

Dead space ventilation gains special importance during a period of 1 h. Dead space increase and reversibility of the increase was investigated during MV with different tidal volumes and during SB. During SB, the dead space volume was 0.21 ± 0.14 ml and increased significantly at MV to 0.39 ± 0.08 ml at a tidal volume of 5 ml/kg and to 0.6 ± 0.08 ml at a tidal volume of 8 and 11 ml/kg. Dead space and wasted ventilation (dead space volume in relation to tidal volume) were determined over a period of 1 h. Dead space increase and reversibility of the increase was mostly reversible by switching back to SB. Surfactant depletion had no further influence on the dead space volume. This increase was mostly reversible by switching back to SB. Surfactant depletion had no further influence on the dead space volume. This increase was mostly reversible by switching back to SB.

IN RESPIRATORY PHYSIOLOGY, the dead space describes that volume part of inhaled air that is not involved in gas exchange. Dead space ventilation gains special importance during a pathophysiological status of the lung at which alveolar regions lose their ventilatory efficiency. This is usually the case in patients suffering from the acute respiratory distress syndrome (ARDS), which is associated with an increased physiological dead space (5). Although this is established knowledge for human patients, where it can be used as a predictor for the outcome of ARDS, no data are available for small animals such as rats (4, 12). However, many ARDS models are established in small animals (2, 13); therefore, knowledge of the pulmonary dead space of rats with and without ARDS is important for a more complete characterization of these models.

Different methods have been established to measure pulmonary dead space (22). Bohr developed a method, which uses a mass-balance equation considering tidal volume ($V_T$) and mixed-expired gas (10). However, especially in critical care, the end-tidal CO2 can be influenced, for example by intrapulmonary shunts, and therefore become an inaccurate number (7). Overcoming this problem, dead space can be determined by the single-breath analysis of carbon dioxide. This method is mainly based on Fowler’s studies and has been further improved up to now (6, 23). Thereby, the expired carbon dioxide concentration is plotted against the total expired volume. Analysis of the graph form then allows determination of dead space volume ($V_D$) as described by Fowler (22).

The most useful tool to measure such single-breath diagram for carbon dioxide is mainstream volumetric capnography. However, mainstream capnography and single-breath analysis have been complicated by the relatively small VT values (<10 ml) that are characteristic for small lungs (26). Common mainstream capnographs inserted directly between Y-piece and tracheal cannula add too much dead space to maintain adequate oxygenation and lack a response time and sample rate adequate for the high-ventilation frequency of small animals.

For the purpose of this study, a novel CO2 sensor specifically designed for mainstream capnography in rats was developed and evaluated. It was the aim of this study to gain reference values for $V_D$ in a widely used small-animal model of ARDS under different respiratory conditions. Therefore, pulmonary dead space was analyzed in healthy and surfactant-depleted rat lungs dependent on VT and time during spontaneous breathing and mechanical ventilation.

MATERIALS AND METHODS

Sensor

We developed a small-animal mainstream CO2 sensor, which is based on infrared absorption of CO2 molecules (Fig. 1). The sensor is composed of a clamp-like housing for the optical components (sensor part) and a small cuvette (external dimensions: 14 mm × 9 mm × 6 mm) with an optical path perpendicular to the mainstream channel. The optical path is limited by two perforations at opposite sides sealed with glass cover slides (Langenbrinck, Emmendingen, Germany). The cuvette was designed to connect the tracheal cannula and the Y-piece of the ventilator. To avoid significant additional dead space resulting from the sensor geometry, the dimensions of the cuvette were minimized, resulting in an apparatus dead space of 0.1 ml. The sensor part was clamped to the cuvette in a way that infrared light, which is emitted by a diode (LED43-SMD3–2, Deep Red Technologies, Harwell, UK) on one side of the clamp can pass through the cuvette onto a photo resistor (PR43, Deep Red Technologies, Harwell, UK) covered by an optical filter (NBP 4.26 μm/180 nm, InfraTec, Dresden, Germany). The diode was pulsed with a frequency of 1 kHz. A measurable voltage signal was achieved by amplification, band-pass filtering (second-order filter with middle frequency = 1 kHz), rectifying, and low-pass filtering (second-order Tschebyscheff filter with cutoff frequency = 45 Hz) of the signal from the photo resistor. The resulting voltage (correlating inversely with CO2 content) was recorded continuously and simultaneously together with the respiratory data (airway pressure, inspiratory and expiratory flow). The sample rate was 500 Hz.

Address for reprint requests and other correspondence: C. Dassow, Dept. of Anesthesiology, Univ. Medical Center Freiburg, Hugstetter Strasse 55, 79106 Freiburg, Germany.

8750-7587/13 Copyright © 2013 the American Physiological Society http://www.jappl.org
and expiratory flow. The CO2 signal is shown as solid line.

...compensated for data analysis.

...phase III reflects the gas exhaled from the terminal airways and the apparatus dead space, expirogram is divided into three phases: phase I represents the volume exhaled from the conducting airways and the apparatus dead space, phase II represents the volume exhaled from the terminal airways and the alveoli with the shortest transit times, and phase III reflects the gas being exhaled from all of the rest of the alveoli (alveolar plateau).

Data Analysis

We used the single-breath diagram method according to Fowler et al. (6, 22) to determine the VD. Thereby, expired CO2 concentration is plotted against expired volume, and the resulting curve can be divided along the volume axis into three phases. Geometrical analysis of the curve allows calculation of the VD (Fig. 2A). Briefly, the CO2 expirogram is divided into three phases: phase I represents the volume exhaled from the conducting airways and the apparatus dead space, phase II represents the volume exhaled from the terminal airways and the alveoli with the shortest transit times, and phase III reflects the gas being exhaled from all of the rest of the alveoli (alveolar plateau).

CO2 was recorded over a period of ~1 min. Data were analyzed using Matlab (Math Works, Natick, MA). To avoid overestimation of the Vd, elasticity and air compressibility of the tubing system were determined and compensated for data analysis.

To overcome the low signal-to-noise ratio of the sensor signal, volume and CO2 data were superimposed and averaged breathwise from start of expiration to end of expiration. All complete breaths in the recorded period were used. Start and end of expiration were automatically detected by using thresholds on the separately recorded inspiratory and expiratory flow signals.

Due to the low-pass behavior of the CO2 sensor system (including electric and pneumatic low-pass behavior), some resulting single-breath diagrams were lacking a discernible third phase. Therefore, a correction of the data was implemented. A deconvolution with the transfer function of the measurement system would have been the optimal method of correction, but was not successful because of the low signal-to-noise ratio. Instead, a template of a single-breath diagram consisting of three straight lines (representing the three phases) was used to numerically approximate the deconvoluted curve form. Therefore, the parameters of the template (start-volume, end-volume, and end-CO2 value of phase two) were changed iteratively in a way that, when the template was convoluted with the transfer function of the measurement system, it matched the measured CO2 data. As an indicator for the fit-quality of the curves, the sum of the squared difference was chosen (least squares method). Fowler’s method was then applied to the resulting template to determine the VD (Fig. 2B).

Physical Lung Model

The sensor was evaluated using a custom physical lung model (Fig. 3A). The model was based on a plastic container (representing the lung’s alveolar compartment) filled with water (150 ml) and room air (175 ml) (Fig. 3A). The gas compartment is continuously flushed with CO2 to maintain a physiological end-tidal CO2 of 38 Torr during ventilation. This was achieved by changing the flow rate of a peristaltic pump (MCP-Pumpantrieb, IsmaTec, Glattbrugg-Zürich, Switzerland). Speed of gas mixture was achieved by installing a small fan (12 V, 25 × 10 mm) in the alveolar compartment. A separate connection allowed ventilation of the alveolar compartment with a small-animal ventilator (FlexiVent, Scireq, Montreal, Canada). The CO2 sensor was attached between the Y-piece and the alveolar compartment. Compliance was adjusted by changing the compressible gas volume via filling a part of the alveolar compartment with water. VD was simulated by inserting a tube (inner diameter = 2.2 mm) between sensor and alveolar compartment. Different VD values are simulated by using tubes with different lengths, resulting in defined volumes (0.2, 0.3, 0.4, 0.5, and 0.6 ml).

Twelve independent CO2 and volume measurements per dead space tube were performed in our model during mechanical ventila-
Anesthesia was induced intraperitoneally with 100 mg/kg ketamine (Ketanest S, Pfizer, Karlsruhe, Germany) /H11001. Airway pressure was measured close to the expiratory tube of the ventilator to allow for flow measurement during ventilator. Custom-made valves were inserted in the inspiratory and expiratory flow rate were inserted separately close to the tracheal cannula. Flow sensors for independent measurement of inner diameter, SIMS Portex, Kent, UK). A research small-animal Non Sterile Polythene Tubing, 0.58 mm inner diameter, 0.96 mm saline/h), as necessary. After tracheotomy for intubation of the trachea, the carotid artery was cannulated with polythene tubing (Portex Non Sterile Polythene Tubing, 0.58 mm inner diameter, 0.96 mm outer diameter, SIMS Portex, Kent, UK). A research small-animal ventilator (FlexiVent, Scireq, Montreal, Canada) was connected to the tracheal cannula. Flow sensors for independent measurement of inspiratory and expiratory flow rate were inserted separately close to the ventilator. Custom-made valves were inserted in the inspiratory and expiratory tube of the ventilator to allow for flow measurement during spontaneous breathing. Airway pressure was measured close to the Y-piece of the ventilator. Animals were ventilated with pressure-limited volume-controlled ventilation with a fraction of inspired oxygen (FIO2) of 1. Ventilation was set to either one of the three following settings: RR of 90 breaths/min at a VT of 5 ml/kg, RR of 70 breaths/min at a VT of 8 ml/kg, and RR of 50 breaths/min at a VT of 11 ml/kg. During the experiments, RR was adjusted to maintain arterial partial pressure of carbon dioxide within the physiological range (35–45 Torr) and to ensure physiological normal breathing of the animals. Positive end-expiratory pressure was set to 2 cmH2O. Inspiratory and expiratory flow rate, airway pressure, and mean arterial pressure were continuously measured and recorded. The rats were killed by exsanguination at the end of the experiment.

Groups and Interventions

Two different experimental protocols were used. During the first protocol, the controlled mechanical ventilation was randomized to one of three VT values (5, 8, and 11 ml/kg) and maintained for 1 h (continuous ventilation). During the second protocol, spontaneous breathing and mechanical ventilation with increasing VT values were alternated to investigate reversibility of the dead space increase (discontinuous ventilation). Induction of spontaneous breathing was achieved by hypercapnia.

For all three VT groups and for the reversibility group, there was an intervention group [surfactant-depleted lung (SDL) group]. Thereby, following a stabilization period of 5 min after animal preparation, lung lavage with saline solution (25 ml/kg) was repeated until criteria for ARDS were fulfilled [arterial PO2 (Pao2)/FIO2 < 200 Torr].

Experimental Protocol

Spontaneous breathing. During both experimental protocols, we switched between spontaneous breathing and mechanical ventilation. To record respiratory parameters (inspiratory flow rate, expiratory flow rate, and airway pressure) and CO2 at spontaneous breathing, we used custom-made back-pressure valves. The valves were inserted in the inspiratory and expiratory branch of our small-animal ventilator system in a way that we could switch promptly between mechanical ventilation with 100% oxygen and spontaneous breathing with room air. Animals breathed spontaneously with an average volume of 5.4 ± 0.9 ml/kg.

Continuous ventilation. Preparation of the animals was performed under spontaneous breathing. Before switching to mechanical ventilation, the CO2 signal was recorded. Animals were randomized to 5, 8, or 11 ml/kg VT and ventilated for 60 min. After switching to mechanical ventilation, measurements were done at 5, 10, 15, 20, 30, 40, 45, 50, and 60 min of ventilation. Mechanical ventilation was switched off after the last measurement, and spontaneous breathing was restored. After a stabilization time of 10 min, another measurement was done.

During the experimental protocol, MAP was recorded every 5 min, and blood-gas analysis was performed every 15 min and once during each spontaneous breathing period.

Compliance (C) was calculated using the airway pressure, and flow, recordings during the CO2 measurements at mechanical ventilation:

\[ C = \frac{V_T}{P_{SE} - P_{EE}} \]

where VT compensated for compliance of the tubing system, PSE is pressure at start of expiration, and PEE is pressure at end of expiration. Median compliances of all breaths per measurement were calculated.

Discontinuous ventilation. Spontaneous breathing and ventilation alternated throughout this protocol. Before switching on mechanical ventilation, a measurement was done. Ventilation with a VT of 5 ml/kg was started and maintained for 10 min. During that period, measurements were done twice (every 5 min). Ventilation was...
showed that it discerns VD values differing by 0.1 ml (P ≤ 0.05).

RESULTS

During mechanical ventilation, the PaO₂/FIO₂ (ratio of PaO₂ and FIO₂) remained over 400 Torr in the control group, while it was lower than 200 Torr in the SDL group. At spontaneous breathing, it was lower than 300 Torr in both groups and lower than 200 Torr in the SDL group after intervention (Table 1). Compliance during mechanical ventilation was significantly lower in the SDL group (P < 0.005; Table 1). No significant differences were found in MAP and RR between the groups.

Evaluating our CO₂ sensor in the physical lung model showed that it discerns VD values differing by 0.1 ml (P < 0.001) with its response time of 25 ms. A linear dependence could be found between the measured dead spaces with a slope of 0.75 and a coefficient of determination of 0.93 (Fig. 3B).

During spontaneous breathing, the average VD in all rats was 0.21 ± 0.14 ml. By switching on controlled mechanical ventilation, VD values increased in all three VT groups and did not change over the mechanical ventilation period of 1 h. Switching back to spontaneous breathing led to a decrease in VD values close to those before mechanical ventilation (0.27 ± 0.12 ml, Fig. 4A).

Absolute VD values during mechanical ventilation were 0.39 ± 0.03 ml at a VT of 5 ml/kg, 0.6 ± 0.08 ml at a VT of 8 ml/kg, and 0.6 ± 0.08 ml at a VT of 11 ml/kg. Compared with spontaneous breathing, absolute VD values increased significantly at mechanical ventilation (P < 0.01; Fig. 4B).

Wasted ventilation (VD/VT) increased significantly in all three VT groups by switching on mechanical ventilation (P < 0.005). This increase was highest in the 5 ml/kg group, followed by the 8 ml/kg group, and lowest in the 11 ml/kg group. A significant difference was found between the 5 ml/kg group and the 11 ml/kg group (P < 0.01). Switching back to spontaneous breathing after 1 h of ventilation, the decrease in wasted ventilation was significant in the 5 ml/kg group (P < 0.001) and the 8 ml/kg group (P < 0.05), but not in the 11 ml/kg group (Fig. 5A).

No significant difference in wasted ventilation was found between normal and surfactant-depleted lungs during mechanical ventilation (Fig. 5B).

Changing from spontaneous breathing to mechanical ventilation every 10 min at increasing VT values led to a diminished efficiency in reversibility in the surfactant-depleted lungs. Wasted ventilation during spontaneous breathing was significantly higher after mechanical ventilation with 8 and 11 ml/kg in the surfactant-depleted lungs compared with healthy lungs (P < 0.005; Fig. 6).

SB, spontaneous breathing; MV 15, MV 30, MV 45, MV 60: mechanical ventilation at 15, 30, 45, and 60 min, respectively; SDL, surfactant-depleted lung; PaO₂/FIO₂, ratio of arterial partial pressure of O₂ to fraction of inspired O₂; MAP, mean arterial pressure.

Table 1. Physiological parameters of 46 rats

<table>
<thead>
<tr>
<th></th>
<th>SB</th>
<th>MV 15</th>
<th>MV 30</th>
<th>MV 45</th>
<th>MV 60</th>
<th>SB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂/FIO₂ Torr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Mean</td>
<td>273.57</td>
<td>416.74</td>
<td>415.25</td>
<td>418.21</td>
<td>407.79</td>
<td>294.43</td>
</tr>
<tr>
<td>Control SD</td>
<td>43.03</td>
<td>91.54</td>
<td>96.53</td>
<td>70.21</td>
<td>75.67</td>
<td>66.24</td>
</tr>
<tr>
<td>SDL Mean</td>
<td>281.22</td>
<td>82.96</td>
<td>96.79</td>
<td>113.13</td>
<td>124.13</td>
<td>194.73</td>
</tr>
<tr>
<td>SDL SD</td>
<td>42.15</td>
<td>42.67</td>
<td>68.65</td>
<td>94.85</td>
<td>104.78</td>
<td>21.23</td>
</tr>
</tbody>
</table>

MAP, mmHg

Control Mean | 121.25 | 120.8 | 118.54 | 109.55 | 100.74 | 87.61 |
Control SD   | 15.52  | 17.95 | 18.40  | 19.55  | 20.74  | 10.07 |
SDL Mean     | 112.92 | 98.25 | 91.25  | 83.25  | 79.83  | 63.8  |
SDL SD       | 13.37  | 16.81 | 15.67  | 19.87  | 15.36  | 14.34 |

Compliance, ml/mbar

Control Mean | 0.21   | 0.2    | 0.2    | 0.2    | 0.19   | 0.01   |
Control SD   | 0.01   | 0.01   | 0.02   | 0.01   | 0.01   | 0.01   |
SDL Mean     | 0.13   | 0.15   | 0.14   | 0.15   | 0.15   | 0.15   |
SDL SD       | 0.02   | 0.03   | 0.03   | 0.03   | 0.03   | 0.03   |

Respiratory rate, breaths/min

Control Mean | 45.94  | 92.68/63.33/41.95 | 56.11 |
Control SD   | 8.96   | 4.74/2.79/2.63    | 22.90 |
SDL Mean     | 43.49  | 96.05/64.6/47.44  | 72.27 |
SDL SD       | 11.30  | 8.45/4.51/3.44    | 40.46 |

Fig. 4. VD values in healthy lungs. A: absolute VD values measured during spontaneous breathing (SB; shaded area) and 1 h of mechanical ventilation (MV) at three different tidal volumes [VT; 5 ml/kg body wt (solid line), 8 ml/kg body wt (dashed line), and 11 ml/kg body wt (dotted line)]. B: averaged VD values over MV (dark shaded bars) and SB (light shaded bars). Values are means ± SD; n = 6–8. *P < 0.05.

J Appl Physiol • doi:10.1152/japplphysiol.00299.2013 • www.jappl.org
been strong evidence that increased VD/VT is related to a higher mortality risk in ARDS (4, 12). For instance, there has been strong evidence that increased Vd/Vt is related to a higher mortality risk in ARDS (4, 12). This was proven even during the early stages of ARDS (4). Since lung protective ventilation is still the most effective therapy to reduce mortality in ARDS, the measurement of Vd/Vt or its subcomponents has gained importance (7). Mainstream capnography has been established in several animal models recently, among others in piglets and rabbits (17, 23). However, no mainstream capnograph is available or has been built for animals smaller than piglets or rabbits. Capnographs that are customized for small rodents all function with side-stream technique (for instance the Type 340 Capnograph by Harvard Apparatus). Nonetheless, rats or mice are more often used as a laboratory model, which raises the need of equipment suitable for those experiments.

We developed a new CO2 mainstream sensor, which is suitable for small lungs with a very small VT (<10 ml). With this newly developed sensor, we found four results that seemed to be of interest. First, using our sensor yields a low apparatus dead space of only 0.1 ml. Second, we were able to discern dead space differences as low as 0.1 ml. Third, the device was successfully applied in rat experiments to determine pulmonary dead space. Fourth, our sensor has a low response time of 25 ms, and we used a sample rate of 500 Hz.

Compared with available sensors, our sensor provides significantly better time resolution, suited for the breathing frequency of a rat. Our sensor could easily be inserted between tracheal cannula and Y-piece of the ventilator system. The results obtained from the animal investigation indicate that our sensor is useful to record capnograms of small lungs, such as rat lungs.

To evaluate the performance of our sensor, we developed a simple physical lung model simulating the alveolar compartment. This model had to fit the properties of a small rat lung, including gas exchange, and the potential to simulate an injured lung. Investigating preset dead spaces in our model, we found that our sensor underestimates the given dead space in a linear manner. This was observed to be independent from VT and compliance and may, therefore, be associated with our automated calculation method. However, the model was suitable for testing the sensor performance. Mechanical ventilation of the physical model resulted in typical capnograms similar to those recorded in vivo. The evaluation of the sensor showed that it is capable to discern between dead spaces that differ by <0.1 ml. It further showed that the difference of the resulting dead spaces and the difference of the set dead spaces have a

DISCUSSION

The concept of respiratory dead space is an old one that still remains relevant. It defines the volume compartment of the airways that is ventilated, but does not participate in physiological gas exchange (6). As a dynamic value depending on pulmonary strain (and therefore on pressure, flow rate, and volume of respiratory ventilation), Vd serves as an important clinical parameter of respiratory physiology. Mainstream sensors are still the most accurate method to measure CO2 in breathing gas (23). The alternative solution is side-stream capnography. However, those sensors have the disadvantage of time-delayed measurement, which only allows an approximation of the capnogram (11). Unfortunately, most mainstream sensors have the disadvantage to add a significant apparatus dead space to the ventilation system. This gains particular importance in small animals with very low absolute VT values. Although mainstream capnography is routinely used in clinical environments since the 1980s (9), in small lungs, such as in newborn lungs, this technique could not be used until the late 1990s (1). The main problem to overcome was the volume size of additional apparatus dead space. However, even modern mainstream capnographs suitable for newborns do not apply for small-animal lungs such as rat lungs.

Capnography is an effective and simple method to determine dead space and monitor lung function (22). Capnographic parameters have been shown to produce good prognostic values, for example, of lung injury (4, 12). For instance, there has been strong evidence that increased Vd/Vt is related to a higher mortality risk in ARDS (12, 15). This was proven even during the early stages of ARDS (4). Since lung protective ventilation is still the most effective therapy to reduce mortality in ARDS, the measurement of Vd/Vt or its subcomponents has gained importance (7). Mainstream capnography has been established in several animal models recently, among others in piglets and rabbits (17, 23). However, no mainstream capnograph is available or has been built for animals smaller than piglets or rabbits. Capnographs that are customized for small rodents all function with side-stream technique (for instance the Type 340 Capnograph by Harvard Apparatus). Nonetheless, rats or mice are more often used as a laboratory model, which raises the need of equipment suitable for those experiments.

We developed a new CO2 mainstream sensor, which is suitable for small lungs with a very small VT (<10 ml). With this newly developed sensor, we found four results that seemed to be of interest. First, using our sensor yields a low apparatus dead space of only 0.1 ml. Second, we were able to discern dead space differences as low as 0.1 ml. Third, the device was successfully applied in rat experiments to determine pulmonary dead space. Fourth, our sensor has a low response time of 25 ms, and we used a sample rate of 500 Hz.

Compared with available sensors, our sensor provides significantly better time resolution, suited for the breathing frequency of a rat. Our sensor could easily be inserted between tracheal cannula and Y-piece of the ventilator system. The results obtained from the animal investigation indicate that our sensor is useful to record capnograms of small lungs, such as rat lungs.

To evaluate the performance of our sensor, we developed a simple physical lung model simulating the alveolar compartment. This model had to fit the properties of a small rat lung, including gas exchange, and the potential to simulate an injured lung. Investigating preset dead spaces in our model, we found that our sensor underestimates the given dead space in a linear manner. This was observed to be independent from VT and compliance and may, therefore, be associated with our automated calculation method. However, the model was suitable for testing the sensor performance. Mechanical ventilation of the physical model resulted in typical capnograms similar to those recorded in vivo. The evaluation of the sensor showed that it is capable to discern between dead spaces that differ by <0.1 ml. It further showed that the difference of the resulting dead spaces and the difference of the set dead spaces have a

Fig. 5. Wasted ventilation in healthy and surfactant-depleted lungs. A: Vd to VT ratio (Vd/VT) at SB and at MV with different VT values. Wasted ventilation was determined at SB before (solid bar) and after (light shaded bars) MV and averaged over the 1-h MV period (dark shaded bars). Values are means ± SD; n = 4–8. *P < 0.05. B: Vd/VT at different VT values (healthy animals = shaded bars; surfactant depleted animals = shaded dotted bars). Values are means ± SD; n = 6–9.

Fig. 6. Reversibility in healthy and surfactant-depleted lungs. Changes from SB (shaded areas) to MV (open areas) with increasing VT values in healthy (solid line) and surfactant-depleted (dashed line) animals. Values are means ± SD; n = 4–11. *P < 0.05.
highly linear relationship. Although dead space was underestimated by our sensor, this error was linear and did not influence the detection of dead space change. Therefore, the sensor performance is sufficient for the measurement of dead space in small animals.

We decided to calculate dead space according to Fowler’s method, which has been improved since its introduction in 1948 (6). Others have reported that this method cannot be applied to small lungs with short exhalation times (<200 ms) due to the low sample rates and high response times of available sensors (17). This often leads to the absence of phase III in the capnogram. We circumvented this deficit in our sensor, but, due to its low-pass behavior, the problem was not resolved completely. In some capnograms, especially in those recorded from surfactant-depleted animals, phase III was not as well-defined as desired. Therefore, we developed an automated method to correct the measured data. Applying this method to our model data, we achieved sufficiently accurate results to discern differences in dead space lower than 0.1 ml.

The main findings of the animal experiments can be summarized as follows: 1) for the first time, pulmonary dead space of rats was measured during spontaneous breathing and during mechanical ventilation in dependence of VT, lung damage, and time; 2) compared with spontaneous breathing, absolute VD values and wasted ventilation (Vd/VT) significantly increased at mechanical ventilation; 3) the change in Vd between spontaneous breathing and mechanical ventilation is reversible; 4) no significant difference in wasted ventilation was found between normal and surfactant-depleted lungs during mechanical ventilation.

The anatomical dead space in mechanically ventilated animals with a VT of 5 ml/kg was increased compared with spontaneously breathing animals, but did not differ significantly. Thereby, the VT values were similar. At higher VT values, there was a significantly higher dead space. Interestingly, this dead space did not increase with VT, but was almost identical at 8 and 11 ml/kg. Absolute dead space values did not change during mechanical ventilation, which may have resulted from the relatively short ventilation time of 1 h. According to the sensor evaluation, absolute dead space values are underestimated linearly by a factor of 0.75 and a constant part of 0.19 ml. Correcting for that, an average absolute dead space of 3.2 ml/kg body wt was found for rats at VT values of 8–11 ml/kg. Other studies have provided dead space data that allow an estimation of 1.36 ml/kg body wt for human dead space (3) at comparable VT values. Therefore, the dead space per kilogram body weight was slightly over two times as high in the rat model compared with human values. This may either result from species differences, a different study design, or a different evaluation system. Considering a species difference, this would indicate that the human lung is significantly more efficient than the rat lung in terms of dead space.

Concerning the underlying physiological processes, the following conclusions can be made. The dead space measured in our study exclusively consists of the volume of the conducting system in the lung (trachea, bronchi, and bronchioles) being the anatomical dead space plus the apparatus dead space. An increase in dead space indicates an expansion of these parts of the lung and an increase of the strain and, accordingly, the stress of the corresponding tissue. This indicates that positive pressure ventilation does always increase stress in the lung compared with spontaneous breathing. However, there is also the phenomenon of the virtually identical Vd values at ventilation with 8 and 11 ml/kg. Since we consider the increase of dead space to be an increase in the volume of the conducting system of the lung, this means that, already at ventilation with 8 ml/kg, the conducting system is extended maximally. Such a maximal extension matches other studies that analyzed pulmonary tissue based on models (24) and based on direct (21) or indirect observation (14). Hence it can be concluded that further increases in VT will then be exclusively used to extend the alveolar compartment.

Usually dead space is not expressed as an absolute value but as proportion of VT (Vd/VT) (4a). In rats, we found a ratio of 0.1 at spontaneous breathing, which is lower compared with that found in humans (normally ~0.3) (4a). We found that wasted ventilation was increased by positive pressure ventilation in all three VT groups compared with spontaneous breathing. This positive effect of spontaneous breathing on Vd/VT is in line with the observation that Vd/VT is lower if spontaneous breathing is allowed during mechanical ventilation in humans (18, 28).

According to the VT and Vd, Brewer et al. measured (3), Vd/VT can be estimated to 0.17 for anatomical dead space in humans during mechanical ventilation. This value is slightly lower compared with the Vd/VT we measured in rats at VT values of 8 and 11 ml/kg. Furthermore, marked increase of Vd/VT during ARDS has been found in humans (15). In our data, no such significant increase of Vd/VT has been observed in the surfactant-depleted rat lungs. However, we used relatively short experiment times with mechanical ventilation periods of 1 h. Even in studies concerning the early phase of ARDS, patients were investigated 10 h after ARDS had developed (15). On that account, prolonging mechanical ventilation may have led to a significant increase in Vd/VT.

Another reason for a missing difference of Vd/VT in surfactant-depleted lungs could be alveolar dead space, which is included in the dead space of the study in humans, but not in our measured dead space. There are other animal models in which Vd/VT has been investigated in small surfactant-depleted lungs, for instance in piglet and rabbit lungs (17, 27), but those studies do not come to a definite conclusion. Some have found an elevation of Vd/VT (27), but there is also evidence that Vd/VT was decreased after surfactant depletion (17).

We took great care in the analysis of the reversibility of the dead space changes by implementing a special protocol for its analysis. Both protocols revealed that the increase in Vd/VT is reversed shortly after spontaneous breathing is reestablished. In the context of our interpretation, this means that the expansion of the conducting system is reversible, at least after a short time of ventilation in noninjured lungs. In the surfactant-depleted lungs, the reversibility was missing. This indicates that, with less surfactant, the expansion of the conducting system based on the positive pressure ventilation was inhibited. It hence leads to one of either conclusions: 1) the mechanical ventilation recruited/opened the conducting system, and this effect lasted after turning the ventilation off; or 2) the missing surfactant led to a higher retraction of bronchioles so that these did expand less due to the ventilation.

Additionally, methodological problems have complicated the evaluation of our data in view of the fact that only one-third of the surfactant-depleted animals could be weaned after me-
We used saline lavage to deplete surfactant in the rat lung. Saline lung lavage is a widely used rat model to investigate ARDS. VD values at mechanical ventilation were significantly distinguished at breathing frequencies as high as 90 breaths/min. The index continuously remained lower than 200 Torr in lavaged animals, whereas in control animals the value was constantly higher than 400 Torr. Surprisingly, under spontaneous breathing, we determined a PaO2/FIO2 between 200 and 300 Torr in both groups. This was probably due to the breathing depressing effect of our anesthetics (ketamine and medetomidine), which may have led to an impaired oxygenation (16). However, VD seems not to be influenced by that. Compliance was significantly lower in lavaged animals, which is a well-known indicator for lung injury (19).

Conclusions

Our study has shown that the newly developed CO2 sensor is able to measure dead space by volumetric capnography in very small lungs, such as in rats. VD values of 0.1 ml could be distinguished at breathing frequencies as high as 90 breaths/min. The data collected in this study add reference information about functional parameters in a widely used animal model of ARDS. VD values at mechanical ventilation were significantly higher compared with spontaneous breathing. This dead space increase was completely reversible in healthy lungs, but not in surfactant-depleted lungs at ventilation with 8 ml/kg body wt or higher VT. Furthermore, the dead space that was measured during mechanical ventilation with VT values in the range of 5 to 11 ml/kg body wt was always higher than during spontaneous breathing. This clearly indicates that positive pressure ventilation is associated with an increase in mechanical stress on the conductive airways tissue.

Grants

This project was supported by the German Research Foundation “Protective Artificial Respiration”: GU 561/7-2.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author Contributions

Author contributions: C.D. and J.G. conception and design of research; C.D. and H.R. performed experiments; C.D. and D.S. analyzed data; C.D., D.S., and J.G. interpreted results of experiments; C.D. prepared figures; C.D. drafted manuscript; C.D., D.S., H.R., and J.G. edited and revised manuscript; C.D., D.S., H.R., and J.G. approved final version of manuscript.

References