NATURE OF DIVIDING NUCLEI IN SKELETAL MUSCLE OF GROWING RATS

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Much is known about the early stages of muscle development—the proliferation of myoblasts, their fusion into myotubes, and the transformation of myotubes into young muscle fibers. However, the mode of growth of muscle in the young animal remains obscure. It is known that muscle growth is extensive (e.g. in the chicken the pectoralis muscle enlarges from 0.7 g at hatching to 300 g in the adult) and is mainly the result of hypertrophy of the fibers (1), with little, if any, increase in their number (2). Moreover, it is generally assumed that, unlike what happens in most other tissues, there is no division of the nuclei and, consequently, no increase in their number (3). This view is supported by experiments showing that, while myoblasts can divide at early stages of development, they lose this ability once they have been incorporated into myotubes (4). Furthermore, during muscle regeneration following injury, myoblasts appear which take up thymidine-^{3}H, indicating their ability to divide, whereas the nuclei of muscle fibers do not (5). Hence the prevalent opinion has been that new nuclei are not produced in skeletal muscle fibers during growth.

This view was questioned when DNA determinations revealed a considerable increase in the number of fiber nuclei during growth in rats (6) and chickens (1), and mitotic figures were observed within the muscle fibers of growing rats (7). Furthermore, after an injection of thymidine-^{3}H, radioautography revealed labeled nuclei within the confines of the basement membrane of muscle fibers (7, 8). In the hope of reconciling these apparently conflicting observations, it was suggested that the muscle nuclei which take up thymidine-^{3}H and undergo mitosis are not true muscle nuclei but belong to "muscle satellite cells" (7). Such cells, which had been observed only in the electron microscope (9), consist of a single nucleus surrounded by scanty cytoplasm and are located between the basement membrane and the plasmalemma of the fibers, whereas true muscle nuclei are within the plasmalemma.

Since satellite cell nuclei are eaily distinguished from true muscle nuclei in the electron microscope, it was decided to examine radioautographs in this instrument following thymidine-^{3}H injection. Six male rats aged 17 days and weighing 25–30 g were given a single injection of 25–60 μCi of thymidine-^{3}H/g body weight and sacrificed at intervals varying from 1 to 72 hr later. The tibialis anterior muscle was fixed by immersion in glutaraldehyde paraformaldehyde (10), postfixed in osmium tetroxide, and embedded in Epon. Thin (silver to gold) sections were prepared for radioautography in the electron microscope with Ilford L4 emulsion (11–13) and poststained with lead citrate (14).

Electron microscopy revealed the existence of two types of nuclei within the basement membrane of the fiber. The nuclei of the first type (85–90% of the total) are in direct contact with the myofibril-containing cytoplasm; they are the true muscle nuclei. The nuclei of the second type are surrounded by a small amount of cytoplasm, which is separated from the myofibril-containing cytoplasm by an intercellular space; these are the satellite cell nuclei; they are usually smaller and darker than true muscle nuclei.

In radioautographs obtained 1 hr after injection of thymidine-^{3}H, approximately 3% of the nuclei within the basement membrane were labeled. These invariably belonged to satellite cells (Fig. 1). Not one of the true muscle nuclei was labeled at that time. The results were similar at 6 and 10 hr after injection (Table I). It was, therefore, concluded that satellite cell nuclei, but not true muscle nuclei, were able to synthesize DNA. Moreover, since it is known that DNA synthesis precedes mitosis (8), it may be concluded that the mitotic figures previously observed in the muscle fibers of growing rats (7) must belong to satellite cells. This conclusion is in accord with a recent report that, after colchicine treatment of 30-g rats, arrested mitoses of the nuclei of satellite cells, but not of true muscle nuclei, may be observed (15).

By 24 hr, a few true muscle nuclei were also labeled (Table I). At 48 hr, the labeling frequency of true muscle nuclei was increased, and by 72

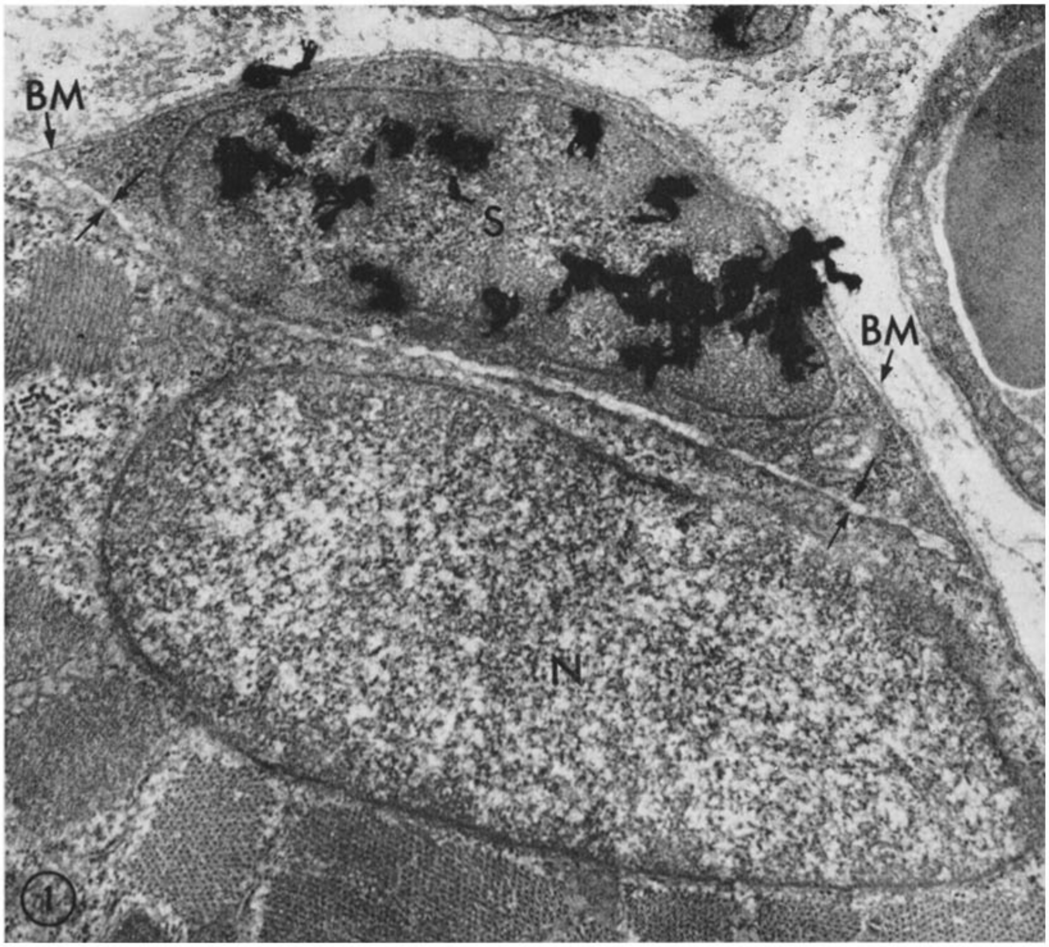

FIGURE 1 Electron microscope radioautograph of the tibialis anterior muscle from a 30 g rat, 10 hr after injection of thymidine-^{3}H. A satellite cell (*S*) identified by the intercellular space (opposing arrows) which separates it from the myofibril-containing cytoplasm is located immediately beneath the basement membrane (*BM*) of a muscle fiber. Below, a true muscle nucleus (*N*) is observed within the plasmalemma of the fiber in direct contact with myofibrils; it is somewhat larger and lighter than the satellite cell nucleus. The satellite cell nucleus is overlaid with silver grains, indicating that it has taken up thymidine-^{3}H during the premitotic DNA synthesis. × 30,000.

hr, it exceeded that of satellite cells (Fig. 2). Since a dose of thymidine-^{3}H is metabolized within a few hours after injection (8), the label found within true muscle nuclei at 24 hr or later must have come from cells which took up the label immediately after injection, i.e. from satellite cells. It was concluded that, after division of the labeled satellite cells had taken place, one or both daughter cells were incorporated into the fiber, thus becoming true muscle nuclei.

In conclusion, the results confirm the classical view that true muscle nuclei do not divide, while providing an explanation for the increase of their number during growth. The satellite cells associated with muscle fibers are capable of undergoing mitosis in growing animals; and such mitoses are followed by the incorporation of one or both daughter nuclei into the associate fiber. Satellite cells thus function as myoblasts.

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TABLE I

*Nuclear Labeling in Tibialis Anterior Muscle of 25–30 g Male Rats at Various Times after a Single Injection of Thymidine-³H, as Seen in Electron Microscope Radioautographs**

Time elapsing between thymidine-^{3}H injection and sacrifice	No. of nuclei labeled	
	Satellite cell nuclei	True muscle nuclei
hr		
1	20	0
6	11	0
10	24	0
24	8	2
48	12	11
72	4	24

* Over 300 nuclei examined/animal.

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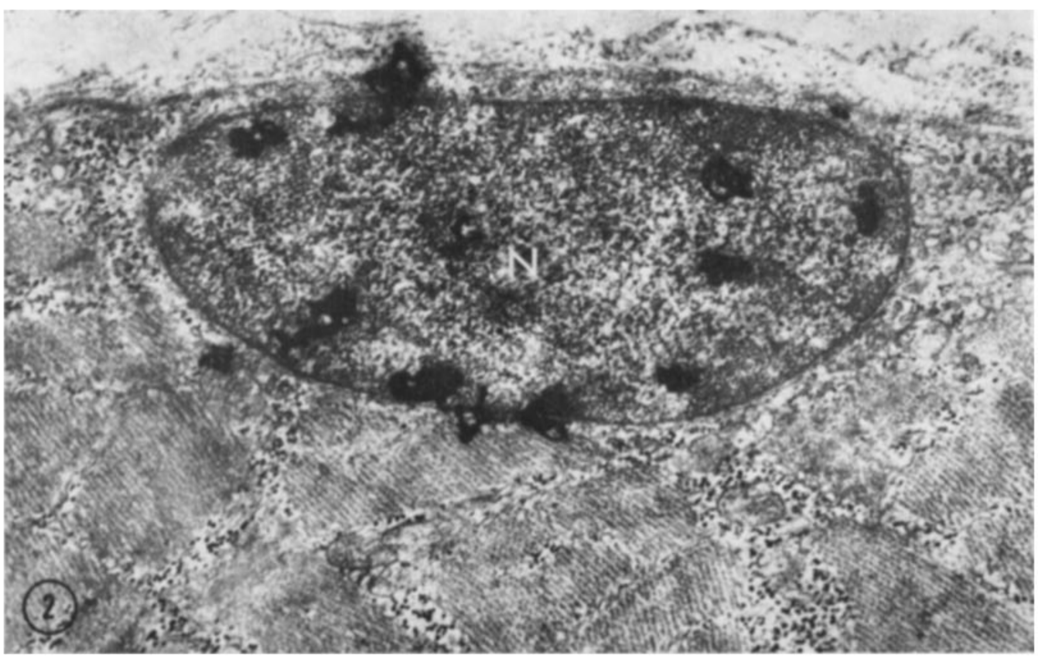

FIGURE 2 Electron microscope radioautograph of the tibialis anterior muscle from a 30 g male rat, 48 hr after injection of thymidine-^{3}H. A true muscle nucleus (*N*), located within the plasmalemma of a fiber, is in direct contact with its myofibril-containing cytoplasm. This nucleus is overlaid with silver grains, indicating that it is the result of mitosis of a nucleus which took up thymidine-^{3}H during DNA synthesis almost 48 hr previously. Since satellite cell nuclei, but not true muscle nuclei, are able to synthesize DNA, it is concluded that this labeled nucleus is the result of incorporation of a satellite cell into the fiber following mitosis. × 30,000.

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