Pre- and Postnatal Lung Development, Maturation, and Plasticity
Invited Review: Pulmonary alveoli: formation, the “call for oxygen,” and other regulators

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Massaro, Donald, and Gloria D. Massaro. Invited Review: Pulmonary alveoli: formation, the “call for oxygen,” and other regulators. Am J Physiol Lung Cell Mol Physiol 282: L345-L358, 2002; 10.1152/ajplung.00374.2001.—The lung’s only known essential function is to provide sufficient alveolar surface to meet the organism’s need for oxygen and elimination of CO2. The importance of the magnitude of alveolar surface area (Sa) to O2 uptake (VO2) is supported by the presence among mammals of a direct linear relationship between Sa and VO2. This match has been achieved, despite the higher body mass-specific VO2 of small organisms compared with large, by a greater subdivision of alveolar surface, not by a larger relative lung volume in small organisms. This highly conserved relationship between alveolar architecture and VO2 suggests the presence of similarly conserved mechanisms that control the onset, rate, and cessation of alveolus formation and alveolar size, which are also influenced by retinoids and thyroid and corticosteroid hormones. Furthermore, the “call for oxygen” is met at a breathing rate and tidal volume at which the work of breathing is lowest. Thus there is a complex, fascinating, but poorly understood, signaling relationship among VO2, the neural regulation of breathing, and lung architecture, composition, and mechanics.

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\[ \text{Vo}_{2}, \text{ the neural regulation of breathing, and lung architecture, composition, and mechanics.} \]

In this paper we review and try to synthesize, interpret, and understand some recent and old findings about the formation of alveoli, its regulation, and the plasticity of the architecture of the lung's gas-exchange region. We do not review development of the fetal lung, for which there are several recent reviews (61, 107, 150); we review only selected insights from studies on mutant animals, and we do not review the lung's architectural response to pneumonectomy.

**FORMATION OF ALVEOLI: TIMING, ARCHITECTURAL METHODS, AND SITES**

To facilitate exposition and to conform to the literature (5, 25, 26, 95), we call the gas-exchange structures of the architecturally immature lung alveolar sacules,

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**Fig. 1.** Interspecies relationship between alveolar surface area (Sa) and total organismal oxygen uptake (\(\text{Vo}_{2}\)). [From Tenney and Remmers (143).]

**Fig. 2.** The distance between alveolar walls (Alveolar Diameter) is plotted against the organism's body mass-specific oxygen consumption (\(\text{Vo}_{2}\)). [From Tenney and Remmers (143).]

**Fig. 3.** The work of breathing in humans as the breathing frequency changes. [From Otis et al. (122).]
their subdivision into smaller units (alveoli) septation, and the period in which septation occurs the period of septation. The formation of alveoli other than by septation of alveolar saccules is referred to as “other,” but the architectural mechanism by which the other occurs is unclear (see below). We discuss evidence that, at least in part, the regulation of septation and other means of forming alveoli may differ.

In all mammals of which we are aware, pulmonary alveoli are formed, in part, by septation of alveolar saccules (3, 5, 17, 25, 26, 28, 33, 41, 43, 44, 46, 83, 154, 156, 157). However, the time in development during which septation occurs varies considerably among species in a manner that greatly reflects the newborn’s activity lifestyle. For example, guinea pigs (33) and range mammals (3, 28, 154), which have great locomotive capacity at birth, septate in uterus; others, e.g., rats (25, 26) and mice (5), with little locomotive capacity at birth, septate after birth. Humans septate during the last month of gestation and during the postnatal period (83, 156, 157). Precisely when septation ends in humans is uncertain, but it seems to continue for at least a few months after birth (83, 156, 157).

Burri et al. (25, 26) published some of the first and most useful modern era studies on septation. Among many important observations, they showed that the distance between alveolar walls (Lm) diminishes during the period of septation (5, 26). In rats, ~70% of this diminution occurs by postnatal (PN) day 7 (26), suggesting that septation is almost complete by then. This interpretation of the 70% fall in Lm was supported and extended by the use of procedures that allow estimation of the volume of individual alveoli. Thus the average volume of individual alveoli in rats falls sixfold between PN days 1 and 6 (125), but only little more over a period twice as long, i.e., between PN days 2 and 14 (95) (Fig. 4). Because the rate at which lung volume increases does not change during the period of septation (24), the virtually identical fold fall in volume over 6 days and 12 days indicates septation is complete, or almost complete, by PN day 7, as was gleaned from the data of Burri et al. (26).

Based on the number and volume of individual alveolar saccules present in 1-day-old rats (before septation) and the number of alveoli in 6-day-old rats (after the onset of septation), Randell et al. (125) calculated septation of alveolar saccules to account for only about one-third of the alveoli formed between PN days 1 and 6. Calculations from similar measurements on rats on PN day 2 and PN day 14 indicate that ~25% of alveoli formed during the period of septation result from septation of the original alveolar saccules (95). These observations support the notion (93) that during the period of septation, alveoli are formed by septation of the original saccules present at birth and by other as yet poorly identified architectural events, which probably occur at the periphery of the lung.

Beginning in the second PN week and accelerating in the third, alveolar walls become thinner (25, 26) due, at least in part, to apoptosis of interstitial cells of the alveolus (7, 22, 136). This timing is stressed to encourage consideration of the role in these processes, e.g., ending septation, alveolar capillary remodeling, and apoptosis, of several molecules, already identified, whose expression peaks in rat lung at approximately PN day 7–9. These molecules include galectin-1 (30, 124), cellular retinoic acid binding protein-I (CRABP-I) (120), rA5D3, a recently cloned gene (15), and cGMP phosphodiesterase (57). Bioinformatic and experimental molecular searches for additional genes whose expression is brief elevated or depressed around PN day 7–10 and that share similar molecular binding sites in their promoter regions should be fruitful.

In rat (13, 95) and mouse (75), species whose gas-exchange region has been studied most completely, alveoli continue to form after PN day 14 until about age 40 days (Fig. 4). However, little about the anatomical process or sites of the postseptation formation of alveoli has been directly shown. Reports (18, 46) that the number of generations of conducting airways diminishes after birth in dogs have suggested these airways may have been remodeled into gas-exchange airways, thereby increasing the number of alveoli (so-called retrograde alveolarization). However, because there are so few terminal airways compared with the number of alveoli, it is unlikely retrograde alveolarization, if in fact it occurs, would produce many alveoli. More importantly, using rigorous sampling techniques and morphometric procedures, Randell et al. (125) did not detect a change in the number of terminal bronchiules in rats during the early PN period. Many additional questions about other means of forming alveoli remain. For example, after the period of septation has ended, are alveoli formed throughout the lung among already formed alveoli, or are they generated, as the thorax enlarges, in a more peripheral location, i.e., in the subpleural zone, much as a tree increases in length and crown size by growth from the peripheral tips of its branches? Reason and indirect evidence support the periphery as the site of postseptation formation of alveoli. Thus if after septation of alveolar saccules is complete and alveoli continue to form throughout the lung by production of septa among alveoli already

Fig. 4. Age-related changes in the mean volume of individual alveoli (V) and in the number of alveoli per rat (N). [Values for ages 2 and 14 days from Massaro and Massaro (95); values for age 44 days from Blanco et al. (13).] Values for age 60 days and age 95 days not previously published.
formed, alveoli should become smaller, not larger, after age 14 days (Fig. 4) unless lung volume increases more rapidly than alveoli are formed, which does not occur (24). The presence of a uniform turnover throughout the lung of extracellular matrix after age 14 days would support the notion of alveolus formation throughout the lung. However, within the limits of the methods used to assess it, matrix turnover is more rapid among subpleural alveoli than among more central alveoli (96). This supports the notion the periphery is the site of the postseptation formation of alveoli. Finally, analysis of changes in alveolar septal border lengths during PN development of ferrets suggests septation of already formed alveoli is not a prominent mechanism for an increase in the number of alveoli and size of alveolar surface area after the period of septation (155). From these findings and considerations, we propose that after the period of septation, alveoli are formed predominantly in the peripheral subpleural region. We extrapolate from these same considerations that during the period of septation, the other means of alveolus formation takes place in the subpleural region.

The cellular and molecular bases for the putative shift in the location of alveolus formation from throughout the lung, including the subpleural region, to only the subpleural region, are unclear. The architectural mechanism(s) for the peripheral formation of alveoli is also unknown. However, if the lung’s gas-exchange region is considered to be a series of branching tubes, continued branching of the distal alveoli and septation of the blunt ends of the branches to generate appropriately sized alveoli as the thorax enlarges are to us, an attractive possibility as a mechanism of alveolus formation after the period of septation (93). Supporting this possibility, cells that store retinol, a precursor of all-trans retinoic acid (ATRA), which can induce alveolus formation (see below), are diffusely distributed in the lung during the period of septation (147) but become concentrated in the subpleural region after that period has ended (81, 96, 119). It will be of interest to test whether treatment with ATRA, which induces alveolus formation in adult rodents (12, 98, 99), causes the in vivo appearance of lipid interstitial cells throughout the lung.

THE “CALL FOR OXYGEN”

Hyperoxia. The clear relationship among O2 need, alveolar size, and the magnitude of alveolar Sa from the smallest to the largest mammals (Figs. 1 and 2) (143) suggests that hyperoxia (excess O2) and hypoxia (or other causes of cellular O2 shortfall) have opposing regulatory effects on the size of the alveolar Sa. Experimental work supports this notion but must be considered in light of the fact that most of it has been carried out in rats and with consideration of the potential harmful effects on cellular function exerted by hyperoxia and hypoxia through the production of O2 radicals (50). For example, among several studies that show hyperoxia diminishes septation (9, 23, 48, 137, 151), those of Randell et al. (125) in which newborn rats were exposed to 95% O2 from PN day 1 to PN day 6 provide the most quantitative information, including the demonstration that hyperoxia depresses other means of forming alveoli as well as septation (125). However, 95% oxygen severely damages the lung (23, 48, 137, 151) and slows body growth (139); it is, therefore, uncertain whether the decreased rate of alveolus formation reflects a need for less gas-exchange surface in an O2-rich environment, the toxic effect of O2 directly on the lung, an effect on the lung of systemic O2 toxicity, or a combination of these possibilities.

The issue of O2 toxicity was somewhat diminished by Burri and Weibel (27), who began much of the early work on the relationship between O2 need and lung architecture. They exposed rats that had already septated but were still growing rapidly to 40% O2, which is much less damaging than 95% O2. A key feature of their experiments is that the rats exposed to 40% O2 increased body mass at the same rate as air-breathing rats of the same age. In spite of identical rates of body growth, which suggests the absence of systemic damage due to hyperoxia, lung growth in O2 rats, as evidenced by total lung volume, alveolar capillary volume, lung tissue volume, alveolar Sa, and alveolar capillary Sa, was ~16% less than in air-breathing rats (27). However, pulmonary oxygen toxicity must still be considered because at any concentration of inspired O2, the lung is exposed to a higher Po2 than other tissues. Nevertheless, our operational conclusion regarding the Burri and Weibel data (27) is that a diminished rate of increase of gas-exchange surface, without a concomitant inhibition of increase of body mass, reflects a need for less alveolar Sa due to a greater delivery of O2 to tissues in an O2-enriched environment.

One of the anonymous reviewers of this manuscript pointed out that the arterial O2 content at 40% O2 is only about 2% higher than at 21% O2 and asks, “Why should that tiny increase in O2 content cause the degree of damage in alveolar architecture reported by Burri and Weibel (27)?” This is a good, thought-provoking question for which we lack an equally good answer. Perhaps the most obvious answer is that our operational conclusion about the Burri and Weibel work is wrong, and the difference between alveolar and peripheral tissue O2 tension at these concentrations of O2 is sufficient to cause alveolar O2 toxicity without O2 toxicity in the peripheral tissues. Conversely, however, if it is peripheral tissue(s) that sounds the call for oxygen, the difference in peripheral tissue Po2 at 20.9% and 40% inspired O2 may be sufficient to signal the need for less lung. Such putative tight regulation would be consistent with the notion of symmorphosis, i.e., sufficient but not excess tissue for functional need (152). The molecular basis for sensing O2 need, the location of the sensor(s), and the signaling path(s) constitute a challenging and exciting area of research.

Hypoxia. The relationship between lung function and a chronically low inspired Po2 has been intensively studied for many years (for a range of reviews, see Refs. 8, 34, 51, 67, 76, 82, 110, and 111), mainly
because of people native to high altitude whose forbears lived for generations at high altitude. We refer to those individuals as highlanders, and we refer to those of the same race, native to sea level, as lowlanders. It is important to point out that among highlander populations in different parts of the world, e.g., South America, the U.S. Rockies, Tibet, and Ethiopia, those populations that have established high altitude residence earliest in evolutionary time seem to be most adapted, perhaps reflecting more time for genetic adaptation (111).

Andean highlanders have a 38% larger residual lung volume (67), larger, more numerous alveoli (134), lower maximum expiratory flow rate per lung volume, and lower upstream airway conductance than lowlanders (20). The last two characteristics contributed to the notion that gestation and PN maturation of humans at high altitude results in dysanaptic lung growth, more specifically, excess growth of the gas-exchange region compared with the conducting airways (20). Also contributing to the notion of dysanapsis in this context is the clear evidence that the adaptive response of healthy pregnant humans and animals (38, 87, 111), while not complete (54, 64, 111), partially protects the fetus from the low Po2 of high altitude, which is, therefore, first fully felt at birth (110, 127). Thus the lung’s conducting zone, which in all mammalian species reported develops mainly during gestation (21), would be less affected by a low atmospheric Po2 than the gas-exchange region of organisms that septate after birth, e.g., rats (25, 26), or mainly after birth, e.g., humans (83, 156, 157). Tenney and Remmers (142) compared alveolar dimensions of guinea pigs bred and raised for many generations at 4,530 m and third-generation sheep resident at 4,390 m with guinea pigs and sheep native to Hanover, NH (altitude 160 m). Both species septate in utero (3, 33). They failed to find intraspecific differences in alveolar dimensions between sea level and highlander animals (Table 1), supporting the notion that atmospheric hypoxia does not have a large effect on alveolar dimensions in organisms that septate in utero.

Dogs brought to high altitude (3,100 m; Leadville, CO) at age 2.5 mo and maintained at high altitude for 14 mo had greater lung distensibility, diffusion capacity for carbon monoxide (DLCO), and lung tissue volume (Vt) 3 mo after return to sea level (Dallas, TX) than did same-aged dogs maintained at sea level (69). It is of some interest that the higher DLCO was due to an increased capillary volume (Vc), not higher membrane diffusion capacity (DMCO). Thus because the elevated Vt could reflect the higher Vc, there is no evidence from this study to indicate the young dogs increased Sa. However, young humans (average age 20 yr) residing at 3,100 m (42) and native or longtime dwellers at 4,500 m (126) have a higher DLCO than lowlanders due to a high DMCO and Vc. The higher DMCO could reflect a larger Sa, but it could also be due to a thinner alveolar membrane. If the difference in DMCO between dogs (69) and humans (42) is due to a higher Sa in human highlanders than in lowlanders, one reason could be that when the need for an elevated diffusion capacity was removed in the dogs during 3 mo at sea level, alveoli were destroyed to match tissue size to functional need, in line with the concept of symmorphosis (152). This notion is supported by the rapid calorie-related loss of alveoli when need for Sa is diminished by a fall of V02 (see below) (94).

We recognize that gestation, birth, and living under the hypoxia of altitude differ from the same events in laboratory-generated hypoxia at sea level. Nevertheless, to examine the effect of hypoxia on the lungs of organisms that septate after birth, we maintained female rats in 13% O2 for at least 3 wk before they were mated; male rats were not maintained in 13% O2 because it was very difficult to breed when both partners, even if acclimated, were kept in 13% O2 (13, 101, 108). Once pregnant, the rats remained in 13% O2 throughout their pregnancies and with their pups for varying periods after birth. At age 2 days, the average volume of alveolar sacculae was larger in 13% O2 rats than in air-breathing rats and was, among the measurements made, the only intergroup difference at that time (Table 2). However, unlike the lack of an effect of hypoxia on septation in guinea pigs and sheep (142), which septate in utero (3, 33), even though the dams were acclimatized to it, hypoxia markedly diminished PN septation (Table 3, Fig. 5). And, unlike Andean humans (134), rat pups exposed to hypoxia had fewer, not more, alveoli. Thus this issue needs resolution. Removal of rats from 13% O2 to room air after the period of septation did not result in spontaneous septation (13, 101). The absence of post hoc septation indicates, as shown earlier (93), that there is a “critical” period for septation as there is, for example, in the development of vision (65). The molecular basis for the critical period in response to 13% O2 and its relationship, if any, to the corticosteroid hormone-induced critical period in

Table 1. The effect of hypoxia on alveolus formation by septation or on “other” means of forming alveoli depends on when hypoxia is experienced and whether septation occurs pre- or postnatally

<table>
<thead>
<tr>
<th>Organism</th>
<th>Septation</th>
<th>Conditions</th>
<th>Alveolus Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>In utero</td>
<td>High altitude</td>
<td>Not affected (142)</td>
</tr>
<tr>
<td>Sheep</td>
<td>In utero</td>
<td>High altitude</td>
<td>Not affected (142)</td>
</tr>
<tr>
<td>Rat</td>
<td>Postnatal</td>
<td>13% O2 during gestation and postnatally</td>
<td>Depressed during period of septation (13, 101); not depressed after period of septation (13)</td>
</tr>
<tr>
<td>Rat</td>
<td>Postnatal</td>
<td>13% O2 from age 23 to 44 days</td>
<td>Alveolus formation not depressed, alveolar size and Sa increased (13).</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate reference numbers. Sa, surface area.
low upstream conductance (20) present in human highlanders, much as occurs in chronic obstructive pulmonary disease (36, 63, 131). We make this comparison recognizing that highlanders can exhibit exceptional exertion at high altitudes and, therefore, have evolved excellent methods of adaptation (67).

Based on the number and average volume of alveolar saccules present at age 2 days and the size of alveoli present at age 14 days, it is clear hypoxia also impairs other means of forming alveoli and to a greater extent than it impairs septation (Table 3). This other component of alveolus formation is not trivial (Table 3). After the period of septation, the rate of alveolus formation in rats remaining in 13% O2 is the same as in air-breathing rats (Table 2, Fig. 5). This similarity reflects a decreased rate of alveolus formation in air-breathing rats after the period of septation, not an increased rate of alveolus formation in 13% O2 rats (Fig. 5). Thus alveolus formation during the period of septation seems to have a hypoxia-depressable component that is not present after the period of septation, i.e., it is confined to ages 2-14 days in rats.

We combined three sets of seminal findings by others in an attempt to explain the age-dependent effect hypoxia has on alveolus formation. First, because of the direct linear relationship between resting VO2 and Sa across the entire span of mammalian body mass (Fig. 1 (143), we think the call for oxygen (79) is the primal, highly conserved regulator of Sa. This regulation may, at least partly, be mediated by retinoids (see below). Second, hypoxia decreases VO2 in rats and in many other organisms, including humans, but the depression diminishes with age (for an excellent review, see Ref. 114). Third, hypoxia-induced increase in minute ventilation (VE) is absent in 2-day-old rats but develops and increases with age (102, 114, 115). We tentatively propose that the markedly lower VO2 of hypoxic newborn rats decreases the need for alveolar Sa and, by as yet unidentified signaling pathways, the formation of alveoli is diminished. Although this occurs without a lower total lung volume in 13% O2 rats, the gas-exchange region of early PN hypoxic rats has less tissue than air-breathing rats of the same age (101). Therefore, in the face of O2 deprivation, and hence less

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**Table 2. Hypoxia and alveolus formation in rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Air</th>
<th>13% O2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 2 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass, g</td>
<td>7.1±0.2</td>
<td>7.0±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Lung volume, ml</td>
<td>0.45±0.02</td>
<td>0.46±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Sa, cm²</td>
<td>146±9.0</td>
<td>150±5.0</td>
<td>NS</td>
</tr>
<tr>
<td>v, μm²×10⁻⁴</td>
<td>9.7±0.3</td>
<td>12.6±0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>N × 10⁻⁶</td>
<td>1.4±0.2</td>
<td>1.4±0.0</td>
<td>NS</td>
</tr>
<tr>
<td>Age 14 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass, g</td>
<td>26.7±0.9</td>
<td>22.7±1.0</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Lung volume, ml</td>
<td>1.57±0.03</td>
<td>1.72±0.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sa, cm²</td>
<td>770±11</td>
<td>629±44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>v, μm²×10⁻⁴</td>
<td>3.0±0.1</td>
<td>7.4±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N × 10⁻⁶</td>
<td>24.2±2.0</td>
<td>9.3±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age 40 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass, g</td>
<td>145±9.0</td>
<td>106±9.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lung volume, ml</td>
<td>5.6±0.3</td>
<td>5.2±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Sa, cm²</td>
<td>2,446±90</td>
<td>1,829±135</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>v, μm²×10⁻⁴</td>
<td>6.1±0.7</td>
<td>9.5±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N × 10⁻⁶</td>
<td>41.0±2.1</td>
<td>25.5±1.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Measurements on lungs of rats carried in utero by dams previously acclimated to 13% O2 that were in, born into, and maintained in 13% O2 until killed. Air pups were born from air-breathing dams and maintained in air after birth. Sa, gas-exchange surface area; v, average volume of an alveolus; N = number of alveoli per rat; NS = P > 0.05. [Values from Massaro et al. (101) and Blanco et al. (13).]

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**Table 3. Hypoxia impairs septation and other means of forming alveoli in rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Air</th>
<th>13% O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N due to septation, age 2–14 days</td>
<td>4.53×10⁶</td>
<td>2.38×10⁶</td>
</tr>
<tr>
<td>N due to other means, age 2–14 days</td>
<td>19.67×10⁶</td>
<td>6.92×10⁶</td>
</tr>
<tr>
<td>N, total, age 14 days</td>
<td>24.2×10⁶</td>
<td>9.3×10⁶</td>
</tr>
<tr>
<td>N/day, age 2–14 days</td>
<td>2.0×10⁶</td>
<td>0.78×10⁶</td>
</tr>
<tr>
<td>Lung volume, ml, age 14 days</td>
<td>1.54</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Females rats were placed in 13% O2 for at least 3 wk before being bred, were maintained in 13% O2 during gestation and with their pups for various times after the pups were born. The pups were killed at age 2 or 14 days, and the data refer to that period (13). N represents the number of alveoli.
available energy (16, 47, 53), the organism’s energy needs are low partly because it makes less lung tissue and partly because there is less to maintain. This response, as the response to calorie restriction (see below) (92), may have provided an evolutionary advantage.

With age, the depressive effect of hypoxia on $V_{O_2}$ diminishes (114), and, therefore, the $O_2$ need increases. The organism compensates, at least in part, by increasing $V_E$. We suggest that the increasing $O_2$ need, perhaps in concert with the mechanical events associated with the higher $V_E$, initiates an undefined signaling cascade that prevents the postseptation slowing of the rate of alveolar formation.

When rats are initially exposed to hypoxia after the period of septation, they increase $S_a$ by increasing the size of individual alveoli, not by making more alveoli (Table 4) (13). Furthermore, they increase lung volume, but, unlike early PN 13% $O_2$ rats, rats exposed to 13% $O_2$ after the period of septation increase gas-exchange $V_t$, more rapidly than air-breathing rats. The larger volume of alveolar tissue does not suggest thinner alveolar walls (13), which would enhance diffusion. This is different from the lower harmonic mean thickness of the alveolar wall of guinea pigs native to high altitudes compared with sea level members of the same species (62) and the high diffusion capacity in highlander humans (42), which suggest better adaptation in animal and human highlanders then in lowlander animals.

It is apparent, although functional effects of dwelling at high altitudes have been studied for many years, that there have been few studies of lung structure in highlander animals or in humans. However, as the resolution of noninvasive lung imaging procedures increases, hopefully supplemented by morphometrically based anatomical studies, the effect on the lung of being a native highlander, of going to altitude, and of aging at altitude, may become more clear.

Alveolar plasticity in response to the endogenous call for oxygen. The use of an altered inspired $P_{O_2}$ to study the effect the availability of $O_2$ has on alveolar dimensions has, as discussed above, provided very useful information. However, as also mentioned, tissue toxicity due to oxygen radicals as a determinant of the alveolar response to an altered inspired $O_2$ is difficult to exclude. Treatment with thyroid hormone, or blocking conversion of thyroxine to triiodothyronine, to alter $V_{O_2}$, is confounded by the action of thyroid hormone beyond its effect on $V_{O_2}$ (6). We believe that a more physiological means of altering $V_{O_2}$, one that occurs in nature (66) and represents an endogenous alteration in the call for oxygen, is calorie restriction (CR) and CR followed by refeeding (CR-RF). CR lowers $V_{O_2}$; refeeding after CR returns $V_{O_2}$ to values present before CR (47, 53).

Sahebjami and Wirman (133) and subsequently others (58, 74, 77) showed that CR in adult rats (58, 77, 133) and hamsters (74) increases $L_m$ and diminishes $S_a$. As may be gleaned from the titles of their articles (58, 74, 77, 133), they felt these changes represented starvation or nutritionally induced emphysema. Sahebjami and Wirman (133) and later Kerr et al. (77) found refeeding reversed these changes but, as nearly as we can tell from their papers, they did not raise the possibility that the diminished $L_m$ reflected alveolar regeneration (77, 133).

The effect of CR and of CR-RF on $V_{O_2}$ and the highly conserved relationship across species between resting $V_{O_2}$ and alveolar dimensions (Figs. 1 and 2) (143) led us to a different interpretation of the early work on calorie intake and the architecture of gas-exchange structures. We propose that the changes in alveolar architecture in response to CR and to CR-RF represent endogenous programs of alveolar destruction and regeneration, i.e., alveolar turnover induced by calorie-related changes in $V_{O_2}$. Therefore, we extended their work (58, 74, 77, 133) to test the hypothesis that CR activates endogenous alveolar destruction and that CR-RF induces alveolar regeneration. In adult mice, CR resulted in ~45% fewer alveoli, less $S_a$ without diminished lung volume, and a 20% decrease in the amount of lung DNA due, at least in part, to apoptosis of alveolar wall cells (94). Refeeding resulted in alveolar wall cell replication, an increase of lung DNA, and alveolar regeneration (94). The loss of alveoli with CR is consistent with the doubled rate of proteolysis in lungs of adult CR rats (145).

We tentatively suggest the following to explain our findings. CR diminishes total organismal (47, 53) and lung (55) $V_{O_2}$. The fall in total $V_{O_2}$, perhaps in concert with the lower lung $V_{O_2}$, signals a need for less $S_a$. For survival during severe CR, the organism requires sub-

Table 4. Average alveolar volume, alveolar number, and gas-exchange $S_a$ at age 23–44 days: air vs. 13% $O_2$

<table>
<thead>
<tr>
<th>Exposure</th>
<th>$V_t$, $\mu m^3 \times 10^{-4}$</th>
<th>$N \times 10^{-6}$</th>
<th>$S_a$, cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Air, killed at age 23 days</td>
<td>3.9 ± 0.3</td>
<td>30.5 ± 0.9</td>
<td>1,199 ± 55</td>
</tr>
<tr>
<td>2 Air, killed at age 44 days</td>
<td>7.2 ± 0.5</td>
<td>39.7 ± 1.4</td>
<td>2,952 ± 154</td>
</tr>
<tr>
<td>3 Air, then 13% $O_2$ from age 23–44 days,</td>
<td>9.8 ± 0.7</td>
<td>42.9 ± 2.6</td>
<td>3,690 ± 167</td>
</tr>
<tr>
<td>killed at age 44 days</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$P$ values

<table>
<thead>
<tr>
<th></th>
<th>1 vs. 2</th>
<th>1 vs. 3</th>
<th>2 vs. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Exposures same as in Table 2. $S_a$, gas-exchange $S_a$. NS, $P > 0.05$. Air then 13% $O_2$ rats were exposed to 13% $O_2$ for the first time at age 23 days. [From Blanco et al. (13).]
strates to maintain muscle mass and increased glu-
cconeogenesis to provide glucose for brain metabolism (135). In this setting, we propose that an endogenous program for increased lung proteolysis (145), which includes alveolar destruction, is activated and helps provide needed substrates. This program also diminishes the mass of lung tissue, thereby decreasing the cost of its maintenance. Refeeding increases the call for oxygen and, by an as yet unidentified signal(s), activates a program of alveolar regeneration. The signals, which may initially be mechanical but must ultimately be molecular, for the calorie-related endogenous programs of alveolar destruction and regeneration, are being sought.

**HORMONAL REGULATION OF ALVEOLUS FORMATION DURING THE PERIOD OF SEPTATION**

**Glucocorticoid hormones.** In contrast to the detailed information about architectural processes of alveolus formation during the period of septation, due mainly to work in Bern (24–27, 156, 157) and which now includes many species (3, 5, 28, 83, 140, 154), little is known about the regulation of septation. Our early thinking was greatly influenced by a consideration of the architectural events needed to form a septum and by the notion that changes in the concentration of systemic hormones (59, 60) might regulate the sequence in which organs, e.g., PN lung and pancreas, attain adult anatomical or functional characteristics (5, 25, 26, 60). We should bear in mind that little work on regulation of alveolus formation has been done other than in rodents.

From the available systemic hormones, we tested the possibility that glucocorticosteroids might affect (inhibit) the formation of septa for three main reasons. First, eruption and elongation of alveolar septa are brought about by forming ridges in epithelial sheets, which in part requires epithelial cell division. In addition, new septa must be filled with capillaries and fibroblasts, which also requires cell replication. Because glucocorticosteroids inhibit cell division in several tissues (86), including the lung (93, 112), they might prevent septation. This idea was strengthened because there is a trough in the serum concentration of the species’ active glucocorticosteroid hormone during the period of septation, whether septation occurs in utero or postnatally (59, 70). Furthermore, the serum concentration of glucocorticosteroid hormones begins to increase as septation ends and as there is acceleration of the thinning of the alveolar wall (25, 26). This suggests the elevated concentration of the hormone initiates the end of septation, the onset of accelerated alveolar wall thinning, and the remodeling of alveolar vessels from a double capillary to a single capillary system (25, 26).

Treatment of rat pups with dexamethasone, a synthetic glucocorticosteroid hormone, during the period of septation prevents septation (14, 93) and diminishes the rate of DNA synthesis and accumulation in the lung (93). The mechanism(s) by which dexamethasone inhibits septation is unknown, and, in view of the many genomic (1) and nongenomic (29) actions of corticosteroid hormones, there is a myriad of possibilities. Nevertheless, in the spirit of guilt by association (Ref. 4 and later in this paper), the PN time course of activity in lung of ornithine decarboxylase (ODC) offers two possible, not mutually exclusive, mechanisms. The activity of ODC peaks in lungs of rats during septation (PN days 4–6) but not in liver, heart, brain, or kidney (146) and is depressed in lung, but not in liver, by corticosteroid treatment (10) during the period of septation (26). ODC, whose activity correlates with DNA synthesis (123), catalyzes the synthesis of polyamines, which are involved in cell replication. Thus to the extent that proliferation of alveolar wall cells is essential to, but not sufficient for, the formation of a septum, the impairment of septation by dexamethasone could reflect its depression of proliferation of alveolar wall cells by its inhibition of ODC activity.

Because polyamines can regulate communication through gap junctions (138), the inhibition of septation by dexamethasone could involve more than depression of cell replication. Dexamethasone might block septation by interfering with intercellular communication through gap junctions. This notion is supported by studies on other organs showing that the expression of gap junction protein is developmentally regulated (19, 71) and that gap junction channels regulate epithelial-mesenchymal transformation during heart development (118). To extend this line of thinking, oxidative stress diminishes the function of gap junctions (78, 90, 116); hypoxia (37), hyperoxia (50), and premature birth (49) cause oxidative stress and impair septation (13, 23, 32, 89, 101, 140). Therefore, if corticosteroids, hyperoxia, hypoxia, and premature birth inhibit septation by diminishing cell-cell communication through gap junctions, it opens the possibility of preventing the inhibition of septation during hypoxia, hyperoxia, or corticosteroid treatment by pharmacologically augmenting communication through gap junctions.

In addition to blocking septation, dexamethasone treatment markedly accelerates alveolar wall thinning and changes the cellular composition of the wall (91). Within 2 days of the onset of treatment of 4-day-old rats with diltuent or dexamethasone, dexamethasone-treated pups have 20% thinner gas-exchange walls, a 32% lower absolute volume of retinol-storing interstitial fibroblasts, and a 1.5-fold higher volume of alveolar type II cells than diluent-treated rats (91). These experiments also provide evidence that dexamethasone 1) diminishes replication of fibroblasts in the alveolar wall, thereby diminishing the number of retinol storage cells (see below for relevance) and 2) impairs conversion of alveolar type II cells to alveolar type I cells, for which there would be less need if the gas-exchange surface is increasing at a slower rate than in diltuent-treated pups.

Once septation has been prevented by corticosteroids, discontinuing them is not followed by spontaneous septation, at least not up to age 60 or 95 days, i.e., 47 (93) and 82 (132) days after stopping the administration of corticosteroids. Failed septation is accompa-
nied by the formation of fewer pulmonary arteries and by pulmonary hypertension (84). Because the gas-exchange Sa is also diminished, a restricted alveolar capillary bed could contribute to the pulmonary hypertension. Furthermore, the magnitude of hypoxia-induced pulmonary hypertension is greater in dexamethasone-treated rats than in diluent-treated rats (84). This important paper (84) clearly demonstrates a long-term functional effect of early events. The effects of dexamethasone on septation and the pulmonary vessels have important implications for prematurely born babies treated with corticosteroids for days or weeks. Such treatment might increase the impairment of septation that occurs even in the absence of glucocorticoid therapy (68, 89, 140). In addition, because of the anatomy of the lung in bronchopulmonary dysplasia, these infants have areas of alveolar hypoxia that could further increase pulmonary vascular resistance.

**Thyroid hormones.** Three considerations led us to the hypothesis that thyroid hormone exerts a regulatory effect on subdivision of the large sacculles that constitute the gas-exchange region of the rat lung at birth. 1) Thyroid hormone concentration in rat serum (113) and thyroid hormone receptor density in rat lung (11, 129) begin to increase just before the onset of septation. 2) Thyroid hormone treatment induces substantial changes in brain architecture without a detectable effect on $V_{\text{O}_2}$ (31, 121). 3) Thyroid hormone treatment increases DNA synthesis in the lung of newborn rats (113). Triiodothyronine ($T_3$) administered to newborn rats at a dose that does alter the developmental increase in body weight (92) and that at even higher doses does not increase $V_{\text{O}_2}$ (141) accelerates the pace of septation, resulting in smaller alveoli and a greater Sa without affecting lung volume (92). The combination of a larger Sa without a large lung volume indicates $T_3$ induces the formation of additional alveoli. Injection of propylthiouracil, which blocks conversion of thyroxine to $T_3$ (35), impairs septation without slowing the developmental increase of body mass or lung volume. Thyroxine treatment, at a lower dose than is required to increase $V_{\text{O}_2}$ (141), overcomes the inhibitory effect of propylthiouracil on septation (92). These findings indicate thyroid hormone does not accelerate septation by increasing $V_{\text{O}_2}$ and raise the possibility that treatment with thyroid analogs that have little effect on $V_{\text{O}_2}$ might induce septation when given alone, or in combination with, retinoids (see below).

**RETINOID REGULATION OF ALVEOLUS FORMATION: MORE GUILT BY ASSOCIATION**

Several lines of evidence available in the late 1980s and early 1990s led to the notion that retinoids might play a key role in the formation of pulmonary alveoli during the period of septation. In rats, this evidence included: 1) the high concentration of cellular retinol binding protein-I (CRBP-I) in lung, but not in liver, during the period of septation (120); 2) treatment of adult rats with ATRA upregulates CRBP-I mRNA, whereas treatment with dexamethasone, which inhibits septation (14, 93, 132), downregulates CRBP-I mRNA (130); 3) the lung’s concentration of CRABP-I mRNA peaks at approximately PN day 9–10 (120) (we now speculate CRABP-I may be an inhibitor of septation or may be involved in conversion of the double to the single capillary system in the alveolus; see below); and 4) fibroblasts rich in vitamin A (retinol) storage granules occupy a large fraction of the alveolar wall throughout the lung during the period of septation (147), a time when alveoli are formed throughout the lung. After the period of septation, these cells become located mainly in the subpleural region (81, 96), the site where, we believe, the postseptation formation of alveoli takes place (96). These observations, as clues that retinoids exert a regulatory effort on septation, were supported by the general knowledge that retinoids play a key role in developmental processes in many tissues (106).

On the supposition of guilt by association, i.e., much lung retinoid activity during the period of septation, we tested the hypothesis that treatment of newborn rats with ATRA might prevent the inhibition of alveolus formation produced by dexamethasone; the hypothesis was not falsified (97). Furthermore, treatment of rat pups with ATRA alone causes the formation of more numerous, but smaller, alveoli without affecting lung volume or alveolar Sa. Briefly (see Ref. 83 for a full discussion of this seeming paradox), the absence of a higher Sa in rats treated with ATRA alone, compared with vehicle-treated rats, suggests the presence of a control mechanism that inhibits the size of alveoli when there is not a $V_{\text{O}_2}$-induced need for additional Sa. On the basis of these findings and the larger alveoli of rats exposed to hypoxia in the postseptation period (Table 2) (13), we envision two processes, differently regulated, in the formation of alveoli: eruption of a septum and subsequent elongation of a septum. We propose that ATRA induces eruption of a septum and determines the distance between septa; other factors, principally $V_{\text{O}_2}$, determine the length of a septum. Thus with excess eruption of septa in ATRA-treated rats, without a need for greater Sa, septum length is curtailed. This notion presupposes a different gradient for the morphogen ATRA among species, or interspecific differences in cellular sensitivity to ATRA, to account for the interspecific differences in spacing of septa (143). Finally, to explain the interspecific differences in alveolar size, we do not exclude a link between $V_{\text{O}_2}$ and alveolar wall ATRA gradients.

Because of the opposing action of ATRA and dexamethasone on CRBP-I in adult rat lung (130), the effect of treatment with ATRA on CRBP-I mRNA and CRABP-I mRNA was examined in rats during the period of septation. ATRA treatment transiently increases the concentration of CRABP-I mRNA but does not prevent the depression of its mRNA by dexamethasone (153). ATRA also increases the concentration of CRBP-I mRNA in the lungs of neonatal rats (153) as it does in lungs of adult rats (130). Of particular interest, ATRA treatment prevents the depression of CRBP-I mRNA induced by dexamethasone (153).
The consequences to septation of the timing of the changes in lung concentration of CRBP-I and CRABP-I and the opposing action of ATRA and dexamethasone on CRBP-I mRNA concentration in lung are unknown, but it may be useful to speculate about this in light of some known and suspected general properties and functions of these proteins. The cellular concentration of retinoid-binding proteins exceeds those of the retinoids to which they bind with high affinity, resulting in exceedingly low intracellular concentrations of free retinoids (117). Binding of retinoids by CRBP and CRABP helps to insure specificity of the interaction of retinoids with cellular dehydrogenases responsible for their metabolism and prevents nonenzymatic isomerization and oxidation of retinoids (117). Indeed, CRBP-null mice exhibit a sixfold faster turnover of retinol than wild-type mice, which is consistent with the notion that retinol is promiscuously metabolized in the absence of CRBP-I (52). CRBP-I increases cell uptake of retinol, decreases its esterification, and increases the generation of ATRA and other biologically active retinoids (117). Therefore, a high concentration of CRBP-I, as occurs in lungs of untreated rats during the period of septation (120), should increase the production of ATRA by lung cells, possibly in a cell-specific manner. Because we think septation is mainly over by PN day 7–8, the peak of CRABP-I about then could bind ATRA and end septation. Furthermore, the high expression of CRABP-I, if it occurred in only some cell types in the alveolar wall, could determine which cells respond to ATRA as the lung begins to remodel the alveolar wall, converting its double capillary system to a single capillary system.

Recent findings expand the potential therapeutic usefulness of ATRA beyond the prevention of failed septation. ATRA partially rescues septation previously inhibited by treatment of rat pups with dexamethasone and in adult mice with a genetic failure of septation (99). Veness-Meehan et al. (149) found ATRA does not prevent the hyperoxia-induced inhibition of septation in rat pups but confirmed ATRA prevents the inhibition of septation by dexamethasone. Two subsequent studies showed that rat pups exposed to hyperoxia and simultaneously treated with ATRA, but not those treated with vehicle, septate a few weeks after removal from hyperoxia without post-O2 treatment with ATRA (39, 148). This suggests that ATRA, perhaps by an antioxidant action, preserves the lung’s ability to septate. Finally, also relevant to potential therapy in humans, ATRA substantially abrogates in adult rats the elastase-induced loss of elastic recoil, increased lung volume, large gas-exchange units, diminished number of alveoli, and low alveolar SaO2, i.e., ATRA induces alveolus regeneration (12, 98, 144). The mechanism(s) and downstream changes in gene expression and protein-protein interaction by which ATRA affects alveolus formation are being actively sought in several laboratories.

The induction of alveolus formation by ATRA, of course, led to studies to identify the retinoid receptors involved. Retinoid receptors are nuclear receptors of two classes: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (88). Three subtypes of RARs and RXRs have been identified: RAR-α, RAR-β, and RAR-γ, and RXR-α, RXR-β, and RXR-γ. At physiological concentrations of ligand, RARs respond to ATRA and 9-cis retinoic acid and RXRs respond to 9-cis retinoic acid.

Retinoid agonists, antagonists, and mutant mice are being used to determine which retinoid receptors are involved in septation. RAR-β−/− mutant mice have early onset of septation and, during the period of septation, form alveoli twice as fast as wild-type mice. As expected from the results from RAR-β mutant mice, a RAR-β agonist blocks septation (100). Thus RAR-β is an endogenous inhibitor of septation. However, RAR-β−/− mice generate alveoli after the period of septation at the same rate as wild-type mice. This supports the notion that alveolus formation, during the period of septation and after, is regulated, at least in part, by different molecular mechanisms. On the basis of the early induction of septation in RAR-β−/− mice, it is possible that treatment of very prematurely born children with a RAR-β antagonist (85) would allow the early onset of septation. Further important information has come from analysis of RAR-γ mutant mice (104). RAR-γ gene deletion diminishes septation, and the additional deletion of one RXR-α allele further impairs septation (104). Because ATRA induces alveolus formation, RAR-β inhibits septation, and RAR-γ mutant mice have impaired septation, combined therapy with appropriate agonists and antagonists might provide the strongest therapeutic induction of alveolus formation. RAR-γ was recently reported (56) to induce “alveolar repair and/or alveolarization in adult rats.” The same report states a RAR-γ agonist “has been shown to induce alveolar repair in two rodent models, pancreatic elastase-induced emphysema in rats, and cigarette smoke-induced emphysema in mice” (56). Alveolar repair, i.e., the reestablishment of the integrity of the alveolar air-tissue barrier after it has been injured (45, 103), occurs spontaneously in many conditions (72, 73), including elastase-induced emphysema (80). Therefore, the “repair” ascribed to RAR-γ must have been induction of the formation of alveoli.

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