Reaction of Target Organs to Androgens in Feminized Rats (35961)

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A considerable body of evidence, mainly from work by Neumann and his group (1), is available to show that 1,2α-methylene-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dion (Cyproterone) possesses antiandrogenic properties. Cyproterone acetate (CA) exerts, in addition, gestagenic effects. Treatment of pregnant rats with CA results in persistent abnormalities of the male urogenital tract, *e.g.*, presence of vagina and rudimentary male accessory sex organs (1–3). The syndrome presented by the male offspring of animals given CA during pregnancy is, notably in dogs, similar to that known to occur in the human and called "testicular feminization" (4).

Male pseudohermaphroditism (Ps) was found among Stanley-Grumbeck rats. Target organs (other than seminal vesicles and prostatic glands, which were absent) of these Ps-rats did not react to testosterone (5). This finding agrees with those made in human testicular feminization (6, 7).

The studies referred to above (1–3) revealed that in spite of the treatment of the mother rats with CA, some of the androgen dependent structures of the reproductive tract developed and though mostly as rudiments persisted into adulthood. The testes often showed an incomplete descensus, reduced weight, and various abnormalities.

The present experiments were performed to see whether (a) the mammary glands of male offspring of CA-treated rats reacted to testicular androgens as is known to occur normally in this species (11); and (b) the rudimentary seminal vesicles and prostatic glands and mammary glands could be stimulated by testosterone given to the rats when adult.

Materials and Methods. Originally highly inbred albino rats kept at the Physiological Institute in Lund as a closed colony were used. A pelleted diet and water were freely available. Adult virgin females were mated and given CA[1] during the last third of pregnancy. Injections of 30 mg of CA/day were given from days 14 or 15 to day 20 of pregnancy and the young were delivered by cesarean section on day 22. Details about the treatment of the mothers with CA or solvents and the procedures to obtain and rear the young may be found in previous papers (2, 3).

The present studies were performed on seven newborn, eleven 10 day old, and 22 adult (3-5 months old) male as well as 17 adult female offspring of CA-treated rats. Eight adult males and five adult females obtained from mothers treated with solvents served as controls. In addition intact rats were studied. Testosterone propionate (Tp) in sesame oil was given subcutaneously in daily doses of 0.05 (4♂, 4♀; 0.02 ml of 2.5 mg/ml), 0.5 (2♂, 2♀; 0.02 ml of 25 mg/ml) or 5.0 mg (5♂, 1♀; 0.1 ml of 50 mg/ml) for 14, 21, or 28 days. Examinations were made 1 day after the last injection. The animals were then 3.5–4 months old. Male (3) and female (2) controls were given 0.1 ml of oil daily. The treatments were randomly distributed among litters. The seminal vesicles were weighed after removal of secretion. Whole mounts and paraffin sections of mammary glands were prepared and assessed as in previous investigations (8, 9). Serial sections for histological studies of the urogenital region were prepared as previously (2, 3).

Results. The observations made on the urogenital region of the newborn or 10 day old males did not reveal any differences between the present and the previous strain of

[1] Kindly supplied by A. G. Schering, Berlin.

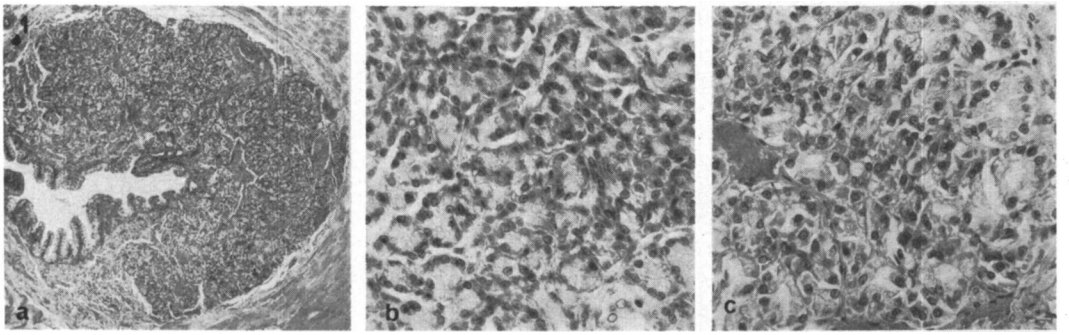

FIG. 1a. Low magnification picture of a cross section through the urethra and the prostate rudiment of a male prenatally exposed to CA and, later in life, injected with sesame oil for 28 days; HE, ×12. (b) Higher magnification of the prostate. Same male as in (a). Note the compact epithelial structure. (c) Detail from the prostate of a male prenatally exposed to CA and, later in life, injected daily with 5.0 mg of Tp for 28 days. The lumen of the glandular ducts is dilated. (b, c) HE, ×206.

rats used (2, 3). A few scattered anlagen of prostatic glands could be found. Seminal vesicles as well as the deferent and ejaculatory ducts were present. The last-mentioned ducts opened into the anterior part of the vaginal anlage, which was separated from the urethra down to the perineum. Uterine horns and fallopian tubes were absent. The adult male offspring of CA-treated rats presented the same abnormalities as those of the previous strain, except that the prostatic rudiment seemed to be larger (Fig. 1).

Except for the presence of nipples in both sexes (10), the mammary glands of 8 adult male offspring of CA-treated rats differed markedly from those obtained from 8 littermate females examined at the same age. Like 8 male controls (mothers treated with solvents) and 3–5 months old intact males [cf. (11)] the 8 males mentioned presented compact glands extending over an area less than half of that in females. The ducts were covered by a dense system of alveoli with large epithelial cells. End buds were absent. The mammary glands of the 8 littermate females consisted, like those of their 5 controls and adult virgin rats, of an extensive, arborized system of ducts with some lobules and end buds (Fig. 2a, b). Testosterone propionate did not clearly modify the histological appearance of the small seminal vesicles of the males prenatally exposed to CA, though the weight of the seminal vesicles increased with the dose of Tp and the length of treatment from about 140 mg to about 250 mg. The secretory epithelium of the seminal vesicles was the same in Tp-treated as in corresponding controls and normal untreated adult rats. A slight stimulation of the rudimentary prostatic glands was obtained. After injections of 5.0 mg of Tp for 2–4 weeks, the lumen of the glandular ducts were more dilated than in those exposed to a lower dose or in controls. A stimulation of the male mammary glands, as indicated chiefly by a distension of the alveoli with secretion, was obtained from the same rats given 5.0 mg of Tp for 14, 21, or 28 days and from one rat given 0.5 mg of Tp for 21 days (Fig. 3). A development of alveoli occurred in the glands of one of the female littermates given 5.0 mg of Tp for 14 days and in 2 others given 0.5 mg of Tp for 14 and 28 days, respectively (Fig. 2c). The effect of 0.05 mg of Tp in another 4 females was doubtful.

Discussion. The present results were uniform and clear. The treatment of mothers with CA did not influence the appearance of the mammary glands in the adult male offspring. As it is known that the development of the mammary glands in male rats is promoted by androgens secreted by the testes (9, 11) the present findings indicate that the same holds true for the CA-exposed males. Alveolar growth, such as seen in the males of the present experiments, does not occur in

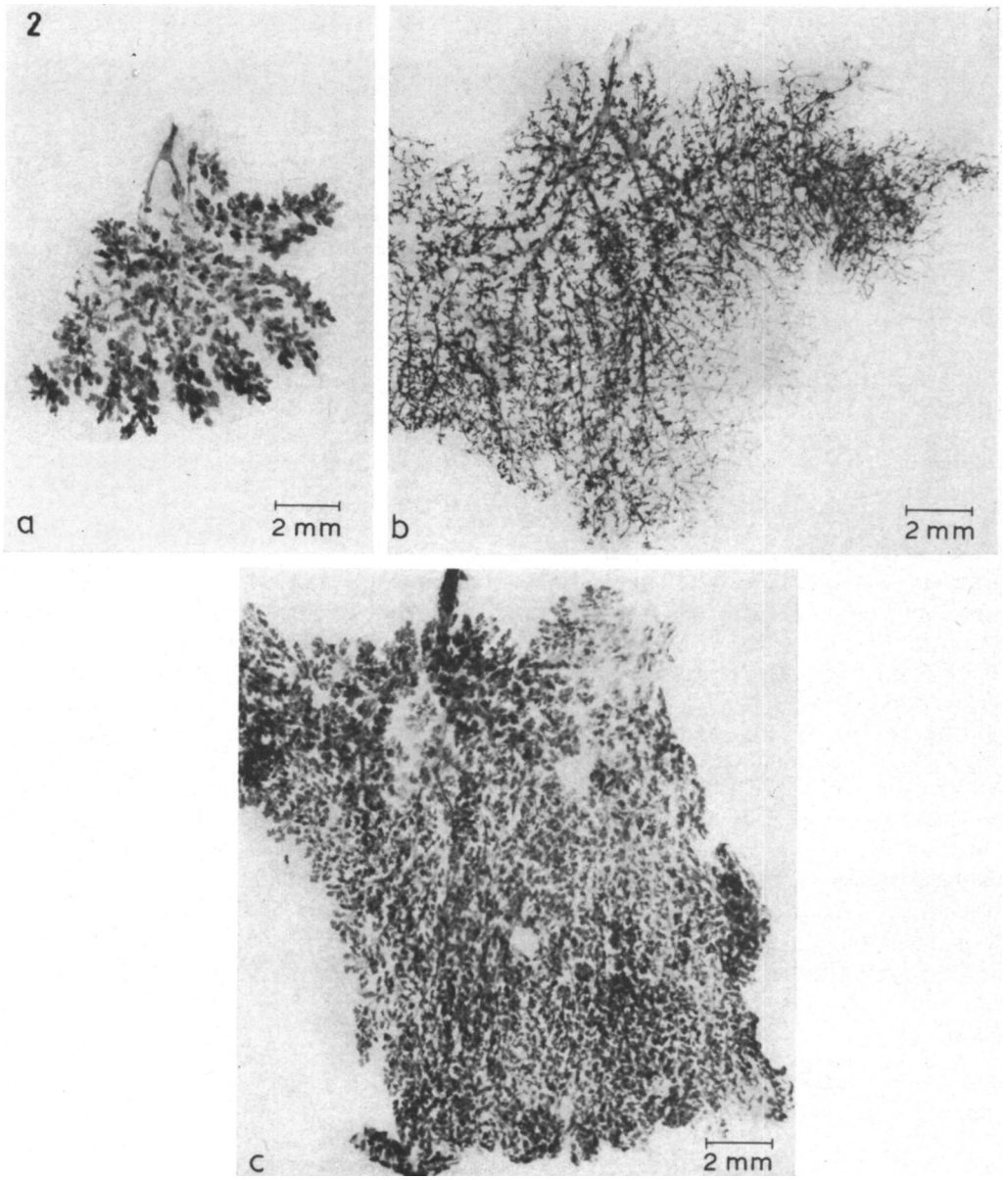

FIG. 2. Difference between the mammary glands of adult male and female offspring of CA-treated rats. Effect of Tp in females. Whole mount preparations stained with gallocyanin-chromalum and photographed with the same magnification: (a) male; (b) female; (c) female injected daily with 5.0 mg of Tp for 14 days.

the absence of androgens. Further support for this conclusion may be derived from the observation that the mammary glands of virgin female littermales showed only ducts but no alveoli. That the glands reacted to hormonal stimuli agrees with data from Neumann *et al.* (12), who, with the use of pituitary and female sex hormones, obtained fully developed lactating mammary glands from castrated adult male rats feminized by the same procedures.

The microscopic appearance of the small seminal vesicles does not seem to exclude some influence of endogenous androgens ei-

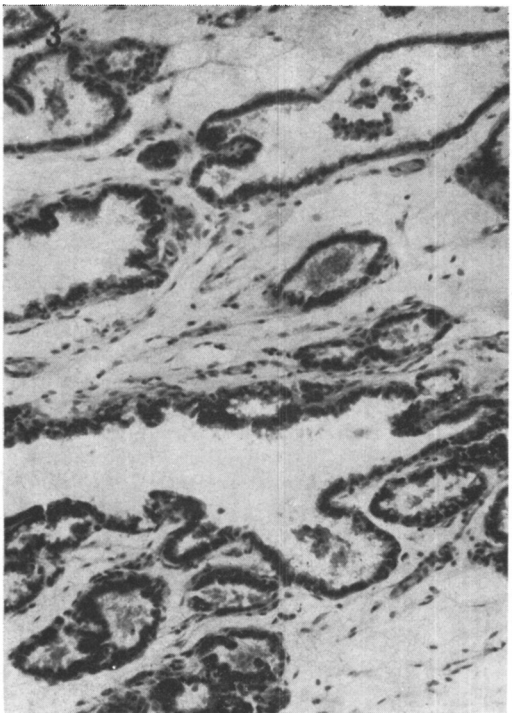

FIG. 3. Effect of 5.0 mg of Tp given daily for 21 days on the mammary gland of an adult male offspring. Stimulation of secretion. Paraffin section, stained with hematoxylin–eosin; × 120.

ther. To judge from the state of the secretory epithelium a further stimulation by exogenous Tp did not occur. A response of the prostatic epithelium to lower doses of testosterone could not be observed. However, the 5.0 mg Tp dose elicited the reaction of the prostatic glands described above. A stimulation of the mammary glands by Tp was obtained from both sexes. Though the methods employed do not permit any accurate assessments (9), it should be recalled that the lowest, 0.05 mg, dose of Tp, which proved ineffective in the present experiments, is regarded as physiologic in mammary stimulation (11).

The present observations indicate that neither androgen secretion by the testes nor responses of target organs were completely inhibited. Even in this respect the abnormal male offspring of CA-treated rats do not present the syndrome of a true testicular feminization (2, 3).

Summary. Adult male rats feminized by treatment of their mothers with Cyproterone acetate had well-developed mammary glands appearing structurally like those of intact adult males of the species. The prostatic glands, but not the epithelium of the seminal vesicles, reacted to high doses of testosterone propionate (Tp). The weight of the seminal vesicles increased. An effect of Tp in high doses was seen on the mammary glands. In the present "feminized" rats neither the hormonal activities of the testes nor the reaction of target organs were inhibited. The conditions prevailing in these rats are not the same as in "testicular feminization."

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