

# Histopathology of Prepubertal Rat Testes Subjected to Various Durations of Spermatic Cord Torsion

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Sixty prepubertal rats were subjected to unilateral spermatic cord torsion for a duration of 0, 1, 3, 5, 9 or 12 hours. At the end of this period, the ipsilateral testes either were removed for immediate processing or subjected to detorsion and orchiopexy, followed by a six-week recovery period prior to histologic study. Twelve histologic parameters were each scored according to the degree of pathologic findings, thus allowing for a quantitative assessment of testicular damage. The sequence of specific histologic degeneration that occurred with spermatic cord torsion is described. These changes were found to be dependent on the duration of torsion, with the greatest damage occurring after three hours or more. In the animals undergoing detorsion followed by a six-week recovery period, severe degeneration was noted for all durations of torsion studied. The extent of this degeneration was significantly correlated with a reduction in fertility.

**Key words:** spermatic cord torsion, rat, testicular pathology.

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In recent years, a great deal of attention has been focused on the effects of unilateral spermatic cord torsion on the contralateral testis and on fertility (for reviews see: Cosentino et al, 1984, 1985; Cosentino and Rabinowitz, 1985). Little is known, however, about the effects of unilateral spermatic cord torsion on the ipsilateral testis.

The first extensive series of studies on testicular

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blood supply and the effects of ischemia on mammalian testicular histology were reported by Harrison and coworkers (Harrison, 1949, 1952, 1956; Harrison and Weiner, 1949; Harrison and Oettle, 1950; Oettle and Harrison, 1952). They observed that in the rat, ischemia of about one hour's duration produced spermatogenic destruction with only Sertoli cells remaining in the seminiferous tubules. However, Steinberger and Tjioe (1969) did not see these changes until after 2 hours of ischemia. Furthermore, Smith (1955), working with dogs, observed that spermatogenic cells were slightly damaged by 2 hours of ischemia, with severe damage occurring only after 4 hours. These apparent discrepancies are probably due to the different methods employed and the different places where ischemia was induced (Harrison, 1949), as well as the various times after ischemia that the tissues were observed (Tjioe and Steinberger, 1970). Cerasaro et al. (1984), for example, recently noted that severe contralateral damage occurred in rats subjected to unilateral torsion, while no damage was seen in those animals undergoing ligation of the spermatic vessels. Since our concern is with spermatic cord torsion, which initially occludes the veins but not the arteries (Skoglund et al, 1970), we induced ischemia in the present study using 720 degrees of spermatic cord torsion. Until recently, experimental evidence for the duration of prepubertal, unilateral spermatic cord torsion that reduces

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subsequent fertility was absent from the literature (Cosentino et al, 1984). In that study, we noted that the sharpest decline in the fertility parameters occurred between 5 and 9 hours of torsion, and that this decline was minimized if the damaged testis was removed at the end of the torsion period. However, the specific histologic changes occurring with spermatic cord torsion over time, and the fate of the ischemic organ after the re-establishment of circulation with detorsion and orchiopexy, is unknown. The present study, therefore, was undertaken to observe the effects of various durations of prepubertal spermatic cord torsion on the histology of the ipsilateral testes of rats, and to note any regeneration that might occur after reduction of the torsion followed by a six-week recovery period.

## Materials and Methods

### Animals

Sixty prepubertal (35-day-old) male and 60 adult female Sprague-Dawley rats were used. The animals were obtained at 25 days of age and allowed to acclimatize for 10 days. All animals were housed at 21 C with a 12-hour light: 12-hour dark ratio, and were fed standard feed pellets (Purina, St. Louis, MO) and water *ad libitum*.

### Induction of Torsion

The animals were randomly separated into six groups, each of which was anesthetized with sodium pentobarbital (2.4 mg/100 g, ip). One testis of each animal was exposed through a scrotal incision and subjected to 720 degrees of unilateral spermatic cord torsion for various periods of time (0, 1, 3, 5, 9, or 12 hours). The testis was replaced into the scrotum and fixed in place both medially and laterally using 6-0 silk suture, and the scrotum was closed with wound clips. Control animals (0 hours of torsion) had one testis exposed for 15 minutes, then similarly replaced into the scrotum with orchiopexy. At the end of the appropriate interval, five animals from each group underwent detorsion of the ipsilateral testis, while the remaining five

animals had the ipsilateral testis removed and processed for histologic examination (short-term). The male rats were then maintained in our vivarium for 21 days after the surgery until they were 56 days old, so as to attain puberty for a 21-day period of fertility testing.

### Fertility Tests

At 56 days of age, each male was housed with two adult female rats for 21 days, thus exposing each male rat to approximately nine female reproductive cycles. At the end of this period, the female rats were killed by an overdose of ether and examined for gravidity. If the male rat impregnated either of his mates he was considered fertile.

### Histologic Scoring

As described above, the ipsilateral testis was removed at the end of the torsion period in one-half of the animals, and was prepared for histologic examination. At the end of the 3-week mating period (77 days after the induction of torsion/detorsion; ie, long-term), the ipsilateral testis of each male rat was removed, sliced, immediately placed in Bouin's fixative, and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin and observed under a microscope. The histologic parameters studied are listed in Table 1. Each testis was scored from 0 to 4 for each parameter, with the higher numbers indicating a more widely distributed pathologic condition for that parameter within the tissue section being studied. We have previously reported a detailed description of this technique (Cosentino et al, 1985). The mean seminiferous tubular diameter was calculated to within 5  $\mu$  for each testis. This was done by averaging the diameters of ten round seminiferous tubules randomly selected in each tissue section. A tubule was considered round (cut in cross section) if the height was within 20  $\mu$  of the width. The mean coefficient of variation for seminiferous tubular diameters within a section was 8.11%  $\pm$  0.41. All specimens were coded to prevent observer bias.

### Statistical Analyses

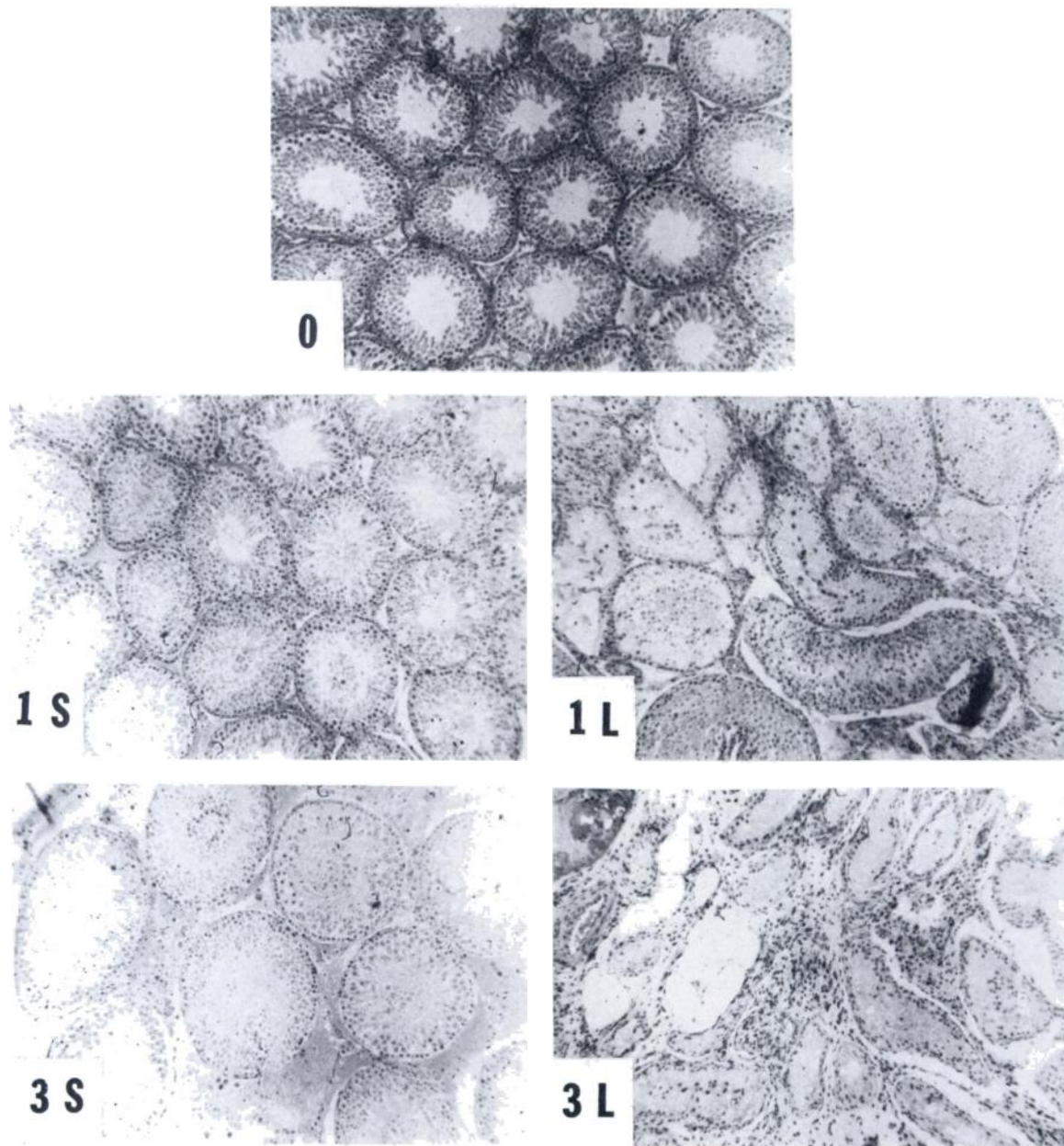
Data were analyzed for differences among groups (different periods of torsion) using analysis of variance followed by Fischer's test for least significant difference. Differences between histologic scores of immediate post-torsion samples and those of testes undergoing subsequent detorsion and a six-week recovery period were analyzed using paired t tests. Analysis of fertility data was done using a binomial chi-square test. Pearson's product-moment correlations were used to analyze relationships between histology scores and periods of torsion or male rat fertility.

## Results

The gross appearance of the traumatized organ after one hour of spermatic cord torsion showed some discoloration due to hemorrhage, which was typically near the dorsal pole of the testes. The degree of hemorrhagic infarction increased with the duration of torsion.

TABLE 1. Histologic Parameters Examined

Seminiferous Tubules
Loss of spermatozoa and spermatids
Absence of germ cell layers
Degeneration of germ cell layers
Disarray of germ cell layers
Rupture of tubules
Leydig cell reactions at ruptured tubules
Thickness of myoid cell layer
Interstitialium
Leydig cell proliferation
Edema
Hemorrhage
Granuloma
Fibrosis



**Fig. 1A.** The microscopic appearance of the rat testis immediately following (S) spermatic cord torsion of various durations and after six weeks following reduction of the torsion (L). Numbers indicate duration of torsion in hours. Short-term (1S and 3S) and long-term (1L and 3L) histologic findings after 0 to 3 hours of torsion (0 = testis of a normal control animal). ( $\times 70$ )

Both the short-term and long-term effects of spermatic cord torsion on testicular histologic findings were studied (Fig. 1). The short-term effects (immediately following the torsion period) consistently displayed damage that increased in severity with longer periods of torsion (Figs. 1A and 1B). This also was the case in those testes undergoing subsequent detorsion and a six-week recovery period (long-term effects) (Figs. 1A and 1B). It is of particular interest to

note that in all cases the long-term changes were consistently more pathologic than the short-term changes.

In order to quantitate the specific histologic changes occurring in the testis with spermatic cord torsion, we used the scoring system described above to obtain a graphic representation of each parameter with respect to the duration of torsion. At the end of the torsion periods, the following parameters had histol-

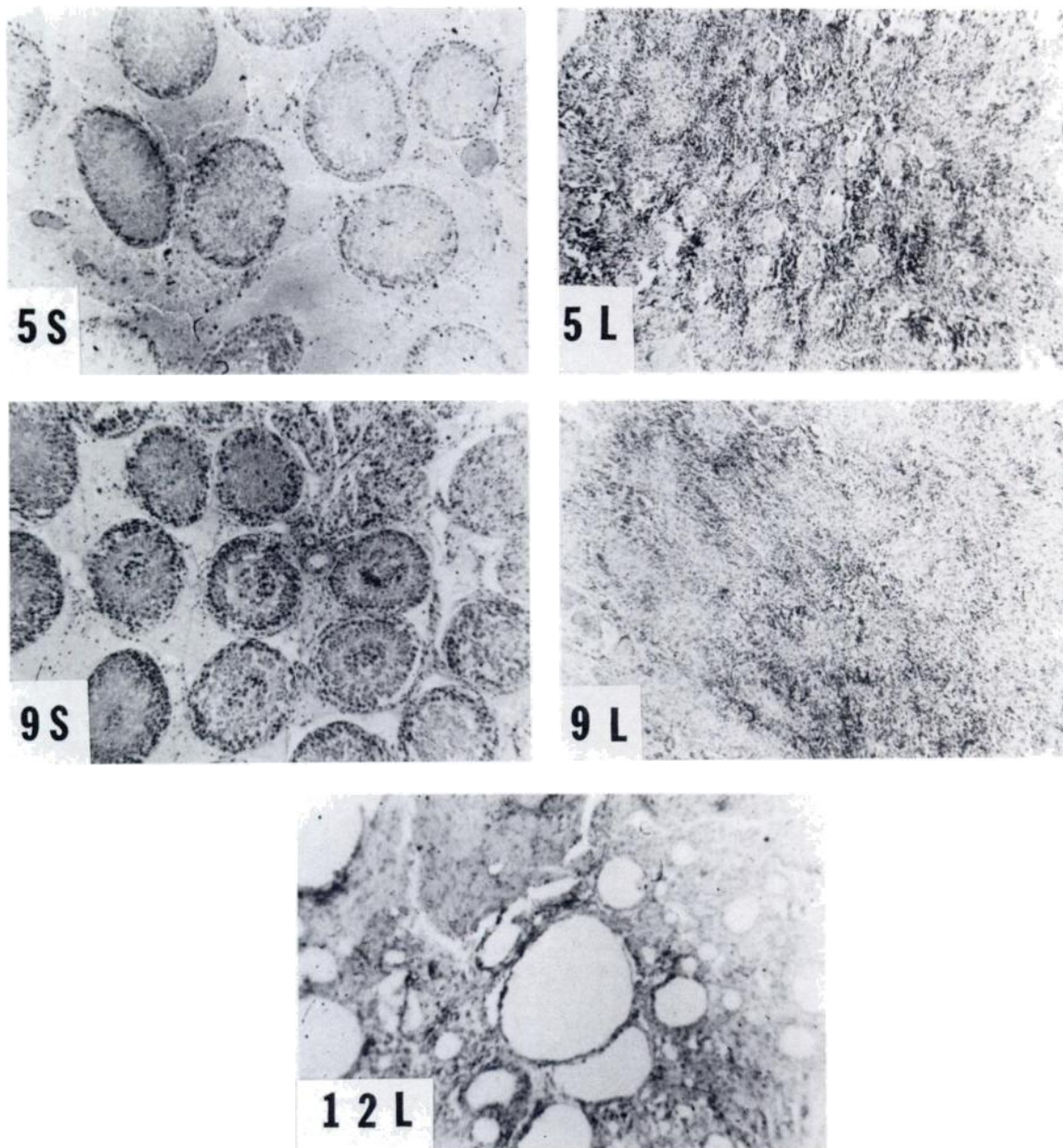
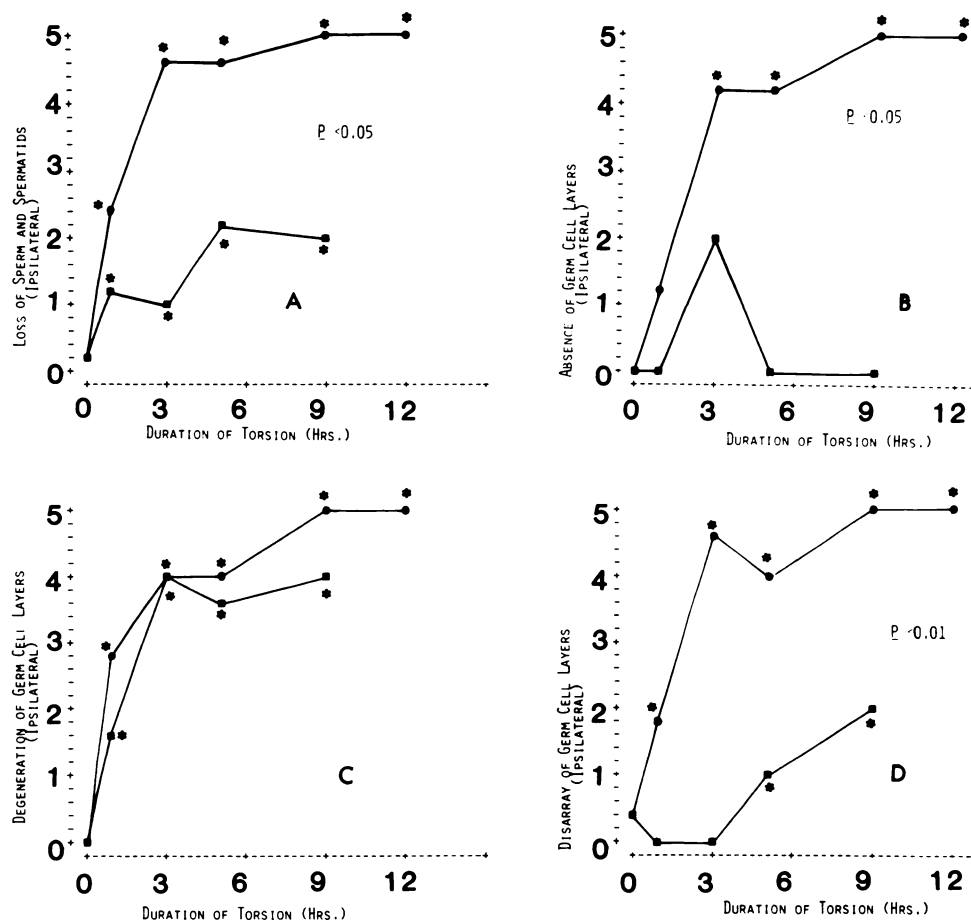


Fig. 1B. Short-term (5S and 9S) and long-term (5L, 9L, and 12L) histologic findings after 5 to 12 hours of torsion ( $\times 70$ ).

ogy scores that were significantly ( $P < 0.05$ ) dependent on the duration of torsion: loss of spermatozoa and spermatids ( $r = 0.798$ , Fig. 2A); degeneration of germ cell layers ( $r = 0.792$ , Fig. 2C); disarray of germ cell layers ( $r = 0.911$ , Fig. 2D); rupture of tubules ( $r = 0.818$ , Fig. 3A); edema ( $r = 0.762$ , Fig. 4A); and hemorrhage ( $r = 0.649$ , Fig. 4B). These findings showed that the longer the duration of spermatic cord torsion, the greater the pathologic findings observed in these six parameters at the end of the

torsion period. Indeed, all six of these parameters showed significantly ( $P < 0.05$ ) higher histology scores for at least one of the durations of spermatic cord torsion tested when compared with control values (Figs. 2-4). Those parameters that showed significant pathology after only one hour of torsion include: loss of spermatozoa and spermatids (Fig. 2A); degeneration of germ cell layers (Fig. 2C); edema (Fig. 4A); and hemorrhage (Fig. 4B). The largest changes occurring after one hour were edema (histo-



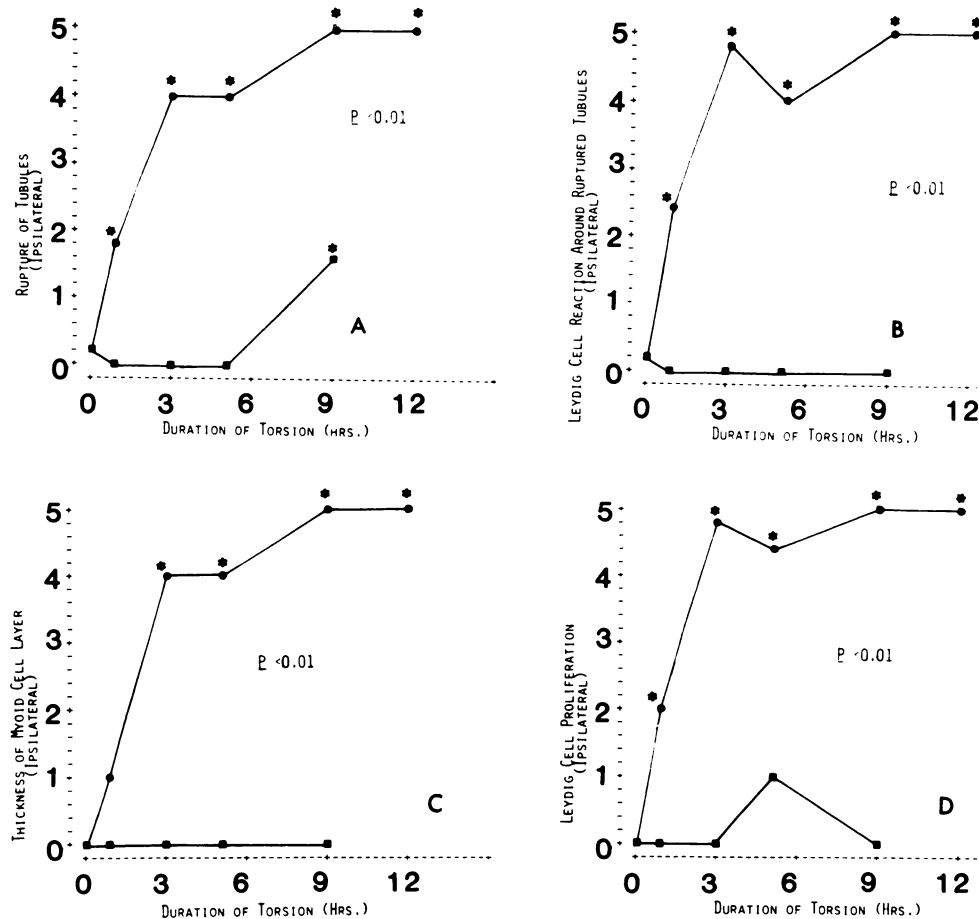
**Fig. 2.** Specific histologic changes in the ipsilateral testis of rats subjected to unilateral spermatic cord torsion for various durations. ■, immediately following the torsion period (short-term changes); ●, after spermatic cord torsion for the indicated period followed by detorsion and a six-week recovery period (long-term changes); ★, significantly different from values obtained at 0 hours of torsion ( $P < 0.05$ ).  $P$  values on figures indicate significant difference between short-term and long-term changes.

logic score = 3.0) and hemorrhage (histologic score = 2.5). The disarray of germ cell layers (Fig. 2D) and rupture of seminiferous tubules (Fig. 3A) did not differ significantly from control scores until after at least 5 and 9 hours of torsion, respectively. The highest histologic scores (most pathologic parameters) obtained immediately following spermatic cord torsion were hemorrhage (Fig. 4B) and degeneration of germ cell layers (Fig. 2C), both of which had scores of 4.0 after 3 hours of torsion.

In order to assess the overall immediate histologic changes that occurred in the testis after spermatic cord torsion of various durations, a total histology score was assigned to each ipsilateral organ. This score was obtained by summing the scores assigned to the 12 histologic parameters for each animal. The total histology score was found to be significantly

dependent ( $P < 0.05$ ) on the duration of torsion ( $r = 0.853$ ), and immediately following a torsion period of only one hour, this score was significantly greater than the control values (Fig. 5).

Of the animals in each group subjected to spermatic cord torsion for the indicated durations, half underwent detorsion and fixation of the damaged organ. After the six-week recovery period, the histologic scores were obtained as noted above and represent the long-term damage occurring in the ipsilateral testis. The ipsilateral testis of these animals showed significant ( $P < 0.05$ ) pathologic changes in each of the 12 histologic parameters (Figs. 3-5). After only one hour of spermatic cord torsion and a subsequent six-week recovery period, the following parameters showed histology scores significantly ( $P < 0.05$ ) more pathologic than the control values: the



**Fig. 3.** Specific histologic changes in the ipsilateral testis of rats subjected to unilateral spermatic cord torsion for various durations. ■, immediately following the torsion period (short-term changes); ●, after spermatic cord torsion for the indicated period followed by detorsion and a six-week recovery period (long-term changes); ★, significantly different from values obtained at 0 hours of torsion ( $P < 0.05$ ).  $P$  values on figures indicate significant difference between short-term and long-term changes.

loss of spermatozoa and spermatids (Fig. 2A); the degeneration of germ cell layers (Fig. 2C); the disarray of germ cell layers (Fig. 2D); rupture of seminiferous tubules (Fig. 4A); Leydig cell reactions around ruptured tubules (Fig. 3B); Leydig cell proliferation (Fig. 3D); and fibrosis (Fig. 4D). The animals exposed to torsion for 3 hours or longer showed significant long-term pathologic changes in all parameters studied ( $P < 0.05$ ). All long-term histologic parameters studied were significantly ( $P < 0.05$ ) dependent on the duration of torsion ( $r = 0.774-0.858$ ). When the scores for all histologic parameters were summed (total histology score) for each animal undergoing torsion, detorsion, and a six-week recovery period, a significant ( $P < 0.05$ ) dependence of these values on the initial duration of torsion was noted ( $r = 0.823$ ; Fig. 5). Furthermore, the overall long-term histologic

damage done to the ipsilateral testes was significant ( $P < 0.05$ ) after only one hour of torsion (Fig. 5).

The long-term damage to the ipsilateral testis was significantly greater ( $P < 0.01$  to  $P < 0.05$ ) than that of the short-term damage for all but three of the parameters (Figs. 2-5). The three parameters not showing this progressive pathology included: degeneration of germ cell layers (Fig. 2C), edema (Fig. 4A), and hemorrhage (Fig. 4B).

The fertility of the male rats undergoing unilateral spermatic cord torsion with subsequent detorsion and a six-week recovery period was significantly reduced ( $P < 0.01$ ) in animals undergoing torsion for 9 or 12 hours (Table 2). Furthermore, the fertility of these animals was inversely proportional to the duration of torsion ( $r = -0.991$ ;  $P < 0.01$ ). The fertility of these male rats was found also to be inversely pro-

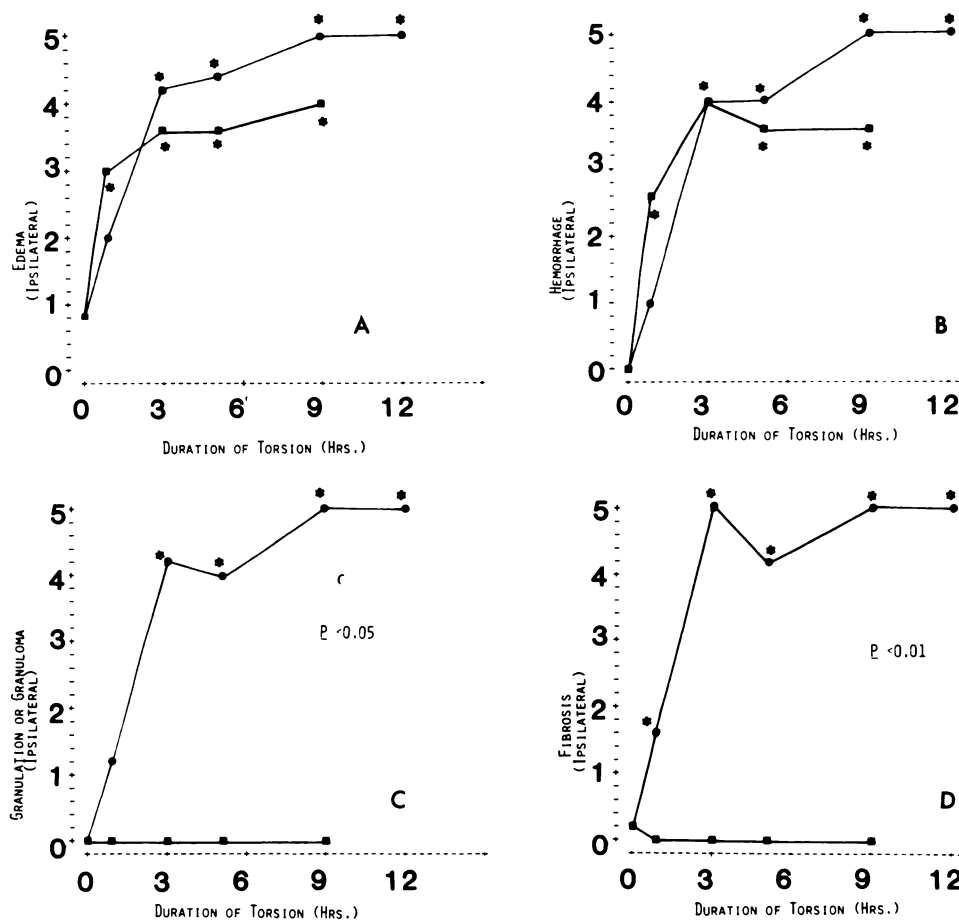


Fig. 4. Specific histologic changes in the ipsilateral testis of rats subjected to unilateral spermatic cord torsion for various durations. ■, immediately following the torsion period (short-term changes); ●, after spermatic cord torsion for the indicated period followed by detorsion and a six-week recovery period (long-term changes); ★, significantly different from values obtained at 0 hours of torsion ( $P < 0.05$ ). *P* values on figures indicate significant difference between short-term and long-term changes.

portional to the total histology score of the insulted organ ( $r = -0.797$ ;  $P < 0.05$ ; Fig. 6).

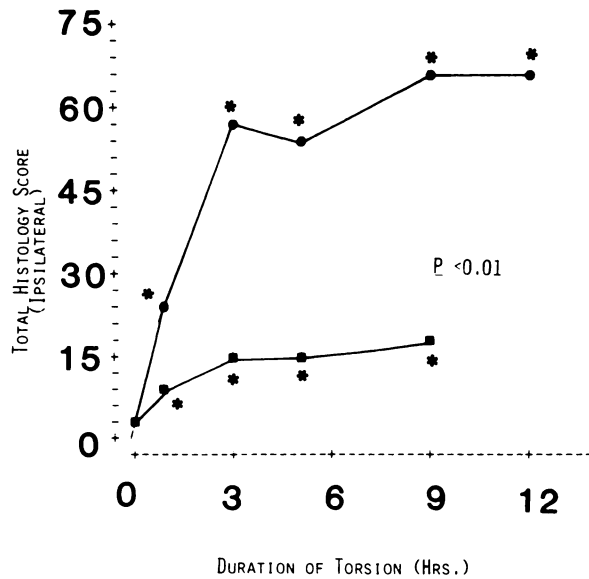
### Discussion

The importance of these observations lies in their relation to infertility problems associated with spermatic cord torsion. Initially, torsion of the spermatic cord occludes the veins but not the arteries, resulting in edema, hemorrhagic infarction, and generalized ischemia of the testis (Skoglund et al, 1970). Since this condition is seen commonly in prepubertal and adolescent boys, we performed the present experiments on animals not yet capable of sexual reproduction (prepubertal). This was done prior to the time spermatozoa appear in the seminiferous tubules of rats (ie, 45 days) (Clermont and Perey, 1957). However, at this stage of development in the rat, spermatids can be found in the seminiferous tubules, which

is also true in humans. Thus, we believe the 35-day-old rat model approximates the situation in the prepubertal and adolescent human.

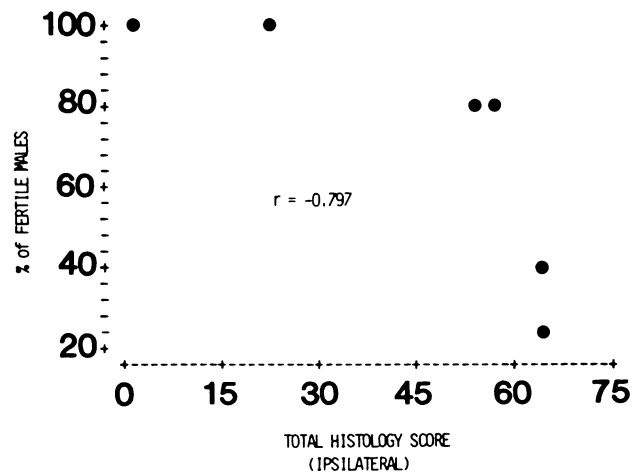
The immediate histologic damage resulting from spermatic cord torsion of various durations was significant in six of the parameters studied. Therefore, those lesions would appear to be primary to spermatic cord torsion, particularly since their extent was dependent upon the duration of torsion. Those lesions appearing only after the six-week recovery period were thus secondary to torsion, and were probably part of the overall degenerative process that was noted (see below). Of the six types of primary lesions observed, some showed significant changes after only one hour of torsion, suggesting they are the most susceptible to this type of insult.

Owing to the sudden circulatory changes occurring with spermatic cord torsion, it was not surprising to



**Fig. 5.** Total histology scores (see text for details) for the ipsilateral testis of rats subjected to unilateral spermatic cord torsion for various durations. ■, immediately following the torsion period (short-term changes); ●, after spermatic cord torsion for the indicated period followed by detorsion and a six-week recovery period (long-term changes); ★, significantly different from values obtained at 0 hours of torsion ( $P < 0.05$ ).  $P$  values on figures indicate significant difference between short-term and long-term changes.

observe edema and hemorrhage as having the highest histology scores after one hour of torsion. However, with respect to changes within the seminiferous tubules, the most rapid changes were degeneration of germ cell layers (Fig. 2C) and a loss of spermatozoa and spermatids (Fig. 2A). Indeed, the exfoliation of spermatids is consistent with the work of Oettle and Harrison (1952), which showed a similar result after 1 hour of ischemia induced by occlusion of the abdominal testicular artery. Those researchers may have observed less severe testicular damage because their procedure did not occlude the vasal artery, which provides a small amount of blood to the testis. The observation that the disarray of germ cell layers (Fig. 2D) and ruptures of seminiferous tubules (Fig. 3A) increased only after extended periods of torsion



**Fig. 6.** Relationship between total histology score (see text for details) and fertility of rats subjected to unilateral spermatic cord torsion for 0 to 12 hours followed by detorsion and a six-week recovery period. The correlation is significant ( $P < 0.05$ ).

suggests that these parameters are somewhat more resistant to the influences that spermatic cord torsion had on the testis.

However, with respect to long-term histologic changes following various periods of torsion and the recovery period, we were somewhat surprised to find that many parameters showed significant pathologic damage after exposure to only one hour of spermatic cord torsion. Indeed, no long-term regeneration occurred in any of the parameters studied (Figs. 1 and 5). Our data indicate that significant degeneration occurred in all but three of the histologic parameters, and those three were the ones showing the most pathologic primary lesions, which could not have degenerated much further. We were therefore particularly concerned with these results, as it is the currently accepted treatment of spermatic cord torsion in peripubertal boys to untwist the cord in the hope that damaged testicular tissue will regenerate and contribute to subsequent fertility. We have shown that the histologic changes observed here correlate well with the fertility of the animals (Fig. 6).

The observations in the present study reveal the

**TABLE 2.** Fertility of Male Rats Undergoing Unilateral Spermatic Cord Torsion for Various Durations Followed by Detorsion and a Six-week Recovery Period

	Duration of Torsion					
	0 Hr (Control)	1 Hr	3 Hr	5 Hr	9 Hr	12 Hr
Fertile male rats (%)	9/9 (100)	5/5 (100)	4/5 (80)	4/5 (80)	2/5 (40)*	1/4 (25)*

\*Significantly different from control values ( $P < 0.01$ ).

sequence of specific histologic damage to the testis occurring with spermatic cord torsion in the prepubertal animal and the lack of regeneration with a subsequent reduction of torsion. We therefore suggest that attempts to salvage a testis after spermatic cord torsion may be futile, and should be critically reviewed.

### Acknowledgments

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