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Production of Cardiac Necroses and Nephrocalcinosis by Stress in Adrenalectomized Rats.* (25729)

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In various animal species, including the rat, large doses of certain corticoids and electrolytes produce massive "infarctoid" myocardial necroses, irrespective of presence of adrenals. Furthermore, small doses of corticoids and electrolytes, although by themselves ineffective, can so prepare or "condition" the myocardium that upon subsequent exposure to stressors (*e.g.*, cold, surgical trauma, restraint or forced muscular exercise) it responds with acute development of a usually fatal, infarctoid cardiopathy. In all these circumstances, the cardiac lesions are accompanied by nephrocalcinosis if Na_2HPO_4 is used as conditioning electrolyte(1). In previous studies, soon after it became clear that numerous manifestations of the alarm reaction result from discharge of ACTH and corticoids, it was shown that not all stress-induced changes are relayed through the hypophyseal-adrenal system, since many of them occur even after hypophysectomy(2) and adrenalectomy(3). It seemed of interest to determine, therefore, whether induction by stress of an infarctoid cardiopathy and of nephrocalcinosis in suitably conditioned rats is dependent upon integrity of the adrenals.

Materials and methods. Eighty female Holtzman rats, with mean initial body weight of 100 g (range: 94-110 g), were subdivided into 8 equal groups and treated as indicated in Table I. Adrenalectomy was performed on first day of experiment, under ether anesthesia. Na_2HPO_4 was given at dose of 1 mM in 2 ml of water twice daily by stomach tube. Triamcinolone§ or 9α-fluorocortisol (F-COL)‖ acetate was administered as microcrystal suspension, subcutaneously, at daily dose of 500 μg in 0.2 ml of water, during first 4 days; then, the daily dose of both these steroids was raised to 750 μg. Treatment with various stressors was initiated on sixth day. The animals were exposed to cold (3°C) 19 hours in a refrigerated room. Motor denervation of all 4 extremities was performed under ether anesthesia. Restraint procedure consisted in tying the rats to boards with adhesive tape, in prone position, for 7 hours. A detailed description of all these technics has been given elsewhere (1). Throughout period of observation the animals were fed exclusively on Purina Fox Chow. The experiment was terminated on eighth day by killing all surviving rats with chloroform. Immediately after autopsy, hearts and kidneys were fixed in alcohol-for-

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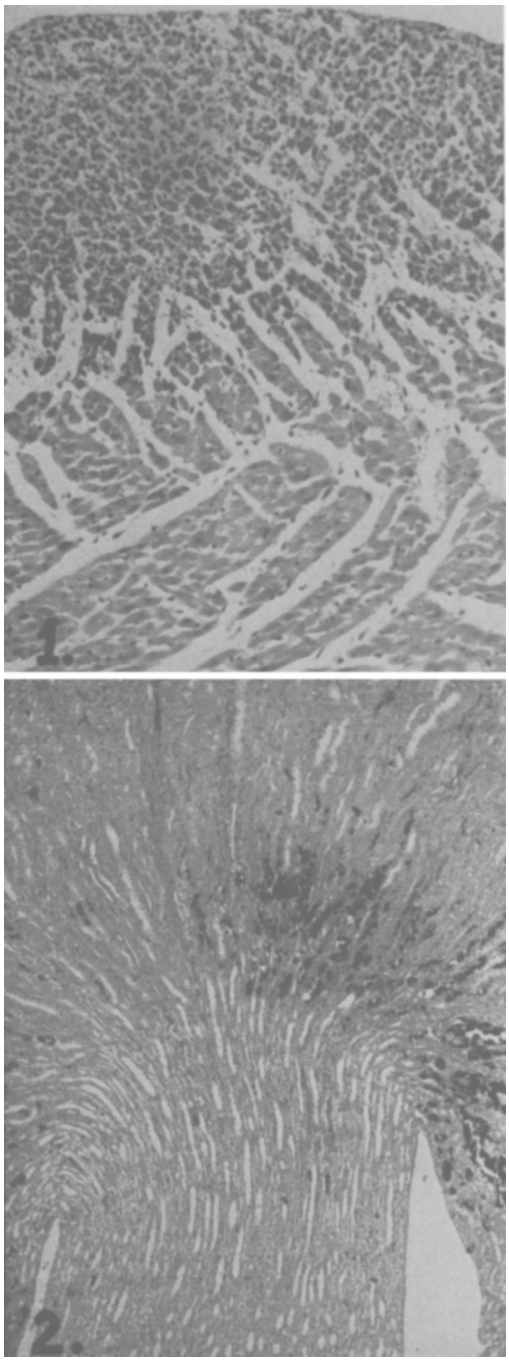

FIG. 1. Extensive, acute subendocardial necrosis with secondary inflammatory reaction in myocardium of rat of Group 2 (hematoxylin-phloxine × 100).

FIG. 2. Widespread nephrocalcinosis at corticomedullary junction line of kidney in rat of Group 3 (von Kóssa technic with hematoxylin-phloxine counterstain × 30).

mol for histological examination. Cardiac lesions and nephrocalcinosis were arbitrarily graded (on the basis of macro- and microscopic observations) in terms of a scale of 0 to 3. The means of these findings (with standard errors) and percentual mortality rate are summarized in Table I.

Results. In agreement with our earlier work with intact rats(1), such short pretreatment with Na_2HPO_4 and F-COL or triamcinolone produced neither cardiac necrosis nor nephrocalcinosis. On the other hand, in rats conditioned by the first 2 agents, exposure to any of the 3 stressors resulted in marked cardiac necrosis, usually accompanied by nephrocalcinosis (Figs. 1 and 2), and a mortality rate that reflected severity of organ lesions. Also in agreement with our previous observations on intact rats(1), triamcinolone, a virtually pure glucocorticoid, was much less effective in this respect than F-COL, which possesses both gluco- and mineralocorticoid properties. Interestingly however, occasionally, stressors did produce minor cardiac and renal changes, even in triamcinolone-pretreated animals.

Discussion. Evidently, in themselves ineffective doses of F-COL plus Na_2HPO_4 suffice to sensitize the adrenalectomized rat to production of cardiac necroses and nephrocalcinosis by 3 essentially different nonspecific stressors. Therefore, these actions of stress cannot be relayed through discharge of corticoids or catecholamines, of adrenal origin. This does not mean that production of cardiac necrosis and nephrocalcinosis by stress is independent of adrenal hormones, since exogenous corticoids were given as part of the conditioning procedure and the possibility of discharge of endogenous catecholamines from extra-adrenal sources has not been excluded. Yet, our findings do prove that, here, the corticoids only play a conditioning role and that this, like so many effects of stress, does not depend upon stimulation of endogenous adrenal hormone production. Our findings also confirm that corticoids that exhibit no clear-cut mineralocorticoid effects (*e.g.*, triamcinolone) possess little or no conditioning ability as regards production of cardiac necrosis or nephrocalcinosis, under these circumstances.

TABLE I. Production of Cardiac Necroses and Nephrocalcinosis by Stress in 8 Groups of Adrenalectomized Rats.

Treatment*	Cardiac necrosis (scale 0-3)	Nephrocalcinosis (scale 0-3)	Mortality (%)
F-COL	.0	.0	0
" + cold	1.3 ± .45	.0	10
" + motor denervation	2.7 ± .20	1.4 ± .30	70
" + restraint	2.3 ± .45	1.1 ± .30	90
Triamcinolone	.0	.0	0
" + cold	.0	.0	0
" + motor denervation	.0	.1 ± .10	30
" + restraint	.6 ± .30	.3 ± .20	0

* In addition to treatments listed in this column, all animals were adrenalectomized and given Na_2HPO_4 by stomach tube, as indicated in text.

Summary. In response to various stressors (cold, denervation, restraint), bilaterally adrenalectomized rats, suitably conditioned by pretreatment with certain corticoids and Na_2HPO_4, develop severe cardiac necroses and nephrocalcinosis. Apparently, these manifestations of stress are not mediated through increased secretion of adrenal hormones.

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Effect of Sodium Ferrocyanide and Bicarbonate on Reabsorption of Inorganic Sulfate in Renal Tubules.* (25730)

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Inorganic sulfate is reabsorbed by renal tubules in the dog by a mechanism exhibiting a transfer maximum (Tm)(7). Reabsorption is sensitive to a number of inorganic anions, such as chloride(2), phosphate and nitrate(6) and thiosulfate(1). This paper reports effects of sodium ferrocyanide and bicarbonate. The ferrocyanide ion was chosen because it is not reabsorbed in tubules in the dog(4). Bicarbonate was studied because of its marked effect on phosphate reabsorption (9).

Methods. Experiments were performed on unanesthetized female dogs in supine position. Suitable blood levels were obtained by intravenous priming injections and sustaining infusions. In each experiment, sulfate reabsorption was measured during 3-4 control periods, then in 3-5 periods during infusions of sodium ferrocyanide or bicarbonate. Filtered sulfate was always above Tm loads. In sodium bicarbonate experiments, arterial blood samples were collected anaerobically under paraffin oil. Venous blood samples were used in other experiments. Creatinine was analyzed by method of Bonsnes and Taussky(5), inorganic sulfate by method of Power and Wakefield (11) and bicarbonate according to Van Slyke and Neill(12). Ferrocyanide ion was determined colorimetrically as ferric ferrocyanide (Prussian blue) on trichloroacetic acid (10%) filtrates of plasma and urine, containing 0.1-0.3 μmoles Fe $(CN)_6^{-4}$/ml. 2 ml filtrate + 2 ml $Fe_2(SO_4)_3$-reagent (1 part 17% $Fe_2(SO_4)_3$ + 200 parts saturated solution of gum ghatti). After 20 minutes optical density was read at wavelength 660 mμ. In one ex-

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