

Influence of High Dietary Sodium Chloride on Aortic Ruptures in Turkeys Induced by Diethylstilbestrol.* (29240)

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Aortic ruptures have been induced in rapidly growing male turkeys by diethylstilbestrol (DES) implantation(1). A high percentage of fatal dissecting aneurysms ensue as a result of this treatment; however, this incidence can be influenced by composition of the diet(2). This report describes the influence of high dietary sodium chloride (NaCl) levels on the incidence of aortic ruptures, serum composition, and blood pressure of turkeys injected with DES.

Materials and methods. Experiment 1. One hundred and twenty-eight, one-day-old Broad-Breasted Bronze male turkey poults were started in electric brooders with raised wire floors and fed a 20% protein commercial-chick-starter mash. At 4 weeks of age the birds were transferred to unheated batteries and fed a 19% protein diet(2) which contained 0.5% NaCl (Diet 1). At 10 weeks of age birds were assigned to concrete-floored pens and separated into 16 groups, each consisting of 8 birds. Four groups were continued on diet 1 and 4 groups were fed the same diet with 1.5% added NaCl (Diet 2). Another 4 groups of birds were changed to a 23% protein diet with 6% added animal fat(2) and 0.5% NaCl (Diet 3). The remaining 4 groups were fed diet 3 with 1.5% added NaCl (Diet 4). At 10 weeks of age, one-half of the birds in each group were injected subcutaneously in the neck region with 30 mg DES in liquid form,[†] and this treatment was repeated weekly until the experiment terminated at 21 weeks of age.

Experiment 2. The management of poults in Exp. 2 was similar to Exp. 1 up to 10 weeks of age, when the birds were divided into 16 groups of 10 birds each. Four groups were fed diet 1. Another 4 groups were fed

diet 1 with 2.5% added NaCl (Diet 5). Another 4 groups were fed diet 3. The remaining 4 groups of birds were fed diet 3 with 2.5% added NaCl (Diet 6). Four birds in each group were injected weekly with 30 mg of DES in liquid form. Four birds in each group were treated with 60 mg of the same drug, and 2 poults in each group did not receive DES and served as controls.

Treatment with DES, weighing, and bleeding initiated at 10 weeks of age was repeated weekly until the experiment terminated at 21 weeks of age. Total serum lipids were determined by the method of Bradstein *et al* (3), total serum protein according to Kingsley(4), and total serum cholesterol according to Pearson *et al*(5).

Direct blood pressures were recorded from the carotid arteries of 18 unanesthetized birds subjected to various treatments as shown in Table I. Siliconized polyethylene tubing having an i.d. of 0.066" and o.d. of 0.095" was inserted into the carotid arteries following local anesthesia of the involved surgical area. At least 3 readings were made on each of the 18 birds when they were 15 weeks of age.

Results. Exp. 1. Four percent of the birds fed diet 1 and treated with 30 mg of DES died of aortic rupture; however, 19% of birds similarly treated and fed diet 2 died of

TABLE I. Blood Pressure of Birds Treated with DES and Fed Diets with Different Levels of Salt (Exp II).

Diet*	Treatment	Mean blood pressure (mm Hg)†‡
3	DES, 30 mg	141
3	" 60 "	146
3	Control	202
6	DES, 30 mg	144
6	" 60 "	157
6	Control	197

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† Caponade, Vineland Laboratories, Vineland, N. J.

* Diet 3 contained 0.5% NaCl and diet 6 contained 3% NaCl.

† At 15 wk of age.

‡ Avg of triplicate measurements for 3 birds per treatment.

TABLE II. Serum Composition of Birds Fed 4 Diets and Treated with DES (Exp II).

Diet*	Treatment	Total cholesterol†			Total lipid†			Total protein‡		
		11§	15	19	11	15	19	11	15	19
1	Control	70	80	103	124	140	124	3.3	2.0	3.5
1	DES	425	700	490	830	835	800	10.0	9.5	10.9
3	Control	126	134	70	140	210	120	2.8	2.1	3.7
3	DES	933	1120	1050	1220	1490	1575	8.0	11.8	12.4
5	Control	140	130	79	175	179	88	3.1	2.4	2.6
5	DES	735	885	999	1409	1225	1230	10.0	10.0	12.1
6	Control	126	124	70	176	179	119	3.3	4.0	3.5
6	DES	1425	770	1051	1505	1123	1425	10.6	9.9	6.2

* Diets 1 and 3 contained low NaCl levels, and diets 5 and 6 contained high levels of NaCl.

† mg/100 ml serum. Avg of duplicate samples for 3 birds per treatment.

‡ g/100 ml serum.

§ Age in wk.

the disease. Forty percent of the treated birds fed diet 3, and 42% of the birds fed diet 4 and injected with DES died of aortic rhexis. None of the controls expired as a result of the syndrome. Birds dying from causes other than aortic ruptures were not used in calculating mortality percentages.

Exp. 2. Thirty-eight percent of the birds treated with DES and fed diet 1 died of aortic ruptures. However, 47% of the turkeys fed diet 3 and 48% of the birds fed diet 5 and treated with the hormone succumbed of the syndrome, while 67% of the hormone-treated turkeys fed diet 6 died of angiorrhexis. It appeared that both 30 and 60 mg of DES were equally effective in producing the condition. Birds fed diets 5 and 6 had wet, loose droppings, but weight gains were not affected.

DES treatment caused a marked lowering of blood pressure. However, the NaCl content of the diet had no influence on blood pressure of control or DES-treated birds (Table I).

Treatment with DES also caused hyperproteinemia, hyperlipemia and hypercholesterolemia. None of these constituents was modified by variations in NaCl or protein content of the diet (Table II).

Discussion. The results of these experiments not only confirmed previous observations(1) that DES was effective in producing aortic rupture in male turkeys, but also indicated that the incidence of aortic ruptures induced by DES treatment could be increased by elevating dietary NaCl levels.

It has been suggested that aortic ruptures occur more commonly in male turkeys than females under natural conditions because the former sex has a higher blood pressure(6). Apparently this reasoning does not explain the mode of action of DES in the induction of aortic ruptures or the increased incidence of the disease in DES-treated male birds fed high dietary levels of NaCl. Neither treatment with DES nor increased NaCl levels in the feed caused elevated blood pressure in these experiments. Therefore, the integrity of the aortic wall during the period of rapid growth is probably the factor most directly involved in the development of the disease because it is during this period that the aorta appears to be susceptible to damaging agents such as DES. As reported by Beall *et al*(1), DES-induced aortic ruptures did not occur after birds were 16 weeks of age, the time at which weight gains declined.

It has been reported that in the male chick DES depressed systolic blood pressure(7); however, 1.5% saline as a source of drinking water elevated the systolic blood pressure of chicks(8).

The significance of hyperproteinemia, hypercholesterolemia, and hyperlipemia in the production of the disease was not resolved in this study. The sera of DES-treated turkeys was white and thick as has been reported in DES-treated cockerels(9). It was at this time that the blood pressure values of turkeys reported here were measured.

Summary. The incidence of aortic ruptures in turkeys induced by injection of DES in a

liquid form was modified by dietary composition including high NaCl levels. Diethylstilbestrol injections also caused hyperproteinemia, hypercholesterolemia, hyperlipemia and lowered blood pressure. High NaCl diets did not affect blood pressure.

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Follicular Fluid Electrolytes in Ovulation.* (29241)

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Although many aspects of the composition of follicular fluid have attracted research interest, the electrolyte composition has been inadequately reported. In the present study Na and K concentrations in the fluid of rabbit follicles were determined at 3 stages of follicular development. These data permit evaluation of the hypothesis that imbalances in cation transport across the follicle wall occur in the ovulatory process(1).

Materials and methods. Ten sexually mature female rabbits of mixed strains were grouped as follows: Group I, 2 animals judged to be in diestrus on the basis of vaginal appearance and refusal to copulate; Group II, 4 animals classed as being in estrus on the basis of tumescent reddish-purple vaginal lining; Group III, 4 animals judged to be in estrus mated with normal males. All animals were lightly anesthetized (approximately 35 mg pentobarbital per kg i.v.), and opened with bilateral flank incisions for visualization of the ovaries. Mated animals were anesthetized 7 to 8½ hours after mating and sampled during the 9th hour.

The exteriorized ovaries were held in place by clamping the mesovaria parallel to the

ovary, in a hemostat equipped at the tip with a spacer which prevented the jaws from closing on the mesovarian vessels. A sample of follicular fluid was taken from each of the large follicles; in the case of unmated estrus rabbits these were follicles which appeared to be sufficiently mature to ovulate if the animals had been mated (avg. diam. $1,600 \pm 38 \mu$), in the case of mated animals the follicles sampled were those in which ovulation appeared imminent (avg. diam. $1,880 \pm 62 \mu$). The largest follicles of the animals in diestrus were also sampled, although they appeared smaller, tougher, and flatter (avg. diam. $1,420 \pm 56 \mu$) than those of the other groups. The sampling procedure was as follows: The follicle to be sampled was punctured by a tared micropipette formed from capillary tubing (1.5 mm OD) ground to a bevel (Fig. 1). With the aid of an aspirator, fluid was drawn into the pipette and it was reweighed. Sample weights ranged from .9 to 3.5 mg and could be reproducibly read to within $\pm .01$ mg. Samples contaminated with blood were not used. After weighing, the pipette was rinsed into 8-9 ml of distilled H₂O in a 10 ml volumetric flask, .5 ml Li₂SO₄ solution was added and the volume brought to 10 ml with distilled H₂O. Plasma samples

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