The Journal of Experimental Biology 205, 3327–3334 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4579

Increased blood oxygen affinity during digestion in the snake *Python molurus*

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Accepted 5 August 2002

Summary

Many snakes exhibit large increases in metabolic rate during digestion that place extensive demands on efficient oxygen transport. In the present study, we describe blood oxygen affinity following three weeks of fasting and 48 h after feeding in the Burmese python (*Python molurus*). We also report simultaneous measurements of arterial blood gases and haematological parameters. Arterial blood was obtained from chronically implanted catheters, and blood oxygen-dissociation curves were constructed from oxygen-content measurements at known oxygen partial pressure (P_{O_2}) values at 2% and 5% CO_2. Arterial pH remained constant at approximately 7.6 after feeding, but digestion was associated with an approximately 6 mmol l^{-1} increase in [HCO_3^-], while CO_2 partial pressure (P_{CO_2}) increased from 2.21±0.13 kPa in fasted animals to 2.89±0.17 kPa at 48 h after feeding. Blood oxygen affinity *in vivo* was predicted on the basis of pH *in vivo* and the blood oxygen-dissociation curves obtained *in vitro*. The blood oxygen affinity *in vivo* increased during digestion, with P_{50} values decreasing from 4.58±0.11 kPa to 3.53±0.24 kPa. This increase was associated with a significant decrease in the red blood cell [NTP]/[Hb_4] ratio (relationship between the concentrations of organic phosphates and total haemoglobin) and a significant decrease in mean cellular haemoglobin content, which is indicative of swelling of the red blood cells. Our data for blood oxygen affinity and arterial oxygen levels, together with previously published values of oxygen uptake and blood flows, allow for a quantitative evaluation of oxygen transport during digestion. This analysis shows that a large part of the increased metabolism during digestion is supported by an increased venous extraction, while arterial P_{O_2} (Pa_{O_2}) and haemoglobin saturation do not vary with digestive status. Thus, we predict that venous P_{O_2} (Pv_{O_2}) is reduced from a fasting value of 5.2 kPa to 1.6 kPa during digestion.

Key words: blood oxygen binding, oxygen transport, arterial blood gases, acid–base balance, feeding, reptile, snake, *Python molurus*.

Introduction

A principal role of the blood is to transport oxygen and nutrients to the tissues while ensuring adequate removal of CO_2 and metabolic waste products. The affinity by which the blood binds oxygen must be sufficiently low to ensure unloading in the tissues, but the affinity must also be sufficiently high to attain loading in the gas-exchange organ (e.g. Willford et al., 1982; Brauner and Wang, 1997). This obviously entails a possible conflict between binding and unloading of oxygen and it seems particularly important that animals exhibit an optimal blood oxygen affinity when metabolic rate is increased. In this context, reptiles, particularly snakes, offer some interesting experimental possibilities because they exhibit large increments in metabolic rate during digestion of large meals that are similar to those observed during muscular exercise (e.g. Secor and Diamond, 1995; Andrade et al., 1997).

During exercise, reptiles often experience a metabolic acidosis resulting from lactic acid production that reduces blood oxygen affinity through the Bohr effect. The changes in acid–base status are opposite during digestion, where an alkalinisation of the blood occurs after net secretion of acid to the stomach lumen (the ‘alkaline tide’). Nevertheless, all species studied reduce the pH changes by increasing the partial pressure of CO_2 (P_{CO_2}) through a relative hypoventilation (see Wang et al., 2001a). Among reptiles, the possible changes of blood oxygen affinity during digestion have only been studied in *Alligator mississippiensis*. In this species, HCO_3^- binds directly to the haemoglobin molecule and acts to maintain a virtually constant blood oxygen affinity during digestion (Bauer et al., 1981; Weber and White, 1986; Busk et al., 2000a).

Pythons exhibit much larger metabolic increments during digestion than crocodilians, but a similar mechanism is not involved because oxygen binding of *Python* haemoglobin is insensitive to HCO_3^- (J. Overgaard and R. E. Weber, unpublished data). However, it is possible that altered acid–base status, the reduction in plasma [Cl^-] that attends the alkaline tide, possible changes in red blood cell volume and the concentration of phosphates might alter blood oxygen binding during digestion. In the present study, we examine the effects of digestion on blood oxygen-binding properties of the snake *Python molurus*. To do this, we compare oxygen-binding

properties of whole blood from fasting animals with that of snakes that were 48 h into digestion, because this is the time where oxygen consumption is maximal (Secor and Diamond, 1995; Overgaard et al., 1999). In addition, we performed simultaneous measurements of arterial blood gases, acid–base status, plasma ions and haematological parameters *in vivo*, which enable a prediction of blood oxygen binding *in vivo*. Finally, based on previous determinations of blood flows and metabolic rate during fasting and digestion (Secor et al., 2000), we predict blood oxygen transport and venous blood gases *in vivo*.

Materials and methods

Animals

Fourteen snakes (*Python molurus*, Linnaeus) were purchased from a commercial supplier and kept at Aarhus University in a 1.5 m × 0.8 m × 1.0 m container. A lamp provided a temperature range between 25°C and 35°C, and the snakes were maintained at a 12 h:12 h L:D cycle with free access to water and shelter. All animals appeared healthy and gained weight during captivity. They were fed mice or rats on a weekly basis, but food was withheld 2–3 weeks before commencing the experiments. At the time of experimentation, each snake weighed between 400 g and 3500 g (1189±312 g; means ± 1 S.E.M.).

Surgical procedure

The snakes were anaesthetised with halothane vapour until they ceased to exhibit reflexes when pinched. A ventro-lateral incision was made 5–6 cm anterior to the anus, and the dorsal aorta was exposed for occlusive cannulation with Bolab catheters (Lake Havasu, AZ, USA) containing heparinised Ringer solution. The catheter was pushed forward 2–3 cm into the aorta in the anterior direction, and the vessel was sealed firmly around the catheter with sutures. The incision was closed, and the catheter was externalised and secured to the skin with two or three sutures. The surgery normally took less than 20 min, and the animals spontaneously resumed voluntary breathing. They were allowed to recover for 24–48 h after surgery. The catheter was flushed daily to avoid blood clotting. After the experiments had been terminated, all animals were killed by an overdose of Nembumal (200 mg kg^{-1}).

Experimental protocol

After surgery, the snakes were placed in individual plastic containers kept in a climatic chamber maintained at 30°C, where they remained for the entire experiment and had free access to drinking water. After 24–48 h recovery from surgery, a 1 ml blood sample was taken for determination of blood gases, haematological parameters and plasma ions in fasting animals. In addition, 3 ml blood was sampled for construction of whole-blood oxygen-dissociation curves *in vitro*. All excess blood cells were re-infused with an appropriate volume of saline. After blood sampling from fasting animals, nine snakes were allowed to feed voluntarily on mice or rats until satiety; these animals consumed 15–35% of their own body mass (27±2%). Blood sampling for *in vivo* measurements and *in vitro* blood oxygen-dissociation curves was repeated 48 h after feeding, when the snakes were in a postprandial state. To investigate the effects of blood sampling, five snakes followed the same protocol but were not allowed to feed between samplings. These animals served as unfed controls.

Measurements of blood gases and haematological parameters in vivo

Blood pH was measured using a capillary pH electrode connected to a PHM 73 meter (Radiometer, Copenhagen, Denmark). Total blood oxygen content (C_{O_2}) was measured as described by Tucker (1967) using the correction pointed out by Bridges et al. (1979). Total CO_2 content of the plasma ($C\mathrm{pl}_{CO_2}$) was measured according to Cameron (1971), and plasma bicarbonate concentration ($[HCO_3^-]$) was calculated as $[HCO_3^-]=C\mathrm{pl}_{CO_2}-(P_{CO_2}\alpha_{CO_2})$, using an α_{CO_2} (CO_2 solubility in blood) value of 0.0366 mmol l^{-1} (Heisler, 1984). Arterial P_{CO_2} ($P\mathrm{a}_{CO_2}$) *in vivo* was calculated on the basis of the Henderson–Hasselbalch equation: $P\mathrm{a}_{CO_2}=C\mathrm{pl}_{CO_2}/[\alpha_{CO_2}(1+10^{(\mathrm{pH}-\mathrm{pK}')})]$. The apparent pK′ for *Python* plasma at 30°C was calculated using the rearranged Henderson–Hasselbalch equation on the basis of $C\mathrm{pl}_{CO_2}$, and the pH that was measured in the tonometers at known P_{CO_2} levels. Correlation analysis of these data resulted in the following relationship between pK′ and pH: pK′= –0.0763×pH+6.7283 (r=0.37); This value is in good agreement with that predicted on the basis of plasma ion composition (Heisler, 1984).

Arterial P_{O_2} ($P\mathrm{a}_{O_2}$) was measured at 30°C with an E5046-0 O_2 electrode (Radiometer, Copenhagen), and haematocrit (Hct) was determined as the fractional red blood cell volume after spinning blood in capillary tubes at 12000 revs min^{-1} for 3 min. Plasma chloride concentration ($[Cl^-]$) was measured using a CMT 10 chloride titrator (Radiometer, Copenhagen), and potassium and sodium concentrations of the plasma ($[K^+]$ and $[Na^+]$, respectively) were determined by flame photometry (FLM 3 Flame photometer, Radiometer, Copenhagen). Osmolality was determined by freezing point depression (Knauer semimicro osmometer; Berlin, Germany).

Construction of blood oxygen-dissociation curves

The freshly collected blood was divided in two Eschweiler tonometers (Kiel, Germany) at 30°C. Each oxygen-dissociation curve (ODC) was constructed *in vitro* at a constant P_{CO_2} (1.87 kPa and 4.67 kPa) from measurements of blood oxygen content at various P_{O_2} levels (Tucker, 1967). Gas mixtures were prepared by Wösthoff gas-mixing pumps (Bochum, Germany) and humidified in glass flasks. Initially, blood was equilibrated to a high P_{O_2} (30% O_2) for 35 min to measure oxygen-carrying capacity (full saturation). The P_{O_2} of the gas mixture was then reduced in steps. Blood was equilibrated to each oxygen level for a minimum of 35 min before measurements, and blood C_{O_2} was always determined

in duplicate. Haemoglobin-bound oxygen ($[HbO_2]$) was calculated as: $[HbO_2]=C_{O_2}-(P_{O_2}\alpha_{O_2})$, where α_{O_2} is the oxygen solubility in blood at 30°C (Christoforides and Hedley-Whyte, 1969). Haemoglobin-oxygen saturation was calculated as $[HbO_2]$ relative to the oxygen capacity measured at 30% O_2. Each ODC was established on the basis of three to four points, with HbO_2 saturations between 20% and 80%. The results were plotted in Hill plots ($\log(Y/1–Y)$ *vs* $\log P_{O_2}$), where Y is the fractional HbO_2 saturation, and a linear regression was applied to the individual data. Values for cooperativity (n) were estimated as the slopes of the regression lines, and P_{50} was determined as the P_{O_2} where $\log(Y/1–Y)=0$.

In all blood samples used for determination of oxygen-binding properties *in vitro*, we measured Hct, pH, $C\mathrm{pl}_{CO_2}$ and [NTP] (the concentration of organic phosphates in the blood) at 30% air (approximately at half saturation). [NTP] was determined spectrophotometrically using a Sigma kit (no. 366) (Sigma-Aldrich, Vallensbæk Strand, Denmark). Total haemoglobin concentration ($[Hb_4]$) was assumed to be equal to $[HbO_2]$ values at full saturation (30% O_2), and red blood cell haemoglobin concentration ($[Hb_4]_{RBC}$) was calculated using Hct and $[Hb_4]$.

In vivo blood oxygen affinity (P_{50}) and cooperativity (n) values were estimated from the Bohr factor ($\Phi=\triangle\log P_{50}/\triangle\mathrm{pH}$), and arterial pH was determined for each individual animal at 0 h and 48 h. From these values and the P_{O_2} measured *in vivo*, HbO_2 saturation *in vivo* could be estimated using the Hill equation: HbO_2 saturation $=(100(P_{O_2}/P_{50})^n)/(1+(P_{O_2}/P_{50})^n)$.

Statistical analysis

The results from 0 h and 48 h measurements were compared using paired *t*-tests (two-tailed) using $P\leq0.05$ as the level of statistical significance between treatments. All results are presented as means ± 1 S.E.M.

Results

Blood gas composition and haematological variables in vivo

Values for *in vivo* blood gases, acid–base parameters, plasma ions, osmolality and Hct are listed in Table 1. In general, arterial blood gas composition and acid–base status did not change with time in the unfed control animals sampled after 48 h, indicating that blood sampling *per se* did not alter arterial blood gas composition. Both unfed and fed snakes showed significant reductions in Hct and C_{O_2}, which may be attributed to blood sampling. Arterial P_{O_2} was not affected by digestive status. Plasma osmolality increased, although not significantly in the control animals.

Although arterial pH remained constant after feeding, digestion was associated with a significant increase in both $[HCO_3^-]$ and $P\mathrm{a}_{CO_2}$. Thus, $P\mathrm{a}_{CO_2}$ increased by approximately 0.7 kPa after feeding, and $[HCO_3^-]$ increased by approximately 6 mmol l^{-1}. Plasma osmolality increased significantly in the fed animals and was accompanied by a proportional increase in plasma $[Na^+]$ (Table 1) and a visually detectable ‘milky’ appearance of the plasma during digestion. Both plasma $[Cl^-]$ and plasma $[K^+]$ decreased significantly during the postprandial period.

Blood oxygen-binding characteristics and haematological variables in vitro

Mean values for oxygen-binding properties and haematological properties at 2% and 5% CO_2 are listed in Table 2 for both fed animals and unfed controls. High P_{CO_2} reduced pH and oxygen affinity in all individuals (Table 2). The calculated Bohr effects (Φ) increased significantly following feeding, but remained unchanged in unfed control animals (Table 2).

Blood obtained from digesting animals had a significantly higher pH at any given P_{CO_2} *in vitro*, indicating an alkaline tide *in vivo* (Tables 1, 2). There was also a small, but significant,

Table 1. In vivo *arterial blood gases, acid–base parameters and haematological parameters of fasting and digesting snakes (*Python molurus*)*

Experiment	Before feeding (0 h)	After feeding (48 h)	Control (0 h)	Control (48 h)
$P\mathrm{a}_{O_2}$ (kPa)	9.98±0.40 (9)	9.54±0.59 (9)	9.49±1.19 (5)	10.22±0.60 (4)
pHa	7.60±0.02 (9)	7.62±0.03 (9)	7.55±0.02 (5)	7.57±0.01(4)
$P\mathrm{a}_{CO_2}$ (kPa)	2.21±0.13 (9)	2.89±0.17 (9)*	2.88±0.27 (5)	2.70±0.20 (4)
$[HCO_3^-]_{pl}$ (mmol l^{-1})	17.0±0.7 (9)	23.3±1.2 (9)*	19.7±1.4 (5)	19.3±0.9 (4)
Hct (%)	19.2±0.8 (9)	16.5±0.7 (9)*	22.1±0.9 (5)	15.8±1.3 (5)*
C_{O_2} (mmol l^{-1})	3.62±0.17(9)	2.97±0.13 (9)*	3.83±0.44 (5)	3.10±0.45 (4)*
Osmolality (mOsm)	277.2±1.2 (9)	296.8±2.0 (9)*	273.0±1.8 (5)	286.3±4.3 (3)
$[Na^+]_{pl}$ (mmol l^{-1})	150.8±3.5 (9)	158.7±2.9 (9)	147.2±4.1 (4)	151.2±5.4 (3)
$[K^+]_{pl}$ (mmol l^{-1})	3.8±0.1 (9)	3.4±0.1 (9)*	3.7±0.1 (4)	3.9±0.2 (3)
$[Cl^-]_{pl}$ (mmol l^{-1})	109.1±1.7 (9)	99.0±1.8 (9)*	109.1±2.2 (5)	112.0±1.8 (3)

Data for snakes that were not fed but sampled at a 48 h interval are also included (unfed control).

$P\mathrm{a}_{O_2}$, arterial O_2 partial pressure; pHa, arterial pH; $P\mathrm{a}_{CO_2}$, arterial CO_2 partial pressure; $[HCO_3^-]_{pl}$, concentration of plasma bicarbonate; Hct, haematocrit; C_{O_2}, total concentration of oxygen in whole blood; $[Na^+]_{pl}$, sodium concentration in plasma; $[K^+]_{pl}$, potassium concentration in plasma; $[Cl^-]_{pl}$, chloride concentration in plasma.

Values are means ± S.E.M. (N).

* signifies statistical difference between 0 h and 48 h values at $P\leq0.05$.

Table 2. *Blood oxygen affinity* in vitro *at two* P_{CO_2} *levels (2% and 5% CO_2) in fasting and digesting snakes (*Python molurus*)*

Experiment	Before feeding (0 h)		After feeding (48 h)		Control (0 h)		Control (48 h)	
CO_2 level	2% CO_2	5% CO_2	2% CO_2	5% CO_2	2% CO_2	5% CO_2	2% CO_2	5% CO_2
pH	7.57±0.02 (9)	7.31±0.02 (9)†	7.65±0.03 (9)*	7.40±0.02 (7)†,*	7.63±0.02 (5)	7.34±0.02 (5)†	7.66±0.03 (4)*	7.37±0.02 (4)†
P_{50} (kPa)	4.67±0.07 (9)	5.36±0.10 (9)†	3.42±0.19 (9)*	4.43±0.31 (7)†,*	4.39±0.12 (5)	5.38±0.19 (5)†	4.40±0.19 (4)	5.38±0.23 (4)†
logP_{50}	0.67±0.01 (9)	0.73±0.01 (9)†	0.53±0.02 (9)*	0.64±0.04 (7)†,*	0.64±0.01 (5)	0.73±0.01 (5)†	0.64±0.02 (4)	0.73±0.02 (4)†
n	2.86±0.16 (9)	2.64±0.14 (9)†	2.42±0.17 (9)*	2.36±0.15 (7)	2.88±0.10 (5)	2.47±0.13 (5)†	2.47±0.20 (4)	2.50±0.02 (4)
Bohr effect (Φ)	–0.23±0.02 (9)		–0.36±0.03 (7)*		–0.31±0.02 (5)		–0.30±0.01 (4)	

Data for snakes that were not fed but sampled at a 48 h interval are also included (unfed control).

pH, pH measured in the tonometers; P_{50}, oxygen tension at half saturation; n, Hills coefficient for cooperativity of oxygen binding; Bohr effect (Φ), calculated as $\triangle\log P_{50}/\triangle$pH. Values are means ± S.E.M. (N).

* signifies statistical difference between values at 0 h and 48 h; † signifies statistical difference between values at 2% and 5% CO_2 within each experimental condition.

increase in pH at 2% CO_2 after 48 h in the unfed control animals, but this was not observed at 5% CO_2 (Table 2). Blood oxygen affinity was significantly increased at 48 h during the postprandial period with a reduction in P_{50} from 4.67 kPa to 3.42 kPa and 5.36 kPa to 4.43 kPa at 2% and 5% CO_2, respectively (Table 2). There were no changes in P_{50} with time in the unfed control animals.

The reductions in haematocrit and [Hb_4] observed *in vivo* were accompanied by similar reductions in the blood samples used for *in vitro* studies (Tables 1, 3). $[Hb_4]_{RBC}$ *in vitro* was significantly reduced from 4.8±0.1 mmol l^{-1} to 4.5±0.1 mmol l^{-1} after feeding, while there were no significant changes in the unfed control animals (Table 3). There was a significant decrease in the [NTP]/[Hb_4] ratio from 2.42±0.15 in fasting animals to 2.21±0.18 after feeding (Table 3). The [NTP]/[Hb_4] ratio also decreased with time in the unfed control animals, although this reduction was not significant (Table 3).

The influence of [NTP]/[Hb_4] and $[Hb_4]_{RBC}$ on blood oxygen affinity is depicted in Fig. 1, which shows the calculated logP_{50} for each animal at a pH of 7.6 (obtained from the individual Bohr effects).

Discussion

Arterial blood gases, acid–base status and plasma ions in vivo

The arterial blood gases and acid–base status of fasting and digesting animals (Table 1) are in good agreement with our previous study on *Python molurus* (Overgaard et al., 1999). Digestion was associated with increased plasma [HCO_3^-] and reduced plasma [Cl^-] following the net transfer of acid into the lumen of the stomach ('the postprandial alkaline tide'; Coulson et al., 1950; Coulson and Hernandez, 1983; Rune, 1965). Plasma [HCO_3^-] of the animals used for feeding experiments was lower than the level measured in the control group at 0 h (Table 1), but we have no explanation for this difference. In spite of this increase in base excess during digestion, arterial pH remained virtually unchanged during digestion because of a simultaneous increase in arterial P_{CO_2} (Table 1). The respiratory compensation of the metabolic alkalosis seems to occur in all reptiles and amphibians, whereby arterial pH is kept constant through ventilatory modulations and, thus, increments in arterial P_{CO_2} (Glass et al., 1979; Overgaard et al., 1999; Busk et al., 2000a, 2000b; Wang et al., 2001a).

Plasma osmolality increased significantly after digestion. The levels of amino acids and nutrients increase during digestion and might contribute to the increase in osmolality (Secor and Diamond, 1997), but plasma osmolality and plasma [Na^+] levels also tended to increase in unfed control animals (Table 1). Thus, it appears that the surgical procedure and/or blood sampling contribute to the increase in osmolality.

Earlier studies on *Python*, using either chronically cannulated animals (Overgaard et al., 1999) or heart puncture

Table 3. In vitro *haematological parameters in whole blood used for oxygen-dissociation curves of fasting and digesting snakes (*Python molurus*)*

Experiment	Before feeding (0 h)	After feeding (48 h)	Control (0 h)	Control (48 h)
Hct (%)	19.2±0.8 (9)	16.6±0.8 (9)*	21.8±0.7 (5)	16.6±2.4 (4)
[Hb_4] (mmol l^{-1})	0.93±0.04 (9)	0.74±0.03 (9)*	1.07±0.05 (5)	0.86±0.14 (4)
$[Hb_4]_{RBC}$ (mmol l^{-1})	4.83±0.09 (9)	4.50±0.15 (9)*	4.92±0.14 (5)	5.10±0.16 (4)
[NTP]/[Hb_4]	2.42±0.15 (9)	2.21±0.18 (9)*	2.60±0.13 (5)	2.45±0.12 (4)

Data for snakes that were not fed but sampled at a 48 h interval are also included (unfed control).

Hct, haematocrit; [Hb_4], total haemoglobin concentration; $[Hb_4]_{RBC}$, concentration of tetrameric haemoglobin in the red blood cells; [NTP]/[Hb_4], relationship between the concentrations of organic phosphates and total haemoglobin. Values are means ± S.E.M. (N). * signifies statistical difference between 0 h and 48 h values at $P \leq 0.05$.

of very large individuals (Secor et al., 2001), have reported a decrease in Hct following digestion. Our study shows similar reductions in both postprandial and unfed control animals, suggesting that this decrease results from blood sampling. Nevertheless, assuming a blood volume of 7% of body mass and removal of 5 ml blood kg^{-1}, blood sampling alone can only account for a reduction in Hct from 19.2% to approximately 17.8%. Hence, other processes must contribute to the changes in Hct, which is in apparent conflict with plasma osmolality. Recent studies show that large fluid shifts account for the increased intestinal mass (Starck and Beese, 2001), and pronounced water shifts between intra- and extracellular stores may occur during digestion. Clearly, this aspect needs further investigation. The decrease in Hct contrasts with the marked postprandial increases observed in dogs and amphibians (Kurata et al., 1993; Wang et al., 1995; Busk et al., 2000a); however, Hct does not change during digestion in alligators (Busk et al., 2000b). Increased oxygen-carrying capacity of the blood would be beneficial when metabolic demands are high, and increased Hct often occurs during exercise in mammals and other vertebrates (Nikinmaa, 1990).

As in previous studies on *Python* and other ectothermic vertebrates, $P_{a_{O_2}}$ was not affected by digestion (Wang et al., 1995; Overgaard et al., 1999; Busk et al., 2000a, 2000b). In contrast, using heart puncture, Secor and Diamond (1995) reported that $P_{a_{O_2}}$ decreases from 16.0 kPa to approximately 3.3 kPa following digestion in *Python*, but this sampling technique is likely to yield a mixture of arterial and venous blood. Hence, in spite of the relative hypoventilation that characterises the postprandial state (Overgaard et al., 1999; Secor et al., 2000), *Python* maintains a high $P_{a_{O_2}}$ during digestion.

Oxygen-binding characteristics

Blood oxygen affinity of fasting *Python* is within the range of the 42 snake species summarised by Pough (1977), including the family Boidae. The Bohr effect of fasting and digesting *Python* (Table 2) was slightly lower than the mean of –0.44 reported for nine snake species by Pough (1980). The Bohr effect of *Python* increased during the postprandial period (Table 2). This may be attributed to relative changes between plasma pH and red blood cell pH (pH_e and pH_i, respectively) following digestion. The decreased red blood cell content of phosphates (a non-diffusable anion) found in this study (Table 3) will increase $\triangle pH_i/\triangle pH_e$ as well as pH_i with elevated pH_e (Wood et al., 1978). Thus, it is possible that pH_i changed more for a given change in pH_e during digestion. In addition, decreased [NTP], together with increased pH_i, may have enhanced the specific effect of CO_2 on oxygen affinity (Duhm, 1976). This would increase the apparent Bohr effect in spite of the decrease in red blood cell [NTP].

Blood oxygen affinity increased markedly during digestion. This effect was present at both 2% and 5% CO_2 levels (Table 2) and also when the P_{50} of each individual animal was estimated at a pH of 7.6 using their respective Bohr effects. The increased affinity was associated with a decrease in the [NTP]/[Hb_4] ratio and a decreased haemoglobin concentration within the red blood cells (Fig. 1 and Table 3). Both of these variables are likely to contribute to the increased oxygen affinity, as a multiple linear regression analysis showed a positive correlation with $\log P_{50}$ ($\log P_{50}=0.134\times[Hb_4]_{RBC}+0.112\times[NTP]/[Hb_4]+0.595$; $r^2=0.62$). This regression analysis, however, does not exclude the possibility that other factors, such as red blood cell pH or [Cl^-], may contribute. The specific effect of [NTP]/[Hb_4] on the $\log P_{50}$ of 0.112 was lower than the values of approximately 0.2 reported for other snakes (Johansen and Lykkeboe, 1979; Ragsdale et al., 1995; Herman and Ingermann, 1996). An influence of cell volume on blood oxygen affinity has been demonstrated in a number of vertebrates. It is generally attributed to reduced interactions between phosphate and haemoglobin molecules but may also be caused by reduced haemoglobin–haemoglobin interactions (Nikinmaa, 1990). In *Python molurus*, it seems that reduced haemoglobin–haemoglobin interactions may indeed have a marked effect on blood oxygen affinity. Thus, haemoglobin solutions with an [Hb_4] of 0.05 mmol l^{-1} have a P_{50} of approximately 1.5 kPa at saturating phosphate concentrations (J. Overgaard and R. E. Weber, unpublished data), which is a considerably higher affinity than reported here for intact red blood cells, where the [Hb_4] is much higher.

Very little is known about the factors that regulate red blood cell phosphate concentrations in ectothermic vertebrates, but humoral factors are likely candidates (Tetens and Lykkeboe,

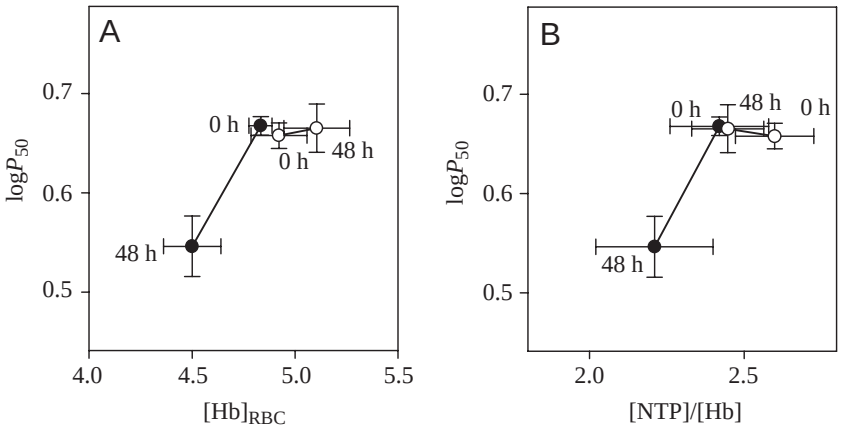

Fig. 1. The relationship between blood oxygen affinity calculated at a pH of 7.6, expressed as $\log P_{50}$, and (A) red blood cell haemoglobin content ($[Hb]_{RBC}$) as an indicator of cell volume and (B) the relationship between the concentrations of organic phosphates and total haemoglobin ([NTP]/[Hb_4] ratio) within the red blood cells. Open symbols represent data from unfed control snakes measured at 0 h and 48 h, while closed symbols represent data obtained from snakes before (0 h) and after (48 h) feeding. Data are presented as means ± 1 S.E.M. (N=9 for postprandial animals and N=4 or 5 for fasting controls).

Table 4. *Oxygen-binding parameters of whole blood estimated at* in vivo *pH in fasting and digesting snakes (*Python molurus*)*

Experiment	Before feeding (0 h)	After feeding (48 h)	Control (0 h)	Control (48 h)
P_{50} (kPa)	4.58±0.11 (9)	3.53±0.24 (9)*	4.65±0.09 (5)	4.68±0.26 (4)
logP_{50}	0.66±0.01 (9)	0.54±0.03 (9)*	0.67±0.01 (5)	0.67±0.03 (4)
n	2.84±0.18 (9)	2.37±0.15 (9)*	2.76±0.09 (5)	2.44±0.17 (4)
Saturation (%)	89.0±1.9 (9)	90.3±1.5 (9)	83.8±6.4 (5)	87.2±1.9 (4)

Data for snakes that were not fed but sampled at a 48 h interval are also included (unfed control).

P_{50}, oxygen tension at half saturation; n, Hills coefficient for cooperativity of oxygen binding. Saturation was calculated on the basis of oxygen-binding properties and *in vivo* P_{O_2}. Values are means ± S.E.M. (N).

* signifies statistical difference between 0 h and 48 h values at $P \leq 0.05$.

1981; Nikinmaa, 1990). In garter snakes *Thamnophis sirtalis*, it has been shown that progresterone increases [NTP]/[Hb_4] ratio and reduces oxygen affinity (Ragsdale et al., 1993). Catecholamines are known to decrease [NTP]/[Hb_4] in teleost red blood cells (Nikinmaa, 1990), but this mechanism has not been studied in reptiles, and it seems unlikely that digestion is associated with increased levels of circulating catecholamines (Wang et al., 2001b). In several vertebrate species, it is well established that the increased blood oxygen affinity during hypoxia correlates with reductions in [NTP]/[Hb_4] (Nikinmaa, 1990; Weber and Jensen, 1988). The low venous oxygen levels during digestion may be involved in the regulation of [NTP]/[Hb_4] ratio in *Python* but is unlikely to result from oxygen limitation to the red blood cell, as red blood cells maintain their [NTP]/[Hb_4] ratio when kept at low oxygen tension *in vitro* (Tetens and Lykkeboe, 1981).

Prediction of blood oxygen affinity and oxygen transport in vivo

P_{50} and cooperativity (n) were estimated at *in vivo* pH (Table 4) using the variation of P_{50} and n with pH for each snake. The estimated *in vivo* oxygen dissociation curves (ODCs) for fasted and digesting snakes are illustrated in Fig. 2. The P_{50} estimated at *in vivo* pH decreased in all nine animals after feeding by an average of approximately 1.07 kPa, while cooperativity decreased slightly (Table 4). The predicted saturation of arterial blood remained high during digestion and in control animals (Table 4).

In conjunction with previously published data on blood flows and rates of oxygen consumption, our data on oxygen-binding properties and arterial blood gases permit analysis of systemic oxygen delivery during fasting and postprandial conditions. In *Python*, systemic blood flow ($\dot{Q}_{sys}$) increases from a fasting value of 19 ml kg^{-1} min^{-1} to 85 ml kg^{-1} min^{-1} during digestion of a meal equivalent to 25% of body mass, while oxygen consumption increases from 0.8 ml O_2 kg^{-1} min^{-1} to 7.2 ml O_2 kg^{-1} min^{-1} (Secor et al., 2000); however, this study did not include measurements of blood flow in the carotid and vertebral arteries, so we have used a $\dot{Q}_{sys}$ of 25 ml kg^{-1} min^{-1} and 100 ml kg^{-1} min^{-1} in fasting and digesting snakes,

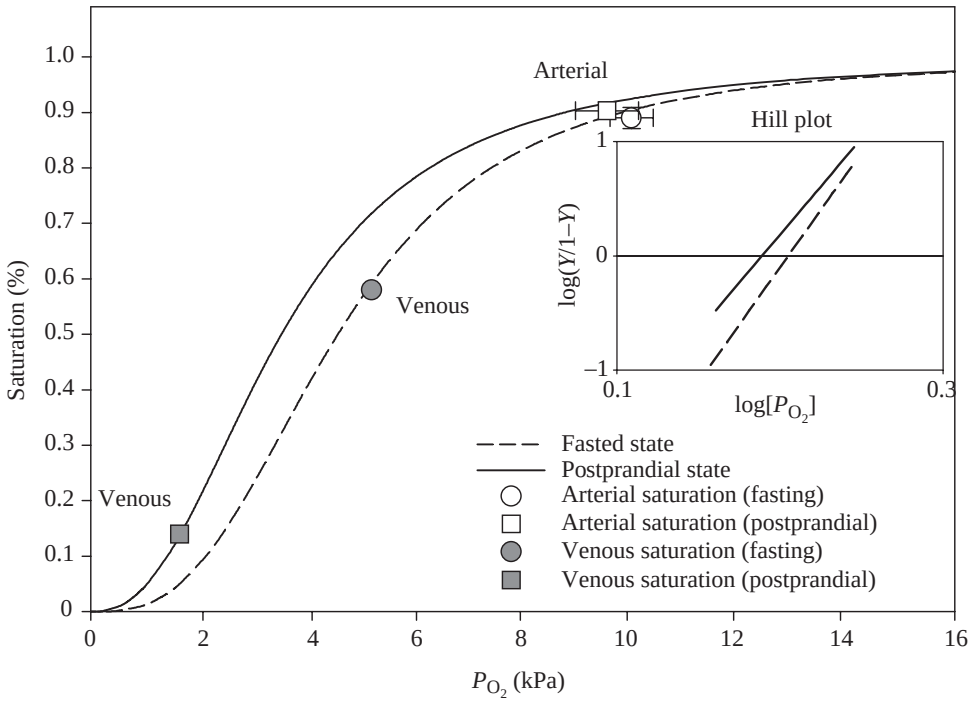

Fig. 2. Predicted blood oxygen dissociation curves *in vivo* for *Python molurus* while fasting (broken line) and during the postprandial period (solid line). These blood oxygen-dissociation curves were predicted on the basis of arterial pH *in vivo* as well as *in vitro* Bohr effects and blood oxygen affinities (see text for further explanation). The open symbols represent the measurements of arterial oxygen partial pressure (P_{O_2}) and haemoglobin-bound oxygen (HbO_2) saturations *in vivo* (given as means ± 1 S.E.M.), while closed symbols represent predicted venous values (circles and squares for fasting and postprandial snakes, respectively). The insert shows the corresponding Hill plots.

respectively, for predictions of oxygen transport. Assuming a blood oxygen-binding capacity of 4 mmol l^{-1} O_2 for both fasting and digesting snakes, we calculated venous HbO_2 saturation and P_{O_2} using $\dot{Q}_{sys}$, our predicted arterial HbO_2 saturation (Table 4) and the *in vivo* blood ODCs. This analysis predicts mean venous HbO_2 saturations of 58% and 14% in fasting and digesting animals, respectively, with corresponding venous P_{O_2} values of 5.2 kPa and 1.6 kPa, respectively (Fig. 2). It seems, therefore, that *Python molurus* extracts oxygen extremely efficiently during digestion, and, because our analysis predicts mixed venous oxygen levels, it must be expected that some tissues extract even more oxygen. No measurements of venous blood gases during digestion in *Python* appear to exist, but, given our predictions, it would certainly be worthwhile obtaining these measurements in future studies. Secor and Diamond (1995) report a P_{O_2} of approximately 3.3 kPa in blood obtained by cardiac puncture, indicating that venous P_{O_2} does indeed reach low values during digestion. In comparison, pulmonary Pa_{O_2} of the Savannah monitor *Varanus exanthematicus* is approximately 3.3 kPa during maximal exercise on a treadmill (Hopkins et al., 1995), and Pv_{O_2} decreases to approximately 2.7 kPa in hard-working human muscles (Pedersen et al., 1999).

In *Python*, the rate of O_2 uptake ($\dot{V}_{O_2}$) increases with meal size, and values in excess of 20 ml O_2 kg^{-1} min^{-1} have been reported (Secor and Diamond, 1997). Our estimated venous oxygen levels at a $\dot{V}_{O_2}$ of 7.2 ml O_2 kg^{-1} min^{-1} leave very little room for further oxygen extraction (Fig. 2). Hence, to sustain a $\dot{V}_{O_2}$ of 20 ml O_2 kg^{-1} min^{-1}, the snakes must increase $\dot{Q}_{sys}$ to values three times greater than those measured by Secor et al. (2000). Alternatively, Hct, and thus blood oxygen-carrying capacity, should be increased to 60%. This seems unlikely given that all studies on *Python* have documented slight decreases in Hct following feeding (Overgaard et al., 1999; Secor et al., 2001; Table 1).

The increased blood oxygen affinity in *Python* during conditions of elevated metabolic rate seems at odds with the general view that a right-shifted dissociation curve would favour unloading of oxygen in the tissues. In fact, as originally proposed by Weber and White (1986), the specific effect of HCO_3^- on haemoglobin oxygen binding in crocodilians compensates for the alkaline tide, such that blood oxygen affinity remains virtually unchanged during digestion (Busk et al., 2000b). Furthermore, Pough (1980) suggested that reptiles possess a low blood oxygen affinity to compensate for low capillary density and large diffusion distances. The low estimated Pv_{O_2} values during digestion (Fig. 2) do not, however, indicate that unloading is impaired. Furthermore, diffusion distances in gastrointestinal organs have not been reported in reptiles, so it is possible that unloading is adequately ensured in these organs. We have previously shown that there is no anaerobic contribution to the SDA response in *Python*, as plasma concentration of lactic acid does not increase (Overgaard et al., 1999). The increased affinity during digestion may, therefore, relate to the loading of O_2 within the pulmonary circulation. It is possible that saturation of pulmonary venous blood is reduced during digestion because of the decreased pulmonary transit time (caused by the elevated pulmonary blood flow) and the greatly reduced systemic Pv_{O_2}. This effect may be particularly important for reptilian lungs that have limited diffusive capacity (Hopkins et al., 1995; Glass, 1991). Under these circumstances, an increased blood oxygen affinity would enhance the P_{O_2} gradient across the lung epithelium and, thereby, increase oxygen saturation.

This study was supported by the Danish Natural Science Research Council. We are grateful to Prof. Roy E. Weber for thoughtful advice and comments.

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