RESPIRATORY PROPERTIES OF THE BLOOD OF CROCODYLUS POROSUS
GORDON C. GRIGG and MICHAEL CAIRNCROSS

Abstract. The blood of Crocodylus porosus has a high oxygen capacity (5.5 mmol l\(^{-1}\) at hematocrit = 28\%). The shape of the oxygen equilibrium curve of the blood is described by ‘n’= 2.7 in the physiological range of P\(_{CO_2}\) and its oxygen affinity is described by the equation:

\[
\log_{10} P_{50} = 0.4163 + 0.0200 T^{ºC} + 0.3763 \log_{10} P_{CO_2}
\]

Thus, the blood has a low oxygen affinity which is strongly sensitive to both temperature and P\(_{CO_2}\). There is a high buffering capacity, 37 mmol (l pH\(^{-1}\)), and a large Haldane effect, 0.93 mmol CO\(_2\) (mmol Hb\(^{-1}\)).

The fixed-acid Bohr effect seems to be much reduced in comparison to the CO\(_2\)-specific Bohr effect. We discuss the possibility that low levels of red cell organic phosphate may be an adaptive strategy to desensitise P\(_{50}\) to changes in plasma pH. The significance of the blood respiratory properties is discussed in terms of the life style of C. porosus, particularly in relation to ectothermy and diving.

Keywords: Bohr effect, Ectothermy, Buffering capacity, O\(_2\)-affinity, Crocodile, O\(_2\)-Hb equilibrium curve, Diving, Red cell organic phosphates.

There have been few studies of the respiratory properties of the blood of crocodilians. Wilson (1939) described a high affinity for oxygen in hemoglobin from Alligator mississippiensis and Dill and Edwards (1931, 1935) determined whole blood oxygen equilibria. Bohr effect, and effect of temperature on P\(_{50}\) in A. mississippiensis and Crocodylus acutus. Information about respiratory and other properties of reptilian blood has been reviewed by Dessauer (1970), Wood and Lenfant (1976) and Howell and Rahn (1976). The only data reported previously on respiratory properties of whole blood from Crocodylus porosus has been a single Value for P\(_{50}\) (Bauer and Jelkman, 1977).

Two features of the life style of C. porosus, ectothermy and diving, suggest particular questions about its blood respiratory properties. The body temperature of C. porosus and other ectotherms is subject to daily and seasonal fluctuations, and if oxygen affinity is strongly temperature-dependent, this would have an impact on gas transport. Most reptiles employ behavioural and physiological strategies to minimise the response of body temperature to changes in ambient temperature (Templeton, 1970). Crocodilians are known to show behavioural thermoregulatory responses (Smith, 1975) and the presence of typical reptilian physiological mechanisms for controlling rates of heat loss and gain has been demonstrated in alligators (Smith, 1976) and C. johnstoni (Grigg and Alchin, 1976). Despite behavioural and physiological mechanisms, body temperature of crocodilians is certain to vary considerably, particularly in smaller animals (Grigg et al., 1979) and because of their amphibious habits. Any sensitivity of the oxygen equilibrium curve to changes in temperature could exert a significant effect on oxygen transport.

Diving is a strategy of great importance to crocodiles for predation and escape. In reptiles and other vertebrates, a large Bohr effect, high O\(_2\) capacity, high O\(_2\) affinity and a large buffering capacity have been regarded as a suite of adaptations to diving (Andersen, 1961, 1966). Within the mammals there are many contradictions to these apparent correlations with diving habit, and the question of their applicability to reptiles requires more information. Bennett and Dawson (1976) have reviewed information about the interplay between aerobic and anaerobic metabolism in reptiles. Assessment of the relative contributions of each during a dive depends partly upon information about the size of the useable oxygen store, the course of changes in levels of oxygen in the blood and lungs, and the respiratory and buffer properties of the blood. Accordingly the present study was undertaken as a background for studies on diving physiology of C. porosus.

Materials and methods
Animals and blood sampling. All analyses were performed on blood samples from two Crocodylus porosus brought to Sydney from Arnhem Land, northern Australia, and housed at Taronga Zoo. The animals were 'pegleg' (2.0 m total length, 15.6 kg, female) and 'Captain Goodvibes' (2.1 m, 18.2 kg, male). Blood samples of 20 nil were taken from the caudal sinus into tubes containing EDTA to prevent clotting.
Access to the sinus was gained with a large bore (14-17G) needle inserted medially from below the tail. Best collection was obtained well posteriorly about two-thirds of the distance between vent and tail-tip, where the ventral projections from the hemal arches are shorter. Samples were taken between August 3 and November 8, 1976, during which time both animals remained healthy, feeding well on thawed fish. Despite the frequent handling and removal of blood samples, hematocrit throughout the study remained at 26-29% in both animals, slightly above the mean hematocrit (24.8%) of 96 *C. porosus* sampled in the field (Grigg, Unpublished data). Not surprisingly, both animals became more and more difficult to handle as the study progressed.

**Oxygen and carbon dioxide equilibrium curves.** O₂ and CO₂ equilibrium curves were determined by a mixing technique similar to that described by Haab, Piiper and Rahn (1960). Tonometry was carried out in a pair of 50-ml round-bottomed flasks rotating at 45° to the horizontal in a water bath in which temperature could be controlled closely. Fully oxygenated and fully deoxygenated paired aliquots of blood at three separate levels of carbon dioxide could be prepared by equilibration in the tonometers to pairs of humidified gas mixtures, as follows:

<table>
<thead>
<tr>
<th>For oxygenation</th>
<th>For deoxygenation</th>
<th>Approximate P&lt;sub&gt;CO₂&lt;/sub&gt; (Torr) (dependent on T °C P&lt;sub&gt;B&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CO&lt;sub&gt;2&lt;/sub&gt; in air</td>
<td>0.5% CO&lt;sub&gt;2&lt;/sub&gt; in N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.5</td>
</tr>
<tr>
<td>2.0% CO&lt;sub&gt;2&lt;/sub&gt; in air</td>
<td>2.0% CO&lt;sub&gt;2&lt;/sub&gt; in N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>16</td>
</tr>
<tr>
<td>8.0% CO&lt;sub&gt;2&lt;/sub&gt; in air</td>
<td>8.0% CO&lt;sub&gt;2&lt;/sub&gt; in N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>61</td>
</tr>
</tbody>
</table>

Oxygen equilibrium curves were set up by measuring the oxygen partial pressure in 0%, 20%, 40%, 50%, 60%, 80%, and 100%, mixtures of deoxygenated and oxygenated blood. The mixtures were made in a 1 ml tuberculin syringe fitted externally with a brass spacer bar to ensure accurate proportions in the mixture. A small pellet of mercury in the dead space allowed easy mixing prior to measurement. Oxygen partial pressures were measured using Radiometer equipment (PHM71), the oxygen electrode being temperature stabilised at blood equilibration temperature. A Radiometer Blood Micro-pH electrode was used for determination of pH in the oxygenated and deoxygenated aliquots. The oxygen capacity of oxygenated samples was measured using the combined O₂ and CO₂ technique of Peters and van Slyke. In early experiments, the accuracy of establishing mixtures was confirmed by measurements of O₂ content. Full deoxygenation of the 0% sample was confirmed in every case, using the same technique. To minimize errors due to the oxygen consumption of red cells, each sample for any mixture was withdrawn separately while the tonometers were stopped briefly. Equilibration was then continued while measurements of P<sub>O₂</sub> (and oxygen content) were made.

From each of the two crocodiles, oxygen equilibria were determined at three levels of P<sub>CO₂</sub> and at 10°, 20° and 30°C, a total of eighteen curves. Additionally, four oxygen equilibrium curves were repeated under the same conditions but on different days and their similarity to the previous determination in each case suggested confidence in the methodology (Table 1).

Carbon dioxide equilibrium curves at each temperature were plotted according to van Slyke determinations of CO₂ content in oxygenated and deoxygenated blood samples equilibrated at 0.5%, 2% and 8% CO₂ which enabled assessment of the Haldane effect.

**Analyses**

*Two-factor and three-factor analysis of variance.* Because the experiments were designed to test the effect of temperature and carbon dioxide on blood respiratory properties, and because blood samples came from either one of two sources, there were three experimental treatments associated with any measurement. These were temperature, P<sub>CO₂</sub> and the animal from which the blood had been taken. The design therefore lent itself to application of three-factor analysis of variance in order to determine the dependence of any measured parameter on these treatments or upon interaction between them (table 1).

**Oxygen equilibrium curves.** Data were transformed according to the Hill equation and 'n' and P<sub>50</sub> determined by linear regression techniques, P<sub>O₂</sub> being the dependent variable (table 1). High values of the correlation coefficients, r<sup>2</sup> were taken to indicate good fit of the data to the Hill equation. For each Curve, the parameters 'n' (expressing shape) and P<sub>50</sub> (oxygen affinity) represent a complete description of the transformed curve between 10% and 90% saturation. Accordingly, each of these parameters was analysed by 3-factor and then 2-factor Analysis of Variance to establish its dependence or independence upon P<sub>CO₂</sub>, T °C and the individual from which the blood sample was drawn. Standard multiple regression techniques were then used in order to produce a generalised equation predicting P<sub>50</sub> given measurement temperature and P<sub>CO₂</sub>. The regression equation enabled a full graphic description of the curves.
Buffering capacity of whole blood. Measured values of total CO\textsubscript{2} content, corrected for dissolved CO\textsubscript{2} using x values (whole blood) from Severinghaus et al. 1956 were plotted against measured pH. The contribution to CO\textsubscript{2} content from carbamino compounds was ignored. The slope of the resultant regression is the buffer capacity, $\beta = \Delta\text{HCO}_3^-/\Delta\text{pH}$ mmol (1 blood - pH unit)$^{-1}$, a unit which, as Dejours (1975) has noted, is uncommonly called the slyke.

**Results**

(1) Oxygen carrying capacity. During the study, hematocrits of each individual remained essentially stable: Goodvibes, 27.4% (SE = 0.50) and Pegleg, 28.4% (SE = 0.18). There were no significant differences between individuals in either hematocrit or O\textsubscript{2} capacity. Pooling the data, hematocrit = 27.9% (SE = 0.30), and O\textsubscript{2} capacity = 5.52 mmol L$^{-1}$ (SE = 0.15 or 12.4 vol%. Based on hematocrit and hemoglobin determinations on 82 wild caught *C. porosus* (Grigg and Gruca, unpublished observations) a hematocrit of 27.9% is equivalent to a hemoglobin concentration of 8.7g%. No cases of methemoglobinemia were found among 110 crocs sampled. Assuming that each gram of Hb binds with 1.34 ml O\textsubscript{2} then hemoglobin concentration of 8.7 g% predicts O\textsubscript{2} capacity = 5.20 mmol L$^{-1}$. Allowing 0.12 mmol L$^{-1}$ for dissolved oxygen, this compares favourably with the mean value of 5.52 mmol L$^{-1}$ determined by direct measurement.

(2) Oxygen equilibrium curves. These are described by the values of n and P\textsubscript{50}, tabulated for each set of CO\textsubscript{2}, and temperature circumstances in table 1. By Analysis of Variance there was no evidence to suggest that the origin of the blood had any effect on either P\textsubscript{50} or ‘n’ (P > 0.05), so data from both animals were pooled and a two-factor Analysis of Variance carried out. As expected, P\textsubscript{50} depends strongly upon both T °C and P\textsubscript{50} (P < 0.001) and there is a significant interaction between them which is, however, eliminated by logarithmic transformation of P\textsubscript{50} (figs. 2, 3). Values of ‘n’ also show significant correlation with temperature and PCO\textsubscript{2} if all levels of each are included (P < 0.005 in each case). Inspection of table 1 suggests that the very high values of n at 10 °C and 0.5% CO\textsubscript{2} (4 Torr) in blood from each animal may be contributing strongly to this result. Indeed, 10 °C and 0.5% CO\textsubscript{2} have little or no physiological realism for *C. porosus* and if the 0.5% CO\textsubscript{2} column is dropped from this analysis the CO\textsubscript{2} effect is removed and there is only a very slight temperature effect. If the 10 °C row is left out also, all significant relationships disappear. At normal physiological levels therefore, x value of n = 2.7 is representative. Graphic analysis of the dependence of ‘n’ on temperature and PCO\textsubscript{2} outside the physiological range shows the correlation between n and temperature at low levels of PCO\textsubscript{2} (fig. 1) which is of theoretical interest. Note also the very slight but significant dependence of n on temperature in the physiological range of P\textsubscript{50} (P < 0.05).

**TABLE 1.** Values of P\textsubscript{50}, n (Hill coefficient), $r^2$ and pH, the correlation coefficient in the Hill plot (See text) at three temperatures and three PCO\textsubscript{2} levels. The values are set out for three-factor analysis of variance.

<table>
<thead>
<tr>
<th>PCO\textsubscript{2} (Torr)</th>
<th>'Goodvibes' male 18.2k g</th>
<th>'Pegleg' female 15.6 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>10°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P\textsubscript{50}</td>
<td>7.9</td>
<td>9.2</td>
</tr>
<tr>
<td>n</td>
<td>4.75</td>
<td>2.26</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>pH</td>
<td>7.85</td>
<td>7.35</td>
</tr>
<tr>
<td>P\textsubscript{s}</td>
<td>11.4</td>
<td>20.6</td>
</tr>
<tr>
<td>n</td>
<td>3.71</td>
<td>2.58</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.95</td>
<td>0.99</td>
</tr>
<tr>
<td>pH</td>
<td>7.65</td>
<td>7.41</td>
</tr>
<tr>
<td>20°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P\textsubscript{50}</td>
<td>14.0</td>
<td>14.5</td>
</tr>
<tr>
<td>n</td>
<td>2.10</td>
<td>2.06</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>pH</td>
<td>7.79</td>
<td>7.47</td>
</tr>
<tr>
<td>30°C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In order to determine predictive relationships, the dependence of \( P_{50} \) upon \( T \) °C and \( P_{CO2} \) was analysed by multiple regression analysis in which each value of \( P_{50} \) (determined by linear regression of Hill’s equation) was associated with its temperature and \( P_{CO2} \) of equilibration. Of the total variability, 91% is described by the equation:

\[
\log_{10} P_{50} = 0.4163 + 0.02 T \degree C + 0.3763 \log_{10} P_{CO2}
\]

The relationships are represented graphically in figs. 2 and 3, along with representative oxygen equilibria generated by Hill’s equation using \( P_{50} \) values generated from the multiple regression equation and \( n = 2.7 \). Note that in generating oxygen equilibria other than those chosen for illustration, one must remember to choose an appropriate value of ‘n’ if \( P_{CO2} = 3.5 \) (fig. 1).

**Effect of temperature on oxygen affinity** The slope of the line \( \Delta \log_{10} P_{50} / \Delta T \) is an expression of the effect of temperature on \( P_{50} \); for \( C. porosus \) \( \Delta \log P_{50} / \Delta T = 0.0200 \).

**Effect of \( P_{CO2} \) and \( pH \) on oxygen affinity.** Whereas the equation given above explained 91% of the variability of \( \log_{10} P_{50} \) including \( pH \) as a variable instead of \( \log_{10} P_{CO2} \) led to the following equation:

\[
\log_{10} P_{50} = 5.38 + 0.02 T \degree C - 0.616 pH
\]

This equation describes significantly less variability (84%) \( (P < 0.001) \), showing that \( \log P_{CO2} \) predicts \( P_{50} \) better than does \( pH \). Given the degree of autocorrelation between these two variables, this implies very strongly that \( pH \) has only a small direct effect on \( P_{50} \). We attempted to separate the effects of \( pH \) and \( P_{50} \), by partial regression, including both variables, and deriving the equation:

\[
\log_{10} P_{50} = 2.69 + 0.02 T \degree C + 0.59 \log_{10} P_{CO2} + 0.39 pH
\]
Fig. 2. The effect of temperature on oxygen affinity and the oxygen equilibrium curve of *C. porosus* whole blood, plotted from equation 1.

The total variability explained by this equation is 93% not significantly more than equation 1 (91%). This would be expected if the autocorrelation between pH and $P_{50}$ were complete. However, equation 3 explains significantly more variability than equation 2, confirming that $\Delta \log_{10} P_{50} / \Delta \log_{10} P_{CO2}$ and $\Delta \log_{10} P_{50} / \Delta pH$ are not equivalent. The crucial experiments to evaluate these separate effects of pH and $P_{CO2}$ were not performed and no values for fixed-acid Bohr effect and specific CO$_2$ effect can be given. Since $P_{50}$ is predicted better by $P_{CO2}$ than by pH, it seems likely that in *C. porosus* the specific CO$_2$ effect predominates (see Discussion).

Fig. 3. The effect of $P_{CO2}$ on oxygen affinity and the oxygen equilibrium curve of *C. porosus* whole blood, plotted from equation 1.
(3) CO₂ transport and blood buffering. Carbon dioxide equilibrium curves are seen in fig. 4. Each point was derived from equilibration of a different blood sample so one might expect considerable variability. By Analysis of Variance it was demonstrated that P<sub>CO₂</sub> and the state of oxygenation both exert a significant influence on CO₂ content, that the source of the blood was not a significant factor, and that equilibration temperature had no effect. This last differs from expectation and the lack of temperature effect may be obscured within the background noise of variability alluded to above. The Haldane effect was calculated at P<sub>50</sub> = 40 Torr (for comparison with human and other data) by interpolation and found to be approximately 5 mmol CO₂ 1⁻¹ or 0.93 mmol CO₂ (mmol Hb)⁻¹, very high compared with a human value of about 0.35. At 30 °C and P<sub>CO₂</sub> = 40 Torr, the mean ΔpH between oxygenated and deoxygenated whole blood was 0.026.

The relationship between pH and blood bicarbonate is shown in fig. 5 and suggests a buffering capacity (ΔHCO₃⁻ /ΔpH) of 37 mmol (1 - pH⁻¹). The large Haldane effect is seen.

![Fig. 4. Carbon dioxide equilibrium curves of whole blood from C. porosus, showing the large Haldane effect. Each point is the mean of six determinations and the vertical bar represents one standard error about the mean. Analysis of variance showed no significant effect of temperature, so data were pooled.](image)

![Fig. 5. Blood buffering capacity of whole blood from C. porosus. By analysis of covariance the intercepts of the regression lines are significantly different (P < 0.001) but not the slopes.](image)

Discussion
Hematocrit and O₂ capacity. Among reptiles, C. porosus has a high blood O₂ capacity, 5.5 mmol 1⁻¹ at a hematocrit of 27.9%. Values of hematocrit in 96 C. porosus sampled in the Liverpool and Tomkinson Rivers, northern Australia, averaged 24.8% (range 19.1-31.3, SE = 0.28, Grigg, unpublished observations).
This suggests an average \( \text{O}_2 \) capacity of 4.9 mmol - 1^{-1} in the wild, in the upper part of the range for reptiles (3.44-5.62 mmol - 1^{-1}) reported by Dawson and Poulson (1962). That high \( \text{BOC} \) contributes to survival in diving reptiles has been questioned by Wood and Lenfant (1976), referring to the frequent occurrence of high levels of methemoglobin among reptilia. However, no cases of methemoglobinemia were found in wild-caught \( C. \ porosus \) and this species has sufficiently high levels of methemoglobin reductase (NADH-MR) (4.43 I.U., SE = 0.14, n = 41) to suggest rapid reversal of methemoglobin as it is formed (Grigg and Gruca, in preparation). It seems likely that high \( \text{BOC} \) in \( C. \ porosus \) can be regarded as an adaptation to diving increasing the oxygen store.

Interestingly, although \( \text{O}_2 \) capacity is high, hematocrit values are lower than those for some reptiles whose \( \text{BOC} \) is lower than that of \( C. \ porosus \). \( Varanus gouldi \), a Varanid well known for its high rates of aerobic metabolism, has a hematocrit of 29% (SD = 2.4) and \( \text{BOC} \) of 3.57 mmol - 1^{-1} (Bennett, 1973). \( Sauromalus obesus \), with a hematocrit of 33% (SD = 1.3) has an \( \text{O}_2 \) capacity of 4.33 mmol - 1^{-1} (Bennett, 1973).

\textbf{Oxygen affinity.} The oxygen affinity of the blood of \( C. \ porosus \) is quite low. At 37 °C, to enable comparison with data presented in fig. 24 of Wood and Lenfant (1976), \( P_{50} = 49 \) Torr (\( P_{\text{CO}_2} = 16 \) Torr, pH = 7.52). High values of \( P_{50} \) are, however, typical of reptiles in general and \( C. \ porosus \) falls neatly on the extrapolated \( P_{50} / \text{weight} \) relationship given by Wood and Lenfant (1976, p. 260). This contrasts with the much higher \( \text{O}_2 \) affinity reported by Anderson (1961) for \( \textit{Alligator mississippiensis} \). Comparable results for the other crocodilian that has been studied, \( C. \ acutus \) (Dill and Edwards, 1931), are difficult to calculate but their '12 foot' animal 'A' would likely have weighed 150-200 kg if there is a similar length-weight relationship as in \( C. \ porosus \). If \( P_{50} \) is similarly temperature sensitive in both species, at 37 °C a \( P_{50} \) of about 45 Torr may be calculated from their data. This falls within the limits predicted by the \( P_{50} / \text{weight} \) relationship.

There has been considerable discussion about adaptive correlation of blood oxygen affinity with environment and life style (e.g. Johansen and Lenfant, 1972) and in fishes such correlations are well marked (Grigg, 1974). Among reptiles, however, the existence of an apparently close relationship between \( P_{50} \) and weight (in lizards and crocodiles at least) shows little evidence for adaptive modification of \( P_{50} \) to suit environment or habits. Modification of \( P_{50} \) has been suggested as an adaptation to diving in reptiles (Andersen, 1961) yet both diving and non-diving species fit the \( P_{50} \) weight relationship equally well.

\textbf{Effects of organic phosphates, \textit{CO}_2, and pH on oxygen affinity.} Grigg and Gruca (1979) have reported low values for red cell ATP (including other nucleoside triphosphates) and 2,3 DPG in \( C. \ porosus, C. \ johnstoni \) and \( C. \ novaeguinea \), confirming earlier reports of low red cell phosphates in crocodilians (Rapoport and Guest, 1941; Bartlett, 1978). Bauer and Jelkman (1977) studied hemolysates for \( C. \ porosus \) and found oxygen affinity to be insensitive to inorganic phosphate and a wide range of organic phosphates. Their values of \( P_{50} \) for hemolysates, are predicted accurately by our equation derived for whole blood, as discussed elsewhere (Grigg and Gruca, 1979). Accordingly, it is unlikely that organic phosphates are involved in the regulation of whole-blood oxygen affinity in \( C. \ porosus \).

Grigg and Gruca (1979) have proposed that low red cell organic phosphates may represent an adaptive strategy to reduce the fixed-acid Bohr effect in animals subject to large changes in plasma pH. Such changes may be associated with a prolonged dive or other behavioural event which depends upon anaerobic glycolysis. In crocodilians, plasma pH is known to fall considerably following a dive (Andersen, 1961) or a burst of activity (Coulson and Hernandez, 1964). At such times, a strong fixed-acid Bohr effect could inhibit \( \text{O}_2 \) loading at the respiratory surface, and a lowered sensitivity of \( P_{50} \) to pH could be regarded as advantageous to crocodilians.

This line of reasoning is given extra weight by our finding that the fixed-acid Bohr effect is reduced in \( C. \ porosus \) in comparison to the \( \text{CO}_2 \) Bohr effect. Further, data presented in a graph by Bauer and Jelkman (1977) showed the \( \text{O}_2 \) affinity of hemolysate (at \( P_{\text{CO}_2} = 40 \) Torr) to be independent of pH over the physiological range.

\textbf{\( \text{CO}_2 \) transport and blood buffering.} The carbon dioxide equilibrium curves (fig. 5) suggest a relatively low plasma bicarbonate in \( C. \ porosus \), 16 mmol - 1^{-1} at \( P_{\text{CO}_2} = 30 \) Torr. This compares favourably with 17 mmol - 1^{-1} (SE = 0.58) in samples of venous plasma drawn from 99 wild-caught specimens (G.C.G. unpublished observations). However, both these values are probably too low because of lactate accumulation due to disturbance of the animals at sampling (see later). Lenfant et al. (1970) found 32 mmol - 1^{-1} \( \text{HCO}_3^- \) \( \text{HCO}_3^- \) (\( P_{\text{CO}_2} = 30 \) Torr) in \( \textit{Chelys fimbriata} \). In \( V. \ niloticus \), Wood and Johansen (1974) recorded a plasma \( \text{HCO}_3^- \) of 30 mmol - 1^{-1} (\( P_{\text{CO}_2} = 25 \) Torr). In alligators, Coulson and Hernandez (1964) reported 19.8 mmol - 1^{-1} plasma bicarbonate at unspecified \( P_{\text{CO}_2} \), similar to \( C. \ porosus \).

The buffering capacity of \( C. \ porosus \) is high (fig. 5), which suggests considerable ability to cope with a large build-up of blood lactate following a dive or a burst of activity. Comparison with values for other
reptiles suggests a degree of acidosis in our experiments. Normal values of pH at these temperatures would be 7.6-7.7 according to Howell and Rahn (1976). Blood lactate analysis of five samples of blood in our study averaged 9.31 mmol -1 (SE = 0.82), confirming the acidosis. This value can be compared with an average of 4.41 mmol -1 (SE = 0.47) obtained from analysis of venous plasma from 42 wild-caught C. porosus (Grigg unpublished observations). Coulson and Hernandez (1964) have discussed the increase in blood lactate following handling of alligators. They report normal values ranging from 'trace' to 1.0 mmol -1 (average 0.7), in alligators kept undisturbed and in a soundproof box prior to sampling.

Effects of temperature. Bennett (1973) found lowered O₂ capacity at high temperatures in both Sauromalus hispidus and Varanus gouldii, particularly at lowered pH. No effect of temperature or P₉₀ on O₂ capacity was found in C. porosus over the ranges studied. Oxygen affinity in C. porosus is, however, strongly temperature-dependent, Δlog P₅₀/dT = 0.0200.

It is interesting to speculate on what, if any, adaptive significance can be attached to the steep dependence of P₅₀ upon temperature. Crocodilians in captivity are known from telemetric studies to undergo substantial daily changes in body temperature (Smith, 1975). Water temperatures in the wild in Arnhem Land vary seasonally by at least 10 °C, while air temperature frequently shows a 15 °C range during a single day. Grigg et al. (1979) provide information about thermal time constants for reptiles heating and cooling in water and air, from which it is clear that crocs less than 100-150 kg are unlikely to maintain any substantial overnight difference between body temperature and water temperature. It seems certain that body temperature of C. porosus less than 100 kg varies considerably both daily and seasonally.

One might suspect, therefore, that C. porosus would show relative independence of P₅₀ on temperature. Indeed, it would seem reasonable to propose that lowered sensitivity of P₅₀ to temperature changes would be common among reptiles because of the large fluctuations in Tₜ which accompany daily basking and retreat. However, reptiles commonly have a steep effect of T on P₅₀ (Pough, 1969; Greenwald, 1971, Wood and Lenfant, 1976). One exception is Varanus niloticus where low temperature sensitivity was found and interpreted as an adaptation to minimise the increase in oxygen affinity which would otherwise occur in lizards going from sun basking to submergence in water (Wood and Johansen, 1974).

C. porosus is another reptile which goes from basking to submergence in water and the finding of strong dependence of P₅₀ upon T is in sharp contrast to the situation in V. niloticus.

Before the adaptive significance of high or low sensitivity of P₅₀ to T can be explained for reptiles, more information is needed about a wider variety of reptiles, but one possible explanation of the strong sensitivity in C. porosus can be proposed. Grigg (1978) reported a strong dependence of metabolic rate on temperature (Q₁₀=2.68, T = 20-30 °C) in juvenile C. porosus. Thus both metabolic rate and P₅₀ are strongly T-dependent in this species. Seasonal changes in temperature are much less in the entirely tropical range of C. porosus than one finds in temperate habitats, yet the habitat of C. porosus is divided sharply into wet (summer), dry (winter) seasons. This seasonality of rainfall has a marked effect on crocodile activity, for C. porosus breeds in ‘the wet’. The marked physiological sensitivity to temperature may allow C. porosus to make the most of what temperature fall there is in the cool (and dry) season as a period of lesser activity, with concomitantly reduced overall energy metabolism. Interestingly, high values of metabolic Q₁₀ particularly at temperatures below the activity range, are not uncommon among reptiles (see review by Bennett and Dawson, 1976). In many species Q₁₀ is low over the temperature range at which they are active, yet higher at lower temperatures. Hoskins and Aleksiu (1973) studied liver, skeletal muscle and cardiac muscle from Garter Snakes (Thamnophus sirtalis), above 20°C, Q₁₀ = 2.4, but from 4-20 °C, Q₁₀~ 5.8. As Bennett and Dawson (1976) have said ‘a high thermal dependence of metabolism at temperatures below the range normally associated with activity might well produce considerable conservation of energy for reptiles during inactivity in cool surroundings’.

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References


