The Journal of Experimental Biology 203, 3765–3744 (2000)
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JEB2951

THE CARDIOVASCULAR RESPONSES OF THE RED-EARED SLIDER (*TRACHEMYS SCRIPTA*) ACCLIMATED TO EITHER 22 OR 5 °C

I. EFFECTS OF ANOXIC EXPOSURE ON *IN VIVO* CARDIAC PERFORMANCE

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Accepted 25 September; published on WWW 14 November 2000

Summary

The extreme anoxia-tolerance of freshwater turtles under cold conditions is well documented, but little is known about their cardiac performance in such situations. Using chronic catheterization techniques, we measured systemic cardiac power output (PO_{sys}), systemic cardiac output ($\dot{Q}_{sys}$), heart rate (f_H), systemic stroke volume ($V_{s,sys}$), systemic resistance (R_{sys}) and mean arterial pressure (P_{sys}) in red-eared sliders (*Trachemys scripta*). The effects of cold acclimation and anoxic exposure were studied. Turtles were acclimated to either 22 °C or 5 °C, and the anoxic exposure was either acute (6 h) at 22 °C or chronic (3 weeks) at 5 °C. Cold acclimation alone decreased PO_{sys} by 15-fold, representing a Q_{10} of 8.8. In addition, f_H and $V_{s,sys}$ decreased significantly, while R_{sys} increased and moderated the arterial hypotension. Acute and chronic anoxic exposures significantly decreased PO_{sys}, $V_{s,sys}$, f_H and P_{sys} and increased R_{sys}. But the changes were qualitatively much larger with chronic anoxia. For example, acute anoxia in 22 °C-acclimated turtles decreased PO_{sys} by 6.6-fold, whereas chronic anoxia in 5 °C-acclimated turtles decreased PO_{sys} by 20-fold. The remarkable cardiovascular down-regulation that accompanies long periods of cold anoxia in these turtles was characterized by comparing cardiovascular status during chronic anoxia at 5 °C with that during normoxia at 22 °C. Cardiac PO_{sys} was reduced 330-fold, through decreases in $\dot{Q}_{sys}$ (120-fold), f_H (24.2-fold), $V_{s,sys}$ (5.7-fold) and P_{sys} (2.2-fold), while R_{sys} was increased 64.6-fold. We also compared cardiac glycolytic rates by assuming that PO_{sys} was proportional to ATP supply and that glycolysis yielded 18 times less ATP per mole of glucose than oxidative metabolism. At 22 °C, the 6.6-fold decrease in PO_{sys} with anoxia suggests that a Pasteur effect was needed in cardiac tissues during acute anoxia. However, this would not be so with chronic anoxia at 5 °C because of the 22-fold decrease in PO_{sys}. We propose that the suppression of the Pasteur effect and the large Q_{10} values for cold acclimation would conserve glucose stores and enable turtles to withstand anoxia much longer under cold than under warm conditions.

Key words: turtle, *Trachemys scripta*, heart, heart rate, stroke volume, cardiac output, cardiac power output, temperature, anoxia.

Introduction

Freshwater turtles can withstand 4 months of anoxia at 3 °C (Ultsch and Jackson, 1982), and this remarkable anoxia-tolerance is accompanied by a five- to tenfold reduction in metabolic rate (Herbert and Jackson, 1985b; Jackson, 1968). Beyond this, details of this metabolic depression are limited largely to biochemical adaptations (Storey, 1996), which provide a largely qualitative view of energy supply and demand. An important quantitative insight into the problem of energy supply and demand during anoxia can be provided by measuring work performed by the heart muscle because cardiac power output can be expressed as an ATP demand, and this ATP demand can be related to rates of glycolysis. However, *in vivo* cardiac power output has not been measured in cold-acclimated turtles chronically exposed to anoxia; information exists only for heart rate and blood pressure. Turtles (*Chrysemys picta belli*) exposed to anoxia at 3 °C for 93 days experienced a 76-fold decrease in heart rate and a 5.6-fold drop in mean arterial blood pressure compared with measurements made in normoxic animals at 22 °C (Herbert and Jackson, 1985b). These observations, combined with measured reductions in stroke volume in anoxic perfused turtle hearts, were used to predict that cardiac power output would be approximately 500-fold lower in cold anoxic turtles than in warm normoxic ones (Farrell et al., 1994). The purpose of the present study was to quantify directly the degree of cardiac down-regulation that occurs *in vivo* in turtles with cold acclimation and with anoxic exposure.

Our chronic catheterization techniques were based on those pioneered by White and Ross (1966) and perfected by Hicks and

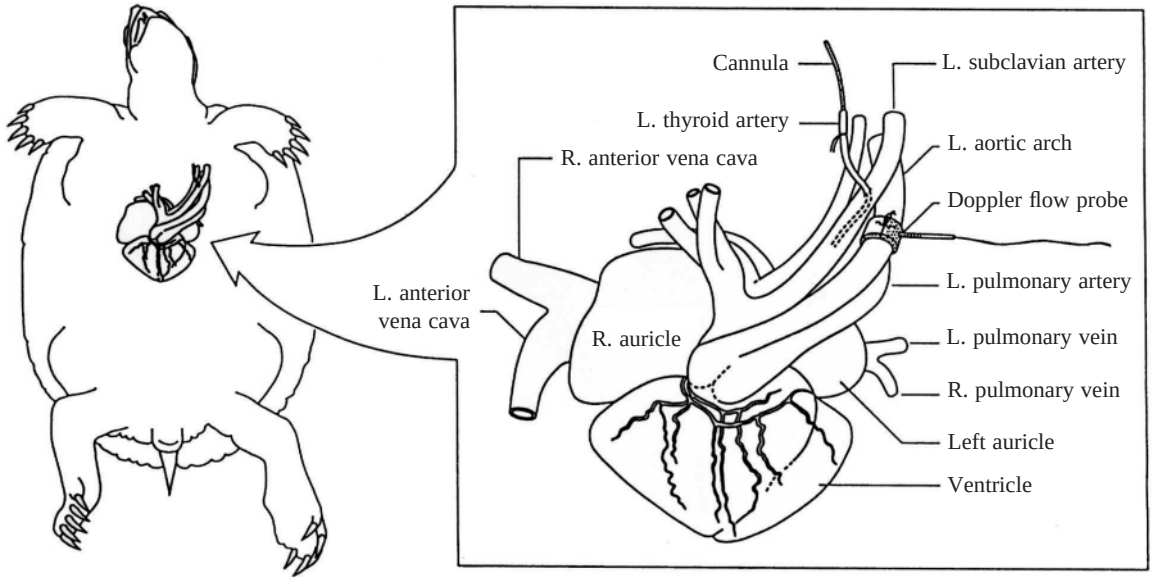

Fig. 1. Ventral view of the surgical area including the sites of cannulation and flow probe placement. L, left; R, right.

Wang (1998) when they successfully measured cardiovascular status in *Trachemys scripta* during a 2 h exposure to anoxia at 25 °C. Hicks and Wang (1998) reported a twofold reduction in systemic cardiac power output (PO_{sys}) for this short anoxic exposure. We anticipated finding much larger decreases in cold-acclimated turtles exposed to chronic anoxia. To assess cardiovascular status, we measured PO_{sys}, systemic cardiac output ($\dot{Q}_{sys}$), heart rate (fH), systemic stroke volume ($V_{s,sys}$), systemic resistance (R_{sys}) and mean arterial pressure (P_{sys}) in *T. scripta*. To dissect out the effects of temperature acclimation from those of anoxic exposure, we used turtles acclimated for 3 weeks to either 22 °C or 5 °C. A sub-group of turtles was then subjected to either an acute, 6 h exposure to anoxia at 22 °C or to a chronic, 3-week exposure to anoxia at 5 °C.

Materials and methods

Experimental animals

Red-eared sliders (*Trachemys scripta* Gray) (body mass 724±71 g, mean ± S.E.M., N=23) were obtained from a commercial supplier (Carolina Biological Supply Co., Burlington, NC, USA) and held indoors in standing water in polypropylene containers with access to basking platforms as well as deep water. Room and basking lights were set for a 12 h:12 h L:D photoperiod. The turtles were fed on commercial pellets (Wardley Laboratories Inc., Secaucus, NJ, USA), small pieces of fish and lettuce at least three times per week. Turtles were fasted for 1 week prior to surgery. Experiments with 5 °C-acclimated turtles were conducted from September 1995 to May 1996, while experiments with 22 °C-acclimated turtles were conducted from June to September 1996. No significant difference was found between the mean body mass of the four experimental groups.

Surgical preparation

Turtles were anaesthetized with 4 % Halothane vaporized in 95 % O_2, 5 % CO_2 and then intubated and ventilated with 2 % Halothane during surgery (Hicks and Wang, 1998). Halothane levels were controlled using a Halothane vaporizer (Fluotec Mark 2, Cypran Ltd, UK). An electric bone saw (Mopec, Detroit, MI, USA) was used to remove a 4 cm×5 cm piece of the plastron, thereby exposing the heart and systemic vessels. A blunt probe was used to gently remove any muscle attached to the plastron, and any minor bleeding was stopped by cauterization. A PE50 polyethylene cannula, filled with heparinized saline (50 i.u. ml^{-1}), was inserted occlusively into the left subclavian artery *via* the thyroid artery and advanced towards the heart (approximately 5 cm) before being secured in place with 3-0 gauge silk thread. The cannula was led out of the shell through a trocar inserted at the base of the left foreleg and secured to the skin with a purse-string suture. A Doppler flow probe (Iowa Doppler Products, Iowa City, IA, USA) was placed around a portion of the left aorta that had been freed of connective tissue (see Fig. 1). Leads from the probe were carried out through the hole in the plastron and secured alongside the cannula onto the carapace with cyanoacrylate and Flexacryl (Lang Dental Manufacturing Co., Wheeling, IL, USA). Prior to the plastron being closed, a penicillin/streptomycin/neomycin solution (Sigma Chemical Co., St Louis, MO, USA) was sprayed over the surgical field. The plastron piece was resealed in place with a layer of Gelfoam sponge (Upjohn Co., Kalamazoo, MI, USA) and sealed with bone dust and cyanoacrylate. The entire area was then covered with Flexacryl.

Following surgery, turtles were ventilated with air until they could actively withdraw their limbs. The animals were then allowed to recover in individual 40 l glass aquaria for 48 h prior to any cardiovascular measurements. Haematocrit was monitored for the first week following surgery and ranged from 14 to 26 %. Turtles with levels below 13 % were killed by decapitation. A number of turtles were not used for the experiments either because the arterial cannula did not remain patent or because the Doppler flow probe failed. All procedures

were approved by the Animal Care Committee at Simon Fraser University.

Instrumentation and terminology

Systemic arterial blood pressure (P_{sys}) was recorded with a pressure transducer (Narco LDI-5, Narco, TX, USA) connected to the cannula. The pressure transducer was calibrated against a static water column before each recording, and pressures were regularly referenced to the water level of the tank in which the turtle was submerged. The flow probe was connected to a Doppler flow meter (Iowa Doppler Products, Iowa City, IA, USA) and the range manipulated for maximum signal strength. This range was recorded and used for all subsequent measurements. Each flow probe was individually calibrated *in situ* at the termination of the experiment by delivering known volumes of diluted blood through the cannulated left aorta. The aortic outflow was connected to a pressure head so that calibrations could be made at physiologically representative pressures. Calibration curves were linear, and r^2 was always greater than 0.87 (typically greater than 0.93). Pressure and flow signals were preamplified and displayed continuously on a Gould chart recorder (model 2400, Cleveland, OH, USA). Heart rate (f_H) was measured by counting the number of systolic peaks over a 1 min period in the 22 °C groups and over a 4 min period at 5 °C. Measurements were taken from post-breath portions of the traces in the normoxic turtles.

Mean systemic arterial pressure (P_{sys}), systemic cardiac output ($\dot{Q}_{sys}$), systemic stroke volume ($V_{s,sys}$), systemic power output (PO_{sys}) and systemic resistance (R_{sys}) were calculated as follows:

$$P_{sys} = (\text{systolic pressure} + \text{diastolic pressure})/2\,, \quad (1)$$

$$\dot{Q}_{sys} = 3.5\dot{Q}_{LAo}\,, \quad (2)$$

$$V_{s,sys} = (\dot{Q}_{sys}/f_H)/M_b\,, \quad (3)$$

$$PO_{sys} = (\dot{Q}_{sys} \times P_{sys} \times \alpha)/M_v\,, \quad (4)$$

$$R_{sys} = P_{sys}/\dot{Q}_{sys}\,, \quad (5)$$

where $\dot{Q}_{LAo}$ is left aortic blood flow (ml min^{-1} kg^{-1}), f_H is heart rate (beats min^{-1}), M_b is body mass (kg), M_v is ventricular mass (g) and α is 0.0167 min s^{-1}, a conversion factor to mW. P_{sys} was measured from pressure traces by calculating the mean of systolic and diastolic pressure. $\dot{Q}_{sys}$ was estimated from $3.5\dot{Q}_{LAo}$ to approximate the contributions of the right aorta, carotid and subclavian arteries to total systemic cardiac output (Wang and Hicks, 1996; Comeau and Hicks, 1994). It is possible that this estimate, which was determined for warm normoxic turtles, does not hold either for anoxic conditions or after cold acclimation. Hicks and Wang (1998) implanted the three flow probes required to measure systemic blood flow directly during much shorter anoxic exposures, but reported only total systemic flow and not the individual arterial blood flows. T. Wang (personal communication) has found that arterial blood flow distribution did not vary markedly during short-term anoxia, so our assumption is probably valid for our acute anoxic exposures. For the chronic studies, we were willing to accept the uncertainty of our assumption rather than increase the surgical procedures substantially (implanting three flow probes rather than one) prior to a chronic anoxic exposure.

Experimental protocol

During experiments, individual turtles were allowed to move freely inside a water-filled aquarium (30 cm×30 cm×60 cm) covered with black plastic to minimize visual disturbance. Continuous cardiovascular recordings were taken for periods of 30 min without the experimenter in the room. For turtles acclimated to 22 °C, cardiovascular status was assessed daily during the 8-day post-surgery recovery period, starting on the second day. After the 8-day recovery period, the 6 h anoxic exposure was started for one group of 22 °C-acclimated turtles by denying them access to surface air by fitting a Plexiglas cover over a plastic mesh grate to the top of the tank and by equilibrating the water with N_2. Turtles that were subsequently acclimated to 5 °C were also allowed an 8-day recovery period after surgery, but during this recovery period they were progressively exposed to 5 °C. The protocol was as follows: 6 h at 5 °C on day 3; 12 h at 5 °C on day 4; 18 h at 5 °C on day 5; no 5 °C exposure on day 6; 24 h at 5 °C on day 7; no 5 °C exposure on day 8; held at 5 °C on day 9. Again, cardiovascular status was recorded at the end of each 're-warming' period prior to the turtles being moved into the 5 °C room. Following the recovery period, the normoxic group of turtles was allowed to acclimate to 5 °C for 5 weeks and cardiovascular status was measured every second day. The anoxic group of turtles was first held at 5 °C under normoxic conditions for 4 days before being submerged for 3 weeks in water bubbled with N_2, as described above. Cardiovascular status was measured every second day. All 5 °C experiments were conducted in a cold room held at this temperature.

Statistical analyses

Cardiovascular variables were determined by averaging three random 2 min sections of the 30 min recording period. In most cases, mean values ± S.E.M. for six animals are presented (N=5 for the 5 °C anoxic group). Differences between means of experimental groups were determined using either one-way or two-way analyses of variance (ANOVAs), where appropriate, while multiple comparisons were performed using Student–Newman–Keuls tests. $P<0.05$ was used as the level of significance. Within-group comparisons used a repeated-measures ANOVA.

Results

Eight-day recovery period from surgery

The cardiovascular changes accompanying the 8-day recovery period from surgery are summarized in Figs 2 and 3. Although there were significant day-to-day changes for some of the variables, there were no consistent trends across all four test groups. From the perspective of recovery, it is important to note that only one of the 24 possible variable/treatment combinations was significantly different between the sixth and eighth days of

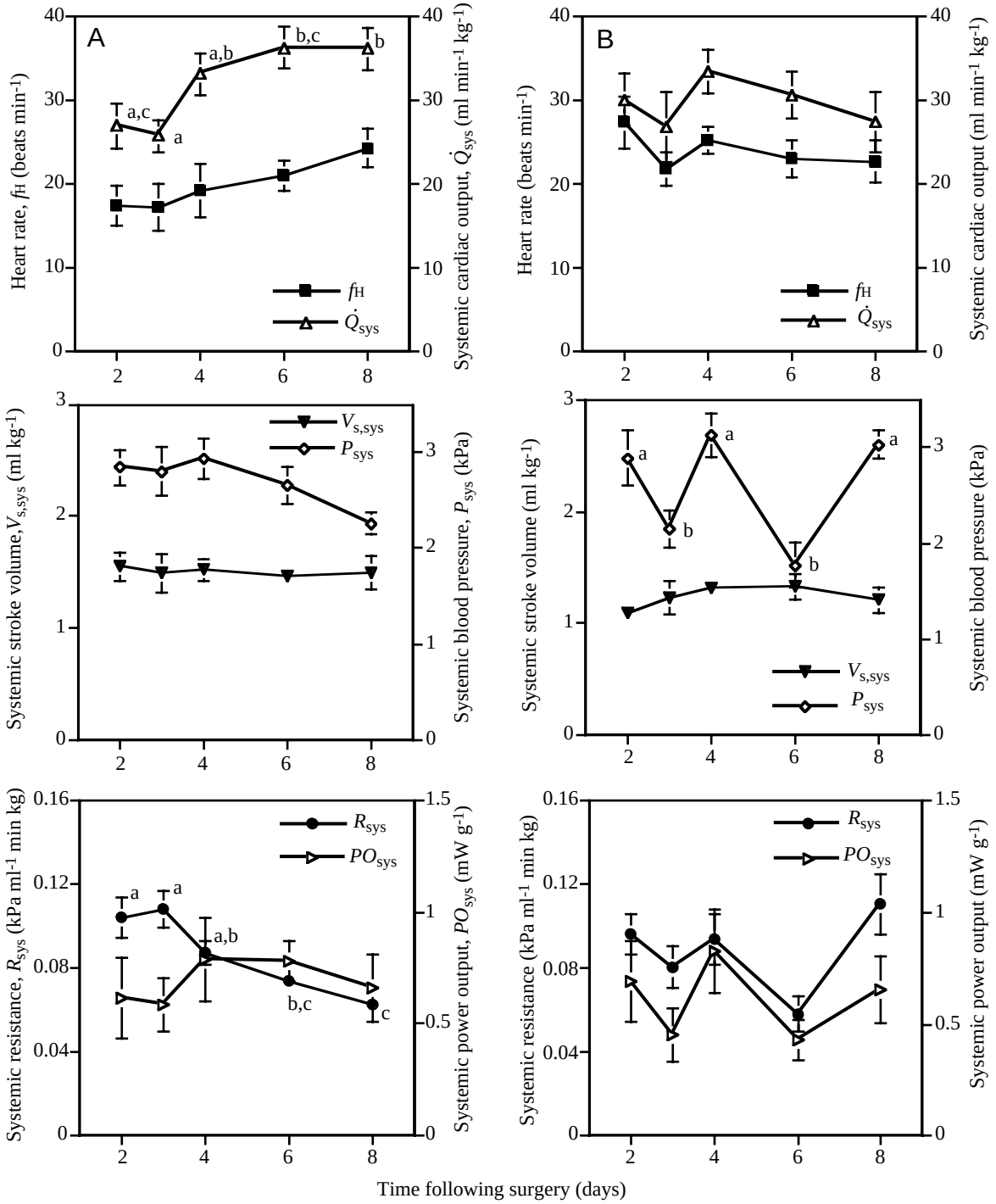

Fig. 2. Post-surgery cardiovascular variables during recovery and measured under normoxic conditions at 22 °C for the groups of 22 °C-acclimated turtles used subsequently for the normoxic (A) and anoxic (B) experiments (N=6). Significant differences (P<0.05) between values are indicated by dissimilar letters. Values are means ± S.E.M.

recovery. In addition, the 5 °C-acclimated groups (Fig. 3) showed less day-to-day variability than the 22 °C-acclimated groups. The routine cardiovascular variables for the four experimental groups of turtles measured on the eighth day post-surgery under normoxic conditions at 22 °C are compared in Table 1. Arterial blood pressure and R_{sys} were significantly higher in the 22 °C anoxic group of animals. Some of this variability may have reflected differences in the winter *versus* summer timing of the 5 °C and 22 °C experiments, as well as the progressive exposure to 5 °C for the cold-acclimated turtles.

Warm-acclimated turtles with and without an acute exposure (6 h) to anoxia

The progressive cardiovascular changes that accompanied

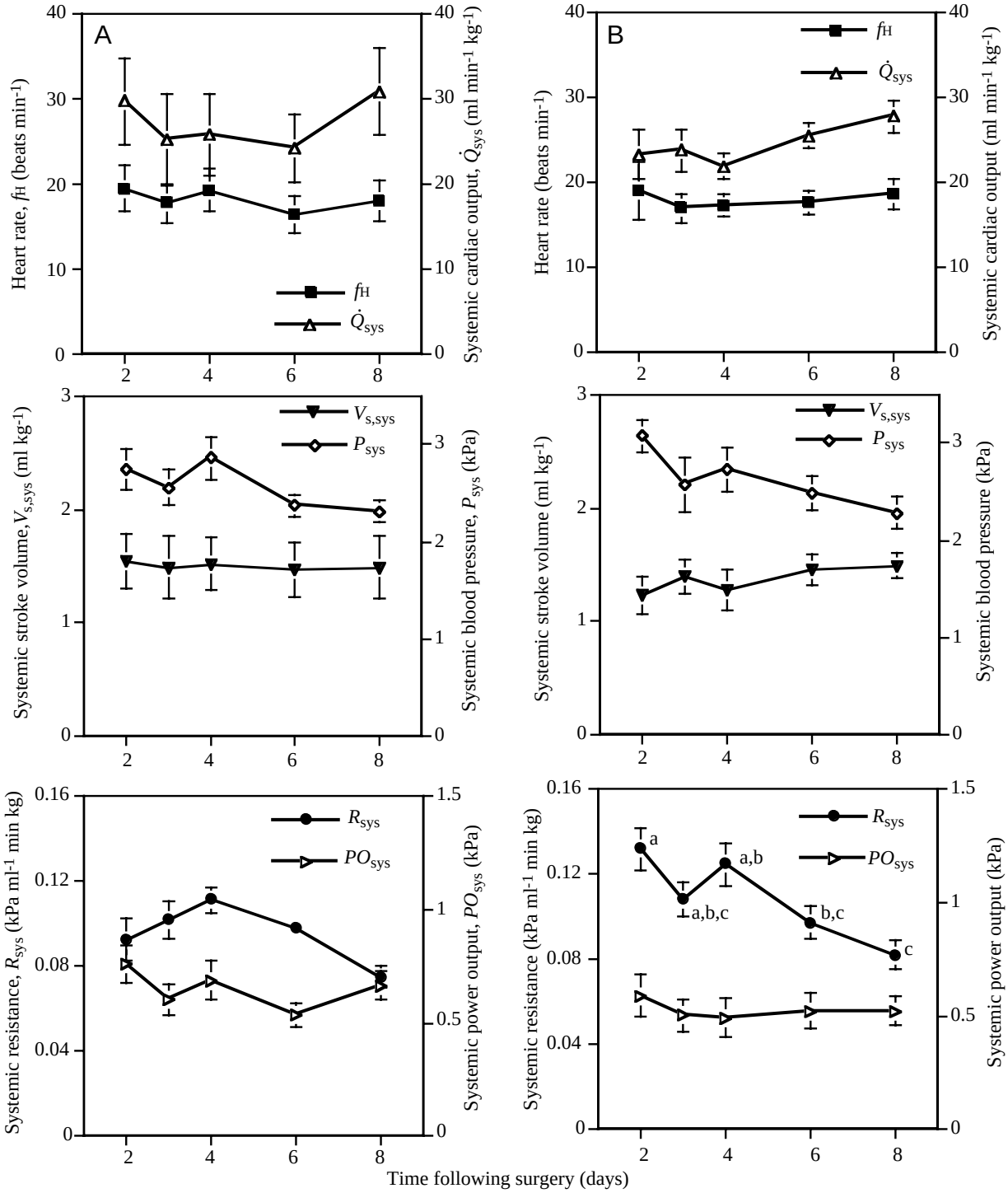

Fig. 3. Post-surgery cardiovascular variables during recovery and measured under normoxic conditions at 22 °C for the groups of turtles subsequently acclimated to 5 °C and used for the normoxic (A, *N*=6) and anoxic (B, *N*=5) experiments. Significant differences (P<0.05) between values are indicated by dissimilar letters. Values are means ± S.E.M.

the acute exposure to anoxia at 22 °C are summarized in Fig. 4A. Most of the cardiovascular adjustments occurred during the first 2 h of the anoxic exposure, and none of the variables changed significantly after this.

Table 2 compares the cardiovascular changes after 6 h of anoxia with the normoxic control values. The approximately twofold decrease in both f_H and $V_{s,sys}$ contributed to the 4.5-fold decrease in $\dot{Q}_{sys}$. The decrease in $\dot{Q}_{sys}$ produced a significant arterial hypotension (a 30 % decrease in P_{sys}) even though R_{sys} more than tripled. As a result of the decrease in

Table 1. In vivo *cardiovascular status for the four experimental groups of turtles measured at 22 °C under normoxic conditions following the 8-day post-surgery recovery period*

Group	Heart rate (beats min^{-1})	Systemic stroke volume (ml kg^{-1})	Systemic cardiac output (ml min^{-1} kg^{-1})	Mean arterial pressure (kPa)	Systemic power output (mW g^{-1})	Systemic resistance (kPa ml^{-1} min kg)
22 °C normoxic	24.2±2.3	1.49±0.15	36.0±2.5	2.26±0.12[a]	0.66±0.10	0.062±0.005[a]
22 °C anoxic	22.6±2.5	1.21±0.11	27.3±3.6	3.03±0.15[b]	0.66±0.15	0.110±0.015[b]
5 °C normoxic	18.0±2.4	1.71±0.28	30.8±5.1	2.31±0.11[a]	0.66±0.06	0.075±0.005[a]
5 °C anoxic	18.6±1.8	1.49±0.11	27.7±1.9	2.28±0.17[a]	0.52±0.06	0.082±0.007[a]

Values are means ± 1 S.E.M. for all experimental groups (N=6, except 5 °C anoxia where N=5).
Significant differences (P<0.05) between groups are indicated by dissimilar letters.

Table 2. In vivo *cardiovascular status for 22 °C-acclimated and 5 °C-acclimated turtles exposed to either normoxic conditions or acute anoxia (6 h) at 22 °C or chronic anoxia (22 days) at 5 °C*

Group	Heart rate (beats min^{-1})	Systemic stroke volume (ml kg^{-1})	Systemic cardiac output (ml min^{-1} kg^{-1})	Mean arterial pressure (kPa)	Systemic power output (mW g^{-1})	Systemic resistance (kPa ml^{-1} min kg)
22 °C normoxic	24.2±2.3[a]	1.49±0.15[a]	36.0±2.5[a]	2.26±0.12[a]	0.660±0.0980[a]	0.06±0.005[a]
22 °C anoxic	10.3±0.9[b]	0.78±0.10[b]	8.0±1.2[b]	1.59±0.16[b]	0.100±0.0320[b]	0.20±0.014[b]
5 °C normoxic	5.2±0.5[c]	0.77±0.13[b]	4.0±0.9[c]	1.32±0.13[b,c]	0.044±0.0068[c]	0.33±0.054[b]
5 °C anoxic	1.0±0.2[d]	0.26±0.06[c]	0.3±0.1[d]	1.02±0.05[c]	0.002±0.0002[d]	3.88±0.450[c]

Values are means ± 1 S.E.M. for all experimental groups (N=6, except 5 °C anoxia where N=5).
Significant differences (P<0.05) between groups are indicated by dissimilar letters.

$\dot{Q}_{sys}$ and the arterial hypotension, PO_{sys} was 6.6 times lower following 6 h of anoxic exposure (Table 2) than in the normoxic group of turtles at 22 °C.

Cold-acclimated turtles under normoxic conditions

The progressive cardiovascular changes that accompanied the 36-day acclimation to 5 °C are summarized in Fig. 4B. Cardiovascular status was altered significantly and appreciably during the first 6 days of acclimation; very few significant changes occurred after day 10. This rapid cold acclimation may reflect, in part, the progressive introduction to 5 °C that occurred during the post-surgery recovery period.

Table 2 compares the cardiovascular variables at day 36 of 5 °C acclimation with those of the 22 °C-acclimated normoxic group. Cold acclimation resulted in significant decreases in all cardiovascular variables except for R_{sys}, which increased significantly. The 4.7-fold decrease in f_H to 5.2 beats min^{-1}, which represented a Q_{10} of 2.7, contributed significantly to the ninefold decrease in $\dot{Q}_{sys}$ to 4.0 ml min^{-1} kg^{-1}. Despite the large decrease in $\dot{Q}_{sys}$, arterial hypotension was limited to a less than twofold decrease in P_{sys} because of a 5.5-fold increase in R_{sys}. Nonetheless, the 15-fold decrease in cardiac PO_{sys} to 0.044 mW g^{-1} with 5 °C acclimation represented a Q_{10} of 8.8.

Cold-acclimated turtles exposed to chronic (22-day) anoxia

The progressive cardiovascular changes accompanying the 22-day chronic exposure to anoxia at 5 °C are summarized in Fig. 4C. The cardiovascular changes reached an apparent steady state after 12 days of anoxia, i.e. none of the cardiovascular variables changed significantly between day 20 and day 28 of the experiment. Compared with normoxic 5 °C-acclimated turtles, chronic anoxic exposure resulted in significant decreases in PO_{sys} (22-fold), $\dot{Q}_{sys}$ (13.3-fold), f_H (5.2-fold) and $V_{s,sys}$ (3.0-fold) and a significant increase (11.8-fold) in R_{sys} beyond the changes produced by 5 °C acclimation alone (Table 2). These changes were qualitatively the same as, but quantitatively much greater than, those produced by acute anoxia at 22 °C.

Compared with normoxic 22 °C-acclimated turtles, chronic anoxic exposure caused a profound depression of cardiovascular status in 5 °C-acclimated turtles (Table 2). There was a 120-fold decrease in $\dot{Q}_{sys}$, as a result of a 24.2-fold decrease in f_H and a 5.7-fold decrease in $V_{s,sys}$. Arterial hypotension was moderated (P_{sys} decreased by only 2.2-fold) by a 64.6-fold increase in R_{sys}. PO_{sys} was reduced 330-fold to 0.002 mW g^{-1} from 0.66 mW g^{-1}. Of this 330-fold reduction in PO_{sys}, a 22-fold decrease could be attributed to the effect of chronic anoxia at 5 °C, while a 15-fold decrease could be attributed to cold acclimation. Chronic anoxic exposure and cold acclimation contributed similarly to the depression of f_H (5.2-fold *versus* 4.6-fold, respectively) and $\dot{Q}_{sys}$ (13-fold *versus* ninefold, respectively) (Table 2). However, chronic anoxic exposure contributed much more to the increase in R_{sys} (11.8-fold) than cold acclimation (5.5-fold). Cardiac depression and increased vascular resistance are clearly significant features that accompany chronic anoxic exposure in cold-acclimated turtles.

Discussion

Cardiac performance under normoxic conditions

In the present study, 22 °C-acclimated *Trachemys scripta* were allowed an 8-day post-surgery recovery period under normoxic conditions. Given that cardiovascular status had stabilized by the eighth day and that there was reasonable agreement in cardiovascular status among the four groups of turtles, despite some seasonal and procedural differences, we are confident in our control values as presented in Table 1. We also followed cardiovascular status during a 36-day period of acclimation to 5 °C and found that the cardiovascular changes associated with cold-acclimation were largely completed by the tenth day. Therefore, we are confident that our measurements are indicative of cold-acclimation. Furthermore, where comparisons are possible, cardiovascular status for warm-acclimated and cold-acclimated turtles in this study compares favourably with that of previous studies. For example, f_H values of 24.2 and 5.2 beats min^{-1} for 22 °C and 5 °C, respectively, are comparable with those reported by Herbert and Jackson (1985b) for *Chrysemys scripta* under normoxic conditions at 20 °C (30 beats min^{-1}) and after 3 months at 3 °C (1.8 beats min^{-1}). Hicks and Wang (1998) reported a f_H of 27.8 beats min^{-1} for normoxic *T. scripta* at 25 °C. However, f_H values reported for *T. scripta* at 22 °C by Hicks (1994) and Comeau and Hicks (1994) were 38 and 43 beats min^{-1}, respectively. Some of these differences probably represent changes in ventilatory state when sampling occurred. For instance, towards the end of apnoea at 19 °C, turtles acclimated to this temperature had a heart rate of 11 beats min^{-1} that increased sharply to 23–30 beats min^{-1} with ventilation (Shelton and Burggren, 1976).

By comparison, the intrinsic f_H values for an *in situ* heart preparation for *C. scripta*, devoid of neural control and acutely exposed to 15 °C and 5 °C, were 23.4 and 8.1 beats min^{-1}, respectively (Farrell et al., 1994). Given that the *in situ* f_H at 5 °C is higher than that *in vivo* for cold-acclimated turtles, cold-acclimation apparently permits the expression of physiological changes that depress f_H. Indeed, isolated perfused hearts of frogs *Rana temporaria* when acclimated to low temperature have lower contraction frequencies when tested over a wide range of test temperature than warm-acclimated hearts (Harri and Tabo, 1975). Possible mechanisms for these changes include the expression of myosin isoforms with lower ATPase activities (Vornanen, 1994) and/or a lengthening of the activation and relaxation phases of cardiac contraction. Autonomic modulation of the heart could also be involved in cold acclimation of turtles; these effects are considered in the accompanying report (Hicks and Farrell, 2000).

There was good agreement between the mean systemic arterial pressures reported here (2.3–3.0 kPa) and in previous studies with turtles. For example, P_{sys} ranged from 2.0 to 4.0 kPa at 20 °C (Comeau and Hicks, 1994; Hicks, 1994; Herbert and Jackson, 1985b), while Hicks and Wang (1998) reported 3.1 kPa for *T. scripta* at 25 °C. Herbert and Jackson (1985b) reported a P_{sys} of 1.9 kPa at 3 °C, whereas our value was 1.3 kPa at 5 °C. There was also good agreement with previous values of $V_{s,sys}$. In the present study, $V_{s,sys}$ was 1.49 ml kg^{-1} at 22 °C, while Shelton and Burggren (1976) and Hicks (1994) reported values of 1.34 and 1.8 ml kg^{-1} for turtles at 20 °C, respectively. The good agreement with the $V_{s,sys}$ value of 1.56 ml kg^{-1} reported by Hicks and Wang (1998) for turtles at 25 °C is especially important because they directly measured almost the entire systemic flow with three flow probes and used only one minor assumption. This agreement adds credence to the assumption we used when we estimated $\dot{Q}_{sys}$ from a measurement of left aortic flow. Unfortunately, we have no means of assessing whether our assumption was equally satisfactory for cold-acclimated turtles.

Given the good agreement between the present and previous studies for f_H, $V_{s,sys}$ and P_{sys} in warm-acclimated turtles, it should not be surprising that $\dot{Q}_{sys}$ and PO_{sys} were also comparable. In the present study, $\dot{Q}_{sys}$ was between 27.3 and 36.0 ml min^{-1} kg^{-1}, while PO_{sys} was between 0.52 and 0.66 mW g^{-1} at 22 °C. Both Comeau and Hicks (1994) and Hicks (1994) reported similar levels of $\dot{Q}_{sys}$ (29 ml min^{-1} kg^{-1} and 24 ml min^{-1} kg^{-1}, respectively) and PO_{sys} (1.4 mW g^{-1} and 0.44 mW g^{-1}, respectively) for turtles at 20 °C. Hicks and Wang (1998) reported somewhat higher values for $\dot{Q}_{sys}$ (43 ml min^{-1} kg^{-1}) and PO_{sys} (1.2 mW g^{-1}) for *T. scripta* at 25 °C. Assuming that $\dot{Q}_{sys}$ is between 1.7 and 1.8 times pulmonary cardiac output (Wang and Hicks, 1996; Hicks and Wang, 1998), then total cardiac power output in our study would have ranged from 0.9 to 1.4 mW g^{-1}. By comparison, maximum cardiac power output has been measured in turtle hearts *in situ* at 2.56 mW g^{-1} (Farrell et al., 1994) and *in vivo* at 2.46–3.02 mW g^{-1} (Shelton and Burggren, 1976; Comeau and Hicks, 1996).

Beyond the general agreement of cardiovascular variables for studies with turtles held at temperatures around 20 °C, the present study provides novel information on the extent and speed with which $\dot{Q}_{sys}$, $V_{s,sys}$, R_{sys} and PO_{sys} are adjusted at 5 °C. Previous studies have only measured f_H and P_{sys}. The adjustments in cardiovascular status were completed within 20 days and, by and large, the major changes occurred within the first 10 days. If further adjustments do occur beyond the 36-day acclimation period used here, then their rates of change would be low.

Cold acclimation produced negative chronotropic and negative inotropic effects since there were sizeable decreases in both f_H and $V_{s,sys}$. The fact that PO_{sys} decreased by 15-fold with a Q_{10} of approximately 9 demonstrates that cold-acclimation involved an active depression of cardiac activity. A similar conclusion can be reached for the 4.7-fold decrease in f_H. This 15-fold reduction in cardiac metabolic rate undoubtedly reflects parallel reductions in the ATP requirements of other tissues and, as such, there would be a sizeable decrease in the rate of fuel utilization and waste accumulation in cold-acclimated turtles. The substantial increase in R_{sys} with cold acclimation probably reflects a prioritization of regional blood flow distribution. Systemic vasoconstriction, passive collapse of vessels due to the lower P_{sys} or some combination of these could have brought about the increase in R_{sys}. The involvement of adrenergic and cholinergic regulation of the cardiovascular system of the turtle with cold acclimation is investigated in a companion report (Hicks and Farrell, 2000).

Cardiac performance under anoxic conditions

We were impressed at the anoxia-tolerance of cold-acclimated turtles. Several turtles were allowed to recover under normoxic conditions at the end of anoxic exposure (data not shown) and were found to be in good condition. Blood pH was 7.17 in our anoxic group (see Hicks and Farrell, 2000). Herbert and Jackson (1985a) previously found that when blood pH fell below 7.0 during prolonged anoxia, recovery of turtles was impaired.

The present study is the first to measure systemic PO_{sys} in cold-acclimated turtles during a chronic anoxic exposure. The 5 °C-acclimated turtles under anoxia had a 330-fold lower PO_{sys} than the 22 °C-acclimated turtles under normoxia (0.002 mW g^{-1} *versus* 0.66 mW g^{-1}). f_H and P_{sys} have been measured previously under cold anoxia (Herbert and Jackson, l985b) and, using these values, total cardiac power output was estimated to decrease from 1.7 mW g^{-1} under 20 °C normoxia to 0.003 mW g^{-1} under 3 °C anoxia, i.e. a 500-fold difference (Farrell et al., 1994). This prediction is very much in line with the present results if we account for the 70–80 % difference (noted above) between PO_{sys} and total cardiac power output that reflects the cardiac work associated with pulmonary perfusion under warm, normoxic conditions. Thus, if we use the estimated range for total cardiac work in normoxic 22 °C-acclimated turtles (0.9–1.4 mW g^{-1}, presented above) and assume that there was no pulmonary flow in anoxic 5 °C-acclimated turtles, then the reduction in total cardiac power output would be between 450-fold and 700-fold. [The assumption that pulmonary blood flow stops during chronic anoxia and that the anoxic heart works only to perfuse the systemic circulation appears to be valid since Hicks and Wang (1998) found that pulmonary blood flow stopped after only 1 h of anoxia.] The general agreement between the two estimates of the reduction in cardiac power output is particularly encouraging because it lends indirect support to our assumption that the distribution of blood flow between the major systemic vessels was not changed in a major way by cold acclimation and by anoxia. However, given the large accompanying changes in R_{sys}, it is possible that the distribution of blood flow did shift somewhat, and so we urge caution in using our values beyond general applications. One possibility is that the cerebral circulation was spared at the expense of the organs perfused by the left aorta. In this case, we would have overestimated the decrease in $\dot{Q}_{sys}$ with our assumption. Further experiments are needed to examine regional blood flow distribution in cold-acclimated and chronically anoxic turtles before this point can be resolved.

Hicks and Wang (1998) reported *in vivo* cardiovascular status for *T. scripta* at 25 °C after a shorter (2 h) anoxic period. In general, their findings were qualitatively similar to ours, but quantitatively the changes were smaller. They reported a 51 % decrease in PO_{sys}, a 26 % decrease in P_{sys}, a 21 % decrease in $\dot{Q}_{sys}$ and a 43 % decrease in f_H. This quantitative difference no doubt reflects a difference in the duration of the acute anoxic period between the two studies. In addition, the post-surgery recovery period was longer in our experiments, and this may have contributed to the difference.

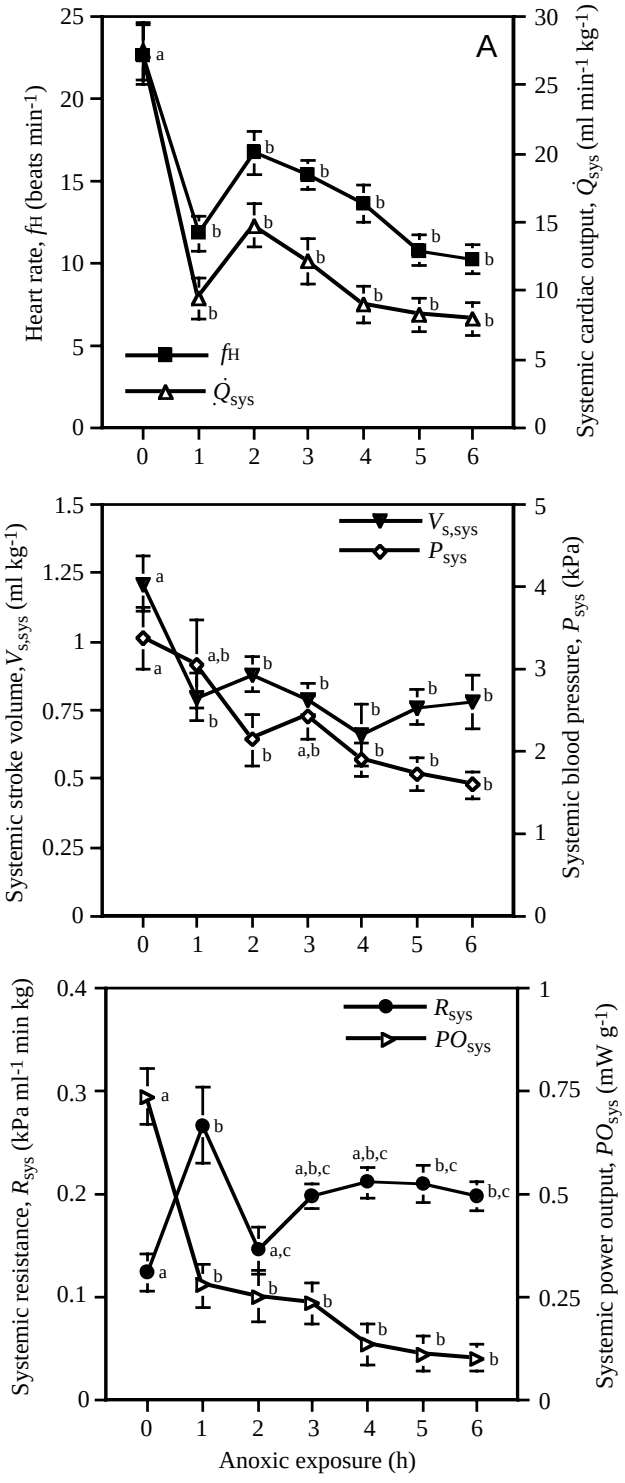

Fig. 4. Cardiovascular variables of turtles: (A) during an acute, 6 h anoxia exposure at 22 °C (N=6); (B) during acclimation to 5 °C under normoxic conditions (N=6); (C) during acclimation to 5 °C and chronic exposure to anoxic conditions beginning on the seventh day of cold acclimation (N=5). Significant differences (P<0.05) between values are indicated by dissimilar letters. Values are means ± S.E.M.

There are no comparable *in vivo* cardiovascular measurements for 5 °C-acclimated turtles. However, total

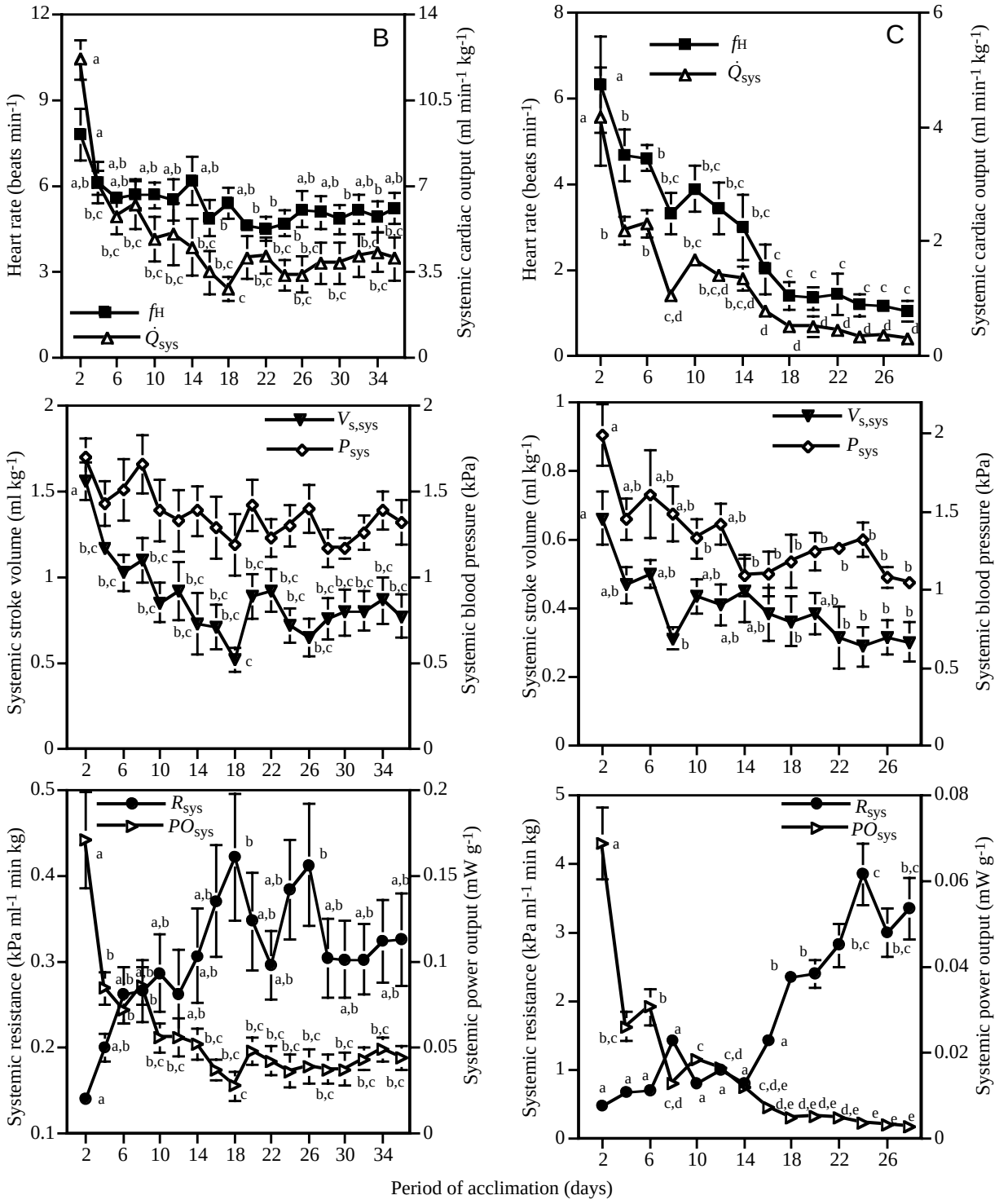

Fig. 4. *Continued*

cardiac power output has been measured in isolated and *in situ* anoxic turtle heart preparations at low temperatures. On the basis of the data of Reeves (1963), Farrell et al. (1994) estimated that the best isolated heart preparation at 22 °C had a maximum anoxic total power output of 0.87 mW g^{-1}. Similarly, total power output was 0.77 mW g^{-1} under anoxia at 15 °C for *in situ* perfused hearts and decreased to 0.17 mW g^{-1} at 5 °C (Farrell et al., 1994). By comparison, it is clear that cardiac performance *in vivo* during acute anoxia under warm conditions (0.58 mW g^{-1} after 2 h, Hicks and Wang, 1998; 0.1 mW g^{-1} after 6 h, present study) can be well below the intrinsic capabilities of the heart. Furthermore, this difference becomes two orders of magnitude for 5 °C-acclimated turtles chronically exposed to anoxia (PO_{sys} was 0.002 mW g^{-1}). The

underlying mechanisms for this chronic depression of cardiac activity *in vivo* await further study.

Anoxic depression of the cardiovascular system in 5 °C-acclimated turtles was clearly quantitatively greater than in 22 °C-acclimated turtles. For example, at 22 °C, the 6 h anoxic exposure produced bradycardia (2.3-fold decrease in f_H), reduced inotropy (1.9-fold decrease in $V_{s,sys}$) and systemic vasoconstriction (3.3-fold increase in R_{sys}). However, these changes were as much as four times larger after the 22-day anoxic exposure at 5 °C. These differences could come about through adrenergic and cholinergic regulatory mechanisms. Autonomic regulation of the cardiovascular system is considered further in an accompanying report (Hicks and Farrell, 2000). Alternatively, they could reflect intrinsic responses to the anoxic and acidotic conditions that prevail. In addition, the arterial hypotension under cold anoxic conditions (a 2.2-fold reduction in arterial blood pressure) could directly affect vascular resistance and modulate blood flow distribution. When transmural vascular pressure falls to a critical pressure and relaxes vascular elastin fibres, the vessel collapses and blood flow stops (Burton, 1972). Thus, if critical closing pressures vary between vascular beds, then peripheral blood flow distribution could vary as a function of the critical closing pressure when arterial blood pressure decreases. Presumably, critical organs such as the brain would have the lowest critical closing pressure if this were the case.

By measuring PO_{sys} for the anoxic turtle heart *in vivo* we can assess the extent to which the glycolytic rate was adjusted in cardiac tissues. Here, we assume that PO_{sys} is proportional to ATP utilisation and that glycolysis supplies 18 times less ATP per mole of glucose than oxidative metabolism. In 22 °C-acclimated turtles, acute anoxia caused only a 6.6-fold decrease in PO_{sys}, so a Pasteur effect would be needed for the heart to compensate for the 18-fold reduction in ATP yield per mole of glucose during the acute anoxic exposure. In contrast, chronic anoxia caused a 22-fold decrease in PO_{sys} for 5 °C-acclimated turtles, so that a Pasteur effect would not be needed to maintain the cardiac ATP supply. Suppression of the Pasteur effect would conserve glucose stores and this, combined with the temperature-mediated depression of cardiac performance (Q_{10}=9), is probably an important reason why turtles can tolerate anoxia much better under cold than under warm conditions.

Our cold-acclimation experiments were performed over the winter months because other ectotherms show important physiological modifications on a seasonal basis. For example, the tortoise *Kinixys speki* has a reduced metabolic rate when held at 5 °C in November, but not in June or August when they are normally active (Hailey and Loveridge, 1997). When kept under constant conditions, *Gopherus agassizii* show reduced activity and food consumption in the autumn (Jackson et al., 1976). Whether seasonal plasticity in physiological state plays an important role in the anoxic tolerance of turtles remains to be determined.

This work was supported by a Natural Sciences and Engineering Research Council of Canada research grant to A.P.F and a Simon Fraser University Graduate Fellowship awarded to J.M.T.H.

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