Chapter 17

NEW COMPARATIVE ASPECTS OF MYOCARDIAL REGENERATION WITH SPECIAL REFERENCE TO CARDIOMYOCYTE PROLIFERATIVE BEHAVIOR

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AS A RULE, both biologists and clinicians believe that cardiac muscle is practically devoid of any capacity to regenerate via mitotic division (hyperplasia) of its cardiomyocytes. This concept seems to be in agreement with studies of experimental and pathological material demonstrating the extreme rarity or even complete absence of mitotic division myonuclei, irrespective of the kind of myocardial injury (see reviews by McMinn, 1969; Polezhaev, 1972a, b; Hudgson and Field, 1973; Rumyantsev, 1977).

However, during the past two decades numerous data were obtained that stimulated new inquiries into the role of cardiomyocyte hyperplasia as a possible factor in myocardial regeneration (for literature, see Rumyantsev, 1966, 1977, 1979; Klinge, 1967; Polezhaev, 1972a, b; Zak, 1973, 1974; Oberpriller et al., 1979).

It has become evident that not all types of terminally differentiated cardiomyocytes fail to synthesize DNA and to divide mitotically. The stable inhibition of both these processes was found to be especially typical of mammalian ventricular myocytes that were used almost universally during the last century for studies of myocardial regeneration. Contrary to popular concepts, less differentiated types of cardiomyocytes, namely ventricular myocytes of the adult lower vertebrates, lizards, and even mammalian atrial myocytes were found to possess a considerable capacity for reactivated hyperplasia (Rumyantsev, 1966, 1973, 1974; Rumyantsev and Mirakyan, 1968; Sulima, 1968; Oberpriller and Oberpriller, 1971, 1974; Bader and Oberpriller, 1978; Becker et al., 1974).

Our results demonstrating these unexpected and profound differences in proliferative behaviour of various types of cardiac myocytes are briefly summarized in this chapter. Detailed descriptions of the material and methods employed are given elsewhere (Rumyantsev, 1961, 1966, 1973, 1974; Niwelinski et al., 1974; Rumyantsev and Kassem, 1976).

The essential points to be emphasized should be clear from the text, illustrations, and tables that follow. We should like to stress the importance of the following:

1. Identification of cells resuming DNA synthesis and mitosis at the ultrastructural level by means of electron microscopic autoradiography with ^{3}H-thymidine as a label.

2. Estimation of the cumulative parameters of reactivated cardiac myocyte hyperplasia using multiple injections of ^{3}H-thymidine.

The experimental models employed are demonstrated schematically in Figure 17-1.

In order to assess myocardial regenerative potential, we must be aware of specific traits of normal histogenesis. As is seen in Figure 17-2, immature cardiac myocytes of embryonic and early postnatal hearts are capable of DNA synthesis and mitosis, despite the presence of well-differentiated myofibrils (for literature, see Rumyantsev, 1977). The latter disintegrate into free myofilament bundles after the onset of metaphase (Rumyantsev, 1967, 1972; Oberpriller and Oberpriller, 1971; Kasten, 1972; Goode, 1975) and reassociate at the beginning of the next mitotic cycle (Fig. 17-2). In contrast to this, myoblasts of skeletal muscle cease to synthesize DNA and to divide mitotically after the appearance of the first differentiated myofibrils. The main source of skeletal myogenesis — a pool of morphologically undifferentiated myoblasts — is practically absent in most stages of cardiac myogenesis (Manasek, 1973). The mature myocardium lacks Mauro's myosatellites (Mauro, 1961), which are frequently regarded as dormant myoblasts providing for regeneration of skeletal muscle (for review, see Carlson, 1973, 1979).

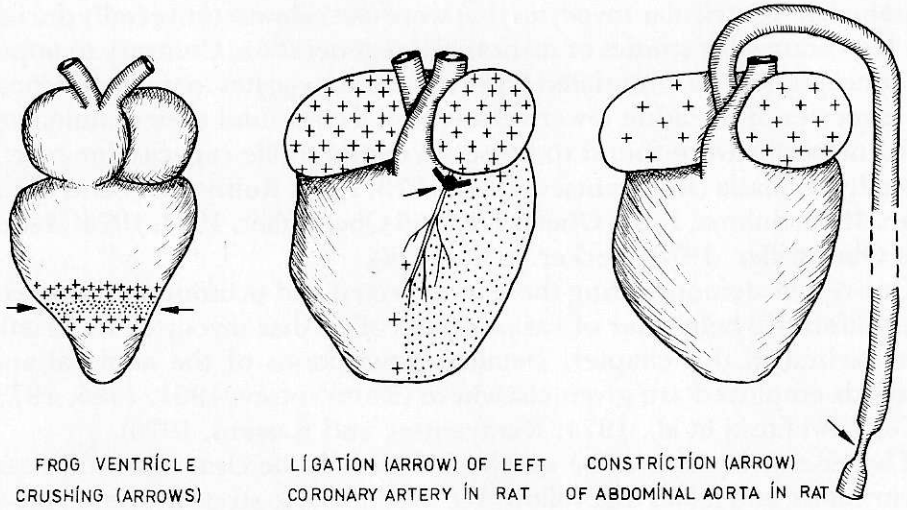


Figure 17-1. Scheme of the experimental models employed in our studies of reactivated proliferation of the adult vertebrate cardiomyocytes. The intensity and distribution of proliferation are indicated by varying density of crosses. Necrotic areas are shown by dots.

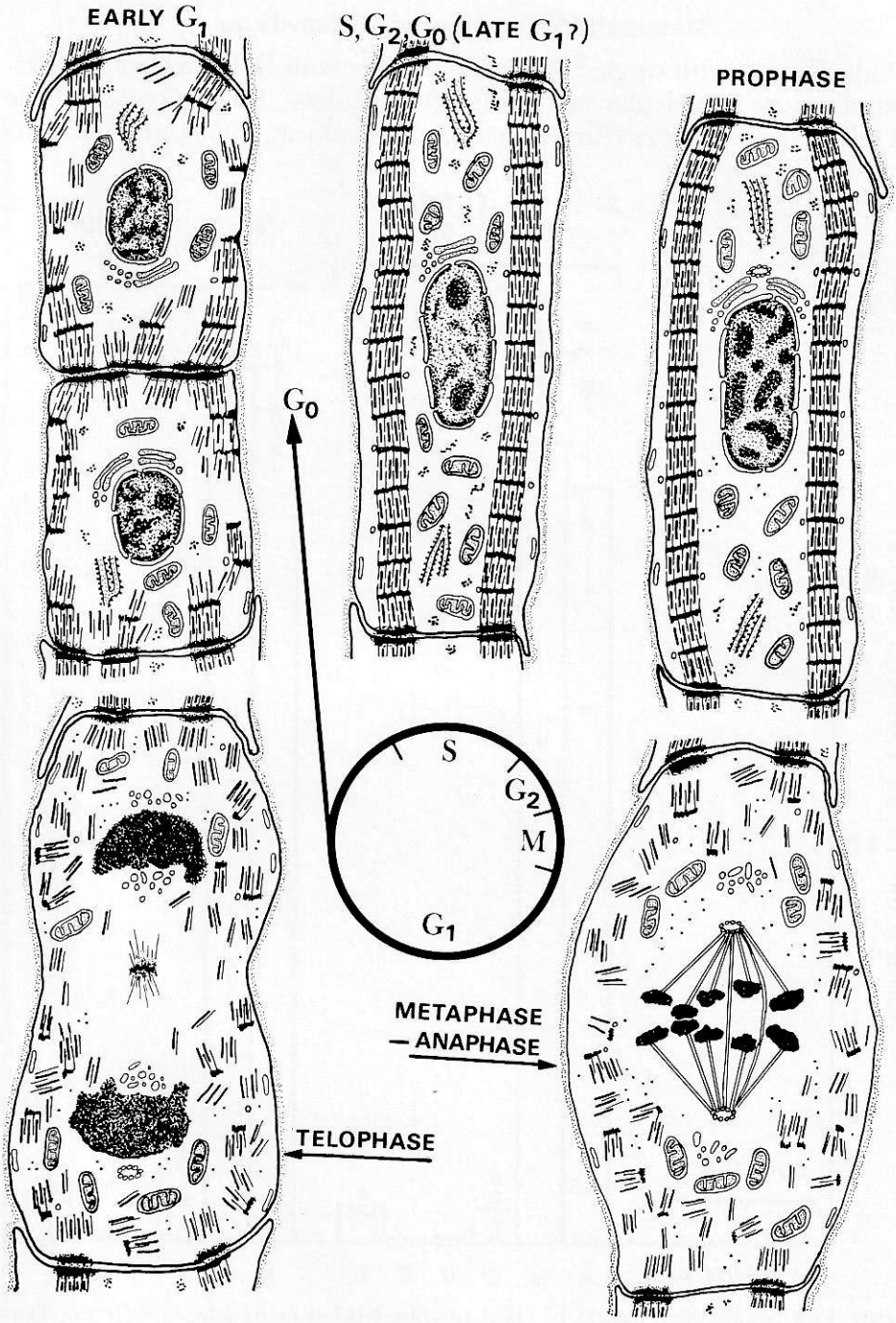


Figure 17-2. Changes in the myofibril ultrastructure at different phases of the developing cardiomyocyte mitotic cycle.

Mammalian Ventricular Myocardium

Experiments with single ^{3}H-thymidine injections following traumatization of mouse ventricular myocardia (Rumyantsev, 1966), ligation of the rat left coronary artery (Rumyantsev and Mirakyan, 1968), and constric-

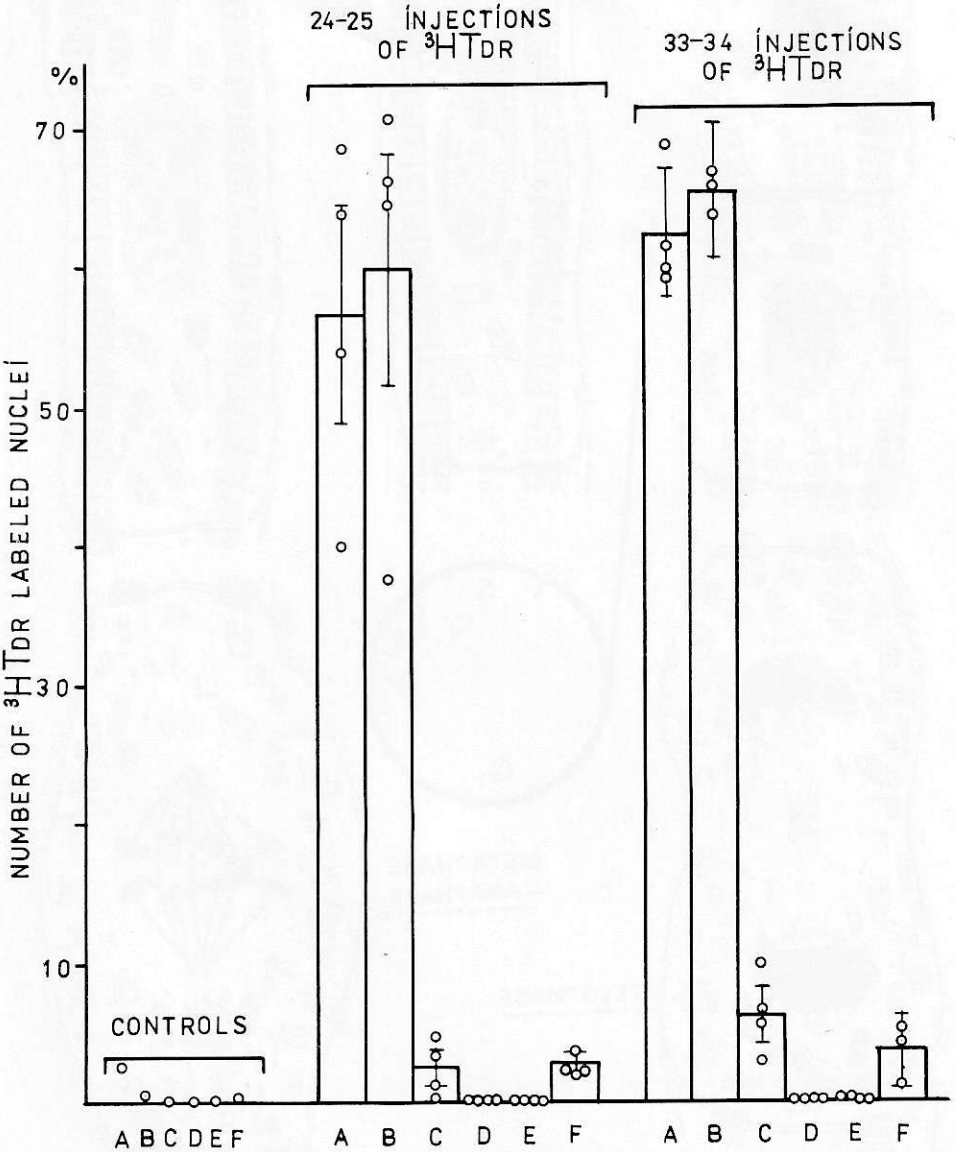


Figure 17-3. Cumulative indices of ^{3}H-thymidine-labeled myonuclei in different heart compartments of rats with left ventricle infarction. Multiple ^{3}H-thymidine injections were made at twelve hour intervals starting from day 3 to 5 after infarction. Each point represents the labeling index for one animal. A, left atrium; B, right atrium; C, perinecrotic ventricular myocardium; D, interventricular septum; E, right ventricle; F, atrioventricular conductive system of the heart. Vertical bars are standard errors of the means.

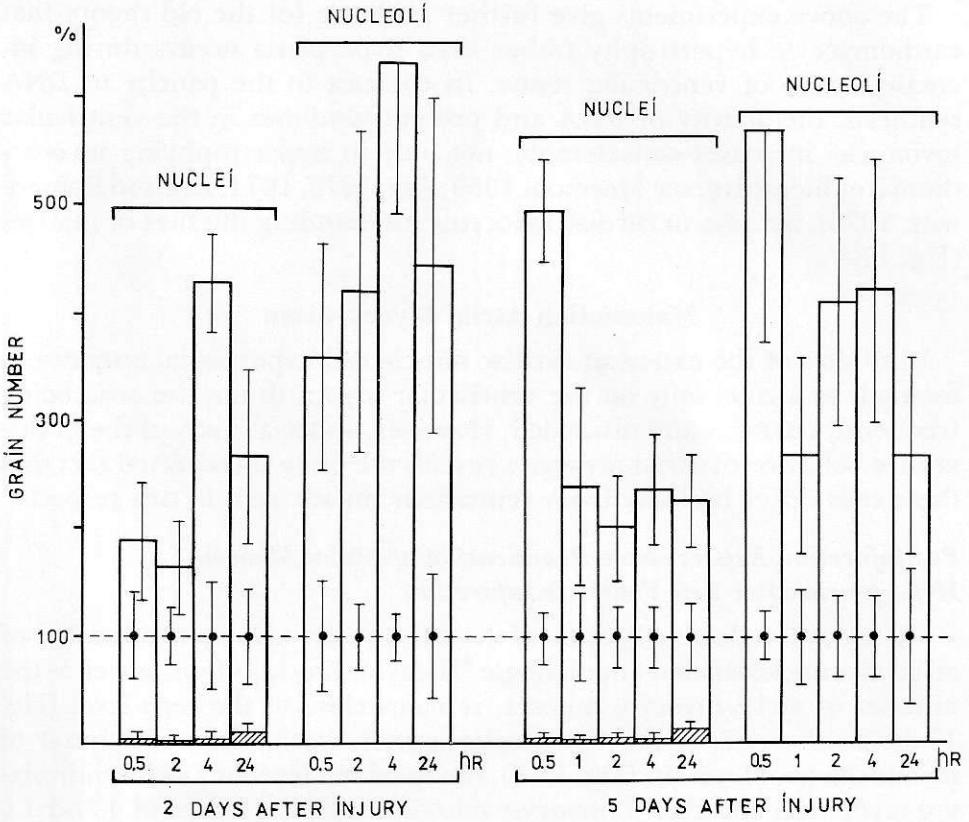


Figure 17-4. The intensity of ^{3}H-uridine labeling of perinecrotic myocyte nuclei and nucleoli after injury to the mouse left ventricular myocardia. The data are expressed as grain count percentages, taking as 100 percent the labeling of nucleoi and nuclei of the ventricular myocytes from uninjured controls (dashed line at the 100 percent level). Time in hours after ^{3}H-uridine administration and postinjury days are indicated below the graphs. Vertical bars are 95 percent confidence limits. Background density (when existing) is indicated by hatching at the basal parts of the graphs.

tion of the rat abdominal aorta (Rumyantsev, 1970) reveal no more than 0.5 percent labeled myonuclei in both the perinecrotic and hypertrophying myocardia irrespective of the postoperative stages. A stable inhibition of DNA synthesis in the bulk of ventricular myocytes is in agreement with the usual observation of the extremely rare occurrence of mitotic division of these cells under experimental and pathological conditions. Even after many sequential ^{3}H-thymidine injections, the number of labeled myonuclei in the perinecrotic myocardium of rats increases only about 2.5 to 6 percent (Fig. 17-3). A similar estimate of the cumulative indices of labeled cardiomyocytes in highly hypertrophied left ventricular myocardium of rats with the same labeling conditions reveals practically no DNA synthesizing myonuclei, while the majority of endothelial and stromal cells are labeled.

The above experiments give further evidence for the old theory that cardiomyocyte hypertrophy rather than hyperplasia occurs during increase in size of ventricular tissue. In contrast to the paucity of DNA synthesis, the activity of RNA and protein syntheses in the ventricular myonuclei increases considerably, not only in hypertrophying myocardium (for literature, see Meerson, 1969; Zak, 1973, 1974; Zak and Rabinowitz, 1979), but also in cardiac myocytes surrounding the sites of injuries (Fig. 17-4).

Mammalian Atrial Myocardium

In studies of the extent of cardiac muscle cell hyperplasia, attention is focused, as a rule, only on the ventricular myocardium, the atria being frequently cut away and discarded. However, a special study of the proliferative behavior of atrial myocytes reveals the quite unexpected fact that these cells differ basically from ventricular muscle cells in this respect.

Postinfarction Repercussive Reactivation of Atrial Myocyte Hyperplasia after Left Ventricle Infarction

Up to day 5 following ligation of the left coronary artery, the number of atrial myonuclei labeled after a single ^{3}H-thymidine injection, as well as the number of atrial myocyte mitoses, remains close to the zero level (Fig. 17-5). At the end of the first postinfarction week, there is a burst of myonuclei proliferation (Fig. 17-6), the maximal level of DNA synthesizing myonuclei and that of myocyte mitoses achieving values of 4 and 1.5 percent, respectively (Fig. 17-5).

According to data obtained by means of curves of labeled mitoses (Fig.

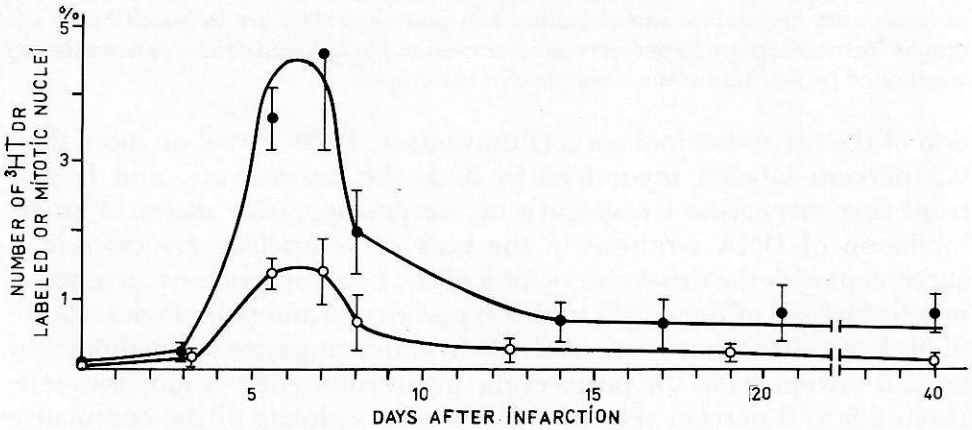


Figure 17-5. Percentages of ^{3}H-thymidine pulse-labeled (filled circles) and mitotically dividing (open circles) myonuclei in the left atria of rats at different postinfarction stages. Vertical bars are standard errors of the means. There are at least three animals per point.

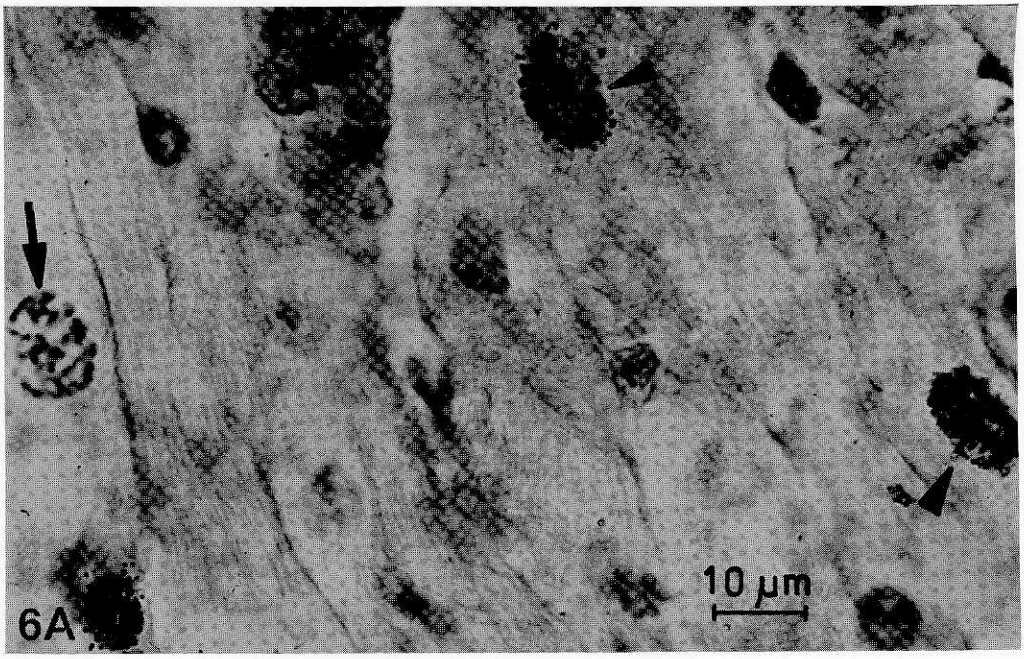


Figure 17-6. Myonuclei proliferation in the left atria of rats by day 7 after ligation of the left coronary artery. *A.* prophase myocyte (arrow) and two myonuclei (arrowheads) labeled after a single ^{3}H-thymidine injection; *B.* telophase myocyte (arrow). PAS-hematoxylin.

17-7), atrial myocytes proliferate about two times more slowly than the connective tissue cells of the myocardium. The number of proliferating atrial myocytes decreases gradually. Even a month later, the left atrium still contains up to 1 percent of DNA-synthesizing or mitotically dividing myocytes (Fig. 17-5). It should be noted that reactivation of DNA synthesis and mitoses proceeds throughout the whole left atrium. However, the above index of the frequency of proliferating myonuclei can be underestimated and considered as being too low.

It seems of special interest therefore to evaluate the extent of accumulation of labeled atrial myonuclei following multiple ^{3}H-thymidine injections. A ten-times repeated precursor administration from day 5 to day 9 after infarction results in the labeling of 51.5 ± 4.5 percent of left atrial myonuclei (Rumyantsev and Kassem, 1976). After many repeated ^{3}H-thymidine injections, the cumulative indices of myonuclei labeling increased additionally by about 10 percent, reaching 62.4 ± 2.3 percent (Figs. 17-3 and 17-8). In some rats, the frequency of labeled atrial myonuclei approaches 70 percent. The fifteenfold increase of labeled myonuclei in these experiments as compared with values obtained after single ^{3}H-thymidine pulses (Fig. 17-5) may be tentatively explained as follows:

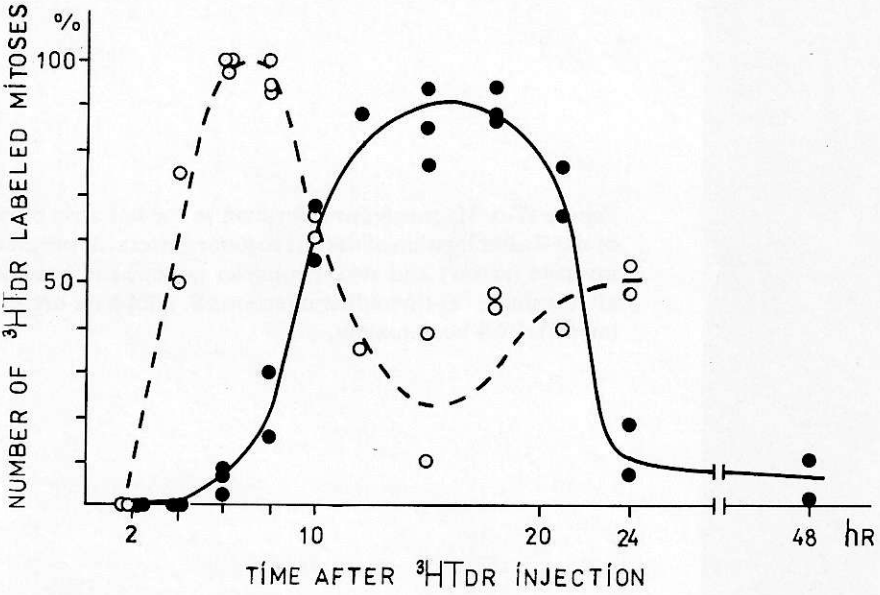


Figure 17-7. Curves of ^{3}H-thymidine-labeled mitoses for left atrium myocytes (filled circles) and connective tissue cells (open circles) from rat hearts with left ventricle infarction. ^{3}H-thymidine was given at day 5 after infarction. One can calculate from these curves that myocytes proliferate about twice as slowly as nonmuscle cells. G_2 and S phases of the mitotic cycle last five to eight and eleven to thirteen hours respectively for the myocytes and three to four and six to seven hours for connective tissue elements.

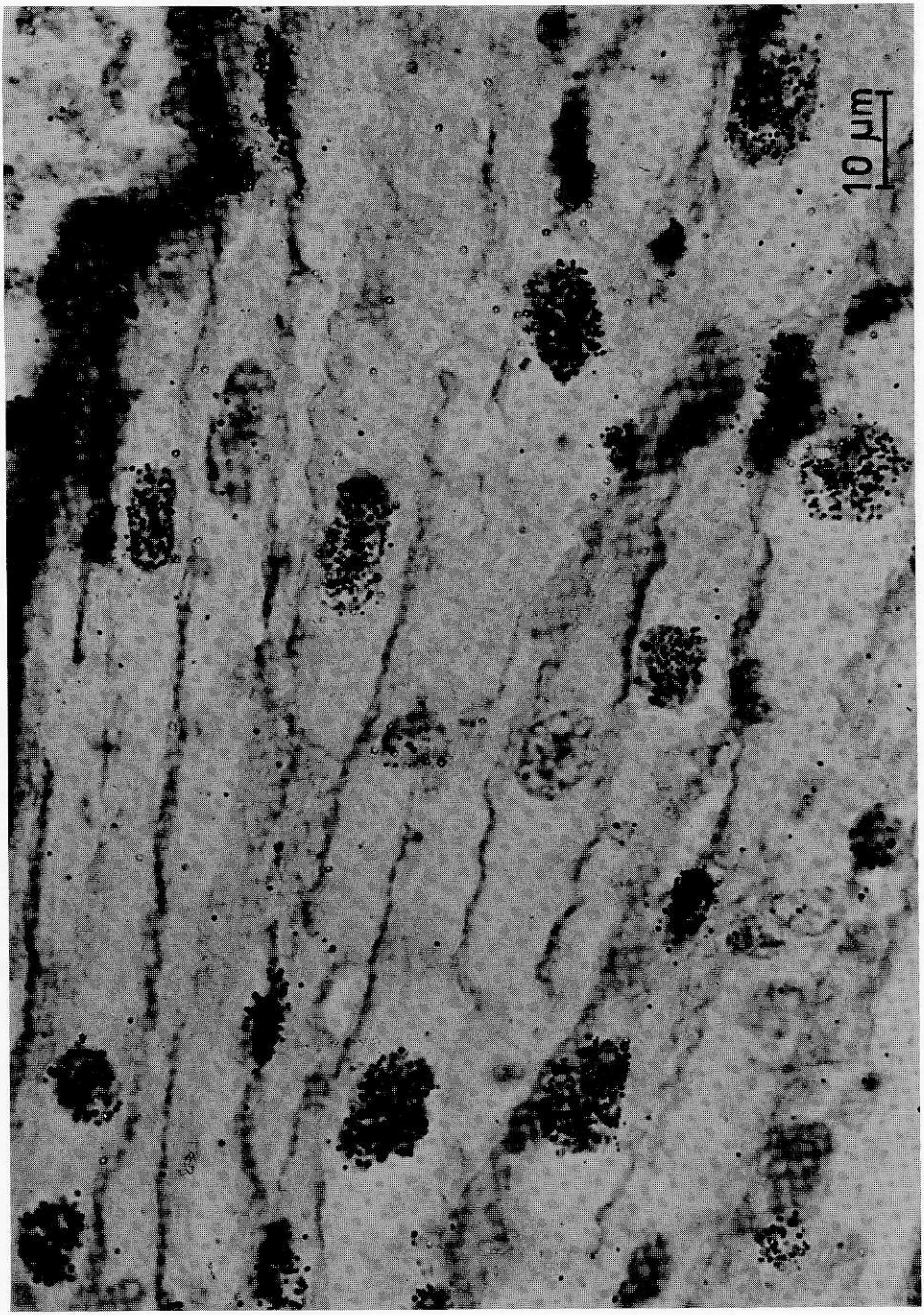


Figure 17-8. Labeling of the majority of myonuclei in the left atrium of rat, nineteen days following ligation of the left coronary artery. ^{3}H-thymidine was injected thirty-four times at twelve hour intervals beginning from day 3 to day 19 after infarction. PAS-hematoxylin.

1. Cumulative indices of labeling include the labeling of all myocytes that reenter the S phase during the whole period of prolonged precursor availability.
2. Mitoses of early labeled myocytes should result in the accumulation in atrium of numerous pairs of labeled daughter myonuclei, which was frequently observed in our radioautographic preparations (see for illustrations, Rumyantsev and Kassem, 1976; Rumyantsev, 1979).

Left ventricle infarction stimulates myonuclear hyperplasia not only in the left but also in the right atrium. Reactivation of DNA synthesis in the right atrium is considerably retarded. However, at later postinfarction stages, the degree of new DNA synthesis in the right atrium increases so that cumulative indices of labeled myocytes are at practically the same level as in the left atrium (Fig. 17-3).

In the above experiments, the most impressive finding is the virtual absence of labeled myonuclei in the adjacent ventricular myocardia of the same rats, the atria of which contain up to 60 percent of ^{3}H-thymidine tagged myocytes.

According to preliminary counts, the total number of myonuclei in the left atria of the rats is found to increase by about 50 percent by the third month after ligation of the coronary artery.

It can be demonstrated by means of Feulgen-DNA cytophotometry that, at advanced postinfarction stages, the majority of atrial myonuclei become polyploid, displaying ploidy classes higher than 2C DNA (Fig. 17-9). This is a consequence of myonuclear DNA endoreduplication rather than normal DNA synthesis occurring before mitosis. Taking into account the current concept of somatic cell polyploidy (Brodsky and Uryvaeva, 1977), we are inclined to think that myonuclear polyploidization, i.e. endoreduplication, may be regarded as one of the mechanisms of myocardial regeneration. This process would provide for an increase in the total cardiomyocyte genome in atrial muscle.

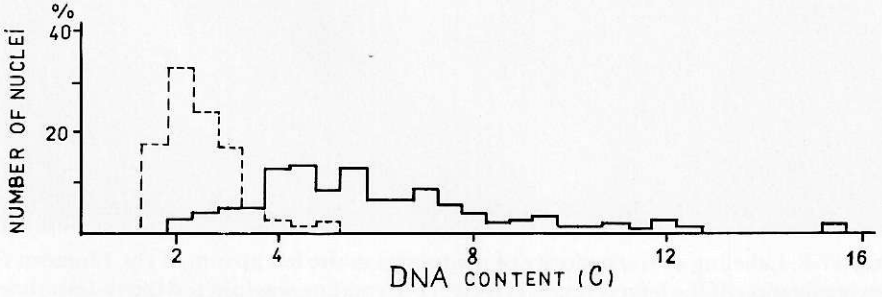


Figure 17-9. Changes in ploidy of the left atrium myonuclei on the fortieth day following left coronary artery ligation (solid line) as compared with ploidy of atrial myocytes of unoperated control rats (dashed line).

Extracardiac Atrial Muscles and Their Behavior after Left Ventricular Myocardial Infarction

In rats, as well as in other rodents, the pulmonary veins are covered by external sheets of typical atrial myocytes, which continue into the muscles of the auricular walls. Despite the fact that these extracardiac myocytes have an ultrastructural organization identical to that of the bulk of "normal" myocytes of atria (Klika and Jarkovska, 1976), the former are rarely involved in reactivated hyperplasia following left ventricular myocardium infarction (Fig. 17-10).

Activation of DNA Synthesis by Atrial Myocytes after Aortic Constriction

To elucidate the nature of the stimulus causing reactivation of DNA synthesis in atrial myocytes, it is interesting to study this question during cardiac hyperfunction independent of myocardial infarction. Cardiac hyperfunction may be induced by severely constricting the abdominal aorta (see Fig. 17-1), according to Beznak's method modified by Kogan (1961). This treatment results in the development of a pronounced cardiac hypertrophy as well as DNA synthesis and mitoses in a small fraction of atrial myocytes on the second postoperation week (Table 17-I). This is demonstrated after a single injection of ^{3}H-thymidine. In a multiple labeling experiment (thirty injections), there is an accumulation of up to 30 percent of labeled myonuclei in the left atria (Table 17-I). The degree of rekindled DNA synthesis correlates well with the increase in the heart weight. The accumulated frequency of labeled myocytes for the left atria surpasses significantly ($p < 0.05$) that for the right atria by 1.8 times (Table 17-I). The abundance of labeled myonuclei in rat atria after multiple ^{3}H-thymidine injections contrasts sharply with the absence of labeled myonuclei in ventricles where only endothelial and stromal cells contain radioactive DNA.

Local Injuries of the Atrial Myocardia

There have been few investigations into the effects of local injuries on the proliferation of atrial myocytes (Kolossova et al., 1970; Galankin and Paltsin, 1975). Our results are based only on preliminary data obtained after single ^{3}H-thymidine injections following local burning or linear crushing of the left auricular appendage. Judging from the data presented in Table 15-II, perinecrotic atrial myocytes respond by a pronounced stimulation of DNA synthesis and mitoses within at least the second and the third postoperative weeks. The fact that single radioisotopic pulses result in labeling of no more than 3.6 percent of the perinecrotic myonuclei cannot be regarded as evidence for low proliferative reactivity of atrial myocytes following local stimuli. In view of the previously described ex-

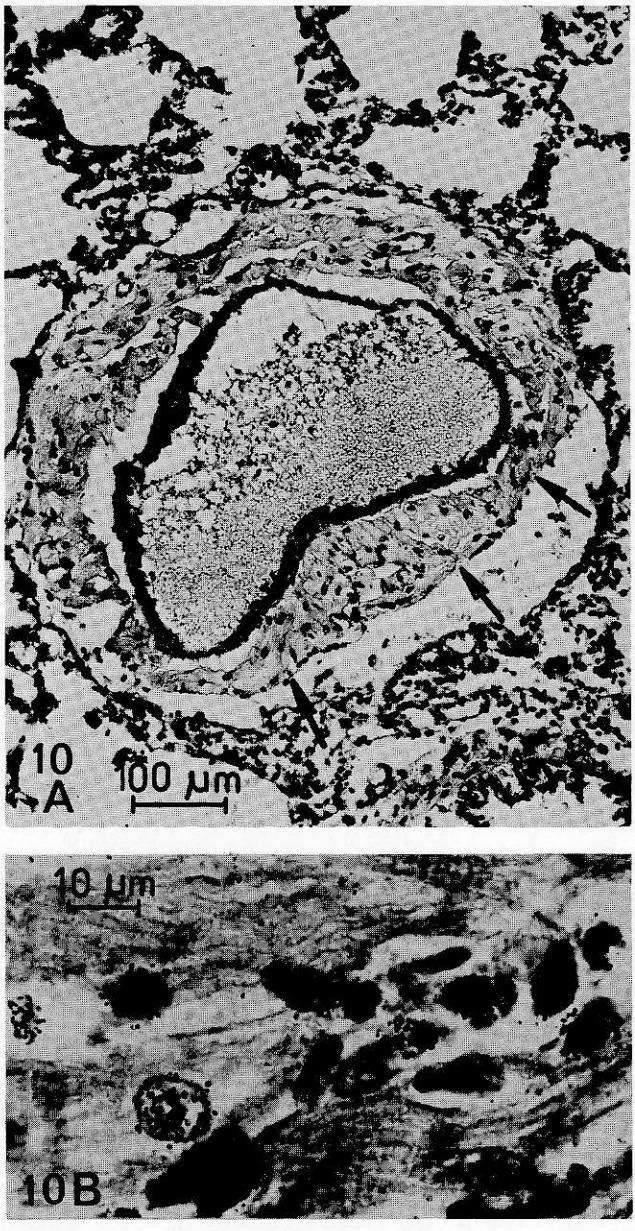


Figure 17-10. General view *A*) of the perivenous sheet of atrial myocardium (arrows) within a rat lung and occasional (*B*) ^{3}H-thymidine labeling of the myonucleus from a similar sheet, (a) nineteen and (b) fifteen days following ligation of the left coronary artery. ^{3}H-Thymidine was given thirty-three times (*A*) and twenty-four times (*B*) beginning from the third postinfarction day. PAS-hematoxylin (a) and hematoxylin-eosin (b).

TABLE 17-I

INDICES OF MYONUCLEI LABELED AFTER ^{3}H-THYMIDINE INJECTIONS (LI IN % ± SEM) and INDICES OF MITOSES (MI IN % ± SEM) IN ATRIA OF RATS FOLLOWING AORTIC CONSTRICTION

Days After Aortic Constriction	*Number of Animals*	*Heart Weight (in % of body weight ± SEM)*	*Number of ^{3}HTdr Injections*	*Left Atrium*		*Right Atrium*	
				LI	*MI*	*LI*	*MI*
0 (unoperated controls)	16	0.387 ± 0.032	1	0.122 ± 0.040	0.049 ± 0.004	0.187 ± 0.074	0.047 ± 0.029
7-10	12	0.487 ± 0.025†	1	0.183 ± 0.082	0.122 ± 0.056	0.121 ± 0.048	0.090 ± 0.090
11-14	6	0.600 ± 0.030†	1	1.600 ± 0.590*	0.960 ± 0.440	1.120 ± 0.492	0.190 ± 0.110
17-18	5	0.546 ± 0.025†	1	0.570 ± 0.520	0.080 ± 0.080	0.470 ± 0.160	0.022 ± 0.022
21-24	16	0.609 ± 0.023†	30‡	29.750 ± 4.650†	0.055 ± 0.024	16.780 ± 3.330†	0.069 ± 0.031

§ Significant difference from control values at $p < 0.05$.
† Significant difference from control values at $p < 0.01$.
‡ Repeated ^{3}H-thymidine injections were made beginning from seventh post-operation day at 12-hour intervals.

TABLE 17-II
3H-THYMIDINE PULSE LABELING AND MITOTIC DIVISION OF MYONUCLEI IN THE PERINECROTIC ATRIAL MYOCARDIUM OF RATS WITH DIFFERENT INJURIES TO AURICLE

Kind of Injury	*Side of Auricle*	*Days After Operation*	*Number of Animals*	*% ± SEM of ^{3}H-Thymidine Labeled Nuclei*	*% ± SEM of Mitoses*
Unoperated controls	Left	0	16	0.122 ± 0.040	0.049 ± 0.004
Unoperated controls	Right	0	16	0.187 ± 0.074	0.047 ± 0.009
Local burning	Left	3	2	0	0
Local burning	Left	17	2	1.340 ± 0.460	1.400 ± 0.100
Local burning	Left	14	2	2.500 ± 1.100	1.020 ± 0.190
Local burning	Left	21	1	1.00	1.00
Linear crushing	Left	4	1	3.40	2.15
Necroses after constriction of aorta	Right	7-10	3	3.950 ± 1.570	0.150 ± 0.090
Necroses after constriction of aorta	Right	11-14	3	3.520 ± 0.600	1.260 ± 0.380
Necroses after constriction of aorta	Right	17-18	4	2.120 ± 0.330	0.500 ± 0.110

periments employing multiple ^{3}H-thymidine injections after myocardial infarction or aortic constriction, one can predict similar results here — namely, that a considerable number of atrial myonuclei will be labeled. A similar stimulation of DNA synthesis is characteristic of myocytes located around necrotic foci that appear in right atria after aortic constriction (Table 17-II). As is true in the case of the injured frog heart, which is described later, scarring occurs in spite of the renewed proliferation of perinecrotic atrial myocytes.

Proliferative Behavior of the Atrioventricular Conductive System Myocytes

In experiments with multiple ^{3}H-thymidine injections following myocardial infarction or aortic stenosis, we demonstrated for the first time that myocytes of the conductive system of the heart are capable of a very limited degree of hyperplasia (Rumyantsev and Kassem, 1976). The relative number of DNA synthesizing myocytes in the atrioventricular part of the conductive system is approximately fifteen to twenty times lower than in the atrium (Figs. 17-3 and 17-11). The occasional presence of mitoses is indicative of the possible division of some specialized myocytes. It should be noted that necroses of the specialized conductive system are absent in rats with either left ventricle infarction or aortic constriction. Since the working ventricular myocardia located far from the infarcted area are practically devoid of DNA synthesizing myonuclei (Fig. 17-3), one can conclude that myocytes of the conductive system are much more responsive in terms of reactive hyperplasia than working ventricular muscle cells. It would be interesting to investigate the degree of reactivated hyperplasia

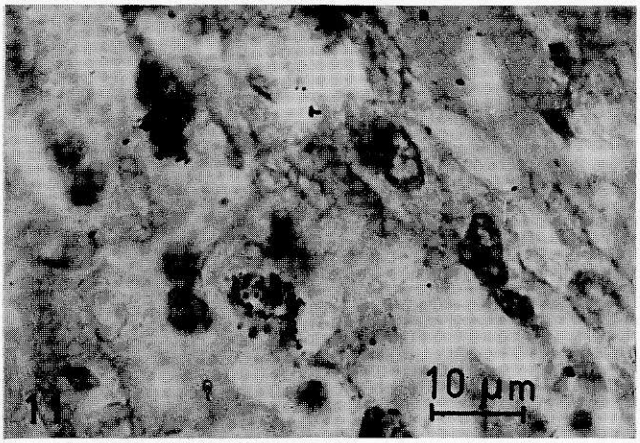


Figure 17-11. Labeling of the myonucleus within atrioventricular bundle of rat. ^{3}H-thymidine was injected ten times from day 5 to day 9 after infarction. PAS-hematoxylin.

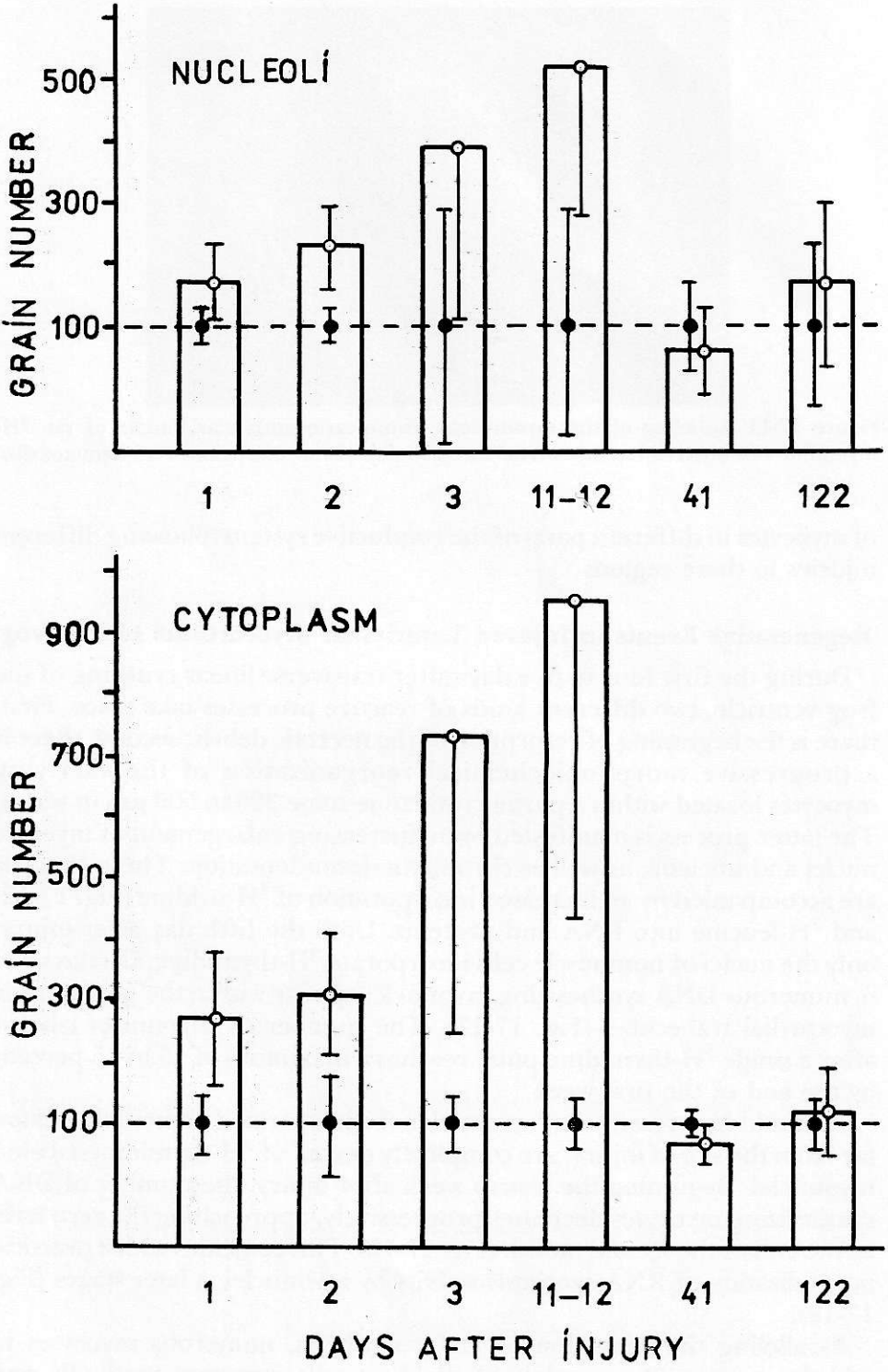
of myocytes in different parts of the conductive system following different injuries to these regions.

Regenerative Events in Injured Ventricular Myocardium of the Frog

During the first four to five days after transverse linear crushing of the frog ventricle, two different kinds of reactive processes take place. First, there is the beginning of resorption of the necrotic debris; second, there is a progressive morphobiochemical reorganization of the surviving myocytes located within a perinecrotic zone some 300 to 500 µm in width. The latter process is manifested by an increasing enlargement of myocyte nuclei and nucleoli, as well as chromatin decondensation. These changes are accompanied by an increased incorporation of ^{3}H-uridine (Fig. 17-12) and ^{3}H-leucine into RNA and proteins. Until the fifth day after injury, only the nuclei of nonmuscle cells incorporate ^{3}H-thymidine. On days 5 to 6, numerous DNA synthesizing myonuclei appear within the perinecrotic myocardial trabeculae (Fig. 17-13). The number of myonuclei labeled after a single ^{3}H-thymidine pulse reaches a maximum of 13 to 15 percent by the end of the first week.

It should be stressed that myocardia of the operated ventricles, located far from the site of injury, are completely devoid of ^{3}H-thymidine-labeled myonuclei. Beginning the fourth week after injury, the number of DNA synthesizing myocytes decreases progressively, approaching the zero level by the end of the second month (Fig. 17-13). This coincides with a practical normalization of RNA synthesis activity by myonuclei at later stages (Fig. 17-12).

Paralleling the activation of DNA synthesis, numerous myocytes in mitosis appear. The number of dividing cells increases gradually and


NUCLEOLÍ
GRAÍN NUMBER
500
300
100
1
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CYTOPLASM
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41
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DAYS AFTER ÍNJURY

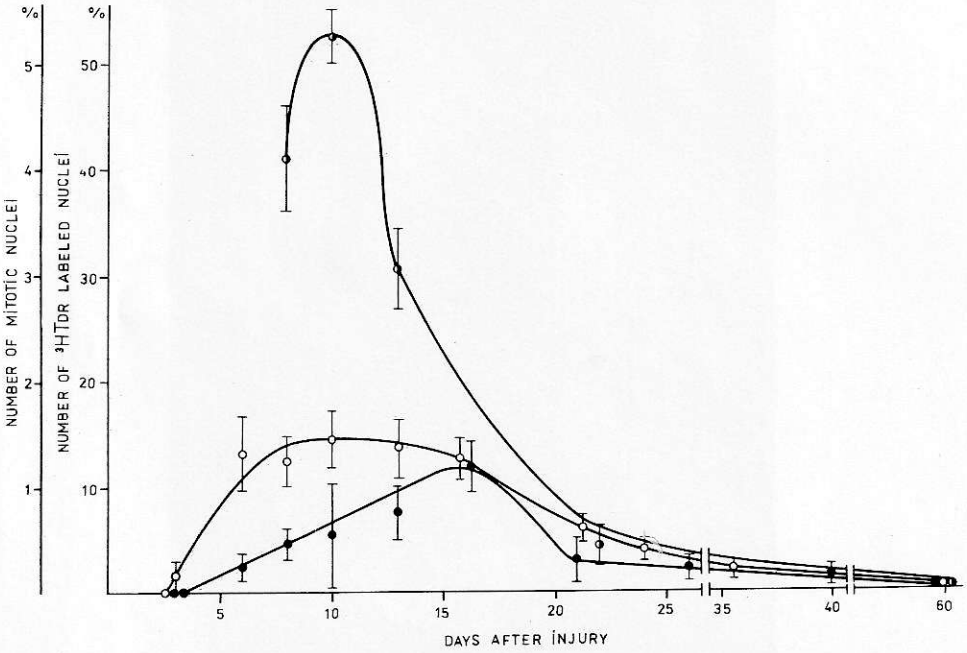

Figure 17-13. Percentages of labeled myonuclei after single pulse (open circles) and after three times repeated ^{3}H-thymidine injections (divided circles). Percentages of mitotically dividing myonuclei are shown by solid circles. All data are obtained from counts within the juxtanecrotic region of injured frog heart ventricles. Vertical bars are standard errors of the means. There are at least three animals per joint.

reaches a maximum of approximately 1.3 percent in the middle of the third week (Fig. 17-13). Later, mitotic activity falls quickly.

To assess the cumulative percentage of proliferating myocytes, a total of three ^{3}H-thymidine injections are given. This experimental approach brings about the labeling of 40 to 60 percent of myocytes exclusively within a perinecrotic reactive zone (Fig. 17-13). When ^{3}H-thymidine is given many times throughout the entire period of most intensive myocyte hyperplasia (i.e. from day 5 to day 18 after injury), the number of labeled myonuclei in this region reaches 94.1 ± 0.78 percent (Fig. 17-14). These last autoradiographic experiments clearly demonstrate the stimulus for

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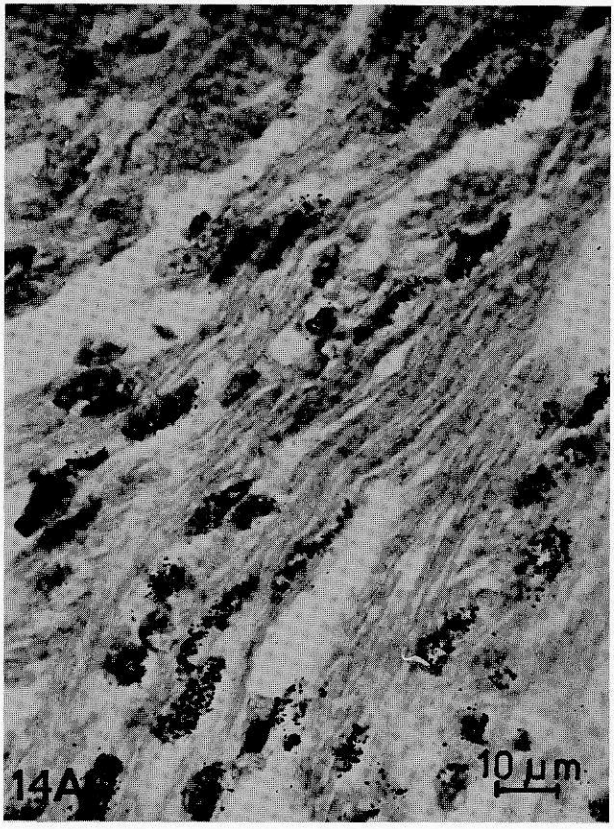
Figure 17-12. The intensity of ^{3}H-uridine labeling of the juxtanecrotic myocyte nucleoli and cytoplasm at different stages after the injury to frog heart ventricle. Isotope was given two to six hours before fixation (nucleoli labeling counts) or twenty-four hours before fixation (cytoplasm labeling counts). The data are expressed as percentages (open circles) from grain counts in controls (filled circles at the 100 percent level). Vertical bars are 95 percent confidence limits.


14A
10 µm


10 µm

the resumption of myocyte proliferation after injury of the frog heart operated upon locally. No labeled myonuclei are ever found in the morphologically normal myocardium beyond the perinecrotic reactive zone.

In spite of the intensive myocyte proliferation at the borders of the necrotic area, this zone is filled mainly with scar tissue. Slender muscle anastomoses appear very late after injury (two to three months) between the basal part of the injured ventricle and its apex isolated by crushing. The majority of these anastomoses are formed in the subepicardial layer (Fig. 17-15). In the above multiple labeling experiments, practically all the myocyte nuclei of regenerated myocardial anastomoses contain silver grains (Fig. 17-14). Regeneration of even the slender muscle anastomoses often permits the establishment of synchronized contractions of the basal and apical parts of operated ventricles that were beating independently at earlier stages (Rumyantsev, 1961).

Ultrastructural Changes Accompanying the Reactivated Hyperplasia of Adult Cardiomyocytes

Our electron microscopic investigations (Rumyantsev, 1973, 1974, 1977, 1979) permit us to conclude that in spite of the evident difference in stimuli (local or distant) leading to cardiomyocyte hyperplasia in frog ventricle and rat atria under different experimental conditions, the ultrastructural reorganization of myocytes is rather similar in both systems.

Rat Atrium Myocardia

During the three to five day period preceding the onset of atrial myocyte proliferation, there is a progressive enlargement of nucleoli and some chromatin decondensation. This is often accompanied by cytoplasmic changes in the largely undifferentiated cytoplasm. These changes include an accumulation of numerous ribosomes as well as hyperplasia of the rough endoplasmic reticulum and Golgi apparatus (Fig. 17-16). Numerous lysosomes appear that give a strong reaction for acid phosphatase (Fig. 17-17). Despite the fact that some myofibrils are loosened, they persist in all reorganized myocytes (Fig. 17-16) together with intercalated discs and specific atrial granules. Many myocytes become enriched in nonmyofibrillar cytofilaments (so-called intermediate-sized filaments 80 to 110 Å in diameter). The above changes are indicative of an increased biosynthetic activity of atrial myocytes as well as of a partial dedifferentiation or "re-

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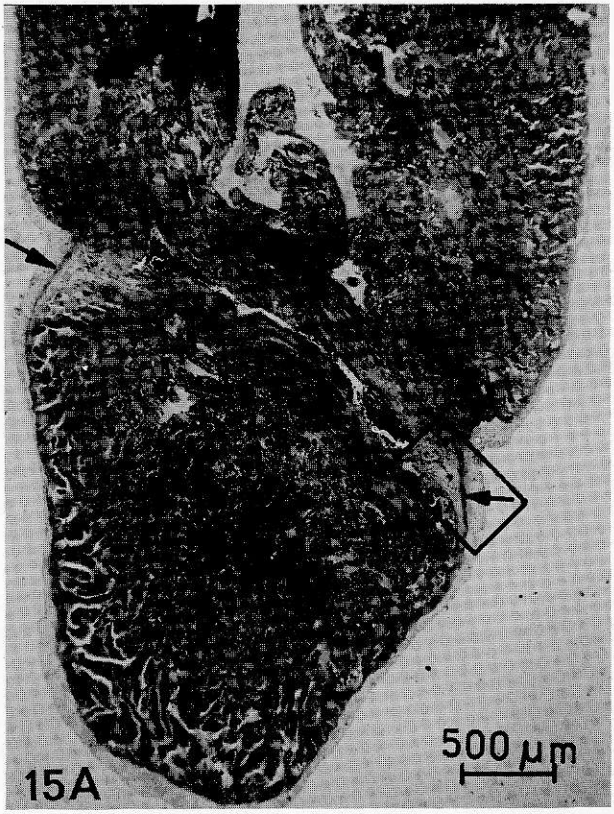
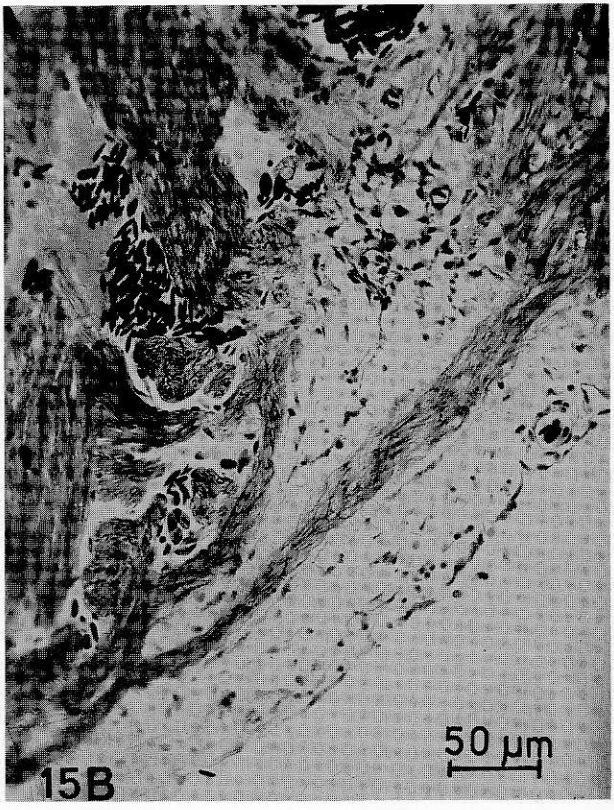
Figure 17-14. Labeling of the majority of myonuclei in juxtanecrotic myocardium (*A*) and in the regenerated myocardial trabecula formed within the crushed zone (*B*) of the frog heart ventricle. Repeated injections of ^{3}H-thymidine were made at twelve hour intervals from day 5 to day 18 following heart injury, fixation being delayed up to the day 73 postoperation. Hematoxylin-eosin.


500 µm
15A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15B

juvenating reorganization" (Rumyantsev, 1974) since a certain resemblance to immature embryonic cardiac muscle cells is achieved. As shown by electron microscopic autoradiography, there is no direct correlation between the degree of myocyte partial dedifferentiation and resumption of the DNA synthesis. ^{3}H-Thymidine labels not only nuclei of most "dedifferentiated" myocytes but also those of moderately changed (Fig. 17-18) or even practically unchanged cells. However, one sees a pronounced nucleoli enlargement and decondensation of chromatin in the majority of ^{3}H-thymidine-tagged myocytes.

Myocytes in prophase differ from their neighbors only because of the appearance of chromosomes. However, metaphase is accompanied by the disappearance of Z-lines, resulting in the release of numerous sarcomeres and myofilament bundles at the subsequent mitotic phases (Fig. 17-19). Such a transitory disintegration of the rigid myofibrils within the perinuclear region seems to be necessary for the separation of chromosomes. A similar disorganization of the contractile machinery is typical of mitotic myocytes in normally developing myocardium (Fig. 17-2). At all phases of mitosis, dividing myocytes remain attached to adjacent muscle cells by means of desmosomes and intercalated discs, which are unchanged at their boundaries. As a rule, mitosis proceeds without cytokinesis, so that a binucleate myocyte is formed (Fig. 17-19).

No evidence is found for the formation of true myoblasts devoid of myofilaments, intercalated discs, and specific atrial granules. Nonmuscle cells are identified as endothelial and connective tissue elements.

Frog Myocardia

The major electron microscope data described above for rat atrial cells are likewise valid with respect to the fine structural changes seen in injured frog myocardia. The main difference during the preproliferative period is the presence in frog tissue of numerous phagocytes that resorb the necrotic material of the myocardium (Fig. 17-20). While nuclear reorganization precedes the burst of DNA synthesis and mitoses as in reactivated atrial myocytes, the cytoplasmic manifestations of "partial dedifferentiation" (accumulation of ribosomes, rough endoplasmic reticulum, etc.) are somewhat delayed until the second to third post-injury week (Fig. 17-21). Dedifferentiation is incomplete so that free myoblasts are not formed. DNA synthesizing (Fig. 17-21) and mitotically dividing (Fig. 17-22) perinecrotic myocytes are characterized by the same ultrastructural fea-

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Figure 17-15. *A*. Slender myocardial anastomoses (arrows) regenerated in the subepicardial region of the crushed zone of the frog heart ventricle, 122 days after injury. *B*. Framed portion of *A* at higher magnification. Iron hematoxylin.

Figure 17-16. Hyperplasia of the rough endoplasmic reticulum (RER) in left atrium myocyte of rat. Day 5 after left coronary artery ligation. G = Golgi apparatus; SG = specific atrial granules; N = nucleus; MF = myofibril.

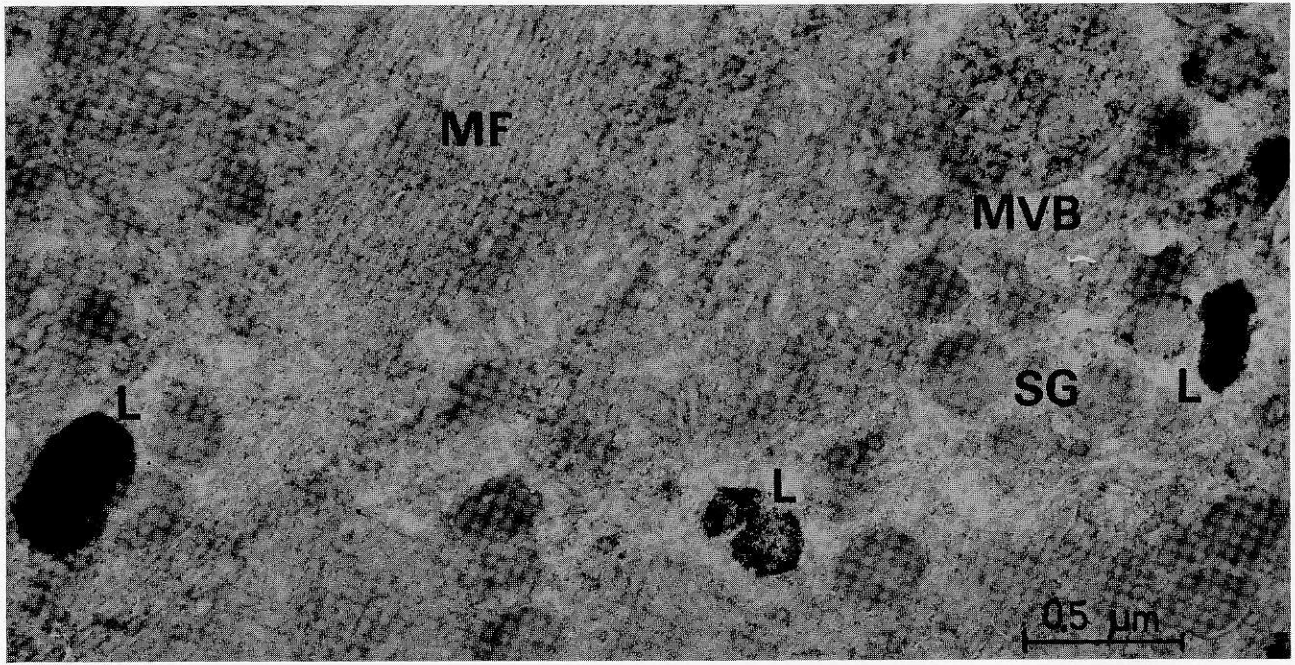

Figure 17-17. Acid phosphatase positive lysosome-like structures (L) in the left atrium myocyte of rat, day 6 after ligation of the left coronary artery. Specific atrial granules (SG) are mostly unreactive. MVB = multivesicular body containing some reaction product; MF = myofibril.

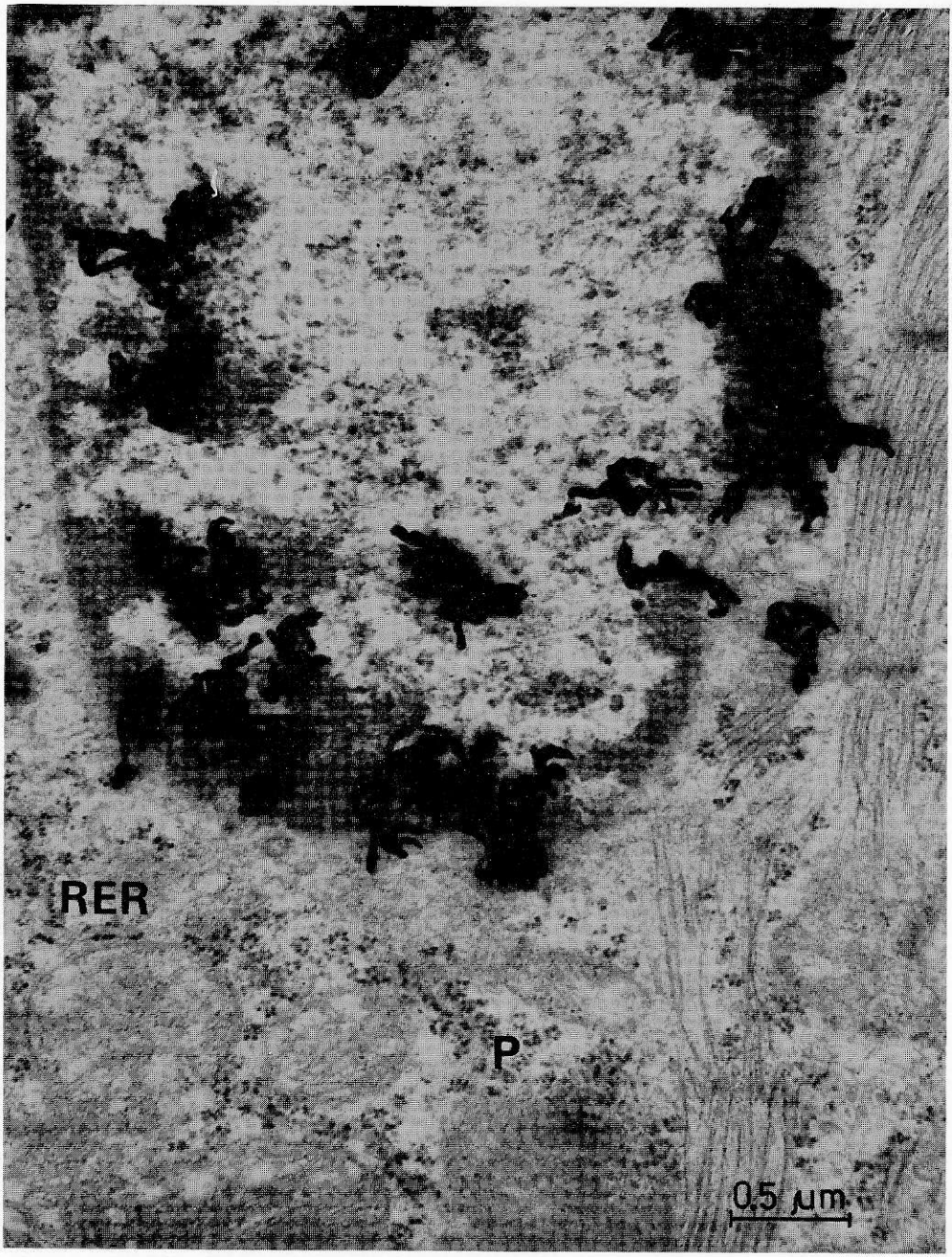

Figure 17-18. ^{3}H-thymidine pulse labeling of the left atrium myocyte of rat; day 6 after ligation of the left coronary artery; two hours after isotope injection. P = polysomes; RER = rough endoplasmic reticulum.

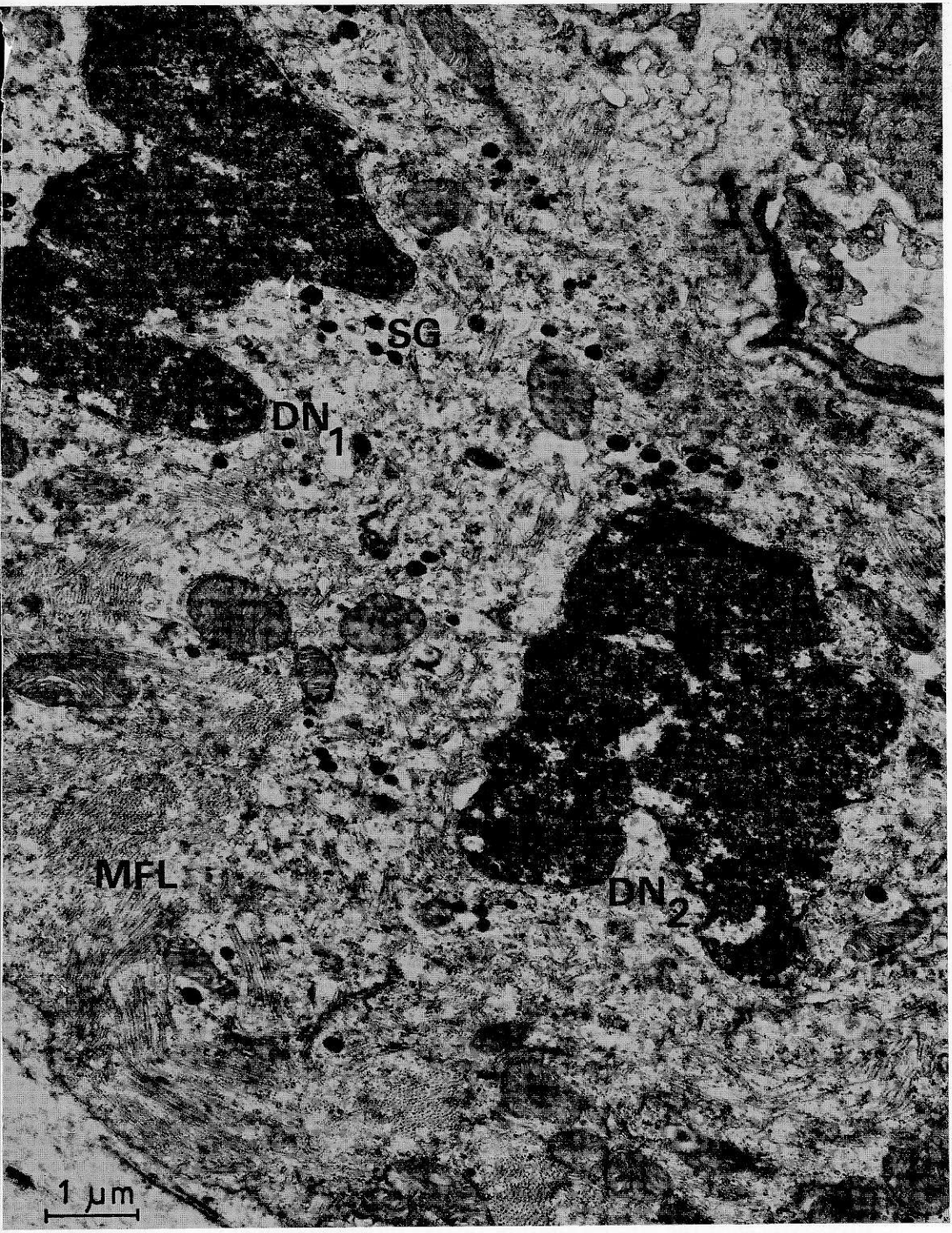

Figure 17-19. Portion of the late telophase myocyte from the left atrium of rat on day 6 after ligation of the left coronary artery. Myofilament bundles (MFL) are distributed randomly in the vicinity of the reappearing daughter nuclei (DN_1 and DN_2). Z-disc material is largely absent. SG = specific atrial granules.

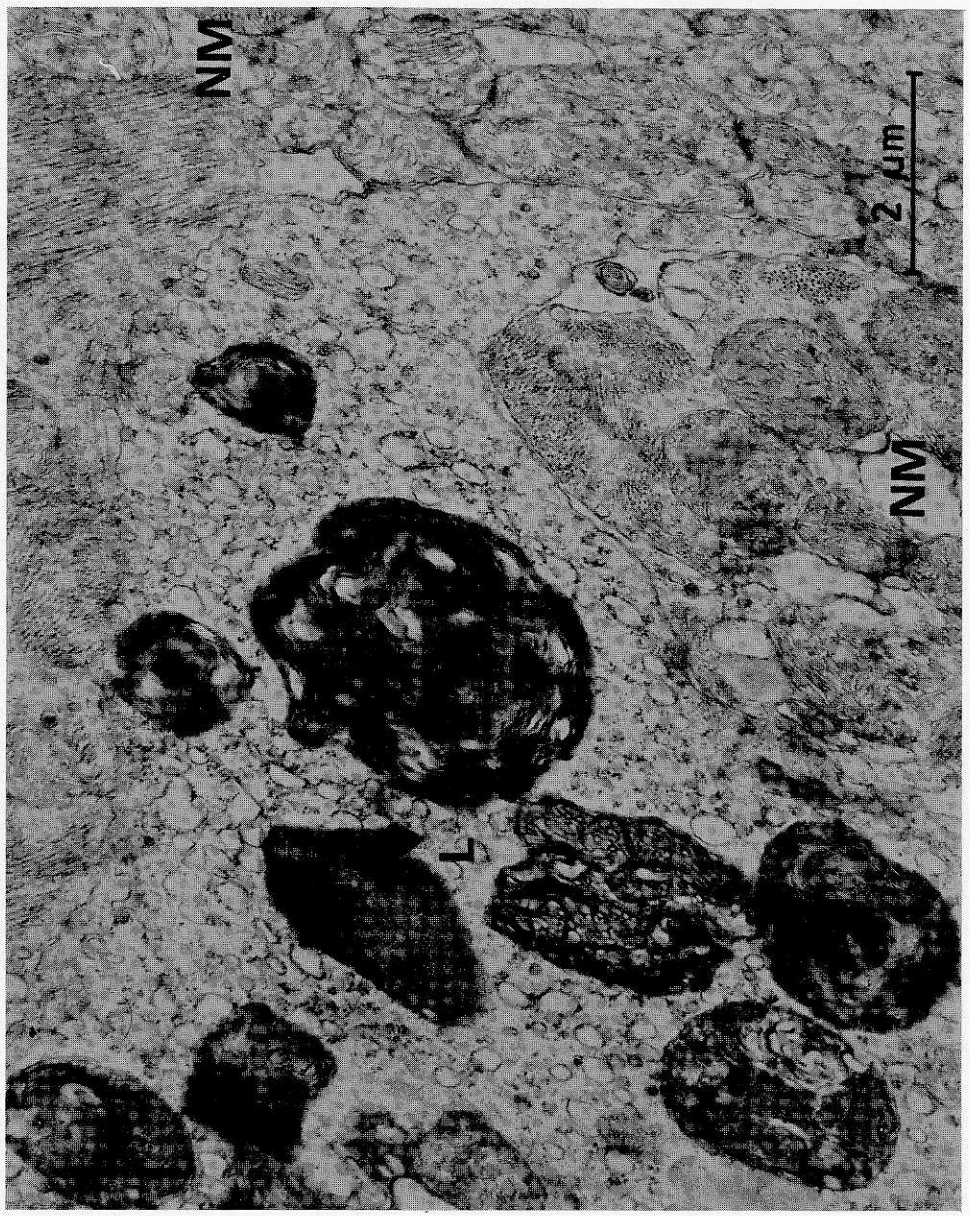

Figure 17-20. Acid phosphatase rich lysosomes (L) in the phagocyte cytoplasm, which is in close contact with necrotized myocytes (NM). Day 3 after the injury to frog heart ventricle.

tures typical of the corresponding phases of the mitotic cycle in the normally developing myocardium (Fig. 17-2). The majority of the above ultrastructural changes are less pronounced or become normalized at the most advanced stages studied (one month or later).

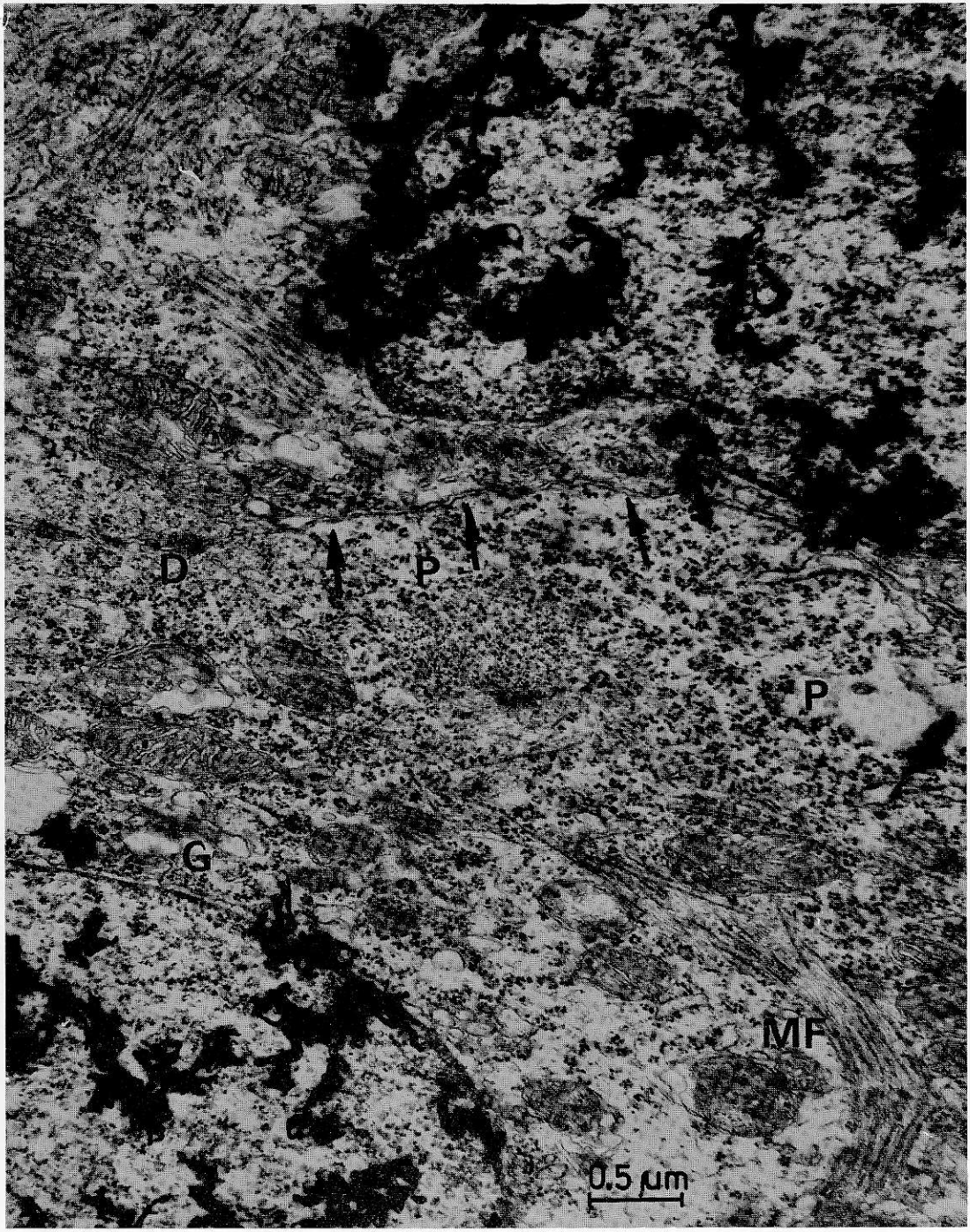

Figure 17-21. ^{3}H-thymidine pulse labeling of myonuclei from juxtanecrotic ventricular myocardium of the frog heart; day 16 after heart injury; two hours after isotope injection. Note the abundance of polysomes (P) and loosening of myofibrils (MF). G = Golgi vesicles; D = desmosome-like structures. Arrows point to the apposed cell membranes.

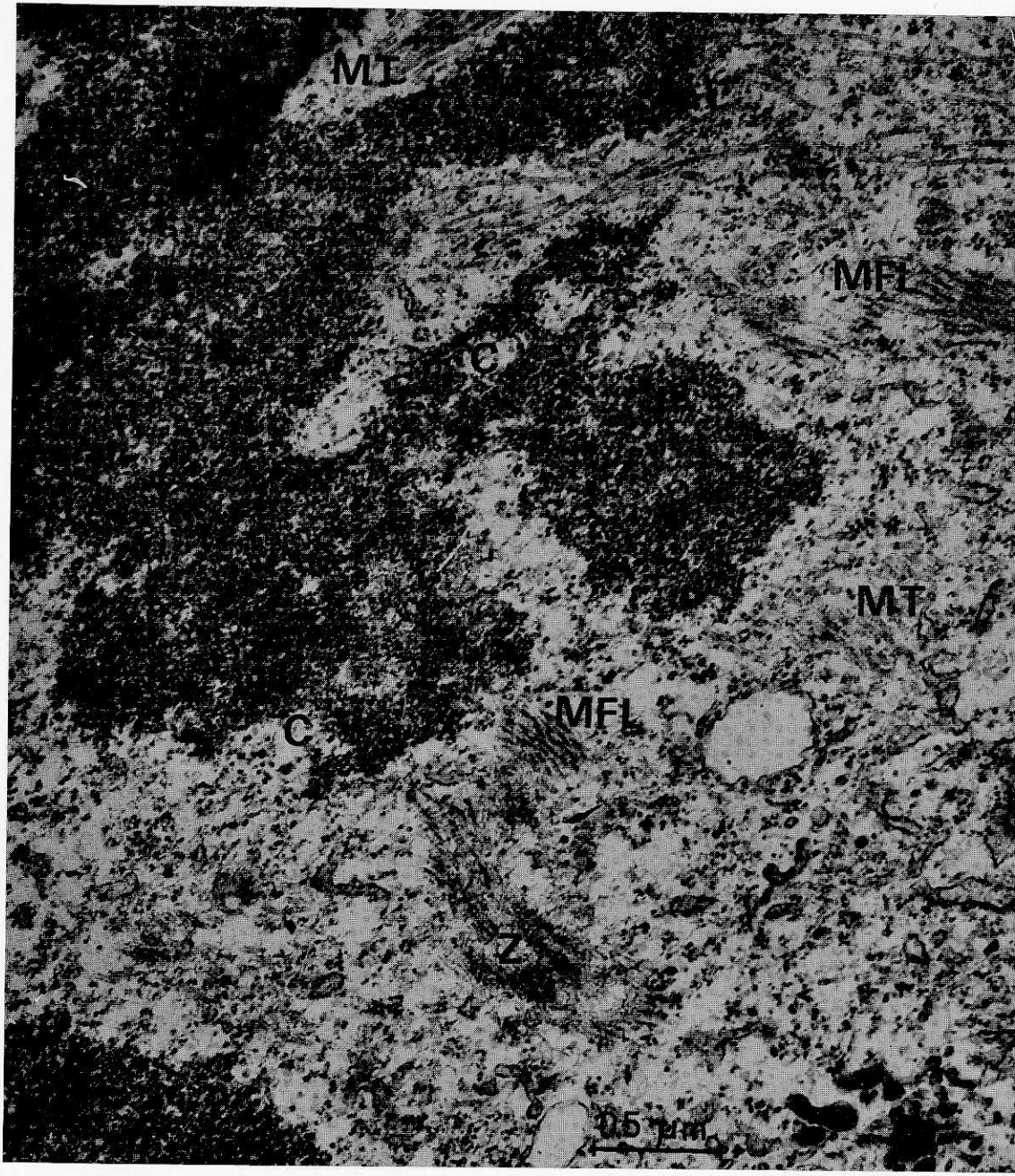

Figure 17-22. Metaphase myocyte from the juxtanecrotic region of the injured frog heart ventricle, day 16 after frog heart ventricle injury. C = chromosomes; MT = spindle microtubules; MFL = scattered myofilament bundles; Z = persisting portion of the Z-disc.

Discussion

The results of our comparative investigations on the regenerative potentialities of cardiac muscle with special reference to the proliferative behavior of myocytes are summarized in the scheme shown in Figure 17-23. We can conclude that all kinds of myocardia studied contain variable percentages of myocytes capable of being reactivated in terms of DNA synthesis and mitosis. The number of reversible postmitotic myocytes reaches 60 percent and more in rat atrium and frog ventricle. The latter is in agreement with data obtained from the newt heart by Oberpriller and associates (Oberpriller and Oberpriller, 1971, 1974; Bader and Oberpriller, 1978; Oberpriller et al., 1979). Irrespective of the kind of myocardial tissue investigated or the systemic position of the animal species studied, we found no evidence for significant dedifferentiation leading to the formation of actively migrating myoblasts devoid of myofibrils (Fig. 17-23).

The incompleteness of myocyte dedifferentiation and the absence of "dormant myoblasts" (comparable to Mauro's satellites of the skeletal

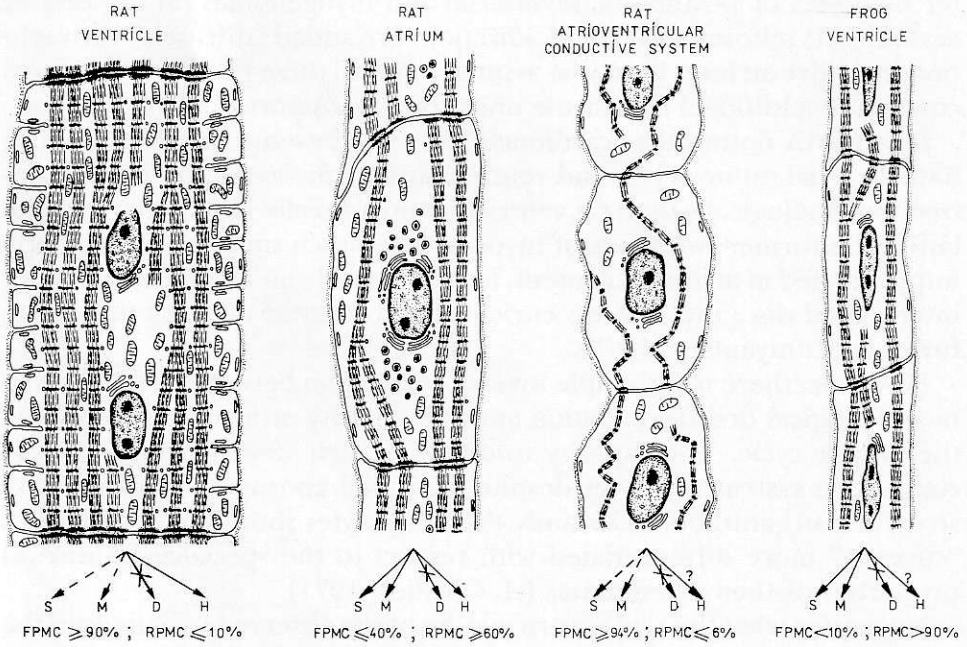

Figure 17-23. Schematic representation of the possible proliferative and nonproliferative responses of different cardiac myocytes to the injury and/or overloading. S = DNA synthesis; M = mitosis; D = complete dedifferentiation (free myoblast formation); H = hypertrophy; FPMC = fixed postmitotic (or static) cells; RPMC = reversible postmitotic cells. Assessment of the FPMC and RPMC percentages is made taking into consideration experiments with multiple ^{3}H-thymidine injections (see text).

muscles) in all adult myocardia studied seem to be responsible for cicatrization at the sites of myocardial necroses in spite of the proliferation of perinecrotic myocytes, which may proceed rather actively (frog heart, atria of rat heart). The outgrowth of proliferating myocytes into the necrotic area is limited by the relatively long preproliferative period (four to five days), by the increased length of the reactivated mitotic cycles in myocardia of the adult animals (Fig. 17-7; cf. Rumyantsev, 1973), and by the maintenance of myocyte-to-myocyte attachments with persistence of specialized junctions and intercalated discs despite partial dedifferentiation of myocytes. All the above factors seem to favor overgrowth by the more rapidly proliferating non-muscle cells within the necrotic myocardial area.

Because of the fixed position of the actively dividing perinecrotic myocytes and overgrowth by connective tissue cells, there is a predominating accumulation of postmitotic muscle cells around but not inside the necrotized areas. This is clearly demonstrated in the experiments with multiple ^{3}H-thymidine injections after injury to the frog heart (Fig. 17-14).

When atrial myocyte hyperplasia is stimulated from a distance, as a result of left ventricle infarction or aortic constriction in rats, new daughter myocytes or postmitotic myonuclei and myogenomes (in the case of acytokinetic mitoses and polyploidization) are added "diffusely" throughout the entire atrium. It may be assumed that all these proliferative events contribute additional contractile units for the support of heart function.

It is worth noting that cardiomyocytes that resume the mitotic cycle, namely atrial rat myocytes and ventricular frog myocytes, are less specialized morphologically than rat ventricular muscle cells. Compared with the latter, the former two kinds of myocytes are much smaller, are relatively impoverished in myofibril content, have practically no T-tubules, and have intercalated discs that are less enriched with contrast material (for literature, see Rumyantsev, 1977).

However, there is no simple inverse correlation between the degree of morphological dedifferentiation and the capacity of myocytes to resume the mitotic cycle. The capacity to divide is fairly low in the case of the conductive system myocytes despite their well-known "primitive" ultrastructural organization. Certainly these myocytes should be regarded as "covertly" more differentiated with respect to the specialized electrical properties of their membranes (cf. Challice, 1971).

No matter what the real nature may be of the observed variations in the capacity for reactivated proliferation among the different kinds of heart myocytes, it is essential that there be a reconsideration of the old classical concept of myocardial regeneration, which takes into account the proliferative behavior of the mammalian ventricular myocytes only.

Our comparative investigations seem to permit the distinguishing of at least two different kinds of stimuli for the reactivation of the adult car-

diomyocyte mitotic cycle. One of these stimuli, which appears to be rather local, is manifested by the reactivated proliferation of perinecrotic myocytes in the injured frog ventricle or rat atria.

Another type of stimulus, which may act from a distance, involves heart hyperfunction. This is evidenced by rat atrial myocyte hyperplasia following ligation of the left coronary artery or coarctation of aorta. Additional evidence of the role of cardiac hyperfunction in the reactivation of the atrial myocyte hyperplasia is found in the fact that the majority of extracardiac atrial myocytes of the perivenous myocardial sheets in lungs do not participate in the postinfarction mitotic burst seen in atrial myocytes. It is highly unlikely that these extracardiac myocytes are involved in hyperfunction after infarction or aortic constriction, in contrast to "cardiac" atrial myocytes which would be affected.

It will be interesting to investigate in the future the question as to whether the observed variations in the activity of left and right atrium myocyte hyperplasia following infarction or aortic constriction may be explained in terms of corresponding changes in circulation dynamics.

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