© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 1090-1092 doi:10.1242/jeb.139220

CLASSICS

How and why pH changes with body temperature: the α-stat hypothesis

Tobias Wang and Donald C. Jackson discuss the impact of Robert 'Blake' Reeves' classic paper 'An imidazole alphastat hypothesis for vertebrate acid-base regulation: Tissue carbon dioxide content and body temperature in bullfrogs', published in *Respiration Physiology* in 1972.

The exquisite maintenance of pH of bodily fluids is a hallmark of homeostatic regulation that occupies numerous lectures of any decent university course in human or comparative physiology. Here, it is typically emphasised that arterial pH is tightly regulated by the concerted effort of transepithelial movements of acid-base equivalents (through structures such as the kidneys, skin or the gills) and the arterial partial pressure of CO_2 (P_{CO_2}) as dictated by ventilation of lungs or gills in relation to metabolic CO_2 production. The delicate regulation of pH, however, does not reveal why pH is regulated at 7.4 in mammals, whilst many ectothermic vertebrates defend a pH much closer to 8. This fundamental question occupied Herman Rahn and Robert Blake Reeves, both at the Department of Physiology at The State University of New York at Buffalo, USA, from the late 1960s. During the ensuing decades, a series of comparative studies, particularly on fish, frogs and turtles, led to the formulation of the α-stat hypothesis. This hypothesis, which suggested that it is the ionisation of proteins, particularly the α-imidazole groups on histidines, that is being regulated must certainly be regarded as one of the major contributions of the comparative approach to general physiology. Nevertheless, the α-stat hypothesis was also met with critiques that were difficult to resolve and may explain why the topic has received remarkably limited attention in recent years.

In the early 1960s, relatively little was known about acid-base physiology of ectothermic vertebrates. C. Ladd Prosser's influential textbook from 1950 merely stated that 'land vertebrates have a blood pH usually near 7.4' (Prosser, 1950), whereas William B. Yapp's contemporary textbook was even less informative: 'The normal pH varies from one animal to another but is usually between 7 and 8' (Yapp, 1961). However, in 1962, Eugene Robin published careful measurements of arterial blood gases from freshwater turtles maintained at 10, 24 and 37°C that revealed a consistent rise in arterial P_{CO_2} and a fall in pH over this temperature range. These data received surprisingly sparse mechanistic interpretation, but Robin remarked, 'it seems clear that the maxim that life can only exist within a relatively narrow pH range is not applicable to the turtle and may only be true for homeothermal animals'. This statement might reflect a view that 'lower ectothermic' vertebrates are endowed with less precise homeostatic control mechanisms – a view that seems to have flourished at the time. Rahn was inspired by these findings and was at the same time outlining the profound differences in arterial P_{CO_2} values between water-breathers and air-breathers (Rahn, 1967).

Rahn and his colleagues then measured arterial acid-base variables in carp, bullfrogs and snapping turtles, and found very similar patterns to those described by Robin (Howell et al., 1970). However, Rahn's group at Buffalo went further and noted that the decrease in pH with increased temperature mirrored the reduction in neutral pH (i.e. the pH where H^+ and OH^- concentrations are the same) owing to the increase in the ionisation constant of water with increased temperature. Thus, it seemed as if these diverse animal groups exhibited tight regulation of the acid-base status in a manner that ensured the same relative alkalinity, i.e. arterial pH remains above neutral, over a broad temperature range. The ectothermic vertebrates were in fact as good at regulating pH as the endotherms and it became clear that arterial pH was indeed regulated at a new set-point when the temperature changed and this regulation was achieved through a relative hypoventilation, where arterial P_{CO_2} rises with increased temperature. The findings would also have obvious direct implications for clinical practice, because one could now argue that arterial pH must be increased in accordance with the shift in neutral pH as temperature was lowered during hypothermic surgery.

Rahn's ideas originally appeared as a well-written perspective in a Ciba Symposium volume in 1967 (Rahn, 1967), to which W. T. Albery and B. B. Lloyd appended a theoretical analysis concluding that (to explain the pH change), 'One should perhaps look for an important buffer equilibrium with pK ~7 and ΔH ~7 kcal' (Albery and Lloyd, 1967). pK is the negative logarithm of the dissociation constant and should be within the physiologically relevant pH range. Rahn's colleague at Buffalo, Reeves, then did just that when he presented his 'α-stat hypothesis' at the Comparative Physiology of Respiration symposium in Göttingen in 1971 and subsequently published his classic paper in *Respiration Physiology* in 1972. In this hypothesis, Reeves redirected the focus from the mere neutrality of water to proteins that are essential for metabolism and physiological functions, and hence emphasised the biological consequence of the temperature-mediated change in pH set point. Aptly, the line showing how pH decreases with temperature is now commonly referred to as the 'Buffalo curve' (Fig. 1).

Theodore B. Rosenthal had already demonstrated in 1948 how the acid-base status of blood that is cooled or warmed without access to air (i.e. a closed system) is dictated by the temperature sensitivity of the major buffer groups, with pK values in the vicinity of the pH. Thus, as a human

Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 1090-1092 doi:10.1242/jeb.139220

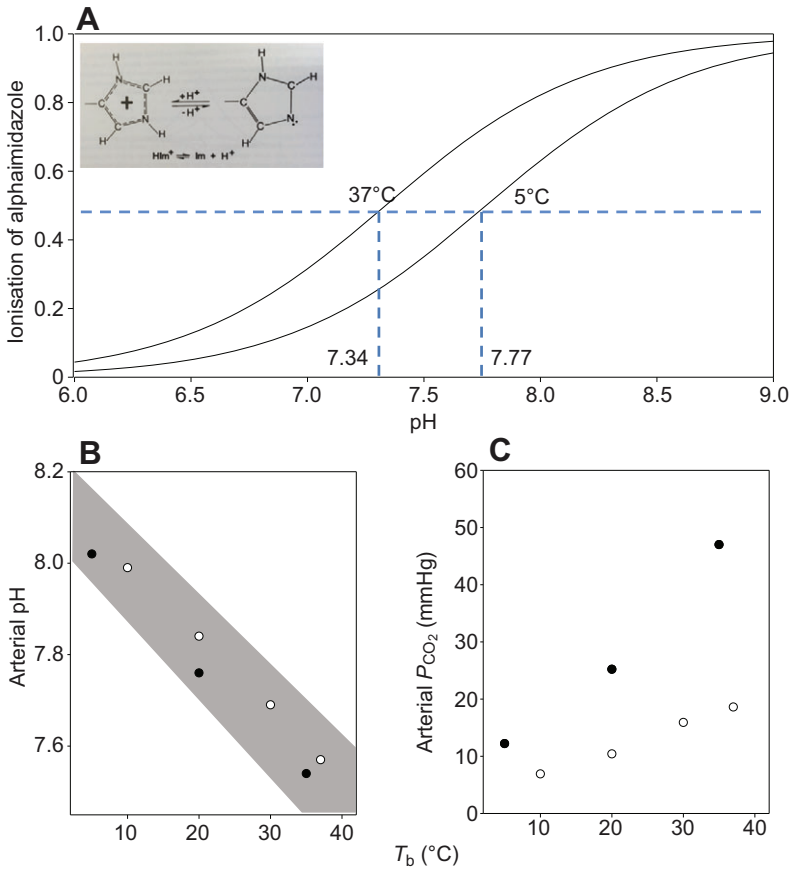

Fig. 1. The α-stat hypothesis for acid-base regulation and the Buffalo curve. (A) A decrease in pH of approximately 0.015–0.018 pH units °C^{-1} (equivalent to ΔH_o of 7 kcal mol^{-1}) will maintain ionisation of histidine if the decrease in pH with rising temperature matches the decrease in p*K* of the α-imidazole group (here shown as a decrease in pH from 7.77 at 5°C to 7.34 at 37°C where alphastat remains unchanged at approximately 0.5). (B,C) Based on *in vivo* measurements of arterial blood gases in toads (*Rhinella marina*, open symbols) and turtles (*Chelydra serpentine*, filled symbols) published a few years earlier by Howell et al. (1970), it was evident that arterial pH decreases as proposed by the α-stat hypothesis – the so-called Buffalo curve (B) – because arterial P_{CO_2} increases (C).

blood sample is cooled anaerobically at constant total CO_2 concentration, P_{CO_2} decreases and pH increases, whereas ionisation of the proteins (the major buffers in blood) remains virtually constant. Aware of this relationship, Reeves now focused on relevant buffers with p*K* values close to normal physiological pH and discovered that the α-imidazole group on the amino acid histidine was a prime candidate for a buffer with such p*K* values. It turned out that p*K* of the α-imidazole group on the histidines changes in the order of −0.015 to −0.020 pH units °C^{-1}. This p*K* change with temperature (i.e. d*pK*/d*T*) was remarkably close to the measured pH change (dpH/d*T*) *in vivo*.

Reeves therefore proposed that air-breathing animals regulate their pH in a manner that resembles Rosenthal's closed system. He suggested, 'a framework which emphasizes the regulation of the degree of ionization (α) of imidazole moieties in proteins not only of the blood, but within intracellular compartments as well. Viewed in this context, mammalian acid-base principles are seen to be an isothermal case of a phylogenetically more comprehensive regulatory pattern' (Reeves, 1972). Thus, when animals change body temperature they alter pH so the ionisation of proteins, such as enzymes, and hence their biological functions, remain intact.

The α-stat hypothesis is also appealing because maintenance of the protein charge alleviates disruptive transmembrane ion movements and hence avoids any associated disturbance to cell volume regulation – a response Reeves demonstrated in careful experiments on isolated red blood cells. Furthermore, because protein charge does not change, the α-stat regulation means that pH is altered exclusively (at least in air-breathers) through ventilatory control of arterial P_{CO_2} without changes in total CO_2 concentration of the bodily fluids. Arterial P_{CO_2} therefore increases with increased temperature because pulmonary ventilation does not increase to the same extent as metabolic CO_2 production. The animal therefore functions as a semi-closed system that mimics the events in the closed 'Rosenthal' system, where P_{CO_2} increases passively in response to the altered p*K* of the major buffer systems. This reduction in pulmonary ventilation relative to metabolism had already been experimentally verified in turtles (Jackson, 1971). A similar pattern was also extended to amphibians where the analysis was more complex because cutaneous CO_2 excretion needs to be taken into account, although ventilation clearly also regulates arterial P_{CO_2} in bimodal breathers (Wang et al., 1998).

Despite its obvious appeal, and the clear link between the observed pH changes to the optimisation of biochemical processes, the α-stat hypothesis received considerable disapproval from some comparative physiologists. In particular, Norbert Heisler in Göttingen, Germany (Heisler, 1986) pointed out that the *in vivo* pH changes in many animals did not precisely match that p*K* change of the α-imidazole group (i.e. dpH/d*T*≠d*pK*/d*T*); in some animals, such as varanid lizards and hibernating mammals, arterial pH does not change at all when body temperature is altered. It has also been observed that plasma HCO_3^- concentration does not always remain constant when body temperature changes (e.g. Stinner et al., 1994). However, is that sufficient to refute the α-stat hypothesis? Here, we agree with Cameron, who pointed out that there is not a single d*pK*/d*T* value for the many α-imidazole groups in the various proteins of the body (Cameron, 1989). Therefore, dpH/d*T* may differ among species or amongst organs in an individual animal.

In the introduction to his original 1972 publication, Reeves emphasised the need for a general theory of acid-base regulation applicable to both endo- and ectothermic vertebrates, while making reference to the then recent observations on 'blood pH and P_{CO_2} in cold-blooded vertebrates' (Robin, 1962; Howell et al., 1970). Importantly, though, he also

Journal of Experimental Biology (2016) 219, 1090-1092 doi:10.1242/jeb.139220

acknowledged 'the neglected pioneer work of Austin, Sunderman and Camack (Austin et al., 1927)'; and J. Harold Austin and colleagues had indeed been neglected! They had measured arterial blood gases in four alligators held at 9 and 35°C and had observed a dpH/dT of −0.018 pH units °C^{-1}. Highlighting this decrease in arterial pH as their most interesting finding, the researchers had also noted that 'this change with temperature is found to be practically the same as the change in pH required to maintain constant [BPr] in a given serum with changing temperature', where BPr was a description for protein ionisation or what Reeves called α-stat regulation. Later in the paper, Austin and colleagues deduce that maintaining constant protein ionisation prevented cell volume changes as ion movements over the cell membrane are avoided. It is difficult to imagine that anybody could have written a more succinct abstract of the α-stat hypothesis; quite an impressive feat given that the paper was written almost 50 years before the α-stat hypothesis was conceived. In the current academic environment, where citations and h-indexes are often the only metrics used by departmental heads and deans to evaluate academic success, it is striking that the paper by Austin and co-workers (Austin et al., 1927) has received only 30 ISI citations in the past 90 years, whereas Reeves (1972) has been cited almost 400 times. Why was Austin's seminal paper overlooked? Perhaps because Austin and collaborators were clinical investigators interested in the chemical properties of blood and not biologists interested in how ectothermic vertebrates, like their alligators, function in nature. Nor were there any active contemporary animal physiologists to exploit their findings. By contrast, Reeves' publication appeared at a time when comparative physiology was in full flower and provided a much needed mechanism that had a significant impact on our understanding of the acid-base physiology of ectothermic vertebrates.

Tobias Wang
Aarhus University
tobias.wang@bios.au.dk

Donald C. Jackson
Brown University
Donald.Jackson@brown.edu

References

Albery, W. J. and Lloyd, B. B. (1967). Variation of chemical potential with temperature. In *Development of the Lung* (ed. A. V. S. de Reuck and R. Porter), pp. 30-33. London: Churchill.

Austin, J. H., Sunderman, F. W. and Camack, J. G. (1927). The electrolyte composition and the pH of serum of a poikilothermous animal at different temperatures. *J. Biol. Chem.* **72**, 677-685.

Cameron, J. N. (1989). Acid-base homeostasis: past and present perspectives. *Physiol. Zool.* **62**, 845-865.

Heisler, N. (1986). Comparative aspects of acid–base regulation. In *Acid–Base Regulation in Animals* (ed. N. Heisler), pp. 397-450. Amsterdam: Elsevier.

Howell, B. J., Baumgardner, F. W., Bondi, K. and Rahn, H. (1970). Acid-base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600-606.

Jackson, D. C. (1971). The effect of temperature on ventilation in the turtle, *Pseudemys scripta elegans*. *Respir. Physiol.* **12**, 131-140.

Prosser, C. L. (1950). *Comparative Animal Physiology*. Philadelphia: W.B. Saunders.

Rahn, H. (1967). Gas transport from the external environment to the cell. In *Development of the Lung* (ed. A. V. S. de Reuck and R. Porter), pp. 3-23. London: Churchill.

Reeves, R. B. (1972). An imidazole alphastat hypothesis for vertebrate acid-base regulation: Tissue carbon dioxide content and body temperature in bullfrogs. *Respir. Physiol.* **14**, 219-236.

Robin, E. D. (1962). Relationship between temperature and plasma pH and carbon dioxide tension in the turtle. *Nature* **195**, 249-251.

Rosenthal, T. B. (1948). The effect of temperature on the pH of blood and plasma *in vitro*. *J. Biol. Chem.* **173**, 25-30.

Stinner, J. N., Nelson, D. L. and Heisler, N. (1994). Extracellular and intracellular carbon dioxide concentrations as a function of temperature in the toad *Bufo marinus*. *J. Exp. Biol.* **195**, 345-360.

Yapp, W. B. (1961). *An Introduction to Animal Physiology*. Oxford: Clarendon.

Wang, T., Abe, A. S. and Glass, M. L. (1998). Temperature effects on lung and blood gases in *Bufo paracnemis*: consequences of bimodal gas exchange. *Respir. Physiol.* **113**, 231-238.