MUSCLE AND LIVER INTRACELLULAR ACID-BASE AND METABOLITE STATUS AFTER STRENUOUS ACTIVITY IN THE INACTIVE, BENTHIC STARRY FLOUNDER PLATICHTHYS STELLATUS[1]

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In addition to an extracellular acidosis in which blood metabolic acid load greatly exceeded lactate load, exhaustive activity in starry flounder resulted in an intracellular acidosis of largely metabolic origin in the white muscle, with intracellular pH dropping from 7.56 to 7.27, as measured by DMO distribution. An accumulation of lactate and depletion of glycogen in addition to a shift of fluid from the extracellular to intracellular space were associated with the postexercise acidosis. Pyruvate levels increased in blood and later in muscle; the relative rise in pyruvate was greater than that in lactate so the lactate:pyruvate ratio declined. The muscle intracellular acidosis was corrected sooner than the extracellular acidosis (4–8 h vs. 8–12 h). The restoration of muscle pHi was associated with an increase in pyruvate, a restoration of glycogen stores, and clearance of the lactate load. It is suggested that both lactate and acidic equivalents (H^+) were cleared from the muscle via in situ oxidation and/or glyconeogenesis and that the rapid correction of the intracellular acidosis through efflux of part of the H^+ load facilitated metabolic recovery. The liver showed a progressive alkalinization after exercise. This alkalinization was of metabolic origin and not associated with lactate accumulation. Except for a short-lived depression 0.5 h after exercise, red cell intracellular pH remained virtually constant.

INTRODUCTION

Intense activity in vertebrates results in the accumulation of lactate and acidic equivalents (H^+) in the working muscle. Both end products appear in the blood space, though, in flounder, lactate does not accumulate to any great extent (1–2 mmol/liter), with blood levels only one-quarter those of H^+ (Wood, McMahon, and McDonald 1977; Milligan and Wood 1987). The classical picture is the Cori cycle: lactate and H^+ leave the muscle and are transported via the blood, to the liver, where they are converted to glucose. The glucose then enters the blood to be taken up by the muscle and utilized to replenish glycogen stores (Newsholme and Leech 1983). However, the fates of lactate and H^+ after exercise in relatively inactive, benthic fish such as starry flounder are unclear. The data of Wardle (1978) and Batty and Wardle (1979) suggest that lactate, and presumably H^+, are not transported out of the muscle but rather are utilized as substrates for in situ glyconeogenesis.

The present study investigates the possible fates of H^+ and lactate after exercise in starry flounder. Using the DMO method for measuring intracellular pH (pHi; Waddell and Butler 1959), we examined the intracellular acid-base and metabolite changes associated with exercise in the white-muscle mass, liver, and blood. In addition, we investigated the effect of the exercise-induced extracellular acidosis on red blood cell (RBC) pHi. This study complements our work on the active, pelagic, rainbow trout (Milligan and Wood 1986*b*) and thus provides insight into the physiological reason(s) for the species-dependent pattern of lactate and H^+ accumulation in the blood.

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MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Starry flounder were captured, held at 11 ± 1 C, and catheterized in the caudal artery as described in the companion paper (Milligan and Wood 1987).

EXPERIMENTAL PROTOCOL

In the present experiments, fish were sampled only once (terminally) rather than sequentially for analysis of blood, muscle, and liver acid-base and metabolite status. Approximately 12 h prior to sampling, fish were infused with a 1 ml/kg dose of 5 μCi/ml ^{14}C-DMO (New England Nuclear; 5,5-dimethyl-2,4-oxazolidinedione; specific activity, 50 mCi/mmol) and 20 μCi/ml ^{3}H-mannitol (New England Nuclear; specific activity, 27.4 mCi/mmol) in Cortland saline (Wolf 1963) adjusted to 160 mmol/liter NaCl, followed by an equal volume of saline. Individual fish (four to six at each time) were terminally sampled prior to ("rest"), immediately after exercise (0 h), as well as at 0.5, 2, 4, and 12 h. Immediately after withdrawal of an arterial blood sample (2,000 μl) from the catheter, fish were removed from the water by one investigator, placed on a sponge, and wiped dry. Ten muscle samples (75–100 mg) were taken by a second investigator by punching 10 prechilled biopsy needles (ID = 4.78 mm) through the dorsal epaxial muscle mass, 8–10 cm posterior from the head. Samples were immediately frozen in the needles on dry ice. The fish were then killed by a cephalic blow, the liver was excised, and small portions (250–350 mg) were frozen on dry ice; larger tissue samples (2–3 g) were taken for determination of total body water. Total elapsed time from first grasping the fish to freezing of muscle was ~15 s, and not more than 60 s elapsed before a liver sample was obtained. Fish did not struggle during the tissue-sampling procedure. The carcass was then weighed and dried to a constant weight at 85 C for determination of total body water.

Arterial blood was analyzed for pH, O_2 tension (P_{O_2}), total CO_2 (in both whole blood and plasma), hematocrit, [hemoglobin], [glucose], [lactate], and [pyruvate]; and RBC pHi was measured directly. Plasma was analyzed for ^{3}H and ^{14}C radioactivity, [protein], and levels of Na^+, Cl^-, K^+, Ca^{2+}, and ammonia. Muscle and liver were analyzed for ^{3}H and ^{14}C radioactivity, [lactate], [pyruvate], glycogen, and total water contents.

ANALYTICAL TECHNIQUES AND CALCULATIONS

Arterial blood pH, total CO_2, [lactate], [glucose], [hemoglobin], hematocrit, and plasma total CO_2, [protein], and levels of ^{3}H and ^{14}C radioactivity were measured using methods described in the companion paper (Milligan and Wood 1987). Arterial P_{O_2} (Pa_{O_2}) was measured with a Radiometer O_2 electrode (type E5036) maintained at the experimental temperature. Whole-blood [pyruvate] was measured on 250 μl whole blood fixed in 500 μl ice-cold 8% perchloric acid. Samples were stored on ice for 5 min, centrifuged for 3 min at 9,000 *g*, then stored at 5 C not longer than 48 h prior to analysis. Pyruvate was assayed on 400 μl of supernatant neutralized with Trizma buffer (1.5 mol/liter; Sigma), using the l-lactate dehydrogenase method described by Segal, Blair, and Wyngaarden (1956) and Sigma reagents. RBC pHi was measured directly by the freeze-thaw technique of Zeidler and Kim (1977) as described by Milligan and Wood (1986*b*).

Plasma concentrations of Na^+, K^+ (by flame photometry), and Cl^- (by coulometric titration) were determined using methods described by McDonald, Hōbe, and Wood (1980). Plasma [Ca^{2+}] was determined colorimetrically on 25-μl aliquots using the cresolphthalein complexone method (Sigma technical bulletin 585). Plasma [ammonia] was measured on 100 μl plasma extracted 4:1 with ice-cold 12.5% trichloracetic acid by a micromodification of the salicylate hypochlorite method of Verdouw, van Echteld, and Dekkers (1978).

For analysis of tissue lactate and pyruvate, frozen tissue samples (80–250 mg) were homogenized on ice in 1 ml of 8% ice-cold perchloric acid with a glass homogenizer for 5 min. The homogenate was then transferred to 1.5-ml centrifuge tubes and centrifuged for 3 min at 9,000 *g*. The supernatant, diluted 1:4 with 8% perchloric acid, was neutralized with Trizma buffer (1.5 mol/liter; Sigma) and analyzed for lac-

tate and pyruvate using the l-lactate dehydrogenase method as described for blood samples. Muscle and liver glycogen were measured on tissues of similar weights using the anthrone method described by Hassid and Abraham (1957). Tissue water content was determined by drying to a constant weight at 85 C, extracellular fluid volume (ECFV) was measured as outlined below, and intracellular fluid volume (ICFV) was calculated by difference. All tissue metabolites were expressed in terms of mmol/liter intracellular fluid (ICF) and corrected for levels in the trapped extracellular fluid (ECF).

^{3}H and ^{14}C radioactivity in muscle and liver were determined by digesting 50–150 mg of tissue in 2 ml NCS tissue solubilizer (Amersham) for 2–4 days until clear. The digests were neutralized with 60 µl glacial acetic acid; then 10 ml fluor (OCS; Amersham) was added. Samples were stored in the dark overnight to reduce chemiluminescence, then counted on a Beckman LS-250 liquid scintillation counter. Dual label quench correction was performed using quench standards prepared from the tissue of interest and the external standard-ratio method or internal standardization, as required (Kobayashi and Maudsley 1974).

The nonbicarbonate buffer capacity (β) of muscle and liver was determined by acid titration of tissue homogenates from six fish, according to a method described by Cameron and Kormanik (1982). Tissue (1–2 g) was frozen on dry ice, pulverized with a mortar and pestle, then suspended in 3 ml 0.9% NaCl. The tissue homogenate was titrated to pH 8.0 with 1N NaOH, allowed to stabilize, then back titrated to pH 6.5 with 0.02N HCl under a nitrogen atmosphere at 11 ± 1 C. A Radiometer G-202 pH electrode and associated acid-base analyzer were used to measure pH. The slope of the curve relating pH versus millimoles HCl added over the pHi range observed in vivo for each tissue (fig. 2) was taken as the buffer capacity in mmol/pH/kg wet weight and then converted to mmol/pH/liter ICF, as for tissue metabolites.

Tissue ECFV was calculated according to the equation

$$\text{ECFV (ml/g)} = \frac{\text{Tissue [}^{3}\text{H-mannitol] (dpm/g)}}{\text{Plasma [}^{3}\text{H-mannitol] (dpm/ml)/Plasma } H_2O \text{ (ml/ml)}}, \quad (1)$$

where plasma water content was calculated from the refractive index.

^{3}H-mannitol proved to be an unsuitable marker for liver ECFV. After 12 h of equilibration, liver ECFV estimates were often as high as 600 ml/kg, when total tissue water was only 725 ml/kg. Therefore, it was obvious that mannitol-derived ECFV estimates were erroneous. In a separate experiment on eight resting fish, liver ECFV was estimated by carboxy-^{14}C-inulin and methoxy-^{14}C-inulin distribution, which yielded values not significantly different from one another and much lower than mannitol values. The inulin was repurified by passing it through a Sephadex G-50 column. The mean of these values, 169.5 ± 38.6 ml/kg (n = 8) was used in place of the mannitol-derived ECFV estimates. In this same experiment, white-muscle ECFV estimates based on inulin were not appreciably different from those based on mannitol. Thus mannitol-derived ECFV estimates for white muscle were assumed to be correct and used throughout.

Liver and muscle pHi were calculated according to equation (5) in the companion paper (Milligan and Wood 1987), where extracellular pH (pHe) was arterial pH, pK_{DMO} was taken from Malan, Wilson, and Reeves (1976), and [DMO]e was calculated from equation (6) in the companion paper (Milligan and Wood 1987) and

$$[\text{DMO}]i \text{ (dpm/ml)} = \frac{\text{Tissue [}^{14}\text{C-DMO] (dpm/g)} - (\text{ECFV (ml/g)} \times [\text{DMO}]e \text{ (dpm/ml)})}{\text{ICFV (ml/g)}}. \quad (2)$$

Estimates of metabolic acid load (ΔH^+m) in whole blood, liver, and muscle were calculated according to equation (1) in the companion paper (Milligan and Wood 1987) using the appropriate values for pH, $[HCO_3^-]$, and β for the compartment in question. As each fish was sampled only once, ΔH^+m was based on group means at each time compared to group means of the rest sample. $[HCO_3^-]$ in liver and muscle was estimated from pHi and plasma arterial CO_2 tension (Pa_{CO_2}), assuming CO_2 tension (P_{CO_2}) was in equilibrium between ECF and ICF. The lactate load (ΔLa^-) in whole blood, liver, and muscle was calculated for comparison with ΔH^+m by subtracting the mean rest concentration from the respective postexercise mean concentration at each sample time.

STATISTICAL ANALYSIS

Means ± 1 SEM (n) are reported throughout, unless stated otherwise. Differences between groups were tested for significance ($P < .05$) with Student's two-tailed t-test, unpaired design.

RESULTS

TISSUE BUFFER CAPACITIES

The buffer capacity of muscle was ~1.6 times that of liver (table 1). These values represent total physicochemical buffer capacity (i.e., nonbicarbonate + bicarbonate). However, since P_{CO_2} was kept low during titration, intracellular $[HCO_3^-]$ would be very low (<1 mmol/liter) and would not contribute significantly to the measured β value.

EXTRACELLULAR ACID-BASE, METABOLITE, AND ELECTROLYTE STATUS

Changes in hematology and extracellular (i.e., plasma) arterial pH (pHa), Pa_{CO_2}, and $[HCO_3^-]$ were qualitatively similar to those described in the companion paper (Milligan and Wood 1987) and thus are not shown. However, in the present study, the P_{CO_2} elevation was corrected much more rapidly (by 0.5 h) and fell below rest values at 2 h, an effect that persisted until 12 h. This in turn caused a significant increase in pHa (pHe), above the rest level, at 12 h (fig. 2). These effects could result from differences between repetitive versus single sampling or between aerated closed experimental chambers versus flow-through chambers. At rest, mean Pa_{O_2}, which was not measured in the companion paper, was 53.3 ± 12.5 torr (n = 5), typical of truly resting starry flounder (Wood, McMahon, and McDonald 1979). Pa_{O_2} fell by ~70% immediately after exercise to a mean of 16.3 ± 3.5 torr (n = 6), remained low at 0.5 h (21.9 ± 5.1 torr; n = 6), but by 2 h had fully recovered (45.8 ± 10.8 torr; n = 6) and remained unchanged through the remainder of the recovery period.

Whole-blood lactate levels were low at rest (0.1–0.2 mmol/liter; fig. 1) and increased after exercise. However, peak levels at 2–4 h were only three- to fourfold rest values. At no time did blood and muscle [lactate] come into equilibrium (fig. 1). At all times, ΔH^+m in blood was much greater than ΔLa^- (fig. 5). This pattern is similar to that described in the companion paper (Milligan and Wood 1987), though the peak blood lactate values attained were even lower in the present study (0.8 vs. 1.3 mmol/liter). Whole-blood [pyruvate] followed a similar pattern (table 2) to that of [lactate], though the absolute levels and elevations were much smaller. Blood levels remained similar to muscle levels throughout (table 2). Whole-blood [glucose] increased after exercise, peaking at 1.6 times rest levels at 0.5–2 h and returning to

TABLE 1

NONBICARBONATE BUFFER CAPACITIES (β) OF LIVER AND WHITE MUSCLE

	β MEAN ± SEM	
TISSUE (n)	mmol/pH/kg	mmol/pH/l ICF
White muscle (6) ...	−33.00 ± 4.56	−47.76 ± 6.83
Liver (6)	−20.89 ± 1.68	−37.46 ± 3.16

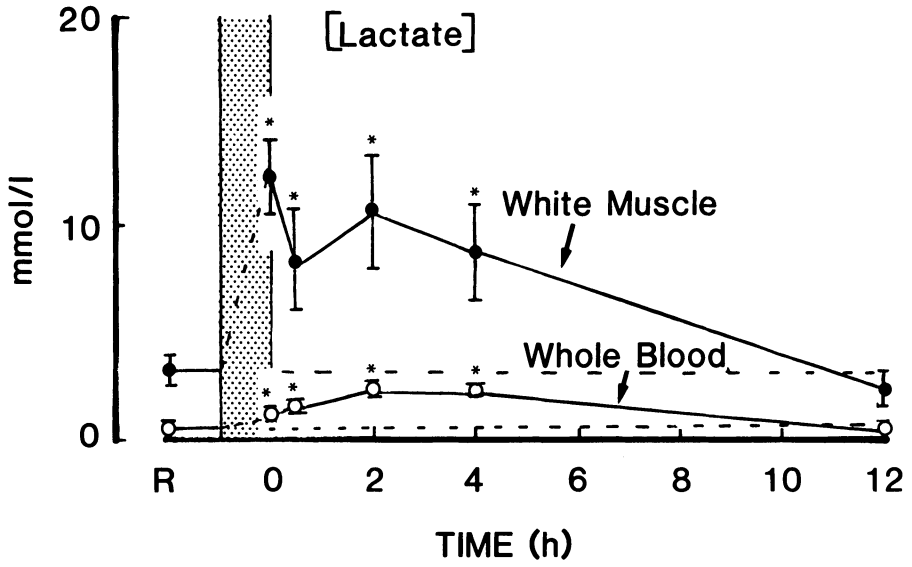

FIG. 1.—Effects of 10 min of enforced activity on white-muscle and whole-blood [lactate] in starry flounder. At all times, muscle levels were significantly higher than blood levels. R indicates rest value; vertical stippled bar indicates a 10-min period of activity; 0 h is immediately after activity. Each point represents the mean value and the vertical bars represent ±1 SEM; 2h, $n = 6$ for all points except R ($n = 5$) and 4 h ($n = 4$). * Indicates a significant difference ($P < .05$) from the corresponding rest value.

preexercise values by 4 h (table 2). Ammonia was rapidly cleared, so that by 2 h levels were not different from rest levels.

Plasma [Na^+], [Cl^-], and [Ca^{2+}] rose 13%–15% after exercise (table 3), similar to the increase in plasma [protein] observed in the companion paper (Milligan and Wood 1987), suggesting that these ion levels increased as a consequence of a reduction in plasma volume. The increase was short lived, though, since by 0.5 h into recovery they had returned to rest levels. Plasma [K^+] responded differently (table 3), increasing to a greater extent (by ~60%), remaining elevated for a longer period (up to 4 h), and returning to rest levels by 12 h.

TISSUE INTRACELLULAR ACID-BASE AND METABOLITE STATUS

At rest, white-muscle pHi averaged 7.56 ± 0.05 ($n = 5$; fig. 2), ~0.23 units below pHe, and fell to a minimum of 7.27 ± 0.02 ($n = 6$; fig. 2) immediately after activity. By 4 h, muscle pHi had returned to a level not significantly different from rest values, and at 12 h it was slightly, though not significantly, above the rest value. The immediate postexercise change in pHe was

TABLE 2

BLOOD AND WHITE-MUSCLE METABOLITES PRIOR TO AND FOLLOWING ACTIVITY IN THE STARRY FLOUNDER

	MEAN ± 1 SEM (n) AT (mmol/liter)					
METABOLITE	Rest (5)	0 H (6)	.5 H (6)	2 H (6)	4 H (4)	12 H (6)
Blood [pyruvate]	.03 ± .01	.10 ± .03[a]	.14 ± .01[a]	.23 ± .03[a]	.23 ± .05[a]	.03 ± .01
Muscle [pyruvate]	.25 ± .11	.12 ± .04	.24 ± .06	.39 ± .07	.51 ± .17[a]	.14 ± .05
Blood [glucose]	1.18 ± .12	1.78 ± .42	1.83 ± .23[a]	1.97 ± .29[a]	1.32 ± .25	1.35 ± .09
Muscle glycogen[b]	38.56 ± 4.87	19.32 ± 2.52[a]	22.71 ± 3.12[a]	35.58 ± 4.57	11.62 ± 2.89[a]	32.58 ± 7.52
Plasma [ammonia] ...	.17 ± .07	.52 ± .12[a]	.37 ± .07[a]	.14 ± .03	.13 ± .05	.09 ± .04

[a] Significantly different ($P < .05$) from corresponding rest value.
[b] Expressed as glucose units.

TABLE 3

PLASMA IONS PRIOR TO AND FOLLOWING ACTIVITY IN THE STARRY FLOUNDER

ION	MEAN ± 1 SEM (*n*) AT (meq/liter)					
	Rest (5)	0 H (6)	.5 H (6)	2 H (6)	4 H (4)	12 H (6)
$[Na^+]$	167.8 ± 4.1	189.0 ± 8.9[a]	163.3 ± 5.4	169.4 ± 3.3	173.5 ± 8.3	160.8 ± 4.1
$[Cl^-]$	151.4 ± 5.1	171.3 ± 8.4[a]	146.2 ± 4.9	154.4 ± 4.0	158.8 ± 13.7	146.3 ± 3.7
$[K^+]$	2.7 ± .1	4.3 ± .3[a]	2.8 ± .2	3.1 ± .1[a]	3.3 ± .3[a]	2.9 ± .1
$[Ca^{2+}]$	5.7 ± .7	6.6 ± .3[a]	5.7 ± .2	6.1 ± .4	6.4 ± .9	5.1 ± .5

[a] Significantly different ($P < .05$) from corresponding rest value.

greater than that in pHi (0.37 vs. 0.29 units), resulting in a reduction in the pHe-pHi gradient from 0.23 to 0.15 pH units. A plot of the muscle data on a pH-$[HCO_3^-]$ diagram (fig. 3) indicated that immediately after exercise the intracellular acidosis was of mixed respiratory and metabolic origin, with the latter factor clearly predominating. Thereafter, the acidosis was completely metabolic. In plotting these data, we assumed that Pa_{CO_2} was representative of intracellular P_{CO_2}. While this assumption may lead to an underestimate of true intracellular P_{CO_2}, it will not appreciably alter the interpretation of the data.

Accompanying this metabolic acidosis

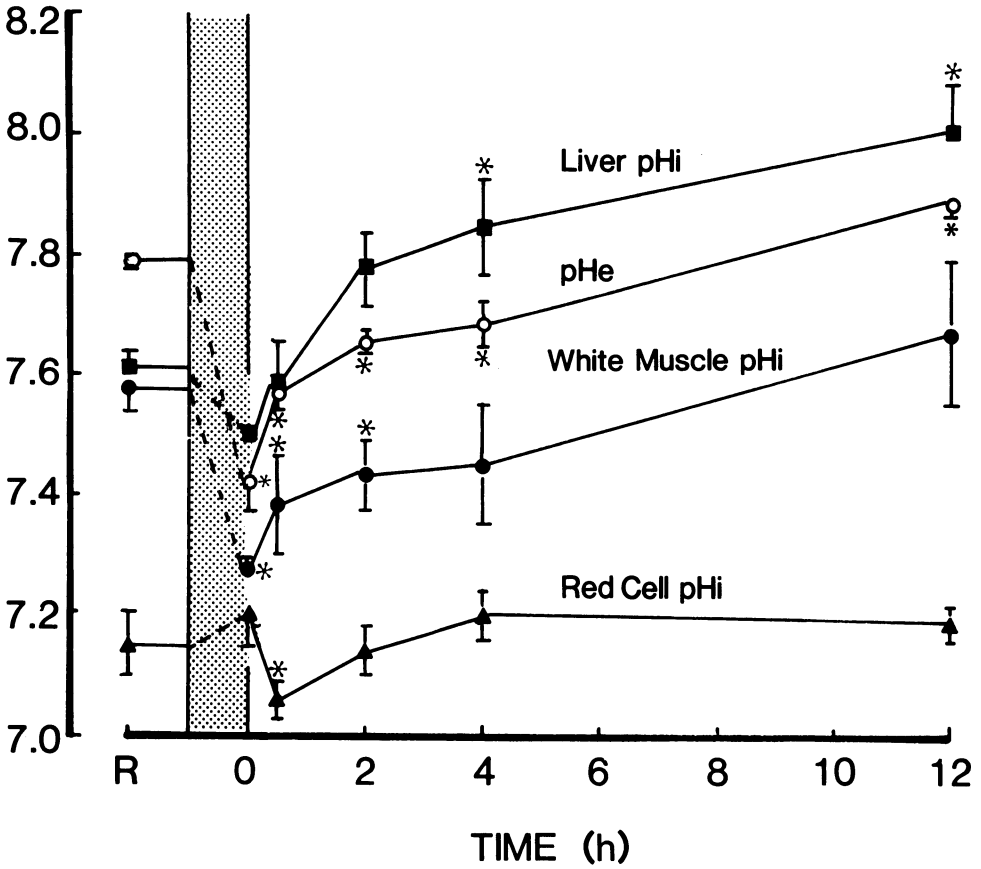

FIG. 2.—Response of liver pHi (■), muscle pHi (●), RBC pHi (▲), and pHe (pHa; ○) to 10 min of activity. Other details are as in the legend to fig. 1.

was a fourfold increase in muscle [lactate] (fig. 1) and an ~50% reduction in glycogen content (table 2). Levels of glycogen were variable, but its disappearance was more than adequate to account for the increase in [lactate]. Muscle [lactate] remained elevated through 4 h, returning to rest levels by 12 h (fig. 1). Glycogen restoration tended to parallel lactate disappearance. Muscle [pyruvate] followed an entirely different pattern from that of [lactate], increasing only at 4 h into recovery, when levels doubled (table 2). By 12 h, [pyruvate] had returned to rest levels.

Liver pHi at rest was slightly higher than muscle pHi at rest, averaging 7.60 ± 0.04 (n = 5; fig. 2). Immediately after exercise, liver pHi tended to fall, though the change was not significant. A pH-[HCO_3^-] diagram analysis indicated that this acidotic tendency was due entirely to the increase in P_{CO_2} (fig. 3*B*). By 2 h, liver pHi began to increase, and by 4 h the elevation became significant, with pHi averaging 7.86 ± 0.08 (n = 4; fig. 2). Metabolic base continued to accumulate (or conversely, metabolic acid was lost; fig. 3*B*), so that by 12 h, liver pHi averaged 8.02 ± 0.08 (n = 6; fig. 2), 0.42 units above the rest level. These changes in liver acid-base status were not accompanied by changes in metabolic status, for neither [lactate], [pyruvate], nor glycogen content changed, though again the data were highly variable (table 4).

By comparison with liver and white-muscle pHi, RBC pHi was quite low at rest, 7.16 ± 0.04 (n = 5; fig. 2). Despite the large drop in pHe and increase in P_{CO_2} immediately after activity, RBC pHi remained fairly constant, showing a significant decline only at 0.5 h and recovering by 2 h. At 12 h, when pHe exhibited alkalosis, pHi was unchanged, further demonstrating the ability of the RBCs to regulate pHi independently of changes in pHe.

FLUID-VOLUME DISTRIBUTION

Immediately following activity, white muscle exhibited a significant cell swelling, with ICFV increasing by 40–50 ml/kg (fig. 4*B*). Since total tissue water did not vary at any time in the experiment (808.9 ± 2.6 ml/kg; n = 40), there was a reciprocal contraction of the ECFV (fig. 4*A*). Neither liver (718.8 ± 8.9 ml/kg; n = 40) nor total body water (784.0 ± 2.9 ml/kg; n = 40) changed as a consequence of exercise.

DISCUSSION

METHODOLOGY

The potential problem of DMO disequilibrium between ICFV and ECFV in the dynamic postexercise situation has been considered and discounted in the companion paper (Milligan and Wood 1987). A second potential source of error in the pHi estimate is the assumption that plasma pHa is representative of the ECF. The white-muscle mass is perfused with both arterial and venous blood, with the true interstitial pH lying between pHa and venous pH (pHv). The teleost liver, however, is perfused mainly by venous blood (Smith and Bell 1976). However, at least in rainbow trout, we have shown that arterial versus venous pH, [DMO], and [mannitol] differences were insignificant except immediately after exercise and that even then they tended to self-compensate, thus having negligible effect on calculated whole-body and tissue pHi (Milligan and Wood 1986*a*, 1986*b*).

Use of mannitol for an ECFV marker in flounder liver proved unsuccessful. A similar difficulty was encountered in estimating liver and heart ECFV with mannitol in rainbow trout (Milligan and Wood 1986*b*). The inulin-derived estimates for liver ECFV in resting flounder were similar to those obtained from inulin distribution (C. M. Wood and S. Munger, unpublished data) and from PEG-4000 and Cl^--K^+ space estimates in trout (Houston and Mearow 1979). In using this correction, it was assumed that exercise did not cause a fluid shift in the liver. In the calculation of pHi by equation (5) in the companion paper (Milligan and Wood 1987), a ±50 ml/kg change in ECFV will alter pHi by ±0.02–0.03 pH units, which is outside the limits of detection of the DMO method.

Adcock and Dando (1983) have recently emphasized the importance of rapid tissue fixation via freeze-clamping to avoid artifactual overestimation of tissue lactate and, to a lesser extent, pyruvate levels through "sampling anoxia." While we did not use freeze-clamping, our biopsy needle–fixation technique probably froze the tissue almost

as quickly and much faster than the classical immersion method. Thus our muscle lactate rest levels are identical and our muscle pyruvate rest levels are actually lower than those obtained by Adcock and Dando (1983) via freeze-clamping in two similar flatfish. They are also much lower than the values obtained by them and other workers via immersion freezing.

EXTRACELLULAR ACID-BASE, METABOLITE, AND ELECTROLYTE STATUS

The postexercise changes in plasma pH, $[HCO_3^-]$, and P_{CO_2} were similar to those described in the companion paper (Milligan and Wood 1987). Whole-blood P_{O_2} fell after exercise. A decline in P_{aO_2} implies a diffusive limitation to gill O_2 transfer, per-

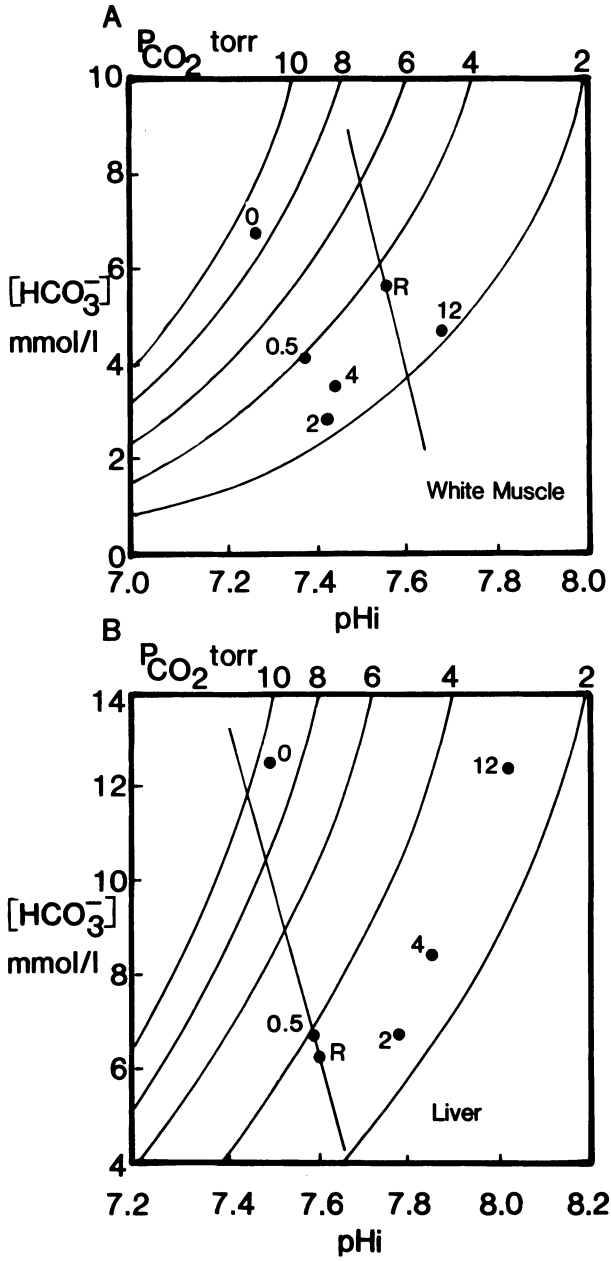

FIG. 3.—pHi-$[HCO_3^-]$ diagrams of changes in intracellular acid-base status after 10 min of enforced activity in white muscle (*A*) and liver (*B*). Straight lines plotted are the tissue in vitro nonbicarbonate buffer capacities from table 1; isopleths are P_{CO_2}. Means are plotted. Other details are as in the legend to fig. 1.

TABLE 4

LIVER METABOLITES PRIOR TO AND FOLLOWING ACTIVITY IN THE STARRY FLOUNDER

METABOLITE	MEAN ± 1 SEM (*n*) AT (mmol/liter)					
	Rest (6)	0 H (5)	.5 (5)	2 H (5)	4 H (4)	12 H (6)
[Lactate] . . .	1.49 ± .44	1.49 ± .23	1.71 ± .54	1.60 ± .38	1.20 ± .32	1.50 ± .28
[Pyruvate] . .	.39 ± .14	.19 ± .10	.49 ± .34	.18 ± .06	.39 ± .17	.38 ± .13
[Glycogen][a]	291.3 ± 56.1	189.5 ± 60.2	128.0 ± 37.7	330.5 ± 70.3	179.6 ± 64.2	177.8 ± 18.0

NOTE.—There were no significant changes in liver metabolites after exercise.

[a] Expressed as glucose units.

haps as a consequence of a reduction in gill-blood transit time owing to an increase in cardiac output (Perry, Daxboeck, and Dobson 1985; Wood and Perry 1985). The fact that Pa_{O_2} fell by ~70% and Pa_{CO_2} more than doubled (from 3.2 ± 0.1 torr [n = 5] at rest to 8.4 ± 0.5 torr [n = 6] at 0 h) would suggest that diffusive limitations existed. The response of Pa_{O_2} to exercise differs between and even within species. Using methodology identical to that of the present study, though with a different batch of starry flounder (collected in 1984 vs. 1982), Wood and Milligan (1987) reported no change in Pa_{O_2}. Similarly, in rainbow trout, Kicenuik and Jones (1977) and Holeton, Neumann, and Heisler (1983) reported no fall in Pa_{O_2} after exhaustive exercise, whereas Primmet et al. (1986) found that Pa_{O_2} declined significantly. Possible explanations include differences in exercise regimes and fish populations, though the underlying physiological reasons are unclear.

Changes in whole-blood [pyruvate] after exercise followed a pattern similar to that of blood [lactate] (fig. 1, table 2). The relative changes in [pyruvate] were greater, which resulted in a reduction in the blood lactate:pyruvate ratio from ~7.5 at rest to ~3.9 at 0 h. Over the same period, the muscle lactate:pyruvate ratio *increased* from 12.9 to 100.2 (table 2). A similar reduction in the blood lactate:pyruvate ratio was observed after exercise in the inactive, benthic flathead sole (Turner, Wood, and Clark 1983*a*). By way of contrast, after exercise in more active fish, such as rainbow trout, there was a four- to fivefold increase in the blood lactate:pyruvate ratio (Turner, Wood, and Hōbe 1983*b*). This species difference is not due to the pyruvate response, for the absolute increases were similar in flounder, sole, and trout, but rather to a difference in the lactate response. After exercise in trout, peak blood [lactate] often reached 20 mmol/liter (Turner et al. 1983*a*), versus 0.5–2 mmol/liter in flounder (fig. 1) and sole. Thus it would appear that, in flatfish, movement of pyruvate from the muscle to blood is not subject to the same restrictions as is lactate movement.

The rather abrupt, short-lived rise in plasma [ammonia] (table 3) was most likely a reflection of increased ammoniagenesis in white muscle (Driedzic and Hochachka 1976). Similar effects have been seen in the rainbow trout and flathead sole (Turner et al. 1983*a*, 1983*b*). Branchial clearance of this ammonia load probably explained the small increase in ammonia excretion to the water after exercise (Milligan and Wood 1987). Whole-blood [glucose] (table 2) increased, presumably as a consequence of breakdown and mobilization of liver glycogen (Driedzic and Hochachka 1975).

The postexercise changes in plasma electrolytes (table 3) were similar to those reported for flathead sole (Turner et al. 1983*b*). Increases in plasma [Na^+], [Cl^-], and [Ca^{2+}] were nearly identical to the postexercise elevation in plasma [protein] observed in the companion study (Milligan and Wood 1987), indicating causation by the reduction in whole-body ECFV (Milligan and Wood 1987). The two- to threefold greater increase in plasma [K^+] is commonly observed after glycolytic activity in vertebrates, owing to a release, induced by acidosis, of K^+ from the muscle ICFV (Woodbury 1974).

INTRACELLULAR ACID-BASE STATUS OF TISSUE AT REST

RBCs had the lowest rest pHi of the tissues examined, similar to pHi values reported for other fish, such as the rainbow trout (Milligan and Wood 1986*a*, 1986*b*; Primmet et al. 1986).

Flounder white-muscle pHi (fig. 2) was 0.2–0.3 units greater than values reported for white muscle from more active pelagic species, such as rainbow trout (Hōbe, Wood, and Wheatly 1984; Milligan and Wood 1986*b*), dogfish (Heisler, Weitz, and Weitz 1976), and eel (Walsh and Moon 1982). However, it was not dissimilar to values reported for the relatively inactive, demersal catfish (Cameron and Kormanik 1982) and sea raven (Milligan and Farrell 1986). These interspecies differences may be related to muscle lactate levels, for the active species with the lower pHi values generally tended to have higher muscle lactate levels (8–10 mmol/kg vs. 1–3 mmol/kg).

On the other hand, the level of flounder liver pHi (fig. 2) was very similar to those reported for eels (Walsh and Moon 1982, 1983) and rainbow trout (Milligan and Wood 1986*b*). From these limited observations in fish and from those in mammals

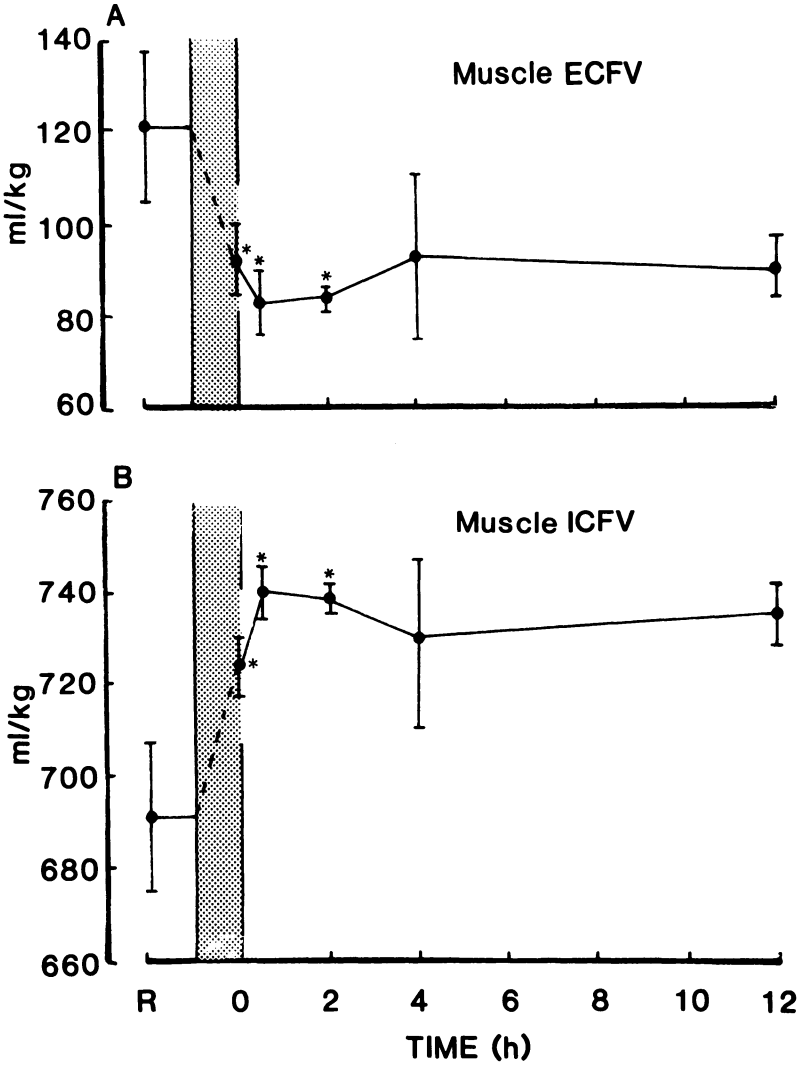

FIG. 4.—White-muscle (*A*) ECFV and (*B*) ICFV prior to and following 10 min of activity. Other details are as in the legend to fig. 1.

(see Roos and Boron 1981), it can be inferred that liver generally has a higher resting pHi than does muscle, which may reflect differences in tissue metabolic state and/or membrane potential at rest.

The tissue buffer capacities followed a different sequence, with that of white muscle greater than that of liver (table 1). RBC buffer capacity was not measured. Similar trends have been reported for fish (e.g., trout [Milligan and Wood 1986*b*] and catfish [Cameron and Kormanik 1982]) and mammals (Roos and Boron 1981). Variations in tissue buffer capacity are related to total amount of histidine-related compounds (HRC) present; those tissues with a greater HRC content have a greater buffer capacity (Abe et al. 1985). The absolute value for flounder white muscle is similar to that of the inactive, demersal catfish (Cameron and Kormanik 1982) but much less than values reported for rainbow trout (Castellini and Somero 1981; Milligan and Wood, 1986*b*) and dogfish (Heisler and Neumann 1980). Since white-muscle buffer capacity is positively correlated with glycolytic potential (as determined by lactate dehydrogenase activity; Castellini and Somero 1981), the relatively low buffer capacity of flounder muscle was to be expected.

POSTEXERCISE CHANGES

After 10 min of strenuous activity, flounder white muscle exhibited a severe drop in pHi, which was mainly due to an accumulation of metabolic protons (figs. 2, 3). The source of metabolic protons produced during glycolysis in vertebrate muscle is adenosine triphosphate (ATP) hydrolysis, which, when tightly coupled to glycolysis, will result in a 1:1 stoichiometry between H^+ and lactate production (Gevers 1977; Hultman and Sahlin 1980; Hochachka and Mommsen 1983). In flounder muscle after exercise, H^+ appeared to accumulate in excess of lactate (fig. 5*B*), suggesting that ATP hydrolysis and glycolysis became uncoupled (i.e., that ATP consumption exceeded production). Since terminal rather than serial samples were taken, with ΔH^+m and ΔLa^- calculated from the mean at each time, the significance of the discrepancy between H^+ and lactate cannot be assessed statistically. However, similar patterns (i.e., ΔH^+m in excess of ΔLa^-) have been observed in rainbow trout (Milligan and Wood 1986*b*) and sea raven (Milligan and Farrell 1986) after exhaustive exercise. In the trout the excess of ΔH^+m over ΔLa^- was equivalent to the decline in ATP levels.

The liver displayed a slight respiratory acidosis after exercise (figs. 3*B*, 5*C*), which was cleared by 0.5 h. Through the remainder of the recovery period, the liver exhibited a pronounced alkalosis of metabolic origin (fig. 5*C*). A similar, though less severe, alkalosis occurred in trout liver after exercise (Milligan and Wood 1986*b*). However, in contrast to the results of the present study (table 4), trout liver accumulated lactate, suggesting that alkalosis was due to an uptake of lactate independent of H^+ and subsequent metabolism. The alkalosis in flounder liver may have been of similar genesis, with lactate turnover being so rapid that no net accumulation was evident. However, the relatively low lactate dehydrogenase activity (a necessary enzyme for lactate metabolism) generally found in the livers of inactive species (e.g., plaice, sole, flounder [Dando 1969] and sea raven [Walsh, Moon, and Mommsen 1985]) would tend to argue against this. Furthermore, if a tissue is metabolizing lactate, an increase in pyruvate is usually observed (Dando 1969); this did not occur in flounder liver (table 4). It is possible that the alkalosis was not due to metabolism but rather arose from transmembrane transport of either H^+ or basic equivalents. The physiological significance of the alkalosis is unclear, though it could be important in controlling hepatic metabolism during recovery from exercise. It is unlikely that the rise in liver pHi was an artifact of the accumulation of DMO by the liver over time, since the experiments were designed such that at each sampling period the DMO had been in the animal for approximately the same length of time (10–12 h). Nevertheless, the result is so unusual that confirmation by an independent technique (e.g., nuclear magnetic resonance, microelectrode) would be worthwhile at some future time.

Unlike liver and muscle pHi, RBC pHi tended to remain constant after exercise, except for the acidosis evident at 0.5 h (fig. 2). This maintenance of RBC pHi after exercise has also been observed in striped bass

(Nikinmaa, Cech, and McEnroe 1984) and rainbow trout (Milligan and Wood 1986*b*; Primmet et al. 1986) and has been attributed to a β-adrenergic influence of catecholamines (Nikinmaa 1982, 1983; Nikinmaa et al. 1984). A similar mechanism is thought to operate in flounder (Wood and Milligan 1987). Deoxygenation of the hemoglobin owing to low Pa_{O_2} may also have helped sustain pHi via the Haldane effect.

THE FATE OF LACTATE AND H^+

Strenuous activity in flounder resulted in lactate production within the muscle, though there was little accumulation in the blood space (fig. 1) compared to that seen in the more active fish (e.g., dogfish [Piiper, Meyers, and Drees 1972] and trout [Turner et al. 1983*a*; Milligan and Wood 1986*b*]). As discussed in the companion paper (Milligan and Wood 1987), this pattern of lac-

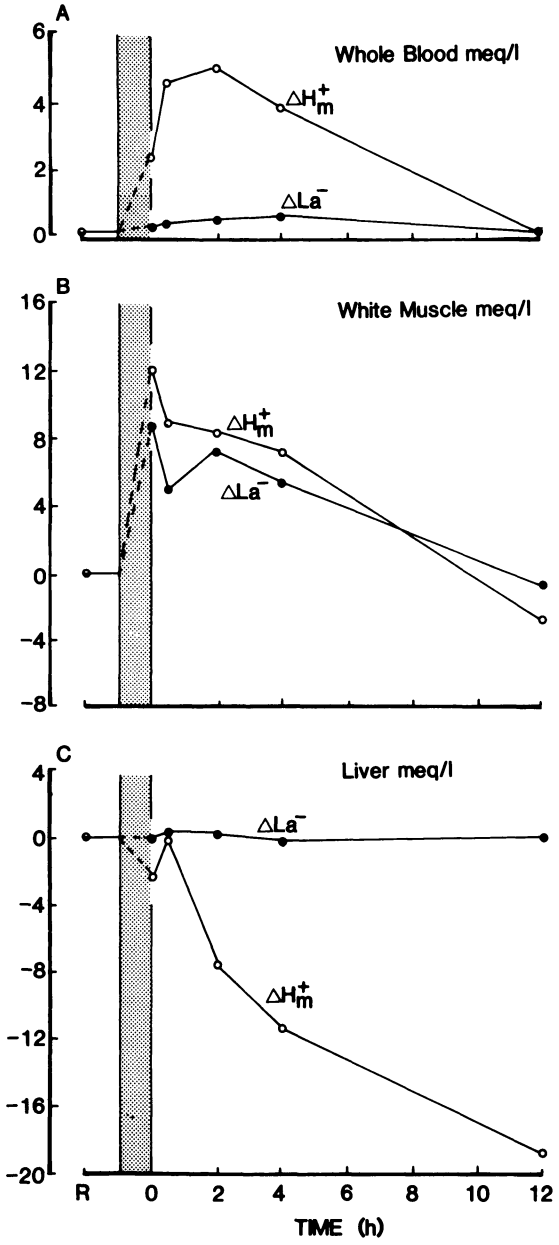

FIG. 5.—ΔH^+m (○) and ΔLa^- (●) after 10 min of activity in whole blood (*A*), muscle (*B*), and liver (*C*) (see text for explanation). Other details are as in the legend to fig. 1.

tate nonaccumulation appears to be characteristic of relatively inactive fish species. It is possible that lactate efflux is perfusion limited, owing to a low blood flow through the white-muscle mass. However, if this were the situation, then ΔH^+m appearance in blood should have been affected to the same extent. Furthermore, in white muscle ΔH^+m declined faster than ΔLa^- (fig. 5*B*), and ~20% of the total H^+ load passed through the ECFV to be temporarily stored in the environmental water (Milligan and Wood 1987). These findings indicate that H^+ efflux from muscle in fact exceeded lactate efflux—and that lactate was retained, as suggested by Wardle (1978) and Batty and Wardle (1979).

We suggest that most of the lactate and the greater proportion of the H^+ remained in the white-muscle mass and were metabolized in situ, i.e., that the Cori cycle was of little importance. The partial efflux of H^+ in combination with this metabolism would explain the rapid correction of muscle pHi and subsequent intracellular alkalosis (Milligan and Wood 1987). Fish white muscle is capable of lactate oxidation, albeit at lower rates than aerobic tissues (e.g., heart and red muscle; Bilinski and Jonas 1972). For flounder muscle to clear ΔLa^- via in situ metabolism would require an average O_2 consumption of 2 mmol O_2/kg/h (assuming that 3 mol O_2 were consumed/mol lactate metabolized; Newsholme and Leech 1983), well within reported O_2 consumption rates for muscle (Gordon 1972). Furthermore, there is a growing body of physiological and biochemical evidence that vertebrate muscles are capable of in situ glyconeogenesis (e.g., in flatfish [Wardle, 1978; Batty and Wardle 1979], frog [Bendall and Taylor 1970; Connett 1979], rabbit [Bendall and Taylor 1970; Dyson, Cardenas, and Barsotti 1975], rat [McLane and Holloszy 1979; Constable et al. 1984], and man [Hermansen and Vaage 1977]). Thus it is not unreasonable to suggest that lactate and H^+ may also have been used as substrates for in situ glyconeogenesis. The observed late rise in muscle [pyruvate] at 4 h (table 2) lends support to this argument.

Regarding trout white muscle after exercise, we have similarly argued that lactate and H^+ are metabolized in situ, initially by oxidation and later by glyconeogenesis (Milligan and Wood 1986*b*). Interestingly, recovery of white-muscle pHi was more rapid in flounder (complete before recovery of pHe, i.e., at 4–8 h) than in trout (complete at 8–12 h), and this faster pHi recovery was associated with a more rapid restoration of muscle glycogen stores (at 12 h in flounder vs. 24 h in trout). A similar pattern of muscle H^+ and lactate clearance was observed after exercise in the sluggish sea raven (Milligan and Farrell 1986). We suggest that restoration of both glycogen stores and the potential for further metabolism in white muscle is of prime importance in the relatively inactive species and that rapid correction of the intracellular acidosis in preference to the extracellular acidosis facilitates this process. The fish's survival depends more on the ability to use the muscle again glycolytically as soon as possible rather than on the capacity for aerobic respiration and blood O_2 transport.

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