# Thyroxin as Sensitizing Agent in Production of Renal and Cardiovascular Lesions with Corticoids.* (22418)

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Soon after it had been discovered that desoxycorticosterone acetate (DOCA) can produce extensive hyalinization of mesenchymal structures, with nephrosclerosis, myocarditis and periarteritis nodosa in the rat, it became evident that certain "conditioning factors" (*e.g.*, unilateral nephrectomy, dietary sodium supplements) are capable of facilitating the development of this syndrome. Concurrent treatment with other hormones can also modify the production of hyalinosis by DOCA and related prophlogistic corticoids. For instance, an antiphlogistic hormone, such as cortisol acetate (COLA), greatly aggravates the renal changes, but inhibits the purely inflammatory manifestations (especially the periarteritis and, to a lesser extent, the myocarditis) of DOCA-overdosage. Crude anterior pituitary extracts, or partially purified somatotrophic hormone (STH) preparations, exacerbate all the manifestations of the DOCA-induced hyalinosis syndrome—indeed they can produce hyalinosis even without concurrent DOCA-treatment—but only in the presence of functioning adrenal tissue. It had therefore been assumed that STH (or some other anterior pituitary principle which contaminates all available STH preparations) induces hyalinosis through the intermediary of the adrenal cortex, perhaps by enhancing the metabolic transformation of DOCA-like corticoids into compounds still more active in producing hyalinosis. The rather voluminous literature concerning the hormonal factors which influence hyalinosis has been discussed elsewhere(1-6).

Thyroxin shares with STH the ability to sensitize the rat to the production of hyalinosis by DOCA, but it was not known whether in this respect, its effect—like that of STH—is dependent upon the presence of adrenal tissue. It also remained to be shown whether the particularly nephrotoxic effect of combined treatment with DOCA and COLA can be further enhanced by concurrent thyroxin-administration. The experiments to be reported below were performed in order to clarify these points.

*Materials and methods.* Sixty female Sprague-Dawley rats, with an initial bodyweight of 100-114 g (avg 107 g) were subdivided into 6 equal groups for treatment, as indicated in Table I. Both steroids† were administered subcutaneously in the form of microcrystals, the total daily dose being suspended in 0.2 ml of water (containing the usual suspending agents). Thyroxin‡ was given as the sodium salt, also in 0.2 ml of water, subcutaneously. All animals were conditioned for the nephrotoxic effect of the hormones by the removal of the right kidney on the day hormone-treatment began. At the same time, in the last 2 groups of this series, the adrenals were extirpated through the lumbar route. All the animals received 1% NaCl as a drinking fluid and "Purina Fox Chow" as the sole source of nourishment. In Table I the mean intensity of cardiac and renal lesions, as well as the degree of fluid-accumulation in pleura and peritoneum, are expressed in a scale of 0 to +++. The fluid-intake listed is the mean of the volume ingested during the last 3 days. The experiment was terminated on the 13th day of hormone-treatment.

*Results.* As indicated in Table I, thyroxin greatly enhanced the production of cardiac and renal lesions, as well as the accumulation of fluid in the large body-cavities. This effect of the thyroid hormone was quite evident, both at the low (*cf.* Groups 1 and 2)

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† Cortisol acetate ("Cortril") was generously supplied by Pfizer Laboratories, and desoxycorticosterone acetate ("Cortate") by Schering Corp.

‡ Obtained from British Drug Houses Ltd.

TABLE I. Sensitization to Certain Corticoid-Overdosage Effects by Thyroxin.

| Group | Treatment in μg/day COLA | DOCA | Thyroxin* | Adrenal | Body-wt loss (in %) | Heart-lesions | Kidney-lesions | Ascites and pleural fluid | Fluid-intake (ml/d) | Mortality (in %) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 500 | 500 | 0 | Intact | 2 | 0 | 0 to + | 0 | 87 | 0 |
| 2 | 500 | 500 | 250 | " | 0 | 2+ | 3+ | 2+ | 130 | 20 |
| 3 | 1000 | 1000 | 0 | " | 11 | + | + | 0 | 130 | 0 |
| 4 | 1000 | 1000 | 250 | " | 5 | 2+ | 3+ | 3+ | 123 | 30 |
| 5 | 500 | 500 | 250 | Adr-X | 13 | 2+ | 3+ | + | 110 | 30 |
| 6 | 1000 | 1000 | 250 | " | 19 | 2+ | 3+ | 3+ | 139 | 30 |

* Dose raised to 500 μg during last 2 days of experiment.

and at the high (*cf.* Groups 3 and 4) level of steroid-treatment, and it was not prevented by complete adrenalectomy (*cf.* Group 2 with 5 and Group 3 with 6).

Histologically, the cardiac lesions were mainly characterized by formation of hyalinized foci, surrounded by inflammatory cells, and by periarteritis nodosa of the heart vessels. In the kidney, there was hyalinization of the glomeruli, occasionally accompanied by hemorrhages into Bowman's capsule. Many tubules were dilated and contained more or less intensely hyalinized protein-casts. Conversely some proximal convolutions were completely collapsed; most of these lost their brush-border and their eosinophilia, but often they contained clear cells in mitotic division. This aspect is quite characteristic of the "endocrine nephrons" seen in kidneys whose excretory function is abolished by constriction of the afferent vessels (Fig. 1). Outside the heart, periarteritis nodosa was not observed in any groups of this experimental series, presumably because of the high dose of anti-inflammatory hormone given.

In addition to the fluid-accumulations in peritoneum and pleura, an enormous and generalized subcutaneous edema had developed in all the rats of Group 4 and, to a lesser extent, in a few of the animals in Group 2. No such edema was seen in any of the adrenalectomized animals receiving the same hormone-treatment, although the cardiac and renal lesions were just as severe here as in the presence of the adrenals.

*Discussion.* The very acute renal and cardiac changes accompanied by pronounced water-retention are rather reminiscent of the "eclampsia-like syndrome," which results when renin is administered to rats pretreated with DOCA(7,8). The latter condition has repeatedly been compared with clinical pre-eclampsia and eclampsia(8,9). Without entering into a discussion of the possible relationship between these experimental and clinical syndromes, it may be pointed out that the changes observed in our rats strikingly resemble those obtained by combined DOCA and renin-treatment. Furthermore, several of the animals in our Group 4 showed nervous manifestations (convulsions, tics), brain-edema and multiple hemorrhages (especially in the heart, kidney, brain and, occasionally, in the peritoneum) all of which have also been reported to occur in the DOCA + renin syndrome. The latter had been thought to be rather specifically due to renin. In rats (similarly conditioned by unilateral nephrectomy, NaCl and pretreatment with DOCA) this "eclampsia-like" condition has also been elicited with hypertensin—the product of enzymatic interaction between renin and its substrate—but not with adrenaline, vasopressin, thromboplastin, ACTH, histamine or crude human placental extracts(8).

The corticoid + thyroxin syndrome described here appears to differ from the DOCA + renin syndrome only in that the former, unlike the latter, does not lead to complete anuria (except perhaps during the last few hours of life). However, in view of the many resemblances, we are nevertheless inclined to regard the 2 syndromes as essentially identical in their manifestations. This does not necessarily invalidate the conclusions of Masson and his colleagues(7,8) concerning the specific pathogenic rôle played by the renal-pressor mechanism. In our rats the many

"endocrine nephrons" might well have supplied an endogenous source of renin or of some related substances.

*Summary.* In rats, conditioned by unilateral nephrectomy and a high NaCl-intake, 12 days of treatment with comparatively small doses of desoxycorticosterone acetate (DOCA) and cortisol acetate (COLA) causes only very mild renal and cardiac lesions, which result in no detectable fluid-accumulation or mortality. Concurrent administration of thyroxin greatly aggravates the cardiac and renal

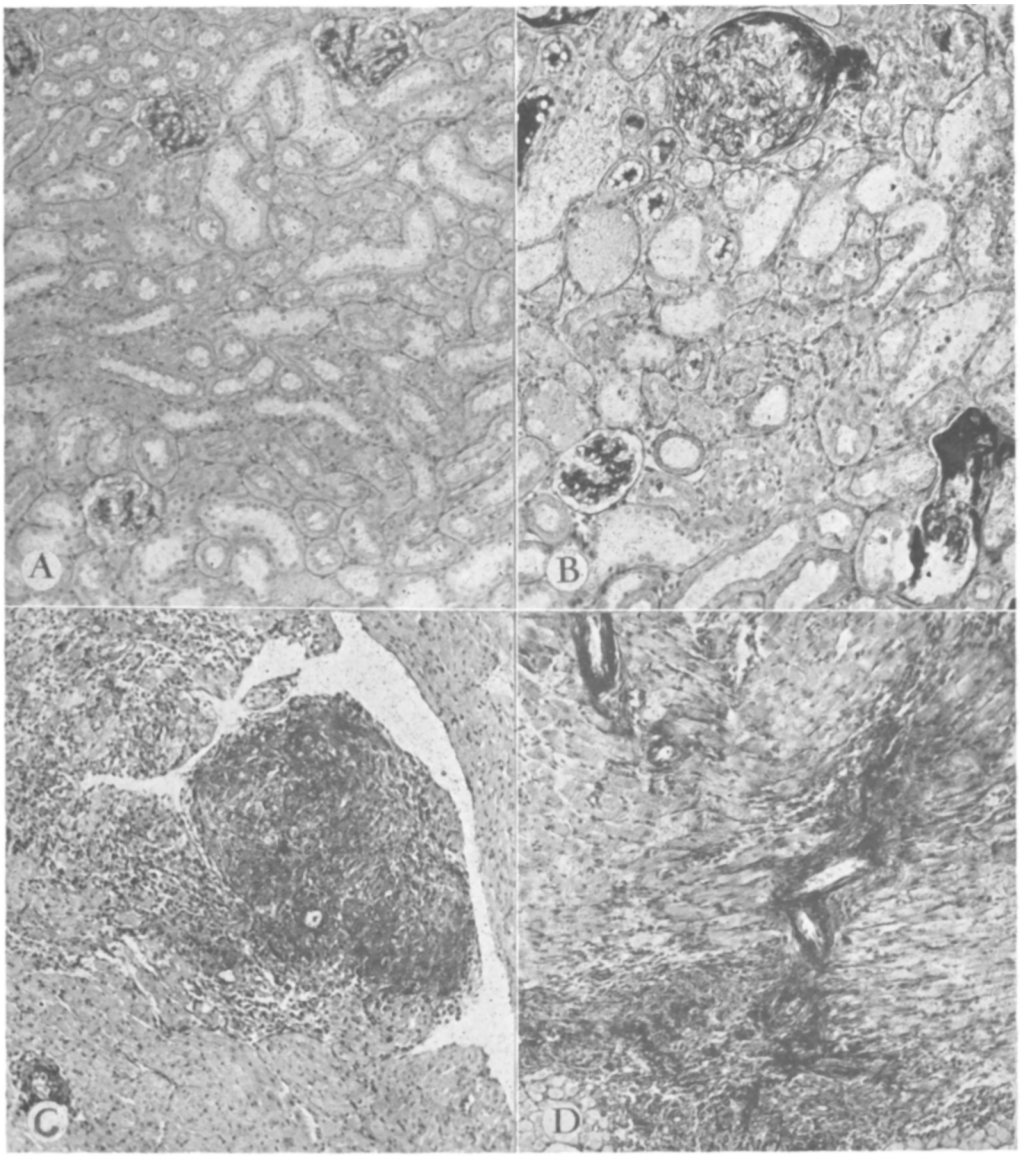


FIG. 1. Sensitization by thyroxin for the production of renal and cardiovascular lesions with corticoids. *A.* Control rat of Group 3 treated with COLA and DOCA. Renal structure is essentially normal at the end of this short-term experiment. *B.* Kidney of a rat from Group 4, which was treated with COLA, DOCA and thyroxin. Note intense renal damage, due to hyalinization of the glomeruli and their afferent arterioles. Some of the tubules are dilated, due to obstruction by hyalin-casts, while others have become solid epithelial cords, reminiscent of the nephrons in the "endocrine kidney." *C.* Region from heart of the animal shown in Fig. B. Largely hyalinized granulomatous nodule. *D.* Other region of heart shown in Fig. C. Several small arteries undergoing hyalin-necrosis.

lesions; at the same time it produces pronounced fluid-accumulations in the peritoneum, pleura and subcutaneous tissues, with multiple hemorrhages, nervous disturbances and a high mortality. The syndrome is regarded as essentially similar to that previously produced by other investigators through combined treatment with DOCA and renin. The ability of thyroxin to produce cardiac and renal damage under these conditions—unlike the comparable effects of STH—is not prevented by adrenalectomy.

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## Influence of 9-Alpha Fluorohydrocortisone on Contractility and Na Content of Isolated Cat Papillary Muscle.* (22419)

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The effect of steroids upon tissues other than the components of the pituitary-adrenal and renal axis has been studied by many workers, including Ingle, Szego and Eisenberger(1-3). Previous studies(4,5) have shown that under certain conditions progesterone and cortisone exert a negative inotropic action (*i.e.* contractility) on the heart. With a simple electronic system perfected in this laboratory, we have measured a previously unreported inotropic effect of 9-alpha fluorohydrocortisone‡ (9-a FF) on isolated cat papillary muscle. This synthetic hormone was chosen for this study because of its marked mineralocorticosteroid and glucocorticosteroid activity(6).

*Methods.* The cat papillary muscle preparation employed was that first described by Cattell and Gold(7). Cats anesthetized with ether were killed by cardiectomy. At least 2 and sometimes 3 papillary muscles, varying from 2-15 mg in weight, were removed from the right ventricle. These muscles were individually dissected, leaving the chorda tendinae and some valvular tissue attached to one end, and a tab of myocardium to the other. The papillary muscle was thus isolated without causing tissue injury. A hook, which also served as one electrode on a muscle holder, was placed through the chorda tendinae. From the other end of the muscle a suture attached to the papillary:myocardial juncture was tied to an extension on the pin of an electronic transducer tube (RCA 5734). The transducer tube transformed slight mechanical displacements into electrical potentials which were in turn picked up by a direct-writing Sanborn EKG instrument. A control and a treated muscle were stimulated in two identical chambers which were attached to the electronic system in such a manner that both control and experimental observations could be recorded simultaneously.

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