Chapter 18

CARDIAC MUSCLE AND ITS POTENTIAL FOR REGENERATION IN THE ADULT NEWT HEART

JOHN O. OBERPRILLER, JEAN C. OBERPRILLER,
DAVID M. BADER, AND TIMOTHY M. MCDONNELL

ONE OF THE MOST SIGNIFICANT health problems facing medical research is that of the myocardial infarct. In order to adequately examine experimental approaches to this problem, it is essential that one understand the proliferative and regenerative capacities of the cardiac myocyte. Surpisingly little work has been done in this area, presumably because the adult mammalian ventricular myocyte appears to have little or no capacity to respond to injury by a proliferative response (24, 25, 30, 39). Since the demonstration by Overy and Priest (23), Rumyantsev and Snigirevskaya (33), Manasek (13), and Weinstein and Hay (38) that a developing cardiac myocyte, containing well-differentiated products such as cytoplasmic myofilaments, may undergo cell division, a number of systems have been exploited for the study of the dividing myocyte. These include both *in vitro* and *in vivo* studies on developing systems (30). Regenerative responses have been studied in mammals by Rumyantsev (27, 29) and Rumyantsev and Kassem (31) and in lower vertebrates *in vivo* in the frog by Rumyantsev (26, 27, 28), Rumyantsev and Schmantzar (32), and Niweliński et al. (19) and in the newt by Oberpriller and Oberpriller (21, 22), Bader and Oberpriller (1, 2), Oberpriller et al. (20), and Becker et al. (3). *In vitro* studies have been performed on frog (30) and larval salamander hearts (16), and recently on the adult newt heart by Nag et al. (17, 18).

This chapter will begin with a brief discussion of the morphology of the newt heart, followed by a discussion of two major types of experiments we have used to examine the regenerative capabilities of the heart of the adult newt, *Notophthalmus viridescens*. In the first type of experiment, we examined the response of the newt heart to ventricular amputation, with emphasis on a study of its proliferative capacity. In the second type of experiment, we examined the characteristics of small miniventricles that form from grafts of minced ventricle placed on the amputation surface of a ventricle, with an emphasis on a study of their proliferative and histogenetic capacities.

Morphology of the Newt Heart

The general gross morphology of the salamander heart has been well described by Davies and Francis (4). The newt heart is a three-chambered

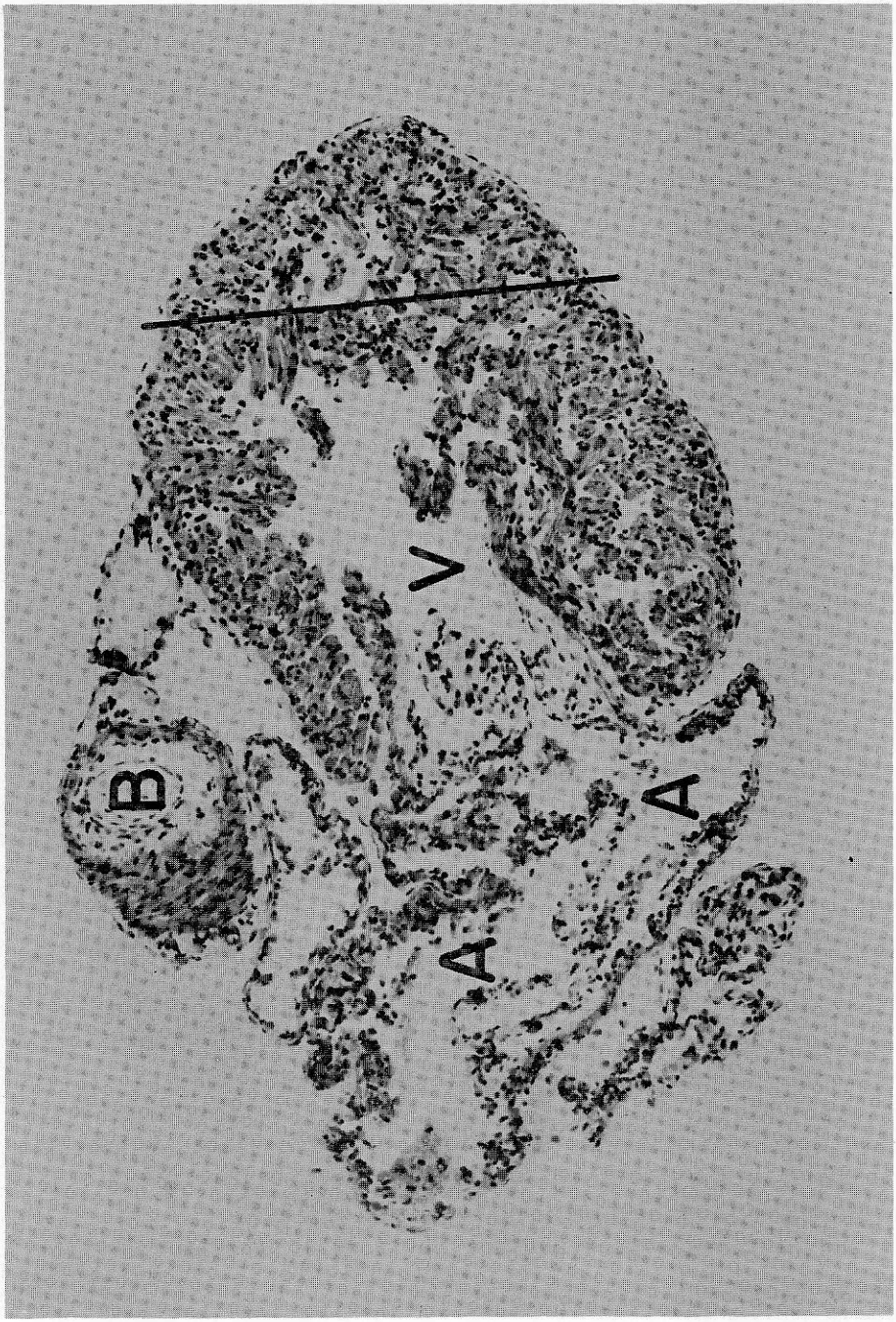

Figure 18-1. Section through the adult newt heart with the two atria (A), the bulbus cordis (B), and the ventricle (V). The line demarcates the approximate amputation plane through the ventricle that was used in the two experimental series. ×95.

heart consisting of two atria, separated by an interatrial septum and a single ventricle, which empties into the bulbus cordis (Fig. 18-1). The atria are composed of a thin wall with only occasional trabeculae extending into the lumen. The atrial wall consists of three major compartments. The endocardium is composed of an endothelium underlain by a very thin connective tissue layer. The myocardium or muscle compartment consists almost exclusively of myocytes. The epicardium consists of a mesothelium and a relatively thick connective tissue layer.

The histology and ultrastructure of the adult amphibian ventricle has been described by a number of investigators (4, 7, 9, 10, 14, 35, 36). The newt ventricle is avascular and consists of a thin outer wall with a meshwork of trabeculae extending into the lumen of the ventricle (Fig. 18-1, 18-2). As in *Necturus* (9), the myocardium or muscle compartment of the outer wall and trabeculae contains few fibroblasts and is composed primarily of myocytes. The muscle compartment consists of two types of myocytes, light and dark, as viewed with the electron microscope (Fig. 18-3). The dark myocytes have a more dense cytoplasm and more myofibrillae than light myocytes (Fig. 18-4). The significance of light and dark myocytes is unknown. However, Hirakow (9), who also described these cells in *Necturus,* suggests that the light myocytes may possibly function in conduction.

The ultrastructure of the ventricular myocytes reveals that they have a basal lamina and routinely contain glycogen, dense granules, pinocytotic vesicles, subsarcolemmal cisternae, and a number of myofibrillae that, as in other forms, are not always distinctly separated. The sarcoplasmic reticulum and Golgi are rather sparse. The myocyte sarcolemma does not have transverse tubules (11). However, it has a number of projections extending from the cell (18) that often interdigitate with neighboring myocytes. At the junction between light and dark myocytes, the dark cells may have narrow projections, which invaginate the light cells at the level of the Z and H bands. Intercalated disc junctions are not as elaborate as observed in mammals and the major junctions appear to be desmosomal in nature (Fig. 18-4). With routine transmission electron microscopy, we have not positively identified gap junctions in the newt ventricle. However, they have been identified in the *Necturus* ventricle by Hirakow (9) and by the freeze fracture method in the *Ambystoma* ventricle by Mazet (15).

The internal surface of the ventricle wall and trabeculae is covered by endocardium. Beneath the endothelium, there is a relatively small amount of connective tissue containing a considerable number of cells with granules that stain metachromatically with toluidine blue and that are probably mast cells (Fig. 18-4). The epicardium consists of a mesothelium underlain by a thicker connective tissue layer than that of the endocardium.

Figure 18-2. Scanning electron micrograph of ventricular trabeculae that project into the ventricular lumen. The bulges on the surface of the trabeculae represent endothelial nuclei. ×410.

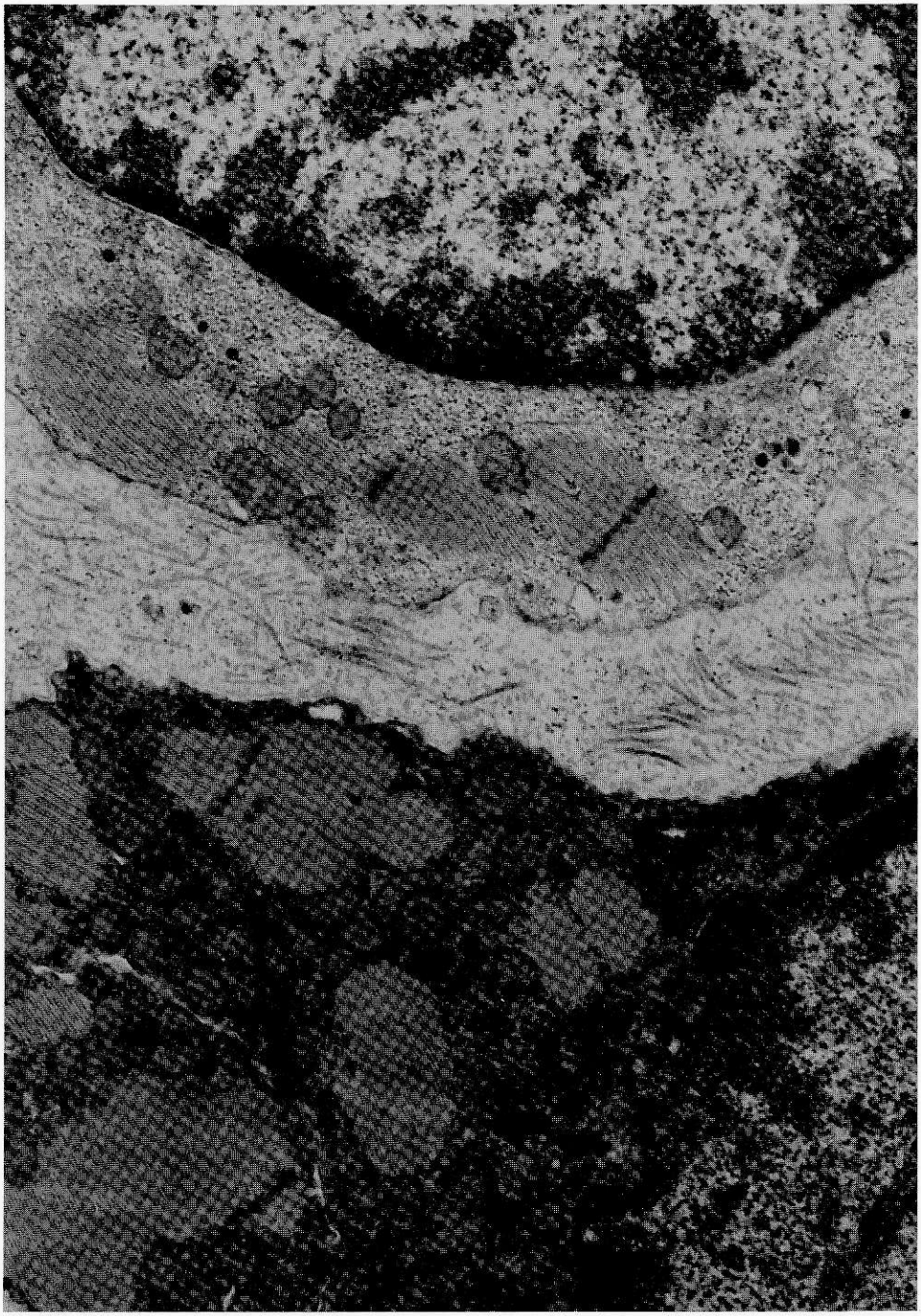

Figure 18-3. Electron micrograph illustrating dark and light ventricular myocytes. $\times 14{,}000$.

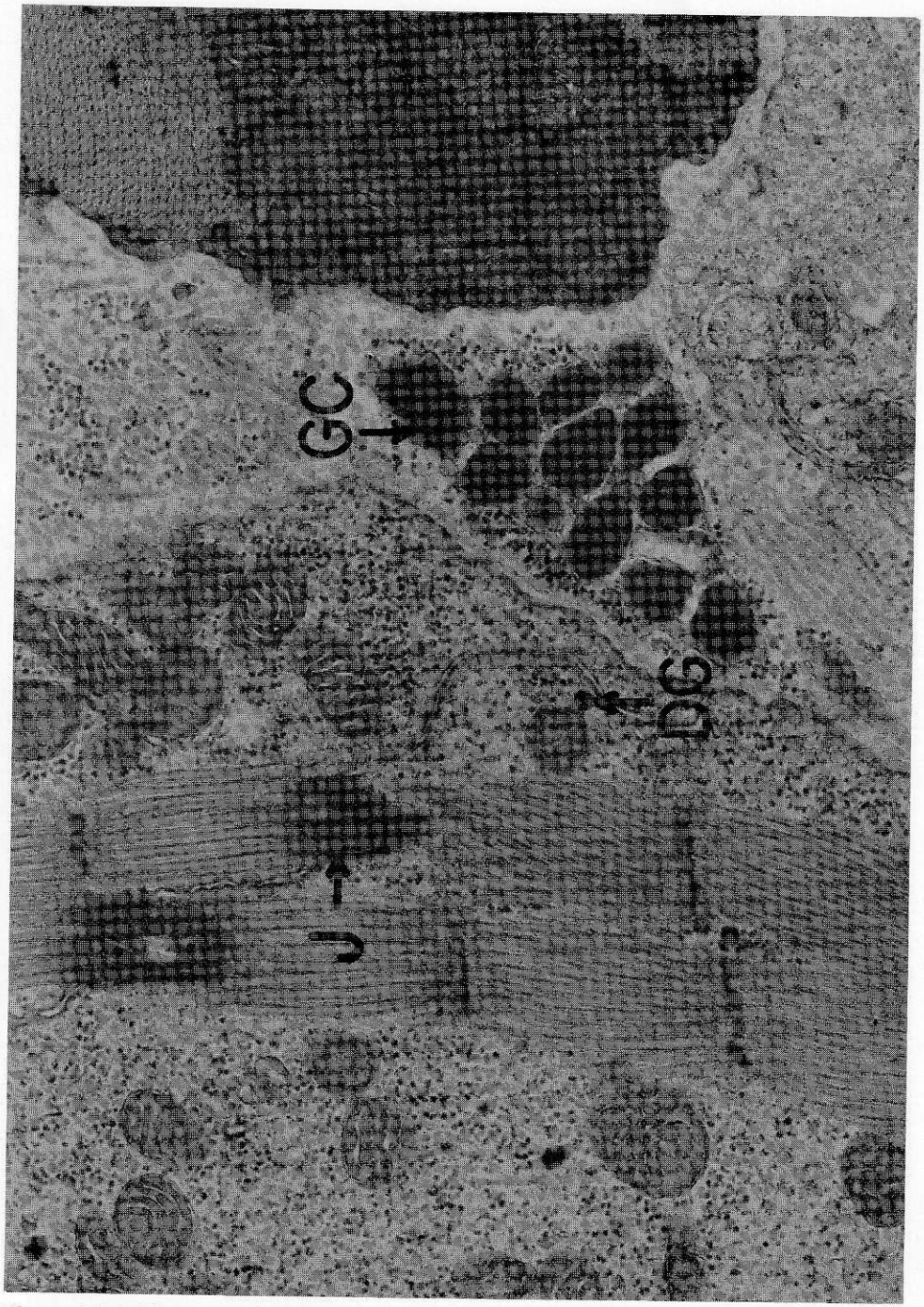

Figure 18-4. Electron micrograph of portions of dark and light ventricular myocytes. A portion of a granule cell (GC) can be observed between the myocytes. In this section, the characteristic features of the myocytes, such as desmosomal type junctions (J), dense granules (DG), and glycogen can be readily observed. ×26,000.

Amputation of the Apical Portion of the Newt Heart

In the ventricular amputation experiments, the apical region of the ventricle was amputated approximately at the region indicated by the line in Figure 18-1. After amputation, the ventricles were studied at various intervals in order to determine cytological and proliferative changes at both the light and electron microscopic levels. Autoradiographic techniques were utilized for the determination of DNA synthetic patterns at both light and electron microscopic levels. The animals used in the autoradiographic studies were given single injections of tritiated thymidine one to three hours prior to fixation. Details of these techniques have been reported previously (21, 22).

During the first week after amputation, the major events are clot formation and degeneration in the trabecular cells immediately adjacent to the plane of amputation. Immediately after amputation of the ventricular apex, the wound was closed by a blood clot that can be seen one hour after amputation in Figure 18-5. The area of this blood clot is also evident in the ventricle five days after amputation (Fig. 18-6). Cellular degeneration and necrosis occurs in both the blood clot and in the trabecular cells adjacent to the amputation area, as seen in Figure 18-6 at five days after amputation. During this period of degeneration, which is prominent into the second week after amputation, macrophages could be observed in the necrotic areas of the trabeculae (Fig. 18-7) and in the area of the blood clot. Myocytes within this necrotic area of the trabeculae contained a considerable amount of lipid and the myofibrillae were observed in various stages of disarray. Except for myocytes, which were in extreme phases of necrosis, the stage at which the process of degeneration was still reversible was difficult to determine. During this same time period, the blood clot area becomes covered on the inside first with a migrating endothelium and slightly later on the outside with a mesothelial covering (Fig. 18-8). As the mesothelial cells of the epicardium migrate over the clot, the cells take on a variety of shapes and many have numerous microappendages and processes (Fig. 18-9). The exact nature of the stimulus and the nature of migration of these cells is not known.

Beginning in the second week after amputation and continuing into the second month, DNA synthesis and mitosis was observed in the muscle compartment of the trabeculae and wall adjacent to the blood clot (Figs. 18-10, 18-11). Ventricular DNA synthesis in the muscle compartment has not been observed in uninjured or sham-operated control animals. The DNA synthetic and mitotic activity in this compartment reached a peak in the third week after amputation (Table 18-I). This proliferative activity appeared to be a localized response to amputation and generally did not extend much beyond 300 μm from the amputation site. In order to determine whether labeled or mitotic cells such as those seen in Figure

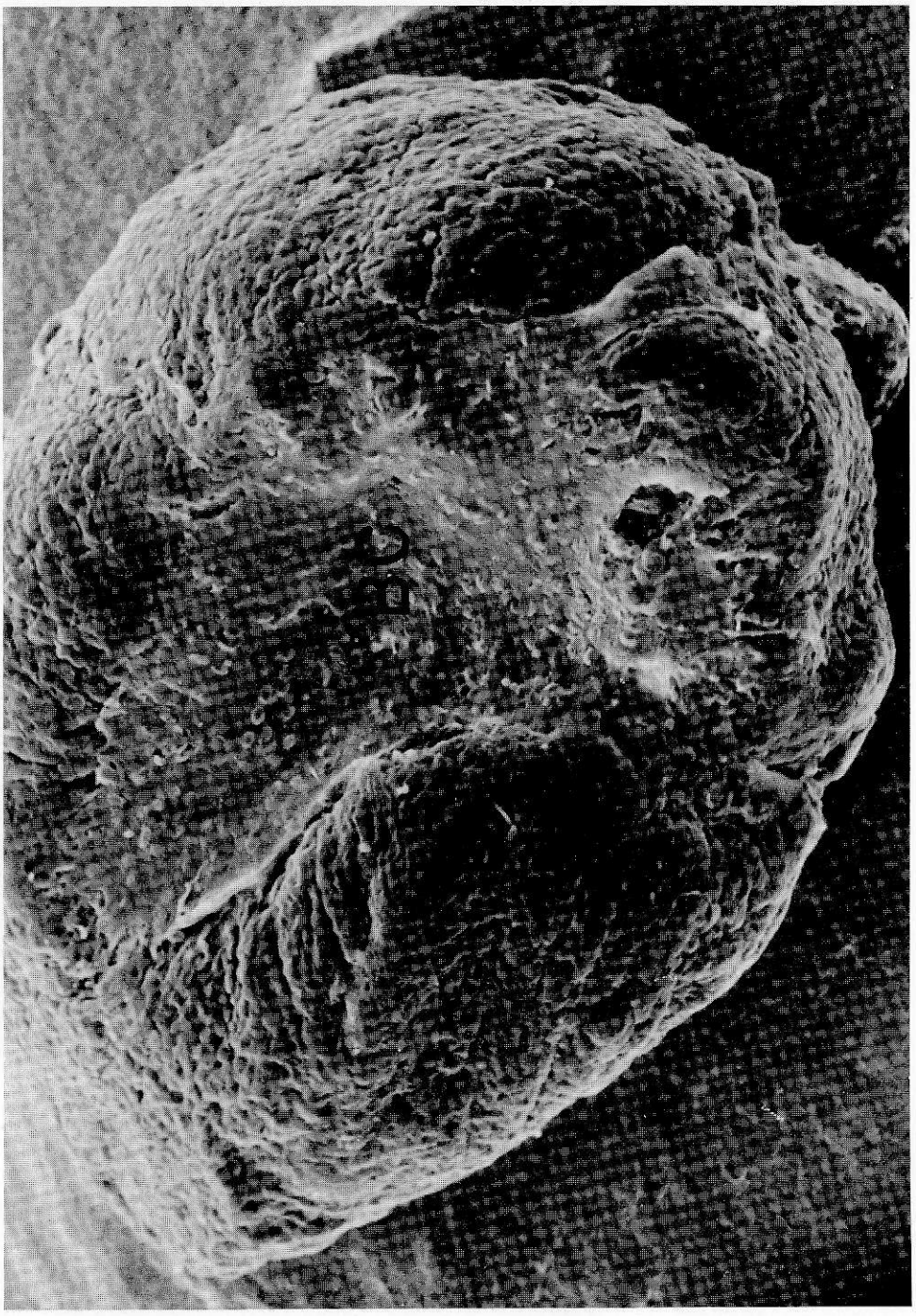

Figure 18-5. Scanning electron micrograph of a ventricle one hour after ventricular amputation. The area in the center is the region of the blood clot (BC) that forms immediately after amputation. ×120.

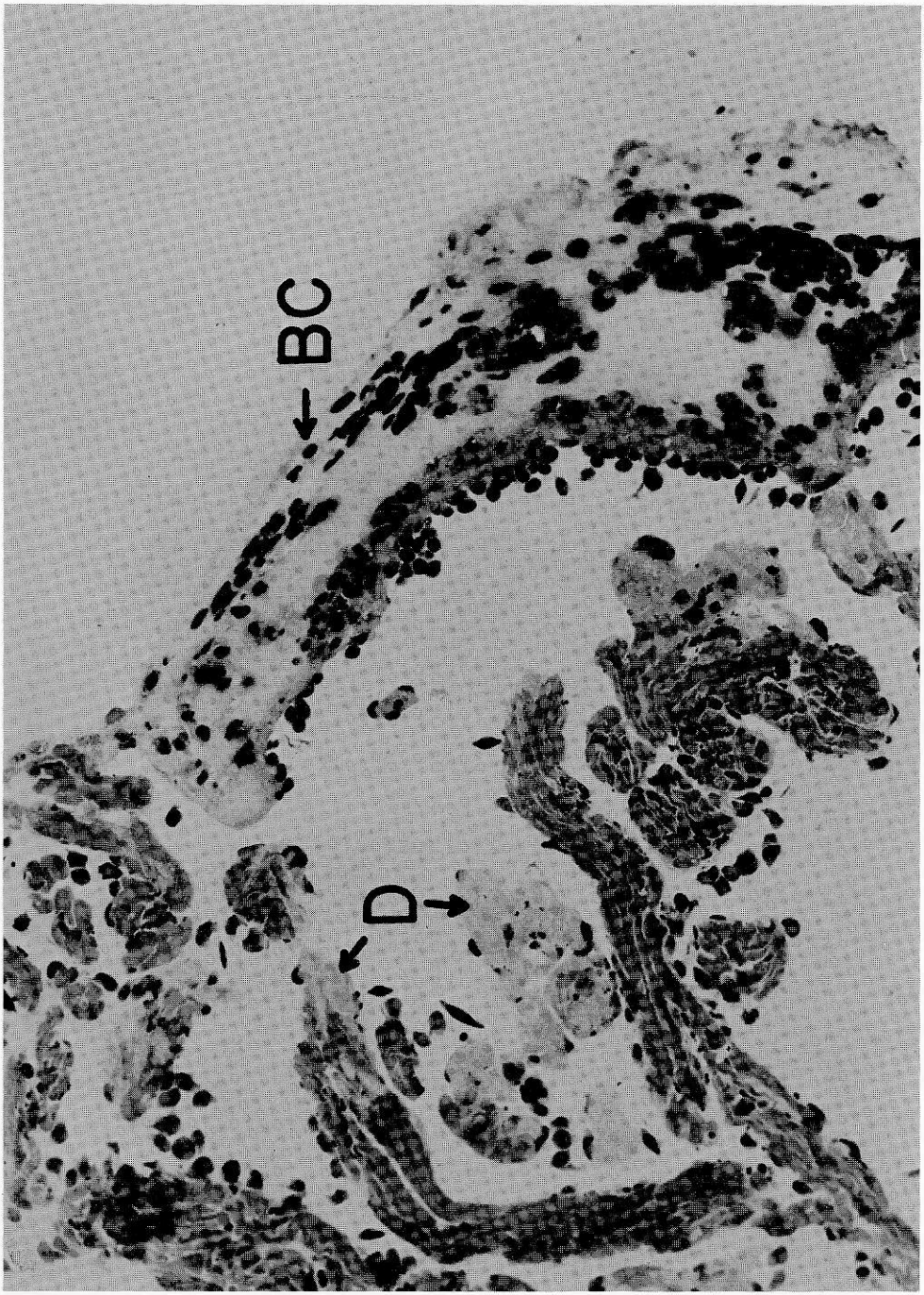

Figure 18-6. Light micrograph of a ventricle five days after amputation showing a portion of the blood clot (BC) that forms across the amputation gap and trabeculae that are undergoing partial degeneration (D) in the area immediately adjacent to the amputation zone. ×200.

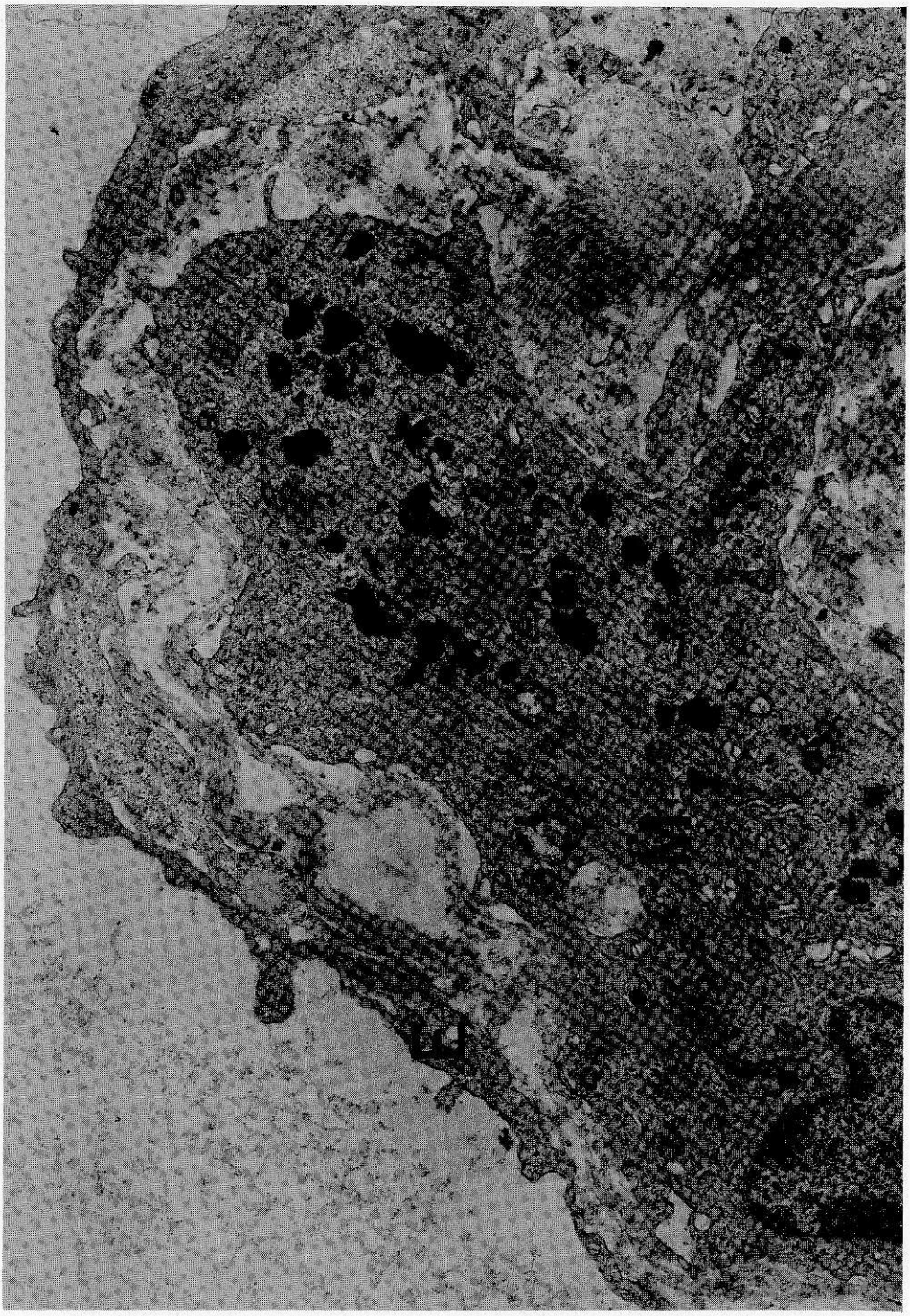

Figure 18-7. Electron micrograph of a portion of a degenerating trabecula six days after amputation. A macrophage (M) can be observed beneath the endothelium (E). ×8,300.

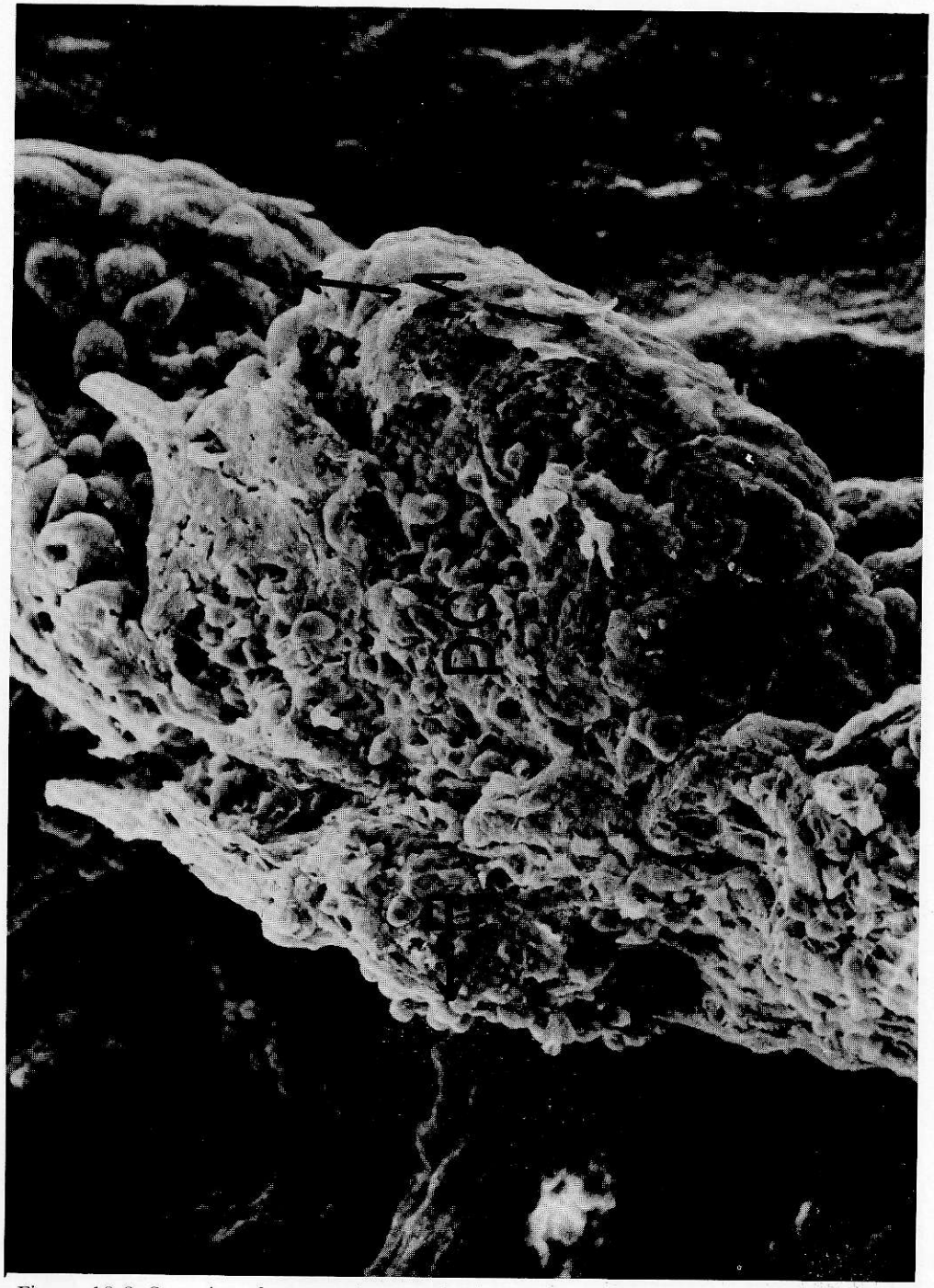

Figure 18-8. Scanning electron micrograph of a sectioned degenerating blood clot (DC), which spans the amputation gap at ten days after amputation. At this time, endothelial cells (E) cover this area on the inside and mesothelial cells (M) of the epicardium can be observed on the outer surface of the clot. ×350.

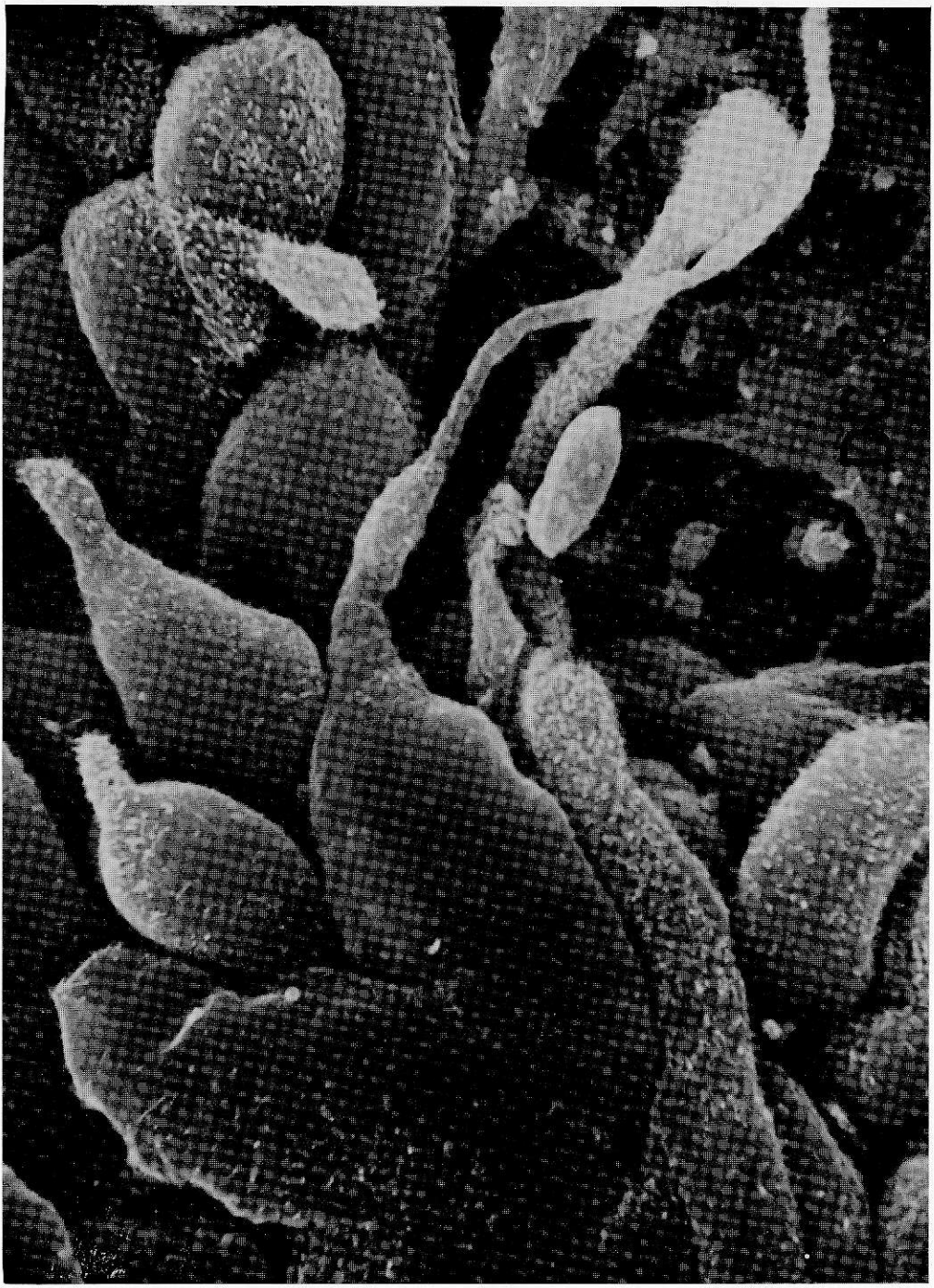

Figure 18-9. Scanning electron micrograph of mesothelial cells, which appear to be migrating over the degenerating blood clot (DC) area at ten days after amputation. The mesothelial cells appear to be very active, as seen by the processes and many microappendages on the surface of the cells. In one cell, the structures evident beneath the surface are typical of cells that have a phagocytic nature. ×1,800.

TABLE 18-I
THYMIDINE INDICES OF CONTROL AND AMPUTATED VENTRICLES*

Group	*Thymidine Index*
Control	0.00 ± 0.00
5 Day	0.00 ± 0.00
10 Day	1.7 ± 0.85
15 Day	5.2 ± 1.41
20 Day	4.1 ± 0.5
25 Day	2.4 ± 0.42

* This table indicates percentages and standard deviations of trabecular cells which have labeled nuclei. In the experimental groups, cell counts were made in the area extending up to 130 μM from the wound margin. At least 1,000 cells, excluding blood and endocardial cells, were counted for each of two animals in each control and experimental group. Each animal used for this study was injected intraperitoneally with 2.5 μC[1] of tritiated thymidine and fixed for light microscopic autoradiography one hour after injection.

18-11 at the light microscopic level are myocytes, sixteen and twenty day postoperative ventricles were studied by using the technique of electron microscopic autoradiography. From these studies, it was found that between 80 and 85 percent of the labeled cells of the muscle compartment were myocytes, in that these cells contained myofibrillae and glycogen and possessed a basal lamina and often desmosomal cell junctions.

In Figure 18-12, a portion of a myocyte is seen with a labeled nucleus and a desmosomal cell junction. The myofibrillae within this cell appear similar to myofibrillae observed in control myocytes. Myocytes observed in the mitotic phases of cell division appear to have undergone considerable changes from cells in DNA synthesis, especially in regard to the myofibrillae. In prophase (Fig. 18-13), one can observe myofibrillae with intact Z bands. However, these bands appear to be somewhat less prominent and the myofibrillae appear to be undergoing disassociation. At a later stage of mitosis (Fig. 18-14), the myofibrillae appear to have undergone more disassociation, often appearing to be separated as sarcomeric units. However, in cross section, even these apparently separated myofibrillar units contain both thick and thin filaments (as can be seen in Fig. 18-14).

Most often, Z bands are not observed in metaphase cells. However, Z bands are occasionally seen in some metaphase cells (Fig. 18-15). This observation that many Z bands are lost in mitotic myocytes following prophase has been made for the adult frog (28), cultured chick heart (5), the chick heart *in vivo* (8), and the adult newt heart. It seems possible that Z band degradation may be necessary for cytokinesis in these cells. However, Kelly and Chacko (12) have demonstrated an extensive number of Z bands present in the mitotic myocytes of cultured chick heart. Thus it appears that the extent of Z band breakdown necessary for cytokinesis is not well understood. Mitotic myocytes remain attached to neighboring myocytes as they progress through cell division (Fig. 18-15). Although we have not

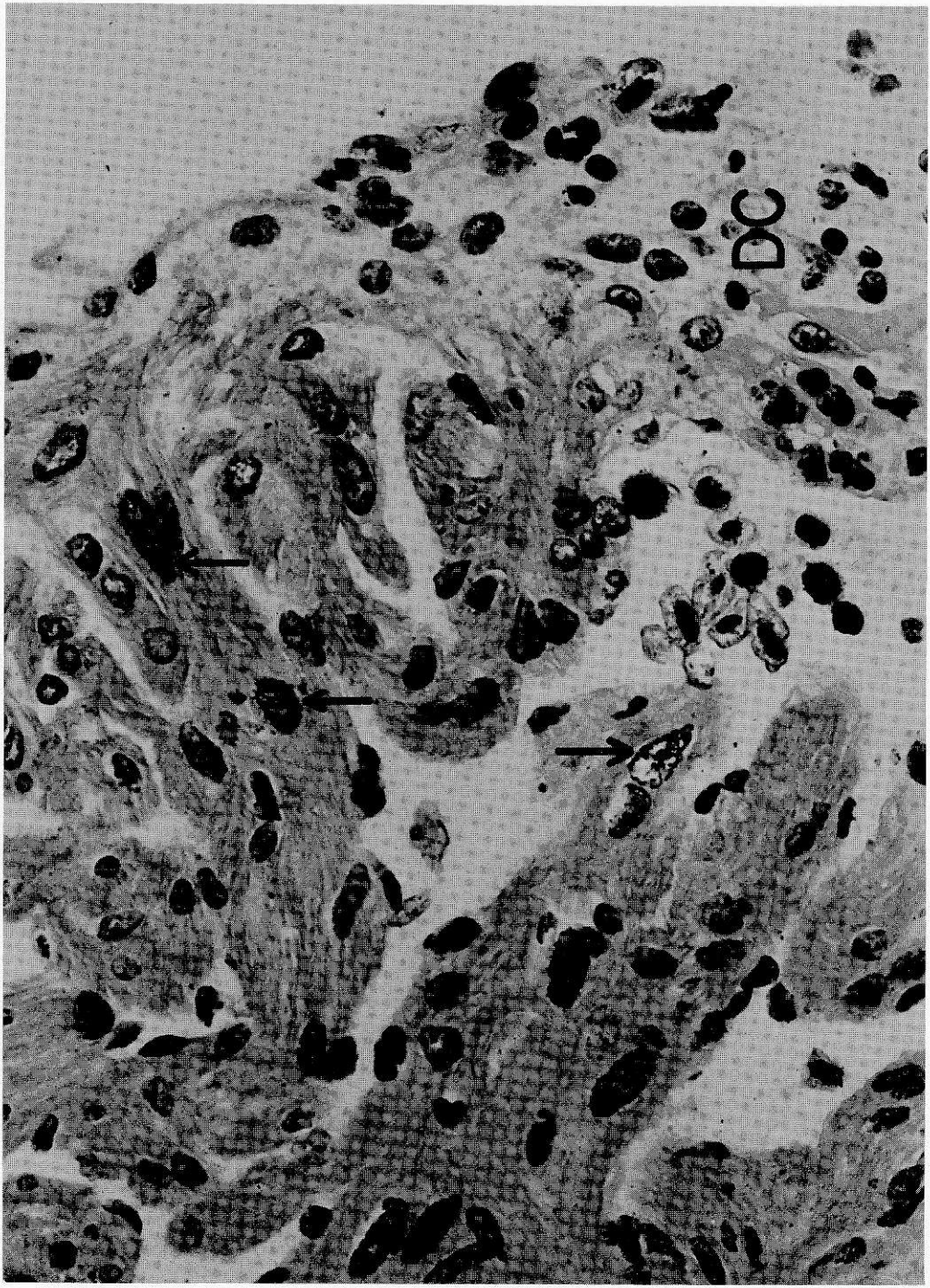

Figure 18-10. Light microscopic autoradiograph of a ventricle twenty days after amputation. A mitotic cell and labeled nuclei, indicative of DNA synthesis (arrows), can be observed in the trabeculae adjacent to the region of the degenerating blood clot (DC). ×200.

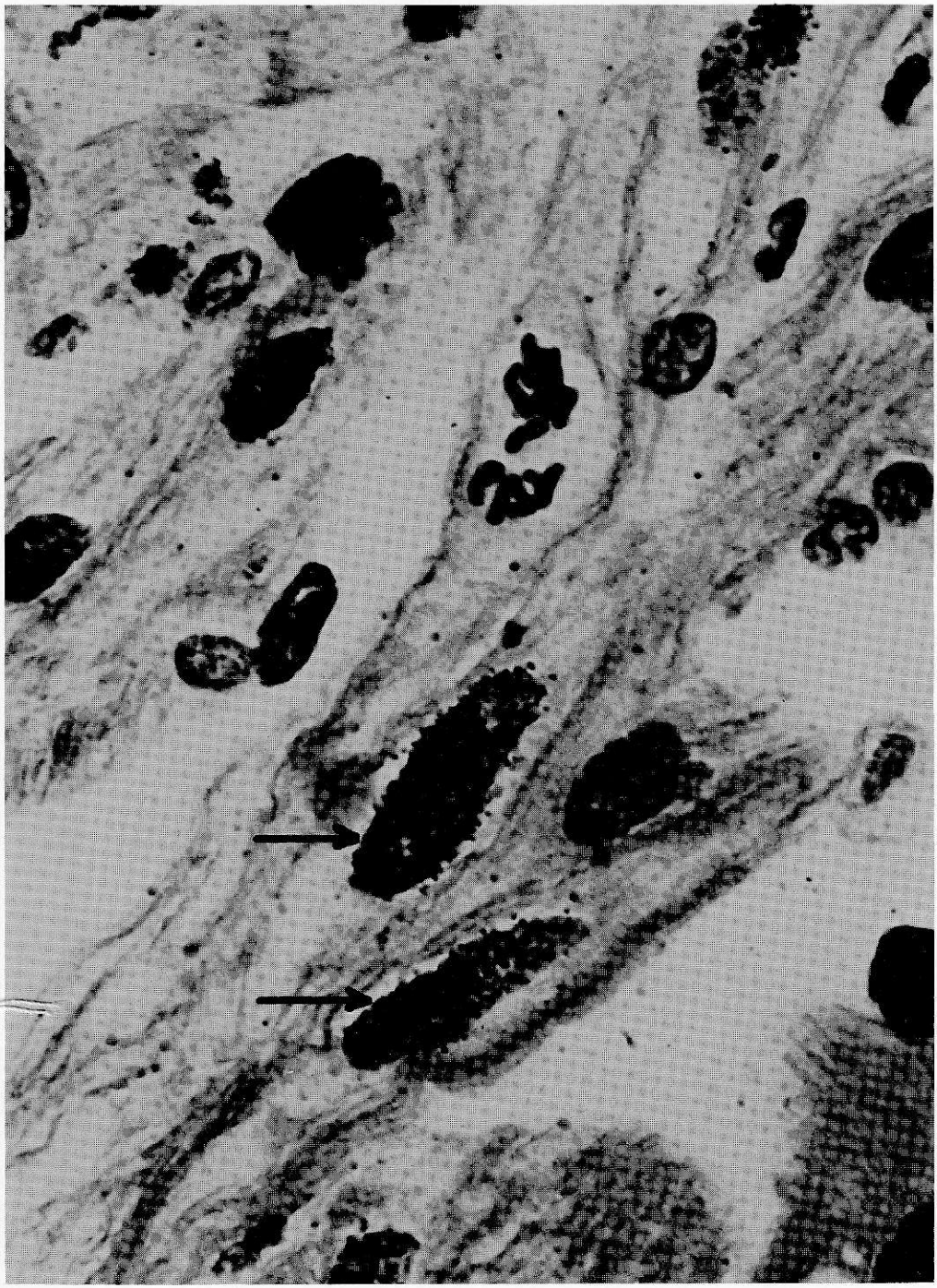

Figure 18-11. Light microscopic autoradiograph of trabeculae adjacent to the amputation site 20 days after amputation. Two labeled nuclei (arrows) and a mitotic cell can be observed within a trabecula. ×1,400. (From J. O. Oberpriller and J. C. Oberpriller, *J. Exp. Zool, 187*:249-206, 1974. Courtesy of the Wistar Institute, Philadalphia.)

Figure 18-12. Electron microscopic autoradiograph twenty days after amputation. A portion of a cell is seen with a labeled nucleus (N), indicating DNA synthesis, and myofibrillae (MF). × 17,000. (From J. O. Oberpriller and J. C. Oberpriller, *J. Exp. Zool.*, *187*:206, 1974. Courtesy of the Wistar Institute, Philadelphia.)

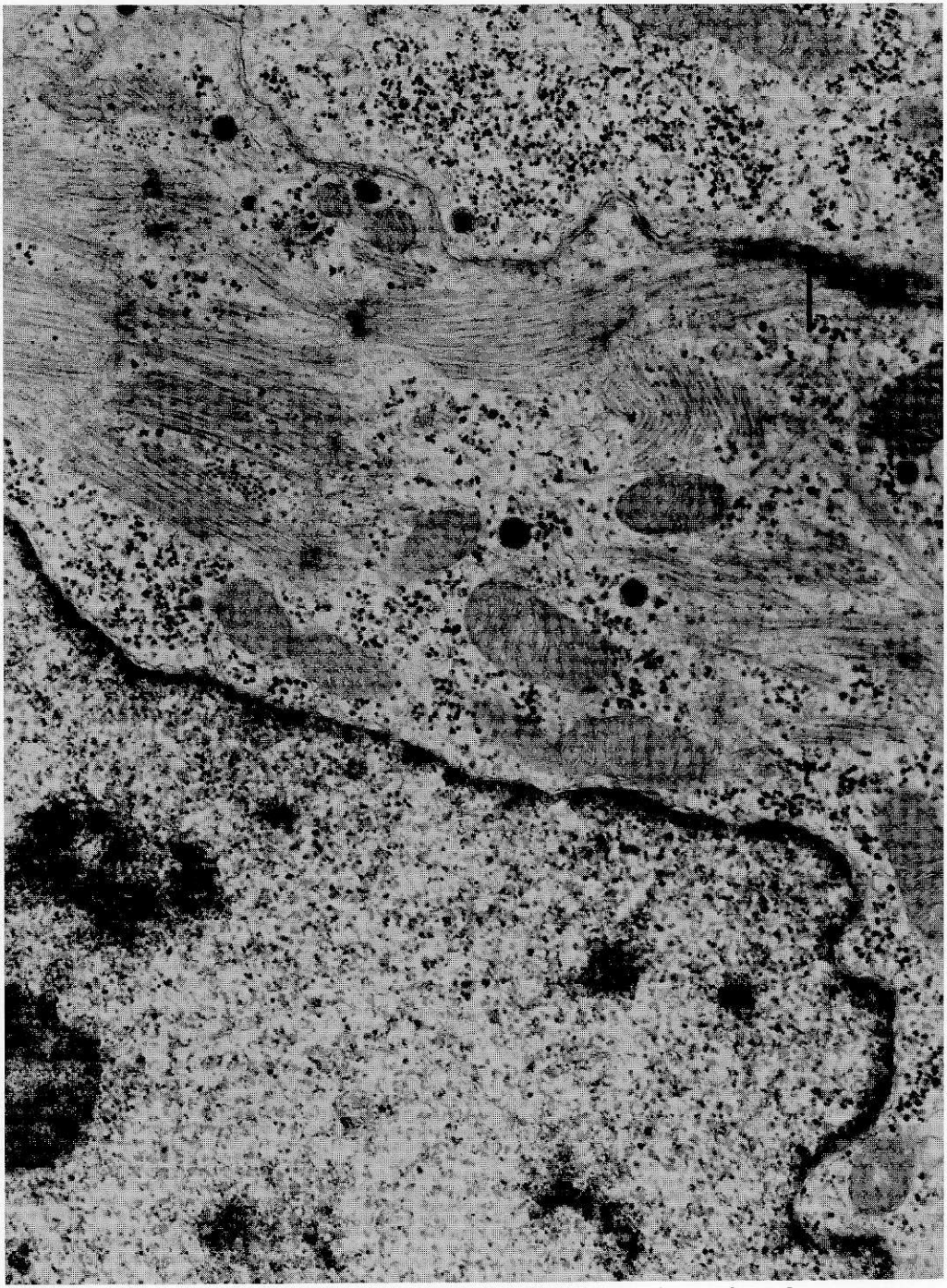

Figure 18-13. Electron micrograph of a myocyte in prophase at sixteen days after amputation. The myofibrillae of this cell appear to have somewhat less structural integrity than those observed in control myocytes. However, they still possess numerous but somewhat smaller Z bands. A desmosomal cell junction can also be seen (arrow). ×24,000.

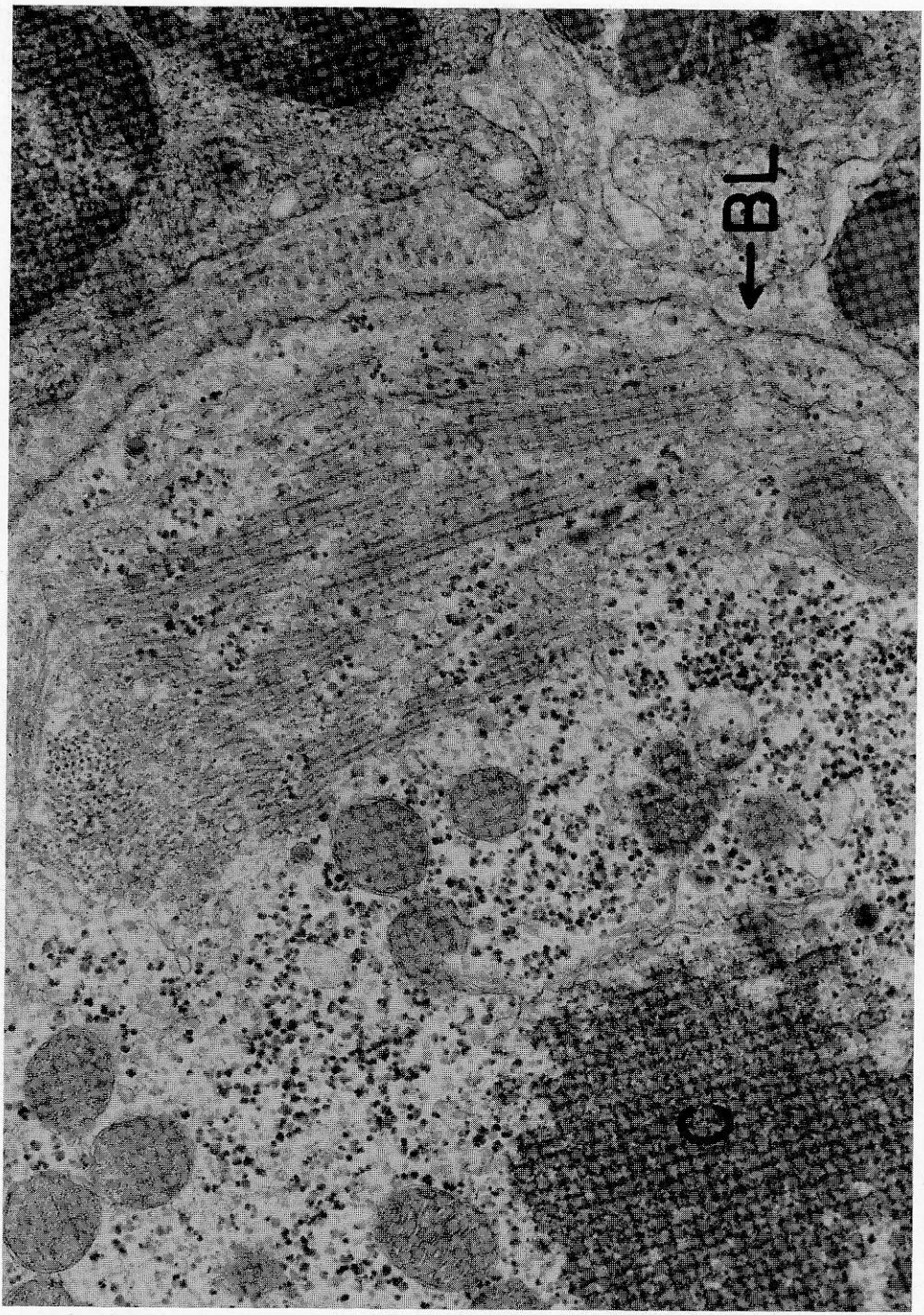

Figure 18-14. Electron micrograph of a mitotic myocyte at sixteen days after amputation. This myocyte contains chromosomes (C), dense granules, glycogen, basal lamina (BL), and myofibrillae with both thick and thin filaments. ×28,000.

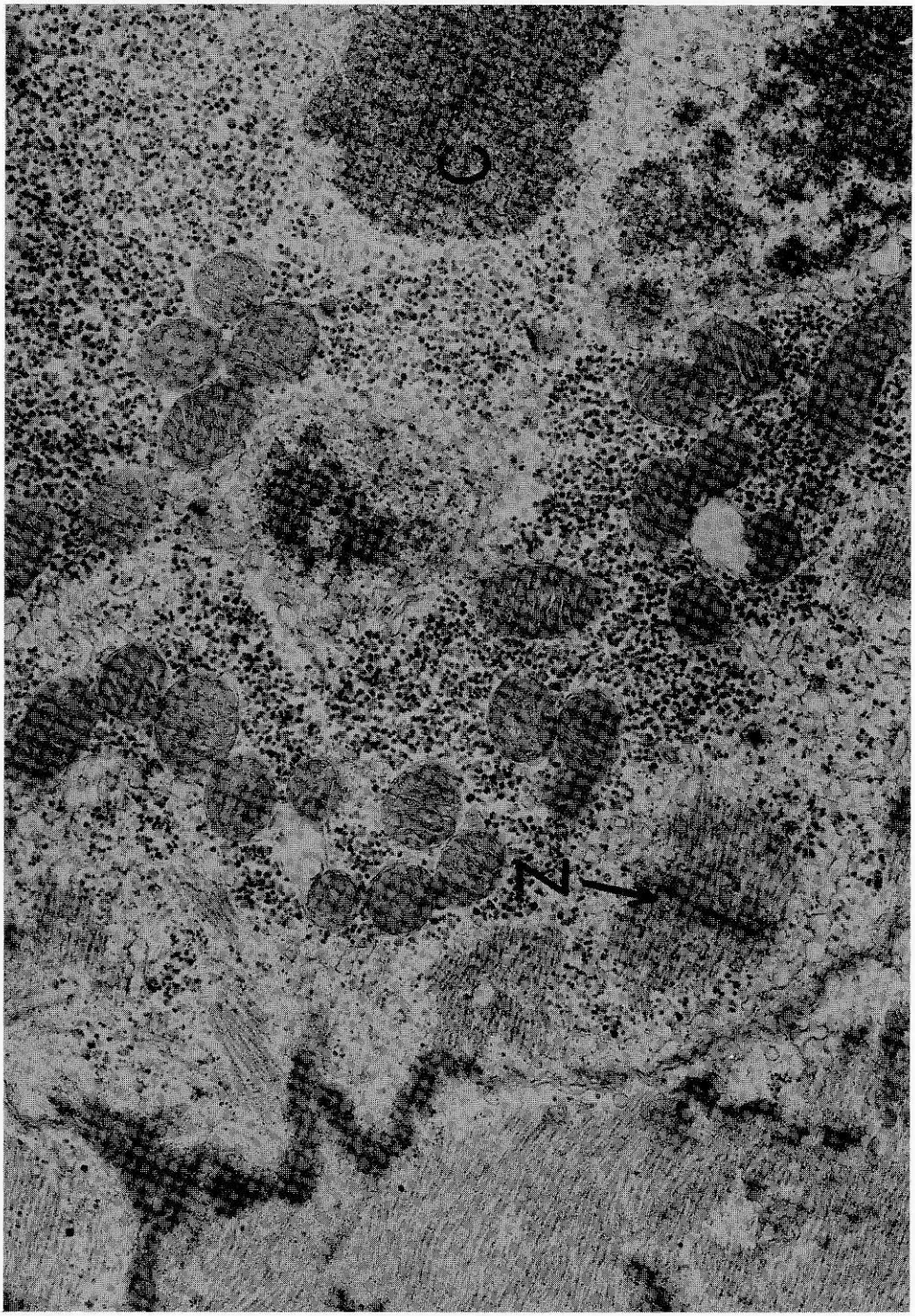

Figure 18-15. Electron micrograph of a mitotic myocyte at sixteen days after amputation. This portion of the cell contains chromosomes, (C) dense granules, desmosomal cell junctions, glycogen, and a portion of myofibrillae with Z bands (Z). ×24,000.

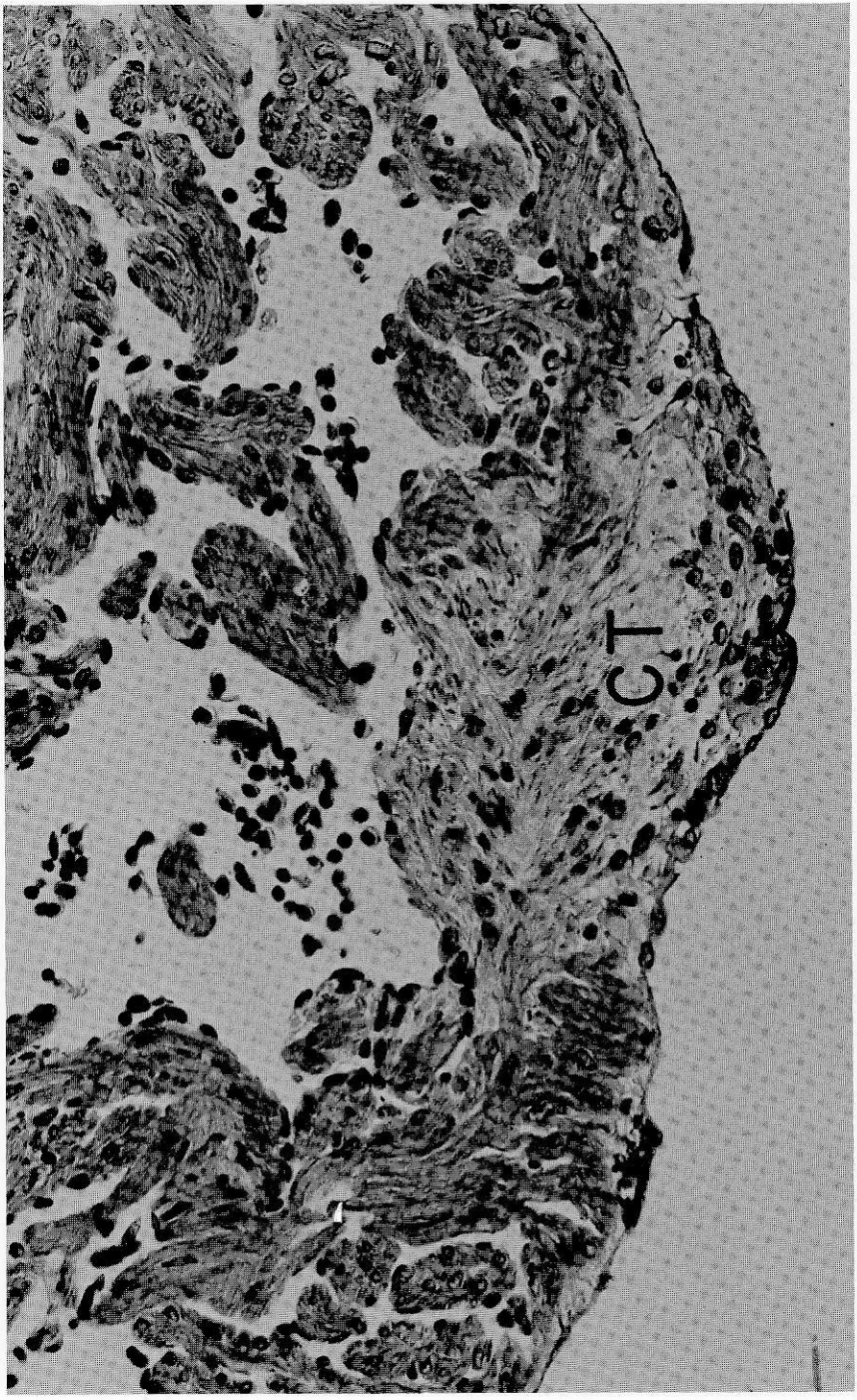

Figure 18-16. Light micrograph of the amputation region of a ventricle forty days after amputation. The region spanning the amputation gap is now mostly composed of connective tissue (CT) with some myocytes within. ×200.

obtained all of the stages of mitosis in our study, we do feel that cytokinesis does occur in this system as it does in the frog (28) and as it appears to do in our minced ventricle experiments. Polyploidy may also account for a portion of this proliferative response; at the present time, we are attempting to study the possible role of polyploidy in this system.

These studies demonstrate that trabecular myocytes adjacent to the amputation site have the capability of DNA synthesis and mitosis. The exact nature of how this response is used by the injured ventricle is unknown since the amputation gap is filled initially with a blood clot that degenerates and by thirty days contains a considerable amount of connective tissue with usually only a few myocytes within. It is thought that this variable number of myocytes may represent differences in the ages of the animals used. At forty days after amputation, the connective tissue in the amputation gap becomes much denser (Fig. 18-16). This type of healing in response to injury has also been observed in the frog by Rumyantsev (26) and in the lizard by Sulima (37). Thus it appears that the amputated newt ventricle can respond to injury by proliferation of myocytes, but that the amputation gap gradually becomes filled largely by connective tissue. The reason for such healing may be that newly formed myocytes have difficulty in penetrating the connective tissue that forms in place of the degenerating blood clot.

In addition to a localized proliferative response in the ventricular muscle compartment to ventricular amputation, a response is also elicited in the atria of the injured heart. Cells within the muscle compartment of the atrial wall undergo DNA synthesis and mitosis as a result of ventricular amputation (Table 18-II). The DNA synthetic response begins sometime during the first week after amputation and reaches a peak at fifteen days postoperative. It gradually tapers off at the end of the fourth week. Although these results have been obtained at the light microscopic level, it

TABLE 18-II
MIOTIC AND THYMIDINE INDICES OF CONTROL ATRIA AND EXPERIMENTAL ATRIA AFTER VENTRICULAR AMPUTATION*

Control	0.0000 ± 0.0000	0.2796 ± 0.2626
5 Day	0.0000 ± 0.0000	0.4249 ± 0.3055
10 Day	0.0771 ± 0.1091	0.7422 ± 0.9145
15 Day	0.3462 ± 0.3648	6.3583 ± 4.2530
20 Day	0.1681 ± 0.0612	3.1230 ± 3.3588
25 Day	0.0000 ± 0.0000	0.4530 ± 0.1005

* This table indicates percentages and standard deviations of mitotic and labelled cells in the muscle compartment of atria. In the experimental groups, approximately one-fourth of the ventricle was amputated. The control groups consisted of unoperated animals. An average of 1,720 cells were counted for each of two animals in each control and experimental group. Each animal used for this study was injected intraperitoneally with 2.5 μC^1 of tritiated thymidine and fixed for light microscopic autoradiography one hour after injection.

appears that most of the cells of the muscle compartment are myocytes. However, confirmation of these results at the electron microscopic level is underway. Statistical studies using analysis of variance indicate that this response in the atrium is a general response throughout the atrium and not a localized type of response as observed in the ventricular response. A similar response of the rat atrium to ventricular injury was first reported by Rumyantsev in 1968. His subsequent ultrastructural work has unequivocally demonstrated that rat atrial myocytes undergo DNA synthesis and mitosis as a response to ventricular infarction (29).

Minced Ventricular Grafts

In order to further study the proliferative and histogenetic capacity of the adult newt ventricle, we devised an experimental situation in which a portion of the ventricular apex was removed, minced into small pieces, and then grafted back onto the amputated ventricle so that the graft spanned the amputation gap. The minced grafts were then studied at various postoperative intervals in order to determine cytological changes with regard to degeneration, proliferation, and histogenesis. The grafts were studied at both the light and electron microscopic levels and DNA synthetic patterns were determined from autoradiographs. Details of these techniques have been reported previously (1, 2).

During the first week after grafting, much degeneration and necrosis occurred within the graft pieces and no reorganization of these pieces was apparent. A number of myocytes undergoing degeneration had pycnotic nuclei and apparently were irreversibly damaged. However, other myocytes, fewer in number, had euchromatic nuclei and presumably remained viable. Degeneration of the graft and phagocytotic activity of macrophages continued into the second and third weeks. During this period, continuity between the main ventricular lumen and graft lumen was established and the graft pieces appeared to have coalesced. The extensive degeneration and initial coalescence of graft pieces is evident in the sixteen day graft in Figure 18-17. In the ten and sixteen day grafts, myocytes had euchromatic nuclei, increased amounts of rough endoplasmic reticulum, polyribosomes, and Golgi complexes, all of which would indicate active protein synthesis. The myofibrillae of these cells were often in a state of disorientation and in the sixteen day grafts appeared to be fewer in number than in the uninjured myocytes.

In the second week after grafting, DNA synthesis and mitosis were first observed in the muscle compartment of the trabeculae and the graft wall as indicated in Table 18-III. In the control group, which consisted of sham-operated animals in which the pericardium was opened and then closed, no uptake of thymidine or mitosis was observed in the muscle compart-

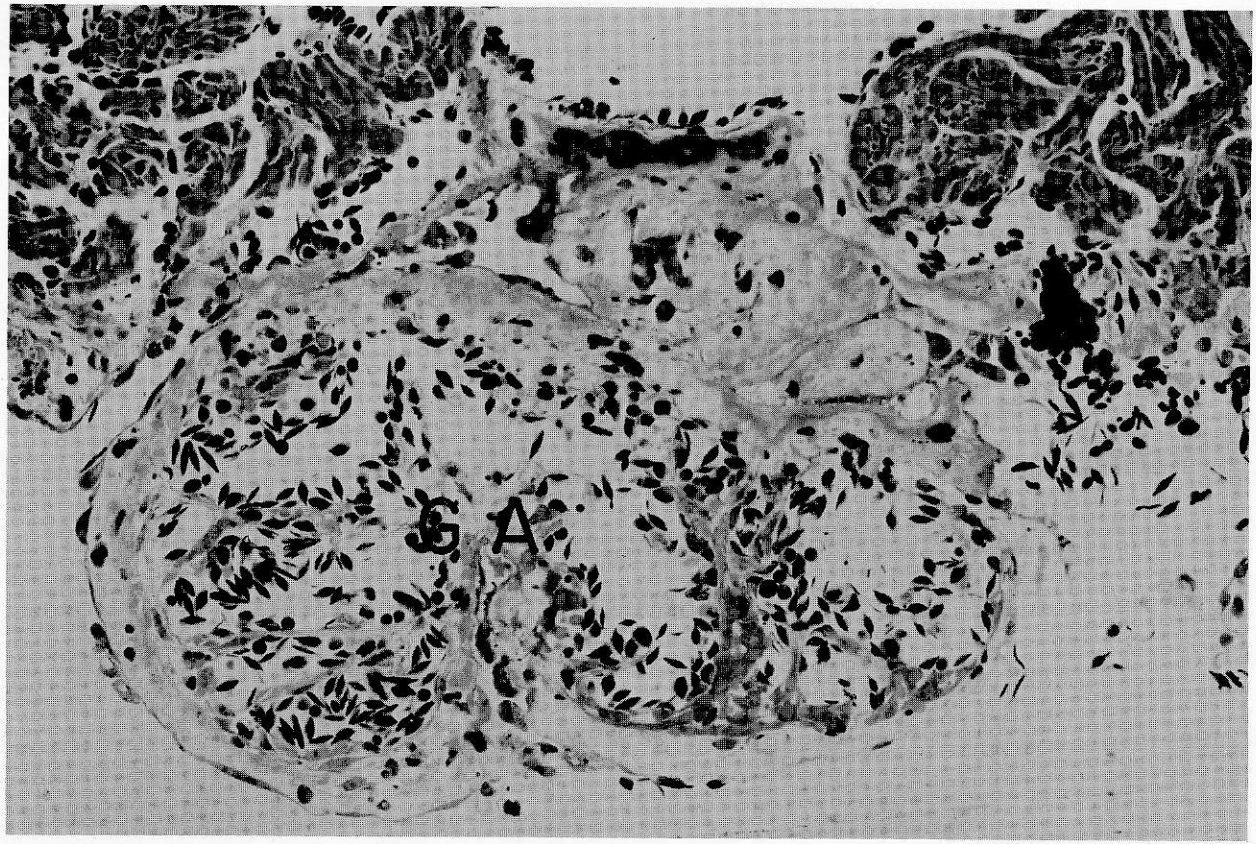

Figure 18-17. Light micrograph of a minced ventricle graft sixteen days after grafting. The graft area (GA) has loosely arranged trabeculae and large spaces in the graft lumen. Many of the original graft myocytes have degenerated. The epicardium covers the graft on the outside. × 180. (From D. Bader and J. O. Oberpriller, *J. Exp. Zool., 208:*177-194, 1979. Courtesy of the Wistar Institute, Philadelphia.

TABLE 18-III
MITOTIC AND THYMIDINE INDICES OF MIXED CARDIAC MUSCLE GRAFTS*

Group	*Mitotic Index*	*Thymidine Index*
Control	0.00 ± 0.00	0.00 ± 0.00
5 day	0.00 ± 0.00	0.00 ± 0.00
10 day	0.18 ± 0.07	2.12 ± 0.34
16 day	2.61 ± 0.27	24.3 ± 0.4
30 day	0.28 ± 0.09	3.02 ± 0.05
70 day	0.00 ± 0.00	0.11 ± 0.09

* This table indicates percentages and standard deviations of cells which are mitotic or have labeled nuclei. At least 1,500 graft cells, excluding blood and endocardial cells, were counted for two animals in each control and experimental group. Each animal used for this study was injected intraperitoneally with 2.5 μC^1 of tritiated thymidine and fixed for light microscopic autoradiography one hour after injection. From D. Bader and J. O. Oberpriller: *J Exp Zoo, 208:*177-194, 1979. Courtesy of The Wistar Press, Philadelphia.

ment at sixteen days after the operation. The peak of proliferative activity is at sixteen days after grafting. At this time, numerous myocytes were observed in mitosis at the ultrastructural level. Twenty-seven mitotic cells in the muscle compartment were observed at various stages of mitosis and of these, twenty-three were myocytes, in that they had myofilaments, glycogen, and, in many cases, an associated basal lamina. The ultrastructure of these mitotic myocytes is similar to that reported in the amputated ventricle section.

The thirty day minced graft contained more myocytes and in general appeared more organized as a continuous miniventricle than did the sixteen day graft. At thirty days, the myocytes were observed in various stages of myofibrillogenesis and contained numerous 10 nm filaments. In this thirty day group, two of five grafts examined demonstrated a functional rhythmic contraction, which was asynchronous with the contraction of the ventricle to which they were attached. This type of asynchronous beat was also observed in some of the fifty day and seventy day grafts. This indicates that the new wall formed by the graft can be functional. The type of stimulus to which the graft muscle responds is unknown. However, it may be that it responds to the filling of the graft lumen after the host ventricle contracts.

By seventy days, the grafts form small, fairly well-integrated miniventricles (Fig. 18-18), with an outer wall and trabeculae consisting primarily of cardiac muscle with a variable amount of connective tissue. The seventy day myocytes have numerous well-organized myofibrillae (Fig. 18-19) and intercellular junctions similar to those observed in uninjured ventricular myocytes.

Cell counts of the muscle compartment of minced cardiac muscle grafts (Table 18-IV) were made in an attempt to determine the changes that

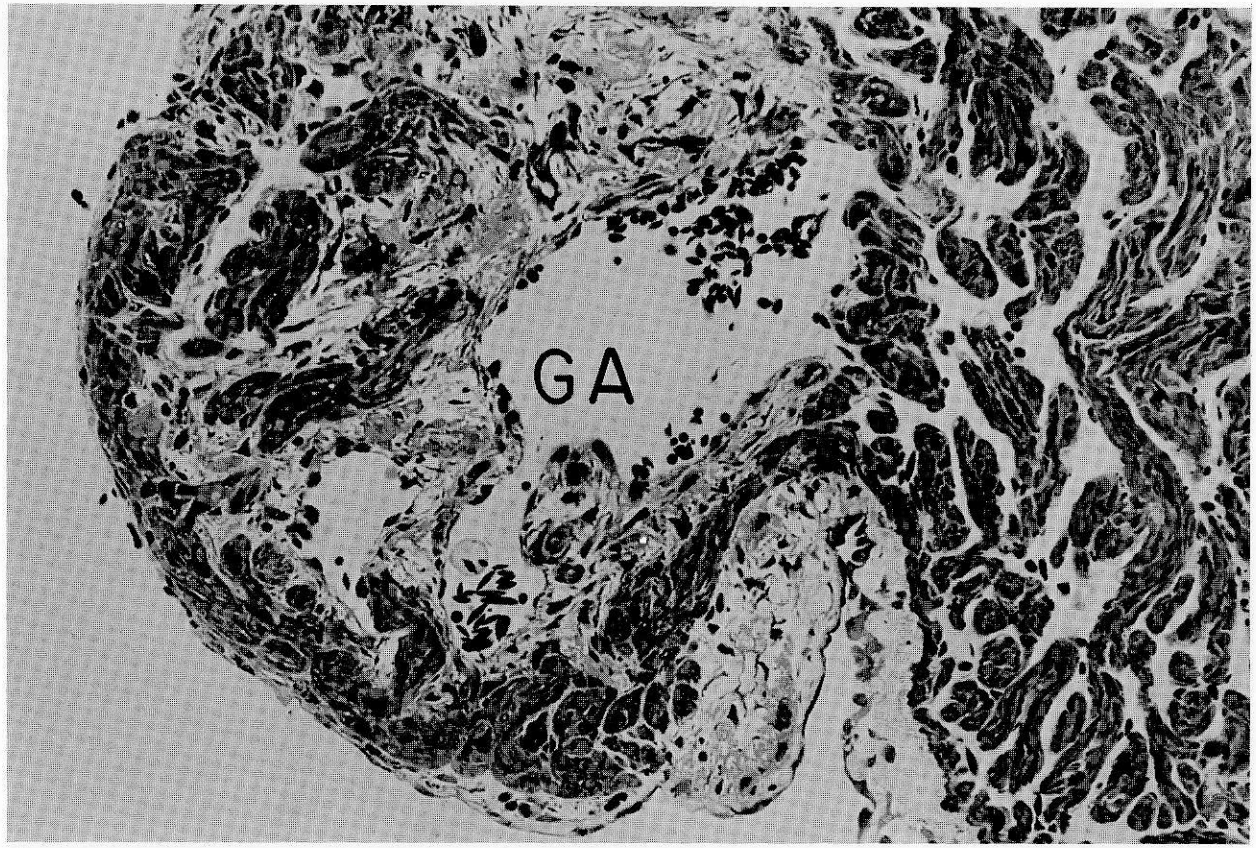

Figure 18-18. Light micrograph of a seventy day minced ventricle graft. The epicardium and endocardium of the wounded ventricle are continuous with that of the graft (GA). Luminal continuity can also be seen. This graft had distinct rhythmic contractions, which were asynchronous with the adjacent host ventricle. × 160. (From D. Bader and J. O. Oberpriller, *J. Exp. Zool., 208:*177-194, 1979. Courtesy of the Wistar Institute, Philadelphia.)

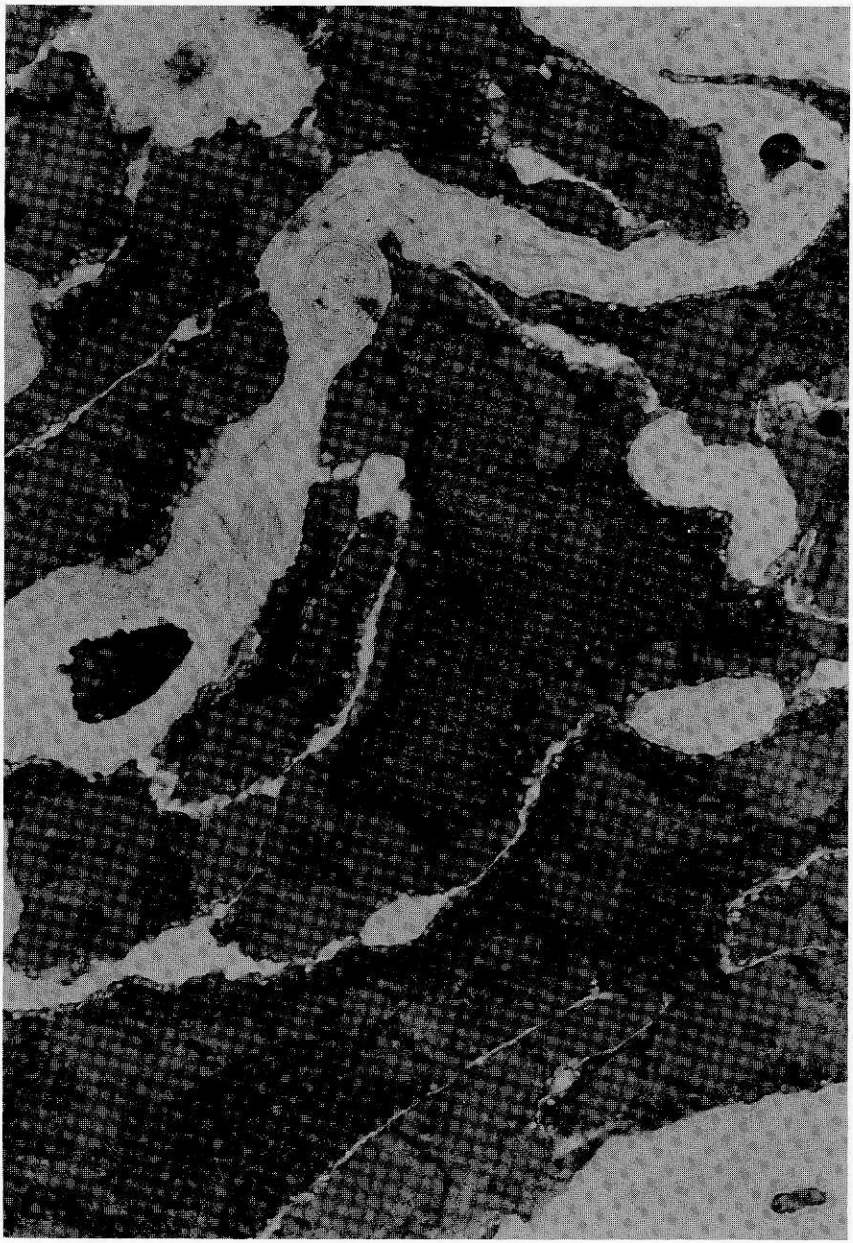

Figure 18-19. Electron micrograph of a portion of a seventy day minced ventricle graft. Numerous myocytes are seen in cross, longitudinal, and oblique section. The myocytes contain many myofibrillae and are similar in structure to control myocytes. ×6,700. (From D. Bader and J. O. Oberpriller, *J. Exp. Zool., 208*:177-194, Philadelphia.)

TABLE 18-IV
CELL COUNTS OF MINCED CARDIAC MUSCLE GRAFTS*

Experimental Group	*Total Number of Graft Cells*	*Percentages*	*Average Graft Area*
0-day group	2,010 cells/mm^2 ± 78	100	0.46 mm^2
5-day group	1,900 cells/mm^2 ± 175	94	0.40 mm^2
16-day group	410 cells/mm^2 ± 87	21	0.39 mm^2
30-day group	1,050 cells/mm^2 ± 110	52	0.44 mm^2
50-day group	1,210 cells/mm^2 ± 151	60	0.43 mm^2

* Averages and standard deviations of cells counted in trabecular network and outer wall of the graft. Endothelial and blood cells were not included in the counts. Grafts of three animals were counted at the widest point of the graft for each experimental group. Also indicated are the percentages of the cells seen in the graft considering 0-day grafts at 100% of the cells grafted. The right hand column represents the average graft area for each experimental group, measured at the widest point of the graft. Three grafts were measured for each experimental group. From D. Bader and J. O. Oberpriller: *J. Exp. Zoo., 208:*177-194, 1979. Courtesy of The Wistar Press, Philadelphia.

occur in the graft as a result of degeneration and proliferation. During the degenerative, proliferative, and redifferentiative phases of the grafts, the cross-sectional areas of the grafts remain relatively unchanged. Therefore, one can get a fairly consistent comparison of the grafts of the various phases. From Table 18-IV, it is obvious that extensive degeneration occurs during the first three weeks, with only approximately 21 percent of the counted cells surviving. Between the proliferative phase at sixteen days and the differentiative phase at fifty days, the number of cells observed within the graft have more than doubled, indicating that a considerable number of new myocytes are formed in the minced ventricle system.

These minced ventricle studies demonstrate that a miniventricle that can be functional can form from minced pieces of ventricle. The formation of such an integrated structure from minced pieces indicates that the newt heart is able to undergo a certain degree of histogenesis.

Discussion

The above experiments indicate that the newt heart is capable of a certain degree of proliferation and histogenesis as a response to injury. Consequently, a number of questions arise in this system. One is that of the origin of the new myocytes that form. Although we have not performed elaborate experiments, it would appear that the cells arise by the cell division of already existing myocytes, after a number of cellular changes. This same opinion is held by Rumyantsev (28) for the frog. However, one has difficulty in ruling out the origin from other cell types, although the sequence of events observed in the cell division of myocytes seems not to appear to require an origin of these cells from other cell types.

Another major question to be considered is what the type of mechanism

might be which serves as the stimulus for initiation of cell division in these cells in the ventricle in response to amputation. Since the response is a localized phenomenon, it may have to do with injury or loss of an adjacent part. However, in the atrial response to ventricular injury, the mechanism appears to be quite different and perhaps represents a physiological response of an overloaded system somewhat similar to the regenerative hypertrophy of internal organs (34).

Finally, another question is why the amphibian ventricle is so much more responsive than the mammalian ventricle to injury. There are essential differences between the two, which might play a role. The amphibian has a lower and variable body temperature. The amphibian heart is highly trabeculated and avascular, with relatively little connective tissue. There are ultrastructural features that differ from the mammalian heart, such as the lack of T-tubules and the presence of dense granules. Goss (6) has suggested that the increased capacity for responding to injury could be due to a greater ability of hearts in lower vertebrates to grow throughout life.

At the present time, there are few answers to these questions. It is reasonable to assume that if one is to stimulate the adult mamalian ventricle to assume a role in proliferation and histogenesis, one must begin by studying the systems that readily demonstrate these capacities. Surely one of the better model systems would be the heart of the lower vertebrates, such as the frog or the newt.

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