CENTRIOLES IN INTRAFUSAL MUSCLE FIBERS

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INTRODUCTION

Centrioles are generally considered to be involved in cell division and are also related to the differentiation of cilia and flagella.

Most skeletal muscle contains stretch receptors called muscle spindles consisting of a bundle of small (intrafusal) muscle fibers which are innervated by both sensory and motor nerves. The fine structure of muscle spindles in a variety of species from amphibian to mammalian has been studied intensively (see Barker, 1973), but centrioles in intrafusal muscle fibers have not been described. A large number of electron microscope studies on skeletal muscle have also been published during the past two decades (Peachey, 1968; Hoyle, 1969; Sandow, 1970). The occurrence of centrioles during myogenesis has been observed, but, to our knowledge, centrioles have not been reported in mature skeletal muscle. On the other hand, differentiated heart muscle cells do contain centrioles (Przybylski, 1971). During a study of the ultrastructure of adult cat muscle spindles, we have unexpectedly observed centrioles in the intrafusal muscle fibers.

This study will describe the fine structure of centrioles and the relationship between centrioles and other cytoplasmic organelles in intrafusal muscle fibers. Some implications of these findings will be discussed.

MATERIALS AND METHODS

Seven muscle spindles completely or partially isolated from tail muscles in four adult cats were investigated. The animals were anesthetized with intraperitoneal sodium pentobarbital 35 mg/kg. Dissection was carried out in mammalian Ringer with the aid of a stereomicroscope at room temperature. Isolated muscle spindles were put on glass cover slips. Then,

each end of the muscle spindle, with extrafusal fibers attached, was lightly stretched by grasping the ends with metal clamps. Some whole muscles which contained muscle spindles were exposed and fixed in situ.

The spindles were initially fixed with 1.5-3.5% glutaraldehyde buffered with 0.0675 M phosphate buffer (pH 7.3-7.5, 354-600 mosM) for 2 h. Subsequent to fixation, they were rinsed for 2-12 h in phosphate buffer and postfixed with 1% osmium tetroxide in the same buffer at 4°C. Rapid dehydration in a graded series of cold ethanol was followed by infiltration and embedding in Araldite (Luft, 1961). Care was taken to embed the tissues so that sections could be cut in either transverse of longitudinal planes.

Of seven muscle spindles, three muscle spindles were cut at random into transverse sections. One muscle spindle was more systematically sectioned. It was cut transversely into alternate thick and thin sections from the end of the capsular region to the midequatorial region. 20 sections with a thickness of about 1 μm were followed by 100 thin sections with a thickness of 50-80 nm. In this case, the total length covered amounted to about 600 μm. Three remaining muscle spindles were longitudinally sectioned.

Thick sections stained with toluidine blue were used for survey purposes. Thin sections stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) were examined in a Siemens Elmiskop 1A electron microscope.

RESULTS

This investigation focused its attention on the presence or absence of centrioles in the intrafusal muscle fibers. Three of the seven muscle spindles examined contained centrioles. A total of four centrioles and two diplosomes (a pair of centrioles) were found in these three spindles. No

centrioles were noted in extrafusal muscle fibers, however, a systematic search was not made.

The fine structure of centrioles in the intrafusal muscle fibers was identical with those of other cells (Fawcett, 1966; Stubblefield and Brinkley, 1967; Fulton, 1971). The centriole had inner and outer diameters of 1,500 and 2,000 Å, respectively (Figs. 1 and 2), and a length of about 2,700 Å (Fig. 6). As is generally known, the wall of a centriole consists of nine longitudinally oriented triplet tubules arranged in pinwheel fashion. Each tubule measured about 250 Å in diameter. Figs. 1 and 2 show seven and four triplet tubules, respectively. Other triplets appear to be obliquely arranged. Fawcett (1966) described that a part of the wall is usually tilted because of the spiral fashion of the wall. The pair of centrioles shown in Fig. 6 exhibit the usual perpendicular orientation. From the aforementioned results it is reasonable to conclude that centrioles exist in the intrafusal muscle fiber of the adult cat. No cilia were found in the intrafusal muscle fiber.

The muscle spindle has two types of intrafusal muscle fibers, namely the nuclear chain and the nuclear bag fibers. Two diplosomes and three centrioles were found in the nuclear chain fibers, and one centriole was found in a nuclear bag fiber. The one found in the nuclear bag fiber was located in the juxta-equatorial region. In the nuclear chain fibers, one diplosome was found in the same region described above (Fig. 5) and the others were located between the juxta-equatorial and intracapsular polar region (Figs. 7 and 1).

In the equatorial and myotubule region of the intrafusal muscle fiber, the nuclei are situated in the center and the myofibrils occupy the periphery. In the polar region the nuclei move toward the periphery, so that the central region becomes occupied by myofibrils. All centrioles and diplosomes were encountered in the sarcoplasm which lay between the myofibrils, particularly around the nucleus. Therefore, the location of centrioles in the intrafusal muscle fiber appears to be variable (Figs. 5 and 7).

The centrioles and diplosomes were found in association with Golgi complexes (Figs. 2-4, and 6) and/or microtubules (Figs. 1 and 2). These relationships are well known in most animal cells. In two cases, so-called "micro-ladders," "ladder-like" structures (Katz, 1961), or "leptomeric organelles" (Karlsson and Andersson-Cedergren, 1968) appeared close to the centriole or diplosome (Figs. 1 and 6). The perpendicular arrangement of fine filaments around the centriolar wall is demonstrated in Fig. 6. Some of these filaments seem to approach the leptomeric organelle. This particular leptomeric organelle of extraordinarily large size can be seen in the low magnification electron micrograph (Fig. 7). It is not clear whether the leptomeric organelles adjacent to the centrioles is metely a coincidence or whether these leptomeric organelles do indeed arise from the centrioles.

DISCUSSION

As has been described in the Results, there is no doubt about the identification of centrioles in intrafusal muscle fibers. Their dimension, the existence of peripheral triplets of tubules arranged in pinwheel pattern, their location in juxta-nuclear region, and the right angle orientation in the cases where a pair appeared, are all typical.

The finding of centrioles in intrafusal muscle fibers of three muscle spindles required the examination of many thin sections. Consequently, the fact that no centrioles were seen in the remaining four muscle spindles was most probably caused by the insufficiency of sampling rather than by the absence of centrioles.

In most animal cells, centrioles are considered to perform important functions in cell division and in the formation of cilia and flagella (Fulton, 1971).

Although early investigators suggested that muscle spindles might be centers for growth (Kölliker, 1862) or regeneration of muscle (see Batten, 1897), it is now clear that this is not so but that spindles are specialized sensory organs, as originally shown by Sherrington (1894). Mitotic figures are not seen in differentiated intrafusal muscle fibers. Further, Marchand and Eldred (1969) showed that intrafusal muscle fibers of newborn to 12-day old rats did not incorporate labeled thymidine into their nuclei. Hence, the centrioles in adult intrafusal fibers do not appear to be involved in cell division.

In the intrafusal muscle fibers of adult cats, the participation of centrioles in ciliogenesis is doubtful because of the absence of cilia. However, according to Fulton (1971) and others, a centriole has the same structure as a basal body of a cilium and flagellum. Centrioles and cilia which arise from centrioles have been observed in embryogenesis of skeletal and heart muscle (Przybylski, 1971). It seems possible that centrioles and cilia exist in the intrafusal muscle fibers in their developmental stage. Therefore, some centrioles in
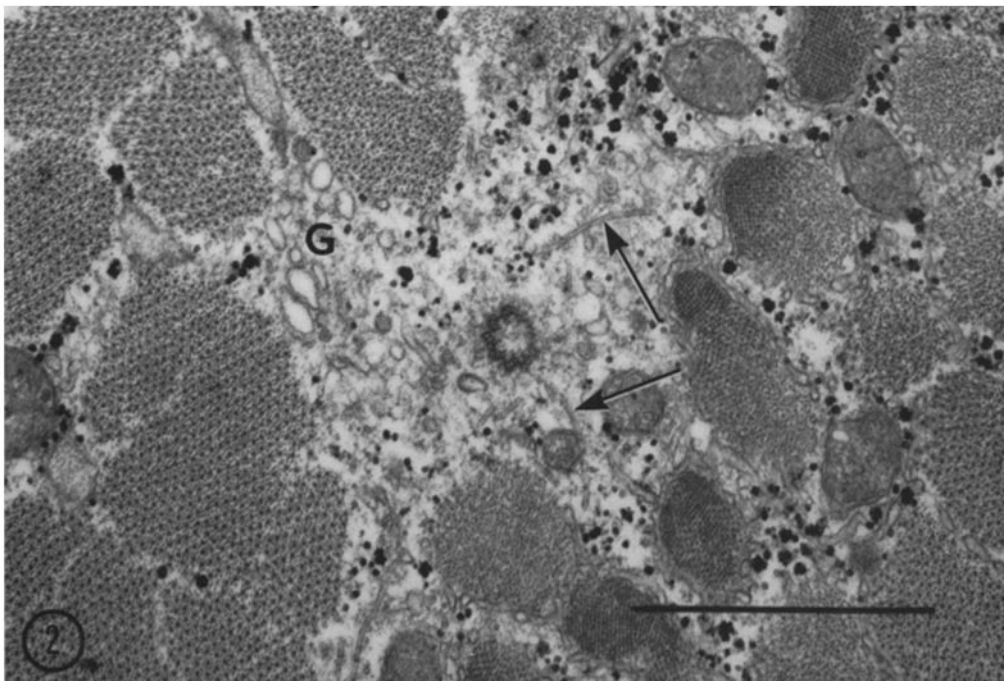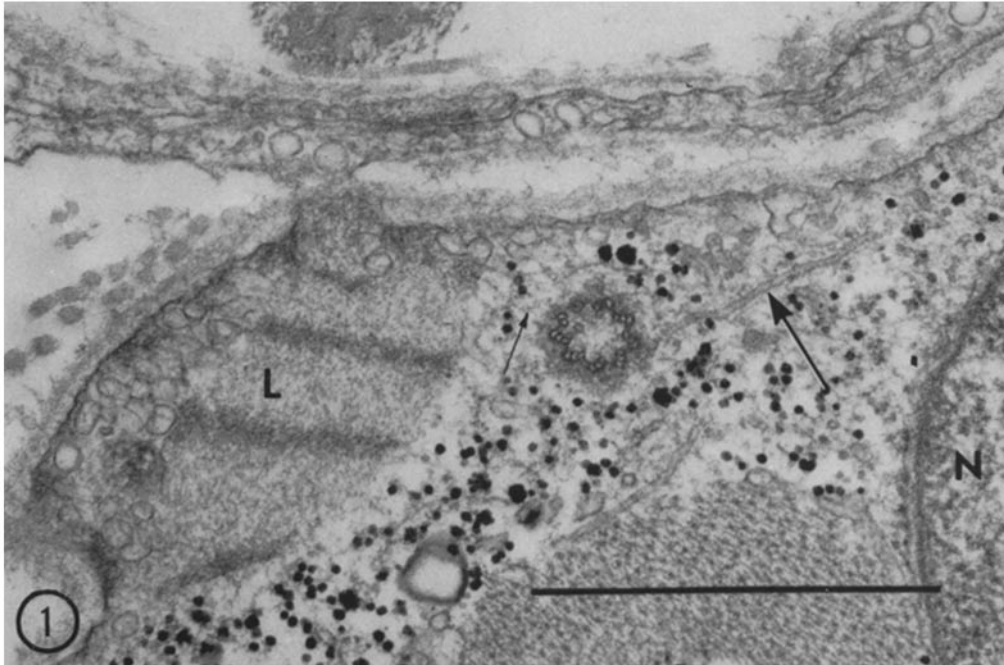
FIGURE 1 A centriole in the intracapsular polar region of a nuclear chain fiber. Thin filaments (thin arrow) reaching between the centriolar wall and the dense band of a leptomeric organelle (L). A microtubule (thick arrow) is present in close proximity to the centriole. N, nucleus. Scale line, 1 μm. × 54,000.

FIGURE 2 A centriole in a nuclear chain fiber from a more proximal region than Fig. 1. A Golgi complex (G) and microtubules (arrows) are present adjacent to the centriole. Scale line, 1 μm. × 40,000.
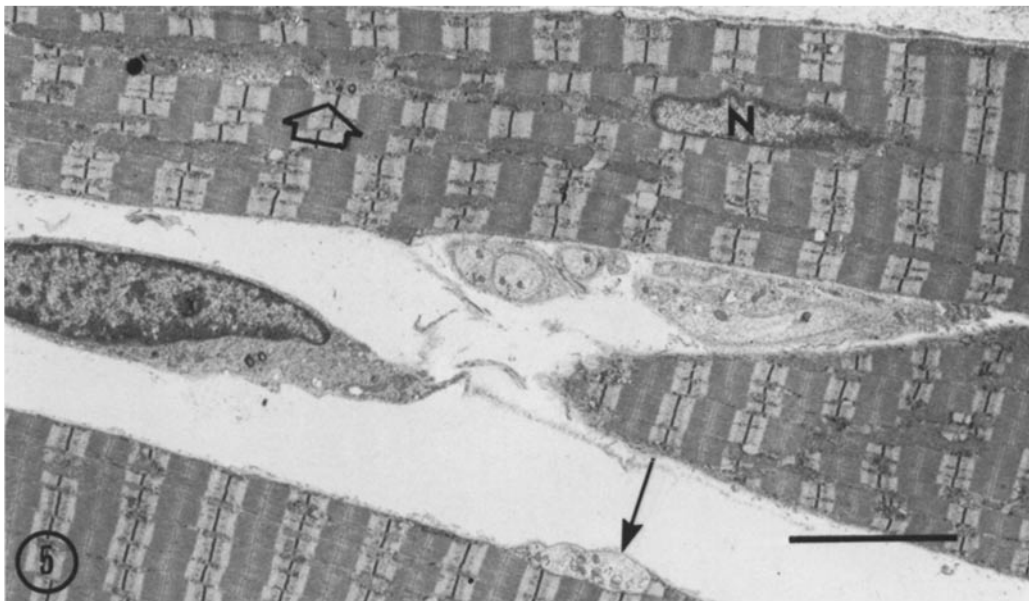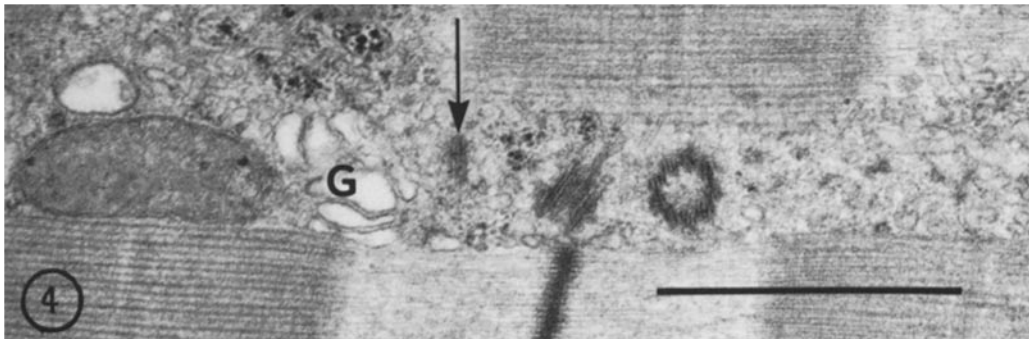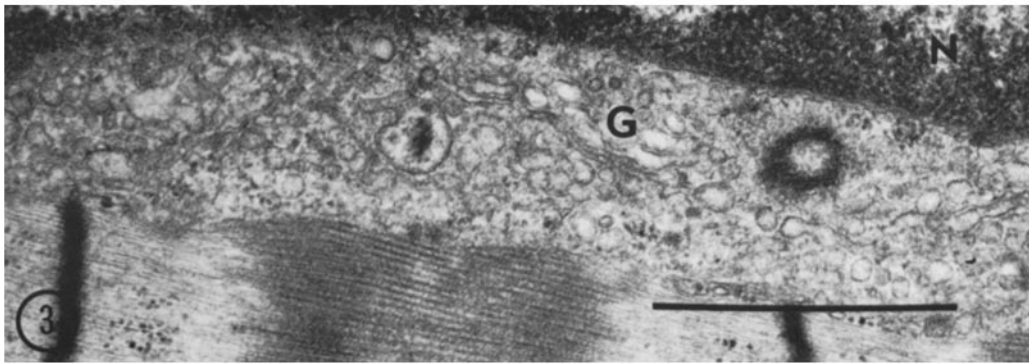
FIGURE 3   A centriole in the juxta-equatorial region of a nuclear bag fiber. It is located close to a nucleus (N) and Golgi complex (G). Scale line, 1 μm. × 40,000.

FIGURE 4   A diplosome and presumably a part of the wall of a daughter centriole (arrow) in the juxta-equatorial region of a nuclear chain fiber. G indicates dilated Golgi sacs. Scale line, 1 μm. × 40,000.

FIGURE 5   A survey picture including the area shown in Fig. 4 (open arrow). A nucleus (N) occupies a central portion of this fiber. A small sensory nerve ending (arrow) terminates on a nuclear chain fiber at bottom. Scale line, 5 μm. × 4,400.
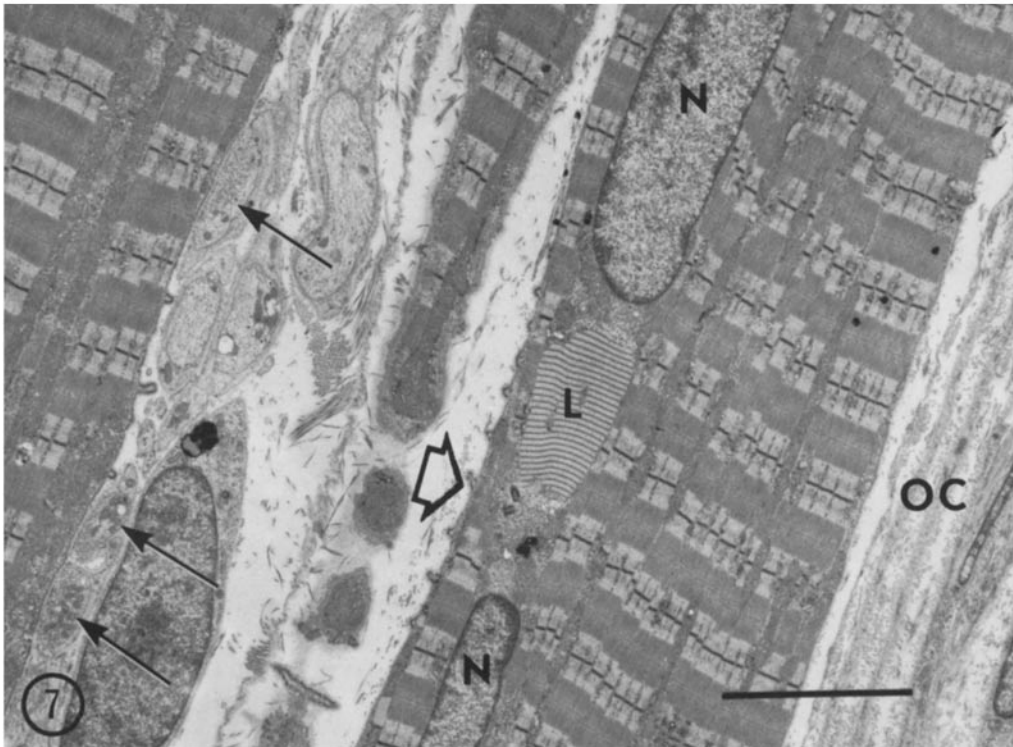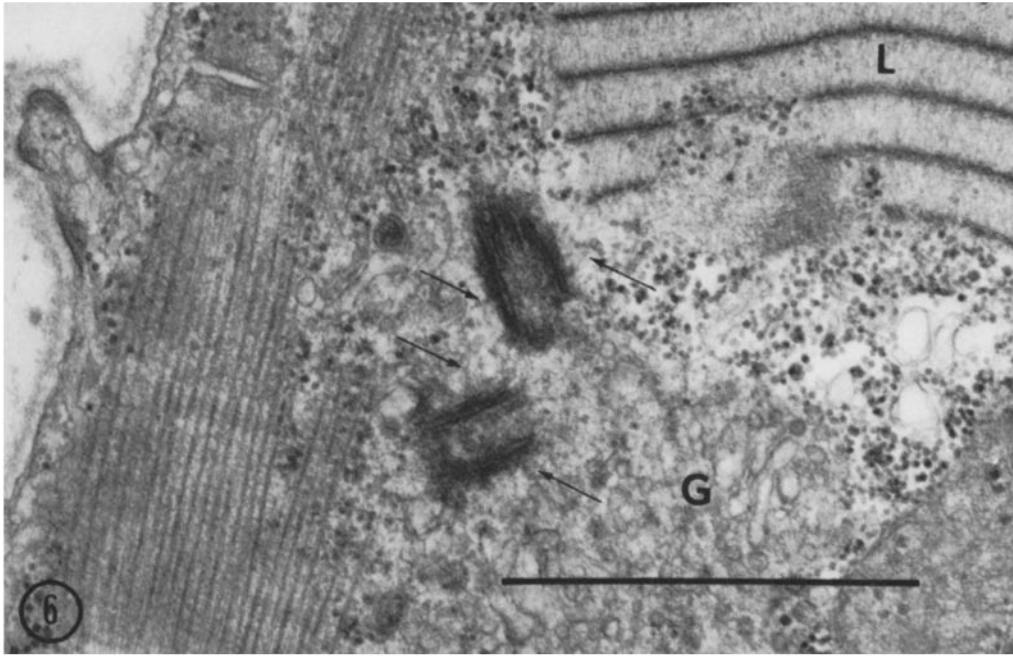
FIGURE 6 A pair of centrioles in the intracapsular polar region of a nuclear chain fiber. They exhibit a right angle orientation. Arrows indicate fine filaments around the centriolar wall. *G*, Golgi complex. *L*, leptometric organelle. Scale line, 1 μm. × 55,000.

FIGURE 7 A survey picture including the area shown in Fig. 6 (open arrow). Parts of capsule cells (*OC*) and three nuclear chain fibers are present. The chain fiber at left is innervated by trail motor nerve endings (arrows). *N*, nucleus. *L*, leptomeric organelle. Scale line, 5 μm. × 5,000.

the adult intrafusal muscle fibers could be rudimentary or abortive cilia.

Other functions ascribed to centrioles are the reproduction of new centrioles (Stubblefield and Brinkley, 1967; Fulton, 1971), microtubule formation (Stubblefield and Brinkley, 1967; Fulton, 1971), and the movement of the nucleus (Bessis and Breton-Gorius, 1967; Rondanelli et al., 1968). Small fragments of tubules are present on the left side of the diplosome in Fig. 4. They may be a part of the wall of a daughter centriole. If so, the centrioles of intrafusal muscle fiber may be capable of producing new centrioles. The relation between centrioles and microtubules is not clear in the intrafusal muscle fiber. Ovalle (1972) found that nuclear chain fibers exhibited a greater abundance of microtubules than the nuclear bag fibers. The distribution of centrioles in intrafusal muscle fibers showed a similar tendency. Only the presence of nuclei in intrafusal muscle fibers suggests that the centrioles may in some way participate in the movement of the nuclei in intrafusal muscle fibers.

Sakaguchi (1965) and Lauweryns and Boussauw (1972) demonstrated that centrioles in various kinds of cells were associated with the striated filamentous bundles. The leptomeric organelles, which also have cross striation, are occasionally encountered in intrafusal muscle fibers (Katz, 1961; Barker, 1973).

This study provides little information about the function of centrioles in intrafusal muscle fibers, however, it clearly shows that some, if not all, adult cat muscle spindles contain centrioles.

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