An Infarct-like Myocardial Lesion and Other Toxic Manifestations Produced by Isoproterenol in the Rat

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Progress in the study of myocardial necrosis has been handicapped by the lack of a simple and reliable method for the production of this lesion in experimental animals. The studies of investigators working to this end for nearly a hundred years have been reviewed recently by Taylor et al.[1] Surgical methods of producing coronary infarcts in the dog were disappointing because of the difficulty in achieving standardized lesions.[2,3] More recent improvements in technique[4-6] have resulted in greater uniformity in the lesions, but the methods are still expensive, time-consuming, and coupled with a high mortality. Although the rat is often more readily available than the dog, only a few authors have attempted to produce surgical lesions in this species.[7-9]

Many attempts were made in Germany, particularly during the late 30's, to produce the morphological equivalent in experimental animals of human angina pectoris. Much of this work centered in the Institute of Büchner.[10] These workers found that hemorrhagic shock,[11,12] orthostatic collapse,[13-15] or low atmospheric pressure[16,17] could produce disseminated cardiac necrosis in the rabbit. The lesions in these animals were small foci of necrosis and inflammatory reaction, particularly prominent in the subendocardial areas of the left ventricle and the papillary muscle. The mechanism by which the lesions were produced under these circumstances was nonspecific, since in other experiments similar lesions were described in this species after only forced restraint[18] or the administration of sodium chloride.[14] According to this school carbon monoxide poisoning,[18,19] anaphylactic shock,[14] insulin shock,[20] histamine shock,[13, 14,18,21] or toxic doses of pentylenetetrazol (pentamethylenetetrazol)[20] would also produce disseminated myocardial necrosis in the rabbit.

Other authors, working with the cat, were able to produce similar lesions in the heart after large doses of epinephrine,[18] vasopressin (Pitressin),[22] or digitalis.[23] Banting and his group were successful in producing myocardial necrosis in the dog and rat by the administration of large doses of acetylcholine[24,25] or by vagal stimulation of unanesthetized or lightly anesthetized dogs.[26] Horswell was unable to produce these lesions in the dog with acetylcholine.[27] Robertson et al.[28] produced a striking focal myocarditis with necrosis by the intravenous injection of papain to rabbits, rats, or mice.

Myocardial necroses related to coronary arterial lesions have been reported after corticotropin (ACTH) administration,[29] with adrenal-regeneration hypertension,[30] with renal hypertension,[31] or after coronary atherosclerosis produced in rats by dietary means.[32,33]

A new approach to the study of myocardial necrosis has been described by Selye.[34] Rats sensitized by the oral administration of monosodium phosphate developed myocardial necrosis following the injection of 2α-methyl-9α-chlorocortisol. Lesions were found in many areas of the heart in these animals but were most frequently located in the wall of the right ventricle and papillary muscles.

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In a recent preliminary publication [35] we have described the production of myocardial necrosis in the rat by a wide range of doses of isoproterenol (1-[3′,4′,-dihydroxyphenol]-2-isopropylaminoethanol) hydrochloride (DIH). This paper describes in detail the gross and microscopic lesions in these animals. It is evident from these data that careful choice of dosage of this compound allows the production of standardized myocardial lesions of predictable severity.

Material and Methods

The animals used in this study were male Wistar rats weighing an average of 258 ±10 gm. and females weighing 200 ±4 gm. They were housed under controlled conditions of temperature and humidity and fed a commercial rat food. As a basis for the choice of doses used in subsequent tests an acute toxicity test was performed to determine the median lethal dose (L. D_{50}). DIH was administered subcutaneously in aqueous solution at four dose levels to groups of 10 animals. The mortality rate present in the groups at 24 hours was recorded and used to calculate the L. D_{50} by the method of Litchfield and Wilcoxon.[36] In the remaining experiments, starting with the L. D_{50}, 16 gradually decreasing doses were tested, each dose being one-half of the previous one. The injections were given subcutaneously to five male and five female rats on two consecutive days; control rats received distilled water. Forty-eight hours after the first injection the rats were killed and autopsied. The hearts were removed and weighed, and the gross lesions were graded according to the following system: Grade 0: no lesions; Grade 1: mottling of the apex and distal parts of the left ventricle caused by intermingled pale and dark red streaks; Grade 2: well-demarcated necrotic areas limited to the apex; Grade 3: large infarct-like necrosis involving at least one-third of the left ventricle and extending to adjacent areas of the interventricular septum and right ventricle; Grade 4: large infarct-like necrosis involving more than half of the left ventricle interventricular septum and extending to the distal portion of the right ventricle.

After fixation in Bouin's fluid frontal sections of the heart, which included both auricles, ventricles, and interventricular septum, were embedded in paraffin. Sections cut at 5μ were stained with hematoxylin and eosin, Cason's trichrome,[37] and periodic acid Schiff (P. A. S.).[38] Frozen sections of the heart were stained with Sudan IV. Microscopically the hearts were graded as follows: Grade 0: no lesions; Grade 1: focal lesions of the subendocardial portion of the apex and/or the papillary muscle, composed of fibroblastic swelling or proliferation and accumulation of histiocytes; Grade 2: focal lesions extending over wider areas of the left ventricle, with right ventricular involvement (lesions included also edema, mottled staining, fragmentation, and segmentation of muscle fibers); Grade 3: confluent lesions of the apex and papillary muscles, with focal lesions involving other areas of the ventricles and the auricles (The lesions included vacuolar and fatty degeneration, granular disintegration, and hyaline necrosis of the muscle fibers. Marked capillary dilatation was present with hemorrhages. Extensive edema, occasionally with a mucoid component, caused sequestration of muscle fibers.); Grade 4: confluent lesions throughout the heart, including infarct-like massive necrosis, with occasionally acute aneurysm or mural thrombi. (The latter lesions were usually apical but also occurred in the papillary muscles or right ventricle. The lesions were similar in character to those in Grade 3.) Histological sections were also made of other organs in which gross lesions were observed, and in two additional animals from each group sections were made of the brain, pituitary, lung, liver, kidney, adrenal, and pancreas.

Results

The L. D_{50} of DIH after subcutaneous administration to the rat was found to be 680 mg. per kilogram. When this dose was administered to a group of rats on two consecutive days the mortality was 80%. Doses of 340, 170, and 85 mg. per kilogram caused deaths of 90%, 50%, and 10% of the animals, respectively, during the experimental period. Sporadic deaths occurred at lower doses: one at 21.2 mg. and two at 1.3 mg. per kilogram. These deaths occurred in animals with particularly severe heart lesions. The animals which died at doses of 170 mg. per kilogram or greater also revealed liver and kidney necrosis, hydrothorax, and hemorrhagic lung edema.

The animals which received doses of 5.25 mg. per kilogram or greater showed characteristic symptoms after injection. Within 20 minutes of treatment, the animals assumed unusual postures. Some lay on their side or back, with the head extended; others supported themselves on the side of the cage and extended their head and front legs; in either position they breathed with their mouth open. Respirations were dysp-

TABLE 1.—*Incidence of Gross and Microscopic Heart Lesions in the Rat After Treatment with 1-(3,4-Dihydroxy Phenyl)-2-Isopropyl Amino Ethanol HCl*

	Grade	Dose, Mg/Kg. 680	340	170	85	42.5	21.2	10.5	5.25	2.6	1.3	0.65	0.33	0.16	0.08	0.04	0.02	Control
Animals showing gross lesions, No.	0	1	1	1	--	--	--	--	--	2	--	4	6	5	6	10	10	10
	1	--	--	--	--	--	--	--	--	1	3	2	2	3	2	--	--	--
	2	1	2	1	--	3	3	6	5	6	6	4	2	1	2	--	--	--
	3	1	--	1	4	4	4	4	3	1	1	--	--	--	--	--	--	--
	4	7	7	7	6	3	3	--	1	1	--	--	--	--	--	0	0	0
Average severity Heart *		3.3	3.2	3.3	3.6	3.0	3.0	2.4	2.5	1.8	1.8	1.0	0.6	0.5	0.6	0.452	0.473	0.342
		--	--	--	0.607	0.557	0.593	0.535	0.519	0.481	0.427	0.464	0.410	0.429	0.432	--	1	10
Animals showing microscopic lesions, No.	0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	4	--
	1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	2	3	1	3	--	--	2	--	--	3	4	5	5	7	9	10	5	--
	3	2	4	--	--	2	1	6	6	4	4	4	3	3	1	--	--	--
	4	5	5	7	10	8	7	4	4	3	2	1	2	--	--	--	--	--
Average severity		3.2	3.4	3.4	4.0	3.8	3.5	3.4	3.4	3.0	2.8	2.6	2.7	2.3	2.1	2.0	1.4	0

* Expressed in gm. per 100 gm. body weight. Values are given only for those animals which survived 48 hr.

neic, irregular, and abdominal. Heart beats were strong at first and later weak and very rapid. At doses greater than 21.2 mg. per kilogram there was a reddish foamy exudate from the nostrils and corneal opacities. Many of these animals also had marked edema of the head, neck, chest, and front legs.

Marked lesions occurred outside the heart at dose levels of 170 mg. per kilogram and greater. These consisted of centrolobular liver necrosis and hemorrhage in 40% to 50% and cortical ischemia or necrosis of the kidney in 10% to 40% of the rats. In all animals, marked congestion with petechiae occurred in the abdominal viscera and leptomeninges and swelling of the brain was present. Pulmonary edema and hemorrhage, as well as hydrothorax, occurred in 40% to 90% of the rats receiving these higher doses.

At doses between 85 and 1.3 mg. per kilogram, no necrotic changes were observed outside the heart, but congestion of the liver, spleen, kidney, gastrointestinal tract, and leptomeninges, with cerebral swelling, was still observed in 30% to 100% of the animals at these doses. Pulmonary edema was occasionally observed in animals treated at doses between 85 and 21.2 mg. per kilogram.

At autopsy the hearts of most of the animals receiving DIH were obviously enlarged; when weighed this increase in size was found, as shown in Table 1, to be significant for those groups receiving doses of 5.25 mg. per kilogram or more. At dose levels lower than this the heart enlargement was less marked.

The cardiac necrosis was usually located at the apical area of the heart; however, when the lesions were severe the greatest part of the left ventricle and the apical portion of the right ventricle were also involved. Macroscopic lesions were occasionally seen in the auricles as indistinct, poorly demarcated, small, longitudinal, yellow streaks. The necrosis of the ventricles, which developed during the 48 hours of the experimental period, consisted of dry,

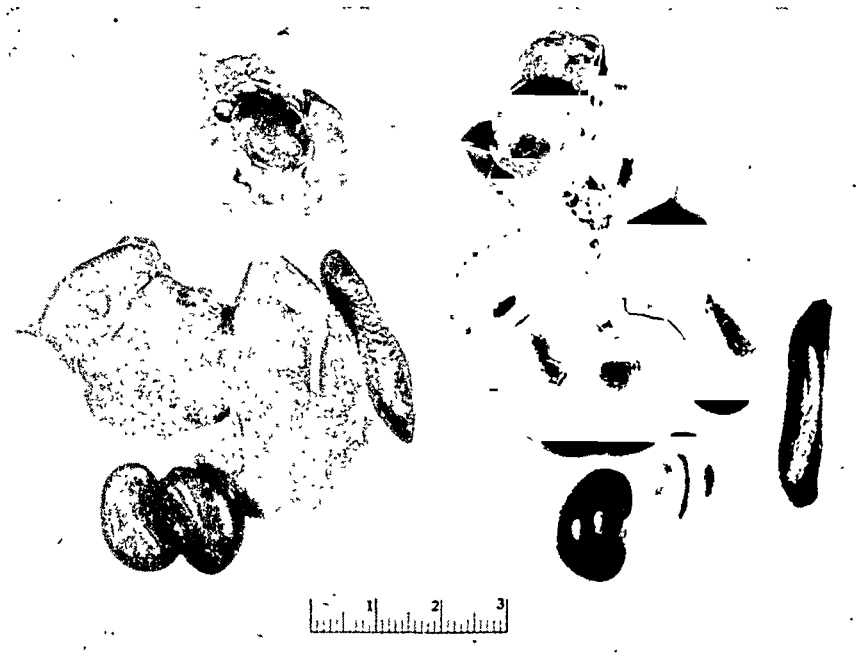
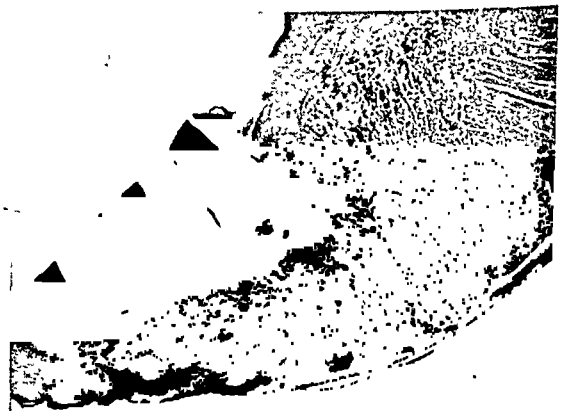


Fig. 1.—Heart, lungs, spleen, liver, and kidneys (left) from a control rat and (right) after 85 mg. of isoproterenol hydrochloride per kilogram subcutaneously on two consecutive days. Necrosis is evident at the apex of the heart, with severe congestion of the lungs and abdominal viscera.

granular, friable, pale grayish-yellow areas, occasionally surrounded by dilated veins and hemorrhage. The cut surface of large infarct-like lesions was homogeneous; however, smaller lesions revealed a mottled or striated cross section. The areas of necrosis of the ventricular wall appeared to be thin and bulging, compared with the surrounding areas which were thickened.

The group treated with 85 mg. per kilogram showed the most marked cardiac enlargement (0.607 gm/100 gm. of body weight). This group revealed also the severest cardiac necrosis, with an average grade of severity in the gross of 3.6, as illustrated in Figure 1. Nine of the rats in this group had infarct-like myocardial lesions involving the apex, the lower part of the left ventricle, the interventricular septum, and occasionally the right ventricle. Groups treated with higher doses had relatively less-pronounced cardiac changes, but 40% to 50% of these animals died after only one treatment with DIH, and the time lapse was not sufficient for development of lesions of maximal severity. Animals which survived long enough, however, manifested large infarcts of hemorrhagic character; in four animals mural thrombi were also seen in acute aneurysms of the left ventricle. The groups treated with doses between 42.5 mg. and 5.25 mg. per kilogram had 100% incidence of cardiac necrosis, with a decrease in size as the dose of the drug was reduced. Rats treated with doses of 2.6 mg. to 0.08 mg. per kilogram also manifested infarct-like apical necrosis; however, gross necrosis was observed only in 20% to 50%. In other instances there was a mottling of the distal portion of the left ventricle, caused by intermingled pale and dark red streaks. Only the groups treated with the 0.04 and 0.02 mg. per kilogram doses and the control group were free from gross cardiac lesion.

Fig. 2.—Necrosis involving the whole thickness of the left ventricle, causing acute aneurysm and mural thrombus. Rat was treated with 680 mg. per kilogram of DIH. Cason's trichrome; ×20.



Microscopic Picture of Heart Lesions.—Microscopically the necroses were focal, confluent, or massive and infarct-like. The latter were found most frequently in the apex at dose levels from 680 mg. to 0.33 mg. per kilogram. The necrosis often involved the whole wall (Fig. 2), but in other cases at lower doses only a partial thickness was necrotic. In the latter case the subendocardial sheet was more involved than the subepicardial portion of the myocardium. At a dose of 85 mg. per kilogram massive necrosis was observed in the apex and/or the papillary muscles and adjacent subendocardial portions of the left ventricle and less frequently the right ventricle in nine rats. The cardiac infarcts, observed grossly, were found on microscopic examinations to be either massive or confluent necrosis. Confluent necrosis differs from the massive lesion in the respect that some relatively unaffected muscle fibers are present in the necrotic areas. Generally muscle fibers lying near the capillaries showed less histolysis than muscle bundles which were located further from the blood supply. This characteristic of the lesion gave a variegated appearance to the confluent necrosis. It had the same localization as the massive lesion; however, unlike the massive infarct-like necrosis, confluent necrosis was also found in the auricles. It was seen in all rats treated at doses from 85 mg. to 10.5 mg. per kilogram. At higher doses the focal necroses were located in the basal portion of the left ventricle, the right ventricle, and the auricles. They may show the same histological characteristics as the confluent lesion; however, the necrosis of the muscle fibers was less pronounced and the reactive changes predominated. At lower dose levels the focal lesions were observed at the apex and papillary muscle of the left ventricle. They were composed of edema, histiocytes, swelling, and proliferation of the fibroblasts, but regressive changes of the muscle fibers were generally mild or absent.

Components of Myocardial Lesions.—The heart lesions had histological components which lent themselves to being grouped into

TABLE 2.—*Incidence of Histological Components of the Myocardial Lesions*

	Dose, Mg/Kg.																
	680	340	170	85	42.5	21.2	10.5	5.25	2.6	1.3	0.65	0.33	0.16	0.08	0.04	0.02	Control
Mottled staining	9	10	10	10	10	10	10	10	7	6	8	8	8	4	1	--	--
Vacuolar degeneration	--	--	3	6	2	2	1	1	--	--	--	--	--	--	--	--	--
Fatty degeneration	7	8	8	10	8	7	1	1	--	--	--	--	--	--	--	--	--
Granular disintegration	10	10	10	10	10	10	10	10	6	7	6	6	5	2	--	--	--
Hyaline necrosis	7	8	9	10	10	9	7	8	4	3	4	2	--	--	--	--	--
Capillary dilatation	8	10	9	9	4	3	3	2	3	2	--	2	1	--	--	--	--
Interstitial edema	9	10	9	10	10	10	10	8	10	8	8	4	5	4	3	3	--
Sequestrating edema	1	3	5	6	5	2	4	2	2	1	1	--	--	--	--	--	--
Leukocytes	9	8	8	10	3	2	3	4	--	--	--	--	--	--	--	--	--
Histiocytes	4	2	5	10	10	8	10	10	8	7	7	6	6	4	4	1	--
Fibroblastic swelling	5	3	4	10	10	8	10	10	10	10	10	10	10	10	10	9	--
Fibroblastic proliferation	2	1	3	9	10	8	10	10	6	5	5	4	2	2	3	3	--

two classifications: first, regressive lesions of the myocardial fibers, and, second, reactive inflammatory changes of the stroma. They occurred, as shown in Table 2, in combinations which allowed correlations to be drawn between the dose of drug administered and the histological features of the cardiac necrosis.

1 Regressive Changes of the Myocardial Fibers: The mildest lesion of the muscle fibers was the eosinophilia of the cytoplasm. The involved fibers were swollen, revealing segmentation and fragmentation. They gave a weak P. A. S. reaction and stained pink with Cason's trichrome. Some groups of muscle fibers showed this change, while neighboring bundles were preserved. The alternating staining characteristics of these areas caused a mottled appearance in the myocardium. The nuclear staining and structure of the muscle fibers was preserved. This change was observed in all animals given doses as low as 5.25 mg. per kilogram, and 80% of the animals had this lesion at a dose level of 0.16 mg. per kilogram. At higher doses mottled, eosinophilic muscle bundles were found at the margin of necrotic areas. However, at lower doses (2.6-0.08 mg. per kilogram) they were the most prominent components of the heart lesion.

At dose levels from 170 to 5.25 mg. per kilogram, 10% to 30% of the rats showed vacuolar degeneration of the muscle fibers. At a dose of 85 mg. per kilogram this change occurred in six animals. The muscle fibers involved were ballooned out, and large vacuoles, as shown in Figure 3, pushed the swollen nuclei to one side of the cytoplasm. The vacuoles did not stain with Sudan IV in frozen sections and gave a negative P. A. S. reaction. This change occurred in small groups of muscle fibers, located most frequently at the apex or beneath the endocardium of the left ventricle, together with extensive hyaline necrosis.

In a severer stage of regressive muscle changes the swollen fibers disintegrate into small granules; the outlines of the muscle fibers are lost, and the nuclear staining disappears. This granular disintegration was found in 100% of the animals receiving doses of 5.25 mg. per kilogram or greater, with a decreasing incidence down to doses of 0.08 mg. per kilogram.

The most characteristic lesion of the muscle fibers was hyaline necrosis. They became homogeneous, strongly eosinophilic, and P. A. S.-positive (Fig. 4), and stained deep red with Cason's trichrome. Striation of the muscle fibers was lost, and the nuclei were either shrunken or disappeared. The

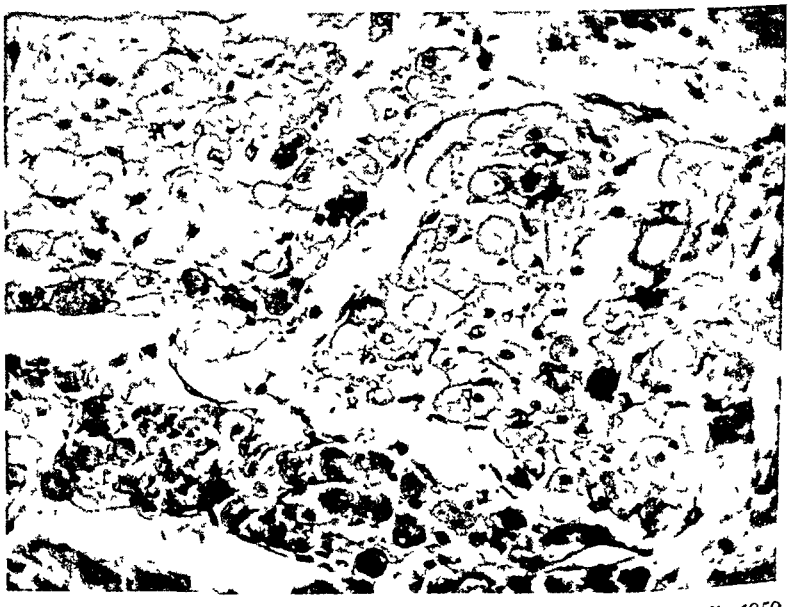


Fig 3 — Vacuolar degeneration of muscle fibers adjacent to an area of hyaline necrosis Periodic acid Schift; × 300

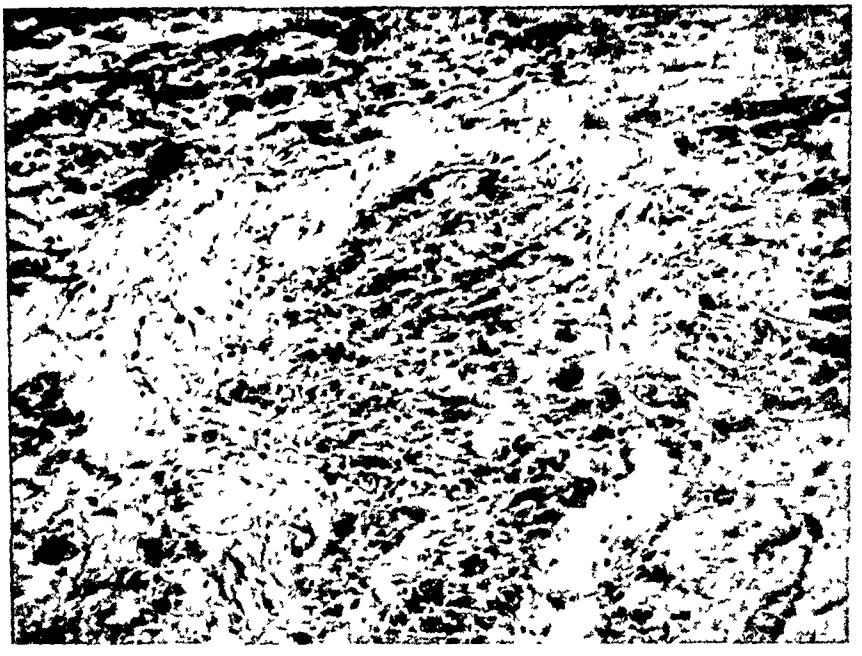


Fig 4—Hyaline necrosis of muscle fibers separated from normal fibers by mucoid edema. Periodic acid Schiff; × 120

necrotic fibers also stained with Sudan IV in frozen section. Fatty changes of the muscle were most prominent at the higher dose levels. Hyaline necrosis was present in 70% to 100% of the hearts of the animals receiving doses of 5.25 mg. per kilogram or greater and was the primary component of the massive infarct-like necrosis. At lower doses hyaline necrosis was also observed in confluent lesions. It did not occur at dose levels below 0.33 mg. per kilogram.

2. Reactive Changes of Stroma: The reactive changes which occurred around the involved muscle fibers consisted of capillary dilatation, interstitial edema, leukocytic and histiocytic infiltration, and fibroblastic changes. Widespread capillary dilatation was observed in 80% to 100% of the animals receiving doses of 85 mg. per kilogram or greater. At lower doses the dilatation was less frequent and restricted to the areas showing regressive changes. As a result of increased capillary permeability edema developed, which diffused throughout the stroma, making it appear loose. The edema, in more pronounced cases, separated the muscle fibers. This sequestrating type of

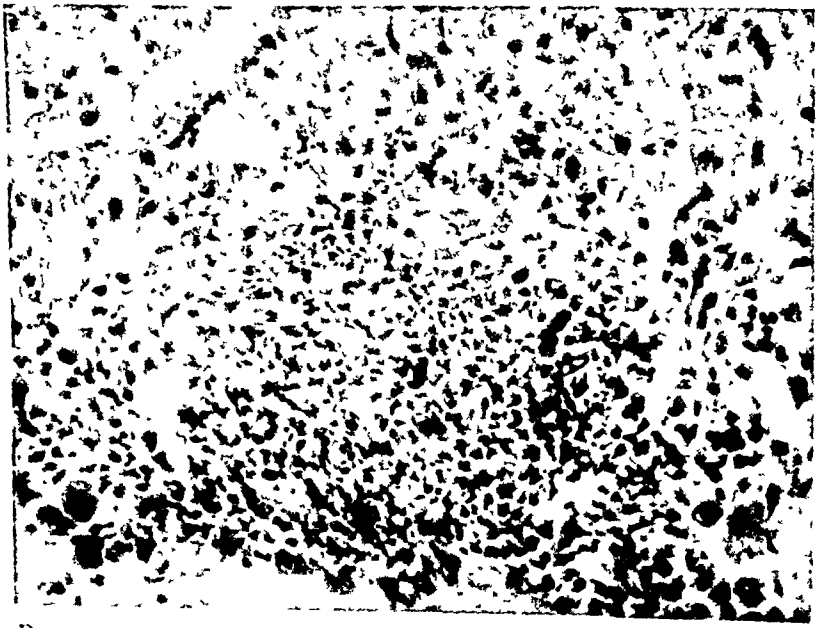


Fig. 5.—Leukocytic reaction at the margin of an area of necrosis Hematoxylin and eosin; × 300

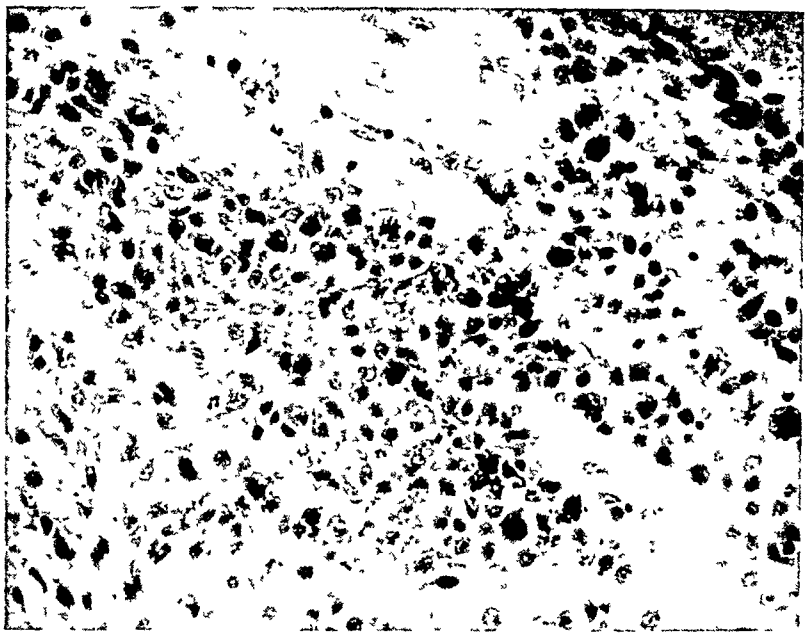


Fig 6—Histiocytic foci in the myocardium The dark granules in the cells are hemosiderin Hematoxylin and eosin; × 300.

edema was more marked at dose levels of 85 and 42 5 mg. per kilogram. The irregular spaces were generally located in the left ventricle, parallel with the muscle fibers, isolating some subendocardially located bundles from the remaining part of the myocardium. The edematous areas were relatively acellular; however, in dose levels from 85 to 5 25 mg. per kilogram they contained many swollen, star-shaped cells. In such cases the intracellular fluid stained light blue with hematoxylin and gave a positive P A. S reaction In two cases (at doses 21.2 and 10.5 mg. per kilogram) some fibrin threads were also observed in the edematous fluid.

The inflammatory cells, forming a reaction around the involved muscles, were leukocytes and mononuclear cells. Leukocytic reaction was frequent in animals treated with higher doses (Fig. 5). It decreased abruptly in animals treated with less than 85 mg. per kilogram. Leukocytes were not found in appreciable numbers around necrotic foci in animals treated with 2.6 mg. per kilogram or less. The mononuclear cells were mostly histiocytes (Fig 6) These contained occasional fat droplets or

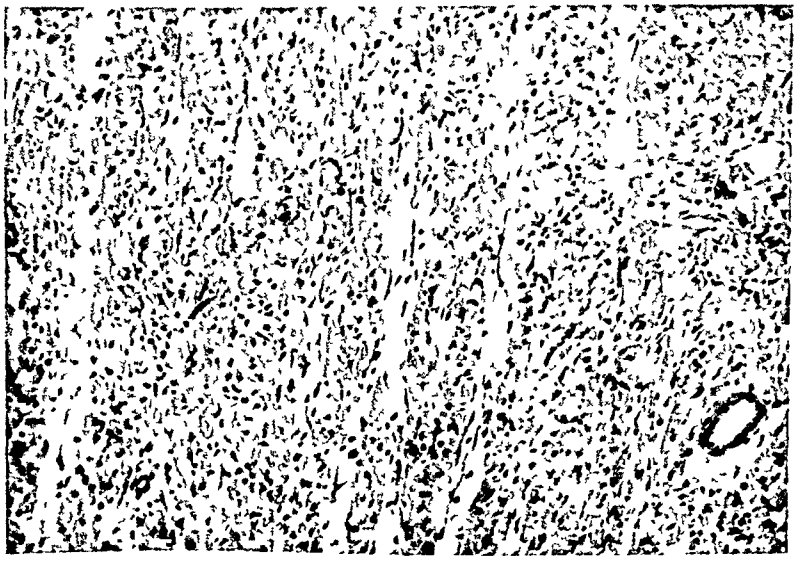


Fig 7—Swelling of fibroblasts between the muscle fibers Hematoxylin and eosin; × 120

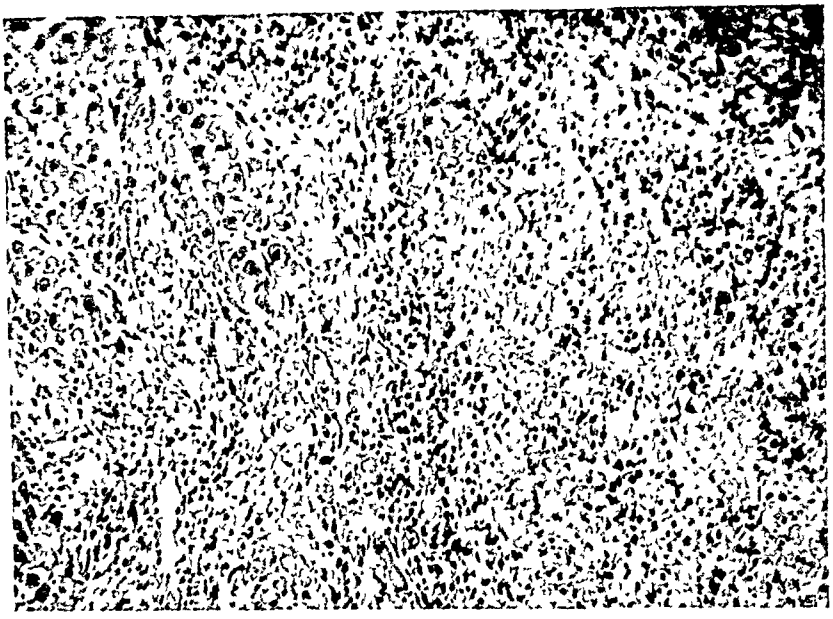


Fig. 8—Fibroblastic proliferation at the border of an area of massive necrosis. Hematoxylin and eosin; × 120

hemosiderin pigment. There were also some lymphocytes and mast cells. At doses below 85 mg. per kilogram the leukocytic reaction was replaced by histiocytes, which were pronounced down to doses of 0.16 mg. per kilogram. At doses of 0.08 and 0.04 mg. per kilogram the focal changes of the myocardium contained histiocytes.

The swelling and proliferation of fibroblasts became marked at 85 mg. per kilogram. The fibroblasts, which are normally flat and hardly visible among the muscle fibers, became swollen, oval, or polygonal (Fig. 7). This change was widespread and was seen even at the lowest doses, when other changes were indistinct or absent. The proliferation of fibroblasts was pronounced around the margins of massive necroses. (Fig. 8). The endothelial cells of the endocardium showed similar changes. Along with the fibroblasts newly formed capillaries penetrated into the necrotized areas, introducing the process of organization. Between dose levels of 85 mg. and 2.6 mg. per kilogram many mitotic figures and multinucleated fibroblasts could be seen (Fig. 9). The cytoplasm of these cells was basophilic. Among them there were groups of cells with elongated nuclei and scanty cytoplasm. The chromatin was accumulated longitudi-

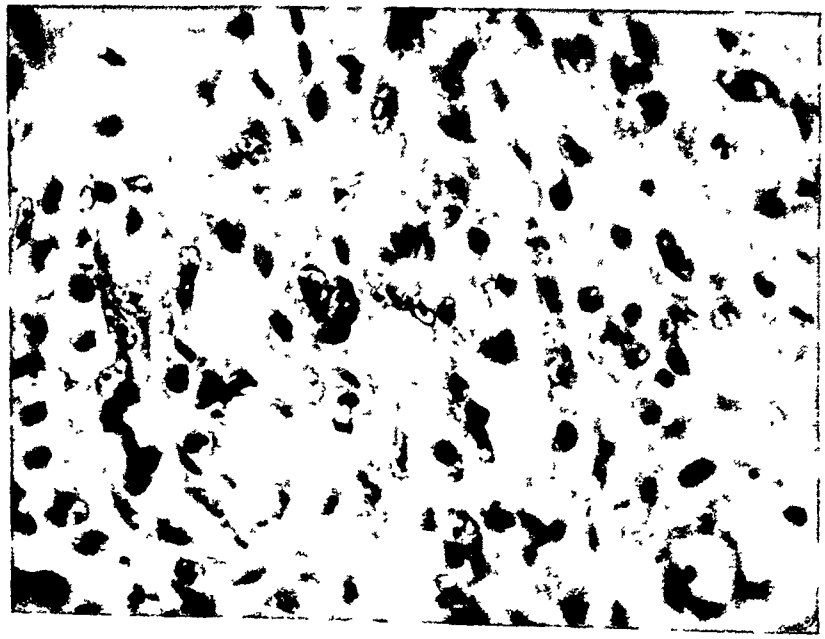


Fig 9 — Fibroblasts, revealing multiple nuclei and mitotic figures Hematoxylin and eosin; × 500

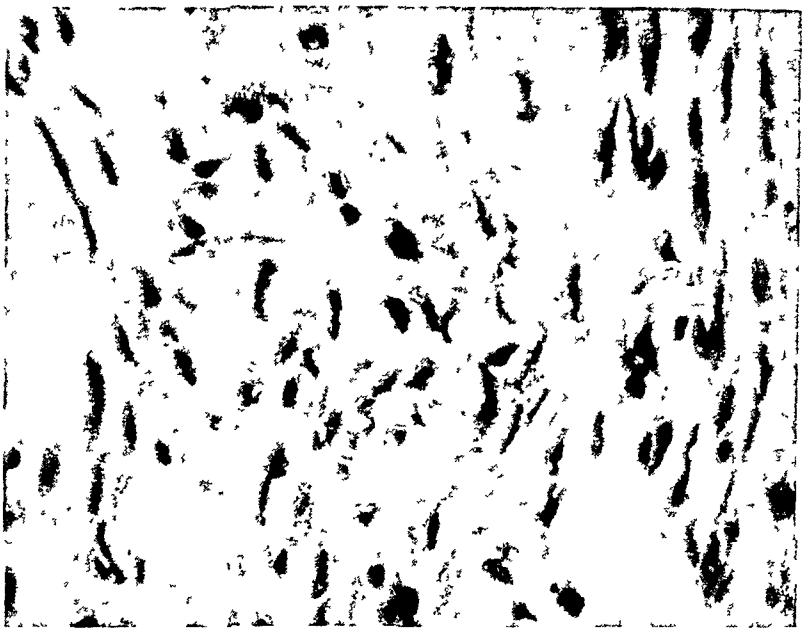


Fig 10 — Large numbers of Anitschkow myocytes between necrotic muscle fibers Hematoxylin and eosin; × 500

nally at the center of the nuclei and formed fine dentation in the direction of the nuclear membrane (Fig. 10). These cells were identical with the Anitschkow myocytes The proliferation of the fibroblasts was also observed independently from necrosis At lower doses (2 6 to 0.02 mg. per kilogram) the foci in the myocardium were composed mostly of fibroblasts. In three instances, at dose levels of 21.2 and 5.25 mg per kilogram, we observed well-circumscribed subendocardial granulomata, whose necrotic centers were surrounded by swollen fibroblasts, giant cells resembling Aschoff giant cells, and histiocytes.

Coronary artery. Apart from the swelling of the wall of the arterioles inside the areas of massive necrosis no changes were observed in the coronary arteries. In one animal only, treated with 680 mg. per kilogram, hyalin thrombi were seen in some small coronary branches, lying inside an apical infarct.

Valves. Our attention was not directed in the present study toward lesions of the valves. Occasionally a swelling of the bicuspid and aortic valves was observed. In four animals, treated with high doses, scattered foci composed mostly of mononuclear cells were observed in the bicuspid valve

Pericardium. The endothelial cells covering the infarcted areas were swollen. Some fine threads of fibrin, forming villi on the pericardium, could also be seen occasionally.

Comment

Our experimental results clearly demonstrate that DIH in a wide range of doses could produce massive cardiac necrosis closely resembling the experimental myocardial infarct which is seen following coronary artery ligation or the myocardial infarct of the human. The fully developed necrosis, like the ischemic necrosis of the myocardium, was localized generally in the apex, less frequently in the papillary muscle, and occasionally in the right ventricle. Histologically the muscle bundles showing granular disintegration or hyalin necrosis were surrounded by a leukocytic demarcation zone. Widespread capillary dilatation and marked edema were observed. Similar changes were reported by Johns and Olson [9] in rats after ligation of the coronary artery. The rapidity of the fibroblastic proliferation and the great number of Anitschkow myocytes [39] seem to be remarkable. Rats killed 48 hours after the first injection revealed a wide zone of fibroblasts around the necrotic muscle fibers. The penetration of the fibroblasts into the necrotic area was followed by an active budding and ingrowth of newly formed capillaries. Fibroblastic foci were

also observed independently from manifest myocardial necrosis. According to Mallory, White, and Salcedo-Salgar,[40] in human cases the proliferation of fibroblasts and the penetration of newly formed blood vessels into the infarcted area begin about the fourth day after coronary occlusion. Karsner and Dwyer[41] reported that in myocardial infarction of the dog the proliferation of fibroblasts starts after 24 hours, following the ligation of the coronary artery, and is marked after 48 hours. Consequently, the rat reacts in a manner similar to the dog, suggesting a greater reactivity of repair processes in these species.

Though the cardiac necrosis produced by DIH corresponds in gross and histological characteristics to that of the ischemic necrosis of man and experimental animals, no changes were observed which suggested a disturbance of coronary circulation. Except in one case, we did not observe any organic lesions in the coronary artery. Since according to Denison, Bardhanabaedya, and Green[42] DIH causes dilatation of the coronary vessels, the basis of the myocardial necrosis does not appear to be vascular spasm or occlusion.

It has been reported[43,44] that DIH administered intravenously to the dog caused a marked fall in the blood pressure and increased the rate and amplitude of the heart beat. According to Solbach,[15] acute coronary insufficiency arises when the balance between the oxygen need and blood supply of the myocardium is disturbed. The immediate result will be hypoxemia of the myocardium, followed in severe cases by myocardial necrosis. It has been shown[14] that in collapse of different origin the coronary circulation is diminished, causing multiple myocardial necroses. Similar necrosis was also produced by epinephrine infusion,[18] which caused myocardial ischemia by increasing the oxygen requirement of the heart muscle, and so in spite of coronary vasodilatation a relative insufficiency of oxygen was produced.[45] In the light of these studies it seems probable that a similar mechanism may play a role in the development of the cardiac necrosis produced by DIH.

This theory is strengthened by the results obtained after low doses of DIH. Doses lower than 5.25 mg. per kilogram seldom produced massive, infarct-like necrosis, but rather multiple disseminated necrosis developed, which closely resembled that obtained by the German authors. Nevertheless, DIH, unlike epinephrine or collapse, also produced massive infarct-like necrosis of the myocardium. Consequently, it possesses some specific characteristic. DIH, like collapse, diminishes the blood supply of the myocardium by lowering the blood pressure. Like epinephrine, DIH has also a direct cardiac action, increasing the oxygen need of the heart muscle. These combined effects of DIH could explain the production of isolated gross cardiac necrosis in all animals at doses as low as 1/500 of the L. $D._{50}$ The morphological effects of DIH on the heart, which have not hitherto been disclosed[43,46] and which are produced at doses below those that cause any other toxic manifestations, suggest the use of this drug as a means of producing experimental cardiac necrosis. Since relative hypoxemia of the heart muscle can cause myocardial infarction in man without coronary occlusion,[47-50] our findings with DIH may have considerable therapeutic importance.

Summary

It is shown that isoproterenol (1-[3',4'-dihydroxyphenol]-2-isopropylaminoethanol) hydrochloride is capable, when administered subcutaneously to the rat, of producing gross and microscopic myocardial necrosis. There is a close correlation between the dose injected and the degree of severity of the necrosis, which makes possible the production of standardized myocardial lesions.

On the basis of the localization and histological characteristics of the myocardial necrosis produced by this drug and the similarity to ischemic necrosis produced by other techniques we tentatively conclude that

the lesions in both cases have a common pathogenetic mechanism. The pharmacologic properties of this drug which would contribute to the production of these lesions are discussed.

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