

Interrelations of the Proliferation and Differentiation Processes during Cardiac Myogenesis and Regeneration

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I. Introduction

Myogenesis has attracted the ever-increasing interest of investigators for more than 100 years. It should be emphasized that interrelationships between cytodifferentiation and the proliferation of myogenic cells have been found to be highly complicated and apparently not identical in diverse types of myogeneses. Thus it is now well established that the proliferation and differentiation of myogenic cells are mutually exclusive processes in developing and regenerating skeletal muscle. Neither DNA synthesis nor mitosis and amitosis occur following the onset of myofibril differentiation and/or myoblast fusion (Konigsberg, 1965; Betz *et al.*, 1966; Holtzer, 1970a,b; Lentz, 1973; Manasek, 1973; Carlson, 1973). However, in developing and regenerating smooth muscle, the cells of which display less complex nonsarcomeric contractile machinery, proliferation and differentiation coexist (Dubinko, 1966; Zavarzin, 1967; Cobb and Bennett, 1970; Jinkine and Rumyantsev, 1970).

It is of importance therefore to establish what kind of interrelationship between proliferation and differentiation is typical of cardiac myogenesis, that is, whether these processes are mutually exclusive or compatible, as in skeletal and in smooth muscle, respectively. Cardiac myogenesis has been much less studied in this respect as compared to skeletal myogenesis (Holtzer, 1970b). During the long history of myogenic studies there frequently has been a tendency to emphasize a basic similarity in the interrelationships between proliferation and differentiation in cardiac and skeletal myogenic cells (Godlewski, 1902; Katznelson, 1936; and Holtzer, 1970b). Several investigators have concluded that developing cardiac muscle cells possessing striated myofibrils are incapable, as in skeletal myogenesis, of synthesis of DNA and mitosis (e.g., Rumery and Rieke, 1967; Shafiq *et al.*, 1968). Based on these observations the possible participation of a pool of structurally undifferentiated myoblasts (or myogenic stem cells) in cardiac myogenesis was repeatedly claimed.

During the past two decades, however, a growing body of evidence has accumulated supporting the idea that the dogma of mutual exclusivity of proliferation and striated myofibril differentiation cannot be applied to cardiac myogenesis (for literature, see Manasek, 1973). If this is true, there are many interesting questions to consider, involving (1) the relative contribution of myoblast and immature myocytes to myogenesis, (2) the behavior of myofibrils and other organelles at different phases in the proliferative cycle and their distribution between the daughter cells, (3) the mechanism of cardiac muscle

cell cytokinesis, (4) the degree of myocyte differentiation still compatible with proliferation, (5) changes in the chronology of the mitotic cycle and in myocyte ploidy at different stages of myogenesis, (6) the stability of myocyte withdrawal from the proliferative cycle, and so on. As concerns skeletal myogenesis, the majority of these questions are not relevant because of the stable suppression of nuclear proliferation in myofibril-containing myoblasts and myotubes.

The study of the proliferative behavior of cardiac myogenic cells is closely related to the investigation of myocardial regeneration. In spite of the numerous conflicting reports concerning this almost century-old problem the majority of workers agree that there is a profound difference in the regenerative capacity of skeletal and cardiac muscles. The former are known to be capable of restoring innumerable muscle fibers (for reviews, see Betz *et al.*, 1966; Carlson, 1973), while the latter are usually considered practically unable to regenerate necrotized or excised parts via proliferation of the remaining myocardial cells (see reviews by Antischkow, 1912, 1913; Mönckeberg, 1924; Hudgson and Field, 1973). In agreement with this conclusion mature cardiac muscle cells were classified as *elementi perenni* (Bizzozero, 1894), or cells of static cell populations (Cameron, 1971). So-called myosatellites found in skeletal muscle fibers and regarded as a possible source of their regeneration were not observed in myocardium (Mauro, 1961; Muir *et al.*, 1965). Unlike skeletal muscle, myocardium is very rarely subject to neoplastic transformation (Saphir, 1960), which is strongly suggestive of a stable inhibition of DNA synthesis and mitosis of the myonuclei and of the absence of a pool of undifferentiated myogenic cells (Holtzer and Sanger, 1972).

Some investigators believe that myocardial cells possess an intrinsic capacity to dedifferentiate and multiply at the borders of necroses which, however, is not manifested overtly because of the lack of permissive conditions and appropriate stimuli or, possibly, because of nonmuscle cell overgrowth (Polezhaev *et al.*, 1965; Hay, 1966; Polezhaev, 1972a,b). There are provocative reports of rather intensive myocardial regeneration (Sinitsin, 1961, 1970) and its effective stimulation by some agents (for review, see Polezhaev, 1972a,b). Since the majority of data on the regenerative capacity of cardiac muscle are based on orthodox histological observations, McMinn (1967) suggested that this problem be reinvestigated using modern cytological methods.

It is surprising that almost all the studies on the regenerative capacity of cardiac muscle deal only with mammalian ventricular myocardium. However, several recent investigations permit the conclusion

that more primitive types of cardiac muscle cells, namely, atrial myocytes in mammals and both ventricular and atrial myocytes in lower vertebrates, display a much greater capacity for reactivated hyperplasia than ventricular myocytes in mammals (Kolossova, 1973; Rumyantsev, 1961, 1966, 1973a,b; Sulima, 1968; Oberpriller and Oberpriller, 1971, 1974).

Several reviews survey different aspects of cardiac myogenesis (De Haan, 1965; Manasek, 1970b, 1973; Zak, 1973, 1974), regeneration (Anitschkow, 1912, 1913; Mönkeberg, 1924; Polezhaev *et al.*, 1965; Polezhaev, 1972a,b; 1975; Sarkisov, 1970; Hudgson and Field, 1973), and hypertrophy (Meerson, 1969; Zak, 1973, 1974) and are of value to anyone interested in these problems.

The aim of this article is to describe the complicated proliferative behavior of cardiac muscle cells both in normal myogenesis and regeneration and its dependence on the differentiative properties of these cells. This seems to be useful for further progress in the study of myogenesis at the cellular level.

II. Differentiative Properties of Cardiac Myocytes

From the vast body of multidisciplinary evidence concerning cardiac myocyte differentiation (for review, see De Haan, 1965; Manasek, 1970b, 1973; McNutt, 1970) we briefly summarize here only findings, common to all species, that seem to be relevant to the subsequent description of the proliferative behavior of these cells.

Before their transformation into myocardium the epithelial cells of the precardiac splanchnomesoderm are interconnected by terminal bars. Their cytoplasm contains a developed Golgi apparatus, sparse small mitochondria and rough endoplasmic reticulum tubules, numerous free ribosomes, and a few polysomes and glycogen granules (Manasek, 1968a; Virág and Challice, 1973). Only thin filaments of a chemically unidentified type were observed, predominantly associated with the terminal bars (Olivo *et al.*, 1964; Dourain *et al.*, 1965; Manasek, 1968a; Virág and Challice, 1973). The appearance of typical thick filaments—the first unequivocal manifestation of muscle differentiation—precedes slightly the onset of rhythmical contractions of the tubular heart. At the beginning of differentiation these filaments are randomly arranged, being interspersed with 60-Å-diameter filaments. Soon after emergence of the irregular dense spots, which transform rapidly into primitive Z bodies, sarcomerogenesis starts. Some of the intercalated disks seem to originate from the terminal bars of splanchnomesodermal cells. At the onset of the tubular heart contractions most of the differentiating muscle cells contain a few

myofibrils which may be composed of only several sarcomeres. In the vicinity of the primitive Z disks the appearance of the sarcoplasmic reticulum vesicles is noted. The rough endoplasmic reticulum proliferates intensively, and the number of large polysomes and glycogen granules increases (Manasek, 1968a; Hagopian and Spiro, 1970; Virág and Challice, 1973). Formation of the first Z bodies is generally observed in the vicinity of the cell membrane (Hagopian and Spiro, 1970; Rash *et al.*, 1970b; Legato, 1972), frequently in connection with desmosomes and/or skeins of 80- to 130-Å-diameter filaments. It is known now that these intermediate-sized filaments occur in most diverse cell types (Ishikawa *et al.*, 1968; Uehara *et al.*, 1971), conceivably representing microtubule precursor material (Wisniewski *et al.*, 1968; Holtrop *et al.*, 1974).

While the majority of investigators claim that the earliest myofibrils are assembled just beneath the cell surface, Manasek (1968a) observed their appearance in different regions of the cytoplasm. Data concerning the spatial interrelationships between large polysomes and arising thick myofilaments (Cedergren and Harary, 1964; Legato, 1972), as well as the mechanisms of myofibril growth, that is, the addition of new myofilaments and entire sarcomeres, remain largely speculative both for skeletal and cardiac myogenesis (cf. Holtzer, 1970b; Lentz, 1973; Manasek, 1973). Little is known about the precise role played by Z disks in sarcomerogenesis (Holtzer, 1970b; Fischman, 1973; Lentz, 1973). Their participation, possibly with the cell cortex, in myofilament bundle alignment and interlinking is not improbable, however (Manasek, 1968a; Hagopian and Spiro, 1970; Rash *et al.*, 1970b). Some workers speculate that Z-disk precursor material spots represent centers of myofilament formation and growth (Wainrach and Sotelo, 1961; Legato, 1972). There are no indications that microtubules, regularly observed in differentiating cardiac myocytes, participate in myofibrillogenesis.

The earliest cardiac muscle cells displaying a unique phenotype owing to the differentiation of myofibrils, intercalated disks, desmosomes, sarcoplasmic reticulum, and the accumulation of glycogen are still often designated cardiac myoblasts (Wainrach and Sotelo, 1961; Olivo *et al.*, 1964). The latter should be better referred to below as immature myocytes (cf. Manasek, 1973). The extensive development of the Golgi apparatus, rough endoplasmic reticulum, intercalated disks, and desmosomes in differentiating cardiac muscle cells represents specific features depending presumably on both their epithelial origin and secretory function (Manasek, 1968a, 1973). Since skeletal myofibrillogenesis proceeds without intensive development of the Golgi apparatus and endoplasmic reticulum, Manasek (1968a, 1970a,

1973) assumed that these organelles are involved in the production of mucopolysaccharides for cardiac jelly and an extracellular sulfated matrix. This was proved by using $^{35}\text{SO}_4$ or galactose- ^3H as a label (Manasek, 1973).

The progressive differentiation of cardiac myocytes proceeding gradually during the course of embryonic and postnatal development implies a step-by-step increase in the size, number, and structural complexity of myofibrils, mitochondria, and sarcoplasmic reticulum elements, and plasma membrane specialization, that is, intercalated disks, desmosomes, and tight junctions. On the contrary, the relative amount of undifferentiated cytoplasm, nonsarcomeric 80- to 110-Å-diameter filaments, microtubules, free ribosomes, Golgi and rough endoplasmic reticulum elements diminishes visibly, especially during the postnatal stages of cardiac myogenesis (Hibbs, 1956; Muir, 1957; Challice and Edwards, 1961; Wainrach and Sotelo, 1961; Olivo *et al.*, 1964; Schiebler and Wolff, 1966; Rumyantsev, 1967; Manasek, 1968a, 1970a; Pager, 1968; McNutt, 1970; Hagopian and Spiro; Rash *et al.*, 1970b). The centrioles regularly observed in the immature cardiac myocytes of embryos (Rumyantsev, 1967; Przybylsky, 1971) become rare after birth, possibly as a result of cell volume increase (Rash, 1969).

As judged from their ultrastructural properties, the ventricular myocytes of the rat reach an almost mature state about 2 weeks after birth (Schiebler and Wolff, 1966; Rumyantsev, 1967; Pager, 1968). Chemo-differentiation of cardiac myocytes in the rat is practically complete 10 days after birth (Hecht, 1971). A sharp rise in the activity of all the oxidative enzymes studied is observed at this stage, while the glycolytic pathway predominates during embryonic and earlier postnatal myocardial development (Tóth and Schiebler, 1967; Diculescu *et al.*, 1969). The literature pertaining to the chemo-differentiation of cardiac myocytes has been reviewed by Beckett and Bourne (1973).

A T system is formed only in mammals and some birds during late postnatal development of the ventricular myocardium (Gossrau, 1968; Hirakow, 1970; Page and Fozzard, 1973). At earlier ontogenetic and phyletic stages of cardiac myogenesis the coupling of the sarcolemma with the interior compartments of the cardiac myocyte is believed to be provided by terminal subsarcolemmal cisternae of the sarcoplasmic reticulum (Sommer and Johnson, 1970; Hirakow, 1970).

Atrial myocyte differentiation has several peculiar features as compared with that of ventricular myocardium. In the rat these cells are much less subject to progressive hypertrophy, which is typical of ventricular myocytes during postnatal development (Pager, 1968). This seems to be due to the relative paucity of myofibrils in atrial myo-

cytes. (Page *et al.*, 1972; Pilny, 1975). Their intercalated disks are not so enriched in contrast material as those in ventricles and remain relatively small even in adult mammals; T tubules do not appear in the majority of atrial myocytes (Hibbs and Ferrans, 1969; McNutt and Fawcett, 1969; Forssmann and Girardier, 1970; Berger and Rona, 1971).

Unlike the organelles involved in the contractile function, the Golgi and rough endoplasmic reticulum elements develop more intensely in atrial myocytes compared to ventricular myocytes. This depends on considerable secretory activity in atrial myocytes, producing numerous "specific" atrial granules (Kisch, 1963; Jamieson and Palade, 1964; see review by Bencosme and Berger, 1971). These granules do not contain catecholamines, being composed mainly of glycoproteins (Bencosme and Berger, 1971; Huet and Cantin, 1974a,b).

Thus the differentiation of atrial myocytes is a good example of highly expressed cell bifunctionality (myosecretory specialization). On the whole it may be said that atrial myocytes are less differentiated than ventricular ones (Pager, 1968), which should be kept in mind in interpreting the different proliferative behaviors of these types of cells in adult mammals (see Section IV,D).

Cardiac myogenesis involves differentiation of the specialized myocytes forming the conducting system of the heart. These highly excitable cells are very poor in myofibrils being rich in nonsarcomeric cytofilaments (De Haan, 1965; Gossrau, 1968; Virág and Challice, 1969; Challice, 1971). The specialized myocytes differ histochemically from working atrial and ventricular muscle cells in their relative paucity in respiratory enzymes, in abundance of glycogen and in high activity of choline esterase and enzymes of glycolysis (Gossrau, 1968; Tóth and Schiebler, 1967). Ultrastructural aspects of their differentiation were studied by Yamauchi (1965) and Nanot and le Douarin (1975).

At the earliest stages of heart formation the population of the arising muscle cells is almost homogeneous, the endothelial and connective tissue elements progressively invading the myocardium only later in the process of development (Manasek, 1968a, 1973; Virág and Challice, 1973).

Despite the claimed participation of the persisting fraction of pre-myoblasts or myogenic "stem" cells in embryonic and postnatal cardiac myogenesis (Wainrach and Sotelo, 1961; Rumery and Rieke, 1967; Shafiq *et al.*, 1968; De Haan, 1971; Goode, 1973; Przybylski and Chlebowksi, 1972; Zak, 1973) no ultrastructural or cytochemical criteria have been established to distinguish these purely hypothetical cells from endothelial and connective tissue elements. The salient

feature of cardiac myogenesis is the absence from all its stages of intra-sarcolemmal cells resembling satellites of skeletal muscle (Mauro, 1961; Muir *et al.*, 1965). Moreover, other kinds of putative myoblasts have not been identified (Rumyantsev, 1967, 1972a; Manasek, 1968a,b, 1973; Weinstein and Hay, 1970), and no cell fusion has been observed in developing myocardium.

It may be concluded from the foregoing that, apart from myofibrillogenesis, differentiation of cardiac muscle cells is characterized by several specific features not encountered in skeletal myogenesis. These peculiarities are better understood if we take into account the proliferative behavior of cardiac myocytes.

III. Cell Proliferation in Cardiac Myogenesis

A. MORPHOLOGY OF DNA-SYNTHESIZING MYOCARDIAL CELLS

1. Light Microscopy

Unlike the evidence concerning somatic myogenesis (Stockdale and Holtzer, 1961) thymidine-³H labels a considerable number of myonuclei in chick and mammalian embryo hearts (Rumyantsev, 1963a, 1965; Wegener *et al.*, 1964; Sissmann, 1966; Zavarzin, 1967; Erokhina, 1968a,b; Jeter and Cameron, 1971; Chacko, 1973; Polinger, 1973). The presence of striated myofibrils and of glycogen in the vicinity of labeled nuclei was strongly suggestive of the capacity of immature cardiac myocytes to replicate DNA after the onset of contractile protein synthesis. Light microscope observations, however, were not conclusive enough in this respect, since closely apposed membranes of differentiating myocytes and hypothetical proliferating pre-myoblasts could be resolved only at the ultrastructural level.

2. Electron Microscope Autoradiography

The absence of mutual exclusivity of DNA synthesis and myofibrillogenesis in developing myocardium was proved unequivocally after numerous thymidine-³H pulse-labeled nuclei were observed in the myofibril-containing myocytes of chick (Weinstein and Hay, 1970; Hay and Low, 1972) and rat embryos and neonate hearts (Rumyantsev, 1973a, 1976; Goldstein *et al.*, 1974) by means of electron microscope autoradiography (Table I). Myofibrils in the majority of labeled cells display a regular sarcomeric organization with well-outlined Z disks and both kinds of hexagonally packed myofilaments (Fig. 1). On the plasma membrane myofibrils are attached to typical

TABLE I
NUMBER OF THYMIDINE-³H-LABELED MUSCLE AND NONMUSCLE CELL
NUCLEI IN DEVELOPING MICE AND RAT HEARTS (ELECTRON
MICROSCOPE AUTORADIOGRAPHY)^a

Species and stage of development	Chamber	Labeled nuclei (%)	
		Myocytes	Nonmuscle cells
Mouse, 13-day embryo	Ventricle	30.6	29.6
Mouse, 18-day embryo	Ventricle	16.2	—
Rat, 1 day old	Ventricle	15.2	28.3
Rat, 3 days old	Ventricle	7.6	18.5
Rat, 5 days old	Ventricle	11.5	19.8
Rat, 5 days old	Atrium	5.5	6.2
Rat, 11 days old	Ventricle	3.5	8.2-9.7
Rat, 11 days old	Atrium	2.3-3.5	8-9.8
Rat, adult (after infarction)	Atrium	4.6-5	2.9-16.2

^a After Rumyantsev, 1978.

intercalated disks. The degree of differentiation of thymidine-³H incorporating myocytes increases notably at more advanced stages of embryonic cardiac myogenesis and especially after birth (Fig. 1). As judged by their myofibril, sarcoplasmic reticulum, intercalated disks, and glycogen content, the DNA-synthesizing myocytes of each given stage of heart development are in all respects similar to the great majority of adjacent unlabeled muscle cells (Rumyantsev, 1973a; Goldstein *et al.*, 1974).

As a rule, in the embryonic rat and chick heart thymidine-³H labels mononucleate myocytes, but in the myocardium of 5- to 11-day-old suckling rats occasional dikaryotic muscle cells were observed with both their nuclei tagged.

DNA synthesis seems to proceed without cessation of myocyte rhythmical contractions, as confirmed by the observation of both contracted and relaxed myofibrils in cells tagged with thymidine-³H (Rumyantsev, 1973a). It is also quite probable that the heterosynthetic activity of replicating myocytes remains mainly undisturbed (Weinstein and Hay, 1970), their voluminous nucleoli being rich in both granular and fibrillar components while the sarcoplasm contains numerous polysomes and rough endoplasmic reticulum tubules (Rumyantsev, 1976). Examples of simultaneously proceeding DNA

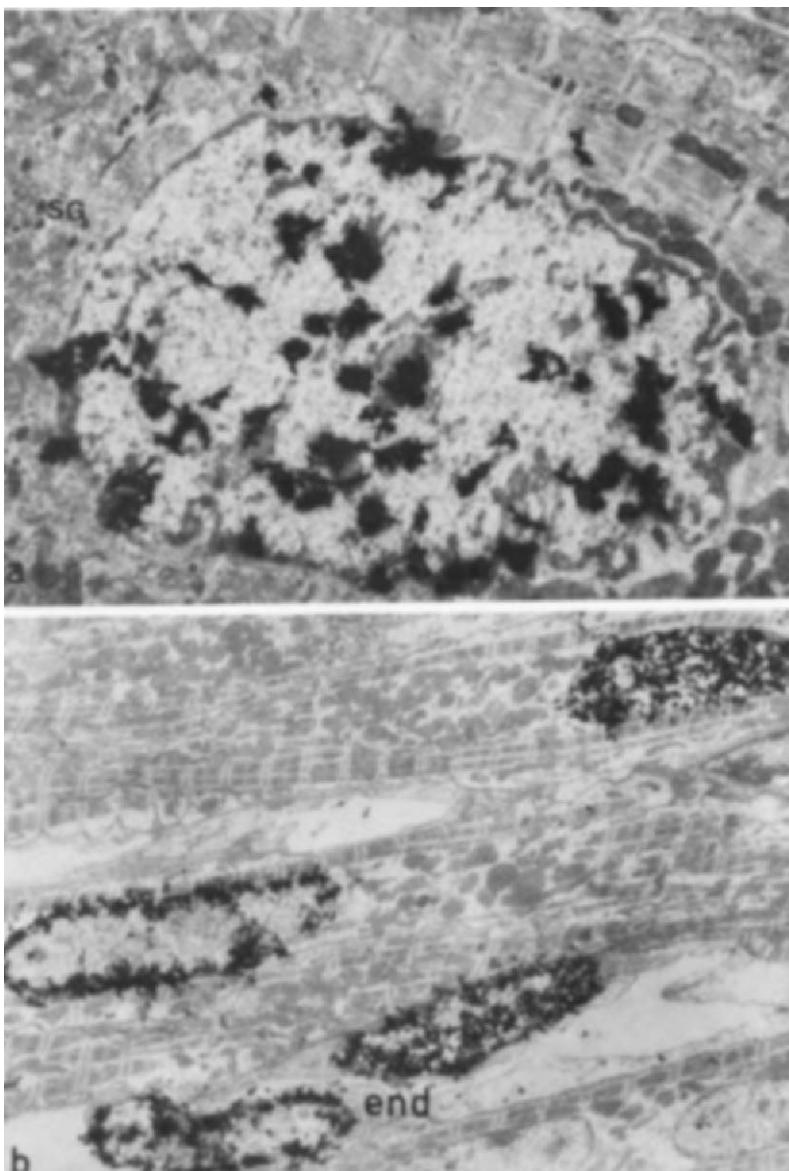


FIG. 1. Thymidine- ^3H pulse-labeled myocytes in suckling rat hearts. (a) Atrial myocyte of 5-day-old rat. SG, Specific granules. $\times 9300$. From Rumyantsev (1973a). (b) Two DNA-synthesizing myocytes and two replicating endothelial cells (end) in the left ventricle of an 11-day-old rat. A portion of the binucleate myocyte is seen in the lower-right-hand corner. $\times 2650$.

synthesis and translation of specific proteins are supported by double-labeling experiments (Cahn and Lasher, 1967).

For 8, 12, 14, 19, 25, and 72 hours following thymidine-³H administration some labeled myocytes in mouse and suckling rat hearts still remain undivided, the pattern of their ultrastructure being practically unchanged (Rumyantsev, 1973a, 1976). This is suggestive of the occurrence of muscle cells with a prolonged G₂ period. The ultrastructural peculiarities of labeled myocytes following their division are described in Section III,B and C.

B. NUCLEAR DIVISION AND CYTOKINESIS

1. Light Microscopy

It is noteworthy that in embryonic cardiac muscle, unlike the situation in skeletal muscle, not amitotic divisions but mitoses of centrally located myonuclei were described predominantly (Godlewski, 1902; Schockaert, 1909; Kurkiewicz, 1910; Tsimbal, 1937; Törö, 1939a; Kotshetov, 1959; Weissenfels, 1962; Rumyantsev, 1963a, 1965; Sasaki *et al.*, 1968b). Godlewski (1902) demonstrated the presence of striated myofibrils in the vicinity of a dividing nucleus. However, Schockaert (1909) and Weissenfels (1962) observed resorption of myofibrils belonging to a dividing myocyte, while other investigators (Rumery and Blandau, 1964; Rumery and Rieke, 1967) emphasized the fact that only structurally undifferentiated myoblasts are capable of mitotic division in developing myocardium. A comparison of skeletal and cardiac muscle from the same rat and rabbit embryo revealed that only the latter displays mitotic figures located, as far as can be judged at the light microscope level, within myofibril-containing muscle cells (Rumyantsev, 1964; Rumyantsev and Sokolovskaya, 1964). All phases of mitosis of the myocyte nuclei were easily observed in embryonic myocardium of chicks (Schockaert, 1909), rats, and rabbits (Rumyantsev, 1964). Cytotomy was well pronounced in less differentiated myocardial fibers of embryo hearts, often being incomplete in the dividing cardiac myocytes of suckling rats (Rumyantsev and Sokolovskaya, 1964).

Descriptions of amitoses as the main type of cardiac myonuclei proliferation in embryos are exceptional, but at the postnatal stages of cardiac myogenesis amitosis-like pictures were observed by numerous investigators (Schiefferdecker, 1916; Staemmler, 1928; Körner, 1935; Törö, 1939a; Hort, 1953; Linzbach, 1955; Robledo, 1956).

It was found in the rat, however, that 7–10 days after birth mitosis of the relatively mature cardiac myocytes often is not followed by cyto-

tomy, the small "twin" daughter myonuclei being reconstructed a short distance from one another. The subsequent postmitotic growth of these twin nuclei can bring them into contact, which simulates amitosis (Rumyantsev, 1963a; Rumyantsev and Sokolovskaya, 1964; Erokhina, 1968b). These amitosis-like pairs of myonuclei generally remain unlabeled after thymidine-³H administration, both the nuclei receiving isotope if material is fixed 48–72 hours later. The formation of pairs of amitotic-like myonuclei via modified mitosis is corroborated by cytophotometric data, since each of the two nuclei as a rule contain diploid amounts of DNA (Erokhina, 1968b).

2. Electron Microscopy

Earlier reports give rather confusing information. Several investigators mentioned the absence of myofibrils from mitotic myocardial cells (Muir, 1957; Wohlfarth-Bottermann, 1959; Wainrach and Sotelo, 1961; Weissenfels, 1962; Shafiq *et al.*, 1968).

It was later unequivocally demonstrated that in the developing chick and rat heart numerous mitotic cells possess typical thick and thin myofilaments (Rumyantsev, 1967, 1972a; Manasek, 1968a, 1973; Rumyantsev and Snigirevskaya, 1968; Weinstein and Hay, 1970; Chacko, 1972; Hay and Low, 1972; Przybylski and Chlebowski, 1972; Goldstein *et al.*, 1974; Goode, 1975; Kelly and Chacko, 1976).

According to Chacko (1972), the myofilament bundles in dividing myocytes are not organized into sarcomeres. However, in prophase (Fig. 2), just as in S phase, there are numerous myofibrils with well-outlined sarcomeres (Rumyantsev, 1967, 1972a; Rumyantsev and Snigirevskaya, 1968). Other organelles are also little changed, the

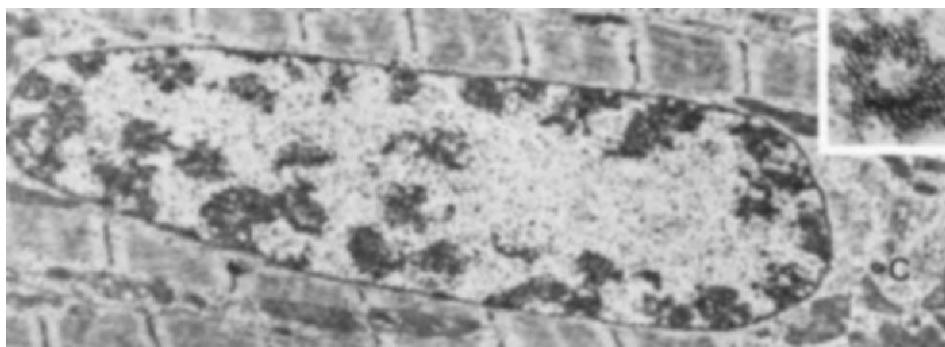


FIG. 2. Prophase myocyte from the left ventricle of a 7-day-old suckling rat. The structure of the myofibrils is still unchanged. $\times 3100$. Inset: Higher magnification of the centriole (C). $\times 44,000$.

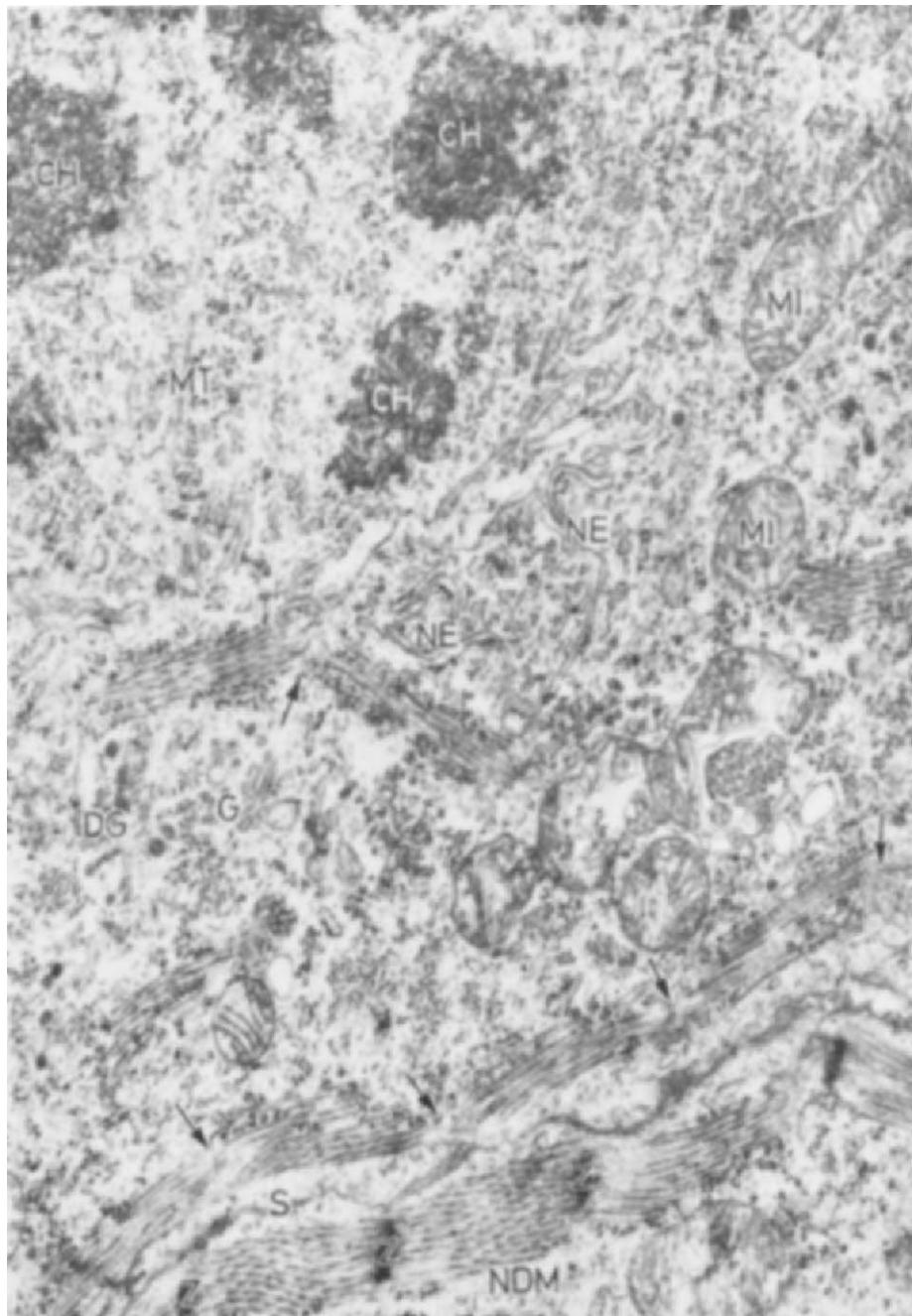
Golgi flattened cisternae being transformed into small vesicles at the end of prophase (Hay and Low, 1972; Rumyantsev, 1972a). Centrioles were encountered at the poles of prophase myonuclei (Fig. 2), while the number of microtubules was still very limited.

In prometaphase myocytes of the embryo heart the majority of Z disks lose their contrast material (Rumyantsev, 1967, 1972a, 1973a; Rumyantsev and Snigirevskaya, 1968; Hay and Low, 1972; Goode, 1975; Goldstein *et al.*, 1974). At the beginning of this process the sarcomeres still maintain their original position (Fig. 3), presumably being interlinked through fine, filamentous material still persisting in the region of the Z disks.

In metaphase and anaphase progressive degradation of the Z lines results in the release and more-or-less random displacement of free sarcomeres and myofilament bundles (Figs. 4 and 5c). Thick filaments demonstrate no visible changes in ultrastructure, but thin filaments in metaphase myocytes of early embryonic myocardium can disappear partly or completely (see insets for Figs. 4 and 6), which may be due to depolymerization (Rumyantsev, 1972a). This is often followed by enlargement of the interspaces between persisting thick myofilaments, and these interspaces are often invaded by free ribosomes. In good agreement with the above data rhythmical contractions of the dividing myocyte ceased after the onset of metaphase (Kasten, 1972, 1975; Goode, 1975), which is disputed, however, by Kelly and Chacko (1976).

In more differentiated mitotic myocytes of the suckling rat heart the process of Z-disk resorption can be incomplete (Fig. 5a and b), involving predominantly myofibrils near the dividing nucleus. Some sacromeres lose Z disks completely, and others from one side of the myofibril only; a few Z lines remain practically unchanged. Przybylski and Chlebowski (1972) observed the persistence of a few Z-disk material in metaphase myocytes of 3- and 20-day-old chick embryo hearts, whereas Kelly and Chacko (1976) found myofibrils with well discernible Z disks in approximately 50% of cultured chick embryo myocytes at different phases of their mitosis.

Unlike Z disks, intercalated disks and desmosomes are not changed after the onset of metaphase (Fig. 5d), despite the known resemblance of their contrast material to that of Z disks. The tubules of the sarcoplasmic reticulum, even near the resorbed Z disks, undergo no considerable alteration (Rumyantsev, 1972a). The rough endoplasmic reticulum tubules may be partly or even completely deprived of attached ribosomes. Free polysomes can be dissociated into isolated ribosomes (Przybylski and Chlebowski, 1972), which is a common fea-



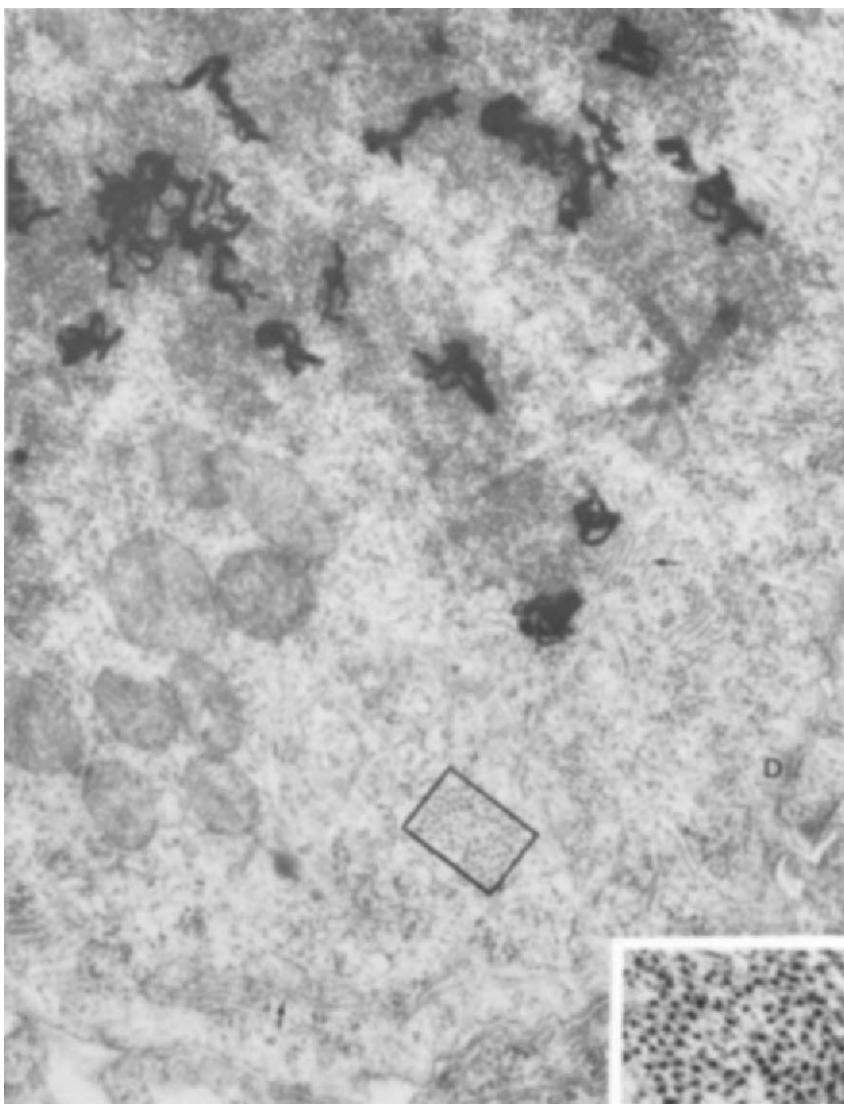


FIG. 4. Portion of a metaphase myocyte with labeled chromosomes from the ventricle of a 15-day-old mouse embryo 8 hours after thymidine- 3 H injection. Myofibril bundles (arrows) released from myofibrils after Z-disk breakdown (cf. Fig. 3) are distributed randomly. D, Desmosome. $\times 29,300$. Inset: Detail of the framed area. $\times 53,500$.

FIG. 3. Portion of a ventricular prometaphase myocyte from an 18-day-old rat embryo. Arrows indicate Z-disk regions lacking contrast material. Note contrast Z disks in a myofibril of the adjacent nondividing myocyte (NDM). CH, Chromosomes; MT, microtubule; NE, nuclear envelope remnants; G, Golgi apparatus; DG, dense granules; MI, mitochondria. $\times 21,200$. From Rumyantsev (1972a).

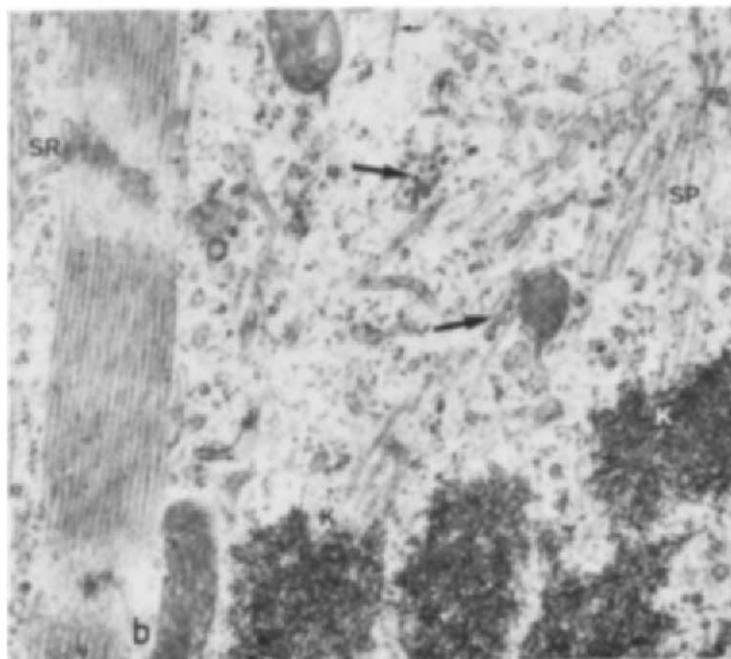


FIG. 5a and b. See page 204 for legend.

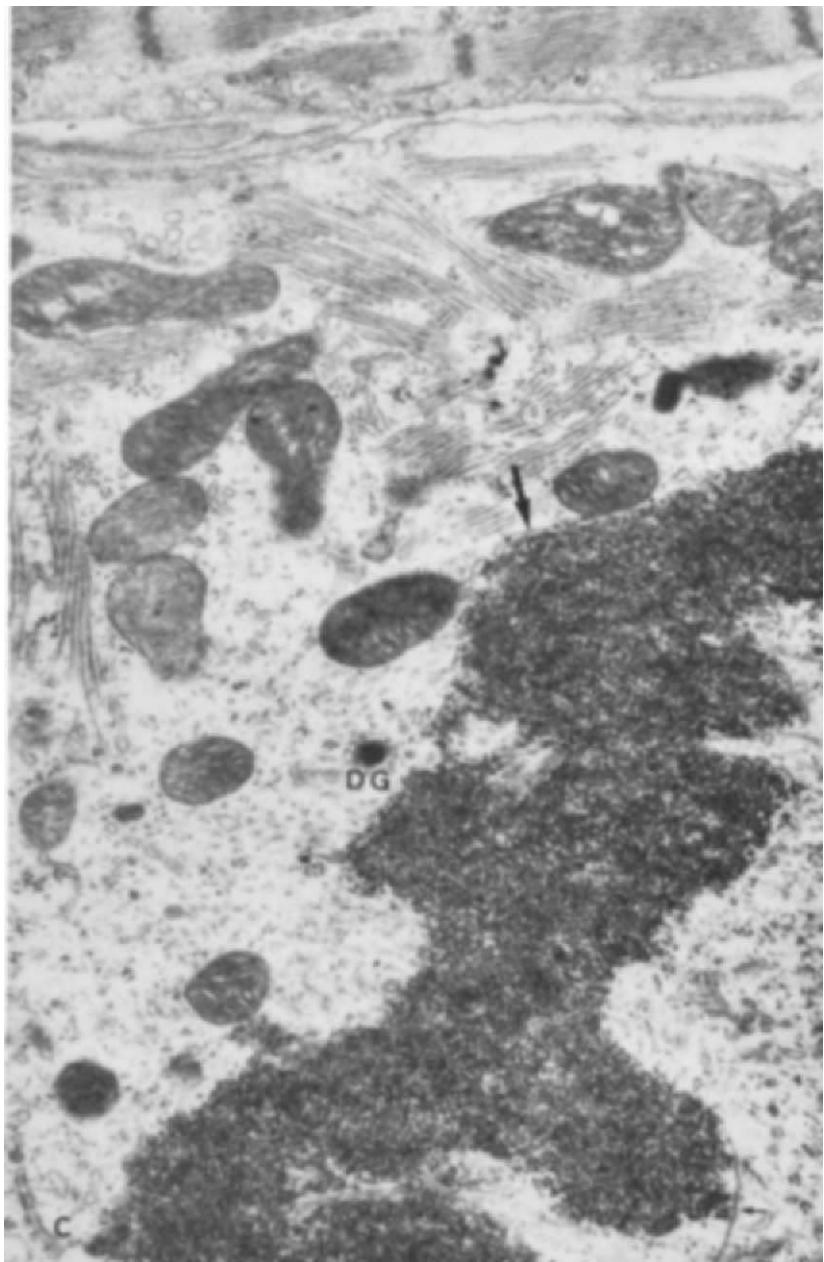


FIG. 5c. See page 204 for legend.

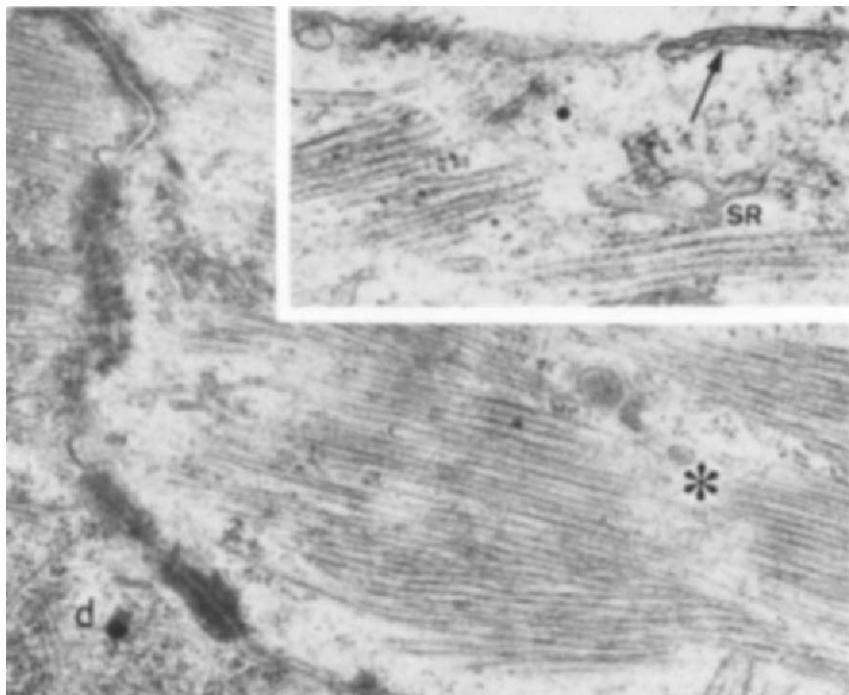


FIG. 5. (a) Anaphase and telophase myocytes from the left ventricle of a 7-day-old suckling rat. Unlike adjacent nondividing muscle cells, both mitotic myocytes display only a few sarcomeres with persisting Z lines (arrows). From Rumyantsev (1972a). $\times 2500$. (b) Detail of framed region 1. Some of abundant spindle microtubules (SP) are attached to kinetochores (K). The upper of two Z disks is preserved, and another is almost resorbed. Sarcoplasmic reticulum tubules (SR) are seen near a myofibril, especially at the level of the Z disks. The small arrow points to an isolated thick myofilament; the two large arrows indicate accumulations of dense vesicles. $\times 27,500$. (c) Detail of framed region 2. Released myofilament bundles are randomly distributed in the vicinity of the telophase chromosomes. Note the complete absence of Z-line material. The small arrow points to an isolated myosin filament, and the large arrow to the nuclear envelope beginning to reappear on the chromosomal surface. The regularly organized myofibrils seen at the top of the figure belong to a nondividing myocyte. DG, Dense granule. $\times 20,500$. (d) Detail of framed region 3. Intercalated disk at the end process of the anaphase myocyte remains unchanged, whereas the Z line (asterisk) is almost completely resorbed. The attachment of myofilaments to the contrast material of an intercalated disk appears to be disrupted, however, in the dividing cell and persists in the adjacent myocyte seen on the left. Note the accumulation of tubular elements between the intercalated disk and the released sarcomeres. $\times 31,000$. Inset: Detail of framed region 4. The sarcoplasmic reticulum elements (SR) persist in the vicinity of free myofilament bundles and beneath the sarcolemma (arrow). Asterisk labels dense spot possibly composed of Z-disk material. $\times 45,000$.

ture of other types of dividing cells (Sharff and Robbins, 1966). Goode (1975), however, observed large helical polysomes in metaphase.

The mitotic apparatus is well developed and contains the centrioles, located near the chromosomes, and abundant microtubules. The chromosomal microtubules are attached to typical kinetochores (Fig. 5b). In anaphase and telophase the majority of myofilament bundles are more or less randomly distributed in the polar processes of the dividing myocytes. The reconstruction of the nuclear envelope (Fig. 5c) and Golgi apparatus proceeds in the same manner as in other types of dividing cells (cf. Robbins and Gonatas, 1964).

Clumping of the microtubular fascicles within the interzonal region frequently results in the formation of stem bodies (cf. Reith and Jokelainen, 1973), which may be surrounded by the numerous small dense granules (Goode, 1975; Kelly and Chacko, 1976). Cytokinesis usually occurs in the latest telophase of poorly differentiated myocytes of embryonic hearts (Fig. 6). Cleavage furrow ingrowth continues up

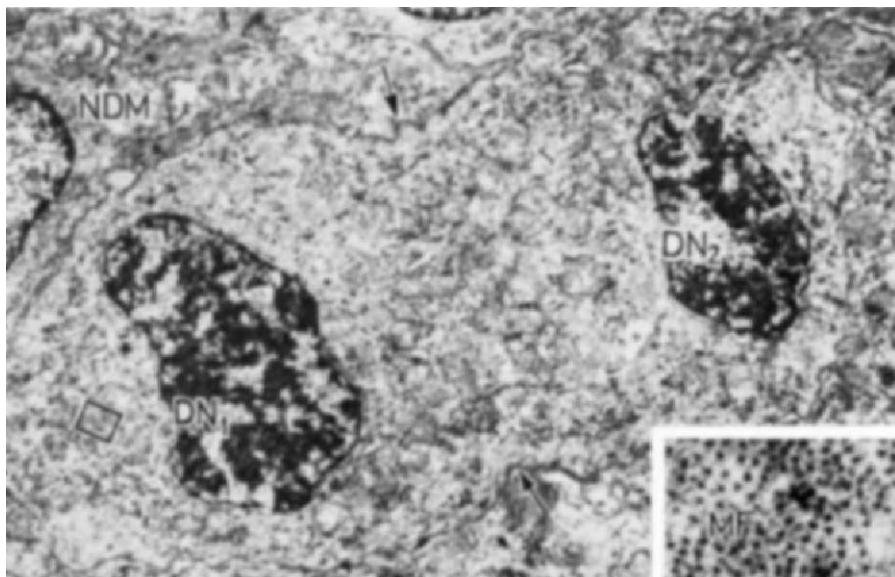
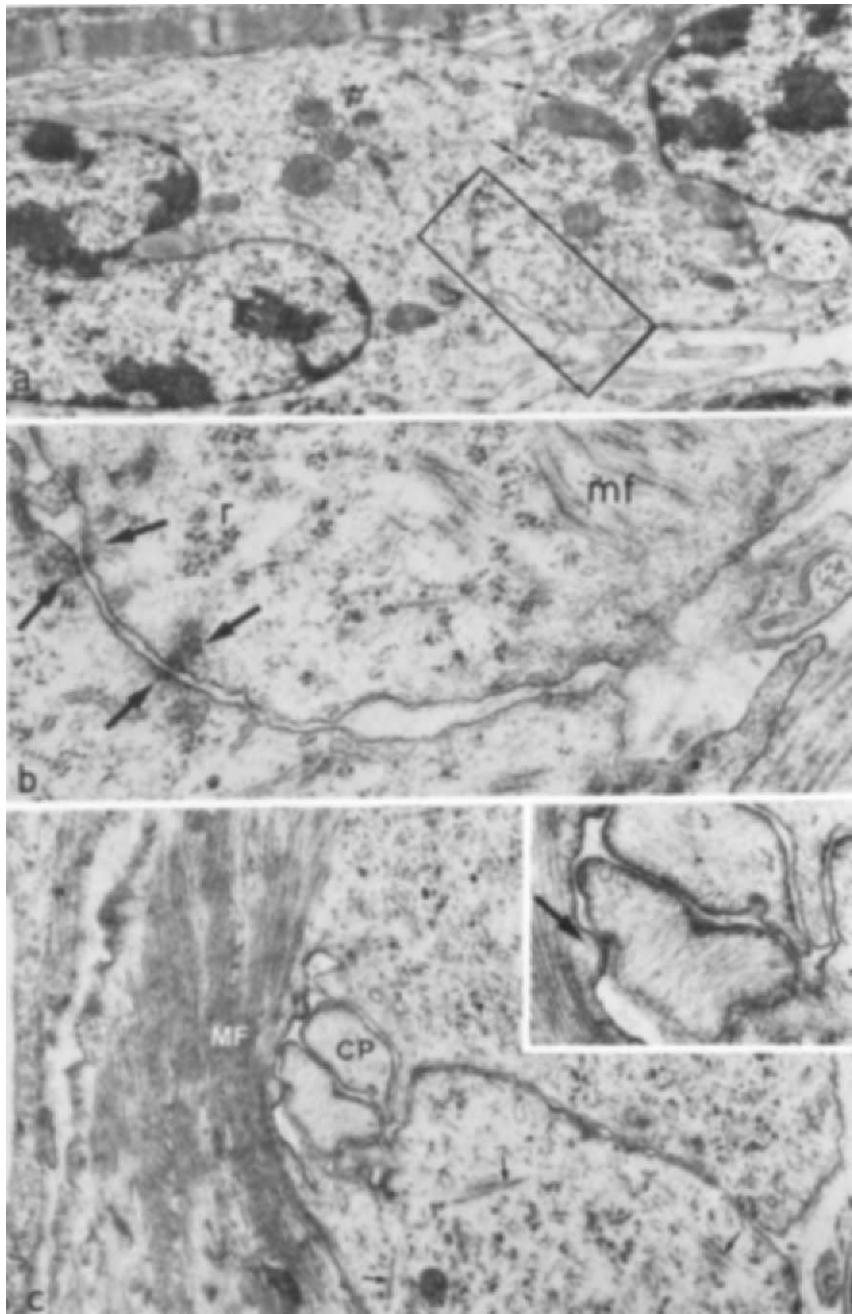


FIG. 6. A late telophase ventricular myocyte from an 18-day-old rat embryo. Arrows point to opposed deepenings of the plasma membrane indicative of furrowing formation. The interzone contains no voluminous myofilament bundles that could arrest furrow ingrowth. DN₁ and DN₂, Daughter nuclei; NDM, nondividing myocyte. $\times 10,600$. Inset: Detail of the framed area. Note the lack of clearly discernible thin threads in the enlarged interspaces between the thick myofilaments (MF). $\times 67,000$. From Rumyantsev (1972a).



to the seemingly complete separation of both daughter cells (Fig. 7a) in case the interzonal region contains no persisting myofibril or voluminous accumulation of myofilaments (Rumyantsev, 1972a; Goode, 1975). Such persistence of myofibrils is characteristic of numerous dividing myocytes at the postnatal stages of cardiac myogenesis (Fig. 7c). The furrowing may be accompanied by a local concentration of filaments about 60 Å in diameter and by the appearance of cytoplasmic protrusions rich in these filaments (Fig. 7c). Thus, as in other dividing cells (Bluemink, 1970; Szollosi, 1970) cytokinesis in the cardiac myocyte seems to involve formation of a subcortical contractile ring. Furrow ingrowth scatters small myofilament bundles which are frequently seen lying along the opposite furrowing membranes (Figs. 7c and 8b). Soon after completion of cytotomy the cytoplasm surrounding these membranes differentiates progressively. This begins with the formation of desmosome-like structures on both furrowing membranes (Fig. 8a). A little later there is a gradual orientation of the myofilaments, previously dispersed in the interzone, toward the opposite membranes of the furrow, where primitive new intercalated disks arise (Fig. 8b). It is worth noting that these morphogenetic processes in the vicinity of furrowing membranes involve the accumulation of polysomes and the appearance of coated vesicles (Fig. 8b).

Complete inhibition of cytokinesis in numerous dividing myocytes at early postnatal stages of cardiac myogenesis results in the progressive accumulation of dikaryotic muscle cells in adult mammalian hearts (Challice and Edwards, 1961; Rumyantsev, 1972a; Zak, 1974). Soon after birth these myodikaryons still continue to proliferate, and both their myonuclei enter S phase (Fig. 9) and mitosis (Solger, 1900; Klinge, 1970; Rumyantsev, 1972a) synchronously. Besides the furrowing, if present, and subsequent differentiative events observed in the cytotomy region, the posttelophase period attracts particular inter-

FIG. 7. Formation of cleavage furrow in early posttelophase ventricular myocytes of a 7-day-old suckling rat. (a) The apposed membranes of the cleavage furrow (arrows) separate completely both the daughter cells in the plane of section. $\times 9000$. (b) Higher magnification of the framed region in Fig. 7a. Note the appearance of the apposed dense spots (arrows), possibly indicative of subsequent desmosome and/or intercalated disk differentiation. The myofilaments (mf) run roughly parallel to the plane of furrowing. Ribosome (r) clusters are abundant. $\times 36,000$. (c) Furrow ingrowth seems to be arrested by the voluminous myofilament bundle (MF). Cytoplasmic protrusions (CP) appear where the furrow stops. Scattered myofilaments are designated by arrows. $\times 18,000$. Inset: Cytoplasmic protrusions at higher magnification. Note the abundance of minute filaments which are cut transversally beneath the plasma membrane (arrow). $\times 34,500$. From Rumyantsev (1972a).

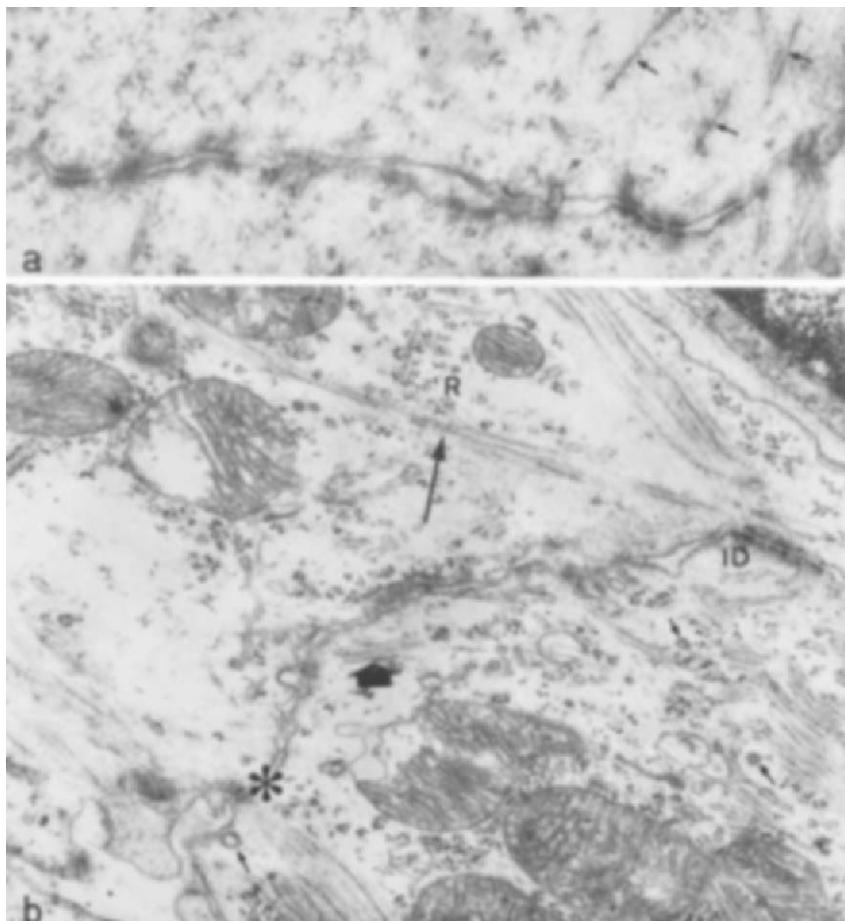


FIG. 8. Further steps in the differentiation of the cleavage furrow area in early posttelophase ventricular myocytes of a 7-day-old suckling rat. (a) Orientation of the free myofilaments (arrows) toward the desmosome-like structure, indicative of the beginning of intercalated disk formation. Similar desmosome-like structures appear along the more centrally located zones of furrowing membranes. $\times 27,500$. (b) Primitive intercalated disks (ID and asterisk) arise in the external regions of the cleavage furrow. The long arrow designates the dense precursor of the Z disk in one of the myofilament bundles converging toward the nascent intercalated disk (ID). Myofilaments located near interior parts of the furrow (thick arrow) remain less oriented. Ribosomes (R) and coated vesicles (small arrows) are numerous. $\times 17,500$.

est because of the beginning of gradual Z-disks restoration proceeding in the following way (Rumyantsev, 1972a, 1973a; Goode, 1975).

Early posttelophase myocytes differ from the majority of nondi-

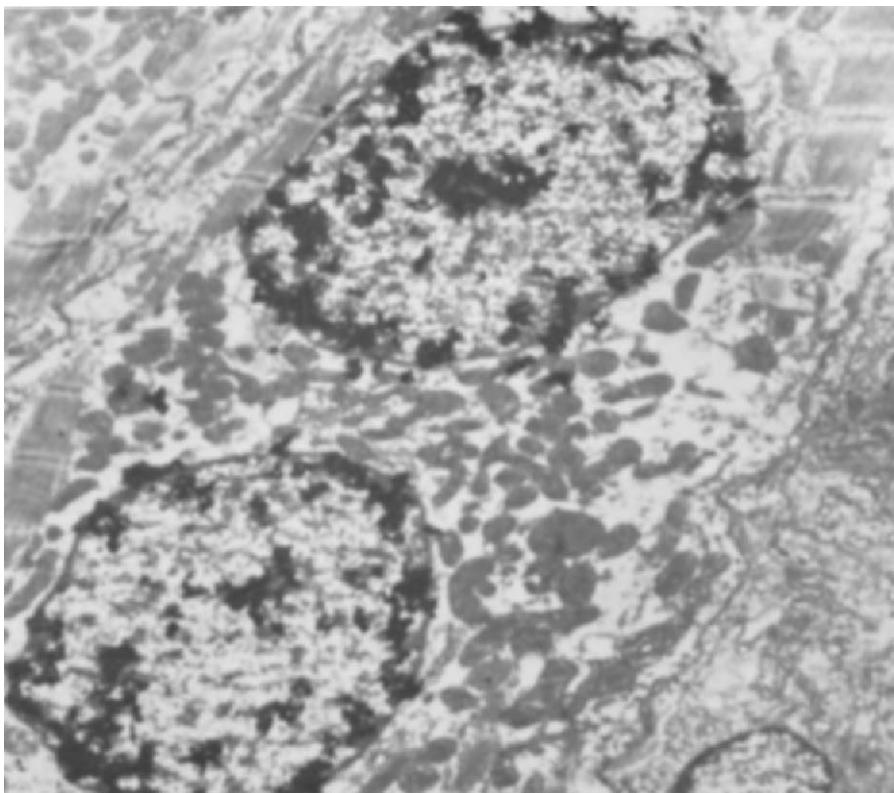


FIG. 9. Synchronous DNA synthesis in nuclei of a dikaryotic ventricular myocyte of an 11-day-old suckling rat. 1.5 hours after thymidine- ^3H injection. $\times 5300$.

viding muscle cells owing to the small size of twinlike daughter nuclei containing incompletely decondensed chromatin (Fig. 10). Instead of the well-organized myofibrils seen in the neighboring myocytes, the posttelophase cells possess a poorly organized network of myofilament bundles, except for some myofibrils persisting in mitosis. Soon after division small, irregular patches of contrast material appear between the ends of adjacent myofilament bundles (Fig. 10). These patches, and networks of minute filaments intimately connected with them, are interpreted as being the precursors of reintegrating Z lines. Some patches of similar dense material arise in contact with the plasma membrane. Polysomes are usually numerous in the vicinity of areas of myofibril restoration. Gradually, primitive Z

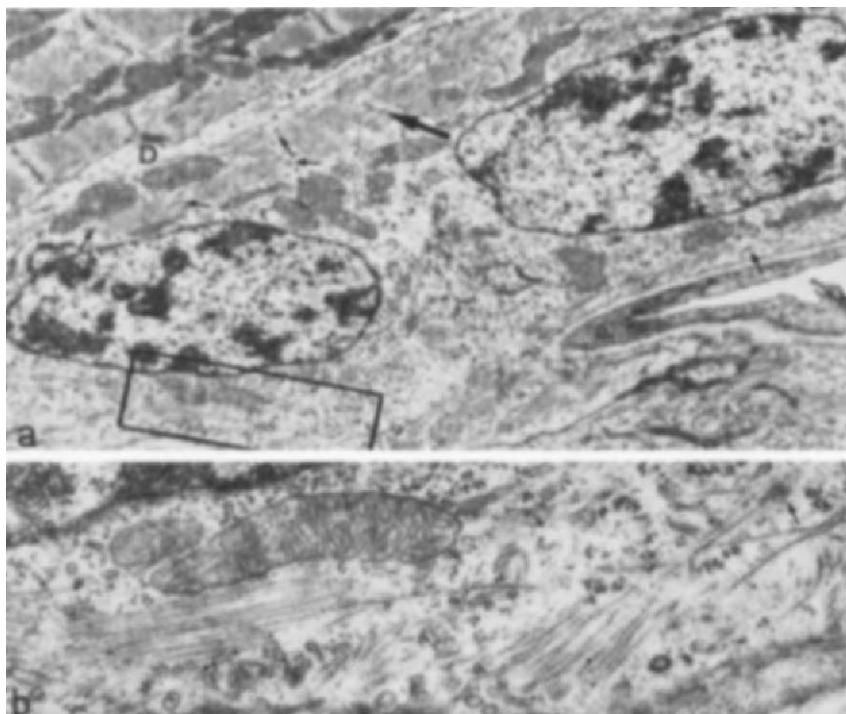


FIG. 10. Typical early postmitotic binucleate ventricular myocyte of a 7-day-old suckling rat. The myofibrils in the upper left corner belong to the adjacent nondividing cell. No conspicuous Z disks are present in myofilament bundles of the binucleate myocyte. Small, dense patches of Z-disk precursor material appear at the sites designated by small arrows. Large arrow points to longer, primitive Z line. D, Desmosome. $\times 7000$. (b) Detail of the framed region. Myofilament bundles are poorly ordered. Only one ZZ body (arrow) is seen in their vicinity. Free ribosomes are numerous. $\times 23,500$. From Rumyantsev (1972a).

bodies grow in size, become narrower, and change their orientation (with respect to the long axis of the restoring myofibrils) from the initially prevailing oblique to a more transverse. Thin myofilaments become more distinctly outlined and attach to the restoring Z lines. After this a progressive reorientation of the immature myofibrils parallel to the long axes of both daughter cells is observed (Fig. 10). Prelabeling of proliferating myocytes with thymidine ^3H at different intervals before fixation (Fig. 11) facilitates tracing of the entire sequence of events involved in the postmitotic reassembly of myofibrils.

Since thymidine- ^3H as a rule labels myocytes with a regular myofi-

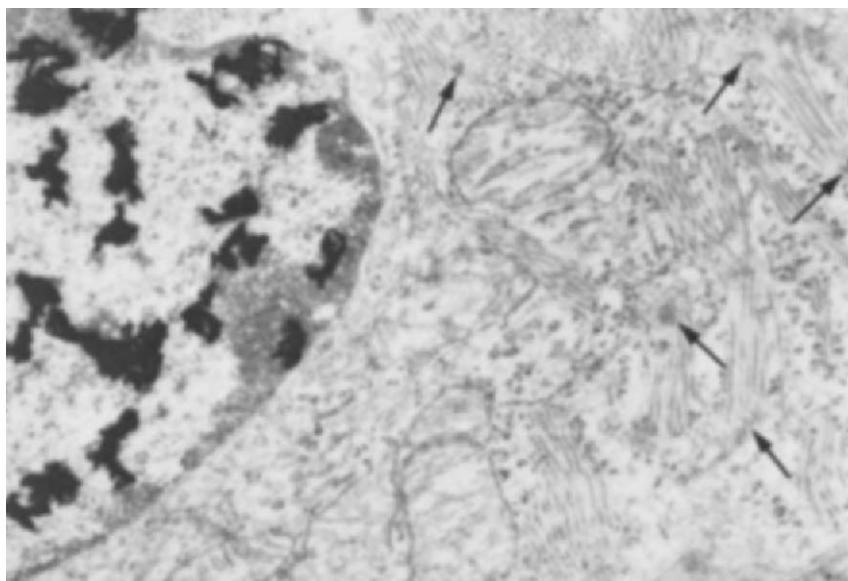


FIG. 11. Formation of numerous Z-band precursors (arrows) in the early posttelophase myocyte of an 18-day-old mouse embryo. The daughter nucleus is labeled with thymidine- ^3H injected 14 hours before fixation. Note the abundance of ribosomes near the restored myofibrils. $\times 19,500$.

bril structure (Fig. 1), one can conclude that posttelophase myofibril restoration is roughly completed before the end of the G_1 period.

Cyclic changes in myofibril ultrastructure during myocyte division are summarized in Fig. 12).

C. MECHANISM AND SIGNIFICANCE OF MYOFIBRIL REORGANIZATION IN THE MITOTIC CYCLE

Numerous questions arise concerning the mechanisms of myofibril partial degradation and restoration during each mitotic cycle. In this context Z-disk behavior attracts special attention. It was supposed that a limited amount of a certain "Z-disk degradation factor" appears (or is activated) at the end of prophase. The incompleteness of Z-disk degradation observed frequently in the dividing cardiac myocytes of suckling rats was interpreted to be the result of a deficiency in this factor in relation to the increased number of myofibrils to be attacked (Rumyantsev, 1972a).

Of special interest therefore is the isolation and partial purification

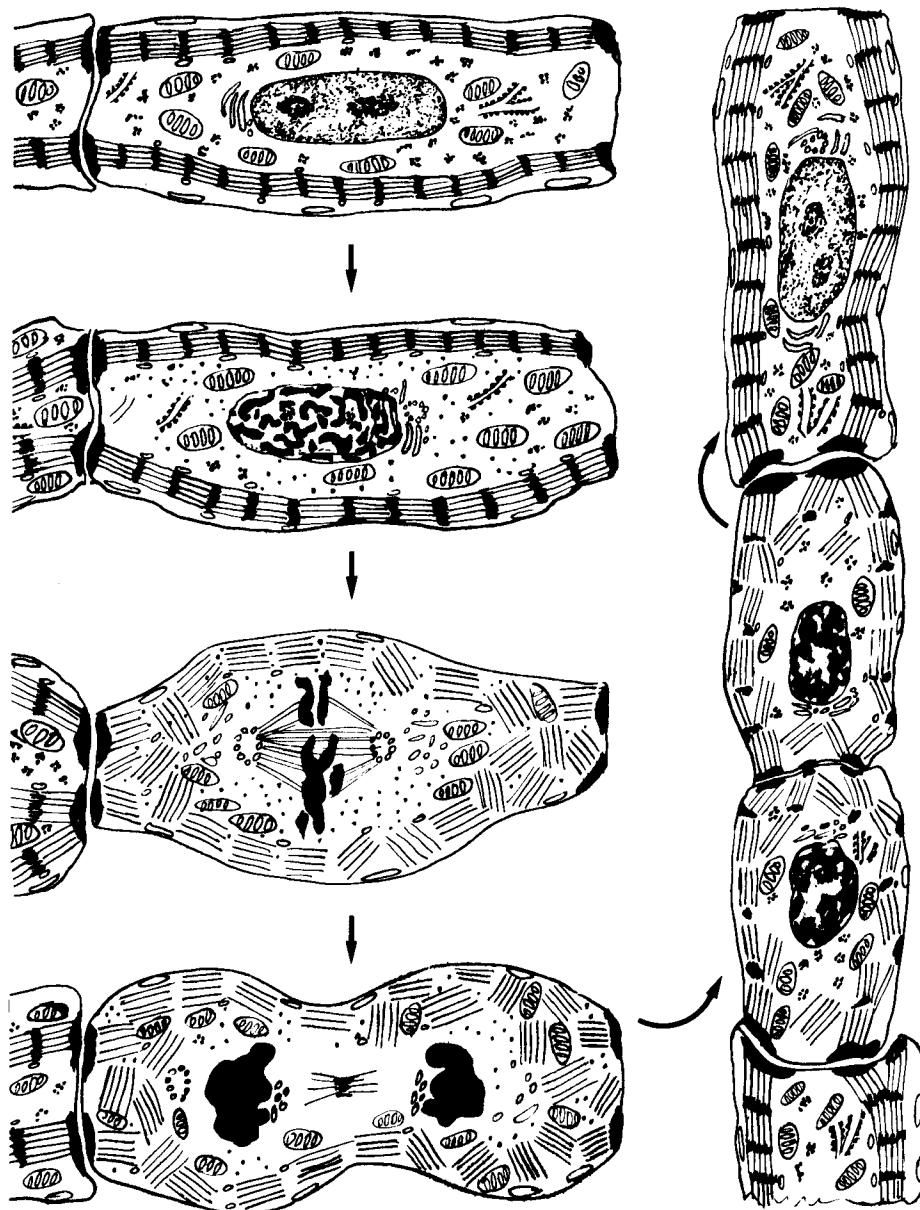


FIG. 12. Scheme of myofibril changes throughout the mitotic cycle (for an explanation see Section III,B,2).

of an endogenous calcium-activated neutral protease from muscle homogenates selectively degrading Z disks (Busch *et al.*, 1972; Reddy *et al.*, 1975). This protease attacks α -actinin, claimed to be one of the major constituents of Z disks (Robson *et al.*, 1970; Zaalishwili, 1971), more or less specifically. It is tempting to speculate that the ionic calcium released in a dividing cell after nuclear envelope degradation in prophase (Tilney and Marsland, 1969) can activate this factor if it exists in the immature cardiac myocyte and cause the above drastic changes in myofibril ultrastructure.

The following experiments also shed some light on the mechanisms that underlie Z-disk breakdown and restoration during each mitotic cycle of the cardiac myocyte. Several workers removed Z lines by means of cytochalasin B (Manasek *et al.*, 1972), sodium deoxycholate (Etlinger and Fischman, 1973; Zak, 1974), and other agents (Walcott and Ridgway, 1967; Harsanyi and Garamavölgyi, 1969; Stromer *et al.*, 1967; Rash *et al.*, 1970a).

The selectively removed Z disks can be restored by treatment of the extracted myofibrils with solubilized material from these disks (Stromer *et al.*, 1967). After trypsinization of myocardium for cell cultures the majority of Z disks are found to be destroyed and myoflament bundles released, as in mitosis; it is worth noting that restoration of Z disks interlinking the previously dispersed sarcomeres occurs in this case even after the inhibition of protein synthesis (Etlinger and Fischman, 1973). Therefore, self-assembly processes can play an important role in the posttelophase restoration of Z disks and of whole myofibrils (Rumyantsev, 1972a, 1976). Changes in biosynthetic activity at different phases in the mitotic cycle also cannot be underestimated. Z-disk degradation occurs roughly simultaneously with other degradative events (disintegration of the nuclear envelope, Golgi apparatus, nucleoli, rough endoplasmic reticulum, etc.), which may depend on a sharp decrease in the level of protein synthesis and the number of large polysomes observed in mitosis (Sharff and Robbins, 1966; Przybylski and Chlebowski, 1972). On the contrary, the number of polysomes and rough endoplasmic reticulum elements at the foci of posttelophase myofibril restoration increases markedly (Rumyantsev, 1972a). It may well be that both kinds of events, that is, activation of the Z-disk degradation factor together with a sharp decrease in protein synthesis, underlie myofibril changes in prometaphase, whereas their restoration after mitosis can involve not only self-assembly but activated translation also.

The cyclic changes in cardiac myocyte myofibril ultrastructure are accompanied by the *de novo* synthesis of contractile proteins during

interphase, which is necessary for a gradual increase in the myofibril content from cycle to cycle.

Manasek's explanation (1968b) of the characteristic intensive development of rough endoplasmic reticulum and Golgi elements in immature cardiac myocytes as compared with somatic myogenesis can be tentatively supplemented as follows. These organelles may not only be involved in cardiac jelly and specific granule secretion (see Section II) but also in the highly complicated cardiac myofibrillogenesis and in differentiation of intercalated disks and desmosomes in the region of cytotomy.

Kasten (1972, 1975) considers the changes in myofibrils in mitosis an expression of transient dedifferentiation or modulation resulting in the cessation of special functions. The latter permits the dividing cell to liberate (mobilize?) the energy required for chromosomal movement. In the dedifferentiating skeletal muscle Z disks appear to be the most resistant parts of the sarcomeres (Hay, 1959; Bullière, 1968; Crossley, 1972), which is at variance with their known vulnerability (Hudgson and Field, 1973) clearly manifested in the dividing cardiac myocyte.

Whatever the dedifferentiative significance of the reversible breakdown of myofibrils in the dividing cardiac myocyte, it seems probable that this process represents a special kind of cellular adaptation allowing chromosomal movements and cytokinesis (Oberpriller and Oberpriller, 1971; Hay and Low, 1972; Kasten, 1972, 1975; Rumyantsev, 1972a; Goldstein *et al.*, 1974) without dissolution of the bulk of myofilaments.

According to Cobb and Bennett (1970), both myofilaments and dense bodies remain unchanged in the mitotically dividing smooth muscle cells of chick embryos. However, the number of myofilaments and dense bodies in the vicinity of the dividing nucleus of these cells is found to be considerably decreased as compared to the situation in smooth muscle cells in S phase (Imai *et al.*, 1970; Rumyantsev, 1976). It remains to be determined whether or not these data are indicative of a basic similarity in the changes in contractile machinery during the mitosis of cardiac and smooth muscle cells.

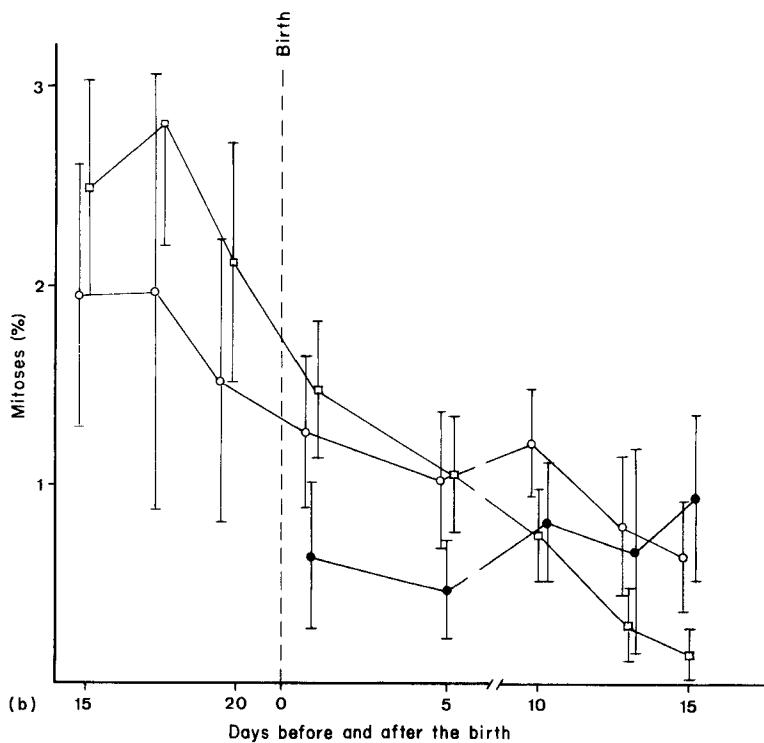
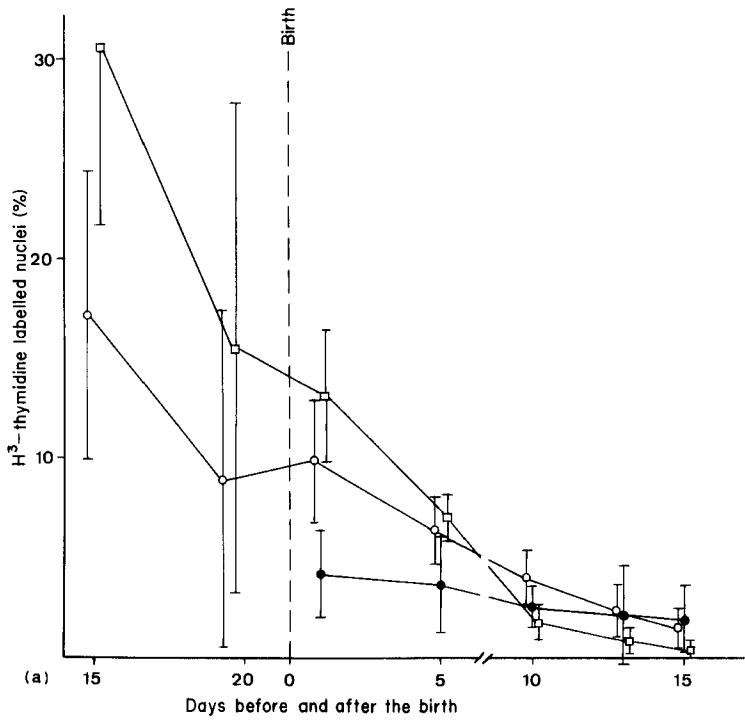
More simple specialized structures such as pigment (Marshak *et al.*, 1972), secretary (Redman and Sreebny, 1970), and specific atrial granules (Rumyantsev, 1974) undergo no visible changes during mitosis, being distributed passively at different phases of division.

Striated myofibrils of cardiac myocytes presumably represent a complex and rigid system which interferes with mitosis during several cell generations without blocking it.

D. KINETICS OF DNA SYNTHESIS AND MYONUCLEI PROLIFERATION

Olivo and Slavich (1930) were the first to follow the gradual decrease in mitotic index values during histogenesis of chick embryo myocardium from 2.25% in the 48-hour embryo, to 0.2% at the moment of hatching, and down to the zero 10 days thereafter. Goerttler (1956) and Grohmann (1961) give somewhat higher values for the mitotic index, demonstrating its topographic differences (see Section III,H). In these studies the differentiative properties of proliferating myocardial cells were not characterized. Later attention was focused on nuclei belonging, as well as can be judged from light microscope evidence, to myofibril-containing cells. Just before the onset of differentiation of precardiac mesoderm and soon after the appearance of the first myofibrils, the number of thymidine-³H incorporating and mitotically dividing cells of the tubular heart of chick and mouse embryos decreases drastically for a short time (Sissman, 1966; Zavarzin, 1967; Erokhina, 1968a; Stalsberg, 1969a,b; Andres *et al.*, 1971). This may be indicative of interference of the initiation of specific protein synthesis with cell proliferation. However, we cannot exclude changes in membrane permeability, in the intracellular thymidine pool, and other events.

All workers describe a rapid rise in the number of DNA-synthesizing and mitotically dividing cardiac muscle cells following the short pause in their proliferation at the beginning of differentiation. In chick embryo myocardium the maximum number of the thymidine-³H pulse-labeled muscle cells (about 30–40%) is observed at the stage 27 described by Hamburger and Hamilton (Cameron and Jeter, 1969; Andres *et al.*, 1971; Jeter and Cameron, 1971), and in mouse embryos similar values for the cardiac myocyte labeling index are achieved by the eleventh day of gestation (Erokhina, 1968a). Thereafter the number of thymidine-³H incorporating and mitotically dividing (Fig. 13) myocyte nuclei gradually decreases and approaches zero at the beginning of the third postnatal week in the case of the rat and mouse (Rumyantsev, 1963a, 1965; Rumyantsev and Sokolovskaya, 1964; Erokhina, 1968a,b; Sasaki *et al.*, 1968a; Achabadze and Olenina, 1972; Achabadze, 1974; Erokhina and Rumyantsev, 1974). For the majority of the stages investigated the ratio between the indexes of thymidine-³H pulse-labeled and dividing myonuclei is close to 10:1 (Rumyantsev, 1963a; Klinge and Stöcker, 1968). Some investigators observed up to 1–2% thymidine-³H pulse-labeled and up to 0.1–0.4% mitotically dividing myonuclei in rat and mice hearts 1 month after



birth and even later (Petersen and Baserga, 1965; Overy and Priest, 1966; Klinge and Stöcker, 1968; Klinge, 1970; Kunz *et al.*, 1972).

The size of the "proliferation pool" of cardiac myocytes at different stages of myocardium histogenesis was estimated roughly by means of prolonged thymidine-³H labeling (Rumyantsev and Sokolovskaya, 1964; Erokhina, 1968a,b; Andres *et al.*, 1971; Jeter and Cameron, 1971). Close to 100% values for the proliferation pool were obtained for the earliest stages of cardiac myogenesis, for example, in 10- to 12-day mouse embryos (Erokhina, 1968a) and in chick embryos younger than 5 days (Andres *et al.*, 1971; Jeter and Cameron, 1971). It is likely that later myocyte withdrawal from the generation cycle progresses gradually, since the index of labeled myonuclei decreases despite the prolonged thymidine-³H availability. Thymidine-³H injections, repeated 10 times at 11-hour intervals, starting on the fifth day of rat postnatal development resulted in the labeling of 82.5% myonuclei in the ventricular myocardium (Rumyantsev, 1978). A similar experiment with adult rats revealed only occasional thymidine-³H labeled ventricular myocytes (Rumyantsev and Kassem, 1976). The increase in the number of labeled myonuclei after continued thymidine-³H administration could also be interpreted to be a result of postmitotic premyoblast or stem cell differentiation (De Haan, 1971; Goode, 1973). This is rather improbable, however, taking into account the evidence presented in Sections II and III,E,I, and K.

In agreement with autoradiographic data morphometry shows that the total number of ventricular myonuclei stops to increase on day 15 of rat postnatal development (Hort, 1953), achieving in adult rats about $20-25 \times 10^6$ (Grimm *et al.*, 1970) or $25-38 \times 10^6$ (Sasaki *et al.*, 1970c). In developing cardiac muscle of mice the mean number of myonuclei grows from 0.8×10^6 on day 15 of embryonic development to 9.6×10^6 by day 15 of postnatal life and after that does not change appreciably (Erokhina, 1968b). Using the method of heart muscle tissue dissociation with potash lye (Schneider and Pfitzer, 1973; Gräbner and Pfitzer, 1974), Belov and his associates (1976) found that the total number of ventricular myonuclei rises from 1×10^6 in hearts of 1-

FIG. 13. The number of thymidine-³H labeled (a) and mitotically dividing myonuclei (b) at different stages of rat cardiac myogenesis. Squares, Ventricular myonuclei; open circles, atrial myonuclei; solid circles, nuclei of myocytes from the atrioventricular system. Ordinate: Percentage of thymidine-³H labeled nuclei (a) and mitoses (b). Abscissa: Day of embryonic and postnatal development. Zero designates the time of birth. The vertical bars represent the 95% confidence limits in Fig. 13a and the standard error of the mean in Fig. 13b.

to 3-day-old mice to only 3.8×10^6 in hearts of 5-month-old animals. A partial loss of cardiac myocytes due to the alkaline dissociation cannot be excluded.

Calculations of the cell number based on a determination of the DNA content in heart homogenates disregard the fact that both muscle and nonmuscle cells are mixed in this procedure, thus resulting in much higher values than those obtained in morphometric studies. In rat myocardium, for example, the number of nuclei was found to grow from 0.6×10^6 in 13-day embryos, to 16.2×10^6 in 14-day animals, and to about 200×10^6 in adults (Winick and Noble, 1965). Similar results for rat cardiac myogenesis were reported by Enesco (1959), Enesco and Leblond (1962), and Leblond (1964), and for the chick by De Haan (1971). It seems highly probable that the increase in the number of myocardial nuclei observed after the practical cessation of thymidine- ^3H incorporation into mice myonuclei, that is, after day 15 of postnatal life, is in fact due to a proliferation of endothelial and connective tissue cells.

E. DURATION OF MITOTIC CYCLE PHASES IN CARDIAC MYOGENESIS

The above-mentioned progressive decrease in the number of DNA-synthesizing myocytes at more advanced stages of cardiac myogenesis (Fig. 13) may be dependent not only on their withdrawal from the mitotic cycle or on depletion of a putative myoblast pool, but also on a lengthening of the cycle phases. The data collected in Table II allow the conclusion that immature cardiac myocytes are characterized by a moderately long mitotic cycle, comparable in terms of the duration of its phases to that of smooth muscle (Dubinko, 1966), some epithelial cells (Zavarzin, 1967), and cells of other tissue. The gradual maturation of cardiac myocytes is accompanied by a considerable lengthening (1.5 to 2 times) of all stages of the mitotic cycle including the S phase, which is especially evident in the case of mouse cardiac myogenesis (Table II). Microcinematographic studies of newborn rat cardiac myocytes *in vitro* demonstrated that their mitosis lasts up to 6 hours (Kasten, 1972). Thus the lengthening of the mitotic cycle phases together with the progressive withdrawal of myocytes from the proliferation pool may play a role in the decrease in thymidine- ^3H labeled cells at later stages of cardiac myogenesis (Fig. 13).

Taking into account the number of thymidine- ^3H labeled myocytes and the length of the mitotic cycle phases at different stages of mouse myocardium development, Erokhina (1968b) calculated the rates of myocyte production and their expected absolute numbers in terms of developmental stages. The curve obtained was found to practically coincide with the curve for the total myocyte numbers as determined by morphometry, thus demonstrating the validity of both approaches.

TABLE II
LENGTH OF MITOTIC CYCLE PHASES IN MYOCYTES OF
DEVELOPING HEARTS OF VARIOUS ANIMALS

Species and stage of development	Duration (hours)					References
	T	G ₁	S	G ₂	M	
Chick (culture of embryonic myocardium)	45	27-33	9	4-6	0.5-0.9	Rumery and Rieke (1967)
Chick embryo, one somite ^a	16	8.6	5.8	2.5	—	Andres <i>et al.</i> (1971)
Chick, 5-day embryo ^a	30	16.5	12.3	1.8	—	Andres <i>et al.</i> (1971)
Mouse, 8-day embryo (pre-cardiac mesoderm)	6.5	0.5	4.5	1.5	—	Zavarzin (1967)
Mouse, 9-day embryo	10	1.5	5	3.5	—	Erokhina (1968a)
Mouse, 10-day embryo	11	2.5	6.5	2	—	Erokhina (1968a)
Mouse, 15-day embryo	14-17	2-5	9	3	—	Erokhina (1968b)
Mouse, 18-day embryo	23-24	5-7	13-14	3.5	—	Erokhina (1968b)
Mouse, 1-week old	30	12	13	2-5	—	Erokhina (1968b)
Rat, 15-day embryo	18.5	7	7.5	3	—	Rumyantsev and Sokolovskaya (1964)
Rat, 18-day embryo	22	8	8.5	3.5	—	Rumyantsev and Sokolovskaya (1964)
Rat, 19.5-day embryo	42	30	8	1.5-5.7	0.8	Wegener <i>et al.</i> (1964)
Rat, 1-2 days old	40	20	13	6	—	Rumyantsev and Sokolovskaya (1964)
Rat, 2 days old ^a	—	—	16	—	8	Sasaki <i>et al.</i> (1970b)
Rat, 3-4 days old (culture of myocardium) ^a	—	—	—	—	6.1	Kasten (1972)
Rat, 5-7 days old	—	—	12	4	—	Jinkine and Rumyantsev (1970)
Rat, 1 week old ^a	—	—	16	—	10	Sasaki <i>et al.</i> (1970b)
Rat adult (atrial myocytes proliferating after infarction)	—	—	12.5	5	—	Rumyantsev and Mirakyan (1968a)

^a Data obtained using methods other than the labeled mitoses curve, as in the case of the other results cited.

It was suggested that the above-mentioned gradual increase in mitotic cycle duration reflects a slowly ripening conflict between the intrinsically antagonistic processes of cell proliferation and differentiation coexisting in developing myocardium (Rumyantsev and Sokolovskaya, 1964). An analogous lengthening of the mitotic cycle phases is also typical of other types of differentiating cells which continue to proliferate after the onset of specific macromolecule synthesis, for example, smooth muscle cells (Dubinko, 1966; Zavarzin, 1967; Jinkine and Rumyantsev, 1970), pigment epithelium cells (Marshak *et al.*, 1972), chondrocytes (Andreeva, 1969), and erythroblasts (Holtzer *et al.*, 1972).

On the contrary, cells that, like myoblasts of skeletal muscle, proliferate only before the onset of differentiation are characterized by a relatively short, stable mitotic cycle (Zhinkin and Andreeva, 1963; Marchok and Herrmann, 1967; Zavarzin, 1967; Andres *et al.*, 1971).

The number of cell generations required for the accumulation of a definitive myocyte population cannot be determined with much accuracy, considering the progressive withdrawal of differentiating myocytes from the proliferation pool, the above-mentioned lengthening of the cycle, and polyploidization of a certain myocyte fraction (see Section III,G). In clones originating from 1 myofibril-containing cell, up to 8 to 20 cardiac myocytes can be observed (De Haan, 1967; Chacko and Joseph, 1974). There is every reason to suggest that *in situ* each cardiac myocyte is capable of undergoing a greater number of generations as compared with the three to four mitotic cycles necessary for the formation of 8 to 20 myocyte-containing colonies in these *in vitro* experiments. Therefore it is tempting to suggest that cardiac myogenesis can be accounted for by the proliferation of immature myocytes arising from the cells of the splanchnic mesoderm without the participation of cells from the hypothetical premyoblast pool at any subsequent stage (Rumyantsev, 1967, 1972a, 1976; Manasek, 1968a, 1973; Jeter and Cameron, 1971). The material described in Sections II and III,I and K is relevant in estimating such a hypothesis.

F. PROLIFERATION OF CARDIAC MYOCYTES AND DNA POLYMERASE ACTIVITY

Changes in DNA polymerase activity may play a certain role in the regulation of eukaryotic cell reproduction. For instance, the cessation of skeletal myoblast proliferation, their fusion, and myotube formation coincide with the decrease in DNA polymerase activity to nearly the zero level (O'Neill and Strohman, 1970; Stockdale, 1970; Wicha and

Stockdale, 1972). In developing rat myocardium also, the decrease in the activity of cytoplasmic 6- to 8S DNA polymerase to the zero level was observed on day 17 of postnatal life (Claycomb, 1973, 1974, 1975c; Doyle *et al.*, 1974), when practically all the myocytes leave the mitotic cycle, according to autoradiographic data (Fig. 13a). The activity of thymidine kinase displays a 70-fold decrease at the same stage of myocardial development (Gillette and Claycomb, 1974). The activities of cytoplasmic DNA polymerase and thymidine kinase and the rate of cardiac myocyte DNA synthesis are inversely related both to respective concentrations of catecholamines, cAMP, NAD⁺, and to poly (ADP)ribose polymerase activity, which increase during postnatal differentiation of cardiac muscle (Claycomb, 1975a,b). It remains unclear, however, whether DNA polymerase is lost or stably inhibited in mature cardiac muscle cells (Zak, 1974). The latter seems to be more probable, taking into account the possible initiation of repair DNA synthesis in nonmitotic nuclei of differentiated skeletal muscle fibers by means of ultraviolet light (Stockdale and O'Neill, 1972). In the rat ventricle myocytes, however, the repair DNA synthesis was observed only at earlier stages of the postnatal development being absent or stably inhibited in myocytes obtained from ventricles of the adult rats (Lampidis and Schaiberger, 1975).

Holtzer and his associates suppose that the synthesis of DNA polymerase is switched off after the onset of contractile protein elaboration in the differentiating cardiac myocyte (Holtzer, 1970a; Holtzer and Sanger, 1972). The latter, however, is thought to be capable of a limited number of divisions until a complete dilution of DNA polymerase occurs. These assumptions would reconcile the theory of quantal mitosis (Holtzer, 1970a) with the evident capacity of cardiac myocytes to proliferate until a certain critical degree (Rumyantsev and Sokolovskaya, 1964) of differentiation is achieved.

G. CARDIAC MYOGENESIS AND PLOIDY OF HEART MUSCLE CELLS

In agreement with autoradiographic data (see Section III,D), cytophotometry reveals numerous myonuclei with a DNA content ranging between 2 and 4C DNA in the heart of mouse and rat embryos and neonates (Table III). The number of such "hyperploid" myonuclei exceeds considerably the percentage of S and G₂ nuclei that might be expected from thymidine-³H labeling indexes characteristic of different stages of cardiac myogenesis (Mirakyan and Rumyantsev, 1968; Erokhina, 1968b). This apparent discrepancy was explained to be a

TABLE III
PERCENTAGES OF THE VENTRICULAR MYONUCLEI ACCORDING TO THEIR
DNA CONTENT IN VARIOUS ANIMAL HEARTS AT DIFFERENT STAGES
OF ONTOGENESIS (FEULGEN-DNA PHOTOMETRY)

Species and stage of development	DNA content				References
	2C	4C	8C	16C	
Frog, adult ^a	90	10	—	—	Mirakyan and Rumyantsev (1968)
Turkey, 4–12 months old	95.1	4.7	0.2	—	Pfitzer (1971a)
Mouse, 19-day embryo ^a	77–89	13–21	2	—	Erokhina (1968b)
Mouse, 1 week old ^a	73–92	7–18	8	—	Erokhina (1968b)
Mouse, 2 weeks old ^a	70	27	3	—	Erokhina (1968b)
Mouse, adult ^a	79	20	1	—	Erokhina (1968b)
Mouse, 5 months old ^a	95	5	—	—	Petersen and Baserga (1965)
Rat, 1 day old ^a	60	40	—	—	Mirakyan and Rumyantsev (1968)
Rat, 1 week old ^a	70	30	—	—	Mirakyan and Rumyantsev (1968)
Rat, 2 weeks old ^a	75	25	—	—	Mirakyan and Rumyantsev (1968)
Rat, adult ^a	90	10	—	—	Mirakyan and Rumyantsev (1968)
Rat, adult	93	7	—	—	Pfitzer <i>et al.</i> (1972)
Rat, adult ^a	98.2	1.8	—	—	Grove <i>et al.</i> (1969)
Pig, adult	82.3	14	3.7	—	Pfitzer (1971b)
Monkey, rhesus, 3–4 years old	90	10	—	—	Pfitzer and Schulte (1972)
Monkey, rhesus adult	35–60	30–55	3–12	—	Pfitzer and Schulte (1972)
Monkey, gorilla, 4 years old	52	34	12	2	Pfitzer and Schulte (1972)
Monkey, chimpanzee, 7 years old	35	55	10	—	Pfitzer (1972a)
Monkey, hamadryas baboon, 20 years old	15	55	25	3	Pfitzer (1972a)
Human, 4 days old	91	9	—	—	Pfitzer (1972b)
Human, 8 years old	30	65	5	—	Pfitzer (1972b)
Human, 38 years old	30	65	5	—	Pfitzer (1972b)
Human adult, heart weight 250–500 gm	20	50	35	5	Kompmann <i>et al.</i> (1966)

^a Measurements made on sectioned myocardium; all others represent measurements made on myocardial smears.

result of the transient withdrawal of G₂ myocyte fraction from the mitotic cycle at different stages of embryonic and postnatal cardiac myogenesis after completion of DNA synthesis. Such a myocyte fraction, corresponding, presumably, to the cells of a G₂ or R₂ population (Gelfant, 1963; Epifanova and Terskikh, 1969), seems to exist in the myocardium of adult vertebrates (Table III) practically devoid of thymidine-³H incorporating myonuclei. While the myocardium of the frog, turkey, mouse, and rat contains small or moderate percentages of hyperdiploid myonuclei (2–16%), in the heart of the adult human, monkey, and pig the content of these myonuclei is much higher or even prevailing (Table III). The problem is complicated by the fact that in species with a high content of polyploid myonuclei the degree of ploidy increases considerably with age (Table III). However, there is no reliable evidence concerning the thymidine-³H uptake or mitotic division of cardiac myonuclei in young and adult humans, monkeys, and swine (Gräbner and Pfitzer, 1974), which makes it difficult to explain the increase in ploidy of these myonuclei via the fusion of telophase chromosome complexes in accordance with Pfitzer's scheme (1972b). Thus the mechanism of progressive cardiac myonuclei polyploidization, both in normal postnatal ontogenesis of the above species and during heart muscle hypertrophy (see Section IV,C), still remains to be elucidated. The study of the formation and subsequent behavior of bi- and multinucleated cardiac myocytes, which accumulate largely after birth (Muir, 1957; Challice and Edwards, 1961; Klinge, 1970; Rumyantsev, 1972a; Schneider and Pfitzer, 1973; Gräbner and Pfitzer, 1974; Belov *et al.*, 1976), can shed some light on the mechanism of polyploid myonuclei formation during the course of postnatal cardiac myogenesis. There is direct (see Section III,B) and indirect (Pfitzer, 1972b; Gräbner and Pfitzer, 1974) proof of the formation of bi- and multinucleated cardiac myocytes via acytokinetic mitosis. The cycle block at the G₂-M boundary, the fusion of daughter myonuclei, and other anomalies of cell division should be responsible for the increasing nuclear ploidy in the heart of humans, monkeys, and swine (Pfitzer, 1972b; Schneider and Pfitzer, 1973; Gräbner and Pfitzer, 1974).

Since the number of nucleoli in cardiac myocytes remains fixed irrespective of the degree of ploidy, it is doubtful if the increase in DNA content represents a replication of all the genes in myonuclei (Bloom and Egli, 1969). The possibility of gene amplification also is not excluded.

Despite the uncertainty mentioned, the widespread unequivocal

presence of greater than 2C DNA nuclei in cardiac myocytes at different stages of ontogenesis is one of the most striking peculiarities of these cells. Skeletal muscles are known to have exclusively diploid myonuclei at all stages of normal or regenerative myogenesis (Lash *et al.*, 1957; Firket, 1958; Strehler *et al.*, 1963; Betz *et al.*, 1966; Mirakyan and Rumyantsev, 1968).

It is noteworthy that somatic polyploidy was also observed in different types of cells (such as pigment epithelial cells, hepatocytes, etc.) which continue to proliferate, like cardiac myocytes, after the onset of cellular differentiation (Brodsky and Uryvaeva, 1974). The coexistence of the intrinsically antagonistic processes of proliferation and differentiation throughout several subsequent generation cycles results in a progressive lengthening of the cycle phases (see Section III,E), cytokinesis blocking, incompleteness of daughter nuclei separation, and so on. These abnormalities seem to be involved in the polyploidization of cardiac myocyte nuclei (Mirakyan and Rumyantsev, 1968; Pfitzer, 1972b; Schneider and Pfitzer, 1973; Gräbner and Pfitzer, 1974) and other types of proliferating differentiated cells (Brodsky and Uryvaeva, 1974).

H. DIFFERENCES IN MYOCYTE PROLIFERATION RATES AND MYOCARDIAL COMPARTMENTALIZATION

Regional differences in myonuclei proliferative activity were first studied to determine their possible role in heart morphogenesis (Sissman, 1966; Stalsberg, 1969a,b) or malformations (Goerttler, 1956; Grohmann, 1961). Careful observations of the local fluctuations in the mitotic activity of cardiac muscle cells permit the conclusion that they do not play a major role in such morphogenetic events as looping of the straight tubular heart, and so on (Stalsberg, 1969a,b). In the ventricles of chick, mouse, and rat embryos the number of proliferating myocytes is two or three times higher in the more basophilic compact superficial layer of the myocardium than in its inner trabeculae (Rumyantsev and Sokolovskaya, 1964; Erokhina, 1968b; Andres *et al.*, 1971; Jeter and Cameron, 1971). This is indicative of a predominantly appositional growth of the peripheral compact ventricular myocardium which develops most intensely in phylogenesis and ontogenesis (Marceau, 1904; Kotschetov, 1959). In agreement with these data the myocytes of this layer appear to be less differentiated, judging from both ultrastructural and cytochemical criteria (Markwald, 1969).

The atrial myocytes in chicks, rats, and mice proliferate during embryonic and early postnatal development a little less intensely than

ventricular myocytes (Goerttler, 1956; Grohmann, 1961; Rumyantsev and Sokolovskaya, 1964; Erokhina, 1968b). However, beginning at the end of the second week after birth these interrelationships are found to be reversed (Fig. 13), and even in the atria of adult rats a small fraction, about 2–3% of the proliferating myocytes, was detected by means of prolonged thymidine-³H administration (Rumyantsev and Kassem, 1976). In ventricles of adult rats, as a rule only endothelial and connective tissue cells were labeled in these experiments, which confirms earlier data (Walker and Leblond, 1958; Messier and Leblond, 1960; Schultze and Oehlert, 1960; Edwards and Klein, 1961; Shorter and Titus, 1962).

The number of DNA-synthesizing and mitotically dividing myonuclei is significantly lower in different compartments of the conductive system of the developing heart in rat and mouse embryos (Fig. 13a) as compared to their working myocardium (Erokhina and Rumyantsev, 1974; Rumyantsev, 1978). However, like the atrial myocardium, the conductive system of young adult rats still contains up to 1–2% tagged myocytes after thymidine-³H administration repeated 10 times at 12-hour intervals (Rumyantsev and Kassem, 1976).

I. PROLIFERATION OF MYOFIBRIL-DEPRIVED CELLS IN DEVELOPING MYOCARDIUM

At the advanced stages of cardiac myogenesis in mice and rats electron microscope autoradiography revealed thymidine-³H nuclear labeling both in myocytes and myofibril-deprived cells (Table I). The latter were referred to as endothelial and connective tissue elements, considering their topology and ultrastructure (Fig. 1b). At 12–14 hours and later, following thymidine-³H administration, most of these labeled cells are found to divide. Their progeny, however, are neither involved in myofibrillogenesis nor attached to the adjacent myocytes with desmosomes or intercalated disks (Rumyantsev, 1978).

Thus myocardial growth may be due to the coordinated, but independent, proliferation of both muscle and nonmuscle cells. The latter proliferate more intensely, providing myocardium vascularization and stroma formation. This explains why in the adult rat heart myocytes represent only about 25% of the total cell population (Zak, 1974). This overgrowth seems to depend on the mitotic cycle of nonmuscle cells in the developing heart, which is twice as short as that of cardiac myocytes. Maintenance of the relatively high values of the proliferative pool of endothelial and/or connective tissue cells during late embryonic and early postnatal stages of myocardial histogenesis certainly plays a role in the overgrowth of these cells (Erokhina, 1968b). The

kinetics of nonmuscle cell proliferation in the developing heart of rat and mouse were studied by several workers (Erokhina, 1968b; Klinge and Stöcker, 1968; Sasaki *et al.*, 1970a; Achabadze, 1974; Kunz *et al.*, 1972; Kranz *et al.*, 1975). Beginning from the day 15 of rat and mouse postnatal development thymidine-³H labels only 0.2–4% of all myocardial nonmuscle cells (Erokhina, 1968b; Sasaki *et al.*, 1970a; Klinge and Stöcker, 1968). However, thymidine-³H injections repeated 10 times at 12-hour intervals label up to 20% of all nonmuscle cells in the adult rat hearts (Rumyantsev and Kassem, 1976). An almost complete absence of labeled ventricular myonuclei in these experiments demonstrates that the mature myocardium is practically devoid of "dormant" myoblasts.

J. EFFECT OF 5-BROMODEOXYURIDINE ON CARDIAC MYOGENESIS

When incorporated into DNA, 5-bromodeoxyuridine (BUdR) is known to inhibit reversibly the initiation of tissue-specific protein synthesis. Thus it prevents the differentiation of cultured skeletal muscle myoblasts (Okazaki and Holtzer, 1965; Coleman *et al.*, 1969; Holtzer, 1970b). Incorporation of BUdR-³H into the nuclei of precardiac mesoderm cells was demonstrated autoradiographically (Chacko and Joseph, 1974). It was shown that BUdR inhibits cardiac muscle cell differentiation only when administered up to stage 7 of chick embryo development. Thereupon the cells of both precardiac mesoderm and arising early cardiac myocytes become insensitive to the incorporated analog. BUdR-treated cardiac myocytes display the same proliferative activity as the controls in culture. Chick embryo hearts reach the same size as the controls and contract vigorously despite the action of BUdR. All these results, obtained by Chacko and Joseph (1974), together with evidence compiled in Sections II and III, oppose any essential role of a hypothetical premyoblast pool in cardiac myogenesis following the initial stages.

IV. Reactivation of Hyperplasia of Cardiac Muscle Cells and Their Participation in Myocardial Regeneration

A most important point in myocardium regeneration studies involves the question whether all kinds of cardiac myocytes in different groups of adult vertebrates are practically unable to resume a mitotic cycle when stimulated by injury. A possible role of myocyte dedifferentiation and activation of hypothetical premyoblasts or myogenic stem cells, if they persist in the hearts of adult animals, should also be investigated in this context. In contrast to the many data concerning

myocardial regeneration in adult mammals (see Section IV,C), there is only limited information about its occurrence in lower vertebrates and birds.

A. LOWER-VERTEBRATE MYOCARDIUM

1. *Light Microscopy*

The first description, made by Zielonko (1875), was not sufficiently clear as regards cell division and hypertrophy in damaged ventricle myocardium in the frog. Comparative studies of myocardial regeneration were not resumed until almost a century later. In fishes (Kolossova, 1961), amphibians (Rumyantsev, 1961, 1966, 1973b; Oberpriller and Oberpriller, 1971, 1974; Nieweliński *et al.*, 1974), and reptiles (Sulima, 1968; Rumyantsev, 1973b), numerous mitoses or paired amitosis-like nuclei were observed within the muscle fiber stumps near areas of myocardial necrosis induced by traumatization of the ventricle. These stumps were enriched in slightly basophilic sarcoplasm, their enlarged myonuclei containing prominent nucleoli. At 3–4 weeks and later following injury to a frog heart a slowly progressing outgrowth of some muscle fiber stumps bordering the granulation tissue that fills up the area of necrosis occurred. This was most demonstrative in experiments involving transverse linear squashing of the frog ventricle to isolate completely the apical myocardium from the bulk of the ventricular muscles (Rumyantsev, 1961; Niweliński *et al.*, 1974). As a result, scarce, slender anastomoses of apparently newly formed myocardial tissue were observed within the scar, filling the gap between the apical and basal parts of the squashed ventricle at later stages (2–3 months following injury) of regeneration. Some workers (Kolossova, 1973; Sulima, 1968; Kolossova and Sulima, 1972) believe that partial regeneration of cardiac muscles in injured lower-vertebrate hearts is carried out at the expense of myoblasts liberated from myofiber stumps.

2. *Electron Microscopy*

Normally cardiac myocytes in the ventricles of lower vertebrates display several features typical of more primitive ultrastructural organization compared with the ventricular myocytes of adult mammalian hearts. The former are smaller in size, contain less numerous myofibrils and only a few sarcoplasmic reticulum tubules; a T system and gap junctions are generally absent, and intercalated disks are smaller and thinner (Sommer and Johnson, 1969, 1970; Gros and Shrevel, 1970; Hirakow, 1970; Page and Niedergerke, 1972). Because of these

peculiarities, and the occurrence of dense membrane-limited granules, ventricular myocytes of lower vertebrates resemble the embryonic ventricular or mature auricular muscle cells of mammals (cf. Section II).

The following description of postinjury myocyte reorganization is based on experiments with adult frog hearts (Rumyantsev and Schmantzar, 1968; Rumyantsev, 1973a,b; Niweliński *et al.*, 1974).

Myocardium distant from the site of traumatization retains its ultrastructure practically unchanged. During the first postoperative week there is progressive enlargement of the nucleoli and some chromatin decondensation in myocytes bordering the necrotized area of the frog ventricle. Numerous phagocytes rich in lysosomes resorb the dead myocytes. By the end of the first week and later, an increasing accumulation of ribosomes is frequently observed in the abundant sarcoplasm of the reactive myocytes with enlarged "euchromatic" nuclei. This is often accompanied by the formation of numerous stacks of rough endoplasmic reticulum tubules, Golgi apparatus hyperplasia (Figs. 14, 15, and 17), an increase in the number of microtubules and intermediate-sized (ca. 80- to 110-Å-diameter) filaments, as well as by the scarcity of glycogen granules in the sarcoplasm. Most reorganized myocytes are characterized by a relative paucity of myofibrils displaying an irregular ultrastructure and small mitochondria which are poor in both cristae and contrast matrix. The centrioles are often observed in the vicinity of the Golgi elements, which give an intensive reaction for thiamine pyrophosphatase (Niweliński *et al.*, 1974).

The above changes are considerably accentuated during the second and the third postinjury weeks and have been referred to as myocyte "parital dedifferentiation" or "rejuvenating transformation" (Rumyantsev, 1973b), considering the striking resemblance of most transformed myocytes to cells of early embryonic myocardium (Figs. 14 and 15).

Dedifferentiation seems never to be complete, and even the most rejuvenated myocytes retain some hexagonally packed myosin and actin filaments, as well as simplified, intercalated disks and desmosomes. No evidence was obtained favoring the hypothesis (Sulima, 1968; Kolossova, 1973) of free myoblast formation.

At advanced postoperative stages (2-4 months after injury) the myocytes bordering the scar tissue are only partly redifferentiated. Ultrastructurally they resemble myocytes of mouse papillary muscles, abutting at the chordae tendineae (Virág and Challice, 1969). Both are rich in sarcoplasm, free cytoplasmic filaments, and dense spots of "hyperplastic" Z-band material, whereas their myofibrils are less regularly organized than normal.

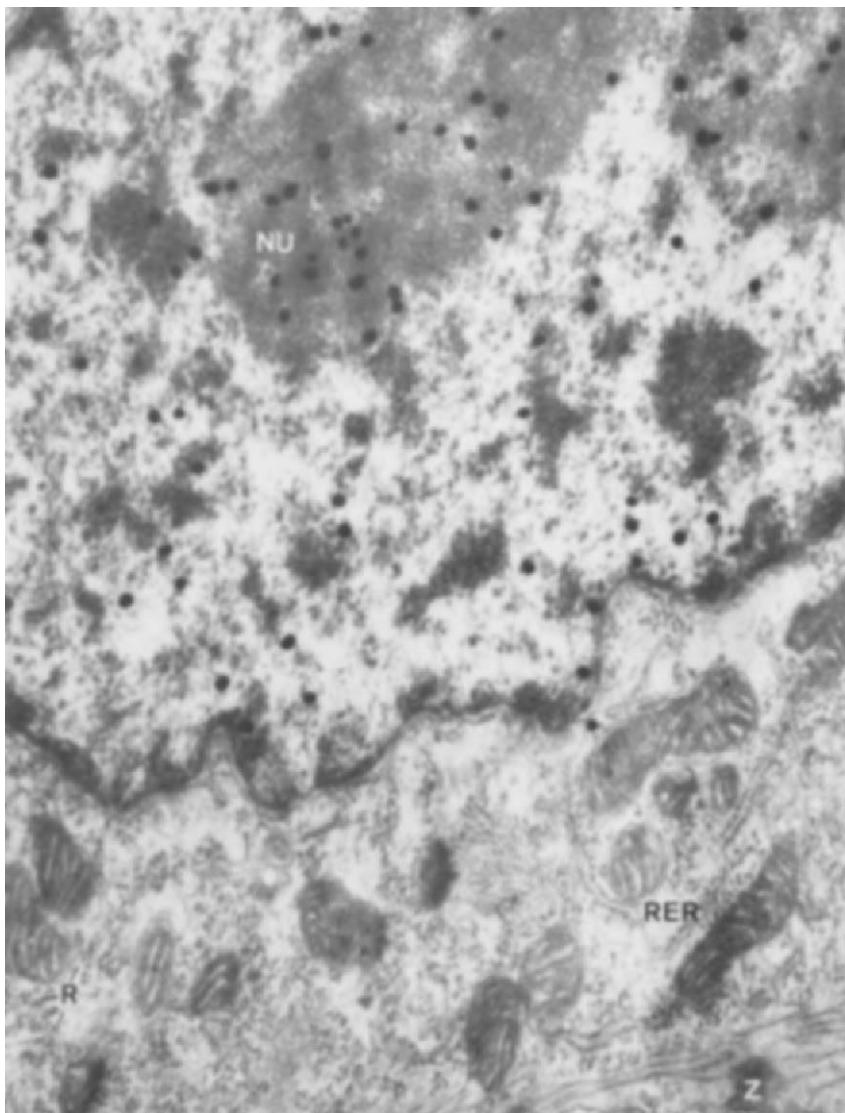


FIG. 14. Uridine- ^3H label over the nucleus of a partially dedifferentiated myocyte 14 days after injury to a frog ventricle, 1.5 hours following isotope injection. The label overlaps predominantly the filamentous regions in nucleoli (NU) and perichromatinic areas of decondensed chromatin. Abundant sarcoplasm with numerous free ribosomes (R) and rough endoplasmic reticulum tubules (RER) still remains unlabeled. Note the primitive Z disk associated with the cell membrane (Z). $\times 29,000$. From Rumyantsev *et al.* (1974).

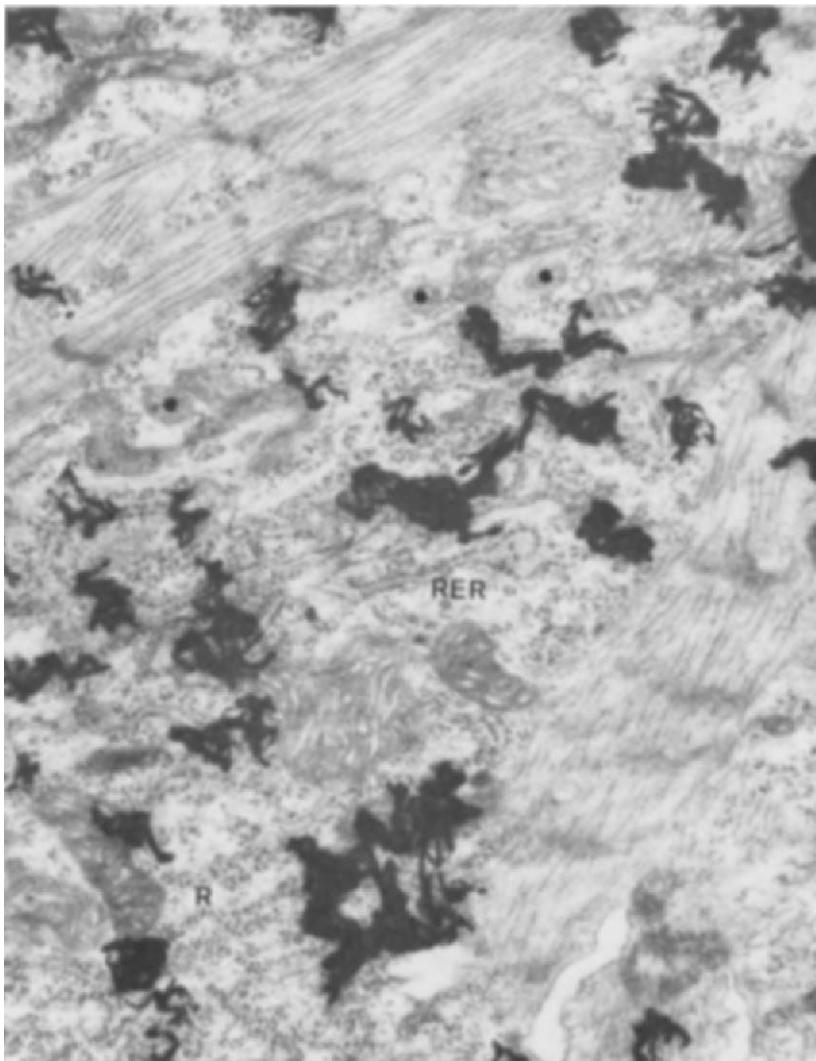


FIG. 15. Portion of a partially dedifferentiated myocyte adjacent to the site of frog ventricle injury 16 days following the operation, 24 hours following isotope injection. Uridine-³H label migrated from the nucleus to the cytoplasm enriched in ribosomes (R) and rough endoplasmic reticulum (RER). Note the presence of very small mitochondria (asterisks). $\times 31,700$.

3. Changes in the Biosynthetic Activity of Myocytes Bordering Injured Ventricular Areas

a. *Synthesis of RNA and Proteins.* One to two days after injury RNA and protein synthesis in perinecrotic myocytes is drastically activated, and this becomes even more evident during the following 2 weeks (Rumyantsev, 1973b; Rumyantsev *et al.*, 1974). This is in agreement with the above ultrastructural evidence on the activation of nuclear and cytoplasmic biosynthetic machinery. RNase eliminates practically all uridine-³H incorporation, demonstrating the specificity of RNA labeling. Electron microscope autoradiography reveals the predominant uridine-³H labeling of the fibrillar zones of the enlarged nucleoli and the localization of grains near the chromatin within the nuclei (Fig. 14). Twenty-four hours after uridine-³H administration an intensive labeling of the sarcoplasm enriched in polysomes and rough endoplasmic reticulum elements is observed (Fig. 15).

Forty-one days following injury and later uridine-³H incorporation is markedly reduced.

b. *DNA Synthesis.* During the first 4 days following frog ventricle injury thymidine-³H labels only the nuclei of endothelial and granulation tissue cells (Rumyantsev, 1966, 1973b; Jinkine and Rumyantsev, 1970). Beginning on the fifth postoperative day and later, numerous thymidine-³H labeled myonuclei appear within the trabeculae bordering the sites of injury of frog myocardium (Figs. 16 and 17). Their number reaches about 13% at the end of the first week (Fig. 18). Similar results are obtained 10 days following injury to the newt heart (Oberpriller and Oberpriller, 1974). The myocardial trabeculae located far from the injured zone, as in the hearts of control animals, are completely deprived of DNA-synthesizing muscle cells. From the fourth week onward the number of thymidine-³H labeled myonuclei in injured frog hearts decreases progressively, approaching zero only by the end of the second postoperative month (Rumyantsev, 1973b). Using three thymidine-³H injections repeated at 24-hour intervals, one can observe up to 30–60% labeled myocyte nuclei during the second postinjury week (Fig. 18). Four weeks after frog heart injury the number of thymidine-³H labeled myonuclei no longer increases considerably, despite the prolonged availability of precursor. Experiments with multiple thymidine-³H injections permit the suggestion that, when appropriately stimulated, at least half if not all of the myocytes in the frog heart can resume the mitotic cycle.

Electron microscope autoradiography confirms the above evidence (Rumyantsev, 1973a,b; Oberpriller and Oberpriller, 1974), demonstrating numerous myofibril-containing myocytes with thymidine-

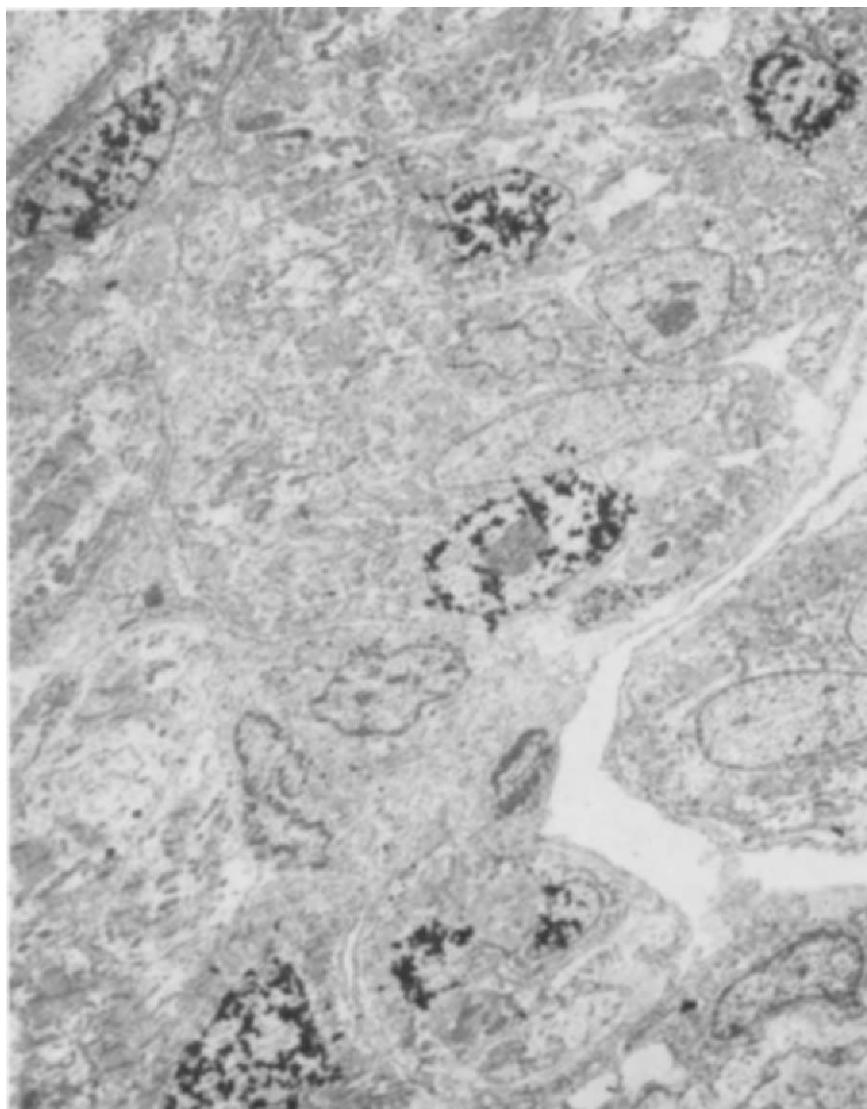


FIG. 16. Thymidine-³H labeling of numerous myonuclei within the trabeculae bordering the site of a frog ventricle injury 14 days following the injury, 5 hours after isotope administration. $\times 2750$.

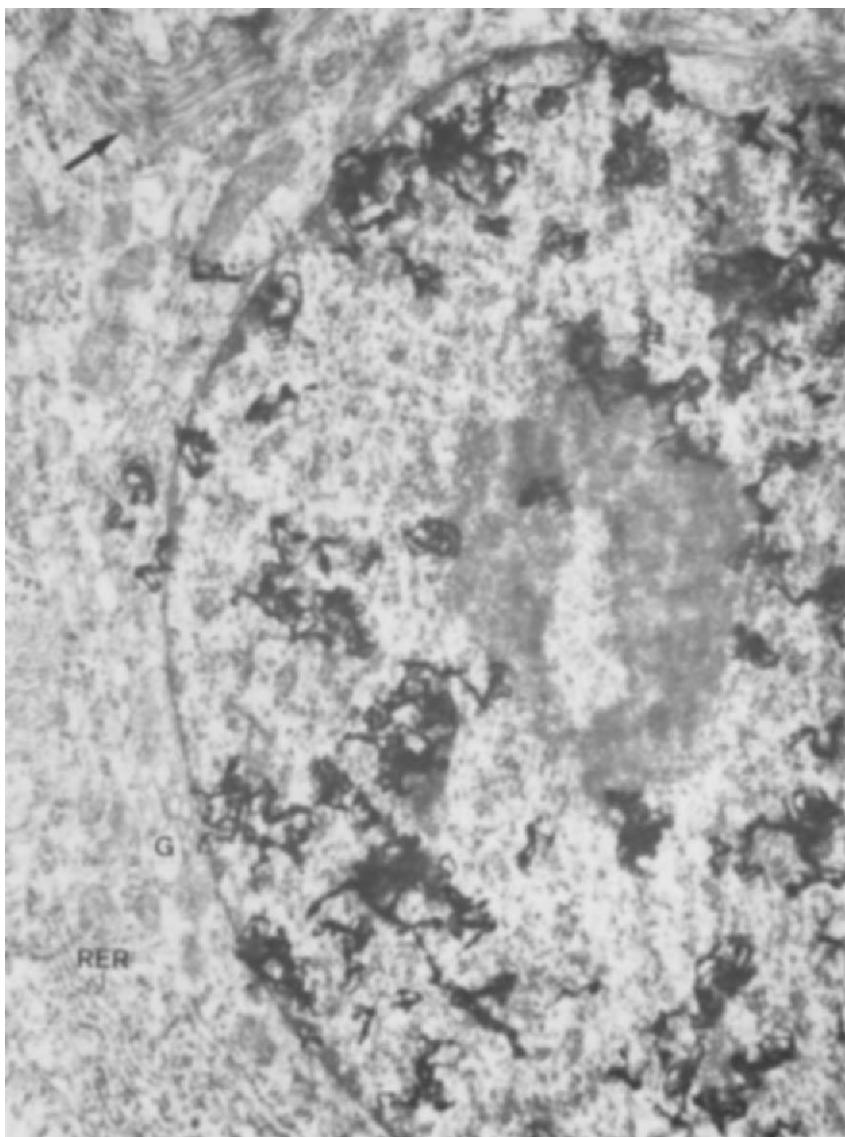


FIG. 17. Portion of a partially dedifferentiated myocyte 16 days following injury to a frog ventricle, 2 hours after thymidine-³H injection. The tag overlaps small patches of more condensed chromatin and nucleolus-associated chromatin. The nucleolus remains largely unlabeled. Note the paucity of myofibrils (arrow), the abundance of rough endoplasmic reticulum (RER), and the juxtapanuclear location of the Golgi apparatus (G). $\times 26,500$.

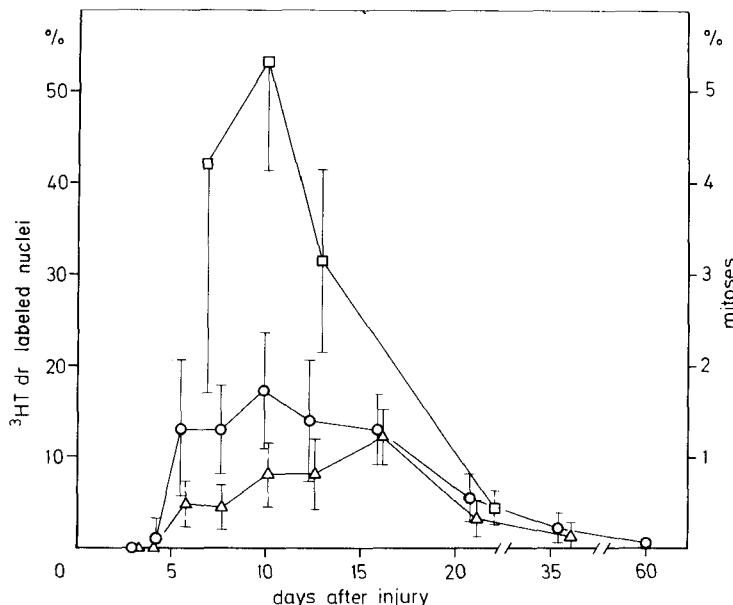


FIG. 18. The number of cardiac myocyte mitoses (triangles) and myonuclei labeled after a single (circles) or three successive (squares) thymidine- ^3H injections. The counts were made in the vicinity of the frog ventricle damaged zone. Abscissa: Days after injury. Ordinate: labeled myonuclei (left) and mitoses (right). Continuous vertical bars are 95% confidence limits, and discontinuous bars are standard errors of the mean. From Rumyantsev (1973b).

^3H -labeled nuclei in the vicinity of the injured heart region (Fig. 16). There is no evident correlation between myocyte partial dedifferentiation and their reentering DNA synthesis. Seven to eight days after frog myocardium injury the majority of thymidine- ^3H labeled myocytes display enlarged nucleoli and euchromatic nuclei, and their cytoplasmic structures are moderately altered. Partial dedifferentiation is much more obvious in numerous thymidine- ^3H tagged myocytes 14–16 days following the injury (Fig. 17).

Among the free cells encountered in areas of myocardial necrosis and cicatrization there are many thymidine- ^3H incorporating cells devoid of typical myofilaments and identified as connective tissue and/or endothelial cells (Rumyantsev, 1973a,b; Oberpriller and Oberpriller, 1974). There is no reason to regard any of them as myoblasts, since intermediate stages of their differentiation into immature muscle cells have never been found, regardless of the time elapsed

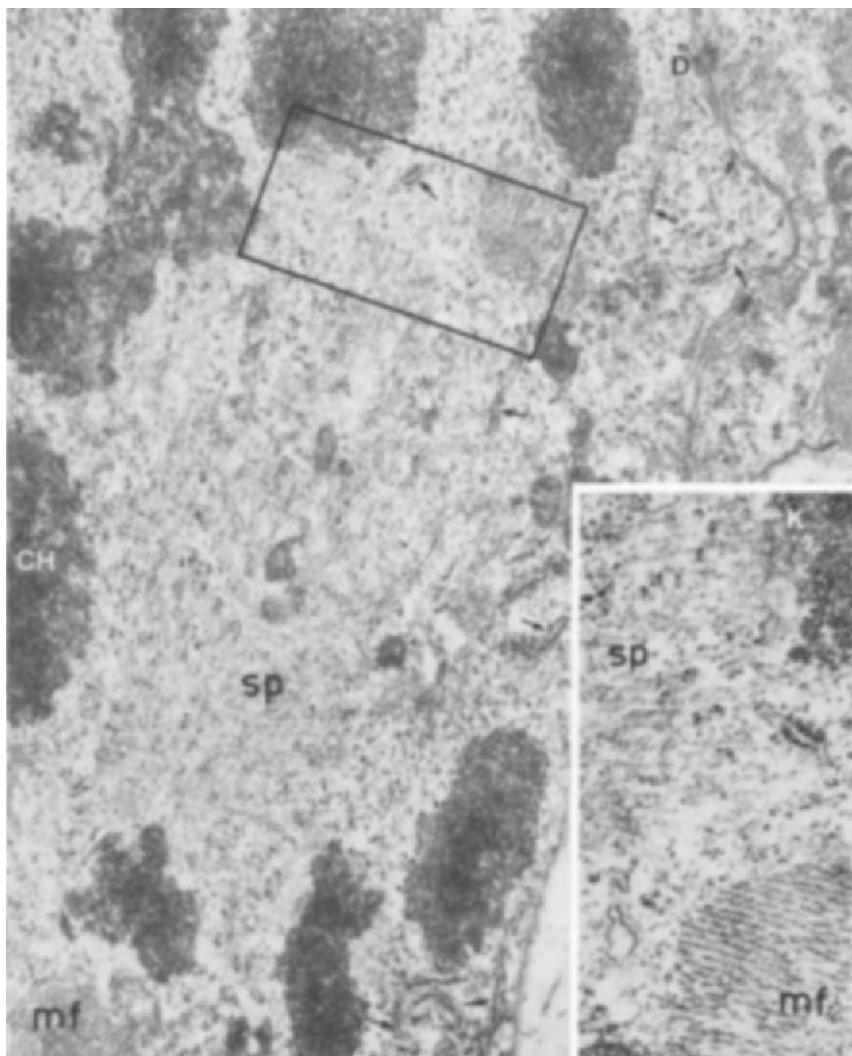


FIG. 19. Portion of a metaphase myocyte 16 days after frog ventricle injury. The area between chromosomes (CH) is occupied mainly by spindle microtubules (sp). Small arrows point to rough endoplasmic reticulum elements. mf, Myofibrils; D, desmosome. $\times 17,500$. Inset: Detail of the framed area, showing spindle microtubules (sp), a kinetochore (K), and a myofibril (mf). $\times 32,500$. From Rumyantsev (1973b).

after thymidine-³H administration. These cells are not attached to myocytes by means of desmosomes or intercalated disks, being separated from the muscle cells by a layer of glycocalyx or connective tissue ground substance.

In agreement with autoradiographic findings the reactivation of DNA synthesis by cardiac myocytes in the adult frog can also be revealed with cytophotometry of Feulgen-strained preparations (Mirakyan and Rumyantsev, 1968).

4. Myocyte Mitotic Activity

In agreement with light microscope evidence electron microscopy reveals numerous mitoses of myocyte nuclei (Fig. 19) in perinecrotic trabeculae of newt (Oberpriller and Oberpriller, 1971) and frog (Rumyantsev, 1973b) hearts. As in normal cardiac myogenesis, the majority of sarcomeres lose their Z disks after the onset of metaphase. Mitosis continues, progressing up to the formation of daughter nuclei. The index of stimulated myocyte mitoses attains a maximum (about 1.3%) in the damaged frog heart in the middle of the third post-operative week. Thereafter the number of mitoses decreases sharply (Fig. 18).

As judged from the curves for thymidine-³H labeled mitoses, cardiac myocytes in the regenerating frog heart proliferate about two times more slowly than cells forming the scar (Rumyantsev, 1973b). This probably is responsible for the incompleteness of frog myocardium regeneration and nonmuscle cell overgrowth.

Incision, burning, and squashing of myocardium in newts, frogs, and lizards result in a similar degree of cardiac mitosis reactivation, thus indicating that neither the type of injury nor lower-vertebrate species variation play an essential role (Sulima, 1968; Oberpriller and Oberpriller, 1971; Rumyantsev, 1973c). The observation of strikingly rapid myocardial regeneration in newts (Becker *et al.*, 1974) cannot be explained satisfactorily on the basis of myocyte mitosis kinetics as described in this section and remains to be investigated.

B. AVIAN MYOCARDIUM

The ultrastructural organization of cardiac muscle cells in avians is rather similar to that in lower vertebrates. The T system and gap junctions as a rule are absent (Sommer and Johnson, 1969, 1970). Along with other features indicating relatively primitive organization, this similarity suggests that the cardiac muscle cells in birds are also capable of resuming more or less intensive hyperplasia. According to Kolossowa (1960, 1973), adult chicken myocardium responds to injury mainly by sequestration of myoblast-like cells which do not, however,

redifferentiate into cardiac myocytes later, being admixed with cells of scar tissue. By the third postinjury day amitotic divisions and occasional mitoses of myonuclei within the perinecrotic myofibers are observed.

The ingenious experiments of Novikov (1973a,b) demonstrate that early embryonic chick myocardium regenerates *in situ* without cicatrization after extended necroses. This is claimed to occur as a result of regulative migration of poorly differentiated myocytes from adjacent myocardial regions. Dediifferentiation of perinecrotic myocytes and formation of myoblasts play rather an insignificant role in such regeneration. No activation of mitoses in myocytes bordering the injured zone was observed, the mitotic activity being equally high (up to 5%) in all parts of the perinecrotic and distant myocardium. The paucity of stromal cells in early embryonic hearts (Manasek, 1968a, 1973) seems to be of great importance for effective myocardial regeneration in Novikov's experiments.

Necrotized areas in hearts of 18-day chick embryos and posthatching chicks are filled predominantly with scar tissue, despite the pronounced dedifferentiation of myocytes around the injured region, activation of their mitoses, and sequestration of myoblasts (Novikov, 1973a,b). Thus connective tissue cell overgrowth seems to be one of the major factors determining the outcome of myocardial regeneration (cf. Hay, 1966). The lack of electron microscope and autoradiographic data makes it premature to draw a definite conclusion about the regenerative capacity of avian myocardium.

C. MAMMALIAN VENTRICULAR MYOCARDIUM

Since the middle of the nineteenth century efforts have been made to study the regenerative capacities of ventricular cardiac muscle. The literature is extensively reviewed by Anitschkow (1912, 1913), Goldzieher and Makai (1913), Mönckeberg (1924), Tähka *et al.* (1957), Polezhaev *et al.* (1965), Klinge (1967), McMinn (1967, 1969), Sarkisov (1970), Polezhaev (1972a,b, 1975), Hudgson and Field (1973), and Sidorova (1976). According to widespread opinion mammalian cardiac muscle responds to injury only with hypertrophy of the surviving myocytes which, like neurons, cannot divide. However, there are many conflicting reports in this respect. Only a few investigations deal with induced myocyte hyperplasia in the atrium and the conductive system of the heart (Section IV,D and E).

1. Light Microscopy

The capacity of cardiac muscle to regenerate was investigated after myocardial traumatization, burning, infarction, different forms of myo-

carditis, myocardial dystrophies, focal myocytolysis, and so on. Numerous workers did not find any manifestation of cardiac muscle regeneration regardless of the nature of the injury or the type of species, both under experimental and pathological conditions (Bode, 1897; Elsberg, 1899; Karsner and Dwyer, 1916; Klose, 1920; Ichteiman, 1934; Moritz and Atkins, 1938; Ageitshenko, 1947; Harrison, 1947; Walls, 1949; Tähka *et al.*, 1957; Warren *et al.*, 1957; Wanick, 1961). Different "attempts" at regeneration were found, however, by other investigators, such as mitotic and amitotic divisions of myonuclei near the sites of lesions, formation of myoblasts, and sprouting of muscle buds from myofiber stumps. All these manifestations, if found, were generally abortive and not sufficient for regeneration of a considerable part of the destroyed muscle. As a result, cicatrization occurs commonly in areas of necrosis of mammalian myocardium.

The present skepticism concerning the reality and a possible role of amitoses in myogenesis (Capers, 1960; Cooper and Königsberg, 1961; Zhinkin and Andreeva, 1963), saves us a detailed analysis of numerous descriptions of direct divisions of myonuclei within cardiac myofiber stumps (Berent, 1892; Oppel, 1901; Antischkow, 1912; Schiefferdecker, 1916; Nieth, 1949; Grundmann, 1951; Tarapina, 1958; Kolossova, 1961; Polezhaev *et al.*, 1965; Survillo and Naumetz, 1966). Whereas the normal left ventricle of the cat contains 1.64% doubled (amitosis-like) myonuclei, their number in the vicinity of old myocardial scars increases up to 4.3% (Grundmann, 1951). Similar evidence is given by Nieth (1949). However, the binuclearity of many cardiac myocytes in adult mammals (Muir, 1957; Challice and Edwards, 1961; Rumyantsev, 1972a; Gräbner and Pfister, 1974) suggests that paired nuclei within the cardiac myofibers bordering the sites of injury are formed not via amitosis but as a result of shifting and the close contact of both myocyte nuclei.

Rare (0–0.2%), or even single, mitotic figures inside the muscle fibers bordering necrotized areas occurred after experimental traumatization of ventricles (Martinotti, 1888; Mircoli, 1889; Bonome, 1889; Rumyantsev, 1955, 1966; Voronov, 1975), after infarctions and ischemic micronecroses (Kolster, 1893; Ring, 1950; Klinge, 1967; Mirakyan and Rumyantsev, 1968; Rumyantsev and Mirakyan, 1968a; Zapryagaev, 1970; Rumyantsev, 1974; Sidorova and Bolshakova, 1967), and after experimental burning of the heart wall (Tarapina, 1958). Descriptions of prophase and metaphase predominate, while anaphase and telophases (Fig. 20) seem to be very rare. This may be due not only to the relative rapidity of the latter phases but also to the abnormalities typical of many of the observed metaphases (Klinge,

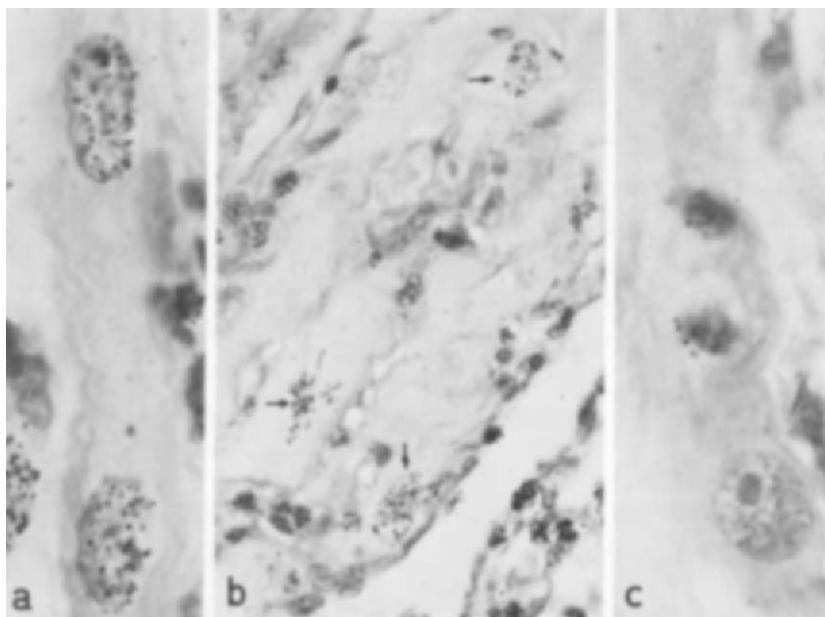


FIG. 20. DNA-synthesizing and mitotically dividing myonuclei in subepicardial, perinecrotic muscle fibers of a rat left ventricle. Ten successive thymidine- ^{3}H injections were given at 12-hour intervals from the fifth to the ninth day following left ventricle infarction. (a) Two labeled myonuclei. $\times 1270$. (b) Two labeled myonuclei and a labeled metaphase (arrows). $\times 480$. (c) A weakly labeled telophase. $\times 1270$. (a) and (c) Hematoxylin-eosin; (b) PAS-hematoxylin. From Rumyantsev and Kassem (1976).

1967). The absence or unipolar formation of the mitotic apparatus, chromosome dispersion, and clumping (pycnomitoses) were not uncommon.

Because of their basophilia, paucity of myofibrils, and euchromatic appearance of enlarged myonuclei with prominent nucleoli, stumps of myofibers have a certain resemblance to the so-called *Muskelknospen* of regenerating skeletal muscle. This evidently prompted several workers to describe partial dedifferentiation and bud growth as the manifestation of abortive myocardial regeneration (Heller, 1914; Warthin, 1924; King, 1941; Ring, 1950; Barboni and Ganassi, 1954). However, these changes can be related not to the actual sprouting of myofiber stumps, but to the progressive cicatrization causing secondary changes in their metabolism and morphology (Tähka *et al.*, 1957).

Descriptions of myoblast sequestration from the ends of surviving myofibers are not uncommon in studies of myocardial regeneration

(see reviews by Polezhaev *et al.*, 1965; Polezhaev, 1972a,b, 1975). Some investigators claim that these myoblasts are capable of limited redifferentiation following the proliferative phase (Skuba, 1968, 1969; Zaprygaev, 1970), while others deny this (Mirakyan and Sperling, 1972).

Following the work of Oppel (1901) and Anitschkow (1912, 1913), a vast body of evidence was compiled to support the theory of "myogenic granulation tissue" formation in areas of myocardial necrosis (for literature, see Murphy, 1963; Polezhaev *et al.*, 1965). According to Anitschkow (1912, 1913), the ends of the surviving myofibers dedifferentiate and give rise to free cells deprived of myofibrils and incapable of redifferentiation. Characteristic condensation of their chromatin in the form of a longitudinal serrate line was believed to be a marker structure for these cells referred to later as "Anitschkow myocytes" (Jacki, 1919; Hesse and Hesse, 1924; Ghosh, 1957; Kolossova, 1960, 1961, 1973; Murphy, 1963; Mikat, 1964; Murphy and Becker, 1966). Similar nuclei were observed, however, in smooth muscle, epithelial, and mast cells (Semsroth and Pool, 1926; Ehrlich and Lapan, 1939; Glawson, 1941; Hopps and Wissler, 1946; Zak, 1947; Rumyantsev, 1957; Tóth, 1958). As a result, the idea of a myocardial origin for Anitschkow myocytes was considerably downgraded (Wenezianowa-Grusdkowa, 1929; Ichteiman, 1934; Rumyantsev, 1957; Tóth, 1958, Rubenstein and Saphir, 1962). Although Tóth (1958) had suggested that the condensation of chromatin characteristic of these cells is indicative of degeneration, electron microscopy reveals a normal appearance of their organelles and intensive development of the rough endoplasmic reticulum (Fig. 21). The incorporation of thymidine-³H into the "caterpillar" nuclei (Rumyantsev, 1966) is not compatible with the interpretation of Anitschkov myocytes as being degenerating cells. Their significance remains to be clarified.

Only a few workers described conspicuous regeneration of ventricular myocardium. Kotshetov (1959, 1970), Zakharievskaya (1946), and Zapryagaev (1970) believe that complete healing of the smallest myocardial necroses involving only 1 to 2 myofibers is possible in adult rabbit hearts via the formation of buds with amitotically and mitotically dividing myonuclei. According to Cellarius and Semenova (1972), however, all kinds of myocytolysis in myocardium, including necrotization of a single myocyte, are followed by focal cicatrization. The majority of these minute scars disappear later as a result of desmolytic processes. Therefore Sinitzin's (1959, 1961, 1970) rather confused description of regeneration of voluminous blocks of myocardial tissue is

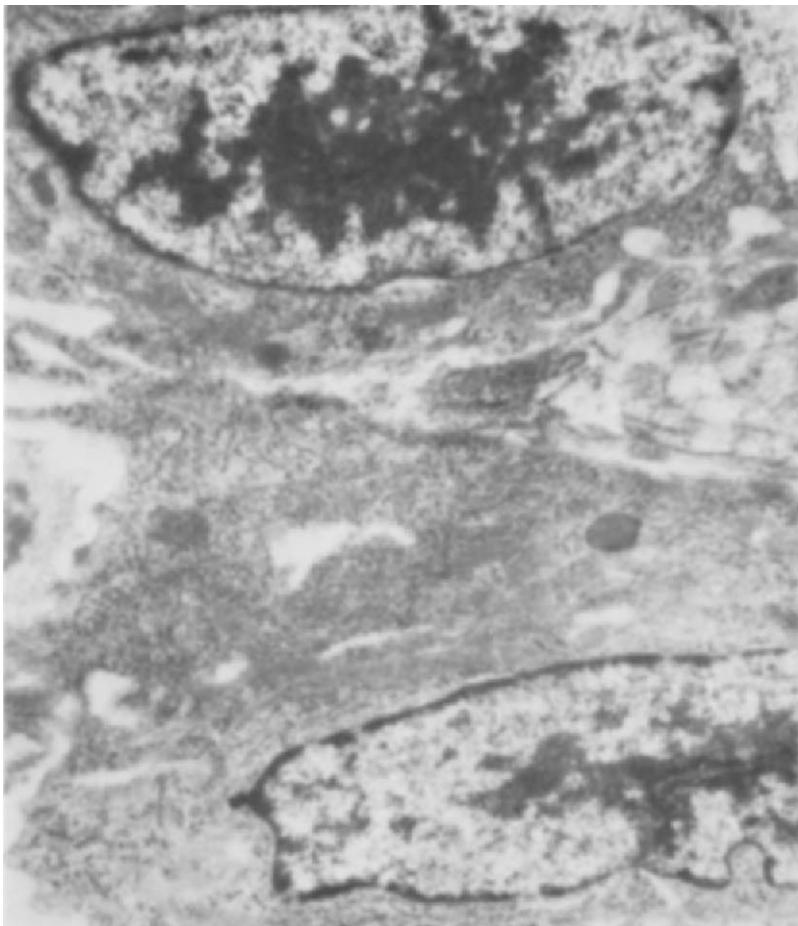


FIG. 21. Two cells with characteristic serrated chromatin typical of Anitschkow myocytes. Left atrium of a rat with a severe ventricular infarction 6 days after left coronary artery ligation. Note the abundance of rough endoplasmic reticulum elements in the cytoplasm of the inferior cell. $\times 11,000$.

generally regarded as not at all convincing (Knorre, 1961; Rumyantsev and Zhinkin, 1967; Sarkisov, 1970, 1973).

The lack of unanimity concerning the extent of cardiac muscle regeneration and its mechanisms in orthodox histological studies obliges us to describe in more detail data based on the use of modern cytological methods.

2. Electron Microscopy

Several investigators observed a dedifferentiative reorganization of cardiac myocytes near areas of necrosis and cicatrization in ventricles. Perinecrotic myocytes were found to be enriched in sarcoplasm, free ribosomes, rough endoplasmic reticulum, and irregularly distributed minute cytofilaments of an unidentified type. The nucleoli and Golgi apparatus were increased in size, whereas myofibrils, intercalated disks, and mitochondria became less regularly organized (Kajihara, 1970; Dusek *et al.*, 1971a; Hatt, 1972). Consistent with these data it was demonstrated that perinecrotic ventricular myocytes are enriched in RNA and display increased activity of glycolytic and pentose cycle enzymes (Belov and Ilnitskaya, 1970; Danilova *et al.*, 1971; Dusek *et al.*, 1971a). The dedifferentiative significance of the above observations emphasized by Kajihara (1970) and Dusek and his associates (1971a) has not yet been proved, despite the obvious similarity of changed cardiac myocytes to cells of immature myocardium (Sarkisov, 1972). Glagoleva and Tshetshulin (1968) described the appearance of myoblasts within granulation tissue and their subsequent differentiation into myofibril-containing cells. A question, however, arises whether the cells described as myoblasts are in fact moderately dedifferentiated myocytes, since at the ultrastructural level the myogenic origin of these cells is revealed by the presence of myofilaments or sarcomeres.

According to Sarkisov (1963, 1970, 1973), nonproliferating mature cardiac myocytes display a capacity for so-called intracellular regeneration, that is, for replacement, restoration, and hyperplasia of their organelles under experimental and pathological conditions. Electron microscopy provides numerous examples of the appearance of newly formed myofibrils, mitochondria, sarcoplasmic and rough endoplasmic reticulum, and other myocyte organelles in injured and overloaded hearts (Richter and Kellner, 1963; Sarkisov and Vtjurin, 1967; Glagoleva and Tshetshulin, 1968; Büchner and Onishi, 1970; Wilcken *et al.*, 1970; Semenova *et al.*, 1971; Cellarius and Semenova, 1972; Hatt, 1972; Rumyantsev, 1972b, 1974). As a rule, new organelle morphogenesis proceeds in myocytes enriched in ribosomes. Danilova and her colleagues (1971) claim that activation of the pentose cycle enzymic pathway is one of the important steps in the initiation of "intracellular" myocardial regeneration. The significance of "organelle hyperplasia" in cardiac myocytes of adult animals and its relation to moderate dedifferentiation is far from being fully understood. It is highly probable, though, that intracellular hyperplasia is involved in

processes underlying the capacity of cardiac myocytes to undergo hypertrophy (Meerson, 1969; Sarkisov, 1970).

Several reviews describe the ultrastructural aspects of myocardial intracellular regeneration (Sarkisov, 1970) and hypertrophy (Meerson, 1969; Büchner and Onishi, 1970; Meessen, 1971).

3. RNA and DNA Synthesis; Ploidy and Division of Ventricular Myocytes

a. *Injured or Infarcted Myocardium.* In agreement with submicroscopic and histochemical findings, the incorporation of uridine-³H into myocytes bordering on the sites of experimental traumatization of mouse ventricles is markedly intensified (Rumyantsev, 1966). This is most pronounced 2 days after heart injury; on the fifth day, when myocyte nuclei and nucleoli enlargement, as well as sarcoplasmic basophilia, reach a maximum, the intensity of uridine-³H labeling diminishes. In regenerating skeletal muscles the most intensive uridine-³H incorporation also precedes the appearance of strong basophilia (Zhinkin and Goryatsheva, 1968).

Unlike RNA synthesis, DNA replication in nuclei of myofibers bordering necrotic tissue in mouse and rat ventricles is only slightly activated if at all (Fig. 22). This is demonstrated irrespective of the postinjury stage and the kind of damage, both by autoradiography of thymidine-³H labeling (Rumyantsev, 1966, 1970, 1974; Walker and Adrian, 1966; Mirakyan and Rumyantsev, 1968; Klinge, 1967; Kranz *et al.*, 1970; Galankin, 1975; Rumyantsev and Kassem, 1976) and Feulgen photometry (Mirakyan and Rumyantsev 1968). Attempts to label as many myonuclei as possible by injecting thymidine-³H three times or more at 8- to 12-hour intervals were as a rule unsuccessful

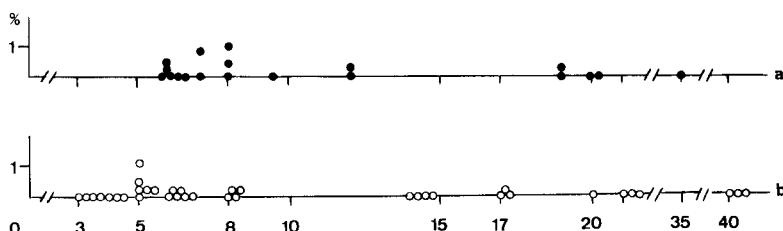


FIG. 22. Percentages of myonuclei labeled after three injections of thymidine-³H given at 11-hour intervals (a), and after a single injection (b). Counts were made in the myofibers surrounding the infarcted area of the rat left ventricle. Abscissa: Days after infarction. Each point is a labeling index for one animal.

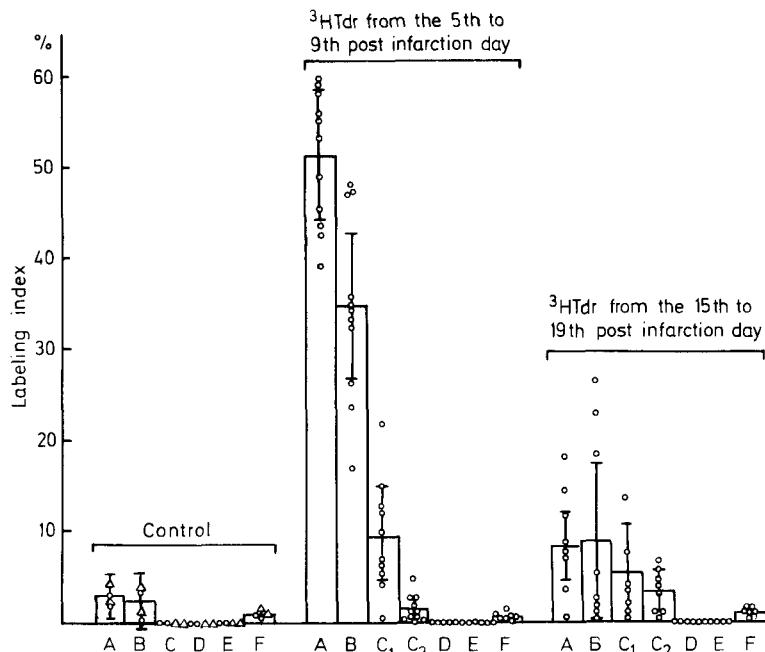


FIG. 23. Percentage of thymidine- ^3H labeled myonucleic acid in different heart compartments of control rats and rats with severe left-ventricle infarctions. Circles, Unoperated animals; triangles, sham operated animals. Both control and experimental rats received 10 successive thymidine- ^3H injections at 12-hour intervals. Each point is a labeling index for one animal. The vertical bars represent 95% confidence limits. A, Left atrium; B, right atrium; C, left ventricle (C_1 , subepicardial perinecrotic myofibers; C_2 , other juxtanecrotic myofibers in the left ventricle); D, right ventricle; E, interventricular septum; F, atrioventricular node together with the bundle of His. From Rumyantsev and Kassem (1976).

(Rumyantsev, 1966, 1974). Only after thymidine- ^3H injections were repeated 10 to 33 times did the number of labeled myonuclei within the myofiber stumps rise maximally to 9% (Fig. 23). Numerous DNA-synthesizing nuclei were observed in the thin, subepicardial layers of surviving myofibers (Rumyantsev, 1970; Rumyantsev and Kassem, 1976). The presence of thymidine- ^3H labeled mitoses within these myofibers (Fig. 20), including several telophases, shows that sluggish DNA synthesis may be followed by occasional myonuclei hyperplasia (cf. Sidorova and Bolshakova, 1976). The possibility of endoreduplication has not been ruled out as well.

b. *Hypertrophied Myocardium.* The total DNA content in hyperfunctioning hearts increases considerably following the initial activa-

tion of RNA synthesis (Rossi and Dianzani-Mor, 1958; Grove *et al.*, 1969; Meerson, 1969; Zak, 1973, 1974). Contrary to earlier suggestions this is due chiefly not to the replication of myonuclei DNA but to the prolifertion of stromal and endothelial cells (Rumyantsev *et al.*, 1967; Mirakyan and Rumyantsev, 1968; Meerson, 1969; Morkin and Ashford, 1968; Grove *et al.*, 1969; Grimm *et al.*, 1970; Rumyantsev, 1970; Mandache *et al.*, 1973). The number of thymidine-³H labeled ventricular myonuclei rises in overloaded mice hearts only from 0.035% in controls to 0.09% (Kunze and Citolier, 1967), and in rats from 0.21 to 0.57% (Guski and Kunz, 1973). Similar results were obtained with hypertensive rats by Sasaki and his associates (1970c). In all likelihood, Wegner and Mölbert (1966), who described up to 8% DNA-synthesizing myocytes in overloaded rat hearts, failed to discriminate between myocyte and nonmuscle cell nuclei.

Results of cytophotometric determinations of DNA content in the ventricular myonuclei of hypertrophied hearts fall into two distinct categories. In good agreement with reliable autoradiographic data there is no significant increase in the number of myonuclei containing greater than 2C DNA in rat hearts (Mirakyan, 1967, 1970; Meerson *et al.*, 1968; Mirakyan and Rumyantsev, 1968; Pfitzer and Kuhn, 1970; Grimm *et al.*, 1970; Sasaki *et al.*, 1970d; Pfitzer *et al.*, 1972). The same is typical of the highly hypertrophied turkey hearts (Pfitzer, 1971).

In contrast, in the human, monkey, and pig, myocardial hypertrophy is accompanied by a progressive accumulation of ventricular myonuclei with 4C, 8C, and even (rarely) 16C DNA content (Sandritter and Scommazzoni, 1964; Kompmann *et al.*, 1966; Eisenstein and Wied, 1970; Pfitzer, 1972a,b). There is no autoradiographic evidence of DNA synthesis in these species, however. Maintenance of the fixed maximum number of nucleoli in myocytes of hypertrophied human hearts suggests that nucleolar organizers at least are not replicated (Bloom and Egli, 1969). Thus the mechanism of an increase in myonuclei DNA content in the hypertrophied heart of the human, monkey, and pig remains to be elucidated. Contrary to the majority of investigators, Capers (1964) and Morishita and his associates (1970) found no increase in the myonuclei DNA content in hypertrophied human hearts. The problem is particularly complex, some workers claiming that extreme hypertrophy of the human heart may be followed by hyperplasia of cardiac myocytes (Linzbach, 1952; Morishita *et al.*, 1970; Sasaki *et al.*, 1970c; Astorri *et al.*, 1971; Adler and Sandritter, 1971). Little is known about the nature of myocyte hyperplasia in the highly hypertrophied human heart. Since mitoses are usually absent in the adult human heart, it was supposed that the splitting of myocytes may occur (Linzbach, 1952).

4. DNA Synthesis and Mitosis in Injured and Hypertrophied Immature Myocardium

There are several reports pertaining to the enhanced regenerative capacity of myocardium in growing animals (Heller, 1914; Kermenli, 1920; Warthin, 1924; Rumyantsev, 1955; Schlesinger and Reiner, 1955; Robledo, 1956; Walters *et al.*, 1965; Malishev, 1975a,b). Marked activation of mitoses in marginal myofibers surrounding traumatized myocardial zones was observed in rabbit embryos (Malishev, 1975a,b) and suckling kittens (Rumyantsev, 1955). Similar data were obtained by burning the ventricular wall in neonatal rats (Robledo, 1956). As a result true myocardial regeneration without scar formation was described in injured embryonic myocardium (Malishev, 1975a,b). Several investigators showed that DNA synthesis and myocyte hyperplasia are clearly activated in the hypertrophied hearts of human infants (MacMahon, 1937; Black-Schaffer and Turner, 1958), puppies (Bishop, 1971, 1973), and weanling rats (Neffgen and Korecky, 1972).

The number of DNA-synthesizing and mitotically dividing myocytes in the vicinity of necrotized areas in the heart of suckling rats and kittens was found not to increase (Dusek *et al.*, 1971b; Achabadze and Olenina, 1972; Achabadze, 1974; Galankin and Vtjurin, 1974; and Galankin, 1975). These workers emphasized that cicatrization of the injured myocardium is a common result, regardless of the degree of its differentiation, with recovery being carried out via intracellular hyperplasia only (see Section IV,C,2).

Despite conflicting reports Sidorova (1976) believes that immature myocardium possesses in fact a markedly enhanced capacity to proliferate after different kinds of lesions and after the onset of stable hypofunction. Additional experiments should indicate to what extent the proliferative response in the injured or overloaded immature myocardium contributes to its restoration or hypertrophy.

5. Stimulation of Myocardial Regeneration

Numerous investigators claim that regeneration of ventricular myocardium in adult mammals may be effectively stimulated using myocardial extracts, hydrolyzates, commercial RNA or DNA, bacterial polysaccharide pyrogenal, vitamins B₆ and B₁₂, and so on (Törö, 1939b; Polezhaev *et al.*, 1961, 1965; Achabadze, 1964; Saidrasulov, 1963; Vitkus, 1969; Skuba, 1970). Stimulated myocardial regeneration is generally reported to be carried out via proliferation and subsequent differentiation of myoblasts migrating into the central region of the necrotized zone. Sprouting of the ends of adjacent surviving

myofibers, enhanced DNA synthesis of their nuclei, and even metaplastic myoblast neoformation have been noted as well. The relevant literature has been reviewed by Polezhaev and his associates (1965) and Polezhaev (1972a,b, 1975).

The stimulatory effect of the above agents on myocardial regeneration and myocyte DNA synthesis was not confirmed, however, in analogous experiments (Mohr, 1952; Mohr and Helmreich, 1954; Karapetyan *et al.*, 1970; Mirakyan *et al.*, 1970; Achabadze, 1971). The cytological evidence of stimulated myocardial regeneration through cardiac myoblast hyperplasia was considered to be not conclusive enough and subject to criticism (Knorre, 1961; Rumyantsev and Zhinkin, 1967; Sarkisov, 1973). Instead of myoblast or myocyte hyperplasia, pyrogenal and myocardial hydrolyzates activated DNA synthesis and mitoses in connective tissue and endothelial cells (Karapetyan *et al.*, 1970; Mirakyan *et al.*, 1970).

D. ENHANCED CAPACITY OF ATRIAL MYOCYTES FOR REACTIVATION OF DNA SYNTHESIS AND MITOSIS

1. *Light Microscopy and Autoradiography*

The well-known profound morphophysiological differences among ventricular, atrial, and specialized cardiac muscle cells have been completely disregarded in the studies of myocardial regeneration performed over the past 100 years on ventricular myocardium. It was stated that the left auricle incorporated into the extended cicatrix after ventricular infarction in the rat is enriched in thymidine-³H labeled and mitotically dividing myonuclei as compared with surviving ventricular myofibers (Rumyantsev, 1968; Rumyantsev and Mirakyan, 1968a,b). Subsequent detailed studies confirmed this observation, demonstrating that atrial myocytes of the adult rat in fact possess a markedly enhanced capacity for reactivated proliferation (Rumyantsev, 1970, 1972b, 1974; Kolossova *et al.*, 1970; Rumyantsev and Kassem, 1976; Sidorova and Bolshakova, 1976). During the first three postinfarction days the bulk of the atrial myocytes in the rat respond by enlargement of their nuclei and nucleoli and by enhanced basophilia of their sarcoplasm. On days 5 to 6 there occurs an outburst of DNA synthesis and mitoses of myonuclei (Figs. 24 and 25), the mean number of thymidine-³H labeled and mitotic myocytes reaching about 4 and 1.6%, respectively. Under conditions of "continuous" labeling employing thymidine-³H injections repeated 10 times at 12-hour intervals, the number of labeled myonuclei increased up to 50–60% (Figs. 23 and 26). When estimated by means of the curve for labeled

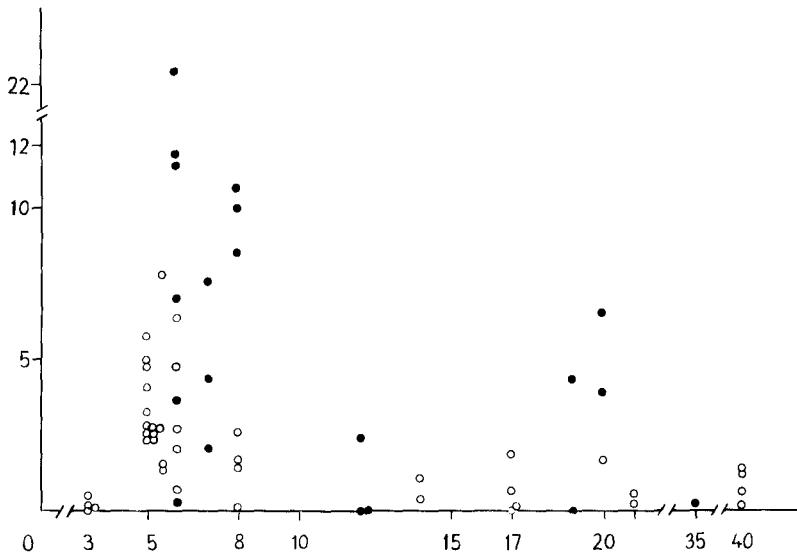


FIG. 24. Percentages of atrial myonuclei labeled with thymidine- ^3H after severe left ventricle infarction. Each point is a labeling index for one rat. Open circles, Single thymidine- ^3H injection; solid circles, precursor injection repeated three times at 11-hour intervals. Abscissa: Days following infarction. From Rumyantsev (1974).

myonuclei mitoses the S and G² periods were found to last 12–13 and 8 hours, respectively (Rumyantsev and Mirakyan, 1968a). This is similar to their length in postnatal myocardium (Table II).

In auricular myocardium stimulated by left ventricle infarction one may easily trace all phases of myonuclei mitoses. However, as in dividing cardiac myocytes of 5- to 10-day-old suckling rats, the anaphase movement of chromosomes is frequently restricted and cytotomy blocked. As a result, an increase in the number of binucleate atrial myocytes is observed 2-3 weeks after infarction. Fixation of material at different intervals following thymidine-³H administration permits one to trace the entire sequence of mitotic events and to state that about half of the myonuclei pairs become labeled 10-21 hours following precursor injection.

It must be stressed that reactivated hyperplasia of atrial myocytes is rather diffusely spread throughout the whole left auricle. Beginning the second week following infarction the number of DNA-synthesizing and mitotically dividing myocytes decreases visibly (Figs. 24 and 25). However, thymidine-³H injections repeated 10 times during postinfarction days 15–20 still labels about 9% of the atrial myonuclei (Fig. 23).

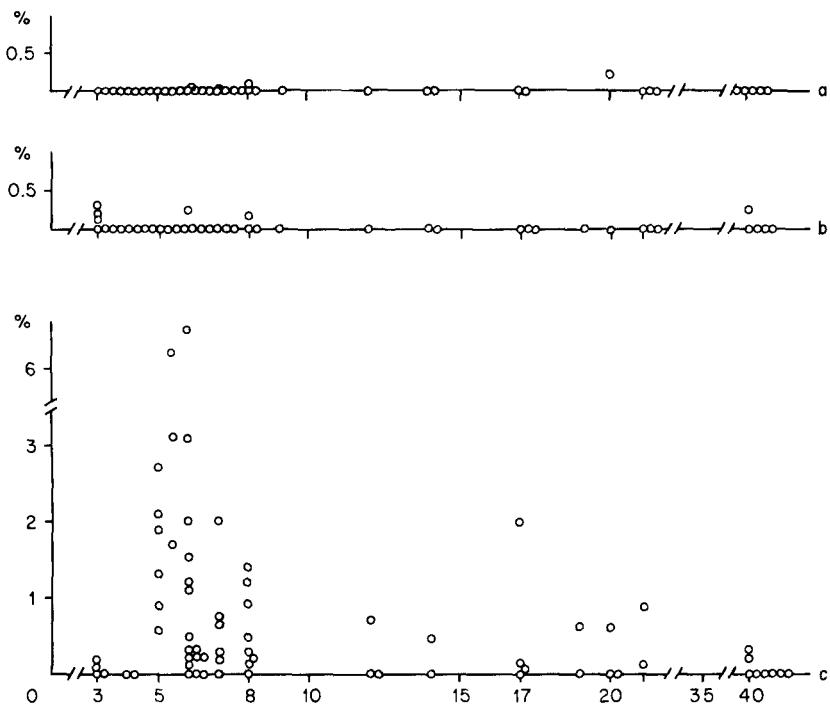


FIG. 25. Percentages of myocyte mitoses in left ventricle some distance from myofiber stumps (a), in left ventricle myofiber stumps (b), and in left atria (c). Each point is a labeling index for one rat. Abscissa: Days after left ventricle infarction. From Rumyantsev (1974).

Cytophotometry of Feulgen-stained preparations revealed a progressive increase in the DNA content of atrial myonuclei at advanced postinfarction stages in rats (Rumyantsev and Mirakyan, 1968a). Along with about 4C DNA myonuclei there occurred nuclei containing about 8C and even more DNA. This is consistent with the accumulation in the atrium of myonuclei that are enlarged, hyperchromic, and frequently bizarre in shape. Similar progressive polypliodization of myonuclei takes place in human auricles in cases of mitral stenosis (Pfizer and Capurso, 1970). The mechanism of the formation of >2C DNA auricular myonuclei remains obscure; a block at the G₂-M boundary, the fusion of adjacent daughter chromosomal complexes during acytokinetic mitosis, and so on, may underlie the increase in DNA content (cf. Pfizer, 1972b; Brodsky and Uryvaeva, 1974).

Concurrently with myocytes of the left atrium those of the right auricle also respond to left ventricle infarction by activation of DNA

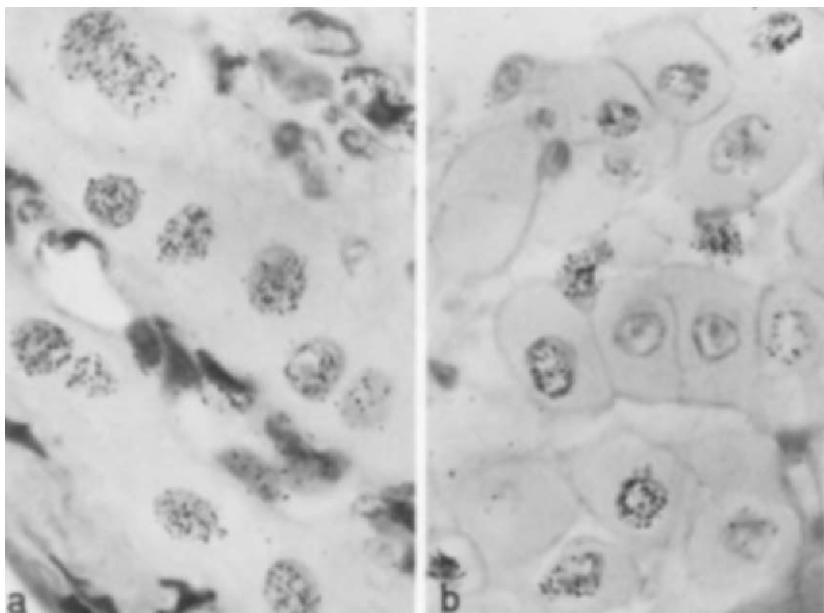


FIG. 26. Thymidine-³H labeling of the majority of nuclei within left atrium myofibers cut longitudinally (a) and transversally (b). Note the twin appearance of the myonuclei pairs, which is indicative of their formation via mitosis (a). Ten successive thymidine-³H injections were given at 12-hour intervals from the fifth to the ninth day following left ventricle infarction in a rat. $\times 930$. (a) Hematoxylineosin; (b) PAS-hematoxylin. From Rumyantsev and Kassem (1976).

synthesis (Fig. 23). The question arises as to the nature of the stimulus inducing the transition of atrial myocytes from the resting state (G_0 or R_1 period; see Epifanova and Terskikh, 1969) to the reproductive state. Involvement of the right atrium suggests that a triggering mechanism is activated by myocyte hyperfunction or by chalone-like diffusible factors rather than by ischemia itself (Rumyantsev, 1974; Rumyantsev and Kassem, 1976). A proliferation pool of atrial myocytes in normal adult rats—about 2–3% (see Section III)—is too small to explain the rapid transition of nearly half of all atrial myocytes to the proliferative state via the recruitment of cells from this pool. Local injuries to the atrial wall (Rumyantsev and Mirakyan, 1968a; Kolossova *et al.*, 1970) and heart hyperfunction after constriction of the abdominal aorta induce less pronounced myocyte proliferation in auricles than left ventricle infarction. Galankin (1975) observed no active DNA synthesis and mitoses in rat atria following infarctions.

2. Electron Microscopy

As emphasized by several workers (Pager, 1968; Rumyantsev, 1972b, 1974; Zak, 1974; Sidorova, 1976), mammalian atrial myocytes are less differentiated ultrastructurally than ventricular ones (see Section II).

Within five postinfarction days preceding the outburst of reactivated hyperplasia and later most rat atrial myocytes undergo more-or-less pronounced "rejuvenating reorganization" (Rumyantsev, 1972b, 1974) similar to that observed in the ventricular myocytes of damaged frog heart (see Section IV,A). There is an enlargement of myonuclei and nucleoli, a decondensation of chromatin, an accumulation of numerous ribosomes and rough endoplasmic reticulum elements within the abundant undifferentiated sarcoplasm, as well as hypertrophy of the Golgi apparatus. The latter shows an increased thiamine pyrophosphatase activity (Figs. 27 and 28a). All these changes are indicative of increased biosynthetic activity in the cells. Ultrastructural reorganization of atrial myocytes is also accompanied by the activation of lysosomes (Rumyantsev, 1974; Rumyantsev *et al.*, 1974), which is confirmed by the acid phosphatase test (Fig. 28b). Thus it is not improbable that lysosomes are involved in preproliferative processes (cf. Hirschhorn *et al.*, 1965; Lane and Becker, 1967). The accumulation of abundant 80 to 110-Å-diameter cyofilaments, possible candidates for microtubule precursor material (Wisniewski *et al.*, 1968; Holtrop *et al.*, 1974), was observed in numerous myocytes; although myofibrils were frequently loosened, they occurred along with intercalated disks and specific atrial granules in all reorganized myocytes (Fig. 27).

In agreement with light microscope evidence autoradiography of ultrathin sections following a single thymidine-³H administration revealed about 3–5% tagged myocytes (Fig. 29). Their ultrastructure was identical to that of the bulk of unlabeled muscle cells. As judged by electron microscope autoradiography, pronounced partial dedifferentiation is not a necessary prerequisite for myocyte transition to the proliferative state (Rumyantsev, 1974).

There was no reason to regard labeled cells devoid of myofilaments, intercalated disks, and atrial granules as myoblasts, since all of them were identified as endothelial and connective tissue cells.

The division of reactivated atrial myocytes proceeds at the ultrastructural level in full accordance with the previously proposed scheme (Fig. 30). Z-disk breakdown occurs in prometaphase, being the most pronounced in myofibrils surrounding the mitotic nucleus.

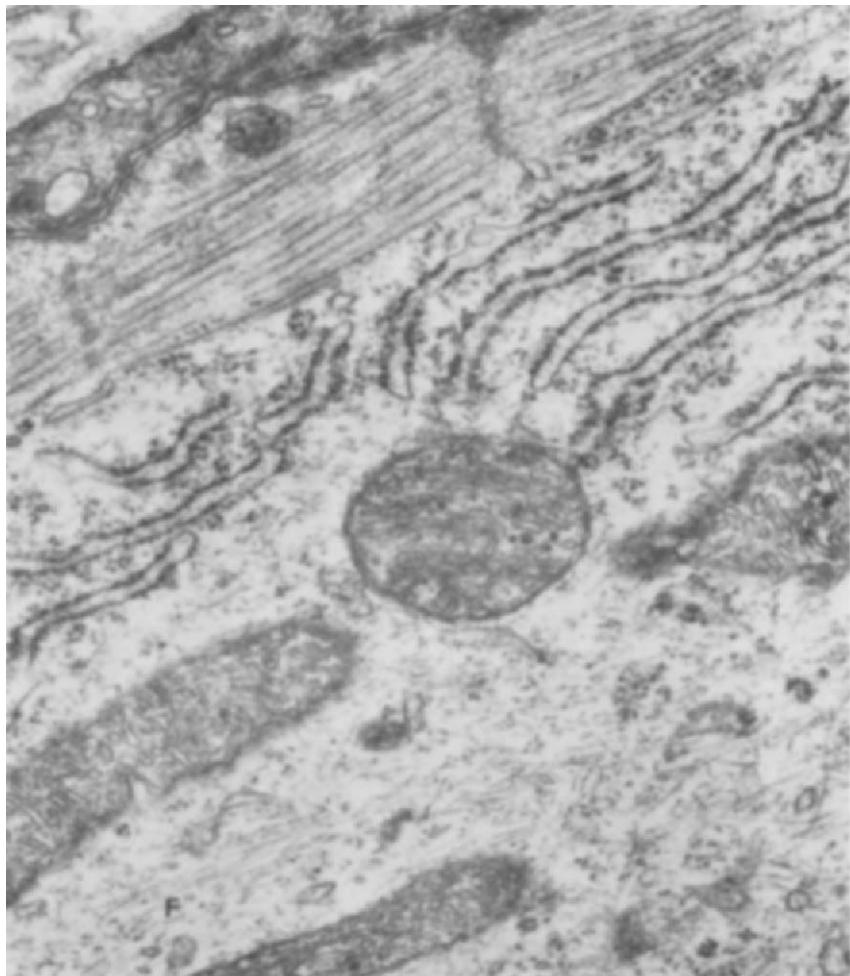


FIG. 27. Portion of a partially dedifferentiated atrial myocyte of a rat 3 days after left ventricle infarction. Abundant sarcoplasm contains slacks of rough endoplasmic reticulum tubules and so-called intermediate cytofilaments (F). $\times 35,500$. From Rumyantsev (1972b).

Released myofilament bundles and free sarcomeres are pushed toward periphery. The incompleteness of myocyte dedifferentiation does not prevent it from producing all the structures of the mitotic apparatus (Fig. 30). Specific granules persist throughout all mitosis phases and frequently touch chromosomes. Reconstruction of the

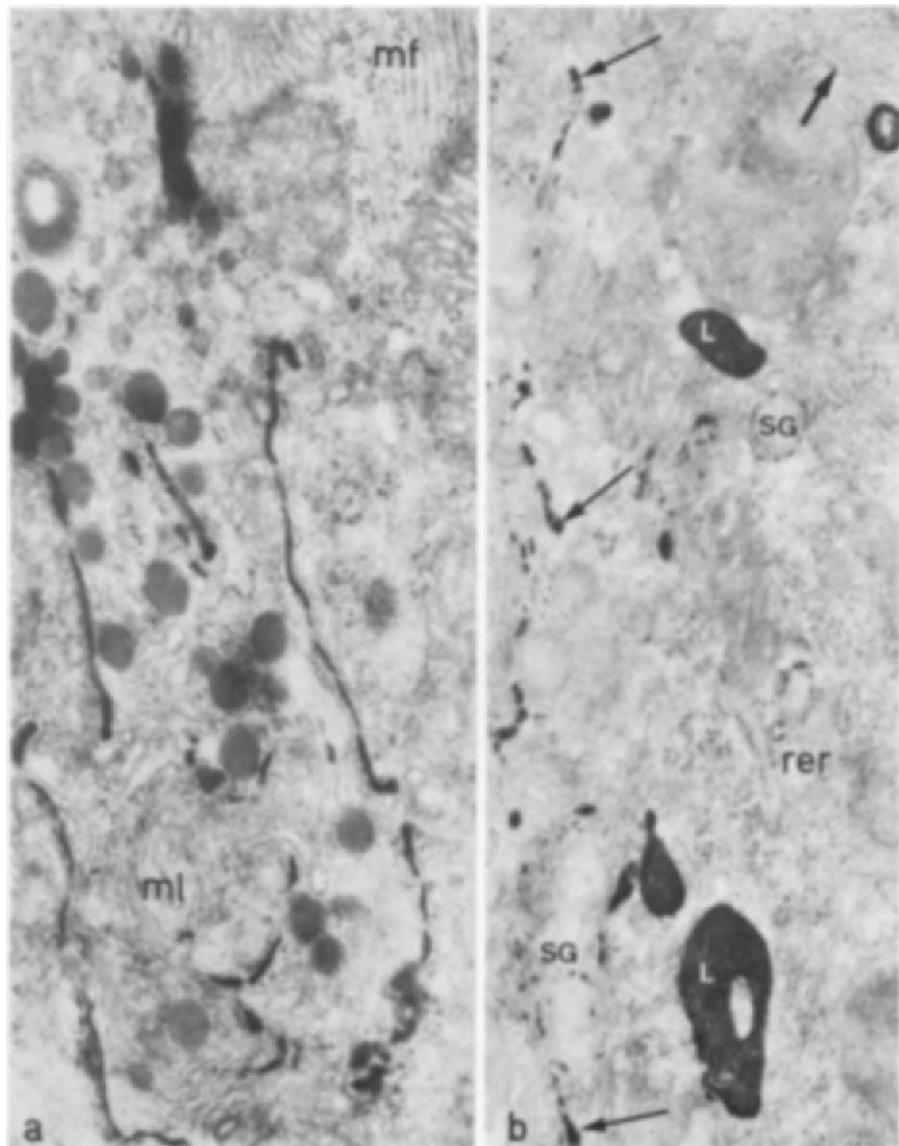


FIG. 28. Portions of left atrium myocytes 6 days following severe infarction of the left ventricle of a rat. (a) Strong reaction for thiamine pyrophosphatase within Golgi apparatus cisternae. mf, Myofilaments; ml, multilamellar body. $\times 39,000$. After Rumyantsev and Seina (1974). (b) Strong reaction for acid phosphatase in lysosomes, and a weaker one in the Golgi apparatus cisternae (thin arrows). Specific atrial granules (SG) and a rough endoplasmic reticulum tubule (rer) are unreactive. Thick arrow points to a myofibril. $\times 39,000$.

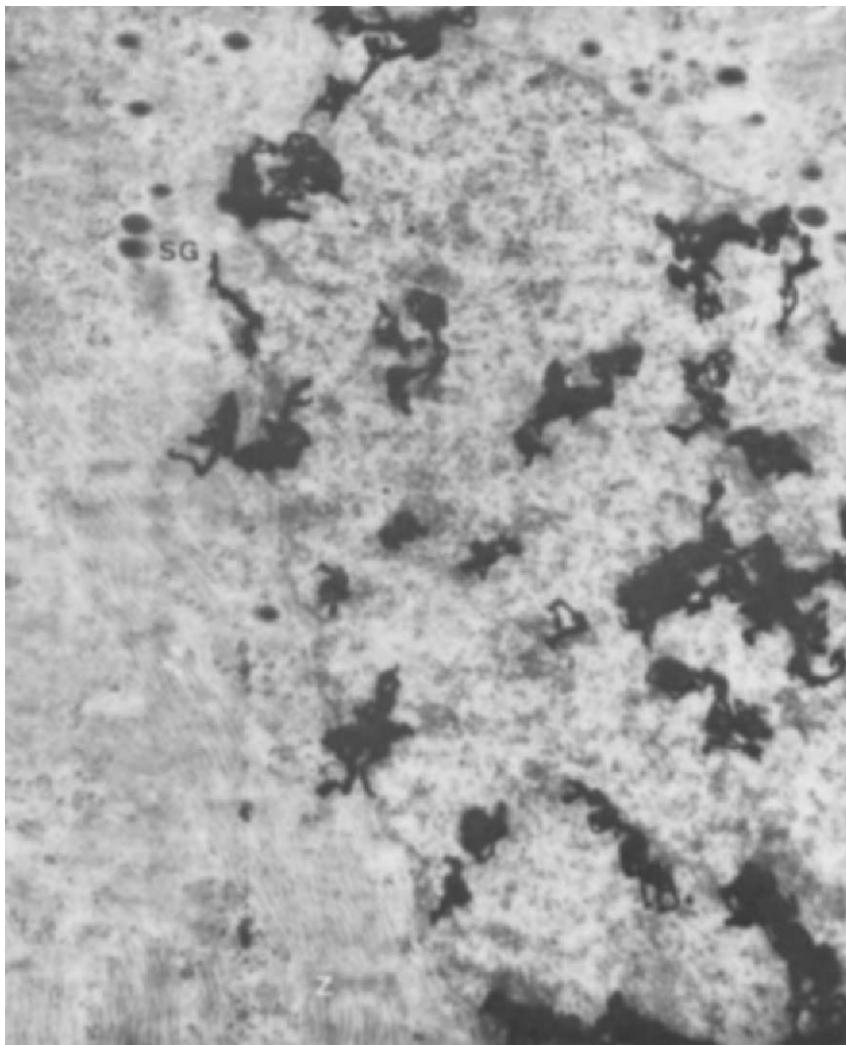


FIG. 29. Thymidine-³H pulse-labeled myocyte from the left atrium of a rat with severe left ventricle infarction 6 days following ligation of the left coronary artery. Note the presence of highly organized myofibrils with easily discernible Z disks (Z), numerous specific atrial granules (SG), and an accumulation of free ribosomes. $\times 22,000$.

daughter nuclei begins in the absence of furrowing. After the reconstruction of small daughter nuclei the beginning of the furrowing may be observed (Fig. 31), but cytokinesis is rarely complete.

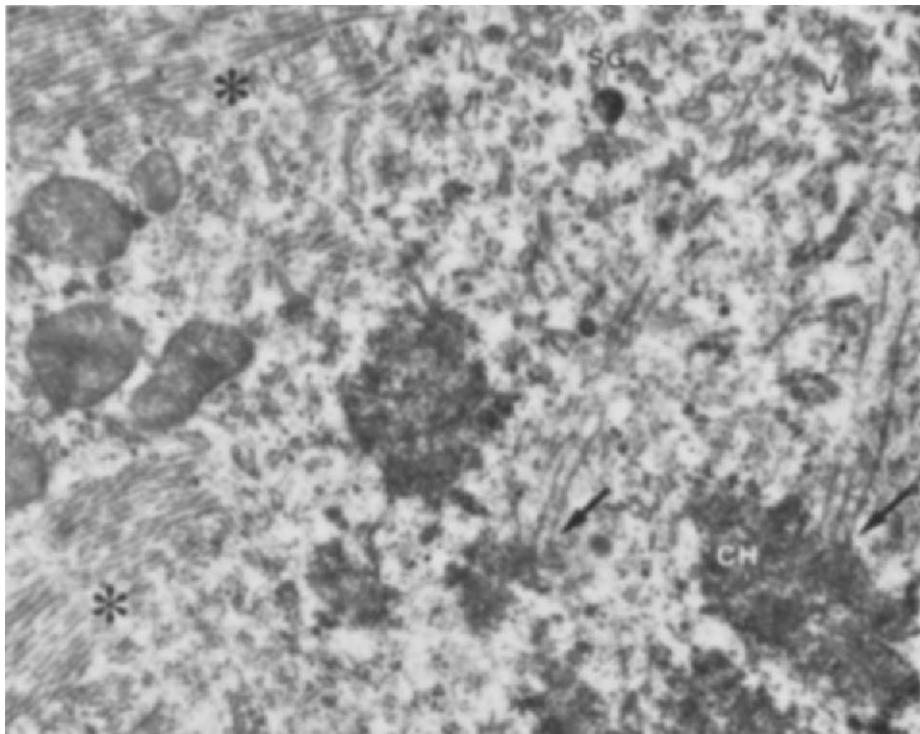


FIG. 30. Portion of a metaphase myocyte from the left atrium of a rat 6 days after left ventricle infarction. Attachment of spindle microtubules to chromosomes is indicated by arrows. The loss of Z-disk contrast material is seen at the points marked by asterisks. Numerous smooth-surfaced vesicles (V) have accumulated in the vicinity of the mitotic apparatus. SG, Specific granule. $\times 24,000$. From Rumyantsev (1974).

E. REACTIVATED HYPERPLASIA OF CELLS IN THE CONDUCTIVE SYSTEM OF THE HEART

It was supposed that cells of the atrioventricular system provide for regeneration of working cardiac muscle (Hofmann, 1902; Retzer, 1920), especially in heart disease (Van der Stricht and Todd, 1919; Todd, 1932; Field, 1951). Zanchi and Lenégre (1955) described degeneration of specialized muscle cells in diseased hearts and assumed that these cells were transformed into histiocytes.

By using multiple thymidine³H injections to estimate the cumulative indexes of labeled cells, it was demonstrated that the number of

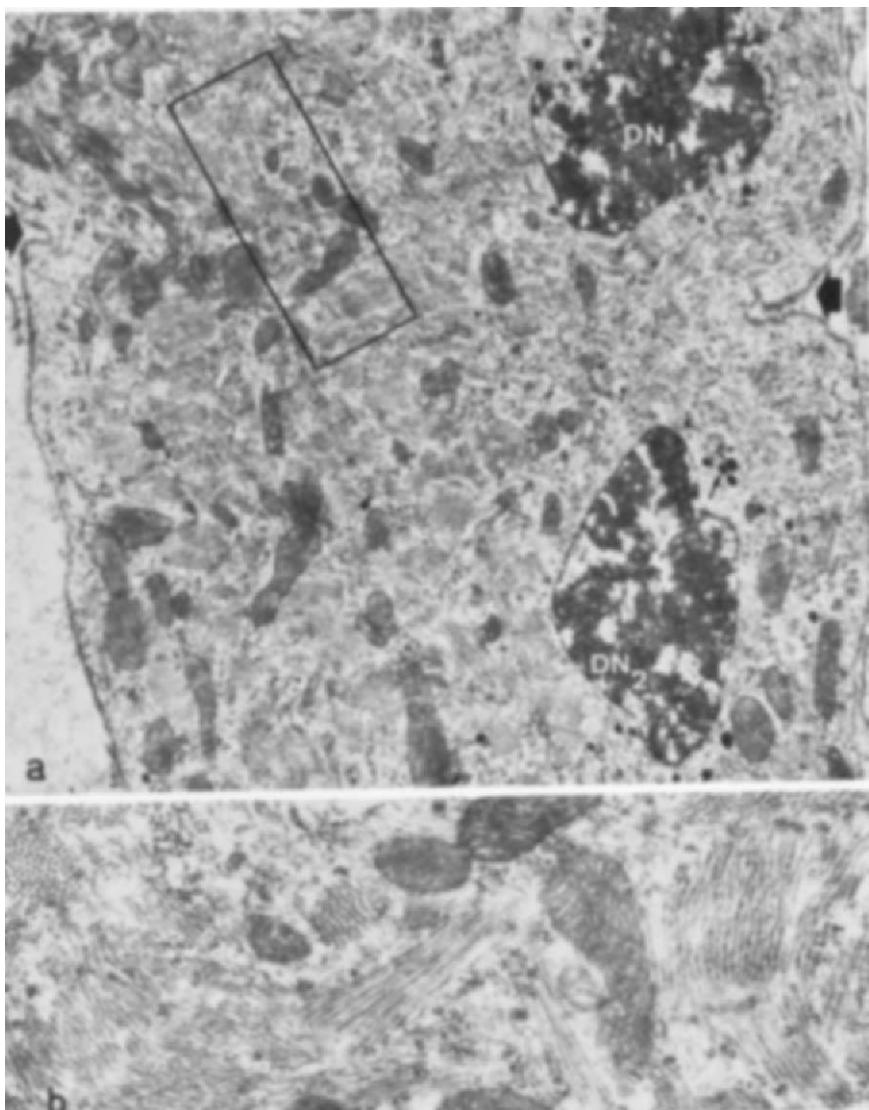


FIG. 31. (a) Portion of an early posttelophase myocyte from the left atrium of a rat 6 days after left coronary artery ligation. Thick arrows indicate the sites of an abortive cleavage furrow formation between both the daughter nuclei (DN₁ and DN₂). The small arrow points to the specific granules. $\times 9100$. (b) Detail of the framed area, showing the random distribution of isolated sarcomeres and myofilament bundles. $\times 26,000$. From Rumyantsev (1974).

DNA-synthesizing muscle cells in the atrioventricular system of rats following left ventricle infarction rises only to 2–5% as compared to about 1% observed in normal adult rats (Rumyantsev and Kassem, 1976). Occasional mitoses of specialized myocytes were found to occur under these experimental conditions. No evidence for the migration of replicating specialized myocytes and their conversion to working cardiac myocytes was obtained.

V. Neoplastic Transformation of Cardiac Myocytes

Unlike skeletal muscle, myocardium was rarely observed to be the source of true rhabdomyoma outgrowth, while rhabdomyosarcomas of cardiac muscle origin were identified only in several dozen cases (Mahaim, 1945; Goldberg and Steinberg, 1955; Saphir, 1960; Worton, 1949; Goodwin, 1968). Presumably this depends on the very low capacity of mature cardiac myocytes to proliferate (Holtzer and Sanger, 1972). It is interesting to note therefore that almost all true cardiac rhabdomyosarcomas were found in atria (Stoll and Lauer, 1955). This is consistent with the characteristic ease with which their myocytes reenter the mitotic cycle as compared to ventricular myocytes (see Section IV,D). Eisenstein and Wied (1970) believe that myocytes of the adult heart are protected from neoplastic transformation by the stable inhibition of mitosis but not of DNA synthesis, since mature myocardium displays numerous myocytes with 4C DNA nuclei.

VI. Proliferative Behavior of Cardiac Myocytes *in Vitro*

Many investigations concerned with cultured cardiac muscles have been repeatedly reviewed (Levi, 1934; Chlopin, 1946; Fell, 1952; Murray, 1965). Here only those relevant to the proliferative and differentiative behavior of cardiac myocytes *in vitro* are examined.

A. TISSUE AND ORGAN CULTURES

Myocytes of explants of early embryonic hearts can retain their striated myofibrils several weeks or months and continue to proliferate via mitosis (Olivo, 1923, 1926; Levi, 1934; Tsimal, 1937; Chlopin, 1946). Enrichment of the cultivated myofibers in sarcoplasm and their paucity of myofibrils were considered to represent moderate dedifferentiation in both orthodox histological (Olivo, 1926; Rumyantsev,

1927; Smirnova, 1927; Levi, 1934) and electron microscope studies (Weissenfels, 1962; Jonek and Stockinger, 1964). However, the degree of differentiation of myocytes of early embryonic hearts may increase in the absence of subculturing and at low doses of embryo extract (Hogue, 1947; Kotshetov, 1952; Engelbart, 1960; Weissenfels, 1962). Explants of myocardium of 25-day-old chickens (Olivo, 1929) and 10-day-old rabbits (Tsimbal, 1937) degenerated rapidly, but those of adult lower-vertebrate myocardium survived up to several months (Morozov, 1928; Rumyantsev, 1963b). In the latter case the myocytes underwent partial dedifferentiation and proliferated actively (Rumyantsev, 1963b), as they do after injury *in situ* (cf. Section IV,A). According to Grigoriyev and Shchelkunov (1974), explants of the adult rabbit myocardium may survive in a pure embryo extract, with sprouting of the myofibril-containing buds observed at their periphery.

The nature of cell outgrowth at the periphery of myocardial explants still remains obscure. The absence of myofibrils from these cells was the reason they were considered fibroblasts (Carrel, 1912) or dedifferentiated myocytes (Olivo, 1926; 1929; Levi, 1934; Tsimbal, 1937; Chlopin, 1946; Olivo and Lucchi, 1965a,b). The presence of thin filaments in the cytoplasm of outgrowing cells (Weissenfels, 1962; Olivo and Lucchi, 1965b) gives no conclusive evidence of their muscle origin, since similar filaments have been observed in a large variety of nonmuscle cells (Wessels *et al.*, 1971). Redifferentiation of the cells in the growth zone and the appearance of striated myofibrils were never observed.

B. CULTURES OF ISOLATED MYOCARDIAL CELLS

After the pioneering work of Cavanaugh (1955) numerous investigators cultivated isolated cardiac muscle cells obtained by trypsinization of embryonic and neonatal hearts to study different aspects of myocardial cytobiology.

The isolation of myocytes by trypsin digestion started with the disjoining of desmosomes and their invagination into the cytoplasm (Fischman and Moscona, 1969). Most myofibrils break down, giving rise to randomly distributed myofilament bundles (Kasten, 1966; Fischman and Zak, 1971; Etlinger and Fischman, 1973). The lack of sarcomeric organization explains the diffuse fluorescence of the cytoplasm of myocytes treated with antimyosin serum soon after trypsinization (Holtzer *et al.*, 1959). The persistence of a large amount of actomyosin after myofibril disaggregation is demonstrated biochemically as well (Harary and Farley, 1963).

The restoration of myofibrils proceeds asynchronously after the attachment of isolated cells to glass, lasting from several hours (Mark and Strasser, 1966) to 1–2 days (Rumery *et al.*, 1961; Cedergren and Harary, 1964; Rumery and Blanday, 1964). Despite the appearance of large (myosinic?) polysomes near regenerating myofibrils (Cedergren and Harary, 1964; Legato, 1972), sarcomerogenesis was not blocked by cycloheximide or a low temperature (4°C), which suggests self-assembly rather than protein synthesis as a basis of myofibril restoration (Fischman and Zak, 1971; Etlinger and Fischman, 1973). After the reassembly of myofibrils, isolated myocytes begin to contract, each with its own rhythm, which can be maintained for several weeks (Cavanaugh, 1955; Harary and Farley, 1960, 1963; Lehmkuhl and Sperelakis, 1963; De Haan, 1965). Adjacent myocytes can aggregate without fusion, forming voluminous myofibers, all cells of which contract with the same rhythm. At the points of contact of aggregating myocytes desmosomes and intercalated disks redifferentiate. The number of myofibrils increases gradually (Cedergren and Harary, 1964; Legato, 1972). Thus in a culture of isolated cardiac myocytes one can follow the entire sequence of events expected to take place in the injured myocardium *in situ* when regeneration occurs via free myoblast proliferation and differentiation. The absence of similar phenomena even in injured myocardium of lower vertebrates (see Section IV,A) indicates a nonmyoblastic proliferative response of cardiac muscle *in situ*.

Several investigators concluded that isolated cells in culture never divide mitotically if they contain myosin or striated myofibrils (Rumery and Blandau, 1964; Rumery and Rieke, 1967). It was later shown, however, that numerous dividing cells display *in vitro* well-organized myofibrils (Mark and Strasser, 1966; De Haan, 1967; Kasten, 1972; Pryzybylski and Chlebowski, 1972; Chacko, 1973; Goode, 1975).

Soon after trypsinization no more than 3% of isolated cultured myocytes containing such markers as myosin or glycogen incorporate thymidine-³H. This is in contrast with about 18% thymidine-³H labeled differentiated myocytes observed in embryonic chick hearts used for explantation (Goode, 1973, 1975; Polinger, 1973). The number of labeled myofibril-containing cardiac muscle cells cultured for 48 hours in the presence of thymidine-³H, rose to 75%. This was interpreted to be a result of the differentiation of premyoblasts or myogenic stem cells (Goode, 1973, 1975). In view of the absence of such elements from embryonic myocardium (Sections II and III), the above phenomenon may be more likely due to a blockage of replica-

tion in early explants (Manasek, 1973) and a subsequent outburst of myocyte proliferation (Agrell, 1965). In this context it should be interesting to study the possible depressing effect of trypsinization on membrane transport of thymidine, on the subsequent steps of its metabolism, on changes in endogenous thymidine content and so on.

The transient disaggregation of polysomes following trypsinization (Hosick and Strohman, 1971), indicative of penetration of the enzyme throughout the cell membrane, can also play a role in the temporary blockage of myocyte proliferation in early explants. Retrypsinization of cardiac myocyte cultures generally produces only myofibril-deprived cells (Murray, 1965), which may be due to the selection of more resistant nonmuscle cells.

Myofibrils of dividing cultured cells undergo the same cyclic changes as those observed *in situ* (see Section III). Z disks are destroyed in metaphase (Kasten, 1972, 1975), or even in prophase, and restored after mitosis parallel to the reassembly of myofibrils (Goode, 1975). Kelly and Chacko (1976) concluded, however, that in the cultured myocytes from the embryonic chick hearts Z-disks disaggregation, sarcomere dislocation and cessation of myocyte rhythmical beating may not be the prerequisites for completion of mitosis and cytokotomy.

The proliferative behavior of isolated myocytes in cultures obtained from the adult animal hearts using trypsin and collagenase digestion (Vahouny *et al.*, 1970; Glick *et al.*, 1974) remains to be studied. Such an approach seems to be promising for further investigation of the dedifferentiative and regenerative potentialities of mature myocardia.

VII. Concluding Remarks

The evidence compiled in this article permits the conclusion that, after the transformation of splanchnomesoderm into a primitive heart is over, cardiac myogenesis proceeds at the expense of myofibril-containing myocytes until a certain critical degree of differentiation is achieved and withdrawal from the mitotic cycle is completed (Rumyantsev and Sokolovskaya, 1964). Unlike the situation in skeletal myogenesis, morphologically undifferentiated myoblasts are absent or do not play significant role at most stages of embryonic and postnatal myocardial development (cf. Manasek, 1973).

Myocytes of the developing heart are seemingly the most structurally specialized cells capable of dividing without sharp dedifferentiation. This capacity appears to be due to at least two major permissive modifications of the cardiac myocyte mitotic cycle. One of them is a

gradual lengthening of the cycle phases in the course of progressive cardiac myocyte differentiation, probably allowing for the completion of all syntheses and intracellular morphogenetic events required for division. Another, more specific, modification is mitosis-dependent Z-disk degradation. The resulting release of myofilament bundles may facilitate their roughly equal distribution between daughter cells, as well as the chromosomal movement and cytotomy. Myofibril restoration was found to be carried out in the G₁ period.

The nonsyncitial nature of vertebrate myocardium results from a repeated series of myocyte cytokineses, the daughter muscle cells being incapable of fusion, unlike the progeny of dividing skeletal myoblasts. Differentiation of the majority of intercalated disks appears to be related to processes accompanying cytokinesis.

At later developmental stages the increased degree of myocyte differentiation interferes progressively with proliferation. The blockage of cytotomy leads to the accumulation of binucleate cardiac myocytes. Disturbances of the normal mitotic cycle may underlie the increase in the ploidy of some of the myonuclei.

The specific features of cardiac muscle regenerative and compensatory reactions are largely due to (1) the absence of a preexisting pool of myogenic cells (comparable, for instance, to Mauro's satellite cells in skeletal muscle) form myocardia of all vertebrates studied, and (2) the incompleteness of myocyte dedifferentiation in the reactive states and the lack of true myoblast sequestration. Such conditions appear to be most unfavorable for a hyperplastic response of "static" cardiac myocytes to lesions or overloading.

Unexpectedly, the myocytes of lower vertebrate hearts and those of adult mammalian auricles proved to possess a pronounced capacity for reactivation of DNA synthesis and mitosis. Therefore these kinds of cardiac myocytes at least should be classified not as elementi perenni or cells of static populations but as "reversible postmitotic cells" (cf. Cowdry, 1942) or cells of "expanding" populations (Leblond, 1964), comparable to hepatocytes, and so on. Even adult mammalian ventricular myocytes displaying the most rigid restraints regarding resumption of DNA synthesis and mitosis cannot be defined without being stipulated as cells of a true static population. Unlike neurons, a limited percentage of these myocytes may overcome these restraints and pass through all phases of the mitotic cycle. If the increase in DNA content characteristic of the myocytes in overloaded primate hearts is due to the replication of all genes but not to the amplification of some or to myonuclei fusion, this phenomenon is then another example of DNA synthesis reactivation in mammalian ventricular muscle cells. It

seems probable that the relative ease with which the mitotic cycle is reactivated in the myocytes of lower vertebrate hearts and atria of mammals depends on their less differentiated state compared to that of mammalian ventricular muscle cells.

The dualism of the cardiac myocyte displaying both muscle and epithelial cell phenotypes (Manasek, 1973) is clearly manifested in its proliferative behavior under normal and reactive conditions. Similar to epithelial cells (cf. Goss, 1967), developing or reactivated cardiac myocytes proliferate without profound dedifferentiation, remaining attached to their nondividing counterparts by means of desmosomes or intercalated disks.

The nonmyoblastic proliferative response of moderately dedifferentiated myocytes may call for derepression of genes coding for the synthesis of DNA polymerase, thymidine kinase, and so on. It is of interest therefore to measure the activity of these enzymes during mitotic outbursts in postinfarction atria.

Undoubtedly the reactivation of intensive thymidine-³H incorporation into myonuclei of lower-vertebrate ventricles or those of rat atrium following, respectively, traumatization or infarction of the ventricular myocardium cannot be explained as a result of the enhanced synthesis of so-called metabolic DNA, reported by several workers in the cardiac myocytes of adult animals (Pelc, 1964; Cameron and Hoage, 1976). Thymidine-³H incorporation claimed to be typical of metabolic DNA synthesis is extremely low (hardly above the background level) and is not followed by mitosis. In this respect the situation observed in the rat atria after ventricular infarction or in injured lower vertebrate myocardium is quite different. It is worth mentioning that Burholt and his associates (1973) give evidence against the existence of metabolic DNA synthesis.

Complicated interrelationships of the proliferation and differentiation processes observed in the development and regeneration of cardiac and smooth muscles dispel the idea that they are mutually exclusive (Peter, 1940; Rush, 1954). Unlike skeletal myogenesis, the intrinsic antagonism of these processes is manifested in cardiac muscle not by the inhibition of replication just after the onset of differentiation but by the hindrance of progressively differentiating myocyte division which increases gradually from cycle to cycle.

Typical features of cardiac myogenesis and the regeneration are difficult to interpret from the viewpoint of the quantal mitosis theory (cf. Weinstein and Hay, 1970). According to this concept the mitotic cycles of differentiating cardiac myocytes (so-called proliferative or postquantal cycles) are very limited in number, possibly because of

the irreversible switching-off of DNA polymerase synthesis following quantal mitoses (Holtzer and Sanger, 1972). At variance with the quantal mitosis theory we see that proliferative cycles may provide for cardiac myogenesis after its earliest, BUdR-sensitive stages (see Section III). Moreover, these cycles are easily reactivated in some types of terminally differentiated cardiac myocytes (Section IV).

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