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The effect of β-adrenergic blockade on the recovery process after strenuous exercise in the rainbow trout, *Salmo gairdneri* Richardson

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Trout fitted with arterial catheters were subjected to 6 min of strenuous exercise, injected with either saline (controls) or the β-adrenergic antagonist propranolol, and monitored over the following 8-h recovery period. Control responses were very similar to those previously reported, except for much higher resting and post-exercise plasma catecholamine levels, and less marked RBC pHi regulation, perhaps due to season (February–May). Trout subjected to prior β-blockade would not exercise. Trout β-blocked immediately after exercise showed a much higher incidence of mortality during the recovery period, but accompanying symptoms were similar to those previously documented in control trout dying after exercise. Specific effects of post-exercise β-blockade seen in both survivors and mortalities were a sustained elevation of arterial P_{CO_2} and an inhibition of blood glucose elevation. There were negligible effects on RBC pHi and volume regulation, blood metabolic acid and lactate dynamics, or plasma ion changes. The results provide little support for the hypothesis that β-adrenergic actions of plasma catecholamines are intimately involved in post-exercise recovery, but must be considered in the context of the 'winter' trout, where β-responses may be diminished.

I. INTRODUCTION

In confirmation of the early studies of Nakano & Tomlinson (1967), there is now abundant evidence that exhaustive exercise causes plasma catecholamine mobilization in teleost fish (Ristori & Laurent, 1985; Primmett *et al.*, 1986; Butler *et al.*, 1986; Milligan & Wood, 1987). Wood & Perry (1985) have argued that this surge of catecholamines into the bloodstream is a key factor integrating the metabolic, acid-base, circulatory, and respiratory responses to severe exercise. To a large extent, the evidence is circumstantial. The best documented phenomenon is the β-adrenergic regulation of red cell pHi and increase in red cell volume which help sustain blood oxygen transport in the face of extracellular acidosis in severely stressed fish (Nikinmaa, 1983, 1986; Nikinmaa *et al.*, 1984; Primmett *et al.*, 1986; Milligan & Wood, 1987). However, even here the story is complicated by seasonal variation (Nikinmaa & Jensen, 1986) and species differences (Milligan & Wood, 1987).

One study which pointed towards another post-exercise role for catecholamines was the demonstration by Wardle (1978) that β-adrenoreceptor blockade increased blood lactate levels in exercised plaice, suggesting that catecholamines were involved in lactate retention in muscle. However, in a similar flatfish, the starry

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flounder, Wood & Milligan (1987) were unable to confirm Wardle's observations or to demonstrate any influence of adrenergic blockade on post-exercise lactate or acid-base dynamics, apart from a small effect on red cell pHi regulation. However, flatfish may not be the best species in which to look for post-exercise adrenergic effects, for they spend much of their time buried in the substrate, are not particularly well-adapted for swimming, and show only a small catecholamine mobilization above their high resting plasma levels (Milligan & Wood, 1987). The rainbow trout may be a much better candidate, for it is well adapted for swimming with high aerobic and anaerobic scope, is known to mobilize high plasma levels of adrenalin and noradrenalin, and shows pronounced β-responses on its red cells.

The post-exercise responses of rainbow trout have been well characterized (Turner *et al.*, 1983; Holeton *et al.*, 1983; Wood *et al.*, 1983; Primmett *et al.*, 1986; Nikinmaa & Jensen, 1986; Milligan & Wood, 1986*a*,*b*, 1987). Recently, Perry & Vermette (1987) and Vermette & Perry (1987*a*,*b*, in prep.) have documented in some detail the responses of resting trout to prolonged infusion of adrenalin into the bloodstream at levels comparable to those seen after strenuous activity. In many respects, the putative β-adrenergic responses to adrenalin infusion resembled those occurring during recovery from exhaustive exercise. Therefore, the goal of the present investigation was to characterize the β-adrenergic role(s) of catecholamines in the recovery process after actual exercise, through the use of the β-blocking agent propranolol.

II. MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND PROCEDURES

Rainbow trout, *Salmo gairdneri* Richardson, (200–400 g) were obtained from Spring Valley Trout Farm, Petersburg, Ontario and held at seasonal temperatures (5–10° C) in flowing, dechlorinated Hamilton tap water. Prior to experimentation, the fish were acclimated to 15 ± 2° C for at least 7 days, the change being made at a maximum rate of 1° C per day. *Ad libitum* feeding with commercial trout pellets was suspended during this period. Experiments were performed over the period February–May 1985. Acclimation and experimental water had the following composition (in mEq l^{-1}): Na^+ 0·6; Cl^- 0·8; Ca^{2+} 1·8; Mg^{2+} 0·3; K^+ 0·05; titration alkalinity (to pH = 4·0) 2·0; total hardness 140 mg l^{-1} as $CaCO_3$; pH 8·0.

To allow repetitive blood sampling with least disturbance to the animal, dorsal aortic cannulae were implanted while the fish were under 1 : 20,000 MS-222 (Sigma) anaesthesia. Following surgery, the animals were allowed to recover for a minimum of 48 h in darkened acrylic chambers flushed with air-equilibrated acclimation water. After withdrawal of the resting blood sample, the fish were transferred to a half-filled 500-l tank (diameter 92 cm; water level 40 cm). Severe exercise was then imposed by manually chasing the fish, vigorously, for 6 min. By the end of this treatment, all trout seemed incapable of further burst performance, but some continued to swim slowly around the tank. This condition probably represented an exhaustion of the largely glycolytic white muscle, with the continued slow activity supported by red muscle (Johnston, 1977; Milligan & Wood, 1986*b*). A time 0 h blood sample was then taken, saline or blocker infused, and then the fish was returned to its individual chamber and monitored for the following 8 h, with blood samples taken at 0·5, 1, 2, 4, and 8 h (if death did not ensue earlier).

Blood samples (500–900 μl) were withdrawn anaerobically via the dorsal aortic catheter into ice-cold, gas-tight Hamilton syringes. Saline replacement was employed. Blood samples were analysed for arterial extracellular pH (pHe), red blood cell intracellular pH (pHi), total CO_2 content in both whole blood and true plasma, haematocrit, haemoglobin, lactate, glucose and plasma levels of sodium, potassium, calcium, chloride, total protein

and, in a few cases, adrenalin and noradrenalin. Not all parameters were measured in all fish.

Immediately after the 0-h blood sample, experimental fish were infused with the β-adrenergic antagonist propranolol HCl (10 μmol kg^{-1}; Sigma) in a volume of 1 ml kg^{-1} trout saline. Control fish were infused with saline alone. Because all trout were treated identically until the end of exercise (the 0-h sample served as a check), these experiments tested the effects of β-blockade on the recovery process. In preliminary experiments, we found it was uninformative to infuse the propranolol before exercise, for β-blocked trout would not swim but, rather, allowed themselves to be pushed around the tank. As documented by Wood *et al.* (1983), exhaustive exercise resulted in the death of some of the fish during the recovery period. In the present experiments, only 1 of the 9 control fish died, whereas 10 of the 18 β-blocked fish died, a significant difference. Therefore, three separate groups were considered: control survivors, β-blocked survivors, and β-blocked mortalities.

ANALYTICAL METHODS

Arterial pHe was determined by injecting approximately 40 μl whole blood into a Radiometer micro-electrode (E5021) thermostatted to the experimental temperature and connected to a Radiometer PHM 71 or 72 acid-base analyser. For measurement of RBC pHi, an RBC pellet was obtained by centrifuging 400 μl of whole blood for 2 min at 9000 × **g**. The plasma was removed, the pellet sealed, and the erythrocytes were lysed by repeated freezing and thawing in liquid nitrogen and warm water (Zeidler & Kim, 1977). Thereafter, the procedure was the same as for pHe. Total CO_2 was measured on 50-μl samples of true plasma and whole blood, using the method of Cameron (1971) and a Radiometer PCO_2 electrode. True plasma was obtained by centrifuging 80 μl whole blood in sealed, heparinized microhaematocrit tubes (Radiometer) at 5000 × **g** for 5 min. The haematocrit was read directly from the tube which was then broken to allow anaerobic aspiration of the plasma into a Hamilton syringe for transfer to the Cameron chamber. Total plasma protein was determined with an American Optical Goldberg refractometer.

Haemoglobin concentration was measured colourimetrically by the cyanmethaemoglobin method using 20-μl samples and Sigma reagents. Lactate was measured on 100-μl of whole blood immediately deproteinized in 200 μl of ice-cold 8% perchloric acid (w/v) and then centrifuged at 9000 × **g** for 3 min. The supernatant was analysed enzymatically for L-lactate, employing Sigma reagents. Whole blood glucose was determined on 100 μl samples, deproteinized in 900 μl ice-cold 3% trichloroacetic acid (w/v), and centrifuged as for lactate; the o-toluidine method of Hyvarinon & Nikkita (1962) and Sigma reagents were employed.

The plasma which was removed in the RBC pHi measurement was stored at −20° C and later used for determination of ion levels. Immediately after thawing, plasma was appropriately diluted for measurements of Na^+, K^+ and Ca^{2+} by atomic absorption spectrophotometry (Varian AA-1275). Cl^- was determined by coulometric titration (Radiometer CMT 10).

For measurements of plasma adrenalin and noradrenalin concentrations, plasma was separated by centrifugation as for pHi, a preservative mix was added (5 μl per 100 μl plasma of 90 mg ml^{-1} EGTA plus 60 mg ml^{-1} glutathione in 4% KOH), and the samples immediately frozen at −80° C for not more than 60 days prior to assay. Analyses were performed using a commercially available ^{3}H-labelled radio-enzymatic assay (Cat-A-Kit, UpJohn Diagnostics).

CALCULATIONS

Calculations of PCO_2 and $[HCO_3^-]$ in whole blood and plasma were performed as described by Wood *et al.* (1983) using the Henderson–Hasselbalch equation, employing αCO_2 and pK' values reported for *Salmo gairdneri* by Boutilier *et al.* (1984). In turn, the HCO_3^- concentrations in whole blood were used in the calculation of blood metabolic acid load (ΔH^+_m), again as described by Wood *et al.* (1983). Over any interval (time 1–2), ΔH^+_m was estimated as

$$\Delta H^+_m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta\,(pHa_1 - pHa_2), \quad (1)$$

where β, the non-bicarbonate buffer capacity of whole blood, was calculated from the blood haemoglobin ([Hb]) at time 2 using the regression relationship determined *in vitro* for *S. gairdneri* blood by Wood *et al.* (1982):

$$\beta = -1{\cdot}073\,[\mathrm{Hb}] - 2{\cdot}48. \tag{2}$$

The total blood metabolic acid load at any time was calculated by summing the ΔH^+_m s, signs considered, from the rest sample onwards. The calculation was performed for whole blood rather than plasma because lactate was also determined in whole blood. The comparable blood lactate load (ΔLa^-) was calculated as the difference between the resting lactate level and that present at any experimental time. Mean cell haemoglobin concentration (MCHC) was estimated from the Hb and haematocrit (Ht) measurements:

$$\mathrm{MCHC} = \frac{[\mathrm{Hb}]}{\mathrm{Ht}}. \tag{3}$$

ANALYSIS OF DATA

Data have been expressed as means ± 1 S.E.M. for each of the three groups: controls ($n=8$), β-blocked survivors ($n=8$), and β-blocked mortalities ($n=10$). Significant differences ($P \leq 0{\cdot}05$) were tested by the paired or unpaired Student's two-tailed *t*-test, as appropriate. As the treatments of the three groups were identical until after the 0-h sample, their data have been combined at rest (R) and at 0 h for the sake of clarity in illustrations. To ensure that this did not introduce bias into the analyses, the three groups were tested for significant between-treatment differences at these times for all parameters. Out of 108 comparisons (18 parameters × 3 groups × 2 times), there were only 5 significant differences, slightly less than that predicted by chance alone ($P \leq 0{\cdot}05$).

III. RESULTS

POST-EXERCISE RESPONSES IN CONTROL FISH

In control animals, most of the responses to 6 min of exhaustive exercise were very similar to those previously documented for identically exercised trout in our laboratory (Turner *et al.*, 1983; Wood *et al.*, 1983; Milligan & Wood, 1986*a,b*, 1987). Briefly, the major feature was a pronounced extracellular acidosis, pHe falling by about 0·4 units immediately post-exercise (Fig. 1). The acidosis was of dual origin, with both metabolic and respiratory contributions. The metabolic component was reflected in decreases in blood and plasma total CO_2 and HCO_3^- concentrations (not shown) and has been quantified in Fig. 2 as ΔH^+_m. While ΔH^+_m and ΔLa^- were initially similar at 0 h, ΔLa^- continued to increase to a level greatly in excess of ΔH^+_m (Fig. 2). The respiratory component comprised a sharp elevation in P_{CO_2} immediately post-exercise, returning to resting levels by 1 h (Fig. 3). Furthermore, significant increases were observed for blood glucose (Fig. 4), all plasma ions (Fig. 5), plasma protein (Table I), haematocrit and haemoglobin (not shown), and there was a pronounced swelling of the RBC's reflected in a decreased MCHC (Table I). Most disturbances were restored within 8 h; only lactate and glucose concentrations in the blood were still elevated after this period.

Two prominent differences from our previous investigations were seen in the RBC pHi and plasma catecholamine data. RBC pHi fell by about 0·1 unit after exercise, rather more than in earlier studies, and remained significantly depressed until 2 h (Fig. 1). Catecholamine levels were measured only at rest and immediately post-exercise. Adrenalin increased from 30 ± 5 to 358 ± 102 nmol l^{-1} ($n=7$), while noradrenalin increased from 29·7 ± 3 to 445 ± 160 nmol l^{-1} ($n=7$). Both rest and

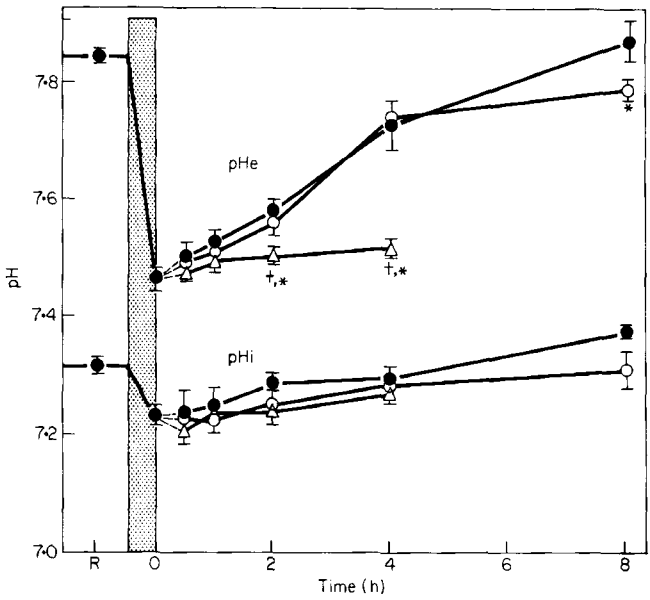

FIG. 1. The effect of β-blockade on changes in arterial extracellular pH (pHe) and RBC intracellular pH (pHi) after 6 min of strenuous exercise in rainbow trout. Means ± 1 S.E.M. Propranolol (10 μmol kg^{-1}) or saline (controls) were injected immediately after the 0 h sample, all groups being treated identically until then ($n = 26$). Thereafter: ●, controls ($n = 8$); ○, β-blocked survivors ($n = 8$); △, β-blocked mortalities ($n = 10$, declining to 5 by 4 h). R, Pre-exercise rest value; stippled bar, exercise period; *, mean significantly different ($P \leq 0{\cdot}05$) from control value at same time; †, mean significantly different from β-blocked survivors value at same time.

post-exercise levels were approximately one order of magnitude greater than recorded previously.

THE EFFECT OF β-ADRENERGIC BLOCKADE ON POST-EXERCISE RECOVERY

A pronounced effect of β-blockade was a greatly increased post-exercise mortality, 10 out of 18 as compared with 1 out of 9 in the control group. The first death was seen between 1 and 2 h, while most occurred between 4 and 8 h. In other studies, we have found that this same dose of propranolol (10 μmol kg^{-1}) causes no mortality in resting trout (C. M. Wood, unpubl. results). The data from β-blocked fish have therefore been separated as survivors and mortalities. For the sake of completeness, it should be noted that the one mortality in the control group, which was excluded from the analysis, showed similar trends to the other fish in this group prior to death at 2 h.

Overall, β-blockade had minimal effect on the metabolic component of the post-exercise acidosis, but a major effect on the respiratory component. Thus P_{CO_2} remained significantly elevated throughout the post-exercise period in both β-blocked groups, rather than returning to resting values by 1 h as in the controls (Fig. 3). This P_{CO_2} elevation was slightly higher in the β-blocked mortalities. The metabolic component, ΔH^+_m was slightly smaller in the β-blocked survivors than in the controls, a difference which was significant at 4 h (Fig. 2). In contrast, the β-blocked mortalities showed a slightly greater ΔH^+_m at this time. Rather similar

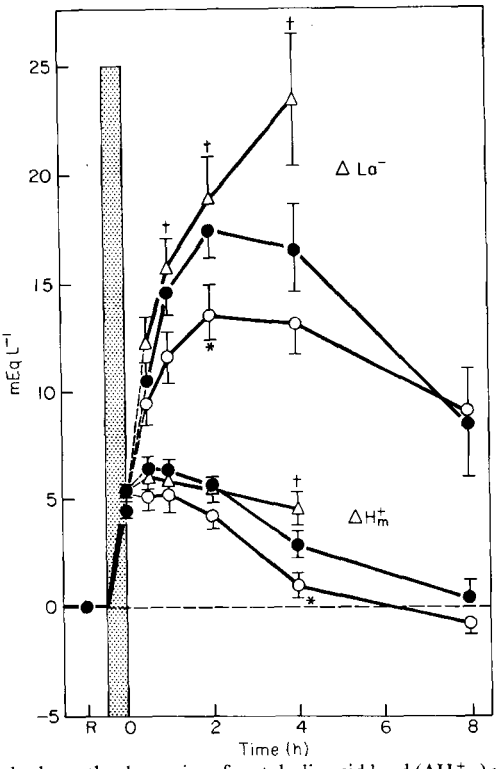

FIG. 2. The effect of β-blockade on the dynamics of metabolic acid load (ΔH^+_m) and lactate load (ΔLa^-) in the bloodstream of rainbow trout after 6 min of strenuous exercise. Details as in Fig. 1.

effects were seen with ΔLa^-, which was lower than control levels by 2 h in the β-blocked survivors, but higher in the β-blocked mortalities (Fig. 2). When the data of the two β-blocked groups were combined, there were no significant differences from control responses in either ΔH^+_m or ΔLa^-.

Changes in pHe in β-blocked survivors were almost identical to those in controls, except at 8 h (Fig. 1). This similarity was due to the combined influence of the larger respiratory acidosis (P_{CO_2} elevation, Fig. 3) and smaller metabolic acidosis (ΔH^+_m elevation, Fig. 2) in the β-blocked survivors. Their lower pHe at 8 h was entirely due to the P_{CO_2} elevation. The β-blocked mortalities showed almost no restoration of pHe, with significantly lower values than the other two groups at 2 and 4 h. This was largely due to the higher ΔH^+m in this group (Fig. 2), with a minor contribution from the higher P_{CO_2} (Fig. 3).

β-Adrenergic blockade had no effect on post-exercise changes in RBC pHi (Fig. 1). While pHi tended to be slightly lower in both β-blocked groups, there were no significant differences from control values, even when the two β-blocked groups were combined. A similar lack of influence of β-blockade was seen with post-exercise changes in haematocrit and haemoglobin (not shown), red cell swelling as indicated by MCHC (Table I), and plasma protein (Table I).

β-Adrenergic blockade had a pronounced influence on post-exercise blood glucose regulation (Fig. 4). Instead of continuing to rise during the recovery period as in controls, blood glucose levels stabilized (survivors) or fell (mortalities) in

TABLE I. The effect of β-blockade (10 μmol kg^{-1} propranolol injected immediately after the 0 h sample) on changes in mean cell haemoglobin concentration (MCHC) and plasma protein levels in the blood of rainbow trout after 6 min of strenuous exercise. Means ± 1 S.E.M.

Time	Rest	0 h	0·5 h	1 h	2 h	4 h	8 h
MCHC (g Hb 100 ml^{-1} RBCs.)							
Control	30·9	25·5	25·8	27·3	27·1	28·2	31·3
($n=8$)	±1·2	±0·5	±0·4	±0·7	±1·3	±0·8	±1·2
β-blocked survivors	30·1	26·4	27·0	27·0	27·9	30·1	30·0
($n=8$)	±0·6	±1·0	±0·7	±0·9	±1·3	±1·3	±1·3
β-blocked mortalities	29·4	24·9	27·4	28·3	27·5	32·4	—
($n=5$–10)	±0·8	±1·3	±1·3	±1·3	±1·3	±1·5	
Plasma protein (g 100 ml^{-1})							
Control	3·17	3·62	3·45	3·15	2·93	2·66	2·63
($n=8$)	±0·24	±0·27	±0·26	±0·23	±0·22	±0·19	±0·21
β-blocked survivors	2·86	3·13	3·14	3·02	2·81	2·68	2·84
($n=8$)	±0·19	±0·30	±0·33	±0·27	±0·21	±0·21	±0·24
β-blocked mortalities	3·15	3·41	3·39	3·23	3·14	3·03	—
($n=5$–10)	±0·11	±0·13	±0·13	±0·12	±0·08	±0·13	

Decreases in MCHC and increases in plasma protein were significant ($P \leq 0.05$) relative to rest values in all groups up to 0·5–2 h. There were no significant differences between groups at any time.

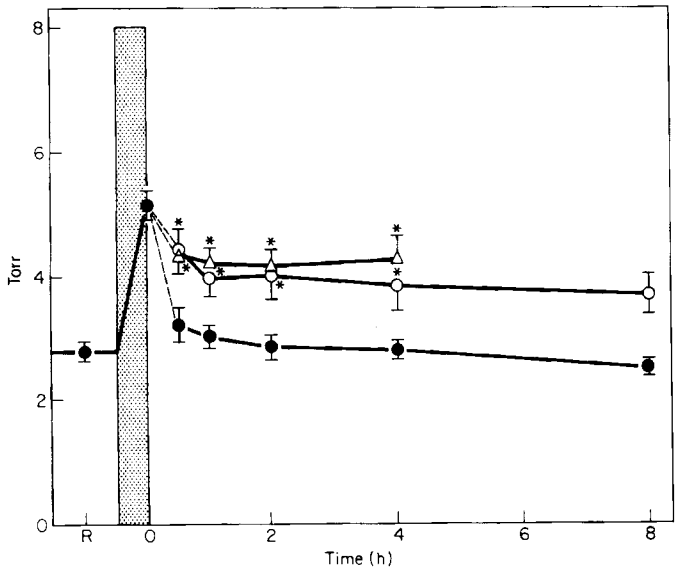

FIG. 3. The effect of β-blockade on changes in the partial pressure of carbon dioxide (P_{CO_2}) in the arterial blood of rainbow trout after 6 min of strenuous exercise. Details as in Fig. 1.

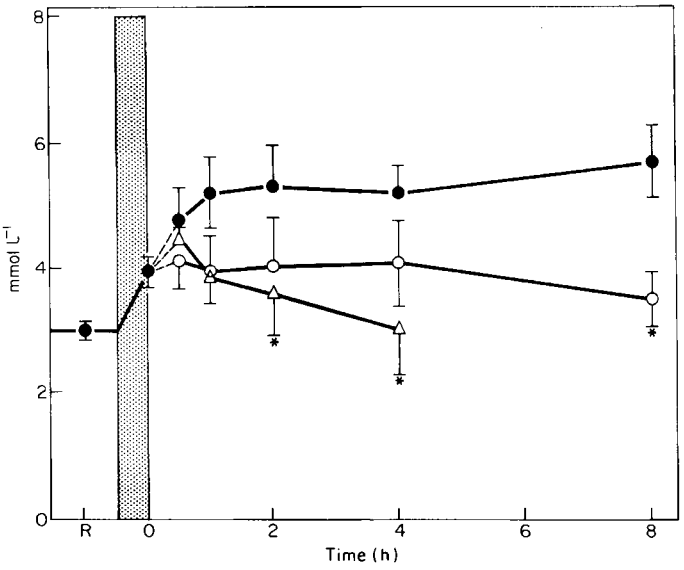

FIG. 4. The effect of β-blockade on changes in blood glucose levels in rainbow trout after 6 min of strenuous exercise. Details as in Fig. 1.

the β-blocked fish. These differences were significant by 2 h in the β-blocked mortalities, by 8 h in the β-blocked survivors, and by 2 h when the data sets were combined.

Post-exercise changes in plasma ions were largely unaffected by propranolol (Fig. 5). The only significant difference was the greater increase in plasma $[K^+]$ at 2 and 4 h in the β-blocked mortalities [Fig. 5(c)]. However, since this effect, together with the pHe depression (Fig. 1) and the greater elevations in ΔH^+_m and ΔLa^- (Fig. 2) were also symptomatic of control fish dying after exercise (cf. Wood *et al.*, 1983), and did not occur in β-blocked survivors, it appears most unlikely that they were specific β-adrenergic effects.

IV. DISCUSSION

METHODOLOGY

Propranolol is a competitive, non-selective β-antagonist, equally effective on both β1- and β2-adrenoreceptors. The relatively high dose of propranolol (10 μmol kg^{-1}) was purposely chosen to ensure complete blockade, especially in view of the high catecholamine levels measured in the bloodstream at the time the blocking agent was injected. At one tenth of the dose levels used here, propranolol produced effective β-blockade against the cardiovascular effects of infused catecholamines in trout (Wood & Shelton, 1980). A similar dose to that used here produced complete β-blockade against the respiratory effects of infused catecholamines in trout (M. G. Vermette & S. F. Perry, in prep.). The β-blockade was clearly effective in the present study, as demonstrated by the pronounced differences in post-exercise P_{CO_2} and blood glucose regulation in the presence of propranolol.

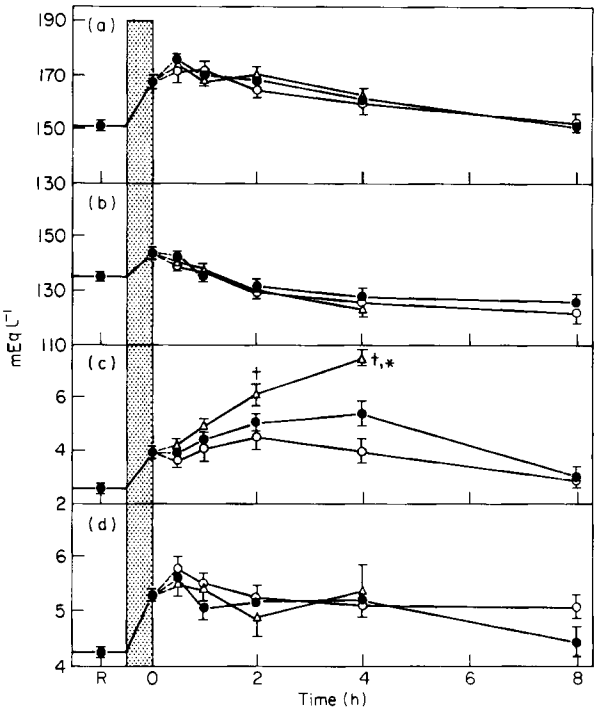

FIG. 5. The effect of β-blockade on changes in the plasma concentrations of (a) sodium, (b) chloride, (c) potassium and (d) calcium in rainbow trout after 6 min of strenuous exercise. Details as in Fig. 1, except that $n=14$ until 0 h. Thereafter: controls, $n=3$; β-blocked survivors, $n=5$; β-blocked mortalities, $n=6$, declining to 4 by 4 h.

This same dose of propranolol had no effect on blood acid-base, respiratory, ionic, or haematological parameters in resting trout (C. M. Wood, unpubl. results; Fievet *et al.*, 1987; M. G. Vermette & S. F. Perry, in prep.), though it did cause a cardiac depression (Wood & Shelton, 1980).

It is unfortunate that the β-blockade could not be instituted prior to exercise. However, the results from such an experiment would be meaningless because we found that β-blocked fish would not swim. This was most probably due to the inhibitory effects of propranolol in depressing cardiac function (Wood & Shelton, 1980) and in antagonizing β-adrenergically-mediated increases in cardiac output and branchial diffusive conductance (Wood & Perry, 1985). These adaptations are critical for increased oxygen delivery to the tissues during exercise in the highly aerobic trout. In contrast, similar β-blockade had negligible influence on exercise performance in the relatively anaerobic starry flounder (Wood & Milligan, 1987).

The resting and post-exercise catecholamine levels in the present study may be questioned because they were so much higher than those reported from similarly cannulated and exercised trout from several laboratories (Ristori & Laurent, 1985; Primmett *et al.*, 1986; Butler *et al.*, 1986), including our own (Milligan & Wood, 1987). Nevertheless, we are confident of the measurements, as they were obtained by identical methodology to other much lower values in trout (Milligan & Wood, 1987). We attribute the difference to seasonality (see below).

POST-EXERCISE RESPONSES IN CONTROL FISH

Most post-exercise responses in the present investigation were very similar to those previously documented for the rainbow trout, and of which the mechanisms involved have been discussed (Turner *et al.*, 1983; Holeton *et al.*, 1983; Wood *et al.*, 1983; Primmett *et al.*, 1986; Milligan & Wood, 1986*a,b*, 1987). However, two differences are noteworthy.

Firstly, RBC pHi fell significantly (by about 0·1 unit) in the face of a 0·4-unit depression in pHe after exhaustive exercise, whereas in most other studies pHi actually increased (Primmett *et al.*, 1986; Milligan & Wood, 1986*b*), remained constant (Milligan & Wood, 1986*a*), or fell only slightly (Milligan & Wood, 1987). However, this does not mean that RBC pHi was completely unregulated in the present study. *In vitro*, the slope (ΔpHi/ΔpHe) for trout blood is variously reported as 0·48–0·59 (Heming *et al.*, 1986; M. G. Vermette & S. F. Perry, in prep.), 0·73 (Milligan & Wood, 1985), 0·90 (Perry & Vermette, 1987) and 1·09 (Nikinmaa, 1983). The maximum *in vivo* slope (rest–0 h) in the present study was 0·23. Interestingly, Nikinmaa & Jensen (1986) found almost no regulation of RBC pHi (*in vivo* slope $\sim$0·80) when trout were exercised in 'winter'. Based on this and other evidence, they hypothesized that 'winter' fish lacked β-adrenergic responses on their RBCs. While Nikinmaa & Jensen (1986) did not specify exact dates, the thermal history of their fish appears similar to that of the current trout which were tested in the period February–May, several weeks after raising water temperature from ambient (5–10° C) to 15° C. Based on the pHi *v.* pHe relationship as well as the catecholamine data and results of β-blockade, we suggest our fish exhibited at least some aspects of this 'winter' pattern.

A second important difference from previous studies was the much higher plasma levels (approximately 10-fold) of adrenalin and noradrenalin both at rest and immediately after exhaustive exercise. While we are aware of no previous measurements taken exclusively from 'winter' trout, we suggest that this may be a seasonal difference. Ristori & Laurent (1985) alluded to a seasonal cycle in plasma catecholamine levels in *S. gairdneri*; however, no direct data were reported. If plasma catecholamine levels are greatly elevated in winter, then this could cause down-regulation of receptor numbers, desensitization, or tachyphylaxis, resulting in the observed loss of the β-adrenergic red cell response. Other β-adrenergic responses of fish also appear to diminish or disappear in winter (Peyraud-Waitzenegger, 1979; Peyraud-Waitzenegger *et al.*, 1980; Part *et al.*, 1982).

THE EFFECT OF β-ADRENERGIC BLOCKADE ON POST-EXERCISE RECOVERY

One of the most obvious effects of β-blockade was an increase in post-exercise mortality. The syndrome of post-exercise mortality has been described previously in control trout, though its proximate cause remains elusive (Wood *et al.*, 1983). The actual symptoms of post-exercise death (greater ΔH^+_m, ΔLa^-, failure to restore pHe, greater increase in plasma $[K^+]$) were both qualitatively and quantitatively similar in dying β-blocked fish and dying control fish (cf. Wood *et al.*, 1983). Thus these symptoms should not be viewed as direct consequences of β-blockade but, rather, as correlates of post-exercise mortality. Propranolol appears to intensify the 'selection' process which results in post-exercise death in some

fish. Wood *et al.* (1983) suggested that this death was associated with a failure of pHi correction in white muscle. Milligan & Wood (1986*b*) have shown that this correction occurs largely via aerobic metabolism, i.e. the removal of H^+_m by the oxidation of lactate and the resynthesis of ATP and glycogen. Thus, the actions of propranolol on cardiac output and branchial diffusive conductance, which could impair oxygen delivery to the tissues, probably contributed to the greater incidence of post-exercise mortality in β-blocked fish.

In light of the above, only those differences from the control pattern which occurred in β-blocked survivors, or in both β-blocked survivors and β-blocked mortalities, can be considered specific actions of the β-blockade. These are the reduction of post-exercise blood glucose elevation and the sustained post-exercise increase in P_{CO_2}.

The former response was expected, for high blood glucose levels after exercise have often been attributed to glycogenolytic effects of plasma catecholamines on the liver (eg. Nakano & Tomlinson, 1967; Mazeaud *et al.*, 1977). In mammals, this response is mediated through both α- and β-adrenoreceptors on hepatocytes (Exton, 1979), with the relative importance of the two varying considerably between species. The same appears true in fish, for while the β-effect is important in the rainbow trout, the α-effect was dominant in the starry flounder (Wood & Milligan, 1987).

The higher P_{CO_2} levels in both β-blocked groups most likely resulted from inhibition of β-adrenergically-mediated increases in branchial diffusive conductance and/or ventilation (cf. Wood & Perry, 1985). The arterial P_{O_2} was not measured, but if these explanations are valid, one would predict a lower P_{O_2} in the β-blocked fish relative to controls. Interestingly, no effects on arterial P_{CO_2} were seen when exercised starry flounder were treated with the same dose of propranolol (Wood & Milligan, 1987), indicating another difference between the two species.

The most surprising aspect of the β-blocking experiments was the lack of effect of propranolol on post-exercise RBC pHi and red cell swelling (as evidenced by MCHC changes), which was in direct contrast to several previous studies (e.g., Nikinmaa, 1986; Primmett *et al.*, 1986). However, the RBC pHi regulation in the present study was less marked than in most previous investigations. Nikinmaa & Jensen (1986) could demonstrate virtually no RBC pHi regulation in exercised 'winter' trout, a phenomenon which they attributed to the absence of a β-adrenergic response on the red cells (though blocking experiments were not performed). Our results, from experiments in February–May, support this interpretation.

Post-exercise lactate (ΔLa^-) dynamics in the bloodstream of trout were also unaffected by β-blockade, in agreement with findings in the starry flounder (Wood & Milligan, 1987). Fievet *et al.* (1987) similarly observed no effect of propranolol on blood lactate changes in trout subjected to acute hypoxia. These results all conflict with the interpretation of Wardle (1978) that lactate release from muscle is under β-adrenergic control in plaice. However, as Wood & Milligan (1987) have pointed out, alternative, non-specific effects may explain Wardle's results.

β-Blockade also had no effect on the dynamics of ΔH^+_m or ionic changes in the bloodstream after exhaustive exercise. A net excretion of H^+_m across the gills to the environmental water via complex modulation of Na^+ and Cl^- exchanges is an important contributor to the regulation of ΔH^+_m in the first 4–8 h after exercise in trout (Holeton *et al.*, 1983; Wood & Perry, 1985; Milligan & Wood, 1986*a*).

Vermette & Perry (1987*a*) showed that adrenalin infusions stimulated net branchial H^+_m excretion in resting trout by a similar mechanism, while other studies suggested this was a β-adrenergic effect (Payan & Girard, 1978; Wood & Perry, 1985). We therefore anticipated that a higher and/or longer-lasting elevation of ΔH^+_m would be seen in the bloodstream of β-blocked trout, but this did not occur. These data again agree with the results of similar experiments in the starry flounder (Wood & Milligan, 1987). As a further test, in preliminary experiments, we have measured post-exercise H^+_m, Na^+, and Cl^- fluxes across the gills of intact trout (P. L. M. van Dijk & C. M. Wood, unpubl. results). Again β-blockade had no significant effect. Similarly α- or β-adrenergic blockade had no influence on H^+_m handling in trout during the compensation of hypercapnic acidosis (M. G. Vermette & S. F. Perry, in prep.), where adrenergic involvement was also expected.

In summary, apart from the documented effects on arterial P_{CO_2} and blood glucose regulation, the present results provide little support for the original hypothesis that the β-adrenergic effects of plasma catecholamines are intimately involved in the post-exercise recovery processes (Wood & Perry, 1985). Rather, we must suppose that other hormones, metabolites, or the blood acid-base status itself, play more important roles. However, one serious *caveat* is that these results may only be representative of 'winter' trout, in which β-adrenergic responses may be diminished. There is a clear need for more comprehensive seasonal studies.

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