

cells, small mucous cells and inner polygonal support cells supports this view. This activity is also seen in the basement membrane of *Mystus vittatus* which supports the view that it may be associated with the fibrous proteins¹⁵ and the passage of metabolites across the cell membranes^{16,17}. Insignificant or negative reaction to alkaline phosphatase activity in large mucous cells and dermis account for their poor metabolic activities in the present study.

Acid phosphatase activity has been regarded as having a lysosomal distribution with an important role in hydrolytic and catabolic wasting processes¹⁸⁻²⁰ and in the cytolysis of keratinized cells³. In whiting the acid phosphatase activity is restricted to a narrow zone at the extreme exterior of the epidermis³.

Since keratinization is not found in the present study and the acid phosphatase activity is confined to the outermost squamous support cells and basal columnar cells, it may have some relation to the hydrolytic and catabolic wasting process.

- 1 Acknowledgment. We are thankful to P. Vishwanatham, Government College, Mhow, and Dr R.S. Shrivastava, Holkar Science College, Indore, for providing laboratory facilities and to the Council of Scientific and Industrial Research, New Delhi, for a fellowship for M.S.
- 2 G.H.O. Burgess, Nature 178, 93 (1965).

- 3 A.K. Mittal and T.K. Banerjee, Microscopie 30, 337 (1974).
- 4 A.K. Mittal and T.K. Banerjee, Can. J. Zool. 53, 833 (1975).
- 5 A.M. Bullock, R.J. Roberts and J.M.D. Gorden, J. mar. biol. Ass. U.K. 56, 213 (1976).
- 6 M. Saxena and S.K. Kulshrestha, Annl Zool. 15, 141 (1979).
- 7 G. Gomori, Microscopic Histochemistry: principles and practice. University of Chicago Press. Chicago 1952.
- 8 A.G.E. Pearse, Histochemistry, theoretical and applied. Churchill Livingstone, Edinburgh, London and New York 1968.
- 9 G. Bevelander and P.L. Johnson, J. Cell comp. Physiol. 26, 25 (1945).
- 10 N.B.B. Symons, J. Anat. 89, 238 (1955).
- 11 M. Mori, W. Yoshika, T. Mizushima and N. Amatusu, Archs Histol. Jap. 20, 513 (1960).
- 12 J.F. Danielli, H.B. Fill and E. Kodicek, J. exp. Path. 26, 367 (1945).
- 13 J. Yamada, Bull. Fac. Fish. Hokkaido Univ. 7, 185 (1956).
- 14 E. Kiguel, Anat. Rev. 151, 267 (1965).
- 15 M.S. Burstone, Enzyme Histochemistry and its Applications in the Study of Neoplasm. Academic Press. New York 1962.
- 16 R. Moong, Biol. Rev. 21, 41 (1946).
- 17 J.G.R. Bradfield, Biol. Rev. 25, 113 (1950).
- 18 H. Zalkin, A.L. Tappel, K.A. Caldwell, S. Shibko, I.D. Desai and T.A. Holliday, J. biol. Chem. 237, 2678 (1962).
- 19 A. Mellors and A.L. Tappel, J. Lipid Res. 8, 479 (1967).
- 20 A. Mellors, A. Tappel, P.L. Sawant and I.D. Desai, Biochem. biophys. Acta 143, 299 (1967).
- 21 G. Emanuelli, G. Satta and G. Perpignano, Clin. chim. Acta 25, 167 (1969).
- 22 A.L. Tappel, K.A. Caldwell, I.D. Desai and S. Shibko, Archs Biochem. 96, 340 (1962).

Increase in muscle fibres in the lateralis muscle (white portion) of Mugilidae (Pisces, Teleostei)¹

E. Carpenè and A. Veggetti

Istituto di Biochimica e Istituto di Anatomia Normale Veterinaria, Facoltà di Medicina Veterinaria dell'Università di Bologna, I-40126 Bologna (Italy), 18 April 1980

Summary. A cycle of postlarval growth is described in the white portion of the lateralis muscle of Mugilidae. The cycle was identified histochemically by the myosin-ATPase reaction.

The postnatal growth of striated muscle in vertebrates is not due exclusively to hypertrophy but also to an increase in the number of muscle fibres. In mammals the increase in fibre number is quite evident shortly after birth²⁻⁸ (as an extension of embryonic tissue differentiation⁹) and during periods of muscle regeneration¹⁰⁻¹⁵. Muscle growth due to an increase in fibre number in the adult animal is more controversial, even though it has been described in the radialis muscle of rat¹⁶ and, more recently, in the sartorius muscle in man^{17,18}. In birds, a postnatal increase in fibre number has been reported in the anterior latissimus dorsi muscle of the adult chicken not only after denervation but also after simple stretching¹⁹. In fish, postlarval growth has been studied very little; however, it appears to be ascribable not only to hypertrophy but also to hyperplasia, as has been reported in the cod and eel²⁰⁻²². During our histochemical research on muscle fibre types²³⁻²⁶, while looking for annual variation in the white, pink and red portions of lateralis muscle fibres in fish, we noted a growth cycle in the white muscle of Mugilidae, which is reported in the present paper.

Our results are based on experiments conducted from November 1978 to March 1980 on 2-year-old Mugilidae of the following species of different length: *Mugil saliens* (9-12 cm), *M. capito* (11-15 cm), *M. chelo* (7-8 cm) and *M. auratus* (15-18.5 cm). The growth rates of these fishes differ markedly, being greatest in *M. auratus* and least in *M.*

chelo. The fish, collected in the northern Adriatic and kept in aquaria containing sea water at room temperature (18-25 °C), were killed weekly by decapitation under tricaine methanesulfonate (MS-222) anaesthesia. The lateralis muscle samples, taken from regions underneath the 1st and the 2nd dorsal fin, were rapidly frozen in isopentane at -80 °C. 10-µm serial transverse sections were cut in a cryostat and stained for succinate dehydrogenase (SDH)²⁷, lactate dehydrogenase (LDH)²⁷, menadione α -glycerophosphate dehydrogenase (M α -GPDH)²⁷ and myosin adenosine triphosphatase (myosin-ATPase) after preincubation for 1-1.5-2 min in 0.1 M acetate buffer at pH 4.0, 4.2, 4.3, 4.6 and in 0.1 M 2-amino-2-methyl-1-propanol at pH 10.1, 10.2, 10.35 with NaOH²⁸⁻³⁰. Between November and August, in all species studied, the white portion of the lateralis muscle (in contrast to the red and pink ones) was composed of a single fibre type (fast-twitch) 'large diameter' (80-100 µm), which had a high myosin-ATPase activity, and which was alkali-stable and acid-labile (figure 1, a), SDH negative and weakly positive to M α -GPDH and LDH. At the beginning of September, with the exception of *M. chelo*, 'small diameter' fibres (18-20 µm) began to appear, which were intimately connected with, and located between, the large diameter fibres, and whose high myosin-ATPase activity was not only alkali-, but also acid-stable. These fibres constituted approximately 20-30% of the total number of white muscle fibres and were unreactive to the other

enzymatic staining methods (SDH, M α -GPDH and LDH). At the end of September the small diameter fibres were not very numerous, whereas 'intermediate diameter' fibres, which showed high myosin-ATPase activity and were weakly acid-stable, were visible throughout the myotome between the large diameter fibres (figure 1, b). Beginning with the deepest layers of the myotome, the intermediate diameter fibres increased in diameter and showed high myosin-ATPase activity, became less and less acid-stable (figure 2), and ultimately showed no activity whatsoever

after acid preincubation, as with the large diameter fibres. The mosaic framework persisted in the most superficial layers of the white muscle in proximity to the pink portion and disappeared completely in November, when all the intermediate and large diameter fibres showed the same alkali-stable and acid-labile myosin-ATPase activity. In the months that followed, the diameters of all white muscle fibres tended toward maximum values.

On the basis of the myosin-ATPase reaction, it was therefore possible not only to observe the appearance of new small fibres, but also to follow their evolution. Their initial acid- and alkali-stable myosin-ATPase activity became less and less acid-stable as the fibres increased in diameter, until it became acid-labile and alkali-stable, as in the fast-twitch muscle^{29,31}. Because their myosin-ATPase activity is stable to the acid and alkali pre-incubation, the small diameter fibres could be interpreted either as adult IIC fibres³² or as undifferentiated fibres similar to those of embryonic and regenerating muscle^{19,33-35}. The ex novo appearance of these fibres of very small diameter, their evolution into type II fibres only³², and the lack of IIC fibres in the white muscle during the other months of the year lead us to believe that the second hypothesis is more likely. Without our entering into a discussion of its meaning, this particular acid and alkali-stable myosin-ATPase activity could be due to the presence of slow and fast myosin in the same fibre^{34,36-38}, to a characteristic embryonic myosin^{39,40}, or a hybrid type of myosin⁴¹⁻⁴³. In conclu-

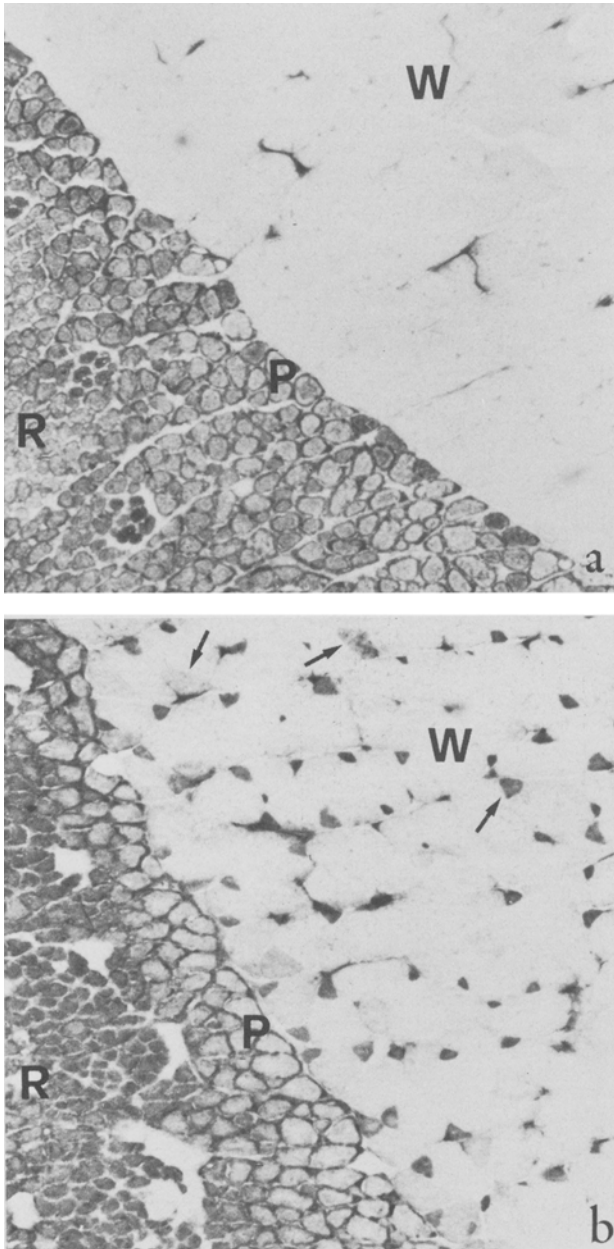


Fig 1. Cross section of the lateralis muscle of the Mugilidae: red (R), pink (P) and white (W) portions. The white portion is composed of 'large diameter' fibres which have acid-labile myosin-ATPase activity (a). At the beginning of September 'small diameter' fibres appear. These fibres enlarge subsequently in diameter ('intermediate diameter' fibres) and, at the end of September, have a high myosin-ATPase activity that is less and less acid-stable (b, arrows). Myosin-ATPase reaction following preincubation at pH 4.2. $\times 69$.

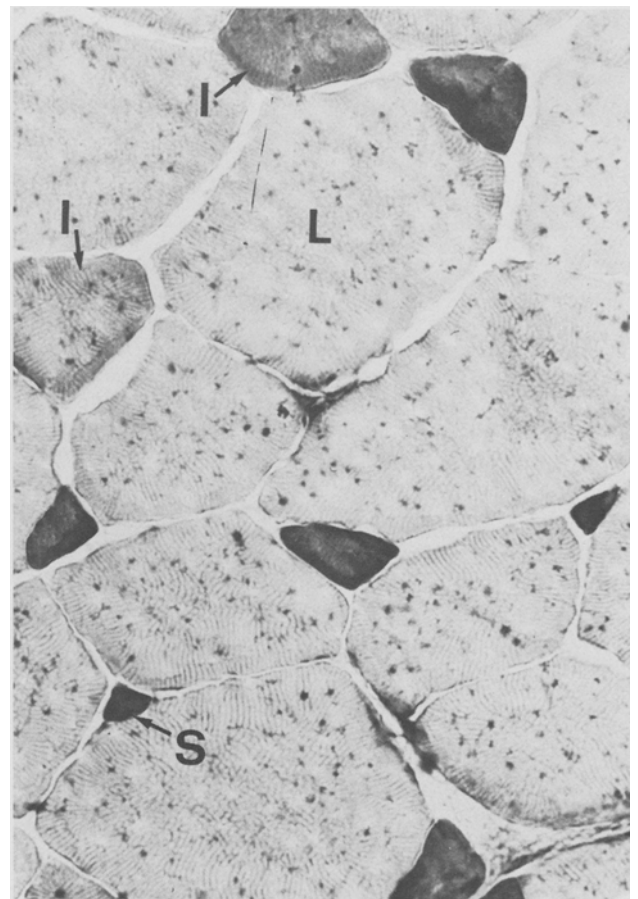


Fig. 2. Cross section detail of the white portion at the end of September. The 'small diameter' fibres, which are strongly acid-stable (S), develop into the 'large diameter' fibres, which are acid-labile (L). The acid stability decreases as the diameter increases (I). Myosin-ATPase reaction following preincubation at pH 4.2. $\times 275$.

sion, in the white muscle of *M. saliens*, *M. capito* and *M. auratus* it was possible to demonstrate a growth cycle as revealed by the appearance of new fibres. Consistent with this cycle was the absence of new small fibres in *M. chelo*, the species with the smallest increase in length during the experiment. The small, new fibres that we found in the Mugilidae were similar in their distribution and diameter to those found by Willemse and Van den Berg in different postlarval stages of the eel with Sudan black B²². The reason for this autumnal growth is not clear; it may be caused by the more favorable conditions of food availability or the higher water temperature or both. We are conducting research to verify whether or not this cycle of growth is annual in the mullet and to clarify the genesis of these newly formed muscle fibres, which could come from satellite cells or which might arise by fibre splitting⁴⁴.

- 1 This work was supported by a CNR grant (Oceanografia e fondi marini project).
- 2 G. Goldspink, Proc. Irish Acad. 62, 135 (1962).
- 3 J.J. Chiakulas and J.E. Pauly, Anat. Rec. 152, 55 (1965).
- 4 D.T. Bridge and D. Allbrook, J. Anat. 106, 285 (1970).
- 5 F.P. Moss and C.P. Leblond, Anat. Rec. 170, 421 (1971).
- 6 J. Stügl, Folia morph. 20, 121 (1972).
- 7 E. Schultz, Anat. Rec. 180, 589 (1974).
- 8 J. Rayne and G.N.C. Crawford, J. Anat. 119, 347 (1975).
- 9 G. Goldspink, in: Structure and Function of Muscle, 2nd edn, vol. I, part 1, p. 179. Ed. G.G. Bourne. Academic Press, New York and London 1972.
- 10 A. Hess and S. Rosner, Am. J. Anat. 129, 21 (1970).
- 11 P.W. Benoit and W.D. Belt, J. Anat. 107, 547 (1970).
- 12 B.E. Walker, Am. J. Anat. 133, 369 (1972).
- 13 P. Hodgson and J. Field, in: The Structure and Function of Muscle, 2nd edn, vol. II, part 2, p. 311. Ed. G.H. Bourne. Academic Press, New York and London 1972.
- 14 M.H. Snow, Anat. Rec. 188, 201 (1977).
- 15 E.C.B. Hall-Craggs, J. Anat. 107, 459 (1970).
- 16 B. Morpurgo, Anat. Anz. 15, 200 (1898).
- 17 R.D. Montgomery, Nature 195, 194 (1962).

- 18 E.D. Hay and C.M. Doyle, Anat. Rec. 175, 339 (1973).
- 19 O.M. Sola, D.L. Christensen and A.W. Martin, Exp. Neurol. 41, 76 (1973).
- 20 M.G. Walker, J. Cons. perm. Int. Explor. Mer 33, 228 (1970).
- 21 J.J. Willemse, Aquaculture 8, 251 (1976).
- 22 J.J. Willemse and P.G. Van den Berg, J. Anat. 125, 447 (1978).
- 23 A. Veggetti and F. Mascarello, Zbl. Vet. Med. Anat. Histol. Embryol. 7, 371 (1978).
- 24 F. Mascarello and A. Veggetti, Bas. appl. Histochem. 23, 103 (1979).
- 25 F. Mascarello, G. Aureli and A. Veggetti, Quad. Anat. prat. 35, 15 (1979).
- 26 E. Carpenè and A. Veggetti, Bas. appl. Histochem. suppl. 23, XII (1979).
- 27 V. Dubowitz and M.H. Brooke, in: Muscle Biopsy: a modern approach, p. 30. Saunders Co. Ltd, London-Philadelphia-Toronto 1973.
- 28 H.A. Padykula and E. Herman, J. Histochem. Cytochem. 3, 170 (1955).
- 29 L. Guth and F.J. Samaha, Exp. Neurol. 25, 138 (1969).
- 30 I.A. Johnston, S. Patterson, P. Ward and G. Goldspink, Can. J. Zool. 52, 871 (1974).
- 31 M. Barany, J. gen. Physiol. 50, 197 (1967).
- 32 M.M. Brooke and K. Kaiser, Arch. Neurol. 23, 369 (1970).
- 33 T. Gordon, R. Perry, T. Srihari and G. Vrbova, Cell Tissue Res. 180, 211 (1977).
- 34 G.F. Gauthier, S. Lowey and A.W. Hobbs, Nature 274, 25 (1978).
- 35 A.S. Colling-Saltin, J. neurol. Sci. 39, 169 (1978).
- 36 G.F. Gauthier and S. Lowey, J. Cell Biol. 81, 10 (1979).
- 37 H. Lutz, H. Weber, R. Billeter and E. Jenny, Nature 281, 142 (1979).
- 38 R. Billeter, H. Weber, H. Lutz, H. Howald, H.M. Eppenberger and E. Jenny, Histochemistry 65, 249 (1980).
- 39 G. Huszar, Nature New Biol. 240, 260 (1972).
- 40 F.A. Sreter, M. Balint and J. Gergely, Devl Biol. 46, 317 (1975).
- 41 N.A. Rubinstein, F.A. Pepe and H. Holtzer, Proc. natl Acad. Sci. USA 74, 4524 (1977).
- 42 J.F.Y. Hoh, Febs Lett. 98, 267 (1979).
- 43 D. Pette, G. Vrbova and R.C. Whalen, Pflügers Arch. ges. Physiol. 378, 251 (1979).
- 44 B.M. Carlson, Am. J. Anat. 137, 119 (1973).

Survival enhanced by skin-wound trauma in mice exposed to ⁶⁰Co radiation^{1,2}

G.D. Ledney, E.D. Exum and P.A. Sheehy

Immunology Division, Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda (MD 20014, USA), 11 June 1980

Summary. A 4%-body-surface skin wound given 24 h before exposure of mice to ⁶⁰Co radiation raised the LD 50/30 from 825 to 975 rads, resulting in a dose reduction factor of 1.2. Enhanced survival of mice wounded before radiation was independent of extramedullary splenic myelocytopenia.

Increased mortality is observed in rodents subjected to wound trauma after midlethal doses of radiation^{3,4}. In animals receiving lethal doses of radiation before wounding, survival times are decreased compared to irradiated controls. Contrary to the decreased survival noted in rodents subjected to wounding after radiation, wounding before radiation may enhance survival. However, the data supporting this are equivocal. In rats, survival was increased⁴ only slightly or not at all⁵ in animals wounded before midlethal radiation doses. In mice, a small increase in number of survivors was noted when midlethal irradiation followed wounding³. The purpose of this report is to establish that wounding before midlethal and lethal doses of radiation enhances survival and that survival is independent of extramedullary splenic myelocytopenia.

Materials and methods. Female (C57BL/6 X CBA)F1 Cum BR mice were obtained from Cumberland View Farms,

Clinton, Tennessee, at 5 weeks of age and quarantined for 2 weeks in groups of 15 mice each. Animals were used only if they were free of histologic lesions of common murine diseases and of *Pseudomonas* sp. Splenectomy was done when indicated on 10-week-old mice under methoxyflurane anesthesia. Wounding and irradiation were performed when the mice were 14–16 weeks of age. All animals were housed 4 per autoclaved plastic filter-topped cage, and they were fed Wayne Lab-Blox diet and chlorinated (12 ppm) water. The mice were kept in controlled environment rooms throughout the study. A 2.0–2.5 cm² circular wound was cut in the anterior dorsal skin fold and underlying panniculus carnosus muscle with a steel punch cleaned by immersion in 70% ethanol. Such a wound constituted about 4% of the mouse total skin surface area. Wounding was done under methoxyflurane anesthesia between 10.00 h and 14.00 h, 24 h before or after ⁶⁰Co exposure. The