

BLOOD GASES OF THE WATER SNAKE, *NATRIX TESSELLUTA*, AND THE DESERT SNAKE, *ERYX COLUBRINUS*

By

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ABSTRACT

1. This comparative study examines O₂ dissociation curves of blood of two colubrine snakes: an aquatic snake, *Natrix tessellata*, and a desert snake, *Eryx colubrinus*.
2. In the case of the whole blood of the aquatic snake, oxygen capacity was higher and P₅₀ was much lower than in that of the desert snake.
3. Measured P₅₀ value of the aquatic snake was nearly the same as that estimated from the equation postulated by Pough (1977), while P₅₀ value of the desert snake was somewhat higher and the constants of the equation had to be changed into P₅₀(mmHg) = 30.54 + 11.92 log W gm).
4. The O₂ dissociation curves of both snakes shifted to the left by low temperature and to the right by high temperature. At low temperature, the rates of shifting ($\Delta P_{50} / \Delta ^\circ C$) were nearly the same in both snakes, while at high temperature, the shifting was much greater in the case of the desert snake.
5. Regarding the Bohr effect, $\Delta P_{50} / \Delta \text{pH}$ was nearly the same at low pH, while at high pH, the rate of shifting of O₂ dissociation curve of the aquatic snake was much higher than that of the desert snake.

INTRODUCTION

Intraspecific differences in oxygen affinity of blood are correlated with body weight in all classes of vertebrates (Burke, 1966). In the case of colubrine snakes, Pough (1977) has calculated the least squares regression of this relationship as $P_{50}(\text{mmHg}) = 21.54 + 7.39 \log W(\text{gm})$.

Snakes, as poikilothermic animals, do not maintain a constant pH as their body temperature changes with that of the environment. The effect of temperature and pH shows variation on their blood oxygen affinity. Such an effect is correlated to the degree of thermophily of the animal; the most thermophilic species had the greatest oxygen affinity (Pough, 1969).

The few previous studies on snakes showed that of blood aquatic species has higher oxygen affinity and greater Bohr effect than that of terrestrial ones (Sullivan, 1967; Greenwald, 1971 and Standaert & Johansen, 1974). The high affinity promotes the utilization of pulmonary oxygen stores during diving and a rapid resaturation of blood during the short breathing periods at the water surface, while the great Bohr effect provides a high unloading of oxygen to tissues during periods of breathholding (Wood & Lenfant, 1976 and Seymour *et al.*, 1981).

The present investigation reports a comparative study of respiratory functions of blood of two colubrine snakes: the dice snake *Natrix tessellata* and a desert snake *Eryx colubrinus*. The dice snake is very aquatic in its habits and spends most of the time in streams and still water, lying on the bottom (Stidworthy, 1969), while the desert snake burrows in non-indurated sand and is widely distributed over the arid areas of Africa (Marx, 1968). The aim of this study is to elucidate the correlation between the affinity of blood for oxygen and the adaptation of the snakes to their habitats. Data are presented on O₂ dissociation curve as a function of body weight, thermal acclimation and pH.

Methods and Technique

The dice aquatic snake *Natrix tessellata* and the desert snake *Eryx colubrinus* are brought from the field, and maintained at laboratory temperature ($25 \pm 3^\circ\text{C}$) and normal day light conditions. The aquatic snakes were kept in aquaria with some water and the desert snakes were kept in wooden boxes with some sand.

Sampling of Blood

All measurements were carried out within two weeks of snake capture. Snakes were anaesthetized with ether and left for some time (10 minutes) for renewal of normal lung ventilation. A sample of blood from each snake was drawn from the heart into a heparinized syringe and one drop of 1% sodium fluoride was added for every 3ml. of blood to reduce the rate of glycolysis and to stabilize the pH. Blood was then kept on ice and the following measurements were carried out:

- a) Blood Gases: for blood gas analysis, the Corning 166 pH blood gas analyzer was used to measure pH, PO₂ and PCO₂. Each determination required only 0.15 - 0.2 ml. blood.
- b) Oxygen Capacity: The O₂ capacity of the blood was measured by fully oxygenating the sample with air at 25°C for 30 minutes equilibrium period, and then the sample was analyzed for O₂ content as partial pressure (mmHg) of oxygen (PO₂). In eighteen out of twenty instances, O₂ capacity changed less than 10% during the course of the experiment.
- c) O₂ dissociation Curve: 2 ml. of the agitated blood were drawn into a 30 ml. graduated syringe, followed by successive amounts of nitrogen, oxygen and carbon dioxide appropriate to the required partial pressures. The needle of the syringe was closed with a short blind length of small-bore rubber tubing and the syringe was rotated for 15 minutes at 37°C in a water bath. Care was taken to ensure that the plunger of the syringe was quite free to move inside the barrel, so that the gas was at atmospheric pressure. After 15 minutes, the syringe was removed from the water bath and a fresh gas mixture of the same composition was introduced into the syringe and the blood re-equilibrated for another 20 minutes. The use of a two-stage equilibrium facilitated control of the final partial pressure in various stages, with the gas-to-blood volume ratio employed (14:1). The pH of the blood was measured and sufficient N-NaOH was added to ensure that the pH would be 7.0, 7.3 and 7.5 in subsequent equilibration to study the Bohr effect on the oxygen dissociation curve.

The gas PO₂ and PCO₂, with which the blood had been brought into equilibrium, was transferred from the syringe into the sample chamber of the pH blood gas analyzer. Each determination is the mean of at least five measurements of PO₂ content.

d) Effect of Temperature: Snakes were divided into three batches, each of which was put in a plastic container which was in turn put in a separate (Lotus) incubator. Each of the three incubators was adjusted at one of the three chosen temperatures, i.e. 10, 25 and 35°C.

RESULTS AND DISCUSSION

Construction of O₂ dissociation curves of blood of both snakes (Fig. 1) showed that at laboratory temperature (25±3°C) and pH = 7.3, the curve of the dice aquatic snake *Natrix tessellata* is located to the left of that of the desert snake *Eryx colubrinus*. The oxygen capacity values were found to be 171 and 157 mmHg, and P₅₀ values were 35±0.34 and 53±0.48 mmHg for the aquatic snake and the desert snake respectively. Such figures reflect the great affinity of blood of the aquatic snake to oxygen in order to promote rapid reoxygenation of its blood during the short breathing periods at the water surface.

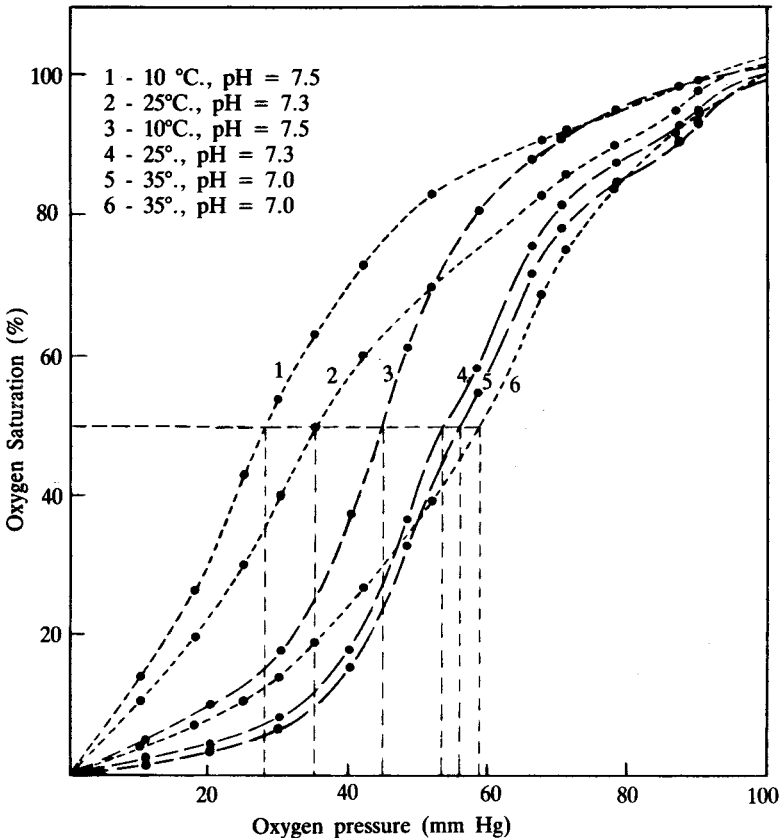


Figure 1: O₂ dissociation curves of the water snake, *Natrix tessellata* (1, 2, 6) and the desert snake, *Eryx colubrinus* (3, 4, 5) at different temperatures and pH.

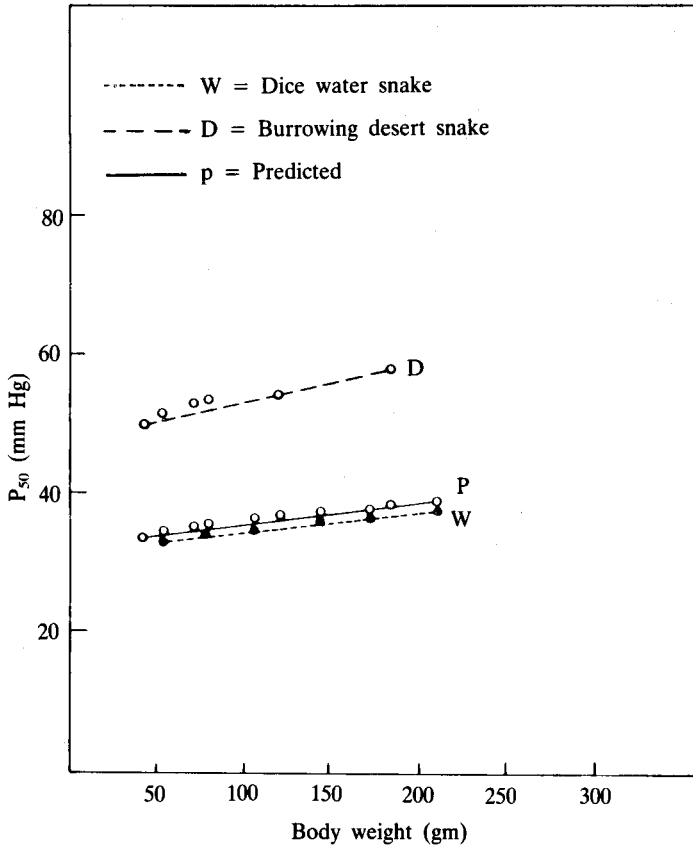


Figure 2: The relationship between the half saturation of blood with oxygen (P_{50}) and body weight of the water snake, *Natrix tessellata* and the desert snake, *Eryx colubrinus*.

a) Oxygen affinity and body weight: Regarding the correlation between oxygen affinity of blood and body weight of the colubrine snakes previously postulated by Pough (1977), it is clear from figure (2) that the measured P_{50} value of the aquatic snake is nearly the same as that estimated from the equation. On the other hand, the P_{50} value of the desert snake is somewhat higher than the one predicted and the constant of the equation have to be changed to P_{50} (mmHg) = $30.54 + 11.92 \log W$ (gm).

b) Effect of temperature: The effect of various temperatures (10, 25 and 35°C) on the oxygen dissociation curves was studied and showed that the curves were shifted to the left by low temperature and to the right by high temperature (Fig. 1). At a low temperature, the rate of shifting of the curves of both snakes was nearly the same ($\Delta P_{50}/\Delta^\circ\text{C} = 0.46$ and 0.7 for the aquatic and desert snake respectively). On the other hand, at a high temperature of 35°C, the

O₂ dissociation curve of the aquatic snake showed a much higher rate of shifting ($\Delta P_{50} / \Delta ^\circ\text{C} = 2.4$) than that of the desert snake ($\Delta P_{50} / \Delta ^\circ\text{C} = 0.3$). This variation in the rate of shifting may reflect the adaptation of both snakes to their habitats: while the aquatic snakes do not expose themselves to high temperatures in their natural habitat, the desert snake is well adapted to the climatic conditions prevailing in the desert.

Table 1

Effect of temperature and pH on the half saturation (P_{50}) of blood with oxygen of the aquatic snake *Natrix tessellata* and the desert snake *Eryx colubrinus*.

Species	P^*_{50} (mmHg)			$\Delta P_{50} / \Delta ^\circ\text{C}$		$\Delta P_{50} / \Delta \text{pH}$	
	10°C (pH=7.5)	25°C (pH=7.3)	35°C (pH=7.0)	10-25°C	25-35°C	pH= 7.3-7.5	pH= 7.0-7.3
Aquatic snake	28±0.34	35±0.34	59±0.35	0.46	2.40	35	80
Desert snake	44.5±0.31	53±0.48	56±0.45	0.70	0.30	42.5	10

*Mean of 12 animals \pm standard error.

Bohr effect: The O₂ dissociation curves of both snakes were studied with respect to different pH values attained at the studied temperatures (Fig. 1). It was found that high pH values (attained at 10°C) shifted the O₂ dissociation curves of both snakes to the left; i.e. decreased the oxygen affinity, while the low pH values (attained at 35°C) shifted them to the right, i.e. increased oxygen affinity. The Bohr effect and temperature effect act in concert to fix the position of the dissociation curves. Such an observation was previously reported in blood of various turtles by Sullivan and Riggs (1967).

The rate of shifting of O₂ dissociation curves (Table 1) of both snakes acclimated to low temperature and at pH 7.5 is nearly the same. On the other hand, at high temperature (35°C) and at pH 7, the rate of shifting in the aquatic snake is much higher than that in the desert snake ($\Delta P_{50} / \Delta \text{pH} = 80$ and 10 respectively). The high Bohr effect gives the aquatic snake an advantage when exposed to a relative oxygen deficiency during diving and also balances the high affinity of its blood for oxygen. These observations were also reported by Standaert and Johansen (1974) in the case of the elephant trunk snake, *Acrochordus javanicus* and by Seymour *et al.* (1981) in the case of *Acrochordus arufurae*.

REFERENCES

- Burke, J.D. 1966. Vertebrate blood oxygen capacity and body weight. *Nature*, London, 212:46.
 Greenwald, O.E. 1971. The effect of temperature on the oxygenation of gopher snake blood. *Comp. Biochem. Physiol.*, 40 A:865.

- Marx, H. 1968.** Checklist of the reptiles and amphibians of Egypt. Special publication, NAMR U-3, Cairo, Egypt.
- Pough, F.H. 1969.** Environmental, adaptations in the blood of lizards. *Comp. Biochem. Physiol.*, 33:885.
- **1977.** The relationship between body size and blood oxygen affinity in snakes. *Physiol. Zool.*, 50 (2):77.
- Seymour, R.S.; G.P. Dobson and J.C. Baldwin 1981.** Respiratory and cardiovascular physiology of the aquatic snake, *Acrochordus arafurae*. *J. Comp. Physiol.*, 144-215.
- Standaert, T. and K. Johansen 1974.** Cutaneous gas exchange in snakes. *J. Exp. Zool.*, 191 (2)-169.
- Stidworthy, J. 1969.** Snakes of the world. Paulhamlyn, London, pp. 160.
- Sullivan, B. 1967.** Oxygenation properties of snake hemoglobin. *science*, N.Y., 157-1308.
- and **A. Riggs 1967.** Structure, function and evolution of turtle hemoglobins III - Oxygenation properties. *Comp. Biochem. Physiol.*, 23-459.
- Wood, S.C. and C.J. Lenfant 1967.** Biology of reptilia, edited by C. Gans and W.R. Dawson, Vol. 5, Academic press.

غازات الدم للثعبان المائي «ناتركس تسيللاتا»

والثعبان الصحراوي «ابركس كولابريناس»

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- يتناول هذا البحث دراسة مقارنة لمنحنى التفكك الأوكسجيني لدم ثعبانين من عائلة كولابرينيدي ، احدهما مائي (ناتركس تسيللاتا) والآخر صحراوي (ابركس كولابريناس) .
- وجد أن قابلية دم الثعبان المائي للانداء بالاوكسجين أكبر بكثير من دم الثعبان الصحراوي .
- بدراسة نصف نسبة التشبع بالاكسجين (P_{50}) لدم الثعبان المائي وجد أنها تساوي تقريباً نفس الكمية المحسوبة من القانون العام للثعابين ، ولكن في حالة الثعبان الصحراوي ، فإن الثوابت يجب أن تتغير .
- بتعرض الحيوانين لدرجة حرارة مرتفعة ، وجد أن منحنى التفكك الأوكسجيني يتجه إلى اليمين ، والعكس عند تعرضها إلى درجة حرارة منخفضة .
- بدراسة تأثير تغير الحالة الحامضية - القاعدية (تأثير بوهر) وجد أن التغير بسيط في منحنى التفكك الهيدروجيني عند درجة الحرارة المنخفضة . ولكنه واضح في حالة الثعبان المائي عنه في حالة الثعبان الصحراوي عند تعرضهما لدرجة حرارة مرتفعة .