Antifertility activity of trimethylphosphate in male rats

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Summary. The toxic effect of trimethylphosphate on rat testes was investigated after oral dosing with 250 mg/kg for either 5 days/week for 30 days or 6 days/week for 60 days. After these prolonged treatments azoospermia resulted.

Evidence for antifertility activity of trimethylphosphate (TMP) was first demonstrated by the absence of progeny following treatment of male rodents[2]. In particular, rats were found to be more sensitive to the sterilizing action of TMP than mice and the minimum oral doses for effective responses were 100 mg/kg and 1 g/kg for 5 consecutive days, respectively. The sterilizing action was reported to be reversible and of a temporary functional type involving post-meiotic cell stages such that motile spermatozoa were produced but lacked the ability to fertilize. It was suggested that the delayed antifertility action of TMP on testicular function was attributable to effects on pre-meiotic cell stages, namely spermatogonial cells. In addition a probable delayed antifertility action through the effects of TMP on spermatogonial cells was also noted.

In a more recent study of TMP-induced sterility involving mice, rats and rabbits, TMP was found to primarily affect epididymal spermatozoa[3]. Rats administered 100 mg/kg, either p.o. or by i.p. injection for 5 consecutive days per week over 1 month, reduced fecundity 29% during the first week following termination of treatment and fertility returned to normal during the next 2 weeks. TMP given at 600 mg/kg over 5 consecutive days reduced fecundity to 0–5% for a 4-week period following cessation of treatment after which fertility returned to normal during the next few weeks. The investigators produced additional data which indicated that the mechanism of TMP-induced sterility involves inhibition of spermatozoon choline acetyltransferase activity; inhibition of this enzyme compromises spermatozoa motility and their ability to fertilize. However spermatogenesis in the test animals was not affected by TMP treatment.

It is known that administration of TMP to *Drosophila* causes disruption of spermatogenesis and leads to azoospermia, through an action on pre-meiotic cell stages[4,5]. These

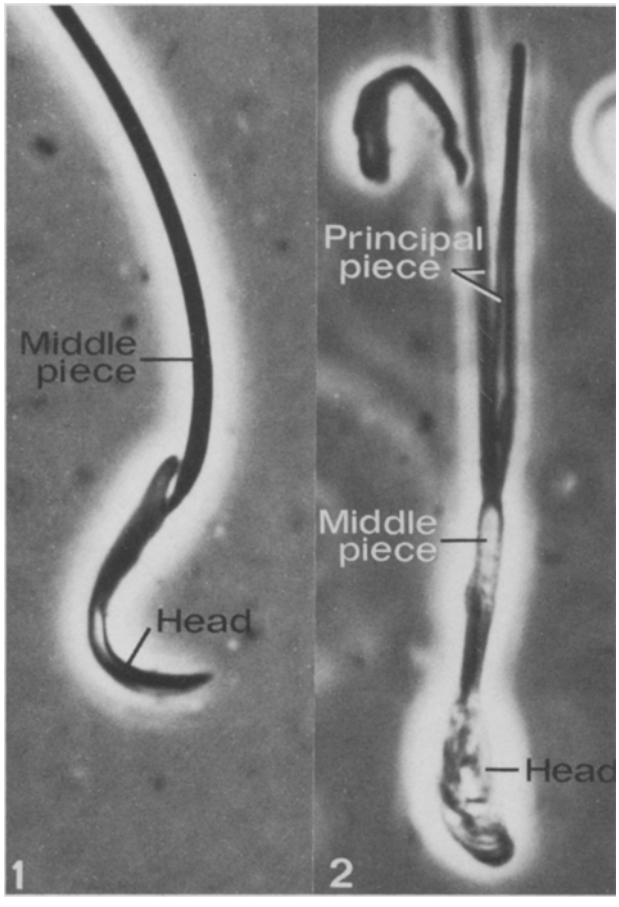

Figure 1. Phase contrast micrograph of a spermatozoon from the epididymis of an untreated rat, illustrating normal morphology. × 1500.

Figure 2. Phase contrast micrograph of a spermatozoon from the epididymis of a rat treated with TMP for 5 days/week for 30 days, illustrating abnormal acrosomal and head morphology together with fusion of 2 principal pieces forming a double-tailed spermatozoon. An abnormal and detached head is also shown. × 1500.

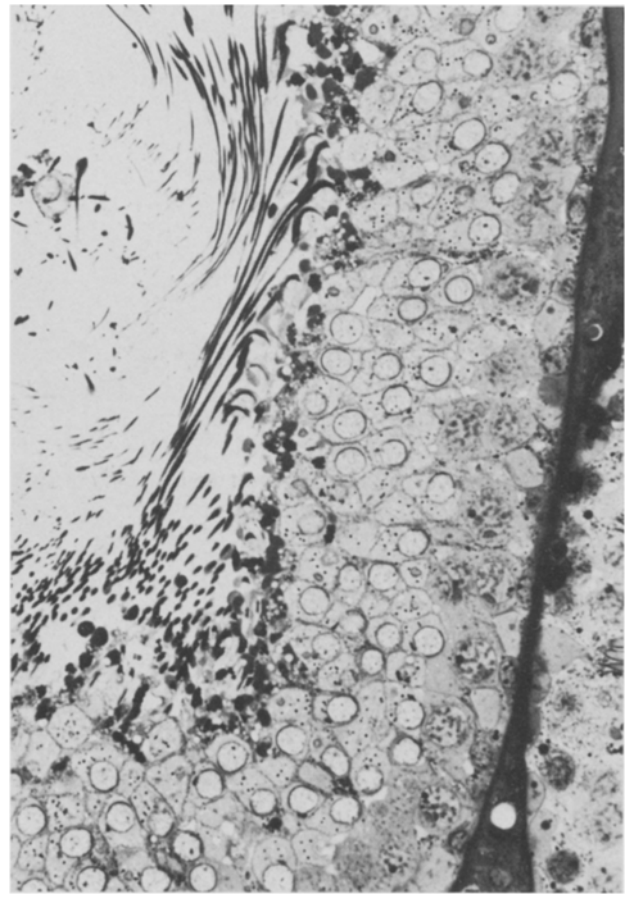

Figure 3. Photomicrograph of seminiferous epithelium of the testis of an untreated rat, illustrating normal spermatogenic activity. × 430.

observations together with the earlier antifertility studies in rodents[2] suggest that cytological studies should be made on spermatogenesis in rats exposed to prolonged doses of TMP.

Materials and methods. Random-bred albino adult Sprague, Dawley rats of approximately 200 g and proven fertility were treated with trimethylphosphate (TMP) obtained from Albright and Wilson Ltd., Warley, U.K. Doses of TMP at 250 mg/kg in distilled water were given p.o. for either 5 days/week for 30 days or 6 days/week for 60 days. Upon cessation of treatment, each rat was placed with 2 mature virgin females to assess fertility. Semen from the corpus epididymis was taken from 2 rats of the 30-day treatment group and examined under phase contrast optics to determine the presence of sperm as well as sperm morphology.

In addition testes of 3 animals from each treatment group, as well as controls, were fixed by vascular perfusion through the thoracic aorta as previously described[6] using a mixture of glutaraldehyde, formaldehyde and trinitrocresol buffered to pH 7.4 with 0.1 M cacodylate buffer. Following fixation, the hardened tissue was cut into 1-mm cubes, postfixed in 2% osmium tetroxide in the same buffer, dehydrated and embedded in a 1:1 mixture of epon araldite. 1-µm sections were cut with an ultramicrotome, stained with toluidine blue and photomicrographs were obtained using a Leitz Orthoplan microscope.

Results and discussion. Spermatozoa obtained from the epididymis of untreated rats exhibited normal morphological features (fig. 1). However, spermatozoa obtained in the same manner from rats treated with TMP for 5 days/week for 30 days were all abnormal. For example, many detached heads were found amongst others exhibiting abnormalities of the head, middle piece and principal piece (fig. 2). These variations in morphology were not seen within normal spermatozoa. Moreover it was noted that the virgin females used to test fertility of rats of both treatment groups after cessation of treatment, lacked vaginal plugs after 2 weeks, thus showing a lack of mating ability on behalf of the treated males. However, vaginal plugs and subsequent progeny were produced in control tests for fertility.

Full spermatogenic activity was observed in testes of untreated rats and the seminiferous epithelium exhibited all stages of germ cell growth and differentiation (fig. 3). Testes of rats which had received TMP 5 days/week for 30 days showed impaired spermatogenesis due to abnormal spermiogenesis and depletion of the numbers of mature spermatids. Round spermatids showed vacuoles within their nuclei and extensive extracellular spaces were observed between the germ cells and Sertoli cells (fig. 4). Germ cells were absent from the seminiferous tubules of rats treated with TMP for 6 days/week for 60 days, resulting in collapse and shrinkage of the tubules and the appearance of the

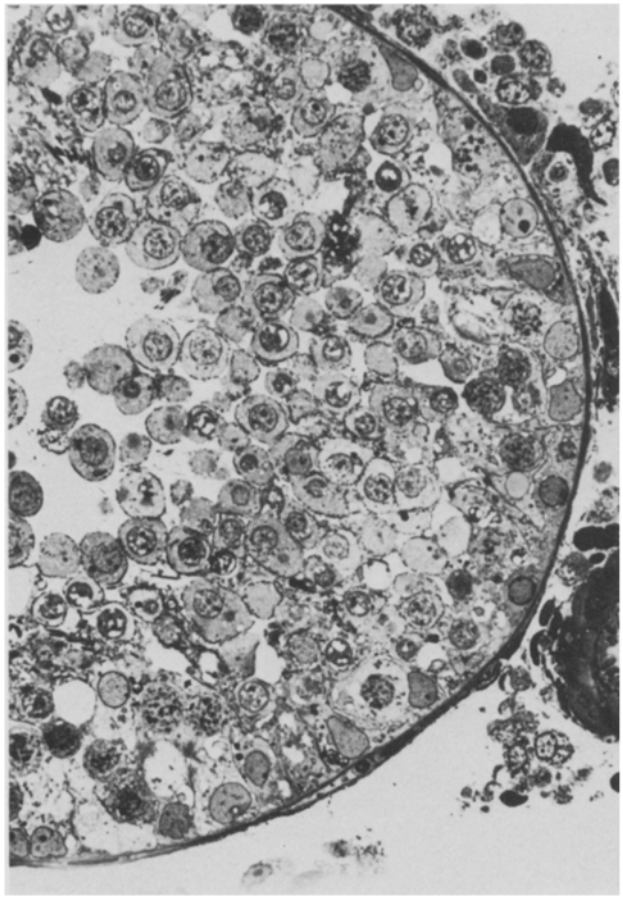

Figure 4. Photomicrograph of seminiferous epithelium of the testis of a rat treated with TMP for 5 days/week for 30 days. Note the absence of mature spermatids which normally occur at this stage of the spermatogenic cycle. Extracellular spaces are seen and vacuolisation of spermatid nuclei is apparent. × 430.

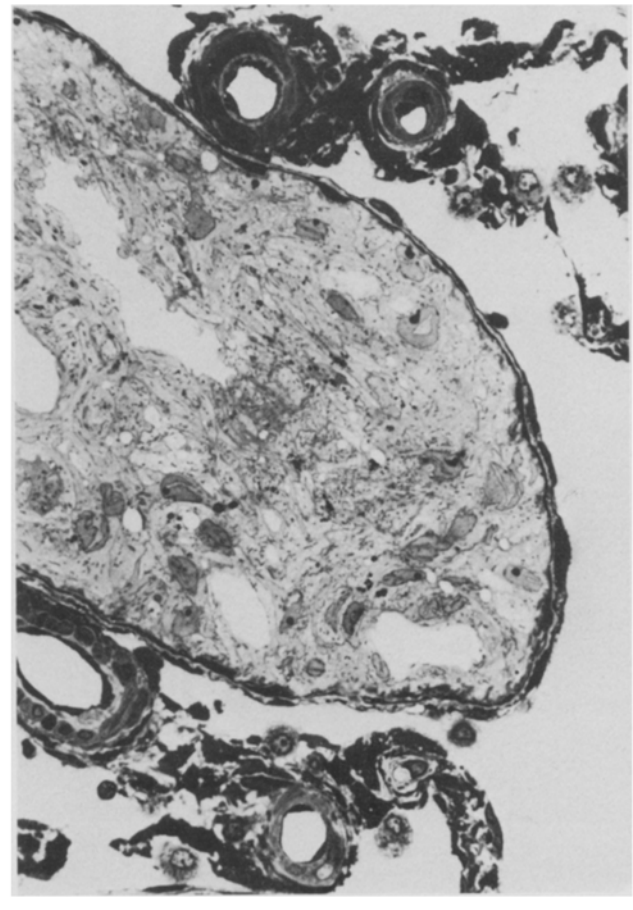

Figure 5. Photomicrograph of seminiferous epithelium of the testis of a rat treated with TMP for 6 days/week for 60 days, illustrating collapse of the epithelium due to elimination of germ cells. The seminiferous tubules are reduced in diameter and contain only Sertoli cells. × 430.

Sertoli-cell-only condition. The lumen of many seminiferous tubules were often filled with processes of Sertoli cell cytoplasm (fig. 5).

The present observations are in contrast to previous claims[7] that high doses of TMP (up to 300 mg/kg/week) produce temporary sterility in rats in which the delay in a return to full spermatogenesis function is simply dependent upon the dose. Our studies have demonstrated complete loss of germ cell activity after prolonged doses of TMP. It is clear that the available data do not permit definition of the action of TMP on the function of the testes. Nevertheless, the results emphasize that agents which interfere with fertility by affecting the formation of mature spermatozoa can also disrupt the growth of germ cells, either directly, or indirectly through the Sertoli cells and Leydig cells. The data suggest that the effects of agents on spermatogenesis should be assessed by examination of testicular function as a whole.

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Vasotocin protects rats against convulsions induced by pentylenetetrazol[1]

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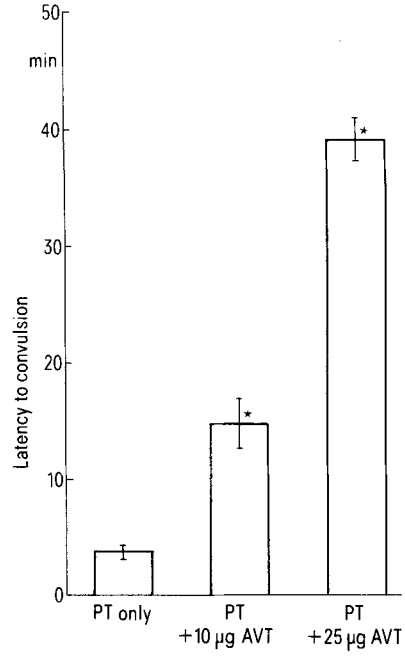
Summary. The AVP analog, vasotocin, administered s.c. effectively antagonized pentylenetetrazol-induced convulsions, supporting the contention that AVP may be a mediator in convulsive disorders.

In recent years, it has been recognized that arginine vasopressin (AVP) may have important functions in the central nervous system (CNS). The many reported behavioral or central physiological effects of AVP provide evidence for a neuromodulator or neurotransmitter role of AVP. Particularly important with regards to this study are the antipyretic effects of AVP when perfused or injected into the septal area of the brain of the sheep and the rat[3-5]. In the former study[4], AVP release in vivo in the septal area of the brain of the sheep was observed during fever. Other studies have observed AVP release into the blood during hyperthermia[6,7]. Interestingly, in contrast to the antipyretic effects of AVP injected directly into the brain tissue of the rat[5], if it is injected as a bolus into the cerebroventricular system, the responses include hypothermia and seizures[8]. This latter effect was dependent on a sensitization process: 1st administration of AVP caused absence-like seizures while subsequent administration caused myoclonic-tonic convulsions even at doses considerably smaller, such as 10 ng. These observations led us to test the hypothesis that AVP might be responsible for febrile convulsions by using a hyperthermia-induced convulsion model in the rat. The results[9], using homozygous Brattleboro rats with genetic absence of AVP, and rats treated with intracerebroventricular anti-AVP antiserum, suggest that AVP may be a mediator of hyperthermia-induced convulsions. Consequently, these experiments were undertaken to determine if an AVP analog, vasocotin (AVT), given parenterally, could influence pentylenetetrazol-induced convulsions. Pentylenetetrazol-induced convulsions are used as an animal model for human convulsive disorders[10,11].

Male, Long-Evans rats of 250–300 g b.wt were used in these experiments. Rats were housed in colony cages with a 12::12 h light-dark cycle at an ambient temperature of 19±0.5 °C. Food and water were available ad libitum. Experiments were performed on 1 rat at a time and each rat was used only once.

Vasotocin (Bachem) (10 µg) was injected s.c. between the shoulder blades of the conscious rat in a volume of 0.5 ml of sterile physiological saline. Pentylenetetrazol-induced convulsions were induced by a s.c. injection of 20 mg of pentylenetetrazol (Sigma) in 0.5 ml sterile physiological saline. This experiment included 3 experimental groups: a) rats which received only pentylenetetrazol, b) rats which received 10 µg of arginine vasotocin (AVT) 10 min before



Pentylenetetrazol-induced convulsions. The bars represent the mean (±SEM) latency to convulsion as assessed by the 1st myoclonic jerk, in response to pentylenetetrazol (20 mg) and arginine vasotocin (10 µg and 25 µg). Each bar represents 5 rats. PT, pentylenetetrazol; AVT, arginine vasotocin. The groups are statistically different from each other by t-test for unpaired samples ($p < 0.01$).