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The Influence of Burst Exercise on the Carbohydrate Metabolism and Blood Acid-Base Status of Northern Pike (Esox lucius L.)

by

Karl Schwalme

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science.

Department of Zoology

Edmonton, Alberta

Fall 1984

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Influence of Burst Exercise on the Carbohydrate Metabolism and Blood Acid-Base Status of Northern Pike (Esox lucius L.>" submitted by Karl Schwalme in partial fulfilment of the requirements for the degree of Master of Science.

W C Mackay
Supervisor

Date July 4, 1984

GENERAL ABSTRACT

The influence of burst exercise on carbohydrate metabolism and blood acid-base status was examined in the northern pike (Esox lucius L.), a fish with a 'sprinter' type of exercise metabolism. Capture by angling was used to exercise pike for the study of carbohydrate metabolism. Glycogen in the white muscle and liver; lactate in the white muscle, liver, and blood; and glucose in the blood were measured at 0, 3, 6, 48, and 96 h after exercise.

Muscle lactate was elevated to high levels (41.8 mmol kg^{-1}) during angling but declined substantially (to 23.2 mmol kg^{-1}) by 6 h of recovery. Capture by angling resulted in large elevation of blood lactate level which reached 15.2 mmol l^{-1} by 3 h but declined to 6.2 mmol l^{-1} by 6 h. Resting blood lactate levels, as indicated by 48 and 96 h values, averaged 1.7 mmol l^{-1}. Burst exercise resulted in moderate changes in liver lactate; peak levels at 3 h averaged 16.1 mmol kg^{-1} while 48 and 96 h levels both averaged 9.2 mmol kg^{-1}. Blood glucose levels rose from 2.53 mmol l^{-1} immediately after exercise to 6.11 mmol l^{-1} at 3 h and by 96 h (4.13 mmol l^{-1}) had still not returned to normal. Liver glycogen concentration of pike was very high (9.8 % of wet wt) and did not change significantly during 96 h of recovery from angling-induced exercise.

Replenishment of white muscle glycogen appeared to be very slow, requiring much longer (96 h) than the time needed to remove excess lactate. This indicates that the main mechanism of lactate removal after exercise is probably oxidation rather than the Cori cycle or in situ lactate glyconeogenesis. Therefore, the amount of lactate removed from pike within 6 h after exercise was estimated to determine if the fish's metabolic rate could account for lactate removal through oxidation. These calculations required that the axial white muscle mass and the variation in lactate concentration within the white muscle mass be determined. The axial white muscle was found to represent 53.8% of total body weight. Brief (1.5 min) burst exercise caused a tripling of lactate concentration and a 2/3 depletion of glycogen throughout the entire axial white muscle mass of pike.

An estimated 9.57 mmol of lactate was removed from pike of 1 kg body wt during 6 h after exercise. A metabolic rate of 153 mg O_2 kg^{-1} h^{-1} is sufficient to account for this rate of lactate removal through oxidation. However, the mass of the red muscle (3.99% of body wt) and the other highly oxidative organs and tissues (gills, liver, kidney, heart, and

spleen) amounts to only 7.82% of a pike's body weight. Therefore, it is unlikely that all of the lactate is oxidized in these organs and tissues because they would need a metabolic rate of more than 1,000 mg O_2 kg^{-1} h^{-1} to oxidize lactate at even 60% of its rate of removal after exercise.

The influence of burst exercise on plasma glucose and blood acid-base status was examined in pike that were chronically cannulated in the ventral aorta and exercised in the laboratory. Within 5 min after exercise blood pH declined from 7.94 to 7.36, plasma HCO_3^- declined from 16.04 mmol l^{-1} to 7.95 mmol l^{-1}, and blood PCO_2 increased from 5.44 mm Hg to 11.29 mm Hg. Thus, the acidosis was initially of mixed respiratory (elevated PCO_2) and metabolic (H^+ release from muscle) origin. Blood pH and plasma HCO_3^- returned to resting levels in 8 h. Since resting levels of PCO_2 were re-established by 2 h, the acidosis after this time was entirely of metabolic origin. At 2 h the increase in blood lactate anion was very large (12.8 mmol l^{-1} above resting levels) and exceeded the metabolic H^+ load (8.1 mmol l^{-1}). Therefore, lactate anions and H^+ ions apparently have different rates of entry and/or exit from the blood, even though they are produced in stoichiometrically equal amounts during exercise.

By 2 h after exercise plasma glucose had increased by 8.8 mmol l^{-1}, from 4.3 mmol l^{-1} to 13.1 mmol l^{-1}, but pre-exercise levels were almost regained by 12 h. This hyperglycemia was not simply a consequence of lactate removal because intra-arterial infusion of a lactate load equivalent to that produced during exercise (9.8 mmol kg^{-1}) caused plasma glucose to be elevated by only 1.7 mmol l^{-1}.

Overall, the physiological responses to burst exercise exhibited by pike are qualitatively similar to those of the better studied salmonids. However, unlike salmonids, pike appear well adapted to cope with exercise stress because they showed low mortality (< 3%) following angling-induced exercise.

ACKNOWLEDGEMENTS

I express sincere gratitude to Dr. W.C. Mackay for his advice and encouragement throughout this study. Drs. L.C.H. Wang and L.P. Milligan contributed many constructive and helpful ideas which are greatly appreciated. Dr. L.C.H. Wang is also thanked for loan of the blood gas analyser. I wish to thank Lauren Turner and Colin Chapman for invaluable assistance with the computer and Jackie Newcomb for assistance with field work. Dr. J.R. Nursall provided useful advice about english grammar and Pat Valastin and Heidi Zwickel provided excellent technical assistance in the aquatic facilities. Financial support through a Boreal Institute for Northern Research grant (# 55-30177) and a graduate teaching assistantship is appreciated.

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Who has seen the wind?

Neither you nor I:

But when the trees bow down their heads,

The wind is passing by.

-Christina Rossetti

I. GENERAL INTRODUCTION

Frequent bursts of vigorous muscular exertion are part of everyday life for many animals. Burst exercise is most often used either to escape predation or to capture prey, but it also serves other purposes. Fish, for example, perform burst exercise when attempting to navigate fish ladders, rapids, and culverts in streams and to escape from anglers, who often release fish anyway to aid conservation. The physiological problems that burst exercise creates for fish and the mechanisms used to deal with these problems are thus of considerable interest.

In most large animals, the explosive acceleration and high rate of energy demand that characterizes maximal burst exercise far exceeds the rate at which oxygen can be supplied to working muscles. Under these conditions, a large part of the energy for muscle contraction is provided by anaerobic glycolysis, the process of ATP production from catabolism of glycogen to lactic acid (Black et al. 1962; Bennett and Licht 1972; Hermansen and Vaage 1977). Unfortunately, and despite its great survival value, burst exercise can be very stressful. Muscle glycogen can be severely depleted following exercise (Stevens and Black 1966) and, especially in fish, the massive lactate accumulation that accompanies burst exercise can cause severe acidosis (Turner et al. 1983a; Wood et al. 1983). These problems are accentuated in fish because of their low metabolic rate (Brett 1972) and low dietary carbohydrate intake (Cowey 1979), which could otherwise supply precursors for glycogen synthesis.

The only fish in which burst exercise has been studied extensively are salmonids (Black et al. 1958,60,62,66; Miller et al. 1959; Turner et al. 1983a,b; Wood et al. 1983). Salmonids swim almost continuously, many species make long migrations, and their scope for aerobic activity exceeds that of most other fish (Jones and Randall 1978); thus they exemplify the 'marathoner' style of locomotion. However, salmonids do not appear particularly well adapted to cope with the stress of burst exercise. Mortalities of 30% often occur in salmonids following strenuous exercise induced by angling or vigorous chasing (Parker and Black 1957; Bouck and Ball 1966; Wendt 1966; Wood et al. 1983). Even when salmonids survive burst exercise, recovery is a slow process. Replenishment of muscle glycogen is especially slow, requiring in excess of 24 h to reach completion

(Black et al. 1962; Wendt 1966). Resting blood and muscle lactate levels are not restored for 8 to 12 h and correction of blood pH is only slightly faster requiring approximately 6 h (Turner et al. 1983a,b; Wood et al. 1983). Burst exercise usually (Wydoski et al. 1976; Perrier et al. 1978; Pickering et al. 1982), but not always (Black et al. 1960), elevates blood glucose level, which subsequently remains high for at least 12 h and often as long as several days.

Very little is known about burst exercise physiology in 'sprinter' type fish, animals that theoretically should be well adapted to cope with exercise stress. Therefore, the present study examines the northern pike (Esox lucius L.), a 'sprinter' fish that performs intense burst exercise routinely when it attempts to capture prey. The study's primary objective was to describe the influence of burst exercise on carbohydrate metabolism and blood acid-base status.

My study is divided into two chapters. The first chapter deals with carbohydrate metabolism and presents an evaluation of the mechanisms pike may use to dispose of lactate and to resynthesize muscle glycogen after exercise. All the pike used for this study were collected by angling; consequently an assessment of the pike's ability to survive this stress is also included. In the second chapter the influence of burst exercise on blood acid-base balance is examined with particular emphasis on the relationship between lactate anion and H^+ ion accumulation in the blood. The hypothesis that post-exercise release of lactate into blood elevates blood glucose level is also investigated. In the overall discussion I briefly compare pike and salmonids according to the physiological responses that accompany burst exercise.

II. INFLUENCE OF ANGLING-INDUCED EXERCISE ON THE CARBOHYDRATE METABOLISM OF NORTHERN PIKE

A. INTRODUCTION

From the late 1950's and early 60's (Miller et al. 1959; Black et al. 1960,62,66) until recent times (Turner et al. 1983a,b; Wood et al. 1983) salmonids have been the favourite fish for studies of burst exercise physiology. There exists however, the disturbing possibility that salmonids are not particularly well-adapted to cope with the stress of severe burst exercise. Salmonids often experience mortalities of 30% after exercise induced by chasing or angling (Black 1958; Bouck and Ball 1966; Wendt 1966), even when they do not suffer physical damage to the gills or other body organs (Parker and Black 1959). Furthermore, many studies (Black et al. 1960,62; Wendt 1966; Turner et al. 1983a,b; Wood et al. 1983) have used hatchery-reared trout which are reputed to be less 'physically fit' than their wild counterparts (Hochachka 1961; Miller and Miller 1962). Therefore, the changes in muscle, liver, and blood carbohydrate levels seen following burst exercise in salmonids (Black et al. 1960,62; Wendt 1966) may be quite different from those shown by 'sprinter' fish such as northern pike (Esox lucius L.) which are probably well adapted for burst exercise. Additionally, muscle glycogen depletion, lactate accumulation, and lactate disappearance following burst exercise in fish has not yet been determined on a whole animal basis. Such information could shed light on the mechanisms fish use to dispose of excess lactate and to resynthesize muscle glycogen following exercise.

Several contrasting hypotheses currently exist to explain the fate of exercise-produced lactate. One hypothesis is that much of the excess lactate is incorporated into blood glucose by the liver, this glucose subsequently being used to synthesize muscle glycogen (ie: the Cori cycle) (Hochachka 1961). Alternatively, the lactate may be oxidized to CO_2, with most of the oxidation occurring in the red muscle, gills, liver, kidney, heart, and spleen since these tissues have much greater oxidative ability than the white muscle (Bilinski and Jonas 1972). There is also some evidence in mammals (Bendall and Taylor 1970) and fish (Batty and Wardle 1979) that lactate incorporation into

glycogen can occur within the white muscle itself.

In the present study, changes in white muscle and liver glycogen and lactate, and blood glucose and lactate were measured during recovery from angling-induced burst exercise in the northern pike, a non-salmonid 'sprinter' type fish. The rate of lactate disappearance from the whole body after angling was estimated to determine whether physiological oxygen consumption rates are sufficient to account for lactate removal through oxidation within:(a) the whole animal, and (b) tissues with high oxidative capacity (ie: red muscle, gill filaments, liver, kidney, heart, and spleen).

Finally, records were kept of all pike caught by angling to further test the findings of Beggs et al. (1980) who reported an angling-induced mortality of 30% in a closely related Esox species, the muskellunge (Esox masquinongy M.).

B. MATERIALS and METHODS

Angling Study

Adult northern pike from Lac Ste. Anne, Alberta were caught by angling, using barbless lures, between July 6 and August 8, 1982. Average body weights and fork lengths were 672±338 g (mean±SD)and 45±7.7 cm (mean±SD) respectively (n=44). All but a few of the largest fish were 'played' until they appeared exhausted; this required an average of 1.4 min. Upon landing, fish were placed in large, water-filled containers and immediately transported by boat to the Lac Ste. Anne Biological Station (transport time averaged 7 min). At the Biological Station, fish were either sacrificed immediately (0 h) or allowed to recover for either 3, 6, 48, or 96 h in 400 to 1000 L holding tanks flushed daily with lake water. Pike were assigned to these recovery times as randomly as possible within the limits of practicality. Water temperature in the lake at time of fish capture and in the recovery tanks was 19±2°C.

Fish were sampled by gently netting or grabbing them with studded gloves and quickly sacrificed by cranial concussion. The 0, 3, 6, and 48 h fish were not anaesthetized prior to sacrifice because anaesthesia stops gill ventilation, which could accelerate anaerobiosis in the tissues. As a simple test of the effects of anaesthesia, the 96 h fish were anaesthetized (before sacrifice) with a 0.2% concentration of 2-phenoxyethanol (first mixed with 2 volumes of ethanol). After sacrifice, approximately 1 g samples of liver and white muscle (from the dorso-lateral musculature just anterior to the dorsal fin) were excised from the fish, chopped with a razor, weighed, and immediately digested in hot 30% KOH until homogeneous. Blood samples were obtained by caudal puncture and immediately deproteinized. Average times, after sacrifice, needed to obtain these samples were: muscle-1.0 min (range 0.6-1.6 min); liver-2.1 min (range 1.1-3.1 min); blood- between 2 and 7.5 min.

Changes in tissue glycogen and lactate levels due to anaerobiosis after sacrifice were investigated by excising three muscle samples from individual fish at regular times after sacrifice. For each fish, differences in glycogen and lactate concentrations between

the first and successive samples were expressed as percent change per minute. Average rates of change were only a few percent per minute and, except for muscle glycogen between the 2nd and 3rd sampling times, were not significantly different from zero (t-test, $p < 0.05$)(Table 1). Therefore, errors in muscle lactate and glycogen measurement due to post-mortem changes are considered negligible.

Body Site Variation

The extent of variation in lactate concentration within the white muscle was evaluated to determine whether the concentration at a single site could be used in calculating the whole body rate of lactate disappearance after angling. White muscle lactate and glycogen were measured in rested and exercised pike at each body site shown in Fig. 1.

After a four-day rest following capture in August, eight pike were lightly anaesthetized and placed individually into submerged chambers made of plastic pipe, 75 cm long and 15 cm inside diameter, with netting at both ends. Water temperature was 19 °C and continuous pumping of fresh water into each chamber prevented oxygen depletion inside. The next morning, while still in darkness, four fish were immobilized in the resting state with anaesthetic (2-phenoxyethanol at 0.4%). The other fish were stimulated to exercise by alternately lifting the cage out of water for 30 sec and then submerging it again for 30 sec; the cycle was repeated three times and the fish anaesthetized as before. Muscle samples were quickly excised from the three body sites and immediately frozen in a dry ice-acetone mixture. Initially, pike lunged violently, but with fatigue their struggling subsided to thrashing or flopping about; this exercise resembles that induced by angling.

Chemical Analyses

Glycogen was analysed using the anthrone method of Seifter et al. (1949) as modified by Van Handel (1965). Aliquots of liver and muscle digests and whole blood were combined with 20 volumes of 6% wt/vol perchloric acid, centrifuged, and the supernatant analysed for lactate by the method of Barker and Summerson (1941). Recoveries of glycogen and lactate added to KOH digests averaged 95±5.53% (mean±SD) and 108±8.60% (mean±SD) respectively (n=10). Whole blood glucose was measured

Table1. Post-sacrifice rates of change in white muscle lactate and glycogen levels. Three muscle samples were taken from individual pike at specified times after death. Percent change from the first sampling time (mean= 1.08 min; range= 0.95 - 1.21 min) was calculated for each fish.

Sampling time: min after sacrifice		Percent change per min from time 1 95 % CI's are in brackets, n=23	
mean	range	Lactate	Glycogen
2.86	2.12 - 3.63	1.7 (-2.7 - 6.1)	7.7 (-1.5 - 16.8)
8.38	6.05 - 13.82	1.0 (-0.1 - 2.1)	-2.5 (-5.0 - -0.1)

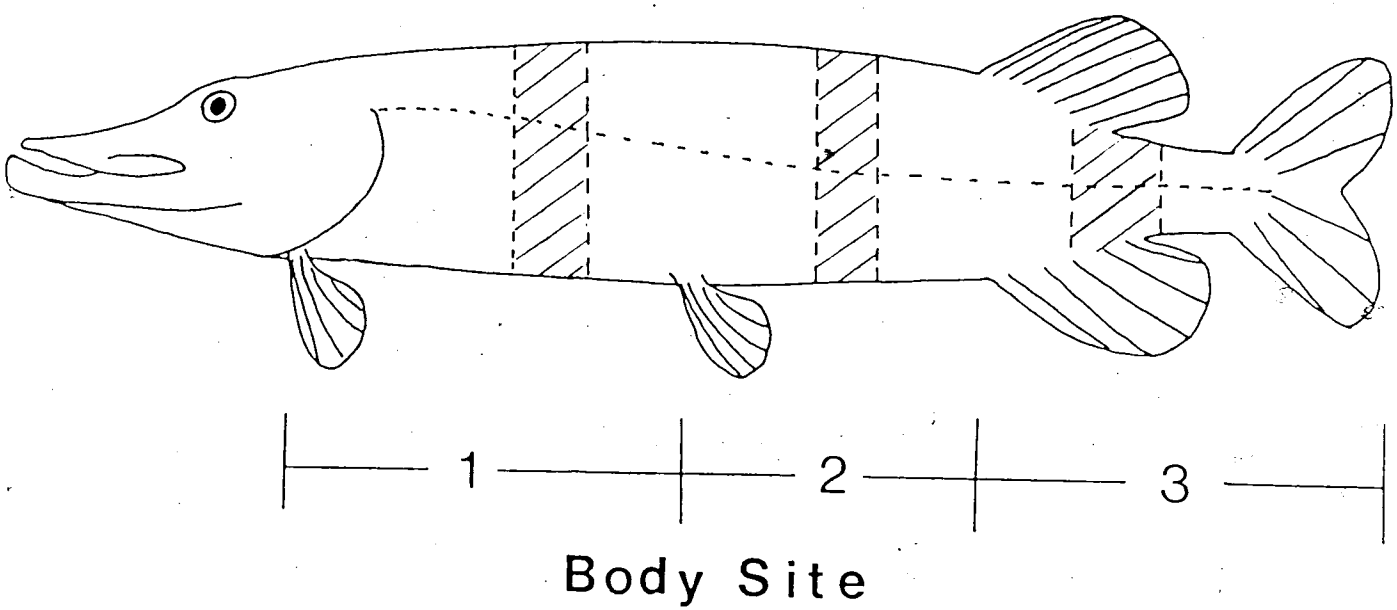


Figure 1

Diagram showing the three body sites of pike which were sampled (hatched areas) to evaluate the extent of variability in lactate and glycogen levels within the axial white muscle mass. Site 1- pectoral fins to pelvic fins. Site 2- pelvic fins to anterior insertion of dorsal fin. Site 3- caudal to anterior insertion of dorsal fin.

with a commercial kit (Sigma Chemical Co. St. Louis, Tech Bull. #510) employing the glucose oxidase-peroxidase method of Raabo and Terkildson (1960). All measurements were performed in duplicate; duplicates agreed within 3-4% on average.

Weights of the White Muscle and Highly Oxidative Tissues

These parameters were measured in freshly killed pike taken from Lac Ste. Anne in November. White muscle mass in each of the three body sites shown in Fig. 1 was determined to calculate the rate of lactate removal following angling. The mass of the gill filaments, red muscle, liver, kidney, heart, and spleen (henceforth termed 'aerobic' tissues) was also needed to calculate the metabolic rate they would require to oxidize the lactate removed. In pike, accurate measurement of the axial red muscle mass from its cross-sectional area is very difficult because much of the red muscle is very thin (approx. 0.5 mm.) and not sharply demarcated from the white fibres. Nevertheless, the cross-sectional area of red muscle in 9 to 15 freshly cut sections per fish was used to estimate the axial red muscle mass, but the measurements were made to give a bias towards over-estimation (by approx. 25%). Muscle in the head and at the base of the pectoral and pelvic fins was dissected free and when the red and white fibres could not be easily separated they were weighed together so as to again overestimate the red muscle mass.

Statistics

All data are expressed as means and 95% confidence intervals. In the angling study, homogeneity of variance could not be demonstrated. Therefore, the Kruskall-Wallis non-parametric procedure (Sokal and Rolf 1969) was used to test for significant differences (at $P<0.05$) between the five sampling times. Comparison of individual samples with the 0 h sample was made with an approximate t test ($P<0.05$)(Sokal and Rolf 1969).

Calculations

The relationship between the post-exercise decline in white muscle lactate concentration and replenishment of muscle glycogen was assessed as follows. The decline in average white muscle lactate level (mmol kg^{-1}) from 0 h until 3, 6, and 48 h was expressed as its equivalent concentration of glycogen glucose units (2 lactate molecules form one glucose unit of glycogen). These equivalent concentrations were each added to the average 0 h muscle glycogen level to give the average muscle glycogen level to be expected if all the lactate disposed of by 3, 6, and 48 h had been incorporated directly back into white muscle glycogen. This assumes that the lowest muscle glycogen levels occurred immediately after exercise (0 h).

The following procedure was used to calculate the amount of lactate removed from the white muscle of pike of a standard weight (1 kg) in 6 h after angling. In the angling study, muscle samples were obtained from site 2 and the lactate levels at sites 1 and 3 (at 0 and 6 h) were estimated from the extent of variation in lactate level within the white muscle. Muscle lactate content at each site was calculated as the product of white muscle mass and lactate concentration, and lactate content of the three body sites was summed to give total white muscle lactate content. The difference in total white muscle lactate content between 0 and 6 h is the amount of lactate removed from the white muscle within 6 h after angling.

Complete oxidation of lactate, via the Krebs cycle and oxidative phosphorylation, is described by the overall equation: $C_3H_6O_3+3O_2 \rightarrow 3CO_2+3H_2O$. The metabolic rate required to oxidize lactate at its rate of removal after angling was calculated as:

$$(La^- / 6) \times 3(\text{molar ratio of } La^- \text{ to } O_2) \times 32(\text{MW of } O_2, \text{ mg mmol}^{-1});$$

where La^- is mmol of lactate removed per kg body wt in 6 h after angling. The total amount of glycogen utilized during angling-induced exercise was calculated from the difference between post-exercise (0 h) and resting (96 h) muscle lactate levels, assuming all the excess lactate came from glycogen.

C. RESULTS

Pike experienced a large increase in blood lactate concentration after exercise; levels at 3 h were about 9-fold greater than at 48 and 96 h (Fig. 2). The elevated blood lactate levels at 0 h were probably due to release of lactate from the muscle into blood whilst the fish were in transit to the Biological Station for sampling. Liver lactate showed a moderate elevation at 3 h but by 6 h had declined to essentially resting (ie: 48 and 96 h) values. As expected, muscle lactate level was very high immediately after angling and showed a large decline in the first 6 h. Blood glucose level more than doubled after angling and remained somewhat elevated even after 96 h of recovery. The similarity of muscle, liver, and blood lactate at 48 and 96 h (Fig. 2) indicates that anaesthetization of the 96 h fish did not noticeably increase anaerobiosis.

Large variation existed in muscle and liver glycogen levels (Fig. 3). White muscle glycogen did not significantly increase above the 0 h level until 96 h after exercise. The dashed lines in Fig. 3 indicate the white muscle glycogen levels expected at 3, 6, and 48 h if all the lactate removed from the muscle by these times had been incorporated directly back into white muscle glycogen. Comparison of the expected glycogen levels with the observed average values (Fig. 3) indicates that white muscle glycogen synthesis after exercise was much slower than lactate removal. Liver glycogen concentration at 48 h was about 34% below the 0 h level, although this difference was not significant ($P<0.05$) according to the Kruskal-Wallis multiple comparison test (Fig. 3).

A trend of increasing muscle lactate and glycogen concentration caudally from site 1 occurred in almost all the rested and exercised fish examined for body site variation (Fig. 4). However, the variation in lactate levels after exercise was relatively small as average levels at site 1 and 3 were 90% and 101% respectively of the levels at site 2 (Fig. 4). Figure 4 also clearly shows that brief burst exercise results in substantial glycogen depletion and lactate accumulation, not just from the tail region, but throughout the entire axial white muscle mass of pike. Table 2 shows the weights of the white muscle and 'aerobic' tissues of a 1 kg pike in November. The increase in gonad size that occurs in pike between mid-summer (when the angling study was performed) and November had very little effect on the values in table 2 because even in November the gonads represent

Figure 2

Changes in muscle, liver, and blood metabolites of pike after angling induced exercise. The Kruskal-Wallis test was significant (at $P<0.05$) for all parameters shown. Means and 95 % confidence intervals are shown; n values are indicated below the mean; * indicates significant difference from 0 h (approximate t-test; $P<0.05$).

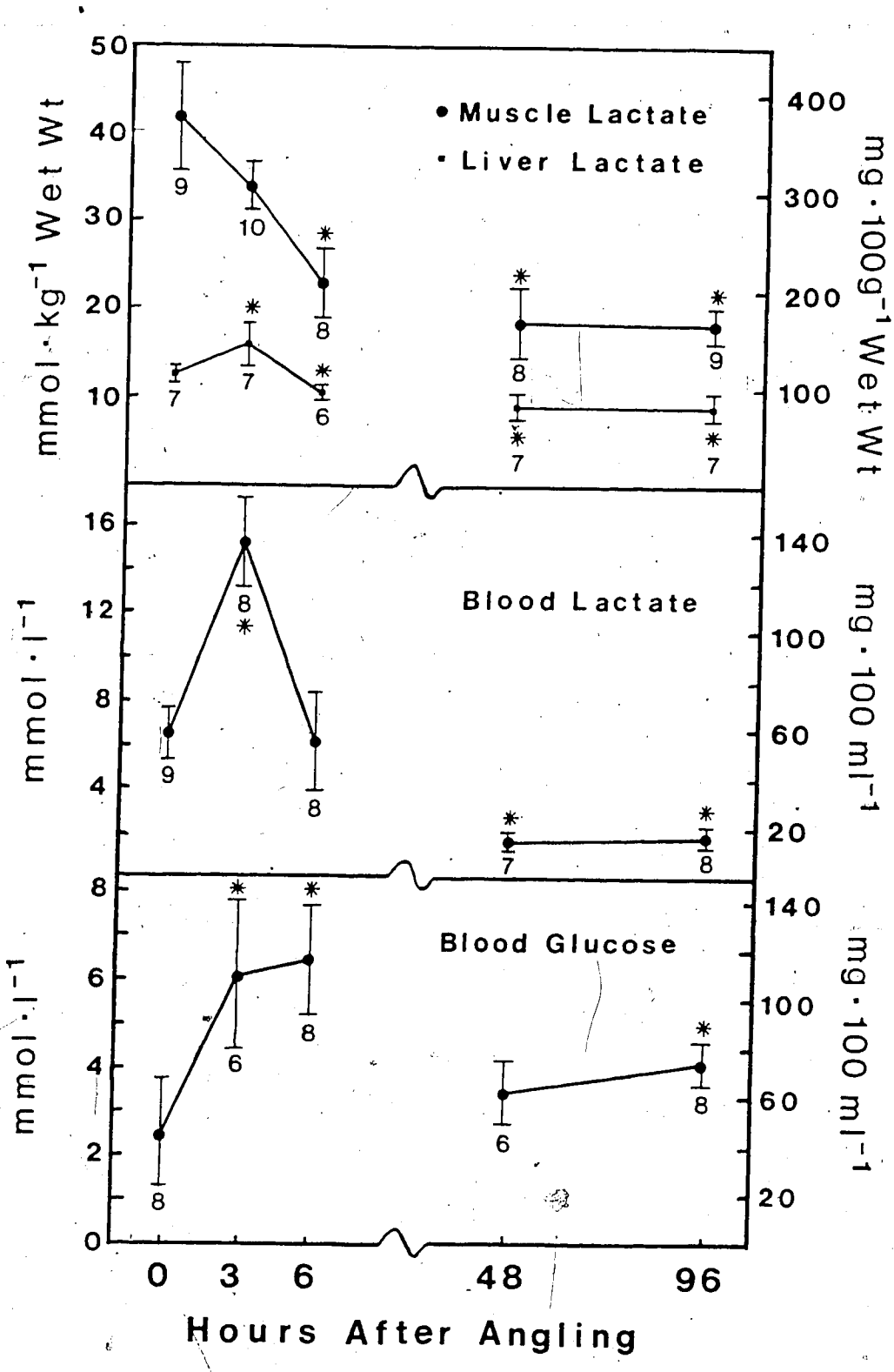

• Muscle Lactate
• Liver Lactate
mmol·kg⁻¹ Wet Wt
mg·100g⁻¹ Wet Wt
Blood Lactate
mmol·l⁻¹
mg·100 ml⁻¹
Blood Glucose
mmol·l⁻¹
mg·100 ml⁻¹
Hours After Angling
0 3 6 48 96

Figure 3

Changes in white muscle and liver glycogen of pike after angling. The Kruskal-Wallis test indicates that significant differences ($P<0.05$) exist between sampling times for muscle glycogen but not for liver glycogen. Dashed lines indicate the muscle glycogen levels expected at 3, 6, and 48 h if all the lactate removed by these times had been incorporated directly back into muscle glycogen (see text). Other details as in Fig. 2.

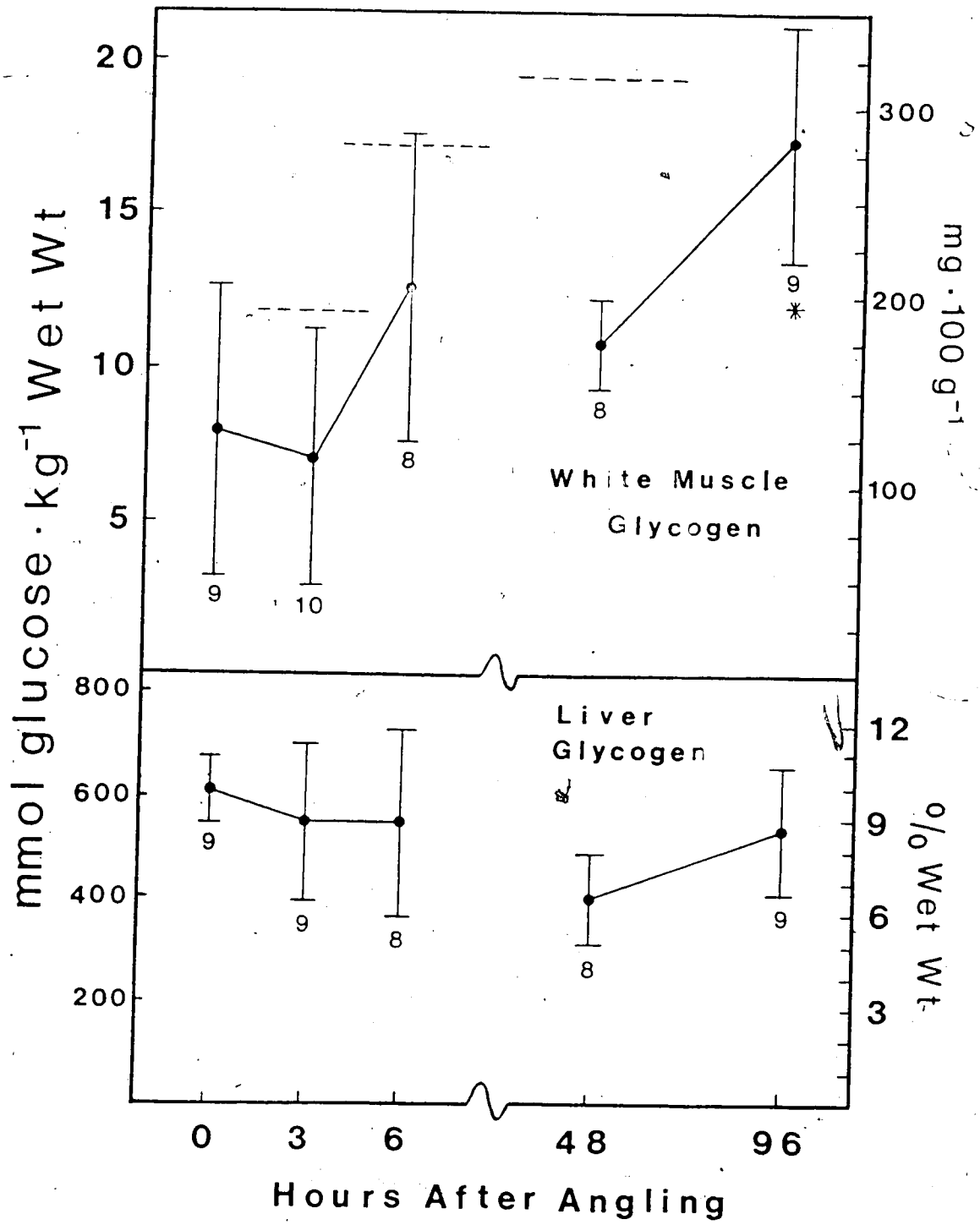

mmol glucose · kg⁻¹ Wet Wt
mg · 100 g⁻¹
% Wet Wt
White Muscle Glycogen
Liver Glycogen
Hours After Angling
0
3
6
48
96
20
15
10
5
800
600
400
200
300
200
100
12
9
6
3
9
10
8
9
8
9
9
8
9
8

Figure 4

Variation of glycogen and lactate levels within the white muscle mass of four rested (R1-R4) and four exercised (E1-E4) pike. Body sites are those in Fig. 1. Muscle samples from each body site were divided into four subsamples before analysis. Means and 95 % CI's shown for R1-R4 and E1-E4 are those for the four subsamples at each body site of an individual fish. The averages and 95 % CI's calculated from between fish variation are shown at the right of each graph. For muscle lactate the average post-exercise values at sites 1 and 3 are expressed as a % of the site 2 level.

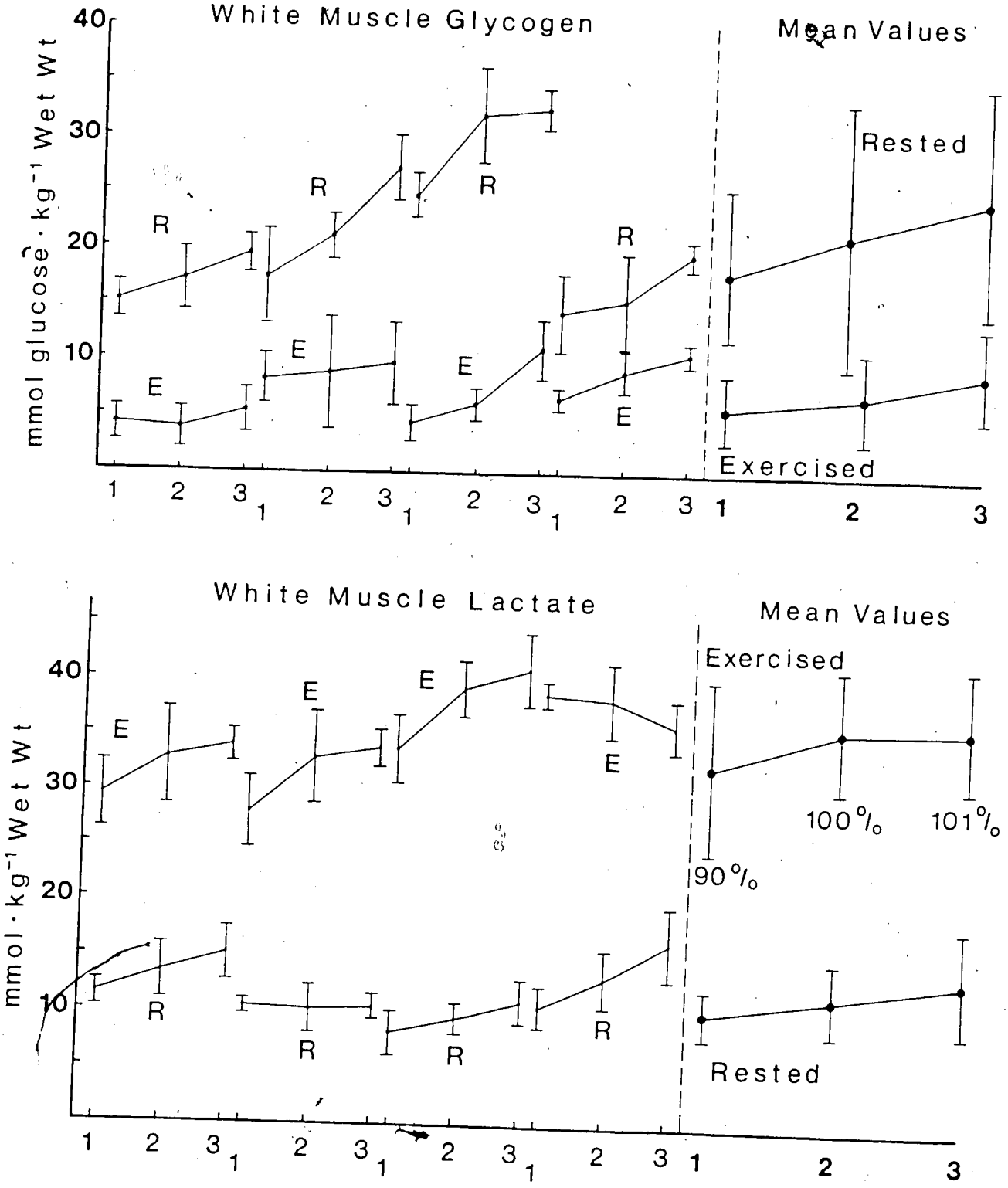

White Muscle Glycogen
Mean Values
mmol glucose · kg⁻¹ Wet Wt
40
30
20
10
R
E
Rested
Exercised
1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
White Muscle Lactate
Mean Values
mmol · kg⁻¹ Wet Wt
Exercised
90%
100%
101%
Rested
Body Site

Table 2. Weights of the white muscle and highly oxidative tissues and organs of pike. n=7

	Mean	95 % CI
Body Wt (g)	1024	(834, 1215)
	Grams per kg body weight	
White muscle		
site 1	247.0	(233.8, 260.2)
site 2	187.8	(170.3, 205.3)
site 3	102.9	(93.9, 111.9)
Total white muscle	537.7	(517.8, 557.6)
1 Red muscle	39.9	(28.9, 50.9)
2 Gill filaments	11.3	(10.2, 12.4)
3 Liver	14.1	(12.0, 16.3)
4 Kidney	9.9	(8.6, 11.2)
5 Heart	1.45	(0.97, 1.93)
6 Spleen	1.52	(0.60, 2.43)
Gonads	36.00	(10.4, 61.6)
Total 'aerobic' tissues (1 to 6)	78.2	(67.8, 88.6)

only 3.6% of total body weight on average.

The total amount of lactate removed from the white muscle of a 1 kg pike during 6 h after angling is calculated to be 9.57 mmol (table 3). This is probably very close to the amount of lactate removed from the whole body because, as indicated by liver and blood levels (Fig. 2), lactate concentrations in non-white muscle tissues are similar at 0 and 6 h. To oxidize lactate as rapidly as it disappears from pike after angling would require a metabolic rate of 153 mg O_2 kg^{-1} h^{-1}. In Fig. 5 this metabolic rate is partitioned, in all possible ways, between the 'aerobic' tissues (red muscle, gills, liver, kidney, heart, and spleen) and the remaining body mass. It is clear that if even 60% of the lactate disappearing from pike in 6 h after exercise is oxidized in the 'aerobic' tissues the metabolic rate of these tissues must be more than 15 times the standard whole body metabolic rate of approximately 70 mg O_2 kg^{-1} h^{-1} at 20 °C (Beggs et al. 1980). Such high metabolic rates would approach the basal metabolic rate of small mammals (approx. 1500 mg O_2 kg^{-1} h^{-1} for white rats). From the difference between 0 and 96 h muscle lactate levels, the amount of glycogen utilized during angling was estimated to be 654 mg (4.04 mmol of glucose units) or 80% of average 0 h liver glycogen content which was 821 mg (5.07 mmol of glucose units).

During the summers of 1982-83, a total of 105 pike were caught by angling. Every pike struggled very vigorously before it could be landed. All fish were held captive for at least 3 hr; 85 were held for more than two days. Of the 105 fish only 3 lost equilibrium after angling. One fish subsequently died, a second regained equilibrium and was sampled at 3 hr, and the third fish was returned to the lake.

Table 3. Calculation of the metabolic rate required to oxidize all lactate removed from the white muscle of pike in 6 hr after angling.

	0 hr			6 hr		
	Body site			Body site		
	1	2	3	1	2	3
[muscle lactate] % of site 2 level[a]	90.5	100	101.1	90.5	100	101.1
[lactate] ($mmol \cdot kg^{-1}$)	37.8	41.8[b]	42.3	21.0	23.2[b]	23.5
Estimated muscle wt[c] $g \cdot kg$ body wt^{-1}	247.0	187.8	102.9	247.0	187.8	102.9
lactate content[d] mmol	9.34	7.85	4.35	5.19	4.36	2.42

Total white muscle lactate content at 0 hr = 9.34 + 7.85 + 4.35 = 21.54 mmol

at 6 hr = 5.19 + 4.36 + 2.42 = 11.97 mmol

Lactate removed from white muscle of a 1 kg pike in 6 hr = 9.57 mmol

= 861 mg

Metabolic rate required to oxidize 9.57 mmol lactate in 6 hr after angling

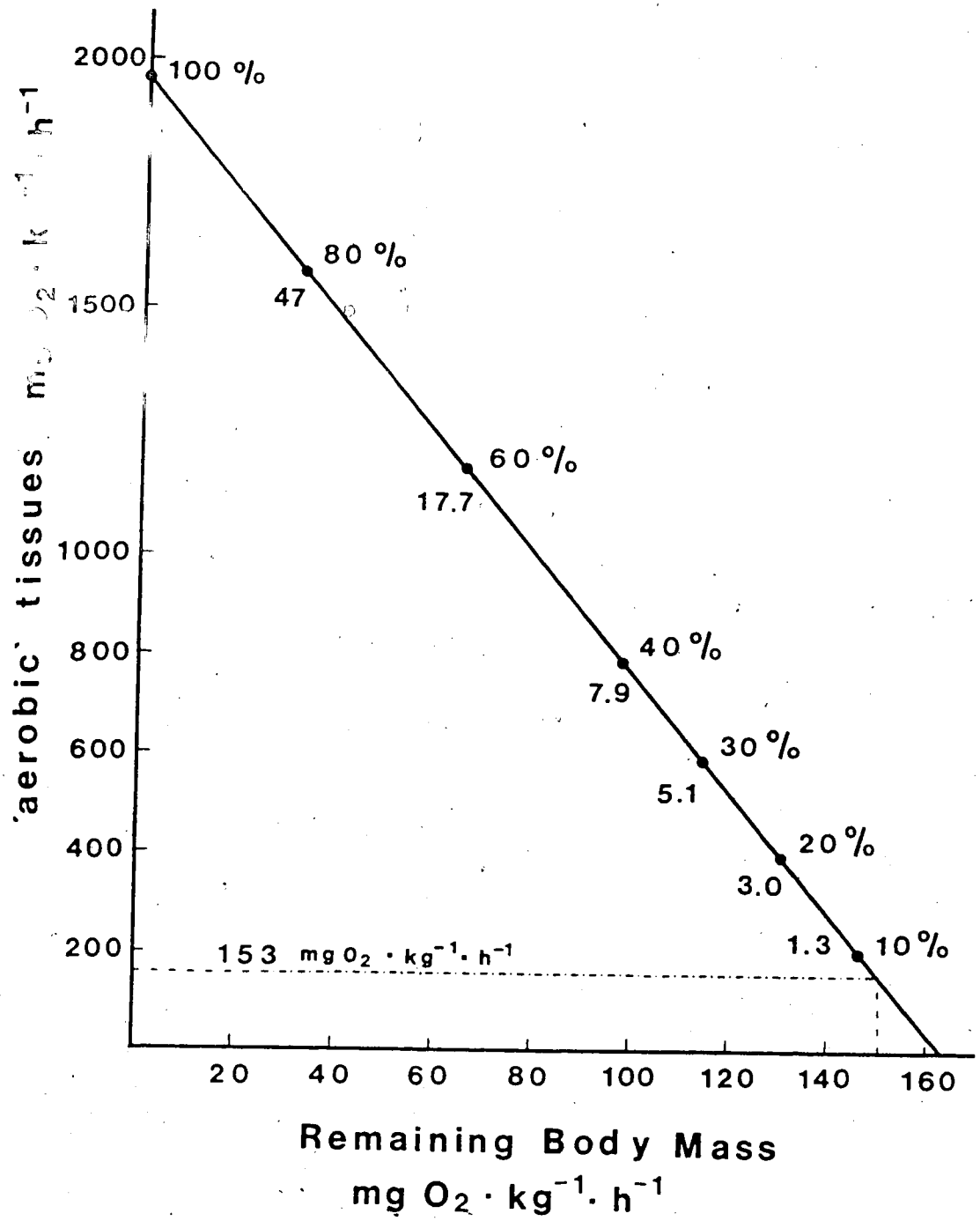
$= (9.57 \text{ mmol} \times 3 \times 32 \text{ mg } O_2 \cdot mmol^{-1}) \div 6 \text{ hr}$ (see text)

$= 153 \text{ mg } O_2 \cdot kg \text{ body } wt^{-1} \cdot hr^{-1}$

a From Fig. 4
b Averages at 0 and 6 hr from Fig. 2
c From Table 2
d Calculated as [lactate] ($mmol \cdot kg^{-1}$) ÷ 1000 x estimated muscle mass (g)

Figure 5

Partitioning of oxygen consumption between the 'aerobic' tissues (gills, red muscle, liver, kidney, heart, and spleen) and the remaining body mass. The diagonal line represents all combinations of metabolic rate that give a whole body O_2 consumption of 153 mg O_2 kg^{-1} h^{-1}. The latter is the rate required to oxidize all the lactate removed from pike in 6 h after angling. Percentage values show the proportion of total lactate removal that could be oxidized in the 'aerobic' tissues if they possessed the metabolic rate indicated. Numbers below the diagonal line are the ratio of 'aerobic' tissue MR to remaining body mass MR.


2000
1500
1000
800
600
400
200
100 %
80 %
47
60 %
17.7
40 %
7.9
30 %
5.1
20 %
3.0
1.3
10 %
153 mg O2 · kg-1 · h-1
20
40
60
80
100
120
140
160
'aerobic' tissues
Remaining Body Mass
mg O2 · kg-1 · h-1

D. DISCUSSION

Northern pike experienced very low mortality (< 3%) after angling-induced exercise when physical damage to the gills and other body organs was avoided. Much higher mortality rates of about 30% have been reported following exercise in salmonids (Bouck and Ball 1966; Wendt 1966; Wood et al. 1983) and also in muskellunge (Beggs et al. 1980). In these studies much of the mortality probably resulted from the use of fish that were in poor health due to hatchery-rearing or the stress of surgery and anaesthesia. In muskellunge (Beggs et al. 1980), surgery and anaesthetization were performed immediately after angling; gill ventilation and oxygen uptake were thus impaired at a critically important time.

Despite the 'sprinter' lifestyle of pike, the magnitude and duration of changes in muscle, liver, and blood carbohydrate levels after burst exercise (Figs 2 and 3) are similar to those seen in salmonids that survived exercise (c.f. Black et al. 1960,62; Wendt 1966; Turner et al. 1983a,b). However, it is surprising that blood lactate levels attained by pike following angling, 15.2 mmol l^{-1} at 3 h (Fig. 2), were much higher than maximum post-angling levels of 4 mmol l^{-1} previously reported for muskellunge (Beggs et al. 1980). Possible explanations for this discrepancy are discussed in the next chapter.

The extreme slowness of white muscle glycogen resynthesis in pike, compared to the rate of lactate removal (Fig. 3), suggests that very little of the exercise-produced lactate is incorporated into white muscle glycogen (either via the Cori cycle or in situ lactate glyconeogenesis). The relatively large amount of variation between fish makes interpretation of Fig. 3 difficult. However, if lactate incorporation into white muscle glycogen is quantitatively important during recovery from exercise, one would have expected better regulation of glycogen levels (ie: less variation) and a clearly discernible trend of increasing muscle glycogen concentration in the time needed to dispose of excess muscle lactate (ie: in 48 h).

Any struggling by the fish during or prior to sampling would have utilized glycogen and slowed down the apparent rate of glycogen replenishment. But struggling during sampling would also have produced lactate in amounts stoichiometrically equal to the glycogen depleted. Therefore, comparison of the expected muscle glycogen levels

(dashed lines in Fig. 3) with the actual values is still valid. The low blood lactate levels at 48 and 96 h (1.7 mmol l^{-1}) indicate that the fish had disposed of the excess lactate produced during any exercise bouts prior to sampling. If white muscle glycogen replenishment occurred as quickly as lactate removal, the muscle glycogen levels of the fish 48 h after exercise should also have been completely replenished; yet they were not (Fig. 3).

Fasting during the recovery period or elevated levels of circulating adrenalin due to 'captivity stress' could have slowed down the rate of muscle glycogen resynthesis. Both fasting and adrenalin are known to inhibit muscle glycogen synthesis in mammals (Newsholme and Start 1973). Nevertheless, under the conditions of the present study, lactate incorporation into muscle glycogen appeared to be of little importance in either removal of excess lactate or resynthesis of white muscle glycogen. A very slow rate of white muscle glycogen resynthesis after exercise was also found in previous studies of salmonids (Black et al. 1962; Wendt 1966).

By 96 h after exercise pike had substantially replenished their white muscle glycogen stores (Fig. 3). If muscle glycogen replenishment after angling occurred through mobilization of liver glycogen, as has been suggested (Love 1970; Shul'man 1974), liver glycogen content would be depleted by 80% (see Results). Such a severe depletion could endanger the obligatorily glucose-metabolizing tissues such as the brain, kidney medulla, and red blood cells which depend on liver glycogen for maintenance of blood glucose level (Newsholme and Start 1973). At most, only a 34% decline in liver glycogen occurred by 48 h after angling with a return to normal by 96 h (Fig. 3). Therefore, a major part of the glycogen resynthesis in white muscle after burst exercise must have occurred through gluconeogenesis, most likely from protein or glycerol.

Although the complete Cori cycle appears to be of minor importance in removal of excess lactate, the post-exercise hyperglycemia that occurs in pike (Fig. 2) and in other fish (Wardle 1972; Mazeaud et al. 1977; Perrier et al. 1978) suggests that exercise-produced lactate may be incorporated into blood glucose. However, this is unlikely to be the primary fate of exercise-produced lactate. At a hematocrit of 25% (normal for pike) the plasma glucose concentration is approximately 4/3 of the total blood level, assuming that intracellular glucose concentration is negligible (Newsholme and Start 1973). If the 0 to 6 h increase in glucose concentration throughout the entire

extracellular fluid (15% of body weight, Holms and Donaldson 1969) is the same as in plasma, this could account for only 1.58 mmol of the 9.57 mmol of lactate removed in 6 h. If much of the lactate is converted into blood glucose, this glucose must be used for energy production since it is not used for net glycogen synthesis in either liver or white muscle. Eventually, this glucose must be used oxidatively because anaerobic utilization would not lead to a net removal of lactate. There appears to be little need for prior conversion to glucose since most fish tissues can oxidize lactate directly (Bilinski and Jonas 1972). When present at high physiological concentrations, lactate is oxidized in preference to glucose by trout hearts (Lanctin et al. 1980).

Based mainly on the slowness of white muscle glycogen synthesis in fish, previous workers (Black et al. 1962; Driedzic and Hochachka 1978) have concluded that oxidation is the primary fate of exercise-produced lactate. My analysis shows that relatively low rates of oxygen consumption (153 mg O_2 $kg^{-1}hr^{-1}$) would be required of pike to oxidize lactate at the rate it disappears after angling. Beggs et al. (1980) reported the oxygen consumption of muskellunge during the first 6 h after angling to be about 100 mg O_2 kg^{-1} h^{-1}, which corroborates reasonably well with the present study. This seems a low metabolic rate for a fish that has just completed vigorous exercise and may reflect the anaesthetization that Beggs et al. (1980) performed on their muskellunge immediately after angling. Salmonids have oxygen uptake levels of 300 to 600 mg O_2 kg^{-1} h^{-1} during sustained swimming, and upon fatigue, require several hours to re-establish resting levels of about 100 mg O_2 kg^{-1} h^{-1}(Brett 1964).

The prevailing dogma seems to be that lactate oxidation in fish must be performed in the red muscle, gills, liver, kidney, heart, and spleen since these tissues have much greater oxidative capability than the white muscle (Bilinski and Jonas 1972). However, Fig. 5 clearly shows that the oxygen uptake rate of these 'aerobic' tissues must be very high, 1,000 to 2,000 mg O_2 kg^{-1} h^{-1}, to oxidize the major portion of the lactate metabolized by pike in 6 h after angling. Surprisingly, 'in vitro' oxygen uptake rates in this range have been recorded by Gordon (1972b) for the red muscle of a wide variety of semi-tropical marine fish. However, his measurements likely over-estimate 'in vivo' rates because even the measurements of white muscle oxygen uptake (100 - 400 mg O_2 kg^{-1} h^{-1}) (Gordon 1972a) are as high or higher than the probable whole body metabolic rates of the fish. It

would be very unusual if the oxygen uptake of all the 'aerobic' tissues of pike (Table 2) were high enough to account for the observed rate of lactate removal.

What then is the primary fate of exercise-produced lactate in pike? The whole body metabolic rate of pike is probably sufficient to account for lactate disappearance through oxidation but that of the 'aerobic' tissues alone is not. I propose that significant oxidation of lactate may occur in the non-red muscle mass. Most of the non-red muscle is composed of white fibres but fish also have so called 'pink' fibres which are intermediate between the red and white fibres in their oxidative ability (Johnston et al. 1975). My measurements were intended to over-estimate only the most highly aerobic or red muscle mass and may not have included all of the 'pink' fibres.

Although they have very low oxidative capacity (Johnston 1982), even the true white fibres may make an important contribution to whole body oxygen uptake because of their large mass (54% of body weight; table 2). Fish white muscle contains most of the tissue blood volume (Stevens 1968), contains mitochondria (Johnston 1982), and consumes oxygen 'in vitro' (Gordon 1972a). Additionally, evidence in fish (Wardle 1979; Turner and Wood, 1983) and in man (Hermansen and Vaage 1977) indicates that only about 10 to 20% of the exercise-produced lactate is actually released from the muscle into the blood. For the 'aerobic' tissues to oxidize 20% of the lactate removed from the white muscle after exercise they would require an oxygen uptake rate of about 400 mg O_2 kg^{-1} h^{-1} (Fig. 5). This seems quite reasonable. Koboyashi and Wood (1980) reported lactate excretion by fish to be negligible and lactate incorporation into lipids is considered insignificant (Krebs et al. 1975). In summary, the most likely fate of exercise-produced lactate in pike appears to be oxidation in both the 'aerobic' tissues and the non-red muscle with only small amounts being incorporated into blood glucose and white muscle glycogen. In most fish, oxygen consumption increases tremendously within minutes after exercise that results in fatigue (Brett 1964,72). It is unlikely that protein or lipid oxidation can be increased this rapidly. The large quantity of lactate produced during exercise is perhaps the most readily available substrate for oxidation.

III. INFLUENCE OF BURST EXERCISE ON BLOOD ACID-BASE AND PLASMA GLUCOSE STATUS OF NORTHERN PIKE

A. INTRODUCTION

During vigorous anaerobic exercise, lactate anions (La^-) and H^+ ions are produced in the muscle in stoichiometrically equivalent amounts (Hochachka and Mommsen 1983). However, in salmonid fish, the increase in blood La^- concentration following exercise (>11 mmol l^{-1}) exceeds the portion of the increased blood H^+ burden that is due to H^+ release from the muscle (ie: the metabolic acid load) (Turner et al. 1983a; Wood et al. 1983). Marine flatfish, in contrast, have very low post-exercise blood [La^-] (<2 mmol l^{-1}) (Wardle 1979), so that the metabolic acid load exceeds the increase in blood [La^-] (Turner et al. 1983a). Turner et al. (1983b) have suggested that flatfish, being sedentary and of low aerobic metabolism, retain lactate for further processing within the muscle, instead of releasing it into the blood. It is of interest therefore, to know the degree of blood lactate elevation following burst exercise in the northern pike (Esox lucius L.) because these fish are also inactive most of the time (Diana 1980) and yet, unlike flatfish, perform explosive burst exercise when capturing prey. Previous studies have indicated that the blood lactate level of pike (Soivio and Oikari 1976) and the closely related muskellunge (Esox masquinongy M.) remains low (<5 mmol l^{-1}) after exercise and that in muskellunge accumulation of H+ in the blood exceeds that of La^- (Beggs et al. 1980). However, these results may not represent normal physiological responses because the fish used were stressed by either four weeks of fasting (Soivio and Oikari 1976) or by anaesthetization and surgery immediately after exercise (Beggs et al. 1980).

Prolonged elevation of plasma glucose level is often seen in fish after exercise or handling stress (Wardle 1972; Wydoski et al. 1976; Perrier et al. 1978). This hyperglycemia may be a response to some stress, such as acidosis, accompanying exercise or may result from lactate removal via the Cori cycle (ie: glucose synthesis from lactate by the liver)(Hochachka 1961). The work of Renaud and Moon (1980a,b) indicated that American eel livers have good ability to perform gluconeogenesis from lactate.

The purpose of the present study was to determine the influence of burst exercise on blood acid-base, lactate, and plasma glucose status in northern pike that were not

anaesthetized and had not undergone prolonged fasting. An additional experiment was performed in which pike were intra-arterially infused with lactate to determine if metabolism of excess lactate produces hyperglycemia when exercise stress is absent. The results show that following burst exercise blood La- accumulation exceeds the metabolic acid load and that lactate removal is not the cause of post-exercise hyperglycemia in pike.

B. MATERIALS and METHODS

Pike were obtained from Lac Ste. Anne, Alberta by angling because, of the methods available, this was the least stressful for the fish. Their body weight averaged 820±182 g (mean±SD,n=14). Pike used for the exercise experiment were collected in early June when lake temperature was 15-16 °C while those for the lactate infusion experiment were collected in late September when lake temperature was 9-10°C. Three or four days after capture the fish were transported to the University of Alberta Aquatic Facilities and treated with malachite green to prevent fungal infection. Within two days after arrival, water temperature was raised to 19 °C, the temperature at which all experiments were performed. Experiments began on most fish between 6 and 13 days after capture. Fish were still in good health at this time, as indicated by resting blood lactate, glucose, and pH levels. Pike did not feed in captivity.

Cannulae consisted of 0.965 mm O.D. polyethylene tubing (Clay Adams, PE50, intramedic tubing) tipped with a 26 gauge needle and filled with heparinized (300 USP units/ml) saline (0.9% NaCl). In preparation for cannulation, pike were anaesthetized with 2-phenoxyethanol (first mixed with 2 volumes ethanol) at 0.1%, then laid on their side and the operculum held open to expose the ventral aorta. The cannula needle was inserted approximately 3 mm into the ventral aorta and sutured in place as shown in Fig. 6. While some swelling of the aorta and surrounding tissues occurred following cannulation, post-mortem examination revealed no evidence of hemorrhage or bleeding at the cannulation site in any fish. After cannulation, pike were placed individually into the submerged chambers described in the previous chapter (see Fig. 6). Pike were allowed to recover in these chambers overnight in darkness and they were exercised or infused with lactate the following day.

Pike were induced to exercise by alternately lifting the chamber partially or completely out of water for 30 sec. and then submerging it for 30 sec. This cycle was repeated three times for a total exercise time of 1.5 min. Initially, pike would lunge violently, but with fatigue their struggling subsided to thrashing or flopping around inside the chamber. Blood samples (500 ul.) were drawn anaerobically before exercise, at 5 min and 0.5, 1, 2, 4, 8, 12, 24, and 48 h after exercise. All samples were analyzed for blood lactate and plasma glucose and all except the 48-h samples for pH, PCO_2, and hematocrit.

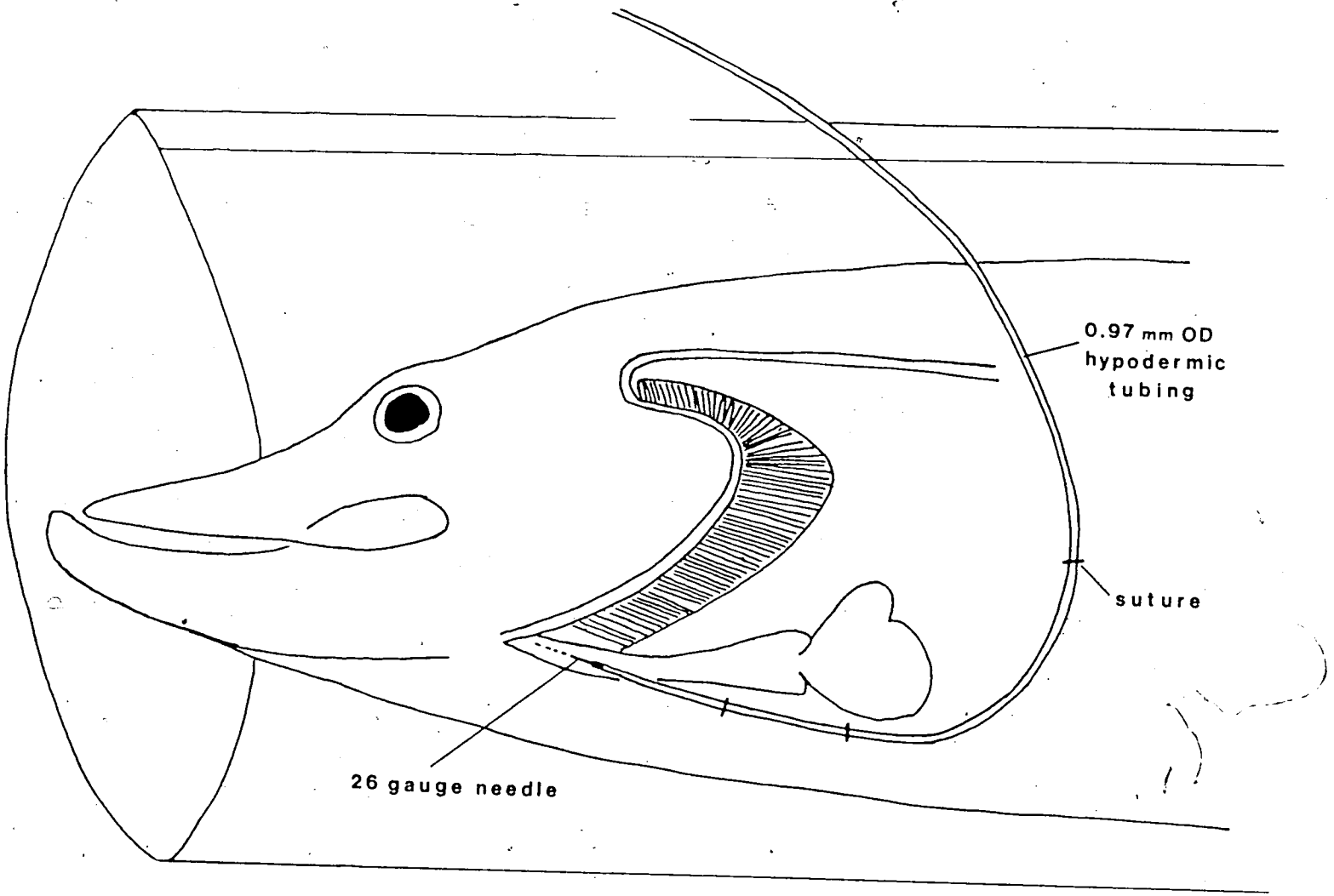


Figure 6

Illustration of the ventral aorta cannulation technique and one of the chambers used to hold pike.

Pike were sacrificed 48 h after exercise, and samples were removed, weighed, and digested in 30% KOH for glycogen analysis.

Lactate solution for infusion was prepared by adding equimolar amounts of L(+)-lactate (Sigma) and NaOH to water and adjusting the final pH to 7.6 with HCl or NaOH. This solution had a final L(+)-lactate concentration of 5 g 100 ml^{-1}. To mimic the quick initial rise in plasma lactate after exercise, approximately 250 mg of lactate (5.0 ml of solution) was infused, by peristaltic pump, into each fish via the cannula over a 10 min period. Following this, lactate was infused at a slower rate (7.5 ml h^{-1}) to sustain plasma lactate at high levels for 2 h. After 2 h, 9.78±1.23 mmol La kg^{-1} body wt (mean±SD, n=6) had been infused. This approximates the amount of lactate a pike produces during a vigorous burst of exercise (see previous chapter). Blood (500 ul) was sampled before, during, and at 3 and 6 h after the start of infusion and analyzed for blood pH, plasma lactate, and glucose. Infusion was stopped one minute before blood sampling to allow infused lactate to be carried away from the cannula tip and thereby avoid contamination of the blood sample. Lactate solution in the cannula and the first drop of blood were discarded prior to removal of blood samples.

Plasma was frozen in sealed vials prior to analysis. Whole blood used for lactate analysis was deproteinized immediately after sampling with 20 volumes of 6% wt/vol perchloric acid; plasma was deproteinized similarly after thawing. Blood lactate, plasma glucose, and liver glycogen were measured using the methods described in the previous chapter. Blood pH and PCO_2 were measured using Radiometer BMS3MK2 electrodes thermostatted to and calibrated at 19 °C and connected to a Radiometer PHM73 pH/blood gas monitor. Plasma bicarbonate concentration was calculated from the Henderson-Hasselbach equation in the following form:

$$[HCO_3] = S \times PCO_2 \times 10 \exp (pH-pk').$$

Values of pK' were taken from Albers (1970) at the appropriate pH and temperature. The value of S at 19°C (0.04789 mmol CO_2 l^{-1} mm Hg^{-1}) was obtained from Severinghaus (1965) by linear interpolation between 15° and 20 °C.

C. RESULTS

The high blood pH (Fig. 7) and low blood lactate and plasma glucose levels (Fig. 8) before exercise indicate that the pike used for experimentation were in good health. Previous experience showed that depressed pH (<pH 7.8) and elevated blood lactate (>2.5 mmol l^{-1}) and glucose levels (>8.0 mmol l^{-1}) were characteristic of pike in poor health. When cannulated, pike had bruised snouts due to collision with holding tank walls but no other evidence of physical damage (abrasion, bleeding, or frayed fins) or traces of fungal growth could be found on any fish. Fish were sacrificed at 48 h after exercise by which time some abrasion of the caudal peduncle was evident and close examination revealed traces of fungal growth on most fish. Pike had ample reserves of liver glycogen which averaged 5.2±2.98 % wet wt (mean±SD,n=8).

Brief (1.5 min) burst exercise resulted in large depressions of blood pH and plasma HCO_3^- and greatly elevated levels of PCO_2 and lactate (Figs. 7,8). Recovery was complete in 2 h for PCO_2, and in 8 h for pH and plasma HCO_3^-. Blood lactate continued to rise in the first hour after exercise while pH had already begun to increase. Return of blood lactate to resting levels required about 8 h (Fig. 8) and coincided with the final increase in plasma HCO_3^- (Fig. 7). Plasma glucose increased tremendously (by 8.8 mmol l^{-1}) in the first 2 h after exercise but by 12 h had returned almost to resting levels (Fig. 8). One fish, with an unusual amount of fungal growth, had a very high pre-exercise plasma glucose level (11.8 mmol l^{-1}) and elevated blood lactate levels (3.3 mmol l^{-1}) at 12 and 48 h after exercise; these values were not included with data from the other fish. Nevertheless, the plasma glucose level of this fish also showed the typical rapid rise after exercise and the subsequent decline to pre-exercise values (not shown). Hematocrit averaged 19.5±1.9% (mean±SD,n=8) before exercise, increased to 23.5±3.3% (mean±SD) at 5 min after exercise and progressively declined thereafter due to red cell removal by sampling. At 12 h average hematocrit was 13.9±2.8% (mean±SD) (data not shown).

Intra-arterial infusion of lactate resulted in a rapid increase in plasma lactate to levels as high as those achieved after burst exercise; these levels were sustained for two hours. By 6 h plasma lactate had declined almost to resting levels (Fig. 9). Blood pH increased by about 0.3 pH units during lactate infusion. During lactate infusion plasma glucose level increased very slowly, the average increase at 2 h (1.7 mmol l^{-1}) being only

about 1/5 of the increase (8.8 mmol l^{-1}) seen after exercise (Fig. 9). Hematocrit did not increase during lactate infusion but instead declined from the average initial level of 19.2±4.2% (mean±SD,n=6) to an average of 15.2±4.5% (mean±SD) at 2 h (data not shown).

Figure 7

Changes in blood pH (top); blood PCO_2 (middle); and plasma HCO_3^- concentrations (bottom) of pike after burst exercise. Exercise occurred at 0 h as described in the text. Shown are the means and 95 % CI's of eight fish, except at 12 and 24 h where n was 7 and 6 respectively.

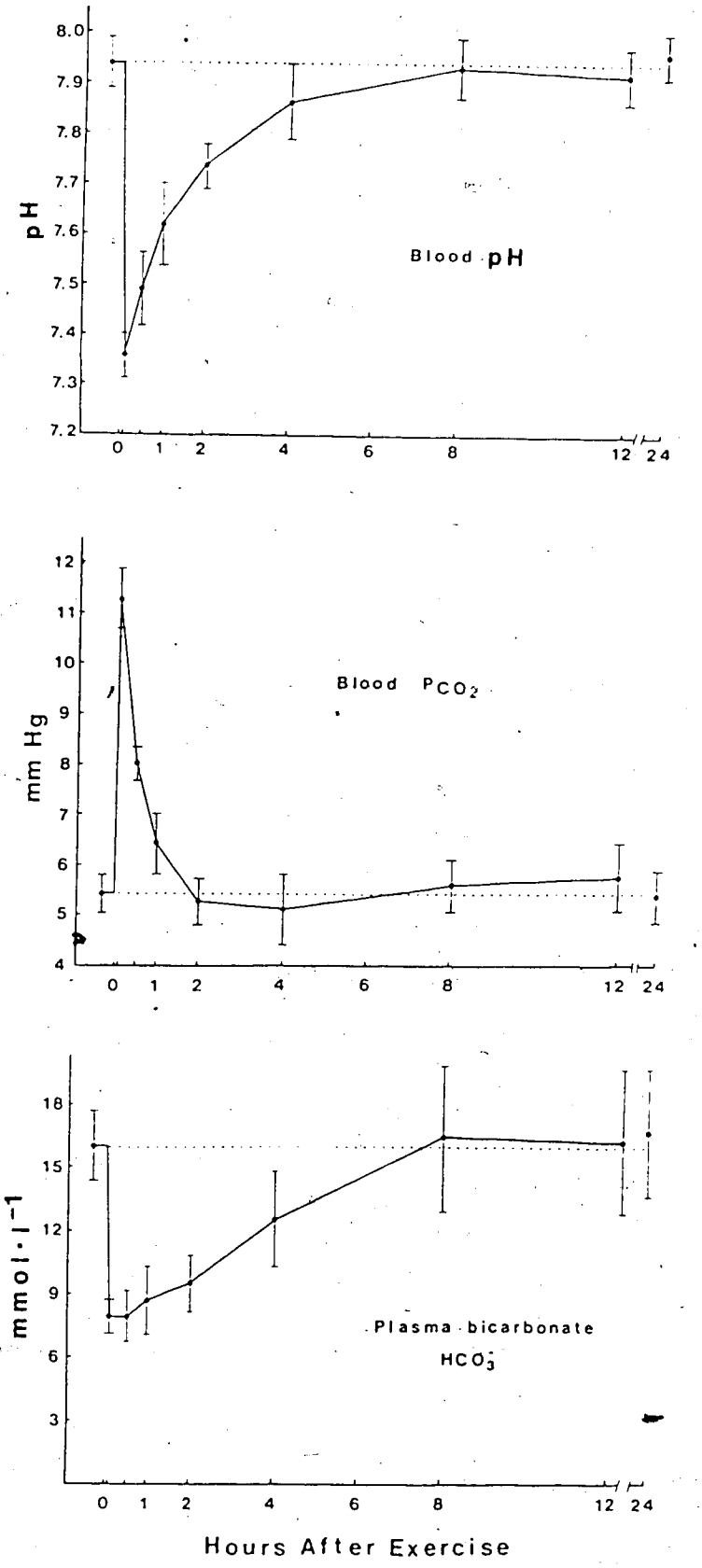

pH
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
Blood pH
mm Hg
12
11
10
9
8
7
6
5
4
Blood P_{CO_2}
mmol·l⁻¹
18
15
12
9
6
3
Plasma bicarbonate
HCO_3^-
0 1 2 4 6 8 12 24
Hours After Exercise

Figure 8

Changes in whole blood lactate (top) and plasma glucose (bottom) levels of pike after burst exercise. Unless otherwise indicated, n is 8 for lactate and 7 for glucose Other details as in Fig. 7.

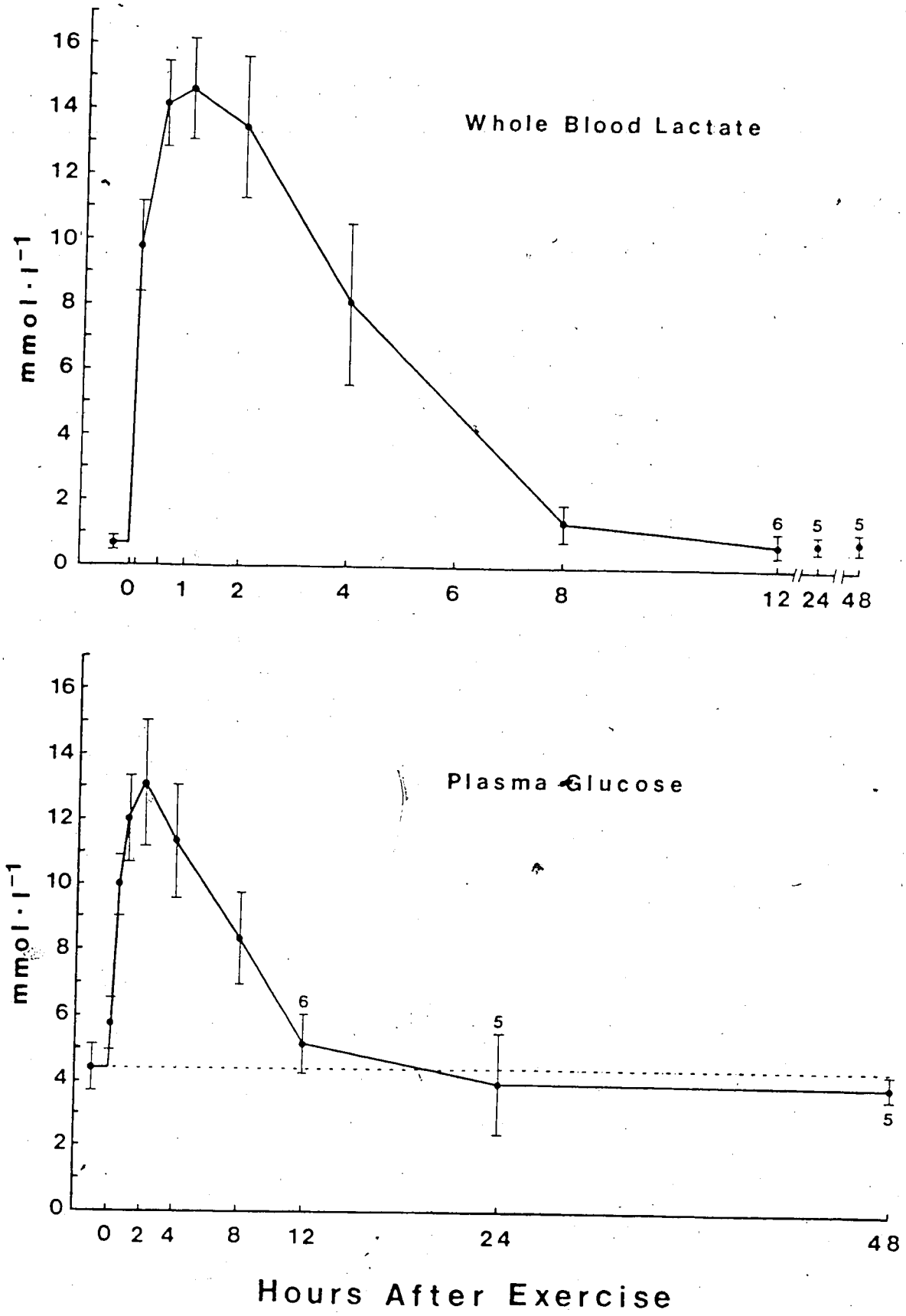
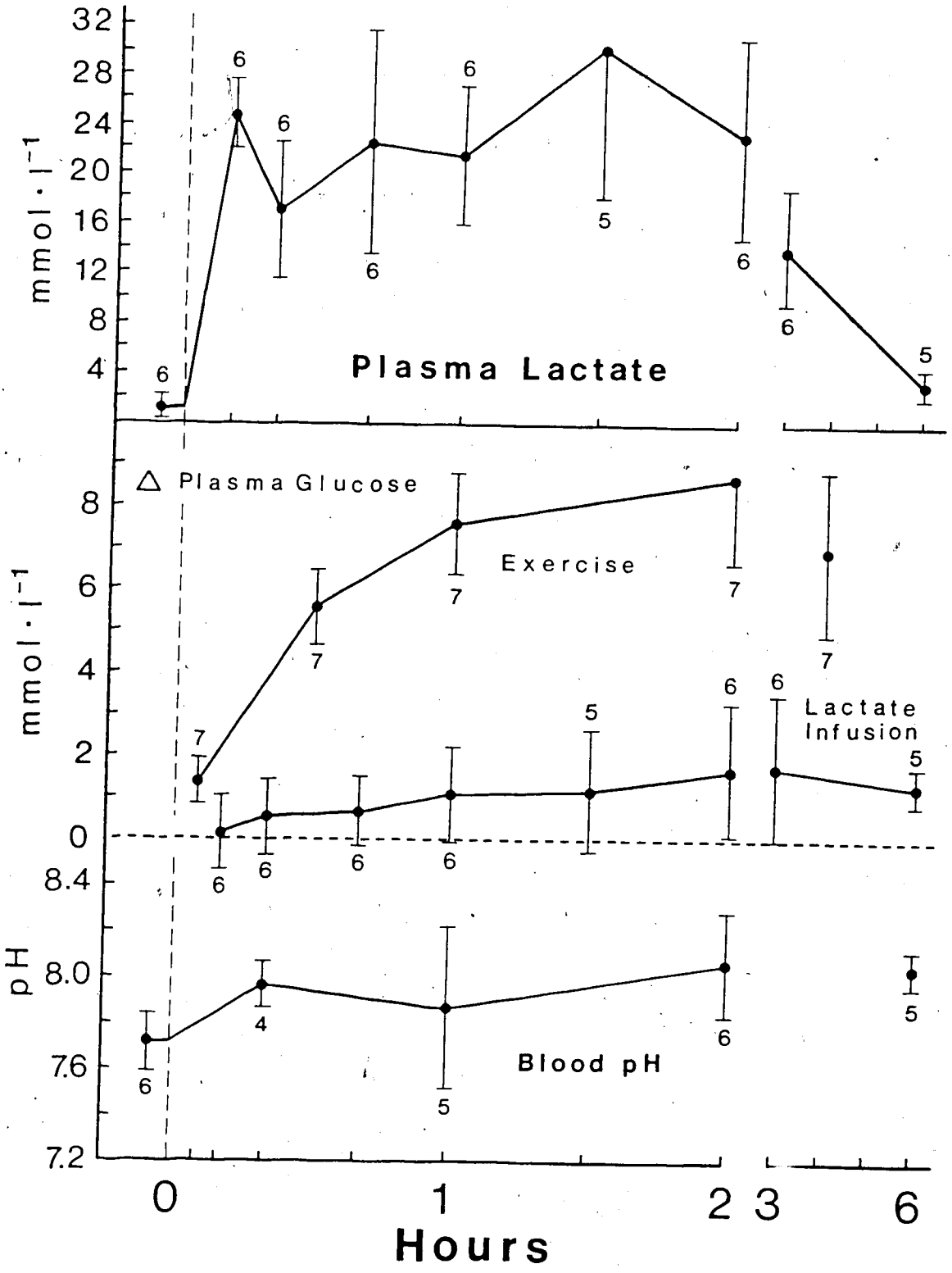

Whole Blood Lactate
mmol·l⁻¹
16
14
12
10
8
6
4
2
0
0 1 2 4 6 8 12 24 48
6 5 5
Plasma Glucose
mmol·l⁻¹
16
14
12
10
8
6
4
2
0
0 2 4 8 12 24 48
6 5 5
Hours After Exercise

Figure 9

Influence of intra-arterial infusion of neutralized L(+)-lactic acid on plasma glucose level of pike. Top and bottom panels show plasma lactate and blood pH respectively before, during, and after infusion. Middle panel shows changes in plasma glucose from the initial level following exercise (upper curve) and following lactate infusion (lower curve). Lactate infusion (9.78 mmol kg^{-1}) occurred between 0 and 2 h. Means and 95 % confidence intervals are shown; n values are indicated.


mmol · l⁻¹
32
28
24
20
16
12
8
4
Plasma Lactate
△ Plasma Glucose
Exercise
Lactate Infusion
mmol · l⁻¹
8
6
4
2
0
pH
8.4
8.0
7.6
7.2
Blood pH
0
1
2
3
6
Hours

D. DISCUSSION

The extremely rapid depression of blood pH following burst exercise was likely due to both elevated PCO_2 (Fig. 7) (respiratory acidosis) and to H+ release from the muscle (metabolic acidosis), as is known to occur in salmonids (Wood et al. 1983). Elevated blood PCO_2 after exercise may have been caused by increased aerobic metabolism, by proton-initiated release of CO_2 from muscle bicarbonate, or by inhibition of CO_2 excretion at the gills. However, after 2 h the acidosis was entirely of metabolic origin because plasma PCO_2 returned to pre-exercise levels (Fig. 7).

The results of this study indicate that, in pike following exercise, La^- accumulates in the blood in excess of the metabolic H^+ load. Excess H^+ ions in the blood are buffered by bicarbonate and non-bicarbonate buffers. At 2 h the quantity of H^+ ions buffered by bicarbonate was 6.49 mmol l^{-1} (Fig. 7). The non-bicarbonate buffer capacity (B) of pike blood is unknown but can be estimated from that of trout whole blood in which the mmol l^{-1} of H^+ ions buffered per pH unit is given by $B = -28.35 \times Ht - 2.59$, where Ht is hematocrit (Wood et al. 1982). With an Ht of 0.195 (average before exercise) the estimated B for pike is -8.1 mmol l^{-1} per pH unit and the amount of H^+ ions bound by non-bicarbonate buffers at 2 h is 1.60 mmol l^{-1}. The total amount of excess protons buffered in the blood, 8.1 mmol l^{-1}, is the sum of bicarbonate and non-bicarbonate buffers. This is considerably less than the 12.8 mmol l^{-1} of excess lactate present in the blood of pike at 2 h (Fig. 8). A similar post-exercise accumulation of blood La^- in excess of H^+ was reported in trout (Turner et al. 1983a,b; Wood et al. 1983). Turner et al. (1983a) concluded that the discrepancy between La^- and H^+ accumulation after exercise results from differential rates of release from the muscle because, when La^- and H^+ are infused into the blood, the ions are removed at similar rates.

Despite the close phylogenetic relationship between pike and muskellunge the pattern of lactate accumulation in the blood after exercise appears to be very different. In contrast to pike, Beggs et al. (1980) found that in muskellunge exercised by angling the increase in blood H^+ load (7.0 mmol l^{-1}) greatly exceeded that of La^- (2.5 mmol l^{-1}). This discrepancy between pike and muskellunge results primarily from the much larger blood lactate accumulation in pike (to 14.7 mmol l^{-1} at 1 hr; Fig. 8) than in muskellunge (to only 4 mmol l^{-1} at 1 h after angling; Beggs et al. 1980). The present results also conflict with the

study of Soivio and Oikari (1976) on northern pike in which maximum post-exercise blood lactate level was also very low (5 mmol l^{-1}). The latter results may simply reflect a diminished ability of the fish to exercise after four weeks of fasting, but the findings on muskellunge are harder to explain. The fish caught by Beggs et al. (1980) probably experienced hypoxic stress from being anaesthetized immediately after angling, a time when their need for oxygen is very great. Therefore, inhibition of lactate release into the blood may have been a response the muskellunge used to cope with hypoxia. This idea is supported by Wardle's (1979) studies of Atlantic plaice (Pleuronectes platessa), which indicated that inhibition of lactate release into the blood after exercise is triggered by stressful environmental conditions and may be mediated by the action of catecholamine hormones.

In esocid fish, non-release of lactate during hypoxia may have survival value during feeding when large food items held in the mouth could disrupt water flow over the gills, thereby inhibiting oxygen uptake and creating hypoxia in the tissues. It is possible that during this hypoxia the heart and visceral organs derive energy from anaerobic glycolysis (Hochachka 1980; Driedzic et al. 1978). Therefore, one purpose of non-release of lactate into blood during hypoxia may be to avoid lactate inhibition of glycolysis in the visceral organs and heart.

The argument of Turner et al. (1983b) that non-release of lactate from muscle after exercise is characteristic of sedentary animals, apparently does not apply to pike. Although pike are sedentary, under normoxic conditions they experience the same degree of elevation in blood lactate level after exercise as do the more active trout. Furthermore, pike can deal effectively with large quantities of lactate in the blood because they require only about 6 h to remove an intra-arterial lactate load equivalent to that produced in the muscle during burst exercise (Fig. 9).

Following exercise or handling stress the blood glucose level of fish remains elevated from 12 h to as long as several days (Black et al. 1960; Wardle 1972; Wydoski et al. 1976; Mazeaud et al. 1977). In pike caught by angling and allowed to recover in captivity, blood glucose remained elevated even after four days (see previous chapter). This prolonged hyperglycemia suggests that blood glucose was influenced by factors in addition to exercise such as fasting or 'captivity stress' and therefore the influence of

exercise 'per se' is not clear. In the present study, pike were fasted and held in captivity for about a week prior to exercise. Figure 8 shows that in pike a large and relatively short-lived hyperglycemia is a response to exercise and is separate from any response to fasting or captivity stress. That a similar pattern of post-exercise hyperglycemia occurred in a fish that already had a very high plasma glucose level further emphasizes this point. Extended (20 min) hypoxia causes a slow increase in blood glucose level (Mazeaud 1977), but since the hypoxia that accompanied exercise in pike was very brief (1.5 min) it likely contributed little to the observed hyperglycemia.

Pike can remove large amounts of lactate from the blood with only a small increase in the plasma glucose level (Fig. 9). This suggests that the post-exercise hyperglycemia in pike is a response to exercise stress and not simply a consequence of lactate removal by conversion into glucose. It also suggests that no large increase in gluconeogenesis occurred to remove the infused lactate, most of which was probably oxidized (previous chapter). Indeed, increased oxygen consumption of pike was indicated by a large increase (about 2x) in gill ventilation rate that occurred during lactate infusion.

Despite the sedentary lifestyle of pike, the changes in blood acid-base status (pH, PCO_2,HCO_3^-, and lactate) that accompany burst exercise are remarkably similar to those of the more active salmonids. Surprisingly, the post-exercise blood lactate accumulation is much greater in pike than in marine flatfish and apparently also muskellunge. A large increase in blood glucose level normally accompanies burst exercise in pike and may function to alleviate stress although the mechanism remains unknown.

IV. GENERAL DISCUSSION

Pike and salmonids display opposite patterns of swimming with salmonids being 'marathoners' and pike being 'sprinters'. Yet, the physiological responses accompanying burst exercise appear remarkably similar in the two fish. Recovery from burst exercise in terms of muscle glycogen resynthesis, lactate removal, and correction of blood pH after exercise is equally slow in both species. When healthy animals are examined under normoxic conditions both pike and salmonids show similar patterns of blood lactate accumulation and post-exercise changes in PCO_2 and HCO_3^- (cf. Wood et al. 1983). Additionally, resting white muscle glycogen levels are similar in pike (Figs. 3 and 4) and in salmonids (c.f. Black et al. 1962; Stevens and Black, 1966) suggesting that the two fish can perform similar amounts of anaerobic work in a single burst.

Levels of exercise stress as indicated by the extent of muscle glycogen depletion, lactate accumulation and depression in blood pH are as great in pike as in salmonids. However, despite equally high stress levels, and the slowness of recovery, pike suffer low mortality rates following burst exercise. The greater survival of pike than salmonids after exercise may have resulted from a better ability to elevate blood glucose levels. Maximum post-exercise elevation in plasma glucose of salmonids (approx. 4.3 mmol l^{-1}) (Wydoski et al. 1976; Perrier et al. 1978; Pickering et al. 1982) is only half as great as in pike (8.8 mmol l^{-1} by 2 hr; Fig. 8). Post-exercise hyperglycemia may have survival value because it is not simply a by-product of lactate removal. Extra plasma glucose after exercise is probably derived from the liver glycogen store which is much greater in pike (9.8% wet wt) than in salmonids (1.5% wet wt; Black et al. 1962) and this may explain the larger hyperglycemia seen in pike.

Possibly, the greater survival of pike after angling results from a better ability to elevate plasma glucose level. Unfortunately, this hypothesis is contradicted by the much smaller hyperglycemia shown by the angled pike (Fig. 2) compared to those exercised in the laboratory (Fig. 8). In part, this discrepancy reflects the use of whole blood in the angled fish and of plasma in the cannulated fish. Since intracellular glucose level is negligible (Newsholme and Start, 1973), glucose concentration will always be higher in plasma (by about 4/3 in pike) than in whole blood. Another explanation may be the use of

different sampling sites; blood glucose level may be lower in the caudal circulation (ie: in the angled fish) than in the ventral aorta (ie: in the cannulated fish). At present, the role of blood glucose in recovery from burst exercise remains unclear and requires further study.

In summary, the major physiological consequences of burst exercise in northern pike, as indicated by this study, are the following. A brief burst of exercise results in large glycogen depletion and lactate accumulation throughout the entire axial white muscle mass. Much of the exercise-produced lactate is probably oxidized within the large non-red muscle mass. Resynthesis of white muscle glycogen relies primarily on gluconeogenesis from protein rather than the Cori cycle, in situ lactate glyconeogenesis, or depletion of liver glycogen.

Burst exercise initiates a dramatic hyperglycemia which probably results primarily from breakdown of liver glycogen, although hormonal stimulation of lactate gluconeogenesis cannot be ruled out.

Blood acidosis occurs extremely rapidly after burst exercise and is of mixed respiratory (elevated PCO_2) and metabolic (H^+ release from muscle) origin. Lactate and H^+ ions apparently have different rates of entry and/or exit from the blood indicating that removal of these ions occurs by processes that are at least partially dissociated. Normally, lactate accumulates to high concentrations in the blood but under certain conditions, such as hypoxia, esocid fish may inhibit lactate release from the muscle.

It is hoped that the preceding ideas will stimulate future research into exercise physiology of fish. I extend best wishes to anyone who decides to pursue further this fascinating topic.

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