A servo-controlled respiration system for inhalation studies in anesthetized animals

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Rosenthal, Frank S., and Changhong Li. A servo-controlled respiration system for inhalation studies in anesthetized animals. J. Appl. Physiol. 83(5): 1768–1774, 1997.—To facilitate aerosol deposition experiments and aerosol exposures in anesthetized animals, a servo-controlled respiration system was developed and tested. The system induces ventilation by varying extrathoracic pressure in a whole body respirator in which an intubated animal is placed. The pressure inside the whole body respirator is varied with a three-way servo-controlled spool valve connected to sources of positive and negative pressure. A computer-based system detects respiratory flow and computes the controlling signal for the valve by using a proportional-integral-derivative algorithm, to achieve desired patterns of flow and volume vs. time. The system was used with dogs and found to accurately induce various single-breath breathing patterns involving constant-flow inspirations and expirations as well as breath-hold periods. A similar system was used to induced repeated breaths with desired parameters for continuous exposure to particles and for ventilation of animals between experiments.

respiration; ventilator; dog; inhalation toxicology; aerosol deposition; negative pressure ventilation

SYSTEMS FOR CONTROLLING respiration by varying extrathoracic pressure have been used for several decades. In particular, negative pressure ventilation has been used to treat and manage diseases in which there is paralysis of respiratory muscles or to provide inspiratory muscle rest (1, 5, 6).

In previous studies of aerosol behavior in the lungs of anesthetized animals, we have used a whole body respirator (“box”) in which transpulmonary pressure is varied by changing extrathoracic pressure with respect to airway pressure (4). This system provided the advantages of being able to provide the aerosol at atmospheric pressure and being able to induce a wide variety of breathing patterns. In the original system, the pressure in the respirator was varied by using a 7-liter manually driven syringe to either withdraw air from or supply air to the body box. Although this system functioned effectively, it had the disadvantages of requiring continuous manual operation, requiring considerable operator training, and resulting in high variability in both instantaneous and average flows. This variability, in turn, probably contributed to variability in experimental results for aerosol deposition and dispersion. The system was further limited by the maximum respiratory volumes, flows, and pressures that could be induced, dependent on the size of the manual syringe and the strength of the operator.

To surmount these problems, a servo-controlled ventilation system to vary chamber pressure and thereby induce respiratory flow was designed and constructed. The system was built with readily available commercial materials at minimal cost and interfaced to a computer control and data-acquisition system. The design and optimization of the system was done in accordance with standard principles and algorithms from elementary control theory. The system was used and tested in single-breath aerosol deposition and dispersion experiments in dogs in which submicron particles were used, and in carrying out exposures of dogs to fluorescent particles for periods up to 2 h.

SYSTEM DESIGN

The system was designed to meet the following criteria:

1) The system should be able to induce arbitrary breathing patterns, specified by the inhaled flow rate vs. time, the exhaled flow rate vs. time, the breath-hold period between inspiration and expiration, and the inhaled and exhaled volumes.

2) The system should be able to induce an approximate square-wave breathing pattern, i.e., constant-flow inspiration and constant-flow expiration, with flow rates between 0.25 and 1.0 l/s.

3) Breathing patterns and flow rates produced by the device in replicated breaths should be highly reproducible.

4) The system should be able to take into account differences between individual animals in the mechanical properties of the respiratory system.

5) The system should be integrated with a data-acquisition system for determining aerosol deposition and dispersion.

6) The system should have a continuous mode in which a specified breathing pattern is repeated, with an arbitrary number of “resting” tidal breaths before the specified breaths. The volume and flow rate of the tidal breaths should also be arbitrarily specified. All parameters should be able to be reset during the experiments.

Servo-controlled respiration system. The ventilation system is shown in Fig. 1. The animal is intubated and placed inside the box. The proximal end of the endotracheal tube is connected to the supply of air, outside of the box, through a connector in the wall of the box. Respiration is induced by varying the pressure in the box. The pressure in the box can be set to room pressure by opening valve 2. The pressure in the box is adjusted by operation of a three-way servo spool
Valve (model 10–1100, Dynamic Valves, Palo Alto, CA). Positive current flowing through the valve coil opens the valve to suction from a "house" vacuum line operating at −10 lb./in.$^2$ (psi), whereas negative current opens the valve to positive pressure from a house compressor system regulated at 15 psi. Both house systems had considerable buffer volumes, as indicated by the fact that source pressures were almost constant during operation of the system. The servo valve requires no minimum operating pressure and opens to maximum flow at either port at 15 psi. Both house systems had considerable buffer volumes. For protection of the valve orifice, positive pressure is fed through a high-efficiency particulate air filter.

Respiratory flow is measured by a Fleisch pneumotachometer connected to a Celesco pressure transducer (0–2 cmH$_2$O) and associated electronics, which provides a signal in the range of ±2.5 V. The flow signal is digitized at 50 Hz, stored in the computer, and used to compute the valve drive signal that controls the position of the servo valve.

The computer stores the command signal, the flow signal, and the servo valve signal for off-line display and analysis of the data and monitoring of the performance of the system.

Two Magnehelic pressure gauges display the positive or negative pressure inside the chamber during the experiments. Transpulmonary pressure is monitored with an esophageal balloon catheter connected to a Celesco pressure transducer, which is connected to an analog direct current voltmeter. A pressure switch connected to a solenoid valve opens the box to room pressure in case a system excursion causes the pressure in the box to exceed safe limits.

Computation of the servo-valve signal. An arbitrary command function $c(t)$, specifies the desired function of flow rate vs. time. This together with actual flow being sensed by the pneumotachometer $s(t)$ is used to compute, in real time, the valve drive signal $v(t)$, which determines the position of the spool in the servo valve. The computation of $v(t)$ follows standard control theory for proportional-integral-derivative (PID) systems. We have

$$e(t) = c(t) - s(t)$$

$$v(t) = K_P e(t) + K_I \int e(t) dt + K_D \frac{de}{dt}$$

where $K_P$, $K_I$, and $K_D$ are constants that are optimized for a given experiment.

In our system, $v(t)$ is computed digitally in a computer program. The integral and derivative in Eq. 2 are approximated by summations and finite differences to provide a value of $v$ at each time point for which $c$, $s$, and $e$ are specified. Positive values of $v$ open the servo valve to positive pressure, and negative values of $v$ open the servo valve to suction. The value of $v$ is inputted to a digital-to-analog converter, which is connected to a current driver that controls the servo valve.

For convenience, the command function and the sensor and error values were expressed in the equivalent volts produced by the pressure transducer connected to the pneumotachometer. The characteristics of the transducers in our system are such that 1 V is equivalent to −0.63 l/s.

Valve performance. The electromechanical performance of the servo valve was tested by providing negative and positive sources of air pressure and by observing the flow through the valve as a function of input current (Fig. 2). It was verified that the valve provided a transition from positive to negative flow at approximately zero current and otherwise performed according to the manufacturer’s specifications.

Determination of the PID control parameters. At the beginning of experiments with a given animal, a “tuning” procedure was used to find values of $K_P$, $K_I$, and $K_D$, to use in the control algorithm. These parameters were obtained by the Ziegler-Nichols method (2). Briefly, this method consists of setting $K_I$ and $K_D$ to zero and increasing $K_P$ until a value of $K_{max}$, where the system just reaches sustained oscillations. The period of oscillation (T) is measured from the computer display of flow vs. time. Values are then set as follows: $K_P = 0.6K_{max}$, $K_I = 2(K_P)/T$, and $K_D = (1/8)(K_P)(T)$. It was found
that system performance was considerably improved by determining separate control parameters for inhalation and exhalation and for each flow rate. These valves were varied in the algorithm computing the valve drive signal, depending on the phase of inspiration and flow rate. In some experiments, a breath-hold period was inserted between inspiration and expiration. During these periods, valves were set appropriately to ensure zero flow, and the PID system was turned off.

Application 1: Single-breath aerosol deposition and dispersion experiments

The servo system was used in conjunction with a single-breath aerosol bolus deposition and dispersion measurement system that has been previously described (3, 4). Briefly, the airway is connected outside the box to a three-way valve that can alternate between room air and a reservoir of monodisperse aerosol, consisting of particles with a mean aerodynamic diameter of 0.7 µm and a geometric SD <1.2, generated by a MAGE (Lavoro Y Ambiente, Bologna, Italy) condensation aerosol generator. The system utilizes a light-scattering instrument for measuring particle concentration and a pneumotachometer and associated pressure transducer for measuring respired flow (see Fig. 1). Both aerosol concentration and flow signals are digitized at 50 Hz and stored in the computer. Valves controlled by the computer determine the interval of the inhaled volume during which aerosol is introduced. For use with the PID system, the original data-acquisition and control program, written in Pascal and implemented on an IBM-compatible computer, was modified as follows: in the programming loop during which data are acquired, computer code was added to compute the valve output function and to output a signal proportional to this value to the amplifier controlling the valve position. Additional code was added to input and record the PID parameters as well as the valve output function for each run.

Before the measurements, animals were premedicated with acepromazine (1–4 mg) and glycopyrollate (5 µg/lb.) and anesthetized with pentobarbital sodium (25 mg/kg). Spontaneous respiration was suppressed by hyperventilating the animals so that end-tidal CO₂ was ~4%. Transpulmonary pressure was measured with an esophageal catheter placed 10 cm proximal to the gastroesophageal junction. Quasi-static pressure-volume curves were measured to determine lung volumes. Total lung capacity (TLC) was defined as lung volume at a transpulmonary pressure of ~25 cmH₂O.

Several breathing patterns were used in the single-breath aerosol deposition experiments. In some experiments, inspiration was from functional residual capacity (FRC) to TLC (determined from quasi-static pressure-volume curves), and exhalation was from TLC to FRC. Other experiments used a similar breathing pattern but with a pause between inspiration and expiration. In other experiments, inhalation was from a specified volume below TLC to TLC, and exhalation was from TLC to residual volume. Residual volume was detected in the computer program by an increase in the error between command and sensor flows.

Fig. 2. Flow performance of servo valve. Negative pressure source = −10 lb/in² (psi); positive pressure source = +10 psi.

Fig. 3. Flow and volume waveforms for a single-breath aerosol deposition experiment. Inspired flow rate = expired flow rate = 0.5 l/s. Inspired volume = 2.3 liters.
All measurements were carried out with square-wave flow functions, i.e., constant inspiratory flow and constant expiratory flow. These flow rates were usually either 0.25 or 0.5 l/s. In a few experiments, flows of 0.75 l/s and above were induced.

In the experiments in which inspiration began at a specified volume below TLC, the initial volume was set manually by varying the box pressure with a 7-liter syringe while the computer monitored exhaled volume. At the beginning of data acquisition, the valve connecting the syringe to the box was closed, and the experiment proceeded under servo control. During breath holds between inspiration and expiration, the servo valve was set to its null position (closed to both positive and negative pressure), and valve 4 was closed. Because the servo valve in its null position was not completely leak tight, an additional solenoid valve (not shown) between the servo valve and the box was closed during the breath-hold period.

Application 2: Exposure of animals to aerosols. The servo-controlled respiration system was used to expose animals to aerosols of fluorescent particles in preparation for an investigation of the microscopic distribution of deposited particles in lungs excised from the animals after the exposure. The particles were produced by nebulization of a water suspension of polystyrene particles with a mean size of 0.75 µm and an SD of 0.01 µm. The animal preparation was identical to that in application 1 described above. For the exposures, a breathing pattern was specified as described above, with the inspiration starting at FRC and a 5-s breath hold between inspiration and expiration. Inhaled volume was between 1.0 and 1.6 liters. Aerosol was introduced during 0.8 liter of the inspired breath. Inhaled and exhaled flow rates were set at 0.5 l/s. This breathing pattern was followed by a series of tidal "resting" breaths during which no aerosol was introduced. The breathing pattern was then repeated, followed by another series of resting breaths, and so on. The procedure continued for a period of up to 2 h, after which the animal was euthanized, the lungs were excised, and tissue sections were prepared for further analysis. For the resting breaths, inspiration was done at constant flow, whereas expiration was done passively by opening the box to room pressure via valve 3 (Fig. 1). This passive exhalation also reset the lungs to FRC and prevented any systematic buildup or decrease of pressure in the box with the repeated breaths. Breaths were switched from aerosol to room air by using valve 2 (Fig. 1). The parameters of the exposure breaths as well as the number of resting breaths, the tidal volume of the resting breaths, and their flow rate were set at the computer keyboard by the system operator and could be changed during the exposure period. In general, the parameters of the resting breaths were adjusted to maintain end-tidal CO2 in the desired range close to that in application 1 described above.

Table 1. Values used to determine control parameters in 4 dogs

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Inhalation</th>
<th>Exhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kmax, V/V*</td>
<td>T, s</td>
</tr>
<tr>
<td>95-4</td>
<td>12</td>
<td>0.13</td>
</tr>
<tr>
<td>95-6</td>
<td>18</td>
<td>0.14</td>
</tr>
<tr>
<td>96-1</td>
<td>15</td>
<td>0.12</td>
</tr>
<tr>
<td>96-2</td>
<td>17</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*1 V = 0.63 l/s.

Table 2. Flow rate in typical experiments

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Command Flow, l/s</th>
<th>Mean Inspired Flow, l/s</th>
<th>Mean Expired Flow, l/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>95-1g</td>
<td>0.250</td>
<td>0.236 ± 0.000</td>
<td>0.247 ± 0.000</td>
</tr>
<tr>
<td>95-1c</td>
<td>0.500</td>
<td>0.476 ± 0.001</td>
<td>0.489 ± 0.001</td>
</tr>
<tr>
<td>95-6k</td>
<td>0.500</td>
<td>0.485 ± 0.002</td>
<td>0.490 ± 0.000</td>
</tr>
</tbody>
</table>

Values are means ± SD.

Fig. 4. Flow and volume waveforms for a single-breath aerosol deposition experiment. Inspired flow rate = expired flow rate = 0.5 l/s. Inspired volume = 2.3 liters. Breath-hold time = 5 s.
to 4%. The fluorescent aerosol passed through the aerosol photometer, and with the use of the aerosol data-acquisition system the inhaled aerosol amount, the exhaled aerosol amount, and the single-breath deposition fraction were computed on a breath-by-breath basis while the exposure was in progress. This information enabled the operator to immediately detect any failure in the exposure system. The system also functioned as a whole body respirator before and after the exposure period.

RESULTS

Four dogs were studied with single-breath studies of aerosol deposition and dispersion by using the servo
system and were subsequently exposed to fluorescent particles also by using the servo system. Table 1 shows the values of $K_{\text{max}}$ and $T$ determined by the tuning procedure in the four dogs. These parameters were determined with a square-wave command signal, utilizing a flow rate of 250 m/s. Similar results were found in experiments done at 500 m/s. Table 2 shows typical values of the mean flows achieved by the system.

Figures 3–7 show the respiratory flows and volumes observed in several experiments.

In many of the waveforms, particularly at the lower flow rate of 250 m/s, a small oscillation with frequency on the order of 100 cycles/min is apparent (Fig. 7A). It was assumed that this was due to flow oscillations caused by the heartbeat, since this frequency was approximately equal to the heart rate, which was also ~100 beats/min in these experiments. To investigate this phenomenon, several experiments were done, in which the servo-respiration system controlled respiration in an isolated lung. The resulting flow waveform showed less oscillation (Fig. 7B) in both inspiratory and expiratory phases, and the oscillations that were present were at a higher frequency than the heart rate. This indicated that a major cause of the flow irregularity in the in vivo experiments was the heartbeat. Both in vivo and isolated-lung studies showed some flow transients in the inspiratory phase associated with opening of the bolus valve (Fig. 7, A and B). These transients were especially pronounced in the isolated-lung studies (Fig. 7B), probably because of the absence of the damping effect of the chest wall recoil on the system. In experiments in which the bolus valve was not actuated, these transients were not seen (Fig. 7C).

**DISCUSSION**

The servo-controlled ventilation system was able to accurately induce the breathing patterns required by the experiments and otherwise satisfied all of the design criteria specified above. The mean flows achieved by the system were highly reproducible in most cases to within better than 1%. For the square-wave flow functions, the mean flow tended to be slightly less than the command flow because of the rounding of the edges of the square wave. This rounding was due to the limitation of the maximum flows that could be supplied to the chamber to change chamber pressure. A given mean flow could easily be achieved by making the command signal slightly greater to correct for the effect of this rounding. The performance of the system was slightly worse at 500 m/s compared with 250 m/s, reflecting the fact that the system was limited by the maximum flows possible, given the source pressures and orifice of the servo valve. Preliminary experiments utilizing two similar servo valves in parallel indicated that flow performance was improved (e.g., less rounding of the

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**Fig. 7.** Comparison of flow waveforms in in vivo and in vitro experiments. A: flow waveform in an intact dog, for a command flow of 250 m/s, inhaled volume of 1.5 liters, bolus inserted between 0.6 and 0.8 liter. B: flow waveform in an isolated lung for a command flow of 250 m/s, inhaled volume of 1.45 liters, bolus inserted between 0.65 and 0.85 liter. C: flow waveform in an isolated lung for a command flow of 250 m/s, inhaled volume of 1.45 liters, with no bolus in inhalation.
edges of the square-wave flow), and flows up to and above 0.75 l/s could be induced.

Experiments with isolated lungs indicated that the deviations from constant flow observed in respiration with intact animals were mostly due to flow or pressure transients caused by the heartbeat. Additional perturbations of constant flow were attributed to pressure or flow changes associated with the opening and closing of the bolus valve. Because the flow was measured via a pressure transducer, it is difficult to know whether the transients observed were true transients in flow rate or whether they were due to artifacts produced by the pressure transducer.

The use of digital real-time computation of the valve drive signal, rather than the use of analog circuits, greatly simplified the design, implementation, and troubleshooting of the system. In particular, it facilitated the use of different PID control parameters for different inhalation vs. exhalation, or turning off of the PID system entirely during a breath-hold period. Although, in principle, these features could be included as part of an analog system, their implementation would likely be considerably more complex and time consuming.

Although we performed no detailed study of the effect of digitizing rate on system performance, the rate commonly used for data acquisition (50 Hz) was found adequate for control purposes. This also simplified the programming of the system design in that data-acquisition and control procedures could be incorporated in a single programming loop.

As an exposure system, the system provided a wider range of breathing patterns than is usually available. In particular, we were able to induce a combination of breath-hold maneuvers and tidal breaths. Real-time measurement of aerosol deposition, inspired and expired volumes, flow rates, and flow and volume waveforms was also useful in monitoring the experiments and in estimating total exposure during the procedure.

Although our use of this system has been limited to aerosol experiments, it is likely that it could be applied to various physiological measurements. Arbitrary flow and volume patterns (e.g., sinusoidal oscillatory flow) could be induced over a range of frequencies. Other parameters such as the esophageal and airway pressure could easily be captured by the system for both data-acquisition and control purposes. Additional uses of this system may be considered for the clinical management of humans and animals undergoing artificial ventilation.

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