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An Evaluation of the Copulatory, Endocrinologic, and Spermatotoxic Effects of Carbon Disulfide in the Rat[1]

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An Evaluation of the Copulatory, Endocrinologic, and Spermatotoxic Effects of Carbon Disulfide in the Rat. ZENICK, H., BLACKBURN, K., HOPE, E., AND BALDWIN, D. (1984). *Toxicol. Appl. Pharmacol.* 73, 275–283. The present study was undertaken to evaluate the endocrinologic and spermatogenic effects of carbon disulfide (CS_2) exposure in the rat. Adult, male rats were exposed to either 600 ppm CS_2 or filtered air for 6 hr/day for 5 days/week for 10 weeks. One week prior to exposure and then at Weeks 1, 4, 7, and 10, males were placed with ovariectomized, hormonally primed females, and copulatory behaviors were scored. Fifteen minutes postcopulation, the female was killed and the ejaculate was recovered from the excised uterine tract along with the semen plug. Sperm counts, sperm motility, and morphology were determined. A blood sample was obtained for analyses of testosterone, follicle-stimulating (FSH), and luteinizing hormone (LH). At the end of the 10th week, five animals in each group were challenged with either human chorionic gonadotropin (HCG, 50 IU/animal, iv) or gonadotropin-releasing hormone (GnRH, 100 ng/animal, iv), and the testosterone or gonadotropin responses were monitored over time. Animals were subsequently killed with one epididymis and testis processed for histology and a sperm count determined from the other epididymis. Analysis revealed that CS_2 exposure produced significant alterations in copulatory behavior and a decrease in ejaculated sperm counts by the fourth and seventh weeks of exposure, respectively. No endocrinologic alterations were observed. Moreover, caudal epididymal sperm counts were not depressed and the testes appeared histologically normal. These data suggest that CS_2 does not exert a direct effect on the testes, but rather may interfere with the processes regulating sperm transport and ejaculation.

Carbon disulfide (CS_2) was first used in 1851 in the manufacture of matches. It has subsequently been utilized as a solvent in the manufacture of a number of compounds. By far the most predominant use today of CS_2 is in the production of viscose rayon fibers.

A number of studies have documented the adverse cardiovascular, neurological, and hepatic effects that accompany CS_2 exposure in industrial workers. However, investigations of the endocrinological and reproductive effects have yielded equivocal results. Two earlier studies reported decreases in sperm counts and altered sperm morphology in workers exposed to CS_2 (Lancranjan, 1972; Lancranjan *et al.*, 1969). In contrast more recent investigations observed no alterations in these parameters (Gondzik, 1976; Meyer, 1981). Endocrinologic data are also inconsistent. Some studies have reported decreases in various gonadotropins and steroid and metabolic hormones (Lancranjan *et al.*, 1969; Cirla *et al.*, 1978; Maugeri *et al.*, 1971), while others have found increases (Wagar *et al.*, 1981) or no changes in these hormones (Cirla and Graziano, 1981). The differences may in part be attributed to differences in exposure levels, which were not well documented. Only a limited number of animal studies have been conducted with alterations in testicular histology being the only

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endpoint evaluated (Gondzik 1971; Cymerski *et al.*, 1973).

A preliminary study by Tepe and Zenick (1982) indicated that inhalation exposure to 600 ppm CS_2 for a 10-week period altered copulatory behavior in male rats and decreased ejaculated sperm counts with only slight effects on basal plasma testosterone concentrations. The present study was undertaken to evaluate more thoroughly the effects of CS_2 on reproductive function in male rats. In addition to the parameters of sperm count and sperm morphology that were measured in the previous study, sperm motility and seminal plug weight were determined to provide a more detailed analysis of sperm function and androgen-dependent accessory gland function, respectively. Moreover, hypothalamic–pituitary–testicular function was assessed by two approaches: (1) measures of circulating basal levels of plasma gonadotropin and testosterone and (2) specific measurements of testicular steroidogenic capacity and hypothalamic–pituitary function. The latter aim was achieved by evaluating the testosterone response to injections of human chorionic gonadotropin (HCG) and the gonadotropin response to injections of gonadotropin releasing hormone (GnRH).

METHODS

Animal and exposure conditions. Male, Long Evans Hooded (LEH) rats, were purchased from Charles River Breeding Laboratory (Wilmington, Mass.) at 70 days of age. Throughout the experiment Purina Chow (5001) and tap water were available *ad libitum*. Animals were housed singly in stainless-steel hanging cages in quarters maintained on a 12:12-hr reverse light:dark cycle with lights on at 2100 hr. Animals were weighed at weekly intervals.

Carbon disulfide-exposed animals were individually placed in stainless-steel holding cages that were placed in a 60-m^3 stainless-steel and glass inhalation chamber. CS_2 vapor was generated by dripping reagent grade CS_2 (Fisher Scientific, Springfield, N.J.) into a heated flask. The vapor was then mixed with HEPA-filtered air and introduced into the chamber. The inhalation chamber and operator's room were monitored by a Baseline gas chromatograph. The Baseline Industries Inc. P1030A gas chromatograph used in this study was controlled by a self-contained microprocessor consisting of a central processing unit and random access memory. Functions were programmed so that the chamber environment and standard were automatically sampled and analyzed every 10 min (sampling time 45 sec, analysis time 2 min, oven temperature 150°C). The response of the detector, oven temperature, and span signals were automatically recorded on a self-contained strip chart recorder. The unit is equipped with a photoionization detector, two ports for sampling, and two stainless-steel columns: 2 ft × 1/8-in. Porapak QS 50/80; 3 ft × 1/8-in. Porapak QS 50/80. Flow rates to the gas chromatograph were: carrier, N_2, 40 cc/min; fuel, H_2, 35 cc/min; air for combustion 250 cc/min. Eight-inch stainless-steel sample lines were approximately 8-ft long and were equipped with inline stainless-steel sintered filters, pore size 15 μm. Standard curves were generated by preparing carbon disulfide standards in Mylar bags for various attenuations. A commercially prepared standard, 600 ppm (Matheson, Joliet, Ill.), was analyzed daily and the chamber concentration adjusted accordingly. Chamber and effluent scrubbing operations were under negative pressure (0.3 in. water). The chamber effluent was mixed with natural gas and incinerated at 600°C. CS_2 concentrations in the chamber averaged 607 ± 47 ppm. Animals were exposed 6 hr/day, 5 days/week, for 10 weeks. Control animals were treated identically to treated animals except that they were exposed to filtered air alone. The CS_2 concentration employed in this study was selected so as to be below that producing neurotoxic impairment. Moreover, pilot work indicated no effects at 350 ppm while 900 ppm resulted in reduced weight gains deemed undesirable (approximately 20% less than controls).

After a 1-week acclimation period in the animal room, the animals were then allowed several mating experiences over a 2-week period to assure mating proficiency prior to the beginning of the study. In these instances, an ovariectomized, hormonally primed female was placed in the male's home cage. The female was left with the male until presence of a copulatory plug was noted. Females had received estradiol benzoate (0.1 mg/0.1 ml corn oil, sc) 48 hr prior to mating, followed by an injection of progesterone (0.1 mg/0.1 ml corn oil, sc) 4 hr prior to mating. Animals were mated once a week throughout the study to maintain a constant abstinence period.

At approximately 120 days of age, all animals were exposed to filtered air and allowed to acclimate to the chambers for 1 week prior to initiation of the study. After the acclimation period, baseline values for copulatory behaviors, semen parameters, semen plug weights, and hormone levels were obtained (see subsequent section for methodology). Animals were rank ordered based on sperm count and assigned to treatment and control groups so that mean sperm counts across the two groups were matched (14 males in control group; 14 males in CS_2-exposed group).

In addition to the preexposure (baseline) sample, animals were evaluated for mating behavior and semen parameters following 1, 4, 7, and 10 weeks of exposure. On these weeks, matings were conducted on the fifth day of ex-

posure. The exposure period on this day was limited to 4 hr and animals were mated 1 hr after removal from the chambers. Seventy-two hours following collection of the semen sample, animals were anesthetized with Metofane (methoxyflurane, Pitman–Moore, Inc.) and approximately 2 ml of blood were drawn via cardiac puncture. These samples were used for analysis of testosterone, LH, and FSH. This time interval was selected to reduce any effects of copulation *per se* on circulating hormone levels.

In addition to these "mated" animals, 10 additional control and CS_2-exposed animals were maintained, but not mated, bled, or otherwise manipulated. The animals from all groups were killed following 10 weeks of exposure. The reproductive organs were excised and weighed.

Mating behavior. Each male rat was placed in a 33 × 28 × 23-cm Plexiglas cage in a room with a red light source and allowed to acclimate for 15 min. After acclimation, an ovariectomized, hormonally primed female was introduced. The time between the introduction of the female and the first mount was recorded (mount latency) as well as the time between the first mount and ejaculation (ejaculation latency). In addition, the total number of mounts and total numbers of intromissions were recorded. Accuracy in determining ejaculation was confirmed by the presence of a semen plug in the vagina of 100% of the females at time of death.

Semen analysis. Mated female rats were killed 15 min postejaculation by CO_2 asphyxiation. A laparotomy was performed, and the uterine horns, cervix, and vagina were excised. The fluid in the uterus was aspirated into a warmed syringe (37°C). The vagina and uterine horns were opened and rinsed free of sperm. The copulatory plug was recovered, rinsed, and weighed.

An aliquot (10 μl) of the uterine contents was placed on a microscope slide and sperm density visually estimated (low, medium, high). Based on this estimate, another aliquot was diluted (generally from 1:5 to 1:10) with warmed (37°C) phosphate buffered saline to yield a density of 30 to 50 sperm per field when viewed at 200× magnification. Fifteen microliters of the diluted sample was placed on a prewarmed slide, and 100 sperm were counted and scored as motile or nonmotile. These counts were used to estimate percentage of motility (number of motile sperm/total number of sperm).

All slides were rinsed with distilled water and the rinsings combined with the original sample. The total volume of the sample was brought to 50 ml, and the number of sperm was counted under light microscopy with a modified Neubauer hemocytometer.

Hormone response to HCG and GnRH challenges. At Week 10, five of the rats in each group were challenged with HCG (50 IU/animal) while five others received GnRH (100 ng/animal). A blood sample was drawn from the jugular vein of each animal under Metofane anesthesia. GnRH or HCG was then injected intravenously. Animals injected with HCG were sampled 1, 2, and 4 hr postchallenge, and the plasma was analyzed for testosterone. GnRH-injected animals were sampled 15 min and 1 hr postchallenge, and plasma was analyzed for LH and FSH.

Termination procedures. Forty-eight to seventy-two hr after the Week 10 mating, rats were killed by CO_2 asphyxiation and their reproductive organs removed and weighed. One cauda epididymis from each rat was minced and filtered through a 60-gauge brass screen; the filtrate was diluted to 100 ml. Sperm counts were performed as described above. The other cauda epididymis and one testis were fixed in Bouin's solution, dehydrated, embedded in paraffin, sectioned, stained with hematoxylin–eosin, and examined by light microscopy.

Hormone assays. Blood was removed as described above and centrifuged; the plasma was frozen (−20°C) and stored until time of analysis. Duplicate samples were assayed for plasma testosterone with a Radioassay Systems Nosolvex testosterone kit (Radioassay Systems, Carson, Calif.).

Plasma LH and FSH samples were analyzed in duplicate with the NIADDK rat LH and FSH radioimmunoassay kits kindly provided by the Pituitary Hormone Agency, NIADDK, NIH. The standard reference preparations were rat LH-RP-1 and rat FSH-RP-1. Logit transformation and unweighted least squares analyses were used to calculate sample values (Bourne and Baldwin, 1980). The intra- and interassay coefficients of variations were 9.1 and 10.8%, respectively, for LH and 10.5 and 11.7%, respectively, for FSH.

Statistical analysis. A repeated measures analysis of variance with one between factor (dose) and one within factor (weeks) was applied to the analyses of body weight, copulatory behaviors, and sperm parameters. All significant effects were further tested by the method of Least Squares Difference (Kirk, 1968). Significance levels were set at $p \leq 0.05$ for all comparisons.

RESULTS

Mean and standard deviation for body weight and the various measures of copulatory behavior and semen parameters are presented in Table 1. Numbers in parentheses represent the mean percentage of baseline. This variable was calculated for each animal as a ratio of his (exposure value/baseline value) × 100 at each time point sampled. Sample size used in statistical analysis varied slightly for each parameter since an occasional missing value on an animal at one time point resulted in the deletion of his data at all time points on that parameter. This method is the only way missing values can be handled in a repeated measures analysis of variance.

TABLE 1

MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT, COPULATORY BEHAVIORS, AND SEMEN PARAMETER

Parameter	Baseline	Week 1	Week 4	Week 7	Week 10
Body weight (g)					
Control ($N = 14$)	382 ± 26	397 ± 29 (104)[a]	444 ± 44 (116)	465 ± 35 (121)	498 ± 45 (131)
CS_2 ($N = 14$)	380 ± 21	391 ± 17 (103)	432 ± 34 (113)	448 ± 42 (118)	463 ± 30 (121)*
Mount latency (sec)					
Control ($N = 14$)	8.7 ± 2.3	5.6 ± 2.0 (83)	3.9 ± 1.6 (69)	5.58 ± 3.5 (78)	5.9 ± 4.0 (99)
CS_2 ($N = 12$)	17.1 ± 2.4	11.0 ± 4.43 (82)	3.8 ± 1.9 (47)	5.8 ± 3.9 (60)	3.5 ± 1.6 (45)*
Ejaculation latency (sec)					
Control ($N = 14$)	321 ± 17	343 ± 24 (102)	372 ± 18 (103)	317 ± 20 (100)	347 ± 23 (102)
CS_2 ($N = 12$)	415 ± 27	330 ± 27 (94)	247 ± 21 (90)*	247 ± 23 (90)*	254 ± 23 (90)*
Mounts ($\sqrt{\ }$)					
Control ($N = 14$)	2.35 ± 0.66	2.09 ± 0.66 (100)	1.63 ± 0.54 (102)	1.44 ± 0.29 (95)	1.40 ± 0.41 (97)
CS_2 ($N = 12$)	2.44 ± 1.13	2.31 ± 0.72 (94)	1.55 ± 0.35 (100)	1.81 ± 0.37 (94)	1.88 ± 0.49 (96)
Intromissions ($\sqrt{\ }$)					
Control ($N = 14$)	3.97 ± 0.84	3.88 ± 0.57 (106)	3.91 ± 0.58 (113)	3.62 ± 0.51 (97)	3.75 ± 0.70 (95)
CS_2 ($N = 12$)	3.81 ± 0.62	3.54 ± 0.71 (90)	3.76 ± 0.70 (107)	3.52 ± 0.70 (93)	3.58 ± 0.59 (94)
Sperm counts ($\times 10^6$)					
Control ($N = 12$)	52 ± 11	65 ± 28 (134)	54 ± 15 (108)	57 ± 16 (115)	54 ± 21 (103)
CS_2 ($N = 12$)	58 ± 18	58 ± 35 (106)	53 ± 14 (97)	39 ± 10 (72)**	35 ± 17 (64)**
Sperm motility (%)					
Control ($N = 14$)	44 ± 13	34 ± 13 (86)	36 ± 9 (97)	36 ± 10 (93)	28 ± 9 (73)
CS_2 ($N = 12$)	41 ± 13	30 ± 17 (94)	26 ± 11 (67)	31 ± 14 (88)	33 ± 15 (87)
Semen plug weight (gm)					
Control ($N = 14$)	0.103 ± 0.02	0.104 ± 0.02 (105)	0.122 ± 0.02 (122)	0.121 ± 0.02 (120)	0.130 ± 0.02 (129)
CS_2 ($N = 12$)	0.096 ± 0.03	0.098 ± 0.02 (121)	0.095 ± 0.02 (115)	0.096 ± 0.02 (113)	0.101 ± 0.02 (121)

[a] Number in parentheses is mean percentage of baseline for that group.

* $p \leq 0.05$ significantly different from controls at that week.

** $p \leq 0.01$ significantly different from controls at that week.

Body Weight

CS_2-exposed animals failed to gain weight as rapidly as the controls between Weeks 4 and 10 of exposure ($p \leq 0.05$). However the final weight decrement at Week 10 was only 10%.

Copulatory Behavior

Latency data were subjected to logarithmic transformation prior to analysis to reduce heterogeneity of variance. Mount and intromission data were square-root transformed for the same reason. These transformations are conventional for behavioral data of this nature (Kirk, 1968). Even with transformation, those variables of brief duration (mount latency) or infrequent occurrence (number of mounts) still exhibited marked variability.

In general, mount latencies initially decreased for all animals; however, the decreases were of greater magnitude in the CS_2-exposed group by Week 4 and significantly different from controls by Week 10. Ejaculation laten-

cies demonstrated similar trends. Whereas the control group's behavior remained relatively stable, the CS_2-exposed animals exhibited approximately a 10% decrease in ejaculation latency, seen as early as Week 4 of exposure ($p \leq 0.05$).

Despite treatment-related decreases in both latency measures, no CS_2-related effects were seen in the number of mounts or intromissions or in the analysis of these behaviors when combined (latter data not shown). Thus CS_2-exposed animals performed the same number of events within an abbreviated time frame.

Semen Evaluation

Although the ejaculated sperm counts recorded in controls remained relatively stable, CS_2-exposed animals exhibited significant depressions in sperm counts at Weeks 7 and 10 of exposure ($p \leq 0.01$). No significant effects were seen in sperm motility, although motility did decline in both groups over the duration of the experiment. Analysis of semen plug weights revealed significant effects ($p \leq 0.05$) resulting from a greater increase in absolute plug weights in the controls over weeks compared to the CS_2-exposed group. This difference was not significant when analyzed as "percentage-change from baseline."

Hormone Analyses

Data from the hormone analyses are presented in Tables 2 and 3. Because of problems in refrigeration, baseline (preexposure) samples were obtained on only 6/13 control animals and 8/13 CS_2-exposed animals. However, separate analysis of data on these subpopulations (raw data and percentage of baseline) reflected essentially identical trends to that seen in the analysis of the raw data on all animals on Weeks 1 through 10.

No treatment-related effects were seen in the analysis of testosterone, FSH, or LH. Neither HCG nor GnRH challenges disclosed any treatment-related differences (Table 3). HCG injection produced a significant rise in testosterone ($p \leq 0.01$) by the first hour that was still seen in the 2-hr sample. Values had returned to baseline by 4 hr postchallenge. GnRH challenge produced a marked elevation in LH and a lesser increase in FSH by 15 min. Values had returned to baseline by 1 hr postchallenge.

TABLE 2

GROUP MEAN AND STANDARD DEVIATIONS FOR HORMONE EVALUATIONS

Parameter	Baseline	Week 1	Week 4	Week 7	Week 10
Testosterone (ng/ml)					
Control	1.07 ± 0.58[a]	1.65 ± 1.07[b]	1.27 ± 0.58	1.15 ± 0.62	1.07 ± 0.53
CS_2	0.93 ± 0.12[c]	1.61 ± 0.81	1.28 ± 0.90	1.21 ± 1.02	1.18 ± 1.05
FSH (ng/ml)					
Control	292 ± 61	166 ± 128	178 ± 150	178 ± 142	172 ± 127
CS_2	278 ± 77	163 ± 132	163 ± 123	159 ± 135	148 ± 119
LH (ng/ml)					
Control	50 ± 12	44 ± 28	47 ± 15	49 ± 22	41 ± 22
CS_2	43 ± 10	37 ± 17	37 ± 15	29 ± 14	33 ± 16

[a] $N = 6$.
[b] $N = 13$ for both groups Weeks 1 through 10.
[c] $N = 8$.

TABLE 3

HORMONE RESPONSES TO HCG OR GnRH INJECTIONS (iv)

	GnRH Injections (100 ng/animal)					
	FSH			LH		
Time:	Baseline	15 min	1 hr	Baseline	15 min	1 hr
Control ($N = 5$)	269 ± 86[a]	301 ± 58 (115)[b]	241 ± 16 (94)	41 ± 22	88 ± 22 (249)	43 ± 24 (109)
CS_2 ($N = 5$)	221 ± 30	245 ± 46 (111)	227 ± 65 (107)	43 ± 82	94 ± 21 (225)	35 ± 9 (87)

	hCG Injections—Testosterone Response (50 IU/animal)			
Time:	Baseline	1 hr	2 hr	4 hr
Control ($N = 6$)	5.57 ± 4.5	12.67 ± 3.88 (468)	8.38 ± 22 (301)	6.78 ± 2.80 (139)
CS_2 ($N = 4$)	5.03 ± 3.3	9.90 ± 2.93 (380)	7.83 ± 2.74 (336)	5.70 ± 3.57 (151)

[a] Mean ± standard deviation.
[b] Percentage change from baseline.

Termination Data

All animals were killed 48 to 72 hr following the Week 10 mating, including the males exposed to CS_2 or filtered air but not mated or bled during the 10 weeks. Analyses contrasted these "nonmated" groups with the "mated" groups. Means and standard deviations for these groups are presented in Table 4. Organ weights were analyzed as well as organ/body weight ratios. The only treatment-related effect was a decrease in prostate weight in the "mated" CS_2 group relative to their "mated" controls ($p \leq 0.05$). A similar trend was seen in the prostate/body weight ratios. Mating experience *per se* produced a significant increase ($p \leq 0.05$) only in epididymal weights (mated animals, $\bar{x} = 0.70 \pm 0.08$ g; nonmated animals, $\bar{x} = 0.61 \pm 0.09$ g).

No treatment-related effects were seen in sperm counts obtained from the cauda epididymis, nor were any treatment-related alterations observed upon histological evaluation of the epididymis or testis.

DISCUSSION

In the present study, rats tolerated exposure to 600 ppm CS_2 extremely well for the 10-week exposure period. The only indication of systemic toxicity was a slight reduction in the rate of weight gain. Animals appeared to be in good health. Their response to handling was normal and during observation of copulatory behaviors, there were no signs of lethargy, disorientation, or psychomotor incoordination.

Primary effects noted in treated animals included alterations in mating behavior patterns characterized by decreased mount and ejaculation latencies as well as depression of sperm count in ejaculated samples. These results confirm earlier observations in this laboratory (Tepe and Zenick, 1982). Unlike our earlier investigation, the decline in sperm count was only observed in the ejaculate and not in cauda epididymal sperm counts. Closer perusal of earlier data revealed that cauda epididymal sperm count differences were a result of ex-

TABLE 4

TERMINATION DATA OBTAINED FOLLOWING 10 WEEKS EXPOSURE TO CS_2 (600 ppm)

	Treatment			
	Control		CS_2	
	Mated (N = 14)	Nonmated (N = 10)	Mated (N = 10)	Nonmated (N = 10)
Testis (g)	2.79 ± 0.20[a]	2.49 ± 0.65	2.65 ± 0.17	2.68 ± 0.16
Ratio[b]	0.54×10^{-2}	0.49×10^{-2}	0.56×10^{-2}	0.46×10^{-2}
Epididymis (g)	0.71 ± 0.06	0.62 ± 0.11	0.69 ± 0.09	0.61 ± 0.06
Ratio	1.39×10^{-3}	1.23×10^{-3}	1.47×10^{-3}	1.31×10^{-3}
Vas deferens (g)	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.01	0.11 ± 0.02
Ratio	2.24×10^{-4}	2.29×10^{-4}	2.34×10^{-4}	2.35×10^{-4}
Seminal vesicle (g)	2.63 ± 0.39	2.39 ± 0.26	2.43 ± 0.34	2.34 ± 0.39
Ratio	5.10×10^{-3}	4.79×10^{-3}	13.0×10^{-3}	5.20×10^{-3}
Prostate (g)	0.65 ± 0.20	0.57 ± 0.15	0.44 ± 0.07*	0.51 ± 0.09
Ratio	1.26×10^{-3}	1.26×10^{-3}	0.94×10^{-3}	1.10×10^{-3}
Sperm count ($\times 10^6$) (g cauda)	432 ± 188	405 ± 251	529 ± 149	480 ± 264

[a] Mean ± standard deviation.

[b] Ratio = organ weight/body weight.

* $p \leq 0.05$ significantly different from "mated" controls.

tremely elevated control values ($11{,}500 \times 10^6$) relative to what is normally observed in this laboratory. On the other hand, values observed in the CS_2-exposed group in that study fell well within the normal range obtained in our laboratory. Thus it does not appear that the decrease in ejaculated sperm count is the result of altered sperm production in the testes or sperm maintenance in the epididymides. The absence of histological lesions in these tissues is consistent with this observation. The possibility does exist that factors involved in sperm transport or contractility of the epididymis or vas deferens may be compromised in CS_2-exposed animals.

The factors that control the contractions of these organs are not clear. The vas deferens and portions of the epididymis are densely populated with adrenergic nerve endings. Some authors contend that noradrenaline release from these nerve terminals is a primary mediator of vasal contraction (Mann and Lutwak-Mann, 1981). In addition, oxytocin appears to play a role in stimulating sperm transport (Mann and Lutwak-Mann, 1981; Hib, 1974).

Ratnasooriya *et al.* (1979, 1980) have shown that sperm concentrations in ejaculated samples can be reduced to near zero without mechanical blockage or effects on spermatogenesis. In their studies they applied a sympathomimetic drug directly to the epididymis. These authors hypothesized that alterations in the contraction of smooth muscle in the vas deferens and/or epididymis may produce decreases in ejaculated sperm count. A similar phenomenon may be occurring in the current study.

Copulatory behavior was assessed 1 hr after the males were removed from the inhalation chambers. Thus the transitory nature of the response as distinguished from a more per-

sistent impairment was not determined. However, we have noted identical disruptions of copulatory behavior when assessment was 8 to 10 hr postexposure (Tepe and Zenick, 1982). The alterations in copulatory behavior would also be consistent with the effects of CS_2 on biogenic amine availability documented for the central nervous system. McKenna and Distefano (1977) have noted that CS_2 blocks dopamine β-hydroxylase producing elevations in brain dopamine levels. Increases in brain levels of dopamine have in fact been associated with decreases in copulatory times (Crowley and Zemlan, 1979; Dewsbury, 1975). An additional possibility is that the abbreviated copulatory period (analogous to premature ejaculation?) may have affected the number of ejaculated sperm. Chester and Zucker (1970) have shown that copulatory behavior can influence the number of ejaculated sperm recovered from the female tract.

To reduce the confounding contribution of copulation to estimates of hormone levels, animals were sampled 72 hr later or essentially at the end of the first day of exposure for that sample week. Hormone data from "nonmated" males at the end of a 5-day exposure week yielded comparable results (data not shown). The results of this study clearly indicate that under the experimental conditions described, CS_2 had no apparent effect on the hypothalamic–pituitary–testicular axis. However, the CS_2-induced decreases in prostatic weight and seminal plug weights suggest a target-tissue insensitivity to androgen action and will require further evaluation.

The possibility that CS_2 may exert a direct toxic effect on the testis at higher doses or for longer lengths of exposure cannot be dismissed. Gondzik (1971) reported testicular atrophy in rats injected with CS_2 (25 mg/kg, ip) on alternate days for 60 days. However, in a subsequent inhalation study, no testicular lesions were observed in rats exposed to 428 ppm CS_2 for up to 28 weeks (Gondzik, 1976). Furthermore, we have failed to see treatment-related effects in cauda epididymal sperm counts or reproductive organ weights in rats exposed to concentrations of CS_2 up to 900 ppm for 12 weeks (data not shown).

The results from this investigation further support the utility of this approach in evaluating male reproductive toxicity. Conventional approaches involve killing independent groups of animals and evaluating sperm samples obtained from the cauda epididymis. Alterations in functional processes (ejaculation, sperm transport, etc.) such as those observed in the present study would not be detected. Moreover many of the spermatogenic endpoints examined in this report can be monitored in the human population.

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