

# Measuring lung function in mice: the phenotyping uncertainty principle

Jason H. T. Bates and Charles G. Irvin

Vermont Lung Center and College of Medicine, University of Vermont, Burlington, Vermont 05405

**Bates, Jason H. T., and Charles G. Irvin.** Measuring lung function in mice: the phenotyping uncertainty principle. *J Appl Physiol* 94: 1297–1306, 2003;10.1152/japphysiol.00706.2002.—Measuring lung function in mice is essential for establishing the relevance of murine models to human lung disease. However, making such measurements presents particular technical challenges due to the small size of the animal, particularly with regard to the measurement of respiratory flows. In this review, we examine the various methods currently available for assessment of lung function in mice and contrast them in terms of a concept we call the phenotyping uncertainty principle; each method can be considered to lie somewhere along a continuum on which noninvasiveness must be traded off against experimental control and measurement precision. Unrestrained plethysmography in conscious mice represents the extreme of noninvasiveness and is highly convenient but provides respiratory measures that are so tenuously linked to respiratory mechanics that they cannot be considered as meaningful indicators of lung function. At the other extreme, the measurement of input impedance in anesthetized, paralyzed, tracheostomized mice is precise and specific but requires that an animal be studied under conditions far from natural. In between these two extremes lie methods that sacrifice some precision for a reduction in the level of invasiveness, a promising example being the measurement of transfer impedance in conscious, restrained mice. No method is optimal in all regards; therefore, the appropriate technique to use depends on the application.

input impedance; unrestrained plethysmography; transfer impedance

MUCH OF WHAT WE KNOW ABOUT both the normal functioning of the lung and mechanisms of lung disease comes from studies utilizing animals. For example, animal systems provided the essential link between inflammation and airway hyperresponsiveness and asthma (71). Mice are now widely used for lung research because of certain advantages thought to be provided by this species (12, 21, 67). These advantages include a well-understood immunologic system, the vast arrays of available reagents, a short reproductive cycle, making genetic studies more feasible, a well-characterized genome, transgenic technology, and perceived economic factors. However, for any animal system to provide useful insight into disease, it must exhibit an appropriate phenotype. Demonstrating this for mouse models of lung disease poses a special challenge because valid assessment of lung function in such a small animal requires that a number of technical challenges be overcome.

Before the end of the 1980s, there existed a limited body of peer-reviewed literature concerning measure-

ment of mouse lung function (4, 11, 28, 47) as well as lung volumes and diffusing capacity (63). However, there were almost no reports of dynamic lung mechanics. This paucity of information no doubt reflected, in part, the difficulty of measuring the necessary respiratory signals, particularly the small gas flows (59). This situation began to change with the work of Martin et al. in 1988 (46), who demonstrated that measurements of pulmonary resistance and compliance could be made in this small species. The studies of Levitt and Mitzner (39, 40) then illustrated the utility of using mice in genetic studies. Subsequently, a number of approaches have been developed for measuring lung function in mice *in vivo*. In this review, we examine these various methods and contrast their respective attributes to demonstrate that each method represents a compromise between accuracy, noninvasiveness, and convenience such that none is optimal in all regards.

## THE PHENOTYPING UNCERTAINTY PRINCIPLE

Physiological investigation *in vivo* consists of a fundamental struggle between the achievement of measurement precision and the maintenance of unperurbed or “natural” conditions. This struggle is embodied, for example, in the relative merits of *in vivo*

Address for reprint requests and other correspondence: J. H. T. Bates, Dept. Medicine, Univ. of Vermont, HSRF, Rm. 228, 149 Beaumont Ave., Burlington, VT 05405 (E-mail: jhtbates@zoo.uvm.edu).

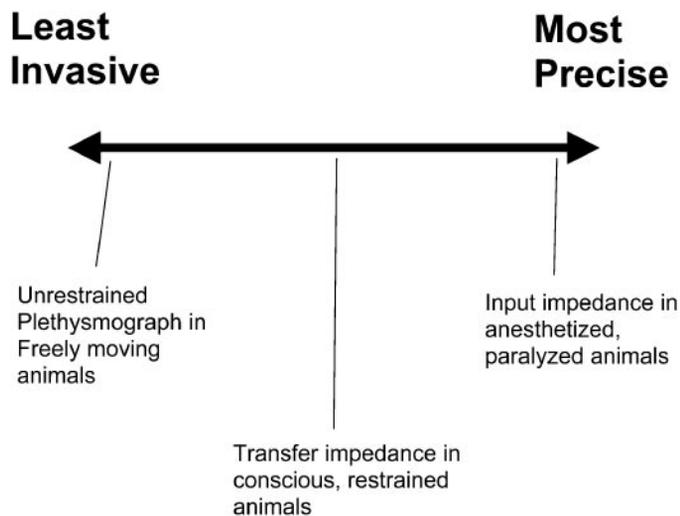


Fig. 1. The phenotyping uncertainty principle: a compromise between the achievement of natural conditions and measurement precision.

vs. *in vitro* experimentation as an approach to solving biological problems. This state of affairs is reminiscent of the famous Heisenberg uncertainty principle of quantum mechanics (22). The Heisenberg uncertainty principle states that it is impossible to know both the position and momentum of a particle simultaneously with perfect precision. Thus, as one gains a more precise knowledge of the position, the more uncertain is the knowledge of the momentum and vice versa. We suggest that a similar principle is at work with regard to the assessment of physiology in an animal: the more precisely one wishes to characterize some aspect of physiology, the less relevant to normal functioning are the conditions under which characterization must be made. We will call this the “phenotyping uncertainty principle” and suggest that it may apply throughout the whole field of physiological investigation. This is no better exemplified than in our present focus, which is the pursuit of improved methods for assessing lung function in mice.

Of the various techniques that have been used to measure murine lung function, two represent the extremes of a continuum along which noninvasiveness or “natural physiological conditions” must be traded off against experimental control and measurement precision (Fig. 1). At one extreme of this continuum is unrestrained plethysmography (UP), which involves having an animal roam freely in a closed container while only the pressure inside the container is measured. Cyclic variations in container pressure occur as gas is inspired and expired, and changes in the nature of these pressure variations are taken as indicative of changes in lung function (9, 30). Unfortunately, these variations in container pressure have an indeterminate and possibly little bearing on mechanical lung function and in fact are much more relevant to the control of breathing as reflected in the respiratory pattern (i.e., breathing frequency and tidal volume). These factors severely limit the specificity and preci-

sion of UP as a means of assessing lung function. At the other extreme of the noninvasiveness-precision continuum is the measurement of input impedance ( $Z_{in}$ ) using the forced oscillation technique in anesthetized, paralyzed, tracheostomized animals (55, 60). This method controls for breathing (oscillatory) frequency, tidal volume, mean lung volume, and volume history and hence provides the greatest precision of any method yet developed for assessing dynamic lung mechanics. However, it does so at the expense of a level of invasiveness that places the animal far from its natural state. In an intermediate position between these two extremes lies a number of other techniques for assessing respiratory mechanics in mice, such as the use of forced oscillations to measure transfer impedance ( $Z_{tr}$ ) in conscious animals (51, 72). We will now examine these various methods in more detail.

#### AT ONE EXTREME: CONTROLLING NOTHING

UP as it is currently practiced represents the smallest degree of animal constraint conceivable for the assessment of lung function. UP was first investigated in 1868 by Bert (7) and further developed in 1955 by Drorbaugh and Fenn (16) as a method for assessing lung function in infants. It was pursued further in a small number of subsequent studies (18, 19, 37, 53, 61) but never gained much of a following until recently. Currently, the quantity enhanced pause (Penh) (see Table 1 for a list of definitions), derived from UP, is being widely used as a means for studying bronchial responsiveness in various animal models of lung disease (e.g., Refs. 5, 10, 14, 23, 24, 29, 30, 52). Figure 2 shows how Penh is calculated from the pressure changes occurring inside a closed chamber containing a spontaneously breathing animal.

Penh is a dimensionless number derived from chamber pressure that serves as a general characterization of its shape. As such, it reflects the drive to breathe and the timing of the respiratory pattern generator. Al-

Table 1. List of symbols and abbreviations

$t$	Time
$P(t)$	Pressure (as a function of time)
$V(t)$	Volume (as a function of time)
$\dot{V}(t)$	Flow (as a function of time)
$R$	Resistance
$E$	Elastance
$P_0$	Baseline pressure
$Z_{in}$	Input impedance
$f$	Frequency
$R(f)$	Real part (resistance) of impedance (as a function of frequency)
$X(f)$	Imaginary part (reactance) of impedance (as a function of frequency)
$I$	Inertance
$G_t$	Tissue resistive parameter from the constant-phase model
$H_t$	Tissue elastic parameter from the constant-phase model
$\alpha$	Parameter from the constant-phase model that links $G_t$ and $H_t$
Penh	Enhanced pause
$Z_{tr}$	Transfer impedance

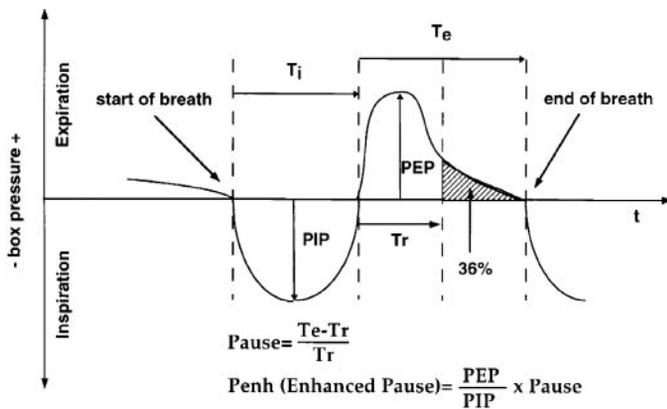


Fig. 2. Calculation of enhanced pause (Penh) =  $(P_1/P_2) \times (T_1/T_2)$ , where P is pressure and T is time. The signal shown is a stylized version of the pressure measured inside a closed plethysmograph in which a mouse was placed. Plethysmographic (box) pressure varies cyclically as the mouse breathes. The inspiratory and expiratory durations ( $T_I$  and  $T_E$ , respectively) are defined on the basis of the upper and lower portions of the pressure curve and do not necessarily correspond to actual inspiration and expiration. PEP, peak expiratory pressure; PIP, peak inspiratory pressure;  $t$ , time;  $T_r$ , time to expire 74% of the tidal volume. (From Ref 30. Reproduced with permission.)

though some studies have shown a good correlation between Penh and other measures of lung function (15, 23, 30), others have shown that Penh does not correlate well with morphometric changes in mice (41). Moreover, Petak et al. (55) have shown that, under some circumstances, Penh may behave in an opposite fashion from a direct measurement of lung mechanics.

The pressure changes occurring inside a closed chamber containing a breathing animal arise from two sources: 1) gas compression and rarefaction resulting from the pressure changes in the thoracic gas that produce inspiratory and expiratory flow and 2) changes in gas humidification and temperature as air moves between the box (ambient conditions) and the lungs (body temperature, saturated). In any given situation, it is not possible to distinguish between these two sources merely from measurements of changes in box pressure. Furthermore, the relative contributions of these two sources change with bronchoconstriction (43) and likely also with any kind of mechanical lung abnormality. The usefulness of Penh in the assessment of lung function is accordingly severely limited.

It has recently been shown that the component of box pressure produced by gas conditioning can be essentially eliminated by heating and humidifying the air in the box to body conditions (43). This substantially reduces the magnitude of the swings in box pressure during breathing (Fig. 3). The remaining swings in box pressure are presumably due to thoracic gas compression and so would theoretically have a direct relationship to airway resistance. However, these residual pressure swings are also determined by absolute lung volume and tidal volume, both of which can be markedly altered during bronchoconstriction by methacholine (43). Thus, unless lung volume and tidal volume can be either controlled or measured independently,

UP is unlikely to be of practical use as a means of obtaining accurate measures of mechanical lung function. This is not to say that UP does not have its place. The convenience and noninvasiveness of the technique makes it suitable for long-term screening for any event that alters the pattern of breathing (8, 65). In this regard, factors that influence breathing pattern, such as chemoreceptor sensitivity, are appropriately assessed by UP. However, UP as it is currently practiced does not provide a meaningful characterization of mechanical lung function. For that, one requires a method that is closer to the other extreme of the noninvasiveness-precision continuum (Fig. 1). Before considering such methods, however, we need to examine in a little more detail the theoretical basis of lung function measurement.

**ASSESSING RESPIRATORY SYSTEM MECHANICS**

Respiratory system mechanics, in the classical view, is embodied in the single-compartment linear model shown in Fig. 4, consisting of a flow-resistive conduit (with resistance R) serving a single elastic compartment (with elastance E). The equation of motion describing this model is

$$P(t) = R\dot{V}(t) + EV(t) + P_0 \quad (1)$$

where  $P(t)$  is the pressure at the entrance to the model,  $\dot{V}(t)$  is the flow of gas into the model,  $V(t)$  is volume of gas in the elastic compartment,  $P_0$  is the resting applied pressure (e.g., positive end-expiratory pressure), and  $t$  is time. Equation 1 typically provides a very accurate description of the respiratory system if it is perturbed with a sinusoidally oscillating  $\dot{V}$  signal (2). The parameters R and E are readily determined by fitting Eq. 1 to records of  $P(t)$ ,  $\dot{V}(t)$ , and  $V(t)$ .

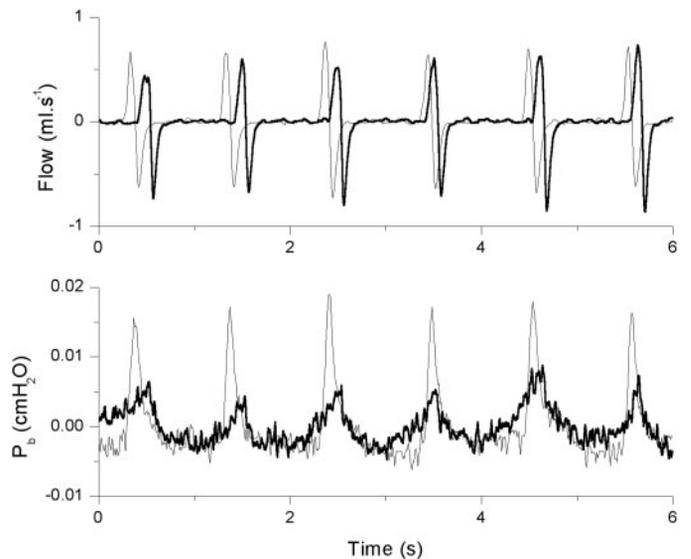


Fig. 3. Records of flow (top) and barometric pressure ( $P_b$ ; bottom) made with a mouse breathing spontaneously while enclosed in the chamber with the gas inside the chamber at 23°C and relative humidity at 29% (thin lines) and after the chamber gas was heated to 37°C and humidified to 85% (thick lines). Inspiratory flow is positive. (From Ref 43. Reproduced with permission.)

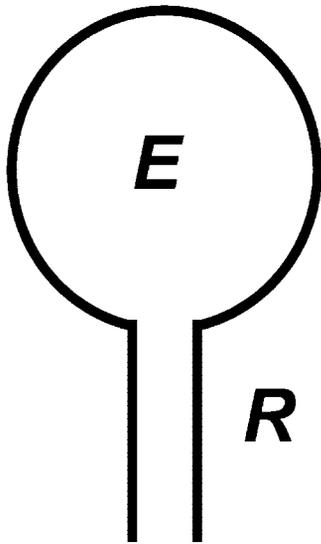


Fig. 4. Single-compartment model of respiratory system mechanics, with resistance ( $R$ ) and elastance ( $E$ ). This model is governed by Eq. 1.

However, the values of  $R$  and  $E$  obtained in this way vary markedly with the frequency at which the respiratory system is oscillated. This frequency dependence of  $R$  and  $E$  can be particularly marked over the range of normal breathing frequencies and is caused by the viscoelasticity of the respiratory tissues together with any regional heterogeneities of mechanical function that might be present throughout the lung (62). Consequently, simply determining  $R$  and  $E$  at a single frequency does not constitute a complete characterization of respiratory mechanics. Considerably more information is obtained if one measures  $R$  and  $E$  over a range of frequencies. Such a characterization is embodied in the respiratory  $Z_{in}$  (54). The usual way of determining  $Z_{in}$  consists of perturbing the respiratory system with a broad-band  $\dot{V}(t)$  waveform that contains many different frequencies simultaneously.  $Z_{in}$  is then determined at each of the frequencies involved by using the Fourier transform to convert the  $P$  and  $\dot{V}$  signals in the time domain to their equivalent representations in the frequency domain.  $Z_{in}$  is a complex function of frequency ( $f$ ), with a real part,  $R(f)$ , and an imaginary,  $X(f)$ , thus

$$Z_{in}(f) = R(f) + iX(f) \quad (2)$$

where  $i$  is the positive square root of  $-1$ .  $R(f)$  is usually termed the resistance because it is nothing more than the value of  $R$  that would be obtained if Eq. 1 were fit to data obtained by oscillating the respiratory system sinusoidally with  $f$ .  $X(f)$  is termed the reactance and is equivalent to the value of  $E$  that would be obtained through Eq. 1, divided by  $-2\pi f$ .  $X(f)$  contains negative contributions from elastic structures in the respiratory system and positive contributions from structures with mass (i.e., those having inertance). At very low  $f$ , only the elastic structures contribute significantly, and so  $X(f)$  is negative. As  $f$  increases, the inertive structures assume increasing importance until they eventually

dominate and  $X(f)$  becomes positive. The value of  $f$  at which the elastic and inertive components are equal and opposite [i.e., when  $X(f)$  is zero] is called the resonant frequency.

Even  $Z_{in}(f)$ , however, does not give a complete characterization of respiratory mechanics because the above theory applies exactly only if the respiratory system behaves dynamically like a linear system. This is never precisely the case in reality, and, if the amplitude at which the respiratory system is perturbed becomes large enough, nonlinear mechanical behavior may become significant. For example, if  $\dot{V}$  is large, then turbulence may occur in the airways and resistive pressure drops will become more dependent on the square of  $\dot{V}$  than on  $\dot{V}$  itself (58). Similarly, if  $V$  is large, the tissues of the lung and chest wall may become overdistracted and  $E$  will depend positively on  $V$  (6, 70). In addition, the magnitude of  $Z_{in}(f)$  is increased if part of the lung becomes atelectatic and is reduced if a sigh is given before measurement (1).

#### BETWEEN THE TWO EXTREMES: CONTROLLING SOMETHING

The above discussion establishes that the mechanical nature of the respiratory system, be it that of humans or mice, depends on the frequency at which it is oscillated, the amplitude of the oscillations, and the volume history of the lung before the measurements. Also, because of the forces of interdependence between airways and parenchyma, the responsiveness of the lung to bronchial challenge is exquisitely sensitive to lung volume (3, 49, 69). These can all become confounding factors in any investigation that seeks to assess how a particular intervention affects lung function. Thus, for example, if  $R$  and  $E$  are measured in a spontaneously breathing animal that is free to choose its own breathing frequency and tidal volume, then one runs the risk of seeing changes in these parameters that result purely from changes in breathing pattern or altered lung volume rather than from any change in mechanics reflective of an alteration in airway caliber. This suggests that more definitive results would be obtained if at least some of the breathing pattern and volume parameters were under more precise experimental control.

A technique that shows promise as a compromise between noninvasiveness and precision is the measurement of  $Z_{tr}$ , which is obtained as the frequency domain relationship between pressure oscillations applied to the body surface and flow measured at the mouth (31, 51, 72). Although, in principle,  $Z_{tr}$  should give similar information about lung function to  $Z_{in}$ , its measurement may be associated with some practical advantages. First, when the distal airways of the lung become significantly constricted, the flow oscillations applied at the mouth to measure  $Z_{in}$  may become shunted to a large degree into the central airways that have a finite compliance (3, 44). If the amount of flow reaching the lungs becomes small to the point that it approaches the noise level of flow measurements at the

mouth, then one effectively loses the ability to probe the lung periphery. In contrast, when pressure oscillations are applied at the body surface, flow is driven from the lung periphery toward the trachea and shunting into the central airway compartment is minimized. This means that all flow measured at the mouth comes from the lung periphery, giving  $Z_{tr}$  a signal-to-noise advantage over  $Z_{in}$  for the investigation of severely constricted lungs. Another advantage of  $Z_{tr}$  is that pressure oscillations can be applied to the body surface by enclosing the thorax in a suitable chamber and oscillating chamber pressure. Provided that the airway opening can be isolated in a separate chamber, airway flow can then be measured with transducers that do not have to actually come into direct contact with the animal (e.g., with a pneumotachograph connected to the head chamber). This makes it possible to measure  $Z_{tr}$  in conscious animals provided they are suitably restrained, although achieving such restraint can be a significant challenge.

Zwart and co-workers (33, 51, 72) exploited these advantages to develop a system for measuring  $Z_{tr}$  in conscious mice. The animals are held in a body plethysmograph and restrained so that their noses are sealed into a small second chamber through which flow into and out of the lungs is measured (Fig. 5). The main body chamber connects to a loudspeaker, which is used to generate controlled oscillations in chamber pressure that act on the animal's thorax.  $Z_{tr}$  is obtained as the Fourier-domain relationship between the  $P$  oscillations generated in the large body chamber and  $\dot{V}$  measured in the small nose chamber. Lundblad and Strom (42) adapted this system to allow more convenient restraining of the animals, which is the major practical problem associated with this technique. They measured  $Z_{tr}$  in mice both under control conditions and after allergen and methacholine challenge and found significant changes in both the real and imaginary parts of  $Z_{tr}$ .

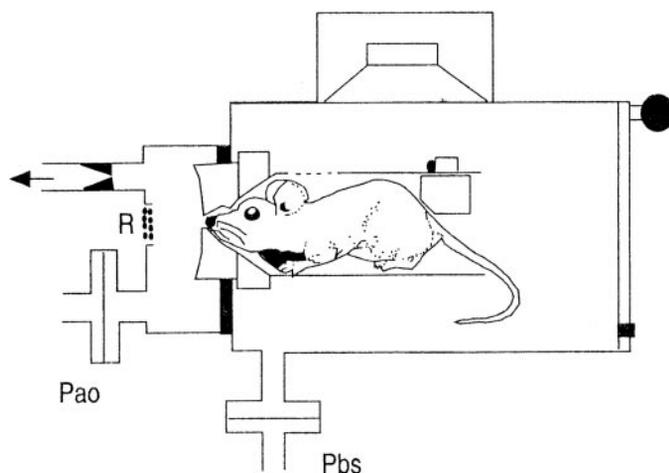


Fig. 5. Experimental setup for measuring transfer impedance ( $Z_{tr}$ ) in mice. A conscious mouse is placed in a body box, with its nose sealed into a separate chamber from which respiratory flow is measured, and through which a bias flow and aerosols are directed. Pbs, box pressure; Pao, airway opening pressure; R, resistance of screen pneumotachograph. (From Ref 72. Reproduced with permission.)

Forced expired flows have also been measured in the mouse (38, 68) in an attempt to mimic standard pulmonary function testing in humans. However, although this procedure is innocuous in human patients, the animals in this study were still anesthetized, paralyzed, and tracheostomized; therefore, there was no gain in noninvasiveness. Presumably, tracheostomy is not essential for this technique, because total body compression could in principle be applied to a spontaneously breathing animal. However, timing it with the end of inspiration could be technically challenging. Another problem with assessing lung function through forced expirations in animals is that it is extremely difficult to make the link between alterations in forced expiratory parameters such as forced expiratory volume in 1 s or forced vital capacity and changes in lung structure. This is because the phenomena that determine limiting flow in the lung are complex and nonlinear, involving a combination of factors such as airway caliber, parenchymal stiffness, airway wall stiffness, and lung volume. Thus, even if an intervention produces a change in forced expiratory volume in 1 s or forced vital capacity, it is difficult to say what structural changes in the lung are responsible. In any event, few data in the literature support the validity of this approach.

A major issue when measuring respiratory mechanics in the intact (i.e., nontracheostomized) animal is that the resistance of the nose constitutes a large component of total respiratory resistance and may change significantly in response to challenge (13, 30, 36). This applies to the  $Z_{tr}$  method, UP, and the double-chamber plethysmographic method (53) and constitutes yet another example of how avoiding an intervention (tracheostomy, in this case) leaves in place something that interferes significantly with the measurement of the quantity of interest.

#### AT THE OTHER EXTREME: CONTROLLING EVERYTHING

In accordance with the phenotyping uncertainty principle, the greatest degree of accuracy and specificity of *in vivo* lung function measurement is made in an animal in which breathing frequency, tidal volume, mean lung volume, and volume history are all under precise experimental control and in which the influence of the upper airways has been eliminated. This requires anesthesia, paralysis, and tracheostomy. One could even go a step further and eliminate the (presumably unwanted) effects of the chest wall by performing a thoracotomy. This all comes at a significance price, of course, in the form of surgical and pharmacological stresses. These stresses may significantly affect lung mechanics through the release of mediators such as catecholamines and the modulation of neural responses.

Measurements in mice under highly invasive conditions began with the miniaturization of techniques originally developed for larger animals and humans. For example, Levitt and Mitzner (39, 40) took changes in peak airway pressure in mechanically ventilated

mice as a simple empirical measure of the pulmonary response to bronchial challenge. More detailed information about lung function would be obtained if  $\dot{V}$  could also be measured, to allow partitioning of this response into its resistive and elastic components (i.e.,  $R$  and  $E$  through Eq. 1). However, it has only been relatively recently that the technical difficulties of measuring  $\dot{V}$  with the necessary accuracy in mice have been overcome. Although miniature pneumotachographs for small rodents have been described (20, 48), such devices tend to be problematic because the magnitude of  $Z_{in}(f)$  is invariably not small compared with the  $Z_{in}$  of the differential pressure transducer used in association with the pneumotachograph (60). Martin et al. (46) got around this problem by placing mechanically ventilated animals in a body plethysmograph.  $\dot{V}$  from the plethysmograph was used in place of tracheal  $\dot{V}$  to calculate  $R$  and  $E$ . However, their approach was limited to providing parameters of mechanics only at the frequency of mechanical ventilation.

There are currently two approaches that have been used successfully to apply broad-band  $\dot{V}$  signals in mice to determine  $Z_{in}$ . One method uses a piston pump controlled by a computer (26, 27, 34). Tracheal pressure and  $\dot{V}$  are determined by correcting the measured volume displacement of the piston face for gas compressibility within the pump cylinder and for resistive and inertive pressure losses along the conduit leading to the trachea. This system allows for precise control of the frequency content and amplitude of the applied  $\dot{V}$  oscillations (60). Because the device also functions as a conventional mechanical ventilator, the volume history before oscillatory measurements can also be controlled. The second method for measuring  $Z_{in}$  in mice uses a wave tube consisting of a thin plastic catheter attached to the trachea at one end and to a loudspeaker at the other (55, 57). The loudspeaker produces oscillatory flow through the tube while the  $Z_{in}$  of the animal is determined from two lateral pressure measurements made at points along the catheter, together with a mathematical model of the acoustic properties of the catheter.

The physiological interpretation of  $Z_{in}(f)$  over a given frequency range requires a suitable mathematical model of lung mechanics. The model most suited to  $Z_{in}$  below 20 Hz in the normal or moderately diseased lung is that of a uniformly ventilated airway subtending a single alveolar compartment, similar to the model described by Eq. 1, except that now the alveolar compartment is not characterized by a single elastic constant. Instead, the tissues of the lung are described by a complex impedance in which the ratio of the real to imaginary parts is independent of frequency, causing it to be referred to as the "constant-phase model." The equation of motion of this model is

$$Z_{in}(f) = R + i2\pi fI + \frac{G_t - iH_t}{(2\pi f)^\alpha} \quad (3)$$

where  $R$  is a Newtonian resistance,  $I$  is an inertance essentially equal to that of the gas in the central

airways,  $G_t$  characterizes viscous dissipation of energy in the respiratory tissues, and  $H_t$  characterizes energy storage in the tissues.  $G_t$  and  $H_t$  are coupled via the equation

$$\alpha = \frac{2}{\pi} \tan^{-1} \frac{H_t}{G_t} \quad (4)$$

where the ratio  $H_t/G_t$  is the inverse of the quantity defined by Fredberg and Stamenovic (25) as hysteresivity. The constant-phase model was first established in larger animals by Hantos et al. (32) but has now been shown to also provide an excellent description of mouse lung mechanics (26, 27, 34, 35, 64, 66). Figure 6 shows an example of the fit provided by the constant-phase model to  $Z_{in}$  from a normal mouse under control conditions and after administration of a methacholine aerosol.

When the lung is investigated alone (i.e., in an open-chest animal), then  $R$  gives a good approximation to the resistance of the conducting airways, whereas  $G_t$  and  $H_t$  characterize the lung parenchyma (34, 66). When the entire respiratory system is involved (i.e., lungs plus chest wall), then  $R$ ,  $G_t$ , and  $H_t$  contain roughly 50% contributions from the chest wall in rats (34) and roughly 30% in mice (1, 66). In either case, however, this model-fitting approach allows a partitioning of respiratory mechanics into a component due

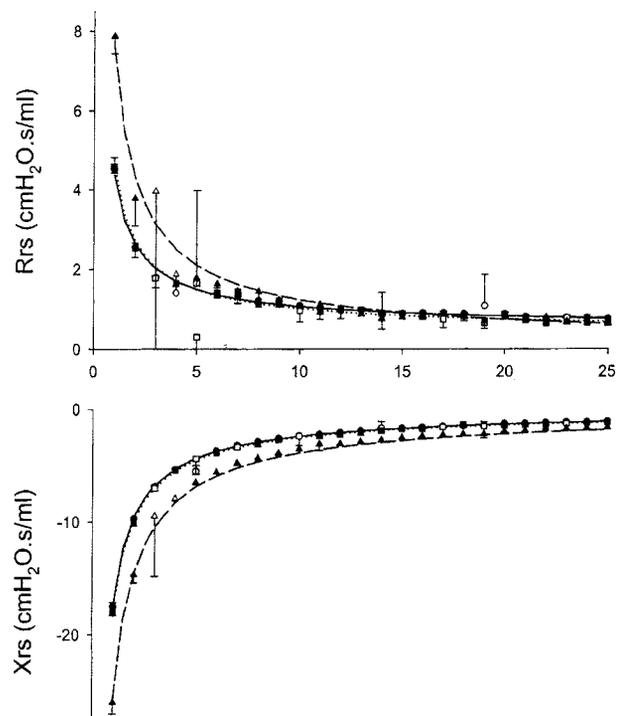


Fig. 6. Examples of real ( $R_{rs}$ : respiratory system resistance) and imaginary ( $X_{rs}$ : respiratory system reactance) parts of impedance obtained from control mice (circles) and mice exposed to hyperoxia for 24 h (squares) or 60 h (triangles). The model fits (Eq. 3) are also shown as lines (solid for control and dotted and dashed for 24 h and 60 h of hyperoxia, respectively). Open symbols were excluded from the model fit due to poor coherence. (From Ref 55. Reproduced with permission.)

largely to gas flow through airways (i.e.,  $R$ ) and a component due to the tissues (i.e.,  $G_t$  and  $H_t$ ). This is much more convenient than the highly invasive alternative of measuring airway resistance directly with alveolar capsules (50, 66).

The parameters  $R$ ,  $I$ ,  $G_t$ , and  $H_t$  are evaluated by fitting *Eq. 1* to measurements of  $Z_{in}$  made over a range of frequencies. The parameters  $R$  and  $I$  provide an overall characterization of the airway tree. For example,  $R$  decreases and  $I$  increases when airway caliber decreases, although the inertive elements of the murine respiratory system are so small that they do not significantly influence  $Z_{in}$  below 20 Hz (26, 27, 34). The parameters  $G_t$  and  $H_t$  characterize the lung tissue. For example, both  $G_t$  and  $H_t$  increase during bronchoconstriction that becomes more pronounced with inflammation (66), due in part to the development of regional heterogeneities in mechanical function throughout the lung (44, 66). Smaller animals have relatively lower airway resistances, conforming to the morphometric observation that their airways are relatively wider than those of larger animals (27), presumably because of the increased ventilatory requirements dictated by their higher basal metabolic rates.

The standard way of measuring bronchial responsiveness in animals is to subject them to progressively increasing doses of a bronchial agonist and then to record the response of the lung to each dose. The agonist can be either injected (17, 56) or aerosolized (66, 70), with corresponding differences in the rate of delivery of agonist to the smooth muscle. Measurement of  $Z_{in}$  is then made at some standard interval after cessation of aerosol delivery. Figure 7 shows how the parameters of the model in *Eq. 1* increase as a function of dose in two groups of BALB/c mice (a control group and a group sensitized to ovalbumin). The sensitized group is clearly more responsive than the control group, as would be expected due to the inflammatory reaction (confirmed by bronchoalveolar lavage) in the former. Interestingly, however, the increase in responsiveness seems to be most marked in the parameters  $G_t$  and  $H_t$ , which characterize the lung tissue, suggesting that more air space closure occurred in the inflamed lungs, in agreement with human studies (45). The major changes in lung mechanics after acute lung injury in mice also occur at the low end of the frequency spectrum in both the real and imaginary parts of impedance (35), again implicating the lung periphery as the major site of the mechanical dysfunction in these study systems.

As a final note, we must point out that complex impedance is not the only useful physiological endpoint one could obtain in mice under highly controlled circumstances. Another extremely important measurement is the pressure-volume (P-V) curve, which is usually obtained by inflating and deflating the lungs in a series of steps over the vital capacity range (28, 47, 70). The P-V curve is constructed from recordings of tracheal pressure and lung volume at the end of each step. Alternatively, the P-V curve can be obtained from a very slow inspiration and expiration (47). The shape

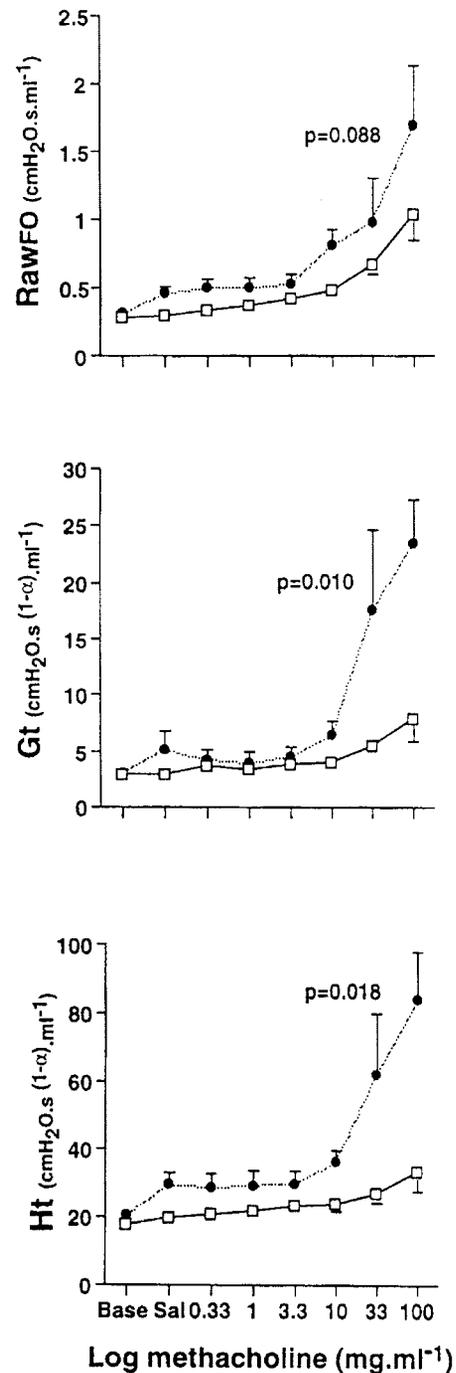


Fig. 7. Dose-response relationship for lung mechanics parameters (*Eq. 3*) in control and ovalbumin-sensitized BALB/c mice exposed to methacholine aerosol. Data are means  $\pm$  SE. ●, Sensitized group ( $n = 8$ ); □, control group ( $n = 8$ ). RawFO, airway resistance;  $G_t$  and  $H_t$ , tissue resistive parameter and tissue elastic parameter, respectively, from the constant-phase model. (From Ref 66. Reproduced with permission.)

of the P-V curve, which can only be obtained in the complete absence of spontaneous breathing efforts, is diagnostic of a variety of lung parenchymal abnormalities. To register the P-V curve to absolute lung volume, a measure of functional residual capacity is also required. Functional residual capacity has been mea-

sured in spontaneously breathing animals with the use of a plethysmographic approach (43) and in paralyzed animals by inert gas dilution (63).

## SUMMARY

The assessment of respiratory system mechanics in rodents has become a major focus in recent years because of the need to phenotype small animals. This presents special problems because these animals are so small, particularly with respect to measuring and controlling respiratory flows. The various techniques available for measuring lung function embody what we call the phenotyping uncertainty principle, whereby precision must be traded off against noninvasiveness; it is likely that these two attributes cannot be optimized simultaneously. At one extreme of this trade-off continuum lies UP in freely moving conscious animals. At the other extreme lies the forced oscillation technique applied to anesthetized, paralyzed, tracheotomized animals. Intermediate between these two extremes is the measurement of pulmonary mechanics in spontaneously breathing animals with an esophageal catheter and the measurement of Ztr in conscious, restrained animals.

It is important to note that the limitations of these various methods are not merely technological. That is, our present inability to measure lung function both precisely and noninvasively at the same time is not simply because we have not yet been clever enough to devise an elegant solution. Rather, it is a fundamental limitation, just like the quantum mechanical situation pertaining to the position and momentum of a particle. For example, regardless of how well we solve the problem of controlling small gas flows and pressures, we will always be faced with the fact that lung mechanics are profoundly affected by breathing frequency. Hence, any behavioral response in a spontaneously breathing animal will significantly alter the mechanical characteristics of the lung. Unless this behavioral response is totally eliminated, it will affect assessment of the effects of an experimental intervention; however, eliminating the behavioral response takes the animal away from its usual or "natural" state.

These considerations are important when choosing a measurement technique for a particular application. Much recent research has tended to sacrifice precision and specificity in favor of convenience through the use of UP. This technique only provides, at best, a very indirect reflection of lung function and should be either abandoned or complemented with more invasive and precise measurements of respiratory mechanics. For studies that explore the mechanisms causing lung dysfunction, more invasive and precise endpoints often provide important insight.

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