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Measurement and analysis of the respiratory system pressure-volume curve obtained by constant and low inflation flow rate in the rat

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Aim. **Since a very low inflation flow rate renders the resistive pressure component of the inflation pressure practically negligible, the quasi-static pressure-volume curve of the respiratory system may be obtained by continuously recording the tracheal pressure. We aimed to validate this method of measurement of the elastic characteristics of the respiratory system in the small rat's respiratory system by comparing pressure-volume data with a validated mathematical approach previously proposed for larger mammals.**

Methods. **Pressure-volume data were interpolated by a fitting polynomial equation and a very good agreement was found between experimental data and the mathematical approach.**

Results. **a) Pressure-volume curves of the respiratory system in healthy rats exhibit a lower and a higher inflection points. The alveolar recruitment process at low volume lasted till a volume of about 0.5-0.7 mL/100 g was inflated (lower inflection point), and the volume at which the elastic properties of the system became increasingly dependent on the mechanical characteristics of collagen fibers of the alveolar wall, rather than of elastin ones, resulted about 1.6-1.8 ml/100 in the rats (higher inflection point). b) A simple method to quantify inspiratory and total lung capacity in experimental animals is proposed. These were estimated respectively to be about 12 and 15-18 ml for the rat.**

Conclusion. **The low inflation flow rate method is confirmed to give reliable results in the small rat's respiratory system too. A simple method to measure inspiratory and total lung capacity in experimental animals is proposed.**

KEY WORDS: Positive-pressure respiration - Rat - Respiratory system.

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The elastic characteristics of the respiratory system are usually described on the basis of the analysis of the static pressure-volume curve.

An interpolating equation of the curve has been proposed,[1] which was recognised to accurately describe the pressure-volume relationship in a variety of experimental and clinical conditions in mammals.

However, they did not include small rodents such as the rat. Moreover, the interpolation by the fitting equation was obtained on curves defined by discrete pressure-volume data points, and not on a continuous record of the entire pressure-volume curve.

Hence, an objective of the present report was to validate the previously proposed equation [1] for the small rat respiratory system also, and to investigate if a continuous record of tracheal pressure during constant, low rate, inflation flow allows to obtain reliable pressure-volume curves. The theoretical basis for this is that, during very slow constant inflation flow, resistive pressures are practically negligible, so that tracheal pressure may be considered a good measurement of the elastic distending pressure of the respiratory system, as already proposed by previous authors.[2-4]

Furthermore, as will be demonstrated, the mathematical analysis of continuously-recorded pressure-volume curves allows to more exactly investigate the appearance of inflation points on the curve, and to define with good approximation the respiratory system's volumes at which the inflection points occur.

The so-called "lower inflection point" represents the end of the alveolar recruitment process which occurs at low inflation volumes in respiratory disease such as ARDS[1], and has been demonstrated in the healthy respiratory system also.[5-8] On the other hand, the "higher inflection point" is commonly considered to occur at the respiratory system's volume at which the elastic properties become increasingly dependent on the mechanical characteristics of collagen fibers of the alveolar wall, rather than of elastin ones. The latter are instead considered to determine the elastic properties of the system in the linear and steeper part of the pressure-volume curve.[9] Thus, it is of general interest to define the respiratory system volumes at which these changes occurs, and the pressure-volume curve flattens and shifts from linearity.

Finally, the previously proposed mathematical approach [1] allows to estimate the higher and lower asymptotic volume values of the elastic pressure-volume curve, which in the present experiments define the inspiratory capacity of the rat. This value has been seldom measured before and, together with data quantifying the functional residual capacity reported in the literature, may be used to estimate the rat's total lung capacity.

Materials and methods

The experiments were carried out on ten albino Wistar rats of both genders (5 males), whose mean weight was 331±23 g. The animals were housed and treated in accordance with the Italian laws on animal experimentation (L. 116/92) and with the European Council Provision 86/609/EEC, which received the "International Guiding Principles for Biomedical Research Involving Animals".

The animals were anaesthetised (chloralose 50 mg/100g i.p.) and laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2 mm i.d, 5 cm long) was inserted through an incision in the second tracheal ring and firmly secured in place. The rats were paralysed (vecuronium 2 mg/Kg i.p.), and positive pressure ventilation was started and maintained for five minutes with tidal volume 10 ml/Kg, frequency of breathing 60/min, positive end expiratory pressure 3 cmH_2O, (Rodent Ventilator 7025, Basile, Italy). The respiratory system was then inflated three times consecutively up to a static pressure on 25-30 cmH_2O to assure a constant volume history. Positive pressure ventilation and positive end expiratory pressure were discontinued, and the tracheal cannula connected to a constant flow pump (SP 2000 Series Syringe Pump sp210iw, World Precision Instruments, USA) set to deliver a 10 ml inflation volume at constant inflation flow of 0.2 ml/s. Lateral tracheal pressure was continuously monitored (142 pc 01d, Honeywell, USA) and recorded (1326 Econo Recorder, Biorad, Italy). The respiratory system inflation by such a low rate flow allows to minimize the resistive pressure, which may be considered practically negligible. Hence, the recorded tracheal pressure signal may be plotted against inflation volume to obtain continuous pressure-volume curves of the respiratory system.

ECG records were obtained before and after each inflation procedure.

Mathematical approach

The inflation volumes were normalised for body weight to reduce the data dispersion. The previously proposed mathematical approach[1] was applied to interpolate the pressure-volume data. The interpolationg sigmoidal equation was:

$$V = a + \left[\frac{b}{1 + e^{-(P-c)/d}} \right] \qquad [1]$$

where V is the inflation volume and P is the elastic pressure. Each of the fitting parameters a, b, c , d has a physiological correlate.[1] The parameter a has units of volume and corresponds to the lower asymptote volume, which in our experiments approximates functional residual capacity. The parameter b, also in units of volume, corresponds to the inspiratory capacity, *i.e.* the total change in volume between the lower and upper asymptotes. The parameter c is the pressure at the point of higher compliance on the curve, and the parameter d is proportional to the pressure range within most of the volume change takes place. The above mentioned parameters were evaluated by minimizing the discrepancy between experimental and model data. Such discrepancy was evaluated by a specific cost function, whose minimization was performed by stochastic-deterministic optimization alghoritms.[10, 11]

The fitting procedure was applied to evaluate average parameters by including all the data points obtained from all of the rats. As predicted[1], the general interpolating curve exhibited a lower and a higher inflection points.

Obviously, the slopes of the tangents to the curve will undergo to substantial changes with the volume of inflation. To allow an estimation of the respiratory system volume at which the inflection points occur, the first derivative of the general interpolating curve was calculated and plotted against inflation volume.

Results

An example of experimental data interpolation for a single representative rat is reported in Figure 1.

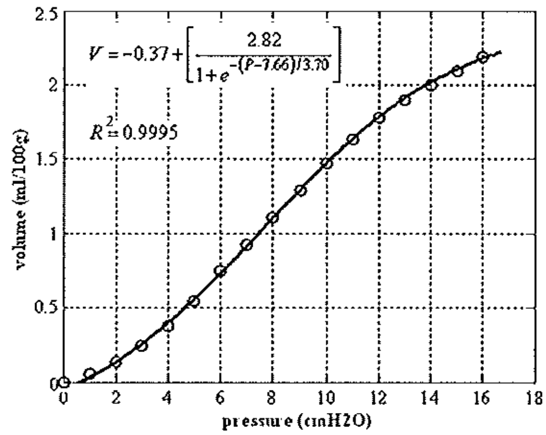

Figure 1.—An example of the interpolation of the pressure-volume data from the respiratory system in a representative rat.

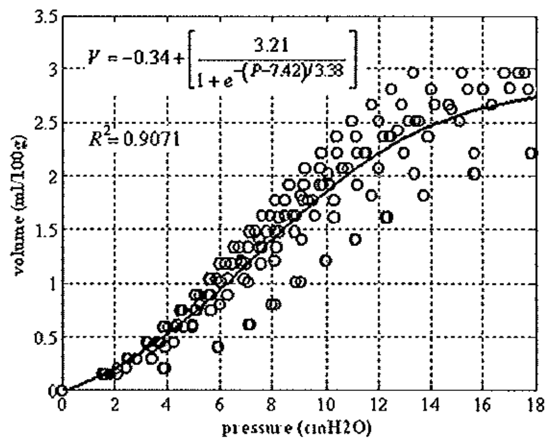

Figure 2.—Mean pressure-volume curve interpolating all the data from all of the rats.

Very good statistical indexes of interpolation were calculated for the other animal also (R^2 values comprised between 0.9982 and 0.9998).

The mean pressure-volume curve interpolating all the data points obtained from all of the rats is reported in Figure 2.

The calculated values of the first derivative of the general interpolating curve (Figure 2) are depicted in Figure 3. It is shown that the lower and higher inflections points occur approximately at inflation volumes of about 0.5-0.7 and 1.6-1.8 ml/100g, while in this range of volume values the curve is almost completely linear, *i.e.* elastance values

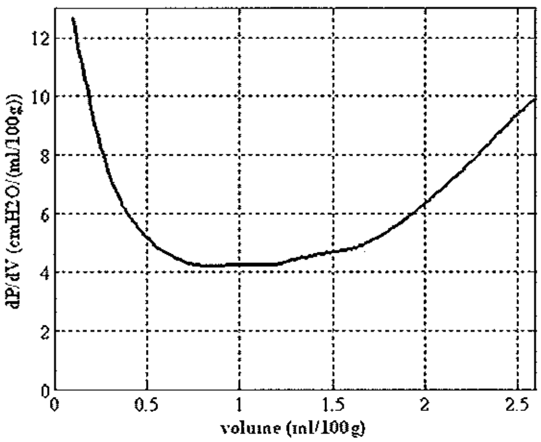

Figure 3.—The calculated values of the first derivative of the general interpolating curve (Figure 2) as a function of the inflation volume. It is shown that the lower and higher inflections points occur approximately at inflation volumes of about 0.5-0.7 and 1.6-1.8 mL/100g respectively, while in this range of volume values the curve is almost completely linear, *i.e.* elastance values do not significantly change with inflation volume.

do not significantly change with inflation volume.

The mean heart rate values before and after the respiratory system inflations resulted 369±26 and 342±22 beats/min respectively, not significantly different.

Discussion

The mean heart rate values we recorded are similar to those previously reported in anaesthetised rats [12-14] and indicate that the general conditions of the animals during the experimental procedure were stable.

Previously reported data from our and other laboratories indicate elastance values for rats of similar weight with respect to those presently studied comprised between 1.8 and 3.3 cmH_2O/mL.[15-21] The inspection of Figure 2 reveals that in the presently studied animals we needed a mean inflation pressure of about 6.2 cmH_2O to inflate the respiratory system with 1 ml/ 100g. Taking into account the mean weight of our animals, from our data mean elastance value results about 1.9 cmH_2O/mL, a very similar results in comparison with previously reported ones.

Furthermore, the statistical analysis of the interpolation of experimental data showed very high R^2 values for all of the rats.

Thus, it may be concluded that the interpolating equation previously proposed[1] may be applied to describe the pressure-volume curves in small rodents too.

On the same basis, and as a confirm of theoretical considerations, we can also indicate that measuring airway pressure during constant and very low flow rate inflation of the respiratory system allows to obtain a valid estimation of the elastic distending pressure. In fact, as previously applied by other authors,[2-4] during very low flow rate inflation the resistive pressure component of airway pressure becomes practically negligible.

Our pressure-volume curve analysis allows to estimate the respiratory system volume at which inflection points occur (Figure 3). Lower inflection point has been attributed to the end of the alveolar spaces recruitment process which starts at very low inflation volumes. This process has been constantly observed in respiratory diseases such as ARDS,[1] but it has been reported for healthy respiratory system too in mammals,[6-8] including rats.[5]

The higher inflection point is commonly considered to occur at the respiratory system's volume at which the elastic properties of the system become increasingly dependent on the mechanical characteristics of collagen fibers of the alveolar wall, rather than of those of elastin. Elastin fibers stress-strain properties account for the linear portion of the curve, in which the tangent does not change with inflation volume (Figure 3). Stress-strain relationship of elastin fibers has indeed been described as highly linear,[22] while non linearity pertains to collagen fibers elastic behaviour.[22, 23] As a result, the respiratory system elastic characteristics are determined by the elastin mechanics at low volumes, and by the collagen mechanics at higher volumes,[9, 24, 25] with the non linearity occurring with the increasing contribution of collagen fibers.[26]

The distance from the higher and lower asymptotes of our general interpolating curve (Figure 2) allows to calculate that the mean inspiratory capacity in our rats, and may be estimated to be about 12 mL. This datum has been seldom measured in the rat, and may be coupled with functional residual capacity measurements to have an estimation of total lung capacity in the rat. Rat's functional residual capacity values reported in the literature for rats of similar weight in comparison to those presently used range from 3.3 to 6.2 mL,[27-30] so that the estimated total lung capacity in our animals would result comprised within 15.3 and 18.2 mL.

This result is in good agreement with previously reported data [30] which indicated a mean total lung capacity value of about 19 mL. Direct measurements of total lung capacity in animals are obviously not possible, and it was assumed that total lung capacity in the rats was achieved when a distending pressure as high as 35 cmH2O was applied.[30] Although often using a little lower distending pressure (30 cmH2O), this experimental approach has been commonly adopted in the scientific literature.[31-33] The presently described estimation of total lung capacity in the rat, based on the mathematical analysis of the pressure-volume curve, indicates a confirmation of the validity of that experimental approach, and suggests an alternative and simple method to assess respiratory volumes in the experimental animals.

In addition, our data allows to estimate that the shift from elastin to collagen fibers in determining the elastic behaviour of the respiratory system (see above) occurs in the rat at a volume a little lower than 50% of the inspiratory capacity, which is a very similar figure in comparison to the human respiratory system.[34]

Our data pertain to the entire respiratory system and do not allow to analyse the separate behaviour of the lungs and the rib cage. However, it was previously reported that the rib cage contribution to the total elastic pressure in rodents,[35] including rats,[36, 37] is very low and almost negligible.

Conclusions

A rather simple mathematical approach may be applied to define the elastic characteristics of the respiratory system in small mammals such as the rats, studied by low inflation flow rate.

The low inflation flow method allows to obtain reliable quasi-static pressure-volume curves.

A simple method to measure inspiratory and total lung capacity in experimental animals is proposed.

Riassunto

Misurazione e analisi della curva pressione-volume del sistema respiratorio nel ratto ottenuta tramite flusso inspiratorio lento e costante

Obiettivo. Un flusso inspiratorio molto lento rende praticamente trascurabile la componente resistiva della pressione di insufflazione. Di conseguenza, si può ottenere la curva pressione-volume quasi-statica del sistema respiratorio registrando la pressione tracheale durante l'inflazione a pressione positiva. Scopo del presente lavoro è di applicare questo metodo di misura anche nel piccolo sistema respiratorio del ratto, verificando che i dati pressione-volume siano adeguatamente descritti da un modello matematico precedentemente validato per animali di taglia maggiore.

Metodi. I dati pressione-volume sono stati interpolati da un'equazione polinomiale e una corrispondenza molto significativa è stata osservata tra i dati sperimentali e l'approccio matematico.

Risultati. A) La curva pressione-volume del sistema respiratorio del ratto presenta un punto di inflessione inferiore ed uno superiore. Il processo di reclutamento alveolare a bassi volumi polmonari dura fino alla inflazione di 0.5-0.7 ml/kg (punto inferiore di inflessione), e il volume al quale le proprietà elastiche del sistema respiratorio divengono significativamente dipendenti dalle caratteristiche meccaniche delle fibre di collagene, piuttosto che da quelle di elastina, risulta circa 1.6-1.8 ml/kg (punto di inflessione superiore); B) viene proposto un semplice metodo per definire le capacità inspiratoria e totale polmonare negli animali da esperimento. Applicandolo ai dati sperimentali, le due capacità sono state stimate a circa 12 e 15-18 ml per il ratto.

Conclusioni. Si conferma che il metodo della inflazione a basso flusso fornisce dati attendibili anche in piccolo animali come il ratto. Si propone un metodo semplice per misurare le capacità inspiratoria e totale polmonare negli animali da esperimento.

PAROLE CHIAVE: Respirazione a pressione positiva - Ratto - Sistema respiratorio.

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