Protection by a Steroid-Spirolactone Against Certain Types of Cardiac Necroses.* (25782)

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Concurrent treatment with desoxycorticosterone and NaCl induces periarteritis-nodosa-like changes in coronary vessels, and destructive lesions of myocardium, in various laboratory animals(1,2). This showed that, in presence of excess sodium, mineralocorticoids can produce morbid structural alterations in the cardiovascular system. Recently, this concept received additional support from the finding that certain corticoids given conjointly with some sodium salts or stress can even produce acute, large, infarct-like, myocardial necroses(3). Sensitization or "conditioning" with mineralocorticoids is an indispensable prerequisite for production of all the experimental cardiac lesions just mentioned; the question arose whether an antimineralocorticoid steroid-spirolactone could offer protection against induction of the "infarctoid" large focal myocardial necroses induced by various means.

Methods. Sixty female albino rats of the Holtzman strain, with a mean initial body weight of 97 g (range: 85-118 g), were subdivided into 6 equal groups and treated as outlined in Tables I and II. 9α-fluorocortisol (F-COL)† was administered in the form of a microcrystal suspension of its acetate, at the daily dose of 750 μg in 0.2 ml of water, subcutaneously. As an antimineralocorticoid we used the steroid-spirolactone 3-(3 keto-7-thioacetoxy - 17β - hydroxy - 4 - androsten - 17 - α-yl) propanoic acid lactone, or "Aldactone"® (SC-9420)‡; this substance was given at the daily dose of 5 mg, also in the form of a microcrystal suspension, in 0.2 ml of water, subcutaneously. The sodium salts (Na_2HPO_4 or Na-acetate) were administered at the dose of 1 mM in 2 ml of water, twice daily, through a stomach tube. To expose our rats to stress, we immobilized them (by tying their legs with adhesive tape to a board in the customary manner) on the eighth day of steroid plus electrolyte treatment, during 24 hours. The first experiment (Table I) was terminated after 13 days, the second (Table II) after 9 days, by killing all surviving rats with chloroform. Immediately after autopsy, hearts and kidneys were inspected with a binocular loupe, then fixed in neutral formalin for subsequent histologic examination of sections stained with hematoxylin-phloxine and the von Kóssa calcium stain. The readings given in the Tables are the means (with standard errors) of macro- and microscopically observed organ lesions expressed in terms of an arbitrary scale of: 0 (no lesion), 1 (slightest detectable lesion), 2 (moderate lesion), and 3 (most severe lesion).

Results. The first experiment (Table I) shows that conjoint treatment with Na_2HPO_4 + F-COL (a steroid possessing both mineralo- and glucocorticoid actions) induces 100% mortality, with intense, infarct-like cardiac lesions and nephrocalcinosis, under our experimental conditions. Since most of our earlier work on production of these necroses had been performed with 2α-methyl-9α-chlorocortisol (Me-Cl-COL), which is difficult to obtain at present, it is noteworthy that the readily available F-COL can be used as a substitute in this type of experiment. The most significant result of this first series of observations was, however, that SC-9420 markedly protects against both the fatal action and the organ lesions that result from conjoint treatment with F-COL + Na_2HPO_4 (Fig. 1).

A second experiment (Table II) was then undertaken to determine whether, in F-COL-conditioned animals, the antimineralocorticoid-spirolactone also protects against development of similar lesions induced by ex-

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TABLE I. Protection by SC-9420 against Toxic Effects of F-COL + Na_2HPO_4.

Group	Treatment	Cardiac necrosis Severity (scale 0-3)	Cardiac necrosis Incidence (%)	Renal calcification Severity (scale 0-3)	Renal calcification Incidence (%)	Mortality (%)
1	F-COL + Na_2HPO_4	2.7 ± .20	100	2.8 ± .15	100	100
2	SC-9420 + F-COL + Na_2HPO_4	.6 ± .23	50	0	0	0

TABLE II. Protection by SC-9420 against Toxic Effects of F-COL + Stress.

Group	Treatment	Cardiac necrosis Severity (scale 0-3)	Cardiac necrosis Incidence (%)	Mortality (%)
1	F-COL + restraint	1.5 ± .30	90	0
2	SC-9420 + F-COL + restraint	0	0	0
3	F-COL + Na-acetate + restraint	3.0 ± 0	100	50
4	SC-9420 + F-COL + Na-acetate + restraint	1.3 ± .40	60	20

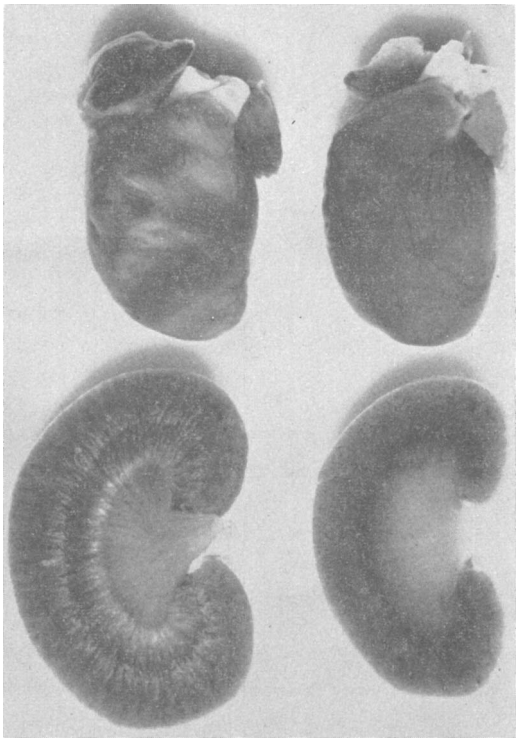

FIG. 1. Typical multiple myocardial necroses and extensive nephrocalcinosis—particularly at corticomedullary junction line—in a rat (Group 1 of first experiment) in which F-COL + Na_2HPO_4 was administered (left). Compare with the apparently normal organs of a rat (Group 2 of first experiment) treated with SC-9420 in addition to F-COL + Na_2HPO_4.

posure to the stress of restraint, with or without concurrent sensitization by an Na-salt. Here, Na-acetate was employed instead of Na_2HPO_4, since concurrent treatment with F-COL + Na-acetate, unlike combined treatment with F-COL + Na_2HPO_4, rarely if ever produces any cardiac necroses in animals not exposed to stress.

SC-9420 also offers considerable protection against induction of mortality and cardiovascular lesions, under these circumstances (Table II). In absence of phosphate treatment, slight renal calcification occurred only in Group 3 (grade 0.3 ± 0.15); hence, this change is not listed in the Table.

Summary. Experiments on rats indicate that the antimineralocorticoid steroid-spirolactone 3-(3 keto-7-thioacetoxy-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone—also known as SC-9420 or "Aldactone"®—offers considerable protection against induction of acute, infarct-like, often fatal myocardial necroses normally produced by fluorocortisol plus some Na-salts and stress, alone or in combination.

1. Selye, H., *Canad. M. A. J.,* 1942, v47, 515.
2. Selye, H., Pentz, E. I., *ibid.,* 1943, v49, 264.
3. Selye, H., *The Chemical Prevention of Cardiac Necroses,* N. Y., Ronald Press Co., 1958.

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