creased prolactin secretion whereas E at 2.0 μg/ml was without effect. These observations indicate that only E, of all steroids tested, can act at the pituitary level to influence prolactin secretion. The effects of other steroid hormones on prolactin secretion *in vivo* are probably mediated *via* pathways other than by a direct effect on the adenohypophysis.

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Sex Differences in a Progeria-Like Syndrome. (29644)

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It has been known for some time that arterial medial calcification is caused by excessive amounts of certain calcific agents such as irradiated ergosterol or vitamin D_2(1,2,3). Under short term conditions of heavy dihydrotachysterol ("DHT"—a vitamin D derivative) overdosage, the occurrence of arterial medial calcification in that rat is altered by certain metabolic conditions [pregnant females and intact males are protected while castrated males manifest lesions(4,5)]. It has further been reported that hydrocortisone administration to mice produces nonvascular myocardial calcification more easily in females than in males and that administration of testosterone to females prevents these calcific cardiac lesions(6).

Just recently it was demonstrated in the adult female rat that chronic treatment with relatively small amounts of DHT induces a unique syndrome which markedly resembles premature senility or progeria. As described elsewhere(7) this progeria-like model is reminiscent of senile changes as observed in animals and man. One exception to this similarity is that osteosclerosis is associated with the progeria-like model, while osteoporosis is the characteristic bone lesion associated with the aging process in man. Certain dissimilar substances such as ferric dextran (Fe-Dex), methyltestosterone and combinations of these agents have been found to be effective inhibitors of the progeria-like syndrome(8,9).

Since very little is known about the condi-

tioning and predisposing factors in the aging process, we thought it of interest to compare intact male and female rats of the same age, strain, and initial body weight in terms of their resistance to the development of an experimental progeria-like syndrome.

Materials and methods. 20 Sprague-Dawley rats (Holtzman Farms), 45 days of age, and of approximately the same body weight were selected from 10 litters (1 male and 1 female from each litter) and subdivided into 2 groups: 10 females with a mean initial body weight of 145 g (range 138-150 g) and 10 males with a mean initial body weight of 146 g (range 136-151 g). This procedure assured a male and female population of the same age and approximately the same initial body weight. From the 45th to the 70th days of age the animals of both groups received dihydrotachysterol ("DHT")* at the dose of 50 μg in 0.5 ml corn oil *per os* daily. The animals were housed in groups of 5 and were maintained on Purina Laboratory Chow and tap water throughout the 4-week treatment period. On the 28th day of experiment (70 days of age) all animals were sacrificed with chloroform and at autopsy the hearts, aortas, and kidneys were fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of 10% formalin) for subsequent embedding in paraffin and staining with the Von Kóssa technique for histochemical demonstration of calcium. The right femur of each animal was fixed in neutral 10% formalin for 48 hours, decalcified, embedded in paraffin, and stained with hematoxylin-eosin for an evaluation of general bone structure. The intensity of the organ lesions (calcium of the soft tissues, sclerosis of the bones) was evaluated histologically in terms of an arbitrary scale: 0 = no lesion, 1 = just visible lesion, 2 = moderate lesions, 3 = maximal lesions. An analysis of variance was performed on 80 observations involving 20 rats equally divided by sex, each animal being examined in four organs. Multiple comparisons were made of sex differences for each organ and 95% confidence limits were obtained for average differences.

* Calcamin®, Dr. A. Wander, S. A., Bern, Switzerland.

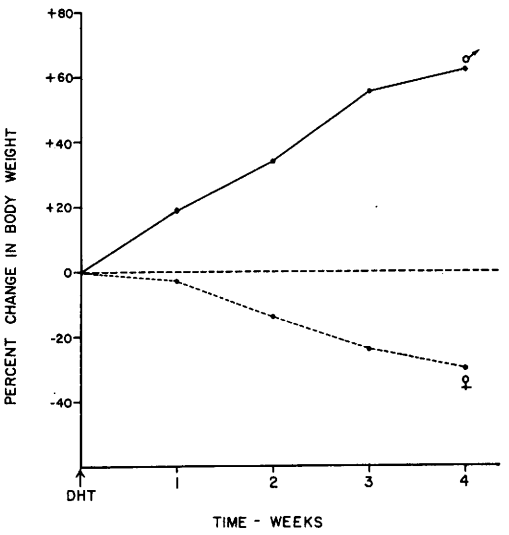

FIG. 1. Sex differences in the percentage change in body weight due to daily low dosage DHT administration.

Results. As indicated in Fig. 1 the percentage change in body weight for the females followed a negative course while that for the males was in a positive direction. This striking sex difference in regard to DHT-induced catabolism was evident as early as the first week after onset of DHT administration and by the end of the 4-week experiment the females manifested a 30% body weight loss while the males gained 62% in body weight. Although the percentage change in body weight for the males is markedly different from that for the females, it should be noted that under our experimental conditions the males are below the body weight range that would be expected of normal untreated Holtzman males at 70 days of age. This finding is not unexpected since previous experimentation revealed that female rats fully protected against the progeria-like syndrome by ferric dextran showed a body weight pattern that was below normal untreated control animals(8). There was no mortality in either group throughout the experiment.

Grossly, all the females manifested pronounced calcification of the coronary arteries, aortas, and kidneys (Fig. 2), severe kyphosis, dental abnormalities (erosions of the gingivae and a separation and lateral drift of

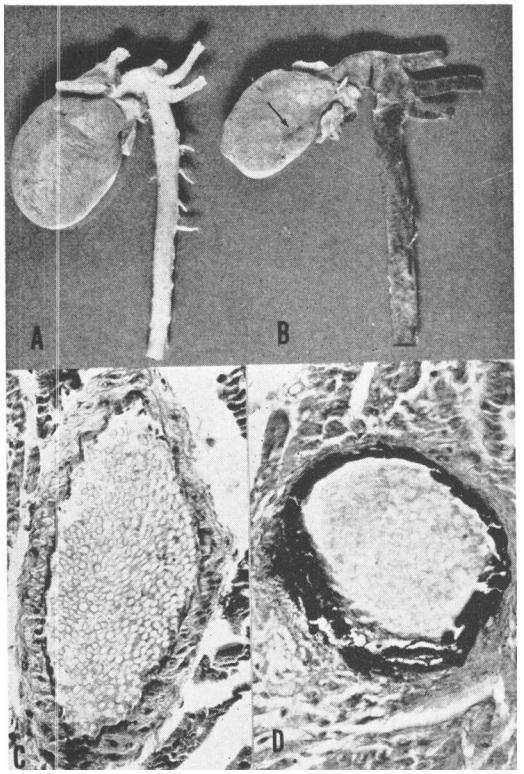

FIG. 2. Sex differences in resistance to arterial calcification in rats treated with DHT (50 y) daily during 4 weeks. A: Gross $AgNO_3$ preparation of heart and aorta from a DHT treated male rat showing complete protection against coronary arterial and aortic calcinosis. B: Comparable gross preparation of an identically treated female rat. Note the heavy calcinosis (black) of descending branch of left coronary artery (arrow) as well as of aorta and its main branches. C: Microscopic section (Von Kóssa × 450) of a coronary artery from right ventricle of a DHT-treated male rat. The vessel is free of pathologic calcification. D: Similar microscopic section from an identically treated female rat. The entire medial layer of the vessel is heavily encrusted with calcium as evidenced by the black staining reaction.

the upper incisors), loss of hair, and atrophy of the skin, skeletal musculature, adipose tissue, and large parenchymatous organs. The males, on the other hand, showed none of these grossly visible pathologic changes and presented no other clinical evidence of progeria-like involvement.

Mean microscopic readings (scale 0-3) and confidence limits for differences between sexes in pathologic calcification of the aortas, coronary arteries, kidneys, and femurs are presented in Table I. All the female animals showed severe medial calcification in the coronary arteries and aorta (Fig. 2), moderate kidney calcification in the interstitial tissue of both the renal cortex and medulla, and extensive osteosclerosis which was especially obvious in the subepiphyseal spongiosa of the femurs. The males were completely protected against aortic and coronary arterial calcinosis, but they manifested a moderate degree of osteosclerosis in the femurs and a just detectable calcific reaction in the interstitial tissue of the renal medulla.

Discussion. It is clear from our results that intact male rats are far more resistant than intact female rats in regard to the development of the experimental progeria-like syndrome. Since age and body weight between sexes were well controlled, it is feasible that endogenous testosterone is responsible for the protection seen in the male animals. If testosterone is the protective factor, it is unlikely that its effectiveness is due only to its anti-catabolic activity. This point is supported by the fact that exogenously administered methyl testosterone is far more potent than Fe-Dex as an anti-catabolic agent. Though both agents are effective inhibitors of this syndrome, the basic mechanism of their action is unknown. Furthermore, if somatotrophic hormone, a powerful anabolic agent, is administered in doses of equal anti-catabolic potency with Fe-Dex, the former is relatively ineffective as an inhibitor of the progeria-like syndrome(9).

TABLE I. Mean Microscopic Readings (Scale 0-3) and Confidence Limits* for Differences Between Sexes in Pathologic Calcification Produced by Chronic DHT Intoxication.

Organ	Calcinosis reading (scale 0-3) ♀	♂	Mean difference Sample estimate	95% Lower bound of sample estimate
Coronary arteries	2.7	0	2.7	2.38
Aorta	2.4	0	2.4	2.08
Kidney	2.0	1.0	1.0	.68
Femur	3.0	2.0	1.0	.68

* A 2-tailed confidence limit was calculated because the direction of the differences was unknown. However, only the lower confidence limit of the avg differences is indicated in Table because this is the bound of greater interest.

Our results are of practical as well as theoretical interest since they are at variance with the widely held belief that arteriosclerotic lesions in the human generally occur more frequently in males than in females. Complex pathologic processes in human disease are often difficult to interpret because one rarely has the opportunity systematically to isolate and study the precise effect of such crucial conditioning factors as sex, diet, age, genetics, stress, and others. It remains to be seen whether the various parameters of aging can be clarified through the use of the experimental progeria-like model and to that end various conditioning factors other than sex are now under investigation in this laboratory.

Summary. Intact male and female rats of the same age, same strain, and approximately the same initial body weight were subjected to a relatively low dose of dihydrotachysterol (DHT) over a period of 28 consecutive days. All the females developed a severe progeria-like syndrome (medial calcification of the coronary arteries and aorta, kyphosis, dental abnormalities, severe body weight loss, osteosclerosis, loss of hair, and atrophy of the skin, adipose tissue, skeletal musculature, and large parenchymatous organs). The males, on the other hand, were protected against this senile-like syndrome with the exception of a moderate degree of osteosclerosis and a minimal calcific reaction in the interstitial tissue of the renal medulla. The significance of these observations are discussed.

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Modification of the Basal Architecture of Renal Tubule Cells in Aged Rats.* (29645)

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Thickening of the peritubular basement membrane has been observed in the kidneys of humans and rats in association with the nephropathies of disease and aging. Farquhar, Vernier and Good(1) reported this change in the kidneys of patients having glomerulonephritis. Gellman *et al*(2) and Freeman and Roberts(3) described a similar observation in cases of diabetic nephropathy and renal glycosuria. Scott and Foltz(4) noted that thickening of the peritubular basement membrane was a consistently occurring feature in normal aged rats.

While investigating the histological changes associated with single essential amino acid deficiencies in aged rats, we observed a definitive morphological alteration in the appearance of the thickened peritubular basement membrane in the kidneys of all rats included in the experiment. Since it was not possible to determine the nature of this modification with light microscopy alone, kidneys of normal aged male rats were examined by electron microscopy.

Method. Kidney sections of 30 aged male

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