Toxicology, 32 (1984) 47—56
Elsevier Scientific Publishers Ireland Ltd.

THE EFFECTS OF CARBON DISULFIDE ON THE REPRODUCTIVE SYSTEM OF THE MALE RAT

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(Received February 13th, 1984)
(Accepted March 27th, 1984)

SUMMARY

Two experimental protocols were employed to determine the effects of carbon disulfide (CS_2) on the reproductive system of the male rat. In the first experiment, adult Long Evans hooded rats were exposed to 0, 350 or 600 ppm CS_2 vapor for 10 weeks (5 h/day, 5 days/week). CS_2 exposure caused no change in reproductive organ weights nor in plasma gonadotropin levels. However, animals exposed to 600 ppm CS_2 had slightly lower epididymal sperm counts and significantly reduced plasma testosterone levels. In order to determine if monitoring hormone levels and sperm status in the same male over time might increase the sensitivity of detecting a toxic reaction, the second protocol was employed. Male rats were exposed to 0 or 600 ppm CS_2. After 0, 1, 4, 7 and 10 weeks of exposure, males were observed for mating behavior, and ejaculated sperm count and plasma hormone levels were determined. Animals exposed to 600 ppm CS_2 had significantly shorter times to mount and to ejaculate and decreased ejaculated sperm counts. Plasma gonadotropin levels were similar in both groups while plasma testosterone levels were marginally depressed in CS_2-exposed animals in the early weeks. These data indicate that CS_2 is a toxin of the male reproductive system resulting in abnormal coital behavior and decreased sperm counts. The second experimental protocol proves to be a sensitive method for assessing adverse effects in the male reproductive system.

Key words: Carbon disulfide; Testis; Toxicology

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Abbreviations: CS_2, carbon disulfide; CV, coefficient of variation; FSH, follicle-stimulating hormone; LEH rats, Long Evans hooded rats; LH, lutenizing hormone.

0300-483X/84/$03.00

Printed and Published in Ireland

INTRODUCTION

Numerous articles have reported the effects of carbon disulfide (CS_2) on various parameters of the male reproductive system. However, the results have not always been consistent. Lancranjan et al. [1] and Lancranjan [2] reported decreased sperm count and increased abnormally shaped sperm in men exposed occupationally to CS_2, while Meyer [3] found no such changes. Cavalleri et al. [4] (1966) observed abnormal androgen metabolism in men working with CS_2, while Cirla et al. [5] and Wagar et al. [6] reported normal androgen levels in their CS_2-exposed male populations. However, Cirla et al. [5] reported decreased serum gonadotropins in CS_2-exposed males, while Wagar et al. [6] found elevated serum gonadotropins in their study. These discrepancies can in part be attributed to the study of populations who were exposed to different concentrations of CS_2 for varying durations. However, information about these parameters is seldom given in sufficient detail to allow the assessment of the total CS_2 exposure for a given group.

Animal studies of the male reproductive system after CS_2 exposure are limited to 2 reports of testicular degeneration in rats intoxicated by CS_2 [7,8].

The present study was designed to further examine the changes in reproductive function accompanying CS_2 exposure using 2 experimental protocols. The first entailed animals being exposed to 0, 350 or 600 ppm CS_2 for 10 weeks prior to sacrifice. Reproductive organ weights, epididymal sperm count and hormone levels were measured. With the second protocol, each animal was mated weekly and had coital behavior, ejaculated sperm count and hormone levels monitored after 0, 1, 4, 7 and 10 weeks of exposure. The later technique offered a non-invasive method of obtaining data from rats (sperm counts, hormone levels) which are similar to those obtained in human studies.

MATERIALS AND METHODS

Treatment protocol

Male, Long Evans hooded (LEH) rats, age 80—90 days (412 ± 25 g) at the start of exposure, were purchased from Charles Rivers Breeding Laboratory (Wilmington, MA). Throughout the experiment, all rats were fed NIH-07 rat chow of defined trace metal content and water ad libitum. Animals were singly housed in stainless steel cages in a facility maintained in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC). Lighting in the room was a 12 h:12 h light/dark cycle commencing at 0500.

CS_2-exposed animals were placed in individual compartments of stainless steel holding cages which 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) 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 automatically every 10 min by a Baseline© gas chromatograph equipped with a photoionization detector and a Porpac Q 50/80 S column. Carbon disulfide concentrations in the chamber averaged 348 ± 27 ppm or 607 ± 47 ppm. These concentrations were chosen as doses which were 10× higher than those which caused reproductive and endocrine alterations in humans [5] but below that which caused neurologic deficits [9]. Control animals were treated identically to CS_2-exposed animals except that they were exposed to filtered air. All animals were exposed for 5 h/day, 5 days/week for 10 weeks.

Experiment I

After 10 weeks of exposure to either 0, 350 or 600 ppm CS_2, the male rats were asphyxiated by CO_2. A cardiac blood sample was obtained and the plasma frozen at −20°C until analysis for lutenizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone levels by RIA (see below). The reproductive organs were removed and weighed. For 5 animals per treatment group the right testis was excised, sliced into sections and placed in buffered glutaraldehyde/paraformaldehyde solution for subsequent light microscopic examination. One cauda epididymis per animal was minced 1 g:50 ml in 0.9% NaCl. The mince was filtered through 60 gauge brass screen and diluted 1:10 with 0.9% NaCl. An epididymal sperm count was obtained by counting an aliquot of the diluted sperm in a hemacytometer. A 0.1-ml aliquot of undiluted sperm was stained with 10 μl 1% Eosin Y, smeared on a microscope slide and examined for abnormal sperm morphology using methods previously used [10].

Experiment II

Males in this study were exposed to filtered air or 600 ppm CS_2 daily for 10 weeks (5 h/day, 5 days/week). The male rats were mated weekly with ovariectomized, hormone-primed females. The females received 0.1 mg/0.1 ml of estradiol benzoate (s.c.) 48 h prior to mating and 0.1 mg/0.1 ml of progesterone (s.c.) 4 h before mating. Males were allowed several mating experiences over a period of 2 weeks before being observed during mating. During observation, several parameters of coital behavior were scored (see below). Evaluations of copulatory behavior and semen were then conducted 1 week prior to exposure (pre-exposure baseline) and after 1, 4, 7 and 10 weeks of exposure. Matings were also conducted during interim weeks to ensure a constant period of abstinence, but no evaluations were done at these times. Based on baseline data, animals were matched for body weight, sperm count and ejaculation latency and assigned to CS_2 or control group.

To observe coital 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 acclimatize to the cage for no less than 15 min. After acclimatization, an ovariectomized,

hormone-primed female was placed in the cage with the male, and the time until the first mount (mount latency) and the time between first mount and ejaculation (ejaculation latency) were recorded. The number of mounts and intromissions were also scored [11]. These tests were carried out during the dark cycle, approximately 6—8 h after removal from the inhalation chambers.

After the male ejaculated, he was removed to his home cage and the female was sacrificed 10—15 min later. A laparotomy was performed and the uterine horns, cervix and vagina were excised. The fluid from the uterine horns was withdrawn by syringe. The vagina and uteri were cut open and, with the vaginal plug, rinsed free of sperm with 0.9% NaCl. The rinsings and the contents of the syringe were combined and stirred to break adhering clumps of sperm. The sperm suspension was diluted to exactly 100 ml and sperm count determined using a modified Neubauer hemacytometer. Sperm morphology was not assessed since no effects on this parameter had been seen in Experiment I.

Approximately 98 h after each evaluated mating, approximately 2.5 ml of blood was drawn from males by cardiac puncture under Metofane (methoxyflurane) anesthesia. After centrifugation the plasma was frozen at −20°C until assayed for LH, FSH and testosterone (see below). After 10 weeks of exposure, the animals were killed and data collected as described in Experiment I.

Hormone assays

Plasma samples were assayed for LH and FSH in duplicate by RIA procedures using NIAMDD Rat LH and FSH kits obtained from the Pituitary Hormone Program of the National Institute of Arthritis, Metabolic and Digestive Diseases, NIH. Reference preparations were RAT-LH-RP-1 and RAT-FSH-RP-1. Tracer was iodinated with ^{125}I using the chloramine-T method. Pooled male rat serum gave estimates of the within-assay coefficient of variation (CV) of 5.4% and 15.2% for LH and FSH, respectively. Between-assay CV were 9.0% for LH and 30.9% for FSH.

Plasma testosterone was determined by RIA using a kit supplied by New England Nuclear (Boston, MA) after methylene chloride extraction. This method provided intra-assay CV of 9.9% and a between-assay CV of 18.8%.

Statistical analysis

One-way analysis of variance (ANOVAS; DMBP Program 1V, 1979) was used for analysis of body and reproductive organ weights, epididymal sperm parameters and hormone values obtained in Experiment I.

Because each mated animal in Experiment II had the same endpoints measured after 0, 1, 4, 7 and 10 weeks of exposure, an analysis of variance (ANOVA) with repeated measures [12] was used to test for differences between groups for mating behaviors, ejaculated sperm count and hormone parameters. One-way ANOVA's [12] were used as post-hoc tests to determine the time point at which individual week values differed from each

other. These data were also analyzed as "percentage change from baseline value" in that each animal's weekly score was divided by its own baseline value and these data analyzed. These analyses yielded virtually the same results as ANOVAs on the raw data and are not shown.

Due to skewedness of distributions, the mount and ejaculation latencies were logarithimically transformed before statistical analysis. Hormone data were transformed by natural logarithm to remove heterogeneity of variance and correlation between mean and variance. These transformations are commonly used with data of this nature.

RESULTS

Table I shows the data for body weight and sperm count and morphology from the Experiment I. Although the groups had similar body weights at the start of the experiment, CS_2-exposed animals had statistically lower body weights at the end of the 10 week exposure period ($P < 0.05$). Despite this difference, reproductive organ weights remained similar in all groups (data not shown). Epididymal sperm counts were similar in all groups, although slightly lower in the 600 ppm CS_2 group ($P < 0.10$). Plasma gonadotropin values were similar among all 3 groups. However, plasma testosterone levels were significantly lower in the animals exposed to 600 ppm CS_2. Mean testosterone levels and standard errors were 3.45 ± 0.60, 5.36 ± 0.80 and 1.75 ± 0.26 ng/ml for animals receiving 0, 350, or 600 ppm of CS_2, respectively.

In light of the slight decrease in sperm count seen in unmated animals exposed to 600 ppm CS_2, Experiment II was conducted to determine if monitoring hormone levels and sperm status in the same, sexually-active

TABLE I

EXPERIMENT I

Body weight and sperm parameters in rats exposed to CS_2 for 10 weeks

	CS_2 concentration		
	0 ppm	350 ppm	600 ppm
Body wt (g)	548 ± 10[a] (29)[b]	518 ± 9 (15)	503 ± 15* (15)
Epididymal sperm count ($\times 10^8$/g cauda)	8.6 ± 0.7 (29)	8.6 ± 0.4 (15)	7.5 ± 0.8 (15)
% Normal sperm morphology	98.8 ± 0.3 (29)	98.8 ± 0.2 (15)	98.3 ± 0.5 (15)

[a]Mean ± S.E.M.

[b]Sample size.

*Body weights lower in CS_2-treated animals ($P < 0.05$) at 10 weeks.

male over time might increase the sensitivity of detecting an adverse reaction to the toxic chemical.

Coital behavior, ejaculated sperm count and plasma hormone levels were monitored after 0, 1, 4, 7 and 10 weeks of exposure. Mount latencies were significantly reduced after 7 weeks of exposure, while ejaculation latencies were significantly decreased by the fourth week of exposure in the CS_2 group ($P < 0.01$, Fig. 1A and 1B). The number of mounts and intromissions remained similar in both CS_2-exposed and control groups (data not shown). This suggests that the events comprising mating behavior in both groups were similar, but that the total behavior took less time in the CS_2-exposed group.

When an ejaculated sperm count was obtained from the same animal on several occasions, there was a significant decrease in count from the CS_2-exposed animals ($P < 0.01$, Fig. 2). A marked decrease in sperm count occurred after 7 weeks of CS_2 exposure and remained depressed throughout the 10 weeks of exposure.

There were no significant treatment-related effects seen in plasma FSH or LH values. However, CS_2-exposed animals had consistently lower plasma testosterone levels (Fig. 3). However, most of this difference can be attributed to the increase in testosterone experienced by the control group during Week 1.

After 10 weeks of CS_2 exposure the animals were killed and data obtained as described for Experiment I. These data are presented in Table II. As with the unmated animals, body weight after 10 weeks of exposure decreased in CS_2 exposed animals although not as severely ($P < 0.06$, 6% decrease in weights), while reproductive organ weights were similar in both groups. Epididymal sperm count was decreased in CS_2 treated animals, a pheno-

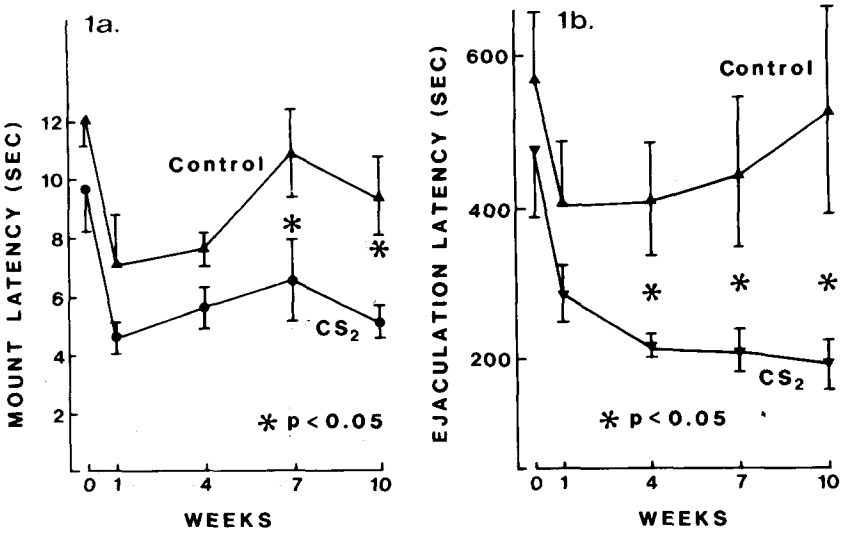

Fig. 1. (a) Mean (±S.E.M.) mount latency in CS_2-exposed and control animals $*P \leq 0.05$. (b) Mean (±S.E.M.) ejaculation latency in CS_2-exposed and control animals $*P \leq 0.05$.

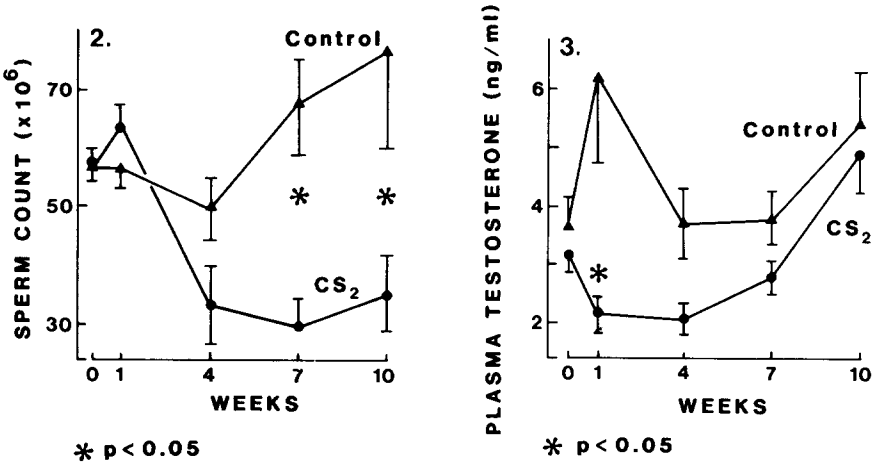

Fig. 2. Mean (± S.E.M.) ejaculated sperm count in CS_2-exposed and control animals. *$P \leq 0.05$.

Fig. 3. Mean (± S.E.M.) plasma testosterone in CS_2-exposed and control animals. *$P \leq 0.05$.

TABLE II

EXPERIMENT II

Body weight, organ weights, sperm parameters and hormonal concentrations in sexually active rats exposed to CS_2 for 10 weeks

	Control (n = 7)	600 ppm (CS_2 (n = 11))
Body wt (g)	507 ± 12[a]	476 ± 12
Testis wt (g)	1.73 ± 0.04	1.86 ± 0.06
Cauda epididymis wt (g)	0.14 ± 0.01	0.13 ± 0.01
Epididymis wt (g)	0.69 ± 0.03	0.65 ± 0.03
Seminal vesicle wt (g)	2.61 ± 0.14	2.61 ± 0.10
Vas deferens wt (g)	0.13 ± 0.01	0.12 ± 0.01
Prostate wt (g)	0.65 ± 0.06	0.80 ± 0.11
Epididymal sperm count ($\times 10^8$/g cauda)	11.5 ± 1.3*	8.0 ± 0.9*
% normal sperm morphology	99.0 ± 0.4	98.4 ± 0.7
Plasma LH (ng/ml)	72 ± 12	75 ± 7
Plasma FSH (ng/ml)	377 ± 38	423 ± 27
Plasma testosterone (ng/ml)	5.38 ± 0.85	4.85 ± 0.64

[a]Mean ± S.E.M.
*$P < 0.05$.

menon also observed in the ejaculated counts (Fig. 2). As the unmated animals did not show this trend (Experiment I), it may imply that secually active animals are more sensitive to reproductive toxins.

Plasma hormone levels were similar in both treated and control groups. Histologic examination of the testes of animals exposed to 350 or 600 ppm CS_2 showed no alterations from normal.

DISCUSSION

This study allows 2 conclusions to be drawn. First, that CS_2 is a toxin of the rat reproductive system which results in abnormal mating behavior and decreased ejaculated serum counts. Secondly, that the method of collecting an ejaculated sperm sample and observing mating behavior is a valuable technique for assessing male reproductive toxicity. The fact that effects are primarily seen in sexually-active animals is consistent with previous reports in other species showing dramatic differences in sperm concentrations as a function of the level of sexual activity (see [13] for a review).

Although the mechanism of CS_2 toxicity cannot be confirmed through this study, the behavioral data do support the theory that alterations in monoamines cause the diverse symptoms of CS_2 intoxification [14]. CS_2 is reported to alter brain monoamine levels, in particular increasing brain dopamine and decreasing brain norepinephrine concentrations, probably through inhibition of dopamine-β-hydroxylase activity [15]. Since increased brain dopamine stimulates mating behavior, i.e. decreases mount and ejaculation latencies [16,17], the data from the present study suggest that the altered mating behavior is due to changes in brain monoamines. It is unlikely that the testosterone values were sufficiently depressed to alter coital behavior [18].

CS_2 exposure had no effect on gonadotropin levels. Moreover, the effects on testosterone were inconsistent and primarily reflected variations in control levels across Experiments I and II as well as between the sexually-active and sexually-rested males used in Experiment II. Recent work in the laboratory has further confirmed limited hormonal influences associated with CS-exposure [19].

The lack of CS_2 effect on testis weight implies that the decrease in ejaculated sperm count is not an effect on spermatogenesis, (as confirmed by histology) but is more likely a post-testicular effect. Epididymal sperm counts in Experiment II were lower in the 600 ppm group relative to the controls. However, this difference was primarily a result of a marked elevation in control epididymal concentrations compared to the values observed for that group in Experiment I or other published data from this laboratory [19,20]. Such observations further support a CS_2-mediated influence on ejaculation producing the decline in ejaculated sperm counts observed. As ejaculation is controlled by the sympathetic nervous system, it is feasible that CS_2 could alter the availability of neurotransmitters to produce incomplete ejaculations. In addition, Matthews and Adler [21] have shown that abnormal mating behavior causes decreased ejaculated sperm counts.

The technique used in this study whereby information on mating behavior, sperm count and hormonal status is obtained in the same animal, should prove useful for analysis of other reproductive toxins. Because the same animals are sampled repeatedly throughout the study, there is reduced variability, thus increasing the probability of detecting an adverse effect, and permitting a smaller sample size to be used. This technique also lends itself to being interfaced with other tests which require mating the animals, i.e. dominant-lethal tests, male mediated teratogenesis, and multiple generation reproduction tests. In addition, because the sperm and hormone data are similar to data obtained in humans, more direct comparisons between animal studies and epidemiology can be made.

In summary, this study shows that relatively high doses of CS_2 have little effect on reproductive organ weights and gonadotrophin levels in rats, but significant alterations are observed in mating behavior and ejaculated sperm count. The technique of observing mating behavior and collecting ejaculated sperm samples proves to be useful in the assessment of potential male reproductive toxins.

ACKNOWLEDGEMENTS

The Research was sponsored by NIOSH RO1-OHO1271. Special thanks to Mr. Frank Grande and Dr. David Baldwin for technical assistance, and to Professor David de Kretser for critical comments on the manuscript.

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