Responses to Drug-Induced Myocardial Necrosis in Rats with Various Degrees of Arteriosclerosis

By Bernard C. Wexler, Ph.D., George W. Kittinger, Ph.D., and Joseph T. Judd, Ph.D.

ABSTRACT

Severe myocardial necrosis was produced in virgin Sprague-Dawley rats without preexisting arteriosclerosis and in breeder Sprague-Dawley rats with various degrees of arteriosclerosis by giving two subcutaneous injections of isoproterenol; the virgin rats received 50 mg/100 g and the breeder rats 25 mg/100 g body weight. Breeder rats withstood the stress and shock of myocardial necrosis with fewer untoward effects than the virgin animals. Changes in the thymus and adrenal glands also indicated that the arteriosclerotic breeder rats responded with a different pattern of adrenocortical activity. There were marked differences between the nonarteriosclerotic and arteriosclerotic rats in the fluctuation of serum transaminases and lactic dehydrogenase levels during the stress of myocardial necrosis. During the acute stages of myocardial necrosis there was intense activation of lipid metabolism with gross and microscopic evidence of intense fatty infiltration of the liver; during myocardial repair this lipid was removed concomitantly with reduction of hypercholesteremia. The dichotomy of response has been ascribed to the possibility that the preexisting arteriosclerosis in the breeder rats led to the development of increased collateral coronary artery circulation which afforded some degree of protection to the myocardium.

ADDITIONAL KEY WORDS

serum lactic dehydrogenase stress adrenal glands thymus aldosterone glucocorticoids serum transaminases serum cholesterol

For the past several years we have been investigating the phenomenon of naturally occurring arteriosclerosis in repeatedly bred rats (1-6). Virgin rats are free of arteriosclerosis. The incidence and severity of arteriosclerosis increases with the number of breedings and the frequency with which the animals are bred. Female breeder rats develop severe, grossly visible arteriosclerosis (1) after four or five pregnancies but they survive. Male breeder rats develop much less severe arteriosclerosis during the time required to raise four or five litters, but their mortality rate is significantly higher than that of the females, and a number of deaths are due to myocardial necrosis.

Rona and co-workers (7) have shown that injection of isoproterenol will produce myocardial necrosis in rats. When we injected virgin rats with isoproterenol, myocardial necrosis developed that was essentially identical to that occurring spontaneously in the arteriosclerotic breeder rats (8). Subsequent investigations showed that virgin rats with no arteriosclerosis respond differently to myocardial necrosis than breeder rats with preexisting arteriosclerosis; e.g., nonarteriosclerotic animals show greater signs of shock.
and a greater increase in alphaketolic adrenal steroid production (8, 12). To compare the effects of preexisting arteriosclerosis on the various adjustments and responses to the drug-induced myocardial necrosis, we studied male rats that had sired four or five litters as subjects having only microscopic arteriosclerosis, females after one or two pregnancies as subjects with moderate or grossly visible arteriosclerosis and females after four or five pregnancies as having advanced or severe arteriosclerosis. We found striking differences in the manner in which these animals responded to the stress of myocardial necrosis.

Materials and Methods

Sprague-Dawley rats were used in all experiments. The animals were closely matched according to body weight for purposes of injecting equivalent doses of isoproterenol, and for comparison of response. The virgin rats were comparable in age to the rats bred once. Female breeder rats were not used until the young had been weaned after at least 23 days of nursing.

The dose of isoproterenol was selected for its ability to produce, with regularity, massive myocardial necrosis with survival of at least 50% to 60% of the animals. Because virgin rats require twice the dose of isoproterenol per 100 g of body weight to produce an area of infarction comparable to that produced in breeder rats (8, 12), they were given 50 mg/100 g body weight, and breeder rats, 25 mg/100 g. Two such doses of isoproterenol were injected subcutaneously, the second 24 hours after the first. Control animals were killed when the study began. On each of 7 days after the injections, equal numbers of male and female virgin and breeder animals were decapitated, and the various chemical analyses and measurements of organ weight were made.

Daily determinations were made of the following: serum glutamic-oxaloacetic (SGOT) and glutamic-pyruvate transaminases (SGPT) by the ultraviolet spectrophotometric method of Karmen and co-workers (9), with a Beckman model DK-1 recording spectrophotometer; serum lactic dehydrogenase (LDH) levels by the UV method of Wroblewski and LaDue (10); and total serum cholesterol by the colorimetric method of Pearson and associates (11).

The final body weight was recorded and the thymus, adrenals, heart (blotted), and kidneys were quickly removed, trimmed free of extraneous tissue, and weighed on a Roller-Smith precision balance.

Systolic blood pressure was determined with the Friedman-Freed microphonic manometer and tail cuff. For histologic studies, the liver and other organs were fixed in 10% buffered neutral formalin. Lipid and other special stains and the method of scoring the severity of the myocardial necrosis have been described (8). Statistical appraisal was by analysis of variance. P values greater than 0.05 were considered nonsignificant.

Results

A. GENERAL OBSERVATIONS

One hour after the first injection of isoproterenol the virgin rats became prostrate and stuporous, their respiratory rate became irregular, and marked tachycardia developed, culminating in apparent shock and congestive heart failure. Those that survived the first day received another injection of isoproterenol on the second day; prostration, irregular breathing, tachycardia and bleeding and frothing around the nose and mouth followed. Pronounced hydrothorax developed rapidly during the first and second day. Although the arteriosclerotic rats developed myocardial necrosis equal in intensity to that observed in rats with normal arteries, they showed no evidence of prostration and shock. During the period of shock and active myocardial necrosis, systolic blood pressure in virgin rats ranged from undetectable to an average of 10 mm Hg, whereas in breeder rats blood pressure averaged 40 mm Hg (normal systolic blood pressure in rats ranges from 90 to 110 mm Hg).

Notwithstanding the disparity between overall response in nonarteriosclerotic rats and that in arteriosclerotic rats, the morpho-
logic changes during the acute onset of myocardial necrosis was virtually the same in the two groups and similar to that already reported (8).

Necrosis involved all layers of the myocardium (Figs. 1 and 2). Leukocytic elements were abundant, but there were surprisingly few histiocytic or fibroblastic elements in or around the sites of cell death in early stages of necrosis. Although in virgin rats myocardial necrosis appeared promptly and reached a maximum by the third day, in breeder rats its onset was later and it reached a maximum by the fourth or fifth day. As reported previously (8), repair of the myocardial damage was rapid in both breeder and virgin rats.

Large areas of myocardial necrosis resolved into microscopically small areas of scar tissue in 5 to 7 days (Fig. 3). Repair was less complete in male rats, both virgin and breeder, than in their female counterparts. Increased staining of lipids within the myocardium at the beginning of the repair phase of necrosis has been a consistent observation in our investigations. However, this change is evanescent and is followed by deposits of acid mucopolysaccharides in the area of infarction. These persist for several days and are eventually replaced by scar tissue.

An outstanding finding in this study is the massive thrombosis and coagulation of blood in the aorta of male breeder rats only (Fig. 4). Large thrombi totally occluded the lumen in virtually all subjects on the first 2 days of the experiment and little or no blood could be obtained from them. Blood flow was more copious from rats decapitated on the third
Aorta of a male breeder rat showing a massive thrombus in the aorta during the acute stage of myocardial necrosis. The edges of the thrombotic mass are rimmed by mucopolysaccharide. A channel, also rimmed by mucopolysaccharide ground substance, is maintained within the thrombotic mass (lower center of photo). Hale stain.

and fourth days, and the number of aortic thrombi was negligible.

B. TIMED SEQUENCE STUDIES OF EVENTS ACCOMPANYING ISOPROTERENOL-INDUCED MYOCARDIAL NECROSIS

Only the detailed data pertaining to the breeder rats are reported here, since those pertaining to virgin rats has already been published (8). The following parameters were compared and correlated with the onset and recovery from the myocardial damage in virgin rats.

Thymus and Adrenal Glands. Breeder rats characteristically have involuted thymi (12-14), and these glands showed none of the dramatic sequence of enlargement and involution observed in virgin rats (8). Instead, they showed progressively more severe involution during the course of the experiment ($P = 0.01$).

During the same period the size and weight of the adrenal glands of breeder rats increased ($P = 0.01$), reached a peak at the time of the establishment of the infarct, and receded during the repair phase (Fig. 5).

Heart and Kidney. The hearts of all of the breeder rats increased in weight progressively during the acute stages of the infarct ($P = 0.01$) (Table 1). This increase reached a peak on the third or fourth day when the necrosis was becoming maximum. During repair and resolution of the necrotic areas of myocardium, the weight progressively decreased.

Similarly, the weight of the kidneys increased during the first 2 days of active myocardial necrosis, when the animals were anuric and had severe pulmonary edema and hydrothorax (Tables 1 and 2). On the third day and thereafter the rats had copious diuresis; their kidneys weighed less and hydrothorax was less severe. Unlike the virgin rats, the breeder rats did not show severe loss of body weight during the stress of myocardial necrosis.

Serum Transaminases (SGOT and SGPT).
**Effects of Isoproterenol on Body and Organ Weights of Male and Female Breeder Rats with Varying Degrees of Arteriosclerosis During the Course of Development and Repair of Myocardial Necrosis**

<table>
<thead>
<tr>
<th>Weight</th>
<th>0 day</th>
<th>1st day</th>
<th>2d day</th>
<th>3d day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. rats</td>
<td>Mean ± SE</td>
<td>No. rats</td>
<td>Mean ± SE</td>
<td>No. rats</td>
<td>Mean ± SE</td>
<td>No. rats</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Heart (mg)</td>
<td>32</td>
<td>1370 ± 25</td>
<td>10</td>
<td>1490 ± 22</td>
<td>10</td>
<td>1730 ± 67</td>
<td>9</td>
<td>1920 ± 60</td>
</tr>
<tr>
<td>Kidney (mg)</td>
<td>32</td>
<td>1604 ± 28</td>
<td>10</td>
<td>1711 ± 56</td>
<td>10</td>
<td>1925 ± 83</td>
<td>10</td>
<td>1811 ± 90</td>
</tr>
<tr>
<td>Final body wt (g)</td>
<td>32</td>
<td>467 ± 5</td>
<td>15</td>
<td>471 ± 6</td>
<td>12</td>
<td>448 ± 10</td>
<td>13</td>
<td>425 ± 9</td>
</tr>
<tr>
<td>Heart (mg)</td>
<td>30</td>
<td>1016 ± 24</td>
<td>9</td>
<td>1199 ± 34</td>
<td>12</td>
<td>1259 ± 47</td>
<td>10</td>
<td>1349 ± 69</td>
</tr>
<tr>
<td>Kidney (mg)</td>
<td>30</td>
<td>1097 ± 55</td>
<td>11</td>
<td>1159 ± 45</td>
<td>10</td>
<td>1321 ± 64</td>
<td>10</td>
<td>1198 ± 69</td>
</tr>
<tr>
<td>Final body wt (g)</td>
<td>33</td>
<td>306 ± 5</td>
<td>17</td>
<td>311 ± 5</td>
<td>15</td>
<td>316 ± 7</td>
<td>15</td>
<td>285 ± 8</td>
</tr>
<tr>
<td>Heart (mg)</td>
<td>32</td>
<td>1142 ± 19</td>
<td>12</td>
<td>1272 ± 30</td>
<td>12</td>
<td>1299 ± 65</td>
<td>10</td>
<td>1377 ± 36</td>
</tr>
<tr>
<td>Kidney (mg)</td>
<td>32</td>
<td>1206 ± 23</td>
<td>12</td>
<td>1323 ± 54</td>
<td>12</td>
<td>1327 ± 50</td>
<td>10</td>
<td>1275 ± 41</td>
</tr>
<tr>
<td>Final body wt (g)</td>
<td>32</td>
<td>322 ± 14</td>
<td>20</td>
<td>331 ± 6</td>
<td>19</td>
<td>323 ± 6</td>
<td>13</td>
<td>299 ± 4</td>
</tr>
</tbody>
</table>

SE = standard error.
The level of serum transaminases was higher, under resting conditions, in breeder rats. In virgin male rats there was a prompt and marked increase in SGOT (200%) with the onset of necrosis (8); in breeder males there was only a modest increase (30%), which fell steadily toward control levels during myocardial repair \((P = 0.01)\) (Fig. 6). Virgin female rats showed a 250% increase in SGOT levels during the acute onset of myocardial necrosis, whereas arteriosclerotic breeder female rats showed an increase of only 40% to 50% during the same phase \((P = 0.01)\) (Fig. 6).

**Serum Lactic Dehydrogenase (LDH).** The resting levels of serum LDH in male and female breeder rats were significantly higher than those in virgin rats. However, instead of the great increase in LDH levels in virgin rats during acute myocardial necrosis (8), there was a fall in these levels in the breeder rats \((P = 0.01)\) during this period (Fig. 6).

**Serum Cholesterol.** During active myocardial necrosis the serum cholesterol level increased in breeder males, but decreased during myocardial repair \((P = 0.01)\) (Fig. 6). Female breeders show significant \((P = 0.01)\) but less marked change in serum cholesterol levels than male breeders. Virgin rats show little or no increase in serum cholesterol during necrosis (8). Fatty infiltration of the liver (Fig. 7) occurred in all rats during active myocardial necrosis; it cleared rapidly during the repair phase.

**Hydrothorax.** This developed with the onset of massive necrosis; it was least severe and had its lowest incidence in the female breeder rats (Table 2). During myocardial repair the hydrothorax receded. A striking exception to this pattern was in male breeder rats with minimal (microscopic) arteriosclerosis. In these animals hydrothorax persisted throughout the 7 days of the experiment (Table 2).

**C. GROSS AND MICROSCOPIC PATHOLOGY**

At autopsy, a search was made for gross evidence of lesions, particularly arterial degeneration. These gross observations were
### TABLE 2
Severity of Myocardial Necrosis and Incidence of Hydrothorax in Male and Female Breeder Rats with Varying Degrees of Arteriosclerosis during Active Destruction and Repair of the Myocardium

<table>
<thead>
<tr>
<th>Test</th>
<th>0 day</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average severity of necrosis*</td>
<td>0</td>
<td>+3</td>
<td>+7</td>
<td>+4</td>
<td>+5</td>
<td>+3</td>
<td>+5</td>
<td>+3</td>
</tr>
<tr>
<td>Incidence of hydrothorax (%)†</td>
<td>0</td>
<td>100</td>
<td>92</td>
<td>50</td>
<td>11</td>
<td>17</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Breeder males with minimal arteriosclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average severity of necrosis</td>
<td>0</td>
<td>+1</td>
<td>+3</td>
<td>+2</td>
<td>+2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Incidence of hydrothorax (%)</td>
<td>0</td>
<td>88</td>
<td>53</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Breeder females with minimal arteriosclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average severity of necrosis</td>
<td>0</td>
<td>+2</td>
<td>+3</td>
<td>+2</td>
<td>+2</td>
<td>+1</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>Incidence of hydrothorax (%)</td>
<td>0</td>
<td>83</td>
<td>67</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Breeder females with severe arteriosclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*This value is the total numerical score of the severity of necrosis divided by the number of animals: 0 = no lesion visible grossly; 1 = red-purple streaks in myocardium; 2-3 = apical necrosis, 4-6 = involvement includes apex and portions of left ventricle; 7-8 = involvement includes apex, left ventricle and right ventricle; 9-10 = all portions of the heart. For further details see ref. 8.

†The amount of fluid in the thorax was measured by aspiration with a syringe.

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**FIGURE 8**
Aorta (arch) of a male breeder rat showing a typical microscopic lesion. The dense black material in the intima is acid mucopolysaccharide, which is eventually replaced by scar tissue. The round, giant-sized cells which appear to be cartilaginous cells, were formerly flattened mesenchymal cells. Note that the media is swollen, individual elastic fibers are thickened, and there is beginning ground substance pooling (dark black splotches). Hale stain.

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checked by histologic investigation. All classes of rats had myocardial necrotic areas that were morphologically the same (Figs. 1 and 2). The virgin rats showed fatty infiltration of the liver, but this was less severe than in breeder rats (Fig. 7). None of the virgin rats showed any gross or microscopic evidence of preexisting arteriosclerosis.

The male breeder rats had no gross evidence of arteriosclerosis of the aorta but 12% of them had small, but grossly visible, calcified plaques in either the right or the left iliac arteries, and 82% had small, focal intimal lesions found microscopically in random samples of arteries. None of the intimal lesions in male breeders were of advanced degree; a lesion usually consisted of endothelial hyperplasia or fibrosis accompanied by deposits of mucopolysaccharide material and, occasionally, thickening of medial elastic fibers (Fig. 8). However, these relatively small lesions were often encountered in such vital places as the ostia of arteries branching from the aorta and in coronary (Fig. 9), cerebral, mesenteric, and renal arteries. This has been a consistent observation in all of our previous studies (1, 4).

Massive aortic thrombi, often recanalized, were found in the aortas of 75% of the male breeder rats on the first day of the onset of
FIGURE 9
Large epicardial coronary artery of a male breeder rat showing the earliest changes that occur in the coronary arteries of these animals. Basophilic acid mucopolysaccharide (black in photo) is deposited within the intimal layer of the artery throughout the entire perimeter of the lumen. Later, this basophilic material becomes calcified. Early intimal cushions of mixed endothelial cells and fibrous tissue can be seen in several foci (arrows). Hematoxylin and eosin.

myocardial necrosis (Fig. 4). On the second day, the incidence of aortic thrombosis increased to 80%; on the third day it was 63% and on the fourth day, 0%. The thrombi were highly metachromatic and stained very positively with the Hale stain, indicating the presence of acid mucopolysaccharides.

The female rats with early arteriosclerosis had no grossly visible aortic arteriosclerosis, but 64% had microscopic lesions similar in severity, morphology and distribution to those found in repeatedly bred male rats. The female breeder rats had no evidence of aortic thrombosis.

Of the female breeder rats with severe arteriosclerosis, 86% had minimal or moderate grossly visible arteriosclerosis of the aorta and all had advanced generalized arteriosclerosis (Fig. 10). Although their main coronary arteries were frequently dilated, distorted, fibrosed and calcified, repair of the myocardial necrosis was most nearly complete in these animals. Grossly, the brains were unusually swollen and edematous; histologi-
cally, 12% of the rats in this group had large cerebral infarcts (Fig. 11); all occurred during the first 3 days of the experiment.

Discussion

One of the most striking findings in these experiments is the great difference between arteriosclerotic and nonarteriosclerotic rats in their overall response to the stress of myocardial necrosis. The arteriosclerotic rats were better able to survive the shock of myocardial necrosis, and they showed less prostration, dyspnea and tachycardia than nonarteriosclerotic rats exposed to the same stress. Perhaps preexisting arteriosclerosis provided the stimulus for the development of an increased collateral system of coronary arteries and this system compensated for the increased stimulation of the heart by isoproterenol. However, the less severe hypotension observed in breeder rats could also explain their better survival. Whatever the specific explanation for this difference in response to myocardial necrosis may be, the condition of preexisting arteriosclerosis should be considered as an important factor.

We believe that the adrenal glands may be intimately involved in the response to the stress of myocardial infarction in these animals. In a previous report (12) we described quantitative differences in the steroid patterns between nonarteriosclerotic and arteriosclerotic rats following the stress of myocardial necrosis. For example, arteriosclerotic rats showed a delayed response in the production of aldosterone following myocardial necrosis with the smallest increase of this steroid occurring in breeder males having only minimal arteriosclerosis. Perhaps the persistent congestive heart failure observed in male breeders is related to this aberrant production of aldosterone. In all of our studies we have been impressed with the fact that preexisting arteriosclerosis, even when minimal, is associated with a different response pattern to the stress of myocardial necrosis.

It has been clearly established that increased adrenocortical activity increases the size of the thymus. An enlarged thymus contemporaneous with decreased adrenal weight following the first dose of isoproterenol is indicative of inadequate adrenocortical secretory response to stress during the acute stages of myocardial necrosis. Our in vitro studies of the steroidogenic capacities of adrenal glands removed from rats similarly treated showed that there was indeed a marked drop in the production of the glucocorticoid variety of adrenal steroids at this particular time (12). Decreased production of glucocorticoids would explain the hypotrophy of the thymus gland, since the glucocorticoids are specifically thymolytic. Catecholamines, e.g., isoproterenol, do not by themselves cause thymus involution, but do so by effects mediated through the pituitary-adrenal axis. The decreased biosynthesis of glucocorticoids during the first stage of myocardial necrosis would also explain the many deaths on the first day due to hypotensive crisis and shock. These changes are reversed on the second and third day, and the adrenal glands respond to the stress of myocardial necrosis. These glands increase in size; their histologic pattern changes; in vitro steroidogenic patterns change; and there is marked involution of the thymus gland. All of these events are manifested most particularly in the nonarteriosclerotic rats. The arteriosclerotic rats do not display such dramatic changes. For example, their thymi are markedly involuted at the outset of the experiment; we think this is due to altered adrenal secretory activity that accompanies arteriosclerosis in these rats (12-15). However, despite the loss of some of their ability to respond to the stimulation of exogenous ACTH, the adrenal cortices can respond to the stress of myocardial necrosis.

Perhaps the later onset of myocardial necrosis and its subsequent more rapid repair in breeder rats can also be ascribed to preexisting arteriosclerosis and the possibility that such animals have increased collateral coronary circulation. The presence of massive aortic thrombosis only in male breeder
rats during the active phase of myocardial necrosis is puzzling. Apparently, after the active stage of necrosis, fibrinolytic activity increased; this would account for the disappearance of the thrombi during the repair phase of the infarct. This sequence of events could also be explained by restoration to normal of severe hemoconcentration.

That initial levels of serum transaminase and LDH were higher in virgin rats than in breeder rats also suggests that preexisting arteriosclerosis and other degenerative changes may have some influence on these enzyme systems. That such differences exist between arteriosclerotic and nonarteriosclerotic animals is further confirmed in the totally disparate pattern of response of these same parameters following myocardial necrosis. One other possible explanation for the difference in enzyme response may be that the liver of breeder rats is invariably damaged either by lipid infiltration, necrosis, or both.

References
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