DIETHYLSTILBESTROL EFFECTS ON ANTLER AND REPRODUCTIVE GLAND MORPHOLOGY IN MALE DEER[1]

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ABSTRACT

Silastic® tube-type implants containing diethylstilbestrol (DES) were placed subcutaneously in five male white-tailed deer (Odocoileus virginianus). *Implants were recovered from four deer and determined to have a mean daily release rate of 205 μg. At this rate DES drastically suppressed antler growth, but the velvet was shed and bone antlers were formed. Treated males had significantly lower testes weights than controls. However, DES affected spermatogenesis but the response was not uniform, ranging from almost complete cessation to limited sperm production. The round spermatid population was significantly ($P < 0.05$) reduced in the DES group. Morphologically, the Leydig cells in treated deer appeared different from those in the control animals. The epididymides were unaffected by treatment. The treatment affected the secretory cells of the Cowper's and prostrate glands in three of the four deer and the secretory cells of the seminal vesicles in all four. Because of abnormal antler development, research with DES as a male deer antifertility agent was discontinued.*

The U. S. Park Service has funded research for the development of a reproductive inhibitor to control the white-tailed deer populations within certain U. S. National Parks. Previous research has been directed against the reproductive capability of the female because of the bizarre antler development that may result when the endocrine system of the male is altered. Since we had experienced little success in controlling the reproductive capability of the female (Matschke 1976a, 1976b, 1977), we sought a method of producing infertile males that would not affect the normal antler cycle. Ideally, such a method should be capable of interfering with spermatogenesis or the accessory sex glands without interfering with the testosterone production responsible for velvet shedding, antler retention, and neck swelling (Wislocki et al. 1947). A synthetic estrogen, diethylstilbestrol (DES), was selected because the primary effect of this exogenous estrogen is to slow the release of the follicle stimulating hormone (FSH) from the anterior pituitary (Lacy and Lofts 1965). We anticipated that lowered FSH levels would interfere with spermatogenesis, but would not completely interfere with the luteinizing hormone (LH) and subsequent testosterone production.

The objective of this study was to determine whether a Silastic® tube-type subcutaneous implant containing DES with an estimated daily release rate of 130 μg per day would alter spermatogenesis or the accessory sex glands without impairing the normal antler cycle.

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PROCEDURES

Penned male deer (yearling or older) were immobilized in April (4 males) and July (1 male) with Pneu-darts[2] containing powdered succinylcholine chloride (Liscinsky et al. 1969). The scrotum was wiped with 70% ethyl alcohol and a small slit was made. A Silastic® tube-type implant (3 cm long x 0.44 cm OD with a wall thickness of 0.064 cm) containing 75 mg of DES was inserted into the scrotum. After the incision was closed with sutures and

[1] A contribution of a cooperative project between the National Park Service and the U. S. Fish and Wildlife Service, U. S. Department of the Interior.

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[2] Manufactured by PNEU-DARTS, Inc., 406 Bridge Street, Williamsport, PA 17701. Use of trade names does not constitute endorsement of the product by the U. S. Government.

sprayed with an antiseptic, the animals were released into a 1 ha enclosure. A blank implant was installed in five control males.

In November all deer were killed. The implants were removed and were assayed by a technique developed by R. E. Crutcher and L. R. Macy (Unpublished—Abbott Laboratories) to determine the daily release rate of the hormone. The animals were weighed and neck diameters were measured at a point 15 cm posterior to the occiput. Antler morphology for all animals was recorded but antler length, tip of the beam to hair line, was recorded only for the April implanted deer. Testes, seminal vesicles, and Cowper's glands were excised, trimmed free of adnexa, weighed, and fixed in Bouin's fluid, as were tissue sections of the prostate and epididymides.

Tissue sections were cut at 5 μm and stained with H & E. Round seminiferous tubule diameters were taken (20 per animal) by averaging two right angle measurements. Testes were systematically scanned; seminiferous tubules (300 per animal) were randomly sampled and, depending on the state of spermatogenesis, categorized as follows: sterile (all germinative cells absent), spermatogonia only, primary spermatocytes only, secondary spermatocytes only, round spermatids, and elongate spermatids. The ratio of tubule to interstitial tissue was obtained by scanning systematically and scoring 300 random points after the method of Chalkley(1943).

The significance of treatment effects on organ weights and other quantitative data was tested through an analysis of variance (Steele and Torrie 1960).

RESULTS

At necropsy it was found that one male had lost its implant; this animal was therefore removed from the study. The other four implants remained in place and were recovered; the mean daily release rate was calculated to be 205 μg ± 40.5 S.D. (range of 153 to 230 μg). The approximate daily dose was 0.004 mg/kg.

Neither body weight nor neck diameters were significantly affected by DES, although neck diameter of the treated group was only 89% of that of the control group. Mean values for the DES-treated group and the control group were 69.8 and 66.8 kg, and 46.0 and 51.8 cm, respectively.

DES had a dramatic effect on preventing antler growth (Fig. 1). Three deer implanted in April had just shed their antlers, and replacement antlers were beginning to form. At the time of implantation none were over 1.5 cm in length. Normal growth and development failed to occur following implantation. All DES males had shed the velvet and had bone antlers. Two deer had small spikes which did not exceed 2.5 cm in length; the third deer had a 0.5 cm spike on the right side, and a spike that measured 5.4 cm on the left. Antlers on all three April implanted males were without the basal burr. The fourth animal (414), which was implanted in July, had a small rack with five points. Although this set of antlers was not measured at implantation time, growth and development were apparently arrested soon after implantation. Antler development was normal in the controls.

Mean testes weights in the DES-treated group (27.8 g ± 5.9 S.D.) were significantly ($P < 0.05$) less than in the control group (59.6 g ± 22.8 S.D.). The mean weights and standard deviations of the seminal vesicles were 5.8 g ± 3.9 S.D. and 11.3 g ± 6.3 S.D., and Cowper's glands were 9.5 g ± 3.0 S.D. and 9.6 g ± 3.9 S.D. for the treated and control groups, respectively. The weight differences were not significant ($P > 0.05$).

DES treatment affected spermatogenesis but the response was not uniform, ranging from almost complete cessation to limited sperm production (Table 1). Male 412 was the most severely affected, with 54% and 39% of the tubules arrested at the primary spermatocyte and round spermatid stages, respectively. Only 1% of the tubules contained elongated spermatids. Round and elongate spermatids were produced by treated males 414 and 323 and the percentage of tubules containing either round or elongate spermatids was similar to the control group (Table 1). However, the population of spermatids per tubule appeared less than in the control animals. Also a higher than normal incidence of head and tail separation among elongate spermatids was observed (Fig. 2). The loss of spermatids was estimated by cell counts of pachytene spermatocytes and round spermatids obtained from 15 round seminiferous tubules in stage I of the eight-stage cycle. The mean population of the round spermatids was 568 for the treated group compared to

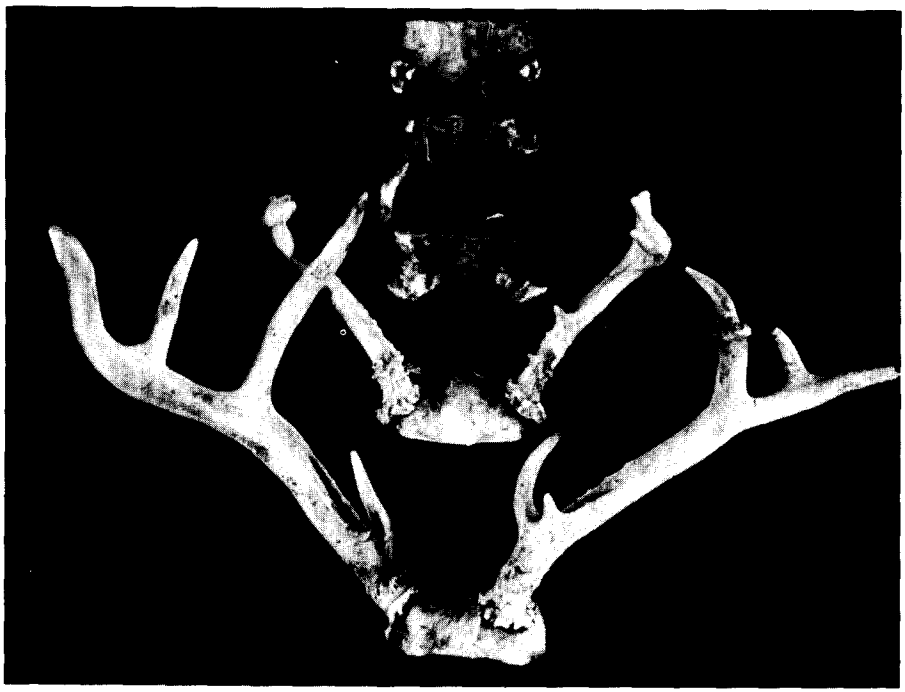

Figure 1. Antler morphology following DES treatment. The top three sets of antlers were from animals implanted in April; the fourth was the animal implanted in July. The bottom antlers were from a control animal.

1,713 for the control group; this difference was significant ($P < 0.05$). However, the difference between the pachytene cell populations of the two groups was not significant ($P > 0.05$). The mean pachytene population count per animal was 398 cells for the treated, and 471 for the control group. The mean ratio of pachytene spermatocytes to round spermatids was 1:1.43 for the treated and 1:3.65 for the control group; this difference was significant ($P < 0.05$). DES-treated male 293 was sterile in 88% of the tubules, but round or elongate spermatids were found in 10% of the tubules. Diameters of seminiferous tubules averaged 64.7 μm in the treated deer and 77.8 μm in the control deer; however, this difference was not significant ($P > 0.05$). The epididymides for both groups appeared histologically similar.

Table 1. Effect of diethylstilbestrol on spermatogenesis in the deer as revealed by quantitative testicular histology. The most advanced germinal cells per individual seminiferous tubule cross section were classified into one of six categories; 300 seminiferous tubules were scored per animal.

Deer number and treatment	*Seminiferous tubules lacking type A spermatogonia*		*Seminiferous tubules containing only:*							
			Type-A spermatogonia		*Primary spermatocytes*		*Secondary spermatocytes*		*Round spermatocytes*		*Elongate spermatids*	
	no.	*(%)*	*no.*	*(%)*	*no.*	*(%)*	*no.*	*(%)*	*no.*	*(%)*	*no.*	*(%)*
DES-412	1	(0)	0	(0)	163	(54)	17	(6)	116	(39)	3	(1)
DES-414	0	(0)	0	(0)	16	(5)	0	(0)	126	(42)	158	(53)
DES-323	1	(0)	0	(0)	2	(1)	0	(0)	114	(38)	183	(61)
DES-293	264	(88)	1	(1)	4	(1)	0	(0)	7	(2)	24	(8)
Controls*	8	(0.5)	2	(0.1)	60	(4)	0	(0)	490	(32.6)	940	(62.6)

*Because of homogenity, the 5 controls were combined.

Figure 2. Tubule cross section of DES-treated testis showing necrotic round spermatids (top arrow) and two spermatozoa with missing tails (bottom arrow). (447X)

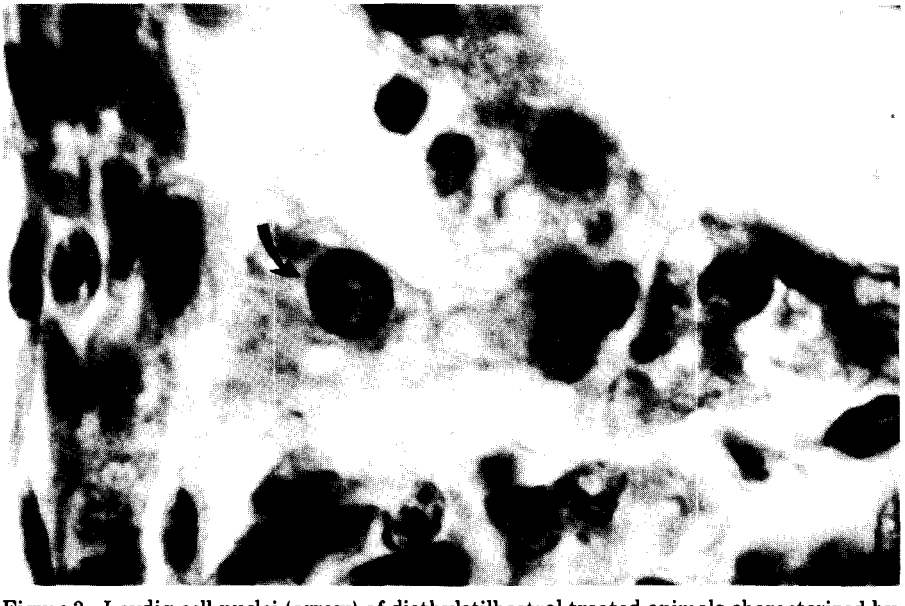

Figure 3. Leydig cell nuclei (arrow) of diethylstilbestrol treated animals characterized by large round nuclei. (447X)

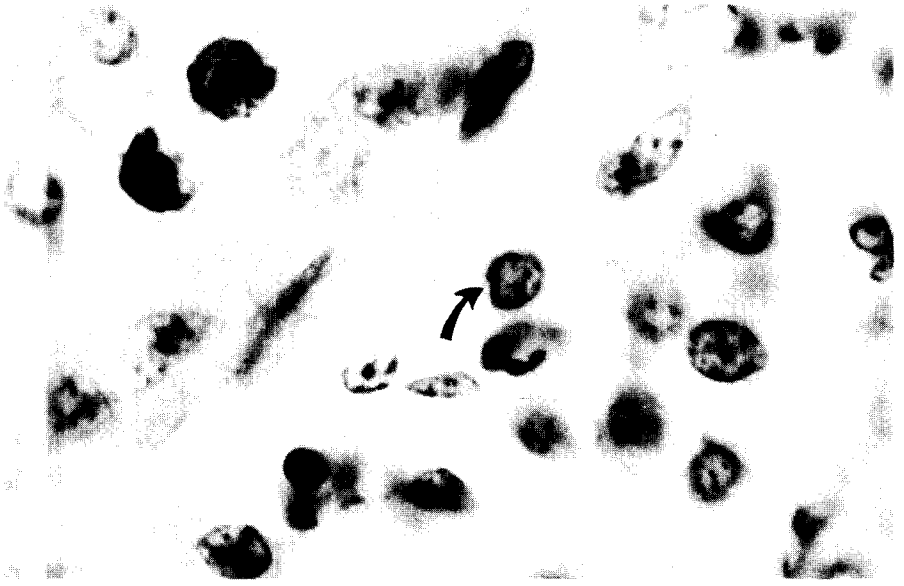

Figure 4. Leydig cell nuclei of control animals shows evidence of regression as nuclear membrane shows signs of being crenated (arrow). (447X)

Leydig cell morphology differed between the two groups. In the DES-treated group most cells had round nuclei (Fig. 3) which did not show evidence of regression, whereas in the control group the nuclei were ovoid in shape, had decreased nuclear volume, and showed slight crenation of the nuclear membrane (Fig. 4). The ratio of interstitial to seminiferous tubule tissue was 25:75 for the treated group and 32:68 for the control group; this difference, however, was not significant ($P > 0.05$).

The seminal vesicles of both groups were secreting plasma; in the treated group, however, areas of nonfunctional cells were present and these areas were without secretory products. The Cowper's glands among the controls had high columnar epithelial cells with large and small lumens containing secretory products; among the treated group, disorganization occurred in some of the secretory cells. All animals had some secretory droplets. The secretory tissue in male 412 was infiltrated with connective tissue, and in male 323 the gland appeared vacuolated. Only one (293) of the four treated animals had a normal prostate gland. The secretory epithelium of the prostates of the other three animals had low columnar to cuboidal cells instead of tall columnar cells.

DISCUSSION

Based on lack of antler growth I postulated that the anterior pituitary tropin(s) responsible for antler growth was suppressed by DES at 153-230 μg daily. At present the tropin(s) responsible for antler growth has not been identified. Wislocki (1943) proposed that the "Antler Stimulating Hormone" may be the growth hormone (STH). Later, Wislocki et al. (1947) proposed that prolactin may be responsible. However, velvet shedding and hardening of the antlers proceeded normally, indicating that some gonadotrophic hormone (LH) and testosterone were being produced (Wislocki et al. 1947). Testosterone is known to be necessary for antler maturation, secondary ossification, shedding of the velvet, and antler retention (Wislocki et al. 1947).

The gonadotrophic hormones responsible for the initiation of spermatogenesis were not completely suppressed even though treatment was applied months before the initiation of

spermatogenesis in this seasonal breeding species. Only male 412 had indications of impeded FSH secretion. This animal had reduced testicular weight and the spermatids failed to complete maturation. DES may also have interfered with LH or testosterone secretion in animal 412, since primary spermatocytes failed to undergo meiosis in 54% of the tubules; testosterone has been shown to be essential for completion of meiotic division (Steinberger 1971). However, some testosterone must have been produced, since the antlers were retained, and the neck was swollen. Leydig cell morphology in deer 412 did not appear to be different from that in the other three treated deer, but the relationship between testosterone production and Leydig cell morphology in this species is unknown. Maximal activity of Leydig cells in mule deer (*Odocoileus hemionus*) is characterized by spherical nuclei, whereas regression is characterized by ovoid nuclei (Markwald et al. 1971).

Sterility, lack of type-A spermatogonia, in 88% of the tubules in male 293 was apparently not treatment related. Gomes (1970) reported that estrogen treatment usually produces tubule atrophy and inhibits spermatogenesis without causing type-A spermatogonia death.

Secretory production of DES-treated animals was impaired in both the Cowper's glands and seminal vesicles, but no evidence exists that any one of the accessory glands is absolutely required for fertility (Price and Williams-Ashman 1961). Whether these males were capable of impregnating a female was not determined. Small sample size is quite likely responsible for the nonsignificance in the weights of the seminal vesicles.

The abnormal antler development seen in this study probably would be esthetically unacceptable to the public. Instead of continuing research on implant geometry that would increase the DES release rate, a search should be conducted for antispermatogenic compounds which do not interfere with the "Antler Stimulating Hormone." Another possibility is vasectomy, which would produce permanent sterility without interfering with antler development or growth.

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