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Spermatogenic Arrest Produced in the Adult Male Rat by 19-Norspiroxenone, a Potent Anti-Estrogen (39007)

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Although the exact modes and sites of action of pituitary gonadotropins and testicular androgens are not well defined, it appears that LH, FSH and/or testosterone are involved in the control of testicular spermatogenesis and extragonadal sperm maturation (1, 2). Administration of estrogens, androgens or progestins (3) can interrupt sperm production, presumably by reducing serum gonadotropins and testicular androgen levels.

In the literature there have been suggestions that estrogens may normally be involved in the control of spermatogenesis. The rat testis does secrete estradiol, and these levels can be enhanced by LH but not FSH (4). In mice, estrogen treatment can initiate spermatogenesis, but the appearance of spermatids is delayed (5). It has been postulated that a product of the germinal epithelium, possibly estrogen, is responsible for selective FSH feedback (6, 7).

In the present investigation we report on the effects of long-term administration of 19-norspiroxenone (2′,3′α-tetrahydrofuran-2′-spiro-17-[estr-4-en-3-one]), a potent anti-estrogen, on fertility in the male rat.

Materials and methods. Adult Sprague–Dawley male rats were given daily subcutaneous injections of 10 mg of 19-norspiroxenone in 0.1 ml of dimethylsulfoxide (DMSO) or 0.1 ml of DMSO for a total of 6 weeks. The rats were decapitated on the day of the last injection, and organs were quickly removed, cleaned and weighed. Samples of various tissues were placed in buffered formalin and prepared for histological examination. All tissues were stained in hematoxylin–eosin, and, in addition, testes were also stained with PAS–hematoxylin.

During the last week of treatment fertility tests were performed on the male rats. On the evening of proestrus a Sprague–Dawley receptive proven breeder female was placed with each test male previously housed in a separate cage for 5 days. The presence of sperm in the vaginal smear or of a copulatory plug in the female on the next morning was taken as evidence of successful copulation. The females were autopsied on day 14 of gestation to determine number of viable fetuses and resorptions as a measure of the fertilizing capacity of the sperm. A maximum of five and a minimum of two different females were tried with each male before assessing the fertility of the male.

Statistical evaluation was Student's t test.

Results. Administration of 19-norspiroxenone for 6 weeks had no significant effect on adrenal, preputial gland or liver weights, or body weight gain but depressed testicular, prostatic and epididymal weights with a corresponding increase in pituitary weights (Table I). Histological examinations of testicular sections revealed spermatogenic arrest in the early stages of meiotic division along with hypotrophic changes in the Leydig cells (Fig. 1). Quantitative evaluation of the sections showed dramatic reduction in the ratio of spermatids to spermatocytes in the seminiferous tubules (Table II). The secretory epithelial cells of the ductus epididymidis, ductus deferens and ventral prostate were reduced in height in males treated with 19-norspiroxenone. Unlike the controls, the ventral prostate acini contained no secretions, and there were few or no spermatozoa in the smaller lumens of the ductus epididymidis and ductus deferens of treated rats. The fertility of 19-norspiroxenone-treated males as measured by number of viable fetuses sired per female impregnated was dramatically reduced to zero (Table II). A few sperm were observed in the vaginal smears of five females placed with one of the 19-norspiroxenone-treated males, attesting to the fact that at least one of the treated males had copulatory behavior. No observations

TABLE I. EFFECTS OF 19-NORSPIROXENONE ON BODY AND ORGAN WEIGHTS.

Treatment	Number of rats	Body weights (g) Initial	Final	Preputial glands (mg/100 g body weight)	Adrenals (mg/100 g body weight)	Testes (g/100 g body weight)	Seminal vesicles (mg/100 g body weight)	Ventral prostates (mg/100 g body weight)	Epididymes (g/100 g body weight)	Pituitaries (mg/100 g body weight)	Liver (g/100 g body weight)
DMSO	8	468.1[a] ±74.9	534.3 ±59.4	20.2 ±8.4	10.9 ±2.2	0.66 ±0.05	101.7 ±17.6	187.4 ±32.5	0.23 ±0.02	2.7 ±0.5	3.0 ±0.2
19-Norspiroxenone	7	497.4 ±61.8	525.0 ±44.6	17.9 ±5.7	10.0 ±1.1	0.22[b] ±0.02	28.0[b] ±8.3	53.1[b] ±8.6	0.06[b] ±0.05	3.6[b] ±0.3	3.1 ±0.3

[a] Mean ± 1 SD
[b] $P < 0.001$.

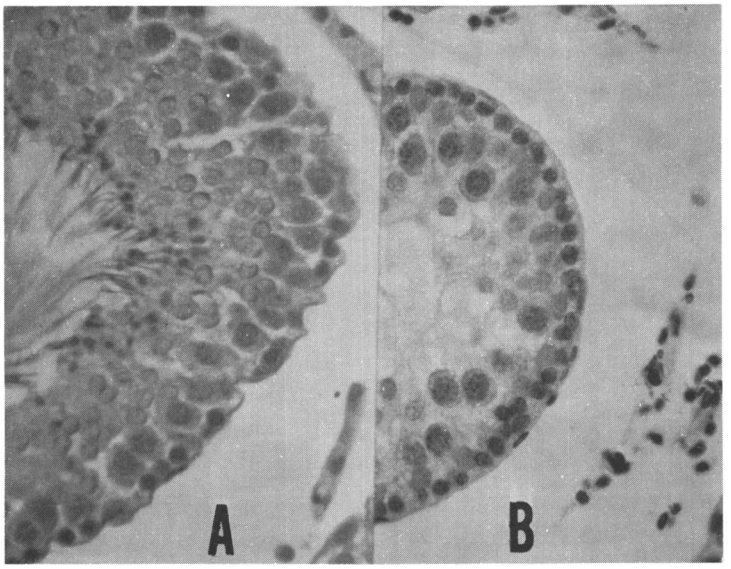

FIG. 1. Effect of 19-norspiroxenone on testes. All stages of spermatogenesis can be seen in tubule of control male (A). However, note that spermatogenesis is arrested in early meiotic division in a much smaller tubule of 19-norspiroxenone-treated male (B). Leydig cells of experimental male are also hypotrophic. × 450.

were made on the sexual behavior of the other males.

Discussion. 19-Norspiroxenone is an exceptionally potent anti-estrogen (8) that can eliminate cornified cells in the vaginae of intact female rats for up to 2 weeks after a single 2-mg injection (unpublished). Furthermore, this compound appears to be unique in having no estrogenic activity (8). Unlike other anti-estrogens (clomiphene citrate, ethamoxytriphetol [MER-25] etc.) whose estrogenic activity produced adrenal enlargement (9), 6 weeks of 19-norspiroxenone treatment had no effect on adrenal weight. Although pituitary enlargement could be indicative of estrogenic stimulation (9, 10), preliminary results from our laboratory indicate that administration of 19-norspiroxenone produces significant accumulation of pituitary prolactin, which in turn, may be the cause of the increased pituitary weight.

Although it is difficult to assess the progestinlike activity of 19-norspiroxenone because such activity is usually synergistic with estrogens (11), it seems unlikely that the compound is a progestin. In the mouse, 19-norspiroxenone has only weak progestinlike activity (8), while in the rat the compound has no uterotrophic activity (unpublished). In man, pharmacologic doses of progesterone can produce azoospermia, but spermatogenesis remains moderately active and there is no effect on the Leydig cells (12).

The castrationlike effects of 19-norspiro-

TABLE II. EFFECTS OF 19-NORSPIROXENONE ON SPERMATOGENESIS AND FERTILITY OF MALE RATS

Treatment	Males No.	Spermatid to spermatocyte ratio[a]	Proestrous females placed with each male (No.)	Males producing vaginal plugs in females (No.)	Viable fetuses (No.)
Control	8	2.80 ± 0.33[b]	2	8	12.8 ± 1.9
19-Norspiroxenone	7	0.02 ± 0.06[c]	5	1	0[c]

[a] Spermatids, stages 10–18, and post-zygotene spermatocytes I were identified according to LeBlond and Clermont (17).

[b] Mean ± 1 SD.

[c] $P < 0.001$.

xenone on the sexual accessory organs suggest that the compound is an anti-androgen. However, 19-norspiroxenone has been reported to be neither an androgen nor an anti-androgen (8), but its androgen-depriving effects may be an indirect result of pituitary inhibition. It has been postulated that a product of the germinal epithelium, possibly estrogen, is responsible for selective FSH feedback (6, 7). In fact, a spermatogenic factor in the rat testis, other than testosterone, regulates pituitary synthesis of FSH (13). Thus, it appears that the dramatic inhibition of spermatogenesis produced by 19-norspiroxenone may be the result of its anti-estrogenic action in blocking at some site of the hypothalamic–pituitary–testicular axis. Indeed, in the male the effects of testosterone on the hypothalamic–pituitary axis may result from conversion of testosterone to estrogen in the hypothalamus (14). Furthermore, the testis of the rat secretes estradiol (4) and there are estrogen receptors in the male pituitary, hypothalamus (15) and testis (16) which may be the mechanism through which estrogen exerts its effects.

Summary. 19-Norspiroxenone, a potent anti-estrogen with no known estrogenic action, produces a striking arrest of spermatogenesis in the early stages of meiosis in the adult male rat with a complete block of fertility.

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