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Report of Failure of Alpha-tocopherol to Prevent Diethylstilbestrol-Induced Purpura in Dogs.*

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Castrodale *et al.*[1] showed that diethylstilbestrol given in large doses parenterally to dogs produced thrombopenia in about 8-14 days and hemorrhagic manifestations several days thereafter. This was accompanied by changes in the peripheral blood, the bone marrow, and in some instances, the liver prior to death. Tyslowitz and Dingemanse,[2] and Crafts[3] described similar results in dogs with the former investigators reporting the development of an aplastic anemia after a latent period of 1-3 weeks. Liver extract injections did not prevent the development of the anemia. Skelton, Shute *et al.*[4] reported that the administration of synthetic alpha-tocopherol acetate (Ephynal Acetate — Hoffman-La-Roche) in oral doses of 200 mg per day corrected the diethylstilbestrol-induced purpura of dogs and caused the platelet counts and capillary fragility to return to normal. If employed earlier, the appearance of frank purpura and blood vascular deficiency was prevented. The study here reported was made in an attempt to confirm the observations of Skelton and his associates.

Methods. Three male and 3 female adult dogs were used as experimental animals. The dogs were observed over a period of 5-11 weeks. The diet consisted of Dickinson Dog Food without additional supplement. The following determinations were done twice weekly throughout the experiment: capillary fragility test, bleeding time, coagulation time, and platelet count. In addition, erythrocyte and leukocyte counts, hemoglobin determinations, and differential counts were done at weekly intervals on Dogs 4, 5, and 6 throughout the investigation, and on Dogs 1, 2, and 3 during the latter part of the investigation. Ten cc blood samples were drawn from the femoral arteries into syringes coated with liquid paraffin. The blood samples were then placed in small paraffined beakers containing 0.5 cc of 2.5% sodium citrate. All blood determinations other than differential counts were done from these specimens. Capillary fragility tests were done by a method modified from Dalldorf[5] which employed a negative pressure of 180 mm mercury for one minute over a 4 cm diameter circular area of the abdominal wall. Bleeding times were done by the Duke method. Coagulation times were determined by the 2-tube method of Lee and White in a 37-degree water bath. Platelets were counted by the direct wet method using Rees-Ecker diluting fluid. The red counts were made using the same diluting fluid. Differential counts were obtained from Wright stained smears of arterial blood. Hemoglobin values were determined by the Haden-Hausser method; hematocrit readings, by the Wintrobe method.

In addition, bone marrow biopsies were taken from the ribs of Dogs 4, 5, and 6 at the beginning and termination of the experiment. In Dogs 1, 2, and 3 biopsies were made only at the end of the experiment. Two or 3 control determinations were made on each dog immediately prior to experimental study.

Four dogs received daily intramuscular injections of approximately 1.5 mg per kg body

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1 Castrodale, D., Bierbaum, O., Helwig, E. B., and MacBryde, C. M., *Endocrinology*, 1941, **29**, 363.

2 Tyslowitz, R., and Dingemanse, E., *Endocrinology*, 1941, **29**, 817.

3 Crafts, R. C., *Endocrinology*, 1941, **29**, 606.

4 Skelton, F., Shute, E., Skinner, H. G., and Wand, R. A., *Science*, 1946, **103**, 762.

5 Dalldorf, Gilbert, *J. Exp. Med.*, 1931, **53**:**2**, 289.

TABLE I.
Effects of Diethylstilbestrol and Diethylstilbestrol Plus Alpha-tocopherol in Dogs.

Dog	mg/K	Day	Pet.	B.T.	C.T.	Plat.	Hb.	RBC	WBC
1 ♀	1.5 diethylstilbestrol	C	0	2.5	6	228			
		18	3	15.<	18	12			
		18	Alpha-tocopherol started 15 mg/K.						
		25	8	15.<	24	18			
		39	0	15.<	30	20	5.25	2.13	
		40	Diethylstilbestrol stopped.						
		44	1	15.<	26	24	3.7	1.23	2.2
		51	Dog expired. Autopsy performed.						
2 ♀	1.5 "	C	1	2.	12	256			
		18	11	19.<	18	24			
		18	Diethylstilbestrol stopped. Alpha-tocopherol started 15 mg/K						
		25	15	15.<	38	20			
		50	0	5.5	12	186	12.5	5.47	9.95
		52	Diethylstilbestrol started 1.5 mg/K.						
		69	0	1.5	15	38	12.0	5.51	38.45
3 ♀	1.0 "	C	0	2.	8	292			
		22	0	5.5	21	44			
		40	0	2.	16	218			
		42	Diethylstilbestrol dosage increased to 2 mg/K.						
		64	0	1.	13	180	9.0	3.88	27.95
		71	0	1.5	11	266	9.5	4.07	35.30
4 ♂	1.5 "	C	1	3.	11	266	13.5	5.62	14.25
		13	0	1.	11	58	12.5	5.93	31.25
		20	0	1.	28	22	12.5	4.80	49.50
		34	0	1.	18	172	10.5	4.70	20.75
		41	0	2.5	20	206	11.	5.39	16.85
5 ♂	1.5 "	C	1	0.5	13	230	12.0	5.27	15.75
	15 alpha-tocopherol	13	0	3.	14	38	10.5	5.39	36.20
		20	0	15.<	41	18	10.	4.91	112.30
		34	0	6.	20	82	8.	3.85	15.60
		41	0	1.	17	94	10.	4.30	10.45
6 ♂	1.0 diethylstilbestrol	C	2	1.	16	196	14.0	6.67	12.6
		17	0	1.	6	10	13.5	6.17	31.2
		24	Diethylstilbestrol stopped.						
		25	6	15.<	23	18	8.5	3.04	55.
		32	Dog expired. Autopsy performed.						

Days numbered from initiation of medication as listed under column "Dog."
C = Control.
Pet = Number of petechiae in 4 cm diameter circle.
B.T. = Bleeding time in minutes.
C.T. = Coagulation time in minutes.
Plat. = Platelets in thousands per cmm blood.
Hb = Hemoglobin in g per 100 cc blood.
RBC = Erythrocyte count in millions per cmm blood.
WBC = Leukocyte count in thousands per cmm blood.

weight, while 2 dogs (No. 3 and 6) received approximately 1 mg per kg of diethylstilbestrol (Eli Lilly & Co.)

Alpha-tocopherol was employed in 3 dogs in a daily oral dose of approximately 15 mg per kg body weight. In Dogs 1 and 2 it was employed 18 days after the initiation of diethylstilbestrol when hemorrhagic manifestations were apparent. In Dog 5 and later in Dog 2 it was employed simultaneously with diethylstilbestrol.

Results. The changes following diethylstilbestrol administration paralleled those previously described by Castrodale,[1] Tyslowitz,[2] and Crafts.[3] There was a marked fall in platelets reaching the lowest level in 15-20 days. Associated with the severe thrombopenia there was a lengthening of the bleeding time in all dogs (less pronounced in Dogs 3 and 4) and an increased capillary

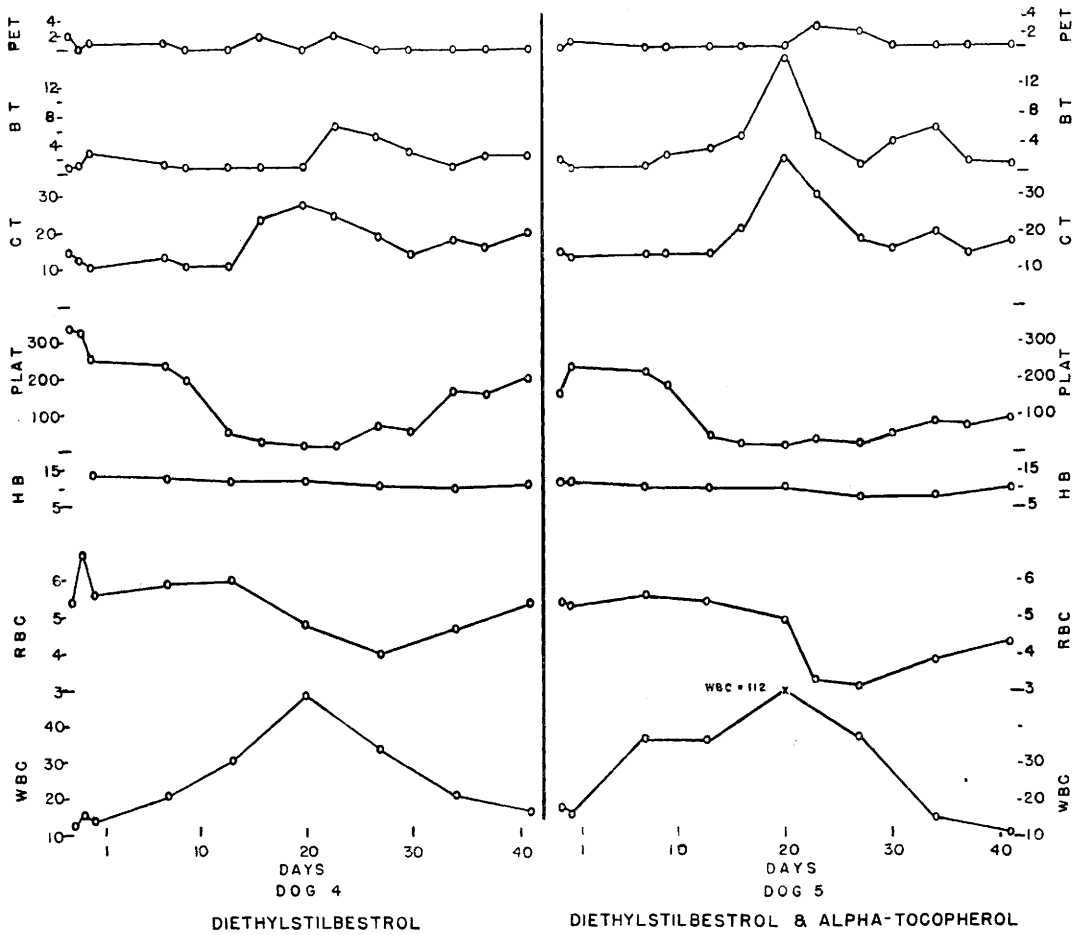

FIG. 1.
Comparative Effects of Diethylstilbestrol Plus Alpha-tocopherol and Diethylstilbestrol in Dogs, Demonstrating Similarity in Nearly All Aspects.
Days numbered from initiation of medication as listed under Table I.
Pet. = Number of petechiae in 4 cm diameter circle.
B.T. = Bleeding time in minutes.
C.T. = Coagulation time in minutes.
Plat. = Platelets in thousands per cmm blood.
Hb. = Hemoglobin in g per 100 cc blood.
RBC = Erythrocyte count in millions per cmm blood.
WBC = Leukocyte count in thousands per cmm blood.

fragility (relatively insignificant in Dogs 3 and 4) which appeared 2-10 days after the fall in platelets. All dogs in which blood counts were done showed a leukocytosis reaching its height about the 20th day and slowly regressing thereafter. In one dog the count reached 113,300 cells per cmm on the 20th day. Concomitantly there was a gradual reduction in the number of erythrocytes and the amount of hemoglobin. It is to be noted that diethylstilbestrol likewise produced a prolongation of the coagulation time with a maximum effect after 20-25 days. To our knowledge there have been no previous reports concerning the effect of diethylstilbestrol on the blood coagulation time.

In 2 dogs (3 and 4) the platelet count, bleeding time and all the blood elements returned to normal despite the continued administration of diethylstilbestrol. The simultaneous administration of alpha-tocopherol did not prevent the occurrence of thrombo-

penia in Dogs 2 and 5 nor did it prevent the appearance of nor modify the severity of purpuric manifestations in Dog 5. In Dog 1 in which there was a fatal outcome, little if any beneficial effect upon the marrow was noted from the use of alpha-tocopherol; while the favorable course of Dog 2 may well have been due to the early discontinuance of diethylstilbestrol. In no instance did alpha-tocopherol tend to delay the appearance of the hemorrhagic tendency nor accelerate the return to normal above that noted in Dogs 3 and 4.

Dogs receiving alpha-tocopherol and diethylstilbestrol showed changes in the erythrocyte count, leukocyte count, hemoglobin values, and hematocrit determinations similar to those receiving diethylstilbestrol alone. Leukocytoses similar to that observed in Dog 4 have been described previously by Castrodale.[1] In the 2 dogs in which death occurred (Dogs 1 and 6) postmortem examinations were done within 12-18 hours and both showed evidence of severe anemia, numerous hemorrhages throughout the body, and liver necrosis which was extensive in Dog 6. There was a marked hypoplasia of all elements of the bone marrow in Dog 1. Rib biopsies performed at the start of the investigations on Dogs 4, 5, and 6 showed normal marrow. Marrow studies postmortem or by biopsy at the conclusion of the studies showed: a markedly hypoplastic marrow in one (Dog 1), hyperplastic marrow in 2 (Dogs 3 and 4), and normal marrow in 3 (Dogs 2, 5, and 6). The type of marrow found appeared to have no significant relationship to the dosage of diethylstilbestrol nor to the administration or nonadministration of alpha-tocopherol.

Summary. Diethylstilbestrol caused a prolongation of the bleeding and coagulation time in dogs, thrombopenia, an increase in the capillary fragility, leukocytosis and a gradual anemia. In 2 dogs death resulted.

The changes in the circulating blood and bone marrow produced by diethylstilbestrol appear to be temporary in some instances. The return to normal, despite the continued administration of the drug, might indicate that a tolerance to diethylstilbestrol had been acquired. This return to normal was as prompt and as great without as with the administration of alpha-tocopherol.

The present studies failed to confirm the report of Skelton, Shute, *et al.* that alpha-tocopherol exerts an antipurpuric action on the hemorrhagic diathesis induced in dogs by diethylstilbestrol.

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Failure of Adrenal Cortical Activity to Influence Circulating Antibodies and Gamma Globulin.*

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Studies attempting to demonstrate a relationship between adrenal cortical activity and circulating antibodies have yielded con-

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