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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[1]

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Exhaustive activity in starry flounder resulted in an acidosis in the whole-body extracellular fluid (ECF) and intracellular fluid (ICF) compartments. In the ECF, the acidosis consisted of a short-lived respiratory component (increase in CO_2 tension [P_{CO_2}]) followed by a longer-lived metabolic component (decrease in [HCO_3^-]). The acid-base disturbance was corrected by 8–12 h. There was little lactate accumulation in the blood, with levels rarely greater than 1–2 mmol/liter, and at all times the blood metabolic acid load (ΔH^+m) was in excess of the blood lactate load (ΔLa^-). Blood [glucose] increased by 50%. Whole-body extracellular fluid volume (ECFV) fell by 17% owing to a shift of fluid into the intercellular fluid volume (ICFV), causing a general hemoconcentration. Exercise also caused an acidosis in the whole-body intracellular compartment, with intracellular pH dropping from a rest value of 7.58 to a low of 7.24. The whole-body intracellular acidosis was corrected ~4 h sooner than the extracellular disturbance and became alkalotic at 8 h, returning to normal at 12 h. Associated with this acid-base disturbance was an increased efflux of acidic equivalents (H^+) to the environmental water, coincident with a large increase in the titratable-acidity flux. Ammonia excretion increased only slightly. Analysis of the distribution of metabolic acid between the ECF, ICF, and environmental water revealed that until 4 h postexercise, the bulk of the acid load remained in the intracellular compartment. Approximately 20% passed through the extracellular fluid and was transiently stored in the environmental water at 4–12 h. This flux of H^+ to the water was associated with an intracellular alkalosis and thus appeared to hasten correction of intracellular acid-base status, perhaps as a means of aiding metabolic recovery.

INTRODUCTION

Strenuous activity in fish results in a pronounced acid-base disturbance in the extracellular fluid (ECF) compartment that has been well characterized in terms of its nature and duration (see Wood and Perry 1985). Pronounced perturbations in both extracellular and muscle intracellular metabolites have also been documented (Black et al. 1960, 1962; Wardle 1978; Batty and Wardle 1979; Turner, Wood, and Clark 1983; Turner, Wood, and Hōbe 1983; Milligan and Farrell 1986). Some portions of the acidic equivalents (H^+) and lactate ions produced in muscle move out into the blood, where their fates are uncertain, as are the fates of the portions remaining in the myotome. The actual pattern of lactate and H^+ accumulation in the blood is species dependent, apparently correlated with the fish's ecology. In the benthic, inactive starry flounder, appearance of lactate in the blood is minimal and the H^+ load is far in excess of the lactate load (ΔLa^-) (Wood, McMahon, and McDonald 1977), a discrepancy opposite that in active species, such as rainbow trout (Turner et al. 1983*a*). Little is known about the mechanism(s) leading to these lactate versus H^+ discrepancies in the blood or about the possible effects of the extracellular acidosis on other tissues. Our knowledge about acid-base changes in muscle, the major site of glycolysis, and

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possible relationships between intracellular pH (pHi) and metabolic events is similarly sparse.

The present study focuses on acid-base changes in the whole-body extracellular fluid (ECF), intracellular fluid (ICF), and environmental water after strenuous activity in the starry flounder, with particular emphasis on transcompartmental exchanges of metabolic acid (i.e., nonvolatile, fixed acid, as opposed to CO_2 [respiratory acid]). A companion paper (Milligan and Wood 1987) documents simultaneous changes in acid-base and metabolite status of individual tissues. A third paper (Wood and Milligan 1987) examines possible adrenergic controls on H^+ and lactate dynamics in exercised starry flounder. These studies complement our previous work on the active, pelagic rainbow trout (*Salmo gairdneri;* Milligan and Wood 1986*a,* 1986*b*). The overall goal was to compare the strategies of two very different fishes that inhabit different environments (seawater vs. freshwater), possess different life-styles (benthic vs. pelagic), and, more important, differ in their exercise performance (relatively inactive vs. active). We have used the DMO method (Waddell and Butler 1959) to investigate the intracellular acid-base disturbance associated with exercise. Recently this technique has been validated for detecting pHi transients in fish over a time course similar to that likely to occur after exercise (Milligan and Wood 1985).

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Starry flounder (*Platichthys stellatus;* 150–1,200 g) of both sexes were collected by otter trawl from East Sound, Orcas Island, and Birch Bay, Washington, from October through December 1982. Fish were held in large (6,800-liter) tanks with sand-covered bottoms supplied with fresh running seawater (29‰) at seasonal temperature (11 ± 1 C) at Friday Harbor Laboratories, University of Washington. During holding, fish fed ad lib. on other small fishes and invertebrates present in the tank. For 3–5 days prior to experimentation, flounder were acclimated to laboratory conditions indoors in Plexiglas, sand-covered tanks supplied with fresh seawater, during which period they were starved.

Caudal artery catheters were surgically implanted according to the method of Watters and Smith (1973) while fish were under MS:222 (1:10,000; Sigma) anesthesia. The caudal artery was exposed by making an incision (~3 cm) in the caudal peduncle just ventral to the lateral line. A nick was made in the artery with a 26-gauge needle, and an ~30-cm length of PE-50 tubing (Clay-Adams) filled with heparinized saline was fed anteriorly into the vessel to the level of the heart. The wound was dusted with oxytetracycline hydrochloride (Syndel Labs, Vancouver), to prevent infection, prior to closing it with silk sutures. Catheters were filled with heparinized (50 IU/ml) Cortland saline (Wolf 1963) adjusted to 160 mM NaCl. Fish were then placed in 15-liter plastic tubs supplied with fresh-flowing seawater (1.5 liters/min) at 11 ± 1 C and allowed to recover for ≥72 h before experimentation. These tubs were fitted with an aeration ring around the perimeter, which allowed them to be operated as low-volume (1 liter/100 g body wt) closed systems for flux measurements. Temperature was maintained at 11 ± 1 C by bathing the tubs in flowing seawater.

Resting values for many physiological parameters are never attained when flounder are held in bare tanks, for the fish lack substrate in which to bury (Wood, McMahon, and McDonald 1979*a,* 1979*b*). Sand could not be used because it prevented thorough mixing and supported microorganism growth that would influence H^+ and ammonia-flux measurements. Instead, a piece of black plastic mesh, under which the flounder could lie, was mounted 5–10 cm from the bottom of the tub. This appeared to mimic the effect of sand satisfactorily, for the fish did not exhibit any undue activity and key physiological parameters, such as arterial pH (pHa) (fig. 1*A*) and arterial O_2 tension (Pa_{O_2}), were typical of those previously reported for resting, buried flounder (Wood et al. 1979*a,* 1979*b*).

EXPERIMENTAL PROTOCOL

These experiments focused on postexercise changes in arterial acid-base and lactate status; whole-body pHi and ECFV; H^+ exchanges with the environment; and ammonia, ^{3}H-mannitol, and ^{14}C-DMO excretion. Parallel experiments were run on two

groups, only one of which ($n = 8$) was subjected to exercise. The other group ($n = 8$) served as a control for sampling effects. These fish were sampled in the same fashion as the exercise group but otherwise were left at rest throughout.

Twenty-four hours prior to exercise, the inflow to the tub was closed, the volume standardized to 1 liter/100 g body wt, and thereafter water was recirculated within the tub by means of aeration. The tub was thoroughly flushed with fresh seawater at experimental temperature (11 ± 1C) for ~20 min at 12 h prior to exercise, at the time of exercise, and then again at 12 and 24 h after exercise, at which point the experiment was terminated. Thus, two 12-h control periods at rest were followed by two 12-h periods after exercise. The water was flushed at 12-h intervals to prevent ammonia levels in the tub from exceeding 500 μmol/liter. At the beginning of the 12-h flux period prior to exercise (i.e., the second control period), fish were infused with a 1 ml/kg dose of 5 μCi/ml ^{14}C-DMO (5,5-dimethyl-2,4-oxazolidinedione; New England Nuclear; specific activity 50.0 mCi/mmol) plus 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. This allowed 12 h for marker distribution before the first blood sample was taken. Water samples for ammonia, titratable acidity, and ^{3}H and ^{14}C radioactivity analysis were taken at the start and end of each 12-h period prior to exercise, immediately after exercise, and at 1, 2, 4, 8, 12, and 24 h after exercise. Thus, net fluxes of ammonia and H^+ as well as losses of markers to the environment could be followed throughout.

To induce strenuous activity, flounder were transferred to a shallow rectangular tank (128 × 98 × 9 cm) and then vigorously chased by hand for 10 min (see Wood et al. 1977). By the end of this period, fish were unable to either burst swim or swim slowly. Fluxes of titratable acidity and ammonia and the loss of markers to the water could not be measured during the exercise period. However, even if such fluxes had occurred at up to five times the immediately postexercise rates during the 10-min exercise period, their influence on all calculated parameters would have been negligible.

Arterial blood samples (500 μl) were drawn prior to ("rest") and immediately after exercise (0 h), as well as at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after activity. Samples were analyzed for pH, total CO_2 (in both plasma and whole blood), hematocrit (Ht), [hemoglobin] ([Hb]), whole-blood [glucose] and [lactate], plasma [protein], and plasma levels of ^{3}H and ^{14}C radioactivity. The volume of blood sampled was replaced with saline.

ANALYTICAL TECHNIQUES AND CALCULATIONS

Arterial blood pH was determined on 40-μl samples injected into a Radiometer pH electrode (type E5021) maintained at 11 C and linked to a Radiometer PHM 71 or PHM 72 acid-base analyzer. Total CO_2 in both whole blood and plasma was measured on 50-μl aliquots by the method of Cameron (1971), using Radiometer CO_2 electrodes (type E5046). CO_2 tension (P_{CO_2}) and [HCO_3^-] in blood and plasma were calculated using the Henderson-Hasselbalch equation, using αCO_2 and pK′ values reported by Boutilier, Heming, and Iwama (1984).

Whole-blood ΔH^+m was calculated in the cumulative fashion described by Turner et al. (1983*a*), using the following equation:

$$\Delta H^+m\,(\text{meq/liter}) = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2) \quad (1)$$

and summing (taking account of the sign) for each period from the rest sample onward. In this equation, [HCO_3^-] was the value for whole blood because whole-blood ΔH^+m (ΔH^+m_{WB}) was compared to whole-blood [lactate] and β, the nonbicarbonate buffer capacity of whole blood, was estimated from the blood [Hb] at each time. The relationship between β, [Hb], and Ht for whole blood and plasma was determined by in vitro CO_2 titrations. Blood was drawn from the arterial catheters of five resting flounder, heparinized at 250 IU/ml, pooled, gently centrifuged to separate plasma from red blood cells, then reconstituted to 6 Ht's ranging from 0% to 35%.

Tonometry, analyses, and estimation of β over the physiological range of pHa (fig. 1*A*) for each [Hb] and Ht were carried out according to the method described by Wood, McDonald, and McMahon (1982).

Ht (by centrifugation), [Hb] (as cyanmethemoglobin), and their ratio (mean cell Hb concentration; MCHC) were measured according to the method of Milligan and Wood (1982). Whole-blood [lactate] was measured on 100 μl whole blood fixed in 200 μl ice-cold 6% perchloric acid. Samples were mixed, placed on ice for 5 min, and then centrifuged at 9,000 *g* for 3 min. The samples were stored at 5 C for not longer than 48 h prior to analysis. The concentration of lactate in the supernatant was analyzed using the l-lactate dehydrogenase/NADH method described by Loomis (1961), using Sigma reagents. Whole-blood [glucose] was analyzed on 100-μl samples deproteinized in 900 μl ice-cold 3% trichloroacetic acid, using the *O*-toluidine method of Hyvarinon and Nikkita (1962) and Sigma reagents. Plasma [protein] was determined using refractometry (American Optical).

For determination of ^{3}H-mannitol and ^{14}C-DMO radioactivity in the ECF, 50 μl of plasma was added to 10 ml scintillation fluid (ACS; Amersham) and counted on a Beckman LS-250 liquid scintillation counter. The injected stock was similarly assayed. Radiotracer loss to the water was determined by counting 5 ml water in 10 ml ACS fluor. Dual label quench correction was performed using the external standard-ratio method described by Kobayashi and Maudsley (1974).

Whole-body ECFV at each time was calculated by the "net retention" method of Hickman (1972):

$$\text{ECFV (ml)} = \frac{^3\text{H-mannitol injected (dpm)} - \Sigma\,\text{sampled (dpm)} - \Sigma\,\text{excreted (dpm)}}{\text{Plasma } [^3\text{H-mannitol}]\,\text{(dpm/ml)}/\text{Plasma } H_2O\,\text{(ml/ml)}}, \quad (2)$$

where plasma water was calculated from the refractive index. Σ Sampled refers to loss of the marker via blood sampling and was calculated as

$$\Sigma\,\text{sampled (dpm)} = \frac{(1 - \text{Ht}) \times \text{vol (ml)} \times \text{plasma } [^3\text{H-mannitol}]\,\text{(dpm/ml)}}{\text{plasma } H_2O\,\text{(ml/ml)}}, \quad (3)$$

where Ht was expressed as a decimal and vol was the volume of each blood sample. Σ Excreted at each time was calculated as

$$\Sigma\,\text{excreted (dpm)} = \text{water } [^3\text{H-mannitol}]\,\text{(dpm/ml)} \times \text{water volume (ml)}, \quad (4)$$

taking into account the exchange of water during flushes. At some sample times, a correction factor was applied to the calculated ECFV estimate to compensate for penetration of mannitol into the intracellular space (see Results). Finally, ICFV was calculated as the difference between total body water and ECFV.

Mean whole-body pHi at each time was calculated according to the equation of Waddell and Butler (1959):

$$\text{pHi} = \text{p}K_{\text{DMO}} + \log\left\{\frac{[\text{DMO}]\text{i}}{[\text{DMO}]\text{e}} \times (10^{[\text{pHe} - \text{p}K_{\text{DMO}}]} + 1) - 1\right\}, \quad (5)$$

where $\text{p}K_{\text{DMO}}$ was taken from Malan, Wilson, and Reeves (1976), pHe (extracellular pH) was plasma pH, and [DMO]e and [DMO]i represented extracellular and intracellular [DMO], respectively. These latter were calculated as

$$[\text{DMO}]e\,(\text{dpm/ml}) = \frac{\text{Plasma } [^{14}\text{C-DMO}]\,(\text{dpm/ml})}{\text{Plasma } H_2O\,(\text{ml/ml})} \quad (6)$$

and

$$[\text{DMO}]i\,(\text{dpm/ml}) = \frac{^{14}\text{C-DMO injected (dpm)} - \Sigma \text{ sampled (dpm)} - \Sigma \text{ excreted (dpm)} - (\text{ECFV (ml)} \times [\text{DMO}]e\,(\text{dpm/ml}))}{\text{ICFV (ml)}}, \quad (7)$$

where Σ sampled and Σ excreted were calculated analogously to equations (3) and (4), respectively. The importance of accounting for marker excretion and loss owing to sampling in whole-body pHi calculations has been demonstrated by Hōbe, Wood, and Wheatly (1984) and Wood and Cameron (1985).

Total ΔH^+m to the whole body at each time was calculated as

$$\Delta H^+m\,(\text{meq/kg}) = \Delta H^+m_{ECF}\,(\text{meq/kg}) + \Delta H^+m_{ICF}\,(\text{meq/kg}), \quad (8)$$

where

$$\Delta H^+m_{ECF}\,(\text{meq/kg}) = BV\,(\text{l/kg}) \times \Delta H^+m_{WB}\,(\text{meq/liter}) + \{(ECFV - PV)\,(\text{l/kg}) \times \Delta H^+m_{ISF}\,(\text{meq/liter})\}. \quad (9)$$

BV and PV were blood and plasma volumes, respectively, for which we used values measured in rainbow trout by Milligan and Wood (1982), in view of the similarity in ECFV between the two species (see Results). ΔH^+m_{WB} was calculated using equation (1). To estimate ΔH^+m_{ISF}, the ΔH^+m to the interstitial fluid (ISF), the β value of the ISF was assumed to equal that of separated plasma and the value of the interstitial $[HCO_3^-]$ was assumed to equal that of true plasma. (For details concerning calculations of ΔH^+m_{ICF}, [ΔH^+m to the intracellular compartment], see Discussion.)

The exchange of H^+ with the environmental water was calculated as the sum of titratable-acidity flux and ammonia flux, signs considered. Titratable-acidity flux was calculated from titratable-alkalinity measurements on 10-ml water samples as outlined in McDonald and Wood (1981). The titratable component reflects the excretion of nonvolatile fixed acid or the uptake of nonvolatile fixed base. The samples were continually aerated to remove CO_2. Water [ammonia] was determined using a micro-modification of the phenol-hypochlorite method of Solorzano (1969).

STATISTICAL ANALYSIS

Means ± 1 SEM (n) are reported throughout, unless stated otherwise. Significant differences ($P < .05$) within groups were assessed using Student's two-tailed t-test, paired design. Regression lines were fitted by the method of least squares, and the significance of the correlation coefficient (r) was determined.

RESULTS

BLOOD BUFFER CAPACITY

In vitro tonometry of whole blood and plasma over the normal range of P_{CO_2} values yielded linear relationships between β, the nonbicarbonate buffer capacity in mmol/liter/pH and [Hb] in g/100 ml or Ht as a decimal. The regression relationships for whole blood were

$$\beta = -1.46 \times [\text{Hb}] - 2.64; \quad (n = 6; r = -0.993; P < .005), \quad (10)$$

TABLE 1

PERCENT OF INJECTED MARKER DOSES EXCRETED TO THE ENVIRONMENT IN EXERCISED AND NONEXERCISED STARRY FLOUNDER

	MEAN ± SEM MARKER DOSES EXCRETED AT (time [h] after injection/experimental time [h])					
	12/0	14/2	16/4	20/8	24/12	36/24
Control:						
^{14}C-DMO	3.71 ± 0.34	5.68 ± 1.14	6.01 ± 1.47	8.42 ± 3.17	11.77 ± 3.01	17.42 ± 4.07
^{3}H-mannitol ...	3.49 ± 0.66	5.79 ± 2.34	5.98 ± 1.49	8.07 ± 2.34	10.08 ± 3.16	16.70 ± 3.73
Exercise:						
^{14}C-DMO	4.93 ± 0.52	5.78 ± 0.75	6.97 ± 1.13	8.93 ± 1.07	10.08 ± 1.34	15.73 ± 2.01
^{3}H-mannitol ...	4.70 ± 0.78	5.95 ± 0.81	7.19 ± 0.76	8.60 ± 0.83	9.91 ± 0.86	14.88 ± 1.58

NOTE.—There were no significant differences at any time between the control and the experimental group. $n = 8$ for all readings.

$$\beta = -40.52 \times \mathrm{Ht} - 2.79; \quad (n = 6; r = -0.993; P < .005); \tag{11}$$

and those for true plasma were

$$\beta = -1.69 \times [\mathrm{Hb}] - 2.08; \quad (n = 6; r = -0.989; P < .005), \tag{12}$$

$$\beta = -46.41 \times \mathrm{Ht} - 2.23; \quad (n = 6; r = -0.993; P < .005). \tag{13}$$

The buffer value for separated plasma was −3.04 mmol/liter/pH.

BEHAVIOR OF ^{3}H-MANNITOL AND ^{14}C-DMO

There was a significant loss of both markers into the environmental water (table 1). Loss rates, while variable, were of similar magnitude for ^{14}C-DMO and ^{3}H-mannitol. Exercise did not affect the rate of marker loss. Total loss owing to repetitive blood sampling was only ~5% of losses by excretion. By the time rest samples were taken (12 h postinfusion) ~95% of the infused marker doses remained, and by the end of the experiment (36 h postinfusion) only ~80%–85% remained. Failure to take these losses into account would have resulted in overestimates of ECFV by ~13 ml/kg (at rest) to ~40 ml/kg (at 24 h) and in whole-body pHi by ~0.04 units (at rest) and ~0.10 units (at 24 h).

A further complication was lengthy marker-equilibration time. At rest (12 h postinfusion) the calculated whole-body ECFV in both groups was ~256 ml/kg (table 2), which agreed closely with estimates, determined by means of different

TABLE 2

UNCORRECTED ECFVs PRIOR TO AND FOLLOWING ACTIVITY IN THE STARRY FLOUNDER

	MEAN ± SEM (ml/kg body wt)									
		Experimental Time (h)								
	Rest	0	.25	.5	1	2	4	8	12	24
Control ...	255.4 ± 15.2	265.1 ± 12.6	265.7 ± 13.3	261.9 ± 12.3	265.7 ± 13.5	280.7[a] ± 18.0	271.3[a] ± 14.2	298.5[a] ± 14.7	297.0[a] ± 14.8	319.0[a] ± 14.8
Exercise ...	258.9 ± 10.7	231.3[a] ± 10.7	237.1[a] ± 9.7	248.4[a] ± 17.9	246.4 ± 9.6	250.2 ± 12.8	275.3[a] ± 13.8	291.4[a] ± 14.2	289.4[a] ± 14.1	308.9[a] ± 15.0

NOTE.—$n = 8$ for all readings.
[a] Significantly different ($P < .05$) from corresponding rest value.

methodology (Milligan and Wood 1982; M. V. E. Attygalle, G. Shelton, and P. C. Croghan, personal communication), in rainbow trout. ECFV rose gradually in the control group, so that by 2 h the change was significant and by 24 h the increase was ~25% (table 2). In the exercise group, ECFV declined significantly for the first 0.5 h after activity and thereafter rose gradually as in the controls. This expansion was probably due to ^{3}H-mannitol penetration of the ICF of the liver and kidney (Hickman 1972) after lengthy equilibration. Clearly, the true postexercise changes in fluid volume distribution were masked. To unmask these changes, rest values (at 12 h postinfusion) were assumed to be correct. The mean increase over rest values in the controls was subtracted from the exercise value at each respective time to yield corrected ECFV estimates (fig. 4*A*). These corrected values were then used for calculation of mean whole-body pHi (fig. 5). In the control group, rest values were employed throughout.

BLOOD ACID-BASE AND METABOLITE STATUS

Ten minutes of enforced activity depressed blood pHa from 7.842 ± 0.024 (n = 8) at rest to 7.509 ± 0.036 (n = 8) immediately after exercise (0 h) and to an even lower value, 7.486 ± 0.032 (n = 8), at 15 min (fig. 1*A*). The immediate acidosis was associated with a doubling of P_{CO_2} to 7.64 ± 0.68 torr (n = 8) at 0 h (fig. 1*B*) and the appearance of a small ΔH^+m in the blood (fig. 2). By 0.25–0.5 h, ΔH^+m peaked (5.28 ± 0.67 meq/liter; n = 8) while P_{CO_2} was beginning to decline. ΔH^+m remained elevated until 8 h (fig. 2), while P_{CO_2} had returned to levels not significantly different from rest levels by 2 h (fig. 1*B*). Analysis based on the principles outlined by Wood et al. (1977) revealed that, immediately after activity, the acidosis was mainly (>90%) of respiratory origin, with little metabolic contribution. However, by 1 h the metabolic component dominated, as indicated by the depression of plasma [HCO_3^-] at this time (fig. 1*C*). By 8 h, both P_{CO_2} and [HCO_3^-] were restored to rest levels, and the acidosis was fully corrected. Acid-base status in the control group was not affected by sampling.

At rest, whole-blood [lactate] was quite low, ~0.25 mmol/liter (fig. 1*D*). After activity lactate levels did increase, but the rise was minimal, with peak levels (at 2–4 h) averaging ~1.3 mmol/liter and rarely >2 mmol/liter in individual fish. Thus, the blood ΔLa^- appeared with both a much slower time course and a much smaller absolute magnitude than did the blood ΔH^+m (fig. 2). Lactate levels also declined slowly, returning to rest values by 8 h. [Lactate] remained constant in the control group (fig. 1*D*).

Further consequences of enforced activity were rapid, significant elevations in Ht, [Hb], and plasma [protein] (fig. 3*A*, 3*B*, 3*D*). These changes most likely reflected a general hemoconcentration due to a fluid shift out of the ECFV and into the ICFV (see below). MCHC (fig. 3*C*) declined only slightly, suggesting that RBC swelling and/or reticulocyte mobilization were of minor importance in the Ht elevation. The hemoconcentration was short-lived, with Ht, [Hb], and plasma [protein] returning to rest levels by 2 h. However, by 4 h the diluting effect of repetitive sampling became evident, as it did in the control group (fig. 3*A*, 3*B*, 3*D*).

Whole-blood [glucose] followed a somewhat different pattern. Though the [glucose] increased by ~50%, the change was not significant until 0.5 h after cessation of activity (fig. 3*E*). Furthermore, repetitive blood sampling did not affect glucose levels in either the control or the exercise group.

WHOLE-BODY FLUID DISTRIBUTION AND pHi

After cessation of activity, whole-body ECFV fell by ~10%–20% and remained depressed until 4 h into recovery (fig. 4*A*). Since total body water remained constant at various times after activity (at 784.0 ± 2.9 ml/kg; n = 40), the reduction in ECFV was a direct consequence of a fluid shift into the ICFV. This resulted in a reciprocal expansion of the ICFV (fig. 4*B*) until 4 h into recovery.

At rest, whole-body pHi in the control group, 7.56 ± 0.04 (n = 8), was virtually identical to that in the exercise group, 7.58 ± 0.05 (n = 8; fig. 5*A*, 5*B*). Immediately after exercise, pHi fell to 7.29 ± 0.04 (n = 8) and then further, to a low of 7.24 ± 0.06 (n = 8) at 15 min, a pattern similar

to that shown by the pHe change. pHi remained depressed until 4 h, when it showed a near complete recovery, despite the fact that pHe was still significantly depressed. By 8 h after exercise, pHi exhibited a significant alkalosis at a time when pHe had just returned to rest levels (fig. 5*A*). The intracellular alkalosis was corrected by 12 h, with resting acid-base status restored. Whole-body pHi remained constant in the control group (fig. 5*B*).

H^+ EXCHANGE WITH THE ENVIRONMENT

The methodology employed does not distinguish between net (H^+) excretion or basic-equivalent uptake, or vice-versa.

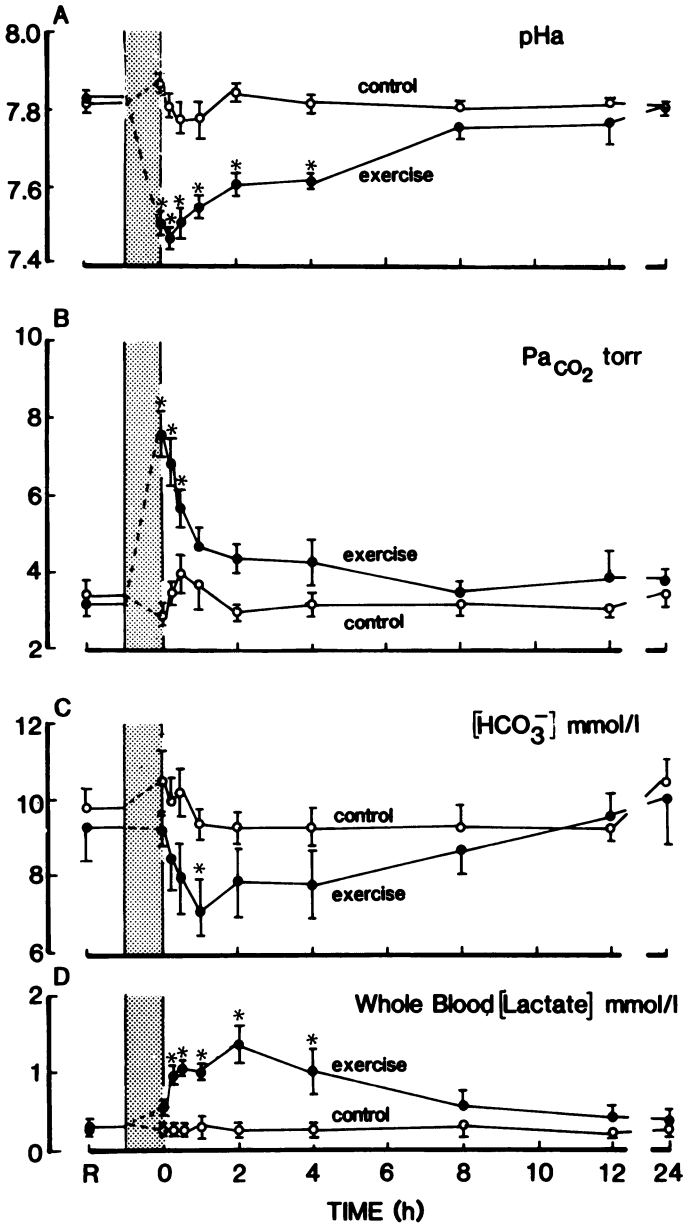

FIG. 1.—Effects of 10 min enforced activity on (*A*) plasma pHa; (*B*) plasma Pa_{CO_2}; (*C*) plasma $[HCO_3^-]$; and (*D*) whole-blood [lactate] in starry flounder. Each point represents the mean, and the vertical bars represent ±1 SEM. ● = Exercise group (n = 8); ○ = control group (n = 8). R indicates rest value; vertical stippled bar indicates 10-min period of activity. Experimental time 0 is immediately after activity. * Indicates a significant difference ($P < .05$) from the corresponding rest value.

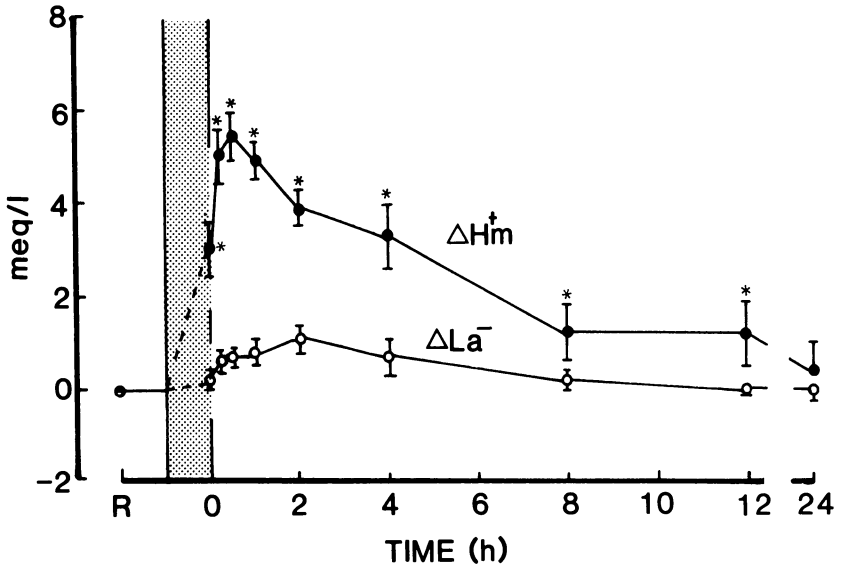

FIG. 2.—Changes in blood ΔLa^- and blood ΔH^+m during recovery from enforced activity. * Indicates that ΔH^+m is significantly different ($P < .05$) from ΔLa^- at that time. Other details are as in the legend to fig. 1.

Fortunately, these distinctions are not important to the net acid-base status of the animal.

During the two 12-h periods prior to activity, net H^+ excretion averaged ~ -160 μeq/kg/h in both the control and exercise groups (fig. 6). This did not change appreciably in the control group during the experimental period. However, in the exercise group net H^+ excretion to the water increased threefold (fig. 6*D*). This was associated with a significant decline in titratable-acidity uptake, from a positive (+70 μeq/kg/h) to a highly negative (−200 μeq/kg/h) value (fig. 6*F*). Ammonia excretion increased at this time (fig. 6*E*), but the change was small. The significance of the increased ammonia excretion is questionable, for similar increases occurred in the control group (fig. 6B), implying that the cause was other than activity related.

Net H^+ excretion returned to baseline values by 8–12 h into recovery, coincident with a similar change in the titratable-acidity uptake. During the final 12 h of recovery, net H^+ excretion declined significantly, to near zero (-7.8 ± 42.6 μeq/kg/h; $n = 8$; fig. 6*D*), representing a period of relative H^+ uptake (or basic-equivalent excretion) compared with the preactivity periods. A similar pattern was not evident in the controls (fig. 6*A*), suggesting that this was not a resetting of basal excretion, but rather a true reversal of H^+ excretion. Once again the change was associated with a similar alteration in the titratable-acidity flux (fig. 6*F*).

DISCUSSION

BLOOD ACID-BASE CHANGES

The arterial acid-base disturbance (fig. 1) after enforced activity in the starry flounder was qualitatively similar to those observed in other teleosts that had contributions from both respiratory and metabolic components (see Wood and Perry 1985).

The rise in P_{CO_2} (respiratory component) was due, no doubt, to both an increased production and efflux of CO_2 from the aerobic tissues and conversion of blood HCO_3^- to CO_2 by H^+ released from the glycolytic muscle mass. This H^+ efflux from the working muscle thereby represented the metabolic component of the blood acidosis that was buffered partly by HCO_3^- and partly by blood proteins (Wood et al. 1977). In most other fish examined to date, the peak in blood ΔH^+m was coincident with the peak in P_{CO_2} (respiratory acid), which occurred immediately after exercise (Wood and Perry 1985). However, in the starry flounder, this differs, for the P_{CO_2} elevation was nearly corrected by the time that the

blood ΔH^+m had peaked (at 1 h into recovery, fig. 3). These patterns were very similar to those in venous blood of exercised starry flounder, as measured by Wood et al. (1977). Thus, there appears to be a temporal separation of respiratory and metabolic acid loading to the blood that will attenuate the overall acidosis. The reason for this separation is not clear, though it may be related to a differential perfusion of the aerobic versus glycolytic tissue mass.

ΔLa^- AND ΔH^+m

In starry flounder, the rise in blood [lactate] after exhaustive exercise was quite small, rarely attaining levels >2 mmol/liter (fig. 1*D*). This minimal appearance of lactate in the blood appears to be characteristic

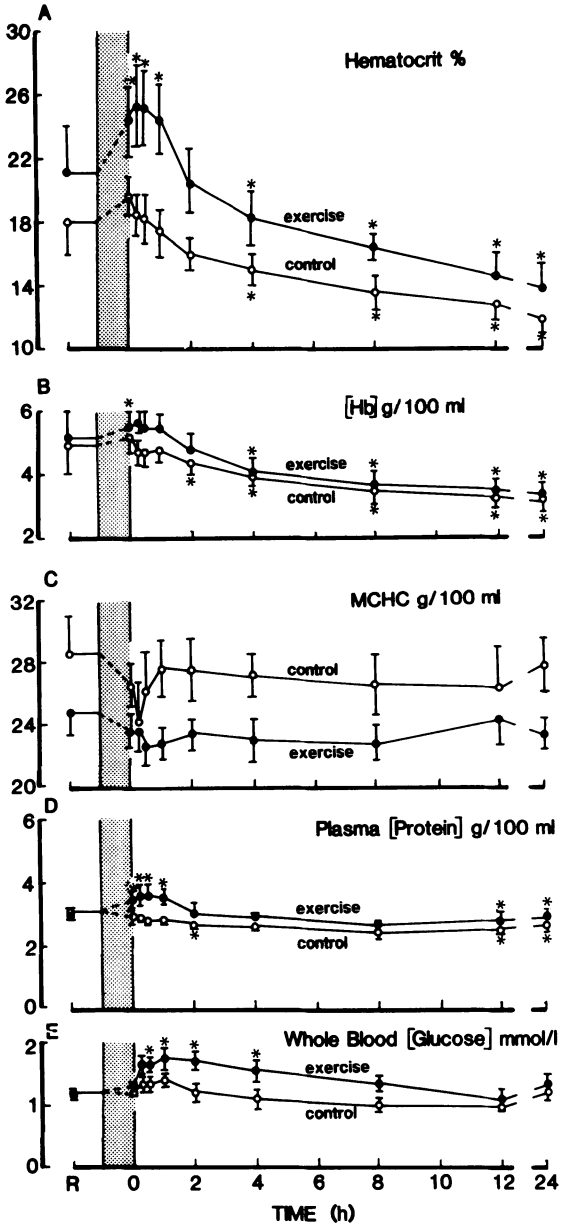

FIG. 3.—Changes in (*A*) Ht, (*B*) [Hb], (*C*) MCHC, (*D*) plasma [protein], and (*E*) whole-blood [glucose] induced by 10 min of activity. Other details are as in the legend to fig. 1.

of relatively inactive, benthic, and demersal species, for it has also been observed in plaice (*Pleuronectes platessa;* Dando 1969; Wardle 1978), various European flounder (Duthie 1982), flathead sole (Turner et al. 1983*b*), sea raven (Milligan and Farrell 1986), and Atlantic skates (C. M. Wood and M. Graham, unpublished observations, cited in Wood and Perry 1985). By way of contrast, postexercise levels of whole-blood [lactate] in active pelagic species (e.g., trout, dogfish, tuna; see Wood and Perry 1985) often attained levels as high as 20–25 mmol/liter, that is, 20–30 times resting levels. In flounder, the postexercise H^+m greatly exceeded the ΔLa^- (fig. 2). The two did not come into equilibrium until 24 h into recovery, at which time they had returned to rest levels. A similar phenomenon has been observed in the other inactive species, but exactly the opposite pattern (i.e., ΔLa^- exceeding ΔH^+m) has been observed in active pelagic fish (Wood and Perry 1985).

In the starry flounder, it is conceivable that differential uptake, from the blood, of lactate in excess of H^+ could contribute to this apparent differential appearance. However, in the flathead sole, the rates of lactate and H^+ removal from the blood were equal after lactic acid infusion (Turner et al. 1983*b*). The low urine flow of marine

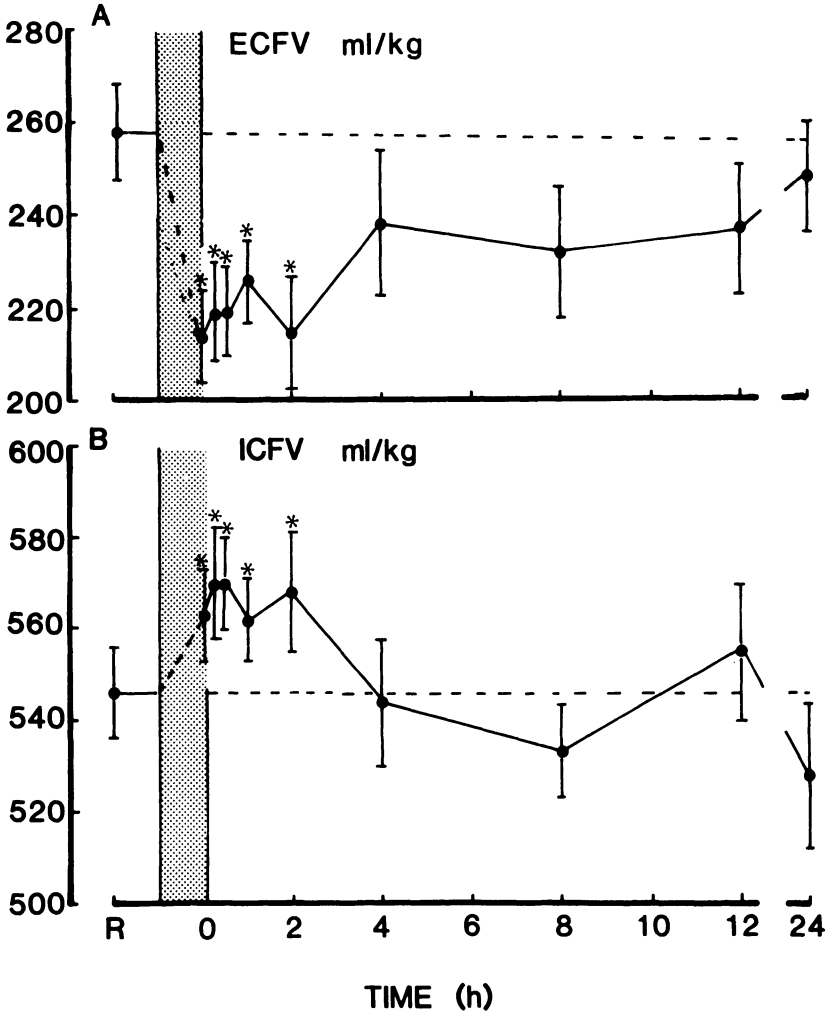

FIG. 4.—Fluid volume distributions prior to and after activity in starry flounder. Corrected values are plotted for (*A*) ECFV and (*B*) ICFV (see text for details). Other details are as in the legend to fig. 1.

flatfish argues against the kidney as a site of significant excretion (McDonald et al. 1982). Lactate excretion at the gills was not measured in the starry flounder, but in the rainbow trout it amounted to <1% of H^+ excretion (Milligan and Wood 1986*a*). On balance, the most plausible hypothesis is that the phenomenon reflects differential lactate and H^+ release from the white-muscle mass, though the controlling factors remain obscure. H^+ movement may be constrained by the "equilibrium limitations" described by Holeton and Heisler (1983), such as pH gradient and the extracellular buffer capacity. Lactate release may be carrier mediated (Hochachka and Mommsen 1983). Wardle (1978) has suggested that, in plaice, a lactate "non-release" mechanism, under the influence of circulating catecholamines, is operative at the level of the muscle. The possible adrenergic influences on lactate and H^+ dynamics after exercise in starry flounder are the subject of an accompanying paper (Wood and Milligan 1987).

FLUID VOLUME DISTRIBUTION

As determined with ^{3}H-mannitol, rest values of ECFV in starry flounder were similar to those reported for other teleosts (e.g., rainbow trout [Milligan and Wood 1982, 1986*a*] and channel catfish [Cameron 1980]) but considerably higher than those

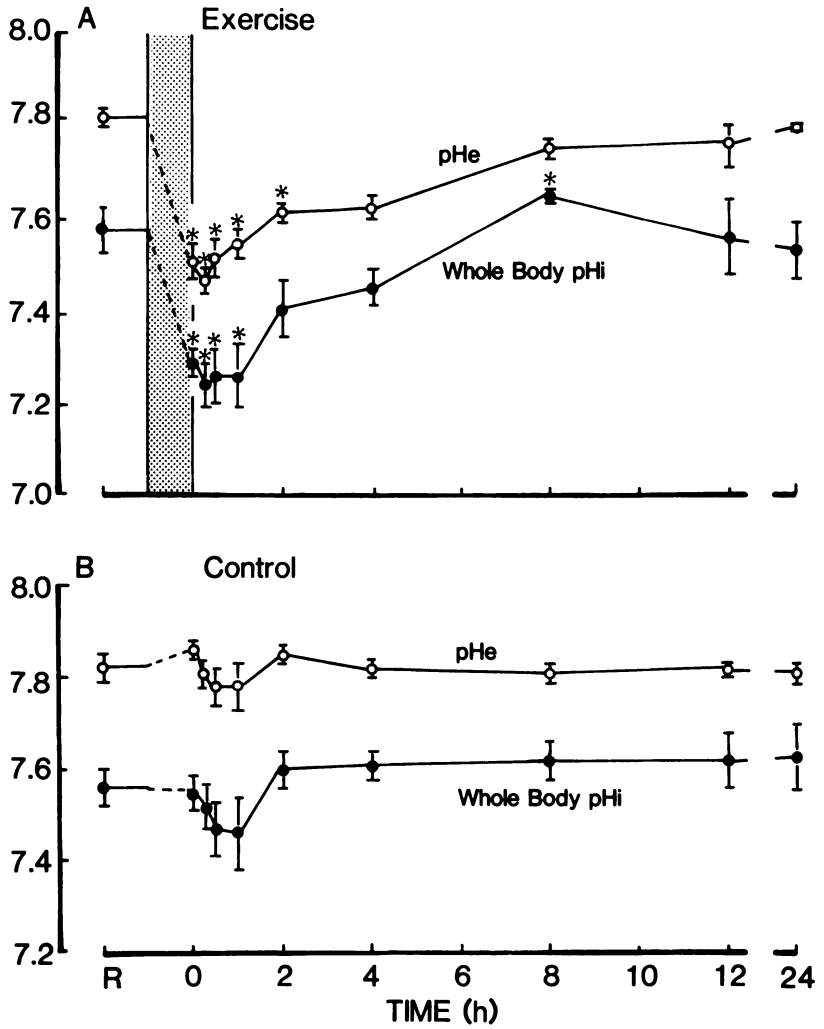

FIG. 5.—Response of whole-body pHi (●) and pHe (○) to exercise (*A*). Data from the control group (*B*) are shown for comparison. Other details are as in the legend to fig. 1.

measured with ^{3}H-inulin (164 ml/kg) in southern flounder (*Paralichthys lethostigma;* Hickman 1972). Inulin is known to give lower estimates than does mannitol (Cameron 1980). Interestingly, however, the artifactual elevation of ECFV due to marker penetration of the tissues starting 12 h postinfusion occurred at the same rate as in Hickman's study (table 2).

Enforced activity resulted in a contraction of the ECFV, and, since totol body water did not change, a reciprocal expansion of the ICFV occurred (fig. 4). This fluid shift occurred almost exclusively into the white

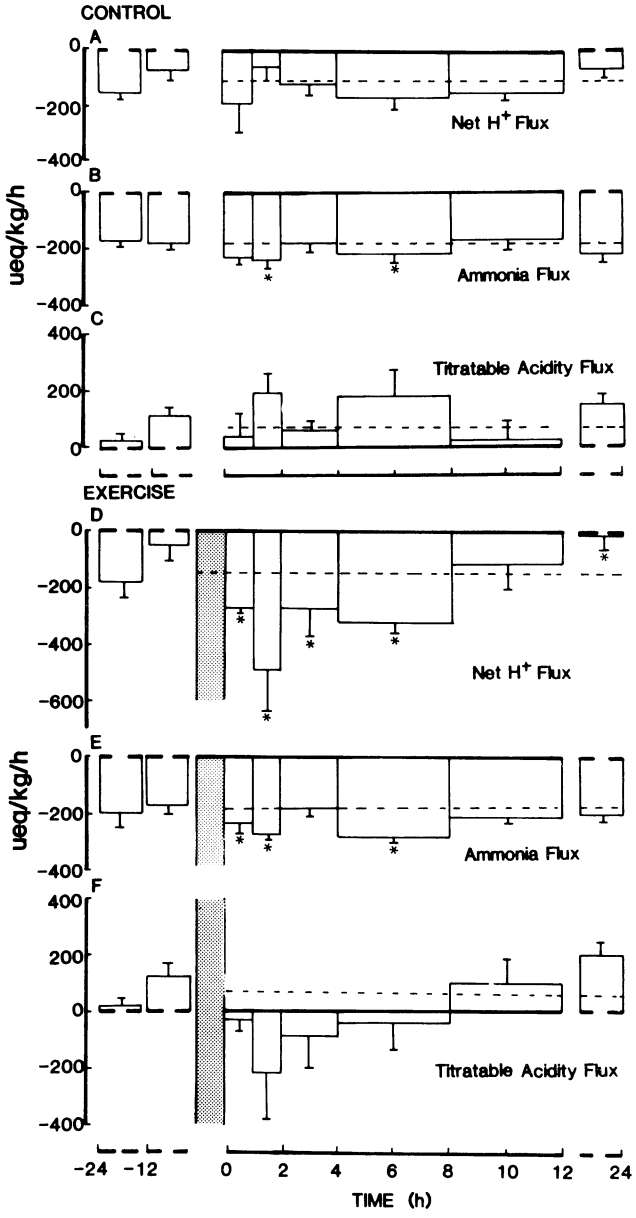

FIG. 6.—Mean fluxes of net (*A* and *D*) H^+, (*B* and *E*) ammonia, and (*C* and *F*) titratable acidity in the control and exercise groups respectively. −24 and −12 indicate two 12-h flux periods prior to the experimental period; the dashed line indicates mean of these two periods; vertical stippled bar indicate period of activity. Experimental time 0 is immediately after activity. Vertical bars represent ±1 SEM. * Indicates a significant difference ($P < 0.05$) from the mean of the respective control periods.

muscle (Milligan and Wood 1987), the redistribution no doubt caused by the breakdown of glycogen into lactate, the increase in other osmolytes (e.g., inorganic phosphate), and the subsequent rise in intracellular osmotic pressure. In turn, the reduction in ECFV produced hemoconcentration, as indicated by the increase in Ht, [Hb], and plasma [protein].

WHOLE-BODY pHi

Mean whole-body pHi in flounder at rest (7.5–7.6) was higher than the 7.2–7.3 observed for rainbow trout (Hōbe et al. 1984; Milligan and Wood 1986*a*) and channel catfish (Cameron and Kormanik 1982). This probably reflected the higher pHi of white muscle in the starry flounder (Milligan and Wood 1987), for this compartment constitutes the bulk of the body mass. After exercise, the drop in whole-body pHi was very similar to that in white-muscle pHi (Milligan and Wood 1987). This intracellular acidosis was corrected much more rapidly than the extracellular disturbance, with intracellular alkalosis occurring at 8 h after exercise. The response of flounder whole-body pHi differs markedly from that of the whole-body pHi of rainbow trout. In the latter, the postexercise whole-body pHi excursion was greater (0.47 units vs. 0.34 units), probably owing to a greater intensity of exercise. Furthermore, in trout whole-body pHi was slower to recover, requiring up to 12 h, and did not exhibit an alkalosis. The implications of this difference are discussed below.

The accuracy of the estimate of pHi in a dynamic situation is dependent on full equilibration of DMO between the ICFV and ECFV. In trout white muscle perfused in vitro, full equilibration is attained within 15 min of a step change in pH (Milligan and Wood 1985); shorter periods were not tested. Thus the reliability of the present 0-h pHi estimate, obtained immediately after the 10-min exercise period, cannot be assessed. All other sample periods met the 15-min criterion. However, some qualitative conclusions can be drawn. The [DMO]i/[DMO]e distribution ratio (of eq. 5) significantly increased after exercise (from 0.57 ± 0.02 at rest to 0.63 ± 0.02 at time 0; $n = 8$). Thus there was a net movement of DMO from ECF to ICF compartments. If, at time 0, this reequilibration was *not* complete, then the pHi estimate at this time would have been too low—and the magnitude of the true pHi change would have been *overestimated.* The disappearance of this disequilibrium during longer recovery periods ($\geq$1 h) would raise calculated pHi substantially. The fact that this did not occur (fig. 5) suggests that disequilibrium, if it occurred at all, was not a serious problem. In any event, it seems likely that true pHi undergoes continuous rather than step changes in vivo, so some blurring of the transients is likely with the DMO method.

MECHANISM OF H^+ EXCRETION

After exercise, starry flounder exhibited a large increase in H^+ excretion to the environmental water, with little change in ammonia excretion (fig. 6). These fluxes undoubtedly occurred across the gills, since in seawater the teleost kidney is relatively insignificant in acid-base regulation owing to its low-volume output (McDonald et al. 1982).

The mechanism(s) of acid excretion in marine fish is (are) poorly understood. Heisler (1982) suggested that acid excretion following exercise is achieved mainly via Cl^-/HCO_3^- exchange in dogfish (*Scyliorhinus stellaris*) and Conger eels (*Conger conger*). However, Evans (1982) has shown that Na^+/H^+ exchange is important in clearing a hypercapnia-induced acidosis in toadfish (*Opsanus beta*) and spiny dogfish (*Squalus acanthias*). In lemon sole (*Parophrys vetulus*), H^+ excretion in response to mineral acid loading was associated with an increased ammonia efflux (McDonald et al. 1982). In the present study, the virtually unchanged ammonia excretion after exercise argues against a prominent role for ammonia in acid excretion. By way of contrast, in the freshwater rainbow trout after exercise, H^+ excretion is associated with greatly increased ammonia excretion (Milligan and Wood 1986*a*).

DISTRIBUTION OF H^+ BETWEEN ECF, ICF AND THE ENVIRONMENT

The data presented allow construction of a ΔH^+m budget in the three compartments: intracellular, extracellular, and environment (fig. 7). Analysis of ΔH^+m in the ECF

was calculated as described in Material and Methods. Calculation of ΔH^+m to the ICF was complicated by the fact that the buffer value (β) for this compartment was unknown. However, ΔH^+m in the ICF was approximated by using the β value of white muscle, −33.00 mmol/pH/kg = −47.8 mmol/pH/liter ICF (Milligan and Wood 1987), and the mean whole-body pHi changes shown in figure 5. We assumed that the bulk of the intracellular buffer capacity resides in the white muscle, which occupies 60% of the body mass, and that contributions from other tissues were negligible. Therefore, the estimate of ΔH^+m in the ICF will tend to err on the conservative side. H^+ loading to the environment was calculated assuming that preexercise excretion rates represented basal production.

The analysis revealed that, up to 4 h after exercise, the bulk of the H^+ load remained intracellular (fig. 7; ΔH^+m_{ICF}). Approximately 20% of the total ΔH^+m passed through the ECF (fig. 7, ΔH^+m_{ECF}) and subsequently appeared in the environmental water (fig. 7; $\Delta H^+m_{H_2O}$). The intracellular H^+ load was cleared rapidly, so that by 8 h into recovery the ICF experienced an alkalosis. The ΔH^+m deficit in the ICF was equivalent to the quantity of H^+ excreted into the water. The alkalosis in the whole-body ICF at 8 h probably reflected an alkalosis in both the white-muscle mass and the liver (Milligan and Wood 1987). By 24 h after exercise, the H^+ excreted to the water was taken back, thereby aiding correction of the intracellular alkalosis. Thus, after strenuous exercise, the starry flounder temporarily stores H^+ in the environmental water to hasten restoration of intracellular acid-base status, perhaps as a means of aiding metabolic recovery (Milligan and Wood 1987).

Total metabolic acid production after exhaustive exercise in the starry flounder (~8 meq/kg; fig. 7) was approximately half that of the rainbow trout, and its disposition between ICF, ECF, and the environmental water was very different between the two species (Milligan and Wood 1986*a*). In trout, only 6% of the total H^+ load (vs. 20% in flounder) appeared in the water, and this transfer appeared to expedite recovery of the extracellular compartment; clearance of H^+ from the intracellular compartment occurred largely via aerobic metabolism rather than via export (Milligan and Wood 1986*a*, 1986*b*). This difference may be re-

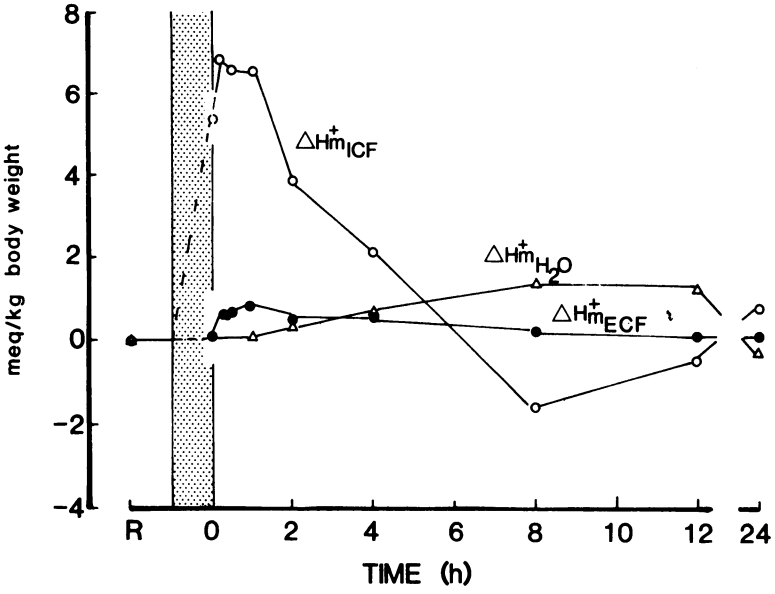

FIG. 7.—Distribution of metabolic acid load in the intracellular space (ΔH^+m_{ICF}; ○) and extracellular space (ΔH^+m_{ECF}; ●) and environmental water ($\Delta H^+m_{H_2O}$; △) after activity in starry flounder. Note that the units are meq/kg body wt. R indicates rest value; vertical stippled bar indicates 10 min of activity. Experimental time 0 is immediately after activity. (See text for details.)

lated to different ecologies. The pelagic rainbow trout possess a strip of aerobic red muscle along the lateral line that allows continual cruising in the water column even after glycolytic exhaustion of the white muscle; the maintenance of adequate O_2 delivery through the bloodstream is critically important. Therefore, the priority lies in correcting the extracellular, rather than the intracellular, acidosis. The flounder, on the other hand, leads mainly a benthic life, possessing little aerobic red muscle. The burden of locomotion is placed on the glycolytic white muscle (Duthie 1982), and even severe anemia has little effect on exercise performance (Wood et al. 1979*b*). Therefore, in flounder, correction of the intracellular acidosis to a level compatible with metabolism and restoration of glycogen stores is more important than correction of the extracellular acidosis and maintenance of O_2 delivery.

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