SUCCINIC DEHYDROGENASE ACTIVITY IN MYOCARDIAL INFARCTION AND IN INDUCED MYOCARDIAL NECROSIS *

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In experimentally induced renal necrosis, histochemically demonstrable succinic dehydrogenase activity in damaged cells is either reduced or disappears completely.[1] Since the heart contains large amounts of this enzyme, it seemed of interest to ascertain whether similar changes in enzymatic activity occur in myocardial necrosis.[2] This report deals with profound changes in the dehydrogenase activity in ischemic heart muscle cells which take place only a few hours after the onset of ischemia. Similar changes could be demonstrated also in the hearts of rats in which necrosis of the myocardium was induced by various drugs.

A marked decrease of microscopically demonstrable dehydrogenase activity was recently described in the heart as well as in other organs of rats following bilateral adrenalectomy.[3] Since the reported findings in the hearts were similar to those in myocardial necrosis, it was deemed advisable to repeat these experiments. However, no such decrease in enzymatic activity in adrenalectomized rats could be detected.

METHODS

Human Material

Blocks were taken from 10 hearts of persons with typical histories of acute myocardial infarction. Blocks were secured also from hearts which at necropsy showed evidence of rheumatic heart disease, and from the hearts of individuals who succumbed to various diseases with no gross or microscopic evidence of cardiac involvement. Necropsies were performed 2 to 15 hours after death. Fresh frozen sections, generally 10 to 15 μ thick, were cut without fixation on a Sartorius microtome. This microtome is equipped with a special cooling device for the cutting knife and was found to be very useful for the preparation of thin frozen sections suitable for histochemical staining.[4] Sections were dipped in tap water for a moment and then immersed in the incubation mixture for 1 to 2 hours at 37° C. under aerobic conditions as previously described.[1] Sections were then washed in tap water, immersed for several hours in 10 per cent formalin, and mounted

* Received for publication, July 10, 1954.

on glass slides covered with glycerin. The coverglass was rimmed with nail polish. Such preparations were usable for many months, if kept in the dark. Occasional sections were counterstained with hematoxylin. In addition, adjacent tissue blocks were fixed in formalin and prepared for routine sections.

Animal Experiments

Cardiac lesions were produced by means of fluoracetate[5] and plasmocid (8-[3-diethylaminopropylamino]-6-methoxyquinoline)[6]* in young male and female rats of the Wistar strain. Fluoracetate was given in a dose of 3 mg. per kg. of body weight intraperitoneally. The injection was repeated after 24 hours and the animals sacrificed 4 hours later. Several other rats received 2 doses of 2 mg. each at an interval of 1 hour instead of the 3 mg. dose. Plasmocid was injected intraperitoneally in 2 divided doses of 6 mg. each with an interval of 1 hour. The animals were sacrificed 22 to 24 hours later. A few animals were permitted to live 48 hours.

Larger rats were used for adrenalectomy. Both adrenal glands were removed under light ether anesthesia. A second group of sham-operated animals was used as controls. In these, the adrenal glands were exposed and the wounds afterwards closed. All animals were given normal saline solution to drink and food ad libitum. They were sacrificed after 4 days. Tissues removed and stained for dehydrogenase activity included the heart, kidney, liver, and brain. In general it was sufficient to leave sections of the heart for 10 to 30 minutes in the incubation mixture.

RESULTS

Distribution in the Normal Human Heart

The adult human heart showed dehydrogenase activity only in muscle fibers (Fig. 1). Connective tissue, vessels, valves, endocardium, and pericardium remained unstained. The reduced tetrazolium was seen as rather coarse granules about 0.5 to 1.5 μ in diameter. The granules were arranged in parallel lines along the myofibrils. They covered the sarcoplasm almost completely, but the sarcoplasm remained unstained. Nuclei showed no activity.

Even in hearts from necropsies performed as late as 15 hours after death, satisfactory preparations could be obtained, although the formazan granules in these preparations sometimes were less intensely colored.

* Obtained from the Laboratory of Tropical Diseases, National Microbiological Institute, Bethesda, Md.

The Influence of Myocardial Infarction

Of the patients with acute myocardial infarction, one died 1½ hours; one, 2 hours; 3, 6 to 8 hours; one, 14 hours; and the remaining 4 patients, 36 to 140 hours after the onset of typical acute symptoms. In all cases, the coronary arteries showed severe sclerosis with marked narrowing. Fresh thrombotic occlusions were found in seven hearts. In all 5 cases in which the symptoms had lasted longer than 8 hours, typical, grossly recognizable infarcts were found. In the 3 cases in which the time interval between onset of symptoms and time of death was between 6 and 8 hours, areas of congestion and slight discoloration suggested the sites of infarction. In the hearts of the 2 individuals who died 1½ and 2 hours, respectively, after the onset of clinical symptoms, no distinct gross findings of acute necrosis were seen. All hearts, however, showed myocardial fibrosis to a varying degree.

In the heart of the patient who survived only 1½ hours, microscopic evidence of acute necrosis was not seen. There were, however, extensive areas of old fibrosis as well as foci in which granulation tissue had replaced dead muscle fibers. These areas were devoid of dehydrogenase activity. In some of the apparently undamaged muscle fibers in the vicinity of these abnormal areas, moderate reduction of dehydrogenase activity was seen.

In the heart of the patient who survived for 2 hours, routine staining showed only a few focal areas of myocardial necrosis. In these necrotic fibers dehydrogenase activity had completely disappeared. Reduction of enzymatic staining, however, was seen also in many muscle fibers that showed no unequivocal microscopic evidence of damage with routine stains (Fig. 2). In some of these the reduced tetrazolium was not deposited in the usual granular form but rather in irregularly shaped, larger particles. The cytoplasm often stained pink. In many muscle fibers with reduced deposition of dye, cross striation appeared entirely normal.

In the hearts from two patients who survived the acute attack for 6 to 8 hours, the changes observed were similar to those described. In the third case, however, necrosis of muscle fibers was extensive on routine staining and in addition there was considerable infiltration by polymorphonuclear leukocytes within the areas of necrosis. The necrotic fibers showed complete loss of enzymatic activity. It is probable that in this instance the onset of myocardial infarction antedated the clinical symptoms, since Mallory and his co-workers[7] have shown that diffuse infiltration by polymorphonuclear leukocytes begins after

about 5 hours but will not become extensive before 24 hours have elapsed.

In the remaining cases in which myocardial infarction had taken place 14 to 140 hours before death, there was extensive necrosis of muscle fibers with complete inactivation of the enzyme (Fig. 3). Deposition of dye was not noticed in polymorphonuclear leukocytes or in other inflammatory cells. In all of these hearts, areas of scarring, subacute fibrosis, and subacute myomalacia showed a complete lack of enzymatic activity. Within areas of subendocardial fibrosis in the hearts of these patients as well as in those with rheumatic heart disease, surviving muscle fibers showed a varying but often reduced degree of enzymatic activity (Fig. 4).

The Effect of Fluoracetate and Plasmocid on the Heart of the Rat

Following the use of fluoracetate and plasmocid, focal areas of myocardial degeneration were seen. These were often located beneath the endocardium. In general, damage was considerably more marked with plasmocid. In several rats that were sacrificed within 24 hours, the damage was discernible in preparations stained with hematoxylin and eosin by some increase in the eosinophilia of the cytoplasm, occasional foci of liquefaction necrosis, and proliferation of mononuclear cells (Fig. 5). In the hearts of animals that survived up to 48 hours, necrosis was much more marked and the proliferation of mononuclear cells more pronounced. Dehydrogenase activity in damaged fibers was either absent or markedly reduced (Fig. 6).

The Influence of Adrenalectomy

No significant decrease in dehydrogenase activity could be detected in the various organs, including the hearts of rats sacrificed 4 days following bilateral adrenalectomy, as compared with sham-operated and with unoperated normal control rats.

Comment

Seligman and his co-workers[8,9] were the first to draw attention to the staining pattern of succinic dehydrogenase in mammalian organs. They found that in various species, including the rat, mouse, dog, rabbit, guinea-pig, and hamster, the heart, kidney, and liver contained the larger amounts of stainable enzymatic activity. The myocardium of the human heart likewise revealed large amounts of stainable succinic dehydrogenase.

The abundance of this enzyme in the heart muscle of all species so

far examined indicates its fundamental importance for the metabolic process which takes place during cardiac contraction. It is now recognized that succinic dehydrogenase is one of the most important enzymes in biologic oxidation. By fractionation of the heart muscle into three components, (1) residue containing myofibril fragments, connective tissue, and nuclei, (2) sarcosomes, and (3) sarcoplasm, it was found that the highest specific activity of respiratory enzymes including succinic dehydrogenase is located in the sarcosome fraction.[10] Since sarcosomes (mitochondria) are arranged along the myofibrils in a longitudinal fashion, it is reasonable to assume that the granular deposits of reduced tetrazolium occur in the vicinity of these structures.

The loss of stainable dehydrogenase in muscle fibers undergoing necrobiotic changes due to anoxia is very striking. Diminution of enzymatic activity apparently exceeds changes which can be demonstrated by usual routine staining. Similarly, in experimentally induced renal necrosis, impending cell death is accompanied by loss of dehydrogenase activity.[1] In contrast to the kidney, however, there is no reappearance of dehydrogenase in necrotic areas in the heart since there is no regeneration of muscle cells. Reduction in dehydrogenase activity occurs also in muscle fibers that are slowly degenerating due to chronic anoxia.

The time relationship between the changes in dehydrogenase activity and gross or microscopic alterations in the myocardium following the exclusion of the blood supply could be studied in an experimental animal, since ligation of branches of coronary vessels can be performed without great difficulty in the dog.[11] For practical reasons, experiments of this type could not be carried out. However, a number of experimental procedures are known which will induce myocardial necrosis in smaller animals. The procedure chosen was the induction of acute myocardial damage by two chemicals, fluoracetate[5] and plasmocid.[6] The latter substance led to more consistent results in our hands. Plasmocid induced a varying, but often marked degree of myocardial damage within 24 hours. In damaged muscle fibers, the reduction of enzymatic activity appeared similar to that in the human heart following the occlusion of coronary vessels. In these experiments, as in those on renal necrosis, alterations in enzymatic activity were more widespread than the changes which could be detected with routine stains.

In contrast, no changes in dehydrogenase activity were found in the heart muscle following adrenalectomy. The different results obtained by us may well be explained by the different method of pre-

paring the sections for enzymatic staining. With thick sections incubated in a mixture without added activators, as used by Bourne and Malaty,[3] only inconsistent staining results were obtained. If, however, activators were added to the incubation mixture and thin sections prepared, no difference could be detected between preparations from adrenalectomized and control rats.

The resistance of histochemically demonstrable dehydrogenase to post-mortem changes is quite striking. In order to simulate conditions prevailing in human necropsy material, a portion of the heart was removed from 3 rabbits immediately after they were sacrificed. The thorax was then closed by sutures and the cadavers left for 3 hours at room temperature and then up to 24 hours in the ice box. No significant changes in the staining pattern could be seen in sections prepared from the heart immediately after death and in those prepared after termination of the experiment.

The fact that dehydrogenase activity remains active at least up to 15 hours after death clearly indicates that tetrazolium salts are not reduced by living tissues only, as was originally assumed. Indeed, under favorable conditions, even tissue extracts can reduce tetrazolium salts. Nevertheless, differences in tissue viability may be detected by their use.[12] It must be assumed that early post-mortem changes have considerably less damaging influence upon the succinic dehydrogenase system than the changes which occur during cellular death intravitam, regardless of the agent responsible for the cellular destruction. These observations clearly indicate that post-mortem changes and necrosis occurring during life cannot be discriminated by simple procedures.

From a practical point of view it is important to realize that post-mortem changes do not interfere with the staining reaction, at least for several hours. This enables one to prepare sections for testing succinic dehydrogenase activity not only from experimental material but also from that obtained by necropsy. Such preparations may be of help in evaluating the degree and distribution of myocardial damage.

Summary

The distribution of succinic dehydrogenase activity was studied in thin frozen sections of the human heart and of the heart of the rat under various experimental conditions. Only myocardial cells showed evidence of enzymatic activity. In post-mortem material, satisfactory staining could be obtained as long as 15 hours after death. In areas of myocardial necrosis following the occlusion of coronary vessels, de-

hydrogenase activity disappeared rapidly. Reduction of enzymatic activity was noticed as early as 2 hours after the onset of acute symptoms and extended to muscle fibers which showed no significant changes with routine staining techniques.

The administration of plasmocid and fluoracetate induced focal myocardial necrosis in the rat heart. In necrotic areas dehydrogenase activity was likewise reduced. In contrast, bilateral adrenalectomy had no influence upon dehydrogenase activity in the heart of the rat.

The use of the histochemical staining reaction for succinic dehydrogenase is suggested for the evaluation of myocardial damage under experimental conditions and in necropsy material.

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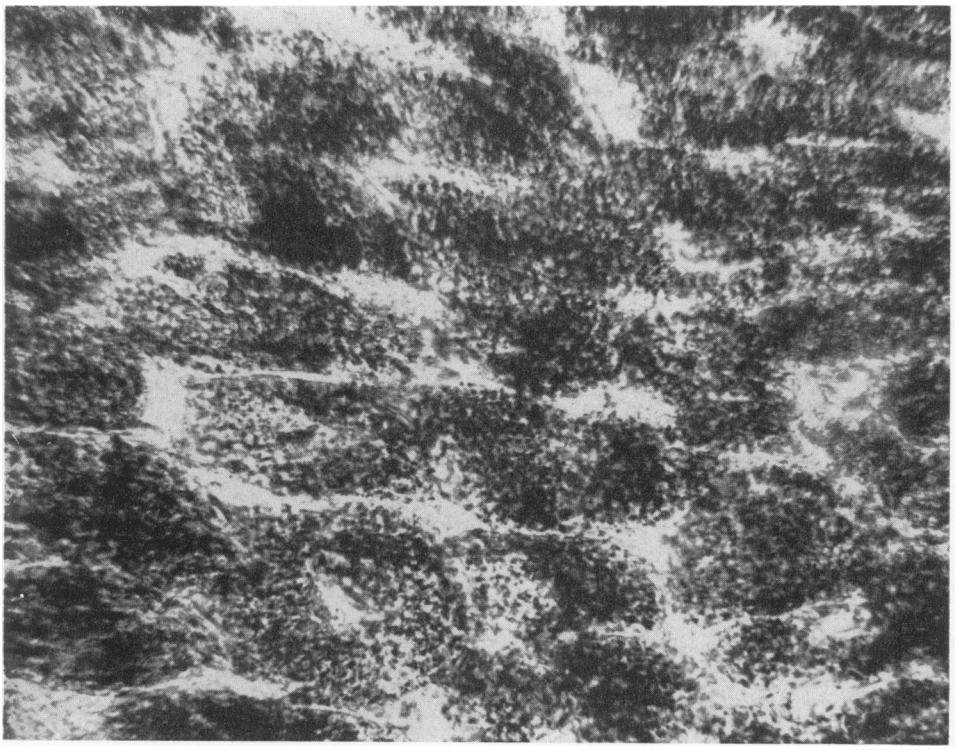
[*Illustrations follow*]

LEGENDS FOR FIGURES

All photomicrographs with the exception of Figure 5 are from fresh frozen sections cut at 10 to 15 μ and prepared for the demonstration of succinic dehydrogenase activity without counterstain.

FIG. 1. Human heart, left ventricle. Dehydrogenase activity is limited to myocardial cells. × 750.

FIG. 2. Section from the left ventricle of the heart of a 55-year-old man who died 2 hours after the onset of the typical clinical signs of acute coronary thrombosis. There is distinct diminution of the staining reaction in many myocardial cells. Sections of the adjacent areas stained with hematoxylin and eosin showed no evidence of acute necrosis. × 250.



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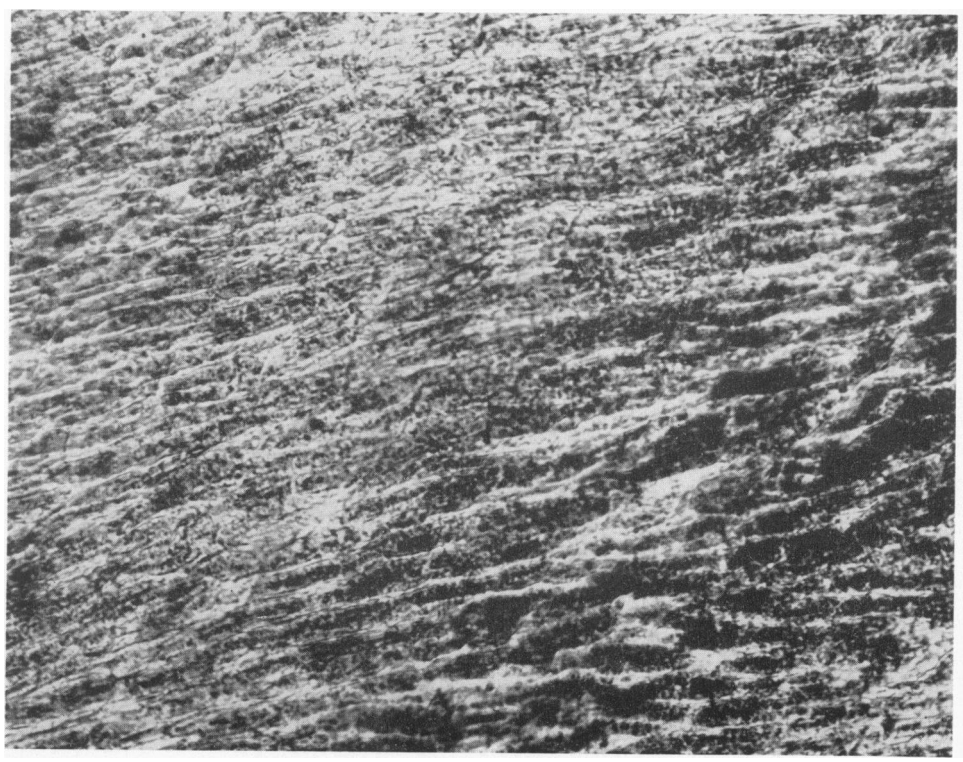

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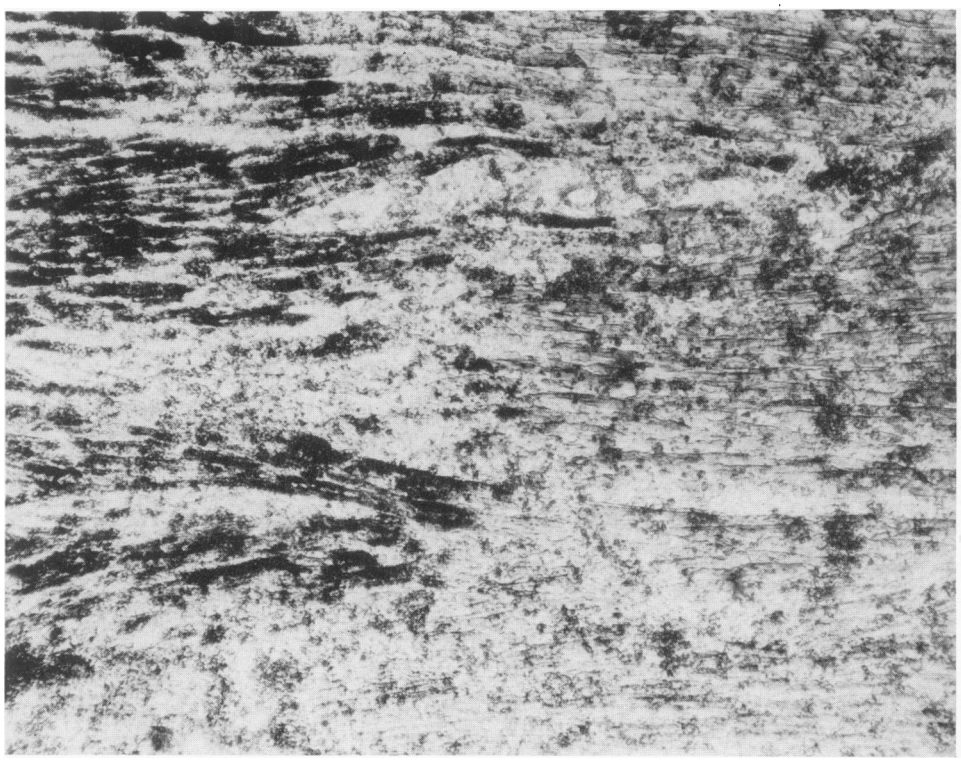
FIG. 3. Section from the left ventricle of a 49-year-old man, who succumbed 48 hours after the onset of the typical clinical signs of acute myocardial infarction. This section is from the edge of the grossly recognized infarct. There is complete loss of enzymatic activity in the necrotic muscle cells. × 125.

FIG. 4. Section through the subendocardial region of the septum of a 49-year-old woman with coronary heart disease who died of a cerebral hemorrhage. There is complete absence of staining in the fibrotic subendocardial area. Surviving muscle fibers show varying degrees of activity. × 125.



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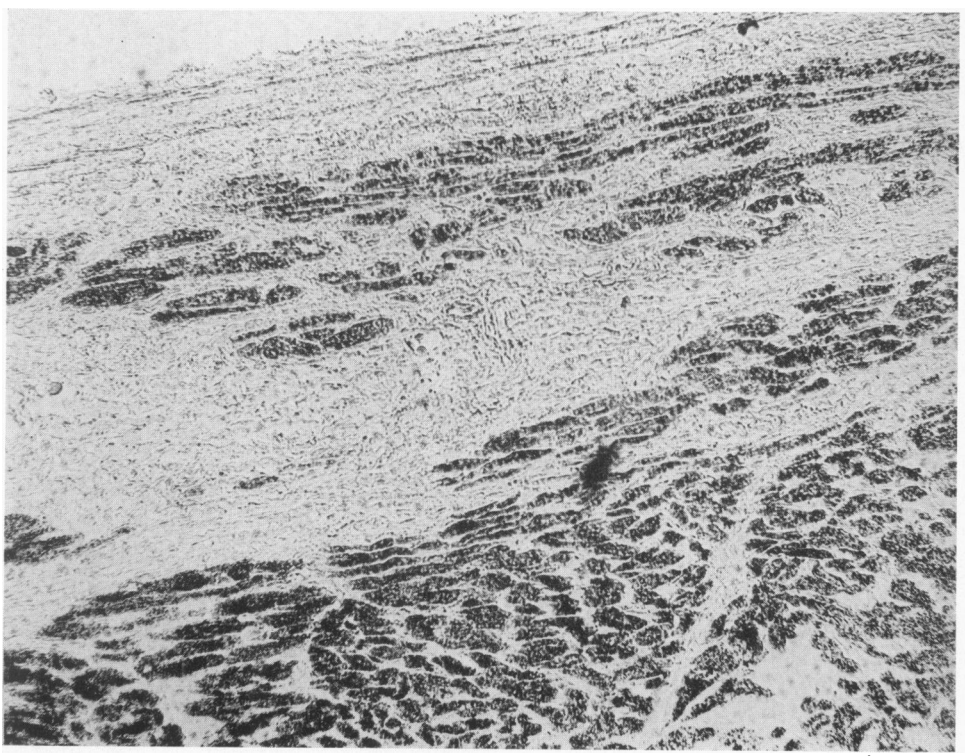

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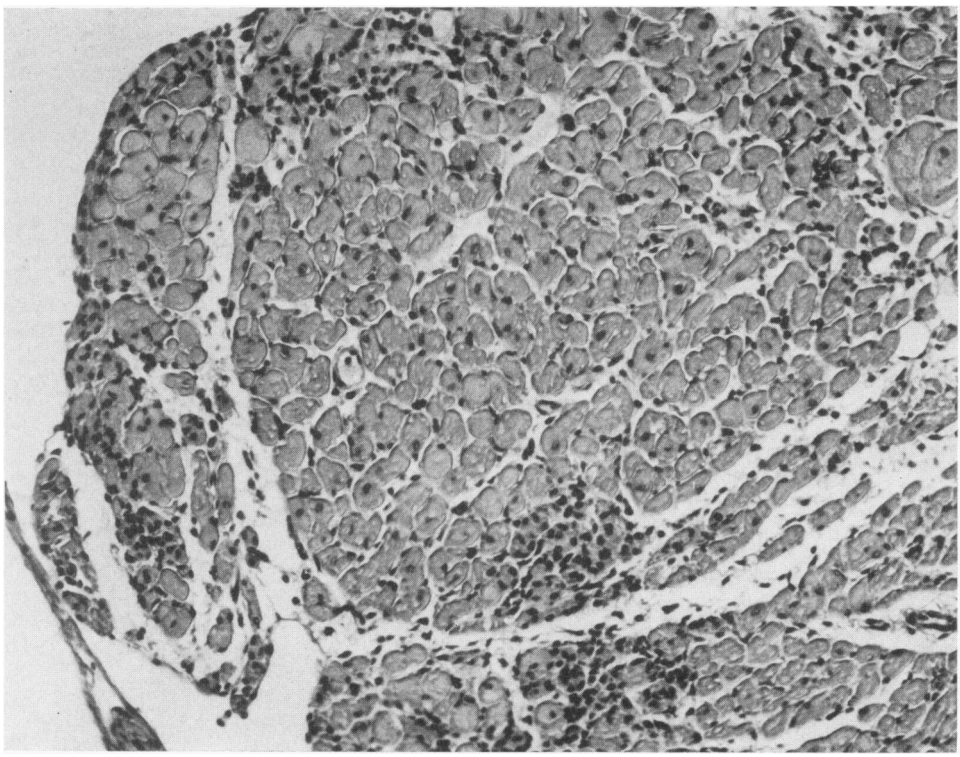
FIG. 5. Rat heart. The animal was sacrificed 22 hours after the second of two intraperitoneal injections of 6 mg. of plasmocid given 1 hour apart. There are focal areas of subendocardial myocardial damage and monocytic proliferation. Hematoxylin and eosin stain. × 250.

FIG. 6. Rat heart. Section was taken from an area of the heart adjacent to that shown in Figure 5. There is very extensive inactivation of the enzyme. × 250.



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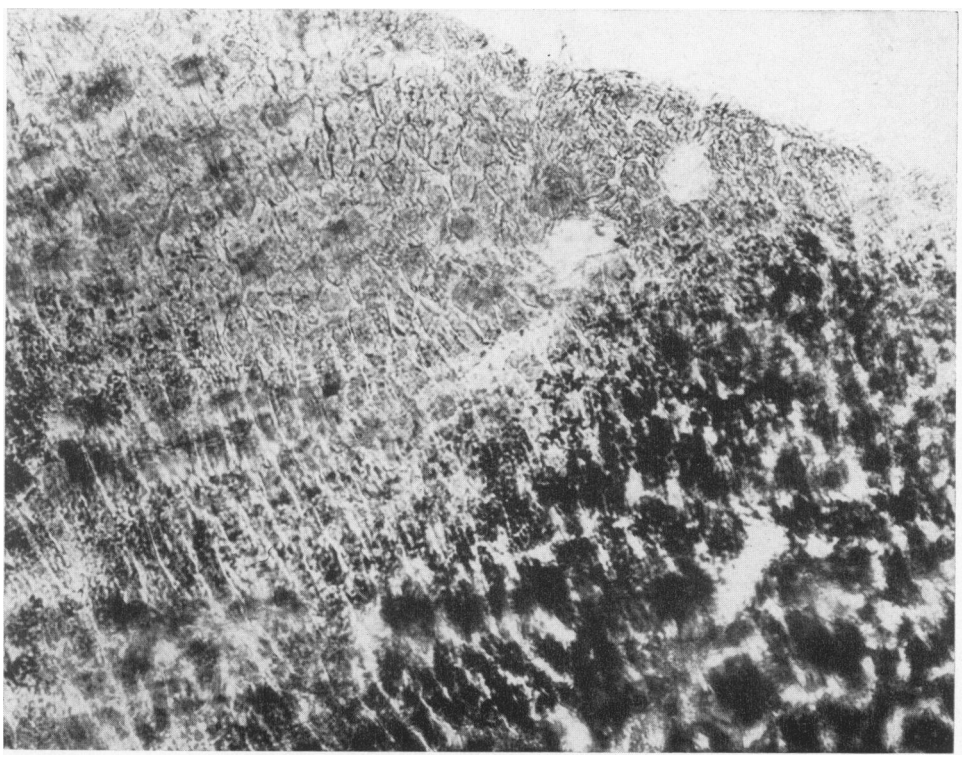

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