THE EFFECT OF INTERMITTENT EXERCISE ON CARBOHYDRATE
METABOLISM IN RAINBOW TROUT (Salmo gairdneri)

by

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ABSTRACT

The purpose of this study was to examine the effects of exercise of short duration, and the effects of re-exercise on carbohydrate metabolism. I feel that the levels of severe exercise studied approximate the levels of severe exercise which a rainbow trout probably experiences in its natural environment.

The level of blood lactate, blood hemoglobin, muscle lactate, muscle glycogen, and liver glycogen were determined in unanesthetized, intact, one and one-half year old rainbow trout acclimated to 10.5°C. Samples were taken immediately after exercise of 3 seconds to 5 minutes, after recovery of 3 minutes to 60 minutes, and after re-exercise of 3 seconds to 5 minutes.

The results indicate that exercise of even the shortest duration studied causes an immediate increase in the level of blood lactate, muscle lactate, and blood hemoglobin. Exercise also causes an immediate decrease in muscle glycogen, but does not cause a change in the level of liver

glycogen. Changes during the 60 minute recovery period are slight. In general, the effects of re-exercise after a 60 minute recovery period are additive.

A correlation analysis between muscle glycogen and muscle lactate indicates that there is a source of muscle lactate other than muscle glycogen at exercise levels of long duration. The source of this muscle lactate does not appear to come from liver glycogen. The energy may be supplied by catabolism of protein or lipid, or by absorption of foodstuffs from the gut.

This study provides evidence that rainbow trout are not well adapted for recovery from severe exercise of short duration.

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INTRODUCTION

There have been many studies of the relationship between severe exercise and concomitant changes in carbohydrate metabolism in fish (von Buddenbrock, 1938; Secondat and Diaz, 1942; Black et al, 1961; and Caillouet, 1964). In almost all of these studies, the fish were continuously exercised for 15 minutes. Exercise of this duration always elevated muscle lactate and blood lactate, depleted muscle glycogen, and in general, produced a marked disturbance which is referred to as muscular fatigue.

There have been very few studies of the effects of discontinuous or intermittent severe exercise in any intact animal, and none to my knowledge in fish. Christensen (1962) has demonstrated that man can do an optimum amount of exercise in a given time by alternate exercise and rest periods of 30 seconds. His results showed that continuous exercise for 9 minutes elevated the level of blood lactate from 7 to 150 mg %, whereas discontinuous exercise for 30 minutes (alternate exercise and rest periods of 30 seconds) elevated the level of

blood lactate to only 20 mg %.

Thus, the present study was an attempt to discover whether or not fish are adapted to tolerate discontinuous exercise as well as man is with respect to accumulation of lactate. In other words, I presumed that energy for exercise is supplied by degradation of muscle glycogen; and when demand for oxygen exceeded the supply, lactate accumulated. It was also the purpose of this study to examine the changes in carbohydrate metabolism during severe exercise of extremely short duration. Moreover, I feel that discontinuous exercise and exercise of short duration probably simulate the exercise which a rainbow trout experiences in its natural environment.

MATERIALS AND METHODS

I. FISH

One and one-half year old rainbow trout, Salmo gairdneri, were reared from eggs obtained from a commercial trout hatchery in McLeary, Washington. They were raised at Summerland Trout Hatchery operated by British Columbia Department of Recreation and Conservation, Fish and Game Branch. Trout were fed twice daily with pelleted fish food (J. R. Clarke Company, Salt Lake City, Utah). The water temperature remained constant throughout the summer, 10.5 to 11.5°C.

At least two days prior to experiment, fish were transferred from outside ponds to standard hatchery troughs (40 x 460 x 12 cm. deep). Fish were not starved before experiment and were kept covered to reduce external stimuli.

At the outset of the experiment furunculosis was prevalent. Diseased fish were not used in the experiment and all fish were treated with sulfamerazine given with the food. Incidence of furunculosis was negligible by July, and treatment was discontinued July 3.

II. EXPERIMENTAL CONDITIONS

Fish were held in covered troughs at least two days prior to experiments, and feeding was continued as usual. Treatments were applied randomly and singly; and each fish used only once.

A. Unexercised condition. The term unexercised rather than resting or basal level is employed since the fish usually manifest some activity during sampling. Fish were captured with as little disturbance as possible, air-dipped to the exercise trough, and sampled immediately.

B. Exercised conditions. Fish were captured with as little disturbance as possible, air-dipped to the exercise trough, and exercised by splashing water in the trough for a prescribed period of time (from 30 seconds to 5 minutes), or for a prescribed distance. Fish were chased by two persons.

C. Recovery condition. Fish were exercised as above and then left to rest a prescribed period of time, from 3 minutes to 60 minutes, in the same trough. Movement was restricted to one-half of the trough and the trough was covered during the recovery period.

D. Intermittent exercised conditions. The fish was

exercised and left to recover as above. After the recovery period, the lid was lifted and the fish was re-exercised. The specific levels of exercise and recovery times studied appear in Table I.

III. SAMPLING METHODS

A. Blood samples. After treatment a fish was captured by hand as quickly and adeptly as possible, placed ventral side up in a v-shaped trough which was kept submerged allowing the fish to respire in water, and a blood sample was obtained by cardiac puncture. Blood was withdrawn into a 2 ml. syringe which had been previously water-proofed with parafin oil, and rinsed with one drop of heparin solution. The blood sample was immediately ejected into a conical test tube; 0.02 ml. of the blood was transferred to 5.0 ml. of 0.1 M hydrochloric acid for determination of hemoglobin concentration, and 0.5 ml. or 1.0 ml. was transferred to 9.0 ml. cold 10% trichloroacetic acid for determination of lactate concentration. Hemoglobin concentration was determined within three hours of sampling. Lactate was determined on the deproteinized filtrate within three months. Time to capture the fish and time to withdraw the blood sample were recorded by stopwatch, and appear in Table II.

B. Tissue samples of muscle and liver. After treatment, the fish was captured as quickly as possible, killed by

breaking the neck, and placed on its side. The muscle sample was obtained by punching through the musculature immediately below the dorsal fin with a #9 cork borer. The liver sample consisted of the whole liver. Both muscle and liver samples were frozen immediately in dry ice and ethanol. Unwanted tissue (fat, skin, and bone) was removed, and the sample weighed on a torsion balance. The muscle sample was divided into two portions. One portion was homogenized with cold 10% trichloroacetic acid (6.0 ml. per gram) and the filtrate analyzed for lactate concentration within three months. The other portion of the muscle sample and the liver sample were digested in 60% potassium hydroxide (3.0 ml. per gram) for four hours at 100°C and the digests analyzed for glycogen concentration within 6 months. Time to capture, kill, obtain the muscle sample, and obtain the liver sample were all recorded by stopwatch, and appear in Table II.

C. After sampling, the fish was weighed, and the sex determined by visual examination of the gonads.

IV. ANALYTICAL METHODS

All analyses were determined using a Klett-Summerson photoelectric colorimeter.

A. Hemoglobin was analysed colorimetrically as acid hematin using a #54 filter and comparing the sample to a standard

supplied by Klett-Summerson. Hemoglobin is reported in g % (grams per 100 ml. blood).

B. Lactate was determined colorimetrically on 1 ml. aliquots from deproteinized filtrates of blood and muscle homogenates by the Barker-Summerson method (1941). Interfering materials in the filtrate were removed by the Van Slyke-Salkowski treatment of copper sulphate and calcium hydroxide (Hawk et al, 1954). Lactic acid is oxidized to acetaldehyde with concentrated sulphuric acid, the aldehyde is coupled with a chromogenic agent, p-hydroxydiphenyl, and compared with a standard of lithium lactate. Lactic acid is reported in mg % (mg per 100 ml. blood, or mg per 100 grams wet weight of muscle).

C. Glycogen was determined colorimetrically by the anthrone method (Carroll et al, 1956) on potassium hydroxide digests of muscle and liver which were diluted to a known volume. Glycogen is precipitated with ethanol, hydrolysed to glucose, and coupled with a chromogenic agent, anthrone. A standard curve is determined for each analysis using three concentrations of glucose, and glycogen concentration determined from the standard curve as glucose. Glycogen is reported in mg % (mg of glycogen per 100 grams wet weight of tissue).

V. HANDLING OF DATA

No samples were pooled, all values were determined

and recorded separately for each fish. Sample size, mean, and standard deviation are given for each variable and each condition. Conditions were applied singly and randomly to each fish. Comparisons between conditions are made using the F-test, ANOV, and Duncan's new multiple range test (Steel and Torrie, 1960). A latin square design (9 x 9 x 9) was used in one set of experiments (3 minutes, 8 minutes, and 30 minutes recovery from 1 minute, 2 minutes, and 5 minutes exercise) in order to determine the effect of sampling sequence during the day, the effect of sampling sequence from day to day, and the effect of experimental conditions. All statistics were calculated by the Computing Center, University of British Columbia.

Table I. Duration of exercise periods, duration of recovery periods, and number of times re-exercised in the present study.

Duration of Exercise	Duration of rest (minutes)	Number of times re-exercised
0 (a)	30, 60	0
3 seconds (b)	30, 60	once after 60 min. rest
15 seconds (c)	30, 60	twice after 60 min. rest
30 seconds	30, 60	twice after 60 min. rest
1 minute	3, 8, 30	once after each rest period
2 minutes	3, 8, 30	once after each rest period
5 minutes	3, 8, 30	once after each rest period
to exhaustion (d)	0	0

(a) unexercised condition

(b) actually exercised 4.6 meters taking approximately 3 seconds (mean 3.4 seconds, range 2.0 to 6.0 seconds)

(c) actually exercised 23 meters taking approximately 15 seconds (mean 15.0 seconds, range 10.0 to 36.0 seconds)

(d) exercised to exhaustion. Typically the behavior of the fish changes following approximately 1 minute of exercise.

At this time the escape behavior changes to a hiding behavior. (mean 67.3 seconds, range 51 to 80 seconds)

Table II. Time to obtain and prepare blood and tissue samples.
 Blood and tissue samples were obtained from separate fish.
 All times are given in seconds.

Sample	Time	n	Mean $\pm$ standard deviation
blood	to catch and position fish	305	13.7 $\pm$ 4.1
	to withdraw blood sample	305	31.4 $\pm$ 23.9
tissue	to catch fish	222	2.9 $\pm$ 1.8
	to kill fish	222	2.8 $\pm$ 1.2
	to remove muscle sample and place in dry ice and ethanol	222	10.4 $\pm$ 2.6
	to remove liver and place in dry ice and ethanol	62	14.1 $\pm$ 6.4

RESULTS AND DISCUSSION

I. HEMOGLOBIN

A. Results.

The mean level of hemoglobin in unexercised fish was 9.15 g %. The effects of exercise, recovery, and intermittent exercise are illustrated in Fig. 1, 2, 3, and 4.

In general, exercise caused hemoconcentration. Fig. 1 illustrates that the hemoconcentration response to exercise is triphasic. Exercise of short duration caused a marked increase in hemoglobin which was followed by a marked decrease, and this in turn was followed by a gradual increase. During recovery the hemoglobin continued to rise (Fig. 2). The hemoglobin level decreased after a long recovery period. For example, the level had almost returned to pre-exercise levels after a 30 second exercise in 60 minutes as indicated in Fig. 4. Re-exercise caused further hemoconcentration as illustrated in Fig. 3 and 4. The triphasic curve persisted but to a lesser extent, when the fish were rechased after recovery periods of 30 minutes or 60 minutes.

Table III. Level of hemoglobin concentration during continuous and discontinuous exercise of the same total duration.

Total duration of exercise (seconds)	Condition			Hemoglobin (g %)
	Exercise (seconds)	Recovery (minutes)	Exercise (seconds)	
30	30	0	0	9.70
	15	60	15	11.00
60	60	0	0	9.75
	30	60	30	10.53
120	120	0	0	10.05
	60	3	60	11.82
	60	8	60	12.96
	60	30	60	12.00

Fig. 1. Changes in levels of blood hemoglobin concentration during 5 minutes severe exercise.

Fig. 2. Effect of rest after severe exercise of 1, 2, or 5 minutes on the levels of blood hemoglobin.

Fig. 3 Effect of exercise and re-exercise on the levels of blood hemoglobin concentration.

Fig. 4 Effect of intermittent exercise of 30 seconds on levels of blood hemoglobin concentration.

B. Discussion

Korzhuev (1964) found that centrifuging the acid hematin solution reduced the hemoglobin values by 18-20% (the turbidity of acid hematin solutions of fish blood is due to the presence of nuclei in the erythrocytes). Thus, the absolute values of hemoglobin concentration reported in this study are probably high, but comparisons between treatments are still valid.

The mean level of hemoglobin in unexercised fish was 1.24 g % higher than the value for 1961 (Black, et al.) and 1.67 g % lower than the value for 1963 (Manning) for unexercised fish raised at the same hatchery, fed the same food, and kept at the same temperature. Schiffman and Fromm (1959) give the hemoglobin level of hatchery raised Salmo gairdneri as 6.31 g %. Some variable other than location, type of feed, or temperature, must affect blood hemoglobin levels. Some possibilities are stage of maturity, furunculosis, and sulfamerazine.

Hemoconcentration associated with exercise of fish has been noted by others, but none has recorded a triphasic response since, none to my knowledge has studied exercise of extremely short duration. Black (1955) found a significant hemoconcentration in largemouth bass, but not in kamloops trout, fine-scaled sucker, carp, squawfish, northern black

catfish, sockeye salmon (Black, 1957c), or lake trout (Black, 1957b) following 15 minutes severe exercise. There are three possible explanations for the observed hemoconcentration:

1. erythrocytes move into circulating blood,
2. water moves out of blood or,
3. both of the above.

Black et al. (1959) attempted to demonstrate the movement of water in order to explain hemoconcentration of exercise, but observed no significant difference between dry weight of blood before and after 15 minutes strenuous exercise. Hemoconcentration of exercise has been observed in species other than fish: in man (Bard 1956), and in rats (Sieter 1958). Best and Taylor (1961), and Rakestraw (1921), indicate that exercise in man results in decreased plasma volume, increased plasma protein, increased red cell concentration, and increased hemoglobin concentration. Gregersen and Rawson (1959) observed a decrease in total blood volume with exercise in man.

Hall et al. (1926) noted increased hemoglobin concentration with asphyxiation in cod. Scholander et al. (1962) noted circulatory shut down with asphyxiation in the grunion since there was an elevated muscle lactate but no elevated blood lactate. (Since lactate was not diffusing from muscle to blood then blood flow to skeletal muscle must have been reduced.) Nakatani (1955) observed a significant increase in hemoglobin level as a result of electric shock in rainbow

trout. Denyes and Joseph (1956) found an increased hemoglobin concentration in goldfish and carp acclimated to higher temperatures. In species other than fish, the following causes of hemoconcentration have been noted: electric shock or insulin shock in man decreased plasma volume 10 to 20% (Bard 1956), and induced fever in the dog increased the hemoglobin level (Kubicek et al. 1959).

Barcroft (1956) observed a triphasic response in blood flow to skeletal muscle of man in response to continuous infusion of adrenaline. Manning (unpublished) noted that severe exercise in rainbow trout causes circulatory shutdown, and thus plasma skimming occurs.

A possible explanation for the observed results is that the three phases of the hemoglobin response to exercise are associated with circulatory changes. The initial rapid increase and decrease are due to circulatory changes in response to an increase and decrease of adrenaline. The rapid increase in hemoglobin is due to plasma skimming, the rapid decrease is due to erythrocyte sludging. The later slow increase in hemoglobin is due to circulatory change in response to the accumulation of metabolites causing the characteristic reactive hyperemic vasodilatation in the muscle, and thus resolving the red cell sludging and restoring a condition of plasma skimming. Alternatively, the later slow increase in hemoglobin could be due to a release of stored erythrocytes from storage

organs. Udvardy (unpublished) has demonstrated that erythrocyte storage organs in salmon are in the spleen and the anterior one-third of the kidney. Moreover, studies in man have shown that release of erythrocytes in exercise occurs slowly, and only in severe exercise. The later slow increase in hemoglobin concentration could also be due to water moving from blood to muscle tissue in response to an osmotic gradient due to the increase in the number of osmotically active particles in the muscle as a result of muscle contraction.

II. LACTATE

A. Results - Blood Lactate.

The mean level of blood lactate in unexercised fish was 7.19 mg % (range 0.66 to 13.57 mg %). The effects of exercise, recovery, and intermittent exercise are illustrated in Fig. 5, 6, 7, and 8.

Exercise, even of the shortest duration, caused an elevated blood lactate. Allowing the fish to rest after the exercise always resulted in a further increase in blood lactate (Fig. 5). Fig. 8 illustrates that a 60 minutes recovery period was insufficient for restoration of unexercised levels of blood lactate after exercise of only 15 seconds. However, the level of blood lactate reached a peak and started to decrease during the 60 minute recovery period.

Table IV. Effect of exercise and re-exercise on the level of blood lactate. In each case fish were sampled immediately after exercise or re-exercise.

Exercise time (seconds)		3	15	30	60	60	60	120	120	120	300	300	300
Recovery time (minutes)		60	60	60	3	8	30	3	8	30	3	8	30
Blood	first chase	9	9	14	15	15	15	24	24	24	55	55	55
Lactate	second chase	16	47	55	49	64	86	79	86	115	86	98	120
mg %	third chase		74	97									

Re-exercise always caused a further increase in blood lactate, as indicated in Table IV.

A comparison of blood lactate levels of fish exercised for the same total length of time reveals that intermittent exercise caused greater increases than continuous exercise (Table V). However, from Table V it can also be seen that the effect of continuous exercise is to cause a greater increase in the level of blood lactate than discontinuous exercise, if the fish is left to rest after the initial chase. The fish also appeared to respond better to chasing, and thus did more work when exercised intermittently than when exercised continuously. Typically the fish responds to chasing by evasive behavior for about 1 minute, and further chasing results in the fish attempting to hide rather than to escape. The effect of this change in behavior is revealed by comparing values for exercise of 2 minutes, rest 3 minutes, (blood lactate of 54 mg %) with 5 minutes of continuous exercise (blood lactate of 55 mg %). These values indicate that the fish's response during recovery from 2 minutes exercise was not as great as during the first 2 minutes of exercise.

Fig. 6a compares the effect of continuous and discontinuous exercise of the same total duration (2 minutes). Examination of Fig. 6a reveals that exercise and re-exercise of 1 minute did not elevate the blood lactate as much as an

Table V. The level of blood lactate concentration during continuous and discontinuous exercise of the same total duration.

Total duration of exercise	Condition			Blood lactate mg %
	exercise	recovery	re-exercise	
seconds	seconds	minutes	seconds	
30	30	0	0	14
	15	60	15	47
	30	60	0	50
60	60	0	0	15
	30	60	30	55
	60	30	0	70
120	120	0	0	24
	60	3	60	49
	120	3	0	54
	60	8	60	64
	120	8	0	74
	60	30	60	86
	120	30	0	100

initial continuous exercise of 2 minutes. But re-exercised fish were sampled immediately after the re-exercise, and had they been left to recover, their blood lactate levels probably would have exceeded those of fish exercised continuously for 2 minutes. Fig. 6b compares the effect of similar total durations of exercise; 5 minutes continuous and 4 minutes discontinuous exercise. Examination of Fig. 6b reveals that exercise and re-exercise of 2 minutes elevated the blood lactate more than initial continuous exercise of 5 minutes, even though re-exercised fish were sampled immediately after re-exercise.

Fig. 7 and 8 illustrate the additive nature of intermittent exercise on blood lactate and the lack of recovery in rest periods of 60 minutes after exercise of only 15 or 30 seconds duration.

B. Results - Muscle Lactate.

The mean level of muscle lactate in unexercised fish was 132.38 mg per 100 grams wet weight (range 57.19 to 199.05 mg %). The effects of exercise, recovery, and intermittent exercise are illustrated in Fig. 9, 10, 11, 12, and 13.

Exercise, even of the shortest duration, caused an immediate elevation in muscle lactate. Restoration of unexercised levels of muscle lactate after a 30 minute rest period was slight (Fig. 9). Fig. 10 illustrates that muscle lactate in

Fig. 5 Effect of rest after severe exercise on the levels of blood lactate. Fish were sampled immediately after exercise, 3 minutes after, 8 minutes after, and 30 minutes after exercise.

Fig. 6. Changes in levels of blood lactate after continuous exercise (continuous curves), and after discontinuous exercise (discontinuous curves). Note that fish exercised intermittently were sampled immediately after the second exercise, whereas fish exercised continuously were sampled in the recovery condition.

Fig. 7. Effect of intermittent exercise of 15 seconds on the levels of blood lactate.

Fig. 8 Effect of intermittent exercise of 30 seconds on the levels of blood lactate.

fish exercised only 1 minute continued to rise sharply for 3 minutes after the exercise, whereas that of fish chased 2 minutes increased slightly, and that of fish chased 5 minutes decreased rapidly.

That muscle lactate failed to return to normal even after exercise of short duration is illustrated in Fig. 9, 11, and 12. This tendency for muscle lactate to remain elevated explains why blood lactate also failed to decrease to normal from short periods of exercise, and must be due to either a diffusion barrier between muscle and blood, or due to impaired blood circulation within muscle.

The effects of re-exercise after 60 minutes rest appeared to be additive. However, there appeared to be an upper limit (approximately 470 mg %) which muscle lactate did not exceed (Fig. 13). The effect of intermittent exercise of short duration, illustrated in Fig. 11 and 12, was to cause further increases in muscle lactate, again indicating the failure of muscle lactate to return to unexercised levels after exercise of short duration. The levels of muscle lactate after intermittent exercise appear in Table VI.

Table VI. Effect of exercise and re-exercise on the level of muscle lactate. Fish rested 60 minutes between exercise periods, and were sampled immediately after exercise or re-exercise.

Duration of exercise		15 seconds	30 seconds	5 minutes
Muscle lactate mg %	first chase	236	291	468
	second chase	276	366	462
	third chase	304	358

Fig. 9. Changes in the level of muscle lactate during 5 minutes severe exercise, and 30 minutes after the severe exercise.

Fig. 10. Changes in levels of muscle lactate during recovery from severe exercise of 1, 2, and 5 minutes.

Fig. 11. Effect of intermittent exercise of 15 seconds on the levels of muscle lactate.

Fig. 12. Effect of intermittent exercise of 30 seconds on the levels of muscle lactate.

Fig. 13. Effect of intermittent exercise of 5 minutes on the levels of muscle lactate.

C. Discussion of Blood and Muscle Lactate.

Blood and muscle lactate are discussed together since muscle tissue is the source of blood lactate, and thus changes in levels of muscle lactate are reflected by changes in levels of blood lactate.

The resting level of blood lactate varies from year to year in the rainbow trout raised at Summerland, even though temperature and feeding conditions are similar. Some probable reasons for this year to year variability include the following: the history of the fish differ (for example the exact type of food), technique of catching the fish and withdrawing the sample vary from experimenter to experimenter; and most important, there are distinct races in the species Salmo gairdneri. Levels of blood lactate in unexercised Salmo gairdneri appear in Table VII. These values are similar to those reported for other species of fish (Seondat and Diaz, 1942; Black, 1955, 1956, 1957a, 1957b; Parker et al., 1959; Caillouet, 1964; Phillips, 1958; Dean and Goodnight, 1964; Lievestad, 1957; Heath and Pritchard, 1962), and similar to those reported for man (Bock et al., 1932; Cook and Hurst, 1933; and Bang, 1936).

The level of muscle lactate in unexercised rainbow trout at Summerland is much more variable than the level of blood lactate. Levels of muscle lactate in unexercised fish appear in Table VIII.

Table VII. Levels of blood lactate in unexercised Salmo gairdneri.

Year	Blood lactate mg%	Age years	Reference
1957	7.2	1	Black <u>et al.</u> , 1959
1958	4.0	$1\frac{1}{2}$	Black <u>et al.</u> , 1962
1958	9.3	$1\frac{1}{2}$	Miller <u>et al.</u> , 1959
1959	8.8	$1\frac{1}{2}$	Black <u>et al.</u> , 1962
1961	5.1	$1\frac{1}{2}$	Manning, 1963
1964	7.2	$1\frac{1}{2}$	present study

Table VIII. Levels of muscle lactate in unexercised fish.

Year	Species	Muscle lactate mg%	Reference
1927	haddock	150	Ritchie
1927	cod	80	
1927	hake	50	
1957	cod	5 to 50	Leivestad <u>et al.</u>
1957	steelhead trout	174	Nakatani, 1957
1958	rainbow trout	66	Black <u>et al.</u> , 1962
1959	rainbow trout	187	Black <u>et al.</u> , 1962
1962	grunion	50	Scholander
1964	rainbow trout	132	present study

The values of muscle lactate in resting fish are much higher than those reported for the frog (8 mg %, Sacks et al., 1954) and for the cat (15 mg %, Sacks and Morton, 1956), but the frog was pithed and the cat was anesthetized, thus eliminating activity during the sampling procedure.

The source of resting blood lactate is probably muscle, but Evans (1934) has stated that in the heart-lung preparation of a dog, the lungs produce enough lactate to account for resting blood lactate production. Cook et al., (1933) stated that the source is glycolysis. Leopold and Bernard (1931) observed that resting blood lactate was not correlated with resting blood glucose in children.

According to Huckabee (1956), the distribution of lactate between the erythrocytes and the plasma is equal. Decker (1942) observed that the distribution is equal only in the resting condition, and that at higher concentrations of lactate, the concentration of lactate is greater in the plasma than in the red cells.

Many variables other than exercise alter blood and muscle levels of lactate. Scholander (1962) using the grunion, Leivestad et al. (1957) using the cod, and Caillouet (1964) using the channel catfish, showed that asphyxia, induced by removing the fish from the water, elevated muscle lactate. They found that the blood lactate did not rise until the fish

was returned to the water and concluded that blood was being diverted away from muscle tissue when the fish was out of water.

In mammals, Himwich (1932) elevated blood lactate with the following: adrenaline injection, alkali injection, evisceration, anaemia, and hemorrhage. Kubicek et al. (1959) showed that induced fever increased blood lactate in dogs. Eggleton and Evans (1930) showed that hypoventilation decreased blood lactate. Huckabee (1958) showed that hyperventilation, bicarbonate infusion, pyruvate infusion, and glucose infusion all elevate blood lactate. Huckabee (1961) also found an elevated blood lactate in many resting hospital patients. The majority of these elevated blood lactates could be attributed to either elevated blood pyruvates or to hypoxemia of respiratory or cardiovascular disease. No explanation was given for the elevated blood lactate in the remaining cases, and each of these patients died of acidosis.

Secondat and Diaz (1942) were the first to show that exercise elevates blood lactate in fish; and Nakatani (1957) first to show that exercise elevates muscle lactate in fish. The lactate response to exercise was first demonstrated by Fletcher and Hopkins (1907). They also proposed that the failure of muscle to contract in spite of adequate stimulation was due to muscle lactate reaching a certain maximal value. Meyerhof (1920) demonstrated that the source of lactate was muscle glycogen.

The response of blood lactate during severe exercise has been studied in many species of fish (Secondat and Diaz, 1942; Black 1957 a, b, c; Nakatani, 1957; Black et al. 1958 - 1960; Miller et al., 1959; Parker and Black, 1959; Parker et al., 1959; Jonas et al., 1962; Black and Barrett, 1957; Caillouet, 1964; Dean, 1962; Barrett and Connor, 1962).

Collins and Elling (1960) and Collins et al., (1964) found that prolonged moderate exercise of steelhead trout (Salmo gairdneri) in an endless fishway increased blood lactate only two fold. Huckabee (1956) reports that even mild exercise elevates blood lactate in man.

Hochachka (1961) analyzed the effect of training on oxygen debt and glycogen reserves in rainbow trout. He found that the trained group could accumulate an oxygen debt three times that of the untrained group, that the trained group used more of their muscle glycogen reserves and resynthesized it faster during recovery, and that it took longer to exhaust the trained fish. Lactate levels were not measure. Anderson et. al., (1960) found that the maximum levels of blood lactate occuring in man after exercise were the same for athletes and non-athletes.

Denyes and Joseph (1956) observed that larger bass tended to produce more lactate than smaller bass, but no significant correlation between size and lactate levels was found

in the present study.

Black et al. (1962) observed a difference in the lactate response to exercise between sexes in rainbow trout. The level of muscle lactate was greater in the female than the male during exercise and recovery. Muscle lactate reached a maximum level after 2 minutes of severe exercise in the female, whereas it took 9 minutes in the male. Anderson (1960) observed that the lactate response was greater in women than men for the same amount of work done. Caillouet (1964) observed no significant differences between sexes in the lactate response during exercise and recovery in the channel catfish. No consistent significant differences were observed between sexes in the present experiment.

The effect of age or stage of sexual maturity on the lactate response to exercise has not been experimentally determined in rainbow trout. Anderson (1960) observed that age was a significant factor in determining the lactate response to exercise in man. In the present study, the gonads of every fish were examined. We observed an obvious increase in gonad development during the summer, but the sexual maturation did not appear to affect the lactate response to exercise in any consistent manner.

Maximal levels of blood and muscle lactate after

exercise vary from species to species throughout the animal kingdom. Most workers studying the lactate response in fish have used 15 minutes continuous severe exercise. Bang (1936) observed that the maximal blood lactate was produced by severe exercise for 1 minute in man. Anderson (1960) observed that maximal blood lactate levels were produced by sprinting 400 meters (approximately 1 minutes), and sprinting further than 400 meters failed to cause a greater increase in blood lactate. Maximal levels of blood and muscle lactate after exercise appear in Table IX.

Others have observed that blood lactate continues to rise after exercise in fish (Black et al., 1962; Nakatani, 1957, Secondat and Diaz, 1942). This has also been observed in man (Anderson, 1960). Black (1957a) observed that blood lactate rises for $3\frac{1}{2}$ hours after 15 minutes after severe exercise, whereas the delayed increase only lasts 2-5 minutes in man (Anderson, 1960). There are at least three possible explanations for the continued rise in blood lactate:

1. that the muscle continues to produce lactate,
2. that there is an efficient diffusion barrier between muscle and blood, or
3. that circulation to the muscle is impaired.

Anderson (1960) has attributed the continued rise of blood lactate in man to post-exercise increased tonus associated

Table IX. Maximal levels of blood and muscle lactate after exercise in fish, frog, and mammals.

Species	Duration of exercise minutes	Maximum blood lactate mg %	Maximum muscle lactate mg %	Reference
rainbow trout	15	77.9	415	Black <u>et al.</u> , 1962
rainbow trout	15	54.0	295	Black <u>et al.</u> , 1962
rainbow trout	15	32.6		Miller <u>et al.</u> , 1959
rainbow trout	15	69.7		Manning, 1963
rainbow trout	15	54.6	468	present study
steelhead trout	8.5		389	Nakatani, 1957
tench	15	53		Secondat and Diaz, 1942
channel catfish	15	59.1		Caillouet, 1964
black catfish	15	34.9		Black, 1955
sucker	15	55.3		Black, 1955
carp	15	54.3		Black, 1955
squawfish	15	94.2		Black, 1955
bass	15	76.2		Black, 1955
sunfish	6 - 45	77		Heath and Pritchard 1962
frog			87	Sacks <u>et al.</u> , 1954
cat			322	Sacks and Sacks, 1956
man	1	126		Bang, 1936.

with hyperexcitability due to acidosis. In the present study muscle lactate continues to rise after exercise of 1 or 2 minutes, but decreased immediately after exercise of longer duration, that is, 5 minutes. Some diffusion barrier must exist between muscle and blood. The rate of diffusion of lactate has only been studied between erythrocytes and plasma. Johnson et al., (1945) showed that lactate diffusion in mammalian blood was passive and unrestricted but extremely temperature dependant (the Q_{10} for diffusion increased from 2 to 30 for a temperature decrease of 25-36 to 0-14 degrees). Margaria et al. (1933) observed that the delay of diffusion of lactate from muscle to blood is greatest during severe exercise. Manning (unpublished) noted that severe exercise in rainbow trout causes circulatory shutdown. It is most probable that the delayed increase in blood lactate is due to all three factors.

Another factor which possibly contributes to delayed increase of blood lactate and to the slowness of recovery is the effect of temperature on the lactate response. Black (1957a) showed no significant differences of lactate production at two temperatures of acclimation in Kamloops trout. Denyes and Joseph (1956) found the highest individual values and greatest variability among fish was associated with fish acclimated to lower temperature in large mouth bass. Dean and Goodnight (1964) found a higher lactate production in white crappie, and black bullhead when acclimated to lower temperatures. In

large mouth bass, blood lactate was lower at lower acclimation temperatures for unexercised fish, but higher at lower acclimation temperatures for exercised fish. In the bluegill, blood lactate was higher at lower acclimation temperatures for unexercised fish, but lower at lower acclimation temperatures for exercised fish. They also concluded that at lower temperatures of acclimation, fish rely on the pentose shunt for energy production. Ekberg (1962) demonstrated that glucose-6-phosphate dehydrogenase is not altered by changing external temperature of the carp, whereas 6-phosphogluconic dehydrogenase and aldolase are more active in cold adapted fish. He also found that resistance to iodoacetate poisoning is greater in gills from cold adapted goldfish, but resistance to cyanide poisoning is greater in warm adapted fish. Hart and Heroux (1954) showed that the lactate response to exercise was the same in deer mice acclimated to 0 degrees as it was in those acclimated to 22 degrees. They found that the lactate response was only altered by temperature at extremely low temperatures, -10 to -40 degrees. Caillouet (1964) found that levels of blood lactate of unexercised channel catfish were higher in fish acclimated to higher temperatures, and that similar trends were noted in exercised channel catfish. Caillouet (1964) also noted higher individual values and great variability among individual carp acclimated to higher temperatures. Activity in fishes (Fry, 1947; Fry and Hart, 1948) and the rate of diffusion of lactate from erythrocytes to plasma (Johnson et al., 1945) have been shown

to increase with increase in temperatures. Hochachka and Hayes (1962) have shown that trout acclimated to low temperatures rely more on the pentose shunt for energy production than the Embden-Meyerhoff pathway. It is probable that activity, metabolism, and diffusion are temperature dependant in the rainbow trout. All of the experiments in the present study were done at the same temperature, 10.5 degrees C.

The major difference between fish and mammals with respect to the lactate response is the rate of recovery of blood lactate. Alpert and Root (1954) showed that 40% of lactate produced by severe exercise in man was utilized within 10 minutes; whereas Black et al., (1962) showed that 40% of the lactate produced by severe exercise in rainbow trout was utilized in approximately 8 hours. The slow recovery of blood lactate in fish has been noted by others (Secondat and Diaz, 1942; Black et al., 1959). In man, Anderson (1960) has shown that blood lactate was the last value to return to resting levels after exercise. Heart rate, oxygen intake, carbon dioxide output, and pulmonary ventilation each returned to unexercised levels before blood lactate.

The fate of lactate produced by strenuous exercise has been the subject of much study. Cori (1929) observed that lactate given either by mouth or subcutaneously enhanced liver glycogenesis. That the liver removes lactate was corroborated

by other workers (Eggleton and Evans, 1930; Evans et al., 1930; Himwich et al., 1930; Ivy and Crandall 1941; Vennesland et al., 1942.) Eggleton and Evans (1930) perfused frog muscle with lactate and observed no increase in muscle glycogenesis. Himwich (1932) and McGinty (1931) showed that the heart oxidizes lactate. Evans (1933), Himwich (1932), and Ashford (1931) showed that brain metabolizes lactate. Hill (1924) described the recovery of lactate as a biphasic process; the initial rapid phase was the oxidation of muscle lactate, and the later slow phase was the oxidation of lactate which escaped from the muscle to the blood. Wells (1957) related the recovery curve of lactate to that of oxygen consumption and on this basis concluded that recovery was a triphasic process.

On the basis of oxygen consumption after strenuous exercise, Meyerhoff (1920) theorized that $\frac{1}{4}$ of the lactate was oxidized, and $\frac{3}{4}$ was resynthesized into glycogen. Both oxidation and glycogenesis are processes which occur in the liver and both require oxygen. Eggleton and Evans (1930) found that the rate of removal of lactate decreased from 2 mg % per minute to 0.6 mg % per minute after removing the liver. Recent observations by Drury and Wick (1956, and 1958) indicate that very little if any lactate is resynthesized into glycogen in the intact rabbit. Most of the lactate appears as expired carbon dioxide. They also observed that lactate is oxidized faster than glucose and they hypothesized that lactate acts as a quick fuel in emergencies.

There are very few studies of intermittent strenuous exercise in man, and a review of the literature revealed none on intermittent strenuous exercise in fish. Christensen (1956) found that alternating periods of exercise and recovery of 30 seconds for one hour did not cause a significant increase in blood lactate, whereas continuous exercise at the same intensity caused exhaustion in 9 minutes and elevated blood lactate to 150 mg %. Christensen also showed that if the duration of work and rest periods was increased, then the blood lactate increased. Some of Christensen's data appear in Table X.

Astrand et al. (1960) using experimental conditions similar to Christensen showed that the major factor determining blood lactate during intermittent exercise was the duration of the exercise period, and that the duration of the recovery period was of secondary importance.

Man can tolerate strenuous intermittent exercise at least 20 times longer than fish, since he recovers from exercise much faster.

Flock et al. (1939) intermittently electrically stimulated the muscle of an anesthetized mouse. They found that a recovery time of greater than 28 minutes was required between electrical stimuli lasting 1 minute to prevent an increase in muscle lactate.

Table X. Blood levels of lactate after intermittent exercise in man from Christensen (1956).

Duration of exercise	Duration of rest period	Duration of total exercise time	Blood lactate mg %
30 seconds	30 seconds	30 minutes	20
1 minute	1 minute	30 minutes	45
2 minutes	2 minutes	30 minutes	95
3 minutes	3 minutes	30 minutes	120
9 minutes		9 minutes	150

Astrand and Christensen explain their results on the basis of myoglobin. Exercise is done aerobically using oxygen bound to myoglobin. When the oxygen store bound to myoglobin is depleted, then the source of energy is anaerobic and the result is lactate formation. On this basis, their results show that the myoglobin will provide sufficient oxygen for 30 seconds strenuous exercise, and that only 30 seconds recovery time is required to reload the myoglobin with oxygen. The results of Christensen and those of the present study demonstrate the marked difference in the response between fish and man to intermittent exercise, and are compared in Table XI.

An explanation of the obvious discrepancy between the response of fish and man to intermittent exercise is difficult. Yamaguchi and Matsuura (1961) have characterized myoglobin of tuna, and concluded that the prosthetic group of hemoglobin and myoglobin of fish in general is probably the same as that of mammals. Matsuura and Hashimoto (1956) have characterized myoglobin in several species of fish (tuna and swordfish) and found that myoglobin of fish is similar to that of horse heart. Matsuura and Hashimoto (1955) demonstrated that the iron content in myoglobin of the red muscle of fish (*Paranthunnus sibi* and *Thunnus orientalis*) is greater than that found in horse heart. Assuming that myoglobin is present in trout muscle, then the long time

Table XI. A comparison of the resistance to fatigue between fish and man during intermittent strenuous exercise as indicated by the level of blood lactate

Animal	Duration of exercise	Duration of rest period	Number of times exercised	Total time exercised	Blood lactate mg %	Reference
man	30 sec.	30 sec.	60	30 min.	20	Christensen
fish	30 sec.	60 min.	3	1.5 min.	97	present study

required for recovery can only be explained by a slower circulation time, or a slower diffusion time of lactate from muscle to blood.

The circulation time is probably much less in fish than in man. Mott (1957) reports that the circulation time in the toadfish is about 2 minutes, whereas that of man is only 15 to 18 seconds (Fishman, 1963). Assuming that myoglobin is a functional component of fish muscle and assuming that the circulation time in fish is 10 times that of man, then recovery from strenuous exercise of 30 seconds should take 10 times as long in fish as it takes in man. But, the results of the present study show that fish can not resist intermittent strenuous exercise when the recovery period is 120 times as long. As present, there appears to be no explanation for the discrepancy between the response to strenuous intermittent exercise between fish and man.

The significant relationship between exercise and mortality in fish has been reviewed by Black (1958). Mortality as a result of hyperactivity has been observed in cod and dab (von Buddenbrock, 1938), tench (Secondat and Diaz, 1942), striped bass (Litt, 1954), chinook salmon fingerlings (Anonymous, 1957), sockeye salmon (Black, 1957c) coho and chinook salmon (Parker and Black, 1959), and channel catfish (Caillouet, 1964). In most of these studies mortality was

associated with high levels of blood lactate. In the present study there were no mortalities produced by exercise. Moreover, a search of the literature has failed to produce any evidence for mortality in rainbow trout as a result of severe exercise. Huckabee (1961) has described a fatal pathological condition in humans in which mortality is associated with lactate acidosis. The elevated blood lactate in this case is not associated with hyperactivity, but rather with impaired peripheral circulation.

Other parameters have been associated with the elevated blood lactate of hyperactivity. Secondat (1950) observed a decreased blood oxygen capacity which was correlated with an elevated blood lactate in the carp. Jonas et al. (1962) showed that mortality in rainbow trout could be produced by injections of lactic acid but not with sodium lactate.

Auvergnat and Secondat (1942) observed that 15 minutes exercise caused a decreased pH in carp blood. The pH did not return to normal after a 6 hours rest period. They attributed this acidification of blood to the release of lactic acid from muscle to blood. A decrease in blood carbon dioxide accompanied the decrease in pH, and the carbon dioxide was still decreasing 6 hours after the exercise. They also noted that the osmotic pressure of plasma was elevated immediately after the exercise, and remained elevated for 4 hours.

The increased osmotic pressure of plasma was attributed to the presence of by-products of muscle metabolism. Black et al. (1959) made similar studies in rainbow trout. They observed that both blood pH and blood carbonate increased during the first 3 minutes of strenuous exercise, and then both decreased during the remaining 12 minutes of exercise. Blood pH continued to fall, the level being significantly depressed 2 hours after the exercise. The highest level of carbonate occurred 8 hours after the exercise. Black noted that the characteristic change in behavior of exercised trout occurred at the same time as the maximum levels of hydrogen ion and maximum levels of blood carbonate. Thus, lactic acid is neutralized at the expense of plasma bicarbonate, but a significant decrease in blood pH still occurs after 15 minutes of severe exercise (Auvergnat and Secondat, 1942).

In summary; the level of blood lactate, and thus of muscle lactate, is the result of a great number of dynamically operating factors. Those factors which probably contribute most to the variability in the observed data are sex and age.

III. GLYCOGEN

A. Results - Muscle Glycogen

The mean level of muscle glycogen in unexercised fish was 251 mg % (mg per 100 grams wet weight of tissue).

The effects of exercise, recovery, and intermittent exercise are illustrated in Fig. 14, 15, 16, and 17.

The level of muscle glycogen in unexercised fish decreased during the course of the summer from 251 to 213 mg %.

Exercise, even of the shortest duration, caused an immediate decrease in muscle glycogen. Fig. 14 illustrates that one-half of the muscle glycogen present in unexercised fish is depleted as a result of only 30 seconds exercise. The rate of utilization of muscle glycogen decreased with further exercise.

Fig. 15 illustrates that muscle glycogen in fish exercised 1 minute and 2 minutes continued to decrease for 3 minutes after the exercise, whereas that of fish exercised 5 minutes decreased only slightly. Muscle glycogen of fish exercised 2 and 5 minutes failed to recover in 30 minutes after the exercise, whereas that of fish exercised 1 minute did recover slightly. That muscle glycogen failed to return to unexercised levels even after extremely short duration is illustrated in Fig. 16 and 17.

The effects of re-exercise after 60 minutes recovery are to cause further decrease in levels of muscle glycogen,

Fig. 14. Changes in levels of muscle glycogen during 5 minutes severe exercise.

Fig. 15. Changes in levels of muscle glycogen during recovery from severe exercise of 1, 2, and 5 minutes.

Fig. 16 Effect of intermittent exercise of 15 seconds on the levels of muscle glycogen.

Fig. 17. Effect of intermittent exercise of 30 seconds on the levels of muscle glycogen.

but there appeared to be a lower limit (approximately 20 mg %) which muscle glycogen did not exceed (Fig. 16 and 17). The levels of muscle glycogen after intermittent exercise appear in the Table XII.

B. Results - Liver Glycogen

The mean level of liver glycogen in unexercised fish was 4.90 g % (g per 100 g wet weight).

The level of liver glycogen decreased during the summer as did muscle glycogen.

The changes in the levels of liver glycogen during exercise, during recovery, and during intermittent exercise were not statistically significant in the present study. Characteristically, the liver glycogen samples have a very large inherent variability. Levels of glycogen after exercise and re-exercise of 30 seconds appear in Table XIII. The changes in levels in liver glycogen during 5 minutes severe exercise appear in Table XIV. The results of an Analysis of Variance on the data in Table XIV appear in Table XV. Changes in levels of liver glycogen during 5 minutes severe exercise were not significant.

Table XII. Effect of exercise and re-exercise on the level of muscle glycogen. Fish rested 60 minutes between exercise periods, and were sampled immediately after exercise or re-exercise.

Duration of exercise		15 sec.	30 sec.	5 min.
Muscle glycogen mg %	first chase	161	113	49
	second chase	111	85	24
	third chase	83	59	

Table XIII. Effect of exercise and re-exercise of 30 seconds on the level of liver glycogen. Fish rested 60 minutes between exercise periods, and were sampled immediately after exercise or re-exercise.

Liver glycogen (grams %)		
	mean	(95% confidence interval)
first chase	2.407	(1.592 to 3.222)
second chase	2.984	(2.225 to 3.743)
third chase	3.027	(3.027 to 4.074)

Table XIV. Effect of 5 minutes severe exercise, and 30 minutes recovery after 5 minutes severe exercise on the levels of liver glycogen.

Condition	liver glycogen (grams %)
	mean $\pm$ standard deviation
unexercised	4.90 $\pm$ 1.40
chase 1 min.	4.54 $\pm$ 2.11
chase 2 min.	4.83 $\pm$ 1.30
chase 5 min.	5.21 $\pm$ 1.46
chase 5 min., rest 30 min.	3.36 $\pm$ 0.88

Table XV. Analysis of variance of liver glycogen data from Table XIV during and after 5 minutes severe exercise.

Source of variation	degrees of freedom	Sum of squares	Mean square	F	F. _{.05}
total	38	95.7162			
treatment	4	15.8475	3.96	1.69	2.65
error	34	79.8687	2.35		

C. Discussion - Muscle and Liver Glycogen.

The levels of muscle glycogen reported in the present study are similar to values in the literature for Salmo gairdnerii. The levels of muscle glycogen in unexercised rainbow trout appear in Table XVI.

Claude Bernard (1876) was the first to report the presence of glycogen in fish. Other early workers also noted glycogen content in fish (Schöndorff and Wachholder, 1914; Kilborn and MacLeod, 1919; and Dill, 1921). In general, the level of muscle glycogen is lower in fish than in mammals (West and Todd report the level of muscle glycogen in man to be 0.4 to 0.6 g %). The level of liver glycogen is similar in man and rainbow trout.

From year to year there is a marked variation in the levels of muscle glycogen among fish treated similarly at Summerland. Miller et al. (1959) have shown that changes in diet can cause changes in level of muscle glycogen. In Kamloops trout, Black et al. (1962) found that the level of muscle glycogen was higher in males than in females during rest, exercise, and during recovery from exercise. It is of significance that Black and his co-workers only demonstrated this sex difference once; in later studies the sex difference was not significant. It is probable that the effect of

Table XVI. Levels of muscle glycogen in unexercised rainbow trout.

Muscle glycogen g. %	Diet	Reference
0.085	ground mammalian liver and fish viscera	Black <u>et al.</u> , 1960
0.118	beef liver	Miller <u>et al.</u> , 1959
0.178	Clark's trout feed	Miller <u>et al.</u> , 1959
0.251	Clark's trout feed	present study

severe exercise or carbohydrate metabolism will produce different results between the sexes only in sexually mature fish.

Hochachka (1961) has shown that age, stage of maturation, and training each affect the level of muscle glycogen in trout.

Moreover, glycolysis during sampling will deplete the muscle glycogen by 30% in 5 minutes at room temperature (Black et al., 1960).

Recently, the analytical method for glycogen has been criticized (Orrell and Bueding, 1964). These workers indicate that values of muscle glycogen obtained with potassium hydroxide digests are ~~not similar to~~ the values obtained by other procedures.

The first experimental study of the depletion of muscle glycogen in fish during exercise was done by Black et al. (1960). Miller et al. (1959) have also studied the effect of exercise on muscle on muscle glycogen in fish. Both Black and Miller observed that one-half of the muscle glycogen is depleted by 2 minutes severe exercise. This is in contrast to the findings of the present study in which one-half of the muscle glycogen was utilized with 30 seconds exercise, and that over two-thirds was utilized in 2 minutes severe exercise. Black et al. (1960) showed that about 80% of the muscle glycogen was utilized in 15 minutes severe exercise, whereas in the present study 80% was utilized in 5 minutes severe

exercise. There are two possible explanations for the results reported above. First, that the previous workers did not determine the level of muscle glycogen after 30 seconds severe exercise. And second, that the level of muscle glycogen in the unexercised fish was higher in the fish that I studied than in the fish studied by Black or Miller.

Black et al., (1961) have related the rapid decrease in muscle glycogen to the rapid increase in muscle lactate and to the change in behavior. They noted that fish rarely swim rapidly after 2 minutes severe exercise. Similar changes in behavior occurred in the present study, but the changes in swimming velocity were not quantitatively determined.

Hochachka (1961) pointed out that muscle glycogen is rarely depleted to zero, no matter how severe or how long the exercise. Muscle glycogen was never completely depleted in the present experiment. A possible explanation for this is that a protective mechanism exists. For example, the presence of lactic acid in the tissues will increase the hydrogen ion content which could cause inactivation of some acid sensitive enzyme in the glycogenolytic or glycolytic chain. Keller and Cori (1955) have shown that the enzyme phosphorylase is inactivated in the presence of high hydrogen ion concentration, and Hers et al. (1964) have shown that amylo-1,6-glucosidase is inactivated at about pH 4.5.

Black et al. (1962) has observed that the level of muscle glycogen in rainbow trout severely exercised for 15 minutes did not return to normal during a 24 hour recovery period. In the present study the level of muscle glycogen did not return to the unexercised level during a 60 minute recovery period from only 15 seconds severe exercise. Black et al. (1960) have also shown that the muscle glycogen recovers faster if the fish are fed during recovery. Muscle glycogen returns to normal faster in mammals than in fish. The slow resynthesis of muscle glycogen in fish could be due to any of the following:

1. Enzyme inhibition due to the elevated hydrogen concentration associated with the prolonged elevation of lactic acid in the muscle.
2. The metabolism of the fish is lower since the temperature is lower than that of mammals.
3. The lack of nutrients required to resynthesize the muscle glycogen due to impaired circulation would slow glycogen synthesis.

To my knowledge, there have been no previous studies on the effect of intermittent exercise on fish, and, no studies on the effect of intermittent exercise on muscle glycogen levels in any intact animal. Flock et al. (1939) intermittently electrically stimulated the leg muscle of mice and measured lactate and glycogen levels. They found that the

muscle glycogen was almost completely depleted after stimulation for 1 minute, and that the level of muscle glycogen changed little thereafter even though the muscle continued to contract. These workers therefore postulated that glycogen serves as the initial source of energy, but that there must also be some other source of energy. If muscle glycogen is the major source of energy for muscle contraction during severe exercise in trout, then the present study indicates that muscle glycogen reserves are probably the limiting factor in exercise. Moreover, the frequency of severe exercise of short duration must be very low--that is, only a few times per day.

Heath and Pritchard (1962) demonstrated that oxygen consumption remains elevated for about 24 hours after severe exercise of 6 to 45 minutes in the bluegill sunfish. They also measured blood lactate levels and observed that the latter returned to pre-exercise levels within 10 hours. It is probable that the elevated oxygen consumption during the first 10 hours is associated with oxidation of the lactate produced by exercise, and that the elevated oxygen consumption from 10 to 24 hours is associated with re-synthesis of muscle glycogen.

IV. The Relationship between Glycogen and Lactate in Muscle.

Meyerhoff (1920) was the first to demonstrate that muscle glycogen was the source of lactate during exercise. Black (1957c) presumed that the source of blood lactate in exercised trout was muscle glycogen. Black et al. (1960) demonstrated that the relationship between muscle glycogen and blood lactate is curvilinear. My data substantiates Black's data since in the present study the relationship between blood and muscle lactate is curvilinear. Thus, an extrapolation from Black's graphs would yield a linear relationship between muscle glycogen and muscle lactate.

To demonstrate the relationship between lactate and glycogen in muscle a correlation analysis was done on the means of 11 samples. The correlation coefficient ($r = -0.934$) indicated that the relationship between these two variables is highly significant. The observed relationship (the calculated regression line) is compared with the theoretical relationship in Fig. 18 assuming that 162 mg. of glycogen appear as 180 mg. of lactate. At lower levels of exercise (that is, low lactate and high glycogen levels) the points fit the theoretical line very well. But, at higher levels of exercise (that is, low glycogen and high lactate levels) all of the points are above the theoretical line. These latter results indicate that at the higher

Fig. 18. The relationship between muscle lactate and muscle glycogen in fish sampled immediately after exercise.

levels of exercise studied in the present experiment there is a source of muscle lactate other than muscle glycogen. The slope of the line of the theoretical relationship lies within the 95% confidence limits of the slope of the calculated regression line, but not within the 99% confidence limits of the slope of the calculated regression line. The slope of the line of the theoretical relationship is -1.11. The 95% confidence limits are -1.05 to -1.91, and the 99% confidence limits are -1.14 to -1.82.

In Table XVII, the percent of lactate not accounted for by the decrease in glycogen is high only for severe exercise lasting for more than one minute. There are four possible explanations for the results recorded in Fig. 18 and Table XVII.

1. Actual glycogen present is greater than that measured.
2. Actual lactate present is less than that measured.
3. Glycogen is being rapidly synthesized during exercise.
4. There is a source of lactate other than glycogen during the later stages of prolonged severe muscular exercise.

Table XVII. The percent of muscle lactate not accounted for by the decrease in the level of muscle glycogen.

Exercise condition	Level of muscle glycogen mg %	Decrease in muscle glycogen levels mg %	Level of muscle lactate mg %	L Increase in muscle lactate levels mg %	T Theoretical increase in level of muscle lactate mg %	$\frac{(T-L)}{L} \times 100\%$
unexercised	251	0	132	0	0	0
15 sec.	161	90	236	104	100	4
30 sec.	113	138	291	159	153	4
15 sec. (twice)	111	140	276	144	156	0
15 sec. (3 times)	83	168	304	172	187	0
1 min.	92	159	306	174	177	0
30 sec. (twice)	85	166	366	234	184	21
30 sec. (3 times)	59	192	358	226	213	6
2 min.	74	177	387	255	197	23
5 min.	49	202	468	336	224	33
5 min. (twice)	24	227	462	330	252	24

$\frac{(T-L)}{L} \times 100\%$ = Percent of muscle lactate not accounted for by the decrease in the level of muscle glycogen assuming that 162 mg of glycogen appear as 180 mg of lactate.

The analytical methods for determination of glycogen have been recently criticized by Orrel and Bueding (1964). These workers reported that extraction with hot alkali yields absolute values similar to other methods of extraction; but that the glycogen isolated with alkali has different characteristics than that obtained by less severe extraction techniques.

The method used to determine lactate concentration was critically examined by Barker and Summerson (1941) in the original paper on this method. It is unlikely that any substances interfere with lactate determination in sufficient quantities to invalidate the evidence for a source of lactate other than muscle glycogen.

There is no evidence, to my knowledge, for or against synthesis of muscle glycogen during exercise in fish. But, measurements of the rate of restoration of muscle glycogen after 15 minutes severe exercise by Black et al. (1960) indicate that muscle glycogen synthesis is a slow process in fish. Therefore it is probable that there is a source of lactate other than muscle glycogen during prolonged muscular exercise.

If liver glycogen is a source of muscle lactate, then I would expect two results:

1. a depletion of liver glycogen during

exercise, and

2. an elevated blood glucose during exercise.

Miller et al. (1959) demonstrated that liver glycogen is in fact depleted during 15 minutes exercise in trout if the initial level of muscle and liver glycogen is low. Dean and Goodnight (1964) found that 15 minutes exercise in bass, crappie, and bullhead decreased liver glycogen significantly after depletion of muscle glycogen stores. The levels of liver glycogen did not change significantly during exercise or during intermittent exercise in the present study.

Elevation of blood glucose during exercise has not been demonstrated in rainbow trout. Secondat (1950) found that exercise usually elevated blood glucose in carp, but that the increase was not consistent. In lake trout Black (1957b) found that blood glucose increased after 15 minutes severe exercise and remained elevated throughout the 24 hour recovery period studied. In bass, crappie, and bullhead, Dean and Goodnight (1964) found that 15 minutes exercise always produced a statistical significant increase in blood glucose level. If glucose does increase in response to exercise, the mechanism probably involves adrenaline. In mammals, adrenaline increases blood glucose levels, and the elevated blood glucose during muscular activity appears to be related to excitement (Peters and Van Slyke, 1946).

Other possible sources of lactate include catabolism lipid and protein from blood or from the muscle itself, and by absorption of foodstuffs from the gut. None of these were examined in the present study. Fontaine and Hatey (1953) have shown that both fat and protein are metabolized during the upstream migration of Atlantic salmon. Blazka (1958) has reported that exercise elevates ammonia and non-protein nitrogen in carp blood. Hypoxia elevates non esterified fatty acids (but does not elevate lactate) in trout blood (Blazka, 1958.)

In summary, the present study provides evidence that there is a source of muscle lactate other than muscle glycogen, and that this source of energy is probably catabolism of lipid or protein.

V. General Discussion

It is evident that in fish the changes of carbohydrate metabolism during severe exercise are still not completely understood. In general, metabolism appears to be similar in fish and mammals in that glycogen is broken down during severe exercise.

When the supply of oxygen does not meet the demand, then the reduced forms of DPNH_2 and FADH_2 accumulate. Thus, the Krebs's cycle fails to operate due to lack of supply of the

oxidized forms of DPN and FAD. In turn, pyruvate backs up in the chemical chain to form lactate. As a result, accumulation of lactate indicates the insufficiency of the oxygen supply and also muscular exertion. Lactate also serves as an hydrogen acceptor for DPNH_2 .

During recovery from severe exercise, lactate is either excreted or converted back to pyruvate. The pyruvate so formed is then oxidized to carbon dioxide and water, or resynthesized to glycogen. Resynthesis of glycogen is probably from blood glucose rather than from muscle lactate, and is a slow process in fish. The above scheme is illustrated in Fig. 19.

It is possible to explain the slowness of recovery from severe exercise on the basis of the circulatory system using 30 seconds severe exercise as an example.

1. Severe exercise for 30 seconds elevates muscle lactate 160 mg per 100 g of muscle above the unexercised level. Since 1 ml of oxygen is required to convert 8.03 mg of lactate to pyruvate, then 160 mg of lactate requires 20 ml of oxygen.

2. In the present study the hemoglobin concentration was sufficient to transfer a maximum of 11 ml of oxygen

per 100 ml blood. Thus to transfer 20 ml of oxygen, 182 ml of blood is required.

3. The cardiac output in fish is small, and is probably about 1 ml/100 g body weight/minute for rainbow trout (Hart, 1943). In order to pump 182 ml of blood/100 g body weight, the fish would require 182 minutes, or approximately 3 hours to recover from 30 seconds severe exercise.

IN THE PRESENCE OF ADEQUATE OXYGEN - UNEXERCISED CONDITION

IN THE PRESENCE OF AN INSUFFICIENT OXYGEN SUPPLY - SEVERE EXERCISE

RECOVERY FROM AN INSUFFICIENT OXYGEN SUPPLY - RECOVERY FROM SEVERE EXERCISE

Fig. 19. The effect of oxygen supply on carbohydrate metabolism.

CONCLUSIONS

Some general conclusions can be made from the present study. In rainbow trout, severe exercise depletes muscle glycogen extremely rapidly. Moreover, in fish, the restoration of muscle glycogen to pre-exercise levels is a slow process. In contrast to this, the rate of depletion of muscle glycogen during moderate exercise is small (Black et al. 1960). Blood lactate continues to rise and remains elevated for at least 30 minutes following exercise of extremely short duration, that is, 3 seconds. Intermittent severe exercise after recovery periods of 60 minutes causes further increases in blood and muscle lactate, and further decreases in muscle glycogen. In conclusion, it appears that rainbow trout are not well adapted to tolerate severe exercise.

SUMMARY

1. The effects of intermittent exercise and exercise of short duration (3 seconds to 1 minute) on carbohydrate metabolism have been studied in rainbow trout.

2. The effect of severe exercise of short duration was to cause the following:

a. an immediate decrease in muscle glycogen levels. One-half of the muscle glycogen was utilized during 30 seconds severe exercise, and 80% utilized during 5 minutes exercise.

b. an immediate increase in muscle lactate levels. Muscle lactate increased more than two fold during 30 seconds severe exercise, and increased more than three and one-half fold during 5 minutes severe exercise.

c. an immediate increase in blood lactate levels. Blood lactate levels increased two fold during 30 seconds severe exercise, and increased more than ten fold during 5 minutes severe exercise.

d. an initial rapid increase in hemoglobin

concentration, followed by a rapid decrease in hemoglobin concentration, and this in turn followed by a slow increase in hemoglobin concentration.

3. During recovery periods of 3 to 60 minutes after severe exercise the following occurred.

- a. Muscle glycogen levels changed very little.
- b. Muscle lactate levels changed very little.
- c. Blood lactate levels continued to rise during the recovery periods even in fish chased 3 seconds. This is in complete contradistinction to findings in mammals.

- d. Hemoglobin levels continued to rise during the recovery periods even in fish chased 3 seconds.

4. The effect of re-exercise was to cause the following:

- a. an immediate further decrease in muscle glycogen levels until the muscle glycogen was depleted to approximately 20 mg %.
- b. an immediate further increase in muscle lactate levels until muscle lactate level reached approximately 20 mg %.
- c. an immediate further increase in levels of blood lactate and hemoglobin concentration.

5. Levels of liver glycogen did not appear to change during exercise, recovery, or re-exercise. Liver glycogen values are characterized by their large variability.

6. No mortalities occurred as a result of severe exercise. No consistent differences were noted with respect to sex, weight, catch time, or sample time.

7. Evidence is presented which indicates there is probably a source of muscle lactate other than muscle glycogen during 5 minutes severe exercise. Liver glycogen does not appear to be the source of this muscle lactate.

AREAS FOR FURTHER INVESTIGATION

It would be interesting to repeat the experiment using steelhead trout, which is a different race of the same species but generally thought to be a more active fish.

Much of the variability in data of the present study is probably due to many of the variables which were not controlled. A more complex design may reveal the effects of age, size, sex, and maturity.

Because many of the differences between findings of the present study and findings in man by Christensen are attributed to differences in the circulatory systems of fish and man, it is of importance that the circulatory system in fish be studied. In particular an accurate estimate of the cardiac output and circulation time are required. To explain the observed changes in blood hemoglobin concentration during exercise, it is essential that the location and control of the venous reservoirs and the location and control of red cell reservoirs be ascertained.

It would also be profitable to study the role of myoglobin in rainbow trout. That is, to determine its concentration and its dissociation curve in order to demonstrate whether or not it is functional in this animal.

To explain the unknown source of muscle lactate during 5 minutes severe exercise, it is necessary to study changes in levels of proteins and lipids in the muscle and the blood. It would also be of interest to determine the rate of turnover of glucose during exercise and recovery.

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APPENDIX

Table XVIII. Sample Size, mean, and standard deviation for each of the variables studied. Blood and tissue samples were obtained from separate fish.

Exercise condition			Blood samples				Muscle samples			
chase min.	rest min.	chase min.	n	weight grams mean $\pm$ Sd	lactate mg % mean $\pm$ Sd	hemoglobin g % mean $\pm$ Sd	n	weight grams mean $\pm$ Sd	lactate mg % mean $\pm$ Sd	glycogen mg % mean $\pm$ Sd
1			8	275 $\pm$ 69.5	15 $\pm$ 3.6	9.75 $\pm$ 1.11	9	223 $\pm$ 27.0	306 $\pm$ 69.4	92 $\pm$ 41.3
2			9	252 $\pm$ 56.9	24 $\pm$ 6.2	10.05 $\pm$ 1.09	9	255 $\pm$ 29.4	387 $\pm$ 88.1	74 $\pm$ 75.4
5			9	219 $\pm$ 27.3	55 $\pm$ 9.5	10.74 $\pm$ 1.09	8	260 $\pm$ 64.8	468 $\pm$ 50.7	49 $\pm$ 15.7
1	3		9	252 $\pm$ 55.0	43 $\pm$ 7.7	10.96 $\pm$ 1.01	9	245 $\pm$ 47.5	362 $\pm$ 107.4	46 $\pm$ 29.7
2	3		9	266 $\pm$ 38.0	54 $\pm$ 8.6	10.42 $\pm$ 1.05	9	220 $\pm$ 40.6	400 $\pm$ 90.0	49 $\pm$ 43.3
5	3		9	256 $\pm$ 57.3	74 $\pm$ 14.9	11.00 $\pm$ 1.04	9	213 $\pm$ 48.4	394 $\pm$ 85.6	33 $\pm$ 43.7
1	8		9	248 $\pm$ 54.6	49 $\pm$ 11.5	11.05 $\pm$ 1.22	9	242 $\pm$ 49.5	272 $\pm$ 75.9	73 $\pm$ 56.3
2	8		9	267 $\pm$ 44.4	74 $\pm$ 5.3	11.20 $\pm$ 0.99	9	270 $\pm$ 54.4	418 $\pm$ 71.0	43 $\pm$ 40.0
5	8		9	274 $\pm$ 39.3	78 $\pm$ 16.7	11.56 $\pm$ 1.75	9	223 $\pm$ 34.3	424 $\pm$ 62.5	38 $\pm$ 45.6
1	30		9	287 $\pm$ 93.6	70 $\pm$ 12.9	11.19 $\pm$ 1.35	9	207 $\pm$ 46.3	256 $\pm$ 55.2	103 $\pm$ 76.1
2	30		9	233 $\pm$ 53.5	100 $\pm$ 7.1	11.47 $\pm$ 1.70	9	244 $\pm$ 59.9	380 $\pm$ 132.7	34 $\pm$ 22.8
5	30		9	260 $\pm$ 47.0	107 $\pm$ 25.3	12.17 $\pm$ 1.10	9	230 $\pm$ 76.5	402 $\pm$ 91.1	35 $\pm$ 24.5
5	60		6	301 $\pm$ 61.1	144 $\pm$ 27.6	10.81 $\pm$ 0.85	6	303 $\pm$ 62.2	403 $\pm$ 41.0	19 $\pm$ 18.6
1	3	1	6	359 $\pm$ 40.2	49 $\pm$ 5.4	11.82 $\pm$ 1.49	-	-	-	-
2	3	2	6	272 $\pm$ 29.2	79 $\pm$ 10.3	12.14 $\pm$ 0.78	-	-	-	-
5	3	5	6	336 $\pm$ 100.5	86 $\pm$ 6.7	12.59 $\pm$ 0.59	-	-	-	-
1	8	1	6	318 $\pm$ 55.0	64 $\pm$ 5.1	12.96 $\pm$ 1.26	-	-	-	-
2	8	2	6	298 $\pm$ 49.7	86 $\pm$ 7.6	11.82 $\pm$ 1.22	-	-	-	-
5	8	5	6	356 $\pm$ 59.5	98 $\pm$ 10.4	12.84 $\pm$ 1.28	-	-	-	-
1	30	1	6	293 $\pm$ 34.9	86 $\pm$ 14.1	12.00 $\pm$ 1.25	-	-	-	-
2	30	2	6	349 $\pm$ 71.8	115 $\pm$ 14.7	11.87 $\pm$ 0.98	-	-	-	-
5	30	5	6	290 $\pm$ 77.3	120 $\pm$ 19.0	12.32 $\pm$ 1.71	-	-	-	-
5	60	5	6	294 $\pm$ 41.1	148 $\pm$ 16.5	11.49 $\pm$ 0.94	6	265 $\pm$ 43.6	462 $\pm$ 42.4	24 $\pm$ 11.0

Table XVIII. (continued).

Exercise condition					Blood samples				Muscle samples			
chase sec.	rest min.	chase sec.	rest min.	chase sec.	n	weight grams mean $\pm$ Sd	lactate mg % mean $\pm$ Sd	hemoglobin g % mean $\pm$ Sd	n	weight grams mean $\pm$ Sd	lactate mg % mean $\pm$ Sd	glycogen mg % mean $\pm$ Sd
3					7	247 $\pm$ 33.6	9 $\pm$ 1.9	10.74 $\pm$ 0.46	-	-	-	-
3	30				8	286 $\pm$ 55.7	24 $\pm$ 4.5	10.83 $\pm$ 0.50	-	-	-	-
3	60				6	265 $\pm$ 38.4	19 $\pm$ 17.5	10.78 $\pm$ 0.78	-	-	-	-
3	60	3			6	262 $\pm$ 53.3	16 $\pm$ 6.9	10.32 $\pm$ 0.71	-	-	-	-
15					6	261 $\pm$ 33.8	9 $\pm$ 2.8	10.15 $\pm$ 0.75	6	259 $\pm$ 42.8	236 $\pm$ 51.0	161 $\pm$ 31.2
15	30				6	306 $\pm$ 56.9	34 $\pm$ 8.0	10.65 $\pm$ 0.76	6	306 $\pm$ 53.3	193 $\pm$ 20.7	148 $\pm$ 64.0
15	60				6	295 $\pm$ 40.9	25 $\pm$ 9.2	10.13 $\pm$ 1.22	6	304 $\pm$ 51.0	240 $\pm$ 114.5	162 $\pm$ 30.3
15	60	15			6	227 $\pm$ 46.2	47 $\pm$ 20.5	11.00 $\pm$ 1.23	6	243 $\pm$ 58.0	276 $\pm$ 56.0	111 $\pm$ 62.9
15	60	15	30		5	289 $\pm$ 61.3	76 $\pm$ 5.8	11.42 $\pm$ 0.61	6	251 $\pm$ 50.4	177 $\pm$ 40.8	134 $\pm$ 42.2
15	60	15	60		7	301 $\pm$ 25.1	63 $\pm$ 20.1	11.53 $\pm$ 1.20	6	302 $\pm$ 44.0	196 $\pm$ 95.9	132 $\pm$ 30.5
15	60	15	60	15	6	298 $\pm$ 40.2	74 $\pm$ 23.0	11.52 $\pm$ 1.07	6	289 $\pm$ 42.9	304 $\pm$ 83.7	83 $\pm$ 44.0
30					7	308 $\pm$ 36.3	14 $\pm$ 3.0	9.70 $\pm$ 1.13	6	287 $\pm$ 65.5	291 $\pm$ 61.3	113 $\pm$ 12.5
30	30				6	270 $\pm$ 34.9	49 $\pm$ 11.1	10.87 $\pm$ 0.98	6	263 $\pm$ 45.7	271 $\pm$ 77.3	127 $\pm$ 56.2
30	60				6	258 $\pm$ 58.8	50 $\pm$ 15.6	10.04 $\pm$ 0.74	6	256 $\pm$ 62.6	179 $\pm$ 32.0	157 $\pm$ 38.5
30	60	30			6	300 $\pm$ 27.1	55 $\pm$ 12.1	10.53 $\pm$ 1.04	6	253 $\pm$ 39.6	366 $\pm$ 70.7	85 $\pm$ 21.1
30	60	30	30		6	308 $\pm$ 58.3	102 $\pm$ 15.5	11.34 $\pm$ 0.97	6	294 $\pm$ 53.5	309 $\pm$ 36.6	81 $\pm$ 25.7
30	60	30	60		6	274 $\pm$ 42.2	88 $\pm$ 13.1	10.82 $\pm$ 0.94	6	304 $\pm$ 19.8	320 $\pm$ 68.9	81 $\pm$ 23.6
30	60	30	60	30	6	292 $\pm$ 40.3	97 $\pm$ 19.1	11.50 $\pm$ 0.54	6	268 $\pm$ 68.3	358 $\pm$ 36.1	59 $\pm$ 11.6
0	30				6	323 $\pm$ 29.7	16 $\pm$ 11.7	10.70 $\pm$ 0.55	-	-	-	-
0	60				6	259 $\pm$ 50.5	14 $\pm$ 7.6	10.60 $\pm$ 1.07	-	-	-	-
to exhaustion					-	-	-	-	10	270 $\pm$ 68.9	388 $\pm$ 75.4	78 $\pm$ 21.2
unexercised					8	310 $\pm$ 53.0	7 $\pm$ 4.1	9.15 $\pm$ 1.40	9	221 $\pm$ 49.2	132 $\pm$ 49.7	251 $\pm$ 48.8

Table XIX. Time to obtain and prepare blood and tissue samples.

Exercise condition			Blood samples (time in seconds)		Muscle samples (time in seconds)			
chase min.	rest min.	chase min.	catch time mean $\pm$ Sd	time to obtain blood mean $\pm$ Sd	catch time mean $\pm$ Sd	kill time mean $\pm$ Sd	muscle time mean $\pm$ Sd	liver time mean $\pm$ Sd
1			14.4 $\pm$ 5.18	74.3 $\pm$ 44.46	2.9 $\pm$ 1.05	2.8 $\pm$ 1.09	14.8 $\pm$ 2.44	16.8 $\pm$ 11.29
2			11.7 $\pm$ 3.67	51.2 $\pm$ 31.12	2.1 $\pm$ 0.33	3.8 $\pm$ 2.53	15.9 $\pm$ 5.33	20.8 $\pm$ 12.69
5			11.8 $\pm$ 4.35	113.2 $\pm$ 67.31	2.0 $\pm$ 0.93	3.0 $\pm$ 1.07	16.1 $\pm$ 5.19	16.5 $\pm$ 5.78
1	3		16.1 $\pm$ 3.26	28.0 $\pm$ 14.88	3.8 $\pm$ 2.82	3.2 $\pm$ 0.67	10.7 $\pm$ 2.35	-
2	3		19.1 $\pm$ 4.37	50.2 $\pm$ 74.66	2.7 $\pm$ 1.00	2.6 $\pm$ 1.24	10.6 $\pm$ 2.65	-
5	3		17.4 $\pm$ 5.98	20.2 $\pm$ 13.17	3.8 $\pm$ 3.77	2.9 $\pm$ 1.05	11.4 $\pm$ 2.50	-
1	8		24.0 $\pm$ 8.90	22.5 $\pm$ 10.55	4.1 $\pm$ 3.02	2.4 $\pm$ 0.88	10.2 $\pm$ 1.20	-
2	8		17.3 $\pm$ 2.45	19.2 $\pm$ 13.76	2.7 $\pm$ 2.83	3.0 $\pm$ 1.58	10.1 $\pm$ 3.10	-
5	8		18.1 $\pm$ 4.65	37.0 $\pm$ 26.62	2.3 $\pm$ 1.00	2.6 $\pm$ 1.13	10.1 $\pm$ 2.80	-
1	30		20.0 $\pm$ 4.44	44.1 $\pm$ 38.92	3.1 $\pm$ 1.83	3.3 $\pm$ 1.12	10.2 $\pm$ 1.30	-
2	30		19.1 $\pm$ 5.37	28.1 $\pm$ 8.10	3.0 $\pm$ 1.32	3.6 $\pm$ 1.33	11.0 $\pm$ 2.06	-
5	30		20.1 $\pm$ 4.31	23.6 $\pm$ 11.17	3.1 $\pm$ 2.15	3.0 $\pm$ 1.00	9.6 $\pm$ 2.83	11.4 $\pm$ 3.16
5	60		9.3 $\pm$ 2.33	16.5 $\pm$ 16.08	1.7 $\pm$ 0.82	2.7 $\pm$ 1.21	8.8 $\pm$ 4.26	-
1	3	1	6.2 $\pm$ 0.75	14.7 $\pm$ 15.38	-	-	-	-
2	3	2	4.7 $\pm$ 1.75	11.8 $\pm$ 2.86	-	-	-	-
5	3	5	4.8 $\pm$ 1.17	10.7 $\pm$ 3.14	-	-	-	-
1	8	1	5.2 $\pm$ 1.33	17.8 $\pm$ 8.28	-	-	-	-
2	8	2	4.8 $\pm$ 0.75	12.5 $\pm$ 6.59	-	-	-	-
5	8	5	6.2 $\pm$ 1.17	16.5 $\pm$ 16.56	-	-	-	-
1	30	1	5.5 $\pm$ 1.22	24.3 $\pm$ 30.94	-	-	-	-
2	30	2	4.7 $\pm$ 0.81	13.0 $\pm$ 5.93	-	-	-	-
5	30	5	5.8 $\pm$ 2.31	13.0 $\pm$ 12.79	-	-	-	-
5	60	5	6.5 $\pm$ 2.07	38.0 $\pm$ 33.64	1.2 $\pm$ 0.41	1.5 $\pm$ 0.84	8.3 $\pm$ 0.52	-

Table XIX. (continued)

Exercise condition					Blood samples (seconds)		Muscle samples (time in seconds)			
chase sec.	rest min.	chase sec.	rest min.	chase sec.	catch time mean $\pm$ Sd	time to obtain blood mean $\pm$ Sd	catch time mean $\pm$ Sd	kill time mean $\pm$ Sd	muscle time mean $\pm$ Sd	liver time mean $\pm$ Sd
3					16.7 $\pm$ 6.37	35.6 $\pm$ 20.03	-	-	-	-
3	30				13.4 $\pm$ 2.62	24.6 $\pm$ 25.63	-	-	-	-
3	60				21.7 $\pm$ 7.99	33.0 $\pm$ 26.25	-	-	-	-
3	60	3			21.3 $\pm$ 13.92	25.5 $\pm$ 25.52	-	-	-	-
15					10.3 $\pm$ 5.20	16.3 $\pm$ 11.32	3.2 $\pm$ 1.47	2.2 $\pm$ 1.17	9.5 $\pm$ 1.87	-
15	30				17.5 $\pm$ 3.56	21.0 $\pm$ 16.05	3.5 $\pm$ 2.07	3.0 $\pm$ 0.89	8.8 $\pm$ 1.33	-
15	60				28.0 $\pm$ 15.62	61.5 $\pm$ 37.57	4.7 $\pm$ 4.76	3.5 $\pm$ 1.64	10.2 $\pm$ 2.48	-
15	60	15			13.8 $\pm$ 2.23	26.7 $\pm$ 22.61	2.8 $\pm$ 0.75	3.2 $\pm$ 1.33	8.2 $\pm$ 1.47	-
15	60	15	30		8.6 $\pm$ 3.51	24.8 $\pm$ 33.69	1.8 $\pm$ 0.75	3.0 $\pm$ 1.67	8.8 $\pm$ 1.47	-
15	60	15	60		10.7 $\pm$ 4.61	24.4 $\pm$ 22.30	6.2 $\pm$ 8.33	3.0 $\pm$ 0.63	9.5 $\pm$ 2.43	-
15	60	15	60	15	12.5 $\pm$ 5.21	17.8 $\pm$ 12.73	3.4 $\pm$ 1.62	3.3 $\pm$ 1.89	7.9 $\pm$ 0.90	-
30					9.3 $\pm$ 2.81	25.7 $\pm$ 28.25	2.7 $\pm$ 1.63	2.3 $\pm$ 0.52	9.2 $\pm$ 2.40	12.3 $\pm$ 3.38
30	30				14.8 $\pm$ 5.00	31.2 $\pm$ 23.05	3.0 $\pm$ 2.28	2.8 $\pm$ 1.72	10.1 $\pm$ 4.49	-
30	60				14.5 $\pm$ 2.17	20.5 $\pm$ 10.77	3.0 $\pm$ 2.28	2.7 $\pm$ 0.82	9.0 $\pm$ 2.97	-
30	60	30			11.8 $\pm$ 3.43	17.8 $\pm$ 19.32	2.3 $\pm$ 1.03	3.0 $\pm$ 1.41	7.8 $\pm$ 1.33	10.3 $\pm$ 1.34
30	60	30	30		7.7 $\pm$ 0.82	20.2 $\pm$ 8.38	5.3 $\pm$ 2.42	2.2 $\pm$ 0.75	8.2 $\pm$ 0.75	-
30	60	30	60		8.8 $\pm$ 1.72	59.2 $\pm$ 64.52	1.7 $\pm$ 1.21	2.7 $\pm$ 1.21	8.5 $\pm$ 2.26	-
30	60	30	60	30	9.2 $\pm$ 2.93	29.3 $\pm$ 22.29	1.8 $\pm$ 0.75	1.7 $\pm$ 0.52	8.5 $\pm$ 1.64	11.1 $\pm$ 1.84
unexercised					19.5 $\pm$ 7.19	47.9 $\pm$ 39.68	2.2 $\pm$ 0.83	3.4 $\pm$ 2.00	13.8 $\pm$ 6.98	11.0 $\pm$ 6.50
0	30				18.2 $\pm$ 3.65	23.8 $\pm$ 17.72	-	-	-	-
0	60				14.8 $\pm$ 4.17	30.5 $\pm$ 19.89	-	-	-	-
to exhaustion					-	-	2.0 $\pm$ 0.67	2.0 $\pm$ 0.67	8.6 $\pm$ 2.27	-

Table XX. Actual time taken to swim 4.3 meters (approximately 3 sec.) and actual time taken to swim 23 meters (approximately 15 sec.)

Exercise condition					ACTUAL TIME TAKEN (seconds)			
					Blood samples			
chase sec.	rest min.	chase sec.	rest min.	chase sec.	first chase mean $\pm$ Sd	second chase mean $\pm$ Sd	third chase mean $\pm$ Sd	
3					2.86 $\pm$ 0.38			
3	30				2.75 $\pm$ 0.71			
3	60				3.33 $\pm$ 1.37			
3	60	3			3.33 $\pm$ 1.97	4.00 $\pm$ 1.27		
15					13.83 $\pm$ 1.17			
15	30				13.00 $\pm$ 2.10			
15	60				14.33 $\pm$ 1.21			
15	60	15			15.33 $\pm$ 4.84	17.83 $\pm$ 2.43		
15	60	15	30		15.60 $\pm$ 5.46	12.60 $\pm$ 2.50		
15	60	15	60		16.43 $\pm$ 2.70	15.29 $\pm$ 2.14		
15	60	15	60	15	14.83 $\pm$ 3.97	14.83 $\pm$ 1.17	16.67 $\pm$ 3.20	
					Muscle samples			
					first chase mean $\pm$ Sd	second chase mean $\pm$ Sd	third chase mean $\pm$ Sd	
15					15.85 $\pm$ 2.48			
15	30				13.35 $\pm$ 0.52			
15	60				17.50 $\pm$ 2.43			
15	60	15			17.50 $\pm$ 1.48	15.00 $\pm$ 1.09		
15	60	15	30		16.65 $\pm$ 1.03	14.15 $\pm$ 0.41		
15	60	15	60		14.50 $\pm$ 1.22	15.85 $\pm$ 1.17		
15	60	15	60	15	15.00 $\pm$ 0.82	15.70 $\pm$ 0.69	15.00 $\pm$ 0.82	

Table XXI. Analysis of variance and Duncan's new multiple range test on the data in the latin square design. The purpose of this design was to demonstrate the effect of variability during any one day, from day to day, and the treatment effect.

A. The experimental conditions.

Treatment number	Condition		Day number	Date June	Time
	exercise minutes	recovery minutes			
1	1	3	1	3	AM
2	1	8	2	4	AM
3	1	30	3	4	PM
4	2	3	4	5	AM
5	2	8	5	5	PM
6	2	30	6	24	AM
7	5	3	7	24	PM
8	5	8	8	25	AM
9	5	30	9	25	PM

B. Analysis of Variance.

source	Calculated F values for each variable					F.05	F.01
	weight	catch	sample	hemoglobin	lactate		
	time	time	time				
during the day	2.31	1.09	1.17	1.43	2.30		
between days	1.56	2.83	1.28	4.65	1.02	2.12	2.87
treatment	0.84	2.13	1.19	1.86	26.65		

B. Duncan's New Multiple Range Test on means with significant F values. Any two means not underscored by the same line are significantly different from each other at the 5% level. Any two means which are underscored by the same line are not significantly different at the 5% level. The tests are applied in the order from the most significant to the least significant.

1. Treatment - lactate - highly significant.

number	1	2	4	3	5	7	8	6	9
mean	43	49	54	70	74	74	78	<u>100</u>	<u>107</u>

2. Between days - hemoglobin - highly significant.

number	3	9	2	1	5	8	7	4	6
mean	9.54	<u>10.46</u>	<u>11.14</u>	<u>11.39</u>	<u>11.54</u>	<u>11.62</u>	<u>11.64</u>	<u>11.78</u>	<u>11.90</u>

3. Between days - catch time - significant.

number	5	3	2	4	1	9	6	8	7
mean	14.1	17.0	<u>17.3</u>	<u>18.4</u>	<u>19.3</u>	<u>19.8</u>	<u>21.5</u>	<u>21.7</u>	<u>22.2</u>

4. During the day - weight - significant.

number	5	2	4	8	7	9	3	1	6
mean	224	232	235	<u>260</u>	<u>262</u>	<u>266</u>	<u>278</u>	<u>288</u>	<u>299</u>

5. During the day - lactate - significant.

number	5	8	9	3	4	2	6	1	7
mean	65	66	67	69	<u>72</u>	<u>73</u>	<u>78</u>	<u>78</u>	<u>84</u>

6. Treatment - catch time - significant.

number	1	5	7	8	6	4	3	9	2
mean	16.1	17.3	17.5	18.1	<u>19.1</u>	<u>19.1</u>	<u>20.0</u>	<u>20.1</u>	<u>24.0</u>