ADRENERGIC ANALYSIS OF EXTRACELLULAR AND INTRACELLULAR LACTATE AND H^+ DYNAMICS AFTER STRENUOUS EXERCISE IN THE STARRY FLOUNDER PLATICHTHYS STELLATUS[1]

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Adrenergic blockade with a selective α antagonist (yohimbine) and β antagonist (propranolol) was employed to test whether catecholamine mobilization was involved in lactate and acidic equivalents (H^+) dynamics following strenuous exercise in chronically cannulated starry flounder. In control fish (not blocked) sampled repetitively for 8 h after exercise, arterial [lactate] remained characteristically low (<2 mmol/liter), and acid-base, hematological, and glucose changes were similar to those seen in other studies. Neither α nor β blockade immediately after exercise nor β blockade before exercise had significant influence on most responses, though hyperglycemia was inhibited by α blockade and prolonged by β blockade. Blood [lactate] was also marginally elevated, and red blood cell (RBC) swelling was reduced by β antagonism. A second series involving single-terminal sampling of cannulated flounder demonstrated that RBC intracellular pH (pHi), [NTP], and arterial P_{O_2} remained unchanged in the face of severe postexercise acidosis; hemoglobin (Hb)-bound O_2 per unit Hb ($[O_2]/[Hb]$) fell by 25% but had recovered by 1 h. White-muscle [lactate] increased to 10–20 times blood levels, and pHi in muscle, brain, and heart fell substantially, changes which were sustained at 1 h. Postexercise β blockade decreased RBC pHi, inhibited RBC swelling, prevented recovery of $[O_2]/[Hb]$, elevated blood [lactate], and increased muscle [lactate] at 1 h. The pHi of muscle, heart, and brain were not significantly affected. In contrast to other investigators, we conclude that catecholamines have little influence on postexercise lactate and H^+ dynamics in flounder but do help to sustain blood O_2 transport in the face of extracellular acidosis. Apparent stimulation of lactate release by β blockade can be explained by increased production of lactate by muscle, owing to hypoxemia.

INTRODUCTION

A remarkable feature of flatfish, order Pleuronectiformes, is the very low levels of lactate (generally <2 mmol/liter) in the bloodstream after exhaustive exercise, despite markedly elevated levels in the muscle (Dando 1969; Wood, McMahon, and McDonald, 1977; Duthie 1982; Turner, Wood, and Hōbe 1983; Milligan and Wood 1987*a*, 1987*b*). Wardle (1978), through the use of adrenergic agonists and antagonists, attributed this effect to plasma catecholamines acting on β-adrenergic receptors in muscle cells to restrict lactic acid efflux to the extracellular compartment. This would represent a novel role for catecholamines, for they are generally thought to augment rather than restrict lactate appearance from muscle in both fish (Larsson 1973) and mammals (Opie 1985; Karlsson 1985). While catecholamine mobilization has not been studied in flatfish, several other teleosts are known to raise plasma adrenaline and noradrenaline levels by 1–3 log units after strenuous exercise and/or physical disturbance (Nakano and Tomlinson 1967; Mazeaud, Mazeaud, and Donaldson 1977; Mazeaud and Mazeaud 1981; Butler 1986; Primmet et al. 1986). The cardiovascular effects of catecholamines have been well documented (Wood and Shelton 1980). Wood and Perry (1985) have argued that catecholamine mobilization is a key factor integrating the metabolic, acid-base, circulatory, and respiratory responses to severe exercise in fish.

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The interpretation of Wardle's (1978) results is open to question, since only some of his fish showed greater lactate appearance in the blood after β-adrenergic blockade, while the highest levels were actually seen after α-adrenergic blockade. In addition, Wardle measured only lactate and not blood acid-base status. Blood lactate measurements in fish do not necessarily represent lactic acid; indeed lactate and H^+ (metabolic acid or acidic equivalents) appearance in the bloodstream may follow very different patterns (see, e.g., fig. 2; Milligan and Wood 1987*a*). In mammals, catecholamines may actually stimulate H^+ efflux from acidotic tissues (Riegle and Clancy 1975; Clancy, Gonzalez, and Fenton 1976; Gonzalez and Clancy 1984). It is also possible that β blockade, through its inhibitory effects on cardiac output (Wood and Shelton 1980), branchial O_2–diffusing capacity (Perry, Daxboeck, and Dobson 1985), and red blood cell (RBC) O_2 transport (Nikinmaa 1982, 1983; Nikinmaa, Cech, and McEnroe 1984) could impede O_2 delivery to the muscle. Thus enhanced lactate release could reflect greater lactate production by hypoxemic muscle tissue rather than greater muscle-cell permeability.

The goal of the present investigation on the starry flounder, *Platichthys stellatus,* was to reevaluate Wardle's (1978) conclusions in light of these concerns. A cannulation approach was employed to avoid the complicating effects of sampling stress, and blood acid-base status and glucose levels were monitored to provide a more complete picture of the postexercise responses. Specific experiments focused on (1) the effects of separate α- and β-adrenergic blockade on blood lactate and H^+ dynamics and (2) the influence of β blockade on RBC O_2 transport, white-muscle lactate production, and intracellular pH (pHi) regulation in several key tissues after strenuous exercise.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Starry flounder (*Platichthys stellatus;* 150–1,400 g) were collected by otter trawl during November and December, 1984, from East Sound, Orcas Island and Birch Bay, Washington, and held at Friday Harbor Laboratories for at least 7 days as outlined in our accompanying paper (Milligan and Wood 1987*a*). These were a completely separate group of fish, collected in a different year (1984 vs. 1982) from those used in our accompanying papers. All fish were fitted with caudal artery catheters and allowed to recover for at least 72 h in tubs fitted with black plastic mesh and served with flowing seawater (9 ± 1 C), according to the procedures described in our accompanying papers (Milligan and Wood 1987*a*).

EXPERIMENTAL PROTOCOL

Series 1.—The effects of three different treatments—α blockade ($n = 6$), β blockade ($n = 6$), and β blockade prior to exercise ($n = 6$)—on the postexercise blood responses were evaluated in comparison to a control group ($n = 8$) similarly exercised but not pharmacologically treated. The standard 10-min chasing procedure described by Wood et al. (1977) was used to induce strenuous exercise.

In the control group, arterial blood samples (500 μl) were drawn prior to ("rest") and immediately after (0 h) exercise and at 0.5, 1, 2, 4, and 8 h after activity. The blood volume sampled was replaced with saline. The protocol was the same in the α- and β-blockade groups except that, immediately after the 0-h blood sample, either yohimbine HCl (Sigma; 10 μmol/kg) or propranolol HCl (Sigma; 10 μmol/kg) were infused via the caudal artery catheter in a volume of 1 ml/kg of flounder saline. Thus, the three groups were treated identically until the end of exercise, for which the 0-h sample served as a check; these experiments therefore tested the effects of α or β blockade on the recovery processes. In the prior or preexercise β-blockade treatment, the protocol was modified so that the propranolol was injected immediately after the rest sample. After 30 min, a second rest sample was taken to check for any effects on preexercise blood parameters. These β-blocked fish were then exercised and sampled at 0, 0.5, 1, 2, 4, and 8 h. This experiment therefore tested the effects of β blockade on the rest, exercise, and recovery processes. Blood samples were analyzed for arterial pH (pHa), total CO_2 (in both whole blood and plasma), [hemoglobin] ([Hb]), hematocrit

(Ht), whole-blood [lactate] and [glucose], and plasma [protein]. Through oversight, glucose measurements were not made in the control group, so the control data for this parameter (fig. 3*A*) were taken from the study described in an accompanying paper (Milligan and Wood 1987*a*).

Series 2.—RBC O_2 transport, nucleoside triphosphate (NTP) levels, acid-base status, white muscle to blood lactate gradients, and pHi in white muscle, heart, and brain were examined in fish sampled either at rest (n = 26), immediately after exercise (i.e., 0 h; n = 17), after 1 h recovery (n = 19), or after 1 h recovery in the presence of β blockade (n = 7). β blockade was induced immediately after exercise as described above.

Not all parameters were measured in all fish, and the samples for tissue pHi were of course terminal. For measurements of tissue pHi, fish were infused with 5 μCi/kg of ^{14}C-DMO and 20 μCi/kg of ^{3}H-mannitol ~12 h prior to terminal sampling, as we have detailed in an accompanying paper (Milligan and Wood 1987*b*). At sampling, an arterial blood sample (2,000 μl) was withdrawn from the catheter, and then needle biopsies (~100 mg each) of the epaxial muscle mass were immediately frozen in liquid N_2. The flounder was then killed by a cephalic blow, and portions of the brain (~100 mg) and heart ventricle (~200 mg) were excised, blotted, and quickly frozen. Larger tissue samples were taken for the determination of water contents. Total elapsed time from first grasping the animal to freezing of muscle was ~10 s; for the other tissues elapsed time was <60 s.

Blood samples were analyzed for arterial pH (pHa), total CO_2 (in both whole blood and plasma), [Hb], Ht, whole-blood lactate and NTP levels, arterial O_2 tension (Pa_{O_2}), arterial O_2 content (Ca_{O_2}), and plasma levels of protein and of ^{14}C and ^{3}H radioactivity. Heart, brain, and muscle were analyzed for ^{14}C and ^{3}H radioactivity and for total water content, with the additional measurement of [lactate] in muscle.

ANALYTICAL TECHNIQUES AND CALCULATIONS

All analyses and calculations were performed as in our accompanying papers (Milligan and Wood 1987*a*, 1987*b*), with the following exception: Ca_{O_2} was determined with a Lex-O_2-Con analyzer (Lexington Instruments) using a sample volume of 50 μl and the recalibration procedure described by Wood, McMahon, and McDonald (1979). To adjust for differences in Hb and physically dissolved O_2 concentrations between fish, Hb-bound O_2 content per unit Hb ($[O_2]/[Hb]$) was calculated as

$$[O_2]/[Hb]\ (\mu mol/g) = \frac{Ca_{O_2}\ (\mu mol/l) - (Pa_{O_2}\ (torr) \times \alpha O_2\ (\mu mol/liter/torr))}{[Hb]\ (g/liter)}, \quad (1)$$

where αO_2 represents the measured O_2 solubility coefficient in blood plasma of *P. stellatus* at 9 C (2.048 μmol/liter/torr; Wood et al. 1979). Blood NTP levels were determined by fixing 200 μl whole blood in 200 μl of 12% TCA and freezing the slurry in liquid N_2. Within 48 h the samples were thawed and immediately analyzed for NTP using a micromodification of a commercial diagnostic kit (Sigma Chemical Company 1983). Since NTP is almost entirely intracellular, NTP levels were expressed as both cellular concentrations (i.e., as [NTP]/Ht) and as contents per unit Hb (i.e., as [NTP]/[Hb]).

STATISTICAL ANALYSIS

Data have been expressed as means ± 1 SEM (n) where n = number of fish. The significance of differences was assessed using Student's two-tailed t-test with either a paired (measurements on the same animal [series 1]) or unpaired (independent measurements [series 1 and 2]) design as appropriate. A 5% significance level was employed, unless stated otherwise.

RESULTS

SERIES 1

The major changes in the blood of the control group following strenuous exercise were extracellular acidosis (fig. 1*A*), arterial CO_2 tension (Pa_{CO_2}) elevation (fig. 1*B*), and a rapid increase in metabolic acid load (ΔH^+m; fig. 2*A*) greatly in excess of a much smaller and slower lactate elevation (ΔLa^-;

fig. 2*B*). These were accompanied by increases in blood [glucose] (fig. 3*A*); hemoconcentration reflected in greater Ht, [Hb], and plasma [protein] (e.g., fig. 3*C*); and a small but significant swelling of the RBCs exhibited as a decreased mean cell Hb concentration (MCHC; fig. 3*B*). Apart from minor quantitative variations, these effects were virtually identical to those that we saw in a different batch of flounder (Milligan and Wood 1987*a*, 1987*b*). Thus the following description addresses only effects attributable to the blocking treatments.

Prior to blockade, there were no significant differences in any parameter between the control and experimental groups either at rest or immediately after exercise (0 h). The influences of α- or β-adrenergic blockade after exercise were surprisingly minor. There were no significant effects on the recovery of pHa (fig. 1*A*), Pa_{CO_2} (fig. 1*B*) or on the time course and magnitude of ΔH^+m

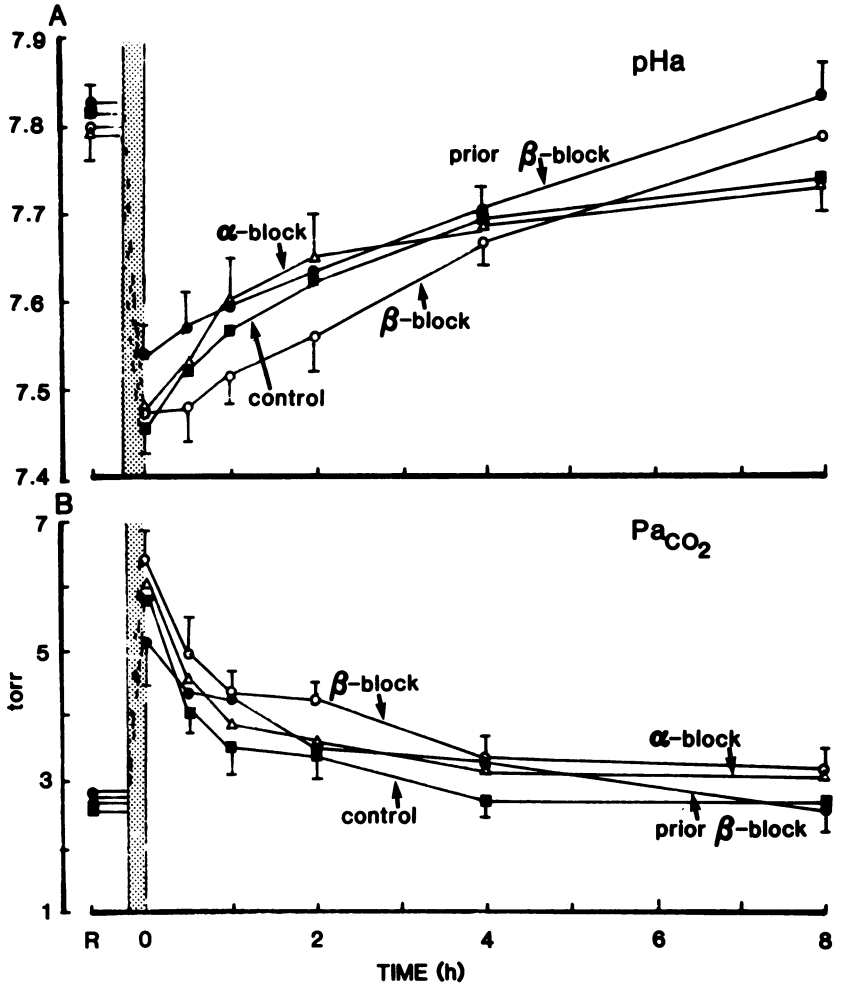

FIG. 1.—Changes in (*A*) pHa and (*B*) Pa_{CO_2} after strenuous exercise in control flounder (■; $n = 8$), fish treated with 10 μmol/kg yohimbine HCl immediately after the 0-h sample (△; *α-block; n* = 6), fish treated with 10 μmol/kg propranolol HCl immediately after the 0-h sample (○; *β-block; n* = 6), and fish treated with 10 μmol/kg propranolol HCl 30 min prior to the rest sample shown (●; *prior β-block; n* = 6). Points plotted are means ± 1 SEM, though only the SE bars of the highest and lowest means at each time are shown for the sake of clarity. R = rest; 0 h = immediately after exercise; vertical stippled bar indicates 10 min of strenuous exercise. With respect to the R values, changes in pHa were significant ($P < .05$) until 4 h in the control, β-block, and prior β-block groups and until 8 h in the α-block group. Changes in Pa_{CO_2} were significant until 2 h in the control and α-block groups and until 4 h in the β-block and prior β-block groups. There were no significant differences ($P < .05$) at any time between the control group and any of the blocked groups.

(fig. 2*A*) and ΔLa^- (fig. 2*B*) appearance in the blood. However, in light of series 2 results (below), it is noteworthy that ΔLa^- (fig. 2*B*) was greatest in the β-blocked group ($.05 < P < .10$ relative to the control group at 2 and 4 h).

After α blockade, there was no significant elevation of blood glucose during the postexercise period (fig. 3*A*). In contrast, after β blockade, the hyperglycemia at 0.5–4 h was similar to that in the controls but persisted at 8 h, by which time the control group had been restored to rest levels. Postexercise changes in MCHC (fig. 3*B*) and plasma [protein] (fig. 3*C*) were generally similar in control, α-, and β-blocked groups. For MCHC, the significant differences from controls in the β-blocked group at 4 h and in the α-blocked group at 8 h were small exaggerations of nonsignificant differences already present at rest (fig. 3*B*).

β blockade prior to exercise was employed to test whether some of the changes already present at the immediate end of exercise (0 h) could be modified by this pretreatment. Establishment of the competitive β blockade might also be more effective at rest when endogenous catecholamine levels are presumably low. However, again the influence of this antagonist was rela-

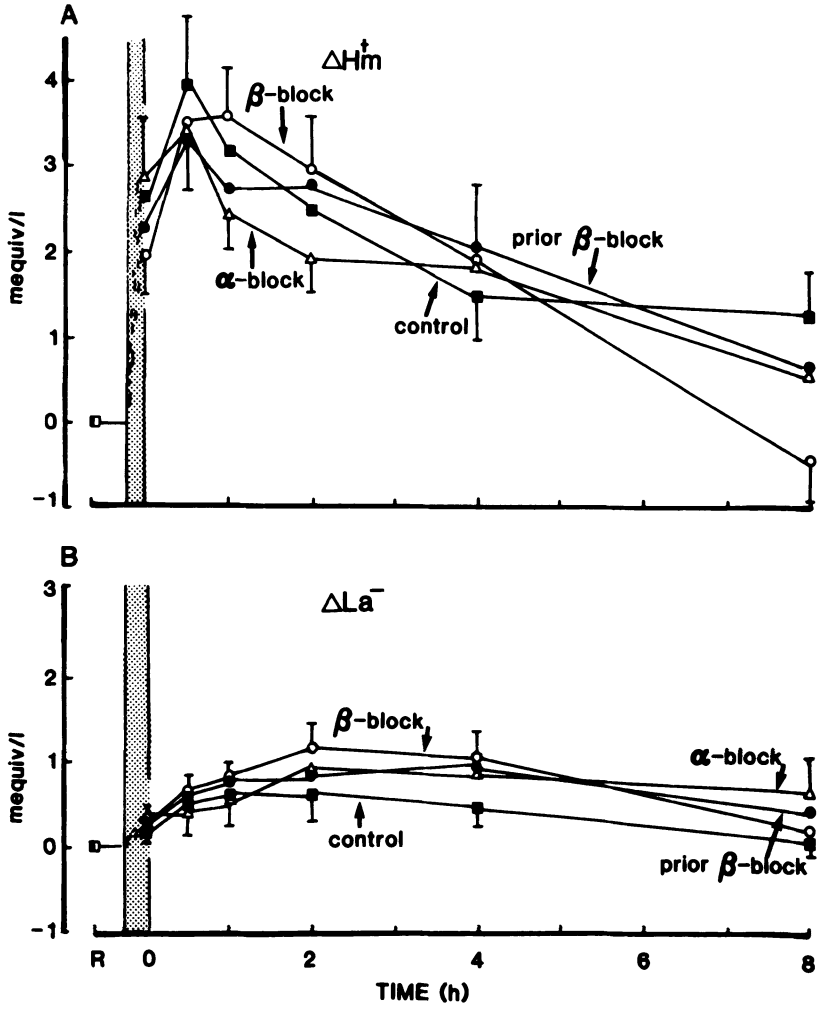

FIG. 2.—Changes in (*A*) whole-blood ΔH^+m and (*B*) whole-blood ΔLa^- after strenuous exercise in control (■), α-block (△), β-block (○), and prior β-block (●) groups. Both ΔH^+m and ΔLa^- were significantly different ($P < .05$) from 0 until 4 h in all groups. There were no significant differences ($P < .05$) in either ΔH^+m or ΔLa^- at any time between the control group and any of the blocked groups. Other details are as in the legend to fig. 1.

tively minor. There was no effect on any measured parameter at rest (data not shown), and our subjective impression was that the preexercise β-blocked fish swam just as vigorously as the untreated animals. Furthermore, with the exception of MCHC, changes in all parameters at 0 h were identical to those in the controls—and remained so throughout the recovery period (figs. 1–3). In contrast to the other groups, there

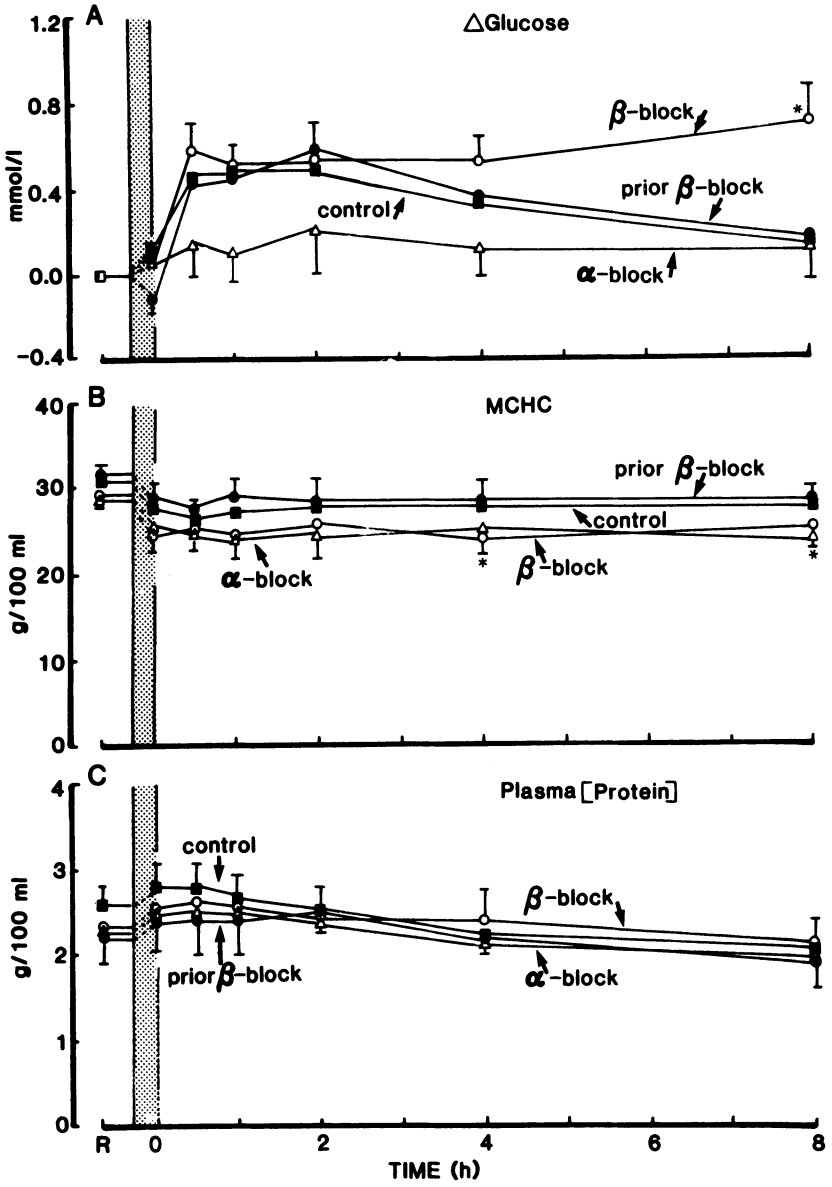

FIG. 3.—Changes in (*A*) whole-blood Δ glucose; (*B*) MCHC; and (*C*) plasma protein concentration after strenuous exercise in control (■), α-block (△), β-block (○), and prior β-block (●) groups. Δ Glucose was not significantly different from zero at any time in the α-block group but was so ($P < .05$) from 0.5 to 2 h in the prior β-block group, from 0.5 to 4 h in the control group, and from 0.5 to 8 h in the β-block group. Relative to the rest value, MCHC did not change significantly in the prior β-block group but was significantly depressed from 0.5 to 2 h in the control group, from 0 to 4 h in the β-block group, and from 0 to 8 h in the α-block group. Plasma protein concentration was significantly elevated at 0 h in the control and α-block groups and at 0 to 0.5 h in the β-block and prior β-block groups. * Indicates the only significant differences ($P < .05$) at any time between the control group and the designated experimental groups. Other details are as in the legend to fig. 1.

was no significant fall in MCHC after exercise in the preexercise β-blocked flounder, though the overall trends were similar (fig. 3*B*).

SERIES 2

The second series examined changes in RBC O_2 transport, muscle lactate levels, and intracellular acid-base status in several key tissues of control fish after severe exercise, as well as the possible influence of β blockade (imposed immediately after 0 h) on the recovery processes for these parameters.

As in the study described in our accompanying paper (Milligan and Wood 1987*b*), RBC pHi remained constant immediately after exercise despite a large extracellular acidosis (fig. 4*A*). At 1 h recovery, RBC pHi was again unchanged in the control animals but had fallen significantly in the β-blocked fish. β blockade also appeared to inhibit the postexercise swelling of the RBCs, for MCHC at 1 h fell significantly in the controls but not in the β-blocked group (fig. 4*B*). This result differed from the postexercise β-blockade data of series 1 but agreed with the preexercise β-blockade results of series 1. There were no apparent metabolic changes in erythrocytic NTP levels, for [NTP]/[Hb] remained constant in all treatments (fig. 4*B*). Mean cellular NTP concentrations reflected the diluting effects of RBC swelling, but again the changes were not significant (rest = 5.96 ± 0.74 μmol/ml [n = 8]; 0 h = 6.10 ± 0.45 μmol/ml [n = 12]; 1 h = 5.03 ± 0.54 μmol/ml [n = 12]; 1 h β-blocked = 5.52 ± 0.86 μmol/ml [n = 7]). Despite the marked rise and subsequent fall in P_{aCO_2} (fig. 4*C*), P_{aO_2} remained relatively constant (fig. 4*D*); neither parameter was affected by β blockade. C_{aO_2} was 2.03 ± 0.20 mmol/liter (n = 8) at rest, 1.55 ± 0.19 mmol/liter (n = 10) at 0 h, 1.99 ± 0.26 mmol/liter (n = 8) at 1 h, and 1.62 ± 0.38 mmol/liter (n = 6) in the 1-h β-blocked group. While none of these changes were significant in themselves, once postexercise [Hb] variations were taken into account, there were clear differences in $[O_2]$/[Hb] (fig. 4*E*). $[O_2]$/[Hb] fell by 25% immediately after exercise and had recovered by 1 h in the control fish but remained significantly depressed in the β-blocked group. As in series 1, blood lactate levels increased slowly after exercise (fig. 4*F*; see fig. 2*B*). However, relative to the control group, in this experiment, β blockade significantly potentiated the appearance of lactate in the blood at 1 h (fig. 4*F*), a difference that had been present but not significant in series 1 (fig. 2*B*). While this ΔLa^- was 100% greater than that in control fish, the absolute levels remained very low (<2 mmol/liter). Since only single blood samples were taken from each animal, ΔH^+m could not be calculated for individual fish in this experiment (see eq. [1] of Milligan and Wood 1987*a*). Based on mean values for each group, ΔH^+m was 2.71 mmol/liter at 0 h and 2.83 mmol/liter at 1 h in the control group versus 4.22 mmol/liter at 1 h in the β-blocked group. The significance of this difference cannot be assessed statistically.

In resting flounder (n = 8–12), tissue fluid volumes, as determined by ^{3}H-mannitol distribution and total water content, were as follows: white-muscle ECFV = 85.1 ± 6.8 ml/kg, ICFV = 719.3 ± 7.6 ml/kg; heart-ventricle ECFV = 224.1 ± 15.5, ICFV = 611.0 ± 14.3; brain ECFV = 73.1 ± 6.8, ICFV = 741.9 ± 11.1. There were no significant changes after exercise and/or β blockade, though white muscle followed the general postexercise trends that we have previously described (Milligan and Wood 1987*b*).

There was considerable variation (7.8–7.3) in mean pHi among different tissues in resting flounder, with brain having the highest value, followed by heart, white muscle, and RBC (figs. 4*A*, 5). In contrast to the constancy of RBC pHi after exercise (fig. 4*A*), pHi declined significantly in the other tissues, with the fall being greatest in brain and least in heart (fig. 5*A*, 5*C*, 5*D*). Intracellular acidoses persisted in all these tissues at 1 h in control fish. Again in contrast to the case with RBC, there was no evidence that β blockade interfered with pHi regulation in these tissues; if anything, pHi's tended to be higher in the β-blocked group at 1 h (fig. 5*A*, 5*C*, 5*D*). However, β blockade significantly elevated muscle lactate concentrations ~60% above both the 0-h and the 1-h levels of untreated animals (fig. 5*B*). Since the absolute levels of lactate were 10–20-fold higher in muscle than in blood, the ~0.75 mmol/liter greater blood [lactate] (fig. 4*F*) in the β-blocked group

could well have resulted from the ~7.5 mmol/liter greater [lactate] in the muscle, *produced after* β *blockade.*

DISCUSSION

RESPONSES UNDER CONTROL CONDITIONS

In control flounder, resting values and postexercise changes were very similar to those reported for an entirely separate batch of *Platichthys stellatus* in our accompanying papers (Milligan and Wood 1987*a*, 1987*b*). The only significant differences at rest were a higher RBC pHi (by ~0.13 units) and lower white-muscle pHi (by ~0.27 units) in the fish studied in the present paper, though relative postexercise trends were similar. The only significant

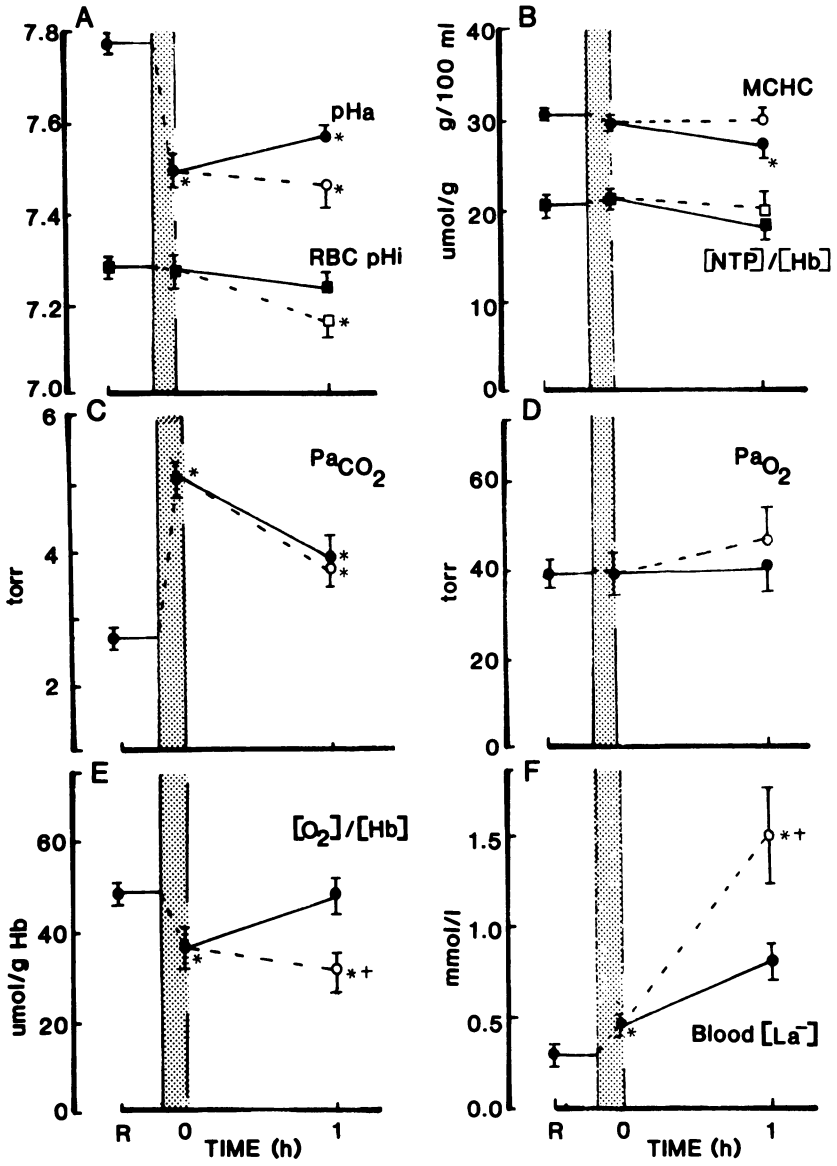

FIG. 4.—Changes in (*A*) pHa and RBC pHi, (*B*) MCHC and [NTP]/[Hb], (*C*) Pa_{CO_2}, (*D*) Pa_{O_2}, (*E*) $[O_2]/[Hb]$, and (*F*) blood $[La^-]$ after strenuous exercise and the effects of β-blockade on these changes. The β-block group (○) were treated with 10 μmol/kg propranolol HCl immediately after exercise (0 h); the other fish (control; ●) were not treated. n = 8–26 at rest, 10–17 at 0 h, 8–19 at 1 h control, and 6–7 at 1 h β-block. * Indicates a significant difference ($P < .05$) from the R value; + indicates a significant difference at 1 h between the control and β-block values.

postexercise difference was the fall of PaO_2 at 0–0.5 h in our previous group of fish (Milligan and Wood 1987*b*) a decrease that did not occur in the group considered in the present paper (fig. 4*D*). Identical exercise protocols and methodology were used in the two studies, so we can only attribute these differences to year-to-year variability in field-collected fish, though the physiological causes are unknown.

Polar nonelectrolytes such as mannitol do not easily cross the blood-brain barrier in higher vertebrates, and we have some preliminary evidence that this is also true in fish (C. M. Wood and M. S. Graham, unpublished data). Thus, the ECFV of brain tissue may have been underestimated in the present study. However, this would have had negligible influence on the calculated pHi values. For example, model calculations show that, even if ECFV were as great as four times the mannitol-measured value, pHi would have been underestimated by only ~0.02 units, which is outside the detection limits of the DMO method.

The current determinations of tissue pHi at rest conform to the basic pattern that is now emerging for a variety of teleost fish. Thus, brain pHi is usually highest, followed by metabolically active tissues such as liver and heart, with RBC and muscle pHi at the lower end of the range (Cameron and Kormanik 1982; Walsh and Moon 1982; Cameron 1985; Milligan and Farrell 1986; Milligan and Wood 1986*a*, 1986*b*). These variations could reflect differences in membrane potential, steady-state metabolism, intracellular compartment heterogeneity, or some combination of these factors.

We selected heart and brain as two representatives of essential tissues in which postexercise regulation of acid-base status

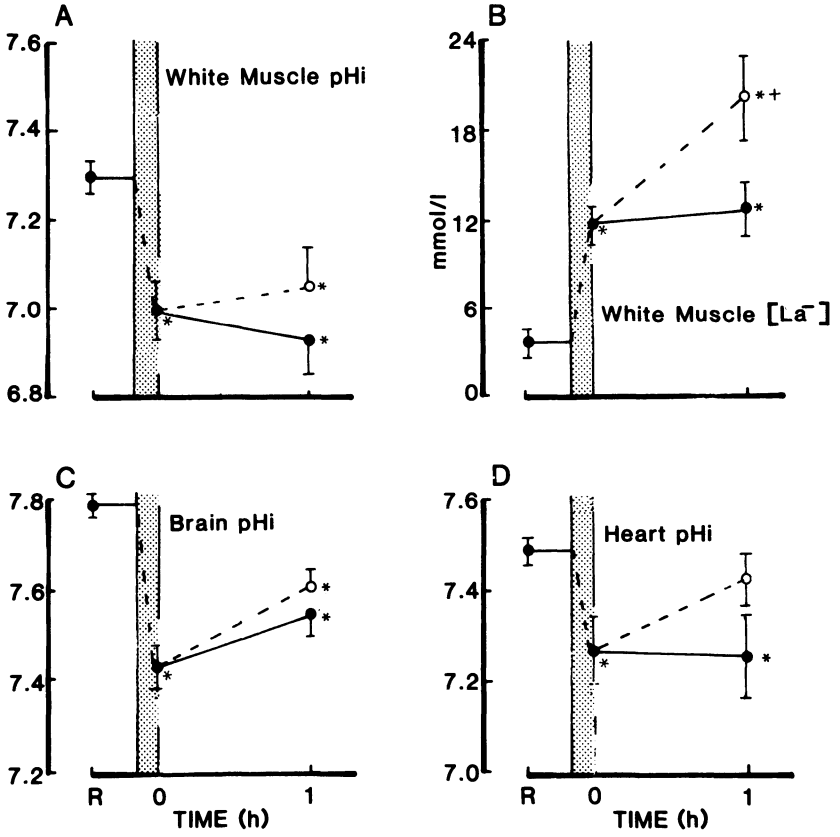

FIG. 5.—Changes after strenuous exercise in (*A*) white-muscle pHi; (*B*) white-muscle La^-, expressed in mmol/liter of ICF; (*C*) brain pHi; and (*D*) heart-ventricle pHi and the effects of β-blockade on these changes. n = 8–12 at rest, 6–9 at 0 h, 7–8 at 1 h control, and 7 at 1 h β-block. Other details are as in the legend to fig. 4.

might be most prominent. Indeed, earlier studies on sea raven (*Hemitripterus americanus;* Milligan and Farrell 1986) and rainbow trout (*Salmo gairdneri;* Milligan and Wood 1986*b*) showed that heart pHi did not fall after exhaustive exercise and actually exhibited alkalosis during the recovery period. In the trout, brain pHi was only mildly perturbed by strenuous activity. However, in the starry flounder, both compartments became markedly acidotic at 0 h and had not recovered by 1 h (fig. 5*C*, 5*D*), indicating the danger in extrapolating between species. In this regard it is significant that, under hypercapnic acidosis in vitro, the myocardium of another flounder (*Pleuronectes flesus*) maintained contractility much better than that of the trout (Gesser and Poupa 1979; Gesser and Jorgensen 1982). This difference was probably related to mobilization of intracellular calcium during acidosis in flounder ventricle but not in trout ventricle. Thus, the flounder may tolerate, rather than prevent, intracellular acidosis because of the presence of other functional protective mechanisms.

Nevertheless, the starry flounder did regulate RBC pHi at a very constant level in the face of the large extracellular acidosis after exercise (fig. 4*A;* see fig. 2 of Milligan and Wood 1987*b*), as do the trout (Primmet et al. 1986; Milligan and Wood 1986*b*) and striped bass (*Morone saxatalis;* Nikinmaa et al. 1984). In the two latter species, in vitro experiments have implicated a β-adrenergically mediated swelling of the RBCs owing to increased cation influx, most importantly a stimulation of Na^+ (entry) versus acidic equivalent (exit) exchange in excess of Cl^- (entry) versus basic-equivalent (exit) exchange (Nikinmaa and Huestis 1984; Cossins and Richardson 1985). In addition to this net extrusion of H^+, the dilution of intracellular organic polyanions (Hb and NTP) by swelling also tends to raise RBC pHi via the resultant shift in the Donnan ratio (Nikinmaa 1982). These factors are thought to help sustain Hb oxygenation in the face of extracellular acidosis after exercise (Nikinmaa 1983; Nikinmaa et al. 1984). The present observations on the flounder in vivo are in general accord with these ideas, for after exercise β blockade resulted in a drop in RBC pHi (fig. 4*A*), a lack of swelling (fig. 4*B*), and a reduction in $[O_2]/[Hb]$ (fig. 4*E*). NTP, which can act as a negative allosteric modifier of Hb O_2 affinity, did not appear to play a significant role in these responses. However, in view of the regulation of RBC pHi and the constancy of Pa_{O_2} after exercise (fig. 4*B*), it is interesting that $[O_2]/[Hb]$ fell at 0 h in the absence of β blockade (fig. 4*E*). Thus, factors additional to those considered here must also influence Hb oxygenation in the flounder.

THE INFLUENCE OF ADRENERGIC BLOCKADE

The specific antagonists used here—and their relatively high dosages—were purposely chosen to ensure complete blockade as well as comparability to the study of Wardle (1978). Mammalian physiology now recognizes separate α1, α2, β1, and β2 receptors, but it is not yet clear whether the same fine classifications can be applied in fish. In mammals, propranolol is a competitive, nonselective β blocker equally effective on both β1 and β2 adrenoreceptors (Gilman et al. 1985). Yohimbine is a competitive α blocker with reported α2 selectivity in mammals (Gilman et al. 1985) but with demonstrated potency on cardiovascular (presumptive α1) adrenoreceptors in fish. Thus at one-tenth the dosage levels used here, these two agents produced effective β and α blockade, respectively, against the cardiovascular effects of infused catecholamines in the trout (Wood and Shelton 1980). The significant actions of propranolol and yohimbine on glucose dynamics, RBC function, and muscle lactate in the present study show that they were also effective in the flounder.

Postexercise hyperglycemia in fish has long been attributed to the glycogenolytic effects of plasma catecholamines on the liver (Nakano and Tomlinson 1967; Wardle 1972; Larsson 1973; Mazeaud et al. 1977). In mammals, this response is mediated through both α and β adrenoreceptors on hepatocytes, with their relative importance varying between species (Exton 1979). In *P. stellatus,* the response appears to be due largely to α adrenoreceptors, for α blockade inhibited postexercise hyperglycemia whereas β blockade prolonged it (fig. 3*B*).

On balance, our results provide little support for the contention of Wardle (1978) that the nonrelease of lactic acid after exercise is due to a β-adrenergic action of circulating catecholamines on the muscle cells; rather, our data indicate that any postexercise increase in blood ΔLa^- associated with β blockade (e.g., figs. 2*B*, 4*F*) was probably caused by greatly elevated lactate production in white muscle (fig. 5*D*), *occurring after β blockade.* In turn, this was probably due to impaired O_2 delivery, for Hb oxygenation fell after β blockade (fig. 4*E*), as discussed above, and cardiac output was likely reduced at this dosage of propranolol (Wood and Shelton 1980). Branchial O_2-diffusing capacity may also have been lowered (Perry et al. 1985), though the Pa_{O_2} data provide no evidence that this occurred (fig. 4*D*).

One possible explanation for this difference is species variation, for Wardle (1978) used the plaice, *Pleuronectes platessa.* However, there are also several anomalies in Wardle's study that render his conclusions open to alternate interpretation. For example, the highest postexercise blood lactate levels in surviving plaice were actually seen in α-blocked fish. Furthermore, β blockade did not cause increased lactate appearance in the bloodstream if the plaice had been well adapted to the experimental conditions (i.e., a holding period comparable to that used in the present study). Even in plaice freshly collected from the wild, β blockade caused elevated blood lactate in only six of 11 exercised animals, and these six all died. Selective β stimulation with isoxuprine did not affect postexercise lactate appearance in the blood for the first 2 h after injection, and thereafter there was considerable overlap with the results for the control group. We suggest that the high-dosage levels of drugs employed by Wardle (1978) may have induced nonspecific toxic effects leading to muscle hypoxemia and elevated lactate production in those fish that were already suffering from other stresses—and that this, rather than a specific adrenergic control on lactate efflux, was the cause of the observed effects. The elevated muscle lactate levels in β-blocked fish of the present study (fig. 5*B*) directly support this interpretation.

Our results also provide little support for adrenergic involvement in postexercise acid-base regulation in either the extracellular (figs. 1, 2) or intracellular compartments (fig. 5) of *P. stellatus,* with the exception of the RBC (fig. 4*A*). Previously, we have argued that catecholamines might play a key role in these processes (Wood and Perry 1985; Milligan and Farrell 1986). These arguments were based largely on in vitro data from salmonids and, in the case of tissue pHi regulation, on an analogy to findings in mammals (see e.g., Riegle and Clancy 1975; Clancy Gonzalez, and Fenton 1976; Gonzalez and Clancy 1984). The present results indicate either that these concepts do not apply to the specific case of *P. stellatus*—or, more generally, that the concepts are not relevant to the in vivo situation in fish. Recently, Farrell and Milligan (1986) were unable to demonstrate any infuence of adrenaline on pHi regulation in the trout ventricle exposed to respiratory acidosis in vitro, despite the fact that we have found heart pHi to be perfectly regulated after a comparable acidosis induced by exhaustive exercise in vivo (Milligan and Wood 1986*b*). Interestingly, a recent study in humans (Katz, Sahlin, and Juhlin-Dannfelt 1985) found no effect of β blockade on H^+ and lactate release from maximally exercising muscle.

The situation is obviously complex, and our understanding at present is fragmentary. The relative lack of effect of adrenergic blockade on postexercise lactate and acid-base dynamics could indicate either that there is little catecholamine mobilization in *P. stellatus* after normal strenuous exercise or that catecholamines are mobilized but exert little influence on these processes. In salmonids, catecholamine levels rise dramatically after exhaustive exercise (Nakano and Tomlinson 1967; Butler 1986; Primmet et al. 1986), but examinations of the effects of adrenergic blockade in vivo have so far been limited only to RBC function (Nikinmaa 1982; Primmet et al. 1986). In both species, there is a clear need for studies relating the actual levels of plasma catecholamines after exercise to a detailed pharmacological analysis of intracellular and extracellular acid-base regulation and lactate metabolism.

LITERATURE CITED

BUTLER, P. J. 1986. Exercise. Pages 18–34 *in* S. NILSSON and S. HOLMGREN, eds. Fish physiology: recent advances. Croom Helm, London.

CAMERON, J. N. 1985. The bone compartment in a teleost fish, *Ictalurus punctatus:* size, composition, and acid-base response to hypercapnia. J. Exp. Biol. **117**:307–318.

CAMERON, J. N., and G. A. KORMANIK. 1982. Intracellular and extracellular acid-base status as a function of temperature in the freshwater channel catfish, *Ictalurus punctatus.* J. Exp. Biol. **99**:127–142.

CLANCY, R. L., N. C. GONZALEZ, and R. A. FENTON. 1976. Effect of beta-adrenoreceptor blockade on rat skeletal muscle pH. Am. J. Physiol. **230**:959–964.

COSSINS, A. R., and P. A. RICHARDSON. 1985. Adrenalin-induced Na^+/H^+ exchange in trout erythrocytes and its effect upon oxygen-carrying capacity. J. Exp. Biol. **118**:229–246.

DANDO, P. R. 1969. Lactate metabolism in fish. J. Mar. Biol. Assoc. UK **49**:209–223.

DUTHIE, G. G. 1982. The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. J. Exp. Biol. **97**:359–373.

EXTON, J. H. 1979. Mechanisms involved in effects of catecholamines on liver carbohydrate metabolism. Biochem. Pharmacol. **28**:2237–2240.

FARRELL, A. P., and C. L. MILLIGAN. 1986. Myocardial intracellular pH in a perfused rainbow trout heart during extracellular acidosis in the presence and absence of adrenalin. J. Exp. Biol. **125**:347–359.

GESSER, H., and E. JORGENSEN. 1982. pHi, contractility, and Ca^{++}-balance under hypercapnic acidosis in the myocardium of different vertebrate species. J. Exp. Biol. **96**:405–412.

GESSER, H., and O. POUPA. 1979. Effects of different types of acidosis and Ca^{2+} on cardiac contractility in the flounder (*Pleuronectes flesus*). J. Comp. Physiol. **131**:293–296.

GILMAN, A. G., L. S. GOODMAN, T. W. RALL, and F. MURAD, eds. 1985. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 7th ed. Macmillan, New York.

GONZALEZ, N. C., and R. L. CLANCY. 1984. Catecholamine effects on HCO_3^-/Cl^- exchange and rabbit myocardial cell pH regulation. Respir. Physiol. **56**:289–299.

KARLSSON, J. 1985. Metabolic adaptations to exercise: a review of potential beta-adrenoceptor antagonist effects. Am. J. Cardiol. **55**:48D–58D.

KATZ, A., K. SAHLIN, and A. JUHLIN-DANNFELT. 1985. Effect of α-adrenoceptor blockade on H^+ and K^+ flux in exercising humans. J. Appl. Physiol. **59**: 336–341.

LARSSON, A. L. 1973. Metabolic effects of epinephrine and norepinephrine in the eel *Anguilla anguilla* L. Gen. Comp. Endocrinol. **20**:155–167.

MAZEAUD, M. M., and F. MAZEAUD. 1981. Adrenergic responses to stress in fish. Pages 49–75 *in* A. D. PICKERING, ed. Stress and fish. Academic Press, London.

MAZEAUD, M. M., F. MAZEAUD, and E. M. DONALDSON. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fisheries Soc. **106**:201–212.

MILLIGAN, C. L., and A. P. FARRELL. 1986. Extracellular and intracellular acid-base status following strenuous activity in the sea raven (*Hemiptripterus americanus*). J. Comp. Physiol. **156B**:593–590.

MILLIGAN, C. L., and C. M. WOOD. 1986*a*. Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. J. Exp. Biol. **123**:93–121.

———. 1986*b*. Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol. **123**:123–144.

———. 1987*a*. Effects of strenuous activity on intracellular and extracellular acid-base status and H^+ exchange with the environment in the inactive, benthic starry flounder *Platichthys stellatus.* Physiol. Zool. **60**:37–53.

———. 1987*b*. Muscle and liver intracellular acid-base and metabolite status after strenuous activity in the inactive, benthic starry flounder *Platichthys stellatus.* Physiol. Zool. **60**:54–68.

NAKANO, T., and N. TOMLINSON. 1967. Catecholamine and carbohydrate metabolism in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. J. Fisheries Res. Board Can. **24**:1701–15.

NIKINMAA, M. 1982. Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, *Salmo gairdneri.* Mol. Physiol. **2**:287–297.

———. 1983. Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. J. Comp. Physiol. **152**:67–72.

NIKINMAA, M., J. J. CECH, JR., and M. MCENROE. 1984. Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. J. Comp. Physiol. B **154B**:365–369.

NIKINMAA, M., and W. H. HUESTIS. 1984. Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. J. Exp. Biol. **113**: 215–224.

OPIE, L. H. 1985. Effect of beta-adrenergic blockade on biochemical and metabolic response to exercise. Am. J. Cardiol. **55**:95D–100D.

PERRY, S. F., C. DAXBOECK, and G. P. DOBSON. 1985. The effects of perfusion flow rate and adrenergic stimulation on oxygen transfer in the isolated saline-perfused head of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **116**:251–269.

PRIMMET, D. R. N., D. J. RANDALL, M. MAZEAUD, and R. G. BOUTILIER. 1986. The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. J. Exp. Biol. **122**:139–148.

RIEGLE, K. M., and R. L. CLANCY. 1975. The effect of norepinephrine on myocardial intracellular hydrogen ion concentration. Am. J. Physiol. **229**:344–349.

SIGMA CHEMICAL COMPANY. 1983. The ultraviolet determination of ATP (adenosine-5'-triphosphate) in whole blood at 340 nm. Sigma Tech. Bull. No. 366-UV. Sigma, St. Louis.

TURNER, J. D., C. M. WOOD, and H. HÖBE. 1983.

Physiological consequences of severe exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*): a comparison with the active pelagic rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **104:** 269–288.

WALSH, P. J., and T. W. MOON. 1982. The influence of temperature on extracellular and intracellular pH in the American eel, *Anguilla rostrata* (Le sueur). Respir. Physiol. **50:**129–140.

WARDLE, C. S. 1972. The changes in blood glucose of *Pleuronectes platessa* following capture from the wild: a stress reaction. J. Mar. Biol. Assoc. UK **52:** 635–651.

———. 1978. Non-release of lactic acid from anaerobic swimming muscle of plaice, *Pleuronectes platessa* L: a stress reaction. J. Exp. Biol. **77:**141–155.

WOOD, C. M., B. R. MCMAHON, and D. G. MCDONALD. 1977. An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus.* J. Exp. Biol. **69:**173–185.

———. 1979. Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus:* a comparison with other teleosts. J. Exp. Biol. **78:**167–179.

WOOD, C. M., and S. F. PERRY. 1985. Respiratory, circulatory, and metabolic adjustments to exercise in fish. Pages 1–22 *in* R. GILLES, ed. Comparative physiology and biochemistry—current topics and trends. Vol. A. Respiration—metabolism—circulation. Springer, Berlin.

WOOD, C. M., and G. SHELTON. 1980. Cardiovascular dynamics and adrenergic responses of the rainbow trout *in vivo.* J. Exp. Biol. **87:**247–270.