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Development of the Urogenital Tract in Male Offspring of Rats Injected during Pregnancy with a Substance with Antiandrogenic Properties (Cyproterone)

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Summary. Detailed examinations were performed of the urogenital tract of newborn males obtained from rats injected daily with 1,2α-methylen-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dion acetate (Cyproterone acetate) from the 14th or 15th day of pregnancy until term. The substance was dissolved in propylene glycol or in sesame oil containing 3.5 per cent benzyl benzoate. A dose of 10 mg Cyproterone acetate was given once, twice or three times per day.

The observations were as follows: The prostatic glands were strongly inhibited, a vaginal anlage was formed from the male urethra and the prostatic utricle. Effects were neither observed in the seminal vesicles nor in the deferent ducts (except in one case). The müllerian ducts degenerated as in normal males.

Possible mechanisms of action of Cyproterone acetate are discussed.

Evidence obtained chiefly from work on castrated mammalian fetuses and organ cultures has shown that the growth and differentiation of male accessory sex organs is stimulated by agents derived from the fetal testis. Apart from stimulating the development of the organs mentioned the fetal testis inhibits the growth of the müllerian ducts. Though the stimulating effects indicate androgenic actions, it is not clear, whether the nature of the hormones produced by the testis is the same in fetal and in adult life (JOST, 1961; PRICE and ORTIZ, 1965).

In a number of investigations NEUMANN and coworkers recently observed that Cyproterone (1,2α-methylene-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dion) and its acetate antagonized effects exerted by testosterone or endogenously produced androgens on a variety of target organs of more or less mature rats (NEUMANN, 1966; NEUMANN and ELGER, 1965; NEUMANN and KRAMER, 1964). The authors also found that rats given Cyproterone during pregnancy delivered males showing abnormalities of accessory and external sex organs, *e.g.* absence of prostatic glands and presence of a vaginal anlage. Uterine horns did not develop. A vagina with an external orifice occurred when the newborn males were treated with Cyproterone even for some time after birth.

The observations made with Cyproterone by NEUMANN and coworkers on male fetal rats appear to be similar to those of previous workers who studied effects of estrogens given to pregnant rats (GREENE *et al.*, 1938, 1939, 1940; BENGMARK, 1958). Furtheron, the masculinizing effect of testosterone on female rat fetuses was counteracted by Cyproterone, but the effects of Cyproterone on fetal males could not be prevented by testosterone (NEUMANN and KRAMER, 1964).

Much is known about the origin and hormone dependency of the organs constituting the urogenital tract in fetal and newborn rats (JOST, 1961; PRICE and ORTIZ, 1965). The effects of Cyproterone on the differentiation and growth of this part of the body have not been studied in detail in rats. However, information of these points might provide further insight into the nature of the actions of Cyproterone, which, in view of the observations by *e.g.* GREENE et al., BENGMARK, and NEUMANN and KRAMER, remains conjectural.

The present report is concerned with a detailed study of the influence of Cyproterone acetate given to pregnant rats on the development of the urogenital tract in their male offspring.

Material and Methods

Sprague-Dawley rats from a closed colony kept at the Institute of Anatomy were used. The diet, which consisted of pellets (Ewos) and tap water, was freely available. Females in prooestrus were caged with males during one night. On the following morning vaginal smears were taken. The presence of sperms was regarded to indicate pregnancy and the day after mating was considered as day 1 of pregnancy. The occurrence of a pregnancy was ascertained by palpation on the 14th or 15th day after mating. Injections were given daily for 7 or 8 days, that is, from day 14 or 15 to day 21. On the 22nd day after mating the young were delivered by cesarean section[1]. The young whose postnatal development should be studied were nursed by foster rats. The remaining ones were fixed in Bouin's fluid after removal of the upper part of the body.

Injections. The preparation used was 1,2α-methylen-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dion acetate, Cyproterone acetate (CA). The solvents were either a mixture of 1) ethyloleate and benzyl alcohol (4:1), 2) sesame oil and benzyl benzoate (10:0.35) or 3) propylene glycol. Before use the sesame oil—benzyl benzoate solutions ('S', see Table) were heated to 140°C for about 10 min, that is until all precipitates had disappeared. Before injection the solutions were cooled to about 35°C. At this temperature the solutions remained clear for several

Table. *Solutions of Cyproterone acetate and numbers and routes of injections given to pregnant rats*

Exp.	No. of rats		Solution[a]	No. of daily injections		CA given per day mg	Start of injections. Day of pregnancy
	pregnant	male young		i.m.	s.c.		
1	5	8	EB	—	1	10	15
2	2	7	S:1	—	1	10	14
3	2	4	P:1	1	—	10	15
4	4	20	P:1	2	—	20	15
5	4	12	P:1	3	—	30	14
6	3	9	P:1	2	—	30	14
			S:1	—	1		
7	3	6	P:2	2	—	30	15[b]
			S:2	—	1		

[a] EB = 50 mg CA/ml ethyl oleate—benzyl alcohol; 0.2 ml. S:1 = 25 mg CA/ml sesame oil—benzyl benzoate; 0.4 ml. S:2 = 50 mg CA/ml sesame oil—benzyl benzoate; 0.2 ml. P:1 = 25 mg CA/ml propylene glycol; 0.2 ml into each hind limb = 0.4 ml. P:2 = 50 mg CA/ml propylene glycol; 0.1 ml into each hind limb = 0.2 ml.

[b] Start in one of the rats on day 14.

[1] Spontaneous deliveries were prevented by the gestational effect of Cyproterone acetate (NEUMANN, 1966).

hours. Fresh solutions were made at least once a week. For the solutions 'P' (Table) a suspension of 50 mg CA/ml propylene glycol was prepared. At 60°C the suspension turned into a clear solution after about 20 min. After cooling, the solution containing 50 mg CA/ml proved unstable. A precipitation occurred less rapidly when the suspension was diluted with propylene glycol before heating, so that the final concentration was 25 mg/ml. The amount of CA administered in one subcutaneous injection was 10 mg. The dose given intramuscularly was divided among both hind limbs (see Table). Further details concerning the treatment of the different groups of rats are given in the Table. Though discussed below, it should be mentioned here that 1) ethyl oleate-benzyl alcohol (EB) damaged the tissues and produced general adverse effects, 2) much of the rather large volume of the oily solution (S) failed to be absorbed and 3) more or less considerable amounts of CA given in propylene glycol (P) remained as precipitates at the injection sites. The solvents 'S' and 'P' were tolerated well by the adult rats, but a constant and quantitative resorption of the CA could not be achieved. Sesame oil—benzyl benzoate, but not the other solvents, proved harmless to the newborn young. Injections of 0.04 ml 'S:1' (Table) were given daily subcutaneously to the young. Newborn controls were obtained from rats treated with the solvent during pregnancy. Controls with sesame oil—benzyl benzoate were not studied. A great number of normal specimens were available.

At autopsy the injection sites were exposed and examined macroscopically.

After fixation in Bouin's fluid, the external and internal genital organs, together with the rectum, were dissected out, dehydrated in alcohol, and embedded in paraffin. The preparations were serially cut transversely in 5-micron sections. The sections were stained in haematoxylin and eosin and mounted in DPX.

Results

Before the observations made on males born by rats subjected to different treatments are dealt with, the normal conditions shall be recalled. These were present in the *controls*.

Newborn Males Obtained from Untreated Rats or Controls Injected with Solvents only

The prostatic glands are well developed at birth. They are divided in a dorsolateral and a ventral group. The seminal vesicles are straight tubules. Just medial to the openings of the ejaculatory ducts into the urethra, the prostatic utricle has a paired contact with the dorsal urethral wall (Fig. 1). The prostatic utricle is a small structure seen only in a few sections between the ejaculatory ducts. It contains a lumen but there is no luminal connection between the utricle and the urethra.

Experimental Rats

From the table it may be seen that the schedule of injections varied and that CA was given in increasing amounts. Rats subjected to the same treatment were combined into a group, here called "Experiment". The procedure was adopted after it had been found that the grouping according to effects, which was made independently by J.-G. F., showed, except for the highest dose of CA, a close correlation to the techniques of injections. The observations will now be described with reference to the experiments indicated in the table.

Exp. 1. Large infiltrations occurred at the injection sites and the general condition of the pregnant rats deteriorated during the treatment. Amongst other things, a loss of body weight and a majority of dead fetuses were recorded. The urogenital tract of the males available at term was no different from that of controls.

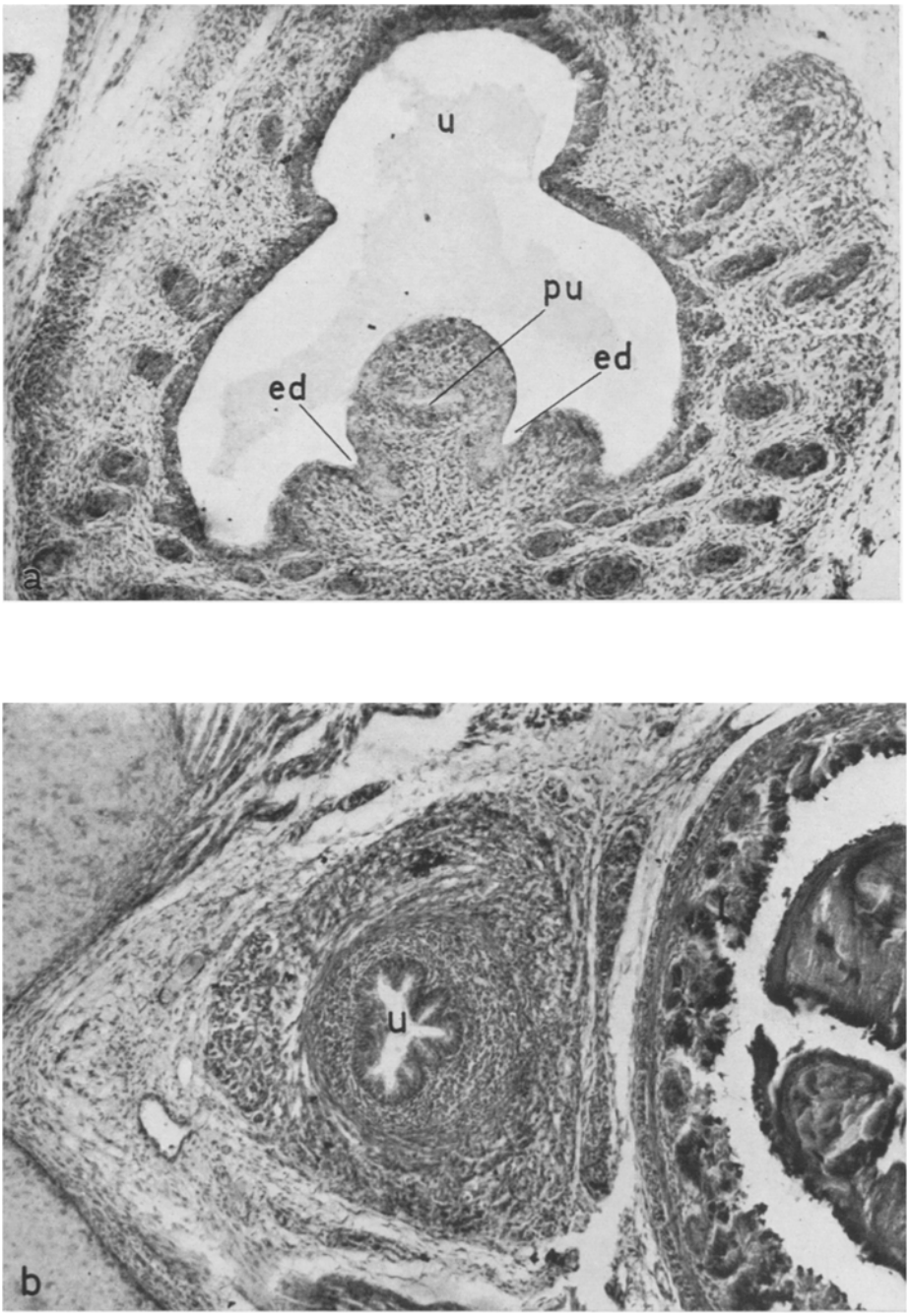

Fig. 1. a Microphotograph of a crossection through the male urethra of an untreated newborn male. Several well developed prostatic glands are seen. b A crossection through the posterior part of the male urethra in an untreated, newborn male. *pu* prostatic utricle; *ed* ejaculatory ducts; *u* urethra. Magnification: a and b: 90×

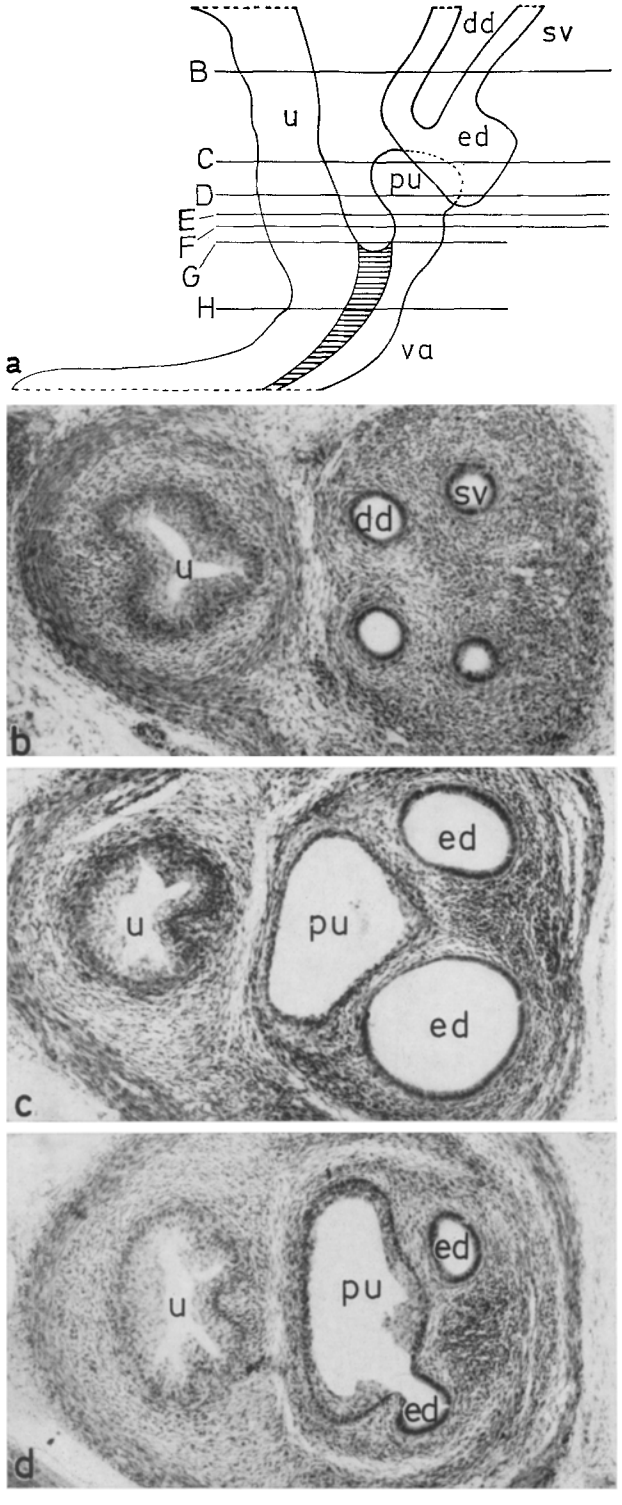

Fig. 2a—d

Fig. 2a—h. Microphotographs of crossections from the genital apparatus in a male obtained from experiment 4. The levels of the crossections are indicated in 'a' which is a graphic reconstruction of the genital region under discussion. Remaining prostatic glands are not shown. The lined area in 'a' shows the furrows on the lateral walls of the urethra. b The anlagen of the seminal vesicles and the deferent ducts have a normal appearance at this level. c The

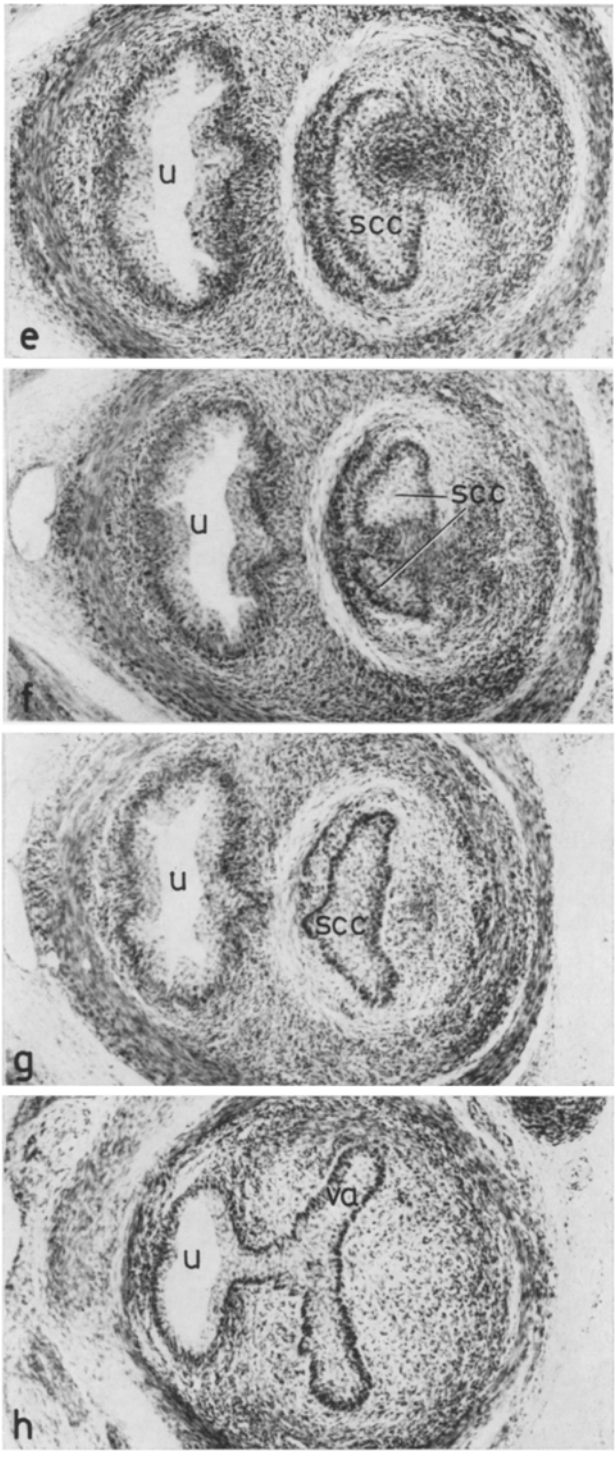

Fig. 2 e—h

prostatic utricle as well as the ejaculatory ducts are dilated. d The ejaculatory ducts open into the prostatic utricle. e f g Posteriorly to the lumen in the prostatic utricle, the "vaginal anlage" is solid (e) and in some sections paired (f). The region of the "vaginal anlage" next to the attachment to the urethra is solid and unpaired (g). h On the lateral walls of the urethra are strongly pronounced furrows indicating the later division into a ventral urethral part and a dorsal vaginal part. *dd* deferent ducts, *ed* ejaculatory ducts, *pu* prostatic utricle, *scc* solid cell cord, *sv* seminal vesicle, *u* urethra, *va* vaginal anlage. Magnification: 90×

Exp. 2. Macroscopically clear oil was found under the skin. At term a total of 25 living and one dead young were obtained from the two rats. The findings on the newborn males were as follows. The male urethra is affected in some cases. Furrows of the type seen at birth on the lateral walls of the female urogenital sinus are present on the lateral walls of the male urethra. (These furrows indicate the division of the urogenital sinus into a ventral urethral part and a dorsal vaginal part.) The contact region between the ejaculatory ducts and the urethra has undergone profound changes. The ducts do not open into the urethra but into the strongly enlarged and dilated prostatic utricle which is paired in the vicinity of the urethra. The ejaculatory ducts open into this paired region. However, there is no connection between the lumen in the utricle and that of the urethra. The alteration is important. It resulted in a modification of the relations between the ejaculatory ducts and the urethra apparently involving a pull into the dorsal direction on the contact region between the structures mentioned. The furrows on the lateral walls of the urethra begin at the attachment of the modified utricle to the urethra and then continue posteriorly. The number and size of the prostatic glands is decreased: the ventral glands are not seen but there are still remnants of the dorso-lateral group. It should be emphasized that some of the males of this group do not show any of the changes described. In other ones, the modifications are small. The conditions described above refer to the animals with the most pronounced changes.

Exp. 3. As judged from the condition of controls as well as of the females from which 13 living young were obtained, the propylene glycol was well tolerated. The dose of CA given was the same as in the preceding experiments, but the effect appeared stronger. All males examined presented the same modifications as those described under Exp. 2 for the minor proportion of rats reacting most extensively. — The young which were nursed by a foster rat and injected with CA in propylene glycol did not survive.

Exp. 4. The greater volume of propylene glycol did not cause any noticeable disabilities. The observations made on the males showed an inhibition of the prostatic glands, but there were still well developed remnants of the dorso-lateral group.

The posterior part of the seminal vesicles, the ejaculatory ducts, and the prostatic utricle are often strongly dilated (Fig. 2). The position of the utricle in relation to the urethra is now still more modified than described above. The utricle is no longer in contact with the urethra and the communication between these two structures is maintained by a solid cell cord containing cells of the same type as those in the urethra. This cell cord seems to have arisen from a frontal division of the anterior part of the urethra into a ventral urethral part and a dorsal "vaginal" part by a deepening of the furrows on its lateral walls. The furrows are also well developed along the whole urethra posterior to the attachment of the solid cell cord. The urethral part ventral to the furrows contains a lumen, the dorsal part is solid. In some cases the ejaculatory ducts open into the prostatic utricle, in others the ducts gain contact with the utricle but there is no luminal connection.

Exp. 5. The injection sites in the hind limbs showed rather much tissue reaction, sometimes haemorrhages and always whitish plaques of precipitated

CA. The young obtained from the four rats were living and apparently in good condition. It was not possible to distinguish males from females by macroscopic inspection. The results of microscopic examinations are the same as in the foregoing group (Exp. 4) except that in most males the prostatic glands are reduced to small buds. Sometimes a few more developed glands are seen.

Exp. 6 and *7*. In order to reduce the amount of propylene glycol, and with this the traumatization of the hind limbs, one third of the daily dose of CA was given subcutaneously in oil (Table). The findings at the injection sites were the

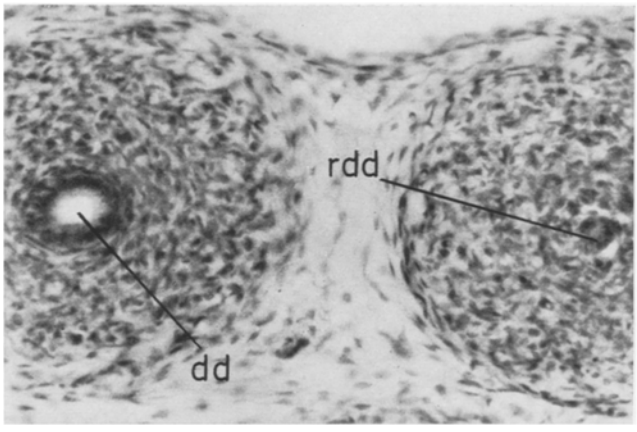

Fig. 3. One of the deferent ducts has degenerated partly in a male belonging to Experiment 6. A remnant of the duct is seen as a small epithelial nest. *dd* deferent duct, *rdd* remnant deferent duct. Magnification: 220×

same as those described above. The microscopic studies on males obtained from the rats of the present groups did not reveal any significant deviations from the results described for Experiments 4 and 5.

It should be mentioned here, that the development of the seminal vesicles apparently proceeded undisturbed in all experiments. None of the males of the present material showed seminal vesicles which were different from those of the controls. The posterior part of the seminal vesicles as well as the ejaculatory ducts are often strongly dilated in males exposed to the higher dose of CA. The deferent ducts are affected in only one animal belonging to experiment 6. Here, a greater part of one of the ducts has degenerated and is represented by scattered epithelial remnants only, the other duct appears as in controls (Fig. 3).

Males Obtained from Exp. 5 and Given 1.0 mg CA Daily Postnatally

The males were injected daily subcutaneously with 0.04 ml of the solution 'S:1' (Table) from 1 or 2 days to 11 or 12 days of age. As indicated by their appearance and body weight, which was approximately the same as that of untreated littermates, the injections were tolerated well. At autopsy oil was found under the skin. The external genital organs were, at 11 or 12 days of age, indistinguishable from those of normal females of the same age. The prostatic

glands are represented by some small buds only. The urethra appears divided down to the perineum into a ventral part, the urethra proper, and a dorsal vaginal part. The latter is to a large extent solid but it contains a lumen in its anterior part. Compared with controls the seminal vesicles and the deferent ducts do not show any significant deviations. The ejaculatory ducts open into the vaginal anlage. Changes occurring in older males of this kind will be reported on later.

Discussion

The observations made on the material presented confirm and extend those of Neumann *et al.* (*e.g.* Neumann and Elger, 1965). The doses of CA administered in the present work are higher than those used by Neumann, who dissolved the substance in a mixture of benzyl benzoate—castor oil (1:5) when concentrations of 50 mg per ml were required. Unpublished own experiments on adult male rats showed that this as well as other solvents of the same kind caused considerable damage to the tissues at the injection sites. The rats lost unduly in body weight. The solvents 'S' and 'P' (Table) proved satisfactory with regard to possible side effects, but, as reported on above, the quantity of CA which was taken up by the body cannot be assessed with any accuracy. Presumably, it was much less than the dose injected. For this reason repeated injections (with intervals of several hours) of the same dose (10 mg) were given in order to study effects of increasing amounts of CA. Considering the points just mentioned it seems reasonable to assume that the doses of CA found to be effective by Neumann and ourselves are, in fact, not very different.

The present findings also confirm the effects of CA observed by Neumann and his group on the prostatic glands and the male urethra. The development of a vagina with an external orifice was also seen in unpublished studies on males derived from the present experiments and injected daily with CA for about 2 weeks after birth.

The transformation of the male urethra into a structure similar to the urogenital sinus in female fetuses will now be considered with regard to the origin of the structures involved. Both the transformed male urethra and the female urogenital sinus are divided into a ventral urethral part and a dorsal vaginal part by furrows penetrating into the lateral walls. In females the dorsal part forms the posterior part of the vagina. Its anterior part is a derivative from the fused müllerian ducts (Forsberg, 1963). In feminized males, the prostatic utricle is added anteriorly to the vaginal part derived from the frontal division of the urethra. According to Bengmark (1958) the prostatic utricle is a müllerian derivate. The vaginal anlage in feminized males then has a dual origin: a small anterior part arising from the müllerian epithelium, and a greater posterior part developing from the epithelium in the urethra.

In view of the well attested obligatory role played by the fetal testis in the development of the male accessory sex organs (*e.g.* Jost, 1961; Price and Ortiz, 1965) and with regard to the antiandrogenic properties of CA reported by Neumann it appeared surprising that the deferent ducts as well as the seminal vesicles escaped any influence of CA. Considering the timing of the injections the situation presents itself as follows. The first injections were given on the 14th or 15th gestational day. The müllerian ducts have grown down to contact the urogenital

sinus at about 16 days. It should be recalled that the ducts begin to degenerate in untreated animals at 17.5 days. The first anlage of the seminal vesicles is seen at 18.5 days and the prostatic glands appear at 19.5 days (BENGMARK, 1958).

The interval between start of injections and the appearance of the anlage of the seminal vesicles should be regarded to have been at least 3 or, mostly, 4 days. The development of the prostatic glands begins about one day later than that of the seminal vesicles. The data given would seem to exclude the possibility that CA did not reach the anlage of the seminal vesicles in time to prevent their differentiation and growth.

Another cause of the failure of the seminal vesicles and deferent ducts to respond to CA in a manner similar to the prostatic glands and the urethra might be a difference in sensitivity of organs derived from wolffian and entodermal epithelium, respectively. This point is being investigated. Recently, ELGER (1967) reported a degeneration of the wolffian ducts, a complete inhibition of the development of the prostatic glands and a feminization of the external genital organs in male rabbit fetuses, whose mothers had been treated with 0.625—100 mg CA/kg body weight/day between day 13—24 of pregnancy.

As mentioned in the introduction the findings of NEUMANN on the male offspring of rats injected with CA during pregnancy recalled results of previous workers (GREENE *et al.*, 1938, 1939, 1940a) who studied effects of estrogens. The results of the present, more detailed analysis of the effects of CA underline the similarities with those obtained with estrogens. Thus BENGMARK (1958) obtained a strong inhibition of the prostatic glands in male fetuses after estradiol injections to the pregnant mother rat. The ventral prostatic glands were most strongly affected. Of the dorsolateral group, remnants were seen. GREENE *et al.* (1938, 1939, 1940a) injected estradiol to pregnant rats and obtained a strong inhibition of the prostatic glands of the fetuses. Higher doses of estradiol resulted in the formation of a vaginal anlage in newborn males, similar to that seen in newborn females. The ejaculatory ducts entered the vaginal anlage in the same way as described in the present investigation. Still higher estradiol doses resulted in an inhibition of the seminal vesicles and the deferent ducts. The estradiol treatment also resulted in a more or less complete maintenance of the müllerian ducts. Similar results were obtained from mice (RAYNAUD, 1950; JEAN, 1965).

Progesterone administered to pregnant rats or rabbits has no influence on the development of the fetal genital tract (JOST, 1947, 1960; REVESZ *et al.*, 1960; SUCHOWSKY *et al.*, 1967). The few cases in which masculinization of human female fetuses have been seen after treatment of the pregnant mothers with progesterone might be explained by an abnormal metabolism of the exogenous progesterone (WILKINS, 1960). However, an androgenic effect of progesterone has been seen in castrated male rats and guinea pigs (LAMAR, 1937; GREENE *et al.*, 1940b; CLAUSEN, 1942; LYSTER *et al.*, 1959; SUCHOWSKY *et al.*, 1967).

It is generally believed that the fetal testis produces two types of hormones, one stimulating the growth and differentiation of the male accessory sex organs and another one which inhibits the growth of the müllerian ducts. It should be recalled now that parts of the male vagina were derived from the müllerian ducts. The major portion of these ducts had disappeared, the remaining portion corresponds to the prostatic utricle in normal males.

From the observations made in the present experiments it is apparent that CA in the amounts circulating in the body did not counteract the activities of the fetal testis entirely. Effects similar to those produced with CA have been reported by others to occur after treatment with estrogens. Since it is well known that different steroids, even those which are produced endogenously, may affect the same effector organ in the same way, it would seem premature to conclude that CA has estrogenic properties, especially as CA has no estrogenic effect in the Allen-Doisy test (cf. Elger, 1967). On the other hand, this possibility cannot be excluded. CA was observed, for instance, to stimulate the growth of the vaginal epithelium in female rabbit fetuses (Elger, 1967). As shown by *e.g.* Junkmann and Neumann (1959) and Suchowsky *et al.* (1967) substances which antagonize or synergize with known hormones usually exert, like the hormones themselves, a variety of actions besides those which are predominant.

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References

Bengmark, S.: The prostatic urethra and prostatic glands. Med. Diss. Lund (1958).

Clausen, H. J.: Effect of progesterone and desoxycorticosterone on accessory sex organs of the castrate male guinea pig. Endocrinology **31**, 187—191 (1942).

Elger, W.: Die Rolle der fetalen Androgene in der Sexualdifferenzierung des Kaninchens und ihre Abgrenzung gegen andere hormonale und somatische Faktoren durch Anwendung eines starken Antiandrogens. Arch. Anat. micr. Morph. exp. **55**, 657—743 (1967).

Forsberg, J.-G.: Derivation and differentiation of the vaginal epithelium. Lund: Thesis 1963.

Greene, R. R., M. W. Burril, and A. C. Ivy: Experimental intersexuality: the production of feminized male rats by antenatal treatment with estrogens. Science **88**, 130—131 (1938).

— — — Experimental intersexuality: the paradoxal effects of estrogens on the sexual development of female rat. Anat. Rec. **74**, 429—438 (1939).

— — — Experimental intersexuality: the effects of estrogens on the antenatal development of the rat. Amer. J. Anat. **67**, 305—345 (1940a).

— —, and D. M. Thomson: Further studies on the androgenicity of progesterone. Endocrinology **27**, 469—472 (1940b).

Jean, C.: Féminisation de la Souris mâle adulte par injection d' œstrogènes à la mère gravide. C. rend. Soc. Biol. **160**, 309—313 (1965).

Jost, A.: Recherches sur la différenciation sexuelle de l'embryon de lapin. (Deuxième partie: Action des androgènes de synthèse sur l'histogenèse génitale). Arch. Anat. micr. Morph. exp. **36**, 242—270 (1946—1947).

— Cited from Wilkins 1960.

— The role of fetal hormones in prenatal development. In: The Harvey lectures, Ser. **55**, 201—226 (1961).

— Gonadal hormones in the sex differentiation of the mammalian fetus. In: Organogenesis, p. 611—628. Ed. De Haan and Ursprung. New York: Holt, Rinehart & Winston 1965.

Junkmann, K., u. F. Neumann: Zum Wirkungsmechanismus von an Feten antimaskulin wirksamen Gestagenen. Acta endocr. **90**, 139—154 (1964).

Lamar, J. K.: Some effects of progestin-containing extracts and progesterone on young male albino rats. Anat. Rec. **70**, Suppl. 45 (1937).

Lyster, S. C., G. H. Lund, W. E. Dubin, and R. O. Stafford: Ability of some progestational steroids to stimulate male accessory glands of reproduction in the rat. Proc. Soc. exp. Biol. (N.Y.) **100**, 540—543 (1959).

Neumann, F.: Methods for evaluating antisexual hormones. In Methods in drug evaluation, p. 548—573. Ed. P. Mantegazza and F. Piccinini. Amsterdam: North-Holland Publ. Co. 1966.

—, and W. Elger: Proof of the activity of androgenic agents on the differentiation of the external genitalia, the mammary gland and the hypothalamo-pituitary system in rats. In: Excerpta Medica Internat. Congr. Ser. No **101**, 168—185 (1965).

—, J. D. Hahn u. M. Kramer: Hemmung von Testosteronabhängigen Differenzierungsvorgängen der männlichen Ratten nach der Geburt. Acta endocr. **54**, 227—240 (1967).

—, and M. Kramer: Antagonism of androgenic and antiandrogenic agents in their action on the rat fetus. Endocrinology **75**, 428—433 (1964).

Price, D., and E. Ortiz: The role of fetal androgen in sex differentiation in mammals. In: Organogenesis, p. 629—652. Ed. De Haan and Ursprung. New York: Holt, Rinehart & Winston 1965.

Revesz, C., C. I. Chappel, and R. Gaudry: Masculinization of female fetuses in the rat by progestational compounds. Endocrinology **66**, 140—143 (1960).

Suchowsky, G. K., E. Turolla, and G. Arcari: Studies of the so-called virilizing effects of steroids in female rat fetuses. Endocrinology **80**, 255—262 (1967).

Wilkins, L.: Masculinization of female fetus due to use of orally given progestins. J. Amer. med. Ass. **172**, 1028—1032 (1960).

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