Sensitization by Dihydrotachysterol (DHT) and Calcium Acetate for the Induction of Cardiac Lesions by Various Agents

H. Selye, M.D., E. Bajusz, M.D., S. Renaud, D.M.V., and Y. Lemire, M.D.*
Montreal, Canada

In a previous paper of this series, we showed that, in rats, the oral administration of NaH_2PO_4 qualitatively alters the cardiotoxic actions of certain vitamin-D derivatives, such as dihydrotachysterol (DHT), so that an acute, purulent myocarditis results.[1] It has been found, furthermore, that the production of an Electrolyte-Steroid-Cardiopathy with Necroses (ESCN) by combined treatment with certain corticoids and "sensitizing sodium salts" is greatly enhanced in rats exposed to various stressor agents or given dietary supplements of fats or carbohydrates.[2]

In this communication, we wish to report upon experiments which show that in rats given calcium acetate instead of sodium phosphate, otherwise ineffective amounts of DHT also produce a suppurating myocarditis, although the pattern of the accompying soft-tissue calcification is somewhat different. It will be seen, furthermore, that the cardiac damage caused by calcium acetate plus DHT—like that of the ESCN that is induced by corticoids plus sodium salts—can be precipitated by various stressors and by the oral administration of corn oil or glucose supplements.

MATERIALS AND METHODS

One hundred sixty female Sprague-Dawley rats, with an average initial body weight of 100 grams (range: 94 to 109 grams), were subdivided into 16 equal groups and treated as indicated in Table I.

Calcium acetate (1 mM.), dihydrotachysterol, or "DHT," (25 μg), and glucose (600 mg.) were all administered in 2 ml. of water, by gavage, twice daily, throughout the experiment. The animals that were to be treated with calcium acetate plus DHT simultaneously received the above-mentioned doses of the two compounds mixed in 2 ml. of water. Corn oil was administered by stomach tube, at the dose of 1 ml., twice daily, throughout the experiment.

Treatment with the various potentially precipitating agents was initiated on the fifth day, since preliminary experiments had shown that by that time, treatment with calcium acetate plus DHT alone produces only minimal cardiac lesions and no mortality. The cold baths consisted in

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From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada.

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immersing the rats in icy water for 5-minute periods, once on the fifth day and twice on the sixth day. Noradrenalin was given subcutaneously, at the dose of 300 μg in 0.2 ml. of olive oil, once on the fifth day and twice on the sixth day. The restraint procedure consisted in immobilizing the rats with adhesive tape, in the prone position on wooden boards for a period of 17 hours, beginning on the morning of the fifth day. Motor denervation of all four extremities was performed, under surgical anesthesia, on the fifth day.

Throughout the period of observation the rats were fed exclusively on Purina Fox Chow*; the experiment was terminated on the seventh day by killing all surviving rats with chloroform. Immediately after autopsy, the hearts were fixed in neutral formalin and stained by von Kossa's technique for the histochemical demonstration of calcium on paraffin-embedded sections. The cardiac lesions were arbitrarily graded in terms of a scale of 0 to 3; the means of these observations (with standard errors) and the percentual mortality rate are summarized in Table I.

TABLE I. SENSITIZATION BY DIHYDROTACHYSTEROL (DHT) AND CALCIUM ACETATE FOR THE INDUCTION OF CARDIAC LESIONS BY VARIOUS AGENTS

GROUP	TREATMENT	CARDIAC LESIONS	MORTALITY (%)
I	None	0	0
II	Cold baths	0.1±0.10	0
III	Noradrenalin	0.4±0.30	0
IV	Restraint	0.5±0.25	0
V	Motor denervation	0	0
VI	Corn oil	0	0
VII	Glucose	0	0
VIII	DHT	0	0
IX	Calcium acetate	0	0
X	DHT + Calcium acetate	0.8±0.15	0
XI	DHT + Calcium acetate + Cold baths	2.5±0.23	10
XII	DHT + Calcium acetate + Noradrenalin	3.0±0	20
XIII	DHT + Calcium acetate + Restraint	2.1±0.28	50
XIV	DHT + Calcium acetate + Motor denervation	1.1±0.17	30
XV	DHT + Calcium acetate + Corn oil	1.8±0.32	80
XVI	DHT + Calcium acetate + Glucose	1.5±0.27	0

RESULTS

Perusal of our data (Table I) indicates that no cardiac lesions occurred either in the controls (Group I) or in those subjected to motor denervation (Group V), treatment with corn oil (Group VI), glucose (Group VII), DHT (Group VIII), or calcium acetate (Group IX), alone. Cold baths (Group II), noradrenalin (Group III), and restraint (Group IV) induced only minimal lesions when given by themselves.

Treatment with DHT plus calcium acetate (Group X) produced definite but mild cardiac lesions, consisting of calcification of the Mönckeberg-sclerosis type in the coronary arteries and calcium deposition within the cardiac muscle fibers or in their surrounding stroma (Fig. 1). Previous experiments have shown that this type of lesion can be produced by various vitamin-D derivatives (including DHT) alone, although without supplements of sensitizing salts much larger doses of the sterols are required to obtain calcifications of similar intensity.[1,3]

*Ralston Purina Company, Ltd., Canada.

In addition to tissue calcification, there were, in the myocardia of most of the animals treated with DHT plus calcium acetate, definite inflammatory, suppurating lesions, with extensive fiber necrosis and massive infiltration with polymorphonuclear leukocytes. These changes cannot be produced even with fatal doses of DHT alone.

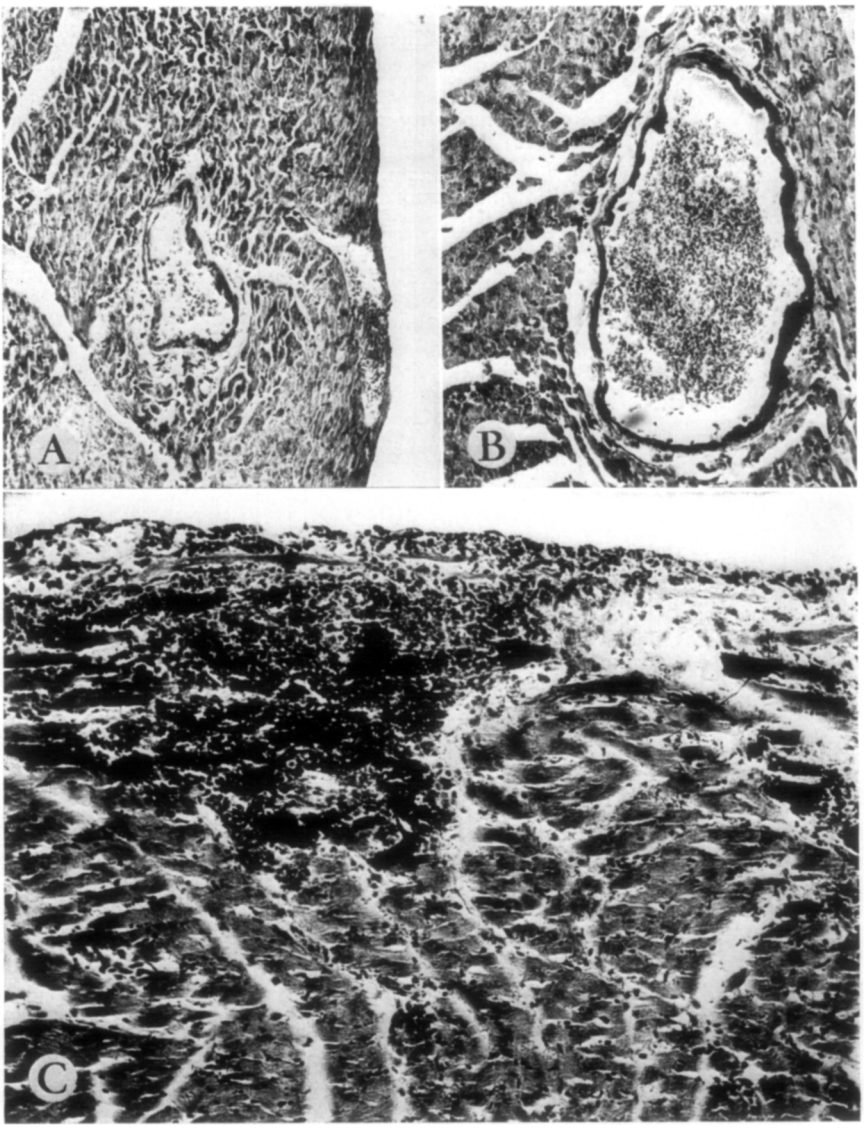

Fig. 1.—Cardiac lesions produced by calcium acetate plus DHT, under various circumstances. *A*, Small artery in the wall of the right ventricle of a rat treated with calcium acetate plus DHT (Group X). *B*, Corresponding coronary vessel of a rat which, in addition to treatment with calcium acetate plus DHT, was exposed to the stress of cold baths (Group XI). The lumen of the artery is dilated, and the wall more heavily calcified than in the previous section. *C*, Heavily calcified and inflamed subendocardial nodule from the heart shown in *B* (all sections, von Kossa technique).

The same type of cardiac and coronary lesions occurred, but with significantly greater intensity in the rats which were treated with cold baths (Group XI), noradrenalin (Group XII) (Fig. 2), restraint (Group XIII), motor denervation (Group XIV), oil (Group XV) or glucose (Group XVI), in addition to the DHT plus calcium acetate.

Neither DHT nor calcium acetate alone produced any appreciable degree of nephrocalcinosis, but the kidneys of the rats treated with DHT plus calcium acetate exhibited extensive calcification, mainly in the stroma of the cortical region. Calcium deposition with the tubules at the corticomedullary junction

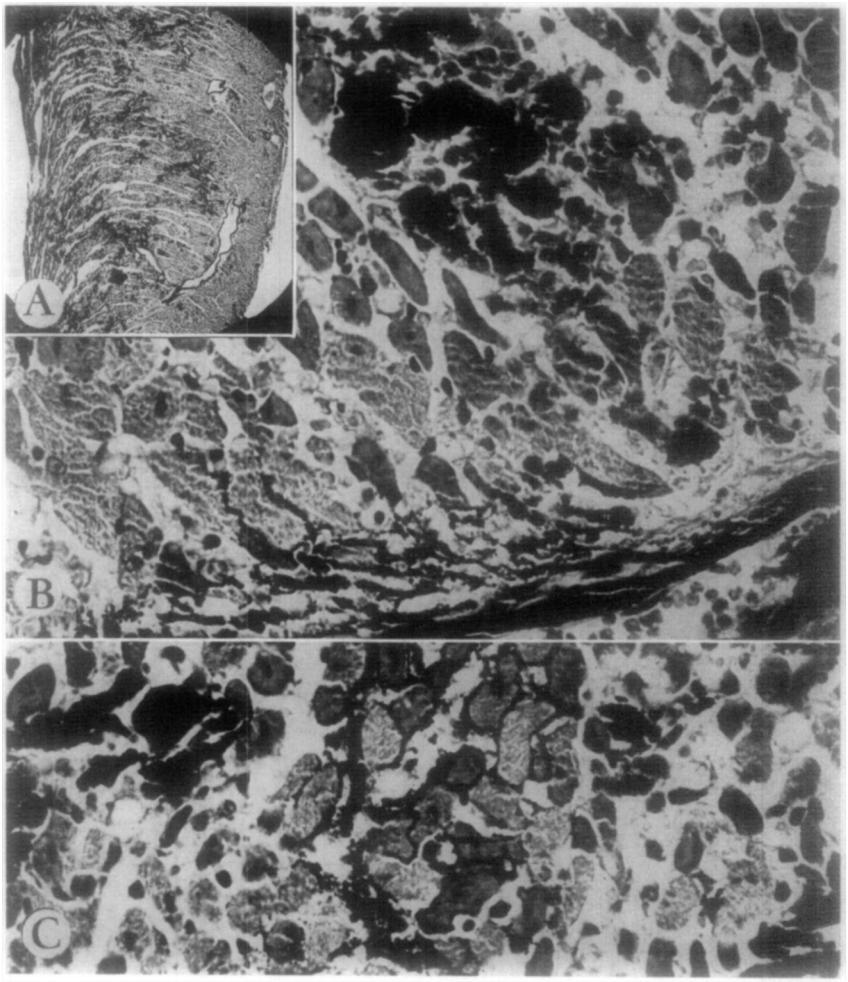

Fig. 2.—Intense calcium deposition in the heart of a rat treated with noradrenalin following sensitization by calcium acetate plus DHT (Group XII). *A*, (Insert) General view of the calcium deposition which affects the arteries and muscle fibers throughout the width of the right ventricle. Note again the dilatation of the rigid, calcified coronary arteries. *B*, High magnification of a region from the heart shown in *A*. Near the bottom of the field there is an artery from which strands of calcified tissue appear to invade the adjacent myocardium. Near the top, the entire muscle fibers are impregnated with calcium. *C*, Another region from the same heart. Here, the muscle fibers are calcified in the left, and the perimuscular connective tissue in the middle, portion of the field (all sections, von Kossa technique).

line—such as occurs in rats treated with DHT plus NaH_2PO_4[1,2]—was absent or negligible in the animals of the present experimental series. In this respect, therefore, calcium acetate cannot replace NaH_2PO_4. The intensity of the nephrocalcinosis produced by DHT plus calcium acetate (Group X) was not significantly altered by any of the agents administered to the rats of Groups XI—XVI; hence, the corresponding figures are not listed in the table.

Deaths occurred only in the groups which, in addition to DHT plus calcium acetate, were treated with one of the agents capable of precipitating the development of cardiac lesions.

DISCUSSION

It is obvious from these observations that calcium acetate can replace sodium phosphate as a sensitizing agent for the production of myocardial and coronary calcification, as well as suppurating myocarditis, in the rat. Still, there is a difference between the two salts as regards their effect upon the course of DHT intoxication: calcium acetate produces a greater degree of soft-tissue calcification and an almost exclusively cortical nephrocalcinosis, owing to the deposition of insoluble calcium salts in the stroma of the renal cortex, while NaH_2PO_4 produces a more intense myocarditis and calcium deposition within the tubular lumina, particularly at the corticomedullary junction line. This difference is apparently due to the fact that although DHT sensitizes tissue for calcification under the influence of an excess of either calcium or phosphate, the two ions have different tissue affinities.

Evidently, after sensitization by DHT plus calcium acetate the most diverse agents can precipitate intense cardiac lesions and cause high mortality. Presumably, the eliciting or precipitating effect is rather nonspecific and largely due to stress. This explanation appears to be particularly probable as regards the precipitation of cardiac lesions by cold baths, restraint, and motor denervation.

Adrenergic hormones are known to produce a Mönckeberg-type of arterial calcification in some species (e.g., the rabbit) but not in the rat. However, threshold doses of DHT plus calcium acetate can so sensitize the cardiovascular system of the rat that this normally insensitive species reacts to noradrenalin as does the naturally sensitive rabbit.

Nothing is known about the mechanism through which oral administration of fat and carbohydrate elicits cardiac lesions in rats conditioned with DHT plus calcium acetate. It is noteworthy, however, that these same dietary supplements are also highly effective in eliciting an ESCN in rats conditioned by otherwise ineffective amounts of corticoids plus sodium salts.[2]

SUMMARY

Calcium acetate can replace sodium phosphate as a sensitizing agent that permits the production of severe cardiovascular and renal lesions with otherwise ineffective doses of dihydrotachysterol (DHT). Still, the effect of the two electrolytes is not the same. Under the influence of DHT, sodium phosphate induces comparatively little cardiac and vascular calcification, much suppura-

ting myocarditis, and a type of nephrocalcinosis that is primarily characterized by the formation of calcium casts within the tubular lumina at the corticomedullary junction line. On the other hand, under the same conditions, calcium acetate induces only mild myocarditic changes, much calcium deposition in the cardiovascular system, and a type of nephrocalcinosis that is almost exclusively limited to the stroma of the renal cortex.

In rats simultaneously treated with comparatively small doses of DHT plus calcium acetate the resulting cardiovascular lesions can be considerably aggravated by a variety of stressor agents (cold baths, restraint, motor denervation). Furthermore, although noradrenalin does not normally produce any cardiovascular calcification in the rat, it is highly effective in this respect when the animal is pretreated with comparatively small doses of DHT plus calcium acetate.

Finally, the oral administration of corn oil or glucose likewise aggravates the cardiovascular effects of DHT plus calcium acetate intoxication; these dietary supplements also act upon the course of the DHT plus calcium acetate syndrome in the same manner as they were previously shown to act upon the cardiopathy induced by corticoids plus sodium salts.

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