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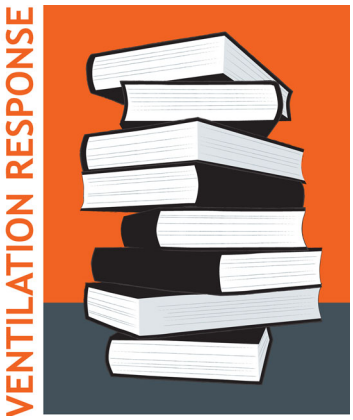
CLASSICS

Is the hypoxic ventilatory response driven by blood oxygen concentration?



Bill Milsom and Tobias Wang discuss the impact of Mogens Glass, Robert Boutilier and Norbert Heisler's classic paper 'Ventilatory control of arterial P_{O_2} in the turtle *Chrysemys picta bellii*: Effects of temperature and hypoxia', published in *Journal of Comparative Physiology* in 1983.

Vertebrates breathe faster and deeper when confronted with low oxygen levels in the inspired water or air. The underlying mechanisms for this ubiquitous hypoxic ventilatory response, and the attendant cardiovascular responses, received considerable attention in the latter half of the 19th century. Indeed, the 1938 Nobel Prize in Physiology or Medicine was awarded to Corneille Jean François Heymans for 'the discovery of the role played by the sinus and aortic mechanisms in the regulation of respiration' (De Castro, 2009). This seminal research established that oxygen-sensitive chemoreceptors within the carotid body, a small and heavily vascularized structure at the bifurcation of the carotid artery, sense oxygen and relay information on arterial blood gas composition to the central nervous system via the afferent fibres of the glossopharyngeal nerve (also called the sinus nerve). This led to renewed interest in hypoxic reflexes by the biomedical community, but precious little was known about the comparative physiology of hypoxia responses by the time of the Second World War.

As part of the 'Golden Era' of comparative physiology (1960–1980), many studies began to characterize the ventilatory responses of different vertebrate classes with descriptions of changes in tidal volume, breathing frequency and overall ventilation pattern. It became clear that the magnitude of the ventilatory response and the threshold at which it was elicited differed considerably amongst species. In many cases, such differences in response could be related to the species' natural environments, i.e. many species living in hypoxic burrows or at high altitude had muted responses that appeared to reflect adaptations that enhance oxygen uptake and delivery. From this, it became clear that the underlying regulation was complex, such that the breathing pattern at any given inspired oxygen level was the end result of a cascade of events (Weibel, 1984). In most cases, the actual stimulus to the oxygen-sensitive chemoreceptors was unknown, because few studies measured blood gases. The lack of blood gas measurements posed a particularly pertinent problem in amphibians and reptiles. This is because their undivided heart allows for large cardiac shunts where some systemic blood bypasses the lungs and re-enters the systemic circulation, which means that the partial pressure of oxygen (P_{O_2}) in the arterial blood can be considerably lower than inspired levels or even the P_{O_2} within the lungs (Shelton and Burggren, 1976). The actual stimulus to the chemoreceptors (i.e. the P_{O_2} at the chemoreceptor sites) therefore remained uncertain and it was clear that any interpretation of similarities and differences in the ventilatory responses amongst vertebrates required that the relationship between inspired O_2, oxygen uptake, blood oxygen levels and acid–base status be clarified.

In the early 1980s, Mogens L. Glass, Robert (Bob) G. Boutilier and Norbert Heisler decided to investigate the nature of the oxygen stimulus during breathing in reptiles by studying the influence of hypoxia on the ventilatory responses of freshwater turtles (*Chrysemys scripta*). During their doctoral research, Glass and Boutilier had independently refined methods for simultaneous measurements of tidal volume and breathing frequency and gas exchange in freely moving turtles, snakes and frogs (e.g. Glass and Johansen, 1976; Glass and Wood, 1983; Boutilier and Shelton, 1986). These aquatic air-breathers were allowed to dive in an aquarium, but forced to air-breathe through a funnel containing a plethysmograph (which measured tidal volume and breathing frequency) and a flow-through system that allowed the inspired and expired gas concentrations to be measured. To monitor blood gas levels, arterial blood samples were collected from a cannula inserted into one of the turtle's arteries. The refined techniques alleviated the stress on the animal caused by constraint. The presence of both Glass and Boutilier in Heisler's laboratory at the Max-Planck-Institut für Experimentelle Medizin in Göttingen, Germany, provided the ideal opportunity for them to precisely measure arterial blood gases while applying their newly developed techniques to measure how the animals breathed in order to clarify the relationship between arterial oxygen levels and the hypoxic ventilatory response. In addition, they constructed *in vitro* blood oxygen equilibrium curves at the three relevant temperatures (10, 20 and 30°C) to address how the rise in ventilation associated with hypoxia correlates with arterial P_{O_2}, arterial oxygen concentration and the oxygen saturation of haemoglobin (Glass et al., 1983), as blood oxygen affinity falls when the temperature rises.

The resulting manuscript (Glass et al., 1983) was a true tour de force and clearly demonstrated that the ventilatory response of *Chrysemys* to hypoxia was flexible, but precisely controlled. This provided authoritative evidence against the widely held perception that pulmonary ventilation of the ectothermic reptiles was erratic and irregular and that reptiles were endowed with a 'sloppy' respiratory control system that allowed for wide swings in blood gases (Glass and Wood, 1983). It also demonstrated that although the almost sevenfold rise in oxygen consumption between 10 and 30ºC was attended by an almost equal rise in ventilation, arterial P_{CO_2} increased with an attendant drop in arterial pH. The haemoglobin in the arterial

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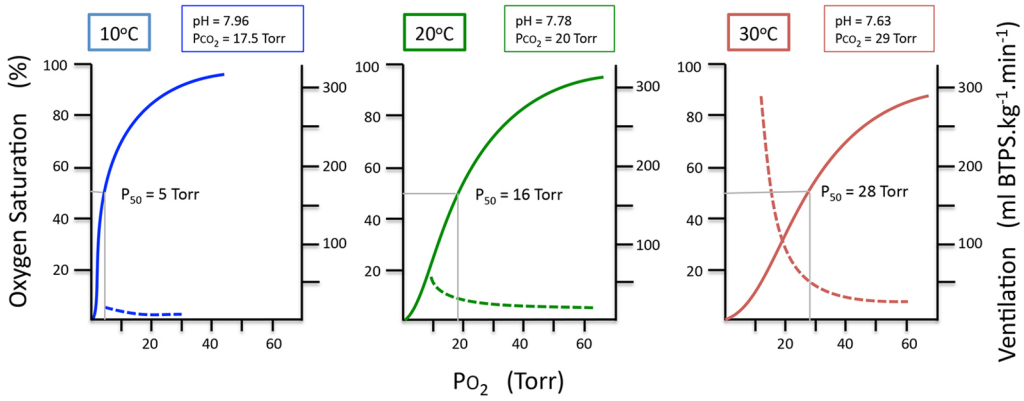

Fig. 1. Glass and colleagues measured pulmonary ventilation and gas exchange in turtles equipped with arterial catheters to measure arterial blood gases at 10, 20 and 30°C. The measurements were performed at various levels of hypoxia. The resulting relationships between arterial P_{O_2} and the measured pulmonary ventilation rates are shown for each temperature. In each panel, Glass and co-workers also depict the oxygen equilibrium curve, and through this representation it is clear that the threshold for the hypoxic ventilatory response coincides with P_{O_2} where the arterial blood becomes less saturated. Glass and colleagues therefore proposed that the receptors responsible for the hypoxic ventilator response may be monitoring oxygen saturation of the blood. Redrawn from Glass et al. (1983).

blood remained about 90% saturated at all temperatures (Fig. 1). Glass, Boutilier and Heisler ascribed the lack of complete saturation to intracardiac shunting, where some of the oxygen-poor blood from the systemic veins recirculates in the systemic circulation (right-to-left cardiac shunt). Although the turtles decreased ventilation relative to metabolism when the temperature rose, Glass and colleagues (1983) showed that arterial P_{O_2} increased from around 30 mmHg at 10ºC to approximately 60 mmHg at 20 and 30ºC. This was a counter-intuitive finding that attracted interest amongst other reptilian physiologists, and contemporary theoretical models developed by James W. Hicks and Steve Wood showed that the 'surprising' rise in arterial P_{O_2} could be explained by the lower blood oxygen affinity with increased temperature (reviewed by Hicks and Wood, 1989).

The most significant discovery, however, appeared during exposure to hypoxia. Donald C. Jackson had previously shown that although the ventilatory responses were much larger at 30°C compared with those at lower temperatures, the threshold for the responses also increased with temperature (Jackson, 1973). Because Glass and co-workers (1983) had also measured the oxygen equilibrium curves of the turtle blood at all three temperatures, they could show that the threshold in arterial P_{O_2} required for ventilation to increase was associated with the level of hypoxia where arterial haemoglobin saturation had fallen to approximately 50%. The authors concluded that the receptor system was monitoring haemoglobin O_2 saturation rather than P_{O_2} of the arterial blood. This was a novel proposal, but one that made perfect sense from a functional point of view. Because of the sigmoidal oxygen equilibrium curve, extra ventilation confers little extra oxygen to the blood when arterial P_{O_2} resides on the flat upper portion of the oxygen equilibrium curve, where haemoglobin is fully saturated and can carry no more oxygen. This important finding revealed a tight match between the various steps of the oxygen transport cascade where pulmonary ventilation is matched to the capacity of the blood to bind oxygen. Numerous later studies revealed that this matching of the hypoxic ventilatory response to haemoglobin O_2 saturation is a virtually universal phenomenon in both ectothermic and heterothermic vertebrates.

However, the observation that ventilation appeared to be driven by reductions in blood oxygen concentration (or O_2 haemoglobin saturation) rather than partial pressure *per se* was problematic, for how are the oxygen-sensitive cells within the carotid body able to sense how much oxygen is bound to the haemoglobin within the red blood cells? Alternative mechanism(s) to the sensing of blood oxygen concentration *per se* had to be considered. Tobias Wang, a graduate student under the supervision of Glass at the University of Sao Paulo in Riberao Preto, Brazil, and colleagues were inspired to manipulate the blood oxygen saturation independent of partial pressure in toads to investigate whether the reduction in arterial oxygen concentration would be enhanced (Wang et al., 1994): but this was not the case. In the same year, Bill Milsom and his students (Garland et al., 1994) conducted similar studies in heterothermic rodents and arrived at different conclusions. Thus, the underlying mechanisms for detecting O_2 binding of haemoglobin in red blood cells remains both debated and unresolved, and discordant views between the authors of this Classics article have spurred a long-lasting and fruitful collaboration, beginning when Tobias Wang joined Bill Milsom at UBC as a postdoctoral fellow. Conflicting views still exist as to whether and how the ventilatory responses of turtles and other ectotherms are related to arterial partial pressure of oxygen or arterial oxygen concentration. The continued debate highlights the extent to which the study by Glass, Boutilier and Heisler (1983) has spurred discussions and has served to formulate hypotheses for experimental investigation, which for us makes this study a true classic.

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