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Effects on Spermatogenesis in the Guppy *Poecilia reticulata* by Prolonged Exposure to Trimethylphosphate

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Trimethylphosphate (TMP) has a number of industrial uses and can cause adverse effects in biological systems. A review article by CONNOR (1979) on the mutagenic, including toxic, carcinogenic, and sterilizing properties of TMP, concludes that TMP is capable of producing a variety of genetic damage in a range of diverse systems. However, these effects are predominantly manifested at relatively high doses and this requirement for effectiveness at high doses (Table 1) is probably attributable to its lack of anti-cholinesterase activity (JACKSON & JONES 1968).

Table 1. Relative toxicity of trimethylphosphate

Test Organism	LD_{50} (single oral doses of TMP) g/kg
rats (Sprague-Dawley)	2.0[a]
rabbits (albino)	1.3[a]
guinea pigs	1.1[a]
rats	0.8[b]
mice	1.5[b]

[a] DEICHMANN & WITHERUP (1946)

[b] Registry of Toxic Effects of Chemical Substances

During investigations to determine whether TMP induced mutations in *Poecilia reticulata* (HANNA unpublished), it appeared that TMP had produced a temporary sterility in males treated with 1000 ppm (1.12 g/L) TMP for 14 days. Progeny from treated males were produced in excess of 60 days after treatment, whereas control fish started producing progeny at 30 days. In addition, FELICE & RASCH (1968) estimated that the duration of spermatogenesis in the species *Poecilia sphenops* is approximately 30 days. On the basis of these data it was decided to study the effects of TMP on spermatogenesis in guppies after prolonged exposure to a relatively high dose of TMP.

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MATERIAL and METHODS

Poecilia reticulata adult males used in this investigation were of the *Flavus* coloration (DZWILLO 1959), proven fertility and approximately 10 months old. All fish were maintained at a temperature of 25±2°C in aquaria of 6.75 L containing distilled water supplemented with Aqua Lab (15 g/L) producing a hardness of about 140 ppm $CaCO_3$ and pH 7.3±0.2. Each aquarium had continuous fluorescent lighting for 12 h/day as well as constant aeration and filtering. Fish were fed daily a diet of Tetramin, freeze dried shrimps and *Daphnia* (Alive-O). Trimethylphosphate (TMP), obtained from Albright and Wilson Ltd., Warley, U.K., was added to the aquarium water to give a concentration of 2000 ppm (2.43 g/L). The test conditions were semi-static in that half of the volume was renewed weekly during the test period. Fish of both the control and TMP treated groups were sacrificed on day 100. The testes were immediately dissected out, their lengths measured, and then fixed for 2 h in 2% v/v glutaraldehyde in 0.1M cacadolate buffer at pH 7.4. They were given three 20 min rinses in buffer, post fixed in 2% OsO_4 in 0.025M buffer at pH 6.8 for 2 h, dehydrated through a graded ethanol series and then infiltrated and embedded in the low viscosity medium of SPURR (1969). Sections were cut at 0.5 µm and stained in toluidine blue (0.05% w/v in benzoate buffer, pH 9.0) over a hotplate and then rinsed in running water (O'BRIEN *et al.* 1964). Permanent mounts were then made.

RESULTS

During the treatment of male fish with TMP there were no deaths. However, towards the end of the treatment all fish exhibited a darkening of colour as well as a slowing down of movements. Furthermore, the length of testes showed a significant decrease in length (Table 2) which indicated a loss in testicular volume. There was no significant difference in body length at the end of treatment.

Table 2. Body and testicular lengths of male guppies treated with 2000 ppm trimethylphosphate for 100 days

	Number	Body length[a] (mm)	Testes length (mm)
Control	11	19.9 ± 0.9	3.1 ± 0.2
Treated	4	20.3 ± 0.9[b]	2.0 ± 0.3[c]

a excludes tail fin
b $t = 0.604$; $p > 0.20$
c $t = 7.696$; $p < 0.001$

Testicular morphology and spermatogenesis in the guppy has been described by VAUPEL (1929), PORTE & FOLLENIUS (1960) and PANDEY (1969). In testes of untreated mature males (Fig. 1), spermatogonia located near the outer testis wall divide mitotically to produce numerous cysts of cells (or acini) which move inward. Following meiosis cysts of spermatids differentiate into sperm

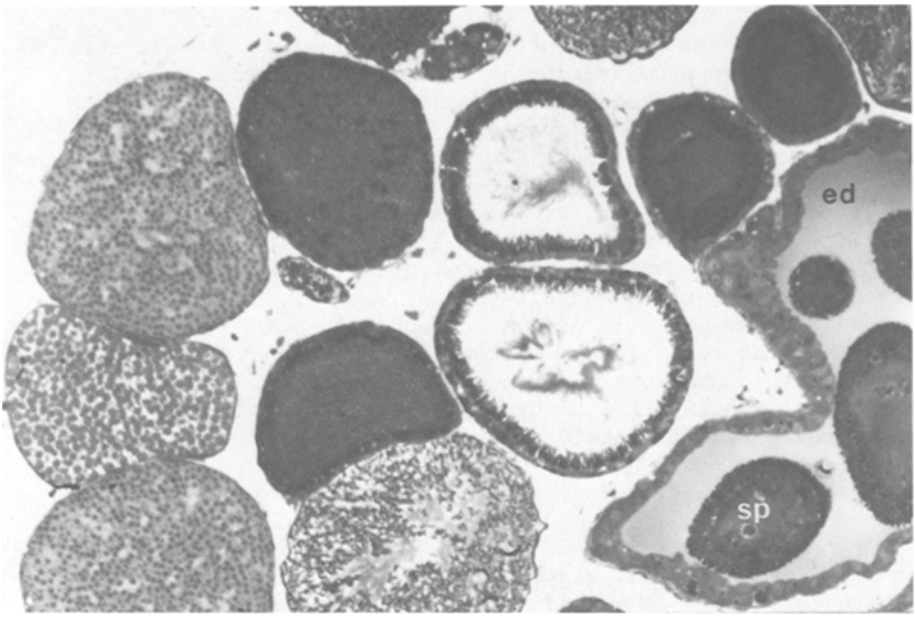

FIG. 1. T.S. of testis of a control fish showing many cysts at different stages of spermatogenesis. sp, spermatophore; ed, efferent duct. X270.

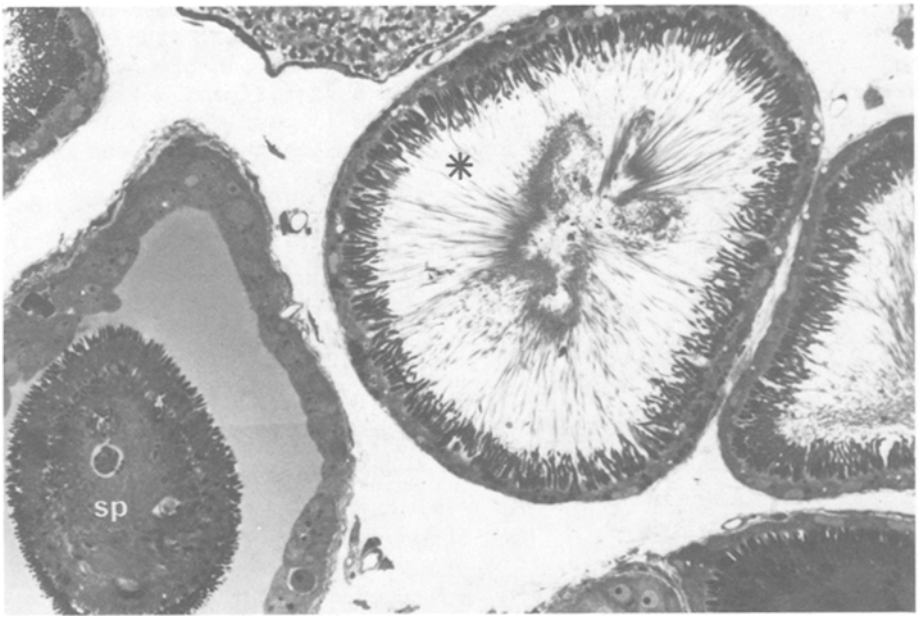

FIG. 2. As per Fig. 1 but H.P. of a cyst of developing sperm (*) in close proximity to an efferent duct (ed). sp, spermatophore. X570.

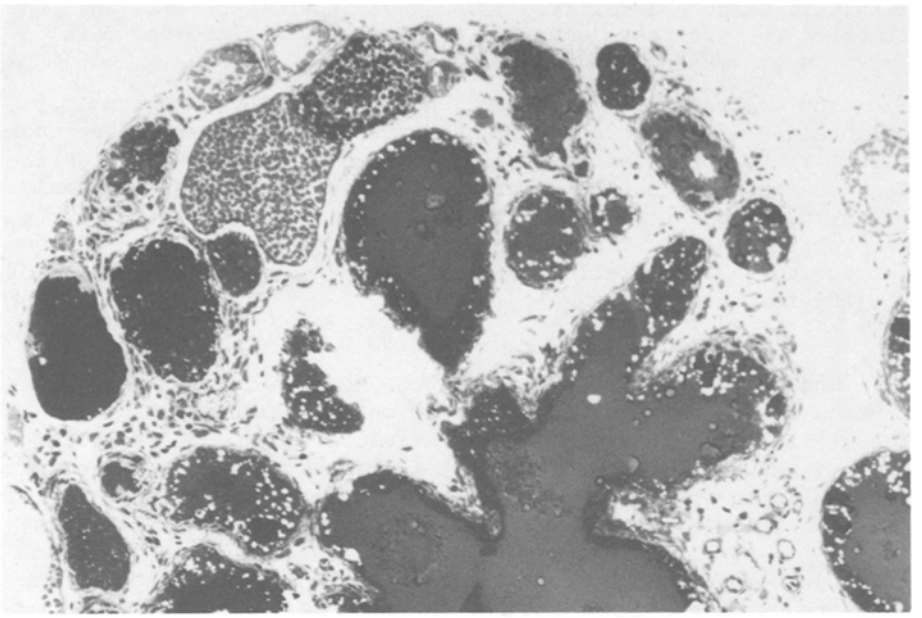

FIG. 3. T.S. of testis of guppy testis after treatment with 2000ppm trimethylphosphate for 100 days. Cyst numbers are much reduced compared with controls. X150.

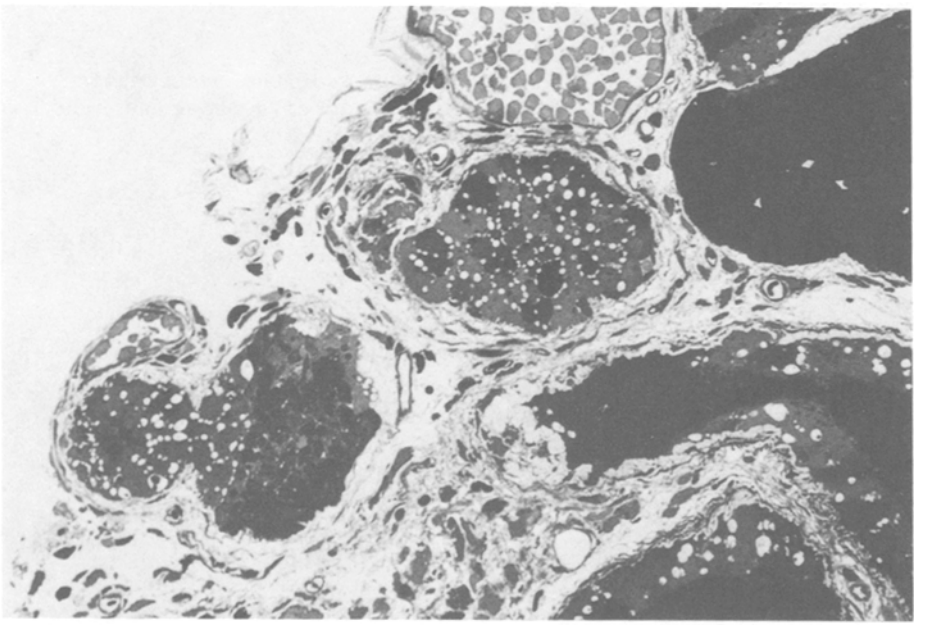

FIG. 4. As per Fig. 3 but H.P. showing degenerating cysts as well as high vacuolarization of different cells within the testes. X430.

(Fig. 2), which are released as bundles (spermatophores) into the lumen of the efferent ducts and eventually pass into the main sperm ducts (vas deferens).

The testes of TMP treated fish exhibited obvious pathological changes which included a marked reduction in cyst numbers compared with control testes (Fig. 3) and accounted for the significant decrease in testicular size. No normal meiotic or post-meiotic cysts were observed, and instead, several degenerating cysts were noted. Furthermore, a high degree of vacuolarization was observed in the degenerating cyst cells and the epithelial cells of the efferent ducts (Fig. 4).

DISCUSSION

These results have shown that TMP, causes in guppies, a disruption to spermatogenesis by acting on pre-meiotic cells with the result that cysts degenerate. As a consequence of the reduction of the total number of cysts, testicular size is reduced. TMP similarly effects fertility in Drosophila (HANNA 1980, 1981) and rats (HANNA & KERR in press), by acting on pre-meiotic germ cells.

Due to the presence of chronologically ordered cysts at various stages in sperm production, the male guppy provides a suitable aquatic organism for determining the stage at which a chemical has an effect on spermatogenesis. As the chemicals temophos and fenitrothion are known to have an effect on guppy reproduction (YASUNO *et al.* 1980), further tests of functional changes of spermatogenesis by organophosphorus compounds in male guppies, is required.

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