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2 October 1973

DNA Synthesis and Mitosis in Well-Differentiated Mammalian Cardiocytes

Abstract. *Incorporation of [^{3}H]thymidine into nuclei of heart cells of 2-day-old rats indicates that neonatal cardiac cells containing well-aligned myofibrils synthesize DNA. In these highly differentiated cells, neither the presence of contractile proteins nor their organization into myofibrils inhibits either DNA synthesis or mitosis.*

The relation between DNA synthesis and mitosis and the manufacture of cell-specific proteins has been the subject of numerous studies. In many tissues, differentiation occurs only in cells that no longer divide (for example, nerve cells, keratinizing cells, and reproductive cells). The suggestion that production of myofibrils in striated muscle may inhibit cell division is based on evidence obtained in studies of skeletal muscle (*1*, *2*). Skeletal muscle cells containing myofibrils do not divide (*3*).

Unlike skeletal muscle, embryonic cardiac cells synthesize DNA and divide after myofibrils have appeared (*4–8*). With autoradiography, DNA synthesis has been demonstrated in differentiating cardiac cells of chick embryos (*4*, *5*). Mitotic figures have been observed in electron micrographs of chick heart cells containing a few myofibrils (*6*, *7*). Chacko (*9*) has observed mitosis in electron micrographs of rat cardiocytes during late gestation and at birth. Therefore, in differentiating embryonic cardiac muscle, the appearance of contractile proteins inhibits neither DNA synthesis (*5*) nor mitosis (*6–8*).

Chacko (*9*) has stated that rat myocardial cells do not undergo mitosis once the myofilaments become organized into well-defined myofibrils. However, postnatal cardiac cells of the rat contain well-defined myofibrils (*10*). In a recent review, Zak (*11*) cites evidence indicating that heart muscle cells divide up through 3 weeks of postnatal life. Claycomb (*12*) has recently reported that DNA biosynthesis in cardiac muscle of the rat ceases by the 17th day of postnatal development, which correlates temporally with an almost total loss of cytoplasmic DNA polymerase activity.

Can neonatal cardiac cells that are more highly differentiated than embryonic heart cells synthesize DNA and divide? To examine this question, we studied the hearts of 2-day-old

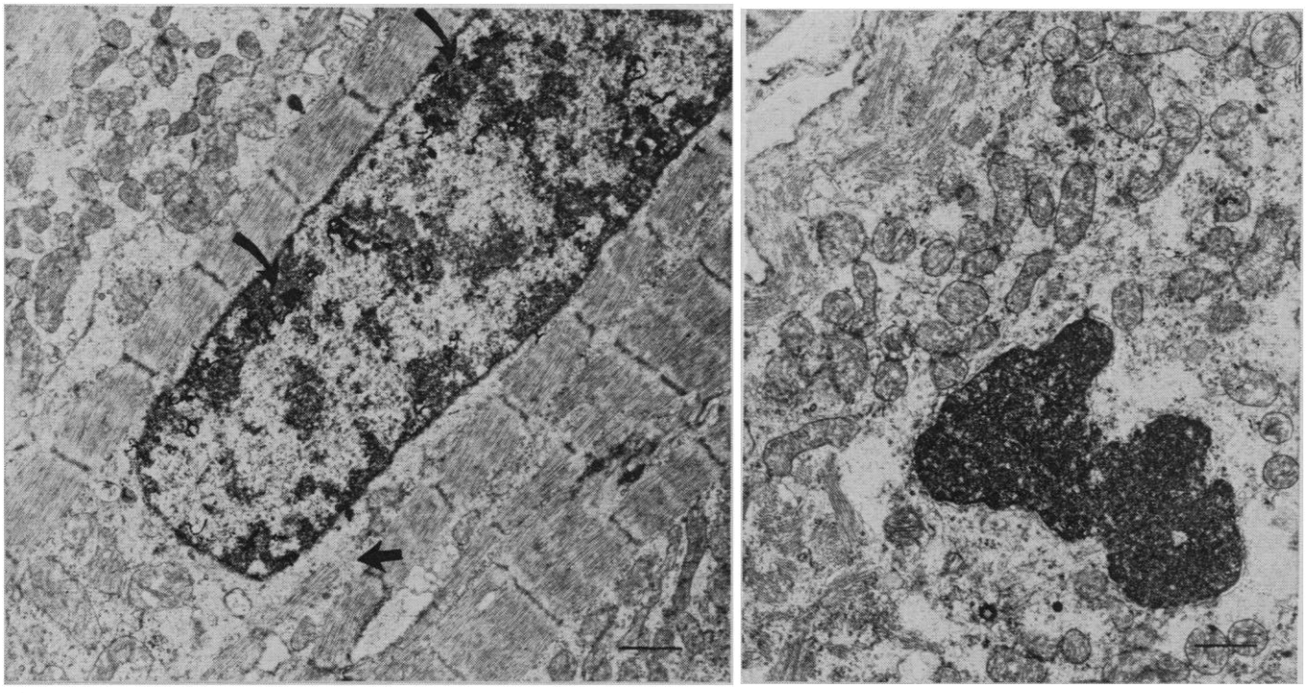

Fig. 1 (left). Typical ventricular myocardial cell of neonatal rat containing mitochondria, granular endoplasmic reticulum, polyribosomes, microtubules, Golgi vesicles, sarcoplasmic reticulum, and numerous myofibrils aligned in the long axis of the cell. The large nucleus contains developed silver grains (curved arrows), indicating incorporation of [^{3}H]thymidine. In one myofibril, the Z band (arrowhead) can barely be seen, although adjacent I and A bands of this myofibril can be seen. Fig. 2 (right). Ventricular cell in telophase. Chromosomes have moved toward the centriole and the nuclear envelope is reforming. Bundles of thick and thin filaments are grouped at random in this mitotic cell. The Z bands are not seen, and the organization of myofilaments into myofibrils is lost. Scale in each figure, 1 μm.

rats because we have observed a large number of both well-differentiated cells and mitoses at this age (unpublished data). White Holtzman rats were given [^{3}H]thymidine (50 μc per gram of body weight) by subcutaneous injection; uninjected rats of this same age served as controls. Four hours after injection, the hearts were fixed in glutaraldehyde-paraformaldehyde (*13*) and processed for electron microscope autoradiography (*14*).

Examination of autoradiographs revealed many myocardial nuclei that had incorporated [^{3}H]thymidine. Specific labeling of DNA was apparent from the very high counts (40 to 100) over the nuclei as compared to the very low background (one grain or less). Both labeled (Fig. 1) and unlabeled cells showed the same degree of differentiation. Only those cells in mitosis showed a lack of organized myofibril structure (Fig. 2).

Loss of myofibril structure may be necessary for mitosis in rat cardiocytes, as suggested by Rumyantsev (*10*). His electron microscopic studies of mitosis in 5- and 7-day-old rats indicate that during prometaphase, myofibrils near the nucleus partially disintegrate (*10*). The Z bands linking myofibril subunits disappear and bundles of thick and thin filaments remain during the various stages of mitosis (*10*). Our observations on the various stages of mitosis in 2-day neonatal rats confirm and extend these findings.

The presence of well-aligned myofibrils in labeled cells (Fig. 1) suggests that the mitotic cell in Fig. 2 has had a temporary loss of myofibril structure and was once as well differentiated as the cell synthesizing DNA (Fig. 1). Support for this interpretation comes from Kasten's (*15*) observation of beating ventricular cells of newborn rat in cultures. Some of these cells undergo mitosis but do not beat during the latter stages of cell division.

We show at the ultrastructural level in vivo synthesis of DNA in highly differentiated mammalian heart cells. The highly organized myofilament structure at the time of DNA synthesis confirms the suggestion of Rumyantsev and Snigirevskaya (*16*) that in rat cardiocytes the presence of well-aligned myofibrils does not act as a mitotic inhibitor or an inhibitor of DNA synthesis.

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13 August 1973; revised 26 September 1973

Miniature End Plate Potentials Recorded from Mammalian Myoneural Junctions in vivo

Abstract. *Fibers of the cat soleus muscle had a mean resting potential of 87 millivolts as measured with an intracellular microelectrode. Miniature end plate potentials had a mean amplitude of 0.95 millivolt, a mean frequency of 1.01 per second, a mean duration of 4.44 milliseconds, and a mean rate of rise of 0.99 volt per second. Two populations of response could be discerned on the basis of rate of rise. Neither α-chloralose nor nerve section had any significant effect on the response pattern. The in vivo preparation appears to provide a superior representation of the physiology of the myoneural junction.*

Most single cell data on synaptic function at the neuromuscular junction has been obtained with intracellular recordings from in vitro preparations (*1*). These studies have been important in developing our present concepts of the events at the neuromuscular junction, but a question about them persists. Are electrophysiological parameters measured in tissue excised and maintained in artificial salt solutions an accurate reflection of those in the living animal? Since the neuromuscular junction is extremely sensitive to hypoxia and to small changes in osmotic pressure (*2*), electrophysiological recordings made in vivo might be different from those made in vitro. The purposes of the present study were to adapt intracellular microelectrode recording techniques to an in vivo preparation of the cat soleus muscle; to establish the characteristics of the spontaneous postsynaptic response recorded under as nearly physiologic conditions as possible; and to compare these values with those recorded in vitro.

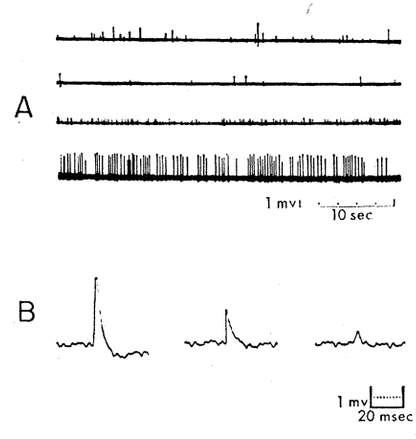

Fig. 1. Typical records of MEPP's recorded from the cat soleus muscle in vivo. The recordings in (A) show the range of amplitude and frequencies, and those in (B), with an expanded time base, show durations and rates of rise.

Cats were anesthetized either with α-chloralose or by C-1 spinal section under vinyl ether. The hindlimb was dissected, exposing the soleus muscle and isolating its nerve and blood supply by the method of Riker *et al.* (*3*). Steel pins were inserted through distal ends of the femur and tibia, and the leg was rigidly mounted in a Brown-Schuster myograph. Intracellular potentials were measured with glass capillary microelectrodes filled with 3*M* KCl (resistance, 8 to 15 megohms). Potentials were monitored on an oscilloscope and recorded on magnetic tape.

When a muscle cell was impaled at a point near the neuromuscular junction, a resting membrane potential of 80 to 90 mv was obtained. The mean, 87.0 ± 1.1 mv, is considerably higher than that reported for in vitro preparations such as the cat tenuissimus, 67 mv (*4*); rat soleus, 77.0 mv (*5*); and rat hemidiaphragm, 73 mv (*6*). The potential is stable; it can be recorded for hours with only 3- to 5-mv decreases.

Spontaneous miniature end plate po-