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LONG-TERM SUBMERGENCE AT 3 °C OF THE TURTLE *CHRYSEMYS PICTA BELLII* IN NORMOXIC AND SEVERELY HYPOXIC WATER

III. EFFECTS OF CHANGES IN AMBIENT P_{O_2} AND SUBSEQUENT AIR BREATHING

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SUMMARY

Western Painted Turtles, *Chrysemys picta bellii* ($N = 5$), were maintained submerged and apneic for 90 days: days 0–21 in severely hypoxic water (P_{O_2} = 0–5 mmHg), days 22–43 in aerated water ($P_{O_2} \sim 160$ mmHg), and days 44–90 again in hypoxic water. From day 90 onward, the water was aerated and the turtles were allowed access to the air; water and air temperatures were maintained at 3 °C.

Arterial blood samples were taken periodically and analysed for P_{O_2}, P_{CO_2}, pH, [Na^+], [K^+], [Cl^-], [lactate$^-$], [glucose] and haematocrit. Plasma [HCO_3^-] was calculated for all samples and total plasma calcium was measured on samples from two animals.

Each exposure to low P_{O_2} water caused progressive lactic acidosis and a transient respiratory acidosis with an accompanying fall in plasma [Cl^-] and rise in plasma [K^+] and [calcium]. During the intervening period in aerated water, blood pH recovered significantly (from 7·33 to 7·74 in 7 days), due primarily to a fall in P_{CO_2} (from 23·5 to 10·6 mmHg), while [lactate$^-$] remained unchanged (at about 50 mM), and [HCO_3^-] rose slightly. Plasma [K^+] promptly returned to nearly normal values.

When permitted to breathe on day 90, the three surviving turtles rapidly restored pH to normal by pronounced hyperventilation ($P_{CO_2} < 5$ mmHg). Metabolic acidosis, however, disappeared slowly with a $t_{\frac{1}{2}}$ for [lactate$^-$] and [HCO_3^-] restoration of about 2 weeks.

We conclude that a wintering turtle can stabilize or even slightly improve its acid-base and ionic status by moving from an anoxic environment to well-oxygenated water. Further improvement can be gained by breathing air, but recovery proceeds at a very slow rate if the animal remains at 3 °C.

INTRODUCTION

Freshwater turtles winter in water for at least 5–6 months in the northern United States and southern Canada. It is generally assumed that most of this time the

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turtles are submerged and do not breathe air (Porter, 1972); this is certainly the case for those periods when the habitat is sealed in ice. The behaviour of turtles while submerged, however, is uncertain. Ernst & Barbour (1972) reported that painted turtles (*Chrysemys picta*) spend a significant portion of the winter buried in mud, which is presumably low in oxygen. In contrast, there are anecdotal reports of turtles of the same species swimming beneath the ice, and a recent radiotelemetric tracking study of wintering painted turtles (D. M. DeLisle & W. W. Burggren, personal communication) has suggested daily movements of these animals throughout the winter. In another recent study using similar techniques, however, snapping tutles (*Chelydra serpentina*) remained buried and immobile throughout the coldest part of the winter (G. R. Ultsch, D. C. Jackson & D. Lee, unpublished observations).

Given this uncertainty regarding the natural behaviour of wintering turtles, laboratory studies can only examine the consequences ot the various possibilities. In previous studies of the Western Painted Turtle, *Chrysemys picta bellii* (Ultsch & Jackson, 1982:; Jackson & Ultsch, 1982), we showed that this animal could survive at 3 °C for as long as 6 months in a virtually anoxic state, but that continuous submergence in aerated water permitted some aerobic metabolism and caused less severe acidosis. These observations, besides revealing the differences in effects of continuous submergence in anoxic or aerated water at 3 °C, also suggest the possibility that turtles wintering without access to air could improve their physiological condition by leaving the mud and temporarily entering the oxygen-rich water above them. In the absence of ice, of course, they could also swim to the surface and breathe.

It was our purpose in this study to extend our previous observations on the physiology of wintering turtles by assessing the effects on blood ionic and acid-base balance in a single group of turtles switched from a severely hypoxic state to conditions in which O_2 at ambient P_{O_2} was available, either from the water only, or from both water and air.

MATERIALS AND METHODS

Western Painted Turtles (*Chrysemys picta bellii*) were supplied by a commercial dealer (Lemberger, Germantown, Wisconsin) from a stock collected in Wisconsin in October 1979. We maintained them at 15–20 °C and a 12–12 photoperiod until mid-January 1980. They were fed dog food and chopped fish twice a week. They were then chronically catheterized in the right subclavian artery and cooled at 1 °C/day in shallow, aerated water at 3 °C. Details of the catheterization procedure and further maintenance of the experimental animals are given in Ultsch & Jackson (1982).

On the first day at 3 °C, blood samples were taken and analysed for arterial P_{O_2}, P_{CO_2}, pH and haematocrit, and for plasma [Na^+], [Cl^-], [K^+], [lactate$^-$] and [glucose]. Plasma [HCO_3^-] was calculated using the Henderson–Hasselbalch equation with pK and CO_2 solubility calculated from Reeves (1976). On 29 January 1981, denoted as day zero of the experiment, the turtles were submerged. The O_2 tension of the water was maintained at a low level (0–5 mmHg) by covering the water surface with 2 cm of heavy-duty paraffin oil and continuously bubbling N_2 through the water. A screen prevented the animals from surfacing and allowed the N_2 to escape. The tanks were covered with opaque plastic that excluded most light. A 12–12 photoperiod w

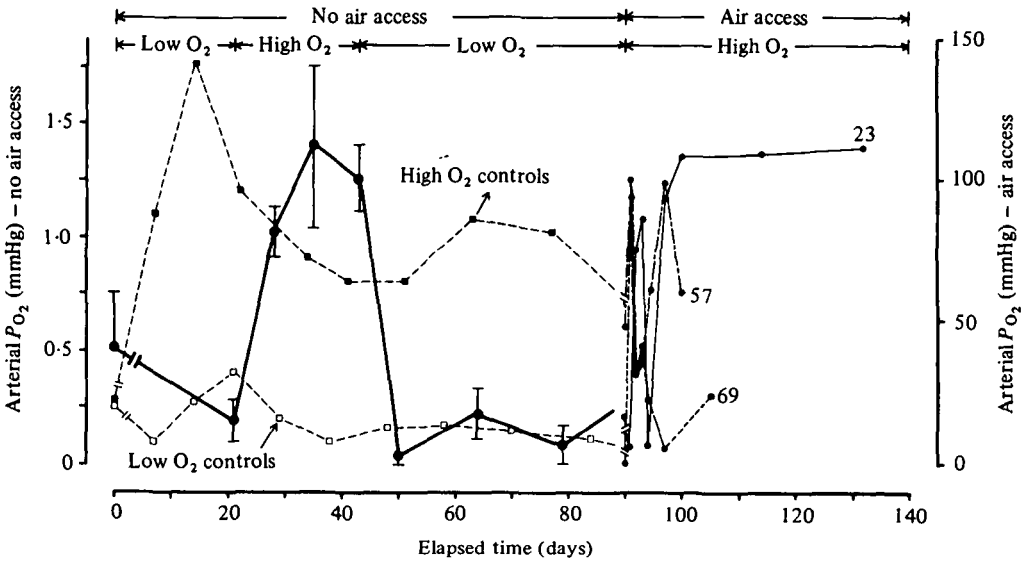

Fig. 1. Arterial P_{O_2} of *Chrysemys picta bellii* at 3 °C as a function of time. Days 0 and >90 are air access data (right-hand ordinate); days 1–90 are submergence data (left-hand ordinate). Time periods of air access and oxygen tensions in the water (low, 0–5 mmHg, high, ~160 mmHg) are indicated at the top of the figure. Controls represent turtles maintained continuously submerged in high or low P_{O_2} water (see Ultsch & Jackson, 1982). Data are given as $\bar{X} \pm$ S.E. for days 0–90; survivors are plotted individually after day 90.

maintained throughout the experiment. On day 21, blood samples were taken, and the gas bubblers were subsequently switched from N_2 to air for the next 3 weeks, which raised the P_{O_2} to approximately 160 mmHg. Additional blood samples were taken on days 28, 35 and 43. The gas input was then switched back to N_2 for the duration of the submergence period. Blood samples were taken on days 50, 64, 79 and 90. Up to day 79, the sample size was 5 turtles; on day 90 it was 4, as one of the turtles died prior to the sampling.

After 90 days of submergence, the water was replaced with shallow, aerated, oil-free water, and the restrictive screen removed, allowing the turtles easy access for air breathing. The temperature was maintained at 3 °C, and blood samples were taken from the surviving turtles on days 91–94, 97, 100, 114, 132 and 134. Only turtle no. 23 survived past day 100; it died on day 142, 52 days after being allowed access to air.

Maintenance of the cold turtles, blood sampling and handling procedures, and the analytical methods are all given elsewhere (Ultsch & Jackson, 1982; Jackson & Ultsch, 1982), except that in the present study calcium concentrations were determined only with the colorimetric method (Sigma Kit 585).

RESULTS

The experiments reported here were conducted simultaneously with others already published (Ultsch & Jackson, 1982; Jackson & Ultsch, 1982) in which similarly

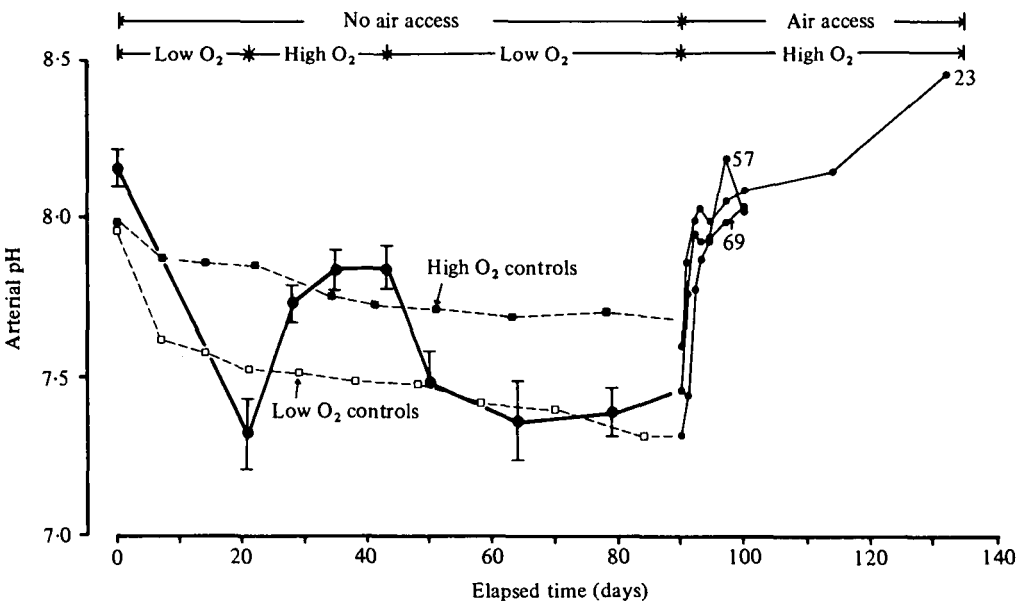

Fig. 2. Arterial pH as a function of time. Data presentation as in Fig. 1.

prepared and treated turtles were submerged under constant conditions in either high or low P_{O_2} water. Therefore the results of the previous studies can serve as controls; that is, the blood values of the turtles which were alternated between high and low O_2 tensions are compared to values of turtles maintained continuously at high or low P_{O_2}. All results, except for calcium where our sample size is only 2, are displayed in this manner.

During the initial 21 days in low O_2 water, arterial P_{O_2} fell predictably to values similar to those of low O_2 controls; during the period of aeration it increased by a factor of approximately 5, and then fell again to low levels when the water was again deoxygenated (Fig. 1). Three weeks in hypoxic water, therefore, had no effect upon the ability of the turtles to take up O_2 from the water when it became available. When the turtles resumed breathing, they immediately established high and variable $P_{a\,O_2}$ values.

Glucose concentrations and haematocrits showed no particular trend with relation to the control groups. Glucose averaged about 10 mM throughout; there was some tendency for an increase during the hypoxic episodes, and a decrease during the period of high ambient P_{O_2}, but the differences were not significant. Similar comments can be made for haematocrit, with the values decreasing from about 25% to 15% during the first 100 days. Neither variable changed in a consistent manner with the advent of air breathing.

The acid-base status of the blood reflected the availability of O_2 and the significant uptake of O_2 that occurred during the period of high P_{O_2}. During the initial hypoxic period, arterial pH fell (Fig. 2) as arterial P_{CO_2} rose (Fig. 3) and plasma [HCO_3^-] decreased (Fig. 4). These changes were largely generated by a large increase (to ov

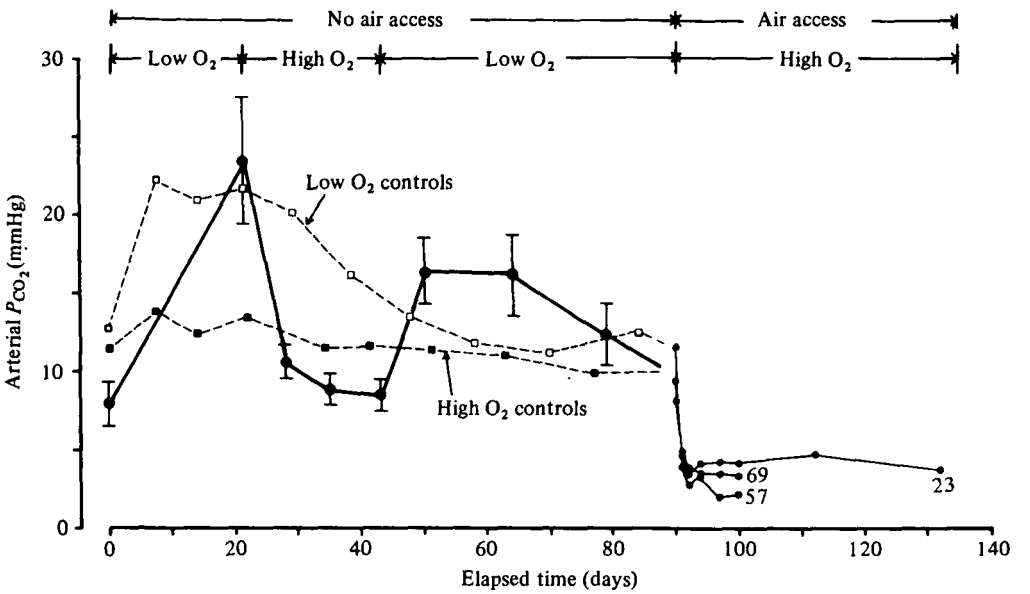

Fig. 3. Arterial P_{CO_2} as a function of time. Data presentation as in Fig. 1.

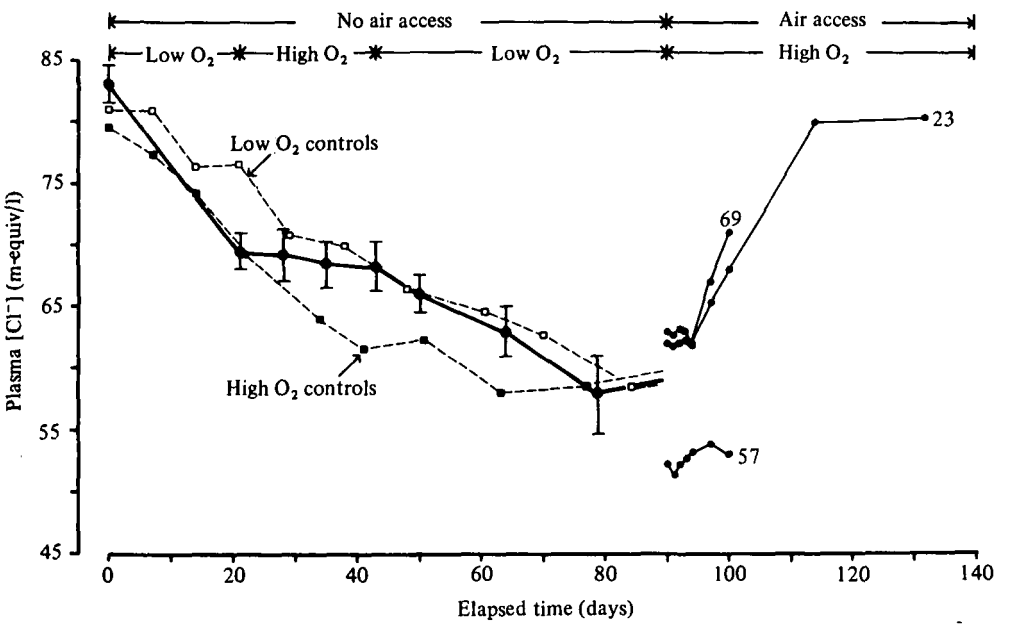

Fig. 4. Plasma bicarbonate concentration as a function of time. Data presentation as in Fig. 1.

50 mM) in plasma lactate concentrations (Fig. 5). These changes approximated those of the low O_2 control group after 21 days. The aeration period was marked by a stabilization of [lactate$^-$], a slight increase in [HCO_3^-], and a marked decrease in P_{CO_2}. The result was a significant restoration of arterial pH to values which matched those of high O_2 controls that had not experienced any hypoxia. Turtle 23 raised its pH

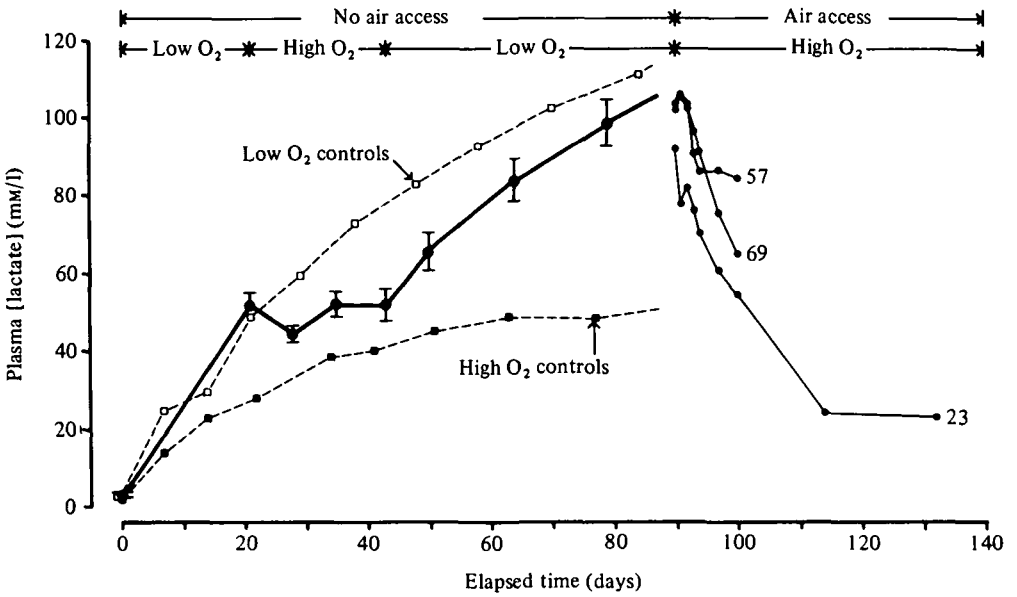

Fig. 5. Plasma [lactate$^-$] as a function of time. Data presentation as in Fig. 1.

from 7·70 to a normal value of 8·02 during this period. During the subsequent hypoxia, the turtles resumed the trend of increasing [lactate$^-$] and decreasing [HCO_3^-]. After an initial fall the arterial pH stabilized near 7·4 as both [HCO_3^-] and P_{CO_2} fell. During the subsequent breathing period, pH was rapidly restored in all three survivors by hyperventilation that lowered P_{CO_2} to less than 5 mmHg (Fig. 3). Restoration of normal [lactate$^-$] and [HCO_3^-] were much slower processes, the latter (but not the former) reaching completion after 42 days of air breathing in turtle 23.

Ion concentrations (excepting [Na^+], Fig. 6), were also dependent upon the availability of O_2. As in our earlier study (Jackson & Ultsch, 1982), [Na^+] was well regulated until an animal was nearing death. Plasma [K^+] (Fig. 7) and [Cl^-] (Fig. 8), on the other hand, changed significantly, apparently in response to the changes in [lactate$^-$]. When [lactate$^-$] increased, [K^+] increased and [Cl^-] decreased. When [lactate$^-$] stabilized during water aeration, so did these two ions. When air breathing was resumed, [K^+] fell rapidly at first, and then more slowly; [Cl^-] did not change at all during the first few days, and then increased slowly in two of three turtles, approaching control levels in turtle no. 23 after 24 days.

As in our previous study (Jackson & Ultsch, 1982), we calculated a plasma 'ion gap' ($= [Na^+]+[K^+]-[HCO_3^-]-[Cl^-]-[\text{lactate}^-]$) on the basis of the ion concentrations thus far presented (Fig. 9). This calculation is based on the assumption of electrical neutrality, so that any apparent gap actually represents unmeasured ions. As in the previous study, a gap resulting from excess anions, that is, a 'cation gap', developed throughout the submergence to values over 50 m-equiv/l. Associated with this gap was an increase in total plasma calcium concentration, measured on two turtles (Fig. 10). During the recovery period, both the cation gap and the plasma [calcium] fell in parallel.

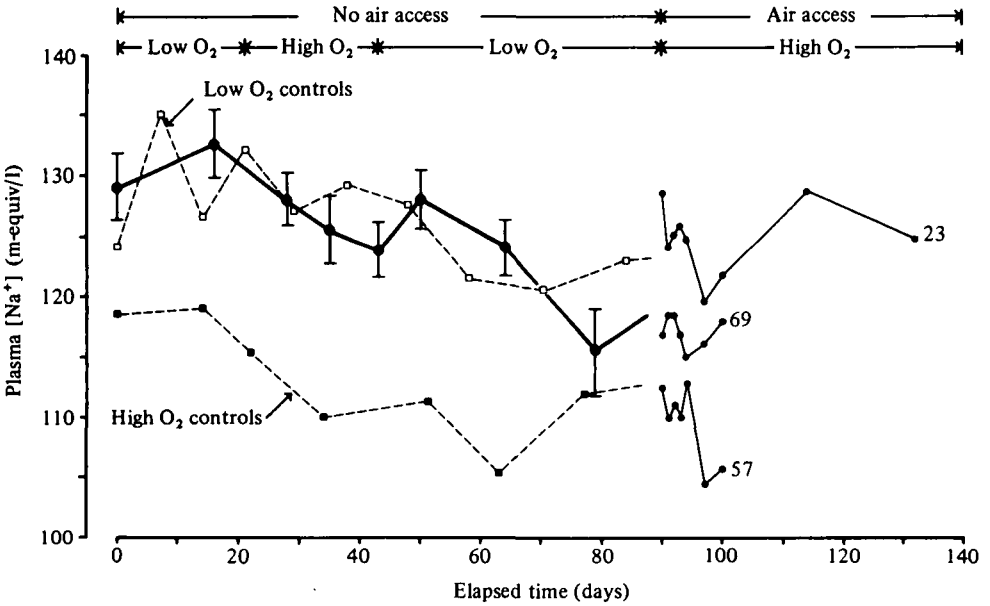

Fig. 6. Plasma [Na^+] as a function of time. Data presentation as in Fig. 1.

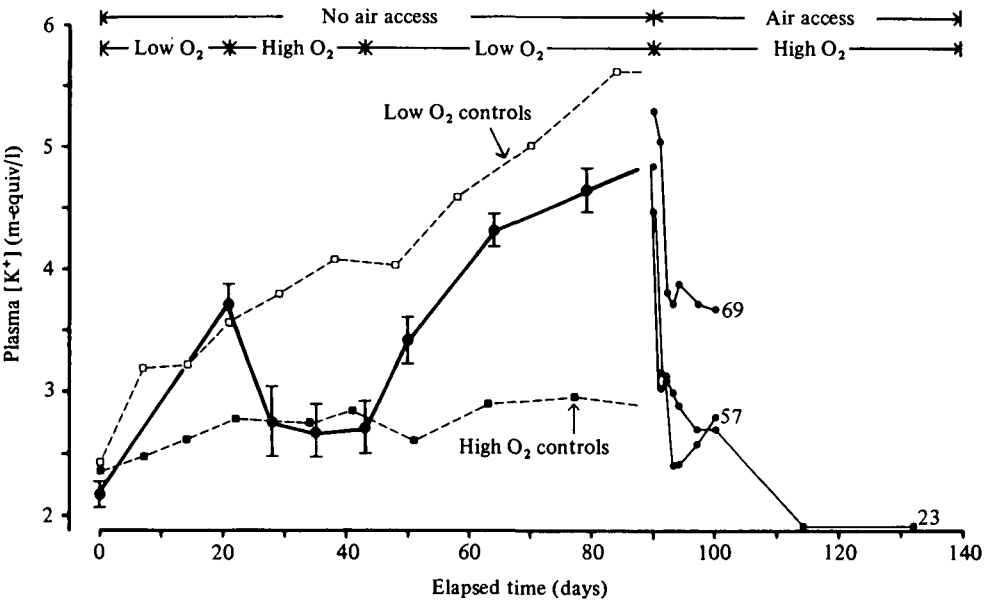

Fig. 7. Plasma [K^+] as a function of time. Data presentation as in Fig. 1.

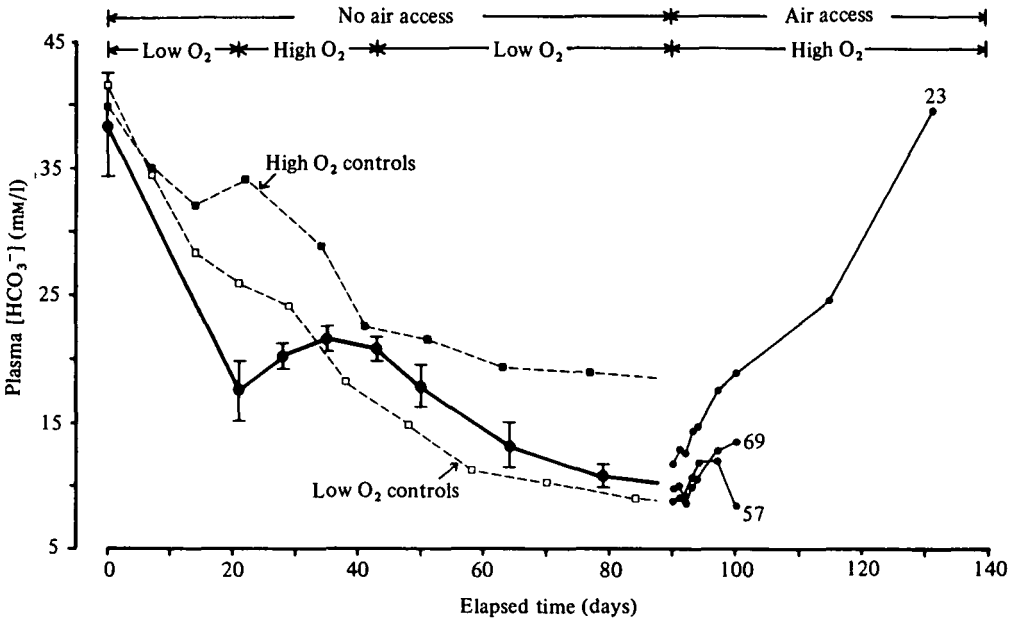

Fig. 8. Plasma $[Cl^-]$ as a function of time. Data presentation as in Fig. 1.

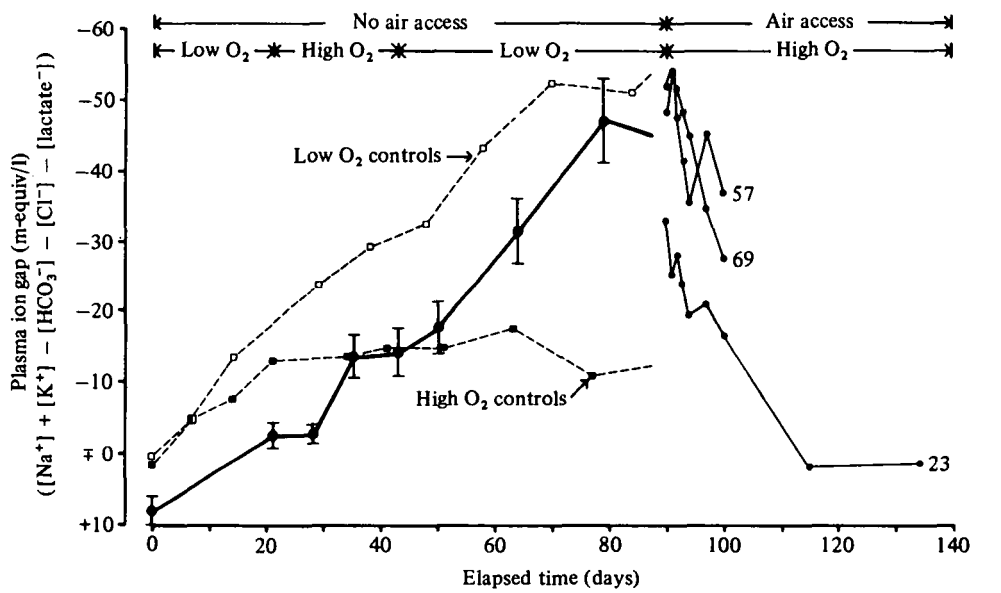

Fig. 9. Plasma ion gap, defined as the difference between positively charged and negatively charged univalent ions, as a function of time. Data presentation as in Fig. 1.

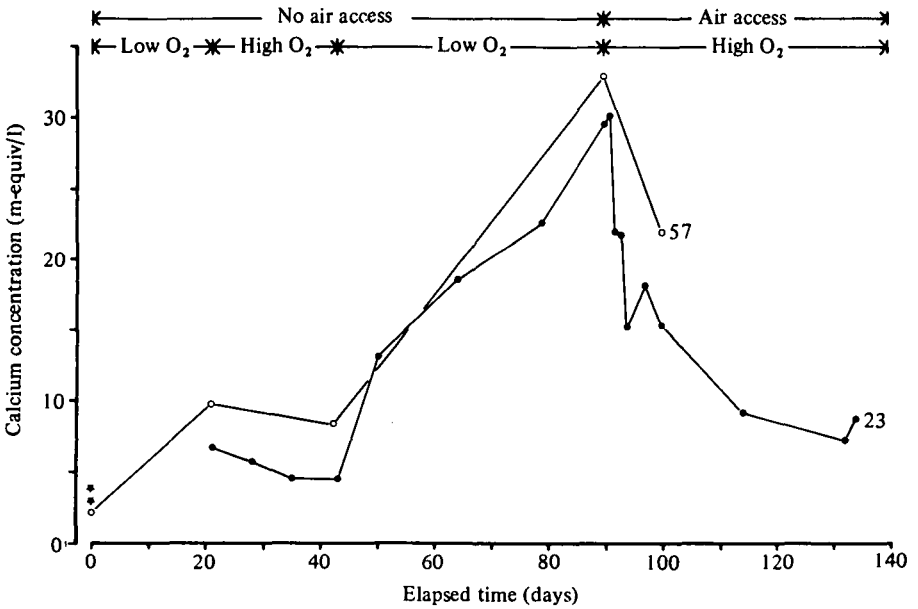

Fig. 10. Total calcium concentrations as a function of time in turtles 57 and 23. Stars are day zero values from two control turtles.

DISCUSSION

In this study we have simulated three possible microenvironments for O_2 uptake by a submerged wintering freshwater turtle at 3 °C: (1) a severely hypoxic environment providing little or no O_2; (2) air-saturated water, but with O_2 only available by means of extrapulmonary aquatic uptake; and (3) atmospheric P_{O_2}, accessible through both pulmonary uptake from air and extrapulmonary uptake from the water.

When subjected to condition 1, which comprised the first 3 weeks and the final 7 weeks of apnoea, the turtles were forced to rely completely upon anaerobic metabolism. We suggest that this essentially anoxic condition simulates the circumstances encountered by a wintering turtle that buries itself in O_2-poor mud. The primary anaerobic metabolite, lactate, accumulated steadily during these weeks, and other symptoms of O_2 deficiency, such as hyperkalaemia, also became apparent. The acid-base consequences of this anaerobic state, discussed in detail earlier (Jackson & Ultsch, 1982), included a primary metabolic acidosis, a secondary but transient respiratory acidosis, and an array of compensatory ionic changes in the blood, including decreased [HCO_3^-], decreased [Cl^-], and increased [calcium]. The acid-base changes during this and subsequent conditions are depicted on a Davenport diagram in Fig. 11.

In condition 2, weeks 4–6 of the apnoeic period, the turtles were able to extract O_2 from the water by uptake through non-pulmonary surfaces. The rise in blood P_{O_2} associated with this condition, however, was quite small (to only 1·0–1·5 mm Hg), but at this low temperature haemoglobin affinity for O_2 is high (L. Maginniss & R. B. Reeves, personal communication), and significant, albeit modest, amounts of O_2 may

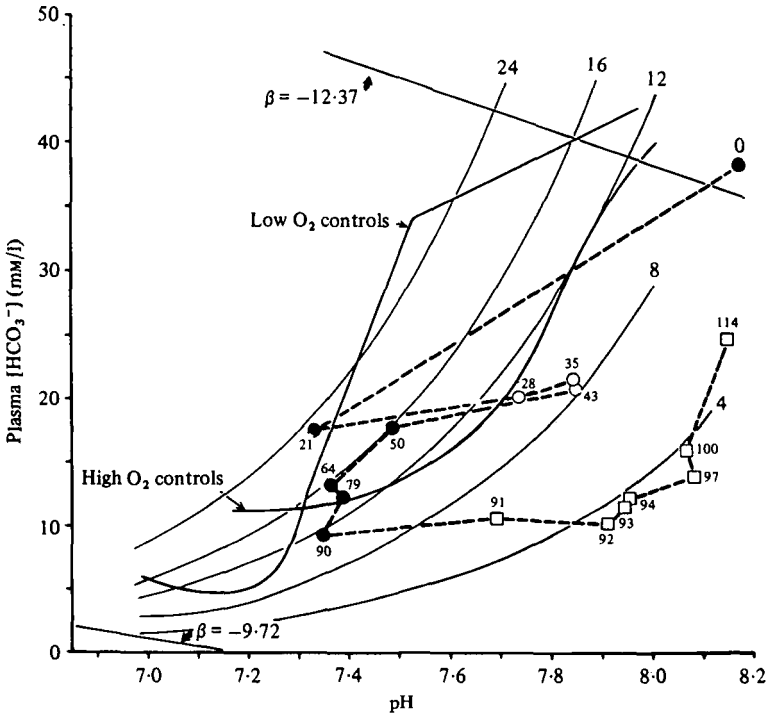

Fig. 11. Plasma [HCO_3^-] *v.* pH plot (Davenport diagram) for turtles at 3 °C. Number of days elapsed is indicated next to each data point. Periods of submergence (after day zero) in low P_{O_2} water are shown as blackened circles, in high P_{O_2} water as open circles, and with access to air as open squares. Buffer values are from a control turtle ($\beta = -12{\cdot}37$) and a long-term anoxic turtle ($\beta = -9{\cdot}72$); these values and the low and high O_2 control values are from Ultsch & Jackson, 1982).

be bound at this low P_{O_2}. Notwithstanding the problems of O_2 transport by the blood, there was clear evidence for a shift from completely anaerobic to largely aerobic metabolism when dissolved O_2 became available. Plasma [lactate$^-$] stabilized during these 3 weeks, a response that indicates that the dissolved O_2 was adequate for a maintenance level of oxidative metabolism, but was not sufficient to oxidize the accumulated lactate. Fig. 11 reveals that the considerable restoration of blood pH during the exposure to aerated water was largely due to a reduction in P_{CO_2} back to control values, but that some metabolic correction occurred as well. Plasma [HCO_3^-] rose slightly during this period, whereas it would have fallen in parallel with the buffer curve in the absence of a metabolic (i.e. strong ion) acid-base change. The most dramatic improvement noted was the nearly complete and rapid restoration of plasma [K^+] (Fig. 7), which may represent the most sensitive indicator of the anaerobic to aerobic shift among our measured variables. Many of the variables, however, did not show an improvement and, in general, the overall effect of the water aeration was to stabilize the body fluid state at its post-anaerobic condition. We suggest that the aerated water treatment simulates the circumstances of a turtle that moves out of the mud into open water where the P_{O_2} is similar to the ambient air value, but at a time

when the surface is ice-covered and no aerial gas exchange is possible. Such behaviour could ameliorate the acid-base status of the blood and prolong the duration of submergence, compared to a continuous anoxia. Our previous study, however, demonstrated that even when turtles are continuously exposed to aerated water, a variable degree of acidosis results (Ultsch & Jackson, 1982), although in a group of apnoeic, non-catheterized animals, kept in aerated water, the acidosis was no worse after 6 months than was that of the turtles in the present study after only 3 weeks of anoxia.

The final gas exchange condition utilized in our study, access to both atmospheric and dissolved O_2 at 3 °C, simulated the natural situation in which the water surface is ice-free and the turtle can freely ventilate its lungs, but while still remaining at low temperature. This therefore does not represent the situation at the end of the winter because the water temperatures rise at that time and turtles typically emerge from the water and bask. Our results indicate that all the observed blood changes reverse back toward normal, but that the process of recovery is very slow. This suggests that a brief period of air access during the winter, lasting even up to several days, may not have an appreciable effect on the blood acid-base and ionic status. For substantial recovery to occur, a breathing period of longer duration, up to several weeks, may be required. This assumes that the O_2 stored in the body at the end of a breathing episode will be exhausted atter a day or so of apnoea. We assume that the recovery process at the elevated temperatures associated with basking occurs much faster, but further experimentation will be required on this aspect.

One striking feature of the final breathing phase of this study was the pronounced and persistent hyperventilation that occurred. Blood P_{CO_2} values fell to below 5 mmHg in all surviving animals wihin 2 days of the initiation of breathing and remained there for the remainder of the experiment. This is approximately half the normal P_{CO_2} and indicates a pulmonary ventilation (relative to metabolic rate) of about twice the normal value. In terms of respiratory control, it is not obvious what constituted the chemical stimulus for this continued hyperventilation because, except for the first day or two, all of the blood variables usually regarded as chemical stimuli (P_{O_2}, P_{CO_2} and pH) had either returned to normal or shifted in the direction of respiratory inhibition. Furthermore, normal respiration at this temperature is probably almost entirely concerned with O_2 uptake because of the effectiveness of extrapulmonary CO_2 loss. At 3 °C, breathing, or the cessation of breathing, have negligible effects upon blood P_{CO_2} and pH except after very long apnoeic periods (Ultsch & Jackson, 1982). But blood P_{O_2}, which could be logically regarded as the normal respiratory stimulant at these low-temperature conditions, was of course abnormally high because of the elevated ventilation. We suggest that the respiratory stimulation was unrelated to blood values but was in some way associated with the persistent metabolic acidosis, perhaps via brainstem chemoreceptors responding to ionic imbalance in the brain environment as has been shown to occur in a related emydid species, *Pseudemys* (= *Chrysemys*) *scripta* (Hitzig & Jackson, 1978). In an earlier study on diving at 24 °C in this latter species, a similar hyperventilation was observed that was unexplainable by the conventional blood chemical stimuli (Jackson & Silverblatt, 1974). If the brain chemoreceptor hypothesis is correct, it would mean that acid-base or related ionic disturbances are potentially paramount in respiratory control even under ambient

circumstances (3 °C) at which the normal acid-base contribution of the lung is negligible.

One of the aspects of the response to apnoea that we observed both in this and in our previous study (Ultsch & Jackson, 1982) was the elevation in blood P_{CO_2} when the turtles were in O_2-poor water (Fig. 3). Although at present we do not know the basis for this hypercapnia, we can assume that it resulted from an imbalance between the rates of CO_2 generation and excretion. The obvious source of endogenous CO_2 generation in this anaerobic state was from the titration by lactic acid of HCO_3^- and CO_3^{-2} ions, the latter possibly deriving from bone and/or shell and associated with calcium elevation (Fig. 10 and Jackson & Ultsch, 1982). However, we cannot exclude possible metabolic sources of CO_2, such as have been reported in anaerobic fish (Kutty, 1968; Shoubridge & Hockachka, 1980; Van den Thillart & Kesbeke, 1978), although there is no evidence to our knowledge for the existence of anaerobic CO_2-producing pathways in Chelonia (Hochachka *et al.* 1975), nor, in view of the observed lactate elevation do we see the need to postulate them. A disturbance in CO_2 excretion is plausible because of the depression of both heart rate and blood pressure during the anaerobic state (unpublished observations). It is of interest to note that when the turtles were submerged in aerated water, P_{a,CO_2} was normal which suggests that under this condition the relationship between CO_2 production and excretion was similar to that in the air-breathing state.

All of the physiological changes in gas exchange, acid-base status and ionic status that we have presented here and in the previous two papers can be viewed as a response to a severe lactic acidosis that develops rapidly in cold apnoeic turtles exposed to hypoxia, and eventually in those in aerated water. While it is clear that emergence from anoxic mud into water of high P_{O_2} is beneficial, and that this benefit can be realized in spite of the previous anoxia, we do not know what the actual behaviour of the various species is during normal wintering. Certainly long periods are spent buried; this may be to avoid predation, aerobic fungal infections, or for other unknown reasons. It is possible that during exceptionally long winters, the ability to utilize dissolved oxygen may be of crucial survival value during the last few weeks before the temperature is high enough to melt surface ice in the most northerly portions of the ranges of freshwater turtles. Such zoogeographical implications of the physiology of wintering in turtles and other aquatic ectotherms are interesting subjects for future studies.

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