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Effects of Testosterone on Genital Organs of Female Rats Treated from Date of Birth.*

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The effect of testosterone propionate (T.p.) upon the ovaries of female rats has been previously reported from this laboratory. It was shown that T.p. prevented the formation of corpora lutea in adult as well as in 21- to 23-day-old immature rats and that follicular development was not arrested.[1] It was also reported that T.p. enhances and maintains the function of corpora lutea of the rat provided early corpus luteum formation is present at the time of the first injection.[2] These observations are in contrast to those of other workers who report the formation of corpora lutea in immature rats[3,4,5] and the findings of cystic corpus luteum tumors in rats treated with T.p. from birth.[6] The present report summarizes our findings in female rats treated with T.p. from the day of birth for periods ranging from 7 to 232 days.

Of a total of 61 female rats of the Wistar strain, 46 survived the treatment. The animals, beginning with the day of birth, were given subcutaneous injections of 1 mg (0.1 cc) of testosterone propionate dissolved in oil 3 times weekly. The pertinent data are given in Table I. At autopsy, the ovaries, tubes, and periovarian tissue were removed *in toto*, and, after fixation and embedding, sectioned serially until the ovaries were demonstrable. Hematoxylin-eosin was used routinely, supplemented by Weigert-van Gieson, Gram, and Sudan III stains. Representative sections of uteri, vaginæ, and other organs were examined.

The ovaries, in serial sections, were found to be atrophic, and follicular development was arrested just beyond the stage of antrum formation although in one animal a few large follicles were found. Corpora lutea were uniformly absent (Fig. 1). There was atrophy of the stromal and thecal cells, some of the

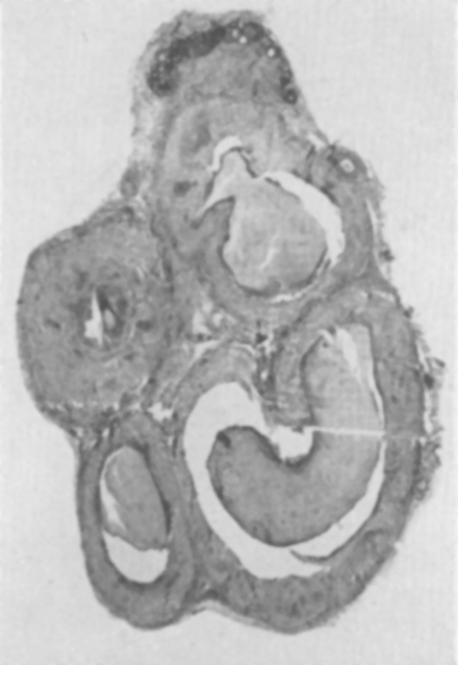

FIG. 1.
Rat 51721, 40 mg in 92 days. Unilateral right tubal abscess. Note atrophic ovary at upper pole.

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine. Testosterone propionate (Perandren) was generously supplied by the Ciba Pharmaceutical Products, Inc., Summit, N.J. My thanks are due to Mrs. Agnes Chinn, Mr. V. Montesclaros, and Mr. P. Lassegues for their technical help.

1 Laqueur, G. L., and Fluhmann, C. F., *Endocrinology*, 1942, **30**, 93.

2 Fluhmann, C. F., and Laqueur, G. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 223.

3 Hohlweg, W., *Klin. Wchnschr.*, 1937, **16**, 586.

4 Salmon, U. J., *Endocrinology*, 1938, **23**, 779.

5 Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 385.

6 Shay, H., Gershon-Cohen, J., Paschkis, K. E., and Fels, S. S., *Endocrinology*, 1939, **25**, 933.

TABLE I.
Incidence of Tubal Abscesses in Female Rats Treated from Birth with Testosterone.

No. of rats	Total dosage, mg	Period of inject., days	Avg wt, g	Tubal abscesses	
				Present	Absent
3	4	7	12.4	0	3
3	8	17	24.7	0	3
3	12	27	58.0	0	3
4	16	36	81.7	2	2
4	20	45	119.7	1	3
3	25	57	143.0	1	2
4	30	68	181.5	1	3
3	40	92	223.7	2	1
3	50	116	186.7	1	2
2	60	143	190.0	1	1
3	70	165	228.0	2	1
2	80	184	244.5	2	0
2	90	207	246.0	1	1
7	100	232	218.0	4	3

latter showing the nuclear derangement described as deficiency cells.[7] With the larger dosages, a significant number of follicles were altered so that they resembled testicular tubules as described in detail by Marx.[8]

Tissue masses of various sizes were found in the region of the adnexa in 18 animals, 12 bilaterally and 6 unilaterally. These lesions grossly resembled those described by Shay *et al.*[6] Although there was no direct proportionality between the total dosage and the presence of these lesions, none were found in the animals receiving less than 16 mg. Histologically, the masses represented various stages of salpingitis, mesosalpingitis, and periovarian cellulitis. In all acute and subacute lesions, Gram stain demonstrated a variety of bacteria. In the older lesions granulation tissue had walled off the abscesses, and innumerable large mononuclear cells, occasionally forming multinucleated giant cells, were seen. Frozen sections stained with Sudan III demonstrated abundant fatty material in the mononuclear cells. Acute as well as chronic stages of salpingitis were seen in the same section, and in more than half of them parts of normal fallopian tubes were found adjacent to the abscess walls.

The ovaries of rats with tubal abscesses were not grossly identified, but histologically they showed the changes already described. In a few instances, however, fibrosis was marked suggesting a previous oophoritis. In 3 rats, this was in the acute stage, the periovarian space being filled with an inflammatory exudate.

In rats with pyosalpinges, the uteri and vaginæ of 6 were distended by secretion, leucocytes, and a few bacteria while in 12 rats there was no uterine involvement. On the other hand, pyometra were found in 3 rats without an accompanying salpingitis. In all animals treated longer than 36 days, the vagina was distended by strongly basophilic mucoid material and leucocytes. This mucoid material was presumably produced by secretory cells which were frequently found among the squamous epithelial cells. Although the vaginal orifice was not regularly inspected during the course of the experiment, it was found at autopsy that in 42 of the 46 rats the vagina was closed.

Examination of the tubes with no abscess formation showed uniform secretory changes in the epithelium accompanied by a mild leucocytic invasion, but no bacteria were demonstrated. Hence, the formation of abscesses was probably secondary, and superimposed upon preceding secretory activity of the tubal epithelium. The source of the bacterial invasion was not conclusively determined. No other inflammatory lesions, even at the site of injection, were found in these animals.

The ovarian changes reported here are in

[7] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1933, **17**, 494.

[8] Marx, L., *J. Exp. Zool.*, 1942, **91**, 365.

accord with those reported by Mazer and Mazer[9] and by us[1] where treatment was begun in slightly older but still immature rats. The development of tubal lesions in rats injected from birth is of interest in connection with the observations of Green and Ivy[10] and Hamilton and Gardner[11] who found pyometra and distention of the vagina in rats subjected to an androgenic stimulus during the last third of intrauterine growth.

Conclusions. 1. Testosterone propionate injected into female rats from the day of birth arrests follicular development beyond the stage of the antrum and prevents corpus luteum formation. 2. These findings extend our previous observations on adult and 21-day-old rats, and it is therefore concluded that testosterone in the doses used prevents luteinization of the rat's ovary at any age. 3. When treatment is initiated on the day of birth, there is an arrest of follicular development and a partial transformation of the follicles into seminiferous tubule-like structures, not observed in our previous study with older rats. 4. Inflammatory lesions representing various stages of salpingitis, mesosalpingitis, and periovarian cellulitis appeared in about 40 per cent of the experimental animals. 5. The formation of tubal abscesses, caused by secondary bacterial invasion, is preceded by the stimulation of secretory changes in the tubal epithelium.

Since the paper was submitted for publication, a preliminary report by H. B. Hale and C. K. Weichert appeared in the March issue of these PROCEEDINGS (1944, **55**, 201), in which the authors report on ovarian tumors in adult rats following prepuberal administration of estrogens. Grossly, these tumors resembled those reported by Shay *et al.*[6]

[9] Mazer, M., and Mazer, Ch., *Endocrinology*, 1939, **24**, 175.

[10] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

[11] Hamilton, J. B., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 570.

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A Method of Blood Replacement in the Rat.

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In the course of experiments in this laboratory it became necessary to remove a large proportion of the blood of a rat and to replace it rapidly with other blood. Preliminary experiments showed that the readily accessible vessels in the rat, such as the jugular, femoral, and tail veins, are not suitable for the rapid withdrawal and replacement of blood. It was found, however, that the inferior vena cava is a very satisfactory site for the performance of this operation, and that rats recover rapidly in spite of the surgical procedure which is necessitated by the use of this vessel.

Method. The rat (wt. 150-250 g) is anesthetized, the abdomen is incised, and the inferior vena cava is exposed. A 20-gauge hypodermic needle which has been bent at an angle of 45° about 0.5 cm from the tip is inserted into the vessel and is held firmly in position by means of a clamp. The rat is heparinized (25 units per 100 g body wt) by attaching to the needle a syringe containing heparin solution (Connaught Laboratories, Toronto; 1000 units of heparin per ml), drawing 1 ml of blood into the syringe and then reinjecting it. An amount of blood not exceeding 5 ml is then drawn into the syringe. The syringe is replaced by another containing the same volume of heparinized blood and this is then injected. After the replacement process has been carried out the required number of times, the vessel around the site of entry of the needle is grasped with smooth blunt forceps and the needle is carefully withdrawn. A few drops of a solution of thrombin*

* The writer wishes to thank Parke, Davis and Company for a gift of a supply of thrombin.