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Thinning of fetal pulmonary arterial wall and postnatal remodelling: ultrastructural studies on the respiratory unit arteries of the pig

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Summary. Adaptation to extra-uterine life and postnatal remodelling of intra-acinar arteries was followed in 34 Large White pigs, from birth to adult life, applying morphometry to light and electronmicroscopic studies. After birth, percentage wall thickness decreased rapidly due to a reduction in overlap of adjacent smooth muscle cells and an increase in smooth muscle cell surface area/volume ratio, ($p < 0.01$ at 12 h), without a reduction in the volume density of smooth muscle cells. Smooth muscle cells appeared immature at birth and synthetic rather than contractile organelles predominated. Between 3 weeks and 6 months myofilament volume density doubled ($p < 0.0001$). At all ages, pericytes, intermediate and smooth muscle cells showed similar volume densities of contractile and synthetic organelles. Thus, the high fetal pulmonary vascular resistance appeared to be due to the shape and arrangement of smooth muscle and other contractile cells within the vessel wall, rather than an excessive contractility of these cells. After birth rapid remodelling of arterial wall structure achieved a reduction in wall thickness by 30 min, continuing during the first week of life. After 3 weeks, remodelling involved an increase in wall thickness, connective tissue deposition with more collagen than elastin ($p < 0.0001$), and smooth muscle cell differentiation.

Key words: Pulmonary circulation – Vascular smooth muscle – Neonatal adaptation

Introduction

In the normal human infant pulmonary vascular resistance falls rapidly at birth as the intrapulmonary arteries dilate and pulmonary arterial smooth muscle mass is reduced (Naeye et al. 1961). The ultrastructural basis of these observations must be studied in experimental animals. We chose to study the pig lung because in pig and man the pulmonary arterial pressure and vascular resistance fall at a similar rate during the first two weeks of life, and at the same time the peripheral pulmonary arteries show a similar reduction in medial thickness (Haworth and Hislop 1981; Haworth and Hislop 1983).

In the present study the ultrastructural changes occurring in the respiratory unit arteries have been investigated in the pulmonary circulation of 34 pigs, 31 of which were aged three weeks or less.

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Materials and methods

Preparation of lung tissue for light and electron microscopic studies. Pulmonary vascular structure was studied in the lungs of 34 Large White pigs (Table 1). The animals in any age group

Table 1. Ages of animals studied

Age	Number of animals
Stillborn	2
1 min	1
5 min	1
30 min	2
2.5 h	2
4.5 h	2
8 h	2
12 h	3
1 day	2
4 days	3
1 week	3
1.5 weeks	4
2 weeks	1
2.5 weeks	2
3 weeks	1
6 months	3
Total	34

came from at least two different litters. The male:female sex ratio was 1.3:1. Immediately after killing the animal, while the heart was still beating, the right ventricle and trachea were cannulated and perfused simultaneously with glutaraldehyde fixative (2.5% glutaraldehyde in 0.1 M cacodylate buffer), the pulmonary artery at a pressure of 100 cm water, and the trachea at a pressure of 30 cm water (Hall and Haworth 1986). The stillborn animals were fresh stillbirths, were not runts, and their lungs were injected in the same manner as those of the animals which were killed. In each animal 30 blocks of tissue were taken from the right lower lobe. In order to ensure systematic sampling of all regions, the lobe was cut horizontally at one and at two thirds of the distance between the hilum and pleura, slices of tissue 2 mm thick were removed and 10 small blocks (2 mm^3) were taken from each slice. In addition, 10 blocks of similar size were taken from the lung tissue distal to the 2 slices.

After osmication, uranyl acetate post fixation and dehydration the tissue was embedded in thin sheets of Epon. One micron thick sections were cut and stained with Toluidine blue. Arteries were identified by their apparent structural characteristics (muscular, partially or non-muscular) and with respect to the type of airway which they were accompanying. Their wall thickness and external diameter was measured (see below). The same arteries were then photographed before thin sectioning proceeded. Thus, all arteries examined ultrastructurally were also studied by light microscopy. Arteries considered suitable for ultrastructural study were those which by light microscopy were circular and cut in transverse section, did not have crenulated elastic fibres and appeared to be fully distended. In each animal at least 6 blocks of tissue were examined ultrastructurally, 2 from each group of 10 blocks. Ultrathin sections were taken using a diamond knife, stained with lead citrate and examined using a Jeol 100CX electron microscope. Photographic plates were taken at a magnification suitable for identification of cell outlines (magnification $\times 5000$) and cytoplasmic components (magnification $\times 12500$), with prints having a final magnification of 10–25000.

Definitions of the type of arteries studied. Because arteries studied ultrastructurally were first identified by light microscopy a light microscopic classification of arterial wall structure has been used throughout – muscular, partially muscular and non-muscular. Arteries which appeared non muscular on light microscopy were, however, seen on electron microscopic examination to contain pericytes (Weibel 1974; Meyrick and Reid 1979). Intrapulmonary arteries were also classified according to the type of accompanying airway, as preacinar (proximal to the acinus or respiratory unit), terminal bronchiolar, alveolar duct or as alveolar wall arteries. In the pig lung the terminal bronchioli are longer and the respiratory bronchioli are correspondingly shorter than in man (Haworth and Hislop 1981). In the present study therefore, the terminal bronchiolar arteries are considered as part of the respiratory unit.

Quantitative Morphometric Techniques

Light Microscopic Studies. Determination of percentage arterial wall thickness, external diameter and structure of arteries accompanying each type of peripheral airway. In each of the 34 animals, using 1 micron sections, the external diameter and wall thickness was measured. Because the elastic laminae were difficult to discern in Toluidine stained thin sections, being thin and interrupted in the peripheral pulmonary arteries of these young animals, percentage arterial wall thickness rather than medial thickness was determined:

$$\% \text{ wall thickness} = \frac{\begin{array}{c}\text{Distance between luminal surface of endothelium and external surface of outermost smooth muscle cell (excluding adventitia)}\end{array}}{\text{External diameter}} \times 100$$

Arteries with a similar external diameter were grouped together and the mean % wall thickness of each size group was calculated. Also, arteries accompanying each type of airway were classified according to wall structure and the mean percentage arterial wall thickness and mean external diameter of each structural type was calculated. Having established that the findings were similar in animals of the same age, the data were pooled according to age: stillborn (2 animals), 1–30 min (4 animals), 2.5–4.5 h (4 animals), 8–12 h (5 animals), 1–4 days (5 animals), 1–3 weeks (11 animals) and >6 months (3 animals). In each age group, the mean number of arteries assessed at alveolar wall level was 25, at alveolar duct level was 26 and at terminal bronchiolar level was 8.6.

Ultrastructural Studies. When considering the appearance of an artery or cell, findings were noted only when consistently present in all electron micrographs of all animals of the same age.

Electromicrographs were analysed using the Reichert-Jung semi-automatic image analysing system MOP/AM03. The following assessments were made: 1) The surface area/volume ratio of the smooth muscle cells in terminal bronchiolar arteries and in small preacinar arteries was determined using a planimetric technique (Weibel 1969). In each animal a mean of 70 measurements was obtained from studies on 6–8 arteries. 2) The volume densities of various structures and organelles were also determined. (The volume density of a structure is the relative concentration of that structure within a unit volume of tissue or cell). The ultrastructural feature of the pericytes, intermediate cells (Meyrick and Reid 1979) and smooth muscle cells (cell membrane, nucleus (7 nm filaments previously identified at a magnification of $\times 35000$), myofilaments, Golgi cisternae, rough endoplasmic reticulum, mitochondria and matrix material) were determined using a Weibel multipurpose point counting grid with 1 cm point spacing, giving a total of 9875 measurements. 3) Similarly, the volume density of smooth muscle cells relative to connective tissue elements was determined in the media of the muscular arteries, giving 1448 measurements in all. 4) The volume densities of the connective tissue elements in the internal elastic lamina, media and external elastic lamina (amorphous elastin, microfibrillar elastin, collagen, basement membrane material and ground substance and debris) were also determined (6320 measurements).

The raw data from both light and electron microscopic studies plus magnification factors were stored in a database (SIR) and retrieval and statistical analysis was carried out using the SIR and SAS systems of the University of London Computer Centre. Specifically, the SAS programme PROC GLM was used for analysis of variance and co-variance (SAS 1982). Because of the variable number of animals in each age group, the analysis of co-variance was non-orthogonal and it was for this reason that the GLM programme was used.

Results

Light microscopic studies

Arteries accompanying all types of peripheral airways showed an abrupt reduction in wall thickness

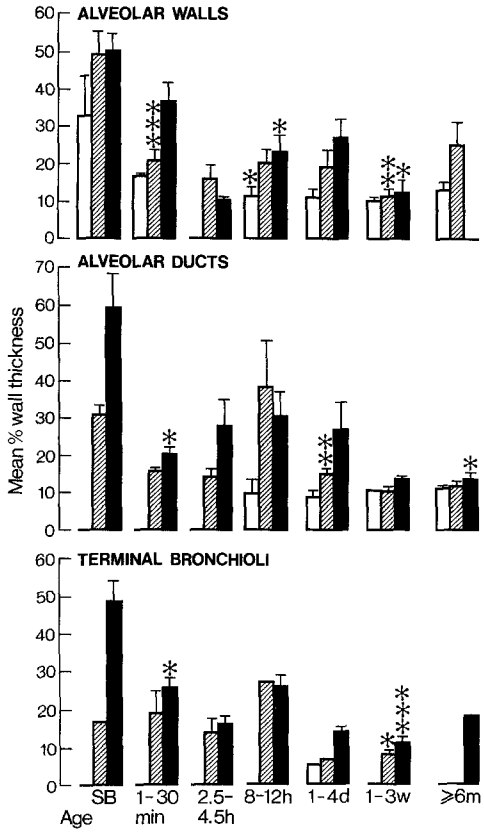

Fig. 1. Mean percentage arterial wall thickness in the non muscular (□), partially muscular (▨), and muscular (■) arteries in the alveolar walls and accompanying the alveolar ducts and terminal bronchioli, in animals of different ages. I – Standard error; asterisk(s) indicates significant reduction in wall thickness after birth and any further significant reduction: * $p<0.05$, ** $p<0.005$, *** $p<0.001$

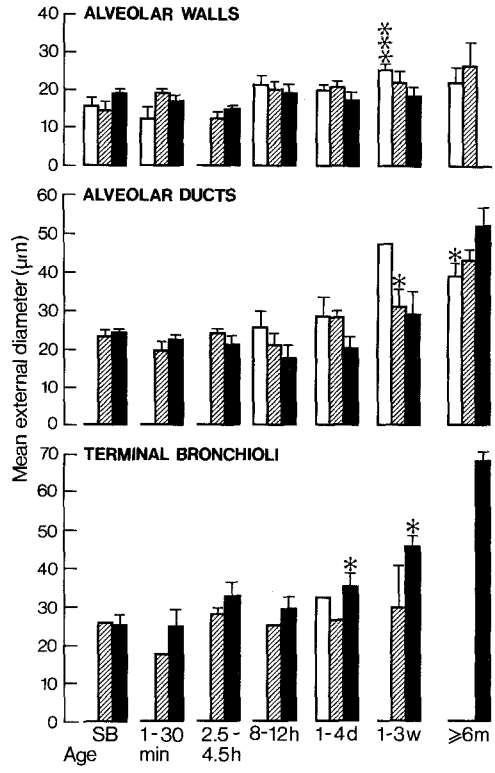

Fig. 2. Mean external diameter (μm) in non muscular (□), partially muscular (▨), and muscular (■) arteries in the alveolar walls, and accompanying the alveolar ducts and terminal bronchioli in animals of different ages. I SE; asterisk(s) indicates significant increase in external diameter after birth and any further significant increase, as in Fig. 1

during the first 30 min of life (Fig. 1). In arteries with an external diameter less than 50 μm, 70% of vessels had a percentage wall thickness >30% before birth and at 30 min 75% had a percentage wall thickness of 15–30%. Further wall thinning occurred more gradually during the first 21 days of life in arteries of all sizes. (In all arteries <1000 μm in diameter the relation between percentage wall thickness and external diameter was similar to that described in a previous light microscopic study (Haworth and Hislop 1981).

The external diameter of arteries accompanying alveolar walls and alveolar ducts did not increase significantly until after the first week of life (Fig. 2). By contrast, the muscular ateries accompanying the terminal bronchioli showed an increase in external diameter at 2.5–4.5 h, and were significantly larger than at birth by 1–4 days. They continued to increase in size during the first three weeks of life, and between 3 weeks and adulthood. The wall thickness of terminal bronchiolar and pre-acinar arteries also increased between 3 weeks and >6 months of age, but the number of smooth muscle cell layers composing the arterial wall did not. The mean wall thickness of small preacinar arteries (3–3.5 layers of smooth muscle cells) increased from 8.8 μm to 15.8 μm, that of arteries one generation proximal to the terminal bronchioli (two layers of cells) increased from 5.1 μm to 7.1 μm and that of terminal bronchiolar arteries (one layer of cells) increased from 2.0 μm to 4.1 μm.

Ultrastructural studies

1. Arrangement of pericytes, intermediate and smooth muscle cells in the media, their shape and relation to endothelial cells. The subendothelial cells were classified according to their relation to the overlying endothelial cell and its basement membrane. Pericytes lay within the basement membrane of the endothelial cell, intermediate cells shared the basement membrane of the endothelial cell, and smooth muscle cells lay within their own

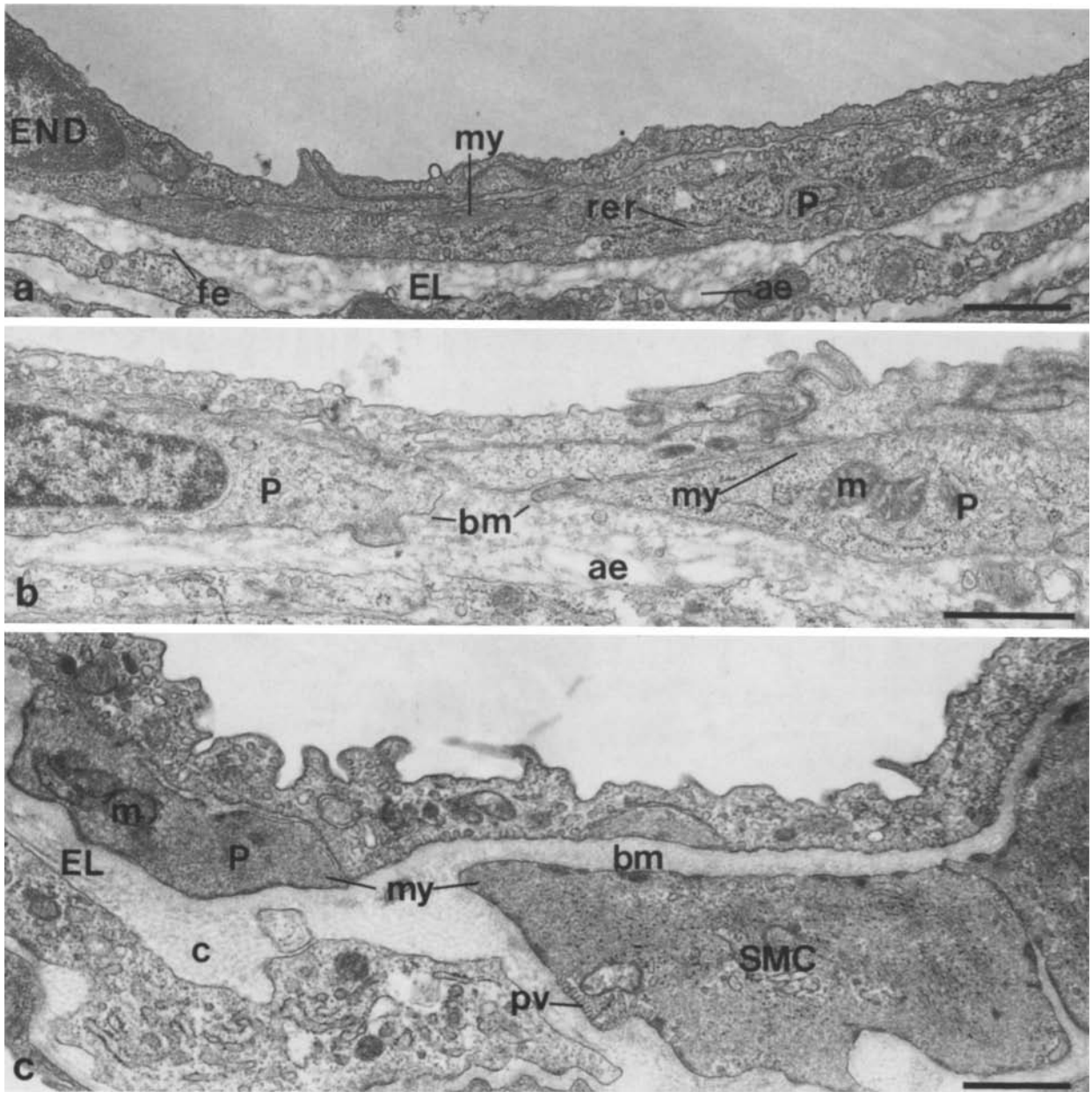

Fig. 3. Electron micrographs of transverse sections through non-muscular intra-acinar arteries, **a** 4.5 h old, **b** 3 days old, **c** adult. *Abbreviations:* ae = amorphous elastin; bm = basement membrane; c = collagen; EL = elastic lamina; END = endothelium; fe = microfibrillar elastin; m = mitochondria; my = myofilaments; P = pericyte; pv = plasmalemmal vesicle; rer = rough endoplasmic reticulum; SMC = smooth muscle cell. Scale – bar line = 1 μm

basement membrane (Weibel 1974; Meyrick and Reid 1979). This was the only criterion used since the appearance of the cells changed with age.

During the first day of life, the pericytes lying in the non-muscular arteries of the alveolar walls and accompanying the alveolar ducts had an elongated profile when cut in transverse section (Fig. 3a). The cell profiles became shorter with age (Figs. 3b, c), a change consistent with the pericytes having acquired a stellate shape, as reported in adult animals of other species (Weibel 1974). At all ages the abluminal surface of the endothelial cells was moulded around any associated pericyte giving a thin cell layer of uniform thickness (Fig. 3a).

After birth the smooth muscle cells of partially and completely muscularised vessels rapidly became thinner, longer and fusiform when viewed in transverse section (Fig. 4). Their surface/volume ratio increased after birth, particularly during the

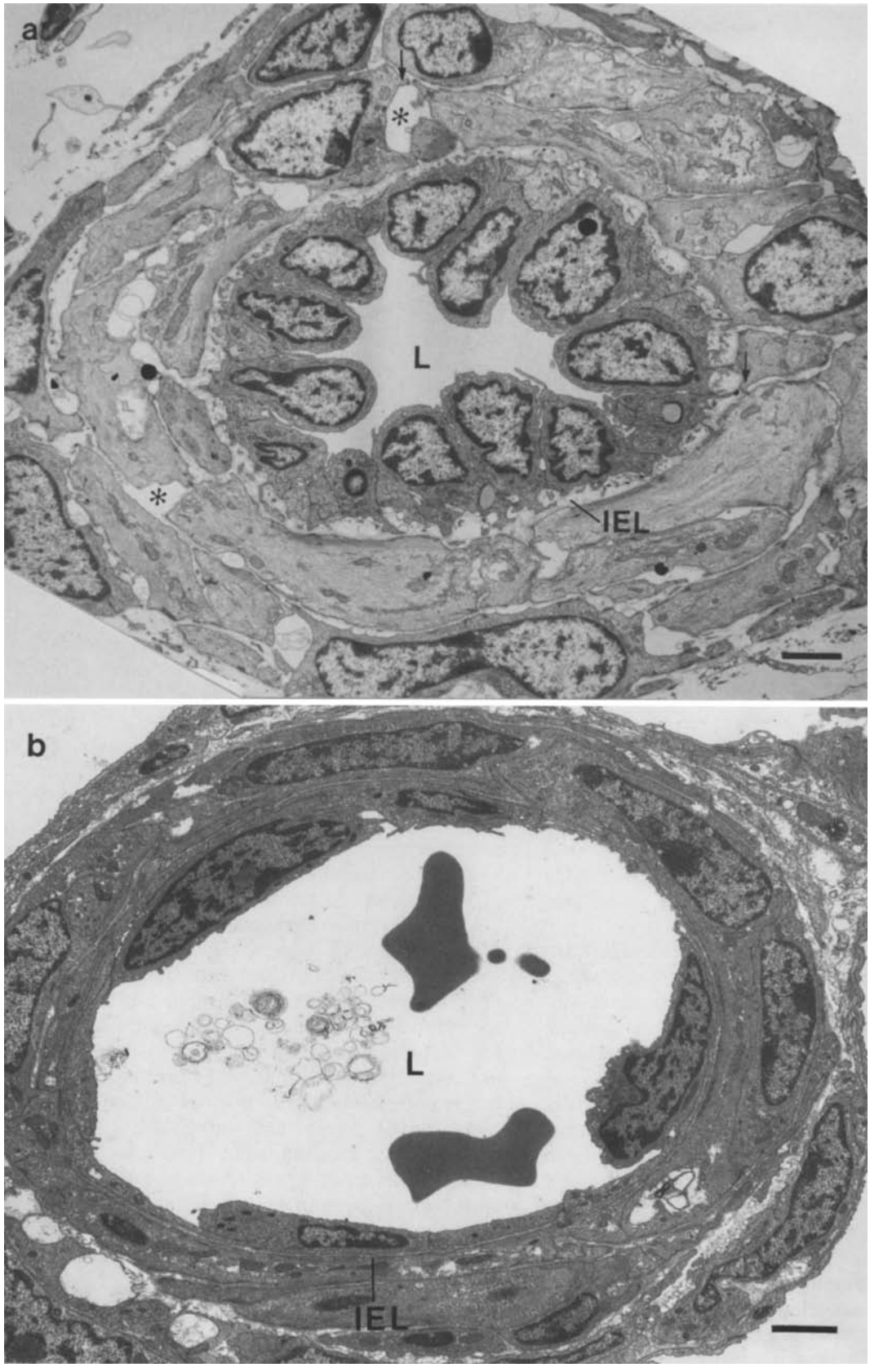

Fig. 4. Electron micrographs of transverse sections through small muscular arteries from animals. **a** stillborn, **b** aged 5 min taken at the same magnification. IEL = internal elastic lamina; L = lumen; *Abbreviations:* ↓ narrow process making contact with adjacent smooth muscle cells; * = "spaces". Scale – bar line = 2 μm

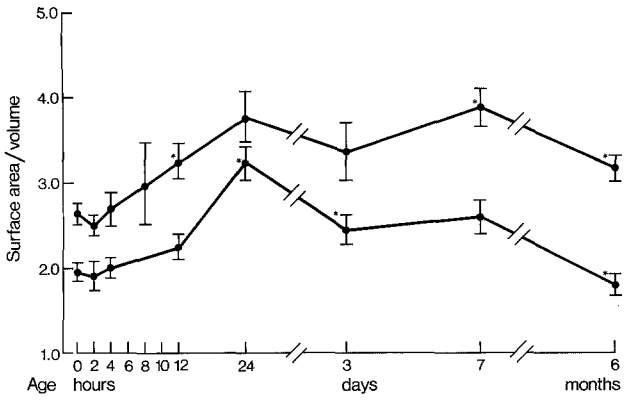

Fig. 5. Mean surface area/volume ratio of smooth muscle cells in terminal bronchiolar (*top line*) and small pre-acinar muscular arteries (*bottom line*), from birth to six months. * = significant difference from the newborn and then from each subsequent significantly different value ($p < 0.05$–0.005). I = SE

first 12 h when wall thickness also decreased most rapidly (Fig. 5). In the terminal bronchiolar arteries the smooth muscle cells formed a complete subendothelial cell sheet at birth and throughout life. During the first few hours of life the smooth muscle cells overlapped each other (Fig. 4). Less overlap was seen by 12 h (Fig. 6a) and during the first 24 h the smooth muscle cells became progressively more elongated and tapered having a brick-like appearance in longitudinal section (Fig. 6b), this appearance being retained throughout life. In the slightly larger muscular arteries containing two smooth muscle layers, the changes in shape and in surface area/volume ratio of the smooth muscle cells with age (Fig. 5) were similar to those seen in the terminal bronchiolar vessels.

Although the smooth muscle cells became thinner after birth, their volume density did not decrease relative to that of the connective tissue elements. At all ages the volume density of smooth muscle cells was 96% (SD 1.6) in arteries composed of one layer of smooth muscle cells and 86.4% (SD 1.4) in those composed of two layers of cells. Thus the postnatal reduction in percentage arterial medial thickness appeared to have been achieved by the smooth muscle cells becoming thinner and increasingly separated from each other, without there being a reduction in the relative amount of vascular smooth muscle.

In the partially muscular arteries, during the first 3 weeks of life regions of endothelial/intermediate or smooth muscle cell apposition were seen, where the intercellular gap was narrow (approximately 50 nm). The gap was wider, approximately doubled (100 nm), in animals >6 months of age. However, small areas of close contact between endothelial and smooth muscle cells with an intercellular gap of approximately 15 nm and without intervening basement membrane were seen at all ages.

Within the alveolar region, many small "undilated" muscular arteries in which a lumen could not be discerned by light microscopy were seen in the stillborn and newborn lung <12 h of age (Fig. 7).

2. Ultrastructural composition of pericytes, intermediate and smooth muscle cells. At birth, the pericytes, intermediate and smooth muscle cells had a similar appearance which changed little during the first 3 weeks of life. All the cells appeared poorly differentiated having only thin sheets of myofilaments at their ad-endothelial surface, with few surface dense bodies. Beneath the myofilament layer the cytoplasm was rich in polysomes, rough endoplasmic reticulum, and mitochondria. Quantitative studies showed that during the first 3 weeks of life the myofilament volume density was similar in the pericytes, intermediate and smooth muscle cells (Fig. 8). Arteries with a single layer of smooth muscle cells showed a transient reduction in myofilament volume density after the first 24 h of life ($p < 0.0001$) (Fig. 8), a change associated with the disappearance of the thin abluminal myofilament sheet present at birth.

In the adult animals the pericytes, intermediate and smooth muscle cells fulfilled the same criteria as in newborn animals with respect to their relation to the endothelial cells, the integrity or otherwise of their basement membrane, and were present in the same location as in newborn animals. However, all the cells now appeared larger and had a more fully differentiated appearance, having arrays of myofilaments and dense bodies throughout the cytoplasm (Fig. 6c). As in the younger animals the volume density of the filaments was similar in pericytes, intermediate and smooth muscle cells but it approximately doubled in all cell types between three weeks and adult life ($p < 0.0001$) (Fig. 8). The mitochondrial volume density also increased between 3 weeks and age >6 months, in all three cell types ($p < 0.03$). By contrast, the volume densities of synthetic organelles, Golgi cisternae and rough endoplasmic reticulum was relatively high at birth and decreased significantly between 3 weeks and adult life ($p < 0.002$).

3. Connective tissue elements. The non and partially muscular arteries contained little connective tissue at any age, but the amount did increase between 3 weeks of age and adult life. At birth the single

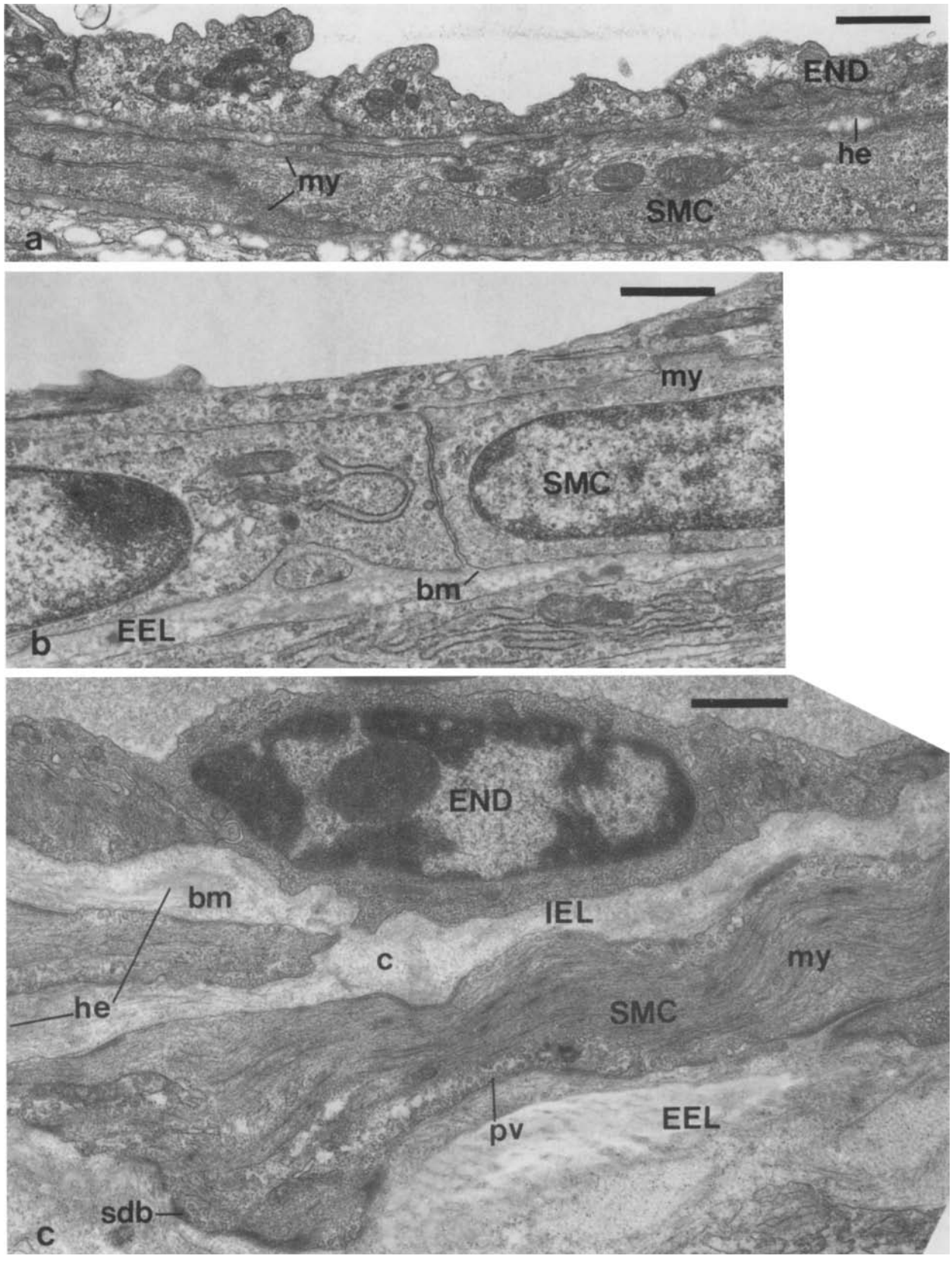

Fig. 6. Electron micrographs of small muscular arteries from **a** 12 h pig, transverse section; **b** 12 h pig, longitudinal section; **c** adult, transverse section, showing the increase in myofilament concentration and in ab- and adluminal and cytoplasmic surface dense bodies with age. EEL = external elastic lamina; sdb = surface dense body; SMC = smooth muscle cell. Other abbreviations as in legends to Figs. 3 and 4

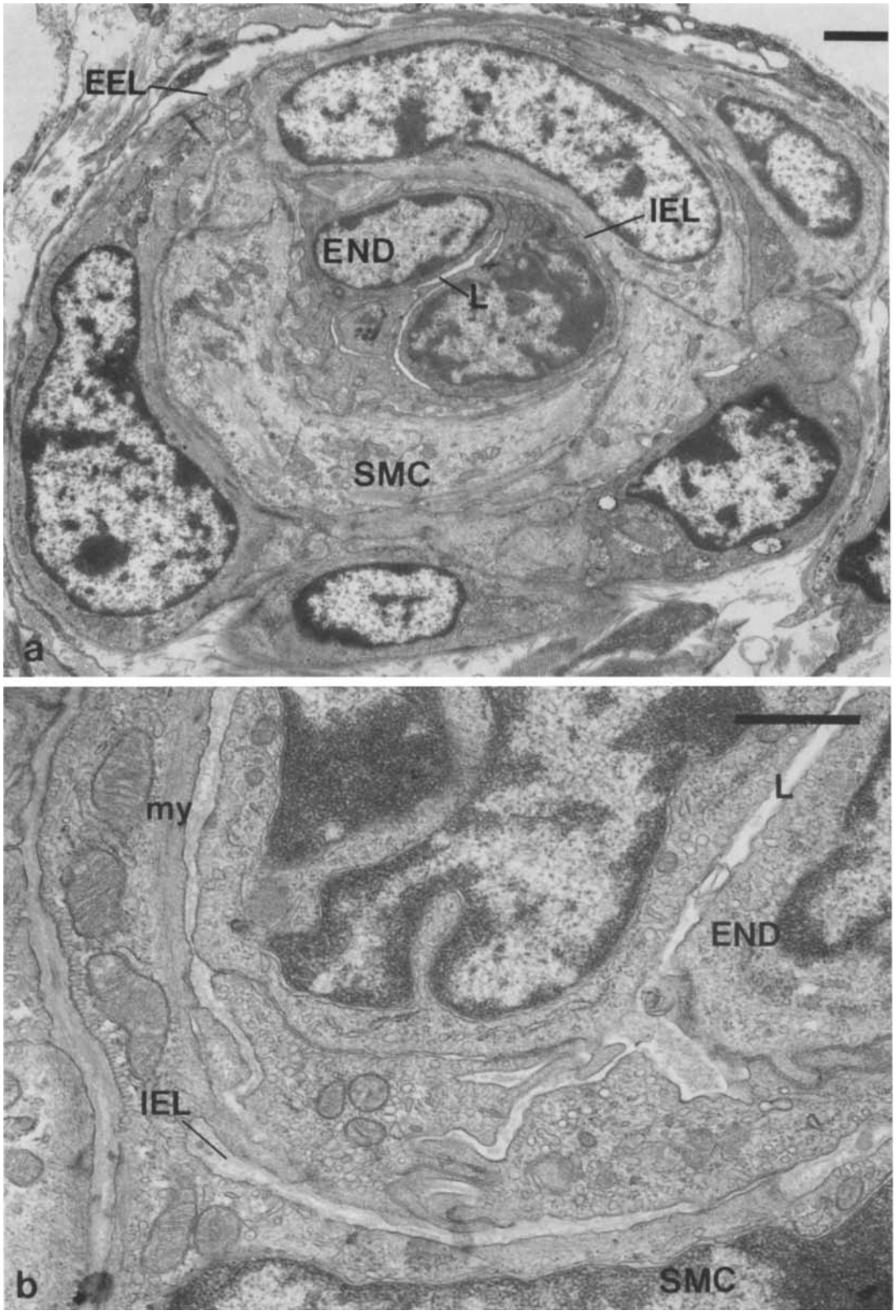

Fig. 7. Electron micrographs of transverse sections of undilated arteries from two stillborn pigs. **a** Low magnification shows endothelium surrounded by a single layer of overlapping smooth muscle cells, the lumen is minute. Scale bar line = 2 μm. **b** Detail of another artery showing gaps between adluminal surface of endothelium, interdigitating junctions between endothelial cells. Scale: bar line = 1 μm. Abbreviations as in legends to Figs. 3, 4, 6

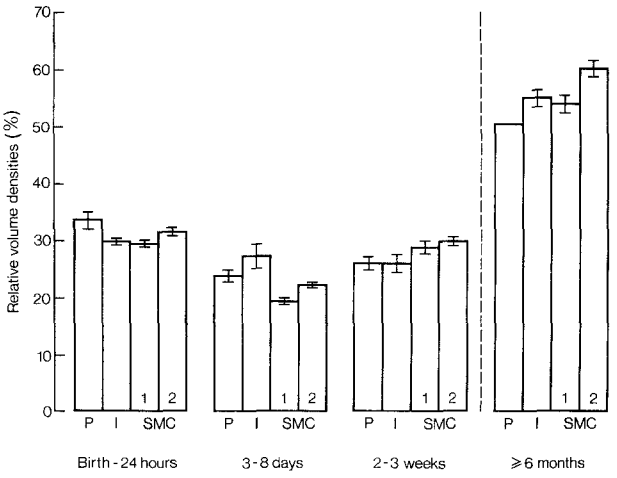

Fig. 8. Histogram of relative volume densities of myofilaments in pericytes (*P*), intermediate cells (*I*), smooth muscle cells in arteries containing one layer of smooth muscle cells (*SMC1*), and those containing two layers (*SMC2*), at different ages. I = Standard error

elastic lamina was composed of basement membrane material and amorphous elastin with its microfibrillar components. It increased in thickness with age, beginning with an increase in basement membrane-like material, followed by an increase in size of amorphous elastin profiles between 3 weeks and adulthood. Only a few collagen filaments were present at birth but swathes of collagen fibres were commonly seen in adult animals (Fig. 3a, b compared with 3c).

The muscular arteries accompanying terminal bronchioli were the smallest and most peripheral arteries to have a distinct, but thin internal elastic lamina at birth (Fig. 6a compared with 6c) (Hall and Haworth 1986). Within the media, there was no obvious increase in connective tissue immediately after birth but between 3 weeks and adulthood extensive deposition of connective tissue lead to separation of the smooth muscle cells which remained in close contact only at their tapering edges (Fig. 6c). The external elastic lamina remained ill-defined and patchy.

As shown above the volume density of total connective tissue relative to that of smooth muscle cells did not change with age. In the adult, the greater absolute amount of connective tissue was associated with a greater volume density of collagen as compared with other connective tissue elements ($p < 0.0001$). Of the different connective tissue components, the volume density of amorphous elastin and its microfibrillar components, collagen and basement membrane material was always greater in the media of arteries with two than in those with one smooth muscle cell ($p < 0.0001$).

Discussion

This ultrastructural study necessarily involved intensive study of a relatively small number of arteries, but these arteries appeared to be representative of the entire pulmonary vasculature. The light microscopic findings in the present study showed that pulmonary arterial wall thickness decreased and external diameter increased at a similar rate and in the same branches of the pulmonary arterial tree in these animals as in the previous more extensive light microscopic study (Haworth and Hislop 1981).

The present study shows that the thick walled pulmonary arterial wall structure characteristic of fetal life is due to the presence of overlapping smooth muscle cells each cell having a low surface/volume ratio. In addition the pericytes, intermediate and smooth muscle cells have been shown to have fewer myofilaments relative to other cytoplasmic organelles at birth than later. Thus, each cell probably has less capacity to vasoconstrict than has been supposed, and the high fetal pulmonary vascular resistance is probably due to the singular arrangement of the cells within the vessel wall, each cell having only a moderate degree of vasoconstrictor tone. Birth was followed by a phase of rapid remodelling during which the cells became thinner and appeared to "spread" within the vessel wall. Their surface/volume ratio increased rapidly during the first 24 h and cell/cell overlap decreased. There was no evidence of a reduction in the amount of vascular smooth muscle. The rapidity with which these changes occured indicate considerable plasticity of the vessel wall, a suggestion compatible with the appearance of the vessels at birth. Relatively large "spaces" separated overlapping cells, and there was little "fixed" connective tissue, collagen and amorphous elastin. Even the terminal bronchiolar arteries did not have a well defined external elastic lamina which might have limited expansion.

The elastic and large muscular pulmonary arteries of the same animals also undergo rapid remodeling after birth (Hall and Haworth 1987). As in the peripheral vessels, an increase in lumen diameter is achieved by the smooth muscle cells becoming thinner without a reduction in vascular smooth muscle. However, in the gas exchanging arteries thinning of the smooth muscle cells occurred more rapidly, within the first day rather than by the fourth day of life. The mechanisms responsible for these adaptative changes are uncertain, but both mechanical factors and release of vasoconstrictor tone are probably important. Im-

mediately after birth a rapid reduction in wall thickness was associated with an increase in external diameter in the muscular terminal bronchiolar arteries, suggesting a reduction in vasconstrictor tone. However, the external diameter remained unchanged in the non and partially muscular arteries, possibly because these vessels were stretched in a longitudinal direction at birth when the lungs were inflated (Hall and Haworth 1986). In all vessels, a reduction in cell contractility may have initiated or facilitated thinning of the smooth muscle cells and a reduction in cell/cell overlap. The temporary reduction in smooth muscle cells myofilament volume density seen in muscular vessels after 24 h of age may reflect a temporary reduction in contractility while rapid changes in cell shape and vessel organisation were taking place.

After birth, having assumed a more definitive position in the vessel wall, the smooth muscle cells appeared to deposit connective tissue around themselves (Ross and Klebanoff 1971), so stabilising the newly organised wall structure. In the newborn lung, smooth muscle cells containing a predominance of synthetic organelles were associated with small amounts of connective tissue while in the adult lung the cells contained more contractile organelles in the presence of much connective tissue. The conducting pulmonary arteries show the same changes (Hall and Haworth 1987), as does the developing aorta (Gerrity and Cliff 1975). In vitro studies indicate that smooth muscle cells do not have secretory and contractile functions simultaneously (Chamley-Campbell et al. 1979) and deposition of connective tissue appears to have precidence in the vasculature of the newborn, irrespective of the intravascular pressure to which the vessel is exposed. In the present study on respiratory unit arteries, collagen predominanted over other connective tissue elements as occurs in the conducting pulmonary arteries of the same animals (Hall and Haworth 1987) and as has been described in the newborn rat aorta (Gerrity and Cliff 1975). Collagen presumably increases the tensile strength of the vessel wall as it is distended by the increasing cardiac output of the growing animal. In the respiratory unit arteries, deposition of connective tissue led to increase in separation of the smooth muscle cells from each other and from endothelial cells, presumably reducing communication between cells. However, gaps remain in the internal elastic lamina of terminal bronchiolar arteries throughout life, through which endothelial and smooth muscle cells contact each other (Hall and Haworth 1986).

Pericytes and intermediate cells are generally considered to be different from smooth muscle cells because they are less specialised, but we found the difference to be one of position and size rather than cytoplasmic composition. These observations are in accord with recent immuno-cytochemical studies which have shown that pericytes contain actin (Herman and D'Amore 1983), myosin (Joyce et al. 1985a), a tropomyosin which is immunologically similar to that of smooth muscle cells (Joyce et al. 1985b), contain cyclic GMP-dependent protein kinase (Joyce et al. 1984) and are potentially contractile cells. However, the concentration of tropomyosin is lower in pericytes than in smooth muscle cells (Joyce et al. 1985b). In the present study, all three cell types had a more differentiated appearance and a higher myofilament volume density in adult than in newborn animals. These findings suggest that characteristic lability of the newborn pulmonary circulation may be determined, not by an excessive ability of the cells to respond to vasoconstrictor stimuli, as has been thought, but by a more moderate capacity to contract by cells which remain in their thick unspread fetal state. The smooth muscle cells of the large conducting pulmonary arteries also show an increase in myofilament concentration with age (Hall and Haworth 1987). In addition, in the elastic and large muscular pulmonary arteries the adluminal smooth muscle cells contain fewer myofilaments relative to other cytoplasmic organelles than do cells closer to the adventitia, and the cytoplasmic composition of the terminal bronchiolar arteries in the present study resembles that of the adluminal cells of the larger vessels. Thus, there appears to be an inner core of smooth muscle cells lying beneath the endothelium which extends from the hilum to the precapillary bed and which in the elastic and large muscular arteries is surrounded by a layer of more differentiated cells. The relationship between smooth muscle cell differentiation at different ages, heterogeneity at all ages and the development of nervous innervation is an intriguing problem. The less differentiated adluminal smooth muscle cells are presumably those which migrate through gaps in the internal elastic lamina to form the "intimal proliferation" seen in pulmonary vascular disease.

The present study on the peripheral pulmonary arteries together with a previously published study on the conducting vessels (Hall and Haworth 1987) form the basis for future work on the developing pulmonary vasculature in health and disease. The striking questions which have emmerged include possible changes in junctional structure, the relation between the development of the different types

of smooth muscle cell contractile proteins and the development of nerves and neurotransmittors, and the relation between such morphological features and pharmacologic responses.

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