Measurement of tidal volume by using transthoracic impedance variations in rats

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¹Department of Human Physiology, School of Medicine, Flinders University of South Australia, and Departments of ²Critical Care Medicine and ³Biomedical Engineering, Flinders Medical Centre, Adelaide, South Australia 5042, Australia

Davidson, K. G., A. D. Bersten, T. E. Nicholas, P. R. Ravenscroft, and I. R. Doyle. Measurement of tidal volume by using transthoracic impedance variations in rats. J. Appl. Physiol. 86(2): 759–766, 1999.—The application of impedance pneumography for monitoring respiration in small animals has been limited by problems with calibration. With improved instrumentation, we describe the calibration of tidal volume in anesthetized rats. The detection of changes in voltage, reflecting the electrical impedance variations associated with respiration, was optimized by using disposable adhesive silver-silver chloride electrodes, advanced circuitry, and analog-to-digital recording instrumentation. We found a linear relationship between change in impedance and tidal volume in individual rats (R² = 98%), which was strongly influenced by rat weight. Consequently, a calibration equation incorporating change in impedance and rat weight was derived to predict tidal volume. Comparison of the predicted and true tidal volumes revealed a mean R² = 98%, slopes of −1, intercepts of −0, and bias of −0.07 ml. The predicted volumes were not significantly affected by either frequency of respiration or pulmonary edema. We conclude that impedance pneumography provides a valuable tool for the noninvasive measurement of tidal volume in anesthetized rats.

METHODS

Design Elements of the Impedance Pneumograph and Recording System

Respiratory movements were monitored by using a modified constant-current impedance pneumograph (IPG) based on a design first reported by Geddes and associates (9) in 1962 (Fig. 1). The IPG was used in the fully floating mode with bipolar electrode connection. Briefly, the complete unit is built on a single circuit board and incorporates alternating-current or direct-current output coupling. Voltage regulators provide stable direct-current power rails of ±15 V, which supply various circuit components. A single transistor Colpitts oscillator produces a low-voltage, high-frequency (50 kHz) current, via a toroidal transformer, between two transthoracic electrodes. Two 10-kΩ resistors in series with the transformer output provide sufficient internal Z to operate the IPG in essentially constant-current mode. Field-effect transistor (FET) input amplifiers with very high-input Z present virtually no loading on the measured circuit. The frequencies around 50–100 kHz are commonly used, as they are high enough to avoid stimulation of tissue, electrode polarization, and problems associated with high skin Z values and, yet, not so high as to encounter problems with radio interference (7).

The signal is amplitude modulated by ΔZ across the thorax, buffered by the two FET input amplifiers, amplified by a differential amplifier, and passed through high- and low-pass filters in series, effectively forming a band-pass filter centered
on 50 kHz. The narrow band of frequencies (44–54 kHz) allowed to pass eliminates unwanted interference, leaving only the 50-kHz carrier waveform modulated by the Z signal. The signal emerging from the filters is then demodulated by a precision full-wave rectifier to remove the carrier, with the final output representing the ΔZ. The original circuit incorporated an electrocardiogram filter and output, and, although this was included in the updated IPG, it was not used.

The modifications to the IPG, including a higher 50-kHz voltage at the transformer output, allows a doubling of the series resistors, affording near-perfect constant-current operation. Modern components, such as the FET input amplifiers, allow absolute minimum loading on the electrode circuit. The offsetting capability was not included because the high-pass filter is effectively alternating-current coupled and the use of a precision LM308 differential amplifier made the offsetting facility redundant. The original circuit omitted a feedback resistor around the final operational amplifier of the precision rectifier; this has been corrected.

The IPG was interfaced with a MacLab system 4 analog-to-digital instrument and Chart software (version 3.5.3; AD Instruments, Sydney, Australia). Data were recorded at 100 Hz.

Anesthesia

Male porton rats (118–480 g) were anesthetized with methohexital sodium (60 mg/kg ip; Brietal, Eli Lilly, Sydney, Australia) and pentobarbital sodium (40 mg/kg ip; Nembutal, Boehringer Ingelheim, Sydney, Australia). Prolonged anesthesia was maintained by occasional injections of Brietal (30 mg/kg ip) and Nembutal (20 mg/kg ip). The thorax and abdomen were shaved, and the remaining hair was completely removed by using hair removal cream (Veet; Reckitt & Colman Pharmaceuticals, Sydney, Australia). Body temperature was monitored by using a rectal thermocouple (Yellow Springs Instruments, Yellow Springs, OH) and maintained at 37°C by placing the rats on a thermostatically controlled pad.

Electrode Selection and Placement

Preliminary experiments in spontaneously breathing rats examined the effect of electrode type and position on the amplitude of the Z signal. Adhesive, disposable solid-gel monitoring electrodes (25 and 50 mm), 3M red dot Ag-AgCl electrodes (3M Health Care, London, Canada), and Neonatal Hydrogel Ag-AgCl electrodes (3M Health Care) were compared with subcutaneous stainless steel needles (0.45 × 13 mm; Becton-Dickinson Medical Products) and stainless steel dental needles (0.40 × 38 mm; Halas Dental, Sydney, Australia). Unlike the subcutaneous electrodes, the disposable electrodes were easy to attach, detected a strong Z signal with high reproducibility, and afforded a high degree of tolerance to changes in rat position. Although all adhesive types proved similarly effective, the 25-mm 3M red dot electrodes were used for the remainder of the study because of their affordability and suitable size.

The position of the electrodes on the chest is reported to influence the magnitude of the signal detected (2, 18). We, therefore, tested several electrode positions and found the strongest ΔZ with the least noise when the electrodes were placed on either side of the chest in the midaxillary line at the xiphoid level, with the outer edges of the electrodes almost meeting along the sternum and the lower edges lining up with the base of the rib cage. Although a ground electrode was theoretically unnecessary in the fully floating mode, one was applied to the lower abdomen to minimize drift. Potentially, this electrode provides more defined capacitive paths from each electrode and associated leads to Earth.

Ventilation and Monitoring Cardiorespiratory Variables

A caudal artery was cannulated for sampling arterial blood and monitoring systemic arterial blood pressure and heart rate via a disposable pressure transducer (Sorenson Transpac; Abbott Critical Care Systems, Chicago, IL) interfaced to the MacLab recording system. The catheter was flushed
continuously (syringe pump, model 355, Sage Instruments, Cambridge, MA) with heparinized isotonic saline (2 IU heparin/ml; David Bull Laboratories, Melbourne, Australia) at a rate of 1 ml/h.

Baseline cardiorespiratory variables were recorded for 1 min in the spontaneously breathing anesthetized rats in both the supine and prone positions. Rats were then tracheostomized, by using a 16-gauge cannula, and were mechanically ventilated, by using a sinusoidal waveform where the inspiratory time/total time for the waveform = 0.5 (flexiVent; SCIREQ Scientific Respiratory Equipment, Montreal, Quebec, Canada), in the prone position at a f of 60 breaths per minute (bpm) (22).

Arterial blood (0.15 ml) was collected into heparinized syringes (PICO; Radiometer, Copenhagen, Denmark), and blood gases were measured on a blood-gas-pH analyzer (model ABL 5; Radiometer). VT and f were adjusted to maintain the arterial Pco2 between 35 and 45 Torr. Respiratory f was determined by using the Chart software ratemeter function.

Calibration

The IPG was calibrated by using both control (n = 20) and edematous (n = 19) rats. Before calibration, the rats were given an additional dose of Brietal (15 mg/kg) via the lateral caudal vein at the hilum of the tail to further suppress respiratory drive. Although cardiac output was maintained for some minutes, this dose was lethal to nonventilated rats. The mechanical ventilator was momentarily set to deliver 0 ml and then programmed to increase VT in 0.5-ml increments up to 4.5 ml. VT was held at each increment for six breath cycles and then progressively lowered in the same manner. The procedure was repeated three times. On each occasion, a f of either 30, 60, or 120 bpm was randomly used. The total procedure took 6 min/rat.

Induction of Edema

A polyethylene catheter (0.2-mm internal diameter × 0.5-mm external diameter) was inserted −4 mm below the tracheostomy and directed to a point just above the carina. Haemaccel (Behring Institute, Marburg, Germany), containing Evans blue (15 mg/ml) as a marker, was instilled intratracheally into ventilated rats at a rate of 3 ml/kg over 30 min (compact infusion pump, model 975; Harvard Apparatus). The rats were placed prone at a 30° angle with the head tilted caudal vein at the hilum of the tail to further suppress reflux of the instillate. Again VT and f were adjusted to maintain the arterial Pco2 between 35 and 45 Torr. Respiratory f was determined by using the Chart software ratemeter function.

Lung Isolation

After calibration, the thorax was opened and the heart and lungs were removed (22). The lobes were resected, weighed, and lyophilized to determine the wet-to-dry lung weight ratio.

Data Calculation

The Z signal was calibrated by using linear regression analysis, solving the change in millivolts (ΔmV) for the VT delivered for each rat (y = intercept + slope·x), where the ΔmV represents the change of magnitude of Z computed directly by using the “Max − Min” software function for the fourth breath cycle at any given VT

\[ ΔmV = \text{slope}_1 \cdot VT \]  

Because there was an obvious weight dependence, intercept1 and slope1 were solved for the weight of each rat

\[ \text{slope}_1 = \text{intercept}_3 + \text{slope}_3 \cdot \log (\text{weight}) \]  
\[ \text{intercept}_1 = \text{intercept}_2 + \text{slope}_2 \cdot \log (\text{weight}) \]

Therefore, the final calibration equation included both rat weight and volume

\[ ΔmV = [\text{intercept}_2 + \text{slope}_2 \cdot \log (\text{weight})] \]  
\[ + [\text{intercept}_3 + \text{slope}_3 \cdot \log (\text{weight})] \cdot \text{VT} \]

Statistics: Validation of Model

For any given ΔmV, a predicted VT was calculated by rearranging the calibration equation

\[ \text{Predicted VT} = \frac{[ΔmV - \text{intercept}_2 - \text{slope}_2 \cdot \log (\text{weight})]}{[\text{intercept}_3 + \text{slope}_3 \cdot \log (\text{weight})]} \]

For each rat, the predicted and actual VT were compared. Then the mean R², slopes, and intercepts ± 95% confidence intervals (CI) were determined within each group (30, 60, or 120 bpm; control or edema). The Bland and Altman (6) method was used to calculate the bias (mean difference) ± 95% CI between the predicted and actual VT within each group.

To establish the validity of the model we used the “PRESS” technique, otherwise known as the “leave one out” approach, as described in Draper and Smith (8), to predict the VT for each rat, at each f, both with and without edema. This technique involves leaving out one observation from the data analysis; the rest of the data is then used to construct a model to predict the observation omitted. This process is repeated for every observation, and, as before, the mean R², slopes, intercepts, and bias ± 95% CI of the predicted vs. actual VT were determined. Unless otherwise stated, results are expressed as means ± 95% CI. Student's t-test was used for all comparisons.

RESULTS

Z Variations

Spontaneous breathing. A typical Z trace from a spontaneously breathing rat is shown in Fig. 2A. Inspiration was associated with increased Z, and expiration with a decrease. Sighs were recorded as large upward deflections occurring at a rate of about one sigh every 3 min. The sensitivity of the detection is illustrated by the small pulsatile deflections, consistent with cardiac oscillations (16), that were superimposed on the major respiratory fluctuations. However, it is also possible that these inflections are due to electrical activity during myocardial contraction. Similarly, breathing impinging on cardiac output, particularly during sighs. The Z signal remained stable with involuntary movement of the rat’s legs, head, or tail, and changes were absent during periods of apnea.

Although the technique appears to work equally well in awake animals (unpublished observations), the animals tend to have a poor behavioral tolerance toward the leads and need to be restrained.
Mechanical ventilation. The ΔZ signal remained remarkably constant at any given VT (Table 1). In individual rats, the ΔZ at any VT was the same regardless of whether VT was being increased or decreased (Fig. 2B). If the lung was held at constant volume, there was little, if any, noise or drift. However, the minor pulsatile deflections, reflecting either cardiac oscillations or electrical activity of the heart, persisted. When the rat was taken off the ventilator, the absolute Z decreased to the level found at 0 ml VT. Presumably, this corresponds to functional residual capacity.

Effect of Body Position

Body position, whether supine or prone, did not affect the ΔZ, as reflected over five breath cycles recorded after 30 s in either position [spontaneous breathing; supine: 1.65 ± 0.114 (SE) mV, prone: 1.59 ± 0.123 mV; n = 39 rats].

Extent and Distribution of Edema

Intratracheal instillation of Haemaccel resulted in a relatively even distribution of Evans blue (Fig. 3). The wet-to-dry lung weight ratio increased from 4.8 ± 0.03 (SE), similar to that reported previously (20), to 6.1 ± 0.12 (P < 0.001).

Derivation of Calibration Equations

A direct, linear relationship was found in each rat between ΔmV and VT (all R² ≥ 97%). This relationship was clearly dependent on weight, with the smallest rats having the greatest ΔZ. Regression analysis was, therefore, used to examine the effect of rat weight on the ΔmV for each given VT (Table 2). Because multiple measures were performed on each rat (i.e., at VT = 0.5, 1.0, 1.5, 2.0 ml, etc.), each data point cannot truly be considered an independent event. Therefore, rather than using standard multiple linear regression analysis to derive the calibration equation, we first solved the millivolts for ventilated VT in each rat and then solved the acquired slopes (slope₁, Eq. 2a) and intercepts (intercept₁, Eq. 2b) for rat weight (Table 3). Clearly, the slope was more dependent on rat weight than was the intercept.

Consequently, the final slopes (slope₂ and slope₃) and intercepts (intercept₂ and intercept₃), which were used to derive the calibration equations (Eq. 3), included both VT and rat weight in their derivation.
Correlation and Agreement Between Predicted and Actual VT

When the calibration equation from each group was rearranged to predict VT (Eq. 4), a high degree of correlation was obtained for all groups (R²: 98–99%), irrespective of respiratory rate or edema (Table 4). The slopes were ~1, and their CI crossed 1. There was minimal offset, as the intercepts were between −0.05 and 0.00 mV and the upper and lower CI crossed zero. The difference between the predicted and actual VT or “bias” was minimal and was between 0.14 and 0.17 ml for the control and between −0.01 and −0.03 ml for the edematous rats. The 95% CI of the bias were −0.09 ml in the control and −0.05 ml in the edematous rats.

Model Validation: The PRESS Technique

When the PRESS technique was applied to each data set (i.e., 30, 60, or 120 bpm; control or edema), comparable R², slopes, intercepts, and bias & 95% CI were obtained (Table 5) to those described above (Table 4). This was also the case when the equations (Eq. 4) derived from the edematous rats were applied to the control data and vice versa (Table 6).

DISCUSSION

Our results demonstrate that Z pneumography can be used to determine VT accurately in small animals independently of f or pulmonary edema. The technique is relatively noninvasive and can be used to monitor breathing in anesthetized animals over prolonged periods.

Improvements in Pneumography

Pneumograph design. At least three IPG circuit designs have been reported (1, 23). Unlike the other circuits, the constant-current IPG requires no balance adjustment and has a linear response with high Z tolerance, and calibration is independent of total subject Z. Because it measures the ΔZ, it is the only circuit suitable for volumetric determination. In addition, the

Table 2. Relationship between ΔZ and rat weight in rats with and without pulmonary edema at various VT and ventilation frequencies

<table>
<thead>
<tr>
<th>VT, ml</th>
<th>Slope</th>
<th>Intercept, mV</th>
<th>R², %</th>
<th>Slope</th>
<th>Intercept, mV</th>
<th>R², %</th>
<th>Slope</th>
<th>Intercept, mV</th>
<th>R², %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>−1.22:−0.98</td>
<td>0.62:0.48</td>
<td>67.70</td>
<td>−1.24:−1.04</td>
<td>0.62:0.51</td>
<td>69.70</td>
<td>−1.41:−1.04</td>
<td>0.64:0.51</td>
<td>72.70</td>
</tr>
<tr>
<td>1.0</td>
<td>−3.44:−2.62</td>
<td>1.75:1.41</td>
<td>81.71</td>
<td>−3.53:−2.79</td>
<td>1.74:1.45</td>
<td>79.76</td>
<td>−3.73:−2.79</td>
<td>1.76:1.44</td>
<td>80.76</td>
</tr>
<tr>
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<td>3.08:2.67</td>
<td>83.86</td>
<td>−6.45:−5.21</td>
<td>3.13:2.67</td>
<td>83.88</td>
<td>−6.90:−5.21</td>
<td>3.23:2.67</td>
<td>80.88</td>
</tr>
<tr>
<td>2.0</td>
<td>−9.58:−7.82</td>
<td>4.67:4.08</td>
<td>84.90</td>
<td>−9.62:−7.77</td>
<td>4.63:3.94</td>
<td>84.91</td>
<td>−9.91:−7.72</td>
<td>4.68:3.94</td>
<td>83.91</td>
</tr>
<tr>
<td>2.5</td>
<td>−13.31:−10.75</td>
<td>6.41:5.55</td>
<td>85.91</td>
<td>−13.17:−10.38</td>
<td>6.29:5.26</td>
<td>84.93</td>
<td>−13.53:−10.38</td>
<td>6.36:5.26</td>
<td>83.93</td>
</tr>
<tr>
<td>3.5</td>
<td>−21.20:−16.42</td>
<td>10.05:8.42</td>
<td>86.93</td>
<td>−20.58:−15.82</td>
<td>9.75:7.95</td>
<td>85.92</td>
<td>−20.16:−15.82</td>
<td>9.55:7.95</td>
<td>85.92</td>
</tr>
</tbody>
</table>

Results were derived by using linear regression analysis to solve ΔZ in mV for rat weight (118–480 g) at various VT and ventilation frequencies (bpm). Results are expressed as ratio of mean to slope, intercept, and R² in nonedematous (control; n = 20) and edematous rats (n = 19).
Toxic and irritant and also affect offset potentials.

Inert. Reactions between the skin or tissue and the stainless steel electrodes is that they are not chemically appear as noise on the trace. Another problem with the electrode offset potential, whereas short-term changes generation of electrode offset potentials (24). Baseline drift and noisy characteristics, similar to that reported needle electrodes produced an erratic baseline with signals with little drift over periods of 10 h (data not shown). In contrast, the stainless steel subcutaneous needle electrodes produced an erratic baseline with drift and noisy characteristics, similar to that reported by others (17, 27). This has been attributed to the generation of electrode offset potentials (24). Baseline drift is normally the result of long-term changes in the electrode offset potential, whereas short-term changes appear as noise on the trace. Another problem with the stainless steel electrodes is that they are not chemically inert. Reactions between the skin or tissue and the electrodes can produce soluble metallic salts, which are toxic and irritant and also affect offset potentials.

**Table 3. Intercepts and slopes used in final calibration (Eq. 3)**

<table>
<thead>
<tr>
<th>Frequency, bpm</th>
<th>Weight vs. slope_1</th>
<th>Weight vs. intercept_1</th>
<th>R^2, %</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
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<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
<td>95</td>
<td>-4.07</td>
<td>-1.14</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>60</td>
<td>94</td>
<td>-4.00</td>
<td>-1.12</td>
</tr>
<tr>
<td>120</td>
<td>120</td>
<td>120</td>
<td>94</td>
<td>-3.81</td>
<td>-1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>92</td>
<td>-3.06</td>
<td>-0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>91</td>
<td>-3.08</td>
<td>-0.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>94</td>
<td>-2.89</td>
<td>-0.64</td>
</tr>
</tbody>
</table>

Results were derived by using linear regression analysis to solve the slopes and intercepts for the log of each rat’s weight. Results are shown at various frequencies (bpm) in rats with (Edema) and without (Control) pulmonary edema.

“Floating system” used avoided subject-ground artifacts (23).

Electrode selection and placement. Type. Several electrodes, including some of those previously reported (17, 21), were evaluated. The adhesive Ag-AgCl disposable electrodes produced stable, reproducible, and noise-free signals with little drift over periods of >10 h (data not shown). In contrast, the stainless steel subcutaneous needle electrodes produced an erratic baseline with drift and noisy characteristics, similar to that reported by others (17, 27). This has been attributed to the generation of electrode offset potentials (24). Baseline drift is normally the result of long-term changes in the electrode offset potential, whereas short-term changes appear as noise on the trace. Another problem with the stainless steel electrodes is that they are not chemically inert. Reactions between the skin or tissue and the electrodes can produce soluble metallic salts, which are toxic and irritant and also affect offset potentials.

**Table 4. Relationship between predicted (Eq. 4) and actual V_t at various ventilation frequencies in rats with and without edema**

<table>
<thead>
<tr>
<th>Frequency, bpm</th>
<th>Control</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

R^2 | 99 | 99 | 99 | 99 | 98 | 98 | 98 | 98 |

±95% CI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Slope | 1.09 | 1.10 | 1.08 | 0.99 | 1.00 | 1.00 |

±95% CI | 0.157 | 0.177 | 0.155 | 0.065 | 0.078 | 0.070 |

Intercept | 0.05 | -0.05 | -0.03 | 0.00 | -0.01 | -0.01 |

±95% CI | 0.089 | 0.088 | 0.079 | 0.055 | 0.053 | 0.054 |

Bias, ml | 0.14 | 0.17 | 0.14 | -0.03 | -0.01 | -0.02 |

±95% CI | 0.082 | 0.093 | 0.083 | 0.039 | 0.047 | 0.043 |

Results were derived by using linear regression analysis to solve predicted vs. actual V_t. Results are expressed as means ± 95% confidence intervals (CI). Mean difference, or bias, was determined within each group. Results are shown at various frequencies (bpm) in rats with (Edema; n = 19) and without (Control; n = 20) pulmonary edema.

Unlike the stainless steel electrodes that store offset potentials and may take as long as 6 min to recover to an acceptable baseline, the offset potential of the adhesive Ag-AgCl electrode is small and decreases exponentially (24), hence producing a stable trace. The electrical stability of the adhesive Ag-AgCl electrodes has also been considerably enhanced through the use of electrolyte gel.

**Table 5. Relationship between predicted (Eq. 4) and actual V_t at various ventilation frequencies in rats with and without edema after employing the PRESS technique**

<table>
<thead>
<tr>
<th>Frequency, bpm</th>
<th>Control</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>60</td>
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<td>60</td>
</tr>
<tr>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

R^2 | 99 | 99 | 99 | 98 | 98 | 98 | 98 |

±95% CI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Slope | 1.16 | 1.16 | 1.12 | 0.97 | 1.00 | 1.00 |

±95% CI | 0.201 | 0.252 | 0.197 | 0.067 | 0.086 | 0.073 |

Intercept | 0.07 | -0.08 | -0.05 | 0.00 | -0.01 | -0.01 |

±95% CI | 0.120 | 0.119 | 0.102 | 0.060 | 0.059 | 0.061 |

Bias, ml | 0.29 | 0.27 | 0.21 | -0.08 | -0.01 | -0.01 |

±95% CI | 0.104 | 0.131 | 0.104 | 0.041 | 0.051 | 0.047 |

Data were derived by using the PRESS technique. See Table 4 legend. n = 19 and 20 for Edema and Control, respectively.

Table 6. Comparison of relationship between predicted (Eq. 4) and actual V_t derived by applying control equations to edematous data and vice versa

<table>
<thead>
<tr>
<th>Frequency, bpm</th>
<th>Control Data Derived Using Coefficients</th>
<th>Edema Data Derived Using Control Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>60</td>
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</tr>
<tr>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

R^2 | 99 | 99 | 99 | 98 | 98 | 98 |

±95% CI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Slope | 1.03 | 1.00 | 1.11 | 1.06 | 1.13 | 0.97 |

±95% CI | 0.086 | 0.084 | 0.097 | 0.117 | 0.169 | 0.106 |

Intercept | 0.00 | 0.00 | -0.10 | 0.07 | -0.08 | 0.07 |

±95% CI | 0.042 | 0.041 | 0.069 | 0.092 | 0.102 | 0.052 |

Bias, ml | 0.07 | -0.01 | 0.15 | 0.06 | 0.20 | 0.01 |

±95% CI | 0.050 | 0.048 | 0.058 | 0.061 | 0.089 | 0.064 |

Results were derived by using linear regression analysis to solve predicted vs. actual V_t. Calibration coefficients from control equations (Table 3) were used to determine predicted volume for edematous data and vice versa. Results are expressed as means ± 95% CI. Mean difference, or bias, was determined within each group. Results are shown at various frequencies (bpm) in rats with (Edema) and without (Control) pulmonary edema. n = 19 rats.
and position of the electrodes on the chest are critical. Bone, lung, heart, and connective tissues comprise a relatively constant contribution to total transthoracic electrical $Z$ such that intrathoracic gas and biological fluids become the main variables. Whereas ionized fluids have a comparatively low resistance, fat and air are highly resistive (19). $Z$ measurements are, therefore, influenced not only by $V_T$, but also by the underlying fat. Therefore, as the position of subcutaneous electrodes may critically change, e.g., with alterations in muscle tension with depth of anesthesia, the large surface area and adhesive properties of the $3M$ electrodes afforded relatively high tolerance to positioning and provided good reproducibility.

Consistent with previous reports (2, 15), we found the greatest amplitude of $Z$ excursions with the electrodes placed on either side of the chest in the midaxillary line at the xiphoid level, with a ground electrode applied to the lower abdomen.

Recording system. Technological advances in analog-to-digital instrumentation and software have led to the development of sensitive, fast multichannel data recorders capable of defining high-fidelity signals. The system used records and displays experimental data on-line with excellent resolution and also has the advantage of computer-based data handling and analysis. These critical advances allow data manipulation and greater sensitivity that was lacking in earlier $Z$ research.

$Z$ Variations in the Rat

Effect of weight and body position on $Z$. The $\Delta Z$ was directly related to $V_T$. Consistent with the findings of Baker et al. (2), Berman et al. (5), and Hamilton et al. (11), who reported no change in the relationship between $Z$ and $V_T$ with body position in humans (2, 11) and dogs (5), body position did not affect $\Delta Z$ in rats. Analogous to humans, the $\Delta Z$ at any $V_T$ was greatest in the smallest animals (2, 3). Consequently, rat weight was included in deriving the calibration equations.

Comparison of predicted and actual $V_T$. We used regression analysis to investigate the strength of the relationship between the ventilated $V_T$ and that predicted from the calibration equation. The bias was summarized for each group (f and edema) by calculating the mean difference between the predicted and actual $V_T$. The precision was reflected by their 95% CI 

Comparison of the predicted and true $V_T$ by linear regression analysis showed $R^2 \approx 98\%$, with slopes and intercepts of $-1$ and $0$, respectively (Table 4). In other words, $\approx 98\%$ of the observed $\Delta Z$ can be explained by the calibration equation. The bias was small, and the CI was narrow. In a 200-g rat, this amounts to an error of $\approx 3\%$ at resting $V_T$.

Effect of frequency. Consistent with the work of Baker and Hill (3), we found that the $\Delta f$ over a wide range of respiratory rates, 30–120 bpm, had no effect on $\Delta Z$ or the relationship between predicted and actual $V_T$. However, at high $f$ the baseline did not immediately return to zero during the expiration phase of the volume calibration maneuver. Presumably, this reflects dynamic hyperinflation, although we have no way of knowing this.

Effect of alveolar edema. In agreement with others (5, 12), the absolute level of $Z$ decreased slightly over the 30-min period of Haemaccel infusion. The decrease in absolute $Z$ may have resulted from changes in regional ventilation, perfusion, and/or increased lung fluid; however, the $\Delta Z$ did not change. Consequently, the correlation between predicted and actual $V_T$ was not affected by edema or $f$. Moreover, there was no deterioration in the bias or the 95% CI. Indeed, comparable $R^2$, slopes, intercepts, and bias $\pm$ 95% CI were obtained when the equations derived from the edematous rats were applied to the control data and vice versa.

In conclusion, we have described a quantitative method for determining $V_T$ in rats based on change in transthoracic $Z$. An equation was derived by using animal weight and $\Delta Z$ to accurately predict $V_T$ over a wide range of $f$. Predicted $V_T$ was not affected by edema. The IPG provides a valuable tool for the continuous, noninvasive measurement of $V_T$ and $f$ in small anesthetized animals.

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