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The Effects of Aging on Satellite Cells in Skeletal Muscles of Mice and Rats*

Mikel H. Snow

Department of Anatomy, University of Southern California, School of Medicine, Los Angeles, California, U.S.A.

Summary. Myosatellite cells were examined and quantified at the fine structural level of resolution during aging of skeletal muscles in mice and rats. Satellite cells in the soleus and gastrocnemius muscles of animals between eight and 30 months of age appeared, according to morphological criteria, metabolically less active than those examined in immature muscles. In the soleus muscle of the mouse, satellite cells decreased in number from 4.6% at eight months of age to 2.4% at 30 months. This decrease appeared to be due to the passage of some satellite cells into the interstitial space as a result of the formation of external lamina material around the entire satellite cell surface.

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

Introduction

Myosatellite cells are small, mononucleated, structurally undifferentiated cells found in a variety of vertebrate skeletal muscle fibers between external lamina and sarcolemma (Reviewed by Muir, 1970). Ever since satellite cells were first described by Mauro in 1961, considerable interest has been directed toward determining their functional significance during various physiologic and pathologic states. Moss and Leblond (1971) have shown that, during muscle growth in the rat, satellite cells divide and one or both daughter cells fuse with an adjacent myofiber, thus contributing to an increase in total myonuclei. A similar recruitment of satellite cells

Send offprint requests to: Mikel H. Snow, Ph.D., Department of Anatomy, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, California 90033, U.S.A.

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has been suggested during compensatory hypertrophy of muscle in the rat (Schiaffino et al., 1976). Recently, Snow (1977b) demonstrated that in uninjured hindlimb muscles of young rats, satellite cells labeled with ^{3}H-thymidine can become regenerating myoblasts following mincing and transplantation.

Although such studies clearly indicate the myogenic capacity of satellite cells in young muscles, information regarding the structure, distribution and function of satellite cells in adult muscle has been sparse and somewhat contradictory. It has been reported, for example, that such cells are extremely rare or absent in uninjured muscles of adult rabbits and mice (Reznik, 1969), adult rats (Elyakova, 1972), and post-metamorphic newts (Hay and Doyle, 1973; Popiela, 1976). In contrast, Gutmann and Hanzlíková (1972) provided fine-structural verification of, but did not quantitate, satellite cells in the levator ani muscle of three-year-old rats. More recently, Schultz (1976) described the cytological features of satellite cells in lumbrical muscles of mice up to 10 months of age, but again, this study did not include quantification of the satellite cell population. Allbrook et al. (1971), on the other hand, examined the frequency of satellite cells during aging of rat and mouse muscle. Their study was primarily concerned with changes in satellite cell numbers during muscle growth, but they did report approximately 4.2 % satellite cells in both the subclavius muscle of 170-day-old rats and the peroneus longus muscle of 140-day-old mice. There are no available studies on the distribution of satellite cells in muscles of rats or mice older than these. The only quantitative data on satellite cell distribution in senile muscle has been provided by Schmalbruch and Hellhammer (1976) who found 0.5 % satellite cells in a limb muscle of a 73-year-old man. It is the purpose of the present study to determine the cytological features and frequency of satellite cells in non-growing, uninjured muscles of adult and senile mice and rats. The findings clearly demonstrate the presence of satellite cells at all ages examined, although in the mouse the number of such cells decreases between adult and senile stages. This decrease appears to be due to the passage of some satellite cells into the interstitial space during normal aging.

Materials and Methods

Fourteen C57 black Iackson mice and four Sprague-Dawley rats of both sexes, obtained from the animal colony maintained by Dr. Caleb Finch at the Andrus Gerontology Center of the University of Southern California, Los Angeles, California, were divided into an eight to ten-month-old group, a 19 to 20-month-old group, and a 29 to 30-month-old group. The four Sprague-Dawley rats were 29 months old. Since the maximal life expectancy of mice and rats is about 36 months, the 29 to 30-month-old animals were considered senile. The soleus and gastrocnemius muscles from both hindlimbs were removed under sodium pentobarbital anesthesia and pre-fixed by immersion in 2.0 % glutaraldehyde plus 2.5 % paraformaldehyde (Karnovsky, 1965) buffered to pH 7.4 with 0.1 M sodium cacodylate. The muscles were post-fixed for one hour in 1 % osmium tetroxide, and dehydrated in ethanols. Thin Epon-embedded sections were mounted on carbon-coated 200 mesh grids, stained with uranyl acetate and lead citrate, and examined with a JEOL 100C electron microscope. Satellite cells and myonuclei were counted on single transverse sections and the grid squares were used as a counting reticle. Identification of satellite cells was verified by means of high magnification electron micrographs, and the frequency of satellite cells was expressed as a percentage of the total number of satellite cell nuclei and myonuclei. Determination of the mean size of satellite cell nuclei and myonuclei was based on measurements of the length, width and height of 14 satellite nuclei and 19 myonuclei in transverse and longitudinal sections of mouse soleus muscle.

Results

Satellite cells in uninjured skeletal muscle of young (one to two-month-old) rats and mice have been described previously (Allbrook et al., 1971; Moss and Leblond, 1971; Schultz, 1974, 1975; Cardasis and Cooper, 1975; Snow, 1977a); therefore only a brief description of such cells will be given here. All satellite cells observed in soleus muscles of young animals were separated from the underlying myofiber by a 20 to 60 nm space devoid of any external lamina material (Fig. 1). Most satellite cells displayed a euchromatic nucleus with a prominent nucleolus and, in general, a high nuclear to cytoplasmic ratio (Fig. 1). The cytoplasm typically contained polyribosomes, mitochondria, some rough endoplasmic reticulum and a predominant Golgi apparatus. Occasionally satellite cells were observed to be undergoing mitosis. Thus, based on nuclear and cytoplasmic morphology, most satellite cells in young muscle appeared metabolically active.

Satellite cells were observed in soleus and gastrocnemius muscles at each of the adult ages examined; their cytological features did not differ markedly between eight and 30 months of age (Figs. 2, 3, 5, 6). However, when compared to those in younger muscles, the adult and senile satellite cells appeared less active according to morphological criteria. Heterochromatin was a typical feature of the nucleus, and nucleoli were rarely observed (Figs. 2, 3, 5). In addition, the nuclear to cytoplasmic ratio appeared higher in the older satellite cells. Profiles of rough endoplasmic reticulum with dilated cisternae were rarely seen, and in general the Golgi apparatus was poorly developed. Microtubules and microfilaments were only occasionally seen and glycogen was not detected. Caveolae seemed to be less abundant on both the internal and external surfaces of the adult satellite cells. Nuclei of satellite cells in the older muscles were approximately the same size (height × width × length) as those in younger animals ($1.5 \times 4.1 \times 12.0$ microns and $2.4 \times 4.3 \times 12.1$ microns, respectively). Although adult satellite cells were never observed to be undergoing mitosis in this study, centrioles were occasionally seen even as late as 30 months of age (Fig. 3). Satellite cells were also observed in association with intrafusal muscle fibers in uninjured, adult, soleus muscle (Fig. 4). Cytologically, satellite cells in muscle spindles appeared similar to those described in extrafusal myofibers.

One of the more interesting present findings was the apparent change with age in the external lamina-satellite cell relationship. Examination of satellite cells from each of the three adult groups revealed several examples of external lamina material in the satellite cell-myofiber interspace (Figs. 5, 6). As mentioned above, this region in younger muscles does not contain external lamina material (Fig. 1). In some of the adult satellite cells external lamina material occupied only a small peripheral portion of the satellite cell-myofiber interspace (Fig. 5), while in others the external lamina occupied most of this interspace (Fig. 6). In all such cases, there was a region of the satellite cell-myofiber interspace which maintained a typical 20 to 60 nm separation between the satellite cell and the myofiber. Occasionally, small mononucleated cells which indented the adjacent muscle fiber surface appeared completely surrounded by an external lamina (Figs. 7, 8a, 8b). Examination of serial sections through such cells verified that these cells could not be classified as satellite cells because the external lamina material filled the satellite-myofiber interspace (Figs. 8a, b). Finally, measurements of the external lamina in adult and senile muscles revealed that it was about twice as thick as in younger animals.

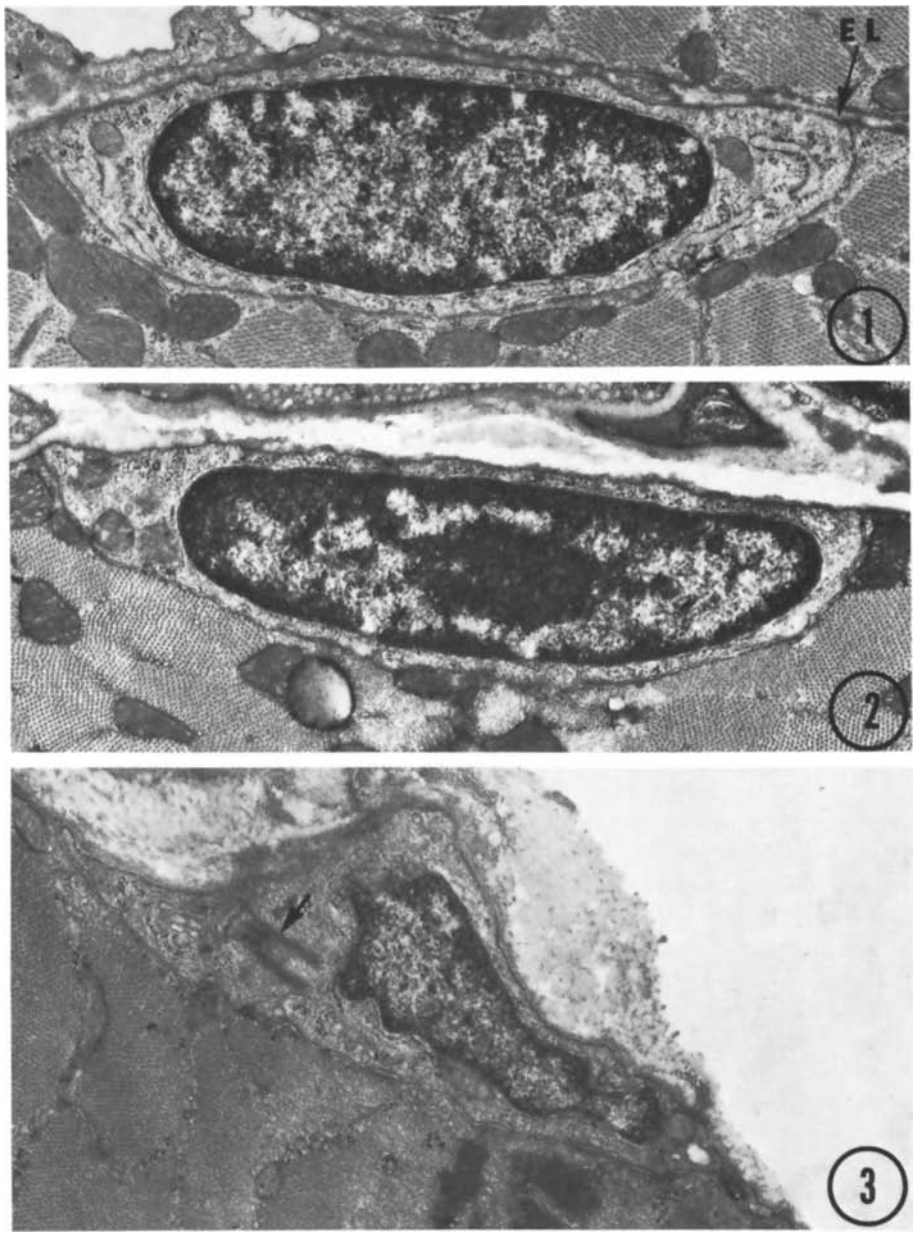

Fig. 1. Satellite cell in soleus muscle of one-month-old rat. External lamina (*EL*) covers outer surface of satellite cell but does not penetrate satellite-myofiber interspace. ×15,000

Fig. 2. Satellite cell in soleus muscle of eight-month-old mouse. Note condensed heterochromatin in periphery of nucleus. ×18,940

Fig. 3. Satellite cell in gastrocnemius muscle of 29-month-old rat. Note centriole (*C*) in cytoplasm. ×17,000

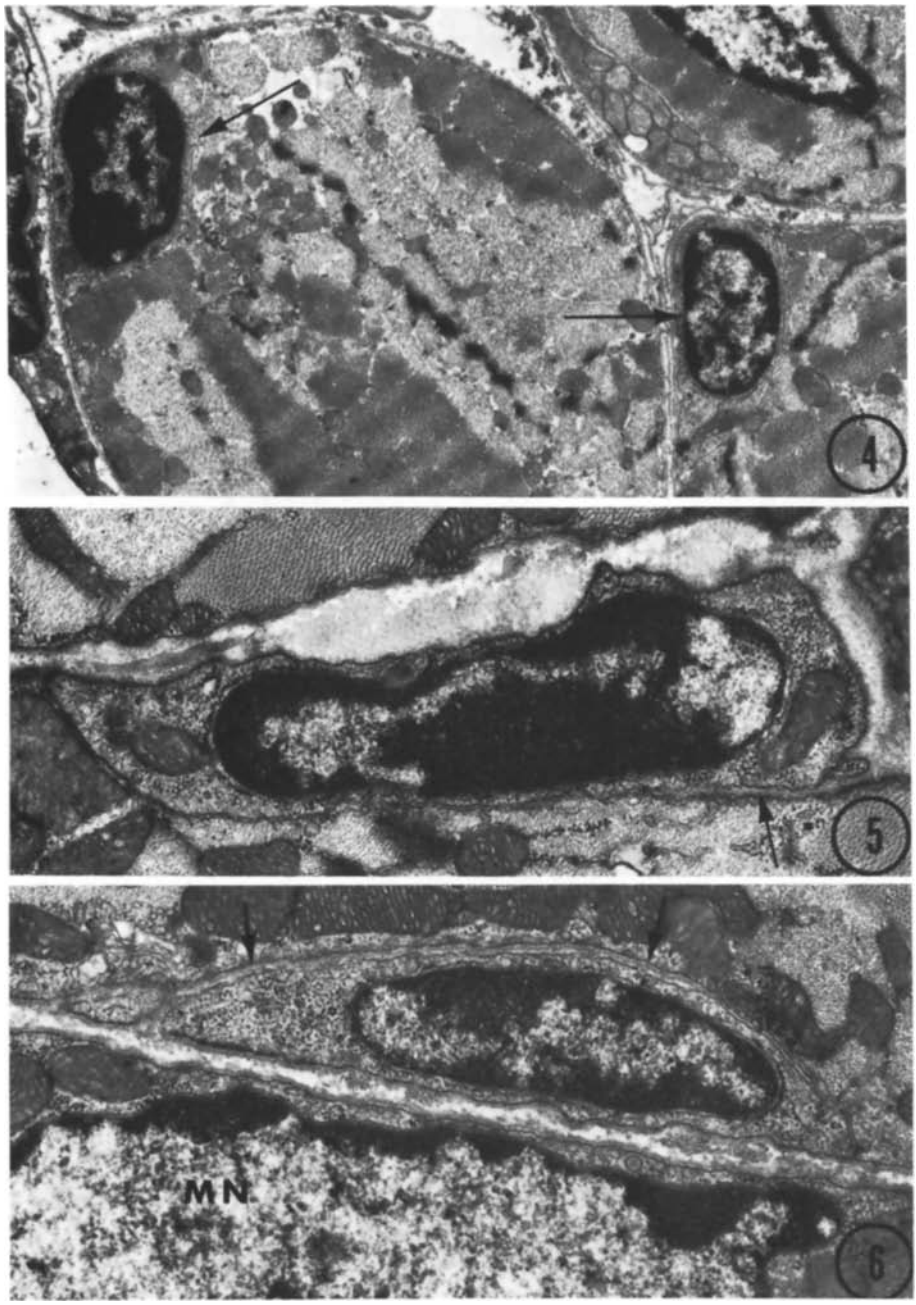

Fig. 4. Cross section of muscle spindle in soleus muscle of eight-month-old mouse. Two satellite cells (arrows) associated with two intrafusal fibers. × 12,000

Fig. 5. Satellite cell in soleus muscle of eight-month-old mouse. Note external lamina material (arrow) in small portion of satellite-myofiber interspace. × 23,400

Fig. 6. Satellite cell in soleus muscle of 29-month-old mouse. External lamina material present in large portion of satellite-myofiber interspace (arrows). Note differences in size and chromatin distribution between satellite cell nucleus and myonucleus (*MN*). × 24,100

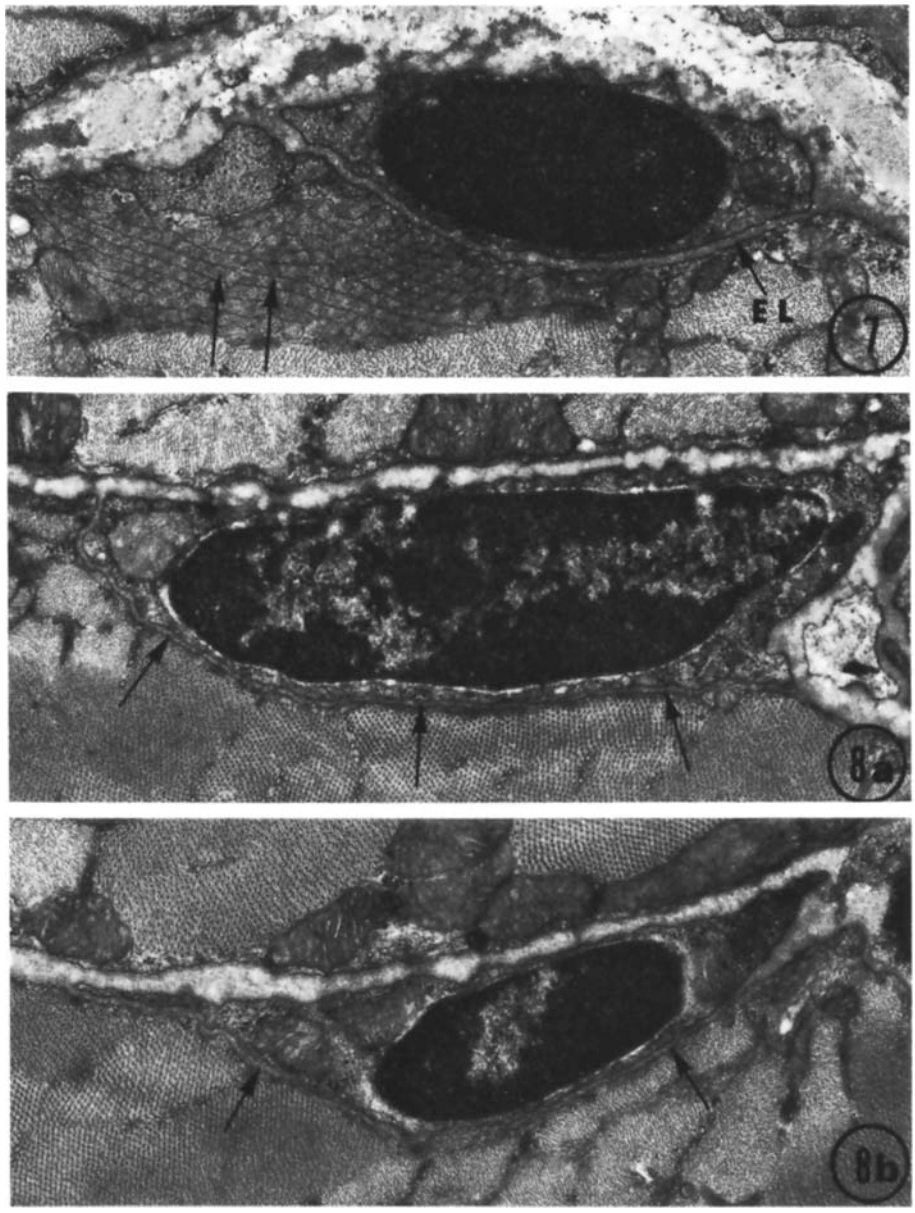

Fig. 7. Undifferentiated appearing cell in soleus muscle of 29-month-old mouse. External lamina material (arrows) completely fills interval between cell and underlying myofiber. ×22,100

Fig. 8. a Undifferentiated-appearing cell in soleus muscle of 29-month-old mouse. External lamina material (arrows) completely fills interval between cell and underlying myofiber. ×22,100. **b** Same cell as in 8a, but 7 μ deep to Fig. 8a. Examination of such serial sections confirms presence of external lamina (arrows) around entire cell. ×22,100

Table 1. Frequency of myosatellite cells in uninjured adult soleus muscle of the mouse

Age	Number of myonuclei observed	Number of myosatellite cell nuclei observed	Percentage of myosatellite cells
8–10 months	387	19	4.6%
19–20 months	264	12	4.3%
29–30 months	498	12	2.4%

The distribution of satellite cells in uninjured soleus muscles of the mouse is summarized in Table 1. Included in these quantitative data are those satellite cells which were partially separated from the underlying myofiber by external lamina material. Those cells which appeared to be completely separated from the myofiber by external lamina were not included. Table 1 shows that a significant decrease in the percentage of satellite cells from 4.6% to 2.4% occurs between eight and 30 months of age.

Although all muscles in this study were uninjured, a variety of pathologic changes, which appeared to progress with age, were noted in a few of the muscle fibers examined. In the sarcoplasm, lipofuscin granules, a few abnormal mitochondria and areas of myofilament dissolution were noted. Tubular aggregates were occasionally seen but only in the gastrocnemius muscle (Fig. 7). Myonuclei frequently displayed varying degrees of heterochromatin around the periphery of the nucleus, and a few myonuclei were centrally located. Motor end-plates occasionally appeared abnormal in that they either contained an abundance of neurofilaments and neurotubules or appeared more dense than normal due to an apparent accumulation of presynaptic vesicles.

Discussion

This study demonstrates that satellite cells are present in uninjured soleus and gastrocnemius muscles of adult and senile mice and rats. Moreover, in the soleus muscle of the mouse, satellite cell nuclei represent about 4.6% of all nuclei counted beneath the external lamina at eight months of age, whereas about 2.4% satellite cells were noted at 30 months of age. The 4.6% figure at eight months is consistent with the 4.2% value calculated by Allbrook et al. (1971) in the peroneus longus muscle of four to five-month-old mice. Although no other reports exist regarding precise quantification of satellite cells in senile muscle, Gutmann and Hanzlíková (1972) have observed satellite cells in the levator ani muscle of three-year-old rats, and Schmalbruch and Hellhammer (1976) observed a single satellite cell in the extensor digitorum muscle of a 73-year-old man. Recently, Ontell (1974) reported 0.7% satellite cells in the tibialis anterior muscle of 800 gm rats. However, it is difficult to correlate these findings with the present investigation since the age of the rats was not given and her calculations were based on the total number of nuclei within the muscle, rather than the number of nuclei within the external lamina. It is possible, however, that the frequency of satellite cells varies between fast-twitch and

slow-twitch muscles as indicated in a study of adult rat muscles by Kelly (1975) who counted 5% satellite cells in the slow-twitch soleus muscle and 0.4% in the fast-twitch extensor digitorum longus muscle.

The role of satellite cells during the regeneration of skeletal muscle has been a subject of much interest and controversy since Mauro (1961) first described satellite cells in adult frog muscle. At present there seems to be little doubt that satellite cells in growing muscles of young mammals can become regenerating myoblasts after injury (Snow, 1977b). However, it has been argued that satellite cells are either absent or extremely rare in undamaged, non-growing, adult muscle, and therefore they cannot be considered a major source of regenerating myoblasts (Reznik, 1970, 1976; Elyakova, 1972; Hay, 1974; Mastaglia et al., 1975). According to these investigators the major source of regenerating myoblasts in adult muscle is from mature muscle fibers which bud off mononucleated cellular fragments following muscle injury. Although the present study does not provide definitive evidence regarding the origin of regenerating myoblasts in adult tissue, it does substantiate that satellite cells are present in significant numbers in adult soleus muscle of mice. Therefore, they may contribute to a regenerative response. At present there is no available evidence which eliminates the possibility that in adult muscle, regenerating myoblasts may be derived from more than one source.

The greater density of adult and senile satellite cell nuclei reported here is consistent with the findings recently published by Schultz (1976) who examined the fine structural features of satellite cells in lumbrical muscles of mice between the ages of one and 10 months. Thus, on structural grounds, older satellite cells appear to be less active than satellite cells in growing muscle. This conclusion appears to be consistent with the fact that satellite cells in young muscles have been shown to be able to divide and then fuse with their adjacent growing muscle fiber (Moss and Leblond, 1972; Snow, 1977b), whereas neither metabolic nor mitotic activity have been demonstrated in satellite cells in non-growing adult muscles.

The appearance of varying amounts of external lamina material in the interspace between eight and 30-month-old satellite cells and their underlying myofiber has also been noted in 10-month-old satellite cells in lumbrical muscles of mice (Schultz, 1976). In adult and senile muscle in this study a few structurally undifferentiated cells appeared to be completely surrounded by an external lamina and, therefore, such cells were not classified as satellite cells, but rather they seemed to resemble most closely pericytes in the interstitial space. Although encroachment into the satellite-myofiber interspace by external lamina material was noted at each of the older ages examined, it seemed to be more frequently encountered in the oldest group. It might be argued, based on examination of the electron micrographs, that mononucleated cells may be passing into the muscle fiber compartment rather than *out* into the connective tissue compartment. The quantitative data presented here tend not to support this view since the percent of satellite cells decreases between eight and 30 months of age. Thus, the evidence suggests that during muscle aging, some satellite cells are sequestered into the connective tissue compartment by the progressive formation of external lamina material around the satellite cell. Finally, the appearance of external lamina material in the satellite-myofiber interspace may be related to the observed thickening of the external lamina around the same muscle fiber. If external lamina

material is being produced during muscle aging, it should not be surprising to find deposition of this material in the satellite-myofiber interspace.

Although other investigators have reported the presence of external lamina material in a portion of the satellite-myofiber interspace, nearly all these studies were of atrophic or diseased muscle. Venable (1966) showed an encroachment of external lamina material in testosterone-dependent levator ani muscles following castration. Similar findings have been reported during denervation atrophy in mice (Schultz, 1974) and rats (Ontell, 1975). Finally, Wakayama (1976) noted a similar change in the external lamina in dystrophic muscles of a nine-year-old boy. The present findings may also reflect a general response of the external lamina to muscle atrophy, in this case senile atrophy, since moderate degenerative changes were noted in some muscle fibers and motor end-plates.

With respect to normal skeletal muscle, Popiela (1976) recently reported external lamina material in the satellite-myofiber interspace in post-metamorphic newts. According to this author, satellite cells present prior to metamorphosis become ‚pericytes' following metamorphosis due to the formation of external lamina material in the satellite-myofiber interspace. If satellite cells in adult newt and mammalian muscle do become ‚pericytes' during muscle aging or muscle atrophy, then the important question becomes, whether such cells retain their myogenic capacity and contribute to a regenerative response.

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