Steroid Pellets and Venous Diameter in Orchidectomized, Ovariectomized, and Ovariectomized–Hysterectomized Mice[1] (37590)

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Ligation of pellets of various steroid hormones to the uterine horns of ovariectomized adult mice may be followed by a significant increase in venous diameter (1, 2). The present research concerns the effect on vein size of steroid pellets in adult orchidectomized, ovariectomized, and ovariectomized–hysterectomized mice.

Materials and Methods. Cylindrical, uniformly compressed, 3.5–4.5 mg pellets of single crystalline steroids without excipient were prepared (3). The steroids were progesterone, estrone, estradiol-17β, testosterone, and dihydrotestosterone (5α-androstan-17β-ol-3-one). Glass pellets of the same size and shape were also made. All mice were Jackson Laboratory B6D2F$_1$ (C57BL/6-J♀ × DBA/2-J♂) adults.

Groups of 10 males under sodium amytal and ether anesthesia were castrated via an abdominal incision. A steroid or glass pellet was secured to the testicular end of the right vas deferens by a ligature around vas and pellet. Groups of 10 virgin females under ether anesthesia were ovariectomized by cautery through dorsal incisions. One week later the abdomens of the same female mice, again under sodium amytal and ether anesthesia, were opened midventrally and a glass or steroid pellet was folded into the right uterine fat mass. In a third series, groups of 10 virgin females under ether anesthesia were ovariectomized by cautery through dorsal incisions. The cephalic portions of the uterine horns were also removed by cautery. One week later these females were re-anesthetized, the remainder of the uterus except its cervical extremity was extirpated through ventral incisions by cautery, and a glass or steroid pellet was folded into the right uterine fat mass. In all cases care was taken not to damage testicular, ovarian, uterine, and other major blood vessels.

Females were killed 21 days and males were killed 28 days after pellet implantation. Death was by ether inhalation. The degree of hair growth on the skin areas that were shaved before surgery was noted. The abdomen was opened, the viscera were inspected, and the pellet was observed to make sure that it was correctly located in the fat mass. Ten intact, untreated adult males were killed, opened and similarly examined. In all mice, selected major veins (Tables I, II) were exposed without touching them and their diameters at standardized locations were measured in the arbitrary units of an ocular micrometer in a dissecting microscope. The measurements for a given vein in a given control or experimental group were averaged. Next, the 10 individual measurements for a given vein in an experimental group were compared with the 10 corresponding measurements in the appropriate control group, and the Wilcoxon rank sum test (4) was applied to determine whether the difference in the two groups was significant ($p < 0.05$). Since comparing several experimental groups with a single control group, although it simplifies statistical analysis, has the effect of producing more significant differences than is strictly appropriate at the 0.05 significance level, differences at this level were regarded as borderline.

Results. Average values for the glass pellet control, experimental, and intact males are indicated in Table I. A blank space indicates an experimental average that did not differ

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TABLE I. Males; Average Diameters of Veins in Castrate (C) Controls, and in Castrate (C) Experimental and Intact (I) Mice When Significantly Different[a] from Those Controls.

	Pellet	Internal spermatic v.		Femoral v.		Inferior vena cava
		Left	Right	Left	Right	
C	Glass	13.6	16.0	18.5	18.8	47.8
C	Progesterone	19.0**	18.5*			
C	Estrone			22.4***	21.9**	
C	Testosterone	17.9**	19.9*	20.4**	21.5**	59.6***
C	Dihydrotestosterone	18.7**				57.1***
I	—	21.5***	21.8***			

[a] * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

significantly from the corresponding glass pellet control average. Study of male mice carrying estradiol-17β was terminated when several of them died in 8–10 days after pellet implantation. Autopsy of these mice revealed bladders distended to a diameter of 1 cm or more. Three of the 10 estrone-treated males also had distended bladders. The seminal vesicles of males treated with dihydrotestosterone were enlarged and full of secretion. Hair regrowth on abdominal shaved areas was complete at autopsy in mice with glass and progesterone pellets and incomplete in all other experimental animals.

Average diameters for female veins appear in Table II. Individual control values for veins in ovariectomized mice were not significantly different from those in ovariectomized–hysterectomized controls. As in the males, hair regrowth in shaved areas was complete in mice with glass and progesterone pellets and incomplete in those with pellets of the other steroids.

Discussion. Estradiol benzoate and testosterone delayed hair regrowth in shaved skin areas in dogs (5), a result similar to that observed in mice. Gross distention of the urinary bladder due to obstruction following administration of estrogens has previously been reported for male and female mice (6, 7).

The observations of others on the effect of steroids on blood vessel size in female rats and mice have been summarized elsewhere; the results of the present experiment extend earlier findings and permit quantita-

TABLE II. Females; Average Diameters of Veins in Controls, and in Experimental Mice When Significantly Different[a] from Controls, after Ovariectomy (O) and Ovariectomy–Hysterectomy (OH).

	Pellet	Ovarian		Uterine		Femoral		Inferior vena cava
		Left	Right	Left	Right	Left	Right	
O	Glass	11.8	11.3	9.0	9.6	19.4	19.7	46.9
O	Progesterone			*7.2***	*7.4****			
O	Estrone			*7.7**	*6.9****	25.4***	25.3***	58.4***
O	Estradiol-17β				10.8*	21.4*	22.1*	53.0**
O	Testosterone	14.0*	14.1***		*8.0****			57.5**
O	Dihydrotestosterone		13.3*	10.9***	10.9**			
OH	Glass	10.1	10.6	9.1	9.3	20.6	19.5	47.2
OH	Progesterone					*17.5***	*17.3**	
OH	Estrone	13.5**	13.8***	14.6***	13.6***	23.7**	25.0**	53.4**
OH	Estradiol-17β	12.7**	14.0**	14.7***	14.8***	26.2***	27.5***	54.2**
OH	Testosterone	16.5***	15.2***	17.4***	17.3***	25.2***	25.0***	59.7***
OH	Dihydrotestosterone	14.6***	13.6***	16.6***	16.6***	25.6***	26.5***	66.5***

[a] * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

tive comparison. When uniformly compressed pellets of various steroids were implanted subcutaneously in rats, 90% absorption of the pellets required from 27 days to over 1 yr, depending on the compound (1, 2). Thus failure of veins to change significantly in diameter in the present study may have been due to insufficient release of steroid from a pellet. However, the response of the femoral veins and inferior vena cava indicates that enough steroid was usually absorbed to reach effective levels in the systemic circulation.

In evaluation of venous response it should also be remembered that while measurements were made in terms of diameter, or $2r$, *functional* enlargement of a vein should be considered in terms of cross-sectional area, or πr^2.

Comparison of venous diameters in orchidectomized mice bearing glass pellets and in intact mice makes it clear that of the veins measured, only the internal spermatic veins were significantly reduced in size following castration. Since several steroids largely restored these veins to their size in intact mice, it would appear that the size of the veins is dependent on hormonal stimulation rather than on the presence of the testis as such. Whether ovarian vein size is under similar endocrine regulation will not be known until a study of vein size throughout the estrous cycle has been completed. However, it is noteworthy that in ovariectomized control mice extirpation of the uterus does not significantly affect uterine vein size, suggesting that the latter does not depend on the continuing presence of the uterus. In the females, progesterone never caused an increase in venous size. Indeed this hormone and estrone in some cases were associated with a reduction in the diameters of veins. The present study, like earlier experiments (1, 2), confirms the ability of steroid compounds to influence venous diameter in castrate female mice and discloses a similar mechanism in males. However, the factors responsible for alteration of venous dimension, whether morphological or physiological or both, remain to be determined.

Perhaps the most puzzling result was the much greater venous response in ovariectomized–hysterectomized compared to ovariectomized mice. It may be postulated that the uterus selectively binds within itself a significant part of the total circulating steroid or that that organ is responsible for some other kind of inactivation of the steroid; either phenomenon would reduce circulating systemic levels of the compound that was administered.

Summary. Pellets of most steroids impeded hair regrowth in shaved areas. Estradiol-17β caused fatal obstruction of the urinary tract in males.

The diameters of the internal spermatic, ovarian, uterine, and femoral veins and the inferior vena cava in the mouse were measured in intact males, in orchidectomized males bearing glass pellets (controls) and steroid pellets, and in ovariectomized and ovariectomized–hysterectomized females with similar pellets. Mice with steroid pellets had significant increases, and sometimes significant decreases, in vein size compared to control mice with glass pellets. Venous response was greater after removal of both ovaries and uterus than after removal of ovaries only. The diameter of the internal spermatic vein appeared to depend on the influence of a steroid rather than on the presence of the testis as such.

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