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Mast Cells in the Testis, Epididymis and Accessory Glands of the Rat: Effects of Neonatal Steroid Treatment

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Mast cells in the testis of control adult rats were found almost exclusively around subcapsular blood vessels. Discrete mast cells were distributed throughout the stroma of the epididymis and sex accessory glands. In neonatally estrogen-treated rats, a greater number of mast cells was present in the testicular interstitium, whereas no signficant increase in the number of mast cells per square millimeter of stroma was found for the epididymis and sex accessory glands, despite stromal proliferation. On the other hand, androgen-treated rats did not have increased mast cell numbers in any organ. These results indicate that the increase in mast cell numbers was estrogen-dependent, specifically related to the testis and did not seem to be a consequence of the increase in the connective interstitial tissue.

Key words: mast cells, testis, epididymis, sex accessory glands, neonatal steroid treatment.

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It has been clearly established during the last few years that mast cells of the female reproductive system are subjected to cyclical changes during the estrous cycle. Variations in both the number and degranulation pattern have been reported in the uterus and ovaries of the rat (Gibbons and Chang, 1972; Jones et al, 1980) and hamster (Harvey, 1964; Brandon and Evans, 1983), and estrogens also have stimulatory effects on mast cells in other locations, such as the peritoneal cavity (Modat et al, 1982). In the hamster ovary, mast cells seem to mediate the vascular response to the gonadotropin surge in proestrus (Krishna and Terranova, 1985). It has been reported recently that mast cells increase in the dominant follicle of the cow (Nakamura et al, 1987).

On the contrary, mast cells of the male reproductive system have received little attention. Recently, increased numbers of mast cells have been reported in the testis of infertile men (Behrendt et al, 1981; Maseki et al, 1981), and treatment of patients with idiopathic infertility with mast cell blockers induced a significant improvement in sperm count and motility (Schill et al, 1986). It is, therefore, of interest to examine the possible role of mast cells in the male reproductive organs, both in normal and pathologic conditions.

In a preliminary study, we reported that neonatally estrogen-treated rats presented increased

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numbers of mast cells in the testis at puberty and adult life (Gaytan et al, 1986a). This increase was correlated with the level of maturational arrest of spermatogenesis and with the relative volume of the interstitial connective tissue. We suggested the hypothesis that mast cells might be increased as a consequence of the increase in this tissue compartment. To determine whether the increase in mast cell number was specifically related to the testis and dependent on estrogens, mast cell numbers were studied in the epididymis and accessory glands, in addition to the testis, in either neonatally estrogen- or androgen-treated rats.

Materials and Methods

Wistar male rats were injected subcutaneously in the first day of life either with 500 μg of estradiol benzoate (Sigma Chemical Co., St. Louis, MO) or 500 μg of testosterone propionate (Sigma Chemical Co.) in 0.1 cm^3 of olive oil (steroid-treated animals) or 0.1 cm^3 of the vehicle only (control animals). Five animals per group were used. The animals were killed at 45 days of age. After decapitation, each testis, epididymis, ventral prostate and seminal vesicle was carefully removed, weighed and processed for histologic examination.

Histologic Procedures

One of each pair of organs (epididymis and seminal vesicle) per animal and half of the ventral prostate were immersed in 2% phosphate buffered glutaraldehyde, cut into small blocks and fixed for 24 hours, washed overnight and embedded in Araldite (Durcupan ACM, Fluka Co., Switzerland) after dehydration. The contralateral organs from two animals per group were fixed in Bouin-Hollande's fluid for 24 h and after dehydration embedded in paraffin wax.

A preliminary study was performed on paraffin-embedded tissues to analyze the distribution of mast cells within the whole organ. It was carried out on 10-μm thick serial sections stained with 0.2% toluidine blue in 70% ethanol. Except for the testis of control and androgen-treated rats, mast cells were distributed throughout the stroma of the different organs studied.

Mast Cell Counting Procedures

Mast cells were counted in plastic-embedded tissues since this material provided greater definition, particularly of the functional status of the cells (ie degranulated or not). Three blocks per organ from each animal were selected at random. From these blocks, 25 sections (1-μm thick and with a surface area ranging from 2-4 mm^2), at least 25 μm apart, were randomly selected per organ from each animal, stained with 1% toluidine blue in 1% borax and examined with the light microscope. These numbers were considered adequate because the variability among sections was less than 10%. The testicular epididymal and seminal vesicle tissues were oriented to obtain sections perpendicular to their long axis, whereas the ventral prostate was oriented at random. For the epididymis, blocks from the caput, corpus and cauda were selected.

Cell counting was carried out under the light microscope with an ×25 objective. Depending on the size of the sections, several microscopic fields were systematically analyzed to score the whole section. In each field, the number of mast cells within the test area was counted with the aid of 100-test point reticle eye piece. Afterwards, the area occupied by the stroma in the same field was obtained as the product of the number of points occurring on the stroma and the area corresponding to each point. Mast cell counts were, therefore, expressed as the number of cells per square millimeter of stroma since mast cells were located exclusively in this tissue compartment, and because of the necessity of correcting for the different volume stroma in the various experimental groups.

Statistical Analysis

Statistical analysis was carried out by the one-way analysis of variance and Tukey's multiple comparison method for comparison among means.

Results

The organ weights of the different groups are shown in Table 1. Estrogen-treated rats presented significantly lower ($p < 0.01$) organ weights than the controls. Androgen-treated rats had intermediate organ weights, which were significantly lower

TABLE 1. Organ Weights in Control and Steroid-Treated Rats*

Organ	Treatment		
	Oil	Estradiol	Testosterone
Testis	981.75 ± 26.26	282.26 ± 42.54†	693.46 ± 48.21†‡
Epididymis	133.44 ± 5.11	45.37 ± 1.07†	98.25 ± 8.50†‡
Ventral prostate	130.08 ± 13.85	34.40 ± 4.91†	104.52 ± 5.80‡
Seminal vesicles	104.10 ± 10.60	33.47 ± 2.68†	54.16 ± 10.55†

*mg, mean ± SEM.
†$p < 0.01$ vs. control rats.
‡$p < 0.01$ vs. estrogen-treated rats.

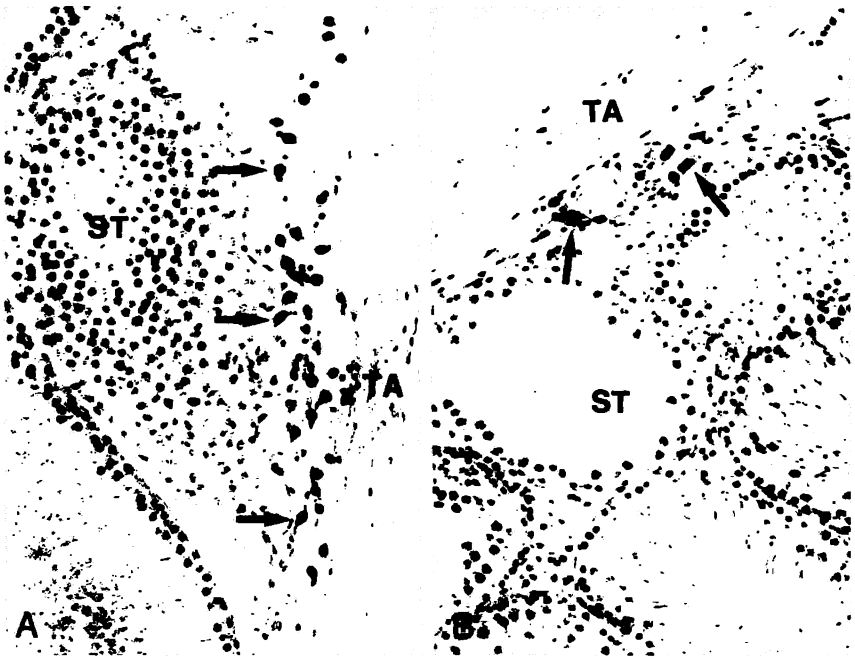

Fig. 1. Paraffin-embedded testicular sections from control (A) and androgen-treated (B) rats, stained with toluidine blue. Metachromatic mast cells (arrows) are located under the tunica albuginea (TA) and are absent from the interstitium among the seminiferous tubules (ST). × 120.

($p < 0.01$) than those of the control rats, except for the ventral prostate, and significantly higher ($p < 0.01$) than the estrogen-treated animals, except for the seminal vesicles.

In the testis of control rats, mast cells were almost exclusively found under the tunica albuginea, mainly in groups around blood vessels (Fig. 1A). Mast cells in the testicular interstitium were only occasionally found, and for practical purposes it may be considered that these cells are absent from the testicular interstitium of the controls. Mast cells in androgen-treated rats were also located under the tunica albuginea (Fig. 1B). These animals presented differentiated Leydig cells and at least qualitatively normal spermatogenesis (Fig. 2A). On the contrary, estrogen-treated rats had impaired spermatogenesis and undifferentiated interstitial cells with elongated nuclei and scarce cytoplasm. The most relevant feature of these animals was the presence of a greater number of mast cells in the testicular interstitium (Fig. 2B). Mast cells had central nuclei and metachromatic secretory granules in the cytoplasm (Figs. 2C,D). No degranulated cells were observed.

Mast cells in the epididymis (Fig. 3), ventral prostate (Fig. 4), and seminal vesicles (Fig. 5) were distributed throughout the stroma, and showed a similar distribution in all groups. These cells were relatively frequent in the ventral prostate, either in control (Figs. 4A,B) or steroid-treated rats (Figs. 4C,D). In the epididymis and seminal vesicles of estrogen-treated rats, mast cells were more frequently found than in other groups due to the relatively increased volume of the stroma (Figs. 3C,D and 5B,C).

Results from mast cell counting are shown in Table 2. In the testicular interstitium mast cells were detected only in estrogen-treated rats. No significant differences were found for the number of mast cells in the epididymis or in the sex accessory glands among the different groups.

Discussion

Mast cells in the testis of control rats were characteristically located around subcapsular blood vessels, being virtually absent in the portion of the testicular interstitium far from the tunica albuginea. Such a location is suggestive of a possible role in the regulation of the blood flow to the testis. Mast cells of the hamster ovary have been reported in a similar location being found exclusively around blood vessels of the hilium. It has been suggested that these cells participate in gonadotropin-induced preovulatory events (Krishna and Terranova, 1985). Although recent reports seem to indicate that the hCG-induced changes in the vascular permeability of the rat testis are not mediated by histamine, and implicate polymorphonuclear leukocytes instead

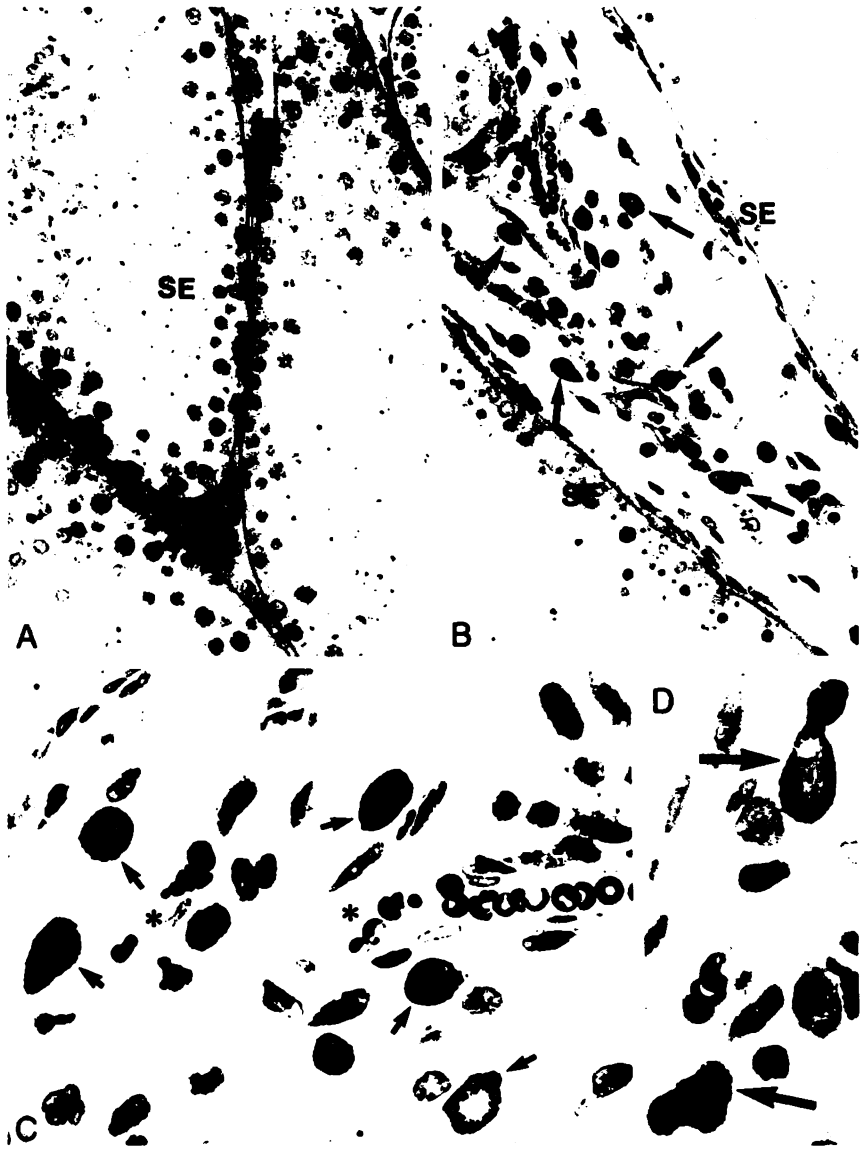

Fig. 2. Semi-thin testicular sections. (A) Androgen-treated rat. Nearly normal seminiferous epithelium (SE) and differentiated Leydig cells in the interstitium (asterisks). × 250. (B) Estrogen-treated rat. Atrophic seminiferous epithelium (SE) and numerous mast cells (arrows) in the interstitium. × 260. (C) Detail of an area of Fig. 2B. Mast cells (arrows) are dispersed in the interstitium near blood vessels (asterisks) and no differentiated Leydig cells are present. × 650. (D) High magnification of mast cells (arrows), showing central nuclei (N) and metachromatic cytoplasmic granules. × 850.

(Bergh et al, 1986, 1987), mast cells do secrete inflammatory mediators and vasoactive factors other than histamine (Schleimer et al, 1984; Lagunoff and Richard, 1987) and their participation in other physiologic conditions cannot be excluded.

In a preliminary study, we reported that neonatally estrogen-treated rats presented increased numbers of mast cells in the testis, the increase being related to the proliferation of the interstitial connective tissue (Gaytan et al, 1986a). The present study was performed to test this hypothesis. Several conclusions can be reached from the present data. Estrogen-treated rats had increased numbers of mast cells exclusively in the testis in a location where normally there are no mast cells. A mast cell increase in a specific organ has a different meaning from a generalized increase in mast cell numbers, and probably involves a different mechanism of production. The responsiveness of mast cells to steroid hormones has been clearly established in the female. Cyclic changes in the number and degranulation pattern of the cells during the estrous cycle have been found in the reproductive organs (Gibbons and Chang, 1972; Krishna and Terranova, 1985; Shinohara et al, 1987), as well as in other locations such as peritoneum (Modat et al, 1982).

Although the precise mechanism of these changes is not clear, it is generally agreed that estradiol acts

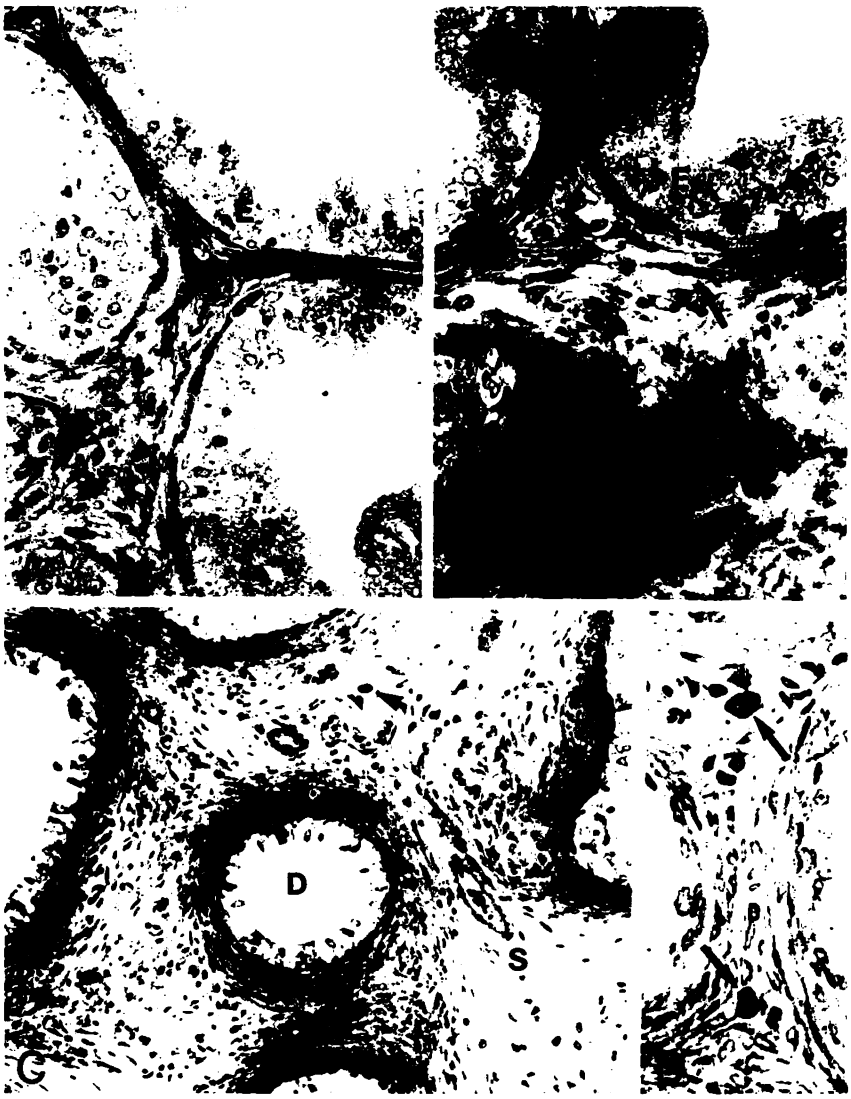

Fig. 3. Semi-thin sections of the epididymus. (A) Control rat. (B) Androgen-treated rat. The epithelium (E) and stroma (S) can be observed. Mast cells (arrows) in the stroma are scarce. × 250. (C) Estrogen-treated rat. A mast cell (arrow) is present in the stroma (S). The connective tissue and the smooth muscle (M) surrounding the epididymal duct (D) are considerably increased. × 225. (D) Mast cells (arrows) in the stroma from an estrogen-treated rat. × 280.

on mast cells (Modat et al, 1982; Krishna and Terranova, 1985). The absence of increased mast cell numbers in the epididymis and accessory sex glands reported here is difficult to explain by the hypothesis that the mast cell increase was due to a direct effect of estrogens. Whereas testicular changes present in neonatally estrogen-treated rats seem to be due to the gonadotropin decrease induced by estrogens (Aguilar et al, 1987a), a direct effect of these hormones on the stroma of the accessory sex organs has been previously reported (Orgebin-Crist et al, 1983; Gaytan et al, 1986b).

The present results suggest that the increase in mast cell numbers, as well as their unusual location in the testicular interstitium, are due to testicular alterations induced by neonatal estrogen treatment. Similarly, an increased number of mast cells in the testis of infertile men has been reported (Behrendt et al, 1981; Maseki et al, 1981) and this has been related to dysfunction of the blood-testis barrier. However, morphologic and functional studies have shown that postnatal estrogen tratment did not interfere with the establishment of this barrier in the rat (Vitale et al, 1973). It is tempting to speculate that altered seminiferous tubules and/or interstitial cells release growth and/or chemotactic factors for mast cells. Recently, Jackson et al (1986) reported that a large number of mast cells appeared in the testicular interstitium of rats treated with ethylene dimethane sulfonate (EDS), which specifically

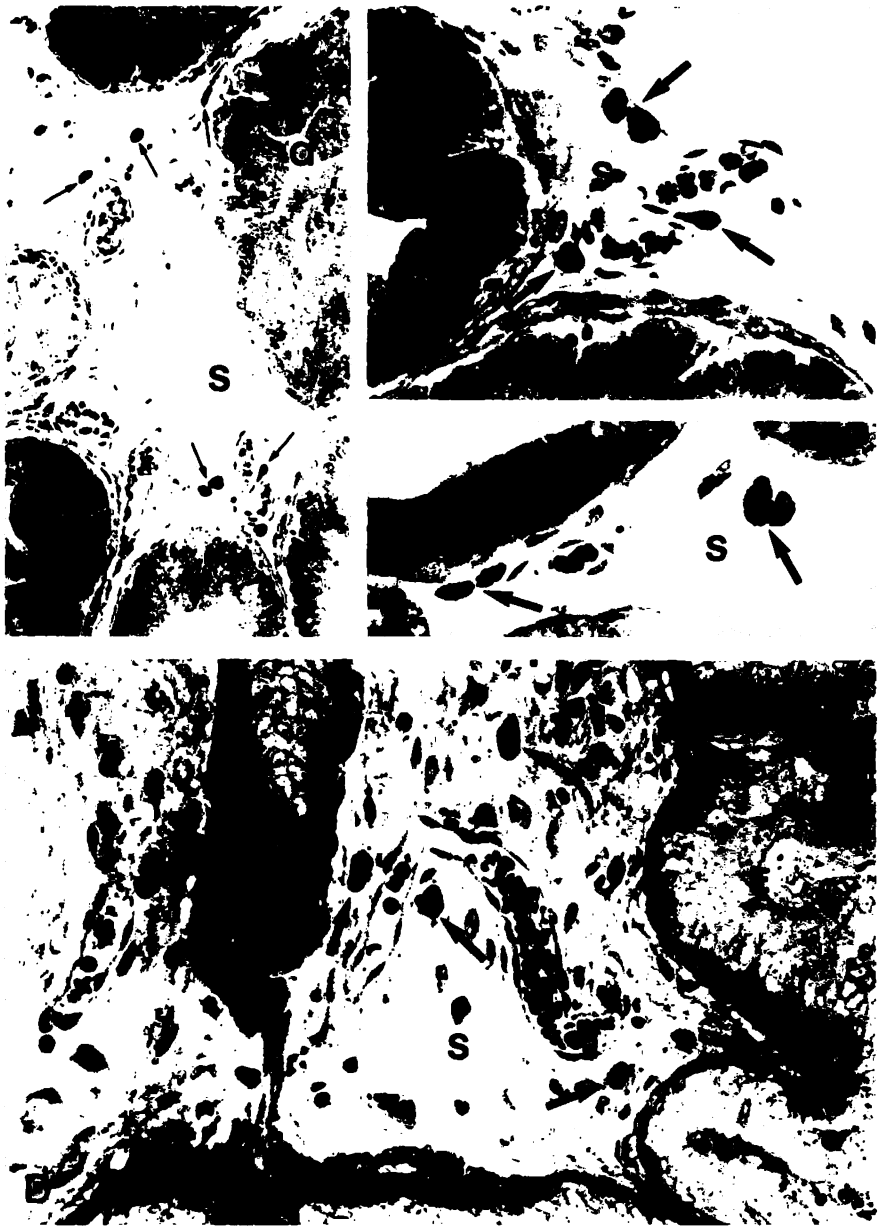

Fig. 4. Semi-thin sections of the ventral prostate. (A) Control rat. Mast cells (arrows) are relatively abundant in the stroma (S) among glandular acini(a). × 200. (B) Detail of an area of Fig. 4A, showing mast cells (arrows) closely related to blood vessels (asterisk) of the stroma (S). E.- epithelium. × 260. (C) Androgen-treated rat. The epithelium (E) has normal features, and mast cells (arrows) can be observed in the stroma (S). × 260. (D) Estrogen-treated rat. Mast cells (arrows) in the stroma (S) that is slightly increased, whereas the epithelium (E) is poorly developed. × 250.

destroys Leydig cells, and that the appearance of mast cells was coincident with the period of time in which a new generation of Leydig cells was developing. Neonatally estrogen-treated rats present undifferentiated interstitial cells that undergo differentiation from 45–90 days of age (Gaytan et al, 1986c). The interrelationships, if any, between these two cell types and the possible role of mast cells in the recovery of damaged interstitial tissue are unknown. The participation of mast cells in vascular and connective cell proliferation has been well-established in different systems (Hatanaka et al, 1986; Norrby et al, 1986).

The previously suggested relation between increased mast cell numbers and interstitial connective tissue proliferation may be rejected from the present data, since the proliferation of the stroma after estrogen treatment is considerably higher in the accessory sex organs (Orgebin-Crist et al, 1983; Gaytan et al, 1986b) than in the testis, without a specific increase in mast cell numbers.

Neonatal androgen treatment did not induce an

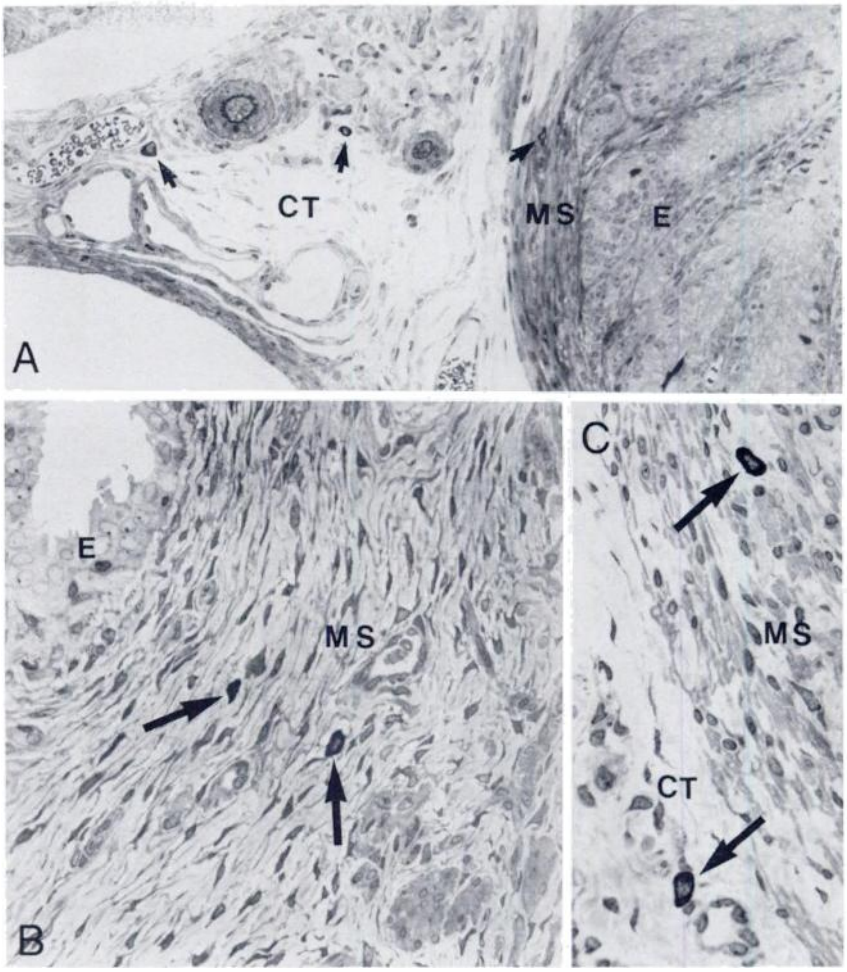

Fig. 5. Semi-thin sections of the seminal vesicles. (A) Control rat. Mast cells (arrows) are present in the connective tissue (CT) and in the smooth muscle sheath (MS) surrounding the epithelium (E). × 200. (B) Estrogen-treated rat. The epithelium (E) is poorly developed and the muscular sheath (MS), in which mast cells (arrows) can be observed, is considerably increased. × 225. (C) Detail showing mast cells (arrows) in the connective tissue (CT) and in the muscular sheath (MS) from an estrogen-treated rat. × 260.

increase in mast cell numbers, perhaps because the testicular changes in these animals were less pronounced than in estrogen-treated rats. Whereas in early postnatal development both estrogen and testosterone have similar effects on the testis (Aguilar et al, 1987b), androgen-treated rats have partially recovered at 45 days of age, as shown by the organ weights and histologic data. On the other hand, the possibility that estrogens have qualitatively different effects than androgens on testicular structure cannot be rejected from the present data. Further studies on this subject are needed.

Since mast cell numbers are specifically increased in the testis after neonatal estrogen treatment, this

TABLE 2. Number of Mast Cells per mm² of Stroma in the Reproductive Organs of Control and Steroid-Treated Rats*

	Treatment		
Organ	Oil	Estradiol	Testosterone
Testis	ND	165.90 ± 30.21	ND
Epididymis	21.11 ± 1.61	23.40 ± 3.10	18.37 ± 1.40
Ventral prostate	73.78 ± 4.62	74.85 ± 7.25	69.22 ± 3.71
Seminal vesicles	18.19 ± 1.00	24.26 ± 5.38	17.12 ± 0.86

*Mean ± SEM. The testis was the only organ with significant differences in the number of mast cells/mm² of stroma.
ND = not detected.

system seems to be a suitable model for studies focused on testicular mastocytosis in infertile men. Due to the abnormal location of mast cells in the testis of estrogen-treated rats, in addition to their increased numers, this system can be easily analyzed either qualitatively or quantitatively.

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References

Aguilar E, Bellido C, Aguilar R, Pinilla L, Tejero A, Galaz CF. Mechanisms in the production of prepuberal reproductive defects in neonatal estrogenized male rats. Andrologia 1987a; 19:22–31.

Aguilar R, Bellido C, Aguilar E. Differences in prepuberal neonatally estrogenized or androgenized male rats. Andrologia 1987b; 19:183–187.

Behrendt H, Hilscher B, Passia D, Hofmann N, Hilscher W. The occurrence of mast cells in the human testis. Acta Anat 1981; (Abstr) III:14.

Bergh A, Widmark A, Damber JE, Cajander S. Are leucocytes involved in the human chorionic gonadotropin-induced increase in testicular vascular permeability? Endocrinology 1986; 119:586–590.

Bergh A, Rooth P, Widmark A, Damber JE. Treatment of rats with hCG induces inflammation-like changes in the testicular microcirculation. J Reprod Fertil 1987; 79:135–143.

Brandon JM, Evans JE. Changes in uterine mast cells during the estrous cycle in the Syrian hamster. Am J Anat 1983; 167:241–247.

Gaytan F, Bellido C, Lucena MC, Paniagua R. Increased mast cell number in the testis of neonatally estrogenized rats. Arch Androl 1986a; 16:175–181.

Gaytan F, Bellido C, Aguilar R, Lucena MC. Morphometric analysis of the rat ventral prostate and seminal vesicles during prepubertal development: Effects of neonatal treatment with estrogen. Biol Reprod 1986b; 35:219–225.

Gaytan F, Pinilla L, Aguilar R, Lucena MC, Paniagua R. Effects of neonatal estrogen administration on rat testis development with particular reference to Sertoli cells. J Androl 1986c; 7:112–121.

Gibbons AFE, Chang MC. Number of mast cells in the rat uterus with special reference to its relation to hormonal treatment and decidual response. Biol Reprod 1972; 6:193–203.

Harvey EB. Mast cell distribution in the uterus of cycling and pregnant hamsters. Anat Rec 1964; 148:507–515.

Hatanaka K, Imakita M, Go S, Yamamoto A. Effects of degranulation of mast cells on proliferation of mesenchymal cells in the mesentery of mice. Cell Tissue Res 1986; 246:53–56.

Jackson AE, O'Leary PC, Ayers MM, de Kretser DM. The effects of ethylene dimethane sulphonate (EDS) on rat Leydig cells: Evidence to support a connective tissue origin of Leydig cells. Biol Reprod 1986; 35:425–37.

Jones RE, Duvall D, Guillette LJ Jr. Rat ovarian mast cells: distribution and cyclic changes. Anat Rec 1980; 197:489–493.

Krishna A, Terranova PF. Alterations in mast cell degranulation and ovarian histamine in the proestrus hamster. Biol Reprod 1985; 32:1211–1217.

Lagunoff D, Richard A. Methods of the study of rat peritoneal mast cell secretion. In: Poisner and Trifaró, eds. In vitro methods for study secretion. Amsterdam: Elsevier Science Publishers BV, 1987; 13–28.

Maseki Y, Miyake K, Mitsuya H, Kitamura H, Yamada K, Mastocytosis occurring in the testes from patients with idiopathic male infertility. Fertil Steril 1981; 36:814–817.

Modat G, Bombarek A, Lalaure M. Variations quantitatives du nombre et de la taille des mastocytes péritonéaux chez la ratte au cours du cycle sexual normal, aprés ovariectomie et administration d'oestro génes. C R Soc Biol 1982; 176:675–681.

Nakumura Y, Smith M, Krishna A, Terranova PF. Increased number of mast cells in the dominant follicle of the cow: relationships among luteal, stromal and hilar regions. Biol Reprod 1987; 37:546–549.

Norrby K, Jacobsson A, Sörbo J. Mast-cell-mediated angiogenesis: a novel expermental model using the rat mesentery. Virchows Arch (Cell Pathol) 1986; 52:195–206.

Orgebin-Crist MC, Eller BC, Danzo BJ. The effects of estradiol, tamoxifen and testosterone on the weights and histology of the epididymis and sex accessory organs of sexually immature rabits. Endocrinology 1983; 113:1703–1715.

Schill WB, Schneider J, Ring J. The use of Ketotifen, a mast cell blocker, for treatment of oligo- and asthenozoospermia. Andrologia 1986; 18:570–573.

Schleimer RP, MacGlashan DW, Peters SP, Naclerio R, Proud D, Adkinson NF, Lichtenstein LM. Inflammatory mediators and mechanisms of release from purified human basophils and mast cells. J Allergy Clin Immunol 1984; 74:473–481.

Shinohara H, Nakatani T, Morisawa S, Matsuda T, Naruse Y. Mast cells in the ovarian bursa of the golden hamster. Biol Reprod 1987; 36:445–450.

Vitale R, Fawcett DW, Dym M. The normal development of the blood-testis barrier and the effects of clomiphene and estrogen treatment. Anat Rec 1973; 176:331–344.