Compensatory alveolar growth normalizes gas-exchange function in immature dogs after pneumonectomy

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Takeda, Shin-Ichi, Connie C. W. Hsia, Eva Wagner, Murugappan Ramanathan, Aaron S. Estrera, and Ewald R. Weibel. Compensatory alveolar growth normalizes gas-exchange function in immature dogs after pneumonectomy. J. Appl. Physiol. 86(4): 1301–1310, 1999.—To determine the extent and sources of adaptive response in gas-exchange to major lung resection during somatic maturation, immature male foxhounds underwent right pneumonectomy (R-Pnx, n = 5) or right thoracotomy without pneumonectomy (Sham, n = 6) at 2 mo of age. One year after surgery, exercise capacity and pulmonary gas-exchange were determined during treadmill exercise. Lung diffusing capacity (D_L) and cardiac output were measured by a rebreathing technique. In animals after R-Pnx, maximal O₂ uptake, lung volume, arterial blood gases, and D_L during exercise were completely normal. Postmortem morphometric analysis 18 mo after R-Pnx (n = 3) showed a vigorous compensatory increase in alveolar septal tissue volume involving all cellular compartments of the septum compared with the control lung; as a result, alveolar-capillary surface areas and D_L estimated by morphometry were restored to normal. In both groups, estimates of D_L by the morphometric method agreed closely with estimates obtained by the physiological method during peak exercise. These data show that extensive lung resection in immature dogs stimulates a vigorous compensatory growth of alveolar tissue in excess of maturational lung growth, resulting in complete normalization of aerobic capacity and gas-exchange function at maturity.

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other dogs were raised to maturity. Physiological and radiological studies were performed at rest under anesthesia during maturation and were previously published (39, 40). One year after surgery, dogs were trained to run freely on a motorized treadmill while wearing a customized leak-free respiratory mask (1) and attachments necessary for ventilatory measurements. Bilateral subcutaneous carotid artery loops were constructed to allow repeated catheterization. Aerobic capacity, ventilation, gas-exchange, and mechanical and hemodynamic function were measured during heavy exercise.

Exercise training. The treadmill speed was kept constant at 6 or 8 miles/h, depending on the preference of the dog. After a warm-up period at 6 miles/h and 0% grade, the treadmill grade was raised by 5% every 3 min up to 60% or 80% of the previously achieved maximal workload. Exercise was sustained for a total of 30 min/day 5 days/wk. Maximal workload was adjusted accordingly. Exercise intensity was varied from day to day during training to prevent dogs from anticipating the upcoming workload.

Respiratory apparatus. The dog breathed through a large two-way respiratory valve (model 2700, Hans Rudolph, Kansas City, MO) connected to a two-way inflatable balloon valve (model 8230, Hans Rudolph) and a 3-liter anesthetic rebreathing bag. The inspiratory port was connected to a screen pneumotachometer (model 3813, Hans Rudolph) and opened to room air or a large meteorologic balloon containing 100% O₂. The expiratory port led to a mixing chamber and a heated screen pneumotachometer. Expiratory air concentrations were sampled continuously from the distal end of the mixing chamber by a mass spectrometer (model MGA 1100, Perkin-Elmer). The pneumotachometer-computer system was calibrated by the method of Yeh et al. (51). Rectal temperature, gas concentrations, and electrocardiogram were continuously monitored during exercise. All signals were digitized by computer at 50 Hz. Ventilation, O₂ uptake, CO₂ production, respiratory rate, tidal volume, and heart rate were calculated from mixed expired gas and averaged over a predetermined number of breaths. Expiratory flow was integrated to obtain volume which was expressed in BTPS conditions.

Catheterization and hemodynamic measurements during exercise. Percutaneous catheterization of the carotid artery was performed in the awake dog under local anesthesia on the day of study. The catheter was connected to a fluid-filled transducer and a carrier amplifier, and the signals were digitized by computer. Maximal O₂ uptake was measured as defined by Seeherman et al. (37), i.e., the point where O₂ uptake no longer increased with increasing workload and was associated with a continuously rising lactate concentration. Hb concentration was measured spectrophotometrically (Beckman Instruments, Fullerton, CA). Hematocrit was determined with a microcapillary centrifuge. Convolutional blood gases were measured (ABL3, Radiometer, Copenhagen, Denmark) and O₂ saturation at body temperature was calculated by using O₂ half-saturation pressures of Hb measured for dog blood.

Rebreathing measurements during exercise. These methods have been reported previously in detail (7, 8). An anesthetic bag was filled with one of two rebreathing gas mixtures containing 9% helium, 0.6% acetylene (C₂H₂), 0.3% C₁₈O₂, and 30% O₂ balance N₂ or 90% O₂. The bag volume was selected from the dog's average tidal volume at a given workload plus 200 ml ATPD to prevent collapse of the bag during rebreathing. Exercise consisted of a 5-min warm-up period at 6 miles/h and 0% grade. Then the workload was increased to a preselected level and sustained for ~4 min. At the end of the 3rd min, the balloon valve was automatically switched at a selected end expiration to allow the dog to rebreathe from the anesthetic bag for 5–10 s, depending on the workload. Rebreathing measurements were repeated at each workload with use of each of the two different rebreathing mixtures. Gas concentrations at the mouth were monitored continuously. Diffusing capacity of the lung (DLCO) and cardiac output were estimated from the exponential rates of disappearance of end-tidal C₁₈O₂ and C₂H₂, respectively, with respect to helium. Lung volume was estimated from helium dilution. All results were corrected for mixing efficiency by using a method adapted from Hook and Meyer (18). In all dogs, 90% mixing was achieved within four breaths. The Bunsen solubility coefficient for C₂H₂ in blood and tissue was corrected to the measured body temperature and hematocrit, as described by Jibelian et al. (29).

Postmortem studies. On completion of physiological measurements (~18 mo after surgery), the dog was deeply anesthetized with pentobarbital sodium (25 mg/kg iv) and intubated via a tracheotomy. The lungs were collapsed through bilateral intercostal incisions. Simultaneously, an overdose of pentobarbital sodium (100 mg/kg iv) was given, and the lungs were immediately reinflated within the thorax by intratracheal instillation of 2.5% glutaraldehyde buffered in 0.03 M potassium phosphate (pH 7.40, 350 mosM) at a constant hydrostatic pressure of 25 cmH₂O above the highest point of the sternum in the supine position. The lungs and heart were then removed en bloc and immersed in 2.5% glutaraldehyde. Major respiratory muscles were dissected completely, trimmed of extraneous tissue, weighed, and processed for separate analysis.

Volume of the intact lung was measured by immersion displacement (46). Each lung was sectioned serially at 2-cm intervals, and each cut surface was photographed using 35-mm Ektachrome color film. Volume of the lung after sectioning was estimated from the photographs by point counting with use of the Cavalieri principle (14). Tissue blocks were collected from each stratum by a systematic, volume-weighted sampling procedure with a random start. Morphometric analysis consisted of four stratified levels (46): gross (level I), low-power light-microscopic (×200, level II), high-power light-microscopic (×400, level III), and electron-microscopic analysis (×11,000, level IV). Each lung was divided into an upper and a lower stratum. The right upper stratum consisted of the upper and middle lobes; the right lower stratum consisted of the lower and cardiac lobes. The left upper stratum consisted of the upper lobe and lingula; the left lower stratum consisted of the lower lobe. For the Sham group, two blocks per stratum were taken for light microscopy (total 8 blocks/dog); three blocks per stratum were taken for electron microscopy (total 12 blocks/dog). For the R-Pnx dogs, four blocks per stratum were taken for light microscopy (total 8 blocks/dog); six blocks per stratum were taken for electron microscopy (total 12 blocks/dog). Samples from each block were embedded in methacrylate for thick sections (5 μm) and stained with hematoxylin and eosin. Volume densities were estimated by point counting using standard test grids. Volume density of coarse parenchyma in lung included all structures >1 mm (level I). Volume density of fine parenchyma in coarse parenchyma included all structures between 20 μm and 1 mm (level II).

Additional systematic random samples from each block were embedded in Epon. These were used to prepare semithin sections (1 mm) to estimate the volume density of alveolar septa in fine parenchyma, including all structures measuring <20 μm (level III), and to estimate volume and surface densities of alveolar structures within the septum, e.g.,
capillaries and alveoli, as well as harmonic mean thickness of the tissue-plasma barrier ($t_{\text{hb}}$) by electron microscopy (level IV). Surface densities were estimated by intersection counting. Measurements were related back to the entire stratum through the cascade of levels; these methodological details have been reported elsewhere (46). A sufficient number of fields was examined to yield a total of 300–400 counted points or intersections per structure per stratum. For electron microscopy (level IV), the numbers of micrographs examined were 30 per stratum per dog for the Sham group and 60 per stratum per dog for the R-Pnx group to account for the fact that the strata were twice as large in the R-Pnx group. All morphometric data were calculated for each stratum separately; a volume-weighted average for the entire lung was then obtained. Absolute volume and surface area of individual alveolar structures were obtained by relating the respective volume and surface densities at each level back through the cascade of levels to the measured volume of the stratum (46).

Diffusing capacity of the lung for O$_2$ and CO estimated by morphometry. Lung diffusing capacity was calculated for O$_2$ ($D_LO_2$) and for CO ($D_OC$) by a modified version (47) of the previously established morphometric model of Weibel (45). The model describes the gas diffusion path from alveolar air to the binding sites on Hb as two serially linked conductance steps: 1) through the tissue and plasma barrier ($D_b$ or $D_{bC}$) and 2) in the erythrocyte ($D_eC$ or $D_{eC}$)

$$D_{L}^{-1} = D_b^{-1} + D_e^{-1}$$

(1)

$$D_b = K_b \frac{(S_a + S_p)}{(2 \cdot t_{hb})}$$

(2)

where

$$D_e = \Theta \cdot V_c$$

(3)

where $S_a$ and $S_p$ are the measured total alveolar and capillary surface area and $V_c$ is the measured total capillary blood volume. $K_b$ is the Krogh diffusion coefficient for O$_2$ or CO, respectively, in tissue and plasma taken from the literature (49). A set of systematic linear test lines was laid over each electron micrograph. The length of all intercepts of the test line with the barrier, from the alveolar air to the nearest red cell membrane, was measured with a logarithmic ruler. Intercepts that did not cross the epithelial and the red cell surfaces were not measured. The $t_{hb}$ in a direction perpendicular to the epithelial surface is given by the mean of all reciprocal intercept lengths

$$t_{hb} = \frac{1}{\sum \frac{1}{n_i}} \sum n_i$$

(4)

where $n$ is the number of linear intercepts of length $l$. The factor $\frac{1}{2}$, derived from stereological principles, was introduced to correct for the mean projection angle (15, 48). The estimate of $t_{hb}$ has been shown to be normally distributed (47).

The term $\Theta$ is the empirical uptake and reaction rate of O$_2$ or CO with dog whole blood. For O$_2$, $\Theta_{O_2}$ (in $\text{ml}$·$\text{ml}$·$\text{Torr}^{-1}$·$\text{s}^{-1}$) was calculated as

$$\Theta_{O_2} = K_{O_2}^{(60\%)} f(T) \cdot (0.0587 \cdot \alpha_{O_2}) \cdot (1 - S_O_2) \cdot (0.001333 \cdot [\text{Hb}])$$

(5)

where $K_{O_2}^{(60\%)}$ is the red cell reaction velocity at 60% saturation. $K_{O_2}^{(60\%)} = 440 \text{mM}·\text{m}^{-1}·\text{s}^{-1}$ is measured by the stop-flow technique and corrected for the effect of the unstirred layer of plasma surrounding the red blood cell (16). $f(T)$ is the temperature factor derived from the Arrhenius equation that corrects $K'$ from the standard 37°C to the core temperature measured at peak exercise. $\alpha_{O_2}$ is the solubility of O$_2$ at the core temperature during peak exercise. $S_O_2$ is the initial fractional saturation of O$_2$ in mixed venous blood entering the lung capillaries. [Hb] is the Hb concentration (in g/dl) of arterial blood measured at heavy exercise. For CO, $\Theta_{CO}$ (in $\text{ml}$·$\text{ml}^{-1}$·$\text{Torr}^{-1}$·$\text{s}^{-1}$) is calculated for dog blood at a body temperature of 40°C (17)

$$\frac{1}{\Theta_{CO}} = 0.929 + 0.00379 \frac{P_{A_CO}}{[\text{Hb}]}$$

(6)

where $P_{A_CO}$ is the mean alveolar PO$_2$ (in Torr) during rebreathing measured at the highest workload for each animal.

Statistical analysis. Values are means ± SE. Physiological data were analyzed with respect to O$_2$ uptake or cardiac output, and the slopes and intercepts were compared between groups by ANOVA. Postmortem data from the remaining lung of R-Pnx animals were compared separately from those in the left lung and both lungs of Sham animals by ANOVA using STATVIEW (version 4.5, Abacus Concepts, Berkeley, CA). Differences among groups were considered significant at $P ≤ 0.05$.

**RESULTS**

Exercise measurements of gas-exchange. Table 1 shows the physiological data during maximal exercise. There was no difference in body weight between groups. Maximal O$_2$ uptake was similar between groups. End-expiratory and end-inspiratory lung volumes were lower in R-Pnx dogs, but only the reduction in end-expiratory lung volume reached statistical significance. There were no significant differences in hematocrit, mean
PAO₂, and arterial blood gases between groups. There were also no significant differences in minute ventilation, cardiac output, stroke volume, and DLCO between groups. Blood lactate concentration increased similarly with increasing O₂ uptake in both groups (Fig. 1). The relationships of arterial O₂ saturation and alveolar-arterial P O₂ difference to O₂ uptake during exercise are similar in both experimental groups (Fig. 2). One Sham animal developed significant declines in arterial O₂ saturation during moderate exercise due to hypoventilation; the alveolar-arterial P O₂ difference of this animal at a given O₂ uptake was in keeping with that of other Sham animals. The relationships of DLCO to pulmonary blood flow, estimated by the rebreathing technique, were also similar between groups (Fig. 3).

Postmortem measurements. Morphometric data were available from three animals in the R-Pnx group and six animals in the Sham group. Two animals in the R-Pnx group died before the terminal experiment, and their lungs could not be adequately fixed. Figure 4 shows the similar light-microscopic appearance of the gas-exchange region in the two experimental groups. Tables 2 and 3 show the morphometric measurements of the remaining left lung in animals after R-Pnx compared with those in the left lung and both lungs of control animals. In both groups, volume of the intact lung measured by immersion displacement was significantly larger than volume of the sectioned lung measured by the Cavalieri principle (Table 3), confirming that alveolar septa were fixed under tension, but the two measurements varied in parallel between the groups. Volume of the left lung after R-Pnx increased more than twofold to equal that of two lungs in control animals (Table 3). Except for a higher type I epithelial volume after R-Pnx, volume densities of septal structures and surface densities of alveoli and capillaries were not different between groups (Table 2). Absolute volume of septal structures and surface areas of alveoli and capillaries after R-Pnx were also not different from those in both lungs of control animals (Table 3). Thus alveolar septal tissue volume of the left lung after R-Pnx exceeded that of the control left lung by a factor of 2.69; this compensatory tissue proliferation involved all tissue components of the septum: volumes of epithelium, interstitium, and endothelium increased 2.6-, 2.76-, and 2.64-fold, respectively. Capillary blood volume was correspondingly higher by 2.7-fold after R-Pnx. The τhb was similar between groups (Table 2). As a result of the increased surface areas and capillary blood volume, DLCO was of the remaining lung after R-Pnx estimated by morphometry increased 2.62-fold to a level similar to that in both lungs of control animals (Table 3). Correlation of DLCO estimated by physiological and morphometric methods. The mean DLCO estimated by morphometry in each group agreed well with the highest DLCO obtained by rebreathing during exercise (Fig. 3, right). In the present study, individual estimates of DLCO by the two methods differed by 5–20%; the mean ratio of DLCO measured by rebreathing to DLCO measured by morphometry for all immature dogs (n = 9) is 1.05 ± 0.05 (SE). In Fig. 5 the correlation...
between the two methods is shown for all animals we have studied to date, including dogs after left pneumonectomy as adults, after R-Pnx as adults, and adult Sham dogs, in addition to the immature dogs reported here. There is a highly significant correlation ($r = 0.803$). The mean ratio of $D_L$CO measured by rebreathing to $D_L$CO measured by morphometry for all dogs ($n = 21$) is $1.10 \pm 0.04$ (SE).

**DISCUSSION**

Summary of results. Immature dogs responded vigorously to major lung resection. One year after removal of 55% of lung by R-Pnx, aerobic capacity and pulmonary gas-exchange function measured up to maximal exercise were completely normal. Normalization of gas-exchange function resulted from a remarkable compensatory lung growth in excess of normal developmental lung growth that involved all cellular compartments of the alveolar septa and returned alveolar tissue volumes and surface area completely to normal. In both groups, estimates of lung diffusing capacity by a morphometric method postmortem agreed well with that by a physiological rebreathing method at peak exercise, supporting the belief that structural changes in the remaining lung mediate the increased capacity for alveolar-capillary gas-exchange. Even though morphometric data were obtained from only three animals after pneumonectomy, the magnitude of change was large and consistent (>2-fold in most measurements) as well as statistically significant.

Compensatory response to pneumonectomy in adult animals. There are three potential mechanisms for augmenting diffusing capacity after pneumonectomy: 1) recruitment of incompletely used pulmonary capillaries, 2) remodeling of existing alveolar-capillary membrane to enhance gas diffusion, and 3) growth of new alveolar tissue and capillaries. After pneumonectomy the entire cardiac output is directed through one lung; hence, effective pulmonary blood flow per unit of lung at any workload is doubled compared with control animals. The greater effective blood flow can open previously collapsed capillaries or distend open capillar-
Morphometric measurements

### Table 2. Absolute volumes, surface areas, and diffusing capacities

<table>
<thead>
<tr>
<th></th>
<th>Left Lung</th>
<th>Both Lungs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>R-Pnx</td>
<td>Sham</td>
</tr>
<tr>
<td>Volume density in lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septum</td>
<td>0.1387 ± 0.0160</td>
<td>0.1141 ± 0.0068</td>
</tr>
<tr>
<td>Septal tissue</td>
<td>0.0634 ± 0.0069</td>
<td>0.0521 ± 0.0031</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>0.0754 ± 0.0093</td>
<td>0.0618 ± 0.0029</td>
</tr>
<tr>
<td>Epithelium</td>
<td>0.0191 ± 0.0019</td>
<td>0.0162 ± 0.0007</td>
</tr>
<tr>
<td>Type I</td>
<td>0.0154 ± 0.0011</td>
<td>0.0126 ± 0.0006</td>
</tr>
<tr>
<td>Type II</td>
<td>0.0038 ± 0.0008</td>
<td>0.0036 ± 0.0004</td>
</tr>
<tr>
<td>Intersitialium</td>
<td>0.0287 ± 0.0039</td>
<td>0.0230 ± 0.0023</td>
</tr>
<tr>
<td>Endothelium</td>
<td>0.0156 ± 0.0011</td>
<td>0.0130 ± 0.0009</td>
</tr>
<tr>
<td>Surface density in lung, cm⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveoli</td>
<td>725 ± 64</td>
<td>620 ± 27</td>
</tr>
<tr>
<td>Capillary</td>
<td>608 ± 53</td>
<td>513 ± 26</td>
</tr>
<tr>
<td>Harmonic mean thickness, µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue-plasma barrier</td>
<td>0.803 ± 0.055</td>
<td>0.825 ± 0.070</td>
</tr>
<tr>
<td>Tissue barrier</td>
<td>0.548 ± 0.011</td>
<td>0.544 ± 0.022</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.527 ± 0.033</td>
<td>0.527 ± 0.031</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values are representative of comparison of R-Pnx and sham groups.

### Table 3. Absolute volumes, surface areas, and diffusing capacities

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<tbody>
<tr>
<td></td>
<td>R-Pnx</td>
<td>Sham</td>
</tr>
<tr>
<td>Terminal body wt, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, ml/kg</td>
<td>28.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
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<tr>
<td>Immersion displacement</td>
<td></td>
<td></td>
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<tr>
<td>Cavalieri method</td>
<td>64.3 ± 3.8</td>
<td>29.7 ± 1.8</td>
</tr>
<tr>
<td>Septum</td>
<td>37.2 ± 1.2</td>
<td>17.0 ± 0.9</td>
</tr>
<tr>
<td>Septal tissue</td>
<td>5.10 ± 0.47</td>
<td>1.92 ± 0.04</td>
</tr>
<tr>
<td>Capillary blood</td>
<td>2.35 ± 0.19</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>Capillary</td>
<td>2.80 ± 0.29</td>
<td>1.04 ± 0.03</td>
</tr>
<tr>
<td>Absolute surface area, m²/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveoli</td>
<td>2.69 ± 0.16</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>Capillary</td>
<td>2.26 ± 0.15</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>Morphometric diffusing capacity for CO, ml·min⁻¹·Torr⁻¹·kg⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DpCO</td>
<td>8.27 ± 0.25</td>
<td>3.26 ± 0.38</td>
</tr>
<tr>
<td>DpCO</td>
<td>3.00 ± 0.44</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>DpCO</td>
<td>2.18 ± 0.24</td>
<td>0.81 ± 0.03</td>
</tr>
<tr>
<td>Morphometric diffusing capacity for O₂, ml·min⁻¹·Torr⁻¹·kg⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DpO₂</td>
<td>10.18 ± 0.30</td>
<td>4.01 ± 0.47</td>
</tr>
<tr>
<td>DpO₂</td>
<td>11.64 ± 1.63</td>
<td>4.36 ± 0.13</td>
</tr>
<tr>
<td>DpO₂</td>
<td>5.38 ± 0.37</td>
<td>2.06 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values are representative of comparison of R-Pnx and sham groups. DpCO and DpCO₂, membrane diffusing capacity for CO and O₂; DpCO₃ and DpCO₂, erythrocyte diffusing capacity for CO and O₂; DpO₂, lung diffusing capacity for O₂.
1 yr after left pneumonectomy or R-Pnx, arterial $O_2$ saturation, diffusing capacity, pulmonary hemodynamics, cardiac output, and pulmonary mechanics at maximal exercise remain abnormal; compensation reached no more than 70–80% of the normal values (19–21, 24–27).

Compensatory response to pneumonectomy in immature animals. Although the physiological reserves of diffusing capacity must also have been recruited in immature dogs after pneumonectomy, their postpneumonectomy adaptive response is primarily characterized by active alveolar proliferation. This compensatory proliferative tissue response has been well documented in rats and rabbits (3–6, 33–36, 38, 42, 44), but the rodent data could not be directly extrapolated to large animals because of the continuous growth pattern of the rodent; i.e., its epiphyses never close (12). A few studies have examined large immature animals (11, 30, 43), but none has previously addressed the long-term postpneumonectomy functional outcome at maturity by exercise studies. Short-term physiological studies by Ford et al. (13), Arnup et al. (2), and Thurlbeck et al. (43) in immature dogs 11–15 wk after left pneumonectomy showed normalization of lung volumes (2, 13) and a marked compensatory increase in alveolar number (43). The only long-term study was a series by Wilcox et al. (50), Pimmel et al. (32), and Davies et al. (11) in immature beagles after left pneumonectomy. At 1 yr after pneumonectomy, lung volume and $D_{LCO}$ measured at rest were normal (50). However, structural studies of the remaining lung performed in these same animals 5 yr after left pneumonectomy demonstrated no increase in the number of alveoli compared with the same lung of control animals (11). Hence, the physiological and structural data from this series of animals are at variance and also differ from the short-term data of Thurlbeck et al. (43). Davies et al. (11) suggested that the acceleration of lung growth early after pneumonectomy may be transient and is not sustained up to maturity. Later studies by Johson et al. (30) in beagles, 7–9 mo after left pneumonectomy as puppies, reported significant increases in resting lung diffusing capacity, alveolar septal tissue volume, and alveolar surface area of the remaining lung consistent with compensatory lung growth; however, the nature of the increase in septal tissue volume and the extent of functional compensation at exercise were not studied.

We previously reported the serial measurements of resting lung function during maturation in the present group of immature dogs (from 4 wk to 1 yr after surgery) (39, 40). Results are as follows. 1) Resting $D_{LCO}$, measured by a rebreathing technique, returned to normal by 8 wk after pneumonectomy and remained normal up to maturity. 2) Volume of fine septal tissue, measured physiologically by a rebreathing technique, returned to normal rapidly. Volume of nonseptal lung tissue, measured by combined rebreathing and computerized tomography techniques, remained below normal up to maturity. 3) Static lung volume-transpulmonary pressure relationship, lung elastic recoil, and total pulmonary resistance remained abnormal up to maturity. 4) Pneumonectomy did not selectively affect growth or development of the thoracic cage. Findings 1 and 2 suggest that compensatory lung tissue growth had occurred, and this interpretation is confirmed by the present morphometric data. The morphology of post-

![Graph](http://jap.physiology.org/)
pneumonectomy compensatory alveolar growth, involving all septal tissue components and leading to restoration of gas-exchange function, is distinct from the reparative growth seen after acute and chronic diffuse lung injury, which leads to eventual fibrosis. The biochemical and molecular signals/mediators evoked after pneumonectomy are also likely different from those evoked in other diffuse lung injury models. These pneumonectomy-induced signals are incompletely defined, although mechanical stretch is believed to play a major role. Findings 3 and 4 suggest that large airways and blood vessels did not grow at the same rate as the parenchyma after pneumonectomy. In addition, the composition of the noncellular septal components may have changed after pneumonectomy, contributing to the altered mechanical behavior. Further studies are necessary to clarify these issues.

Comparison of response in immature and adult dogs. We demonstrate here that postpneumonectomy compensation in gas-exchange is indeed sustained in immature dogs, eventually yielding a functional capacity of the remaining lung more than twice that of the control left lung at maturity; alveolar-capillary gas-exchange is restored completely to normal (Fig. 2). Not only is gas-exchange normal at rest, but the pattern of recruitment of pulmonary capillaries with increasing blood flow during exercise is also normal (Fig. 3). These results provide evidence that the adequacy of end-capillary O₂ saturation according to the Bohr integral (31). In contrast, postpneumonectomy adaptive mechanisms restore only 50% of the functional deficit in DLCO at a given workload in adult dogs after R-Pnx (23); these animals show persistent reductions in DLCO and DLCO/Q̇c at peak exercise, causing arterial O₂ saturation to fall prematurely as exercise load increases (26).

The maturity-related differences in postpneumonectomy morphological response are shown in Table 4 with use of previous data from adult dogs studied by similar techniques 16 mo after R-Pnx (23). Compared with the left lung of the respective control group, the relative increase in most structural parameters was consistently greater in dogs pneumonectomized as puppies than as adults. One important exception is that specific volume of type II epithelium increased to the same extent in adult and puppy pneumonectomized groups (2.1- and 2.3-fold, respectively). On the other hand, specific volume of type I epithelium increased by 1.6- and 2.7-fold in these respective groups (P < 0.0001). This comparison suggests that patterns of regenerative alveolar growth in the puppy are different from those in the adult animal. In the puppy, type I and type II cells are stimulated equally in regenerative growth. In the adult dog, regenerative growth of type II cells exceeds that of type I cells. Reasons for this difference are not clear. In rats, which continue to grow throughout their life span, early response to left pneumonectomy is characterized by a greater increase in the number of type II cells than type I cells (36, 41). Presumably these type II cells develop into type I cells, and as the stimulus for accelerated cell proliferation diminishes with time, the observed early differential cellular response may not be sustained. Available evidence suggests that the response to pneumonectomy in adult dogs is slow and protracted over many months; hence, the persistent differential type I vs. type II cellular response in pneumonectomized adult dogs may reflect continued active epithelial proliferation. The adult-puppy comparison also demonstrates that enhanced regenerative alveolar-capillary growth is responsible for the superior functional capacity of the immature dog after pneumonectomy.

Correlation of DLCO measured by physiological and morphometric methods. Diffusing capacity estimated from structural parameters by a morphometric model (45, 47) is thought to represent the maximum possible diffusing capacity on the basis of the assumptions that 1) the alveolar-capillary anatomy determines the upper limit of gas-exchange and 2) the distribution of capillary red blood cells found postmortem is similar to the one in vivo distribution during exercise. Because the alveolar-capillary bed is not fully recruited at rest, DLCO estimated by the morphometric model is consistently higher than that measured by physiological methods at rest (9, 10). We showed previously in adult dogs that when morphometric DLCO is compared with physiological DLCO measured at peak exercise in the same animals, the agreement is much closer (22, 28). Combining all mature and immature dogs studied by these two techniques (Fig. 5), we found that physiological estimates are on average 10% higher than morphometric estimates. This is a remarkable agreement, considering the disparate approaches and assumptions involved in the two techniques.

Conclusions. We conclude that the loss of >50% of lung tissue as a result of surgical pneumonectomy in immature animals leads to strong compensatory growth of the remaining alveolar tissue that exceeds normal maturational growth and fully reconstitutes the size of the pulmonary alveolar-capillary network. On reaching somatic maturity, alveolar gas-exchange capacity during exercise is fully maintained.

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REFERENCES


