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The reproductive toxicity of the organophosphate pesticide O, O-dimethyl O-4-nitrophenyl phosphorothioate (methyl parathion) in the male rat

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*Methyl parathion (MP) is a pesticide widely used to protect crops but also illegally used in many countries for spraying homes and businesses to contain insects. The present study was planned to investigate the effects of MP on the male reproductive organs in the rat. Male Wistar rats (13–14 weeks old) were treated with MP and sacrificed as follows. Experiment 1: 0 (water vehicle), 1.75, 3.5 or 7 mg/kg (*i.p.*) for 5 days and sacrificed on day 14; experiment 2: 0, 0.5 or 1 mg/kg (*i.p.*) for 12 days and sacrificed on day 130; experiment 3: 0, 0.5 or 1 mg/kg (*i.p.*) for 12 days and sacrificed on day 77; experiment 4: 0, 0.75 or 1.5 mg/kg (*i.p.*) for 25 days and sacrificed on day 17; experiment 5: 0 or 3.5 mg/kg (*p.o.*) for 25 days and sacrificed on day 17 after the last exposure. The reproductive organs were removed, weighed and processed for histopathological analysis. Structural changes, for example the morphology of the epithelium and the lumina of the organs, were observed in all animals. Biochemical estimates of acid phosphatase (ACP), cholesterol, total protein, uric acid, and vitamin C were conducted in the epididymes. The weight of the epididymes increased in experiment 2 in a dose-dependent pattern ($p < 0.01$) and decreased in experiments 4 and 5 ($p < 0.01$). The weight of the ductus deferens decreased in experiment 3 at 1 mg/kg dose level ($p < 0.001$) and increased in experiment 5 ($p < 0.05$). The weight of the seminal vesicle decreased in experiment 3 at both 0.5 mg/kg and 1 mg/kg dose levels ($p < 0.001$), and increased in experiment 5 ($p < 0.01$). The weight of the prostate decreased in experiments 4 (in a dose-dependent pattern) and 5 ($p < 0.001$). ACP levels decreased in experiment 4 ($p < 0.001$) with a greater effect at 0.5 mg/kg than at 1 mg/kg. In experiment 5 ($p < 0.01$) cholesterol levels decreased to less than 50% of the control level for this experiment ($p < 0.01$) and protein levels also decreased ($p < 0.01$). Vitamin C levels decreased in a dose-dependent pattern in experiments 4 ($p < 0.001$) and 5 ($p < 0.01$). There were no effects on uric acid level. Sperm density was decreased in the epididymes of the rats treated and the epithelium of the epididymis*

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and ductus deferens showed cellular necrosis, brush-border disruption and nuclear pyknosis. Nuclei were haloed, except in experiment 2 and the 0.5 mg/kg group of experiment 3. Methyl parathion did not induce significant changes in the structure of the seminal vesicle and prostate, except that epithelial folding was shorter than in the control. In conclusion, MP is a reproductive toxicant in the male rat and causes deterioration in the structural integrity of the reproductive organs and also the biochemical parameters in the epididymis.

Key words: pesticides, organophosphates, gonadotoxicity, reproductive toxicity, fertility, endocrine disruptors

INTRODUCTION

If the merits of pesticides include enhanced economic potential for their use in agriculture to contain pests, on the debit side are the serious health implications for man and animal life [4]. Inadvertent pesticide exposure may take place as a result of illegal domestic use to control insects. There have been several instances of the misuse of pesticides to spray homes, such as that in the United States in 1990s, when two unlicensed users sprayed a number of houses and businesses to eradicate cockroaches [39, 40, 46]. Methyl parathion (MP) is toxic in humans, and its toxicity profile include headache, dizziness, and confusion, loss of consciousness, difficulty in breathing, loss of co-ordination, muscle twitching, tremor, nausea, vomiting, abdominal cramps, diarrhoea, blurred vision and excessive perspiration and salivation [12, 40]. In non-agricultural countries the population may be exposed to pesticide residues in food stuffs, especially fruit and vegetables, imported from other countries [17] or as a result of bioaccumulation in the food-chain, including marine products [29]. Moreover, MP or its metabolite, *p*-nitrophenol, is one of the pesticides commonly detected in the urine samples of the general population in the United States [3].

Methyl parathion is known to have different mechanisms of toxicity on non-target organisms, but the most important one is inhibition of the activities of the enzyme acetylcholinestarase, thus making it a potent neurotoxin by enhancing the accumulation of endogenous acetylcholine [22, 27, 43]. Furthermore, MP is known to affect other biochemical parameters, such as glutamate dehydrogenase, alanine aminotransferase, aspertate aminotransferase, lactate dehydrogenase and malate dehydrogenase in the brain, liver, muscle and plasma [10]. At very low dose levels, MP has affected the open-field behaviour, auditory responses, and plaque-forming cell count in the rat spleen [16]. It also has the ability to cross the placental barrier, affecting the foetal brain, growth and survival in rats [17, 25]. MP is embryotoxic in chickens and induces skeletal deformities in the survivors [19, 45] as well as retarded growth, reduced body weight and body length and shortening of the long bones, in addition to various other developmental deformities [23]. Daily *i.p.* injections of 1 or 1.5 mg/kg MP to pregnant rats during gestation days 6–19 decreased the maternal and foetal protein synthesis in a dose-dependent pattern [13, 14]. At dose levels of 5–20 μg/100 g body weight/day, MP induced testicular structural changes in adult male white-throated munias (*Lonchura malabarica*) [28]. Testicular weight was also reduced significantly, albeit only after 10 days at the two higher dose levels, but a significant decrease in the number of tubules containing healthy germ cells occurred after even a single administration of MP at the lowest dose [28]. These authors confirmed that MP may impart antigonadal effects by impairing the cholinergic functions of the brain and/or testes [28]. The effects of MP on the ovaries and uterus have been extensively studied in animals. Briefly, the effects were decreased compensatory weight gain of the ovaries in hemi-castrated rats, decreased healthy follicles [11] and a decrease in the number of oestrous cycles and the duration of each phase [2, 41]. MP is also known to have severe effects on the structure and function of the placenta [25] and is known to affect the protein, phospholipid, total lipid and cholesterol levels in the ovaries [21].

Treatment of MP at dose levels of 9.4–75 mg/kg for 5 days significantly increased sperm abnormalities but without any effects on sperm count when this was examined 1 or 5 weeks following the last exposure [31]. In another study it was observed that very low doses of MP (0.5–1.5 mg/kg; *i.p.*; 12 or 25 treatments) induced the formation of abnormal sperm and decreased the sperm count in direct relation to

decreased ascorbic acid levels in the testis. In this study there were no direct effects on the fertility of the male rats treated, nor any marked effects on F_1 generation [32]. On the other hand, very few studies have been conducted to evaluate the effects of MP on the male reproductive system in general, and, in particular, there have been no studies exploring the effects on the accessory reproductive organs in the male. In view of this, the present study was planned to investigate the adverse effects of MP on the accessory reproductive organs of the male rat.

MATERIAL AND METHODS

Animal models

Male Wistar rats (13–14 weeks old) were bred and maintained in the central animal house with a 12:12 light-dark cycle. Then animals were housed in polypropylene cages with paddy husk bedding at a temperature of 28 ± 2°C and 50 ± 5% humidity. They were fed on laboratory chow (Gold Mohur; Lipton India, Ltd) and water *ad libitum*. At the end of the experimental period the animals were anaesthetised (Pentobarbitone sodium, 45 mg/kg, *i.p.*) and then sacrificed by terminal dose of the same. All experimental procedures and animal maintenance conformed strictly to the guidelines of the Institutional Animal Ethics Committee and to the Federal laws governing the use of animals in experiments.

MP treatment schedule and basis of dose selection

The pesticide MP is manufactured and marketed with the brand name of Metacid 50 by Bayer India Ltd., Mumbai. It is an emulsifiable insecticide containing 50% w/w MP (O-O-dimethyl O-4-nitrophenyl phosphorothioate). The commercial form of MP was procured from licensed dealers. The experimental design used in this study is shown in Table 1.

In experiment 1, 1.75, 3.5 and 7 mg/kg, equivalent to 1/4, 1/2 and 1 LD_{50} (*i.p.*) respectively, were administered [17]. In a screening study planned to find out the first effects of MP to become visible in the reproductive organs structural changes were only noticeable on day 14. Hence the sampling day for experiment 1 was established as day 14 after the last exposure. In this experiment animal mortality was observed and three rats died during the treatment period and replacement animals were used.

Experiments 2 and 3 were designed to evaluate the effects of MP after a long period following the cessation of treatment of relatively smaller doses. No rats died in these two experiments.

Table 1. Schedule of MP treatment and animal sacrifice

Experiment number	MP [mg/kg] (route)	No. of rats/ /group	No. of treatments	Sacrifice day after the last treatment
1	0 (water-vehicle; *i.p.*)	5	5	14
	1.75 (*i.p.*)	5	5	14
	3.5 (*i.p.*)	5	5	14
	7 (*i.p.*)	5	5	14
2	0 (*i.p.*)	5	12	130
	0.5 (*i.p.*)	5	12	130
	1 (*i.p.*)	5	12	130
3	0 (*i.p.*)	5	12	77
	0.5 (*i.p.*)	5	12	77
	1 (*i.p.*)	5	12	77
4	0 (*i.p.*)	5	25	17
	0.75 (*i.p.*)	5	25	17
	1.5 (*i.p.*)	5	25	17
5	0 (*p.o.*)	5	25	17
	3.5 (*p.o.*)	5	25	17

Experiment 4 was designed to investigate the long-term effects of small doses after *i.p.* exposure. In this experiment also no animals died. The doses selected in experiments 2–4 were arbitrary but were very small. Although the *i.p.* route is not the one by which MP exposure takes place, we have selected this route because of the simpler handling, the accurate administration of prescribed doses and the easy and quick absorption from the peritoneal cavity [17, 31, 32].

Experiment 5 was designed to evaluate the long--term effects of *p.o.* administration of higher dose of 3.5 mg/kg, which is equivalent to half LD_{50} [17] on the reproductive system. In this experiment three further animals died during treatment and were replaced with new ones.

Assessment of qualitative structural damage to the reproductive organs

Laparotomy was conducted in each animal to expose the reproductive system. The reproductive organs (the epididymis, ductus deferens, ventral prostate, and seminal vesicles) were removed and placed in phosphate buffered saline. The weights of the reproductive organs were recorded and further processed for light microscopical observation after paraffin embedding, as per the standard procedure [7]. Sections of 5 μm thick were stained with haematoxylin and eosin. The qualitative changes induced by MP were recorded. In the lumen of the epididymis sperm density was

observed and was graded as normal (+++), moderately decreased (++) or severely decreased (+), depending on the concentration of spermatozoa in the tubular cross-sections. Structural changes in the epididymis and ductus deferens such as epithelial necrosis, nuclear pyknosis and degeneration, brush-border disruption, vacuoles in the epithelium or the presence of immature germ cells were noted and a grade "+" was assigned if these features were present and a grade "–" if they were absent. Similarly, qualitative changes were also observed in the seminal vesicle and prostate.

Assessment of the biochemical effects on the epididymis

The epididymis of one side was homogenised in 5 ml phosphate buffered saline. The homogenate was used for biochemical analysis as described below.

Acid phosphatase (ACP)

The activity of this enzyme was assayed by the calorimetric method of Kind and King [44]. Briefly, 2 ml of buffer substrate was added to two test tubes (test and control) and kept in a water bath at 37°C for a few minutes. Then 0.1 ml of homogenate was added to the test and incubated for 1 h at 37°C. Both the test tubes were removed from the water bath. 0.8 ml of sodium hydroxide and 1.2 ml of sodium bicarbonate were added and 0.1 ml of homogenate was also added to the control. Then 1 ml each of antipyrene and potassium ferricyanide was added to both tubes. The reading was taken at 520 nm against the reagent blank. The activity of ACP was expressed as IU/L.

Cholesterol

The cholesterol level was estimated by Zak's method [44]. For 0.1 ml homogenate in a test tube 9.9 ml of $FeCl_3$ acetic acid solution was added. The mixture was kept at room temperature for 10–15 min, and then centrifuged, and 5 ml supernatant was taken for the test. A standard graph was plotted from which the values of the unknown were calculated. The colour intensities measured at 540 nm were directly proportional to the concentration of cholesterol. The cholesterol level was expressed as mg/dl.

Protein

The total protein level was estimated by the biuret method [44]. For 0.1 ml homogenate in a test tube, 2.9 ml of NaCl was added and mixed. Then 3 ml of biuret reagent was added and kept for 10 min and the colour intensity was read at 540 nm to express the total protein level as g/dl.

Uric acid

The uric acid level was quantified by the Caraway method [44]. 4.5 ml of tungstic acid was added to a beaker containing 0.5 ml of homogenate. The solution was mixed well and centrifuged. 3 ml of the supernatant obtained was used as a test solution. 3 ml dilute standard and 3 ml water were used as the blank. All the tubes were further processed by adding 0.6 ml sodium carbonate and 0.6 ml dilute phosphotungstate, mixed and placed at 25°C in a water bath for 30 min. The colour intensity was read within 15 min at 700 nm or red filter. Uric acid level was expressed as mg/dl.

Vitamin C

The vitamin C level was estimated by the 2, 4-dinitrophenyl-hydrazine method [20]. Briefly, 0.5 ml of homogenate was added to 2 ml of freshly prepared metaphosphoric acid, mixed well in a vortex mixer and centrifuged for 10 min at 2500 rpm. 1.2 ml of the clear supernatant was taken. In seven other tubes 0.1, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml, respectively of working standards were taken and the volume made up to 1.2 ml with metaphosphoric acid. To the blank 1.2 ml of metaphosphoric acid was added. 0.4 ml of dinitrophenyl-hydrazine-thiourea-copper sulphate was added to all the tests, mixed and the tubes were incubated in a water bath at 37°C for 3 h with glucuronic acid and ascorbate-2-sulphate. The tubes were removed from the water bath and chilled for 10 min in an ice bath. 2 ml of chilled sulphuric acid (12 mol/l) was added to all the tubes and mixed well in a vortex mixer at room temperature. The absorbance was read at 520 nm against the reagent blank to express the result in mg/dl.

Statistical analysis

Data were represented as means ± SD from 5 animals/group. The differences were compared for statistical significance by one-way ANOVA followed by Bonferroni's (post hoc) test. However, the data from experiment 5, which involved only two groups, were analysed by the Mann-Whitney U-test. In all cases the significance level was set at $p < 0.05$. Data analysis was carried out by the SPSS package version 10.

RESULTS

Table 2 shows the weights of the reproductive organs in the control and MP-treated rats. MP did not affect the weights of the reproductive organs in experiment 1, but the weights of the epididymes increased in the 0.5 and 1 mg/kg groups in experiment 2 on day 130. The latter effect was exerted in

Table 2. Reproductive organ weight in control and MP-treated rats

Experiment no.	Groups	Epididymis	Ductus deferens	Seminal vesicle	Prostate
1	Control	0.53 ± 0.02	0.14 ± 0.03	0.96 ± 0.05	0.84 ± 0.03
	1.75 mg/kg	0.56 ± 0.05	0.13 ± 0.03	0.97 ± 0.01	0.79 ± 0.02
	3.5 mg/kg	0.56 ± 0.03	0.10 ± 0.01	0.98 ± 0.04	0.83 ± 0.02
	7 mg/kg	0.58 ± 0.07	0.11 ± 0.01	0.93 ± 0.02	0.85 ± 0.01
2	Control	0.44 ± 0.09	0.12 ± 0.02	0.95 ± 0.05	0.85 ± 0.04
	0.5 mg/kg	0.55 ± 0.01^{**b}	0.12 ± 0.02	0.88 ± 0.12	0.79 ± 0.07^{a}
	1 mg/kg	0.57 ± 0.02^{**b}	0.13 ± 0.03	0.87 ± 0.06	0.87 ± 0.02^{a}
3	Control	0.62 ± 0.12	0.22 ± 0.02	0.91 ± 0.06	0.85 ± 0.01
	0.5 mg/kg	0.60 ± 0.06	0.20 ± 0.03^{b}	0.71 ± 0.03^{***c}	0.82 ± 0.05
	1 mg/kg	0.68 ± 0.07	0.13 ± 0.03^{***b}	0.75 ± 0.05^{***c}	0.88 ± 0.05
4	Control	0.65 ± 0.05	0.19 ± 0.03	0.92 ± 0.02	0.88 ± 0.05
	0.75 mg/kg	0.57 ± 0.01^{**a}	0.15 ± 0.01	0.98 ± 0.10	0.69 ± 0.02^{***b}
	1.5 mg/kg	0.52 ± 0.02^{***a}	0.17 ± 0.03	1.06 ± 0.14	0.48 ± 0.05^{***b}
5	Control	0.65 ± 0.05	0.19 ± 0.03	0.92 ± 0.02	0.88 ± 0.05
	3.5 mg/kg	0.50 ± 0.07**	0.25 ± 0.04*	1.17 ± 0.19**	0.66 ± 0.15**

Data are represented as means ± SD from 5 animals/group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ control *vs.* treated; and $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, inter-group differences.

a dose-dependent pattern with inter-group differences ($p < 0.01$). On day 77 in experiment 3 the ductus deferens had a lower weight in the 1 mg/kg dose group ($p < 0.001$) and the weights of the seminal vesicle also decreased in both the 0.5 and 1 mg/kg groups ($p < 0.001$; Table 2). However, there was no effect on the weights of the epididymis and prostate. In experiment 4 the weights of the epididymes and prostate decreased in a dose-dependent pattern in the 0.75 mg/kg and 1.5 mg/kg treated groups with inter-group differences ($p < 0.01$–0.001). In experiment 5 the weights of the ductus deferens and seminal vesicle increased in the 3.5 mg/kg group ($p < 0.01$), whereas that of the epididymis and prostate decreased ($p < 0.01$; Table 2). Long-term *p.o.* treatment of 3.5 mg/kg in experiment 5 was more effective in reducing the weights of the epididymis and prostate and in increasing that of the ductus deferens and seminal vesicle. After a long period of 130 days in experiment 2 the small *i.p.* doses were still effective in keeping down the weights of the epididymis (Table 2). The *i.p.* doses also decreased the weights of the ductus deferens and seminal vesicle during recovery phase on day 77. However, long-term *i.p.* treatment of 0.75 mg/kg or 1.5 mg/kg in experiment 4 was able to reduce only the weights of the epididymis and prostate and not those of other organs (Table 2).

Methyl parathion induced structural changes in the epididymis. One conspicuous finding was reduced sperm density in the lumen of the epididymal tubule (Table 3; Fig. 1). The epididymes from the control animals of all groups showed normal structure

Table 3. Qualitative assessment of damage in MP-treated epididymis

Experiment no. (sample point)	Dose [mg/kg]	Sperm density	Epithelial necrosis	Nuclear degeneration	Pyknosis	Germ cells in the lumina
1 (14 day)	1.75	+++	+	+	+	–
	3.5	++	+	+	+	–
	7	++	+	+	+	–
2 (130 day)	0.5	+++	–	–	–	–
	1	+++	–	–	–	–
3 (77 day)	0.5	++	–	–	–	–
	1	+	+	–	+	–
4 (17 day)	0.75	+	+	+	+	+
	1.5	+	+	+	+	+
5 (17 day)	3.5	++	+	–	+	–

Control rats did not show any of the above-mentioned qualitative changes. The sperm density in the control groups was normal (+++) (not shown in the table).
+ severaly decreased, ++ moderately decreased and +++ normal sperm density. "+" presence and "–" absence of a particular pathological change.

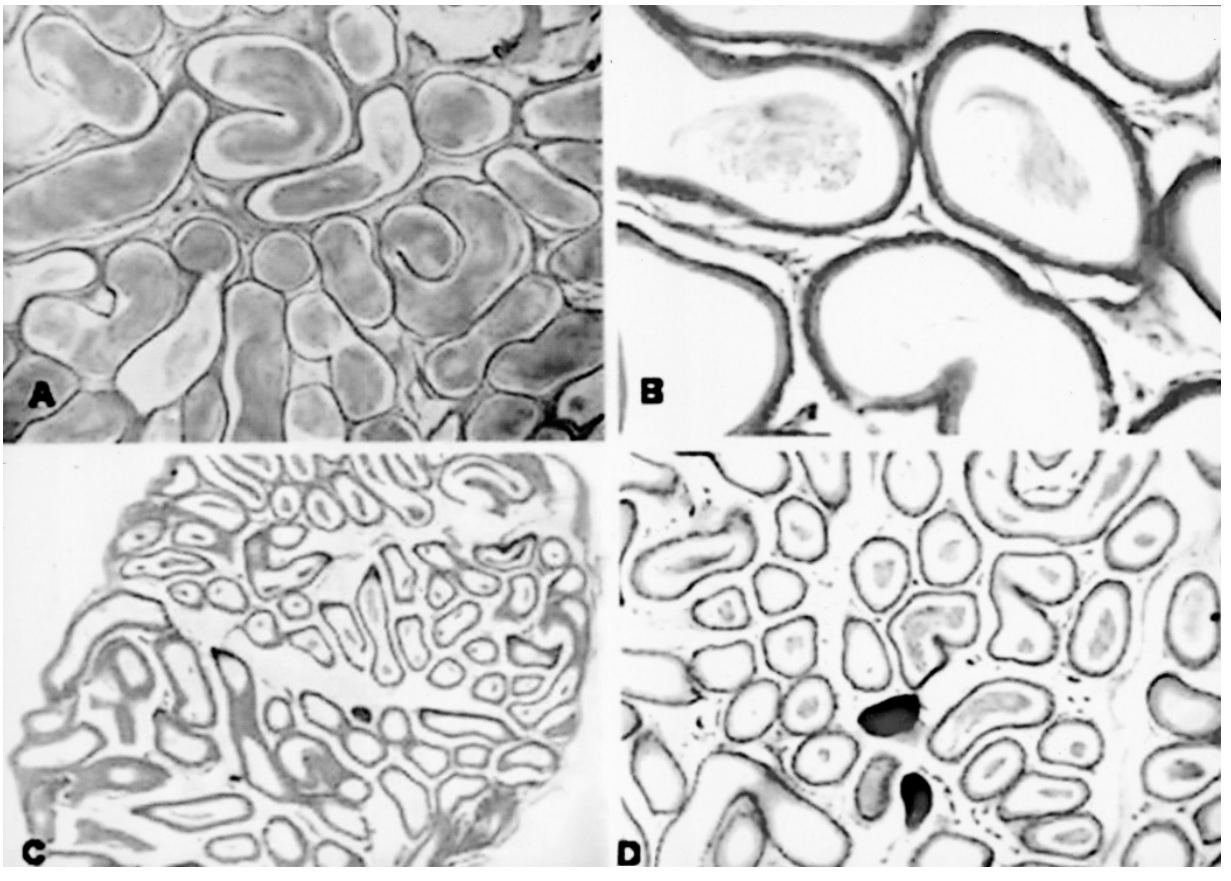

Figure 1. Photomicrographs of epididymes showing sperm density in the lumina of tubular sections; **A.** From control rat showing sperm (+++) in the lumina completely filled except for a very few tubular sections. H&E, 100×; **B.** From rat exposed to 12 treatments of 1 mg/kg on day 77. Note that sperm density is low (+). H&E, 400×; **C.** From a rat exposed to 25 *i.p.* treatments of 0.75 mg/kg on day 17 (+). Note that many tubules are empty and others have fewer spermatozoa. H&E, 40×; **D.** From a rat exposed to 3.5 mg/kg (*p.o.*) showing moderate (++) sperm density on day 17. H&E, 100×.

and normal sperm density (+++; Fig. 1A). However, sperm density was found to be decreased in the experimental groups (Fig. 1B–D). In the 3.5 mg/kg and 7 mg/kg dose groups in experiment 1 the sperm density was moderately decreased (++), whereas in experiment 2 at both dose levels sperm density was normal. Sperm density was moderately decreased (++) at 0.5 mg/kg, and severely decreased (+) at 1 mg/kg dose levels in experiment 3 (Table 3). In experiment 4 both 0.75 mg/kg and 1.5 mg/kg dose groups showed the same effects on sperm density (+), whereas in experiment 5 a moderate decrease (++) was seen. Intra-epithelial nuclei (+; Fig. 2A), epithelial necrosis (+; Fig. 2B), immature germ cells in the lumina of tubular cross sections (+; Fig. 2A) and nuclear changes (+; Fig. 2C, D) were observed in the epididymis, except in experiment 2 and the 0.5 mg/kg group of experiment 3 (Table 3).

The ductus deferens from the control group showed a normal brush border of the epithelium (Fig. 3A), but the same occurred in all the treated groups of experiments 1 and 4 (Fig. 3B), although not in experiments 2, 3 and 5 (Table 4). The immature germ cells were seen in the lumen of the epididymis from the treated groups belonging to experiment 4 (Table 4; Fig. 3B). Pyknotic nuclei (in all experiments, except the 1.75 mg/kg group of experiment 1 and the 0.5 mg/kg group of experiment 3), and bubbling of the eosinophilic bodies (in experiment 4; Fig. 3C) were seen in the treated epididymis. The vacuoles in the epithelium were seen in epididymes from experiments 1 and 4 (Fig. 3D).

Analysis of the structure of the seminal vesicle revealed no major changes except the nuclear pyknosis and a smaller number of epithelial folds in the treated groups (Fig. 4A, B). These changes were observed in all experiments except 2 and 3. There were no major changes in the structure of the prostate of the treated rats as compared to the control (Fig. 4C, D) although pyknotic nuclei and small folliculi were seen. In the treated rats the folliculi appeared to be empty compared to those in the control.

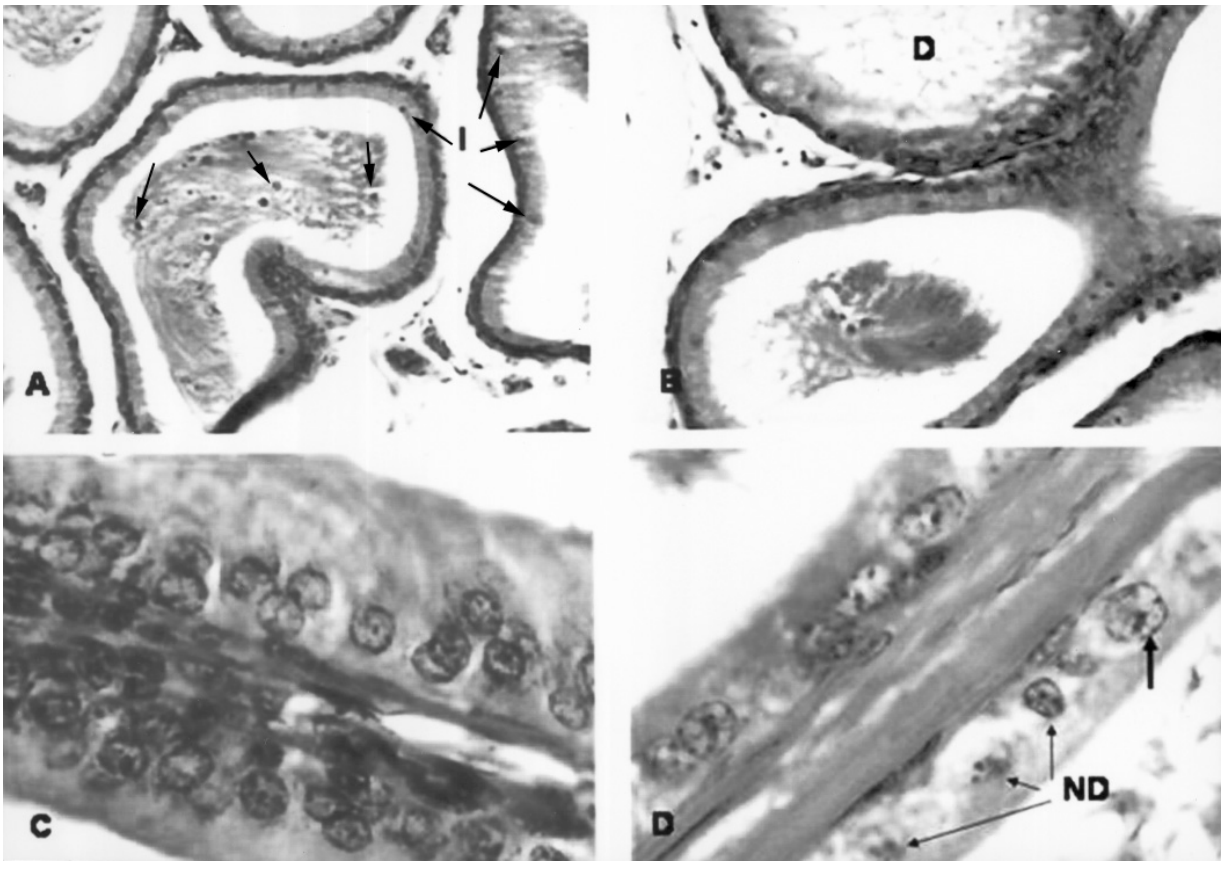

Figure 2. Photomicrographs of epididymes from control and treated rats; **A.** The exfoliated germ cells in the lumen (arrows) and intra-epithelial nuclei (I) in the epididymis from a rat exposed to 25 *i.p.* treatments of 1.5 mg/kg on day 17; **B.** Epithelial degeneration (D) in the epididymis of another rat from the same group. Note that the intra-epithelial nuclei are visible but are undergoing degenerative changes. H&E, 400×; **C.** Epithelium of an epididymal tubule from a control rat; **D.** Epithelium of the epididymis from a rat treated with 3.5 mg/kg on day 17. Note that the nuclei are undergoing degenerative changes (ND). H&E, C, D: 1300×.

Acid phosphatase concentration decreased in both experiments 4 and 5 (Table 5). The decrease was greater at 0.75 mg/kg compared to that at 1.5 mg/kg in experiment 4 ($p < 0.001$). The *i.p.* treatment was associated with a greater decrease in the ACP level in experiment 4 than the *p.o.* treatment with the higher dose of 3.5 mg/kg in experiment 5 ($p < 0.01$; Table 5). Cholesterol and total protein levels decreased only in experiment 5 ($p < 0.01$), whereas vitamin C level decreased in both experiments ($p < 0.01$–0.001), with greater effect in experiment 4. MP showed no effect on uric acid concentration in the epididymis.

DISCUSSION

To our knowledge, this is the first study to report the toxic effects of MP on the reproductive organs. Previous toxicity studies of MP concentrated mainly on the female reproductive system and all these studies indicated the damage caused to the ovaries and uterus [2, 11, 41]. The studies that have evaluated the effects on the male reproductive functions have revealed toxic effects on testis function in a rare experimental model on white-throated munias [28]. Other studies revealed that MP affected sperm morphology in mice [31] and sperm count and morphology in the rat, with a positive relationship with a decreased level of ascorbic acid level in the testis [32]. The present results indicate that MP also affects the accessory reproductive organs in the rat, a fact that has not yet been explored. We were unable to observe any effects, except some structural changes, up to day 14 after the last exposure to high *i.p.* doses (1.75–7 mg/kg), an observation also supported by the absence of any significant variation in organ weights by that time (Table 1). This observation also demonstrates that MP does not cause any structural changes in accessory reproductive organs within a short time of *i.p.* exposure, regardless of the strength of the dose. A strange finding was that the weights of the epididymes decreased in experiments 4 and 5,

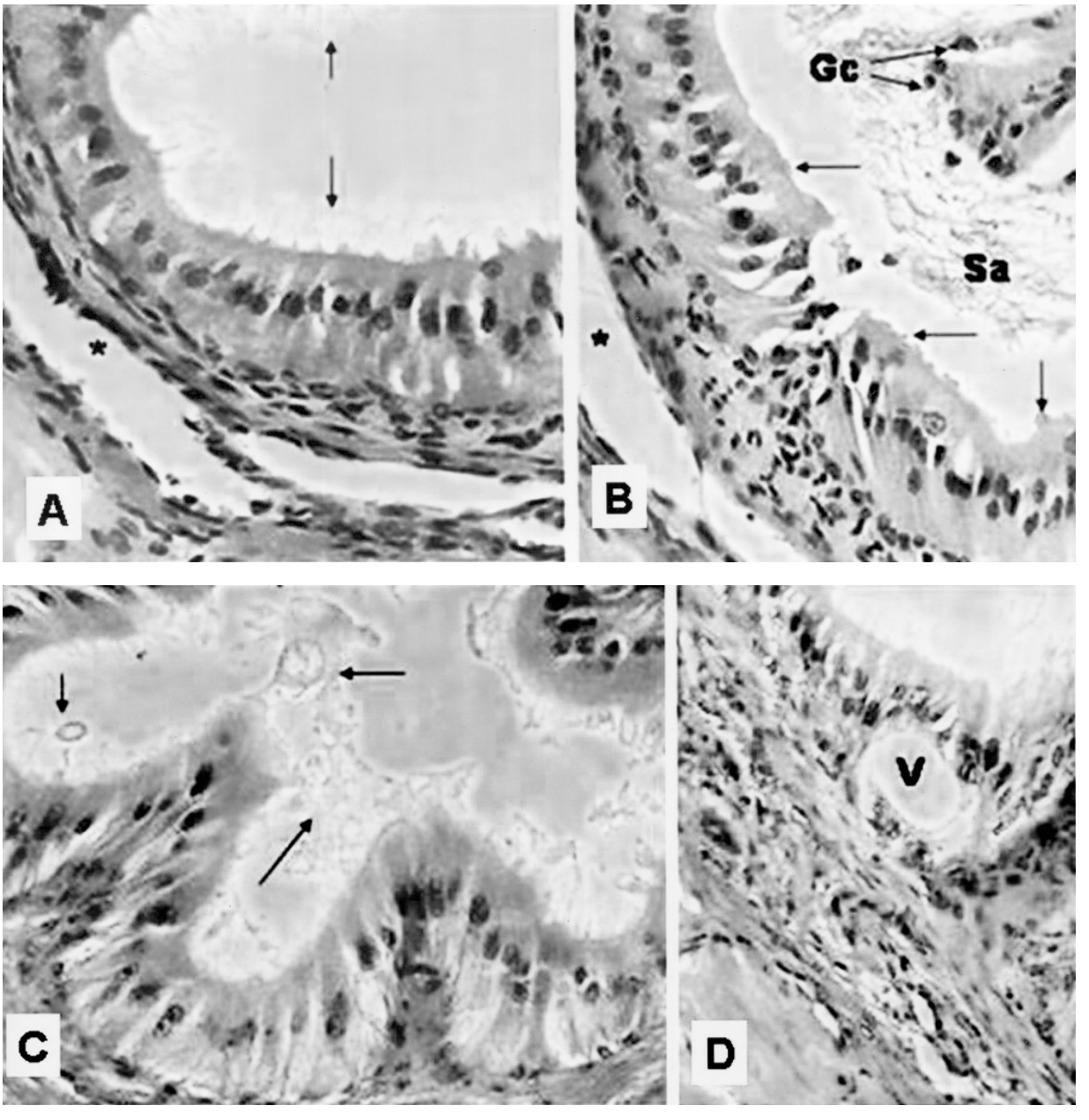

Figure 3. Structure of the ductus deferens in control and MP-treated rats; **A.** From a control rat, showing normal epithelium with stereocilia (arrows); a gap formed by a preparation defect has been indicated in this as well as in the next illustration (*); **B.** From a 0.75 mg/kg (*i.p.*) treated rat on day 17, showing spermatozoa (Sa) and exfoliated germ cells (Gc) in the lumen. Note that the stereocilia are very few or are absent from the epithelium. H&E, 400×; **C.** From a rat treated with 1.5 mg/kg on day 17, showing stereocilia disruption and the bubbling of eosinophilic bodies (arrows); note also the pyknotic nuclei in the epithelium; **D.** From a rat treated with 3.5 mg/kg on day 14, showing a large vacuole in the epithelium (V). H&E, 400×.

although the routes of exposure and doses used were no way comparable. The decrease in epididymal weight may be partially, if not completely, due to reduced protein quantity in the organ. However, it is not possible to extrapolate the same concept to weight changes in other organs, since we did not estimate the protein quantity in them. The epididymal weights increased in experiment 2 on day 130, exceeding those on day 77 in experiment 3. The absence of any significant effects on day 77 may be due to recovery from the toxicity of MP. From observation of the structural changes in the epididymes of the treated rats of experiments 4 and 5, we believe that the decreased epididymal weight was largely due to lowered sperm density, degenerative changes and decreased protein quantity. For unknown reasons there was an early increase in the weight of the ductus deferens in

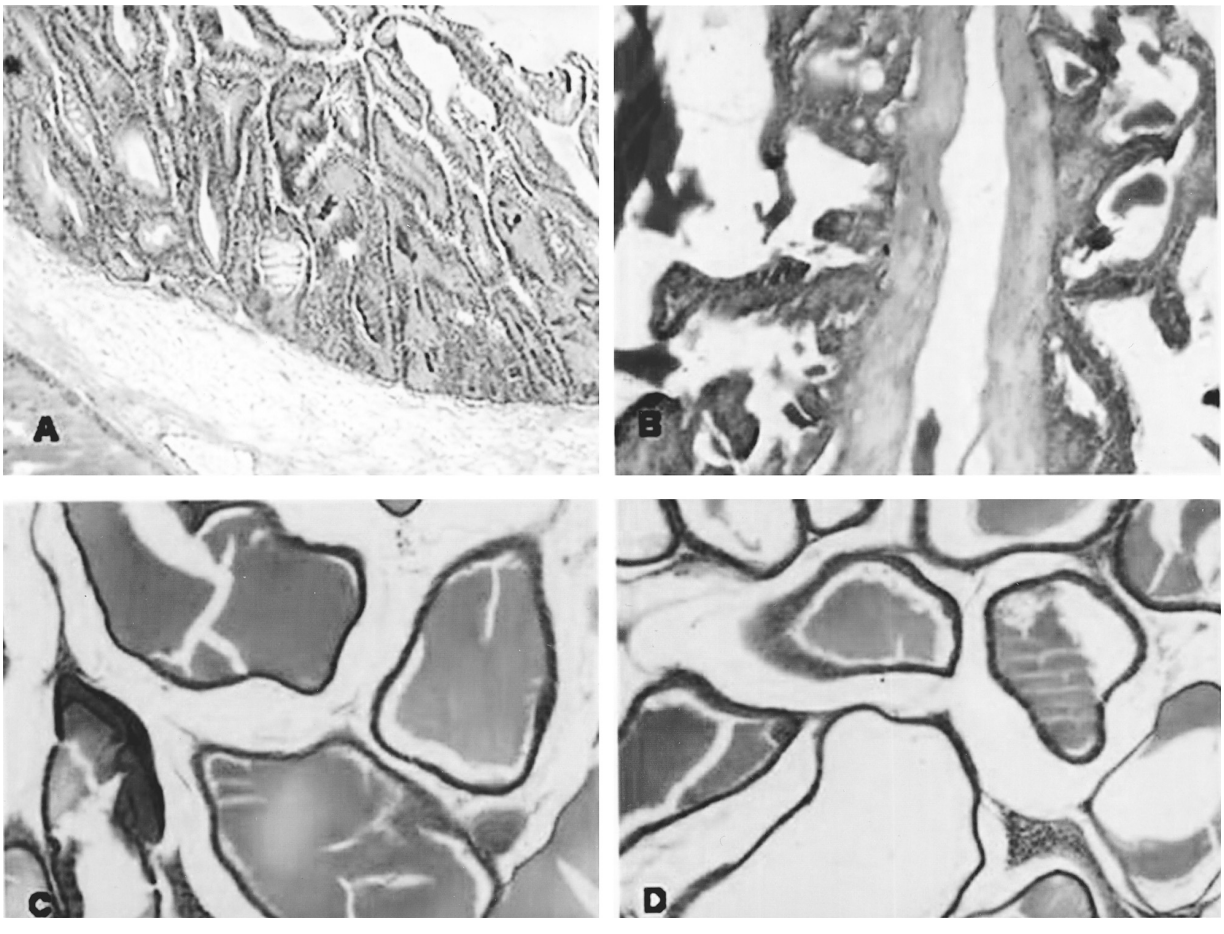

Figure 4. A. Photomicrograph of a seminal vesicle from a control rat, showing normal structure; **B.** From a rat treated with 1.5 mg/kg on day 17, showing a smaller number of epithelial folds and pyknosis of epithelial cell nuclei. H&E, 400×; **C.** A section of the prostate of a control rat; **D.** From a rat treated with 1.5 mg/kg on day 17. H&E, 100×.

Table 4. Qualitative assessment of damage in MP-treated ductus deferens

Experiment number (sample point)	Dose [mg/kg]	Pyknosis	Brush border disruption (stereocilia)	Sperm in the lumen	Vacuoles in the epithelium	Germ cells in the lumen
1	1.75	–	+	–	+	–
	3.5	+	+	–	+	–
	7	+	+	+	+	–
2	0.5	+	–	–	–	–
	1	+	–	–	–	–
3	0.5	–	–	–	–	–
	1	+	–	+	–	–
4	0.75	+	+	+	+	+
	1.5	+	+	+	+	+
5	3.5	+	–	+	–	–

There were no qualitative changes in the structure of the ductus deferens in the control groups (not shown in the table). "+" presence and "–" absence of a particular feature.

experiment 5 at 3.5 mg/kg on day 17. However, on day 77 in experiment 3 the weight of the ductus deferens was decreased at 1 mg/kg, which may be due to long persistent degenerative effects of MP. Similarly, the weights of the seminal vesicle decreased in experiment 3 at 0.5 and 1 mg/kg dose levels on day 77 and increased in experiment 5 at 3.5 mg/kg on day 17 (Table 2). These observations

Table 5. Biochemical effects of MP on the epididymis

Experiment no.	Groups	ACP [IU/L]	Cholesterol [mg/dl]	Total protein [g/dl]	Uric acid [Mg/dl]	Vitamin C [mg/L]
4	Control	131.40 ± 2.07	44.50 ± 10.99	4.13 ± 0.37	10.19 ± 0.55	10.12 ± 1.02
	0.75 mg/kg	94.39 ± 5.46^{***c}	41.04 ± 12.47	4.22 ± 0.37	10.59 ± 0.97	4.11 ± 1.21^{***c}
	1.5 mg/kg	110.24 ± 7.39^{***c}	49.32 ± 12.44	4.06 ± 0.42	10.97 ± 1.12	3.13 ± 1.01^{***c}
5	Control	131.17 ± 2.34	73.29 ± 7.14	4.76 ± 0.83	11.29 ± 0.64	11.29 ± 1.32
	3.5 mg/kg	102.14 ± 12.71**	34.26 ± 4.20**	1.72 ± 0.41**	9.17 ± 1.22	5.15 ± 0.61**

Data are represented as means ± SD from 5 animals/group; $^{**}p < 0.01$, $^{***}p < 0.001$, control *vs.* treated; $^{c}p < 0.001$, inter-group differences.

indicate that *p.o.* treatment of 3.5 mg/kg increases the weights of the ductus deferens and seminal vesicle and that *i.p.* exposure decreases the weights for a long time after the cessation of treatment. Long-term *i.p.* treatment of 0.75 mg/kg and 1.5 mg/kg MP in experiment 4 and *p.o.* treatment of 3.5 mg/kg in experiment 5 decreased the weight of the prostate in a dose-dependent pattern, indicating that both routes of exposure affected the prostate structure, although the effect appeared to be greater after *i.p.* exposure than after *p.o.* exposure (Table 2). Maintenance of the weights of the accessory reproductive glands depends on testosterone level [18], and several pesticides such as atrazine [42] or methoxychlor, have reduced the weights by affecting either the hypothalamus or pituitary or both [24, 35]. MP increased the testosterone level in the testis (data not shown) in both experiments 4 and 5, although the weights of the epididymes and prostate decreased, indicating that MP acted as an antioestrogen in the testis, as seen with other chemicals [47]. Generally, the decline in prostate weight is due to a decrease in the level of testosterone, since this is an androgen-dependent organ [34]. Studies in the rat have shown that neonatal and pre-pubertal steroids are of critical importance in setting the long-term growth regulation of the prostate [33]. That is, it has a high affinity to the androgen receptor and binds to the same, hence exerting effects on some of the androgen-regulated endpoints such as the weight of accessory reproductive organs. The reason for the decrease in prostate/epididymal weight in the present case may be the non-availability of testosterone because of competitive binding of MP or its metabolites to the androgen receptors. This aspect of MP toxicity, however, needs to be explored further. However, similar observations have been made in rats exposed *in utero* to endocrine disruptors 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) or p, p-DDE, which increased the testosterone level but decreased the prostate weight as a result of decreased androgen receptors in the latter [26]. Vinclozolin, a pesticide, also exhibited such effects in adult rats, in which it increased the testosterone level, but the weights of prostate and other accessory organs decreased [47].

Decreased sperm density in the epididymis is an indicator of reduced spermatogenesis as a result of the toxicity of any agent [37]. The sperm density was moderately decreased (++) in experiment 1 at 3.5 mg/kg and 7 mg/kg, which may be due to the direct spermicidal effects of MP on sperm in the epididymis (Table 3). In addition, after prolonged exposure to 3.5 mg/kg in experiment 5 on day 17, there was only a moderate decrease (++; Fig. 1D) in sperm density, which means that *p.o.* treatment was not very effective in decreasing this. In contrast, *i.p.* exposure of 0.75 mg/kg (Fig. 1C) or 1.5 mg/kg in experiment 4 on day 17 or 1 mg/kg in experiment 3 on day 77 severely affected (+; Fig. 1B) the sperm density, indicating that *i.p.* exposure of even smaller doses had greater and more prolonged spermatotoxic effects. The present results are in disagreement with an earlier report, in which sperm count was not affected after MP exposure of mice at very high dose levels [31], but in agreement with the results of another study in rats [32]. However, the results obtained on day 130 in experiment 2 indicated that there was a complete recovery from the spermatotoxic effects of MP (Table 3).

The histopathological changes in the epididymis were intra-epithelial nuclei, immature germ cells in the lumen, epithelial necrosis, and nuclear changes such as pyknosis, and degeneration (Fig, 2A–D), which, when taken together, indicate epithelial degeneration [6]. However, such effects were not seen in experiment 2 on day 130, indicating a recovery. MP is a well known inhibitor of acetylcholinesterase activity [39], and some authors [28] have reported that the testicular damage in MP-treated munias may have some relation to inhibited acetylcholinesterase

in the testis or brain. It is unknown at present whether or not any effect of MP on the nerves supplying these reproductive organs mediates the structural changes observed in the present study. MP affected the ACP concentrations in the epididymis in experiments 4 and 5 (Table 5), and in both cases it was decreased, although the *i.p.* treatments of 0.75 mg/kg and 1 mg/kg in experiment 4 had a greater effect than the *p.o.* treatment of 3.5 mg/kg in experiment 5. MP-induced cell damage resulted in the release of ACP into the blood stream, hence reducing its level in the epididymis [1], and this action of MP is similar to that of the pesticide quinalphos [36]. Cholesterol and protein levels decreased only in experiment 5 after *p.o.* treatment of 3.5 mg/kg, indicating that the oral exposure is effective in reducing cholesterol storage and protein synthesis in the epididymis. Vitamin C, which decreased in experiments 4 and 5 (Table 5), is an important antioxidant that scavenges the free radicals [30] is important in maintaining the structural integrity of reproductive organs, including the epididymis [5]. Hence the structural damage observed in the reproductive organs may also be due to decreased vitamin C levels in them. Degenerating sloughed-off immature germ cells were seen in the lumina of the epididymal tubule and ductus deferens in experiment 4, indicating testicular dysfunction [38], and many agents have been known to induce similar effects [8, 9, 15]. This observation indicates that the epithelial sloughing was induced early in the testis after *i.p*. exposure rather than after *p.o.* exposure.

Methyl parathion also induced structural changes in the ductus deferens, except in experiment 2, in which only nuclear pyknosis was observed in the epithelial cells. Pyknotic nuclei were also seen in other experiments (Fig. 3C), except at 1.75 mg/kg of experiment 1 and 0.5 mg/kg of experiment 3. Brush-border disruption was induced on day 14 in experiment 1 and on day 17 in experiment 4 (Fig. 3B, C), indicating that these changes appear earlier after *i.p.* exposure to MP (Table 3). Another degenerative change observed was in the vacuoles in the epithelium, which may be a generalised phenomenon prior to cell degeneration. Like the epididymis, the ductus deferens also showed immature sloughed-off germ cells in the lumen only in experiment 4 on day 17, indicating that long-term *i.p.* exposure was associated with the early induction of germ cell sloughing in the testis. The structure of the seminal vesicle (Fig. 4A, B) and prostate (Fig. 4C, D) showed no significant changes except pyknotic nuclei and shorter epithelial folding. These effects may be secondary to the decrease in their weight owing to disturbed testosterone function.

We conclude that MP affects the structure and biochemical functions of the reproductive organs in the rat. These effects were found after exposure to very small doses of MP. The users of MP should therefore be aware of the fact that this pesticide is a toxicant, which affects the structure and functions of the reproductive organs.

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