A reevaluation of the validity of unrestrained plethysmography in mice

LENNART K. A. LUNDBLAD, CHARLES G. IRVIN, ANDY ADLER, AND JASON H. T. BATES. A reevaluation of the validity of unrestrained plethysmography in mice. J Appl Physiol 93: 1198–1207, 2002. First published May 3, 2002; 10.1152/japplphysiol.00080.2002.—Presently, unrestrained plethysmography is widely used to assess bronchial responsiveness in mice. An empirical quantity known as enhanced pause is derived from the plethysmographic box pressure \( P_b(\tau) \), where \( \tau \) is time and assumed to be an index of bronchoconstriction. We show that \( P_b(\tau) \) is determined largely by gas conditioning when normal mice breathe spontaneously inside a closed chamber in which the air is at ambient conditions. When the air in the chamber is heated and humidified to body conditions, the changes in \( P_b(\tau) \) are reduced by about two-thirds. The remaining changes are thus due to gas compression and expansion within the lung and are amplified when the animals breathe through increased resistances. We show that the time integral of \( P_b(\tau) \) over inspiration is accurately predicted by a term containing airway resistance, functional residual capacity, and tidal volume. We conclude that unrestrained plethysmography can be used to accurately characterize changes in airway resistance only if functional residual capacity and tidal volume are measured independently and the chamber gas is preconditioned to body temperature and humidity.

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spiratory mechanics rather than simply the pattern of breathing.

**Glossary**

- El: Lung elastance
- f: Frequency
- FRC: Functional residual capacity
- G: Tissue dissipation parameter
- H: Tissue stiffness parameter
- Iaw: Airway inanerance
- IPPI: Inspiratory plethysmographic pressure integral
- P0: Estimate of end-expiratory pressure
- Pa0(t): Airway opening pressure
- Pb(t): Pressure of gas in the box around the animal (relative to atmospheric)
- Penh: Enhanced pause
- P H2O: Water vapor pressure of air inside box (mmHg)
- Pl(t): Pressure of gas in the thorax (relative to atmospheric)
- Raw: Airway resistance
- Rl: Lung resistance
- t: Time
- Tb: Temperature (°C) inside box
- UP: Unrestrained plethysmography
- Va: Volume of animal, not including thoracic gas
- Vb: Volume gas inside plethysmograph chamber
- VL(t): Thoracic gas volume
- VN(t): V(t) normalized to have a peak-peak excursion of 1.0
- VN*(t): V(t) normalized to have a peak-peak excursion of 1.0
- V(t): Gas volume, referenced to FRC, inspired into lungs
- ΔV(t): Change in lung volume
- V(t): Flow into airway
- VT: Tidal volume
- Zin: Input impedance

**METHODS**

**Theory of UP**

The Glossary lists the symbols and abbreviations used in the following mathematical development.

We consider an animal breathing spontaneously inside a closed chamber of volume Vb. Suppose that a volume V(t) of gas is inspired into the lungs, starting from functional residual capacity (FRC). In the absence of both gas compression and gas conditioning effects, the new lung volume would be

$$\Delta V(t) = V(t)$$

(1)

where VL(t) is thoracic gas volume. The volume of gas in the chamber, but outside the animal, would then decrease by the same V(t). Thus the reduction in Vb would be perfectly offset by the increase in body volume of the animal, and there would be no change in chamber pressure (Pb). However, inspired gas becomes saturated with water vapor and is heated to body temperature, causing the inspired V(t) to increase in volume by an amount

$$\Delta V(t) = V(t) \left[ \frac{310}{273 + T_b} \left(1 + \frac{47 - P H_2 O}{760}\right) - 1 \right]$$

(2)

where Tb and P H2O are the temperature and water vapor pressure, respectively, inside the chamber, ΔV is change in lung volume, and we assume that 310 K is body temperature for the mouse and 47 mmHg is the vapor pressure of water in saturated air at body temperature. Our expression for total lung volume thus becomes

$$VL(t) = FRC + V(t) + \Delta V(t)$$

(3)

The increase in lung volume [V(t) + ΔV(t)] is now greater than the loss in chamber gas volume [V(t)], so the remaining chamber gas becomes compressed by an amount ΔV(t), and its pressure rises accordingly.

However, when gas flow (V) is nonzero, VL(t) is further changed as it is compressed and decompressed in response to changes in alveolar pressure according to Boyle’s law. The alveolar pressure is

$$P_L(t) = P_b(t) - V(t) Raw = -V(t) Raw$$

(4)

where $P_L(t)$ is gas pressure in the thorax and Raw is airway resistance, so the lung volume in Eq. 3 now becomes

$$VL(t) = [FRC + V(t) + \Delta V(t)] \left[ \frac{1}{1 - \frac{V(t) Raw}{P_b(t)}} \right]$$

(5)

The difference between the $VL(t)$ in Eq. 5 and that which would have occurred if neither gas conditioning nor compression had taken place (Eq. 1) equals the amount by which the gas inside the box, but outside the animal, becomes compressed. This difference is

$$\Delta V(t) = \frac{V(t) Raw \left[FRC + V(t)\right]}{1 - \frac{V(t) Raw}{P_b(t)}}$$

(6)

The $V_b$ around the animal, before compression or decompression of thoracic gas, is $V_b - V_a - FRC - V(t)$, where $V_a$ is the volume of the animal’s body, excluding the volume of gas in the lungs. This volume of gas becomes compressed by the amount given in Eq. 6, so invoking Boyle’s law and using Eqs. 2 and 6, the box pressure is

$$P_b(t) = \frac{V(t) \left[ \frac{310}{273 + T_b} \left(1 + \frac{47 - P H_2 O}{760}\right) - 1 \right]}{1 - \frac{V(t) Raw}{P_b(t)}} \left[V_b - V_a - FRC - V(t)\right]$$

(7)

$P_b(t)$ thus consists of two components: the first term in Eq. 7 is in phase with $V(t)$ and describes changes in $P_b(t)$ due to gas conditioning, whereas the second term is in phase with $V(t)$ and is the change in $P_b(t)$ due to Boyle’s law.

It is possible, in principle, to eliminate the effects due to gas conditioning by heating and humidifying the air in the box (25). Equation 7 then reduces to
\[
\begin{align*}
P_b(t) &= \frac{V(t)\text{Raw}[\text{FRC} + V(t)]}{[1 - \frac{V(t)\text{Raw}}{P_b(t)}][V_b - V_a - \text{FRC} - V(t)]} \\
\text{where } T_i \text{ is inspiratory time. We denote this quantity the } \text{inspiratory plethysmographic pressure integral (IPPI). In the experimental part of our study described below, we used IPPI to validate our theory of UP. Specifically, we determined IPPI from the lefthand side of Eq. 10 by measuring } P_b(t) \text{ in spontaneously breathing mice. We then compared it to IPPI calculated from the righthand side of Eq. 10 using independent measurements of } V_t, \text{ Raw, and FRC.}
\end{align*}
\]

**Experimental**

The experimental component of our study was performed in two parts: 1) an initial study in which we investigated how methacholine challenge affects the relationships among \( P_n \), \( V \), and \( V_N \); and 2) a validation study in which we pursued the experimental verification of the theory outlined above by conditioning the inspired gas and determining IPPI using both sides of Eq. 10. These studies were approved by the Institutional Animal Care and Use Committee.

**Initial Study**

**Experimental.** We studied female mice (26.6–30.4 g) of the A/J strain \((n = 4)\), which is known to be intrinsically hyperresponsive \((6, 22, 23)\). The mice were anesthetized with an intraperitoneal injection of 70 mg/ml pentobarbital (Nembutal, Abbott, North Chicago, IL). After tracheostomy and tracheal cannulation with a 20-gauge Luer stub adaptor (Intramedic, Franklin Lakes, NJ), the mice were placed in a 300-ml body box (Buxco, Sharon, CT). \( P_b \) was measured (MP45, Validyne, Northridge, CA) with respect to a reference chamber ported to atmosphere. The box had a leak to atmosphere, giving it a time constant of 1.76 s. The frequency response of \( P_b \) to changes in \( V_b \) was assessed between 0.5 and 20 Hz with a piston pump (flexiVent, SCIREQ, Montreal, QC) and found to be flat to within 6% over this frequency range. \( V \) was measured at the tracheal cannula using a custom-designed pneumotachograph similar to that described by Mortola and Noworaj. The pneumotachograph had a constant resistance of 0.060 ± 0.006 cmH2O·s·ml⁻¹ up to a flow of 4 ml/s. Esophageal pressure \((P_e)\) was measured with a blood pressure transducer (Cobe, Lakewood, CO) connected to a 15-cm length of PE-50 saline-filled polyethylene tubing (Intramedic, Parsippany, NJ) positioned in the esophagus via the mouth so as to give maximum signal excursion during breathing. \( P_{H2O} \) was measured with a humidity sensor (Humidal, Colton, CA), and \( T_b \) was measured with a thermocouple (Omega, Stamford, CT).

With the box at room temperature \((28.1 ± 1.0°C)\), we subjected the mice first to an aerosol of saline and then to sequentially doubling doses of methacholine aerosol, up to a concentration of 100 mg/ml. After each dose, \( V, P_n \), and \( P_e \) were monitored for 2 min.

**Data analysis.** The theory outlined above predicts that, when a mouse breathes spontaneously inside a box at room temperature, there will be two contributions to the resulting \( P_b(t) \): one due to gas conditioning and one due to gas compression. **Equation 7** shows that the first contribution will be in phase with \( V(t) \), whereas the second will be in phase with \( V(t) \). Because we measured \( V(t) \) directly at the tracheal opening, and by numerical integration obtained \( V(t) \), we were able to determine these phase relationships. We made this determination in a 10-s epoch of regular breathing data at each methacholine dose, as follows. We scaled the \( V \) and \( V_N \) signals from each epoch so that each signal had a peak-peak amplitude of unity, yielding the normalized signals \( V_N \) and \( V_N \), respectively. We then determined the parameters \( A, B, \) and \( C \) of the regression equation

\[
P_b(t) = AV_N(t) + BV_N(t) + C \tag{11}
\]

**Equation 11** is an empirical description of \( P_b(t) \) in terms of the component proportional to \( V_N \) and a component proportional to \( V_N \). The parameters \( A, B \) in Eq. 11 thus represent the fractions of the respiratory variations in \( P_b(t) \) that can be attributed to \( V_N \) and \( V_N \), respectively. The parameter \( C \) takes into account the mean value of \( P_b(t) \). Consequently, the quantity \( A/(A + B) \), which we call the flow phase fraction of \( P_b(t) \), is a measure of the fractional contribution of \( V_N \) to the variations in \( P_b(t) \). If \( V_N \) and \( V_N \) were perfect sine waves, the flow phase fraction would be an exact estimate of the fraction of \( P_b \) in phase with \( V_N \). This was not the case because the spontaneous breathing wave forms of \( V_N \) and \( V_N \) were not sinusoidal. In some cases, the maximum in the \( V_N \) power spectrum was at the fundamental, whereas in others the first or second harmonics were maximal (particularly when the breathing rate was slow). Therefore, the quantity \( A/(A + B) \) only gives an approximation to the fraction of the respiratory variations in \( P_b \) attributable to \( V_N \).

From the same data epochs, we also calculated lung resistance \((R_l)\) as a measure of the degree of bronchoconstriction caused by the inhaled methacholine. This was done by fitting the equation

\[
-Pes(t) = RL\dot{V}(t) + EI\dot{V}(t) + P_0 \tag{12}
\]

where \( E \) is lung elastance and \( P_0 \) is an estimate of end-expiratory transpulmonary pressure.

**Validation Study**

**Experimental.** We studied BALB/c female mice \((17–20 g, n = 4)\) obtained from Charles River. The animals were acclimatized in our animal facility for at least 1 wk before the experiments. The mice were anesthetized with intraperitoneal pentobarbital sodium \((90 \text{ mg/kg})\), and the trachea was dissected free of surrounding tissue and cannulated with a
Comparing chamber pressure to airway pressure gave a reflected gas decompression within the lung. The walls of the mouse chamber contained a circulating water jacket so that the inside temperature could be controlled (Fig. 1). Wet paper lining the bottom of the chamber kept the internal environment saturated with water vapor. A horizontal support fixed to the removable front face of the chamber allowed the animal to be conveniently prepared and installed before the chamber was sealed. A horizontal support fixed to the removable front face of the chamber allowed the animal to be conveniently prepared and installed before the chamber was sealed. P_0 was measured with a sensitive piezoresistive differential pressure transducer (SC-24, SCIREQ) referenced to a second chamber with a long time constant (>6 s) leak to atmosphere. Airway opening pressure (Pao) was measured with another piezoresistive transducer. The temperature and humidity in the chamber were monitored by an electronic sensor (Digital Thermo-Hygro, Radio Shack).

The inspiratory line to the animal was controlled by a four-way stopcock outside the chamber. The stopcock either closed the inspiratory line completely, opened the inspiratory line to the outside, or opened the inspiratory line to the chamber interior. These three stopcock positions allowed the system to operate in three different ways, as follows.

When the inspiratory line was open to the outside of the chamber, the animal either breathed outside air spontaneously or was mechanically ventilated by a ventilator connected to the inspiratory line. When the inspiratory line was completely closed, the animal attempted to breathe against a closed airway. Chamber pressure then reflected gas decompression within the lung. Comparing chamber pressure to airway pressure gave a measure of the FRC as determined by Boyle’s law.

When the inspiratory line was open to the chamber interior and chamber pressure was recorded, the system operated essentially like a conventional unrestrained plethysmograph, except that the upper airways were bypassed. At the same time, Pao gave a recording proportional to respiratory flow, with the constant of proportionality being the resistance of the conduit between the lateral pressure tap and the box interior. The conduit resistance was determined in a separate calibration experiment by connecting a flexiVent small-animal ventilator to the port through the front face of the chamber (i.e., in the position usually occupied by an animal). Known flows were delivered through the port to the conduit, and the pressure drop across the conduit was measured. The ratio of pressure to flow yielded conduit resistance. We employed three different conduits in this way: one with a resistance of 0.76 cmH_2O·s·ml^{-1} (comparable to the Raw of a normal mouse), one with a resistance of 2.03 cmH_2O·s·ml^{-1}, and one with a much higher resistance of 11.0 cmH_2O·s·ml^{-1}.

Our experimental procedure consisted of first setting the stopcock in the first configuration described above and connecting a flexiVent small-animal ventilator to the inspiratory line. The animal was mechanically ventilated by the flexiVent at 200 breaths/min with a VT of 0.20 ml. After 3 min of regular ventilation, the animal was allowed to exhale for 1 s, and then a 2-s volume perturbation signal was delivered to the lungs by the flexiVent. The volume signal consisted of the superposition of 13 sine waves with frequency spaced roughly evenly over the range of 1–20.5 Hz. The piston displacement and cylinder pressure of the flexiVent were recorded while the perturbation was applied and stored for later analysis. Next, the flexiVent was disconnected, and the animal was allowed to breathe spontaneously from the air outside the box for several minutes to allow the normal drive to breathe to be reestablished. At end expiration, the stopcock was then set in the second position described above for a period of ~15 s, while the animal made spontaneous inspiratory efforts against a closed airway. Pao and P_0 were recorded during this period and stored for later calculation of FRC. Finally, the stopcock was set in the third position described above, and the animal was allowed to breathe spontaneously from the air inside the chamber while Pao and P_0 were recorded for 15-s periods. This was first done with chamber temperature maintained at 23–25°C and a relative humidity of 27%–34%. The measurements were then repeated with the chamber temperature at 35–39°C and a relative humidity of 85–93%. Measurements were made with the animal breathing through the low-, medium-, and high-resistance conduits, to simulate normal conditions and moderate and severe bronchoconstriction, respectively. All recorded signals were low-pass filtered at 30 Hz and sampled at 128 Hz.

Data analysis. We determined Raw from the data collected when the flexiVent was used to apply volume oscillations to the lungs, as follows. Lung input impedance (Zin) was calculated as described previously (15, 19). This included employing a dynamic calibration procedure to account for the phys-

![Fig. 1. The plethysmographic chamber used to measure input impedance, functional residual capacity, and unrestrained plethysmography.](http://jap.physiology.org/)


**RESULTS**

**Initial Study**

Figure 2 shows the flow phase fraction vs. RL for the four mice studied when challenged with saline and the various doses of methacholine. Three of the mice gave data-similar plots and showed a strong increasing relationship (combined $r^2 = 0.73$) between the flow phase fraction and $R_L$. The fourth mouse also showed a strong relationship ($r^2 = 0.93$), but the flow phase fraction was elevated compared with that of the other animals.

**Validation Study**

Table 1 shows the values of Raw and FRC obtained in the four mice studied.

The thin lines in Fig. 3 show typical records of five consecutive breaths of $P_b(t)$ and $V(t)$ obtained from one of the mice during spontaneous rebreathing from within the chamber. The temperature of the air in the box was 23°C, and its relative humidity was 29%. Both that the FRC obtained reflected the imposed changes in the amounts of air in the lungs to within an average of ±10%.

### Table 1. Values of Raw and FRC determined for each mouse

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Raw, cmH$_2$O·s·ml$^{-1}$</th>
<th>FRC, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.40</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>0.56</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>0.29</td>
<td>0.33</td>
</tr>
<tr>
<td>4</td>
<td>0.59</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Airway resistance (Raw) does not include the resistance of the tracheal cannula or any other connecting tubing. Functional residual capacity (FRC) does not include the 0.37 ml of gas between the site of airway opening pressure measurement and the tracheal opening.
respiratory and cardiogenic variations are clearly visible in both signals. The thick lines in Fig. 3 show five breaths from the same mouse when the gas in the box was heated to 37°C and humidified to a relative humidity of 85%. The breathing pattern, as evidenced by $V(t)$, is about the same as before, but the excursions in $P_b(t)$ are markedly diminished.

Figure 4 shows the ensemble average of $P_b(t)$ plotted against the ensemble average of $V(t)$ for each of the four mice when they breathed spontaneously through the low-resistance conduit with the gas in the chamber at a temperature of 23–25°C and a relative humidity of 27–34%. The signals are almost completely out of phase in this situation, so $P_b(t)$ is in phase with $V(t)$, which is consistent with the phenomenon of gas conditioning. When the gas in the box was conditioned to a temperature of 35–39°C and a relative humidity of 85–93%, the excursions in $P_b(t)$ were greatly reduced.

Fig. 3. Records of gas flow (top) and gas box pressure ($P_b$; bottom) made with a mouse breathing spontaneously while enclosed in the chamber, with the gas inside the chamber at 23°C and relative humidity 29% (thin lines), and after the chamber gas was heated to 37°C and humidified to 85% (thick lines). Inspiratory flow is positive.

Fig. 4. Ensemble average of $P_b(t)$ (where $t$ is time) vs. ensemble average of flow into airway [$V(t)$] for each of the 4 mice in the validation study. The mice breathed spontaneously through the low-resistance conduit with the gas in the chamber at a temperature of 23–25°C and a relative humidity of 27–34% (thin lines) and with the gas in the box conditioned to a temperature of 35–39°C and a relative humidity of 85–93% (thick lines). Inspiratory flow is positive.
and much more in phase with flow (Fig. 4). When the animals breathed conditioned gas through the medium-resistance and high-resistance conduits, the swings in $P_b(t)$ became appreciable again and were clearly in phase with $V(t)$ (Fig. 5), consistent with compression and decompression of thoracic gas.

Finally, we used the measured values of $R_{aw}$, $V_T$, and $FRC$ to calculate IPPI, according to Eq. 10, and compared the results to IPPI determined from the integrated ensemble-averaged $P_b(t)$ signal. This calculation was performed in each mouse by using four to six different 6-s data segments obtained with each of the three resistive conduits, when the gas in the box was heated to body temperature and humidified. Figure 6 shows an identity plot of the corresponding estimates of IPPI.

**DISCUSSION**

UP was first investigated in 1868 by Bert (2) and then further developed in 1955 by Drorbaugh and Fenn (10) as a method for assessing lung function in infants. It was pursued further in a small number of subsequent studies (11, 12, 21, 28, 30) but never gained much of a following until recently. Now the quantity $Penh$, derived from UP, is being widely used as a means for studying bronchial responsiveness in various animals models of lung disease (1, 5, 7, 13, 14, 16, 17, 27). Although some studies have shown a good correlation between $Penh$ and other measures of lung function (8, 13, 17), others have shown that $Penh$ does not correlate well with morphometric changes in mice (24). Some degree of correlation between $Penh$ and bronchoconstriction is expected, because anything that affects lung mechanics is likely to also affect the breathing pattern; $V_T$ is probably just as good an index to follow as $Penh$ in this regard (25). However, just because two quantities are correlated does not mean

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**Fig. 5.** Ensemble average of $P_b(t)$ vs. ensemble average of $V(t)$ for each of the 4 mice in the validation study. The mice breathed spontaneously through the medium-resistance conduit (thick lines) and the high-resistance conduit (thin lines) with the gas in the chamber conditioned to a temperature of 35–39°C and a relative humidity of 85–93%.

**Fig. 6.** Inspiratory plethysmographic pressure integral (IPPI) determined by integrating the ensemble-averaged $P_b(t)$ signal (horizontal axis) vs. its determination by the formula given in Eq. 10 (vertical axis). The different symbols represent the 4 mice in the validation study. $R_{aw}$, airway resistance; $V_L$, lung volume; $V_T$, tidal volume; $V_{s}$, gas volume inside box.
that one can be used as a surrogate for the other. Also, Petak et al. (29) have just shown that, under some circumstances, Penh may behave very differently from more direct measures of lung mechanics. Thus, as Drazen et al. (9) point out, changes in Penh need to be confirmed with an independent method of assessing airway obstruction based on appropriate physical principles.

Given the present interest in using Penh to assess lung function in mice, we felt that a detailed study of the physics and physiology behind UP and its relation to lung mechanics was warranted. We began our investigation with an initial study aimed at determining how P_b relates to V and V in a relevant experimental situation. We chose to examine the hyperresponsive A/J strain of mouse challenged with methacholine because of the substantial changes in lung mechanics induced. Each mouse produced a strong, positive correlation between the flow phase fraction and R_L (Fig. 1), showing that the proportions of P_b in phase with V changed substantially as the animals became bronchoconstricted. By the theory encapsulated in Eq. 7, this means that the relative contributions to P_b of gas conditioning and gas compression also changed. This is not surprising for the following reasons. Under baseline conditions, we would expect most of the respiratory fluctuations in P_b to be due to gas conditioning, because only small changes in alveolar pressure are necessary to generate flow along unobstructed airways. However, when the airways become constricted, greater changes in alveolar pressure, and hence greater degrees of gas compression and decompression, are required to generate respiratory flows. This causes the proportional contribution from gas compression to increase progressively as an animal becomes constricted, as illustrated by the results in Fig. 2. An increase in breathing frequency would also increase the flow phase fraction. However, in the mice we studied, breathing frequency did not increase with methacholine challenge and by the highest doses had even decreased considerably. Thus the increases in flow phase fraction with R_L that we see in Fig. 2 likely underestimate the increased contributions to P_b from gas compression.

The results of our initial study lead us to follow up with a validation study in order to gain a deeper understanding of the physics underlying UP and to test our theory outlined above in METHODS. As in the initial study, the follow-up study showed that, when a normal mouse breathes air at room temperature and humidity, the respiratory fluctuations in P_b(t) are dominated by warming and humidification of the gas as it enters the lungs (Fig. 3). About two-thirds of these fluctuations in P_b(t) disappeared when the gas in the chamber was preconditioned to be at body temperature and humidity (Figs. 3 and 4). The validation study also confirmed that the fluctuations that remain after preconditioning are due to gas compression and decompression in the lung, because they were amplified by having the animal breathe through an increased external resistor (Fig. 5).

The quantitative validation of our theory of UP is demonstrated by the assessment of IPPI. When IPPI was calculated by integrating P_b(t) over inspiration and compared with its theoretical equivalent given in Eq. 10, a tight relationship was obtained (Fig. 6). Of course, equating IPPI to the right-hand side of Eq. 10 assumes that the values of Raw and FRC, measured through independent maneuvers, still pertained during the period of spontaneous breathing from which V_T and IPPI were calculated. We have no reason to suspect that Raw should have changed throughout the course of the experiment, because the values of Raw used in Eq. 10 were dominated by the added conduit resistances, which were fixed. Furthermore, FRC was determined several times in each mouse used in the validation study, both with and without the additional conduit resistances, and the variations found were neither systematic nor large (±10%). We, therefore, conclude that IPPI accurately embodies the physical processes responsible for the respiratory fluctuations in P_b(t) when an animal breathes spontaneously inside a closed chamber.

Only those changes in P_b due to gas compression have direct relevance to the mechanical properties of the lungs. As Raw increases, these changes are combined in an indeterminate and variable way with the changes in P_b(t) due to gas conditioning, which are not relevant to lung mechanics. Such findings have major significance for the use of UP, as it is presently practiced in mice, by delineating the problems of assuming that Penh is a valid measure of lung function. Not only is Penh limited to be a reflection of the pattern of breathing, but also the signal on which it is based is a variable composite of two phenomena, one of which is relevant to respiratory mechanics. Whereas an animal may change its breathing pattern as it becomes bronchoconstricted, it seems unreasonable to expect that the nature and extent of these changes should precisely mirror the alterations in lung mechanics. Therefore, whereas a change in Penh may be useful as a general indicator that some reaction has taken place in an animal, it seems unlikely to be a meaningful measure of changes in the mechanical properties of the lung.

We have shown that UP can provide accurate reflections of mechanical lung function, provided gas conditioning effects are eliminated and both V_T and FRC are known. Only if these conditions prevail does a change in IPPI reflect a change solely in Raw (Eq. 10). Preconditioning the chamber air, as we have done, is a practical solution to eliminating the effects of heating and humidification on P_b(t), even if animals have difficulty maintaining thermal equilibrium in a heated and humidified box for long periods of time. However, monitoring V_T and FRC was only possible in our experiments because the animals were anesthetized and tracheostomized. Unfortunately, this does not make this version of UP practical, because the major virtue of
UP is that it can be undertaken on conscious, unrestrained animals. Following changes in VT and FRC accurately under such conditions is problematic yet is required because both quantities are subject to change as the airways constrict. In the validation experiments, we found that altering the conduit resistance that the mice breathed through had very little effect on FRC. However, using the same technique as described above for the validation study, we also measured FRC in the mice in the initial study that was challenged with methacholine. FRC in these animals increased progressively with methacholine dose, reaching >50% above baseline by the time the methacholine aerosol had reached a concentration of 12.5 mg/ml. This indicates that the lungs of these animals experienced a substantial amount of gas trapping. We might expect changes in FRC to be even more marked in intact, conscious animals because they breathe significantly more quickly than anesthetized animals (33) and so would be expected to experience a greater degree of dynamic hyperinflation. Also, conscious animals have the potential for glottic regulation of end-expiratory lung volume. Equation 7 shows that a change in FRC produces a proportional change in P_b(t). This leads to a proportional change in Penh, independent of any changes in Raw, which further argues against any usefulness of Penh as a meaningful standalone measure of bronchial responsiveness.

The animals examined in the follow-up study also changed their VT a great deal as external resistance was added to their breathing circuit. With the low-resistance conduit, mean VT for all determinations in all animals was 0.064 ml, with a standard deviation of 0.024 ml. With the medium-resistance conduit, VT increased substantially to 0.164 ml, perhaps due to the respiratory stimulation of the added resistance. The effect was quite reproducible, with the standard deviation of VT being still small at 0.032 ml. When the high-resistance conduit was used, VT decreased somewhat to 0.147 ml, but the standard deviation was markedly increased at 0.80 ml, possibly reflecting the difficulty that the animals experienced in breathing through such a severe constriction. These results thus show that constriction in spontaneously breathing mice may cause marked changes in the breathing pattern. Furthermore, our animals were anesthetized, so their changes in VT may have been less marked than those that would occur in conscious animals. Interestingly, the changes in breathing pattern that can occur with increased respiratory rate have also been applied to the diagnosis of lung disease in humans (20). In any case, it is clear that much of the change in P_b(t), and hence in Penh, that occurs during bronchoconstriction may be due to a change in VT. This has nothing to do with any changes in lung mechanics. It must also be noted that the values of VT obtained with the medium- and high-resistance conduits were not particularly small compared with FRC (Table 1), which is one of the conditions on which Eq. 10 is based. Equation 8 shows that a large VT should increase P_b(t), and hence increase IPPI, over that calculated through Eq. 10. We do not see such an effect in Fig. 6, which suggests that either it was small or else it was canceled out by some other systematic effect.

In conclusion, we have shown that P_b(t) obtained during UP is determined in large part by gas conduction when normal mice breathe spontaneously inside a closed chamber in which the air is at room temperature and humidity. When the air in the chamber is preconditioned to body temperature and humidity, the changes in P_b(t) are greatly reduced. The remaining changes are due to gas compression and decompression within the lung and can be amplified by having the animals breathe through increased resistances. The gas compression and decompression effect is also increased when mice are bronchoconstricted with methacholine. When the chamber gas is appropriately conditioned, we have shown that IPPI, the time integral of P_b(t) over inspiration, is accurately predicted by a term containing Raw, FRC, and VT as factors. This validates our quantitative theory of UP and shows that we have identified the important parameters and variables that determine P_b(t). Our study also shows that, unless these various quantities can be either controlled or measured, UP is not likely to be of practical use as a means of obtaining accurate measures of mechanical lung function. This applies in particular to Penh, which we conclude should not be used as a means of assessing bronchial responsiveness.

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