# DNA Synthesis and Mitosis in Adult Amphibian Cardiac Muscle Cells in vitro

Abstract. *High-resolution autoradiography and fine structural analysis of adult newt heart tissue in long-term culture revealed that tritiated thymidine was concentrated in the nuclei of dedifferentiated myocardial cells. Mitotic chromosomes were observed in some of these cells. This demonstrates that adult amphibian myocardial cells in vitro are capable of DNA synthesis and mitosis.*

Myocardial diseases—particularly ischemia in adult human beings—destroy the contractile and conducting elements of the heart, resulting in permanent fibrotic scarring. There is little subsequent regeneration, which requires DNA synthesis and mitosis of adult cardiac myocytes. The growth of the heart in the vertebrate embryo is primarily the result of an increase in cell number without an appreciable change in the ratio of protein to DNA in the tissue (*1*). Soon after birth, cell multiplication stops; subsequent enlargement of the heart is simply the result of an increase in cellular size. This phenomenon of an embryonic mitotic phase followed by postnatal cellular growth without significant cell division has been demonstrated (*1*-*5*) for vertebrates. Synthesis of DNA in mammalian myocardia in vivo in response to induced renal hypertension (*6*) and administration of Adriamycin (*7*) has been observed by light microscopy, yet the potential of adult cardiac myocytes in vitro for DNA synthesis and cell replication has not been established. We have developed a long-term culture method for adult newt heart explants (*8*) that has enabled us to examine the cellular integrity and contractility of adult cardiac myocytes in vitro.

Our electron microscopic studies (*9*) revealed that the cardiac myocytes in culture exhibited progressive alterations over time in the contractile apparatus (the myofibrils). The alterations consisted of disruptions of the compact myofibrils, resulting in the distribution through the cytoplasm of scattered patches of myofibrils and myofilaments, structural features that resemble those of embryonic cardiac myocytes. Cells altered in this way were designated as dedifferentiated cells, the number of which gradually increased during the 4-week culture period.

Since adult newt cardiac myocytes in vivo possess a negligible potential for DNA synthesis as evidenced by our tritiated thymidine incorporation experiments, we examined the capability of adult cardiac myocytes in vitro for DNA synthesis and cell replication. Our high-resolution autoradiographic studies on

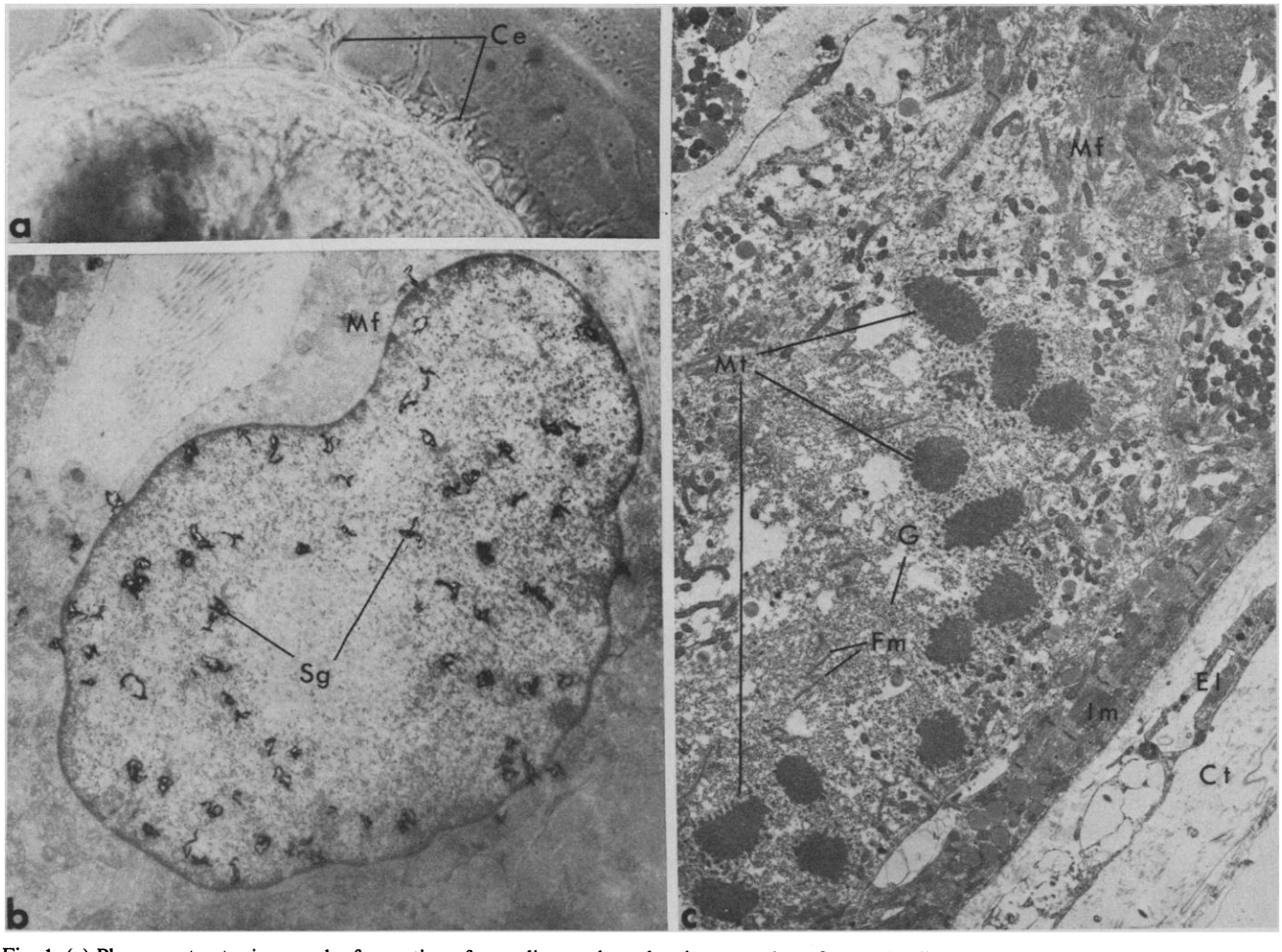


Fig. 1. (a) Phase-contrast micrograph of a portion of a cardiac explant showing smooth surface and cellular extensions (*Ce*) to the culture dish (× 200). (b) Autoradiograph of a 7-day culture showing incorporation of tritiated thymidine into the nucleus of a dedifferentiated cardiac myocyte as exhibited by the presence of silver grains (*Sg*) on the nucleus; *Mf*, myofibril (× 10,500). (c) Mitotic chromosome (*Mt*) shown in a dedifferentiated cardiac myocyte of a 14-day culture. Note the presence of patches of myofibrils (*Mf*), free myofilaments (*Fm*), and abundant glycogen (*G*) in the cell; *Im*, intact muscle cell; *El*, epithelial cell layer; *Ct*, loose connective tissue layer (× 4200).


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cardiac explants demonstrated that cardiac myocytes can undergo DNA synthesis and mitosis in vitro while maintaining their contractility in long-term culture conditions.

Cell biologists have studied DNA synthesis and cell division of larval and embryonic cardiac myocytes in monolayer and organ cultures (*10, 11*). In our study, adult newt ventricles were cut into several pieces (0.5 to 1.0 mm) and cultured for 4 weeks at 25°C in a modified Leibovitz medium (L-15) supplemented with 10 percent fetal calf serum and 1 percent penicillin streptomycin. Since this growth medium contained a high concentration of basic amino acids as free bases (*12*), the amino acids substituted for the usual bicarbonate-$CO_2$ buffer system so that a constant *p*H was maintained in free gas exchange with the atmosphere. The explants (Fig. 1) were examined daily under a phase-contrast microscope for evidence of contractility of the cardiac myocytes. The ventricular explants were labeled for 24 hours with [$^3$H]thymidine (1 $\mu$Ci/ml) at 7, 14, 21, and 30 days after culture initiation; the labeling time was found to be suitable for sufficient access of tritiated thymidine to the cells for autoradiography. The explants were then cut into thin sections and processed for electron microscopic autoradiography.

The first interesting observation was the survival of the adult heart pieces in culture as evidenced by the beating of the pieces throughout the culture period. In the first week of culture, one third of the pieces established pulsation rates from 3 to 67 beats per minute. These rates did not change until the end of the culture period. The cut surfaces had become smooth and covered with flat cells and a layer of loose connective tissue, indicative of healing (Fig. 1, a and c). Examination of the autoradiograms revealed that dedifferentiated cardiac myocytes can undergo DNA synthesis and mitosis. The radioactivity in the nuclei of the cardiac myocytes was initially detected in a 7-day culture (Fig. 1b). The number of labeled nuclei in the cardiac myocytes gradually increased until a maximum was observed in the third week of culture, beyond which there was a decline in incorporation of [$^3$H]thymidine. This implies that DNA synthesis in the cardiac myocytes began in the first week and gradually increased until a peak was reached in the third week, after which synthesis declined. Some of these cardiac myocytes exhibited mitosis, as is evidenced by the presence of mitotic chromosomes (Fig. 1c). Mitotic cells were observed throughout the culture period. The trend of mitosis was similar to that of DNA synthesis. The maximum number of mitotic chromosomes was observed in the third week of culture, which was followed by a decline in the fourth week.

The cardiac nonmuscle cells, mostly fibroblasts and endothelial cells, also incorporated [$^3$H]thymidine in their nuclei. The number of labeled nonmuscle cell nuclei was highest in the first week of culture but decreased gradually thereafter. The nonmuscle cells underwent mitosis and, as for the myocytes, the profile of mitosis was similar to that of DNA synthesis.

Since the mitotic activity is restricted to the dedifferentiated cells, which possess features characteristic of the embryonic cardiac muscle cells, the basic research question concerns the reprogramming of the adult cardiac myocytes with embryonic features of DNA synthesis and cell division. The reprogramming due to dedifferentiation is very important in the regeneration of the adult myocardium, since adult cardiac myocytes do not undergo DNA synthesis and mitosis under normal situations. The present findings provide evidence that the adult amphibian cardiac myocytes can undergo DNA synthesis and mitosis when explanted and cultured.

This study and our previous studies (*8, 9*) have cast light on the autorhythmicity of adult cardiac myocytes. Although the cells of our cardiac explants underwent dedifferentiation, the explants did not change the beating rate that they established when first placed in culture. What is the relationship between the morphological alterations in myofibrils and the pattern of heartbeat in cardiac explants? Evidently the alterations that occurred were not great enough to affect heartbeat. Since embryonic cardiac muscle cells with patches of myofibrils and free myofilaments are capable of maintaining a sustained pulsation rate, dedifferentiated cardiac muscle cells, reminiscent of the embryonic cells, possessed the same kind of potential for maintaining sustained contractile properties. There is probably a need for a minimum amount of contractile proteins to maintain the contractile property of the cardiac muscle cells.

ASISH C. NAG
CHRISTOPHER J. HEALY
MEI CHENG
*Department of Biological Sciences, Oakland University, Rochester, Michigan 48063*

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## Sociobiology of Bank Swallows: Reproductive Strategy of the Male

Abstract. *Male bank swallows pursue a mixed reproductive strategy. As previously documented, they form monogamous pair bonds with females with whom they will share parental duties of nest-building, incubation, and feeding of the young. In addition, however, they routinely seek promiscuous copulations with other females, both before and after pair-bonding.*

Trivers suggests that a monogamous male should be under selective pressure to pursue a "mixed" reproductive strategy when conditions permit: "to help a single female raise young while not passing up opportunities to mate with other females whom he will not aid" (*1*, p. 145). As a corollary, in such species the male should sequester his mate, that is, protect her from insemination by other promiscuous males. In the course of a long-term study of the bank swallow, *Riparia riparia* (*2*), we have discovered that males of this species appear to routinely and actively pursue a mixed reproductive strategy (MRS). Some of the behavior patterns we have observed have been noted before and interpreted differently (*3, 4*), but we believe that our observations in sum can be more par-


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