EFFECT OF 1,3–DINITROBENZENE ON PREPUBERTAL, PUBERTAL, AND ADULT MOUSE SPERMATOGENESIS

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Exposure of prepubertal, pubertal, and adult mice to 0, 8, 16, 32, 40, or 48 mg 1,3-dinitrobenzene (m-DNB)/kg body weight and measuring responses 1–25 d posttreatment (dpt) demonstrated significant effects on testicular function only at 48 mg/kg dosage. m-DNB had no effect on body or testis weights with the exception of reduced adult mouse testis weights at 22 dpt with 48 mg/kg ($p < .05$). None of the exposures resulted in detectable levels of germinal epithelial cells in the ductus epididymis. Exposure of prepubertal and pubertal mice to m-DNB caused only minimal nonsignificant changes in the relative percent of testicular cell types present up to 25 dpt. The adult mice testicular cell type ratios, in particular the round and elongating spermatid populations, changed significantly at doses of 48 mg/kg. Also, a reduction in the percent tetraploid cells occurred at d 1, suggesting these cells may be a primary target of m-DNB action.

Caput and caudal sperm from mice exposed to m-DNB prior to puberty did not demonstrate an increased susceptibility to DNA denaturation when analyzed by the sperm chromatin structure assay. However, in pubertal mice, m-DNB exposure further exaggerated the abnormal chromatin structure that normally characterizes sperm during the onset of sperm production. In adult mice, 48 mg/kg resulted in increased susceptibility to DNA denaturation of caput sperm chromatin at 11 dpt ($p < .05$) and in caudal sperm at 22 dpt ($p < .01$). The abnormal chromatin structure of cauda sperm from adult mice was highly correlated with sperm head morphology abnormalities (ABN; 0.82 to 0.95, $p < .01$, 11 and 22 dpt, respectively), but showed lower correlations with dose (0.60 to 0.79, $p < .01$, 11 and 22 dpt, respectively). For pubertal mice, a positive relationship was also observed between the variation of sperm chromatin structure abnormalities and ABN. The effects of m-DNB on testicular function in prepubertal and pubertal mice appear to be less pronounced than in adult mice. Furthermore, following exposure to the same dosage, the effect of m-DNB is less severe in adult mice than that observed for adult rats as reported in the companion paper.

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INTRODUCTION

Previous (Cody et al., 1981; Blackburn et al., 1985; Foster et al. 1987; Linder et al., 1988; Hess et al., 1988) and current studies (Evenson et al., companion paper, p. 81 of this issue) have shown that m-DNB has a negative impact on spermatogenesis in adult rats. In the present study, reproductive toxicology experiments employing m-DNB were extended to mice, another commonly used experimental animal. In addition to effects on adult (85-d-old) animals, the effects of m-DNB exposure on prepubertal (11–19 d of age) and pubertal (26 d of age) mice were investigated; the latter is an extension of previous work (Janca et al., 1986) on flow cytometer characterization of germ-cell differentiation in developing mouse testes.

METHODS AND MATERIALS

Experimental Animals

Male B6C3F$_1$/J mice between 21 and 55 d postpartum (dpp) were obtained from Jackson Laboratories (Bar Harbor, Me.). The same strain of prepubertal (1–28 dpp) males was produced at our facilities by mating C57BL/6J females with C3H/HeJ males (Jackson Laboratories). An age of 26 dpp was chosen as descriptive of pubertal mice because it represented the age at which the pubertal rise in haploid mouse testicular cells (spermatids), evidenced by FCM, was at its midpoint (Janca et al., 1986). Litter sizes were limited to 10 pups by culling excess female siblings. Pups were weaned at 21 dpp.

Weanling and older mice were maintained on an ad libitum diet of Purina Rodent Cow (Ralston Purina Co., St. Louis, Mo.) and deionized water. Animals were housed in plastic cages with wire tops, bedded on pine shavings, maintained at a room temperature of 21 ± 2°C, and kept on a 0700–1900-h lighting schedule.

Chemical Treatment

Stock and diluted working solutions of m-DNB were prepared as described in the companion paper on rats. Mice were administered corn oil/acetone carrier either alone (control) or mixed with a m-DNB to deliver 8, 16, 32, 40, or 48 mg/kg body weight. Adult and pubertal mice were administered m-DNB via oral intubation using an 18-gauge, 1 1/2-in curved feeding needle with 2 1/4-mm ball tip; a 22-gauge, 1 1/2-in straight feeding needle with 1 1/4-mm ball tip was used for the prepubertal mice (Popper and Sons, New Hyde Park, N.Y.).

Sixty-four adult mice at 85 d post partum (dpp) were randomly assigned to 5 groups and exposed to 0, 8, 16, 32, or 48 mg m-DNB/kg body weight ($\bar{X}$ = 27.8 g). Sixty-four pubertal (26 dpp) mice were randomly as-

signed to 5 groups and exposed to 0, 8, 16, 32, or 48 mg m-DNB/kg body weight ($\bar{X}$ = 11.3 g). Forty-eight prepubertal (11–19 dpp) mice were exposed to m-DNB at dosages of 0, 40, or 48 mg/kg body weight; the mean body weight was 6.0 g but dosages were adjusted for the weight of individual mice. The adult mice were fed 1.0 ml m-DNB solution or corn oil/acetone carrier (control). The pubertal and prepubertal mice were fed a volume of m-DNB solution or corn oil/acetone that was in proportion to the weight of the adults; that is, pubertal mice ($\bar{X}$ = 11.3 g) were fed 0.4 ml and prepubertal mice ($\bar{X}$ = 6.0 g) were fed 0.2 ml.

Tissue Sampling

Timing of sample collection was varied from the companion rat study to correspond to differences in timing of spermatogenesis between rat and mouse (Clermont and Perry, 1957; Oakberg, 1956; Kluin et al., 1984). Two to four randomly selected animals from each dosage group were killed by cervical dislocation on days post treatment (dpt) 1, 3, 11, and 22 for adult and pubertal mice. Two to three prepubertal mice were sacrificed at 19 dpp (group 1) and 32, 34, and 36 dpp (group 4) after treatment 11 dpp; at 23, 24, and 25 dpp after treatment 14 or 15 dpp (group 2); and at 27, 28, and 29 dpp after treatment 18 or 19 dpp (group 3).

Immediately following cervical dislocation, the testes, epididymides, and vas deferens were excised and immersed in Hanks balanced salt solution in separate petri dishes placed on ice. Testis and epididymal cells were isolated and prepared for flow cytometry measurements as described in the companion paper except that sperm cells were not sonicated.

Cell Measurements

Evaluation of eosin-stained sperm by light microscopy for sperm head morphology and flow cytometric (FCM) measurements of testicular cells, and caput and caudal lumen cells were performed as described in the companion paper.

Statistics

Data were analyzed using the general linear models procedure (GLM) contained in the Statistical Analysis System (SAS; Goodnight et al., 1988). For adult and pubertal mouse data, independent variables were dose m-DNB, mouse nested within dose, day post treatment, and the interaction of dose with day. Mouse nested within dose was used as the error term for dose, while the residual was used to test the remaining effects. For prepubertal mice, independent variables were group (see tissue sampling section), mouse nested within group, treatment (m-DNB treated or control), and the interaction of group with treatment. Mouse nested

within dose tested dose, and the residual tested the remaining effects. Some data were transformed using the method of Kirk (1982) to select the appropriate transformation. Data for adult mouse body weight, diploid testicular cells, round spermatids, elongated spermatids, $SD\alpha_t$ for caput and cauda epididymal, and abnormal sperm were transformed using 1/observation; tetraploid testicular cell data were transformed using $\log_{10}$(observation + 1). Pubertal mouse data were transformed using $\log_{10}$(observation + 1) for tetraploid testicular cells, round spermatids and elongated spermatids, $\sqrt{(\text{observation} + 0.5)}$ for testis weight and $SD\alpha_t$ of cauda sperm, $\sqrt{\text{observation}}$ for body weight, $\log_{10}$(observation) for abnormal sperm, 1/observation for $SD\alpha_t$ of caput sperm. Prepubertal mouse data were transformed using $\log_{10}$(observation + 1) for tetraploid testicular cells and elongating spermatids, $\sqrt{\text{observation}}$ for diploid testicular cells and elongated spermatids, $\log_{10}$(observation) for testis weight, and 1/(observation + 1) for body weight. Preplanned comparisons were performed using orthogonal contrasts. Results were reported as least-square means ± standard errors; transformed means are reported as such unless otherwise indicated. Pearson product-moment correlations were obtained using the CORR procedure contained in SAS (Delong, 1985).

RESULTS

Effect of m-DNB on Body and Testis Weights

Adult Mice There were no significant differences from control values in body weight of mice killed 1, 3, 11, or 22 d post treatment (dpt) (n = 3 or 4 for each group). When data were pooled across m-DNB doses, body weights were significantly greater at 22 dpt than at 1, 3, or 11 dpt (30.2 ± 0.5 g vs. 26.7 ± .4, 27.5 ± .4, and 27.9 ± .4 g, respectively; $p < .01$). No dosage of m-DNB resulted in testis weights significantly different from control at any day post treatment. When data were pooled across dpt, testis weights at 48 mg/kg were significantly lower compared to those at 0, 8, 16, or 32 mg m-DNB/kg (96.3 ± 2.1 mg vs. 106.4 ± 1.9, 105.1 ± 1.9, 110.5 ± 1.9, and 105.1 ± 1.9 mg, respectively; $p < .01$).

Pubertal Mice Total body weights of mice killed at 1, 3, 11, or 22 dpt were not significantly different from controls (n = 3 or 4 for each group). Pooling of data across dpt showed significantly lower body weights at 32 and 48 mg/kg as compared to 0, 8, or 16 mg/kg (4.17 ± 0.6 and 4.15 ± 0.07 vs. 4.38 ± 0.07, 4.27 ± 0.07, and 4.29 ± 0.07 g, respectively; $p < .05$). Pooling across dose showed successively greater body weights on 1, 3, 11, and 22 dpt (3.5 ± 0.1, 3.8 ± 0.1, 4.6 ± 0.1, and 5.1 ± 0.1, respectively; $p < .01$). All testis weights were not significantly different ($p < .01$) from controls at any time point sampled. Pooling across doses

revealed successively greater testis weights on 1, 3, 11, and 22 dpt (6.4 ± 0.1, 6.9 ± 0.2, 8.3 ± 0.2, and 9.6 ± 0.1, respectively; $p < .01$).

Prepubertal Mice In our first experiment using a dosage of 48 mg/kg body weight, only 9/22 mice survived by 22 dpt. As a result, m-DNB dosage was reduced to 40 mg/kg, which yielded 90% survival at 22 dpt. Thus data were pooled across the two m-DNB dosages for comparison to control. Treatment with m-DNB resulted in body and testis weights that did not differ from control values at any day post treatment. Figure 1 shows the increasing body and testis weights when data were pooled across treatments (m-DNB and control) for mice killed 8–25 dpt (19–36 dpp).

Effect of m-DNB on Presence of Abnormal Germ-Cell Types in the Caput Epididymis

All samples of ductus epididymis contents obtained from the caput of pubertal and adult mice 1, 3, 11, and 22 dpt of dosages of 0, 8, 16, 32, and 48 mg/kg were analyzed by FCM for the presence of abnormal germ cell types by adjusting photomultiplier settings to those used for measuring testicular cells as described in the companion paper. Cells with the acridine orange (AO) staining characteristics of testicular germinal epithelia were not detected above the levels (essentially nondetectable) observed in control samples.

Effect of m-DNB on Relative Percent of Cell Types Present in Testis

Adult Mice Adult, 85 dpp mice were exposed to 0, 8, 16, 32, or 48 mg m-DNB/kg body weight and then killed at 1, 3, 11, or 22 dpt. Testicular cell suspensions were analyzed by FCM to determine the percentage of

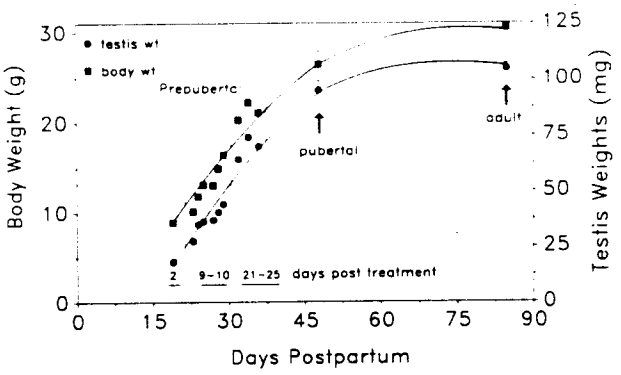

FIGURE 1. Effect of 40 or 48 mg m-DNB/kg body weight exposure during prepuberty (11–19 dpp) on subsequent body and testis weight at 2–22 dpt. The data points at 2 and 9–10 dpt correspond to 40 mg/kg and the points at 21–25 dpt correspond to 48 mg/kg. The adult (85 dpp) values are from control mice.

cell types present as described in the companion paper. The proportion of diploid testicular cells did not differ between treatment and control for any m-DNB dose at any day post treatment. There was, however, a significant dose by day interaction for proportions of tetraploid cells, total haploid cells, round spermatids, and elongating spermatids. Significantly lower than control levels of tetraploid testicular cells resulted on 1 dpt with 48 mg m-DNB/kg, which by 11 dpt was significantly greater than control for both the 16 and 48 mg/kg treatment levels (Fig. 2). Of the reduction observed on d 1, 47% originated from the population of cells with the lower RNA content, which are likely pachytene spermatocytes (Janca et al., 1986). Proportions of total haploid cells were significantly lower than control values 3 and 11 dpt with 48 mg m-DNB/kg (Fig. 2). Of the haploid testicular cells, proportions of round spermatids significantly greater than control resulted at 11 dpt with 48 mg/kg (Fig. 2), while levels of elongating spermatids significantly lower than control resulted at 3 and 11 dpt with 48 mg/kg (Fig. 2). Proportions of elongated spermatids

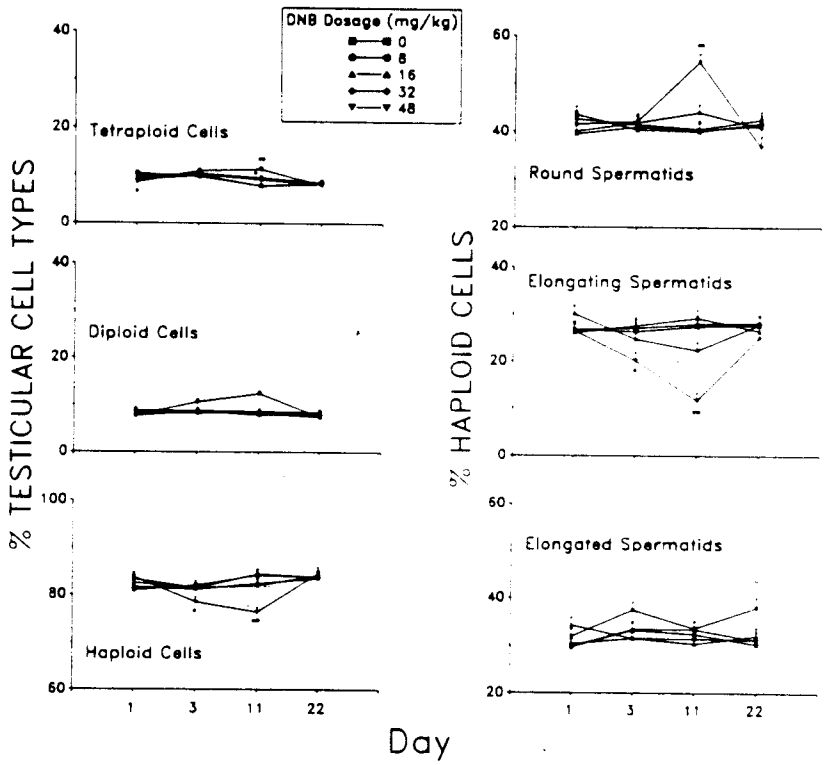

FIGURE 2. Effect of 0, 8, 16, 32, or 48 mg m-DNB/kg weight on relative percent of tetrapoloid, diploid, and total haploid (subdivided into round, elongating, and elongated spermatids) cells present in testis biopsies from control (corn oil/acetone vehicle alone) and adult mice 1-22 dpt.

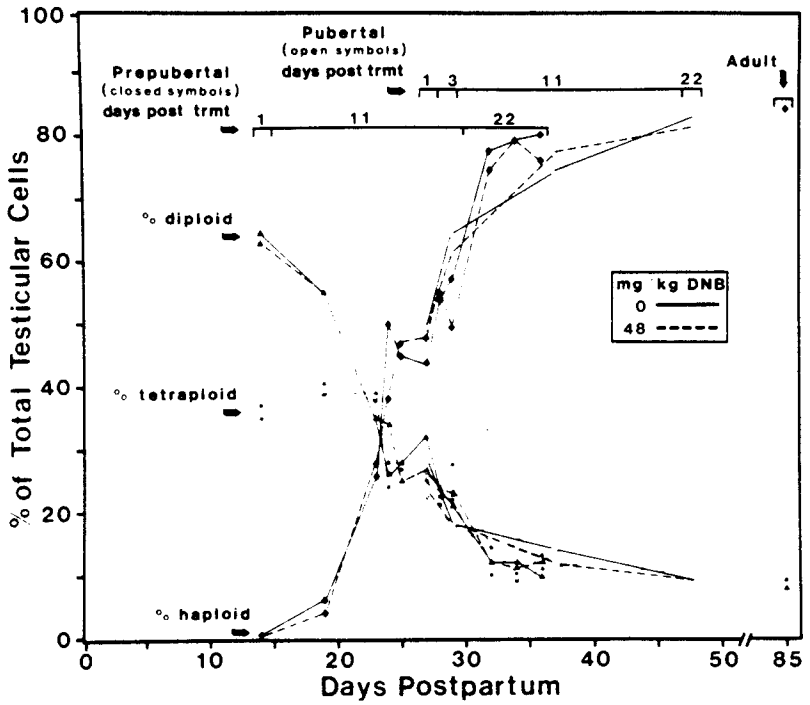

FIGURE 3. Effect of 0 (corn oil/acetone vehicle alone) and 48 mg m-DNB/kg body weight on relative percent haploid, diploid, or tetraploid cells present in testis biopsies 2-25 dpt for prepubertal mice and 1-22 dpt for pubertal mice. Values corresponding to the 2 and 8-10 dpt time points were from prepubertal mice treated with 40 mg/kg. The adult values are from control mice.

did not differ between treatment and control for any m-DNB dosage at any dpt.

Pubertal Mice Pubertal, 26 dpp mice were exposed to the same m-DNB dosages and killed at the same dpt as the adults. Mice at 26 dpp were selected for exposure since this time period corresponds to the dramatic shift that occurs in germ-cell ratios, which result from the increased wave of meiotic daughter cells (round spermatids). Round and elongated spermatids appear at 18 and 30 dpp respectively, and an adult pattern occurs after 38 dpp (Janca et al., 1986). There were no differences between treatment and control values for proportions of any testicular cell type, indicating that exposure up to 48 mg/kg did not significantly alter the timetable for initiation of spermiogenesis relative to that observed in the controls studied here and that previously observed (Janca et al., 1986). When data were pooled across DNB doses, proportions of diploid and tetraploid cells were significantly reduced at each successive dpt, while total haploid cells were significantly increased at each dpt (Fig. 3; $p < .01$). Of the haploid cells, levels of round spermatids were signifi-

TABLE 1. Effects of m-DNB Exposure on Mouse Testicular Cell Type Ratios[a]

	Days post treatment											
	1						3					
	% Haps			% Total			% Haps			% Total		
Dose (mg)	Rd	Eg	Ed	H	D	T	Rd	Eg	Ed	H	D	T
Adults (d 85)												
0	43.6	26.8	29.7	81.0	8.6	10.4	40.4	26.4	33.2	81.9	8.4	9.8
	2.3	3.3	1.0	0.5	0.7	0.6	3.1	1.0	3.2	0.5	1.1	1.0
8	42.6	26.8	30.4	83.3	7.7	9.0	41.5	27.1	31.5	81.4	8.6	10.0
	2.5	3.8	1.1	0.3	0.6	0.5	1.0	1.3	1.0	0.7	0.8	0.7
16	39.5	26.9	34.3	82.5	8.1	9.4	41.0	27.6	31.5	81.4	8.2	10.4
	1.0	5.3	4.6	2.1	0.9	1.2	1.4	1.0	1.0	0.8	0.4	0.4
32	40.1	30.1	29.9	81.6	8.7	9.7	41.9	25.6	31.7	81.1	8.7	10.3
	1.2	1.7	1.8	0.5	0.2	0.4	2.0	0.6	1.4	2.5	1.1	1.6
48	41.6	26.5	31.9	83.7	7.8	8.5	42.1	20.2	37.5	78.4	10.6	11.0
	1.0	2.3	1.3	2.0	1.6	0.5	6.0	8.6	2.9	4.0	2.4	1.7
Pubertals (d 26)												
0	93.2	5.9	0.9	49.0	28.4	22.7	80.2	19.1	0.7	64.0	18.1	17.9
	1.6	0.7	0.8	2.3	2.6	0.3	10.0	9.6	0.6	2.7	1.7	1.2
8	92.1	7.3	0.6	53.6	23.8	22.6	80.4	19.0	0.6	58.9	20.6	20.5
	3.0	2.9	0.8	5.1	2.5	2.6	7.8	7.9	0.3	2.1	2.8	1.6
16	91.4	8.1	0.5	49.3	26.3	24.4	88.3	11.3	0.4	59.6	20.1	20.3
	6.8	6.3	0.4	2.2	5.3	3.1	1.4	1.5	0.1	4.2	2.0	2.4
32	86.8	11.4	1.8	48.8	26.0	25.1	84.8	14.7	0.5	60.4	21.1	18.4
	5.1	4.0	1.7	6.0	2.9	3.3	2.4	2.4	0.1	2.2	0.5	1.6
48	93.0	6.4	0.6	49.1	24.4	26.5	82.9	16.5	0.6	61.4	18.8	23.3
	3.0	2.8	0.3	3.3	3.3	0.9	9.3	9.1	0.3	3.9	1.6	8.6

	Age treated–age killed, prepubertal mice											
	11–19						12–14					
	% Haps			% Total			% Haps			% Total		
Dose (mg)	Rd	Eg	Ed	H	D	T	Rd	Eg	Ed	H	D	T
0				6.2	54.6	39.2				0.9	64.5	34.6
				0.6	3.5	2.8						
40				4.3	55.2	40.5				0.5	62.7	36.8
				0.8	1.8	2.0						
	15–25						18–27					
0	96.9	2.5	0.6	44.5	28.3	27.2	98.1	1.6	0.3	43.2	31.9	24.9
40	97.6	2.0	0.4	47.0	24.7	28.3	98.3	1.6	0.2	48.3	27.0	24.7
	0.9	0.8	0.1	3.7	2.4	1.3	0.4	0.6	0.2	3.2	2.9	1.2
	11–32						11–34					
0	56.2	32.6	11.2	77.3	12.5	10.2	46.1	27.3	26.6	78.8	11.8	9.4
48	59.8	31.1	9.2	73.9	11.7	14.4	51.2	27.6	21.2	78.8	10.7	10.5
	0.7	2.1	1.7	1.7	1.2	0.9	1.9	2.9	1.2	0.6	0.6	0.8

[a]Effects of 0, 8, 16, 32, or 48 mg/kg m-DNB body weight exposure to pubertal and adult mice on testicular cell type ratios at 1, 3, 11, and 22 dpt. Prepubertal mice were exposed to 40 or 48 mg/kg body weight at 11, 12, 14, 15, and 18 dpp and killed at ages 14–36 dpp. The percent haploid cell types (haps) included round (Rd), elongating (Eg), and elongated (Ed) spermatids. The percent total

TABLE 1. Effects of m-DNB Exposure on Mouse Testicular Cell Type Ratios[a] (*Cont.*)

	Days post treatment											
	11						22					
	% Haps			% Total			% Haps			% Total		
Dose (mg)	Rd	Eg	Ed	H	D	T	Rd	Eg	Ed	H	D	T
Adults (d 85)												
0	40.1	27.5	32.4	84.1	8.0	7.8	41.9	27.8	30.3	83.8	7.5	8.6
	1.7	2.3	3.5	0.7	0.5	0.7	3.2	3.9	1.5	2.3	1.1	1.3
8	40.6	28.0	31.4	84.4	7.8	7.8	42.6	26.2	31.3	83.7	8.1	8.2
	0.8	3.1	2.4	1.9	0.7	1.3	0.9	0.9	0.9	0.1	0.4	0.4
16	40.3	29.3	30.4	81.9	8.6	9.5	41.4	26.5	32.1	84.1	7.4	8.5
	3.6	2.1	2.6	0.5	0.5	0.8	1.6	2.6	1.0	1.2	0.9	0.6
32	44.1	22.4	33.4	82.3	8.5	9.1	40.9	27.9	31.3	83.5	8.3	8.2
	2.7	5.7	4.4	1.3	1.1	1.0	2.6	0.9	3.3	1.0	0.5	0.5
48	54.7	11.7	33.6	76.3	12.3	11.3	39.0	23.4	37.7	83.1	8.5	8.5
	9.6	5.1	5.4	5.3	4.0	1.6	2.3	0.1	2.5	2.8	1.2	1.6
Pubertals (d 26)												
0	55.1	23.3	21.7	73.8	14.3	11.8	42.7	26.4	30.9	82.7	9.0	8.4
	5.8	8.1	2.5	8.7	5.3	3.4	5.7	3.0	4.6	1.7	0.6	1.2
8	51.8	26.0	22.1	75.9	11.8	12.3	42.7	28.7	28.6	82.0	9.3	8.7
	0.9	2.2	1.9	3.0	2.8	0.3	4.2	4.4	1.6	0.5	1.1	1.0
16	52.8	26.4	20.8	75.9	12.3	11.5	42.8	27.5	29.6	81.4	9.3	9.3
	0.5	1.6	1.2	2.1	1.7	1.6	3.9	2.0	2.8	1.4	1.3	0.7
32	52.1	24.7	23.2	70.8	15.4	13.7	44.3	29.3	26.4	81.1	9.2	9.7
	9.9	3.9	6.1	5.9	4.1	1.8	0.9	4.8	4.0	1.2	0.5	1.3
48	56.0	22.5	21.5	76.6	11.9	11.4	43.8	28.6	26.7	80.7	9.4	10.0
	5.4	4.8	2.7	1.6	1.9	0.7	2.3	0.6	1.5	1.1	1.5	0.5

	Age treated-age killed, prepubertal mice											
	14–23						15–24					
	% Haps			% Total			% Haps			% Total		
Dose (mg)	Rd	Eg	Ed	H	D	T	Rd	Eg	Ed	H	D	T
0	96.1	4.0		26.1	35.1	38.8	96.8	3.1	0.2	49.6	26.2	24.2
	0.9	0.9		5.7	3.0	2.7	0.9	0.7	0.2	3.8	2.2	1.6
40	94.6	5.4		27.4	35.0	37.6	97.8	1.8	0.4	37.9	34.2	27.9
	0.6	0.6		0.6	1.4	0.8	0.5	0.2	0.3	1.8	1.4	0.4
	19–28						19–29					
0	95.3	4.3	0.4	53.7	23.8	22.5	88.8	10.6	0.6	56.9	21.3	21.8
40	94.0	5.5	0.5	55.2	23.6	21.1	95.2	4.4	0.4	49.1	23.4	27.5
	1.2	1.4	0.3	5.7	2.5	3.2	1.5	1.4	0.2	3.1	1.4	2.4
	11–36						Adult controls					
0	53.3	22.5	24.2	79.5	9.5	10.9	41.5	27.1	31.4	82.7	8.2	9.2
	1.3	2.1	3.1	1.6	1.0	0.6	2.7	2.5	2.6	1.7	0.9	1.3
48	58.6	23.0	18.5	75.2	12.2	12.6						
	4.0	3.9	0.1	0.6	0.8	0.2						

included haploids (H), diploids (D), and tetraploids (T). The upper and lower numbers in each set are the percentage and standard deviations, respectively. Blank spaces indicate no cells present of that particular type.

cantly reduced at 11 and 22 dpt compared to 1 and 3 dpt (1.73 ± .01 and 1.65 ± .01 vs. 1.95 ± .01 and 1.92 ± .01, respectively; $p < .01$), while successive proportions of elongating spermatids and were significantly increased from 1 to 11 dpt, then did not change to d 22 (10.1 ± 1.8%, 16.4 ± 1.9%, 24.9 ± 1.9%, and 27.9 ± 1.6%, respectively; $p < .01$), and levels of elongated spermatids did not differ between 1 and 3 dpt but successively increased between 3 and 22 dpt (0.26 ± 0.03 vs. 0.19 ± 0.03, $p < .05$; 0.19 ± 0.03, 1.36 ± 0.03, and 1.47 ± 0.03, respectively; $p < .01$).

Prepubertal Mice Mice at 11, 12, 14, 15, 18, or 19 dpp were exposed to 0 (control), 40, or 48 mg/kg m-DNB and then killed at 8–25 dpt (19–36 dpp). There were no significant differences between treatment and control values for proportions of any testicular cell type, indicating exposure to m-DNB during prepubertal periods did not cause any major alterations in the relative percentage of testicular cell types present up to 25 dpt. When data were pooled across treatment, levels of diploid and tetraploid testicular cells were significantly decreased with time posttreatment, while proportions of haploid testicular cells were significantly increased (Fig. 3; $p < .01$). Of the population of haploid testicular cells, proportions of round spermatids successively increased as dpp of mouse sacrifice increased from 19 to 23–25 to 25–27 dpp ($p < .01$), then did not change ($p > .05$) to 32–36 dpp (0.1 ± 1.4, 54.1 ± 0.9, 96.6 ± 0.9, and 94.9 ± 1.0, respectively). Elongating spermatid levels increased with increasing dpp, with values at 23–25 dpp and 25–27 dpp not differing ($p > .05$), but were greater ($p < .01$) than those of 19 dpp and smaller ($p < .01$) than those at 32–36 dpp (0.60 ± 0.04 and 0.69 ± 0.04 vs. 0.04 ± 0.07 and 1.44 ± 0.04, respectively). Elongated spermatid levels were significantly greater in mice sacrificed 32–36 dpp than in mice sacrificed 19 dpp, 23–25 dpp, or 25–27 dpp, which did not differ (4.33 ± 0.14 vs. 0.32 ± 0.22, 0.45 ± 0.15, and 0.60 ± 0.16, respectively; $p < .01$). Values of all percentages of testis cell types in all mice are presented in Table 1.

Effect of m-DNB on Adult and Pubertal Mouse Sperm Chromatin Structure

Caput and caudal epididymal sperm were isolated from all pubertal and adult mice treated with 0, 8, 16, 32, and 48 mg/kg m-DNB and killed at 1, 3, 11, and 22 dpt. The sperm were analyzed by the sperm chromatin structure assay (SCSA) for abnormalities of chromatin structure.

Adult Mice Treatment and control values for standard deviation of α_t ($SD\alpha_t$) of caput epididymal sperm did not differ for any dose m-DNB at any day post treatment. When data were pooled across dose m-DNB, $SD\alpha_t$ at 11 dpt was larger ($p < .01$) than at 1 and 3 dpt but not different from that at 22 dpt (0.025 ± 0.001 vs. 0.029 ± 0.001, 0.030 ± 0.001, and 0.027 ± 0.001, respectively). Pooling across day post treatment showed $SD\alpha_t$ to be greater at 48 mg/kg than at 0, 8, 16, or 32 mg/kg ($p < .05$), which

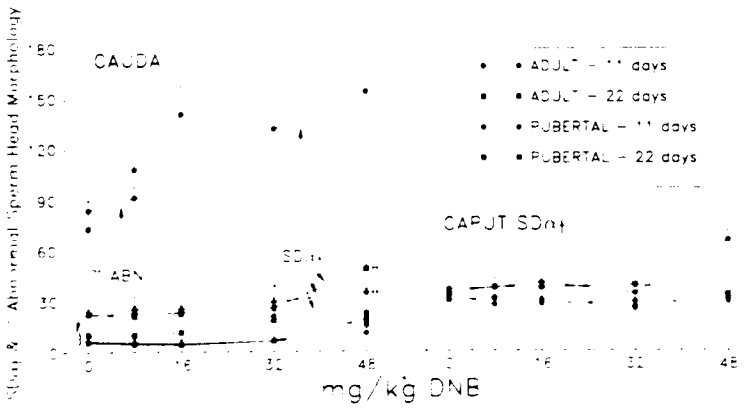

FIGURE 4. Effects of 0, 8, 16, 32, or 48 mg m-DBN/kg body weight on pubertal or adult mice caput and cauda sperm chromatin structure expressed as $SD\alpha_t$ and on percent cauda sperm head morphology abnormalities expressed as %ABN.

did not differ (0.024 ± 0.001 vs 0.031 ± 0.001, 0.028 ± 0.001, 0.028 ± 0.001, 0.030 ± 0.001, respectively).

The interaction of dose m-DNB with day post treatment had a significant effect on $SD\alpha_t$ of cauda epididymal sperm. A dosage of 16 mg/kg resulted in significantly greater than control $SD\alpha_t$ values at 1 dpt (26.5 ± 2.2 vs. 21.6 ± 2.2, $p < .05$), while 32 and 48 mg/kg yielded greater than control $SD\alpha_t$ values at 11 dpt (Fig. 4). By 22 dpt, only 48 mg/kg resulted in greater than control $SD\alpha_t$ values.

Pubertal Mice No sperm were present in the pubertal caput and cauda epididymides at 1 and 3 dpt; thus results are reported only for 11 and 22 dpt. Neither m-DNB dose, day post treatment, nor the interaction of dose with day post treatment had a significant effect on the $SD\alpha_t$ of caput epididymal sperm. Similarly, there were no significant differences between treatment and control values for $SD\alpha_t$ of cauda epididymal sperm for any dose m-DNB at any day post treatment. However, when data were pooled across days post treatment, $SD\alpha_t$ progressively increased with increased m-DNB dose such that 48 mg/kg yielded greater ($p < .05$) values than control (6.7 ± 0.5, 7.4 ± 0.5, 8.3 ± 0.5, 8.1 ± 0.5, and 8.7 ± 0.5 for 0, 8, 16, 32, and 48 mg/kg, respectively). When data were pooled across m-DNB doses, on the other hand, significantly lower $SD\alpha_t$ values resulted in 22 versus 11 dpt (4.7 ± 0.3 vs. 10.9 ± 0.4, respectively; $p < .01$).

Correlation between Caudal Sperm $SD\alpha_t$, Percent Abnormal Sperm Head Morphology, and m-DNB Dosage for Adult and Pubertal Mice

Cauda epididymal sperm from pubertal and adult mice treated with 0, 8, 16, 32, and 48 mg m-DNB were scored for abnormal sperm head

TABLE 2. Correlation Coefficients of Cauda Sperm $SD\alpha_t$ with Percentage Abnormal Sperm Head Morphology (%ABN) and DNB Dosage for Adult and Pubertal Mice

	Adult			Pubertal		
	n	%ABN	Dose	*n*	%ABN	Dose
11 dpt	15	0.82**	0.60**	4	0.99*	0.69
22 dpt	14	0.95**	0.79**	13	0.10*	0.05
11 and 22 dpt	29	0.91**	0.69**	17	0.90*	0.27

*$p < .05$.
**$p < .01$.

morphology. Correlation coefficients were obtained between $SD\alpha_t$ values and percentage abnormal spermheads and m-DNB dosages and are presented in Table 2. Analysis of variance showed that treatment and control values for percent abnormal sperm did not differ from either adult or pubertal mice, regardless of DNB dosage or day post treatment. For adult mice, when data were pooled across day posttreatment, 48 mg/kg resulted in greater ($p < .01$) levels of abnormal sperm than did 0, 8, or 16 mg/kg DNB. The 32 mg/kg dose yielded results not significantly different from both the lower and higher dosage results (0.18 ± 0.02, 0.21 ± 0.02, 0.22 ± 0.02, 0.17 ± 0.02, and 0.11 ± 0.02, respectively for 0, 8, 16, 32, and 48 mg/kg).

DISCUSSION

m-DNB has been shown to cause severe testicular dysfunction in adult rats exposed by oral gavage to 48 mg/kg body weight (Linder et al., 1988; Hess et al., 1988; Evenson et al., companion paper). Hess et al. (1988) suggest that a primary target of m-DNB may be Sertoli cells, which lose their function in supporting development of germ cells. Response to m-DNB exposure and recovery in rats (Linder et al., 1988; Hess et al., 1988; Evenson et al., companion paper), is variable as was observed in this study on mice.

For adult mice, the present study showed that exposure of 48 mg/kg m-DNB also caused abnormal spermatogenesis and resulted in reduced testicular weights, altered testicular germ-cell type ratios, abnormal chromatin structure as defined as an increased susceptibility to acid-induced DNA denaturation, and an increase in abnormal sperm head morphology. The effects were not as pronounced, however, as those observed in rats exposed to m-DNB (companion paper). In this study of mice, no evidence of germinal epithelial cells was found in the epididymis, and alteration of testicular cell types, as well as alterations of chromatin structure and sperm head morphology, were much less dramatic than in rats.

Furthermore, an alteration of chromatin structure was seen at 32 mg/kg dosage in adult rats, whereas in adult mice an alteration was seen only at 48 mg/kg.

m-DNB exposure had no significant effect on body and testicular weights of pubertal mice. The lack of a strong effect is confirmed by the observation that exposure up to 48 mg/kg did not alter the normal timetable for initiation of spermatogenesis. Exposure to 48 mg/kg causes an increased level of sperm chromatin structure abnormality (Fig. 4) significantly above the level of abnormality found in untreated pubertal controls. Similar to observations in the companion rat study, a higher correlation was found between m-DNB dose and $SD\alpha_t$ values than between dose and percentage abnormal sperm heads; likewise, a higher correlation existed between $SD\alpha_t$ values and abnormal sperm head morphology than between dose and sperm head abnormality. Thus, as in the companion study, the present study suggests that the SCSA is an indicator of equal or greater sensitivity of toxic induction of sperm nuclear alterations than is the evaluation of sperm head morphology.

Of particular interest in the present study was the observation that exposure of prepubertal mice to m-DNB had little effect on total body weight gain or testicular growth and development. Exposure at 11–19 dpp to 40 or 48 mg/kg did not produce any major changes in the relative percentage of testicular cell types present 2–25 dpt (Table 1). Apparently neither pachytene spermatocytes nor Sertoli cells were significantly affected; the latter continued to proliferate and apparently retained their protective function for developing germ cells.

The reasons for lack of major effects of m-DNB on prepubertal and pubertal mouse spermatogenesis relative to adult mice and rats (companion paper) are not clear. That m-DNB was absorbed is evidenced by the lethal effects observed in the prepubertal mice at 48 mg/kg; however, these results strongly indicate that this compound apparently had no specific testicular effects on prepubertal mice and minimal effects in pubertal mice.

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