Effect of Vitamin E and Methyltestosterone Upon the Progeria-Like Syndrome Produced by Dihydrotachysterol

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IN THE RAT, chronic intoxication with certain vitamin D derivatives, such as dihydrotachysterol (DHT), produces organ lesions reminiscent of premature aging. Among the changes characteristic of this "progeria-like syndrome" are the following: (1) a type of generalized Mönckeberg arteriosclerosis with calcification in the cartilaginous portions of the ribs, trachea and larynx; (2) a concurrent loss of body weight and atrophy of the sex organs, liver, kidney, thymicolymphatic apparatus, fat and connective tissue; (3) loss of skin elasticity and many wrinkles; (4) dental anomalies similar to those seen in senile rats; and (5) greatly shortened life span. Rarely cataracts are observed, but pretreatment with dihydrotachysterol sensitizes the rat for the production of cataracts by other agents. Although these changes are reminiscent of senility, the associated skeletal lesions are not. Osteosclerosis is seen rather than osteoporosis, although the newly formed bone tissue is brittle. This entire progeria-like syndrome can be prevented by calciphylactic desensitization with ferric dextran or by treatment with an anabolic androgen such as methyltestosterone.[1-3]

Calciphylaxis is a reaction by which the organism can selectively send calcium to certain organs and thereby induce them, by deposition of hydroxyapatite crystals, to undergo virtual petrification. On the other hand, calciphylactic desensitization (reverse calciphylaxis or "anacalciphylaxis") can prevent the formation of pathologic soft-tissue calcifications.[4] This protective effect is responsible for the aforementioned prevention with ferric dextran (a calciphylactic challenger) therapy of abnormal soft-tissue calcification in the progeria-like syndrome. However, the mechanism of the simultaneous inhibition of senility-like changes unassociated with calcification (loss of skin elasticity, atrophy of various organs) remains to be elucidated.

Recently, we observed that vitamin E (*d*-α-tocopheryl acetate) can prevent the development of various forms of calcinosis normally induced in dihydrotachysterol-sensitized rats by the administration of certain calciphylactic challengers.[5]

These points raised the question whether or not the progeria-like syndrome, elicited by chronic dihydrotachysterol intoxication, could likewise be prevented by vitamin E and if so, whether or not this prophylactic action could be further enhanced by the administration of methyltestosterone.

MATERIALS AND METHODS

One hundred and forty female Sprague-Dawley rats of the Holtzman strain, with a mean initial body weight of 100 gm. (range 96 to 104 gm.), were divided into fourteen equal groups for two experimental series (Tables I and II and Fig. 1 and 2). The data corresponding to the untreated control animals are repeated in the tables and figures of both series for comparison.

In the first series, vitamin E (*d*-α-tocopheryl ace-

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The experimental work described was supported by Distillation Products Industries, Division of Eastman Kodak Company.

TABLE I

Effect of Increasing Doses of Vitamin E Upon the Syndrome of Chronic Dihydrotachysterol Intoxication

Group	Vitamin E Treatment* (mg.)	Calcification		Final Body Weight (gm.)
		Heart	Kidney	
I†	0	0	0	187 ± 5.54
II	0	1.6	0.7	90 ± 3.69
III	1	0.4	0.3	97 ± 3.82
IV	10	0.8	0.6	96 ± 5.12
V	50	0.3	1.0	120 ± 4.81
VI	100	0	0	140 ± 10.24
VII	250	0	0	186 ± 2.83
VIII	500	0	0	174 ± 8.90

* In addition, all animals except those in Group I received dihydrotachysterol as indicated in the text.

† Absolute control animals.

TABLE II

Effect of Vitamin E plus Methyltestosterone Therapy Upon the Syndrome of Chronic Dihydrotachysterol Intoxication

Group	Vitamin E Treatment* (mg.)	Calcification		Final Body Weight (gm.)
		Heart	Kidney	
I	0	0	0	187 ± 5.54
II	0	1.4	0.8	86 ± 4.26
III	0	0.3	0	124 ± 5.67
IV	1	0.3	0.2	117 ± 9.17
V	10	0	0.5	121 ± 8.65
VI	100	0	0	157 ± 9.74
VII	250	0	0	186 ± 4.98

* In addition, all animals except those in Group I received dihydrotachysterol as indicated in the text, and methyltestosterone was given to Groups III through VII at the daily dose of 100 μg. as indicated in the text.

tate N.F.)* was administered in 2 ml. of water by stomach tube to Groups III through VIII twice daily throughout the experiment at the dose levels indicated in Table I. One drop of Tween® 80† was added to emulsify 10 ml. of the suspension. In addition, all animals received dihydrotachysterol (Calcamin®),‡ 50 μg. in 0.5 ml. of corn oil, by stomach tube once daily, starting on the fifth day of the experiment.

In the second series, all animals were given dihydrotachysterol as already described with the exception of Group I which received no pathogenic or protective pretreatment and acted as absolute control animals. Groups II through VII were given methyltestosterone (Me-T) (Oreton-M®)§ subcutaneously, 100 μg. in 0.2 ml. of water per day, throughout the experiment. In addition, Groups III through VII received vitamin E as in the first series at the dosages indicated in Table II.

The rats were kept on cubes of Purina Laboratory Chow‖ for the first fifteen days of dihydrotachysterol treatment. After this, the development of dental anomalies made it difficult for the unprotected animals to chew hard cubes; therefore, during the second half of the experiment, this food was replaced by ground Purina Chow in all groups, for the sake of uniformity. Tap water was given to all animals throughout the experiment.

All rats were killed with chloroform on the thirtieth day, and the organ lesions were gauged with a dissecting loupe in terms of an arbitrary scale described elsewhere in which 0 = no lesion, 1 = just detectable, 2 = moderate and 3 = most severe lesion.[4] Representative specimens of heart, aorta and kidney were fixed in alcohol-formal (4 parts of absolute alcohol and 1 part of 10 per cent neutral formalin) for subsequent embedding in paraffin and staining with the von Kóssa stain for demonstration of calcium phosphate deposits. The bones were fixed in neutral formalin, decalcified with formic acid and stained with the periodic acid-Schiff stain.

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§ Schering, Bloomfield, New Jersey.

‖ Purina Co. of Canada.

RESULTS

The growth curves in the first experimental series (Fig. 1) and especially the final body weights (given in Table I with standard errors) indicate that the depression of growth induced by dihydrotachysterol is not significantly counteracted by treatment with 1 and 10 mg. of vitamin E, but the effect of therapy with 50 mg. or more proved to be statistically significant ($p < 0.01$). Almost complete protection was obtained with doses of 250 and 500 mg. of vitamin E per day; indeed, the apparent difference in the final body weight of these groups and in that of the untreated control animals was not statistically significant.

Calcification in the heart and kidney was pronounced in the rats receiving dihydrotachysterol alone (Group II), and this was only partially suppressed in the animals which,

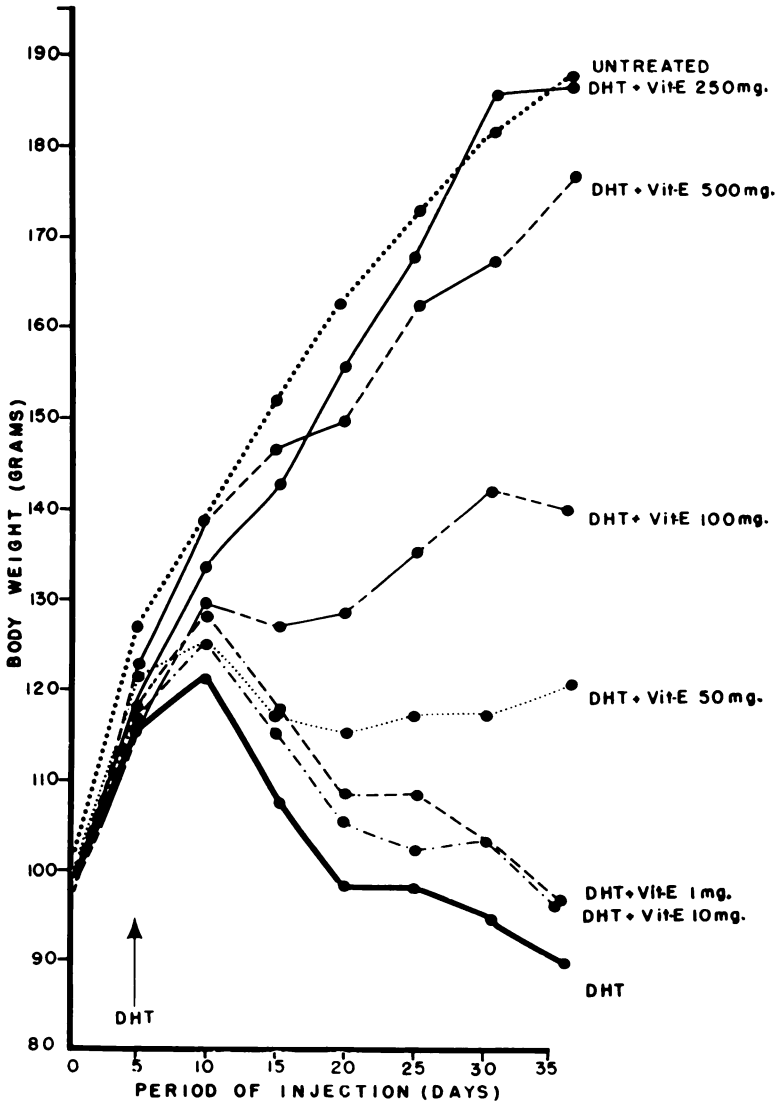

FIG. 1. Effect of various doses of vitamin E upon the body weight loss normally induced by dihydrotachysterol.

in addition, received 1 to 50 mg. of vitamin E. On the other hand, the administration of 100 mg. or more of vitamin E gave complete protection against cardiac calcification and nephrocalcinosis. The osteosclerosis induced by chronic dihydrotachysterol intoxication was similarly influenced, but since it does not lend itself as well to quantitative estimation, we did not include it in the tables.

The action of methyltestosterone is essentially similar to that of vitamin E (Fig. 2 and Table II), but we could find no evidence of any synergism between these two agents. Methyltestosterone alone offered as much protection as methyltestosterone plus 1 or 10 mg. of vitamin E, although methyltestosterone given in conjunction with 100 or 250 mg. of vitamin E yielded growth curves not statistically significantly different from those obtained with the corresponding amounts of vitamin E alone. We know from our earlier work that complete protection against dihydrotachysterol intoxication can be obtained under similar conditions with higher doses of methyltestosterone;[2]

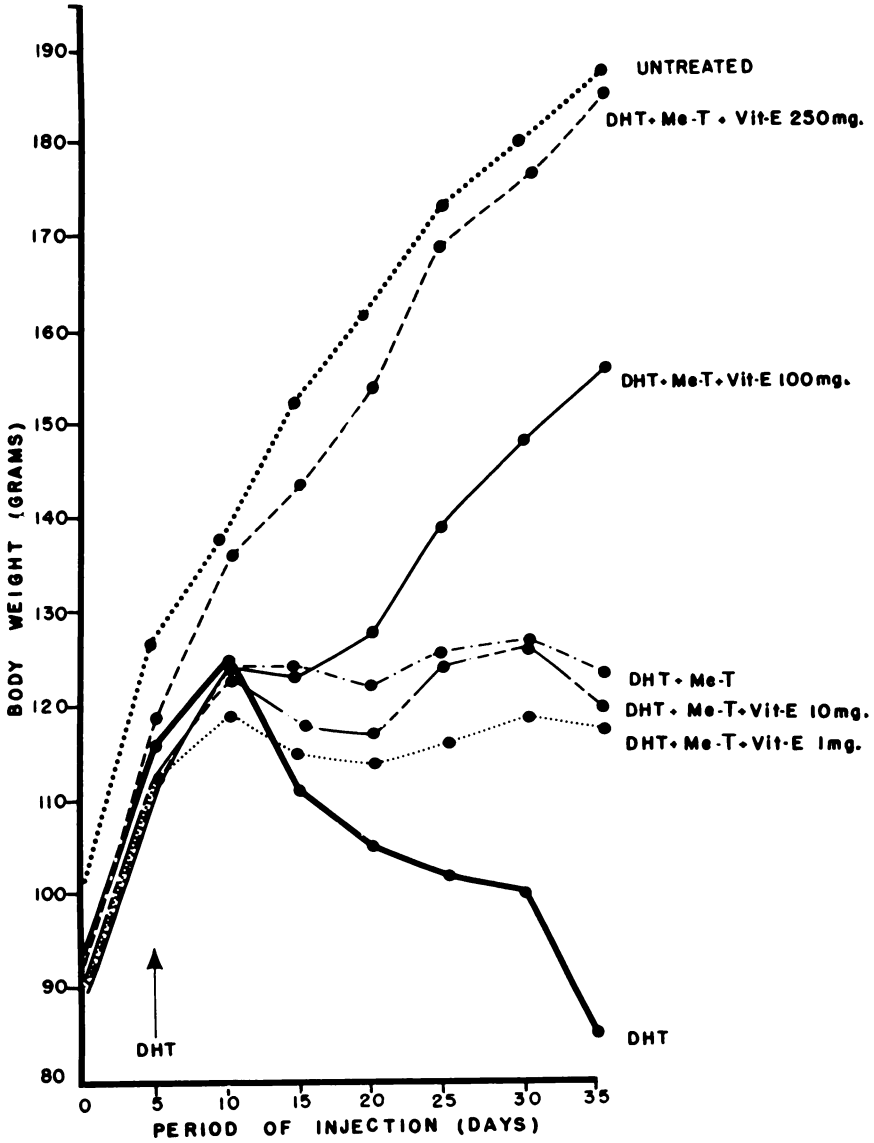

FIG. 2. Effect of methyltestosterone and vitamin E upon the body weight loss normally induced by dihydrotachysterol.

therefore, in the present series, we administered this androgen in an amount which gives only partial protection, subject to further enhancement. Under these circumstances, doses of vitamin E, ineffective in themselves, do not increase the moderate protection obtained by the low dose of methyltestosterone, although administration of amounts of vitamin E which offer definite protection in themselves, exhibit no synergism with these threshold doses of methyltestosterone.

Here again, the prevention of the dihydrotachysterol-induced osteosclerosis by methyltestosterone therapy alone or in combination with vitamin E treatment corresponded essentially to the protection offered by these agents against cardiac or renal calcification, as well as the depression of the body weight gain.

COMMENTS

In interpreting the significance of these observations, it should be clearly stated that,

although the progeria-like syndrome induced by dihydrotachysterol greatly resembles the changes characteristic of aging, we have no proof of any fundamental similarity in the mechanisms responsible for them. However, it is of interest that various compounds can antagonize both the catabolic and calcifying effects of dihydrotachysterol. Our earlier work has shown that ferric dextran is efficacious in this respect and that its action can be enhanced by concurrent treatment with methyltestosterone. The present observations indicate that vitamin E also acts as a dihydrotachysterol antagonist, but—at least under our experimental conditions—its effect is not enhanced by the androgen.

Among many agents tested, so far we have been able to find only the following three which can antagonize the production by dihydrotachysterol of the progeria-like syndrome: (1) ferric dextran, a calciphylactic challenger commonly used in clinical medicine to stimulate hemopoesis; (2) methyltestosterone, an anabolic androgen; and (3) *d*-α-tocopheryl acetate (an antisterility vitamin). It is difficult to see how these agents could exert their protective effect through a common pathway, but it is possible, of course, that all three act through different mechanisms. Theoretically, it appears probable that ferric dextran acts through calciphylaxis by virtue of its strong challenging potency, even though methyltestosterone may antagonize at least the catabolic action of dihydrotachysterol as a consequence of its known anabolic effect. It remains to be shown, however, why, on the other hand, ferric dextran counteracts not only calcification but also catabolism, whereas, on the other hand, methyltestosterone prevents pathologic calcification in addition to catabolism. In any event, both these agents appear to act systemically since, given parenterally, they are effective against enterally administered dihydrotachysterol. On the other hand, vitamin E may act locally in the alimentary tract, by interfering with the intestinal absorption of dihydrotachysterol, since to date we have not been able to duplicate its dihydrotachysterol-neutralizing action unless both compounds were given orally.

SUMMARY

Experiments on the rat indicate that the progeria-like syndrome induced by chronic intoxication with dihydrotachysterol can be prevented by concurrent *d*-α-tocopheryl acetate treatment.

Methyltestosterone is equally effective, but no synergism could be demonstrated between vitamin E and the anabolic androgen.

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