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A critical evaluation of automated blood gas measurements in comparative respiratory physiology

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ABSTRACT

Precise measurements of blood gases and pH are of pivotal importance to respiratory physiology. However, the traditional electrodes that could be calibrated and maintained at the same temperature as the experimental animal are increasingly being replaced by new automated blood gas analyzers. These are typically designed for clinical use and automatically heat the blood sample to 37 °C for measurements. While most blood gas analyzers allow for temperature corrections of the measurements, the underlying algorithms are based on temperature-effects for human blood, and any discrepancies in the temperature dependency between the blood sample from a given species and human samples will bias measurements. In this study we review the effects of temperature on blood gases and pH and evaluate the performance of an automated blood gas analyzer (GEM Premier 3500). Whole blood obtained from pythons and freshwater turtles was equilibrated in rotating Eschweiler tonometers to a variety of known P_{O2}'s and P_{CO2}'s in gas mixtures prepared by Wösthoff gas mixing pumps and blood samples were measured immediately on the GEM Premier 3500. The pH measurements were compared to measurements using a Radiometer BMS glass capillary pH electrode kept and calibrated at the experimental temperature. We show that while the blood gas analyzer provides reliable temperature-corrections for P_{CO2} and pH, P_{O2} measurements were substantially biased. This was in agreement with the theoretical considerations and emphasizes the need for critical calibrations/corrections when using automated blood gas analyzers.

1. Introduction

Any experimental study within respiratory physiology is entirely dependent on the ability to perform precise measurements of blood gases, i.e. partial pressures of O_2 and CO_2 (P_{O2} and P_{CO2}, respectively) as well as pH. Incorrect measurements were the culprits for the famous controversy regarding active oxygen secretion, where Christian Bohr explained his measurements of higher arterial P_{O2} compared to alveolar P_{O2} by active oxygen transport across the lung epithelium. August and Marie Krogh could refute this claim by virtue of new and better measurements of blood P_{O2}, and thus conclusively showed that "the absorption of oxygen and the elimination of carbon dioxide in the lungs takes place by diffusion and diffusion alone" (Bohr, 1909; Krogh, 1910; Wang, 2011).

While good pH electrodes have existed for more than a century, reliable electrodes for determination of blood P_{O2} and P_{CO2} were not developed until the 1950s (Stow and Randall, 1954; Clark, 1956; Severinghaus and Bradley, 1958) and were quickly integrated into blood gas analyzer units (Astrup and Severinghaus, 1986; Severinghaus, 2004). Particularly the BMS series from radiometer has been used extensively within comparative physiology. These blood gas systems had the great advantage that measurements could be performed at the same temperature as the experimental animal, thus avoiding errors associated with appropriate temperature correction, which differ between species. However, with the subsequent development of alternative measurement techniques, such as optodes, and the rapid advance of computerized control of measuring devices, blood gas analyzers have become increasingly automated, eradicating the production of the traditional radiometer electrodes. While this automation has greatly eased measurements and lessened operational errors, the ability to control the temperature of the electrodes has become increasingly difficult, such that correct temperature correction has become of pivotal significance. However, many of the automated blood gas analyzers rely on algorithms derived from human blood when generating the reported values, and it is not always obvious which parameters are actually measured and which of the reported parameters are merely calculated. There is, accordingly, a need to investigate the reliability of these automated blood gas analyzers for comparative studies, particularly on ectothermic vertebrates where the temperature differences are large and where the nucleated red blood cells may affect measurements.

In the present study, we review how and why P_{O2}, P_{CO2} and pH change when the temperature of a blood sample is increased during

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measurements. This introduction serves to address the potential problems of using automated blood gas analyzers for comparative studies. Furthermore, we evaluate the performance of a GEM Premier 3500 automated blood gas analyzer (Instrumentation Laboratory, Bedford, MA) by direct comparison with tonometered blood samples from freshwater turtles (*Trachemys scripta*) and ball pythons (*Python regius*), as well as a few additional species of snakes, thereby extending a similar study on shark and trout blood (Gallagher et al., 2010; Harter et al., in press). Herein we compared the temperature corrected blood gas values provided by the GEM Premier 3500 to the set blood gas values in the tonometers equilibrated to gas mixtures prepared with a Wösthoff pump. The pH values measured by GEM Premier 3500 were compared to pH measurements with a radiometer capillary pH electrode. We hypothesize that while an automated blood gas analyzer may suffice to correct for temperature when measuring pH and P_{CO2} at a temperature different from 37 °C, this will not necessarily be the case when measuring P_{O2} due to inter-specific differences in temperature dependency.

2. The nature of the problem—what happens to pH, P_{CO2} and P_{O2} upon heating?

Measurements of blood gases have traditionally been performed with electrodes kept at the same temperature as the experimental animal, but most new automated analyzers (Radiometer ABL series, GEM premier series, iSTAT etc.) either heat the samples to 37 °C, the normal body temperature of humans, or measure at room temperature, whereupon the values are automatically corrected to 37 °C using empirical equations derived for human blood. Both the changes in temperature and the corrections can lead to erroneous results and interpretations of the measurements if the sample temperature dependency differs from that of human blood (see Fig. 1)

A blood sample drawn into a syringe without air bubbles can be considered a closed system with no mass exchange with the surroundings. When such a blood sample is injected into a blood gas analyzer with a temperature that differs from that of the experimental animal, blood gases and pH will change as a consequence of the direct effects of temperature on the many chemical reactions in the blood. Chemical reactions that either produce heat (i.e. exothermic with negative enthalpy (ΔH °)) or consume heat (i.e. endothermic with positive ΔH°) are directly influenced by temperature in accordance with the principle of Le Chatelier, and the effect on the equilibrium constant (K) can be quantified using the Van't Hoff equation (Wyman, 1939):

$$\frac{dpK}{dT} = \frac{-\Delta H^o}{R \cdot T^2}, \quad (1)$$

where T is the kelvin temperature and R is the universal gas constant (see Table 1). Hence, upon heating of the blood sample within the analyzer, the various equilibria are displaced in the endothermic direction (i.e. the heat consuming reaction).

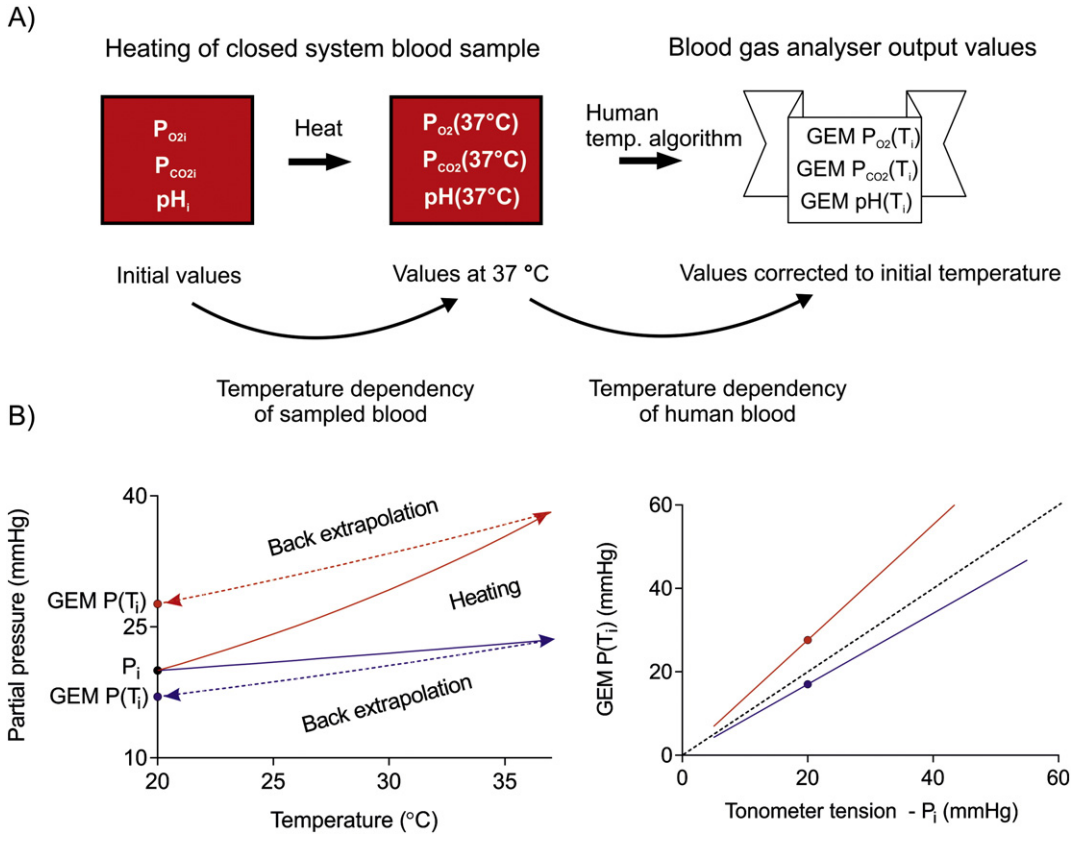

Fig. 1. Manifestation of errors in temperature correction. Panel A illustrates heating of a blood sample in a syringe that can ideally be considered a closed system so that no mass exchange takes place. In the blood gas analyzer, the blood sample with pH, P_{CO2} and P_{O2} at the initial tonometer temperature (P_{O2i}, P_{CO2i}, pH_i) is heated to 37 °C. The degree to which pH, P_{CO2} and P_{O2} change upon heating is a function of the intrinsic temperature dependency of the sampled blood. The blood gas analyzer then back extrapolates the measured values to the initial temperature via a temperature correction algorithm based on empirical equations derived from human blood. The left graph in panel B shows two exponential courses for the increase in blood gas tension upon heating from the partial pressure P_i at 20 °C to 37 °C (full red and blue curve, see Eq. (B2) in Appendix B). The full red curve is calculated for a temperature constant twice that of the human one incorporated for P_{CO2} in the blood gas analyzer (Ashwood et al., 1983), whereas the full blue curve represents a temperature coefficient half the value of the human constant. The broken curves illustrate the back extrapolation incorporated by the blood gas analyzer and it is seen that the temperature corrected values (i.e. GEM $P(T_i)$) differ from the initial (i.e. P_i). When plotting the temperature corrected values as a function of the initial tonometer values (see Eq. (B3) in Appendix B) a line displaced above the line of identity prevails if the sample temperature dependency exceeds the human and vice versa.