

MYOCARDIAL INTRACELLULAR pH IN A PERFUSED  
RAINBOW TROUT HEART DURING EXTRACELLULAR  
ACIDOSIS IN THE PRESENCE AND ABSENCE OF  
ADRENALINE

By A. P. FARRELL

*Department of Biological Sciences, Simon Fraser University, Burnaby, BC,  
V5A 1S6, Canada*

AND C. L. MILLIGAN

*Department of Biology, McMaster University, Hamilton, Ontario, Canada*

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SUMMARY

Myocardial intracellular pH was measured in a perfused rainbow trout, *Salmo gairdneri*, with DMO (5,5-dimethyl-2,4-oxazolidinedione), to test the hypothesis that catecholamines promote active regulation of myocardial pH in order to protect contractility during a respiratory acidosis comparable to that observed after exercise. Under control conditions (extracellular pH = 8.0;  $P_{\text{CO}_2}$  = 2 Torr), myocardial pH was  $7.53 \pm 0.01$  ( $N = 5$ ). Acidosis (extracellular pH = 7.45;  $P_{\text{CO}_2}$  = 8.6 Torr) reduced contractility, mechanical efficiency and intracellular pH ( $7.25 \pm 0.04$ ), but did not affect myocardial  $\text{O}_2$  consumption. The addition of  $0.5 \mu\text{mol l}^{-1}$  adrenaline during extracellular acidosis prevented the loss of contractility, restored mechanical efficiency, but did not change intracellular pH significantly. Thus, adrenaline enabled cardiac contractility to recover, without intracellular pH regulation, possibly by modulation of sarcolemmal calcium changes. The absence of a myocardial acidosis after exercise *in vivo* is discussed with respect to possible intracellular pH regulation *via* lactate uptake and metabolism.

INTRODUCTION

An extracellular acidosis of both respiratory and metabolic origin normally occurs after exhaustive exercise in teleosts (Black, Chiu, Forbes & Hernslip, 1959; Wood, McMahon & McDonald, 1977; Graham, Wood & Turner, 1982; Holeyton, Neumann & Heisler, 1983; Turner, Wood & Clark, 1983a; Turner, Wood & Høbe, 1983b; Milligan & Farrell, 1986). Typically, the respiratory component predominates during the initial phase of recovery (0–2 h), producing about a 0.5 pH unit reduction in extracellular pH ( $\text{pH}_e$ ) (see Wood & Perry, 1985). *In vitro* experiments clearly demonstrate that an extracellular acidosis of this magnitude depresses cardiac performance by reducing both cardiac contractility and the intrinsic heart rate (fH) (see reviews by Gesser & Poupa, 1983; Farrell, 1984). Respiratory acidosis decreases

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myocardial intracellular pH ( $\text{pH}_i$ ) in isolated ventricle strips from trout (Gesser & Jorgensen, 1982), and the lower  $\text{pH}_i$ ; probably reduces cardiac contractility, as is the case in the mammalian myocardium (Williamson *et al.* 1976). However, *in vivo* myocardial  $\text{pH}_i$  does not fall in trout and sea raven despite the pronounced extracellular acidosis after exercise; in fact, the myocardium tends to become alkalotic (Milligan & Farrell, 1986; Milligan & Wood, 1986b). Since a physiological concentration of adrenaline protects the normal range of *in vitro* cardiac performance during extracellular acidosis (Farrell, MacLeod, Driedziec & Wood, 1983; Farrell, MacLeod & Chancey, 1986), it is possible, therefore, that the increased level of circulating catecholamines associated with exercise stress (Primmatt, Randall, Mazeaud & Boutilier, 1986) promotes some form of active regulation of myocardial  $\text{pH}_i$ ; and hence prevents the deleterious effect of acidosis on contractility.

This hypothesis was tested using the DMO (5,5-dimethyl-2,4-oxazolinedione) method of Waddell & Butler (1959) to measure  $\text{pH}_i$  in perfused hearts from rainbow trout, *Salmo gairdneri*, exposed to an extracellular acidosis of respiratory origin in the presence and absence of adrenaline.

#### MATERIALS AND METHODS

The experimental procedures and equipment for the perfused trout heart were essentially those fully described in the accompanying work (Farrell *et al.* 1986). The perfusate was delivered to the sinus versus *via* a stainless steel cannula placed in the hepatic vein. The ductus cuvier was ligated. The ventricle pumped the perfusate against the output pressure head *via* a stainless steel cannula placed in the ventral aorta. The preload and diastolic afterload to the heart were set so that the heart generated a power output similar to that of a resting fish, during the equilibration period with non-recirculating control perfusate ( $10^\circ\text{C}$ ). Cardiac output ( $\dot{V}_b = 15 \text{ ml min}^{-1} \text{ kg}^{-1}$  fish weight) was set by the intrinsic  $\text{fH}$  and manipulation of stroke volume ( $\text{SV}_H$ ) *via* the preload. The mean output pressure was  $45 \text{ cmH}_2\text{O}$ . The major procedural difference was the recirculation of the experimental perfusate after the usual equilibration period with non-recirculating, control perfusate. The volume of the experimental perfusate was 100 ml, to which  $0.5 \mu\text{Ci } [^{14}\text{C}]\text{DMO}$  (New England Nuclear, specific activity:  $50 \text{ mCi mmol}^{-1}$ ) plus  $2.0 \mu\text{Ci } [^3\text{H}]\text{mannitol}$  (New England Nuclear, specific activity:  $27.4 \text{ mCi mmol}^{-1}$ ) were added.

The equilibration of DMO and mannitol was assessed using recirculation times of 10, 15, 20 and 25 min with condition I below (14 heart preparations). At the end of each test period, inflow and outflow perfusate ( $700 \mu\text{l}$ ) was sampled and analysed for  $\text{pH}$ , total  $\text{CO}_2$ ,  $\text{P}_{\text{O}_2}$  and levels of  $^3\text{H}$  and  $^{14}\text{C}$  radioactivity. The ventricle was removed, blotted dry, frozen in liquid  $\text{N}_2$  and later analysed for levels of  $^3\text{H}$  and  $^{14}\text{C}$  radioactivity and total water content.

Having established that marker equilibrations were complete within 20 min (see Table 1), the following test conditions were examined over a 20-min perfusion period:

Condition I. Control:  $\text{pH}_e = 8.05 \pm 0.04$ ,  $\text{P}_{\text{CO}_2} = 1.67 \pm 0.17$  Torr ( $N = 5$ ).

Condition II. Control +  $0.5 \mu\text{mol l}^{-1}$  adrenaline:  $\text{pH}_e = 8.00 \pm 0.02$ ,  $\text{P}_{\text{CO}_2} = 1.86 \pm 0.08$  Torr ( $N = 6$ ).

Condition III. Acidosis:  $\text{pH}_e = 7.39 \pm 0.03$ ,  $\text{P}_{\text{CO}_2} = 8.7 \pm 0.6$  Torr ( $N = 6$ ).

Condition IV. Acidosis +  $0.5 \mu\text{mol l}^{-1}$  adrenaline:  $\text{pH}_e = 7.36 \pm 0.01$ ,  $\text{P}_{\text{CO}_2} = 8.5 \pm 0.3$  Torr ( $N = 8$ ).

Thus, a typical experiment consisted of a 20-min equilibration period, during which a heart was perfused with non-recirculating control saline, followed by a 20-min 'test' period, during which one of the above conditions was examined using a recirculating perfusate containing [ $^{14}\text{C}$ ]DMO and [ $^3\text{H}$ ]mannitol. The preload and afterload were not changed throughout the 20-min test period. At the end of each test period, input and output samples (1000  $\mu\text{l}$ ) of perfusate were analysed for the parameters listed above, with the additional measurement of lactate concentration in the output sample. The heart was treated as described above.

The analytical techniques and calculations are fully described elsewhere (Milligan & Wood, 1985; Farrell *et al.* 1986).  $^3\text{H}$  and  $^{14}\text{C}$  radioactivity were measured in duplicate, with 100  $\mu\text{l}$  samples of perfusate in 10 ml ACS fluor (Amersham) and 50 mg samples of ventricle digested in 2 ml NCS tissue solubilizer (Amersham), neutralized and counted in 10 ml OCS fluor (Amersham). All samples were counted on a Beckman LS-9000 liquid scintillation counter.  $\text{P}_{\text{O}_2}$  and  $\text{pH}_e$  were determined with Radiometer microelectrodes at  $10^\circ\text{C}$  and total  $\text{CO}_2$  was measured using the method described by Cameron (1971).  $\text{P}_{\text{CO}_2}$  and  $[\text{HCO}_3^-]$  were calculated from total  $\text{CO}_2$  using the Henderson-Hasselbalch equation and values for  $\alpha\text{CO}_2$  and  $\text{pK}'$  reported by Boutilier, Heming & Iwama (1984). Myocardial  $\text{pH}_i$  and ECFV (extracellular fluid volume) were calculated as described by Milligan & Farrell (1986) using the inflow  $\text{pH}$ , and [ $^3\text{H}$ ]mannitol and [ $^{14}\text{C}$ ]DMO concentrations for estimates of  $\text{pH}_e$ , [ $^3\text{H}$ ]mannitol $_e$  and [DMO] $_e$ , respectively. The perfusate lactate concentration was determined enzymatically (lactate dehydrogenase method; Sigma bulletin no. 826) on 500  $\mu\text{l}$  perfusate extracted with 500  $\mu\text{l}$  ice-cold 8% perchloric acid. The cardiovascular variables were analysed as in previous work with the aid of analogue-to-digital conversion using an APPLE microcomputer (Farrell *et al.* 1986). Myocardial oxygen consumption ( $\dot{M}_{\text{O}_2}$ ,  $\text{nmol s}^{-1} \text{g}^{-1}$ ) was  $[\text{input } \text{P}_{\text{O}_2} - \text{output } \text{P}_{\text{O}_2}] \times \alpha\text{O}_2 \times [\dot{V}_b/60 \text{ (ml s}^{-1} \text{g}^{-1})]$ , where  $\alpha\text{O}_2 = 1.695 \mu\text{mol O}_2 \text{ ml}^{-1}$  (Altman & Dittmer, 1974). Mechanical efficiency of the heart was calculated from  $[\text{power output (mW g}^{-1})] \times 2.22/\dot{M}_{\text{O}_2}$ . Preparations showing abnormal cardiac performance during the initial equilibration period were not used. Estimates of the ventricular non-bicarbonate buffer capacity were made according to the methods outlined by Milligan & Farrell (1986) using an IL pH electrode. Hearts from four fish were pooled and the slope of the pH *versus* mmol HCl relationship over the pH range 6.9 to 7.7 was taken as the buffer value of the tissue, in  $\text{mmol pH}^{-1} \text{ l}^{-1}$  intracellular fluid (ICF). The relationship was determined in duplicate.

Mean values  $\pm$  1 s.e.m. are reported throughout unless otherwise stated. Significant differences ( $P < 0.05$ ) were determined using a Student's two-tailed *t*-test.

## RESULTS

*Preparation stability: cardiovascular variables and marker equilibration*

After 10, 15, 20 and 25 min of recirculating perfusion, cardiovascular variables were generally unchanged compared to values recorded after the equilibration period with open-circuit perfusion (Table 1). The slight decrease in  $\dot{f}H$  was not reflected in  $\dot{V}b$  or power output because of compensatory changes in  $SVH$ . The difference between input  $P_{O_2}$  and output  $P_{O_2}$  ( $\Delta P_{O_2}$ ) was usually 30–50 Torr. Myocardial oxygen consumption ( $\dot{M}_{O_2}$ ) ranged from a low of  $13.9 \pm 5.5$  ( $N = 4$ ) after 10 min of equilibration to a high of  $24.3 \pm 7.7$   $\text{nmol s}^{-1} \text{g}^{-1}$  ( $N = 4$ ) after 25 min of equilibration (Table 1), though these values were not significantly different. Equilibration of [ $^{14}\text{C}$ ]DMO in the ventricle was quite rapid, as indicated by the constancy in the ratio of  $\text{DMO}_i/\text{DMO}_e$ —and hence the estimate of  $\text{pH}_i$ —from 10 min onwards (Table 1). [ $^3\text{H}$ ]mannitol equilibration was slightly longer, requiring up to 15 min for complete equilibration. The 'test' period of 20 min, therefore, allowed complete marker equilibration and adequate time for the heart to come into a new steady-state with respect to performance parameters monitored in response to the acidosis and/or adrenaline challenges.

*Condition I*

Under control conditions ( $\text{pH} = 8.05 \pm 0.04$ ,  $P_{\text{CO}_2} = 1.67 \pm 0.17$  Torr,  $N = 5$ ), cardiovascular variables remained unchanged after 20 min of recirculating perfusate, compared to measurements taken at the end of the open-circuit circulation (Fig. 1). A net acidic equivalent excretion from the heart occurred in all preparations, since the outflow  $\text{pH}_e$  tended to be lower than the inflow  $\text{pH}_e$  by 0.07–0.10 pH units. There was no evidence of lactate excretion into the perfusate. Under these conditions myocardial  $\text{pH}_i$  averaged  $7.53 \pm 0.01$  (Fig. 2).

*Condition II*

With control perfusate, the presence of  $0.5 \mu\text{mol l}^{-1}$  adrenaline ( $N = 6$ ) improved with resting power output and  $\dot{V}b$  through an increase in  $\dot{f}H$  (Fig. 1).  $SVH$  and systolic pressure were unchanged at the constant preload, even though  $\dot{f}H$  had increased. While there was a modest stimulation of the cardiac performance compared to that which occurred in condition I, myocardial  $\text{pH}_i$ ,  $\Delta P_{O_2}$  and  $\dot{M}_{O_2}$  were unchanged, but mechanical efficiency was improved (Fig. 2; Table 2). No lactate was detected in the perfusate and there was a small, though significant, increase in the ECFV at the expense of ICFV (Fig. 3).

*Condition III*

Extracellular acidosis ( $\text{pH}_e = 7.390 \pm 0.029$ ,  $P_{\text{CO}_2} = 8.7 \pm 0.6$  Torr,  $N = 6$ ) reduced the resting cardiac performance. Under the imposed conditions of constant preload and diastolic afterload, there were significant decreases in  $\dot{V}b$  and power output (Fig. 1). The primary change was a decrease in the intrinsic  $\dot{f}H$ . Impaired

Table 1. Stability of cardiovascular variables during the test period and stability of pH<sub>i</sub> measurements as a function of the equilibration time during the test period

Variable	10 min (N=4)		15 min (N=5)		20 min (N=4)		25 min (N=4)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Power output (mW g <sup>-1</sup> )	0.89 ± 0.09	0.86 ± 0.12	1.09 ± 0.10	1.08 ± 0.09	1.12 ± 0.10	1.11 ± 0.12	0.96 ± 0.04	0.98 ± 0.03
Cardiac output (ml min kg <sup>-1</sup> )	12.70 ± 1.04	12.15 ± 1.45	14.93 ± 0.74	14.84 ± 0.73	15.69 ± 0.68	14.84 ± 1.34	13.97 ± 0.58	14.00 ± 0.58
Heart rate (beats min <sup>-1</sup> )	47.2 ± 7.9	54.8 ± 10.8	63.4 ± 6.6	57.3 ± 4.7	75.4 ± 1.8	73.5 ± 2.1	70.8 ± 2.9	67.3 ± 5.7
O <sub>2</sub> consumption (nmol O <sub>2</sub> s <sup>-1</sup> g <sup>-1</sup> )	13.9 ± 5.4		21.5 ± 4.8		18.6 ± 3.9		24.3 ± 7.7	
pH <sub>i</sub>	7.58 ± 0.01		7.56 ± 0.02		7.53 ± 0.01		7.57 ± 0.02	
DMO <sub>2</sub> /DMO <sub>0</sub>	0.38 ± 0.05		0.38 ± 0.02		0.35 ± 0.02		0.34 ± 0.03	
Water content (l kg <sup>-1</sup> )	0.832 ± 0.008		0.828 ± 0.008		0.830 ± 0.012		0.829 ± 0.011	
ECFV (l kg <sup>-1</sup> )	0.308 ± 0.018		0.252 ± 0.021		0.240 ± 0.015		0.232 ± 0.016	

Initial and Final refer to beginning and end of perfusion period.

contractility, usually observed with acidosis (see Farrell *et al.* 1986), was only indicated by the absence of a compensatory increase in SVH as FH decreased (see condition I). The increase in  $\Delta P_{O_2}$  without a change in  $\dot{M}_{O_2}$  reflected the decrease in  $O_2$  delivery (a reduced  $\dot{V}b$ ). Nevertheless, the heart was less efficient during extracellular acidosis (Table 2).

Myocardial  $pH_i$  decreased significantly to  $7.25 \pm 0.04$  (Fig. 2). This acidosis was of mixed respiratory and metabolic origin (Fig. 4). There was no change in fluid distribution (Fig. 3) compared to condition I and lactate was detected in only one of the six preparations ( $0.14 \text{ mmol l}^{-1}$ ).

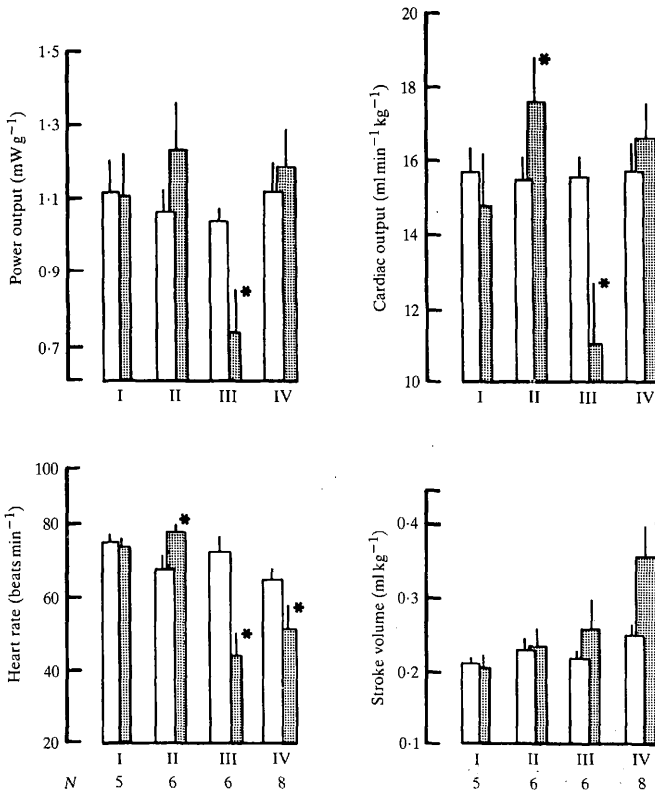


Fig. 1. Initial (open bar) and final (stippled bar) values for the cardiovascular variables of perfused trout hearts under the four test conditions. A significant difference between the initial and final values for a given condition is denoted by an asterisk.  $N$  = number of preparations.

## Condition IV

The presence of  $0.5 \mu\text{mol l}^{-1}$  adrenaline during extracellular acidosis ( $\text{pH}_e = 7.366 \pm 0.013$ ,  $\text{P}_{\text{CO}_2} = 8.53 \pm 0.30$  Torr,  $N = 8$ ) prevented the loss of cardiac performance normally associated with acidosis (condition III) (Fig. 1; Table 2). This was achieved by partially preventing the negative chronotropy associated with acidosis and by improving contractility so that SVH could increase when FH was reduced. Systolic pressure was also increased significantly from  $49.4 \pm 0.4$  to  $53.6 \pm 0.7$  cmH<sub>2</sub>O ( $1 \text{ cmH}_2\text{O} = 98.1 \text{ Pa}$ ).

Myocardial  $\text{pH}_i$  was unchanged compared to condition III (Fig. 2), indicating that  $\text{pH}_i$  regulation was not involved in the myocardial protection afforded by adrenaline under the present conditions. Furthermore, the myocardial intracellular

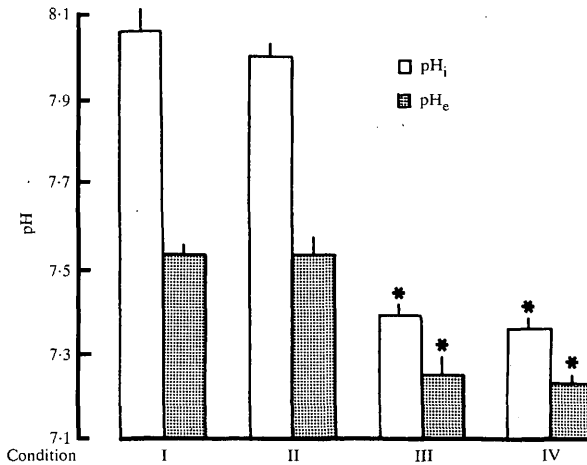


Fig. 2. A comparison of intracellular ( $\text{pH}_i$ ) and extracellular ( $\text{pH}_e$ ) pH measurements in the perfused trout heart at the end of the four test periods. An asterisk denotes a significant difference from the control condition I.

Table 2. Effect of extracellular acidosis with and without  $0.5 \mu\text{mol l}^{-1}$  adrenaline on oxygen consumption and mechanical efficiency

	Perfusion conditions			
	I ( $N = 5$ )	II ( $N = 6$ )	III ( $N = 4$ )	IV ( $N = 8$ )
$\dot{M}_{\text{O}_2}$ ( $\text{nmol s}^{-1} \text{g}^{-1}$ )	$22.0 \pm 3.0$	$22.7 \pm 7.7$	$28.0 \pm 7.7$	$26.9 \pm 3.7$
Mechanical efficiency (%)	$11.0 \pm 3.8$	$16.4 \pm 2.6^*$	$7.3 \pm 1.2^*$	$10.3 \pm 0.9^\dagger$
$\Delta \text{P}_{\text{O}_2}$ (mmHg)	$37.0 \pm 3.8$	$32.2 \pm 6.8$	$63.0 \pm 8.7^*$	$43.2 \pm 3.9^\dagger$

\* Significantly different from condition I.  
† Significant difference between conditions III and IV.

acid-base status was not appreciably different from that of condition III, and the response of the heart to the increased  $P_{CO_2}$  was passive (Fig. 4). While the presence of adrenaline did not affect  $O_2$  consumption,  $O_2$  delivery was enhanced, as indicated by the fall in  $\Delta P_{O_2}$  to a level comparable with condition I. Mechanical efficiency was also restored (Table 2). No lactate excretion from the heart was detected. There was a 10% increase in the ECFV, and, as total water content was unchanged, a reciprocal reduction in ICFV (Fig. 3). This increase was slightly greater than that observed in condition II.

#### DISCUSSION

The stability of the preparation was demonstrated by the control experiments (condition I).  $\dot{M}_{O_2}$  has not been measured previously in a perfused trout heart, but

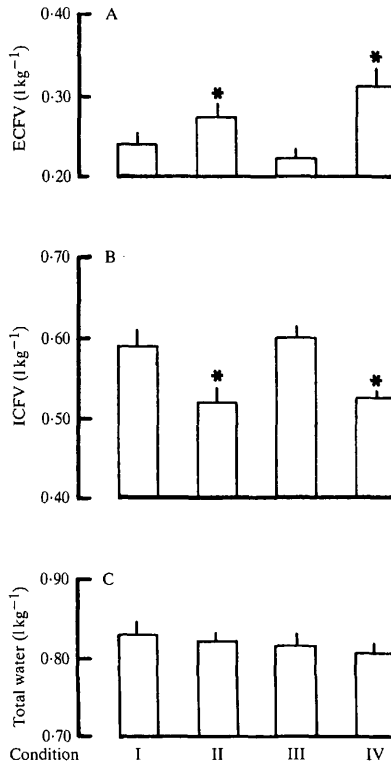


Fig. 3. Comparisons of fluid distributions in the perfused trout heart under the four test conditions. An asterisk denotes a significant difference from the control condition I.



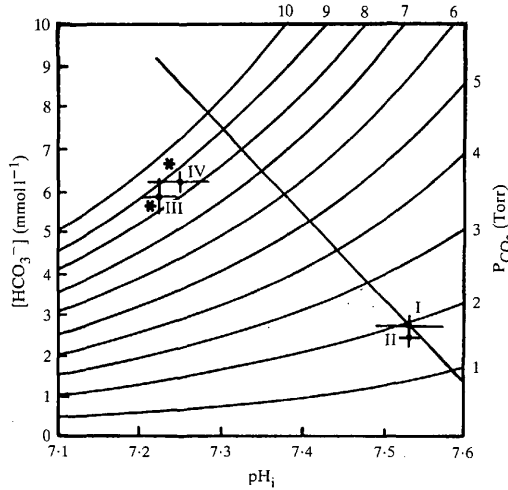


Fig. 4. A  $\text{pH-HCO}_3^-$  diagram indicating the intracellular changes under control conditions (I), control plus adrenaline (II), extracellular acidosis (III) and extracellular acidosis plus adrenaline (IV). The non-bicarbonate buffer line for the trout heart is indicated. The gradient of this line,  $\beta$ , was  $-22.58 \text{ mmol pH}^{-1} \text{ kg}^{-1}$  wet tissue (correlation coefficient = 0.987) or  $-27.54 \text{ mmol pH}^{-1} \text{ l}^{-1}$ . The points significantly off the buffer line are denoted by an asterisk.

the average value for all measurements with control perfusate ( $N = 34$  fish) of  $23.2 \pm 2.30 \text{ nmol s}^{-1} \text{ g}^{-1}$  and a mechanical efficiency of  $13.1 \pm 1.2\%$  at an average power output of  $1.06 \pm 0.05 \text{ mW g}^{-1}$  compare favourably with values determined for perfused sea raven hearts (Farrell, Wood, Hart & Driedzic, 1985). In the sea raven,  $\dot{M}_{\text{O}_2}$  and mechanical efficiency were, respectively,  $15 \text{ nmol s}^{-1} \text{ g}^{-1}$  and  $16\%$  at a power output of  $1.1 \text{ mW g}^{-1}$ . The measurement of  $\dot{M}_{\text{O}_2}$  in the trout heart may be an underestimate, since the ventricle could remove oxygen from the superfusate as well as the perfusate. This underestimate is likely to be small ( $<5\%$ ) based on recent experiments (M. S. Graham & A. P. Farrell, unpublished results) in which deoxygenated superfusate was found to have a minimal effect on  $\dot{M}_{\text{O}_2}$  and maximum cardiac performance. This also means that the oxygenated perfusate probably provided an adequate  $\text{P}_{\text{O}_2}$  gradient to supply oxygen to the outer, compact layer of myocardium, which is normally perfused *via* the coronary circulation. Hypoxic and, presumably, acidotic compact myocardium would not, therefore, have biased the  $\text{pH}_i$  measurements appreciably. Indeed, there was good agreement with *in vivo* estimates of trout ventricle  $\text{pH}_i$ . Furthermore, if there was an appreciable amount of hypoxia, it is unlikely that the perfused heart would have been able to generate workloads comparable to maximum levels observed in swimming trout (Farrell *et al.* 1986).

The equilibration of DMO over the 20-min test period was confirmed by the similar DMO distribution ratios, and hence  $\text{pH}_i$  values, for the 15- and 25-min equilibration times. The experiments with a 10-min equilibration time produced slightly higher  $\text{pH}_i$  values, indicating that full equilibration probably had not been attained after 10 min. Under conditions I and II, the DMO distribution ratios ( $[\text{DMO}]_i/[\text{DMO}]_e$ ) were identical, namely  $0.35 \pm 0.02$  ( $N = 4$ ) and  $0.34 \pm 0.04$  ( $N = 6$ ), respectively. This suggests that DMO was also fully equilibrated after 20 min under condition II. Under conditions III and IV, the DMO distribution ratio increased significantly, to  $0.75 \pm 0.03$  ( $N = 6$ ) and  $0.77 \pm 0.04$  ( $N = 8$ ), respectively, indicating that in the 20 min equilibration period DMO was being redistributed in response to the acidosis.

Fish myocardial  $\text{pH}_i$  has not been measured previously in a perfused heart preparation. The  $\text{pH}_i$  of 7.53 at  $\text{pH}_e = 8.0$  is in good agreement with DMO measurements of  $\text{pH}_i$  in the hearts of intact fish at rest [sea raven:  $\text{pH}_i = 7.50$ ,  $\text{pH}_e = 7.90$  (Milligan & Farrell, 1986); trout:  $\text{pH}_i = 7.34$ ,  $\text{pH}_e = 7.70$  (Milligan & Wood, 1986a,b)]. In isolated ventricle strips from trout,  $\text{pH}_i$  was 7.3 at  $\text{pH}_e = 7.6$  (Gesser & Jorgensen, 1982; Nielsen & Gesser, 1984).

There was no effect of adrenaline on  $\text{pH}_i$  of the trout heart during exposure to extracellular acidosis under the present experimental conditions. The fall in  $\text{pH}_i$  was greater than that predicted from the *in vitro* non-bicarbonate buffer capacity. This suggested that a metabolic acidosis accompanied the respiratory acidosis (Fig. 4). The presence of adrenaline did not appreciably change the myocardial intracellular acid-base status, although cardiac performance was restored. Gesser & Jorgensen (1982) found that reducing  $\text{pH}_e$  from 7.6 to 6.9 (3%  $\text{CO}_2$  to 13%  $\text{CO}_2$ ) reduced the  $\text{pH}_i$  of trout ventricular strips from 7.34 to 6.99, and reversed the  $\Delta\text{pH}_{e-i}$  gradient. The  $\Delta\text{pH}_{e-i}$  gradient was not reversed in the present experiments.

In the presence and absence of adrenaline, an increase in  $\text{P}_{\text{CO}_2}$  led to a shrinkage of the intracellular compartment and a reciprocal expansion of the extracellular compartment. A similar shift of fluid from the intracellular to the extracellular compartment in response to anoxia has been observed in isolated trout ventricle strips (Nielsen & Gesser, 1984). These responses are the opposite of those shown by fish erythrocytes, which swell in response to an increased  $\text{P}_{\text{CO}_2}$  and the swelling is exacerbated in the presence of adrenaline (Nikinmaa, 1982). The mechanism(s) responsible for the myocardial cell shrinkage is (are) unclear, as are the reasons for the difference with fish erythrocytes.

Since the present work provides no support for catecholamine-mediated myocardial  $\text{pH}_i$  regulation in trout, the absence of major myocardial  $\text{pH}_i$  disturbance in intact fish following exhaustive exercise (Milligan & Farrell, 1986; Milligan & Wood, 1986b) remains unexplained. Because there is evidence of active  $\text{HCO}_3^-$  accumulation in mammalian myocardium which is stimulated by circulating catecholamines (Clancy, Gonzalez & Fenton, 1976; Gonzalez & Clancy, 1984), the teleost myocardium may, therefore, differ in this regard. An alternative mechanism which might regulate myocardial  $\text{pH}_i$  in teleosts may involve uptake of lactate ions from extracellular fluid and subsequent utilization of the lactate as a fuel with the associated

utilization of intracellular hydrogen ions. In trout after exhaustive exercise, the myocardium experiences a significant alkalosis which is accompanied by an accumulation of lactate in the myocardium (Milligan & Wood, 1986b). A similar myocardial lactate uptake and oxidation may account for the intracellular accumulation of base observed in the sea raven heart after exercise (Milligan & Farrell, 1986). Both trout and sea raven hearts are capable of using lactate as an adequate substrate to fuel cardiac activity (Lanctin, McMorren & Driedzic, 1980; Driedzic & Hart, 1984). Thus, the present experiments may have prevented active pH<sub>i</sub> regulation by not including lactate in the perfusate. Whether this hypothesis is true must await further experiments. The lactate measurements in the present study provided no insight into the problem because tissue lactate levels were not measured and lactate was only detected in the perfusate of one preparation. Driedzic, Scott & Farrell (1983) observed that lactate release into the perfusate from sea raven hearts receiving oxygenated perfusate was low and not even detectable over a 50 min perfusion period in half the preparations. Using the trout preparation we could consistently detect lactate levels in the perfusate when ion exchange antagonists were added to the acidotic perfusate (A. P. Farrell & C. L. Milligan, unpublished results). Thus, it would seem that the non-detectable levels of lactate under the present experimental conditions probably reflected low levels of lactate production and/or release.

Myocardial protection is also afforded by an increase in the intracellular calcium pool, which offsets the negative effects of intracellular acidosis (see Gesser & Poupa, 1983). Passive sarcolemmal calcium influxes, resulting from an increase in extracellular calcium, can improve contractility under control conditions, as well as during acidosis (Farrell *et al.* 1986). However, these effects are never as large as those produced by adrenaline with constant extracellular calcium (Farrell, Hart, Wood & Driedzic, 1984; Farrell *et al.* 1986). Catecholamine stimulation of the acidotic myocardium without pH<sub>i</sub> regulation may result from adrenergic modulation of sarcolemmal calcium channels in the trout heart. In mammalian and amphibian hearts,  $\beta$ -adrenoceptors can mediate an increase in the inward calcium current by increasing the probability of a given calcium channel entering an open state (Reuter, 1983). If this occurs in the trout heart during respiratory acidosis, then the rise in intracellular calcium could protect contractility.

It must be stressed, however, that there are important species differences in the way teleosts recover myocardial contractility during acidosis. Flounder ventricular strips, for example, recover spontaneously through a release of calcium from intracellular stores such as mitochondria, without any apparent involvement of transsarcolemmal movement of calcium from the extracellular space (Gesser & Poupa, 1981, 1983). This intracellular calcium release does not occur in trout ventricular strips, and although it occurs in the Atlantic cod, it does not restore contractility (Gesser & Jorgensen, 1982). Extracellular calcium is less effective at restoring contractility in the Atlantic cod and in the sea raven (Farrell *et al.* 1984), compared to the situation in trout. Therefore, adrenergic modulation of sarcolemmal calcium channels may be important in trout, but not necessarily in all teleosts.

In summary, catecholamines restored contractility in the trout heart, in the absence of  $\text{pH}_i$  regulation under the present perfusion conditions. The mechanism for this recovery may be related to adrenergic modulation of sarcolemmal calcium channels. Further studies on these exchanges are clearly warranted, as are studies on myocardial lactate uptake and metabolism, which might explain the enigma of constant myocardial  $\text{pH}_i$  during the post-exercise respiratory acidosis observed in trout and sea raven.

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