EFFECT OF AN ANTI-ANDROGEN ON THE 
DIFFERENTIATION OF THE INTERNAL GENITAL 
ORGANS IN DOGS

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Summary. From experiments with anti-androgens, it can be concluded which processes in the sex differentiation of dogs are androgen-dependent and which are not. In contrast to rats but as in rabbits, retrogression of the Wolffian ducts can be induced in the treated members of this species compared to their controls, but retrogression of the Müllerian ducts is uninfluenced in both treated and control animals. From this, it is concluded that the latter process is not androgen-dependent.

INTRODUCTION

The most potent known anti-androgen was discovered by its ability to feminize male rat foetuses (Hamada, Neumann & Junkmann, 1963). It was found in subsequent experiments that this compound is capable of inhibiting effects of endogenously produced as well as exogenously administered androgens (Junkmann & Neumann, 1964; Neumann, Richter & Günzel, 1965; Neumann, 1966). Cyproterone acetate proved to be a valuable tool for the exploration of the biological rôle of androgens, especially when the surgical approach is difficult or even impossible, e.g. in investigations regarding the effects of testosterone within the testis itself (Neumann & von Berswordt-Wallrabe, 1965, 1966) or in foetal sex differentiation processes (Elger & Neumann, 1966; Neumann & Elger, 1966; Neumann, Elger & Kramer, 1966; Neumann & Kramer, 1964).

The first species in which the entire genital tract was studied was the rabbit (Elger, 1966). In addition to feminization of the external genital organs, the following results were of special importance in male foetuses: the gonads were differentiated and descended normally; retrogression of the Müllerian ducts could not be prevented; above a critical dose level (5 mg/kg/day i.m.), involution of the Wolffian ducts was almost complete.

This result was considered to be a proof of Jost’s theory postulating that, besides androgens, other factors of the male gonad play a rôle in sex differentiation (Müllerian duct inhibiting hormone).

Initial studies on the rat foetus were confined to the differentiation of the external genital organs, where more or less complete feminization was found (Hamada et al., 1963; Junkmann & Neumann, 1964). In subsequent studies,
the results obtained in rabbits after relatively low doses of cyproterone acetate could never be achieved in rats, although excessive doses were administered (Jost, 1967; Forsberg, Jacobsohn & Norgren, 1968; Elger, Neumann & von Berswordt-Wallrabe, personal communication). In spite of maximal feminization of the external genitalia, retrogressed Wolffian ducts were never found.

The reason for these species differences is unknown and an extension of the experiments to other species seemed desirable in order to find out whether one or other reaction is the rule or the exception.

MATERIAL AND METHODS

Two beagle bitches were used as experimental animals. The bitches were mated once daily on 3 consecutive days, the middle day being regarded as Day 1 of pregnancy, when this condition had subsequently been established.

Cyproterone acetate was injected i.m. as an oily solution (benzyl benzoate/caster oil 1: 10) in a daily dose of 10 mg/kg from the 23rd to the 44th day of pregnancy. The foetuses were removed from the uterus by Caesarean section on the 45th day. After fixing in Bouin’s solution, they were embedded in paraffin and serial sections were made at 0-005-mm intervals, every twentieth section being mounted on microscope slides. Thus, two successive sections represented a distance of 0·1 mm.

RESULTS

Three male foetuses were examined from each of the two litters. As controls, two male and two female untreated foetuses of the same age were used. The internal genital organs of treated males and untreated controls are shown schematically in Text-fig. 1, after reconstruction from serial sections.

Gonads

In treated animals as well as in male and female controls, the gonads were still located in the immediate vicinity of the caudal kidney pole. No abnormal gonads were found.

Müllerian ducts

In all males, whether treated or not, the Müllerian ducts had retrogressed to minute epithelial and mesenchymal traces. These remnants, however, were found almost constantly throughout the whole length of the genital tract (Pl. 1, Fig. 1). Only in the close proximity of the urogenital sinus did a caudal remnant (Pl. 1, Fig. 2), which ended blindly in the forepart, persist in three treated males (967/1, 967/4, 722/2).

Wolffian ducts

All treated foetuses had degenerated Wolffian ducts, with the exception of one foetus (967/1) in which the ducts were not affected by the anti-androgen. In the remaining five foetuses, the Wolffian ducts had retrogressed either unilaterally or bilaterally and short sections of discontinuity were also found, particularly in the immediate vicinity of the urogenital sinus (722/4 and 722/7).
Fig. 1. Mesogenital folds (MGF) of a male dog foetus whose mother had been treated with cyproterone acetate. R = rectum. Both Müllerian and Wolffian ducts have retrogressed. The previous position of both ducts is marked by mesenchymal condensations.

Fig. 2. Genital cord of a feminized male dog foetus in the vicinity of the urogenital sinus. A segment of a Müllerian vagina (MV) can be seen between the neck of the urinary bladder (NUB) and the rectum (R). Wolffian ducts are definitely absent at this level.

Fig. 3. Feminized male dog foetus. Partition of the urogenital sinus into a smaller ventral (U, urethral) and a wider dorsal (V, vaginal) portion. The prostate development is almost completely suppressed. RPB = rudimentary prostatic bud.

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TEXT-FIG. 1. Scale drawings of the internal reproductive tract of anti-androgen-treated dog foetuses.
Urogenital sinus

In all treated male foetuses, a separation of the anterior part of the sinus, resulting in the formation of a (sinus–) vagina (Pl. 1, Fig. 3), was observed, as in the female controls. The prostatic development was also suppressed to some extent (Pl. 1, Figs. 2 and 3).

External genital organs

The external genital organs had reached a similar stage of development in the treated males as in female foetuses of the same age.

Genital cord

In the feminized male foetuses, the distance from the fusion of the urogenital folds to the sinus orifice of the genital ducts was relatively long compared with the male controls. The small number of controls, however, does not allow any definite conclusions to be drawn.

DISCUSSION

Under the influence of cyproterone acetate, the sexual differentiation in dogs is essentially similar to the pattern already seen in rabbits, thus confirming the androgen-dependence of the Wolffian ducts, the prostate and other structures and differentiation processes. Since the high degree of feminization of the male genital tract, including retrogression of the Wolffian ducts, was not accompanied by a substantial influence on the Müllerian ducts, these experiments further support Jost’s theory of sexual differentiation. It can be presumed that the suppression of the Müllerian ducts by a specific testicular activity is the rule in mammals, but in our own experiments in rats, no retrogression of the Wolffian ducts was obtained by anti-androgen treatment. There are also inconsistent results in vitro with cultures of the genital tract where spontaneous retrogression of the Müllerian ducts in the absence of the foetal gonad was observed (Price, Ortiz & Zaaijer, 1967). In recent experiments, we were able to stabilize the Wolffian ducts in female rat foetuses by methyl testosterone treatment of pregnant rats, an effect which could be completely abolished by concomitant cyproterone acetate treatment (Elger, Steinbeck, Cupceancu & Neumann, 1970). It seems, therefore, that those hormonal factors (androgens?) which physiologically stabilize the Wolffian ducts in rats differ from synthetic androgens with regard to their biological action.

At this time, it is not possible to give a sound general interpretation of the hormonal dependence of the Müllerian and Wolffian ducts. It can only be stated that androgens are not involved in Müllerian duct retrogression (Jost, 1967) and that anti-androgens, consequently, do not prevent this process.

REFERENCES


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