MITOSIS IN DEVELOPING CARDIAC MUSCLE

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INTRODUCTION

The myocardium of the developing chick undergoes a rapid increase in size and complexity during embryonic life. Cell division occurs throughout the myocardium during this period, but decreases as embryonic development progresses. The mitotic indices for different regions of the developing heart have been determined in the light microscopic observations of Grohmann (1) for the entire period of embryonic life.

Several studies of developing embryonic myocardial cells have suggested that cells containing myofibrils do not divide. Working with cultured myocardial cells, Rumery and Blandau (2) and, more recently, Rumery and Rieke (3) were unable to detect mitotic figures in cells containing myofibrils visible under the light microscope. The electron microscopic study of Wainrach and Sotello (4) suggested that the developing myocardium may contain a population of undifferentiated cells and that mitoses are limited to this group. These workers observed no mitotic figures in cells with myofibrils. Mark and Strasser (5), however, observed a mitotic figure in cultured rat heart cells containing striated myofibrils visible under the light microscope. More recently, DeHaan (6), working with cultured embryonic chick heart cells, observed an increased rate of mitosis after feeding embryo extract to cell cultures containing actively contracting cells. He suggested that differentiated, actively contracting cells, as well as noncontracting cells, are able to divide under these conditions.

Similar work with developing skeletal muscle strongly suggests that cells of this type containing myofibrils do not divide. Przybylski and Blumberg (7), in their recent electron microscopic study of developing chick skeletal muscle, failed to detect cell division in myotubes. Immunochemical and radioautographic studies of Stockdale and Holtzer (8) and Okazaki and Holtzer (9) suggested that developing skeletal muscle cannot simultaneously synthesize contractile protein and DNA, supporting the concept that cell division and differentiation are mutually exclusive events. Indeed, it has been suggested that other cell systems exhibit the same dichotomy between mitosis and synthesis of proteins characteristic of the differentiated state (10).

The present report presents morphological evidence that mitosis and the presence of myofibrils are not incompatible in the developing chick heart. The myocardium of embryonic chicks in various stages of development was extensively studied with the electron microscope. This report records observations on the occurrence of mitotic division among chick heart cells that contain myofibrils. Other findings relevant to the differentiation of myocardial cells will be the subject of a longer paper.

MATERIALS AND METHODS

Fertile white Leghorn chicken eggs were incubated at 38°C for various periods of time to yield embryos ranging from Hamburger-Hamilton (11) stage 8 to stage 37. Eggs were then carefully opened to expose the embryos which were removed and placed in cold (0°C) glutaraldehyde-formaldehyde fixative (12), buffered to pH 7.6 with 0.2 M cacodylate buffer. Younger embryos were fixed whole, but the hearts of larger embryos were dissected free. All material was fixed at 0°C for 15 min. After a brief rinse in cold 0.2 M cacodylate buffer, pH 7.6, the tissues were placed in 1% OsO_4 at 0°C for a period of 2 hr. Following osmication, some specimens were block-stained with uranyl acetate (13) prior to dehydration and embedding in Araldite. Sections were cut with diamond knives on a Huxley microtome. They were mounted on uncoated copper grids and stained with lead citrate (14). Sections were examined in a RCA EMU-3F microscope.

RESULTS AND DISCUSSION

Dividing muscle cells were observed in hearts of embryos ranging through stage 37 (11 days), the oldest examined in this series. Contrary to a previous report (4), no evidence was found in the present study for the existence of a pool of undifferentiated cells in the developing myocardium. Although there was some variability in the number of filaments in individual cells, in no instance were the dividing cells significantly retarded in this respect relative to the surrounding cells. Myocardial cells of older embryos contained large accumulations of glycogen and well developed myofibrils comprised of both thick and thin filaments. Glycogen was equally abundant in the dividing cells (Fig. 1), and the myofibrils were frequently displaced to the periphery (Figs. 2, 3). The peripheral disposition of myofibrils is presumed to be due to the presence of the spindle apparatus near the center of the cell (Fig. 3).

In a cinematographic study depicting an actively contracting rat heart cell in a state of division, Mark and Strasser (5) noted that the myocardial cell did not round up prior to division and did not separate from its neighbors during cytokinesis. The present study corroborates this earlier light microscopic observation by demonstrating the presence of desmosomes and developing intercalated discs attaching dividing myocardial muscle cells to their neighbors. Cells in various stages of mitosis were observed, and in no instance were any cell attachments separated.

The occurrence of mitoses among cells containing myofibrils and the absence of an undifferentiated myocardial cell population indicate that the increase in muscle cell numbers in the develop-

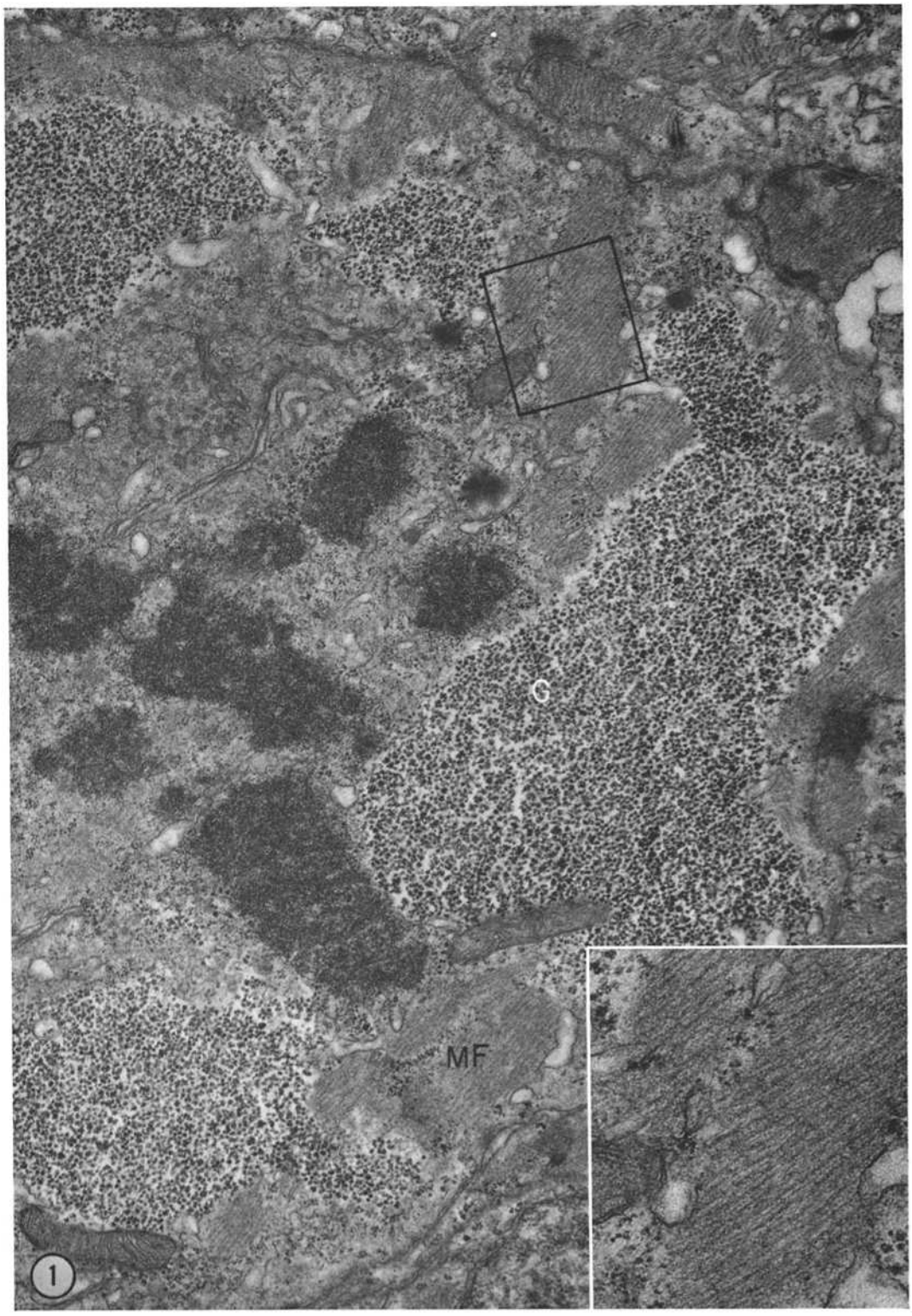

FIGURE 1 This dividing cell from the ventricle of an 11-day-old chick embryo exhibits a normal state of differentiation. Large accumulations of glycogen (*G*) are present and the cytoplasm contains numerous myofibrils (*MF*) which are cut tangentially. A desmosome and a developing intercalated disc are present. Chromosomes are prominent, and the cell has retained its Golgi complex. *Inset:* Higher magnification of the tangentially cut myofibrils demonstrating thick and thin filaments.

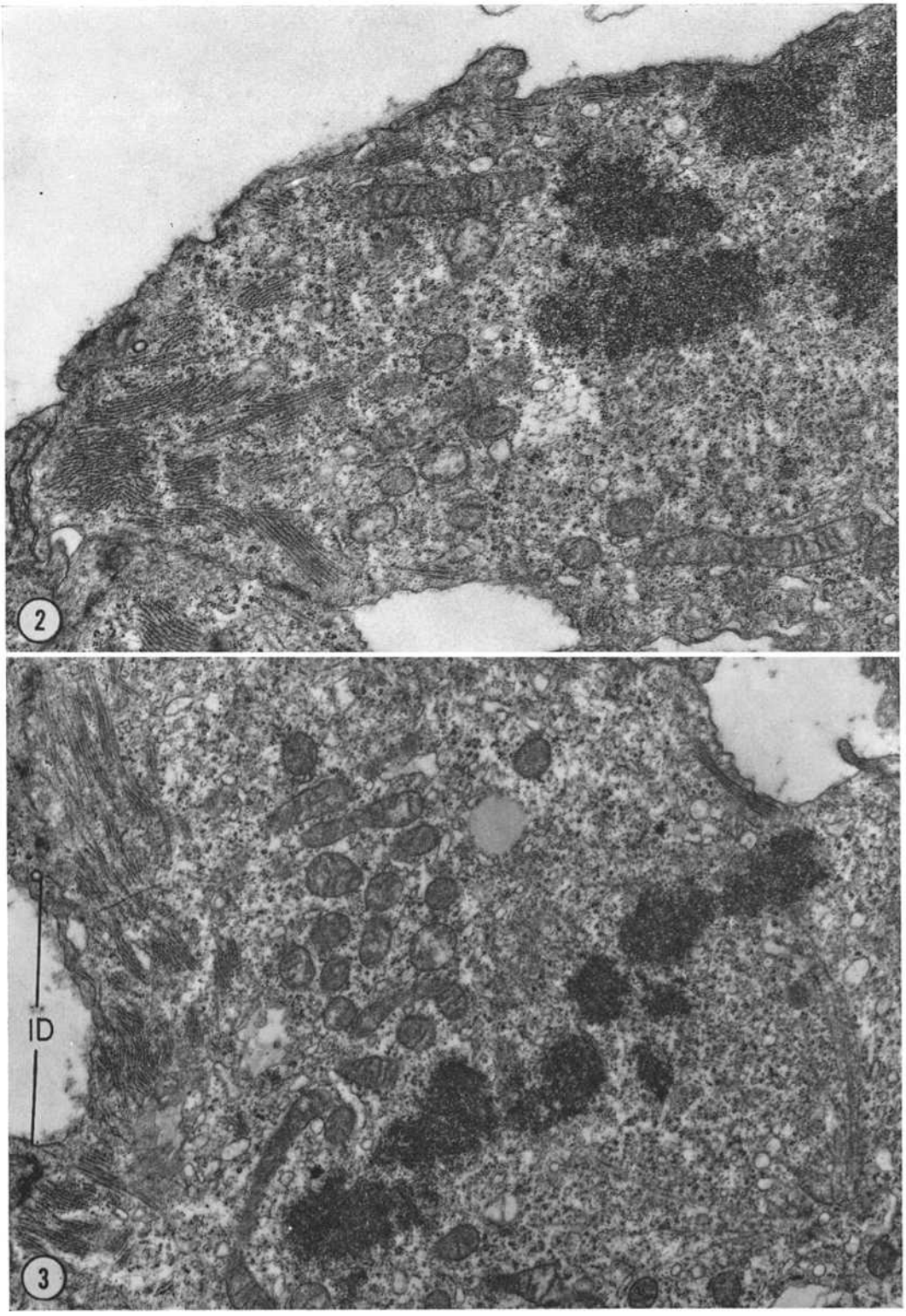

FIGURE 2 In this dividing myocardial cell from the ventricle of a 7-day-old embryo the myofibrils are not so well developed as those in the 11-day-old embryo of Fig. 1. In this cell, their appearance appears to be restricted to the periphery of the cell.

FIGURE 3 This dividing ventricular cell was found in the same heart as the one in Fig. 2. The mitotic spindle apparatus is visible in the lower right. The myofibrils in this cell, too, are primarily found in the peripheral cytoplasm. Two developing intercalated discs (*ID*) are seen in the left-hand portion.

ing heart is the result of divisions in the general population of developing muscle cells, and is not to be attributed to differentiation from a population of proliferating precursors. The reported increase in ventricular mitotic index from 1.5 at 30 hr of incubation to a peak of about 3.2 by the 4th day of incubation (1) therefore suggests that mitotic events actually become more frequent as the cells comprising this tissue initially accumulate myofibrils.

These observations appear to be in conflict with studies on other developing muscle systems. The work of Stockdale and Holtzer (8) indicated that developing skeletal muscle cells growing in culture no longer synthesize DNA once their cytoplasm contains myosin. The findings of these workers are in agreement with the results of other investigators who failed to detect cell division in the syncytial myotube stage (7, 15) and have been interpreted as suggesting that the production of myofibrils may be an event which inhibits cell division.

The observations reported here show that the cessation of cell division in the mononucleate cardiac cells is not necessarily correlated with the appearance of cytoplasmic myofibrils. If the mechanisms governing mitosis in cardiac and skeletal muscle are similar, then the cessation of division in skeletal muscle myotubes may not be related to the presence of myofibrils, but rather to some other developmental event. Certainly, the present observations suggest that an extrinsic control of mitosis in this cell type remains a real possibility. Although mitotic activity in the intact myocardium differs greatly in embryos of different ages (1), Chaytor (16) reported that cells grown from donor hearts of different ages exhibited similar mitotic indices after a short period of tissue culture. This also would seem to suggest that in the intact embryo mitotic restraint with advancing age may be a consequence of the organization of the cells into myocardial tissue, rather than a loss of the ability of these cells to divide as they become more specialized.

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