The measurement of thoracic gas volume (TGV) in mice is hampered by the problems relating to the instrumentation required for such a small animal (7, 9), and by the weakening of the respiratory drive during anesthesia or the absence of muscular activity due to paralysis (9), which precludes the estimation of TGV by Boyle’s principle (3). This latter problem has been addressed by Lundblad et al. (9), who proposed mechanical compression of the chest wall during occlusion of the airway opening in mice paralyzed to ensure the apneic conditions required by low-frequency measurements of respiratory impedance. The potential disadvantages of this method are the expiratory direction of the chest deformation, which may facilitate closure of the airways connecting the gas compartments, and the nonuniformity of the pleural pressure change; indeed, these factors may have led to unreliable TGV values at functional residual capacity (FRC) in this study (9).

In this report, we describe an alternative plethysmographic technique to obtain TGV values in apneic mice at any level of lung inflation. Inspiratory effort against an occluded airway opening was generated by electrical impulse stimulation of intercostal muscles. TGV values estimated by stimulation (TGVst) were compared with those obtained during the spontaneous inspiratory effort (TGVsp) occurring during the initial phase of the experiments. The method was checked by comparing the difference in TGVst measured before and after injection of a known volume of gas. Following the validation studies, TGV was measured at end expiration in two additional strains of mice.

METHODS

Animal Preparation

Male CBA/Ca mice (N = 19) were anesthetized with an intraperitoneal injection of pentobarbital sodium [70 mg/kg body weight (BW)], tracheostomized, and cannulated with a 0.8-mm inner diameter polyethylene tube. The animals were placed in the supine position in a custom-built body plethysmograph (160 ml volume) and ventilated transmurally with a small-animal respirator (Harvard Apparatus, South Natick, MA) at a rate of 160 breaths/min, a tidal volume of 0.25 ml, and a positive end-expiratory pressure of 2 cmH2O. Supplemental doses of pentobarbital sodium (5 mg/kg) were administered when indicated, generally at the beginning of the measurements. The animal handling and study protocol were approved by the Institutional Animal Care and Use Committee of the University of Szeged.

Female BALB/c mice (N = 6) and female C57 BL/6 mice (N = 6), obtained from the Animal Resource Centre (Murdoch, Western Australia), were prepared similarly to the CBA/Ca mice, except that anesthesia was induced by intraperitoneal injection of 0.1 ml/10 g BW

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of a mixture of 40 mg/ml ketamine and 2 mg/ml xylazine (Parnell Laboratories, NSW, Australia), and that a flexiVent small-animal ventilator (SCIREEQ, Montreal, PQ, Canada) was used at a rate of 450 breaths/min and a tidal volume of 8 ml/kg. The experimental procedures were approved by the Institute’s Animal Ethics Committee and conformed to the guidelines of the National Health and Medical Research Council of Australia. The data on age and BW for the three groups of mice are given in Table 1.

**Measurement of TGV**

The measurements of TGV were performed as follows. The respirator was stopped, and the tracheal cannula and body box were opened to the atmosphere for ~2 s to equilibrate the transrespiratory pressure (Prs) with the ambient pressure at end expiration. The box and airway opening were then closed, and the corresponding pressures [Pbox and tracheal pressure (Ptr)], measured with miniature pressure transducers (model 8507C-2, Endevco, San Juan Capistrano, CA), were recorded for 10 s. TGV was calculated from either the spontaneous breathing effort against the closed airway or the stimulated breathing effort, i.e., when intercostal muscles were stimulated via a pair of thin-wire electrodes arranged diagonally between the upper and lower chest regions, with single impulses 8–12 V in amplitude and 0.1 ms in duration (model 544, Grass Instruments, Quincy, MA), repeated five to six times in a 10-s recording interval. The lung volume was raised from FRC in two ways: first, by lowering Pbox via the vacuum line to either −10 or −20 cmH2O, governed by the height of a limiting water column, while the tracheal cannula was opened to the atmosphere, and, second, by injecting a known amount of nitrogen into the lungs (see below). Apart from the respirator type, the same experimental setup (Fig. 1) was employed in the two study sites.

The signals of Pbox, Ptr, and flow were low-pass filtered at 50 Hz and digitized at a rate of 256 Hz by an analog-digital board of a personal computer. The recordings of Pbox were corrected for the thermal characteristics of the plethysmograph; this was necessitated by the markedly different rates of the volume changes in the spontaneous and evoked respiratory muscle contractions. To this end, the pressure-flow relationship, i.e., the impedance of the plethysmograph \( Z_{box}(\omega) \), where \( \omega \) is the angular frequency, was measured with forced oscillations between 0.5 and 50 Hz, as the load impedance on a wave tube (6), with an apneic and occluded airway, average-sized mouse in the box. The pressure-volume relationship, i.e., the elastic modulus (E), was obtained as \( E(\omega) = j\omega Z_{box}(\omega) \), where \( j \) is the imaginary unit, and the correction function \( c(\omega) = E_0/E(\omega) \) was applied to the Pbox recordings in the frequency domain to obtain the corrected \( c(\omega) \) box pressure Pbox, \( c(\omega) \) as Pbox, \( c(\omega)Pbox/\omega \). \( E_0 \) corresponds to the isothermal elastic modulus of the box and hence can be calculated as \( P_0/Vbox \), where \( P_0 \) is the atmospheric pressure and Vbox is the volume of gas in the plethysmograph. The application of \( c(\omega) \), therefore, corrected the Pbox signal for the deviations from the isothermal compression of the box, and, since it was based on the actual thermal characteristics of the plethysmograph, it was a more accurate and less troublesome solution compared with the use of a mass of copper wool in the box (20). With this correction, Boyle’s law for an isothermal process can be assumed in both gas compartments during the respiratory effort against the occluded airway, where the change in box volume is equal but opposite in sign to that in thoracic volume. The respiratory efforts were identified on the basis of the changes in Ptr: when Ptr exceeded the threshold level set corresponding to the magnitude of the cardiogenic oscillations, the data points above the level were included in the estimation of the Pbox, vs. Ptr relationship by linear regression. With the assumption that the pressure changes are small relative to the atmospheric pressure (Patm) and with the use of the slope of the regression line \( s = \Delta Pbox/\Delta Ptr, TGV \) was calculated as TGV = \( −s\beta(Vbox − Vm) − Vds \), where \( \beta = (Patm − P_H_2O)/Patm \) is a correction factor accounting for the partial pressure of water vapor (P_H_2O) in the alveolar gas (3), Vm is the volume occupied by the mouse in the box, and Vds is the instrumental dead space between the tracheal cannula and the site of occlusion (0.05 ml).

**Study Protocol**

Comparison of TGV values calculated from the spontaneous and the stimulated breathing effort. TGVsp and TGVst were calculated within a single data epoch by recording both the stimulated and the spontaneous breathing effort against an occluded airway in the CBA/Ca mice. The recordings were first made at the FRC, where the mice were apneic at the beginning of the data epoch and the stimulated breathing effort was registered. The spontaneous breathing effort returned later in the same occlusion period, allowing the estimation of TGVst and TGVsp at the same end-expiratory lung volume. Subsequently, Prs was raised to either 10 or 20 cmH2O before the spontaneous breathing effort returned (whenever necessary, the appearance of the spontaneous breathing activity was delayed by the addition of

![Image](https://i.imgur.com/7G5Q5JG.png)

**Fig. 1. Schematic arrangement of the plethysmographic measurement of thoracic gas volume (TGV).** During mechanical ventilation, stoppers A and C are open to the respirator and the atmosphere, respectively, and stopper B is closed. All stoppers are closed for the estimation of TGV during the spontaneous or the stimulated breathing effort. During the increase in lung volume, stoppers B and C are open to the atmosphere and a vacuum line, respectively, while stopper A is closed. The maximum transrespiratory pressure [tracheal pressure (Ptr) − box pressure (Pbox)] of 10 or 20 cmH2O is set by a limiting water column. The equipment dead space between the tracheal cannula and stoppers A and B is 0.05 ml, which is subtracted from the measured values of TGV.

**Table 1. Characteristics and TGV values at end-expiration in the 3 strains of mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>Age, wk</th>
<th>Body weight, g</th>
<th>TGVst, ml</th>
<th>TGVsp, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA/Ca</td>
<td>19</td>
<td>16</td>
<td>35.0±2.7</td>
<td>0.29±0.05</td>
<td>0.24±0.05</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>6</td>
<td>8</td>
<td>19.1±1.8</td>
<td>0.34±0.08</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td>6</td>
<td>8</td>
<td>18.6±0.6</td>
<td>0.28±0.06</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; N, no. of mice. TGV, thoracic gas volume. TGVst and TGVsp are obtained from the stimulated and the spontaneous contractions of respiratory muscles, respectively.
small supplemental doses of anesthetics). At the end of the inflation, the box and the airway opening were again closed for \( \sim 20 \) s, and the occluded breathing effort was recorded. With this timing and maintenance of the appropriate level of anesthesia, it was possible to record both the stimulated and the spontaneous effort at the same elevated lung volume. The baseline (FRC) measurements of TGVst and TGVsp and the inflation maneuvers to a Prs of 10 or 20 cmH\(_2\)O were all repeated three times in each animal. The readings of TGVsp and TGVst were pooled for each animal and each Prs level to obtain mean values and determine the variability of the TGV estimates.

Comparison of TGV values measured with different resident gases. In four CBA/Ca mice, TGVst and TGVsp were determined at the FRC as above. The animals were then ventilated with a mixture of 20% oxygen and 80% helium for 5 min, and the occlusion maneuvers were recorded. Subsequently, ventilation with air was resumed for 5 min, and the measurements of TGV were repeated. The values of TGVst and TGVsp obtained with air as the resident gas before and after the helium-oxygen breathing were averaged to compensate for any temporal change in TGV during the protocol.

Comparison of the change in TGV following injection of known amounts of gas. In six CBA/Ca mice, TGVst was determined at FRC and after the lung volume was increased by injecting 0.2, 0.4, or 0.6 ml of nitrogen from a calibrated syringe into the lungs. The change in lung volume (\( \Delta V \)) was corrected for the temperature and humidity differences between injected gas and alveolar air. This maneuver was repeated three times at each \( \Delta V \) in each mouse (except two in which the 0.6-ml measurements were not made), and the corresponding differences were averaged. The values of \( \Delta V \) were compared with the corresponding differences in TGVst obtained before and after the injection (\( \Delta \)TGVst).

Estimation of baseline values of TGV in apneic BALB/c and C57 BL/6 mice. TGVst was measured during end-expiratory pauses of ventilation in six animals each in these two strains of mice (before further experiments unrelated to this study). The animals were mostly apneic in this phase, and values of TGVsp were, therefore, not available.

RESULTS

Typical recordings of Ptr and Pboxc for spontaneous and stimulated maneuvers are illustrated in Fig. 2. The stimulated contractions were much shorter than the spontaneous efforts (\( \sim 50 \) vs. \( \sim 200 \) ms) and of a more sinusoidal shape because of the more limited harmonics content. Typically, Pptr led Pboxc in the stimulated maneuvers at FRC [the phase angle was 13.9 \( \pm \) 9.8 (SD) \(^\circ\)], whereas it was more often absent or even opposite at higher lung volumes, manifesting in a significantly smaller mean phase angle at Prs = 10 cmH\(_2\)O (6.5 \( \pm \) 8.9\(^\circ\), \( P < 0.05 \)) and 20 cmH\(_2\)O (2.6 \( \pm \) 11.4\(^\circ\), \( P < 0.01 \)). The Pboxc vs. Pptr relationships did not exhibit any looping for the slower spontaneous maneuvers, which in turn were more often distorted by the baseline drift or environmental disturbances in Pbox.

TGVsp was more variable than TGVst within the same recording at the FRC, the coefficient of variation being 6.7 \( \pm \) 3.6 vs. 3.8 \( \pm \) 3.0%. The coefficients of variation from the two techniques were closer in measurements at higher lung volumes (2.1 \( \pm \) 1.2 vs. 2.9 \( \pm \) 1.1% and 2.0 \( \pm \) 1.1 vs. 2.5 \( \pm \) 0.1%, respectively, at 10 and 20 cmH\(_2\)O). Figure 3 displays the relationship between TGVst and TGVsp for the three levels of Prs. It should be noted that the pairs of TGV values were obtained from successive maneuvers in each mouse. While the relationship is strong, the values of TGVst are systematically higher than those of TGVsp at every level of Prs, with an average value of \( \sim 16\% \) obtained on the pooled data. The relative difference between TGVst and TGVsp was larger at FRC (25 \( \pm \) 16%) than at the Prs of 10 cmH\(_2\)O (14 \( \pm \) 7%; unpaired \( t \)-test: \( P < 0.05 \)) or 20 cmH\(_2\)O (17 \( \pm \) 10%, not significant). There was no difference in the values of FRC obtained in the four mice ventilated with both air and the

![Graph](http://example.com/graph.png)
helium-oxygen mixture (0.32 ± 0.02 vs. 0.33 ± 0.01 ml for TGVsp and 0.29 ± 0.03 vs. 0.30 ± 0.02 ml for TGVst). The ratios TGVst/TGVsp for air (1.09 ± 0.08) and helium-oxygen (1.11 ± 0.08) were not statistically significantly different either (paired t-test).

Comparison of values of ΔTGVst and ΔV obtained from the injections of nitrogen (Fig. 4) revealed a strong correlation between these variables (r² = 0.983). Linear regression on the pooled data resulted in the relationship ΔTGVst = −0.046 + 1.12 ΔV, which reflected a slight underestimation and overestimation of the injected volume by ΔTGVst, at the smallest and highest ΔV, respectively.

The TGV values measured at the FRC in all CBA/Ca mice (i.e., including the four animals that participated in the resident gas study) with both the spontaneous and the stimulated effort, and the TGVst values obtained at the FRC in the C57BL/6J and BALB/c mice, are listed in Table 1.

**DISCUSSION**

The results of the present study show that it is technically feasible to measure TGV in apneic mice at any time, i.e., level of inflation by means of a plethysmographic technique. The respiratory drive may decrease for longer periods during anesthesia in mice, which makes it possible to utilize the apneic periods for the measurement of low-frequency oscillation mechanics during the suspension of mechanical ventilation at different levels of Prs for a few seconds (16) or even during slow, deep inflations (5), without the need for muscle paralysis. However, in the absence of inspiratory effort, the alternative to the plethysmographic technique in the measurement of absolute lung volume remains a method of dilution (8) or degassing (airway occlusion following 100% oxygen breathing) and subsequent inflation (17), both of which are time consuming and, therefore, unsuitable under dynamic conditions. In the present study, electrical stimulation of intercostal muscles anteriorly produced an inspiratory effort against an occluded airway opening that was adequate for measurements of TGV.

If these measurements are performed in conjunction with slow inflation and deflation maneuvers (8) during the same data epoch, the absolute lung volume from the FRC to the TLC, and a description of the absolute volume-pressure relationships of the lungs would be obtained in vivo and under physiological conditions in mice. The fact that the estimation of TGV does not have to rely on spontaneous breathing activity may acquire particular significance when the measurements of TGV are to be accurately timed, e.g., during the expected rapid changes in lung volume in response to pharmaceutical interventions. In this regard, the technique of external chest compression recently proposed by Lundblad et al. (9) furnishes a similar solution, although the two approaches differ in the direction (compression vs. expansion) and perhaps also in the homogeneity of the deformation of the alveolar units.

The comparison of the TGV values resulting from the different maneuvers revealed that the difference between TGVst and TGVsp was higher at the FRC than at elevated lung volumes (Fig. 3); however, the difference was slight and statistically significant only between the FRC and Prs = 10 cmH2O. By contrast, Lundblad et al. (9) observed a marked (~50%) difference between TGVsp and TGVst obtained with chest compression at the FRC, despite the use of a thermal correction procedure, which completely eliminated the differences at higher Prs levels. Those authors argued that the partial closure of lung units at low lung volume was the most likely explanation for the discrepancy at the FRC, and that the validity of the chest compression method was acceptable at a Prs of 2 cmH2O and above. In our study, involving chest expansion, the difference between TGVst and TGVsp was more balanced over the volume range studied and does not substantiate any radical change in lung unit closure at the FRC. Since the stimulated contractions of the respiratory muscles were approximately four times faster than the spontaneous efforts, we hypothesize that the systematic differences between TGVsp and TGVst are related to the rate of the breathing effort.

The influence of the breathing frequency on the estimation of TLC in normal and asthmatic subjects was studied by Rodenstein and Stănescu (13), who observed no change in TGV in the normal subjects with increasing panting frequency, whereas there was a slight increase and a marked elevation, respectively, in the asthmatic subjects before and after an induced bronchospasm. Bohadana et al. (1) considered a number of potential factors that would lead to a frequency dependence of the TGV estimates in patients with chronic airway disease, including abdominal gas compression, inhomogeneous pleural pressure swings, nonzero flow between the alveoli and the airway opening, and deviation from isothermal conditions in the alveoli at high expansion rates. The conclusions drawn in that study (1) cannot be translated directly so as to relate to TGV measurements in mice, and the experimental and simulation tools employed by those authors would also be difficult to adapt to this much smaller and far less investigated species. Nevertheless, we have attempted to clarify whether or not the rate dependence of TGV can be attributed to deviations from the isothermal process in the alveolar gas. As suggested by Mead and Collier (11), we replaced the diatomic gas (nitrogen) by a monoatomic gas (helium), in a content of 80% in the resident gas, and reasoned that the change in specific heat ratio from 1.4 to 1.66 would lead to an ∼15% increase in

![Fig. 4. Increases in the TGV estimated from the stimulated breathing effort (ΔTGVst) following injections of 0.2, 0.4, and 0.6 ml nitrogen into the lungs, corrected for BTS (V) conditions (ΔV). The different symbols correspond to values obtained in individual mice (2 animals, TGVst was measured only at 0.2 and 0.4 ml). The dashed line is the line of identity.](http://jap.physiology.org/DownloadedFrom/10220.33.4/on April 26, 2017)
TGV if adiabatic conditions pertain in the lungs, whereas, in the case of the isothermal process, the TGV values should be the same for both gases. While it is unrealistic to assume that an approximately fourfold increase in the expansion rate between the spontaneous and stimulated breathing efforts would cover the whole transition from isothermal to adiabatic conditions and result in an ~20% increase in TGV, these measurements reveal no systematic difference between the TGV estimates with the two gases at either rate. This indicates that, similarly as in humans (1; 12) and dogs (11), the alveolar gas undergoes isothermal expansion at spontaneous contraction rates of the respiratory muscles in mice, a situation that does not change noticeably if faster (stimulated) deformation occurs.

The discrepancy between TGVst and TGVsp can thus perhaps be explained most plausibly on the basis of pressure equilibration within the respiratory system. The pressure transmission between the alveolar space and the tracheal opening may not be instantaneous because of the nonzero resistance of the tracheobronchial tree and a proximal compliant compartment (1, 14, 18). These components could possibly be considered responsible for the attenuation of the alveolar pressure swings at the site of the PTr measurement and for the corresponding overestimation of TGV, but their effect is inconsistent with the delay between PTr and Pbox that we often observed during the fast maneuvers. Additionally, it might be expected that, with increasing lung volume, the attenuation would decrease because of the decreasing airway resistance demonstrated in the same range of Prs in mice (5, 16). This assumption is not supported in view of the lack of any significant decrease in the difference between TGVst and TGVsp in the present investigation. It is more reasonable then to assume that the change in pleural pressure is not uniform when only a few groups of intercostal muscles participate in the inspiratory effort, and the stimulated muscles expand a lung region that communicates rapidly with the trachea, resulting in the earlier fall in PTr, whereas the lung regions underlying the other intercostals and the diaphragm equilibrate only subsequently. Such a situation may result in an overestimation of TGV, with a delay in Pbox relative to PTr, whereas, if the interregional equilibration is faster, the overestimation of TGV would be associated with Pbox leading PTr. Overall, although the mechanisms causing the rate-dependent estimates of TGV and different looping patterns are not completely clear, it should be pointed out that the systematic difference between TGVsp and TGVst was associated with a very strong relationship between these quantities and amounted to an average of only 16%.

Interestingly, the tendency of TGVst to overestimate the conventionally obtained TGVsp was confirmed by the nitrogen injection measurements only at the highest volume increase (Fig. 4); at the ΔV of 0.2 ml, the changes in TGVst were even slightly smaller than the injected volume, which may be explained on the basis of larger measurement errors associated with the smaller changes in volume. Overall, the relationship between ΔV and ΔTGVst was very strong (r² = 0.98).

Comparison of our data (Table 1) with the few values of end-expiratory lung volume reported for mice in the literature is impeded by the differences in strain, sex, and BW. For the CBA/Ca mice utilized in the methodological part of this study, no data are available for comparison. In BALB/c mice, the plethysmographic FRC was measured by Lundblad et al. during the breathing effort, and values of 0.33–0.35 ml (10) and 0.31 ± 0.01 (SE) ml (9) were obtained; the chest compression in mice of the same strain in the present work resulted in a similar value (0.30 ± 0.02 ml). The volume data of Lundblad et al. (9) are slightly higher than our TGVst estimates, probably because the former were determined at Prs = 2 cmH2O. The single-breath washout method employed by Schulz (15) in paralyzed animals resulted in a value of FRC [0.41 ± 0.03 (SD) ml] higher than that in the present study. For C57BL/6J mice, an estimate similar to ours was obtained by Schulz with the washout technique [0.34 ± 0.02 (SD) ml], whereas the values measured by Tankersley et al. (19) with the degassing technique [0.25 ± 0.01 (SE) ml] and by Lai and Chou (8) using Ne dilution [0.25 ± 0.01 (SE) ml] are appreciably lower. The variety of techniques covered by this small number of investigations may well contribute to the relatively wide range of FRC values reported. The present study was not designed to systematically examine strain differences in lung volumes.

In conclusion, the combination of classical plethysmography with the respiratory muscle stimulation described in the present study resulted in FRC values reasonably consistent with the few earlier reports on three strains of mice. Although comparison of the volume estimates relating to the spontaneous and the stimulated breathing effort revealed a small but systematic difference, the measurement of TGVst is rapid and easy to time, as it is independent of the changing respiratory drive in the anesthetized mice.

GRANTS

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