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

I. SURVIVAL, GAS EXCHANGE AND ACID-BASE STATUS

BY GORDON R. ULTSCH* AND DONALD C. JACKSON

Division of Biology and Medicine, Brown University,
Providence, Rhode Island 02912, U.S.A.

SUMMARY

Survival and blood acid-base status were measured on freshwater turtles (*Chrysemys picta bellii* Gray) that were submerged at 3 °C in either aerated (high O_2) or N_2-equilibrated (low O_2) water. Results from catheterized turtles, without access to air under these conditions, and from non-catheterized turtles which were either apnoeic (in high O_2 and low O_2 water) or in high O_2 water with access to air, are compared.

Under the most adverse conditions (catheterized, submerged, low O_2), survival duration was 126 ± 14 ($X \pm$ S.E.) days, and 2 of the 10 turtles so treated were still alive after 177 days, although their condition was poor. Apnoeic, high O_2 turtles generally survived longer and were in better condition despite a skin fungus condition that selectively affected these animals. Six of ten non-catheterized high O_2 turtles were still alive after 189 days without breathing.

All apnoeic turtles developed an acidosis which, except for a transient hypercapnia in low O_2 turtles, was a metabolic acidosis attributable to elevated lactic acid. Acidosis was most severe in low O_2 turtles in which peak plasma lactates exceeded 200 mM. High O_2 turtles, as judged by higher blood P_{O_2} and lower lactate concentrations, were able to extract dissolved O_2 from the water and support a significant portion of their metabolic requirements by aerobic metabolism. Our data indicate that wintering turtles can remain alive for up to 6 months, even while totally anoxic and severely acidotic, but that the acid-base state and probably the recovery potential are significantly improved if dissolved O_2 is available for extra-pulmonary uptake.

INTRODUCTION

Freshwater turtles spend most of their time under water and, except for periods of basking, only occasionally come to the surface to breathe (Belkin, 1964). At summer temperatures these apnoeic periods may last for minutes or hours (Lucey & House, 1977; Burggren & Shelton, 1979), but during the winter, when the water temperature

* Usual address: Department of Biology, University of Alabama, University, AL 35486, U.S.A.

is near freezing, turtles may remain continuously submerged for weeks or even months (Carr, 1952). The capacity for an animal that is normally dependent on aerial respiration to suspend breathing for this length of time is clearly a problem of considerable physiological interest. From an ecological viewpoint, it is possible that the northern distribution of turtles is limited by their physiological tolerance to prolonged submergence in cold water, particularly in areas where the habitat can be ice-covered for months at a time.

The physiological and biochemical adaptations accounting for the diving ability of turtles have been extensively studied, but except for descriptive investigations of diving duration, these studies have been conducted at temperatures above 15 °C. At these temperatures, gas exchange is severely curtailed during a dive and internal O_2 supplies are sufficient for at most several hours (Robin et al. 1964; Jackson, 1968; Burggren & Shelton, 1979). However, once O_2 supplies are depleted, turtles exhibit a remarkable ability to survive for many additional hours utilizing anaerobic glycolysis (Belkin, 1963; Robin et al. 1964, Jackson, 1968; Clark & Miller, 1973). During a dive of this duration an initial respiratory acidosis that develops during the period of oxidative metabolism is combined with a metabolic acidosis due to the accumulation of lactic acid, the primary end-product of anaerobic glycolysis. Blood lactic acid concentrations typically reach 20–50 mM in experimental dives of long duration (Robin et al. 1964; Jackson & Silverblatt, 1974; Penney, 1974), and the combined respiratory and metabolic disturbances may result in blood pH values as low as 6·7.

Low temperatures slow the rate at which these changes occur and may also affect the character of the changes, leading to a dramatically increased tolerance of long-term submergence. Musacchia (1959) reported survival times up to 155 days in *Chrysemys picta* submerged at 1·5 °C. However, no physiological measurements were made in this study, and other studies that do provide physiological data for low temperatures (Hutton & Goodnight, 1957; Rapatz & Musacchia, 1957; Frankel, Steinberg & Gordon, 1966) were conducted on turtles that were out of water. Therefore, although both field observations and laboratory studies reveal that turtles can and do survive long periods of submergence in cold water, there is no information concerning their physiological state and no analysis of the mechanisms making their extraordinary behaviour possible.

Hence it was our purpose to describe and interpret the physiological consequences of prolonged submergence at low temperature in a freshwater turtle, focusing particularly on aspects of gas exchange, acid-base balance and ion regulation.

METHODS

Animals. Western painted turtles (*Chrysemys picta bellii* Gray) of both sexes and ranging in weight from 476 to 930 g were obtained from Lemberger Associates in Germantown, Wisconsin. The animals were collected in October in Wisconsin shortly before shipment. We housed them at 15–20 °C with a 12:12 photoperiod in large tanks with basking platforms. They were fed several times a week with dog chow and chopped fish.

Catheterization procedure. Turtles were cooled for at least 12 h at 3 °C and then

catheterized in the right subclavian artery with PE 90 tubing, using a procedure modified from Jackson, Palmer & Meadow (1974). Access to the artery was gained through a 2 cm hole trephined through the plastron. The catheter was led out of the animal through the skin at the base of the neck and then through a hole drilled in the overlying lip of the carapace, where it was anchored with silicone cement. The hole in the plastron was plugged with a snug-fitting plexiglas disk sealed with dental acrylic. After a prophylactic injection of chloramphenicol (100 mg/kg), the catheter was flushed with a heparinized (100 units/ml) glucose-free balanced salt solution (modified from McDonald, 1976) and sealed with a stainless-steel plug. Turtles were allowed to recover overnight in air at room temperature before being submerged, with access to air, in the experimental holding tanks at 15 °C. These tanks were situated in a cold room with controlled lighting (12:12 photoperiod).

Experimental groups. The catheterized turtles, as well as a number of non-catheterized turtles, were cooled from 15 °C at 1 °C/day to the experimental temperature of 3 °C. During the second day at 3 °C, denoted as day zero in the results, they were grouped (10–11 per group) and treated as follows:

(1) High O_2, catheterized, no air access. Control blood samples (see below) were taken while these animals still had access to air. Then a rigid plastic screen (1·5 cm mesh) was secured about midway in the 30–40 cm water depth; this prevented the animals from surfacing to breathe. Aerators were placed above the mesh to mix and aerate the water. The conditions in this tank were intended to simulate a natural open-water environment under the ice.

(2) Low O_2, catheterized, no air access. This group was treated similarly to group 1, except that the surface of the water was covered with 2–3 cm of heavy-duty paraffin oil, and the water was continuously bubbled with nitrogen. This kept the water P_{O_2} at 0–5 mmHg, which was intended to simulate the hypoxic conditions surrounding a turtle hibernating in the mud.

The remaining three groups served as controls.

(3) High O_2, non-catheterized, no air access. Except for the lack of surgery and periodic blood sampling, these animals were treated similarly to those of group 1. Blood samples were taken at the end of the submergence period for comparison with the similarly treated catheterized turtles.

(4) Low O_2, non-catheterized, no air access. As in group 3, these animals served as controls, in this case for group-2 turtles.

(5) High O_2, non-catheterized, air access. Groups 3 and 4 served as controls against the effects of surgery; this group served as a control against the effects of a lengthy exposure to low temperature. The animals were not operated upon and had access to O_2 via both the aerial and aquatic modes of gas exchange.

The water in all experimental tanks was tapwater and was changed at least every 3 weeks, and more frequently if the water did not appear to be clean. Water volume was 8–13 l/turtle and water depth in all tanks was about 30 cm. The sides and tops of the tanks were covered with cardboard or plastic sheets, so that except during blood sampling and viability checks, the animals were in dim light during the 12 h light period.

Blood sampling and analyses. Blood was sampled from each catheterized turtle at

day zero (just prior to forced submergence) and periodically thereafter until the death of the animal. The original catheters remained patent for the duration of the study (up to 178 days) without special precautions other than filling with the heparinized saline solution after each sampling session. The absence of a clotting problem was of considerable advantage to us and presumably helps to maintain blood fluidity in the sluggish circulation of cold turtles. There are reports that the clotting time of turtle blood is considerably slowed at low temperature because of reduced prothrombin levels (Brambel, 1941) or elevated endogenous blood heparin levels (Kupchella & Jacques, 1970).

Two blood aliquots were taken at each sampling time. The first (0·3 ml) was collected anaerobically into a heparinized glass syringe and was immediately analysed for pH, P_{CO_2} and P_{O_2}, using a Radiometer BMS Mk2 Blood Micro System in combination with a Radiometer PHM 73 pH/Blood Gas Monitor and a PHM 84 Research pH Meter. With this system, P_{O_2} was read directly from the Monitor, P_{CO_2} was determined with the pH channel of the Monitor (because of the inadequate sensitivity of the P_{CO_2} channel), and pH was read from the PHM 84 meter. All electrodes were thermostatted to 3 °C. Blood gas electrodes were calibrated with zero O_2 solutions and with gas mixtures produced by gas-mixing pumps (Wösthoff M301 a/F). The pH electrode was calibrated with Radiometer precision buffers. Meter outputs for the gas electrodes were monitored continuously with a stripchart recorder; in practice, equilibration of the electrodes with the samples required about 12 min and was facilitated by using calibration gas mixtures with partial pressures near those of the blood samples. Plasma [HCO_3^-] was then calculated from the Henderson–Hasselbalch equation using constants for pK' (6·346) and CO_2 solubility (0·0808 mmol/ l.mmHg^{-1}) given by Reeves (1976). Van Slyke analyses of total CO_2 were made on two plasma samples as a check on this method and the agreement was within 0·8 and 1·2 mM.

The second aliquot of blood (0·5 ml) was collected into either a glass or plastic syringe without heparin. A portion of this blood was used for a haematocrit determination and the remainder was centrifuged to obtain the plasma. Some of this plasma was immediately deproteinized with 8 % perchloric acid, centrifuged, and stored at 3 °C in a capped Eppendorf vial, as was the remaining plasma. Determinations of lactate, glucose, Na$^+$, K$^+$ and Cl$^-$ concentrations were performed in the following 2–4 days, and any remaining plasma stored at -12 °C.

Lactate analyses of the deproteinized plasma were performed with an enzymatic test kit (Sigma no. 826-UV) with absorption changes measured on a Beckman Model DUR Spectrophotometer. Standards were run for each series of analyses. Because of high lactate concentrations, samples usually had to be diluted (sometimes as much as 50 or 100:1) to bring the absorbance into the acceptable range. Glucose analyses were performed with a colorimetric analysis kit (Sigma no. 510) and sample dilution, when necessary, did not exceed 2·5:1.

Ion analyses were performed with a flame photometer (Instrumentation Laboratories Model 143) for Na$^+$ and K$^+$, and with a chloride titrator (Radiometer CMT 10) for Cl$^-$.

Surviving turtles from groups 3–5 were killed after 155–189 days so that their body

fluids could be analysed. They were prevented from breathing as they were removed from the water and were immediately decapitated. The plastron was rapidly removed, and blood samples (via cardiac or arterial puncture) and body fluid (urine, pericardial fluid and peritoneal fluid) samples were taken. The blood sampling and analytical protocol was the same as described above for the catheterized turtles. The entire collection sequence was completed in about 10 min at 3 °C. The lungs were examined in these turtles and in the dead catheterized turtles for the presence of gas.

Data on plasma $[Na^+]$, $[Cl^-]$ and $[K^+]$ and the analyses of body fluids other than blood are presented in a second paper (Jackson & Ultsch, 1981).

Evaluation of viability. The condition of each turtle in the study was periodically assessed by observing the reaction to mechanical stimulation. The withdrawal of the head or limbs away from a stimulus was taken as evidence that the animal was alive. In addition, catheterized turtles were occasionally evaluated by connecting the arterial catheter of each animal to a pressure transducer and recording blood pressure on a polygraph.

Buffer curves. CO_2 buffer curves were constructed on blood taken from turtle 63 after it had been submerged for 178 days in low O_2 water at 3 °C, and on blood from a control turtle that had been held for 16 days in a tank of aerated water at 3 °C and with access to the atmosphere. Blood from the control turtle (haematocrit = 22 %) was used to construct buffer curves for oxygenated and deoxygenated blood. Only a deoxygenated buffer curve was measured on the blood of turtle 63 (haematocrit = 7 %) because we were interested in the buffering capacity of the blood of an anoxic turtle. In order to evaluate the effect of low haematocrit, we adjusted a second sample to a haematocrit of 22 % by centrifuging the blood, removing the appropriate amount of plasma, and resuspending the cells in the remaining plasma. The blood was then deoxygenated and a second buffer curve was constructed. We were thus able to compare, in these two animals, the effects of oxygenation, haematocrit and prolonged anoxia on the blood buffering capacity.

The blood samples were equilibrated in a tonometer (Instrumentation Laboratories Model 237) at 3 °C with gas mixtures supplied by Wösthoff gas-mixing pumps. Three P_{CO_2} mixtures (approximately 5, 16 and 31 mmHg) were used for each curve. After equilibration, samples were taken from the tonometer sequentially and the pH measured (Radiometer PHM 84 Research pH Meter and BMS MK2 Blood Micro System) until agreement within 0·01 pH units was obtained. Plasma $[HCO_3^-]$ was calculated as described earlier. A least-squares regression line was fitted to the three points obtained for each curve, and the slope gave the buffer value. The correlation coefficients (r) for all curves were greater than 0·999.

RESULTS

Survival. The data on survival of each of the experimental groups are given in Table 1. Statistical comparisons between the groups were not attempted because of a complicating cutaneous fungal infection that eventually affected most of the turtles in aerated water. Most of the high O_2, non-catheterized turtles (groups 3 and 5) eventually responded to treatment, as evidenced by a visually determined remission of

Table 1. *Survival of turtles (days) (mean $\pm$ 1 $\times$ S.E.M.) at 3 °C with and without O_2 available in air or water*

	High O_2			Low O_2	
	Air access intact (5)*	Apnoeic		Apnoeic	
		Catheterized (1)	Intact (3)	Catheterized (2)	Intact (4)
Initial					
N	10	11	10	10	10
Non-survivors					
N	4	11	4	8	6
Duration	109±31·4	95·2±11·8	131±28·1	113±13·9	134±10·3
(Range)	(25–176)	(44–155)	(68–181)	(56–167)	(86–162)
Survivors					
N	6	0	6	2	4
Day terminated	189	–	189	177, 178	155–168

* Experimental group number (see Materials and Methods).

the extent of fungus, but the catheterized, high-O_2 animals did not respond to treatment and all died by 155 days; this was the only group with no survivors. The physiological condition of the catheterized turtles deteriorated once the disease became apparent (see below) and a primary effect of the disease may have been to hinder cutaneous gas transfer. No turtles in low O_2 water (groups 2 and 4) showed symptoms of the condition.

It is instructive to compare the results of groups paired according to treatment.

(1) Non-catheterized and submerged: groups 3 and 4. The turtles in aerated water (group 3) survived longer and the survivors were clearly in better condition than the turtles in low-O_2 water (group 4). All four of the group 4 survivors were weak and torpid at the time of sacrifice, while only 1 of the 6 survivors in group 3 was in poor condition. By day 165, 6 of the original 10 animals in group 4 had died, while only 2 of the 10 animals in group 3 had succumbed in spite of the fungal problems of the latter group.

(2) Low O_2 and submerged; groups 2 and 4. There was no significant difference in the maximal survival times of catheterized and non-catheterized turtles in low O_2 water. The three early deaths in the catheterized groups may have been related to surgery, but the remaining five deaths occurred at an average of 134 days, similar to that of the non-catheterized controls (group 4).

(3) High O_2 and non-catheterized; groups 3 and 5. Turtles in aerated water survived equally well in this study whether or not they had access to atmospheric air. Six turtles in each group survived 189 days. This indicates that aquatic gas exchange alone is sufficient for at least 6 months at 3 °C when O_2 is dissolved at ambient P_{O_2}.

Gas exchange and acid-base status. Arterial P_{O_2}, initially similar in both catheterized groups (22·7 ± 7·4 mmHg in high O_2, 20·7 ± 4·6 mmHg in low O_2), fell sharply to low values during apnoea in both groups, although the turtles in high O_2 had consistently higher P_{O_2} levels (Fig. 1). The low-O_2 turtles probably had zero P_{O_2} in the blood, and the average of about 0·3 mmHg for all samples is probably due to contamination occurring during the sampling and measuring process. The P_{O_2} of the high O_2 turtles
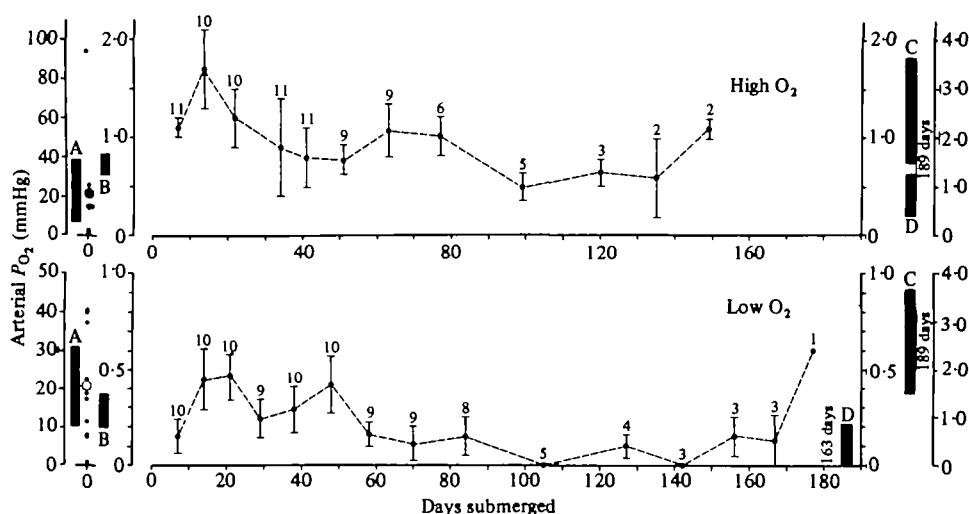
Fig. 1. Arterial P_{O_2} of turtles at 3 °C submerged in either aerated water (upper panel) or N_2-equilibrated water (lower panel). Sequential values (mean ± S.E.) are shown for each group of catherized turtles, and the number above each value is the number of turtles surviving and sampled at that time. The vertical black bars in this and the subsequent figures represent the 95 % confidence intervals for the following: (A) pre-dive control values of the catheterized turtles; (B) the last values before death of the catheterized turtles; (C) values of five control turtles after 189 days at 3 °C with access to air; this bar is the same in each panel; (D) values for control turtles after prolonged apnoeic submergence; six high-O_2 turtles were sampled after 189 days, four low-O_2 turtles were sampled between 155 and 168 days. The left-hand axis applies only to bar A, the central axes to bar B and to the sequential values, and the right-hand axis applies to bars C and D.

was also low, but was always at or above 0·5 mmHg, indicating a small, but significant, uptake of O_2 from the water. Turtles with access to air (C in Fig. 1) had much lower P_{O_2} (2·6 ± 1·1 mmHg) than pre-dive controls, indicating that aerial respiration was much reduced after more than 6 months at 3 °C.

Arterial P_{CO_2} also was initially similar in both catheterized groups (11·4 ± 0·59 mmHg in high O_2; 13·2 ± 0·71 mmHg in low O_2) but the effect of apnoea was quite different in the two groups (Fig. 2). In the high O_2 animals, mean P_{a, CO_2} initially rose only slightly and then fell to the pre-dive range and remained there until a terminal increase was observed. In contrast, P_{a, CO_2} in the low O_2 turtles increased notably in the early weeks of the dive, then gradually returned to and even fell below the pre-dive value. An increase in the final sample was likewise seen in several members of this group. The P_{CO_2} values of all three non-catheterized groups were in or below the pre-dive range.

Blood pH, similar in the catheterized groups in the pre-dive condition (7·99 ± 0·02 in high O_2; 7·96 ± 0·02 in low O_2), was highly variable in the high O_2 group in the early weeks of apnoea (Fig. 3). In the low-O_2 group there was a uniform steep decline initially which continued in three turtles but which slowed considerably in the other 7. All animals in this group were distinctly acidotic within 1 week after the onset of apnoea, whereas three of the high O_2 animals remained within or above the normal pH range after 42 days of apnoea. These three turtles did not at that point appear to
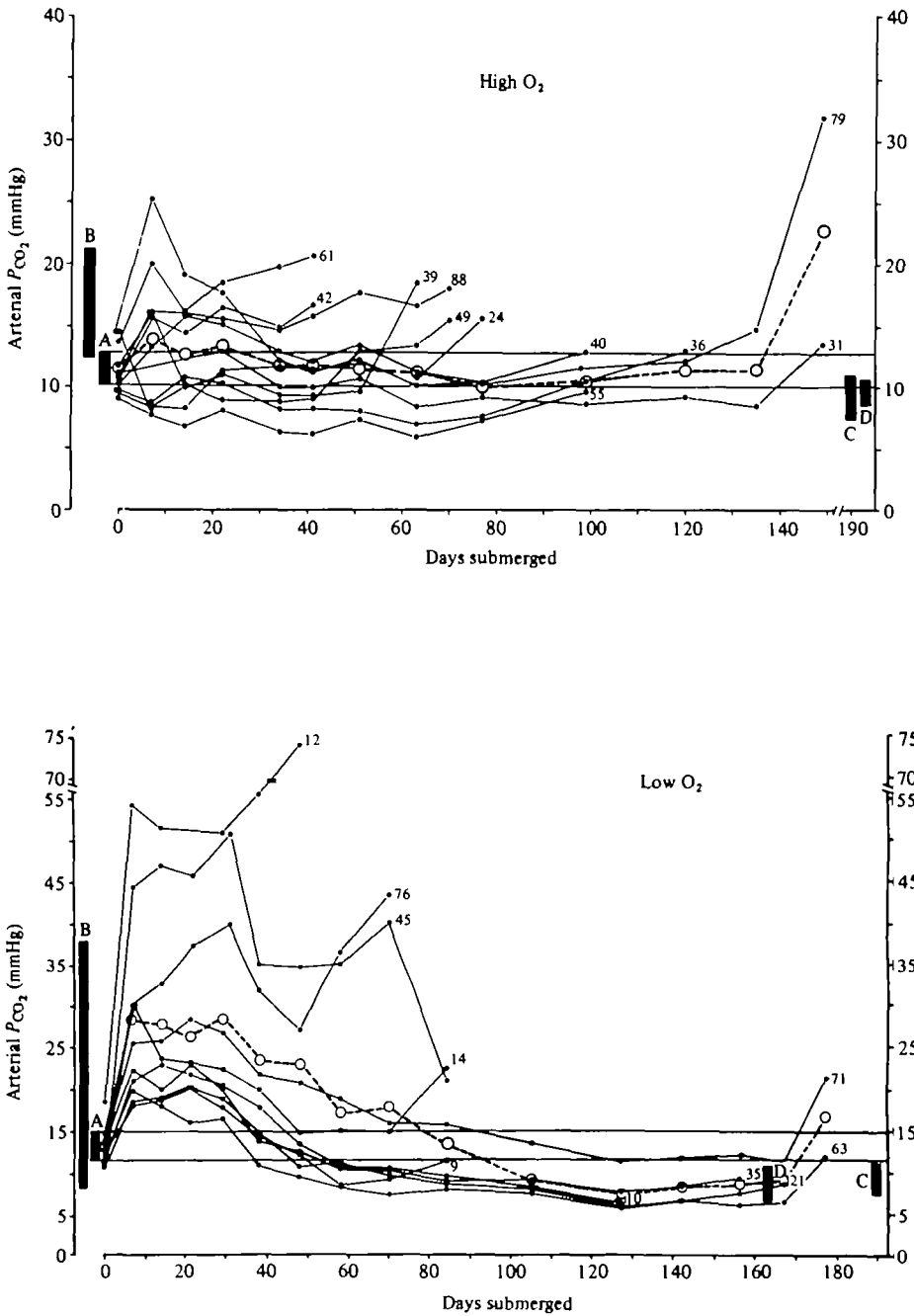
Fig. 2. Arterial P_{CO_2} of turtles at 3 °C in high-O_2 water (upper panel) and in low-O_2 water (lower panel). See Fig. 1. legend for explanation of bars A–D. The horizontal band delimited by two parallel lines is the extension of bar A, the 95 % confidence interval for the pre-dive values. In this and in the following figures, individual values for all catheterized turtles are shown and each animal is identified by its number at its final sampling period. The open circles, connected by the dotted line, are the mean values for surviving animals.
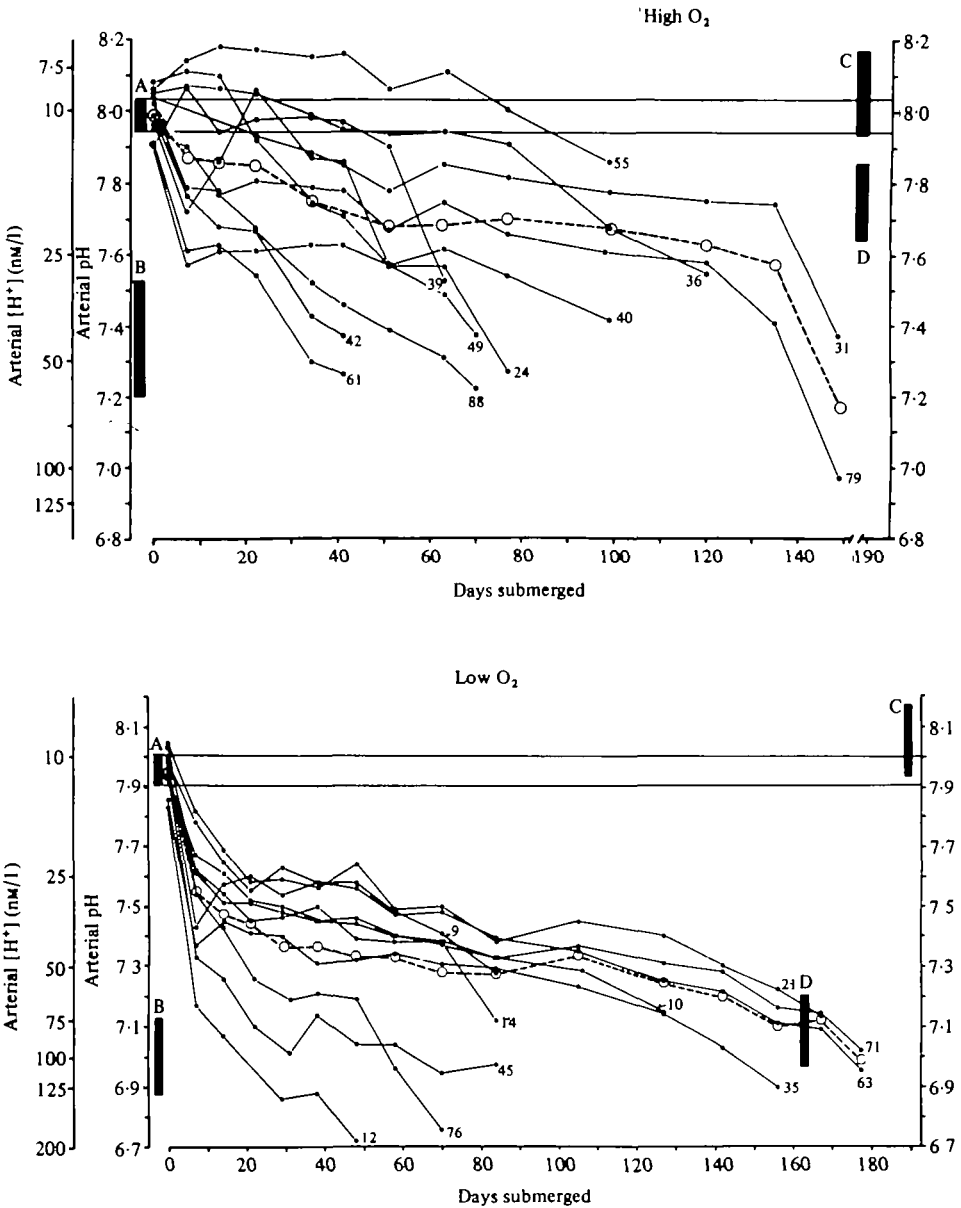
Fig. 3. Arterial pH (and [H⁺]) of turtles at 3 °C. For explanation of graph format see legends for Figs. 1 and 2.

be infected by the fungus. The relatively high pH range of the unoperated, submerged, high-O_2 turtles (D in Fig. 3) after 189 days of apnoea further substantiates the capacity of these animals to resist acidosis when dissolved O_2 is available.

Plasma $[HCO_3^-]$ (Fig. 4) fell steadily in the low-O_2 group throughout apnoea, and although there were some initial delays, the values generally declined in the high-O_2
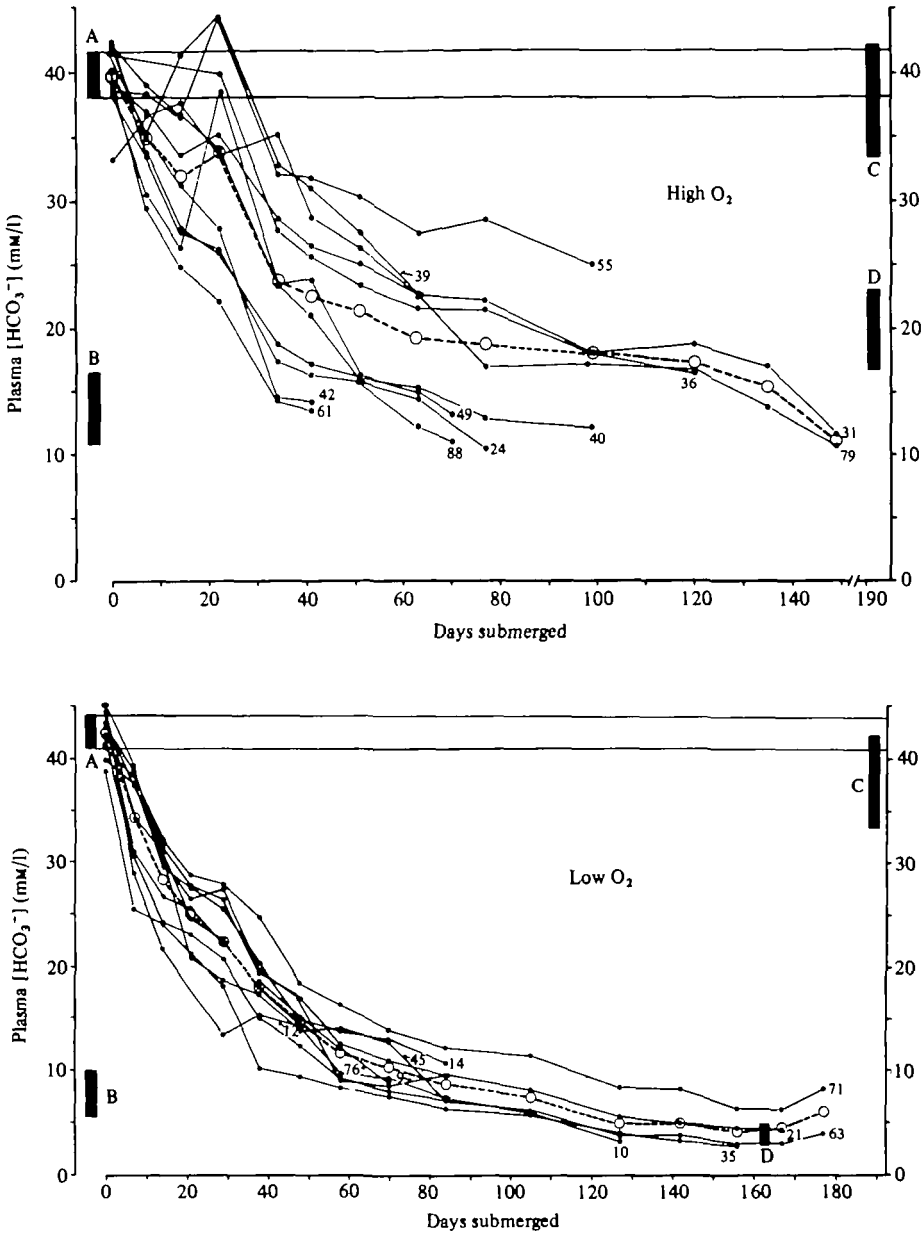
Fig. 4. Plasma [HCO₃⁻] of turtles at 3 °C. For explanation of graph format, see legends for
Figs. 1 and 2.

group as well, but the final range of values was higher. The unoperated high O₂
apnoeic group, however, had even higher values, which may again reflect their relative
freedom from the fungus infection.

Plasma [lactate⁻] exhibited a uniform and dramatic increase in the low-O₂ turtles
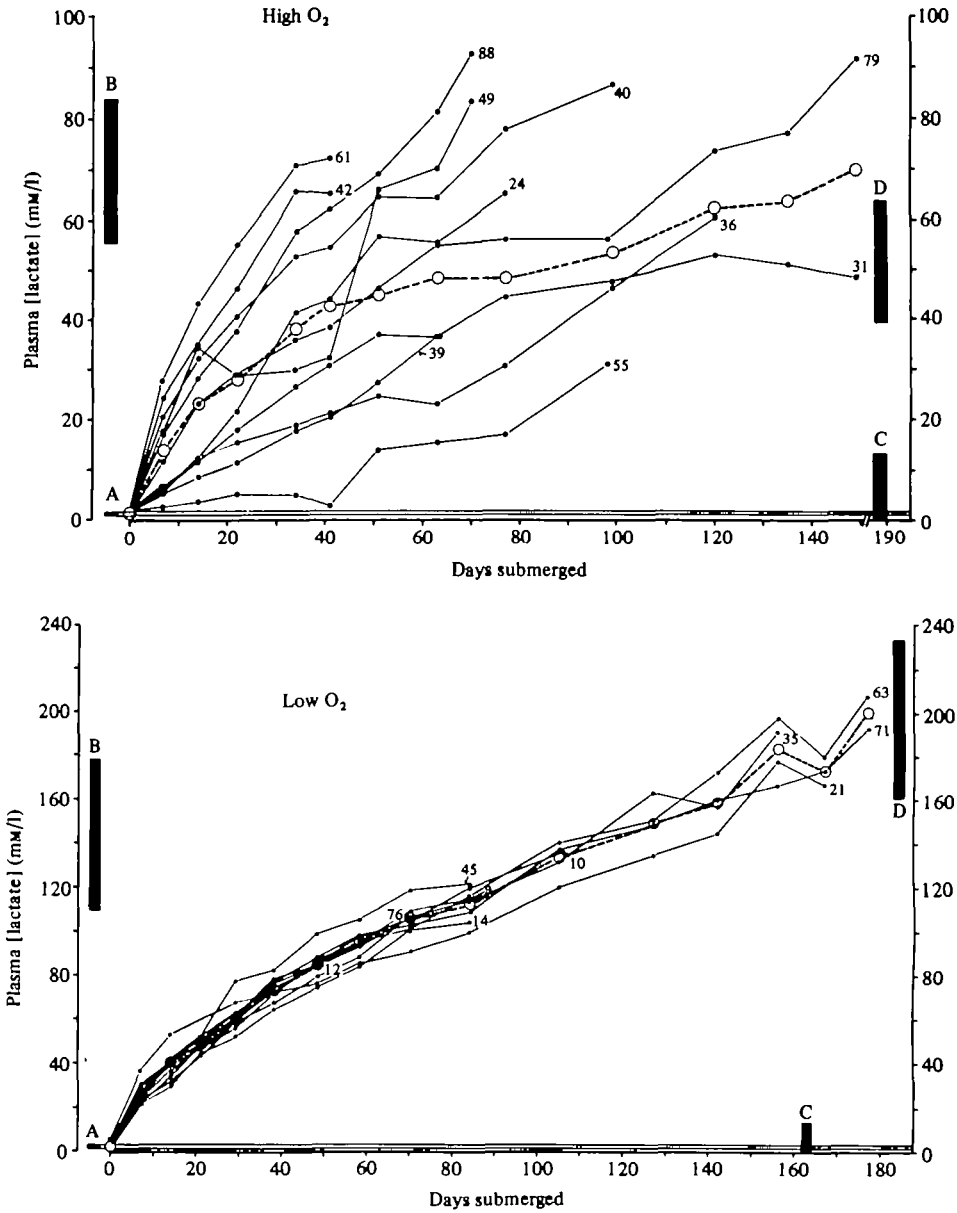to peak values that exceeded 200 mM in both catheterized and non-catheterized

Fig. 5. Plasma [lactate⁻] of turtles at 3 °C. For explanation of graph format, see legends for Figs. 1 and 2.

turtles (Fig. 5). The high O_2 turtles also had increased lactate, but again there was considerable scatter. The lactate values of the high O_2 non-catheterized apnoeic turtles (D) resembled the final low range of values in the catheterized group. No turtles in high O_2 achieved lactate values as high as 100 mM. Plasma glucose concentration, in contrast to previous reports of diving (Penney, 1974) or N_2 exposure
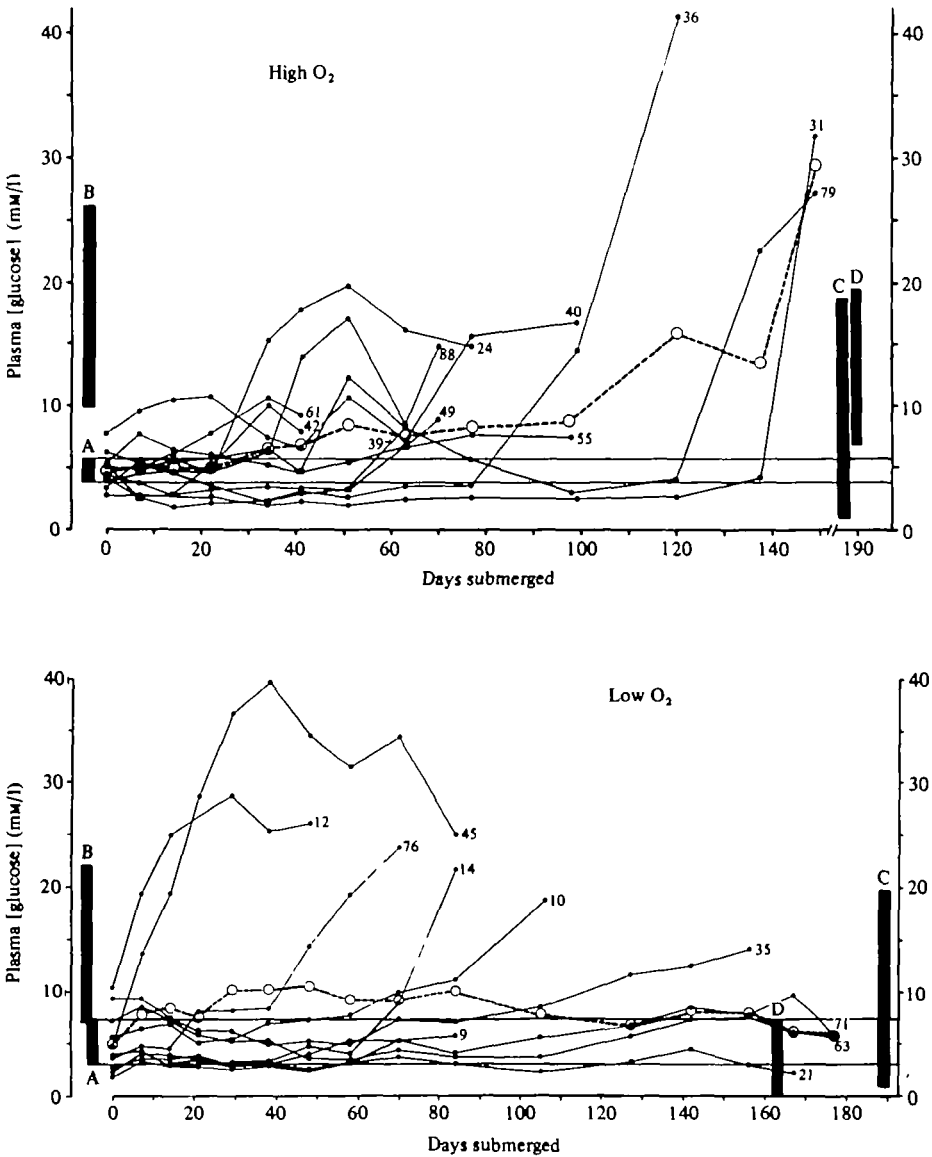
Fig. 6. Plasma [glucose] of turtles at 3 °C. For explanation of graph format, see legends for Figs. 1 and 2.

(Clark & Miller, 1973; Johlin & Moreland, 1933) in turtles, did not increase consistently in either group, although a sharp increase was frequently observed as a turtle's condition deteriorated (Fig. 6).

Buffer values. The slopes of the CO_2 buffer curves are given in Table 2. Oxygenation state had no effect on slope but lactic acidosis appeared to reduce the buffering capacity. The turtle tested had a plasma lactate concentration at the time of sampling of over 200 mequiv/l. Reduced haematocrit predictably lowered buffering capacity.

Table 2. *CO_2 buffer values of turtle blood at 3 °C as a function of oxygenation, acid-base state and haematocrit*

	Haematocrit (%)	P_{O_2} (mmHg)	Buffer Value ($\Delta[HCO_3^-]/\Delta pH$)
Control turtle	22	224	−12·38
	22	0	−12·37
Acidotic turtle	22	0	−9·72
	7	0	−4·56

Lung volume. There was a clear distinction between the turtles submerged in aerated water, in which the lungs were invariably collapsed, and the turtles submerged in N_2-equilibrated water, in which the lungs were typically inflated with 20–60 ml of gas. Several analyses indicated that the P_{O_2} and P_{CO_2} values of this gas were the same as blood.

DISCUSSION

This study has confirmed that freshwater turtles can survive long periods of apnoea at low temperature. The limits of survival that we observed exceed those previously reported in experimental diving at 1–2 °C (Musacchia, 1959; Smith & Nickon, 1961) and approximate to the maximum expected time these animals would ever have to remain submerged in nature. Although the comparisons of survival between the different treatment groups was confounded by the fungus problem, the turtles in aerated water were clearly less stressed than those in low-O_2 water. This was particularly evident in the non-catheterized groups, in which the turtles in aerated water survived longer and were in better condition. However, even under the most adverse conditions that we imposed, which was submergence in O_2-poor water with periodic blood sampling from surgically-placed catheters, two of ten turtles survived for nearly 6 months. The condition of these anoxic animals was quite poor, however, at this extreme stage.

It is important to emphasize that a primary object of this study was to define the limits of survival. We kept each turtle submerged and continued to make measurements as long as it remained alive. All surviving non-catheterized turtles were killed in order to provide data for comparison with the periodically sampled animals. Therefore, we do not know the limits of survival (as defined in this study) or of acid-base disturbance compatible with full recovery. It is possible, however, that the advantages of water aeration will become even more apparent when this important information is available.

The superior performance of turtles submerged in aerated water resulted from their ability to extract O_2 from the water, even though related emydid species are not thought to have effective extrapulmonary respiration at higher temperatures (Belkin, 1968; Robin *et al.* 1964; Jackson & Schmidt-Nielsen, 1966). In addition to the circumstantial evidence of longer survival and better physical condition in aerated water, direct evidence for aquatic O_2 uptake was provided by the significantly higher blood P_{O_2} values in the high-O_2 turtles (Fig. 1), and by the less severe increases in

lactate levels (Fig. 5). The turtles in aerated water relied considerably less upon anaerobic metabolism than did the low-O_2 turtles, and presumably were able to utilize aerobic pathways for a significant portion of their ATP production. Because of the lower rate of rise in plasma lactate, blood pH decreased more slowly in the high O_2 turtles (Fig. 3).

The advantage of aeration can perhaps be best appreciated from the remarkably little effect of diving on the blood acid-base states of individual high-O_2 turtles prior to the onset of the fungus condition. Consider, for example, turtles 31, 36 and 55. These animals had the slowest rates of lactate rise and had little or no change in pH or P_{CO_2} for up to 80 days of apnoea. Turtle 31 still had a blood pH over 7·7 after 134 days. These results, although exceptional within the treatment group, are significant because they reveal what a turtle under these conditions can do; furthermore, their condition extrapolated to 189 days is similar to that of the six non-catheterized turtles examined at this time (Bar D in Figs. 3–5). Thus, these seemingly exceptional turtles may be actually representative of painted turtles that are unaffected by the skin fungal infection.

It is evident that extrapulmonary gas exchange is much more important in turtles at 3 °C than at higher temperatures. It is known that in many air-breathing ectotherms with access to air, there is greater reliance on extrapulmonary avenues of gas exchange when temperatures are low (for example: Whitford & Hutchison, 1965). This also has been observed in the box turtle, *Terrapene ornata* (Glass, Hicks & Riedesel, 1979) with respect to CO_2 loss. In *Chrysemys* (= *Pseudemys*) *scripta*, breathing cycles are dramatically reduced at 5 °C compared to higher temperatures (Lucey & House, 1977). Under the conditions of our study, gas exchange was possible only across extrapulmonary surfaces, which in turtles include the integument, the buccal cavity and the cloacal structures (Gage & Gage, 1886; Root, 1949; Dunson, 1960). The effectiveness of this exchange at low temperature can be attributed in part to the very low metabolic requirements (Penney & Kavanaugh, 1976) and to the high affinity of haemoglobin for O_2. The P_{O_2} of blood supplying the tissues in the high O_2 turtles in our study was always less than 2 mmHg; however, according to recent measurements of O_2 binding at 3 °C in blood from turtles in this study (L. Maginniss & R. B. Reeves, personal communication), haemoglobin was about 6 % saturated at 1 mmHg, and P_{50} under the *in vivo* conditions was 3·7 mmHg. Although 6 % saturation is low, it represents an O_2 concentration that is some 100 times higher than the dissolved O_2 at a P_{O_2} of 1 mmHg. The study of O_2 transport at very low temperatures, with very small P_{O_2} differences for diffusion, high blood O_2 affinity and slow circulation time should prove to be a fruitful area of study. Our study provides evidence that significant amounts of O_2 can be transported between the environment and the tissues under these seemingly unfavourable conditions.

The control turtles that had continuous access to air throughout the study apparently also relied to a considerable extent on extrapulmonary gas exchange. Although we did not systematically monitor their behaviour, we never observed one swim to the surface to breathe. The P_{O_2} of these animals averaged only 2·6 mmHg, far below the pre-dive P_{O_2} of the catheterized turtles, but still significantly higher than the P_{O_2} of the apnoeic turtles in aerated water. The latter comparison indicates that some aerial

respiration occurred although not enough to prevent a hypoxic state sufficient to produce a relatively small increase in lactate. Despite this modest metabolic acidosis, the blood pH and plasma $[HCO_3^-]$ values were not significantly different from the pre-dive controls.

In the low-O_2 group the turtles became anoxic in less than 7 days and exhibited a relatively uniform and progressive deterioration in their acid-base state. However, these changes occurred very slowly over a period of months before reaching levels of acidosis that have proven fatal in turtles at higher temperatures. In this study at 3 °C, pre-dive pH values were close to 8·0 and the final values before death in the low O_2 turtles averaged about 7·0. Blood pH in turtles (genus *Pseudemys*) submerged at 16–18 °C fell from about 7·54 to 6·7, but this took only 24–120 h (Robin *et al.* 1964). At 24 °C, blood pH of *Chrysemys* (= *Pseudemys*) *scripta* decreased during diving from 7·6 to 6·8 in 2–4 h (Jackson & Silverblatt, 1974). Low temperature greatly attentuates the time course of acidosis in the diving turtle. The final pH values reached (about 6·7–7·0) are quite similar in these studies, however, and may represent a lower limit to survival.

Although the level to which pH fell in this study was not exceptional in comparison to other studies, the changes accounting for the acidosis were strikingly different both from the previous studies on turtles at higher temperatures and from studies of other diving vertebrates. As mentioned in the introduction, the typical pattern during diving is a combined respiratory and metabolic acidosis due to CO_2 retention and anaerobic lactate production. The pattern of acid-base change during prolonged submergence at 3 °C is illustrated graphically for the two catheterized groups on a HCO_3^-–pH (Davenport) diagram (Fig. 7). In the anoxic turtles at 3 °C there was a transient respiratory acidosis that subsided after several weeks and a metabolic acidosis that increased progressively throughout the dive. In the high-O_2 turtles, the acidosis was almost entirely metabolic throughout. The difference in the two populations of cold turtles, therefore, was the absence (eventually) of a respiratory component to the acidosis and a far more severe lactic acidosis.

The transient elevation of blood P_{CO_2} in low-O_2 turtles is of interest in view of the probable reduction, or even elimination, of metabolically produced CO_2 in this virtually anoxic condition. We attribute the rise primarily to the reaction of body fluid HCO_3^- and, possibly, CO_3^{-2} with lactic acid which generated molecular CO_2. The carbonate ions may have been mobilized from bony tissues in association with calcium and magnesium (Jackson & Ultsch, 1981). Reduced peripheral blood flow associated with the depressed, anoxic state may also have contributed to the hyper-capnia by interfering with CO_2 excretion. The unlikelihood of a metabolic CO_2 component is based on the predominance of glycolysis as the anaerobic pathway in Chelonians (Hochachka *et al.* 1975; P. W. Hochachka, personal communication).

The maintenance of normal or nearly normal blood P_{CO_2} in the turtles submerged in aerated water reveals that pulmonary ventilation, while necessary for normal O_2 uptake, is not necessary for normal CO_2 loss at 3 °C. It is of interest that the control blood pH of about 8·0, the maintenance of which appeared to be independent of pulmonary CO_2 loss, falls on the pH–temperature curve characteristic of ectothermic vertebrates (Howell *et al.* 1970). Apparently the turtle at 3 °C, like predominantly
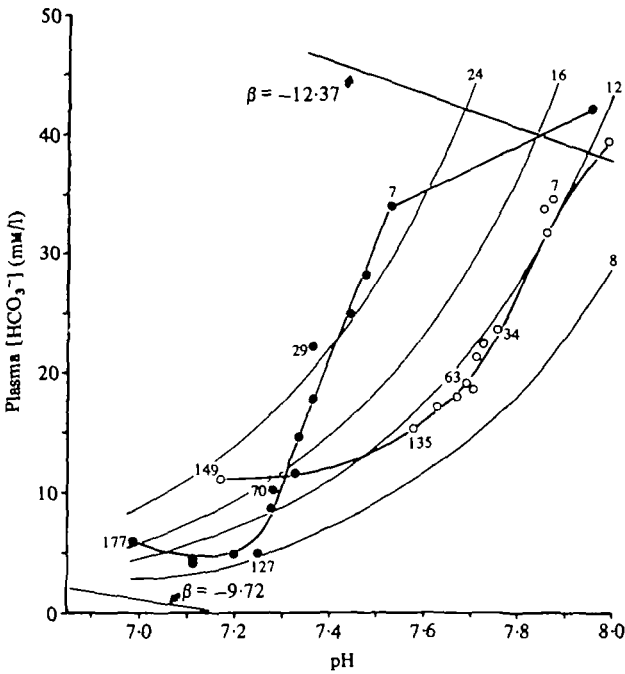
Fig. 7. Plasma [HCO$_3$-] v. pH plot (Davenport diagram) of blood data for turtles submerged at 3 °C in low-O$_2$ water (solid circles) and high-O$_2$ water (open circles). Each plotted point is the mean value of the surviving turtles with the number of days submerged specified for selected points. Curves are drawn by eye. Buffer values shown are from control turtle ($\beta = -12\cdot37$) and anoxic turtle ($\beta = -9\cdot72$), each at an haematocrit of 22 % (see Table 2). Note that high-O$_2$ turtles follow a P_{CO_2} isopleth (12 mmHg) for most of the dive (pure metabolic acidosis), while in the low-O$_2$ animals, a respiratory acidosis initially accompanies the metabolic acidosis.

skin-breathing salamanders at higher temperatures (Moalli *et al.* 1981), can control its blood pH in the typical ectotherm manner using the largely passive extrapulmonary gas-exchange mode.

The major cause of acidosis in the anoxic turtles was an increase in plasma lactic acid concentration. In the extreme situation reached in this study, concentrations of over 200 mM were observed which is at least 4 times higher than previously observed in diving turtles, although levels exceeding 100 mM were reported in turtles following exposure to nitrogen for over 24 h (Johlin & Moreland, 1933). At 3 °C, lactic acid ($pK = 4\cdot16$) is 99·9 % dissociated and, in terms of body fluid acid-base chemistry, is a strong acid. An aspect of this study which is of considerable interest, therefore, concerns the mechanisms by which the turtle buffers an acid load of this magnitude. The lactate concentration of 200 mM is more than the pre-dive concentration of total anions, and is far more than the pre-dive concentrations of weak acid anions. In the previous studies of diving turtles at higher temperatures, an acidaemia of comparable magnitude was reached with a much smaller rise in lactate (Robin *et al.* 1964; Jackson & Silverblatt, 1974), and even though a respiratory acidosis in the latter studies contributed to the low pH, additional mechanisms for buffering the lactate must have been involved in the 3 °C turtles. In the following paper (Jackson & Ultsch, 1981) we report

the ionic mechanisms, including an unexpected increase in plasma calcium and magnesium, which enabled the cold turtles to tolerate such high lactate levels.

In conclusion, *C. picta bellii* can survive long periods of submergence at 3 °C even when anoxic; however, survival is apparently prolonged and the acid-base state of the blood is significantly improved if O_2 is available in the water for extrapulmonary uptake. These observations are relevant to our understanding of the behaviour of turtles during their long underwater periods in cold weather. If the turtle spends this time buried in the mud at the bottom (Ernst & Barbour, 1972), in what is probably an anoxic environment, long survival is possible but at the cost of a severe metabolic acidosis and deep torpor. The maximum duration that can be withstood under these conditions may even influence the northerly distribution of turtles. If, however, the turtles avail themselves of the dissolved O_2 by moving into the open water, as Eastern painted turtles (*C. picta*) were observed to do in a recent telemetry study (D. DeLisle & W. W. Burggren, personal communication), then our results indicate that longer dives could be tolerated and a less severe metabolic acidosis would probably improve the chances for normal recovery in the spring. Recovery, however, must require that the lungs be inflated from a collapsed state.

REFERENCES

BELKIN, D. A. (1963). Anoxia: tolerance in reptiles. *Science, N.Y.* **139**, 492–493.

BELKIN, D. A. (1964). Variations in heart rate during voluntary diving in the turtle *Pseudemys concinna*. *Copeia*, pp. 321–330.

BELKIN, D. A. (1968). Aquatic respiration and underwater survival of two freshwater turtle species. *Respir. Physiol.* **4**, 1–14.

BRAMBEL, C. E. (1941). Prothrombin activity of turtle blood and the effect of a synthetic vitamin K derivative. *J. cell. comp. Physiol.* **18**, 221–232.

BURGGREN, W. W. & SHELTON, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. exp. Biol.* **82**, 75–92.

CARR, A. (1952). *Handbook of Turtles*, p. 217. Ithaca, N.Y.: Cornell University Press.

CLARK, V. M. & MILLER, A. T. (1973). Studies on anaerobic metabolism in the fresh-water turtle (*Pseudemys scripta elegans*). *Comp. Biochem. Physiol.* **44** A, 55–62.

DUNSON, W. A. (1960). Aquatic respiration in *Trionyx spinifer asper*. *Herpetologica* **16**, 277–283.

ERNST, C. H. & BARBOUR, R. W. (1972). *Turtles of the United States*, p. 141. Lexington, Kentucky: University of Kentucky Press.

FRANKEL, H. M., STEINBERG, G. & GORDON, J. (1966). Effects of temperature on blood gases, lactate, and pyruvate in turtles, *Pseudemys scripta elegans*, in vivo. *Comp. Biochem. Physiol.* **19**, 279–283.

GAGE, S. H. & GAGE, S. P. (1886). Aquatic respiration in soft-shelled turtles: a contribution to the physiology of respiration in vertebrates. *Am. Naturalist* **20**, 233–236.

GLASS, M. L., HICKS, J. W. & RIEDESEL, M. L. (1979). Respiratory responses to long-term temperature exposure in the box turtle, *Terrapene ornata*. *J. comp. Physiol.* **131**, 353–359.

HOCHACHKA, P. W., OWEN, T. G., ALLEN, J. F. & WHITTOW, G. C. (1975). Multiple end products of anaerobiosis in diving vertebrates. *Comp. Biochem. Physiol.* **50** B, 17–22.

HOWELL, B. J., BAUMGARDNER, F. W., BONDI, K. & RAHN, H. (1970). Acid-base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600–606.

HUTTON, K. E. & GOODNIGHT, C. J. (1957). Variations in the blood chemistry of turtles under active and hibernating conditions. *Physiol. Zoöl.* **30**, 198–207.

JACKSON, D. C. (1968). Metabolic depression and oxygen depletion in the diving turtle. *J. appl. Physiol.* **24**, 503–509.

JACKSON, D. C., PALMER, S. E. & MEADOW, W. L. (1974). The effects of temperature and carbon dioxide breathing on ventilation and acid-base status of turtles. *Resp. Physiol.* **20**, 131–146.

JACKSON, D. C. & SCHMIDT-NIELSEN, K. (1966). Heat production during diving in the fresh water turtle, *Pseudemys scripta*. *J. cell. Physiol.* 67, 225–232.

JACKSON, D. C. & SILVERBLATT, H. (1974). Respiration and acid-base status of turtles following experimental dives. *Am. J. Physiol.* 226, 903–909.

JACKSON, D. C. & ULTSCH, G. R. (1981). Long-term submergence at 3 °C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. II. extracellular ionic responses to extreme lactic acidosis. *J. exp. Biol.*

JOHLIN, J. M. & MORELAND, F. B. (1933). Studies on the blood picture of the turtle after complete anoxia. *J. biol. Chem.* 103, 107–114.

KUPCHELLA, C. E. & JACQUES, F. A. (1970). The effects of induced cold torpor and time of year on the plasma acid mucopolysaccharide levels in *Pseudemys scripta elegans* and *Chrysemys picta bellii*. *Comp. Biochem. Physiol.* 36, 657–668.

LUCEY, E. C. & HOUSE, E. W. (1977). Effect of temperature on the pattern of lung ventilation and on the ventilation–circulation relationship in the turtle, *Pseudemys scripta*. *Comp. Biochem. Physiol.* 57 A, 239–243.

McDONALD, H. S. (1976). Methods for the physiological study of reptiles. In *Biology of the Reptilia* (ed. C. Gans and W. R. Dawson), Physiol. A, vol. 5. New York: Academic Press.

MOALLI, R., MEYERS, R. S., ULTSCH, G. R. & JACKSON, D. C. (1981). Acid-base balance and temperature in a predominantly skin-breathing salamander, *Cryptobranchus alleganiensis*. *Respir. Physiol.* 43, 1–11.

MUSACCHIA, X. J. (1959). The viability of *Chrysemys picta* submerged at various temperatures. *Physiol. Zoöl.* 32, 47–50.

PENNEY, D. G. (1974). Effects of prolonged diving anoxia on the turtle, *Pseudemys scripta elegans*. *Comp. Biochem. Physiol.* 47 A, 933–941.

PENNEY, D. & KAVANAUGH, J. (1976). Thyroxine and oxygen consumption in *Pseudemys scripta elegans*. *Comp. Biochem. Physiol.* 54 A, 443–445.

RAPATZ, G. L. & MUSACCHIA, X. J. (1957). Metabolism of *Chrysemys picta* during fasting and during cold torpor. *Am. J. Physiol.* 188, 456–460.

REEVES, R. B. (1976). Temperature-induced changes in blood acid-base status: pH and P_{CO_2} in a binary buffer. *J. appl. Physiol.* 40, 752–761.

ROBIN, E. D., VESTER, J. W., MURDAUGH, H. V. JR. & MILLEN, J. E. (1964). Prolonged anaerobiosis in a vertebrate: Anaerobic metabolism in the freshwater turtle. *J. cell. comp. Physiol.* 63, 287–297.

ROOT, R. W. (1949). Aquatic respiration in the musk turtle. *Physiol. Zoöl.* 22, 172–178.

SMITH, H. M. & NICKON, D. C. (1961). Preliminary experiments on the role of the cloacal bursae in hibernating turtles. *Chicago Acad. Sci. nat. Hist. Misc.* 178, 1–8.

WHITFORD, W. G. & HUTCHISON, V. H. (1965). Gas exchange in salamanders. *Physiol. Zoöl.* 38, 228–242.