J. exp. Biol. **109**, 55–62 (1984)
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THE CONTRIBUTION OF THE ALKALINE PERICARDIAL FLUID OF FRESHWATER TURTLES TO ACID BUFFERING DURING PROLONGED ANOXIA

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Accepted 17 October 1983

SUMMARY

The role of buffering of the high pericardial fluid [HCO_3^-] of the turtle, *Chrysemys picta bellii* Gray, was evaluated during prolonged anoxia at 3 and 10 °C. At 3 °C, pericardial fluid samples were collected from groups of animals after 0, 1, 2, 4 and 8 weeks of anoxia, and the ionic composition of these samples was compared to plasma values from the same animals. At 10 °C, pericardial and plasma samples were taken from normoxic turtles and from turtles after 11 days of anoxia. The samples were analysed for total CO_2 (C_{CO_2}), [Cl^-] and [lactate$^-$]. At 3 °C, the fall in pericardial [HCO_3^-] and the rise in [lactate$^-$] lagged behind the same changes in the plasma, until after about 8 weeks of anoxia the composition of pericardial fluid became identical with that of plasma. At 10 °C, pericardial [HCO_3^-] fell significantly after 11 days of anoxia but was still above plasma [HCO_3^-], while [lactate$^-$] was essentially the same in both fluids. We conclude that the pericardial fluid does participate in the buffering of lactic acid during prolonged anoxia. However, its involvement is delayed, possibly until the energy supply for the active carrier-mediated transfer processes responsible for the high [HCO_3^-] gradient breaks down as a consequence of the prolonged anoxia. Analysis of the overall buffering in the body reveals that the contribution of the pericardial fluid is minor.

INTRODUCTION

Many freshwater turtles possess a highly alkaline pericardial fluid. Smith (1929) reported that the total CO_2 content in this fluid (primarily HCO_3^-) exceeded 100 mM in specimens of the following species: *Emys blandingii, Chelydra serpentina, Graptemys geographica, Chrysemys marginata bellii* (probably the present *Chrysemys picta marginata*) and *Pseudemys elegans* (now called *Chrysemys scripta elegans*). In these same turtles, the peritoneal, or perivisceral, fluid also had elevated total CO_2

Key words: Pericardial fluid, buffering, lactate, prolonged anoxia.

content, lower than pericardial fluid, but still about twice the plasma concentration. Plasma total CO_2 in freshwater turtles is high compared to the values in other vertebrates, and in Smith's study ranged between 33 and 52 mM.

In view of their specialized composition, Smith speculated that the pericardial and peritoneal fluids may serve as buffer reserves under conditions of excess organic acid production, such as during diving anoxia. Support for this suggestion came from the observation that when lactic acid was administered by an unspecified route, lactate entered the peritoneal fluid of *Chrysemys scripta elegans* (Murdaugh, Robin, Pyron & Weiss, 1965). Similarly, Jackson (1969*a*) found that prolonged metabolic acidosis in the same species, induced by daily intragastric infusion of dilute HCl for 2 weeks, caused the $[HCO_3^-]$ of both pericardial and peritoneal fluid to fall to values that were below the reduced plasma $[HCO_3^-]$.

Results of studies conducted to test the buffering role of these fluids during acute experimental diving acidosis, however, have not supported the hypothesis. Jackson & Silverblatt (1974) reported no significant change in the $[HCO_3^-]$ of either compartment in *Chrysemys scripta elegans* after 4 h of diving at 24 °C, or at 1 or 24 h following such a dive. A small increase in pericardial [lactate] but not peritoneal [lactate] was noted 1 h after completion of diving. Likewise, Penney (1974) observed no significant increase in peritoneal [lactate] in the same species after 24 h submersion at 22 °C, although plasma lactate was increased from 1·0 to 37·0 mM. More recently, on the other hand, Ultsch & Jackson (1982) have found that very long-term anoxia (up to nearly 6 months) in *Chrysemys picta bellii* at 3 °C resulted in the same low $[HCO_3^-]$ and the elevated [lactate] in pericardial fluid, peritoneal fluid and plasma.

In the present study, also conducted on *Chrysemys picta bellii*, we have followed the time course of $[HCO_3^-]$ and [lactate] in pericardial fluid and plasma during prolonged anoxia at 3 °C in an attempt to reconcile these disparate results. In addition, measurements were made at 10 °C on one group that was normoxic and on a second group after 11 days of anoxia.

METHODS

Animals

Western painted turtles (*Chrysemys picta bellii* Gray) obtained from Lemberger Associates, Germantown, WI, U.S.A. (now: Kons Scientific), were shipped to Germany or Rhode Island for the experimental studies.

Animal preparation and experimental protocol

The bulk of the present investigation was part of a larger study concerned with intra- and extracellular acid-base response to prolonged anoxia at 3 °C. Details of the experimental methods have been described previously (Jackson & Heisler, 1982) and only the aspects pertaining to the present study will be given here.

Turtles were surgically fitted with chronic arterial catheters and were slowly acclimated from room temperature to 3 °C over a period of 2 weeks at a rate not exceeding −1·5 °C per day. Ten turtles were studied as normoxic 3 °C controls, and the remaining turtles were submerged in anoxic water and studied in groups of seven after 1, 2, 4, 8 and 12 weeks of anoxia.

Triplicate blood samples from each turtle, while still in water, were collected anaerobically in heparinized tuberculin syringes and analysed for pH, P_{O_2} and P_{CO_2} using Radiometer electrodes. Plasma obtained from one of these samples was analysed for total CO_2 concentration (C_{CO_2}) using a modified version of the method described by Cameron (1971). Plasma from an additional blood sample was deproteinized in two parts of 0·8 N (8 %) perchloric acid and the supernatant was analysed for lactic acid concentration using the enzymatic method (Boehringer, Mannheim).

After being administered an overdose of Nembutal, the turtle under study was removed from the water and its plastron was removed to expose the body cavity. The pericardial membrane was carefully opened and the pericardial fluid was withdrawn into a tuberculin syringe. A portion of this fluid was analysed for C_{CO_2} using the Cameron method. Pericardial fluid [HCO_3^-] was calculated by subtracting dissolved CO_2 from C_{CO_2}. Dissolved CO_2 was calculated as $\alpha_{CO_2} \times P_{CO_2}$, where $\alpha_{CO_2} = 0{\cdot}0808\ \text{mmol}\ l^{-1}\ \text{Torr}^{-1}$ (Reeves, 1976). A further portion of the pericardial fluid sample was mixed with two parts 0·8 N perchloric acid and analysed for lactic acid, and the balance was analysed for Na^+ and K^+ using a flame photometer (Instrumentation Laboratory, Model 343) and for Cl^- using a chloride titrator (Radiometer, Model CMT 10).

Two groups of turtles were studied at 10 °C. The first group ($N = 6$) had continuous access to air and are denoted as normoxic controls. The second group ($N = 6$) was submerged in N_2-equilibrated water for 11 days prior to study. This anoxic duration is about the maximum that these turtles can withstand and still recover normal function (C. V. Herbert & D. C. Jackson, unpublished observation). The procedure for collecting samples was identical for animals in both groups. The turtle being studied was killed by decapitation and its plastron was removed, as described above, exposing the heart and other viscera. A pericardial fluid sample was collected into a tuberculin syringe, and a blood sample was taken by heart puncture into a second tuberculin syringe. The pericardial fluid and plasma were analysed, as described above, for C_{CO_2}, [lactate$^-$] and [Cl^-]. No acid-base measurements were made on whole blood from these animals. Measurements of brain ions were made on these animals, but these data will be reported elsewhere.

RESULTS

Series I: 3 °C

Control animals had variable concentrations of bicarbonate in pericardial fluid ($\bar{x} \pm$ S.E. = 80·4 mM ± 10·4; range: 43·9–121·8 mM. In all turtles, however, pericardial [HCO_3^-] exceeded plasma [HCO_3^-], in the peak cases by more than threefold (Fig. 1). Plasma [HCO_3^-] was 36·9 ± 0·8 mM. An inverse linear relationship existed between the pericardial concentrations of HCO_3^- and Cl^- as described by the equation: $[Cl^-] = 127{\cdot}1 - 0{\cdot}98\ [HCO_3^-]$, $r = 0{\cdot}97$. Because the slope was close to 1·0, the sum of the two anion concentrations averaged 127·1 mM in the control animals. This agreed closely with the sum of the pericardial concentration of measured cations, Na^+ and K^+, which in the same animals was 126·5 ± 2·8 mM. Control pericardial [lactate$^-$] was 0·5 ± 0·04 mM compared with a significantly higher plasma value of

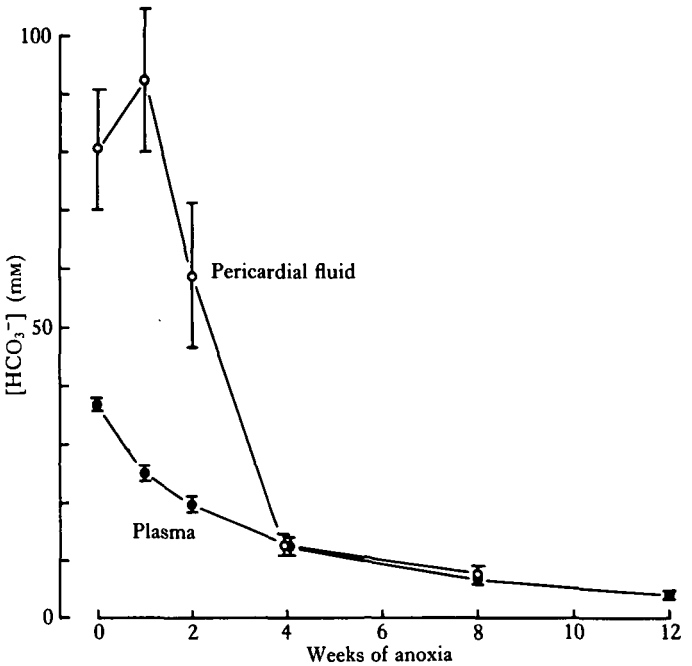

Fig. 1. Plasma and pericardial fluid $[HCO_3^-]$ of turtles during 12 weeks of anoxia at 3 °C. Means ± S.E.

1·6 ± 0·2 mM. Most turtles had only trace quantities of lactate in their pericardial fluid but one animal with 3·1 mM raised the group mean.

Anoxia caused a progressive rise in plasma $[lactate^-]$ and fall in plasma $[HCO_3^-]$ that was apparent after 1 week, the earliest sampling time in this study. Pericardial values, however, changed more slowly. Pericardial $[HCO_3^-]$ was not significantly reduced below control values, even after 2 weeks of anoxia (Fig. 1). By the fourth week sample and thereafter, though, plasma and pericardial $[HCO_3^-]$ were the same. Insufficient pericardial volume was obtainable on the 12-week animals to permit analysis.

The increase in pericardial $[lactate^-]$ also lagged behind the rise in plasma $[lactate^-]$ (Fig. 2), but by 1 week the pericardial value had increased significantly above the control pericardial value (t-test, $P = 0{\cdot}025$). The pericardial and plasma $[lactate^-]$ were not different statistically before exposure to anoxia for 4 weeks. At 8 weeks the plasma and pericardial lactate concentrations were identical.

Series II: 10 °C

After 11 days of anoxia, which is close to the maximum duration from which turtles can recover at this temperature (C. V. Herbert & D. C. Jackson, unpublished observations), pericardial $[HCO_3^-]$ was significantly reduced from the normoxic value, but was still higher than normoxic plasma $[HCO_3^-]$ (Table 1). The fall in pericardial $[HCO_3^-]$ was approximately similar to the rise in pericardial $[lactate^-]$, which was the same as the rise in plasma $[lactate^-]$. The chloride concentration, on the other hand, rose in the pericardial fluid and fell in the plasma.

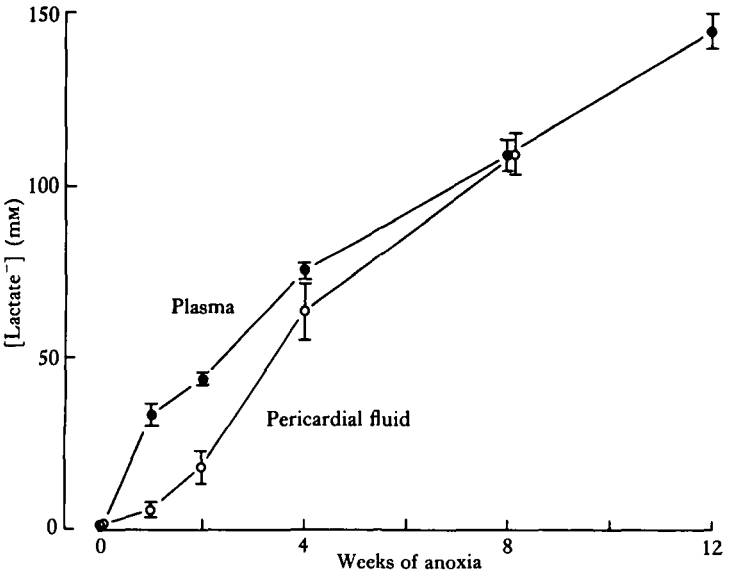

Fig. 2. Plasma and pericardial fluid [lactate$^-$] of turtles during 12 weeks of anoxia at 3 °C. Means ± S.E.

Table 1. *Plasma and pericardial fluid anion concentrations of normoxic turtles and turtles after 11 days of anoxia at 10 °C (mM, $\bar{x}$ ± S.E.)*

	Plasma			Pericardial fluid		
	C_{CO_2}	[Cl$^-$]	[Lactate$^-$]	C_{CO_2}	[Cl$^-$]	[Lactate$^-$]
Normoxic	46·6 ± 1·9	77·5 ± 2·7	1·5 ± 0·1	112·7 ± 8·7	23·7 ± 8·2	0·3 ± 0·04
Anoxic	22·0 ± 1·5	67·7 ± 1·7	67·7 ± 3·3	52·6 ± 2·8	63·8 ± 6·2	61·1 ± 2·5

DISCUSSION

These results reconcile the previous conflicting reports concerning the participation of the pericardial fluid in the buffering of fixed acid during diving. Clearly, this fluid does participate in acid buffering but the apparent H^+ penetration into the pericardial space is slow; under the circumstances of the present study, a period of 2–4 weeks was required before a significant fall in [HCO_3^-] was observed at 3 °C. At 10 °C, an equilibrium between pericardial and plasma [lactate$^-$] was observed after 11 days of anoxia, but pericardial [HCO_3^-] was still significantly higher than plasma [HCO_3^-]. This probably explains the lack of an apparent buffering role of pericardial or peritoneal fluid following dives of 1 day or less at 22–24 °C in *Chrysemys scripta elegans* (Jackson & Silverblatt, 1974; Penney, 1974). These are bulk fluids that apparently equilibrate slowly with blood perfusing the pericardial or peritoneal membranes.

This demonstrated contribution of the pericardial fluid to buffering of the lactacidosis during prolonged anoxia at 3 and 10 °C does not necessarily mean that the contribution is quantitatively an important one to the anoxic turtles. Although the

concentration of pericardial HCO_3^- is high, the pericardial fluid volume is small, and the absolute amount of lactate buffered by the decrease in pericardial HCO_3^- is also small. This point can be documented by considering the HCO_3^- or total CO_2 contents in various body compartments in a hypothetical 1 kg turtle. For the purposes of this analysis, available data from various emydid species were employed. In *C. scripta*, pericardial fluid volume and peritoneal fluid volumes averaged 1·9 ml kg^{-1} and 16·6 ml kg^{-1}, respectively, while [HCO_3^-] was 109 mM and 76 mM (Jackson & Silverblatt, 1974). For a 1 kg animal, this gives a total of 1·47 mmol of HCO_3^-, of which only 0·21 mmol is in the pericardial fluid. The plasma [HCO_3^-] of these turtles is about 36 mM (Jackson & Silverblatt, 1974; present study, Fig. 1) and, assuming the same concentration throughout the extracellular fluid (ECF) and an ECF volume near 200 ml (Stitt, Semple & Sigsworth, 1971), the total ECF HCO_3^- content is 7·2 mmol. The intracellular [HCO_3^-] has recently been calculated for skeletal muscle, cardiac muscle and liver from tissue pH data (Jackson & Heisler, 1983), and values range from 7 to 12 mmol kg^{-1} of cell water. Assuming an intracellular fluid volume of 500 ml kg^{-1} and an average [HCO_3^-] of 10 mmol kg^{-1} water, the total intracellular HCO_3^- is about 5 mmol in a 1 kg animal. Thus, within these several fluid compartments, a 1 kg turtle has a whole body bicarbonate pool of about 14 mmol. Recent measurements indicate that the bicarbonate in each of these sites is largely depleted during prolonged anoxia at 3 °C (Ultsch & Jackson, 1982; Jackson & Heisler, 1982, 1983), so that the pericardial fluid, despite its high [HCO_3^-], contributes less than 2 % of the total HCO_3^- buffering ($0{\cdot}21/14 \times 100 = 1{\cdot}5$ %). This does not take into account that, judged from measurements in other orders of animals (Heisler & Piiper, 1971, 1972; Heisler & Neumann, 1980; Heisler, 1984), buffering by the intracellular and extracellular non-bicarbonate buffers would be larger than bicarbonate buffering by a factor between 2 and 20 (depending on the change in plasma pH from 8 to nearly 7).

An even larger possible reservoir for carbonate buffer substances, is the bone and shell of the animal. In recent unpublished measurements, we have found that powdered shell of *C. picta* contains about 1300 mmol of acid-extractable CO_2 per kg of shell wet weight. The shell of these turtles represents about 30 % of total body weight (Jackson, 1969*b*), so that a 1 kg turtle has some 390 mmol of total CO_2 within its shell, an enormous reserve which is 20–30 times the total amount found in the rest of the body. It is not clear at present how much of this CO_2 (presumably in the form of CO_3^{2-} or HCO_3^-) is mobilized during prolonged anoxic acidosis, although it is possible that the major mobilization of Ca^{2+} and Mg^{2+} we have observed during anoxia (Jackson & Ultsch, 1982; Jackson & Heisler, 1982) is derived from shell and bone in association with CO_3^{2-} or HCO_3^-.

We conclude, therefore, that the pericardial fluid plays at best a minor role in the response of freshwater turtles to anoxic metabolic acidosis. Firstly, the buffer reserve of this fluid is only tapped during extremely prolonged anaerobic states that probably do not occur except at low winter temperatures (Gatten, 1981). Then lack of energy leads to failure of the active pump mechanisms that, under normal conditions, are required to maintain the large pericardial fluid/plasma gradient. At higher temperatures, the maximal possible duration of anoxia is too short to cause energy shortage and the pericardial fluid buffering properties are not exploited. There a

considerable doubts that long anaerobic dives normally occur at summer temperatures (Gatten, 1981). Secondly even if this HCO_3^- reserve were fully utilized, it is depleted together with much larger reserves elsewhere in the body, so that the contribution of the pericardial fluid is minimal.

The possibility still remains that the specialized composition of the pericardial fluid has functional significance to the cardiac tissue which it bathes. However, only the outer surface of the heart is exposed to pericardial fluid while the bulk of the heart muscle is bathed in blood or an ultrafiltrate of blood *via* the coronary vessels. It remains to be tested whether changing the composition of the pericardial fluid from its normal alkaline state would affect cardiac function.

Whatever the role of these fluids, it is clear that their formation must involve active anion transport as originally pointed out by Smith (1929). No study to date has examined either the site or the nature of this transport process. It appears that severe acidosis, whether induced by gastric infusion of inorganic strong acid under normoxic conditions (Jackson, 1969*a*) or by endogenous production of 'weak' lactic acid under anoxic conditions (this study), causes a loss of the distinctive alkaline composition of the pericardial fluid and equilibration with plasma. It is not clear how this equilibration occurs, although it could be due either to an inhibition (possibly by lack of energy) of the transport process or to an enhancement of anion permeability. The mechanism of formation and modification of this interesting fluid deserves further study.

The authors acknowledge the expert technical assistance of Günther Forcht and Sylvia Glage in Göttingen and of Theodore Schlegel in Providence. The study was supported in part by NSF Grant PCM78-22333 and by Deutsche Forschungsgemeinschaft.

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