CHAPTER 1

The growing lung: normal development, and the long-term effects of pre- and postnatal insults

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At first sight, adult physicians may feel that intra-uterine lung development is of little interest and importance to them. Paradoxically however, the developing lung impacts more directly on adult practice during foetal life than at any time until the foetus becomes an adult; this is truly an area of interface between adult and paediatric pulmonologists. The lung develops within a foetus which is (usually) within the uterus of an adult, who may be a smoker, or a substance abuser, or may also be receiving treatment for a respiratory condition such as asthma or cystic fibrosis (CF). Some legitimate drug treatment of the mother may adversely impact on foetal lung development. Events around birth and early childhood may also leave a permanent legacy, the fruits of which may appear only in middle age. An appreciation of normal development is fundamental to understanding the effects of the diseases which are described elsewhere in this monograph. The study of events during development may allow new insights into pathophysiological processes in later life, such as foetal ion transport and CF. This chapter therefore emphasizes themes where pathophysiology and disease states are linked.

The aim of this chapter is to describe how the lung develops, with a particular emphasis on conditions relevant to adult physicians, rather than trying to give a comprehensive summary of this huge topic. In particular, the many individual mediators and receptors which have been implicated as important in lung development will not be listed in detail. Interested readers are referred to a recent monograph [1]. However, at the moment it is not possible to give a coherent synthesis of the many disparate pieces of evidence accumulating about normal lung growth. It should also be noted that many mediators which have been implicated in pathological processes, such as airway remodelling, also have important roles in normal growth (*e.g.* transforming growth factor-β). Long term, attempts to promote lung health must begin with the unborn baby, by physicians working with parents.

Normal development of the lung before birth

The airways and lung parenchyma

The broad rules of lung development were described by REID [2], although with new data some minor modifications have to be made. These rules are: 1) the bronchial tree is developed by week 16 of gestation; 2) alveolar development is largely after birth and;

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Table 1. – Summary of the stages of normal lung development

Stage of lung development	Morphometric correlates
Organogenesis: the embryonic period (1–7 weeks)	Lung buds appear as outpouching of foregut. Mesenchyme driven branching of airways. First appearance of primitive pulmonary arteries and veins.
Pseudoglandular stage (5–17 weeks)	Virtually the complete branching structure of the future bronchial tree is laid down. Cellular differentiation (cartilage, neuronal tissue, ciliated cells, smooth muscle) commences from proximal to distal. The pre-acinar vascular pattern is fully mature at the end of this period.
Canalicular stage (16–26 weeks)	Early acini become visible under the light microscope. Capillaries form a meshwork within the mesenchyme. Type-1 and -2 cell differentiation.
Saccular or terminal sac stage (24 weeks to term)	Blind saccules start to divide, and alveolarization commences. The capillary network becomes closer together, and the walls between the sacs contain a double capillary network.
Postnatal: alveolar stage	Formation of alveoli, mostly in first 6 months, virtually complete by 2 yrs.
Postnatal: microvascular maturation	Formation of new double capillary layers, followed by remodelling to form the mature single layer.

3) pre-acinar arteries and veins follow airway development, intra-acinar vessels follow the alveolar development (the acinus contains the respiratory bronchiole, which by definition contains no cartilage in the wall, the alveolar ducts and the alveolar sacs). The different phases of lung development are summarized in table 1, and described in more detail in the following sections.

The embryonic period (weeks 1–7). On day 26, the lung buds appear as a ventral outgrowth of the primitive foregut, invading the mesenchyme. The buds maintain a connection with the foregut through the primitive hypopharynx. By 4–5 weeks, the lobar structure is evident, and subsegmental branching is evident by 7 weeks. There is clear-cut animal experimental data that the branching pattern is driven by signals from the mesenchyme to the budding airway [3]. For example, chick lung mesoderm transplanted to mouse epithelium results in a chick, not mouse, lung branching pattern. The initial vascular supply to the developing lung is from the aorta, however, during this stage the pulmonary arteries are derived from the sixth pair of aortic arches, and the pulmonary veins from outgrowths of the left atrium, thus, cardiac smooth muscle is found in the central branches of the pulmonary venous tree.

The pseudoglandular stage (weeks 5–17). During this period, virtually the complete branching structure of the future bronchial tree is laid down, giving rise to 20 generations [4, 5]. There is controversy as to the extent of the epithelial mass of the prospective acinus laid down, with recent studies suggesting that as much as one-half may be formed by the end of this stage [5, 6].

It is in the pseudoglandular period that cellular differentiation commences from proximal to distal. Cartilage appears before the 10th week, and reaches the last airway generations by week 25 [4]. Primitive ciliated cells appear at approximately week 10. The *in utero* function of these early ciliated cells is obscure; there certainly seems no reason to need a mucociliary escalator this early in development. The bronchial wall is much more complex at an early stage than had been appreciated. It contains smooth muscle and is innervated from very early on (discussed later). By the end of this period, the pre-acinar vascular pattern is fully mature.

Table 2. – Space constraints which may impede lung development

Intrathoracic	Intra-abdominal	Chest wall	Amniotic
Pleural effusion	Ascites	Thoracic cage disease (*e.g.*	Oligohydramnios (*e.g.*
Tumours	Tumour	Jeune's asphyxiating dystrophy)	Potter's syndrome)
Diaphragmatic hernia		Amniotic bands	

Cannalicular stage (weeks 16–26). The final two generations of the bronchial tree are laid down early in the cannalicular period [5]. At this stage, the early acini become visible under the light microscope. They consist of an airway stem and an array of short tubules, delineated by mesenchyme. Capillaries form a meshwork within the mesenchyme. The primitive cuboidal cells which hitherto predominated in this part of the lung differentiate into type-2 cells containing the lamellar bodies which form the intracellular storage bodies of surfactant [7] and type-1 epithelial cells. The development of the surfactant system is described in more detail later.

Saccular stage (24 weeks to term). Each airway ends in a blind saccule. At this stage, these saccules start to divide, and alveolarization commences. These transitory structures give rise to the alveolar ducts and sacs. The capillary network becomes closer together and the walls between the sacs contain a double capillary network.

Antenatal influences on lung size

The lungs need space in which to develop, intraluminal fluid secretion and the stimulus of foetal breathing movements to develop normally. Constraints of space for development are classified in table 2. The lung is an actively secreting organ during embryogenesis. By term, the foetus produces 5 $mL \cdot kg^{-1} \cdot h^{-1}$ of lung fluid, which contains chloride at a higher concentration than plasma, *i.e.* chloride is actively secreted against a concentration gradient. This lung fluid is absolutely necessary for foetal lung development [8]. The important subject of ion transport in the foetal lung is discussed in a separate section.

The final influence on lung development is foetal breathing movements [9]. These can be observed using ultrasound in the second half of pregnancy. Any cause of impairment of foetal breathing (table 3) will result in pulmonary hypoplasia [10]. One not uncommon cause is antenatal onset of myopathy (*e.g.* myotonic dystrophy or severe spinal muscular atrophy).

The pulmonary circulation

The basic principles were also described by REID [2]. During budding, the rudimentary epithelial outpouching is accompanied by loose connective tissue associated with foregut-derived vasculature. By the end of the embryonic period, this loose tissue is connected up to the system of bronchial arch arteries and the primitive pulmonary veins, which are

Table 3. – Causes of reduced foetal breathing movements

Muscle disease	Anterior horn cell disease	Nerve disease	Central nervous system disease
Myotonic dystrophy inherited from mother	Spinal muscular atrophy	Failure of phrenic nerve conduction for any reason	Agenesis of phrenic nuclei, brainstem disease

outgrowths of the left atrium. Central bronchial arteries develop, which subsequently regress to be replaced by the definitive bronchial arteries, which extend down to the level of the terminal bronchioles by the end of this period. In the cannalicular stage, the distal pulmonary circulation starts to develop. Pre-acinar arteries are present by 28 weeks, but continue to muscularize until term. Just before birth, smooth muscle extends further, distally along the pulmonary vascular tree, and consists of a larger percentage of the thickness of the vascular wall at any given level, than at any time during subsequent development. A double capillary network develops by secondary septation, and near birth the capillary networks fuse to start to produce the mature blood-air barrier.

Functional differentiation in the maturing lung

There are more than 30 different cell types within the mature lung. This section can only describe a few important changes during development. Areas relevant to human disease will also be highlighted in this section.

Development of the nervous system within the respiratory tract

Just as in the mature lung, the function of the nervous system in the developing lung is largely obscure. Nervous tissue is present around the primitive lung bud from ~5 weeks gestation (embryonic period), and nonspecific enolase is detectable by 8 weeks, suggesting maturation of primitive neural crest cells. This neuronal tissue ensheathes smooth muscle from an early stage [11]. The nervous system comes to innervate the airways, glands and vasculature. In contrast to reports from adult lungs [12], many ganglia are found in the foetal lung, in particular from weeks 16–18 (early cannalicular phase) as nerve trunks become larger and more compact [11]. Neurotransmitters appear sequentially and early in the developing lung. Cholinergic nerves (10–12 weeks) are more prominent than the sparser adrenergic system (20 weeks). The nonadrenergic, noncholinergic system is represented, with substance P, galanin and vasoactive intestinal peptide acting as neurotransmitters (probably from 16 weeks) [13].

Airway smooth muscle

Myoblasts develop from mesodermal cells, and smooth muscle cells staining for α-actin cover the branching epithelial tubules from early gestation [10]. The control of this process involves the extracellular matrix, in particular, with cytokines and growth factors acting as autocrine or paracrine signals. α-smooth muscle actin, the isoform characteristically expressed in airway smooth muscle, is regulated in a temporal and tissue-specific manner and is present in the first trimester in humans [14, 15]. Myosin heavy chain isoforms are also developmentally regulated [16]. Structural studies, showing early expression of the contractile apparatus, have been confirmed by evidence that the bronchial smooth muscle is spontaneously contractile from early on in gestation, both spontaneously and, in the pig at least, in response to cholinergic stimulation [17–19]. The pressures generated by these waves of contraction have been shown to be ~2–3 cmH_2O in sheep [20] and 1.4–4.2 cmH_2O in the mouse [21].

Although the developmental importance of these contractions is obscure, it is speculated that transmission of these pressure changes to the parenchyma stimulates growth. Certainly *in vitro* pulsatile forces are more growth promoting than nonpulsatile [22, 23]. Hyperoxia, as well as affecting alveolar development, also results in increased airway responsiveness and remodelling. Airway relaxation may also be affected [24]. The

interactions of prematurity and hyperoxia on the airway are controversial. However, the direct effects of oxygen (O_2) on the developing airway may be relevant to the changes seen in the short and long term in chronic lung disease of prematurity.

Developmental biology of surfactant

Pulmonary surfactant is produced by type-2 cells, and stored in lamellar bodies. It is crucial for maintaining the functional integrity of alveoli. The main morbidity of extreme prematurity is due to surfactant deficiency causing neonatal respiratory distress, the treatment of which has been transformed by the availability of exogenous surfactant.

The components of surfactant are developmentally regulated. Surfactant protein (SP)-B and SP-C are detectable early in gestation, before recognizable mature type-2 cells or surfactant phospholipid can be detected. Type-2 cells with lamellar bodies appear between 20–24 weeks gestation [25]. SP-A and SP-D synthesis parallels that of phospholipid; note that although SP-A is required for tubular myelin formation [26] these "surfactant" proteins have little or no surface active properties and are more properly part of the collectin family with mannose-binding lectin. Indeed, SP-D is not even located in the lamellar bodies in type-2 cells. Collectins bind to non-host carbohydrate moieties, and have immune functions within the airway. Surfactant-containing lamellar bodies are detectable in the lung first during the cannalicular stage, at 20–24 weeks. SP-A and SP-D messenger ribonucleic acid (mRNA) can be detected earlier than this [27] suggesting that type-2 differentiation occurs earlier in development. Later in gestation, surfactant begins to be secreted into the airway lumen. The functional maturation of the surfactant system can be accelerated therapeutically.

Although the commonest disorder of surfactant is temporary, and causes neonatal respiratory distress which is reversible provided the baby survives, rare congenital surfactant deficiencies have been described recently. The commonest is congenital SP-B deficiency. The SP-B gene is located on chromosome 2, and a number of different mutations have been described [28, 29]. The disease is inherited as an autosomal recessive and presents as respiratory distress in the term baby, usually relentlessly worsening and rapidly fatal. The histology is of pulmonary alveolar proteinosis. Surfactant studies usually reveal complete absence of SP-B, sometimes with pro-SP-B expression, and sometimes but not invariably, defects in SP-C expression [28]. Similar effects have been described with absent lamellar bodies but with normal SP-B [30], and also with mutations in the granulocyte macrophage colony stimulating factor (GM-CSF)/interleukin-3/interleukin-5 receptor β chain [31]. A series of infants with SP-B deficiency associated with misalignment of lung vessels has been described [32]. It is likely that other hereditary deficiencies or malfunction of SP may be detected in the future. Possible treatment options, largely based on animal studies, include exogenous GM-CSF and adenovirus-mediated GM-CSF gene transfer [33, 34]. Lung transplantation has also been successful in these infants [35].

Ion transport in the developing lung

The foetal lung is a secretory organ, and fluid secretion is essential for normal development. Just before birth, the lung actively secretes a chloride rich fluid at a rate of 5 $ml \cdot kg^{-1} \cdot h^{-1}$, which is essential for normal development [8]. At birth, secretion must be switched off, and fluid absorbed across the epithelium. Maternal circulating catecholamines are hypothesized to be important in this process, and at least two possible mechanisms have been described *in vivo* [36]. The lung epithelial sodium channel (ENaC)

is the ion channel which has been studied in most detail, although without doubt many other ion channels are important.

ENaC contains three subunits, α, β, and γ, which are differentially regulated during foetal development [37]. A recent study in human foetal lung rather surprisingly showed αENaC mRNA expression even in the embryonic phase in the foetal lung bud, and it is widely distributed in epithelial tissues. By late gestation, expression followed the distribution of the type-2 cell [38]. There is a surge in αENaC expression late in gestation in the rat and mouse [37, 39]. The physiological importance of αENaC in man is unclear. On the one hand, the amiloride-sensitive drop in nasal potential difference, a surrogate for ENaC activity, is decreased in those who go on to develop newborn [40], however, those with mutations in the αENaC gene resulting in pseudohypoaldosteronism do not have a history of neonatal respiratory distress [41, 42]. A recent study [38] showed that αENaC mRNA is readily detectable in preterms with respiratory distress. This surprising finding could be explained by post-transcriptional modification of αENaC, or defects in the β- or γ-subunits, or the importance of other channels, for the existence of which there is evidence from biochemical [43] and electrophysiological [43–45] evidence.

Aquaporins (AQP) are channel proteins which facilitate membrane water transport. Together with the upsurge in αENaC expression around birth, AQP-4 mRNA expression dramatically rises in epithelial cells [46] 2 days before birth, peaking on the first postnatal day. AQP-4 gene expression is higher *in vitro* in 21% O_2 compared with 3%, suggesting that the postnatal surge may be related to lung oxygenation at birth.

Chloride secretion is also important in the developing lung. Inhibition of chloride secretion by bumetanide or frusemide resulted in reduction of airway calibre with no change in branching pattern [47] in the embryonic rat. It is not known whether maternal diuretic treatment in the human has any important effects on airway calibre.

It is interesting to speculate how active chloride secretion is achieved in the foetus with CF. CF transmembrane regulator (CFTR) mRNA is widely expressed in the lung from the pseudoglandular stage [48]. The signal is gradually localized to the distal airways and appears in the submucosal glands, the earliest site of changes in CF only postnatally. However, despite the wide expression of CFTR in the normal foetal lung, the lungs of the newborn with CF are virtually normal [49]. Still more paradoxically, changes in tracheal submucosal glands may be found *in utero*, before expression of CFTR mRNA [50]. This implies that there must be other chloride channels which can take over the function of CFTR *in utero*, which could possibly be exploited therapeutically later in life. Unfortunately, although other chloride channels have been characterized postnatally, little is known about potential candidate chloride channels in the foetal lung.

Functional changes around the time of birth and their consequences

Just prior to birth, the placenta is the organ of respiration. Virtually all the venous return to the right heart is shunted away from the lungs through the oval foramen and the arterial duct. Pulmonary vascular resistance (PVR) is maintained at a very high level by the muscular precapillary vessels (discussed earlier). At birth, the umbilical cord is tied and cut, and the lungs must take over all respiratory function within seconds or the baby will perish. The lung must convert from a secretory to an absorptive organ. The mechanisms of these dramatic changes are obscure. However, it is clear that the first breath results in vasodilatation by at least two mechanisms. Firstly, the mechanical effect of traction on the vasculature as the chest wall expands pulls open the vessels. Secondly,

O_2 entering the lungs for the first time results in pulmonary vasodilatation. However, in the experimental situation, PVR does fall even if the foetus is delivered into an atmosphere of pure nitrogen [51]. Undoubtedly other mediators are important, including cyclo-oxygenase metabolites. The gene for cyclo-oxygenase-1 (but not -2) in endothelium and vascular smooth muscle shows enhanced expression in late foetal and early postnatal life [52]. Endothelin receptor expression increases around the time of birth, implying a role for this system in postnatal adaption [53]. The role of nitric oxide in postnatal adaptation is controversial. As in other areas, there are likely to be important species differences. Nitric oxide synthase is more abundant in young compared with mature animals [54, 55]. Smooth muscle sensitivity to nitric oxide may be greater at birth than in older animals [56]. It should be noted that the effects of mediators differ ante- and postnatally. For example, endothelin-1 causes vasodilatation in the foetal sheep, but vasoconstriction postnatally [57].

Postnatally, the pulmonary circulation undergoes three stages to become fully mature. Firstly, there is passive stretching as above. Secondly, in the normal infant, smooth muscle starts to regress. Finally, there is adaptive structural remodelling, this last process takes several weeks. The development of new vessels alongside that of new alveoli also contributes to the fall in PVR. Concomitantly, there is first functional and then structural occlusion of the arterial duct. The oval foramen is closed functionally by the rise in left atrial pressure compared with right atrial pressure. It remains at least probe patent in approximately one-third of normal people.

Immediately after birth, before structural remodelling has taken place, PVR may rise steeply if the baby becomes even minimally hypoxic or acidaemic. The foetal shunts (oval foramen, arterial duct) reopen, and right-to-left shunting causes profound hypoxaemia, "persistent foetal circulation". The occurrence of this syndrome in the babies of women treated with indomethacin in pregnancy suggests that prostaglandins may be very important in pulmonary vascular control in this period [58]. Most of these babies either die or recover very rapidly, although a few are left with chronic lung disease secondary to the intensive ventilatory support required in the newborn period. RAINE *et al.* [59] described two babies who appeared to have an overlap syndrome, with pulmonary hypertension developing in the neonatal period, running a more indolent course than typical persistent foetal circulation, but dying of pulmonary hypertension within 3 months of birth. At autopsy there was marked distal extension of smooth muscle, and enlarged endothelial cells, which combined to occlude or reduce the arterial lumens. Alveolar development was normal. It is probable that PVR never fell to normal levels after birth. These cases may represent an as yet poorly characterized overlap syndrome with primary pulmonary hypertension of onset in later childhood.

Normal postnatal development of the lung

After birth, alveoli continue to multiply and enlarge and airways continue to both enlarge and elongate. To make sense of this from birth to adulthood, it may be useful to draw an analogy with KARLBERG's [60] three-phase mathematical model of height development in postnatal life (fig. 1) and apply it to lung growth.

In this model, the first infant nutrition-dependent phase comprises a very rapid, but rapidly decelerating, growth lasting until the end of the second year. In the lung this equates with the phase of alveolar mutiplication terminating by the end of 2 yrs. From the end of the first year, the second, growth hormone-dependent, childhood phase begins and growth increases almost linearly until ~10 yrs of age which if not overtaken by the pubertal phase, would then continue but slowly peter out by ~20 yrs of age. In the lung

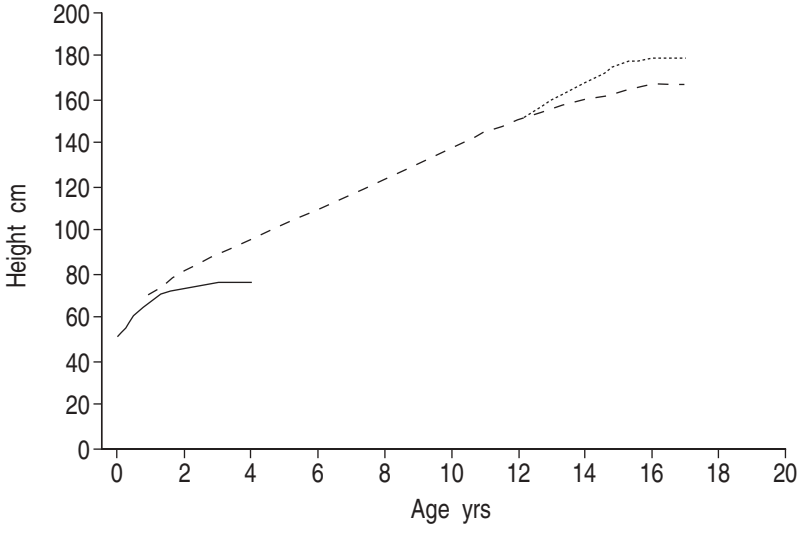

Fig. 1. – A three phase infant, child, pubertal model of lung growth. ——: infancy=alveolar multiplication; - - -: childhood=airway enlargement; ·····: puberty=sex dysanapsis. Modified from KARLBERG [60].

there is no further multiplication of alveoli or change in airway numbers, merely enlargement and elongation producing linear changes in lung function with height. The third, pubertal, sex steroid-dependent phase causes a rapid change in height, plateauing off at adult height. This sudden change is exactly mirrored in the lung with three-dimensional enlargement but without multiplication.

Each of these phases are discussed further, later in the chapter, however, when considering them it is important to remember that whereas antenatal lung growth is described by histological, cytochemical or molecular mechanisms, at present, postnatal growth of the human lung can only be assessed from either structure or function with a paucity of cellular or molecular data. Structure and function are of course related, but neither consistently nor linearly. Thus, conclusions drawn, for example, from such histological studies that exist may not readily translate into functional differences. Furthermore, different functional studies depending on what is measured will often lead to different conclusions. For example, static lung volume measurements may produce different outcomes to those in studies examining forced expiratory manoeuvres. The lungs are also bounded by the thoracic cage whose physical size must determine that of the lung. However, respiratory musculature may develop at a different rate compared with chest wall size particularly in puberty.

The effects of sex and race often have not been determined and so descriptions are often only approximations. These caveats are crucial to understanding what is and is not known of lung growth.

The first two years of life

The number of alveoli present at birth (variously estimated as $0–5\times10^7$) and the age at end of alveolization (estimated at 2–20 yrs) is controversial [61, 62]. The current consensus is that alveolar formation starts at 26 weeks, that by term only 15% of the adult component are present and that the bulk of the process of forming new alveoli

is completed by 6 months and virtually complete by 2 yrs. This has profound implications for our understanding of the effects of disease.

The formation of millions of alveoli is accomplished by a complex process of folding and division. The first stage is the outgrowth of ridges from the sides of the saccule walls, forming primitive alveoli. The secondary septa contain a double capillary layer, and further new alveoli are formed by the infolding of one of these layers in order to further subdivide the air spaces. The double capillary networks then undergo remodelling to form the familiar single capillary sheet around each alveolus.

Airways also undergo profound postnatal changes, with increased smooth muscle [63] and bronchoconstrictor responsiveness [64] but no increase in airway number. The principle functional adaptations are to cope with gas exchange and minimize the resistance to airflow in the small infant. As laminar airflow is inversely proportional to the fourth power of the radius and turbulent airflow is inversely proportional to the seventh power of the radius, very small changes in airway size have profound effects on airflow.

The chest wall is far more compliant in infancy and if there was no pulmonary compensation for this increased compliance, little ventilation and gas exchange would occur. Infant airways are large compared to the parenchyma they serve [65, 66]. For example, the internal radius of a newborn human trachea is ~2 mm, rising only three-fold by adulthood, compared with a 20-fold rise in weight and an eight-fold rise in body surface area. Functional studies [67] have also shown that the ratio of peripheral-to-central airway resistance is no different between infants, children or adults. Histologically, opinions differ. Postnatally, the muscular coat of the bronchioles thins and is lost altogether in the terminal and respiratory bronchioles. This is thought to occur in a manner similar to fingers extending through a mitten glove, namely that the muscle coat stays where it is as the airways grow through them. HORSFIELD *et al.* [68] examining bronchial casts, suggested that peripheral airways in infancy are relatively large and indeed of adult size by age 1 yr. HISLOP et al. [69], in contrast, concluded that growth of the airways was symmetrical with the rest of the lung, something so rare in biology as to be somewhat implausible. Thus, by the third year of life alveolar numbers are virtually complete and peripheral airways probably near adult size.

Childhood

This phase is now solely concerned with alveolar enlargement, peripheral airway elongation and both elongation and enlargement of the central airways. Nearly all information in this age group is derived from functional studies.

It has been reported that peripheral resistance formed a greater proportion of the whole in children than in adults, the opposite of data by JONES *et al.* [66] on infants. Females may have larger airways than males, the evidence coming from females having greater forced expiratory airflows per unit lung volume than males [70]. Nevertheless, the differences between the sexes for a given height, though statistically significant, are small but represent an example of dysanapsis [71] *i.e.* a true sex difference. Forced expired lung function and static lung volumes bear a strong linear relationship with standing height during this period.

Puberty

At the end of puberty, lung function formerly nearly equal between the sexes, will be ~25% greater in males than in females of identical height. Thus, it is important to consider why this divergence occurs. As mentioned earlier, there is no new airway or

alveolar development therefore changes in lung function must be secondary to elongation and growth. There is little objective evidence however to confirm or refute this.

During puberty, it is truncal rather than limb growth which dominates, the reverse of the prepubertal pattern, particularly in males. As the lung lies within the thoracic cage, then considerable changes in lung volume and flow during puberty are to be expected. The early part of a forced expiration is partly dependent on thoracic muscle power. The pubertal growth in muscle power and bulk occurs ~14 months after the growth spurt [72], hence the "tall and gangly" period followed by the "filling out" period, and therefore the changes in lung function during this period are the result of complex interactions.

Nonlinear changes in lung function during adolescence were first observed in a cross-sectional study in the USA [73] where lung function suddenly appeared to increase at 153 cm in both sexes. Although pubertal stage was not measured in this study, it was assumed to be the reason for the sudden change. Peak flow showed a sudden marked increase in males during late puberty (Tanner stages 4 and 5 [72]) and is related to testosterone levels [74]. In the UK, a discontinuity was observed [75] in the linear increase in lung function with height between 160–165 cm in males and 150–155 cm in females (fig. 2). This corresponds well with the period of maximal linear growth during the pubertal growth spurt in both sexes as judged from a standard UK growth chart. In males particularly, children in late puberty had superior lung function to those in early puberty even if the latter were of equal height. This is confirmed by ENGSTROM *et al.* [76] who reported children having superior lung function to younger children of the same height.

The reason why prepubertal females of the same height as prepubertal males have very similar lung function, but postpubertal females do not, is related to different pubertal patterns of thoracic growth between the sexes. DEGROODT *et al.* [77] measured the thoracic dimensions and lung function of 477 males and 149 females every 6 months for

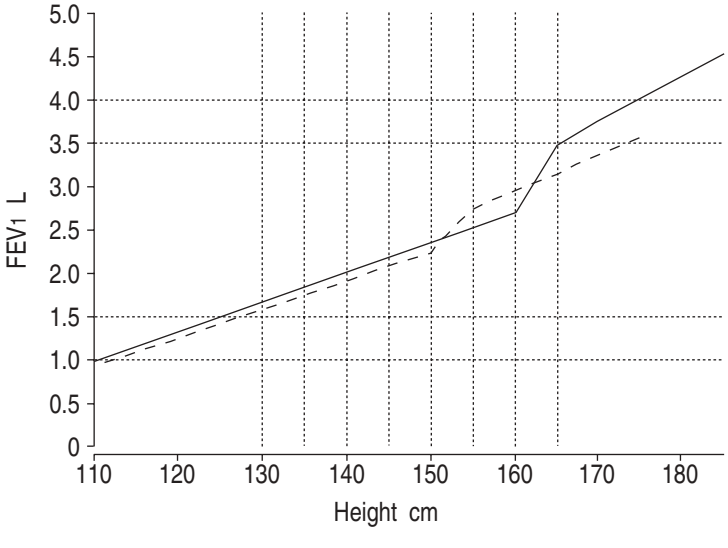

Fig. 2. – Comparison of forced expiratory volume in one second (FEV_1) against height for both males (—) and females (- - -). Note that in young children, females have slightly (<5%) lower FEV_1 than males (phase 1). Females then overtake males during their adolescent growth spurt (phase 2) but again are lower once the male growth spurt begins (phase 3). Note that all other forced expiratory measures of lung function have a very similar pattern.

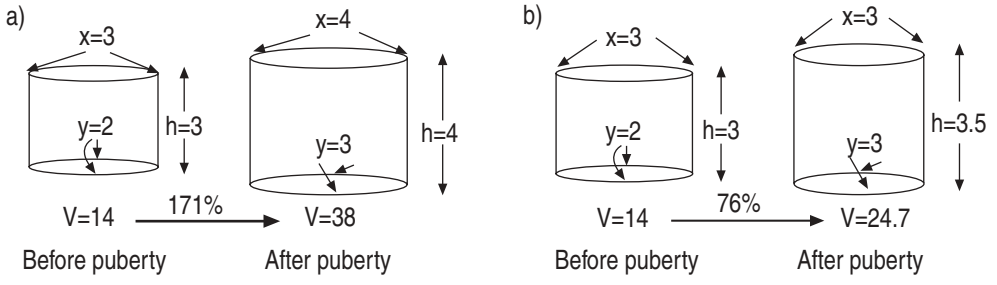

Fig. 3. – The growth of the thorax during a) male and b) female puberty. It is assumed that the thorax is an elliptical cylinder of volume (V): (πxyh)/4. The values used are arbitrary and are given to illustrate the large difference in V that occurs with comparatively small changes in each dimension.

8 yrs. It was observed that thoracic width in females did not change during adolescence whereas in males it did, but at only one-half of the rate as that observed for thoracic length. As thoracic length increased twice as fast in males than females, the overall effect on lung volume in females compared to males was profound.

To use an analogy, suppose the prepubertal thorax is an elliptical cylinder of arbitrary dimensions (fig. 3). The prepubertal volumes are essentially equal between the sexes. During puberty comparatively small differences in dimensions between the sexes produce large differences in the resulting volume. In the example shown, the postpubertal sex difference in volume is 53%, two-thirds of which is due to the lack of change in thoracic width in females in puberty, while the rest is due to the increased thoracic length in males compared to females.

This analogy is confirmed by cross-sectional data [75] where for males (but not females) the ratio, for example, of the forced expired volume in one second (FEV_1) to the product of maximum inspiratory thoracic circumference squared and sitting height was constant. The square of the circumference of an ellipse is proportional to its cross-sectional area. Width and depth are used to calculate the circumference. Thus, it is more accurate than squaring a single transthoracic dimension (width or depth) because the thorax is elliptical rather than circular in cross-section. In females, the ratio of FEV_1 to the product of the square of chest depth at maximum inspiration and sitting height was also constant. Note that depth rather than width was more useful, as DeGroodt *et al.* [77] had demonstrated that thoracic width did not change during female puberty.

This assumes that the lung merely passively changes as thoracic size changes. Clearly, this is unlikely when one considers the other factors involved, such as muscle power. Sherrill *et al.* [78] measured 1,024 subjects at least three times over a minimum of 4 yrs to construct a composite pattern of lung function changes during adolescence and subsequent senescence. Because longitudinal data was available, measures of lung function growth velocity could also be evaluated.

In males, peak height velocity occurred at 13.1 yrs (table 1) but the peak growth in forced vital capacity (FVC) was nearly 1 yr later and the peak increase in mid-expiratory flow (the average flow during expiration between 25–75% of FVC) was 1.3 yrs later. Whether this was related to the later development of muscle power during puberty is plausible but unproven. In females the lung function growth velocities all tended to be later (up to 1 yr) than peak height velocity but not significantly so. The age at which lung function peaked, *i.e.* when lung function growth velocity became zero, occurred 2 yrs earlier (17.8 yrs) in females than males (19.8 yrs).

To summarize, therefore, postpubertal females of identical height have lower lung function than males because of the lack of thoracic width changes in puberty and a lesser increase in muscle power.

The transfer factor for carbon monoxide as a measure of "lung growth"

The carbon monoxide diffusing capacity of the lung ($D_{L,CO}$) has come to be regarded as a useful tool for assessing lung growth [79–81]. It measures the loss of CO from a respired test gas mixture. For this to occur, the gas must reach an alveolus surrounded by capillaries containing haemoglobin (assuming there has been no recent pulmonary haemorrhage). Each alveolus has its own capillary network and assuming normal haematology, the $D_{L,CO}$ is a good measure of functional integrity of the alveolar-capillary network. It can be corrected for accessible lung volume (VA) to calculate the transfer constant, K_{CO} ($D_{L,CO}$/VA). During exercise, for example, $D_{L,CO}$ rises indicating that more functioning alveolar-capillary units are being used *i.e.* there has been recruitment. If $D_{L,CO}$ is normal at rest but there is no rise on exercise, it may indicate that all functioning alveolar-capillary units are being used already, suggesting a defect in alveolar-capillary development.

In vitro experiments have produced the equation:

$$\frac{1}{D_{L,CO}} = \frac{1}{D_{m,CO}} + \frac{1}{(h \times V_c)} \quad (1)$$

where $D_{m,CO}$ is the transfer constant of the alveolar-capillary membrane (regarded as small), h is the rate of reaction of haemoglobin with CO and V_c is the pulmonary capillary blood volume taking part in gas exchange [82–85]. The former two are thought to be reasonably constant so that a change in $D_{L,CO}$ reflects changes in V_c. The utility of these measurements is discussed in a latter section of this chapter.

Adverse and therapeutic influences on antenatal lung growth

Antenatal administration of corticosteroids

Corticosteroids are frequently and appropriately administered to mothers in preterm labour to mature the foetal lung. In animal models, steroids certainly speed up the maturational process, but ultimately at the cost of smaller lungs [86]. The relevance of the observation of reduced lung size to the human is unclear, since these preterm babies have many other reasons for impaired lung growth, and dissecting out the antenatal effects of corticosteroids is very difficult. Steroids upregulate SP-B and SP-C production at the transcriptional level [87]. A further benefit of steroid therapy may be the stimulation of direct transcription of the ENaC genes [88, 89], favourably modulating fluid absorption at birth. In the current state of knowledge, it must be said that any possible risks of antenatal steroids are far outweighed by the immediate benefits to the foetus. However, although there is sound evidence for administering a single dose of steroid to mature the lung, the practice of giving repeated courses has no evidence base, and has given rise to concern not merely about effects on lung development but also about effects on the developing brain [90].

Effect of thyroid hormones

Thyrotropin (TRH) has been used in humans therapeutically to try to promote lung maturation. In animals, TRH promotes morphological maturation, and the appearance of surfactant. Unfortunately, after early promise, the therapeutic use of TRH in humans prior to preterm delivery has not been helpful [91].

Natural obstruction and surgical manipulation of the developing airway

The lungs are a secretory organ before birth. Experimental tracheal obstruction in the foetal lamb results in pulmonary hyperplasia and insulin-like growth factor-2 release [92]. If the human airway is obstructed naturally (*e.g.* bronchial atresia) the lung distal to the obstruction enlarges and ultimately becomes destroyed. This was exploited in an attempt to correct the pulmonary hypoplasia associated with congenital diaphragmatic hernia. A clip was applied to the foetal airway, and the foetus returned to the uterus. Unfortunately, controlled studies showed no benefit from this ingenious approach [93].

Effects of tobacco smoke

There is no doubt that the adverse effects of cigarette smoke impact on the foetal airway. Studies in newborns soon after birth have shown evidence of airflow obstruction in the babies of mothers who have smoked during their pregnancies [94–96]. Interestingly, the same effect was seen in babies of atopic mothers and, completely unexplained, in one study in mothers who had hypertension in pregnancy [95]. This was associated later with viral-induced, wheezing lower respiratory tract illness [97–99]. As is predictable from knowledge of airway development, the adverse effects of tobacco smoke are most marked in the second half of pregnancy [100], hence, efforts to dissuade pregnant women from smoking should continue throughout pregnancy.

Effects of maternal allergen ingestion and inhalation

There is no doubt that the immediate postnatal period is a time of vulnerability to allergic sensitization. There is increasing research interest in the possibility of antenatal sensitization of the foetus, not merely to ingested allergens but even possibly to aeroallergens [101]. Such sensitization might set the scene for subsequent T-helper cell type-2-mediated airway inflammation and obstruction. At the moment the implications of this are still being worked out. Exposure of the foetus to small amounts of allergen may potentially induce a beneficial tolerance rather than allergy. A more detailed discussion of these issues can be found in a later chapter of this monograph.

Effects of profound maternal malnutrition

There is no doubt that maternal malnutrition in rats affects foetal lung growth. There is also evidence that rats malnourished before birth may be more vulnerable to postnatal injury. A recent paper followed up nearly 1,000 adults born during a severe famine in Holland [102]. Perhaps, as might be expected, there was a stronger association with obstructive airways disease in those who were exposed to the effects of famine later, rather than earlier, in pregnancy. There was no association with lung function or immunoglobulin-E levels. Unfortunately, CO transfer, a marker of alveolar-capillary membrane size was not measured in this group.

Adverse postnatal effects on lung growth

Neonatal respiratory distress

The most dramatic adverse effects on lung growth are seen in the context of neonatal respiratory distress after preterm birth. Bronchoalveolar lavage studies have established

that infants who go on to develop chronic lung disease (CLD) have early and prolonged upregulation of pro-inflammatory cytokines, adhesion molecules and profibrotic cytokines [103, 104]. In addition, the baby is exposed to the toxic effects of O_2-enriched mixtures and positive pressure ventilation. There is compelling animal evidence that postnatal parenteral corticosteroid administration interferes with secondary alveolarization [105, 106], and these infants are frequently given corticosteroids to try to wean them from ventilation. Many of the effects of these insults are predictable from knowledge of normal lung development, namely pulmonary hypoplasia, pulmonary hypertension and airflow obstruction. Morphometric studies have indeed demonstrated profound alveolar-capillary hypoplasia in infants dying with CLD [107]. In the short-term, one study showed that although oxygen saturation and saturation variability became normal, airway obstruction, far from exhibiting catch-up recovery, actually worsened and fell away from centile lines [108]. More long-term studies have also failed to show evidence of catch-up growth with evidence of airway obstruction [109] and reduced pulmonary capillary blood volume [110] even in mid-childhood. These studies provide compelling evidence that interference with alveolar growth results in permanent and lifelong change. There is no compensatory growth later on.

It is not merely positive pressure ventilation which exerts long-term adverse effects. Prematurity even without the need for respiratory support, and oxygen therapy without ventilation, are also associated with airflow obstruction in mid-childhood [111]. In one small study, surfactant therapy made no difference to airflow obstruction in mid-childhood [112]. Thus, it is unlikely that advances in neonatal intensive care will prevent long-term pulmonary complications, although the fact that ever smaller babies are being salvaged means that the pattern of lung disease may change in today's survivors compared with those who have currently reached their teenage years. This subject is discussed in more detail in another chapter of this monograph.

Effects of pneumonialpertussis on lung growth

Respiratory infections are discussed in more detail in a later chapter of this monograph. Childhood pneumonia is a potential adverse influence on lung growth, even without causing bronchiectasis or obliterative bronchiolitis [113–116]. Retrospective studies cannot unpick whether there was a pre-existing abnormality which preceded the development of the pneumonia and predisposed the child to it, and it is this pre-existing abnormality, and not the effects of pneumonia, which is being detected in follow-up studies. For example, BARKER *et al.* [116], on the basis of death certificate data, suggested that events in the first year of life predisposed children to chronic obstructive airways disease later. It is now known that events before birth are more likely the cause of what happened in the first year of life (pneumonia and wheezing), and it is likely these that have marked out the future chronic bronchitic.

There are a total of four studies which have reported on lower respiratory tract infection in childhood and later lung function in adults [113–116]. Three are confounded by lack of knowledge of any history of previous wheezing, and only one had lung function data on young adults. Thus, the findings of a recent birth cohort study are particularly interesting [113]. A total of 1,392 British people were prospectively followed up from birth in 1958 until their mid-thirties. The history of pneumonia or pertussis had been obtained from an interview at age 7 yrs. Interestingly, only one-half gave the same history at age 34–35 yrs, underscoring the unreliability of retrospective recollection. Spirometry was measured at age 34–35 yrs. A history of pneumonia was associated with small deficits in FEV1 (mean±SD: 102±73 mL) and FVC (173±70 mL), persisting after inhalation of salbutamol. The deficit was larger in subjects with no history of wheezing,

than those with current wheeze, and not significant in those with past wheezing. The deficits for pertussis were smaller, and only significant for FVC (FEV_1: 41±70 mL; FVC 81±76 mL). There was no effect on the timing of pneumonia or pertussis (before or after age 2 yrs). The effect was not lost after adjustment for multiple confounding factors. The loss of lung function probably represented a failure of growth rather than accelerated ageing. However, the study did not have any figures for pre-illness lung function, so it could not distinguish whether pneumonia occurred in children with premorbid poor lung function, or whether pneumonia caused a decline in growth. The absence of an effect of the timing of pneumonia on subsequent lung growth suggests the former.

Effect of airway inflammation on airway growth

This a controversial area, in which a number of observations have been made, but it is difficult to combine them into a coherent whole. These are: 1) in adults with asthma, delay in initiating treatment with inhaled corticosteroids results in suboptimal improvement in lung function. This has been demonstrated in a randomized controlled trial [117]; 2) in adults with asthma, bronchoscopy and biopsy show changes including subepithelial fibrosis, referred to collectively as "airway remodelling" [118, 119] and; 3) in adults with asthma, there is at least some evidence that aggressive control of asthma with high-dose inhaled corticosteroids results in regression of at least some of the changes of remodelling [120]. These observations have led to the model that the airway inflammation characteristic of asthma causes secondary fibrosis (possibly as part of a regeneration/repair process in the airway), *i.e.* that there is no remodelling without preceding inflammation.

In children the evidence that delay in initiating inhaled steroid therapy for asthma prejudices lung function, rests on an uncontrolled observational study [121], the results of which have been accepted somewhat uncritically. The paradigm that inflammation causes remodelling has also been subsumed into paediatric thinking. However, this assumption is beginning to be challenged.

Firstly, a bronchoscopic study in children with nonspecific respiratory symptoms showed evidence of airway remodelling at an early stage, long before asthma could be diagnosed [122]. Although another group have challenged this [123], the authors' own preliminary observations [124] do not show a close association between evidence of inflammation and remodelling in children with severe asthma. Thus, there is some evidence in the paediatric literature to suggest that inflammation and remodelling may in fact be processes which proceed at different rates, possibly driven by the same underlying factor, but by no means is it certain that remodelling is driven directly by inflammation. Indeed, there is evidence that an abnormality in the pulmonary extracellular matrix may drive inflammation [125], leading to the intriguing possibility that asthma is a connective tissue disease and not an airway disease at all. Secondly, the interactions between remodelling and normal growth are completely unexplored. It is likely that the same cytokines and growth factors are involved in both processes, and what may appear to be irreversible in an adult may not be so in a growing child.

In conclusion, structural airway changes are seen in small children with asthma [122, 124] and CF [126], and probably other diseases as well. The fear is that they will persist, resulting in the child entering adult life with fixed airflow obstruction. Undoubtedly this happens in some cases. However, there are many important issues which have barely been researched in this field [127, 128]. In particular, the uncritical assumption that the airway is the same in an adult and a small child, and the diseases thereof should be treated the same way, must firmly be resisted.

Congenital heart disease

Even though before birth, very little blood flows to the lung and the nature of any heart defect would little change its O_2 content, claims are made for antenatal pulmonary changes. For example, WAGENVOORT *et al.* [129] reported that the medial surface area index (ratio of the pulmonary arterial medial smooth muscle surface area to the lung parenchymal surface area) was markedly raised in foetuses found to have atrial septal defects, although it is difficult to envisage the foetal haemodynamic differences of a patent foramen ovale and secundum atrial septal defect as being more than minor.

Postnatally, however, the effects of a high pulmonary blood flow and pressure on the lung and its vasculature are well known [130–133] and are characterized initially by an abnormal extension of vascular smooth muscle into the peripheral pulmonary arteries (grade A, RABINOVITCH *et al.* [133]), progressing to an increase in percentage arterial wall thickness (grade B), finally leading to a reduction in the total number of small pulmonary arteries (grade C). These grades bear a strong correlation to the pulmonary artery pressure and vascular resistance.

In contrast, conditions leading to a low postnatal pulmonary artery flow/pressure have been researched less extensively. Neonates with pulmonary atresia histologically possess fewer, smaller and thinner pulmonary arteries though the longitudinal distribution of their muscularization is normal [134]. Thus, they have a raised alveolar-to-arterial ratio. Their bronchial arterial supply however was normal. In contrast, older children with tetralogy of Fallot have increased intra-acinar arterial and venous vessels [135] with a normal branching pattern. The larger arteries were of reduced calibre with a thicker muscular coat and there was also a reduced alveolar number and total lung volume. Whether these effects are different to those in pulmonary atresia because of the older age group or palliative surgery in some is uncertain. In the situation of inadvertent surgical ligation of the pulmonary artery in infancy, at death 3 yrs later, histology showed small alveoli with thickened walls [136].

In any event, pathologists in this field [137] admit that "There is little more to be gained in correlating structure and function in the traditional manner. The emphasis is shifting to *in vivo* assessment...".

Functional studies so far have failed to distinguish between the effects of congenital heart disease on antenatal or postnatal development. Respiratory mass spectrometry and a rebreathing technique was used at rest and on exercise, using a bicycle ergometer to measure VA (sulphur hexaflouride), effective pulmonary blood flow (acetylene) and pulmonary capillary blood volume (CO^{18}). Changes in $D_{L,CO}$ and KCO are considered to reflect the functional status of the alveolar-capillary network. It is likely that both occur given the histological changes that appear to be present early in the neonatal period. In children investigated several years after a surgically corrected, isolated pulmonary stenosis which prior to treatment would have reduced blood flow to the lungs, there was a reduced (28%) KCO (transfer factor/unit lung volume) at rest which rose in proportion during exercise and as a result remained abnormal [81]. The implication of this is that the number of functioning alveolar capillary units at rest are fewer than normal but during exercise the proportion recruited is normal, however, the absolute number remains subnormal. This effect was not significantly affected by age at surgery implying an antenatal or early postnatal origin of these changes. These findings are similar to those in untreated adult patients [138] where a lower vital capacity, total lung capacity, FEV_1 and specific lung compliance but no difference in functional residual capacity or residual volume was found together with a significantly reduced transfer factor and KCO. However, the relationship of the lung volume expressed as a percentage of total lung capacity to static recoil pressure was normal, implying that the lungs in pulmonary stenosis were small but normal. It was inferred that an abnormality occurred in lung

parenchymal growth, and that alveoli decreased more in size than in number. Functionally, postnatal pulmonary artery obstruction leads to a reduction in radiological lung volume which is restored after its relief [136].

In children, investigated long after surgical correction for an isolated secundum atrial septal defect [139] which would produce a raised postnatal, pre-operative pulmonary flow, transfer factors were normal (cf pulmonary stenosis). An adult has been described with early onset severe pectus excavatum and exercise intolerance, and a total lung capacity of 60% predicted but who had a normal $D_{L,CO}$ and a high (141%) K_{CO} at rest which did not alter with exercise. The interpretation was that maximum pulmonary capillary recruitment maintained normality at rest but there was no residual ability to respond to exercise.

Clearly, therefore, blood flow and thoracic shape affects postnatal lung development. As yet these measurements have not been made in the very young which might yield more informative results.

The importance of the perinatal history

In paediatric practice, it is routine to ask the parents about perinatal history. Many adults will be able to recall what their parents told them about their birth, and it is worth asking about their knowledge. For example, respiratory symptoms present literally from the first day of life and, in particular if severe enough to warrant admission to neonatal intensive care, are never due to asthma or immunoglobulin deficiency. Rather, they suggest in particular primary ciliary dyskinesia [140] or a congenital lung malformation. The perinatal history does not cease to be useful just because the patient is an adult.

Conclusion

The growing lung is vulnerable to disease and complications of therapy. Damage in the phase of rapid alveolar development is irreversible, and will have life-long effects. Every care must be given particularly in the vulnerable phases of development. Public health measures should be directed at allowing the optimum environment for future lung health.

Summary

Lung growth begins shortly after conception and has an adult number of airways by 16 weeks gestation, complete alveolar numbers and peripheral airway calibre by age 3 yrs and lung function which evolves over a further 15 yrs. Such a process can be considered on a molecular, histological or functional basis, each of which provides complementary but incomplete information. Disturbances to this process due to maternal smoking or malnutrition, delivering preterm, having congenital heart disease or early life viral illness all have potentially life-long consequences which need to be remembered by physicians treating adults with respiratory disease.

Keywords: Lung growth, lung development, puberty, origins of adult respiratory disease.

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