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QUANTITATIVE STUDIES OF MUSCLE GLYCOGEN UTILIZATION DURING SUSTAINED SWIMMING IN CRUCIAN CARP (*CARASSIUS CARASSIUS* L.)

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INTRODUCTION

The swimming musculature of teleosts consists of three main types of muscle fibre which are often arranged into anatomically discrete regions. Superficial red muscle forms a thin sheet of aerobic tissue running adjacent to the lateral line along the whole length of the trunk. The remainder of the myotomal bulk consists of two types of fibre with high myofibrillar ATPase activity (Johnston, Frearson & Goldspink, 1972; Nag, 1972; Johnston *et al.* 1973). The superficial red muscle in contrast has been shown to have a much lower myofibrillar ATPase (Johnston, Frearson & Goldspink, 1972). Other differences in the electrical, contractile, ultrastructural, and mechanical properties of these fibres have been documented (Hidaka & Toida, 1969; Patterson & Goldspink, 1972; Stanfield, 1972). The proportions of these different types of fibre is dependent on species and appears to be related to the locomotory behaviour of the fish (Boddeke, Slijper & van der Stelt, 1959). The metabolic adaptations shown by these fibres have been the subject of numerous studies (George & Bokdawala, 1964; Bone, 1966; Boström & Johansson, 1972). In general, the superficial red fibres have a predominantly oxidative metabolism, being rich in myoglobin, vascular supply, mitochondria and the enzymes of oxidative phosphorylation and the respiratory chain (Boström & Johansson, 1972; Hamoir, Focant & Distèche, 1972; Patterson, Johnston & Goldspink, 1973). Of the remaining two fibres with high myofibrillar ATPase by far the greater proportion consist of large-diameter fibres which have a poor capillary network, few mitochondria and high concentrations of glycolytic enzymes and are thought to function primarily anaerobically (Boddeke *et al.* 1959; Bone, 1966; Hamoir *et al.* 1972; Patterson & Goldspink, 1972). The remaining type of fibre, which is intermediate in position between the so-called red and white muscles has an intermediate type of enzyme distribution (Patterson *et al.* 1973). Not only are there differences in physiological and metabolic adaptation between these fibre types, but they are thought to have somewhat different functions during swimming. Recently there have been several studies on glycogen metabolism in the different muscles of swimming fish (Pritchard, Hunter & Lasker, 1971; Johnston & Goldspink, 1973*a*, *b*). These, however, deal with changes in glycogen concentration in the muscles in fish forced to swim for sustained periods at a

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particular speed. Little can be inferred as to the quantitative utilization of glycogen at different levels of swimming, since under these conditions the swimming muscles are in equilibrium with other sources of carbohydrate reserve, such as the liver. In addition the pattern of glycogen distribution and utilization among the various parts of the myotome is unknown. In the present study a technique is described which allows quantitative studies to be made on glycogen utilization in these muscles for short periods of swimming.

MATERIALS AND METHODS

Crucian carp (*Carassius carassius*) of similar length (11–14 cm) and of both sexes were maintained in tanks of circulating tap water regulated to $7{\cdot}0 \pm 0{\cdot}8$ °C for one week before experimentation. They were fed on a diet of commercial pellet fish food given daily.

Exercise apparatus. Exercise was carried out as described previously (Johnston & Goldspink, 1973*a*) in a flume of similar design to that of Walker (1971). The swimming chamber consisted of a trough 2·42 m long and 25 × 25 cm in cross-section, through which water could be moved at speeds ranging from 15–100 cm/sec. Variation in water speed was obtained by means of valves on the two electrical pumps (0·75 and 2·0 hp) circulating the water and by altering the height of the weir at the end of the trough. Turbulence was reduced to a low level by a series of silk screens placed in the stilling tank and by a streamlined trumpet fitted between the stilling tank and the exercise chamber. The stilling and reservoir tanks together with the trough were constructed of $\frac{3}{4}''$ marine plywood and all surfaces treated with Araldite. The apparatus was contained in an aquarium fitted with a thermostated air-cooling unit; further temperature control was achieved by a refrigeration coil placed in the reservoir tank. The rate of water flow was calculated by introducing dye particles into the stilling tank of the flume and measuring the time taken for the particles to travel through a known length of the trough.

Swimming tests. Fish were introduced into the trough in pairs, and the water speed was increased so that it was just sufficient to discipline their movements. This is equivalent to a swimming speed of just under 1 L/S. Following an introductory period of 30 min, necessary to acclimatize the fish to swimming in the flume, the water velocity was then steadily increased over 1·5 min until it reached the test velocity. At this point, provided both fish had settled down to steady sustained swimming, one fish was removed and plunged into cooled (−160 °C) liquid Freon (Arcton 12 ICI) and the other was allowed to swim on for a further 3 min before being similarly treated. A total of 20 fish were used for each of the swimming speeds investigated. The lengths and weights of all the individuals were measured and recorded.

Biochemical analysis. Muscle and liver samples were obtained from the fish while still frozen after allowing the fish to increase in temperature to −20 °C by placing it in a deep-freeze one hour before dissection. After removal of the skin and external myocommata the superficial red muscle was dissected from each side of the fish. The whole of the muscle was taken in each case and immediately re-frozen in liquid nitrogen (−170 °C) and stored separately. The fish was then re-frozen in liquid

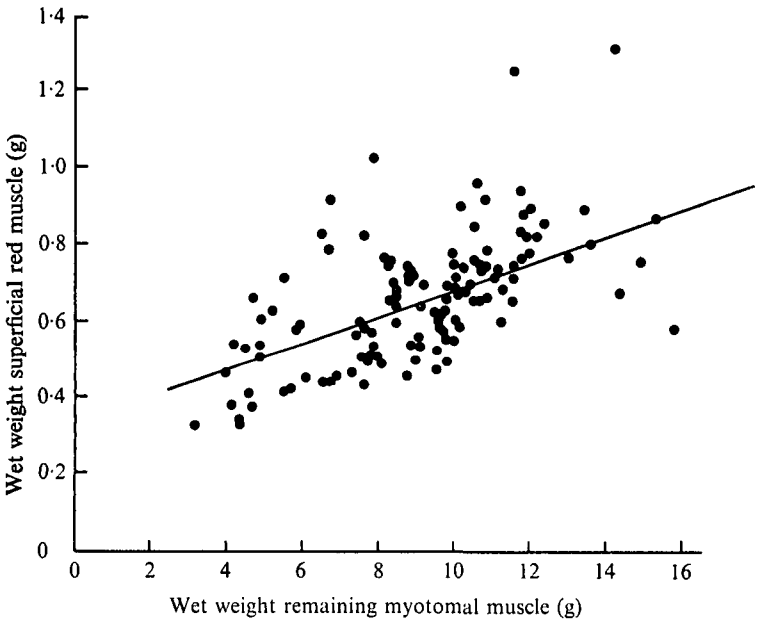

Fig. 1. A graph of the wet weight of superficial red musculature of the crucian carp (g) plotted against the wet weight of the remaining musculature (g).

nitrogen (−170 °C) and placed in a refrigerated cabinet and allowed to reach −20 °C. The whole of the remaining myotomal musculature was then carefully dissected from the carcass from each side of the fish. The whole of the liver was then dissected free of the intestine and gonads. Both these samples were immediately re-frozen in liquid nitrogen (−170 °C) and stored in Polythene tubes in an insulated refrigerated container (−45 °C) until analysis. The glycogen content of the samples of myotomal muscle from the left-hand side of the fish together with that of the liver were determined following digestion of the whole of the weighed sample in 30% KOH at 100 °C for 15 min. Glycogen was precipitated with ethanol from an aliquot of the digest according to the method of Seifter *et al.* (1949) and the precipitated glycogen was assayed by the anthrone method of Carroll, Longley & Roe (1956).

Statistical analyses. The significance between groups of data was calculated using a Student's t test. In cases of unequal variance between sets the Brehrens–Fisher test was utilized (Bliss, 1967). The regression between the wet weights of superficial red muscle and the remaining myotomal musculature was computed using a method for the best-fit relationship between two variables both subject to error (Davies, 1971).

RESULTS

The relationship between the wet weight of superficial red muscle and the remaining myotomal muscle is shown in Fig. 1. A straight line relationship of the form $y = 0{\cdot}036x + 0{\cdot}339$ ($< 0{\cdot}01$) was obtained for carp of length 11–14 cm. The mean weight of superficial red muscle amounted to 7·4% of the total musculature.

The differences between the glycogen concentrations in these muscles for control and test groups of fish swimming for 3 min are shown in Table 1. Only at the lowest

Table 1. *Glycogen utilization by the swimming muscles of Crucian carp* (Carassius carassius *L.*)

Swimming speed L/S	Mean length (cm) ± S.E.	Mean weight (g) ± S.E.	Glycogen utilized/g muscle/sec: Red muscle	Glycogen utilized/g muscle/sec: Remaining myotomal muscle	Glycogen utilized/swimming musculature/sec† : Red muscle	Glycogen utilized/swimming musculature/sec† : Remaining myotomal muscle
2·0	12·6 ± 0·2	43·9 ± 2·2	NS	NS	NS	NS
2·2	12·8 ± 0·2	43·7 ± 1·9	0·557*	NS	0·370	NS
2·8	12·1 ± 0·3	39·1 ± 2·5	0·547*	0·250*	0·371	2·263
3·2	11·9 ± 0·2	36·3 ± 4·6	0·993*	NS	0·622	NS
3·6	12·1 ± 0·2	36·3 ± 1·9	1·170*	0·526*	0·779	4·769
4·2	12·6 ± 0·2	44·13 ± 2·4	2·650***	0·869***	1·765	7·878

† Calculated from mean muscle weights of 120 fish. * = $P < 0.05$. *** = $P < 0.001$. NS = Not significant at $P < 0.05$ level.

swimming speed of 2 L/S was there no significant difference between the glycogen content of the superficial red muscle of test and control fish. At all higher speeds a statistically significant drop in glycogen concentration occurred in this muscle after three minutes of swimming. The $\log_{10}$ of the glycogen utilization (mg glycogen utilized/g muscle/sec) in the superficial red muscle was found to be linearly related to swimming speed ($P < 0.05$) and of the form $y = 0.349x + 0.655$. Glycogen utilization from the remaining trunk musculature only occurred at the higher speeds studied (Table 1). The glycogen utilization by the red muscle/g of tissue was some 2–3 times higher at these swimming speeds. However, when the total glycogen utilization from the combined swimming musculature is calculated, the red muscle only accounts for 15–20% of that metabolized. Even at the highest swimming speed studied no statistically significant differences could be detected in the concentration of liver glycogen between control fish and those that had swum for a further 3 min. The concentrations of glycogen in the liver of control and test groups ($N = 20$) were 12,528 ± 901 and 12,996 ± 1173 (mg glycogen/100 g tissue) respectively.

DISCUSSION

There are three main types of muscle fibres occurring in the fish myotome. The most studied have been the superficial red and deep white muscles, since relatively homogeneous samples can be easily dissected and made available for biochemical analyses. The superficial red muscle is known to be a highly aerobic tissue possessing a well developed capillary network, numerous mitochondria, high concentrations of myoglobin and cytochromes, and the enzymes associated with oxidative metabolism (Braekkan, 1956; Boddeke *et al.* 1959; Boström & Johansson, 1972; Patterson & Goldspink, 1972). The red muscle contains more lipids than white muscle (George & Bokdawala, 1964; Pritchard *et al.* 1971) and has been shown to have a much greater ability to oxidize fatty acids (Jonas & Bilinski, 1964; Bilinski, 1963). The metabolism of white muscle in contrast is thought to be primarily anaerobic since the vascular supply to this tissue is poorly developed (Boddeke *et al.* 1959). Several studies have shown that the levels of glycolytic enzymes are higher in the white than the red muscle (Hamoir *et al.* 1972; Boström & Johansson, 1972). It thus seems likely that

red muscle in fish functions mainly aerobically, oxidizing fats and carbohydrates, whereas the white muscle has a mainly anaerobic type of metabolism, utilizing carbohydrates. A recent histochemical study on the fibres intermediate in position between the red and white muscles has shown them to have high contents of enzymes of both oxidative and glycolytic types (Patterson *et al.* 1973). The relative amounts and distribution of this fibre type would appear to vary considerably between different species.

The relative contribution of the different types of swimming musculature is dependent on swimming speed. At very low speeds fish can propel themselves mainly by use of the pectoral fins (Smit, 1965). The adductor muscles of the pectoral fins have been shown to consist of slow red fibres (Nishihara, 1967). On increasing its linear speed the fish starts to make use of myotomal contractions; in the case of goldfish (*Carassius auratus*) this occurs at a speed of about 0·6 bodylengths/s (Smit, 1965). At these low speeds there is some evidence for the use of the red muscle alone in some species (Bone, 1966; Rayner & Keenan, 1967; Johnston & Goldspink, 1973*a*, *b*). The involvement of both muscle types over a whole range of sustained swimming speeds has now been firmly established (Smit *et al.* 1971; Pritchard *et al.* 1971; Johnston & Goldspink, 1973*a*, *b*; Walker & Pull, 1973). The relative contribution of each muscle type and the parts played by lipid and carbohydrate metabolism in meeting the energy requirements of the muscle are not known for any swimming speed. The processes involved in the utilization and mobilization of lipids and carbohydrates are precisely regulated by a variety of mechanisms which allow the fish to adapt to different types of work (Drummond, 1971). The relative importance of any one type of metabolism is probably dependent on the intensity and duration of the exercise. It is known, for example, that body fat is an important energy source in fish such as eels and salmon that undergo long and arduous spawning migrations (Fontaine & Hatey, 1953; Idler & Clemens, 1959; Mayerele & Butler, 1971). Extensive work on changes in carbohydrate intermediates in the white muscle of salmonids during bursts of strenuous swimming have implicated glycogen as the major source of energy for this type of locomotory activity (Black *et al.* 1960; Black, Robertson & Parker, 1961; Black *et al.* 1962; Black, Bosomworth & Docherty, 1966).

There have been several studies on glycogen metabolism in red and white myotomal muscles at various swimming speeds (Pritchard *et al.* 1971; Johnston & Goldspink, 1973*a*, *b*). These investigations, however, only deal with qualitative changes occurring in muscle glycogen reserves since the muscle was in equilibrium with other sources of carbohydrate reserve during sustained swimming. The transport of glucose by the bloodstream to these muscles is likely to be different as it has been shown that replenishment of glycogen following muscular effort takes place more readily in the red muscle (Johnston & Goldspink, 1973*b*). It is not possible therefore to make any deductions on the relative utilization of carbohydrates by these muscles from that type of experiment.

Preliminary experiments showed that even at the highest speed studied in the present investigation no significant change occurred in the liver glycogen content of fish swimming for 3 min. Black *et al.* (1962) have similarly shown that liver glycogen remained unchanged in the rainbow trout (*Salmo gairdneri*) during short periods of strenuous swimming. It seems likely that under these conditions the time available

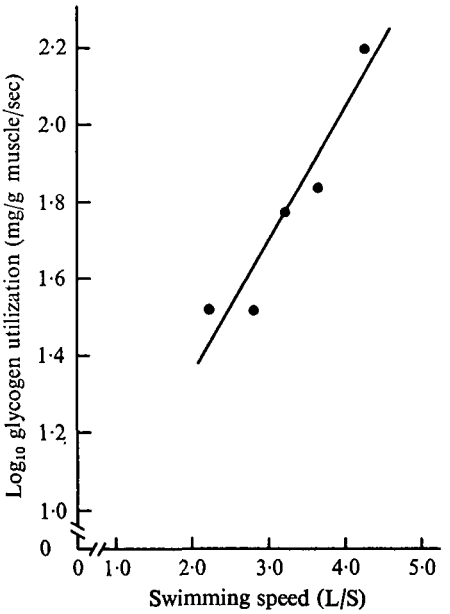

Fig. 2. Glycogen utilization (mg/g muscle/sec) by the superficial red muscle of crucian carp (*Carassius carassius* L.) at different swimming speeds.

for degradation of glycogen and transport of glucose by the circulation is insufficient to affect the processes occurring in the muscles. Therefore if a strenuous exercise load is suddenly imposed on the fish for a short period changes in glycogen content in the muscles will approximately represent the mean glycogen utilization of the muscles. In the present experiments, following an introductory period at a very low speed, the water was suddenly accelerated until it attained the desired speed. At this point some of the fish were removed as controls and plunged immediately into liquid nitrogen (-170 °C). This serves to arrest the metabolism and reduces the chance of recovery from exercise which might occur if the fish were killed in some other way. Other fish were allowed to swim for a further 3 minutes before being similarly treated. Since the pattern of glycogen utilization in accelerating fishes may well be different from normal cruising, only fish that swam steadily for the whole of the experiment were used for analysis. Fish that fell back on to the restraining barrier and darted or struggled to regain their swimming position were discarded. In order to avoid the difficulties arising from unknown differences in the utilization and distribution of glycogen by different regions of the swimming musculature and liver, the whole of these tissues were taken for analysis.

The concentrations of glycogen in the muscle fibres of non-exercising fish is higher in the superficial red fibres than in the deep white fibres (Bone, 1966; Pritchard *et al.* 1971; Johnston & Goldspink, 1973*a*, *b*, *c*). In the Crucian carp a ratio of 2·5 times has been reported (Johnston & Goldspink, 1973*b*). The results obtained in the present study show that over the range of speeds studied the $\log_{10}$ of the glycogen utilization by red muscle (mg glycogen/g muscle/ sec) was linearly related to swimming speed (Table 1, Fig. 2). Significant changes in the glycogen content of the remaining

myotomal muscle which consists principally of white fibres, did not in contrast take place until speeds of about 3 bodylengths/s. This result is in close agreement with a study by Smit *et al.* (1971) on *Carassius auratus*, a closely related species. Smit *et al.* (1971) concluded on the basis of measurements of the oxygen consumption and efficiency of swimming that when the fish exceeds a speed of about 3·4 bodylengths/s it probably generates some of its energy requirements anaerobically. In the present study at speeds in excess of around 3 bodylengths/s the superficial red muscle utilized glycogen (mg glycogen/g muscle) at about 2–3 times the rate of white muscles (Table 1). However, since the superficial red muscle only constituted 7·4% of the total muscle by weight, its contribution to the total glycogen utilized by the swimming musculature was only about 15–20%.

It is not possible to decide how much each muscle contributed to the total power output from these results since the part played by lipid metabolism, changes in the level of glycolytic intermediates and the immediate energy supply to the muscle is unknown. Also, even though the proportion to which other sources of lipid and carbohydrate contribute to the muscle's metabolism has been minimized under these conditions, it will still be continuing at a low level and presumably differently in the two types of muscle. The extent to which carbohydrate metabolism contributes to the total energy requirements of the swimming muscles may also vary according to the duration of the swimming, and fish swimming for longer periods might be able to utilize alternative energy reserves.

The values obtained for glycogen utilization in white muscle of 0·53 mg glycogen/g muscle/min at 3·6 bodylengths/s and 0·87 mg glycogen/g muscle/min at 4·2 bodylengths/s are of similar magnitude to the changes observed in this metabolite under comparable conditions in several other studies. Black *et al.* (1962) obtained changes in white muscle glycogen concentration equivalent to 0·7 mg glycogen/g/min for rainbow trout (*Salmo gairdneri*) swimming 'strenuously' for 2 min. Pritchard *et al.* (1971) observed a mean change of 1·29 mg glycogen/g muscle in Jack mackerel (*Trachurus symmetricus*) swam at 9 bodylengths/s until exhaustion.

These experiments suggest a possible experimental approach to estimating work rates at speeds above the fish's maximum oxygen balance. A summation of the free energy changes associated with differences in the concentration of lipids, tricarboxylic acid and glycolytic intermediates, phosphoryl creatine and adenosine triphosphate together with measurements of oxygen consumption would allow a physiological estimate of the energetic cost of swimming under these conditions.

SUMMARY

1. The relationship between the wet weight of superficial red muscle and the wet weight of the remaining trunk musculature was found to have the form $y = 0{\cdot}036x + 0{\cdot}339$. The average weight of superficial red muscle accounted for 7·4% of the myotomal mass.

2. A technique is described which allows quantitative studies to be made on glycogen utilization in these muscles for short periods of swimming. A total of 120 Crucian carp (*Carassius carassius* L.) were studied at six different swimming speeds ranging from 2·0–4·2 bodylengths/s.

3. The $\log_{10}$ of the total glycogen utilization of the superficial red muscle was found to be linearly related to swimming speed.

4. Statistically significant glycogen utilization from the remaining myotomal musculature only occurred at speeds in excess of around 3 bodylengths/s. At these speeds the superficial red muscle only accounted for 15–20% of the total glycogen mobilized by the swimming musculature.

5. The significance of these results is discussed in relation to the division of labour between the myotomal muscles of fish.

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