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Antiandrogens and reproductive development

By F. Neumann, W. Elger and H. Steinbeck
Department of Experimental Endocrinology, Schering AG, Berlin, West Germany

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[Plates 19 to 23]

The most undesirable side effect of a new gestagen intended for the treatment of pregnancy disturbances is the virilization of female foetuses. In 1961, our drug company developed a new highly effective gestagen, cyproterone acetate (figure 1) (Wiechert & Neumann 1965). We were given the task to test this substance in animal experiments for desirable and undesirable effects, particularly for the occurrence of virilization. As this drug was intended for the treatment of pregnancy disturbances, such as threatening or habitual abortion, it had to be free of virilizing side effects.

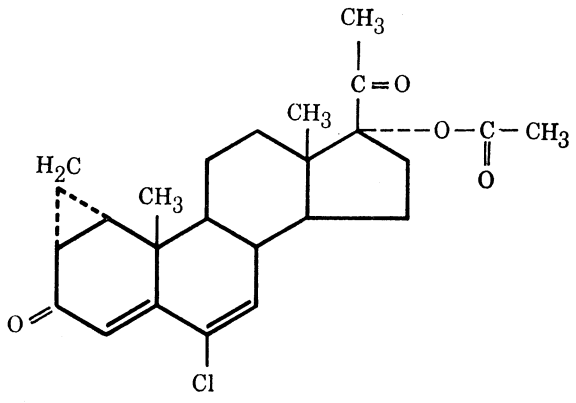


Figure 1. 1,2α-Methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate (cyproterone acetate).

In one routine experiment we treated pregnant female rats from gestation day 17 to 20. Autopsies of the foetuses were performed on the 22nd day. Usually, external inspection of the foetuses shows whether or not the test substance has produced virilization. For instance, the distance between anus and genitalia (the width of the perineum) is considerably greater in male than in female animals; in females it becomes larger under the influence of virilizing substances. In our experiment, no indications of virilization were found. On the contrary, at first we were amazed not to find any male foetuses at all. This unlikely result was disproved after opening the abdomen of these foetuses: some of them had testicles and thus were males.

Because of their historical interest I would like to show you the histologic sections of our very first test (figure 2, plate 19): these are sagittal sections of rat foetuses. Next to each other you see a male, a female, and the male offspring of a treated mother. Without going into details—which will be discussed later—one notices immediately that there seems to be hardly any differences between the normal female animal and the male whose mother was treated with

antiandrogen. The width of the perineum in this male is precisely as small as in the female animal; instead of a penis he has a clitoris, etc.

At this juncture, we had not been greatly interested in the hormonal regulation of sex differentiation. Nevertheless, we understood that we might be dealing with a new side effect produced by a gestagen, and we used the term feminization for this side effect in our first publication (at the request of the referee, at that time, we substituted 'anti-masculine influence' for the simpler expression 'feminization') (Hamada, Neumann & Junkmann 1963). By now, we know that we had discovered something more than a mere gestagen side effect, i.e. a substance which later proved to be the strongest antiandrogen known thus far (Neumann, von Berswordt-Wallrabe, Elger & Steinbeck 1967; Wiechert, Steinbeck, Elger & Neumann 1967).

As a consequence of this finding we became more interested in the hormonal regulation of sex differentiation. We began to study the fundamental writings by Jost (1947*a*, *b*). In one of his papers, based on deductive analogies, he postulated the type of male intersexuality which would result if androgens do not become effective in sex differentiation (Jost 1965). Actually, this situation occurred under the influence of a strong antiandrogen; and, indeed, the type of intersexuality observed by us in male rats corresponded—largely but not entirely—to the type postulated by Jost. While the animals had female external genitalia and even a vagina, accessory sex glands were not developed or very rudimentary; however, Wolffian ducts had developed. From the Wolffian ducts, as we know, the seminal ducts and the epididymis develop at a later stage. According to Jost's theory, this development—the stabilization of the Wolffian ducts—is strictly dependent on androgens. If this theory were right, the Wolffian ducts should have retrogressed under the influence of an antiandrogen.

In this context, I would like to present some illustrations of cross-sections of rat foetuses one day before birth. The mothers had been treated between days 13 and 22 of gestation with subcutaneous doses of 100 μg cyproterone acetate. A cross-section of the genital duct is shown in figure 3, plate 19). The anlage of Wolffian ducts is clearly seen.

Figures 4*a* and *b*, plate 20, show cross-sections through the upper part of the genital cord. The Wolffian ducts and seminal vesicle anlagen can be seen. The junction of the two deferent ducts with the seminal vesicle anlagen to the ejaculatory duct can be seen in figure 4*c*. It should be noted that the anlage of the prostate is completely inhibited. In a corresponding cross-section of a normal male at the same level many prostatic buds can be seen.

It is noteworthy that rudiments of the Müllerian ducts, also called 'prostatic utricles', are always present (figure 4*d*). In normal male animals, they are relatively rare and, if observed at all, not as distinct as under the influence of cyproterone acetate.

After junction of the two deferent ducts with the seminal vesicle thus forming the ejaculatory duct, the ducts continue into these caudal rudiments of the Müllerian ducts (figure 4*d* to *f*). The opening of the ejaculatory duct into the prostatic utricle is also shown in a higher magnification in figure 5, plate 19 (note the different types of epithelial cells).

In animals treated with relatively high antiandrogen dosages, these ducts end in a solid cell cord, which corresponds exactly to the vaginal part originating in the urogenital sinus of normal female animals. This is shown in figure 4*g*.

The solid epithelial cell cord which is homologous to the caudal vaginal part of female animals unites more or less caudally—also depending on dosage—with the urethra. Union with the urethra is beautifully demonstrated in figures 4*h* and *i*.

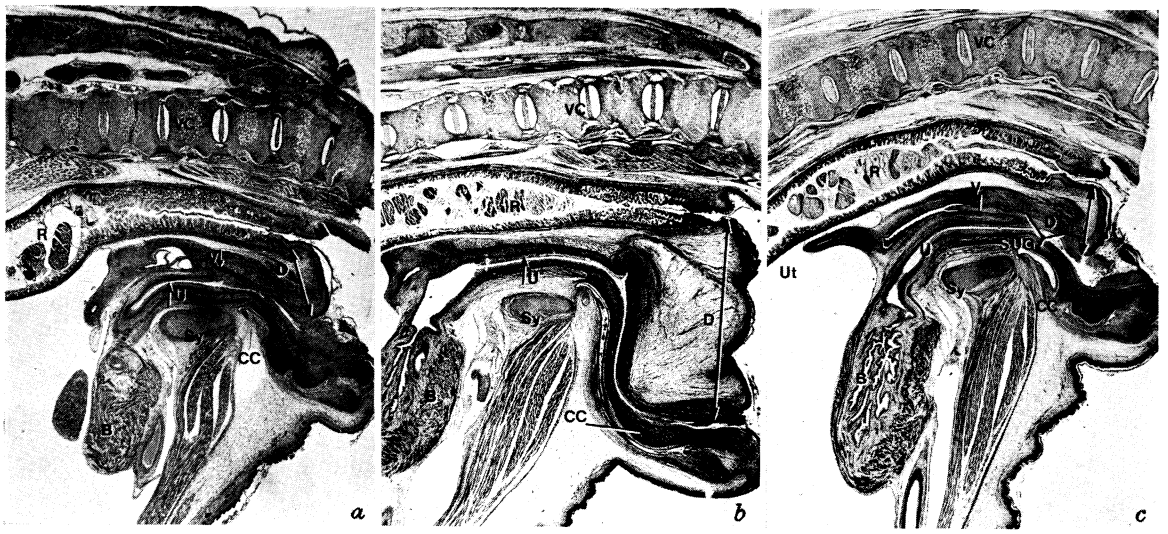


FIGURE 2. Sagittal sections of rat foetuses on the 22nd day of embryonal development. (*a*) Feminized male foetus (mother treated with 10 mg cyproterone acetate/day from 17th to 20th day of gestation); (*b*) normal male foetus; (*c*) normal female foetus. B, urinary bladder; CC, corpus cavernosum; D, anogenital distance; R, rectum; SUG, sinus urogenitalis; Sy, symphysis; U, urethra; Ut, uterus; V, vagina; VC, vertebral column. (Magn. × 8.)

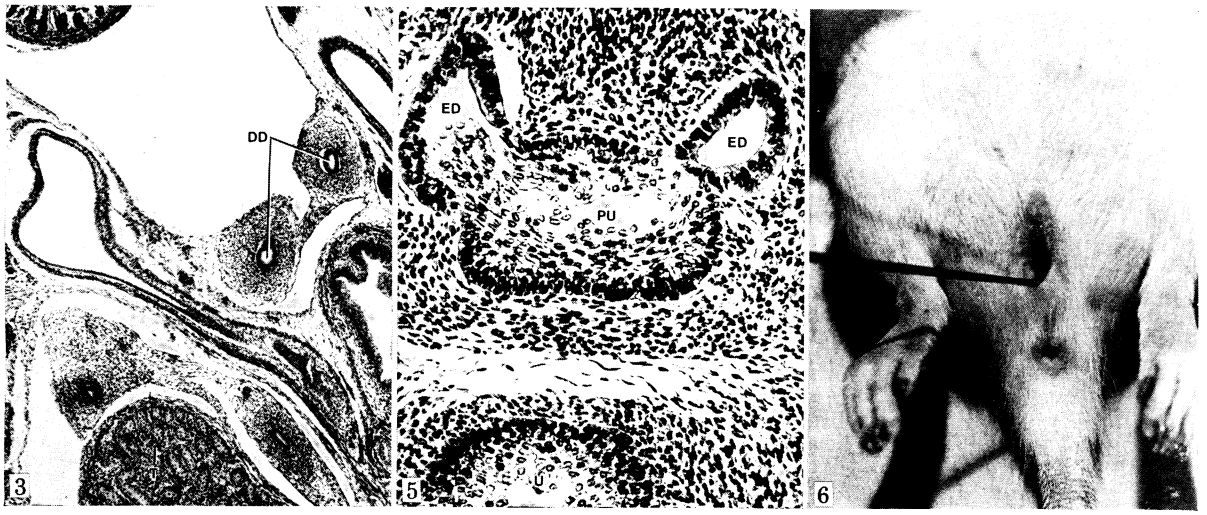


FIGURE 3. Cross-section of a male rat foetus, removed from the uterus at the 22nd day of embryonal development (mother treated from day 13 to 21 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). DD, ductus deferens (derived from the Wolffian duct); T, testis. (Magn. × 37.)

FIGURE 5. Cross-section of a male rat foetus on the 22nd day of embryonal development (mother treated from day 17 to 20 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). Opening of the ejaculatory ducts (ED) into the prostatic utricle (PU). Note the different types of epithelial cells. (Magn. × 93.)

FIGURE 6. Feminized male rat after treatment with 1.0 mg oestradiol daily for 1 week (mother treated with 10 mg cyproterone acetate i.m. from the 13th to the 22nd day of gestation; the newborn received 0.3 mg of the antiandrogen per day subcutaneously until the 21st day of life). Note the presence of a vagina. ($\frac{2}{3}$ actual size.)

(*Facing p.* 180)

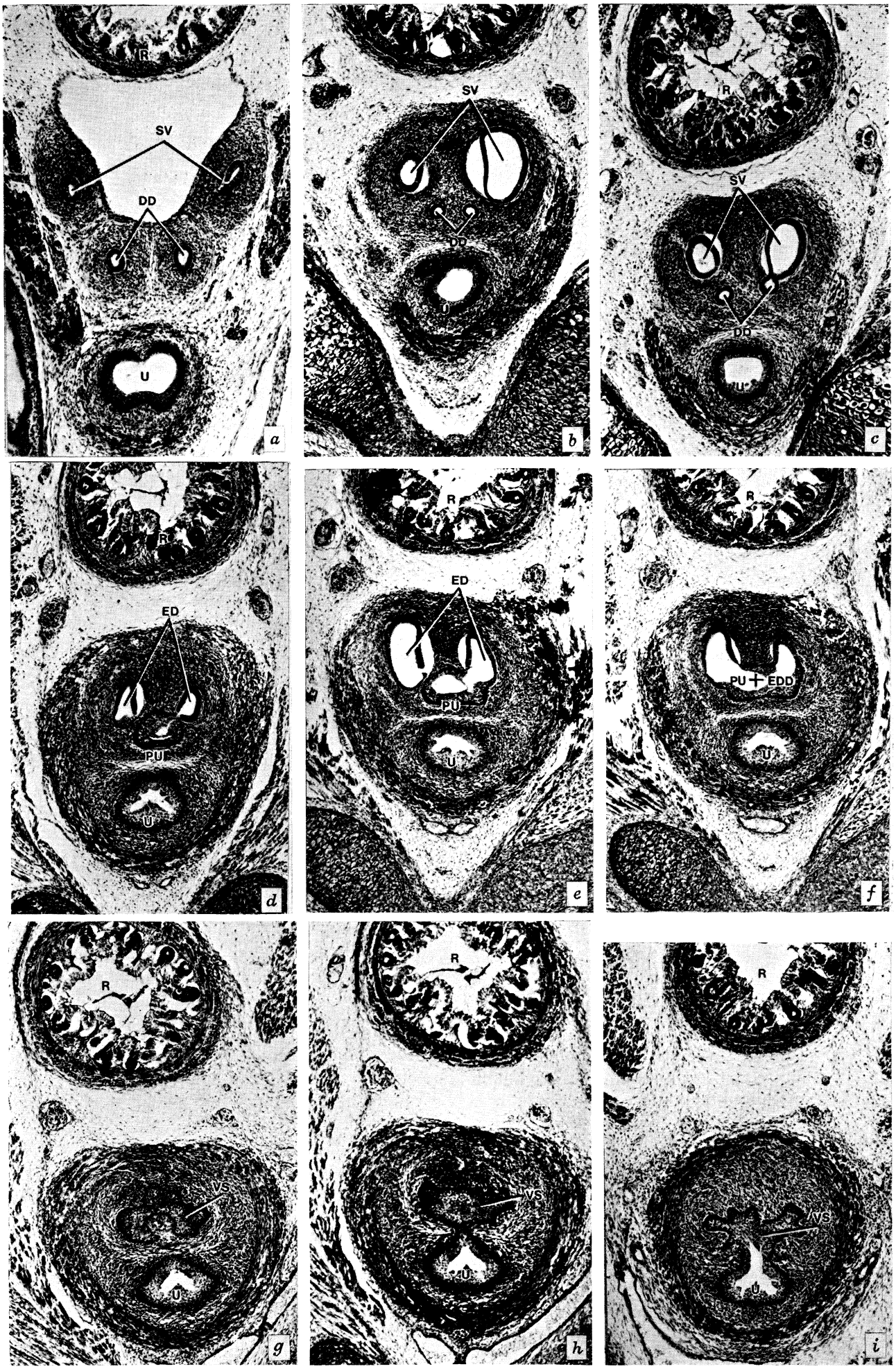


FIGURE 4. For legend see plate 21.

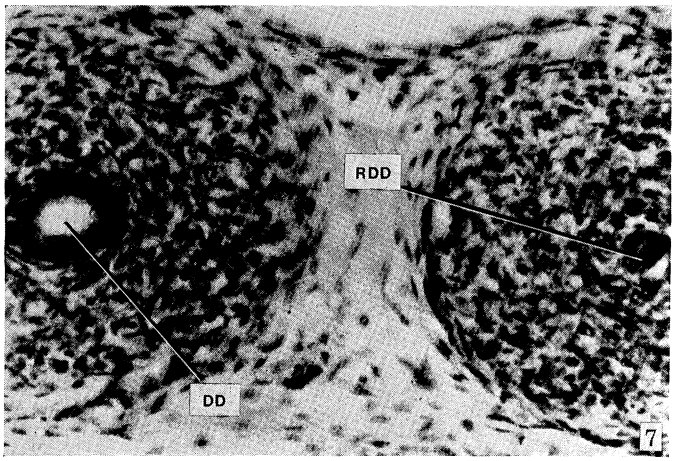


FIGURE 7. Partly retrogressed Wolffian duct of a male rat foetus under the influence of cyproterone acetate (from Forsberg *et al.* 1968). (Magn. × 165.)

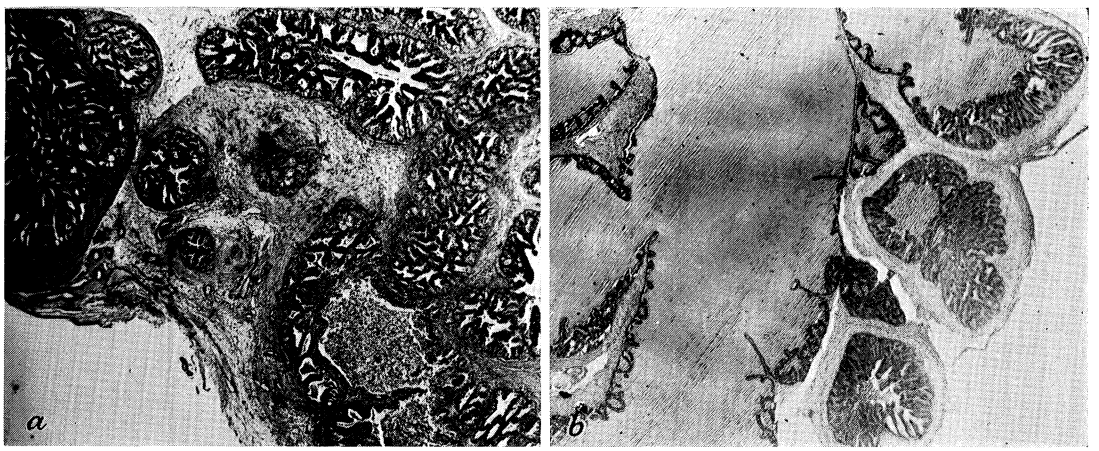


FIGURE 9. Seminal vesicle of: (*a*) a normal adult male rat and (*b*) a feminized adult male rat (mother treated from day 17 to 20 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). Note the atrophy of the glandular tissue. (Magn. × 56.)

DESCRIPTION OF PLATE 20

FIGURE 4. Cross-sections of male rat foetuses on the 22nd day of embryonal development (mother treated from day 17 to 20 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). (Magn. × 42.) DD, ductus deferens; ED, ejaculatory duct; PU, prostatic utricle; PU + EDD, duct of the prostatic utricle and the ejaculatory ducts; R, rectum; SV, seminal vesicles; VS, vagina (sinus part of the vagina); U, urethra. (*a*) and (*b*), anlage of seminal vesicles (SV) and the deferent ducts (DD); (*c*), fusion of the deferent duct (DD) and the seminal vesicle anlage (SV) (right side) thus forming the ejaculatory duct (ED); (*d*) and (*e*), note the appearance of the prostatic utricle (PU) (rudiment of the Müllerian ducts) between the urethra (U) and the ejaculatory ducts (ED); (*f*), fused ejaculatory ducts (ED) and the prostatic utricle (PU)—note the absence of prostate; (*g*), vagina (VS), solid cord of epithelial cells, deriving from the sinus urogenitalis; (*h*) and (*i*), opening of the vagina (V) into the urethra.

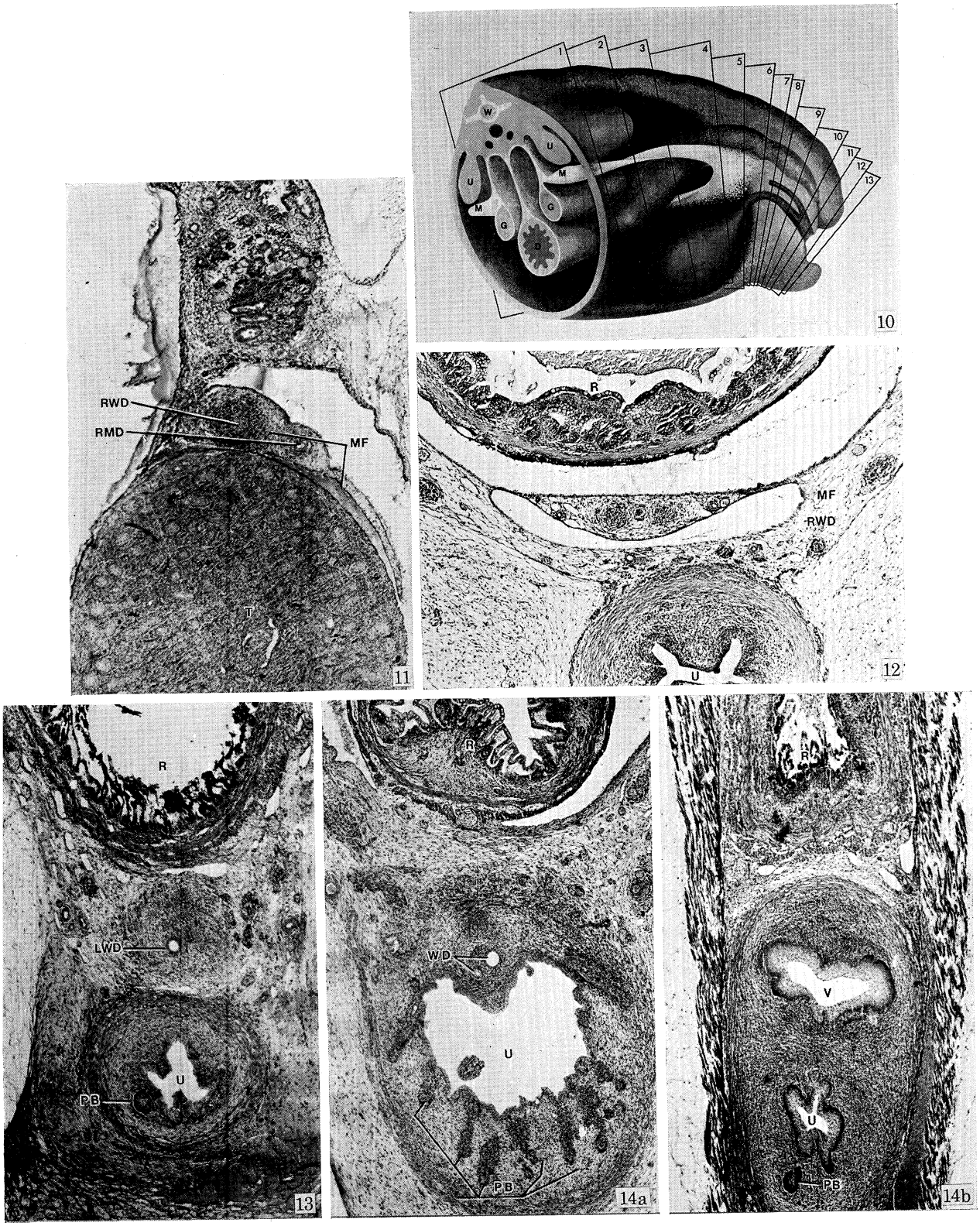


FIGURE 10. Semischematical reconstruction of a feminized male dog foetus (44th day of embryonal development, mother treated from day 23 to 43 of pregnancy with 10 mg/kg cyproterone acetate daily i.m.). Note the absence of the internal genital duct system.

FIGURES 11 TO 14*a*, *b*. Cross-sections of dog oetuses, 44th day of embryonal development. 11 to 13 and 14*b*, feminized males; mothers treated from day 23 to 43 of pregnancy with 10 mg/kg cyproterone acetate daily i.m. 14*a*, normal male. (All magn. × 42.) 11, Gonadal level. Note the normal developed testis (T) and the retrogressed Wolffian ducts (RWD). 12, Cross-section at a lower level than in figure 13. Note the rudiments of the Wolffian ducts (RWD) in the mesogenital fold (MF). 13, Retrogression of the right Wolffian duct, the left duct (LWD) is present. 14, Section at the prostatic level. Note the prostatic buds in the normal male (figure 16*a*). In the feminized male only one prostatic bud can be seen. Note the anlage of a vagina. LWD, left Wolffian duct; MF, mesogenital fold; PB, prostatic buds; R, rectum; RMD, rudiments of the Müllerian ducts; RWD, rudiments of the Wolffian ducts; T, testis; U, urethra; V, vagina; WD, Wolffian ducts.

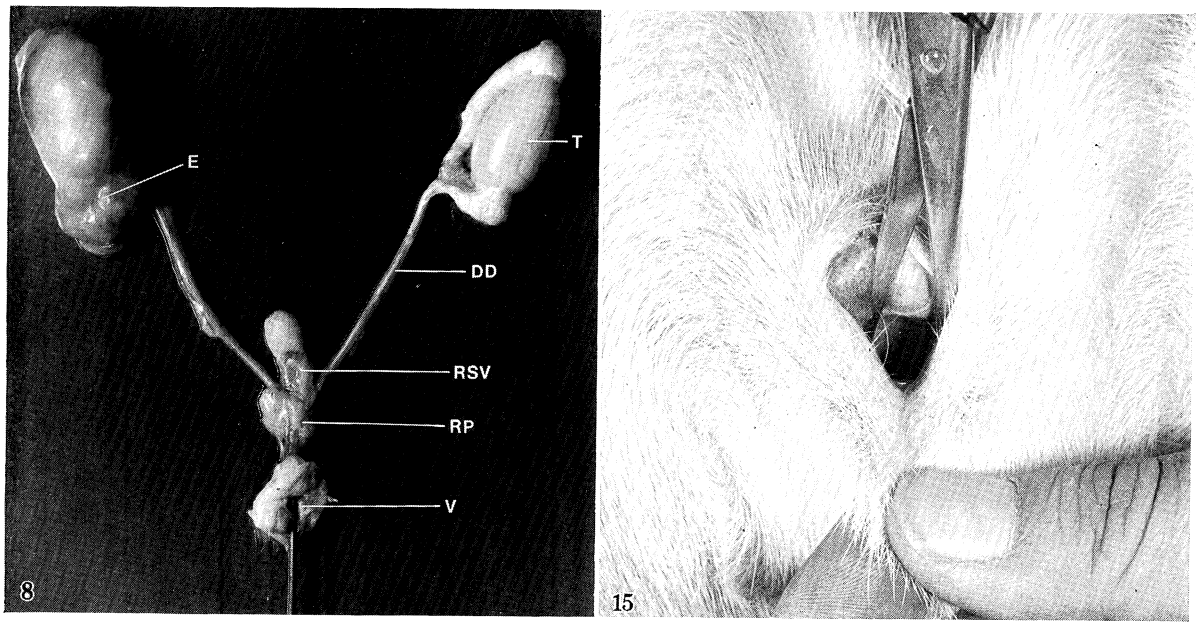


FIGURE 8. Sexual organs of an adult feminized male rat. The mother has been treated from day 13 to 22 of pregnancy with 30 mg cyproterone acetate/animal daily i.m. The newborn males received 1.0 mg cyproterone acetate/animal daily i.m. for the first 3 weeks of life. DD, ductus deferens; E, epididymis (note the dilation of this organ and the testes); RP, rudiments of the prostate; RSV, rudiments of seminal vesicle; T, testis; V, vagina. ($\frac{2}{3}$ actual size.)

FIGURE 15. Adult feminized male dog (mother treated from day 23 of gestation to birth with 10 mg/kg cyproterone acetate daily i.m.). Note the well developed vagina.

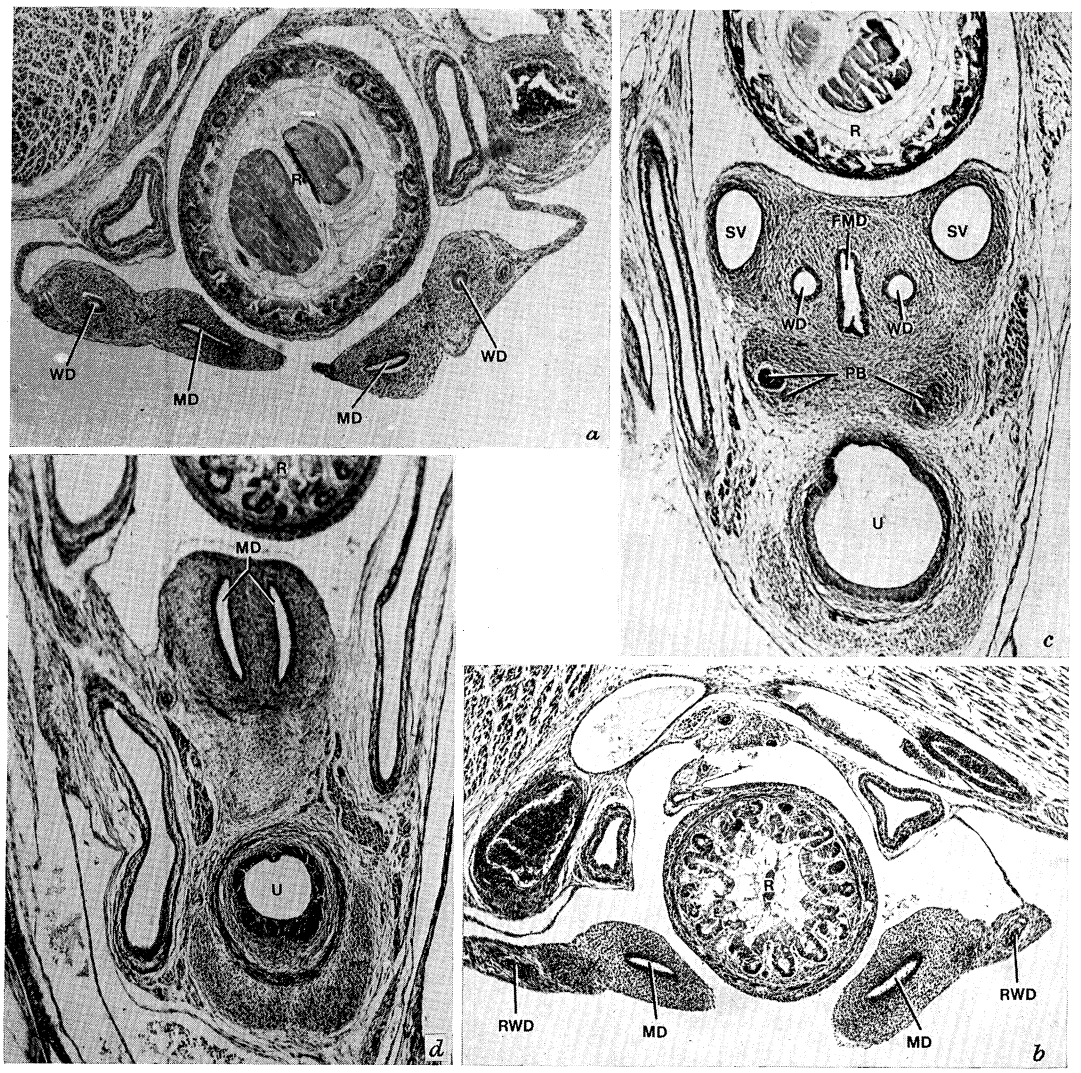


FIGURE 17. Cross-sections of female rat foetuses removed from the uterus at the 22nd day of embryonal development. (Magn. × 42.) (*a*) and (*c*), mother treated from day 15 to 21 of pregnancy with 1.0 mg methyltestosterone/animal daily i.m.; (*b*) and (*d*), mother treated from day 15 to 21 of pregnancy with 1.0 mg methyltestosterone and 30 mg cyproterone acetate/animal daily i.m. FMD, fused Müllerian ducts; MD, Müllerian duct; PB, prostatic buds; R, rectum; RWD, rudiments of the Wolffian duct; SV, seminal vesicle; U, urethra; WD, Wolffian duct.

Sometimes a complete separation of the urogenital sinus into two parts, vagina and urethra, has occurred, i.e. hardly any vestibulum vaginae exists. Under the influence of antiandrogen, the vagina anlage develops in the same manner as in female animals. These changes are definitely irreversible (Neumann, Elger & Kramer 1966). Figure 6, plate 19, shows a vagina in one of such animals in adulthood.

Administration of smaller antiandrogen dosages produces only the faint outline of a vagina. After junction with the remnants of the Müllerian ducts (prostatic utricles), the ejaculatory duct leads directly into the urethra. Yet, sometimes the connexion between testicle and urogenital sinus seems interrupted somewhere, a finding suggested by the autopsy findings in adult feminized animals. A frequent observation was of testicular atrophy caused by blockage of secretion. Such blockages were pronounced in the epididymal region.

To sum up the results of the rat experiments:

Jost's hypothesis was corroborated largely but not entirely. As a rule, the anlage of Wolffian ducts was observed, even after administration of extremely high dosages of antiandrogen which otherwise produced complete feminization of the male animals.

Experiments with mice produced the same results as with rats.

Forsberg, Jacobsohn & Norgren (1968) also studied the influence of cyproterone acetate on sex differentiation in the rat, with very similar results. They observed only a single case of retrogression of a Wolffian duct (figure 7, plate 21). They also concluded that generally one cannot suppress either the Wolffian ducts or the formation of seminal vesicles by administration of cyproterone acetate.

Our own experiments with rats confirm this finding with respect to the Wolffian ducts but differ as far as seminal vesicles are concerned (Neumann, Richter & Günzel 1965). Although seminal vesicles are present in feminized male rats at birth they are rudimentary in adults. We raised feminized animals and sectioned *in toto* the sex organs of the adult animals. One such section is shown in figure 8, plate 23.

As can be seen, the anlage of the seminal vesicles is only rudimentary, and histologic sections of these rudimentary seminal vesicles showed a strikingly strong involution of the glandular tissue as well as relatively increased interstitial tissue. It may also be assumed that in these animals the secretory duct system of the seminal vesicles was not intact (figures 9*a*, *b*, plate 21).

A frequent observation is of interest. We often saw testicular atrophy caused by blockade of secretion, so we can assume that the duct system was interrupted somewhere (see figure 8, left side).

We then examined other species, first rabbits, then dogs, and more recently sheep.

The experiments with rabbits, mainly carried out by Dr Elger (1967), corroborated Jost's hypothesis completely. We obtained the precise type of male intersexuality postulated by Jost, a type which hitherto nobody had succeeded in producing experimentally. Under the influence of antiandrogen the Wolffian ducts also retrogressed.

Permit me to elaborate on this finding by demonstrating some experiments with dogs. All of the following illustrations show cross-sections of 44-day-old dog foetuses. Those whose mothers had been treated received a daily intramuscular injection of 10 mg/kg cyproterone acetate from day 23 to 43 of their pregnancy. By means of semischematic reconstruction of a 'feminized' dog foetus (figure 10, plate 22), I want to demonstrate to you the most frequent type of male intersexuality resulting from such treatment. The differentiation of the gonads is

undisturbed, the Müllerian ducts as well as the Wolffian ducts have retrogressed, i.e. the mesogenital space is empty. To phrase it differently: in many cases, the connexion between gonads and urogenital sinus no longer exists. As to the urogenital sinus, there is no anlage of male accessory sex glands (the only one present normally in dogs is the prostate), and the animals have a vagina and female external genitalia. This result is produced by maximal feminization.

Actually, in most cases—eight feminized dog foetuses have been systematically examined so far—we still note remnants of the Wolffian ducts, mostly anteriorly near the gonads. Unilateral retrogression was observed in some cases, and then, interestingly, it was always the right Wolffian duct which had retrogressed.

Figure 11, plate 22, shows a cross-section at the gonadal level of a dog foetus treated with antiandrogens on the 44th day of embryonic development. The testicle is just as developed as in untreated male control animals, but the mesogenital fold is empty. Rudiments of the retrogressed Wolffian duct are still noticeable.

A more distal section, shortly after junction of the mesogenital from both sides, is shown in figure 12, plate 22. The rudiments of the retrogressed Wolffian ducts can be distinguished. As mentioned before, retrogression of the Wolffian ducts occurs sometimes unilaterally, i.e. on the right side. This phenomenon, however, seems merely a question of dosage. As shown in our rabbit experiments, sufficiently high dosages always produced complete retrogression of the Wolffian ducts. The dosage we administered to dogs (10 mg/kg) might have been too low to achieve this result. One such unilateral case is demonstrated in figure 13, plate 22, the Wolffian duct, retrogressed on the right, is still present on the left.

Figure 14*a*, plate 22, is a section of a normal dog foetus, at the level of the prostatic region. Located close to the urethra can be seen the two Wolffian ducts which lead into it separately, and numerous prostatic buds. Figure 14*b* shows a section at the same level of a feminized male dog foetus. Instead of prostatic buds, which are missing, one notes very clearly the anlage of a vagina between urethra and rectum. Again, the external genitalia are completely female. This needs no further elaboration as we had the same finding in our experiments with rats.

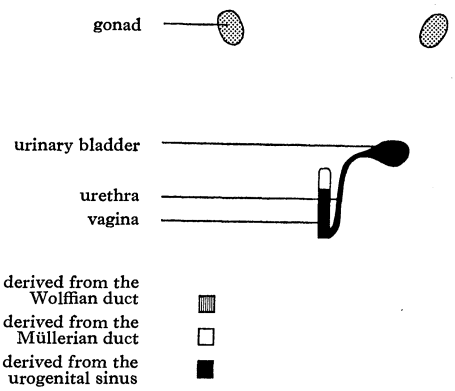
Of course, these changes are equally irreversible, as can be seen in figure 15, plate 23 (external genitalia of a male dog, mother treated with cyproterone acetate during pregnancy beginning on day 23). The vagina, approximately 8 cm long, is well developed. The animal has no penis but a clitoris which, however, is somewhat hypertrophied. This may be explained by the fact that the animal still has its testicles and is thus exposed to androgen influence.

Thus, experiments with rabbits and dogs completely corroborate Jost's theory according to which an additional factor other than androgen must be involved in sex differentiation. Among other things, this other factor is responsible for the retrogression of the Müllerian ducts in the male sex. If retrogression of Müllerian ducts, i.e. of the female duct system, were dependent on androgen, they should be preserved under the influence of an antiandrogen. In other words, these animals, upon reaching adult age, should have had fallopian tubes and uteri. This, however, was not the case.

Next, we have a diagrammatic illustration (figure 16) showing schematically the type of intersexuality produced by administering antiandrogen to pregnant dogs and rabbits. The result lends support to Jost's hypothesis, which really is no longer a hypothesis, at least with regard to these species (rabbits and dogs). This illustration demonstrates the processes in sex differentiation which are definitely dependent on androgen, and those which, definitely or probably, are not, but are regulated by other factors, or one other factor at least.

Under the influence of antiandrogens, i.e. when the effects of androgens are suppressed, the Wolffian ducts are destroyed. No differentiation of accessory sex glands, and no differentiation of male external genitalia occurs. Instead, among other things, the anlage of a vagina forms.

Retrogression of the Müllerian ducts is definitely not dependent on androgen. Likewise it is probable that testicular descent and testicular differentiation itself are also not dependent on androgen. This form of intersexuality occurs spontaneously in the human and is known as testicular feminization.



androgens not active	1. destruction of the Wolffian ducts 2. no differentiation of accessory sexual glands 3. no differentiation of male external genitalia 4. differentiation of a vagina
factor X active	1. regression of the Müllerian ducts 2. (descent of the gonads?) 3. (differentiation of testes?)

FIGURE 16. Androgen-dependent differentiation processes in the dog.

TABLE 1. COMPARISON OF THE SYNDROME OF 'TESTICULAR FEMINIZATION' WITH FEMINIZED DOGS

'testicular feminization'	'feminized' dogs
chromosomal sex ♂	chromosomal sex ♂
gonads ♂	gonads ♂
external genitalia ♀, development of a vagina	external genitalia ♀, development of a vagina
absence of uterus and tubes	absence of uterus and tubes
absence of epididymis and ductus deferens	absence of epididymis and ductus deferens
absence of male accessory sexual glands	absence of male accessory sexual glands
incomplete descensus testiculorum	incomplete descensus testiculorum (scrotal development inhibited)

As early as 1965 (Neumann & Elger 1965), I showed slides comparing the results of antiandrogen treatment of rats with the characteristics of testicular feminization in the human. The similarities are even more striking if one compares feminized dogs with the characteristics of this syndrome (see table 1). This is not surprising. In this form of spontaneous intersexuality, it is assumed that the receptors for androgens are missing, i.e. that in the last analysis dysfunction at the enzymic level inhibits the androgen effect. In our case, this effect is blocked by an antiandrogen. Consequently, we produce an equal or at least similar result.

I would now like to take the liberty of discussing again our rat and mouse experiments, because it is a pity that our results with these species do not quite fit this beautiful theory.

It may be that in these species the physiological stabilization of the Wolffian ducts is achieved by another androgen, the effect of which cannot be inhibited by cyproterone acetate. However, this seems unlikely in so far as the effects of all androgens or anabolics investigated hitherto were abolished. Also, it does not seem very likely that essential processes in sex differentiation are triggered by different hormonal steering mechanisms in different species.

Another explanation may be that in these species the Wolffian ducts react so sensitively to androgens—in the sense of stabilization—that the antiandrogen dosages which would be needed to counteract the androgen are prevented from taking effect. In order to test this hypothesis we started a new experiment: we treated pregnant rats from day 15 to term with 1.0 mg methyltestosterone, a dosage just adequate to stabilize the Wolffian ducts in female foetuses. We hoped that by simultaneous administration of an antiandrogen it might become possible to stop this process.

The results of these experiments, which have been recently completed, are gratifying. In all those ten female foetuses, whose mothers were injected with methyltestosterone alone, Wolffian ducts were formed as can be seen from figures 17*a* and *c*, plate 23. Figure 17*a* is a cross-section near the gonadal level, figure 17*c* is a cross-section immediately above the junction of the Müllerian ducts. An entirely different situation is obtained in those female foetuses whose mothers were injected with 1.0 mg methyltestosterone and 30.0 mg cyproterone acetate simultaneously. In eight of a total of 12 animals Wolffian ducts were not established, two foetuses possessed only one Wolffian duct on the left side. Only in one out of these 12 animals Wolffian ducts on both sides were found (see figure 17*b* and *d*).

In conclusion, I would like to mention very briefly some other organs and organ systems which undergo feminization under antiandrogen influence. Among them are the mammary glands (Elger & Neumann 1966; Neumann & Elger 1966*a*) and also certain nerve centres which regulate the mode of gonadotrophin secretion as well as certain behavioural functions (Neumann & Elger 1966*b*). Under antiandrogen influence, the mammary glands as well as the related brain centres become totally feminized. Time is lacking today to discuss this phenomenon in more detail.

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Discussion on paper by F. Neumann et al., p. 179

A. JOST: The effects of cyproterone acetate on sex differentiation of the foetus give results which depend upon the animal species. I could repeat Dr Elger's experiments on rabbits, and I also found some unexpected effects in females (Jost 1966). But in rats, even with large doses injected into the mother, I did not obtain regression of the Wolffian ducts or inhibition of the seminal vesicles (Jost 1967) while masculinization of the external genitalia was prevented. Since the Wolffian structures are determined in the foetus before day 18, it should be ascertained whether the antiandrogen is transferred in large amounts across the placenta at that stage of pregnancy. Since the compound is weakly soluble it is difficult to introduce it in large quantities into the foetus itself, unless dry powder is used. There are also alternative explanations for the failure of cyproterone acetate to prevent Wolffian stabilization, such as differences in the nature of the foetal testicular secretion according to age or very high local concentrations of testicular androgens, at the level of the Wolffian ducts.

Finally, it should be noticed that cyproterone acetate does not prevent initial testicular differentiation, which would suggest that this process is not governed by androgens.

F. NEUMANN: The role of the placental barrier for antiandrogens in rats before day 13 of pregnancy is unknown. But in mice, mammary gland differentiation was influenced by anti-androgen treatment on day 13, i.e. at the same time that the Wolffian duct should also be influenced. This was not the case. So, the ineffectiveness of antiandrogens on the development of the deferent ducts in mice cannot be explained on the basis of placental transfer.

Another explanation for the ineffectiveness of antiandrogens on the developing deferent ducts in rats and mice may be that in these species the Wolffian ducts react so sensitively to androgen —in the sense of stabilization—that the antiandrogen dosages which would be needed to counteract the androgen are prevented from taking effect. It may also be that in rats and mice the physiological stabilization of the Wolffian duct is achieved by another androgen, the effect of which cannot be inhibited by cyproterone acetate.

There are now other antiandrogens with no progestational effects at all, although they are less active than the original compound. Nevertheless the newer compounds produce the same kind of intersexuality in rabbits: rudiments of the Müllerian duct were not found in this species, but all of the other effects were similar. Until now the newer compounds have not been injected directly into the foetus, and they were also rather insoluble.

A. MUNCK: Is the action of cyproterone acetate to block a receptor in tissue?

F. NEUMANN: We believe the mode of action is by a competitive antagonism at the target organ, although some authors believe that both competitive and non-competitive antagonism occur. Very recent studies have shown that the compound competes in the protein binding of dihydrotestosterone.

R. L. GARDNER: An experiment that could be of help is provided by Ischida's experiments on the teleost fish *Medaka*. He injected oestradiol in olive oil into the newly fertilized egg and achieved sex reversal in every case. The oestradiol was labelled, and it could be detached in the gonad. Our work with mouse blastocysts shows that normal development can follow injection

of a 30 μm droplet of paraffin into the 90 μm blastocoele. This would be an excellent way of applying steroids at this stage of development.

References

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Jost, A. 1967 *Res. steroids* **3**, 207.

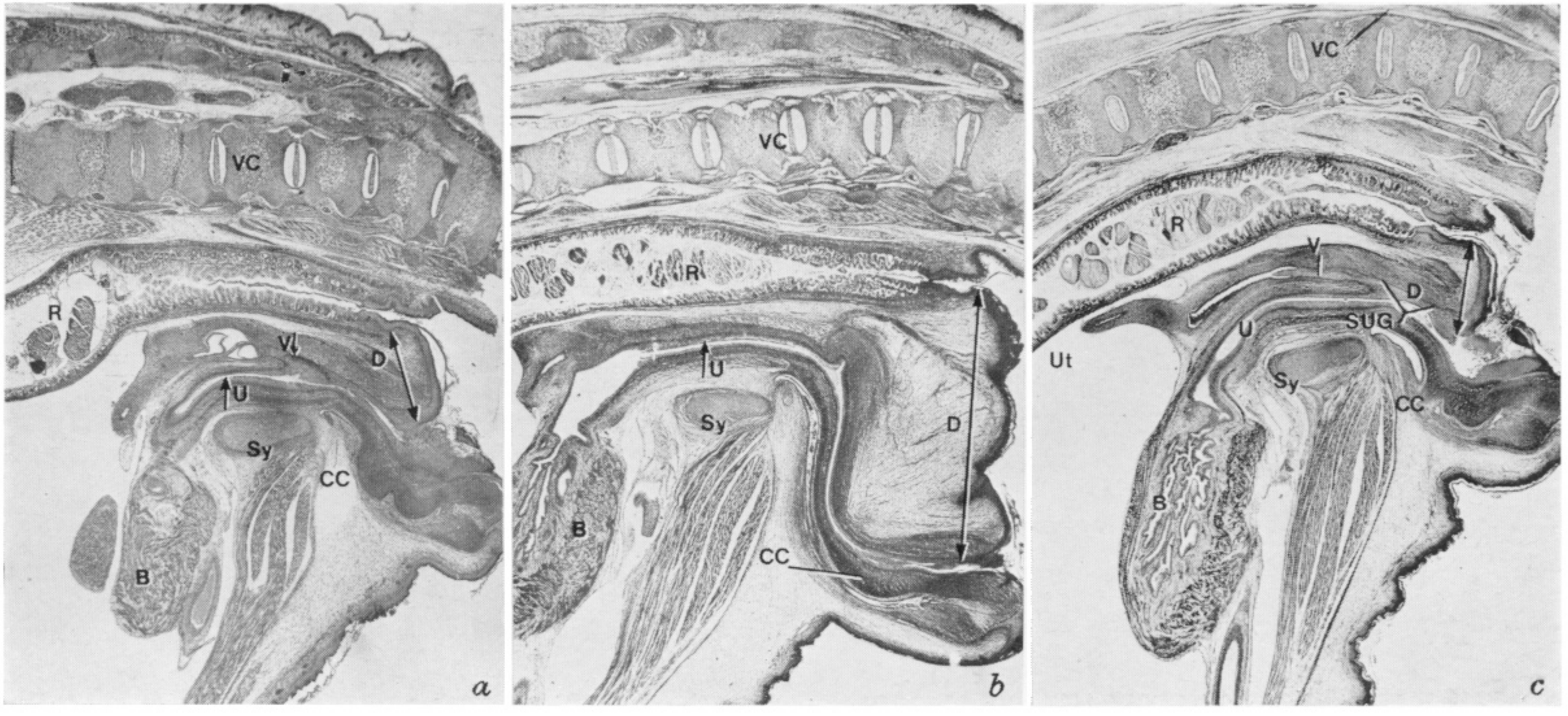
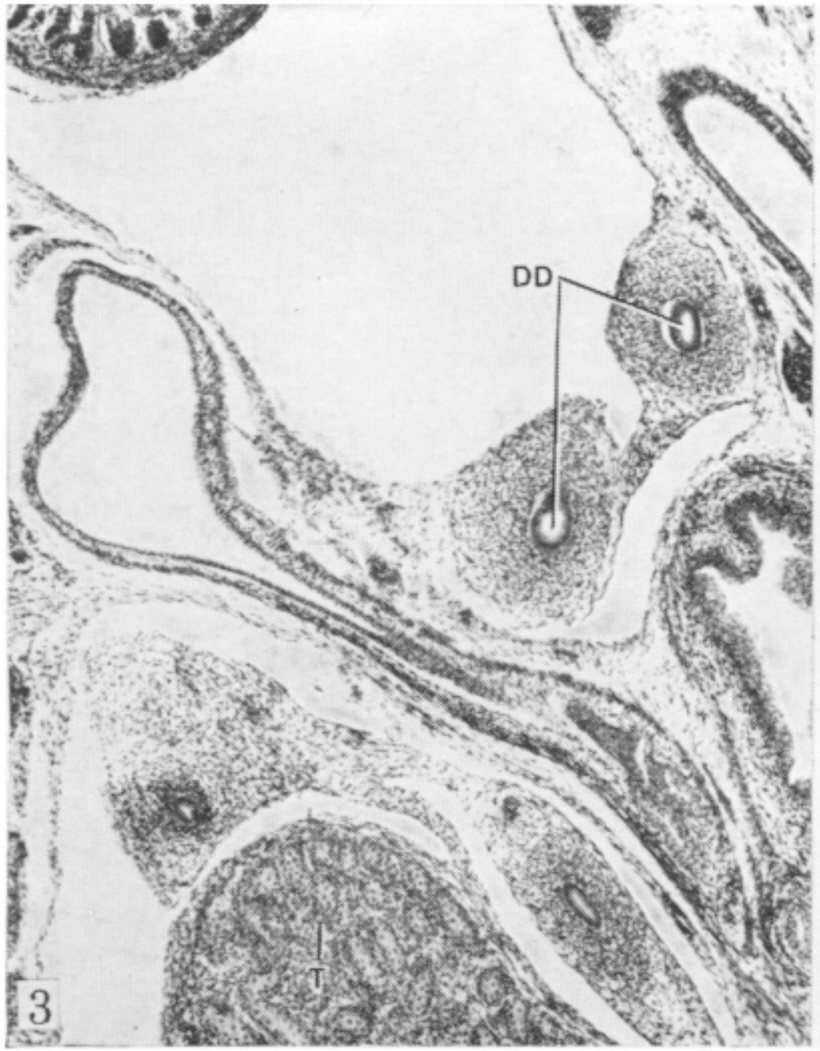
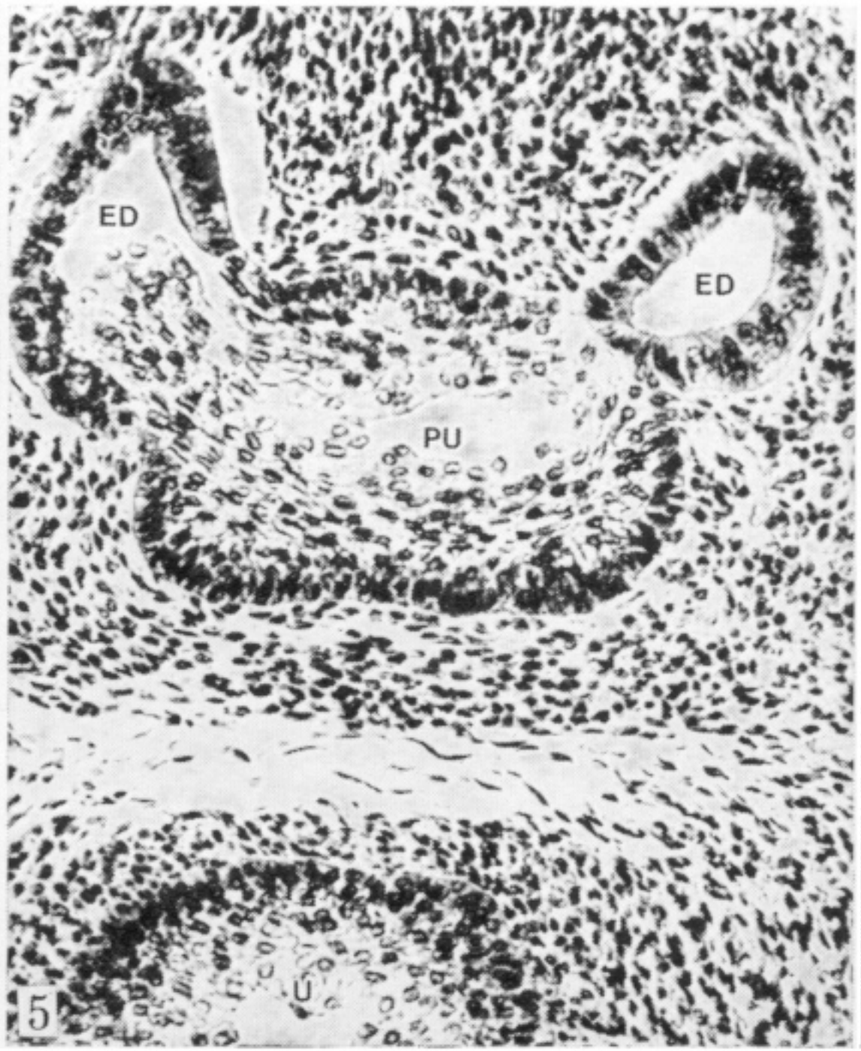


FIGURE 2. Sagittal sections of rat foetuses on the 22nd day of embryonal development. (*a*) Feminized male foetus (mother treated with 10 mg cyproterone acetate/day from 17th to 20th day of gestation); (*b*) normal male foetus; (*c*) normal female foetus. B, urinary bladder; CC, corpus cavernosum; D, anogenital distance; R, rectum; SUG, sinus urogenitalis; Sy, symphysis; U, urethra; Ut, uterus; V, vagina; VC, vertebral column. (Magn. × 8.)





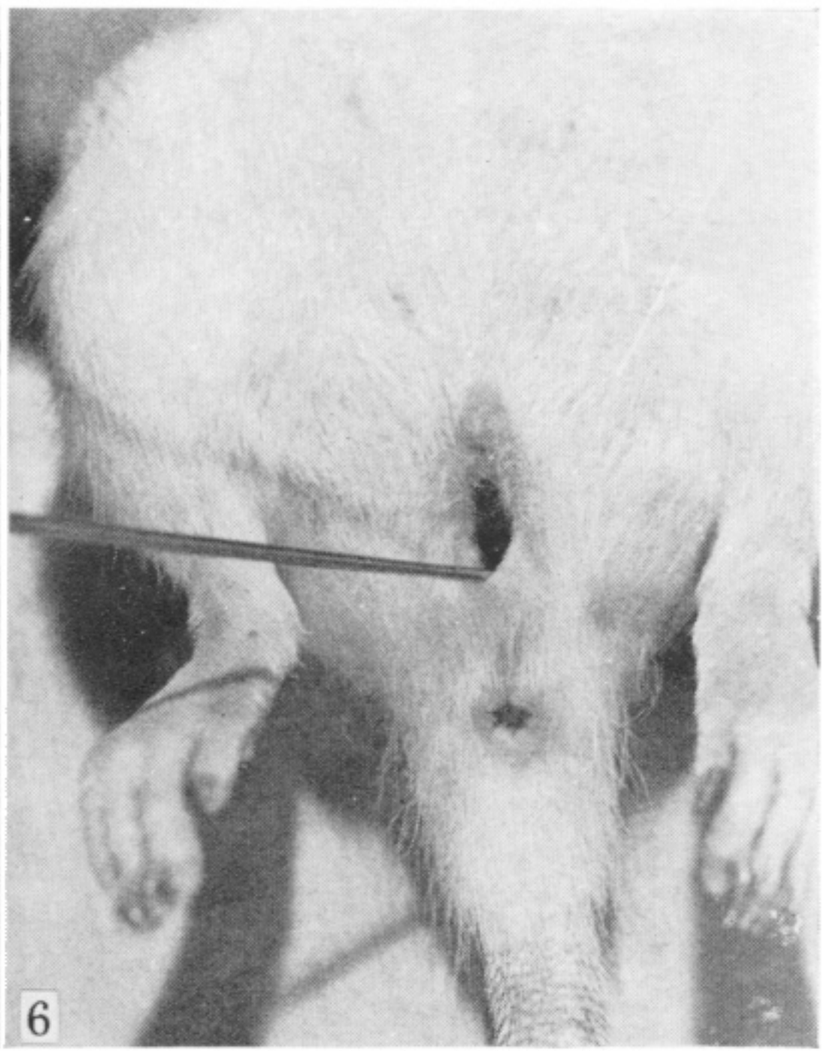
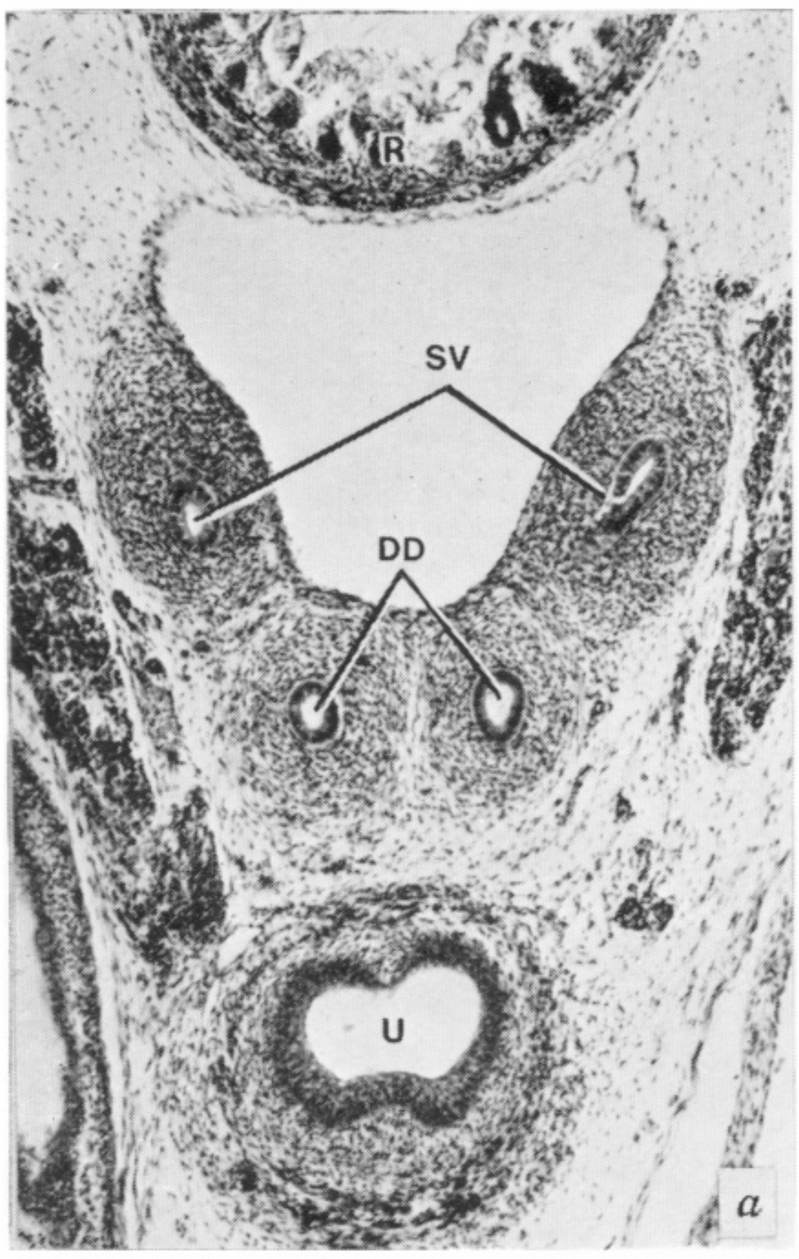
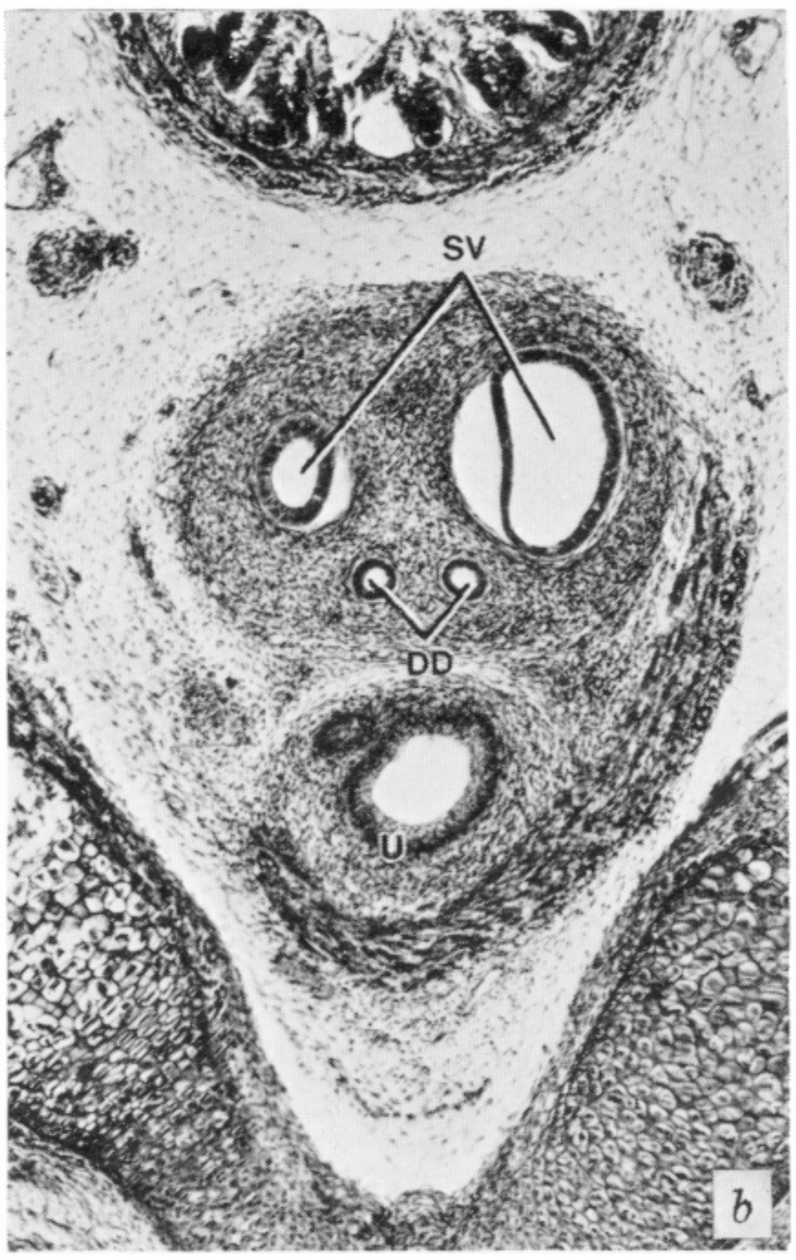


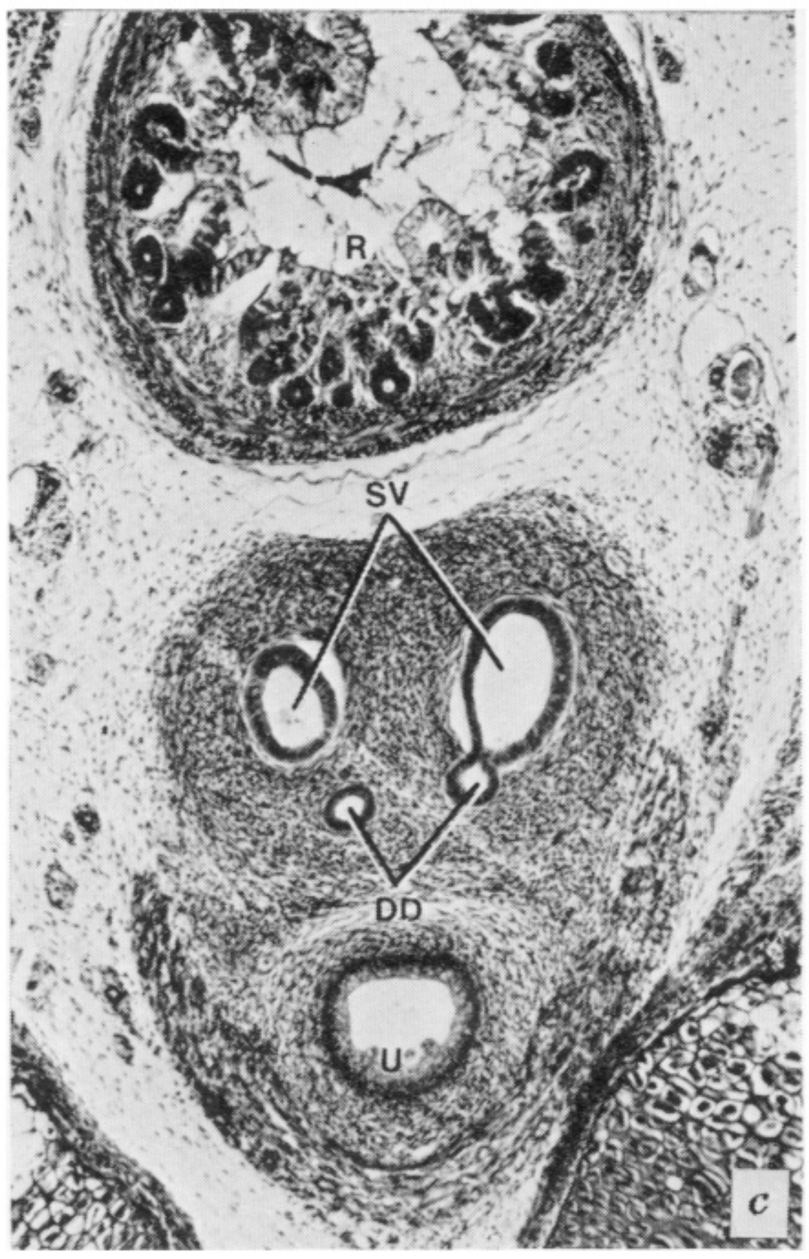
FIGURE 3. Cross-section of a male rat foetus, removed from the uterus at the 22nd day of embryonal development (mother treated from day 13 to 21 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). DD, ductus deferens (derived from the Wolffian duct); T, testis. (Magn. × 37.)

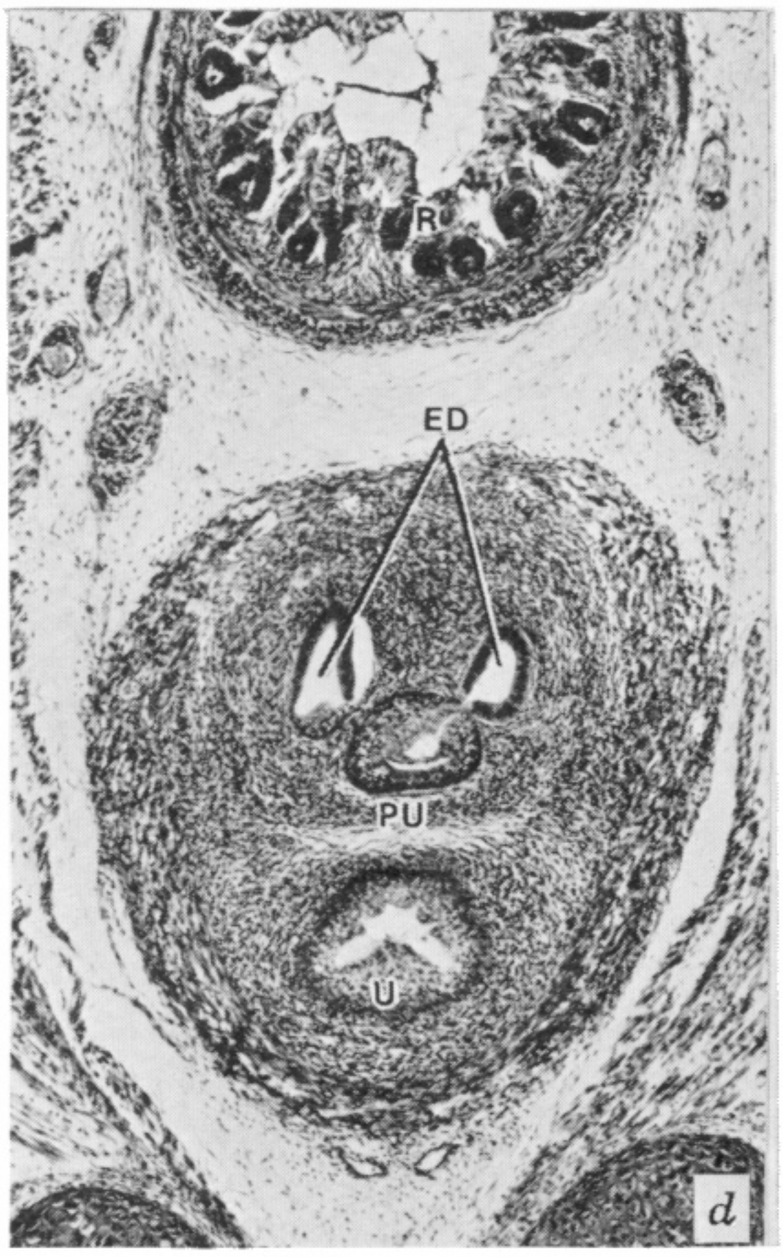
FIGURE 5. Cross-section of a male rat foetus on the 22nd day of embryonal development (mother treated from day 17 to 20 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). Opening of the ejaculatory ducts (ED) into the prostatic utricle (PU). Note the different types of epithelial cells. (Magn. × 93.)

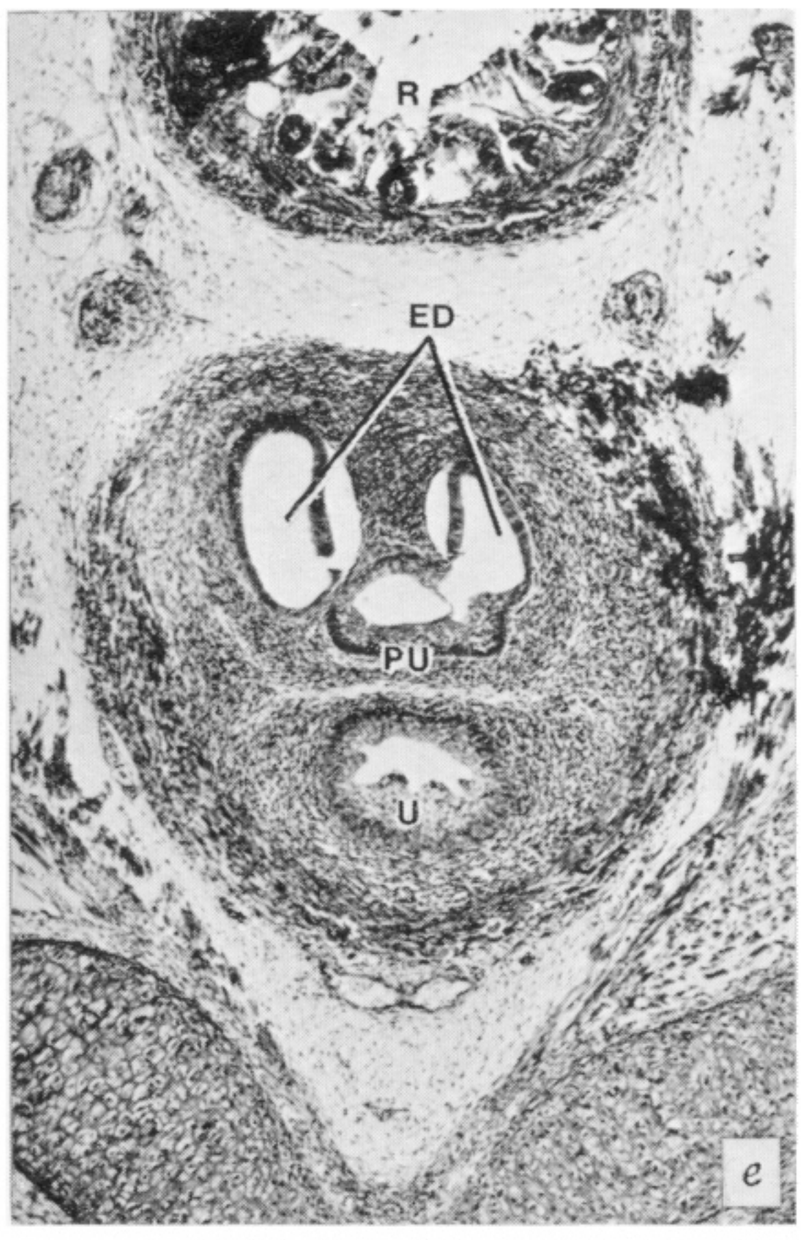
FIGURE 6. Feminized male rat after treatment with 1.0 mg oestradiol daily for 1 week (mother treated with 10 mg cyproterone acetate i.m. from the 13th to the 22nd day of gestation; the newborn received 0.3 mg of the antiandrogen per day subcutaneously until the 21st day of life). Note the presence of a vagina.(($\frac{2}{3}$ actual size.)

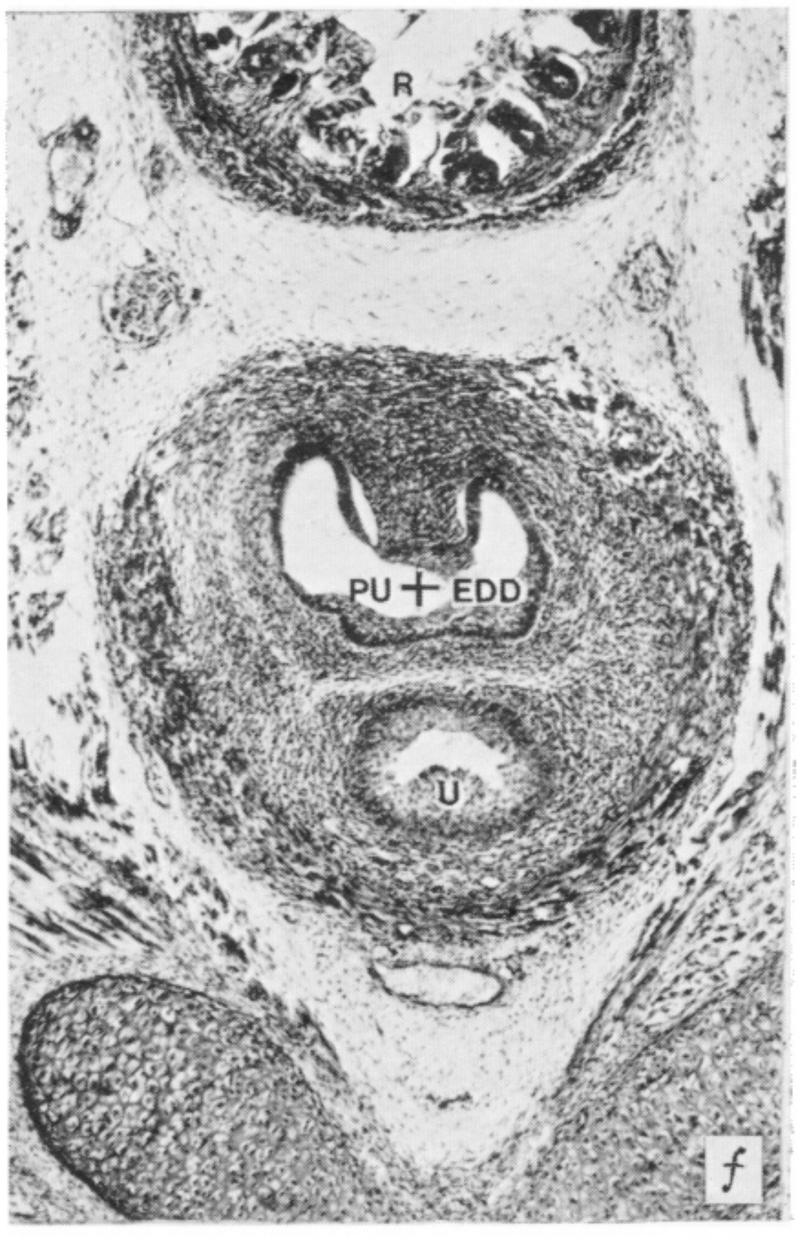


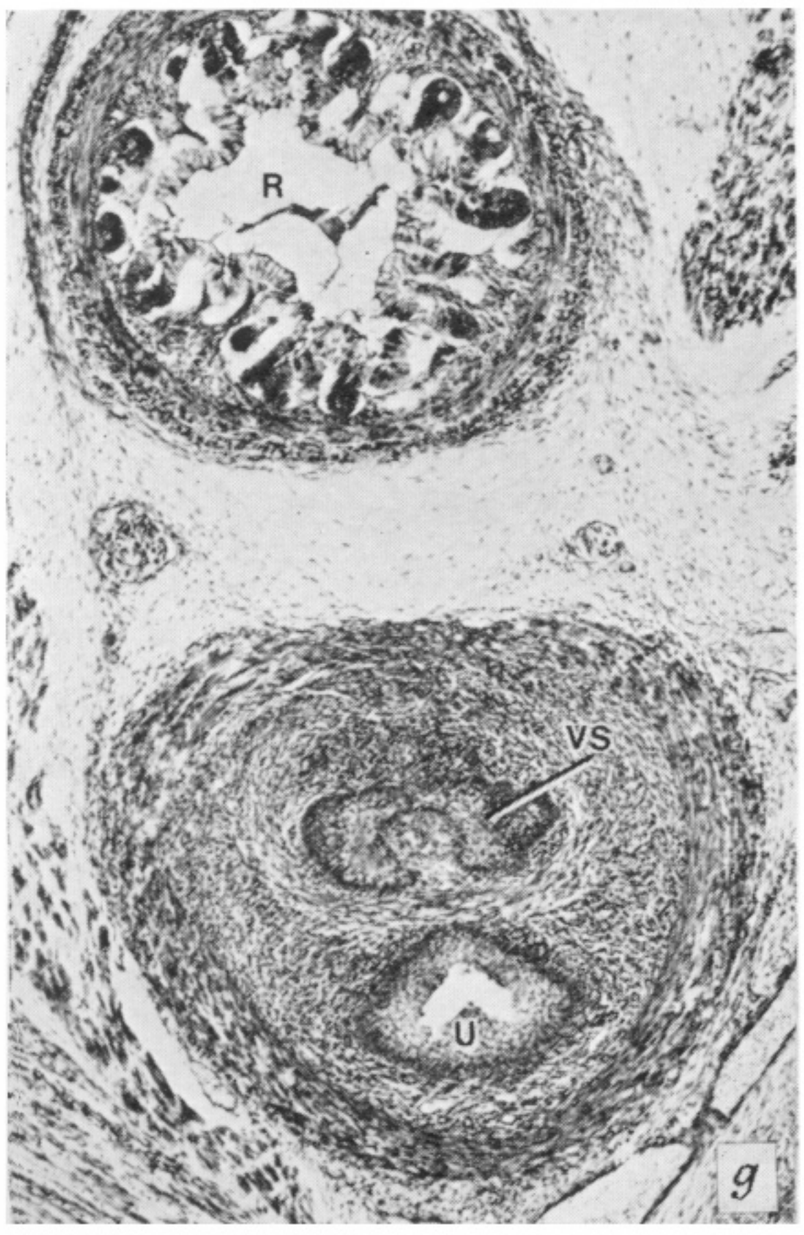


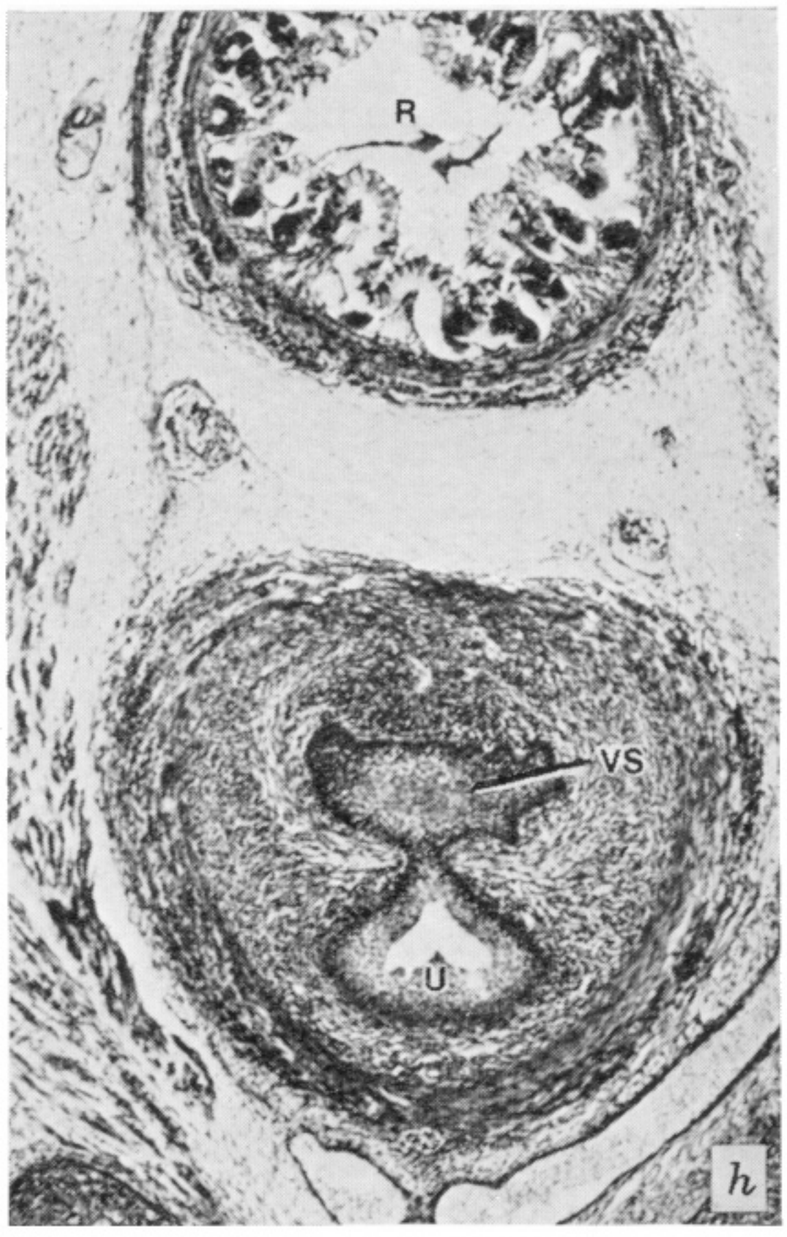












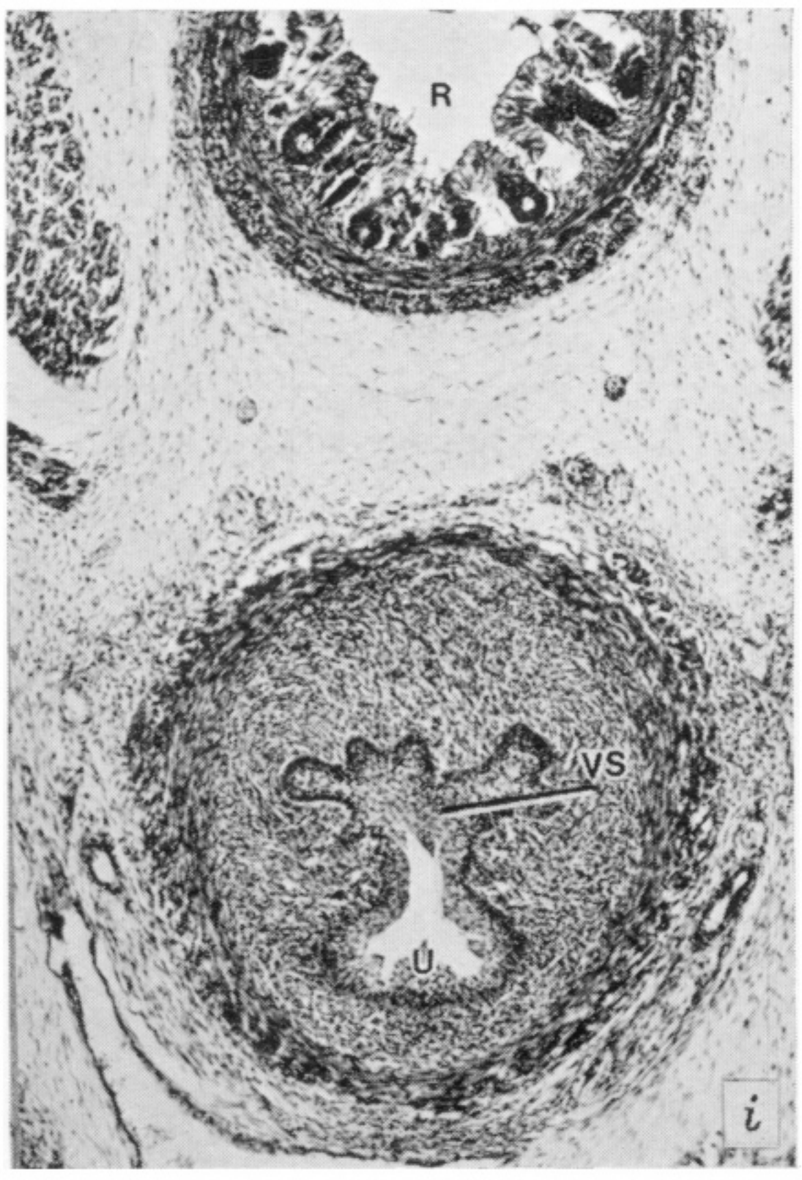


FIGURE 4. For legend see plate 21.

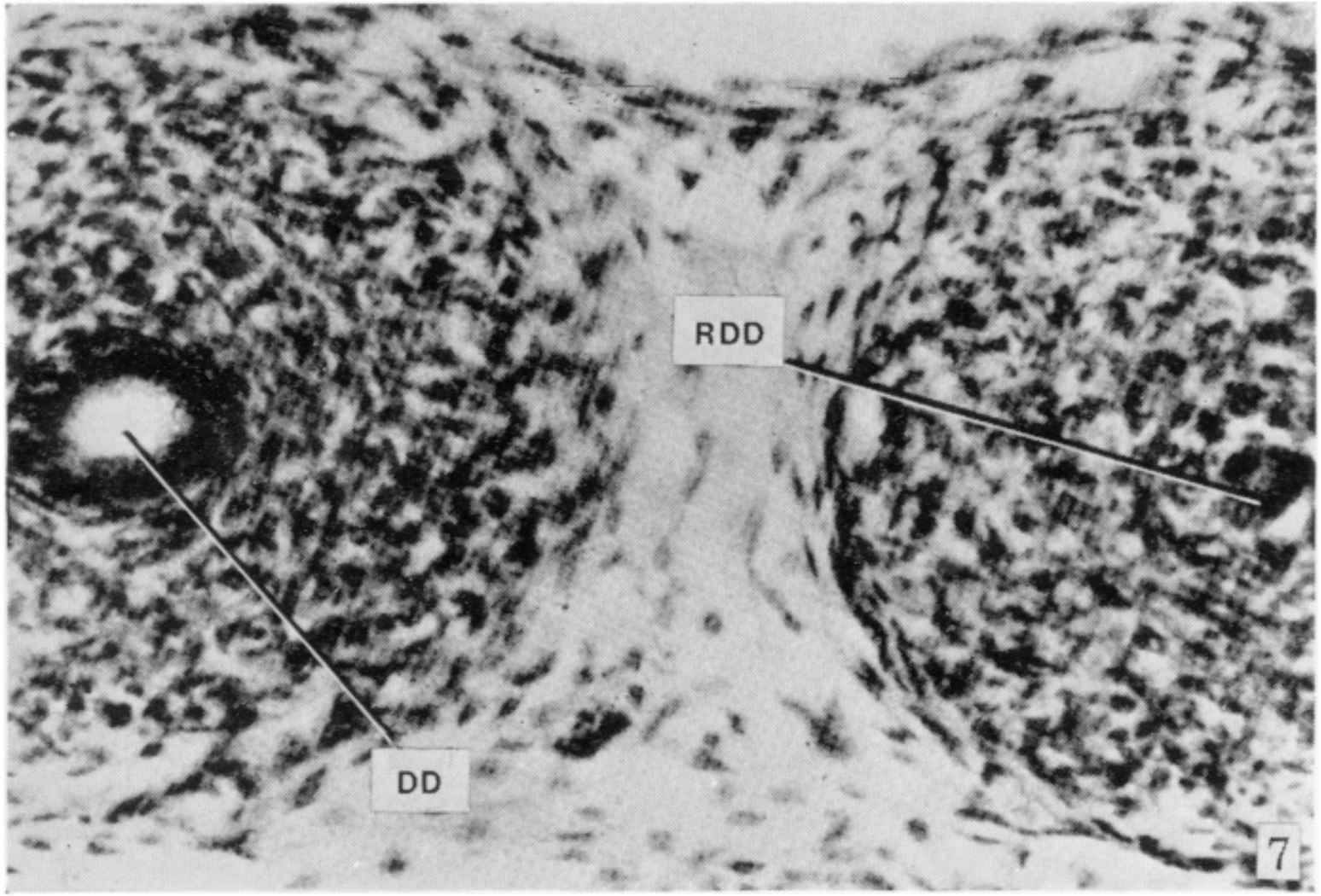


FIGURE 7. Partly retrogressed Wolffian duct of a male rat foetus under the influence of cyproterone acetate (from Forsberg *et al.* 1968). (Magn. × 165.)

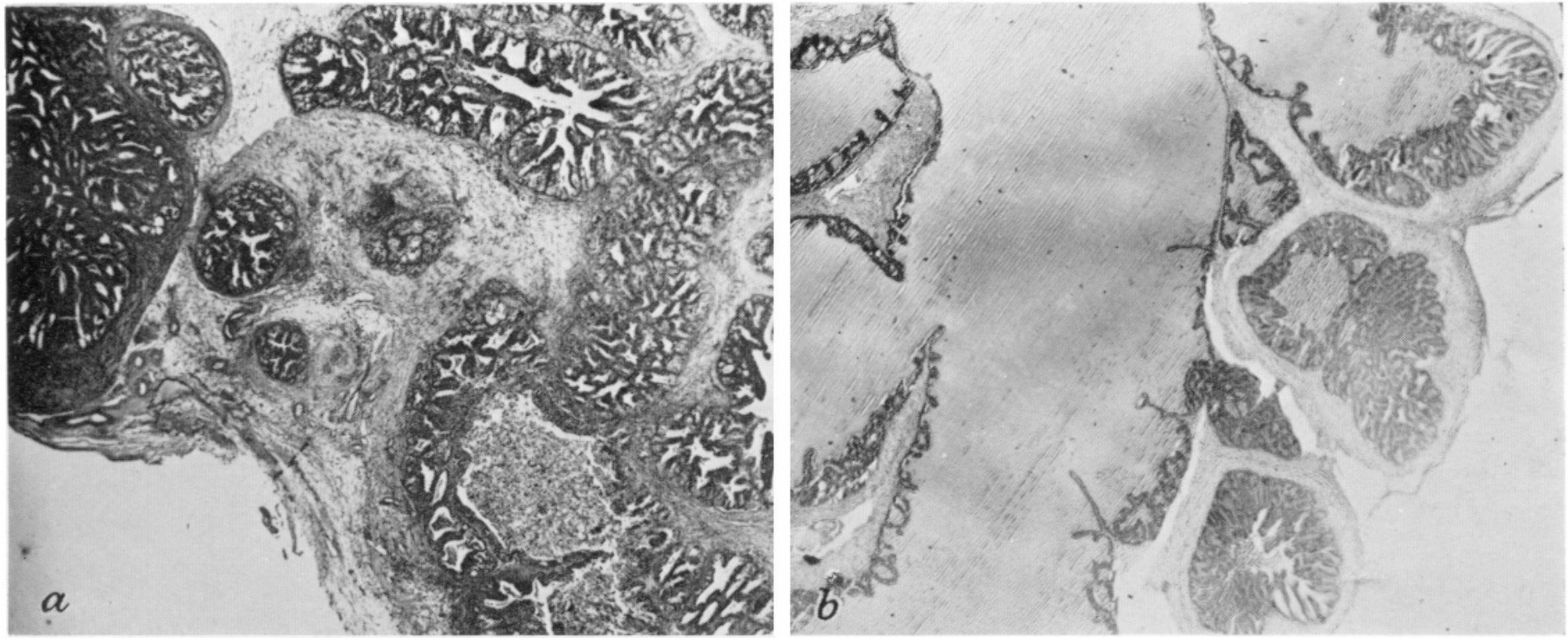
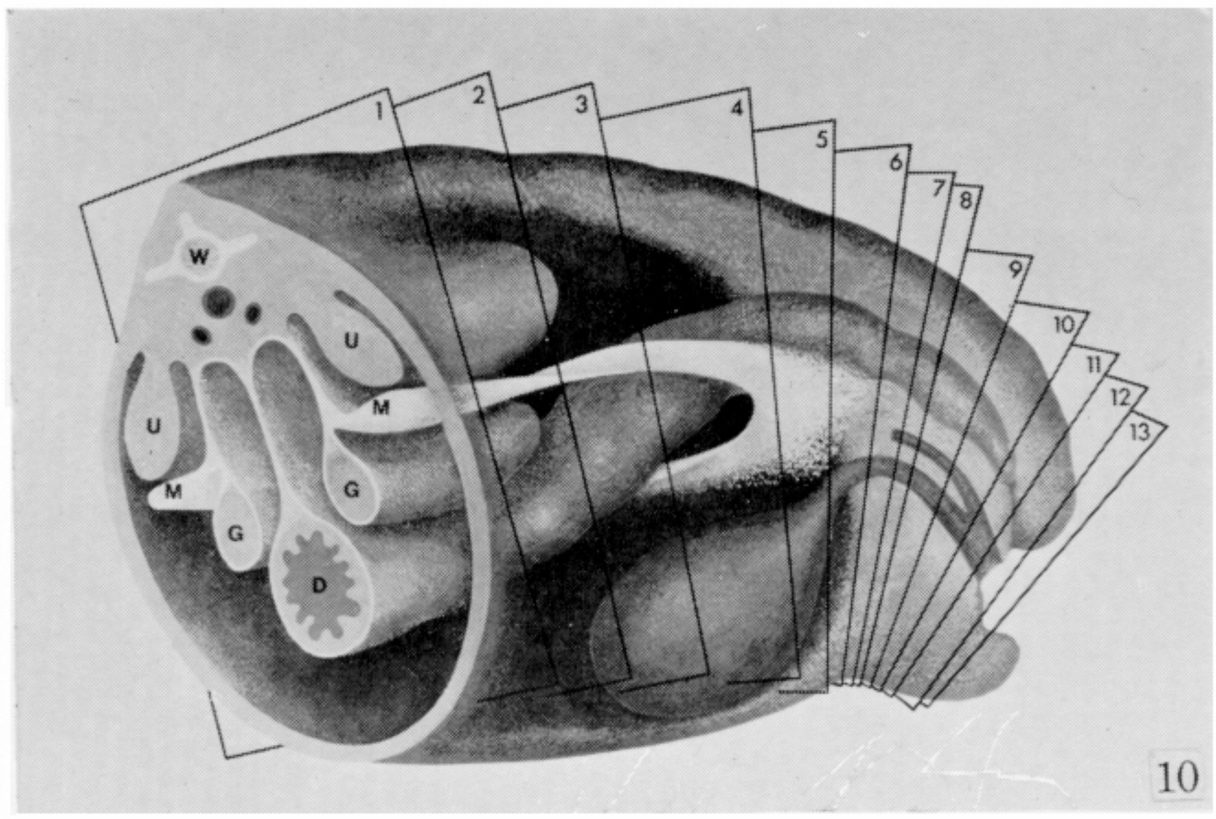


FIGURE 9. Seminal vesicle of: (*a*) a normal adult male rat and (*b*) a feminized adult male rat (mother treated from day 17 to 20 of pregnancy with 30 mg cyproterone acetate/animal daily i.m.). Note the atrophy of the glandular tissue. (Magn. × 56.)



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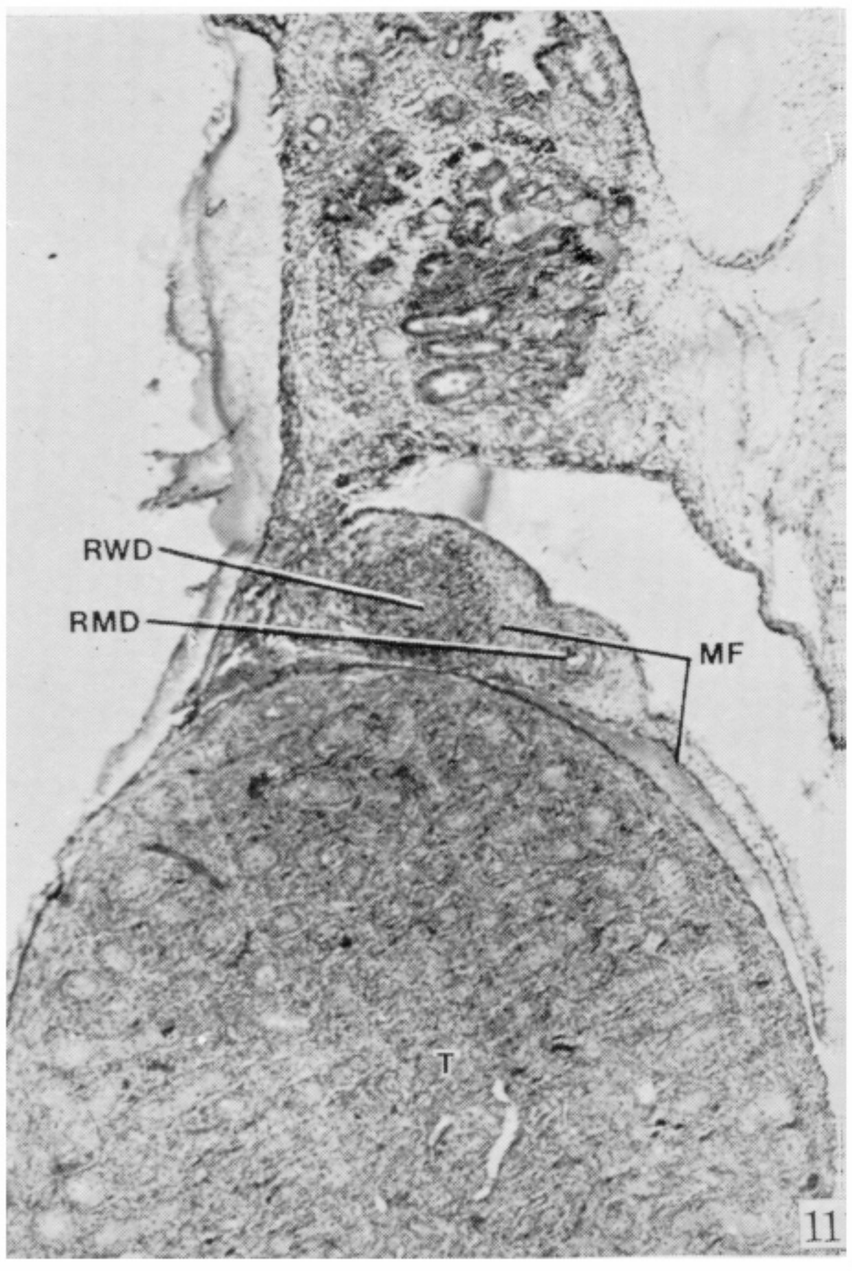

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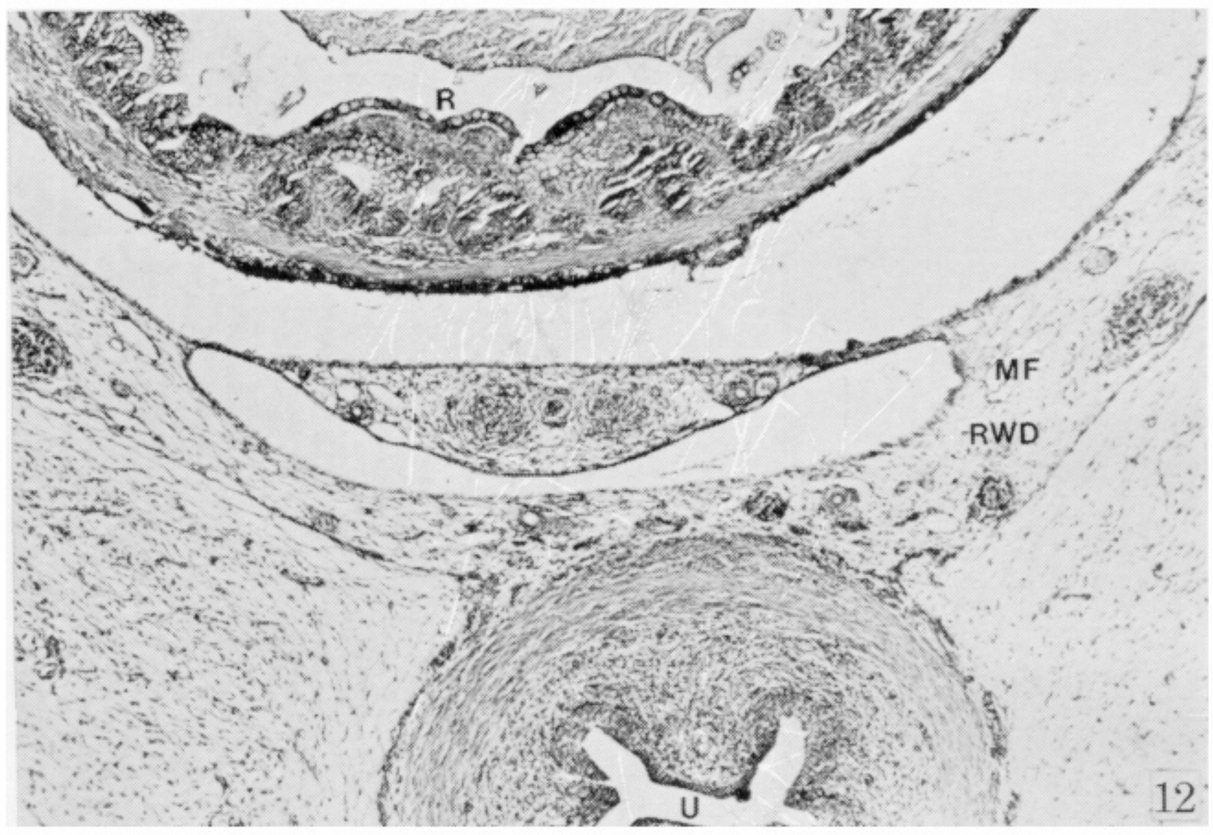

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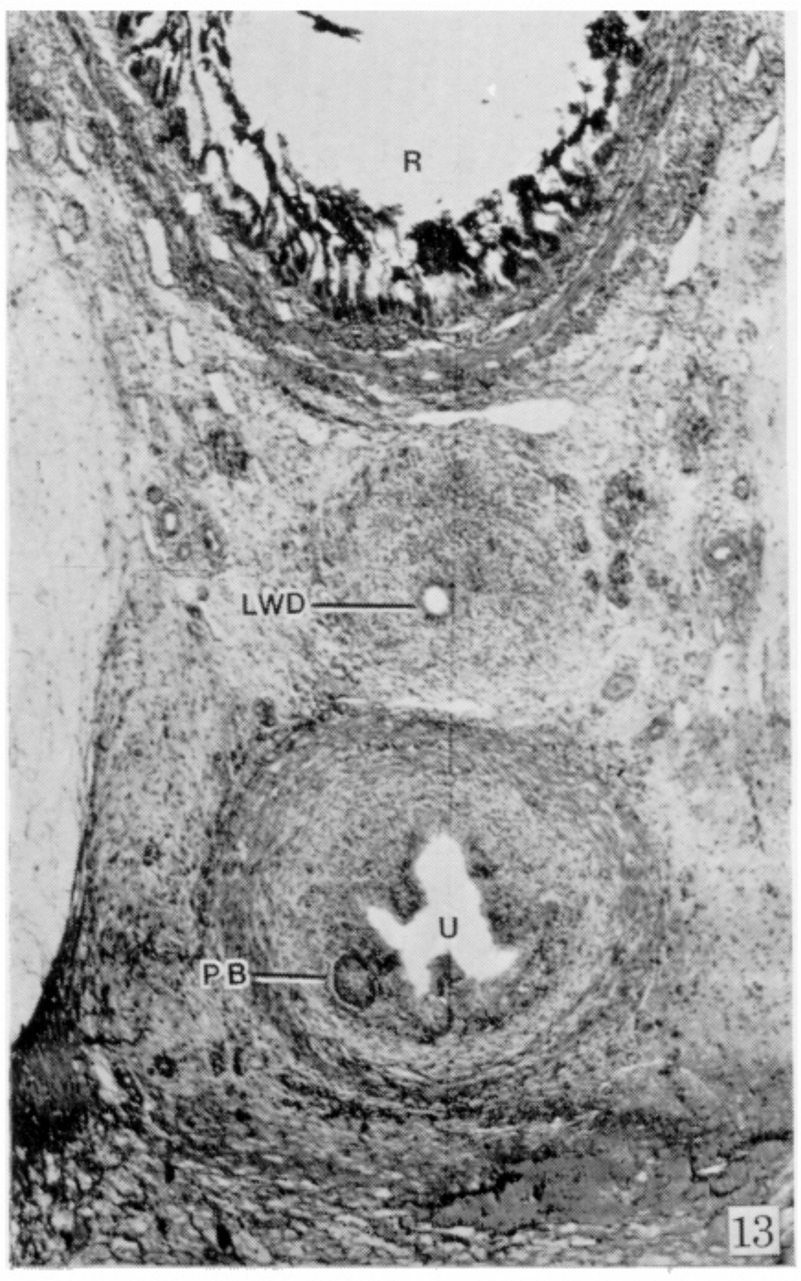

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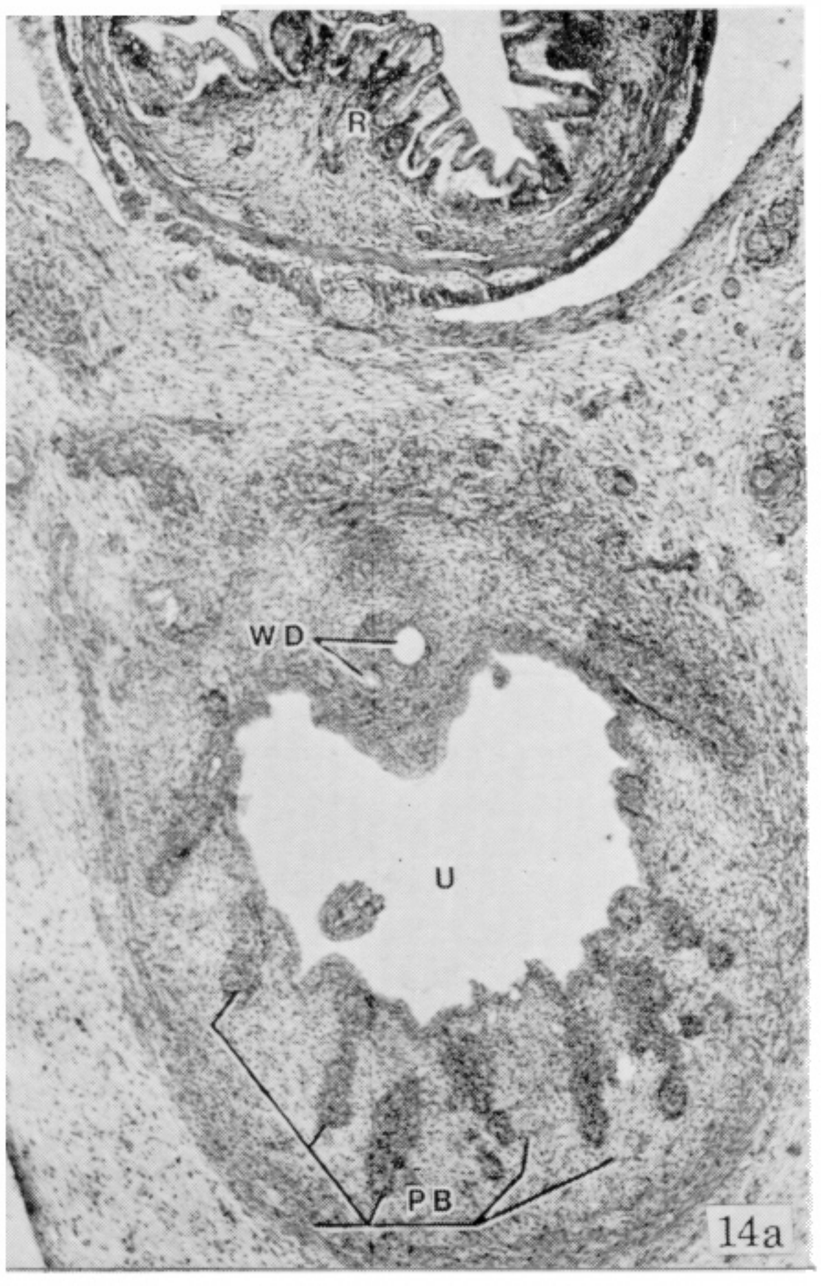

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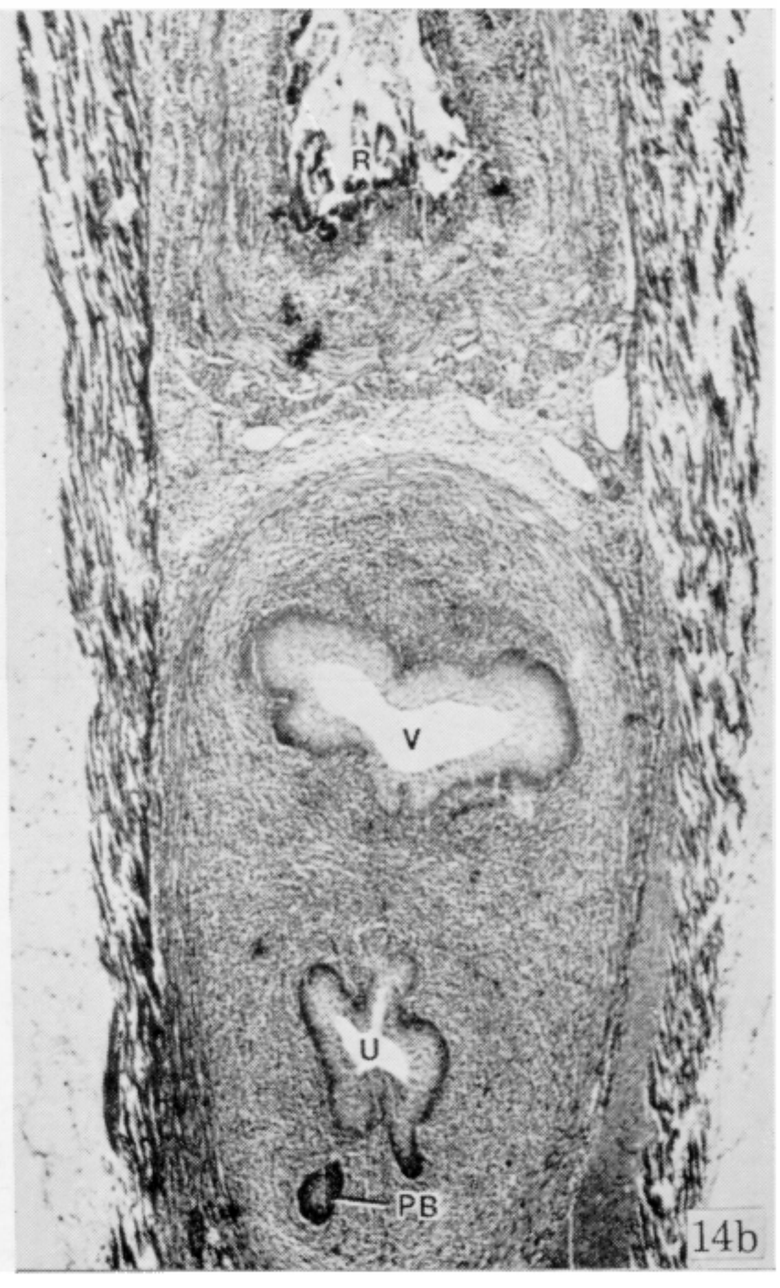
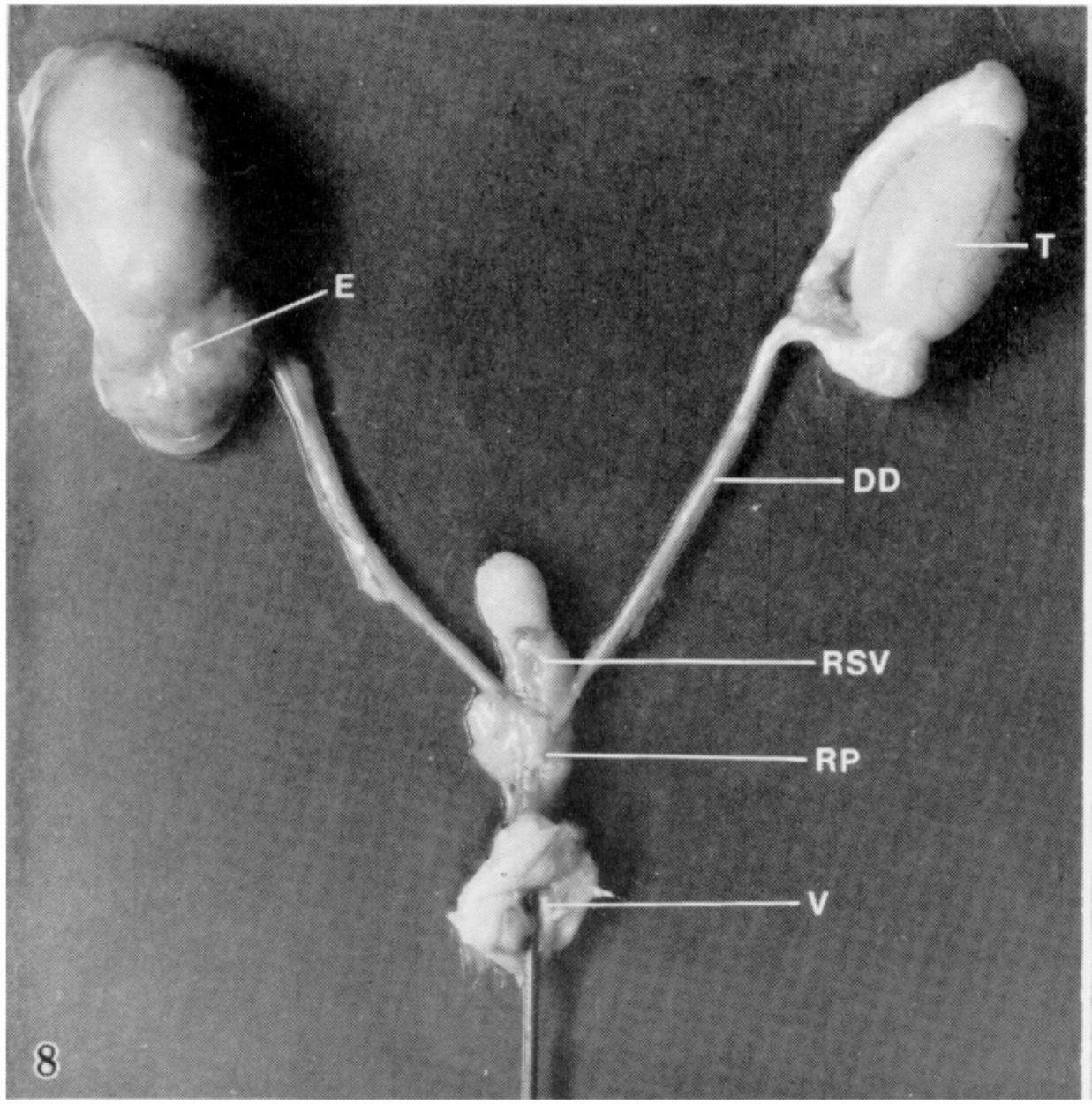


14b

FIGURE 10. Semischematical reconstruction of a feminized male dog foetus (44th day of embryonal development, mother treated from day 23 to 43 of pregnancy with 10 mg/kg cyproterone acetate daily i.m.). Note the absence of the internal genital duct system.

FIGURES 11 TO 14*a*, *b*. Cross-sections of dog oetuses, 44th day of embryonal development. 11 to 13 and 14*b*, feminized males; mothers treated from day 23 to 43 of pregnancy with 10 mg/kg cyproterone acetate daily i.m. 14*a*, normal male. (All magn. × 42.) 11, Gonadal level. Note the normal developed testis (T) and the retrogressed Wolffian ducts (RWD). 12, Cross-section at a lower level than in figure 13. Note the rudiments of the Wolffian ducts (RWD) in the mesogenital fold (MF). 13, Retrogression of the right Wolffian duct, the left duct (LWD) is present. 14, Section at the prostatic level. Note the prostatic buds in the normal male (figure 16*a*). In the feminized male only one prostatic bud can be seen. Note the anlage of a vagina. LWD, left Wolffian duct; MF, mesogenital fold; PB, prostatic buds; R, rectum; RMD, rudiments of the Müllerian ducts; RWD, rudiments of the Wolffian ducts; T, testis; U, urethra; V, vagina; WD, Wolffian ducts.



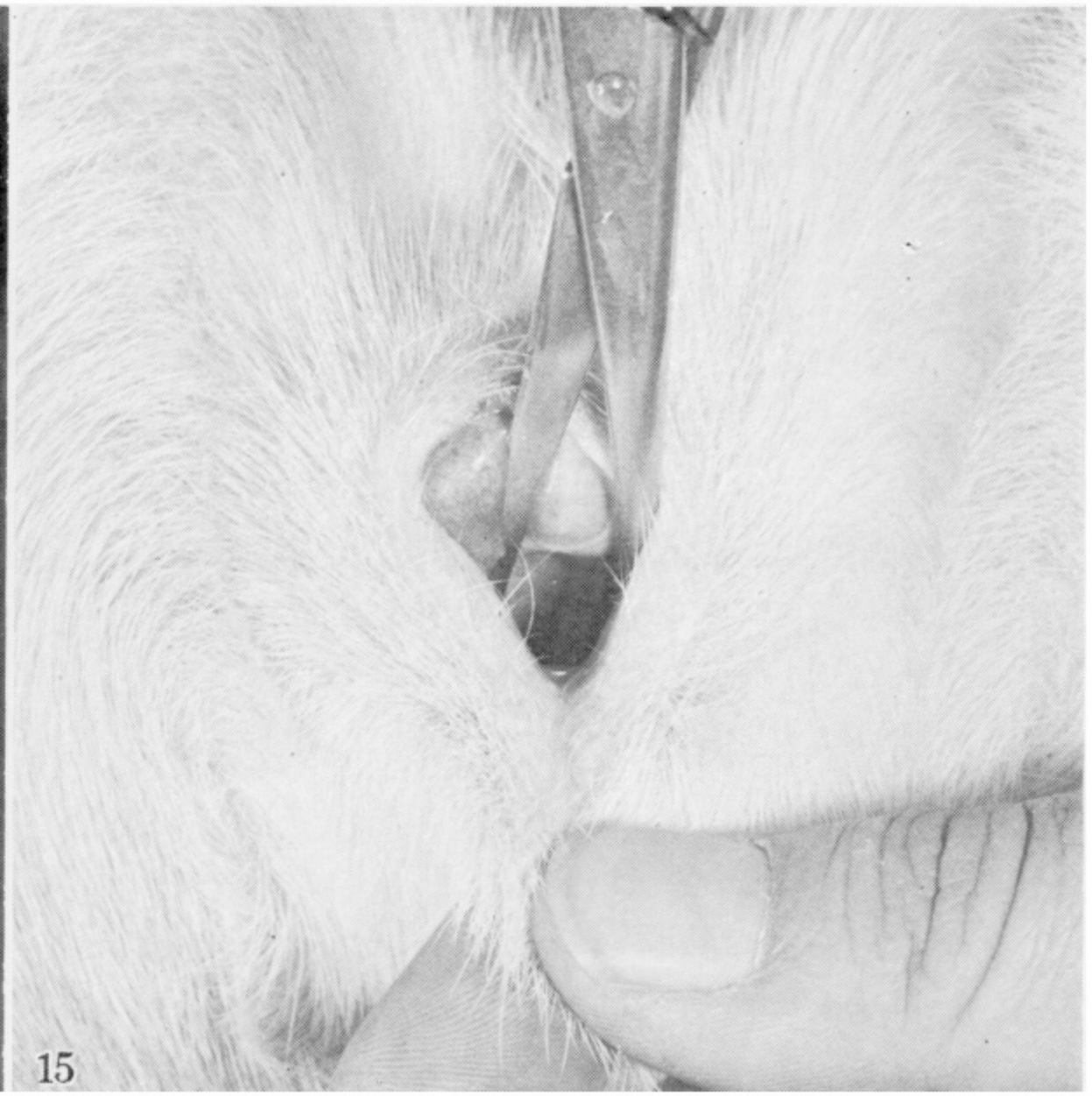
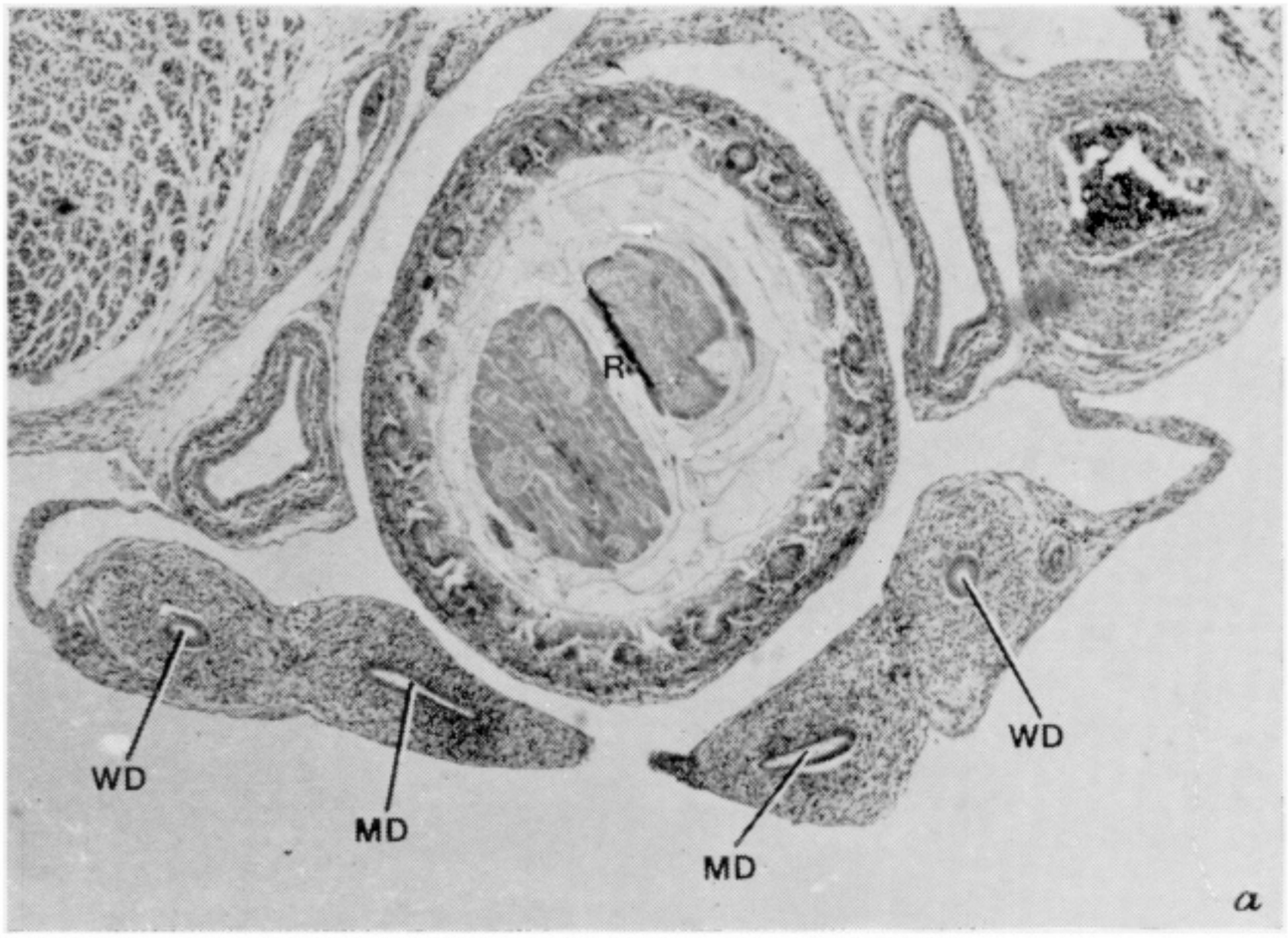
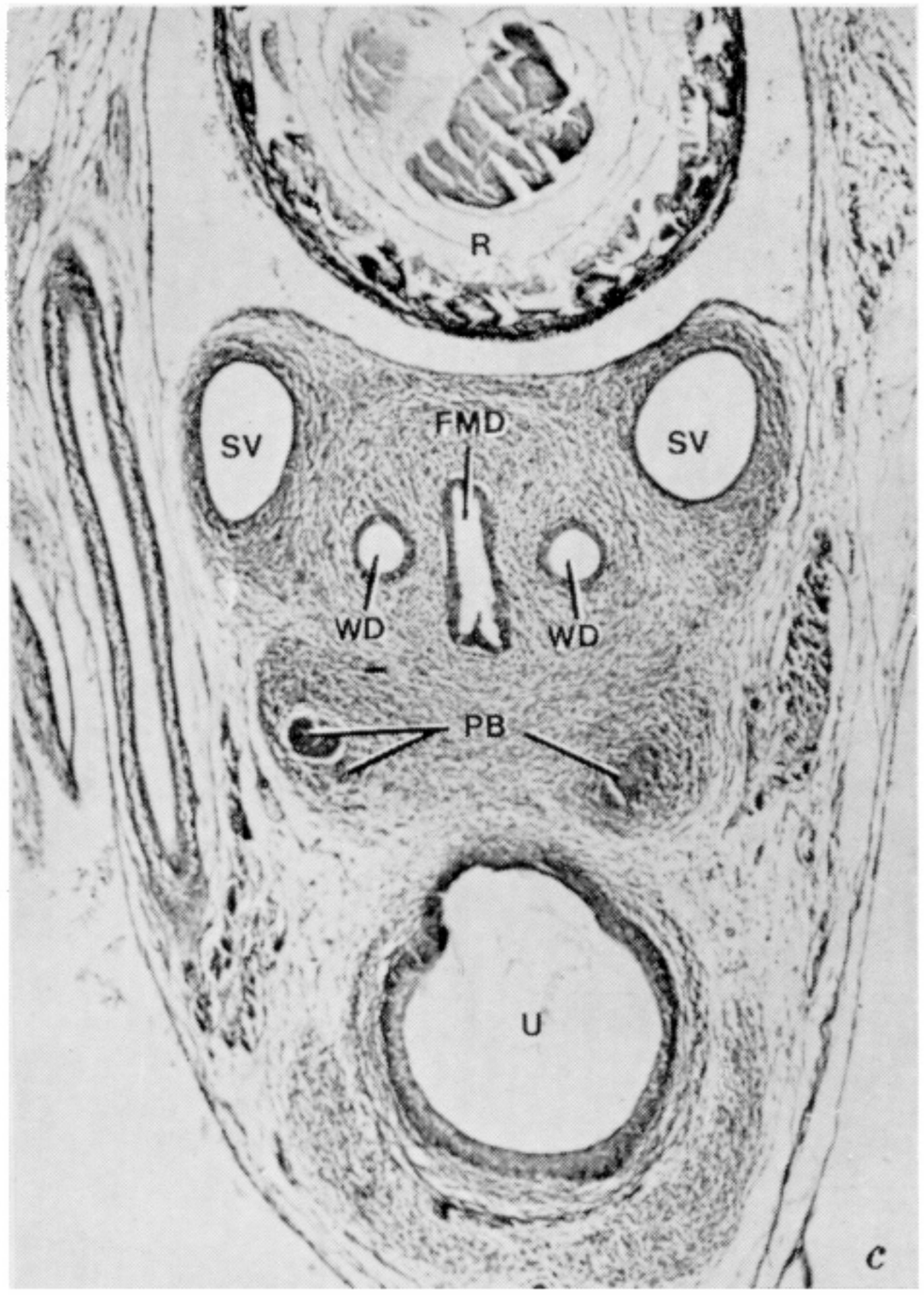
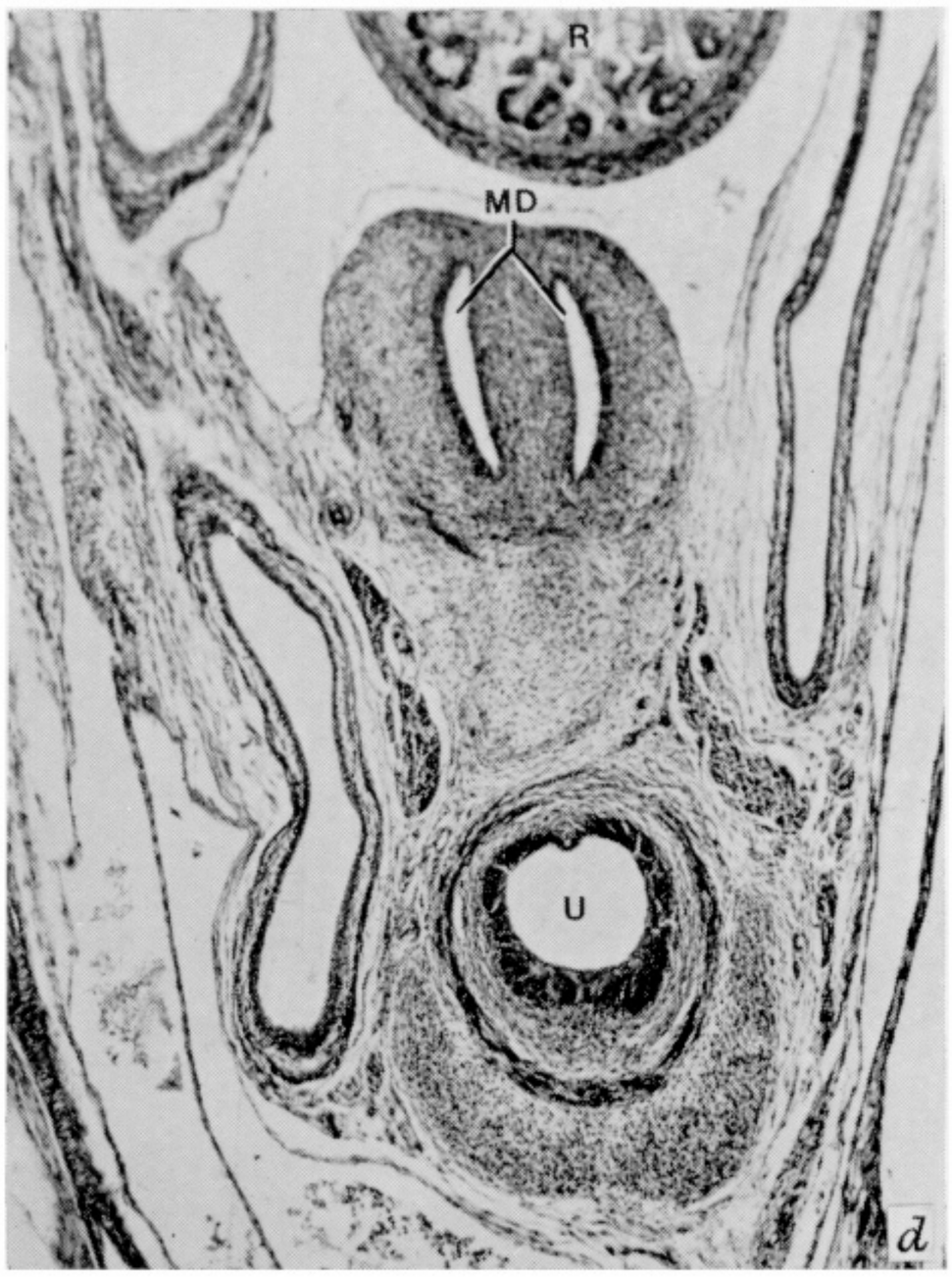


FIGURE 8. Sexual organs of an adult feminized male rat. The mother has been treated from day 13 to 22 of pregnancy with 30 mg cyproterone acetate/animal daily i.m. The newborn males received 1.0 mg cyproterone acetate/animal daily i.m. for the first 3 weeks of life. DD, ductus deferens; E, epididymis (note the dilation of this organ and the testes); RP, rudiments of the prostate; RSV, rudiments of seminal vesicle; T, testis; V, vagina. ($\frac{2}{3}$ actual size.)

FIGURE 15. Adult feminized male dog (mother treated from day 23 of gestation to birth with 10 mg/kg cyproterone acetate daily i.m.). Note the well developed vagina.







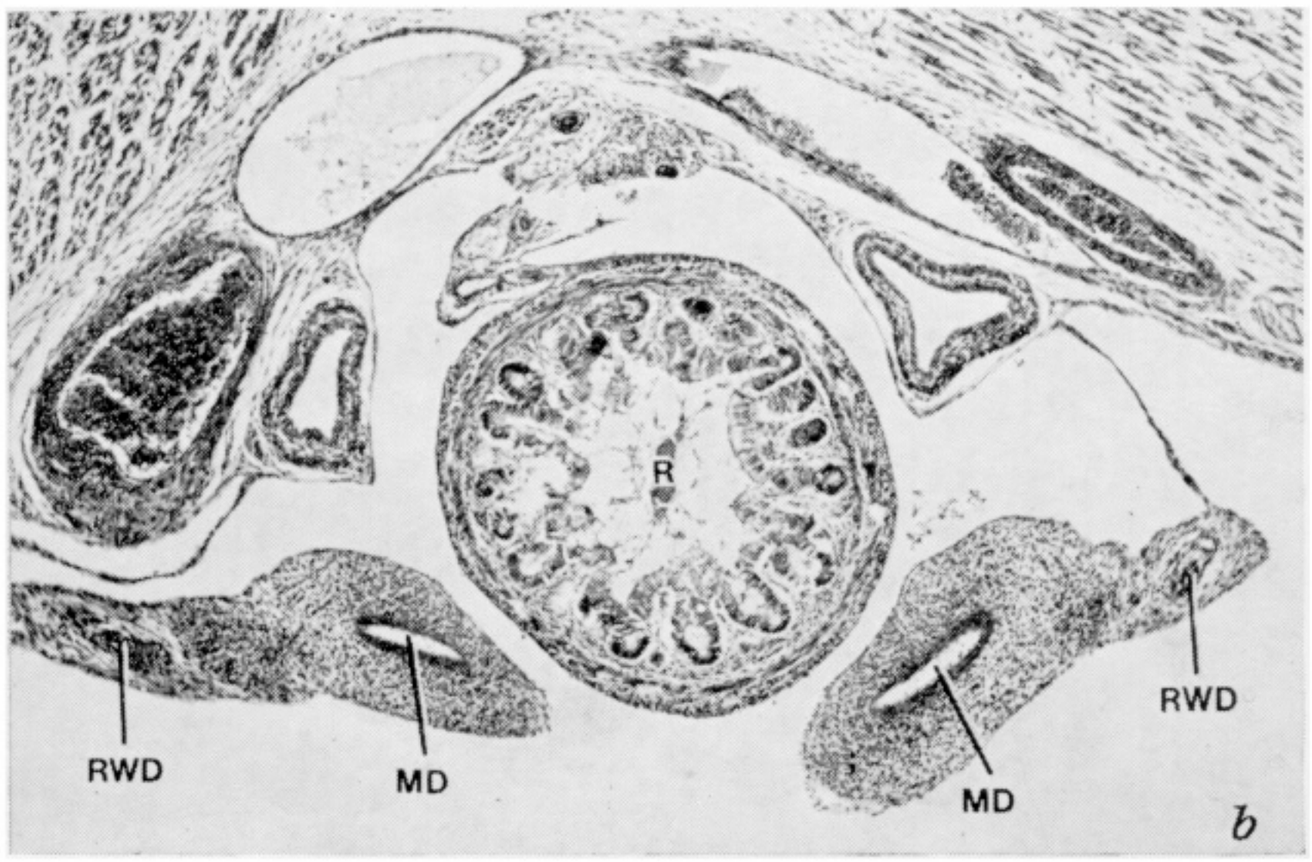


FIGURE 17. Cross-sections of female rat foetuses removed from the uterus at the 22nd day of embryonal development. (Magn. × 42.) (*a*) and (*c*), mother treated from day 15 to 21 of pregnancy with 1.0 mg methyltestosterone/animal daily i.m.; (*b*) and (*d*), mother treated from day 15 to 21 of pregnancy with 1.0 mg methyltestosterone and 30 mg cyproterone acetate/animal daily i.m. FMD, fused Müllerian ducts; MD, Müllerian duct; PB, prostatic buds; R, rectum; RWD, rudiments of the Wolffian duct; SV, seminal vesicle; U, urethra; WD, Wolffian duct.