

# Cytotoxic Effects of Chemotherapeutic Drugs on Mouse Testis Cells<sup>1</sup>

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## ABSTRACT

Studies of testicular cell killing in mice by several chemotherapeutic drugs have been performed to evaluate the harmful effects of oncolytic agents on reproduction. Seven drugs, Adriamycin, 1- $\beta$ -D-arabinofuranosylcytosine, bleomycin, cyclophosphamide, hydroxyurea, vinblastine, and vincristine, given as single injections, were cytotoxic to differentiated spermatogonia. Adriamycin was also highly effective in killing stem cells. The other drugs produced little or no stem cell loss even at doses toxic to the animals. Negligible killing of spermatocytes and spermatids was noted at any dose level. The results demonstrated that oncolytic agents preferentially killed cells at specific stages of the spermatogenic pathway in mice at doses within the clinical range for humans.

## INTRODUCTION

The effect of chemotherapeutic drugs on fertility in man has become an increasingly important medical problem. Because of the difficulty in performing human studies, we used an animal system to test drugs. Mice were chosen since cell kinetics, cell types, and stages of the spermatogenic cycle are well established (5, 18, 20, 21). The features of spermatogenesis relevant to the present study are summarized below.

Spermatogonial stem cell renewal has been extensively studied (2, 3, 8, 9, 22, 25). In the model used here (8, 22), the stem cells are slowly cycling cells designated as  $A_s$  spermatogonia. They divide to form either new  $A_s$  cells or pairs of cells ( $A_{pr}$ ) and subsequently chains of  $A_{ai}$  spermatogonia that differentiate into  $A_1$  cells. The successive generations of  $A_2$ ,  $A_3$ ,  $A_4$ ,  $I_n$ , and  $B$  spermatogonia undergo differentiation and division to form spermatocytes, spermatids, and eventually spermatozoa. Since the differentiated cells are continuously renewed, it is only the stem cells which are at risk in the induction of any long-term effect on spermatogenesis.

An important tool for studies of spermatogenesis has been the 12 characteristic patterns of cell associations or "stages of the cycle of the seminiferous epithelium." These stages have been described in tubular cross-sections of the mouse testis (20). Four generations of spermatogenic cells separated in the developmental sequence by 8.6 days are always found together in a tubule. The morphological appearance of the spermatid usually provides an unambiguous determination of the stage of the cycle. For example, normal tubules, which contain

spermatids in Step 12 of development, are said to be in Stage XII and always contain layers of spermatocytes in meiotic division, zygotene spermatocytes and  $A_3$  spermatogonia.

Furthermore, spermatogenesis is a highly synchronous process, and the kinetics cannot be perturbed by cytotoxic treatments. Neither radiation (5, 19), nor cytotoxic drugs (26, 28), nor hormones (14) can alter the kinetics of spermatogenesis. The survival of specific types of spermatogonia can therefore be determined by counting their progeny at a later time.

In this study, the cytotoxic effects of 7 chemotherapeutic drugs on the stem and differentiated spermatogonia were investigated.

## MATERIALS AND METHODS

### Mice

C3HHeB/FeJ mice, purchased from The Jackson Laboratory, were 8 to 9 weeks old at the start of the experiments.

### Drugs

Seven chemotherapeutic drugs were used in the experiments: ADR<sup>4</sup> (doxorubicin hydrochloride; NDC 38242-874), Adria Laboratories, Inc., Wilmington, Del.; ara-C (Cytosar), The Upjohn Company, Kalamazoo, Mich.; BLM (Blenoxane, NDC 0015-3010), Bristol Laboratories, Syracuse, N. Y.; VLB (Velban; NDC 0002-1452-01) and VCR (Oncovin, NSC 67574), The Eli Lilly and Co.; HU (NSC 32065), Ben-Venue Laboratories, Inc., Bedford, Ohio and CP (Cytoxan), Mead Johnson Laboratory, Evansville, Ind.

Drugs were administered in single injections, usually i.v. (0.01 ml/g body weight). When the drug concentrations were insufficient to reach the LD<sub>50</sub> for the mouse by i.v. injection, as was the case with HU, CP, BLM, and ara-C, i.p. injections of larger volumes were used. No differences were observed between the 2 modes of injection, and the data were pooled. Control animals received 0.9% NaCl solution injections.

### Analysis of Testicular Cell Killing

The protocol for the experiments outlined in Chart 1 is presented below in detail.

(a) **Eleven Days after Injection.** One mouse from each dose point was sacrificed for histological analysis of cell killing in the testes. Testes were fixed in Bouin's solution, subjected to routine histological processing and sectioning, and stained with periodic acid-Schiff-hematoxylin.

The sections were analyzed as follows. The stage of the cycle of the seminiferous epithelium was determined from the spermatid morphology in each tubular cross-section. Then the

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<sup>4</sup> The abbreviations used are: ADR, Adriamycin; ara-C, 1- $\beta$ -D-arabinofuranosylcytosine; BLM, bleomycin; VLB, vinblastine; VCR, vincristine; HU, hydroxyurea; CP, cyclophosphamide; LDH-X, X-isozyme of lactate dehydrogenase; LD<sub>50</sub>, 50% lethal dose.

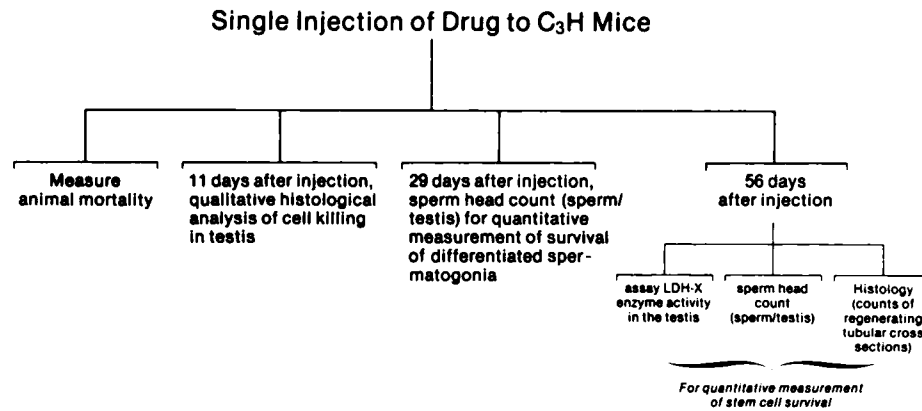


Chart 1. Outline of experimental protocol.

presence or absence of each cell type that was supposed to be found at that stage of the cycle was scored as either: +, present in apparently normal numbers; ±, markedly reduced in numbers; or -, completely absent or nearly completely absent. We then calculated at what stage of spermatogenesis the cells were at the time of treatment from the kinetics of spermatogenesis (18, 20, 21). Table 1 shows the relationships between the stage of spermatogenesis at which a particular cell was at the time of sacrifice and the stage of that cell at the time of injection. For example, a reduction of pachytene primary spermatocytes in a given stage of the cycle indicated partial killing of a specific type of differentiated spermatogonia. This method provided only a qualitative measure of cell survival. However, the cell stages at which the cells were killed were precisely determined.

**(b) Twenty-nine Days after Injection.** Three mice from each group and 6 control mice were sacrificed, and testes were homogenized and sonicated in distilled water (15). Counts of sperm heads (*i.e.*, the nuclei of elongated spermatids) were performed with a hemacytometer and a phase-contrast microscope. The number of sperm heads per testis was calculated and expressed as a fraction of the values obtained in control animals. Since 29 days were required for type A<sub>1</sub> through B spermatogonia to differentiate to the elongated spermatid stage, the number of sperm heads was a measure of the number of surviving differentiated spermatogonia (17). This method provided a quantitative measure of cell survival; however, the precise stages which were killed could not be determined.

**(c) Fifty-six Days after Injection.** The presence of differentiated spermatogenic cells 56 days after treatment can be used as a measure of stem cell survival based on the following 3 factors (15): (a) spermatogenic cells which were already differentiating at the time of treatment would have left the testis within 56 days; (b) sufficient time is allowed for surviving stem cells to progress through the differentiation pathway and produce a new generation of spermatozoa; (c) this interval is short enough to ensure that only a minimal regeneration of stem cell number occurs. The presence of the differentiated spermatogenic cells was assayed by measuring the number of sperm heads and the activity of LDH-X. Since LDH-X is present only in the pachytene spermatocytes and spermatids of the testis (16), it represents an absolute marker for these cells.

Three mice from each group and 6 control mice were sacrificed. Testes were handled as above for sperm head counts.

Table 1  
Correspondence between stage of cell at time of injection and stage of same cell at the time of sacrifice 11 days later

At time of sacrifice (Day 11)		At time of injection (Day 0)	
Stage of cycle (start of stage)	Cell type	Stage	Cell type
I	Step 1 spermatid	Late IX	Leptotene
XII	Diakinesis	Mid VIII	Preleptotene
XI	Diplotene	Mid VII	Preleptotene
X	Pachytene	Early VII	Preleptotene
IX	Pachytene	Early VI	B
VIII	Pachytene	Late IV	B
VII	Pachytene	Early III	In
VI	Pachytene	Early II	A <sub>4</sub>
V	Pachytene	Mid I	A <sub>4</sub>
IV	Pachytene	Late XII	A <sub>3</sub>
III	Pachytene	Early XII	A <sub>3</sub>
II	Pachytene	Early XI	A <sub>2</sub>
I	Pachytene	Late IX	A <sub>1</sub>
XII	Zygotene	Mid VIII	A <sub>1</sub>
XI	Zygotene	Mid VII	A <sub>1</sub>
X	Leptotene	Early VII	A <sub>1</sub>
IX	Leptotene	Early VI	A <sub>1</sub> (A <sub>11</sub> )
VIII	Preleptotene	Late IV	A <sub>1</sub> (A <sub>11</sub> )
VII	Preleptotene	Early III	A <sub>11</sub>
VI	B	Early II	A <sub>11</sub>
V	B	Mid I	A <sub>pr</sub> , A <sub>11</sub>
IV	In	Late XII	A <sub>pr</sub>
III	In	Early XII	A <sub>pr</sub>
II	A <sub>4</sub>	Early XI	A <sub>pr</sub>
I	A <sub>3</sub>	Late IX	A <sub>11</sub>

These samples were prepared for the assay of LDH-X by centrifuging down the sperm heads and heating the supernatant (15, 17). LDH-X activity was assayed by the oxidation of NADH in the presence of α-ketoglutarate. The numbers of sperm and the LDH-X activity per testis were calculated and expressed as a fraction of the values obtained in control animals. At 56 days, stem cell survival was also measured by a histological method (34). The testes were processed, sectioned, and stained with hematoxylin and eosin. Counts of repopulated and total tubular cross-sections were made, and the ratio of these 2 values was designated the repopulation index (10). Tubules showing repopulation of spermatogenic cells must contain surviving stem cells. Therefore, the stem cell survival index was calculated from the repopulation index by applying the Poisson distribution (15, 34).

**(d) Animal Survival.** The LD<sub>50</sub> was calculated from the ratios of the numbers of surviving mice to total tested mice at each dose by the logit method of analysis.

RESULTS

**Animal Mortality.** The dose ranges were chosen to include the animal LD<sub>50</sub>. These LD<sub>50</sub>'s were calculated as shown in Table 2.

**Qualitative Histological Analysis.** Testes were prepared for histological analysis 11 days after drug injection in order to obtain an overall picture of drug-induced histopathology as well as to determine the specific cell stages at which cells were killed by the drugs. There were no apparent changes in the interstitial tissue or in the structure of tubules (Fig. 1). Degrating cells were rarely observed, indicating that the cells that were killed were lost within 11 days after treatment.

The presence or absence of cells at particular stages of differentiation was recorded, and by using Table 1 we calculated the cell types killed at the time of treatment (Chart 2). All such effects appeared to occur uniformly throughout the cross-section of the testis.

ADR produced no apparent loss of cells at doses up to 0.4 mg/kg. The most sensitive cell type was the A<sub>2</sub> spermatogonia, which was completely killed at 1 mg/kg. Spermatogonia in stages A<sub>1a</sub> through A<sub>1</sub> and in A<sub>3</sub> through In were only partially killed at this dose. Increasing doses resulted in more loss of these cells (Chart 2; Fig. 1). The A<sub>1a</sub> through In spermatogonia were completely killed above 8 mg/kg. However, even at the highest doses, B spermatogonia and Pl spermatocytes were unaffected.

Both ara-C and HU exhibited identical patterns of cell killing which differed markedly from those produced by ADR. Partial killing of A<sub>1</sub> spermatogonia through Pl spermatocytes was seen after HU (350 to 3500 mg/kg) (Chart 2) and ara-C (300 mg/kg) treatment. This finding was consistent with the S-phase specificity of HU and ara-C. However, a loss of this specificity was noted with very high doses of both drugs, with complete killing of some spermatogonia (Chart 2).

CP resulted in partial killing of spermatogonia at 67 mg/kg with no detectable effects below that dose. At 200 mg/kg, the A<sub>2</sub> through B spermatogonia were completely killed, and the A<sub>1</sub> spermatogonia and Pl spermatocytes were partially killed

(Chart 2). The pattern of cell killing with CP was similar to that of ara-C or HU, with cells possessing an S phase being most sensitive.

BLM produced another pattern of cell killing; the A<sub>6</sub> through B spermatogonia were most sensitive (Chart 2). This set of stages is later than those sensitive to ADR, but earlier than those sensitive to HU, ara-C, and CP.

The *Vinca* alkaloids (VCR and VLB) produced, at doses that were not toxic, less spermatogonial killing than did most of the other agents used (Chart 2). At 1.7 mg/kg, VCR caused only partial killing of A<sub>2</sub> through In spermatogonia. VLB resulted in no observable loss of spermatogonia, even at 7 mg/kg. It also was surprising that these agents, which show phase-specific cytotoxicity for mitotic cells (1), also did not significantly kill meiotic spermatocytes.

The most apparent effect of these drugs on testicular cells was killing of the differentiated spermatogonia and preleptotene primary spermatocytes. Losses of later primary spermatocytes or of early spermatids were not observed.

A few giant round or early elongating spermatids were seen at 11 days after treatment. These cells were about the same size as secondary spermatocytes. They were likely to be 2c spermatids (i.e., with 2 times the DNA content of a haploid cell) resulting from abnormal meiotic division of primary spermatocytes exposed to the drug treatment. The apparent 2c spermatids were observed most frequently after exposure to VCR, VLB, CP, and BLM. Increased frequencies of abnormally shaped sperm (elongated spermatid nuclei) were found 11 days after treatment with CP, VCR, and VLB. These 2 types of aberrations may be indicative of mutational events as discussed later.

In addition, after VCR treatment, tubules identified as being in Stage XII (presence of the meiotic divisions) showed uncondensed spermatids characteristic of step 9. However, no other abnormal cellular associations were observed, indicating that the cycle of the seminiferous epithelium was preserved. Thus we concluded that spermatocytes treated with VCR must have been blocked or delayed in their elongation and condensation during spermiogenesis.

Table 2  
LD<sub>50</sub> values of mice and testis cells to acute doses of chemotherapeutic agents

Endpoint	Cytotoxic Agents						
	BLM (units/kg)	VCR (mg/kg)	VLB (mg/kg)	ADR (mg/kg)	CP (mg/kg)	ara-C (mg/kg)	Hu (mg/kg)
Animal mortality	230	1.7	9.5	16	330	6400	5800
Differentiated spermatogonia	130	0.7	4.5	1.0	30	16	100
Stem spermatogonia	>200	>2	>6.7	3	>200	>7000	>5000

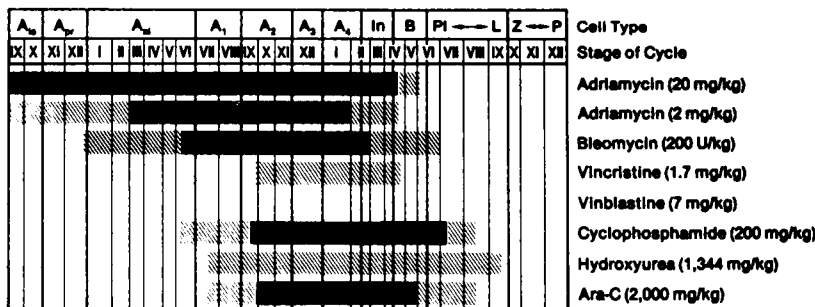


Chart 2. Spermatogenic cells killed by various drugs. Eleven days after drug injection, histological sections were analyzed for cell killing in testis at time of treatment. ■, completely killed; ▨, partially killed; □, no effect.

**Quantitative Measurement of Survival of Differentiated Spermatogonia.** Testicular sperm head counts obtained 29 days after injection (Charts 3 and 4) indicated that the differentiated spermatogonia showed varying degrees of sensitivity to the drugs tested.

By comparing the LD<sub>50</sub> for differentiated spermatogonia with the LD<sub>50</sub> for the animal (Table 2), it becomes evident that the differentiated spermatogonia are more sensitive to ara-C, HU, ADR, or CP than to BLM, VCR, or VLB.

Several types of survival curves for differentiated spermatogonia were obtained (Charts 3 and 4). One group of drugs, HU, ara-C, and BLM, produced survival curves with plateau regions. The plateau indicated that 2 subpopulations of spermatogonia were present: one was entirely killed by doses within this region; the other was resistant to these doses. In the cases of HU and ara-C, the sensitive populations should correspond to S-phase cells. With another group of drugs, VCR, VLB, and CP, the spermatogonia were resistant to low doses and showed a dose-dependent toxic response to increasing drug dose.

**Quantitative Measurement of Stem Cell Survival.** Stem cell survival was determined by sperm head counts, LDH-X activity, and counts of repopulated tubular cross-sections in the testes 56 days after treatment.

Results of sperm head counts are shown in Charts 3 and 4. VCR, VLB, and CP produced no significant decline in sperm head counts below the normal control level at 56 days at any dose level tested. ADR, however, caused extensive stem cell killing as evidenced by markedly reduced sperm head counts.

When the data obtained over a wide range of doses were averaged, both ara-C (33 to 2200 mg/kg) and HU (7 to 7000 mg/kg) resulted in slight but significant ( $p < 0.025$ ) declines in sperm head counts. The average sperm head counts for ara-C and HU-treated mice were  $0.958 \pm 0.016$  (S.E.) and  $0.943 \pm 0.014$  of control, respectively. High doses of HU or BLM produced further small declines in sperm head counts.

The LDH-X enzyme activity paralleled the sperm head counts in all cases; for example, only ADR produced an appreciable

decrease in LDH-X activity (Chart 5).

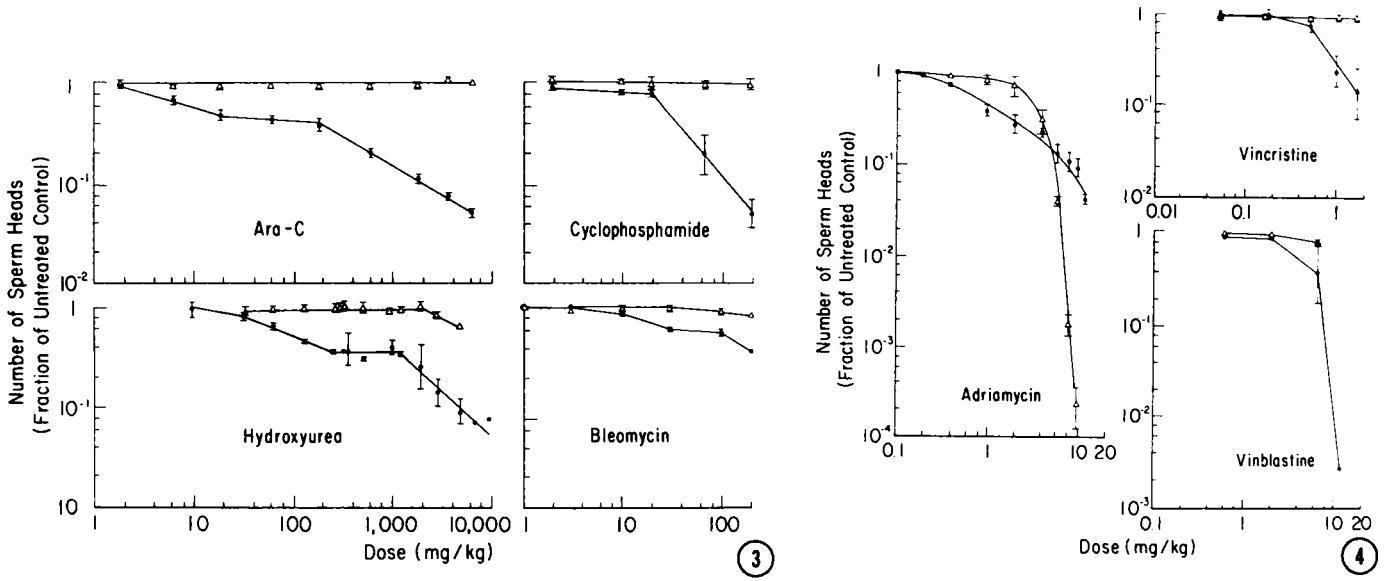
Counts of repopulated tubular cross-sections were performed on histological preparations of testes from the drug-treated mice. Complete spermatogenesis was seen in all tubular cross-sections with 6 of the 7 drugs. Only ADR treatment resulted in many empty tubular cross-sections (Fig. 2). Quantitative counts yielded a stem cell survival curve with a  $D_0$  (1/e dose) of 1.3 mg/kg (Chart 6). For comparison, the  $D_0$ 's for LDH-X activity (Chart 5) and sperm head counts (Chart 4) were 1.2 and 0.8 mg/kg, respectively. Thus, the sensitivity of the stem cell obtained from the counts of repopulated tubular cross-sections was in qualitative agreement with that obtained from sperm head counts and LDH-X enzyme assays.

**DISCUSSION**

Previous studies with chemotherapeutic drugs have demonstrated that ADR (27), CP (28), VCR (11), and ara-C (12) produce biochemical changes as well as cytotoxic effects on rodent testes. However, those studies did not provide quantitative values or dose-response curves for killing of specific spermatogenic cells.

We have used, in the present study, several new assays to quantitatively determine the cytotoxicity of chemotherapeutic drugs on specific spermatogenic cells of the mouse testis. These assays have several advantages: (a) they provide a quantitative measure of the killing of specific types of testicular cells; (b) they measure not only the survival of the cell but also the ability of the cell to differentiate into more mature spermatogenic cells. The latter is more relevant to the ultimate function of the testis; (c) the sperm head counts are simple and rapid; and (d) the assays for stem cell survival measure this important but rare cell without difficult histological counts.

The assays for stem cell survival appear to have the shortcoming that they may be insensitive to a small amount of cytotoxicity. Implicit in the LDH-X and sperm head count assays is the assumption that the number of spermatocytes and sper-



Charts 3 and 4. Log-log plot of the relationship between the testicular sperm head count per mouse (fraction of untreated control) and the injected dose of various drugs. Sperm head counts obtained 29 days after injection (●) are a measure of the number of surviving differentiated spermatogonia. Sperm head counts obtained at 56 days (Δ) are a measure of the surviving stem cells. Values are presented as geometric means and S.E. (bars). The doses used for BLM were expressed in units/kg, but 1 unit is approximately equal to 1 mg.

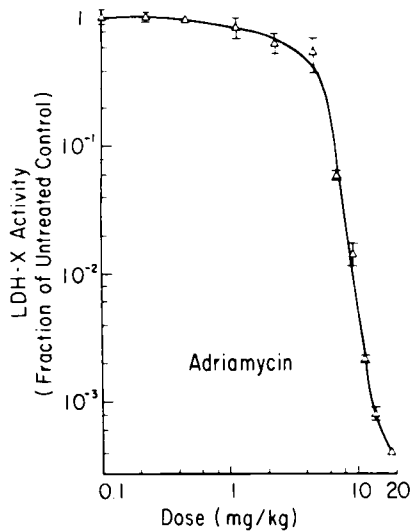


Chart 5. Log-log plot of the relationship between testicular LDH-X enzyme activity per mouse (fraction of untreated control) and the injected dose of ADR. Bars, S.E.

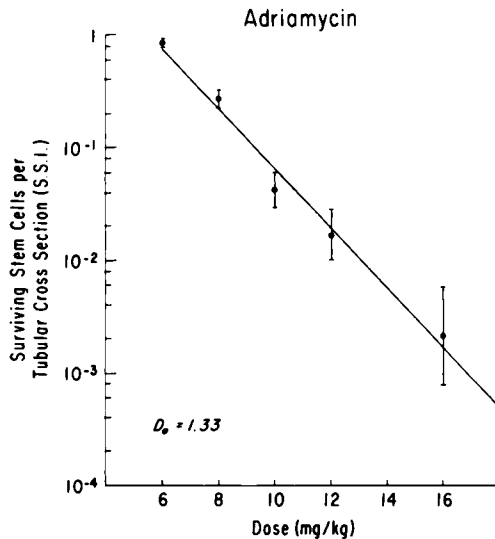


Chart 6. Surviving stem cells per tubular cross-section (SS1) as function of dose were measured from histological sections of testes from ADR-treated mice. S.S.I. =  $\log_e (1 - R1)$ . Bars, S.E.

matids produced are proportional to the number of surviving stem cells. However, the rate of production of spermatocytes from each stem cell could be modified by 3 factors: (a) the cell degeneration which normally occurs during maturation of  $A_1$  to  $A_3$  spermatogonia (2, 22) could be reduced in the treated animals; (b) the  $A_{2b}$  spermatogonia undergo a variable number of divisions to produce chains of  $A_{2b}$  spermatogonia (9), and an additional division of  $A_{2b}$  cells could occur in treated animals; (c) the cell cycle of the slowly cycling stem cells may decrease after treatment and produce differentiating cells more frequently. Thus, it is possible that a small amount of stem cell killing might be masked by these compensatory mechanisms; our preliminary data with neutron irradiation indicate that such mechanisms can raise the apparent survival of stem cells by at most a factor of 4 when using these assays. Counts of repopulated tubular cross-sections are even less sensitive since

at least 90% of the stem cells must be killed before empty cross-sections appear.

The differentiated spermatogonia were the most sensitive cells to all of the drugs tested. The sensitivity of the differentiated spermatogonia was undoubtedly related to their short mitotic cycle involving DNA synthesis and cell division. Differences were observed among the various spermatogonial stages, in sensitivity to different drugs, probably reflecting some changes in the cell metabolism with maturation. Spermatocytes (past preleptotene) and spermatids appeared to be resistant to killing by these drugs. Stem spermatogonia were resistant to 6 of the drugs, but after ADR treatment the stem cells displayed a sensitivity intermediate between the differentiated spermatogonia and the spermatocytes or spermatids. The same qualitative pattern was noted after ionizing radiation (17, 21, 23, 24).

We considered that the resistance of spermatogenic cells to these drugs may have resulted from a lack of drug penetration across the blood-testis barrier. However, the differentiated spermatogonia also are located in the basal compartment of the seminiferous tubules adjacent to the stem spermatogonia. Thus, the greater sensitivity of the differentiated spermatogonia to these drugs (Table 2) ruled out the possibility that the resistance of the stem cell was due to lack of drug availability in the testis. On the other hand, the greater sensitivity of the rapidly cycling differentiated spermatogonia as compared with the slowly cycling stem cells to VLB and CP was consistent with the greater sensitivity of rapidly dividing lymphoma cells as compared with slowly proliferating hematopoietic stem cells to these drugs (1).

The production of abnormal spermatids from cells treated as spermatocytes demonstrates that the drugs, VCR, VLB, CP, and BLM penetrate to these cells. Other studies have shown that the early spermatid stages were most sensitive to CP as measured by the induction of translocations (31) and the production of DNA damage resulting in unscheduled DNA synthesis (32). Furthermore, both CP and VCR have biochemical effects on spermatids, significantly reducing RNA and protein synthesis (11, 12). VCR also produces degeneration of some meiotic cells (11). Thus, the resistance of spermatocytes and spermatids to killing could not be only a result of the lack of drug penetration.

We believe that these studies have relevance to the human population treated with these drugs. The doses used in various clinical protocols are compared in Table 3 with the toxic doses for spermatogonia. If human and mouse spermatogonia respond similarly to these drugs, treatment of patients with ADR, CP, ara-C, and HU would produce extensive killing of differentiated spermatogonia. Such killing would result in transient reductions in sperm counts and temporary sterility. The human testis is indeed sensitive to many chemotherapeutic treatments and transient reductions in sperm counts are often observed (30).

Only ADR should produce significant stem cell killing at clinical doses. For example, treatment of mice with ADR [10 mg/kg (33 mg/sqm)] reduced testicular stem cell survival to  $2 \times 10^{-4}$  of controls. The same stem cell survival was observed after 1250 rads of  $\gamma$ -radiation, which resulted in permanent sterility in mice (15). Thus, even a single clinical course of ADR should produce long-term oligospermia and permanent sterility

Table 3  
Comparison of sensitivity of spermatogonia with clinically used doses of chemotherapeutic drugs

Drug	LD <sub>50</sub> for differentiated spermatogonia in mice		Clinical dose <sup>a</sup> / (mg/sq m)
	mg/kg	mg/sq m <sup>b</sup>	
BLM <sup>c</sup>	130	430	20
VCR	0.7	2.3	2
VLB	4.5	15	20
ADR	1.0	3	40
CP	30	100	500
ara-C	16	53	400
HU	100	330	10,000

<sup>a</sup> Typical dose per course of chemotherapy (7, 13); courses may be repeated at 2- to 4-week intervals.

<sup>b</sup> Calculated by multiplying by 3.3, which is the ratio of weight to surface area for a 26-g mouse (6). Expression of doses on a mg/sq m basis provides a better extrapolation of toxic effects between species than does expression on a mg/kg basis (6).

<sup>c</sup> Dosage expressed in units instead of mg; 1 unit is approximately equal to 1 mg.

in humans. The effect of ADR on humans appeared to be less drastic than on mice. Two of 4 patients who received at least 400-mg/sq m doses of ADR still possessed sperm counts of over 2 million/ml (4).

On the other hand, long-term treatment of men with CP (about 20 g total dose corresponding to 12,000 mg/sqm) resulted in azoospermia lasting up to 19 months (30). Such a persistent effect indicated that significant stem cell killing occurred. The lack of any stem cell killing in mice may have been a result of the lower dose, limited to 660 mg/sqm by the toxicity of a single injection. Since testis stem cells regenerate more slowly than in other stem cell systems (15, 34), it is possible that multiple injections could kill stem cells without toxicity to the animals. Combination treatment with nitrogen mustard, VCR, procarbazine, and prednisone produced azoospermia for up to 4 years in humans (29). Six courses of this drug combination included VCR (12 mg/sqm) which was not expected to produce stem cell killing. The resulting azoospermia may be produced by the other drugs used. From our data, we conclude that there is not yet enough information to extrapolate our results directly to humans.

In addition to their cytotoxic effects, these drugs can be mutagenic to the germinal cells. The observation of diploid spermatids is indicative of irregular chromosome segregation at meiosis (33). The presence of abnormal sperm nuclei has also been correlated with mutagenesis (35). The results of the present study therefore imply that VCR, VLB, CP, and BLM may be mutagenic to mouse testis cells.

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Fig. 1. Cross-section of testis treated with Adriamycin (6 mg/kg) and excised 11 days after injection. Note the absence of pachytene spermatocytes in tubules at Stages I through VI (tubules marked *a*) and the presence of pachytene spermatocytes but absence of the young primary spermatocytes in tubules at Stages VIII through XII (tubules marked *b*).  $\times 320$ .

Fig. 2. Cross-section of testis treated with Adriamycin (6 mg/kg of body weight) and excised 56 days after injection. Note empty tubules containing only Sertoli cells (tubules marked -) and tubules showing repopulation with spermatogenic cells (tubules marked +).  $\times 320$ .



