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Measurements of air ventilation in small vertebrates

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ABSTRACT

The breathing act is usually quite noticeable in most vertebrates; hence, the measurement of breathing frequency (f) rarely poses a serious problem. Differently, the quantitative assessment of tidal volume (V_T) (from which air ventilation, $\dot{V}_E = f \bullet V_T$, is computed) can be a major challenge. This article reviews the most common experimental approaches to quantify V_T in adult or young vertebrates of small body size. In these animals, techniques commonly used in adult humans are unsuitable. Furthermore, physiologically meaningful data necessitate techniques with minimal disturbance to the subject under investigation. During the last fifty years numerous and ingenious approaches have been developed and refined. Although none of them can be considered ideal or totally error-free, for specific tasks and/or species there is an optimal approach to measure tidal volume.

1. Introduction

The measurement of the quantity of air passing into the lungs over a period of time (minute ventilation, $\dot{V}_E$, the product of the air inhaled with each breath, tidal volume, V_T, and of the number of breaths per minute, breathing frequency, f) is part of the quantitative analysis of the breathing pattern, often necessary for clinical or research purposes. In air breathing vertebrates the f component of $\dot{V}_E$ can be measured quite easily by visual inspection of the chest motion, as long as the respiration-related movements can be differentiated from locomotion and other non-respiratory movements of the chest. In many specimens, particularly those breathing fast or shallow like some small birds and mammals and other small-size vertebrates, visual counting of the breathing acts can be performed off-line during slow-speed play back of video recordings (e.g., Mortola and Limoges, 2006; Mortola and Seguin, 2009). Quite differently, the quantification of V_T can pose serious challenges, especially in small animals. The measurement of V_T represents the focus of this article.

The simplest and most obvious approach to measure V_T is to measure the volume of air exhaled into a leak-proof calibrated bag. This is what was done for many centuries, probably since almost two millennia ago, when the Greek philosopher and physician Galen collected expired air into a bladder. One likely source of error at the time must have been the change in temperature between subject and atmosphere. Gas laws were understood some fifteen centuries later and are now taken into consideration in the measurement of lung volumes. The spirometer, conceived in its original water-sealed version by John Hutchison in the middle of the nineteen century (Kiraly, 2005), still represents the gold standard for the measurements of V_T in adult humans, against which other techniques are compared for validation. However, in animals of small size the spirometer is impractical because of its substantial mechanical inertia; in addition, even in miniaturized format, the spirometer offers a significant dead space. Similarly, the recently introduced turbine wheels, the speed of which is proportional to airflow, are difficult to miniaturize and are known to underestimate the volume when flow rates are small. Over the years, several alternative methodologies have been developed to quantify V_T and $\dot{V}_E$ in small vertebrates. The abundance of techniques testifies to the creativity and ingenuity of many investigators, but also indicates that none are universally accepted, ideal or error-free.

This article presents some methodologies designed to quantify V_T (and therefore $\dot{V}_E$) in small animals, the size of a newborn human infant (3–4 kg) or less. Preference is given to familiar methodologies most commonly used in conscious newborn or adult laboratory animals, noting that methods that involve anaesthesia or restraint (even visual disturbance) will affect the breathing pattern. This is not to imply that other approaches may not be better choices for particular experimental circumstances. Emphasis is placed on advantages and limitations; technical details can be traced from the representative references provided.

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2. Plethysmography

A plethysmograph (from Greek *plethusmos* enlargement and *graphein* to write) is a device that measures changes in volume; in its application to breathing, usually it consists of a leak proof container that encompasses either the whole body ('body box') or the head compartment ('reversed plethysmograph'). For use in small-size vertebrates, most often the plethysmograph is built *ad hoc*, according to specific needs and animal size, although a few models are commercially available.

2.1. Pressure-plethymography

Head chambers that could be sealed have been used to measure ventilation in reptiles (Templeton and Dawson, 1963; Rosenberg, 1973); pressure fluctuations within the sealed chamber reflect the ratio of V_T to the volume of the chamber. This method clearly will be associated with rebreathing and the associated effects of asphyxia on ventilation. Further, if the chamber volume is not large with respect to V_T the decreased pressure within the chamber during inspiration can affect breathing pattern, presumably due to pulmonary mechanoreceptors invoking a Hering–Bruer reflex (Clark et al., 1978). Needless to say, this method is not commonly used but it serves to illustrate a potential problem with any method in that care must be taken not to affect the breathing pattern.

Normally, in pressure-plethysmography the animal's body is inside the plethysmograph while the head remains outside, separated by a pliable neck collar of latex or paraffin-sealing film (Parafilm®) (Fig. 1(A)). During inspiration, the pressure in the box (P) increases by a magnitude that depends on the size (air compliance) of the box, and is detected by a sensitive P-transducer. A graduated syringe connected to the box permits the P signal to be calibrated for V. Attention should also be paid to the frequency response of the plethysmograph, especially the adiabatic changes in temperature associated with rapid changes in pressure (Bates, 1989). In some instances, the reversed pressure-plethysmograph is a more efficient choice, by which the P oscillations during breathing are recorded from the head-chamber. This allows easier access to the animal's body, for skin or rectal temperature monitoring, EKG electrodes or other needs (Fig. 2(A)).

The pressure-plethysmograph, in its body or reversed format, was popular for the studies of an infant's respiration some fifty years ago (Cook et al., 1957; Karlberg et al., 1960). Adult conscious laboratory animals tolerate the collar poorly; hence, it is a suitable approach only for anesthetized or heavily sedated specimens. An important exception is represented by the newborns of some common laboratory species (mouse, rat, hamster), which usually rest quietly or fall asleep. In these cases a good separation between the body and head compartment is obtained with the pliable collar placed on the back portion of the animal's snout, rather than around its neck. The box should be sufficiently large to limit the P-build up during inspiration to <0.5 cm H_2O to minimize the external elastic load on the respiratory system.

2.2. Airflow-plethymography

The animal is positioned as in the pressure-plethysmograph; however, the body compartment (Fig. 1(B)) (or the head chamber in case of the reversed plethysmograph, Fig. 1(C)) is connected to a miniature pneumotachograph (see Section 4.1). Because the box is open to atmosphere, the pressure swing during breathing is minimal. The V-calibration is done by injecting and withdrawing air in a known amount from a graduated micro-syringe while the animal is breathing (Fig. 1, insert). The reversed plethysmograph operates essentially as a mask as it measures actual inspired and expired air (see Section 4.1). Very small leaks around the collar are acceptable as long as they remain constant throughout the period of the experiment; this can be checked by periodic repeats of the volume calibration. However, large leaks result in significant pressure loss, though this can be corrected providing the time-decay of pressure within the plethysmograph is known and the recorded signal appropriately corrected (see Section 2.3.3). Similarly to pressure-plethysmography, airflow-plethysmography is suitable for animals that readily accept the collar, like some newborn mammals (Mortola, 1984), bird hatchlings (Szdzuy and Mortola, 2007) and snakes (Bartlett et al., 1986).

2.3. Whole body plethysmography – the barometric technique

The barometric technique enables the measurement of the breathing pattern of unrestrained animals that are contained within a chamber; for this reason it is extremely appealing and widely adopted. The method is based on the idea, originally proposed by Chapin (1954) and validated by Drorbaugh and Fenn (1955), that the pressure P within a sealed chamber with an animal inside must oscillate owing to the changes in temperature and humidity with each breathing act. In fact, during inspiration, the bolus of air inhaled expands because of the difference in temperature and humidity (usually higher in the airways than in the chamber); the opposite occurs in expiration[1]. The small P oscillations within the chamber that are synchronous with breathing can be monitored by a sensitive P-transducer. For the detection of the P-oscillations, and to facilitate the computation of V_T, the chamber should be small relative to animal's size, and must be sealed (an exception is discussed later, see Section 2.3.3); hence, the recordings are relatively brief to avoid important changes in gas composition. As long as the key variables (ambient and body temperatures and humidity, barometric pressure, chamber compliance) are measured correctly, the conversion of the P-oscillations into V_T is straightforward according to gas laws. Over the years, the theory, practical implementations and potential pitfalls of the methodology have been discussed in detail (Malan, 1973; Epstein and Epstein, 1978; Jacky, 1978, 1980; Epstein et al., 1980; Fleming et al., 1983; Mortola and Frappell, 1998). The technique is so simple (Bartlett and Tenney, 1970), or deceptively so, that it has been applied to many experimental conditions and animals. Here, we highlight solely the main assumptions and potential sources of errors when the technique is applied to small vertebrates.

2.3.1. The 'mechanical' component

Owing to the principle at the core of the technique, if temperature and humidity were not different between chamber and animal there should be no breathing-related P-oscillations within the chamber. In reality, also in these conditions breathing-related P-oscillations would be detectable, especially in animals breathing at high rates, because the P-oscillations are a function not only of V_T but also of airflow ($\dot{V}$). In inspiration and expiration alveolar pressure, respectively, drops and increases by a magnitude that, for a given $\dot{V}$ and V_T, depends on airway resistance. These changes in alveolar pressure cause some lung volume expansion and compression, respectively, in inspiration and expiration, responsible for some increase and decrease of the chamber P. Hence, the P-oscillations synchronous with breathing are due to two factors, a *mechanical* component dependent on $\dot{V}$ and resistance, and a *barometric* component dependent on V_T, temperature and humidity. Based solely on the P recording, it is not possible to separate

[1] In reality, thermal phenomena in expiration do not correspond to those in inspiration. Because of this, the change in pressure is less in expiration than in inspiration, introducing an inspiratory drift in the pressure-waveform. The issue is discussed in detail in Mortola and Frappell (1998).