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OXYGEN AND CARBON DIOXIDE TRANSPORT CHARACTERISTICS OF THE BLOOD OF THE NILE MONITOR LIZARD (*VARANUS NILOTICUS*)

BY JAMES W. HICKS*, ATSUSHI ISHIMATSU† AND NORBERT HEISLER

Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin, Göttingen, FRG

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SUMMARY

Oxygen and carbon dioxide dissociation curves were constructed for the blood of the Nile monitor lizard, *Varanus niloticus*, acclimated for 12 h at 25 and 35 °C. The oxygen affinity of *Varanus* blood was low when P_{CO_2} was in the range of *in vivo* values (25 °C: $P_{50} = 34{\cdot}3$ at $P_{CO_2} = 21$ mmHg; 35 °C: $P_{50} = 46{\cdot}2$ mmHg at $P_{CO_2} = 35$ mmHg; 1 mmHg = 133·3 Pa), and the oxygen dissociation curves were highly sigmoidal (Hill's $n = 2{\cdot}97$ at 25 °C and 3·40 at 35 °C). The position of the O_2 curves was relatively insensitive to temperature change with an apparent enthalpy of oxygenation (ΔH) of $-9{\cdot}2$ kJ mol^{-1}. The carbon dioxide dissociation curves were shifted to the right with increasing temperature by decreasing total C_{CO_2} at fixed P_{CO_2}, whereas the state of oxygenation had little effect on total blood CO_2 content. The *in vitro* buffer value of true plasma ($\Delta[HCO_3^-]_{pl}/-\Delta pH_{pl}$) rose from 12·0 mequiv pH^{-1} l^{-1} at 25 °C to 17·5 mequiv pH^{-1} l^{-1} at 35 °C, reflecting a reversible increase of about 30 % in haemoglobin concentration and haematocrit levels during resting conditions *in vivo*.

INTRODUCTION

Although various studies have been made upon the aerobic performance of varanid lizards (Mitchell, Gleeson & Bennett, 1981; Gleeson, 1981; Gleeson, Mitchell & Bennett, 1980; Bennett, 1972) and upon their cardiopulmonary function (Millard & Johansen, 1974; Burggren & Johansen, 1982; Heisler, Neumann & Maloiy, 1983; Johansen & Burggren, 1984), the information available on the gas transport properties of varanid blood is limited to a few studies closely focused on certain aspects. Data have been provided on: the blood oxygen affinity at the preferred body temperature of the savannah monitor lizard (*Varanus exanthematicus*) (Wood,

* Present address: Physiological Research Laboratory, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, USA.

† Present address: Nomo Fisheries Station, Nagasaki University, Nomozaki, Nagasaki 851-05, Japan.

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Johansen & Gatz, 1977); the *in vitro* oxygen dissociation curve and the Bohr effect at 25°C; and the *in vivo* O_2 dissociation curve at 30°C during voluntary diving of *Varanus niloticus* (Wood & Johansen, 1974). The present study was intended to characterize the *in vitro* O_2 dissociation curves and carbon dioxide transport properties of *Varanus* blood in more detail and at two different temperatures, providing the basic information required for an extensive quantitative analysis of cardiopulmonary gas transport in this species (J. W. Hicks, A. Ishimatsu & N. Heisler, in preparation).

MATERIALS AND METHODS

Animals

Nile monitor lizards, *Varanus niloticus*, were purchased from an animal dealer in the United States and airfreighted to West Germany. They were kept in a large (5×7 m) room with access to a small diving tank. Room temperature was maintained at 30°C. Infrared heat radiators were provided throughout the room to allow behavioural body temperature regulation by the animals towards their preferred body temperature of 35°C. They were regularly fed on chopped beef liver and kidney, and chicken meat, and supplied with live laboratory mice at intervals of about 1 week. Food was withheld for 3 days prior to surgery.

Surgical preparation and blood collection

Nine specimens of *Varanus niloticus* (mass 2–6 kg) were anaesthetized with halothane and nitrous oxide. Anaesthesia and oxygen supply were maintained during surgery by artificial ventilation (rate: 12–20 min^{-1}) with a humidified gas mixture of 67% N_2O, 30% O_2 and 3% CO_2 *via* a soft rubber tube which was sized to the trachea and then introduced into it. The gas mixture was passed through a halothane vaporizer (Dräger, Lübeck, FRG) set to 1·5 vol%. After an initial period of about 10 min the inspired halothane level was reduced to 0·5–1·0%. Five non-occlusive cannulae (PE 50) were implanted: into the right and left aortic arches, right and left atria and pulmonary artery. These sites were required for subsequent studies of pulmonary and cardiovascular function (J. W. Hicks, A. Ishimatsu & N. Heisler, in preparation; A. Ishimatsu, J. W. Hicks & N. Heisler, in preparation). Following surgery each cannula was filled to its final volume with a heparinized (200 i.u. ml^{-1} sodium heparinate) solution of PVP (polyvinylpyrrolidone; 0·5 g ml^{-1} in distilled water) (Brown & Breckenridge, 1975). The PVP gel prevents blood from entering the cannula and clotting in it, thus obviating the need for daily flushes with heparinized saline. After surgery and before the experiments the animals were allowed to recover for 72 h in a thermostatted (±1°C) chamber with access to drinking water. Each animal was kept at the experimental temperature (25 or 35°C) for 12 h before samples were taken for determination of O_2 and CO_2 dissociation curves.

Experimental approach

Prior to each experiment an arterial blood sample from the left aortic arch was analysed for blood gases, pH, haematocrit (Hct), haemoglobin concentration ([Hb]) and O_2 capacity. The blood required for curve analyses was withdrawn from the same site, and the blood loss during the experiments was kept as low as possible by reinfusion of unused blood. Whole blood O_2 dissociation curves were determined *in vitro* by one of two methods: either by the mixing technique described by Haab, Piiper & Rahn (1960) and detailed by Scheid & Meyer (1978), or by determination of the oxygen content of whole blood equilibrated at a certain P_{O_2} with the Lex-O_2-Con apparatus (Lexington Instruments, Waltham, MA, USA).

Two 1-ml samples of blood were equilibrated for 10 min with humidified gases at $P_{O_2} = 0$ mmHg or $P_{O_2} = 220$ mmHg at the same P_{CO_2} in intermittently rotating cuvettes. The gas mixtures were provided by precision gas mixing pumps (Wösthoff, Bochum, FRG), and P_{CO_2} was adjusted to values of 21 or 35 mmHg at 25 °C and 35 or 49 mmHg at 35 °C. The lower P_{CO_2} at each temperature approximates normal *in vivo* P_{CO_2} values (Table 1). Predetermined fractions of blood equilibrated with $P_{O_2} = 0$ or 220 mmHg were mixed in gas-tight tuberculin syringes and the P_{O_2} of the mixture (P_{mix}) was measured in duplicate in a thermostatted electrode system (±0·05 °C) as described by Bridges (1983). The pH of each mixed blood sample was determined using a Radiometer BMS3 and PHM 64 pH meter. Haematocrit was measured by microcentrifugation, and [Hb] determined by the cyanmethaemoglobin method.

The blood oxygen saturation (S_{O_2}) was calculated from the equation of Scheid & Meyer (1978):

$$S_{O_2} = V_1(1 + \alpha_{O_2}P_{eq}/C_{O_2tot}) - \alpha_{O_2}P_{mix}/C_{O_2tot}, \quad (1)$$

where V_1 is the fractional volume of oxygenated blood, P_{eq} is the equilibrating P_{O_2} (in this series 220 mmHg), C_{O_2tot} is the oxygen capacity of blood, α_{O_2} is the solubility of O_2 in plasma and P_{mix} is the P_{O_2} of the final mixture.

The solubility of O_2 in plasma was determined at both temperatures by equilibration with 50 % O_2 for 10 min and duplicate determination of the O_2 content. As a validity check of our mixing technique, the saturation of each mixture was compared to values determined directly from O_2 content measurements. Saturation values determined using equation 1 were not significantly different from those determined directly.

Determination of P_{mix} may potentially be biased by two factors. First, the metabolic rate of reptilian nucleated red blood cells is several times higher than that of mammalian cells (Bridges, 1983). Second, nucleated red blood cells may exhibit much higher gas/blood factors than reported for human blood (Bridges, 1983; N. Heisler, personal observation). Both of these factors would result in P_{mix} values lower than the actual P_{O_2} at the time of mixing, and in a comparatively left-shifted oxygen dissociation curve.

To account for these sources of error, additional oxygen dissociation curves were generated at 25 and 35 °C by equilibrating separate 1-ml heparinized blood samples of a blood pool each for 10 min at a number of different P_{O_2} values (29–101 mmHg) at

the same P_{CO_2} as described above. Following tonometry, the blood was analysed for P_{O_2}, P_{CO_2}, pH, [Hb], Hct and O_2 content. The saturation of individual samples was determined from O_2 content measurements, taking differences in [Hb] between samples into account. The gas/blood factor determined for each sample was used for correction of raw blood P_{O_2} data.

CO_2 dissociation curves were determined on heparinized samples (1 ml) of either fully oxygenated or deoxygenated blood at 25 and 35°C by equilibration (10 min) with various levels of CO_2 (1, 2, 3, 5 and 7 %) in N_2, or 30 % O_2 (balance N_2). Three samples of the blood were transferred into haematocrit tubes, total CO_2 was immediately determined from one of them, and the others were centrifuged after being sealed gas-tight for duplicate determinations of haematocrit. pH and C_{CO_2} were determined in the supernatant plasma, using standards bracketing unknown samples, and C_{CO_2} was determined according to the method of Cameron (1971). The erythrocyte total CO_2 content (C_{CO_2ery}) was calculated from whole blood C_{CO_2} (C_{CO_2tot}) and plasma C_{CO_2} (C_{CO_2pl}):

$$C_{CO_2ery} = \frac{C_{CO_2tot} - (1 - Hct/100) \times C_{CO_2pl}}{Hct/100} \quad (2)$$

Plasma bicarbonate concentrations were determined taking physically dissolved CO_2 into account ($[CO_2]_{phys} = \alpha_{CO_2} \times P_{CO_2}$, where the solubility of CO_2 in plasma, α_{CO_2}, was calculated according to the general formula of Heisler, 1984, 1986*a*. [Note: the sign of the last line term of the α_{CO_2} formula in Heisler (1984) is misprinted and should read +.]). The buffer value ($\beta = \Delta[HCO_3^-]/-\Delta pH$; mequiv $pH^{-1}l^{-1}$) was determined from the linear regression of individual data sets.

Data analysis

Oxygen dissociation curves were transformed according to the Hill equation (between 10 and 90 % O_2 saturation), and the cooperativity constant (n) and P_{50} were determined by least-squares linear regression analysis ($r > 0{\cdot}95$). Significant effects of temperature and C_{O_2} on the cooperativity constant n were determined using paired t-tests. The CO_2–Bohr coefficients ($\Delta \log P_{O_2}/\Delta pH$) were determined from Hill plots for each animal from 10 to 90 % saturation. Significance between variables at both temperatures was determined using paired or unpaired t-tests where applicable.

RESULTS

Blood data

In vivo resting acid–base and haematological parameters of varanid blood at 25 and 35°C are summarized in Table 1. Both Hct and [Hb] increased by 30 % as temperature increased. The temperature-induced changes in Hct and [Hb] were fully reversible. The Pa_{CO_2} was lower at 25°C and there was no change in plasma $[HCO_3^-]$. Arterial plasma pH fell with rising temperature by $-0{\cdot}007$ units $°C^{-1}$, which is much less than expected on the basis of the alphastat hypothesis (Reeves,

Table 1. In vivo *acid–base and haematological parameters in systemic arterial blood (left aortic arch) of* Varanus niloticus *at 25 and 35°C*

Parameter	Temperature (°C) 25	35	*P*	*N*
pH	7·59 ± 0·03	7·52 ± 0·06	<0·01	9
P_{CO_2} (mmHg)	24 ± 1	31 ± 3	<0·0001	9
Plasma [HCO_3^-] (mmol l^{-1})	38 ± 5	35 ± 6	>0·05	9
P_{O_2} (mmHg)	60 ± 10	75 ± 7	<0·001	9
Hct (%)	12 ± 2	16 ± 3	<0·001	9
Total [Hb] (g 100 ml^{-1})	3·6 ± 1·0	4·6 ± 1·1	<0·05	9
C_{O_2tot} (ml O_2 100 ml^{-1})	5·7 ± 1·0	7·9 ± 1·1	<0·01	4 and 5

Mean ± S.D.; levels of significance determined by paired *t*-test.

1972), but close to values generally found in reptiles, in particular varanids (reviewed by Heisler, 1986*b*).

Oxygen dissociation curves

The solubility of oxygen in *Varanus* plasma was found to be 0·00276 ± 0·0001 ml O_2 100 ml^{-1} at 25°C ($\bar{x}$ ± S.D., $N = 4$) and 0·00245 ± 0·00006 ml O_2 100 ml^{-1} at 35°C ($N = 5$). The gas/blood factor of the analytical set-up used in combination with varanid blood of the stated haematological parameters was found to be 1·29 ± 0·02 ($\bar{x}$ ± S.D., $N = 129$). Temperature and level of P_{CO_2} did not affect this value. All raw P_{O_2} data were corrected accordingly.

The combined effects of temperature and P_{CO_2} on the oxygen dissociation curve of *Varanus* blood are shown in Fig. 1. P_{50} was 34·3 ± 4·5 mmHg ($\bar{x}$ ± S.D., $N = 4$) at 25°C and $P_{CO_2} = 21$ mmHg and rose to 46·2 ± 2·4 mmHg ($N = 5$) at 35°C and $P_{CO_2} = 35$ mmHg. The cooperativity constant (n) was 2·97 ± 0·40 at 25°C, and increased significantly to 3·40 ± 0·26 at 35°C ($P < 0·001$).

The CO_2–Bohr coefficient ($\Delta \log P_{O_2}/\Delta pH$) was independent of the level of oxygenation at 25°C (Fig. 2). At 35°C the CO_2–Bohr coefficient was dependent on saturation, with the Bohr slope doubling between 10 and 90 % saturation (Fig. 2). The $\Delta \log P_{50}/\Delta pH$ values were −0·300 ± 0·190 at 25°C and −0·662 ± 0·200 at 35°C.

Temperature had little effect on the Hb–O_2 affinity. The apparent enthalpy of oxygenation (ΔH_{app}) was calculated to be −9·2 kJ mol^{-1} from the van't Hoff isochore

$$\Delta H = 2{\cdot}303R(\Delta \log P_{50})/\Delta(1/T), \quad (3)$$

where R is the gas constant and T is absolute temperature (K).

Carbon dioxide dissociation curve

The carbon dioxide dissociation curve of varanid blood was shifted to the right and downwards because of a fall in blood total CO_2 content with rising temperature (Fig. 3). The relationship between C_{CO_2} and P_{CO_2} can be characterized by the general

equation $C_{CO_2} = A + B \log P_{CO_2}$ (Fig. 3). This type of logarithmic equation provides the best fit of the data, but does not reflect any theoretical background of the CO_2 transport properties. The relationships between C_{CO_2} and P_{CO_2} were not significantly

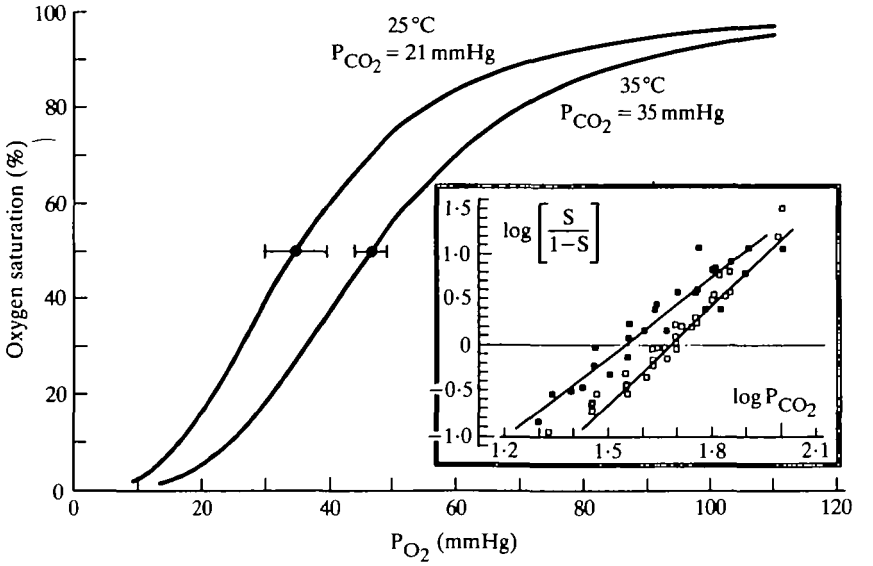

Fig. 1. Oxygen equilibrium curves of *Varanus niloticus* whole blood at 25 and 35°C, generated by direct content determination, and by the mixing method (see text) at P_{CO_2} values of 21 (25°C) and 35 mmHg (35°C). Half-saturation P_{O_2} values (P_{50}) were 34·3 and 46·2 mmHg at 25 and 35°C, respectively (horizontal bars represent ±S.D., $N = 4$ and 5, respectively). Insert: Hill plot of individual measurements.

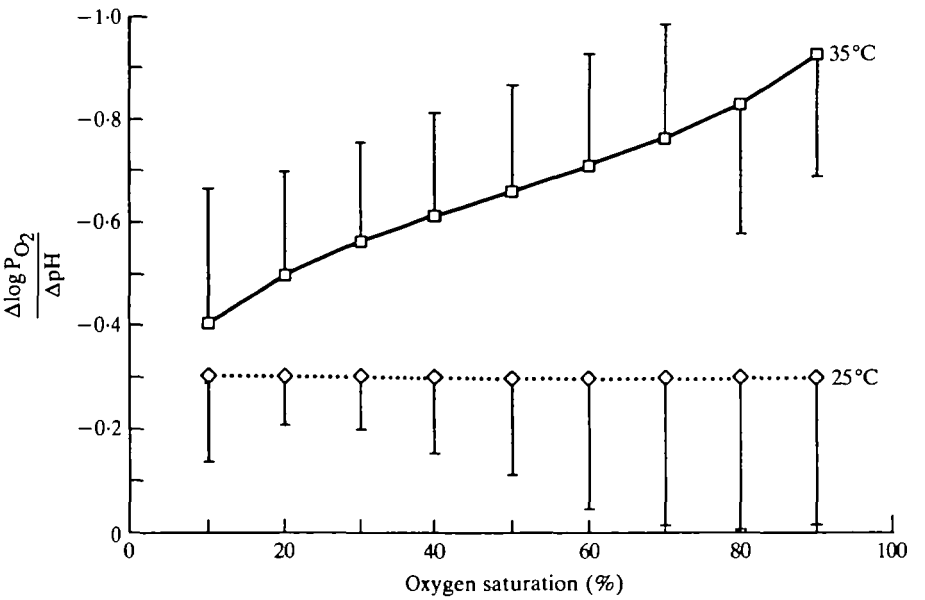

Fig. 2. CO_2–Bohr coefficients of *Varanus* blood at 25 and 35°C as a function of Hb–O_2 saturation ($\bar{x}$ ± S.D., $N = 4$–5).

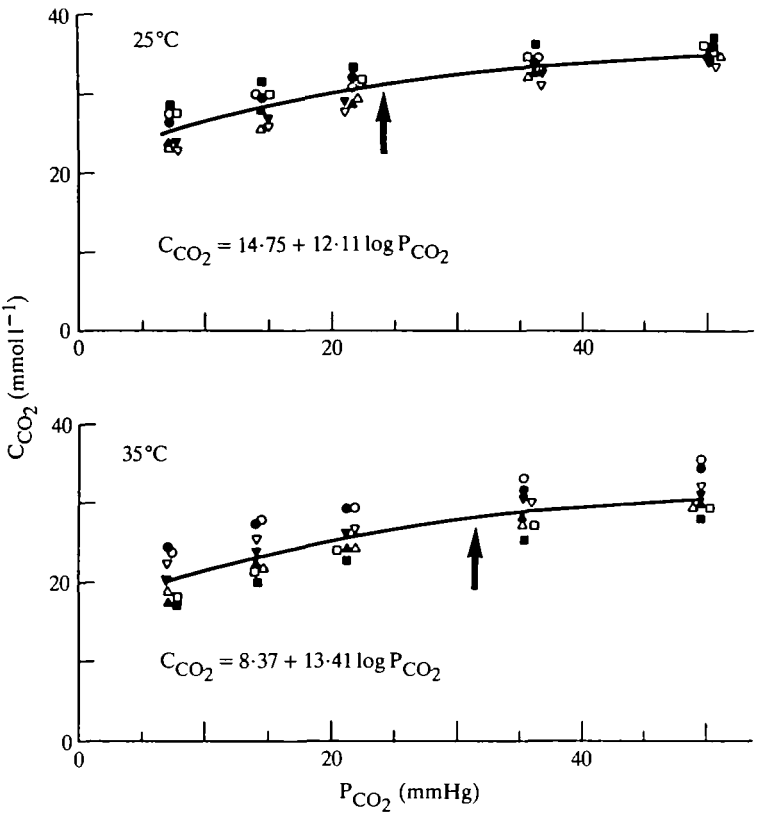

Fig. 3. Carbon dioxide dissociation curves for oxygenated (open symbols) and deoxygenated blood (solid symbols) at 25 and 35°C. Lines represent the indicated least-squares regressions ($C_{CO_2} = A + B \log P_{CO_2}$) for pooled data sets of oxygenated and deoxygenated blood. *In vivo* levels of P_{CO_2} are indicated by arrows.

different for oxygenated and deoxygenated blood (see Fig. 3). At individual P_{CO_2} values a marginal significance could only be detected for 7 % CO_2 at 25 °C ($P < 0{\cdot}05$). Apparently, the blood of *V. niloticus* has no significant Haldane effect, at least not in the physiological P_{CO_2} range.

Blood buffering and CO_2 distribution

There were no significant differences between buffer values (β) for oxygenated and deoxygenated blood at either temperature. The overall β values were $12{\cdot}0 \pm 1{\cdot}9$ at 25 °C and increased to $17{\cdot}5 \pm 3{\cdot}2$ mequiv $pH^{-1}l^{-1}$ at 35 °C ($\bar{x} \pm$ S.D., $N = 8$) (Fig. 4). The lower buffer value at 25 °C reflects the influence of temperature on Hct and [Hb]. This is evident when the buffer value is expressed as the specific buffer value (β/[Hb]), which is independent of temperature: $2{\cdot}4 \pm 0{\cdot}8$ at 25 °C and $2{\cdot}7 \pm 0{\cdot}4$ mequiv $pH^{-1}l^{-1}$ $(g\%Hb)^{-1}$ at 35 °C.

The total CO_2 determined in whole blood originated mainly from the plasma fraction. Only 7–8 % was contained within red blood cells (Fig. 5). These values are close to those expected on the basis of mammalian data, taking the lower Hct into

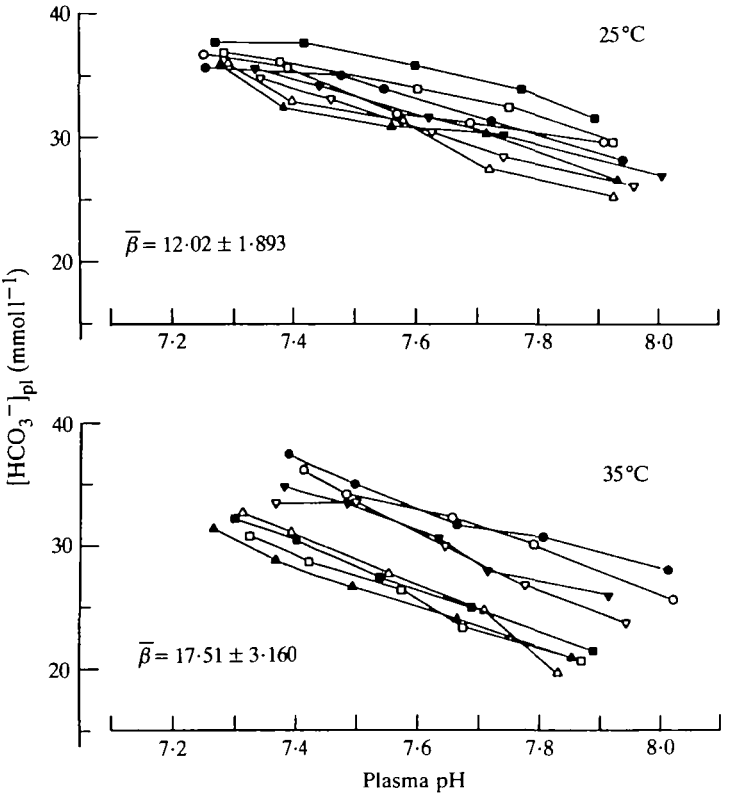

Fig. 4. Relationship between true plasma bicarbonate concentration and plasma pH. Solid symbols represent deoxygenated blood and open symbols represent oxygenated blood. The average buffer value (β; $\Delta[HCO_3^-]_{pl}/-\Delta pH$, mequiv $pH^{-1}l^{-1}$) is based on a regression analysis for all individual data points.

account. There was a tendency for an increase in the erythrocyte fraction with rising P_{CO_2}, as well as for higher values in deoxygenated blood. However, statistically significant differences were not found. Also, temperature had no effect on this parameter.

DISCUSSION

Oxygen affinity

The blood oxygen dissociation curve of the Nile monitor lizard, *Varanus niloticus*, is highly sigmoidal, has a low affinity for oxygen, and is relatively insensitive to temperature. P_{50} values of 35·3 (25 °C) and 42·4 mmHg (35 °C) found at *in vivo* pH values of 7·59 and 7·52 agree with earlier investigations. Correcting the P_{50} at 25 °C (present study) to a pH of 7·45 results in a value of 40·0 mmHg, which is close to the P_{50} of 42·4 mmHg reported by Wood & Johansen (1974) for this temperature and pH. Also the Hill coefficient of 3·1 reported by Wood & Johansen (1974) is compatible with our value of 2·97 at 25 °C.

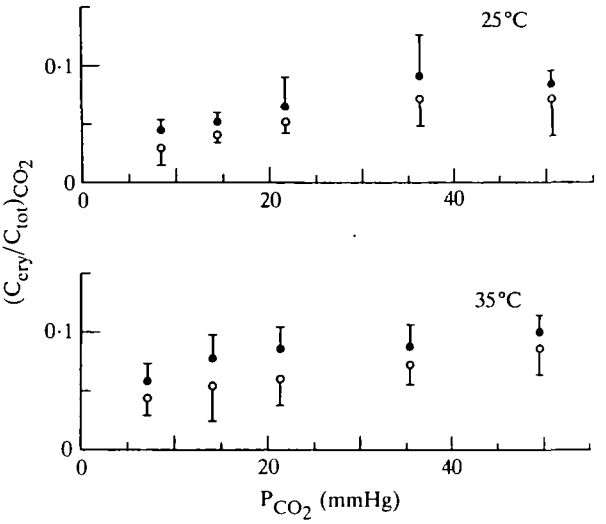

Fig. 5. Distribution of total CO_2 between erythrocytes and plasma in oxygenated (open circles) and deoxygenated (solid circles) whole *Varanus* blood. For details see text.

The cooperativity constant of *Varanus* is modified by both temperature and CO_2. The increase in n as temperature increases produces a saturation-dependent temperature coefficient ($\Delta \log P_{O_2}/\Delta T$). The increase in $\Delta \log P_{CO_2}/\Delta t$ with falling saturation levels is similar to the saturation dependence of the temperature coefficient in pig (Willford & Hill, 1986). The CO_2–Bohr factor, at least at 35°C, increases with saturation. This results from the decrease of n with rising P_{CO_2} (3·4 at $P_{CO_2} = 35$ mmHg to 3·1 at $P_{CO_2} = 49$ mmHg). Similar CO_2-induced pH effects on n and resulting saturation-dependence of the CO_2–Bohr coefficients have been reported in blood of sea turtles, *Chelonia mydas* and *Caretta caretta* (Lapennas & Lutz, 1982) and freshwater turtles, *Pseudemys scripta* and *Chrysemys picta* (Maginniss, Song & Reeves, 1980; Maginniss, Tapper & Miller, 1983). The effects of changing temperature and pH on the shape of reptilian oxygen dissociation curves are in contrast to the relationships in man where CO_2–Bohr slopes and temperature coefficients are independent of saturation (Hlastala, Woodson & Wranne, 1977; Reeves, 1980). The effects of temperature and CO_2 on haemoglobin cooperativity may reflect the multiple haemoglobin systems found in reptiles (Maginniss *et al.* 1980).

Carbon dioxide transport and blood buffering

The plasma $[HCO_3^-]$ at *in vivo* P_{CO_2} values (35 and 38 mmol l^{-1} at 25 and 35°C, respectively) are higher than those reported for most lizards, snakes and crocodilians (10–25 mmol l^{-1}, e.g. Howell & Rahn, 1976; Weber & White, 1986; Grigg & Cairncross, 1980; Glass & Heisler, 1986; for references see Heisler, 1986*b*) and more comparable to those for diving turtles and other diving lizards (e.g. Wood & Moberly, 1970; Howell & Rahn, 1976; Jackson & Heisler, 1982, 1983). The *in vitro* non-bicarbonate buffer value of *Varanus niloticus* blood is similar to that in other

diving reptiles (see Wood & Johansen, 1974), and is little influenced by the blood oxygenation state. Because of the relatively high bicarbonate concentration, the total blood buffer value is relatively high and probably provides an additional reserve for periods of anaerobic diving.

The apparent lack of a significant Haldane effect cannot immediately be understood. Since Bohr and Haldane effects are both considered to be due to negative heterotropic allosteric ligand interaction, any haemoglobin exhibiting a Bohr effect would also be expected to show a Haldane effect. As pointed out by Dill, Edwards & Florkin (1932), however, the differences between C_{CO_2} of oxygenated and deoxygenated blood would be minimized at low [Hb] values. The lower [Hb] and Hct levels may, therefore, be responsible for the failure to detect a small Haldane effect (see Fig. 5) during the present experiments. Wood & Johansen (1974) have reported a Haldane effect for *V. niloticus* blood. The average [Hb], however, was significantly lower in our animals ($3{\cdot}6$–$4{\cdot}6\,g\,100\,ml^{-1}$) than in theirs ($7{\cdot}1\,g\,100\,ml^{-1}$). The reason for a lower [Hb] and Hct in our study is unclear: the animals were apparently healthy, free of parasite infestation, and surgery resulted in little if any blood loss. Also, [Hb] and Hct remained stable throughout the experiments. Haldane factors in reptiles are reported as being quite variable. As recently reported (Weinstein, Ackerman & White, 1986), oxygenation of *Pseudemys* blood decreases C_{CO_2} by $1\,mmol\,l^{-1}$ at *in vivo* P_{CO_2} levels at both 25 and 35 °C ([Hb] = $5{\cdot}8\,g\,100\,ml^{-1}$). This ΔC_{CO_2} is similar to the mean level of change calculated for varanid blood, and *Pseudemys* blood also exhibited a temperature-dependent change in C_{CO_2} at any given P_{CO_2}, similar to that for *Varanus*. In sharp contrast, Grigg & Cairncross (1980) found a fairly large Haldane effect in the Australian crocodile, *Crocodylus porosus*, with a ΔC_{CO_2} of $5\,mmol\,l^{-1}$ at *in vivo* P_{CO_2} of 40 mmHg ([Hb] = $8{\cdot}7\,g\,100\,ml^{-1}$), but no temperature-related shift of the CO_2 dissociation curve, which, as stated, may have been due to their small number of measurements. At present, a final decision as to whether the lack of a significant Haldane effect reflects a unique physicochemical property of varanid haemoglobin, or whether the low [Hb] makes it technically difficult to measure a significant effect, requires further investigation.

Temperature and [Hb]

Body temperature has a pronounced effect on Hct and [Hb] in *Varanus niloticus*. In fact, the lower buffer value at 25 °C reflects the decrease in [Hb], as shown by the constancy of the specific buffer value (β/[Hb]) at both temperatures. The precise mechanism for the temperature dependence of Hct and [Hb] cannot be deduced from the present study. It is possible that, at lower temperatures with associated lower cardiac output, more red blood cells physically settle within the vascular system. As temperature and cardiac output increase more red cells are physically mixed with the plasma. Kooyman *et al.* (1980) have observed large fluctuations in the arterial [Hb] in Weddell seals during periods of rest and immediately following a dive, with [Hb] being 30 % higher following a dive. They suggest that this increase results from the physical effects of increased cardiac output on erythrocytes which

have settled in venous sinuses. Periods of activity may also increase Hct and [Hb] in *V. niloticus*. An increase in Hct of between 20 and 40 % has been observed at both 25 and 35 °C during periods of submaximal activity (J. W. Hicks, A. Ishimatsu & N. Heisler, personal observation). An alternative mechanism would be active mobilization from the reticuloendothelial system during periods of exercise, as occurs in higher vertebrates. Due to the significant effect of [Hb] on both the O_2 and CO_2 transport characteristics of blood, this phenomenon requires further investigation.

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