

## MITOSIS IN ADULT NEWT VENTRICLE

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### INTRODUCTION

Two recent electron microscope studies have demonstrated mitosis in embryonic cardiac fibers. In the chick (3) and the rat (8), both myofibrils and mitotic chromosomes were seen within the same cells. In the normal adult heart, however, cardiac fibers do not usually undergo cell division (2, 7). The ability of adult cardiac fibers to divide after injury has been a matter of controversy (reviews 4, 9). A number of studies at the light microscope level have indicated that newborn rat heart (6) and adult frog (7) and rat (1) cardiac muscle can respond to injury by undergoing DNA synthesis and mitosis. However, in light micrographs, it is difficult to identify with certainty the cell type of these mitotic cells. The present electron microscope study demonstrates that mitotic cells in a wounded area of the newt heart do possess characteristics of a cardiac fiber.

### MATERIALS AND METHODS

The observations of the present investigation were made on adult newts, *Diemictylus vividescens*, which had been collected in Tennessee. The hearts of the animals were surgically exposed and a small portion of the ventricle near the apex of the heart of each animal was removed with iridectomy scissors. In one series, hearts were taken for fixation at 16, 18, and 22 days after the wound was made. In a second series, the animals received 0.05 mg of colchicine 6 hr before fixation at 20 days after the injury. Normal, uninjured ventricles were also fixed for electron microscopy.

Tissues taken for electron microscopy were fixed for 2 hr in cold 3% glutaraldehyde, buffered at pH 7.4 with 0.1 M phosphate buffer. After a buffer wash, the tissues were postosmicated for 2 hr in 1% OsO<sub>4</sub>, buffered at pH 7.4 with Millonig phosphate buffer. Tissue blocks were dehydrated through a series of graded ethanols and propylene oxide, and were embedded in Epon 812. Thick sections were cut for

light microscopy and were stained in 1% toluidine blue in 1% borax. Thin sections were cut and mounted on 200-mesh copper grids and were double stained with uranyl acetate and lead citrate (11). Tissue sections were examined with RCA EMU-3F and Philips EM-200 electron microscopes.

#### OBSERVATIONS

Mitotic figures were observed in thick sections of hearts which were fixed 16, 18, 20, and 22 days after the wounding of the ventricle. All of these mitoses were located adjacent to the wound area and none were seen in sections of uninjured adult newt ventricle. Few of these mitotic cells were seen in thin sections with the electron microscope.

In Fig. 1 is seen a mitotic cell from a heart fixed 20 days after the ventricle was injured. This cell is almost completely surrounded by myofibers. No characteristics were apparent at this resolution which permitted a positive identification of cell type. An electron microscope examination of the cytoplasm of this cell revealed that it contained a group of filaments which had the characteristics of myofilaments (Fig. 2). Although some of the filaments were arranged haphazardly, others showed a more orderly arrangement and appeared to be approximately the length of a normal sarcomere. In the section seen in Fig. 2, the thick filaments, which measured approximately 130 Å in diameter, were cut in cross-section. In addition to these thick filaments, thin filaments were also observed in the group of filaments near the cell membrane.

Mitotic cells were also seen in the wound area of hearts fixed at 16 days. A portion of one of these cells is seen in Fig. 3. A number of fibrils, which appeared to be myofibrils approximately the length of a sarcomere, were found at the periphery of the cell. The peripheral myofibrils of another area of this cell are enlarged in Fig. 4. The filaments appear to have the same dimensions as the thick filaments in the myofibrils of an adjacent nondividing cell. Thin filaments were not apparent in this mitotic cell. Z bands were not observed in either of the cells which were studied. Desmosomes or portions of intercalated discs were seen at the junctions of the mitotic cell with the neighboring cardiac fibers (Fig. 4).

Two other characteristics typical of cardiac fibers are shared by these mitotic cells. They both contain glycogen and there is a basal lamina in association with each (Figs. 2 and 3).

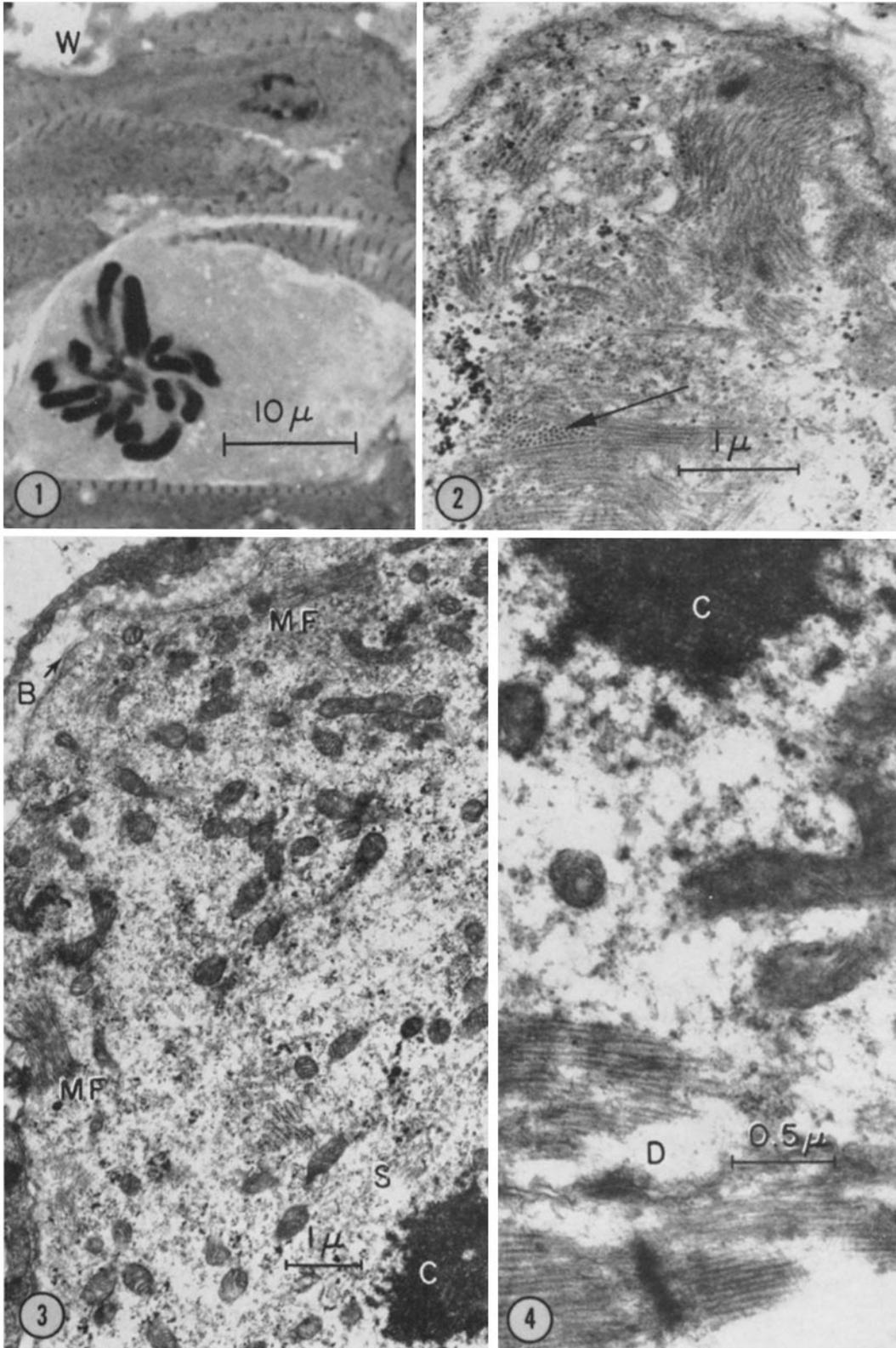
#### DISCUSSION

The dividing cells which were observed in this study of injured adult cardiac muscle are remarkably similar to those which were observed in the embryonic cardiac muscle of the chick by Manasek (3) and of the rat by Rumyantsev and Snigirevskaya (8). In the observations of the latter investigators, as in those of the present study, a polar arrangement of myofilaments was found predominantly at the periphery of the dividing cells. In the mitotic cells of the injured newt heart, the bundles of myofilaments sometimes had a disordered arrangement, similar to the bundles in the cells of the embryonic rat heart which Rumyantsev and Snigirevskaya described.

The myofibrils observed in the present study appeared to be approximately the length of a sarcomere. No Z bands were observed. A similar subdivision of myofilament bundles of the embryonic rat heart was observed by Rumyantsev and Snigirevskaya (8), who reported that some rarefactions were seen in place of Z bands. The same study also indicated that a partial disappearance of some of the Z bands might play an important role in the disordered orientation of myofilament bundles. In the chick embryo, Z bands were found in the DNA synthesis phase of the cell cycle (10). Although Rumyantsev and Snigirevskaya found Z bands with regularity in prophase cells in the embryonic rat, the Z bands were rarely observed in the later stages of cell division. The present study in injured adult newt heart describes cells only in later stages of mitosis, and the presence or absence of Z bands at other stages of cell division remains to be demonstrated in the adult newt. On a physical basis, such a disruption of the myofibrils in a cardiac fiber may be a factor which allows for the process of cytokinesis.

Recent investigators (3, 8) observed the preservation of desmosomes or primitive intercalated discs throughout mitosis in cardiac fibers. In the adult newt heart, although areas without cell junctions were found, a number of intact desmosome-like structures were present between mitotic cells and adjacent cardiac fibers.

Besides myofibrils, the dividing cells of this study illustrated two other features typical of cardiac fiber. A basal lamina was associated with the mitotic cells and glycogen granules were found within the cells. In the uninjured newt myocardium, cardiac fiber seems to be the only cell type having an associated basal lamina. Glyco-



gen was demonstrated in cardiac mitotic cells of the chick (3) and the rat (8). According to a study in the chick embryo by Polinger (5) and also according to observations on uninjured adult newt heart, cardiac fiber seems to be the only cardiac cell type that contains glycogen.

Mitosis in the adult newt heart seems to be a reaction to injury, since the mitotic cells were found only in the area near the wound. This mitotic response involves cells which have the characteristics of cardiac fibers. These characteristics suggest that the origin of such cells may be from adult cardiac fiber, although the possibility that they may have their origin from other cells of the heart must also be considered. In either case, the observations of the present study and those of recent investigators (3, 8, 10) do support the concept that the presence of myofibrils does not preclude cell division.

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FIGURE 1 Portion of a ventricle 20 days after injury. The cell in mitosis is located near the wound (W). It is nearly surrounded by myofibrils which appear to be in neighboring cells.  $\times 2000$ .

FIGURE 2 A group of myofilaments found in the cytoplasm of the mitotic cell in Fig. 1. The thick filaments correspond in size to the myosin filament of a cardiac myofibril. Thin filaments can be seen in the group of filaments close to the cell membrane. Cross-sections of the thick filaments (arrow) can also be seen in this section.  $\times 18,000$ .

FIGURE 3 A portion of a mitotic cell from a ventricle 16 days after injury, containing chromosomes (C) and peripheral myofibrils (MF). A basal lamina (B) is associated with the cell. Spindle fibers (S) of the mitotic apparatus are also present.  $\times 11,500$ .

FIGURE 4 An electron micrograph of an area of the mitotic cell adjacent to the area seen in Fig. 3. A peripheral myofibril is located just above the cell membrane, while myofibrils of the adjacent cell are below the membrane. A portion of a chromosome (C) is located in the upper end of the figure. At the junction between the nondividing cell and the mitotic cell is seen a desmosome (D).  $\times 31,000$ .