Virchows Arch [Cell Pathol] (1982) 39:339–349

Virchows Archiv B

An Autoradiographic Study of the Role of Satellite Cells and Myonuclei During Myogenesis in vitro

Gene L. Trupin[1], Linda Hsu[2], and Gail Parfett[1]

[1] Department of Anatomy, Rutgers Medical School-CMDNJ, Piscataway, N.J. 08854, USA
[2] Department of Anatomy, New Jersey School of Osteopathic Medicine-CMDNJ, Piscataway, N.J. 08854, USA

Summary. Satellite cells and myonuclei of neonatal rat muscles were differentially labeled with ^{3}H-thymidine according to the procedure of Moss and Leblond (1971). Minced muscle fragments containing either labeled satellite cells or labeled myonuclei were cultured until multinucleated myotubes grew out from the explants. Reutilization of isotope released from degenerating nuclei was competitively inhibited by using a culture medium containing excess (0.32–0.41 mM) cold thymidine. After an 8–10 day growth period, the explants were fixed and prepared for autoradiographic (ARG) examination to determine whether labeled satellite cells or myonuclei had contributed to the myonuclear population of the developing myotubes. Counts were made of the number of labeled myotubes in the explants and compared with the number of labeled satellite cells and myonuclei in samples of the original muscle tissues fixed at the time of explantation. The original muscles showed a mean satellite cell labeling index of 51.7% and gave rise to myotubes with a mean labeling incidence of 40%. In contrast, myonuclear labeling in the original muscle tissues showed no correlation with subsequent myotube labeling. Only 3.4% myotube labeling was found in explants in which over 30% of the original tissue myonuclei had been labeled. Under conditions controlled for isotope reutilization, these observations confirm results of in vivo ARG studies indicating that satellite cells are the only significant source of regenerating myoblasts in injured muscle tissue.

Key words: Muscle – Regeneration – Satellite cells – Tissue culture – Autoradiography

Introduction

Recent autoradiographic (ARG) studies on the origin of myogenic cells in regenerating skeletal muscle have traced the fate of satellite cells and myonuclei

Offprint requests to G.L. Trupin at the above address

0340-6075/82/0039/0339/$02.20

differentially labeled with ^{3}H-thymidine (^{3}H-TdR) prior to mincing injury. These studies (Snow 1977a, b) demonstrate that a significant population of satellite cells can survive severe mechanical injury, while adjacent myonuclei appear to undergo rapid degeneration. Subsequent experiments (Snow 1978) in which regeneration was allowed to go to completion indicate that ^{3}H-TdR labeled satellite cells give rise to labeled myonuclei in the regenerated muscle fibers. The primary purpose of the present study was to reexamine this ARG evidence under in vitro conditions controlled for the reutilization of ^{3}H-label. The need for such an approach was made apparent by observations that reutilization of ^{3}H-labeled substances released by injured cells can produce extensive artifactual labeling of regenerating muscle fibers (Steen 1968, 1970; Trupin 1973). Similar reutilization problems have also been recognized in other types of regeneration in which the fate of potential stem cells was traced by ^{3}H-TdR labeling prior to tissue injury (Thornton 1968; Rose and Burnett 1970; Reyer 1971).

A second purpose of this study was to evaluate more fully the potential role of myonuclear dedifferentiation in the process of muscle tissue regeneration. Previous ARG studies on the origin of regenerating myogenic cells have focused primarily upon the role of the satellite cell (Gutmann et al. 1976; Snow 1978; Lipton and Schultz 1979). Furthermore, injury used to induce regeneration in vivo will also damage the vascular supply, causing ischemia and other physiological alterations (LeGros Clark 1946) that may preclude myonuclei from taking part in the regenerative process. In the present study, we have utilized an in vitro system capable of maintaining multinucleated myofibers so as to minimize physiological impediments to myonuclear dedifferentiation. To trace the fate of potential myogenic precursors, fragments of neonatal rat muscle containing satellite cells or myonuclei labeled with ^{3}H-TdR were grown in culture until multinucleated myotubes grew out from the tissues. Isotope reutilization was competitively inhibited by culturing the explants in a medium supplemented with excess cold TdR. The cultures were then examined by ARG to determine whether labeled satellite cells or myonuclei had contributed to the myonuclear population of the newly formed myotubes. The results of this investigation confirm that satellite cells are the only significant source of myogenic elements active during mammalian muscle tissue regeneration.

Materials and Methods

Satellite cells and myonuclei of neonatal Sprague Dawley rats were differentially labeled with ^{3}H-TdR according to the procedures of Moss and Leblond (1971). The labeling protocol rests upon the observation that although only satellite cells incorporate ^{3}H-TdR, label will appear in myonuclei by 18 h after injection as the labeled satellite cells fuse with adjacent myofibers. Subsequent cycles of satellite cell proliferation and fusion have the dual effect of producing numerous labeled myonuclei, while diluting label within the residual satellite cells through repeated mitotic divisions.

Two rat pups, randomly selected from a litter within 8 h after birth, were given a series of 3 subcutaneous injections of ^{3}H-TdR (New England Nuclear, Methyl-^{3}H-thymidine, Specific activity, 20 Ci/millimole) at a dosage of 2 Ci/gm body weight per injection. The injections were administered at 4 h intervals over an 8 h period. These rats (referred to as long-term animals) were then left undisturbed to allow myonuclear labeling to proceed. At intervals from one to 8 days after the first pups were injected, a second pair of littermates received an identical series of 3 injections at the same dosage. To obtain muscle explants containing only labeled satellite

cells, these rats (referred to as short-term animals) were killed nine hours after receiving their first injection. The gastrocnemius, soleus and plantaris muscles were removed, minced into 1 mm^3 fragments and explanted onto collagen coated overslips (Ehrmann and Gey 1956). Within 1 h after sacrificing the short-term animals, tissues containing labeled myonuclei were removed from the long-term littermates and used to prepare a parallel set of explants. Both sets of cultures were fed with growth medium consisting of 80% Basal Medium Eagle's, 15% heat-inactivated horse serum and 5% chick embryo extract supplemented with 0.32–0.41 mM cold TdR. This TdR concentration was found to competitively block isotope reutilization with no apparent cytotoxic effect (Bischoff and Holtzer 1969; Hsu et al. 1979). The cultures, which were grown in sealed Maximow chambers at 35° C, were fed every 2–3 days with the same medium. After a growth period of 8–10 days, the cultures were fixed in 10% neutral buffered formalin, rinsed in distilled water, air dried and dip coated in Kodak NTB-3 emulsion diluted with 2 parts of distilled water. The autoradiographs were exposed in a dried atmosphere for 35 days at −80° C, developed in Kodak D-19 for 4 min at 10° C, stained with hematoxylin and eosin and mounted for light microscopic examination. Counts were made on 236 explants from 14 short-term (satellite cell-labeled) animals and 219 explants from 12 long-term (myonuclear-labeled) animals to determine the percent of labeled myotubes (i.e., the percent of myotubes with at least one labeled myonucleus). Positive labeling was defined as the presence of at least four silver grains directly above a myonucleus. A labeled myonucleus was counted only if it offered the following features: a) an unobstructed view of the location of the nucleus within the myotube; b) presence of a characteristically enlarged nucleolus; c) a parallel alignment of the long axis of the nucleus and the myotube; or d) complementary folds with the borders of adjacent myonuclei.

To confirm the reliability of the labeling procedures, muscle fragments from long and short-term animals were fixed at the time of explantation in 2.5% glutaraldehyde in 0.1 N sodium cacodylate buffer, pH 7.4, containing 5% sucrose. The tissues were post-fixed in OsO_4 and embedded in Epon-Araldite. One micron thick sections were mounted on microscope slides and used to prepare autoradiographs as described above. After development, the ARG thick sections were stained with 1% toluidine blue in 1% sodium borate and examined with the light microscope to identify labeled satellite cells and myonuclei. Labeling was again defined as the presence of four or more silver grains above a nucleus. Confirmation of cell identification was made on adjacent thin sections which were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope. Counts were made of the number of labelled and unlabelled satellite cells and myonuclei the muscle fragments. The identification of all cells and nuclei used in these counts was confirmed by examination of adjacent thin sections.

Results

Labeling of Satellite Cells and Myonuclei in Original Muscle Tissues

EM and ARG examination of muscle fragments fixed at the time of explantation confirmed the reliability of the labeling protocol. In tissues from short-term animals killed within 9 h after the initial injection of ^{3}H-TdR, label was found only in satellite cells (Figs. 1 and 2) and other mononuclear forms such as fibroblasts and endothelial cells. No myonuclear labeling was observed in the muscles of these animals. The incidence of satellite cell labeling (Table 1; Fig. 8) varied from 47.4% to 58.6%, with a mean satellite cell labeling index of 51.7% during the first eight days of postnatal growth. There was no systematic change in percent labeling of satellite cells during this 8 day period ($r=0.501$; $t=1.27$; $df=6$; $p>0.1$). Labeling of individual satellite cells was generally heavy, often with 15–30 silver grains overlying a nucleus.

Muscle fragments from long-term donors showed both extensive myonuclear labeling and labeling of the residual satellite cell population (Figs. 3 and 4). The satellite cells were usually only lightly labeled (2–6 silver grains), indicating

Table 1. Short-term animals. ^{3}H-TdR labeling of satellite cells in explanted muscle fragments and in myonuclei of developing myotubes in vitro

Littermate[a] designation	Age of rats at time of sacrifice (hours)	Explanted muscle fragments				Developing myotubes in vitro		
		Number of labeled satellite cells	Total number of satellite cells	Labeled satellite cells (%)	Total number of myonuclei	Number of myotubes with labeled myonuclei	Total number of myotubes	Labeled myotubes (%)[b]
A	10	37	71	52.1	156	53	115	46.1
B	33	44	75	58.6	158	39	82	47.6
C	59	31	58	53.4	138	58	120	48.3
D	82	37	78	47.4	177	18	48	37.5
E	121	29	55	52.7	128	42	92	45.7
F	154	35	73	47.9	176	17	60	28.3
G	194	33	60	50.8	170	6	25	24.0
Totals		246	476	51.7	1,103	233	542	43.0

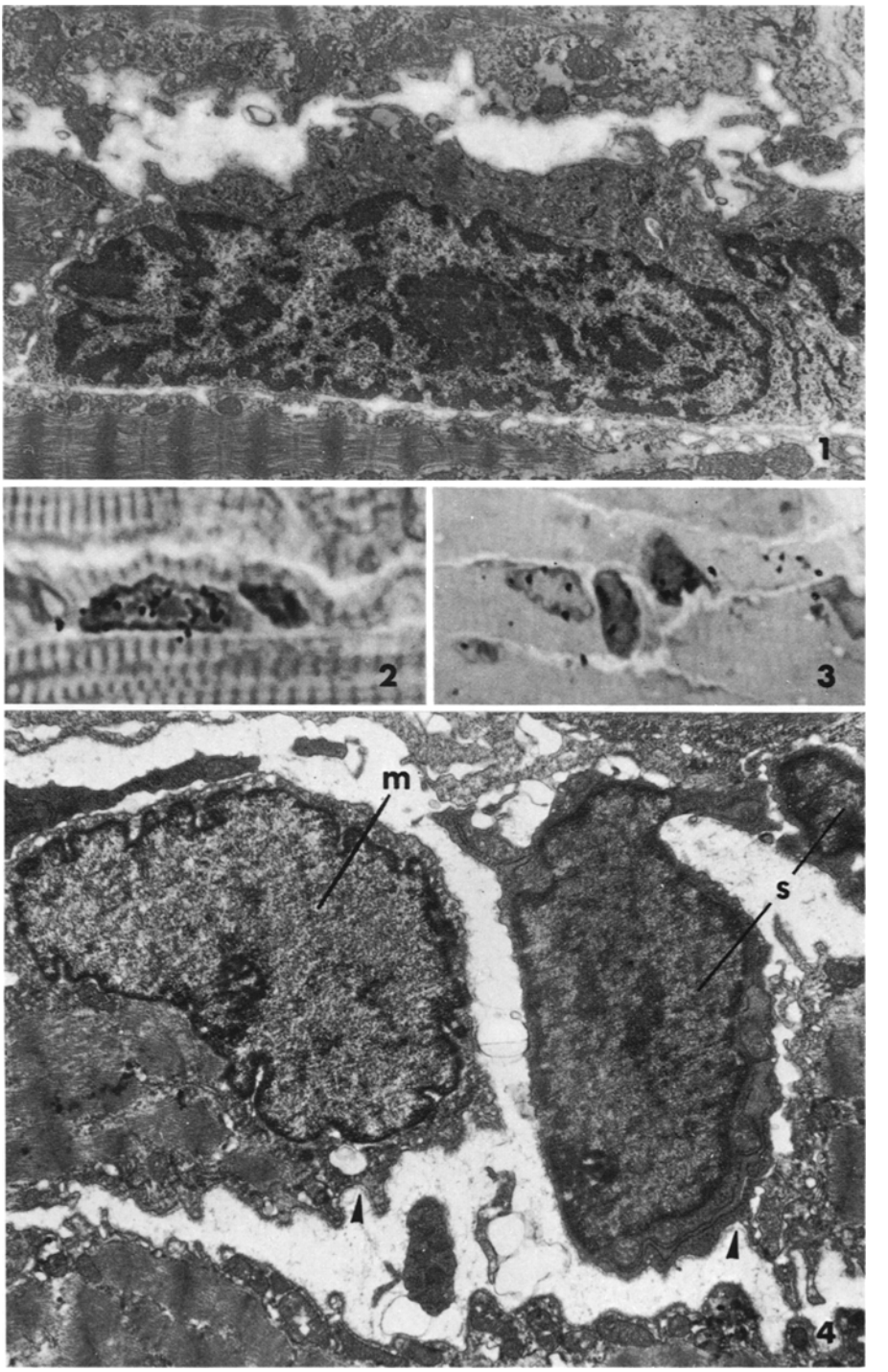
[a] Littermates are identified by the same letter designation in Tables 1 and 2
[b] The percent of myotubes with at least one labeled myonucleus

Table 2. Long-term animals. ^{3}H-TdR labeling of myonuclei in explanted muscle fragments and in myonuclei of developing myotubes in vitro

Littermate designation	Time interval between first injection of ^{3}H-TdR and sacrifice (hours)	Explanted muscle fragments			Developing myotubes in vitro		
		Number of labeled myonuclei	Total number of myonuclei	Labeled myonuclei (%)	Number of myotubes with labeled myonuclei	Total number of myotubes	Labeled myotubes (%)[b]
A	No long-term animals[a]						
B	32	9	101	8.9	32	86	37.2
C	54	16	91	17.6	25	90	27.8
D	80	19	79	24.1	27	144	18.7
E	118	27	85	31.8	19	117	17.1
F	149	31	104	29.8	7	84	8.3
G	190	29	95	30.5	1	29	3.4

[a] Only short-term animals could be taken from litter A, which was sacrificed 10 h after injection
[b] The percent of myotubes with at least one labeled myonucleus

that ongoing mitosis had produced dilution of label in this stem cell population. Myonuclear labeling in these animals showed a rapid increase during the first five days after injection of ^{3}H-TdR (Table 2; Fig. 9). Label was initially found in 8.9% of the myonuclei after a cell division and fusion period of 32 h. As this fusion period was lengthened, the percent of labeled myonuclei rapidly



Figs. 1 and 2. A ^{3}H-TdR labeled satellite cell in an electron micrograph and an ARG of an adjacent thick section. This cell was found in muscle tissue from a short-term animal which received its first injection 9 h prior to its sacrifice at 82 h of age. A second unlabeled satellite cell is visible to the right in both the electron micrograph and ARG. Fig. 1, ×9,900; Fig. 2, ×2,750

Fig. 3 and 4. A labeled myonucleus (*m*) and satellite cells (*s*) in an ARG and matched electron micrograph. The tissues were taken from the long-term littermate of the donor of the tissues seen in Figs. 1 and 2. This animal received its first injection of ^{3}H-TdR 80 hours prior to sacrifice. The 2 satellite cells show only 2–4 silver grains, suggesting dilution of label due to mitotic division during this 80 h period. Arrowheads mark the external lamina. Fig. 3, ×1,700; Fig. 4, ×9,300

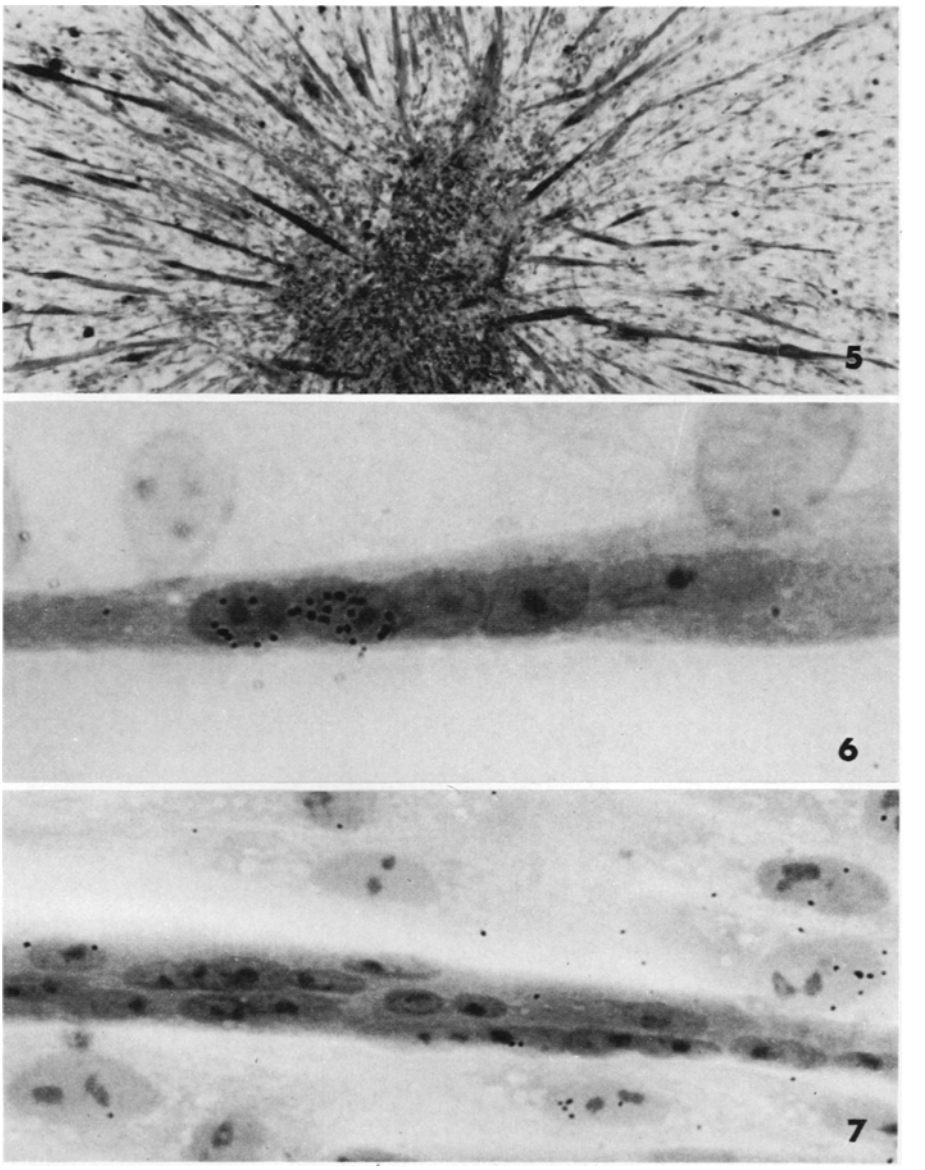
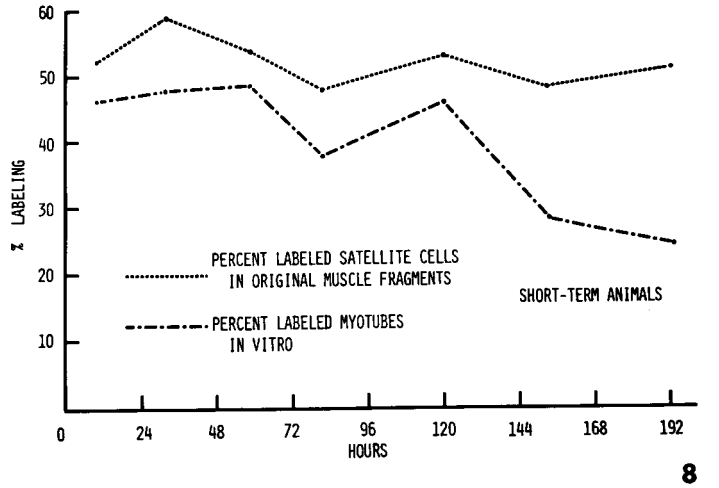


Fig. 5. An 8-day old culture showing myotubes growing from a muscle explant taken from a neo-natal rat 59 h after birth. The background population of mononuclear cells consists largely of fibroblasts. ×90

Fig. 6. A ^{3}H-TdR labeled myotube in an explant from a short-term animal. The donor received its first injection 9 h prior to its sacrifice at 59 h of age. The myonuclei show moderately heavy labeling, with little evidence of background labeling. This explant was taken from the littermate of the donor of the long-term explant shown in Fig. 7. ×1,900

Fig. 7. A myotube in an explant from a long-term animal sacrificed 54 h after the initial injection of ^{3}H-TdR. This animal was the long-term littermate of the donor of the explant in Fig. 6. The myotube shows a light scattering of silver grains, but is not considered to be labeled since none of its nuclei show a minimum of 4 silver grains. ×1,550



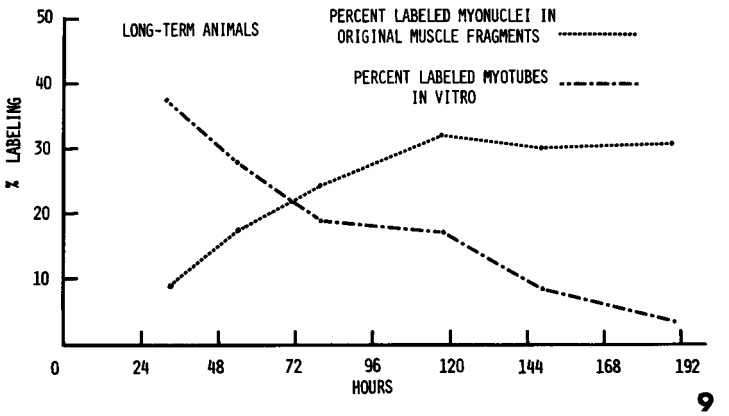


Fig. 8. Percent labeling of satellite cells and of myotubes in vitro from short-term animals. The abscissa is plotted to show age of the rats in hours at the time of explantation

Fig. 9. Percent labeling of tissue myonuclei and of myotubes in vitro from long-term animals. The abscissa is plotted to show number of hours between time of first injection and explantation

increased, leveling off at approximately 30% by 5 days after injection. This asymptotic curve was not unexpected for, as the total number of myonuclei increased, each cycle of satellite cell fusion produced a progressively smaller increase in the percent of labeled myonuclei.

Labeling of Myotubes in vitro

Myotubes began to grow out from the explants about 2 days after explantation, and by 4 days formed well developed radial arrays extending outward from the original muscle fragments (Fig. 5). The ability of the explants to support myogenesis declined with increasing age of the donor. This decline was first seen in explants from 6 day old animals, and by 8 days after birth only 12% of the explants formed myotubes. For this reason, animals older than eight

days were not used in the study. The decline in myogenesis appeared to be correlated with the presence of increasing amounts of connective tissue that may have hampered myotube formation and outgrowth. The decline was unrelated to either the proliferation rate or size of the satellite cell population since these parameters did not change during the first week of postnatal development (Table 1).

Developing myotubes from short and long-term animals showed distinct differences in labeling (Figs. 6, 7). Myotube labeling (the percent of myotubes with at least one labeled myonucleus) in explants from short-term animals roughly paralleled satellite cell labeling in the original muscle fragments, although at a reduced mean labeling incidence of 43% (Table 1; Fig. 8). This myotube labeling varied from 37.5–48.3% in explants taken from animals up to 121 h of age, declining to 24%–28% in explants from older animals (154 and 194 h). However, even in the two older groups, the myotube labeling index was appreciably higher than that of explants from long-term littermates. Explants from long-term animals displayed a myotube labeling pattern which was the reverse of that found in the myonuclear population of the original muscle fragments (Table 2; Fig. 9). The highest myotube labeling index of 37.2% occurred at 32 h after the first injection of ^{3}H-TdR, when only 8.9% of the original tissue myonuclei were labeled. As the period for labeling of the original tissues myonuclei was lengthened, the myotube labeling index declined. At 190 h after injection, only 3.4% of the myotubes were labeled, although over 30% of the myonuclei in the original tissues were labeled at this time. Thus, myotube labeling in explants from long-term animals exhibited a continuous decline despite the rapid increase in myonuclear labeling in the original muscle tissues.

Discussion

The primary hypothesis underlying this investigation is that ^{3}H-TdR labeling of myogenic precursors will produce a similar occurrence of labeling in muscle fibers formed by these precursor elements. Thus, changes in the incidence of labeling in myogenic elements should be reflected by parallel changes in labeling of the developing myotubes. Such comparable labeling was found only in satellite cells and myotubes from short term animals (Fig. 8). Satellite cell and myotube labeling in these groups remained relatively constant and parallel throughout most of the study, showing deviation only in the older animals subject to reduced myogenesis. In contrast, myotube labeling in explants from long term animals (Fig. 9) was essentially the reverse of myonuclear labeling in the original tissues. This reversed pattern of labeling establishes that there was no causal relationship between labeling of the original tissue myonuclei and subsequent myotube labeling. The finding that the lowest incidence of myotube labeling (3.4%) occurred in explants from long-term animals having the greatest number of labeled tissue myonuclei (30.5%) demonstrates more specifically that myonuclei made no significant contribution to myogenesis. This labeling pattern also provides further evidence of the causal relationship between satellite cell and myotube labeling, since the progressive decline of myotube labeling in explants from long-term animals (Fig. 9) can be correlated with the mitotic dilution

of ^{3}H-label in the residual satellite cell population. This decline in myotube labeling cannot be correlated with a similar dilution of labeling in the myonuclear population of the original long-term muscle tissues. Although each cycle of satellite cell mitosis halves the label in the daughter cells destined to become myonuclei, as well as in the residual satellite cells, labeled myonuclei undergo no further mitotic dilution of label once fusion has occurred (Lash et al. 1957). Thus, the first 2 or 3 cycles of satellite cell mitosis and fusion occurring after injection of ^{3}H-TdR will produce a permanent, heavily labeled population of myonuclei. If myonuclear dedifferentiation were a real factor in myogenesis, these labeled myonuclei should have prevented the decline and virtual extinction of labeling in the myotubes developing in the long-term explants. Thus, under conditions controlled for isotope reutilization, these data confirm that: a) satellite cells are the primary source of myogenic elements in regenerating muscle tissue; and b) myonuclear dedifferentiation is not a significant factor during muscle tissue regeneration.

The incidence of satellite cell labeling in the short-term animals remained constant during the first 8 days of postnatal development. This finding is consistent with previous reports of a uniform satellite cell labeling index of 21–23% following single injections of ^{3}H-TdR into young rats (Moss and Leblond 1971; Allbrook et al. 1971; Snow 1977b). The relatively high incidence of satellite cell labeling (51.7%) observed in short-term animals in the present study appears to be a product of 2 factors: the 9 h delay between injection of ^{3}H-TdR and sacrifice; and the use of multiple injections of isotope. Calculations performed on the data of Moss and Leblond (1971) reveal that the 21% incidence of satellite cell labeling found 1 h post injection had increased to over 35% after 10 h due to ongoing mitotic division.[1] The maximal labeling observed in the present study was produced by summing this mitotic effect with the labeling from the two additional pulses of ^{3}H-TdR. The additional 2 injections had a smaller effect upon the labeling index, however, since satellite cells labeled by these pulses could not have completed mitosis by the time the animals were sacrificed.

A variety of experimental approaches have been used to examine the role of satellite cells and myonuclei during muscle tissue regeneration. Substantial evidence that satellite cells are the primary source of regenerating myoblasts was initially obtained through phase microscopic examination of the regenerative response of single myofibers in vitro (Bischoff 1975; Konigsberg et al. 1975). These studies also indicated that the myonuclei within these isolated fibers underwent degeneration, a conclusion supported by in vivo ARG findings that only satellite cells survived a total mincing injury known to induce extensive muscle regeneration (Snow 1977b). The latter investigation had a significant advantage over the in vitro studies of single myofibers in that it sampled a much larger population of myonuclei. However, because mincing injury produces total disruption of the vasculature, this in vivo study (Snow 1977b) did not

1 Moss and Leblond (1971) observed 4,060 nuclei per 50 mm of fiber of which 12% (or approximately 487 nuclei per 50 mm) belonged to satellite cells. One h after ^{3}H-TdR injection, 103 satellite cells per 50 mm were labeled (21.1% labeling index), while at 10 h, 174 satellite cells per 50 mm were labeled (35.7% labeling index)

eliminate the possibility that potential myonuclear dedifferentiation had been blocked by vascular insufficiency. Such disruption of the vascular supply will itself produce a total ischemic necrosis of supplied myofibers (LeGros Clark 1946), and even in vitro a slight reduction of oxygen tension will cause myogenic cells to undergo degenerative changes (Lipton 1977). Additional evidence that satellite cells are the source of regenerating myoblasts has also come from a study of renewed myogenesis following trypsinization and mechanical disruption of myotubes from primary cultures of embryonic chick muscle (Pullman and Yeoh 1978). This study demonstrated that myotubes depleted of mononuclear cells by cytosine arabinoside were incapable of supporting further myogenesis after mechanical disruption. The relevance of these observations to the separate issue of myonuclear dedifferentiation is not entirely clear, however, since the cell disruption procedures were deliberately harsh, often reducing myotubes to individual, isolated myonuclei that were unlikely to participate in further myogenesis. The severity of these procedures becomes evident when one considers that the 1 mm^3 muscle fragments used as explants in the current study contained numerous, largely undamaged, segments of myofibers, each containing a hundred or more myonuclei. The present investigation has attempted to unify these diverse observations by: a) utilizing an in vitro system to provide adequate physiological support for potentially dedifferentiating myofibers; b) examining large, defined populations of labeled satellite cells and myonuclei; and c) using an experimental model designed to closely parallel muscle tissue regeneration in vivo. As with previous ARG studies on the origin of regenerating myoblasts (Snow 1977b, 1978), this investigation has used a system in which new myofibers developed from minced muscle fragments. This approach has been predicated upon the assumption that muscle fragments in culture will give rise to new myofibers by the same mechanisms operative during muscle tissue regeneration. This view is supported by the findings that myotubes formed by minced muscle explants are histologically similar to those found in minced muscle regenerates in vivo, and develop in an identical time period of 2–4 days (Carlson 1968). Thus, with respect to the nature of the injured tissues, means of producing injury, histological features and time required for myogenesis, the formation of myotubes from muscle fragments in vitro is identical to the process of minced muscle regeneration in vivo.

Acknowledgments. We wish to thank Nancy Hayden, Jacqueline Salomon and Joanne Hutchens for secretarial assistance, and Dr. Ronald Cody for his advice on statistical analysis. This study was supported in part by GRS grant 27-1990 to the Rutgers Medical School and grants from the Muscular Dystrophy Association and NIH.

References

Allbrook DB, Han MF, Hellmuth AE (1971) Population of muscle satellite cells in relation to age and mitotic activity. Pathology 3:233–243

Bischoff R (1975) Regeneration of single skeletal muscle fibers in vitro. Anat Rec 182:215–236

Bischoff R, Holtzer H (1969) Mitosis and the processes of differentiation of myogenic cells in vitro. J Cell Biol 41:188–200

Carlson BM (1968) Regeneration of the completely excised gastrocnemius muscle in the frog and rat from minced muscle fragments. J Morphol 125:447–472

Ehrmann RL, Gey GO (1956) The growth of cells on a transparent gel of reconstituted rat-tail collagen. J Natl Cancer Inst 16:1375–1403

Gutmann E, Mareš V, Štichová J (1976) Fate of ^{3}H-thymidine labelled myogenic cells in regeneration of muscle isografts. Cell Tissue Res 167:117–123

Hsu L, Trupin GL, Roisen FJ (1979) The role of satellite cells and myonuclei during myogenesis in vitro. In: Mauro A (ed) "Muscle regeneration". Raven Press NY, pp 115–120

Konigsberg UR, Lipton BH, Konigsberg IR (1975) The regenerative response of single mature muscle fibers isolated in vitro. Dev Biol 45:260–275

Lash JW, Holtzer H, Swift H (1957) Regeneration of mature skeletal muscle. Anat Rec 128:679–697

LeGros Clark WE (1946) An experimental study of the regeneration of mammalian striped muscle. J Anat 80:24–36

Lipton BH (1977) A fine-structural analysis of normal and modulated cells in myogenic cultures. Dev Biol 60:26–47

Lipton BH, Schultz E (1979) Developmental fate of skeletal muscle satellite cells. Science 205:1292–1294

Moss FP, Leblond CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec 170:421–436

Pullmann WE, Yeoh GCT (1978) The role of myonuclei in muscle regeneration: an in vitro study. J Cell Physiol 96:245–252

Reyer RW (1971) DNA synthesis and the incorporation of labeled iris cells into the lens during lens regeneration in adult newts. Dev Biol 24:533–558

Rose PG, Burnett AL (1970) The origin of secretory cells in *Cordylophora caspia* during regeneration. Wilhelm Roux' Arch 165:192–216

Snow MH (1977a) Myogenic cell formation in regenerating rat skeletal muscle injured by mincing. I. A fine structural study. Anat Rec 188:181–200

Snow MH (1977b) Myogenic cell formation in regenerating rat skeletal muscle injured by mincing. II. An autoradiographic study. Anat Rec 188:201–218

Snow MH (1978) An autoradiographic study of satellite cell differentiation into regenerating myotubes following transplantation of muscles in young rats. Cell Tissue Res 186:535–540

Steen TP (1968) Stability of chondrocyte differentiation and contribution of muscle to cartilage during limb regeneration in the axolotl (Siredon mexicanum). J Exp Zool 167:49–78

Steen TP (1970) Cell differentiation during salamander limb regeneration. In: Mauro A, Shafiq SA, Milhorat AT (eds) Regeneration of striated muscle, and myogenesis. Exerpta Medica Amsterdam, pp 73–90

Thornton CS (1968) Amphibian limb regeneration. Adv Morphogenesis 7:205–249

Trupin GL (1973) An ultrastructural study of the minced gastrocnemius muscle in the frog. Ph.D. Thesis, Univ. of Michigan, Ann Arbor, MI

Accepted February 2, 1982