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EXPERIMENT K-6-16

MORPHOLOGICAL EXAMINATION OF RAT TESTES

THE EFFECT OF COSMOS 1887 FLIGHT ON SPERMATOGONIAL POPULATION AND TESTOSTERONE LEVEL IN RAT TESTES

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SUMMARY

Testes from rats flown on Cosmos 1887 for twelve and a half days were compared to basal control, synchronous control and vivarium maintained rats. When the mean weights of flight testes, normalized for weight/100 gms, were compared to the vivarium controls they were 6.7% lighter. Although the flight testes were lighter than the synchronous, the difference is not significant. Counts of spermatogonial cells from 5 animals in each group revealed a 4% decrease in flight compared to vivarium controls. In both cases the t-Test significance was <0.02. The serum testosterone levels of all animals (flight, synchronous and vivarium) were significantly below the basal controls.

INTRODUCTION

The flight of Cosmos 1887 Biosputnik provided an opportunity to investigate the effects of a 12.5 day space flight. The testes have been shown to be affected by space flight, immobilization, irradiation and increased gravity. Two male dogs were flown on Cosmos 110 for 22 days (Fedorova, 1967). Fedorova reported an increase of 30 to 70% atypical spermatozoa consisting of tail curling and the absence of a tail. Seventy- five days after the flight, the abnormalities had decreased to their high normal range of 30%. Mating of these dogs after this period of time produced normal offspring. Rat flight testes from SL-3 showed a 7.5% decrease in stage six spermatogonia and an average weight difference of 7.1% when compared to controls (Philpott et al., 1986). In earlier studies of rats flown aboard Cosmos 690 (Plakhuta-Plakutina, 1977) and Cosmos 605 (Portugalov et al., 1976), no specific changes in the testes, directly attributed to flight, were reported.

Cannon (1914) first developed the idea that organisms react to unfavorable conditions with highly integrated metabolic activities. Selye (1950) summarized the manifestations of physiological response to nonspecific stress indicating that atrophy of the gonads always occurred. Many papers have been written on the effects of social interaction, crowding, peck order and confinement. Flickinger (1961, 1966) showed delayed testicular development in subordinant roosters influenced by group numbers, social rank and social status. Christian and Davis (1964) and Christian et al. (1965) reported that increasing population size in mice resulted in adrenal hypertrophy, inhibition of reproductive maturation and loss of reproductive function in adults. Sex organ weights also declined. Huygens et al. (1939) observed transient atrophy of the testes when dogs were confined. A decrease in testicular weight and total body weight occurred in rats and mice spun on a centrifuge at 2 X g for 8-9 weeks (Crockett, unpublished, 1969).

Immobilization, applied for a short or long period, is considered a form of physiological stress, and induces a decrease in plasma testosterone levels (Charpenet et al., 1981; Armario and Castellanos, 1984; Collu et al., 1984). While most reports indicate no change in the morphology of differentiating germ cells as a result of immobilization (McGrady and Chakraborty, 1983), a "striking" arrest of spermatogenesis in a primate has been reported (Zemjanis et al., 1970).

The sensitivity of the testes to radiation is well known and well studied. The details of spermatogonial effects of irradiation in mice and rats (Huckins, 1978; Van Alphen et al., 1988; Cunningham and Huckins, 1978; Cattanach and Crocker, 1979; Hugon and Borgers, 1966; Lu et al., 1980; Oakberg and Clark, 1961; Oakberg, 1962; Whithers et al., 1974) and primates (Zemjanis et al., 1970) have been the subject of studies utilizing X-irradiation and gamma irradiation. Our team has reported the results of cosmic (HZE) type irradiation on spermatogonial cell counts (Philpott et al., 1981, 1983a, 1983b, 1985a, 1985b). Alpen and Powers-Risius (1981) have quantitated HZE effects using testes weight loss. The Alpen and Philpott papers have reported an increase in RBE (radiobiological effect) after HZE irradiation. Philpott et al. (1983) have shown that doses of less than 0.5 rads can be detected using an assay method that concentrates on cell

numbers in spermatogonial stage six (Huckins, 1978). This sensitive response makes the testes a possible candidate as a biological dosimeter.

METHOD

The left and right testes from five rats flown on Cosmos 1887 provided material for weight determination, testosterone assay and spermatogonial cell loss quantification. Another fifteen animals were maintained on the ground, i.e., five synchronous controls, five vivarium controls and five sacrificed at the time of flight to provide material for basal control. The flight lasted twelve and a half days and landed in Siberia. The animals were transported by bus, airplane and van to the animal facility. Sacrifice occurred two days after the rats returned from space. The testes were removed, weighed, immediately slit open and immersed in cold Triple Fix (Philpott et al., 1980). The specimens were kept at four degrees C, shipped to Ames and refrigerated until time for processing. Samples were treated with 1% osmic acid for one hour, dehydrated in ascending concentrations of acetone, infiltrated with Epon-Araldite and polymerized at 60 degrees C for 48 hours. Six blocks were produced from each testis. Two-micron cross sections of the blocks were cut on a Porter-Blum ultramicrotome and mounted on glass slides. The sections were stained with Toluidine Blue. Alternate sections containing maturation stage six were used to count the surviving spermatogonia (Figure 1). Testosterone measurements were made on the rat plasma samples using Coat-A-Count kits from Diagnostic Products, Inc.

RESULTS

The mean weights for the testes were: flight 1.19 gm, synchronous 1.34 gm, and vivarium 1.44 gm. The average weight difference of the Cosmos flown rat testes was 6.7% as compared to vivarium controls when normalized for weight/100 grams. This difference is significant. However, the difference between the flight and synchronous control animals is not significant. Basal control weights were not available.

Counts of surviving spermatogonia (Table 1) per tubule cross section indicated an average of 38.79 spermatogonia for flight animals, 40.20 for the synchronous controls and 43.75 for the vivarium controls. The decreases of spermatogonia in flight tissues is a significant number as compared to the synchronous control (P<0.02, 1 tail; P<0.005, 2 tail) and vivarium control (P<0.0002, 1 tail; P<0.005, 2 tail). Rats flown on SL-3 experienced a similar decrease in number of spermatogonia (Figure 2). Preliminary counts of Sertoli cells per tubule cross section indicate no significant difference (P>0.05) when vivarium control animals are compared to either synchronous controls or to flight animals. This consistency in Sertoli cell numbers per tubule cross section demonstrates their stability under the adverse conditions of space flight and indicates that the minor volumetric changes in tubular epithelium due to spermatogonial cell loss do not affect the relative numbers per tubule cross section. Spermatogonial cell loss can be quantitated per number of Sertoli cells or per tubule cross section. Changes in spermatogonial cell populations are indicative of actual cell loss and are not significantly influenced by volumetric changes in the tubules (Fig. 3,a,b,c,d).

Serum testosterone (Table 2) was measured for flight animals, basal, vivarium, and synchronous controls. When means were compared between the basal controls and all other experiments, the differences were significant at the <0.001 level for flight, at the <0.05 level for synchronous control, and at the <0.01 level for the vivarium control. The mean for the flight testosterone level was lower than the mean for synchronous or vivarium controls but not significant when a t-Test was applied. These results are somewhat similar to those from rats flown on SL-3 (Figure 4).

DISCUSSION

While it is general knowledge that the testes are very sensitive to certain environmental factors including stress and irradiation, not all reports agree on the extent and nature of morphologic

changes in the seminiferous epithelia (McGrady and Chakraborty, 1983). Basically, most reports indicate that stress will decrease testosterone levels (Charpenet et al., Armario and Castellanos, 1981; Collu et al., 1984) but does not cause any morphological changes in the seminiferous tubules (McGrady and Chakraborty, 1983). Interestingly, in human beings, stress may cause either an increase or decrease in serum testosterone depending upon whether the stress is perceived as a threat to dominance/control (increased testosterone) or as a loss of control (decreased testosterone) (Collu et al., 1984). On the other hand, irradiation, depending on the dosage, can result in the depletion of all spermatogonial cells except a few of the stem cells (Philpott et al., 1983; Van Alphen et al., 1988). At the same time testosterone levels do not seem to be affected either in the serum or intratesticular tissue by irradiation (Cunningham and Huckins, 1978).

Previous space flight investigations prior to SL-3 have not reported changes in seminiferous epithelium while simulated conditions, at least in some investigators' labs, result in changes (Portugalov et al., 1976). Data obtained from rats flown on Cosmos 1887 indicated significantly reduced numbers of spermatogonia when compared to both synchronous control animals and vivarium control animals. Differences in cytological populations were significant (11% decrease compared to vivarium, 4% decrease compared to synchronous) and were generally similar to results obtained from rats flown on SL-3 (Philpott et al., 1986; Sapp et al., 1988). Our assay procedure provides excellent quality specimens and sections thin enough to provide morphological differentiation of each spermatogonial cell class, and precise quantitation.

Rats in the synchronous control group were exposed to all environmental parameters, except micro-G, and the increased G- forces of lift-off and landing, encountered in the Cosmos 1887 space flight. Vivarium controls were exposed only to similar temperature, feeding and lighting conditions. Our data indicate a significant difference in spermatogonial population when these two control groups are compared. This difference may be caused by the stresses encountered under simulated space flight conditions, or to as yet unexplained responses. A similar decrease in spermatogonia was seen in rats subjected to suspension in the simulated SL-3 flight (Figure 2).

We postulate that the decrease (4%) in spermatogonia observed in rats actually flown on Cosmos 1887 when compared to synchronous controls is due to space flight conditions not adequately duplicated in the ground based synchronous experiment. One possible factor is radiation. Dosimetry reports from Cosmos 1887 indicate a dose factor of 0.313 rad at the dosimeter location within the space craft. Dosimeters were not located proximal to the animals, therefore the exact dosage received in that area is not known. Low radiation levels do not produce gross changes in morphology; however, spermatogonia near the first meiotic division are reduced in number. This is not unexpected and many investigations, including our own, substantiate these results (Philpott et al., 1983). We have shown with X-rays and with HZE particles the extreme sensitivity and predictable response of the testicular epithelium to irradiation. The loss of cells not accounted for except by space travel could have resulted from radiation, especially since any particles penetrating the space craft would have been galactic and of similar energy to Iron. Our previous experiments (Figures 5, 6) indicate that irradiation with HZE particles of Iron at the 0.5 rad level caused significant decreases in spermatogonia in mice (Philpott et al., 1985b) and these changes could be detected down to the 0.1 rad exposure level (unpublished).

Previous work in our laboratory led us to conclude that the testicular seminiferous epithelium is a good model for radiation sensitivity studies. It has been shown to be very susceptible to radiation damage in general. It is composed of cellular populations which vary in individual radiosensitivities and is indicated by the multiple slopes seen in spermatogonial survival curves (Philpott et al., 1983b). These characteristics of the testicular epithelium provide an accurate means for biological dosimetric assessment of radiation exposure.

Data collected from this flight (Cosmos 1887) as well as from the earlier SL-3 flight indicate that the biological effects of space flight are multifaceted. Impact on the morphophysiology of the

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testes through a number of different pathways, i.e. decreases in serum testosterone and testes weight loss, is observed in the animals described in this report. Stress related gonadal dysfunction and possible galactic radiation exposure, along with other possible factors, apparently contribute to the significant decrease in spermatogonial cell numbers observed in rats flown in space. Various changes in the environment can alter testicular integrity. The site of action of these various environmental impacts and the mechanisms by which they interfere with both spermatogenesis and steroidogenesis need further investigation. These important investigations should be repeated with longer flights and a shorter time span between recovery and specimen preparation. When it becomes possible, fixation in flight will remove any doubt about the effect recovery may have.

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TABLE 1. TOTAL SURVIVING SPERMATOGONIA PER STAGE 6 SEMINIFEROUS TUBULE PROFILE[A]

Treatment Group Data	Individual Animals[B]	Mean +/- SEM[C]	SL-3[D]
Flight	37.90 +/- 0.15		
	38.77 +/- 0.12		
	39.36 +/- 0.15	38.79 +/- 0.06[**]	39.75 +/- 0.14
	39.08 +/- 0.12		
	38.83 +/- 0.13		
Synchronous	40.44 +/- 0.10		
	41.13 +/- 0.15		
	39.08 +/- 0.19	40.20 +/- 0.06[*]	N/A
	40.00 +/- 0.12		
	40.35 +/- 0.12		
Vivarium	43.15 +/- 0.17		
	44.36 +/- 0.13		
	43.58 +/- 0.16	43.75 +/- 0.07	42.71 +/- 0.17
	43.55 +/- 0.20		
	44.12 +/- 0.17		

[A] Tubules identified according to Huckins (Anat. Rec. 190:905, 1978), cross section only.
[B] 200 tubules counted per animal; mean +/- Standard Error.
[C] 5 animals per treatment; total = 1000 tubules counted.
[D] SL-3 data shown here for comparison, see Philpott et al., EMSA 44:248, 1986).
  Mean +/- Standard Error, 200 tubules scored.

[*] Significantly different from vivarium control, $P<0.001$.
[**] Significantly different from vivarium control, $P<0.0005$.

TABLE 2. SERUM TESTOSTERONE (Cosmos-1887) ng/ml

Animal	Flight	Basal	Vivarium	Synchronous
6	0.18	4.50	2.50	0.62
7	0.18	4.20	1.20	3.50
8	0.62	3.40	0.43	0.85
9	0.10	2.40	0.43	1.85
10	0.62	2.00	0.24	0.77
Mean	0.34	3.30	0.97	1.52
SD	0.26	1.09	0.93	1.21
SE	0.12	0.49	0.42	0.54

	t	p
Basal vs Flight	5.91	<0.001
Basal vs Syn	2.45	< 0.05
Basal vs Viv	3.64	< 0.01
Flight vs Syn	2.13	N S
Flight vs Viv	1.46	N S
Syn vs Viv	0.80	N S

SPERMATOGONIAL CYCLE ($A_1 \rightarrow B$) — 8.6 days

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV		STAGE (ACROSOMAL)	
	15	15	15	15	15	16	20	20	16	14	12	9.6	9.6	9.6		TIME, hr	

MATURATION OF SPERM. TOZOA — A

ACROSOME FORMATION TO HEAD ELONGATION — G

PACHYTENE (P) DIAKINESIS (DI) SECONDARY SPERMATOCYTE — F

PRELEPTOTENE (PL) LEPTOTENE (L) ZYGOTENE (Z) — E

STAGE (SPERMATOGONIA)		
$A_4 \rightarrow I_N \rightarrow B$	D
$A_1 \rightarrow A_3$	C
A_{AL}	B
$A_S \rightarrow A_{PR}$	A

STAGE (SPERMATOGONIA)	4	5	6	1	2	3
TIME, hr	29	29	29	62	29	29

SPERMIOGENESIS

MEIOSIS

SEMINIFEROUS EPITHELIUM CYCLE

Figure 1. Schematic, showing how mature sperm develop after division of the stem cells. The spermatogonial population is counted in stage six.

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Figure 2. Preliminary comparison of volume density counts from Cosmos 1887, SL-3 and a 7 day suspension study (R + 0).
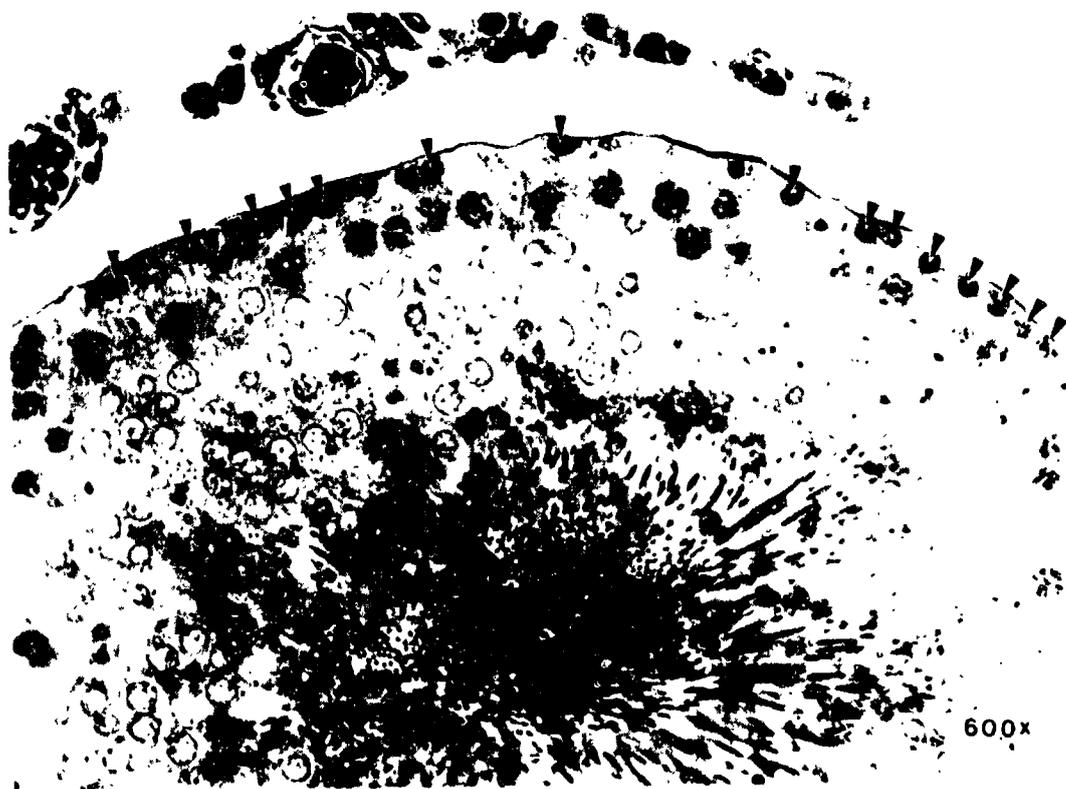
Figure 3 (a) Vivarium control. Arrows indicate spermatogonia, 600X.

Figure 3 Continued. (b) Synchronous control. Arrows indicate spermatogonia, 600X.
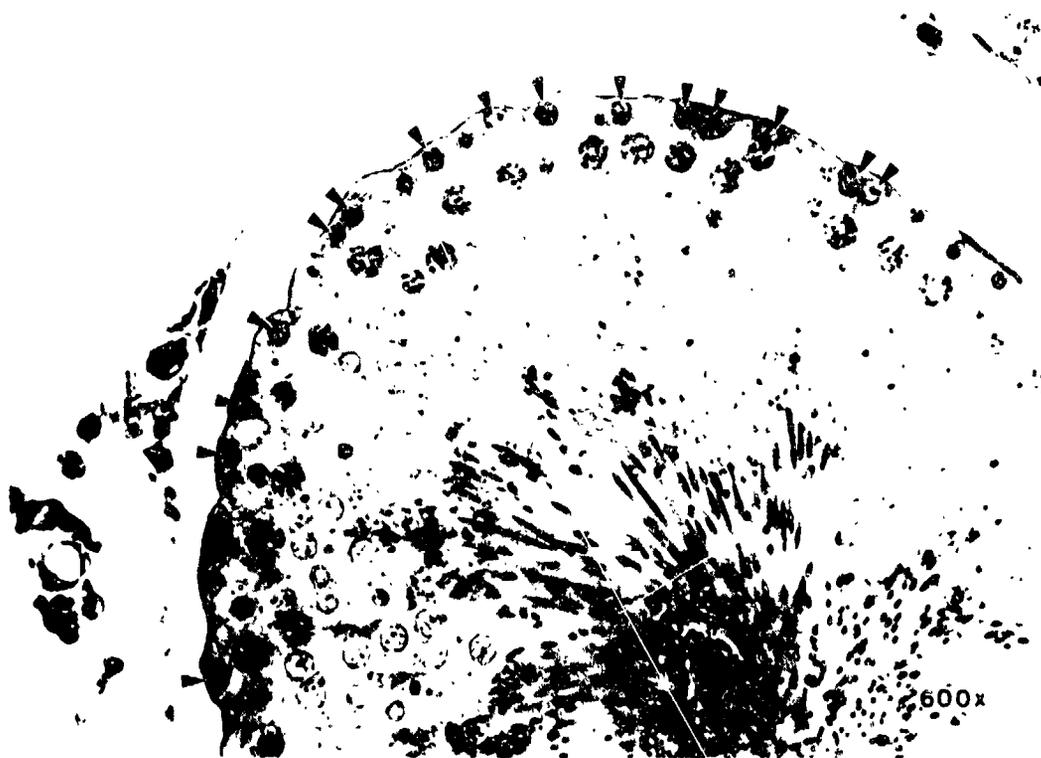
Figure 3 Continued. (c) Cosmos 1887 flight. Arrows indicate spermatogonia, 600X.
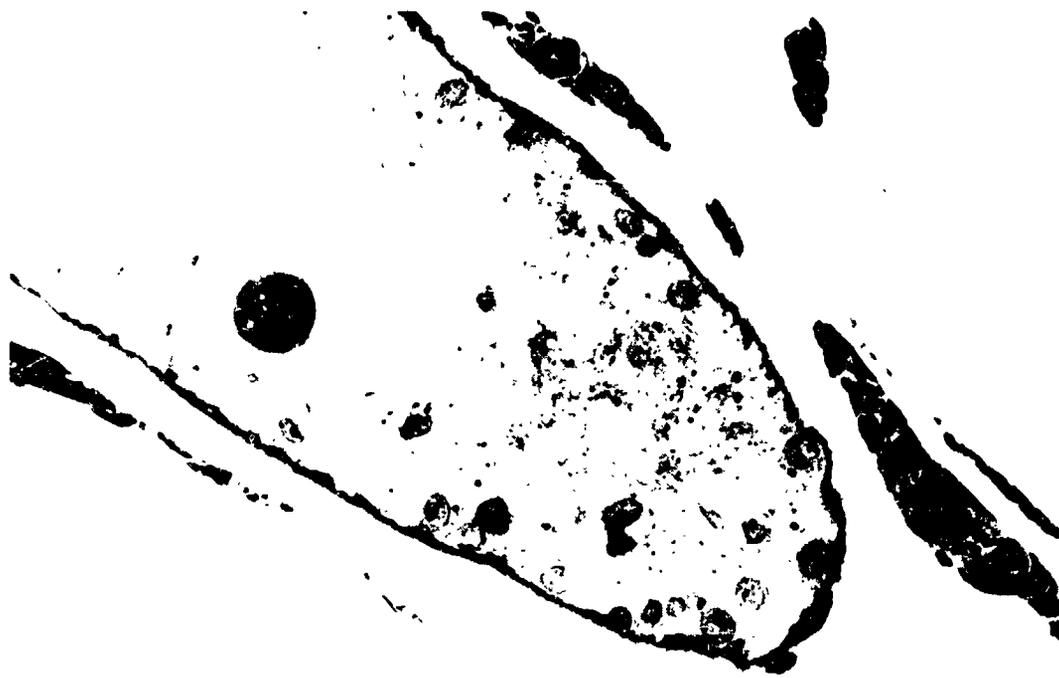
600ˣ

Figure 3 Concluded. (d) Cosmos 1887 flight. Abnormal tubule profiles noted in one animal. Testes weight in this animal was two-thirds of the other flight, 600X.
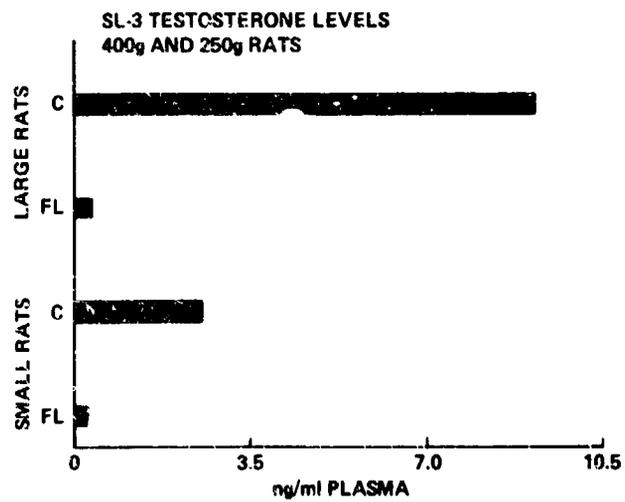
Figure 4. SL-3 testosterone levels.
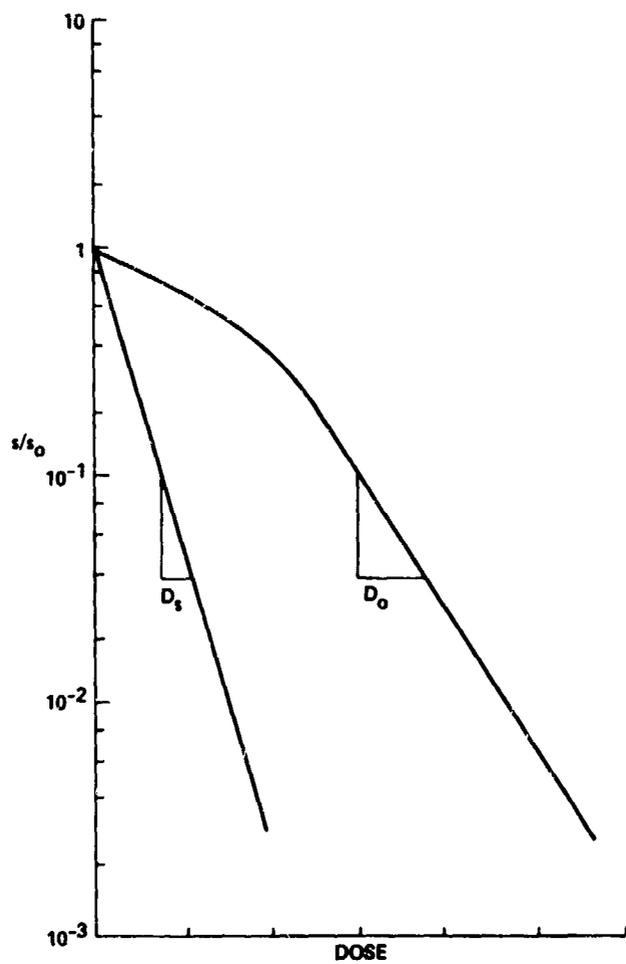
Figure 5. Representative dose response curves for spermatogonial survival after X-rays (curved line) and high energy particles (HZE) (straight line). The curved line plotted from the response to X-rays demonstrates that repair is taking place in the low dose range. The straight line (HZE particles) signifies no repair. Note that the curves show response at very low doses.
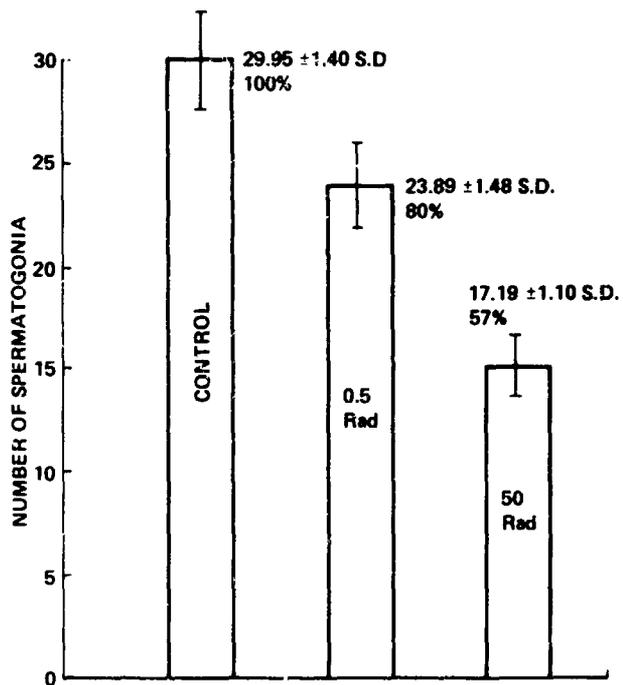
Figure 6. Spermatogonial cell counts six months after 0.5r and 50r of iron particles. Note how long suppression of counts remains after HZE irradiation.