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Phasic Distribution of Seminiferous Tubules in Rats Treated with Triphenyltin Compounds

Ronald L. Snow and Ruth L. Hays*

Zoology Department, Clemson University, Clemson, SC 29631

Reports concerning the effects of triphenyltin compounds on animal reproductive tissue are numerous. Triphenyltins have been used for reproductive control of the German cockroach Blatella germanica (L.), the confused flour beetle Tribolium confusum Jacquelin du Val, and the house fly Musca domestica L. (KENAGA 1965). Triphenyltin acetate (TPTA) and triphenyltin chloride (TPTC) specifically have been shown to be house fly chemosterilants by other investigators (FYE et al 1966; KISSAM & HAYS 1966). NEWTON & HAYS (1968) reported that treatment of female albino rats (42-46 days old) at a dosage of 20 mg/kg/day produced marked effects on ovarian tissue after only 5 days of treatment. Among these effects were a decreased number of mature follicles, an increased incidence of atresia in early follicle development and a pronounced decrease in the number of corpora lutea present. This latter effect indicated a decreased ovulation, thus a decreased fertility. Other studies revealed a decreased spermatogenesis in 35-40 days old albino rats treated for 19 days with TPTA and TPTC at a dosage of 20 mg/kg/day (PATE & HAYS 1968).

In this paper we report the phasic distribution of spermatogenic stages of a representative number of seminiferous tubules in treated rats in an attempt to determine at what point TPTA and TPTC may affect spermatogenesis. We include also a determination of testicular recovery following withdrawal of treatment.

MATERIALS AND METHODS

Thirty-nine Holtzman albino male rats (Holtzman, Madison, Wisconsin), age 29 days with a weight range of 67-87 g, were separated into 3 groups of 13 animals each; controls, TPTA-treated and TPTC-treated. The animals were caged singly and maintained on Purina® Rat Chow and fresh water. Environmental conditions were kept at 22-26°C with a 10 hr/day photoperiod. Treatment was administered in the food at a dosage rate of 20 mg/kg body weight/day for 20 days (PATE & HAYS 1968). On each treatment day, the compounds were dissolved in acetone and added to the food in measured amounts according to the body weight of each animal. The acetone was allowed to evaporate overnight before the treated food was fed to the animals. Control animals received food which had been treated with equivalent amounts of acetone and dried in a similar manner.

*Correspondence and offprint requests

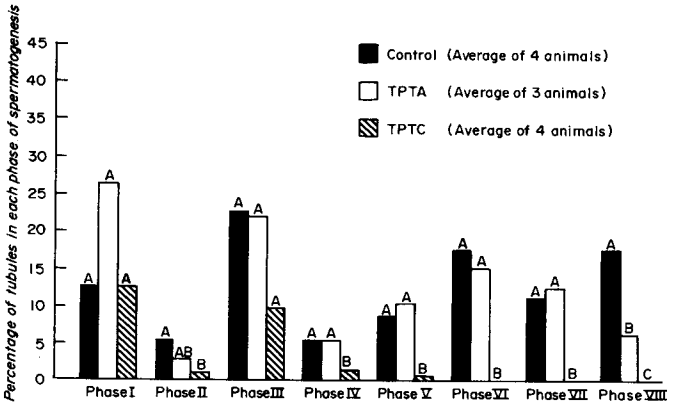

Figure 1. Spermatogenic phases in control, triphenyltin acetate (TPTA)-treated, triphenyltin chloride (TPTC)-treated rats. Dosage = 20mg/kg/day for 20 days. Similar letters over adjacent columns = NSD; dissimilar letters denote significant difference ($p<.05$).

Four animals from each group were randomly selected on day 21 of the treatment period and sacrificed by cervical dislocation. Testes were removed, fixed in Bouin's solution, processed automatically, and embedded in paraffin. Serial cross-sections were cut from the mid-portion of the testis at a thickness of 7 μm, stained with Hematoxylin and counterstained with Eosin. Stained slides were examined to determine the presence or absence of sperm in the seminiferous tubules, percentage of tubules with cell associations characteristic of 8 phases of spermatogenesis as described by ROOSEN-RUNGE & GIESEL (1950) and any abnormality with respect to phase or condition of cells within the tubule lumen or epithelium itself. The percentages of 8 spermatogenic phases present in 100 circular tubules were determined using a percentage calculator for differential blood films. Results were expressed as percentages of each phase present then averaged for each treatment group.

Treatment was continued on the remaining 27 animals until day 25 of the treatment period. Thereafter, the animals were placed on regular food for an additional 70 days, at which time they were sacrificed. The same procedures were followed as with the animals sacrificed on day 21 of the treatment period.

RESULTS AND DISCUSSION

Results of the distribution of phases of spermatogenesis following 20 days of treatment appear in Fig. 1. Averages of percentages of tubules in each phase are shown.

The phasic distribution was consistent among control animals, and was considered normal for 50-day-old rats reared under the conditions specified. All 8 phases were found in each control animal. A full complement of sperm were present in phases V-VIII, and phase VIII tubules displayed a typical swirl of mature sperm.

In TPTA-treated animals, all 8 phases were seen. However, there was a general paucity of mature sperm and the distribution showed a relative predominance of the earlier phases, especially phase I tubules. Sperm deficiencies were noted in phases III-V even though the phase distribution was similar to controls. The number of tubules in phase VIII was 1/3 that observed in controls, and many showed sperm depletion and lacked the characteristic swirl of mature sperm. Statistical analysis of the phase distribution data by Duncan's Multiple Range Test confirmed the significant difference between control and TPTA-treated animals in the percentage of phase VIII tubules. Phase VII tubules are characterized by the movement of spermatozoa toward the periphery of the tubule. Phase VIII tubules are characterized by the return of the spermatozoa to the lumen forming a characteristic swirl. A significant decrease in the number of phase VIII tubules indicates a possible effect of TPTA on Sertoli cells since these latter cells have a functional role in the spermatozoa migration process. While a large difference in percentage of Phase I tubules in control and TPTA-treated animals seems apparent, this difference was not detected statistically due to an unaccountable variation leading to a large error term. There were no significant differences in control and TPTA-treated animals in the percentage of phase II-VII tubules.

In TPTC-treated animals, major spermatogenic anomalies were observed in histological sections. The tubules contained few sperm and did not show the normal phase distribution characteristic of control animals. Complete closure of tubule lumina and a high incidence of necrotic multinucleated cells characterized the tubules (Fig. 2). Sperm depletions were pronounced in phases II-V (Fig. 3). The majority of tubules were phases I and II and no tubule advanced beyond phase V (Fig. 1). Seventy-two % of the tubules were abnormal to the extent that phases could not be determined.

Data analysis revealed a significant difference between control and TPTC-treated animals in the percentage of phase II tubules, and in Phase IV-VIII tubules the TPTC-treated animals differed from both controls and TPTA-treated animals. Because of unaccountable variation, there was no statistically detectable difference between control and TPTC-treated animals in the percentage of phase I or phase III tubules. phase IV is characterized by the beginning of the first and end of the second maturation division. It is at this phase that the TPTC-treated animals differed significantly indicating an interruption in the meiotic process.

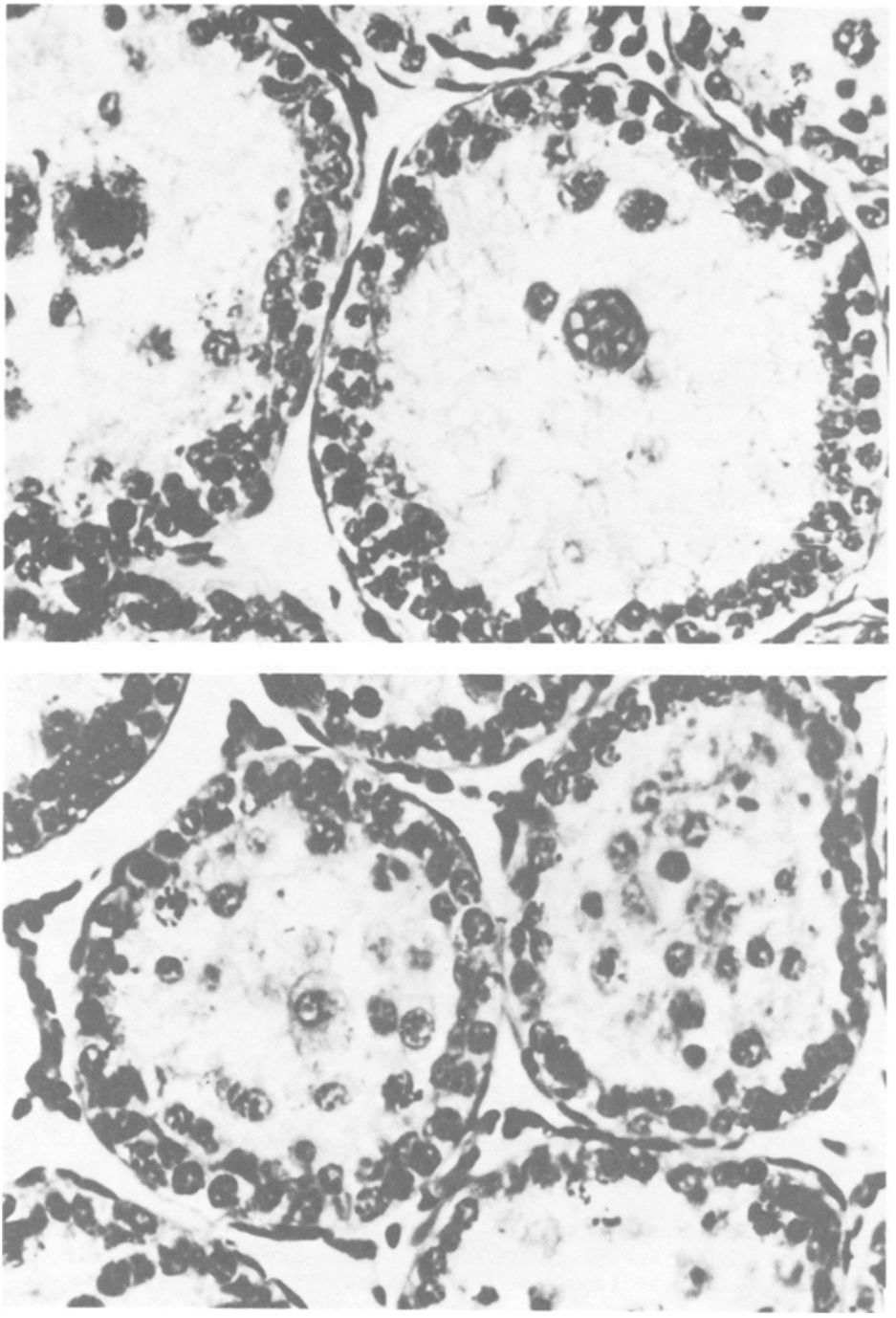

Figure 2. Cross-sections of seminiferous tubules of albino rats treated with triphenyltin chloride for 20 days at 20mg/kg/day. Note closure of tubule lumina and cellular necrosis (x500).

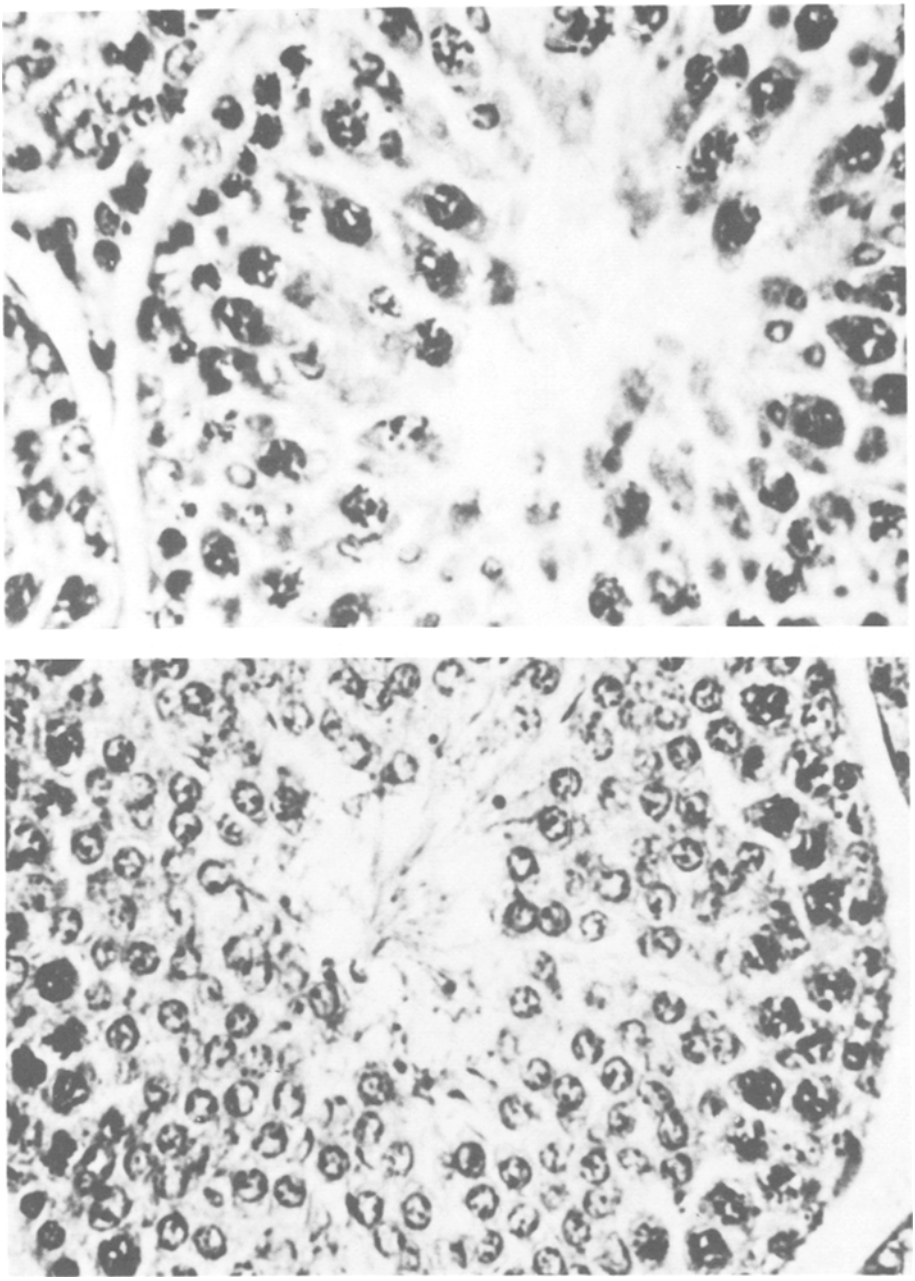

Figure 3. Cross-sections of seminiferous tubules of albino rats treated with triphenyltin chloride for 20 days at 20mg/kg/day. Above: Phase III tubule (x500). Below: Phase V tubule (x500).

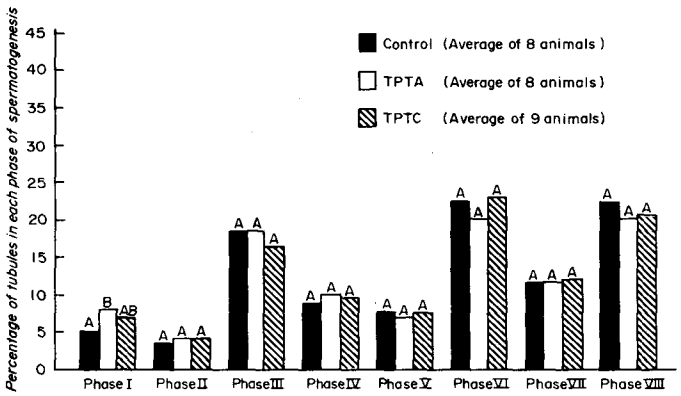

Figure 4. Spermatogenic phases 70 days after discontinuing triphenyltin acetate (TPTA) and triphenyltin chloride (TPTC) treatment (20mg/kg/day for 25 days). Similar letters over adjacent columns denote NSD; dissimilar letters denote significant differences ($p<0.05$).

After a normal diet for 70 days, the remaining 9 animals from each group were sacrificed. Distribution of phases is shown in Fig. 4. In control animals, all 8 phases were observed with a shift toward the more advanced stages of development because of the more sexually mature state of the animals.

The TPTA-treated animals displayed a pattern of spermatogenesis similar to controls. Average phasic distributions were nearly identical, and the cells within the tubules appeared normal with no noticeable sperm deficiency or evidence of necrosis. Statistical analysis revealed a significant difference between control and TPTA-treated animals in the percentage of phase I tubules, but detected no difference in control and TPTA-treated animals in the percentage of phase II-VIII tubules. There was apparently complete recovery from any impairment of the spermatogenic process produced directly or indirectly from TPTA. (Fig. 5).

In the TPTC group, spermatogenesis was similar to controls in all respects. Statistical analysis of the phasic distribution confirmed these findings in that there were no significant differences between control and treated animals in phase I-VIII tubules. All phases appeared normal and mature sperm were present in phases IV-VIII. The phasic distribution and tubule appearance was indicative of complete recovery of the spermatogenic epithelium from the treatment, with no evidence of necrosis or sperm deficiency. (Fig. 5).

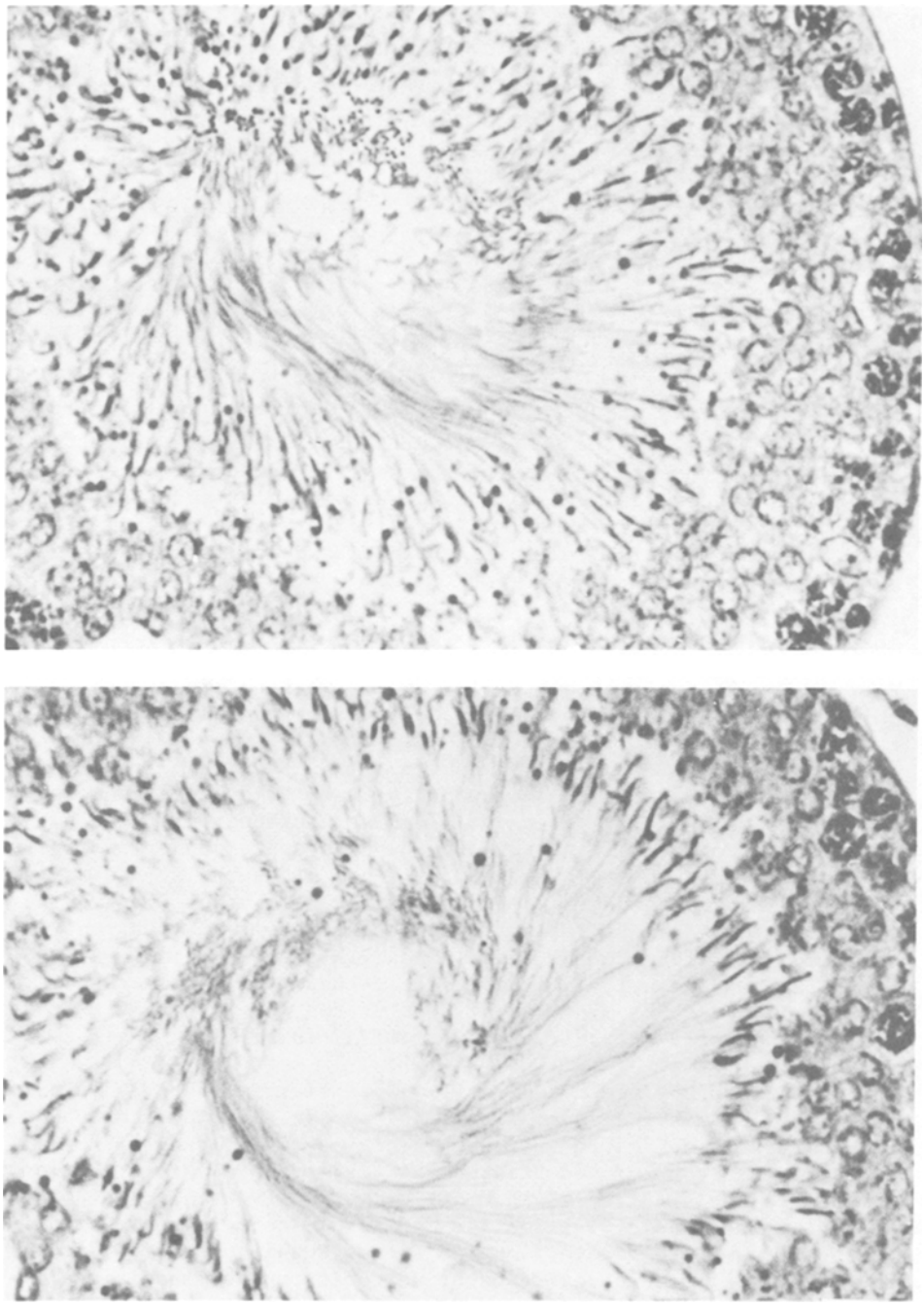

Figure 5. Representative cross-section of phase VIII seminiferous tubules of albino rats 70 days after discontinuing triphenyltin acetate (TPTA) and triphenyltin chloride (TPTC) treatment (20mg/kg/day for 25 days). Above: Recovery from TPTA treatment (x500). Below: Recovery from TPTC treatment (x500).

A decreased fertility in rats treated with triphenyltin hydroxide has been attributed to a failure of the animals to gain weight properly caused by a presumed decreased palatability of treated food and decreased food consumption (GAINES & KIMBROUGH 1968). The treated animals in the present study ate on the average about two-thirds as much food as the controls, thus it is possible that the observed effects were due in part to a deficiency in nutritional balance since malnutrition is known to precede gonadal dysfunction and even atrophy (RIBELIN 1963). However, the possibility of a direct effect of the triphenlytin moiety on inhibition of electron transport and oxidative phosphorylation, ion exchange across membranes and inhibition of ATPase enzymes cannot be ruled out (SELWYN 1976). If the observed effect is due to a direct action of the compound, then the difference in the effects of the two triphenyltins on the phasic distribution of cell associations in seminiferous tubules may be due to the actual amount of the triphenyltin moiety reaching the active site, since the cation portion of the 2 compounds is the same. The anion portion of the compound may be significantly involved in transporting the compound to the reactive site (POLLER 1976). The question of reduced food intake being the sole cause of reduced fertility must remain open also because of observed effects on fertility in female rats being present after only 5 days of treatment, before any significant differences in weight gain were apparent (NEWTON & HAYS 1966). In addition, there should have been no differences in palatability of the food in the two treatments used in the present study, yet differences in distribution of spermatogenic phases in TPTA-treatment and TPTC-treatment were noted. This should detract from the argument that a decreased palatability of treated food resulting in decreased consumption and starvation is the only factor contributing to the observed effects on spermatogenesis.

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