ESTIMATION OF GUINEA PIG SPECIFIC AIRWAY RESISTANCE FOLLOWING EXPOSURE TO COTTON DUST MEASURED WITH A WHOLE BODY FLOW PLETHYSMOGRAPH

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Abstract

This study analyzes a method to estimate the specific airway resistance of guinea pigs exposed to cotton dust using a whole body flow plethysmograph. It is based on a theoretical model, is simple to apply to small unrestrained laboratory animals, and appears to reflect changes in specific airway resistance due to cotton dust exposure more accurately than other indices measured with a whole body plethysmograph.

Introduction

In the past, the whole body plethysmograph has been used to estimate the breathing pattern of unrestrained laboratory animals following exposure to toxic aerosols such as cotton dust. One of the first attempts to quantify whole body measurements was made by Drorbough and Fenn (1955). They described the theoretical considerations that are required to relate tidal volume to the pressure fluctuations in a closed plethysmograph caused by the warming-humidification and the cooling-condensation of air entering and leaving the lungs during tidal breathing. Later, Epstein and Epstein (1978) corrected the implicit assumptions of Drorbaugh and Fenn (1995) that the heating-humidification and cooling-condensation events that occur during breathing equally affect chamber pressure. They pointed out that the situation is more complex since during inflation the gas within the lungs quickly reaches body temperature, but during expiration the phasic cooling of expired air is due only to nasal conditions. Further cooling and condensation of expired gas occurs over a time period much longer than the respiratory cycle within the plethysmograph. Jacky (1980) proposed corrections to the Drorbaugh and Fenn relationships to account for the theoretical considerations of Epstein and Epstein (1987). In more recent years, the whole body plethysmograph has been modified to measure flows into and out of the chamber rather than pressure under constant volume conditions. This method has several advantages since the measurements are not nearly as influenced by thermal drift.

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It should be noted that tidal volume measurements computed solely from measurements of heating and cooling of inspired-expired gas can be significantly in error when gas compression occurs in the thorax as a result of a significant increase in specific airway resistance. Under these conditions a phase difference between thoracic flow and the flow of cooled nasal air increases contributing to an increase in plethysmograph flow.

Due to the complexities involved in the analysis of plethysmograph flow, many investigators have tried to use a form of pattern recognition to correlate changes in airflow measurements made in a flow plethysmograph with changes in airway resistance made with a second, more specific, technique. Some examples of flow parameters that have previously been measured include: breathing frequency, inspiratory time/expiratory time, relaxation time (which has been defined as the time between the beginning of expiration and the time at which 70% of the total volume has entered the plethysmograph during expiration, maximum expiratory flow, time of peak flow during deflation time/ total deflation time, and Penh an index defined by BUXCO Inc.(Schwarze *et al*, 1996). In most cases these parameters have had limited success since they appear to depend heavily on the type and history of exposure experienced by the animal.

The objective of this study was to develop a flow measurement that reflected changes in specific airway resistance in a more general way so that the guinea pig response to cotton dust can be better understood.

Methods

Theoretical Considerations

An example of a whole body flow plethysmograph is illustrated in fig. 1. As the animal breathes, air enters and leaves the plethysmograph through a screen separating the inside and outside of the chamber. The pressure difference across the screen is linearly proportional to airflow. As the animal exhales, the thorax decreases in volume, gas within the chest is compressed, and gas leaving the alveoli through the airways and nose cools while liquid is removed from the gas by condensation. Both these processes tend to reduce gas volume within the thorax and plethysmograph and results in gas flowing into the plethysmograph through the screen. In contrast, when the animal inhales, the thorax increases in volume, gas within the lungs expands, and the gas entering the lungs is warmed and humidified in the nasal passages and airways. During this phase of the respiratory cycle the volume of gas within the lungs and plethysmograph chamber increases and gas flows from the plethysmograph through the screen.

A theoretical model of a guinea pig breathing within a plethysmograph is shown in fig. 2. As the thorax decreases in volume, warmed gas enters the plethysmograph through the airways. The flow of warm moist air through the

airways and nose (V'_N) can be written in the form of a differential equation as:

$$d(V'_N)/dt + V'_N/R_aC_g = V'_T/R_aC_g \quad (1)$$

where V'_T represents the rate of change of volume of the thorax or thoracic flow and R_aC_g represents specific airway resistance. In this expression R_a represents airway resistance and C_g, represents the compliance of the gas in the thorax. C_g can be defined in terms of the average volume of gas in the thorax (V_{TG}), the average absolute pressure within the alveoli (P_B), and the partial pressure of water vapor at alveolar temperature (PH_2O_A) as:

$$C_g = V_{TG} / (P_B - PH_2O_A). \quad (2)$$

A portion of the flow resulting from reducing the volume of the thorax results in gas compression within the lungs. This flow component (V'_{comp}) equals the difference between thorax flow and nasal flow as shown in eq. 3.

$$V'_{comp} = V'_T - V'_N \quad (3)$$

Once the gas leaves the alveolar region of the lungs it cools in the upper airways, nasal cavity, and plethysmograph chamber. In the past, while analyzing airway resistance measurements in humans, Peslin *et al.* (1995) has argued that the cooling component of flow could be approximated by a simple first order differential equation shown in eq. 4.

$$d(V'_C)/dt + V'_C/\theta = G\,V'_N \quad (4)$$

where θ represents the time constant of the cooling process and G represents a gain factor that depends upon the absolute temperature in the nasal cavity (T_N) and the alveoli (T_A), along with the partial pressure of water vapor within the nasal cavity (P_B-PH_2O_N) and the alveolar gas (P_B-PH_2O_A):

$$G = (1 - [T_P/T_A][(P_B - PH_2O_A)/(P_B - PH_2O_N)) \quad (5)$$

On inflation, the temperature (T_P) and partial pressure of water vapor within the plethysmograph (P_B-PH_2O_P) are used in place of the nasal conditions. The flow of gas leaving the lungs corrected to include the effects of cooling (V'_{NC}) is equal to the difference between warm moist nasal air flow and the component of flow due to cooling as described in eq. 6

$$V'_{NC} = V'_N - V'_C. \quad (6)$$

Now, the flow of gas entering the plethysmograph during lung deflation, which is the flow normally measured with the flow plethysmograph, can be written in terms of thoracic flow and the flow of cooled gas from the lung as:

$$V'_{pl} = V'_T - V'_{NC}. \quad (7)$$

A similar analysis of the events occurring in the plethysmograph can be formulated during lung inflation.

Experimental Methods

Laser analysis

An experiment was performed to illustrate that air enters the flow plethysmograph as an animal exhales and leaves the plethysmograph as the animal inhales. A guinea pig was placed in a flow plethysmograph as shown in fig. 3, and pressure fluctuations proportional to flow entering and leaving the plethysmograph were recorded. A laser target was attached to the thorax of the guinea pig and by measuring the distance between a thorax and laser located outside the plethysmograph it was possible to measure the displacement of the chest wall during inspiration and expiration. Chest wall movements were compared with flow measurements to determine plethysmograph flow direction during different phases of the respiratory cycle.

Model Analysis

The system of equations described in the Theoretical Analysis were solved employing a digital simulation of an analog computer written in terms of a MATLAB program. The values of the equation coefficients are given in Table 1. A diagram of the analog simulation of the system of differential equations is given in fig. 4. This system of equations was used to examine various flow components to determine how the flow components were altered by changes in airway resistance.

Inhalation Studies

A group of guinea pigs (N=4) was exposed to cotton dust (30 mg/M^3) for 4 hrs. The exposure system and the characterization of the aerosol have been described previously (Frazer *et al.*,1987). The animals' breathing pattern was measured with a whole body flow plethysmograph after they had breathed 10% CO_2 in air for 3 min. The plethysmograph chamber was constructed of acrylic tube enclosed at each end with an acrylic plate. One end of the chamber had a circular port, 0.8 cm^2 in area, that was covered with a 400 mesh stainless steel screen. Pressure variations across the screen, generated by the breathing animal, were measured with a pressure transducer (Setra, model#239). These breathing patterns were recorded pre-exposure and at hourly intervals up to 18 hrs post exposure on a digital signal analyzer (B&K, model# 2033) and transferred to a digital computer for analysis. The temperature and humidity of the chamber were also determined.

Results and Discussion

Laser Analysis

Results of the laser displacement study are presented in fig. 5. The (——) line represents the plethysmograph flow measurement where positive values represent the magnitude of flow leaving the plethysmograph and negative values represent flows entering the plethysmograph. By definition, the integral of the flow signal, the (·····) line, represents the volume of gas that has entered or left the

plethysmograph. Positive volume values represent the volume of gas leaving the plethysmograph while negative values represent the volume entering the plethysmograph. The (-- -- -- --) line is the laser displacement signal. A positive signal indicates a displacement toward the laser which occurs during lung inflation while a negative signal indicates a displacement away from the laser that occurs during lung deflation. These results illustrate that during lung inflation when the laser signal is positive the volume signal is also positive proving that gas leaves the plethysmograph during lung inflation. In other words, as the guinea pig inhales, gas leaves the plethysmograph, and when the guinea pig exhales, gas enters the plethysmograph.

Model Analysis

Figure 6 shows calculated components of plethysmograph flow during a simulated breathing cycle. Positive components of flow represent flow in a direction that would leave the plethysmograph, and negative components of flow represent flows in a direction that would enter the plethysmograph. Considering the flow components during lung exhalation, the (·—·—·) line represents thoracic flow or the rate of change of the thorax volume while the (——) line represents the flow of warm moist air from the alveoli. The difference between these two curves, (eq. 3), represents the flow resulting from the compression of gas within the thorax and is indicated by the (· · · · ·) line. The nasal airflow after cooling has taken place is given by the (— —) line. The difference between thoracic flow and nasal airflow taking into account the cooling effect is equal to the flow of air into and out of the plethysmograph; the (- - - -) line represents the parameter normally measured with a whole body flow plethysmograph.

Figure 7 illustrates the effects of changes in specific airway resistance on flow measurements recorded with a flow plethysmograph. The top and bottom panel show results of the model under identical conditions with the exception that specific airway resistance was 0.010 in panel A and 0.015 in panel B. It can be seen that peak plethysmograph flow, indicated by the (— — —) line, is greater in magnitude in panel B than in panel A, primarily due to an increase in flow resulting from gas compression in the thorax. The RMS (root mean squared) value of the periodic plethysmograph flow was calculated to compare flow magnitudes using the following relationship:

$$RMS = \sqrt{[ave(V'_{pl}{}^2)]}. \qquad (8)$$

The RMS value of a periodically varying flow is that value which is equivalent from an energy viewpoint to a steady flow of the same magnitude. The RMS flow was 0.14 in panel A and 0.21 in panel B and is illustrated by the horizontal (· - · - · -) lines in fig. 8A and 8B. This simple example illustrates that the RMS value of plethysmograph flow reflects differences in specific airway resistance. The next portion of the study was to examine the RMS values of plethysmograph flow that was measured for guinea pigs exposed to cotton dust.

Inhalation Studies

The breathing rate of guinea pigs inspiring 10% CO_2 in air, following a 3 min equilibration period is shown prior to exposure and at 1 hr internals up to 18 hrs post exposure in fig 8A. It can be seen that the breathing rate increases immediately post exposure, reaches a maximum at approximately 12 hrs post exposure, then tends to decrease slowly. These breathing rate values are consistent with similar results that have been reported in the past (Ellakkani *et al*. (1985), Castranova *et al*., (1987))

Figure 8B shows values of RMS flow calculated for the same flow patterns used to determine respiratory rate. It can be seen that the RMS flow increases immediately following cotton dust exposure, decreases approximately 3 hrs post exposure, remains low until 16 hrs post exposure, and then increases slightly once more.

Table 2 shows several additional indexes computed for the breathing patterns of guinea pigs exposed to cotton dust in this study. These indexes are commonly used as an index of airway obstruction computed from whole body plethysmograph flow measurements.

We have attempted, both theoretically and experimentally, to relate RMS flow into and out of a plethysmograph to the specific airway resistance of an unrestrained guinea pig breathing 10% CO_2 in a plethysmograph. It should be noted that similar results have been reported for the time course of airway resistance using gas trapping measurements (Frazer *et al*.,1989) and more complicated two chamber plethysmograph techniques (Johnson *et al*., 1987, Hara *et al*. (1988)). It is also important to recognize that other previously reported techniques used to quantify airway obstruction with a whole body flow plethysmograph may not be well suited for evaluating airway obstruction in animals exposed to cotton dust.

Summary

In summary, both theoretical and experimental results indicate that the RMS value of plethysmograph flow, normalized with respect to thoracic flow, has the potential to be a useful estimate of specific airway resistance in small laboratory animals.

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Table 1. Values of equation coefficients used in the simulation of flow components shown in fig. 6.

CONSTANT	VALUE	REFERENCE
θ	0.087 sec	Peslin *et. al.*, (1995)
R_aC_g	0.010 sec	Agrawal (1981)
G	0.09	Hankinson *et. al.*, (1983)

Table 2. Analysis of different indexes used to measure specific airway resistance in guinea pigs exposed to cotton dust.

Time (hrs.)	A	B	C	D	E	F	G
-2	1.70	139.2	0.69	0.13	2.86	0.17	1.14
-1	2.01	153.8	0.69	0.13	3.59	0.16	1.15
0	1.78	241.7	0.77	0.08	2.66	0.27	0.70
1	2.09	227.1	0.74	0.08	3.33	0.26	0.78
2	2.17	219.7	0.75	0.09	3.20	0.27	0.69
3	2.23	249.0	0.72	0.08	3.14	0.26	0.63
4	1.89	227.1	0.72	0.09	2.65	0.26	0.61
5	1.82	256.3	0.76	0.08	2.63	0.29	0.62
6	1.79	285.6	0.76	0.07	2.51	0.32	0.53
7	1.46	300.3	0.76	0.07	1.99	0.33	0.49
8	1.67	300.3	0.74	0.07	2.21	0.31	0.48
9	1.79	293.0	0.78	0.07	2.43	0.35	0.49
10	1.89	300.3	0.78	0.07	2.42	0.33	0.42
11	1.75	293.0	0.78	0.07	2.35	0.34	0.52
12	1.77	307.6	0.74	0.06	2.47	0.35	0.55
13	1.73	300.3	0.73	0.07	2.36	0.36	0.50
14	1.71	293.0	0.75	0.07	2.37	0.35	0.51
15	1.74	271.0	0.72	0.08	2.37	0.32	0.54
16	1.85	271.0	0.72	0.07	2.62	0.32	0.58
17	2.03	263.7	0.74	0.08	2.81	0.33	0.59
18	2.02	285.6	0.78	0.07	2.77	0.35	0.58

A = RMS Flow (ml/sec)
B = Breathing Frequency (br/min)
C = Inspiratory Time / Expiratory Time
D = Relaxation Time (sec)
E = Maximum Expiratory Flow (ml/sec)
F = Time of Max Expiratory Flow / Expiratory Time
G = Penh

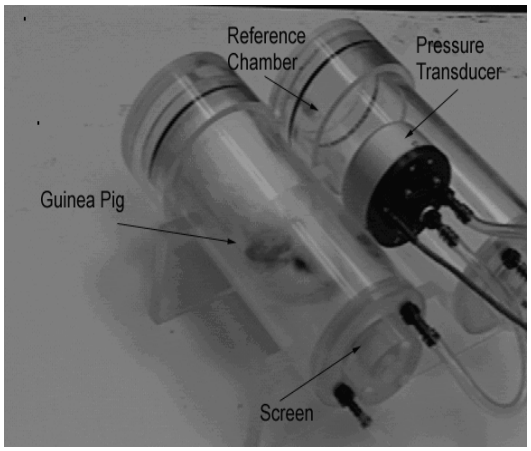

Figure 1. Illustration of a whole body flow plethysmograph.

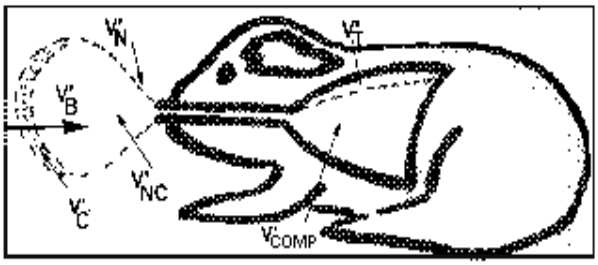

Figure 2 Theoretical model of a guinea pig breathing in a flow plethysmograph.

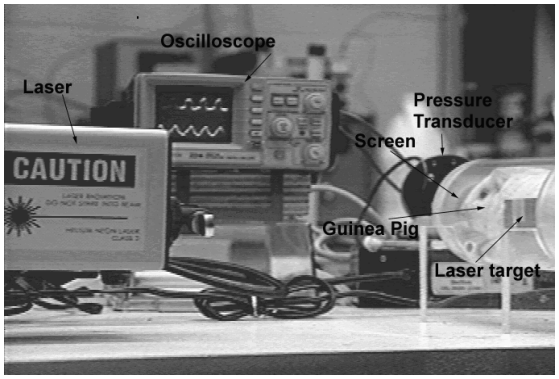

Figure 3. A guinea pig n a plethysmograph during laser displacement measurements.

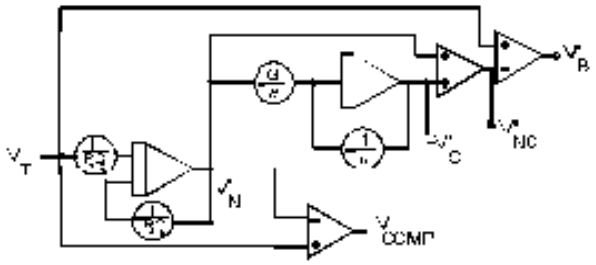

Figure 4. Analog implementation of a system of differential eqautions representing a guineapig in a plethymograph.

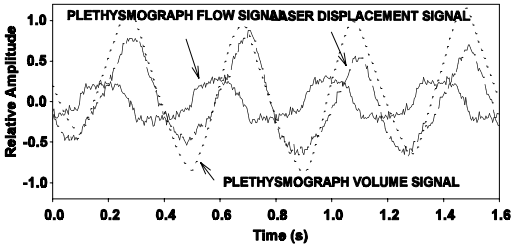

Figure 5 . Plethysmograph flow and volume along with the laser displaced signal as a guinea pig breathes within a whole body flow plethymograph. A positive flow and volume indicates gas leaving the plethysmograph and a positive laser displacement signal indicates an in the size of the throax during luring inflation.

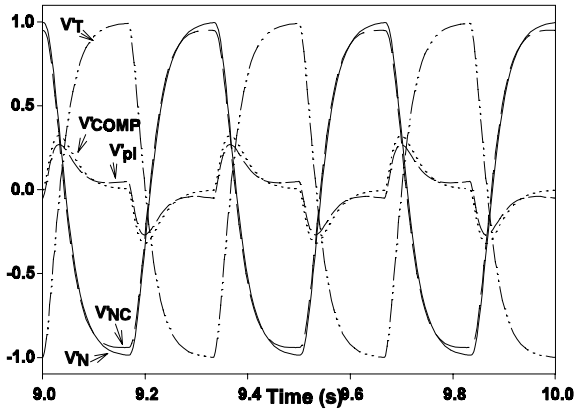

Figure 6. Calculated components of flow generated that simulate a guniea pig breathing in a whole body plethysmograph.

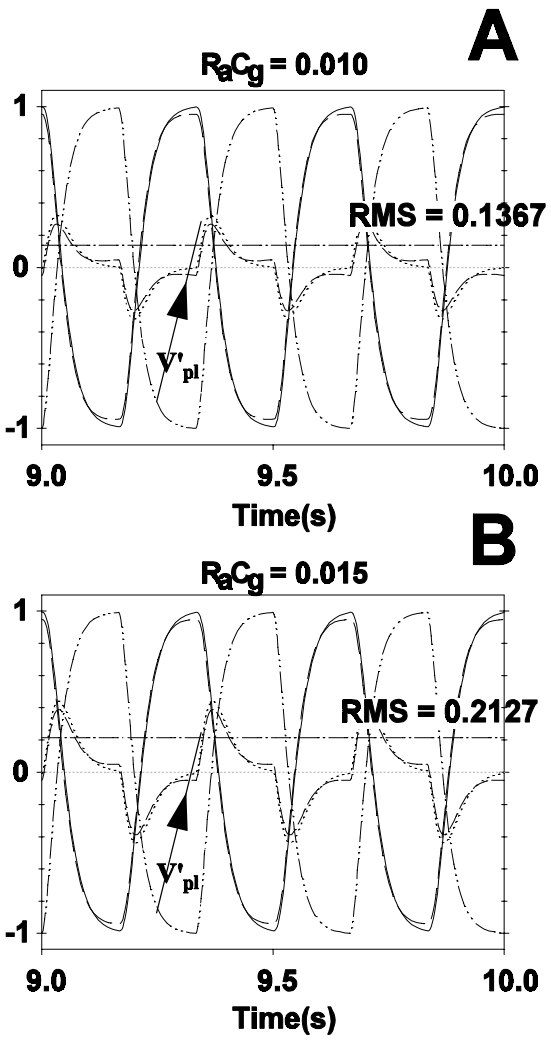

Figure 7. Panel A shows the results of the theoretical analysis when the specific airway resistance was 0.010 and panel B shows the results when specific airway resistance was 0.015. The RMS value of the plethysmograph flow is indicated in each panel. In this example, the Rms value of the flow increased as specific airway resistance increased.

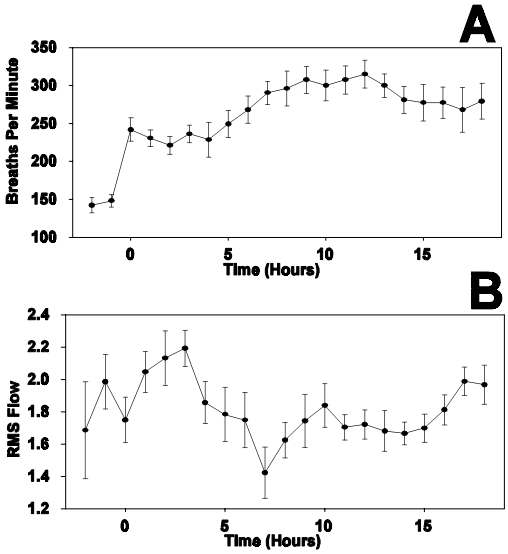

Figure 8. Guinea pigs were exposed to cotton dust (30 mg/M^3 for 4 hrs). Shows the breathing rate of guinea pigs equilibrated in 10% CO2 and air for measurements made pre-exposure and at one hour intervals up to 18 hrs post exposure. B) Shows the RMS value of plethymograph flow at the same time periods desribed in A.