

Germ Cell Degeneration in the Contralateral Testis of the Guinea Pig with Unilateral Torsion of the Spermatic Cord

Quantitative and Ultrastructural Studies

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This study evaluated the long term effects of unilateral torsion of the spermatic cord on the contralateral testis of guinea pigs, employing both fine structural and quantitative studies. Young, adult Hartley strain guinea pigs were divided into six experimental groups (12 animals per group). The first three groups consisted of 36 animals in which unilateral torsion was surgically induced. In group I (torsion maintained), unilateral torsion of the spermatic cord was maintained until the day of sacrifice; in group II (torsion and untwist), torsion of the spermatic cord was maintained for 8 to 12 hours, then the spermatic cord was untwisted and the testis was retained until the day of sacrifice. In group III (torsion and orchiectomy), testes were removed after 8 to 12 hours of spermatic cord torsion. The second three groups consisted of 36 animals: group IV (unilateral orchiectomy), group V (unilateral sham operation), and group VI (pentobarbital injection alone), which served as controls. One half of the animals from each group were killed after 4 months and the other half were killed after 8 months. The most frequently observed histologic changes in the contralateral testes of the experimental animals were focal disorganization and exfoliation of immature germ cells into the lumen. Severe damage, with almost complete absence of germ cells, was noted only in an occasional tubule. Quantitative evaluation of the germ cells of the contralateral testis revealed significant loss of germ cells in groups I, II, and III after 4 months, and in groups I and II after 8 months. Germ cell degeneration was progressive in groups I and II, as demonstrated by the lower germ cell count in the testes of animals of the 8-month group in comparison with the 4-month group. However, in group III animals, a higher germ cell count was recorded at 8 months, which was similar to those of control values. The present study

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confirms our earlier findings that unilateral torsion of the spermatic cord has adverse effects on the contralateral testis. However, since these effects are subtle and inconsistent, a systematic germ cell quantitation is needed for critical assessment of the deleterious effect of unilateral spermatic cord torsion on the contralateral testis.

Key words: testis, torsion, quantitation, germ cell, guinea pig, reproduction.

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In the last 3 to 4 decades, most of the emphasis in the management of spermatic cord torsion has been on improving the salvage rate of the affected testis. Some investigators have suggested that even a seemingly nonviable testis, resulting from spermatic cord torsion, should be retained in the body after the untwisting of the spermatic cord, as this testis may maintain hormonal and some spermatogenic functions (Skoglund et al, 1970; Moyad et al, 1975; Jhunjunwala et al, 1976).

We have demonstrated previously that retention of a damaged testis in the body for a variable time period can cause varying degrees of spermatogenic disruption in the contralateral testis of both humans and guinea pigs (Chakraborty et al, 1980, 1983, 1985; Chakraborty and Jhunjunwala, 1982; Jhunjunwala et al, 1984). Our findings in the guinea pig have been substantiated by other investigators in experimental studies on both prepubertal and adult rats

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(Nagler and de Vere White, 1982; Merimsky et al, 1982; Cosentino et al, 1984; York and Drago, 1985) and rabbits (Cerasaro et al, 1984). Clinical studies also have indicated impaired fertility in patients following unilateral torsion of the spermatic cord (Krarup, 1978; Bartsch et al, 1980; Mastrogiacomo et al, 1982).

Recent studies on various strains of albino rats and strain II guinea pigs, however, cast some doubt on the validity of these findings (Saltzman et al, 1984; Turner, 1985). Saltzman et al (1984) observed occasional histologic alterations in the contralateral testis as a result of ipsilateral testicular torsion, while Turner (1985) did not find any appreciable alteration in the contralateral testis of rats following unilateral torsion of the spermatic cord. The present report deals with further systematic studies employing both light and electron microscopic investigations and germ cell quantitation, which evaluate the effects of unilateral torsion of the spermatic cord on the contralateral testes of guinea pigs after a period of 4 or 8 months following surgical induction of torsion of the spermatic cord.

Materials and Methods

Experimental Design

Seventy-two Hartley strain young, adult male guinea pigs weighing 600 to 700 gm were exposed to various experimental procedures. In 36 animals, unilateral torsion of the spermatic cord was induced surgically by making a scrotal incision and twisting the spermatic cord through 540 degrees (Chakraborty and Jhunjunwala, 1982). The animals were subdivided equally into three groups. In group I (TM) animals, unilateral torsion of the spermatic cords was maintained until the day of sacrifice. Group II (TU) animals were operated on again 8 to 12 hours after the induction of torsion and the spermatic cords were untwisted and returned to the scrotum. In group III (TO) animals, excision of the testes (torsion side only) was carried out 8 to 12 hours after the induction of torsion.

The remaining 36 animals were also subdivided equally into three groups that served as controls: in group IV (UO), animals underwent unilateral orchietomy; in group V (SHAM), a unilateral sham operation was performed by scrotal incision and gentle handling and exposure of testes for 5 minutes; in group VI (PENT) animals, one intraperitoneal pentobarbital injection was given. All unilateral procedures were performed equally on the right and left sides.

All animals were housed in the animal facility with controlled temperature and a 12-hour light: 12-hour dark cycle. All procedures were done with intraperitoneal pentobarbital injection using sterile technique. Six animals from each group were sacrificed after 4 months, and the remaining six after 8 months.

At autopsy, total body weights, as well as the weights of the testis, the epididymis, prostate, and seminal vesicles, were recorded for each animal. The volume of each testis

was measured by volume displacement. Each testis was divided into five equal parts. One piece from each part, ie, a total of five pieces from each testis, were processed for epon embedding. The remaining tissues were fixed in Zenker-formal and processed for routine paraffin embedding.

Light and Electron Microscopic Procedures

Tissues collected from the animals were immediately fixed by immersion in 2.5% buffered glutaraldehyde for 16 to 18 hours, postfixed in 1% O_5O_4 for 1 hour, and processed for routine epon embedding. Semithin (1- μ m) sections were cut from each of five blocks and stained with 1% toluidine blue for light microscopic examination and quantitative study. Altogether, five blocks were sectioned from each testis of each animal. Thin (90 to 100-nm) sections were cut from the well-preserved regions of the block, stained with lead citrate, and examined with a Philips EM 300 electron microscope.

Quantitative Analysis

Germ cell quantitation was performed using a Nikon OptiphotTM microscope at 800 \times magnification from semithin, toluidine blue-stained sections. For germ cell counts, pachytene spermatocytes, round spermatid nuclei, and Sertoli cell nuclei were counted in round cross sections of the seminiferous tubules during stages I-VIII of the seminiferous epithelial cycle (Sinha Hikim and Chakraborty, 1985). The identification of various germ cells and Sertoli cells was based on the typical morphology of these cells (Clermont, 1960; Sinha Hikim and Chakraborty, 1985). Cell counts finally were expressed as the number of germ cells per Sertoli cell (Chakraborty et al, 1983, 1985). Data obtained were based on the count of germ cells in relation to at least 500 Sertoli cells from each testis (Chakraborty et al, 1983). No correction for section thickness or shrinkage due to tissue processing was done, as 1- μ m semithin sections from regular epon-embedded blocks were used (Eins and Wilhelms, 1976; Haug, 1980).

The diameter of at least ten randomly selected seminiferous tubules was measured for all animals from all groups, using an ocular micrometer and a 20 \times objective lens, as previously described (Chakraborty et al, 1985).

Statistical Analysis

The paired Student's *t* test was used for statistical evaluation. *P* values < 0.05 were considered to be significantly different.

Results

Body Weight and Organ Weight

No significant changes in average body weight or in the weights of the epididymis, prostate, and seminal vesicles were recorded in any of the experimental groups as compared with controls. No change in contralateral testicular weight (Fig. 1) or volume (Fig. 2) was found in any of the experimental groups of guinea pigs when compared with controls. There

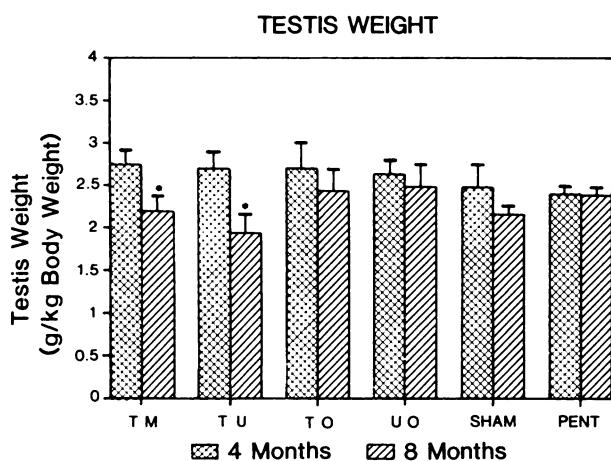


Fig. 1. Contralateral testicular weight of guinea pigs under various experimental conditions. Mean \pm SE (vertical bar). Significantly lower testicular weights were recorded in group I (TM) and group II (TU) animals after the 8-month period in comparison with group I (TM) and group II (TU) animals after the 4-month period (* $P < 0.05$).

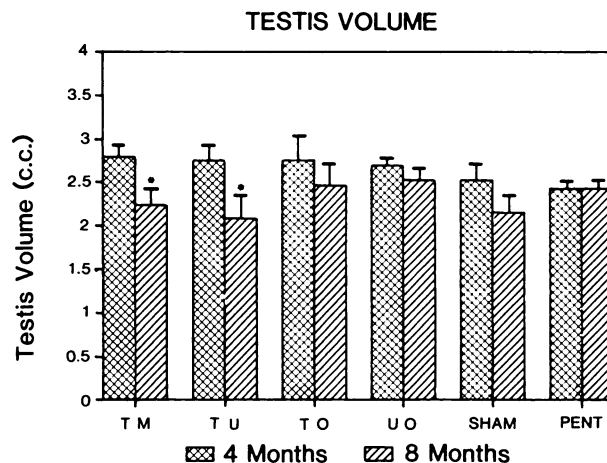


Fig. 2. Contralateral testicular volume expressed as mean \pm SE (vertical bar). Significant decreases (* $P < 0.05$) in testicular volume were recorded in group I (TM) and group II (TU) animals after 8 months, in comparison with the 4-month groups (I and II) of animals.

was, however, a significant decrease in contralateral testicular weight and volume in group I (TM) and II (TU) animals that were killed at 8 months in comparison with those killed at 4 months.

Light Microscopic and Ultrastructural Observations

Torsion Side Testes: In group I (TM), no testicular tissue was found at autopsy in five of 12 animals. In the remaining seven animals, the testis and spermatic cord were found in a twisted position; four revealed severe histologic damage and three revealed mild to moderate damage. In group II (TU), the testis was grossly atrophic in three of 12 animals, while in the remaining nine the testis, although appearing grossly normal, revealed moderate to severe histologic damage, as indicated by the presence of degenerative germ cells in the seminiferous tubules or a total loss of germ cells. In group III, where the testis had been removed 8 to 12 hours after induction of spermatic cord torsion, varying degrees of testicular changes were noticed in all 12 animals. These changes were graded from 1 to 6 based on gross appearance, such as edema and discoloration. Eight testes had damage that was graded 5 to 6, and was characterized by a dark red to blue-black color, while the other four revealed damage that was graded 2 to 4, and was characterized by swelling and a pinkish to dark red appearance. The histology of these testes revealed varying degrees of damage.

Contralateral Testis: Varying degrees of histologic alteration were recorded in the contralateral testis of the experimental group of animals (Figs. 3A-C). The most frequently observed changes in the contralateral testis of the experimental animals for both the 4- and 8-month periods included focal disorganization and exfoliation of immature germ cells in the lumen and the presence of intraepithelial vacuoles of varying sizes, sometimes extending from the basal lamina to the lumen (Fig. 3A). Sertoli cells of these affected tubules also sometimes contained numerous vacuoles, indicating the premature extrusion of the germ cells (Fig. 3B). This accumulation of vacuoles was never found in the control specimen. However, the majority of the tubules appeared normal, containing Sertoli cells and germ cells at various stages of development. Tubular profiles containing each of the 12 stages of the seminiferous epithelial cycle could be identified. When these sections were examined under the electron microscope, however, many of the apparently normal-looking tubules contained degenerative germ cells near the basal lamina and within the Sertoli cell cytoplasm. It was only in an occasional tubule that severe damage with almost complete absence of germ cells (Fig. 3C) was noted.

Quantitative Analyses

Germ Cell Number: Enumeration of the germ cells revealed a significant reduction of the germ cell population in the contralateral testes of groups I (TM), II

Fig. 3A. Light micrograph of a portion of a seminiferous tubule from the contralateral testis of an animal 8 months after unilateral torsion of the spermatic cord, showing prominent vacuoles (V) extending from the basal lamina to the tubule lumen (L) ($\times 900$).

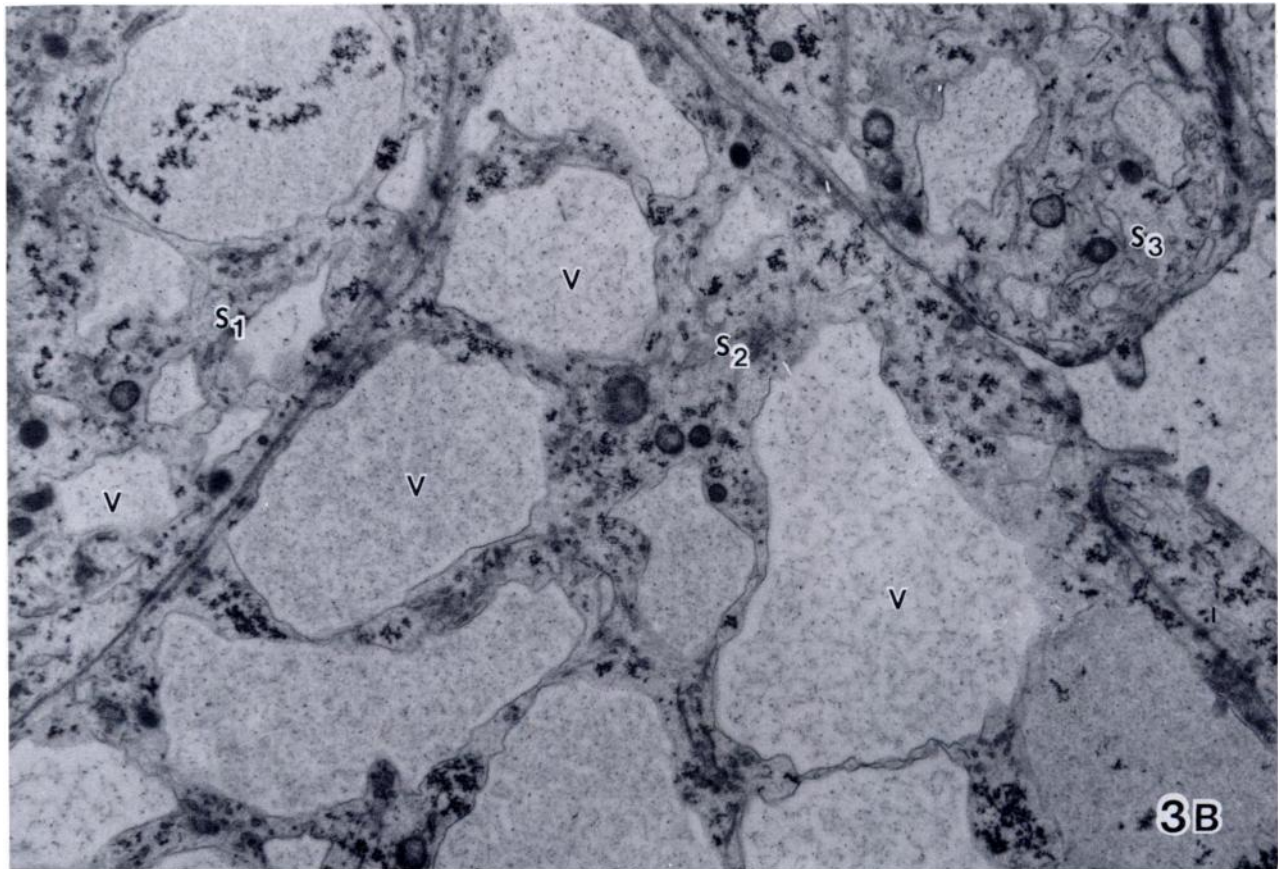
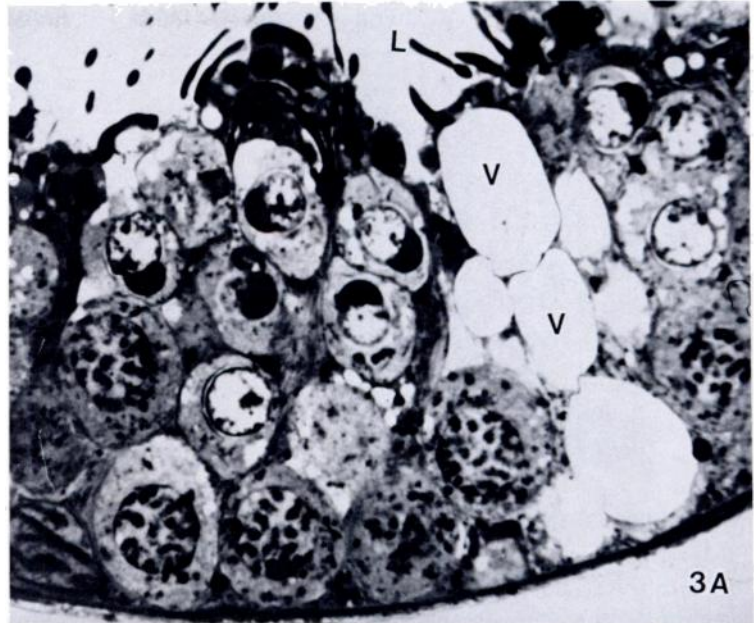


Fig. 3B. Representative electron micrograph of a portion of a seminiferous tubule showing portions of three Sertoli cells (S₁, S₂ & S₃). Note the presence of numerous vacuoles in the cytoplasm of each cell. ($\times 14,000$)

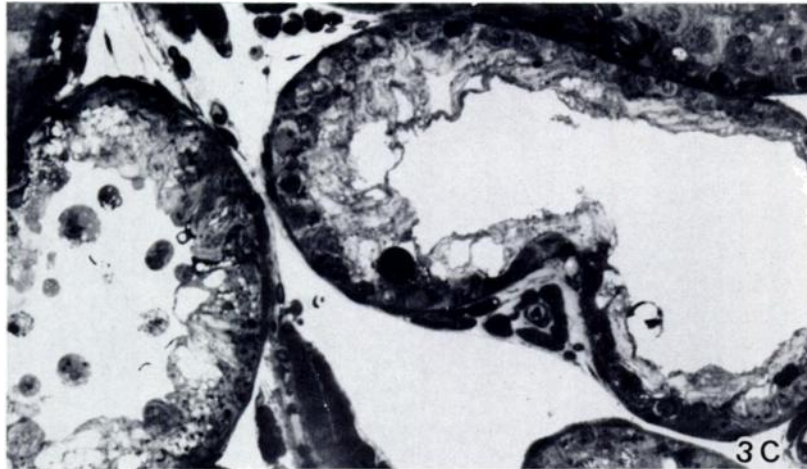


Fig. 3C. Light micrograph of parts of seminiferous tubules from the contralateral testis of an animal 8 months after unilateral torsion of the spermatic cord, showing severe germ cell degeneration (X 240).

(TU), and III (TO) animals for 4 months, and groups I (TM) and II (TU) for the 8-month period in comparison with the respective controls (Table 1). The germ cell count in the contralateral testis of group III (TO) animals approximated the control values by 8 months (Table 1).

Seminiferous Tubule Diameter: No significant change in seminiferous tubule diameter was recorded in the contralateral testis of all experimental groups of guinea pigs as compared with the control groups (Fig. 4).

Discussion

In our previous study, we found that the damage to the contralateral testis of animals with unilateral torsion was variable in different animals subjected to similar experimental conditions as well as in different areas of the same testis (Chakraborty et al, 1980; Chakraborty and Jhunjhunwala, 1982). It was felt, therefore, that simple histologic examination of focal areas of the testis may not provide accurate information about the extent of germ cell degeneration in the

TABLE 1. Germ Cell Counts of the Contralateral Testis of the Guinea Pig Under Various Experimental Conditions

Groups	Germ Cell Number/Sertoli Cell			
	Spermatocytes		Spermatids	
	4 month*	8 month*	4 month	8 month
I (TM)†	2.04 ± 0.13‡	1.70 ± 0.01	4.47 ± 0.38	3.66 ± 0.15
II (TU)†	2.17 ± 0.09	1.83 ± 0.10	4.19 ± 0.21	3.91 ± 0.20
III (TO)†	1.96 ± 0.09	2.25 ± 0.13	4.53 ± 0.13	5.42 ± 0.28
IV (UO)†	2.49 ± 0.12	2.55 ± 0.17	5.64 ± 0.26	5.83 ± 0.53
V (SHAM)†	2.51 ± 0.08	2.52 ± 0.11	5.65 ± 0.17	5.69 ± 0.22
VI (PENT)†	2.42 ± 0.10	2.47 ± 0.06	5.77 ± 0.22	5.96 ± 0.19
	Statistical Evaluation			
TM vs. PENT	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.02	<i>P</i> < 0.001
TM vs. SHAM	<i>P</i> < 0.02	<i>P</i> < 0.001	<i>P</i> < 0.02	<i>P</i> < 0.001
TM vs. UO	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.01
TU vs. PENT	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
TU vs. SHAM	<i>P</i> < 0.02	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
TU vs. UO	NS	<i>P</i> < 0.01	<i>P</i> < 0.01	<i>P</i> < 0.01
TO vs. PENT	<i>P</i> < 0.01	NS‡	<i>P</i> < 0.001	NS
TO vs. SHAM	<i>P</i> < 0.001	NS	<i>P</i> < 0.001	NS
TO vs. UO	<i>P</i> < 0.01	NS	<i>P</i> < 0.01	NS

*Duration of torsion.

†TM = torsion maintained; TU = torsion untwist; TO = torsion and orchiectomy; UO = unilateral orchiectomy; SHAM = sham operated; PENT = pentobarbital injected.

‡Mean ± SEM; NS = not significant.

contralateral testis. Systematic germ cell quantitation at least would provide some analytic data, irrespective of the presence or absence of histologic evidence of damage (Chakraborty et al, 1983). Our current findings clearly demonstrate a significant decrease in the relative number of germ cells (Table 1) in the contralateral testis of group I (TM) and group II (TU) animals, in comparison with the controls (groups IV, V, and VI). The present study, therefore, confirms our earlier reports that the retention of a damaged testis within the body has detrimental effects on the contralateral, so-called unaffected testis. These subtle changes in the focal areas of the testis should be evaluated by a germ cell quantitation procedure.

In addition to histologic examination, alteration in the mean diameter of the seminiferous tubules is another parameter used by some investigators to establish contralateral testicular damage caused by unilateral torsion (Nagler and de Vere White, 1982; Wallace et al, 1982; Cosentino et al, 1984; York and Drago, 1985). Our results do not corroborate these findings. In the present study, the diameters of the seminiferous tubules of the contralateral testes of all experimental groups for both time periods were similar to those of the controls (Fig. 4). It is possible that the seminiferous tubules of rats (used as experimental animal models by these investigators) may respond differently from those of guinea pigs. However, it must be noted that Turner (1985) did not observe any appreciable change in the contralateral testis of Sprague-Dawley rats following unilateral spermatic cord torsion. It should be mentioned here that the autoimmune response to the testicular autoantigen is different in various strains of guinea pigs and rats (Tung et al, 1981). Guinea pigs are susceptible to the testis cell-sperm differentiating autoantigens (TSDA). According to Tung et al (1981), about 50% of outbred Hartley strain guinea pigs that had been vasectomized and none of this strain that were sham-operated develop autoantibodies to TSDA. Therefore, if germ cell degeneration in the contralateral testis of guinea pigs is an autoimmune response, it is likely that this response will be different in some strains (ie, Hartley strain) of guinea pigs, when compared with the response in Sprague-Dawley rats (Bigazzi, 1977).

It is evident from the present study that even untwisting the spermatic cord within 8 to 12 hours after torsion does not protect the contralateral testis, since a significant decrease in the number of germ cells was noted in the contralateral testes of both group I (TM) and group II (TU) animals. These

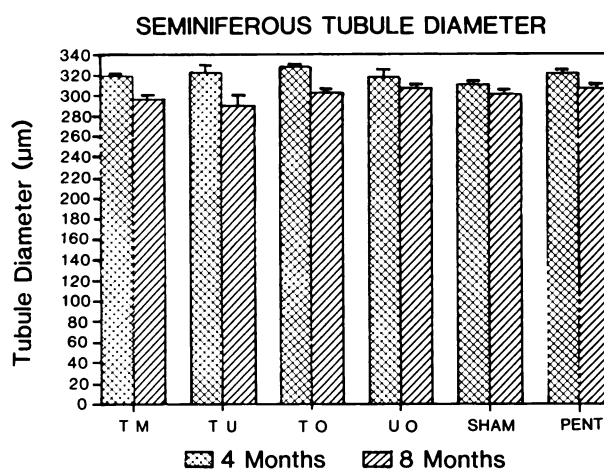


Fig. 4. Seminiferous tubule diameter in experimental and control groups of animals. No significant change was recorded in any of the groups.

degenerative changes were progressive in nature, with lower germ cell counts recorded in the 8-month group compared with the 4-month group. Thus, our findings suggest that a damaged testis, when retained in the body, continues to exert detrimental effects on the contralateral testis. These degenerative changes, however, probably are reversible after orchietomy, since in group III (TO) animals, a higher germ cell count was obtained at 8 months in comparison with 4 months. In a small number of documented human cases, the beneficial effect of early orchietomy has been controversial. In a long-term follow-up study, Bartsch et al (1980) and Mastrogiacomo et al (1982) found early orchietomy to be beneficial, while Krarup (1978) and Danner et al (1982) did not have similar experiences. These investigators were able to study only a few patients, and their impressions were based on only one or two semen analyses.

The present study shows that at least in our guinea pig model, the early removal of a damaged testis may be protective to the contralateral testis. Although other investigators feel that removal of the damaged testis will not have any beneficial effect on the contralateral testis (Saltzman et al, 1984; Turner, 1985), further long-term studies in experimental animals and humans are needed to clarify the controversy.

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International Meeting on Hormonal Therapy of Prostatic Diseases: Basic and Clinical Aspects

An international Symposium on "Hormonal Therapy of Prostatic Diseases: Basic and Clinical Aspects" will be held at the Michelangelo Hotel (Milano, Italy) from May 21 to May 24, 1986. The meeting will be planned by an International Scientific Committee formed by: Bartke A. (USA), Bruchowsky N. (Canada), Geller J. (USA), Motta M. (Italy), Robinson J. (U.K.), Serio M. (Italy), Voigt K.D. (Germany). The program will include invited lecturers as well as sessions of free communications and/or poster presentations on the following topics: The normal prostate: morphological, biochemical and hormonal aspects; The pathological prostate (BPH, Carcinoma, etc.): morphological, biochemical and hormonal aspects; Therapeutic approaches in prostatic diseases (animal and human studies). For further information regarding the program, please contact the Scientific Secretaries. For registration, travel and logistic information, please contact the Organizing Secretariat.

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