Protection, by Coronary Ligature, Against Isoproterenol-Induced Myocardial Necroses.* (25830)

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Multiple focal myocardial necroses can be produced in various experimental animals by catecholamines, *e.g.*: adrenaline(1,2,3,4) or noradrenalin(5); they are particularly constant and severe after treatment with isoproterenol(6). As we shall see, in rats in which a large infarct is produced by ligature of the left coronary artery, the remaining myocardium becomes unusually resistant to production of focal necroses by subsequent treatment with isoproterenol.

Methods. One hundred female Sprague-Dawley rats, with mean initial body weight of 230 g (range: 180-339 g), were subdivided into 5 equal groups and treated as indicated in Table I. *Ligature of left coronary artery* was performed near the origin of the vessel—without pressure respiration, according to a simplified technic described elsewhere(7)—on the first day of experiment. *Sham coronary ligature* was carried out by the same procedure, except that the ligature was placed in cardiac muscle adjacent to the left coronary artery. Since the sham coronary ligatures do not produce myocardial infarction, they are not as stressful as is occlusion of the coronary vessel. Consequently, additional groups of controls were submitted to particularly severe stress to determine the possible effect of intense systemic damage as such. These rats were subjected either to *forced restraint* by tying them to a board for 24 hours on the first day and 7 hours on the fifth day according to a previously described technic(5), or to denervation of their 4 extremities by *transection of the motor nerves* on the first day of experiment, by using a procedure also described elsewhere(5). *Isoproterenol* (Winthrop Laboratories, N. Y.) was administered at the dose of 100 mg/100 g body weight, in 0.5 ml of water, subcutaneously, twice daily, on sixth and seventh days of experiment. The diffuse lesions caused by isoproterenol cannot always be seen clearly on fresh specimens; hence, the *severity of the changes* was appraised on histologic sections stained with the PAS procedure. Intensity of the focal necroses in right ventricle and septum was graded in terms of an arbitrary scale of: 0 (no lesion), 1 (slightest detectable lesion), 2 (moderate lesion), and 3 (most severe lesion). The means of these values are given in Table I with their standard errors.

Results. The lesions produced by isoproterenol in the rats that received no other treatment (Group 1) were constant and severe; they consisted of multiple foci of muscle necrosis secondarily infiltrated by inflammatory cells, particularly macrophages and fibroblasts. The lesions varied in size and location, but showed a definite predisposition for subendocardial layers (Fig. 1). There was also hyaline necrosis. In the walls of many larger arteries, there appeared homogeneous PAS-positive deposits (Fig. 2) and edema developed within the myocardium, especially around the affected arteries. Lesions of essentially the same character and intensity were seen in the rats with sham coronary ligatures (Group 3) and in those previously exposed to the stress of forced restraint

TABLE I. Protection, by Coronary Ligature, against Isoproterenol-Induced Myocardial Necroses, in 5 Groups.

Treatment*	Disseminated cardiac necrosis (scale 0-3)	Mortality (%)
None	1.7 ± .20	30
Coronary ligature	.2 ± .10	10
Sham coronary ligature	2.0 ± .19	25
Restraint	2.0 ± .17	0
Motor denervation	2.3 ± .17	20

* In addition to treatments listed in this column, rats of all groups received *isoproterenol* as indicated in text.

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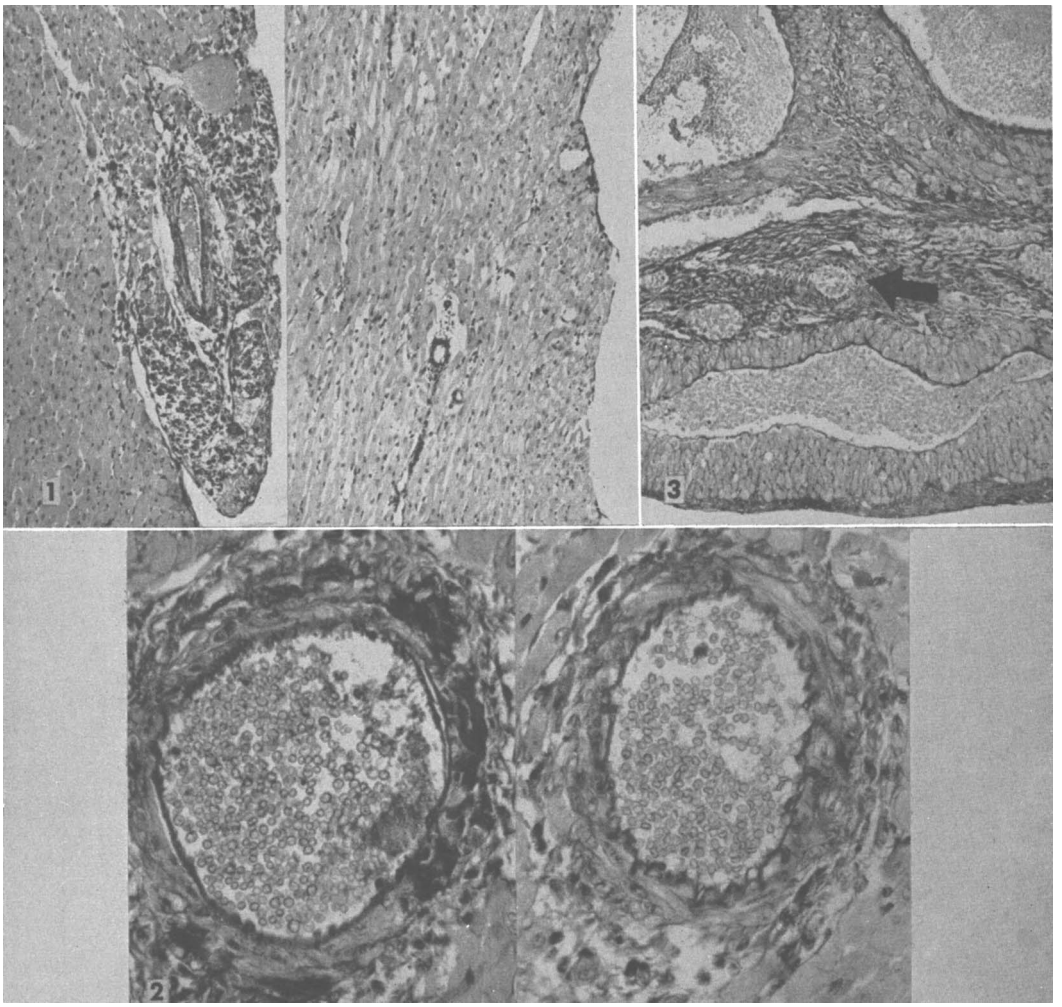

FIG. 1. Subendocardial muscle strata, following treatment with isoproterenol. *Left,* necrosis with inflammatory infiltration, in otherwise untreated rat. *Right,* no necrosis in corresponding region of myocardium, in rat with coronary ligature (PAS × 73).

FIG. 2. Medium sized coronary artery, following treatment with isoproterenol. *Left,* strongly PAS-positive (dark) homogeneous deposits, in an otherwise untreated rat. *Right,* essentially normal arterial structure, in a rat with coronary ligature (PAS × 266).

FIG. 3. Section through the entire thickness of left ventricular wall, in a rat treated with isoproterenol after ligature of left coronary artery. Almost the entire myocardium has been replaced by scar tissue, except for a thin subendocardial layer (top) and a narrow cuff around the large vein (bottom). Around the artery (arrow), no normal myocardium persists (PAS × 73).

(Group 4) or motor denervation (Group 5).

In rats in which the left coronary artery had been occluded 6 days before isoproterenol treatment, virtually the entire wall of the left ventricle underwent coagulation necrosis. Only a narrow layer of surviving myocardium (about 3-5 myofibrils in thickness) remained as an uninterrupted lining along the subendocardial surface of the infarcted area. A similar but less regular lining was also noted just underneath the epicardium that covered the necrotic patch, as well as around the veins (but not around the arteries) within the infarct itself. As indicated elsewhere(7), these muscle strata probably survive because they derive nutrition from the free blood within the ventricle, pericardial vessels, and veins that merely traverse the infarct (Fig. 3). Dis-

seminated necrosis or arterial hyalinization, characteristic of isoproterenol overdosage, was not observed anywhere in the surviving myocardium, except in 2 of the 20 animals in Group 2, and even here, the lesion was barely detectable. Mortality due to isoproterenol intoxication occurred only on 7th day; it was not markedly altered by coronary ligature, perhaps because the detrimental effect of the large infarct nullifies protection against focal necrosis.

Discussion. Evidently, ligature of the left coronary artery near its origin offers considerable protection against subsequent induction of diffuse myocardial lesions by isoproterenol. This protection dose not appear to be due to handling the heart at time of operation, since sham coronary ligatures are ineffective in this respect. Systemic stress may also be excluded as the protective agent. Rats recover from such coronary ligatures almost completely within one or 2 days, as judged by their appetite and general clinical condition; besides, neither forced restraint nor motor denervation —both of which elicited much more pronounced manifestations of stress (adrenal enlargement, thymic involution, loss of body weight)—offers protection against isoproterenol-induced disseminated cardiac necroses. After coronary ligature, systemic blood pressure remained essentially within normal limits; hence, a pre-existent hypotension likewise could not be held responsible for the isoproterenol resistance of surviving myocardium.

The mechanism of this protection remains obscure. Perhaps, when in the state of compensatory hypertrophy (such as occurs after infarction), cardiac muscle becomes insensitive to toxic action of isoproterenol. The resistance may also be due to hemodynamic changes within the myocardium and its vessels, or to absorption of chemical compounds from the infarcted muscle. It remains to be seen, furthermore, whether this protection is specific for isoproterenol and related catecholamines. Additional work will be necessary to clarify these points, but the protective effect of coronary ligature appears to be well established.

Summary. Experiments on rats indicate that, following occlusion of the left coronary artery near its origin, the myocardium outside resulting infarct becomes unusually resistant to production of diffuse cardiac necroses by isoproterenol.

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Biological Activity of Some 6α-Fluoro and 16α-Methyl C-21 Steroids.* (25831)

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Discovery that cortisone was effective as treatment for inflammatory conditions(1) has led to attempts to increase efficacy by chemical modifications. Notable among these have been Δ^1, 2α-methyl, 6α-methyl, 9α-halo, 16α-hydroxy and, recently, 16α-methyl(2,3,4,5,6,7,8,9,10,11). These changes have also been used in combination in attempts to alter some of the undesirable biological effects resulting from some of the single modifications. The present communication describes the influence of the 6α-fluoro and 16α-methyl groups, alone

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