 112 um.

The supernumerary arteries were all smaller than adjacent conventional arteries and since there were no aberrant arteries they were all much the same size with an average of 32.4 μm and a range of 15 - 87 μm (S.D. - 32.8; S.E. - 5.18). The average diameter for supernumerary vessels in the last 11 generations of the axial pathway was 29.3 μm.

The branching pattern of a lateral pathway seems essentially the same as that of an axial artery. This lateral pathway was almost the same as the main pathway if judged over a similar length from the end of the axial pathway, with the same number of branches and of similar dimension. There seems to be a set pattern within a lung for the number of branches over a given length of pathway from the capillary bed to its junction with an axial pathway. Thus

TABLE III 16

Diameter of Artery Branches and Axial Vessel of a Lateral Branch in a 19 Week Fetus

Conventional Branches and Axial Artery

Generation	Diameter of Branch (μm)	Diameter of Axial Artery (μm)	Distance from Main Artery (mm)
1	240	360	0.49
2	155	335	1.96
3	105	290	2.1
4	200	-	2.8
5	155	170	2.94
6	70	125	3.5
7	50	112	3.64
8	70	90	3.99
9	37	75	4.13
10	60	60	4.27
11	35	37	4.76

Supernumerary Arteries

Generation	Diameter (μm)	Distance from Hilum (mm)	Generation	Diameter (μm)	Distance from Hilum (mm)
0	87	0.07	5	37	3.29
0	75	0.28	5	20	3.36
0	40	0.42	6	20	3.5
1	30	0.49	6	30	3.53
1	25	0.56	6	35	3.57
1	50	1.05	7	20	3.67
1	20	1.12	7	25	3.71
1	62	1.54	7	20	3.85
1	60	1.54	7	45	3.92
1	25	1.68	7	20	3.92
1	37	1.82	8	20	4.06
3	55	2.17	8	50	4.06
3	37	2.59	8	25	4.11
3	20	2.73	10	20	4.27
4	20	2.8	10	20	4.34
4	25	2.87	10	15	4.48
5	20	3.15	10	15	4.55
5	20	3.15	10	37	4.62
5	37	3.22	10	20	4.62
5	25	3.22	10		

a long lateral pathway is equivalent to a considerable extent of the end of an axial pathway.

Branching Pattern of the Posterior Basal Segment of the Right Lung

In addition to the reported work on the left lung, a serial reconstruction was made of the posterior basal segment of the right lung in a fetus of 40 weeks gestation: this was compared with the left lung of the 38 week old fetus, this being the closest in age.

The total reconstructed length of the artery to this segment from the hilum was 35 mm. There were 22 airway generations from the hilum to the terminal bronchiolus, of which 14 contained cartilage. Accompanying the airways were 22 conventional arteries. Several of these arteries arose some distance from the bronchial branch which they eventually accompanied. There were in this case 69 supernumeraries which gave a ratio of supernumerary to conventional arteries of 3.13:1, which is within the normal range for the left lung in this series and very close to that for the 38 week fetus which was 3.1:1. The results for this case are shown in Table III 17 and 18.

The pattern is essentially the same as that seen in all other cases with the branching of airways and conventional and supernumerary arteries becoming more frequent towards the periphery. The first seven generations occupied half of the total length as seen in the left lung, over this length there were only 10 supernumeraries. From the proximal half of the pathway arose one third of the conventional branches and one seventh of the supernumerary arteries.

TABLE III 17

Diameter and Branching Pattern of Conventional Arteries in the Right Lung of a Fetus of 40 Weeks Gestation

Artery Generation	Diameter of Branch (μm)	Diameter of Axial Artery (μm)	Distance from Hilum (mm)	Distance from Last Branch (mm)
1	2010	4300	0.42	
2	3400	2600	3.15	2.73
3	1300	2600	3.36	0.21
4	1300	2000	6.79	3.43
5	1700	1700	12.11	5.32
6	1400	1450	14.98	2.87
7	600	1300	18.76	3.78
8	510	1200	20.09	1.33
9	320	1150	22.19	2.1
10	610	1100	23.38	1.19
11	600	850	25.55	2.17
12	390	820	26.81	1.26
13	580	750	27.72	0.91
14	300	550	29.33	1.61
15	450	530	29.47	0.14
16	180	490	29.89	0.42
17	350	500	30.38	0.49
18	290	390	32.2	1.82
19	125	290	32.41	0.21
20	112	160	34.3	1.89
21	100	105	34.72	0.42
22	113	100	34.93	0.21

The arteries of both types decreased in diameter towards the distal end of the pathway and the axial artery showed a diminution in size towards the periphery. It was 4300 μm at the hilum and reduced to 550 μm at the level of the last bronchus. The artery at the level of the terminal bronchiolus was 100 μm. The diameter seven millimetres from the periphery, that is with the 13th generation, was 750 μm.

The average size of the conventional arteries was 760 μm with a range of 100 - 3400 μm (S.D. - 778; S.E. - 165.8). The average at 38 weeks was 741 μm. The average size of the supernumerary arteries was 95.7 μm in comparison with 110 μm in the 38 week old. The range in this case was 25 - 860 μm with a standard error of 11.62.

These results are all very similar to those seen in the left lung of the 38 week old fetus. It seems that the left and right lung follow a similar pattern of development.

RECONSTRUCTION OF THE INTRA-ACINAR BRANCHING PATTERN OF THE ARTERIAL AXIAL PATHWAY

The acinus is defined as that region supplied by a single terminal bronchiolus and is the respiratory portion of the lung. For the purpose of this study the terminal bronchiolus is included with the acinus and measurements have been made from the proximal end of the terminal bronchiolus.

Before birth the intra-acinar region was not traced precisely since even in the later stages of intra-uterine life it was difficult to differentiate between the various airway structures and artery filling scarcely extended beyond the terminal bronchiolus. In fact the acinus is very small at birth and its main development takes

TABLE III 18

Diameter and Branching Pattern of Supernumerary Arteries in the Right Lung of a Fetus of 40 Weeks Gestation

Artery Generation	Diameter (μm)	Distance from Hilum (mm)	Artery Generation	Diameter (μm)	Distance from Hilum (mm)
0	860	0.35	19	32	32.41
3	310	3.57	19	190	32.48
4	350	7.21	19	50	32.48
4	280	12.11	19	30	32.97
5	212	12.25	19	40	33.04
6	87	15.54	19	37	33.11
6	155	15.82	19	30	33.11
6	100	18.34	19	45	33.25
6	75	18.41	19	32	33.32
6	182	18.69	19	60	33.32
7	155	19.74	19	25	33.37
7	290	19.95	19	40	33.51
8	225	20.16	19	37	33.51
8	112	21.28	19	30	33.58
8	137	22.05	19	25	33.65
9	200	23.17	19	30	33.72
9	320	23.31	19	60	33.86
10	42	24.08	19	60	34.0
10	100	25.27	19	75	34.07
11	40	25.55	19	55	34.07
11	172	25.83	19	25	34.23
12	125	27.44	20	48	34.37
13	40	28.21	20	40	34.44
13	40	28.35	20	25	34.51
13	40	29.05	20	37	34.51
15	50	29.75	20	20	34.58
17	50	30.59	20	30	34.58
17	50	30.73	20	55	34.65
17	30	30.87	20	25	34.65
17	50	30.94	20	25	34.72
17	100	31.29	21	25	34.86
17	62	31.5	21	25	34.93
17	40	31.64			
17	30	31.64			
17	35	31.78			
17	48	32.06			
17	50	32.13			

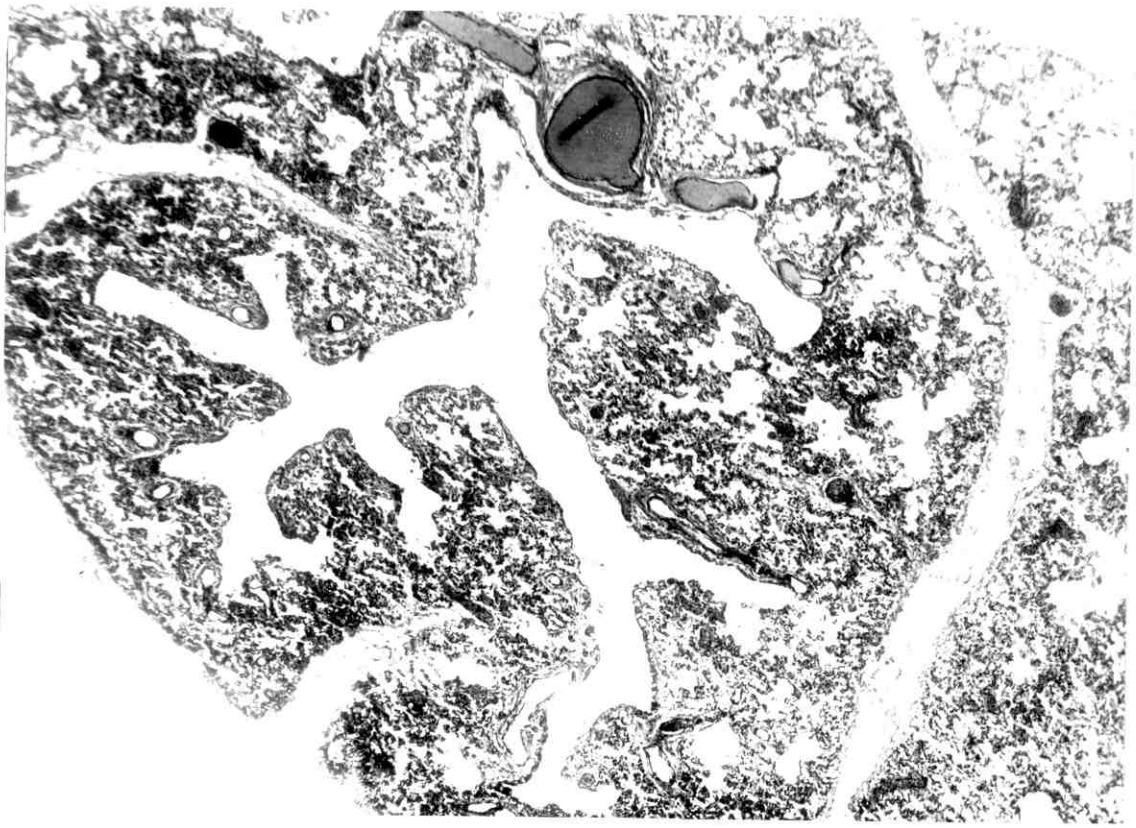
place after birth (Boyden and Tompsett, 1965).

The Branching Pattern at Birth

In the 38 week old fetus it was possible to trace the arteries within the acinus. The distance between the proximal end of the terminal bronchiolus and the pleura was 1520 μm and of this pathway the terminal bronchiolus represented only 70 μm. It was possible to trace the airway and arterial branching pattern to within 400 μm of the pleura. The intervening area was alveolar and since the air spaces were not fully inflated it was impossible to reconstruct their branching. The branching pattern of the airways within the acinus is shown in Figure III 28. The airways have been cut in longitudinal section. There were six airway generations before the pathway terminated in alveolar tissue, four of these being respiratory bronchioli and the two distal ones, alveolar ducts. The main pathway only became an alveolar duct in the last generation traced. Accompanying the airways were six conventional arteries and in addition 10 supernumerary arteries, eight of which were between the first two conventional arteries. This gave a ratio of supernumerary to conventional arteries for this region of 1.65:1 which is very much lower than was seen in the pre-acinar region of the lung.

The diameter of the axial pathway and of its branches are shown in Figure III 29 and Table III 19. The axial artery was 250 μm in diameter at the terminal bronchiolus and reduced to 75 μm at the last respiratory bronchiolus; filling did not extend beyond this. As in the pre-acinar region the conventional branches were smaller than the axial artery having an average diameter of 72.9 μm but larger than the adjacent supernumerary arteries with an average of 25.2 μm.

Figure III 28



Photomicrograph of a section through the acinus reconstructed in the newborn infant of 38 weeks' gestation. The terminal bronchiolus is accompanied by a filled artery cut in transverse section. Respiratory bronchioli and alveolar ducts can be seen, the latter end in poorly inflated alveolar region. (x 50)

Figure III 29

Branching pattern of the arteries within the acinus and in the immediate pre-acinar region at birth. The diameter of the axial pathway and the conventional and supernumerary vessels is shown. The actual length of the pathway traced is 2.17 mm.

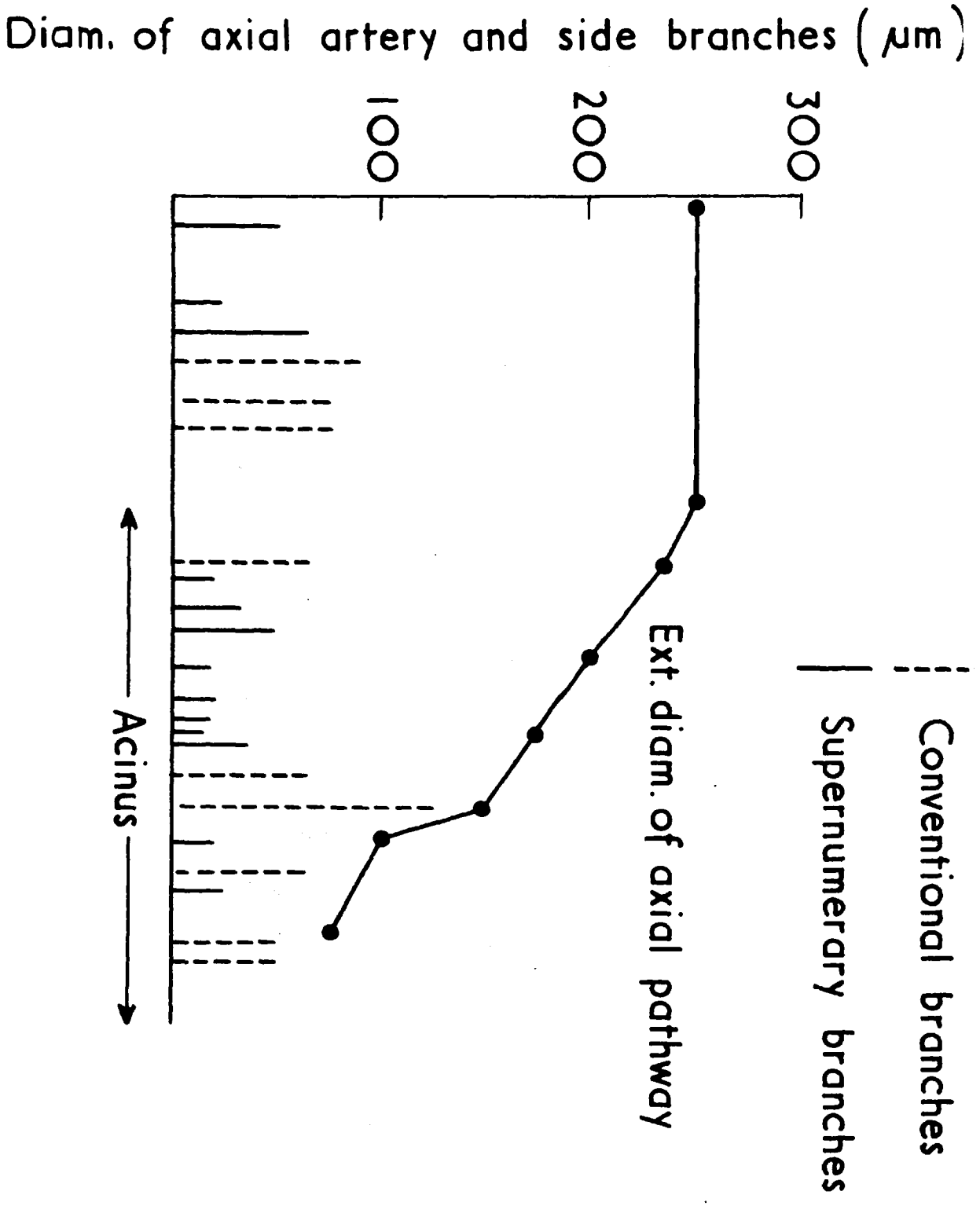

Diam. of axial artery and side branches (μm)
100
200
300
Acinus
Ext. diam. of axial pathway
Supernumerary branches
Conventional branches

TABLE III 19

External Diameter of Axial Artery and Conventional and Supernumerary Branches within the Acinus at Birth (μm)

With Respiratory Bronchiolus	Conventional Arteries	Supernumerary Arteries	Axial Artery
1	65	19	175
1		32	
1		48	
1		18	
1		20	
1		18	
1		15	
1		37	
2	65		175
3	125	20	150
4	62	25	75

No filling at the level of alveolar ducts.

The Branching Pattern During Childhood

The pattern of branching within the acinus was traced during childhood by reconstructing the peripheral centimetre of the axial pathway. The six cases used were aged three days, four months, 18 months, five years, 10 years and 11 years. In all cases this centimetre of tissue included a whole lobule and some of the pre-lobular generations. The results are summarised in Table III 20.

Length of Intra-Acinar Axial Pathway

In the three day infant the total length of the axial bronchus from the proximal end of the terminal bronchiolus to the distal edge of the acinus was 1330 μm, which is similar to the length seen at birth. Individual alveoli were more clearly discerned as separate entities. At four months of age the length of artery from the beginning of the terminal bronchiolus to the end of the alveolar duct was still only 1410 μm. By 18 months this length was increased to 2520 μm and by five years to 5180 μm. This last figure seems high and is because the terminal bronchiolus was particularly long, being 2380 μm in length. At ten years the acinus was only 3500 μm in length and at 11 years it was even smaller, being only 2950 μm. It seems that there are acini of variable length; this is often related to the length of the terminal bronchiolus. If measurements of the acinus are only made from the distal end of the terminal bronchiolus there is a more uniform increase with age (see Table III 20). However there is still some variation and this can be explained by the different shapes of acini found in the lung (Reid, 1967b). The alveoli multiply up to the age of eight years and increase in size as the chest increases in size up to adulthood (Davies and Reid, 1970). It is therefore expected that the length of the acinus would increase

TABLE III 20

Branching Pattern within the Lobule during Childhood

Age	Birth	3 day	4 mth	18 mth	5 yr	10 yr	11 yr
Length of Acinus (μm)	1520	1330	1410	2520	5180	3500	2950
Length of Respiratory Region (μm)	1450	1290	1130	2240	2800	3150	2460
Length of Intralobular Pre-Acinar Region (μm)	210	1470	980	5430	2380	4340	2240
Number of Respiratory Bronchioli	4	4	2	2	4	5	2
Number of Alveolar Ducts	2	4	3	5	5	6	4
Number of Conventional Arteries in Acinus	6	8	5	7	9	10	6
Number of Supernumerary Arteries in Acinus	10	7	6	26	36	34	11
Ratio of Supernumerary to Conventional Artery in Acinus	1.66	0.87	1.16	3.7	4	3.4	1.8
Number of Terminal Bronchioli	3	5	4	4	3	6	2
Number of Conventional Arteries in Lobule	3	5	3	4	2	4	2
Number of Supernumerary Arteries in Lobule	1	12	5	20	6	22	3
Ratio of Supernumerary to Conventional Arteries in Lobule	0.33	2.4	1.6	5	3	5.5	1.5

with age.

Length of Intra-Lobular Pre-Acinar Pathway

At three days this part of the pathway was 1470 μm in length, at four months it was 980 μm. There was no apparent increase with age, this length varying between cases, being at its longest in the case of 18 months when it was 5430 μm. This pre-acinar portion of the lobule may be shorter or longer than the intra-acinar region.

Number of Generations within the Lobule

In the three day child there were eight airway branches within the acinus of which four were respiratory bronchioli and four alveolar ducts (Table III 20). There were five intra-lobular, pre-acinar bronchial branches (i.e. terminal bronchioli). Accompanying these 13 airway generations were 13 conventional arteries. There were in addition, in the pre-acinar region, 12 supernumerary arteries giving a ratio of 2.4:1 and in the acinar region seven supernumeraries giving a ratio of 0.87:1. The ratio in the pre-acinar region was higher than that seen in the case at birth, but lower in the acinar region. This probably reflects individual variation.

In the four month child there were five airway generations within the acinus, the branches being two respiratory bronchioli and three alveolar ducts. There were four intra-lobular pre-acinar branches. There were eight conventional arteries, five within the acinus. There were in the acinus, six supernumerary arteries giving a ratio of 1.16:1 and five in the pre-acinar region, a ratio of 1.6:1. This was a rather low number of supernumerary vessels particularly for the pre-acinar region.

At 18 months there were two respiratory bronchiolar branches and five alveolar ducts within the acinus. There were four terminal bronchioli branching from the pre-acinar region of the lobule. There was therefore a total of five acini in this lobule. All airway branches were accompanied by conventional arteries. It was possible to trace artery branches direct to the alveoli in the region distal to the alveolar ducts. These did not accompany airways while the main vessel was still running with an alveolar duct and have therefore been included as supernumerary vessels to the level when the axial artery came to lie within the alveolar wall. There were 26 supernumerary vessels within the acinus giving a ratio of supernumerary to conventional arteries of 3.7:1, a marked increase over that at four months. There were 20 supernumerary arteries in the intra-lobular pre-acinar region leading to a ratio of 5:1 again an increase over the four month child.

At five years there were four respiratory bronchioli and five alveolar ducts before branching of the bronchiolus became indistinct. There were three other acini within the lobule. There were within the acinus nine conventional arteries and 36 supernumeraries, a ratio of 4:1. There was therefore an even larger number of supernumeraries than at 18 months. In the pre-acinar region there were two conventional and six supernumerary arteries giving a 3:1 ratio.

In the ten year old a tracing was made to the end of the filling which was at the end of the alveolar ducts. Within the acinus were five respiratory bronchioli and six alveolar ducts. All but one, a respiratory bronchiolus had an accompanying conventional artery. There were 34 supernumerary arteries giving a ratio of 3.4:1. Within the lobule in the pre-acinar region there were four conventional

branches and 22 supernumerary arteries, with a ratio of 5.5:1.

Within the acinus there had been a gradual increase in the number of conventional arteries particularly those accompanying alveolar ducts since these appear to increase slightly with age. The supernumerary arteries increased to a greater degree after four months of age, so that the ratio increased. The number of conventional and supernumerary arteries in the intra-lobular pre-acinar region was variable but the ratio increased with age.

Surprisingly the 11 year old did not follow the trends just described. There were only two respiratory bronchioli and four alveolar ducts with six conventional arteries and 11 supernumeraries, giving a ratio of only 1.8:1. There were two terminal bronchioli from the pre-acinar region with only three supernumerary arteries and two conventionals, a low ratio of 1.5:1. This lung was normal and it is likely that the small number of arteries reflects variation between acini.

In summary the total number of respiratory and terminal bronchioli remains much the same throughout childhood. The number of alveolar ducts increases with age. The number of conventional arteries within the lobule is the same as the number of airway branches and therefore increases with age. The number of supernumerary arteries within the acinus increases with age but to a greater extent than the conventional arteries so that the ratio increases. In the pre-acinar intra-lobular region there is an even greater increase in the number of supernumerary arteries. Elliott (1964) found a similar number of conventional and supernumerary vessels in the adult as have been seen here in the ten year old.

It seems that the number of supernumerary arteries increases during childhood as the alveoli multiply. Since there is an increase in the number of alveolar ducts during childhood as part of acinar growth, the conventional arteries also increase. There is an increase in the total length of the acinus with age. The part of the pre-lobular region traced, in all these cases, showed a similar configuration to that seen during fetal life.

Diameter of Intra-Lobular Arteries

The diameter of the axial artery at the level of the terminal bronchiolus increased from 250 μm at birth to 510 μm at 11 years (see Table III 21). The diameter at the end of the axial respiratory bronchiolus increased from 75 μm at birth to 300 μm at ten years. The 11 year old had a slightly reduced diameter in comparison with the ten year old, being 225 μm. There was an increase in diameter at the level of the distal alveolar duct from 40 μm to 137 μm between birth and 11 years. And so at all levels there was an increase with age. In each case there was a decrease in size of the axial pathway towards the periphery. This decrease can be seen in Figures III 30 and 31 which show the diameter of the axial artery and the conventional and supernumerary branches and their branching pattern in the four month and five year old cases. The gradient (diameter/length) of decrease of the axial diameter was much the same in all cases being between 0.096 and 0.23. The lung of three days showed the steepest, and of five years the smallest gradient.

As seen in the pre-acinar region in fetal life the conventional arteries were always smaller than the axial pathway at their branching and were larger than adjacent supernumerary arteries. The diameters

Figure III 30

Reconstruction of the arterial branching pattern within the lobule and in the immediate pre-lobular region in the lung of the four month old. The external diameter of the axial pathway and the diameter of conventional and supernumerary vessels is shown.

The total length of the pathway traced was 5.1 mm.

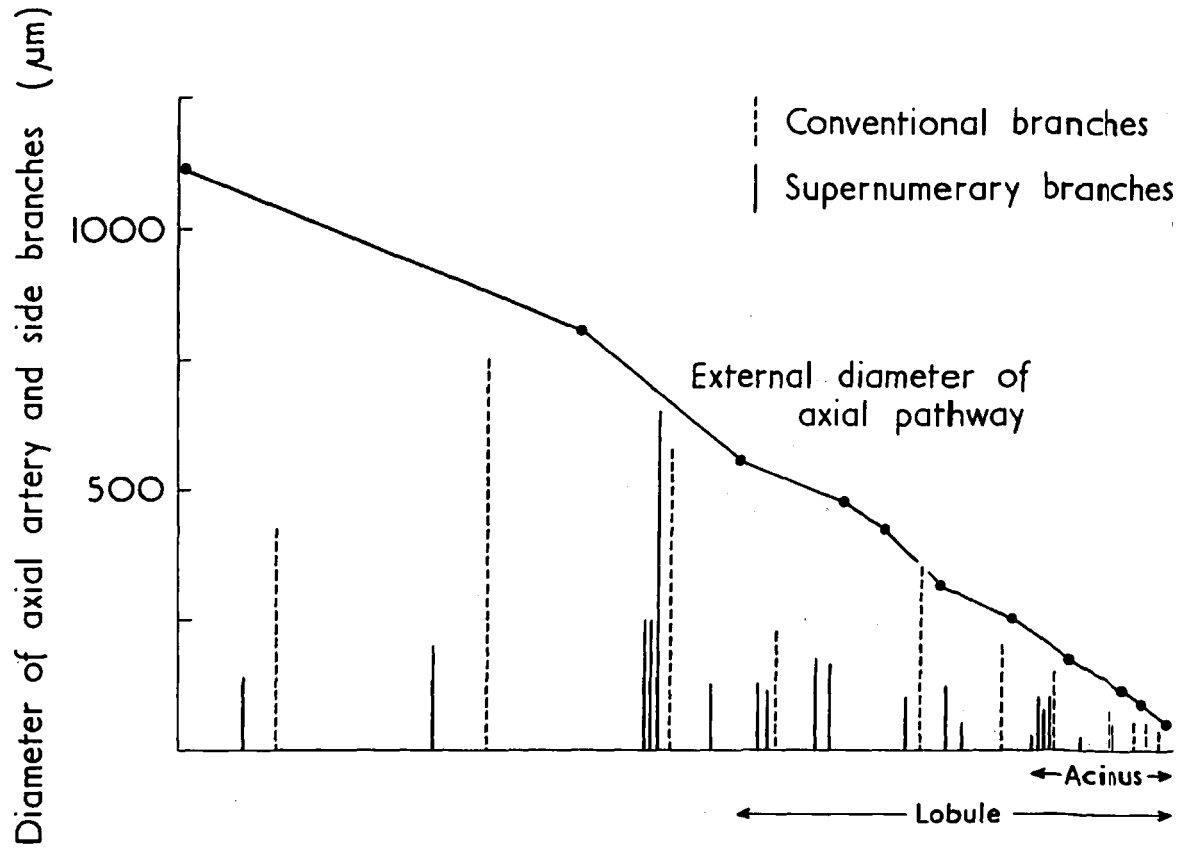

Diameter of axial artery and side branches (μm)
1000
500
Conventional branches
Supernumerary branches
External diameter of axial pathway
Acinus
Lobule

TABLE III 21

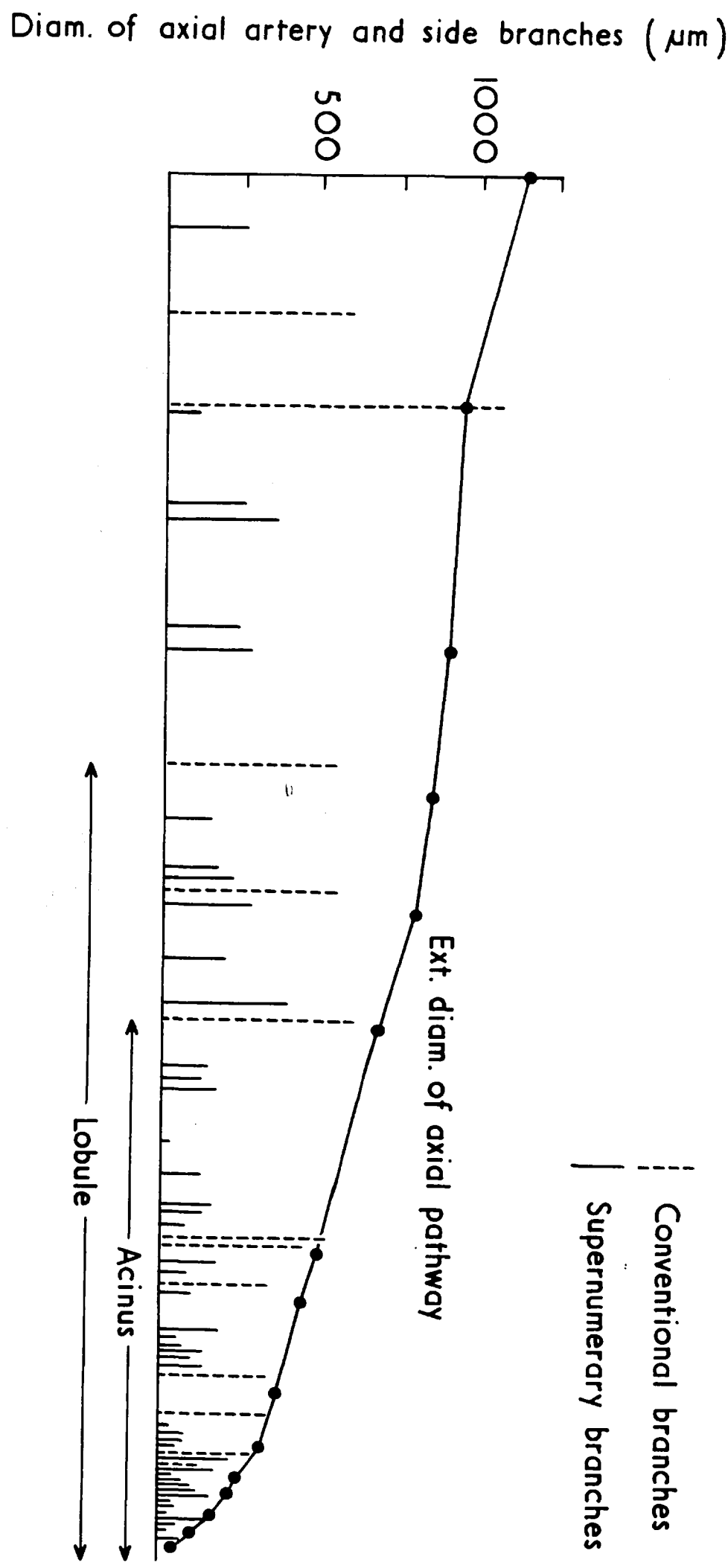
Diameter of Axial Artery within the Lobule During Childhood (μm)

Age	Birth	3 dy	4 mnth	18 mnth	5 yr	10 yr	11 yr
Diameter at Terminal Bronchiolus	250	310	250	430	500	460	510
Diameter at Respiratory Bronchiolus	75	110	85	225	250	300	225
Diameter at Alveolar Duct	-	40	50	75	105	100	137
Gradient (Diameter/ Length)	0.16	0.23	0.17	0.17	0.096	0.13	0.19

Figure III 31

Reconstruction of the arterial branching pattern within the lobule and in the immediate pre-lobular region in the lung of the five year old child. The external diameter of the axial pathway and the diameter of the conventional and supernumerary arteries is shown.

The total length of the pathway traced was 1.26 cm.


Diam. of axial artery and side branches (μm)
500
1000
Ext. diam. of axial pathway
Lobule
Acinus
Conventional branches
Supernumerary branches

of the conventional arteries accompanying terminal and respiratory bronchioli and alveolar ducts are shown in Table III 22. The average for each group at each age is shown. In all three groups the average size of the artery increased with age. At all levels the five year old had rather large arteries; this is probably due to acinar variation. At all ages the average diameter of the vessels decreased towards the periphery. However there were some arteries accompanying respiratory bronchioli of larger diameter than those with terminal bronchioli in the same case.

The mean size of the supernumerary arteries in each generation is shown in Table III 23. There was a tendency to a decrease in diameter towards the periphery in most cases, though there was a marked variation in size as between successive conventional arteries. The mean size of the supernumeraries in each case increased with age from 30.25 μm at birth to 119 μm in the 11 year old.

ARTERIES AND ALVEOLI IN A UNIT AREA OF LUNG

The development and multiplication of the arteries within the acinus can also be studied on random sections by counting the number of small vessels in a unit area of lung.

Number of Arteries per Unit Area

The total number of arteries below 200 μm diameter in one square centimetre of intra-acinar lung is shown for 13 cases in Table III 24, together with the percentage of each size range up to 200 μm external diameter. The results for five representative cases are shown in Figure III 32. In all cases there were far more

TABLE III 22

Diameter of Conventional Arteries within the Lobule During Childhood (μm)

With Terminal Bronchioli	Birth	3 dy	4 mnth	18 mnth	5 yr	10 yr	11 yr
1	90	137	225	340	520	410	750
2	75	240	350	380	600	540	400
3	77	210	200	270	-	420	400
4		150		310		490	320
5		200					
Mean	80.66	187	258	325	560	465	467
S.D.	8	42	80	46	56	61	192
S.E.	4.6	18.8	46.2	23	39.7	30.7	96
With Respiratory Bronchioli							
1	65	100	150	220	530	300	340
2	65	162	80	175	440	285	240
3	125	110			350	450	
4	62	100			350	215	
Mean	79	118	115	197.5	417.5	312.5	290
S.D.	30	29	49	31.8	86.1	98.9	70
S.E.	15	14.5	34.7	22.5	43.1	49.4	49.6
With Alveolar Ducts							
1	-	95	40	115	290	125	95
2	-	37	45	225	280	155	240
3		80	25	105	125	100	100
4		50		175	100	120	120
5				162	137	112	
6						100	
Mean		65	36	156.4	186.4	118.6	138
S.D.		26	10.4	48.5	91.16	20.5	68
S.E.		13	6	21.7	40.8	8.4	34

TABLE III 23

Diameter of Supernumerary Arteries within the Lobule During Childhood

(μm)

With Terminal Bronchioli	Birth	3 day	4 mnth	18 mnth	5 yr	10 yr	11 yr
1	50	61	145	-+	181	141	250
2	-	100	91	76	117	242	62
3	-	78.3	67	82	-	162	48
4		52		45		138	95
5		-					
With Respiratory Bronchioli and Alveolar Ducts							
1	26	65	25	-	-	-	157
2	-	30	50	75	126	101	77
3	20	-	-	106	110	98	-
4	25	75	25	55	-	85	-
5	-	-	-	69	54	85	150
6	-	-		30	150	41	
7		24		62	25	-	
8		29			-	54	
9					125	40	
10						82	
Mean	30.25	57.14	67.1	66.6	111.0	105.75	119.8
S.D.	13.4	25.8	45.7	22.1	50.0	57.8	70.9
S.E.	6.7	8.6	18.7	7.4	17.7	16.7	26.9

+ No supernumerary arteries in these generations

arteries in the groups up to 100 um than in the size range 100 - 200 μm. The highest percentage was seen in the size range 25 - 50 μm followed by the 50 - 75 μm group; the 75 - 100 μm group had considerably fewer. There was therefore a continuous drop in the percentage of arteries with increasing size in all cases studied aged between 26 weeks gestation and 10 years.

During fetal life there was variation in the figures for each case but no trend could be seen. It appears that the fetal lungs have a higher percentage of small vessels than the children. As age in childhood increased there was a drop in the percentage of small arteries particularly those in the 25 - 50 μm group with a corresponding rise in the number of arteries over 100 μm. This was particularly seen at five and ten years. The group of 50 - 75 μm arteries maintained about the same percentage from 26 weeks gestation to ten years. There was a rise in the 75 - 100 μm group after birth.

The total number of arteries within one square centimetre was variable during fetal life. This was partly due to injection difficulties in the cases of 38 and 40 weeks gestation but may also be related to the degree of inflation. If the lung is more distended there will be fewer arteries per unit area. Distension does not affect the percentage figures.

Distension must be taken into account after birth. Since all lungs are fully inflated under the same conditions it is hoped that differences are minimal. It is not possible, as in the adult, to check this by counting the number of alveoli in a given area since these vary in size with growth and multiplication that occurs during childhood as well as with the degree of inflation. Results of

TABLE III 24

Number of Arteries per Square Centimetre of Tissue in Acinar Region and Percentage in each Size Range

Age	Total Number	% in each size range (μm) 25-50	50-75	75-100	100-150	150-200
26 wk gest	139	68.3	26.6	2.1	2.1	0.7
28 wk gest	84	54.7	22.6	7.1	10.7	4.7
36 wk gest	144	51.3	30.5	8.3	6.9	2.7
38 wk gest	60	45.0	40.0	8.3	5.0	3.33
40 wk gest	58	72.4	18.9	3.4	3.4	1.7
3 dy	188	50.5	24.4	13.8	7.4	3.7
4 mnth	116	34.4	31.8	18.1	12.0	3.4
10 mnth	287	45.6	29.9	12.5	8.3	3.8
18 mnth	364	52.1	24.1	9.3	11.5	2.7
3 yr	250	39.6	31.2	14.8	11.6	2.8
4 yr	233	39.9	29.1	15.8	10.4	4.0
5 yr	195	44.6	20.0	11.7	14.8	8.7
10 yr	179	32.9	29.6	13.9	16.2	8.3

absolute figures must therefore be interpreted with caution since they may not be accurate, and although it is worth studying total numbers percentages are more likely to be reliable.

After four months of age there was a marked increase in the number of arteries in a square centimetre. There was a gradual rise through ten and 18 months of age and in the three and four year old the numbers were still high. This increase was in all size ranges so that the percentages remained much the same. After four years there was a drop in the number of arteries and at five and ten years total number was similar to that seen at three days, but with the proportions changed. Throughout childhood this number is higher than during fetal life.

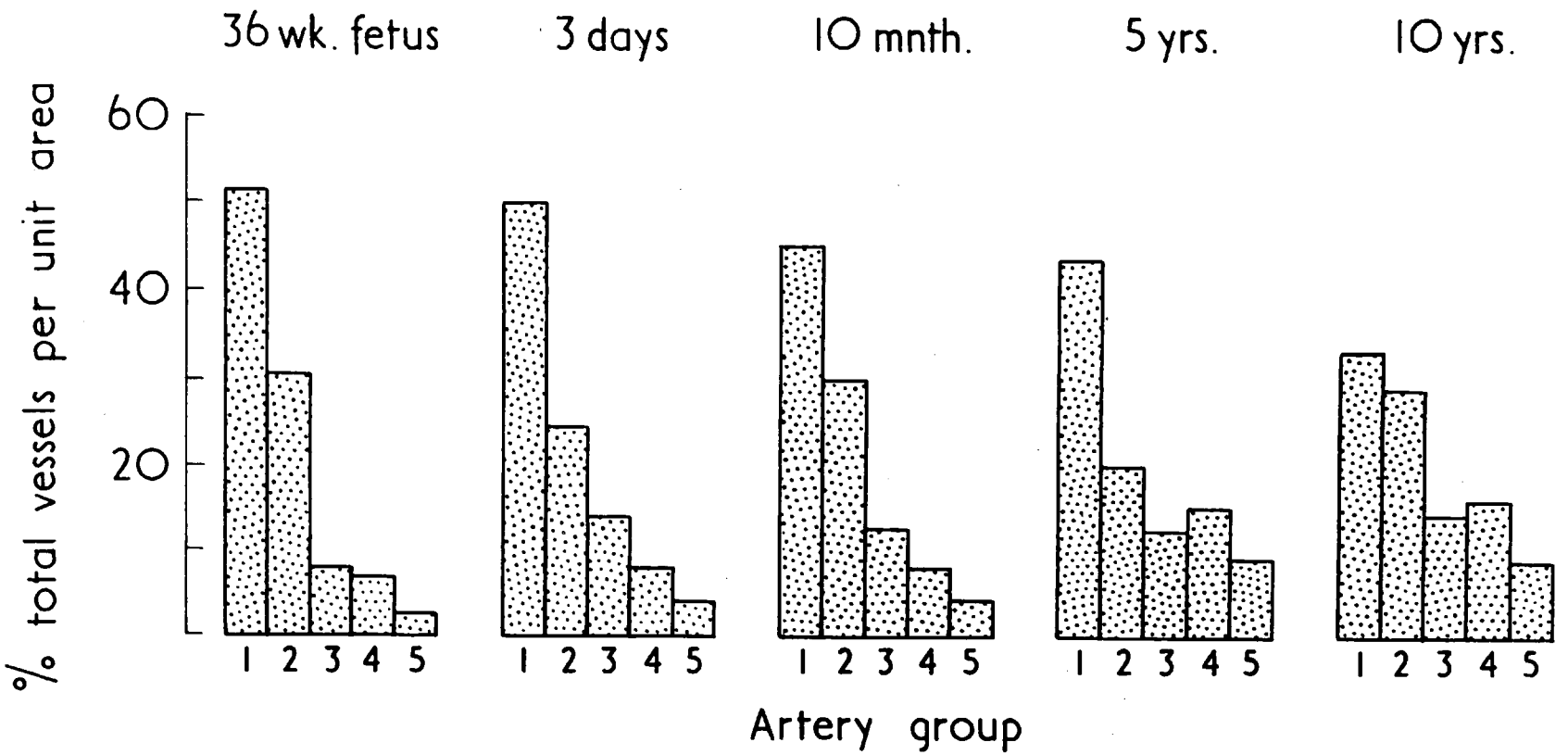
There seems to be a rapid increase in the number of arteries by multiplication, after four months. This corresponds to the rapid increase in the number of alveoli at this time (Dunnill, 1962b). In the five and ten year old the number has dropped since the alveoli are growing in size rather than multiplying (Davies and Reid, 1970) and the arteries are thus more widely separated. This is a time of increase in size rather than number. This is also demonstrated by the increase in the percentage of larger arteries at this age.

Number of Alveoli per Unit Area

By estimating the number of alveoli in a square centimetre it was possible to relate artery number to alveolar number in these cases. The number of alveoli per square centimetre and the ratio of alveoli to arteries is shown in Table III 25. It was not possible to count alveoli in the lungs of less than 40 weeks gestation. There was an increase in the number of alveoli per unit area from birth

Figure III 32

The percentage of arteries in each size range, up to 200 um external diameter, in a unit area of lung tissue of the alveolar region. The results are shown for a 36 week fetus, a three day infant and children aged ten months, five years and ten years.



Artery group	Diameter μm
1	25 – 50
2	50 – 75
3	75 – 100
4	100 – 150
5	150 – 200

TABLE III 25

Number of Alveoli per Square Centimetre and Ratio of Alveoli to Arteries

Age	Alveoli per square centimetre	Ratio Alveoli/ Arteries
40 wks gest	8,227	141
3 dy	4,689	25
4 mnth	11,315	97.5
10 mnth	12,418	43.26
18 mnth	10,477	28.7
3 yr	12,903	51.6
4 yr	9,529	40.8
5 yr	9,123	46.8
10 yr	8,580	47.9

to four months. The number then remained about the same until three years. In the four year old there were fewer, as in the five and ten year old lung. The early increase in number indicates a decrease in alveolar size due to multiplication (Boyden, 1965). From four months to three years the size remains constant though at this time there is still rapid alveolar multiplication (Dunnill, 1962; Davies and Reid, 1970). After three years of age there appears to be an increase in size of alveoli while multiplication is still continuing though at this time evidently not as fast relative to the thoracic cage. These estimates confirm the findings on alveolar size and number of Davies (1969).

Ratio of Alveoli to Arteries (Table III 25)

At birth there seems to be a high ratio of alveoli to arteries per unit area; this may be an artefact due to incomplete filling of arteries or it may be that most of the arteries are below 25 μm in diameter and therefore do not fill. At three days there is a low ratio suggesting multiplication of arteries without change in the number of alveoli. The air spaces at this time were still at the saccule stage (Boyden, 1965). By four months there were true alveoli which seemed to have multiplied faster than the arteries since there is now a high ratio. The total number of arteries at this age was rather low which may be due to incomplete filling.

Between ten months and ten years the ratio is similar, showing a similarity in growth rate of arteries and alveoli. Between ten months and three years there was a marked increase in the number of alveoli but the arteries also multiplied, evidently to keep pace. Later when the alveolar multiplication slows and their size increases the arteries also increase in size as seen by the increase in the percentage of larger vessels in the five and ten year old.

There appear to be two main phases in the growth of the alveoli and arteries. Up to the age of three years the alveoli are multiplying rapidly as the thoracic size increases. They are not at this stage growing in size as the multiplication is sufficient to maintain growth in relation to thoracic size. At the same time the arteries multiply. A second phase is seen between four and ten years. At this time the rate of multiplication is slowing since the full adult complement of alveoli is almost present. Since the thoracic cage is still increasing in size, the alveoli start to increase in size. At the same time the arteries stop multiplying so rapidly and start increasing in size. In the present series the case of four years seems to be at the critical point in this cycle. The number of alveoli per unit area is lower than at three years so the alveoli are growing in size. However the arterial number is still high so that these are still multiplying. The change in the behaviour of the arteries may follow that of the alveoli. There may be a similar lag at four months where the alveoli have started to multiply rapidly although the rate of arterial multiplication has not changed.

These results show, as did the serial reconstructions, that the intra-acinar arteries develop during childhood as the alveoli multiply and then increase in size.

BRONCHIAL ARTERIES

While studying the pulmonary artery, observations were made on the bronchial arteries to establish their presence and any filling by the radio-opaque medium injected into the pulmonary artery gave evidence of pre-capillary pulmo-bronchial anastomoses, since the medium will not pass through vessels smaller than 15 μm.

In the 12 week fetus bronchial arteries were present in the walls of bronchi near the hilum, sometimes only as groups of cells without a patent lumen. Similar small vessels were present in the 14 week fetus. By 16 weeks red blood corpuscles could be seen in the lumen of these vessels. Patent vessels of increasing calibre were present from 16 weeks gestation to birth in the walls of bronchi. At 38 and 40 weeks gestation though there were, throughout the walls of bronchi and bronchioli, numerous bronchial arteries, none had any filling of barium gelatine and all were packed with red blood corpuscles. There were evidently no connections between the two circulations with channels greater than 15 μm. In the newborn of 40 weeks gestation there were examples where the bronchial artery came very close to a filled pulmonary artery which seemed to be supplying a bronchiolar wall. It is possible that this was a position where a pre-capillary anastomosis could open up.

Injection of the pulmonary veins led to filling of the bronchial veins in the lungs of all the fetuses examined in this way. Apparently bronchial veins drain to the pulmonary venous system through vessels larger than 15 μm in diameter even at 20 weeks gestation. At this time the smaller vessels between the epithelium and the cartilage were not filled.

During study of the hilar bronchi of childhood lungs by dissection it was possible to determine the presence of filled bronchial vessels by naked eye examination. Filled vessels could be seen through the bronchial epithelium in all cases of children and adults, thus demonstrating the presence of pulmo-bronchial pre-capillary anastomoses at all ages. Two smaller lungs were examined microscopically. In a case of three days there were a few filled bronchial arteries but in a case of five days there was no visible filling. All cases over four

months of age had bronchial artery filling.

It seems that there is a bronchial and pulmonary circulation during fetal life but there are no patent pre-capillary anastomoses. These must open up immediately after birth since some filling was found at three days. It seems that there may be a lag since there was no filling in the case of five days.

SUMMARY OF RESULTS

1. With a barium sulphate gelatine mixture and using the same methods as used in the adult it was possible to inject the arteries of fetuses from 12 weeks gestation to birth. The same procedure was also used for all childhood cases.

2. Lung volume increased with age, the rate of increase being faster during fetal life than during childhood.

3. Arteriograms of all the cases injected were used to study branching pattern. At 12 weeks gestation the pattern was not complete but at 20 weeks the arteriogram was essentially the same as that for an adult but in miniature. A background blush did not develop until after birth.

4. Arteriograms of siblings showed that they were similar and there is probably some hereditary control of arterial branching pattern.

5. Measurements on arteriograms showed that, with age, the vessels increased in length and in diameter at all levels, the increase being more rapid during fetal life.

6. The diameter at the hilum was directly related to the length of the artery and the volume of the lung. Arteries one centimetre

from the periphery were always of similar diameter, therefore the lumen diameter was related to the volume of lung supplied by it.

7. The hilar arterial diameter increased by a greater proportion than the peripheral diameter. The gradient therefore increased with age. This may relate to increase in vessel number at the periphery.

8. Serial reconstruction was used to trace the branching pattern of the airways and arteries in the pre-acinar region during fetal life and in the intra-acinar region during childhood.

9. At birth the pre-acinar branching pattern was the same as that seen in the adult and confirmed the appearance on the arteriograms.

10. By 19 weeks gestation all pre-acinar arterial branching was complete, both conventional and supernumerary vessels.

11. Between 12 and 19 weeks, at the distal end of the pathway the supernumerary and conventional vessels were growing together. No extra branches appeared proximally as the pathway grew in length.

12. The axial pathway increased in length with age as did each generation.

13. The ratio of conventional and supernumerary arteries was the same throughout fetal life and similar to that of the adult.

14. The diameter of the pathway decreased steadily towards the periphery and at any point there was an increase in diameter with age.

15. The diameter of conventional branches was smaller than of the axial and this decreased towards the periphery.

16. Supernumerary arteries were smaller than conventional arteries and decreased in size towards the periphery: these increased in size with age.

17. Lateral side branches showed the same type of branching pattern as the axial pathway. The branching pattern of an artery of a given size was the same whether it was an axial or lateral branch. Thus the branching pattern and size of vessel depended upon its distance from the capillary bed.

18. The arterial branching pattern was essentially the same in the right and left lung. Although the segmental pattern was different the branching within the segments was of the same type with a similar number of conventional and supernumerary arteries.

19. The intra-acinar arteries developed mainly during childhood while the alveoli were multiplying. At birth only respiratory bronchioli and one or two alveolar ducts could be identified and they had accompanying arteries.

20. During childhood the number of alveolar ducts increased and distance between arterial branches increased. With this growth supernumerary arteries developed and the ratio of supernumerary to conventional arteries also increased.

21. Within the acinus the diameter of the axial pathway, the conventional arteries and the supernumerary arteries increased with age.

22. There was a higher percentage of small arteries below 100 um in diameter during fetal life than in childhood. At about five years the larger arteries increased as a percentage of the total. This occurred when speed of alveolar multiplication had reduced. The total number of arteries per unit area increased while the alveoli were multiplying so that the ratio of one to the other remained the same.

23. Bronchial arteries were present in the lungs from at least 12 weeks gestation (the youngest studied). During fetal life pre-capillary anastomoses (that is greater than 15 μm diameter) were not demonstrated between pulmonary and bronchial arteries. During childhood they were apparent.

CHAPTER IV

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Introduction

The structure of the pulmonary artery wall during fetal life and childhood, up to the age of eleven years, is described in this chapter. The structure of a single axial artery and its side branches, both conventional and supernumerary, are reported for the cases already described in the previous chapter. The structure of the arteries throughout the lung and at additional ages has also been established by several other methods - the thickness of the artery medial wall, the distribution of the types of artery by size and the structure and external diameter of arteries accompanying identifiable airways. These methods are used to assess the amount and extension of muscle in the pulmonary artery walls.

The definitions of structural types of artery referred to in this chapter have been described in detail in Chapter II page 107.

STRUCTURE ALONG AN AXIAL PATHWAY

Pre-Acinar Region (Hilum to Terminal Bronchiolus)

In the adult, as it reduces in diameter to the periphery, the axial pathway changes structure (Elliott, 1964). The largest and most proximal arteries are elastic in structure and these give way successively to transitional, muscular, partially muscular and non-muscular arteries. These changes in wall structure are related to the diameter of the artery.

While tracing the branching pattern of the posterior basal artery during fetal life, as described in Chapter III, it was possible

to determine the structure of the artery wall along the length of the pathway and also to describe the structure of side branches.

Elastic Arteries - General Description

Elastic arteries are the largest arteries found in any lung. At 12 weeks gestation the elastic within the media was not as clearly defined as in the adult. At this age the largest arteries within the lung were still muscular in that they had fewer than four elastic laminae between the internal and external elastic laminae. However these laminae were very closely packed together and may have had the same function as an elastic wall. Further support was probably gained by the fact that the wall was particularly thick in relation to the external diameter. An artery of similar size in an older fetus would have a thinner wall with less elastic. The internal elastic lamina at this age was definite but the external and medial laminae were thin and poorly defined.

By 14 weeks the hilar arteries now qualified as elastic in structure and furthermore the elastic fibres stained more readily. The elastic fibres of the media were still close packed but the percentage wall thickness was lower. There were cells visible between the elastic laminae but they still did not show the staining characteristics of muscle. The elastic fibres were not in concentric rings and tended to run into one another.

The 16 week fetus had rather small arteries at the hilum but their walls were very thick and elastic in structure. The wall thickness of these elastic arteries was particularly high for the diameter and would be more appropriate for an artery three times the size. It was still impossible to discern stained muscle fibres

between the elastic laminae, however there were nuclei present.

With increasing age the elastic gradually became more regular in appearance, concentric rings and even staining were seen. At 23 weeks gestation the muscle cells between the elastic laminae could be discerned due to their brown staining with a van Gieson stain. From this time the muscle became more obvious and the elastic laminae were more widely separated than in the younger fetuses. By 40 weeks gestation there was well defined muscle and collagen between the well spaced and defined elastic fibres. At this time the elastic laminae were 2 μm thick and 2 - 5 μm apart, while at 19 weeks they were 1 μm thick and 1 - 3 μm apart.

Extent of Elastic Arteries

At 12 weeks gestation the elastic arteries were not found in the intrapulmonary region even at the hilum. By 14 weeks an elastic artery structure extended to the second generation within the lower lobe (Table IV 1). At 16 weeks of intra-uterine life this structure had reached the fourth generation but in the lung of the 17 week fetus it did not extend beyond the second generation. At 18 weeks the elastic structure was found as far as the fifth generation. In all cases over 20 weeks gestation the elastic extended close to the level of the seventh generation, the same generation seen by Elliott (1964) in the adult.

The smallest diameter at which an elastic wall was seen varied from case to case though there was a general increase in this diameter with age. At 17 weeks the smallest artery of this type had a diameter of 1000 μm, similar to the smallest seen at

20 weeks. However in the 17 week lung this was only at the level of the second generation and in the 20 week it was the sixth generation. There appears to be no difference in diameter at 28 and 36 weeks, but the elastic extends to a more peripheral level at 36 weeks.

The total length of the elastic artery structure along the axial pathway increased with age. This was not due only to the increase in length of the whole axial pathway, since the percentage of the pathway occupied by the elastic structure also increased (Table IV 1). During fetal life the elastic portion was never more than 43% of the total pathway. In the adult it was 48.6% (Elliott, 1964).

It seems that by 20 weeks of gestation the extent of the elastic region of the axial pathway is delineated and there are only individual variations between this time and adulthood. The diameter at which the elastic structure ends increases with age to 3000 μm in the adult.

Transitional Arteries - General Description

Along an axial pathway arteries with a transitional structure lay between the elastic and muscular region. The media was made up of muscle with an internal and external elastic lamina and five or six additional elastic laminae between.

A transitional wall structure was not identified in this series along the axial pathway until the case of 19 weeks gestation, however side branches of this structure were seen at 16, 17 and 18 weeks. This structure could not be identified either in side branches or along the main pathway in the cases of 20 and 23 weeks

gestational age. The lack of a transitional wall was in part because there were no vessels of the particular size for this structure along this pathway. The change from an elastic to a muscular structure was abrupt in these two cases since at the critical level of size the axial artery showed dichotomous branching into two smaller vessels rather than a gradual reduction in the axial artery size. In the remaining cases a transitional structure was found (Table IV 2), though in varying amount. In the longer pathways found in the older fetuses the transitional structure is relatively more common.

Extent of Transitional Arteries

The greatest number of generations with a transitional structure was in the 19 week fetus where there were four (Table IV 2). The percentage of the axial pathway covered was similar to the other three cases, where there were one, two or three generations; these were always between the sixth and ninth generation. The transitional zone was variable in length but covered only 20% of the pathway, less than half that covered by the elastic arteries. The arterial size range showing a transitional structure increased with age.

It was not possible to draw such detailed conclusions about the transitional zone since this structure was found in so few cases. It does seem to follow the trend seen for the elastic zone. By 19 weeks gestation the adult distribution along the axial pathway of this structure is set: the size range then increases with age.

Muscular arteries - General Description

The large majority of arteries in the lung were muscular.

TABLE IV 1

Distribution of Elastic Structure along an Axial Pathway in the Pre-Acinar Region during Fetal Life

Age in Weeks	Generation reached	Smallest diameter (µm)	Length (mm)	% of Total Pathway to Terminal Bronchiolus
12	-	> 470	-	0
14	2	850	0.7	9
16	4	400	1.4	20
17	2	1000	0.7	6
18	5	920	3.22	27
19	6	780	5.74	36
20	6	1050	5.6	33
23	7	880	9.1	43
28	7	1200	10.78	38.5
36	8	1200	13.58	40
38	7	1700	12.6	42
40	6	1700	14.98	43

TABLE IV 2

Distribution of Transitional Structure along an Axial Pathway in the Pre-Acinar Region during Fetal Life

Age in weeks	Generation	Diameter Range (µm)	Length (mm)	% of Total Pathway to Terminal Bronchiolus
12, 14, 16, 17, 18	No transitional structure seen			
19	6-9	620-780	2.73	17
20, 23	No transitional structure seen			
28	7-9	850-1200	5.53	18
36	No transitional structure seen			
38	8-9	1400-1700	4.2	14
40	6	1300-1700	3.78	11

As in the elastic arteries the laminae were not well defined during fetal life and the muscle did not stain with a special muscle stain until about 23 weeks gestation. Before this the vessels could be identified by their elastic laminae.

Extent of Muscular Arteries

The number of arterial generations along the axial pathway with a muscular wall varied considerably as did the generation level at which they were found. This depended mainly on variation in the length of the elastic region. The smallest number of muscular generations was at 18 weeks (Table IV 3) where only seven were muscular, however this structure was found over 61% of the total length: the largest number was seen in the 36 and 40 week fetuses with 17 arterial generations having a muscular structure although these only covered 58 and 46% of the length respectively.

The length of the muscular portion increased with age. This was related to the change in total length since the percentage of the pathway involved dropped in the older fetuses. At all ages the muscular structure was found over a greater arterial length than the elastic. In those cases with a transitional region the muscular structure covered less than 50% of the total pathway. In the adult the muscular region was 42%.

During fetal life the smallest artery diameter found to have a muscular structure along the axial pathway was in the 17 week fetus and was 97 μm; in this case the largest was 1000 μm. The largest muscular artery seen during fetal life was 1400 μm seen at 38 weeks gestation, the end of the muscular region being 175 μm. The largest muscular artery in the adult was 2000 μm (Elliott, 1964).

TABLE IV 3

Extent of Muscular Structure along an Axial Pathway in the Pre-Acinar Region during Fetal Life

Age (weeks)	Generation	Diameter Range (µm)	Length (mm)	% of Total Pathway to Terminal Bronchiolus
12	1-8	125 - 470	4.2	60
14	2-10	112 - 850	6.0	75
16	4-12	∠112 - 400	4.2	60
17	2-14	97 - 1000	10.8	90
18	5-12	200 - 920	7.3	61
19	9-20	125 - 620	6.6	41
20	6-16	175 - 1050	10.6	63
23	7-23	125 - 880	11.2	53
28	10-24	120 - 850	10.9	39
36	8-25	112 - 1200	20.41	58
38	9-25	175 - 1400	13.3	44
40	6-23 (T.B.)	105 - 1300	16.2	46

T.B. - Terminal bronchiolus

The largest range of size was seen in the 40 week fetus where a muscular structure was found between 105 and 1300 μm arterial diameter.

Partially Muscular Arteries - General Description

The partially muscular arteries (see page 110) in cross-section appear to have a crescent of muscle in their walls. This may cover almost the whole wall or only a small arc. Any artery with muscle which is incomplete around the circumference is called a partially muscular artery. Where they first appear they are difficult to identify as there may be only a very small part of the wall free from muscle. The muscular part will have an internal and external elastic lamina while the non-muscular part has only one elastic lamina.

As in the other vessels muscle cells could only be identified by their staining properties in fetal tissue over 23 weeks of age. In the 12 and 14 week fetuses vessels, in the position of partially muscular, had a wall made up of more than one layer of cells but true muscle cells and elastic fibres could not be identified. These may be termed cellular vessels and can be easily distinguished from the non-muscular vessels. It is possible that these are a primitive artery type with no true muscle cells.

Similar arteries were observed in this series at 17 and 18 weeks but not in the older fetuses. These were found nearer the periphery and were also smaller than those at 12 and 14 weeks. (The nature of these cells is referred to again on page 358). By 17 weeks the partially muscular arteries could be identified by a crescent of muscle bounded by an internal and external elastic lamina. Such vessels were then found throughout fetal life, and became easier to identify as the muscle stain became effective.

Extent of Partially Muscular Vessels

With age and as the arteries enlarged the muscular structure extended towards the periphery. The partially muscular structure was therefore found in more distal generations. However the size range in which the structure was found remained the same. By 38 weeks gestation a partially muscular structure along an axial vessel was only found within the acinus (Table IV 4). The number of generations with a partially muscular wall varied but never more than four. In the 17, 18, 20 and 23 week fetus the partially muscular structure was found in the smallest injected arteries and may have extended further. In the latter two cases, as in the 36 week lung, the partially muscular vessels extended to the level of the terminal bronchiolus and probably further.

The size range of the partially muscular region of the axial pathway varied, but was similar for all ages. The lowest size range was at 17 weeks when it extended between 51 and 97 μm and the highest range at 18 weeks when the partially muscular region extended over an arterial diameter of 105 - 200 μm.

The partially muscular structure was found over the greatest distance in the 12 week fetus where it covered 1.17 mm. This was 16% of the total pathway. In all other cases the partially muscular region composed only 5% or less of the pathway and was always less than one millimetre in length. There is no percentage given for the 38 week fetus since the partially muscular region was within the acinus, but the distance covered was similar to that seen in other cases.

TABLE IV 4

Extent of Partially Muscular Structure along an Axial Pathway in the Pre-Acinar Region during Fetal Life

Age (weeks)	Generation	Diameter Range (μm)	Length (mm)	% of Total Pathway to Terminal Bronchiolus
12	8-9	85-125	1.17	16
14	10-14	70-112	0.42	5
16	-	Unfilled	-	-
17	14-17	∠ 51- 97	0.56	3
18	12-14	∠ 105-200	0.56	5
19	20	80-125	0.49	3
20	16-20 (T.B.)	∠ 110-175	0.63	4
23	23-24 (T.B.)	∠ 55-125	0.49	3
28	24-26	90-120	0.84	3
36	25-26 (T.B.)	80-112	0.35	2
38	T.B.-R.B.3	100-175	0.56	-
40	Acinus	-	-	-

T.B. - Terminal bronchiolus
R.B. - Respiratory bronchiolus

Non-muscular Arteries - General Description

The non-muscular arteries are the smallest pre-capillary arteries and are found at the periphery of all arterial pathways. They have no muscle in their wall, which is made up of a layer of endothelium with or without an elastic lamina. These arteries were identified in all ages studied.

Extent of Non-muscular Arteries

The non-muscular part of the axial pathway was not reached by the filling medium in the fetuses of 16, 17, 18, 20 and 23 weeks gestation. At 12 weeks the last six generations were non-muscular and at 14 weeks the last three. At 19 weeks the last five pre-acinar vessels were non-muscular, but by 28 weeks it was only the last three (Table IV 5). At 36 weeks the non-muscular structure along the axial pathway was only found within the acinus and by 38 weeks gestation it was only at alveolar duct level.

The largest non-muscular artery was seen in the case of 19 weeks gestation where it was 125 μm. At 14 weeks the non-muscular wall did not appear until the artery was 70 μm in diameter. In all other cases the non-muscular phase began at a diameter between these two extremes.

The distance covered by this wall type was greatest at 12 weeks gestation where it was 1.54 mm and comprised 24% of the pre-acinar pathway. The percentage of the artery length occupied by the non-muscular region decreased with age. The length of this region dropped with age as muscle approached the terminal bronchiolus. If the whole of the pathway to the distal edge of the acinus were

TABLE IV 5

Extent of Non-Muscular Structure along an Axial Pathway in the Pre-Acinar Region during Fetal Life

Age (weeks)	Generation	Diameter (μm)	Length (mm)	% of Total Pathway to Terminal Bronchiolus
12	10-15	< 85	1.54	24
14	14-16	< 70	0.7	9
16		Unfilled		
17		Unfilled		
18		Unfilled		
19	21-25 (T.B.)	<125	0.42	3
20	Acinus	<75	-	-
23	Acinus	Unfilled	-	-
28	26-28	<90	0.56	1.5
36	Acinus	<80	-	-
38	Alveolar Ducts	<100	-	-
40	Acinus	-	-	-

T.B. - Terminal bronchiolus

considered there would not be such a large drop.

Structure Within the Acinus

The branching pattern of arteries and airways within the acinus has been traced in several children using serial reconstruction. It was possible at the same time to describe the structure of the axial pathway and its side branches. Within the acinus there were no elastic or transitional arteries even in the oldest children. The largest artery at all ages was muscular in structure.

Extent of Muscular Arteries

In the newborn baby of 38 weeks gestation there were no muscular arteries within the acinus. In the three day infant muscular arteries were found to the third generation of respiratory bronchioli, but at four months these only extended to the level of the terminal bronchiolus. In the child of 18 months there was a muscular structure to the artery accompanying the first generation respiratory bronchiolus. In the first few months there would seem to be much variation, and it seems likely that there will be variation between acini. This was demonstrated using other methods of describing muscularity within the acinus (page 350). In the case of five years muscle extended to the level of the fourth respiratory bronchiolus and in the children of ten and 11 years a muscular structure had extended to the level of alveolar ducts. In later childhood there had been an extension of muscle to the periphery.

The diameter of these muscular arteries is shown in Table IV 6. The largest diameter is taken at the level of the terminal bronchiolus and does not therefore represent the largest muscular

artery. The diameter at this level increased with age. The diameter at the distal end of the muscular region shows the true extension of the muscular structure. The smallest diameter varied from 137 to 300 μm and there was no trend related to age. The largest of these diameters was at 18 months which means that at this age there is least extension of muscle. The smallest wholly muscular arteries in the child lungs studied in this way were found at four months and 11 years.

The length of the muscular region varied though showed some trend to increase with age. The longest was seen in the five year old which also had the longest total intra-acinar length. These figures are of doubtful significance since the total length and shape of the acinus is very variable (Pump, 1969).

The percentage length of the pathway that was muscular is of interest. At birth there was no muscle in the acinus but in the case of three days there appeared to be muscle through 53% of the acinus (51% of the respiratory region). It seems likely that this acinus was unusual and as will be seen later, the majority of the acini in this case had few muscular arteries. In the case of four months only 19% of the acinus and none of the respiratory region had a wholly muscular artery wall. By 18 months the percentage of the respiratory region with muscle had increased to 33% and by five years to 68% and ten years, 83%. There was therefore with age an extension of muscle to the periphery.

Extent of Partially Muscular Arteries

In the child, partially muscular arteries were easily identified between the muscular and non-muscular part of the axial

TABLE IV 6

Extent of a Muscular Structure along an Axial Pathway within Acinus during Childhood

Age	Generation	Size Range (μm)	Length (mm)	% Distance from end of Terminal Bronchiolus
Birth	Pre-acinar	> 175	0	0
3 days	T.B.-R.B.3	200-250	0.63	51
4 months	T.B.	160-250	0.28	0
18 months	T.B.-R.B.1	300-460	1.03	33
5 years	T.B.-R.B.4	250-650	4.22	68
10 years	T.B.-A.D.2	200-520	3.15	83
11 years	T.B.-A.D.4	137-540	2.31	74

T.B. - Terminal bronchiolus
R.B. - Respiratory bronchiolus
A.D. - Alveolar Duct

pathway. At birth this structure was found as far as the third respiratory generation having first appeared at the terminal bronchiolus. At three days this type of artery was found accompanying a fourth generation respiratory bronchiolus. In the cases of four and 18 months, muscle extended only as far as the first and second respiratory bronchiolar generations. At five and ten years a partially muscular structure extended to the alveolar duct level.

The smallest vessel with a partially muscular structure was found at birth and ten years where it extended to a diameter of 100 μm (Table IV 7). At 18 months and five years there was no muscle at all in the axial artery where it was less than 225 μm and 155 μm respectively. The range of diameter was greatest in the newborn where it was 100 - 175 μm. The partially muscular structure never covered a length of artery with diameter drop of more than 100 μm.

The actual length of the partially muscular region was variable though never more than 0.7 mm. This length was similar to that seen in the axial pathway during fetal life though at this time it was in the pre-acinar region. The percentage of the respiratory region occupied by a vessel with a partially muscular wall was variable the greatest being 37%.

Extent of Non-muscular Arteries

At birth the non-muscular region of the axial pathway was not reached until the level of the alveolar duct. In the cases of three days, four and 18 months it was reached at a progressively more proximal point in the branching pattern (Table IV 8). At

TABLE IV 7

Extent of Partially Muscular Structure along an Axial Pathway within the Acinus during Childhood

Age	Generation	Size Range (µm)	Length (mm)	% Distance from end of Terminal Bronchiolus
Birth	T.B.-R.B.3	100-175	0.56	37
3 days	R.B.4	110-200	0.14	12
4 months	R.B.1	110-160	0.217	19
18 months	R.B.1-R.B.2	225-300	0.7	31
5 years	Alveolar Ducts	155-250	0.56	21
10 years	Alveolar Ducts	100-200	0.56	17
11 years	-	-	-	-

T.B. - Terminal bronchiolus

R.B. - Respiratory bronchiolus

five years it was again reached at alveolar duct level and at ten and 11 years in the alveolar region.

The largest non-muscular vessel was found at 18 months and five years and in all cases they were larger than those seen during fetal life in that they were all over 100 μm in diameter.

The length of the non-muscular region varied with age being shortest at five years. This intra-acinar measurement is less significant than the others since the shape and size of an acinus varies widely and it is difficult, while tracing at this level to define the edge of an acinus without wax reconstruction models. These figures for length have only been taken to the last alveolar duct and by five years there are a large number of alveoli peripheral to this, so increasing the length of the non-muscular region of the artery.

The percentage of the intra-acinar region occupied by the non-muscular structure is of interest. At birth this percentage is 63% but by three days only 37%. It is likely that this axial pathway is more muscular than most at this age since at four months 81% of the respiratory portion of the acinus was non-muscular and the remaining 19% partially muscular. After four months the percentage of non-muscular region of the axial pathway dropped with age.

It was clear that the extent of muscle changed during childhood. In the first few years muscle did not extend far into the acinus. In the newborn partially muscular vessels extended to the end of the respiratory bronchioli though this, in the particular acinus studied, was only 37% of the acinar region. At this age the respiratory bronchiolar region was short and it was likely that this

TABLE IV 8

Extent of Non-Muscular Structure along the Axial Pathway within the Acinus during Childhood

Age	Generation	Size Range (μm)	Length (mm)	% Distance from End of Terminal Bronchiolus
Birth	Alveolar Ducts	< 100	0.81	63
3 days	R.B.5-Alveoli	< 110	0.42	37
4 months	R.B.2-Alveoli	< 110	0.92	81
18 months	R.B.2-Alveoli	< 225	0.79	36
5 years	A.D.5-Alveoli	< 155	0.28	11
10 years	-	-	-	-
11 years	Alveoli	< 100	0.64	26

R.B. - Respiratory bronchiolus

A.D. - Alveolar Duct

region increases in length due to growth and centripetal alveolication, so that in the cases of early childhood where muscle appeared to have receded to higher levels it is likely that this is an increase in the number and length of respiratory bronchioli without extension of muscle down the artery wall. The percentage of the acinus with an axial vessel containing muscle in its wall increased after 18 months as muscle extended into the vessels accompanying alveolar ducts. At five years the alveolar region was only 32% of the acinus while at birth it had been 63%. Therefore though the muscular arteries were still with the respiratory bronchioli they were found in a larger percentage of the acinus.

STRUCTURE OF THE CONVENTIONAL ARTERIES

The structure of the conventional arteries and supernumerary arteries in the pre-acinar and intra-acinar region is described here in detail for the 38 week old fetus. The structure of these vessels in the other fetal cases in the pre-acinar region is summarised as is that of the intra-acinar arteries of the children.

Pre-Acinar Region (Hilum to Terminal Bronchiolus)

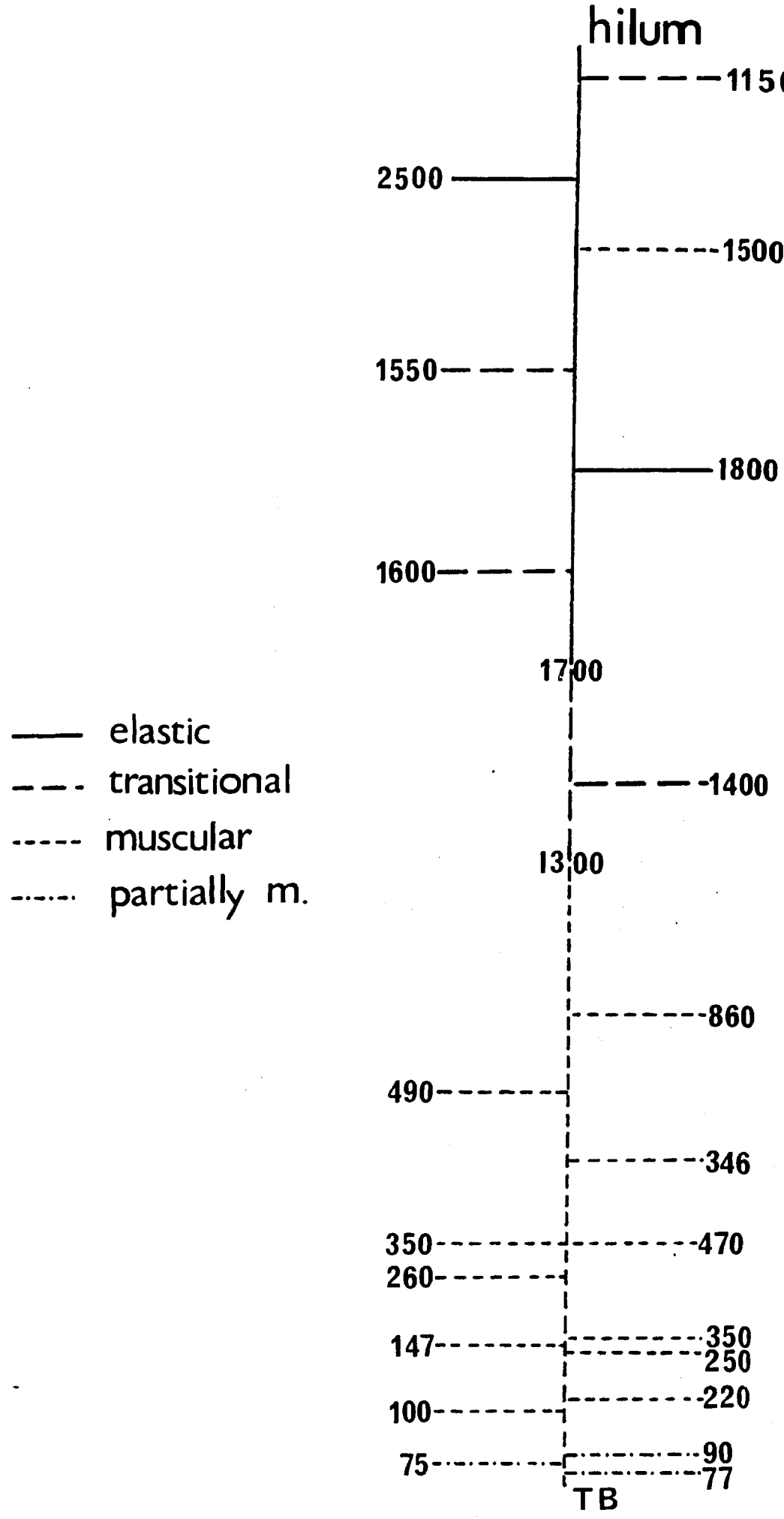
Conventional Arteries at 38 Weeks Gestation (Newborn)

Figure IV 1 shows the structure of the axial pathway to the posterior basal segment in the 38 week fetus, together with the conventional arteries. The diameter of each side branch is shown. As previously reported the first six generations of the axial artery were elastic in structure as far as the arterial diameter of 1700 μm.

Figure IV 1

Diagram to show the structure of the conventional artery branches and their diameter along the axial pathway to the posterior basal segment in the fetus aged 38 weeks. The branching pattern of this pathway has been described in detail in Chapter III.

T.B. = Terminal bronchiolus.


hilum
1150
2500
1500
1550
1800
1600
1700
elastic
transitional
muscular
partially m.
1400
1300
860
490
346
350
470
260
147
350
250
220
100
90
75
77
TB

The first conventional branch was not as might be expected elastic in structure, but was muscular, having a diameter of 1150 μm. The second conventional branch was larger being 2500 μm in external diameter and had an elastic media. The third branch was again of muscular type being only 1500 μm in diameter. The fourth arterial branch had a transitional wall structure and was 1550 μm in diameter. The fifth branch was elastic and 1880 μm in diameter and the sixth transitional and 1600 μm. It seems that the structure of the conventional arteries does not relate to their position in the lung or the structure of the parent artery but depends upon the size of the branch. During fetal life the structure will differ from case to case in arteries of the same diameter, as was seen for the axial pathway. Thus structure of the conventional arteries is size and age dependant until adulthood.

In this case of 38 weeks gestation no elastic structure was seen in arteries below 1700 μm which was the end of the elastic structure along the axial pathway, therefore conventional branches smaller than this were either muscular or transitional in structure. Transitional artery structure was seen in arteries of 1300 - 1700 μm diameter and any artery smaller than this had a muscular wall structure. Thus the elastic artery gave rise to three types of artery, the structure of any one depending upon its diameter.

Only one conventional artery arose from the axial pathway over its transitional region and this was transitional in structure since it was 1400 μm in diameter.

Along the axial pathway where it was less than 1300 μm and as far as the terminal bronchiolus the wall was muscular in structure. In this zone there were 14 conventional artery branches of which 11 were muscular in structure. The distal three branches were

partially muscular. This region would not give rise to elastic or transitional arteries because of the size range of the branches. The partially muscular arteries were 90, 75 and 70 μm in diameter. The muscular axial artery in this case had given rise to muscular and partially muscular arteries depending upon their external diameter.

Conventional Arteries During Fetal Life

Having described one case in detail, in the other fetuses the structure of the conventional arteries can be summarised together. Table IV 9 records the structure of each conventional side branch in the 12 cases studied previously (page 179). These were aged 12 to 40 weeks of gestation.

The findings can be summarised in the following points.

1. The structure of the successive side branches did not follow a pattern. In some cases near the hilum, elastic, transitional and muscular arteries alternated, for example in the lungs at 36, 38 and 40 weeks gestation. At the periphery not all muscular branches were found before partially muscular and non-muscular occur: these structures may also alternate, for example cases at 23, 26 and 36 weeks gestation.

2. The axial artery could give rise to arteries of different structure to itself, however an artery did not give rise to a structure of more proximal type than itself, that is a non-muscular artery would not give rise to a muscular daughter branch.

3. The structure was related to the diameter of the artery. At all ages an elastic structure was found in larger arteries than was the muscular structure.

4. The structure of the conventional arteries was related to diameter in the same way as for the axial vessel. Thus at 12 weeks there were no conventional elastic arteries. At 14 weeks the axial pathway showed an elastic structure only as far as its diameter level of 850 μm since no conventional side branch was larger than 800 μm there were no elastic side branches. At 16 weeks the first elastic conventional artery was seen; it measured 420 μm and the end of the elastic region of the axial pathway was 400 μm. An exception to the rule relating structure to size was seen in the 19 week lung: the end of the elastic region along the axial pathway was 780 μm while two side branches near the hilum of 590 and 460 μm had elastic walls.

5. The diameter at which the structure of conventional side branches changes, altered with age as it did along the axial pathway.

6. Until 28 weeks as well as change in structure with diameter, the generation number to which elastic conventional branches were found progressed to the periphery with age. After 28 weeks the elastic did not extend beyond generation five though the diameter increased - it seems that during fetal life each artery passes progressively through structural types until it reaches its adult structure. Conventional branches therefore changed their structure with age as did the axial pathway. The fifth generation in the lung of the 12 week fetus was non-muscular in structure, but in those between 14 and 23 weeks was muscular in structure. In the 28 week fetus this generation was elastic which was the same as in the older lungs up to adulthood.

7. The change in structure with age was not due only to increase

TABLE IV 9

Structure of Conventional Arteries during Fetal Life (12-40 weeks)

Age (weeks)	12	14	16	17	18	19	20	23	28	36	38	40
Artery Generation												
1	M	M	M	M	M	E	M	E	E	E	M	T
2	M	M	M	T	E	E	E	E	E	E	E	E
3	M	M	E	M	E	M	M	E	E	M	M	M
4	M	M	M	M	E	E	M	E	E	T	T	T
5	N	M	M	M	M	M	M	M	E	E	E	E
6	P	M	M	M	M	M	M	M	M	M	T	T
7	N	M	M	M	M	M	M	M	M	M	T	M
8	N	N	N	M	M	M	M	M	M	M	M	M
9	N	N	M	M	M	M	M	M	M	M	M	M
10	N	P	N	P	M	M	M	M	M	M	M	M
11	N	P	N	M	N	M	M	M	M	M	M	M
12		N	N	P	N	M	M	P	M	M	M	M
13		N	N	P	N	P	M	M	M	M	M	M
14		N	N	N	N	P	N	P	M	N	M	M
15		N	N	N	N	P	N	M	P	M	M	M
16		N	N	N	N	P	N	P	M	N	M	M
17			N		N	M	N	P	P	N	M	M
18					N	N	N	P	M	N	M	M
19					N	N	P	M	M	M	P	M
20					N	N		P	N	M	P	M
21					N	N		P	N	N	P	M
22					N			N	P	N		M
23					N			N	N	M		
24									N	M		
25									N	N		
26									N	N		

E - Elastic
T - Transitional
M - Muscular
P - Partially Muscular
N - Non-muscular

in diameter. An artery of over 1000 μm in a 19 week lung was elastic in structure but by 38 weeks such an artery would be muscular.

8. The final structure was established before the adult diameter was achieved. The fifth generation at 28 weeks, when it became elastic, was only 1040 μm in external diameter while the smallest elastic artery in the adult would be 3000 μm (Elliott, 1964).

9. There was variation between cases in the structure of a generation branch. Conventional artery branch number three in the 28 week fetus was elastic in structure while in a case 8 weeks older it had a muscular structure. This was not due to a regression with age to a more 'primitive' structure but to variation between cases in branching pattern. In fact generation three was smaller at 36 than 28 weeks and must therefore supply a different region of the lung.

10. There were similar changes in structure at the periphery of the lung with non-muscular arteries becoming partially and then wholly muscular in structure (see generation 13 in Table IV 9). There was more age variation at the distal end than the proximal probably because of the greater individual variation in the smaller arteries. Near the hilum there was a more stereotyped pattern because of the segmental pattern. Muscular arteries were found closer to the periphery in the older fetuses and at 40 weeks gestation the muscular arteries extended to the terminal bronchiolus.

The extent of the elastic and transitional region both along the axial pathway and the conventional branches, throughout fetal life, depended upon the external diameter of the artery. The

diameter of the conventional arteries with an elastic structure depended upon the axial pathway. If the age is known the diameter at which an elastic structure is found can be predicted since the diameter changes with age. Up to 19 weeks the diameter varies as the arteries grow and the elastic structure extends into more peripheral generations; this extension is not just related to size. After 19 weeks the elastic structure has reached its ultimate extent and the changing diameter with age is due only to growth. In later fetal life and up to adulthood it is possible to predict structure of these arteries by position in the lung.

The proximal extent of the muscular region of the axial pathway depends upon the extent of the elastic and transitional zone. This will alter with age. The conventional vessels, muscular in structure, will depend upon the diameter related to the axial pathway. At the distal end of the muscular region structure is directly related to the diameter of the vessel throughout fetal life and the diameter involved is similar at all ages. The level in the lung at which the change occurs changes with age as the arteries grow in diameter.

Thus diameter, age and position each influence the structure of any artery.

Intra-Lobular Region

A brief description of the intra-lobular structure of the conventional arteries in the case of the newborn of 38 weeks gestation is given first. This was the youngest case in which this region was studied and the only fetal case. All the children studied including the newborn are then considered together.

Conventional Arteries in the Newborn (38 weeks gestation)

The structure of the intra-lobular pre-acinar arteries is shown in the previous section. Within the acinus of the stillborn there were six conventional arteries, all were non-muscular in structure, the largest being 125 um in external diameter. The axial pathway giving rise to these vessels was, for the first four generations, partially muscular and then became non-muscular.

Conventional Arteries During Childhood

The structure of the conventional arteries accompanying terminal bronchioli, respiratory bronchioli and alveolar ducts in the stillborn and children from three days to 11 years is shown in Table IV 10. In the newborn lung those accompanying the terminal bronchioli were partially muscular, in the three day old lung, two were muscular and three partially muscular. At four months, five, ten and 11 years all intra-lobular, pre-acinar conventional branches were muscular. In the case aged 18 months there were fewer muscular arteries, an apparent loss in muscle. This was not in fact a loss in muscle at this age, but due to this case being particularly non-muscular in the acinar region probably as a result of individual variation. Generally in childhood in the conventional arteries there was an increase with age in muscle as external diameter increased.

A similar trend was seen in those arteries accompanying respiratory bronchioli but muscular arteries were not present until five years rather than three days. In the case of 18 months there were still non-muscular vessels at this level.

Non-muscular vessels were found accompanying alveolar ducts

TABLE IV 10

Structure of Conventional Arteries during Childhood within the Lobule

Age	Birth	3 day	4 mth	18 mth	5 yr	10 yr	11 yr
Generation							
Terminal bronchiolus							
1	P	P	M	P	M	M	M
2	P	M	M	M	M	M	M
3	P	M	M	N		M	M
4		P		P		M	N
5		P					
Respiratory bronchiolus							
1	N	N	P	N	M	M	M
2	N	N	N	N	M	M	M
3	N	P			M	M	
4	N	P			P	M	
Alveolar duct							
1	N	N	N	N	N	P	N
2	N	N	N	N	P	M	M
3		N	N	N	N	N	N
4		N		N	N	N	M
5				N	N	N	
6						N	

N - Muscular
P - Partially muscular
N - Non-muscular

even at 11 years and from a total of 29 conventional arteries accompanying alveolar ducts in all the cases studied only five had any muscle.

There was an increase in the muscularity with age with an extension of the muscular wall structure to the periphery. This extension lagged behind that of the muscle of the axial pathway possibly because of their smaller diameter. The extension of muscle is not only related to diameter since arteries of average diameter 187 μm accompanying terminal bronchioli at three days were muscular and partially muscular in wall structure while arteries of diameter 197 μm accompanying respiratory bronchioli at 18 months were non-muscular and those of 186 μm diameter with alveolar ducts at five years were nearly all non-muscular. The extension of muscle into the artery wall with age lagged behind the increase in diameter.

STRUCTURE OF SUPERNUMERARY ARTERIES

The structure of the supernumerary vessels is described first for the pre-acinar region in the fetus and then for the intra-lobular region in the child.

Pre-Acinar Region (Hilum to Terminal Bronchiolus)

Supernumerary Arteries at 38 Weeks Gestation (Newborn)

Figure IV 2 represents the axial pathway of the posterior basal segment at 38 weeks gestation with the supernumerary artery branches. The structure of each is shown.

There were no elastic or transitional supernumerary arteries. The largest, the first branch from the axial pathway, was 950 μm in diameter and had a muscular wall structure. The main pathway at this point was elastic in structure. This rather large supernumerary vessel was of the aberrant type described by Elliott (1964), since it eventually came to run alongside a bronchus and further subdivide parallel to it: it did not immediately supply the alveolar walls. A further five supernumerary branches arose in the elastic region of the axial pathway. Four of these were muscular in structure being 500, 370, 330 and 200 μm in diameter. These arteries eventually came to run with small bronchioli before entering an acinus but did not accompany an airway for much of their length. The fifth of these supernumeraries was only 87 μm in diameter and had a partially muscular wall. Therefore the main artery with an elastic wall could give rise even to a partially muscular vessel.

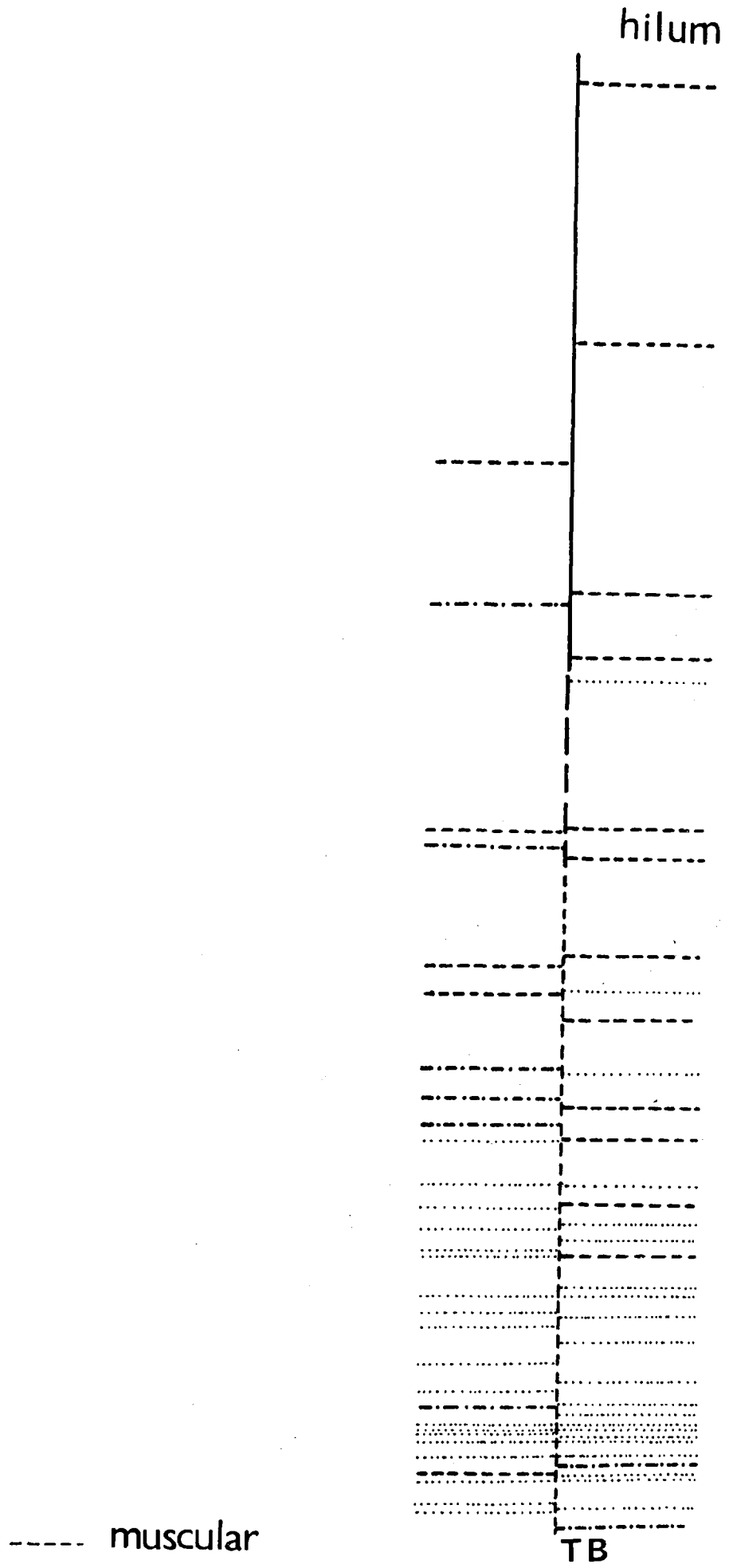
The first supernumerary artery in the transitional zone and the seventh of the axial pathway was a non-muscular artery with a diameter of 75 μm. The next two arteries were muscular followed by a partially muscular and another muscular artery. Arteries of different structure could therefore alternate, structure depending upon the vessel's size. In this case the partially muscular vessel was 112 μm while the three muscular vessels were over 260 μm.

The remainder of the supernumerary arteries arose from the muscular part of the axial pathway. The structure of these branches varied being muscular, partially muscular or non-muscular. There was no set pattern for the branching of the three types. The muscular vessels were on average the largest and the non-muscular the smallest but there was an overlap with the smallest muscular vessels being 97 μm and the largest partially muscular, 112 μm. The smallest

Figure IV 2

Diagram to show the structure of the supernumerary artery branches along the axial pathway to the posterior basal segment in the fetus of 38 weeks gestation.
The branching pattern of this pathway has been described in detail in Chapter III.

T.B. = Terminal bronchiolus


hilum
TB
muscular
partially m.
non-m.

partially muscular vessel was 37 μm and the largest non-muscular 75 μm. There was a progressive increase in the number of non-muscular arteries towards the periphery as might be expected since overall the arterial diameters decreased. Over the total pre-acinar pathway in this case the average size of the muscular supernumerary arteries was 298.9 μm, of the partially muscular, 70.25 μm and of the non-muscular, 46.7 μm. From the axial pathway a total of 17 muscular branches, seven partially muscular and 40 non-muscular vessels arose.

Structure of the Supernumerary Arteries During Fetal Life

The trends described in the 38 week fetus were seen throughout fetal life with the largest supernumerary arteries being muscular and found closer to the hilum. These were followed by partially muscular and non-muscular vessels, however there was no set pattern to the numbers of each type or to the order of their branching. Table IV 11 shows the total number of each type of vessel arising from the posterior basal axial artery for the 12 cases studied. There was a definite increase with age, in the number of muscular supernumerary arteries and a slighter increase in the number of partially muscular: the total number of non-muscular ones also increased but to a lesser degree. An increase in the total number of supernumerary vessels therefore occurs with age. When the numbers were expressed as a percentage of the total as in Table IV 12, there was an increase in the muscular portion, while the partially muscular arteries maintained a similar proportion throughout fetal life and the percentage of non-muscular vessels fell.

It would seem therefore that there is an extension of muscle during fetal life: the increase in muscular arteries being associated with the overall increase in arterial size.

TABLE IV 11

Structure of Supernumerary Arteries during Fetal Life

(12-40 weeks gestation)

Age (weeks)	12	14	16	17	18	19	20	23	28	36	38	40
Number of Arteries												
Muscular	2	3	1	2	3	6	8	9	12	9	17	13
Partially muscular	0	4	2	5	2	5	4	4	0	3	7	6
Non-muscular	39	33	43	33	40	47	31	44	63	64	40	50
Total	41	40	46	40	45	58	43	57	75	76	63	69

Table IV 12

Structure of Supernumerary Arteries during Fetal Life

(12-40 weeks gestation)

Age (weeks)	12	14	16	17	18	19	20	23	28	36	38	40
Percentage of total Supernumeraries												
Muscular	5	8	2	5	7	9	18	16	16	12	26	19
Partially muscular	0	10	4	12.5	4	9	9	7	0	4	11	9
Non-muscular	95	82	94	82.5	89	82	73	77	84	84	63	72

Intra-Lobular Region

At birth (38 weeks gestation) and three days all the supernumerary arteries within the lobule were non-muscular. By four months there were two muscular arteries and one partially muscular artery in the pre-acinar, intra-lobular region but in the intra-acinar region all supernumerary arteries were non-muscular (Table IV 13). With age there was an increase, in the region of the terminal bronchioli, in the number of supernumerary arteries with a muscular or partially muscular coat. Partially muscular vessels only appeared in the respiratory and alveolar duct region at ten and 11 years, and even at this age formed a very small proportion of the total number of supernumeraries in this region. As with the conventional arteries there was a trend towards greater muscularity with age, this being related to artery size.

PERCENTAGE WALL THICKNESS

To assess the degree of muscularity of the arteries during fetal life and childhood measurements were made of the thickness of the media and related to the external diameter of the artery. Such measurements were made of the axial pathway along its whole length in the cases where a serial reconstruction had been made. The side branches were also measured at their origin from the axial pathway. The wall thickness was expressed as a percentage of the total diameter using the formula:

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

In addition to the measurements of the axial pathway measurements were made on the arteries included in sections from all areas of the lungs.

TABLE IV 13

Structure of Supernumerary Arteries during Childhood

	Birth	3 day	4 mth	18 mth	5 yr	10 yr	11 yr
Number of Supernumerary Vessels with:							
Terminal Bronchioli							
Muscular	-	-	2	-	2	9	1
Partially muscular	-	-	1	1	5	8	1
Non-muscular	1	12	6	22	6	7	4
Respiratory Bronchioli							
Muscular	-	-	-	-	-	2	-
Partially muscular	-	-	-	-	-	4	-
Non-muscular	8	4	2	5	9	9	2
Alveolar Ducts							
Muscular	-	-	-	-	-	-	-
Partially muscular	-	-	-	-	-	1	2
Non-muscular	2	3	1	18	20	16	4

- None present

Pattern at Birth

Pattern along Axial Pathway

The actual measurements of external diameter and wall thickness plus the percentage wall thickness of the axial pathway to the posterior basal segment of the left lung at 38 weeks gestation is shown in Table IV 14 and are represented in Figure IV 3, where the percentage wall thickness is plotted against external diameter. Each point represents a single measurement. A distinctive curve can be seen which has two regions to be considered. In the larger arteries there is a horizontal line where the percentage wall thickness hardly changes while the diameter decreases. The second region is in the range of the smaller arteries where there is a rapid rise in the percentage wall thickness. The first part of the curve will be called the 'flat region' and the second part the 'steep region'. In this case the percentage wall thickness at the hilum was 4.36 for an artery diameter 4.5 mm. From this size down to a diameter of 250 μm the percentage wall thickness falls within a small range of 2.81 to 5.65 with an average of 4.16, this is the flat region of the curve.

Below 250 μm external diameter there was an increase in the percentage wall thickness. Over this steep region there was an increase in percentage wall thickness from 4.5 at 250 μm to 11.11 at 90 μm. This curve was the same shape as that described by Elliott (1964) for the adult and Davies (1969) for the child, but the flat region was at a higher percentage than that seen in the child over four months and the adult where the flat part of the curve is as low as 1 - 2%.

TABLE IV 14

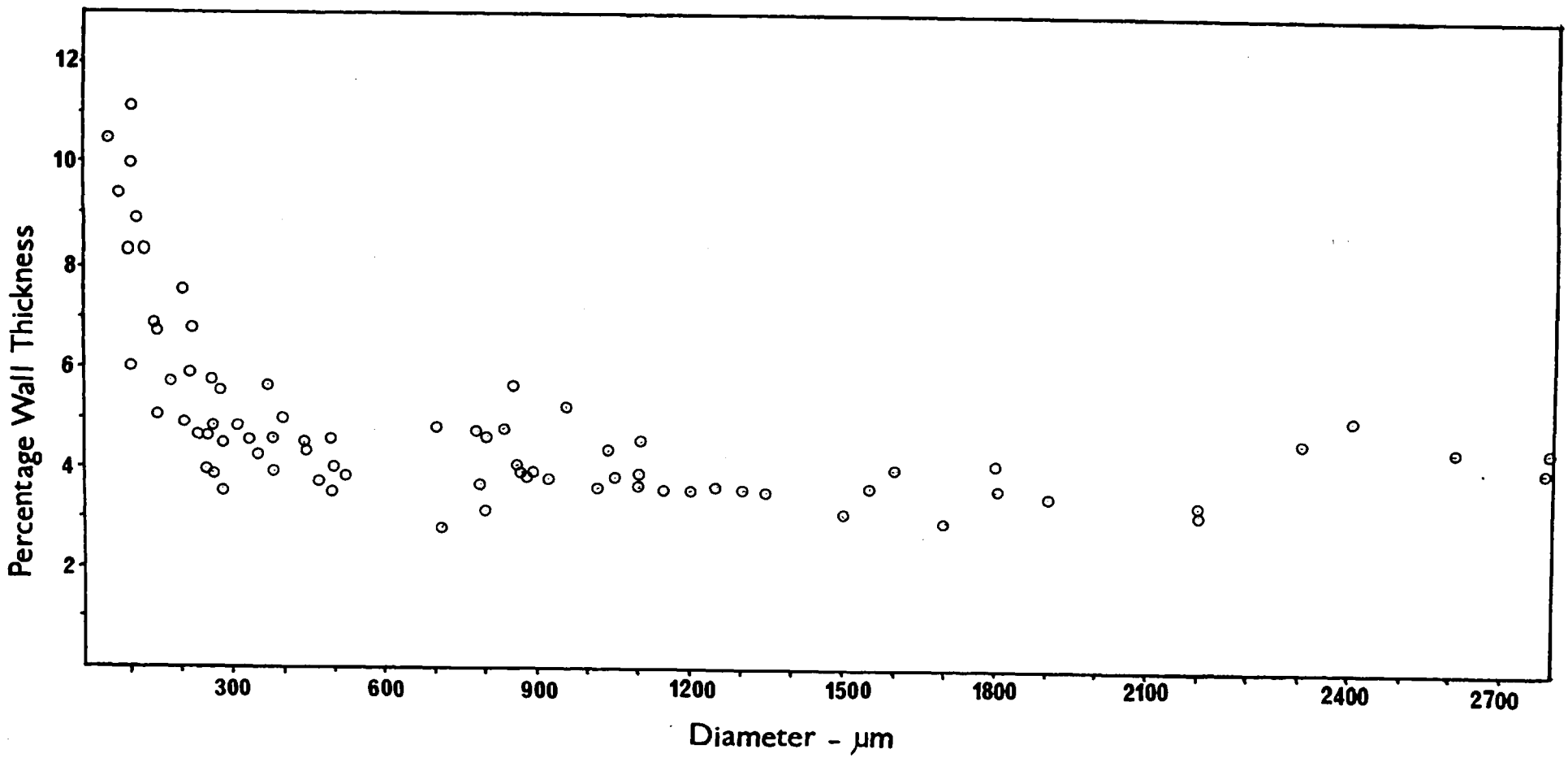
Percentage Wall Thickness along Axial Pathway at 38 weeks

External Diameter (µm)	Wall Thickness (µm)	Percentage Wall Thickness	External Diameter (µm)	Wall Thickness (µm)	Percentage Wall Thickness
4500	98.25	4.36	710	10	2.81
4000	83.5	4.17	700	17	4.85
2500	63	5.04	500	10	4.0
2700	59.5	4.4	500	8.75	3.5
2400	56	4.66	490	11.25	4.59
2700	43.5	3.22	470	8.75	3.72
2200	36	3.27	440	10	4.5
1900	33	3.47	400	10	5.0
1800	37	4.11	380	8.75	4.6
1700	32	3.55	380	7.5	3.94
1700	25	2.94	370	10.5	5.67
1600	32	4.0	350	7.5	4.28
1550	28.5	3.67	330	7.5	4.54
1500	23	3.07	310	7.5	4.83
1350	24	3.55	280	6.25	4.46
1300	23	3.54	280	5	3.5
1250	23	3.68	270	7.5	5.55
1200	21.5	3.58	260	7.5	5.76
1150	20.3	3.53	260	6.25	4.8
1100	20	3.63	260	5	3.84
1100	25	4.54	250	5.75	4.6
1100	21.5	3.9	250	5	4.0
1050	20	3.8	220	7.5	6.81
1040	22.5	4.32	212	6.25	5.89
1020	18.5	3.62	200	7.5	7.5
950	25	5.26	175	5	5.71
920	17.5	3.8	150	3.75	5.0
890	17.5	3.93	148	5	6.75
880	17	3.86	120	5	8.33
870	17	3.9	112	5	8.92
860	17.5	4.06	100	5	10.0
850	24	5.64	100	3	6.0
830	20	4.81	90	3.75	8.33
800	12.5	3.12	90	5	11.11
800	18.5	4.62	75	2.5	9.33
790	15	3.7	48	2.5	10.41
780	18.5	4.74			

Figure IV 3

Percentage wall thickness related to the external diameter, for the axial artery at points along its length and its side branches, in the posterior basal segment of the fetus of 38 weeks gestation.

Each artery measured is plotted.


Percentage Wall Thickness
12
10
8
6
4
2
300
600
900
1200
1500
1800
2100
2400
2700
Diameter - µm

Percentage Wall Thickness Throughout the Lung

Measurements were made on 167 arteries taken at random from throughout the left lung of the fetus at 38 weeks gestation. The percentage wall thickness was estimated for each artery. These were then grouped according to external diameter as in Table IV 15 and the average and standard error calculated for each group. The groups contained arteries in 50 μm ranges up to 300 μm then in 100 μm ranges. The results are shown in Figure IV 4.

The smallest arteries, in the group up to 50 μm diameter, had an average percentage wall thickness of 14.99 (S.E. 1.065). The standard error was high due to considerable variation at this level. Between 50 and 100 μm the percentage wall thickness had dropped to 8.4 and by 100 - 150 μm was only 5.6. There was therefore a rapid drop in the wall thickness in the first 150 μm. There was a further drop to 4.52 by the time the external diameter of the arteries was 300 - 400 μm, the standard error at this diameter had dropped to 0.67 since there was less variation between arteries. In arteries of external diameter greater than 300 μm the percentage wall thickness remained steady between 4.62 and 3.1. The largest artery measured was 2750 μm in external diameter.

This pattern was essentially the same as that seen for the axial pathway over a similar size range. In the general population there was a higher percentage wall thickness in the smallest arteries below 50 μm. Along the axial pathway only one artery below 50 μm had any muscle in its wall while there were six arteries measured in the general population. It seems that the single artery was at the lower end of the range for arteries of this size.

TABLE

Percentage Wall Thickness

Diameter

	0-50	50-100	100-150	150-200	200-250	250-300	300-400
Left Lung							
Mean	14.99	8.4	5.8	5.65	5.19	5.09	4.52
S.E.	1.06	0.32	0.29	0.18	0.17	0.32	0.21
No.	6	41	23	12	17	12	10
Left Upper Lobe							
Mean	15.99	8.26	5.46	5.38	4.9	4.89	4.66
S.E.	0.48	0.46	0.56	0.39	0.21	0.44	0.3
No.	5	20	10	5	9	7	5
Left Lower Lobe							
Mean	10	8.55	6.06	5.84	5.5	5.38	4.38
S.E.	-	0.45	0.3	0.13	0.25	0.51	0.32
No.	1	21	13	7	8	5	5

IV 15

in 38-week Fetus

Range (μm)

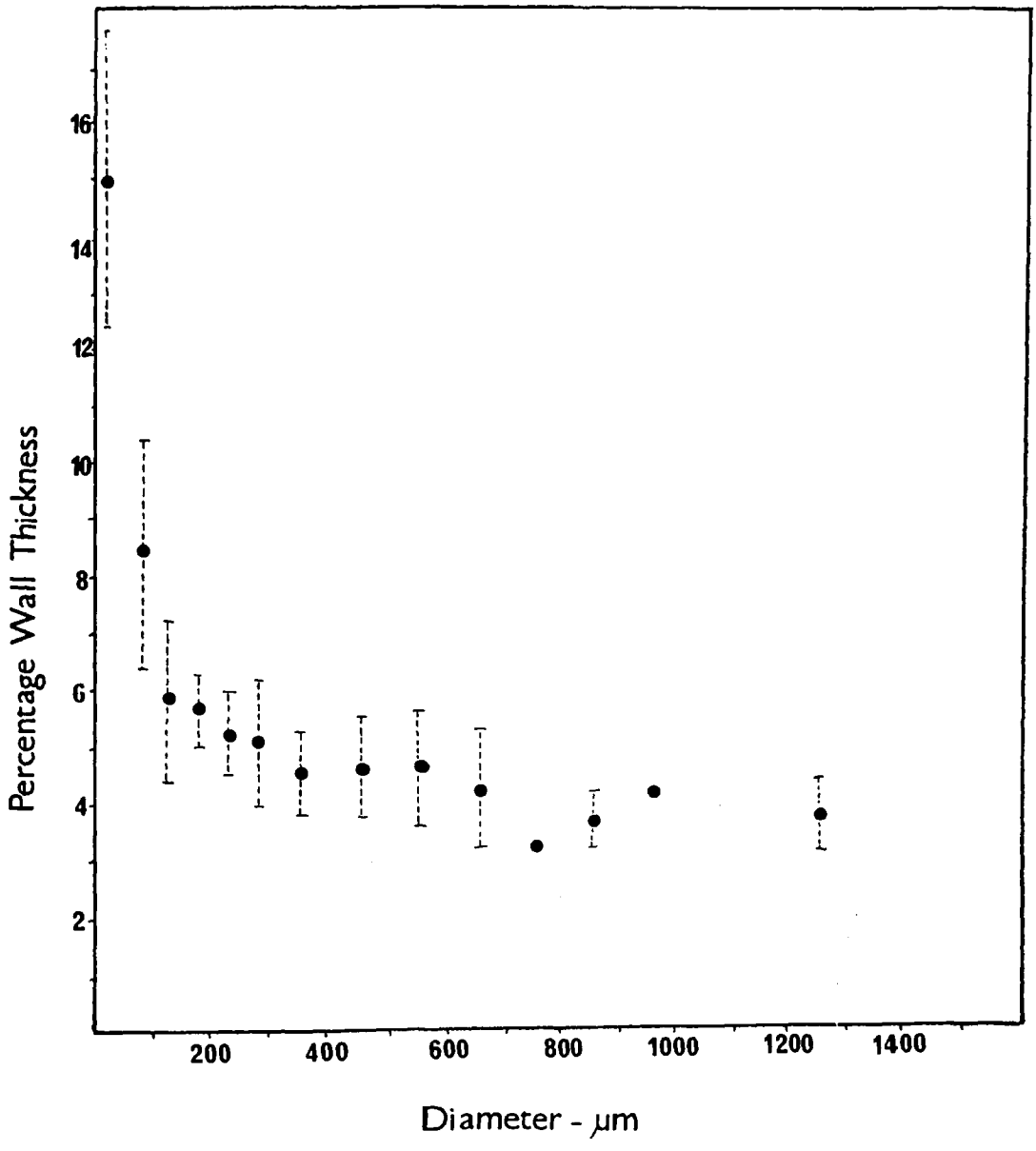
400-500	500-600	600-700	700-800	800-900	900-1000	>1000
4.58	4.62	4.22	3.1	3.6	4.1	3.68
0.33	0.51	0.61	-	0.22		0.14
7	4	4	1	6	1	24
4.2	3.85	4.0	3.1	3.25	-	3.7
0.5	0.35	0.68	-	0.05		0.35
4	2	3	1	2	0	12
5.1	5.4	4.9	-	3.78	4.1	3.83
0.17	0.5	-		0.29	-	0.13
3	2	1	0	4	1	12

S.E. - Standard error of the mean

No. - Number of vessels measured

Figure IV 4

Percentage wall thickness related to external diameter for arteries from throughout the lung of the fetus of 38 weeks gestation. The mean and standard deviation for each size range of arteries is shown.


Percentage Wall Thickness
16
14
12
10
8
6
4
2
200
400
600
800
1000
1200
1400
Diameter - μm

The results for the upper and lower lobe have also been calculated separately and these are also shown in Table IV 15. There is no statistical difference between the two lobes.

It seems that the wall thickness along any axial pathway is the same as that seen in the general population. The following results therefore show the figures for the main pathways and the general population combined.

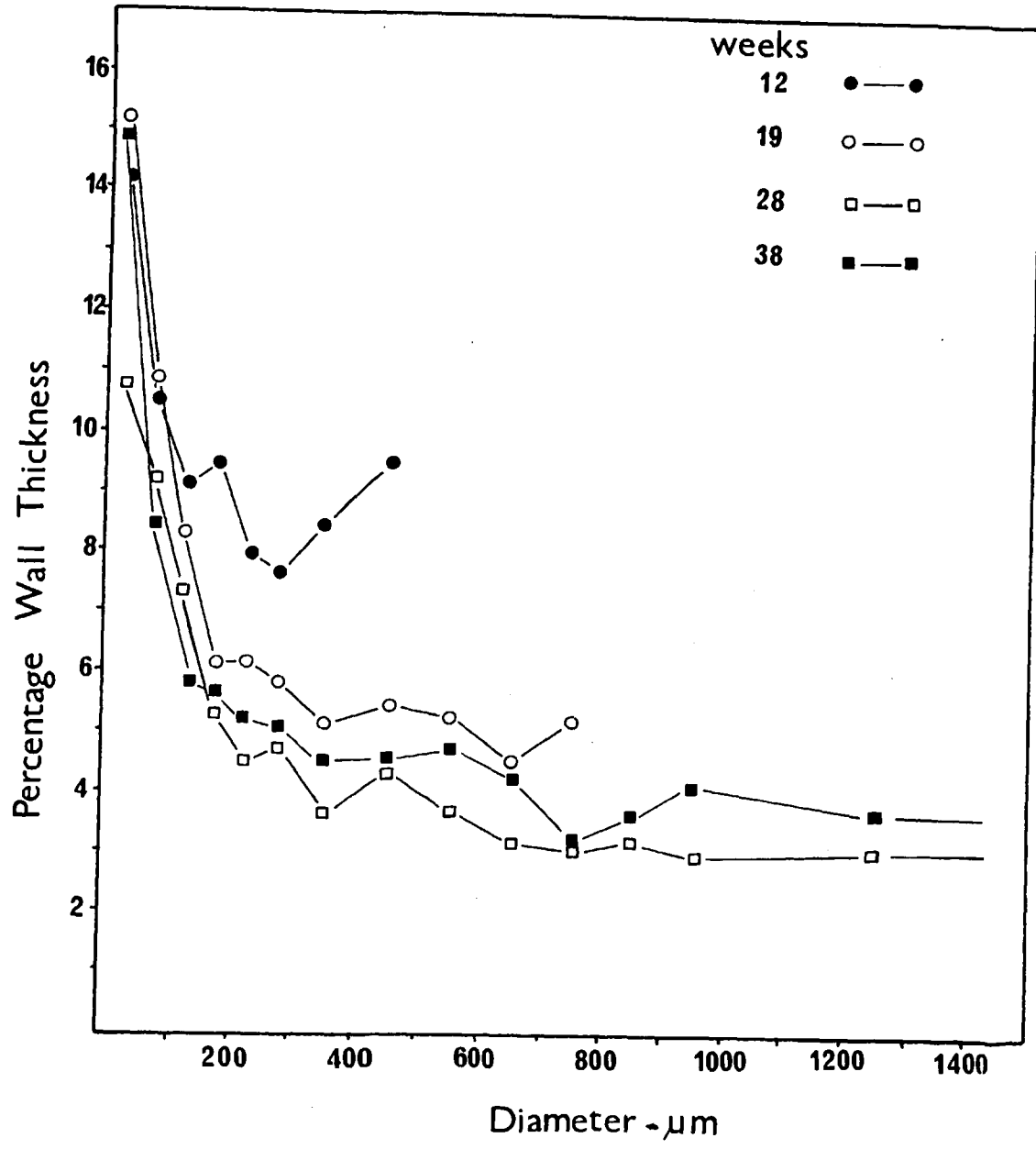
Percentage Wall Thickness During Fetal Life

Figure IV 5 shows the average percentage wall thickness in each size range for the left lung for four fetal ages, 12, 19, 28 and 38 weeks of gestation. These figures, together with the standard error and number of arteries measured, are tabulated in Table IV 16. Each case showed the same shaped curve with a marked increase in percentage wall thickness in arteries below 200 μm external diameter. At 12 weeks the smallest arteries had a mean percentage wall thickness of 14.15 while at 38 weeks it was 14.99 and 19 weeks, 15.22. However at 28 weeks the highest mean value was only 10.75. The mean value in arteries above 200 μm - those included in the flat region - was higher at 12 weeks than at any other age. At this age the percentage wall thickness of arteries between 200 and 500 μm in external diameter varied between 7.98 and 9.57. There were no figures for arteries of greater diameter since the hilar vessel and therefore the largest vessel in the lung was only 470 μm in diameter.

In the case of 19 weeks gestation the percentage wall thickness in the flat region varied between 4.53 and 6.21. At 28 weeks the range was between 3 and 4.7 and at 38 weeks between 3.1 and 5.19. These were probably variations around the same level since all cases

Figure IV 5

Percentage wall thickness related to external diameter of arteries from lungs of fetuses aged 12, 19, 28 and 38 weeks gestation. The mean for each diameter range for each case is shown.


weeks
12
19
28
38
Percentage Wall Thickness
16
14
12
10
8
6
4
2
200
400
600
800
1000
1200
1400
Diameter - µm

TABLE

Percentage Wall Thickness in Diameter

Age (wks)		0-50	50-100	100-150	150-200	200-250	250-300	300-400
	Mean	14.15	10.54	9.15	9.42	7.98	7.65	8.44
12	S.E.	1.51	0.733	0.813	0.518	0.492	2.95	0.24
	No.	4	17	10	7	8	2	10
	Mean	10.14	10.3	7.53	5.89	4.89	4.46	3.54
14	S.E.	0.138	0.657	0.334	0.473	0.217	0.327	0.148
	No.	3	28	29	12	27	13	17
	Mean		5.91	5.31	7.06	7.67	10.5	9.32
16	S.E.	-	0.363	0.352	0.375	0.302	0.643	0.336
	No.		5	14	8	14	6	8
	Mean	11.42	9.69	7.41	6.00	5.58	4.85	5.17
17	S.E.	1.25	0.61	0.345	0.294	0.312	0.274	0.322
	No.	5	25	28	14	19	11	16
	Mean		10.96	7.32	5.54	6.28	5.37	4.84
18	S.E.	-	0.917	0.343	0.304	0.569	0.574	0.275
	No.		17	15	15	10	8	14
	Mean	15.22	10.88	8.28	6.13	6.21	5.85	5.12
19	S.E.	1.61	0.418	0.385	0.235	0.321	0.181	0.223
	No.	3	50	28	18	27	23	26
	Mean		9.53	5.75	4.57	3.53	3.07	3.04
20	S.E.	-	1.05	0.812	0.306	0.385	0.242	0.106
	No.		7	8	9	5	5	8
	Mean		8.94	5.76	5.05	4.73	4.41	3.87
20	S.E.	-	0.443	0.289	0.332	0.13	0.213	0.165
	No.		21	22	11	13	9	14

IV 16

Left Lung of Fetal Ages 12-20 weeks

Range (μm)

400-500	500-600	600-700	700-800	800-900	900-1000	>1000
9.57						
1						
2.96		3.26	3.36	2.52	3.2	
0.176	-	0.213	0.415	0.166		
9		3	4	4	1	
9.08		8.45	8.68			
0.686	-					
5		1	1			
4.5	4.33	3.25	3.37	3.16	2.8	3.62
0.196	0.458	0.114	0.205	0.08	0.104	0.96
3	7	6	2	12	3	2
4.68	4.02	4.45	4.08	4.3	4.87	4.27
0.45	0.39	0.553	0.582	0.702	0.144	0.189
4	2	2	5	2	3	7
5.49	5.25	4.53	5.2			
0.26	0.248	0.555	0.205			
23	14	4	4			
2.97	2.71	3.18	2.81	2.95	3.11	3.81
0.2	0.178	0.445	0.209	0.063	0.025	0.088
7	7	3	6	3	4	8
3.17	3.08	2.96		2.67		3.26
0.18	0.14	0.205	-		-	0.32
9	9	4		1		4

TABLE

Percentage Wall Thickness in

Diameter

Age (wks)		0-50	50-100	100-150	150-200	200-250	250-300	300-400
	Mean		10.89	7.53	6.15	5.59	5.23	4.48
23	S.E.		0.628	0.339	0.313	0.203	0.176	0.218
	No.		25	27	13	14	12	19
	Mean		8.18	4.91	4.20	4.43	3.36	3.03
26	S.E.		1.36	0.275	0.336	0.081	0.152	0.179
	No.		5	10	9	8	5	3
	Mean		5.74	5.11	4.52	4.73	3.80	4.18
26	S.E.			0.479	0.471	0.369	0.215	0.257
	No.		1	3	6	3	4	7
	Mean		6.77	5.16	3.66	3.77	3.48	3.41
28	S.E.		1.855	0.419	0.288	0.273	0.208	0.166
	No.		4	12	14	9	11	8
	Mean	10.75	9.22	7.36	5.31	4.52	4.70	3.66
28	S.E.	0.347	0.92	0.475	0.347	0.1007	0.233	0.189
	No.	2	10	15	11	15	13	14
	Mean		12.49	6.64	5.65	4.91	4.74	4.38
32	S.E.		0.945	0.479	0.303	0.225	0.241	0.221
	No.		4	12	6	3	8	6
	Mean	14.0	8.11	5.68	3.95	3.97	3.07	3.45
33	S.E.		1.07	0.394	0.283	0.251	0.277	0.136
	No.	1	11	13	11	5	3	15
	Mean	12.40	7.40	5.92	5.34	4.75	4.50	4.24
36	S.E.	2.86	0.434	0.313	0.308	0.167	0.184	0.22
	No.	3	23	17	8	19	11	9

IV 16 cont'd

Left Lung of Fetal Ages 23-36 weeks

Range (μm)

400-500	500-600	600-700	700-800	800-900	900-1000	> 1000
4.29	4.12	3.58	2.84	2.78	2.19	3.37
0.186	0.26	0.346	0.114	0.114	0.063	0.221
17	5	9	11	6	2	18
3.47	3.18	3.17	3.04	2.95	3.72	2.61
0.219	0.159	0.204	0.482	0.248		0.168
5	6	6	2	3	1	6
4.23	4.21	3.85	3.72	2.71		3.67
0.404	0.18	0.115	0.347		-	0.26
3	8	4	2	1		3
3.48	3.03	3.71	2.85	2.62	3.57	2.92
0.256	0.345	0.212	0.152	0.147		0.111
11	4	7	5	6	1	11
4.31	3.67	3.13	3.15	3.21	3.0	3.04
0.341	0.122	0.145	0.09	0.088	0	0.1004
11	6	10	9	8	2	21
4.12	4.14	3.79	3.54	3.01	3.26	2.84
0.178	0.86	0.276	0.139	0.07		0.135
7	7	2	5	2	1	6
3.70	3.09	3.29	2.51	2.75	3.00	3.15
0.241	0.28	0.367	0.381	0.11		0.255
8	4	5	3	4	1	8
4.02	3.73	3.22	3.21	3.13	3.17	2.93
0.191	0.122	0.161	0.06	0.113	0.173	0.09
13	6	5	4	7	3	25

S.E. - Standard error of the mean

No. - Number of vessels measured

over 12 weeks fell within this range, except the 16 week old case which seemed to be an exception, in its wall thickness, since the branching pattern was normal.

It was noted that the diameter at which there was a change in slope was much the same in all four cases, that is, between 150 and 250 μm external diameter. This applied to all the fetal cases studied as can be seen from Table IV 16. Elliott (1964) showed this rise to be at 100 - 200 μm in the adult and the present figures for the child seem to confirm this size range. There was the same pattern at all ages though the arteries involved with the increased wall thickness were in more proximal regions in the younger lungs. There was a stereotyped pattern of structure for diameter.

The average percentage wall thickness of each size range has been taken and the mean for the left lung of all cases estimated: the results are shown in Figure IV 6 with standard deviation and Table IV 17 with standard error and number of vessels. A typical curve was produced with the rapid increase in percentage wall thickness in the small arteries. The standard errors are high because of the particularly high average figures for the cases of 12 and 16 weeks gestation.

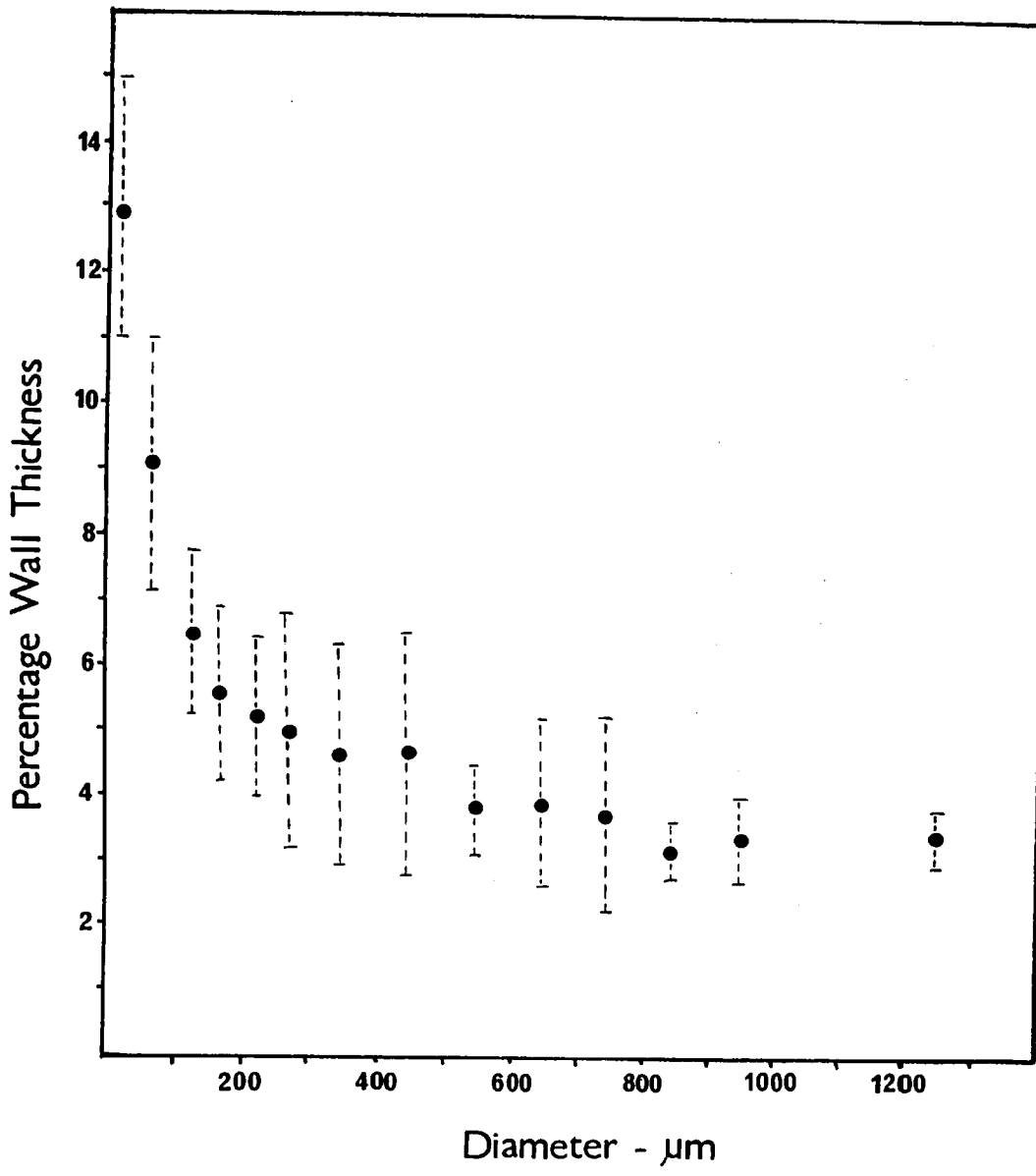
Percentage Wall Thickness in the Right Lung During Fetal Life

The percentage wall thickness has been estimated in the left lung of 17 cases. In order to show any difference between the right and left lungs the right lungs of four cases were also studied in the same manner. The left lung had been studied in three of these cases.

Measurements were made on arteries from the right lung of cases

Figure IV 6

Percentage wall thickness related to external diameter for arteries from all fetal cases ranging in age from 12 to 40 weeks gestation. The mean and standard deviation for the means in each size range of each case are shown.


Percentage Wall Thickness
14
12
10
8
6
4
2
200
400
600
800
1000
1200
Diameter - µm

TABLE

Mean Percentage Wall

Diameter

Age		0-50	50-100	100-150	150-200	200-250	250-300	300-400
12-38 wks L.lung	Mean	12.88	9.06	6.51	5.53	5.22	4.95	4.63
	S.E.	0.69	0.46	0.303	0.32	0.3	0.44	0.42
	No.	27	294	286	184	216	156	204
19-40 wks R.lung	Mean	9.52	8.97	6.00	5.16	4.70	4.30	4.08
	S.E.		0.83	0.73	0.19	0.14	0.21	0.23
	No.	1	66	60	55	43	35	55

IV 17

Thickness during Fetal Life

Range (μm)

400-500	500-600	600-700	700-800	800-900	900-1000	> 1000
4.62	3.80	3.88	3.70	3.03	3.33	3.32
0.45	0.19	0.33	0.39	0.12	0.19	0.13
143	89	84	64	65	23	144
3.79	3.43	3.44	3.19	3.22	2.77	3.69
0.18	0.24	0.45	0.38	0.69	0.29	0
34	35	13	11	7	8	34

S.E. = Standard Error of the Mean of the Means

No. = Total number of vessels measured

aged 19, 20, 23 and 40 weeks. The last was the case in which only the right lung was available for study. The average for each size range together with the range and standard error for each case are shown in Table IV 18. The average figures for all four cases are shown in Table IV 17.

The results showed the same curve that was seen in the left lung. In two cases (20 and 23 weeks) there was no statistical difference between the figures from the right and left lungs. In the case of 19 weeks the right lung had a significantly lower percentage wall thickness than the left.

The right lungs of all four cases studied had very similar percentage wall thicknesses and the standard error of the means was low in comparison with those of the left lung. When the average for all the right lungs was compared with that for the left it was seen that the average for the right lung fell within the range of the left but in all but two diameter ranges it was below the average for the left. This can be explained by the addition in the left lung of the high figures for the 12 and 16 week fetuses. It would seem that there was no true difference between right and left lung during fetal life since the difference in the 19 week case may be explained by technical differences. In the adult, Simons and Reid (1969) found that the arteries from right and left lung were the same.

Percentage Wall Thickness During Childhood

Results on the percentage wall thickness of cases of children aged four months, five years and 11 years have been previously reported by Davies (1969). These specimens were re-examined in the present study using fresh histological material. The results obtained were essentially the same as those reported by Davies and

TABLE

Percentage Wall Thickness in

Diameter

Age (wks)		0-50	50-100	100-150	150-200	200-250	250-300	300-400
	Mean		11.04	6.98	5.40	4.33	4.05	3.93
19	S.E.		0.686	0.668	0.535	0.213	0.405	0.241
	No.		10	14	8	3	6	11
	Mean	9.52	9.39	7.18	5.27	4.73	3.86	3.52
20	S.E.		0.489	0.525	0.324	0.276	0.228	0.128
	No.	1	21	13	20	15	12	19
	Mean		8.33	4.00	4.59	4.69	4.46	4.27
23	S.E.				0.177	0.286		0.336
	No.		1	2	2	9	1	6
	Mean		7.13	5.83	5.39	5.05	4.82	4.61
40	S.E.		0.279	0.239	0.278	0.22	0.227	0.151
	No.		34	31	25	16	16	19

IV 18

Right Lung of Fetal Ages 19-40 weeks

Range (μm)

400-500	500-600	600-700	700-800	800-900	900-1000	> 1000
3.84	3.61					
0.203	0.291					
9	5					
3.78	3.08	3.04	2.81	2.52		
0.272	0.267	0.109	0.312			
7	14	3	2	1		
3.31	2.98	2.95			2.47	
0.075	0.233	0.254				
4	5	3			1	
4.22	4.03	4.34	3.57	3.91	3.06	3.69
0.203	0.205	0.473	0.23	0.352	0.159	0.092
14	11	7	9	6	7	34

S.E. - Standard error of the mean

No. - Number of vessels measured

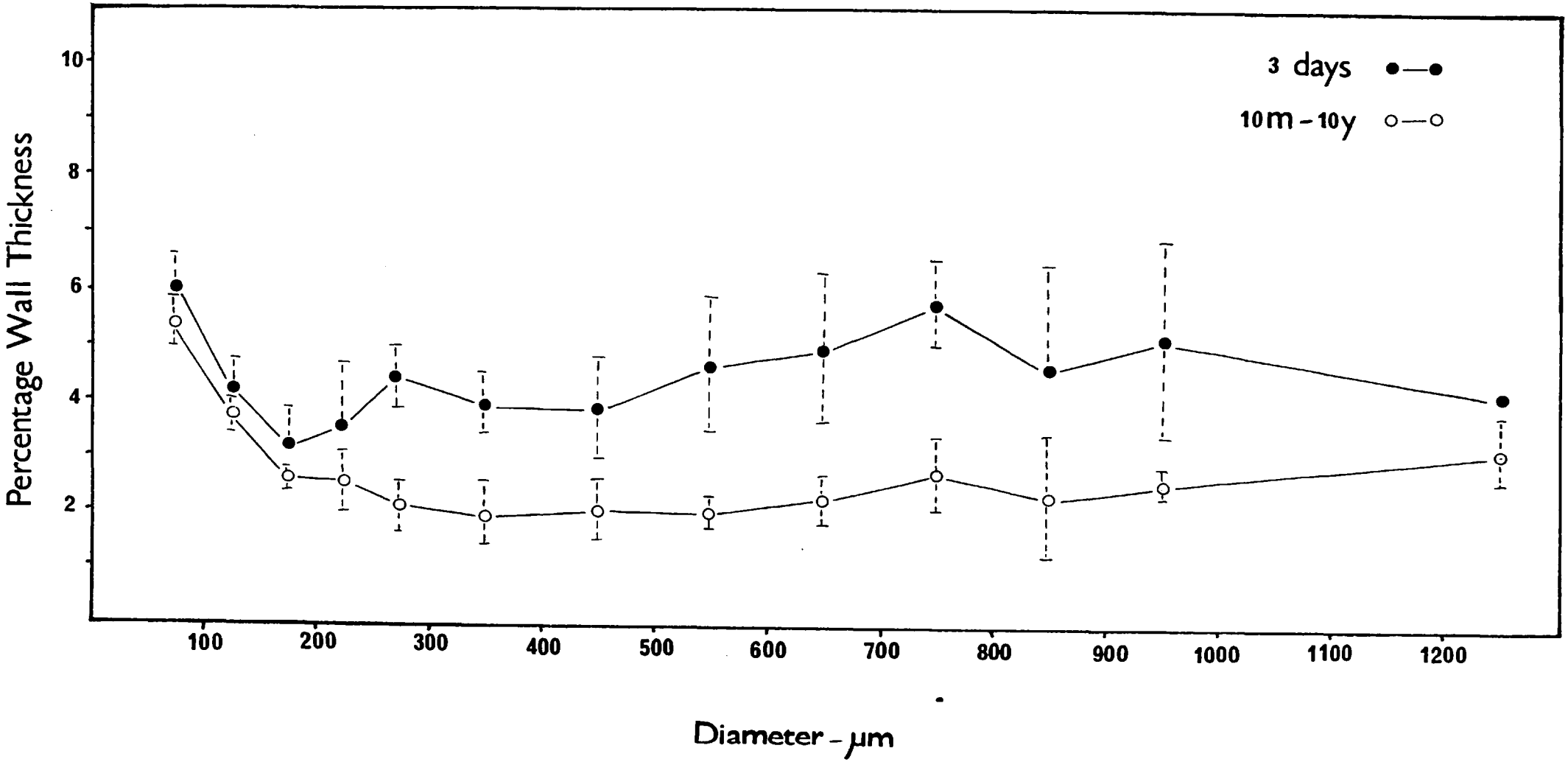
are therefore not included here. In addition to these cases, six others were studied aged three days, ten months, 18 months, three, four and ten years. The results grouped in size ranges are shown in Table IV 19.

In the case of three days (Figure IV 7) the results were in the same range as those seen in fetal life with the percentage wall thickness in the larger arteries being 3 - 5%. The smaller arteries below 200 um external diameter had a reduced percentage wall thickness in comparison with the fetus. Davies showed that by four months the percentage wall thickness was dropping to adult levels, the larger arteries now having a value of 2 - 3%. In the case studied here of ten months there had been a further decrease with figures only just above 2% except in the larger arteries over 700 μm. The 18 months, three and four year olds had a percentage wall thickness slightly lower than the ten months, being mainly below 2% but above 1%. There may have been further thinning of the walls after ten months in the larger arteries but by 18 months it seemed to be complete. It seems that thinning of the larger arteries may take longer than previously reported (Davies, 1969). However it seems likely that all cases over four months were within the normal adult range (Elliott, 1964) and the trend seen is purely individual variation. This was borne out by the fact that the flat region at ten years was higher than at ten months. The larger arteries in the ten month child had a higher percentage wall thickness than any others and any adult. It may be that these were thinning more slowly.

The mean figures for the five cases aged ten months to ten years have been averaged and are plotted with standard deviation in Figure IV 7. The average and standard error are shown in Table IV 20. The resulting curve was very similar to those of

Figure IV 7

Percentage wall thickness related to external diameter for the arteries in the lung of a three day old infant and in the children aged ten months to ten years. The mean and standard deviation in each size range are shown. In the older cases the figures are the mean for the group.


3 days
10m - 10y
Percentage Wall Thickness
10
8
6
4
2
100
200
300
400
500
600
700
800
900
1000
1100
1200
Diameter - μm

Elliott on the adult where there was an average between 1 and 2%. On the same graph are shown the figures for the three day old child. There is an obvious drop in the percentage wall thickness of the larger arteries between three days and the older cases, however in the arteries below 200 μm there had been only a small decrease. Over this period most of the decrease has occurred therefore in the larger arteries: between birth and three days the decrease was in the smaller arteries not in the larger.

It is probable that the thinning of the small arteries is by distension. The larger arteries take longer to reduce their muscle thickness which must occur either by true atrophy or by growth of the lumen without growth in muscle. The latter seems likely since a lag in muscle development as the new arteries grow has already been demonstrated.

The percentage drop in wall thickness is ultimately greater in the small arteries where in the 50 - 100 μm group the percentage wall thickness drops from 9.06 in fetal life to 5.13 in the ten year old and in the 500 - 600 μm group the drop is only from 2.8 - 2.41. However the actual drop in wall thickness is about 50% in all size ranges.

Evaluation of Increase in Percentage Wall Thickness in Small Arteries

In all cases studied there was an increase in the percentage wall thickness in the arteries below 200 μm external diameter. An increase of this kind was also noted in the adult (Elliott, 1964). Simons and Reid (1969) showed that this increase in the adult was

TABLE

Percentage Wall Thickness

Diameter

Age		50-100	100-150	150-200	200-250	250-300	300-400
	Mean	6.0	4.16	3.26	3.54	4.45	3.97
3 days	S.E.	0.375	0.221	0.364	0.446	0.306	0.213
	No.	3	6	3	7	3	6
	Mean		3.99	2.75	2.43	2.13	1.86
10 months	S.E.		0.26	0.103	0.113	0.133	0.159
	No.		3	5	8	9	7
	Mean		3.33	2.66	2.21	1.8	1.47
18 months	S.E.			0.104	0.053	0.053	0.1106
	No.		1	3	5	6	6
	Mean			2.35	2.09	1.85	1.45
4 years	S.E.			0.2007	0.1106	0.0316	0.022
	No.			7	6	10	5
	Mean	5.71	3.86	2.54	2.47	1.97	2.02
3 years	S.E.	0.566	0.282	0.17	0.25	0.113	0.144
	No.	3	6	4	10	10	12
	Mean	5.13	3.85	2.85	3.49	2.91	2.88
10 years	S.E.	0.127	0.114	0.112	0.315	0.174	0.028
	No.	2	11	5	8	5	3

TABLE

Mean Percentage Wall

	Mean	5.42	3.76	2.63	2.54	2.13	1.94
10 months to 10 years	S.E.	0.29	0.145	0.085	0.25	0.21	0.261
	No.	5	21	24	37	40	33

IV 19

during Childhood

Range (μm)

400-500	500-600	600-700	700-800	800-900	900-1000	7 1000
3.90	4.70	4.96	5.79	4.6	5.16	4.2
0.52	0.858	0.78	0.531	0.945	1.226	
3	2	3	2	4	2	1
2.22	2.17	2.2	3.89	3.08	2.89	3.57
0.116	0.17	0.248				0.159
9	7	2	1	1	1	7
1.74	1.60	1.69	2.46	1.52	2.27	
0.134	0.25	0.179	0.318	0.304		
8	6	3	5	2	1	
1.55	2.03	2.27	2.09			3.68
0.173	0.1	0.147	0.152			1.16
3	4	5	5			3
1.86	2.06	2.15	2.66		2.58	2.92
0.134	0.652	0.14	0.106		0.063	0.167
8	2	4	2		3	3
2.92	2.41	2.90	2.56		2.50	2.38
0.185	0.168	0.042			0.134	0.125
7	5	2	2		2	4

IV 20

Thickness during Childhood

2.06	2.05	2.24	2.73	2.30	2.56	3.14
0.242	0.129	0.192	0.305	0.781	0.129	0.305
35	24	16	15	3	7	10

S.E. - Standard error of the mean

No. - Number of vessels measured

due to the wall thickness remaining the same while the external diameter reduced. At 200 μm external diameter the wall had reached the smallest thickness recorded.

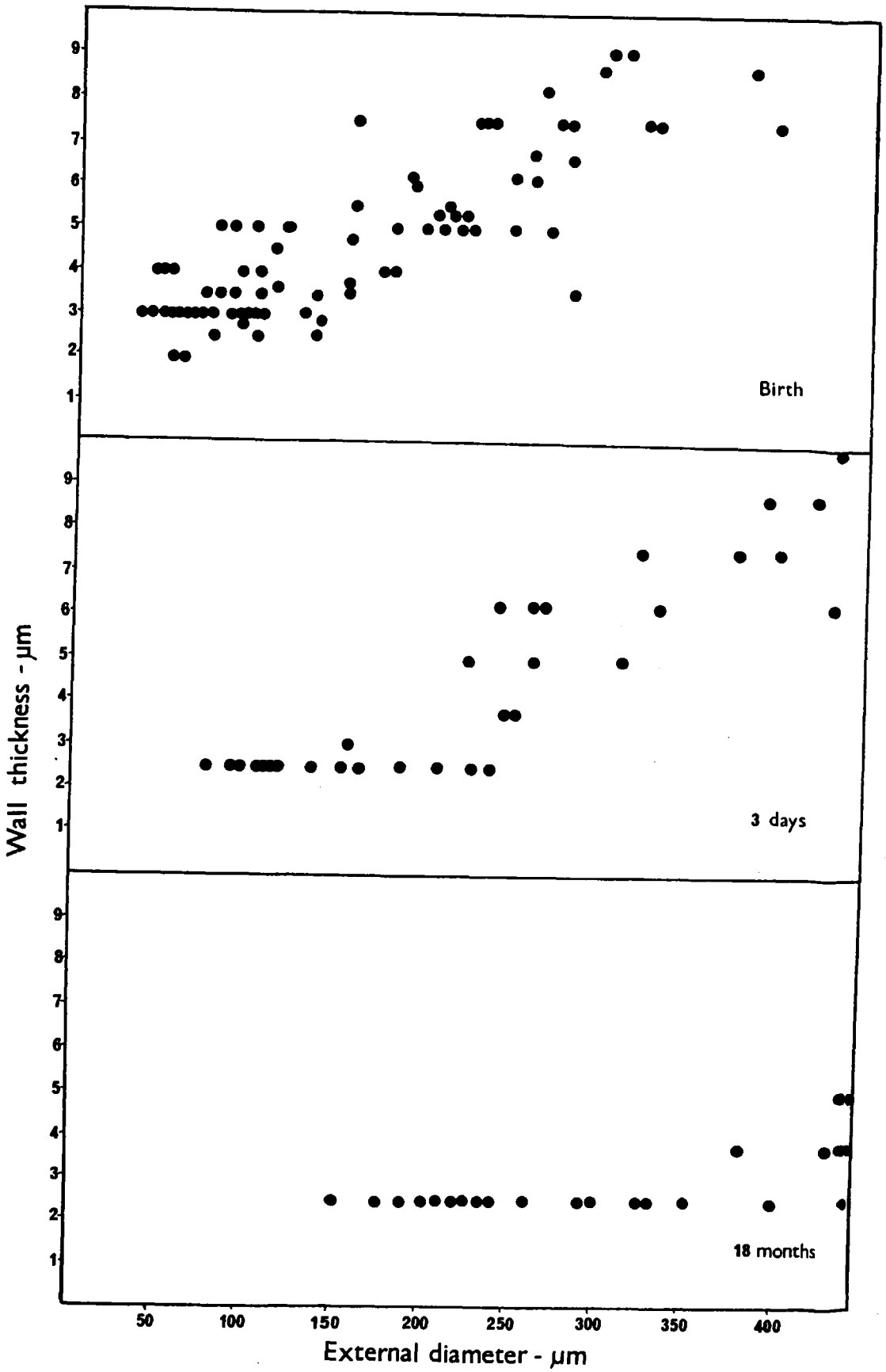
In the present study the absolute wall thickness has been plotted against external diameter for the cases studied. Figure IV 8 shows the graph for a full term fetus, three day infant and 18 month old child representative of all the cases. In all the children over 3 days all arteries up to a diameter of 250 μm had a wall thickness of 2.5 μm as seen in the adult. It was this which had caused the increase in percentage wall thickness. In the fetuses the increase in percentage wall thickness also occurred at about 250 μm external diameter but a wall thickness of only 2.5 μm had not been reached in all arteries of this size. In all fetal cases there was a range of wall thickness at any diameter down to below 50 μm external diameter. The increase in percentage wall thickness was not due to the lack of smaller muscle cells but maintenance of the relatively thick wall.

At all diameters the wall thickness was higher during fetal life than in the children. The rate of reduction in wall thickness was therefore faster during fetal life and the smallest wall thickness was not reached until a smaller external diameter. It was therefore only found over a small range of diameter.

The case of the three day old infant was interesting in that it appeared to embody both types of curve. In the arteries over 200 μm the wall thickness was the same as in the fetus while in arteries below 200 μm the wall thickness was like that of the child with a wall thickness of only 2.5 μm. There was a rapid rise in absolute wall thickness above 200 μm and by 250 μm a wall thickness of 6.25 μm had been reached.

Figure IV 8

Actual wall thickness related to external diameter for arteries in lungs from cases at birth, three days and 18 months.


Wall thickness - µm
External diameter - µm
Birth
3 days
18 months
1
2
3
4
5
6
7
8
9
50
100
150
200
250
300
350
400

Though the wall thickness reduces more rapidly during fetal life it is likely that there is no greater decrease per unit length of vessel since the external diameter decreases less rapidly during fetal life (see Chapter III).

In the fetus there is a greater resistance to flow since, at any size range, there are thicker walls than in the adult particularly in the small vessels. The increase in wall thickness occurs not only because the muscle walls cannot get thinner but because there are vessels with more muscle cells.

In the adult the increase in percentage wall thickness will increase resistance but it is a physical property of the muscle that the wall cannot become thinner. This may be overcome by the spiral of muscle where part of the wall will have no muscle and can therefore reduce the resistance.

In the fetus the percentage wall thickness increases when there is more than one muscle cell present in the wall so that there is a greater resistance in these vessels than in those at the same diameter in the child. This increase cannot be offset by the fact that they are partially muscular since some at this diameter are wholly muscular.

MEDIAL AREA

The percentage wall thickness can be affected by the degree of distension of the artery. If the artery is not fully distended the percentage wall thickness will increase. To ensure that the increase seen in all cases at the periphery was not due to under-distension it was necessary to measure medial area. The area of muscle will not alter with the distension of the vessel and is a

true measure of the amount of muscle in the artery.

Estimation of medial area was made at points along the axial artery of the posterior basal segment of the lung in all fetal cases studied by serial reconstruction. These have been taken as representative of the whole lung. Figure IV 9 shows the medial area plotted against external diameter for the 38 week old fetus. The results are tabulated in Table IV 21. There was a rapid and continuous decrease in medial area as the artery diameter decreased down to 250 μm. The percentage wall thickness over this range remains stable so that the decrease followed the reduction in size of the artery. Below 250 μm diameter the medial area fell less rapidly suggesting that there was not such a rapid loss of muscle as the diameter decreased. This related to the diameter when the percentage wall thickness increased. There was therefore a true increase in muscularity of the peripheral arteries.

The results from the other nine cases aged 12 to 40 weeks gestation were grouped together with the 38 week fetus and the average and standard error for the medial area in each size range estimated. The results are shown in Table IV 23 and the average and standard deviation on Figure IV 9. The graph showed a similar configuration to the 38 week old fetus on its own. There appeared to be a double slowing in the fall of medial area, the first between 650 and 350 μm and the second below 240 μm. The former was probably not significant since it was not very marked.

The slowing in the rate of reduction of medial area in the small vessels showed that there was a relative thickening of the walls at this point in all cases during fetal life. There was a large standard deviation due to the very large range in medial area.

Figure IV 9

Medial area in square μm related to external diameter for the axial artery to the posterior basal artery in the 38 week fetus at a number of levels; and the mean and standard deviation in each size range for arteries from nine cases aged 12 to 40 weeks gestation.

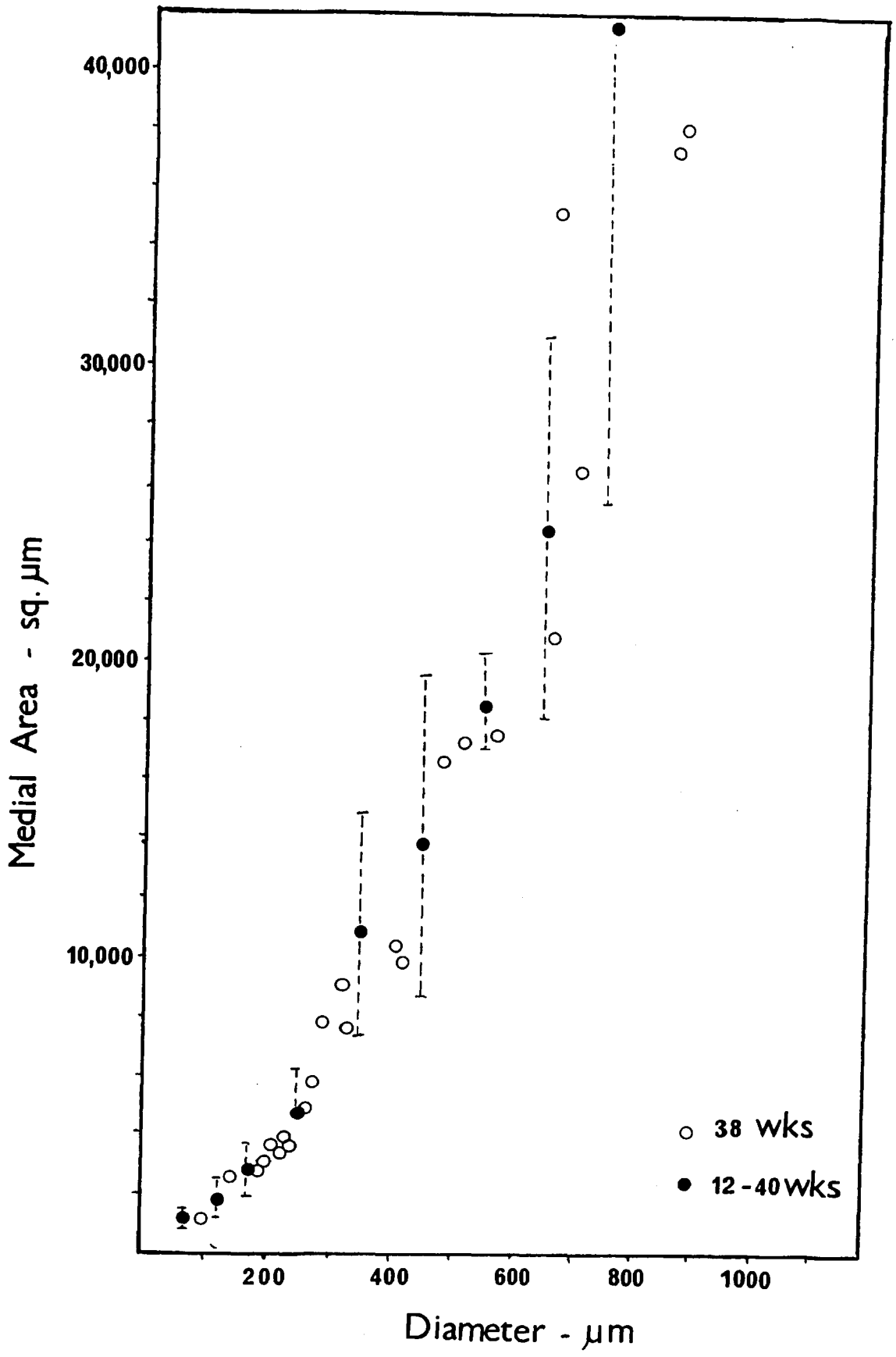

40,000
30,000
20,000
10,000
Medial Area - sq. µm
200
400
600
800
1000
Diameter - µm
38 wks
12 - 40 wks

Table IV 21

Medial Area along the Axial Pathway in a 38-week Fetus

External Diameter (μm)	Wall Thickness (μm)	Medial Area (sq. μm)
102	3.5	1083.2
152	5.5	2531.6
175	5.0	2670.7
206	5.0	3157.7
212	5.5	3568.5
228	5.0	3503.3
238	5.33	3896
240	5.0	3691.8
260	6.16	4913
280	6.66	5719.8
290	8.75	7732.2
318	9.16	8888.6
330	7.5	7599.7
410	11.25	14094.8
420	7.66	9924
480	11.25	16569.1
520	10.83	17325.9
570	10.0	17595.2
650	17.5	34778
660	10.16	20744.6
670	12.5	25823.3
860	14.0	37213.8
870	14.16	38076.9
1020	20.0	62840
1030	15.83	50442.6
1080	13.12	43980
1200	22.5	83243.3
1300	21.5	86366.5
1400	26.0	112244.8
1650	40.0	202344.8
1700	40.6	211681.6

Table IV 22

Medial Area of Pulmonary Arteries during Fetal Life

External Diameter of Artery	Medial Area			
	Mean (sq. μm)	Range (sq. μm)	Standard Error	Number of Vessels
50-100	1091	745-1493	124.6	6
100-150	1770	962-2239	170	13
150-200	2712	1552-3954	274	10
200-300	4785	3158-7732	251.5	26
300-400	10880	6833-18562	941.5	16
400-500	13825	7467-17287	1363.7	16
500-600	18537	16977-21123	500.7	9
600-700	24409	15150-38980	1783.6	13
700-800	41480	22636-72626	4397.3	14
800-900	42669	26409-89682	4092.5	16
900-1000	51006	35664-75063	5844.6	7
1000-2000	128542	43980-261580	4881.8	40
> 2000	448167	211399-1061414	27457	9

This was partly due in the smaller size ranges to the thicker wall already noted in the case of 12 weeks gestation, but the main reason is that within 100 μm diameter groups the amount of muscle increases greatly and the ranges will increase with external diameter increase.

POPULATION COUNTS

The size of artery showing a particular type of structure and the percentage of each type in any one size range can be estimated by measuring all the arteries in a given area of lung and designating their type. As previously shown the elastic arteries are only found above a given diameter, that varies according to age. Distally the structure of the vessel wall is muscular. In the small arteries there is a mixed population of muscular, partially muscular and non-muscular structure - each may be found in the same sized artery. It is the distribution of these which is important and demonstrates the extent of muscle into small arteries.

Population Count at Birth

Figure IV 10 shows the population count in a stillborn fetus of 40 weeks gestation. Each point represents the percentage of each type of vessel in the given size range. In arteries larger than 200 μm the wall will always be muscular, that is 100% of the arterial population of this size has a muscular wall. Below 200 μm partially muscular arteries appear and in the range between 150 and 200 μm comprise 9% of the population. In fact as can be seen from Table IV 23 the largest partially muscular artery was 160 μm. The number of partially muscular arteries increases relatively while the number of muscular arteries decreases. The

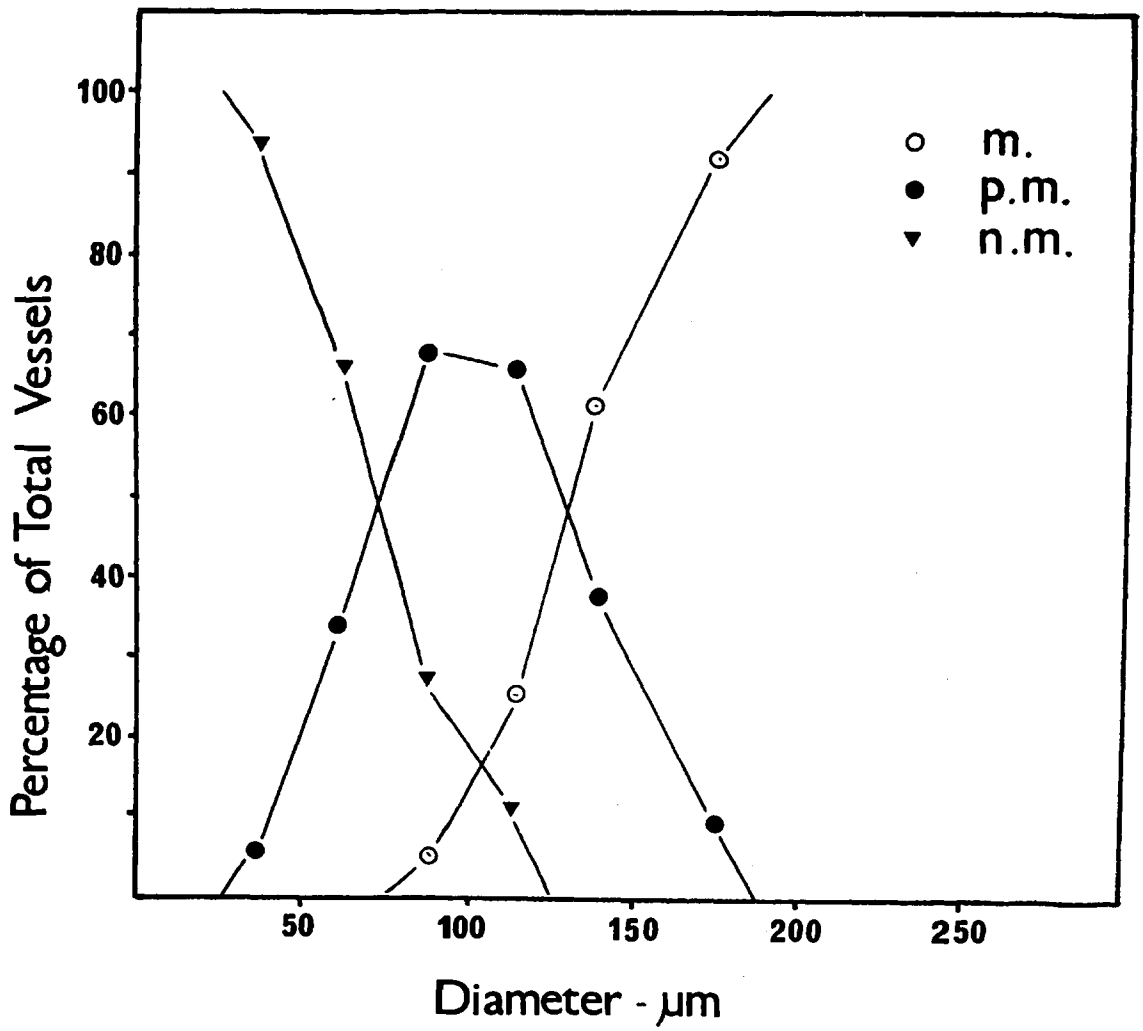
Figure IV 10

Population distribution curves for a fetus of 40 weeks gestation. The percentage of the total vessels with a given structure in each size range is shown.

m. - muscular arteries

p.m. - partially muscular arteries

n.m. - non-muscular arteries


100
80
60
40
20
Percentage of Total Vessels
50
100
150
200
250
Diameter - µm
m.
p.m.
n.m.

smallest diameter range in which a wholly muscular artery was found lay between 75 and 100 μm and from the table it can be seen that the smallest was in fact 95 μm. In this range also the partially muscular arteries reach their peak in the total population, now comprising 68% of the total population at this diameter. Below this diameter fewer partially muscular arteries are found as the percentage of non-muscular arteries increases. Non-muscular arteries appeared in this case between 100 and 125 μm, the largest being 107 μm. They comprised 100% of the population below 37 μm which was the size of the smallest partially muscular vessel seen.

Similar distribution was seen in the adult by Elliott (1964) and in the child by Davies (1969). Davies showed that the diameter at which the different types of artery appeared varied with age and may vary with disease.

Population Counts During Fetal Life

Population counts of this type were made on all the fetal lungs studied microscopically and the results are tabulated in Table IV 23, which shows the largest non-muscular vessel, the range in which partially muscular vessels were found and the smallest wholly muscular artery. In addition the diameter at which it occurs and the height of the peak of the partially muscular arteries is shown. Figure IV 11a and b shows small scale diagrams of the population distribution and the percentages of each type of vessel in each size range for each fetus is shown in Table IV 24.

The figures for all the fetal lungs were similar, showing that throughout fetal life muscle extended to arteries of about

TABLE IV 23

Distribution of Structure by Size in Small Vessels during Fetal Life

Age (wks)	Largest non-muscular (μm)	Range of partially muscular (μm)	Smallest muscular (μm)	Peak of partially muscular arteries (μm)	% Peak
12	77	43-175	85	87.5	68
14	112	48-137	80	87.5	54
16	120	62-137	100	112.5	44
17	75	40-187	80	87.5	82
18	112	50-235	110	125	75
19	87	70-150	50	87.5	60
20	100	52-160	82	112.5	56
20	105	60-162	80	112.5	55
23	95	42-155	85	87.5	67
26	112	35-130	77	112.5	53
26	120	50-160	90	112.5	80
28	175	60-198	130	137.5	70
28	172	40-150	60	112.5	64
32	87	25-125	77	87.5	66
33	131	43-235	130	112.5	71
36	90	37-130	75	87.5	66
38	77	30-150	75	87.5	62
40	107	37-160	95	87.5	68
Mean	109	43.9-163.1	86.7	64.5	102.08
S.E.	6.74	2.42-7.64	4.85	2.29	3.81
Adult (Elliott, 1964)	120	30-130	30	63	72.5

the same size. The figures for the adult are also included in Table IV 23, taken from the work of Elliott (1964). The figures for the largest non-muscular and the range of partially muscular vessels are very similar to the results for the fetus. In the adult however the wholly muscular structure was found in smaller arteries. Though the same wall structure is seen in the same size arteries both throughout fetal life and in the adult, the position in the lung of these arteries is different. As the lung grows in size these arteries were found closer to the periphery. This was demonstrated along the axial pathways. In the 12 week fetus arteries without a continuous muscle wall appeared at a diameter of 175 μm which is at the level of the 10th generation. By 40 weeks gestation the same diameter was at the level of the 21st generation but still proximal to the acinus. In the adult the largest partially muscular artery of 130 μm is found either accompanying alveolar ducts or in the alveolar wall.

Population Counts During Childhood

Davies (1969) found that the curves in the child lung were not the same as those of the adult. The curves were shifted to the right showing that larger arteries were deficient in muscle. In a stillborn lung he found similar results to those reported here in the fetus. It was decided to look at further cases of children to describe more accurately the change in structure or the small arteries during childhood. The results are shown in Table IV 25 and 26 and Figure IV 12a, b and c. The cases of four months, five and eleven years have been previously reported by Davies (1969), but the present results were obtained from new material from these cases. The new cases included were aged three days, ten months,

Figure IV 11a

Population distribution curves for fetuses aged 12 - 20 weeks gestation. The percentage of each vessel type at any external diameter is shown. Fetal age in weeks is shown in the top left hand corner of each square.

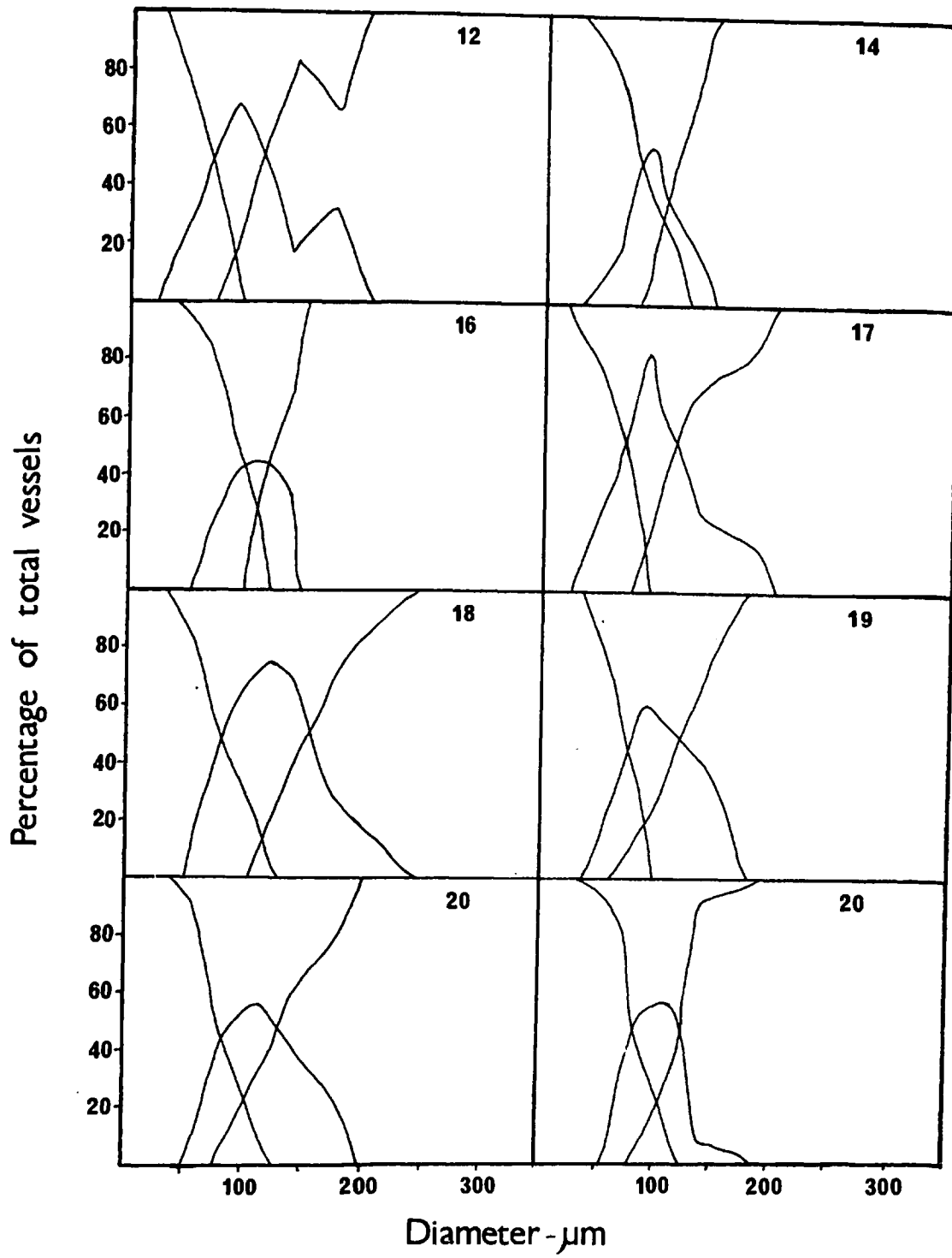

12
14
16
17
18
19
20
20
Percentage of total vessels
80
60
40
20
100
200
300
Diameter - µm

Figure IV 11b

Population distribution curves for fetuses aged 23 - 38 weeks gestation. The percentage of each vessel type at any external diameter is shown. Fetal age in weeks is shown in the top left hand corner of each square.

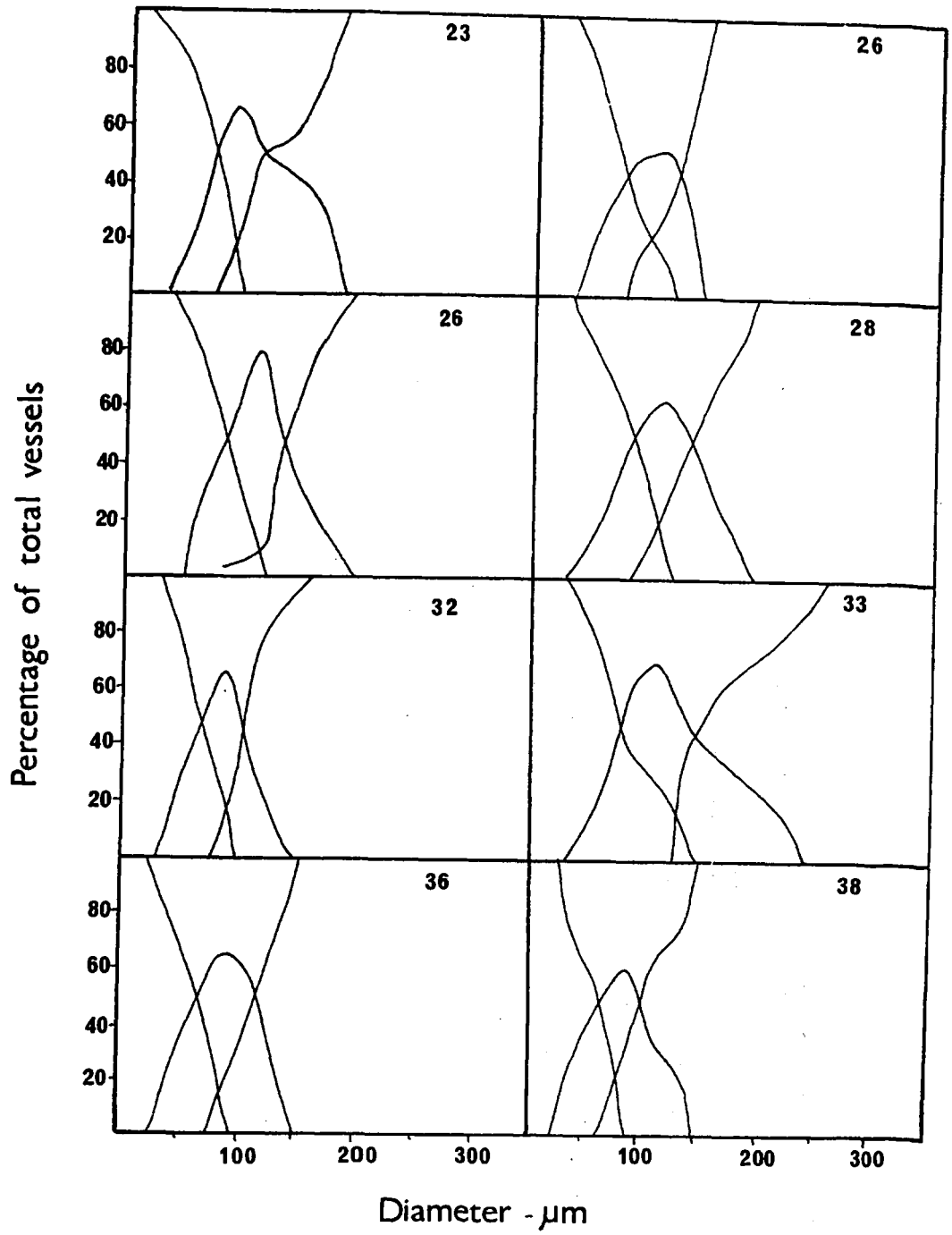

23
26
26
28
32
33
36
38
80
60
40
20
100
200
300
Percentage of total vessels
Diameter - µm

TABLE IV 24

Percentage of each Type of Vessel in each Size Range during Fetal Life

Age (wks)	Percentage	Diameter Ranges (μm)						
		25-50	50-75	75-100	100-125	125-150	150-200	200-250
	n	88	62	16	0	0	0	0
12	p.m	12	38	68	50	17	33	0
	m	0	0	16	50	83	67	100
	n	97	81	41	20	0	0	
14	p.m	3	19	54	30	12	0	
	m	0	0	5	50	88	100	
	n	100	92	60	23	0	0	
16	p.m	0	8	40	44	33	0	
	m	0	0	0	33	67	100	
	n	86	61	5	0	0	0	0
17	p.m	14	39	82	52	28	20	0
	m	0	0	13	48	72	80	100
	n	100	78	43	0		0	0
18	p.m	0	22	57	75		30	6
	m	0	0	0	25		70	94
	n	99	68	23	0	0	0	
19	p.m	1	30	60	46	97	0	
	m	0	2	17	54	3	100	
20	n	100	91	41	10	0	0	0
	p.m	0	9	52	55	8	4	0
	m	0	0	7	35	92	96	100
20	n	100	85	41	11	0	0	0
	p.m	0	15	50	56	45	25	0
	m	0	0	9	33	55	75	100
	n	93	68	20	0	0	0	0
23	p.m	7	32	67	50	44	6	0
	m	0	0	13	50	56	94	100

TABLE IV 24 cont'd

Percentage of each Type of Vessel in each Size Range during Fetal Life

Age (wks)	Percentage	Diameter Ranges (μm)						
		25-50	50-75	75-100	100-125	125-150	150-200	200-250
	n	94	67	30	14	0	0	
26	p.m.	6	33	52	53	22	0	
	m	0	0	18	33	78	100	
	n	100	75	44	13	0	0	0
26	p.m.	0	25	52	80	43	10	0
	m	0	0	4	7	57	90	100
	n	94	71	44	12	0	0	0
28	p.m.	6	25	53	64	50	19	0
	m	0	4	3	24	50	81	100
	n	92	67	20	0	0	0	
32	p.m.	8	33	66	32	10	0	
	m	0	0	14	68	90	100	
	n	97	76	39	29	10	0	0
33	p.m.	3	24	61	71	50	36	18
	m	0	0	0	0	40	64	82
	n	85	54	8	0	0	0	
36	p.m.	15	46	66	57	22	0	
	m	0	0	6	43	78	100	
	n	77	53	4	0	0	0	
38	p.m.	23	45	62	36	25	0	
	m	0	2	34	64	75	100	
	n	94	66	27	11	0	0	0
40	p.m.	6	34	68	66	39	9	0
	m	0	0	5	13	61	91	100

n - non-muscular
p.m - partially muscular
m - muscular

18 months, three, four and ten years.

Non-Muscular Arteries

It was found that in all cases save the ten year old the largest non-muscular vessel was greater in diameter than at any fetal age, that is arteries of a greater diameter had a non-muscular wall.

Partially Muscular Arteries

The smallest partially muscular arteries were of a similar size to those seen during fetal life though on average a little larger than those in the adult. The largest partially muscular artery varied from case to case. At four months it was 170 µm which is similar to the fetus but at five years it was 370 µm. In the child the number and size of arteries which have at least part of their wall without muscle is larger than in the fetus and the adult. The partially muscular structure curve extended over a greater size range and its peak was higher than at any other time suggesting that there is a greater proportion of partially muscular in the modal size as well as there being a greater size range. The peak of the partially muscular arteries had moved to the right.

The percentage of muscular, partially muscular and non-muscular vessels in the total population of arteries counted during the population study was estimated for all the cases both fetal and during childhood. The results are tabulated in Table IV 27. In all the fetal cases except the one at 36 weeks gestation the percentage of partially muscular vessels was always under 35%, the average

TABLE IV 25

Distribution of Structure by Size in Small Vessels during Childhood

Age	Largest non-muscular (μm)	Range of partially muscular (μm)	Smallest muscular (μm)	Peak of partially muscular (μm)	% Peak
3 day	152	40-180	120	112.5	66
4 mth	130	40-170	75	112.5	68
10 mth	125	55-220	112	137.5	81
18 mth	245	100-300	185	225	82
3 yr	126	52-295	77	112.5	83
4 yr	160	50-320	135	175	83
5 yr	202	50-370	155	175	75
10 yr	87	23-195	94	112.5	88
11 yr	160	50-320	148	175	82

TABLE

Percentage of each Type of Vessel in

Age	Percentage	Diameter			
		25-50	50-75	75-100	100-125
	n	92	78	51	31
3 days	p.m	8	22	49	66
	m	0	0	0	3
	n	97	67	31	8
4 months	p.m	3	33	61	68
	m	0	0	8	24
	n	100	97	75	32
10 months	p.m	0	3	25	61
	m	0	0	0	7
	n	100	100	100	75
18 months	p.m	0	0	0	25
	m	0	0	0	0
	n	100	66	25	6
3 years	p.m	0	34	72	83
	m	0	0	3	11
	n	100	95	75	31
4 years	p.m	0	5	25	69
	m	0	0	0	0
	n	100	86	60	45
5 years	p.m	0	14	40	55
	m	0	0	0	0
	n	84	57	9	0
10 years	p.m	16	43	82	88
	m	0	0	9	12
	n	100	65	61	38
11 years	p.m	0	35	39	62
	m	0	0	0	0

IV 26

each Size Range during Childhood

Range (µm)

125-150	150-200	200-250	250-300	300-350	350-400
13	4	0			
65	33	0			
12	63	100			
2	0	0			
47	5	0			
51	95	100			
5	0	0	0		
81	63	17	0		
14	37	83	100		
67	66	6	0	0	0
33	34	82	80	22	0
0	0	12	20	78	100
4	0	0	0	0	
72	68	50	34	0	
24	32	50	66	100	
14	3	0	0	0	0
81	83	55	33	16	0
5	14	45	67	84	100
40	15	4	0	0	0
60	75	63	60	73	78
0	10	33	40	27	22
0	0	0			
53	35	0			
47	65	100			
18	3	0	0	0	0
76	82	74	31	25	0
6	15	26	69	75	100

n - non-muscular
p.m - partially muscular
m - muscular

being 28.3%. The greatest proportion of the total was the non-muscular vessels, with the least being the muscular arteries. After birth and during childhood the percentage of partially muscular vessels was never less than 43% except in the ten month old child. The average for the nine cases was 45.77 which was considerably higher than the percentage during fetal life. In these cases the partially muscular vessels now made up the largest fraction followed by the non-muscular and muscular. During childhood there was also a trend to a larger percentage of partially muscular vessels with age, the largest being seen at 11 years.

In the adult, using figures from Elliott (1964), it was found that the percentage of partially muscular vessels over the same size range was much lower, only 15 and 20% in two cases. This was similar to that seen in the fetus.

The percentage of partially muscular vessels is therefore much greater in the children than during fetal life or in the adult. This was partly because of the larger size range over which they were counted and partly because in each size range counted there was a greater proportion. In the adult there was only a small proportion of partially muscular vessels in the lung. More muscle must have developed in these small vessels.

Muscular arteries

The size of the smallest muscular artery varied from case to case. In the 18 month, four year, five year and 11 year old child the size was particularly high, never below 135 μm. In all cases the diameter was higher than in the adult where the smallest muscular artery was 30 μm (Elliott, 1964). This means that muscle does not extend into such small arteries as in the adult.

Table IV 27

Percentage of Each Type of Vessel in Total Population

Age	Total number of vessels	Percentage		
		Non-muscular	Partially muscular	Muscular
12 weeks	93	36	34	30
14 "	176	61	17	22
16 "	100	58	18	24
17 "	383	45	34	21
18 "	325	51	32	17
19 "	487	54	27	19
20 "	328	64	17	19
20 "	99	49	24	27
23 "	301	52	31	17
26 "	150	52	29	19
28 "	220	59	29	12
28 "	172	62	24	14
36 "	220	41	40	19
38 "	184	37	34	29
40 "	275	49	35	16
3 days	203	43	43	14
4 months	429	36	43	21
10 "	180	49	34	17
18 "	123	45	45	10
3 years	206	27	49	24
4 "	232	40	44	16
5 "	442	39	49	12
10 "	171	25	51	24
11 "	179	33	54	13
Adult	225	73	15	12
"	298	63	20	17

Figure IV 12a

Population distribution curves for children aged three days, four months and ten months. The percentage of each type of vessel in each size range is shown.

m. - muscular arteries

p.m. - partially muscular arteries

n.m. - non-muscular arteries

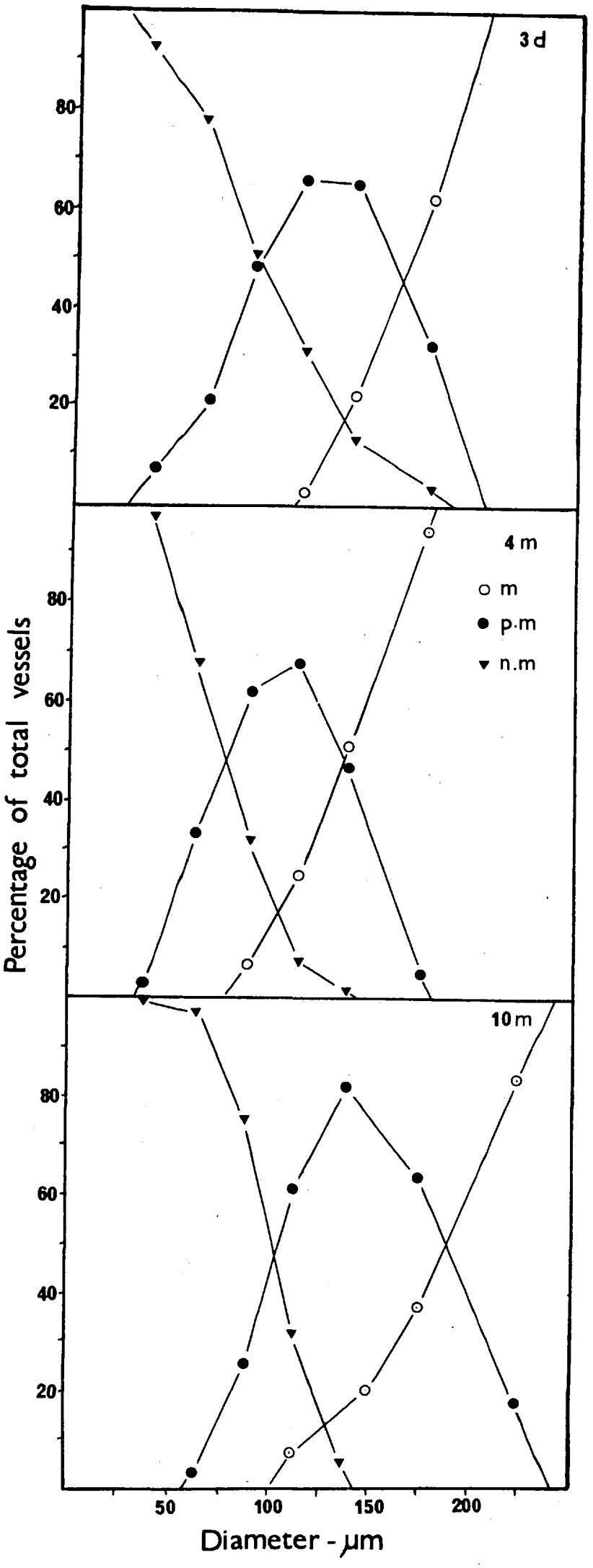

3 d
4 m
10 m
o m
● p.m
▼ n.m
Percentage of total vessels
80
60
40
20
50
100
150
200
Diameter - μm

Figure IV 12b

Population distribution curves for children aged 18 months, three years and four years. The percentage of each type of vessel in each size range is shown.

m. - muscular arteries

p.m. - partially muscular arteries

n.m. - non-muscular arteries

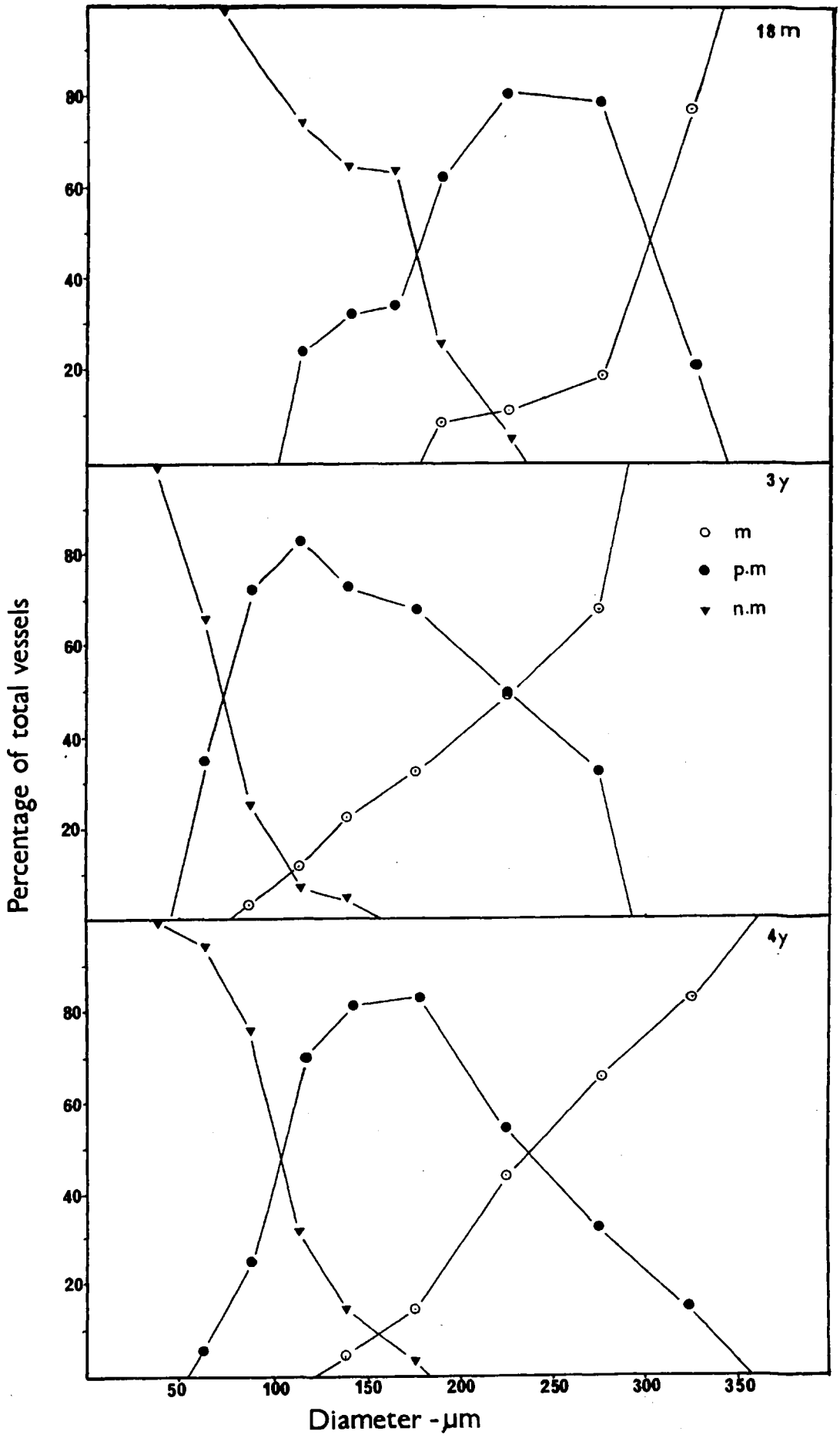

18 m
3 y
4y
m
p.m
n.m
Percentage of total vessels
Diameter - µm
20
40
60
80
50
100
150
200
250
300
350

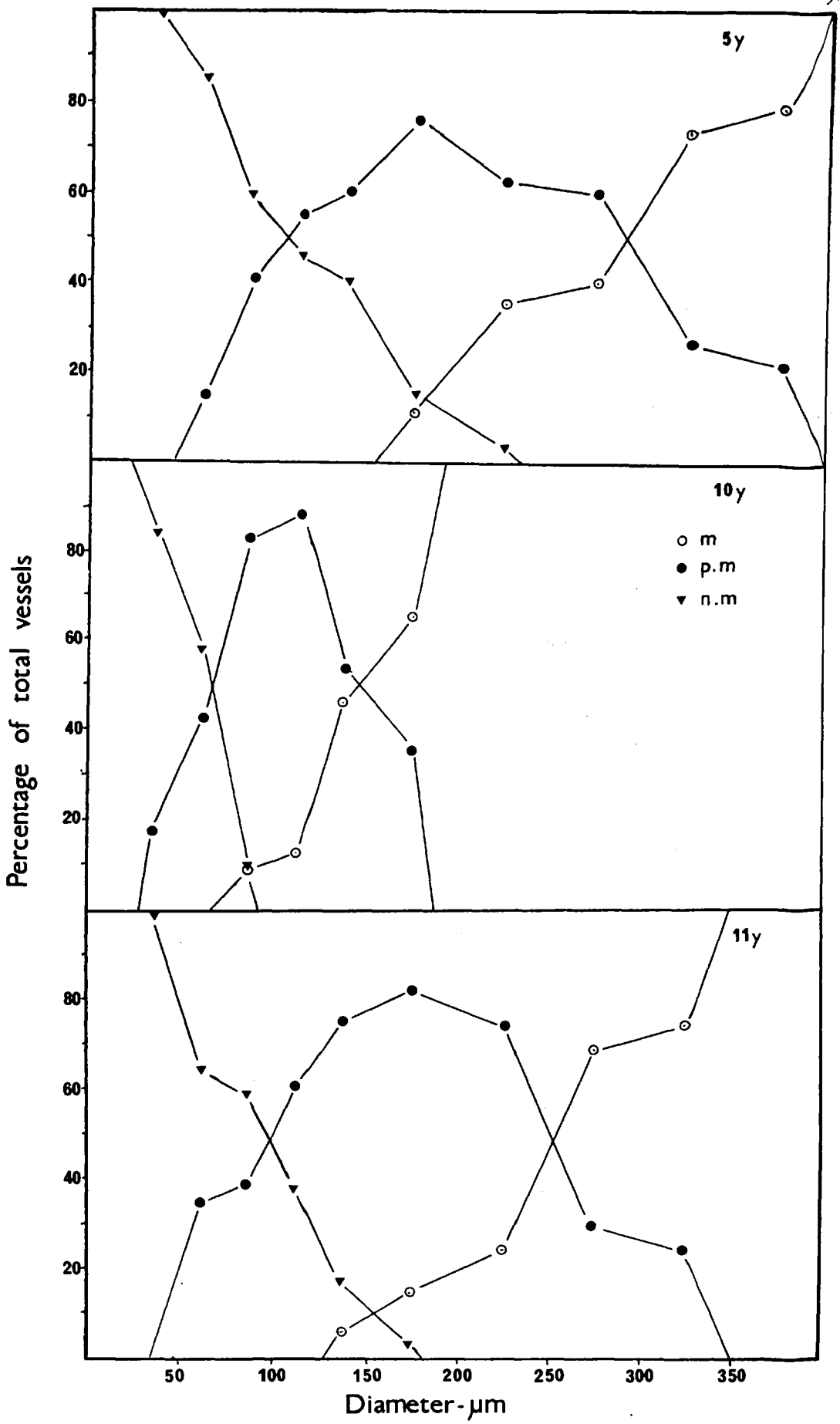
Figure IV 12c

Population distribution curves for children aged five years, ten years and 11 years. The percentage of each type of vessel in each size range is shown.

m. - muscular arteries

p.m. - partially muscular arteries

n.m. - non-muscular arteries


5y
10y
11y
m
p.m
n.m
Percentage of total vessels
Diameter-µm
20
40
60
80
50
100
150
200
250
300
350

The figures and the graph for the ten year old child showed that muscle was present in smaller vessels than in the other cases. It may be that this lung was approaching the adult pattern. The fact that the 11 year old is like the other children may mean that the ten year old was advanced for age or the 11 year old retarded. Without further cases of similar age it is impossible to decide.

The results for all the cases demonstrate a shift of the pattern to the right during the first year of life and then from 18 months the pattern maintained until ten years when there may be a reversal and the curves move back to the left and the adult form. It is unlikely that this shift to the right is due to muscle disappearing in the larger vessels but that muscle does not grow into the small arteries as they grow in diameter.

In each case the distribution of structure along one axial pathway can be compared with the population count for the entire lung. Along the single pathway each structural type was found within the diameter range seen on the total population. In no case did the figures for the axial pathway fall at the extreme limits of the size range for a given structural type.

The partially muscular structure along the axial pathway was found over a much smaller size range than was seen in the general population and fell near the centre of the range though towards its upper limits. The partially muscular structure always falls between the muscular and non-muscular so that there can be no overlap and hence there is a lack of the extreme sizes. Though the partially muscular vessels covered a wider size range in the children than the fetus, surprisingly the structure was found over the same length of pathway in child and fetus. The diameter drop at this level must have been slower in the fetus than the child, which may

be related to the higher wall thickness in the fetus.

ARTERIES WITH AIRWAYS

While studying random sections it is possible to identify and measure arteries running with particular airways. The arteries are therefore being 'landmarked' by the airways they accompany and results between cases can be compared. Respiratory bronchioli can be identified in cross-section by their interrupted cuboidal epithelium. Terminal bronchioli can then often be identified since they give rise to these respiratory bronchioli. Alveolar ducts also have accompanying arteries. Elliott (1964) found that in the adult there was a range of artery diameters running with any airway type. This would be expected since there is more than one generation of each type of airway.

Diameter of Arteries with Airways

Measurements of the range of arterial size with each type of airway were made on stillborn babies of 28, 36, 38 and 40 weeks gestation and infants and children of three days, four, ten and 18 months, and three, four, five, ten and 11 years. Table IV 28 and Figure IV 13 show the average diameter of the arteries accompanying terminal bronchioli, respiratory bronchioli, alveolar ducts and alveoli for each case. In addition in the table the range of diameter is shown.

In only four cases are figures given for the larger arteries found in the alveolar region. In this group were included those arteries surrounded by a definite connective tissue sheath found where three or four alveoli are in apposition.

Figure IV 13

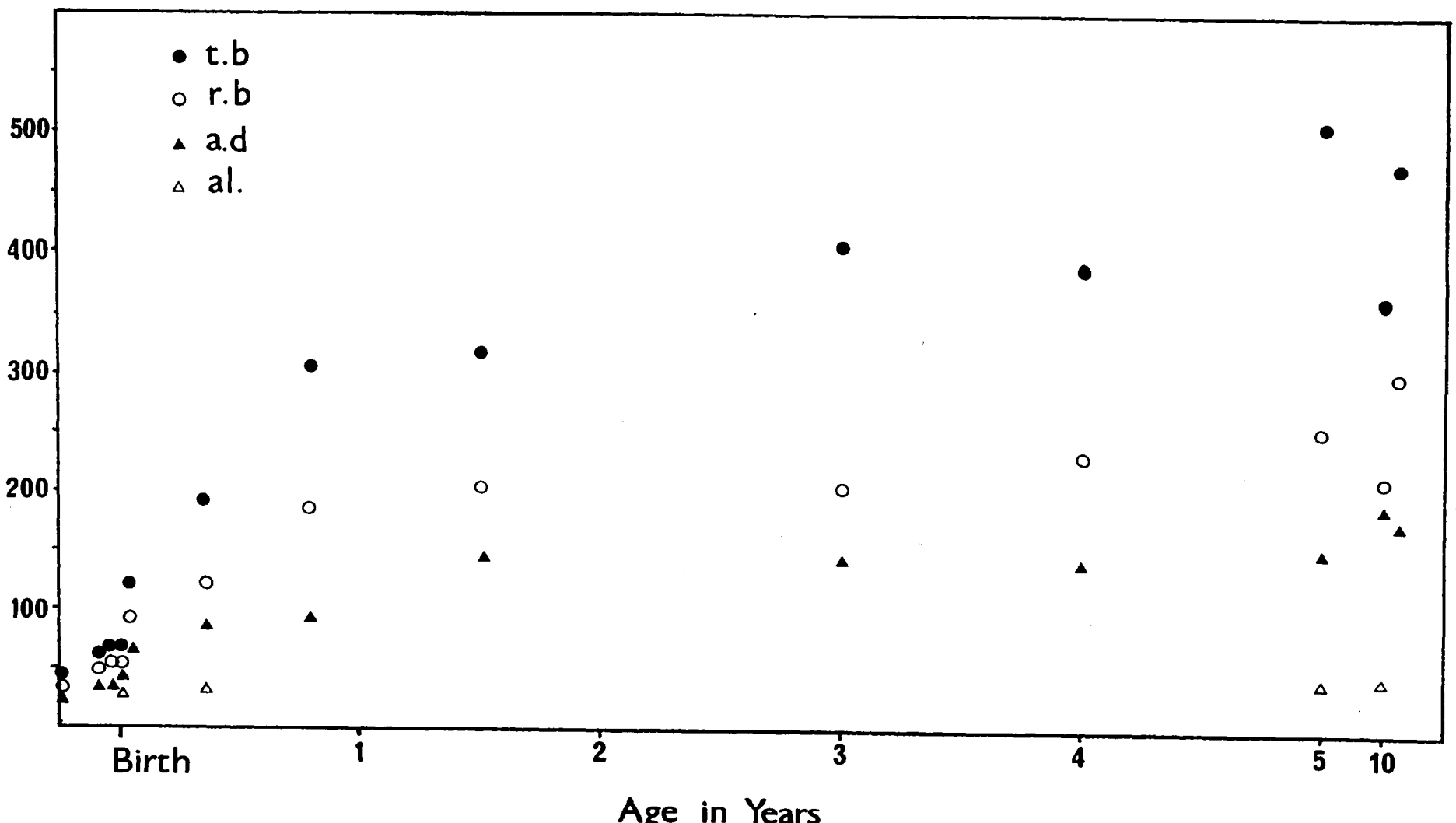
The external diameter of arteries accompanying airways within the acinus related to the age of the lung.

t.b. - arteries with terminal bronchioli

r.b. - arteries with respiratory bronchioli

a.d. - arteries with alveolar ducts

al - arteries with alveoli


t.b
r.b
a.d
al.
500
400
300
200
100
Birth
1
2
3
4
5
10
Age in Years

Arteries with Terminal Bronchioli

At 28 weeks gestation, the youngest case studied in this way, the average diameter of arteries with terminal bronchioli was 48.8 μm with a range of 35 - 62 μm. With age there was a marked rise in average diameter as well as in both the upper and lower limits of the range, up to ten months. The rise then continued less rapidly up to 11 years. This change in the rate of increase was also noted on the measurements of diameter from the arteriograms. The arteries were particularly large in the five year old as was also found in the serial reconstruction of one axial pathway in this case. This increase is likely to be due to individual variation. There was a ten fold increase in diameter between 28 weeks gestation and 11 years.

Measurements made during serial reconstruction of these cases showed that the main pathway at the level of the terminal bronchiolus had a larger diameter than the average figures found here, but they did fall within the range. Most of the arteries measured on random sections would be at the distal end of the terminal bronchiolus, since it was only where they gave way to respiratory bronchioli that they could be identified. The arteries along the main pathway were all measured at the proximal end of the airway and this might explain the discrepancy. In any one lung the range of size even at the distal ends of the terminal bronchiolus is great.

Arteries with Respiratory Bronchioli

The average diameter of arteries running with respiratory bronchioli was, at 28 weeks gestation, 43.5 μm with a range of 25 - 50 μm. The average was smaller than that of those accompanying terminal bronchioli but the ranges overlapped. As before there was a

Table IV 28

Diameter of Arteries with Airways

Age		Diameter of Arteries accompanying Airways (μm)			
		Terminal Bronchiolus	Respiratory Bronchiolus	Alveolar Duct	Alveoli
28 wk	Mean	48.8	43.5	25	-
	Range	35-62	25-50		
36 wk	Mean	60.2	47.7	33.3	-
	Range	37-87	30-77	25-40	
38 wk	Mean	69	52.8	-	-
	Range	50-82	40-85		
40 wk	Mean	67.37	51.25	40.3	32.09
	Range	45-110	27-75	25-62	25-42
3 day	Mean	118	92.7	65	-
	Range	87-175	45-145	60-70	
4 mth	Mean	197.3	122.3	86.5	33.3
	Range	127-265	70-182	70-115	25-40
10 mth	Mean	301	184	90.5	-
	Range	175-360	98-250	75-145	
18 mth	Mean	321.25	206.3	145.7	-
	Range	250-400	125-300	105-225	
3 yr	Mean	406	208	144	-
	Range	320-530	112-320	130-165	
4 yr	Mean	391.5	234	140	-
	Range	270-550	120-410	110-225	
5 yr	Mean	513	257	151	43.4
	Range	430-600	80-530	100-280	25-85
10 yr	Mean	321	215	188	46
	Range	220-430	105-415	75-177	35-52
11 yr	Mean	475	301	175	-
	Range	390-750	162-500	140-190	

more rapid rise in diameter during fetal life and the first ten months of post natal, but the rise did continue to 11 years, there being a seven fold increase in all. The ten year old child had a rather small average diameter as did the arteries with the terminal bronchioli in this case. In all cases the average size of the arteries with respiratory bronchioli was smaller than those with the terminal bronchioli but the ranges overlapped. Since there were several generations of respiratory bronchioli there was a particularly large range of size. The difference in size between these arteries and the previous group increased with age but the percentage difference remained much the same.

In all cases the average figures along the main pathway found by serial reconstruction fell within the normal range, and in only two cases, the 38 week fetus and three day infant, were there any arteries measured which fell outside the normal range using the present method. This closer relationship between the general population and one axial pathway can be explained by the fact that all levels of respiratory bronchioli can be identified and included. Also a greater number of respiratory bronchioli was identified and measured.

Arteries with Alveolar Ducts

At this level the arteries were smaller than those with respiratory bronchioli but the ranges overlap considerably. In three cases (three days, three and five years) the smallest alveolar duct artery was larger than the smallest respiratory bronchiolar artery. There was a gradual increase in the mean size with age, the greatest rate of increase being before 18 months. The total increase from 28 weeks gestation to 11 years was seven fold as with the respiratory bronchiolar

arteries. The ranges were wide because of the number of alveolar duct generations. The figures from random blocks were very similar to those obtained from arteries included in serial reconstruction with the average of these falling within the normal range of the present method.

Arteries with Alveoli

In the four cases measured there was a slight increase with age though only of 50% in 10 years. It is probable that the arteries accompanying the alveoli are always of similar size. This is because they are pre-capillary in position and the closer one gets to the capillary bed the more uniform will be the size of the vessels.

Structure of Arteries with Airways

When the artery accompanying a given airway was measured its structure was also determined, whether it be muscular, partially muscular or non-muscular. The percentage of each type of artery running with a given airway could then be estimated.

The percentages are shown in Table IV 29. At 28 weeks gestation 95% of the arteries running with terminal bronchioli were non-muscular, the other 5% being partially muscular. At the level of the respiratory bronchioli all arteries were non-muscular. At 36 and 38 weeks gestation the types are mixed, the majority of the arteries with the terminal bronchioli being partially muscular with some non and some wholly muscular. At the level of the respiratory bronchioli there were no muscular arteries but still about half were partially muscular. At 40 weeks gestation there were no wholly muscular arteries but there were still two thirds partially muscular arteries with the respiratory bronchioli. At no time during fetal life were

wholly muscular arteries found within the acinus. By three days 72% of the arteries with terminal bronchioli were partially muscular and the rest muscular, however there were still no wholly muscular vessels within the acinus but partially muscular vessels were found to the level of alveolar ducts. By four months all the arteries with terminal bronchioli were wholly muscular and this was so throughout childhood except in the case of 18 months where only 50% were wholly muscular.

At four months muscular arteries extended as far as alveolar ducts where there was a completely mixed set of arteries with a large percentage of each type of artery. At ten and 18 months wholly muscular arteries were not found with alveolar ducts and very few with respiratory bronchioli, the majority of vessels within the acinus being partially muscular. The apparent regression in these last two cases may be due to growth of new respiratory bronchioli and alveolar ducts and the increase in the four month case to centripetal alveolisation without increase in number of branches distally. On the other hand this may be individual variation.

From three years onwards there were no non-muscular arteries running with respiratory bronchioli and the percentage of partially muscular arteries decreased with age particularly over five years. At three years the percentage of muscular arteries with alveolar ducts was higher than at four and five years. However by ten and 11 years the majority of arteries with alveolar ducts were muscular in structure. In the ten year old partially muscular arteries could be seen running with alveoli as they do in the adult (Elliott, 1964).

There is a gradual extension of muscle into the acinus as the child gets older. This is not due only to increase in size since

Table IV 29

Structure of Arteries with Airways

Age	Percentage of each Type of Vessel with Airway											
	Terminal Bronchiolus			Respiratory Bronchiolus			Alveolar Duct			Alveoli		
	n	p.m	m	n	p.m	m	n	p.m	m	n	p.m	m
28 wk	95	5	0	100	0	0	100	0	0		-	
36 wk	18	71	11	59	41	0	100	0	0		-	
38 wk	11	56	33	41	59	0		-			-	
40 wk	50	50	0	84	16	0	100	0	0	100	0	0
3 day	0	72	28	30	70	0	66	34	0		-	
4 mth	0	0	100	3	30	67	35	18	47	100	0	0
10 mth	0	0	100	11	72	17	50	50	0		-	
18 mth	13	37	50	22	72	6	60	40	0		-	
3 yr	0	0	100	0	55	45	0	66	34		-	
4 yr	0	0	100	0	58	42	38	50	12		-	
5 yr	0	0	100	0	65	35	66	17	17	100	0	0
10 yr	0	0	100	0	21	79	0	36	64	66	34	0
11 yr	0	0	100	0	26	74	0	33	67		-	

n - non-muscular
p.m - partially muscular
m - muscular

at four months an artery of 197 μm is likely to be muscular, at ten months partially muscular, at 18 months partially muscular or non-muscular and by ten years muscular again.

These general figures can be compared with the extent of muscle along the main pathway, derived by serial reconstruction. In the younger cases muscular arteries did not extend beyond the first respiratory generations in either the general population or the axial pathway. By five years the muscle extended to the distal respiratory bronchioli along the axial pathway and in the general population some was found with the alveolar ducts. By ten and 11 years muscle was found in the arteries accompanying alveolar ducts by both methods. Partially muscular arteries showed the same trend as the muscular - with increasing age they are found more distally. At 10 and 18 months they form the greater proportion of the arteries in the acinus. Along the axial pathways non-muscular arteries were not found with respiratory bronchioli after 18 months; this was also seen in the general population. It seems that making measurements from random sections leads to similar results to those from serial reconstruction.

EXTENSION OF MUSCLE INTO THE ACINUS

Having established that the results from the random sections are comparable with those from serial reconstruction, it is possible to gain an idea of the distribution of muscle in the acini from throughout the lung. Serial reconstruction is time consuming and will only give results on one or two acini in a single part of the lung.

The results from the population counting and the size and structure of arteries associated with particular airways are especially valuable in estimating the extent of muscle into the acinus both along the main

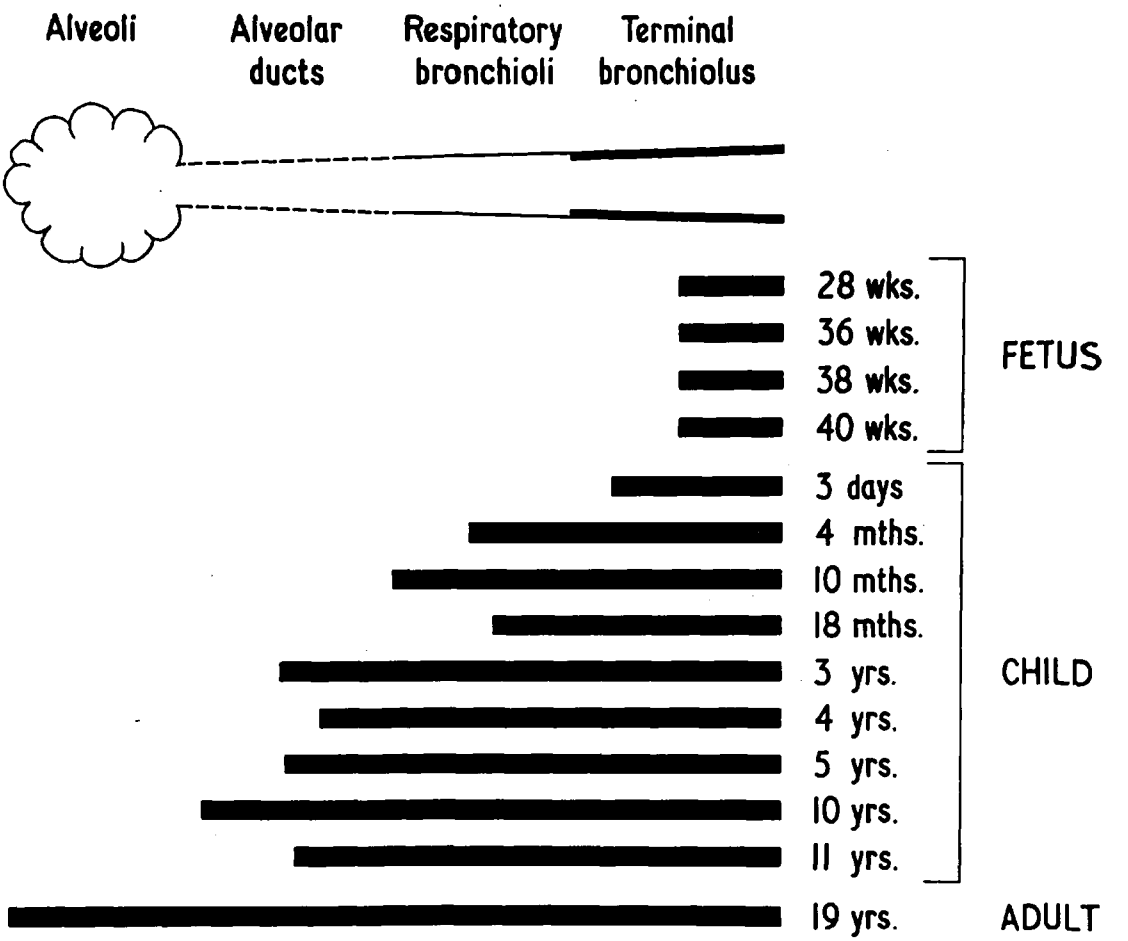
pathway and the side branches, whether conventional or supernumerary. The method of landmarking deals solely with conventional arteries while population counts also include all supernumerary vessels.

At 28 weeks of fetal life, the youngest case studied using both methods of landmarking and population counting, the diameter of arteries accompanying terminal bronchioli was 48.8 μm and of these 5% were partially muscular and the rest non-muscular. The percentage of partially muscular vessels at 48 μm seen in the population count was 14%. At the level of the respiratory bronchiolus there were no partially muscular arteries but from the population counts a few might be expected. It seems that the results from the arteries with airways and population counts are quite similar and this was also found in the other cases. This suggests that the conventional and supernumerary vessels have the same structure for external diameter as the conventional arteries.

A representation of the extent of muscle into the acinus in all 13 cases studied using both methods is shown in Figure IV 14 together with figures for the adult derived from those of Elliott (1964). The figure shows an acinus with the extension of muscle into the arteries at this level. The end of the muscle has been represented by the diameter at the peak of the partially muscular arteries in the population count. At this point there is still a small percentage of wholly muscular arteries and some non-muscular arteries. Most of the arteries less than this in diameter will have at least part of their wall with no muscle. This figure is then plotted for each age using the diameter of arteries with each type of airway as a landmark. For example at three days the peak of partially muscular arteries is at 112.5 μm diameter. This is at the distal end of the

Figure IV 14

Diagram illustrating the extension of muscle in the walls of arteries within the acinus. The end of the muscular region, shown by the black lines, represents the diameter at the peak of the partially muscular population and the position in the acinus that an artery of this diameter is likely to be found.


Alveoli
Alveolar ducts
Respiratory bronchioli
Terminal bronchiolus
28 wks.
36 wks.
38 wks.
40 wks.
FETUS
3 days
4 mths.
10 mths.
18 mths.
3 yrs.
4 yrs.
5 yrs.
10 yrs.
11 yrs.
CHILD
19 yrs.
ADULT

terminal bronchiolus, since the average diameter of arteries with terminal bronchioli was 118 μm. At three years the peak was also at 112.5 μm, though at this time these vessels were at alveolar duct level.

It can be seen from the figure that during fetal life there was no muscle within the acinus in the artery walls. This was due to the fact that the arteries were all of small diameter since the acinus was immature and small. During childhood the acinar arteries were still relatively non-muscular though they had increased in size as the acinus had grown, by lengthening of existing airways and multiplication of alveoli. The muscle in the walls of the arteries had not extended into the new vessels as soon as they had formed nor extended into non-muscular vessels as they had increased in size. The degree of muscle extension varied from case to case during childhood, but there was an extension to the periphery with age. The muscle was found in the most peripheral arteries in the case of ten years but even at this time muscular arteries were not found running with alveoli as they were in the adult, represented in this series by a 19 year old male.

During childhood the acinar arteries are relatively less muscular than in the adult.

VENTRICULAR WEIGHTS IN NORMAL FETUSES

Hearts from 31 fetuses of different ages, ranging from 12 to 40 weeks gestation, were dissected and weighed using the method of Fulton et al (1952). The total ventricular weight, right ventricular weight (RV) and left ventricular weight plus septal weight (LV + S) were all measured. The ratio of LV + S to RV

was estimated. The results are shown in Table IV 30.

The total ventricular weight increased with age, there being a greater increase in the older fetuses. The rate of increase was greater in the younger fetuses. There was a ten fold increase from 12 to 20 weeks while over the next eight weeks only a two to three fold increase and over the following eight weeks a three to four fold increase. The most rapid phase of growth was therefore from 12 to 20 weeks.

At all ages the left ventricle plus septum was heavier than the free right ventricle; they both increased at the same rate. Fulton and his colleagues stated that the normal adult ratio of LV+S to RV was 2.3 - 3.3. In none of these fetuses was this ratio attained and the average for the group was 1.454 (S.E. 0.048) with a range of 1.06 to 2.09. The right ventricle was proportionately greater in the fetus than it is in the adult. Davies (1969) found a ratio of 1.2 in a stillborn and showed that by ten months the adult ratio had been attained.

THE DEVELOPMENT OF MUSCLE CELLS

During the period of this study a lung from a 14 week old fetus was received in a condition fresh enough for electron microscopic study. This fetus was obtained from a termination of pregnancy carried out because the parents had already produced a child with cystic fibrosis.

During study of the lung Miss B. Meyrick identified cells which were of muscular type but primitive. Figure IV 15 shows one such cell (magnification x 20,000). This cell can be identified as a muscle cell since it has a basement membrane and includes

Table IV 30

Ventricular Weights in Normal Fetuses

Fetal age (weeks)	Total Ventricles (gms)	R.V. (gms)	L.V. + S (gms)	$\frac{LV + S}{RV}$
12	0.142	0.068	0.074	1.08
14	0.093	0.038	0.055	1.44
14	0.105	0.042	0.063	1.5
14	0.147	0.064	0.083	1.29
14	0.224	0.091	0.133	1.46
14	0.266	0.093	0.173	1.86
15	0.151	0.056	0.095	1.69
16	0.332	0.16	0.172	1.07
17	0.539	0.212	0.327	1.54
17	0.608	0.228	0.38	1.66
18	0.444	0.196	0.248	1.25
18	0.650	0.294	0.356	1.21
19	0.651	0.273	0.378	1.38
19	0.74	0.294	0.446	1.51
19	0.926	0.384	0.542	1.4
19	1.075	0.485	0.59	1.21
20	1.599	0.649	0.95	1.46
23	1.688	0.708	0.98	1.38
24	2.260	0.805	1.455	1.807
26	2.082	0.866	1.216	1.404
26	2.09	0.674	1.414	2.09
26	2.878	1.173	1.705	1.453
28	2.598	0.955	1.643	1.72
28	3.003	1.289	1.714	1.33
28	4.829	1.658	3.171	1.91
31	4.214	1.916	2.298	1.19
32	4.271	1.667	2.604	1.56
34	6.149	2.141	4.008	1.87
36	11.892	5.774	6.118	1.06
39	12.178	6.355	5.823	1.09
40	12.3	5.6	6.7	1.2

myofibrils. It also contains large amounts of glycogen and the cytoplasm has an electron density greater than the surrounding cells. It is possible that this cell was associated with a blood vessel.

Figure IV 16 shows the wall of a small blood vessel from this lung (magnification x 10,000). An endothelial cell lining the blood vessel can be seen. Outside this is a cell with cytoplasm of the same density as the developing muscle cell in Figure IV 15. There is some evidence of primitive myofibrils and a basement membrane to this cell. It is likely then that this is an even earlier stage in a developing muscle cell - this one being in the wall of a small blood vessel. This cell has some of the charadteristics of a smooth muscle cell but is not fully developed when compared with a muscle cell from a small vessel as siin in the normal adult lung. Such a cell (Figure IV 17 x 10,000) has the characteristic basement membrane and myofilaments.

The immature cell described here may be the cell type seen in the cellular arteries of the fetus at 12 and 14 weeks (see Figure IV 18). Such a cell would explain the lack of staining of muscle cells until late in fetal life both in these small vessels and in the larger ones near the hilum (Figure IV 19).

The cellular arteries described here in the young fetus at the periphery of the lung could be type I embryonal arteries as defined by Kaufman (1965) and reported at all ages of fetal life. In the youngest fetuses he described them at all levels of the lung, later only at the periphery since evidently they were precursors of the mature structure. At 12 weeks he described them only in the intralobular region. In the present study these arteries were

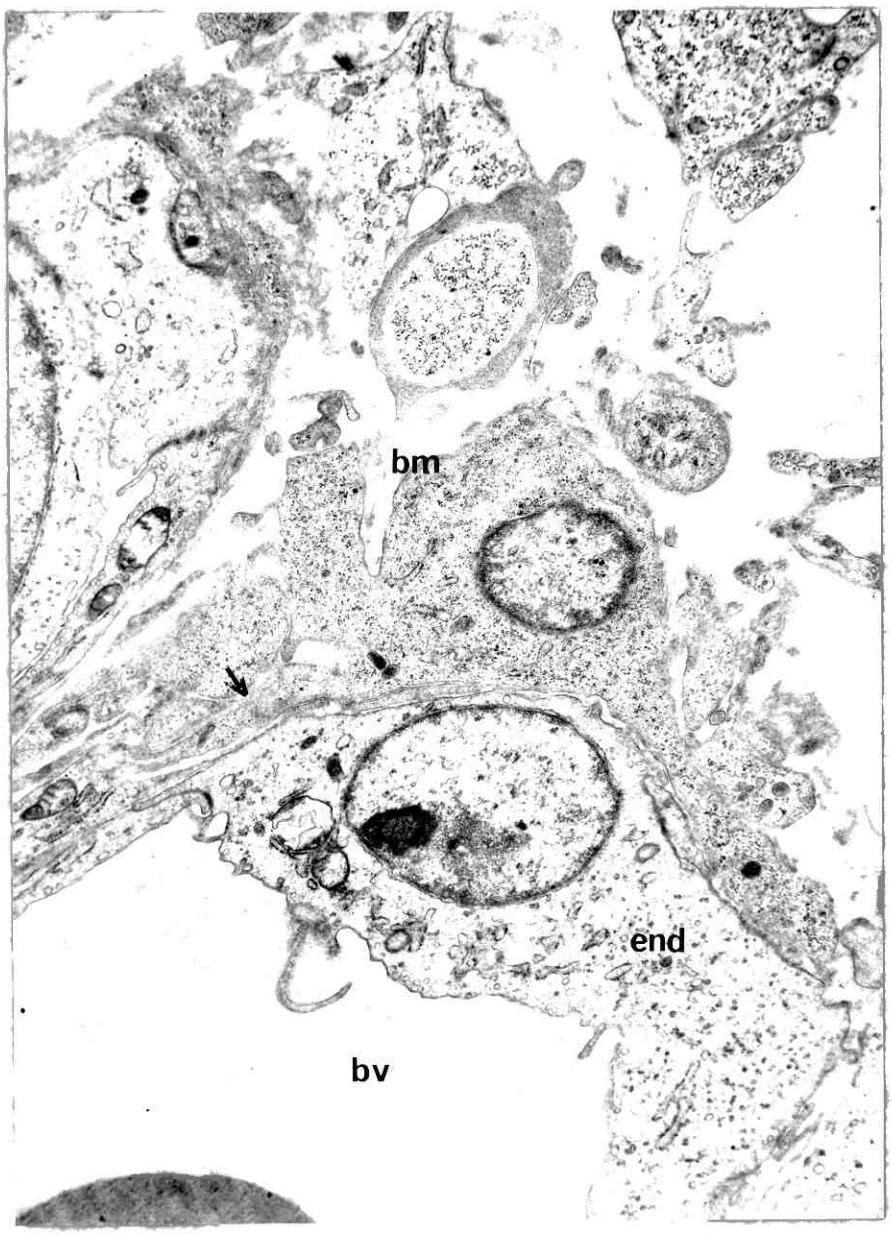
Figure IV 15

Electron micrograph from the lung of a fetus of 14 weeks gestation. This shows a primitive muscle cell identified by its basement membrane - bm and myofibrils - m. It also contains glycogen and the cytoplasm has a density greater than surrounding cells. (x 20,000)


bm
m

Figure IV 16

Electron micrograph from the lung of a fetus of 14 weeks gestation. This shows the wall of a small blood vessel. There is an endothelial cell - end lining a blood vessel - bv; outside this is a cell with denser cytoplasm, with basement membrane - bm and some myofibrils at arrow (x 10,000).


bm
end
bv

found only in lungs of fetuses aged 12, 14, 17 and 18 weeks. Vessels of this type may be present in older fetuses and be overlooked because they are so small. This is unlikely since in the present cases these vessels were usually quite large and were in the size range and position of the partially muscular arteries seen in older cases. These are easily identified in older fetuses and children and also all pathways in the fetuses could be traced to non-muscular vessels and capillaries without seeing these primitive vessels. If then as seems likely the cellular vessels seen in this study were not the embryonal arteries described by Kaufman, such cells were not identified.

An explanation of the unusual staining reported here in the larger vessels and the cellular vessels seen in the young cases is that the muscle cells throughout the arterial system are immature in the young fetuses and that the appearance of a spiral of muscle forming a crescent in cross section happens only when the muscle cell is mature; at this time the cellular vessels disappear. It seems that the transition from muscular to non-muscular structure is different in the younger and older cases and the cellular vessels are purely an embryonic type. It is possible that all the muscle cells in the younger lung are of the same primitive type but in the larger vessels they are overlooked because elastic laminae delineate the media and aid its identification as a muscular vessel. It is to be remembered that the larger vessels also did not take up the stain for muscle in these young fetuses.

Figure IV 17

Electron micrograph from the lung of a normal adult. This shows a small vessel - bv with endothelial cells - end and external to these mature muscle cells - mc. There is the characteristic basement membrane - bm and myofilaments - m. (x 10,000)

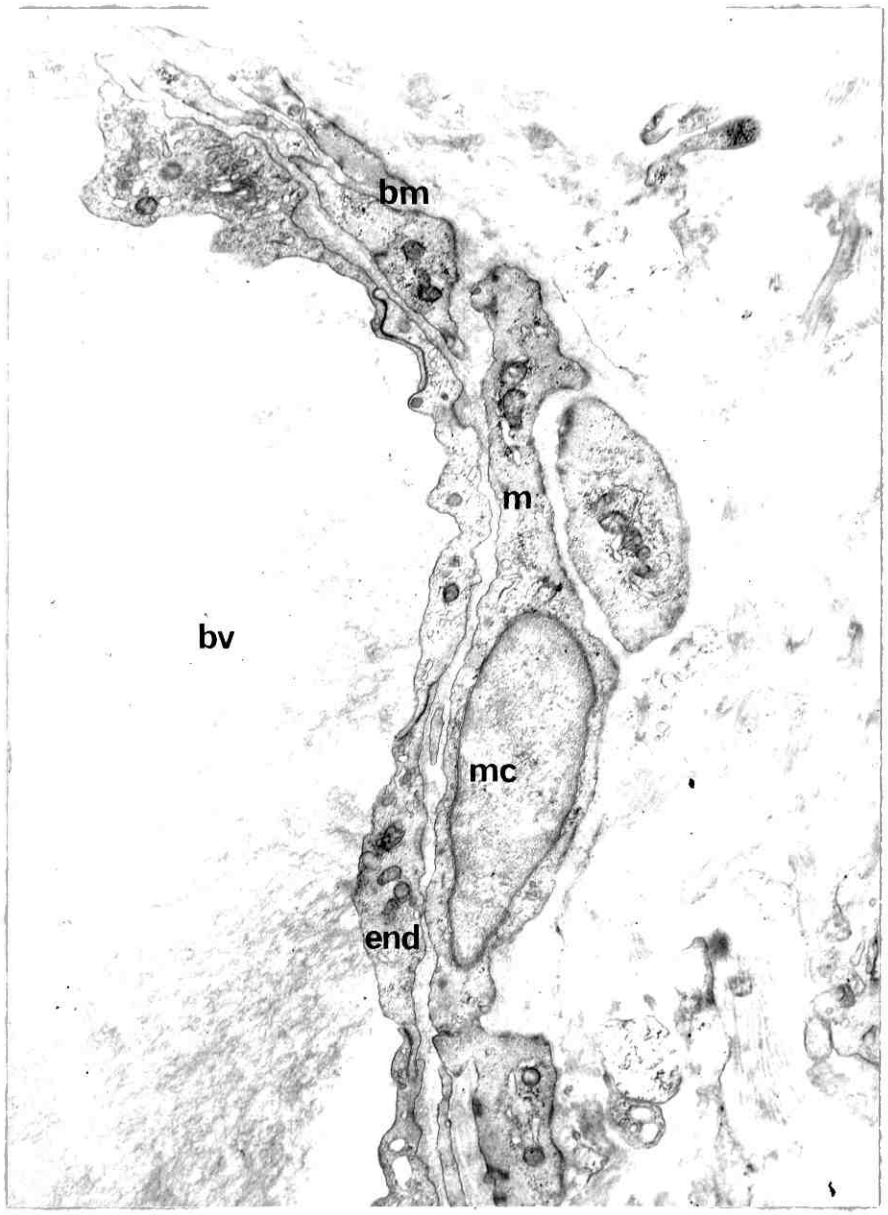

bm
m
bv
mc
end

Figure IV 18

Photomicrograph of a small cellular artery from a fetal lung of 12 weeks gestation. (x675)

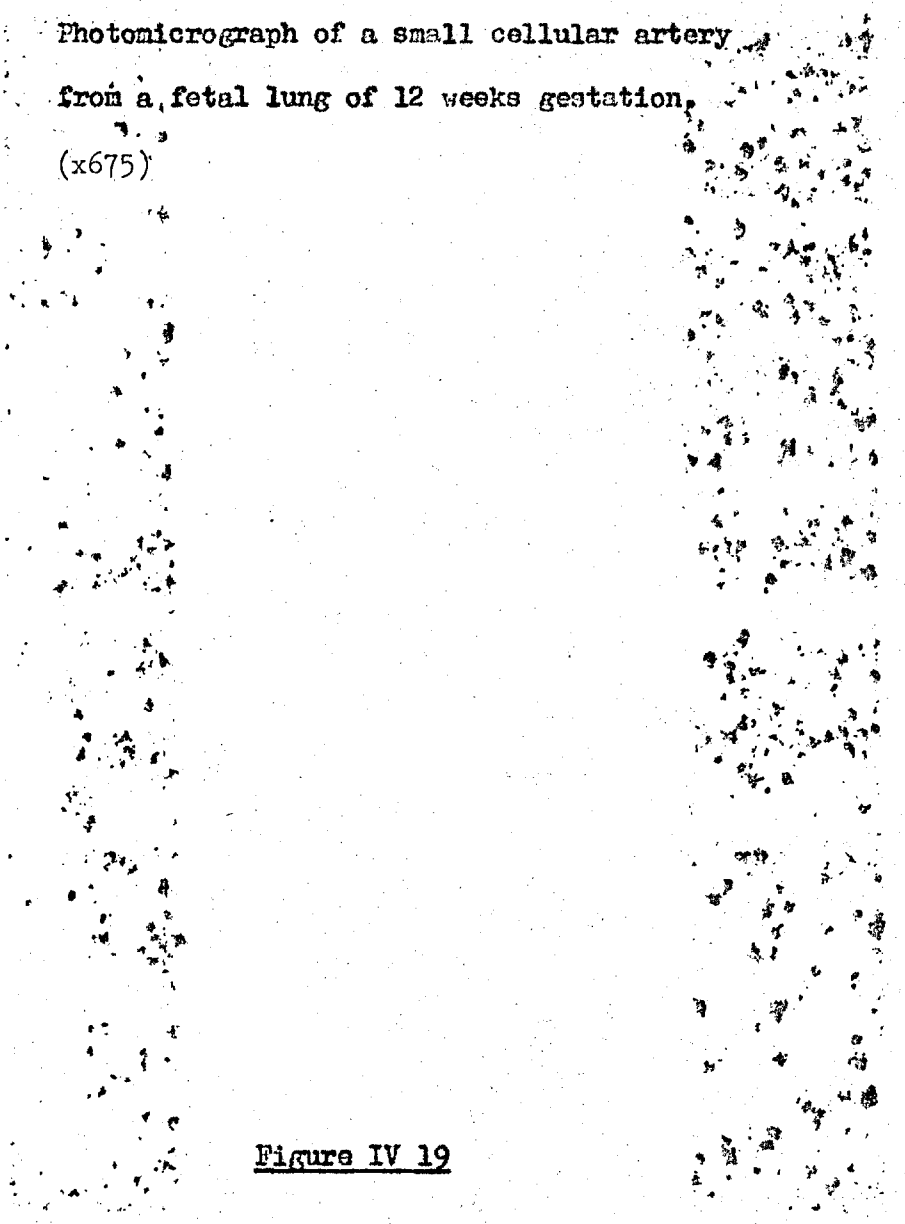
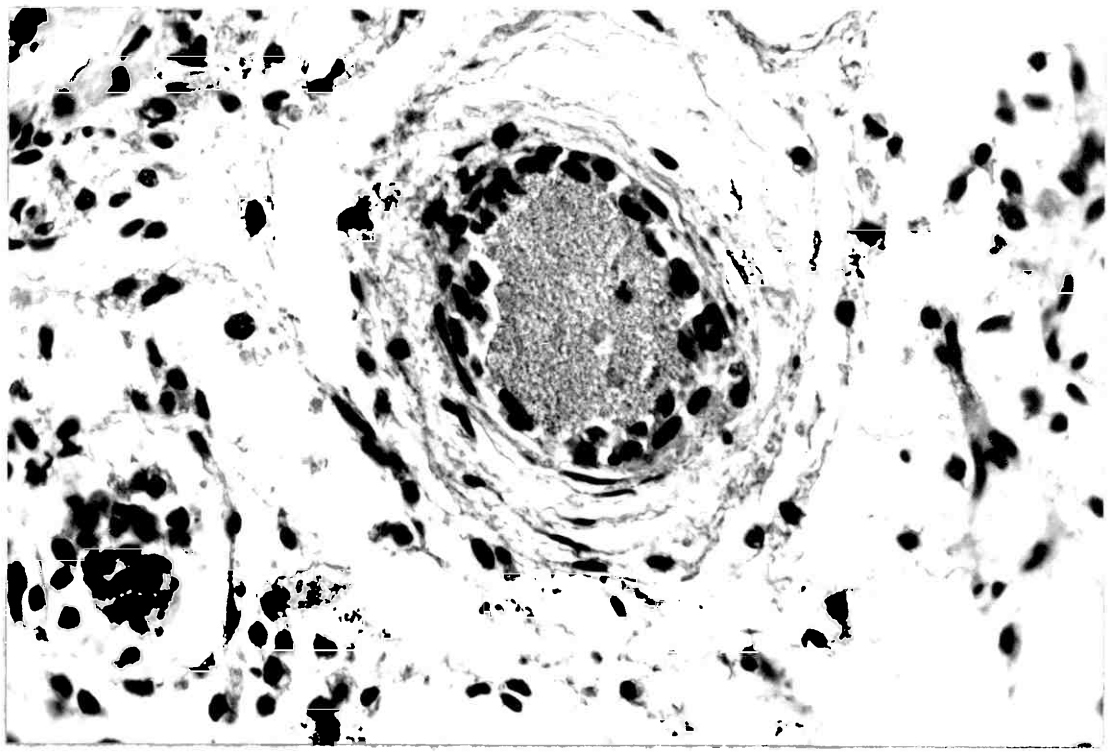
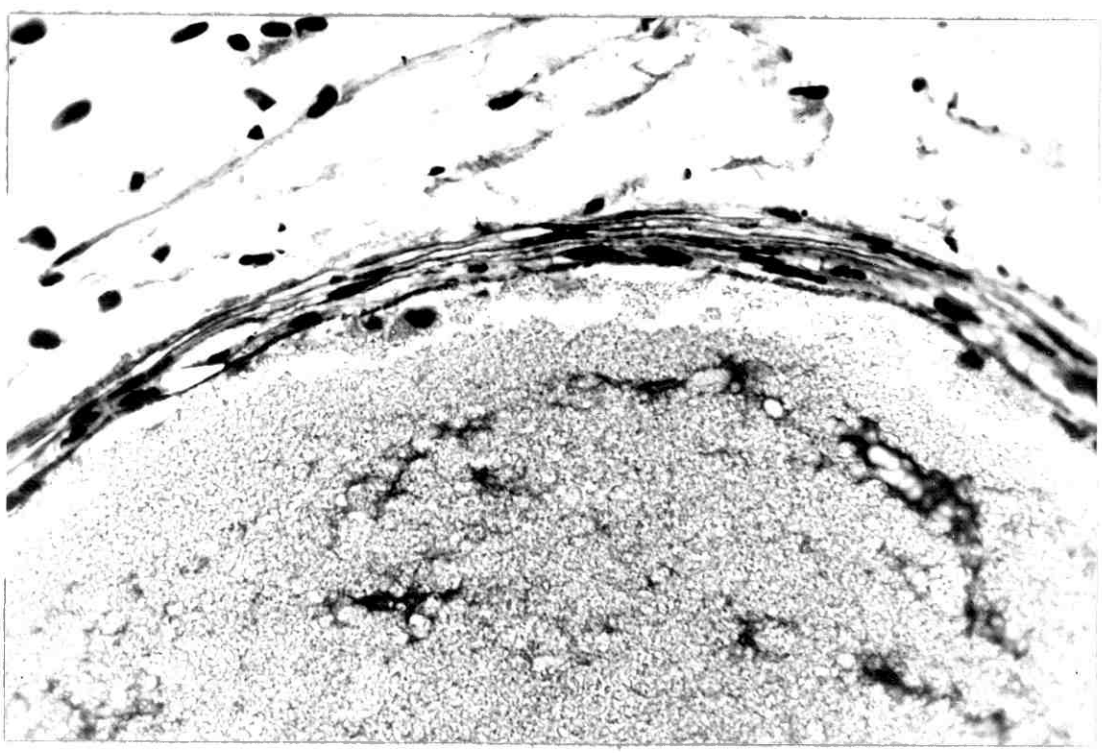


Figure IV 19

Photomicrograph of a muscular artery wall from a lung of a fetus at 12 weeks gestation. The elastic laminae are very fine and between them are cells which are unstained. (x675)





SUMMARY OF RESULTS

1. Having traced the axial pathway by serial reconstruction it has been possible to describe the structure of the whole length of the pre-acinar arterial region in fetuses aged 12 to 40 weeks of gestation and the intralobular arterial structure in a newborn lung and in lungs from children aged three days to 11 years.

Pre-Acinar - Axial Pathway

2. The appearance of elastic arteries changed during fetal life, with the elastic laminae becoming more distinct.

3. Up to 20 weeks gestation elastic arteries were found progressively closer to the periphery. At this age they were found to the same airway level as in the adult. They were smaller in diameter than those of the adult: with age there was an increase in size of the smallest elastic artery.

4. A transitional wall structure was identified only after 19 weeks gestation at which time it was in the position found in the adult. The diameter of arteries in which this structure was found increased during fetal life.

5. Most of the vessels in the fetal lung were muscular in structure. Their proximal extent as judged by size varied during fetal life depending upon the extent of the elastic structure. Their distal extent by size was the same throughout fetal life and the same as in the adult.

6. Partially muscular arteries were found between muscular and non-muscular arteries and by 38 weeks gestation were only found within the acinus: this is as in the adult. In younger fetuses they were more proximal. Along any pathway at any fetal age they were found in a similar size range. A similar length of pathway had a partially muscular structure at all ages on average between 0.5 and 1.0 mm.

7. With age the non-muscular wall occupied a reduced percentage of any given artery length. Throughout fetal life the non-muscular structure appeared at a similar diameter.

Intra-Acinar - Axial Pathway

8. Muscular arteries were not found within the acinus of a newborn. There was a gradual extension of muscle into the acinus with age. Muscle did not extend into such small arteries in the child as in the newborn.

9. A partially muscular structure was found distal to the muscular and was found more distally with age. This structure never covered a diameter range of more than 100 μm. It covered a similar length of pathway throughout childhood and in the fetus.

10. A non-muscular structure was found distal to the partially muscular. The percentage of the pathway occupied by this structure dropped with age. The extent of muscle into the acinus changed during childhood and in the first few years of life there was less muscle.

Conventional Arteries

11. In the pre-acinar region during fetal life and in the intra-acinar region during childhood the structure of the conventional artery branches was related to their external diameter. The diameter ranges altered with age in the same way as for the axial pathway. The conventional arteries were dependent for their structure on the axial artery. Thus diameter ranges for all five artery structures found in conventional arteries were the same as those for the axial artery at the same age.

Supernumerary Arteries

12. The largest supernumeraries during fetal life were muscular in structure. Within the acinus during childhood the largest seen was muscular in structure. Some were partially muscular and others non-muscular. These were independent of the structure of their axial pathway of origin. Their structure depended upon diameter, the size ranges of each type being similar throughout fetal life and changing during childhood as did the conventional arteries. The number of muscular supernumerary arteries increased during fetal life and childhood associated with the increase in size of these vessels.

General Population

13. The percentage wall thickness at 12 and 16 weeks gestation was higher than during the rest of fetal life. The average perdentage wall thickness during fetal life was higher in all diameter ranges than in the child and adult. The percentage wall thickness was the same in right and left lungs during fetal life. There was a drop in

percentage wall thickness and actual wall thickness after birth, at first in the small arteries and then in the larger ones. By ten months of age the wall thickness was the same as it is in the adult.

14. The medial area of an artery decreased with decreasing external diameter. In the small arteries of less than 250 μm external diameter the decrease was not so rapid. A similar medial area was seen throughout fetal life for a given sized artery.

15. In the small arteries there was a mixed population of three types of wall structure - muscular, partially muscular and non-muscular. Throughout fetal life all arteries over 200 μm in diameter were muscular. Below 200 μm arteries of each structure were found. The distribution curves of the artery types were similar throughout fetal life and were similar to those in the adult.

16. During childhood the same three types of artery wall were seen in the smallest arteries. However muscle was not found in such small arteries as in the fetus. In the child the number and size of arteries with at least part of their wall without muscle was larger than in the fetus or adult. Arteries of a greater diameter had a non-muscular wall in the child than the fetus or adult, therefore there were more non-muscular pre-capillary vessels.

17. Arteries within the acinus could be identified by the airway they accompany. The arteries accompanying terminal bronchioli were larger than those with respiratory bronchioli and these were larger than those with alveolar ducts. With age there was an increase in the diameter of arteries accompanying each type of airway. The most rapid increase was from 28 weeks gestation

(when these vessels could first be identified) to one year.

18. The vessels in the acinus at 28 weeks gestation were all non-muscular in structure. During later fetal life and childhood there was an extension of muscle into the acinus. During the whole of childhood the vessels of the acinus were less muscular than in the adult, where muscular arteries are found running with alveoli.

19. By weighing the ventricles of the hearts it was shown that during fetal life the total weight increased with age. The left ventricle plus septum were heavier than the right ventricle and both increased at the same rate. The right ventricle was proportionately greater in the fetus than in the adult and the child over ten months of age.

20. The walls of the arteries in early fetal life contained primitive muscle cells which could be identified with the electron microscope.

CHAPTER V

DEVELOPMENT OF THE PULMONARY VENOUS SYSTEM

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The pulmonary venous system was studied using similar methods to those used for the arterial system. After injection of the pulmonary veins from the left atrium the developing venous pattern was examined (i) on radiographs (ii) by microscopic examination of serial sections and (iii) by study of wall structure and thickness on random sections from throughout the lung.

Successful venous injection was made in the lungs of five fetuses, one at 20 weeks gestation, two at 28 weeks and one each at 34 and 39 weeks. In addition the veins of one lung for each of two children, aged three and ten years, were injected using the routine laboratory method. From these specimens the changing drainage pattern and structure of the vein wall were studied.

THE DRAINAGE PATTERN

The drainage pattern was studied both by measurements made on venograms taken after injection with radio-opaque medium and by serial reconstruction of an axial pathway in one segment of the lung.

Analysis of Venograms

Venograms were available for all the cases injected and measurements were made on the main pathways to each lobe as described in Chapter II.

Figure V 1 shows a venogram of the left lung of a fetus of 28 weeks gestation. There is an appearance of converging axial pathways of similar size which join together close to the hilum. They gradually increase in size towards the hilum. Tributaries

Figure V 1

Venogram of the left lung of a fetus of 28 weeks gestation. (X 1)

Figure V 2

Venogram of the left lung from a three year old normal child. (X 0.9)

Figure V 1

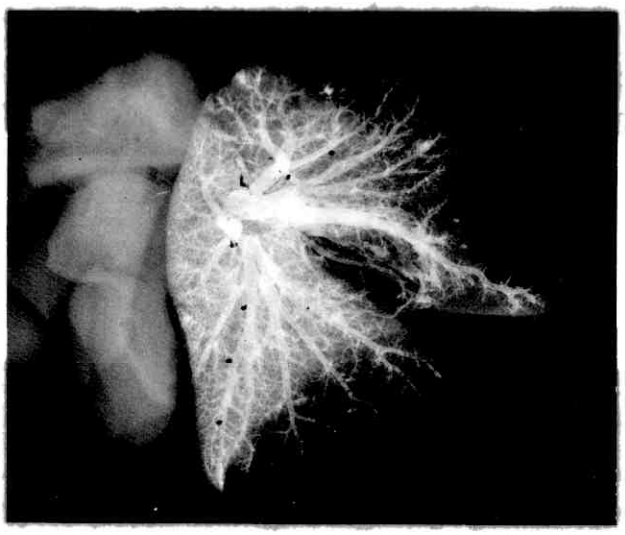
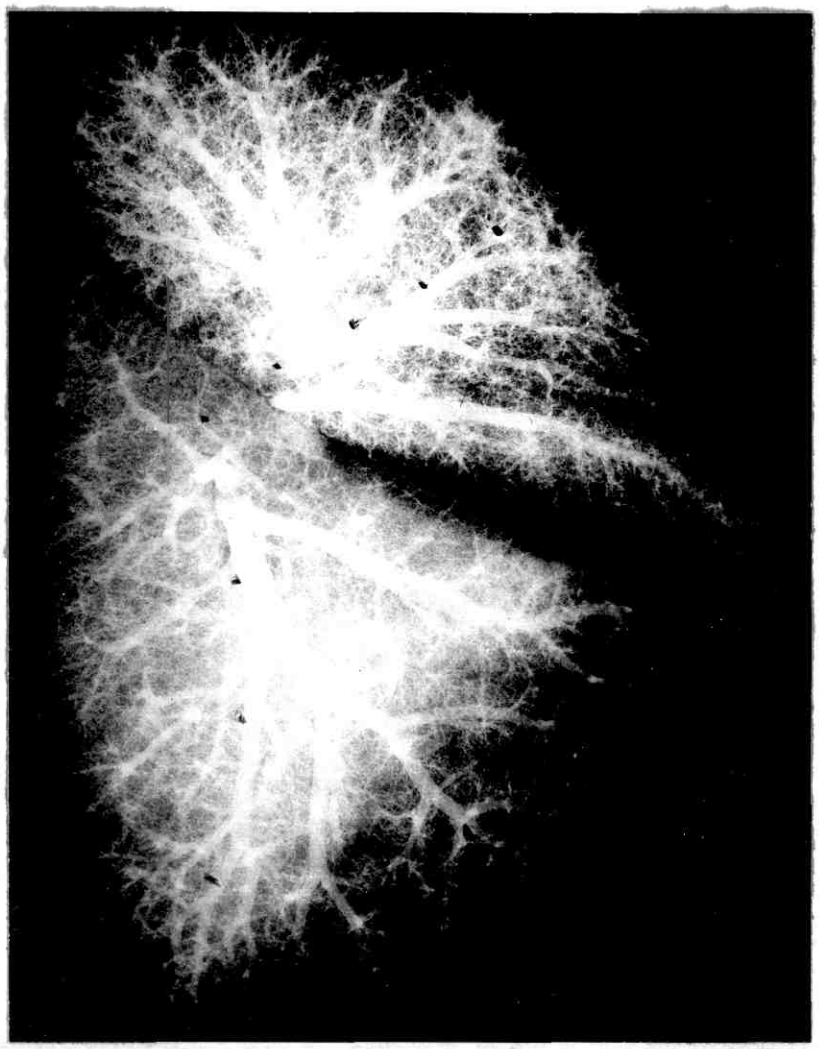


Figure V 2



join the main pathways at right angles in the mid zone of the lung and at the periphery they enter at acute angles. The tributaries to these vessels enter at right angles. There is a fine background haze of small vessels, more dense than the haze of arteriograms at this age.

Figure V 2. is a venogram of the left lung from a normal child of three years. Again the main veins converge at acute angles but small tributaries enter at right angles. The background haze is now very dense, denser than seen in most arteriograms. A similar picture of the venous drainage pattern was seen at ten years of age.

Measurements from Venograms

Measurements were made at intervals along the main pathway in each lobe. The results are shown in Table V 1 and are represented in Figure V 3. Because of filling difficulties in the small fetal lungs it was not always possible to measure both upper and lower lobes. Since in the child lung the veins of each lobe were cannulated separately at the hilum, it was not possible to measure the veins at this level.

In the cases where both upper and lower lobe veins were measured the upper lobe vein was shorter than the lower and had a smaller lumen diameter at all levels. There was an increase in the lumen diameter with age at all levels along the axial pathways both for the upper and the lower lobe. It is not possible to compare hilar diameters as had been done in arteriogram measurement and so the measurement made 25% of the distance from hilum to periphery has been used. At this point in the case of 20 weeks gestation the lumen diameter of the lower lobe was 0.7 mm, in the

Figure V 3

Diagram to illustrate the measurements made on venograms along the axial pathways of upper and lower lobes from 20 weeks gestation to ten years. Age in weeks gestation and years is shown in each square.

Lower Lobe - closed circles

Upper Lobe - open circles

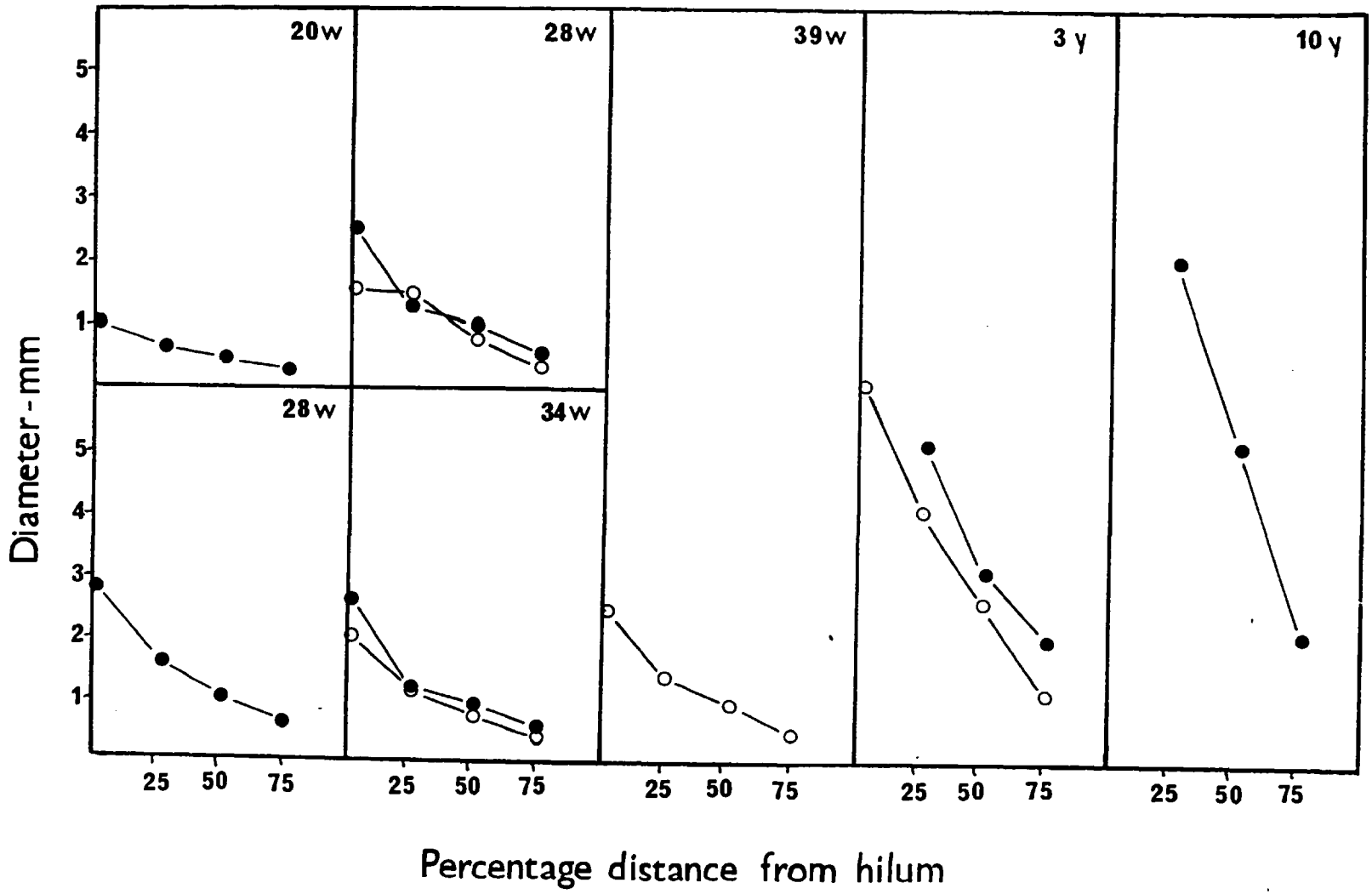

20w
28w
39w
3 y
10 y
28w
34w
Diameter - mm
1
2
3
4
5
25
50
75
Percentage distance from hilum

TABLE V 1

Measurements from Venograms

Age	Length (cm)	Distance (mm) at % distance from hilum				at 1 cm from pleura
		0	25	50	75	
UPPER LOBE VEIN						
20 wks	-	-	-	-	-	-
28 wks	2.4	1.6	1.5	0.9	0.4	0.8
28 wks	-	-	-	-	-	-
34 wks	3	2.0	1.1	0.7	0.4	0.7
39 wks	2.5	2.5	1.3	0.9	0.4	0.7
3 yrs	5	6	4	2.5	1.0	0.9
10 yrs	-	-	-	-	-	-
LOWER LOBE VEIN						
20 wks	2	1	0.7	0.5	0.3	0.6
28 wks	3	2.5	1.3	1.0	0.6	0.8
28 wks	4.3	2.5	1.5	1.0	0.5	0.9
34 wks	3.5	2.5	1.1	0.8	0.5	0.7
39 wks	4.5	-	-	-	-	0.7
3 yrs	9	-	5	3	1.8	0.9
10 yrs	10.2	-	8	5	2	1.0

case of ten years it was 8 mm, an 11 fold increase. The lumen diameter at the periphery increased over the same period from 0.3 mm to 2 mm, only a seven fold increase. The proximal zone would seem to grow relatively more than the periphery. The same applied to the upper lobe veins.

There was an increase in the length of the axial pathway with age. At 20 weeks gestation the lower lobe pathway was 2 cm and at ten years was 10.2 cm, a five fold increase. For the upper lobe the increase was from 2.4 cm in the case of 28 weeks gestation to 5 cm at three years.

There is a linear relationship between the diameter at the 25% point of the lower lobe vein and the total volume of the lung. The length of the pathway increases as the diameter at the hilum increases, in a similar manner to the arteries.

The diameter of veins one centimetre from the periphery of the lung was variable and ranged between 0.6 and 0.9 mm during fetal life and between 0.9 and 1.0 mm in childhood. These are similar diameters to those seen for arteries. It would seem that, at any age, 1 cm from the periphery the artery and vein are similar in size although they increase with age. Diameter of a given vessel would seem to relate to the volume of lung it drains.

Microscopic Reconstruction of the Venous System

The pattern of drainage of the venous system was reconstructed by microscopic examination of serial sections. Four cases were studied in this way. The veins from the periphery to the hilum were studied in the lungs from two fetuses of 20 and 28 weeks gestation. A complete reconstruction of a main pathway from each was made. The

pattern of the proximal veins of the three and ten year old children could be seen on the venograms and showed the same overall pattern as those from fetal life. A block approximately one centimetre thick was taken from the base of the lower lobe in each case and a serial reconstruction made of the axial pathway through the block. From these reconstructions it has been possible to describe the general pattern of venous drainage and the changes with age.

General Considerations

The drainage pattern of the venous system cannot be conveniently described by reference to the airways or in the same terms. The arteries are found closely associated with the airways and usually within the same connective tissue sheath, their pattern of branching is intimately related to that of the airway system. Arteries can always be landmarked and identified by the airway they accompany. The veins, on the other hand, run independently of bronchus and artery and within their own connective tissue sheath. The artery and airway run centrally in an acinus while the veins run at its periphery.

The venous system is closely related to the connective tissue framework of the lung. Arteries are never found within the pleura or connective tissue septa of the lung while veins invariably are. It is not possible to describe the veins without defining the limits of connective tissue septa and sheaths.

The pleura, made up of collagen, is in direct continuity with the connective tissue septa. These sheets of collagen penetrate into the lung for varying distances and in cross-section form boxes around the acini. They do not penetrate to the hilum of the lung

and the boxes are open on one side and allow collateral drift of air (Van Allen and Jung, 1931). Once the periphery has been left the connective tissue is found only as sheaths surrounding arteries, veins and airways. These sheaths are thickest at the hilum where the structures are largest.

Microscopically a septum can be identified and defined as a connective tissue sheet in direct continuity with the pleura and running independently of any vessel. A vein may be present within a septum. A connective tissue sheath refers to the layer of connective tissue enclosing a vein. A vein is always surrounded by such a structure which varies in thickness depending upon vein size. In longitudinal section of such a vein this sheath may be continuous with the pleura, but the sheath is only present because of the vein within it and over no part runs without a vein as do the septa; they are therefore not to be confused.

Reid and Rubino (1959) showed that the pattern of septa in the adult was present by 18 - 20 weeks gestation. In this case at 20 weeks gestation all the collagen sheaths seem connected with the pleura so that throughout the lung there was a box like appearance. In the case of the 28 week fetus septa were only found in the peripheral two millimetres. In this eight weeks the collagen of the sheaths had become more widely separated and the appearance of a box like subdivision of the lung had been lost; the true septa were found only at the periphery. At three and ten years of age the septa were still present, but now up to one centimetre from the pleura, at least proximal to the lobules at the periphery. The increase from 28 weeks gestation to this time is probably due to growth of the lung, in the adult the septa extend 1 - 2 cm into the lung (Reid, 1967b).

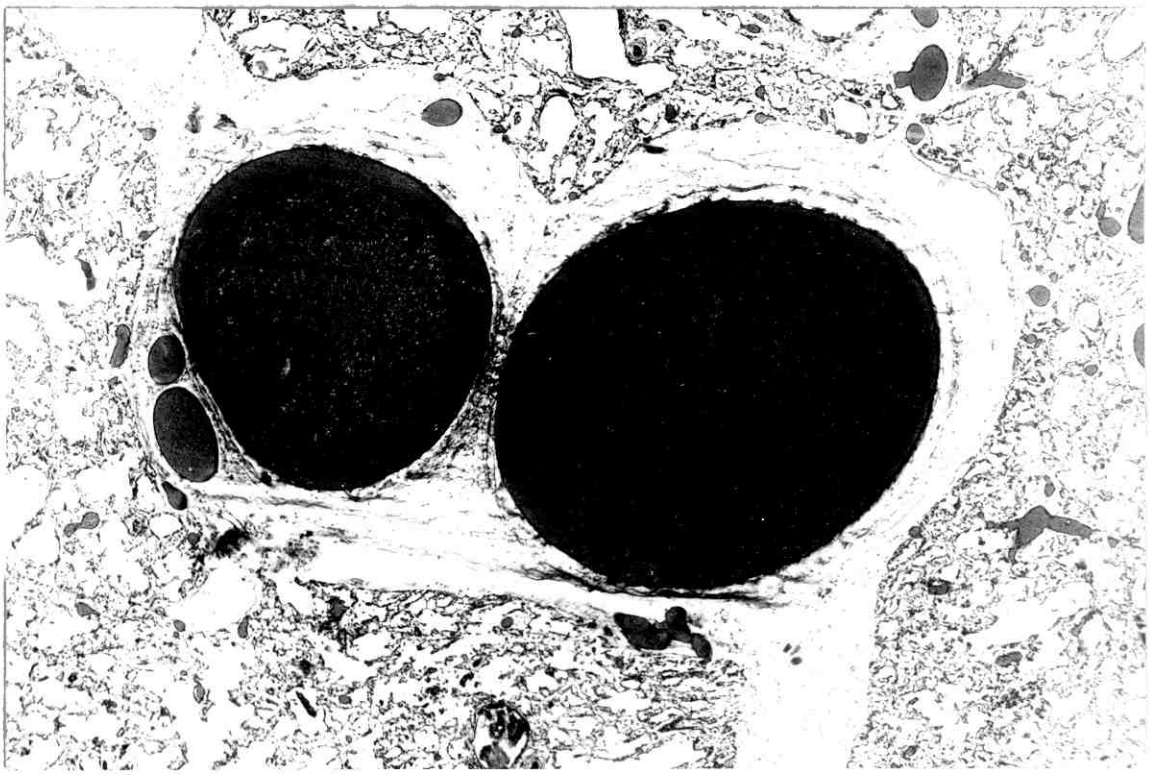
The main veins run from the periphery of the lung to the hilum, receiving tributaries along their whole length. There is an increase in size from the periphery as more blood is collected. The side tributaries to a main pathway increase in size towards the hilum as they themselves are draining a larger volume of lung. The veins do not each collect from a similar unit and therefore are all different in size. There is cumulative collection of blood to correspond with the distribution by the arterial system. The connective tissue sheath around each vein increases in size as the vein enlarges, and near the hilum may link with the sheaths of arteries and airways. At the periphery the veins appear isolated from any other structure except of course where they run in septa.

Arteries can be identified by the airway they accompany. Veins can only be related to the septa and to their distance either from the hilum or from the periphery.

Types of Vein

Four types of vein were recognised during this study. An axial vein was considered to be a main pathway running from the periphery to the hilum by a roughly straight course. This vessel was larger than surrounding vessels. Draining into the axial pathways were conventional veins (Figure V 4). These veins were found, like the axial vessels, within a large connective tissue sheath and were nearly as large as the axial vessel into which they are drained. They entered the axial vein at an acute angle and it was possible to trace them in the same plane as the axial pathway for some distance through the lung. These veins were draining a

Figure V 4



Photomicrograph of a conventional vein running close beside an axial vein before joining with it in a 28 week old fetal lung. The veins are filled with an injection medium and each is surrounded by a connective tissue sheath. (x50)

large volume of tissue and had received many side vessels. When they joined the main vessel they increased its size.

In addition to these conventional veins there were two other types of tributary to the main pathway. These were both smaller than the conventional veins and could be termed supernumerary veins. These vessels entered the main vein at right angles and did not increase the size of this vessel. They varied in size depending upon the distance they had travelled from the capillary bed. These supernumeraries could be divided into two types. The first type - Type I - entered directly from the lung tissue surrounding the axial vein and may have received one or two tributaries but these were never larger than post-capillary in size. These vessels did not have a connective tissue sheath though there may have been some collagen in the wall. They passed straight through the axial vein sheath and into the vein at right angles (Figure V 5).

The second type - Type II - was larger than the first in external diameter. They had travelled a greater distance from their origin in the capillary bed and had been supplied by numerous smaller veins often entering directly from the capillary bed. By the time these vessels reached the axial pathway they were large enough to have a thin connective sheath which was continuous with the sheath of the axial vessel which they were entering. These vessels entered the main stem at right angles and their sheath was much thinner than the main one. They could therefore be distinguished from the conventional veins. The Type II veins showed a rapid increase in size from origin to junction with main vein (Figure V 6). These veins may enter the axial vein immediately or run within its

Figure V 5

Photomicrograph of a Type I supernumerary vein entering an axial vein in the lung of a 28 week old fetus. It is entering the vein at a right angle directly from the surrounding tissue. (x145)

Figure V 6

Photomicrograph of a Type II supernumerary vein entering an axial vein in a lung of a 28 week old fetus. This vein has its own connective tissue sheath and is receiving blood from small tributaries. It is entering the main vein at a right angle. (x145)

Figure V 5



Figure V 6



connective tissue sheath for a short distance before entering it. At this time they may receive veins from the surrounding alveolar tissue or from the connective tissue.

Both types of supernumerary vein were found anywhere between the periphery and the hilum. The Type I was always below a certain diameter determined by the age of the child while the Type II varied in size, the largest being closest to the hilum. The more proximal Type II vessels were larger than the peripheral conventional veins hence it was the pattern not the size which was important in distinguishing them. The different types could be identified by their origin and method of opening into the main veins.

These three types of side branches could all be identified in the cases of three and ten years of age and at 28 weeks gestation but not at 20 weeks gestation, where only conventional and supernumerary could be separated. Type I and II could not be distinguished.

Peripheral Origins of Veins

In the lung of the three and ten year old children it was possible to define several types of origin for a vein. Along one pathway it was not possible to see each type of origin, but in each lung, of any age these origins could be found.

Origin of Veins at Ten Years

The axial pathway traced in the ten year old showed examples of several types of origin.

1) Terminal Bronchiolar Wall The main pathway traced in this ten year lung originated in the wall of a terminal bronchiolus as a venule of 37 μm diameter. This received two small tributaries, of 37 and 62 μm diameter, from the bronchiolar wall and two, of 25 and 37 μm, from the alveoli. They all entered the main vein at right angles. This vein, now with a diameter of 95 μm, joined with a conventional vein of 75 μm draining a respiratory bronchiolus, a branch of the terminal bronchiolus. This vein had in its turn received small post-capillary vessels from the wall of the respiratory bronchiolus. The main pathway then received two small post-capillary vessels of 35 and 25 μm diameter direct from alveolar walls and then another, of 37 μm, from the wall of the bronchiolus.

The next tributary was a Type II supernumerary vein of 100 μm diameter arising between two alveolar ducts and receiving at right angles several small tributaries from alveolar walls. This was followed by another conventional vein (150 μm) draining another respiratory bronchiolus. The axial vein was now 180 μm in external diameter.

The next four tributaries drained, in turn, the bronchiolar wall; two arose from between alveolar ducts and one from an alveolus. The vein draining the bronchiolus was the largest, followed, in size, by those from the alveolar ducts. The tributary from the alveolus was only 30 μm being immediately post-capillary. The main vein was still at this time running within the connective tissue of the terminal bronchiolus and was 240 μm in diameter. At this point it was joined by two conventional veins running within their own connective sheath and draining other areas of the acinus. The vein was still central in the acinus, but now running in its own sheath which was connected with that of the bronchiolus and artery. This

larger vein then received another branch from the bronchiolar wall, one from between alveolar ducts and one direct from the alveolar region though larger than post-capillary. This axial vein eventually joined with another running in a connective tissue septum connected with the pleura. At this level this vein was at the edge of the acinus. Even at this point it received a small vein directly from the bronchiolar wall, which had passed through the alveolar region in a thin connective tissue sheath. After this the main vein continued in its own sheath towards the hilum receiving veins of increasing size and of the three types of tributary vein.

2) Pleura One of the lateral tributaries entering the main pathway nearer the hilum was also traced from its origin. It originated in the pleura at the apex of the lung and received blood from the pleura and adjacent alveoli by tributaries all of post-capillary size. This vessel remained within the pleura and was joined when at a diameter of 200 μm by a conventional vein from a septum. This vessel had received blood from the alveolar ducts, alveoli and the pleura on the other side of the lung - at this point the lung was wedge shaped and only 0.85 mm across. Other tributaries arose in the alveolar region, septa, pleura and between alveolar ducts. It was possible to see that the last type of vein began in connective tissue at the junction of the two alveolar ducts. The main vein remained in the pleura for seven millimetres before joining the pathway described above. Over this length it only increased in size 260 μm and received only two large tributaries.

In summary, at ten years it was possible to identify several sites of origin of venous tributaries - 1) pleura 2) connective tissue septa 3) junction of alveolar ducts 4) alveolar walls and 5) walls of bronchioli.

It seems that quite large tributaries of veins join at divisions of bronchioli, for example where respiratory bronchioli leave terminal bronchioli. Veins at more proximal regions will also join at a division of the bronchiolus. At these branching points the veins seem to be closer to the bronchioli, certainly when the branches are within the lobule. The tributaries entering at this level are conventional veins. Nearer the hilum there are large veins leaving the walls of bronchioli and running to the main veins; again this only occurs close to a bifurcation of an airway. These vessels enter the main axial vein as Type II supernumerary veins. This type of tributary was seen even at the level of the small bronchi where there was some distance for the vein to travel before joining the axial pathway in which length it became quite large.

Origins of Veins at Three Years

At three years of age the veins arose at similar sites to those of the previous case. The main pathway traced arose on the pleura at the diaphragmatic surface and ran into the lung via a connective tissue sheath between acini. This vessel received small tributaries from the walls of the alveoli and from pleura and septa.

Origins of Veins at 28 weeks Gestation

At 28 weeks gestation the acinar region of the lung is not yet differentiated. In these lungs the small respiratory bronchioli open into saccules lined by thin epithelium overlying capillaries. There are no true alveoli and the future respiratory region is described here as pulmonary 'parenchyma'. It was possible to trace

veins from three separate sites in this case. The first from the pulmonary parenchyma, the second from the connective tissue, septa and pleura and the third from the walls of airways.

The veins draining the parenchyma either followed a short course receiving only a few tributaries - Type I supernumerary veins, or they followed a longer course joining with other similar veins before entering the main vein - Type II supernumerary veins. Unlike the pattern in the older children, it was not possible to differentiate veins draining different structures within the alveolar region. As in the older cases the veins draining the airways entered the main vein only via larger tributaries that drained the alveolar region as well as the airway. The short direct tributary seen in the ten year old was not seen in this case.

Venules draining the pleura and septa followed either a direct or indirect course to the main vein depending on their distance from it. At the periphery there were small post-capillary vessels entering the axial stem direct from the pleura.

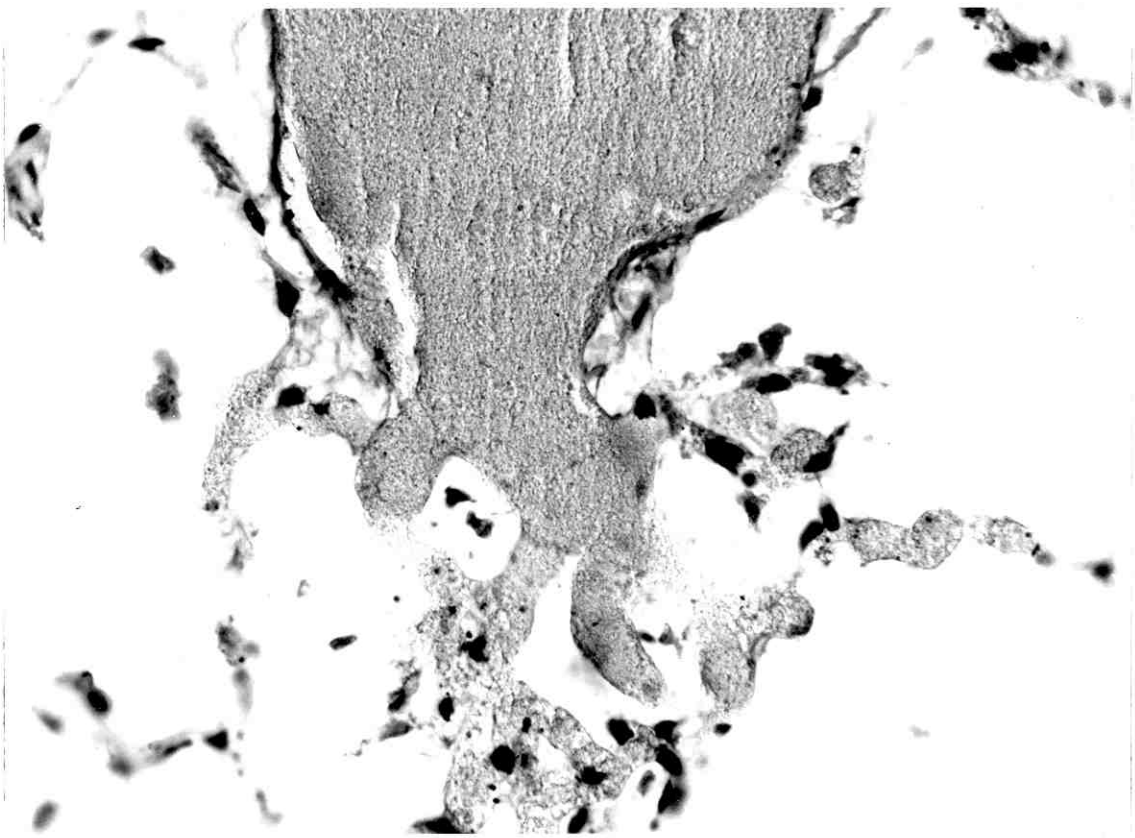
It was particularly noticeable at this age that very small post-capillary veins entered directly into large veins (Figure V 7).

Origins of Veins at 20 weeks Gestation

This was the youngest case in which it was possible to inject the veins successfully. By this age the full complement of pre-acinar arteries and airways is present, however the acinar region is still undifferentiated mesenchyme here referred to as lung 'parenchyma'.

The three main pathways traced in this case all originated in lung parenchyma at the periphery of the lung and immediately ran laterally into the pleura and then back into the lung via a septum.

Figure V 7



Photomicrograph of small post-capillary veins entering directly into large veins. (x600)

Post-capillary vessels arose from the parenchyma, pleura and airway walls as in the case of 28 weeks gestation.

Figure V 8 shows a photomicrograph of the peripheral origin of one of the veins traced and Figure V 9 is a representative diagram of this. There are small vessels draining the pleura and parenchyma and entering the main pathway. There is also a vessel within the septum draining from the connective tissue and the lung parenchyma which joins the main vessel. This in its turn joins a conventional vein (A) which has already received vessels from the periphery of the lung.

The Changing Venous Pattern with Age

The pathways traced in two of the cases studied are shown in Figures V 10 and 11. At 20 and 28 weeks gestation the pathway was traced from periphery to hilum while in the three and ten year old lungs only the peripheral centimetre was traced.

Length of Axial Pathway

In the case of 20 weeks gestation three pathways from within the left lower lobe were traced. These all drained into the same hilar vein. Pathway 1 was 7.3 mm in length, pathway 2 was a tributary to this vessel of 9.31 mm length to the hilum having joined the main pathway 0.5 mm from the hilum, this being the longest pathway in the lung. Pathway 3 in its turn was a tributary to pathway 2 and was 3.43 mm in length. From its origin to the hilum it was 9.03 mm.

In the case of 28 weeks gestation the main pathway draining the posterior basal segment was 18.2 mm long, a marked increase

Figure V 8

Photomicrograph of the peripheral origin of one of the pulmonary veins traced in the fetus aged 20 weeks. Small filled vessels drain the pleura, septa and parenchyma and run in the connective tissue surrounding each lung unit. (x 50)

Figure V 9

Representative diagram of the photomicrograph in Figure V 8. Small vessels drain the pleura, septa and parenchyma and enter an axial vessel which runs in a connective tissue septa. This joins a conventional vein (A) which has already received vessels from the periphery of the lung.

Figure V 8

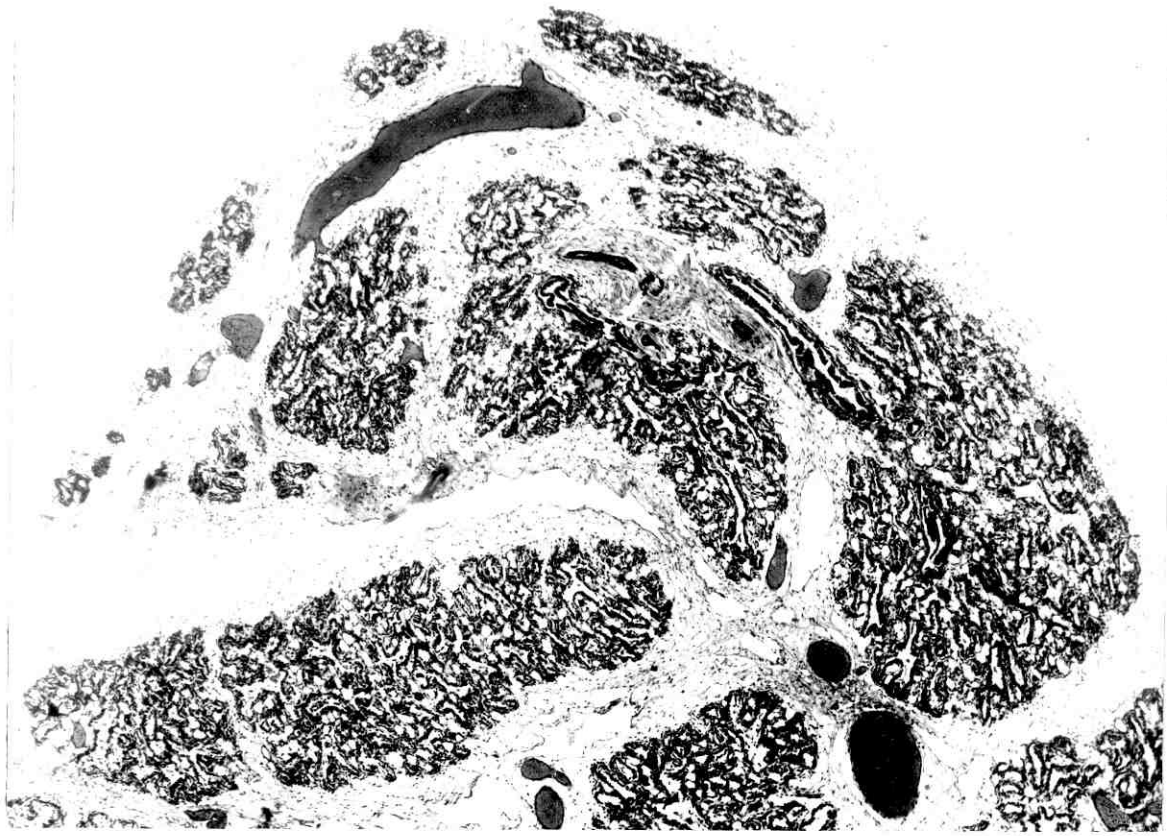
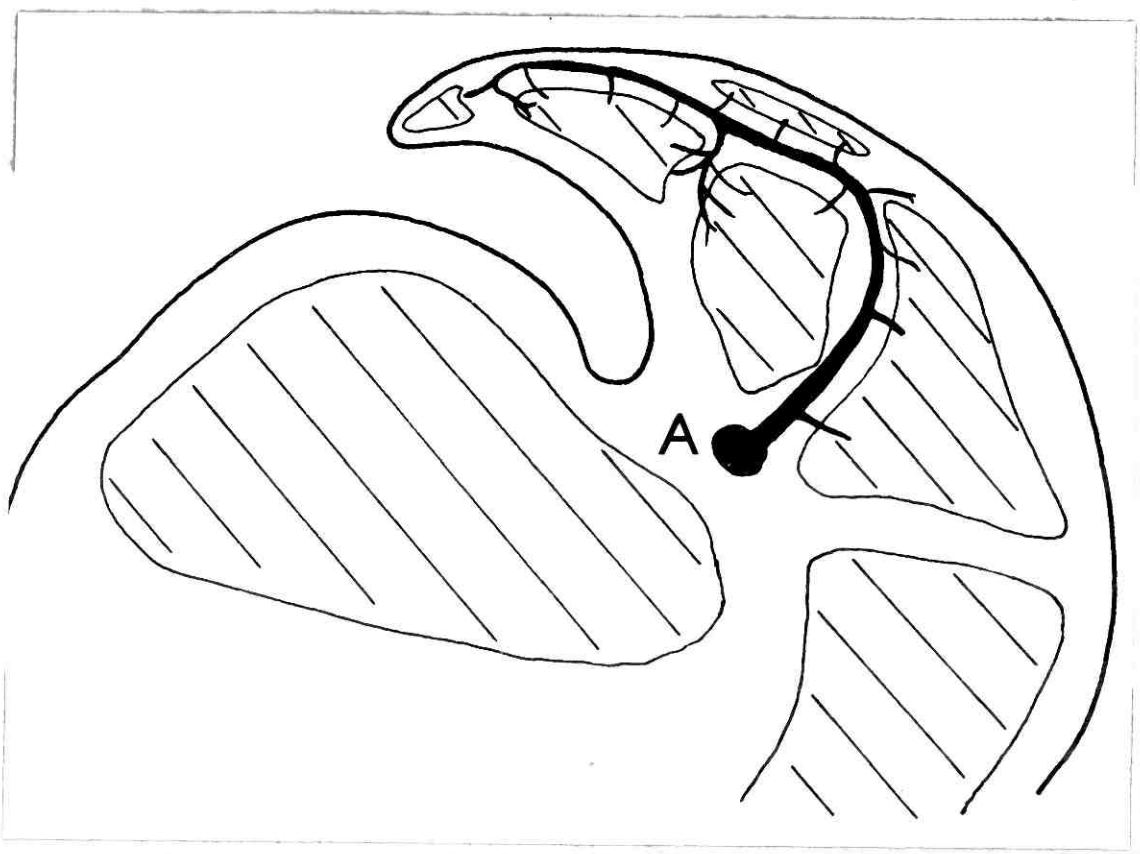


Figure V 9



over that in the case of 20 weeks. In both cases the length was reduced in comparison with the measurements on the venograms. This was due to shrinkage during processing.

At three years the main pathway to the posterior basal segment was 9 cm, as measured on the venogram. In the case of ten years the pathway to the right lower lobe was 10.2 cm. In these cases only a peripheral block was reconstructed microscopically. At three years a pathway was traced for 11.07 mm from the periphery and at ten years 16.1 mm.

Number of Conventional Veins

At 20 weeks gestation, along the longest pathway (2), there were 21 conventional veins. The other two pathways had a total from periphery to hilum of 15 along pathway 1 and 20 along pathway 3.

At 28 weeks the case studied had 25 conventional veins. This increase may be due to multiplication, indicating that by 20 weeks growth of the veins was not complete, or it may be that there was considerable variation in the total number of tributaries along an axial pathway, as there was in the arterial system. The latter seems more likely since both artery and airway multiplication in the pre-acinar region is complete by 16 weeks gestation, and a lag in the venous drainage pattern would seem unreasonable. There was a similar number of conventional veins in both cases to pre-acinar airways, the latter range in this segment up to 25 with an average of 22 (Bucher and Reid, 1961).

In the three and ten year old lung only the periphery was traced and this cannot be directly compared with the fetal pathways. In the 11.07 mm traced in the three year old lung there were 11

conventional veins, and in the 16.1 mm length of the ten year old, 16, a similar arrangement. In these two cases most of these veins were in the intra-acinar region and cannot be compared to the 25 generations of the 28 week fetus. Between three and ten years there was not only an increase in size of the lung but an increase in the number of alveoli and arteries which will lead in turn to an increase in the number of venous branches within the acinar region. It can be postulated that there will be no further increase after ten years since alveolar multiplication is finished. The adult pattern will be the same though the vessels will be larger and further apart. The vessels were the same distance apart in the three and ten year old because multiplication was still proceeding.

The conventional vessels at three and ten years were further apart than those at the periphery of the 28 week fetus (Tables V 3 - 6). Those at the hilum in the two older cases are likely to be even further apart, due to increase in length along the whole pathway without multiplication of vessels. The vessels at three and ten years were much the same distance apart. This is because the total length of the pathway measured on the venogram had only increased from 9 to 10.2 cm so that there will have been little increase between branches particularly at the periphery. It is likely that between three and ten years there had been an increase in the number of vessels within the acinus as the alveoli had multiplied so maintaining the same distribution of vessels.

Considering only the peripheral 11 mm of the pathways (the longest length available for all four) at 20 weeks gestation there were 21 conventional veins, at 28 weeks 19, at three years 11 and at 10 years 12. The vessels at the periphery are found further apart with increasing age. Considering only the first 11 generations from the periphery, they cover 3.0 mm at 20 weeks gestation, 2.8 mm

at 28 weeks gestation, 11 mm at three years and 9.1 mm at 10 years. This shows again that the branches were further apart in the older cases.

Number of Supernumerary Veins

The total number of supernumerary veins along the main pathway (2) at 20 weeks gestation was 79 giving a ratio of supernumerary to conventional veins of 3.76:1. In the case of 28 weeks gestation there were 91 supernumerary veins, a ratio of 3.64:1. At three years there were 42 supernumeraries over the pathway reconstructed giving a ratio of 3.81:1 and at 10 years there were 69, a ratio of 4.31:1.

The ratio of supernumerary to conventional veins was similar at all ages though there was some variation from case to case. There is also variation between pathways of the same lung. At 20 weeks gestation the other two pathways studied had ratios of 3.8:1 and 2.5:1. In all four cases the supernumerary vessels were more frequent at the periphery of the lung than at the hilar region. This is illustrated in Figures V10 and Table V 2. At all levels the supernumerary veins were more frequent than the conventional veins.

In the lung of the 20 week fetus it was not possible to separate type I and type II supernumeraries since they were all very small. In the case of 28 weeks, of the 91 supernumerary vessels, 64 were of type I a percentage of 70.3. These were more frequent at the periphery and the type II vessels relatively more frequent at the hilum. In the case of three years there were out of 42 supernumeraries 24 type I, the percentage being 57.1. In the case of ten years the percentage of type I vessels was 60.8

TABLE V 2

Distribution of Supernumerary Veins

Generation	Age			
Periphery	20 wks	28 wks	3 yr	10 yr
0	3	1	6	4
1	2	0	2	4
2	6	0	3	4
3	0	0	4	0
4	0	2	1	7
5	2	5	3	3
6	4	1	7	7
7	10	9	6	8
8	2	3	5	0
9	15	11	1	8
10	0	10	1	3
11	10	7	3	2
Total to 11th generation	54	49	42	50
12	1	0		9
13	3	9		1
14	2	0		0
15	8	2		10
16	0	0		
17	4	3		
18	0	5		
19	0	15		
20		3		
21		2		
22		3		
23		0		
24		0		
Hilum				

Distribution of Vessels

In all four cases conventional tributaries joined the axial pathway at an increasing distance from each other as the hilum was approached. Figures V 10 and 11 which show the branching pattern at 20 weeks and three years demonstrate this and Tables V 3 - 6 give the distances. In the two fetal cases when the pathway was traced completely as far as the hilum, it can be seen that there was not a steady increase in the distance between branches as there was along the arterial pathways. At the periphery the conventional veins joined close together as they drained from the acini and then lobules. Towards the hilum they were further apart; this can be detected on the venogram as the region where the veins join at right angles. Close to the hilum the vessels joined closely together again; this is at the level of the radiating pattern on the venograms and is virtually at segmental level, each one of the veins draining one segment.

The venogram of the three year old (Figure V 2) did not have a radiating pattern near the hilum and it may be that serial reconstruction would reveal a pattern similar to that seen in the arteries with the conventional veins being progressively further apart at the hilum.

As has been shown in Figures 10 and 11 the supernumerary veins are certainly more frequent at the periphery though there is a variable number between each conventional vein with no recognisable pattern (Table V 2). The main increase towards the periphery is because of the general change in the frequency of tributaries.

It is only possible to see the changes with age by comparing the first, that is the peripheral, 11 generations of the axial vein. This region was the same regardless of age and despite the fact that

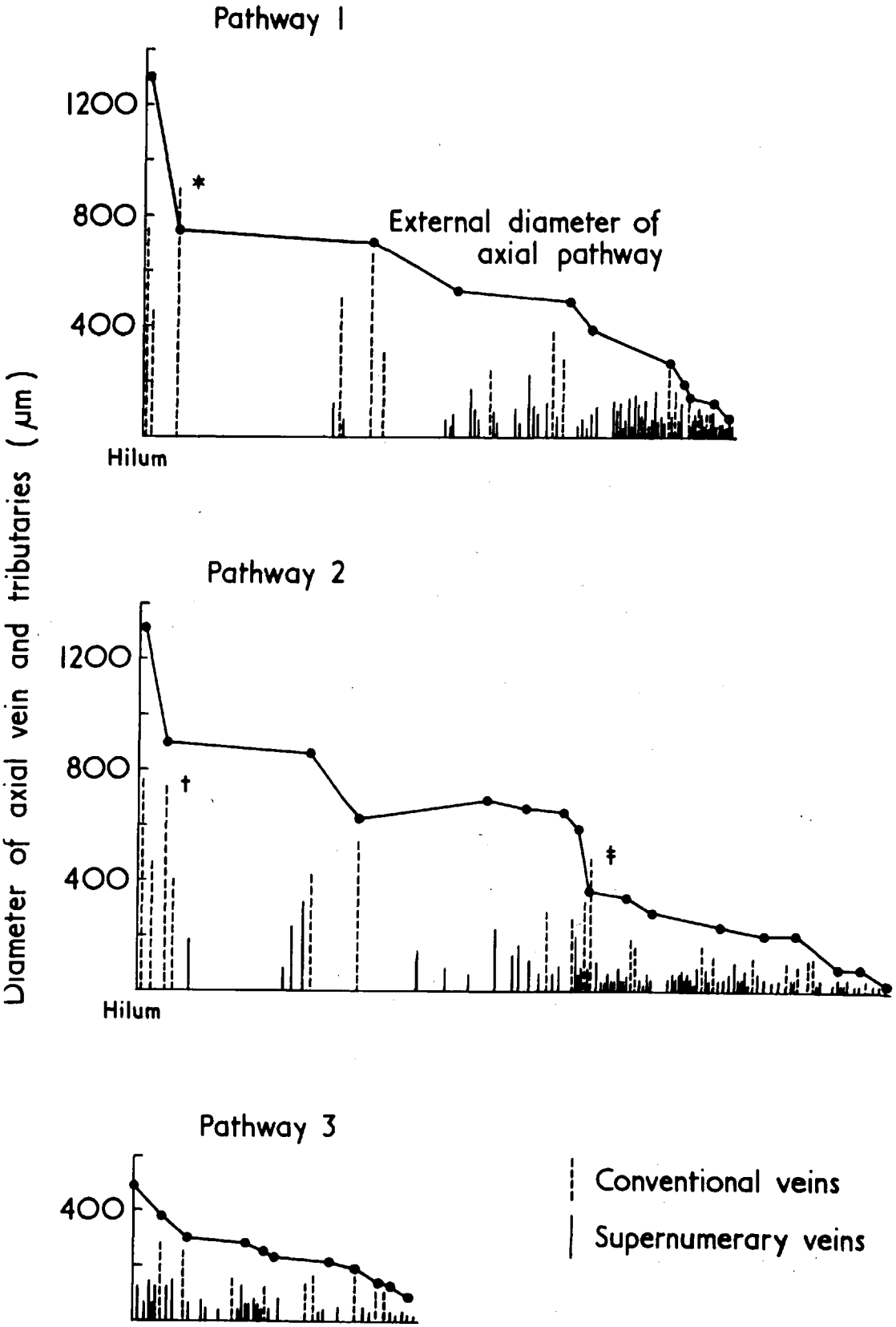
Figure V 20

Diagram representing the drainage pattern and diameter of axial veins and their tributaries both conventional and supernumerary, in the 20 week fetus. Pathway 2 is 9.31 mm in length.

* Point where pathway 2 joins pathway 1

\+ Point where pathway 1 joins pathway 2

∓ Point where pathway 3 joins pathway 2


Pathway 1
1200
800
400
*
External diameter of
axial pathway
Hilum
Diameter of axial vein and tributaries (μm)
Pathway 2
1200
800
400
†
‡
Hilum
Pathway 3
400
Conventional veins
Supernumerary veins

a different part of the lung was involved. In the child this is mainly the acinar region but in the two fetuses the pre-acinar. There was a similar number of supernumerary vessels associated with the conventional veins, between 42 and 53, leading to a ratio of between 3.81 and 4.81. The only significant change with age was one of total length; these veins covered a longer length in the older cases and were therefore further apart and also larger.

The pattern of vessels is like the arteries in that the relative numbers remain the same, the changes being in dimension and distance between tributaries.

Diameter of the Axial Pathway

The diameter of the axial pathway in all four cases is shown in Tables V 3 - 6. Figures V 10 and 11 represent the diameter of the axial pathways in the 20 week fetus and in the three year old, together with the tributary diameters. In all cases there was an increase in the diameter of the axial pathway towards the hilum. This was a continuous rise at a similar rate at all ages but to a larger diameter in the older cases. In the 20 week fetus the diameter at the hilum was 1300 μm, this was reached at a point 9.45 mm from the origin of the vein at the periphery of the lung, there having been 21 generations. At 28 weeks the same diameter was reached in 14 mm and 21 generations. At three years it took 11 mm and 11 generations and at ten years 12 mm and 13 generations. The lateral pathways also showed a similar rate of increase. In all the three pathways traced at 20 weeks gestation the diameter of 800 μm was reached by 7 mm from the periphery.

It seems then that the axial vein diameter depends on the distance from the capillary bed and not upon the number of

Figure V 11

Diagram representing the drainage pattern at the periphery of the lung of a three year old. The diameter of the axial pathway and the conventional and both types of supernumerary tributaries is shown. The actual length of this pathway is 11.07 mm.

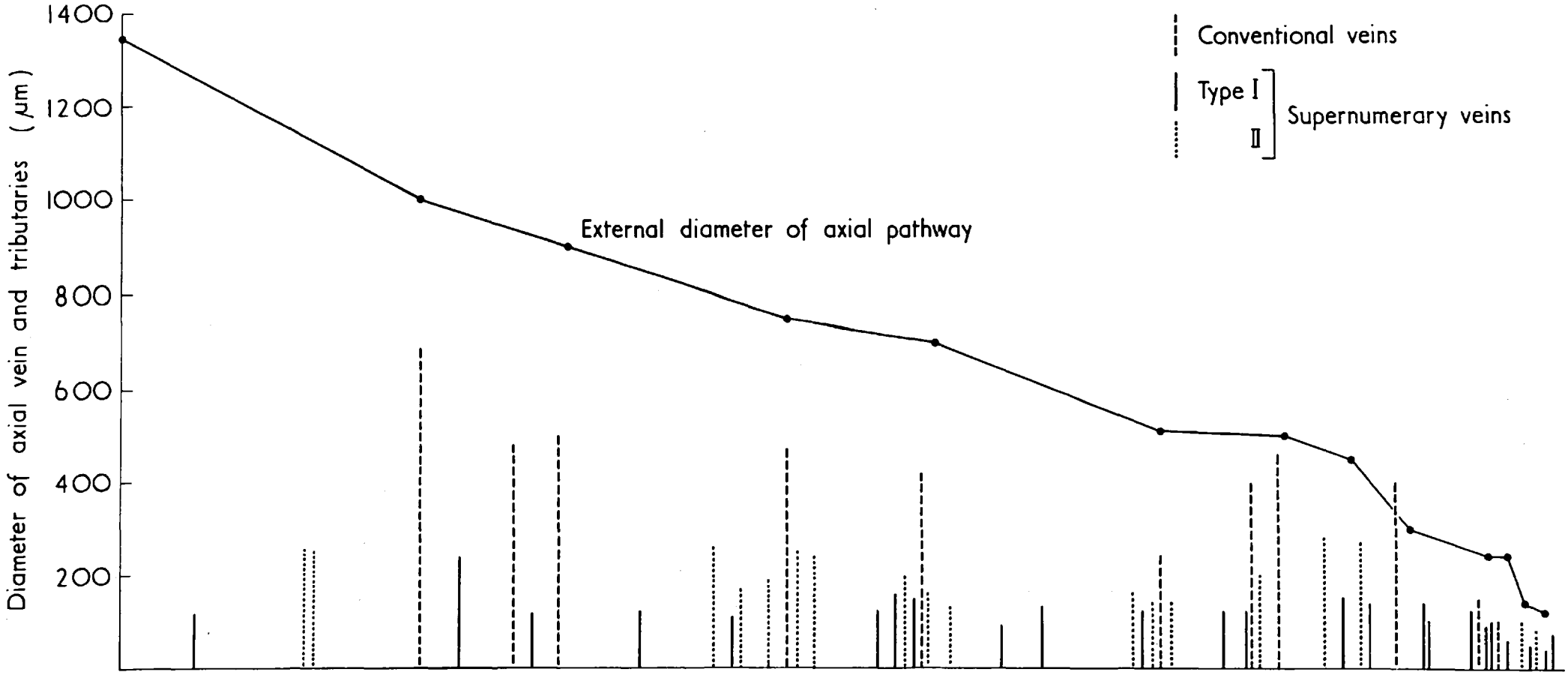

1400
1200
1000
800
600
400
200
Diameter of axial vein and tributaries (μm)
External diameter of axial pathway
Conventional veins
Type I
II
Supernumerary veins

TABLE V 3

Diameter of Axial Vein and Conventional Tributaries and their Arrangement along Pathway 2 of the 20-week Fetus

Generation	Diameter of Conventional Vein (μm)	Diameter of Axial Vein (μm)	Distance from Hilum (mm)	Distance from last Tributary (mm)
1	45	55	9.31	
2	55	75	9.03	0.35
3	135	75	8.68	0.63
4	100	-	8.61	0.07
5	125	200	8.61	0.00
6	100	200	8.47	0.14
7	135	230	8.19	0.28
8	137	250	7.70	0.49
9	170	300	7.35	0.35
10	187	340	6.42	0.89
11	165	340	6.44	0.02
12	480	360	5.74	0.70
13	320	600	5.60	0.14
14	260	650	5.11	0.49
15	290	670	4.76	0.35
16	530	700	2.70	1.96
17	420	850	2.21	0.49
18	400	930	0.56	1.75
19	750	1300	0.49	0.07
20	450	1300	0.14	0.35
21	760	-	0.00	0.14
Mean	286.38			0.48
S.D.	213.2			0.52
S.E.	46.55			0.115

tributaries. This is comparable with measurements made on the venograms and also resembles observations made on the arteries.

Diameter of Conventional Veins

The conventional veins are usually smaller than the axial pathway which they join. The tributaries increase in size towards the hilum. The diameters are illustrated in Figures V 10 and 11 and Tables V 3 - 6. The first conventional vein on these tables is the one closest to the origin of the vein.

The average diameter of the conventional veins increases with age. In the lungs of 20 and 28 weeks gestation the whole pathway to the hilum is considered while at three and ten years only the periphery. Comparisons can therefore only be made on the first, that is the most peripheral, 11 conventional tributaries. At 20 weeks the average diameter is 161.72 µm, at 28 weeks it is 187.72 µm, at three years 393.6 µm and at ten years 385 µm.

Diameter of Supernumerary Veins

The supernumerary veins are smaller on average than the conventional veins though there are some at the hilum larger than peripheral conventional veins. The Type II veins are larger than the Type I. The size of the supernumerary veins varies widely along the pathway but generally speaking smaller vessels are more likely to be found at the periphery. The diameters of the supernumerary veins for all four cases are shown in Table V 7. For the 20 week fetus they have been shown as one group, for the other three cases Type I and II vessels have been listed separately.

TABLE V 4

Diameter of Axial Vein and Conventional Tributaries and their Arrangement in a 28-week Fetus

Generation	Diameter of Conventional Vein (μm)	Diameter of Axial Vein (μm)	Distance from Hilum (mm)	Distance from Last Tributary (mm)
1	160	120	18.06	
2	190	140	17.92	0.14
3	100	160	17.85	0.07
4	140	170	17.71	0.14
5	145	200	17.64	0.07
6	175	260	17.19	0.35
7	125	280	17.05	0.14
8	200	320	16.63	0.42
9	190	320	16.56	0.07
10	380	380	15.93	0.63
11	260	450	15.23	0.70
12	220	520	14.34	0.91
13	200	520	14.27	0.07
14	450	600	13.15	1.12
15	300	620	13.01	0.14
16	440	660	12.52	0.49
17	260	660	12.45	0.07
18	350	740	11.47	0.98
19	460	800	10.49	0.98
20	840	1000	7.63	2.86
21	940	1100	5.53	1.96
22	880	1400	3.57	1.96
23	1250	1200	2.31	1.26
24	640	1800	1.26	1.05
25	470	2400	0.21	1.05
Mean	390.6			0.73
S.D.	300.4			0.73
S.E.	60.07			0.14

TABLE V 5

Diameter of Axial Vein and Conventional Tributaries and their Arrangement at the Periphery of the 3-year old Lung

Generation	Diameter of Conventional Vein (μm)	Diameter of Axial Vein (μm)	Distance from Origin (mm)	Distance from last Tributary (mm)
1	100	240	0.42	
2	150	260	0.56	0.14
3	400	340	1.19	0.63
4	470	500	1.89	0.70
5	400	500	2.1	0.21
6	240	510	2.8	0.7
7	420	700	4.62	1.82
8	470	750	5.67	1.05
9	500	900	7.42	1.75
10	480	900	7.77	0.35
11	700	1000	8.47	0.7
Mean	393.6			0.8
S.D.	171.5			0.58
S.E.	51.8			0.175

TABLE V 6

Diameter of Axial Vein and Conventional Tributaries and their Arrangement at the Periphery of the 10-year old Lung

Generation	Diameter of Conventional Vein (μm)	Diameter of Axial Vein (μm)	Distance from Origin (mm)	Distance from Last Tributary (mm)
1	75	100	0.35	
2	150	150	0.84	0.49
3	180	240	1.82	0.98
4	190	250	1.89	0.07
5	340	310	2.38	0.49
6	460	550	3.08	0.70
7	620	580	5.11	2.03
8	520	1000	6.51	1.4
9	650	1000	6.58	0.07
10	550	960	7.77	1.19
11	500	-	8.89	1.12
12	460	-	9.38	0.49
13	1200	1200	11.90	2.52
14	640	1600	12.46	0.56
15	1000	-	12.53	0.07
16	800	1560	15.89	3.36
Mean	520.9			1.03
S.D.	306.9			0.95
S.E.	76.7			0.25

It can be seen (Figures V 10 and 11) that the more proximal Type II vessels can be larger than the peripheral conventional veins. It is the pattern of branching that is important, not merely size of vessels, when identifying vessel types. There are also some Type I veins which are larger than some Type II veins.

The average size of both types of supernumerary vein increases with age. The average for the case of 20 weeks gestation was only 63.2 μm, this was for both types of vessels. In the 28 week fetus the smaller Type I vessels had a diameter of 68.7 μm, an increase over the value at 20 weeks even in the smallest veins. The small average at 20 weeks was because there were more smaller veins than larger and that the smallest veins were significantly smaller. The range at 20 weeks was 10 - 310 μm and for the Type I supernumerary veins at 28 weeks was 30 - 150 μm. At 28 weeks the supernumerary Type II veins were larger on average than the Type I, the average being 160.5 μm. With a range of 90 - 410 μm there was an overlap in the size of the two types.

Since only the peripheral few generations in the three and ten year lungs were traced their average cannot be compared directly. Both showed that the Type II vessels were larger on average than the Type I. The Type I supernumerary veins had a similar average at three and ten years suggesting that these small veins with their limited drainage do not increase in size. However the range has increased to include larger vessels in the ten year old suggesting that the average is the same because of an increase in the number of small vessels. The total ratio of supernumerary to conventional veins had also increased suggesting that this is so. The average diameter of the Type II veins increased with age, due to growth

without increase in number.

If only the peripheral 11 generations or conventional tributaries are considered it is possible to compare the diameter ranges between the fetal ages and the children. These figures are also shown in Table V 7. There is a rise in diameter of the Type I vessels from 28 weeks gestation to three years but between the cases of three and ten years there is little change in diameter. There is however a continuous rise in the average diameter of the Type II vessels with age.

STRUCTURE OF THE VEIN WALL

The structure of the vein wall was studied using the random sections taken in each case from throughout the lungs.

At 20 weeks gestation there appeared to be no muscle in the walls of even the largest veins in the lung. Their wall consisted of a layer of endothelial cells and external to this collagen fibres (Figure V 12). There were occasionally, small elastic fibres within the collagen but these never formed a continuous sheet or lamina as in the pulmonary arteries at this age. Small veins under 50 μm in diameter had no collagen in their wall which was made up solely of an endothelium. In the case of 28 weeks gestation the same structure was noted. It was felt that overdistension due to the high pressure injection may have obscured the muscle cells in the veins of these young fetuses. Therefore veins in three lungs of two cases aged 28 and 34 weeks of fetal life were injected respectively at pressures of 50, 60 and 80 cm of water. At these pressures there was not complete filling and the vessels were not fully distended.

TABLE V 7

Size of Supernumerary Veins in the Four Cases Studied

Age	20 weeks	28 weeks		3 years		10 years	
Type	I & II	I	II	I	II	I	II
Generation number	21	25		11		16	
Total number Supernumeraries	79	64	25	24	18	42	27
Mean Diameter (μm)	63.2	68.7	160.5	113.19	189.5	120	273.8
Range (μm)	10-310	30-150	90-410	40-175	90-280	25-240	95-700
S.D.	53	27.1	70.6	35	59.5	66.9	134.8
S.E.	6.03	3.4	14.1	7.2	14.03	10.3	26
Generation Number	11	11		11		11	
Number of Supernumeraries	53	42	7	24	18	35	15
Mean Diameter (μm)	42.4	57.5	115.7	113.19	189.5	113.1	242
Range (μm)	10-92	30-115	90-140	40-175	90-280	25-230	95-470
S.D.	21.7	20.1	27.6	35	59.5	67.3	130.6
S.E.	2.97	3.1	10.5	7.3	14.03	11.4	33.7

In both these cases it was possible to discern in the undistended larger vessels an occasional bundle of muscle cells in the wall. However these did not form a continuous layer and there were no elastic laminae. It was not possible to make measurements of this muscle. Figure V 13 shows such a wall in an undistended vessel from the 28 week fetus.

In the lung of the 39 week fetus with the veins injected at the usual pressure of 100 cm water it was possible to discern a continuous layer of muscle between the endothelium and collagen but there was no internal or external elastic laminae. Rather than having a defined outer edge muscle was found between collagen fibres. Wall thickness was measured from the epithelium to the last muscle fibre (see Figure V 14).

In the case of three years the wall structure was better developed. The post-capillary vessels or venules, had an endothelial wall continuous with that of the capillaries. These gave way to larger vessels with an internal elastic lamina and an occasional muscle fibre; these can be termed partially muscular veins. Larger veins had a continuous muscle layer which increased in thickness as the vein increased in external diameter. There was no definite external elastic lamina and elastic and collagen fibres were found alternating with muscle cells. The wall thickness was therefore measured from the internal elastic lamina to the most external muscle cell. The adventitial layer was made up of collagen and elastic with no muscle cells.

In the lung of the ten year old child the structure was much the same as that seen in the three year old, though elastic laminae

Figure V 12

Photomicrograph of the vein wall in a vessel of 800 um external diameter in the lung of a fetus aged 20 weeks. The wall is made up of a layer of endothelial cells and collagen. (x 675)

Figure V 13

Photomicrograph of a vein wall in an undistended vessel from a case of 28 weeks' gestation. An occasional muscle cell can be seen (yellow). External to this is collagen (pink).
Stain - Elastic van Gieson (x 800).

Figure V 14

Photomicrograph of a vein wall from the lung of a fetus aged 39 weeks. A layer of muscle cells (yellow) has on its outside collagen. Some elastic fibres but no definite laminae can be seen.
Stain - Elastic van Gieson (x 800).

Figure V 12

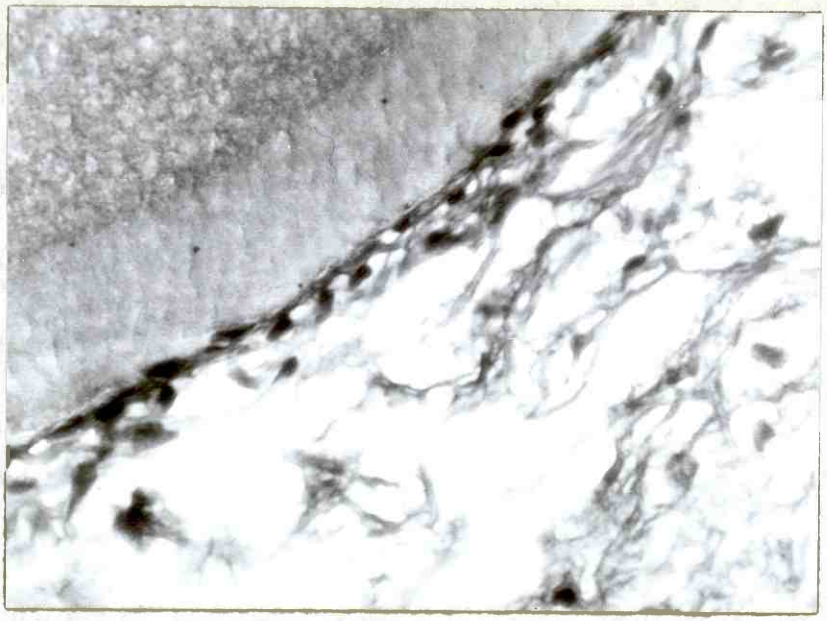
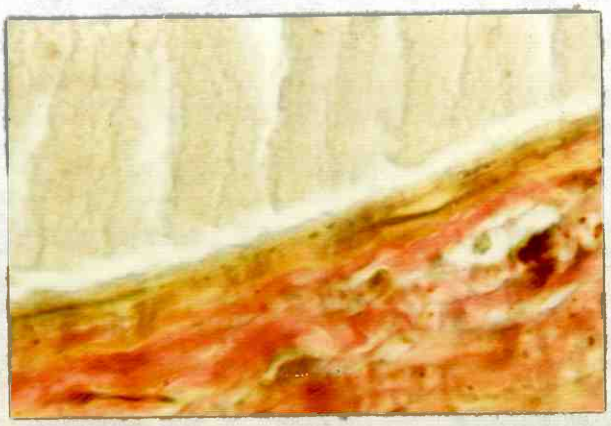


Figure V 13



Figure V 14



were more frequent and the outer limit of the media more clearly differentiated.

Percentage Wall Thickness of Veins

The percentage wall thickness is the wall thickness expressed as a percentage of the external diameter. It was found that this could only be estimated in the fetus of 39 weeks gestation and the three and ten year old children from this series. It was not possible to measure medial thickness in the younger fetuses.

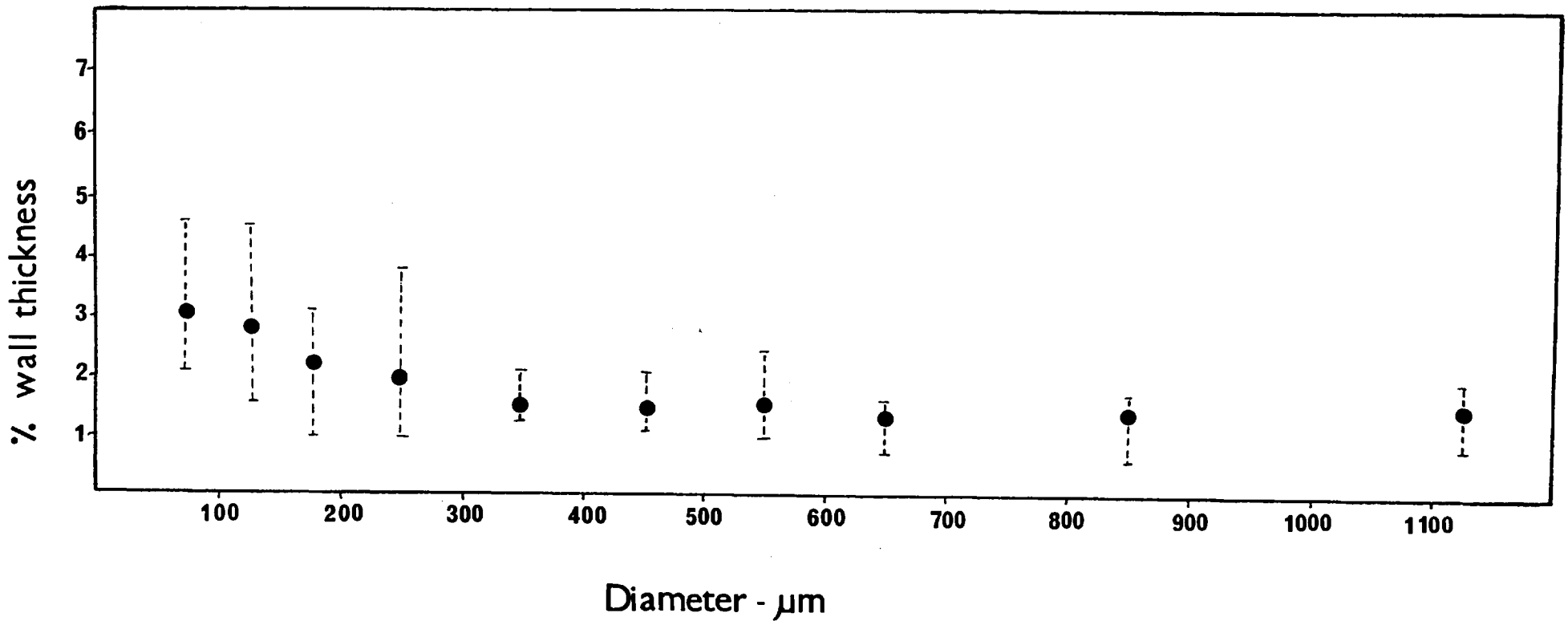
In the three cases the percentage wall thickness was the same. From the largest veins at the hilum down to veins of 300 μm external diameter the percentage wall thickness was similar, with an average of 1.5% and a range of only 0.5 - 2.4%. In veins below 300 μm diameter there was a gradual increase in percentage wall thickness with decrease in external diameter. The maximum wall thickness of 4.65% was reached in a vein of 85 μm diameter. In the veins below 300 μm the range of percentage wall thickness was much greater. This upward trend is similar to that seen in the small arteries of the lung although in the veins it is not as marked. It is partly due to a wall of a single muscle fibre being present in veins of a wide range of diameters. The average wall thickness in different size ranges for all three cases is shown in Figure V 15.

Extension of Muscle

Since there were no true limits to the extent of the muscle in the walls of the veins in the case of 39 weeks gestation it was only possible to detrmine whether muscle was present or not. It was not

Figure V 15

Percentage wall thickness related to external diameter for veins from lungs of cases aged 39 weeks of gestation, three years and ten years. The mean and range for each size range are shown.


% wall thickness
7
6
5
4
3
2
1
100
200
300
400
500
600
700
800
900
1000
1100
Diameter - µm

possible to separate muscular and partially muscular vessels. The smallest vessel in this case with a measurable muscle wall was 105 μm in external diameter. Vessels up to 120 μm were found with no muscle in their walls. There was an overlap - a vessel of the same size may or may not have muscle in its wall.

In the three year old lung it was possible to define three types of structure in the small veins. The first was non-muscular with no muscle in the wall, the second partially muscular with an incomplete muscle coat and the third muscular with a complete muscle coat. The smallest muscular vein at this age was 215 μm in diameter. The largest non-muscular vein was 200 μm in diameter and partially muscular vessels were found in a diameter range of 130 - 260 μm. This means that between 130 μm and 260 μm diameter there was a mixed population of the three types of vessel.

At ten years of age there was also a mixed population, in this case in veins between 70 and 280 μm diameter. The smallest muscular artery was 200 μm, the largest non-muscular was 127 μm. The ranges in the three and ten year old were very similar. All the vessels over 260 μm in the three year old and 280 μm in the ten year old were muscular in structure. When the percentage of each type of vessel on a given size range was plotted, similar curves to those of the arterial population counts were seen. In these cases there was no overlap of muscular and non-muscular veins.

SUMMARY OF RESULTS

1. The pulmonary venous system has been studied after injection with a barium sulphate-gelatine solution, both during fetal life and in children.

2. There was a distinctive appearance of the drainage system when studied on a venogram. Axial pathways increased in size towards the hilum and received tributaries which often entered at right angles.

3. The lumen diameter in upper and lower lobes increased with age, the latter always being the larger.

4. The lumen diameter measured at the hilum on the venogram was related to the length of the vessel and hence to the volume of the lung drained.

5. The axial pathway to the left lower lobe was reconstructed using serial sections in four cases, at 20 and 28 weeks of gestation and at three and ten years post-natal age.

6. The veins ran in connective tissue sheaths first between acini and lobules and then between segments. There were three types of tributaries to the axial veins. The first type have here been called conventional veins: they ran in their own connective tissue sheath and on joining the axial vein increased its size. The second type - supernumerary Type I - were small and entered from the lung tissue around the axial vein. The third tributary - supernumerary Type II - was larger than the Type I but smaller than conventional veins. Type I supernumeraries only drained a small amount of tissue while Type II received several

tributaries before entering the main vein.

7. The veins of the lung originated in the pleura, connective tissue septa, alveolar walls, junction of alveolar ducts and walls of bronchioli.

8. The pre-acinar drainage pattern was probably complete by 20 weeks gestation and there were a similar number of conventional veins and conventional arteries along an axial vessel.

9. The ratio of supernumerary to conventional veins was similar in all cases. There were always more supernumerary than conventional veins and there were more supernumerary veins than supernumerary arteries.

10. Conventional veins joined the axial pathway at varying intervals but usually closer together at the periphery. Supernumerary veins of both types were found closer together at the periphery: their ratio to conventional veins did not increase towards the periphery.

11. The axial pathway increased in diameter towards the hilum at a similar rate at all ages. Diameter was related to distance from the capillary bed.

12. The conventional veins increased in size towards the hilum and increased in size with age.

13. Type I supernumerary veins were smaller than Type II supernumerary veins. The smaller vessels of both types were found nearer to the periphery. Both types showed an average increase in diameter with age.

14. Only after 28 weeks gestation was muscle found in the walls of the veins and at this time was only in discreet bundles.

15. By 39 weeks gestation it was possible to measure a continuous layer of muscle cells making up the wall. The muscular media became more defined with age and elastic laminae increased in number.

16. The percentage vein wall thickness was the same at birth, three years and ten years. It was higher in the small veins.

17. Muscle extended to vessels of a similar diameter in the three older cases. In the small veins there was a mixed population of wholly, partially and non-muscular vessels.

CHAPTER VI

CHANGES IN THE STRUCTURE OF THE PULMONARY CIRCULATION IN CONGENITAL HEART DISEASE

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SUMMARY OF RESULTS 577

Congenital heart lesions may affect the normal circulation of the blood either to the lungs or to the rest of the body and may affect the structure of the vessels within the lungs. By studying the changes produced by different heart lesions on the vessels of the lung it may be possible to correlate haemodynamic changes during childhood with changes in lung structure. It may also be possible to throw further understanding on the factors controlling the normal growth of the lung by assessing changes in an abnormal system.

The lung changes in two heart lesions have been studied each representing a different haemodynamic change - the first causing an increase in flow of blood to the lungs due to a defect in the systemic - pulmonary partition, and the second causing a decrease in blood flow due to a pulmonary stenosis. As examples of the first group lungs from five cases of children whose lesions included a ventricular septal defect and one with a patent ductus arteriosus have been studied, of the second group six cases of tetralogy of Fallot were examined.

Since the results for the cases of tetralogy of Fallot showed the changes to be uniform regardless of age, these are described first, the cases of ventricular septal defect showed complex changes.

A. TETRALOGY OF FALLOT

The pulmonary arteries to the lungs of five children in whom tetralogy of Fallot had been diagnosed were injected using the standard laboratory methods. In a further case the veins were injected in the same way. These children had all died as a result of their heart lesion and had no other abnormalities. In only three cases, T.F.1, 2 and 6, were both lungs available, in the others only the right.

The full histories of the six cases are given in Appendix B, but the salient features are summarised in Table VI 1. The patients ranged in age from one to eight years, but their clinical features were similar with respect to heart lesion, radiographic appearance and angiographic and catheter findings.

In all cases but one, where they were on the small side of the normal range the intrapulmonary vessels were decreased in size as seen on the plain radiograph and the angiograms. In all the cases the right ventricular pressure was at systemic level. In only two cases was the pulmonary artery pressure measured and in these it was reduced. In one of these cases the resistance was increased and in the other decreased. In all except one case (T.F.5) the total pulmonary flow was low with a right to left shunt and systemic arterial desaturation.

Throughout this section the quantitative results on normal children aged ten months, four, five and ten years have been used for comparison with the abnormal cases and they are shown in the tables.

TABLE VI 1

Clinical Features of Cases with Tetralogy of Fallot

Case	Age (yr)	Artery size seen on X-ray	Pulmonary Artery Pressure (mm Hg)	Resistance	Pulmonary/ Systemic Flow	Flow Litres/ minute	Systemic Saturation %
T.F.1	1	Small	Not known	Not known	0.7:1	2.0	82
T.F.2	3¾	Small	Not known	Not known	0.6:1	Not known	81
T.F.3	4¼	Small	Not known	Not known	0.3:1	1.7	64
T.F.4	6¼	Small	Not known	Not known	0.6:1	3.1	85
T.F.5	8	'Small normal'	17/11	Decreased	1:1	5.8	97
T.F.6	4½	Small	12/5	Increased	0.2:1	1.8	54

Volume of Lungs

The volumes of the lungs of the six cases are shown in Table VI 2. It would appear that the lungs of all cases of tetralogy of Fallot are reduced in lung volume in comparison with a normal of the same age. Even the case aged eight years had only the lung volume of a normal at four years.

Macroscopic Appearance of the Lungs

Case T.F.1 had lungs of normal lobation, normal on palpation. There was little dust deposition. Along the hilar surface of the lungs and the apical edge of the left lower lobe there were leashes of filled, tortuous arteries, perhaps bronchial, beneath the pleura. The bronchial arteries within the wall of the bronchi were also filled with injection medium showing the presence of pre-capillary broncho-pulmonary anastomoses.

Case T.F.2 was already sliced when received for examination, however it could be seen that there was normal lobation. The lung was rather more solid than is normal and had a rubbery texture. There were some tortuous vessels, probably derived from the bronchial system, in the pleura close to the hilum but not elsewhere over the lung.

Only the right lung of case T.F.3 was available for investigation. This was normal in appearance and texture, though it was particularly pale with little dust deposition. There was normal lobation. There was a leash of filled, tortuous vessels, probably bronchial, on the apical edge of the lower lobe and other filled vessels were seen on the hilar surface of the upper lobe.

Again for case T.F.4 only the right lung was available for

TABLE VI 2

Volume of Lungs in Tetralogy of Fallot

Case	Age (yr)	Left Lung (cc)	Right Lung (cc)	Total (cc)
T.F.1	1	100	150	250
T.F.2	3¾	Not available		
T.F.3	4¼	-	210	420
T.F.4	6¼	-	330	660
T.F.5	8	-	350	700
T.F.6	4½	380	450	830
Normal				
10 months		-	-	320
4 years		300	380	680
10 years		-	-	1065

study. This had normal lobation and was of normal texture. A few pleural vessels were filled with contrast medium, particularly near the hilum, and the bronchial vessels in the bronchial walls were well filled.

The left lung of case T.F.5 could not be inflated since there were adhesions on the pleura, probably as a result of the left sided Blalock anastomosis. The right lung was inflated and injected; it had normal lobation and showed a considerable amount of dust deposition. Near the hilum under the pleura a few bronchial arteries were filled.

Both lungs of case T.F.6 had normal lobation with no adhesions. Some pleural vessels were filled in both lungs after the venous injection. There were no filled bronchial vessels close to the hilum as in the other cases with arterial injection.

Microscopic Appearance of the Lungs - General

Case T.F.1 The lungs were well inflated with no oedema or inflammation. The bronchi were also clear of infection. The alveolar walls were normal in thickness and structure. The bronchial vessels of the bronchi and pleura were enlarged and filled with the injection medium, confirming the presence of pre-capillary anastomoses. In the proximal regions connections between the bronchial vessels and alveolar wall capillaries could be seen. There was no filling in the distal airways. The arteries and veins appeared normal with no intimal thickening.

Case T.F.2 The lungs of this case were very congested with oedema of the alveoli and lymphatic vessels, though the airways were clear. The alveoli were not fully inflated and the alveolar ducts and respiratory bronchioli stood out as inflated. The bronchial vessels

were not enlarged but some of the vessels were filled in the large bronchi, however there were no obvious connections visible. The veins were normal but there was a small degree of intimal thickening in the medium sized pulmonary arteries.

Case T.F.3 These lungs were well inflated with no oedema of the alveoli, though there was some shedding of epithelium into the alveolar spaces. The bronchioli and bronchi were normal. There was filling of some of the bronchial vessels in the hilar bronchi, but none in the bronchioli. No obvious connections could be seen. The veins appeared to be normal in structure and there was no evidence of intimal thickening in either arteries or veins.

Case T.F.4 The lung was well inflated with normal alveolar walls and no oedema. The bronchi were normal. There was bronchial artery filling in the walls of bronchi and bronchioli but there was no increase in size of these vessels. The pulmonary arteries and veins were normal in appearance with no intimal thickening.

Case T.F.5 Though the lung was fully inflated there were considerable areas of oedema, often strongly eosinophilic, in the alveolar region, some alveoli being blocked by secretion and shed epithelium. The connective tissue septa were increased in thickness by lymphatic dilatation. There was bronchial artery filling mainly in the walls of proximal bronchi and these vessels were enlarged. There was considerable intimal thickening in both arteries and veins and, in the arteries, thrombotic lesions, often recanalised. This recanalisation with web formation may have followed the increase in flow to the lung after the Blalock operation.

Case T.F.6 The lungs were fully inflated with a little oedema patchily distributed but no consolidation. The airways were normal. The connective tissue septa were thickened due to dilated lymphatic vessels. The arteries were normal in appearance with no intimal thickening. The veins had been injected and in many places the medium had passed into the capillaries of the alveolar walls but not into the arteries. There was some bursting of these vessels so that injection medium was found in alveoli. The bronchial vessels were filled with injection medium in the walls of bronchi and bronchioli.

Arteriograms

The arteriograms of cases T.F.1 - 5 are shown in Figures VI 1 - 5. In all five cases there was very good filling of all regions injected. In case T.F.3 only part of the upper lobe was injected; this was due to the lumen of the artery being mechanically occluded, in error, during injection.

The arterial branching pattern in all cases seemed normal but there was a very dense background haze. This was particularly noticeable in cases T.F.1 and 2. Bronchial arteries could be seen near the hilum in case T.F.1, they run a more tortuous course than the pulmonary vessels. It was also possible to see a filled bronchial artery running along the edge of the middle lobe in case T.F.3. This was in connection with the pulmonary artery at the hilum (see arrow). Bronchial vessels could not be discerned on the other three arteriograms but they may have been masked by the pulmonary artery filling.

Measurements from Arteriograms

Measurements were made on the arteriograms at 25% intervals along the axial pathway to the upper and lower lobes. The total length of each pathway was measured.

Length of Pathway

In all five cases as in the normal the lower lobe artery was longer than the upper lobe artery. In all cases the length of each pathway seemed shorter than the normal at the similar age (Table VI 3). This was related to the fact that the total lung volume was also reduced.

Diameter of Lumen along Axial Pathway

The diameters along the axial pathways of upper and lower lobes are shown in Figure VI 6 and Table VI 3. In all five cases the diameter at the hilum of the axial pathway was smaller than the normal of the same age in both upper and lower lobes. At the point 25% from the periphery case T.F.1, 3 and 4 were normal in diameter while cases T.F.2 and 5 had a reduced lumen diameter. In all cases throughout the lung the lumen diameter was smaller than the normal for that age.

None of the changes were gross although it seems that they were greater at the hilum than at the periphery. The oldest case, T.F.5, showed the greatest change from normal and the youngest case, T.F.1, the least.

The diameter at the hilum did not directly relate to the length of the pathway as it did in the normal, the hilar diameter was in fact narrow for the length of pathway that it was supplying.

Figure VI 1

Arteriogram of case T.F.1 (x 0.7).
The arteries appear slightly smaller than normal and there is a dense background haze. Bronchial arteries can be seen close to the hilum at arrow.

Figure VI 2

Arteriogram of case T.F.2 (x 0.5).
The main arteries are reduced in size and there is a dense background haze.

Figure VI 1

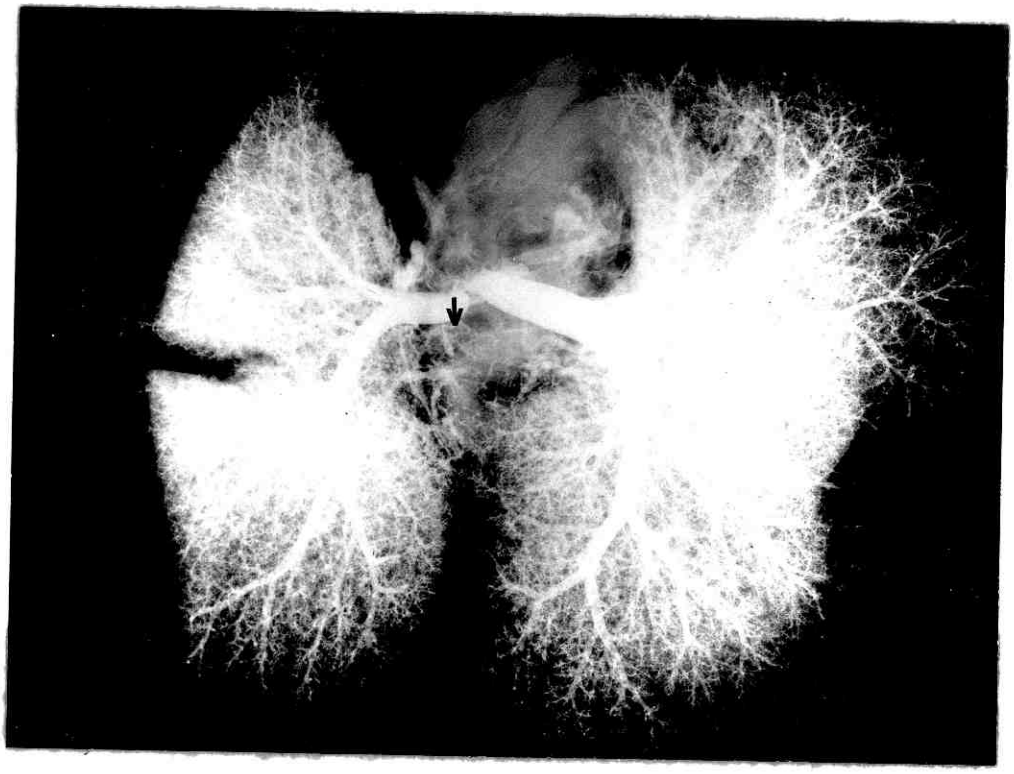


Figure VI 2

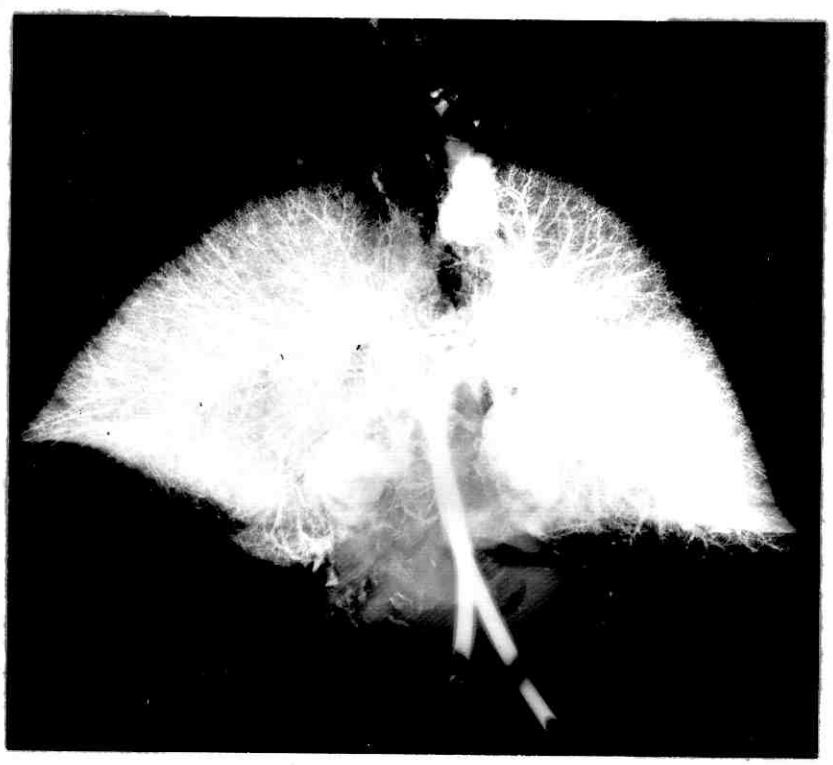


Figure VI 3

Arteriogram of right lung of case T.F.3 (x 1). Only part of the upper lobe is injected due to mechanical occlusion of the main artery. There is a filled bronchial artery along the edge of the middle lobe and joining the pulmonary artery at arrow.



Figure VI 4

Arteriogram of the right lung of case T.F.4 (x 0.7). The arteries are reduced in size and there is an increased background haze.

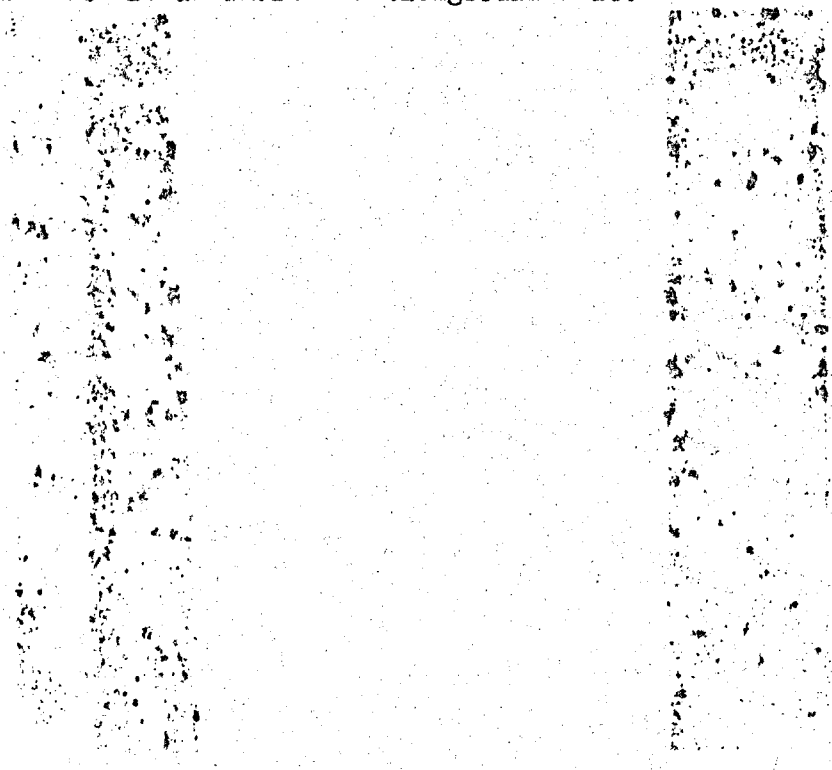


Figure VI 3

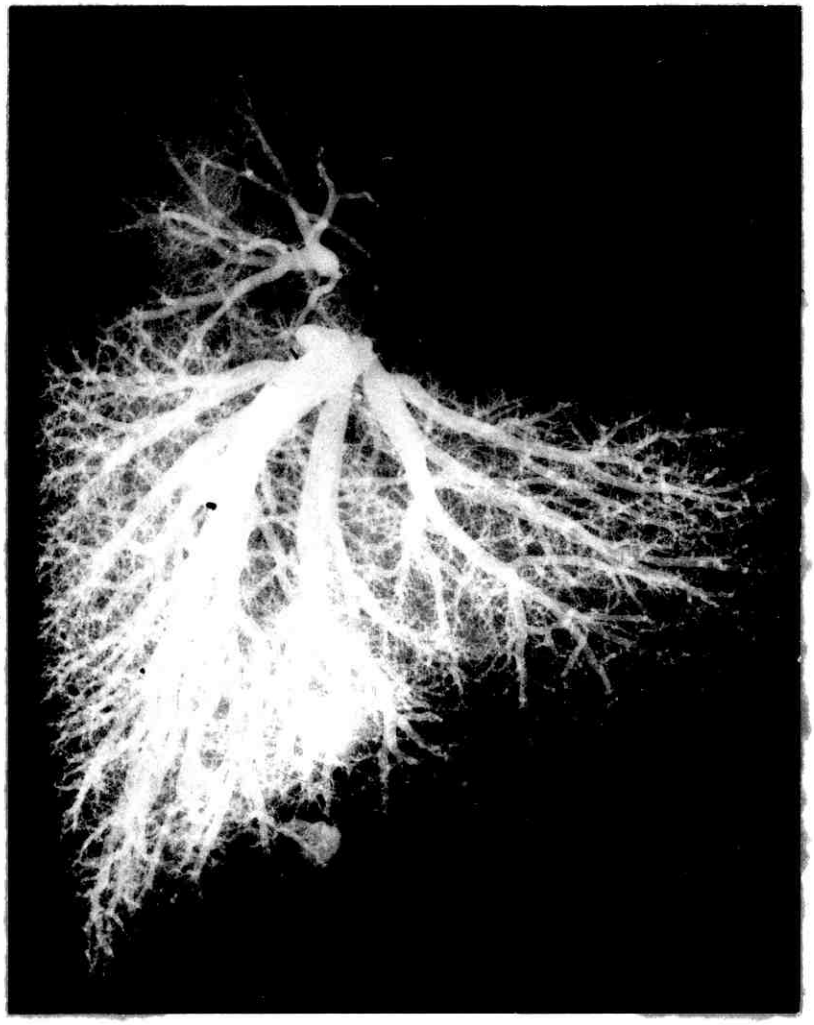


Figure VI 4

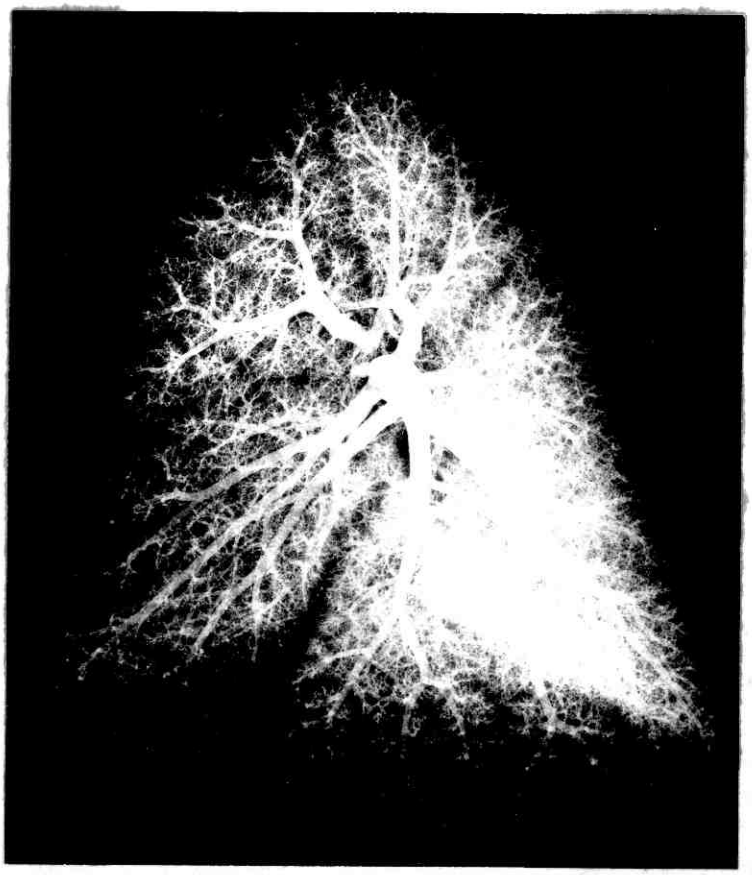
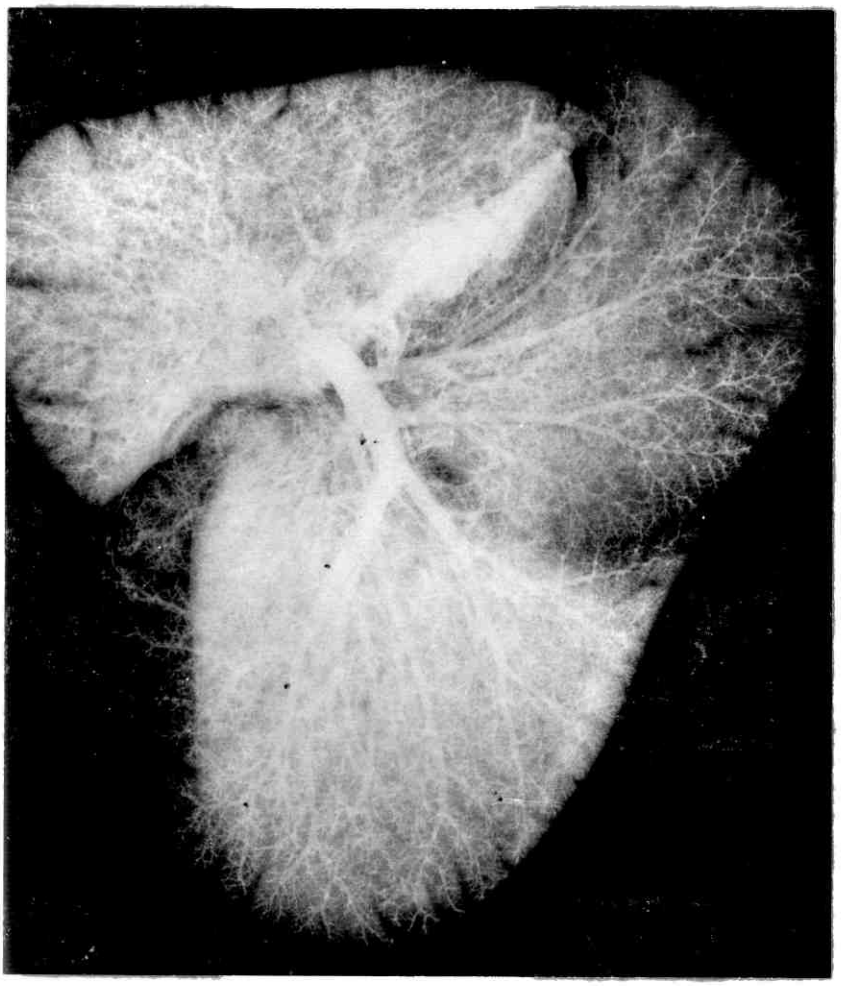


Figure VI 5



Arteriogram of the right lung of case T.F.5 (x 0.75). The main arteries are reduced in size and there is a marked background haze of small vessels.

Gradient of the Pathway

The gradient of the fall along the axial pathways was estimated in terms of the actual length of a pathway and of those pathways when reduced to a hundred equal units. By either method the gradient was reduced in cases T.F.2 - 5 in comparison with the normal (Table VI 4). Case T.F.1, only one year old, had a near normal gradient: thus the diameter of the lumen was decreased more than the length of the axial pathway.

Other Measurements

Measurements were also made in these five cases of the lumen diameter of the main pulmonary artery, of the artery to each lobe and of arteries one centimetre from the periphery in each lobe. The results with the normals of similar age are shown in Table VI 5.

The left and right pulmonary artery were smaller than the normal for age except in the case of T.F.3 where the right pulmonary artery seemed normal. Case T.F.5 had particularly small vessels since the lumen diameter for this eight year old was smaller than the normal of ten months. Similarly the lobar lumen diameters were small for the age of the child except possibly in case T.F.3. Again they were particularly small in case T.F.5. The diameter of the arteries one centimetre from the periphery was in the normal range for age in all five cases. It seems that the diameter was nearer normal at the periphery than the hilum.

In summary it seems that in all five cases there was a reduction in size of the arteries. In all cases this was more marked at the hilum than the periphery. The least affected case was the youngest and the most affected the oldest. The measurements of all five cases were similar which is surprising since there was a wide range of ages.

Figure VI 6

Diagram representing the measurements of lumen diameter made on the axial arteries to the upper and lower lobes of the cases of tetralogy of Fallot together with those of the normal at ten months and five years. The diameter is shown in millimetres for the percentage distances along the pathway.

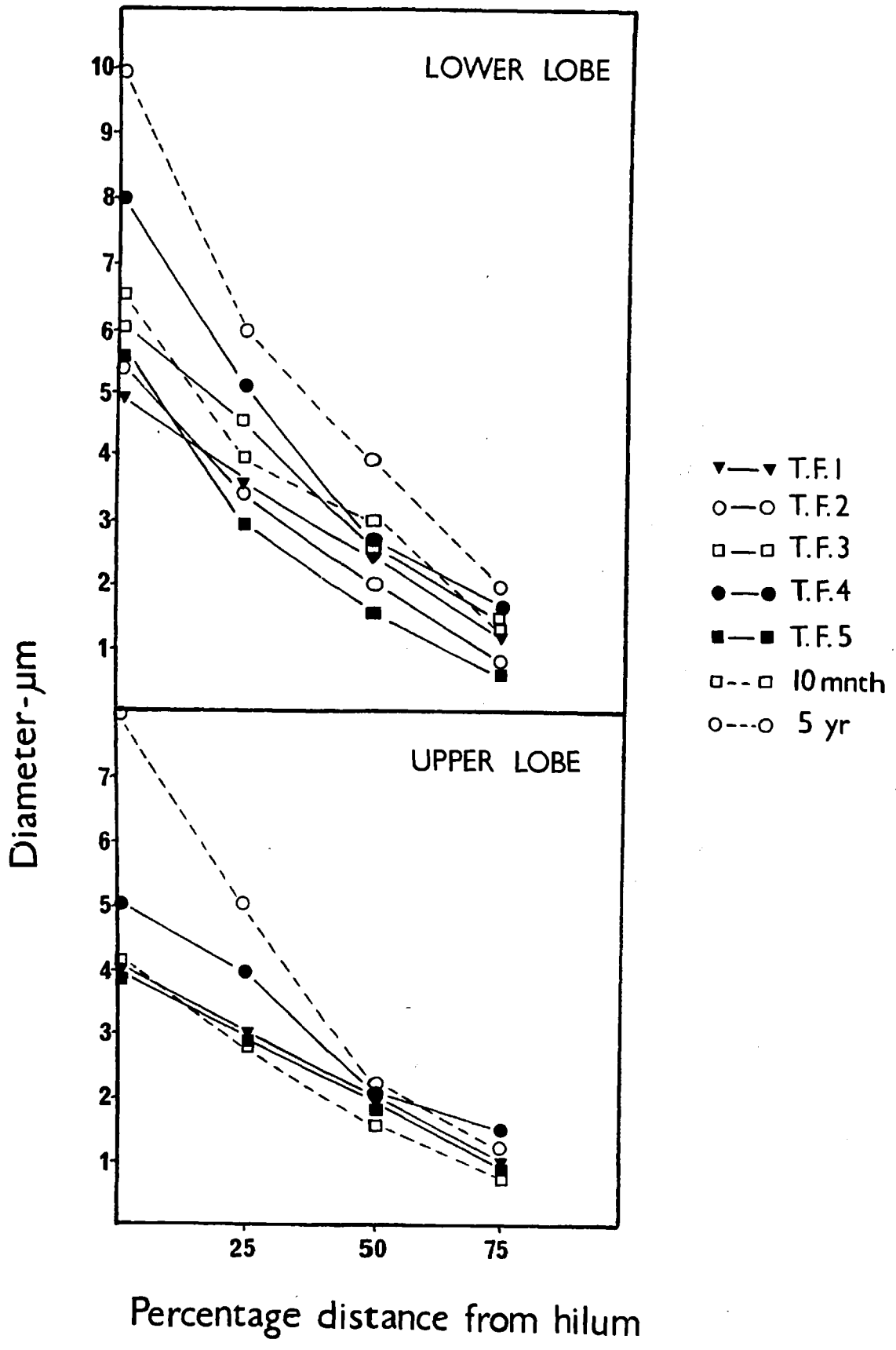

LOWER LOBE
UPPER LOBE
10
9
8
7
6
5
4
3
2
1
7
6
5
4
3
2
1
25
50
75
Diameter-µm
Percentage distance from hilum
T.F.1
T.F.2
T.F.3
T.F.4
T.F.5
10 mnth
5 yr

TABLE VI 3

Measurements on Arteriograms of Axial Pathways

Case	Lower Lobe					Upper Lobe				
	Length (cm)	Diameter (mm) % distance from hilum				Length (cm)	Diameter (mm) % distance from hilum			
		0	25	50	75		0	25	50	75
T.F.1	7	5	3.6	2.5	1.2	6	4	3	2	1
T.F.2	9.5	5.4	3.5	2.0	0.8	-	-	-	-	-
T.F.3	9.5	6	4.5	2.5	1.5	-	-	-	-	-
T.F.4	10.5	8	5	2.5	1.6	5	5	4	2	1.5
T.F.5	8.5	5.6	3	1.6	0.7	6	4	3	2	1
Normal										
10 mths	8.4	6.5	4	3	1.2	4.2	4	3	1.5	0.8
5 yrs	11.2	10	6	4	2	6.2	8	5	2	1.2

TABLE VI 4

Gradiant along Axial Pathway to Lower Lobe in Cases of Tetralogy of Fallot

Case	Gradient	
	Diameter/Actual Length	Diameter/Percentage Length
T.F.1	0.714	1.25
T.F.2	0.568	1.35
T.F.3	0.631	1.5
T.F.4	0.761	2.0
T.F.5	0.658	1.4
Normal		
10 months	0.773	1.625
5 years	0.89	2.5

TABLE VI 5

Measurements from Arteriograms

Case	T.F.1	T.F.2	T.F.3	T.F.4	T.F.5	10 mth	5 yr
Diameter (mm)							
Left P.A.	5.5	7	-	-	-	8	11
Right P.A.	6.5	7	10	9	6	8	10
Lobar							
L.U.L.	3	3	-	-	-	5	7
L.L.L.	4	6.5	-	-	-	5	10
R.U.L.	4	-	-	4	4	5	5
R.M.L.	-	-	-	4	3	4	-
R.L.L.	5	5	5	8	5.5	6.5	8
1 cm from periphery							
L.U.L.	1	0.7	-	-	-	1	0.9
L.L.L.	0.8	1	-	-	-	0.5	1
R.U.L.	0.7	0.7	-	1	-	0.8	0.9
R.M.L.	-	-	0.7	1	1	-	1
R.L.L.	0.8	0.7	1	0.9	1	0.8	1

Branching Pattern of Arteries by Serial Reconstruction

The overall pattern of branching was determined by observations on the arteriograms. The segmental pattern was normal and although the branches were small they seemed to be normal in distribution. It can be assumed that the ante-natal development of airways and arteries was normal since the effect of the heart lesion would only be felt after the onset of the adult haemodynamic pattern. During fetal life there is little blood flow through the lungs and there is also blood available to the lungs via the ductus arteriosus. Only the peripheral centimetre of the lung, which includes the intra-acinar region, which develops after birth, was therefore studied in detail by serial reconstruction.

A block approximately one centimetre deep was taken from the diaphragmatic edge of the posterior basal segment of the right lower lobe in case T.F.4. The bronchial branching pattern to the level of this block was investigated by blunt dissection. The pathway was 7.5 cm long from the hilum to the top of the block and over this distance there were 13 bronchial generations, that is there were 13 conventional side branches. It was not possible to see cartilage in the walls beyond the tenth generation. Down to this level filled bronchial arteries could be seen in the walls of the bronchi. Accompanying each bronchial branch was a conventional artery and there were identified in addition many extra arterial branches from the axial pathway, the supernumerary arteries. The branching pattern of arteries and airways to this point seemed normal.

The pattern of branching at the periphery found by serial reconstruction is shown in Figure VI 7. The length of this pathway to the level of the alveoli, where the arteries came to lie in the alveolar walls was 15.12 mm. The total length of the posterior

basal artery was therefore approximately 9 cm. The length on the arteriogram was greater than this - 10.5 cm. The difference doubtless being due to shrinkage and loss from cutting. In the portion of the lung reconstructed there were 10 bronchial branches before the terminal bronchiolus. Four of these were intra-lobular. The total for the whole pathway was therefore 23 airway branches, within the normal range.

Accompanying the ten bronchioli were ten conventional arteries and in addition 45 supernumerary arteries. This gave a ratio of supernumerary to conventional vessels of 4.5:1. This is higher than seen in the normal over the whole of the pre-acinar region, however there are always more supernumerary vessels at the periphery and in the last ten generations of the normal there was the same ratio.

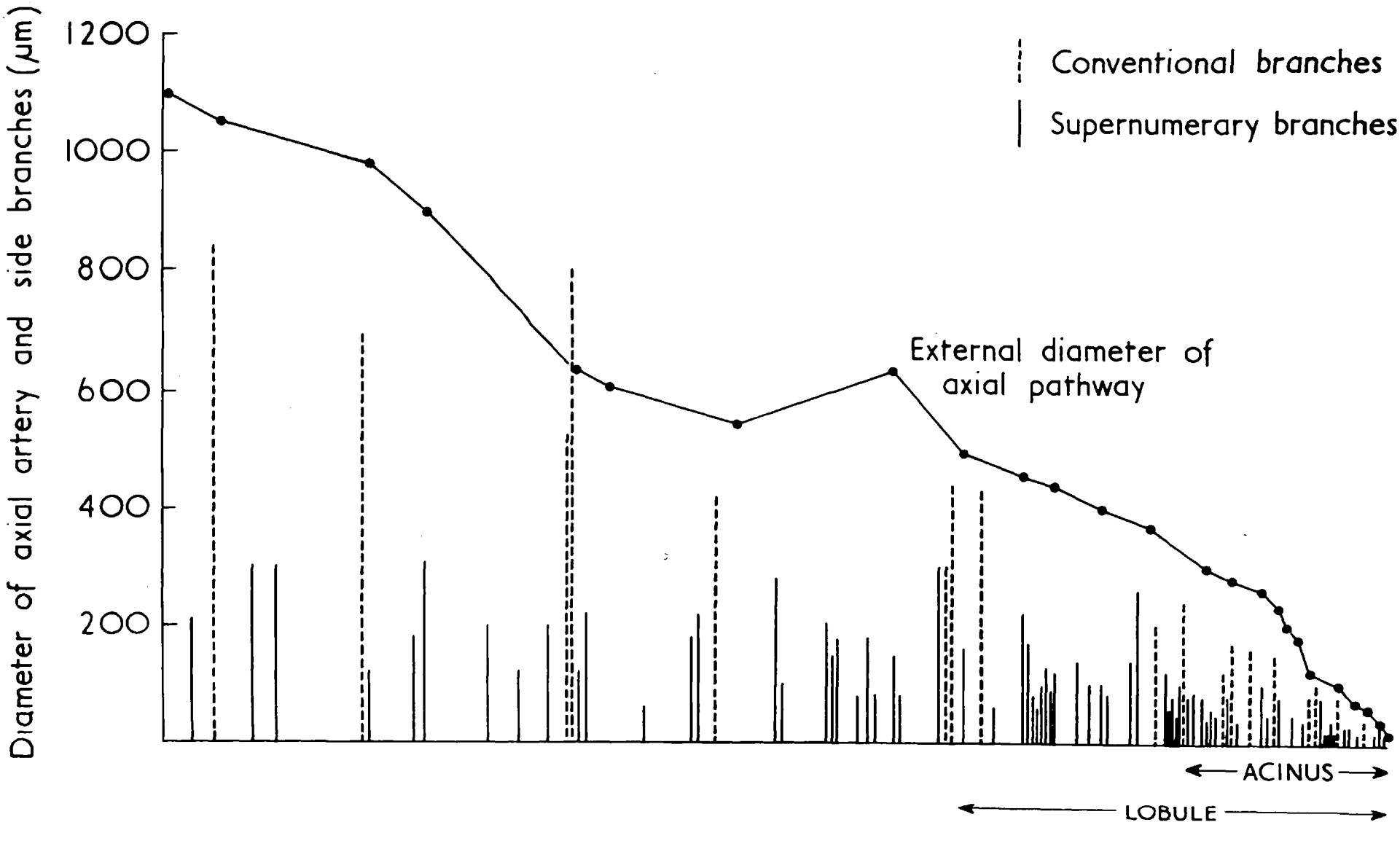
The length of the acinus from the proximal end of the terminal bronchiolus was 2.45 mm, the terminal bronchiolus being 490 μm long. Within the acinus were three respiratory bronchiolar branches and five alveolar duct branches before the main pathway came to lie in an alveolar wall. Running with each of these airways was a conventional artery. With the total of eight intra-acinar conventional arteries were 23 supernumerary vessels, a ratio of 2.87:1. These figures are slightly reduced in comparison with those of the five year old normal child whose acinus was traced in a similar manner, though this could be individual variation.

Diameter of Axial Pathway

The diameter of the axial pathway studied by serial reconstruction is also shown in Figure VI 7 and Table VI 6. In the figure are also shown the diameters of all the side branches. The diameter of the

Figure VI 7

Diagram representing the branching pattern and the diameter of the axial pathway and conventional and supernumerary arteries in the peripheral 15.12 mm of the posterior basal artery of case T.F.4. The extent of the lobule and the acinus is shown.


Diameter of axial artery and side branches (μm)
1200
1000
800
600
400
200
Conventional branches
Supernumerary branches
External diameter of axial pathway
ACINUS
LOBULE

axial pathway decreased towards the periphery. There were no sudden drops since there were no particularly large side branches. The diameter at the beginning of the serial was 1100 μm and dropped to 300 μm at the level of the terminal bronchiolus, this over a distance of 11.9 mm. The diameter at the end of the respiratory bronchiolar region was 225 μm and at the end of the alveolar duct, 80 μm. In this case of 6 years, within the acinus, at the three levels just mentioned, the artery was smaller than the normal at five years. The figures are closer to the normal at 18 months.

Diameter of Conventional Arteries

The diameter of the conventional arteries is shown in Figure VI 7 and Table VI 6. As in the normal the conventional arteries were smaller than the axial pathway at the point of division. They decreased in size towards the periphery though not in absolute order. The average diameter of all the conventional vessels in this pathway was 322.66 μm. At the periphery arteries categorised by their accompanying airways are more comparable. The diameter of the arteries accompanying terminal bronchioli was 297.5 μm, those with the respiratory bronchioli 153 μm and with the alveolar ducts, 87.8 μm. In all these groups the figure was much reduced in comparison with a normal five year old and the figures were similar to those of the normal between four and 18 months. They were relatively smaller than the axial pathway.

Diameter of Supernumerary Arteries

The diameter of the supernumerary arteries is shown in Figure VI 7 and Table VI 7. The supernumerary arteries were smaller than the conventional arteries. The average for the whole of this pathway being 115.29 μm. They decreased in size towards the periphery,

TABLE VI 6

Diameter of Axial Artery and Conventional Arteries and their Special Arrangement at the Periphery in Case T.F.4

Bronchial Generation	Diameter of Conventional Artery (μm)	Diameter of Axial Pathway (μm)	Distance from previous branch (mm)
1	830	1050	-
2	700	980	1.75
3	530	-	2.38
4	800	640	0.07
5	430	570	1.54
T.B.1	300	500	2.73
T.B.3	440	-	0.07
6	430	460	0.35
T.B.2	200	370	2.03
T.B.4	250	300	0.35
R.B.1	127	-	0.49
R.B.2	170	280	0.07
R.B.3	162	260	0.21
A.D.1	155	225	0.28
A.D.2	70	180	0.42
A.D.3	100	112	0.07
A.D.4	77	90	0.28
A.D.5	37	80	0.28
Mean	322.66		

though there was a marked variation in size between successive arteries. The average diameter of the arteries of this type within the lobule was 52.5 μm, less than half the average diameter in the normal five year old. They were smaller towards the distal end of the acinus, those in the intra-lobular, pre-acinar region having an average of 80.58 μm, those in the respiratory bronchiolar region averaging 67.5 μm and those with alveolar ducts, 33.33 μm.

In this case the intra-lobular branching pattern seemed normal for age: the diameter of all vessels in this region was much smaller than the normal at five years and appeared to be equivalent to those seen in a child less than 18 months of age. The volume of this lung and the arteriogram showed some reduction in size being equivalent to a normal child aged four years: the reduction in size of the peripheral vessels was therefore greater than that of the proximal vessels.

Structure of the Axial Pathway

The axial pathway at the most proximal point traced was 1100 μm in diameter, and had a muscular wall structure. This structure extended to the level of the first respiratory bronchiolus at 280 μm. Muscle did not extend as far along the axial pathway as it did in the case of the normal five year old where muscle extended to a diameter of 250 μm at the level of the fourth generation of respiratory bronchioli. Muscle therefore extended to a similar diameter but since in the present case the arteries were smaller muscle did not extend so far into the acinus.

A partially muscular structure was found with the first generation respiratory bronchiolus and as far as the third generation

TABLE VI 7

Diameter of Supernumerary Arteries at Periphery of Axial Pathway in Case T.F.4

Bronchial Generation	Diameter (μm)	Bronchial Generation	Diameter (μm)
0	225	6	95
1	300	6	85
1	310	6	150
2	120	6	260
2	190	T.B.2	130
2	310	T.B.2	60
2	200	T.B.2	90
2	122	T.B.2	45
2	205	T.B.2	100
4	120	T.B.4	75
4	212	T.B.4	85
4	62	T.B.4	77
4	180	T.B.4	35
4	220	T.B.4	50
5	285	T.B.4	60
5	95	R.B.1	80
5	210	R.B.2	37
5	150	R.B.3	105
5	185	R.B.3	48
5	90	A.D.1	75
5	152	A.D.1	52
5	72	A.D.1	25
5	300	A.D.1	37
T.B.3	160	A.D.3	80
6	65	A.D.3	12
6	220	A.D.3	40
6	175	A.D.3	15
6	75	A.D.3	15
6	65	A.D.4	25
6	95	A.D.4	30
6	135	A.D.4	20
6	95	A.D.5	12
6	137	A.D.5	37
6	140	A.D.5	25
6	102		

Mean - 115.29

alveolar duct here with a diameter of 112 μm. This means muscle extended into smaller arteries than in the normal five year old but to a similar level in the pattern of branching. The rest of the axial pathway was non-muscular.

Structure of Conventional Arteries

The conventional arteries as far as the beginning of the lobule were all muscular in structure and ranged in size from 430 to 830 μm. All of the four arteries accompanying terminal bronchioli were muscular in structure, the smallest being 200 μm in diameter. In the normal five year old also these vessels were muscular. In this case the arteries accompanying the respiratory bronchioli were all partially muscular, ranging in size from 127 to 162 μm diameter: in the normal five year old four out of the five arteries at this level were wholly muscular. At this level there was rather less muscle than in the normal therefore.

Of the five vessels accompanying alveolar ducts only one, the first, was partially muscular, the rest were non-muscular: this is similar to the normal at five years.

Save for the somewhat anomalous findings with the respiratory bronchioli it seems that muscle extended to a similar level of conventional arteries as in the normal.

Structure of Supernumerary Arteries

Of the 26 pre-lobular supernumerary arteries seen along this pathway, six were muscular in structure, 12 partially muscular and eight non-muscular. The muscular varied in size from 185 - 310 μm,

the partially from 122 - 285 μm and the non-muscular from 62 - 152 μm. The size ranges overlap as in the normal and a similar range was seen in the conventional and axial arteries. In the intra-lobular, pre-acinar region there were 21 vessels of this type; one of 260 μm diameter was muscular, seven ranging from 95 - 220 μm, were partially muscular and the rest were non-muscular ranging in size from 50 - 137 μm. There is a greater proportion of non-muscular arteries at the expense of both muscular and partially muscular, than in the normal five year old. Within the acinus there was one partially muscular artery, 105 μm in diameter, the remaining 23 being non-muscular. These vessels are of similar muscularity to those in the normal at a similar age.

Serial Reconstruction of Case T.F.5

The peripheral centimetre in a further case, T.F.5 was also traced, since it was a case which had been operated upon earlier in life and the affects of this could be studied. The axial pathway had a normal branching pattern within the lobule which was of normal length for the age of the child. There were four acini within the lobule and in one acinus the axial bronchiolus gave rise to four respiratory bronchioli and six alveolar ducts. Each of these had an accompanying conventional artery. Within the acinus were 40 supernumerary arteries giving a ratio of supernumerary to conventional of 4:1, a normal ratio. The number of vessels in the intra-lobular, pre-acinar region was also normal.

The diameter of the axial pathway was about normal but the conventional arteries were rather reduced in size. The discrepancy may be due to dilatation of the axial vessel by thrombus. There

was intimal thickening along the axial artery which was continuous with a thrombus through the whole length of the acinus to the sixth alveolar duct generation. There was a web of intimal tissue forming septa and also thrombus which had been recanalised (Figure VI 8). The wall was reduced in thickness at this point.

Wall Thickness of Arteries Throughout the Lung

The muscularity of the arteries in these cases was assessed by measuring the percentage wall thickness of arteries found throughout the lung. The results for the five cases are shown in Table VI 8. The arteries have been grouped into size ranges according to external diameter, in the smallest at 50 μm intervals and in larger vessels at 100 μm intervals. The average percentage wall thickness for each size range is shown, as well as the standard error of the mean and the range. The mean and range have been plotted for each case and are shown in Figures VI 9 - 13.

In all five cases the percentage wall thickness at all diameter ranges was higher than in the normal. All five cases were in the age range where the adult level of wall thickness would be expected, this is 1 - 2%. In cases T.F.1 and 4 the increase was only slight. In case T.F.3 there was a particularly wide range in the smaller arteries due to one or two arteries with particularly high wall thicknesses, which have increased the average to above normal. In the larger arteries the percentage wall thickness was within the normal adult range. Cases T.F.2 and 5 had a higher percentage wall thickness, particularly case T.F.5 which was at the same level as the normal at birth. This was possibly due to the increase in flow which had occurred in this case after a Blalock anastomosis at five years of age.

Figure VI 8



Photomicrograph of an artery from case T.F.5 (x 145). There is a thrombus which has been recanalised and the filling medium can be seen. The artery wall is reduced in thickness in comparison with other vessels of this case.

TABLE

Percentage Wall Thickness of Arteries from

Diameter

		50-100	100-150	150-200	200-300	300-400	400-500
T.F.1	Mean	-	3.67	2.94	2.62	3.06	3.26
	S.E.		0.29	0.15	0.15	0.36	0.71
	No.		2	8	23	10	3
	Range		3.6-3.7	2.5-3.8	1.7-4.5	1.5-5.4	2.0-4.5
T.F.2	Mean	5.0	3.52	3.02	2.54	2.81	3.53
	S.E.		0.09	0.13	0.16	0.19	0.42
	No.	1	5	12	11	10	6
	Range		3.3-3.8	2.5-4.0	1.8-3.4	1.5-3.5	2.6-5.5
T.F.3	Mean	-	4.58	3.23	3.25	2.1	2.43
	S.E.		0.41	0.42	0.35	0.42	1.13
	No.		2	9	28	9	4
	Range		4.2-5.0	1.9-6.2	1.7-8.0	1.1-5.3	1.1-5.8
T.F.4	Mean	-	3.5	2.86	2.14	2.31	2.38
	S.E.		0.29	0.22	0.24	0.20	0.15
	No.		8	9	12	9	5
	Range		1.7-4.5	1.3-3.7	1.7-2.4	1.3-3.0	1.9-2.7
T.F.5	Mean	7.29	4.61	3.67	3.14	3.66	3.73
	S.E.	0.78	0.30	0.28	0.18	0.21	0.36
	No.	7	18	19	43	29	11
	Range	4.8-11.0	3.4-6.9	2.5-6.3	1.9-7.4	1.6-7.1	2.4-7.0

VI 8

Cases with Tetralogy of Fallot

Range µm

500-600	600-700	700-800	800-900	900-1000	> 1000
2.52	3.84	3.44	3.01	4.33	3.01
0.09	1.39	0.07			0.20
7	2	2	1	1	13
2.2-2.9	2.4-5.2	3.4-3.5			2.1-4.3
2.63	3.39	3.96	3.82	-	4.57
0.14	0.25	0.81	1.32		0.32
3	4	3	3		8
2.3-2.8	2.8-4.0	3.0-5.6	1.7-6.3		2.9-5.6
1.32	1.23	1.48	1.54	1.92	1.79
0.21	0.12	0.09	0.18	0.17	0.24
3	3	2	3	2	9
1.1-1.7	1.1-1.5	1.4-1.5	1.2-1.8	1.7-2.1	0.8-2.9
2.64	1.81	2.89	2.1	-	2.99
0.32	0.20	0.31			0.26
7	2	4	1		12
1.7-4.2	1.6-2.0	2.1-3.5			1.9-4.8
3.71	4.07	4.04	3.89	4.18	4.35
0.22	0.21	0.47	0.44	0.45	0.15
8	4	3	3	4	7
2.8-4.2	3.6-4.5	3.5-5.0	3.0-4.5	3.2-5.3	3.9-4.8

S.E. - Standard Error of the mean

No. - Number of vessels measured

The lumen diameter in this case was particularly small partly due to this increase in wall thickness.

In all five cases a similar curve was seen. In the small arteries below 200 μm diameter there was a rise in the percentage wall thickness similar but not as great as that seen in normal children, save in case T.F.5 where the highest point reached was 7.39%, higher than is seen in the normal at this age. The beginning of the rise was at the same diameter range in all cases and in the normal.

In the first four cases the percentage wall thickness had dropped from the normal at birth. They had either dropped to the normal level and then increased or they had not completed the decrease. In case T.F.5 which was still at the fetal level it is likely that there was a drop after birth and that the increase was the result of the Blalock operation.

It seems that low blood flow in three cases had not led to atrophy of muscle in the arterial walls as had previously been postulated. In fact there was some increase in all five cases and particularly in the two in which an artificial increase in flow had been produced by operation.

Population Counts

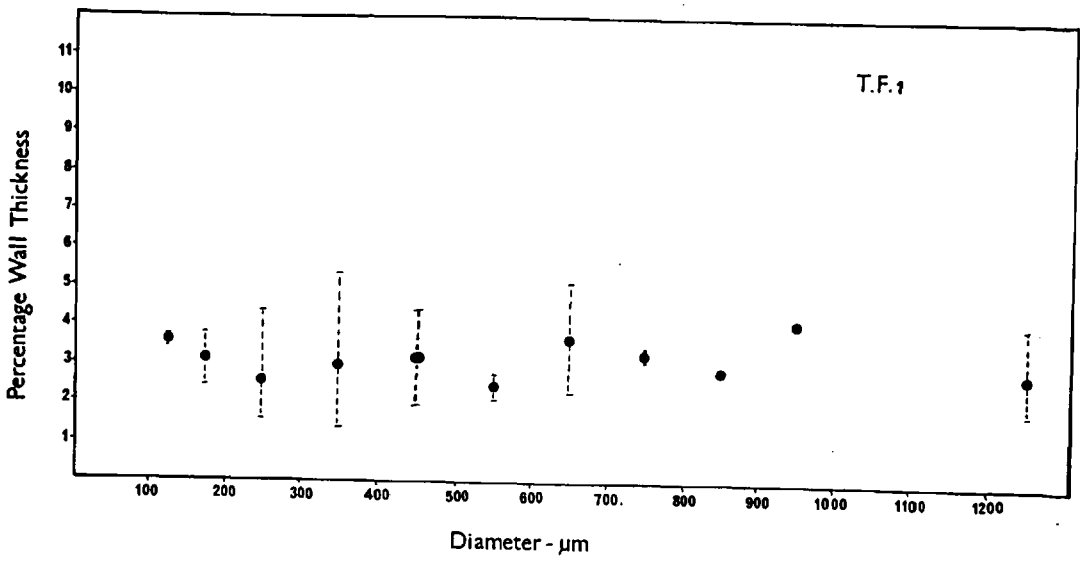
Population counts were made on random sections throughout the lungs of all five cases. These gave an estimation of the extent of muscle into small arteries and the type of vessels seen in any size range. These were then compared with the normal cases of the same age. Figure VI 14 and Tables VI 9 - 11 show the results of the population counting in the present five cases.

Figure VI 9

Percentage wall thickness related to external diameter for arteries from case T.F.1. The mean and range for each diameter range is shown.

Figure VI 10

Percentage wall thickness related to external diameter for arteries from case T.F.2. The mean and range for each diameter range is shown.


T.F.1
Percentage Wall Thickness
Diameter - µm

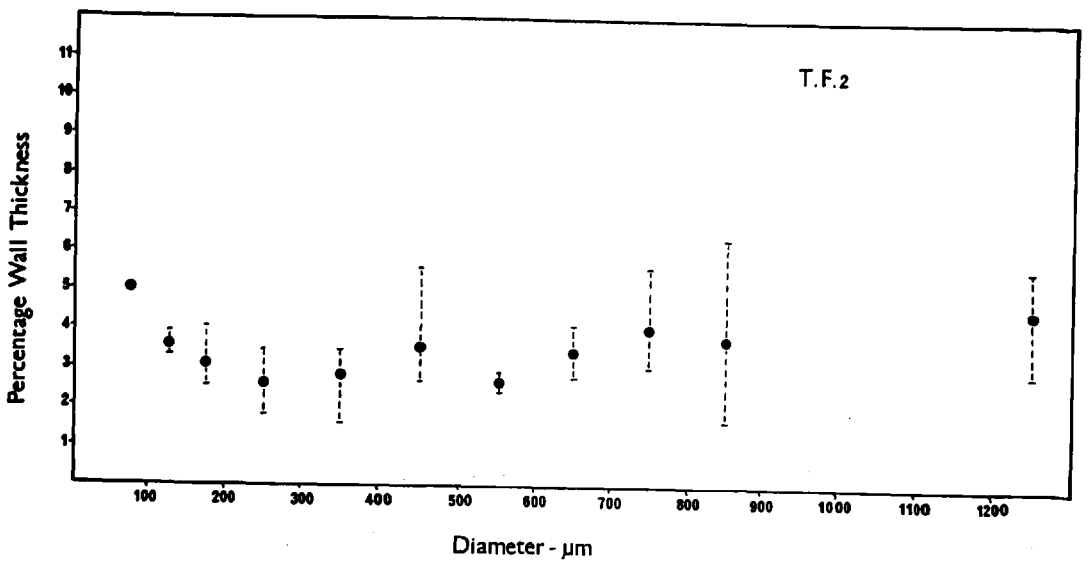

T.F.2
Percentage Wall Thickness
Diameter - µm

Figure VI 11

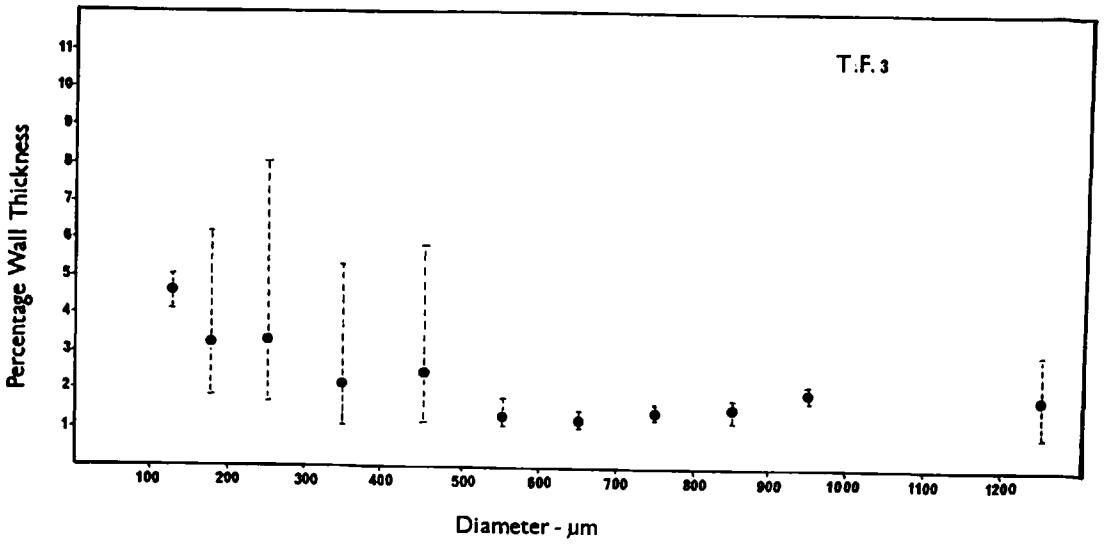
Percentage wall thickness related to external diameter for arteries from case T.F.3. The mean and range for each diameter range is shown.

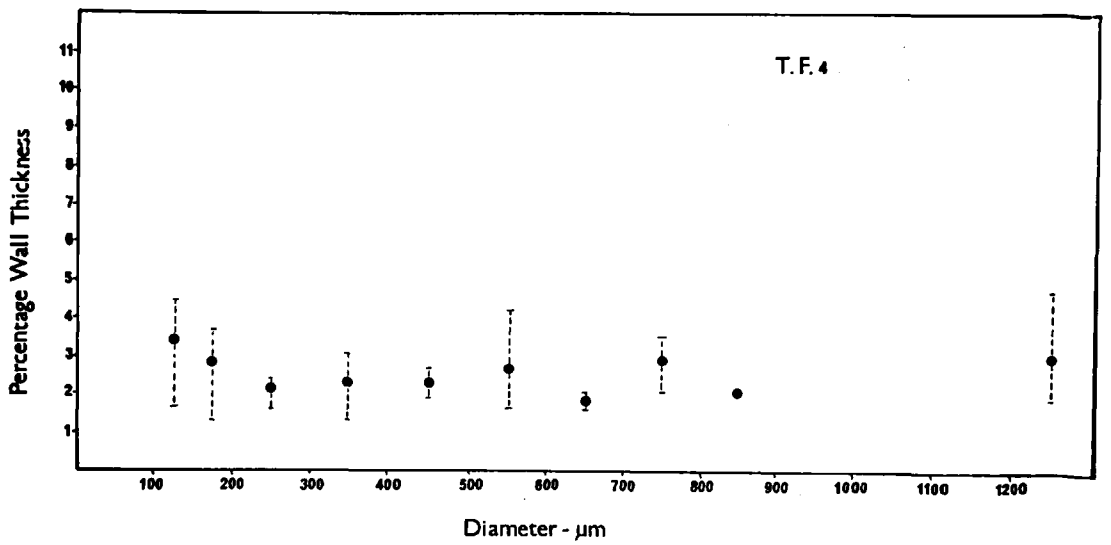
Figure VI 12

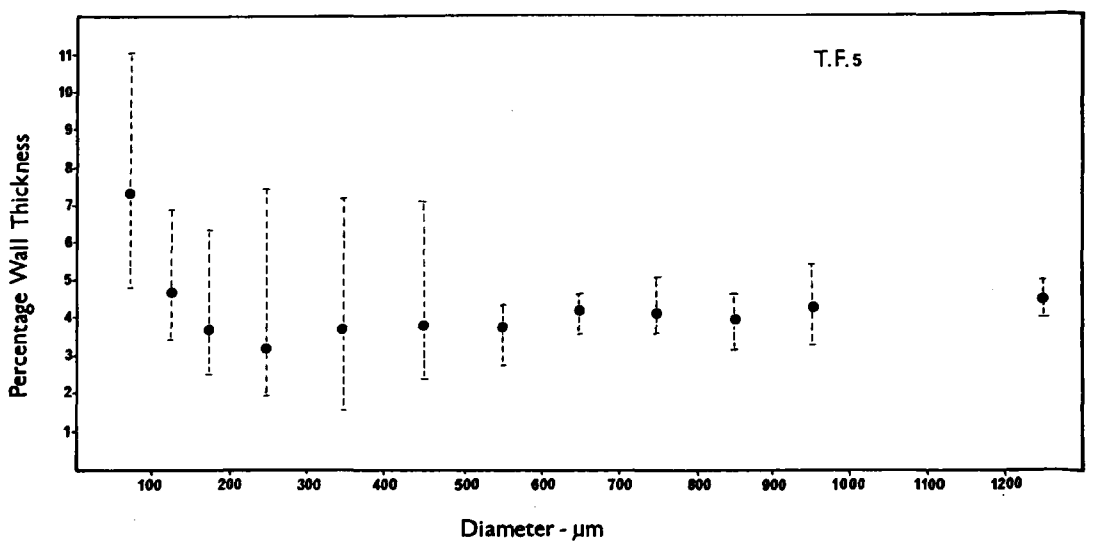
Percentage wall thickness related to external diameter for arteries from case T.F.4. The mean and range for each diameter range is shown.

Figure VI 13

Percentage wall thickness related to external diameter for arteries from case T.F.5. The mean and range for each diameter range is shown.


T.F. 3
Percentage Wall Thickness
Diameter - µm


T. F. 4
Percentage Wall Thickness
Diameter - µm


T.F. 5
Percentage Wall Thickness
Diameter - µm

A mixed population of small arteries was seen in all cases of this series and with a roughly similar distribution to the normal: some showed an increase in muscularity. In case T.F.1, aged one year, the diameter ranges for the types of artery are very similar to those of the normal at ten months (see Table IV 25). The peak of the partially muscular curve is at the same point though it is higher.

The other four cases (from $3\frac{3}{4}$ to 8 years) can be compared with the normal curves of a four and a five year old child. The population curves of case T.F.2 are shifted to the left in comparison to the normal, that is muscle was found in the smaller vessels than in the normal. The smallest muscular artery was only 78 μm in diameter as opposed to 135 μm in the normal at four years. The largest non-muscular vessel was 112 μm as opposed to 160 μm, and the partially muscular arteries covered a smaller range from 50 - 185 μm as opposed to 50 - 320 μm.

Case T.F.3 shows curves very similar to the normal; there was a normal distribution of muscle in this case. Cases T.F.4 and 5 both show a shift to the left, with the smallest muscular artery being smaller than in the normal. The first vessel to have even part of the wall with no muscle was smaller in diameter than in the normal. In these cases muscle extended into smaller arteries than in the normal of five years. In the ten year old normal there is a shift to the left and these cases of six and eight years may be approaching the adult structure. It is more likely that this is an abnormal shift since in the 11 year old normal the muscle still did not extend into smaller arteries and this is probably the age of change.

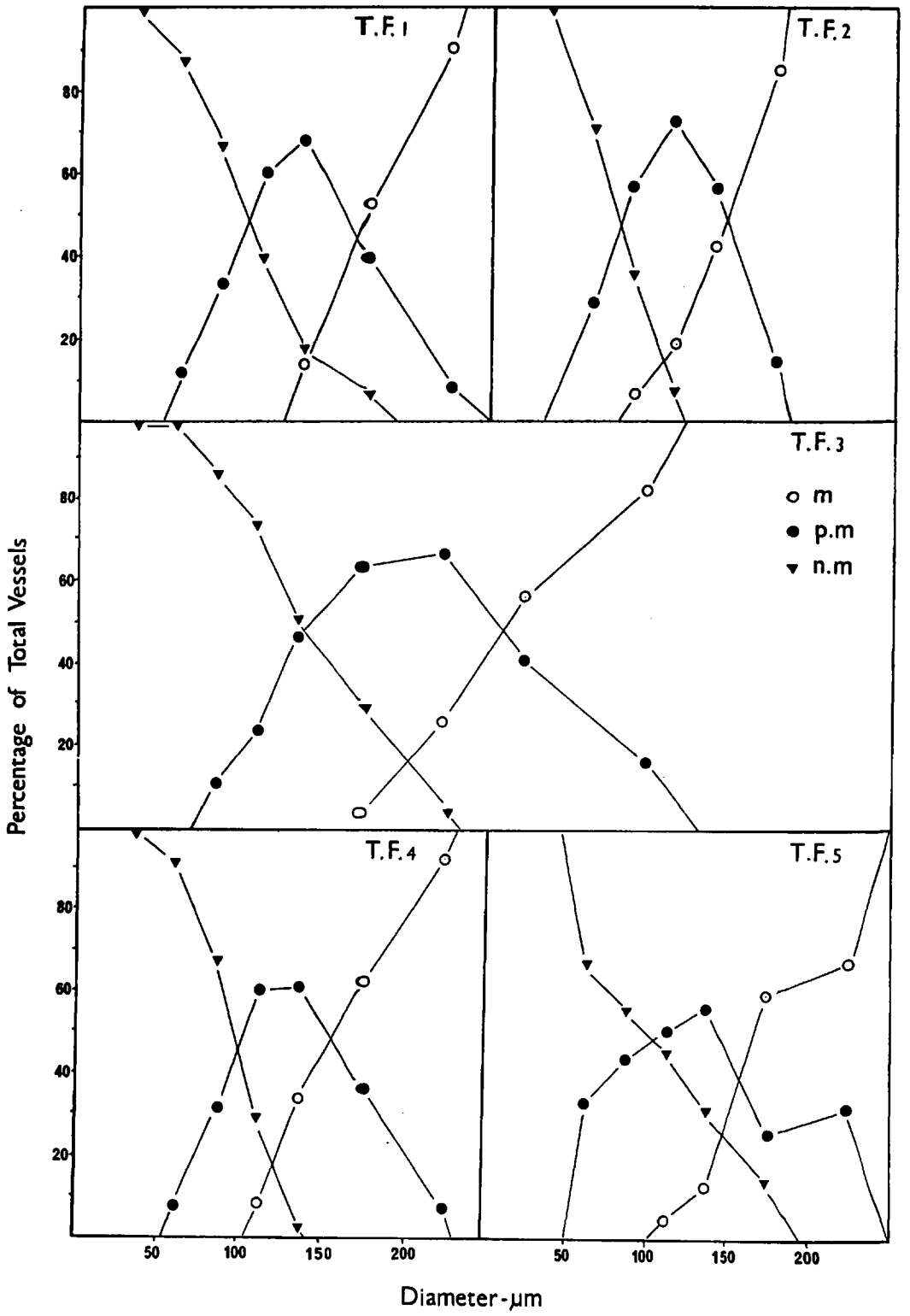
Figure VI 14

Population distribution curves for the five cases with tetralogy of Fallot. The percentage of the total vessels with a given structure in each size range of vessels is shown.

m - muscular arteries

p.m - partially muscular arteries

n.m - non-muscular arteries


T.F.1
T.F.2
T.F.3
T.F.4
T.F.5
o m
● p.m
▼ n.m
Percentage of Total Vessels
Diameter-µm
20
40
60
80
50
100
150
200

It seems that cases T.F.1 and 3 had a normal extension of muscle while the other three cases had muscle extending into smaller vessels than normal for their age.

The percentage of the vessels measured in this study with muscular, partially muscular and non-muscular artery structure was estimated for each case. The results are shown in Table VI 11. The percentages for case T.F.1 were similar to those of the normal at ten months. The other four cases all showed a reduction in the percentage of partially muscular vessels and an increase in the percentage of muscular, in comparison with the normal child. The proportions were similar to those seen in the perinatal period. The percentage of partially muscular was higher than in the younger fetuses and the adult (see Chapter IV).

Arteries with Airways

Measurements of external diameter and the structure of the vessels accompanying identifiable airways within the lobule were also made for the five cases of tetralogy of Fallot.

Diameter of Arteries with Airways

In each case the average diameter of arteries within the lobule accompanying airways decreased towards the periphery, as it does in the normal. The largest arteries accompanied terminal bronchioli. In nearly all cases the range of diameter of the arteries with each type of airway overlapped at each level.

The average size at each level was compared in each case

Table VI 9

Distribution of Structure by Size in Small Vessels in Cases with Tetralogy of Fallot

Case	Smallest muscular (μm)	Range of partially muscular (μm)	Largest non-muscular (μm)	Peak of partially muscular (μm)	%
T.F.1	130	60-225	162	137.5	68
T.F.2	78	50-185	112	112.5	73
T.F.3	160	75-360	208	225	68
T.F.4	100	62-220	137	137.5	62
T.F.5	125	50-230	160	137.5	56
Normal					
10 mths	112	55-220	125	137.5	81
4 yrs	135	50-320	160	175	83
5 yrs	155	50-370	202	175	75

TABLE VI 10

Percentage of Vessels of each Type in each Size Range

Case	%	Diameter Ranges (μm)							
		25-50	50-75	75-100	100-125	125-150	150-200	200-250	250-300
	n	100	88	67	40	18	7	0	0
T.F.1	p.m	0	12	33	60	68	40	9	0
	m	0	0	0	0	14	53	91	100
	n	100	71	36	8	0	0	0	0
T.F.2	p.m	0	29	57	73	57	15	0	0
	m	0	0	7	19	43	85	100	100
	n	100	100	88	75	52	30	5	0
T.F.3	p.m	0	0	12	25	48	65	68	42
	m	0	0	0	0	0	5	27	58
	n	100	92	68	30	4	0	0	0
T.F.4	p.m	0	8	32	61	61.5	37	7	0
	m	0	0	0	9	34.5	63	93	100
	n	-	67	56	45	31	14	0	0
T.F.5	p.m	-	33	44	50	56	26	33	0
	m	-	0	0	5	13	60	67	100
Normal									
	n	100	97	75	32	5	0	0	0
10 mth	p.m	0	3	25	61	81	63	17	0
	m	0	0	0	7	14	37	83	100
	n	100	95	75	31	14	3	0	0
4 yr	p.m	0	5	25	69	81	83	55	33
	m	0	0	0	0	5	14	45	67
	n	100	86	60	45	40	15	4	0
5 yr	p.m	0	14	40	55	60	75	63	60
	m	0	0	0	0	0	10	33	40

n = non-muscular
p.m = partially muscular
m = muscular

TABLE VI 11

Percentage of Each Type of Vessel in Total Population

Case	Total number of vessels	Percentage		
		non-muscular	partially muscular	muscular
T.F.1	194	44	35	21
T.F.2	206	31	40	29
T.F.3	144	53	34	13
T.F.4	184	42	35	23
T.F.5	110	38	38	24
Normal				
10 months	180	49	34	17
5 years	442	39	49	12

TABLE VI 12

Diameter of Arteries with Airways

Case		Diameter of Arteries Accompanying Airways (μm)			
		Terminal Bronchiolus	Respiratory Bronchiolus	Alveolar Duct	Alveoli
T.F.1	Mean	242	148	101	53
	Range	180-320	95-225	60-165	37-70
T.F.2	Mean	216	157	99.7	43
	Range	162-260	102-220	62-162	32-55
T.F.3	Mean	259	158	96.6	68.5
	Range	205-350	72-245	62-162	67-70
T.F.4	Mean	385	211.9	126	64
	Range	325-400	127-310	70-187	35-102
T.F.5	Mean	457	231.5	132.6	68.6
	Range	390-500	150-330	75-212	35-135
Normal					
10 mth	Mean	301	184	90.5	-
4 yr	Mean	391.5	234	140	-
5 yr	Mean	513	257	151	43.4

with a normal of the same age. In all five cases at all levels the arteries were of smaller diameter than in the normal (Table VI 12). They were all reduced by a substantial and similar amount. In the first three cases the arteries were smaller than the normal ten month child. The two older cases of six and eight years had arteries of smaller size than those for a normal four year old, except those accompanying the terminal bronchioli in the eight year old which were larger than those of a normal four year old but smaller than those of a five year old.

The peripheral vessels showed the same trend as the hilar vessels, measured on the arteriograms, in being smaller than normal.

Structure of Arteries with Airways

The percentage of each type of vessel structure accompanying each identifiable airway is shown for all cases in Table VI 13, together with the results of the normal of ten months, four and five years. In all five cases the arteries with the terminal bronchioli were 100% muscular as they are in the normal of similar age. Accompanying the respiratory bronchioli and alveolar ducts was a mixed population of all three types of vessel, in varying proportion. The arteries running with alveolar ducts were less muscular than those with the respiratory bronchioli. Those arteries with the alveoli were all non-muscular except in the case T.F.4 where a small percentage was partially muscular.

Case T.F.1 was less muscular than the normal at ten months, having a greater percentage of non-muscular vessels with the respiratory bronchioli and alveolar ducts. Case T.F.2 was of about normal muscularity while case T.F.3 was slightly less muscular

TABLE VI 13

Structure of Arteries with Airways

Case	Percentage of Each Type of Vessel with Airways											
	Terminal Bronchiolus			Respiratory Bronchiolus			Alveolar Duct			Alveoli		
	n	p.m	m	n	p.m.	m	n.	p.m	m	n.	p.m	m
T.F.1	0	0	100	41	41	18	80	20	0	100	0	0
T.F.2	0	0	100	0	24	76	34	59	7	100	0	0
T.F.3	0	0	100	14	38	48	50	50	0	100	0	0
T.F.4	0	0	100	3	38	59	37	42	21	86	14	0
T.F.5	0	0	100	0	64	36	41	59	0	90	10	0
Normal												
10 mth	0	0	100	11	72	17	50	50	0		-	
4 yr	0	0	100	0	58	42	37	50	13		-	
5 yr	0	0	100	0	65	35	66	17	17	100	0	0

n - non-muscular
p.m - partially muscular
m - muscular

than normal. Case T.F.4 had perhaps slightly more muscle than the normal. Case T.F.5 had similar muscle distribution to a normal five year old and was therefore probably less muscular than a normal at eight years.

In none of the cases was there a great difference from the normal and the results may well lie within the limits of individual variation. The extension of muscle revealed in the population counts of cases T.F. 2, 4 and 5 was not seen, perhaps because of the reduction in size of the arteries within the acinus.

Ventricular Weights

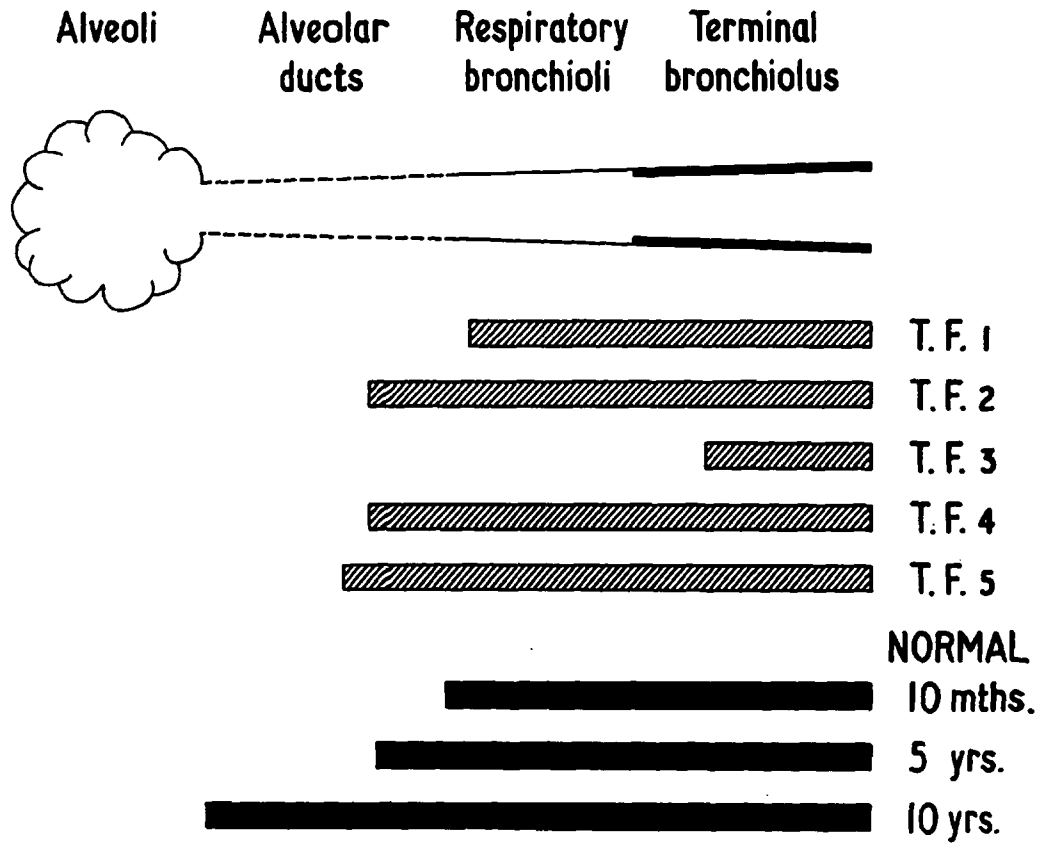
The hearts of cases T.F. 1, 2 and 5 were available for dissection. These were dissected and weighed using the method of Fulton, Hutchinson and Jones (1952). The ratio of left ventricle plus septum to right ventricle was 1.24:1 in case T.F.1, 0.87:1 in case T.F.2 and 1.35:1 in case T.F.5. Normal children of the age of these children would be expected to have the normal adult ratio of 2.3 - 3.3:1. In all three cases there was therefore right ventricular hypertrophy. The hearts of the other two cases were not available for study, but electro-cardiographic findings had indicated right ventricular hypertrophy.

Extension of Muscle into the Acinus

Using the results already described it is possible to estimate the changes in the structure of the vessels within the lungs of these cases. It is particularly important to estimate the extent to which muscle is found within the arteries of the acinus. Figure VI 15

Figure VI 15

Diagram illustrating the extension of muscle in the walls of arteries within the acinus in the five cases of tetralogy of Fallot and three normal cases. The end of the lines represents the diameter at the peak of the partially muscular population and the position in the acinus that an artery of this diameter is likely to be found.


Alveoli
Alveolar ducts
Respiratory bronchioli
Terminal bronchiolus
T. F. 1
T. F. 2
T. F. 3
T. F. 4
T. F. 5
NORMAL
10 mths.
5 yrs.
10 yrs.

shows the extent of muscle in these five cases as well as in the normal lungs of similar age. The end of the muscular portion has been taken as the diameter of the arteries at the peak of the partially muscular curve of the population count. Below this diameter there will be very few muscular vessels and there are only a few non-muscular vessels larger than this. The average diameter of the arteries accompanying the airways in the acinus are used as landmarks.

The muscle in case T.F.1 did not extend quite as far as it did in the normal at ten months. This may be due to individual variation and not be significant. In cases T.F.2 and 4 there was apparently normal extension of muscle in comparison with the normal of five years. In case T.F.3 the muscle did not extend into the acinus as far as in the normal. In case T.F.5 the muscle extended further than in the normal five year old but not as far as in the normal at ten years. Since this child was eight years old it is likely that the extension was normal.

In summary, in two cases there was reduction in the amount of muscle in the acinar arteries and in three cases there was normal distribution.

Arteries and Alveoli per Unit Area of Lung

Alveoli per Unit Area of Lung

The total number of alveoli in one square centimetre was estimated and the results are given in Table VI 14. Case T.F.1 had a normal number of alveoli/sq cm for age. The other four cases all had a smaller number of alveoli than the normal for the same age,

that is the alveoli were larger, and there was therefore a slight degree of emphysema in these cases. Since the total volume of these lungs was less than normal the overall number of alveoli was reduced - that is there was alveolar hypoplasia.

Arteries per Unit Area of Lung

The number of arteries, up to a diameter of 200 μm, in one square centimetre of alveolar region was estimated. The arteries were divided into size ranges from 25 - 200 μm first at 25 and then at 50 μm intervals. The total number and the percentage in each size range is shown in Table VI 15. The total number can be compared with the normal of the same age. Case T.F.1 would seem to have a number falling between the normal 10 and 18 month figures and was therefore probably normal. Case T.F.2 would seem to have a total number similar to the normal four year old while cases T.F.3, 4 and 5 had an increased number over those normals of four, five or ten years. The two oldest cases had nearly double the normal number.

The distribution of the vessels by size range was near normal in cases T.F.1 and 2 but in the other three cases there was a greater proportion of the small arteries between 25 and 75 μm. They showed a similar distribution to the two younger cases and to younger normals. The increase in vessels in the three older cases was almost all in the smaller vessels below 75 μm, so that all five cases were very similar in their total number and distribution, despite the difference in age.

TABLE VI 14

Number of Alveoli in One Square Centimetre of Lung Tissue and the Ratio of Alveoli to Arteries

Case	Alveoli/sq.cm	Ratio Alveoli/Arteries
T.F.1	10,541	31.37
T.F.2	7,462	19.95
T.F.3	8,644	23,11
T.F.4	7,251	20.89
T.F.5	7,313	22.16
Normal		
10 mth	12,418	43.26
4 yr	9,529	40.8
5 yr	9,123	46.8
10 yr	8,580	47.9

Ratio of Alveoli to Arteries

The ratio of alveoli to arteries is shown in Table VI 14. In all, except case T.F.1, it was reduced below normal because of the relative decrease in the number of alveoli and the increase in the arteries. Case T.F.1 was probably normal because it was the youngest in the series and changes had not yet occurred.

In summary there was in four of the five cases a relative increase in the number of small arteries in the acinar region of the lung both in total and particularly in comparison with the reduced number of alveoli.

Venograms

In only one case of tetralogy of Fallot were the veins injected with radio-opaque medium using the usual laboratory technique. This case, T.F.6, was aged 4½ years. Injection of only the lower lobes was technically adequate. Figure VI 16 shows the venogram of the left lower lobe. It is difficult to see the axial pathways in this venogram because of the very dense background filling, not seen in the normal at this age. Some leaks had occurred from the veins into alveoli and this may have contributed in small part to the background haze. The tributaries to the main veins seemed to be smaller than normal.

Measurements on Venograms

The measurements made at 25% intervals along the axial pathways to the lower lobes are shown in Table VI 16 together with the figures for the normal three year old. The lumen diameter was less than that of the normal three year old along the whole length

Table VI 15

Number of Arteries per Square Centimetre of Lung Tissue and the Percentage in Each Size Range

Case	Total Number	% in each size range					
		25-50	50-75	75-100	100-150	150-200	(μm)
T.F.1	336	52	26	10	9	3	
T.F.2	231	44	27	10	14	5	
T.F.3	374	57	24	10	7	2	
T.F.4	347	50	26	10	11	3	
T.F.5	330	48	30	11	8	3	
Normal							
10 mth	287	45.6	29.9	12.5	8.3	3.8	
4 yr	233	39.9	29.1	15.8	10.4	4	
5 yr	195	44.6	20.0	11.7	14.8	8.7	
10 yr	179	32.9	29.6	13.9	16.2	8.3	

of the pathway, but the length of the pathway was the same as the normal three year old. Therefore both diameter and length were reduced for age, the diameter more than the length. This drop in diameter is related to the decreased lung volume in this case. The diameter one centimetre from the periphery was in the normal range, therefore the reduction was greater at the hilar region.

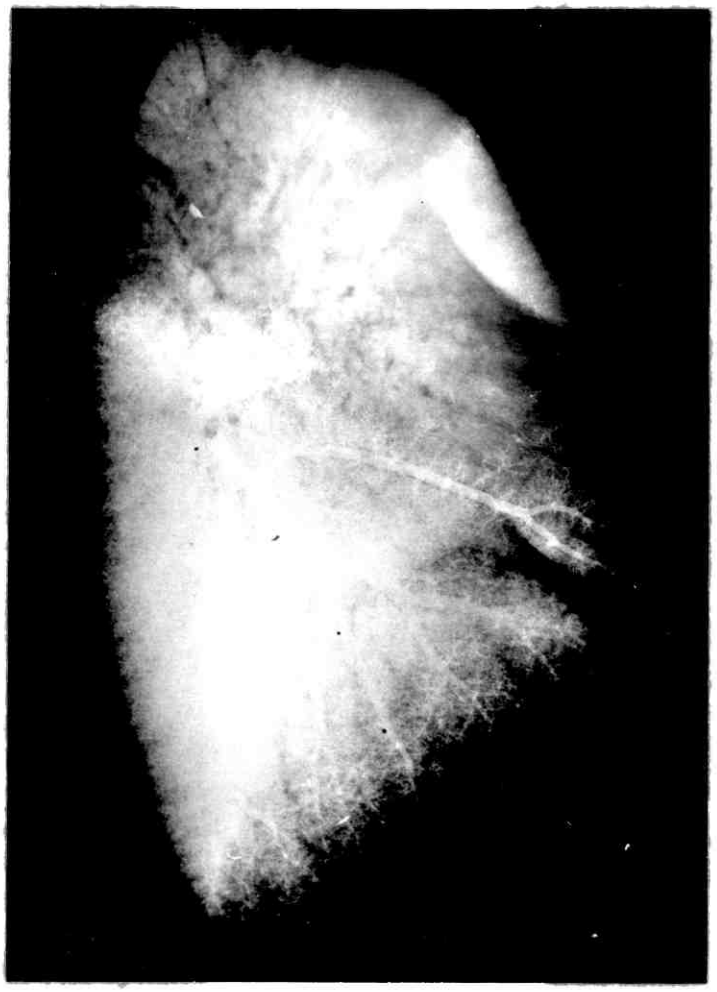
Drainage Pattern of Veins

The drainage pattern can be seen from the venogram. The overall pattern is normal though the tributary veins seem small. By tracing over a short distance (2 mm) near the periphery of the lung, using serial sections, it was possible to see that conventional and supernumerary veins were present. Both types of supernumerary were seen and the three types of vessel were in the normal proportions.

Veins in a Unit Area of Lung

The number of veins and alveoli in one square centimetre of acinar region was established using the same methods as for the arteries. The percentage of each size range of vein from 25 - 200 μm was calculated. The results are shown in Table VI 17 together with the results for the normal three year old child. The total number of veins was similar to the normal though slightly lower. The distribution between size ranges was abnormal in that there was a larger percentage of veins in the size range between 25 and 75 μm. A similar change to that seen in the arteries.

Figure VI 16



Venogram of the left lower lobe of case T.F.6 (x 0.65). There is a very dense background haze almost obscuring the main pathways which seem smaller than normal. The upper lobe is not injected save for part of the lingula.

TABLE VI 16

Measurements from Venograms of Axial Pathway

	Length (cm)	Diameter (mm) % distance along pathway				
		0	25	50	75	1 cm from periphery
Case T.F.6						
Right Lower Lobe	8	-	3.2	2.5	1.8	0.9
Left Lower Lobe	9	7	4	2.5	1.5	0.8
Normal 3 yr						
Left Lower Lobe	9	-	5	3	1.8	0.9

The total number of alveoli was reduced as in the other cases studied and the alveoli were therefore emphysematous. The ratio of alveoli to veins was reduced below normal because of the reduction in alveoli. There was therefore an overall increase in the relative numbers of veins particularly those below 75 μm.

Structure of Vein Walls

The veins in case T.F.6 had been injected and it was possible to compare them with the normal injected cases. They were of normal structure, with the vessels having muscle in the walls which increased with the size of the veins. As in the normal the limits of the media were ill defined particularly to the outside. It was also possible to study the structure of the vein walls in the other five cases in which the arteries had been injected. Like case T.F.6, cases T.F.2 and 3 had vein walls of normal appearance. Case T.F.1 on the other hand seemed to have less muscle in the media than normal. Case T.F.4 showed an increase in elastic in the media and this was in the form of laminae rather than discreet pieces; there was a change towards an arterial wall appearance. Case T.F.5 had veins with normal elastic and muscle but there was intimal thickening.

Measurements of Wall Thickness of Muscular Veins

The smallest veins with a muscular wall in the injected specimen were 90 μm in external diameter. The largest vessel with no muscle in its wall was 155 μm. The wall thickness of the muscular vessels could be measured accurately in case T.F.6 since it had been injected under pressure. The percentage wall thickness of these vessels plotted against diameter are shown in Figure VI 17. The

TABLE VI 17

Veins and Alveoli per Unit Area

	% of veins in each size range (μm)					Total/ sq.cm	Total Alveoli/ sq.cm	Ratio Alveoli/ Veins
	25-50	50-75	75-100	100-150	150-200			
T.F.6 Normal	47	38	10	4	1	312	5,920	18.97
3 yr	43	32	13	10	2	379	8,557	22.57

percentage wall thickness in all the veins measured was similar to the normal wall thickness. There was a slight increase in the percentage wall thickness in the veins less than 300 µm in external diameter, as in the normal.

The percentage wall thickness was also measured on the uninjected veins of the other five cases. Though this is less accurate than measurements on injected material it is possible to see trends. The results from the five cases together with figures from a normal uninjected specimen are shown in Figure VI 18. All five cases had much the same percentage wall thickness and were similar to the normal. Case T.F.5 had slightly thicker walls in the small vessels but there was a reduction in the larger vessels. There was a greater percentage wall thickness in the smaller vessels in all cases.

It seemed that the structure of the vein walls in cases of tetralogy of Fallot was normal. The main changes in the veins as a result of this lesion were a reduction in lumen diameter and a relative increase in the number of small veins in the acinar region.

Figure VI 17

Percentage wall thickness related to external diameter for veins from case T.F.6. Each point represents the measurement from one vein.

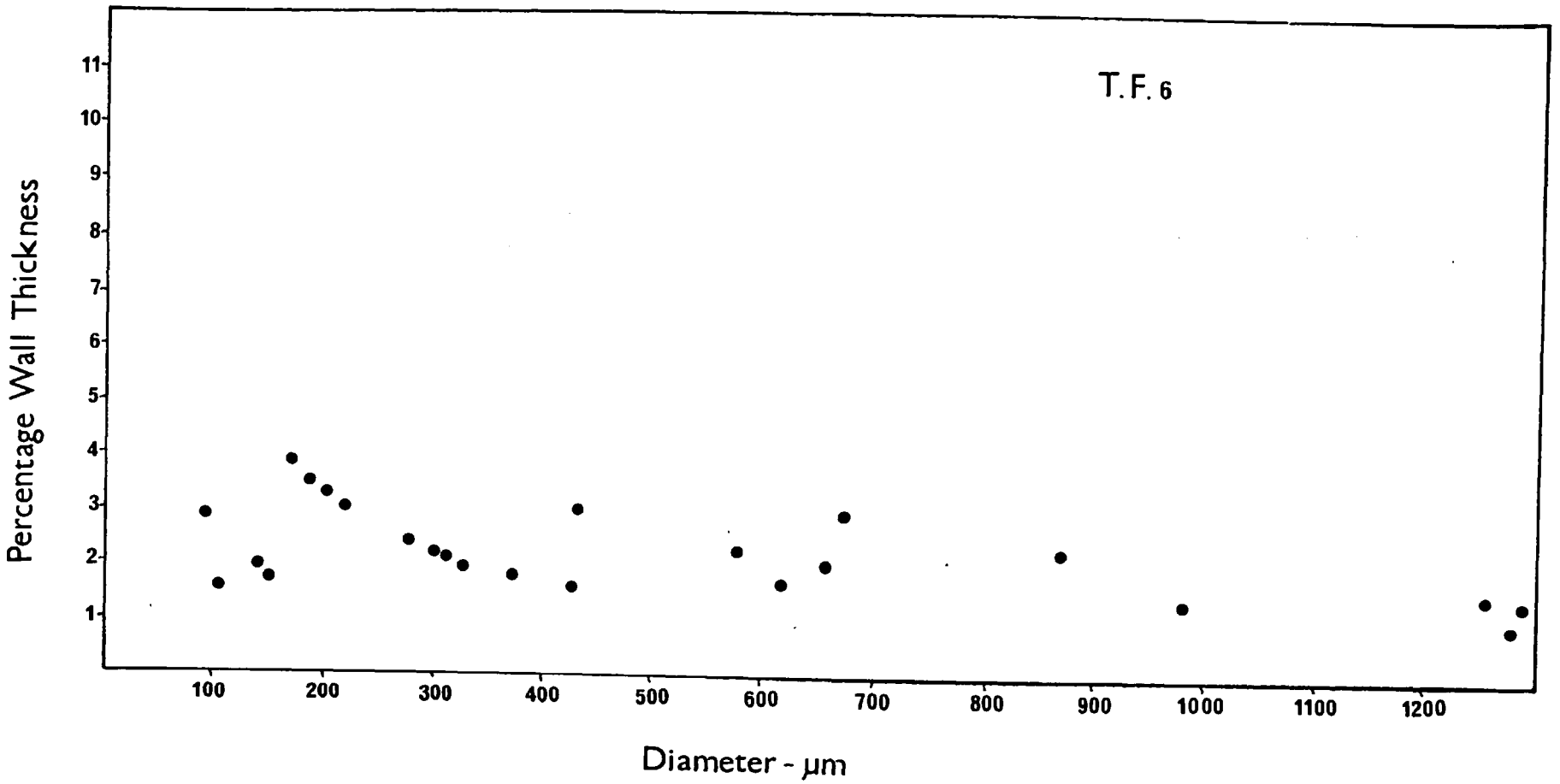
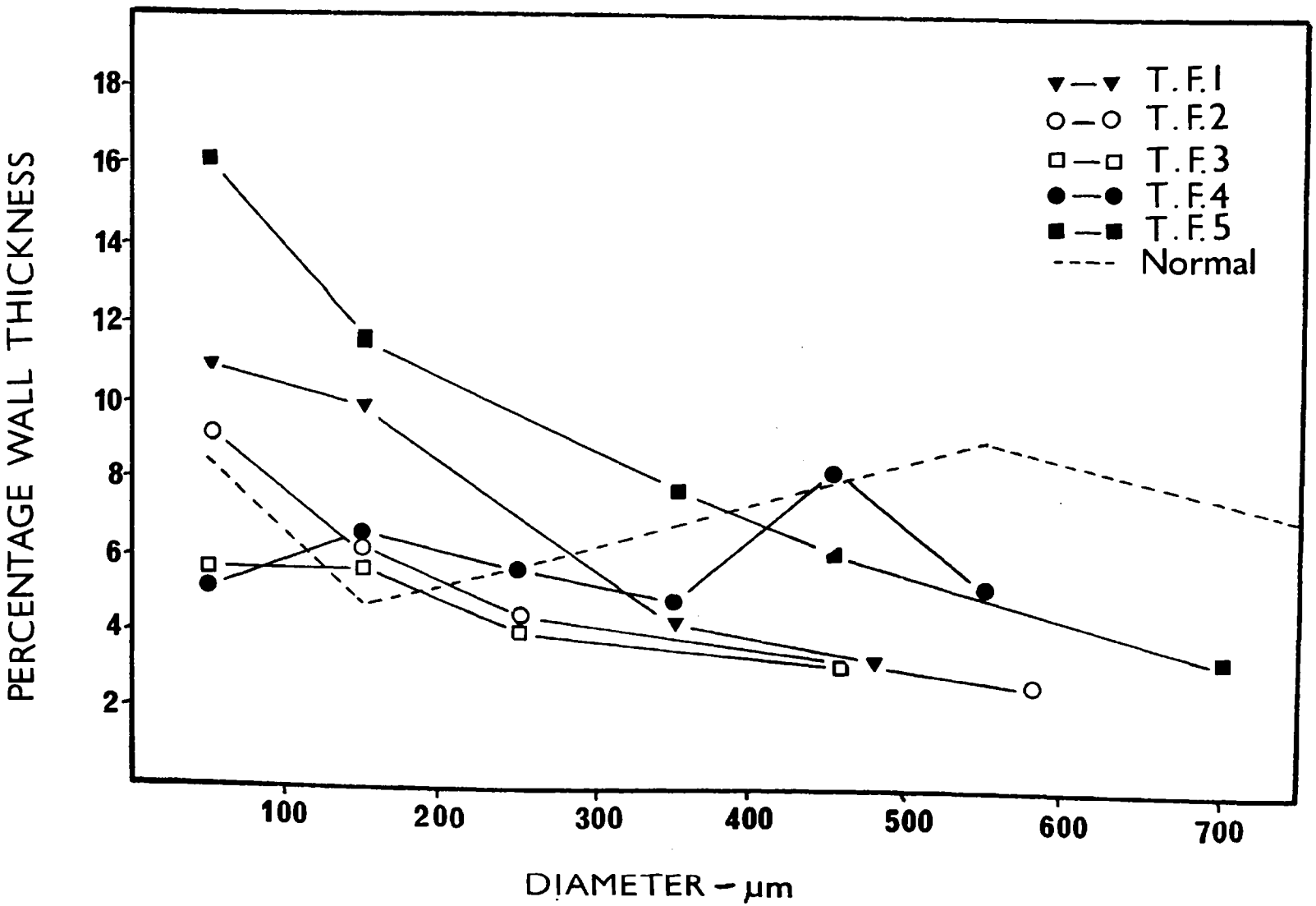

T.F. 6
Percentage Wall Thickness
11
10
9
8
7
6
5
4
3
2
1
100
200
300
400
500
600
700
800
900
1000
1100
1200
Diameter - µm

Figure VI 18

Percentage wall thickness related to external diameter for the uninjected veins of cases T.F.1, 2, 3, 4 and 5. Each point represents a mean value for the size range. The normal line represents measurements made on uninjected veins from a number of cases of children.


PERCENTAGE WALL THICKNESS
18
16
14
12
10
8
6
4
2
100
200
300
400
500
600
700
DIAMETER – µm
T.F.1
T.F.2
T.F.3
T.F.4
T.F.5
Normal

Structural Features in Tetralogy of Fallot - Summary of the Six Cases

The structural findings in the six cases studied can be summarised briefly (see Table VI 18).

Case T.F.1

This case, aged 13 months, had a normal branching pattern on the arteriogram, though all the arteries had a narrower lumen than in the normal. Microscopically the external diameter of the vessels was also reduced. There was a very slight increase in percentage wall thickness which would contribute only little to the reduction in lumen diameter. There was a normal distribution of muscle by size of arteries. The arteries of the acinus were hypoplastic and therefore were less muscular than normal. The number of vessels in the acinar region was normal. The normal muscle distribution was seen in a case with marked right ventricular hypertrophy.

Case T.F.2

This case was aged $3\frac{3}{4}$ years. The arteriogram had a normal branching pattern though the vessels were small throughout the lung. The vessels accompanying distal airways were also smaller than normal. There was an increase in muscle in the walls of the artery, there being an increase in the percentage wall thickness. There was also muscle found in smaller vessels than in the normal. Since muscle extended into smaller vessels, the narrower vessels of the intra-acinar region were of normal muscularity and muscle extended to a normal distance. The number of arteries within the acinus was normal but in a rather high proportion to the reduced

TABLE VI 18

Summary of Structural Changes in the Arteries in Cases with Tetralogy of Fallot

Arteriograms	T.F.1	T.F.2	T.F.3	T.F.4	T.F.5
Pattern	N	N	N	N	N
Lumen Diameter	-	-	-	-	-
Wall Structure					
% wall thickness	+	++	(+)	+	++
Muscle extension into small vessels	N	+	N	+	+
Muscle extension into acinus	-	N	-	N	N,+
Diameter of arteries with airways	-	-	-	-	-
Structure of arteries with airways	-	N	-	+	N
Right ventricular hypertrophy	+	+	+	+	+

N - Normal
+ - Increase in size or muscle
- - Decrease in size or muscle

number of alveoli.

Case T.F.3

This child of similar age to case T.F.2 ($4\frac{1}{4}$ years) had a normal arteriogram save for the reduction in size of the lumen throughout the lung. The external diameter of the distal arteries measured microscopically was also reduced. There was no increase in muscularity since there was a normal percentage wall thickness and no extension of muscle into smaller vessels. There was therefore less muscle in those arteries accompanying acinar airways than in the normal and muscle did not extend so far into the acinus. This case was similar to case T.F.1, but in this case there had been an increase in the number of small arteries and the number of alveoli was low.

Case T.F.4

This case, aged six years, had an arteriogram of normal pattern with reduced lumen diameter. There was also a reduction in external diameter. There was an increase in muscle in the arteries of the lung since there was a raised percentage wall thickness and extension of muscle into smaller vessels than normal. The vessels accompanying intra-acinar vessels were also more muscular than normal, but the overall extension of muscle into the acinar arteries was normal. The vessels within the acinus were increased in number and the alveoli reduced.

Case T.F.5

The pattern of branching in this case, aged eight years, also

was normal, though the lumen diameter was markedly reduced. The wall thickness was greatly increased and there was extension of muscle into smaller vessels than normal. However since the vessels in the acinus were small there was no abnormal extension of muscle. This was a similar pattern to that seen in cases T.F.2 and T.F.4. The vessels in the intra-acinar region, particularly the non-muscular ones were increased in number. There was a reduction in the number of alveoli.

Case T.F.6

In this case, aged 4½ years, the veins had been injected. The venogram showed a normal drainage pattern and this was confirmed microscopically. The lumen diameter of the veins was reduced at all levels. There was no change in the structure of the vein walls and muscle was found in vessels of normal diameter. There was an increase in the relative number of small veins, mainly as a result of a decrease in the number of alveoli. The arteries did not show any obvious structural changes.

B. VENTRICULAR SEPTAL DEFECT

The arteries to the lungs from five cases of ventricular septal defect were injected under the usual laboratory conditions. In only one of these cases was the ventricular septal defect the only anomaly. In one case there was also an atrial septal defect and in three cases an atrial septal defect as well as a patent ductus arteriosus. At a later date it was decided to study the veins after injection. Since no case of ventricular septal defect was available at this time, the veins of a patient with a patent ductus arteriosus (hereafter called V.S.6) were studied since this lesion also causes an increase in flow to the lungs. The veins were injected in only the right lung of case V.S.6 but in all other cases both lungs were available for study.

The full histories of the six cases are given in Appendix B, but the relevant features are summarised in Table VI 19. The patients ranged in age from two weeks to three months, but their clinical features and results of investigation were very variable despite the similar heart lesions.

On the clinical radiographs and angiograms the intrapulmonary vessels were normal in size in two cases, decreased in one and increased in three. In all cases the right ventricular pressure and pulmonary artery pressure were increased to systemic levels, though the degree of elevation varied. In only five cases were catheter results available. In all of these cases there was a degree of systemic arterial desaturation but this varied from case to case. In three cases there was an increase in flow to the lungs and in two a decrease. The three with the increase had

TABLE VI 19

Clinical Features of Cases with Ventricular Septal Defect

Case	Age	Diagnosis	Artery size seen on X-ray	Pulmonary Artery Pressure (mm Hg)	Resistance	Pulmonary/ Systemic Flow	Flow Litres/ minute	Systemic Saturation %
V.S.1	3 wk	V.S.D.	Normal	60/5	Increased	0.3:1	7	37-49
V.S.2	2 wk	ASD, VSD, PDA	Large	84/38	Not known	1.4:1	Not known	78
V.S.3	2 mth	ASD, VSD	Normal	45/7	Normal	9.3:1	32	98
V.S.4	3 mth	ASD, VSD, PDA	Small	75/40	Increased	0.2:1	4	45
V.S.5	3 mth	ASD, VSD, PDA	Large	55/15	Increased	1.1:1	4	75
V.S.6	3 wk	PDA	Large	Not available				

VSD - Ventricular septal defect
ASD - Atrial septal defect
PDA - Patent ductus arteriosus

normal or large vessels on the radiographs. It is likely that there is an increase in flow in case V.S.6 since the arteries are increased in size. The resistance was increased in all save case V.S.3 where flow was greatly increased and pressure increased only to a lesser degree.

In all the studies made on these cases the results from the normal children aged three days and four months have been used for comparison. They are shown in the tables along with the abnormal results.

Volume of lungs

The volumes of the lungs from all six cases are shown in Table VI 20. In comparison with the normal it would seem that cases V.S.1, 2, 4 and 5 are within the normal range while case V.S.3 has a rather high lung volume and case V.S.6 a rather reduced one.

Macroscopic Appearance of the Lungs

Case V.S.1 had an unusual pattern of lobation in that there was separation of the fourth lobe on the hilar surface of the right lung. When dissected it was found that this was in fact a subsegment of the right middle lobe. The left lung had three well defined lobes. There were no pleural adhesions and no visible filled bronchial vessels. The lung was spongy when cut.

TABLE VI 20

Volume of Lungs in Ventricular Septal Defect

Case	Age	Left Lung (cc)	Right Lung (cc)	Total (cc)
V.S.1	3 wk	90	65	155
V.S.2	2 wk	60	70	130
V.S.3	2 mth	145	170	315
V.S.4	3 mth	95	110	205
V.S.5	3 mth	80	110	190
V.S.6	3 wk	-	55	110
Normal				
3 days		-	-	155
4 months		108	115	223

Case V.S.2 had normal lobation: the lung was firm but with no pleural adhesions. Case V.S.3 also had normal lobation and the lungs were very firm on cutting. The connective tissue septa appeared obvious on the cut surface of the lung. Case V.S.4 had normal lobation but the lingula was tongue shaped and there was a partial fissure between it and the rest of the upper lobe. After cutting there were a large number of large air spaces visible at the periphery of the lung in the alveolar region.

The lungs of case V.S.5 were rather unusual in shape. The lingula of the left lung was a long extension and the oblique fissure hardly perceptible although the right middle lobe was almost completely separated from the other two lobes of this lung. The lung was firm with obvious connective tissue septa. The lung of case V.S.6 was of normal lobation and texture. The venous filling was visible through the pleura but there was no filling of pleural or other bronchial vessels.

Microscopic Appearance of the Lungs - General

Case V.S.1 The lung was not fully inflated and the saccules were still the main respiratory unit, there being few alveoli. The alveolar walls were thicker than in the adult and resembled those of fetal life. There was some cellular infiltrate mainly of macrophages in the alveoli, and scattered areas of oedema, but the airways were clear. The connective tissue septa were very prominent with enlarged lymphatic vessels. There were no bronchial vessels filled even at the hilum. The veins and arteries appeared to be particularly thick walled, and the artery walls had the fetal elastic configuration of marked laminae.

Case V.S.2 Though the lungs were fully inflated the alveolar walls were still thicker than in the adult; there were still saccules present with small alveoli. There was no oedema and the airways were normal. There were no filled bronchial arteries and the veins and arteries appeared to have a normal wall structure.

Case V.S.3 The right lower lobe was fully inflated and in this region the alveoli were mature in shape though had rather thick walls. There was no oedema and the airways were normal. In the right upper lobe there were areas of consolidation. The veins and connective tissue septa were normal and there was no bronchial filling even at the hilum. The arteries were very thick walled with marked elastic laminae.

Case V.S.4 The lung was fully inflated and in spite of this the alveoli were still thick walled, normal at this age. There were some areas of oedema and congestion of the alveoli but the airways were clear. The pleura and the connective tissue septa were thick and the lymphatics were large and empty. The arteries and veins were very thick walled with marked elastic laminae. There was no bronchial artery filling and therefore no pre-capillary anastomoses.

Case V.S.5 The lungs were fully inflated and the alveoli were thin walled with scattered oedema. There were inflammatory cells in the alveoli but the airways were clear. The pleura and connective tissue septa were increased in size and prominent with dilated lymphatics. A few bronchial arteries were filled with injection medium close to the hilum. The veins and arteries were normal in appearance.

Case V.S.6 The lung was fully inflated but the alveolar walls were still quite thick. There was evidence of oedema and inflammatory regions in the alveoli and there were regions of cellular infiltration in the bronchioli. The unfilled arteries appeared to be particularly thick walled, and some seemed very large in comparison with the bronchi they accompanied. The injected veins seemed normal in appearance. A few pre-capillaries had been filled but not a large number of alveolar capillaries. Bronchial arteries and veins were filled with injection medium.

Arteriograms

The arteriograms of cases V.S.1 to 5 are shown in Figures VI 19 - 23. In all five cases there was adequate filling of all lobes of the lung. Some of the vessels in cases V.S.1, 2 and 3 were not fully filled since they showed square endings due to premature setting of the injection medium. This happens in the normal but not very often and it is possible that these vessels were harder to fill than the normal. In all these cases some pathways were completely filled and there was always complete filling of the small vessels close to the hilum.

The arteries in case V.S.1 seemed smaller in diameter than normal but the branching pattern was normal. The arteriogram of case V.S.2 showed that the axial pathways were increased in size giving a crowded appearance. The arteries at the periphery were tortuous. The vessels in case V.S.3 were of normal density but seemed increased in size particularly at the hilum. There was some tortuosity of the peripheral vessels. The arteriogram of case V.S.4 was crowded with vessels of small calibre but with no pruning or tortuosity of peripheral vessels. The arteriogram

of case V.S.5 was the most abnormal having very large hilar and axial vessels. There appeared to be a reduction in the number of small vessels at the periphery and there was tortuosity of the vessels both proximally and distally, so increasing the length as well as the diameter of the vessels.

Measurements from Arteriograms

Measurements were made at 25% intervals along the axial pathway to the posterior basal segment of the left lower lobe and to the apical segment of the left upper lobe. The total length of each pathway was also measured.

Length of Pathway

In all cases the upper lobe pathway was shorter than the lower as in the normal. In all the length of this pathway was similar to that of the normal at the *sage age* (Table VI 21).

Diameter of Lumen along Axial Pathway

The diameter of the upper and lower lobe arteries at 25% intervals along the axial pathways is shown in Table VI 21. The results are plotted together with the normal figures in Figure VI 24.

In the upper and lower lobe of case V.S.1 the hilar diameter was slightly smaller than the normal of three days. At the periphery of the lung it was smaller in the lower lobe but larger in the upper.

In the upper lobe of case V.S.2 the lumen diameter at the hilum was as large as a normal at four months and was even larger at the

Figure VI 19

Arteriogram of case V.S.1 (x 1). The branching pattern is normal but the main pathways are rather narrower than normal. The cut off of the injection medium along some of the pathways may be technical.

Figure VI 20

Arteriogram of case V.S.2 (x 1.2). The axial pathways are increased in size giving a crowded appearance. The arteries at the periphery are tortuous and there is a dense background haze.

Figure VI 19

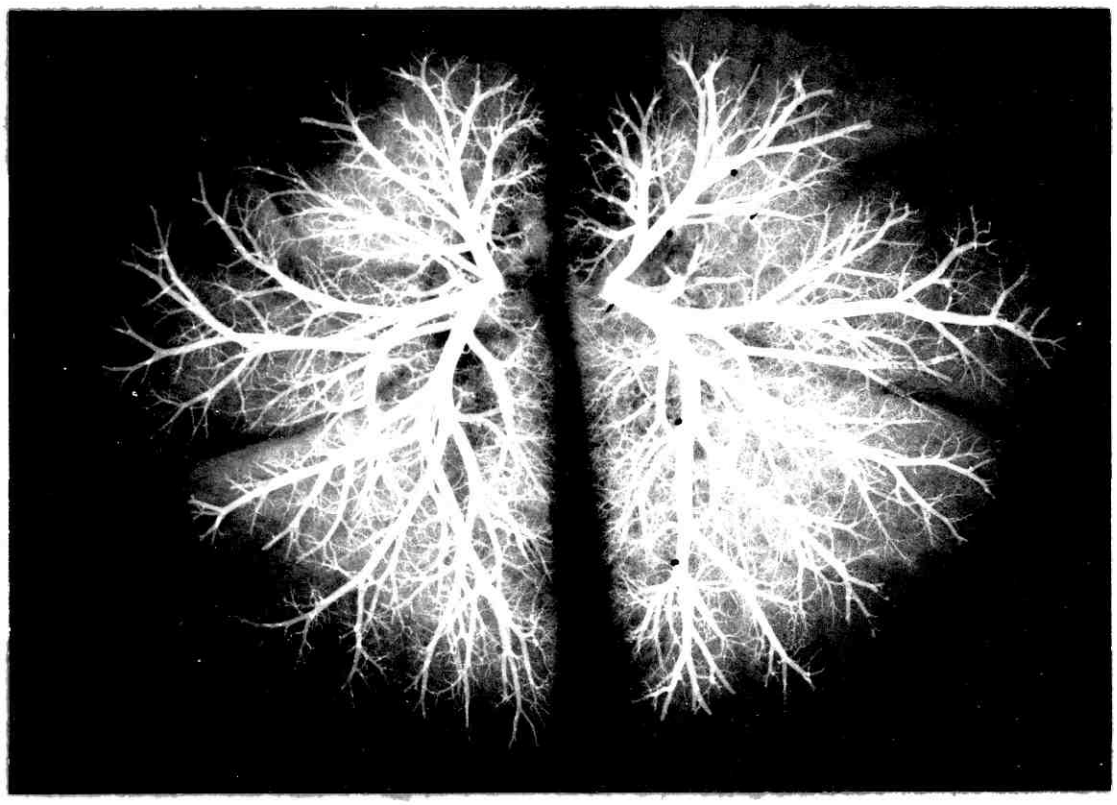


Figure VI 20

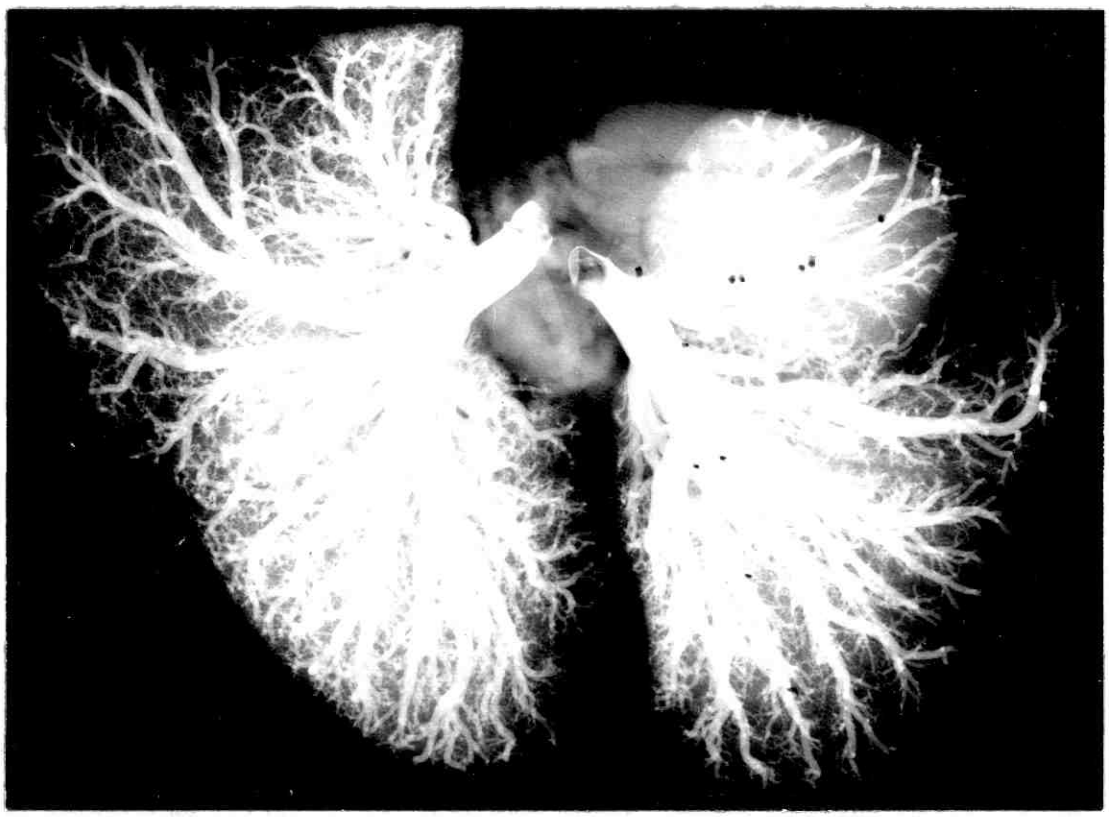


Figure VI 21

Arteriogram of case V.S.3 (x 0.75). There is a normal branching pattern and the vessels are of normal density. The vessels are increased in size particularly at the hilum. There is some tortuosity of the peripheral vessels.

Figure VI 22

Arteriogram of case V.S.4 (x 0.9). There is dense vessel filling but no increase in size of vessels. There is no tortuosity of the peripheral vessels.

Figure VI 21

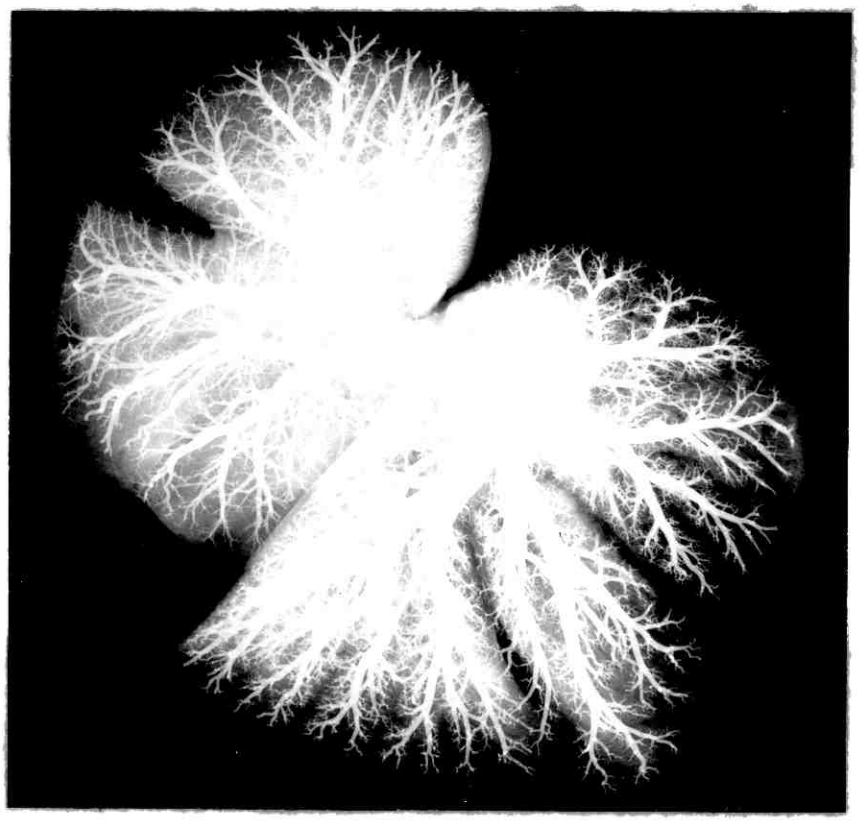


Figure VI 22

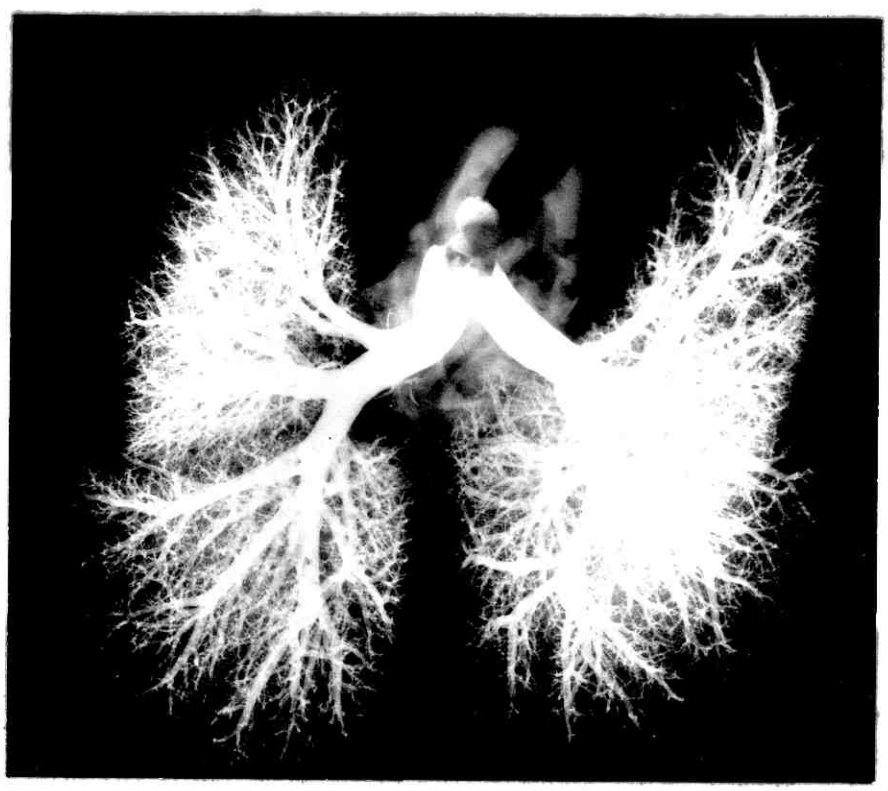
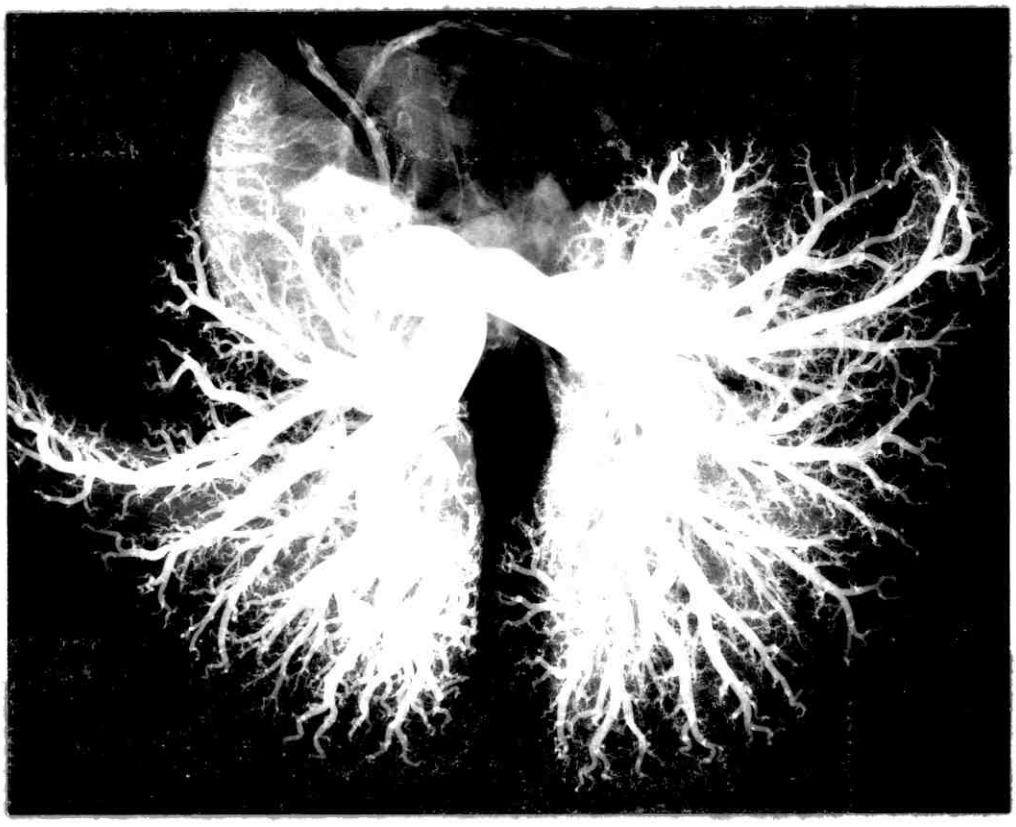


Figure VI 23



Arteriogram of case V.S.5 (x 0.75). There are very large hilar and axial vessels which are also tortuous. There is a reduction in the number of vessels at the periphery and there is tortuosity of those present.

periphery. In the lower lobe the lumen diameters were higher than those of the three day old but slightly lower than those of the four month normal lung, they are probably larger than the normal at two weeks of age.

Case V.S.3 has diameters along the whole length of the pathway in upper and lower lobe higher than the normal at twice its age. Since the volume of this lung was also increased this may be related, however the ten month lung had the same volume but smaller diameter arteries. Therefore there was a true increase in diameter for age and volume.

Case V.S.4 had arteries that were too small for the age of the child and since the lung was normal in volume, too small for the volume of the lung. This decrease was at all levels of the pathway.

Both in the upper and lower lobes case V.S.5 had arteries of a lumen diameter 50% larger than the normal of the same age along the whole pathway.

There is therefore variation in the changes in the arteriograms of cases with ventricular septal defect. Arteries in some were too small for age and volume and in others were too large. In case V.S.5 though increase in diameter at the hilum was more than at the periphery, there was a greater percentage increase at the periphery both in upper and lower lobes. This also applied to cases V.S.2 and 3. The percentage decrease in case V.S.4 is greater at the hilum than the periphery in the lower lobe, while in the upper lobe there was a percentage decrease at the hilum and an increase at the periphery.

Figure VI 24

Diagram representing the measurements of lumen diameter made on the axial arteries to the upper and lower lobes of the cases of ventricular septal defect, together with those of the normal at three days and four months. The diameter is shown in millimetres for the percentage distances along the pathway.

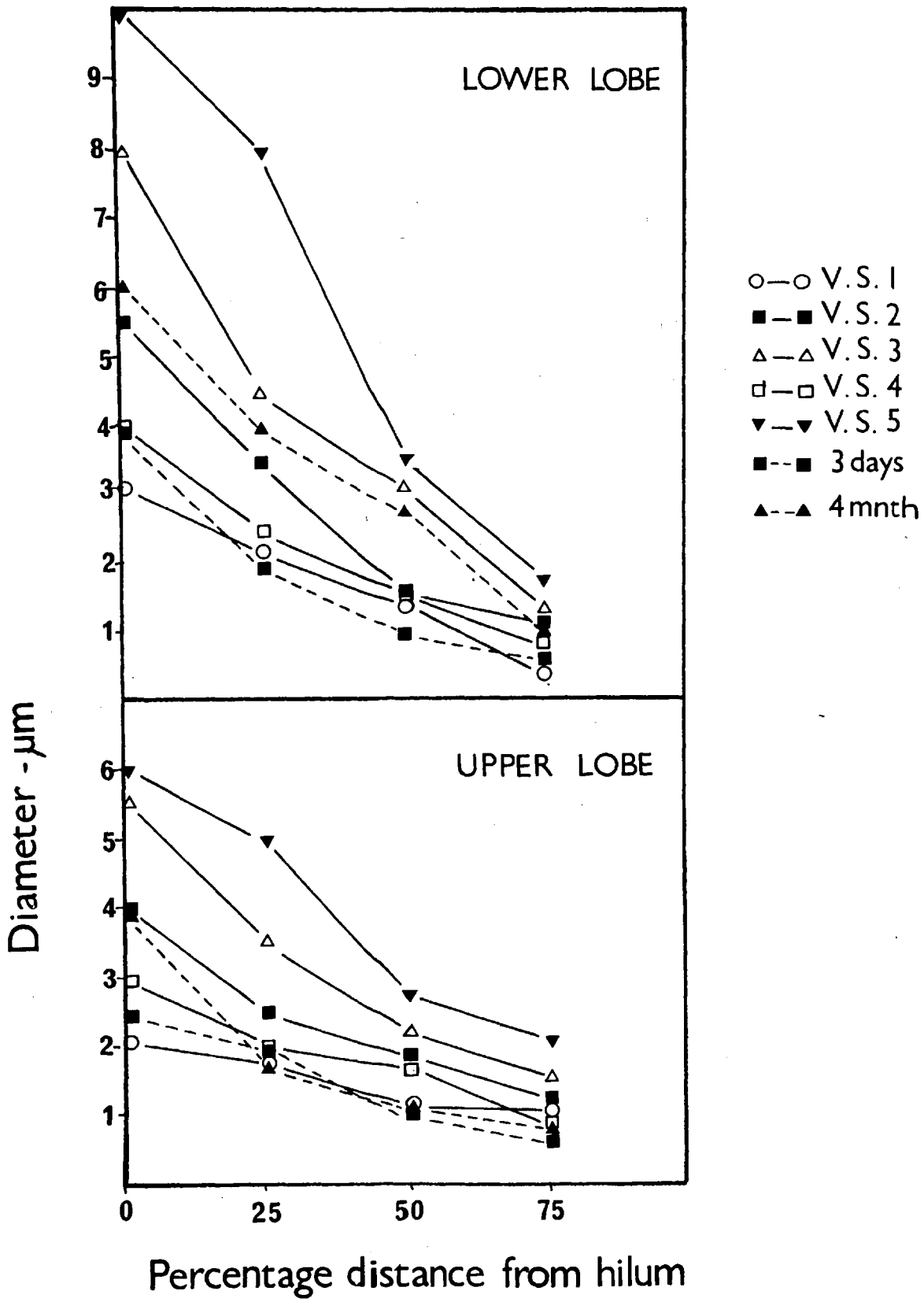

LOWER LOBE
UPPER LOBE
Diameter - µm
Percentage distance from hilum
0
25
50
75
1
2
3
4
5
6
7
8
9
V.S. 1
V.S. 2
V.S. 3
V.S. 4
V.S. 5
3 days
4 mnth

TABLE VI 21

Measurements on Arteriograms

Case	Lower Lobe					Upper Lobe				
	Length (cm)	Diameter (mm) % distance from hilum				Length (cm)	Diameter (mm) % distance from hilum			
		0	25	50	75		0	25	50	75
V.S.1	6.5	3.2	2.3	1.5	0.5	4.0	2.0	1.8	1.2	1.0
V.S.2	6.0	5.5	3.5	1.6	1.2	4.0	4.0	2.5	1.8	1.2
V.S.3	6.0	8.0	4.5	3.1	1.4	5.5	5.5	3.5	2.2	1.5
V.S.4	6.0	4.0	2.5	1.6	1.0	4.0	3.0	2.0	1.7	0.9
V.S.5	6.0	10.0	8.0	3.6	1.8	4.1	6.0	5.0	2.7	2.1
Normal										
3 days	5.9	4.0	2.0	1.0	0.7		2.5	2.0	1.2	0.7
4 mth	6.6	6.0	4.0	3.8	1.0		4.0	1.8	1.2	0.8

Gradient of the Pathway

When the slope from hilum to periphery was estimated from percentage length and diameter and compared with the normal, the gradient increased in those lungs with an increase in lumen diameter (cases V.S.2, 3 and 4) and decreased in those with a reduced lumen diameter (cases V.S.1 and 4). This was borne out by the similarity of peripheral diameter in all the cases. When the gradient was estimated from the actual length and diameter at the hilum it had again increased quite considerably in cases V.S.3 and 5 and had decreased in cases V.S.1 and 4. Case V.S.2 which showed an increase with percentage length showed no change in absolute gradient from the normal.

Other Measurements

In addition to those along the main pathway, measurements were made of the main pulmonary arteries and those to each lobe. Arteries one centimetre from the periphery were measured in each lobe. The results together with those of the normals are shown in Table VI 22.

The left and right pulmonary arteries seemed normal in diameter in cases V.S.2 and 4, but they were decreased in case V.S.1 and increased in cases V.S.3 and 5. The diameters at the lobar hilum show similar trends.

One centimetre from the periphery cases V.S.1 and 4 were normal for age, the other three cases all showed an increase in comparison with the normal.

In summary it seems that - case V.S.1 showed a decrease in lumen size particularly at the hilum; case V.S.2 had a slightly raised lumen diameter particularly at the periphery of the lung;

TABLE VI 22

Measurements from Arteriograms

Diameter of: (mm)	Case						
	V.S.1	V.S.2	V.S.3	V.S.4	V.S.5	3 days	4 mths
Left P.A.	4	6.0	10.0	7.0	11.0	5.0	8.0
Right P.A.	-	6.0	10.0	7.0	10.0	5.0	7.0
Lobar							
L.U.L.	1.8	3.0	6.0	4.0	-	2.5	3.0
L.L.L.	3.0	6.0	8.0	5.5	9.0	3.5	6.0
R.U.L.	2.0	3.0	5.0	3.0	5.0	2.5	5.0
R.M.L.	2.2	4.0	5.5	4.0	5.0	3.0	4.0
R.L.L.	2.2	5.0	8.0	4.5	10.0	3.0	6.2
1 cm from periphery							
L.U.L.	0.8	1.2	1.0	0.7	2.0	0.7	0.5
L.L.L.	0.7	1.1	1.0	0.8	1.0	0.7	0.6
R.U.L.	1.0	1.0	1.5	0.6	1.5	0.7	0.9
R.M.L.	0.6	0.9	1.0	-	1.0	0.7	0.5
R.L.L.	0.7	1.1	1.0	0.8	1.25	0.7	0.8

cases V.S.3 and 5 showed a marked increase in lumen diameter throughout the lung while case V.S.4 showed a reduction more marked in the proximal and central part of the lung.

Branching Pattern of Arteries by Serial Reconstruction

Arteriograms gave an overall impression of the branching pattern in these cases. Case V.S.5 seemed to be the most abnormal with marked tortuosity and apparent pruning. It was therefore decided to study this case in more detail.

The pre-acinar bronchial and arterial branching pattern is developed completely before birth and the effect of the ventricular septal defect is felt only after birth since until this time the two circulations are normally in communication. Serial sections were therefore only made of the peripheral centimetre of an axial pathway since this would include the whole of the lobule, the part in which change might be expected after birth.

A block was taken from the diaphragmatic surface of the left lower lobe to include the peripheral part of the axial vessel to the posterior basal segment. The proximal part of this pathway was traced by blunt dissection. From the hilum to the level of the block was 4.5 cm of pathway. Along this part of the pathway were seven bronchial branches, accompanying these were seven conventional arteries and in addition there were several supernumerary vessels. No filled bronchial arteries were seen in the walls of the bronchi.

From the block the pathway was traced further by serial reconstruction - here it was 11 mm long to the level of the end of

the alveolar ducts. The total length of the pathway was therefore 5.6 cm. In the peripheral region there were 15 airway generations before the terminal bronchiolus giving a total of 22 along the whole pathway, within the normal range. The first six of the 15 had cartilage in their walls therefore the total number of bronchi along the pathway was 13, again within the normal range. Accompanying the airways were 15 conventional arteries and in addition there were 37 supernumerary vessels. The ratio of supernumerary to conventional arteries was 2.47:1, the ratio over the same generations in the normal at birth was 3.6:1, there was therefore a reduction in the number in this case.

Within the acinus there were three respiratory bronchiolar branches and before the end of the arterial filling, one alveolar duct. There were four conventional arteries accompanying these. The only supernumerary arteries seen within the acinus were three branching from the artery accompanying the terminal bronchiolus.

Within the lobule there were three terminal bronchioli with three conventional arteries and only one supernumerary artery. This is the same low number as seen at birth in the normal. The intra-lobular, pre-acinar length was 280 μm and the intra-acinar length from the proximal end of the terminal bronchiolus 700 μm, the terminal bronchiolus being 280 μm long. There had been little growth in this case when it is compared with the normal at birth. Within the acinus there were only three supernumerary arteries, a smaller number than seen in the normal at birth but the same as the normal at four months. The intra-lobular, pre-acinar region in this case at three months had failed to grow since this region in

the four month old normal was larger and had more branches. The whole of the lobule was similar to the normal at birth and had not grown by three months as the normal would.

Diameter of Axial Pathway

The diameter of the axial pathway is shown in Figure VI 25 and Table VI 23. The diameter at the beginning of the serial reconstruction was 1150 μm. This decreased steadily towards the periphery. The diameter at the level of the terminal bronchiolus was 175 μm and at the end of the last respiratory bronchiolus 95 μm. These last two figures are similar to those for the normal cases at birth, three days and four months. There is little change in the normal at this age and this case lies within the normal range.

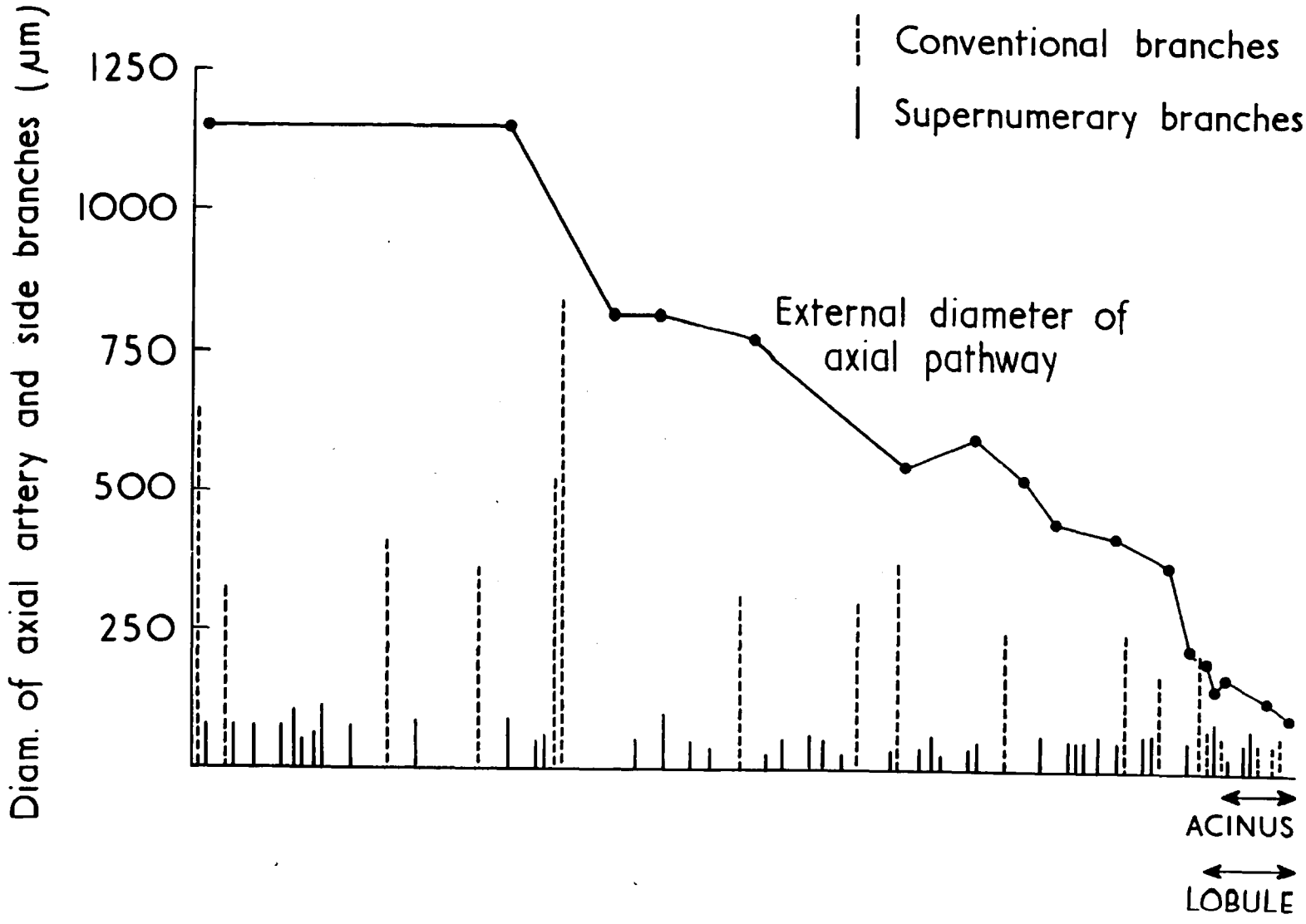
Diameter of Conventional Arteries

The diameter of the conventional arteries is shown in Figure VI 25 and Table VI 23. The conventional arteries were smaller than the axial vessel at the branching point and they also decreased in size towards the periphery. This was not a steady decline since there was variation between successive branches. The average diameter for the pre-acinar part of the pathway was 338.1 μm. That for the peripheral 15 generations in the stillborn infant was 291 μm. There would seem to have been little increase in size with age.

The average diameter for the vessels accompanying terminal bronchioli was 109 μm and those with the respiratory bronchioli 43.3 μm. These are similar in size to the normal at birth and smaller than the normal either of three days or four months. In

Figure VI 25

Diagram representing the branching pattern and the diameter of the axial pathway and conventional and supernumerary arteries in the peripheral 11 mm of the posterior basal artery of case V.S.5. The extent of the lobule and the acinus is shown.


Diam. of axial artery and side branches (μm)
1250
1000
750
500
250
Conventional branches
Supernumerary branches
External diameter of
axial pathway
ACINUS
LOBULE

this case there seems to be a sudden drop in the size of the conventional arteries and the axial pathway within the lobule, this is not seen in the normal.

Diameter of Supernumerary Arteries

The diameter of the supernumerary vessels is shown in Figure VI 25 and Table VI 24. They were very much smaller than the conventional arteries and were much the same size over the length of pathway studied. They had an average of 54.85 μm and the average of those within the lobule was 52.25 μm. This was similar to the normal at three days in the respiratory region but, in the pre-acinar region, smaller than in the normal at three days. They were smaller than those seen in the normal at four months.

In this case it seems that all the vessels within the intra-lobular region and probably those in the distal 15 pre-acinar generations are smaller than normal. This is surprising since the arteriogram of this case showed the proximal vessels to be greatly increased in size. In the three months that the child had lived there had been little growth at the periphery of the lung, since the pathway was similar in size and branching pattern to the normal lung at birth. In the normal newborn studied the first seven generations covered 1.54 cm while in this case they covered 4.5 cm. The remaining 15 pre-acinar generations covered 1.43 cm at birth and 1.029 cm in this case of three months. It seems that increase in length and diameter had occurred in the proximal regions but that in the peripheral centimetre there had been no growth. In the normal at four months the last six pre-acinar generations occupied 4.2 mm while in this case they occupied 2.14 mm and at birth 2.51 mm. In the adult the first seven

TABLE VI 23

Diameter of Axial Pathway and Conventional Arteries along the Pathway traced in Case V.S.5

Bronchial Generation	Diameter of Conventional Artery (μm)	Diameter of Axial Pathway (μm)	Distance from Previous Branch (mm)
1	650	1150	-
2	320	1150	0.28
3	390	1150	1.61
4	360	1150	0.91
5	530	-	0.77
6	830	800	0.07
7	310	770	1.75
8	300	-	1.26
9	370	550	0.42
10	250	520	1.05
11	260	430	1.19
12	175	370	0.35
T.B.1	210	190	0.42
T.B.2	55	-	0.07
T.B.3	62	175	0.14
R.B.1	40	120	0.35
R.B.2	40	-	0.14
R.B.3	62	95	0.07
A.D.1	-	-	0.07

generations occupy almost half the total length and in this case three quarters. In the other four cases the first seven generations occupied respectively 45% in V.S.1, 36% in V.S.2, 63% in V.S.3 and 51% in V.S.4. The normal at birth is 52%.

Structure of Axial Pathway

The axial pathway at the most proximal part traced was 1150 μm in diameter and had a muscular wall. This is the structure expected for an artery of this size at this age. It was at the level of the eighth generation and therefore probably the normal structure for this position. A muscular structure was found as far as the alveolar duct level where the filling ended, this was at a diameter of 95 μm. In the normal of four months of age the muscular wall extended only to the terminal bronchiolus and had a diameter of 160 μm. In this case muscle had extended into the acinus and also into smaller arteries. Normally an artery of 95 μm running with an alveolar duct would be non-muscular in structure but no partially or non-muscular structure was found along this axial pathway as far as it was traced.

Structure of Conventional Arteries

The conventional arteries to the level of the lobule were all muscular in structure ranging in size from 175 - 830 μm. Of the three conventional arteries accompanying terminal bronchioli, one was muscular and 210 μm in diameter, one was partially muscular and 55 μm in diameter and one 62 μm and non-muscular. In the normal four month child all arteries accompanying terminal bronchioli were muscular but these arteries were also larger. Two of the conventional arteries accompanying the respiratory bronchioli in this case were non-muscular and one partially muscular; this is

TABLE VI 24

Diameter of Supernumerary Arteries along Axial Pathway in Case V.S.5

Bronchial Generation	Diameter (μm)	Bronchial Generation	Diameter (μm)
1	75	7	25
2	75	8	25
2	72	9	30
2	65	9	62
2	90	9	20
2	45	9	37
2	52	9	50
2	115	10	62
2	48	10	50
3	90	10	40
4	90	10	40
4	40	10	60
4	60	10	50
6	50	11	65
6	100	11	60
6	37	12	50
6	30	T.B.2	87
7	25	T.B.3	12
7	55	T.B.3	40
7	60	T.B.3	70
7	40		

Mean - 54.2

similar to that seen in the normal four month old child.

Structure of Supernumerary Arteries

In the pre-acinar region studied there were 38 supernumerary arteries. At the proximal end one of 90 μm diameter was muscular. There were five partially muscular vessels found along the length of the pathway, one within the lobule, with a size range of 60 - 115 μm. The remaining 32 vessels were all non-muscular and ranged in size from 25 - 100 μm. As in the normal there was an overlap of diameter for the different structures. The ranges of each type were normal for age. There were fewer muscular vessels in this case than in the normal lung of four months. Within the acinus all three supernumerary vessels were non-muscular which is normal at this age.

It seems that though there was an extension of muscle along the axial pathway, the conventional and supernumerary vessels were not more muscular than normal, and in the pre-acinar region may have been less muscular, though this was probably due to the decrease in size of these vessels.

Wall Thickness of Arteries Throughout the Lung

To assess the degree of muscularity in these cases the percentage wall thickness was estimated for arteries over a large range of external diameter. In each case arteries were measured on sections from throughout the lungs. The results are shown in Figures VI 26 - 30 and Table VI 25.

In cases V.S.2, 3 and 5 the shape of the curves seen was similar

to those seen in all normal cases, that is there was a rise in percentage wall thickness in the smaller arteries below 300 μm. The diameter at which this rise occurred however was not as constant as in the normal. In cases V.S.1 and 4 the highest percentage wall thickness was seen in the largest arteries, those over 900 μm in external diameter. Between 200 and 900 μm diameter the percentage wall thickness was low. Below 200 μm there was a rise as seen in the other cases but not to the level of the large arteries.

The percentage wall thickness at any one diameter range varied from case to case. Each case must be compared with a normal of the same age. Case V.S.1, at three weeks, had a slightly greater wall thickness at all diameters than the normal at three days, particularly in the larger arteries; this increase was more marked when compared with a normal of four months. In arteries below 200 μm diameter the percentage wall thickness was similar to the normal of either three days or four months. It seems that the part of the decrease in wall thickness that occurs after birth had taken place, is that which occurs in the small arteries. This seems to occur first in the normal and is probably as a result of dilatation. The larger arteries had a wall thickness higher than the normal for fetal life, therefore there had either been hypertrophy after birth or the arteries were abnormal during fetal life in this case.

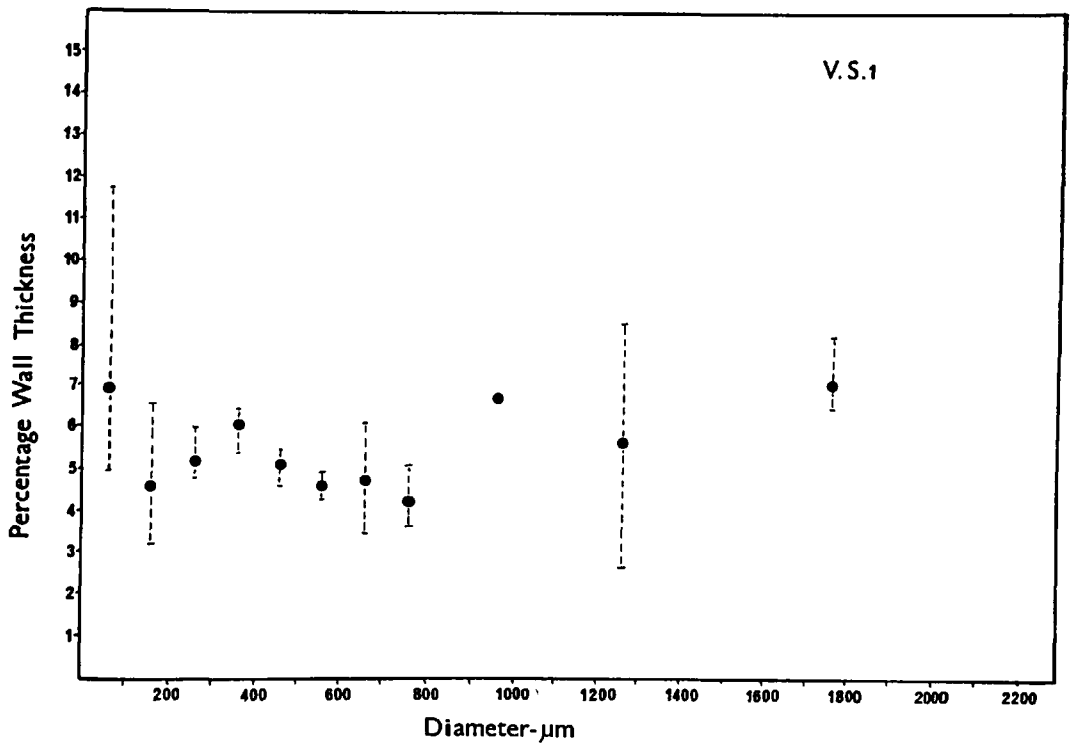
Case V.S.2 had a percentage wall thickness similar to the normal seen in a four month old child over all size ranges. The percentage wall thickness was lower than the normal at three days, therefore the decrease to adult levels normally seen by four months has occurred by two weeks in this case. It could be that the drop in wall thickness in this case had been too rapid and led to dilatation of the vessels. The drop in wall thickness had not been

Figure VI 26

Percentage wall thickness related to external diameter for arteries from case V.S.1. The mean and range for each diameter is shown.

Figure VI 27

Percentage wall thickness related to external diameter for arteries from case V.S.2. The mean and range for each diameter range is shown.


V.S.1
Percentage Wall Thickness
Diameter-µm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
200
400
600
800
1000
1200
1400
1600
1800
2000
2200

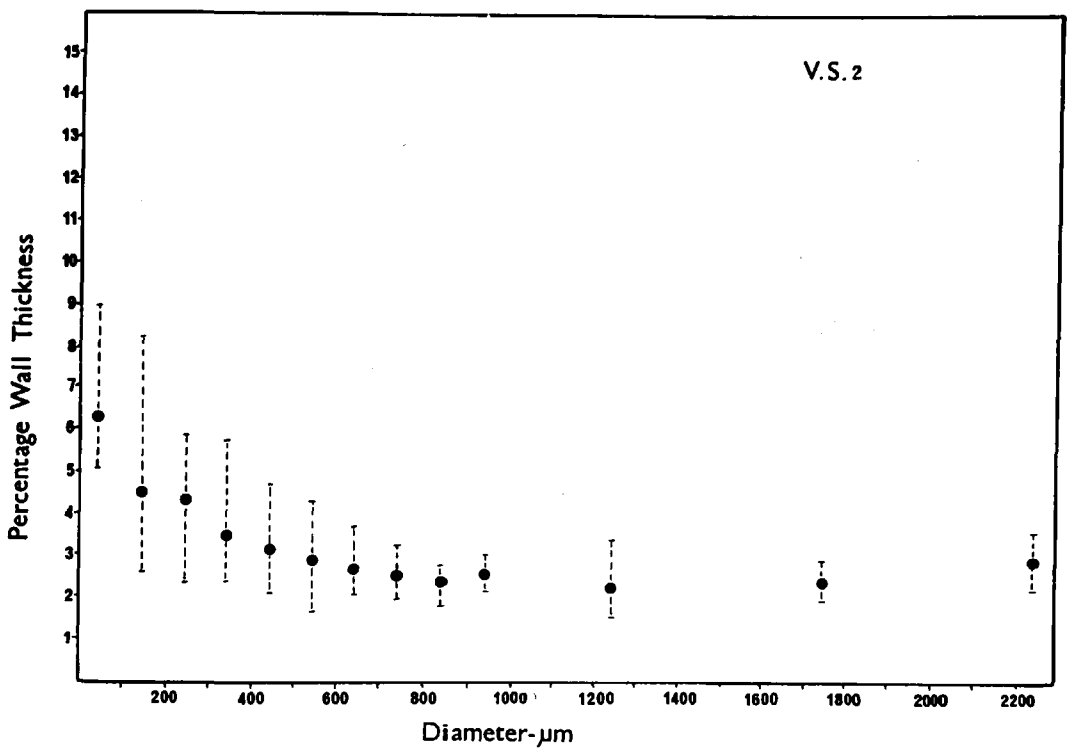
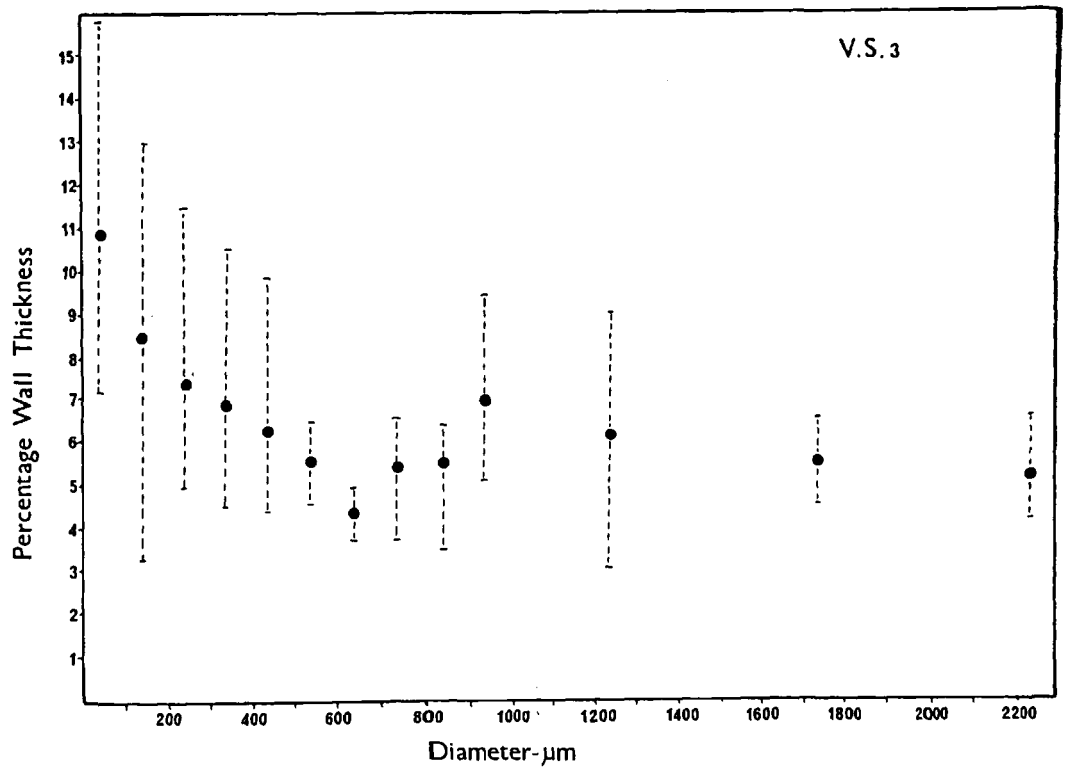

V.S.2
Percentage Wall Thickness
Diameter-µm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
200
400
600
800
1000
1200
1400
1600
1800
2000
2200

Figure VI 28

Percentage wall thickness related to external diameter for arteries from case V.S.3. The mean and range for each diameter range is shown.

Figure VI 29

Percentage wall thickness related to external diameter for arteries from case V.S.3. The mean and range for each diameter range is shown.


V.S.3
Percentage Wall Thickness
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
Diameter-µm

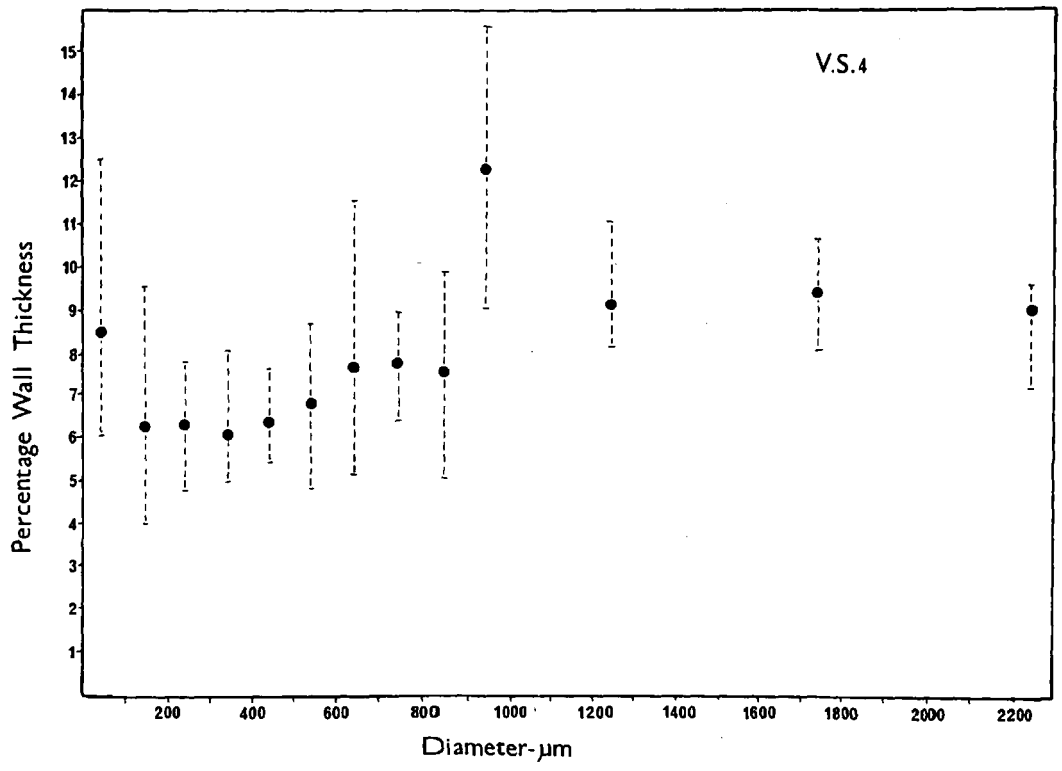
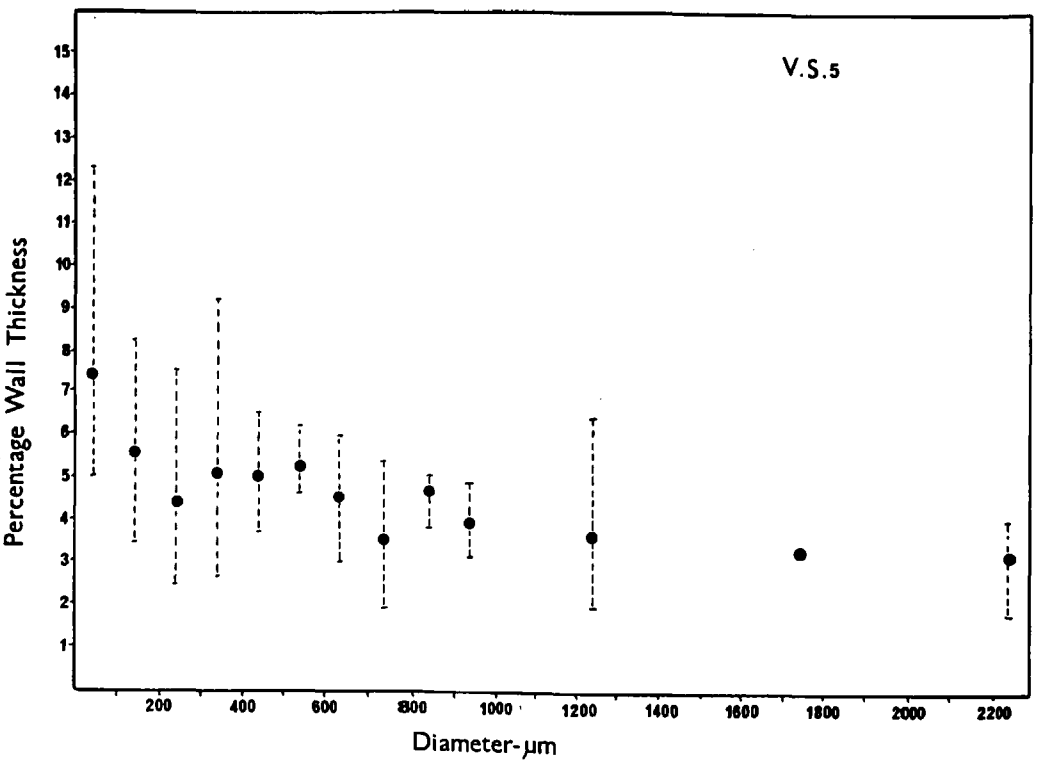

V.S.4
Percentage Wall Thickness
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
Diameter-µm

Figure VI 30

Percentage wall thickness related to external diameter for arteries from case V.S.5. The mean and range for each diameter range is shown.


V.S.5
Percentage Wall Thickness
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
Diameter-µm

accompanied by a loss of muscle from the small vessels as seen in the normal.

Case V.S.3 at two months of age had an increased wall thickness in all arteries up to a diameter of four millimetres, when compared with a normal of four months or with a normal at birth. There had been an increase in muscle in the walls of all arteries after birth. There was a much greater range of percentage wall thickness in any one size range than in the normal and in all but one size range all arteries had a wall thickness above the normal average.

Case V.S.4 had a higher percentage wall thickness in the larger arteries than case V.S.3 but in those arteries below 400 μm the wall thickness was lower though still considerably higher than the normal either at birth or four months. There had been an overall increase in wall thickness particularly in vessels over 200 μm in diameter.

There was an increase in percentage wall thickness in case V.S.5 in vessels below 700 μm in comparison with the normal of four months. At 2000 μm the vessels had normal wall thicknesses. The vessels below 700 μm were similar to those seen in the normal at birth and it seems that there had been no decrease in wall thickness after birth, though this could have been secondary thickening of the walls.

In summary there was one case in which the fetal wall thickness had been maintained (V.S.5), one in which there had been a drop greater than normal (V.S.2); there was one case where there had been an increase in the percentage wall thickness of the larger

TABLE

Percentage Wall Thickness of Arteries from

Diameter

		0-100	100-200	200-300	300-400	400-500	500-600
V.S.1	Mean	6.99	4.59	5.18	6.12	5.16	4.67
	S.E.	0.89	0.35	0.14	0.32	0.12	0.25
	No.	9	8	8	2	7	2
	Range	5.0-11.8	3.2-6.6	4.8-6.0	5.4-6.5	4.6-5.5	4.3-5.0
V.S.2	Mean	6.46	4.65	4.46	3.55	3.27	2.99
	S.E.	0.61	0.39	0.28	0.42	0.29	0.31
	No.	6	15	14	8	8	8
	Range	5.3-9.1	2.8-8.3	2.4-6.0	2.5-5.9	2.2-4.9	1.8-4.4
V.S.3	Mean	10.76	8.3	7.29	6.79	6.27	5.41
	S.E.	1.03	0.6	0.48	0.5	0.65	0.21
	No.	10	16	18	12	8	9
	Range	7.1-15.6	3.1-12.8	4.8-11.3	4.4-10.4	4.2-9.7	4.5-6.3
V.S.4	Mean	8.5	6.28	6.34	6.01	6.38	6.83
	S.E.	0.57	0.27	0.30	0.39	0.33	0.62
	No.	13	28	9	8	7	5
	Range	6.1-12.5	4.0-9.5	4.8-7.8	5.0-8.1	5.4-7.6	4.8-8.6
V.S.5	Mean	7.6	5.7	4.54	5.22	5.14	5.4
	S.E.	0.57	0.32	0.37	0.42	0.58	0.28
	No.	13	22	16	16	4	5
	Range	5.2-12.5	3.6-8.3	2.6-7.7	2.8-9.4	3.8-6.7	4.8-6.4

VI 25

Cases with Ventricular Septal Defect

Range µm

600-700	700-800	800-900	900-1000	1000-1500	1500-2000	> 2000
4.78	4.24	-	6.79	5.76	7.15	-
0.39	0.44			0.63	0.27	
6	3		1	8	6	
3.5-6.1	3.7-5.1			2.7-8.6	6.6-8.3	
2.78	2.69	2.42	2.66	2.35	2.47	3.05
0.23	0.23	0.19	0.37	0.22	0.11	0.21
7	5	4	2	8	8	6
2.2-3.8	2.1-3.3	1.9-2.8	2.3-3.0	1.7-3.4	2.1-2.9	2.3-3.6
4.26	5.37	5.46	6.93	6.14	5.41	5.2
0.29	0.39	0.62	0.65	1.23	0.43	0.4
3	9	3	6	4	4	6
3.6-4.8	3.6-6.5	3.4-6.3	5.1-9.4	3.0-8.9	4.5-6.6	4.3-6.6
7.66	7.75	7.57	12.33	9.1	9.47	9.01
1.11	0.65	0.99	3.27	0.36	0.47	0.41
5	4	4	2	7	4	7
5.1-11.6	6.4-8.9	5.0-9.9	9.1-15.6	8.1-11.0	8.1-10.6	7.2-9.5
4.57	3.69	4.81	4.06	3.71	3.33	3.2
0.41	0.78	0.18	0.26	0.53		0.29
6	5	5	7	10	1	7
3.2-6.1	2.1-5.5	4.0-5.2	3.3-5.0	2.0-6.5		1.9-4.1

S.E. - Standard Error of the mean

No. - Number of vessels measured

vessels while the smaller arteries had probably behaved normally (V.S.1); and two cases where there had been an increase in the wall thickness from birth (V.S.3 and 4).

Population Counts

Population counts were made on the five cases with their arteries injected, to estimate the extension of muscle into small arteries and to show any changes in structure of the small arteries when compared with the normal. In the normal, whether at birth, three days or four months of age the population of small arteries has much the same structure with the mixed population appearing at about 200 μm external diameter and the smallest wholly muscular artery being found between 75 and 100 μm. The smallest partially muscular vessel fell between 25 and 50 μm. Figure VI 31 and Tables VI 26 and 27 show the results of the population counting in the cases of ventricular septal defect.

In all five cases similar curves to the normal were seen. The partially muscular vessels did not appear until below 150 μm diameter. This is at a lower diameter than at birth, but there were several cases during fetal life where this was the normal diameter, and therefore these cases cannot be considered abnormal in that respect. In case V.S.4 partially muscular vessels were only found below 125 μm and this is considerably lower than in the normal.

The smallest partially muscular artery was found between 25 and 50 μm in cases V.S.1, 2 and 5 which seems to be within the normal limits. In cases V.S.3 and 4, however, partially muscular vessels were found in smaller vessels than this. There was therefore

Figure VI 31

Population distribution curves for the five cases with ventricular septal defect. The percentage of the total vessels with a given structure in each size range of vessels is shown.

m - muscular arteries

p.m. - partially muscular arteries

n.m - non-muscular arteries

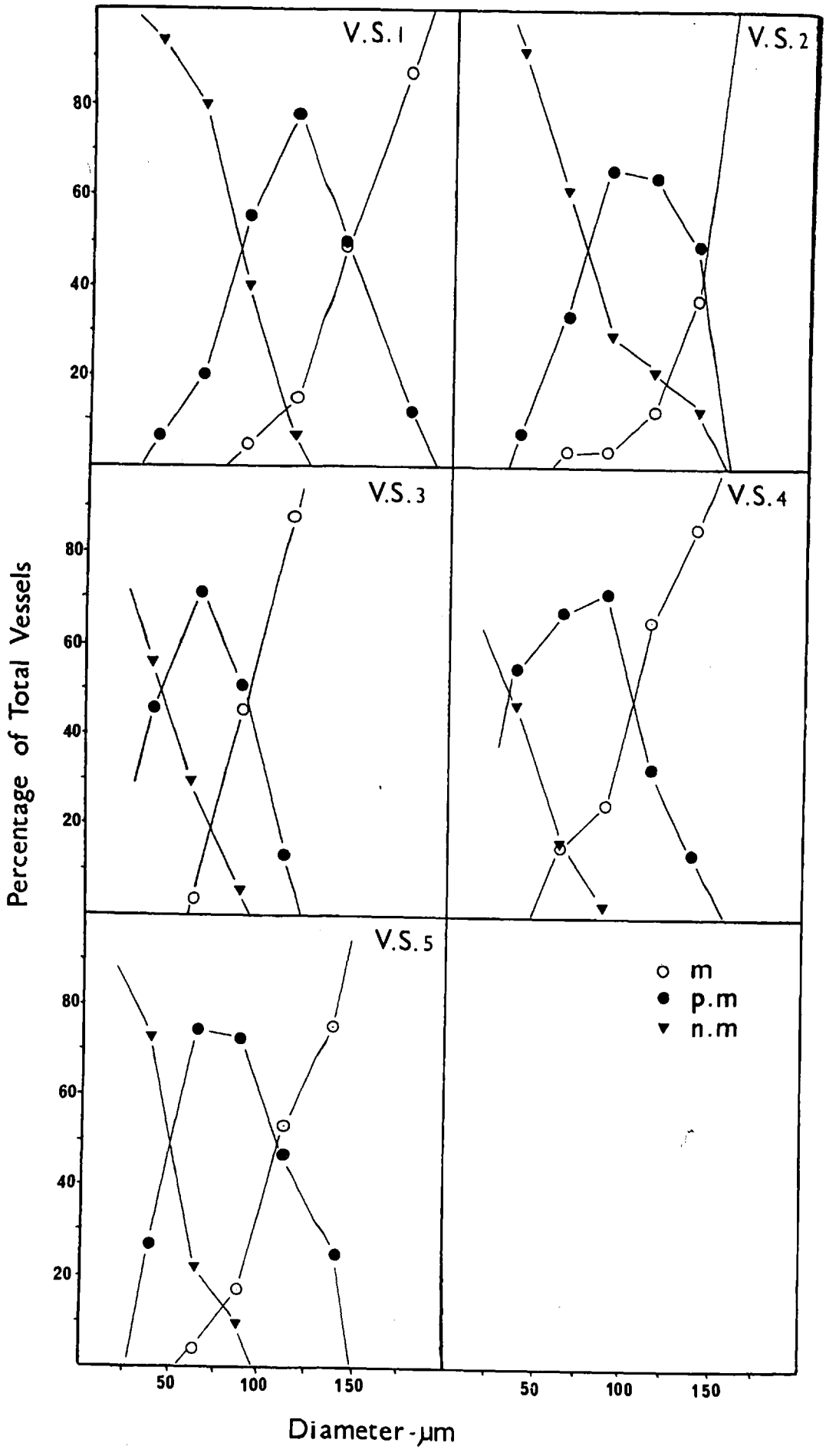

V.S.1
V.S.2
V.S.3
V.S.4
V.S.5
m
p.m
n.m
Percentage of Total Vessels
Diameter - µm
20
40
60
80
50
100
150

TABLE VI 26

Distribution of Structure by Size in Small Vessels

Case	Smallest Muscular (um)	Range of Partially Muscular (um)	Largest Muscular (um)	Peak Partially Muscular (um)	% Peak
V.S.1	98	48-150	105	112.5	78
V.S.2	72	37-148	148	87.5	66
V.S.3	65	35-112	87	62.5	70
V.S.4	55	40-140	75	87.5	72
V.S.5	52	32-130	95	62.5	74
Normal					
3 days	120	40-180	152	112.5	66
4 months	75	40-170	130	112.5	68

TABLE VI 27

Percentage of Vessels in each Size Range

Case	%	Diameter Ranges (μm)					
		25-50	50-75	75-100	100-125	125-150	150-200
V.S.1	n	94	80	40	7	0	0
	p.m	6	20	55	78	50	12.5
	m	0	0	5	15	50	87.5
V.S.2	n	92	62	30	22	12.5	0
	p.m	8	34	66	65	50	0
	m	0	4	4	13	37.5	100
V.S.3	n	55	27	5	0	0	0
	p.m	45	70	50	12	0	0
	m	0	3	45	88	100	100
V.S.4	n	45	16	3	0	0	0
	p.m	55	68	72	33	14	0
	m	0	16	25	67	86	100
V.S.5	n	73	22	10	0	0	0
	p.m	27	74	73	47	25	0
	m	0	4	17	53	75	100
Normal 3 days	n	92	78	51	31	13	4
	p.m	8	22	49	66	65	33
	m	0	0	0	3	12	63
4 mths	n	97	67	31	8	2	0
	p.m	3	33	68	68	47	5
	m	0	0	8	24	51	95

n - non-muscular
p.m - partially muscular
m - muscular

extension of muscle into smaller vessels than normal in these cases.

In all cases but case V.S.1 the smallest muscular vessels were smaller than is normal. The largest non-muscular vessel was also smaller in all cases and particularly in cases V.S.3, 4 and 5.

The overall impression was of an extension of muscle into smaller vessels than normal, particularly in V.S.3, 4 and 5. These were the older cases and it is possible that there had not been time for this to occur in the other two cases.

The percentage of each type of vessel present in the population counted is shown in Table VI 28. Cases V.S.1 and 2 had similar distribution of the vessels to normal. Cases V.S.3, 4 and 5 had a normal percentage of partially muscular vessels but had a smaller percentage of non-muscular and very much higher percentage of muscular than is normal. This is consistent with the extension of muscle into smaller vessels than is normal, a finding particularly marked in these three cases.

Arteries with Airways

Measurements and determination of structure were made on arteries accompanying identifiable airways within the lobule to investigate further the extent of muscle into the acinus and the size of arteries involved.

TABLE VI 28

Percentage of Each Type of Vessel in Total Population

Case	Total number of vessels	Percentage non-muscular	partially muscular	muscular
V.S.1	86	52	35	13
V.S.2	109	43	39	18
V.S.3	104	19	42	39
V.S.4	124	8	48	44
V.S.5	130	21	49	30
Normal				
3 days	203	43	43	14
4 months	429	36	43	21

Diameter of Arteries with Airways

Arteries were measured at four levels, the terminal bronchiolus, respiratory bronchiolus, alveolar duct and alveolus. The results are shown in Table VI 29 which gives the average and range of diameter at each level.

As in the normal there was a decrease in the average diameter of arteries towards the periphery of the acinus in all cases. At all levels case V.S.1 had arteries smaller than the normal of three days. Case V.S.2 had arteries of smaller diameter than normal at the level of the respiratory bronchioli and alveolar ducts. The arteries with the terminal bronchioli were slightly larger than those at three days but since the patient was two weeks old at death it is likely that they too were small for age. In this case the vessels measured on the arteriogram were all larger than normal. The last measurement on the arteriogram must therefore have been proximal to the terminal bronchiolus. There must have been a sudden reduction in size just proximal to the acinus and at some point the lumen must be of normal diameter. It is of interest that the most proximal vessels measured microscopically are those nearest normal diameter.

Case V.S.3, at two months of age had arteries that were larger than the normal even at four months; the arteriogram in this case also showed an increase in vessel size.

Case V.S.4 had arteries accompanying the terminal bronchioli which were probably normal in diameter, however those with the respiratory bronchioli and alveolar ducts were as small as those of a three day old normal and were therefore too small for the age of the child. The arteriogram also showed arteries decreased in size.

TABLE VI 29

Diameter of Arteries with Airways

Case		Diameter of Arteries Accompanying Airways (μm)			
		Terminal Bronchiolus	Respiratory Bronchiolus	Alveolar Duct	Alveoli
V.S.1	Mean	78	62.2	40	-
	Range	62-100	45-112	30-50	
V.S.2	Mean	123	62.1	44.8	30
	Range	80-200	35-95	32-77	
V.S.3	Mean	300	134.5	105.6	-
	Range	-	75-210	82-130	
V.S.4	Mean	161.4	99.7	63.8	41
	Range	87-260	37-160	37-100	37-45
V.S.5	Mean	182	102.5	93.3	37.5
	Range	105-237	80-130	55-130	35-40
Normal					
3 days	Mean	118	92.7	65	-
4 mths	Mean	197.3	122.3	86.5	33.3

Case V.S.5 had arteries of near normal diameter for age, which is surprising since the arteriogram showed lumen diameter greatly increased. It appears that in this case the peripheral vessels did not enlarge as the proximal. This case was similar to case V.S.2. This anomaly was also seen in the serial reconstruction of case V.S.5 where the vessels were small for age.

The vessels in these cases at the acinar level were normal in size in one case, reduced in three and increased in one.

Structure of Arteries with Airways

The percentage of each type of vessel, muscular, partially muscular or non-muscular, accompanying the identifiable airways is shown in Table VI 30, together with the normal figures for the relevant ages.

Case V.S.1 was less muscular than the normal at three days, there being non-muscular vessels accompanying terminal bronchioli and only non-muscular vessels with the alveolar ducts. As in the fetus there were no muscular vessels within the acinus.

Case V.S.2 was more muscular than case V.S.1 with muscular vessels accompanying 50% of the terminal bronchioli. However there were fewer partially muscular vessels within the acinus than in the normal of three days and it seems that again muscle did not extend as far as in the normal. Cases V.S.3, 4 and 5 were quite similar to each other with regard to the number of non-muscular arteries. At the level of the terminal bronchiolus and respiratory bronchiolus there were few non-muscular arteries, as in the normal. Unlike the normal however there were still very few at the level of the alveolar duct and it seemed that the muscle was extending further into the

TABLE VI 30

Structure of Arteries with Airways

Case	Percentage of each type of vessel with airways											
	Terminal bronchiolus			Respiratory bronchiolus			Alveolar duct			Alveoli		
	n	p.m	m	n	p.m	m	n	p.m	m	n	p.m	m
V.S.1	28.6	71.4	0	80	20	0	100	0	0		-	
V.S.2	0	50	50	73	27	0	91	9	0	100	0	0
V.S.3	0	0	100	0	8	92	0	17	83		-	
V.S.4	0	0	100	3	21	76	6	50	44	100	0	0
V.S.5	0	0	100	0	61	39	0	92	8	100	0	0
Normal												
3 days	0	72	28	30	70	0	67	33	0		-	
4 months	0	0	100	3	30	67	35	18	47	100	0	0

n - non-muscular
p.m - partially muscular
m - muscular

acinus than in the normal. In case V.S.4 there were no non-muscular arteries accompanying alveoli, this was partly due to lack of sampling since there were few of these vessels filled. In case V.S.5 again all arteries with alveoli were non-muscular. This case was less muscular than the other two since there were fewer wholly muscular vessels than in the others at the level of the respiratory bronchioli and alveolar ducts.

The extension of muscle further distally in these last three cases was also seen in the population counts. There was in fact less extension than normal in cases V.S.1 and 2 but this was not seen in the population counts. This was because the arteries with airways were also smaller than normal and would therefore tend to be less muscular. Although the arteries in case V.S.4 were also small there was still extension of muscle into the acinus since the muscle extension into smaller vessels was considerable.

In case V.S.5 a reconstruction had been made along an axial pathway and a muscular structure was found to the level of the alveolar duct at a diameter of 95 μm. The average size of arteries with alveolar ducts was 93.3 μm in this case and there were some muscular vessels though only 8% of the total. The side branches are probably less muscular than the axial pathway which would account for this discrepancy. The side branches of the traced pathway were in fact less muscular since there were non-muscular vessels accompanying respiratory bronchioli: this was not seen in the general population. It is probable that the smallest respiratory bronchioli had been missed. There does seem to be some discrepancy in the results from the two methods and it may be advisable to use both, at least in diseased lungs.

Ventricular Weights

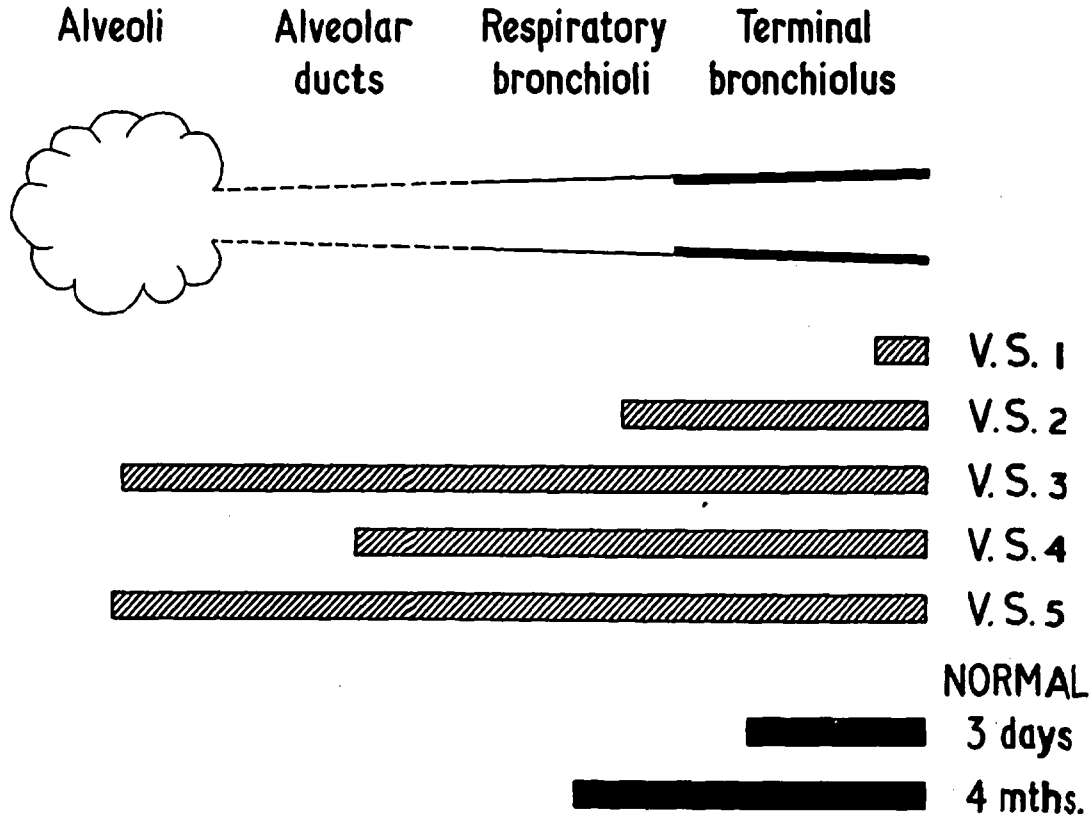
The hearts of three cases, V.S.2, 4 and 5, were dissected and weighed using the method of Fulton, Hutchinson and Jones (1952). The ventricular weights were used to estimate the ratio of left ventricle plus septum to right ventricle. In case V.S.2 it was 1.12:1, in case V.S.4, 1.8:1 and in case V.S.5, 1.159:1. In the adult Fulton et al showed the normal ratio to be 2.3 - 3.3:1. Recavarren and Arias-Stella (1962) showed that the adult ratio was reached by four months and this was confirmed by Davies (1969). Cases V.S.4 and 5 were three months old and therefore should have almost reached adult ratios. Since they had low ratios it is likely that there was right ventricular hypertrophy or at least maintenance of the fetal state of right ventricular preponderence. Case V.S.2 was only two weeks old and it is not possible to say whether the low ratio was due to the normal continued presence of the fetal ratio or whether there had been right ventricular hypertrophy or abnormal maintenance of the fetal state. The hearts from cases V.S.1 and 3 were not available for study but the post mortem report on case V.S.1 stated that the right ventricle was hypertrophied.

Extension of Muscle into the Acinus

Using the results already described it is possible to build up a composite picture of the change in structure of the arterial vessels in the lungs of cases with a ventricular septal defect. It is particularly important to estimate the degree of extension of muscle into the acinus. The cases in this series each showed a different response to a similar haemodynamic change. Figure VI 32 shows a representation of the extension of muscle into the acinus in the five cases. The end of the muscle is represented by the diameter at the

Figure VI 32

Diagram representing the extension of muscle in the walls of arteries within the acinus in the five cases with ventricular septal defect and two normal cases. The end of the lines represents the diameter at the peak of the partially muscular population and the position in the acinus that an artery of this diameter is likely to be found.


Alveoli
Alveolar ducts
Respiratory bronchioli
Terminal bronchiolus
V.S. 1
V.S. 2
V.S. 3
V.S. 4
V.S. 5
NORMAL
3 days
4 mths.

peak of the partially muscular vessels as seen by population counting. The average diameters of the arteries running with airways have been used as landmarks.

In case V.S.1 the muscle did not extend as far into the acinus as it did in the normal, in fact this case was close to the appearance during late fetal life. Though there was a slight extension of muscle into smaller vessels in this case, because the vessels in the acinus were hypoplastic this did not represent extension to the periphery. Case V.S.2 was close to the normal at three days and four months and the extension of muscle into small vessels had been offset by the reduction in size of vessels within the acinus. In the other three cases there was extension of muscle to the periphery of the lung particularly in cases V.S.3 and 5, where muscle extended almost as far as in the adult.

Arteries and Alveoli per Unit Area of Lung

Alveoli per Unit Area of Lung

The total number of alveoli in a square centimetre of alveolar tissue was estimated in all the cases. The results are shown in Table VI 31. The number was similar to normal in cases V.S.1 and 2. In cases V.S.3, 4 and 5 the number was much reduced in comparison with the normal at four months but was higher than the normal at three days. It may be that the burst of multiplication of alveoli has not yet occurred in these cases, though it is likely that it should have done.

TABLE VI 31

Number of Alveoli in one square Centimetre of Lung Tissue and the Ratio of Alveoli to Arteries

Case	Alveoli/sq.cm	Ratio Alveoli/Arteries
V.S.1	4,328	21.0
V.S.2	3,789	20.8
V.S.3	6,550	90.97
V.S.4	5,157	36.8
V.S.5	5,731	33.3
Normal		
3 days	4,689	25.0
4 months	11,315	97.5

Arteries per Unit Area of Lung

The number of arteries below 200 µm diameter was counted in one square centimetre of lung tissue. The total number and the percentage in each size range from 25 - 200 µm is shown in Table VI 32. In four of the five cases the total number and the percentage of each size range was similar to that seen in the normal lung of three days or four months. In case V.S.3 the total number was reduced. It is probable that this was due to lack of filling of the smaller vessels, though unfilled vessels could not be seen microscopically. This lack of filling of small vessels may have been due to a change in structure of the artery wall, since this case did have the highest percentage wall thickness and extension of muscle into small vessels. In cases V.S.2 and 5 there was pruning of vessels seen on the arteriograms but there was no lack of vessels microscopically.

Ratio of Alveoli to Arteries

The ratio of alveoli to arteries was similar to the normal of three days in cases V.S.1 and 2 (Table VI 31). The ratio of case V.S.3 was similar to the normal at four months since both arteries and alveoli were reduced in number. It may be that the lack of arteries in this case was due to over distension. Cases V.S.4 and 5 had a reduced ratio in comparison with the normal at four months but similar to the normal at three days.

There is no reason to suppose that any of these cases are significantly different from the normal, either in the total number of arteries or alveoli or in the ratio of alveoli to arteries.

TABLE VI 32

Number of Arteries per square centimetre of Lung Tissue and the Percentage in each size Range

Case	Total number	% in each size range				
		25-50	50-75	75-100	100-150	150-200 (μm)
V.S.1	206	47	32	9	9	3
V.S.2	182	53	27	12	6	2
V.S.3	72	25	28	25	16	6
V.S.4	140	42	23	14	16	5
V.S.5	172	47	23	12	11	6
Normal						
3 days	188	50.5	24.4	13.8	7.4	3.7
4 months	116	34.4	31.8	18.1	12	3.4

Venograms

In only one case where there was a connection between the systemic and pulmonary circulation of the heart, were the veins injected. This was case V.S.6, aged three weeks, with a patent ductus arteriosus. There was increased flow to the lungs demonstrated on the clinical radiograph. Figure VI 33 shows the venogram of the right lung in this case. The filling was complete and the drainage pattern seemed normal. The main vessels stand out more clearly than normal particularly in the upper and middle lobes. It is possible that there is less background haze.

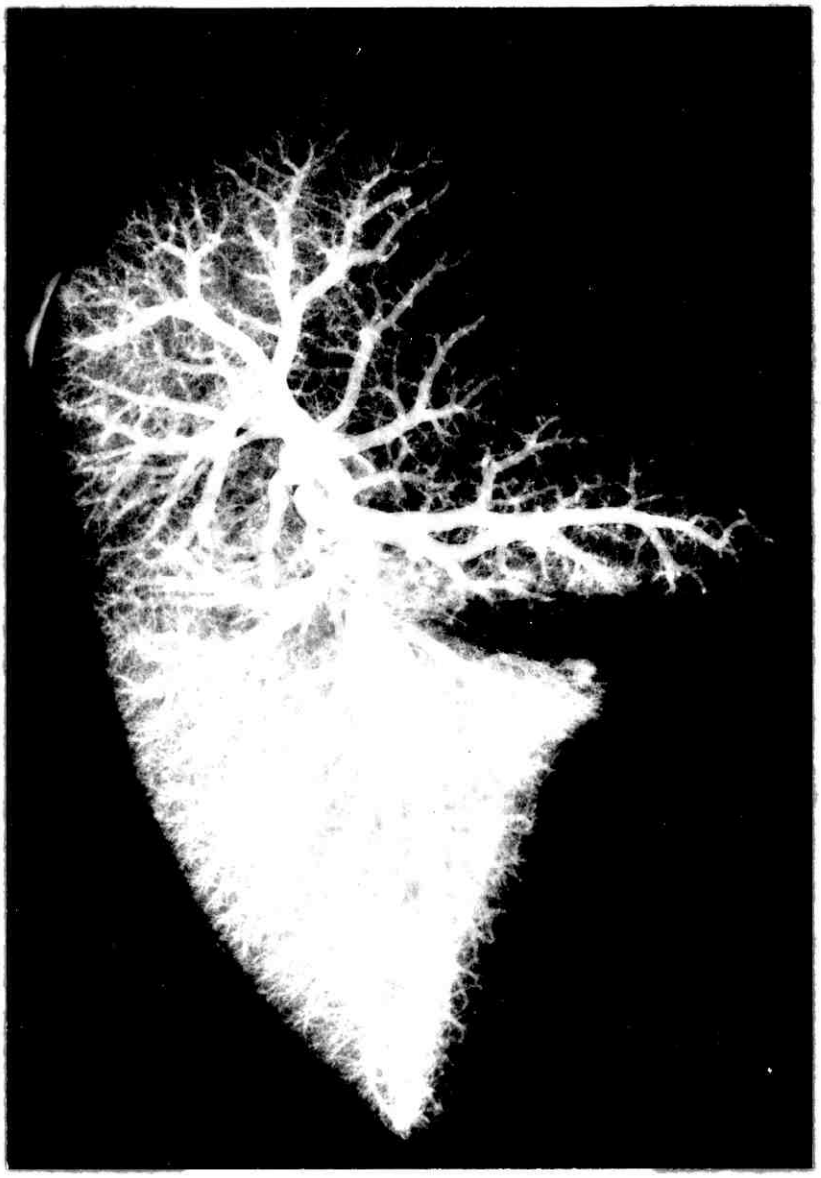
Measurements on Venogram

The measurements made along the axial pathway to the upper and lower lobes at 25% intervals are shown in Table VI 34 together with the normal upper lobe at 39 weeks gestation and the upper and lower lobes at three years. The upper lobe was increased along the whole length of the pathway in comparison with the normal at birth. This increase seems to be greater than would be expected particularly if the normal three year old is considered. There had been an increase in lumen diameter of the veins. The length of the axial pathways was near normal. The diameter of the veins one centimetre from the periphery in each lobe was increased in comparison with the normal cases. It seems likely that the veins throughout the lung were increased in lumen diameter.

Drainage Pattern of Veins

The drainage pattern of the veins can be seen from the venogram. The overall pattern was apparently normal and the tributaries near the hilum were of normal distribution. Serial

Figure VI 33



Venogram of the right lung of case V.S.6 (x 1.3). The main veins are increased in size but the drainage pattern is normal. It is possible that there is a slight reduction in background haze in comparison with the normal, since the main pathways stand out.

TABLE VI 34

Measurements from Venograms

Case	Upper Lobe					Lower Lobe					
	Length (cm)	Diameter (mm) % distance along pathway				Length (cm)	Diameter (mm) % distance along pathway				1 cm from periphery
		0	25	50	75		0	25	50	75	
V.S.6	3	3.5	2.5	2	1	5.4	4	3	2	1.3	1.1
Normal											
39 wks	2.5	2.5	1.3	0.9	0.4	4.5	-	-	-	-	0.7
3 yrs	5	-	4	2.5	1.0	9	6	5	3	1.8	0.9

sections were traced through 2 mm of tissue in the peripheral region. It was possible to see that conventional and both types of supernumerary vessels were present and in the normal proportions.

Veins per Unit Area of Lung

The number of veins and alveoli in a square centimetre was established in the same way as the number of arteries in other cases. The veins were grouped into size ranges from 25 - 200 μm external diameter. The percentage of the total number in each size range was calculated. Since the normal case at birth had not been fully inflated it was not possible to use this method on this case. The figures from the normal three year old were therefore used for comparison. The results are shown in Table VI 35. The total number of veins was decreased in comparison with the normal. The total number of alveoli was also reduced, as would be expected even if a normal case of three weeks were compared with a normal three year old. The ratio of alveoli to veins was increased in comparison with the normal suggesting a total reduction in the number of veins. The percentage of veins in each size range was normal.

Structure of Vein Walls

Only in case V.S.6 were the veins injected so that this was the only case which could be compared with normal injected material. The veins were of similar structure to the normal of 39 weeks gestation, with muscle increasing as the vessels increased in size. There were no true elastic laminae and muscle fibres were found between groups of collagen as in the late fetal cases.

TABLE VI 35

Veins and Alveoli per Unit Area

Case	% of veins in each size range (μm)					Total veins/ sq.cm	Total alveoli/ sq.cm	Ratio alveoli/ veins
	25-50	50-75	75-100	100-150	150-200			
V.S.6	37	31	15	13	4	211	6,368	30.18
Normal								
3 yrs	43	32	13	10	2	379	8,557	22.57

It was possible to look at the structure of the vein walls in the other five cases in which the arteries had been injected. Cases V.S.2 and 5 had veins with a normal appearance, like those of case V.S.6. Case V.S.3 however showed a slight change in wall structure. Some of the vessels were normal while others showed a more obvious amount of elastic tissue in the form of laminae both in the media and at its outside edge. Cases V.S.1 and 4 had even more marked elastic laminae, the vein structure more resembling that of arteries (Figure VI 34). Arterialisation of this type has been noted by Wagenvoort (1970) in cases with venous hypertension.

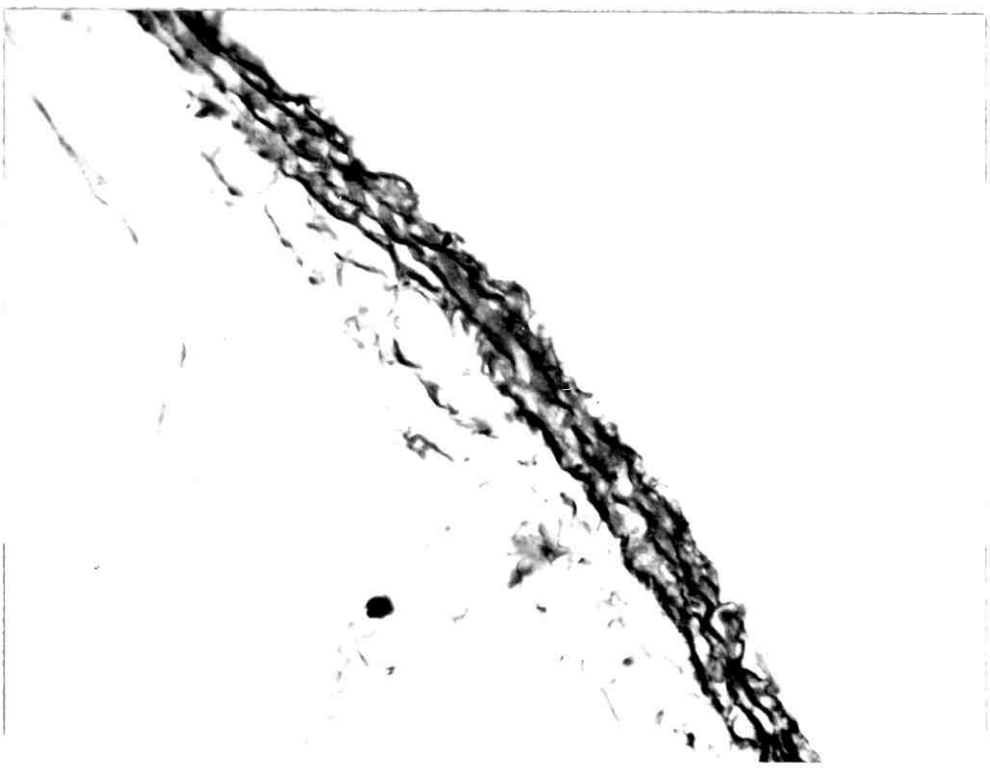
Measurements of wall Thickness of Muscular Veins

The smallest vessel in the injected case V.S.6 with a muscular wall was 105 μm in diameter. The largest vessel with no muscle in its wall was 130 μm in diameter. These figures are very similar to the normal at birth.

The wall thickness could only be measured accurately in the lung with injected veins. The percentage wall thickness for this case is plotted against external diameter in Figure VI 35. The curve produced is almost identical to that seen in the normal cases with an increase in percentage wall thickness in the small veins below 300 μm. The wall thickness fell within the normal range at all diameters.

The percentage wall thickness was also measured on the uninjected veins of the other five cases. Though this is less accurate than measurements on injected material it is possible to see trends. The figures from the five cases together with average results on a series of normal uninjected cases are shown in Figure VI 36. Even in the normal there is a wide scatter of wall

Figure VI 34



Photomicrograph of the vein wall of an uninjected vessel from case V.S.4 (x 675). There are marked elastic laminae within and delineating the media and there is little elastic in the adventitia. The wall of this vein is 'arterialised' in comparison with the normal (see Figure V 13).

thickness and only the means have been plotted for all cases. As with injected material there was a rise in percentage wall thickness in the smaller vessels both in normal and abnormal cases. In cases V.S.2 and 5 the percentage wall thickness was about normal, while in the other three cases it was increased. These three cases were those with the 'arterialisation' of the vein walls and those with the highest percentage wall thickness in the arteries.

It seems that the structure of the vein wall in cases of ventricular septal defect varies. Some show an increase in muscle and elastic and some remain normal. This probably relates to the changes in the walls of the arteries. There would seem to be an increase in lumen diameter of the veins and a possible decrease in the number of small vessels.

Figure VI 35

Percentage wall thickness related to external diameter for the veins from case V.S.6. The mean and range for each diameter range is shown.

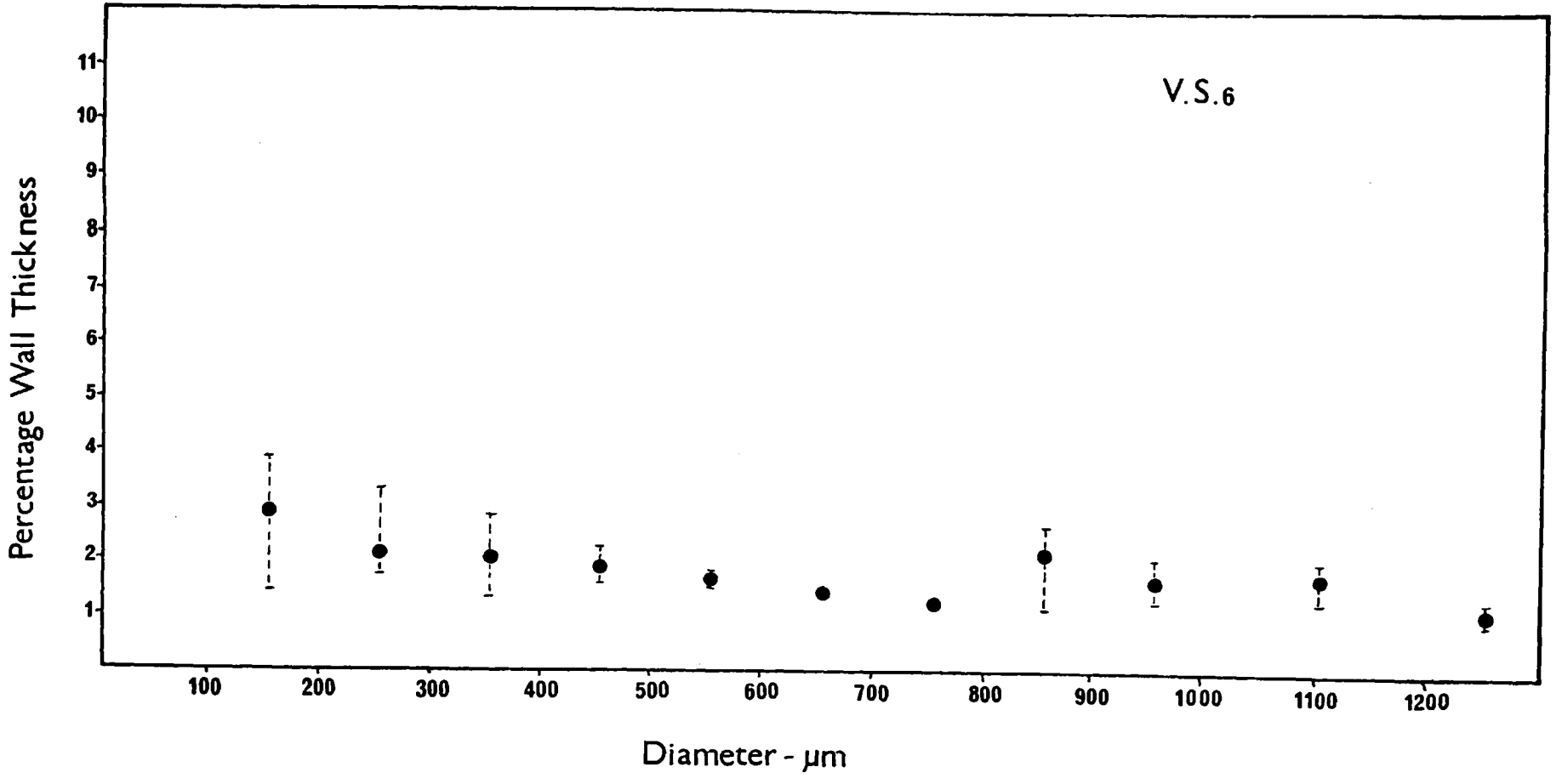
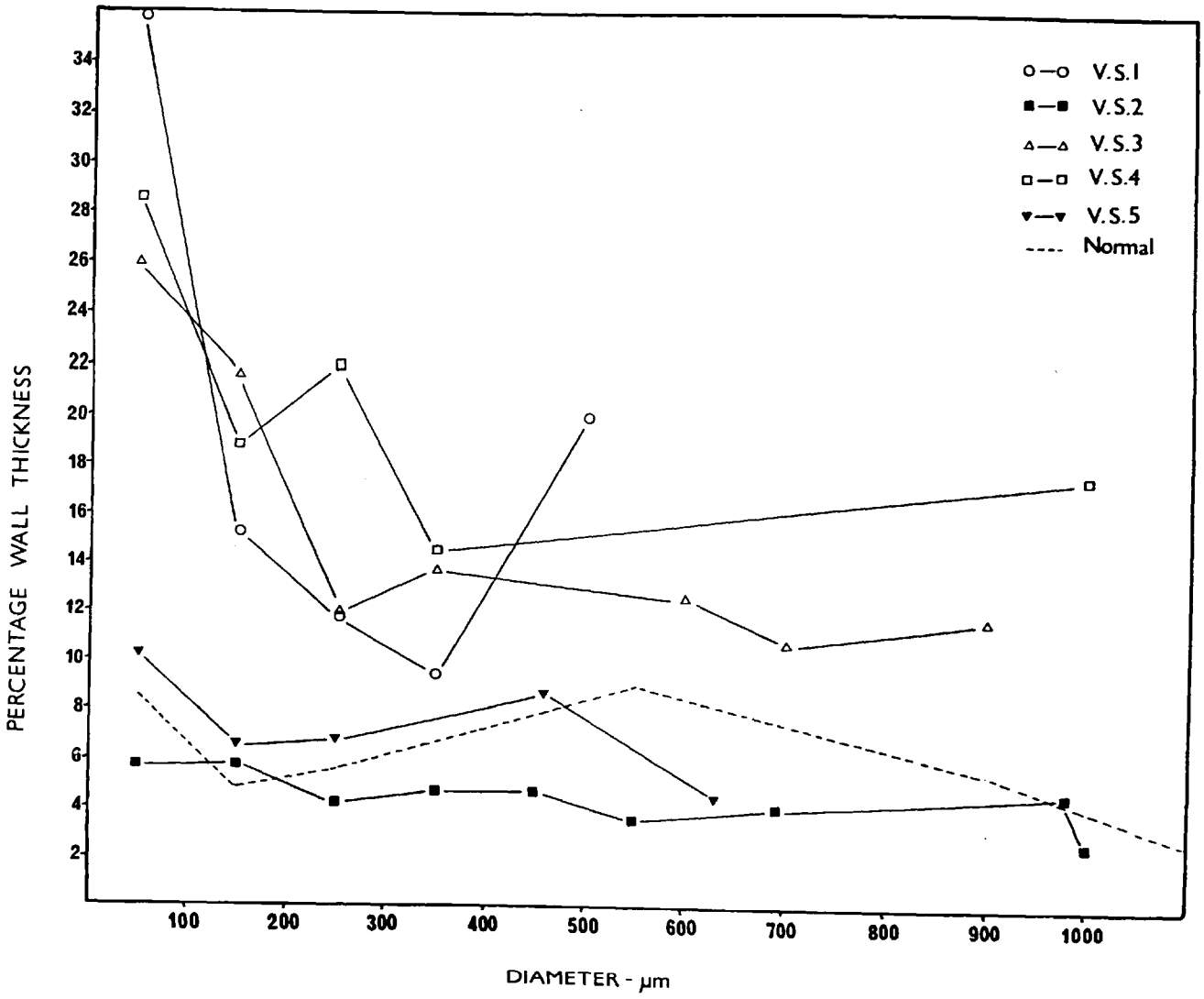

V.S.6
Percentage Wall Thickness
11
10
9
8
7
6
5
4
3
2
1
100
200
300
400
500
600
700
800
900
1000
1100
1200
Diameter - µm

Figure VI 36

Percentage wall thickness related to external diameter for the uninjected veins of cases V.S.1, 2, 3, 4 and 5. Each point represents a mean value for the size range. The normal line represents measurements made on the uninjected veins from a number of children. Cases V.S.1, 3 and 4 have a raised percentage wall thickness.


PERCENTAGE WALL THICKNESS
DIAMETER - μm
V.S.1
V.S.2
V.S.3
V.S.4
V.S.5
Normal
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
100
200
300
400
500
600
700
800
900
1000

Structural Features in Ventricular Septal Defect - Summary of the Six Cases

The structural findings in the six cases can be summarised briefly and are shown in Table VI 36.

Case V.S.1

This case aged three weeks had right ventricular hypertrophy associated with an increase in percentage wall thickness of the larger arteries although the smaller vessels had a normal wall thickness for age. There was extension of muscle into smaller vessels than in the normal but, the vessels in the acinar region being smaller than normal, there was no extension of muscle into the acinar arteries, in fact it was slightly less muscular than the normal. The reduction in size of the vessels was seen throughout the lung as the lumen diameter on the arteriograms was reduced at hilum and periphery. There was no tortuosity seen on the arteriograms and no pruning and the number of vessels per unit area of lung was normal. The veins were increased in wall thickness and were arterialised in structure.

Case V.S.2

This case had right ventricular preponderance but at the age of three weeks this may not be abnormal, particularly as there was a reduced percentage wall thickness. Muscle extended into slightly smaller vessels than normal but since the arteries within the acinus were hypoplastic this did not represent extension of muscle into the acinus. Though the arteries at the periphery were reduced in size, the vessels near the hilum had an increased lumen diameter

TABLE VI 36

Summary of Structural Changes in Arteries in Cases of Ventricular Septal Defect

	V.S.1	V.S.2	V.S.3	V.S.4	V.S.5
Arteriograms					
Tortuosity	N	+	N	N	+
Lumen diameter	-	+	+	-	++
Wall Structure					
% wall thickness	+	-	++	++	+
Muscle extension into small vessels	+	+	++	++	++
Muscle extension into acinus	-	N	+	+	+
Diameter of arteries with airways	-	-	+	-	N
Structure of arteries with airways	-	-	+	+	+
Right ventricular hypertrophy	+	+	+	+	+

N - Normal
\+ - Increase in size or muscle
\- - Decrease in size or muscle

on the arteriogram: at all levels the vessels were tortuous. The number of vessels per unit area seen microscopically was normal despite pruning on the arteriogram. The veins of this case were normal in structure and wall thickness.

Case V.S.3

This case aged two months at death showed a marked increase in muscularity of the arteries with a rise in percentage wall thickness and an extension of muscle into smaller vessels than normal. Since the vessels within the acinus were increased in size this represented a marked extension of muscle to the periphery of the acinus. The more proximal vessels were also increased in diameter but there was no tortuosity. The number of small vessels in the acinar region was reduced. The veins of this case had an increase in muscularity shown by an increase in wall thickness and elastic laminae.

Case V.S.4

This case aged three months, had right ventricular hypertrophy demonstrated by weight, with a marked increase in percentage wall thickness and extension of muscle into smaller vessels. Though the arteries within the acinus were smaller than normal there was still extension of muscle to the periphery of the acinus. In this case there was also a reduction in the size of the proximal and peripheral vessels on the arteriogram, with no tortuosity. The number of arteries per unit area was normal. The veins showed an increase in wall thickness and arterialisation of their structure.

Case V.S.5

This case aged three months, also showed right ventricular hypertrophy with an increase in percentage wall thickness, though not as great as that seen in cases V.S.3 and 4. There was an extension of muscle into smaller vessels and since the vessels accompanying acinar structures were of normal diameter this meant an extension of muscle to the periphery. Though the intra-acinar vessels were of normal calibre those of the pre-acinar region, measured on the arteriogram, were larger than normal and more tortuous. Though there was pruning seen on the arteriogram, there was a normal number of arteries in the acinar region. The number of branches from the axial pathways of the acinar region was however reduced. The veins were of normal wall structure and thickness.

Case V.S.6

In this case of three weeks the veins had been injected. The venogram showed a normal branching pattern and this was confirmed microscopically. The lumen diameter of the veins was increased. There was no change in the appearance of the vein wall nor in its thickness or the extent of muscle into small vessels. The number of small veins per unit area of lung was slightly reduced. The arteries of this case seemed increased in muscularity but not to a gross degree.

SUMMARY OF RESULTS

Tetralogy of Fallot

1. The volume of the lungs in all six cases studied was reduced by comparison with normal lungs of the same age.

2. The arteries measured on the arteriograms were all reduced in length and diameter along their whole length; the gradient along the pathway was also reduced.

3. The segmental branching pattern seen on the arteriogram was normal. There was a dense background haze suggesting a relative increase in small vessels.

4. Serial reconstruction of the peripheral part of an axial pathway in one case (T.F.4) confirmed that the branching pattern was normal.

5. The vessels traced, axial, conventional and supernumerary were all smaller in diameter than the normal of similar age.

6. These vessels were of normal muscularity for their position in the lung, but increased for size.

7. There was an increase in percentage wall thickness in all cases and particularly those which had an increased flow as a result of operation.

8. Two out of five cases (T.F.1 and 3) had a normal distribution of muscle in the small vessels, the other three cases had extension of muscle into smaller vessels than normal.

9. In all five cases the arteries within the lobule were smaller in external diameter than in the normal of similar age.

10. These arteries within the lobule were of normal muscularity as judged by position within the lobule.

11. Right ventricular hypertrophy was demonstrated in all six cases.

12. There was an increase in the number of small arteries in the acinar region associated with a decrease in the number of alveoli. The ratio of alveoli to arteries was therefore decreased.

13. In one case (T.F.6) the veins were injected. Measurements made on the venogram showed them to be reduced in length and diameter.

14. The drainage pattern appeared to be normal on the venogram and this was confirmed by serial reconstruction.

15. The total number of veins per unit area of acinar tissue was increased.

16. In this case and those with the arteries injected the veins had a normal structure with normal percentage wall thickness and extension of muscle.

17. Summaries of the structural features showed that although the cases varied considerably in age the changes in the arteries and veins were similar in all cases.

Ventricular Septal Defect

1. The volumes of the lungs from the six cases studied were normal.

2. The arteriograms of the five cases with the arteries injected were all different in appearance and all abnormal. Two cases (V.S.1 and 4) showed a decrease in lumen diameter, one case a slightly raised diameter (V.S.2) and two cases a marked increase in lumen diameter (V.S.3 and 5).

3. The arteriograms suggested that the branching pattern and size of the arteries were abnormal, there being apparent pruning of side and distal branches in some cases.

4. Blunt dissection showed that distribution of the branches was altered in some cases, though the total number of branches was normal. In two cases (V.S.2 and 5) the first seven generations occupied a greater percentage of the axial artery length than normal.

5. Serial reconstruction of the periphery of an axial pathway in case V.S.5, the case most affected by abnormal growth, showed that though the proximal part of the pathway was increased in length and diameter, the peripheral centimetre of this pathway was reduced in size both in length and diameter. There was no extra branching from the appearance at birth in this case of three months. There would seem to have been a lack of growth in this region of the lung.

6. The arteries along the axial pathway in the case reconstructed were more muscular than normal.

7. Four of the five cases showed a wall thickness greater than the normal for their age. The increase varied in degree. In the fifth case (V.S.2) there had been an excessive reduction in wall thickness.

8. In all of the five cases there was extension of muscle into smaller vessels than is normal.

9. The arteries within the lobule were of normal external diameter in one case (V.S.5), too large in one case (V.S.3) and too small in three cases (V.S.1, 2 and 4).

10. The vessels within the lobule were less muscular than normal in two cases (V.S.1 and 2) and more muscular than normal in the other three cases.

11. Muscle did not extend to the normal level of the acinus in one case (V.S.1), was to the normal level in one (V.S.2) and in the other three cases was found right to the periphery of the acinus, abnormal at this age.

12. The number of small arteries and alveoli in the acinar region was near normal in all five cases.

13. It is likely that there was right ventricular hypertrophy in all cases.

14. In one case the veins were injected. Measurements made on the venogram showed that there was an increase in lumen diameter at all levels.

15. The drainage pattern appeared to be normal and when traced microscopically was found to have the normal proportions of conventional and supernumerary veins.

16. There was a drop in the number of intra-acinar veins when related to the number of alveoli.

17. In this case the veins were of normal structure and wall thickness, as they were in two of the other cases. In three of the six cases (V.S.1, 3 and 4) there was an increase in percentage wall thickness and in the amount of elastic in the media.

18. The summaries of the structural features of the six cases showed that although the patients were similar in age the structural changes varied considerably.

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DISCUSSION

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It is only possible to look at changes in childhood and infant disease and in congenital defects, if the normal structure of the lung is known. Between early fetal life and adulthood there are gross changes in size and structure of the lung and it is necessary to compare any abnormal lung with a normal of the same age.

The normal adult pattern of the pulmonary artery - both the structure and the three dimensional relationship of branches - was described, using precise quantitative methods, by Elliott (1964). Davies (1969) used similar methods of study on children though he did not study the branching pattern. The purpose of this work was to extend these studies both to an even earlier age by reconstructing the branching pattern of the arteries during fetal life and in childhood and by determining the structure related to the length of the axial pathway as well as to diameter. From this work a longitudinal study of ante and post natal development has been made and made available results over a complete age range for comparison with abnormal lungs.

The venous drainage pattern has in the past only been described by structure in the adult with scant reference to the drainage pattern. In the present study the development of the drainage pattern and the structure of the vein walls has been described.

By comparison with these results it is possible to analyse accurately changes in arteries and veins as a result of childhood disease. It must be stressed that all features must be compared to the normal of the same age. It is also possible to decide in an abnormal lung the equivalent normal age and in some cases to determine the age of onset of the changes.

Changes brought about by disease will often give information on the normal development of the lung and its control. Two features of particular importance in the blood vessels of the lung are pressure and flow of blood which are affected by congenital heart defect. Examples of a defect potentially reducing or increasing the blood flow and pressure have been studied in this series.

Before reviewing the results on the normal and abnormal lungs, the accuracy and application of the methods must be mentioned.

TECHNIQUES

Injection Technique

Though the advantage of injecting the pulmonary arteries with a radio-opaque medium has been appreciated by many workers, the pressure at which the injection should be made has been disputed. Short (1956) and Evans, Short and Bedford (1957) used a hypertensive pressure of 50 - 100 mm mercury while Wagenvoort, Heath and Edwards (1964) used pressures approximating to pulmonary systolic pressures in life (25 mm) and warned about higher pressures causing damage to the blood vessels. A high pressure of 100 cm of water was used by Elliott (1964) with no damage to the arteries. He found that all vessels were fully expanded at this pressure. Davies (1969), Anderson (1971) and Semmens (1970) have used this pressure on children and diseased adult and child lungs with no damage to vessel walls.

The lungs of fetuses have a high pressure in the pulmonary arteries, closer to the level of the high injection pressure. Since these lungs were to be treated in the same way as the previous work

in this department by Elliott et al on adults and children and the results were to be comparable it was decided to inject the fetal lungs at the same pressure. This pressure provided good filling in all the fetal lungs and also in the hypertensive cases of children with a congenital heart lesion. There was no bursting of vessels in even the youngest fetal lungs where the muscle was not fully formed in the artery walls.

In all cases the medium extended into arteries as small as 15 μm in diameter but did not extend into the capillary bed. This was similar to the findings in the adult lung. At this pressure all elastic laminae were flattened suggesting full distension. It has been possible therefore, using the same technique, to inject the arteries in lungs from fetuses of 12 weeks gestation up to adult lungs of old age. Our technique was not applicable to younger fetal lungs because of the small size of the arteries at the hilum. Smaller lungs would require cannulation under the microscope and hand injection probably in situ. These lungs would therefore not be comparable.

The veins in the lung have a lower pressure and it might be expected that a lower pressure would be required for injection. After testing lower pressures in fetal lungs it was found that full injection to a diameter of 15 μm with flattening of the laminae was only obtained when a pressure of 100 cm of water was used. This pressure led in the older case of ten years to some leakage into the surrounding tissue but it was still possible to see the overall picture. There was no bursting in the fetal lungs where there was less muscle.

Fixation Technique

In the adult (Elliott, 1964) and the child (Davies, 1969) the same head of pressure of injecting fluid was used to inflate all lungs to the same amount, that is until the pleural surface was tense. It was decided to use the same technique for all cases studied here, so that it could be assumed that in all the total lung volume and alveolar size and number could be compared. Since cases prepared by Davies were also used in the present study it was preferable that all cases were treated in the same manner. Fetal lungs of under 28 weeks gestation and those that had not breathed were not inflated since this would seem unrealistic and was not necessary for adequate fixation. It was found that in all older cases the fixation was adequate and inflation did not cause rupture of the pleura nor of the bronchioli or alveoli.

Staining

Fetal tissue of any kind is difficult to stain, probably because of the high water content and the possible chemical immaturity of the tissue. The fetal lung was found to be particularly difficult to stain especially the muscle of the pulmonary arteries. Different stains have been used in an attempt to give a more efficient means of identification of muscle, unfortunately with little success. It was found that the best stain for all lung tissue, fetal or adult, was Verhoeff's elastic stain counter stained with Van Gieson's stain. Using this method it was not possible to identify muscle during early fetal life. Since the walls of the arteries were relatively thicker than in the adult and child, this was not because the walls were too delicate to see or because the walls were stretched to their full

limit. Cells could in fact be seen between the elastic laminae in the arterial media but these did not stain specifically for muscle.

In later fetal life it was possible to identify stained muscle cells. It seems likely that there is a primitive muscle cell which does not have the staining characteristics of adult muscle. Cells in the walls of small blood vessels with some of the features of muscle cells have been described by Miss Meyrick, a colleague in this department, using the electron microscope (see page 358). Kaufman (1965) described a primitive vessel wall cell which he considered formed an embryonic wall structure and a new type of vessel. He found this new structure only in the smallest vessels which later had a normal wall structure. The primitive muscle cells seen in the present study were found at all sizes of artery and are not comparable with his embryonic vessels. Cellular vessels which were seen only in the 12 week fetus may be of Kaufman's embryonic type.

Immature muscle cells have been described by Yamauchi and Burnstock (1969) and in the vas deferens of the mouse, and Rhodin (1968) has described pericytes as precursors of muscle cells in the small post-capillary venules of the rabbit skin.

Arteriograms and Venograms

The fact that the injection medium is radio-opaque enables a record of the overall branching pattern to be obtained by radiography. It is also possible to see whether injection has been complete and aids identification of particular pathways. Any gross abnormalities in the blood vessel system are obvious,

for example the loss of vessels to one segment or the presence of a thrombus occluding wholly or partially a vessel, and also tortuosity or pruning as a result of changed blood flow. It is also possible to record changes in length and diameter of vessels by measurement, firstly changes with age and then changes due to abnormalities in the heart or lungs. In this way they are an aid to further microscopic study. On the other hand the arteriogram did not offer a satisfactory way to trace accurately the branching pattern along a single pathway as an alternative to serial sectioning even using very fine grain film.

In the adult a background 'blush' or haze is seen in the arteriograms; this masks the main pathways to some extent. This haze was not seen in the fetal lungs nor in children up to the age of four months. It is made up from the numerous filled small vessels throughout the lung. In the fetus though there is filling to the same size of vessel there are not the large number of small vessels. The blush only develops with the rapid multiplication of vessels within the acinus as these grow by alveolar multiplication. During fetal life there are only a limited number of vessels along the main pathways to the terminal bronchiolus, with few vessels within the acinus. The total number of vessels smaller than 200 μm in external diameter, per unit area is less during fetal life and the first few weeks of life than in the older children. Age determines the amount of background blush seen on arteriograms though it seems to be similar in late childhood and the adult. The blush is not due only to the lung increasing in thickness since the blush is present in small lungs. This blush is not apparent in some diseased lungs which would suggest a loss of small vessels. This was noted by Semmens (1971) in cases with emphysema. In the cases with a

ventricular septal defect in the present series there was little background haze, particularly in cases V.S.3 and 5. In these the number of arteries per unit area of tissue, related to alveoli in the acinar region was on the low side of normal. In the cases of tetralogy of Fallot in this series there was an increase in the density of the background haze which is reflected in the increase in number of small vessels per unit area of lung seen in these cases.

Venograms like arteriograms showed the general pattern of the drainage system. Again there was an increase in background haze with age, and in the oldest cases it was difficult to discern the main venous drainage pattern. In the diseased lungs there was an apparent lessening of the haze in the case with a patent ductus arteriosus while there was an increase in the case with tetralogy of Fallot. This correlated with the number of veins per unit area counted microscopically.

It seems then that the arteriograms and venograms can give some idea of the state of the main vessels by measurement and the small vessels by amount of blush and tortuosity.

Percentage Wall Thickness and Medial Area

Many workers have made measurements of the wall thickness related to the external diameter or lumen diameter, however these do not take into account the amount to which the medial coat is stretched which will vary from case to case and within a lung. The injection technique used in the present study ensures that all vessels are fully distended. This is shown by the flattened

elastic laminae. Though this may not be the condition of the vessels during life, at least they are all in the same condition. Any measurements made on these vessels will be comparable throughout the lung and between lungs.

Accuracy of Measurement

Using these precise methods it is possible to produce accurate and comparable figures as between cases. Furthermore these methods are repeatable both at a later date by the same investigator and by different workers. In the present study identical results were achieved on two separate occasions three years apart, for a serial reconstruction. Measurement of percentage wall thickness made on the same material at intervals gave the same overall result but with slight variations in the individual measurement. Results obtained in the present study on the same cases as those used by Davies (1969), though from different blocks gave very similar results both for percentage wall thickness and population counts.

In the normal the range of percentage wall thickness seen in any one size range is very small at artery diameters over 300 μm. Measurements on an abnormal lung will be statistically valid if one artery in every 100 μm size range is measured, providing that they are all in the same direction from normal (Benjamin, personal communication).

Measurements of percentage wall thickness in all cases in this study and in those of others have shown a relative increase in wall thickness in small arteries at all ages. This increase is always in the same size range. It could be as a result of inadequate distension in the small arteries only. However

calculation of medial area which does not alter with distension showed that there was a relative increase in muscularity. This calculation is tedious and since it only confirmed the results of percentage wall thickness was only used for a few arteries and not in each case.

The increase in percentage wall thickness starts at an external diameter of about 300 μm in all lungs whether fetal or during childhood. Elliott found the increase at a similar diameter in the adult (1964). In the child this is the diameter at which the smallest measurable wall thickness was found. This was a wall thickness of 2.5 μm. It can be assumed that this is not completely accurate since it is the equivalent of one unit on the graticule scale using the x 40 objective. However a similar figure was obtained using the x 100 objective. It is probable that this is the diameter of a single extended muscle cell. Yamauchi and Burnstock (1969) showed using the electron microscope that a smooth muscle cell nucleus in the mouse vas deferens was about 2.5 μm in diameter.

If this is the smallest muscle cell then there can be no further thinning of the muscular wall. As the external diameter decreases the wall will form a larger percentage. In the child the wall one muscle cell thick was found in arteries between 50 - 300 μm external diameter. The percentage wall thickness will rise over this size range. At the end of the pathway there is only a spiral of muscle and though the thickness will still be the same there may not be a greater resistance to flow since only part of the wall is muscular. Though the increase is fortuitous it is probably still important in raising resistance.

In the fetus the rise in percentage wall thickness is

greater and is associated with a wall more than one muscle cell thick. The increase is still at the same external diameter but at this age the wall thickness may be 5 μm or more. Because of the rise in percentage wall thickness there is a greater drop in lumen diameter and perhaps a higher resistance. Such vessels are more proximal in the fetal lungs.

Arteries with Airways

Measuring identifiable arteries, that is arteries that can be landmarked by the airway they accompany is a method that has been used by several authors (Short, 1956; Arias-Stella and Saldana, 1963; Arias-Stella and Castillo, 1966). It is a more accurate method than measuring arteries at random since those from different cases can be compared knowing that they are at a comparable level. This is particularly important when deciding if an artery is hypo or hyperplastic. Elliott (1964) showed that the size of arteries with a given airway covered a wide range in the adult. This was also true in the fetus and child. This is due to the fact that there are several generations of each type of airway along an axial pathway and the artery decreases in size steadily towards the periphery. When measurements of this type are made it is essential to have a large sample and when comparing cases to take into account the range as well as the mean. This method is one of the most useful for replacing serial reconstruction since much information can be gathered from it - size of vessels, wall thickness and extent of muscle into the acinus. It has been found that results from this method are very similar to those from serial reconstruction.

Number of Alveoli and Arteries

Dunnill (1962b) and Weibel (1963) used similar and precise methods (Weibel and Gomez, 1962) to estimate the total number of alveoli in a lung. Their figures are similar for the number of alveoli in the adult, which according to Dunnill is attained by eight years. Davies (1969) found similar results to Dunnill for his series of normal children, though his figures were all about 10 per cent higher. He showed that a useful assessment of alveolar size was given by the number of alveoli per cc of tissue. An emphysematous lung had fewer alveoli per cc. In the present cases it has been possible to simplify the method of estimating alveolar size by counting the number of alveoli in one square centimetre of acinar region. This figure though not leading to the total number of alveoli so precisely can be used for comparison between cases. The present results in the normal fitted well the pattern of multiplication described by Dunnill and Davies. In the cases of tetralogy of Fallot however a difference has been demonstrated, the alveoli are larger than normal. Though not as precise as full quantitative analysis this simple method has much to commend it as it is quicker and can be more widely applied. It is therefore useful as a screening test.

For an estimation of the number of small arteries per unit area it is necessary to take into account the number of alveoli since size and inflation may be a factor affecting the number of arteries. The method of inflation used gives a constant degree of inflation so it is only growth which is important. As the alveoli grow in size with age it seems that there are fewer arteries per unit area - this is due to the alveolar number decreasing as they increase in size. The ratio of alveoli to arteries remains the same. It is therefore necessary to estimate

both arteries and alveoli and use the ratio for comparison. This also allows for any artefacts that may be brought about by inflation.

Serial Reconstruction

There are obvious advantages in making a serial reconstruction by microscopic means of the branching pattern. A three-dimensional picture of the branching pattern can be built up and at the same time the structure of the vessel can be studied. Casts show the branching system but the lung is lost for histological investigation. Random sections show structure related to the size of the artery but do not relate this to the position in the lung or the branching system. Serial reconstruction allows study of the branching pattern and structure and their relationship to each other. This however is a very tedious method. Elliott and Reid (1965) described the adult pattern of the arteries and the present study has shown that from 16 weeks gestation the pattern of branching is essentially the same from the hilum to the terminal bronchiolus, the only changes being of size and structure of the vessels. There is of course individual variation. It seems likely from the present study that the venous drainage system is also complete during early fetal life. Having established this it is unnecessary to make reconstructions of all cases unless a developmental anomaly at an early stage is suspected. The structure of arteries related to generation number also seems to be established early.

A set of methods can be used routinely to study cases and obtain the maximum information without serial reconstruction. Arteriograms show an overall impression of the arterial branching pattern while measurements on these show changes in diameter and length. At any age there is a particular size range for the

arteries and any abnormality is shown up when comparable ages are used.

It is also possible to see any gross abnormality on the branching pattern by blunt dissection of the airways and arteries. Any disease with its onset after birth will only affect the intra-acinar branching pattern. It is therefore worthwhile in some cases to make a serial reconstruction of the peripheral centimetre which will include the acinar region. This is particularly important if the arteriogram seems abnormal, as in the case of V.S.5. It was found in this case that the development was retarded in this region. Dissection will show if there is correct spatial arrangement of airways and arteries but it is not possible to discern supernumerary vessels. If a pre-natal growth disturbance is suspected a complete serial reconstruction is still required, for example in cases of Congenital Diaphragmatic Hernia (Kitagawa, Hislop, Boyden and Reid, 1971).

Information on the structure and size of the arteries can be obtained using random sections from the lung. Measuring arteries with identifiable airways gives further landmarking of structure and position. Population counts show any extension of muscle into smaller vessels than usual and percentage wall thickness any change in the amount of muscle in the wall. It is also possible to determine the range of diameter for elastic, transitional and muscular structure. These can then be positioned in the lung by reference to the arteriogram. From random samples it is also possible to estimate alveolar number. An overall impression of the lung is therefore obtained using these methods. Only the relative numbers of supernumerary and conventional

arteries is unavailable and normally this seems to be much the same at all ages.

Having described the normal pattern of the venous system by serial reconstruction it has been found to follow similar laws to the arteries. It is therefore possible to study it in similar manner to the arteries.

BRANCHING PATTERN OF ARTERIES - DEVELOPMENT

Arteriograms

The arteriograms showed the changing pattern of the arterial branching system with age. At first there was an increase in the complexity at the hilum and in the proximal regions as the main arteries branched. By 16 weeks the adult pattern of branching of the main pathways could be seen in miniature. However the background was clear and it remained so until after birth when the blush representing the intra-acinar arteries developed.

The pattern in each lung, though similar, was different. Each case had its own definite pattern but since the lungs from siblings were so much more similar than those of different families it seemed that the branching pattern must be regarded as genetically determined.

Serial Reconstruction

The changing pattern of arterial branching was confirmed by serial reconstruction. In the cases up to 18 weeks of intra-uterine life there was an increase in the number of branches in the

pre-acinar region of the posterior basal segment. After 18 weeks there was variation but no upward trend. The number found in these cases from the hilum to the terminal bronchiolus was similar to the number seen in the adult by Elliott (1964). It seems that the pattern of the adult is present by the 16-18th week of fetal life. This must relate to the fact that the pre-acinar bronchial branching pattern is complete throughout the lung by 16 weeks gestation (Bucher and Reid, 1961). These two systems would seem to grow together. This is borne out by the fact that at 12 weeks gestation there were the same number of conventional arterial branches and airway branches and that growth was from the distal end of both pathways. The branching pattern described here confirms the suggestion put forward by Loosli and Potter (1951) that arteries to the level of the terminal bronchiolus are present by the fourth month of gestation.

Like the adult the fetus has conventional and supernumerary vessels, the latter appear at the same time as the conventional vessels and maintain a similar course throughout life. It seems that all vessels must develop at the same time though it seems unlikely that there is much blood flow through the supernumeraries during fetal life, since they are usually small and thick walled and would require a high pressure to keep them patent.

Though all cases studied over 18 weeks gestation had a similar number of branches there was some variation. Conventional arteries varied from 19 to 26 and supernumerary arteries from 43 to 76. The former are similar variations to those seen by Bucher and Reid (1961) for the airways. The ratio of supernumerary to bronchial branches was similar whether before or after the branching pattern was complete with an average of 2.42 and a standard error of the

mean of only 0.105. This is similar to the pre-acinar ratio seen in the adult of 2.77 (Elliott, 1964). These facts emphasise the similarity between cases as well as the considerable individual variation. It is particularly interesting that the ratio is the same when the pathway is incomplete, whether the right or left lung is involved and along different axial pathways.

In the incomplete pathway of 12 - 18 weeks there was still the normal ratio though at the periphery there were more supernumereries. It seems that there may be a loss in supernumerary arteries in the proximal zone to maintain the ratio. There is some factor which maintains the ratio. Over a certain distance from the periphery that is the capillary bed, there is a similar branching pattern. This is confirmed by tracing of lateral pathways. They run a shorter path to the periphery and their branching pattern is almost identical to that over the similar distance at the periphery of the axial pathway. The branching pattern is dependent on the distance from the periphery.

The supernumerary arteries are of importance during adult life as they could carry between 20 and 45 per cent of the total blood flow if necessary, as assessed by cross-sectional area of the branches. They provide a double method of blood supply to the acinar structure and also a collateral circulation. In the fetus there is a very small blood supply due to the high resistance in the lung vessels. These small vessels are therefore not required to help flow. However they may in fact cut flow further since as they are small they have a higher resistance to flow.

There seems to be no new development of supernumerary arteries in the pre-lobular region after birth. But within the

acinus as the main artery extends with airway growth, conventional arteries accompany and supply side branches and at the same time supernumerary arteries appear. It seems likely though that the conventional arteries appear a little ahead of the supernumerary vessels. In the intra-acinar region of the newborn there were already six conventional arteries but only supernumeraries between the first two branches. After birth the number of supernumerary arteries increasesmore rapidly than the conventional arteries. It seems also that new supernumerary vessels can form in the intra-lobular pre-acinar region during the growth period of childhood. Intra-acinar growth after birth is mainly of the supernumerary arteries except some of the conventional vessels accompanying alveolar ducts.

The supernumeraries may play an important part at birth, since they are smaller than surrounding conventional arteries. As will be discussed later and as was described in this study and by Wagenvoort, Neufeld and Edwards (1961) the smaller arteries seem to dilate at birth while the larger arteries only have a reduced wall thickness after some months. It is probable that there could be considerable flow through these small vessels immediately after birth.

The arteries grow within the acinus as the respiratory structures form, mainly the alveolar ducts and alveoli after birth. The total number of branches within an acinus increases with age. The five year old and ten year old lungs had similar numbers to that seen in the adult lung by Elliott (1964). Dunnill (1962b) and Davies (1969) have shown that alveolar multiplication is complete by eight years. The adult intra-lobular pre-acinar configuration of branching was also reached by ten years of age.

DIMENSIONS OF ARTERIES DURING GROWTH

Using methods of measurement both on arteriograms and microscopically it has been possible to study the changing dimensions of the arteries in the lungs during fetal life and childhood. Certain relationships have emerged between (i) artery diameter and (ii) length, (iii) age and (iv) lung volume and also (v) number of side branches. The most important correlations are dealt with below.

1. The length of axial pathways increases with age and with lung volume: the relationship is curvi-linear.

Measurements made on the arteriograms showed that there was a continuous increase in the length of the axial pathway to the upper and lower lobes. The length of the lower lobe artery was always greater than the upper lobe artery in the same case. The increase was most rapid during fetal life and up to 18 months of childhood. After this the rate of increase slowed considerably.

2. The diameter of an axial pathway increases with age and with lung volume: the relationship is curvi-linear.

Measurements made on the arteriograms showed that there was an increase with age in the diameter of the arterial lumen at all levels along its length. The increase was at similar rate in upper and lower lobes though the upper lobe arteries were always smaller. The rate of increase was at its most rapid during fetal life and early childhood and, as for 1, slowed after 18 months of age. Measurements made microscopically on the arteries within the acinus showed a progressive increase in size with age in diameter, again the rate of increase was greater during fetal life than childhood.

3. A linear relationship exists between the lumen diameter of an artery at the hilum and its length.

It is apparent from 1 and 2 that the increase in arterial length and diameter showed the same relationship to age. It can be deduced and has been shown directly that length of a pathway bears a linear relationship to its diameter at the hilum. A further relationship between diameter and length of vessel is demonstrated by the fact that the diameter of the vessels one centimetre from the periphery was the same at all ages. In the young fetus a vessel one centimetre from the pleura supplied a large proportion of the lung while in the older children it was only the acinar region. Therefore despite the type of distal tissue the diameter relates to the length of pathway to be supplied.

4. A linear relationship is found between the total lung volume and age.

Though volume and age did not show a linear relationship to diameter or length of pathway they did show a linear relationship to each other, both in fetal life and childhood.

5. The cube of the length of the axial pathway and of its diameter at the hilum are each related in a linear manner to (i) age and (ii) lung volume.

Though the diameter and length of the axial arteries did not relate in a linear manner to age and volume (1 and 2), the cube of these showed an almost linear relationship. It seems that the 'volume' of the artery is directly related to the volume of lung which it is supplying, that is to the capacity of the lung. Since the flow to the lung is related to the square of the diameter of

the area and this did not show a linear relationship with age or lung volume it seems that blood flow to the lung is not related in a linear way to the vessel size.

6. In the pre-acinar vessels, as measured on the arteriogram, the proximal lumen diameter increases by a greater percentage than the distal.

During fetal life the increase in diameter measured on the arteriogram is ten fold at the hilum but only six fold at the periphery (that is at a point 75% along the main pathway). After birth the increase is three fold, both proximally and distally. It seems that during fetal life when growth and multiplication is pre-acinar the increase in size is greater proximally. After birth when this multiplication was complete growth is symmetrical, proximally and distally.

7. In the intra-acinar vessels during childhood the diameter of proximal arteries increases by a greater percentage than of the distal.

Microscopically, it could be seen in the vessels of the acinus that those accompanying terminal bronchioli increased by nine fold from birth to 11 years while, over the same length of time, those at the level of the respiratory bronchiolus increased by six fold and at the alveolar ducts by three and a half times. There was therefore a greater increase proximally than distally. It is possible that this is related to the growth and multiplication of the peripheral vessels at this time. After multiplication is complete growth may be more symmetrical as in the pre-acinar region.

8. The gradient changes along the main pathway with age but not in a linear fashion.

Since there is a more rapid increase in the lumen diameter of proximal than of distal arteries during fetal life the gradient from hilum to periphery becomes steeper over this period. During childhood in the pre-acinar region it remains much the same. The low flow of blood through the lungs during fetal life is probably related to the shallow gradient at this time. The gradient in the upper lobe at all ages was steeper than in the lower lobe. This suggests a higher velocity to the upper lobe assuming blood volume to be equal or a greater flow to the lower lobe. An increase in flow to the lower lobes can be demonstrated physiologically.

9. The gradient within the acinus increases with age.

In the intra-acinar region since, during childhood, the proximal vessels increased by a greater amount than the peripheral vessels there was an increase in gradient within the acinus. This would lead to an increase in velocity toward the periphery. Other factors being constant the pre-acinar region will have the same velocity during childhood but at the distal end of the pathways this will increase with age.

10. During fetal life the increase in diameter of the conventional arteries shows a linear relation to age. During childhood it is curvi linear, the increase slowing after 18 months of age.

The conventional artery average diameter for the pre-acinar

region is shown in Figure VII 1 together with the regression line. There is a linear relationship between diameter and age and the overall increase from 10 - 40 weeks was seven fold. In the child the increase in diameter of the conventional arteries was at a slower rate with only a two fold increase from three days to 11 years. The increase was more rapid during the first 18 months of life. The curve was similar to that seen for the vessels measured on random sections.

11. During fetal life the supernumerary arteries increase in diameter, but less than the conventional arteries: during childhood the rate of increase again slows after 18 months of age.

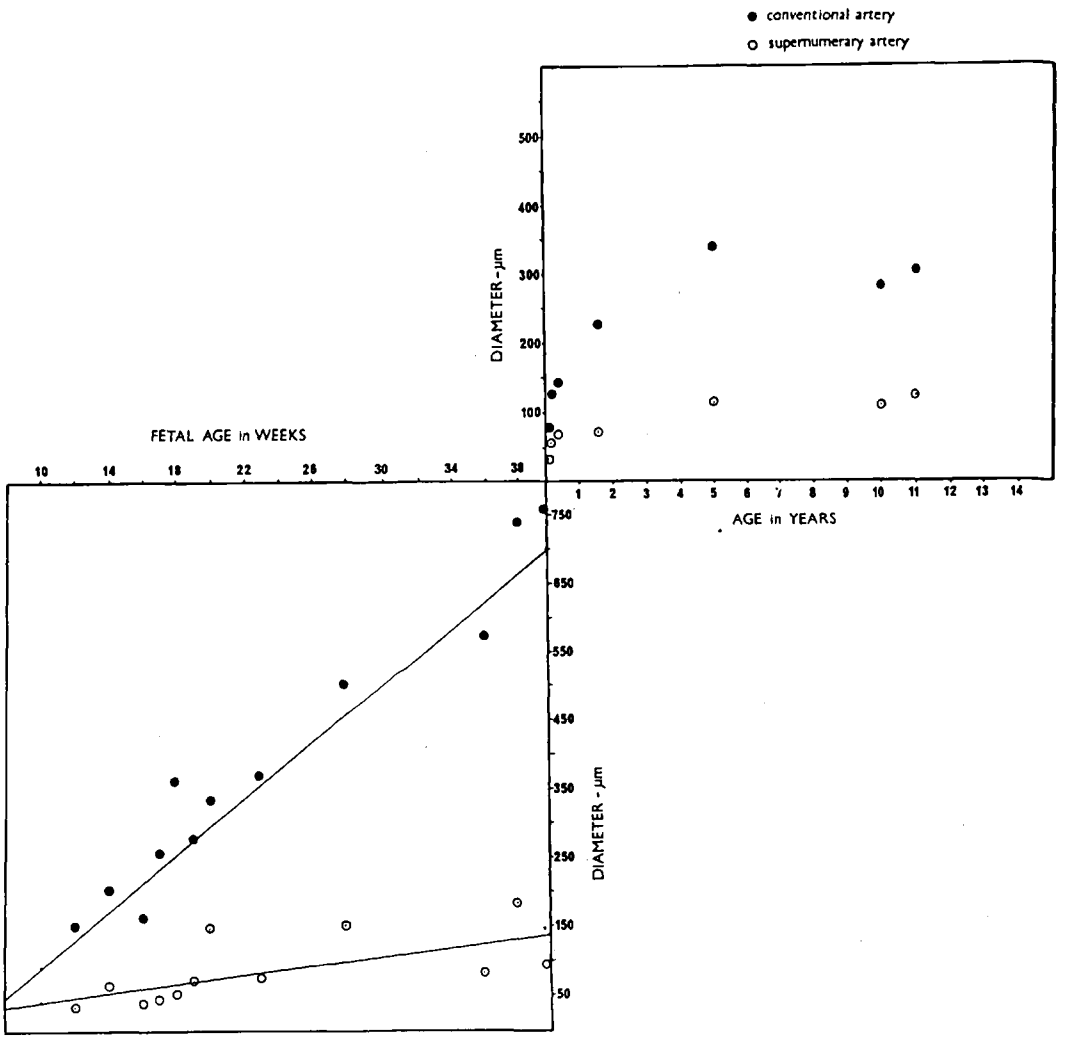
The average diameter of the supernumerary arteries during fetal life and those of the intra-acinar region during childhood are shown in Figure VII 1. The regression line for those of fetal life is shown. There was a three fold increase from ten to 40 weeks gestation, showing a linear increase. The overall increase was less than that seen in the conventional arteries. During childhood the supernumerary arteries are smaller than the conventional but increase at the same rate, the final increase being two fold. As with the conventional arteries there is a more rapid rise in the first 18 months of life.

12. There is a change in the pattern of growth after 18 months of age.

Artery length and diameter, both in the pre and intra-acinar region, show a change in the rate of increase with age at about 18 months. After this age the rate of multiplication of arteries and of alveoli also is slower. The more rapid increase in diameter during fetal life and early childhood is probably related to the

Figure VII 1

Diagram representing the change in diameter of conventional and supernumerary arteries during fetal life and childhood. The mean diameter of the pre-acinar branches along an axial pathway to the posterior basal segment for each fetal age is shown with the linear regression line for each type of vessel. The mean diameter of the intra-lobular conventional and supernumerary vessels along a main pathway to the lobule is related to childhood age in years.


● conventional artery
○ supernumerary artery
DIAMETER - µm
500
400
300
200
100
AGE in YEARS
1 2 3 4 5 6 7 8 9 10 11 12 13 14
FETAL AGE in WEEKS
10 14 18 22 26 30 34 38
DIAMETER - µm
750
650
550
450
350
250
150
50

rapid multiplication of new vessels. While this is occurring there must be increase in size of proximal vessels to allow for the distal increase in number.

Comment of Functional Implications

The blood flow within the lung increases with age as the child increases in size. The volume of the lung increases linearly with age and it might be expected that the blood flow would also show a linear relationship to the volume of the lung. The flow to a lung relates to the area of the blood vessel. In this series there was not a linear relationship between the area of the vessel at the hilum and the volume of the lung. During childhood the increase in cross section area of the artery at the hilum increases more slowly than lung volume. Therefore either flow is not increasing with volume or there is an increase in the velocity at the hilum.

It may be that flow is not related to the total volume but to each individual acinus. The number of these remains the same from 16 weeks gestation (Bucher and Reid, 1961) but they increase in size. The increase in each acinus is probably related to total volume and therefore not to flow. The cross-sectional area at the hilum is related linearly with volume for the first 18 months of life. During this first 18 months there is the same relative growth in size but there is a burst of multiplication of alveoli at this time with a corresponding increase in numbers of arteries in the acinar region. It would seem then that in the first 18 months the flow relates to the number of arteries in the intra-acinar region. After this time there is a relative slowing in the area related to lung volume and it is only the cube of the diameter or the volume of

the artery which is related to total lung volume. This suggests that if the volume of blood passing to the lungs increases linearly with age and lung volume there must be an increase in the velocity of the blood through the lobar artery.

There is a linear relationship between either the cube of the diameter at the hilum or the length of the axial pathway and the volume of the lung. If the proportion of the lung occupied by the blood vessels is the same throughout life this suggests that the volume of blood vessels or the capacity of the lung for blood relates to the diameter of the hilar vessels. This can be seen to be true using the point counting results of Dunnill (1962b). The ratio of arteries to alveoli as seen in the present study also remains much the same throughout life.

This suggests that the change in diameter at the hilum and the changing length of the main pathways is related to the number of vessels in the lung as well as lung volume, and the size of the peripheral vessels. Any deductions about the changes in flow and velocity of blood to the lung must take into account the changes due to multiplication as well as growth in size.

STRUCTURE OF PULMONARY ARTERIES

During fetal life, at least after 12 weeks gestation, the pulmonary arteries have walls of the same type of structure as seen in the adult. Elastic, transitional, muscular, partially muscular and non-muscular vessels were present in most cases, though the diameter range and position in the lung of these vessels was not

the same as in the adult. The special embryological wall structure described by Civin and Edwards (1951) and Kaufman (1965) was not seen in the present study, nor was the progressive change in appearance of the vessel wall throughout fetal life described by Kaufman.

Structure Along an Axial Pathway

In the adult Brenner (1935) and Elliott (1964) showed that the structure of the artery was related to its external diameter. Elliott also related structure to position in the lung. In the fetus these two factors did not apply. During fetal life the smallest elastic artery found became progressively larger and at birth was still smaller than those seen in the adult. This is in contrast to the results of Sylora et al (1968) who considered that all elastic arteries were in the same size range from birth to adulthood and therefore showed extension to the periphery with age. Since the elastic arteries were smaller than normal the large muscular arteries seen in the adult were not present. In

In early fetal life up to 20 weeks gestation the level to which an elastic structure was seen along the axial pathway varied and progressed peripherally with age. At 20 weeks the elastic structure was found to the same level as in the adult. In later fetal life and in childhood structure of the larger arteries relates only to position. Kaufman (1965) showed progressive development of elastic structure to subsegmental level by 14 weeks gestation, which agrees with the present study. He does not describe any of the levels between this and the beginning of the lobule that does not become elastic at any time. However he reports change in structure of

more peripheral vessels - this was not seen in this study.

The elastic structure is present in the fetus in a much smaller vessel than in the child or adult. This is not only because the walls are relatively thicker during fetal life, since in a fetus a full elastic structure is fitted into a thinner wall. It may be that the elastic structure is needed for support of the arterial system since it is these elastic arteries which have the greatest resistance because of their high tensile strength (Burton, 1954). The tension counteracts the distending tendency of the blood pressure and also maintains the vessels open. The elastic structure may be comparable with the cartilage support in the bronchi which is also complete by the 24th week of gestation, a similar time to the final position of the elastic structure. The structure of the vessel wall seen in the adult appears later than the adult branching pattern. The fetal lung is capable of similar distension to the adult lung because of the same proportion of the muscular region. Throughout life the elastic region is less than 50 per cent of the total length with a constant percentage of muscular region.

The transition from elastic to muscular (transitional and muscular are considered together) occurs at the same position throughout life. Muscular arteries appear at a smaller diameter during fetal life than in the adult; however the smallest muscular artery is at the same diameter in fetal life and in the adult and the partially muscular and non-muscular region of the axial pathway are also at the same diameter. But because of the difference in size of the lungs these vessels are more proximal in the fetus. A non-muscular structure is reached before the acinar region in late fetal life but at the periphery of the acinus in the adult.

The structure seems to be reached at the same distance from the periphery, that is from the capillary bed, in both the fetus and adult. The length of the partially and non-muscular portion of the lung is much the same at both ages. The partially muscular region is always the shorter.

During childhood the features are not the same. The partially muscular structure appears at a larger diameter but covers the same distance along the axial pathway and finishes at a larger diameter so that the extent of non-muscular region increases. This must lead to a greater potential area for gas transfer.

It seems that in the fetus and the child the partially muscular structure is present over the same length of artery. In all children the muscular arc of the partially muscular vessels is only one muscle cell thick, whereas in many fetal arteries the partially muscular vessels have a wall more than one cell thick. It is surprising therefore that the partially muscular phase is found over the same distance since there is a greater wall thickness to be lost.

In the more proximal region and the region of the partially muscular region there is a more rapid drop in external diameter in the child than the fetus. The slower drop in external diameter was to overcome the greater drop in wall thickness and a relatively greater increase in the lumen diameter.

Though the diameter of change from muscular to partially muscular is the same in fetus and adult it changes during childhood. Despite its appearance in larger vessels in all the children studied the partially muscular structure did not appear until within the acinus and there was a gradual extension with age of the muscle to

the periphery, because of increase in vessel size.

The partially muscular structure appearing in larger vessels during childhood caused the acinar arteries to be less muscular than in the adult. The vessels grew in diameter but muscle did not extend into them. This lag in development of muscle is discussed further (page 625).

Structure of Conventional Arteries

The structure of the conventional arteries is dependent upon the size of the vessel. However the size at which the structure changes from elastic to transitional and muscular depends upon the site of this change along the axial pathway so that the structure of these vessels is dependent upon the axial pathway. Size is important, since a small vessel leaving an elastic main pathway will be muscular in structure. Over 20 weeks the structure by position is set as with the axial pathway, only size alters.

The smallest muscular and non-muscular arteries are entirely size dependent for their structure and show the same size ranges as the axial vessel and are the same throughout fetal life and compare with the adult. In the child there is a change at acinar level where the mixed population appears in larger vessels so that the diameter of change is age dependent, and the same as the axial artery.

Structure of Supernumerary Arteries

In the fetus since all the supernumerary vessels are small in diameter, structure is directly related to external diameter as in the adult. In the child they are also diameter dependent but the diameter changes with age since the transition fron muscular to partially muscular and non-muscular changes as it does along the axial pathway.

It can be seen that when studying cases of children from the point of view of wall structure it is essential to compare with a child of the same age and not the adult.

Percentage Wall Thickness

In the fetus and the child the same shape curve for percentage wall thickness plotted against external diameter is achieved and this is the same as that seen by Elliott (1964) in the adult and Davies and Reid (1970) in other children. In the fetus the percentage wall thickness at any external diameter is higher than in the child and adult. In fact the actual wall thickness is about double that seen in the adult for the same size artery. This increase is not due to lack of distension because the high pressure injection has ensured that the vessels are fully distended and the evidence for this is the flattened elastic laminae.

The percentage wall thickness was the same throughout fetal life with two exceptions both under 16 weeks gestation, in one case (16 weeks) this was probably due to some abnormality. It may be that the vessels in the very young cases for example the 12 week lung are thicker walled. The overall increase in wall thickness during fetal life has been described by several authors. Civin and

Edwards (1951) thought that the walls were thicker than the lumen probably because they used uninjected material. Naeye (1961a) reported an increase in the medial area in cases over 20 weeks gestation; this was not seen in this study and cannot be explained. O'Neal et al (1957) and Wagenvoort, Neufeld and Edwards (1961) showed that muscle extended into smaller vessels in later fetal life, the former group commented on the extension of muscle into arterioles and the latter group found that there was no increase in wall thickness but an increase in muscle per unit area of lung. This extension was not seen in the present study either by measurement of wall thickness or by population counting.

The cases of 12 and 16 weeks gestation had particularly high wall thicknesses. In the first case this was probably not artefact but due to particularly thick walls in this the youngest fetus studied. The muscle cells were not fully developed and seemed to be more rounded. It is possible that they did not distend fully, or there may be a true increase. The case of 16 weeks was an exception to the rule. Though the branching pattern was normal, the arteries were small both in external diameter as well as lumen. The wall structure was normal for age but very thick. There is no explanation for this except that this was an abnormal lung as a result of some congenital abnormality. However this case was not abnormal in any other way and it may be simply individual variation.

Decrease in Wall Thickness after Birth

All authors working on fetal and child lungs have noted a reduction in wall thickness of the pulmonary arteries after birth. Davies (1969) using the methods of the present study showed that in

a child of four months the adult pattern was almost reached. In a child of ten months in the present study the adult range had been reached.

In the children studied the actual wall thickness was half that of the fetus, for each artery diameter. This could have been attained by thinning of existing muscle cells or by reduction in the total number of muscle cells in the wall or by lack of growth with increase in diameter. Since at all ages it seemed that the muscle cells were about the same size, 2.5 μm, there is probably loss of some muscle cells rather than overall reduction in size of individual cells. Naeye (1966a) using planimetry to measure cell areas demonstrated a reduction in the number of muscle cells relative to intimal cells by four months after which the ratio remained the same.

The reduction in muscle cells could be a true loss or due to a lack of increase in muscle cells as the vessels increased in size. The latter seems the most likely since four months is taken for completion of the reduction. This is also supported by the lag in development of muscle in new vessels demonstrated using population counts. This can be further borne out by studies on medial area. In the first four months the actual wall thickness of any vessel falls to half its fetal level. This means that the vessel can double in diameter and still maintain the same medial area. While the vessel is increasing in size to double its original size there is no need for any development of muscle cells. The vessels seem to more than double in size at the periphery in the first four months so that some new muscle must develop. It seems likely that there is not atrophy but lack of growth of muscle cells as the vessel grows followed by growth. The vessels at the hilum increase in diameter by less than double and their wall thickness

decreases by half so that there is a decrease in medial area in the arteries at the hilum, indicating some atrophy. In the mid zone of the lung by the same token there is also a degree of atrophy.

Wagenvoort, Neufeld and Edwards (1961) demonstrated a high percentage wall thickness in fetal life which fell rapidly in the first two weeks after birth and then gradually for 18 months. In the first four weeks of life they found that rather than the smallest arteries having the higher wall thickness they had the lower; this was later reversed.

In this study, in vessels less than 250 μm in diameter, the three day child had a much reduced percentage wall thickness as well as actual wall thickness, while the larger arteries were still at fetal level for both. There has evidently been a rapid response to the changing blood flow at birth by the small arteries below 250 um within the acinus but not by the larger vessels.

By ten months the larger arteries also had a reduced wall thickness. It is probable that the initial drop is as a result of dilatation while the later reduction is due to growth changes.

In summary the changes at birth with respect to the muscle of the artery walls is of three types (see Table VII 1). In the smallest vessels there is rapid dilatation within the first few days of life. In the mid lung and intra-lobular pre-acinar region there is a lag in growth of new muscle cells while the vessels increase in size; this is followed by a gradual growth of muscle cells but still maintaining a thinner wall thickness. The third type of change is seen in the larger vessels where there is definite atrophy of muscle cells since the wall thickness drops to a greater degree than can be accommodated by the increase in size of the vessels. The

second and third type of change occurs within the first four months. After four months there is development of muscle to maintain adult wall thickness.

Something must initiate the dilatation of the small vessels. Larroche et al (1959) showed a rapid drop in wall thickness within ten days of birth and showed that it was as a result of the birth and was not a developmental feature since it occurred in a newborn after only five months gestation. The reduction in pressure at birth possibly caused by this dilatation will eventually lead to loss in pulmonary arterial muscle. Dammann, McEachen, Thompson, Smith and Muller (1961) showed this when they reduced pressure by causing a pulmonary stenosis in cases with a ventricular septal defect and this is probably the sequence of events in the larger arteries after birth.

In 1953 Dawes, Mott, Widdicombe and Wyatt noted that flow increased after birth due to a drop in resistance in fetal pulmonary arteries associated with expansion with air, oxygen or nitrogen and that it was not a mechanical feature since expansion with saline would not cause the drop in resistance. It is possible that changes in gas tensions may cause changes in the arterial wall causing dilatation by altering the behaviour of the muscle cells.

Adams (1966) concluded that the flow was under neuro-humoral control since drugs could cause vasoconstriction or dilatation in the fetal lung. Asphyxia always caused vasoconstriction and it is likely that the increase in oxygen at birth causes the change in resistance. A possible mediator of this change is bradykinin. Melmon, Cline, Hughes and Nies (1968) showed that in lambs bradykinin constricted the umbilical artery and vein and the ductus arteriosus

TABLE VII 1

CHANGES IN PULMONARY ARTERIES DURING FIRST YEAR OF LIFE

	SMALL VESSELS $< 250\mu m$	MEDIUM VESSELS $> 250\mu m$ (subsegmental)	HILAR VESSELS
AT BIRTH	Dilatation with decrease in wall thickness	-	-
EARLY MONTHS	Increase in external diameter. Lag in muscle development	Increase in external diameter. Lag in muscle development	Increase in external diameter. Atrophy of muscle cells
AFTER 4 MONTHS	Development of muscle to maintain adult percentage wall thickness		

but produced dilatation of the pulmonary vasculature. In the human they found that bradykinin was at a higher level in the cord blood of newborn infants than in the adult. The blood also contains an inactive kinin precursor and a kinin releasing enzyme. A decrease in temperature and a release of neonatal granulocytes (found in increasing numbers before birth) together with the oxygen tension found in a newborn, which are greater than those of a fetus, caused these two factors to become active. They suggested that the bradykinin released by these changes caused dilatation of the vessels.

A possible site for the production of kinins was put forward by Lauweryns and Peuskens (1969). They found using fluorescence and silver staining a cell in the epithelium of bronchi and bronchioli called an Argyrophil or Kultschitzky cell. They believed that this cell produced kinins and catecholamines which related to the changes taking place in the vessels of the newborn.

Further evidence that dilatation is effected by drugs and oxygen is seen in some cases of newborns who maintain the features of a fetal circulation. Gersony, Gabriel and Sinclair (1969) described two such cases and showed that when priscoline (Adrenergic) was administered there was dilatation and oxygen uptake increased. A further case has been described by Burnell (personal communication, pathological material studied personally) in which the fetal resistance was relieved by administration of 90% oxygen. The infants all died when the symptoms could not be relieved by addition of oxygen. The lung of the last case showed an increase in muscularity over the normal at birth in both small and large arteries.

Ventricular Weights

The method of dissection of Fulton, Hutchinson and Jones (1952) has been applied for the first time to fetal hearts. The results showed that the right ventricle was always smaller than the left, as found by de la Cruz et al (1961). The relative weight of the right ventricle was greater than in the adult. Muller (1883) had shown that after 28 weeks gestation the right ventricle with part of the septum was in fact larger than the left. This was not seen in the present series, nor was any change in the ratios before and after 28 weeks as he had described. Patten (1930) also believed that the right ventricle was heavier than the left at birth.

It is difficult to compare the present results with those of the large series of Keen (1955) who only measured the free walls. He found that they were equal. It is possible that if the septum were deducted from the left ventricle the right and left might then be equal.

Though using different methods all investigators have shown an increase of the right ventricle mass relative to the left in comparison with the adult state. The amount varies depending upon the method. All showed a relative drop in the right ventricle after birth. Using Fulton's method Davies (1969) found the normal adult ratio in a 10 month child. The left ventricle had increased more than the right since birth.

It was not possible to say whether there had been a loss in heart muscle in the right ventricle after birth since there were not any young cases available, and by ten months both ventricles had increased in size. The right ventricle had only increased by 25 per cent in

ten months while the left ventricle plus septum by 300 per cent. There is represented either much slower growth by the right ventricle or a reduction after birth.

The right ventricular prepondetence is related to the high resistance of the fetal pulmonary blood vessels and the increase in the percentage wall thickness. Immediately after birth resistance falls and there is a gradual reduction in muscle up to ten months when the adult pattern is reached. This corresponds with the normal ratio being reached in the ten month heart.

The pressure and resistance have dropped to adult levels by 11 days (Rowe and James, 1957) therefore the structural features take longer to accommodate to the new state.

Population Counts

The population counts show the distribution of muscle within the small vessels without serial reconstruction. In the fetus and the adult the muscle extends to arteries of the same size. These arteries however are not in the same position in the lung. In the adult the non-muscular arteries are only found accompanying alveoli while in the fetus they are found in the pre-acinar region. In the younger fetuses where no acinus is present they accompany small bronchioli. It seems that the structure relates directly to artery diameter irrespective of position in the lung. It might be expected that the same would apply during childhood. However as seen by Davies (1969) in his series of cases and in the present series, the distribution is different. The wholly muscular structure did not extend into such small vessels as in the adult and partially muscular and non-muscular vessels appeared at a larger diameter. There was also a greater percentage of partially muscular vessels

in the children up to ten years than in the adult or fetus.

This apparent regression of muscle from small vessels and increase in the number of non-muscular was not due to atrophy of the muscle. The landmarked arteries within the acinus became no less muscular, in fact there was, with age, a steady increase of muscle. The decrease in muscle was in fact due to the increase in size of the vessels. It seems that as the small non-muscular vessels increase in size the growth of muscle has been slower so that larger vessels are non-muscular. This time lagein the growth of the muscle continues at least until the age of five years, by ten years the distribution was close to that of the adult and muscle cell extension had obviously caught up with increase in vessel size. This lag in extension of muscle into smaller vessels lasts for a longer time than the lag responsible for the thinning of the walls of the vessels in the first four months of life. In fact the increase in number of vessels with little or no muscle does not begin until after four months.

There is a gradual change in development of muscle after four months which is seen in the case of ten months. The cases of 18 months, three, four and five years all had similar distribution of muscle in the small vessels. The eleven year old was similar but the ten year old showed the return to the adult distribution. It is possible that the ten year old was advanced for age or the eleven year old retarded. It is more likely that this is about the age of change and there is individual variation.

The lag in the development of muscle was at the time of the greatest multiplication of small arteries within the acinus. However it continued when the vessels were slowing their multiplication.

It is therefore not just an inability of the muscle cells to multiply at the same rate as other cells which causes the lag.

Extension of Muscle into the Acinus

Despite the fact that muscle is not found in smaller vessels during childhood, there is still an extension of muscle into the acinus when arteries accompanying airways are considered. Part of this extension may be as a result of terminal bronchioli becoming alveolised (Boyden, 1965) so that larger arteries come to be with respiratory bronchioli, but this only occurs in the first four months. In addition there is an increase in size due to growth of these arteries and this may outgrow the lag of muscle development. Though there is a gradual extension of muscle into the acinus during childhood, found in the present series and by Davies (1969), as a result of the lag in muscle development shown by population counting the vessels within the acinus are not as muscular as would be expected for the size of the vessels. Even in the cases of ten and eleven years muscle does not extend to the periphery of the alveolar region as it does in the adult.

At birth there are no muscular arteries within the acinus. By four months they have extended to the respiratory bronchiolar region. There is then a gradual extension of muscle into the acinus. The acinus during childhood has a relatively non-muscular arterial supply. This must affect the physiological response to changes in oxygen tension and the exchange of gases. It is possible that since there is a smaller capillary bed in the children the small non-muscular arteries can be used for gas transfer and increase the surface area available for this. Since the arteries grow with the alveoli the larger non-muscular vessels would be

accompanying alveolar ducts and respiratory bronchioli and unless there is gas transfer through the walls of these the extra non-muscular vessels will be superfluous. However, it is likely that there is easy diffusion through the tissue and therefore through airway walls. The partially muscular vessels are also available for gas transfer and they are present in large numbers. Staub (1961) showed in the frozen sections of cat lung that there was gas transfer through walls of arterioles up to 200 μm external diameter when breathing 100% oxygen. These must have had muscular walls. In normal air breathing conditions oxygen transfer did not occur in vessels over 100 μm, this may have been the level of the largest non-muscular vessels. Children with more non-muscular vessels may have an advantage in normal or low oxygen tensions.

These extra non-muscular vessels may also have some effect on the reaction of the lung vessels to changes in oxygen tensions on the lung. Bergofsky, Lehr and Fishman (1962) showed, in dogs and man, that increased carbon dioxide tensions or decreased oxygen tensions in the air breathed caused vasoconstriction and increased pressure in the pulmonary arteries. In 1968 using the cat lung Bergofsky, Haas and Porcelli experimented to determine the sites from which hypoxia and hypercapnia elicit rises in pulmonary artery pressure. They found that the veins were not reactive to either. The pressure was raised to a greater extent by hypoxia than hypoxaemia. They therefore concluded that oxygen lack was most important at alveolar level acting upon the pre-capillary vessels adjoining the air spaces. Hypercapnia had more effect at the level of larger pre-capillary arteries. They believed therefore that there may be two different responses to hypoxia and hypercapnia either of which was under nervous control.

von Euler and Liljestrand (1946) also working on cats showed that the hypoxic reaction took place within the alveoli directly onto muscle cells. It was also shown using casts that after hypoxia in rabbit lungs there was a constriction of the muscle at the level of the alveoli shown by the marks of spiral muscle cells giving a gnarled appearance to vessel endings (Burton, 1959). Kato and Staub (1966) showed that with hypoxia there was a reduction in lumen diameter at the level of the respiratory and terminal bronchioli using rapid frozen sections. In an earlier paper Staub (1963) had described the site of the affect of hypoxia as the arteries at the level of alveolar ducts. If constriction is taking place at this level there must be some way that this is taken back to the larger vessels which are known to constrict. Hilton (1959) demonstrated in a cat leg that dilatation of an artery wall due to exercise could be conducted from muscle cell to muscle cell without nervous control, and that the muscle cell changes could be effected by mechanical stimulation of the muscle or by addition of acetylcholine or bradykinin. In 1962 he suggested that this dilatation was mediated by a plasmakinin produced in the tissue spaces and made active by enzyme action.

The reaction by vasoconstriction to hypoxia leads to a reduction in blood flow to the portion of the lung affected. Using dogs Bechtel (1963) showed that if one lung was given 100% nitrogen the flow to that lung reduced by 25% and Conroy, Finley and Bonica (1963) showed a reduction in flow in an atelectatic or hypoxic lung of 12 - 14%. They showed that addition of chlorpromazine invalidated this effect but Bechtel showed that cutting the vagus nerve increased the effect.

It may be that in the child there is less reaction to hypoxia since there are not so many muscle cells in direct contact with alveoli for the initial reaction to take place. This reaction to hypoxia has not been studied by physiologists in the child. The reaction to hypoxia of vasoconstriction is advantageous while in the child it may be a handicap in that a reduction in flow may lead to less uptake of oxygen. In the adult this feature allows for closing down of the blood supply to a badly oxygenated area produced by airways disease so that blood can be channelled to a better supplied area of lung.

Changes in Arteries at Birth

During fetal life all pulmonary arteries are thick walled with a further increase in percentage wall thickness in the small arteries. Muscular arteries are found only in the pre-acinar arteries and to a diameter of around 100 μm.

Immediately after birth there is a dilatation of the small vessels causing a decrease in resistance and increase in flow to the lung. The arteries within the acinus multiply by branching as the new alveolar ducts and alveoli develop. At the same time as the multiplication there is an increase in size of the arteries accompanying airways and of the supernumerary arteries.

As the vessels increase in size and multiply there is a lag in the development of muscle shown by a lack of extension into the new small vessels and a maintenance of the non-muscular state in the vessels increased in size. There is also a lack of growth of muscle cells in the existing muscular vessels leading to a decrease in percentage wall thickness as the arteries increase in size.

Eventually the muscle cells begin to grow but only maintain a reduced wall thickness and then show a gradual extension of muscle into smaller vessels in children over five years. In the larger vessels there is a loss of muscle cells by atrophy in response to the reduced pressure in the lung.

The overall decrease in muscle in the arteries allows a greater volume to pass through the lung as the volume of blood increases with age. The vessels also increase in size though in the first 18 months the capacity of the lung is increased also by rapid development of new blood vessels within the acinus. This continues through childhood at a slower rate. There is an overall increase in the volume of the lung as blood vessels and alveoli multiply.

DEVELOPMENT OF THE PULMONARY VEINS

The structure of the pulmonary veins has been in the past neglected. At the present there is increasing interest in the functional importance of the veins. The present study has revealed details of their structure and also the drainage pattern and origin of the vessels. In addition the development of the veins during fetal life and childhood has been described.

Drainage Pattern of Veins

Venograms

The overall drainage pattern of the veins can be demonstrated by venograms. These showed the pattern well known from angiograms of main veins returning to the hilum and corresponding to the main

branches of the pulmonary artery, although the hilar arrangement of the arteries and veins is different. The veins were of similar size to the arteries but always slightly smaller, particularly at the hilum. This is probably because the arteries branch more distal to the point where the main veins join so that proximally the arteries are larger. At a point one centimetre from the periphery of the lung arteries and veins were much the same size. Therefore the volume drained affected the size of the veins in the same way that the arterial diameter depended upon the volume of lung to be supplied.

Tributaries joining the axial veins entered nearly at right angles so that a different appearance is seen between the arteriogram and venogram. Arteries branch at a more acute angle from the axial and are normally larger than equivalent venous tributaries. The main vessels do not stand out so readily on the venograms since there seems to be a greater background haze. If only one is available it may be difficult to identify whether a radiograph shows arteries or veins but it is possible. If both are available however, they can be identified quite easily (compare Figures III 13 and V 2). The increase in background haze correlates with the greater number of veins than arteries per unit area in the acinar region. There is also a greater number of supernumerary tributaries along the axial pathways. It seems that the smaller size of the veins is made up for by their greater number.

The pattern of vessels draining the acinar region is similar to that of those supplying the acinus, in that there are veins of different sizes draining into each other to form a larger vessel, like the tributaries of a river system. There is also direct drainage from acini around the main veins via supernumerary veins.

In the two cases of congenital heart disease in which the veins had been injected the venograms were abnormal and the changes were similar to those observed on arteriograms of other cases. There was a reduction in venous background haze in the cases of ventricular septal defect and an increase in the cases of tetralogy of Fallot. The veins seem to follow the arteries for their pattern. The abnormal venogram findings corresponded with microscopic changes.

Measurements made on the venograms also showed similar trends to those seen on the arteriograms both in normal and abnormal lungs. The diameter and length of axial veins increased with age. Because of the greater increase in diameter at the hilum than at the periphery there was an increase in gradient with age. Because of the small number of cases it was not possible to see if the gradient became stable in later childhood as was possible with the artery. The diameter and length of the main vessels showed a similar relationship to volume and age as did the arteries with a more rapid increase before birth than after. It is probable that the arteries and veins follow the same rate of increase and grow parallel to each other.

As in the arteries the linear relationship was between the cube of the vein diameter at the hilum and the volume, suggesting that size related to the capacity of the lung vessel bed and not to flow.

Microscopic Reconstruction

It seems that the venous system develops at the same time as the arterial and airway branching system and is probably complete to the level of the acinus soon after 16 weeks gestation. It also seems likely that the venous system grows in the same way as the arteries with conventional and supernumerary tributaries appearing together as the vein grows at the periphery. The number of conventional tributaries to the veins corresponds to the number of conventional arteries and perhaps each drain roughly the same volume of lung as supplied by one conventional artery.

The supernumerary vessels were in greater number than the conventional but were smaller and seemed to drain a smaller volume of lung, in that they had travelled less distance from the capillary bed than adjacent conventional veins. Like the arterial supernumerary vessels they were more frequent at the periphery of the lung. There was a greater number of these veins than of the corresponding type of artery and the ratio of supernumerary to conventional veins was higher. This could be related to the increased background haze seen on the venograms, and also to the increased number of veins per unit area, in comparison with arteries. The discrepancy in number of vessels may reflect the smaller size of the vessels. In the 20 and 28 week fetuses the average diameter of the veins, both conventional and supernumerary, was less than the average diameter of arteries in fetuses of the same age.

In the venous system two types of supernumerary vessels have been identified, the Type I draining tissue immediately surrounding the axial veins and the Type II running some distance before entering the main vein. These are probably not the equivalent of the accessory and aberrant supernumerary arteries described in the adult

by Elliott (1964). His aberrant arteries were far less numerous than the Type II supernumerary veins and the accessory arteries seemed to supply much larger areas of lung than is drained by the venous Type I vessels. However these two venous types would seem to have the function of draining blood away from the 'potentially dry areas' of the edge of the acinus as described by Elliott, which he found supplied by accessory arteries. These extra veins can also connect with any artery formed during collateral circulation of an injured area and may come into play during vessel recruitment.

It seems that there are more supernumerary veins than supernumerary arteries so that there must be a larger number of vessels leaving the capillary bed than entering from the pulmonary artery. The increase may be partly due to the presence of the additional blood from the bronchial arteries draining via the pulmonary vein. On the other hand the increase in number may reflect the lower pressure in the venous system since the lower pressure in cases of tetralogy of Fallot is associated with an increase in the number of small arteries and veins.

Origins of Veins

Miller (1947) described four sources for the veins, the pleura, capillary bed of alveoli, capillary network of the alveolar ducts and the bronchial branching point. In the present study these four origins were noted and in addition veins were seen to arise from walls of terminal bronchioli and respiratory bronchioli at points other than their branching point. These were doubtless draining the zone supplied by bronchial arteries. In addition small vessels drained the connective tissue sheaths surrounding

the vessels and the connective tissue septa. It was only possible to identify all these origins in the lungs of the three and ten year olds. In the fetal lungs studied the alveolar region was not fully differentiated and veins could only be identified draining pulmonary parenchyma, connective tissue septa and pleura, and walls of airways. As noted by Knisely (1960) the veins leaving the capillary bed entered larger veins at right angles as opposed to the angular branching pattern of the pre-capillary arterial vessels. In this way very small side branches joined larger vessels directly rather than taking a more circuitous route.

Structure of Veins

The structure of the vein walls in the three year old and ten year old lungs described here was similar to that described by Wagenvoort (1970) with the first post-capillary vessels having an endothelium with a single elastic membrane. He found these thin walled vessels up to a diameter of 40 - 100 μm in adults, which is similar to that seen in the ten year old child in this series. In the three year old child lung in the present study non-muscular vessels were found up to a diameter of 200 μm. Since the vessels in the present study have been distended this may be in the same range as Wagenvoort though in this case the figures are more accurate.

Wagenvoort describes muscle in the adult lung first appearing in veins of 40 - 50 μm diameter. On the other hand Brenner (1935) did not find muscle until veins of 150 μm while Takino and Miyake (1936) found muscle in venules of 50 - 80 μm. In the ten year old child, the oldest case studied in this series, muscle appeared at 70 μm but at this time was only in part of the wall and such

vessels have been called partially muscular, and are similar to arteries of the same structural type. These are possibly the same vessels described by Takino and Miyake (1936) as having a 'string of beads' appearance. That muscle has been found in vessels only of a larger diameter than Wagenvoort reported is probably due to the distension of the vessels by injection. Brenner does not describe the presence of muscle in such small veins, which cannot be because of lack of distension. Elliott (1964) found that Brenner did not find muscle in arteries as small as he had using the Department method. Brenner may have only called a vessel muscular when there was a complete muscle coat. It was found in this study that the smallest wholly muscular vein was 200 μm in diameter in the ten year old.

In the three year old muscle did not extend into such small vessels. This may have been individual variation since muscle was found in smaller vessels in the case of 39 weeks gestation. On the other hand this does correspond with the lack of muscle in small arteries in children of about three years. However, unlike the arteries, there was little muscle at birth and it would seem odd to have developed at birth and then be lost again by the third year.

In vessels over 200 μm in diameter there is a progressive increase in the amount of muscle which is found in the wall. At the same time there was an increase in elastic tissue. Measurements of the media were made difficult by the fact that there was no external elastic lamina delineating the external extent of the muscle and elastic was found in the adventitia. This difficulty in measurement was also noted by Wagenvoort (1970). In some abnormal cases this difficulty was not found since there had been

arterialisation of the vein wall (Samuelson, Becker and Wagenvoort, 1970).

Muscle was not seen at all in the cases studied of 20 weeks gestation; no other authors have studied veins as young as this. On the cases of 28 and 34 weeks though muscle was present it was not possible to measure it accurately since it seemed to be thinned and the fibres separated. Samuelson et al (1970) had apparently found it possible to measure vessels at this age but their cases were uninjected. They found a slightly lower percentage wall thickness in the lungs under 34 weeks which would agree with the present findings.

The percentage wall thickness at 39 weeks gestation, three years and ten years was much the same with the vessels over 300 μm having an average percentage wall thickness between 1.3 and 1.6 per cent. The increase from birth to adulthood described by Dammann and Ferencz (1956a) was not seen. Samuelson et al (1970) found a mean value of 3.3 per cent for all the cases they studied up to two years of age. This is probably higher because of lack of distension. In the present study there was a slight rise in the percentage wall thickness in the veins below 300 μm external diameter. This was comparable with the rise in arterial percentage wall thickness in arteries of this diameter. This was partly due to a reduction in external diameter with no reduction in wall thickness but partly due to there being a variety of thickness of wall, that is, there was a true relative increase. The actual wall thickness was much lower in the small veins dropping to 1 μm while in the arteries the thinnest wall was 2.5 μm. There may therefore be some difference in the quality of the muscle cells.

Though the veins are muscular they are less muscular than the arteries and in fetal life particularly there is discrepancy between arteries and veins. During fetal life the arteries are at their most muscular and the veins at their least. In the adult the pressure within the pulmonary veins is about half that of the pulmonary arteries. This is related to the reduction in muscle. The muscle will increase in the veins if there is an alteration in pressure. Smiley, Jaques and Campbell (1966) showed that reversal of the pulmonary blood flow in dogs, causing arterial pressures in the veins, led to medial hypertrophy. Samuelson et al (1970) showed medial hypertrophy in cases with Mitral Stenosis and anomalous pulmonary venous drainage. In the present study it was found that there was no increase in wall thickness when there was an increase in flow and pressure due to a patent ductus arteriosus (Case V.S.6). However there was arterialisation of the venous wall. Rudolph (1962) produced an increase in resistance in the veins with drugs. This was independent of flow and was controlled by the sympathetic nervous system. Hyman (1969) showed that the response to drugs was mainly in the small pulmonary veins and was affecting smooth muscle of these veins rather than the cardiac muscle of the atrial sleeve.

In fetal life the pulmonary arteries are more muscular than in the adult and the pulmonary veins less muscular. It is possible that the flow is so reduced in the lungs because of the high resistance in the pulmonary arteries that little muscle is needed in the pulmonary veins until the flow increases after birth. The increase in muscle cannot be just as a response to an increased flow since there is a continuous increase during fetal life while flow remains much the same. There is as much muscle at birth

as in the 10 year old and possibly the adult. The muscle in the veins would seem to be slow in developing.

THE THIRD LAW OF LUNG DEVELOPMENT

This states that 'Blood vessels are remodelled and increase, certainly while new alveoli are forming and probably until the growth of the chest is complete' (Reid, 1967a). This study has shown this to be true though it is now possible to be more precise. The pre-acinar vessels, arteries and veins, develop alongside the airways and are complete at the end of the fourth month of gestation. The intra-acinar vessels, both arteries and veins, develop mainly after birth along with the alveoli. They increase most rapidly at the stage of most rapid alveolar multiplication. After the alveoli are complete there is increase in size of existing vessels and new pre-capillary ones must continue to form as the alveoli increase in size. The vessels during childhood in the acinar region are less muscular than in the adult, muscle development lags behind arterial multiplication.

Fetal life is concerned with the development of the pre-acinar region while childhood is concerned with intra-acinar development. Later changes are only of size and structure.

CHANGES IN CONGENITAL HEART DISEASE

Knowledge of the normal structure of the lung and its development during fetal life and childhood offers a basis for assessment of abnormal lungs. Since there are such complex changes during childhood it is essential to compare any abnormal case with a normal of similar age. All cases should be prepared and studied with the same precise quantitative methods. From the results it may be possible to estimate the onset of abnormality and possibly to define its pathogenesis.

Two types of lesion were chosen for inclusion in this study since they demonstrated different physiological effect on the circulation. They are at the two extremes of the various changes which can occur. Ventricular septal defects usually lead to an increase in flow with an increased pulmonary artery pressure and pulmonary vascular resistance while a tetralogy of Fallot will lead to a reduction of pulmonary blood flow with normal or reduced pressure and resistance. Reduced pulmonary resistance, pressure and flow are only seen in transposition of the great vessels with pulmonary stenosis. No case of this diagnosis was available for study. The two groups chosen for study are quite common and so six of each group were available to be studied in detail.

A. TETRALOGY OF FALLOT

Though the lungs from only six cases of tetralogy of Fallot were available for study they covered a wide range of age from one to eight years and two cases had had some type of corrective operation performed. Using the precise methods of this study it is possible to draw valid conclusions on the changes

in the disease despite the small number of cases. This is particularly so in this series since the changes were much the same in all the cases regardless of age.

Hypoplasia of the Lungs

The general metabolic rate in children with tetralogy of Fallot is below normal (Bing, Vandam and Gray, 1947) and the children are usually small for their age when seen clinically; this was so in the present series. Hypoplasia is seen also in the lungs; the volume of the lungs in all six cases was below the normal value for age. The percentage reduction increased with age. The reduction is probably related to the reduced metabolic rate and to cyanosis.

There was also a hypoplasia of the number of alveoli judged both by age and by lung volume. In one case the total number was estimated quantitatively and found to be much reduced, only 200 million in a six year old child, this being the normal value for a 30 month child. The total lung volume was not reduced by as much, and the alveoli were therefore larger than normal in all cases.

The hypoplasia was evident on the arteriograms and venogram. In all lungs the total length of the main pathways was reduced related to the volume. The diameter of the lumen at the hilum was also reduced though slightly more than the length. In these measurements also the relative hypoplasia increased with age suggesting that the affect of the lesions becomes greater with age.

Arteriograms

The main points of interest in the arteriograms were the small size of the proximal vessels and the increase in number of the small vessels.

Size of Main Vessels

In all cases the calibre of the main arteries was reduced in comparison with the normal. It is likely that the vessels were normal at birth since the youngest case has vessels larger than the normal at birth. This hypoplasia is probably due to a reduced blood flow from birth. However Naeye (1961d) showed a reduction in medial area before birth. The hypoplasia increases with age the smallest vessels, relatively, being seen in the oldest case T.F.5. This case had had a normal flow since a corrective operation at five years of age but the arteries remained hypoplastic. On the clinical radiograph and angiogram the left lung had even more hypoplastic vessels than the measured right lung because of silting up after the Blalock operation. It seems that once the vessels are small they remain so. Part of the reduction in lumen in this case may have been due to encroachment on it by the medial muscle hypertrophy and by intimal thickening.

There are no previous reports on the reduction in vessel size on arteriograms but Castellanos and Hernandez (1967) showed a reduction in size of the main vessels on angiograms. Using radiographs of their cases Wilson and Amplatz (1967) showed a relatively smaller left upper lobe than right upper lobe artery: this was not seen in the present series either on radiographs or arteriograms. Measurements made on the arteriograms in the present

series confirmed in all cases but one the reduced vessel size seen on the radiographs and angiograms; in the exception the angiogram had suggested normal vessels although in the specimen they were seen to be reduced. The reduction in size of the vessels is related to the reduced flow demonstrated by Dollery, West, Wilcken and Hugh-Jones (1961) by a method using measurement of loss of carbon dioxide from the lungs.

The reduction in size seen in the pre-acinar region on the arteriograms was also seen microscopically in the intra-acinar region where in fact the hypoplasia was greater. It is probable that the intra-acinar and pre-acinar regions are affected differently since there is growth by multiplication of the former as well as change in size.

Background blush All five arteriograms showed an increase in the background blush. Wagenvoort, Heath and Edwards (1964) noted this as a 'mosslike' appearance but they thought it due to dilatation of the peripheral vessels. In this series there is no such dilatation at the periphery and the increase in blush was due to an increase in the number of small vessels apparent when the arteries per unit area of alveolar tissue were counted microscopically. It is possible that this increase is due to extra supply from the bronchial artery circulation. No background blush was seen on angiograms or radiographs. The increase in number is only seen at the periphery, there being no increase in the number of proximal branches nor any increase in size of these vessels.

The broncho-pulmonary anastomoses were only demonstrated in the central part of the lung since there were no bronchial arteries filled after pulmonary artery injection beyond the level of the

cartilage. Filled vessels were only seen on the pleural surface near the hilum. The acinar region may be supplied, in the area around the bronchi, directly but the distal regions must be supplied via the main pulmonary vessels. It is therefore surprising that there is no increase in size of these vessels. Speed of flow may be increased in the proximal region to allow for this. The bronchial supply must increase the oxygen in the pulmonary artery system and it may be this that increases the number of small vessels. It still seems surprising that the number of small arteries related to alveoli is increased to above normal and it may be that the low flow causes an increase in vessel number.

Appearance of Bronchial Arteries

There was no obvious increase in the bronchial supply to the lungs, with age, as was suggested by Bing, Vandam and Gray (1947). The youngest case in this series, aged one year, had dilated bronchial vessels in the walls of bronchi and a large number of bronchial vessels on the pleural surface. The older cases had no greater number visible on the surface or microscopically. Ferencz (1960a) after microscopic study of uninjected lung material, showed that the collateral circulation was opened up by 17 days. There is a very rapid response to the decrease in flow in the pulmonary artery. It may be that growth hormone is needed for the development of these vessels and this is only present in large amounts in early childhood. Meffert and Liebow (1966) showed that the bronchials would develop more rapidly in puppies than in adult dogs. However they do develop in adult humans with an acquired stenosis.

The bronchial collateral circulation once present remains even if there is an artificial increase in flow as in cases

T.F.2 and 5. However in case T.F.5, three years after correction, there was some evidence of hyalinisation of some of the bronchial arteries. This may have been evidence of the beginning of obliteration of these vessels.

Physiological Importance of an Increased Capillary Bed

The increase in the capillary bed may lead to a larger area for blood-gas interphase and therefore a greater chance of oxygenation for the small amount of blood in the lungs. The increase in number of vessels will lead to a slower flow through each vessel which may also aid oxygenation.

Since both the arteries and veins of the pre-acinar region are decreased in size and normal in number the increase in the capillary bed and intra-acinar region must be independent of the fate of the pre-acinar region.

Branching Pattern of Arteries

The branching pattern of the arteries in cases of tetralogy of Fallot has not been described previously. From the arteriograms it could be seen that it was normal save for the increase in small vessels. Dissection and serial reconstruction have confirmed this fact. It was expected that the pre-acinar branching pattern would be normal since it is complete by the sixteenth week of intra-uterine life. Since there is less blood flow to the lung it might be expected that the intra-acinar region would not develop fully, however the branching pattern within the acinus in the two cases traced was normal, though the arteries were reduced in size. As already described, the vessels beyond the alveolar duct were

increased in number particularly in the older cases. These arteries would normally grow with the alveoli. The increase in arteries is even more excessive when related to the reduced alveolar number.

The peripheral vessels when traced microscopically were reduced in size to a greater extent than the proximal vessels measured on the arteriograms, again suggesting that the two regions are independent.

Structure of Artery Walls

The muscularity of the arteries can be estimated by wall thickness and by the distal extent of muscle along the walls of small arteries, the artery being judged either by size or by its position in relation to the airways. In these cases of tetralogy of Fallot wall thickness and muscle extension into smaller vessels were linked together although intra-acinar extension did not parallel the other two features as it would in the normal. In all but one case there was an increase in wall thickness and an extension of muscle into smaller vessels than normal, a muscle hyperplasia.

Previous studies on uninjected material from cases of uncorrected tetralogy of Fallot have reported either a normal (Granston, 1958; Dammann and Ferencz, 1956a; Ferencz, 1960a) or reduced (Best and Heath, 1958; Naeye, 1961d; Wagenvoort and Edwards, 1961) muscularity. They believed this to be due to the low flow and pressure within the pulmonary artery.

In only three of the present series were the details of both pressure and flow available. In two they were reduced and

in the other normal, due to a Blalock anastomosis. In the other three cases the flow was reduced and it can be assumed that there was a low pressure. Nadas (1963) states that in cases of tetralogy of Fallot flow and pressure are low or normal and the calculated pulmonary vascular resistance is low. In other cases studied by catheter in Brompton Hospital this has been confirmed. In this series then low flow and pressure have been associated with an increase in muscle. The greatest increase in muscle was in the two cases in which an attempted correction had been made and the resultant higher flow may explain the further increase in these two cases, though in only one were the haemodynamic conditions restored to near normal. Ferencz (1960b) and Daoud et al (1966) have demonstrated an increase in wall thickness after correction but in cases when the flow became greater than normal.

There is no apparent reason for the increase in wall thickness in these cases, it is certainly not as a result of increased flow or pressure as has previously been assumed. It may be due to some change in relative flow occurring during growth which had simulated the artificial increase seen in cases T.F.2 and 5 after operation.

Despite the abnormal wall thickness and extension of muscle, there is less muscle than in the fetus as judged by wall thickness related to external diameter and some post-natal thinning has occurred. This has either been arrested before completion or the increase is secondary. The latter is probably so in the corrected cases.

Muscle within the Acinus

Though the arteries in these cases are more muscular judged by size, muscle does not extend unduly into the acinar arteries, that is the normal non-muscular arterial pattern characteristic of childhood is found within the acinus. This is because of the small size of the vessels within the acinus. In cases T.F.1 and 3 the acini are in fact less muscular than normal for age. The extension of muscle into smaller vessels than is normal may be to compensate for the smaller vessels within the acinus so that the normal distribution through the lung is maintained, and the physiological reaction to hypoxia can take place at the same level. Any increase in muscle by extension is accompanied by an increase in wall thickness and this may explain the increased wall thickness in these cases.

Thrombi

One of the frequent findings in other series of tetralogy of Fallot has been thrombotic lesions of the arteries and veins.

These changes are normally related to the increase in viscosity of the blood due to polycythaemia and slow flow (Rich, 1948). In this series the only evidence of thrombosis was in the case T.F.5 with a normal flow and polycythaemia. This was also the oldest patient and this may explain this finding. Although Ferencz (1960a) found thrombi frequently in young children and De Mattéis (1969) found, in a series of uncorrected cases, that 61 per cent of those under 10 years had thrombi, in both these series thrombi were more frequent in the older cases. The other five cases in this series had a low flow but no thrombi.

This may have been related to the treatment they received as four of the five had polycythaemia, but the latter is not an indispensible factor for thrombosis (Rich, 1948).

In previous work it has been reported that an artificial increase in flow has led to recanalisation and disappearance of thrombi (Heath, Dushane, Wood and Edwards, 1958; Ferencz, 1964). In this series the case with thrombi in both arteries and veins was the one with correction of the abnormality and a normal flow. Correction had been made three years before death and thrombi were still present. There was no silting up in this lung as in the left. Associated with the thrombi was intimal thickening which was also seen to a small extent in case T.F.2, one that had a slight increase in flow during life. The change in flow may have led to these changes at the same time as to the increase in medial wall thickness.

Veins

Little work has been reported on the structure of branching pattern of the veins in tetralogy of Fallot save as to the presence of thrombi.

This study has shown that the veins, essentially, follow the same trends as the arteries. The pre-acinar region was visible on a venogram in one case and showed the same features as the arteriograms - a reduction in length of the main pathways and in lumen diameter along their whole length. This result contrasts with Wagenvoort's (1970) who, using a microscopical technique, considered that the veins were dilated. The branching pattern was essentially normal when studied microscopically, with conventional and supernumerary vessels in the normal proportion. This pointed to normal ante-natal development with post natal

hypoplasia. Since arterial size was normal at birth, so probably was venous size.

Intra-acinar Region

On the venogram there was a very marked background haze which almost obscured the main vessels. This was comparable with the increased blush on the arteriograms. Microscopically the number of veins per unit area was increased over the normal despite the fact that the number of alveoli was reduced. This is consistent with the increase in small arteries. It is surprising that though the number of small arteries and veins had been increased, probably because of collateral circulation from the bronchial system, the veins in the pre-acinar region were hypoplastic. It is possible that the rate of flow increases through the veins though there is no evidence for this.

Structure of Veins

There was no increase in muscle such as that seen in the arteries. Case T.F.6 in which the veins had been injected had vessels of normal wall structure and muscle distribution. As judged in uninjected vessels all six cases had a normal wall thickness, though in one case there was an increase in elastic tissue as is associated with an increase in flow, or in venous hypertension (Wagenvoort, 1970). The increase in wall thickness in the arteries was not reflected in the veins, but since the artery changes were so small it is possible that the veins had yet to be affected or that they are slower to react to the stimulus for muscle increase.

In only one case was intimal thickening seen in the veins, the one with marked thrombotic lesions in the artery.

Summary of Changes in Tetralogy of Fallot

In tetralogy of Fallot it can be said that overall the lung shows hypoplasia. The total lung volume is reduced and also the number of alveoli, even when these are related to the reduced lung volume. These changes are probably due to reduced blood flow. An increase in blood supply will cause giantism for example with an arterio-venous fistula of a limb: with decrease in blood supply growth reduces to a level that can be supported. It would seem that the blood supply was below normal for age and also for the reduced lung volume.

The arteries and veins of the pre-acinar region are reduced in size - probably this hypoplasia developing after birth since at birth the vessels were normal in branching pattern and probably also in size. It is not known whether flow and pressure of the pulmonary artery are normal before birth.

The intra-acinar or alveolar region develops after birth when the blood pressure and flow are known to be reduced. Also within the acinus the branching pattern of both arteries and veins to the level of the alveoli is normal but judged by size both systems are hypoplastic. Within the alveolar region an excessive multiplication of the small vessels, both arteries and veins has occurred. It would seem that the intra-acinar region has been able to respond to the changed dynamic state while the pre-acinar region was stabilised and established before birth. The increase in number of small vessels can be related to a collateral circulation developing from the bronchial artery system though since there are no anastomoses within the acinus the increased number of vessels does not represent collateral channels. It may be that the higher oxygen tension in the pulmonary artery blood causes a multiplication.

Another perplexing finding is that, although the intra-acinar vessels have multiplied the venous system is still hypoplastic though apparently carrying extra blood, since an increase in flow in the capillaries has been demonstrated physiologically by Bing, Vandam and Gray (1947). Another unexplained change seen in these cases was the increase in wall thickness and extension of muscle along the arteries although blood pressure and flow within them were reduced. There was no evidence of atrophy of muscle as has been described by most authors.

B. VENTRICULAR SEPTAL DEFECT

The six cases used in this series were of similar age all being infants under four months of age. Ventricular septal defect has a marked effect on the child at an early stage and death is more likely to occur in younger children than it does with tetralogy of Fallot, unless the defect is small in which case the child may live with little retardation or deterioration with age (Stanton and Fyler, 1961). Small defects may in fact close spontaneously (Hoffman and Rudolf, 1965). Though the number in this study is small, all cases are in the same clinical group having suffered gross effects of an increased flow rather than 'maladie de Roger'. The lungs have been examined by the detailed and precise pathological methods so that features are accurately described.

In this series there is only one case with a simple ventricular septal defect, the other cases are complicated either by atrial septal defects or patent ductus arteriosus or both. These three lesions all have the same potential for disturbing the vessels of

the lung, that is they can lead to increase in blood flow to the pulmonary circulation and it is possible to consider them as a single group.

Despite the similarity in age and haemodynamic situation the pathological features of the lungs vary and were not related to the size of the defect or the nature of the associated deformity. It can be assumed that all six cases had large connections between the systemic and pulmonary circulations so that there was a site available for a left to right shunt giving high flow to the lungs. This was found in all but two cases - V.S.1 and 4 in which there was a right to left shunt with low flow to the lungs.

Arteriograms

Dilatation of proximal pulmonary arteries with reduction in size of the lobular arteries was reported by Harrison (1958) in each of the arteriograms of ten cases of ventricular septal defect with a right to left shunt. These changes were similar to those seen on arteriograms by Evans and Short (1958) on a similar group of patients. In the present series three cases had dilatation of proximal vessels, but these were those with a left to right shunt. These three also showed tortuosity at the periphery indicating an increase in length of the arteries in addition to the increase in their diameter, not a reduction in size of these vessels. Thus the capacity of the vessels in these cases was increased, though the volume of the lungs was normal. The changes in the three cases were different in degree.

The other two cases in this series showed reduction of the lumen diameter throughout the lung. This hypoplasia was probably

present before birth since in one case, V.S.1, the lumen diameter was smaller than is normal at birth and it is unlikely that this had reduced in size so considerably in two weeks; again the lungs were of normal volume.

Thus in the present series more than one type of abnormal arteriogram was seen, in fact for each case the arteriogram was different but it is possible to impose two separate patterns.

Reactions to Increased Flow

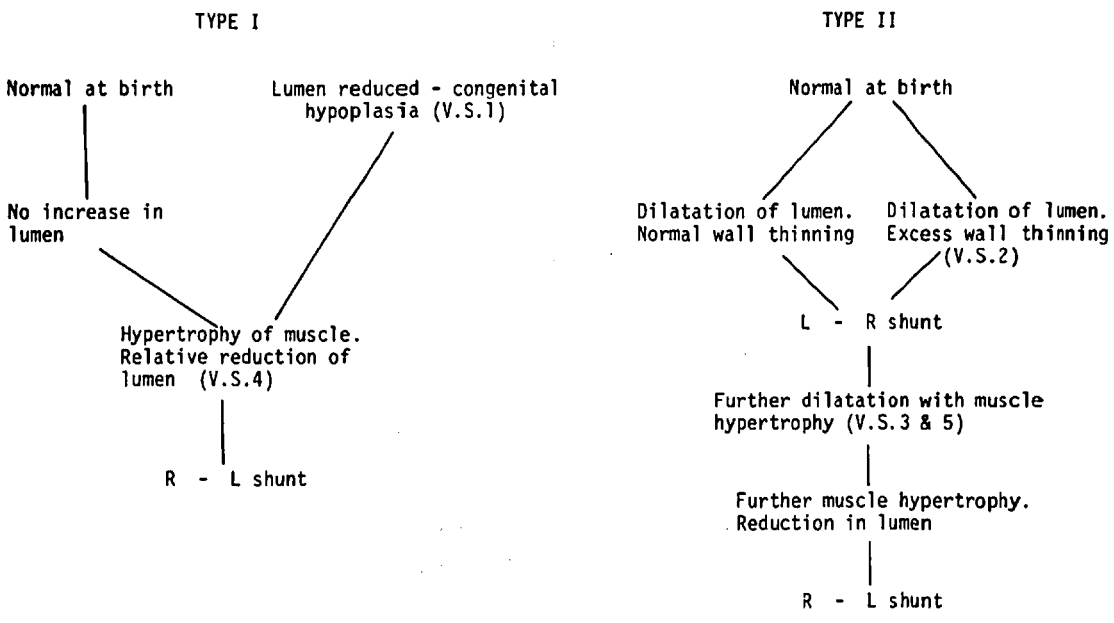
Broadly these results can be regarded as suggesting that there are two patterns of response to the increase in flow and pressure resulting from a ventricular septal defect (Table VII 2).

The first type of reaction seems to be to maintain the fetal state and a high resistance in the pulmonary arteries that probably results from the thick media of the arterial wall. Such high resistance would maintain the right to left flow seen during fetal life. This reaction can be seen in case V.S.1. That the lumen is in fact hypoplastic suggests that this must have been present before birth. This congenital anomaly may be the necessary precursor for this reaction or this case may be an exception.

Older cases such as V.S.4 can be regarded as the later stage of the same type of reaction. They show an increase in muscle thickness above values found at birth and a lumen diameter smaller than can be explained by this muscle increase, showing that the artery has failed to grow as much as normal. This could increase the resistance and cause a marked right to left shunt. Such increase in muscle might continue and later be associated with intimal thickening.

TABLE VII 2

CHANGES IN VENTRICULAR SEPTAL DEFECT



The second reaction suggested from the findings in this series as exemplified by cases V.S.2, 3 and 5. The increase in pulmonary flow that normally occurs at birth leads to dilatation of vessels with thinning of the media: this may be excessive as a result of the abnormally high flow (case V.S.2) and may be followed by further dilatation with muscle hypertrophy: this structural stage is typified by cases V.S.3 and 5. Excessive thinning need not necessarily occur and may not have done in the latter two cases. Later further hypertrophy of muscle may occur increasing resistance and intimal thickening may follow and eventually cause a right to left shunt.

Eventually either of these two reactions lead to the high pressure, high resistance and right to left shunt seen in the Eisenmenger situation. The first reaction or persistence of the fetal type will lead to this much faster than the other.

Dammann, Thompson, Sosa and Christlieb (1960) studied eight cases of ventricular septal defect using radiographic and catheter techniques, serially over several years. They showed changing pressures and flows consistent with Type II changes described here.

The stages seen in the five cases here are all in the first stage of Heath and Edwards (1958) series and it is probable that they would eventually go on to the later stages with intimal thickening and arteriosclerosis. These authors believed that a right to left shunt was only attained at stages five and six: in the two cases reported here it was attained with muscle hypertrophy only.

It seems possible that the determination of which of the two reactions will operate after birth in response to the haemodynamic

changes at birth will be controlled by the intra-uterine development. The first reaction (Type I) may be the change brought about by the increased blood flow on an artery which is hypoplastic as a result of a congenital anomaly. Type II reaction is the response of a normal pulmonary artery at birth to the increased flow. The response to the increased flow by dilatation or by constriction may be genetically determined. It seems likely that the congenital anomaly of the hypoplastic artery will lead to a constrictive reaction, while the normally developed artery will react initially by dilatation.

Intra-Acinar Changes

The size of the pre-acinar vessels was assessed on the arteriograms, the intra-acinar vessels by microscopic measurement. The intra-acinar vessels followed some of the trends seen in the pre-acinar region. In the two cases showing a Type I reaction the intra-acinar arteries were hypoplastic as were the pre-acinar. Only one of the three cases in the Type II group showed the dilatation within the acinus, of the pre-acinar region (V.S.3). In the other two cases, V.S.2 and 5, they were reduced in size. There must have been a sudden decrease in the size of vessels at the lobular region in these cases.

The pre-acinar and intra-acinar region would seem to behave as two separate 'compartments' of the pulmonary vascular system.

A reduction in the background blush was seen in the intra-acinar region of all the arteriograms, suggesting a reduction in vessel number. This was not confirmed by a count of the number of vessels per unit area within the acinus: this was found to be

within the normal range but on the low side. There was a lack of development of the intra-acinar branching in case V.S.5. There is no obvious explanation for the lack of small vessels on the arteriogram. Short (1959) believed it to be due to obstruction to filling in arteries between 0.1 and 1 mm diameter, in this series, however, vessels were well filled.

In the cases of tetralogy of Fallot it was suggested that the increase in number of small vessels was due to an increase in oxygen in the blood. In these cases with a left to right shunt there would also be an increase in oxygen in this region but there is a normal number of vessels. The high flow in these cases may have led to a reduction in small vessels.

Branching Pattern of Arteries

In only one case, V.S.5 was the branching pattern from hilum to periphery studied in detail. In this case growth of the pre-acinar was disproportionate to that of the intra-acinar region. The pre-acinar region had a normal branching pattern suggesting that growth in utero was normal: after birth this region had increased in diameter and length to a greater extent than normal. The intra-acinar region on the other hand had failed to grow at all, either in size or by branching, in the three months that the infant lived. This lack of growth may be the cause of the lack of background haze, which is not seen during fetal life. The number of vessels per unit area of lung suggested that multiplication had taken place but since in the normal the numbers are so variable in the first few months and are similar to those of fetal life it may be that this part of the lung has not developed. This is supported by the finding that the alveoli were not fully mature.

They were still of similar size to saccules.

The other four cases were not studied in such detail but they did show the normal proportion by length between the pre-acinar and intra-acinar region suggesting that growth at least was normal. Multiplication of arteries and alveoli may or may not have taken place within the acinus.

Structure of Artery Walls

All six cases showed changes in the structure of the artery wall. They showed the fetal type of elastic configuration described by Heath, Dushane, Wood and Edwards (1959) and D'Cruz, Lendrum and Novak (1964) which was associated with high pressures and flow. This structure is more similar to a systemic artery structure than the normal pulmonary artery and the pulmonary trunk is described as being of 'aortic type'.

In all the other features of wall structure the cases varied and fitted with the trends and reactions described with reference to the arteriograms.

Muscle Hypertrophy

Four of the five cases in which the percentage wall thickness of arteries could be measured showed an increase in muscle both by increase in the thickness of the wall and extension of the muscle into small arteries (the extension within the acinus is considered separately later). Each showed a different degree of change. The fifth case (V.S.2) had a reduction in wall thickness but still a slight extension of muscle into small arteries. Previous reports have suggested that there is always an increase in muscle except in

cases with a high flow and normal pressure when the walls have been reported as being particularly thin (Wagenvoort, Nauta, van de Schaar, Weeda and Wagenvoort, 1968). In this series the case with the thin wall had a high pressure and only slightly increased flow (V.S.2) while the case with high flow and normal pressure had a particularly high wall thickness (V.S.3). It would seem then that pressure had not directly led to an increase in wall thickness, nor in fact had high flow, since the two cases with low flow had a high wall thickness. This again suggests that there is a genetic basis for the changes that occur - the pattern of response is determined, at least to some extent, by factors other than congenital ones.

Hypertrophy of muscle may affect the size of the vessel lumen. It would seem that muscle hypertrophy may or may not encroach on the lumen. When fetal wall thickness is maintained followed by hypertrophy the lumen is in part reduced by the muscle increase (V.S.1 and 4). As resistance increases with increase in muscle with age flow will decrease as the lumen narrows (Auld, Johnson, Gibbons and McGregor, 1963). In the other three cases in this series muscle hypertrophy did not cause a reduction in lumen in the proximal vessels since at the same time there was dilatation. However in case V.S.5 at the periphery the intra-acinar vessels were reduced in external diameter due to hypoplasia and lumen diameter due to this, and in addition to possible encroachment. In case V.S.2 muscle hypoplasia was present on its own. In case V.S.3 there was still a large lumen at the periphery though a high percentage wall thickness. Here the hyperplasia was to the outside or to the inside but masked by dilatation. Using dogs in whom an increase in pulmonary flow and pressure had been produced,

Esterly, Glagov and Ferguson (1968) showed, using the electron microscope, that muscle hypertrophy by hyperplasia of the media and intimal changes caused encroachment of the arterial lumen within one month. No comment was made on the arterial lumen size. They considered the changes to be the reaction to increased pressure.

Muscle hypertrophy has been found with a reduction in external diameter or with an increased external diameter and increased lumen diameter.

Intimal Fibrosis

In none of the six cases studied was there evidence of intimal thickening, probably because all these cases were young. Wagenvoort, Neufeld, Dushane and Edwards (1961b) found no intimal thickening until over one year. Edwards (1962) believed that there was no increase in resistance unless there was intimal thickening, regarding muscle hypertrophy on its own as insufficient. This hypothesis was disproved by this series of cases.

Muscle within the Acinus

In the older four of the five cases in whom the arterial system had been injected there was extension of muscle further into the acinar vessels than normal. The extension could not be related directly to the degree of arterial resistance. In the youngest case, V.S.1, the muscle extended into vessels of appropriate size but these were not as far within the acinus as is normal.

When there is extension of muscle the number of small non-muscular vessels will be reduced since the total number of vessels in these cases is normal. This will have an effect on the function

of the lung. The vessels may react more rapidly to hypoxia since this has its effect at the level of the small pre-capillary arteries of the alveolar region (Kato and Staub, 1966) and will cause constriction of muscle if it is present in the artery walls (Burton, 1959). In babies with a raised pulmonary artery pressure due to congenital disease, the pressure can be reduced by increasing the oxygen content in the lung.

The extension of muscle possibly also leads to a smaller area for gas transfer. This may be off set by the greater flow of blood through the lungs so that not such a large area is required. In the cases of tetralogy of Fallot where the flow is reduced there is an increase in the number of small non-muscular vessels.

The extension of muscle to the periphery in these cases of ventricular septal defect may represent the basis of the increase in resistance although it is not found in case V.S.1. The increase in muscle is associated with the maintenance of the right ventricular preponderance of fetal life.

Veins

The veins in cases of ventricular septal defect, as in the cases of tetralogy of Fallot, showed similar changes to those exhibited by the arteries. The lumen diameter of the veins measured on the venogram was increased in size as were the pre-acinar arteries of the high flow cases (Type II reaction). In the case in which the veins were injected there was a high flow as judged by the radiographs. The drainage pattern was essentially normal with the correct proportion of conventional and supernumerary vessels. There was a reduction in the background haze of the

acinar region and this was reflected in a slight drop in the number of veins per unit area. Since the pre-acinar arteries and veins are both enlarged while the acinar vessels are normal or reduced in number, there must be a relative increase in speed of flow through the capillaries. The response of the pre-acinar region is different from that of the intra-acinar region in both artery and venous systems.

The rapid flow through the capillaries may lead to a decrease in oxygenation and possible hypoxaemia. However, the pulmonary artery in the cases with left to right shunt already has an increased oxygen content and the arterial to air gradient is therefore reduced. High flow might contribute to reduced oxygenation and this may be accentuated by the reduction in number of small vessels with a non-muscular wall.

Structure of Veins

The structure of the veins seemed to change in similar manner to that of the arteries. In only the one case was it possible to make accurate measurements on injected veins and in this case the wall thickness was normal. It was possible to study the appearance of the vein wall in all six cases. In three of these, V.S.1, 3 and 4 there was 'arterialisation' of the vein wall. This change was described by Wagenvoort (1970) - an increase in the amount of elastic in the media of the veins causing them to look more like arteries. The three cases showing this change were those with the highest wall thickness in the arteries. There was a measurable increase in percentage wall thickness of the veins even on uninjected material in these cases. Cases V.S.2, 5 and 6 showed no 'arterialisation' and no increase in vein wall thickness. In

cases V.S.2 and 5 there was little or no increase in artery wall thickness and in V.S.6 the arteries did not appear very thick walled in the uninjected state. The slight discrepancy between veins and arteries was also seen in tetralogy of Fallot. It seems that the structure of the veins closely follows that of the arteries with veins not increasing their muscle either as much or as rapidly as did the size and branching pattern.

Summary of Changes in Ventricular Septal Defect

Although all the cases in this series were very young they already showed variety in their pathological changes. It is therefore difficult to summarise the findings.

It is possible to define two reactions to the increase in flow. The first, Type I, seen in cases V.S.1 and 4 is probably as a result of a congenital abnormality leading to a hypoplastic pulmonary artery with a very high wall thickness. These cases show a marked increase in muscle both by increase in wall thickness and by extension of muscle into smaller arteries than is normal. Only in the older case was there an extension of muscle to the periphery. The second response, the Type II group including cases V.S.2, 3 and 5, showed the possible reaction of a normal pulmonary vasculature to changed haemodynamics. They showed an increase in lumen diameter at the hilum but not necessarily at the periphery, and an extension of muscle to the periphery of the acinus of varying degree. There was not always an increase in wall thickness. Even within the two groups there were a variety of changes.

As in the cases of tetralogy of Fallot the pre-acinar and intra-acinar regions seemed to behave as separate compartments

within the lung. Structural changes were similar in the two regions but multiplication and change in size seemed retarded in the intra-acinar area.

The pulmonary veins followed closely the changes of the pulmonary arteries both in the pre and intra-acinar region. Dilatation of the main vessels was seen in cases with a high flow. 'Arterialisation' of the vein walls and increase in their wall thickness followed increase in muscle in the arteries. The number of veins in the acinar region was reduced comparable with the artery changes.

HAEMODYNAMIC CONSIDERATIONS

From these studies on two types of congenital heart disease producing two different haemodynamic situations it is possible to make certain deductions about the changes brought about by flow or pressure.

Flow

Flow seems to affect mainly the lumen diameter of arteries and veins and has little influence over the structure of the walls.

Increase in Flow

An increase in flow through the lungs leads to an increase in lumen diameter of arteries shown on the arteriogram. It is the pre-acinar vessels which are involved in this. But since the greatest increase in lumen diameter was not associated with the highest flow there must be other factors involved. The increased

flow led to a degree of tortuosity particularly at the periphery of the lung; this is due to an increase in length of the vessels. The overall increase in length was not sufficient to prevent an increase in gradient of the dropping diameter from hilum to periphery in these cases. This would suggest a relative increase in velocity towards the periphery.

Only in one case did the increase in flow lead to an increase in diameter within the acinus. In the others there was in fact a decrease. At some point in the immediately pre-acinar region there was a sudden drop in size of vessels; this would increase velocity further. This region will form a type of stopcock.

An increase in flow affected the pre-acinar veins by dilatation of the vessels seen on the venogram.

Decrease in Flow

A decrease in flow seen either in cases of ventricular septal defect with a right to left shunt or cases with tetralogy of Fallot gave rise to arteries and veins of small lumen diameter in the pre-acinar region. The diameter in the intra-acinar region was also reduced when there was a low flow. This reduced flow may be primarily as a result of a pulmonary stenosis or secondary as a result of vaso-constriction and hypertrophy of muscle, probably genetically determined. It was possible to distinguish the two types on the arteriograms. In the former there was an increase in background haze and in the latter pruning of peripheral vessels.

Normal Flow

A normal flow produced artificially as in case T.F.5 does not always produce a normal lumen diameter. Once the lumen is

hypoplastic it remains so. Under these circumstances the change in flow did cause a change in wall thickness. Flow may therefore affect wall thickness secondarily. The lack of change of lumen as a result of change in flow once it has been established may explain why the lumen in case V.S.5 is very large although the flow is nearly normal. It is possible that there had been an earlier high flow.

Effect of Flow on Wall Thickness

Flow seems to have little effect on wall thickness of arteries or veins since the muscle may be increased or decreased whether the flow is up or down. All four combinations have been seen in this series. Low flow may be due to a high wall thickness or muscle may be present as a result of flow or incidental to it. One particularly interesting case was V.S.2 where the increase in flow was associated with a decrease in wall thickness. This is similar to the findings of Swan, Zapata-Diaz, Burchell and Wood (1954) and Heath and Whitaker (1957) in cases with atrial septal defects. In their cases however there was no increase in pressure. On the other hand, in the present series the case with no increase in pressure and high flow had a very high wall thickness (V.S.3).

Pressure

An increase in pulmonary artery pressure may or may not give an increase in wall thickness in arteries or veins. It is generally assumed that an increase in pressure is associated with an increase in muscle (Edwards, 1950). This was not the case in V.S.2 of three weeks of age and with a large lumen, where the pressure was particularly high and the wall thickness less than normal.

Pressure and Lumen

An increase in pressure may or may not be associated with an increase in lumen diameter at the pre-acinar level. At the acinar level those with an increased pressure had a reduced external diameter to the arteries and one case with a normal pressure had an increased lumen. On the other hand decreased pressure in the cases of tetralogy of Fallot also gave a reduced diameter.

Pressure and Wall Thickness

Though raised pressures were usually associated with an increase in wall thickness and extension of muscle into smaller vessels there was no correlation with the degree of muscle hypertrophy and the pressure level in the pulmonary artery.

A reduction in pulmonary artery pressure in the series of tetralogy of Fallot also gave a slight rise in wall thickness though in this condition the lumen was reduced. Also since in one case with normal pressure there was a marked increase in muscle some other factor must have been involved.

No pressure level gave an atrophy of muscle as has been described by Naeye (1961d).

An increase in pre-acinar wall thickness was associated with increased muscle within the acinus; this means that here also muscle extension was not related to increase in arterial pressure.

Though there was an increase in muscle at all pressures there was only extension to the periphery of the acinus in those cases with a high pressure. However there was no direct relationship

between the degree of extension and the pressure. There was no extension at low pressures but there was a case of normal pressure with extension and a case of high pressure with no extension.

The role of pulmonary artery muscle in the elevation of pulmonary vascular resistance and pressure is by no means clear.

Small vessels with a high wall thickness will require a higher pressure to maintain them open because the critical closing point is higher (Burton, 1959). This will particularly apply in the cases with hypoplastic lumen and very high wall thickness, that is V.S.1 and 4. In these cases the pressure was increased but not to the highest levels of the series.

If the increase in resistance is not related to pressure or flow there must be another factor involved. Rudolf (1965) suggested that it was related to an elevated left atrial pressure. Using dogs he showed that if this was raised by shunts from the aorta there was also a rise in the pulmonary artery pressure. He believed it might be mediated by local hypoxia due to oedema and broncho-constriction produced by elevated left atrial pressure. In this series in only one case was this pressure available (V.S.2). It was raised considerably and there was a high pulmonary artery pressure but no increase in wall thickness. The high pressure in the left atrium was not reflected in the venous wall thickness in this patient, which was also normal.

The increase in pressure may be due to a reduction in the number of small vessels so increasing the resistance to flow at this level. This was a theory formulated by Griswold, Bing, Handelsman, Campbell and Lebrun (1949) after a series of catheter studies on cases with ventricular septal defect, but with no histological studies. Davies (1969) found microscopically a

reduction in the number of small vessels below 200 μm in cases of cystic fibrosis with right ventricular hypertrophy but not in cases without hypertension. Semmens (personal communication) found a relationship between right ventricular hypertrophy and a reduction in small vessels in chronic airways disease. In the present series there was no significant change in the number of vessels in cases with hypertension and it was probably the hypoxia in the cases of cystic fibrosis and bronchitis which caused the change.

GENETIC INFLUENCE ON CHANGES IN CONGENITAL HEART DISEASE

The changes in ventricular septal defect are obviously more diverse than has been previously supposed. There are at least two reactions to the change in flow and pressure as a result of the defect. The pattern of response taken may be determined before birth since the different reactions manifest themselves so soon after birth. Case V.S.1 aged two weeks was abnormal before birth and therefore not just affected by post-natal increase in flow. Case V.S.2 of three weeks showed an entirely different reaction. Wagenvoort, Neufeld, Dushane and Edwards (1961b) believed that there were no changes up to five weeks of age. This was certainly not the case in this series.

It is possible that the reaction in tetralogy of Fallot reflects in part a congenital defect of the pulmonary artery. The cases in this series may well have been normal at birth and then each case has responded in the same way with hypoplasia of the vessels. However correction on case T.F.5 to produce a normal flow did not alter the hypoplasia. It may be that the pre-acinar pattern is determined before birth and cannot be altered later.

In both tetralogy of Fallot and ventricular septal defect the capillary bed has made adjustments for the changing flow but the pre-acinar veins follow the arterial changes and have not been able to adjust to the change in the acinar region. This suggests that the factors determining structural response of the pre-acinar region are established before birth.

In cases of mitral stenosis some cases respond by pulmonary artery hypertension meaning that the pulmonary oedema caused by a rise in the pulmonary venous pressure may not occur. The vaso-constrictive response may be genetically determined.

In all cases studied in the present series it seems that the pre-acinar and intra-acinar regions behave as separate functional compartments. The pre-acinar branching pattern is determined before birth and it is only able to respond to the changed haemodynamics by changes in size and structure. The intra-acinar region is able to respond by changes in the branching pattern and multiplication of the small vessels as well as by changes in their size and structure.

MUSCLE INCREASE

In this study an increase in muscle in the walls of arteries has been demonstrated both in cases with increased and decreased pressure and flow. This increase may be as a result of hypertrophy of individual muscle cells or of hyperplasia, development of new muscle cells.

Using the precise methods of measurement of the present

study, it was possible to show that in all cases with an increase in muscle this was due at least in part, to an increase in the number of muscle cells. This was demonstrated by the extension of muscle into smaller vessels than in the normal. It was also possible in the cases of ventricular septal defect to see that there were more nuclei in the media of vessels of the same size with medial hypertrophy than without. In the cases with tetralogy of Fallot, the muscle increase was not as great and it was not possible to see by inspection a larger number of nuclei. However there was extension of muscle into smaller vessels than normal.

The muscle increase in these cases was probably the result of abnormal flow and pressure on what was otherwise a normal blood vessel. By contrast Anderson (1971) using the same precise methods found that in primary pulmonary hypertension, probably an intrinsic vascular disease, the increase in wall thickness was due to an increase in size of each muscle cell and that there was no increase in the number of cells. No extension was seen and wall thickening in large muscular arteries was by increase in cell size since there was no increase in the number of nuclei. It seems that the response is different in these cases. This may have been due to an intrinsic abnormality in the cases of pulmonary hypertension leading to hypertrophy rather than hyperplasia. Alternatively this may have been because the changes were occurring in the adult phase after multiplication was complete, in the Anderson series.

Davies (1969) found that in cases of scoliosis with right ventricular hypertrophy and cases of cystic fibrosis with or without hypertension there was an extension of muscle into smaller

vessels suggesting medial hyperplasia rather than cellular hypertrophy.

Naeye (1966b) using methods of planimetry and cell count considered that in cases with ventricular septal defect the increase in muscle was due to an increase in size of muscle cells. This was not the case in the present series. On the other hand he found that in cases with hypoxaemia due to high altitude there was muscle hypertrophy and hyperplasia (1962). Hasleton, Heath and Brewer (1968) put forward the idea that the changes due to hypoxia are different to those of other hypertensive diseases. They found that with hypoxia there was only extension of muscle into small vessels without increase in wall thickness and that there was evidence that this was reversible. In this they did not agree with Naeye (1962) who found muscle hypertrophy in similar cases. Davies also found increase in wall thickness in cases of kyphoscoliosis which are hypoxic and Semmens (1971) in cases with emphysema. Heath and Best (1958) found increase in medial thickening only in cases with vascular disease; those with hypoxic lung disease showed no change.

It is apparent that there have been conflicting reports on the type of muscle change in disease but they all point to the likelihood that there may be two different reactions, producing hypertension. Using the precise methods of measurement employed in this department on different disease states it seems that extension of muscle is associated with an increase in wall thickness whatever the disease and that the increase in muscularity in these cases is therefore due, at least in part, to increase in number of muscle cells. On the other hand, using these same methods, Anderson has shown that increase in wall thickness may be found

without extension of muscle and is then due mainly if not entirely to muscle cell hypertrophy. No disease state has been found with extension of muscle and no increase in wall thickness. The results from this department all refer to actual increase in muscle and not an increase in wall thickness due only to constriction as this is overcome by the injection technique. It is likely that the early stages of increase in resistance are the result of vaso-constriction in the small vessels as demonstrated by Kato and Staub (1966). At this time the resistance can be lowered by addition of dilatation agents. Later as the changes become permanent as a result of hypertrophy or hyperplasia these have no effect. The increase in resistance in all the cases studied in the present series have been permanent. It has been shown that the extension of muscle to the periphery could be relieved by removing the hypoxia (Hasleton, Heath and Brewer, 1968), or administration of acetyl choline.

FACTORS AFFECTING GROWTH

It seems that arteries develop in parallel with airways, the branching of both being complete to pre-acinar level by approximately the end of the fourth month of gestation. The supernumerary vessels grow at the same time as the conventional and their numbers relate to the number of conventional branches regardless of the space available. In the small fetal lungs they are much closer together than in the older cases. At first there would seem to be too many for the amount of lung supplied. However they are smaller than in the older lungs. The adult pattern is present though in miniature. Supporting evidence for the fact that arteries and airways are interdependent is seen in a case of congenital diaphragmatic hernia (Kitagawa,

Hislop, Boyden and Reid, 1971) where the number of conventional arteries was the same as the number of bronchial branches though in both lungs these were reduced in number as a result of the lesion. In one lung the ratio of supernumerary to conventional arteries was normal. This normal relationship of vessels and airways was also seen in a congenitally hypoplastic lung in a case of nine months of age where there were only 10 bronchial generations in the posterior basal segment rather than the normal of about 23 (Henderson, Hislop and Reid, 1971).

Multiplication is from the distal end of the axial pathways. There is the same number of supernumerary arteries found between the conventional arteries when the generation develops as when it is in the adult lung. Though the actual branching pattern is probably not altered once it is laid down, the size and structure of the vessels will alter. The relative growth of the arteries and airways can be affected. In the normal growth would seem to be symmetrical once the main pathways are established, the first seven generations forming half the pathway. In two of the cases with ventricular septal defect there had been abnormal growth in length of the proximal part of the pathway at the expense of the distal region which in one of the cases had not grown in the three months from birth.

Growth in size can be affected at all stages by various factors either congenital or environmental. In three cases with ventricular septal defect the arteries were increased in size presumably as a result of the changed haemodynamics. In two other cases there had been a decrease in size, possibly genetically controlled. In the cases of tetralogy of Fallot there was a reduction in size of vessels as a result of the low flow and

pressure. Davies (1969) also found changes in size in cases with cystic fibrosis and kyphoscoliosis where hypoplasia and atrophy both played a part in the reduction in size of the vessels in child lungs.

The number of airways appear to control the number of arteries in the pre-acinar region. In the developing acinus during childhood the airways also influence the development of the arteries. As the respiratory bronchioli and alveolar ducts divide and multiply so do the conventional arteries and the attendant supernumerary vessels. In this region the supernumerary arteries seem to grow slightly later than the conventionals and probably develop to supply the alveoli as they grow. Before birth there are few supernumeraries in the intra-lobular pre-acinar region but they increase after birth and have the highest ratio in the adult. Within the acinus the ratio increases with age suggesting that the supernumerary arteries multiply for longer.

The number of vessels within the alveolar region can be estimated by the method of counting the number per unit area as well as by serial reconstruction. The arteries multiply with the alveoli, but as the alveoli cease to multiply and start growing in size so do the arteries. Within the acinus the arteries follow the growth of the respiratory structures. The arterial supply is related also to the number of capillaries in the walls of alveoli and thus indirectly to the alveolar surface area. An exception to this was seen in a case of childhood lobar emphysema where there was a vast increase in the number of alveoli with no increase in the number of arteries so that the ratio of alveoli to arteries was increased. There must have been a reduced blood supply for the number of alveoli present since the arteries had not followed the

alveolar overgrowth (Hislop and Reid, 1970). The arteries in this case were related to the number of acini which was normal rather than to the number of alveoli. This is not a constant pattern since the number of arteries was increased relative to the number of alveoli, in the cases of tetralogy of Fallot where the alveolar number was reduced but in fact the arteries were increased above normal and so were even more increased per alveolus. This possibly represented a response to the low flow. In a case in which the alveoli were increased in size but normal in number the vessels were of normal number but the blood supply would be too small for the alveolar surface area therefore had not increased when required. The vessels were again in this case independent (Hislop and Reid, 1971).

In the normal the number of alveoli per acinus increases with age. This was shown by Emery and Mithal (1960) using a radial acinar count. It seems that in abnormal cases the number of alveoli per acinus is more nearly normal for age and change in the total alveolar number is due to an abnormal number of acini (Henderson, Hislop and Reid, 1971; Kitagawa, Hislop, Boyden and Reid, 1971). By the same token alveoli will develop normally even when the acinar number is abnormal. An exception to this was the case with too many alveoli per acinus already mentioned (Hislop and Reid, 1970). Since the arteries within the acinus are controlled by the alveoli and these by the acinus the airways seem to influence all arterial development.

Bronchial arteries according to Boyden (1970) grow as the cartilage develops. In the youngest fetus studied in this series they were found to the level of the last cartilage plate but it was possible that they were not patent at this time. In the cases with

tetralogy of Fallot in this series the bronchial vessels had increased in number and calibre in the walls of airways and there were leashes of bronchial vessels near the hilum on the pleura of the lungs. These were supplying extra blood to the lungs. There is probably some humoral control for their development as a result of the reduced blood flow.

The veins in the lung seem to develop at the same time as the arteries and seem influenced by them. In the youngest case in which the veins were studied, aged 20 weeks, the pre-acinar branching pattern was complete, the number of conventional veins being similar to the number of conventional arteries. There was development within the acinus as alveoli and arteries multiplied. It is difficult to define the acinar level for the veins and therefore quantification was not exact. However there seem to be more venous tributaries than arterial branches. The number per unit area of alveolar tissue is also increased. Associated with this increase in number of veins is a rather smaller average size. Changes in the veins as a result of haemodynamic abnormalities followed closely the changes in the arteries. In the case of patent ductus arteriosus the pre-acinar veins were increased in size and reduced in number per unit area. In the case of tetralogy of Fallot the pre-acinar vessels as seen in the venogram were reduced in size and increased in number. They followed closely the artery changes of structure. The veins in the cases with ventricular septal defect showed muscle increase as did the arteries.

Factors affecting change in structure have been discussed on pages 665 and 671.

Age of Onset of an Abnormality

With such a precise method of describing changes with age it is possible to gain some idea of the age at which a lung has been affected in its growth. Since we now know that all pre-acinar branching of the airways, arteries and veins is complete at least by 20 weeks gestation, any alteration in the branching pattern must be due to changes at an early stage of development. This has been seen in congenital diaphragmatic hernia (Areechon and Reid, 1962; Kitagawa, Hislop, Boyden and Reid, 1971) where lack of space at around 12 weeks gestation has caused an arrest in airway branching pattern. A further case with an abnormal branching pattern has also been seen and again the onset must have been before birth (Henderson, Hislop and Reid, 1971). Other cases with hypoplasia of one lung, for example MacLeod's syndrome, are found to have a normal pattern of branching but are reduced in size (Reid and Simon, 1962). In this disease hypoplasia is due to a single episode of airway disease in childhood rather than a congenital hypoplasia. Similarly though the lungs are abnormal in shape, in cases of scoliosis the number of bronchial and arterial branches is normal (Elliott, 1964) since the onset of the disease is after birth.

Disease in early childhood will affect the growth of alveoli and arteries within the acinus and it is possible to determine both the age to which any given structure pattern is appropriate as well as to determine how it varies from the normal of the same age. Even though the former, the 'appropriate' age is known it does not mean that the onset of the condition was at this time since

the abnormality may arise from reduction in the speed of lung growth rather than its sudden interruption. On the other hand a lung may develop normally and then as a result of the disease atrophy to a state of a younger child. Davies,(1969) reported atrophy in cystic fibrosis and scoliosis. It is important when comparing normal and diseased lungs to take such factors into account. There are occasions when the onset of the abnormality is known and it may therefore be possible to see how the previously normal lung has been affected.

In the cases of ventricular septal defect it is probable that there was normal development of the lung until birth and that any changes are post-natal. The exception is case V.S.1 which had an abnormally small pulmonary artery, probably connected with the heart lesion, particularly since there seemed to be a functional pulmonary stenosis but no anatomical lesion, and also a pulmonary artery smaller than the normal at birth. Only the acinar region will be affected post-natally from the point of branching. This study has shown that the region of the acinus has become retarded in its post-natal development as a result of the heart lesion. In one case aged three months it had not changed from the pattern at birth. The blood supply has affected the artery and airway development. There are also changes in size and structure related directly to the changed haemodynamic situation.

In the cases of tetralogy of Fallot all the lungs were hypoplastic from the point of artery size, lung volume and alveolar number. These features were probably post natal in origin since T.F.1, the youngest, was the least affected and was larger than the normal at birth in all respects.

FUTURE STUDY

Throughout this study the precision of the methods used and hence their value has been stressed. It has been found that some changes are only present with one type of congenital heart lesion while other changes are found as a result of several different lesions. A study on the larger number of lungs from cases with different types of congenital heart disease would greatly add to the understanding of the effect of the lesion on the lungs, particularly of the changes due to flow and pressure. Since they are time consuming it would be too much to consider making complete studies as have been made in the present series on all lungs as a routine procedure. However from the present study it is possible to pick out the methods of examination which give the most information. The smaller amount of work could then be done on a larger number of cases.

Injection of the pulmonary vessels - either arteries or less often veins - is particularly important and should be made on all cases. This ensures complete distension of the vessels so that all measurements are comparable. The arteriograms or venograms obtained after injection offer much valuable information and it is possible to classify the lungs on this feature along. Unusual cases can be identified readily for special study.

The arteriograms show the size and the overall branching pattern of the pulmonary artery bed. Any gross abnormality of the branching pattern suggesting a congenital abnormality can be seen. Measurements show the vessel lumen diameter and this can be compared with the normal values of the same age and the same lung volume. Cases with an increased flow usually show an

increase in lumen diameter with tortuosity of the vessels at the periphery while a decrease gives rise to a small lumen diameter with either an increase or decrease in the amount of peripheral filling but no tortuosity. Venograms do not give as much information as arteriograms since the veins are affected less but in a similar manner to the arteries. Venous injection is worthwhile when there are two lungs available for study from the same case.

Having classified the lungs by their arteriograms it is then possible to gain most necessary information on the artery structure from one or two sections from the lungs. By measurement of wall thickness in vessels over the wide size range seen on one section it is possible to detect medial hypertrophy or atrophy. A 'population count' of small vessels will show the extension of muscle into them and in this way also indicate muscle hypo or hyperplasia as apparent. Study of arteries accompanying airways will show any vessel size change and also the extension of muscle into the acinus. Even in uninjected veins it is possible to demonstrate increase in muscle by 'arterialisation' of the vein wall.

These studies give a wide picture of the changes in the pulmonary vessels with little work. It would seem particularly important to do such a study on cases in which accurate and reliable catheter and angiographic studies have been made so that the relationships of structure and function can be more satisfactorily correlated.

Animal Studies

Despite the precise methods of analysis the interpretation of changes in the lungs caused by increase or decrease in pressure and flow due to congenital heart lesions is not clear. The changes are complex and it is still not always clear using clinical material whether changes in flow or pressure lead to the changes seen in the lungs. The two are closely related clinically and results from the present study have shown that either may play a part. It has been assumed that hypertension has been related to an increase in muscle in the arteries but it is particularly interesting that in the cases of tetralogy of Fallot there was muscle hypertrophy without increase in pressure or flow.

Also of interest are the various features of muscle increase associated with hypertension. Increase in wall thickness by muscle cell hypertrophy or hyperplasia is not always associated with extension of muscle into smaller vessels as was seen by Anderson (1971) in primary pulmonary hypertension, and in the present series of tetralogy of Fallot. This increase in muscularity was associated with right ventricular hypertrophy. Hasleton, Heath and Brewer (1968) found hypertension as a result of hypoxia only with peripheral extension of muscle. This extension with no increase in wall thickness was also seen in this series (case V.S.2). In scoliosis on the other hand both increase in wall thickness and extension of muscle to the periphery are only found when there is right ventricular hypertrophy (Davies and Reid, 1971). Surprisingly in cases with cystic fibrosis increase in wall thickness and extension of muscle were present in cases with no right ventricular hypertrophy (Davies, 1969).

Using animals as an experimental model it should be possible

to make further analysis of these complex features. It is important when using animals to use the precise methods of injection and measurement used for the human. Thus differences can be more accurately assessed and minor changes discerned.

The precise methods applied to animal studies may make it possible to resolve the differences found using human material. Experimental work could with advantage be along the following lines, to throw light on the relation between muscle development and pulmonary artery hypersention:

1. Mechanical production of hypertension
   e.g. systemic to pulmonary artery shunt.
2. Hypertension produced by other agents such as humoral agents, drugs and hypoxia.
3. Reversibility of hypertension either by withdrawal of the stimulus or by vaso-dilator drugs.

1. Experimental Work on Pressure and Flow

Ferguson and Varco (1955) made systemic-pulmonary artery shunts in dogs and recorded pressures and flows. They found that the development of lesions in the pulmonary arteries depended upon increase in pressure rather than flow and that an end to end anastomosis was more effective than an end to side, suggesting that the pulsatile thrust from the heart was an important factor. An increase in pressure caused by banding the veins of dogs also led to changes in the media of the arteries (Ferguson, Berkas and Varco, 1959).

Pool, Averill and Vogel (1962) described a child with an absent right pulmonary artery, in which the blood flow to the left lung was therefore increased but, they presumed with no increase in pressure. They found medial hypertrophy and hypertension at her death. They then produced the same effect by ligating the left pulmonary artery one day after birth in puppies and calves. In all medial artery hypertrophy in the contralateral lung and right ventricular hypertrophy was produced as a result of the increase in flow.

McCrady, Rosenberg, Hallman, McNamara and Vogel (1968) also using calves, found that ligation of one of the pulmonary arteries gave hypertension in the contralateral lung. This was determined by catheter studies and in the fatal cases there was medial hypertrophy. In cases with an artificial atrial septal defect or an aortico-pulmonary shunt there was no evidence of hypertension. Further work along these lines may show that depending upon the site of the increase in flow there is a different reaction in the lung.

Experiments have also been carried out in which the pulmonary blood flow is reduced. Liebow (1962a) ligated the pulmonary artery in rats and found that a collateral circulation developed with bronchial arteries connected with pulmonary arteries and bronchial veins with pulmonary veins. No reports on the structure of the veins and arteries under these conditions are available and are therefore still needed.

No experimental work using the precise methods employed in the present study had been reported. The extension of muscle to the periphery is particularly important functionally since it will affect oxygen uptake and contractility of small vessels in response to hypoxia.

2. Production of Hypertrophy Using Hypertensive Agents

As well as hypertrophy developing as a result of haemodynamic changes it is possible to induce medial hypertrophy by hypertensive agents. Naeye (1965) studied the effects of hypoxia on cows and dogs by ligating one bronchus. He found smooth muscle hypertrophy and hyperplasia in the small arteries and in the calves in the veins. These changes he found to be reversible. Tanaka et al (1966) showed constriction only at the level of the respiratory bronchioli in a hypoxic guinea pig lung; this could eventually lead to hypertrophy. In 1967 Naeye did some further work on the changes with hypoxia using mice kept at decreased pressure. He found that there was an increase in muscle due to hypertrophy of muscle cells and hyperplasia but he did not state at what level.

Using precise methods it should be possible to define more clearly the level and extent of hyperplasia as a result of hypoxia.

Kay and Heath (1967) have produced experimental muscle hypertrophy of the pulmonary arteries of rats by the injection or feeding of seeds of Crotalaria spectabilis. They reported individual muscle cell hypertrophy and extension of muscle into small arterioles and right ventricular hypertrophy suggesting hypertension. No work has been reported on the reversal of these changes or on the affect of drugs on these animals. Nor have precise measurements been made.

Sympathetic drugs cause vaso-constriction of the pulmonary vessels. Sturgess (1970) showed that there was development of right ventricular hypertrophy suggesting hypertension in rats injected with isoprenaline. It seems likely that the arteries will be affected by muscle hypertrophy. Other sympathomimetic drugs may

show a similar effect. Tests with β blockers and drugs causing dilatation such as acetyl choline and tolazoline would also be of interest.

3. Reversibility of Lesions

Clinical trials have shown that there can be reversal of muscle hypertrophy by correction of the lesion. Experimental reversal by creating a shunt and then correcting the lesion, after determining the presence of hypertension by catheter, could be of value. Using dogs Ferguson and Varco (1955) showed that regression after correction of a left to right shunt was very slow even when medial thickening was present without any intimal changes. Hypertension caused by hypoxia is easily reversible both by drugs (Kato and Staub, 1967) and by increasing the oxygen (Naeye, 1965).

Dammann, McEachen, Thompson, Smith and Muller (1961) used a form of clinical treatment as a vicarious experiment on reversibility. By producing a pulmonary stenosis in cases of high flow disease they found a decrease in muscularity on biopsy material at a second operation for total correction. The catheter studies showed a fall in pulmonary artery resistance. In some cases which died after the second operation they found that the tortuosity at the periphery was reduced in comparison with an uncorrected case of the same age. Vogel, McNamara and Blount (1967) reported that hypoxic hypertension could be relieved by tolazoline, both in cases living at high altitude and in those with cystic fibrosis.

Hypertension found in cases with a left to right shunt can be reversed if there are no secondary intimal changes (Leading Article, British Medical Journal, 1969). It should be possible

having induced hypertension with various agents to study the reversal over a period of time. Precise methods would ascertain whether cells have only reduced in size or have disappeared. In the child after birth there is a loss of whole cells rather than muscle cell cytoplasm (Naeye, 1966a).

SUMMARY

SUMMARY

The detailed anatomical results have been summarised at the end of each chapter (pages 233, 370, 425, 577). The main points of interest are given here.

It has been possible to study in detail, using quantitative methods, the structure and branching pattern of the arteries and veins during development. The precise results have been made possible by the use of a standard injection technique. Surprisingly the veins had to be injected under the same high pressure as the arteries, even during fetal life. The smallest lung which it was possible to inject, in this way, was from a fetus of 12 weeks gestation.

The arterial branching pattern of the pre-acinar region is complete by the end of the first four months of gestation, the arteries developing parallel with the airways and at the same rate. Conventional arteries are found accompanying bronchial branches and supernumerary arteries branch from the axial artery at the same time and in the same number as in the adult.

The pre-acinar venous drainage pattern is also fully developed at most by the end of the 20th week of gestation. The venous system would seem to follow the development of the artery and airway system, though running independently. It has been possible to identify conventional veins in similar number to conventional arteries along comparable axial pathways. In addition there are supernumerary veins which correspond to the supernumerary arteries. However the former are in greater number but of smaller diameter. There would seem to be two types of supernumerary vein each found along the entire length of a pathway.

During fetal life growth is mainly in the pre-acinar region, the intra-acinar region being developed during childhood. In this latter region too, the arteries follow the airway and alveolar development and the veins develop in parallel. Both conventional and supernumerary vessels are found in both systems at this level. In childhood new supernumerary arteries develop in the intra-lobular pre-acinar region in addition to the intra-acinar vessels.

During the phases of multiplication, both pre and intra-acinar, there is a rapid increase in the size of proximal vessels. As multiplication slows at about 18 months of age the rate of increase in size slows but continues until adult life.

From half way through fetal life the proximal pre-acinar pulmonary arteries have a structure related to their position in the lung and this is the same as in the adult. The structure of the arteries therefore remains the same while they increase in size, but muscle always extends distally to arteries of the same size and therefore gradually extends to the periphery. However within the acinus, during childhood, the structure of the small vessels changes both when related to external diameter and position. During multiplication and growth of these vessels the development of muscle cells lags behind so that muscle is not found in such small arteries as in the fetus or the adult. This means that from birth the arteries within the acinus are relatively non-muscular. There is a gradual penetration of muscle into these vessels but even by 11 years the arteries are still less muscular than in the adult.

The arteries during fetal life have a particularly high wall thickness which is lost within the first four months partly

by dilatation, partly by lack of muscle development as vessels increase in size and partly by muscle cell atrophy.

The pulmonary veins do not follow the changes in structure of the arteries in normal development. During fetal life there is little muscle in the walls of veins and a measurable wall thickness is only found by birth. At this age there is the same amount of muscle both by wall thickness and extent as in the child of ten years and probably the adult as there are no changes during childhood.

Two types of congenital heart disease have been studied in an attempt to correlate haemodynamic changes with structural changes and to throw light upon the factors affecting growth. In tetralogy of Fallot a reduced flow and pressure in the pulmonary circulation has led to uniform changes in the six cases studied, though they are of a wide range of ages. In ventricular septal defect where there is a potential increase in flow the changes are complex and varied despite the fact that all six cases are of a similar young age.

In both diseases the pre and intra-acinar regions have adapted independently of each other. The pre-acinar region, being complete by birth, only exhibited changes in size and wall structure while in the intra-acinar region there has been change in the number of vessels. In tetralogy of Fallot there has been an over multiplication of arteries and veins in the alveolar region and in ventricular septal defect there has been a reduction in the number of vessels, both arteries and veins, in the acinus together with a retardation of airway growth in this region.

In cases of tetralogy of Fallot the decreased flow is associated with a reduction in size of vessels throughout the

lung, both arteries and veins. The increase in flow seen in some cases of ventricular septal defect had led to an increase in size of the pre-acinar vessels but not always of the intra-acinar arteries. Other cases show a decreased lumen diameter which is associated with a decreased flow due to a right to left shunt at the level of the heart.

The changed flow and pressure also led to changes in the muscle of the arteries and veins. Surprisingly there is an increase in muscle of the arteries in cases with tetralogy of Fallot. In some cases with a ventricular septal defect there is marked increase in muscle while in others it is normal or reduced. The change in muscle can not be related to flow or pressure.

Changes in the arteries are closely parallelled by changes in the veins. An increase or decrease in lumen diameter of arteries is associated with the same modification to the veins. An increase or decrease in acinar arteries is associated with similar changes in the number of veins. As with the arteries the post and intra-acinar regions would appear to be independent since in cases with tetralogy of Fallot the veins are reduced in calibre though there has been an increase in the number of vessels in the intra-acinar region in adaption to the low flow. In the cases with a markedly increased arterial wall thickness the veins show an increase in muscle leading to 'arterialisation' of the wall and an increase in wall thickness.

The findings for the cases with ventricular septal defect suggest two separate pathways of vessel adaptation: it may be that in any given patient the pathway followed is genetically determined. In at least one case the arteries were congenitally abnormal.

APPENDICES

APPENDIX A

Details of the methods and solutions for injection, fixation and staining are given below.

Injection Solution - Barium Sulphate and Gelatine

Micropaque (Dammancy and Company Ltd., Slough) - 500 ml

Gelatine - 50 grm

Distilled Water - 200 ml

Phenol - a few crystals

The gelatine is dissolved in the distilled water in a 37°C oven. This is added to the Micropaque and mixed thoroughly. A few crystals of phenol are added. The mixture is solid at room temperature and is kept in a stoppered flask to avoid contamination by bacteria.

The mixture is reheated to 60°C in a water bath before use.

Formol Saline - Neutral and Buffered

Formaldehyde (40%) - 1000 ml

Distilled Water - 8000 ml

Sodium Chloride - 81 grm

Sodium di-hydrogen phosphate - 31.5 grm

Di-sodium hydrogen phosphate - 58.5 grm

The buffer salts can be dissolved in some heated distilled water before adding to formaldehyde. They do not dissolve easily in cold water.

Verhoeff's Elastic Stain (Culling, 1957)

5% Alcoholic haematoxylin - 20 ml

Lugols iodine - 8 ml

10% Ferric chloride - 8 ml

The ferric chloride is added to the haematoxylin followed by Lugols iodine.

Sections are taken to 70% alcohol and then stained in solution for 8 - 15 minutes. They are then washed in running water for a few minutes. Differentiation is in 2% ferric chloride and is checked microscopically. The sections are washed for at least 30 minutes before counter staining with Van Gieson.

Van Gieson Stain (Department Modification)

7% Acid fuchsin - 15 ml

Saturated aqueous picric acid - 985 ml

Sections are stained for two to three minutes in this mixture, then rinsed in 100% alcohol and blotted dry, then cleared in xylol. They are then mounted in Canada Balsam.

Ehrlichs Haematoxylin and Eosin (Culling, 1957)

Haematoxylin - 2 grm

95% alcohol - 100 ml

Glycerine - 100 ml

Ammonium or potassium alum - 3 grm (an excess)

Acetic acid - 10 ml

Distilled water - 100 ml

This solution is kept for three to four months before use.

Eosin in a 1% aqueous solution is used for counter staining.

Hart's Modification of Weigert's Elastic Stain (Culling, 1957)

Basic fuchsin - 2 grm
Resorcin - 4 grm
Distilled water - 200 ml

These are placed in a porcelain bowl and brought to the boil; 25 ml of 30% ferric chloride is slowly added, stirred and boiled for a further 2 - 5 minutes. The solution is cooled and filtered and the filtrate discarded. The paper and precipitate is placed into the original flask and 200 ml of 95% alcohol added and heated gently until the precipitate dissolves. The filter paper is removed and 4 ml concentrated hydrochloric acid is added and filtered. The volume is made up to 200 ml by adding 95% alcohol.

This solution is diluted 5 - 20% with 1% acid alcohol. Sections are brought to alcohol and then stained overnight at room temperature. They are differentiated with acid-alcohol and washed in water before counterstaining.

French's Modification of Weigerts Elastic Stain

(Drury and Wallington, 1967)

Crystal violet (Gurr's or Revector) - 2.5 grm
Basic fuchsin - 2.5 grm
Dextrin - 1.0 grm
Resorcin - 10 grm
Distilled water - 500 ml
30% aqueous ferric chloride - 62 ml

It is essential that the stain be made up in the following manner.

Place the distilled water in a large evaporating basin and heat until nearly boiling. Mix the dyes and dextrin and then dissolve in the hot water. Add the resorcin and bring the whole to the boil stirring continuously with a glass rod. It is important that the mixture be continuously boiling though not too vigorously. Continue to boil and stir for a minute or two to coarsen the precipitate. Cool, filter through a Buchners funnel and filter flask attached to a suction pump. Wash the deposit with distilled water until the runnings are colourless (8 - 10 litres). After drying overnight in the incubator the deposit is scraped off and dissolved in 550 ml of ethyl alcohol by gently simmering on a hotplate for about 50 minutes. A few drops of hydrochloric acid increase the stability of the dye. Cool, filter and add 20 ml of hydrochloric acid and allow to stand 24 - 48 hours before use.

The stain is diluted in equal parts with 1% acid alcohol. The sections are stained in this for 4 hours at room temperature having first been placed in 0.25% potassium permanganate for 5 minutes and 5% oxalic acid for 2 - 3 minutes and then washed in running water. Differentiation is in acid alcohol.

Martius Yellow, Brilliant Crystal Scarlet and Aniline Blue

(Lendrum et al, 1962)

Celestin blue and Mayer's haemalum

0.5% Martius yellow in 2% phosphotungstic acid (made in 95% alcohol)

Brilliant crystal scarlet (1% aqueous)

Phosphotungstic acid (1% aqueous)

0.5% Aniline blue in 1% acetic acid

Sections are brought to water. The nuclei are stained by celestin blue for 5 minutes followed by Mayer's haemalum for 5 minutes. Differentiation is in acid alcohol. After rinsing in distilled water the sections are stained in Martius yellow for two minutes and rinsed in tap water. They are then stained in brilliant crystal scarlet for ten minutes and mordanted in 1% phosphotungstic acid for five minutes. This is followed by counterstaining with aniline blue for ten minutes then dehydration, clearing and mounting.

Lendrum's Acid Picro-Mallory (Culling, 1957)

Celestin blue

Mayer's haemalum

Picro-Orange - Dissolve 0.2 grm of Orange G in 100 ml of 80% alcohol which has been saturated with picric acid

Acid fuchsin - 1% in 3% trichloracetic acid

Sections are brought to water and then stained with celestin blue for 3 - 5 minutes. Then rinsed in tap water and stained in Mayer's haemalum for 5 minutes. The sections are washed in tap water and then 95% alcohol. Then they are stained in picro-orange for 2 minutes and acid fuchsin for 5 minutes and rinsed in water. They are dipped into equal parts of picro-orange and 80% alcohol and then differentiated in 1% phosphotungstic acid for 5 - 10 minutes and rinsed in water. Finally they are counterstained with aniline blue (2% in 2% acetic acid) for 2 - 10 minutes and dehydrated, cleared and mounted.

Masson's Trichrome (Culling, 1957)

The tissue should be fixed in Bouin's or Zenker's fluid, formol-fixed sections may be mordanted in Zenker's fluid for 30 minutes.

Sections are brought to water and then the mercury pigment removed with iodine-sodium thiosulphate if necessary. After nuclear staining the sections are washed in water until blue. They are then stained in 1% ponceau-fuchsin in 1% acetic acid for 5 minutes and then rinsed rapidly in distilled water. Sections are mordanted in 1% aqueous phosphomolybdic acid for 5 minutes. The slide is drained and then counterstained with aniline blue or light green for 5 minutes. Differentiation is in 1% acetic acid followed by dehydration, clearing and mounting.

APPENDIX B

Case Histories

Case T.F.1 (S.W.)

A girl, born after 41 weeks gestation during which the mother had high blood pressure. The birth weight was 7 lb 9 oz. Onset of respiration was difficult and the child was blue from birth, slow with feeding though her weight gain was satisfactory. On transfer to the Brompton Hospital at three weeks cyanosis was general but more severe on crying. There was moderate tachypnoea, a quiet heart with a grade 2/6 ejection murmur and normal peripheral pulses: the liver was not enlarged.

An electrocardiogram showed an axis of $+90^{\circ}$ and an adult R/S pattern, which in a child might suggest left ventricular hypertrophy.

Cardiac catheterisation and angiocardiography led to the diagnosis of tetralogy of Fallot: the child was discharged but it was planned to operate at a later date. At the age of one year she was re-admitted to Hospital, distressed and with evidence of congestive heart failure - cyanosis, dyspnoea, tachycardia and an enlarged liver. Within 12 hours she became unconscious with evidence of cerebral oedema and deteriorating cardiac function. She died the next day.

X-ray Report

A chest radiograph at four weeks showed the diaphragm level was normal. Details of the main pulmonary artery and heart were unsatisfactory. The hilar vessels and intra-pulmonary

vessels were all small. The veins were normal and there were no other abnormal features.

Angio-cardiographic Report

Injection through the right ventricle filled the pulmonary artery though there was infundibular pulmonary valve stenosis. The main pulmonary hilar vessels and intra-pulmonary vessels were small but present in normal numbers. A ventricular septal defect led to filling of the left ventricle and aorta. No bronchial arteries were visible. The veins were normal in appearance and the speed of flow through the lungs was normal. The diagnosis made was of tetralogy of Fallot.

Catheter Report

There was systemic arterial desaturation (82%). The right ventricular pressure was at systemic level, the pulmonary artery pressure was not measured. The pulmonary to systemic flow was 0.7:1 with the percentage of venous blood in the systemic arteries being 36%. The flow through the body was 2 litres/minute. These results were consistent with the diagnosis of tetralogy of Fallot.

Post-mortem Report

The child died at one year and one month. There was a large thrombus in the right carotid artery extending around the circle of Wilis. Examination of the heart confirmed the diagnosis of tetralogy of Fallot.

Case T.F.2 (J.R.)

This girl was delivered by Ceasarian section at 40 weeks gestation and weighed 7 lb 14 oz. Cyanosis was first noted at five months and dyspnoea on exertion at 11 months. Angiography at 19 months revealed a small pulmonary artery and severe infundibular stenosis. A year later the outflow tract of the pulmonary artery was dilated with an apparent increase in pulmonary artery blood flow. Symptomatically there appeared to be only slight improvement. One year later when the child was centrally cyanosed and had clubbing of the fingers and toes, angiography showed some increase in the size of the outflow tract of the right ventricle but this was mainly due to a bulge on the left and superior aspect of the infundibulum. The filling of the lungs had been considerably improved. Total correction was recommended and this was performed at $3\frac{3}{4}$ years of age. There was a high, large ventricular defect associated with small main, left and right pulmonary arteries. The pulmonary valve was represented by a fibrous diaphragm and small hole. The ventricular septal defect was repaired and the main pulmonary artery enlarged by a gusset. Two days post-operatively she was taken out of the ventilator but one hour later sudden respiratory distress led to cardiac arrest. Open cardiac massage, tracheostomy and bronchoscopy were performed but she failed to improve and died the following day after another cardiac arrest.

X-ray Report

The chest radiograph at three years showed that the diaphragm level was normal. The heart was of normal size with a small pulmonary artery and a right-sided aorta. The hilar and

intrapulmonary arteries were small as were the veins. There were no other abnormalities in the chest X-ray. The manubrium sterni was in two pieces.

Angio-cardiographic Report

Injection was made via the right ventricle. The outflow tract was small. The right major arteries were smaller than the left at corresponding levels and all were too small. There was no collateral filling. The flow through the lung was gross, this may have been because of the dilatation made earlier.

Catheter Report

There was systemic arterial desaturation (72%). The right ventricular pressure was at systemic levels. The pulmonary artery pressure was not determined. The pulmonary to systemic flow was 0.61:1 giving a right to left shunt through the ventricular septal defect of 40%. The flow through the lungs was not estimated.

Post-mortem Report

The heart showed the features of a tetralogy of Fallot.

Case T.F.3 (A.M.)

This girl was born, weighing 5 lb 10 oz after an induced labour at 38 weeks gestation. From birth she gained in weight slowly. She was pink from birth but had recurrent colds. At nine months she had rubella and cyanosis was recognised for the first time. From the age of three years she squatted especially after activity. At four years after four episodes of cyanotic attacks she was admitted to hospital. She was cyanosed at rest

and had moderate clubbing of fingers and toes. There was slight dyspnoea at rest. The pulses were normal with quiet heart with a grade 3/6 ejection murmur. The liver was palpable 1 - 2 cm below the costal margin.

The electrocardiogram showed an axis of $+150^{\circ}$ and the newborn pattern. Cardiac catheterisation showed a state typical of tetralogy of Fallot with a mainly valvar stenosis. A closed pulmonary valvotomy was performed. Two days after operation she had a further cyanotic attack and was resuscitated with difficulty. She was kept on a ventilator for six days, under hypothermia. When she was warmed she went into complete heart block from which she did not recover.

X-ray Report

The radiograph at four years showed that the diaphragm level was normal. The heart shape and size were normal for age. The main pulmonary artery and the hilar and intrapulmonary vessels were all small.

Angio-cardiographic Report

Injection was made via the right ventricle. The pulmonary artery and intra-pulmonary arteries were hypoplastic. There was a ventricular septal defect and medium passed into a large aorta. There was infundibular and valvar stenosis of the pulmonary artery. There was a right broncho-pulmonary anastomosis to the lower lobe from the aorta and one to the posterior segment of the right upper lobe or the apical lower lobe from an internal mammary artery. There was slow filling and emptying of the lung. This was a low pressure, slow flow, pulmonary circulation.

Catheter Report

There was severe arterial desaturation (64%). The right ventricular pressure was at systemic levels. The pulmonary artery pressure was recorded. The ratio of pulmonary to systemic blood flow was 0.26:1. There was therefore a right to left shunt. Sixty nine per cent of the venous blood was in the systemic arteries. The flow through the lungs was 1.7 litres/minute.

Post-mortem Report

The child died aged four years. The lungs were normal anatomically but congested and oedematous. The liver was congested with fatty change and the spleen and kidneys were also congested.

The right ventricle of the heart was reported to be hypertrophied. The foramen ovale was not closed. The mitral valve was normal. There was a large ventricular defect with an overriding aorta. The pulmonary valve was tricuspid. The outflow tract was obstructed by a cylindrical bundle of muscle about 0.75 cm in diameter which crossed it. The diagnosis and cause of death was tetralogy of Fallot.

Case T.F.4 (P.H.)

A heart murmur was first heard in this boy at the age of four. There was cyanosis and shortness of breath on running with occasional squatting. There was little deterioration in the next two years. On examination at five and three quarters there was mild cyanosis at rest with possibly early clubbing of the fingers

and toes. All peripheral pulses were normal. A long systolic ejection murmur with a single second sound could be heard. The electrocardiogram showed an axis of $+170^{o}$ and a tracing indicative of moderately severe right ventricular hypertrophy. Using X-ray and angiographic and catheter studies a diagnosis of tetralogy of Fallot was made. Five months later the child was admitted for operation. The ventricular septal defect was closed and an infundibular stenosis with fibrous trabeculae across the outflow was excised. Immediately after the operation he went into heart block which was corrected. Two hours later he suddenly became hypertensive and had bradycardia and heart block. His heart stopped and did not respond to resuscitation.

X-ray Report

The chest radiograph at six years showed the diaphragm level to be normal, the heart was minimally enlarged. The blood vessels in the hilar and proximal lung fields were too small. There were no abnormal vessel shadows.

Angio-cardiographic Report

Injection was made through the right ventricle which was anterior and coarsely trabeculated. The pulmonary valve was thickened and showed pulmonary valve stenosis. The contrast medium passed through a ventricular septal defect to fill the left ventricle and normal sized aorta. The pulmonary artery and the intrapulmonary vessels were normal in diameter. There was an angiographic appearance of tetralogy of Fallot with valvular but no infundibular stenosis.

Catheter Report

The right ventricular pressure was at systemic level. The pulmonary artery was not entered. There was a systemic arterial saturation of only 85%. The ratio of pulmonary to systemic flow was 0.6:1 with a total pulmonary blood flow of 3.1 litres/minute. There was a right to left shunt of 42%.

Post-mortem Report

The kidneys, spleen, liver and lungs were congested but other organs were normal. The heart was enlarged by hypertrophy of the right ventricle. The pulmonary artery was about half the diameter of the aorta. The mitral, aortic and tricuspid valves were normal. The ventricular septal defect had been closed by a Teflon patch and the pulmonary outflow tract had been corrected by resection of infundibular muscle. The cause of death was tetralogy of Fallot and the operation for correction of the defect.

Case T.F.5 (G.D.)

This boy was known to have heart disease from birth. When he was five years old a Blalock operation was carried out in India and his condition improved. On examination at eight years he was centrally cyanosed with clubbing of fingers and toes. Pulses were normal but there was a continuous murmur and a single second heart sound. An electrocardiogram showed right axis deviation and right ventricular hypertrophy. Cardiac catheterisation showed the appearance of severe Fallot's tetralogy with a functioning Blalock anastomosis. He was discharged and then readmitted seven months later for operation. The surgeon found a ventricular septal defect 2.5 by 1.5 cm. There was a tight infundibular

stenosis which was resected. Four hours after operation there was a decrease in cardiac output. Later there was cardiac arrest from which he recovered after resuscitation but after a further arrest three hours later he died.

X-ray Report

The chest radiograph at eight years showed that the diaphragm level was normal and the heart was normal in size but boot shaped. The aorta was enlarged, the pulmonary artery was small. The intra-pulmonary vessels were small to normal in diameter in the left lung and small in the right. The veins were normal, there were no collateral vessels visible. There were no abnormal shadows.

Angio-cardiographic Report

Injection was made through the right ventricle. There was an intra-cristal ventricular septal defect with an overriding aorta. The pulmonary artery was less than 50% of the size of the aorta. The intrapulmonary veins were normal in size and the speed of filling and emptying was normal. It was not certain if the pulmonary artery filled from the functioning Blalock anastomosis or through the pulmonary valve. The right basal artery was larger than the left and the mid lung vessels of the right lung were small. There was little contrast medium in the left lung because the vessels had silted up as a result of the Blalock anastomosis.

Catheter Report

The right ventricular pressure was at systemic level, the pulmonary artery pressure was 17/11. There was no arterial desaturation, the oxygen saturation in the descending aorta being

97%. There was no significant shunt and the total cardiac output was 5.8 litres/minute.

Post-mortem Report

The lung, liver, spleen and kidneys were congested. The heart was enlarged by hypertrophy of the right ventricle. The aorta was right sided. The pulmonary artery was reduced in size and appeared to be thin walled. The subclavian pulmonary artery anastomosis was identified. The foramen ovale was not closed. The tricuspid, mitral and aortic valves were normal. Muscle had been resected from the infundibulum giving a good pulmonary outflow tract. There was a large ventricular septal defect. Other organs of the body were normal.

Case T.F.6 (J.J.)

This boy was born after a gestation of 39 weeks and weighed 8 lb 15 oz. From one month he was cyanosed when tired. A heart murmur was detected at six months when cyanosis was slight but always present and his activity was restricted. At age $3\frac{3}{4}$ years cardiac catheterisation was performed and a diagnosis of tetralogy of Fallot with severe infundibular stenosis confirmed. Nine months later he was readmitted and a 'total correction' was planned. At operation the appearances were those of a severely stenosed valve ring. The ventricular septal defect was high and about two centimetres in diameter. A complete correction was performed. A few hours after operation there was increasing bleeding from the chest and then a cardiac arrest. He was resuscitated, but then slowly deteriorated and died the following day.

X-ray Report

The appearance of lung and heart were within normal limits although the main pulmonary arteries were not well shown.

Angio-cardiographic Report

Injection through the right ventricle led to opacification of the aorta and the pulmonary artery, the former due to a ventricular septal defect. There was severe infundibular pulmonary stenosis and the pulmonary artery was about half the size of the aorta.

Catheter Report

The pressure in the right ventricle was at systemic level but the pressure in the pulmonary artery was 12/5. The systemic arterial saturation of oxygen was only 54%. There was a right to left shunt of 83%, with a pulmonary to systemic flow ratio of 0.2:1 and a total pulmonary blood flow of 1.8 litres/minute.

Post-mortem Report

The brain, lungs, mucosa of the stomach, liver and spleen were all congested. The heart was enlarged by hypertrophy of the right ventricle with some dilatation of the right atrium. The pulmonary outflow tract would now admit one finger. The tricuspid, mitral and aortic valves were all normal. The ventricular septal defect had been repaired by a Teflon patch.

Case V.S.1 (W.T.)

A boy born, at term, after a normal pregnancy with a birth weight of 6 lb 4 oz. During the first hour of life his skin became dusky and cold; he was then placed in an incubator with added oxygen. He continued to be cyanosed and was treated with Digoxin and diuretics which led to some improvement. At two weeks he was still cyanosed at rest and showed slight jaundice. There was no oedema and the peripheral pulses were of good volume. There was a grade 1/6 short systolic murmur. Breathing was shallow and rapod (40 per minute). The liver was palpable by half a centimetre below the costal margin. The electrocardiogram showed an indeterminate axis.

The baby's condition remained poor - he was dyspnoeic, tachypnoeic and there was peripheral desaturation with congestive heart failure. After cardiac catheterisation and angiocardiography the tentative diagnosis of pulmonary stenosis (Taussig Bing complex) was made but at operation there was no evidence of stenosis and post-operatively the baby became cyanosed and died after two incidents of cardiac arrest.

X-ray Report

A chest radiograph of this case showed the diaphragm level was normal and the heart small with a normal aorta. There were no abnormal vessel shadows or rib notching and the intrapulmonary arteries and veins were normal in size and distribution. There was evidence of diffuse pulmonary oedema.

Angio-cardiographic Report

Injection of the circulation was made from a catheter in the right ventricle: both the aorta and pulmonary artery filled and a ventricular septal defect was demonstrated. The aorta, pulmonary artery and the intrapulmonary arteries and veins were all small and there was no collateral circulation. The lung transit time was unduly long. In the posterior-anterior angiogram film the pulmonary valve could be seen to be abnormal and was probably too small. The infundibulum however did not stay narrowed throughout the run of the angiogram.

Catheter Report

There was severe systemic desaturation (oxygen saturation 37 - 49%). Right ventricular pressure was at systemic level and the pulmonary artery pressure 60/5. The pulmonary to systemic flow ratio was 0.3:1 with the percentage of venous blood in the systemic arteries being 76% and the percentage of systemic arterial blood in the pulmonary arteries being 24%. There was a total systemic flow of 7 litres/minute. Functionally this represents a single ventricle with a high resistance in the pulmonary circuit, so that three times as much blood goes to the body as to the lungs. The blood in the pulmonary artery has the same composition as that in the aorta. There is severe pulmonary hypertension.

Post-mortem Report

The child died aged three weeks. There was complete absence of the spleen. The rest of the body was normal save that the heart showed a large ventricular septal defect with hypertrophy

of the right ventricle: the aorta was right sided but the origin of both aorta and pulmonary artery was normal. There was no pulmonary stenosis.

V.S.2 (M.R.)

This boy was born at 36 weeks gestation after a normal pregnancy, with a birth weight of 6 lb 5 oz. There was no difficulty at the onset of respiration but he fed poorly and required tube feeding. He was mildly jaundiced from the second day. After one week he was found to have a heart murmur and two days later was in heart failure with tachypnoea, tachycardia, crepitations at his lung bases and a liver palpable 3 - 4 cm below the right costal margin: there was no cyanosis and the peripheral pulses were probably normal. A grade 4/6 pansystolic murmur was heard and there was a bilateral chest bulge. The electrocardiogram showed a right ventricular preponderance with right axis deviation and a 'newborn' R/S pattern. Banding of the pulmonary artery was attempted at 10 days but the heart became asystolic and resuscitative measures were unsuccessful.

X-ray Report

The diaphragm was low but not flat and the heart enlarged overall. The pulmonary artery, its intrapulmonary branches and the veins were increased in size. There were no abnormal vessel shadows. On the left side an extra rib was present. The sixth rib was too small on both sides and the eighth deformed on the right. The lower half of the sternum was prominent and the manubrium more horizontal than normal.

Angio-cardiographic Report

Injection was made into the left ventricle which was enlarged. The medium entered the small posterior pulmonary artery and the large anterior aorta almost simultaneously since there was a fairly low ventricular septal defect. The main pulmonary artery and the hilar arteries were dilated. The intrapulmonary vessels were of normal number with a slight increase in size. There was no collateral circulation. There was intramyocardial injection of the contrast medium in the left ventricle. The anatomical deformity was a ventricular septal defect.

Catheter Report

The pulmonary artery pressure was at systemic level (84/38). The blood in the pulmonary artery and aorta was of the same saturation with systemic desaturation (78%). This is indicative of a complete mixing situation which was not so on the angiogram. The desaturation may have been a reflection of pulmonary venous desaturation consequent upon under ventilation. There may have been mixing via a patent ductus arteriosus, however this was not seen on the angiogram.

The ratio of pulmonary to systemic flow was 1.4:1, an overall left to right shunt - 58% of the systemic blood passing into the pulmonary circulation and 42% of the pulmonary blood in the systemic circulation.

Post-mortem Report

The child died aged two weeks. The liver, spleen and lungs were congested. All other organs normal. The heart was enlarged by hypertrophy of the right ventricle. The pulmonary artery

was twice the diameter of the aorta. In the lower path of the interventricular septum was a 3 mm defect. There was a large atrial septal defect (foramen ovale type) about three millimetres in diameter. The ductus arteriosus was patent, the heart valves and aorta were normal.

Case V.S.3 (L.F.)

A girl born at term after a normal pregnancy with a birth weight of 8 lb 10 oz. She was quite well until the age of three weeks when it was noted that she was dyspnoeic particularly when feeding. She was found to have dextrocardia with a conduction defect and cardiac failure. On admission to Brompton Hospital she had central cyanosis, a breathing rate of 80 per minute and a bilateral chest bulge. There was a grade 4/6 pansystolic murmur accompanied by a thrill. The liver was palpable 3 - 4 cm below the costal margin.

An electrocardiogram showed an axis of $+60^{\circ}$ with biventricular hypertrophy. After catheterisation which showed an increase in flow to the lungs, banding of the pulmonary artery was performed at 18 days of age. This reduced the pulmonary artery pressure but she could not be removed from the respirator because of severe respiratory distress. She developed signs of vena caval thrombosis and, following progressive bradycardia and hypotension, died.

X-ray Report

The diaphragm level was normal. There was dextrocardia without situs inversus the heart being of normal shape but increased

in size. The intrapulmonary vessels were tortuous and dilated at the hilum. There were no abnormal vessel shadows. There was pulmonary oedema.

Angio-cardiographic Report

Catheter injection into the anterior ventricle made via the aortic valve showed course traleculation of a right ventricle: this communicated with an anterior aorta. The main pulmonary artery did not fill, although the intrapulmonary vessels were slightly opacified and normal. They were probably filled via a ventricular septal defect or an atrial septal defect.

Catheter Report

There was a gross systemic to pulmonary shunt with a pulmonary flow 9.3 times that of the systemic being 32 litres/ minute. The shunt was probably through the atrial septal defect. Of the pulmonary flow 90% was from the systemic circuit. There was only a small right to left shunt and little pulmonary hypertension, the pressure in the right (pulmonary) ventricle being 45/7. Oxygen saturation in the systemic circulation was high (98%).

Post-mortem Report

The child died aged two months. The aorta was small, about one centimetre in diameter and arose from an anterior ventricle. The pulmonary artery was about three times the size of the aorta and lay immediately behind it.

The final diagnosis was of dextrocardia, atrial septal defect, ventricular septal defect and superior vena caval thrombosis.

Case V.S.4 (R.S.)

A boy born eight days post-mature after a normal pregnancy. The neonatal period was apparently normal. At about eight weeks rapid respirations were noted and he was found to be in heart failure. He had moderate cyanosis, his chest bulged bilaterally with subcostal retraction and tachypnoea. There was a grade 3-4/6 pansystolic murmur. The liver was palpable three centimetres below the costal margin. The electrocardiogram showed right atrial P waves with biventricular hypertrophy.

After cardiac catheterisation the baby was transfused and later developed staphylococcal infection. He gradually deteriorated and died at three months.

X-ray Report

The diaphragm level was normal, the heart was slightly enlarged but of normal shape. There were small hilar and lung vessels but no lung plethora.

Angio-cardiographic Report

Injection was made into the right ventricle. Over half of the filling medium entered the aorta via a large ventricular septal defect two centimetres in diameter. The main pulmonary artery and hilar vessels were enlarged. The intrapulmonary vessels, both arteries and veins, were small. The flow through the lungs was slow and there was poor peripheral filling, features suggesting a high resistance. The ductus arteriosus was patent but small.

Catheter Report

The oxygen saturations and pressure records were open to considerable doubt in this case because it seemed that the equipment was faulty during the investigation. It appeared that there was very severe pulmonary hypertension, the ventricular and pulmonary artery pressures being above normal systemic level (75/40). There was a net right to left shunt so that the ratio of pulmonary to systemic flow was 0.2:1. There was a very low flow in the lungs (4 litres/minute) with considerable mixing at atrial level and a left to right shunt. A right to left shunt took place at the level of a large ventricular septal defect. The findings suggested a common ventricle or a large ventricular septal defect with a patent ductus arteriosus. There was severe systemic desaturation (45%).

Post-mortem Report

The brain, liver, spleen and kidneys were all congested. The lungs showed normal lobation with consolidation of the right middle lobe and of the posterior segment of the right upper lobe. The heart was enlarged by hypertrophy of the ventricles, the right probably being larger than the left. There was an atrial septal defect about three millimetres in diameter and a large ventricular septal defect. The ductus arteriosus was large and patent. Pulmonary venous drainage and the valves were normal. All other organs were normal.

Case V.S.5 (M.K.)

This girl had a birth weight of 7 lb 5 oz after a normal pregnancy. There was no difficulty in onset of breathing and

the child was essentially normal for the first six weeks of life. At this time she had a chest infection and possible heart failure. On admission she was not obviously cyanosed but was tachypnoeic, with intercostal recession and grade 3-4/6 pansystolic murmur. The liver was palpable 2 - 3 cm below the costal margin. Catheter studies suggested a diagnosis of common ventricle, atrial septal defect and patent ductus arteriosus. She was treated with Digoxin and Lasix was given as required. Her condition gradually deteriorated and she died at two months of age.

X-ray Report

The diaphragm level was normal. The heart had an enlarged ventricle with the left upper border greatly enlarged. The main pulmonary arteries, the hilar vessels and intrapulmonary arteries were all increased in size and tortuous. The veins could not be seen. There were no abnormal vessel shadows.

Angio-cardiographic Report

Injection was made through the right ventricle. The right and left pulmonary arteries were dilated and intrapulmonary vessels increased in size. There was only feeble filling of the veins which also were increased in size. The aorta filled but the site of mixing could not be determined.

Catheter Report

There was systemic desaturation (70 - 76%) and severe pulmonary hypertension, the right ventricular pressure being at systemic level (55/15). There was an almost equal pulmonary and systemic

flow, with almost equal left-to-right and right-to-left shunts. The former occurred mainly through the atrial septal defect but the site of the latter was not certain. The total pulmonary flow was 4 litres/minute.

Post-mortem Report

The brain, liver and kidneys were congested. The lungs were anatomically normal but showed areas of consolidation. The heart was enlarged by hypertrophy and dilatation of the right atrium and both ventricles. The pulmonary artery arose anteriorly and was approximately three times the diameter of the aorta. The ductus arteriosus was patent and there was a large atrial defect of secundum type. There was a high intraventricular septal defect, approximately 0.5 cm diameter. Other organs were normal.

Case V.S.6 (A.F.)

The delivery of this baby was normal and at full term: he weighed 6 lb 6 oz. Cardiac failure was present from the age of eight days. This failed to respond to treatment and he developed skin infection and oral thrush. On admission to Brompton Hospital there was generalised systemic vaso-constriction, tachypnoea and marked rib recession. There was a continuous murmur heard throughout the chest. The liver was palpable 4.5 cm below the costal margin and the spleen 2 cm below. The electrocardiogram showed an axis of -150°. This indicated right ventricular hypertrophy with possible left ventricular hypertrophy. During cardiac catheterisation there were three episodes of ventricular fibrillation, from the last of which he failed to recover.

X-ray Report

The diaphragm level was normal. The heart and pulmonary artery were enlarged, while the intrapulmonary arteries were slightly enlarged. There were no abnormal vessel shadows and no other abnormal shadows. The radiograph was suggestive of a case with hypertension and a patent ductus arteriosus.

Angio-cardiographic Report

Injection was made via the aorta which was normal in size. The pulmonary artery filled via a large ductus arteriosus and the hilar arteries and intrapulmonary arteries were enlarged with considerable plethora. There was no collateral circulation.

Catheter Report

No catheter studies were performed.

Post-mortem Report

The child died aged three weeks. The lungs were deeply congested and oedematous and the liver and spleen were congested. The heart was enlarged by hypertrophy and dilatation of the right ventricle. All valves were anatomically normal. The foramen ovale was widely patent, the ductus arteriosus was 3 mm in diameter and widely patent. Other organs were normal. The diagnosis was patent ductus arteriosus.

APPENDIX C

The laboratory numbers for the cases used in the present study are listed below with their age.

Fetal Lungs - Arteries Injected

Age	Bx Number
12 weeks	2108
14 weeks	2285
16 weeks	2603
17 weeks	2084
18 weeks	2109
19 weeks	2074
20 weeks	2479
20 weeks	2310
23 weeks	2150
26 weeks	2526
26 weeks	2506
28 weeks	2423
28 weeks	2437
32 weeks	2505
33 weeks	2204
36 weeks	2429
38 weeks	2195
40 weeks	2458

Fetal Lungs - Veins Injected

Age	Bx Number
20 weeks	2583
28 weeks	2508
28 weeks	2538
34 weeks	2537
39 weeks	2582

Child Lungs - Arteries Injected

Age	Bx Number
3 days	2666
4 months	2061
10 months	2655
18 months	2639
3 years	2656
4 years	2600
5 years	2058
10 years	2657
11 years	2103

Child Lungs - Veins Injected

Age	Bx Number
3 years	2687
10 years	2657

Lungs from Cases with Congenital Heart Disease

Case	Bx Number	Case	Bx Number
T.F.1	2554	V.S.1	2527
T.F.2	2555	V.S.2	2526
T.F.3	2484	V.S.3	2524
T.F.4	2553	V.S.4	2525
T.F.5	2552	V.S.5	2528
T.F.6	2706	V.S.6	2626

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