Postnatal lung function in the developing rat

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Bolle I, Eder G, Takenaka S, Ganguly K, Karrasch S, Zeller C, Neuner M, Kreyling WG, Tsuda A, Schulz H. Postnatal lung function in the developing rat. J Appl Physiol 104: 1167–1176, 2008. First published January 10, 2008; doi:10.1152/japplphysiol.00587.2007.—Little is known about lung function during early stages of postnatal maturation, although the complex structural changes associated with developing rat lung are well studied. We therefore analyzed corresponding functional (lung volume, respiratory mechanics, intrapulmonary gas mixing, and gas exchange) and structural (alveolar surface area, mean linear intercept length, and alveolar septal thickness) changes of the developing rat lung at 7–90 days. Total lung capacity (TLC) increased from 1.54 ± 0.07 to 16.7 ± 2.46 (SD) ml in proportion to body weight, but an increase in body weight exceeded an increase in lung volume by almost twofold. Series dead space volume increased from 0.21 ± 0.03 to 1.38 ± 0.08 ml but decreased relative to TLC from 14% to 8%, indicating that parenchymal growth exceeded growth of conducting Airways. Diffusing capacity of CO (DCO) increased from 8.1 ± 0.8 to 214.1 ± 23.5 mmol·min⁻¹·hPa⁻¹, corresponding to a substantial increase in surface area from 744 ± 20 to 6,536 ± 488 cm². DCO per unit of lung volume is considerably lower in the immature lung, inasmuch as DCO/TLC in 7-day-old rats was only 42% of that in adult (90-day-old) rats. In humans, however, infants and adults show comparable specific DCO. Our functional and structural analysis shows that gas exchange is limited in the immature rat lung. The pivotal step for improvement of gas exchange occurs with the transition from bulk alveolarization to the phase of expansion of air spaces with septal reconstruction and microvascular maturation.

The rat lung expands from birth, and, on day 4, cells of the interairspace walls show peak proliferation rates (3). Primary inter-air-space walls consist of two capillary layers and a central, highly cellular sheet of connective tissue. These structural features form the basis for the rapid alveolar formation from days 4 to 13, which has been termed “bulk alveolarization.” A high gain in capillary volume, as well as alveolar and capillary surface area, is achieved during this bulk alveolarization stage. During bulk alveolarization, but mainly thereafter, the septa with double capillary networks are restructured to the mature form with a single capillary network interwoven with connective tissue strands, which stabilize the interalveolar wall. This process results in a reduction of tissue mass and alveolar septal thickness, as well as the total number of fibroblasts and epithelial type II cells (11). With finalization of septal restructuring at around day 21, lung structural change is considered complete and the lung enters a period of equilibrated growth, during which capillary growth by intussusception still plays an important role in further optimization of gas exchange (3, 4, 28, 33).

Although the structural changes of the maturing lung have been described in detail, limited information is available about the consequences of rapid alveolarization and reconstruction of septal morphology on lung functions such as intrapulmonary gas mixing and gas exchange. Data on lung function during adolescence and adulthood (28, 35) and also during aging (1, 6, 9) are available for several inbred rat strains. Respiratory mechanics during lung maturation have been assessed by Gomes et al. (9) in Sprague-Dawley rats from 10 days to 3 mo of age. A broader spectrum of lung function parameters is provided by Stevens et al. (22) for the phase of equilibrated lung growth, also in Sprague-Dawley rats. Although these studies supply some information on the postnatal functional development of the lung, important data, such as the development of dead space volume compared with lung parenchymal volume, the efficiency of gas exchange during bulk alveolarization, and the impact of the substantial morphological changes of lung parenchyma on the efficiency of intrapulmonary gas mixing, are missing. Since the growing rat is often used as a model for humans, differences and similarities of postnatal lung functions between these two species must be acknowledged. Hence, the rationales of this study are 1) to analyze the correspondence between morphological lung growth and maturation and the development of lung function, 2) to assess functional similarities of lung development between humans and rats, and 3) to make the newly obtained data available.

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available to the scientific community. Even though all the information concerning postnatal lung function described here may not be new for the scientific community, a complete set of lung function parameters are provided for the sake of a comprehensive view.

A lung function unit for anesthetized mice (17, 20) and another specifically developed for the adolescent and adult rat were applied to measure lung function during and at the end of bulk alveolarization (in 7- and 14-day-old Wistar rats, respectively), at the completion of septal reconstruction (in 21-day-old rats), and during subsequent equilibrated lung growth (in 35- and 90-day-old rats). Lung and airway size were assessed from measurement of total lung capacity (TLC), its subdivisions, and series dead space volume (Vs), as a measure of conducting airway size. Parameters of respiratory mechanics were static and dynamic compliance and resistance. The quality of intrapulmonary gas mixing was assessed from the slope of the alveolar plateau (phase III) of He expirograms (SHE) and the quality of alveolar-capillary gas exchange from diffusing capacity of CO (Dco). Morphometric measurements of alveolar size, alveolar surface area (SA), and alveolar septal thickness were carried out at corresponding ages.

We were able to show that, within the time period studied, lung size (TLC) increased by a factor of 11 between 7 and 90 days of age, but the increase in body weight (BW) exceeded the increase in lung size (TLC) by a factor of ~2. Growth of alveolar volume (VA) exceeded growth of the conducting airways, shown by a decline from 14% to 8% in Vs/TLC. The most significant change was a gain in alveolar gas exchange capacity by a factor of 25 between 7 and 90 days of age. SA increased by a factor of ~10; thus gas exchange capacity per unit of surface area and, similarly, per unit of lung volume was found to be limited in the immature compared with the mature lung, inasmuch as Dco/TLC in 7-day-old rats was only 42% of that in 90-day-old rats.

**MATERIALS AND METHODS**

**Animals and Anesthesia**

Specific pathogen-free Wistar-Kyoto (WKY) rats (Janvier, Le Genest-St-Isle, France) were bred and raised at the Institute for Inhalation Biology at the Helmholtz Zentrum München German Research Center for Environmental Health until they reached the appropriate age for lung function testing. Rats were kept under a 12:12-h light-dark cycle, with water and food available ad libitum. For the first 3 wk after birth, each female was housed with its pups in a single cage in an isolated ventilated rack. Only males at 7, 14, 21, 35, and 90 days of age were used for lung function measurements (8 animals per group). The study was conducted under German federal guidelines for the use and care of laboratory animals and was approved by the Government of the District of Upper Bavaria and by the animal care and use committee of this research center.

Anesthesia for lung function measurements was induced by inhalation of isoflurane (5%) in O2 for 1 min in a whole body box and maintained by an intraperitoneal injection of a mixture of medetomidine (0.15 mg/kg), midazolam (2 mg/kg), and fentanyl (0.005 mg/kg). The animals were intubated with a plastic catheter relative to the size of the trachea: for <14-day-old pups, a 1.7 × 50 mm catheter (dead space = 150 μl) was inserted orally into the trachea; with increasing age, catheters with increasing diameters (from 2.2 × 50 to 2.35 × 50 mm) and Vs (from 203 to 274 μl) were used. The animal was then attached to the ventilator, and ventilation was adjusted to physiological end-expiratory CO2 and O2 concentrations, whereby individual ventilation parameters were set, so that respiratory rate was ~120 min−1 for younger rats and 90 min−1 for 90-day-old rats. Tidal volume was adjusted so that the tracheal peak pressure was 12–15 cmH2O. Anesthesia during lung function testing was maintained by 1.5% isoflurane. An injection of pancuronium (0.2 mg/kg ip) was given for complete muscle relaxation 5 min before the first lung function measurement. The rectal temperature and the electrocardiogram were monitored continuously. A remote-controlled heating pad and an infrared lamp were used to maintain the body temperature. After lung function testing was finished, the animals were killed by an overdose of pentobarbital sodium.

**Experimental setup.** The design of the experimental setup was the same as that developed for lung function tests in mice (18, 20, 21). To account for the increasing lung size with age, in this study, two identical functioning piston-type servo ventilators were applied: one (ventilator volume = 3 ml) for 7- to 21-day-old rats and another (ventilator volume = 20 ml) for 35- and 90-day-old rats. The computer-controlled ventilator provides for positive-pressure ventilation and for defined respiratory maneuvers for lung function tests. Respiratory flow signals were given from movements of the respiratory piston. Concentrations of the respiratory and the test gases (He and C18O) were measured by a magnetic sector field mass spectrometer. Gas samples were taken close to the end of the tracheal tube through a 1-m heated capillary at a sampling rate of 0.1 ml/s. For 7- to 21-day-old rats, this rate is too high for continuous sampling, so the inlet of the capillary was controlled by the computer via a magnetic valve. This procedure allows sampling at defined time points and synchronization of sampling with the breathing maneuver. A miniaturized pressure transducer enables the measurement of airway opening pressure (Pao). A second pressure transducer, located at the end of a thin-walled, water-filled tube that is connected to an esophageal cannula (size adjusted to the age of the animal), allows monitoring of esophageal pressure. The differential frequency response was determined for both systems and found to be similar up to the respiratory rate used; minute differences did not practically affect the Cdyn measurements in the range studied. The flow signal, Pao, esophageal pressure, and gas concentration signals were continuously recorded on a multichannel recorder. Additionally, during the lung function measurements, all signals of interest were digitized and recorded on a personal computer for subsequent data analysis. For data analysis, gas signals were corrected for the lag time (delay and 50% of the 5–95% response time), which was determined for each lung function test separately.

**Lung Function**

**Lung volumes.** Lung function testing strictly followed a standardized protocol. According to the individual ventilation parameters, the lungs were inflated at regular intervals to open any collapsed alveoli. Inspiratory capacity (IC) was defined as the volume slowly inspired over 10 s from the level of a completed, relaxed expiration to a tracheal pressure of +30 cmH2O [functional residual capacity (FRC) corresponds to relaxation volume in the present setting]. To account for differences in lung size between the different ages, the duration of inspiration, rather than the inspiratory flow rate, was standardized during this and all other test maneuvers. Expiratory reserve volume was defined as the volume slowly expired over 10 s from FRC to a tracheal pressure of ~10 cmH2O. The amount of gas that was found in the lungs at ~10 cmH2O was defined as residual volume. For the determination of FRC using the He dilution technique, a rebreathing volume of 75% IC labeled with 1% He in 21% O2-balance N2 was applied at a rebreathing frequency of 50 min−1 for 12 cycles. After 12 cycles, ventilation was stopped at the end-inspiratory level, and the valve controlling the capillary inlet was opened, allowing analysis of the mixed He concentration by mass spectrometry. The inspiratory (1%) and mixed He concentration was used for the calculation of
For the estimation of parenchymal volume density ($V_{V_{pa}}$) and alveolar surface density ($V_{S_A}$), the left lobe was cut perpendicular to the anterior-posterior axis of the lung into 3-mm-thick slices. Three or four slices were systematically selected and embedded in paraffin, and 5-µm-thick sections were stained with hematoxylin and eosin. Linear shrinkage from the tissue blocks to the tissue sections was estimated to be 19.4 ± 3.1%. For the estimation of alveolar septal thickness, six small portions of the left lobe of each rat were postfixed by 1% osmium tetroxide in 0.1 M cacodylate buffer and embedded in Epon, and semithin (1-µm-thick) sections were stained by toluidine blue.

Standard procedures (29, 26) were used to estimate volume and surface densities ($V_{V_{pa}}$ and $S_A$), mean linear intercept length ($L_m$), and $S_A$ from paraffin sections as described below. The point-counting approach was used to measure $V_{V_{pa}}$ (air spaces and tissue) and nonparenchymal volume density [$V_{V_{np}}$ (bronchi, bronchioli, >20-µm-diameter blood vessels, and larger connective tissue strips)], with the whole lobe used as reference space (28). Primarily, digital images of the entire surface of the section were obtained using a ×125 objective lens and printed at 12.9 × 16.2 cm. A square-grid system (B100) (29) was used to count points falling on the lung tissue (reference space). Then the number of points falling on nonparenchymal lung structures was determined under a microscope with a ×10 net micrometer (MC, Zeiss, Oberkochen, Germany) and ×5 and ×10 objective lenses.

On the basis of point counts, $V_{V_{pa}}$ was determined as the number of points falling on nonparenchyma × the number of points falling on reference space.

$$V_{V_{pa}} = \frac{1}{I_A}$$

where $I_A$ is number of intersections and $L_t$ is total test length.

The calculated $L_m$ of air spaces was obtained using the following equation

$$L_m = 4/S_A$$

where $S_A$ is the surface density of air spaces, i.e., alveolus and alveolar sac (30).

Total $S_A$ was calculated by the following equation

$$S_A = S_{V_{pa}} \times V_{V_{pa}} \times V_{morph}$$

where $V_{morph}$ is the volume of the fixed lung as determined by the saline-displacement method (18).

In addition, alveolar wall thickness was estimated from six semithin Epon-embedded sections of each animal. Under a microscope with a ×10 net micrometer (MC) and ×40 objective lens, points falling on the septum within the region of interest were counted. The number of intersections of the septum with horizontal and vertical lines was counted, and $S_A$ was determined as follows

$$S_A = 2I/sL_t$$

where $I_A$ is number of intersections and $L_t$ is total test length.

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$$\tau = 2 \times V_{vpa}/S_{vpa}$$

where $V_{vpa}$ is volume of alveolar walls and $S_{vpa}$ is surface area of alveolar walls (9, 13).

Statistical Analysis

Statistical analyses were performed using the commercial statistical package Statgraphics (Statistical Graphics, Rockville, MD).
Values are means ± SD. Differences between age groups were evaluated by multiple-sample comparison by ANOVA with age as a factor, and the Tukey-Kramer multiple-range test was applied to determine the means that are significantly different from each other. Differences were considered statistically significant at an error probability level of 5% (P < 0.05). Linear regression analysis was used to establish a linear model that describes the relationship between two variables under consideration, e.g., lung size as a function of BW. The linear relationship was considered significant at P < 0.05.

RESULTS

Body Weight and Lung Volumes

The lung function parameters and BW with increasing age are summarized in Table 1, and selected parameters are displayed in Fig. 1. From 7 to 90 days of age, BW increased by a factor of ~20 (from 22 to 420 g) in a linear way (BW = 4.90 × age −17.8, r² = 0.98, P < 0.001). During the same period, TLC increased by a factor of 11, indicating that gain of BW clearly exceeds gain of lung size. Accordingly, specific lung size (TLC/BW) decreased with increasing age by a factor of 7.33, BW clearly exceeds gain of lung size. Accordingly, specific lung size (TLC/BW) decreased with increasing age by a factor of 11, indicating that gain of BW and TLC are occurring in proportion over time. The volume of the conducting airways at full lung inflation as inferred from changes in VD increased during the observation period by a factor of 6, from 0.21 to 1.38 ml. However, the relative size of Vd (Vd/TLC) decreased from 14% to 8% (P < 0.001) with age, demonstrating that growth of the gas-exchanging region exceeds growth of the conducting airways. Although absolute values for FRC increased continuously with age, the relative FRC (FRC/TLC) decreased from 37% to 25% (P < 0.001) in 7- to 90-day-old rats, suggesting that the balance determining the relative volume is changing with age.

Respiratory Mechanics

Changes in static compliance of the respiratory system (C) and the lung (Cl) with age were found to behave differently. Absolute values for C increased by a factor of 8 (from 89 to 732 μl/cmH2O) during the observation period. However, specific values (C/TLC) dropped by ~20% (from 61.2 ± 2.5 to 49.9 ± 4.7 μl/cmH2O/ml TLC), P < 0.05) during the first 3 wk of postnatal lung maturation and leveled off with increasing age at 43 μl/cmH2O/ml TLC. Absolute values for C, also increased with age, but specific values (C/TLC) were independent of age and varied ~55 μl/cmH2O/ml TLC. Throughout lung maturation, the chest wall contributes little to C, which is 5.8 and 3.5 times higher than Cl in 14-day-old and adult rats, respectively.

Cdyn at a breathing rate of 130 min⁻¹ increased in proportion to TLC. Specific values (Cdyn/TLC) were not significantly different between the ages studied. Respiratory

Table 1. Functional and morphological features of the developing rat lung

<table>
<thead>
<tr>
<th></th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>35 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>22 ± 1.4</td>
<td>34 ± 6.5</td>
<td>76 ± 8.9</td>
<td>165 ± 13.3</td>
<td>417 ± 22.6</td>
</tr>
<tr>
<td>Lung volume</td>
<td></td>
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<tr>
<td>TLC, ml</td>
<td>1.54 ± 0.07</td>
<td>1.9 ± 0.46</td>
<td>4.6 ± 0.26</td>
<td>7.8 ± 0.83</td>
<td>16.7 ± 2.6</td>
</tr>
<tr>
<td>TLC/BW, μl/g</td>
<td>72.4 ± 5.4</td>
<td>58.9 ± 10.4</td>
<td>61.0 ± 6.1</td>
<td>47.0 ± 3.5</td>
<td>40.0 ± 4.7</td>
</tr>
<tr>
<td>IC, ml</td>
<td>0.97 ± 0.05</td>
<td>1.25 ± 0.39</td>
<td>3.5 ± 0.3</td>
<td>5.5 ± 0.8</td>
<td>12.6 ± 1.6</td>
</tr>
<tr>
<td>FRC, ml</td>
<td>0.56 ± 0.03</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>FRC/TLC</td>
<td>0.37 ± 0.01</td>
<td>0.38 ± 0.04</td>
<td>0.24 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>ERV, ml</td>
<td>0.26 ± 0.06</td>
<td>0.42 ± 0.06</td>
<td>0.77 ± 0.06</td>
<td>0.80 ± 0.14</td>
<td>1.98 ± 0.6</td>
</tr>
<tr>
<td>Vd, ml</td>
<td>0.21 ± 0.03</td>
<td>0.25 ± 0.03</td>
<td>0.64 ± 0.03</td>
<td>0.82 ± 0.06</td>
<td>1.38 ± 0.08</td>
</tr>
<tr>
<td>Vd/TLC</td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.08 ± 0.01</td>
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<tr>
<td>Compliance</td>
<td></td>
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<tr>
<td>Static</td>
<td></td>
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<tr>
<td>C, μl/cmH2O</td>
<td>89 ± 10</td>
<td>104 ± 16</td>
<td>228 ± 27</td>
<td>303 ± 93</td>
<td>732 ± 156</td>
</tr>
<tr>
<td>C/TLC, 1/cmH2O × 10⁻³</td>
<td>61.2 ± 2.5</td>
<td>55.9 ± 7.5</td>
<td>49.9 ± 4.7</td>
<td>42.0 ± 2.48</td>
<td>43.4 ± 3.9</td>
</tr>
<tr>
<td>Cdyn, μl/cmH2O</td>
<td>ND</td>
<td>121.9 ± 18.4</td>
<td>270.2 ± 37.6</td>
<td>381.7 ± 59.4</td>
<td>940.1 ± 158.5</td>
</tr>
<tr>
<td>Cdyn/TLC, 1/cmH2O</td>
<td>ND</td>
<td>65.4 ± 11.1</td>
<td>59.1 ± 7.3</td>
<td>49.0 ± 5.6</td>
<td>55.2 ± 12.2</td>
</tr>
<tr>
<td>Dynamic</td>
<td></td>
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<tr>
<td>Cdyn, μl/cmH2O</td>
<td>36.9 ± 5</td>
<td>54.0 ± 11.6</td>
<td>114.8 ± 15</td>
<td>195.3 ± 25.5</td>
<td>363.1 ± 51</td>
</tr>
<tr>
<td>Cdyn, μl/cmH2O</td>
<td>ND</td>
<td>63.5 ± 14</td>
<td>131.1 ± 17</td>
<td>220 ± 30</td>
<td>403 ± 76</td>
</tr>
<tr>
<td>Respiratory system resistance</td>
<td></td>
<td></td>
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<tr>
<td>R, cmH2O/ml/s</td>
<td>0.64 ± 0.1</td>
<td>0.51 ± 0.1</td>
<td>0.24 ± 0.08</td>
<td>0.13 ± 0.03</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>sR, cmH2O/ls</td>
<td>0.36 ± 0.06</td>
<td>0.30 ± 0.09</td>
<td>0.27 ± 0.13</td>
<td>0.29 ± 0.10</td>
<td>0.56 ± 0.11</td>
</tr>
<tr>
<td>Intrapulmonary gas mixing</td>
<td></td>
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<tr>
<td>S16c, mmHg/ml</td>
<td>-1.04 ± 0.1</td>
<td>-0.80 ± 0.1</td>
<td>-0.23 ± 0.03</td>
<td>-0.18 ± 0.03</td>
<td>-0.07 ± 0.03</td>
</tr>
<tr>
<td>Pulmonary diffusing capacity</td>
<td></td>
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</tr>
<tr>
<td>Dco, μmol/min/HpA</td>
<td>8.1 ± 0.8</td>
<td>13.1 ± 3.7</td>
<td>40.5 ± 9.9</td>
<td>109.3 ± 18.1</td>
<td>214.3 ± 23.5</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Surface area, cm²</td>
<td>744 ± 20</td>
<td>1,175 ± 114</td>
<td>1,648 ± 188</td>
<td>3,571 ± 490</td>
<td>6,536 ± 488</td>
</tr>
<tr>
<td>Lm, μm</td>
<td>69 ± 1.4</td>
<td>55 ± 2.6</td>
<td>59 ± 8.6</td>
<td>67 ± 2.4</td>
<td>69 ± 6.4</td>
</tr>
<tr>
<td>τ, μm</td>
<td>13.4 ± 1.8</td>
<td>8.1 ± 0.6</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.8</td>
<td>6.4 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. Differences between age groups were evaluated by multiple-sample comparison by ANOVA with age as a factor, and the Tukey-Kramer multiple-range test was applied to determine the means that are significantly different from each other. Differences were considered statistically significant at an error probability level of 5% (P < 0.05). Linear regression analysis was used to establish a linear model that describes the relationship between two variables under consideration, e.g., lung size as a function of BW. The linear relationship was considered significant at P < 0.05.

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system resistance derived from the same breathing maneuver dropped from $0.64 \pm 0.1 \text{ cmH}_2\text{O/ml/s}$ in 7-day-old animals to almost one-fifth of that level ($0.14 \pm 0.03 \text{ cmH}_2\text{O/ml/s}$) in 90-day-old rats, but specific values ($R/FRC$) were highest in 90-day-old rats ($0.56 \pm 0.11$ vs. $0.36 \pm 0.06 \text{ cmH}_2\text{O/s}$ in 7-day-old rats, $P < 0.05$). Some-what lower values were detected at 21 days of age ($0.27 \pm 0.13 \text{ cmH}_2\text{O/s}$, $P =$ not significant (NS)).

**Intrapulmonary Gas Mixing and Gas Exchange**

The slope of the alveolar plateau (phase III) derived from the single-breath washin trace of He ($S_{\text{He}}$) was applied to assess the quality of intrapulmonary gas mixing within the alveolar region of the lung. Noticeably higher values (by a factor of $10–15$ relative to the developed lung) were detected in the younger (7- and 14-day-old) animals. However, when we accounted for differences in $V_A$ with lung growth by assessing the slope over the entire alveolar space [$S_{\text{He}} \times (TLC – V_D)]$, slope values appeared to be much less affected with respect to age. The extreme values were detectable at 7 and 21 days of age: $-1.36 \pm 0.17$ and $-0.90 \pm 0.09 \text{ mmHg/V}_{A}$ in 7- and 21-day-old rats, respectively ($P < 0.05$). In adult rats, the value was $-1.09 \pm 0.11 \text{ mmHg/V}_{A}$. This suggests that the quality of gas mixing for the well-diffusible gas He does not appear to
simply decrease with age but, rather, changes in a complex manner.

Alveolar-capillary gas-exchanging capacity increased by a factor of 25 during the observation period. Dco was $8.1 \pm 0.8$ and $214.1 \pm 23.5 \text{ mcmol/min/cmH}_2\text{O}$ in 7- and 90-day-old rats, respectively; thus it considerably exceeded the gain in lung volume by a factor of 2.5. Lung volume growth and increase in Dco did not occur in proportion, which is reasonable in view of the structural changes in the postnatal period (Fig. 2). $S_A$ increased by a factor of $\sim 10$: from $774 \pm 20 \text{ cm}^2$ in 7-day-old rats to $6,536 \pm 488 \text{ cm}^2$ in 90-day-old rats (Fig. 3A). With the formation of secondary alveolar septa, $L_m$ decreased in the first 3 wk of structural development (from $69 \pm 1.4 \text{ mcm}^2$ at 7 days of age to a minimum of $55 \pm 2.6 \text{ mcm}^2$ at 14 days of age) and then increased to its initial value ($69.0 \pm 6.4 \text{ mcm}^2$) at 90 days of age (Fig. 3B).

The alveolar septum thinned substantially, from $13.4$ to $5.4 \text{ mcm}$, during the first 3 wk of life and then leveled off at $6 \text{ mcm}$ (Fig. 3C). When we relate structural and functional values of gas exchange to each, we find that transfer capacity per unit of surface area ($Dco/S_A$) is considerably limited in the developing lung (Fig. 3D). The specific diffusing capacity at 7 days of age is about one-third of that determined in the mature lung. The parallel decrease in alveolar wall thickness and increase in specific diffusing capacity suggest that the thicker tissue barrier at an early age is an important factor in the limitation of gas exchange.

DISCUSSION

The rat is widely used as an animal model to study respiratory physiology, environmental health-related concerns, and experimental medicine. Although selective lung function parameters in rats during lung development have been surveyed by a number of investigators (1, 9, 14, 22, 25, 27), important data, e.g., the efficiency of gas exchange during the phase of bulk alveolarization, the development of the dead space volume compared with lung parenchymal volume, or the impact of the substantial morphological changes of lung parenchyma on the efficiency of intrapulmonary gas mixing, are missing. Our data show that TLC increased by a factor of 11 between 7 and 90 days of age, but gain of BW exceeded gain of lung size (TLC) by a factor of $\sim 2$ in WKY rats. Despite the substantial morphological changes in the rat lung during maturation, the quality of intrapulmonary gas mixing is less limited in newborn rats, as primarily assumed. The most significant change was the gain in alveolar gas exchange capacity, by a factor of 25, between 7 and 90 days of age. Since $S_A$ increased by a factor of $\sim 10$, gas exchange capacity per unit of surface area and, similarly, per unit of lung volume was found to be clearly limited in the immature compared with the mature lung. Dco/TLC in 7-day-old rats was less than one-half of that in 90-day-old rats. In humans, infants (5) and adults (34) show comparable specific diffusing capacity values.

Lung Function in Adult Rats

The present lung function data obtained for adult male WKY rats are in reasonable agreement with those previously published for WKY rats (7, 28) but are distinctly different from data for other strains, such as Fischer 344 and Sprague-Dawley rats (18). Focusing on WKY rats, Yokoyama (33) and Dormans et al. (7) described lung function in 60- to 85-day-old animals. Dormans et al. defined TLC as air retained in the lung at 25 cmH$_2$O tracheal pressure. Yokohama related TLC to lung volume at 30 cmH$_2$O transpulmonary pressure and reported values for TLC and FRC of 11.3 and 3.9 ml, respectively. Their TLC values are $\sim 32\%$ lower and FRC values are $5\%$ lower than those obtained in 90-day-old rats in the present study. Since their animals were somewhat younger, it is difficult to judge whether these lung volume data are within the range of the present data. A possible approach to this dilemma would be reference to specific values found in 35- and 90-day-old rats. In our study, specific values for TLC in 35- and 90-day-old rats were 47 and 40 ml/g, respectively ($P = NS$); therefore, the specific values for TLC reported by Yokohama (36 ml/g) are within the range observed in the present study. Static lung compliance values reported by Yokohama were $50\%$ lower ($610 \text{ mcm/cmH}_2\text{O}$) than those found in 90-day-old rats in the present study. This is mainly due to differences in TLC, because the specific lung compliance value reported by Yokohama ($C_L/TLC = 54 \text{ l/cmH}_2\text{O}$) is in agreement with the present findings in 35- and 90-day-old rats: 49.0 and 55.2 l/cmH$_2$O, respectively ($P = NS$). Some variations may be related to differences in anesthesia and body posture; also, Yokoyama used urethane in prone male animals and Dormans et al. conducted the measurements postmortem.

Lung Function in Developing Rats

Lung function has not been fully studied during bulk alveolarization, and parameters such as VD and slope of the alveolar plateau have not previously been described in the developing rat lung. Stevens et al. (22) studied Fischer 344 rats beginning at 21 days of age, when bulk alveolarization and alveolar wall remodeling are mostly completed. Because of strain differences, the absolute values reported by Stevens et al. are clearly different from those found in the present study. For example, TLC (as defined in the present study) was $1.98$ and $8.63 \text{ ml}$ for 21- and 70-day-old Fischer rats, respectively, and $4.6$ and $16.7 \text{ ml}$ for 21- and 90-day-old Wistar rats, respectively. However, comparison of relative changes with age reveals a number of similarities between these two strains. TLC increased between 21 and 70 days of age by a factor of 4.4 in Fischer rats and by a factor of 3.6 in Wistar rats. TLC/BW decreased from 60.4 to $36.8 \text{ ml/g}$ in Fischer rats and from 61 to $40 \text{ ml/g}$ in Wistar rats, i.e., by $39\%$ and $34\%$, respectively. CL increased between 21 and 70 days of age by a factor of 5 in Fischer rats, whereas CL/TLC was not affected by lung maturation. Comparable results were found in the present study for Wistar rats. Interestingly, Stevens et al. reported that Dco/TLC increased by a factor of 1.6 between 21 and 42 days of age, i.e., during the transition from equilibrated lung growth to the phase of expansion of preexisting air spaces, and then remained unchanged until 70 days of age. Similarly, Dco/TLC increased by a factor of 1.6 between 21 and 35 days of age in Wistar rats and remained constant until 90 days of age. Hence, despite obvious differences in absolute lung function values in Fischer and WKY rats, this estimation suggests that development of lung function is comparable, at least between these two strains.

Several studies point to considerable changes of mechanical properties in the rat lung with maturation (9, 25, 27). In the present study, static and dynamic compliances increased pro-
portionally with age. Comparison of C and Cl indicates a minor contribution of the chest wall to the compliance of the respiratory system throughout lung development. C/TLC and Cdyn/TLC decline during the remodeling phase and then stabilize after completion of bulk alveolarization. Since Cl/TLC appeared to be relatively constant throughout the observation period but C/TLC declined continuously, our findings support the concept that the chest wall becomes somewhat

Fig. 2. Structural changes of alveolar region from an early age (7 days) to developed lung (90 days). Size of parenchymal air spaces decreased with lung maturation and was minimal at the end of the alveolarization period [14 days of age; cf. mean linear intercept (Lm) in Fig. 3B]. Thereafter, air space size increased with age, reaching values similar to those in 7-day-old animals at 90 days. Substantial alveolar septal thinning occurs within the first 3 wk of life and then levels off (cf. τ in Fig. 3C). Left: hematoxylin-eosin-stained sections. Scale bar, 200 μm. Right: toluidine blue-stained sections. Scale bar, 50 μm.
stiffer with age. The resting expiratory position (FRC/TLC) and the specific residual volume declined with age, from 37% to 25% of TLC and from 20% to 13% of TLC, respectively, perhaps as a result of improved mechanical interdependence between airways and parenchyma, as suggested by Gomes et al. (9). They measured resistance and elastance by the forced-oscillation technique at different levels of positive end-expiratory pressure in the developing rat lung (10–90 days of age) at frequencies of 0.9 and 4.8 Hz. Resistance measured at 0.9 Hz reflects a combination of airway and lung tissue resistance, whereas resistance measured at 4.8 Hz is close to pure airway resistance. Elastance and resistance, at both frequencies, decreased considerably with age, which is in agreement with the observations of the present study. To account for lung growth, Gomes et al. normalized their elastance and resistance values to the amount of lung tissue and found that normalized elastance and resistance values decreased progressively with age, which we did not detect when compliance was normalized to lung volume.

Morphological Analysis of the Gas-Exchanging Region

Morphometric analysis was based on single histological sections to assess changes in surface area, alveolar septal thickness, and air space size with lung growth. As a measure of the latter, we used $L_m$, although three-dimensional, more sophisticated reconstructions have been introduced, and several authors have indicated the limitation of this parameter (13, 31, 32). The use of $L_m$ is flawed by the fact that alveolar air spaces cannot reliably be distinguished from alveolar duct air spaces on single histological sections; therefore, $L_m$ represents, to an unknown extent, the combination of alveolar air space and alveolar duct air space. The degree of this ambiguity can vary during lung development, so that the sensitivity of this method as a measure of alveolar size is diminished. Although the present morphological data on $S_A$, alveolar wall thickness, and $L_m$ changes during lung maturation are in good agreement with those published previously (2, 9, 16), $L_m$ data must be interpreted with caution and with respect to results obtained from three-dimensional reconstructions of the gas-exchanging area during lung growth or in adult rats (13, 15). The average volume of an alveolar sacculus at 2 days of age was $9.7 \times 10^4 \mu m^3$, and the average volume of an alveolus was $3.0 \times 10^5 \mu m^3$ and $6.1 \times 10^4 \mu m^3$ at 14 and 40 days of age, respectively (13). Thus basic changes in $L_m$ in the present study are within the range of these values, but the extent differs substantially because of the technical limitations mentioned above.

Development of Gas Exchange Capacity

The data of the present study show that, from an early age to adulthood, BW increased by a factor of 20 while TLC almost doubled in every age group measured. However, TLC/BW decreased by ~50% between 7 and 90 days of age, i.e., from bulk alveolarization to adulthood. This apparently different rate of growth (TLC vs. BW) must be viewed in light of the substantially structural changes during this period: we observed an increase in gas-exchanging surface area by a factor of $10$ and a reduction of the alveolar septal thickness by a factor of $\frac{1}{2}$. Moreover, the double-layer capillary network in the primary saccular wall at birth develops with maturation into an advanced capillary mesh with a single-layered capillary network at the center of the alveolar septum. These structural changes allow gas exchange in adult lungs on both sides of the capillaries (28). As a result, transfer capacity per unit of volume or surface area ($D_{CO}/TLC$ or $D_{CO}/S_A$) increases substantially during the first weeks of life and counterbalances the relatively smaller increase in lung size with respect to body growth. Indeed, it shows that gas exchange is notably limited in the immature, but not the mature, rat lung (Fig. 3D). The greatest gain in specific exchange capacity takes place between 14 and 35 days of age, when the initial phase of intensive cell production and structural reorganization, termed bulk alveolarization and occurring between 4 and 13 days of age, is complete. Therefore, the pivotal step for improving gas exchange capacity in the rat lung occurs with the transition from bulk alveolarization to the phase of expansion of preexisting air spaces with septal reconstruction and microvascular maturation.
One interesting finding is that growth of the $V_A$ exceeded growth of the conducting airways, because $V_d/TLC$ decreased continuously from 14% to 8% with age. Specific airway resistance increased by a factor of $>2$ during lung maturation. These findings suggest that alveolar ventilation per breath becomes more efficient with age at the cost of increased work of breathing.

The quality of intrapulmonary gas mixing is another parameter that may affect gas exchange. The slope of phase III of the single-breath washout trace is considered to reflect mainly ventilation inhomogeneities at the periphery of the mammalian lung. According to model simulations, gas-mixing mechanisms involved in inhomogeneous ventilation are classified as solely convection-dependent inhomogeneities and as those requiring diffusion-convection interactions. Remarkably high values for phase III of the $He$ expirogram were determined in the youngest animals when slope values were considered per milliliter of expired air. With increasing age, the slope value per milliliter of expired air decreased to only 7% in 90-day-old rats. At first glance, this suggests a substantial improvement in alveolar gas-mixing efficiency with age. However, this straightforward assessment is complicated by the fact that the lung volume changed notably. The estimated $V_A$ ($TLC - V_d$) is 1.29 and 15.3 ml in 7-day-old and adult rats, respectively. Thus, from the physiological point of view, it is more appropriate to estimate the pressure difference of $He$ throughout the volume of the alveolar space and compare the slope over the entire alveolar space between the different ages, i.e., $S_{He} = (TLC - V_d)$. In doing so, the change in the slope values does not simply and monotonically decrease with age but, rather, behaves with respect to age in a more complex fashion: the maximum and minimum slope values, $−1.36$ and $−0.90$ mmHg/alveolar space, occur at 14 and 21 days of age, respectively, and the slope value of adult rats ($−1.09$ mmHg/alveolar space) is between these values. Hence, the quality of intrapulmonary gas mixing, at least for the well-diffusible gas $He$, is not simply limited in newborn rats as primarily assumed.

Comparison of Lung Function Development in Humans and Rats

Morphological studies point to striking similarities in lung growth from birth to adulthood between rats and humans (35). The enlargement factor for lung volume was 23.4 in humans and 23.5 in rats, although the parenchymal air space volume rises slightly more significantly (by a factor of 30) in humans. Surface area increased in both species a little less, by a factor of 21. Morphological estimates of pulmonary diffusing capacity for $O_2$ reveal an increase by a factor of 33 in both species. In rats, Tschanz et al. (28) described a progression from the pseudoglandular to the canalicular stage during the last week of pregnancy; therefore, the newborn rat lung is in its saccular stage until 4 days postpartum, whereas humans are born at the transition of the saccular stage to the alveolar stage. Alveolar development and septal reconstruction are completed by 21 days of age in rats and not until the 3rd yr of life in humans. Subsequently, equilibrated lung growth and late alveolarization occur in rats until adulthood, whereas humans remain in a state of late alveolarization and growth until puberty. Therefore, the selected time points allow comparison of lung function between rats until 21 days postpartum and humans until the 3rd yr of life, between 35-day-old rats and human adolescents, and between 90-day-old rats and human adults.

Despite these morphological similarities between species, comparison of lung function development reveals some remarkable differences that must be acknowledged when the growing rat is used as a model. In humans, the development of lung volume from birth to adulthood shows a slightly overproportional increase with body length and a disproportionately high increase of $FRC$ (from 25 to 40 ml/kg), so that the resting expiratory position, $FRC/TLC$, increases to 45–50% with age (22, 35), whereas a decrease from 37% to 25% was found in rats. Growth of lung volume exceeded $BW$ gain in humans, so that specific lung size, $TLC/BW$, increases by a factor of $>2$ until adulthood and reaches $>80$ ml/kg (23, 34), whereas $TLC/BW$ in rats at 90 days of age is one-half of that at 7 days of age and finally reaches $40$ ml/kg, indicating that the specific lung size in humans is twice as large as in rats. Hart et al. (10) showed that, during equilibrated lung growth, $V_d$ and $FRC$ develop in humans in proportion, whereas in rats, lung volume growth exceeds airway growth, even when the corresponding phase between 21 and 90 days of age is considered. These findings are consistent with the observation that airway conductance in humans is proportional to thoracic gas volume in infants (24) and to $FRC$ in adolescents (34), so that the specific airway conductance in humans remains almost constant throughout lung maturation. The increase of specific airway resistance observed in the present study indicates that the opposite is true in the developing rat lung. Nearly the same increase in $Dco$ observed for rats in the present study (by a factor 25) is reported for humans from infancy to adulthood. Castillo et al. (5) reported $Dco$ of 2 and 40 ml min$^{-1}$ mmHg$^{-1}$ in newborns and adults, respectively. In humans, $Dco$ increases in proportion to $TLC$ during childhood, i.e., the phase of equilibrated lung growth, whereas Castillo et al. reported that $Dco$ in infants is proportional to body length. $Dco/TLC$ is slightly higher in newborns than in adults (6–8 ml min$^{-1}$ mmHg$^{-1}$/TLC) vs. 5–7 ml min$^{-1}$ mmHg$^{-1}$/VA, suggesting that gas exchange in the immature human lung is not limited, as in the immature rat lung.

In conclusion, there are striking similarities in pulmonary morphology, physiology, and lung maturation between rats and humans, but the present study also shows notable differences between these species that must be recognized when the growing rat is used as a model for the human lung from infancy to adulthood.

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