The Mammalian Respiratory System and Critical Windows of Exposure for Children's Health

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The respiratory system is a complex organ system composed of multiple cell types involved in a variety of functions. The development of the respiratory system occurs from embryogenesis to adult life, passing through several distinct stages of maturation and growth. We review embryonic, fetal, and postnatal phases of lung development. We also discuss branching morphogenesis and cellular differentiation of the respiratory system, as well as the postnatal development of xenobiotic metabolizing systems within the lungs. Exposure of the respiratory system to a wide range of chemicals and environmental toxicants during perinatal life has the potential to significantly affect the maturation, growth, and function of this organ system. Although the potential targets for exposure to toxic factors are currently not known, they are likely to affect critical molecular signals expressed during distinct stages of lung development. The effects of exposure to environmental tobacco smoke during critical windows of perinatal growth are provided as an example leading to altered cellular and physiological function of the lungs. An understanding of critical windows of exposure of the respiratory system on children's health requires consideration that lung development is a multistep process and cannot be based on studies in adults. *Key words*: branching morphogenesis, canalicular, cellular differentiation, embryogenesis, environmental tobacco smoke, fetal, pseudoglandular, saccular. — *Environ Health Perspect* 108(suppl 3):457–462 (2000).
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Development of the human respiratory system involves the differentiation and proliferation of over 40 different cell types as well as the formation of a highly ordered airway branching system with 25,000 distinct terminations giving rise to more than 300 million alveoli. The transition of the lungs from a simple protruding bud of tissue from the foregut into a highly organized, integrated, complex structure that is innervated, ventilated, and vascularized is a multistep process.

The development of the lungs begins with the evagination of an avascular epithelial bud and subsequent growth into surrounding mesenchymal tissues. After embryogenesis, the fetal lungs in all mammalian species undergo three anatomically distinct stages of growth termed pseudoglandular, canalicular, and saccular (*1*). Although the lungs have developed sufficiently to sustain life at birth, growth is far from complete. Approximately 80% of alveoli in the adult lung arise postnatally. In essence, lung development is a continuum from embryogenesis through early adolescence.

The stages of lung development are controlled by a variety of factors that modulate the timing and pattern of cellular proliferation and differentiation as well as branching morphogenesis. Recent reviews by Hackett and Gitlin (*2*), Shannon and Deterding (*3*) and Ramon (*4*) stress the importance of a number of transcription factors, molecular signals, and soluble factors in orchestrating the developmental process of the respiratory tract. These molecular signals are expressed in both temporal and spatial patterns to facilitate normal lung development and to regulate epithelial–mesenchymal interactions, cellular proliferation, extracellular matrix deposition and composition, growth factor and receptor expression, and cell-to-cell interactions.

Exposure to a variety of toxicants and/or conditions during lung development has the potential to significantly affect the overall growth and function of the respiratory system. The target of a toxic insult to the lungs during development is likely to involve the disruption and/or alteration of a specific molecular signal or transcription factor, but to date, little information is available as to the precise effect of such exposures. However, timing of exposure during development appears to be critical in the subsequent effects observed. For example, maternal malnutrition during gestation may significantly retard fetal growth and the development of the lungs, leading to compromised lung function throughout life. In contrast, exposure to environmental toxicants such as secondhand cigarette smoke may actually accelerate the maturation of specific cell types in the fetal lung (*5*), but the effects of such a change on overall lung function are unknown in the newborn through adulthood.

A number of studies suggest that the processes of cellular differentiation, branching morphogenesis, and overall lung growth can be affected by exposure to chemicals. The effects of exposure, however, are likely to be different for each period of development. For example, during embryogenesis and fetal development, cell number, cell type, and cell function of the airways and alveoli may be significantly affected by exposure to a diverse number of substances and/or conditions. Both embryogenesis and fetal gestation represent critical periods of cellular differentiation and branching morphogenesis. However, because cells continue to differentiate and divide during the postnatal period, chemical exposure during the postnatal period is also likely to affect the respiratory system but in a different manner based on changes in the process of differentiation and morphogenesis (*6*). Because growth is essentially complete by the end of adolescence, exposure to chemicals and other factors are likely to have completely different consequences in the adult compared to children (*6–8*).

Abnormal developmental changes that occur in the perinatal period because of exposure to a variety of chemicals and/or conditions may have long-term effects persisting into adult life. Exposure to substances during critical windows of development may have profound effects that would not be seen if the same exposure were to occur in the adult. Because lung development occurs over the entire perinatal period, exposure effects can have significant consequences whether they occur during the pre- or postnatal period of life. However, our current understanding of these changes is extremely limited.

The purpose of this paper is to examine critical windows of exposure affecting the respiratory system and children's health. We describe each stage of the respiratory system during pre- and postnatal development, beginning with embryogenesis and fetal gestation. Postnatal development through puberty will be examined. We discuss health outcomes as a result of exposure during these

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time periods, and we consider a comparison of similarities and differences between humans and animals and gaps in our knowledge.

Lung Development

The beginning of lung growth occurs in embryogenesis. In humans, the formation of the lungs starts near the end of the first month of gestation, and the pseudoglandular, canalicular, and saccular stages of development follow in sequential periods of approximately 10–12 weeks each, respectively (Table 1) (*9,10*). A similar pattern of lung development is also observed in other mammalian species, although the timing and onset of each stage are considerably different based on the actual length of the gestational period and the relative degree of lung maturation at birth (*10,11*).

Embryogenesis

The lungs in humans first appear at the end of the first month of gestation as an evagination of epithelium from the foregut. The bud rapidly divides as a series of branching tubes in a dichotomous pattern. These tubular branches invade and interdigitate with mesenchymal tissues. Branching morphogenesis during this period forms the most proximal portions of the future tracheobronchial tree. As these tissues grow, they push into the future pleuroperitoneal cavity of the embryo.

During embryogenesis, transcription factors play an important role in gene expression and regulation. Transcription factors are essential in both the stimulation and inhibition of gene expression to regulate the proper temporal and spatial patterning of lung development. Hepatocyte nuclear factor-3 (*12*) and the homeobox gene *TTF-1* (*13*) are examples of transcription factors serving as important regulators of early differentiation of the pulmonary epithelium during this period.

Lung development is also highly dependent on interactions between the epithelium and mesenchyme. This dual origin of lung tissues is critical in development. Removal of mesenchyme from the tip of a lung bud during early phases of development with transplantation to the side of a higher ordered segment abolishes further branching at the site of removal while stimulating growth of a new branch at the site of transplantation (*14*). Lung vascularization arises from budding of vessels from the aortic arches and connection to the vascular plexes forming within the pulmonary mesenchyme.

Fetal Lung Development

Lung development during fetal gestation is categorized into three stages: pseudoglandular, canalicular, and saccular.

Pseudoglandular stage. Tubular branching of the human lung airways continues from the fifth to the seventeenth week of gestation. As early as 2 months of gestational age, all segmental bronchi are present. During this period, the lungs take on the appearance of a glandlike structure. This stage is the most critical for the formation of all conducting airways. During this period, the airway tubular structures are lined with tall columnar epithelium, whereas the more distal structures are lined with cuboidal epithelium.

A number of signals arising from epithelial–mesenchymal interactions during this time continue to modulate cellular proliferation temporally as well as spatially (*4*). These regulatory signals lead to further branching morphogenesis by affecting the rate of cellular proliferation (*15*). The presence of extracellular matrix molecules, including collagen, fibronectin, laminin, glycosaminoglycans, and proteoglycans, as well as cell membrane-bound integrins, also plays an important role in directing lung development by influencing the rates of cellular proliferation and differentiation (*3,16,17*). Mechanical distention exerted on the lung as well as on specific cell types can also significantly affect gene expression and, ultimately, lung growth and development (*4*). A variety of growth factors and growth factor receptors are also important in controlling cellular functions (*3*). Epidermal growth factor, transforming growth factor-α, and retinoic acid all act to affect branching morphogenesis and cellular differentiation (*18,19*).

Epithelial differentiation of ciliated, goblet, and basal cells first appears in the most central airways during this stage of development. Cartilage and smooth muscle cells are also first noted in the trachea and extend more peripherally with progressive growth of the lungs. During this stage of development, the vascular tree develops along with the bronchial tree. Pulmonary arteries follow the branching pattern of the airways, whereas the veins run within the mesenchyme to define the margins of future lung segments and subsegments.

Canalicular stage. This stage lasts from week 16 to week 24 in the human fetus. Lung morphology changes dramatically during this time because of differentiation of the pulmonary epithelium, resulting in the formation of the future air–blood tissue barrier. Surfactant synthesis and the canalization of the lung parenchyma by capillaries begin. During this stage, the future gas exchange regions can be easily distinguished from the future conducting airways of the lungs.

Saccular stage. The saccular stage of lung development in humans lasts from week 24 to near term. The most peripheral airways form widened airspaces, termed saccules. These saccules widen and lengthen the airspace, in large measure by the addition of new generations. During this stage, the future gas exchange region expands significantly. Populations of fibroblastic cells also undergo differentiation during this stage. These fibroblast-like cells are responsible for the production of the extracellular matrix, collagen, and elastin. It is also presumed that they play an important role in epithelial differentiation and control of surfactant secretion in connection with the growth of the gas exchange region during this stage. The vascular tree also grows in length and diameter during this time.

Branching morphogenesis. Branching morphogenesis reflects the pattern of fetal lung development continuing into the postnatal period, characterized by the enlargement of the airways and gas exchange regions by repeated branching and outgrowth of tissues from rapidly dividing epithelial buds. This growth occurs in a radial pattern beginning from the original tracheobronchial mass and extending outward to form multiple future conducting airways before forming the gas exchange portions of the lungs. Branching morphogenesis is a continuous process, playing a critical role in all stages of fetal lung development. As reflected in the stages of development (i.e., pseudoglandular, canalicular, and saccular), conducting airways as well as the ventilatory units forming the gas exchange portions of the lungs can only be formed through a process of branching growth.

The branching of future airways and gas exchange regions of the lungs is critically dependent on factors expressed within the mesenchyme into which these epithelial buds invade (*4,15,16*). Budding and branching processes involve differential factors that regulate focal cell proliferation, as well as changes in epithelial shape and differentiation

Table 1. Development of fetal lungs.

Species	Gestational age (days)				
	Term	Embryonic	Pseudoglandular	Canalicular	Saccular
Human	280	< 42	52–112	112–168	168
Mouse	20	< 9	16	18	19
Rat	22	< 13	16–19	19–20	21
Rabbit	32	< 18	21–24	24–27	27
Sheep	150	< 40	40–80	80–120	120
Primate	168	< 42	57–80	80–140	140

Modified from Meyrick and Reid (*9*) and Fanucchi and Plopper (*10*).

(*4,19*). Factors within the mesenchyme, particularly those present in the extracellular matrix, are influential in directing branching morphogenesis. Fibronectin, laminin, and tenascin are specific extracellular matrix components thought to play critical roles in this process (*20–22*). Growth of the lungs by tubular epithelial branching may be regulated by a variety of substances such as epidermal growth factor, transforming growth factor-α, and retinoic acid (*4,19*).

Organized interaction between the epithelial, interstitial, and vascular compartments forming the gas exchange portion of the lungs is critical to the overall growth, development, and formation of alveolar structures. The synthesis and laying down of extracellular matrix including collagen and elastin are critical in creating the scaffolding on which epithelial cells and the capillary bed intertwine to form alveolar shapes that are maintained throughout life (in the absence of disease).

The process of alveolarization is an extension of saccular formation begun in the late gestational period, identified by the formation of secondary alveolar septa arising from primary alveolar septa (Figure 1). In all species, alveolarization continues postnatally. In species with short gestational periods, birth occurs before the formation of true alveoli in the lungs. Therefore, in some species alveolarization can be considered only a postnatal event (Table 2). In species with longer periods of gestational development, saccular formation is superseded by the formation of new alveoli before birth. In these species, true alveolar structures are present at birth. Although the timing of alveolarization differs among species, the formation of new alveoli continues as a postnatal process in all mammalian species.

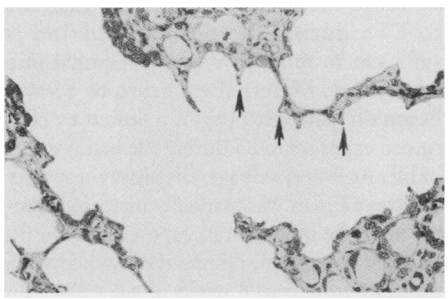

Figure 1. Centriacinar region from the fetal lung of a near-term pig with the parenchymal walls undergoing the process of alveolarization. Short buds of secondary alveolar septa (arrows) extend out from thick primary alveolar septa to form a future alveolar duct.

Table 2. Formation of alveoli in perinatal lungs.

Species	Onset	Completion
Human	252 DGA	730 DPN (2 years)
Mouse	1–2 DPN	28 DPN
Rat	1–4 DPN	28 DPN
Rabbit	30 DGA	?
Sheep	120 DGA	?
Primate	155 DGA	365 DPN (1 year)

Abbreviations: ?, unknown; DGA, days gestational age; DPN, days postnatal age.

Postnatal Lung Development

The formation of new alveoli is critical for continued growth of the lungs into early adulthood. An increase in body mass from birth to adulthood is accompanied by increasing metabolic demands of the organism. Therefore, to meet the metabolic needs of the organism during postnatal growth, lung growth must parallel increases in body mass. A number of studies have demonstrated a strong allometric relationship among lung volume, alveolar surface area, and body mass over a wide range of mammalian species (*23*). Allometric relationships support the concept that there is a close relationship among these parameters and that they must be maintained in a similar manner to ensure the proper exchange of gases between the environment and the organism.

Cellular differentiation. Cellular differentiation of the lungs continues throughout the lifespan of all species; however, this process is most evident during the fetal and early postnatal periods. Little is known about the mechanisms that regulate cellular differentiation. Cellular differentiation begins in the proximal airways and progresses in a radial fashion down the growing airways. Differentiation of the tracheal epithelium has been most extensively studied in the respiratory system. A summary of this differentiation process and the timing for the appearance of a variety of epithelial cell types within the developing trachea is shown in Figure 2 (*24*).

Columnar cells that are undifferentiated characterize the first epithelial cells lining fetal lung tubules. The first epithelial cells to differentiate in the trachea are neuroendocrine cells, followed closely by ciliated cells, and finally basal and secretory cells in rapid sequence. This process of differentiation covers a developmental period ranging from days to months. In rodents including the mouse, rat, and hamster, complete

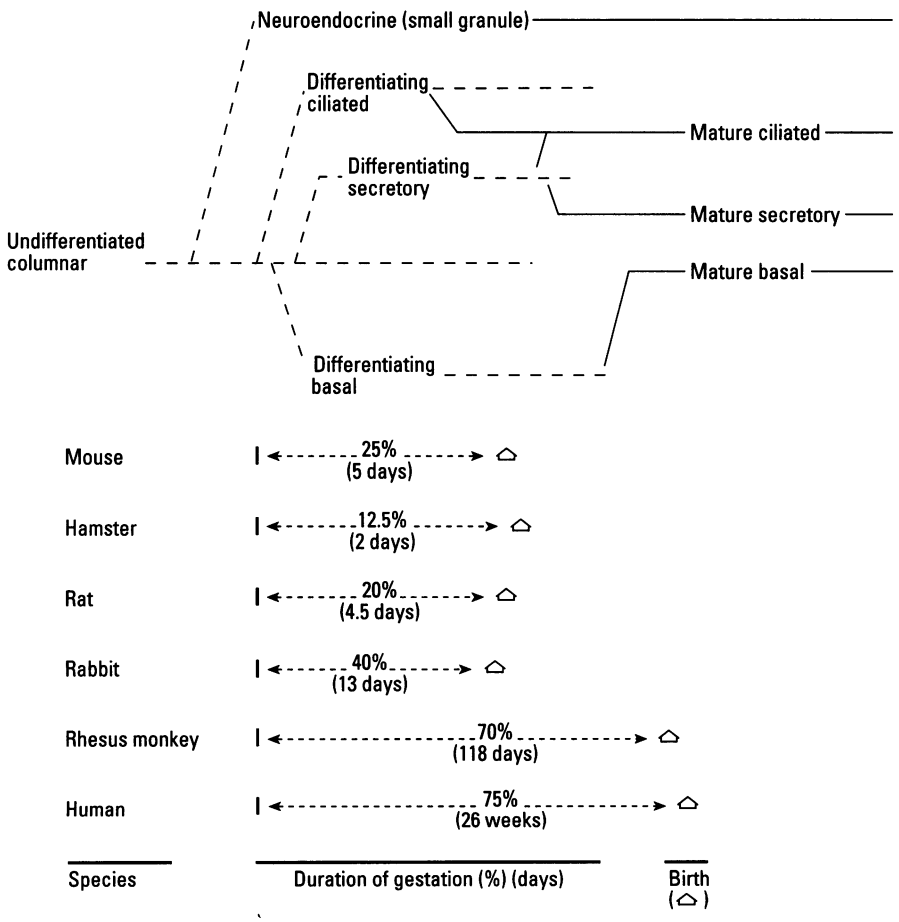

Figure 2. Comparative analysis of cellular differentiation of the tracheal epithelium. Reproduced from Plopper et al. (*24*), with permission of the American College of Chest Physicians. This analysis is based on a summary of studies in six species: mouse, hamster, rat, rabbit, rhesus monkey, and human. In these species, cells undergoing ciliogenesis occur before the beginning of secretory and basal cell differentiation. The absolute length of time (in days) and the percentage of gestation during which the process occurs prenatally is estimated based on the time of first appearance of ciliated cells.

epithelial differentiation of the trachea occurs in as little as 2 days. In primate trachea, cellular differentiation takes up to 6 months to be complete. In most species, epithelial cell differentiation of the trachea usually is not complete until just before birth. For more peripheral airway generations, cellular differentiation is likely to continue into the early postnatal period.

Fetal epithelial cells are typically filled with glycogen that is gradually replaced with a granular cytoplasm filled with numerous organelles during cellular differentiation. These glycogen-filled cells are found throughout the tracheobronchial tree as well as into the most peripheral saccules. Differentiation of the epithelium is highly site specific, giving rise to more than 10 different cell types. For example, within the saccules of the lungs, cells lining these surfaces differentiate to form both squamous type 1 cells as well as cuboidal type 2 cells. The presence of glycogen within these cells may persist into early postnatal life.

Because cellular differentiation occurs in a radial fashion beginning at the trachea, the more distal airways undergo cellular differentiation at a later time during gestation. A number of cells in the more distal airways possess specific metabolic functions including the nonciliated bronchiolar epithelial (Clara) cell. In the fetal state these cells contain abundant amounts of glycogen. Maturation of this cell is characterized by the formation of abundant amounts of smooth and rough endoplasmic reticulum in the basolateral and apical regions of the cell cytoplasm that occur almost exclusively during the early postnatal period. Recent studies suggest that changes in the subcellular structure of cells coincide with the first expression of metabolic function. These sequences of cellular differentiation and maturation reflect the fact that the newborn respiratory system is far from being developed and completely functional at birth. Changes must continue through the postnatal period before the lungs attain characteristics of the adult lungs.

Development of lung xenobiotic metabolizing enyzme systems. Many of the enzymes that play a critical role in lung metabolism are not fully developed at birth. A number of these enzymes are responsible for both the activation and detoxification of xenobiotic compounds. Among the most important enzyme systems to develop during the mid to late gestational periods through early childhood are the glutathione *S*-transferases (*25*) and epoxide hydrolases (*26*). Antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase also appear during this period (*27–29*).

A highly critical family of isozymes involved in the bioactivation and detoxification of xenobiotic compounds is the cytochrome P450 monooxygenase system. The developmental profile of the cytochrome P450 monooxygenase system closely coincides with changes in subcellular composition of Clara cells and endothelial cells during the late gestational and early postnatal period of development. Although this family of isozymes may first appear near the end of gestation, they are more likely to develop during the postnatal period. A number of studies showed that early postnatal exposure to environmental toxicants can alter the developmental profile for these isozymes (*30*).

Potential Targets of Toxic Agents during Lung Development

The effects of many toxicants on the respiratory system have been well characterized in the adult, but less is known in the developing lung. A number of toxicants affect the developing lungs. These include environmental tobacco smoke (ETS) (*5,30–33*), bioactivated compounds (*6–8*), and oxidant gases (*34,35*). The target for a number of these compounds is in large measure airway epithelial cells undergoing maturation and/or rapid proliferation. However, the precise mechanism leading to greater sensitivity of these cells in the neonate compared to the adult is still unknown.

Administration of steroids during lung development can also alter the normal process of lung maturation (*36,37*). The most likely target cell is the epithelial cell that may be altered by an accelerated maturation and a reduction in the rate of proliferation. Our knowledge of the precise effects of chemicals on lung development is still extremely limited. In addition to toxicants, it is also important to keep in mind that nutritional deficiency can also significantly alter lung development (*38*).

ETS

The lungs are extremely sensitive to a large number of inhaled toxicants (*39*). ETS is an excellent example of an airborne pollutant that contains many of these toxicants. ETS is defined as a combination of exhaled mainstream smoke and sidestream smoke (SS) given off from the smoldering end of a cigarette. A strong relationship has been demonstrated between respiratory illness in young children and ETS exposure (*31*).

Exposure to ETS is also associated with significant risks in the development and/or exacerbation of asthma, airway hyperresponsiveness, and other respiratory symptoms such as cough, wheeze, and mucus production. Epidemiological studies suggest that exposure to smoke during the perinatal period may have adverse effects on lung function that can persist into adulthood. However, the mechanisms leading to this process are unknown.

Recent studies from our laboratory (*32*) have used various exposure regimens to aged and diluted SS as a surrogate to ETS to determine the existence of critical windows of exposure during the perinatal period of development. Timed pregnant rats were exposed to environmentally relevant concentrations of SS using a concentration of 1 mg/m^3 total suspended particulates. We examined timed pregnant dams under the following four exposure regimens: *a*) exposure to SS only during the *in utero* period, *b*) exposure to SS only during the postnatal period, *c*) exposure to SS during both *in utero* and postnatal periods, and *d*) exposure to filtered air during both *in utero* and postnatal periods. All rat offspring were examined at 7–10 weeks of age (*32*). Animals exposed to SS during both the *in utero* and postnatal periods exhibited marked alterations in airway sensitivity (Figure 3) and pulmonary neuroendocrine cell (PNEC) frequency (Figure 4) in contrast to animals that had been exposed to SS only during the prenatal period or only to SS during the postnatal period but not both. We hypothesized that these changes are due to SS exposure during critical windows of lung growth and development that include both fetal and early postnatal periods of life. Such exposure conditions are not uncommon in humans and appear to be essential for airway hyperresponsiveness to develop.

Because differences noted in this study could also be attributed to the duration of exposure rather than the timing of exposure, we tested the hypothesis that early exposure to ETS during the perinatal period is sufficient to produce a lasting response into adulthood. Maternal exposure to SS was begun on gestational day 5, followed by continued exposure to SS during the first 3 weeks of life. At 8 weeks of age, the airway reactivity of these animals was markedly increased compared to the lungs of rats exposed only to filtered air (Figure 5), despite the absence of SS exposure from 3 to 8 weeks of age (*33*). This model appears highly analogous to the development of pulmonary function decrements in children exposed to tobacco smoke during the perinatal period (*40,41*). Therefore, this animal model affords the opportunity to examine more precisely critical periods during lung development when exposure to SS leads to increased airway responsiveness and whether changes in PNEC number and/or function might be responsible for these changes. Critical windows of exposure during the perinatal period that affect airway reactivity are clearly defined in these experimental studies, but the long-term effects of such perinatal exposures into adulthood are not known (Figure 6).

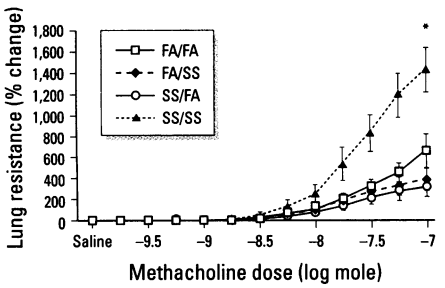

Figure 3. Methacholine-induced changes in lung resistance after differential timing of exposure to ETS during the perinatal period of life. Abbreviations: FA, filtered air; SS, sidestream cigarette smoke. Rats were exposed to FA and/or aged and diluted SS using four different timing regimens: *a*) exposure to FA during both *in utero* and postnatal periods (FA/FA), *b*) exposure to SS only during the postnatal period (FA/SS), *c*) exposure to SS only during the *in utero* period (SS/FA), and *d*) exposure to SS during both *in utero* and postnatal periods (SS/SS). Reproduced from Joad et al. (*32*), with permission of Academic Press, Inc.

*Repeated measures analysis of variance on log-transformed data demonstrated a statistically significant difference ($p < 0.05$) between SS/SS and all other exposure groups.

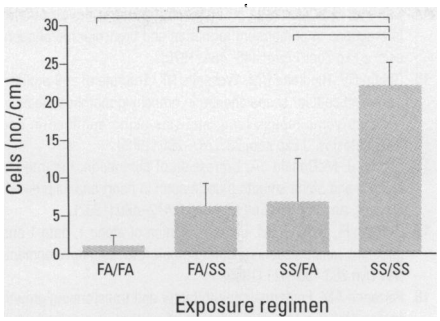

Figure 4. Pulmonary neuroendocrine cell number per centimeter basal lamina length in the airways of rats after differential exposure to ETS. Abbreviations: FA, filtered air; SS, sidestream cigarette smoke. Neuroendocrine cells were identified by neuron-specific enolase staining (*32*). Labeling of each exposure group is identical to that found in Figure 3.

Those animals exposed to SS/SS had significantly greater numbers of neuroendocrine cells compared to all other groups, as indicated by the brackets ($p < 0.01$).

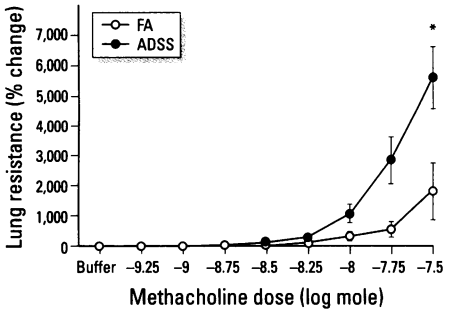

Figure 5. Methacholine-induced changes in lung resistance with perinatal exposure prenatal and 3 weeks postnatal to aged and diluted sidestream smoke (ADSS) or to FA followed by 5 weeks of exposure to FA. Repeated measures analysis of variance on log-transformed data demonstrated persistent airway hyperresponsiveness in animals exposed to ADSS compared to animals exposed only to FA ($p < 0.02$). Reproduced from Joad et al. (*33*), with permission of Academic Press, Inc.

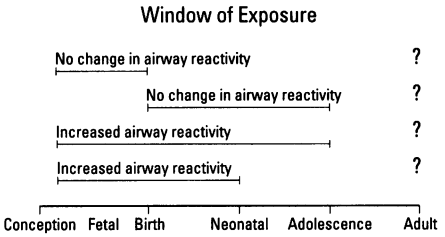

Figure 6. Diagram of critical windows of exposure to ETS during lung development. Horizontal lines represent timing of exposure to ETS. In rats, exposure began as early as gestational day 5 (fetal) and continued through postnatal day 60 (adolescence). Some exposures ended by 21 days postnatal age (neonatal). Airway reactivity in all instances was measured at 60 days postnatal age. The results of these experimental studies suggest that the timing of exposure to cigarette smoke is critical to the biological outcome of increased airway reactivity when measured in adolescence. This critical period of exposure includes both *in utero* and early postnatal periods of life. However, the consequences of perinatal exposure to cigarette smoke in the adult are unknown.

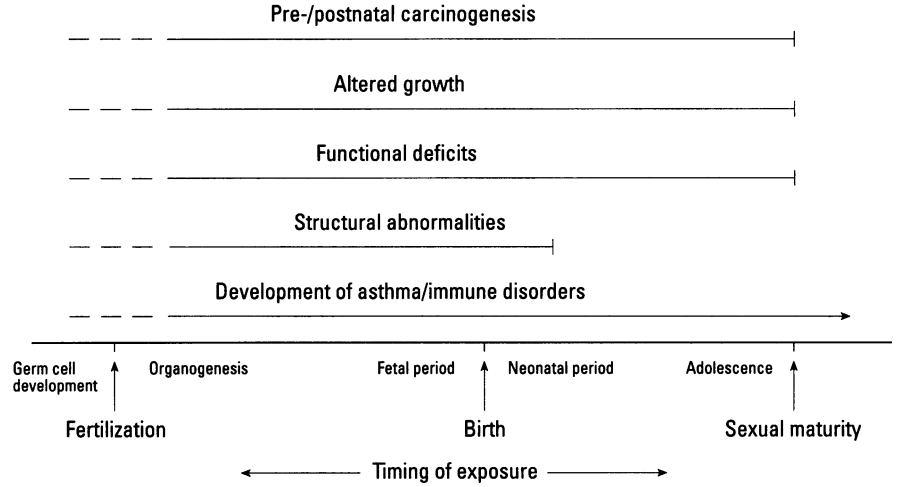

Figure 7. Respiratory effects associated with timing of exposure. Although little information is currently available, a variety of respiratory health outcomes may occur with perinatal exposure to environmental and chemical toxicants and be manifested throughout life. For each respiratory health outcome shown, the solid horizontal line represents the potential window of exposure leading to this effect.

Conclusions

To better understand the potential effects of critical windows of exposure in children on the respiratory system, it is important to consider the following factors that characterize the process of lung development. First, lung development is a multievent process that is not restricted to prenatal life. Although the lungs undergo dramatic changes during the embryonic, pseudoglandular, canalicular, and saccular stages, the majority of changes to the lungs continue postnatally during the process of alveolarization. Second, only a limited number of maturational events must be finished at birth for successful survival of the organism. Third, cellular differentiation, branching morphogenesis, and overall growth define lung development during both the pre- and postnatal periods. Finally, all of these developmental events occur in the presence of an increasing mass of total cells.

Gaps in Our Knowledge

The susceptibility of children to exposure to a wide range of environmental toxicants cannot be based on studies in adults. Cellular differentiation, cellular proliferation, and cellular physiological function of the lungs are continually changing during gestational and postnatal growth. The sensitivity of these cells and their responses to environmental insults are likely to be completely different compared to those found in the adult. The route of delivery of an environmental toxicant to the respiratory system is completely different during the fetal period compared to the postnatal period. Influences of passage through other organ systems and the vasculature as well as through maternal organ systems must be taken into consideration. Our knowledge base regarding perinatal exposure and critical windows is negligible (Figure 7). Without question, there is still much to be learned about the effects of toxicants on gene regulation, molecular signaling, and growth factors during lung development. Without the careful control and proper timing of expression for these factors, growth could become misdirected and chaotic. Future studies must be designed to address these critical windows of exposure to provide meaningful answers for the benefit of healthy children into adulthood.

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