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# Quantitative Studies on Muscle and Liver Glycogen Utilization during Sustained Swimming in Freshwater Mullet, *Rhinomugil corsula* (Hamilton)

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In the freshwater mullet, *Rhinomugil corsula* the glycogen levels of muscle (white and red) and liver reduced drastically during the 1st hr of swimming and continued to decrease at a lesser rate towards the later phase of 5 hr exercise. However, the muscle and blood lactate showed the reverse-trend. The values of glycogen utilization at 20 and 77 cm/sec during the 1st hr of exercise were 1359.7 and 2943.1 mg/kg fish/hr, and during the 5th hr utilization the corresponding values were 159.9 and 112.1 mg/kg fish/hr. The values of unaccounted glycogen during the 1st hr swimming were found to be more (47.5 & 57.3% for 20 & 77 cm/sec respectively) and less (27.5 & 0%) during 5th hr of exercise.

**Key Words:** *Rhinomugil corsula*, **Glycogen, Lactate, Blood glucose**

## Introduction

A perusal of literature (Black et al 1960, Black et al 1962, Black et al. 1966, Brett 1973, Jhonston & Goldspink 1973, Jhonston 1975) reveals that no study has been specifically done to measure quantitative utilization of glycogen connected with long-term swimming activity of fish. In the present study besides the changes in glycogen concentration in muscle (white and red) and liver and blood glucose, quantitative estimation of glycogen utilization and lactate production in mullet, exercised for 5 hr at 20 and 77 cm/sec., were also investigated.

## Materials and Methods

The freshwater mullet, *Rhinomugil corsula*, collected from Vagai Reservoir near Madurai, were acclimated to $30 \pm 0.5°C$ in glass aquaria. Twenty-eight fish of total length $17.8 \pm 0.38$ cm (mean ± SE) and weight $40 \pm 1.95$ g (mean ± SE), were used for the present study. The fish were fed once daily with rice bran and groundnut oil cake in the ratio of 2:1. The acclimation of fish was continued for a minimum of 10 days prior to the commencement of the actual experiments. The tests were carried out in the acclimation temperature.

The apparatus used and the method of exercising the fish in the present study was as dsecribed by Sukumaran and Kutty (1977). After exercise, the fish were killed by stunning it with a hit on the head with a hammer. Blood sample was taken in a heparinized syringe by direct heart puncture and transferred to a vial, under liquid paraffin. The fish was immediately kept in between the ice cubes and the required tissues were dissected out. The superficial red muscle was carefully separated out under dissection microscope. The white muscle and liver samples were removed and kept in deep freeze immediately. A set of muscle, blood and liver samples from unexercised (control) fish was also taken for analysis.

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The glycogen of the samples of myotomal muscle and red muscle together that of liver was precipitated following the method of Seifter et al. (1949) and the glycogen and blood glucose were assayed by anthrone method of Carroll et al. (1956). The lactic acid content was analysed following the method of Barker and Summerson (1941), as outlined by Oser (1965).

## Results

It is observed from table 1 that during 1st hr of swimming there was a sudden drop of glycogen level in white muscle, red muscle and liver for the two swimming speed tested, whereas blood glucose level showed the opposite trend in both cases.The decrease of glycogen in white and red muscles and liver were more marked at the high speed than the low speed. It is also noted that while there was a decrease of glycogen in the later hours of swimming in white muscle and red muscle, there was a slight increase of glycogen level in liver during the 5th hr of exercise. The blood glucose level of fish at both swimming speeds showed the same trend i.e. an increase at the end of 1st hr and remained at the same level during later phase of exericse. However, a slight decrease was noted at the 5th hr of exercise at 77 cm/sec.

The lactate levels of white and red muscle and liver showed the same trend for both speeds (table 2). During the 1st hr of swimming the increase was steep in all the tissues, except in liver of fish exercised at 20 cm/sec where there was a lag in accumulation, but here also during 3rd hr of exercise, a conspicuous increase in the concentration of lactate was noticed. In both exercises during the 5th hr there was decrease in the concentration of lactate in liver. The pattern of muscle lactate was also similar in both the speeds. During 1st hr of swimming a high accumulation of lactate in white muscle was observed in both cases. During later hours of exericse the lactate concentration increased gradually to a maximum value at the 5th hr. The trend of lactate in red muscle was about the same as that of the white muscle, but during later hours of exercise the accumulation of lactate in red muscle was higher than in the white muscle. The trend of blood lactate was also the same in both speeds tested with an initial steep increase during short-term exercise and subsequently showing a gradual increase to a maximum value at the 5th hr. The concentration of lactate in liver was slightly higher than that in blood of unexercised mullet, while at the end of 1st hr the blood lactate value exceeded the liver lactate value in both the speeds tested. But during the 3rd hr of exercise the accumulation of lactate in liver was higher than in blood. However, there was a decrease in lactate concentration in liver during the 5th hr of swimming.

The glycogen utilization and lactate production by the whole fish was estimated considering the total weight of muscles, liver and blood volume and the values are given in figure 1. The glycogen utilization during 1st hr swimming at 20 and 77 cm/sec was 1359.7 and 2943.1 mg/kg fish/hr respectively, while during 5th hr it was 159.9 and 112.1 mg/kg fish/hr respectively. The lactate production also showed the same trend. Data obtained in respect of glycogen utilization for production of lactate and for maximum possible oxidation at the end of one hr and 5 hrs of swimming at 20 and 77 cm/sec are shown in table 3.

## Discussion

The concentration of muscle glycogen decreases with the intensity of exercise (Wittenberger & Diaciuc 1965, Dean & Goodnight 1964, Black et al. 1966, Stevans & Black 1966, Jhonston & Goldspink 1973). Just as muscle glycogen, liver glycogen also decreases with duration of exercise in *R. corsula*. Black et al. (1966) observed a slight decrease in liver glycogen level after short-term exercise, while Stevans and Black (1966) could find no change. It seems likely that under these conditions, the time available for degradation of glycogen and transport of glucose by the circulation is insufficient to affect the processes occurring in liver. It is supported by the fact that in *Salmo gairdneri*, there was a significant reduction in liver glycogen, when the fish was subjected to swim for 24 hrs continuously (Miller et al. 1959). It may also be pointed out that a marked reduction in liver glycogen occurred in cutthroat trout and blue gill sunfish, exposed to hypoxia (Health & Pritchard 1965). In *R. corsula* exercised continuously for 5 hrs the liver glycogen decrease (244, 299 mg/kg fish for 20 and 77 cm/sec respectively) was so drastic that it could not be accounted for the formation of blood glucose (37.5, 36.0 mg/kg fish for 20 and 77 cm/sec respectively) alone and it appears certain that it has moved into the muscle as substrate for energy as shown in elasmobranchs by Suyama et al. (1960).

The glycogen utilization was more during 1st hr and less during the 5th hr of swimming in both swimming speeds (figure 1). The lesser utilization during 5th hr of exercise at the highest speed (figure 1) may be due to the non-availability of adequate amount of glycogen as substrate, since the depletion of muscle glycogen is high. It is also likely that the energy derivation is predominantly from some other substrate, apparantly protein, which is known to be utilized more during later phase of exercise as evidenced by increased nitrogen excretion (Sukumaran 1976).

It has been well established that lactate accumulation in skeletal muscle increases with the intensity of exercise (Wittenberger & Diaciuc 1965, Burt & Stroud 1966, Black et al. 1966, Nakatani 1957, Stevens & Black 1966, Tomilson et al. 1961, Karuppannan 1972).

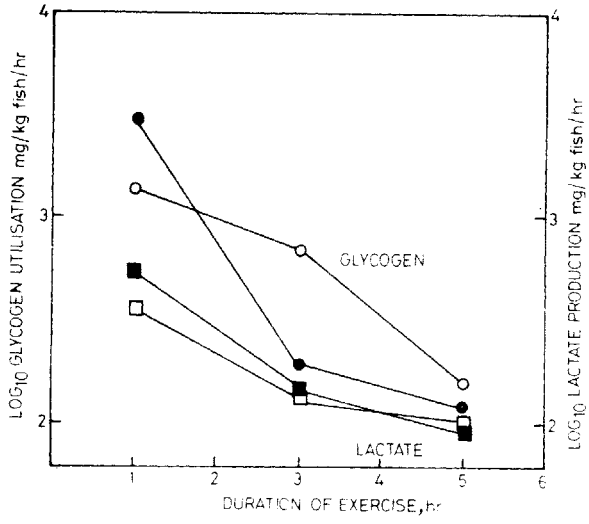


**Figure 1** Glycogen utilization and lactate production in relation to duration of exercise in *Rhinomugil corsula* forced to swim continuosuly up to 5 hr at 20 and 77 cm/sec. The open circles and squares represent glycogen utilization and lactate production at 20 cm/sec, while the closed circles and triangles represent glycogen utilization and lactate production at 77 cm/sec respectively

The total lactate production was more in the 1st hr corresponding to glycogen utilization (figure 1), suggesting the increased anaerobic metabolism during the initial phase of exercise. This is also evidenced by the increased R.Q. values of *R. corsula* under same conditions (Sukumaran & Kutty 1979).

It is also noted in *R. corsula* during the short-term exercise, the increase in concentration of lactate is greater in white muscle than in red muscle (table 2). But in long-term exercise the level of lactate in red muscle exceeds that in white muscle, apparently suggesting production of more lactate in red muscle than in white muscle. It is interesting to note that blood maintains a much lower concentration of lactate than that of muscle (table 2), indicating the existence of a possible barrier between muscle and blood for keeping blood lactate content lower apparently helping the maintenance of blood *p*H. Dando (1969) has postulated the possible existence of a barrier for uptake of lactate from blood under these conditions. Further observations are needed to prove the existence of a barrier between muscle and blood of *R. corsula*.

Another point that comes out of the present study is that about half of the total glycogen utilized during 1st hr of exercise remained unaccounted (table 3), while the unaccounted percentage of glycogen was low in the entire 5 hr period of swimming at 20 cm/sec and nil at 77 cm/sec. It is worth mentioning here that the entire oxygen consumed by the fish was assumed to be utilized only to oxidise glycogen. But it is certain that other substrates were also involved in the oxidative metabolism, since major part of energy, especially at later hours of exercise is derived from protein oridation (Sukumaran 1976, Sukumaran & Kutty 1979), The unaccounted glucose at all the levels might be utilized for the production of end products other than lactate (Hochachka et al. 1974). Kutty and Peer Mohamed (1975) suggested that in mullet, *R. corsula* anaerobic energy is derived under hypoxia through pathways other than that of conventional glycolysis. The unaccounted glycogen (glucose) in exercised mullet might have also been used for deriving energy efficiently through some anaerobic pathway other than the conventional glycolysis .

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**Table 1** *Glycogen levels in white muscle, red muscle and liver and blood glucose in relation to duration of exercise in mullet* Rhinomugil corsula. *exercised continuously at 20 and 77 cm/sec for five hr. Acclimation and test at 30°C*

| Swimming speed | Hours of Exercise | Glycogen in mg% (mean ± SE) | | | |
|---|---|---|---|---|---|
| | | White muscle | Red muscle | Liver | Blood |
| 20 | I | 407.5 ± 35.15 | 529.4 ± 23.50 | 1783.7 ± 186.00 | 159.0 ± 15.13 |
| 20 | III | 142.9 ± 6.20 | 340.5 ± 16.30 | 815.2 ± 37.30 | 127.7 ± 12.80 |
| 20 | V | 89.7 ± 4.30 | 177.7 ± 8.21 | 857.0 ± 40.95 | 130.5 ± 23.55 |
| 77 | I | 120.1 ± 11.16 | 134.7 ± 5.78 | 687.6 ± 50.38 | 163.35 ± 4.39 |
| 77 | III | 52.3 ± 8.44 | 70.6 ± 4.97 | 289.1 ± 46.35 | 134.70 ± 3.57 |
| 77 | V | 9.5 ± 0.94 | 54.9 ± 2.84 | 307.5 ± 38.43 | 120.00 ± 5.03 |
| | | * 640.6 ± 103.88 | * 684.6 ± 65.75 | * 5507.2 ± 401.99 | * 85.5 ± 3.81 |

* — values for unexercised fish

**Table 2** *Concentration of lactatic acid in white muscle, red muscle, liver and blood in relation to duration of exercise in mullet,* R. corsula. *exercised continuously at 20 and 77 cm/sec for five hrs. Acclimation and test at 30°C*

| Swimming speed cm/sec | Hours of Exercise | Lactic acid in mg% (mean ± SE) | | | |
|---|---|---|---|---|---|
| | | White muscle | Red muscle | Liver | Blood |
| 20 | I | 152.0 ± 3.64 | 132.9 ± 5.70 | 38.9 ± 7.41 | 80.2 ± 6.80 |
| 20 | III | 200.2 ± 7.35 | 213.2 ± 3.36 | 116.9 ± 4.50 | 84.0. ± 4.77 |
| 20 | V | 243.2 ± 41.30 | 272.3 ± 9.99 | 73.1 ± 8.20 | 89.5 ± 18.80 |
| 77 | I | 193.8 ± 2.83 | 147.9 ± 17.10 | 61.1 ± 7.90 | 70.4 ± 7.98 |
| | III | 248.6 ± 13.30 | 210.9 ± 24.30 | 139.7 ± 20.75 | 77.3 ± 4.93 |
| | V | 283.1 ± 13.26 | 333.8 ± 22.30 | 85.2 ± 3.04 | 96.7 ± 5.17 |
| | | * 84.1 ± 3.98 | * 83.1 ± 6.61 | * 45.2 ± 3.10 | * 28.3 ± 1.07 |

* — values for unexercised fish.

**Table 3** *Glycogen (glucose) utilization in millimoles/kg fish of* Rhinomugil corsula *exercised continuously for 5 hrs at 20 and 77 cm/sec. Percentage of total utilized are indicated in brackets.*

| Swimming speed cm/sec. | Duration of exercise | Glycogen (glucose) utilized for lactate production | Glycogen (glucose) utilized for maximum possible oxidation | Unaccounted glycogen (glucose) | Total glycogen (glucose) utilized by the fish |
|---|---|---|---|---|---|
| 20 | 1 Hour | 2.06 (30.3%) | 1.50 (22.1%) | 3.22 (47.5%) | 6.78 (99.9%) |
| 20 | 5 Hours | 4.82 (31.0%) | 6.46 (41.5%) | 4.28 (27.5%) | 15.56 (100%) |
| 77 | 1 Hour | 3.18 (21.6%) | 3.10 (21.1%) | 8.43 (57.3%) | 14.71 (100%) |
| 77 | 5 Hours | 6.01 (33.7%) | 11.78 (66.2%) | Nil (0%) | 17.79 (99.9%) |

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