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PROPERTIES OF BLOOD OXYGEN TRANSPORT IN THE TURTLE *PSEUDEMYS SCRIPTA* AND THE TORTOISE *TESTUDO GRAECA*: EFFECTS OF TEMPERATURE, CO_2 AND pH*

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Abstract. Properties of oxygen–haemoglobin binding have been investigated in the aquatic turtle *Pseudemys scripta* and the terrestrial tortoise *Testudo graeca*. Haematocrit (30–35%) and haemoglobin concentration (12–14 g/100 ml blood) were similar in both species. P_{50} at physiological levels of P_{CO_2} (20–25 mm Hg) was 21 mm Hg in *Pseudemys*, compared with 23 mm Hg in *Testudo*. The Bohr shift of the blood of both the turtle and the tortoise was almost identical at −0.28. The heat of oxygenation, ΔH, reflecting the temperature sensitivity of O_2–Hb affinity, was −10.55 in *Pseudemys* and −8.12 kcal/mol in *Testudo*.

These data on whole blood do not support previous generalizations in the literature suggesting marked differences in oxygen–haemoglobin binding between aquatic and terrestrial chelonian reptiles.

Bohr effect — Oxygen transport by blood
Diving reptiles — P_{50}
Oxygen affinity — Temperature

Lung ventilation in the aquatic turtle *Pseudemys* is characterized by 2-min to 2-hr periods of apnoea punctuated at irregular intervals by a series of closely spaced ventilatory movements (Belkin, 1964; Burggren, 1975). In contrast, in the terrestrial tortoise *Testudo* 30–60 sec periods of apnoea are interrupted at quite regular intervals by single breaths (Burggren, 1975). These animals have necessarily adapted physiologically to the constraints and demands on respiratory gas exchange that accompany such divergent breathing patterns and lifestyles.

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Properties of blood oxygenation have been extensively investigated from a comparative and adaptive viewpoint (see Johansen and Lenfant, 1972, for review), and O_2 availability, whether limited by the environment or by such patterns of intermittent lung ventilation, has been demonstrated to exert great selective pressure on both blood morphology and Hb–O_2 interactions. It might thus be anticipated that some particular aspect of the O_2 transport properties of the blood of the aquatic turtle or terrestrial tortoise has been more strongly selected for. Certainly Gaumer and Goodnight (1957) noted that haemoglobin concentrations tended to be higher in more aquatic Chelonia, and Lenfant *et al.* (1970) have suggested that lower blood O_2 affinities are to be found in aquatic compared to terrestrial chelonians. We have thus investigated the respiratory properties of the blood of the turtle *Pseudemys scripta* and the tortoise *Testudo graeca* to elucidate whether their blood respiratory properties reflect the marked differences in their physiology, behaviour and the environments which they inhabit.

Methods

Oxygen dissociation curves, haematocrit, haemoglobin concentrations and blood O_2 capacity were determined with fresh blood repeatedly sampled from chronically implanted cannulae in the femoral artery of 4 *Pseudemys scripta* and 6 *Testudo graeca*. Equal volumes of heparinized blood drawn from each of the turtles or the tortoises were routinely pooled to make up a blood sample for each species of approximately 7 ml, the volume required to make a single dissociation curve. All animals ranged in weight from 1.2 1.5 kg, and since total blood volume is approximately 90 ml/kg body weight in chelonians (Semple, 1960), up to 20 ml of blood could be collected from each animal over an 8–32 hr period without inducing a severe hypovolaemia in any individual. In any case, there was little change in haematocrit or blood O_2 capacity in the pooled samples or in any particular animal during the course of the blood sampling period (table 3).

Oxygen dissociation curves on whole blood were made utilizing a technique first described by Duvelleroy *et al.* (1970) and recently modified and described in detail by Hahn and Foëx (1975) and Hahn *et al.* (1976). The technique consists in principle of exposing a known volume of deoxygenated whole blood to a known volume of oxygen gas, and then dynamically monitoring the changes in blood and gas P_{O_2} which develop. Blood O_2 content (vol %) was calculated from these data by analogue instrumentation (Hahn and Foëx, 1975) and continuously plotted as a function of blood P_{O_2} on an X-Y recorder. A tangent to the line relating blood O_2 content and blood P_{O_2} at a high P_{O_2} (100–300 mm Hg) drawn back to the Y-axis was required to determine O_2 capacity at 100 % blood saturation, since the electrical correction for O_2 dissolved in human blood (Hahn *et al.*, 1976) was not adjusted for turtle blood during the present experiments.

A single 'run' with this apparatus yielded both the oxygen dissociation curve and

the blood O_2 capacity. Changes in the blood pH as the oxygen dissociation curve was drawn were also plotted on a second X-Y recorder. The P_{CO_2} of the blood sample was determined with a Radiometer CO_2 electrode and gas analyser before the blood sample went into the apparatus and after completion of the curve. Oxygen dissociation curves were determined at 20, 25 and 30 °C over a wide range of P_{CO_2} and pH. Blood was tonometered at the appropriate temperature with the desired N_2–CO_2 gas mixture for 20 min, after which time blood P_{CO_2} and gas P_{CO_2} were identical. A pooled blood sample was normally used in the construction of only one O_2 dissociation curve. Duplicate runs were initially performed, however, and confirmed the highly reproducible performance of the apparatus. O_2 dissociation curves from representative runs on individual pooled blood samples constitute the data presented in the figures.

Results

Data on haematocrit, blood haemoglobin concentration, and mean corpuscular haemoglobin concentration determined for *Pseudemys scripta*, *Testudo graeca* and other chelonian reptiles are presented in table 1.

Blood O_2 dissociation curves determined at 25 °C under near-identical conditions of P_{CO_2} and pH are illustrated for *Pseudemys scripta* and *Testudo graeca* in fig. 1. The dissociation curve of the turtle has a slightly more hyperbolic shape than in the tortoise. The actual shape of an O_2 dissociation curve is determined to a large extent by the degree of haem–haem interaction. This interaction can be expressed by 'n', which is the slope of the line on a Hill plot of log $SO_2/(100-SO_2)$ as a function of log P_{O_2}. The more sigmoid the curve, the higher the 'n' value. 'n' is 2.01 for *Testudo graeca* compared with 1.71 in *Pseudemys scripta* (fig. 2, table 2).

TABLE 1
Selected haematological properties of chelonian blood

Species	Haematocrit (%)	Haemoglobin (g/100 ml blood)	Mean corpuscular Hb concentration (g/100 ml RBC)	Reference
Pseudemys scripta	30.5	11.8	38.7	Present investigation
Pseudemys scripta	35.0	11.1	31.6	Gaumer and Goodnight (1957)
Pseudemys elegans	23.5	5.7	24.2	Sheeler and Barber (1964)
Chrysemys picta	27.5	10.1	36.6	Gaumer and Goodnight (1957)
Chelys fimbriata	26.0	6.4	24.6	Lenfant *et al.* (1970)
Testudo graeca	34.5	14.4	41.8	Present investigation
Terrapene carolina	24.9	11.2	45.0	Gaumer and Goodnight (1957)

Aquatic species constitute the upper group of this table, while data for terrestrial chelonians are given in the lower group.

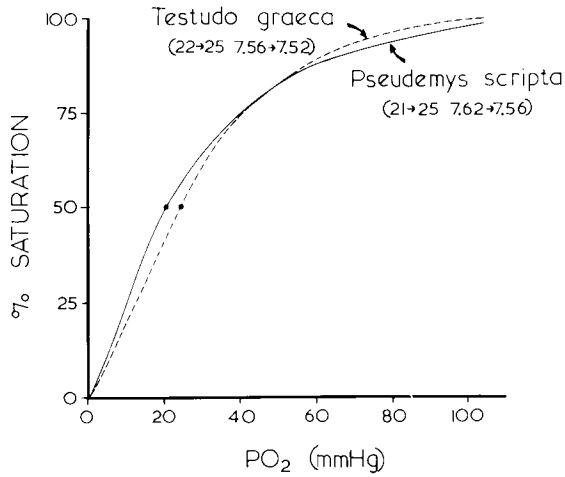

Fig. 1. Oxygen dissociation curves of fresh whole blood at 25 C from *Pseudemys scripta* and *Testudo graeca*. The first set of figures in brackets refers to the change in partial pressure of CO_2 in mm Hg from the beginning of the construction of the curve (0% saturation) to the finishing of the curve (100% saturation). Similarly, the second set of figures refers to changes in blood pH from the beginning to the end of construction of the oxygen dissociation curve.

Blood oxygen capacity in *Pseudemys scripta* (8.7 vol. %) was slightly lower than in *Testudo graeca* (9.8 vol %). The oxygen affinity of the blood of *Pseudemys scripta* (P_{50} = 21 mm Hg at P_{CO_2} = 22 mm Hg) was just slightly higher than in *Testudo graeca* (P_{50} = 23 mm Hg at P_{CO_2} = 22 mm Hg). Oxygen dissociation curves of *Pseudemys scripta* compared favourably with blood oxygen affinities reported for other members of this genus under similar (though non-physiological) conditions of P_{CO_2} and pH (table 2). Figure 3 presents whole blood O_2 dissociation curves for the turtle and tortoise made under conditions of constant temperature (25 C), but with varying initial values of P_{CO_2}. Table 3 is an accompaniment to these curves, and documents the blood parameters pertaining to each O_2 dissociation curve. Oxygen affinity of the blood of both species progressively decreased as P_{CO_2} increased and pH decreased. This phenomenon can be quantified by determining the Bohr shift, where:

$$\text{Bohr shift} = \frac{\Delta \log P_{50}}{\Delta \text{ pH (at } S_{O_2} = 50\%)}$$

A plot of log P_{50} as a function of pH for blood from *Pseudemys scripta* and *Testudo graeca* is presented in fig. 4. The Bohr shifts, represented by the slopes of the lines in fig. 4, are virtually identical in these two chelonians. No tendency for the development of a reduced O_2 capacity with decreasing pH (Root effect) was evident in the blood of either *Pseudemys scripta* or *Testudo graeca* (table 3).

Oxygen dissociation curves for whole blood from the turtle and tortoise determined

TABLE 2
Respiratory properties of chelonian blood

Species	T (°C)	P_{50} (mm Hg)	O_2 capacity (vol %)	'n'	Bohr shift	ΔH (kcal mol)	Conditions of P_{CO_2} and pH under which values determined	References
Pseudemys scripta	25	24.5	8.7	1.71	−0.275	−10.55	P_{CO_2} 37 mm Hg	Present investigation
Pseudemys scripta	25	16	–	–	–	–	P_{CO_2} 40 mm Hg	Gaumer and Goodnight (1957)
Pseudemys scripta	15–20	–	–	–	–	−13.0	Average of values between pH 6.0 and 7.0 (Hb solution)	Sullivan and Riggs (1967)
Pseudemys concinna	25	19.5	6.6–10.8	–	–	–	P_{CO_2} 40 mm Hg	Southworth and Redfield (1926)
Chrysemys picta	25	14	6.5 6.8*	–	–	–	P_{CO_2} 40 mm Hg	Gaumer and Goodnight (1957)
Chelydra serpentina	25	28	–	–	–	–	P_{CO_2} 40 mm Hg	Gaumer and Goodnight (1957)
Chelydra serpentina	15 20	–	–	–	–	− 9.4	Average of values between pH 6.0 and 7.0 (Hb solution)	Sullivan and Riggs (1967)
Chelys fimbriata	26	26	10.1	2.65	−0.56	–	pH 7.6	Lenfant *et al.* (1970)
Pelomedusa subrufa	–	40	8.3	3.13	−0.45	− 5.50	Not specified	Wood and Johansen (1974)
Testudo graeca	25	28	9.8	2.01	−0.285	− 8.12	P_{CO_2} 40 mm Hg	Present investigation
Testudo pardalis	25	32	10.0	2.50	−0.30	− 5.87	pH 7.4	Johansen and Burggren (unpublished)
Testudo tabulata	26	13	–	–	–	–	Not specified	Lenfant *et al.* (1970)
Terrapene carolina	25	12	5.4 6.3*	–	–	–	P_{CO_2} 40 mm Hg	Gaumer and Goodnight (1957)

* Payne and Burke (1964).

All data are for fresh, whole blood unless indicated otherwise. Aquatic species constitute the upper group in this table. Data for terrestrial chelonians are given in the lower group.

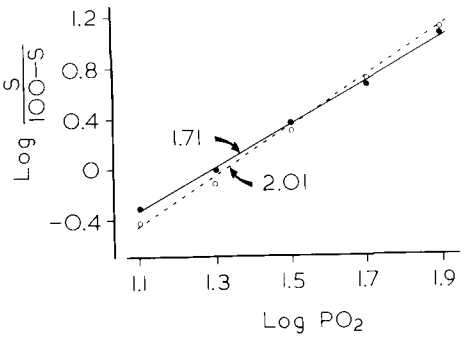

Fig. 2. A Hill plot derived from data on whole blood at 25 C from *Pseudemys scripta* (solid circles) and *Testudo graeca* (open circles). The S value on the ordinate represents the percent saturation of the blood. The indicated slope of the linear regression line drawn for each set of data is expressive of 'n' (see text). The coefficient of correlation for both linear regression lines is 0.99.

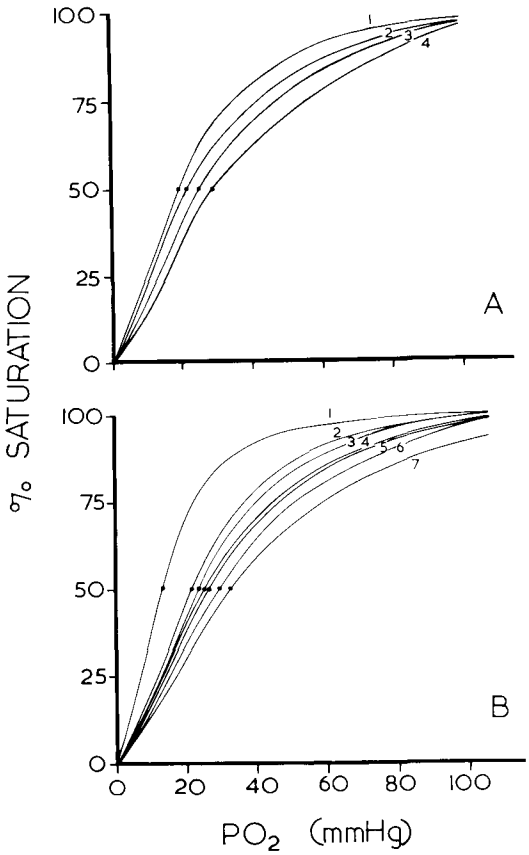

Fig. 3. Oxygen dissociation curves of whole blood at 25 C (A) from *Pseudemys scripta* and (B) from *Testudo graeca*, determined over a wide range of P_{CO_2} and pH. Ascending curve numbers reflect an increasing blood P_{CO_2}. Table 3 should be consulted for details of experimental conditions pertaining to each curve.

TABLE 3

Blood parameters pertaining to the oxygen dissociation curves presented in fig. 3.

Species	Curve number	P_{50}	Initial P_{CO_2}	Final P_{CO_2}	Initial pH	Final pH	Haematocrit (%)	Haemoglobin (g 100 ml blood)	O_2 capacity (vol %)
Pseudemys scripta	1	19.5	15	–	7.74	7.65	–	–	8.2
	2	21.5	22	–	7.62	7.55	–	–	9.2
	3	24.5	37	–	7.50	7.34	–	–	8.9
	4	28.5	73	–	7.11	7.05	–	–	10.8
Testudo graeca	1	13.0	0	0	8.24	8.13	–	–	10.3
	2	21.5	15	19	7.68	7.65	33.5	13.6	9.6
	3	23.5	22	25	7.56	7.52	34.5	14.4	9.8
	4	25.0	28	30	7.48	7.45	32.0	13.1	9.5
	5	26.0	32	34	7.47	7.41	32.5	13.0	9.6
	6	29.5	43	45	7.34	7.31	33.0	13.1	9.8
	7	32.5	54	56	7.22	7.21	32.0	11.7	10.6

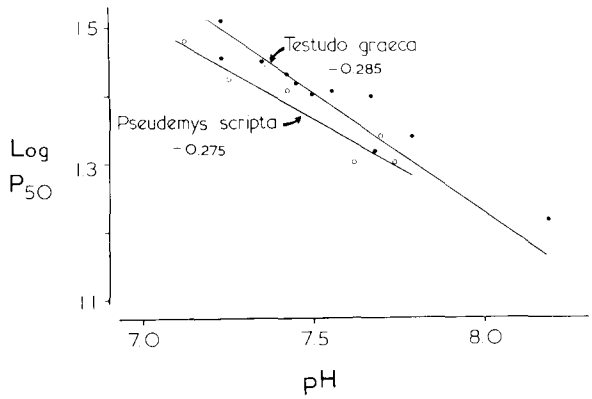

Fig. 4. Relationship between oxygen affinity and pH of whole blood at 25 °C from *Pseudemys scripta* and *Testudo graeca*. The indicated slope of the regression lines is expressive of the Bohr shift.

under conditions of very similar P_{CO_2} and pH, but at 20, 25 and 30 °C, are given in fig. 5. The temperature coefficient of oxygen affinity ($\Delta \log P_{50}/\Delta T$) was approximately 0.018 in *Pseudemys scripta* and 0.017 in *Testudo graeca*. Temperature effects on blood oxygen affinity can be alternatively quantified by calculating the apparent heat of oxygenation, ΔH (kcal/mol), from the van 't Hoff equation (Wood and Johansen, 1974), and values of this parameter for several chelonians are given in table 2. Blood oxygen capacity was not affected over the temperature range of 20–30 °C in either *Pseudemys scripta* or *Testudo graeca*.

Discussion

Data from this investigation as well as previous studies (table 1) indicate no correlation of blood haematology with a terrestrial or aquatic lifestyle. Meaningful comparisons of published haematological data may, however, be frustrated by seasonal variations in haematocrit and haemoglobin in reptiles (Kaplan and Rueff, 1960; Banerjee and Banerjee, 1969). As a result of small differences in their respective haematocrits and haemoglobin concentrations, blood O_2 capacity in *Testudo graeca* is approximately 10 % higher than in *Pseudemys scripta* (table 2). A high blood O_2 capacity could be expected to have considerable adaptive advantage in reptiles diving for long periods of time. However, diving lizards generally have similar blood O_2 capacities to those of terrestrial forms (Wood and Johansen, 1974), and the data on chelonian blood O_2 capacity presented in table 2 similarly show no apparent tendency for elevated O_2 capacity in diving forms.

McCutcheon (1947), working with stripped haemoglobin solutions, noted that the blood of more active, marine chelonians had a lower O_2 affinity than did freshwater species, which in turn was still lower than in terrestrial forms. McCutcheon

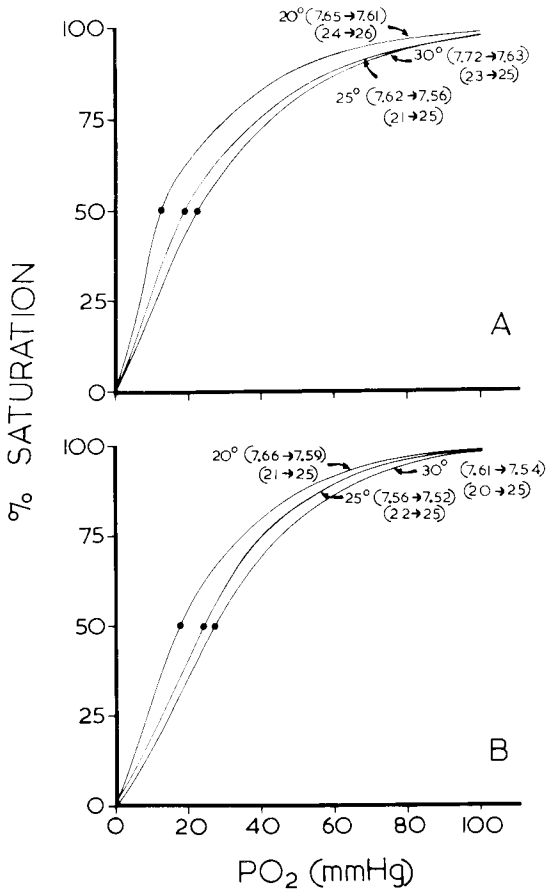

Fig. 5. Effect of temperature on the oxygen dissociation curves of whole blood (A) from *Pseudemys scripta* and (B) from *Testudo graeca*. Initial and final values of blood P_{CO_2} and pH are indicated as explained in the legend accompanying fig. 1.

evoked an argument based on an activity gradient from marine to terrestrial chelonian species, and suggested that a low affinity haemoglobin which unloaded O_2 at a high P_{O_2} best suited the needs of an actively swimming marine turtle. Sullivan and Riggs (1967) reported that over the physiological range of pH 6.5–7.5 there was a tendency for the stripped haemoglobin solutions of terrestrial Chelonia to have a higher O_2 affinity, but there were many exceptions among the 50 species and subspecies examined. Lenfant *et al.* (1970) also reported higher O_2 affinities in terrestrial chelonians, but in their brief survey these authors did not consider all available data. In fact, when the present data from the high CO_2 runs and previously published data for the respiratory properties of chelonian fresh whole blood determined under very similar conditions of temperature, P_{CO_2} and pH are compared (table 2), a correlation of Hb–O_2 affinity with a terrestrial or aquatic lifestyle fails to emerge. It must be emphasized that the data presented in table 2 should be treated with some reservation.

While these O_2 dissociation curves have been constructed over physiological ranges of temperatures, the employed partial pressures of CO_2 were much higher than is now known to occur normally *in vivo* (Lenfant *et al.*, 1970; Burggren, 1976) and blood pH's were correspondingly too low. Much previously published chelonian O_2–Hb affinity data is now of limited use in making analysis of *in vivo* gas transport.

The Bohr shift was virtually identical in both the turtle and tortoise, and at approximately -0.28 was somewhat lower than has been reported for many other intermittently breathing reptiles (Wood and Johansen, 1974; Johansen and Lenfant, 1972). A prolonged dive by *Pseudemys scripta* of 25 min, which would produce an increase in Pa_{CO_2} from approximately 24 to 31 mm Hg (Burggren, 1976), would result in a shift to the right in P_{50} of only 1 2 mm Hg. In the more regularly breathing *Testudo graeca* changes in arterial P_{CO_2} during even the longest periods of apnoea are almost insignificant (Burggren, 1976), and changes in Hb–O_2 affinity during intermittent breathing in this tortoise will be accordingly small. Hence it would appear that the Bohr shift must play only a small role in modifying Hb–O_2 affinity as apnoea progresses in these chelonians.

Sullivan and Riggs (1967) reported that the apparent heat of oxygenation of haemoglobin solutions from 12 chelonian species varied between 9 and 14 kcal/mol. The O_2 affinity of the Nile monitor *Varanus niloticus* is comparatively insensitive to temperature, for $\Delta H = -3$ kcal/mol in this lizard (Wood and Johansen, 1974). These authors suggest that in this instance there is an adaptive importance in having a small temperature effect on Hb–O_2 affinity, for the body temperature of diving lizards may drop precipitously when sun basking is followed by entry into water (Moberly, 1968). Basking behaviour followed by diving also occurs in *Pseudemys* in its natural environment, yet the temperature sensitivity of Hb–O_2 affinity in this turtle is much greater than in the Nile monitor (table 2). While a higher temperature reduces the oxygen affinity of the blood, in poikilotherms it also increases the metabolic rate. Hence it could be equally argued that a great Hb–O_2 affinity temperature sensitivity might promote a greater unloading of O_2 from the blood to the tissues when O_2 consumption becomes elevated by a rise in body temperature.

In light of the foregoing discussion, what adaptive modifications are apparent in the respiratory blood properties of *Pseudemys scripta* and *Testudo graeca*? In point of fact, very few. Oxygen affinity is higher in the turtle than in the tortoise, but the differences in P_{50} are small compared to the differences in P_{50} between swamp and river fishes (Willmer, 1934), water and air breathing amphibians (Lenfant and Johansen, 1967; Johansen and Lenfant, 1972), low and high altitude mammals (Hall *et al.*, 1936) or fetal and adult animals (Metcalfe *et al.*, 1967). Values of Hill's 'n' are slightly lower in *Pseudemys scripta* than in *Testudo graeca*, but the O_2 dissociation curves of both animals are of similar shape when compared to the variations among the chelonian reptiles. Similarly the Bohr effect, the haematological properties of the blood and blood O_2 capacity vary little between these two animals compared to the wide range among other species. There is a difference between *Pseudemys scripta* and *Testudo graeca* in the temperature sensitivity of Hb–O_2 binding, but, as outlined

above, the adaptive advantages of modifications in this blood property remain equivocal at present. In summary, then, the data presented in this study indicate that the evolution of very distinct patterns of intermittent lung ventilation and gas exchange in aquatic as opposed to terrestrial chelonian reptiles does not necessitate striking physiological adaptations in blood respiratory properties.

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