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Metabolic Response After Isoproterenol-Induced Myocardial Infarction in Arteriosclerotic Breeder vs Nonarteriosclerotic Virgin Intact and Gonadectomized Male Rats (38712)

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Repeatedly bred, male and female rats will develop arteriosclerosis, spontaneously (1–4). The female breeder rat develops severe, grossly visible arteriosclerosis but survives significantly longer than its male breeder counterpart. The male breeder rat develops hyperglycemia, hyperlipemia, hypercalcemia, and hypertension just like the female breeder, but does not survive long enough to develop the advanced, grossly visible arterial disease found in the female breeder, i.e., the arterial lesions in the male breeder are of microscopic proportions. Despite their comparatively innocuous-appearing arterial lesions, the male breeder rats often succumb due to a myocardial infarct. We have challenged nonarteriosclerotic (virgin), male and female rats and arteriosclerotic (breeder), male and female rats to myocardial infarct-inducing doses of the potent beta adrenergic stimulating agent, isoproterenol (5–8). Paradoxically, the breeder rats with preexisting arteriosclerosis survived better and with less evidence of pathophysiologic changes than those with no vascular disease (virgins). However, male rats, with or without arteriosclerosis, are the least able to survive or affect repair of the myocardium when compared with female rats, with or without arteriosclerosis.

In an attempt to delve into the apparent paradox of superior survival of arteriosclerotic animals vs nonarteriosclerotic animals and females over males, we investigated the pathophysiologic sequelae which attend an isoproterenol-induced myocardial infarct in breeder females with advanced arteriosclerosis vs virgin females with no arteriosclerosis vs virgin females which had been ovariectomized prior to the induction of myocardial infarction. We found very definite differences in the usual pathophysiologic sequelae which attends acute cardiac damage, e.g., serum insulin, glucose, free fatty acids, depending on the hormonal state of the animals, i.e., breeder vs virgins vs gonadectomized females (to be published). Therefore, in this study, we compared the pathophysiologic response of male breeder rats with microscopic aortic lesions vs intact male virgin rats with no arterial disease vs nonarteriosclerotic male virgin rats which had been castrated, to determine whether reduction of androgen levels would in any way affect the usual pathophysiologic response pattern, survival, and repair of isoproterenol-induced myocardial infarction.

Materials and Methods. Virgin and breeder male rats were obtained from ARS/Sprague–Dawley Farms, Madison, WI. The animals were maintained in our Animal Colony for a minimum of 3 wk to adjust to their new environment. Three groups of male rats were used in this experiment: (a) nonarteriosclerotic virgin male rats 100–140 days old; (b) castrated, nonarteriosclerotic virgin male rats 115–140 days old; and, (c) arteriosclerotic, breeder male rats 120–180 days old. These male breeder rats had been kept in breeding cages, with females, since they were 90 days old and were active breeders until used in this experiment. Lighting was maintained on a 14-hr light, 10-hr dark schedule. The animals were fed regular rat chow (Teklad) and water, ad lib. The nonarteriosclerotic virgin males which were bilaterally gonadectomized were allowed to recover for a minimum of 30 days before being subjected to an acute myocardial infarction along with the other animals.

Experimental animals received one or two subcutaneous injections of isoproterenol (Winthrop Labs, New York) using the same protocol previously described (5–8). All animals were sacrificed by decapitation as follows: 2, 4, 8, 12, and 24 hr after the first

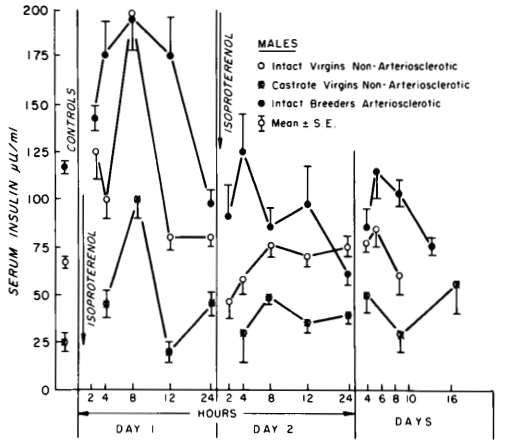

FIG. 1. Changes in serum insulin levels (mean ± standard error) in male, nonarteriosclerotic, intact and castrated, virgin, Sprague–Dawley rats vs arteriosclerotic, breeder, Sprague–Dawley rats. Each point is the mean value of a group of five animals. The animals were sacrificed at various time intervals, after two subcutaneous injections of isoproterenol spaced 24 hr apart. Myocardial necrosis is an ongoing event and becomes maximal between the second and third day. A similar protocol applies to Figs. 2 and 3.

injection (Day 1), 2, 4, 8, 12, and 24 hr after the second injection (Day 2), and Days 4, 8, 12, and 15. Controls were given the injection vehicle for isoproterenol (saline) and then sacrificed randomly at different times throughout the experiment.

Serum insulin was measured by the double-antibody method of Hales and Randle (9) using kits purchased from Schwarz/Mann. However, crystalline rat insulin, generously provided by The Novo Research Institute, Copenhagen, was used as the standard. All serum samples were measured in duplicate and in at least two dilutions. Glucose and free fatty acids (FFA) were measured using the Auto-Analyzer (Technicon). Pertinent organs, particularly the hearts and aortae were fixed in 10% buffered neutral formalin for histopathologic examination. Eighty-six percent of the male breeders were found to have microscopic aortic lesions by random sampling. Virgin rats do not develop arterial lesions until they are senile, i.e., 2-½ to 3 yr of age. Analysis of variance and biostatistical analyses follow the procedures and tables cited in the text by Snedecor and Cochran, (Statistical Methods, 6th edition, Iowa State Univ. Press, 1967).

Results. Insulin. Serum insulin levels rose significantly above base values in all groups during the first 8 hr after the first injection of isoproterenol (Fig. 1). All but the arteriosclerotic breeder males had relatively normal levels by 12 hr. After the second injection of isoproterenol, however, the nonarteriosclerotic, virgin, intact, and castrate males manifested little or no increase in circulating insulin for the duration of the experiment. The arteriosclerotic breeder males did respond with an acute rise in insulin after the second injection of isoproterenol.

Glucose. All of the animals displayed a prompt and marked hyperglycemia after the first injection of isoproterenol (Fig. 2). The serum glucose levels reached a zenith at 4 hr after which there was a precipitous drop. After the second injection of isoproterenol, all of the groups manifested mild hyperglycemia but became normoglycemic by the end of Day 2. Serum glucose levels remained normal from this point until the close of the experiment.

Free fatty acids. The intact, nonarteriosclerotic (virgin) males manifested extreme hyperlipidemia after the first injection of isoproterenol. The hyperlipidemia was maintained after the second injection, remaining at supernormal levels for the duration of the experiment (Day 10). The nonarteriosclerotic, gonadectomized males were the least responsive to the stimulus of isoproterenol in terms of any increase in FFA. In fact, 24 hr after the second injection, the FFA values in castrate males were below control levels and remained below normal for the duration of the experiment. After the second injection of isoproterenol, the arteriosclerotic breeder males displayed normal FFA values by Day 4.

Mortality. The nonarteriosclerotic castrated male rats had a statistically significant higher survival rate than any of the other males (Table I). In this experiment, as in previous experiments (1–8), arteriosclerotic breeder rats were heavier than their nonarteriosclerotic, virgin counterparts. As expected, the castrated males were considerably heavier than their intact counterparts (Table I).

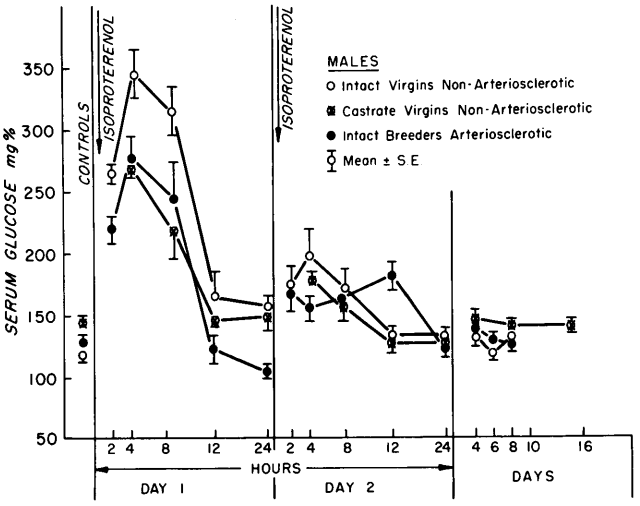

FIG. 2. Changes in serum glucose.

TABLE I. BODY WEIGHT AND SURVIVAL PERCENTAGES FOR ARTERIOSCLEROTIC, BREEDER VS NONARTERIOSCLEROTIC, VIRGIN, INTACT VS CASTRATE MALE RATS SUBJECTED TO AN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION.

	Body wt (g)	% Survival	(*N*)
Breeder males (arteriosclerosis)	452 ± 4	51	(86/170)
Virgin males (no arteriosclerosis)	321 ± 4	42	(88/210)
Castrate virgin males (no arteriosclerosis)	373 ± 2	79*	(79/100)

* $P < 0.01$ by chi-quare test.

Pathology. Because these animals were autopsied on a temporal basis, we were able to follow the progressive development and repair of their acute myocardial infarction. The gross and microscopic myocardial changes in these animals were essentially identical to those which we have observed and described previously (5–8), i.e., massive myocardial necrosis appearing first in the apex of the heart and extending into the left and right ventricles. The necrosis is confluent rather than patchy and histologically begins first in the endocardium and eventually involves all layers of the myocardium. By gross and histologic observation, the castrated males manifested a definite reduction in the severity of the myocardial infarction, usually encountered with this dose of isoproterenol (Day 1). However, after the second injection of isoproterenol (Day 2) there was little or no discernible, grossly visible difference between the three types of experimental animals. On Day 3, when myocardial necrosis reaches a zenith, all of the animals displayed equally severe myocardial infarction. Al-

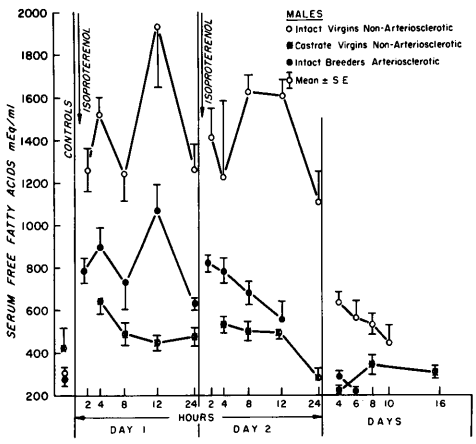

FIG. 3. Changes in serum free fatty acids.

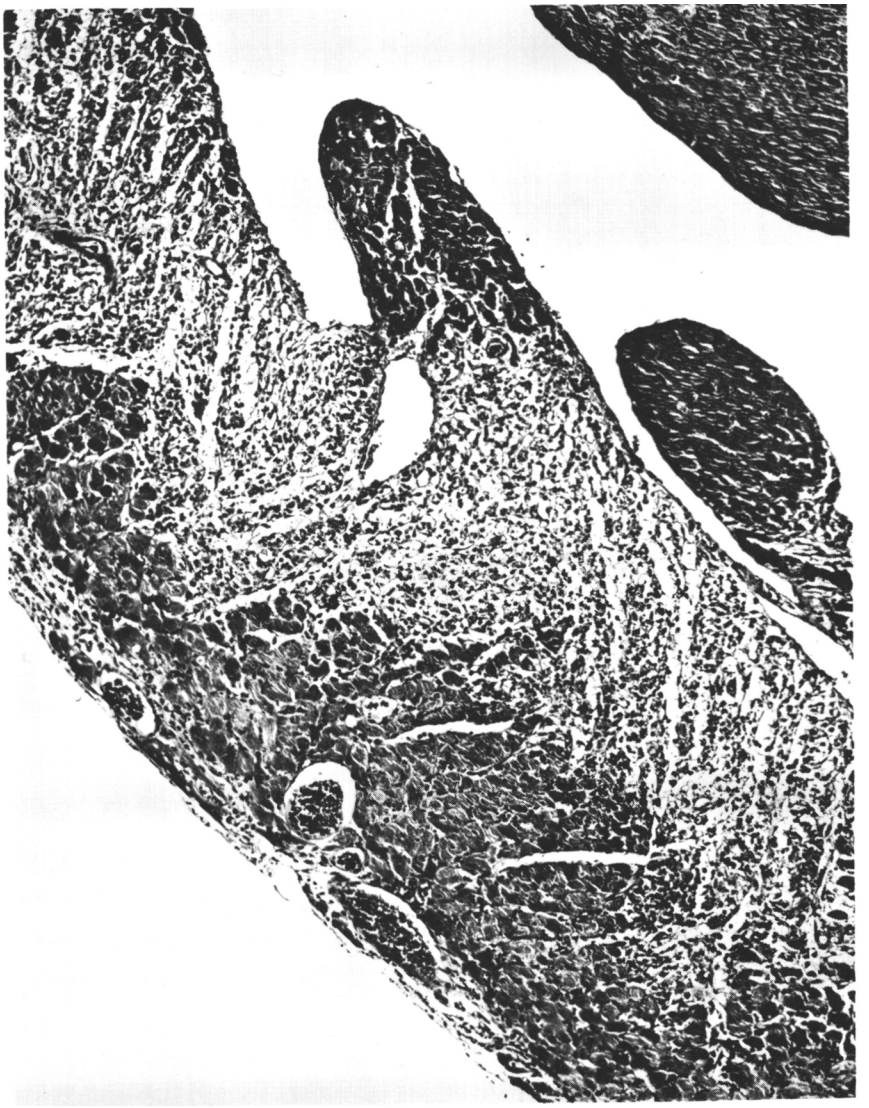

FIG. 4. Left, ventricle of a nonarteriosclerotic virgin male rat, 2 wk after an isoproterenol-induced myocardial infarct. There is extensive and persistent myocardial necrosis with little or no evidence of fibroplasia or repair H & E, × 75.

though all of the animals manifested hydrothorax, due to the congestive heart failure which accompanies the massive myocardial infarction in these animals, the castrated males showed the least amount of fluid accumulation. During the repair phase (Days 4 through 16), although these male rats manifested their usual slow and ineffectual myocardial repair (compared to females), the castrated males exhibited certain, distinctive histopathologic differences not found in the nonarteriosclerotic intact virgin or arteriosclerotic, breeder rats. Instead of persistent foci of necrosis (Fig. 4), calcification and ventricular aneurysms, found in intact virgin and arteriosclerotic breeder rats, the castrate males showed extensive endocardial fibrosis (Fig. 5) and little or no myocardial calcification or aneurysm formation.

Discussion. The findings reported here suggest that the lack of androgens, i.e., castration, may have an ameliorative effect

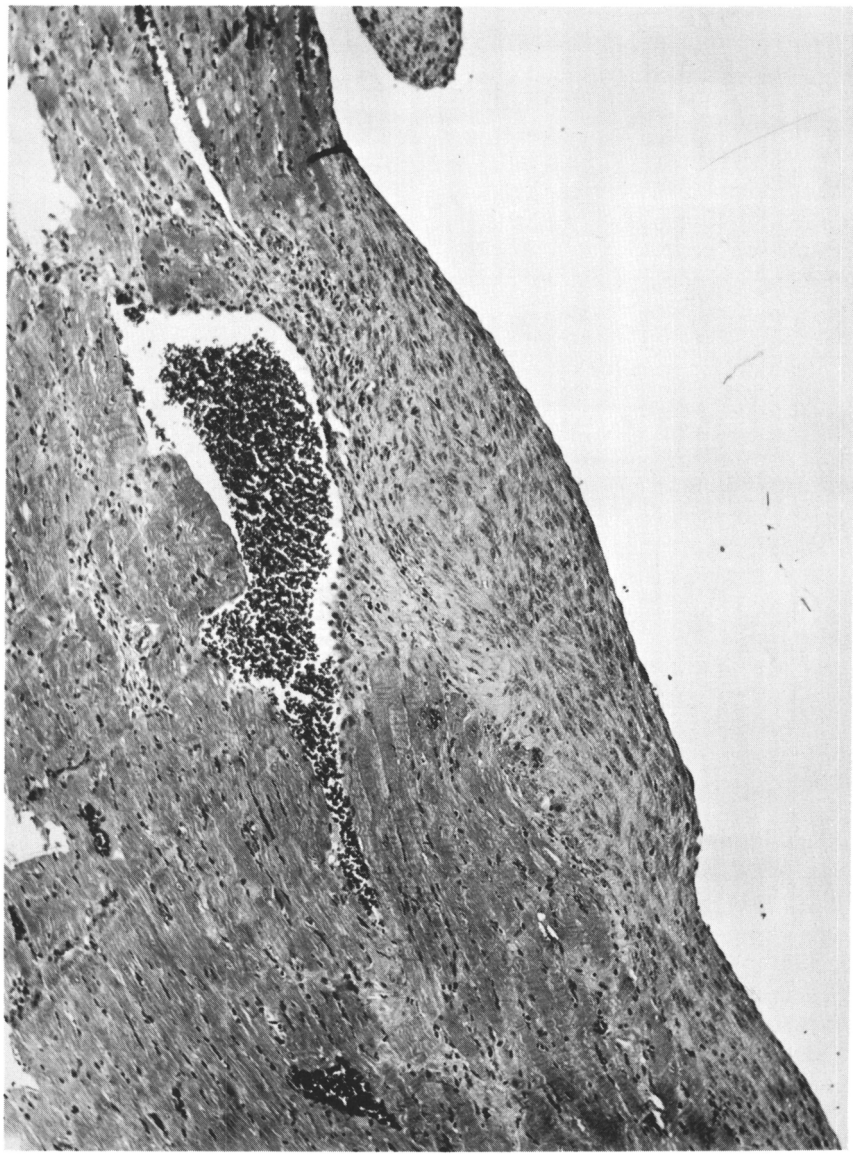

FIG. 5. Left ventricle of a nonarteriosclerotic virgin castrate male, similarly treated. Note that there is no residuum of necrosis and the active fibroplasia of the endocardium. H & E, × 100.

on the pathogenesis of acute myocardial infarction in rats. That is, in castrated male rats, the definitely superior survival, the reduced severity of congestive heart failure, reduced free fatty acid levels, and the appearance of endocardial fibrosis instead of persistent necrosis, all point to a possible protective effect of reduced androgen levels.

Although catecholamines are known to have lipid-mobilizing and glucose-regulating effects, per se, we believe that the dynamic temporal pattern of change observed in these animals is related largely to the myocardial infarct-inducing effects of isoproterenol rather than to its catecholamine properties per se. The pattern of ECG changes (10, 11), enzymes, lipids, glucose, and corticosterone (5–8), which we have observed in isoproterenol-treated rats coincide with the acute necrosis and repair of the myocardium. It

may appear contradictory that both circulating insulin and glucose are concomitantly elevated during the early stages of myocardial infarction (Day 1). Our isoproterenol-treated rats invariably manifest hyperinsulinemia (and hyperglycemia) and their beta cells consistently show complete degranulation indicative of the active discharge of insulin (5, 12, 13). Although we believe that the hyperinsulinemia and hyperglycemia is characteristic of the pathophysiologic response pattern to myocardial infarction, it is true that isoproterenol per se will elevate insulin levels by stimulation of the beta-adrenergic nerve endings in the pancreas (14). It is also possible that the transient hyperglycemia is a result of the transient hyperlipidemia (Randle effect) or to the gluconeogenic effects of the increased glucocorticoid production which attends the acute myocardial ischemia in these animals (5–8). In the latter cases, the hyperinsulinism would be in response to the induced hyperglycemia. The blunting of the hyperinsulinemia and hyperglycemia after the second injection of isoproterenol is also characteristic of the response pattern of these animals. Serum enzymes, e.g., CPK (15) and lactate (16) and other parameters also manifest little or no response at this time. It is of interest, that intact, nonarteriosclerotic (virgin), ovariectomized, and arteriosclerotic breeder female rats also manifest this same pattern of hyperinsulinemia and hyperglycemia when subjected to an isoproterenol-induced myocardial infarction (17).

Under normal conditions, the heart derives a substantial amount of its contractural energy from the oxidation of fatty acids. However, under conditions of anoxia, the amount of glucose vs fatty acids utilized by the heart, may become critical, i.e., excess utilization of free fatty acids by the anoxic heart may predispose toward fatal arrhythmia (18, 19). The intriguing possibility exists that the excess availability of free fatty acids was deleterious in that the castrated males survived best followed by arteriosclerotic breeders and then intact males concomitant with least, moderate, and greatest increase in free fatty acids, respectively.

Our observation of less overt myocardial damage in the castrated animals on Day 1, is in keeping with their superior survival and myocardial repair and also is in conformity with our observation that rats are able to withstand and adjust to the first injection of isoproterenol. However, after a second injection of isoproterenol, within 24 hr of the first injection, the animals lose their ability to adjust to the second bout of myocardial ischemia and their hearts proceed to inexorable necrosis. It is intriguing that these male rats, nonarteriosclerotic and arteriosclerotic, displayed their usual proclivity toward poor myocardial repair (Days 4–11). However, the castrate males manifested unusually good resolution of their myocardial necrosis and, in addition, showed unusual proliferation of fibroblasts, particularly of the endocardium, which is the most vulnerable layer of the myocardium to isoproterenol-induced infarction.

Summary. Male, nonarteriosclerotic (virgin) intact and castrated, Sprague–Dawley rats and male, arteriosclerotic (breeder) rats were subjected to an acute and massive myocardial infarct, by treating them with two large, subcutaneous doses of isoproterenol, spaced 24 hr apart. Serum insulin and glucose rose abruptly after the first injection of isoproterenol, but not after the second injection. Free fatty acids rose, most markedly, in the intact, nonarteriosclerotic rats, less in the arteriosclerotic breeders, and least in the castrates. These changes in free fatty acids coincided with numerical survival, i.e., greatest number of survivors in castrates. The castrated males also manifested the least amount of congestive heart failure and showed the greatest capacity to affect myocardial repair. It is suggested that reduced androgen levels may have an ameliorative effect on the usual pathogenesis of isoproterenol-induced myocardial infarction in rats.

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