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Suppression and Recovery of Spermatogenesis Following Spinal Cord Injury in the Rat

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ABSTRACT: Recently, we reported that changes in spermatogenesis in adult rats during acute phase (within 2 weeks) of spinal cord injury (SCI) were associated with a suppression of pituitary-testis hormone axis, and these effects mimic those that occur after hormone deprivation. In this study, we examined the long-term (>4 weeks) effects of SCI on spermatogenesis and its recovery. Results of this study reveal that while serum follicle stimulating hormone, luteinizing hormone, and testosterone levels in SCI rats recovered within 1 month after the injury, their spermatogenesis continued to regress. By 3 months, spermatogenesis in 70% of SCI rats has totally regressed, characterized by the absence of proliferating spermatogonia; these effects could not be prevented by an otherwise effective regimen of testosterone treatment. Sertoli cells in the regressed seminiferous tubules exhibited unusual behavior, characterized by the formation of multiple cell layers and/or aggregates that extended into the tubular lumen. Active spermatogenesis was observed in nine of the 19 SCI rats by 6 months, seven of which had complete spermatogenesis, but with persisting abnormalities. These results demonstrate that SCI results in total, but reversible, regression of spermatogenesis. Failure to prevent such effects by an otherwise effective exogenous testosterone regimen suggests that nonendocrine factors are involved in the SCI effects on spermatogenesis. The unusual Sertoli cell localization in the regressed testes may have been triggered by the loss of proliferating spermatogonia and may be involved in subsequent spermatogenic recovery.

Key words: Sertoli cells, Leydig cells, testosterone, follicle stimulating hormone, luteinizing hormone.

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Fertility of men is often impaired after spinal cord injury (SCI) (Linsenmeyer and Perkash, 1991). Analysis of the semen of SCI men has revealed numerous abnormalities, including an increase in sperm with abnormal morphology and an overall decrease in sperm motility (Perkash et al, 1985; Ohl et al, 1989). Although two recent papers showed that sperm counts per ejaculate of SCI men was not different from those of normal, able-bodied men (Brackett et al, 1994a,b), some of the SCI men in the studied group were azoospermic with elevated serum follicle stimulating hormone (FSH) and luteinzing hormone (LH). These findings and reports of a regression of the seminiferous epithelium in testicular biopsies of SCI men (Leriche et al, 1977; Hirsch et al, 1991) indicate persisting effects of SCI on spermatogenesis. Recently, we reported that in the rat spermatogenesis became impaired after SCI (Linsenmeyer et al, 1994) and that these effects occurred at the time when testicular testosterone and serum gonadotropin levels were acutely suppressed (Huang et al, 1995). It was postulated that a suppression of the pituitary-testis hormone axis may be responsible for the acute SCI effects on spermatogenesis. In this study, we examined the long-term effects of SCI on spermatogenesis in adult rats. In addition, attempts were made to prevent the SCI effects on spermatogenesis by implantation of 10-cm testosterone capsules, which is effective in maintaining and restoring complete spermatogenesis in hypophysectomized rats (Huang et al, 1987, 1991).

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Materials and Methods

Animals

Sprague-Dawley male rats (250–275 g) purchased from Charles River (Charles River, Massachusetts) were individually caged in a light-controlled, air-conditioned animal room for 2–4 weeks until their body weight reached 375–385 g. Animals were fed Purina rat chow and water *ad libitum*.

Experiment

A total of 75 rats in three cohorts were subjected to spinal cord transection at the level of the ninth thoracic vertebra by the procedures described previously (Linsenmeyer et al, 1994). The

Table 1. *Effects of spinal cord injury on body and testis weights (mean ± SEM)*

Time after spinal cord injury†	Body weight (g)†				Testis weight (mg)	
	Sham		Spinal cord injury			
	Initial	Sacrifice	Initial	Sacrifice	Sham	Spinal cord injury
3 weeks	385 ± 18 (7)	505 ± 7	381 ± 9 (7)	410 ± 10*	1,741 ± 54	1,554 ± 101*
1 month −TC	389 ± 5 (4)	508 ± 17	403 ± 14 (6)	451 ± 10*	1,748 ± 26	1,287 ± 138**
1 month +TC	400 ± 9 (3)	496 ± 9	391 ± 6 (5)	432 ± 16**	1,623 ± 11	923 ± 134**
2 months	398 ± 4 (4)	596 ± 29	402 ± 6 (5)	611 ± 22	1,884 ± 41	1,339 ± 184**
3 months −TC	397 ± 8 (8)	647 ± 14	392 ± 4 (10)	636 ± 26	1,972 ± 45	1,033 ± 85**
3 months +TC	389 ± 10 (4)	535 ± 25	391 ± 4 (3)	576 ± 23	1,504 ± 62	701 ± 6**
6 months	396 ± 4 (17)	678 ± 22	382 ± 9 (19)	525 ± 15*	1,890 ± 57	1,037 ± 77**

* $P < 0.05$, ** $P < 0.01$, compared to sham control rats.
† Number of animals shown in parentheses.
‡ −TC, without 10-cm testosterone capsule implant; +TC, with 10-cm testosterone capsule implant.

control animals ($n = 50$) were sham operated without laminectomy. The testes of these animals were palpated periodically after the surgery to monitor the status of spermatogenic activity. Animals were killed by decapitation 2 weeks to 6 months after the surgery, and trunk blood was collected. Serum was separated by centrifugation and stored at −70°C for the measurement of FSH, LH, and testosterone. In addition, seven sham control rats and 10 SCI rats were given subcutaneous implants of 2 × 5-cm testosterone capsules (TC) (Huang et al, 1987; 1991) immediately after the surgery. These rats were killed 1 or 3 months later. The animal procedures were approved by the Institutional Review Board of both UMD-New Jersey Medical School and East Orange V.A. Medical Center.

Testicular Histology and Morphometry

One-half of each testis with 3–5 cuts on the capsule was fixed in Bouin's solution for 2–4 hours. The middle one-third of the testis was subsequently dissected and fixed in the same fixative for 2–4 days and processed for histology. Five-micron-thick sections were stained with periodic acid Schiff's reagent (PAS) and counterstained with hematoxylin (Preece, 1972). The stages of the seminiferous epithelium were identified according to the development of PAS-positive acrosomes on spermatids (Leblond and Clermont, 1952). The normality of spermatogenesis was evaluated in sections obtained from two or three locations on each testis. The number of tubules in such section was estimated to be between 200 to 400. The criteria of evaluation include completeness of cellular associations in each seminiferous stage and the location and gross appearance of spermatogenic cells. In addition, testicular tissues from rats killed 4 weeks after surgery were also processed for whole mounts of seminiferous tubules (Huckins, 1971). The type B spermatogonia and type A1 spermatogonia preleptotene spermatocytes in stages V–VI and VII–IX tubules, respectively, were identified according to the criteria described by Huckins (1971) and enumerated as reported previously (Huang and Nieschlag, 1986; Huang and Boccabella, 1988).

Serum Hormone Measurement

Serum concentrations of FSH and LH were determined by double-antibody radioimmunoassays as described previously (Huang et al, 1987, 1991). Reagents, provided by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (NIADDK, Bethesda, Maryland), and NIADDK rat FSH RP-2, FSH-I5, anti-rat FSH S-11, rat LH RP-2, rat LH I-6, and anti-rat LH S-7 were used for FSH and LH assays. The sensitivities of the assays (95% binding) were 0.15 ng/ml for LH and 2.6 ng/ml for FSH. The inter- and intraassay coefficients of variation were approximately 12 and 8%, respectively, for both assays. To avoid inter-assay variation, all samples from two identical experiments were measured for FSH or LH in single assays.

Serum testosterone concentrations were determined by radioimmunoassay in ether extracts without chromatography (Huang et al, 1991), using antiserum provided by ICN Immunobiological (Lisle, Illinois). The sensitivity of this assay was 2 pg/tube, and the intra- and inter-assay coefficients of variation were 5 and 12%, respectively.

Statistics

Serum hormone data were analyzed to determine that they were normally distributed and then analyzed with a 4 (time points) × 2 (treatment groups) analysis of variance. When the treatment-by-time interactions were significant ($P < 0.05$), Dunn's tests were used to determine the significance of differences among the treatment groups. Student's t-test was used to determine the significance of the difference in spermatogonial number between SCI rats and sham-operated controls.

Results

Body and Testis Weight

During the first month after the surgery, the body weight of SCI rats at the time of sacrifice was significantly lower than that of sham-operated control rats ($P < 0.05$, Table 1). Thereafter, body weight of the SCI rats at sacrifice was comparable to that of sham control rats at 2 and 3 months ($P > 0.10$) but became lower ($P < 0.05$) at 6 months.

SCI surgery resulted in a significant decrease in testis weight during the first 3 months ($P < 0.05$), and this effect was facilitated by implantation of 10-cm TC that also resulted in a 10 and 25% decrease in testis weight

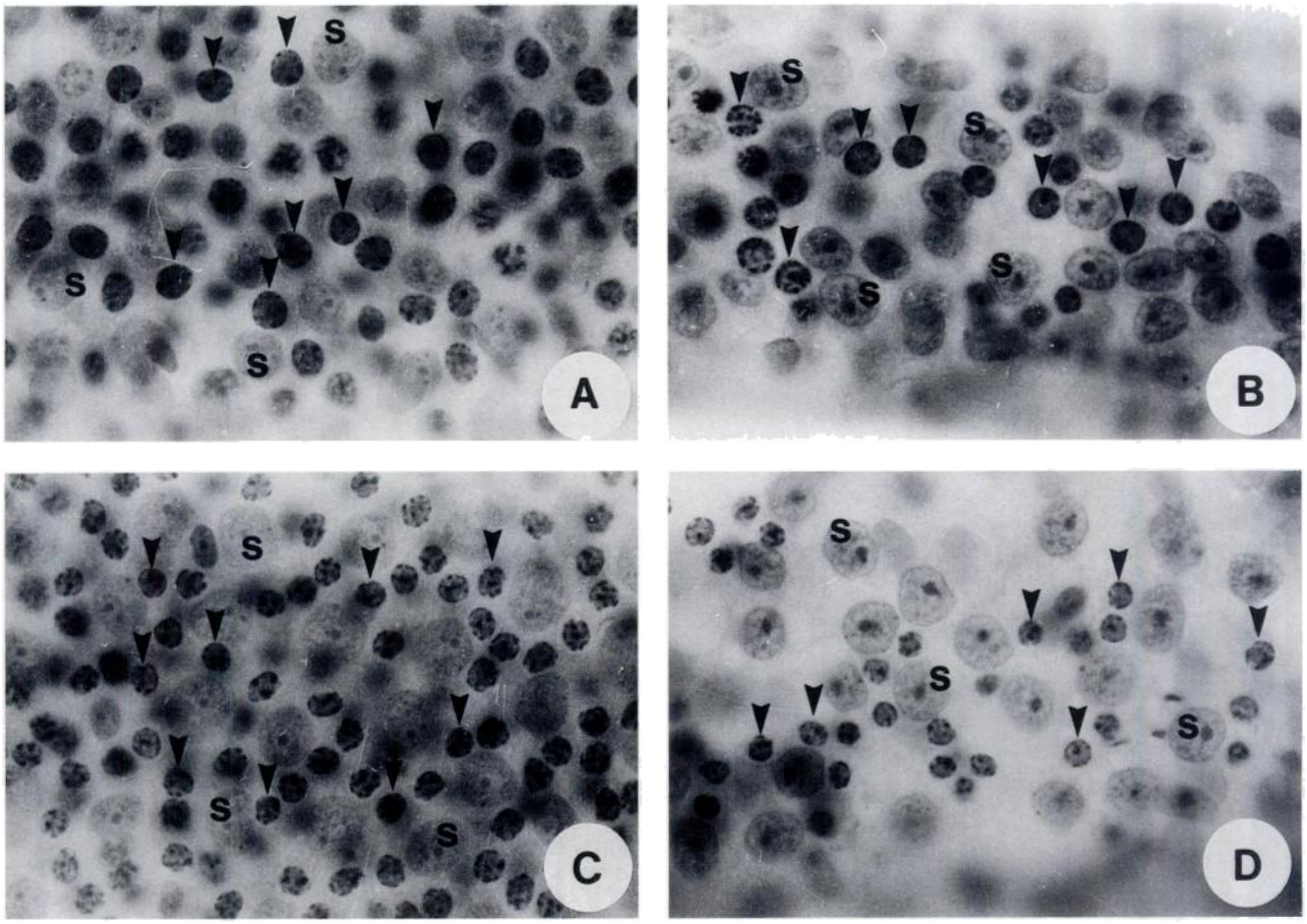

FIG. 1. Whole mount preparation of seminiferous tubules. **(A)** Sham-operated control rat showing the distribution of type B spermatogonia (arrow heads). S, Sertoli cell nucleus. **(B)** Rat killed 1 month after spinal cord injury (SCI). Note that the number of type B spermatogonia (arrow heads) was apparently reduced. S, Sertoli cell nucleus. **(C)** Sham-operated control rat showing the distribution of preleptotene spermatocytes (arrow heads). S, Sertoli cell nucleus. **(D)** Rat killed 1 month after SCI. Note that the number of preleptotene spermatocytes (arrow heads) was apparently reduced. S, Sertoli cell nucleus. 350×.

in sham-operated controls at 1 and 3 months, respectively (Table 1). Six months after the surgery, the mean testis weight of SCI rats was not different from that of SCI rats killed at 3 months. However, there was wide variation among animals as well as between two testes of the same animal. The weight of testes in which seminiferous epithelium remained totally regressed (779 ± 24 mg, n = 25) was not different from that of the regressed testes of SCI rats killed at 3 months (807 ± 58 mg, n = 8). The weight of testes showing active spermatogenesis at 6 months (1,575 ± 90 mg, n = 13) was significantly higher than the weight of those that remained regressed ($P <$ 0.01, unpaired t-test) and the weight of regressing testes measured at 3 months (1,244 ± 70, n = 12, $P < 0.05$).

Spermatogenesis

Complete spermatogenesis was maintained in most of the SCI rats 1 month after the injury. However, spermatogenic lesions that we have reported previously (Linsenmeyer et al, 1991; Huang et al, 1995), such as delayed spermiation, vacuolization of germ cell nuclei, incomplete spermatogenesis, and, frequently, regression of seminiferous epithelium, were also noted in the tubules of these animals. Examination of whole mounted seminiferous tubules of SCI rats 4 weeks after the surgery revealed a marginal decrease ($P < 0.10$) in the number of type A1 spermatogonia and a significant decrease in the number of type B spermatogonia and preleptotene spermatocytes in SCI rats ($P < 0.05$, Fig. 1; Table 2).

Three months after SCI, palpation of the testes revealed a greater than 50% reduction in testicular size in all SCI rats, and all testes became flaccid. At least one testis in seven of the ten SCI rats killed at this time exhibited total regression of the seminiferous epithelium in >95% of the tubules observed. In these tubules, spermatogenic cells, including the six generations of proliferating spermatogonia (A1–B), were no longer present (Fig. 2A); Sertoli cells, few spermatogonia, and some

Table 2. *Effect of spinal cord injury on the number of spermatogonia and preleptotene spermatocytes 4 weeks after the surgery†*

	n	A1 spermatogonia	B spermatogonia	Preleptotene spermatocyte
Spinal cord injury	5	11.4 ± 1.2*	123 ± 9**	239 ± 9**
Sham control	5	15.5 ± 1.2	151 ± 3	290 ± 7

* $P < 0.10$, ** $P < 0.05$, compared with sham control rats.
†Numbers of spermatogonia and preleptotene spermatotcytes expressed as mean ± standard error of the mean per 100 Sertoli nucleoli.

residual preleptotene spermatocytes were that all remained (Figs. 2B, 3B). In the remaining testes, including those of the other three SCI rats, spermatogenesis was regressing, or had regressed, in most tubules even though spermatogenesis was seen in some tubules (Fig. 2C). The regressing epithelium was characterized by disorganized and incomplete cellular associations, vacuolization of epithelium, and the presence of polynucleated cell mass with pyknotic nuclei and cellular debris. Implantation of 10-cm TC maintained qualitatively complete spermatogenesis in sham control rats (data not shown) but failed to prevent the regression of the seminiferous epithelium in SCI rats (Fig. 2D). In fact, spermatogenic regression in SCI rats may have been facilitated by exogenous testosterone, because the decrease in testis weights in SCI rats was enhanced by TC implantation.

Six months after the injury, active spermatogenesis, ranging from reappearance of proliferating spermatogonia and meiotic cells in well-organized epithelium to the presence of qualitatively complete spermatogenesis (Fig. 3A), were observed in 9/19 rats in three cohorts of SCI animals. The frequencies of tubules showing active spermatogenesis were estimated to be between 16 and 100% (56 ± 10%) among animals. The extent of spermatogen-

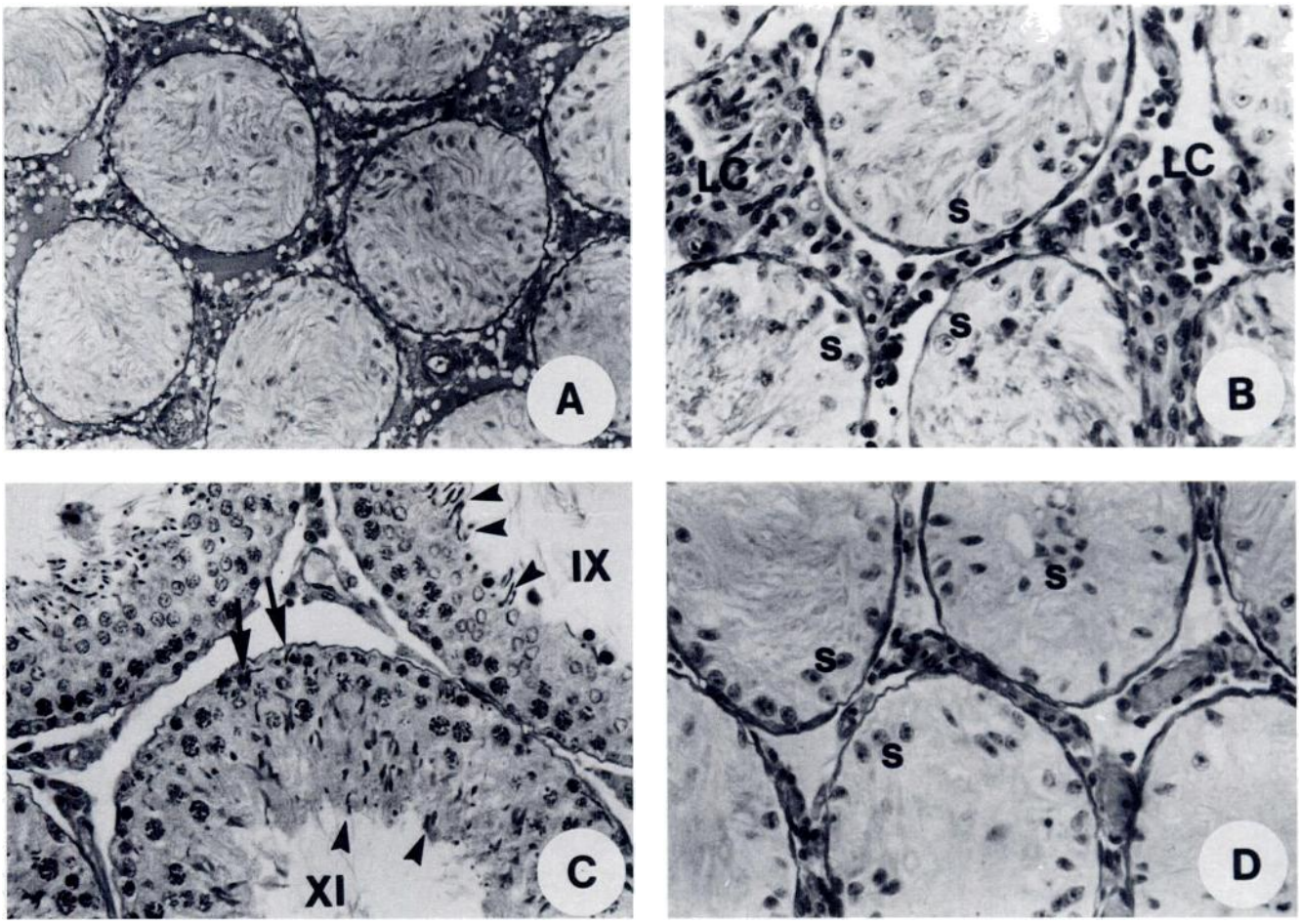

FIG. 2. Rats with spinal cord injury (SCI) killed 3 months after the operation. (**A**) Seminiferous tubules of a rat showing total regression of the seminiferous epithelium. 40×. (**B**) Enlarged view of the seminiferous tubules of another SCI rat showing the absence of proliferating spermatogonia. LC, Leydig cells; S, Sertoli cells. 80×. (**C**) Seminiferous tubules of another SCI rat showing the persistence of spermatogenesis. The presence of mature spermatids (arrow heads) at the luminal edge of stages IX and XI epithelium illustrates a failure in normal spermiation. The presence of sperm heads at the basement membrane (arrows) further suggests that some of the mature spermatids may have been phagocytosed. 80×. (**D**) Seminiferous tubules of an SCI rat with 10-cm testosterone capsule implant showing the lack of preservation of spermatogenesis. S, Sertoli cells. 80×.

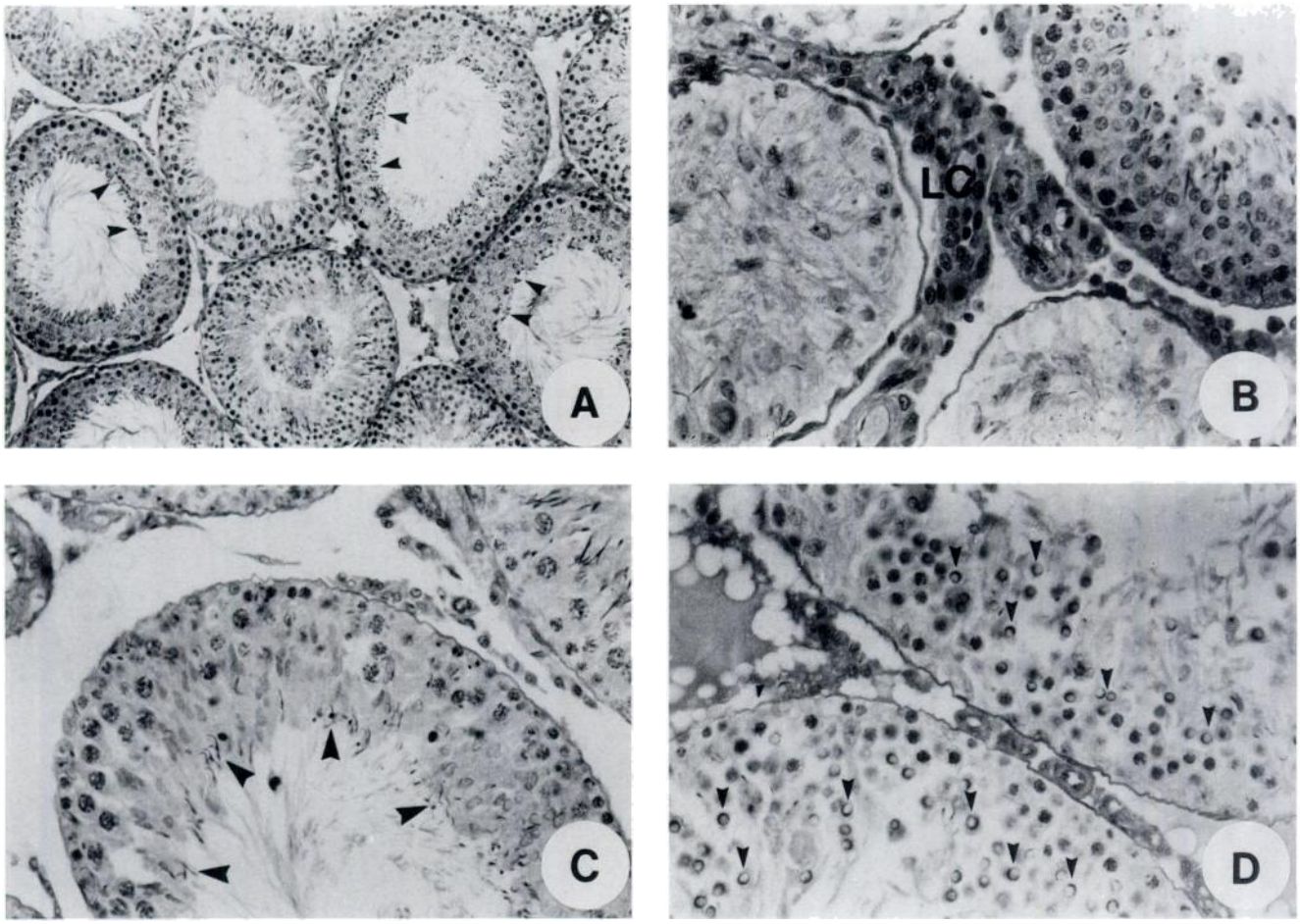

FIG. 3. Restoration of spermatogenesis in rat with spinal cord injury (SCI) killed 6 months after the injury. (**A**) The presence of mature spermatids (arrow heads) at the luminal edge of stages VII–VIII epithelium illustrates the restoration of completion of spermatogenesis. 25×. (**B**) Another SCI rat showing the partial recovery of spermatogenesis. LC, Leydig cells. 80×. (**C**) Portion of a stage X tubule showing the presence of mature spermatids at the luminal edge (arrow heads). This denotes a failure in normal spermiation. 100×. (**D**) Two adjacent stages VII–VIII tubules showing nuclear vacuolization of the young spermatids (arrow heads). The absence of mature spermatids in these tubules demonstrates failure in the second half of spermiogenesis. 100×.

ic recovery within the same testis also varied widely (Fig. 3B). In addition, abnormalities, including delay or failure of spermiation, as suggested by the presence of mature spermatids in the luminal edge of stages IX–X epithelium (Fig. 3C), vacuolization of spermatid nuclei, and incomplete cellular association (Fig. 3D), persisted. Spermatogenesis in four SCI rats of the second cohort remained totally regressed 12 months after the injury (data not shown).

Sertoli Cells

Regression of spermatogenesis in chronic SCI rats was associated with unusual behavior of the Sertoli cells. In many (20–30%) of the regressed tubules, Sertoli cells appeared in multiple layers at the peripheral of the tubules or extended toward the lumen of the tubules (Fig. 4A,B). In addition, Sertoli cells formed aggregates or clusters that frequently extended into, and sometimes across, the lumen of the tubule (Fig. 4C,D). This phenomenon occurred randomly among neighboring tubules and was observed in all seven SCI rats showing total regression of seminiferous epithelium 3 months after injury and in all 10 SCI rats lacking active spermatogenic activity 6 months after the injury.

Serum Hormone Levels

Serum gonadotropin levels in SCI rats at various times after injury are presented in Table 3. Serum concentrations of FSH, LH, and testosterone in SCI rats were not different from those in the sham-operated controls at any time points. Implantation of 10-cm TC resulted in a threefold to fivefold increase in serum testosterone and a 20–50% decrease in serum FSH and LH levels ($P < 0.05$) in both sham-operated control and SCI rats.

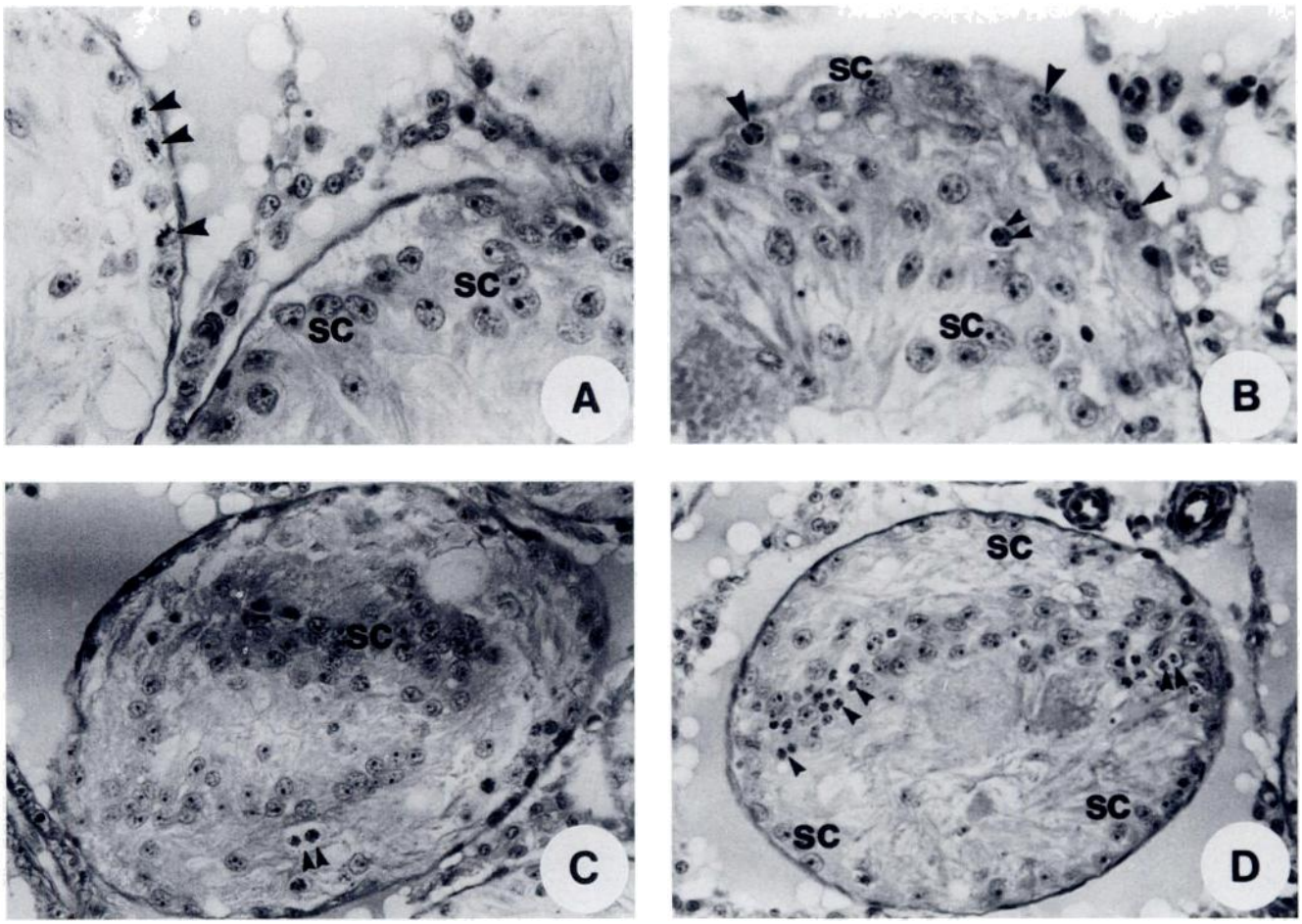

FIG. 4. Unusual Sertoli cell behavior in rats with spinal cord injury (SCI). **(A)** Portion of a tubule showing multiple layers of Sertoli cell (SC) in the periphery of the tubule. The cell type showing the mitotic figures (arrow heads) in the adjacent tubule cannot be identified. 450×. **(B)** Portion of a tubule showing the protrusion of multiple layers of SCs toward the center of the tubule. Note the presence of preleptotene spermatocytes (arrow heads) in the periphery of the tubule and among SCs departing from the basement membrane of the tubule (double arrow heads). 450×. **(C)** Within this cross section, there were at least 40 Sertoli cells SCs in the aggregate that extended into the lumen of the seminiferous tubule. Note the presence of dividing cells (double arrow heads) among these SCs. 200×. **(D)** This micrograph shows a significant number of spermatogenic cells, presumabely preleptotene spermatocytes (arrow heads) and dividing cells (double arrow heads) that were associated with SC aggregate extending across the tubule. The presence of a single layer of SCs in the periphery of the tubule indicates that the SC aggregate was not an artifact. 200×.

Discussion

The extent of spermatogenic regression in SCI rats, including the disappearance of proliferating spermatogonia, is far more severe than that occurring after hypophysectomy or androgen deprivation (Huang et al, 1987, 1991; Huang and Boccabella, 1988). This regression occurred in the presence of relatively normal serum gonadotropins and testosterone and was not prevented by an otherwise effective exogenous testosterone regimen. These results suggest that nonhormonal factors are involved in the SCI-related regression of spermatogenesis. Nevertheless, enhancement of spermatogenic regression in the presence of reduced serum FSH, LH, and testosterone in SCI rats receiving TC implants suggests that hypogonadotropic hypoandrogenism during the acute phase of SCI may have also contributed to the regression of spermatogenesis.

The presence of a reduced number of type B spermatogonia and preleptotene spermatocytes 1 month after the injury demonstrates the persistence of impaired spermatogonial proliferation, consistent with our earlier findings (Linsenmeyer et al, 1994). Previously, it was reported that in intact rats, 25% of normal intratesticular testosterone concentration was sufficient to support normal numbers of A_{al} and A_1 spermatogonia in the presence of 50% normal serum FSH levels (Huang and Nieschlag, 1986; Huang and Boccabella, 1988). On the other hand, 25% normal testicular testosterone maintained only 50–60% of the normal number of A_{al} and A_1 spermatogonia in hypophysectomized rats without detectable serum FSH (Huang et al, 1987). These results suggest that FSH may be important for the proliferation of the stem cells or undifferentiated spermatogonia when testicular testosterone level is drastically reduced. It is postulated that an acute

Table 3. *Chronic effects of spinal cord injury on serum levels of gonadotropins and testosterone*†

	FSH		LH		Testosterone	
Time after SCI	Sham	SCI	Sham	SCI	Sham	SCI
3 weeks	9.1 ± 0.7 (5)	8.1 ± 0.3 (6)	0.32 ± 0.02	0.32 ± 0.03	1.6 ± 0.3	1.6 ± 0.4
1 months −TC	8.0 ± 1.3 (7)	10.9 ± 0.5 (7)	0.33 ± 0.01	0.30 ± 0.02	2.4 ± 0.5	2.1 ± 0.2
1 month +TC	6.2 ± 0.7 (3)*	5.7 ± 0.5 (5)*	0.23 ± 0.02*	0.22 ± 0.02*	7.8 ± 0.3*	6.0 ± 0.3*
2 months	10.5 ± 0.7 (4)	12.8 ± 0.4 (4)	0.38 ± 0.02	0.41 ± 0.01	1.9 ± 0.7	1.6 ± 0.4
3 months −TC	10.7 ± 0.4 (6)	9.9 ± 0.5 (5)	0.42 ± 0.02	0.40 ± 0.02	1.9 ± 0.4	2.6 ± 0.5
3 months +TC	4.2 ± 0.7 (4)*	3.9 ± 0.3 (3)*	0.22 ± 0.02*	0.21 ± 0.02*	10.3 ± 0.9*	6.4 ± 1.1*
6 months	10.9 ± 0.4 (13)	10.7 ± 0.4 (14)	0.50 ± 0.06	0.42 ± 0.02	1.2 ± 0.1	1.0 ± 0.1

FSH, follicle stimulating hormone; LH, luteinizing hormone; SCI, spinal cord injury; −TC, without 10-cm testosterone capsule implant; +TC, with 10-cm testosterone capsule implant.

* Significantly different from those without TC implants at the same time point; $P < 0.05$.

†Serum levels expressed as mean ± standard error of the mean (ng/ml). Number of animals shown in parentheses.

decrease in serum gonadotropins and testosterone after SCI (Huang et al, 1995), in conjunction with the lack of normal neural impulses and perhaps a decrease in the availability of other serum factors resulting from decreased testicular blood flow (Linsenmeyer et al, 1996), may alter the renewal and proliferation of the stem cells. This may result in a loss of undifferentiated spermatogonia and eventual disappearance of proliferating spermatogonia and regression of seminiferous epithelium. A slight but significant decrease in the number of A_1 spermatogonia (among preleptotene spermatocytes) in SCI rats 4 weeks after the injury supports this possibility.

Reappearance of active and qualitatively complete spermatogenesis in SCI rats 6 months after the injury is consistent with early findings in testicular denervated animals (Clark, 1933; King and Langworthy, 1940). These results demonstrate the persistence of viable stem cells that apparently were responsible for the repopulation of the seminiferous epithelium in chronic SCI rats. The source of variation in spermatogenic recovery among animals and among tubules within animals is obscure, but the extent of variation is consistent with sperm production among SCI men (Perkash et al, 1985; Ohl et al, 1989) and heterogenicities in histologic appearance among testicular biopsies of SCI men (Leriche et al, 1977; Hirsch et al, 1991). Although all SCI rats were paralyzed, they were able to move inside the cage, as did the control animals. Although the stress-related factors may contribute to spermatogenic regression and repopulation among animals, variations in spermatogenic activity among tubules within the same testis indicate otherwise. In addition, all SCI rats gained weight after the surgery, and their body weights at the time of sacrifice were not significantly different from those of age-matched, sham-operated control rats 2–3 months after the injury. Therefore, the variation in spermatogenic recovery cannot be ascribed to differences in the extent of stress, motor functions, or nutritional factors. In animals exposed to ionizing radiation or chemotherapeutic agents, the delayed and randomized restoration of spermatogenesis (Meistrich et al, 1978; Meistrich, 1982) is believed to be dictated by survival of the stem cells and their subsequent proliferation and differentiation. Recently, Meistrich et al (1996) found that suppression of testicular testosterone prior to chemotherapy may facilitate protection of the stem cells against chemotherapy. These findings suggest that modification of Sertoli cell paracrine function may be involved in the maintenance of stem cell survival, renewal, and differentiation after chemotherapy (Meistrich et al, 1996). Thus, variations in spermatogenic recovery in SCI rats may also be dictated by the survival, renewal, and subsequent proliferation/differentiation of the stem cells, or the extent of Sertoli cell damage and recovery, or a combination of both.

The persistence of abnormal spermiogenic cells in chronic SCI rats after an extensive recovery period demonstrates a prolonged effect of SCI on spermatogenesis. This likely results from dysfunctioning Sertoli cells, because genetically defective spermatogenic cells are usually eliminated prior to spermiogenesis. Previously, Tash et al (1980) and Welsh et al (1980) reported modulation of Sertoli cell cAMP and protein kinase inhibitor production by isoproterenol, a β adrenergic agonist, suggesting that neural factors may be involved in Sertoli cell regulation. Thus, Sertoli cells in SCI rats perhaps were unable to support normal spermatogenic differentiation due to the lack of normal neural impulses. These abnormal spermatids may result in sperm with abnormal morphology, low motility, or poor viability. This may explain the poor sperm motility, the increase in sperm with abnormal morphology, and the overall decrease in sperm count commonly seen in the semen of SCI men (Perkash et al, 1985; Ohl et al, 1989; Brackett et al, 1994).

Protrusion of clusters of Sertoli cells into the tubular lumen has not been observed in other animal models with severe spermatogenic regression, such as during chronic vitamin A deficiency, or after chemotherapeutic treatment. This phenomenon cannot be explained by the

shrinkage of the tubules, because a single layer of peripheral Sertoli cells remained in most of such tubules (see Fig. 4D). Although this phenomenon may reflect an artifact resulting from tissue processing, this possibility is rather remote because it occurred randomly among the neighboring tubules in areas at least 2 mm away from the original cutting sites. The presence of dividing cells (presumably spermatogenic cells) among these Sertoli cell aggregates suggests that these aggregates may be functional. This unusual Sertoli cell morphology may have been triggered by the loss of proliferating spermatogonia or the absence of normal neural impulses. Unusual Sertoli cell morphology, described as "hyperplasic," in the testes of SCI men had been reported previously (Leriche et al, 1977).

Recent studies demonstrated that complex paracrine feedback mechanisms involving growth factors, cytokines, neural peptides, and steroid metabolites play an important role in the regulation of cellular activities in both spermatogenic cells and somatic (Sertoli and Leydig) cells (Spiteri-Grech and Nieschlag, 1993). In the testes of SCI rats, the disappearance of proliferating spermatogonia and the absence of normal neural impulses may perturb the activities of Sertoli and Leydig cells. These changes may result in or are associated with an altered local paracrine balance, which in turn may facilitate the renewal and/or proliferation of the stem cells and subsequent restoration of spermatogenesis. In this regard, both Sertoli and Leydig cells produce activin (de Jong and Robertson, 1985; Shaha et al, 1989) that was reported to stimulate the proliferation of spermatogonia (Mather et al, 1990) as well as the proliferation and differentiation of the stem cells in various tissues (de Kretser and Robertson, 1989; Yu et al, 1989).

In summary, this study demonstrates that surgically induced SCI in the rat results in total regression of the seminiferous epithelium, characterized by the disappearance of proliferating spermatogonia and unusual morphology of Sertoli cells. Active spermatogenesis was noted in 47% of SCI rats 6 months after SCI surgery, demonstrating that the SCI-induced spermatogenic regression is reversible. The extent of spermatogenic recovery in SCI rats may be dictated by the survival and renewal of the stem cells, and paracrine feedback mechanisms among Sertoli cells and spermatogenic cells may be involved in these processes.

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