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Masculinization of Adult Female Rabbit Following Injection of Testosterone Propionate.

C. K. Hu and C. N. Frazier.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College, Peiping, China.

Lipschütz,[1] working in Steinach's laboratory, observed that the transplantation of the testis into spayed guinea pigs produced growth of the corpora cavernosa of the clitoris, and transformed this structure into a penis-like organ. Since then, as pointed out by Moore,[2] some of the many effects of the various chemical androgens indicate that any masculine rudiments, or homologues of male structures that remain during sexual differentiation, may develop typical masculine responses.

Although the effect of testosterone propionate on the female genital organs has been studied by a number of workers,[3, 4] chiefly in immature rats and mice, no observation seems to have been made either on the immature or adult rabbit. Moore and Price[5] found that the the injection of androsterone into young rats reduced testicular weights, whereas in mature animals, the testes were not injured. This last observation might be taken as indicative of a difference in the effect of the substance on the sexual organs of immature and mature animals.

To our knowledge only de Jongh and Mulder[6] have studied the masculinizing effects of "male hormone" on the adult animal, in this case, the guinea pig. Furthermore, the influence of testosterone on psycho-sexual behavior, except in birds,[7] has apparently not attracted the attention of previous workers. It may be of interest, therefore, to record the observations we have made on adult female rabbits treated with this substance.

Material and Method. To each of 3 adult female rabbits between

1 Lipschütz, A., *Arch. f. Entwickl.-Mech.*, 1918, **44**, 196.

2 Moore, C. R., *Sex and Internal Secretions*, edited by E. Allen, 2nd edition, Williams & Wilkins, Baltimore, 1939, **7**, 420.

3 Lacassagne, A., and Raynaud, A., *Compt. rend. Soc. de biol.*, 1937, **125**, 351.

4 Greene, R. R., Burrill, M. W., and Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 4.

5 Moore, C. R., and Price, D., *Endocrinol.*, 1937, **21**, 313.

6 de Jongh, S. E., and Mulder, J. D., *Endokrinol.*, 1932, **11**, 161.

7 Shoemaker, H. H., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 299.

8½ and 15½ months of age, testosterone propionate* was injected subcutaneously in the dosage of 1 mg per day, 6 days a week. The treatment lasted from 21 to 32 days. Examination of the animals for the physiological effects of the preparation was carried out at intervals of 3-7 days. Attention was paid especially to anatomical changes in the external genitalia, the nipples, and the dewlap, a secondary sexual character of the female rabbit.[8] The behavior of the masculinized animals when confronted with normal female and male rabbits was also studied. One of the 3 animals was ovariectomized 6 months after the last injection. Both ovaries together with the surrounding fat and part of the fallopian tubes were removed under ether anesthesia. At the time of operation, all the changes induced by androgenic treatment had disappeared, the animal having returned to an apparently normal female status. Beginning a week after operation, testosterone was again administered.

Effect on Somatic Sexual Characters. The response to treatment was strikingly uniform in the 3 animals. Change in appearance of the clitoris and labia was noticeable within 3 or 4 days after the beginning of treatment. Between 10 and 14 days after the first injection of testosterone, the clitoris enlarged, and the labia became red and swollen, and then began to fuse posteriorly. The process of hypertrophy continued until the phallus resembled a typical penis in so far as the corpora cavernosa of the clitoris were concerned. When viewed from the anterior surface the glans-like structure could not be distinguished from the penis of a normal rabbit. A prepuce-like structure also developed which could be retracted with better exposure of the newly formed glans. The vaginal opening, although reduced to a small size, never completely closed. This was visible as a longitudinal slit posterior to the penis-like structure. The urethral opening could be seen just within the vaginal orifice between and at the base of the hypertrophied corpora cavernosa of the clitoris. The corpus cavernosum urethrae was absent so that the organ gave the impression of a hypospadic penis. No change resembling a scrotal sac developed in the skin of the genital zone. The maximum degree of masculinization occurred between 15 and 21 days after the first injection of androgenic substance.

After discontinuing the injections, the genitalia reverted to the normal female type. The initial change in reversal of type was noticeable between 11 and 18 days after the last injection, and the process was completed within 39 to 52 days.

* The testosterone propionate, dissolved in sesame oil and marketed as perandren—Ciba, was supplied by Ciba (China) Ltd., Shanghai.

8 Hu, C. K., and Frazier, C. N., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 116.

The nipples and dewlap were not affected by treatment. In one animal there was a suggestive enlargement of the nipples from which a drop of thin fluid could be squeezed, but the normal female rabbit, especially during pseudopregnancy, which might have been induced in this case, may show the same response. In 2 animals a dewlap was present before the administration of testosterone, but this was not modified along with the masculinization of the genitalia. In the third rabbit, dewlap was not present before treatment and none developed after the injection of testosterone.

Effect on Psycho-sexual Behavior. The sexual behavior of the treated females was remarkably masculine in character. In making this statement we are fully aware of the sexual habits of the rabbit, and of the dual rôle both sexes frequently attempt to play when in the presence of the same sex. With females, the masculinized animals were vigorous and aggressive and made repeated attempts at copulation. One of the treated females was placed successively with 5 normal males, and on each occasion it attempted copulation, playing the part of a male. Only one of the normal males attempted to jump the masculinized female, who sat tight in the cage without moving. The change in sexual behavior was evident 2 weeks after the first dose of testosterone when the clitoris was just beginning to enlarge, and persisted until the external genitalia had returned to the female type. In this connection, Shoemaker[7] observed that the female canary, when treated with testosterone propionate, developed the courtship behavior and singing qualities of the male bird. The masculinizing

FIG. 1.
Ventral view of genitalia showing beginning enlargement of clitoris and swelling of vulva, 2 weeks after the first injection of testosterone propionate.

FIG. 2.
Dorsal view of genitalia showing the hypertrophied clitoris and prepuce-like structure, 17 days after the first injection of testosterone propionate.

effect of a testicular graft on the psycho-sexual behavior of the guinea pig was known to Steinach,[9] who reported his observations in 1912.

Effect of Ovariectomy. Ovariectomy made no difference in the response, showing that the effect of testosterone on the lower vaginal zone and clitoris is independent of the ovary. Similar observations were made by Rubinstein and coworkers[10] on the vaginal opening of spayed rats.

The ovariectomized animal, when under the influence of testosterone, showed the same modification in psycho-sexual behavior as had the intact masculinized females.

Summary and Conclusions. Each of 3 adult female rabbits, 8½-15½ months of age, was injected subcutaneously with testosterone propionate, 1 mg per day. Masculinization of the external genitalia followed in about 2 weeks, the clitoris enlarging to form a structure resembling a hypospadic penis, and the labia fusing to partially close the vaginal opening. The anatomical modifications were reversible when treatment was suspended. The psycho-sexual behavior of treated animals became masculine in character. The same response to treatment with respect to somatic sexual characters and psycho-sexual behavior was also induced in a spayed adult female rabbit.

9 Steinach, E., *Pflüger's Arch. f. Physiol.*, 1912, **144**, 71.

10 Rubinstein, H. S., Abarbanel, A. R., and Nader, D. N., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 20.