TOXICOLOGY AND APPLIED PHARMACOLOGY 92, 18–27 (1988)

Rat Testis during 2,5-Hexanedione Intoxication and Recovery

I. Dose Response and the Reversibility of Germ Cell Loss[1]

KIM BOEKELHEIDE

Department of Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island 02912

Received March 31, 1987; accepted September 2, 1987

Rat Testis during 2,5-Hexanedione Intoxication and Recovery. I. Dose Response and the Reversibility of Germ Cell Loss. BOEKELHEIDE, K. (1988). *Toxicol. Appl. Pharmacol.* **92,** 18–27. The histopathology of the testicular injury induced by 2,5-hexanedione (2,5-HD) exposure was examined in the rat. Charles River CD rats (200 g) were intoxicated by consuming 1% 2,5-HD in the drinking water or by intraperitoneal injection of the toxicant. Both neurotoxic and subneurotoxic exposures were studied, the total dose ranging from 40 to 211 mmol/kg. The following results were obtained: (1) there was a time delay between administration of the toxicant and development of the testicular injury, (2) Sertoli cell vacuolation in stages associated with the meiotic metaphase was the first histological sign of cellular injury at all doses, (3) subneurotoxic doses produced selective defects in germ cells in stages I–VIII of the spermatogenic cycle, (4) both subneurotoxic and neurotoxic doses produced germ cell necrosis and generalized sloughing of germ cells, and (5) intensive intoxication followed by a 17-week recovery period resulted in an absence of all postspermatogonial germ cells from the seminiferous epithelium of three of five treated rats. These data demonstrate that 2,5-hexanedione-induced testicular atrophy occurs at exposure levels below those producing clinical neurotoxicity and that, within the time frame of this study, the testicular injury is at least partially irreversible.

The recognition of a symptomatic peripheral neuropathy associated with occupational exposure to *n*-hexane and methyl *n*-butyl ketone led to stricter industrial regulation of these solvents in the late 1970s (Couri and Milks, 1982; Spencer *et al.*, 1980). The testicular toxicity resulting from hexacarbon exposure was identified during animal studies after the control of major exposures (O'Donoghue *et al.*, 1978). Testicular toxicity caused by hexacarbon exposure would likely be an asymptomatic disease process, and, indeed, no human cases of hexacarbon testicular injury have been reported. The question remains, however, whether previous severe occupational exposures which produced clinical neurotoxicity and ongoing sporadic subclinical exposures carry a risk of testicular injury.

2,5-Hexanedione (2,5-HD)[2] is the ultimate *in vivo* toxicant derived from *n*-hexane and methyl *n*-butyl ketone metabolism (Krasavage *et al.*, 1980). Hexacarbon-induced testicular toxicity has been mentioned during studies of the nervous system toxicity and explored biochemically (Chapin *et al.*, 1982; Gillies *et al.*, 1981) and morphologically (Chapin *et al.*, 1983) during intense acute intoxication. No thorough animal studies have

[1] This work was supported by Grant R01 OH02191 from the National Institute for Occupational Safety and Health of the Centers for Disease Control and a Pharmaceutical Manufacturers Association Foundation Faculty Development Award in Toxicologic Pathology.

[2] Abbreviations used: 2,5-HD, 2,5-hexanedione; H & E, hematoxylin and eosin; PAS, periodic acid Schiff and hematoxylin.

0041-008X/88 $3.00

been conducted to evaluate the testicular effects of low dose hexacarbon exposure or the reversibility of the testicular injury.

In this report, we show that both neurotoxic and subneurotoxic doses of 2,5-HD caused extensive germ cell loss in the rat. Following a 17-week recovery period, three of five highly exposed rats (total dose, 211 mmol/kg) had only stem cell spermatogonia and Sertoli cells within their seminiferous tubules. Moderate exposure levels (total dose, 90–138 mmol/kg) resulted in seminiferous tubule depopulation followed by variable germ cell repopulation. Low dose exposures (total dose, 40 mmol/kg) induced Sertoli cell vacuolization followed by focal loss of germ cells. These studies also provide the baseline information and morphological data needed to support an investigation of the mechanism of testicular injury described in the companion article (Boekelheide, 1988).

MATERIALS AND METHODS

Charles River CD rats received food (Pro-Lab Rat, Mouse, and Hamster Chow No. 3000) *ad libitum* and were housed in hanging wire cages at a constant temperature (70 ± 2°F) in 35–70% humidity with a 12-hr alternating light–dark cycle. After 6 days acclimatization, rats (200 g) were randomly assigned to control and treatment groups. Body weight was determined on a weekly basis. Clinical neurotoxicity was determined as previously described (Boekelheide, 1987a) with the additional assignment of quantal numerical values to levels of clinical performance. Thus, an average hindlimb retraction time of less than 1 sec was assigned the value of 1, an average hindlimb retraction time of 1–10 sec was assigned the value of 2, and an average hindlimb retraction time of greater than 10 sec was assigned 3 for a numerical value. Using this approach, the clinical neurotoxicity of the entire population was assessed on a weekly basis. Rats were killed by intraperitoneal injection of pentobarbital followed by cardiac puncture. For statistical purposes, the two testis weights from each animal were averaged to provide an average testis weight per rat. Data were analyzed for mean and standard error and compared by the two-tailed Student t test with significance at $p < 0.05$.

Low dose exposure. Rats were injected intraperitoneally with aqueous 2,5-HD (>98% pure, Eastman Kodak Co., Rochester, NY) at 4 mmol/kg/day for 5 days/week for 2 weeks for a total dose of 40 mmol/kg. Groups of rats (n = 4 or 5) were killed at 2, 4, and 7 weeks after beginning dosing for determination of testis weight and histology.

Moderate exposure. Rats were exposed *ad libitum* for 2 or 3 weeks to a 1% drinking water solution of 2,5-HD (v/v) yielding average total doses of 90 or 138 mmol/kg, respectively. Groups of rats (n = 5 or 6) were killed at 7 and 22 weeks after beginning dosing for determination of testis weight and histology.

High dose exposure. Rats were exposed *ad libitum* for 5 weeks to a 1% drinking water solution of 2,5-HD (v/v) yielding an average total dose of 211 mmol/kg. For this experiment, 60 rats were divided into 10 groups of 5 animals each and a reserve group of 10 animals. During the course of the experiment, 8 rats were killed because of poor clinical status (defined as the loss of >40% of initial body weight) and were excluded from consideration. Group size was maintained by replacement of the terminated rats with randomly selected reserve group rats. Groups of rats were killed at 2, 4, 5, 6, 7, 8, 10, 12, 16, and 22 weeks after beginning dosing for determination of testis weight and histology. In addition, preparations from these animals and controls were used for the biochemical studies presented in the companion article (Boekelheide, 1988).

Controls. Groups of five or six *ad libitum*-fed control rats were killed at 0, 4, 5, 7, and 22 weeks for the determination of testis weight and histology.

Histopathology. A portion of each right testicle was prepared for light microscopy by glycol methacrylate embedding, 2-μm sectioning, and staining with periodic acid Schiff and hematoxylin (PAS) as previously described (Boekelheide, 1987a) or, in the case of the low dose intraperitoneal injection experiment, by paraffin embedding, 6-μm sectioning, and hematoxylin and eosin (H & E) and PAS staining of alternate sections. The content of differentiating germ cells, defined as nonstem germ cells committed to differentiation, was estimated by scoring seminiferous tubule cross sections for the presence of viable germ cells with a nuclear morphology of at least the maturity of intermediate-type spermatogonia. Because of their more darkly staining nuclei, intermediate-type spermatogonia were easily distinguishable from Sertoli cells and could be visualized, along with more mature germ cells, while scanning slides at 100×. In the evaluation of testes from highly exposed animals, an average of 211 seminiferous tubule cross sections were examined per rat (range 50–459). Stem cell content was determined in testes (n = 5) in highly exposed rats which lacked differentiating germ cells by counting total Sertoli cell and stem cell nuclei in an average of 149 seminiferous tubule cross sections per rat (range 75–264).

RESULTS

The Effect of Food Intake upon Body and Testis Weight

The effect of dietary restriction upon body and testis weight was investigated. Charles

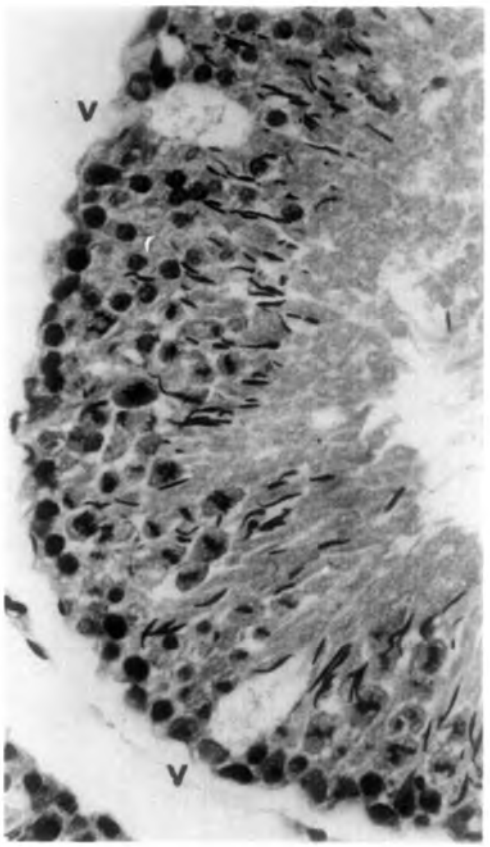

FIG. 1. Sertoli cell vacuoles were the first morphological sign of testicular injury. Large, basally located, Sertoli cell vacuoles (V) were seen in stages XIII, XIV, and I at 4 weeks following low dose exposure to 2,5-HD. H & E, 400×.

River CD rats (200 g) receiving a granulated feed (35-553 NIH 07 Rat and Mouse Ration) were divided into 2,5-HD-exposed and pair-fed control groups with five animals in each group. The exposed group received 1% 2,5-HD in the drinking water for 4 weeks (a total dose of 177 mmol/kg); all rats were killed at 7 weeks for determination of testis weight and histology. These 2,5-HD-exposed and pair-fed control groups were compared to the 7-week *ad libitum*-fed control group. Body weights of 2,5-HD rats (333.2 ± 5.2 g), pair-fed control rats (352.0 ± 4.3 g), and *ad libitum* controls (432.5 ± 14.8 g) all differed significantly from each other at 7 weeks. Testis weights of the pair-fed control rats (1.68 ± 0.08 g) were similar to those of *ad libitum* control rats (1.71 ± 0.05 g), while both differed significantly from the testis weights of 2,5-HD rats (0.71 ± 0.02 g, $p < 0.0001$). Testis histology of the pair-fed and *ad libitum* control rats was indistinguishable. This confirms the work of previous investigators who demonstrated no difference in testis weight and histology between pair-fed and *ad libitum* controls during a 6-week 1% 2,5-HD intoxication of Fischer 344 rats (Chapin *et al.*, 1982; Gillies *et al.*, 1980).

Low Dose Exposure to 2,5-HD

Rats received intraperitoneal injections of 2,5-HD over a 2-week period for a total dose of 40 mmol/kg. No clinical signs of neurotoxicity were apparent in these exposed rats. At 2 weeks, testis weight and histology were nor-

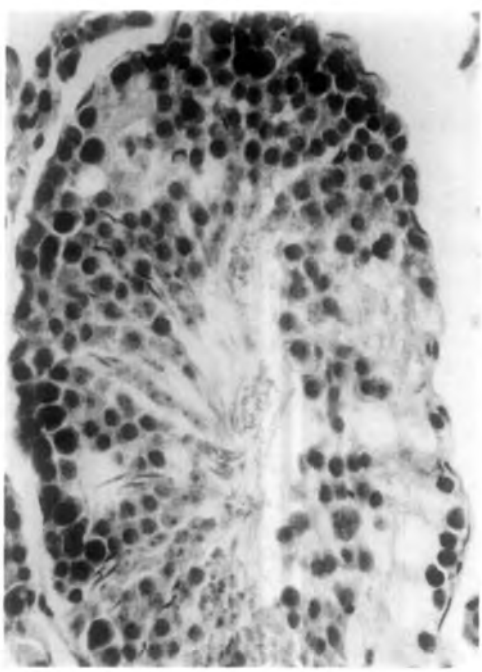

FIG. 2. Focal alterations occurred in stage I–VIII seminiferous tubules at 7 weeks following low dose 2,5-HD exposure. Loss of elongated spermatids, spermatocytes, and round spermatids resulted in areas occupied largely by Sertoli cell cytoplasm (right side of seminiferous tubule). H & E, 360×.

TABLE 1

GERM CELL REPOPULATION AND TESTIS WEIGHT AT 22 WEEKS AFTER MODERATE 2,5-HEXANEDIONE EXPOSURE

Total dose	Testis weight (g)	Depopulated tubules	Repopulated tubules
90 mmol/kg	1.07	97% (233)	3% (6)
	0.69	94% (133)	6% (9)
	0.77	41% (67)	59% (97)
	1.59	0% (0)	100% (224)
	1.81	0% (0)	100% (319)
138 mmol/kg	0.63	100% (49)	0% (0)
	1.12	99% (211)	1% (2)
	0.80	73% (163)	27% (61)
	1.66	0% (0)	100% (106)
	1.81	0% (0)	100% (179)

Note. Rats were intoxicated with 1% 2,5-HD in the drinking water for 2 or 3 weeks for a total dose of 90 or 138 mmol/kg, respectively. Testis histology was examined at 22 weeks and scored for the presence of a differentiating germ cell population (see Materials and Methods). Shown are the average testis weights and the percentages (numbers) of seminiferous tubules from individual rats with (repopulated tubules) and without (depopulated tubules) differentiating germ cells. The difference from the mean of the two testis weights per rat averaged 3.6% (range 0.3–10.3%).

mal. At 4 weeks, 2 weeks after the end of dosing, testis weight was normal. By histological examination, all animals demonstrated the presence of increased numbers of large basally located Sertoli cell vacuoles (Fig. 1). These vacuoles occurred exclusively in stages XIII, XIV, and I, those stages of the spermatogenic cycle related to the meiotic metaphase (Leblond and Clermont, 1952).

At 7 weeks, testis weight was normal (treated testis weight, 1.60 ± 0.06 g; control testis weight, 1.71 ± 0.05 g). Histologically, the majority of seminiferous tubules was normal. Occasional stage I–VIII seminiferous tubules showed loss of germ cells (Fig. 2). Elongated spermatids were the most frequent germ cells lost, followed, in decreasing order of susceptibility, by spermatocytes and round spermatids. Coalescence of this injury resulted in focal germ cell deficits characterized by intact spermatogonia beneath an abundant Sertoli cell cytoplasm. Occasional multinucleated giant cells were identified. Rarely, a markedly atrophic seminiferous tubule contained only Sertoli cells and early germ cells. Sertoli cell vacuolization was no longer prominent.

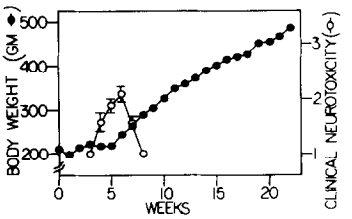

FIG. 3. Rats were intoxicated with 1% 2,5-HD for 5 weeks followed by a 17-week recovery period. Shown is the mean body weight of all surviving rats (in all cases, SE ≤ 14 g). Clinical neurotoxicity was assessed by extending the hindlimbs three times and averaging the times required for repositioning: normal, <1 sec (quantal value 1); moderate toxicity, 1–10 sec (quantal value 2); severe toxicity, >10 sec (quantal value 3).

The Effects of Moderate Exposures to 2,5-HD

Rats were intoxicated with 1% 2,5-HD in the drinking water for 2 or 3 weeks, an average total dose of 90 or 138 mmol/kg, respectively. Rats exposed for 2 weeks showed no clinical signs of nervous system dysfunction while rats exposed for 3 weeks showed an unsteady gait but no deficit in hindlimb retraction after extension.

Groups of rats were killed at 7 and 22 weeks after beginning intoxication. At 7 weeks, 2 and 3 weeks of oral intoxication pro-

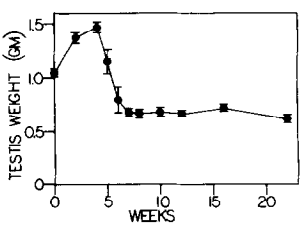

FIG. 4. Groups of five rats were killed at selected time points during and after high dose 2,5-HD exposure. Testis weights fell to atrophic levels at 7 weeks and did not increase during recovery.

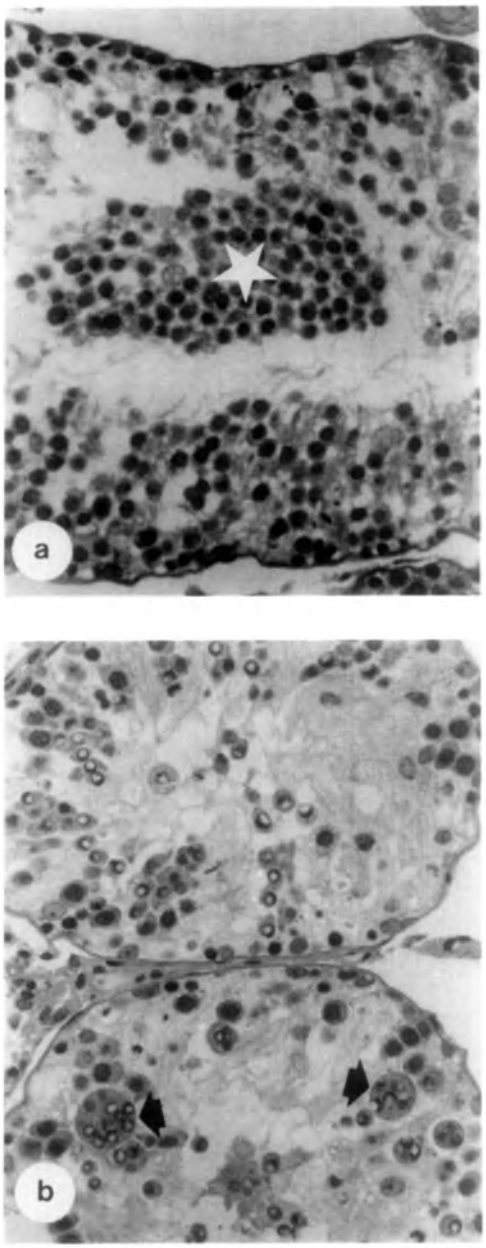

FIG. 5. Sloughing and necrosis were present after 5 weeks of continuous 1% 2,5-HD exposure. (a) Elongated spermatids and spermatocytes were notably decreased in number in a loose and disorganized epithelium. Loss of round spermatids occurred by sloughing of groups of cells into the lumen (star). PAS, 285×. (b) Both individual spermatids and multinucleated giant cells (arrows) contained altered nuclei indicative of cell death. Note the disorganization of the epithelium and the generalized loss of all germ cells. PAS, 285×.

duced a similar degree of severe testicular atrophy manifested as decreased testicular weights (testis weights: 2-week intoxication, 0.79 ± 0.13 g; 3-week intoxication, 0.62 ± 0.04 g; control, 1.71 ± 0.05 g; $p < 0.0001$). At 22 weeks, the testis weights of the 2- and 3-week intoxicated rats were highly variable, ranging from 40% of control to control values (testis weights: 2-week intoxication, 1.18 ± 0.22 g; 3-week intoxication, 1.20 ± 0.23 g; control, 1.71 ± 0.02 g).

Histologic examination of the 2-week intoxicated rats at 7 weeks showed a variable degree of testicular injury. Half of the rats had testes with seminiferous tubules which were severely depleted of germ cells. The testes with less severely damaged seminiferous tubules often demonstrated the histological patterns of injury described above following low dose intraperitoneal injection: selective germ cell loss (elongated spermatids > spermatocytes > round spermatids) in stages I–VIII of the cycle with focal coalescence of these deficits. Occasional spermatocytes showed nuclear karyorrhexis and an increased cytoplasmic staining characteristic for cell necrosis. The 3-week intoxicated rats at 7 weeks demonstrated loss of germ cells resulting in seminiferous tubules containing only Sertoli cells and spermatogonia.

At 22 weeks, following a recovery period of 133–140 days, both the 2- and 3-week intoxicated rats showed variable germ cell repopulation of seminiferous tubules (Table 1).

A High Dose Intoxication and Recovery Study

Rats received 1% 2,5-HD in the drinking water for 5 weeks, a total average dose of 211 mmol/kg. This produced moderate-to-severe clinical neurotoxicity (Fig. 3). Body weight remained fairly constant during intoxication and then rose steadily during a subsequent 17-week recovery period (Fig. 3).

Groups of five rats were killed at selected time points throughout the experiment. Testicular weight rose initially in association

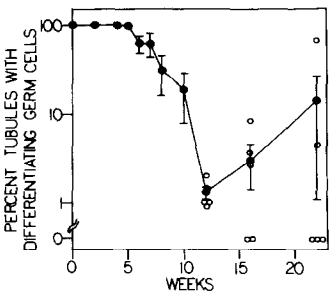

FIG. 6. The percentage of seminiferous tubules with differentiating germ cells (log scale) was determined throughout the time course of high dose 2,5-HD intoxication and recovery. By 12 weeks, only 1% of seminiferous tubules contained differentiating germ cells. The differentiating germ cell content is shown for individual rats at weeks 12, 16, and 22 (open circles).

with maturation of the animals and then fell to levels equivalent to 40% of control testis weight at 7 weeks (Fig. 4). Testis weight remained at these low levels throughout the recovery period.

Histological examination revealed two alternative pathways of germ cell dynamics within the seminiferous tubule: (1) germ cell loss, and (2) germ cell repopulation. Basally located vacuoles first appeared in Sertoli cells at 4 weeks (see Fig. 1). Occasional seminiferous tubules in the 5-week intoxicated rats showed the morphological picture of elongated spermatid > spermatocyte > round spermatid loss from stages I–VIII of the cycle as previously described for low and moderate exposures (see Fig. 2).

The vast majority of seminiferous tubule cross sections showed progressive depletion of differentiating germ cells. Germ cells were lost through both cell necrosis and sloughing. Germ cell sloughing accompanied a generalized "loosening" and disruption of the seminiferous epithelium (Fig. 5a). Cell necrosis was most obvious in spermatids where central nuclear clearing associated with condensation of chromatin at the nuclear rim often

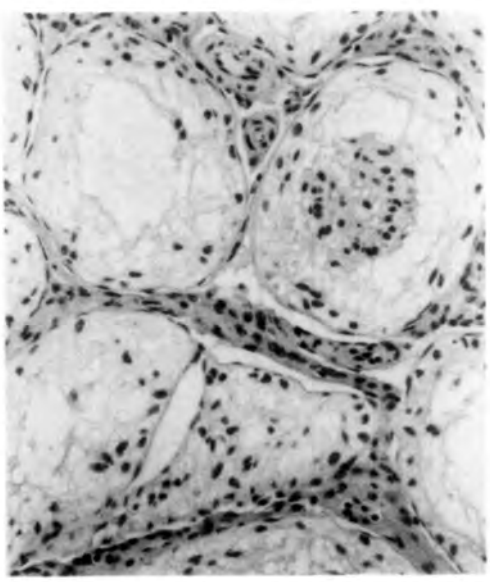

FIG. 7. At 22 weeks, most seminiferous tubules contained empty lumens or aggregates of Sertoli cells. Note the apparent increase in interstitial cell mass. PAS, 175×.

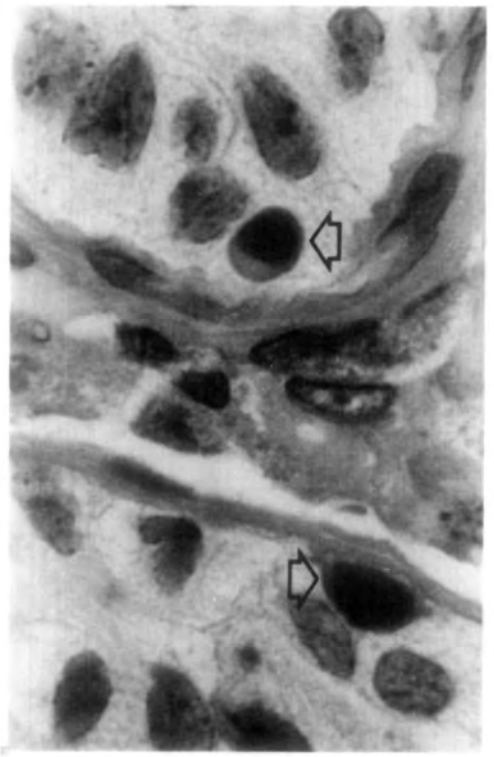

FIG. 8. Seminiferous tubules without differentiating germ cells contained stem cell spermatogonia (arrows). Shown are numerous Sertoli cells in two seminiferous tubules (top and bottom) separated by interstitium. PAS, 1400×.

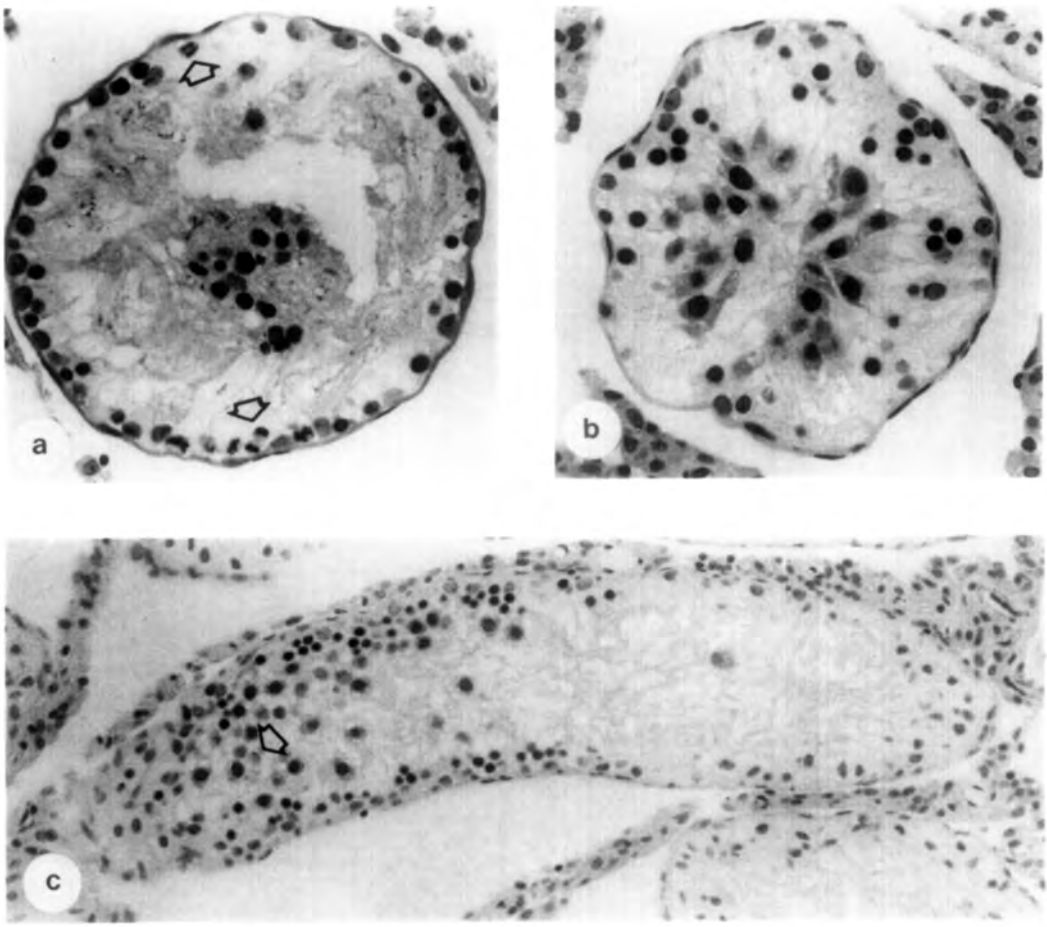

FIG. 9. Germ cell repopulation occurred during recovery in occasional seminiferous tubules. (a) Multiple spermatogonial mitoses were identified in this 8-week seminiferous tubule (arrows). PAS, 295×. (b) Further germ cell differentiation resulted in seminiferous tubules loosely populated by spermatogonia and spermatocytes. Week 12, PAS, 295×. (c) Rapid transitions occurred between depopulated and repopulating regions of seminiferous tubules. Note the cap-phase spermatid (arrow). Week 16, PAS, 195×.

accompanied multinucleated giant cell formation (Fig. 5b). Spermatocyte necrosis was also apparent. Spermatogonia were the most persistent of the differentiating germ cells and could be identified at a low level in all testes examined as late as 12 weeks (Fig. 6). At later time points, testes from 50% of the rats examined showed no remaining differentiating germ cell elements.

The seminiferous tubules which no longer contained differentiating germ cells were of decreased diameter compared with those of control. The basement membrane was more prominent and appeared to thicken slightly during the time of observation. The interstitial space showed an apparent increase in Leydig cell content during the time course of the experiment. Occasional seminiferous tubules contained calcified debris filling the lumenal space. Sertoli cells in seminiferous tubules totally depleted of differentiating germ cells acquired an altered morphology. The Sertoli cell nuclei often assumed a nonbasal location. Aggregates of Sertoli cells totally filled a seminiferous tubule cross section, or, alternatively, the Sertoli cell cytoplasm was

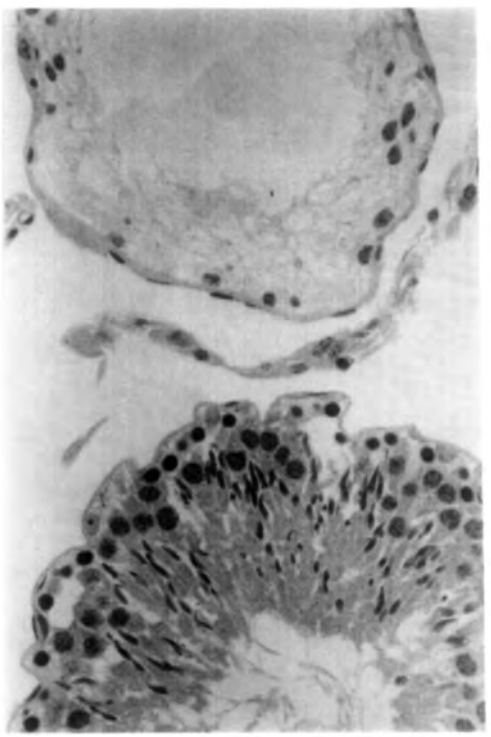

FIG. 10. The most recovered testis at 22 weeks contained a mixture of depopulated and repopulated seminiferous tubules. PAS, 275×.

present as only a thin rim of cytoplasm surrounding an empty seminiferous tubular lumen (Fig. 7).

Close inspection of seminiferous tubules which were devoid of differentiating germ cells revealed the presence of a non-Sertoli cell population with features consistent with stem cell spermatogonia (Huckins, 1971). These cells were most often located basally adjacent to the basement membrane and most often occurred singly with occasional pairs. Morphologically, they had round or oval nuclei with a diameter approximately two-thirds that of the Sertoli cell, a diffuse chromatin pattern which was darker than that of the Sertoli cell, inconspicuous nucleoli, and a smooth, darker staining nuclear contour (Fig. 8). Also helpful in distinguishing these cells from Sertoli cells was the presence of a well-defined cytoplasm and cytoplasmic border. The ratio of the diameter of the nucleus to the cytoplasm was less than 1:1.5.

The number of stem cells relative to Sertoli cells was determined in testes without differentiating germ cells; at 16 weeks, there were 1.86 ± 0.24 ($n = 2$) stem cells per 100 Sertoli cells; at 22 weeks, there were 1.38 ± 0.44 ($n = 3$) stem cells per 100 Sertoli cells. The stem cell content was not significantly different between 16 and 22 weeks.

Morphological features consistent with germ cell repopulation were observed in a minority of seminiferous tubules. Focal intense spermatogonial mitotic activity was observed as early as 8 weeks (Fig. 9a). Progressively more mature germ cells appeared at subsequent time points (Fig. 9b). Adjacent areas of seminiferous tubules demonstrated germ cell depletion and repopulation with a spectrum of maturing germ cells in between (Fig. 9c). The testis with the most extensive germ cell repopulation at 22 weeks weighed 0.80 g (less than 50% of control testis weight) and showed normal-appearing seminiferous tubules interspersed among seminiferous tubules containing only Sertoli cells and stem cells (Fig. 10).

Summary of Intoxicating Dose and Testicular Weights

Testicular weight at 7 weeks decreased after exposure to 90 mmol/kg 2,5-HD (Fig. 11). At 22 weeks, moderate exposure levels (90–

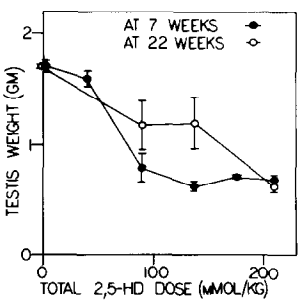

FIG. 11. Testis weights at 7 and 22 weeks after beginning intoxication with a range of 2,5-HD exposures lasting 2 to 5 weeks (total dose 40–211 mmol/kg).

138 mmol/kg) produced highly variable testicular weights ranging from 40% of control to control values. A high total dose of exposure, 211 mmol/kg, was required to produce uniformly severe testicular atrophy at 22 weeks.

DISCUSSION

The time delay between intoxication and development of the testicular injury was most apparent following low dose exposure. A total dose of 40 mmol/kg 2,5-HD administered over 2 weeks produced Sertoli cell vacuolation at 4 weeks and focal loss of germ cells at 7 weeks. This suggested that chemical modification of the testicular target was just the first of a series of events resulting in germ cell loss. A wide range of germ cell types were affected by 2,5-HD intoxication. Because of the delay in manifestation of the chemical injury and the range of germ cell types affected, it is unlikely that the germ cells themselves were the primary target of this intoxication. Rather, given Sertoli cell vacuolization as the first morphologic sign of injury, this supportive cell was the most likely indirect mediator of eventual germ cell damage, as previously suggested (Chapin *et al.*, 1982, 1983).

Focal losses of elongated spermatids, spermatocytes, and round spermatids from early stages of the spermatogenic cycle were noted following low dose 2,5-HD exposure. The elongated spermatids were most susceptible to loss, a phenomenon consistent with "sloughing" caused by typical microtubule disrupting agents (Russell *et al.*, 1981). We have described abnormalities in the microtubule assembly kinetics of both testis tubulin purified from 2,5-HD intoxicated rats and testis tubulin purified from naive rats incubated *in vitro* with 2,5-HD (Boekelheide, 1987a,b, 1988). We hypothesize that the germ cell loss following hexacarbon exposure results from dysfunction of the Sertoli cell cytoskeleton, as discussed in the companion article (Boekelheide, 1988). The total dose of exposure would then determine the extent of Sertoli cell cytoskeletal disruption and consequent germ cell loss.

The 17-week recovery period following high dose 2,5-HD exposure approaches the 12 cycles of the seminiferous epithelium (154 days in the rat) recommended by Amann (1982) for evaluation of reversibility of germ cell damage. During this recovery time, no increase in testicular weight was noted from severely atrophic levels, although histologic examination revealed partial restoration of germ cell production in two of five rats. The depleted seminiferous tubules were devoid of differentiating germ cells, but contained spermatogonial stem cells.

The kinetics of stem cell renewal and germ cell repopulation have been examined for numerous germ cell toxicants (Lu and Meistrich, 1979; Meistrich *et al.*, 1978, 1982). With germ cell toxicants, the length of time required to repopulate the seminiferous tubule is a function of the number of stem cells killed (Meistrich, 1986). However, the dynamics of germ cell restoration in severely atrophic seminiferous tubules depopulated by Sertoli cell toxicants have not been investigated. About 1.6 stem cells per 100 Sertoli cells were identified in germ-cell-depleted testes of highly exposed rats at 16 and 22 weeks. No change in stem cell content was apparent between the 16- and 22-week time points, but the numbers examined were few. This stem cell content is approximately that reported for normal rat seminiferous tubules (Dym and Clermont, 1970; Erickson, 1976). Therefore, in the case of the Sertoli cell toxicant 2,5-HD, factors other than reconstitution of stem cell mass may be involved in the failure of seminiferous tubule repopulation during recovery.

Clearly, 2,5-HD intoxication at both subneurotoxic and neurotoxic levels resulted in an extended period of germ cell depopulation. Whether such an injury remains partially irreversible or eventually becomes fully reversible remains to be determined.

REFERENCES

AMANN, R. P. (1982). Use of animal models for detecting specific alterations in reproduction. *Fundam. Appl. Toxicol.* **2**, 13–26.

BOEKELHEIDE, K. (1987a). 2,5-Hexanedione alters microtubule assembly. I. Testicular atrophy, not nervous system toxicity, correlates with enhanced tubulin polymerization. *Toxicol. Appl. Pharmacol.* **88,** 370–382.

BOEKELHEIDE, K. (1987b). 2,5-Hexanedione alters microtubule assembly. II. Enhanced polymerization of crosslinked tubulin. *Toxicol. Appl. Pharmacol.* **88,** 383–396.

BOEKELHEIDE, K. (1988). Rat testis during 2,5-hexanedione intoxication and recovery. II. Dynamics of pyrrole reactivity, tubulin content, and microtubule assembly. *Toxicol. Appl. Pharmacol.* **92,** 28–33.

CHAPIN, R. E., MORGAN, K. T., AND BUS, J. S. (1983). The morphogenesis of testicular degeneration induced in rats by orally administered 2,5-hexanedione. *Exp. Mol. Pathol.* **38,** 149–169.

CHAPIN, R. E., NORTON, R. M., POPP, J. A., AND BUS, J. S. (1982). The effects of 2,5-hexanedione on reproductive hormones and testicular enzyme activities in the F-344 rat. *Toxicol. Appl. Pharmacol.* **62,** 262–272.

COURI, D., AND MILKS, M. (1982). Toxicity and metabolism of the neurotoxic hexacarbons *n*-hexane, 2-hexanone, and 2,5-hexanedione. *Annu. Rev. Pharmacol. Toxicol.* **22,** 145–166.

DYM, M., AND CLERMONT, Y. (1970). Role of spermatogonia in the repair of the seminiferous epithelium following X-irradiation of the rat testis. *Amer. J. Anat.* **128,** 265–282.

ERICKSON, B. H. (1976). Effect of ^{60}Co γ radiation on the stem and differentiating spermatogonia of the postpubertal rat. *Radiat. Res.* **68,** 433–448.

GILLIES, P. J., NORTON, R. M., BAKER, T. S., AND BUS, J. S. (1981). Altered lipid metabolism in 2,5-hexanedione-induced testicular atrophy and peripheral neuropathy in the rat. *Toxicol. Appl. Pharmacol.* **59,** 293–299.

GILLIES, P. J., NORTON, R. M., AND BUS, J. S. (1980). Effect of 2,5-hexanedione on lipid biosynthesis in sciatic nerve and brain of the rat. *Toxicol. Appl. Pharmacol.* **54,** 210–216.

HUCKINS, C. (1971). The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. *Anat. Rec.* **169,** 533–558.

KRASAVAGE, W. J., O'DONOGHUE, J. L., DIVINCENZO, G. D., AND TERHAAR, C. J. (1980). The relative neurotoxicity of methyl-*n*-butyl ketone, *n*-hexane and their metabolites. *Toxicol. Appl. Pharmacol.* **52,** 433–441.

LEBLOND, C. P., AND CLERMONT, Y. (1952). Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Ann. N.Y. Acad. Sci.* **55,** 548–573.

LU, C. C., AND MEISTRICH, M. L. (1979). Cytotoxic effects of chemotherapeutic drugs on mouse testis cells. *Cancer Res.* **39,** 3575–3582.

MEISTRICH, M. L. (1986). Critical components of testicular function and sensitivity to disruption. *Biol. Reprod.* **34,** 17–28.

MEISTRICH, M. L., FINCH, M., DA CUNHA, M. F., HACKER, U., AND AU, W. W. (1982). Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res.* **42,** 122–131.

MEISTRICH, M. L., HUNTER, N. R., SUZUKI, N., TROSTLE, P. K., AND WITHERS, H. R. (1978). Gradual regeneration of mouse testicular stem cells after exposure to ionizing radiation. *Radiat. Res.* **74,** 349–362.

O'DONOGHUE, J. L., KRASAVAGE, W. J., AND TERHAAR, C. J. (1978). Toxic effects of 2,5-hexanedione. *Toxicol. Appl. Pharmacol.* **45,** 269.

RUSSELL, L. D., MALONE, J. P., AND MACCURDY, D. S. (1981). Effects of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat. *Tissue Cell* **13,** 349–367.

SPENCER, P. S., SCHAUMBURG, H. H., SABRI, M. I., AND VERONESI, B. (1980). The enlarging view of hexacarbon neurotoxicity. *CRC Crit. Rev. Toxicol.* **7,** 279–356.