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BLOOD GAS TENSIONS AND ACID-BASE REGULATION IN THE SALT-WATER CROCODILE, *CROCODYLUS POROSUS*, AT REST AND AFTER EXHAUSTIVE EXERCISE

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SUMMARY

1. Salt-water crocodiles, *Crocodylus porosus* Schneider, were catheterized and P_{O_2}, P_{CO_2}, pH and lactate concentration ([lactate]) were measured in arterial blood during rest and after forced exhaustive activity at 30 °C.

2. Gas exchange ratio (R), calculated from blood P_{O_2} and P_{CO_2}, decreased from about 1·0 to 0·3 during resting voluntary breath-holding and indicated CO_2 sequestration in the body fluids. The mean value for R in undisturbed animals was 0·6, which substantiates the hypothesis that some CO_2 excretion is extrapulmonary.

3. *In vitro* buffer value of true plasma was $-23{\cdot}5 \pm 1{\cdot}9$ mmol $HCO_3^- l^{-1} pH^{-1}$. *In vivo* buffer value, determined by short-term self titration with metabolic CO_2, was $-12{\cdot}2 \pm 4{\cdot}7$ mmol $HCO_3^- l^{-1} pH^{-1}$.

4. Exhaustive activity for 5 min in laboratory animals resulted in pronounced metabolic lactacidosis: pH decreased approximately from 7·43 to 7·11 while lactate concentration increased from 1·2 to 20–30 mmol l^{-1}. The acidosis was reduced by respiratory compensation during the first hour of recovery and by metabolic adjustments that were practically complete after the third hour. Greater acidosis (pH down to 6·4) in larger field-captured animals was resolved over a longer period.

5. During recovery, lactate and proton fluxes between the blood and other tissue were uncoupled.

6. The virtual absence of the fixed-acid Bohr effect in *C. porosus* blood is adaptive in large individuals because it facilitates continued O_2 uptake from the lung into blood which may be acidified by as much as 1 pH unit as a result of physical activity.

INTRODUCTION

Much crocodilian behaviour is slow and peaceful. Like other aquatic reptiles, resting crocodilians usually have ventilatory periods separated by longer periods

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of apnoea (Naifeh *et al.* 1970). Gas exchange in diving reptiles has been termed 'cyclic' because the patterns of CO_2 excretion and O_2 uptake are temporally distinct (Ackerman & White, 1979; Burggren & Shelton, 1979). Whereas O_2 is recruited from the lung more or less constantly, most of the CO_2 accumulates in the bicarbonate buffer system of the body fluids during apnoea and is released during the ventilatory period. This phenomenon has been demonstrated in the alligator, *Alligator mississippiensis* (Andersen, 1961) and in the Nile crocodile, *Crocodylus niloticus* (Glass & Johansen, 1979), and we have studied it in the salt-water crocodile, *Crocodylus porosus*.

The languid existence of crocodiles, however, can be punctuated by fierce activity during predation, defence and escape. Crocodiles often feed on large terrestrial animals that they pull into the water and drown (Guggisberg, 1972). Thus, they must be capable of powerful physical activity and they must also be adequate divers. Both intense activity and diving behaviour promote a reliance on anaerobic metabolism. The magnitude of anaerobiosis in reptiles may exceed that of any other animal group and the build-up of lactate and protons in muscle and blood can lead to severe acid-base disturbance (Bennett, 1978, 1982). The dynamics of metabolite appearance and disappearance in blood following exercise has been studied in various animal groups, but the reptiles deserve more attention because of their extreme anaerobic capacity. Crocodilians, in particular, are of interest because of the severity of the acidosis produced by relatively short bouts of intense exercise (Coulson & Hernandez, 1979; Bennett, Seymour, Bradford & Webb, 1985). We examined acid-base regulation in *C. porosus* during voluntary diving and following provoked activity in the laboratory and field.

MATERIAL AND METHODS

Animals

Seven *Crocodylus porosus* (mean mass = 2·74 kg; range = 0·43–7·0 kg) were captured in the Adelaide River region, near Darwin, in the Northern Territory during November 1983. They were collected by standard methods (Webb & Messel, 1977) under permit No. SL41/83 issued by the Conservation Commission of the Northern Territory. Captive crocodiles were held in large concrete bins (Webb, Buckworth & Manolis, 1983) at approximately 30 °C for a few days until they were used. They were not fed but were released in good condition within a week.

Blood sampling

The right exterior carotid artery was occlusively catheterized in six animals under cold anaesthesia (McDonald, 1976). Animals were placed in an ice-water bath with only their heads held out of water. The latter precaution was taken as previous observations on tropical snakes indicated that death could result from head submersion in cold water. Animals were submersed until rectal temperature fell to approximately 5 °C, when they became completely unresponsive to external

stimuli. They were then transferred to a bed of ice which was manipulated to keep body temperature constant. Catheters, fashioned from polyvinyl chloride tubing (0·58 × 0·96 mm or 1·0 × 2·0 mm and 1·0–1·5 m long) and filled with heparinized saline (250 i.u. ml^{-1}, were implanted *via* a small ventral incision and exteriorized on the dorsal side of the neck. After surgery, the crocodiles were artificially ventilated during post-operative warming until spontaneous ventilation was resumed. They recovered for at least 18 h in isolation before measurements were taken.

Crocodiles are notoriously sensitive to disturbance, which may alter their heart rate and ventilatory pattern (Gaunt & Gans, 1969; Gans & Clark, 1976). To measure completely resting animals, we placed each individual in shallow water (30·0 ± 1·0 °C) in containers of sufficient size to permit them to submerge and move around. The only restraint was a cord securing the jaws together; this cord did not impede normal ventilation. So that the animals were not disturbed by the investigators, the containers were covered and the measurements were carried out quietly. Ventilation rate was observed in isolated animals, by impedance plethysmography (Parks Model 270 Plethysmograph).

Experimental protocol

All measurements were made from 10.00 to 18.00 h local time. A series of blood samples was withdrawn from catheterized crocodiles under three experimental conditions. First, samples were obtained from isolated animals during normal ventilatory cycles, including voluntary apnoea. Second, the top of the container was removed and the animal was allowed to view a person. The crocodiles responded by changing their ventilatory pattern, often undergoing longer periods of voluntary apnoea. We refer to this treatment as 'intimidation'. Finally, they were gently held under water until they began to struggle and then were permitted to surface.

The next day, each catheterized crocodile was exercised and permitted to recover from the bout of activity. Blood samples were first taken from a resting animal prior to activity. Then it was placed in a bathtub half full of water and prodded by hand on the tail and limbs for 5 min. The crocodile responded with intense bouts of struggling and rolling for the first 1–2 min, but the level of activity diminished thereafter. It was then returned to its covered container to rest undisturbed during the recovery period. Blood samples were taken as soon as possible after the cessation of activity (approx. 2–3 min) and at 10, 20, 30 min and 1, 2, 3 h of recovery.

Additional blood samples were obtained by heart puncture from crocodiles exercised to exhaustion in the field (Bennett *et al.* 1985).

Sample analysis

Blood from catheterized crocodiles was obtained according to the methods described by Seymour & Webster (1975), in which gas tensions are measured in samples subsequently returned to the animal to avoid depletion of blood volume.

Blood was aspirated into a Radiometer model BMS 3 Mk 2 blood microsystem connected to a model PHM 72 acid-base analyser, maintained at $30{\cdot}0 \pm 1{\cdot}0$ °C. Several times the volume of the catheter tubing and electrode cell were flushed through the system before gas tension was read at prevailing blood pressure. The P_{O_2} electrode was calibrated with Radiometer P_{O_2} zero solution and water equilibrated with air, accounting for water vapour pressure. After determining the difference in electrode readings of air and water with the same P_{O_2}, the electrode was routinely calibrated with gas from a Radiometer GMA 2 gas mixing apparatus. Reported P_{CO_2} has been increased 7 % to account for the approximate difference between the electrode's responses to blood and water (Heitmann, Buckles & Laver, 1967). The P_{CO_2} electrode was calibrated with the GMA 2 and the pH electrode with precision buffers. The barometric pressure varied less than ± 2 Torr during the experiments and was assumed to be constant at 758 Torr.

In vitro blood buffer values were measured by equilibrating approximately 0·4 ml samples of whole blood to each of the humidified gases supplied by the GMA 2 ($P_{CO_2} = 27{\cdot}3$ and 54·8 Torr). Samples were rotated in a tonometer consisting of two, 2-ml glass vials thermostatted to pH electrode temperature. Equilibration occurred within 10 min and after that time the samples were aspirated into the pH electrode. Plasma [HCO_3^-] was calculated with the Henderson-Hasselbalch equation. To deal with blood pH values as low as 6·4, the pK′ was calculated from a second order polynomial equation fitted to data at 30 °C from Severinghaus (1965); the equation ($pK' = 5{\cdot}2493 + 0{\cdot}2953\,pH - 0{\cdot}0238\,pH^2$) accounts for pH dependence and yields a value within 0·004 units of that calculated at pH = 7·4 by Reeves (1976). Solubility of CO_2 was taken as 0·0356 mmol l^{-1} $Torr^{-1}$ (Severinghaus, 1965). Values of [HCO_3^-] represent true plasma, unless otherwise stated.

Acid-base balance was examined by determining the 'base deficit' in crocodiles that were recovering. This term is defined as the change in [HCO_3^-] at a standard pH, here 7·431 (Table 1), and is calculated according to Woodbury (1974) using *in vitro* buffer values and then correcting for uneven distribution of HCO_3^- between plasma and red blood cells. Base deficit equals all of the protons added to the blood by non-carbonic acid (e.g. lactic acid).

For determination of blood lactate concentration, 0·100-ml samples of blood were deproteinized in 0·200 ml of 0·6 mol l^{-1} perchloric acid and the supernatant was analysed against lactate standards (Boehringer-Mannheim No. 125440) with an enzymatic test kit (Boehringer-Mannheim No. 149993) and a Varian Superscan 3 spectrophotometer.

Mean values $\pm$ 95 % confidence limits for the mean are reported.

RESULTS

Blood gas tensions

Resting crocodiles in shallow water ventilated with series of breaths separated by periods of apnoea. In two undisturbed animals, ventilatory periods averaged 0·33 min and consisted of 6·0 breaths; the non-ventilatory periods averaged

1·78 min. Thus breath-holding accounted for 84 % of the time at 30 °C. This pattern of breathing is common to most aquatic reptiles (Wood & Lenfant, 1976; Seymour, 1982) including the Nile crocodile, *C. niloticus*, which holds its breath for about 80 % of the time (Glass & Johansen, 1979). Unfortunately, it was not possible to coordinate blood measurements with phase of the short ventilatory cycles.

During undisturbed rest, *C. porosus* maintained arterial P_{O_2} at about 102 Torr and P_{CO_2} at about 33 Torr (Table 1). The values of P_{CO_2} and P_{O_2} indicate an overall gas exchange ratio (R) of about 0·6 for resting crocodiles (Fig. 1).

Crocodiles intimidated by the investigators had more variable non-ventilatory periods, sometimes voluntarily holding their breath for as long as 16 min, at other times breathing often. The range of ventilation is evident in the wide distribution of P_{O_2}, encompassing the data from undisturbed animals as well as resting animals held under water (Fig. 1). The effect of breath-holding on P_{CO_2} was much less and the apparent respiratory exchange ratio (R) progressively decreased with increased breath-holding time. The least squares regression of all data is $P_{CO_2} = -0{\cdot}22P_{CO_2} + 56{\cdot}8$ ($r = 0{\cdot}78$, $N = 108$, $P < 0{\cdot}001$, S.E. of slope = 0·017).

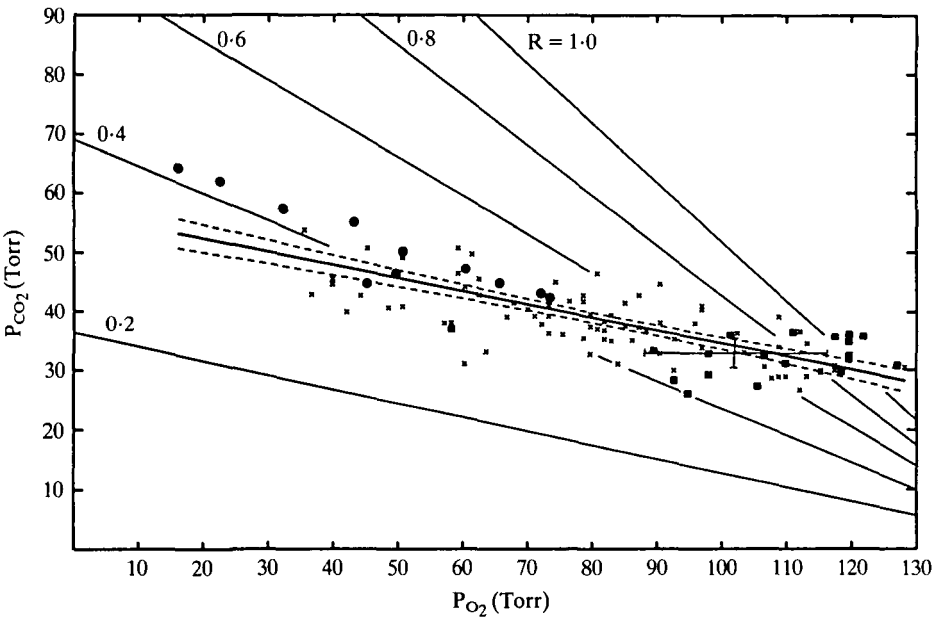

Fig. 1. Relationship of P_{CO_2} to P_{O_2} in the arterial blood of resting *Crocodylus porosus* at 30 °C. The data represent three conditions: resting animals undisturbed by investigators (squares), animals viewing investigators (crosses), animals gently held under water without struggling (dots). The mean and 95 % confidence internal for the resting undisturbed animals is indicated by the large cross. A regression of all data is given as a solid line with its 95 % confidence interval as broken lines. Selected respiratory quotient lines (R), calculated according to Rahn & Fenn (1955), are also indicated.

Respiratory properties of the blood

Mean values of selected respiratory properties of the blood of resting *C. porosus* at 30 °C are presented in Table 1. Haematocrit and haemoglobin concentration are similar to values for other crocodilians (Pough, 1979; Carmena-Suero, Siret, Callejas & Carmena, 1979; Grigg & Cairncross, 1980). Blood pH at 30 °C was about 7·43 (Table 1). Our pH values are slightly lower than predicted by data from other reptiles (Rahn & Garey, 1973; Reeves, 1977), but are similar to the interpolated values from resting *Alligator mississippiensis* (Davies, 1978). We are certain that pH in our animals was not reduced by metabolic acid. Blood lactate concentration in resting *C. porosus* averaged 1·2 mmol l^{-1}. This value is well within the range (0·44–2·22 mmol l^{-1}) for other resting reptiles (Bennett & Dawson, 1976), and is only slightly above the average (0·7 mmol l^{-1}) in resting, undisturbed alligators (Coulson & Hernandez, 1983). Other pH values previously reported for *C. porosus* are admittedly affected by elevated lactate levels (Grigg & Cairncross, 1980).

In vitro *blood buffering*

The buffer value of true plasma for *C. porosus* was −23·53 mmol $HCO_3^- l^{-1} pH^{-1}$ (Table 1). This value is considerably less than that of −37·0 given for the same species by Grigg & Cairncross (1980). A minor part of this difference is explicable by a slightly higher haemoglobin (Hb) concentration in their animals, but the haemoglobin-specific buffer value in their animals was −0·43 mmol $HCO_3^- gHb^{-1} pH^{-1}$, whereas our value is −0·33, or similar to data from *Crocodylus acutus* (−0·28, Dill & Edwards, 1931) and *Alligator mississippiensis* (−0·25; Dill & Edwards, 1935).

In vivo *buffering*

Blood pH varied within a range of 0·14–0·24 units and P_{CO_2} varied by 12–38 Torr during periods of undisturbed rest and quiet intimidation in five

Table 1. *Selected respiratory properties of the whole blood of* Crocodylus porosus *at 30 °C*

Parameter	Units	Mean	95 % Confidence interval	Range	Number of animals
Haematocrit	%	22·2	1·9	19·8–26·3	7
$[Hb]_{blood}$	g 100 ml^{-1}	7·09	0·32	6·75–7·65	7
$[Hb]_{cells}$	g 100 ml^{-1}	32·06	1·91	29·12–33·77	7
Buffer value*	mmol $HCO_3^- l^{-1} pH^{-1}$	−23·5	1·9	−20·7 to −26·6	6
Buffer/[Hb]	mmol $HCO_3^- gHb^{-1} pH^{-1}$	−0·33	0·03	−0·30 to −0·39	6
pH		7·431	0·037	7·387–7·479	6
P_{CO_2}	Torr	32·8	2·5	29·7–36·4	6
P_{O_2}	Torr	102	14	58–127	6
$[HCO_3^-]$*	mmol l^{-1}	23·5	2·2	20·0–26·2	6
[Lactate]	mmol l^{-1}	1·20	0·32	0·74–1·58	6

*True plasma value.

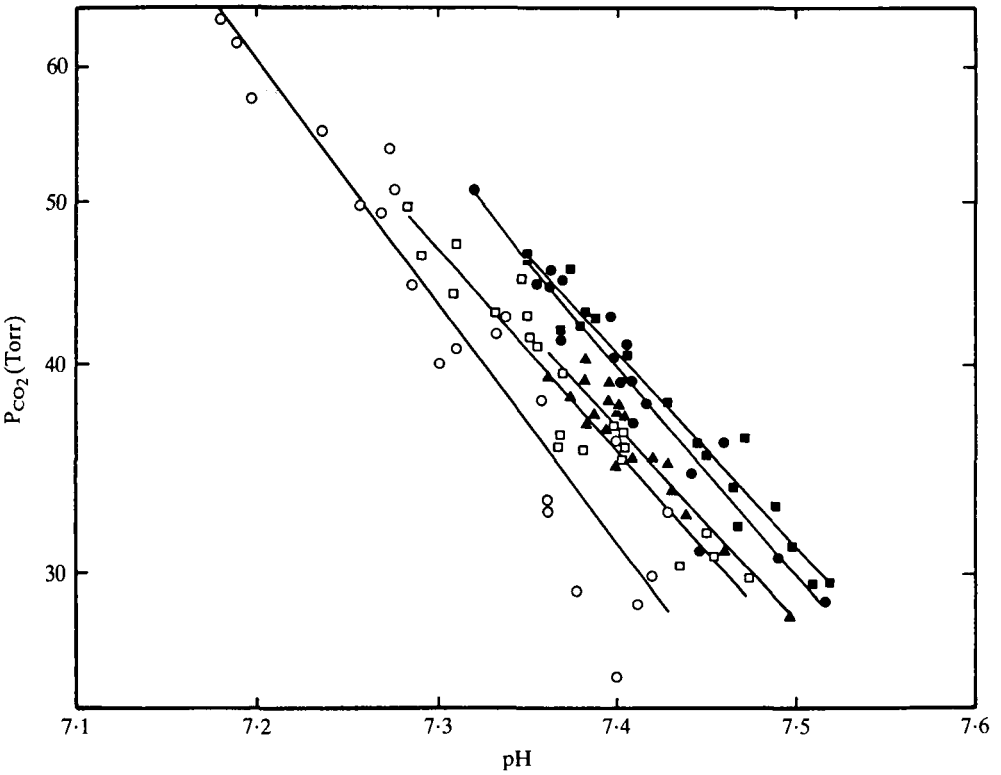

Fig. 2. Least squares regression between $\log P_{CO_2}$ and pH in the arterial blood of five resting *Crocodylus porosus* at 30 °C. The measurements were taken from undisturbed and unintimidated animals.

crocodiles (Fig. 2). Analysis of covariance of the relationship between $\log P_{CO_2}$ and pH indicated that the slopes of different individuals were not significantly different (common slope $= -1{\cdot}29 \log_{10} P_{CO_2} pH^{-1}$), the intercepts were significantly different ($P < 0{\cdot}001$), and the correlation was highly significant ($P < 0{\cdot}001$).

Bicarbonate concentration was calculated from simultaneous measurements of P_{CO_2} and pH during undisturbed rest and intimidation (Fig. 3). Because the animals were titrating the body with metabolically-produced CO_2 during breath-holding, the slope of the relationship between HCO_3^- and pH (regression lines in Fig. 3) indicates the short-term buffer value of all available fluid compartments, before major renal or metabolic adjustments are made. This buffer value represents the combined effects of a redistribution of HCO_3^- between extracellular and erythrocyte compartments and a change in blood HCO_3^- due to the oxygenation of haemoglobin. Although titration with elevated inspired CO_2 for long periods may be required to reach complete equilibrium between all compartments (Woodbury, 1974; Jackson, Palmer & Meadow, 1974), the present values are pertinent to the short-term changes in ventilation immediately after exhaustive exercise. They are therefore useful in correcting blood $[HCO_3^-]$ for changes in P_{CO_2}. Analysis of covariance showed that the slopes of plasma

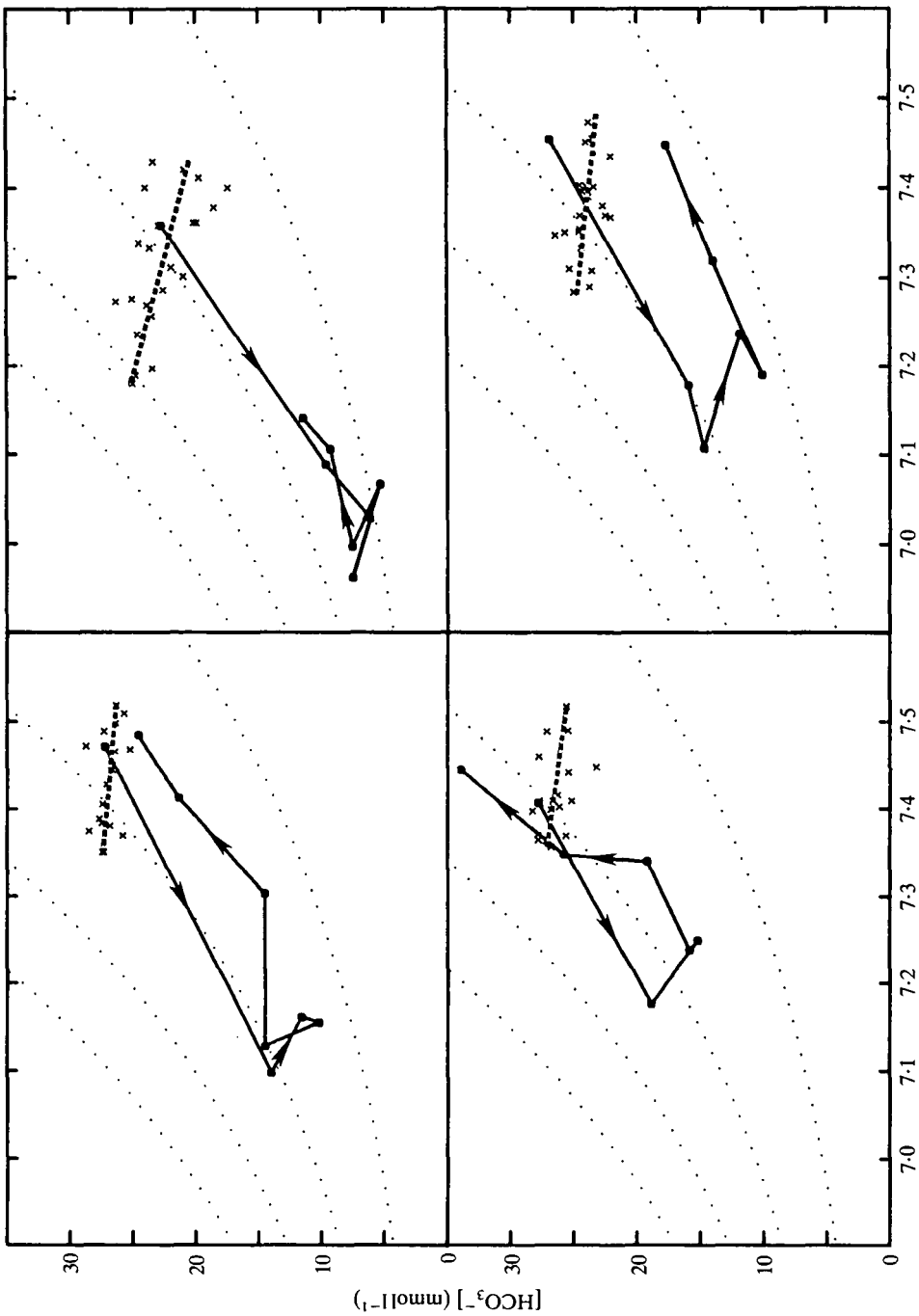

Fig. 3

$[HCO_3^-]$ on pH in the five animals were not significantly different, whereas the correlation was highly significant ($P<0.001$). The common slope from five animals was -12.16 ± 4.66 mmol HCO_3^- l^{-1} pH^{-1}. This buffer value is significantly below that obtained by CO_2 equilibration *in vitro* (-23.53 mmol HCO_3^- l^{-1} pH^{-1}, Table 1), and points to a lower buffering capacity of extracellular fluid in comparison with blood. A similar difference has been observed in the turtle, *Chrysemys scripta* (mean *in vivo* $= -15.35$ mmol HCO_3^- l^{-1} pH^{-1}, Jackson *et al.* 1974; *in vitro* $= -20.3$, Wilson, 1939).

Acid-base balance during and after exercise

Forced exercise resulted in pronounced lactacidosis in laboratory animals (Fig. 4). Blood pH decreased significantly from 7.43 ± 0.06 to 7.11 ± 0.08 immediately after exercise ($P=0.003$ by Student's paired *t*-test). Blood [lactate] increased significantly ($P=0.001$) from 1.3 ± 0.4 to 24.5 ± 5.6 mmol l^{-1}. Mean P_{CO_2} changed from 35.1 ± 6.7 to 43.7 ± 11.2 Torr, but this increment is not significant ($P=0.2$). Similarly, arterial P_{O_2} did not change significantly during activity (from 107 ± 27 to 102 ± 34 Torr; $P=0.75$). Meanwhile plasma $[HCO_3^-]$ decreased significantly ($P=0.002$) from 25.2 ± 3.6 to 14.6 ± 4.2 mmol l^{-1}. These values obtained as a result of laboratory exercise were similar to those from similarly sized (< 5 kg) crocodiles exhausted in the field (Bennett *et al.* 1985). The 5-min period of laboratory exercise was established to ensure exhaustion in the animals. Small field animals became exhausted and unresponsive during a similar period when they were captured.

The initial removal of lactate and protons from the blood of laboratory animals was not exponential. During the first 0.5 h, [lactate] and pH remained fairly constant (Fig. 4), presumably due to a gradually shifting balance of tissue washout and clearance of these ions from the blood. Although this pattern suggests that muscular [lactate] remains considerably higher than blood [lactate] for some time, blood samples taken 10–20 min after cessation of activity yield maximal blood [lactate] values. The time course of lactate appearance in crocodile blood is similar to that reported for other reptiles (Moberly, 1968; Bennett & Dawson, 1976). In man, however, blood lactate reaches its peak more quickly, in 5–10 min after exhaustion (e.g. Crescitelli & Taylor, 1944; Bergström, Guarnieri & Hultman, 1971) and in fish it may take hours (e.g. Black, 1957; Black, Connor, Lam & Chiu, 1962; Turner, Wood & Clark, 1983*a*; Turner, Wood & Hōbe, 1983*b*).

Bicarbonate-pH diagrams of laboratory-exercised crocodiles demonstrate the pronounced metabolic acidosis, with plasma $[HCO_3^-]$ immediately decreasing along the CO_2 isopleths (Fig. 3). During the first hour of recovery, an initial

Fig. 3. Bicarbonate-pH diagrams of four *Crocodylus porosus* at rest and during recovery from exhaustive exercise. Upper points (x) and regression line represent auto-titration with respiratory CO_2 in undisturbed and intimidated resting animals. Lower points (●) and connecting line follow severe metabolic acidosis immediately after exercise and during recovery. The line passes sequentially through data taken at 10, 20, 30 min and 1, 2 and 3 h of recovery. The data are superimposed on P_{CO_2} isopleths.

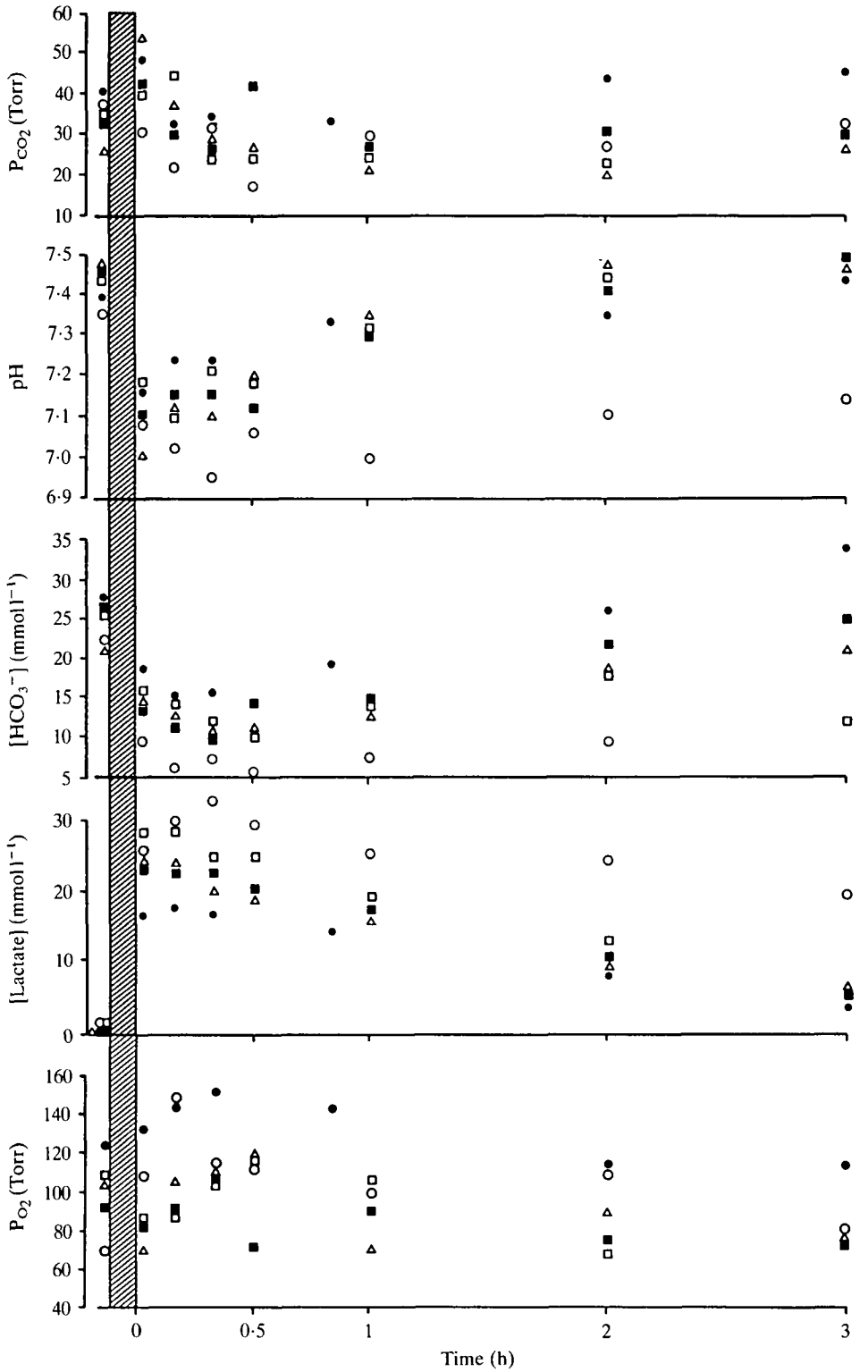

Fig. 4

respiratory compensation is apparent in Fig. 3, and is also indicated by a rising trend in arterial P_{O_2} and a drop in P_{CO_2} in the face of low pH (Fig. 4). However, the major adjustment was metabolic removal of lactate which was nearly complete in four of the five animals after the 3-h period of recovery (Figs 3, 4).

Animals exhausted in the field generally showed a two-component recovery. At first, a respiratory compensation reduced blood P_{CO_2} to below normal values and then recovery continued with metabolic removal of lactate and protons (Fig. 5). The notable exception was animal number 4 (Table 2). It became more severely acidotic during the first 2 h of recovery, with pH dropping to the extremely low value of 6·4. It survived, however, and acid-base balance was gradually restored to normal during the next day.

DISCUSSION

P_{CO_2}-P_{O_2} diagram

Normally the P_{CO_2}-P_{O_2} diagram is based on alveolar gases (Rahn & Fenn, 1955). Our data (Fig. 1) are derived from blood samples from the right systemic arch in crocodiles. Nevertheless, we feel that the values represent alveolar gas as closely as

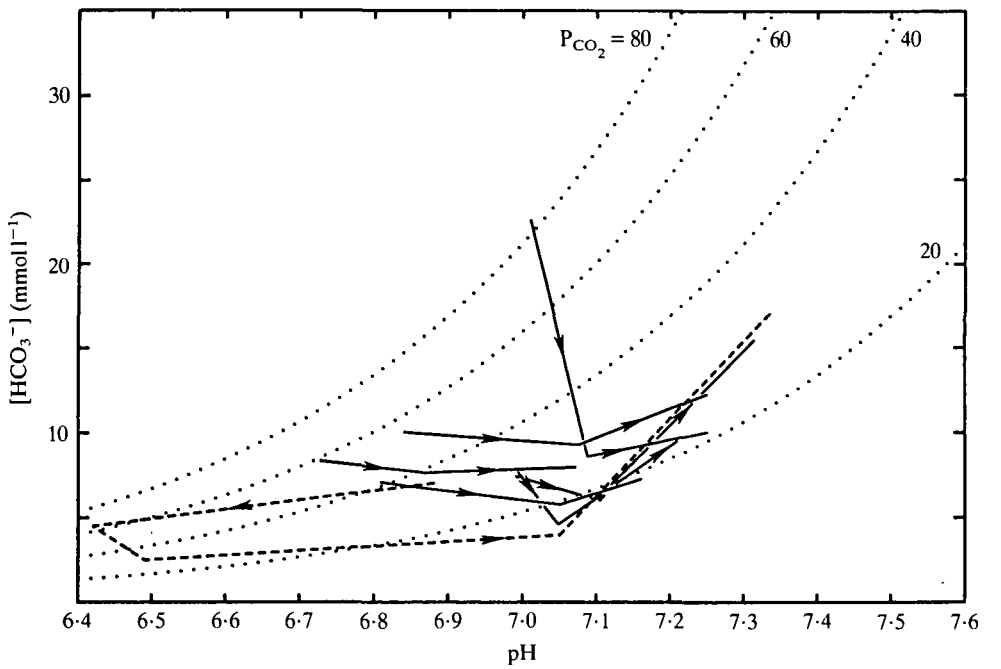

Fig. 5. Bicarbonate-pH diagram of exhausted and recovering *Crocodylus porosus* captured in the field. Data indicate values immediately after capture, followed by 2 and 4 h of recovery. The dashed line shows the protracted recovery of animal number 4, the points being 0, 2, 4, 14 and 29 h after capture (see text).

Fig. 4. Selected physiological variables during recovery from 5 min of exhaustive exercise in five *Crocodylus porosus* at 30 °C.

Table 2. *Acid-base properties of the blood of a 32-kg crocodile (animal number 4) after capture and exhaustion (see text)*

Time after capture (h)	[Lactate] (mmol l^{-1})	$[H^+]$ (μmol l^{-1})	pH	P_{CO_2} (Torr)	$[HCO_3^-]$ (mmol l^{-1})
0·1	49·1	0·135	6·87	36	6·69
2	47·7	0·380	6·42	70	4·51
4	69·1	0·342	6·49	33	2·50
14	42·7	0·089	7·05	14	4·00
29	5·0	0·046	7·34	30	17·24

the gas samples taken directly from the lumina of reptilian lungs. The crocodilian heart is capable of virtually complete separation of arterial and venous blood (White, 1956; Webb, 1979) and central right-to-left shunting does not develop except during long forced dives (White, 1969). In any case, if some shunting occurred centrally or in the lung (Seymour, 1983), it would tend to increase P_{CO_2} and decrease P_{O_2} from the conditions in the lung. The former would increase the slope of the P_{CO_2}-P_{O_2} line and the latter depress it. Although these effects do not exactly counteract each other, because of different shapes of the CO_2 and O_2 equilibrium curves, venous admixture mainly shifts the data points along the line. Such a shift was demonstrated in an aquatic snake, *Acrochordus arafurae*, in which there was little difference in the data derived from the pulmonary artery and vein (Seymour, Dobson & Baldwin, 1981).

The mean resting R (respiratory exchange ratio) value of 0·6 (Fig. 1) is lower than expected, but low R values (0·49) have been reported in *C. porosus* (Grigg, 1978). Grigg attributed low R to the ability of crocodiles to lose over one-third of the CO_2 production as urinary ammonium bicarbonate. A similar low R has been observed in Nile crocodiles (Glass & Johansen, 1979), but Davies (1978) reported higher values (0·73) in *Alligator mississippiensis*.

A declining R during breath-holding indicates buffering of CO_2 in the body and possibly non-pulmonary CO_2 loss, for example in the urine or through the skin. It is an inevitable result of breath-holding (Mithoefer, 1965) and has been observed in several diving reptiles (Wilson, 1939; Berkson, 1966; Lenfant, Johansen, Petersen & Schmidt-Nielsen, 1970; Seymour & Webster, 1975; Ackerman & White, 1979; Burggren & Shelton, 1979; Seymour *et al.* 1981), including crocodilians (Andersen, 1961; Glass & Johansen, 1979). The slope of the diagram is inversely related to the capacity of the body to absorb metabolically-produced CO_2. A shallow slope indicates that much of the CO_2 is hydrated to HCO_3^- plus H^+. The pH change caused by this respiratory acid load depends on the efficacy of *in vivo* buffer value in reptiles. It is possible that diving species have greater *in vivo* buffer values and lower P_{CO_2}-P_{O_2} slopes than non-divers, but, with one exception, all reptilian P_{CO_2}-P_{O_2} data come from divers. It is of interest that the exception, the terrestrial tortoise, *Testudo graeca*, has a significantly higher P_{CO_2}-

P_{O_2} slope (Burggren & Shelton, 1979) than any of the diving species (see references above), and that the blood buffer values in terrestrial tortoises (*Testudo* sp.) are remarkably low (Lenfant *et al.* 1970; Rahn & Garey, 1973). Although it has been pointed out that *in vitro* buffer value is not related to diving behaviour in reptiles (Seymour, 1982), *in vivo* buffering needs to be examined.

Effect of activity on acid-base balance

It is surprising that the *in vitro* buffer value is not higher in these crocodiles considering the extreme lactate concentrations produced during activity, particularly in large individuals (Bennett *et al.* 1985). However, our *in vitro* buffer value ($-23{\cdot}5\,mmol\,l^{-1}\,pH^{-1}$) represents only small animals (0·43–7·0 kg). The possibility that buffer value may be size-dependent is suggested by the data from slightly larger animals (15·6–18·2 kg), which had a reported value of $-37\,mmol\,l^{-1}\,pH^{-1}$ (Grigg & Cairncross, 1980).

In the absence of net changes in ATP in muscle, the production of each lactate molecule results in one proton (Hochachka & Mommsen, 1983). In fact, ATP decreases in active muscle thereby producing additional protons. If each lactate molecule entering the blood is accompanied by a proton, one would expect the change in the base deficit to be equal to the increase in [lactate]. However, base deficit in exhausted and recovering crocodiles falls considerably short of the increase in [lactate]; some protons are shown to be missing (Fig. 6). Thus, the

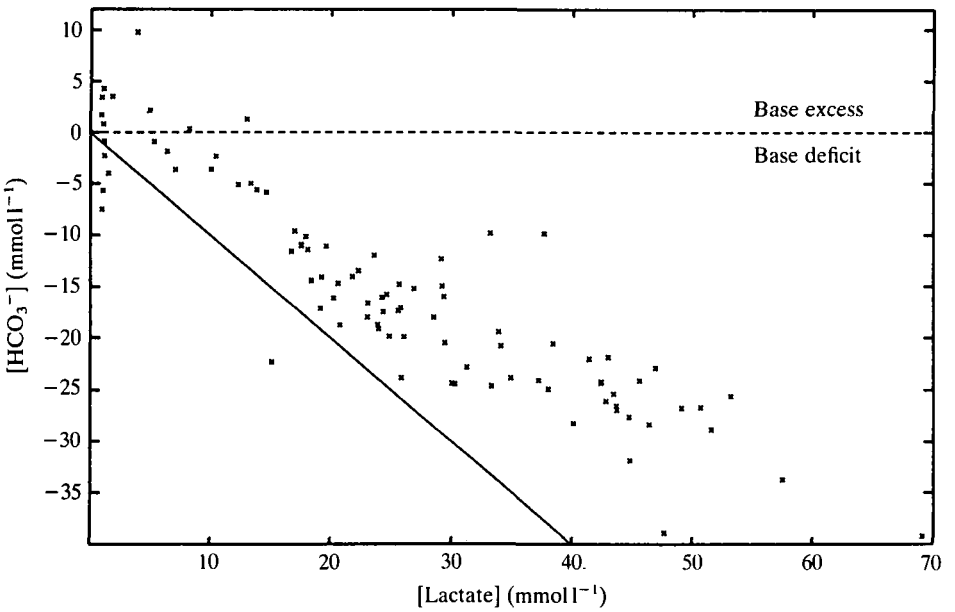

Fig. 6. The relationship between base deficit (calculated at pH = 7·43) and [lactate] in field and laboratory *Crocodylus porosus* during recovery after exhausting exercise. The solid line represents equimolar concentration of bicarbonate removal and lactate addition; the dotted line represents mean bicarbonate concentration in resting animals.

fluxes of lactate and protons in crocodile blood are temporally uncoupled, with protons appearing (as base deficit) at about half the concentration of lactate. Approximately equimolar changes in [lactate] and base deficit following anaerobic metabolism have been observed in alligators, *Alligator mississippiensis* [pH and bicarbonate data from Hernandez & Coulson (1958) and blood buffer value from Dill & Edwards (1935)], turtles, *Chrysemys scripta* (Wilson, 1939; Jackson & Silverblatt, 1974) and lizards, *Varanus salvator, Varanus exanthematicus* and *Iguana iguana* (Mitchell, Gleeson & Bennett, 1981; Gleeson & Bennett, 1982).

Various patterns of proton and lactate concentrations in the blood may be explained by a combination of (1) differential release of lactate and protons by the muscle, possible due to buffering of protons in the muscle, (2) differential clearance of these ions from the blood or (3) addition of metabolic base to the blood.

There has been much conflicting evidence concerning the kinetics of proton and lactate release from exercising muscle. Benadé & Heisler (1978) report that protons may be released much more quickly than lactate from rat and frog striated muscle. Protons can appear in the blood more quickly than lactate in certain amphibians (Boutilier, McDonald & Toews, 1980; McDonald, Boutilier & Toews, 1980), but the opposite may be true for dogs (Hirche *et al.* 1975). In fish, lactate and protons can be retained in muscle for considerable periods and released at different rates, lactate being sometimes higher and sometimes lower, depending on species. Turner & Wood (1983) have shown that proton release from isolated fish preparations depends on the acid-base status of the perfusing fluid; at low pH, proton release is retarded. Thus in some fish, blood base deficit is considerably less than [lactate] (Piiper, Meyer & Drees, 1972; Turner *et al.* 1983*a*). In other fish species, however, lactate is apparently retained preferentially in the muscle while protons are released (Turner *et al.* 1983*b*).

Without knowledge of the fate of artificially-infused lactic acid, we cannot assess the differential rates of proton and lactate clearance. However, Turner *et al.* (1983*a,b*) recently showed that both ions are removed from fish blood at equal rates and suggested that they were effectively eliminated as lactic acid, not lactate. The possibility that some metabolic base is added to the blood during activity and recovery, cannot now be assessed.

Effect of acidosis on O_2 transport

The extreme shift in pH following activity in our crocodiles offers an opportunity to demonstrate the significance of the Bohr effect in O_2 delivery to tissues. The classical value of the Bohr effect, determined by CO_2 titration, has two components, a fixed-acid effect (a rightward shift in the Hb-O_2 equilibrium curve due to protons alone) and a CO_2 effect (a rightward shift due to CO_2, not the protons it may form). There is good evidence that the fixed-acid effect is remarkably small and the CO_2 effect correspondingly large in crocodilian blood (Bauer & Jelkmann, 1977; Jelkmann & Bauer, 1980; Grigg & Cairncross, 1980). Grigg & Gruca (1979) suggest that the low concentration of red cell organic

phosphates found in *C. porosus* and other crocodiles greatly reduces the fixed-acid Bohr effect. Although the magnitude of the fixed-acid Bohr effect should decrease at very low pH, an initially low value is adaptive because it facilitates continued O_2 uptake from the lungs during or after bouts of anaerobic exercise or diving. The large CO_2 effect, on the other hand, is not greatly disadvantageous from the standpoint of O_2 loading of haemoglobin during recovery because rapid ventilatory adjustments quickly make the blood normocapnic or slightly hypocapnic.

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