MYOCARDIAL REGENERATION IN YOUNG RATS *

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The problem of the regenerative capacity of heart muscle has been under discussion for the last hundred years; many papers have been written, but little has been contributed to challenge the conclusion that the myocardium takes a minimal share in the healing of cardiac injuries.

Histologic studies of the hearts of those dying of diphtheria or with cardiac hypertrophy, chiefly children,[1-3] have suggested that there may be true regeneration of cardiac muscle fibers, both by longitudinal splitting of the fibers and by mitotic division. This suggestion, the postulate that in infants regeneration of the myocardium may be possible due to persisting physiologic development, and the fact that the literature contains no data concerning the experimental production of myocardial lesions in newborn animals, have been the factors in my decision to explore the possibility of myocardial regeneration in newborn rats.

REVIEW OF THE LITERATURE

There have been several reviews of the rather extensive literature about hyperplasia and regeneration of the myocardium, of which the more important were by Goldemberg[4] (1886), Goldzieher and Makai[5] (1912), Karsner and Dwyer[6] (1916), Karsner, Saphir, and Todd[7] (1925), MacMahon[3] (1937), and Ring[8] (1950).

Goldemberg,[4] in 1886, brought out the first review of the literature since 1845, and dealt mainly with the problem of hypertrophy of the myocardium. He discussed the earlier papers of Vogel,[9] von Kölliker,[10] Förster,[11] Lebert,[12] Hyrtl,[13] von Rokitansky,[14] Heschl,[15] Hepp,[16] Robin,[17] Wedl,[18] Becquerel,[19] Friederich,[20] Wilks and Moxon,[21] and Rindfleisch.[22] He pointed out that the controversy concerning myocardial hypertrophy had become well established. Some believed that hypertrophy of the heart is due to simple enlargement of the individual muscle fibers, while others accepted, and described, true hyperplasia and even longitudinal splitting of the myocardial fibers (Wilks and Moxon[21] and Rindfleisch[22]).

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Zielonko,[23] in 1874, under the supervision of Virchow, did the first experimental work in this field. He studied hearts of rabbits and frogs and came to the conclusion that hypertrophy is due to the enlargement of individual muscle fibers, and probably also to cellular hyperplasia. Goldemberg[4] concluded as a result of his work that hypertrophy of the heart is due to increase in size of individual muscle fibers rather than to true hyperplasia. Karsner *et al.*,[7] in 1925, after measuring cardiac muscle fibers of normal, hypertrophic, and atrophic hearts, concluded that cardiac enlargement is due to hypertrophy of individual muscle fibers without increase in their number. MacMahon,[3] in 1937, studied 3 cases of cardiac hypertrophy in infants (6, 12, and 20 months of age), due to different causes. He found numerous mitotic figures.

The regenerative capacity of the myocardium has been discussed controversially also from the point of view of the healing of toxic myocarditis, myocardial infarction, and wounds of the heart. Saltykow,[24] in 1905, published a work on diffuse myocarditis in which he stated that the muscular elements themselves play an important part in healing by forming new fibers. Heller,[1] in 1913, described the regeneration of heart muscle fibers in diphtheritic myocarditis by their longitudinal splitting. Warthin,[2] in 1924, studied 16 cases of diphtheritic myocarditis and found evidence of signs of regeneration in 3 of them. His description was as follows:

> Near the necrotic or degenerated portions of the heart muscle, the nuclei of the muscle show a great variety in size and form. They increase in length and show many evidences of longitudinal splitting in every possible stage of such a division. The living muscle substance bordering on the injured area also undergoes a longitudinal splitting into muscle bands containing nuclei; these bands grow into the perimysial tubes, filling these up, replacing the cell detritus, and connecting with the living muscle on the other side of the defect. Muscle bands without nuclei in their substance but accompanied by myoplastic nuclei also extend into the tubes occupied by the dead muscle substance. These bands lie at the periphery of the tube, and in some cases appear to form a hollow cylinder enclosing the remains of the dead muscle substance. . . . We have seen all of the appearances illustrated by Heller in his article on the regeneration of the heart muscle. The defects in the muscle caused by hyaline necrosis may ultimately be bridged by a number of new muscle bands apparently uniting with the living muscle on the other side of the defect. The sharp delimitation of the muscle in perimysial or sarcolemmar tubes was shown in our cases as in those of Anitschkow and Heller. These appearances, as Heller has pointed out, seem contradictory to the present accepted view that the heart muscle has no true sarcolemma. There is a close similarity between the process of regeneration of the heart muscle and that of the peripheral nerve trunks.

MacMahon,[3] in 1937, studied the heart of a child 6 years old, who died several days after onset of diphtheria, and he likewise described numerous mitotic figures in myocardial fibers which were considered

by him to be evidence of true myocardial regeneration. His conclusions were:

Evidence is presented in the form of mitotic division of the nuclei of heart muscle fibers to indicate first, that in cardiac hypertrophy of infants a proliferation of heart muscle fibers can take place, and secondly, that in severe myocardial injury in children, regeneration of myocardial elements can occur.

Mallory, White, and Salcedo-Salgar,[25] in 1939, published a very interesting work on the speed of healing of myocardial infarction, based on the study of 72 human cases. They described the pathologic changes at different periods and compared the rate of healing in their cases with that seen in experimental infarctions. They did not mention regeneration of heart muscle fibers.

King,[26] in 1941, described changes in the muscle fibers around recent wounds in human hearts, consisting of proliferation of nuclei, mitotic figures, and outgrowth of protoplasmic masses. He wrote: "From an examination of heart wounds, the writer is convinced that some regeneration of muscle takes place." This same author, referring to the changes in myocardial infarction, stated: "Observations which strongly suggest regenerative or hyperplastic growth of muscle cells are to be made in some cases of coronary occlusion."

In the experimental field much has been done by inflicting cardiac injuries of different types, such as experimental infarction by ligation of branches of the coronary arteries, traumatic lesions of the myocardium by blows, application of heated instruments, diathermy needles, stab wounds, and experimental myocarditis produced by the intravenous injection of sparteine and adrenalin. The paper by Cohnheim and von Schultess-Rechberg[27] in 1881 was one of the first to discuss the pathologic anatomy of experimental myocardial infarction in dogs. Kolster's publication[28] (1893) was based on experimental myocardial infarction in 11 dogs; he described mitotic figures in heart muscle fibers, connective tissue, and adventitia of blood vessels at the periphery of the necrotic areas, but he concluded that no true regeneration of the myocardium occurred. Fleisher and Loeb,[29-31] in 1909 and 1910, made a series of studies of experimental myocarditis in rabbits. They found that small doses of sparteine (0.012 gm. per kg. of body weight), or caffeine (0.025 gm. per kg.), followed by the intravenous injection of a small quantity of adrenalin (0.2 ml. of a 1 to 1,000 solution), produced microscopic changes in the hearts of rabbits, located chiefly at the base of the left ventricle. They described the microscopic changes of the myocardium at different periods after injection and, although they did not find actual signs of regeneration of heart muscle

fibers, they wrote: "It appears that the myocarditic lesion actually heals and that in many cases the repair is not due to the replacement of the injured parenchyma by fibrous tissue, but by a recovery of certain muscle cells." Christian, Smith, and Walker,[32] in 1911, published a paper concerning the experimental production of cardiorenal disease in rabbits; they repeated Fleisher and Loeb's experiments and described the pathologic lesions in heart, kidney, and liver. No regeneration of the heart muscle fibers was found.

Goldzieher and Makai,[5] in 1912, as a result of their experiments and review of the literature concerning myocardial regeneration, came to the opinion that myocardial lesions probably are repaired only by connective tissue scars. They believed that the regenerative proliferation of muscle fibers, if it occurs at all, is limited to a very slight depth at the edges of a wound. Karsner and Dwyer,[6] in 1916, described the findings in experimental infarction in 13 dogs, and gave an account of the histologic changes. They did not find evidence of myocardial regeneration. Moritz and Atkins,[33] in 1938, produced cardiac contusions in 32 adult dogs by opening the thorax and striking the heart one or more forcible blows. The animals were killed at varying intervals up to 6 months. The degeneration of muscle cells due to the trauma was described, but regeneration was not found.

In 1947 Harrison[34] produced myocardial lesions in 6 rabbits by applying a diathermy needle for 10 seconds, thereby causing an area of necrosis 3 to 4 mm. wide. The animals were killed 24 hours and 3, 5, 7, 9, and 14 days after the operation. He described the changes produced by the injury but there was no regeneration. Walls,[35] in 1949, induced lesions in the lower third of the right ventricle of rabbits' hearts by the application of the head of a red-hot nail. The animals were allowed to survive for periods ranging from 3 days to 1 month and the injured areas were then studied for evidence of regeneration of the muscle fibers, but none was found. He thought that the injury was too severe, so that the reaction to it did not allow myocardial regeneration.

Ring,[8] in 1950, using rabbits and cats, 35 animals in all, produced myocardial infarction by ligation of the left coronary artery. Biopsy specimens taken 2 days to 2 months after the operation were studied for evidences of regeneration of heart muscle fibers. He observed interesting alterations at the ends of the surviving muscle fibers, especially during the period of 7 to 9 days after infarction. These consisted in loss of transverse striation, accentuation of longitudinal striation, and exaggeration of basophilism. At the same time the nuclei were

arranged in pairs and occasional mitotic figures were seen. There was expansion of the ends of muscle fibers, which sent out short processes into the surrounding tissue. These changes were interpreted as early regeneration, which did not proceed beyond the budding stage. He suggested that this was a frustrated attempt at regeneration, probably due to the absence of a sarcolemmal framework along which the new fibers could grow.

Studitskii,[36] in 1954, transplanted fragments of myocardium of young chickens and rabbits into previously made spaces in voluntary muscle of other animals of the same species, and described the changes in the graft at different periods after the operation. The most conspicuous finding was the formation of myoblasts from the heart muscle fibers of the implant. Myoblasts appeared between the fifth and seventh days after transplantation, in the form of cells of two different types, spherical and spindle-shaped. The spherical cells contained one or two nuclei with large nucleoli, and the cytoplasm was coarsely vacuolated. The nuclei of the spindle-shaped cells were oval or elongated and possessed large nucleoli, and the cytoplasm contained numerous small vacuoles parallel to the long axis of the cells, as well as elongated fibrillar structures. Numerous mitotic figures were present in these spindle-shaped cells. Later a mass of spindle cells was formed; the individual cells increased in size and contained numerous fibers which could be seen distinctly between the vacuoles. These cells were able to contract and Studitskii believed that they were actually myoblasts.

Other references to the experimental production of cardiac lesions have been omitted because they do not deal with the problem of myocardial regeneration.

From a review of the literature, the preponderant opinion seems to be that true regeneration of the myocardium does not occur in adults, but that its occurrence in infants is probable.

Material and Methods

The general experimental procedure was to expose the heart of a newborn white rat under general anesthesia, inflict a small burn, repair the incision, and allow the animal to live for a certain period. It was then killed and the reparative processes in the myocardium evaluated.

Eighty rats were used in this study, of which 58 survived for periods which made them suitable for histologic examination. Twenty-two died, their deaths being attributable mainly to the anesthesia, which, in the early stages of the experiment, had not been assayed correctly.

Some were killed by their mothers after being returned to the cages. The hearts of 8 animals which died under anesthesia before the myocardium was burned were used as controls, especially in respect to the presence of mitotic figures in myocardial fibers. The age of the animals at the time of operation was between 4 and 7 days. They were anesthetized with nembutal intraperitoneally. Under sterile precautions a small, left, parasternal incision was made through the skin, the muscles were dissected, and the 6th costochondral junction was excised. Though this small hole the heart was touched for 1 second with a wire 1 mm. in diameter which had been heated to a red heat. This small burn usually was made at the apex of the heart or on the lower third of the anterior surface of the left ventricle, the diameter and depth being very uniform in most instances (Figs. 1 and 2). The pericardium is so thin that it was easily perforated by the heated wire. The wound was closed with a single stitch at the muscular level and the skin edges were kept in apposition by a thin film of celloidin. Tracheotomy or tracheal intubation was not necessary. The pneumothorax was not aspirated. Following the operation, the animals were kept warm, and after recovery from anesthesia (in 3 to 4 hours) were returned to the cage with the mother rat. Beginning 12 hours after the operation, they were sacrificed in pairs, in an ether chamber, at daily intervals until the 15th day; then a pair was sacrificed every 5 days until the 75th day.

Necropsy was performed immediately after death and the heart was fixed in 10 per cent formalin. A block including the lesion was embedded in paraffin, and four to twelve serial sections were cut from each block. Half of them were stained with hematoxylin and eosin and half by Masson's trichrome method. In 16 cases additional sections were stained by von Kossa's method to determine the presence of calcium deposits in the lesions. All preparations were examined microscopically and described; search for mitotic figures was made with an oil immersion objective.

Results

The evolution of the lesion was followed day by day until the 15th day, and then every 5 days until the 75th day. For each period, two hearts were studied and for several intervals a third was added in order to resolve some doubts in the interpretation of the microscopic findings.

Since the gross findings added but little of interest and were very constant, because of the uniformity of the lesions, detailed gross descriptions will not be made in every case.

12 Hours after Injury (Rats 1 and 49). Grossly, after 12 hours, the lesion consisted of a 1 mm. circular, grayish defect covered by fibrinous exudate. Microscopically, coagulation necrosis, vasodilatation, congestion, margination of leukocytes, hemorrhages by diapedesis, interstitial edema, and a few polymorphonuclear leukocytes in the neighborhood of the lesion were noted. In myocardial fibers beneath and around the lesion, granular and vacuolar degeneration was present, and still deeper cloudy swelling of variable degrees was seen. On the surface there were a few threads of fibrin.

24 Hours after Injury (Rats 23, 24, and 42). After 24 hours, the necrotic tissues were infiltrated by polymorphonuclear leukocytes, a few lymphocytes, and macrophages. The myocardial fibers at the border of the lesion showed marked granular and vacuolar degeneration of the sarcoplasm, which was beginning to be removed by phagocytes, leaving empty tubes. The nuclei of these fibers showed pyknosis and karyolysis in various stages, the severity of these changes decreasing in the deeper portions. Interstitial edema, congestion, hemorrhages by diapedesis, and some leukocytic infiltration were present in the neighborhood of the lesion. In the deeper myocardial fibers it was possible to identify several mitotic figures, an observation which was confirmed by staining with Masson's trichrome method, and examining under the oil immersion objective. The interstitial connective tissue around the lesion showed several mitotic figures. On the surface there was fibrinous exudate, and fibrinous adhesions to the lung were present in rat 24.

2 Days after Injury (Rats 35 and 36). After 2 days the necrotic tissues had been partially removed leaving a considerable defect in the heart wall (Fig. 1). The area of the lesion was infiltrated by polymorphonuclear leukocytes, lymphocytes, and abundant macrophages loaded with cellular detritus; the extravasated erythrocytes were becoming hemolyzed. The interstitial connective tissue was beginning to proliferate and with Masson's trichrome stain chains of fibroblasts could be seen advancing from the edges of the defect into the remaining necrotic tissues. At the same time, angioblastic proliferation was beginning. The surviving myocardial fibers at the border of the defect showed variable degrees of granular and vacuolar degeneration, depending on their proximity to the burned area. In the fibers closest to the lesion the degenerated sarcoplasm had been removed already, leaving empty tubes. In deeper portions the muscle nuclei showed great activity, represented by all phases of mitotic division, and in such fibers there was loss of striations and the sarcoplasm appeared finely granular (Fig. 17).

3 Days after Injury (Rats 3 and 38). The lesion after 3 days was similar to that seen on the preceding day. Macrophages were more abundant and the proliferation of the interstitial connective tissue at the borders of the defect was more conspicuous, with formation of a network which was advancing into the defect. Mitotic figures were abundant in the deeper myocardial fibers and in some of them columns of two or three nuclei were seen. Young connective tissue adhesions to the costal wall had formed already in rat 38. In the adhesions there was active angioblastic proliferation with the formation of small capillaries. In this animal, also, there were small deposits of lime salts in necrotic myocardial fibers at the edges of the lesion.

4 Days after Injury (Rats 25 and 26). After 4 days there had been progress in the removal of the necrotic tissues in which there were many macrophages. Proliferation of the interstitial connective and angioblastic tissues had increased. The degenerated muscle fibers at the borders showed heavy calcareous deposits, proved to be calcium phosphate by von Kossa's reaction. In the deeper zones the myocardial fibers still showed varying degrees of granular and vacuolar degeneration, and there were macrophages and a few polymorphonuclear leukocytes and

lymphocytes. Still deeper, the myocardial fibers showed many mitotic figures (Fig. 6).

5 Days after Injury (Rats 2 and 16). Remnants of necrotic tissue were still to be seen after 5 days, and macrophages, loaded with blood pigment and cellular débris, were more abundant. There were also a few polymorphonuclear leukocytes and lymphocytes. Interstitial fibroblastic and angioblastic proliferation had increased. Dead muscle fibers at the margin of the lesion showed heavy calcification, forming a band of calcium salts (Fig. 3). Beneath this band, the surviving muscle fibers showed granularity of their sarcoplasm with loss of striations, and thin processes had progressed from the ends of these fibers into the network of connective tissue. Columns of two or three nuclei and several mitotic figures were present in these fibers. In deeper portions, many mitotic figures were seen in myocardial fibers (Fig. 10).

6 Days after Injury (Rats 44 and 51). In rat 44, fibrous adhesions to the costal wall had formed by 6 days after injury. In both hearts, the myocardial defect was almost completely filled by young connective tissue which contained many small capillaries. The necrotic tissue had been largely removed, and there were abundant macrophages loaded with cellular detritus, as well as polymorphonuclear leukocytes, lymphocytes, and a few eosinophils. The surviving heart muscle fibers at the border of the lesion showed evident signs of proliferation: the ends were progressing into the connective tissue interdigitating with it; the cytoplasm showed varying staining intensity; there were thin, deeply eosinophilic fibers with columns of two or three nuclei; many fibers and their nuclei showed longitudinal splitting (Fig. 14); several muscle fibers had fused to form multinucleated cytoplasmic masses; mitotic figures were seen frequently in other muscle fibers. In rat 51 no adhesions to the costal wall had formed. The remains of necrotic tissue were more abundant than in rat 44 and, while regenerative processes were present, they were less marked.

7 Days after Injury (Rats 17, 18, and 52). In rats 17 and 18 heavy adhesions to the costal wall had formed in 7 days (Fig. 2). The necrotic tissues had been removed completely in all 3 animals and there were abundant blood pigment-laden phagocytes and mononuclear infiltration. The defect was now filled completely with connective tissue containing abundant capillaries. Rat 17 showed several giant cells of foreign body type in the adhesions. These contained small fragments of black substance, probably carbon from the hot wire. The most interesting findings were at the border of the defect, where the ends of surviving muscle fibers were continuing their progress into the connective tissue. These fibers showed signs of regeneration by both mitotic and amitotic division. Mitotic figures in all phases were seen within myocardial fibers (Fig. 7). The fibers in which the nuclei were undergoing mitotic division had lost their striations, and their cytoplasm had become finely granular. Such fibers were easily distinguished from connective tissue elements by their thickness, and by the color of their cytoplasmic granules when stained by Masson's trichrome method. Amitotic division was represented by longitudinal splitting of the cytoplasm and nucleus in many fibers. In deeper zones, also, the myocardial fibers showed abundant mitotic figures.

8 Days after Injury (Rats 19 and 20). On the eighth day the findings were very similar to those seen on the seventh day, except that the surviving muscle fibers had pushed further into the connective tissue.

9 Days after Injury (Rats 31 and 37). In rat 31, after 9 days, the heart was heavily attached to the costal wall at the lesion by fibrous adhesions. In rat 37 the heart was not attached. Regenerative processes were much more advanced in the animal in which adhesions had formed than in the other. This was believed to be due to the increased blood supply provided the region of the lesion by the

capillaries contained in the adhesions. Nevertheless, in both hearts regenerative activity of myocardial fibers was evident. The ends of surviving muscle fibers at the border of the lesion were progressing into the previously formed network of connective tissue, and longitudinal splitting of their cytoplasm in some areas had given them a frayed appearance. In these fibers the nuclei showed variation in staining intensity, size, and shape, and columns of two or three nuclei were frequently seen in one fiber. Thin, deeply eosinophilic fibers with rod-shaped nuclei were conspicuous. In fibers situated even several millimeters from the lesion and in papillary muscles adjacent to the damaged area (Fig. 16), mitotic division figures in all stages were very abundant. A few macrophages and mononuclear leukocytes were present in the area of the lesion. The interstitial connective tissue gave evidence of proliferation by mitotic figures, mainly around blood vessels.

10 Days after Injury (Rats 33 and 34). No adhesions had formed on either of the hearts examined after 10 days. The defect was completely filled with connective tissue infiltrated by mononuclear leukocytes and a few macrophages. At the edges of the lesion, the muscle fibers showed calcification of various degrees; in some cases this was slight, with only a little granular, basophilic material in the cytoplasm, whereas in others there were large deposits of lime salt. In preparations stained by von Kossa's method, the earlier small deposits were found to be positive (calcium phosphate), but the older and larger deposits were negative (probably another calcium salt). The living ends of the muscle fibers were arrested in some areas by these calcium masses; in fact, very little progress of the fibers into the scar tissue was seen. Nevertheless, mitotic and amitotic division was observed in many fibers.

11 Days after Injury (Rats 21, 22, and 53). In none of the rats examined after 11 days was the heart attached to the costal wall. The defect was filled with well vascularized young connective tissue. No necrotic tissue was seen, and calcification had not occurred in these animals. Blood pigment-laden phagocytes and mononuclear leukocytes were still present in the area of the lesion. The surviving myocardial fibers at the border were progressing into the connective tissue, and amitotic and mitotic division was taking place in many of these fibers. In deeper myocardial fibers also, regenerative activity was obvious from the mitotic figures in different phases.

12 Days after Injury (Rats 5, 27, and 28). The hearts of rats 5 and 28 were attached to the costal wall when seen after 12 days. In all three the defect was filled with young, well vascularized, connective tissue. Lymphocytes and plasma cells were present at the area of the lesion, as well as a few blood pigment-laden phagocytes. The ends of the surviving muscle fibers at the border of the defect were sending thin processes into the connective tissue, and in many of these fibers it was possible to see longitudinal splitting and nuclear changes in the form of rod and horseshoe shaped nuclei. In deeper myocardial fibers abundant mitotic figures in all phases were seen.

13 Days after Injury (Rats 39 and 40). In both rats 39 and 40 the heart was firmly attached to the costal wall at the level of the lesion at 13 days. Well vascularized, young, connective tissue filled the defect. Lymphocytic infiltration and macrophages were still present in the area of the lesion. Thin collagen fibers now were beginning to appear in the connective tissue. The heart muscle fibers at the defect showed evident advancement into the connective tissue, and in some areas these new formed muscle fibers were seen progressing into the adhesions several millimeters beyond the actual border of the heart (Fig. 12). In other areas masses of cytoplasm with several nuclei ("muscle giant cells") were seen (Fig. 15). Longitudinal splitting of myocardial fibers was present at the edges of

the defect. Many nuclear changes, such as columns of two or three nuclei, horseshoe and rod-shaped forms, vesicular nuclei, and mitotic figures were present in myocardial fibers near the lesion (Fig. 9).

14 Days after Injury (Rats 29, 41, and 55). In all 3 animals examined after 14 days, the heart was attached to the costal wall. The defect was filled with connective tissue which was maturing, the fibroblasts were becoming fibrocytes, and collagen fibers were more abundant. Newly formed blood vessels were numerous and endothelial cells in columns were passing from the costal wall through the scar into the myocardium. Lymphocytic and plasma cell infiltrations, as well as macrophages, were still present. Heart muscle fibers penetrating the scar tissue were seen, and amitotic division was evident in some of these fibers, but mitotic activity was greatly decreased, as compared to that in hearts seen somewhat earlier.

15 Days after Injury (Rats 7, 30, and 56). After 15 days, the connective tissue filling the defect was of a more adult type, with collagen and elastic fibers producing retraction of the lesion. Some lymphocytic and plasma cell infiltrations were present. No active regeneration of heart muscle was seen, but in the connective tissue there were many newly formed heart muscle fibers.

20 Days after Injury (Rats 46 and 57). In the 2 rats examined at 20 days, the hearts were not attached to the costal wall. The scar consisted of adult connective tissue containing abundant small blood vessels and limesalt deposits. There was still some mononuclear infiltration. Some of the muscle fibers at the border of the lesion also showed calcification. The scar was somewhat retracted. No regenerative activity of muscle fibers was seen.

25 Days after Injury (Rats 4, 6, and 43). In none of the animals examined after 25 days was the heart attached to the costal wall. The scar was made up of connective tissue with some mononuclear infiltration; few bundles of myocardial fibers were seen in the scar tissue. Small deposits of lime salt were present in the area of the lesion in rat 43.

30 Days after Injury (Rats 8 and 47). The scar was more fully retracted after 30 days and the connective tissue showed more collagen and elastic fibers. Some mononuclear infiltration was still present. There were several small limesalt deposits in the scar.

35 Days after Injury (Rats 9 and 45). An adult connective tissue scar, with abundant blood vessels and bundles of heart muscle fibers within the scar, was found in the rats examined after 35 days.

40 Days after Injury (Rat 48). At 40 days there was a small, somewhat retracted scar, made up of adult connective tissue with abundant blood vessels. A few lymphocytes and mast cells were infiltrating the scar, and bundles of heart muscle fibers were embedded in it.

45 Days after Injury (Rat 50). No changes from the 40-day lesion were seen at 45 days.

50 Days after Injury (Rats 10 and 11). At 50 days there was a very small, somewhat retracted scar, made up of adult connective tissue in which there were small limesalt deposits. Around these, a few giant cells of the foreign body type were seen. Only a few lymphocytes were present.

55 Days after Injury (Rat 54). A mature connective tissue scar with blood vessels was present at 55 days. There were pericardial adhesions with lymphocytic infiltration. Myocardial fibers bridged the defect.

60 Days after Injury (Rats 12 and 13). A small scar, formed by adult connective tissue in which there were bundles of myocardial fibers, marked the site of the lesion in each of the hearts examined after 60 days.

65 Days after Injury (Rat 58). Heavy adhesions to the costal wall had formed

at the level of the lesion in the rat examined after 65 days. In these adhesions there was a large bundle of myocardial fibers which protruded several millimeters beyond the actual border of the heart (Fig. 13). Numerous blood vessels passed from the costal wall through the scar to the myocardium.

70 Days after Injury (Rat 32). A mature connective tissue scar with a few myocardial fibers embedded in it was found in rat 32 which was examined after 70 days. In this animal no adhesions had formed.

75 Days after Injury (Rats 14 and 15). After 75 days there remained only a very small scar made up of adult connective tissue in which there were small deposits of lime salts and a few lymphocytes and mast cells. The pattern of myocardial fibers beneath the lesion was somewhat disorderly.

Controls. Eight newborn rats, 4 to 7 days old, were used as controls, especially in searching for mitotic figures in myocardial fibers. No evidence of nuclear division was found in any of these uninjured hearts.

Summary of Histopathologic Changes in Experimental Animals

Degenerative Changes. Twelve hours after injury the retrogressive changes of the alterative phase of inflammation were apparent. Coagulation necrosis was present in the area of the burn, and in adjacent regions in which the injury was less severe there were granular and vacuolar degenerations of myocardial fibers. In deeper and less damaged fibers, variable degrees of cloudy swelling had developed. Besides these, there were the expected circulatory changes of vasodilatation, congestion, margination of leukocytes, hemorrhages by diapedesis, migration of polymorphonuclear leukocytes through the vessel wall, and extravasation of fluids giving rise to interstitial edema, marking the beginning of the exudative phase of inflammation.

Reparative and Regenerative Processes. After 24 hours the alterative-exudative phase was still more conspicuous, as shown by the presence of necrotic and degenerating tissues, more marked interstitial edema, and infiltration of polymorphonuclear leukocytes, lymphocytes, and macrophages. The early reparative phase of inflammation was represented by beginning removal of necrotic tissues, mitotic activity of the nuclei of interstitial connective tissue at the borders of the lesion, and a few mitotic figures in the nuclei of deeper and less damaged myocardial fibers.

At 48 hours the reparative processes were more conspicuous: the removal of necrotic tissues was progressing rapidly, the proliferation of the connective tissue was more marked, and chains of fibroblasts, many of them showing mitotic figures, were present. At the same time, angioblastic proliferation was evident at the border of the lesion. The mitotic activity in the deeper myocardial fibers was more abundant.

Three and 4 days after the operation, the defect in the heart muscle, which was being created by removal of the débris of damaged tissues,

was beginning to be filled in by fibroblastic and angioblastic proliferation. The macrophages were greatly increased in number and contained engulfed granules of blood pigment and cellular débris. The polymorphonuclear leukocytes were more abundant, and lymphocytes and plasma cells were present. Deposits of calcium salts in damaged heart muscle fibers had already formed. The deeper, less damaged myocardial fibers continued to show regenerative activity in the form of mitotic figures.

Five and 6 days after the operation the necrotic tissues were almost completely removed. This was true particularly in those animals in which adhesions to the costal wall had formed, due probably to a better blood supply to the damaged area provided by new-formed blood vessels in the adhesions. Connective tissue and angioblastic tissue was filling the defect, forming a network in which pigment-laden phagocytes and mononuclear cells were present. The surviving heart muscle fibers at the edges of the defect showed evident signs of regeneration such as the formation of thin processes at their ends which were beginning to progress into the network of connective tissue, the presence of thin, deeply eosinophilic muscle fibers with columns of two or three nuclei, the fusion of muscle fibers forming cytoplasmic masses with several nuclei, the longitudinal splitting of cytoplasm and nuclei of many myocardial fibers, and abundant mitotic figures in all phases of development. All the findings described by Heller[1] and Warthin[2] in diphtheritic myocarditis, as well as abundant mitotic figures in myocardial fibers in the neighborhood of the lesion, were seen in these experimental heart lesions. Mitotic figures in myocardial fibers were seen in the deeper, less damaged fibers at 24 hours after the injury.

During the period from 6 to 14 days after the operation, the necrotic tissue was completely removed. The connective tissue now filled the defect and progressively became adult, with collagen fibers beginning to appear at the 13th day. The ends of the surviving myocardial fibers continued to progress into the new-formed connective tissue and, in some cases in which adhesions to the costal wall were formed, newly formed myocardial fibers were seen, progressing into the adhesions several millimeters beyond the actual border of the heart (Fig. 12). Regenerative activity was seen in all injured hearts during this period, but by the 13th day it was greatly decreased.

Adult Scar. Between 15 and 20 days after the injury, the connective tissue of the scar became of almost adult type, with more abundant collagen and elastic fibers giving some retraction. A few blood pig-

ment-laden phagocytes and some other mononuclear cells were present. Bundles of previously formed myocardial fibers were seen progressing into the scar. Regenerative activity of myocardial fibers apparently had ceased. Limesalt deposits in the scar tissue were seen in several cases. Hearts with parietal adhesions showed abundant blood vessels passing from the costal wall through the adhesions to the myocardium.

From 20 days until 75 days after the injury the scar showed few further changes. The connective tissue filling the defect included abundant collagen and elastic fibers. A few lymphocytes and plasma cells were infiltrating the area even 75 days after the injury, and in some cases a few mast cells were present. Limesalt deposits occurred frequently in the scar, and a few giant cells of foreign body type were seen around them. In almost all of these hearts, bundles of myocardial fibers, which had formed during the period of regeneration, were found bridging the scar. Other muscle fibers were embedded in the scar tissue but did not pass through it and some protruded several millimeters into the adhesions beyond the original epicardial level (Fig. 13).

Discussion

Lesions produced by the application of a piece of heated metal a few millimeters in diameter have been used previously for the study of regeneration of voluntary muscle[37] and cardiac muscle.[35] The advantages are: a fairly uniform lesion can be produced; the difficulties occasioned by the beating of the heart tend to be overcome; and a gradient in respect to degree of damage results, since the deeper fibers suffer less injury, which makes it possible to study several phases of repair and regeneration in each specimen.

The present investigation established that the healing of experimental lesions of this type in young rats proceeds more rapidly than the healing of experimental myocardial infarction in dogs as described by Karsner and Dwyer,[6] and much faster than the healing of human myocardial infarction.[25] This accelerated healing is probably due to a greater power of reparative and regenerative proliferation in young animals and, particularly, to the fact that the myocardial circulation suffers no significant damage. The speed of healing is increased when adhesions to the costal wall are formed, due to the better blood supply thus provided.

The removal of necrotic tissues begins as early as 12 hours after the injury and is completed by the 6th day. Polymorphonuclear leukocytic infiltration is seen in the area of the lesion after 12 hours. It increases in intensity in the next few days and disappears about the

6th or 7th day after the injury. Lymphocytic infiltration begins 48 hours after the injury and is present even 75 days after the operation. Macrophages are observed in the area of the lesion 24 hours after the injury; they contain small fragments of necrotic tissues and blood pigment, and are a constant finding until the 13th day.

Proliferation of the interstitial connective tissue is found 24 hours after the injury at the edges of the lesion and is made evident by fibroblasts showing mitotic figures. They increase rapidly to form a network which progressively fills the defect. On the 13th day, collagen fibers begin to appear and between the 15th and 20th days the connective tissue is sufficiently mature to produce some retraction. Angioblastic proliferation is evident 48 hours after the lesion; it follows the proliferating connective tissue which becomes richly vascularized. When adhesions to the costal wall are formed, small blood vessels pass through them from the costal wall to the myocardium.

Limesalt deposits are formed very often in damaged myocardial fibers at the edges of the lesion, and also in the connective tissue filling the defect. In one heart they were observed as early as 3 days after the injury. This dystrophic calcification is more common when adhesions to the costal wall are not formed. This suggests that probably it is favored by relative local anoxia, which is doubtless more severe if there are no adhesions. Calcium deposits are seen also in the connective tissue of adult scars. The early calcium deposits in myocardial fibers give a positive von Kossa's reaction, establishing that the material is calcium phosphate; the older calcium deposits in the connective tissue of the scar give a negative reaction. Apparently they are not a phosphatic salt of calcium.

The most important finding is the evidence of myocardial regeneration, as demonstrated by mitotic figures in the nuclei of myocardial fibers, by amitotic division of cytoplasm and nuclei of heart muscle fibers around the lesion, and by the advancement of the ends of these fibers into the newly formed connective tissue and even beyond the actual border of the heart into fibrous adhesions between the heart and the costal wall. Myocardial mitotic figures are first observed 24 hours after the injury in the deeper, less damaged fibers of the lesion. They increase in number during the following days and reach a maximum on the 9th day. During this period they are very abundant, four or five being seen in a single low-power field (Fig. 4). Five days after the operation, mitotic figures are seen in the surviving muscle fibers at the edges of the lesion. Mitotic activity is greatly decreased by the 14th day and ceases after the 15th day. Amitotic division of heart

muscle fibers is seen very constantly between the 6th and 14th days. It is made evident by longitudinal splitting of the sarcoplasm and nuclei, and all stages of this process can be observed. Growth of the ends of the surviving myocardial fibers into the network of newly formed connective tissue is first seen 5 days after the injury. This is in the form of thin connective tissue. In older scars, bundles of these newly formed myocardial fibers are seen embedded in the scar tissue, or bridging the defect, or even protruding into the adhesions. Formation of multinucleated cytoplasmic masses ("muscle giant cells") is observed very seldom.

Further experiments are indicated to determine the age to which the regenerative capacity of myocardial fibers is maintained, and also to learn if it is possible to induce regeneration in adult myocardium by injection of extracts of embryonic hearts.

Summary

The literature concerning regeneration of the myocardium is reviewed.

Experimental myocardial lesions were produced in 58 rats, 4 to 7 days old, by the application of a red-hot wire. The animals were killed at intervals from 12 hours to 75 days after injury and the lesions were studied histologically.

Regeneration of myocardial fibers by both mitotic and amitotic division was observed in the period from 24 hours to 14 days after the injury. Newly formed fibers progressed into the connective tissue laid down in the lesion and into the fibrous adhesions to the costal wall which were formed in some of the animals.

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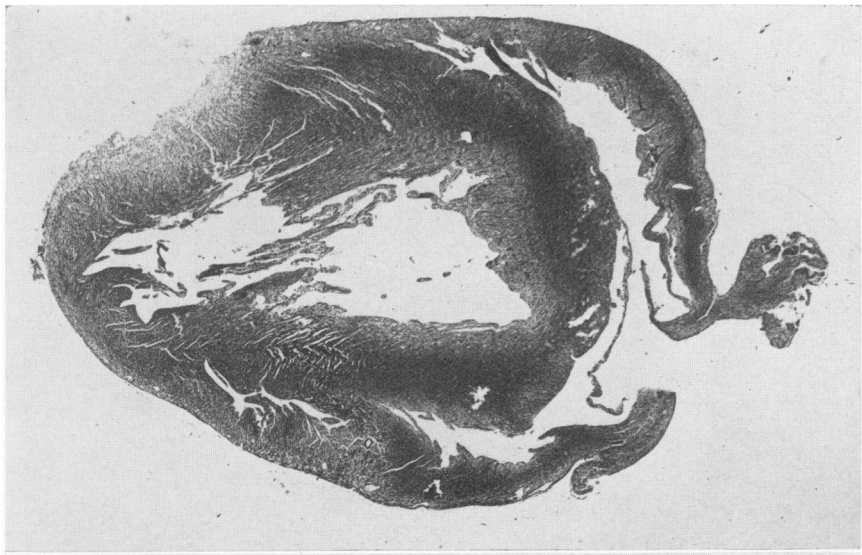
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Heart of rat 35, sacrificed 2 days after the injury. Defect on the anterior surface of the left ventricle near the tip. Hematoxylin and eosin stain. × 15.

FIG. 2. Heart of rat 17, 7 days after the injury. Lesion on the anterior surface of the left ventricle near the tip, firmly attached to the costal wall, which is represented by two pieces of cartilage, voluntary muscle, and connective tissue. Hematoxylin and eosin stain. × 10.

FIG. 3. Rat 2, 5 days after the injury. Calcium deposits in necrotic myocardial fibers at the border of the lesion. Von Kossa's reaction, counterstained with safranine. × 100.



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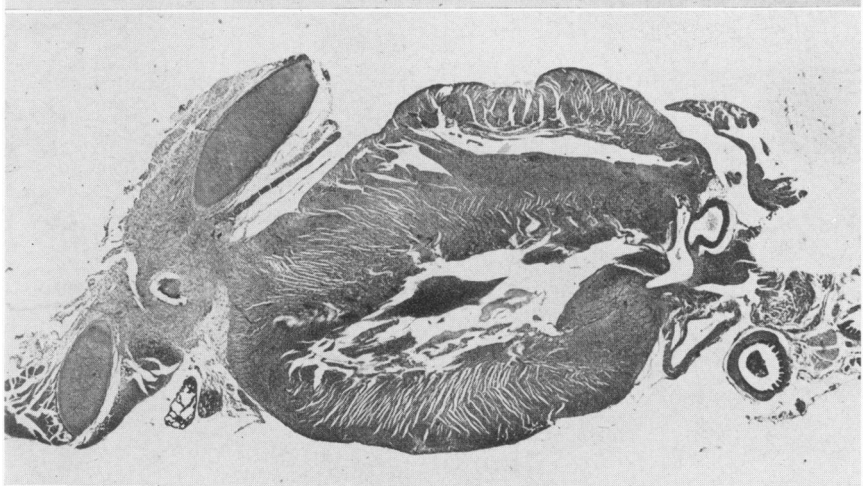

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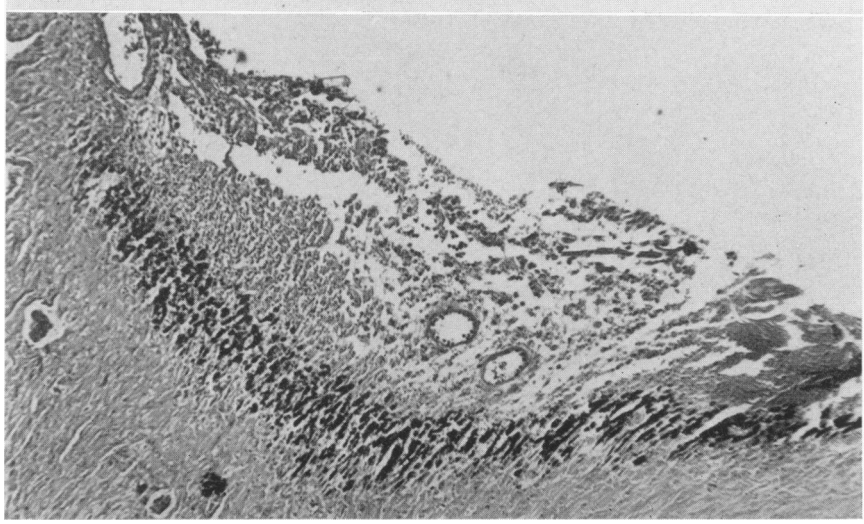

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FIG. 4. Rat 37, 9 days after the injury. Four mitotic figures in myocardial fibers adjacent to the lesion. Masson's trichrome stain. × 420.

FIG. 5. Three of the four mitotic figures of Figure 4 at higher magnification. The chromatin appears as rods and granules. The cytoplasm at the site of nuclear division is clear and granular. Masson's trichrome stain. × 1,350.

FIG. 6. Rat 26, 4 days after the injury. Mitotic prophase in the nucleus of a myocardial fiber, near the lesion. Masson's trichrome stain. × 1,350.

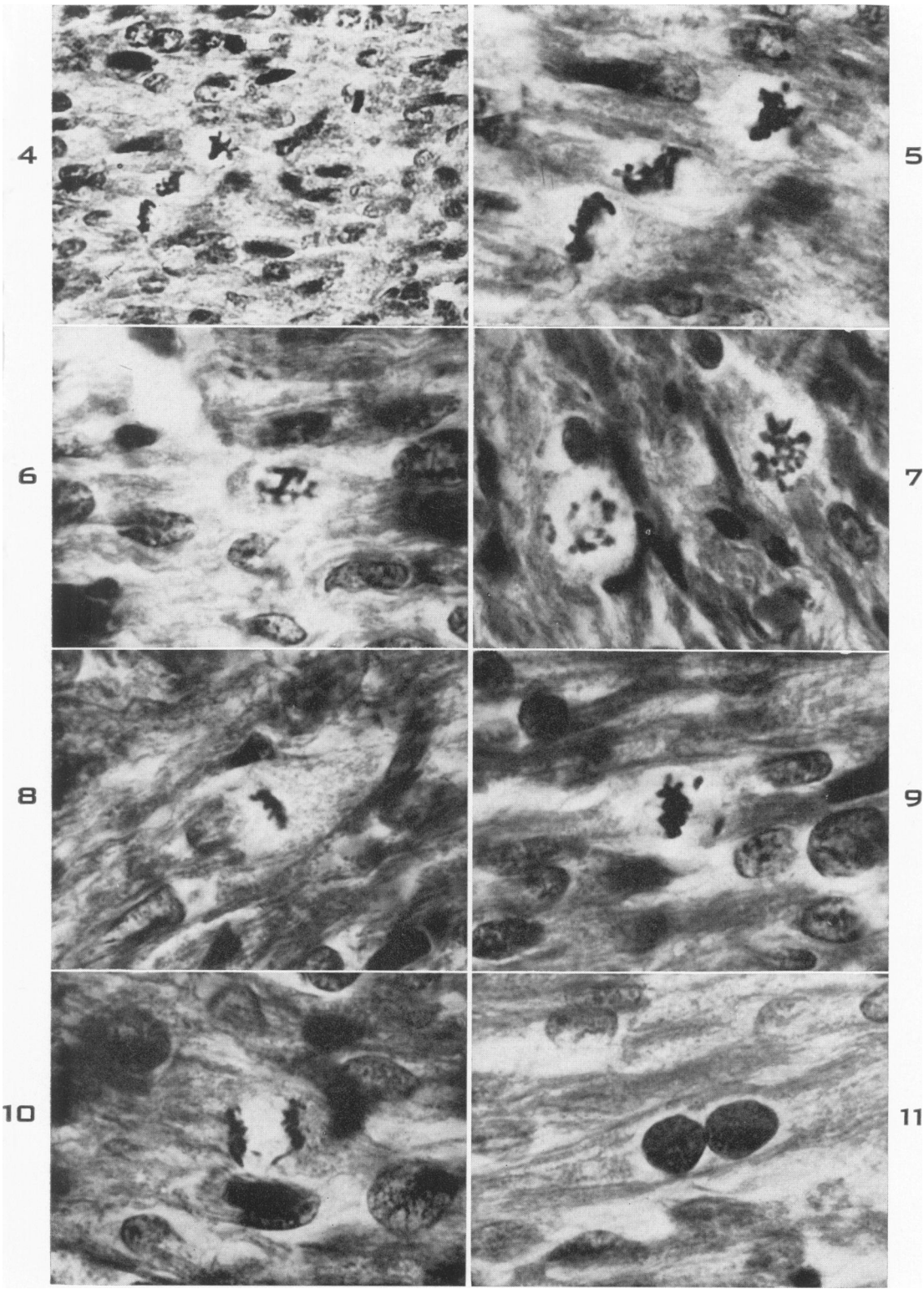
FIG. 7. Rat 7, 7 days after the injury. Metaphase in the nuclei of two myocardial fibers near the lesion. Masson's trichrome stain. × 1,350.

FIG. 8. Rat 36, 2 days after the injury. Mitotic figure in a myocardial fiber located in the deeper portion of the lesion. Masson's trichrome stain. × 1,350.

FIG. 9. Rat 40, 13 days after the injury. Mitotic figures and vesicular nuclei in myocardial fibers near the lesion. Masson's trichrome stain. × 1,350.

FIG. 10. Rat 16, 5 days after the injury. Anaphase in the nucleus of a myocardial fiber. Masson's trichrome stain. × 1,350.

FIG. 11. Rat 37, 9 days after the injury. Myocardial fiber in a zone adjacent to the lesion, showing two nuclei, probably after mitotic division. Masson's trichrome stain. × 1,350.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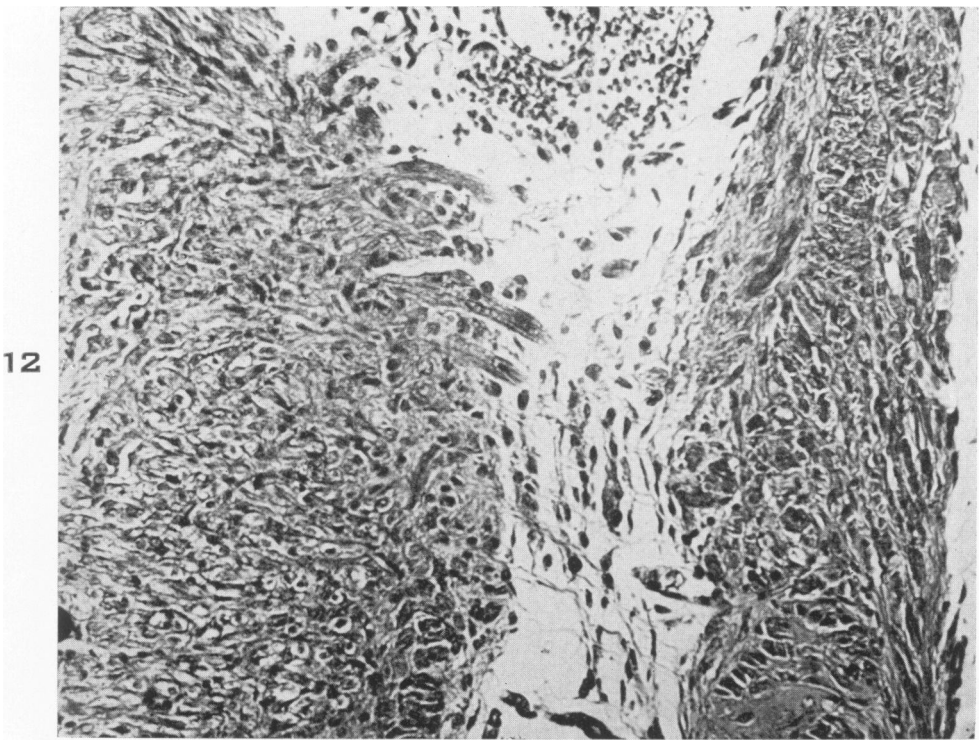
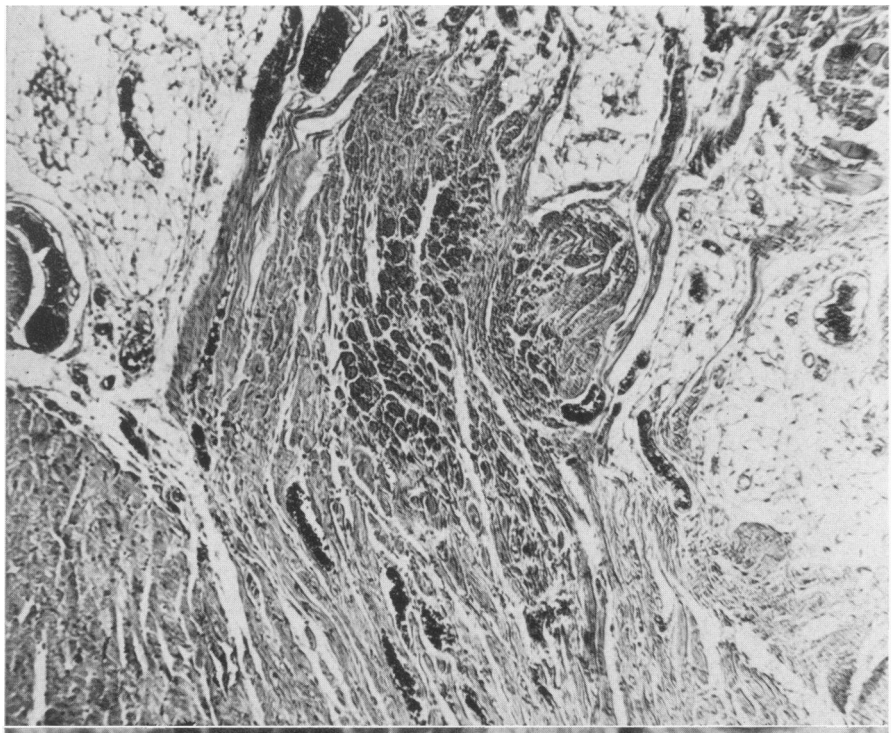


FIG. 12. Rat 40, 13 days after the injury. The heart is at the left, the costal wall at the right; between them are young fibrous connective tissue adhesions. Several myocardial fibers are progressing into the adhesions beyond the actual border of the heart. Masson's trichrome stain. × 330.

FIG. 13. Rat 58, 65 days after the injury. A large bundle of myocardial fibers protrudes beyond the border of the heart into the adhesions. Columns of blood vessels pass from the costal wall (upper right corner) through the adhesions to the myocardium. Masson's trichrome stain. × 330.

FIG. 14. Margin of the lesion of rat 44, 6 days after the injury. The ends of the surviving myocardial fibers show thin processes extending into the young connective tissue filling the defect. There is longitudinal splitting of some myocardial fibers. Masson's trichrome stain. × 700.



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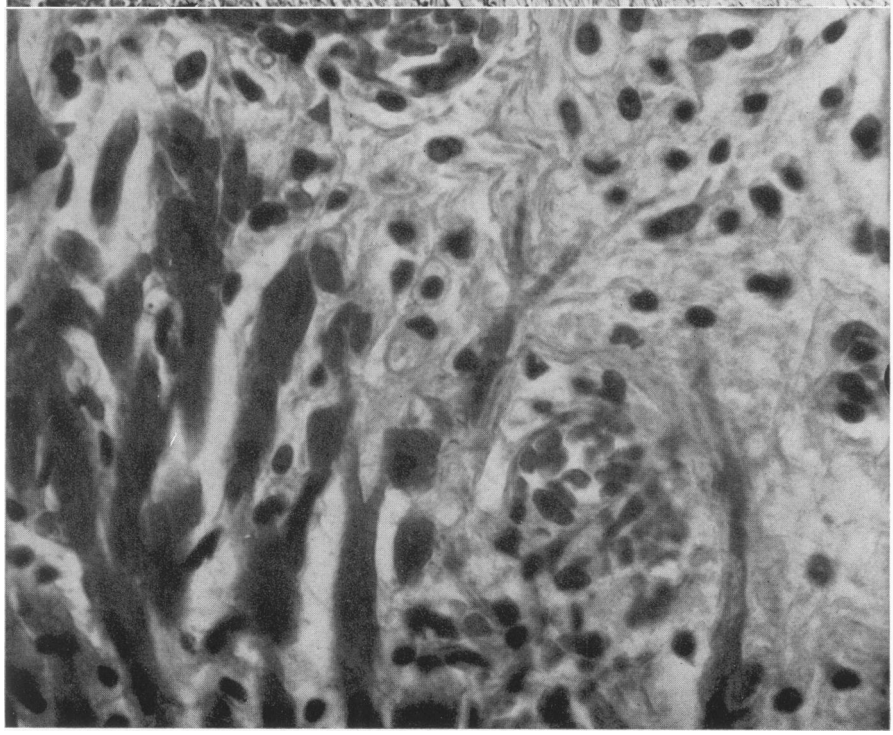

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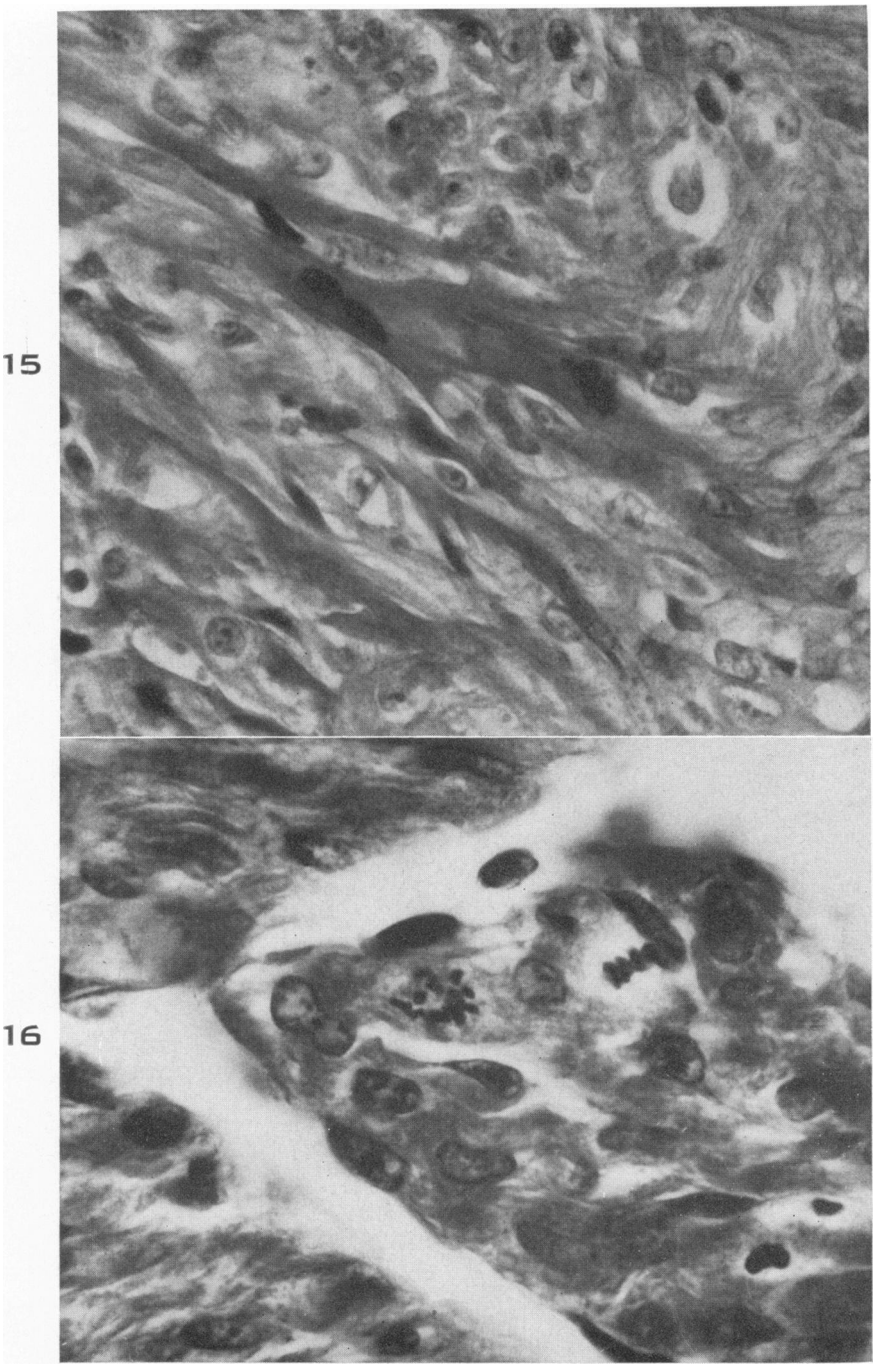

FIG. 15. An area of the margin of the lesion of rat 39. There has been fusion of several myocardial fibers to form a cytoplasmic mass containing several nuclei, one of which shows mitotic division. Masson's trichrome stain. × 700.

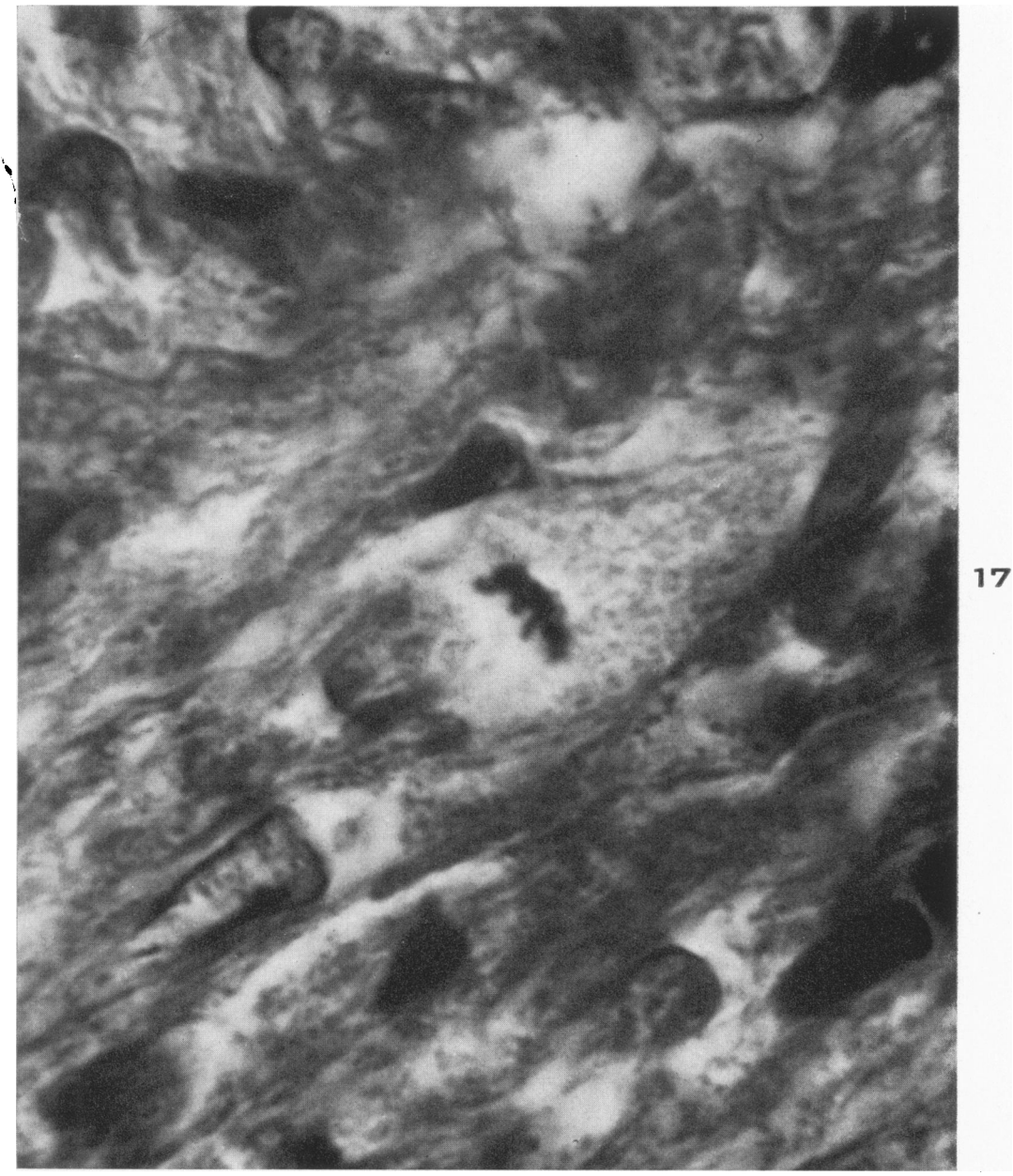

FIG. 16. Rat 37, 9 days after the injury. Two mitotic figures in a papillary muscle adjacent to the lesion. Masson's trichrome stain. × 1,350.

FIG. 17. Rat 36, 2 days after the injury. Higher magnification of Figure 8. The sarcoplasm at the site of nuclear division is clear and finely granular. Masson's trichrome stain. × 2,700.