Myocardial Necroses Produced in Domesticated Rats and in Wild Rats by Sensory and Emotional Stresses.* (29338)

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The widespread belief among clinicians and pathologists that lesions of the coronary arterial system are a necessary prerequisite for development of degenerative changes of the heart muscle is contradicted by numerous experimental observations: hemorrhages, necrotic foci and scars appear in animals with normal coronary vessels, especially in the subendocardial layers of the myocardium, after injection or infusion of catecholamines (1), after prolonged stimulation of the cardiac sympathetic nerves(2,3) and the cardiac vagus(4,5,6) (the latter possibly due to participation of accompanying sympathetic fibres), after experimental stimulation and lesions of the brain stem(7,8,9), and after general faradization(10). More extensive necroses occur under the influence of either extrinsically administered catecholamines or of catecholamine-liberating physical stresses after pretreatment with myocardial vulnerability—augmenting adrenal steroids(11,12, 13,14).

Necroses and scars in the heart muscle in the absence of vascular lesions have been observed as the result of mixed sensory and emotional stresses in baboons which had been exposed to neurosis-producing prolonged interferences in their living habits and their social environment(15,16), and in others after shorter periods of captivity(17). Some of Selye's myocardium-necrotizing stress experiments in rats(11), especially those with section of the motor nerves of the limbs and those with restraint, presumably involved also a strong element of emotional frustration.

Our own experiments were carried out in an attempt to evaluate the susceptibility of domesticated white rats and of wild gray Norwegian rats to myocardial necrosis formation under primarily sensory and emotional stresses.

Materials and methods. Both Sprague-Dawley white rats (weight 100 to 150 g in the acute experiments, growing to about 200 g in the extended experiments), and wild Norwegian rats (weight 150 to 360, average 236 g) were used. The experimental wild rats were caught intact by farmers and grain store personnel with "Havahart" traps, and kept isolated in single cages for 30 to 104 (average 57) days; the wild control rats were killed instantly by shooting at a city dump.

Sensory and emotional stresses were produced by: (a) Exposure to tape recordings of hissing cats and squealing rats for periods of 15 minutes each at 15 minute intervals during 3 or 7 days respectively (Table I, groups III-VI, XI); (b) Electrical doorbells attached to the cages, ringing for periods of 15 minutes each at 15 minute intervals during 3 days (Table I, group VII); (c) Frustration due to the necessity of running and jumping over an electrically increasingly charged metal grid on 7 successive days to obtain food (Table I, group VII); (d) Nine weeks of frustrating training to jump several times daily for food over a gradually widened gap and through one of 2 holes in a vertical black screen, both open during the first 3 weeks then one or the other randomly closed, the second being covered with a movable, hinge-suspended white lid, whereby the animals were driven each time from behind by blasts of air (Table I, groups VIII, IX); (e) Added frustration on the last day of experiment by having to jump repeatedly during 4 hours toward the holes, both of which were now covered confusingly with gray lids, one movable, the other fixed (Table I, group IX).

The white rats (except groups I, III and V) received subcutaneous injections of 2-

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TABLE I

Exp group No.	Type of stress	No. of specimens	No. days of treatment with fluorocortisol	No. of stress days	No. rats with myocardial lesions in % of group	Avg degree of myocardial lesions
	White rats (all female except Group VII)					
I	None	3,606*	0	0	0	0
II	None	555†	7	0	0	0
III	Cat-rat noise	12	0	3	0	0
IV	*Idem*	12	7	3	25	.2
V	"	12	0	7	8	.1
VI	"	12	7	7	0	0
VII	Frustration (electr. grid jumping, bell noise)	9	7‡	7+3‡	33	.4
VIII	Frustration (gap and holes jumping) (Fig. 1, 2)	10	7§	63	50	.5
IX	*Idem* plus final confusion	10	7§	63	50	.5
	Wild grey rats (both sexes)					
X	None	31	0	0	0	0
XI	Isolation plus cat-rat noise (Fig. 3, 4)	13	0	avg 57 +7	69	1.2

* 39 controls obtained in Burlington, plus 3,567 obtained at Inst. of Exp. Med. & Surgery, Montreal (one single specimen showed spontaneous myocardial necrotic foci).

† 16 fluorocortisol-treated controls obtained in Burlington, 539 in Montreal.

‡ On 7 days both frustrating jumping over electric grid for food, and injections of fluorocortisol, followed by 3 days of bell noise.

§ 7 days injection of fluorocortisol at the end of total period of frustrating jumping for food over distances and through confusing holes.

alpha-methyl-9-alpha-fluorohydrocortisone (fluorocortisol) in sensitizing doses (500 μg in 0.2 ml water on 7 successive days) preceding the final 3- or 7-day acoustic stress periods (groups IV, VI), and during the last week of the frustration periods (groups VII, VIII, IX). In group II, fluorocortisol was given alone for control purposes. No fluorocortisol was used on the wild rats.

Grouping and time sequence of the experimental arrangements are shown in Table I.

Microscopic examination of the specimens was done "blindly" without knowledge of the conditions to which the rats had been exposed. The hearts were fixed in formalin for subsequent embedding in paraffin. Three sections prepared from each heart at different levels were stained with hematoxylin-phloxine-saffron for routine morphological studies. The lesions were graded from 0 to 3, as described elsewhere(11,12).

Results. The ineffectiveness of fluorocortisol *per se* in producing demonstrable myocardial lesions was reconfirmed (group II). In white rats not sensitized with fluorocortisol, the frightening noise of hissing cats and squealing rats provoked myocardial necroses only after exposure of 7 days, and only to a slight degree (groups III, V). Corticoid-pretreatment seemed to augment the effect (group IV), but in another group of pretreated rats (VI) no necroses were found despite longer exposure (7 *vs* 3 days).

Non-sensitized wild rats, on the other hand, when exposed to the same frightening noise for one week, displayed a relatively high incidence and degree of necrotic lesions, and an occasional subendocardial hemorrhage (group XI) (Fig. 3, 4).

The noise of electric bells during 3 days, following a 7-day period of frustration (running and jumping for food over an electrically charged grid) elicited mild lesions in one-third of the white rats (group VII).

Most frequent, albeit not severe, necrotizing effects were obtained in this same species (sensitized with fluorocortisol during the final 7 days) after prolonged frustration periods, involving the jumping for food over increasing distances and against increasingly confusing obstacles (group VIII) (Fig. 1, 2). Additional intense frustration on the last day of the experiments did not further aggravate these lesions (group IX).

Discussion. As discussed previously(1,12), the origin of scattered myocardial lesions under stress in the absence of vascular abnormalities is to be ascribed essentially to localized states of anoxia in the ventricular tissue under the metabolic (increased oxygen consumption) and microcirculatory (subendocardial vascular compression, shortened diastone) influence of liberated adreno- and sympathogenic catecholamines.

Discharges of catecholamines into the blood and their augmented urinary excretion are known to occur under sensory(18) and emotional stresses(19-27). The acutely injurious effect of exaggerated catecholamine action on the myocardium manifests itself in alterations of its intermediary metabolism (28), in changes of the S-T segment and T-wave of the electrocardiogram(29), and in a high incidence of ischemic heart disease

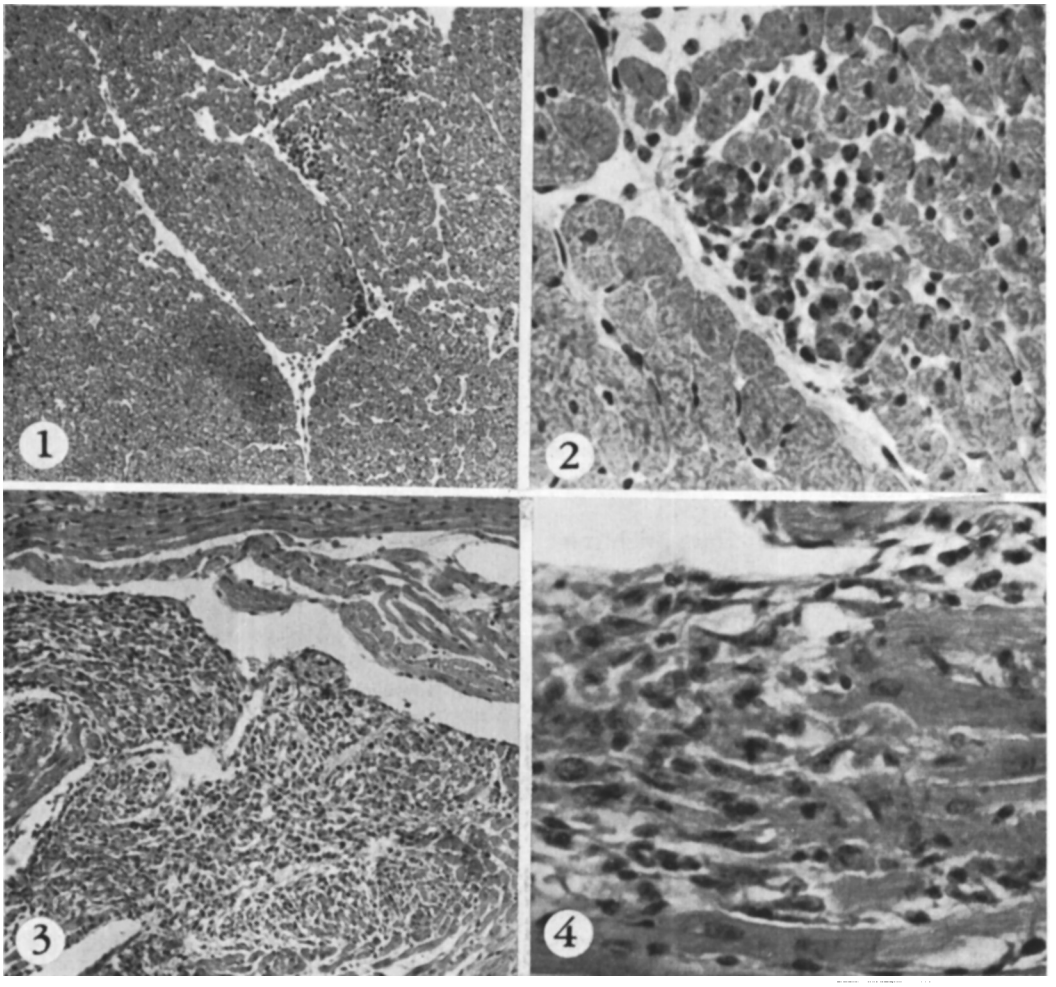

FIG. 1. Subendocardially located small necrotic foci in the cross-sectioned heart of a rat exposed to frustration (involving jumping for food over increasing distances and against increasingly confusing obstacles), and finally treated with fluorocortisol (Group VIII).

FIG. 2. Higher magnification of a degenerating area from the same heart seen in Fig. 1. Note myocardial fibers in various stages of necrosis and inflammatory infiltration in affected region.

FIG. 3. Severe myocardial necrosis in longitudinally-sectioned heart of a wild rat exposed to frightening noises (tape recording of cat and rat fight) (Group XI).

FIG. 4. Higher magnification of a part of the heart seen in Fig. 3 reveals the presence, in a mixed form, of 2 distinct types of myocardial degeneration: myolysis and necrosis with secondary inflammation.

in emotionally tense, excitable individuals(21, 30,31).

Whether or not the augmented secretion of adrenal 17-hydroxycorticosteroids which accompanies sensory(32) and emotional(27,33, 34) stimulations suffices to aggravate the potentially cardiotoxic catecholamine action under such conditions remains to be investigated. It is of interest that adrenal cortical hypertrophy, which develops in rats during isolation(35), is associated with a greatly increased cardiac toxicity of the injected catecholamine isoprenaline(36). This factor may have contributed to the maximal development of myocardial lesions in our isolated but not extrinsically corticoid-pretreated wild rats.

It can be assumed that in wild rats the motor and cardiac flight and fight reflexes are less blunted than in domesticated white rats, and that the suppression of the musculomotor outlet in isolating cages may have rendered more toxic the concomitant adrenergic effects on the heart muscle. Similar conditions are suspected to contribute to the augmented myocardial vulnerability of civilized, immobilized man under sensory and emotional frustrations and stresses(37).

In the domesticated white rats, frightening noises were much less effective even after artificial corticoid-sensitization, whereas frustrating situations produced necrotic lesions in 33 to 50% of these animals.

Summary. Wild rats exposed after periods of isolation to frightening noises (tape recording of hissing cat and squealing rat) displayed myocardial necroses in nearly 70% of the experiments. In non-isolated, domesticated white rats, analogous frightening stimuli proved much less cardiotoxic, even after pretreatment with a sensitizing corticoid (fluorocortisol). Frustrating situations (compulsory jumping for food against increasing and confusing obstacles), on the other hand, elicited myocardial lesions in one-third to one-half of corticoid-sensitized white rats. With the exception of one white rat, none of the numerous untreated and corticoid-treated controls showed any myocardial lesions. The presumable joint role of adrenosympathogenic catecholamine liberation and of adrenal corticoids in the myocardium-necrotizing mechanism of sensory and emotional stresses, and its clinical implications, are briefly discussed.

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Anabolic Responses of Diaphragm Muscle to Insulin and to Other Pancreatic Proteins.* (29339)

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No knowledge exists as to the chemical nature of the glucose barrier in the muscle cell, nor is there any real understanding of how this barrier is removed by insulin. The present study was initially undertaken in the hope that partial enzymatic hydrolysis of some cell surface constituent might yield chemical information about this barrier. Alternately, pretreatment of muscle with hydrolases might be expected to abolish a subsequent response to insulin; from this, information concerning the nature of insulin action on the membrane might be obtained. Neither of these objectives was realized. Instead, it was unexpectedly observed that hydrolases, applied to rat diaphragm muscle, acted themselves like insulin. The work reported here characterizes the insulin-like action of several proteins of the exocrine pancreas on sugar uptake, amino acid accumulation, and glycogen formation in rat diaphram.

Methods and procedure. Male rats of the Holtzman strain, weighing 80-120 g, were anesthetized with ether and the "intact" (caged) diaphragms were excised(1). After rinsing in several changes of Krebs bicarbonate buffer(2) they were placed in individual Erlenmeyer flasks in a metabolic shaker at 37°C. Incubation was done in 13 ml Krebs buffer containing the various substrates,† insulin (or enzymes) and the nonpenetrant raffinose, under a water-saturated gas phase (95% oxygen - 5% carbon dioxide). The frequency of shaking was 110 cycles per minute, the amplitude of the stroke 3 cm. After incubation, the diaphragms were removed from the flasks, blotted, dissected free at the insertions, and weighed. Analyses of muscle for the penetrants were made on extracts prepared by boiling the muscle in 2 ml of distilled water for 15 minutes and deproteinizing with zinc hydroxide(3). Samples of medium after incubation were deproteinized in the same way. D-xylose was estimated with the pentose method of Roe and Rice(4). Extracellular water was determined by raffinose in each diaphragm. A value of 77% of the wet weight was taken as the volume of total tissue water. Raffinose was assayed after hydrolysis, using resorcinol (5). Nelson's method(6), with Somogyi's modified reagent(7), was used to determine 3-0-methyl glucose. L-proline was measured with the low pH ninhydrin method(8). Glycogen was estimated with anthrone, according to the procedure of Seifer *et al*(9) for rat

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† D-xylose or 3-0-Methyl Glucose, 33 mM; L-proline, 1 mM.