

A new ventilator for monitoring lung mechanics in small animals

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Volgyesi, G. A., L. N. Tremblay, P. Webster, N. Zamel, and A. S. Slutsky. A new ventilator for monitoring lung mechanics in small animals. *J Appl Physiol* 89: 413–421, 2000.—Researchers investigating the genetic component of various disease states rely increasingly on murine models. We have developed a ventilator to simplify respiratory research in small animals down to murine size. The new ventilator provides constant-flow inflation and tidal volume delivery independent of respiratory parameter changes. The inclusion of end-inspiratory and end-expiratory pauses simplifies the measurement of airway resistance and compliance and allows the detection of dynamic hyperinflation (auto-positive end-expiratory pressure). After bench testing, we performed intravenous methacholine challenge on two strains of mice (A/J and C57bl/bj) known to differ in their responses by using the new ventilator. Dynamic hyperinflation and a decrease in compliance developed during methacholine challenge whenever respiratory rates of 60–120 breaths/min were employed. In contrast, if dynamic hyperinflation was prevented by lengthening expiratory time, (respiratory rate = 20 breaths/min), static compliance remained constant. More importantly, the coefficient of variation of the results decreased when lung volume shifts were prevented. In conclusion, airway challenge studies have greater precision when dynamic hyperinflation is prevented.

mouse; airway challenge; methacholine; airway resistance; total compliance; animal models; asthma

AS OUR UNDERSTANDING OF THE genetics of many diseases increases explosively, the need for animal models and simple testing methods is becoming more important. Research in this area has compelled researchers to shift from the well-characterized guinea pig and somewhat less developed rat model to the mouse. Scaling of ventilators and monitoring apparatus to such a small animal is not a trivial matter, as the precision of both volume and flow control must be improved. A particularly important tool in research is the airway challenge, which involves the monitoring of changes in respiratory function in response to the administration of various agonists and/or antagonists.

The utilization of constant-flow inspiration and end-inspiratory pause (EIP) for the assessment of airway function in vivo is well documented (3, 23). Ewart et al. (11) used a low-dead-space ventilator with constant

inspiratory flow to measure respiratory system mechanics in mice by the end-inspiratory occlusion technique. They performed dose-response studies to assess pulmonary responses to a range of cholinergic challenges in hyper- and hyporesponding mice. Unfortunately, the authors of this publication provided very little information regarding the design and testing of their ventilator.

The magnitude of both the resistance and the compliance of different components of the respiratory system is strongly dependent on the lung volume range at which they are measured (1, 2, 23). If we wish to draw conclusions about observed differences in resistance or compliance, either in response to increasing doses of agonists or between different populations of subjects, it is imperative, therefore, to make measurements at comparable lung volumes. Unfortunately, administration of airway constrictors such as methacholine (MCh) often leads to dynamic hyperinflation (25, 31), and, therefore, the effects of the shifts in lung volume may well affect observed resistance and compliance changes. The most serious problem with many previous studies (5, 7, 8, 14–17, 21, 29–31) is that there is no way to ensure that the functional residual capacity remains constant during challenge. The challenge-induced dynamic hyperinflation is usually undetectable without an end-expiratory pause (EEP) or an assessment of absolute volume.

We have developed a new ventilator that makes accurate and continuous respiratory function monitoring of small animals more practical. The ventilator provides constant inspiratory flow and delivers fixed and accurate tidal volumes, despite changes in respiratory system impedance in the animal. An adjustable EIP simplifies the assessment of resistance and compliance by the occlusion method, and an adjustable EEP permits the detection of dynamic hyperinflation during airway challenge (22). Dynamic hyperinflation may be minimized by lengthening the expiratory time, which can be controlled independently.

In this paper, we describe the rationale underlying this ventilator, present data based on bench tests, and summarize in vivo studies by using two strains of mice

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known to have different degrees of airway responsiveness. Based on our results, we also show that the major effect of airway challenge is an increase in airway resistance, with little to no effect on the elastic properties of the respiratory system. We speculate that the significant decrease in dynamic compliance during airway challenge previously reported (14, 15, 20) may be the result of a temporary shift to higher volumes during dynamic hyperinflation and was not caused by changes in tissue properties.

METHODS

The Ventilator: Principles of Operation

Our goal was to develop a ventilator that would deliver a relatively constant flow, irrespective of changes in impedance of the respiratory system of the animal. We achieved this by interposing a 24-cm-long capillary tube with a very small internal diameter (0.025 cm) between a high-pressure gas source and the animal. The flow resistance of the capillary tube was previously found to be constant with flows in the range of 2–10 ml/s (see Fig. 3B). With the very high capillary resistance ($240 \text{ cmH}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{s}$) compared with the resistance of the respiratory system of the animal (estimated to reach a maximum $10 \text{ cmH}_2\text{O}\cdot\text{ml}^{-1}\cdot\text{s}$ during MCh challenge), inspiratory flow depends mainly on the source pressure and capillary resistance and is effectively independent of the properties of the respiratory system. The inspiratory flow can, therefore, be controlled by means of an adjustable pressure regulator, and the flow signal can be obtained by monitoring the pressure difference between the ends of the capillary tube. The pressure transducers used in the ventilator (MPX series, Motorola, Phoenix, AZ) have small internal volume ($<0.2 \text{ ml}$) and provide linear response over the pressure ranges used. The inspired volume is obtained by electronic integration of the flow signal. The timing is controlled by a set of electronically actuated solenoid valves (Clippard Minimatic, Cincinnati, OH). These solenoid valves have no sliding parts and have extremely small dead space ($<0.1 \text{ ml}$ including connector) and fast response (5–10 ms). The animal is connected to the ventilator via a miniature Y piece fitted with a male luer tip (dead space 0.02 ml) and plastic tubes of low compliance (1.6 mm ID). One tube is for inspiration, the second one is for expiration, and the third is for connection to a pressure transducer in the ventilator for monitoring airway opening pressure (Pao). The compressible volume of the entire breathing circuit including the solenoid valves and pressure transducers is $<3 \text{ ml}$. To the extent that the resistance of the expiratory circuit (Rexp) is approxi-

mately linear in the flow ranges used, expired volume can be estimated by integrating expiratory flow. The electronic control circuit, employing discrete and integrated analog and digital components, is mounted on a single circuit board ($13 \times 13 \text{ cm}$). The ventilator is housed in a metal cabinet that is 30 cm long, 14 cm high, and 20 cm deep and is completely self-contained, requiring no external equipment to function.

Figure 1 is a schematic representation of the four phases of ventilation, which are described below.

Inspiration. During inspiration (Fig. 1A), the inspiratory solenoid valve is open, and the expiratory solenoid valve is closed. Compressed inspiratory gas, whose pressure can be adjusted by the pressure regulator, enters the animal at a constant flow, the magnitude of which is determined by the pressure differential between the high-pressure source [gas pressure (P_{G})] and the Pao and the resistance of the inspiratory capillary tube. Because P_{G} is much higher than Pao and the resistance of the inspiratory capillary tube is constant, the inspiratory flow is approximately proportional to P_{G} . The inspiratory volume is obtained by electronic integration of the inspiratory flow. Inspiration continues until the volume reaches the set tidal volume in the volume cycle mode or until the Pao reaches the maximum pressure set in the pressure cycle mode.

EIP. At the end of inspiration, the airways are clamped by closing both solenoid valves (Fig. 1B). This feature makes it possible to monitor airway resistance and static compliance by allowing time for redistribution of gases in the lungs and for stress relaxation to take place. The EIP is continuously variable from 0 to $\sim 4 \text{ s}$.

Expiration. After the elapsed time for the EIP, the expiratory solenoid valve opens and allows passive expiration to occur (Fig. 1C). The expiratory flow depends on the viscoelastic properties of the lungs and the Rexp. Because Rexp is nearly constant, expiratory flow is approximately proportional to Pao and is electronically integrated to provide a value proportional to expiratory volume.

Because inspired and expired volumes are virtually identical during steady-state ventilation, expiratory flow can be calibrated by observing the volume trace and adjusting a gain control knob to match the expired volume to the inspired volume. This calibration has no effect on the operation of the ventilator and is necessary only if estimates of expiratory volume are required. The duration of expiration is controlled by two alternative methods. In the first mode, the minimum Pao during expiration may be preset on the ventilator [positive end-expiratory pressure (PEEP) control]. In this mode, expiration continues until Pao equals preset PEEP. Because the precise time at which Pao decays to zero during passive expiration is indefinite, zero end-expiratory pressure cannot

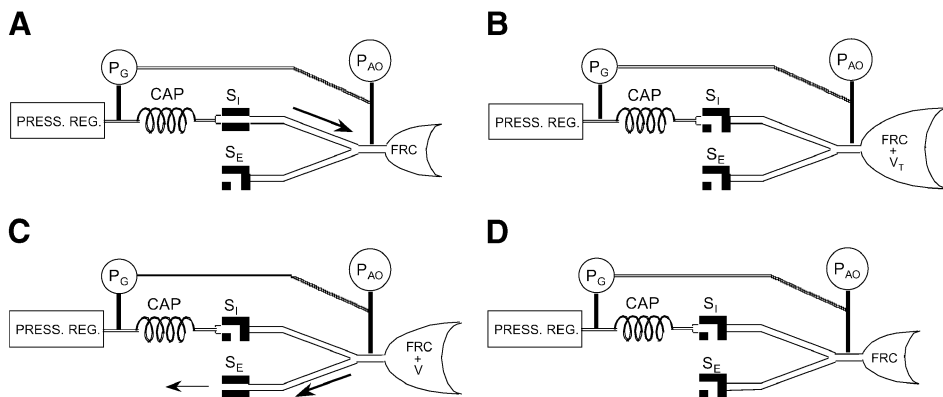


Fig. 1. Schematic representation of the 4 phases available from the ventilator: inspiration (A), end-inspiratory pause (B), expiration (C), and end-expiratory pause (D). P_{G} and P_{AO} are pressure transducers for gas pressure (P_{G}) and airway opening pressure (Pao), respectively. S_{I} and S_{E} , inspiratory and expiratory solenoid valves, respectively; CAP, capillary tube; FRC, functional residual capacity; V, volume; V_{T} , tidal volume.

be used reliably in this mode. A timed expiration mode has, therefore, been incorporated into the ventilator to allow expiration to proceed for a fixed time, independent of the Pao. This feature allows zero end-expiratory pressure or external PEEP to be employed. The latter can be implemented by bubbling the expiratory flow through a water column of the required height or by using a PEEP valve. Expiratory flow, however, cannot be accurately monitored by using the approach discussed above when external PEEP is used because the flow resistance of external PEEP devices is inherently alinear.

EEP. At the end of expiration, both solenoids are again closed to allow for gas redistribution within the respiratory system (Fig. 1D). This pause (variable from 0 to ~ 4 s) allows the adjustment of the respiratory rate without changing other parameters and also makes possible the detection and estimation of auto-PEEP.

Figure 2 indicates the general shape of the Pao during a complete respiratory cycle. The labels for the various time and pressure components are included to clarify the text.

Bench Testing

The fundamental requirement of the ventilator is that the flow delivered to the animal be independent of changes in lung mechanics. The flow resistance of the capillary tube at various flows was determined by the following method. The pressure regulator was first adjusted to supply air at certain fixed pressure. The ventilator was then set to deliver a fixed tidal volume, which was monitored by a water-displacement spirometer. The EIP and EEP were each set to provide ~ 1 s of delay to make the volume estimation using the spirometer easier. The tidal volume was chosen to require a sufficiently long time for its delivery to render the solenoid opening and closing delays negligible. The flow was then calculated by dividing the tidal volume by the inspiratory time obtained from the recording of the flow signal. This procedure was then repeated for different source pressures from 2 to 30 lb./in.². The results were then plotted with the flow on the x -axis and source pressure on the y -axis. Each point on the graph represents the average of three measurements.

The effect of changes in airway resistance on the delivered tidal volume was investigated by using specially constructed high-resolution water-displacement spirometers. The ventilator was set to deliver a fixed tidal volume to the spirometer

through various-sized needles, and the actual volume delivered was noted in each case. The resistance of each needle was calculated by dividing the pressure drop across the needle by the flow through the needle. The two different tidal volumes (10 and 0.4 ml) span the practical range required by rats and mice, respectively. The effect of changes in compliance on delivered tidal volume was also investigated by using a similar spirometer. Changes in compliance were simulated by changing the level of the water in the volume-indicator column in a stepwise fashion.

To characterize rapid events, such as the drop in pressure that occurs with cessation of flow at end-inspiratory occlusion, it is essential that the forcing step function stop and start abruptly. The solenoid response is limited by electrical inductance and mechanical inertia. The solenoid valve is energized by a coil of wire, whose inductance limits the step change in current flowing through the wire. When a steady voltage is suddenly applied to the coil, a certain time is required for the current to increase to the threshold level sufficient to initiate the opening of the solenoid valve. At the cessation of the actuating voltage, there is another delay before the current decreases sufficiently to initiate the closing of the solenoid valve. When the diaphragm of the solenoid valve snaps to the open or closed position, the small changes in coil inductance cause detectable changes in the shape of the current waveform. The delay times for the initiation of solenoid opening and closing and the time required for the solenoid to go from the fully closed to the fully open or from the fully open to the fully closed position were estimated from the waveform of the current flowing through the inspiratory solenoid valve (obtained by monitoring the voltage difference across a small resistor placed in series with the solenoid coil). For the determination of solenoid timing characteristics, we used a sampling rate of 2,000 samples/s to allow detection of events with 0.5-ms resolution.

The linearity of the expiratory flow measurement was assessed by measuring the pressure drop across the entire expiratory circuit with several different flows spanning the range likely to be encountered in vivo.

In Vivo Testing

After obtaining institutional animal care committee approval, two series of experiments were performed to assess bronchial hyperresponsiveness in A/J and C57bl/bj mice, two strains known to differ significantly in this respect (6, 9, 18). For each experiment, the selection of mice from one group or the other was decided by the flip of a coin. Nine C57bl/bj and eleven A/J mice were used for the initial series of experiments (age 4–10 wk, weight 20–30 g). Each mouse was weighed and anesthetized with an intraperitoneal injection of a mixture of xylazine and ketamine (10 and 150 mg/kg, respectively). Once an appropriate depth of anesthesia was reached, the trachea was intubated via a tracheostomy by using a blunt 18-gauge needle and ventilated with a tidal volume of ~ 8 ml/kg at a rate of 80 breaths/min. EIP of sufficient duration to provide stable plateau pressure was employed to allow the subsequent estimation of airway resistance and compliance. To permit the longest EIP while maintaining the respiratory rate, no EEP was used. An internal jugular vein was punctured with a 1-cm-long 25-gauge needle, the blunt end of which was force fit into a flexible plastic capillary tube catheter 6–8 cm in length. The other end of the capillary tube was inserted tightly into the male luer end of a gas-tight Hamilton microsyringe (#1710).

Electronic signals representing Pao, airway flow, and airway volume were continuously displayed and recorded with a

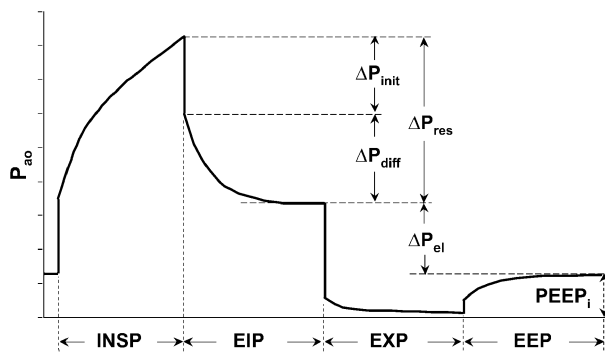


Fig. 2. Theoretical Pao trace during a single respiratory cycle exhibiting dynamic hyperinflation [indicated by the intrinsic auto-positive end-expiratory pressure (PEEP_i)]. INSP, inspiration; EXP, expiration; EIP, end-inspiratory pause; EEP, end-expiratory pause; ΔP_{init} , change in initial pressure; ΔP_{res} , pressure difference between peak inspiration and plateau at end of EIP; ΔP_{diff} , change in pressure difference; ΔP_{el} , difference between end-inspiratory and end-expiratory plateau pressures.

computerized data-acquisition system (Labview, National Instruments, Austin, TX) at a sampling rate of 250 samples/s. To ensure that the sampling rate was adequate for the experiments, 22 consecutive breaths obtained during steady-state ventilation were analyzed. If the sampling rate was too low, the magnitude of the peaks in the reconstructed pressure signal would exhibit considerable scatter. As the standard deviation of the peaks was only 1.2% of their mean value, this sampling rate was deemed adequate for reliably detecting subtle changes in peak pressure.

For each airway challenge, acetyl- β -methylcholine chloride (MCh) was dissolved in 0.9% saline and was administered via the 100- μ l microsyringe into the internal jugular vein. The capillary tube was clamped with a small arterial clip between injections to prevent blood from entering it. Each dose was administered in a volume of 1 μ l/g over 3 s, with extra volume added to compensate for the volume of the capillary tube (7 μ l). Geometrically increasing doses of MCh were given at 5-min intervals or each time the P_{ao} returned to within 10% of the baseline value.

Based on the results of the initial series of experiments during which dynamic hyperinflation was detected during MCh challenge, we performed a subsequent series of experiments in which we tried to minimize dynamic hyperinflation by increasing the duration of expiration. Five mice from the same two strains (age 40–50 wk) were used. During control conditions between challenges, the mice were ventilated with a tidal volume of 10 ml/kg at a rate of \sim 50 breaths/min, and an EEP was employed to detect dynamic hyperinflation. One minute before each MCh challenge, the respiratory rate was reduced to 20 breaths/min by increasing the expiratory time, leaving all other parameters constant. On several occasions, the expiratory time was suddenly returned to the control value just after the peak response to document the effect of this maneuver on the pressure waveform. In some cases, the expiratory time was alternately changed several times from control (short) to challenge (long) duration. Chest wall dis-

placement, estimating lung volume changes, was monitored in several experiments by using a flex sensor (28). This device, originally designed to provide positional feedback of fingers in virtual reality gloves, depends on bending-induced resistance changes for transduction. Although the signal output of this sensor is not linear over a wide range of tidal volumes, subtle changes in functional residual capacity are clearly observable.

Several experiments were also performed by using a standard Harvard rodent ventilator (model 683) and chest wall displacement monitoring for comparison. While we monitored chest wall displacement with the flex sensor and maintained tidal volume at 8 ml/kg, the ventilatory rate was first increased from 60 to 140 breaths/min to determine whether dynamic hyperinflation occurs when minute ventilation is increased and expiratory time is decreased during control conditions. MCh challenge was then performed with the Harvard ventilator set at a respiratory rate of 120 breaths/min and tidal volume of 8 ml/kg (approximating the usual settings in previous studies).

After each experiment, the mouse was killed by an overdose of barbiturate followed by cervical dislocation.

Data Analysis

Total respiratory system resistance was calculated by dividing the pressure difference between peak inspiration and the plateau at the end of the EIP by the inspiratory flow (Fig. 2). The resistance of the tracheostomy cannula, obtained previously at the same flow rates used during the measurements, was subtracted from each resistance value. Total respiratory system compliance was calculated by dividing the tidal volume by the difference between the end-inspiratory and end-expiratory plateau pressures (Fig. 2). Each pressure value used for the calculations was obtained by averaging three consecutive values. Dose-response data were plotted

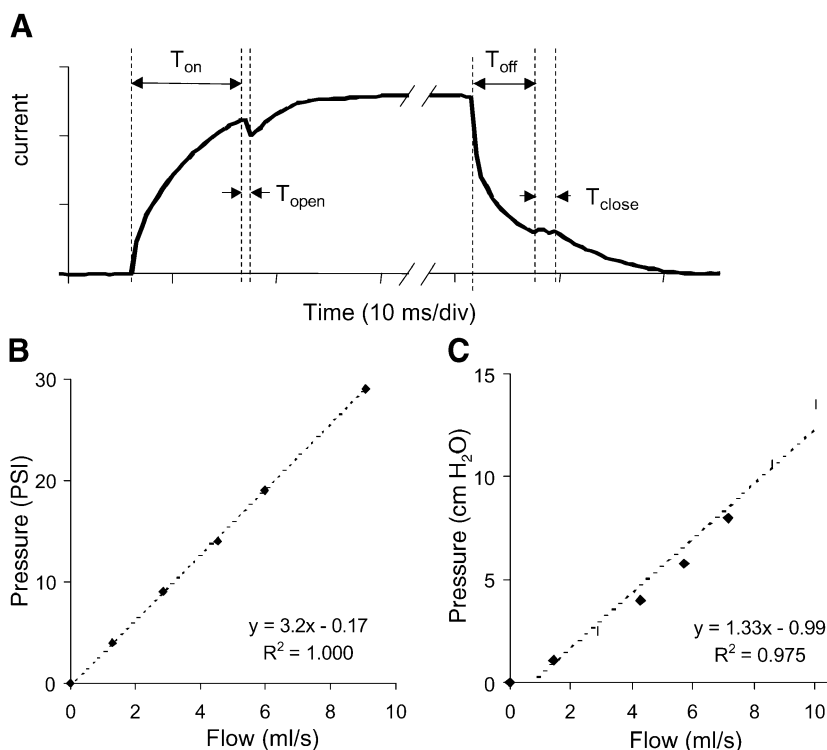


Fig. 3. A: typical current waveform used for the estimation of the time delay for initiating the opening (T_{on}) and closing (T_{off}) and the time required to fully open (T_{open}) or close (T_{close}) the solenoid valves. B: flow through capillary at various source pressures. C: pressure drop across expiratory circuit with different flows. psi, lb./in.²; div, division (distance between consecutive short markings on x-axis).

Table 1. *Effect of resistance changes on the delivered tidal volume*

Nominal Flow, ml/s	V _T Set, ml	Needle, gauge	Pressure, cmH ₂ O	Resistance, cmH ₂ O · ml ⁻¹ · s	V _T Delivered, ml
6.7	10	12	0.4	0.1	10.0
6.7	10	16	6.5	1.0	10.0
6.7	10	18	24.9	3.7	10.1
6.7	10	19	34.7	5.2	10.1
6.7	10	20	93.3	13.9	10.2
6.7	0.40	14	1.1	0.16	0.40
6.7	0.40	18	6.6	1.0	0.40
6.7	0.40	20	25.1	3.76	0.40
6.7	0.40	21	36.3	5.44	0.40
6.7	0.40	23	107	16.0	0.39

V_T, tidal volume.

with a logarithmic scale on the *x*-axis (MCh dose) and linear scale on the *y*-axis (response).

RESULTS

Bench Results

The pressure-flow characteristics of the capillary tube are shown in Fig. 3B. The results indicate that the resistance of the capillary tube (represented by the slope of the line) is constant for flows in the range of 2 to ~9 ml/s.

The delay in initiating the opening of the solenoid valves from the application of the actuating voltage was ~14 ms, and the delay in initiating closing after its cessation was ~7 ms (Fig. 3A). These delays were later equalized to ~7 ms by increasing the actuating current to the solenoids and by modifying the position of the occluding diaphragms. Further examination of the solenoid current waveform indicated that full opening or closing of the solenoid valves occurred within 2 ms. Although the pressure drop across the expiratory circuit was not strictly proportional to flow at the full range of flows used, it provided an approximate indication of expiratory flow (Fig. 3C).

As shown in Tables 1 and 2, the tidal volume delivered by the ventilator remained reasonably constant, despite resistance increases or compliance decreases beyond the physiological range (during MCh challenge).

In Vivo Results

In all cases, copious salivary discharge was observed at the mouth and nares after MCh administration. The fluid secretions, however, seemed to be restricted to the upper airways, as we never observed fluid emanating from the tracheostomy tube. Both blood pressure and heart rate (monitored on several occasions) decreased significantly during MCh challenge but usually returned to near baseline values within 5 min after lower doses of MCh. With the highest doses, some mice sustained irreversible drops in blood pressure. The characteristic shape of the pressure waveform during the EIP changed significantly after MCh challenge. In the prechallenge control waveform, there was an immediate pressure drop [change in initial pressure (ΔP_{init})] after cessation of inspiratory flow followed by a gradual

decline to a plateau [change in pressure difference (ΔP_{diff})]. During response to MCh challenge, ΔP_{init} gradually disappeared, resulting in a smooth decline that could be resolved into the sum of two exponentials.

The results of the experiments performed with the Harvard ventilator indicated no significant dynamic hyperinflation during control conditions even at a rate of 140 breaths/min. During airway challenge, however, dynamic hyperinflation clearly occurred at the traditionally utilized rate of 120 breaths/min (Fig. 4).

The dynamic hyperinflation and the reversibility of the relative responses of the resistive and elastic components of the respiratory system during airway challenge employing the two methods are shown in Fig. 5. The only parameter adjusted at the solid arrows was the expiratory time. The differences in response to MCh challenge between the two groups of mice using the two different methods are shown in Fig. 6. In the second series of experiments, in which a longer time was provided during expiration to allow the respiratory system to return to static equilibrium, the changes in compliance previously seen during MCh challenge greatly diminished or disappeared completely. The increase in peak expiratory flow during MCh challenge concurrent with dynamic hyperinflation (Fig. 6A) is absent without it (Fig. 6B). More importantly, the increase in airway resistance, the usual parameter monitored during airway challenge, separates the two groups of mice with much less uncertainty (smaller coefficient of variation) when dynamic hyperinflation is prevented.

A comparison of dose response to MCh challenge between our later results (no hyperinflation) and those

Table 2. *Effect of compliance changes on the delivered tidal volume*

V _T Set, ml	Plateau Pressure, cmH ₂ O	Compliance, ml/cmH ₂ O	V _T Delivered, ml
5	9.6	0.52	5
5	11.9	0.42	5
5	13.8	0.36	5
5	15.2	0.33	5
5	17.2	0.29	5
5	20	0.25	4.9
5	22.7	0.21	4.8
5	25.4	0.19	4.8

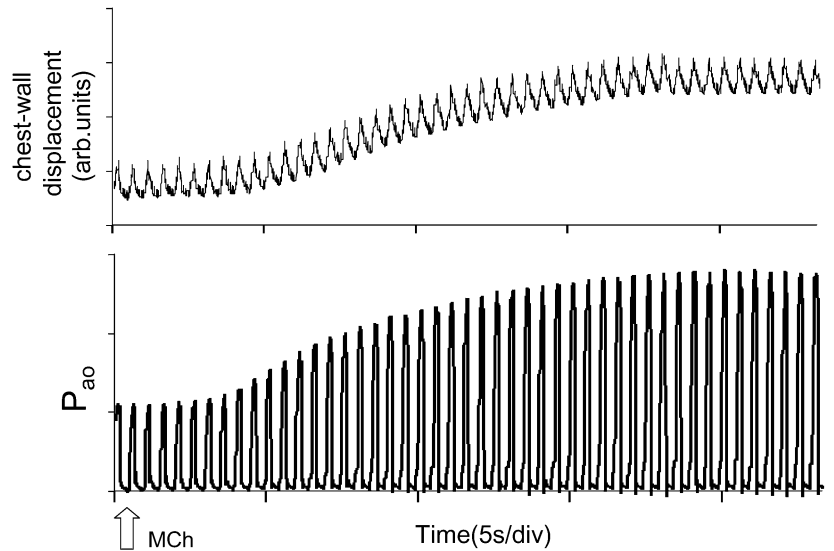


Fig. 4. Dynamic hyperinflation during methacholine (MCh) challenge of a high-responder mouse ventilated with a Harvard ventilator at a rate of 120 breaths/min.

of Martin et al. (20) (Fig. 7) indicates fairly good agreement between the two experimental methods as far as conductance changes are concerned. However, we observed no change in compliance, and it appears that our standard errors are much smaller.

DISCUSSION

The measurement of pulmonary function in small animal models is becoming increasingly important with the development of transgenic and knockout mice (10). In the present study, we present a new ventilator to make such measurements and data indicating the importance of auto-PEEP in the interpretation of bronchial hyperresponsiveness in mice. The temporary drop in blood pressure observed during the adminis-

tration of higher doses of agonist may be responsible for the biphasic shape of the dose-response curve. The decrease or even reversal of the effect of increasing MCh doses may be caused by the drop in peak concentration of the drug getting to the site of action because of a temporary decrease in arterial blood pressure.

Because ΔP_{init} became undetectable during airway challenge, we used the sum of ΔP_{init} and ΔP_{diff} for calculating total respiratory system resistance. Although this method yields highly reproducible results, it precludes the simple separation of total resistance into its components (central and peripheral). One disadvantage of our technique is that it requires the use of general anesthesia, an incision for the tracheostomy, and exposure of a vein, precluding the practicality of

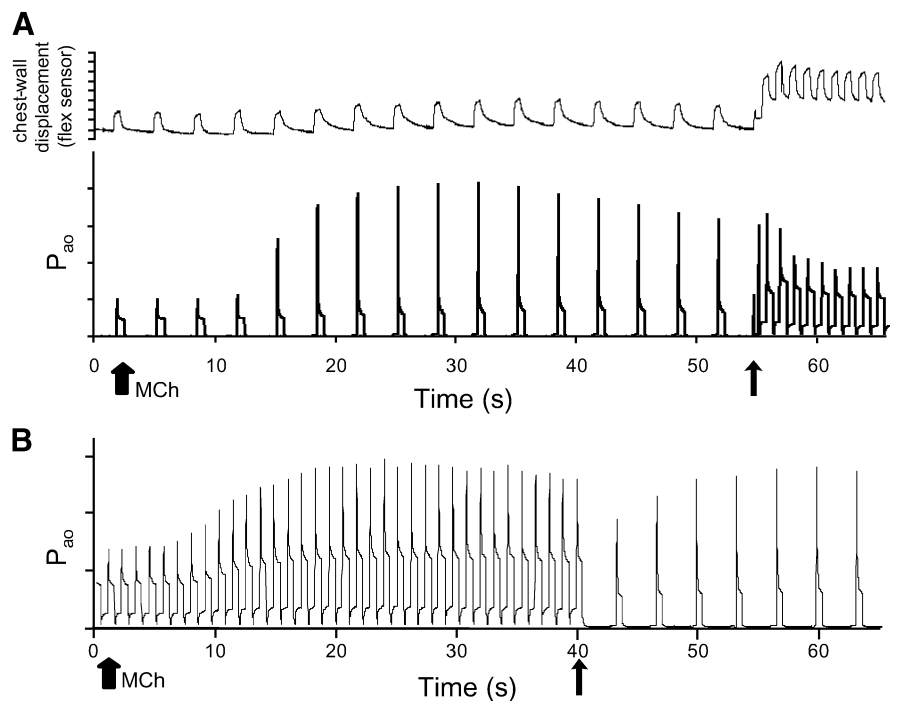


Fig. 5. A: development of dynamic hyperinflation and its effects during MCh challenge when expiratory time is suddenly shortened (thin arrow). B: elimination of the effects of dynamic hyperinflation during MCh challenge by the sudden lengthening of expiratory time (thin arrow).

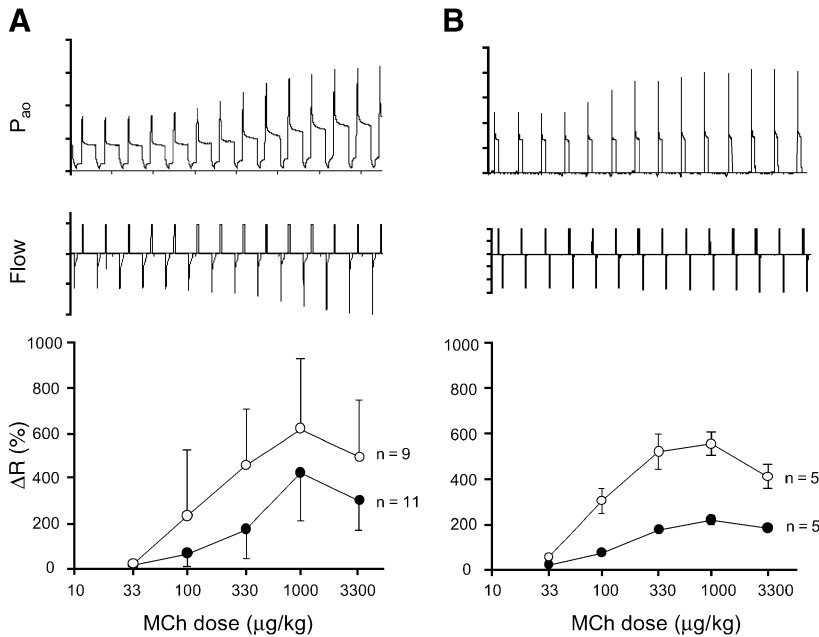


Fig. 6. Typical pressure and flow waveforms and combined results of resistance changes (ΔR ; means \pm SD) with short (A) or long (B) expiratory times during MCh challenge. \circ , High responders; \bullet , low responders. Note the increase in peak expiratory flow in A that is nearly absent in B.

longitudinal studies on the same animal. This problem may be overcome by intubation and the use of a tail vein for repeated studies (4).

Schuessler and Bates (23a) developed a ventilator that permits the application of a computer-controlled forcing function to the airways. By the introduction of small-amplitude oscillations to the airway and the analysis of the complex impedance of the respiratory system, reproducible estimates of airway resistance and dynamic compliance can be obtained. However, ventilation must be briefly and frequently interrupted if continuous documentation of the time course of changes is required.

Another method, the so-called Buxco box, developed by Buxco Electronics for monitoring airway hyperreac-

tivity in spontaneously breathing animals, also employs a whole body plethysmograph. It allows the monitoring of changes in a derived parameter, enhanced pause, which has been shown to correlate with airway resistance in small, awake animals (7). The physiological significance of the derived enhanced pause parameter, however, is somewhat obscure, because it does not appear to be directly related to normally understood respiratory parameters (10). In addition, this method requires the averaging of many breaths to yield stable results.

Ventilating mice with an inspiratory-to-expiratory ratio of 1:1 at a rate of 120–150 breaths/min, the usual protocol in many previous studies (5, 8, 14–17, 21, 29–31), provides only 250 or 200 ms, respectively, for expiration. Although this expiratory time is adequate for normal healthy mice to exhale completely during control conditions, it becomes progressively less adequate as airway resistance increases during MCh challenge, leading to dynamic hyperinflation as seen in Figs. 4 and 5. Thus challenges performed at higher doses of MCh may be performed at higher volume ranges, where compliance is decreased.

Based on the findings in this study, the decrease in compliance during airway challenge may be explained purely on the basis of concurrent volume changes. Our results are in agreement with those of Lauzon and Bates (17a), who found that, in some animals, the change in elastance in response to airway challenge was secondary to dynamic hyperinflation caused by increased airway resistance. Fig. 5 of their publication indicates the presence of hyperinflation similar to that observed in our study. Support for this conclusion can also be inferred from a study by Bates et al. (2). If, in Fig. 4 of their study, the observed compliance changes are extrapolated back to a frequency of zero (static

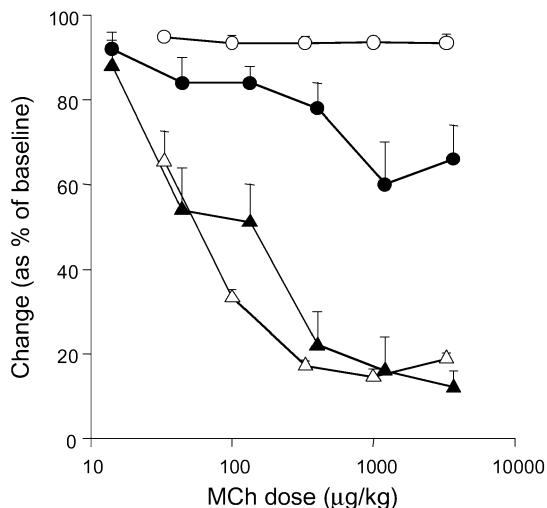


Fig. 7. Comparison (means \pm SE) between our results (open symbols, $n = 5$) and those of Martin et al. (20) (solid symbols, $n = 7$). Dose responses of compliance (circles) and conductance (triangles) changes in response to MCh challenge are shown.

compliance), it appears that there is no change in this parameter.

The fact that most studies fail to control lung volume during airway challenge makes comparisons among them problematic. Variations in lung volumes during challenge may also explain the wide variability of results and the relatively large variance. Clearly, to minimize hyperinflation, long expiratory times are required. To maintain adequate ventilation under these conditions, it is important to minimize inspiratory time. With the hyperresponsive group of mice, the expiratory time was too short to allow complete expiration during higher doses of MCh, even at a respiratory rate as low as 20 breaths/min, and the resulting rise in lung volume caused a small decrease in compliance.

In summary, we have presented technical and functional details of a new ventilator that allows evaluation of respiratory parameters during ventilation by combining constant-flow inspiration with airway occlusion both at end inspiration and end expiration. This ventilator allowed us to demonstrate that the apparent decrease in compliance observed in previous studies (7, 12–16, 19, 24, 26, 27) may have been caused by dynamic hyperinflation and not by alterations in lung tissue properties. Correction of this problem in our study reduced the intrastrain variability of responses and thus made differentiation between strains easier. This methodology should allow more precise detection of differences in airway responsiveness in small animals.

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