Title: MULTIPLEXED MASS SPECTROMETRY FOR SMALL ANIMAL RESEARCH

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Introduction: In order to help ensure scientific rigor, the standards for monitoring anesthetized research animals should be even more stringent than routine clinical standards. Until now, breath-by-breath analysis of multiple simultaneous gas and anesthetic waveforms has not been feasible for such small animals as rats. Our objective was to design and validate a new mass spectrometric (MS) system to service concurrently four anesthesia small animal research laboratories

Methods and Materials: Gas is drawn from the tracheal tube into a capillary *via* an adaptor connected to a pressure-cycled non-rebreathing ventilator circuit. Samples traverse 2–24 m at a rate of 60 $ml \cdot min^{-1}$, and are analyzed by a MS (Perkin-Elmer MGA-1100) for: CO_2, O_2, N_2, N_2O, halothane, enflurane, and isoflurane concentrations. Gas is removed from the animal only during each 5 to 20s sampling period.

Using software specifically designed for this laboratory system, an IBM PC/XT computer simultaneously digitizes the 7 analog gas concentration outputs, applies the appropriate calibration curve to each gas, and displays the data in graphical (waveform) and numerical (inspired, end-tidal, and mean concentration) format at the appropriate station on-line. All gas data are stored in computer memory, permitting immediate re-display of any pair of gas waveforms. 'Bad' breaths are eliminated using a numerical algorithm. Remote stations are IBM PC's with RS232 connections. Up to four stations may be active at once.

Each station can perform its own calibration independently using local gas standards, and can activate sampling at fixed intervals. All numerical data is stored to disk as a summary table suitable for entry into the laboratory notebook. Optionally, the concentration *vs.* time waveforms for each gas are stored to disk for off-line examination, printing, plotting, or re-analysis.

We measured *in vitro* the accuracy of MS end-tidal CO_2 analysis using a phasic respiratory simulation device. The ventilator and breathing circuit alternately supplied 100% O_2 or 5% CO_2 in O_2. An in-line infrared analyzer (Hewlett-Packard) was positioned at the site of MS gas withdrawal, and the MS and infrared-derived waveforms were compared with the known *actual* gas composition. In this way, gas sampling and transmission errors were determined at a variety of rates and I:E ratios.

The *in vivo* experiments involved halothane-anesthetized Sprague-Dawley rats (361-690g) during mechanical ventilation *via* tracheotomy or oral tracheal tube. MAC was measured using the method of Quasha *et al.*[1] with a tail-clamp stimulus and 0.2% atm halothane increments. Linear regression and paired t-tests with $\alpha = .05$ were used for analysis.

Results: Figure 1 shows the effects of ventilator rate and I:E ratio on the absolute error associated with end-tidal PCO_2 ($P_{ET}CO_2$) measurement by MS using a 2m capillary. At a given ventilator rate, the error increases as the expiratory phase shortens. MS data (open symbols) showed only slightly larger errors than the infrared data (closed symbols). The inset graph demonstrates the maximal ventilator rates achievable at various I:E ratios while keeping the MS error <0.5% atm.

In vivo rat experiments showed that the difference between the arterial PCO_2 (P_aCO_2) and the $P_{ET}CO_2$ ($\Delta PCO_2 = P_aCO_2 - P_{ET}CO_2$) was not significantly different from zero, and the correlation between $P_{ET}CO_2$ and P_aCO_2 was strong (Figure 2). Continuous gas sampling for periods up to 5 min did not affect either ΔPCO_2 or airway pressures (mean $\Delta PCO_2 = -1.45 \pm .49$ torr, $P > .20$ initial *vs.* final sample, n=6). Using MS-derived end-tidal halothane concentrations in rats, the MAC of halothane was 0.97±0.04 percent atmospheric (n=14, mean±SEM).

Conclusions/Discussion: We demonstrated that accurate and simultaneous measurements of phasic respiratory concentrations of anesthetic and respiratory gases are possible in small animals, and that non-invasive airway monitoring closely reflects blood-phase CO_2 tension. Our technique simplifies the determination of MAC in rodents, and may increase accuracy over previously-reported methods. The system is adaptable to mass spectrometers of various manufacturers. Use of a non-rebreathing pressure-cycled ventilator permitted sustained gas sampling without interfering with respiratory homeostasis, making it unnecessary to utilize a single-breath sampling technique,[2] as recommended for infants and children using partial rebreathing circuits.

The routine monitoring of inspired and end-tidal concentrations of multiple anesthetic and respiratory gases has become commonplace in clinical anesthesiology.[3] For the first time, the equivalent caliber of monitoring can be applied routinely to the maintenance of respiratory homeostasis and constant alveolar anesthetic levels in small, inexpensive, animals from multiple laboratories. This new technology permits increased rigor in physiological and pharmacological research.

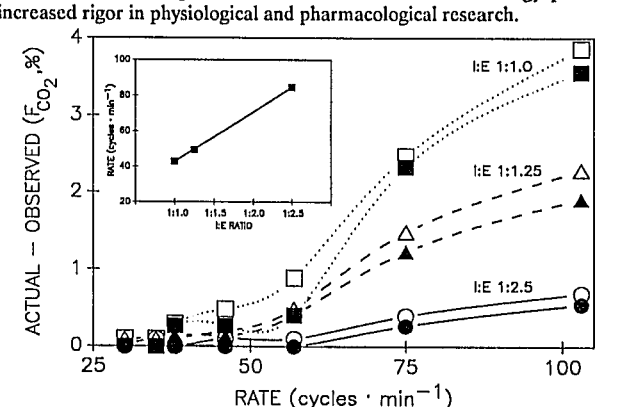

Figure 1: Effect of ventilator rate on absolute end-tidal PCO_2 measurement by mass spectrometer (open symbols) and infrared analyzer (closed symbols). Inset: maximal rates achievable with error < 0.5% atm.

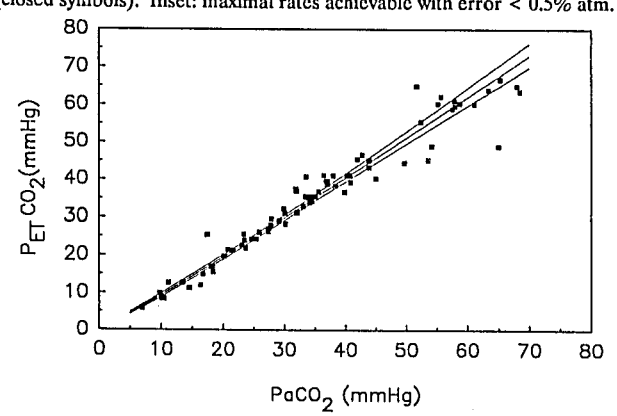

Figure 2: Correlation of arterial and end-tidal PCO_2 in rats; regression line and 95% prediction intervals are shown ($r = 0.97$, $P < .0001$; n=13).

References:

1. Quasha AL, Eger EI, Tinker JH: Determination and applications of MAC. Anesthesiology 53:315-334, 1980

2. Bissonnette B, Lerman J: Single breath end-tidal PCO_2 approximates arterial PCO_2 in infants in children. Anesth Analg 66:S13, 1987

3. Ozanne GM, Young WG, Mazzei WJ, Severinghaus JW: Multipatient anesthetic mass spectrometry. Anesthesiology 55:62-70, 1981