EFFECTS OF HEXAMETHYLPHOSPHORAMIDE ON RAT SPERMATOGENESIS AND FERTILITY

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Summary. Hexamethylphosphoramide, an important industrial solvent, has been investigated because of its damage potential to reproductive cells. Male rats of proven fertility were given consecutive daily doses at prescribed levels and fertility data were obtained by serial mating. Aspermia in rats followed oral treatment with twenty-one doses of 100 mg/kg and five doses of 500 mg/kg. The former dose induced sterility between the 6th and 12th weeks and fertility did not return in a majority of animals. The histological results at both dose levels were consistent with an antispermatogenic action and correlated with the effects observed on fertility. In the pituitary glands of the sterilized males, considerable enlargement of basophil cells occurred. Simple modifications in the chemical structure of HMPA resulted in loss of the sterilizing action.

INTRODUCTION

Hexamethylphosphoramide(hexamethylphosphoric triamide, Hempa, HMPA, I)

is a compound with widespread uses in industry so that an appraisal of its damage potential to reproductive cells is an important matter, apart from any possible application in the field of chemosterilization. By Normant (1967), it is considered as 'the most remarkable aprotic solvent' and described as a very versatile compound on account of its powerful solvent properties, especially for gases and a wide range of polymers. HMPA is incorporated into polyolefins as a stabilizer against deterioration from exposure to ultra-violet light; in plastics, it

is used to impart antistatic properties and also added to motor fuels for the removal of deposits from internal combustion engines. Suggested applications in the biological sphere include incorporation into systemic insecticides, while addition-compounds formed with many organic chemicals are considered to have potential as antiseptics and germicides.

This paper presents a further contribution to the limited information available on the effects of HMPA upon male reproductive cells and fertility in experimental rodents (Kimbrough & Gaines, 1966; Jackson & Craig, 1966, 1969).

MATERIALS AND METHODS

Hexamethylphosphoramide was obtained from various sources and its effect on fertility compared with that of material purified by preparative gas-liquid chromatography. Other compounds used in this work were prepared by methods described or referred to elsewhere (Jones & Jackson, 1968).

The rats used were of a Wistar strain originally obtained from the United States which have maintained a high reproductive rate over many years. They were fed on standard pellet diet with no supplements.

Fertility data were obtained by a serial mating procedure applied to treated males of proven fertility (Bock & Jackson, 1957; Jackson, Fox & Craig, 1961).

For histological examination, the testes and epididymides were fixed in Bouin's solution for 24 hr then cut transversely across the equator and transferred to 70% alcohol for processing in the usual way. Sections were cut at 6 μ and stained with haematoxylin. Pituitary glands were fixed in 7% formol-saline, processed, and sections stained with Mallory's phosphomolybdic stain.

RESULTS

Six consecutive doses of HMPA (500 mg/kg by mouth) were just tolerable in the American Wistar strain of rat. This course of treatment rapidly induced sterility in male rats due to aspermia and, as in the rabbit (Jackson & Craig, 1966), this was not accompanied by loss of mating activity. Material obtained from two commercial sources gave essentially the same anti-fertility pattern as that purified by preparative gas chromatography (Table 1). The sterilizing ability rapidly declined with dose (Table 1) but the compound was mildly cumulative and striking effects were produced by 3 weeks of daily oral treatment (100 mg/kg; Table 2). Although continuing to gain weight, all animals became sterile in the 6th week and remained so for almost 3 months (Table 2); in fact only three of eight males recovered their fertility after 6 months (Table 3). One half of this dose rate did not produce consistent phases of sterility in the treated males and 25 mg/kg by mouth daily for 1 month had no effect on fertility (Table 2).

The histological results are consistent with an antispermatogenic action and correlate with the effects observed on fertility. Gonads from the 50 mg/kg treatment group provided no significant evidence of damage to the seminiferous epithelium whereas animals receiving 100 mg/kg showed severe damage to the testes by the end of treatment (3 weeks). Most of the tubules were depopulated

TABLE 1
AVERAGE WEEKLY LITTER SIZE FROM MALE RATS TREATED WITH ALKYL PHOSPHORAMIDES

Compound	Treatment (mg/kg)	No. of males	Weeks from first dose																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Hexamethyl- phosphoramide	6 × 500	8	5	6	5	1	0	0	0	0	0	0	0	0	0	0	0	0	
	Exp. 1		—	—	2	4	3	0	1	2	0	6	4	5	5	5			
	Exp. 2	8																	
	6 × 500	5	1	5	0.4	0	0	0	0	0	0	0	0	0	0	0	0		
Pentamethyl- phosphoramide	Exp. 3																		
	Exp. 4	6	9	1	7	0	0	0	0	0	0	1							
	5 × 500	5	—	10	4	7	4	6	9	12	4	3	7	5	8	8	2	1	10
	3 × 1000	5	—	3	4	5	0.4	2	4	8	5	11	3	5	6	11	9	6	9
Hexamethylthio- phosphoramide*	3 × 250	7	7	8	0	0	2	7	3	4	5								

HMPA for Exp. 4 was further purified by gas-liquid chromatography.

* Of seven rats, four died in first 2 weeks.

TABLE 2
AVERAGE WEEKLY LITTER SIZE FROM MALE RATS TREATED WITH HEXAMETHYL- AND HEXAETHYL-PHOSPHORAMIDES

Compound	Treatment (mg/kg)	No. of males	Weeks from first dose																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hexamethyl- phosphoramide	21 × 100	8	—	—	—	5	1	0	0	0	0	0	0	0	0	0	0	1	2	3	2	4	3
	20 × 100	4	—	—	—	10	1	0	0	0	0	0	0	0	0	2	0	0	3				
	20 × 50	4	—	—	—	8	10	1	3	7	8	5	2	0.5	5								
	30 × 25	8	8	4	7	8	8	8	10	8	5	9	10	10									
Hexaethyl- phosphoramide	21 × 100	5	—	—	—	12	3	2	6	2	7	1	7	8	9	4	3						
	21 × 50	4	—	—	—	1	5	5	9	3	10	8	4	4									

HMPA was further purified by gas-liquid chromatography.

PLATE 1

FIG. 1. Testis. 20×100 mg/kg; 3 weeks after first dose; depopulation of tubules, giant cells visible in some. $\times 72$.

FIG. 2. Testis. 20×100 mg/kg; 18 weeks after first dose; complete recovery in most tubules. $\times 72$.

FIG. 3. Testis. 20×100 mg/kg; 18 weeks after first dose; complete recovery in some tubules, little or no recovery in others. $\times 72$.

FIG. 4. Testis. 5×500 mg/kg; 14 weeks after first dose; majority of tubules contain only Sertoli cells, but a few show signs of repopulation. $\times 72$.

PLATE 2

Pituitaries, $\times 455$. Basophil cells in pars anterior of the pituitary are indicated as follows:
 G—granular; LG—losing granules; H—hyalinized; V—vacuolated. A—acidophil cell
 (Mallory's acid fuchsin-aniline blue-orange G stain).

FIG. 1. Control.

FIG. 2. 20×100 mg/kg; 18 weeks after first dose.

FIGS. 3 and 4. 5×500 mg/kg; 14 weeks after first dose.

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and giant cells were frequent (Pl. 1, Fig. 1). However, rat testes examined 18 weeks after the first dose showed full spermatogenesis in some tubules (Pl. 1, Fig. 2) although others remained depopulated and contained only Sertoli cells (Pl. 1, Fig. 3). Dosage of 500 mg/kg daily for 5 or 6 days produced drastic changes in the testes of all animals. The tubules remained small after 14 weeks, the majority containing only Sertoli cells (Pl. 1, Fig. 4), although some showed evidence of spermatogenic activity. Interstitial tissue appeared normal.

TABLE 3
AVERAGE WEEKLY LITTER SIZE FOR EIGHT MALE RATS TREATED WITH HEXAMETHYLPHOSPHORAMIDE

No. of males	Weeks from first dose*																						
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23			
3	4	1	0	0	0	0	0	0	0	0	0	0	4	4	6	6	9	9	7	6			
5	5	1	0	0	0	0	0	0	0	0	0	0	0	0†	0	0	0	0	0	0			
No. 'aspermic'	2	4	—	7	7	6	8	8	8	6	7	8	5	4	1	4	4	4	3	4			
No. fertile	6	2	0	0	0	0	0	0	0	0	0	0	1	3	2	2	4	3	3	2			

* Twenty-one consecutive daily doses of 100 mg/kg by mouth in water.

† One male died.

The selective nature of the action of HMPA is evident from general autopsy data regarding organ weights and is consistent with a direct action upon the spermatogenic cells. The testis weight, 18 weeks after treatment with 100 mg/kg, was reduced by 50%, while the weights of accessory sexual organs, adrenals, thyroid and pituitary were in the normal range. No histological change was observed in the pituitaries of animals examined at the conclusion of treatment. However, in the 20×100 mg/kg and 5×500 mg/kg groups there was, after 18 weeks, enlargement of basophil cells associated with coarse granulation, loss of granules, hyalinization and vacuolation (Plate 2). It has long been known that destruction of the seminiferous epithelium by radiation is followed by enlargement in basophil cells (Mottram & Cramer, 1923) and there is other evidence in favour of a feed-back mechanism from the seminiferous epithelium to the pituitary (Johnsen, 1964; Lacy, 1967). The changes described above might therefore be ascribed to a disturbance of pituitary function following the elimination of seminiferous cells by HMPA, leaving interstitial cell activity unaffected. Removal of the testes is known to induce basophilic 'castration' cell formation in the pituitary.

With these courses of treatment, no changes were observed in the white cell count, but Kimbrough & Gaines (1966) reported, and we have confirmed, that oral treatment of rats with HMPA from two commercial sources for 6 weeks at 100 mg/kg produced multiple lung abscesses. Neither pentamethylphosphoramide (Table 1) nor hexaethylphosphoramide (Table 2) showed sterilizing activity in the rat. Hexamethylthiophosphoramide was more toxic than HMPA, and the survivors after three doses of 250 mg/kg showed sterility in Weeks 3 and 4 (Table 1).

DISCUSSION

The antispermatogenic and antifertility activity of HMPA are obviously of interest from viewpoints of industrial hazards to man and possible use as a male chemosterilizing agent in the control of biological populations. In the present study, sterility was induced in rats by 100 mg/kg daily for 3 weeks; some effect was perceptible at 50 mg/kg daily but 25 mg/kg daily for 1 month produced no effect on weekly litter size. Whilst this seems reassuring from the hazard point of view, it is important to bear in mind that fertility is a crude assessment of testicular function as even a small amount of spermatogenic tissue can maintain a normal level of fertility. More prolonged courses of treatment would be required to provide a thoroughly convincing answer to the question of cumulative damage to reproductive cells.

The discovery of the action of HMPA in the housefly (Chang, Terry & Borkovec, 1964) and the seeming resemblance between its structure and that of triethylenephosphoramidate (TEPA, II) led to studies of structure/activity

relationships with the synthesis of compounds intermediate between HMPA and TEPA. Interchange of the alkylamino groups led to an inhibition of the biological activity against the housefly whilst the effects of mixtures of HMPA and TEPA were only additive (Chang & Borkovec, 1966).

The antifertility effects of HMPA and TEPA correlate in male insect and male rodent so far as relative potency is concerned. However, the action on spermatogenic cells of the two chemical types is very different in rodents. HMPA operates essentially by producing aspermia following destructive effects on spermatids and spermatocytes and the action can extend to spermatogonia, resulting in protracted sterility. Basically, TEPA causes the production of morphologically intact, motile spermatozoa which are functionally incompetent, as do certain other aziridines like tretamine (TEM) (Jackson *et al.*, 1961).

The need for relatively high dosage of HMPA to produce chemosterilization of houseflies (Chang *et al.*, 1964) or sterility in rats, raised the possibility that the action could be due to some metabolite. In fact, pentamethylphosphoramidate, the major metabolite in the rat (Jones & Jackson, 1968) and sole metabolite in the housefly (Chang, Terry, Woods & Borkovec, 1967), did not produce sterility in the rat.

Hexamethylthiophosphoramidate (thioHMPA) showed sterilizing activity but proved to be considerably more toxic than HMPA to the rat, results which paralleled those obtained in the housefly (Terry & Borkovec, 1965). Finally, hexaethylphosphoramidate was found to be ineffective following 3 weeks of

daily treatment (Table 2). Thus the presence of six methyl groups in the phosphoramidate molecule is essential for the antifertility action. It is possible that one of these groups is operative in the antispermatogenic mechanism (e.g. by alkylation) although the functional type of sterility produced by the methyl donors, methylmethanesulphonate (Jackson *et al.*, 1961) and trimethylphosphate (Jackson & Jones, 1968), render this less likely.

The interesting antifertility activity of the HMPA molecule is seen to be highly specific and provides little scope for systematic structural modifications. At present, it is not possible to predict structure/activity relationships but the specific actions of known compounds implies that a more intensive search will lead to further discoveries in this field.

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