Characteristics of mosaic muscle growth in rainbow trout *Salmo gairdneri*

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Summary. There are 4 stages in the growth of mosaic (axial) muscle, which is the predominating tissue in trout; the stages are distinguishable by the modal values and range of their fibre diameters over the size range 2.3 to 50+cm fork length. Up to 50 cm, most of the increase in mosaic muscle is attributable to continuous recruitment of new fibers. Beyond 50 cm, further growth is a result of increase in diameter of existing fibers.

Approximately 30-75% of the live weight of fish consists of muscle composed of fibers which are specialized, post-mitotic structures of several types, histologically, functionally and biochemically distinct[1-3]. Surprisingly, few studies have considered somatic growth of fish in terms of the 'population dynamics' of the numerous fibers that comprise their muscles[4-10], though this would seem a necessary approach in attempts to understand the properties of growing muscle tissue. In rainbow trout, 'mosaic' muscle predominates in the axial musculature, accounting for 95% of the muscle mass of the body[11]. In mosaic muscle white (glycolytic) fibers considerably outnumber 'red' fibers which are of smaller diameter and are biochemically distinct from the narrow lateral bands of red muscle which are superficially obvious on removal of the skin[2]. Our studies[8-10] tend to confirm the views of others[3,12] that the 'red' fibers of mosaic muscle are the younger growth stages of a single fiber type, recognized as 'white' fibers as they grow older and larger. Certainly, all fibers evidently originate from the myosatellite cell system or its progenitors[6,7,13] a system that also seems able to exert some controlling influence on fiber growth in mammalian muscle[14].

In our studies, fingerling (approximate mean fork length 7.5 cm)[8] and yearling (mean length 15.5 cm)[9] rainbow trout were grown at different rates as a result of different laboratory regimes of ration size and temperature; some groups were also stimulated to maximum growth by bovine growth hormone administration[8]. We sampled epaxial muscle at the 20th myomere from the last caudal, and also obtained data on fish dry weights (% of wet wt); details of experimental design, techniques, including the method of calculating muscle fiber diameters, are given elsewhere[8,9]. We also studied hatchery-reared rainbow trout obtained over the range 2.3-61.3 cm fork length[10]. The table assembles the more important findings from these various data sources.

Among trout of 2.3-19.9 cm fork length, persistent presence of mosaic fiber diameter modes in the 0-19.9 µm or 20-39.9 µm class indicates the major factor in muscle increase in fish of this size range is recruitment of new fibers[8,10]. A modal increase in fiber diameter occurs as trout exceed 20 cm length. After this, constancy of fiber diameter frequency classes from 25 to 50 cm (table) indicates that fiber recruitment is nearly as important in the growth of muscle in fish of this size range as in trout of less than 20 cm. Marked reduction in frequency of small fibers in the larger fish length range does, however, indicate either that newly recruited fibers remain small in diameter for a shorter time in these trout than in smaller ones, and are therefore less readily detectable[10], or that there is a different method of origin - e.g. fiber splitting[15], which would be very difficult to detect; certainly we have not observed such splitting.

Fiber input dynamics appears somewhat more labile in trout of 20-30 cm length[9] than in smaller trout[8]. In very fast-growing trout in this larger size range the fiber diameter: fish length ratio was less than for slower-growing trout[9]. We can add no information to what is already available concerning the origin of new fibers[6,7,13]. However, during growth from 5 to 20 cm the frequency of fiber diameters including the modal value, remains essentially constant, regardless of major differences in somatic growth induced experimentally[8]. This fact implies that fiber input dynamics are highly responsive to the demands of somatic growth, yet under precise control[8]. The effect of very rapid growth on fiber diameter in trout of 20-30 cm length does, however, imply that this control may be more relaxed in trout of this size range[9].

Apart from the constancy of fiber modal diameter among trout of less than 20 cm - i.e. its independence of somatic growth rate, it also appears independent of the differences in body dry weight and condition ($K=(W\times 10^2)/L^3$, where

Growth dynamics of mosaic muscle fibers in rainbow trout during different phases of somatic growth

Approximate fish length range	Facts concerning mosaic fibers	Postulated mechanisms of increase in muscle	Remarks
2-5 cm	All fibers <40 µm diameter; mode at 20-39.9 µm[10].	Recruitment of new fibers (from myosatellite cell system?) responsible for most of increase in muscle[10].	
5-20 cm	Fiber diamether mode still at 20-39.9 µm; range extends to 119.9 µm; some reduction in 0-19.9 µm class[8-10].	Recruitment of new fibers (from myosatellite cell system?) responsible for most of increase in muscle; assist from diameter range extension at 5 cm[8-10].	Little change in % frequency of diameters in response to great differences in somatic growth rate, condition, % dry weight[8].
20-50 cm	Fiber mode shifts to 40-79.9 µm at 20 cm; little further change in % frequency of fiber diameters; range extends to 159.9 µm[9,10].	Recruitment of fibers presumably from myosatellites[9,10].	Recruitment dynamics still largely responsible for increase in muscle[9,10], though apparent effect of very fast growth rate in increasing recruitment in 20-30 cm range[9].
50+ cm	Fiber mode to >120 µm with increasing fish length; range extends to >200 µm. All small fibers (<40 µm) disappear[10].	Recruitment (from myosatellites?) presumed ended. Further growth from increasing diameter of existing fibers[10].	Increase in fiber diameter (to >200 µm?)[10].

K = condition, W = weight, L = length)[16] which accompany the different growth rates resulting from differences in ration sizes, temperatures, or growth hormone administration[8]. Rainbow trout in the 20–30 cm length range did, however, have mosaic fibers of smaller diameter in winter than in summer[10]. This is an especially significant observation since it was made not on wild populations, but on fish from 2 hatcheries in which food availability and temperature were essentially constant all year, and consequently in which condition (K) did not change significantly with season as it would have done under natural conditions[10]. The possible adaptive significance of this phenomenon in reducing winter protein requirements has already been considered[10].

From our studies[8,9] and others[5,7], it seems that high growth rates and possible attainment of large final size may be inversely related to mean fiber diameter. This would imply that some, at least, of the observed interspecific differences in growth rate among fish are consequences of inherent differences in ability of muscle to continue to recruit new fibers.

The suggestion that the length range of 20–30 cm is of special significance for the characteristics of muscle growth in rainbow trout could be further investigated for fish in this size range by: a) administering large doses of bovine growth hormone and insulin, both of which stimulate growth in fish[8,17], to trout receiving ad libitum rations; b) feeding a 'superdiet' of rations very high in proteins, minerals and vitamins; c) a combination of (a) and (b).

Finally, we note that the rainbow trout appears to be an excellent subject for experimental studies of vertebrate growth. The somatic growth of the species is highly labile – i.e. responsive to differences in food, temperatures, etc., while the predominant mosaic muscle mass is relatively simple in structure and its characteristic growth dynamics are now broadly accounted for.

1 Q. Bone, J. mar. biol. Ass. U. K. *46,* 321 (1966); R.M. Alexander, J. mar. biol. Ass. U. K. *49,* 263 (1969); R.E. Waterman, Am. J. Anat. *125,* 457 (1969); J.J. Willemse, Z. Morph. Ökol. Tiere *81,* 195 (1975); S. Patterson, I.A. Johnston and G. Goldspink, J. Fish Biol. *7,* 159 (1975); M. Greer-Walker and G.A. Pull, J. Fish Biol. *7,* 295 (1975); W.C. Hulbert and T.W. Moon, J. Fish Biol. *13,* 527 (1978); H. Korneliussen, H.A. Dahl and J.E. Paulsen, Histochemistry *55,* 1 (1978); J.J. Willemse and A. DeRuiter, Aquaculture *17,* 105 (1979).
2 I.A. Johnston, P.S. Ward and G. Goldspink, J. Fish Biol. *7,* 451 (1975).
3 Q. Bone, in: Fish Physiology, vol. 7, p. 361. Ed. W.S. Hoar and D.J. Randall. Academic Press, New York 1978.
4 R.M. Love, The Chemical Biology of Fishes. Academic Press, London 1970; J.J. Willemse, Aquaculture *8,* 251 (1976).
5 M. Greer-Walker, J. Cons. perm. int. Explor. Mer *33,* 228 (1970); H. Kryvi and A. Eide, Anat. Embryol. *151,* 17 (1977).
6 A.C. Nag and J.R. Nursall, Cytobios *6,* 227 (1972).
7 J.J. Willemse and P.G. Van Den Berg, J. Anat. *125,* 447 (1978).
8 A.H. Weatherley, H.S. Gill and S.C. Rogers, Can. J. Zool. *58,* 1535 (1980).
9 A.H. Weatherley, H.S. Gill and S.C. Rogers, Can. J. Zool. *57,* 2385 (1979).
10 A.H. Weatherley, H.S. Gill and S.C. Rogers, J. Fish Biol. *17,* 603 (1980).
11 A.C. Nag, J. Cell Biol. *55,* 42 (1972).
12 M. Greer-Walker and L. Emerson, J. Fish Biol. *13,* 475 (1978).
13 H. Kryvi, Anat. Embryol. *147,* 35 (1975).
14 G. Goldspink, in: The Structure and Function of Muscle, vol. 1, p. 179. Ed. G.H. Bourne. Academic Press, London 1972.
15 C.W. Greene, Bull. Bur. Fish., Wash. *33,* 73 (1913).
16 A.H. Weatherley, Growth and Ecology of Fish Populations. Academic Press, London 1972.
17 E.M. Donaldson, U.H.M. Fagerlund, D.A. Higgs and J.R. McBride, in: Fish Physiology, vol. 8, p. 455. Ed. W.S. Hoar, D.J. Randall and J.R. Brett. Academic Press, New York 1979.

Degradation of type I collagen fibrils synthesized by human dental pulp cells in explant culture exposed to *Actinomyces viscosus.* Electron microscope immunotyping[1]

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Summary. Actinomyces viscosus Be 66, added to pulpal cells in culture, does not cause apparent cellular damage. The extracellular matrix consists of altered collagen fibrils and thin filaments, immunochemically identified as type I collagen. They probably represent the first steps of collagen degradation.

In carious dentine, the demineralization process is associated with degradation of the organic matrix, probably caused by the microbial enzymes present in the lesion[2,3]. This proteolysis has also been described in the pulpal tissue (free of bacteria) beneath advanced[4] or early dentine lesion[5]. Thus, the disruption of pulpal collagen fibrils might be correlated with the microbial enzymes found in carious dentine. Indeed, many strains of oral bacteria have been shown to cause disintegration of collagen fibrils or films in vitro[6,7]. However, little attention has been given to the proteolytic activity of *Actinomyces viscosus,* a gram positive microorganism isolated from deep human carious dentine[8] or root surface caries[9]. In the present paper, the collagenolytic activity of *Actinomyces viscosus* on the matrix synthesized by human dental pulp cells in explant cultures is described.

Material and methods. Explant cultures, obtained from children's permanent tooth germs removed for orthodontic reasons, were grown in Leighton tubes and suspended in Eagle basal medium supplemented with 10% calf serum, penicillin, streptomycin and ascorbic acid as described previously[10]. The cultures, incubated 3 weeks at 37 °C were suspended in Eagle medium without antibiotics 2 days before the inoculation with bacteria. Other cultures, without bacteria, served as controls. The strain *Actinomyces viscosus* Be 66, kindly supplied by Prof. Edwardsson