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Comparative action of cyproterone and cyproterone acetate on pituitary and plasma gonadotropin levels, the male genital tract and spermatogenesis in the growing rat

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Summary. In growing male rats, chronic treatment for 28 days with two anti-androgens, cyproterone and cyproterone acetate, affected the weight of testis and accessory sex organs, and induced modifications in spermatogenesis and gonadotropin functions. Anti-androgenic properties of cyproterone acetate explain the large regression in genital tract weight, and might be related to the increase in the total number of type A spermatogonia/testis. Gestagenic properties of cyproterone acetate which inhibit this anti-androgenic activity on the hypothalamo-pituitary axis, did not induce any modification in the gonadotropin levels, but drastically reduced the yield of germ cells during spermatogenesis. On the other hand, cyproterone which is known to be a pure anti-androgen, inhibited the action of testosterone both at the central and peripheral levels and induced an increase in pituitary and plasma gonadotropin levels, together with a decrease in the weight of accessory organs. Furthermore an increase in testicular weight, in the total number of type A spermatogonia, and also in the total number of Sertoli cells/testis was observed.

Introduction.

The influence of treatment with anti-androgens like cyproterone and cyproterone acetate on accessory glands has been studied by numerous investigators (Neumann *et al.*, 1970), but the action of these drugs on the gonadotropin levels and on the testis has been little investigated in prepubertal male rat (Walsh *et al.*, 1972).

We have previously observed an increase in the plasma LH level and testis weight after cyproterone treatment, while no such variation was observed with cyproterone acetate (Viguier-Martinez and Pelletier, 1972).

The object of the present experiment was to analyse the effects of the two anti-androgens on the intertubular tissue and the seminiferous epithelium and to compare their effects on the accessory glands and the pituitary and plasma levels of gonadotropins.

Materials and methods.

Three groups of 5 male Wistar rats each received daily subcutaneous injections : cyproterone (10 mg/day), cyproterone acetate (2.5 mg/day) (Schering AG, Berlin, West Germany), or solvent alone (0.2 ml arachis oil/benzyl alcohol 9:1 v/v) from 23 to 51 days of age. All rats were slaughtered by decapitation the morning after the last injection. Blood and pituitaries were collected from each animal. Pituitaries were pooled for each group of 5 rats, lyophilizated and analysed for LH and FSH by radioimmunoassay. After centrifugation at 4 °C, blood samples were kept frozen until they were analysed for FSH and LH by radioimmunoassay. The body, testis, ventral prostate and seminal vesicles were weighed. After removal the testes were fixed in Bouin-Hollande solution.

The relative volume of intertubular tissue and seminiferous tubules was determined with a 25-point ocular integrator (Hennig, 1957) on 40 fields for each animal. The total volumes of intertubular tissues and seminiferous tubules were then calculated.

The diameters of the tubules were measured with an ocular micrometer on 20 cross sections of tubules/testis. The total length of seminiferous tubules/testis was calculated from the above data by the formula of Attal and Courot (1963).

The area of the nuclei of Sertoli cells was estimated from the weight of *camera lucida* drawings of twenty nuclei/animal.

The stages of the cycle of the seminiferous epithelium were classified as described by Roosen-Runge and Giesel (1950). Sertoli cells, type A spermatogonia, leptotene primary spermatocytes and round spermatids were counted in 5 μm thick cross sections of 10 tubules at stage 7-8/animal. The corrected numbers of germ cells/tubular cross section were calculated by the formula of Abercrombie (1946) as modified by Ortavant (1958) from germ cell diameter measurements. Total numbers of uncorrected Sertoli cells and of corrected type A spermatogonia were calculated from the total length of seminiferous tubules and the mean number of these cells/5 μm thick tubular cross section. The yield of spermatogonial divisions was calculated from the corrected number of primary spermatocytes at leptotene, divided by the corrected number of type A spermatogonia, per 5 μm thick tubular cross section respectively. The yield of meiosis and beginning of spermiogenesis was calculated from the corrected number of round spermatids divided by the corrected number of primary spermatocytes at leptotene per 5 μm tubular cross section.

The daily production of round spermatids/testis was calculated as described by Ortavant (1958).

Pituitaries and plasma were analysed for FSH content using a specific radioimmunoassay kit for rat FSH (NIAMDD). The results are expressed in terms of standard NIAMD Rat FSH-RP_1, one unit of which is equivalent to 2.1 units NIH-FSH-S_1. Pituitaries and plasma LH content were assayed by radioimmunoassay using the technique previously described by Kerdelhué *et al.* (1969). Preparation, specificity and sensitivity of antisera to ovine LH has been described by Pelletier (1971). The purified hormone for radio-iodination and standard, LH RP_1, was provided by Courte (1970) ; one unit of LH RP_1 is equivalent to 1.10 unit of NIH-LH-S 11.

Results.

All the results are shown in figures 1 to 16, and expressed as mean ± SEM. The percentage of variation data are given only for significant results.

1. *Male genital tract.* Cyproterone treatment increased the testis weight by 35 p. 100 as compared to that of the control animals, while cyproterone acetate had no effect (fig. 1). Ventral prostate and seminal vesicle weights were reduced more by cyproterone acetate treatment (75 p. 100) than by cyproterone (38 p. 100) (fig. 2, 3).

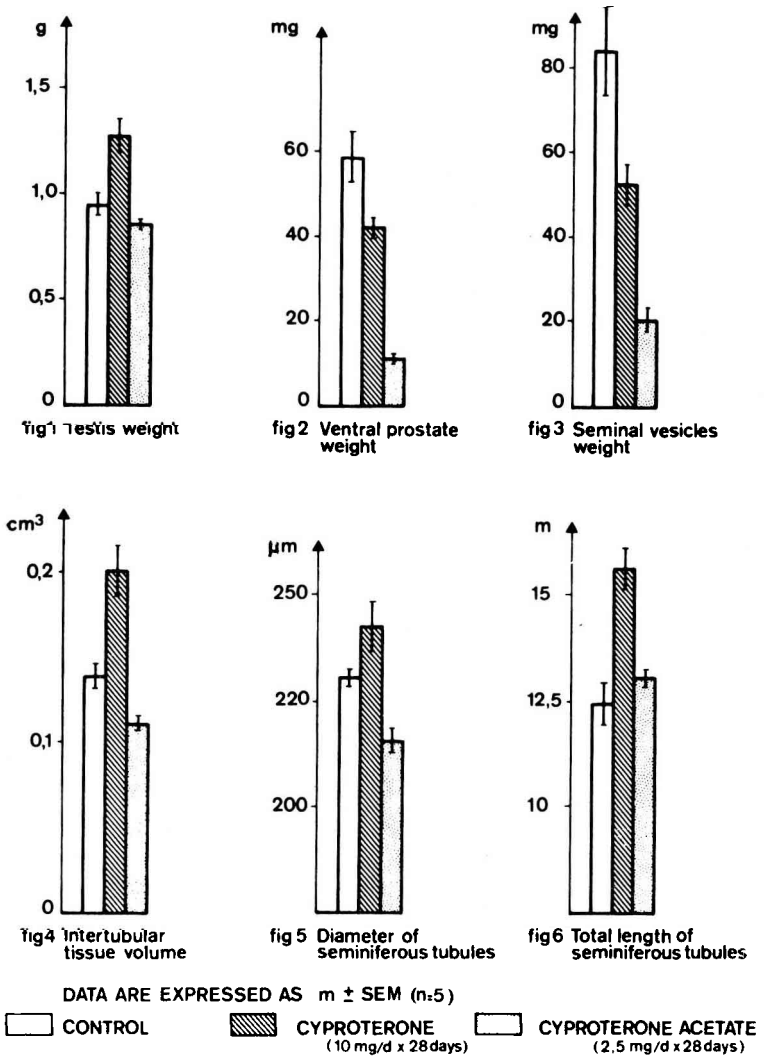

fig 1 Testis weight
fig 2 Ventral prostate weight
fig 3 Seminal vesicles weight
fig 4 Intertubular tissue volume
fig 5 Diameter of seminiferous tubules
fig 6 Total length of seminiferous tubules

FIG. 1 to 6. — *Influence of cyproterone acetate treatment on genital tract of male rat.*

2. *Testis structure.* The intertubular tissue volume, the diameter of seminiferous tubules and the total length of seminiferous tubules/testis increased by 46 p. 100, 5 p. 100 and 26 p. 100 respectively after cyproterone treatment, as compared to the control rats (fig. 4 to 6). Cyproterone acetate treatment did not modify the intertubular tissue volume or the total length of the seminiferous tubules while the seminiferous tubule diameter was significantly reduced by 6 p. 100 (fig. 5).

3. *Seminiferous epithelium.* *a*) Sertoli cells : the total number of Sertoli cells/testis increased significantly (30 p. 100) only after cyproterone treatment. The mean area

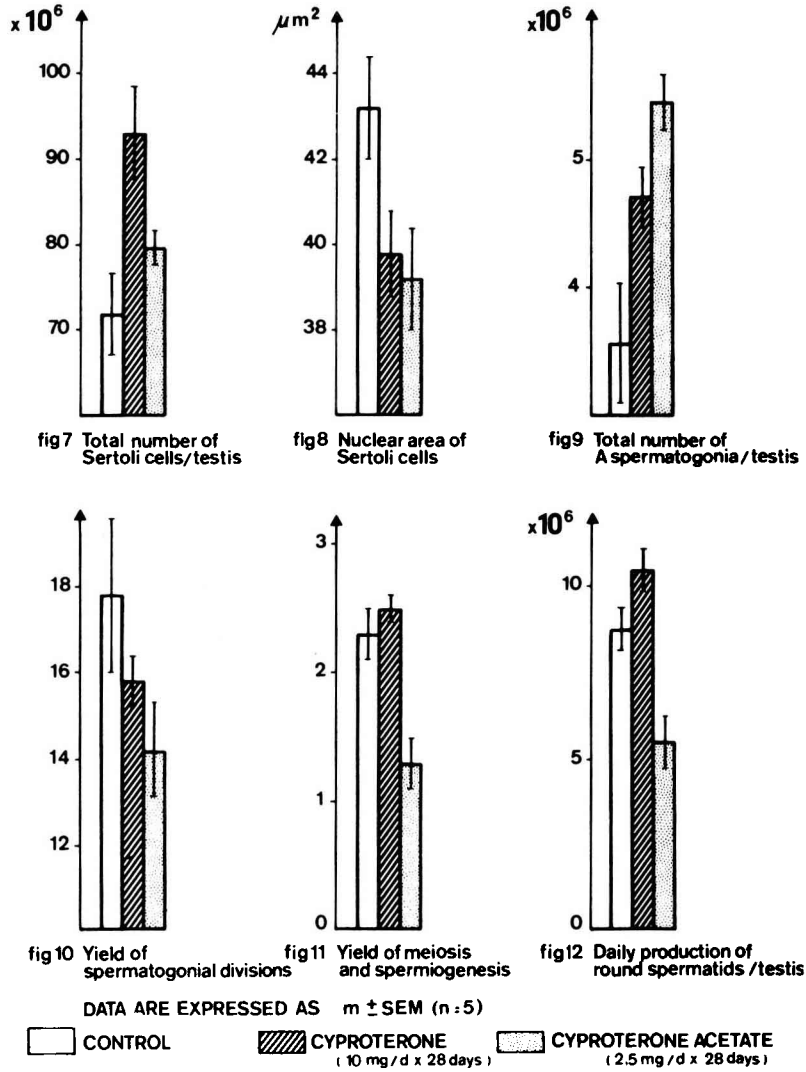

FIG. 7 to 12. — *Influence of cyproterone and cyproterone acetate treatment on spermatogenesis and Sertoli cells of male rats.*

of Sertoli cell nuclei, compared to that of control rats, was reduced by 8 p. 100 after cyproterone, and by 9 p. 100 after cyproterone acetate treatment.

b) Germ cells : both cyproterone and cyproterone acetate treatment increased by 32 p. 100 and 53 p. 100 respectively the total true number of type A spermatogonia/testis as compared to the control rats (fig. 9). The yield of spermatogonial divisions was not significantly reduced after either treatment (fig. 10). Cyproterone did not significantly affect the yield of meiosis and beginning of spermiogenesis, or the daily production of round spermatids/testis (fig. 11, 12), while cyproterone acetate treatment drastically reduced these two indices by 43 p. 100 and 38 p. 100 respectively.

4. *Gonadotropin levels.* Pituitary and plasma levels of LH exhibited a significant increase (178 p. 100 and 120 p. 100) after cyproterone treatment while cyproterone acetate did not modify these values as compared to the control animals (fig. 13, 15).

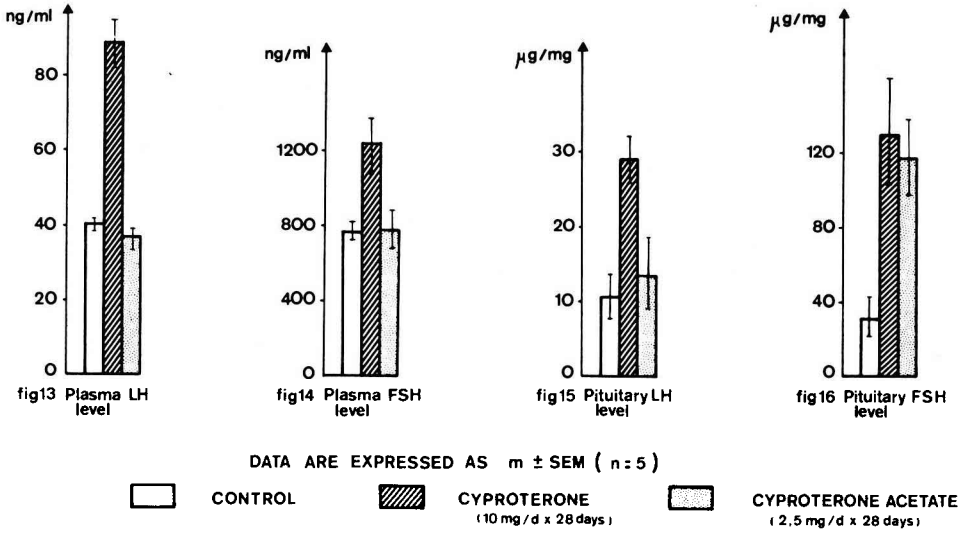

FIG. 13 to 16. — *Influence of cyproterone and cyproterone acetate treatment on plasma and pituitary gonadotropin levels of male rats.*

Pituitary content of FSH was increased after both anti-androgens (314 p. 100 for cyproterone and 274 p. 100 for cyproterone acetate), while plasma FSH levels were significantly increased by 60 p. 100 only after cyproterone treatment (fig. 14, 16).

Discussion.

After cyproterone acetate treatment the testis weight, the intertubular tissue volume, the total length of seminiferous tubules and the total number of Sertoli cells/testis did not differ from that of normal animals. Diameter of seminiferous tubules, nuclear area of Sertoli cells, yield of spermatogonial divisions and of meiosis and daily production of round spermatids were reduced by cyproterone acetate treatment as compared to control animals. Such actions have been previously observed (Neu-

mann and Von Berswordt-Wallrabe, 1966 ; Heinert and Taubert, 1973 ; Flickinger and Loving, 1976). Total numbers of type A spermatogonia/testis were significantly increased.

Plasma LH and FSH levels were not modified by cyproterone acetate treatment, as has previously been shown for LH in the rat (Viguier-Martinez and Pelletier, 1972), and for LH and FSH in man (Brotherton, 1974). Pituitary LH level was unchanged after cyproterone acetate treatment, although pituitary FSH level increased. This could be explained by the gestagenic properties of cyproterone acetate blocking anti-androgenic stimulation of the hypothalamus-pituitary axis thus inhibiting LH synthesis, and release of FSH and LH.

After cyproterone treatment testis weight, intertubular tissue volume, diameter of seminiferous tubules, total length of seminiferous tubules/testis and total number of Sertoli cells were increased resulting from the increase in plasma gonadotropin levels. Some of these variations, i.e. testis weight, intertubular tissue have been previously observed (Mietkiewsky and Lukaszyk, 1969 ; Steinbeck *et al.*, 1971 ; Heinert and Taubert, 1973).

Nuclear area of Sertoli cells was decreased after treatment with both anti-androgens and this could reflect an inhibition of androgen action on Sertoli cells (Dorrington *et al.*, 1975 ; Weddington *et al.*, 1975). Alterations of subcellular structures in Sertoli cells after cyproterone treatment have been reported by Aumüller *et al.* (1975).

The observed increase in the total number of Sertoli cells/testis was not due to a swelling of their nuclei as their nuclear area decreased. Mitoses of supporting cells giving rise to Sertoli cells have been said to stop by the 15th day of life in the growing rat (Steinberger and Steinberger, 1971 ; Nagy, 1972) but some residual divisions were observed *in vitro*, after FSH supplementation between 20 and 30 days of age (Dorrington *et al.*, 1975). In hypophysectomized 28-day old rats a degeneration of 50 p. 100 of Sertoli cell-stock/testis was observed, and FSH treatment partially inhibits this phenomenon (Courot *et al.*, 1971). Increase in plasma levels of gonadotropin and especially FSH after cyproterone treatment, as previously shown by Von Berswordt-Wallrabe and Neumann (1967, 1968), could explain this phenomenon. Pituitary increase of gonadotropins, previously observed for LH by Walsh *et al.* (1972), Viguier-Martinez and Pelletier (1972), could be explained by the inhibition of the negative feedback control of testosterone on the hypothalamus-pituitary axis, since cyproterone is devoid of gestagenic effect (Neumann *et al.*, 1976).

Total numbers of type A spermatogonia/testis increased significantly after both cyproterone and cyproterone acetate treatment. This could be due to the anti-androgen acting directly on stem spermatogonia, or by Sertoli cell-stem cell interaction. The total number of type A spermatogonia/testis increases greatly during the growing process (Attal and Courot, 1963).

The large increase observed here could result from either a stimulation of stem cell stock formation, or a blockage of differentiation of stem cells into more differentiated spermatogonia. In immature rats inhibitory effects of HCG and testosterone on spermatogonial numbers has been thought to be due to inhibition of endogenous FSH by androgens (Chemes *et al.*, 1976). But as we obtained an increase in stem cell stock per testis with both anti-androgens and with either high or normal levels of plasma FSH, androgens could be directly implicated in this phenomenon.

The decreased yield of spermatogonial divisions after both anti-androgens could be related to the large increase in stem cells and compensation for this. Daily production of round spermatids per testis was only slightly increased after cyproterone treatment.

Variations in length of seminiferous tubules, in total numbers of Sertoli cells, and in stem spermatogonia have been observed in our experiments as we have worked with growing rats in which these parameters can be modified.

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Résumé. Un traitement chronique de 28 jours par deux anti-androgènes, la cyprotérone et l'acétate de cyprotérone, provoque chez le rat mâle prépubère des modifications pondérales au niveau du tractus génital, ainsi qu'une altération de la spermatogenèse et des fonctions gonadotropes. Les propriétés anti-androgènes de l'acétate de cyprotérone permettent d'expliquer la régression importante du tractus génital, à laquelle pourrait être liée l'augmentation du nombre total des spermatogonies A par testicule, alors que les propriétés progestatives de l'acétate de cyprotérone, inhibant cet effet anti-androgène au niveau central ne permettent pas de mettre en évidence une modification du taux des gonadotropines, mais provoqueraient la diminution de l'efficacité de la spermatogenèse. Par contre, la cyprotérone, anti-androgène pur, inhibant la testostérone tant au niveau central qu'au niveau périphérique, provoque d'une part une augmentation du taux des gonadotropines hypophysaires et plasmatiques, ainsi qu'une augmentation du poids testiculaire, d'autre part, une diminution du poids des glandes annexes, ceci s'accompagnant d'une augmentation du nombre total de spermatogonies par testicule, et du nombre total de cellules de Sertoli par testicule.

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