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Potentiation of Diethylstilbestrol Induced Aortic Ruptures of Turkeys with Thiouracil.* (32060)

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A high incidence of aortic ruptures can be induced in turkeys by feeding(1) diethylstilbestrol(DES). Hyperlipemia, hypercholesterolemia, and hypotension are accompanying features of the syndrome. It has been reported that thiouracil counteracted the protection afforded by estrogens in cholesterol induced coronary atherosclerosis in cockerels(2). Because of this report, the experiments in the present paper were conducted in an attempt to modify, by use of thiouracil, the incidence of DES induced aortic ruptures of turkeys.

Materials and methods. Broad-Breasted Bronze and Broad-Breasted White male turkeys were raised by conventional methods and fed a 20% protein commercial-chick-starter mash until 4 weeks of age. At this time they were changed to a 19% protein diet containing 0.5% NaCl(3). They were changed again at 6 weeks of age to a basal diet containing 23% protein, 6% animal fat, 3% NaCl(3), and various supplements.

Three separate trials were conducted. In each trial the turkeys were randomized into 7 groups of 8 birds each at 6 weeks of age. The following supplements were added to the basal diet and were fed to 3 groups of poults in each trial: (1) 16 g DES†/100 lb of feed; and (2) 16 g DES/100 lb of feed plus 0.1% thiouracil. One group of turkeys was fed unsupplemented basal diet and served as controls. Since 3 trials were conducted, a total of 9 groups were fed each supplemented diet, and 3 groups the unsupplemented diet. In addition, 1 group of turkeys in the third trial was fed basal diet supplemented with 0.1% thiouracil.

Indirect systolic blood pressure measurements were made on 4 poults per group in each trial at 10 weeks of age(4). Total plasma lipid(5) and cholesterol(6) determinations were recorded on 4 poults per group in each trial at 11 weeks of age. The experiment was terminated when the turkeys were 11½ weeks of age, at which time body weights were recorded.

Small pieces of thyroid glands were fixed in 10% neutral formalin at the conclusion of the trials. Sections of this material were stained with hematoxylin-eosin stain for histologic examination. Small pieces of abdominal aortas from turkeys that died from aortic rupture and from poults that were sacrificed at termination of the experiments were similarly fixed and stained prior to histologic examination. Frozen sections of abdominal aortas were also prepared and stained with oil red O-luxol fast blue stain.

Results. As there were no significant interactions between trials and treatments, the results of the 3 trials were combined. Thirty-seven percent of the poults fed DES died of aortic rhexis; however, when DES was fed with thiouracil, mortality was significantly increased to 69%. Rhexis occurred only in the abdominal aorta, and was a consequence of

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† Stilbosol, Eli Lilly Co., Greenfield, Ind., contained 20 g/lb in a carrier of polyethylene glycol 200.

TABLE I. Influence of DES and DES-Thiouracil on Aortic Ruptures, Blood Pressure, Plasma Composition, and Body Weights of Turkeys (Avg of 3 Exp).

Treatment	% Aortic ruptures	Systolic blood pressure* (mm Hg)	Total lipid† (mg/100 ml)	Total cholesterol† (mg/100 ml)	Body wt‡ (lb)
0	0[a]	224[a]	691[c]	185[c]	6.91[a]
DES	37[b]	175[b]	13,764[a]	1,066[a]	5.28[b]
DES-thiouracil	69[c]	167[c]	10,926[b]	839[b]	3.96[c]

* At 10 wk of age. † At 11 wk of age. ‡ At 11½ wk of age.

Means with different superscripts are significantly different according to Duncan's Multiple Range Test.

the development of dissecting aneurysms. It was observed by histologic observations that there were no differences in aortic lesions of turkeys fed DES alone and DES plus thiouracil. There was lipid deposition in the tunicae intima and media of aortas along with proliferation of the tunica intima and rupture of the internal elastic membrane. In sections through medial hemorrhage, it was evident that the contents of dissecting aneurysms were erythrocytes, proteinaceous fluid, and lipid.

Feeding of DES caused a significant lowering of systolic blood pressure. The addition of thiouracil to feed containing DES also resulted in a further significant decrease in systolic blood pressure, as compared to DES fed turkeys (167 *vs* 175 mm Hg). The systolic blood pressure of control birds was 224 mm Hg at this time (Table I).

Total plasma lipid and cholesterol values were higher in both treated groups than the control group of turkeys (Table I). Even though hyperlipemia and hypercholesterolemia were significantly lowered by the addition of thiouracil to feed containing DES, total plasma lipid and cholesterol values were greatly elevated in DES-thiouracil fed turkeys, as compared with controls.

Feeding of DES significantly decreased body weights (Table I). Addition of thiouracil to DES resulted in a further significant reduction in body weight. The diameter of the lumen and thickness of the wall of the abdominal aortas of DES-thiouracil fed turkeys appeared to be less than those of DES and control turkeys.

Thyroid size was influenced by feeding of both DES and thiouracil. The gland was mildly enlarged in DES fed turkeys and markedly enlarged in DES-thiouracil and thiouracil fed turkeys. Histologically the thyroid gland of control birds consisted of well developed follicles which contained dense, uniform staining colloid, and the follicles were lined by low epithelial cells (Fig. 1). The thyroids of DES fed poults contained small follicles of irregular shape and size which were lined by hypertrophic epithelium and contained pale staining and sometimes precipitated colloid (Fig. 2). The thyroids of DES-thiouracil fed poults contained follicles which were lined by hyperplastic, hypertrophic and folded epithelium and follicular lumens were distorted, slit-like and contained only small amounts of precipitated colloid (Fig. 3). The thyroids of turkeys fed thiouracil alone contained follicles which were lined by greatly swollen epithelial cells having vesicular nuclei and prominent nucleoli, and the lumens of follicles did not contain colloid (Fig. 4).

Discussion. These results confirm an earlier study in which it was reported that aortic atherosclerosis and dissecting aneurysms in turkeys were produced by DES treatments (11). Since no microscopic differences were observed in aortic lesions of turkeys fed DES alone and DES plus thiouracil, it would appear that the ability of thiouracil to potentiate DES-induced aortic ruptures did not result directly from the influence of thiouracil on the aortic wall.

In the present study, hypothyroidism resulted from feeding of diets containing DES, DES-thiouracil, and thiouracil. These data agree with a previous study(7) in which the thyroid glands of chicks were made goitrous by thiouracil feeding, as indicated by hyperplasia and hypertrophy of follicular epithelial cells.

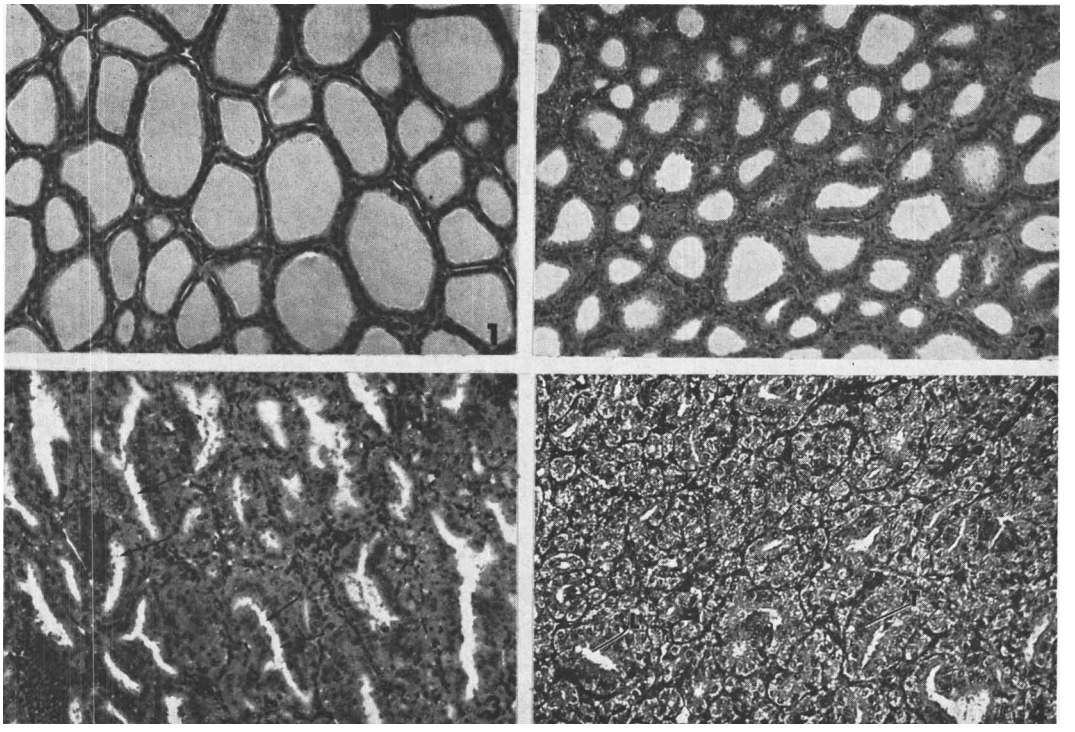

FIG. 1. Thyroid of control turkey. Follicles are large, lined by low epithelial cells, and contain dense staining colloid. H & E. × 83.

FIG. 2. Thyroid of DES fed turkey. Follicles are small, irregular in shape, lined by hypertrophic epithelial cells, and contain pale staining colloid. H & E. × 83.

FIG. 3. Thyroid of DES-thiouracil fed turkey. Follicles are distorted, lined by hypertrophic and hyperplastic epithelial cells, and have slit-like lumens (arrows). H & E. × 83.

FIG. 4. Thyroid of turkey fed thiouracil alone. Follicular epithelial cells are tall (T) and only a few lumens (L) are devoid of colloid. H & E. × 83.

Total plasma lipid and cholesterol values were reduced by adding thiouracil to DES supplemented feeds in our trials, but mortality from aortic ruptures was increased. This would tend to substantiate the finding that hypothyroidism in cockerels induced by thiouracil feeding was associated with loss of ability of estrogens to protect coronary vessels against cholesterol induced atherosclerosis(8). However, in contrast to the present work, thiouracil has been reported to cause elevation of blood cholesterol levels in cockerels(9).

The relationship of increased hypotension to increased incidence of aortic ruptures in DES-thiouracil fed turkeys, as contrasted to DES fed poults, is not clear. However, it is interesting to note that 2 deaths from aortic ruptures occurred in humans 5 months following total thyroidectomy, and a third death from aortic rhexis occurred 31 months after total thyroidectomy(10). It was suggested that hypothyroidism following thyroidectomy caused mucoid degenerative changes to develop in the aortic wall, with resulting rupture of the vessel.

Summary. Thirty-seven percent of turkeys fed DES died from aortic ruptures, while 69% of poults fed DES and thiouracil died from the disease. Total plasma lipid and cholesterol values were elevated by both treatments, but more so by DES alone. Turkeys fed DES or DES-thiouracil were hypotensive, but systolic blood pressure was lowest in turkeys fed the 2 combined drugs. There was pronounced hypertrophy of the thyroid gland of turkeys fed DES-thiouracil or thiouracil, and mild hypertrophy of the gland of poults fed DES. Body weights were depressed and the diameter of the lumen and thickness of the wall of ab-

dominal aortas appeared to be reduced by DES-thiouracil feeding, as compared to feeding of DES alone.

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Metabolism of Puromycin Aminonucleoside in the Normal, "Pre-Nephrotic," and Nephrotic Rat.* (32061)

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Administration of the aminonucleoside of puromycin (PA)† to the rat produces a nephrotic syndrome characterized by proteinuria, hypoalbuminemia, hypercholesterolemia, edema and ascites(1-4). Electron microscopic examination of kidney tissue revealed damage to the glomerular epithelial cell, the earliest manifestation being swelling of the foot processes, and the tubules(5). The biochemical lesion underlying aminonucleoside induced nephrosis is unknown. The rat is unique in its susceptibility to the nephrotoxic action of PA. The human and monkey are mildly susceptible while the guinea pig and mouse are resistant to the nephrotoxic action of this drug. The differential species toxicity may be related to differences in the metabolism of the drug in the various species. It was shown that the major urinary metabolites of PA are the monodemethylated analog, MMPA, and allantoin(6,7). Since certain drugs when administered chronically influence their own metabolic degradation by induction of enzymes of the hepatic microsomes(8), and experimental nephrosis by PA is generally produced by chronic administration of low doses (15 mg/kg), it was of interest to study the metabolism of PA at different time periods during the course of its chronic administration. It is possible that in non-susceptible species, enzymes may be induced which appreciably alter the metabolism of PA, qualitatively or quantitatively, such as to render it non-toxic. Alternatively, toxic metabolites may accumulate in susceptible species as a result of such enzyme induction. This report describes the metabolism of 8-C^{14}-PA in a rat, a susceptible species, at 3 different times during the course of administration of standard doses of PA, *i.e.*, at the normal, "pre-nephrotic" and nephrotic stages.

Materials and methods. Purification of PA. 8-C^{14}-PA was very kindly made available to us by Dr. Ralph Barclay, Sloan-Kettering Institute for Cancer Research, New York. Two-dimensional chromatography of the 8-C^{14}-PA

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† Abbreviations: PA, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl) purine; MMPA, 6-monomethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine; APA, 6-amino-9-(3′-amino-3′deoxy-β-D-ribofuranosyl)purine; IPA, 6-hydroxy-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine.