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An Autoradiographic Study of Satellite Cell Differentiation into Regenerating Myotubes Following Transplantation of Muscles in Young Rats*

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Summary. Satellite cells were traced autoradiographically during the regeneration of skeletal muscle in young Sprague-Dawley rats. Approximately 31% of the satellite cells in uninjured muscles appeared labelled after three injections of tritiated thymidine; none of the myonuclei were labelled in the same muscles. Four to six days after transplanting the radioactive muscles to non-radioactive littermates, regenerating myotube nuclei in the host appeared labelled. Thus, this study confirms that satellite cells in young rats can differentiate into multinucleated myotubes following muscle injury.

Key words: Skeletal muscle – Satellite cells – Regeneration – Electron microscopy – Autoradiography.

Introduction

Injury to skeletal muscle often results in the regeneration of new muscle fibers from mononucleated myoblasts which are believed to arise locally within the damaged muscle (Reviewed by Carlson, 1972). Precisely how such regenerating cells are derived from the injured muscle has been the subject of numerous morphologic studies which in general have supported one or the other of two hypotheses. According to one concept, regenerating mononucleated myoblasts can bud off from damaged muscle fibers (Hay, 1959, 1974; Reznik, 1969; Kahn and Meyer, 1970; Teräväinen, 1970; Elyakova, 1972; Mastaglia et al., 1975), whereas the alternate hypothesis maintains that myosatellite cells (Mauro, 1961) can survive a muscle injury to become regenerating myoblasts (Shafiq and Gorycki, 1965;

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Church et al., 1966; Jirmanová and Thesleff, 1972; Bischoff, 1974, 1975; Kahn and Simpson, 1974; Snow, 1977a).

Since morphologic criteria alone have not been successful in establishing the origin of newly formed regenerating myoblasts, recent attempts have been made to utilize autoradiographic techniques to trace nuclear populations during a regenerative response. Snow (1977b) demonstrated that myosatellite cells which had been labelled with tritiated thymidine in uninjured muscles of young rats appeared as labelled regenerating myoblasts 16–24 h after transplanting the radioactive muscles. However, the eventual myogenic fate of such surviving myosatellite cells was not verified in this study. The present study was designed to determine whether satellite cells labelled with tritiated thymidine prior to muscle injury could differentiate into cross-striated myotubes following muscle transplantation. The results establish that myosatellite cells in young rat muscle can regenerate into new muscle fibers following muscle injury.

Materials and Methods

Six twenty-day-old Sprague Dawley rats of both sexes received three intraperitoneal injections of 2.0 microcuries/gm body weight of tritiated thymidine (Specific Activity: 6.7 Ci/mM; New England Nuclear) administered at 4 h intervals over an 8 h period. 1 h after the final injection the triceps surae muscle of the left leg was removed and minced according to the technique described by Carlson (1972). The radioactive minced muscles were then immediately transplanted to the site of the extirpated left triceps surae muscles of six nonradioactive littermates. Between the second and seventh days after injury the host rats received three I.P. injections of "cold" thymidine to minimize reutilization of the isotope. The right triceps surae muscles from the radioactive animals served as controls for monitoring the labelling pattern in uninjured muscle.

The transplants were allowed to regenerate to the myotube stage (5–7 days) at which time they and the control muscles were removed and processed for electron microscopy and autoradiography as described previously (Snow, 1977 b). The presence of labelling over a nucleus was determined by light microscopic examination of autoradiographs prepared from 1-micron sections, while verification of the identity of the labelled nucleus was determined by electron microscopic examination of the same cell on adjacent ultrathin sections.

Table 1. Distribution of labelled nuclei in nonregenerating and regenerating muscle from three-week-old rats which had received three injections of tritiated thymidine one hour prior to fixation or transplantation

Nuclear classification	Number of nuclei observed	Labelling index
Nonregenerating muscle:		
Unlabelled satellite cells	111[a]	
^{3}H-satellite cells	50[a]	31%
Unlabelled myonuclei	1384	
^{3}H-myonuclei	None	
Regenerating muscle:		
Unlabelled myotube nuclei	1004	
^{3}H-myotube nuclei	76	7%

[a] Total satellite cell population represents 10.4% of all nuclei observed beneath the external laminae

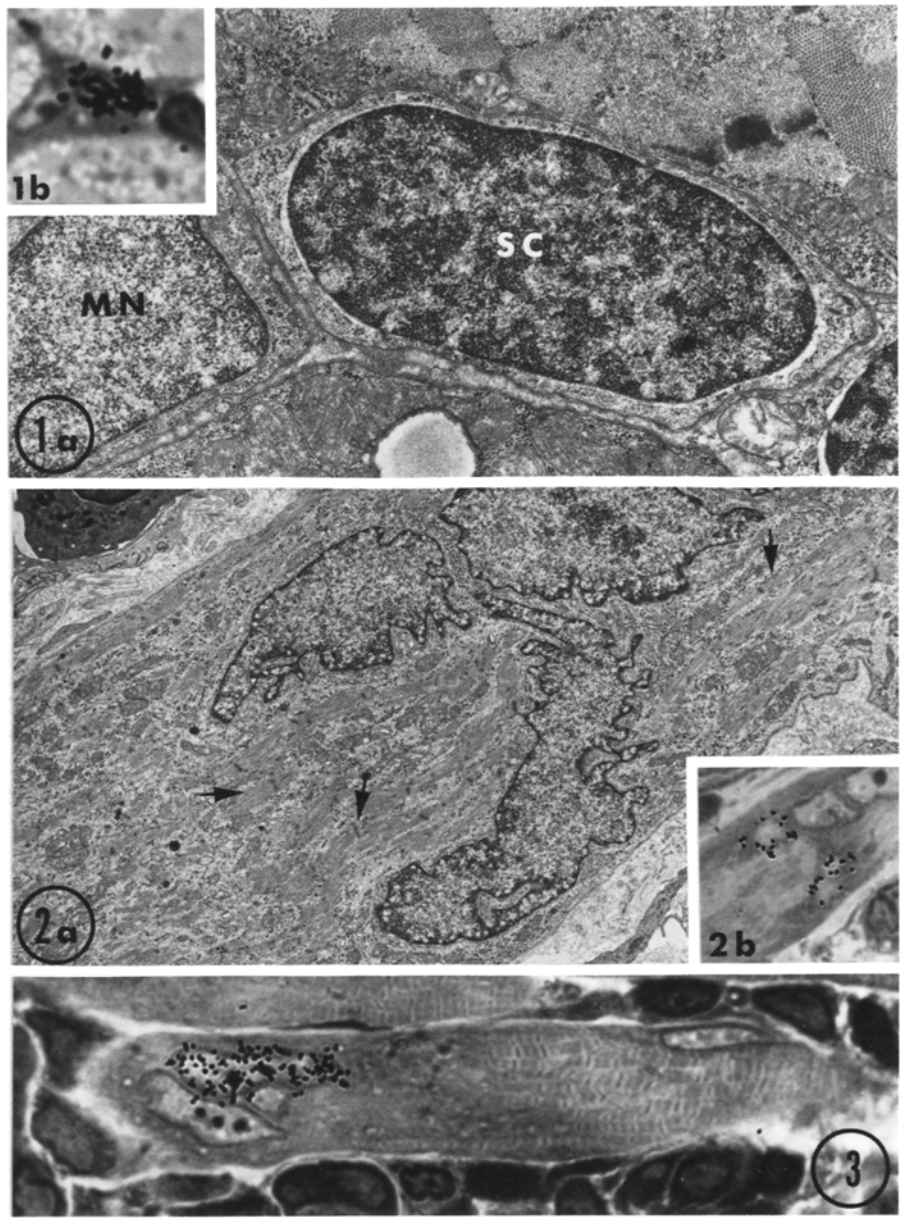

Fig. 1. a Electron micrograph of satellite cell (*SC*) in uninjured soleus muscle of young rat which had received tritiated thymidine prior to fixation. Note myonucleus (*MN*) in adjacent muscle fiber. × 18,630. **b** Light microscopic autoradiograph of section adjacent to Figure 1a. Note silver grains over satellite cell nucleus and absence of label over myonucleus. × 2484

Fig. 2. a Electron micrograph of multinucleated myotube in 5-day regenerate. Note developing myofibrils in sarcoplasm (arrows). × 5085. **b** Light microscopic autoradiograph of section adjacent to Figure 2a. Note silver grains over two nuclei in regenerating myotube. × 1330

Fig. 3. Light microscopic autoradiograph of multinucleated myotube in 6-day regenerate. Note heavily labelled nucleus and cross-striations in sarcoplasm. × 1420

Results

Satellite cells observed in uninjured muscle in this study were cytologically similar to those described in previous reports (Snow, 1977a). Approximately 10–11 % of all nuclei beneath the external laminae of soleus muscle fibers in three-week-old rats were satellite cell nuclei (Table 1).

Light microscopic examination of autoradiographs of nonregenerating muscles from rats which had been exposed to ^{3}H-thymidine revealed many radioactive nuclei (Fig. 1b). Fine structural examination of adjacent sections of these labelled nuclei verified that satellite cells (Fig. 1a), fibroblasts, pericytes, and endothelial cells were radioactive, whereas none of the 1,384 myonuclei observed in these autoradiographs appeared labelled. Table 1 indicates that about 31 % of the satellite cell nuclei were labelled. The number of silver grains per nucleus ranged from four to about 60. Approximately 40 % of the satellite cells were considered lightly labelled (4–10 grains per nucleus), 40 % moderately labelled (11–20 grains per nucleus), and 20 % heavily labelled (over 20 grains per nucleus).

Five to seven days after transplanting the minced radioactive muscles to nonradioactive littermates, many myotubes containing myofibrils and centrally located nuclei were noted in the regenerates. A more detailed fine structural description of myotube formation in minced muscle regenerates in the rat has recently been published (Snow, 1977a). Light microscopic examination of autoradiographs at this stage revealed many radioactive nuclei in cross-striated myotubes (Figs. 2b, 3). Ultrastructural examination of adjacent thin sections of such regenerating cells confirmed that they were labelled muscle nuclei (Fig. 3a). Approximately 7 % of the observed nuclei were labelled (Table 1). The number of silver grains per labelled myotube nucleus ranged from four to about 60 and nearly 66 % of these nuclei were considered lightly labelled (4–10 grains per nucleus), 21 % were moderately labelled (11–20 grains per nucleus), and 13 % were heavily labelled (over 20 grains per nucleus). Examination of longitudinal sections revealed that typically only one or two of the nuclei per myotube were labelled. None of the myonuclei in uninjured muscle fibers of the host rats were labelled.

Discussion

The present investigation confirms that during regeneration of transplanted skeletal muscle, myogenic precursor cells originate from within the donor tissue. Similar results have been reported by Neerunjun and Dubowitz (1975) in an autoradiographic study of muscle transplantation in the mouse.

It has been suggested by Bateson et al. (1967) that regenerating myoblasts may stem from circulatory cells. Although the present study can not eliminate this possibility, the labelled regenerating myotubes observed here could not have been derived from labelled circulatory cells since the host tissues were not radioactive. In addition, it might be argued that labelled fibroblasts, pericytes and endothelial cells which were present in the donor tissue may also have contributed to a pool of regenerating myoblasts after transplantation. This possibility seems unlikely since

available evidence indicates that connective tissue cells can not be transformed into muscle cells following injury (Bischoff, 1975; Dunis and Namenwirth, 1977). Furthermore, several fine structural studies of muscle regeneration have shown that regenerating myoblasts initially appear beneath the external lamina of injured muscle fibers, and therefore involvement of connective tissue cells in the regenerative response would necessitate their migration through the external lamina within 24 h after injury (Price et al., 1964: Shafiq and Gorycki, 1965; Church et al., 1966; Reznik, 1969; Mastaglia et al., 1975).

Since the present fine structural and autoradiographic data show that satellite cell nuclei were labelled in nonregenerating muscles whereas myonuclei were not, it is concluded that the labelled regenerating myotubes seen after injury were derived from the satellite cell population. These findings are consistent with a recent autoradiographic study (Snow, 1977b) showing that, during the first 24 h after muscle injury in the rat, labelled satellite cells remained viable whereas labelled myonuclei appeared pyknotic. If a process does occur by which a myonucleus and its adjacent sarcoplasm can bud off from an injured muscle fiber to become a regenerating myoblast, it does not seem to be a major source of regenerating cells in young mammals. Reutilization of radioisotope by myoblasts which had budded off from the transplanted muscle fibers may also have accounted for the labelled myotubes reported here. However, any available pool of radioisotope should have been significantly diluted since the host rats received multiple injections of "cold" thymidine during the period of myoblast proliferation and fusion. Furthermore, in a recent examination of similarly labelled muscles during the first 24 h after transplantation, very few labelled, pyknotic nuclei which could have provided radioactive metabolites for reutilization were noted (Snow, 1977b).

Approximately 31 % of the satellite cell nuclei were labelled in the nonregenerating muscle, but only about 7 % of the myotube nuclei were labelled. This apparent discrepancy was expected since the majority of labelled satellite cells had from four to 20 silver grains per nucleus and most of these probably diluted out their label through mitosis prior to myoblast fusion. For example, a satellite cell with fewer than 16 grains would only have to undergo two mitotic divisions to reduce the number to less than four grains, which was the minimum required in this study to be counted as labelled. It seemed likely, therefore, that only the most heavily labelled satellite cells (approximately 20 % of all the labelled satellite cells) might retain some of their radioactivity during myotube differentiation. It is also possible that some of the more heavily labelled satellite cells diluted out their label as a result of more than two mitoses prior to fusion. Finally, since a few myotube nuclei appeared as heavily labelled as satellite cell nuclei, these findings suggest that at least in some cases, satellite cells fused to form myotubes without undergoing mitosis after muscle injury.

The results recorded here provide the most definite evidence to date that satellite cells can become regenerating myogenic cells after injury in young rats. Although satellite cells have been shown to exist in adult mammalian muscles, they are present in fewer numbers than in younger animals and their morphology suggests that they may be less metabolically active (Schultz, 1976; Snow, 1978). It remains to be determined whether or not such cells may serve as a reserve source of regenerating myoblasts for the repair of damaged or diseased muscle in adult animals.

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