

## THYMIDINE-<sup>3</sup>H UPTAKE BY SATELLITE CELLS OF REGENERATING SKELETAL MUSCLE

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The satellite cell (6) is a mononucleated cell that is wedged between the multinucleated muscle syncytium and the basal membrane of the myofiber. These cells are described in skeletal muscle of amphibians (6, 7) and mammals (3, 12-14). From data in the literature it is clear that satellite cells are most abundant during embryonic life, though it is not obvious that they participate in the normal myogenesis (3).

In young animals, however, it is possible that satellite cells are involved in the growth of skeletal muscle. This concept is suggested by the electron microscopic demonstration of mitosis in satellite cells of 3-wk-old rats treated with colchicine (13) and by the results of light microscopic radioautographic study showing uptake of tritiated thymidine by some nuclei inside the sarcolemmal sheath (5). It must be emphasized, however, that mitotic division of nuclei was never observed in mature myofibers, nor were labeled nuclei ever found in adult muscle after injection of radioactive DNA precursor (8).

In normal adult striated muscle, satellite cells are very rare (3), and their function is unknown, although one of the proposed theories is that satellite cells are potential myoblasts capable of regenerating myofibers when the main muscle fibers in which they lie are destroyed (2, 3). This hypoth-

esis has gained some support from the following two recent observations related to the biology of satellite cells in regenerating muscle of adult animals: (a) light microscopic radioautography reveals that some nuclei, presumably in the sarcolemmal sheath of a damaged myofiber, incorporate tritiated thymidine (10); and (b) electron microscopic investigations show that satellite cells are more numerous in the myofibers that are near an area of necrotic muscle (11).

To obtain more detailed information on the development of such satellite cells and their possible function as precursors of myoblasts, an electron microscopic radioautographic study was performed during experimental muscle regeneration in normal, adult, white mice (NIH, GP).<sup>1</sup> Prior results obtained with light microscopic radioautography (10) indicate that the most favorable time to study the proliferation of the presumptive myoblasts is 60-72 hr after muscle necrosis. Therefore, a single intraperitoneal injection of tritiated thymidine (4-5  $\mu$ c/g of animal weight) was given 66 hr after cold injury of the gastrocnemius muscle. At different intervals of time after the injection, the muscles

<sup>1</sup> The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this study.

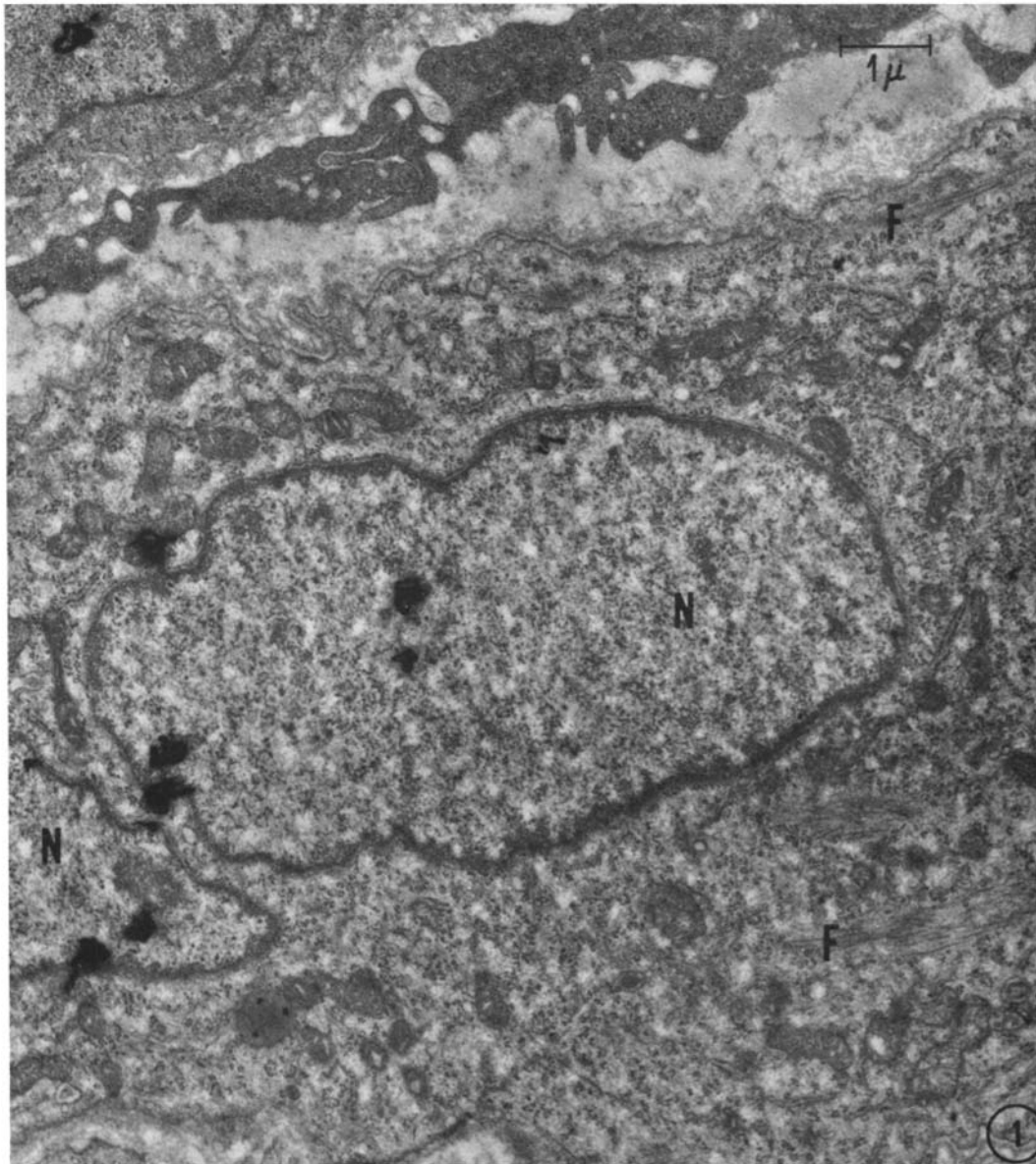


FIGURE 1 Two labeled nuclei (*N*) in a myotube containing some myofilaments (*F*). Another cell with a labeled nucleus is seen at the top left of the picture. 90 hr after injury and 24 hr after administration of tritiated thymidine. Uranyl-lead. Armed Forces Institute of Pathology Negative No. 68-6416.  $\times 17,000$ .

were fixed in glutaraldehyde and osmium tetroxide and embedded in Araldite. Blocks from the regenerating area were selected. Silver sections were collected on grids coated with a collodion film backed by a thin carbon layer, Ilford-L 4 emulsion was used and applied to the sections with the loop

technique (1). After appropriate delay the sections were processed in Microdol X (Eastman Kodak Co., Rochester, N.Y.), washed in water, and fixed in acid fix (4); they were stained by the uranyl-lead method and examined with an RCA-3F or RCA-3G electron microscope.

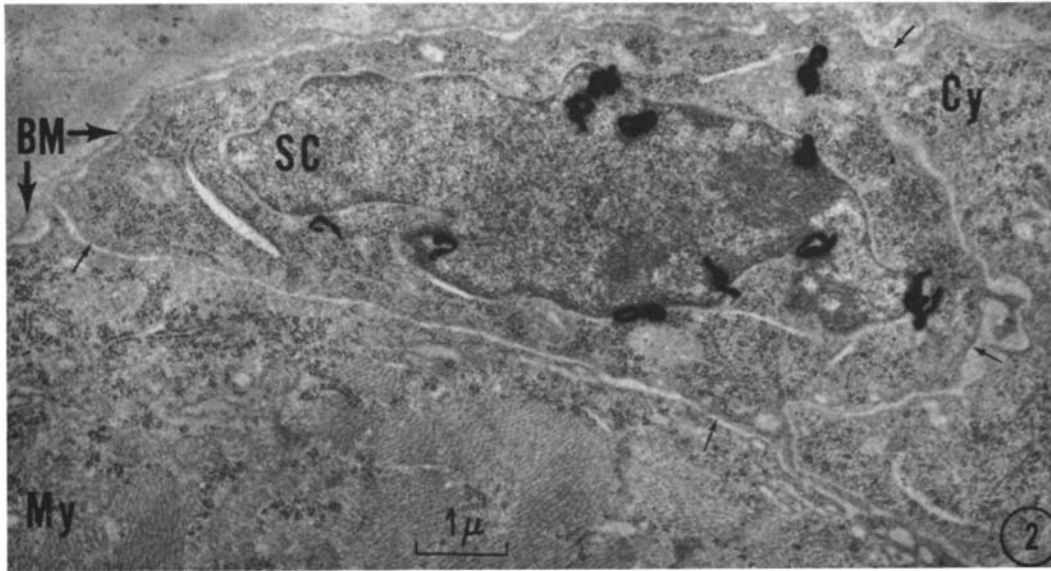


FIGURE 2 Satellite cell (SC) with a labeled nucleus is wedged between the basal membrane (BM) of the myofiber (My), the muscle sarcoplasm, and another satellite cell (Cy). The limits of the labeled satellite cell (SC) are indicated by the small arrows. 72 hr after injury and 6 hr after administration of tritiated thymidine. Uranyl-lead. Armed Forces Institute of Pathology Negative No. 68-6332-1.  $\times 17,000$ .

The results confirm earlier light microscopic radioautographic observations to which the reader is referred for qualitative and quantitative data on this matter (10). 1–3 hr after its administration to the animal, tritiated thymidine is detectable only in endothelial cells and in mononucleated cells, such as fibroblasts, some macrophages, and the presumptive myoblasts. Labeled nuclei in myotubes are often found by 12–24 hr (Fig. 1) but never less than 8 hr after the injection of the radioactive compound.

One of the most interesting findings is the discovery of satellite cells with a labeled nucleus 2–6 hr after the injection of tritiated thymidine. Fig. 2 illustrates such a cell in an area of muscle regeneration seen 72 hr after the cold injury and 6 hr after the injection of the DNA precursor. This labeled mononucleated cell is wedged between the well-preserved basal membrane of the myofiber, the partially damaged muscle sarcoplasm, and another similar cell. The cytoplasm of this mononucleated cell is moderately abundant; it contains large quantities of ribosomes, some rough endoplasmic reticulum, and a few small mitochondria. The ultrastructure of this labeled cell is similar to that of presumptive myoblasts adjacent to the basal

membrane of the sarcolemmal tube of a damaged myofiber (9, 11).

These observations undoubtedly indicate that satellite cells incorporate DNA precursor during experimental muscle regeneration and confirm, indirectly, that they can divide mitotically.

Whether all satellite cells are preexisting dormant myoblasts (2, 6) or are produced locally near the site of damaged muscle (11) must still be analyzed. Nevertheless, the present results give credence to prior postulations and suggest the following conclusions. Whatever their origin, the mononucleated satellite cells appear to be numerous beneath the basal membrane of myofibers that are close to a zone of muscle necrosis; they seem to function as “stem cells” that divide mitotically and provide myoblasts for the regeneration of skeletal muscle tissue in adult animals.

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