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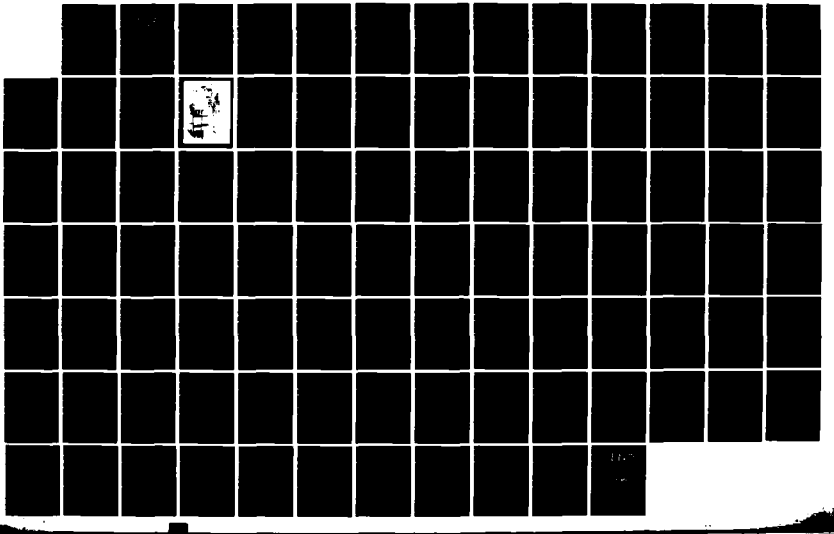

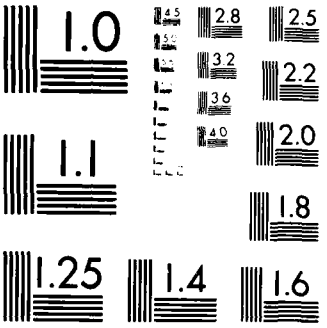

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ASSESSMENT OF ANTIRADIATION DRUG EFFECTIVENESS TO FISSION NEUTRON IRRADIATION

ANNUAL AND FINAL REPORT

CURTIS P. SIGDESTAD, PH.D.

September 1~~984~~
1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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University of Louisville School of Medicine
Louisville, Kentucky 40292

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This report details scientific results of contracted research testing the ability of newly synthesized anti-radiation drugs to protect critical normal tissues from the adverse effects of fission neutron irradiation. Fission neutrons were obtained at the Health Physics Research Reactor (HPRR) at Oak Ridge National Laboratory, Oak Ridge, TN. The neutron beam had an average energy of 0.9 MeV and a dose-rate of about 55 Rads per minute.

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C57B1/6J male mice were used throughout this study. They were irradiated at two meters from the unshielded reactor core with whole body doses which varied from 0 to 600 Rads. The organ systems studied were the hematopoietic and gastrointestinal stem cell populations. The neutron effects on the former was studied using the LD50(30) lethality and the endogeneous spleen colony assay. The gastrointestinal tract effects were studied by determining the LD50(6) and measuring the crypt cell survival using the microcolony assay developed by Wither's et al.

The protectors examined in this study were originally synthesized under the direction of Walter Reed Institute for Research. They were mostly the newer class of protectors, namely the phosphorothioates which have the greatest chance of improving survival after exposure to radiations which would be experienced in atomic weapon detonation. The drugs covered in this report are: WR-347, WR-1065, WR-2529, WR-2721, WR-3689, WR-44923, WR-109342, WR-151327 and WR-168643. They were injected intraperitoneally at a dose equivalent to the one-third the toxic LD50, 30 minutes prior to irradiation.

The results indicate that these agents vary considerably in their ability to protect normal tissues from the adverse effects of neutron irradiation. The study did, however, identify several agents which were surprising in their ability to protect against this high LET radiation.

It is recommended that further studies be initiated with high LET radiations using the protectors WR-151327, WR-3689, WR-2721 and WR-2529.

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SUMMARY

This report represents the total effort in the Contract DAMD17-81-C-1070 screening new anti-radiation drugs for toxicity and testing for their ability to protect critical organ systems from the adverse effects of high LET radiations. Initially, data was also obtained using the low LET radiation source Cobalt-60 for comparison purposes. The critical organ systems included in this screening procedure were the bone marrow and the gastrointestinal tract. The bone marrow sensitivity was tested using thirty day lethality and the endogenous spleen colony assay systems. The gastrointestinal sensitivity was tested using the six day lethality and the microcolony assay for stem cell survival. Preliminary experiments were performed to assess the ability of the intestinal crypt cell to repair sublethal damage after fission neutrons.

The protectors examined in this study were originally synthesized under the direction of the Walter Reed Institute for Research. They were mostly the newer class of protectors, namely the phosphorothioates which have the greatest chance of improving survival after exposure to radiations which would be present in atomic weapon detonation. The drugs covered in this report are: WR-347, WR-1065, WR-2529, WR-2721, WR-3689, WR-44923, WR-109342, WR-151327 and WR-168643.

The drugs and their respective dose modification factors (DMF) for fission neutron gastrointestinal lethality following intraperitoneal administration are, in decreasing order of effectiveness: WR-151327 (2.23), WR-44923 (1.77), WR-2529 (1.47), WR-109342 (1.47), WR-1065 (1.42), WR-2721 (1.39), WR-3689 (1.36) and WR-168643 (1.23).

For hematopoietic neutron radiation lethality the DMF's following i.p. administration were: WR-2529 (1.40), WR-151327 (1.34), WR-168643 (1.25), WR-44923 (1.22), WR-109342 (1.21), WR-2721 (1.20), WR-3689 (1.04) and WR-1065 (1.04).

Using an intestinal microcolony assay system the following drugs provided the listed DMF's against neutron radiation after i.p. injection: WR-3689 (1.24), WR-151327 (1.15), WR-2721 (1.15), WR-44923 (1.14), WR-2529 (1.08), WR-347 (1.05), WR-1065 (1.02) and WR-168643 (1.01).

The protective effect from fission neutron irradiation of the compounds involved in the screening process, utilizing the endogenous spleen colony assay resulted in DMF's as follows: WR-168643 (1.19), WR-3689 (1.18), WR-2529 (1.15)

follows: WR-168643 (1.19), WR-3689 (1.18), WR-2529 (1.15) WR-1065 (1.14), WR-2721 (1.10), WR-151327 (1.07), WR-44923 (1.02), WR-3689 (1.18) and WR-347 (.094).

It is clear from the results obtained that there are agents other than WR-2721 which are good radiation protective compounds. Specifically, WR-151327 appears to have the best protective effect against fission neutron irradiation. The results also indicate that there is a difference in the protective effect depending upon the LET of the radiation, the endpoint used (lethality or stem cell survival) and the organ system tested (gastrointestinal tract or bone marrow).

It is recommended that further studies be initiated with high LET radiations using the protectors WR-151327, WR-44923 and WR-2529. In addition, the results obtained in the preliminary experiments involving repair of sublethal damage after fission neutron irradiation showed some promise for further investigation, especially in the determination of the effect of these protective compounds in the repair process.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. 78-23, Revised 1979).

The author would like to express his deep appreciation to Dr. A. M. Connor whose diligence was invaluable in the successful completion of this project. In addition, the author would like to thank CL. David E. Davidson, Jr. who acted as liaison between this Institution and the funding agency. His extensive knowledge in the area in chemical radiation protectors was of extreme value to the studies performed.

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INTRODUCTION

The efficacy of sulphydryl compounds as radioprotective agents was first demonstrated by Patt in 1949 (1). Subsequent to these studies, many compounds have been tested for their ability to protect against the effects of ionizing radiation. The decarboxylated cysteine derivative, cysteamine (mercaptoethylamine, MEA, WR-347) was found to be the best protector in the sulphydryl class, giving dose reduction factors (DRF) of approximately 1.3 for intestinal death (2) and 1.8 for hematopoietic death (3). In 1959 Akerfeldt (4) reported the synthesis of a thiophosphate derivative of cysteamine which was characterized by a phosphate group covering the sulphydryl. These phosporothiotic acids were shown to provide significant increases in radioprotection as compared to the compounds containing sulphydryl groups alone (5,6). Further synthesis and screening of phosphorothiotic acids demonstrated the most effective radioprotector to be WR-2721 (7). It is not only less toxic than cysteamine (8), but it also protects irradiated skin and bone marrow preferentially over tumor (9) and provides differential protection in several other normal tissue-tumor systems (10,11,12,13). However, there are problems of toxicity and less than adequate protection of some dose-limiting critical organs, such as kidney, lung, and central nervous system (8,14).

More recently, other phosphorothiotic compounds have been synthesized which may provide either decreased toxicity or increased protection as compared to WR-2721. Some of these drugs, such as cysteamine phosphate (WR-638) and WR-77913 have shown radioprotection comparable to that of WR-2721 in the small intestine (2,15,16). Davidson (17) has recently reported that WR-3689 is better tolerated and has better protective activity in mice than WR-2721. The present study extends the number of thiophosphate compounds investigated as potential radioprotective agents against either Cobalt-60 gamma radiation or fission neutron radiation.

MATERIALS AND METHODS

1. ANIMALS

The animals used in all experiments were male C57/Bl/6 mice (Charles River), 70-77 days of age at the time of exposure to drugs and/or radiation. Prior to beginning any experiment, the mice were allowed one week to adapt to the local animal care

FIGURE 3

Daily Mortality Following Fission Neutron Irradiation

Percent mortality on a daily basis after high LET radiation exposure. Mice were followed for time of death up to 30 days post irradiation. The dashed line indicates the arbitrary cut-off point for gastrointestinal syndrome lethality experiments.

gastrointestinal and hematopoietic lethality. From six to thirty days after irradiation there is a distribution of mortality which is somewhat skewed to the left (skewness = 1.5963, kurtosis = 1.7308). Following fission neutron irradiation, however, the daily mortality distribution is somewhat different. As can be seen in Figure 3, there is a broad peak of lethality around the six day demarcation, occurring out to nine days following exposure to radiation. After nine days, the distribution is somewhat different than that seen with gamma irradiation (skewness = 0.6387, kurtosis = 1.3388), indicating that deaths due to gastrointestinal causes may be occurring later than six days following fission neutron irradiation. The LD50(6) for Cobalt-60 radiation was 1065 rads. This is somewhat lower than the previous study, which used 4 MeV X-rays (22), but can perhaps be accounted for by the fact that female C57Bl/6 mice were used in these experiments instead of the male C57Bl/6 mice used before, there being a significant difference in the radiation response of mice of different strains to low LET radiation. There may also be a differential response due to the different energies of the radiations employed. The LD50(30) for Cobalt-60 irradiation was 738 rads and the ratio of LD50(6):LD50(30) was 1.47.

The RBE (relative biological effectiveness) for the LD50(6) was calculated as the ratio of the LD50(6) for gamma rays to the LD50(6) for neutrons. This resulted in an RBE of 4.23 for death in the gastrointestinal lethality dose range. A similar calculation for LD50(30) resulted in an RBE of 3.08, which represented death in the hematopoietic syndrome dose range. The phenomenon of a higher RBE for gut death as compared to marrow death, coupled with the low LD50(6):LD50(30) ratio for neutrons suggests a greater sensitivity of the intestine to neutrons than to gamma radiation, a fact which has been alluded to, but not explained, in earlier studies (24). Figures 2 and 3 illustrate the variation of mortality with time for the radiation doses used in both the low LET and high LET radiations. In addition to the lethality studies performed with fission neutrons and Cobalt-60 gamma rays, other information was gleaned from the hematopoietic and gastrointestinal lethality experiments. When the mean survival time is plotted as a function of radiation dose curves such as those seen in Figure 4 are obtained. Delineated in this Figure are the respective survival time versus radiation dose curves for both fission neutrons and gamma rays. The portions of the curves used to obtain LD50's for gastrointestinal and hematopoietic lethalities are shown to have a considerable overlap,

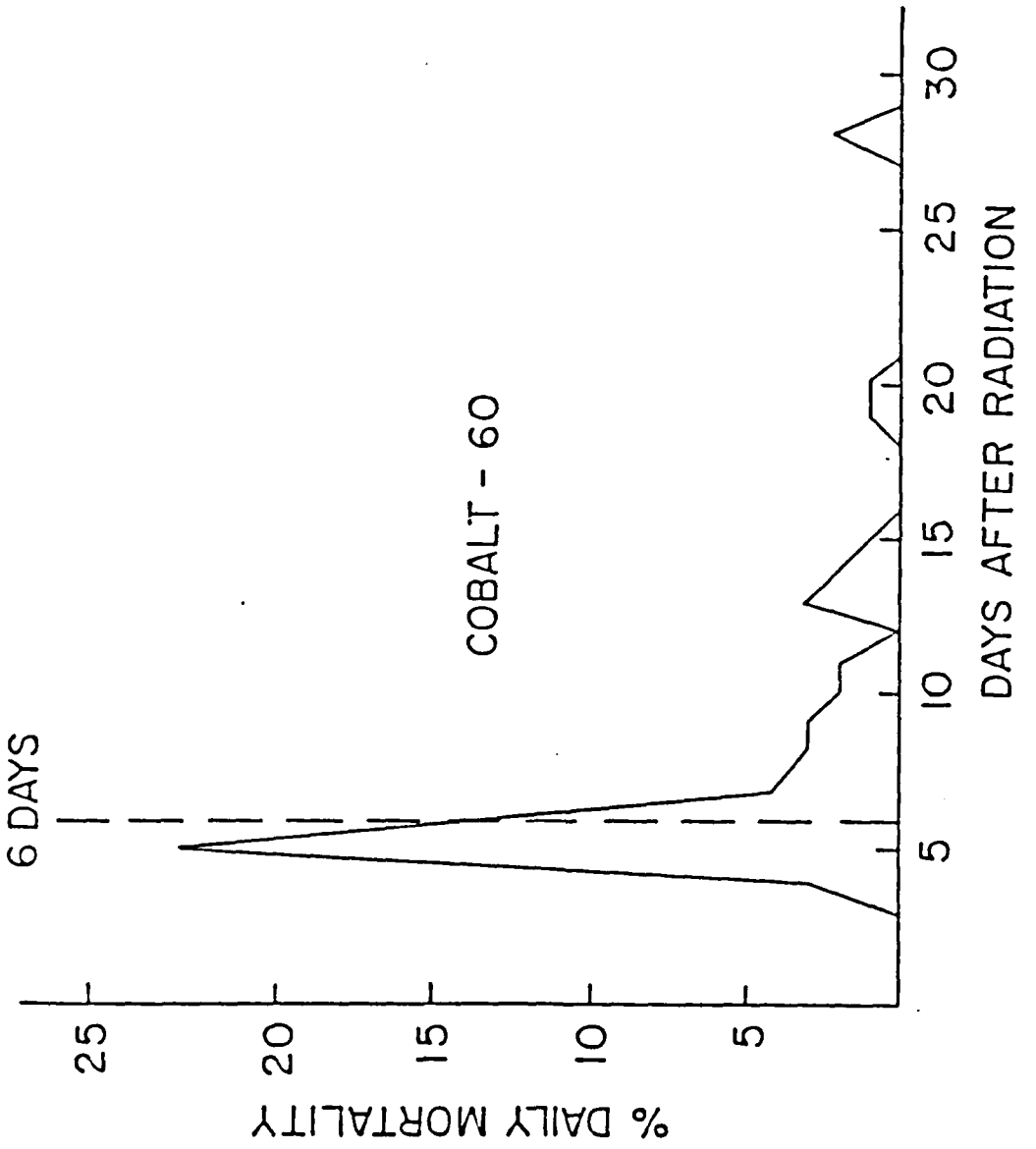

% DAILY MORTALITY
25
20
15
10
5
6 DAYS
COBALT - 60
5
10
15
20
25
30
DAYS AFTER RADIATION

FIGURE 2

FIGURE 2

Daily Mortality Following Cobalt-60 Irradiation

Percent mortality on a daily basis after low LET radiation exposure. Mice were followed for time of death up to 30 days post irradiation. The dashed line indicates the arbitrary cut-off point for gastrointestinal syndrome lethality experiments.

TABLE 2

NEUTRON LETHALITY

DRUG	ROUTE	DOSE (mg/kg)	LD50(6)* (RADS)	DMF	LD50(30)* (RADS)	DMF
NONE	--	--	252 (239-266)	--	220 (217-264)	--
WR-1065	ip	251	358 (102-601)	1.42	250 (98-395)	1.14
WR-2529	ip	1944	370 (336-403)	1.47	327 (272-381)	1.49
WR-2721	ip	741	351 (343-358)	1.39	287 (280-293)	1.30
WR-3689	ip	966	337	1.34		
WR-3689	po	1216	343 (246-439)	1.36	250 (180-314)	1.14
WR-44923	ip	517	447 (422-472)	1.77	293 (247-339)	1.33
WR-109342	po	38	371 (323-420)	1.47	290 (264-315)	1.32
WR-151327	ip	677	562 (554-571)	2.23	321 (300-343)	1.46
WR-168643	ip	852	310 (274-346)	1.23	300 (198-401)	1.36
WR-168643	po	765	330 (303-374)	1.31	332 (101-412)	1.51

* Values in parentheses are 95% confidence limits.

TABLE 1
SALIENT DRUG INFORMATION

DRUG	STRUCTURE	VEHICLE	LD_{50} (mg/kg)* i.p.		p.o.	
WR-347	NH_2-CH_2-CH_2-SH	WATER	498	(457-542)		
WR-2529	$NH_2CO(CH_2)_2NH(CH_2)_2SH$	WATER	2901			
WR-2721	$NH_2(CH_2)_3NH(CH_2)_2SPO_3H_2$	WATER	1108	(1064-1154)	1301	(1135-1491)
WR-3689	$CH_3NH(CH_2)_3NH(CH_2)_2SPO_3H_2$	WATER	1449	(1378-1525)	1816	(1520-2110)
WR-44923	$NH_2(CH_2)_3NH(CH_2)_3SPO_3H_2$	WATER	773	(583-1024)		
WR-109342	 $-CH_2NHCCH_2SH$ (NH)	WATER	37.1	(35.2-39.0)	58.0	(48.6-70.8)
WR-151327	$CH_3NH(CH_2)_3NH(CH_2)_3SPO_3H_2$	WATER	1011	(983-1039)		
WR-168643	$NaOS(CH_2)_4SSS(CH_2)_4SONa$	WATER	1272	(1255-1290)	1142	(999-1278)
WR-176542	$NH_2(CH_2)_4CHNH_2CH_2SPO_3H_2$	WATER	649	(629-671		

*Values in parentheses are 95% confidence limits

RESULTS AND DISCUSSION

Pertinent toxicity data for the drugs tested are presented in Table 1. This includes the LD50 for i.p. and p.o. administration as mg of drug per kg of animal weight (mg/kg). The dosage of drug used in radiation experiments was generally two-thirds of the toxic LD50 value unless precluded by increased toxicity during the irradiation procedure. If this occurred, subsequent use of the drug was at one-half of the previously determined toxic LD50. Use of the nylon tubes (described above) in neutron irradiation procedures sometimes resulted in animal lethalities which were unexpected as related to the earlier drug toxicity data. These lethalities (which were expecially prevalent during the summer months) were probably due to the physical and physiological constraints inherent in the use of these tubes. Following the switch to the better-designed Butyrate tubes far fewer "tube" lethalities were encountered. Table 2 shows the results of the radiation lethality experiments for fission neutron irradiation.

1. RADIATION LETHALITY EXPERIMENTS

A. Control (Untreated)

The LD50(6) in the control animals for fission neutron irradiation was determined to be 252 rads. The 95% confidence limits were 239 to 266 rads. This is identical to the value previously reported in a similar study (22). The LD50(30) for fission neutrons was 220 rads with 95% confidence limits of 217 rads to 264 rads as determined by Probit Analysis. The proximity in values for the LD50(6) and LD50(30) was not unlike the results of earlier studies (23), the ratio of LD50(6) to LD50(30) being 1.05. This demonstrates that with fission neutrons the dose needed to kill mice by the bone marrow syndrome and that necessary to kill by the gastrointestinal syndrome are not greatly different, perhaps, in this case by a factor which is nearly unity. This suggests that a relatively high RBE for gastrointestinal damage, but not for hematopoietic damage, would introduce gastrointestinal deaths in the thirty day lethal dose range used to define hematopoietic death following whole-body exposure. The phenomenon is illustrated in Figures 2 and 3, where the daily mortality is shown as a function of time following irradiation. With the low LET Cobalt-60 radiation (Figure 2), it is clearly seen that there is a peak of lethality prior to the six-day cut-off point used as the demarcation between

nodules on the spleen. In order to obtain numbers of colonies which would regress linearly with dose, a modified logarithmic transform was applied to the colony counts. The following transformation was used:

$$Y = (\text{antilogSlog}(x+1)/n)-1$$

where x is the individual spleen colony count and n is the number of counts made. The transformed number of nodules (colonies) were then plotted as a function of radiation dose to obtain the cell survival curve.

9. SPLIT-DOSE EXPERIMENTS

Split-dose experiments are ones in which the radiation response is determined with a single acute dose of whole body radiation and the results are compared to the response where the same dose is split into two equal fractions separated in time. The time between fractions can vary between one-half and 24 or more hours. Experiments of this type will give an indication of the ability of the tissue to repair sublethal damage. Split dose experiments were performed with low LET radiation (Cobalt-60) as well as high LET radiations (DOSAR, fission neutrons) using the intestinal microcolony assay system.

The low LET split-dose experiments used two doses of 600 rads each, separated by times of 2, 4, 18, and 24 hours. The high LET split-dose experiments used two neutron doses of 150 rads each, separated by times of 0.5, 1, 1.5, 3, 4.5, 6, 18, and 24 hours. Ten mice were used at each experimental point.

as follows:

DMF = LD50 treated/LD50 untreated

7. INTESTINAL MICROCOLONY SURVIVAL

Wither's microcolony assay was used to determine crypt survival in the intestine (25). Three and one-half days following irradiation, a section of the jejunem 1-2 cm distal to the ligament of Treitz was excised from each treated mouse and placed in formol saline for fixation. Following fixation, the specimens were histologically processed and thin (3-5 um) transverse sections made and mounted on microscope slides. The tissue was then stained with hematoxylin and eosin and microscopically examined to count the surviving cryptal microcolonies. In each transverse section, the number of regenerating crypts was examined microscopically and scored (crypts/circumference) and the proportion of crypts destroyed by radiation ascertained. From these data dose-survival curves for control and drug-treated animals were obtained and the survival curve parameters compared by multiple-regression analysis. In the normal jejunem of these mice there were about 137 crypts per circumference. Therefore, fewer than 137 crypts will be counted in sections which have been irradiated. Crypts may be plotted as a function of dose to give a crypt survival curve. However, in order to obtain a circumference cell survival curve one must assume independent survival of cells and that the survival of one cell is sufficient to repopulate the surviving crypt that is counted three and one-half days following irradiation. Using Poisson statistics, the average number of surviving cells per circumference were calculated as follows:

> If the number of cells surviving per circumference is n, the average crypt survival per jejunal circumference is:
>
> $$[137(-\ln(137-n/137)].$$

8. ENDOGENOUS SPLEEN COLONY ASSAY

This assay was based on the method described by Smith et al. (26). Endogenous spleen colony forming units were determined by removing the spleens of irradiated mice 10 days following exposure, fixing them in Bouin's fixative, and utilizing a dissecting microscope at 20X magnification to count the visible

5. DRUG TOXICITY EXPERIMENTS

Drugs were administered to the mice either intraperitoneally (i.p.) or per os (p.o). Intraperitoneal injections were performed with one ml tuberculin syringes and 25 gauge 5/8 needles. Oral administrations of the drugs were done with a six cm x 1.5 mm esophageal cannula which allowed deposition of the compounds in the gastric compartment. Ten mice per dose group were used in each lethality experiment and lethalities were recorded for ten days after drug administration, although no deaths occurred later than two days post-injection with any of the drugs used in these experiments. The Probit method of Finney (21) was used to calculate the lethal drug dose for 50% of the population (the LD50).

6. RADIATION LETHALITY EXPERIMENTS

Four to ten mice were used in each radiation dose group. Where 0% or 100% lethal response was expected, fewer numbers of animals were used in order to ensure higher efficiency in numbers of mice used per significantly-weighted data point without altering the reliability of the response curve. This was possible because in Probit Analysis less weight or significance is given to responses that are at or near either 0% or 100%, no matter how many organisms are used in the assay at these response points. Lethalities were recorded each day for thirty days following radiation exposure and were scored either as LD50(6) (lethalities occurring within the first six days post-irradiation) or LD50(30) (lethalities occurring within 30 days post-irradiation). The LD50(6) is usually regarded as a measure of death due to damage to the gastrointestinal system while death occurring in the seven to thirty day time-frame is due to damage to the bone marrow and blood forming system (hematopoietic death). LD50's were determined using the method of Finney (21).

Intercomparison analyses between control lethalities (non-protected mice) and drug-protected mice were calculated according to the dose-modification factor (DMF). The DMF is defined as the ratio of equally effective radiation doses which are needed to produce an identical radiation response. In this case, the equally effective radiation doses were the LD50's for either gastrointestinal or hematopoietic death. The DMF's for lethality were calculated at the LD50 value

FIGURE 1

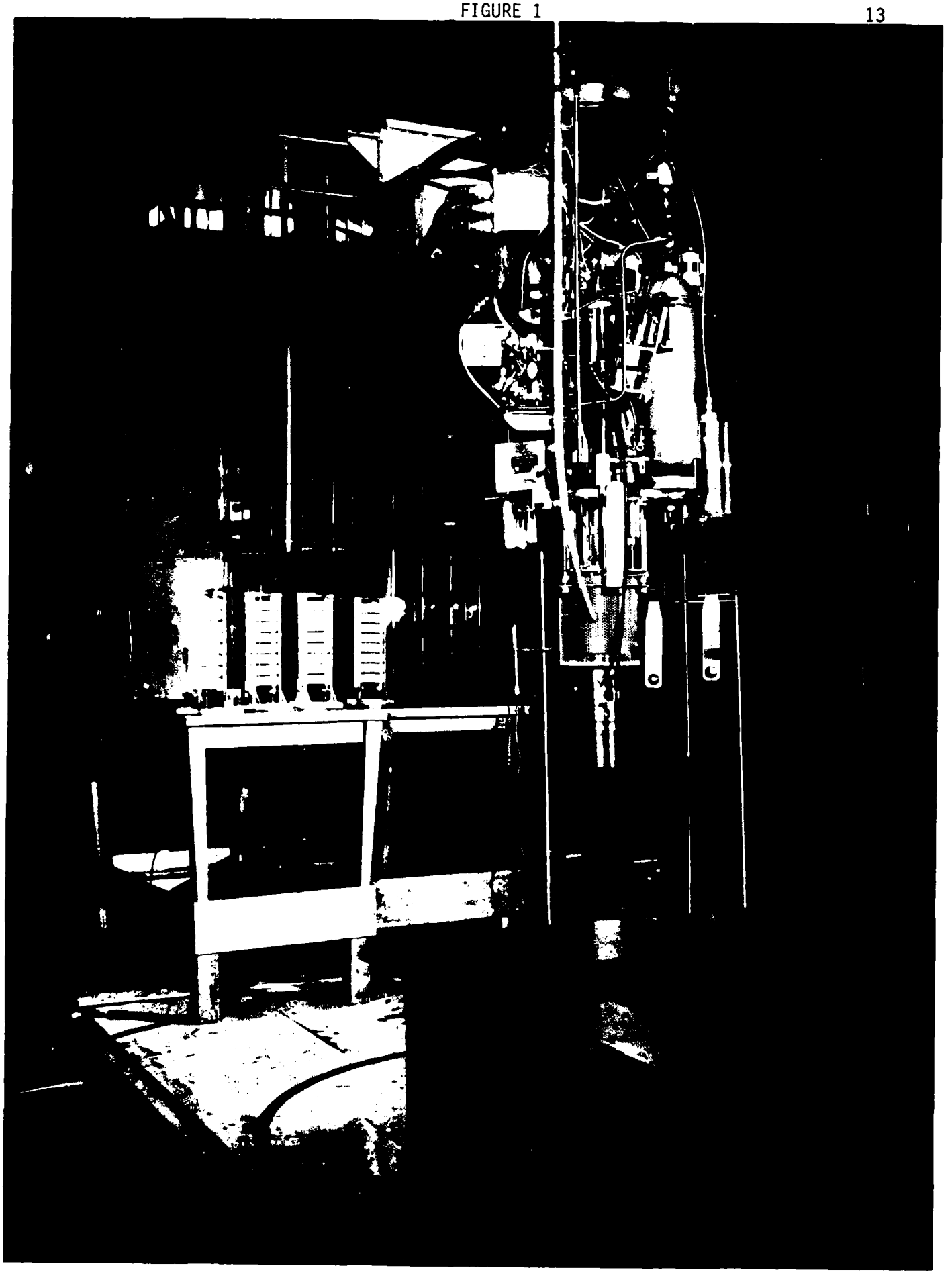


FIGURE 1

DOSAR Neutron Radiation Facility and Procedure

The Health Physics Research Reactor, Oak Ridge National Laboratory, Oak Ridge, TN. This figure depicts the irradiation procedure for all experiments involving high LET radiations. The mice were placed in individual tubes located two meters from the unshielded reactor core. The reactor was operated at 2 kW for steady-state operation. Limited experiments were performed with the reactor operated in a pulse mode.

tubes. It was determined that these nylon tubes attenuated the neutrons by a factor of 0.972 and appropriate adjustments were made in the dosimetry. Beginning May 11, 1982, the mice were irradiated in 1.6 mm thick butyrate tubes and holders of our own design. These tubes and holders were substantially easier to handle and in addition provided a less stressful irradiation environment for the mice. Dosimetry measurements made with the new tubes indicated that there is no measurable attenuation due to their use. The tubes were placed in lucite racks (for the nylon tubes) or red oak racks (for the butyrate tubes) two meters or more from the unshielded core. Figure 1 shows the irradiation setup with nylon tubes in racks on tables at various distances from the reactor. Power level was set at 2 kW with a dose rate of about 30-40 rads/min. Gamma contamination usually amounted to about 15% of the dose for the irradiation protocols used in these experiments. A good discussion of the dose and LET distribution in small animals using this reactor was presented by Willhoit and Jones in 1970 (19). Sigdestad (20) has described the RBE for this radiation procedure.

4. RADIOPROTECTIVE COMPOUNDS

The radioprotective compounds used throughout this investigation were: WR-347, WR-2529, WR-2721, WR-3689, WR-44923, WR-109342, WR-151327, WR-168643, and WR-176542. Pertinent information concerning these drugs is given in Table 1. All drugs were either water-soluble or formed fine suspensions in water after vortex mixing which were suitable for intraperitoneal (i.p.) or per os (p.o.) adminstration. The homogeneity of the solutions or suspensions was demonstrated by the linear results of the drug toxicity experiments. Drugs were administered 30 minutes prior to gamma radiation exposure following an i.p. injection and 30-45 minutes prior to neutron irradiation after i.p. administration. For oral administration, the drugs were given 45 minutes prior to low LET radiation or neutron radiation although the time of drug administration to irradiation was more variable in the neutron radiation due to reactor operation constraints. There were delays in the time from drug administration to reactor-on time due to the fact that the mice were prepared in the reactor control building, transported to the reactor site, and irradiated following the standard muster call, badge check, and reactor start-up procedure.

facility's environmental conditions. They were kept either on wood-chip bedding in plastic mouse boxes (28 x 17 x 12.5 cm) with stainless steel wire tops (five animals per box) or in hanging drawer cages (30 x 30 x 30 cm). The mice were maintained on standard mouse chow (Purina) and HCl acid water (15 ppm) ad libitum. The acid water served to control intestinal flora, the growth of which could serve as a source of error in gastro-intestinal lethality experiments. This was a change from the previous use of chlorinated water, which controlled bacterial growth to the same extent but had to be replaced more often (approximately every two days versus every three or four days for the acid water) due to the sublimation of the chlorine. The animal room was maintained at 22°C and with a 12/12 hour light-dark cycle (lights on at 0600 hours Eastern Standard Time and lights off at 1800 hours EST).

2. LOW LET RADIATION

In all low LET irradiation procedures performed until September 1, 1981, mice were exposed to whole-body gamma radiation with a Cobalt-60 teletherapy unit (Picker C-10000). A 20 x 20 centimemter field was used with a source-to-subject distance (SSD) of 95 centimeters. The dose rate varied slightly from experiment to experiment, due to radioacitve decay, but was generally in the 95-100 rads/minute range. An average exposure rate was determined with a thimble chamber and a Victoreen condenser R-meter. A plexiglass container was used to hold the ten mice which were used at each dose level. After September 1, 1981, due to the change of the project locale from the Radiation Center to the James Graham Brown Cancer Center, low LET irradiations were done with an AECL Therac 780 Cobalt Teletherapy Unit. A 35 x 35 centimeter field was used with a SSD of 80 centimeters. This provided a dose rate of approximately 200 rads/minute. Dosimetry was performed as described above.

3. HIGH LET RADIATION

Fission neutron facilities for mouse irradiation were furnished by the Health Physics Research Reactor (DOSAR) at Oak Ridge National Laboratory. The reactor facility has been previously described in some detail (18). The fission spectrum has a peak energy of 0.9 MeV with a mean energy of 1.2 MeV. In all irradiation procedures performed until May 11, 1982, the mice were irradiated in 3.0 mm thick nylon

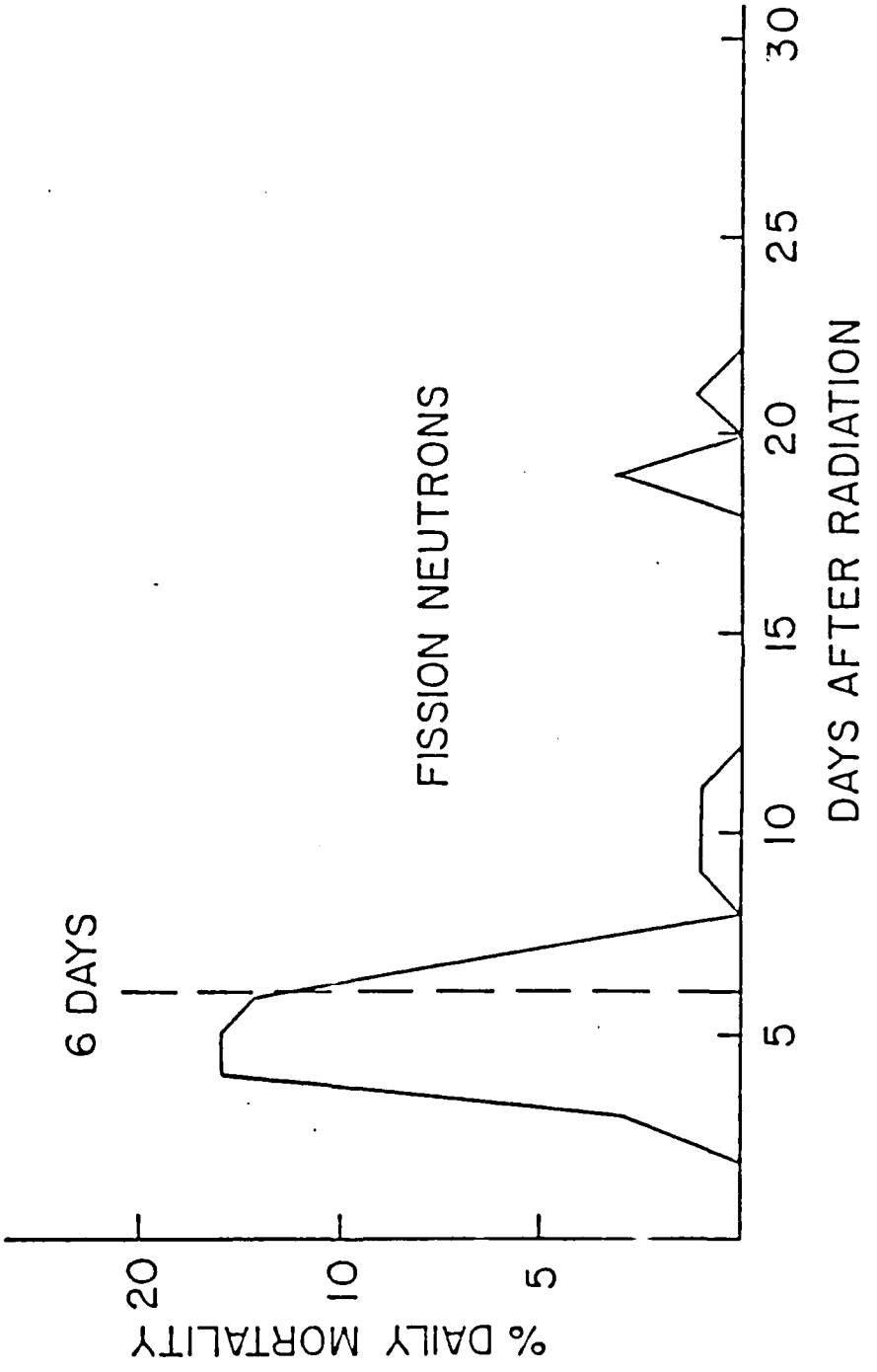

% DAILY MORTALITY
20
10
5
6 DAYS
FISSION NEUTRONS
5
10
15
20
25
30
DAYS AFTER RADIATION

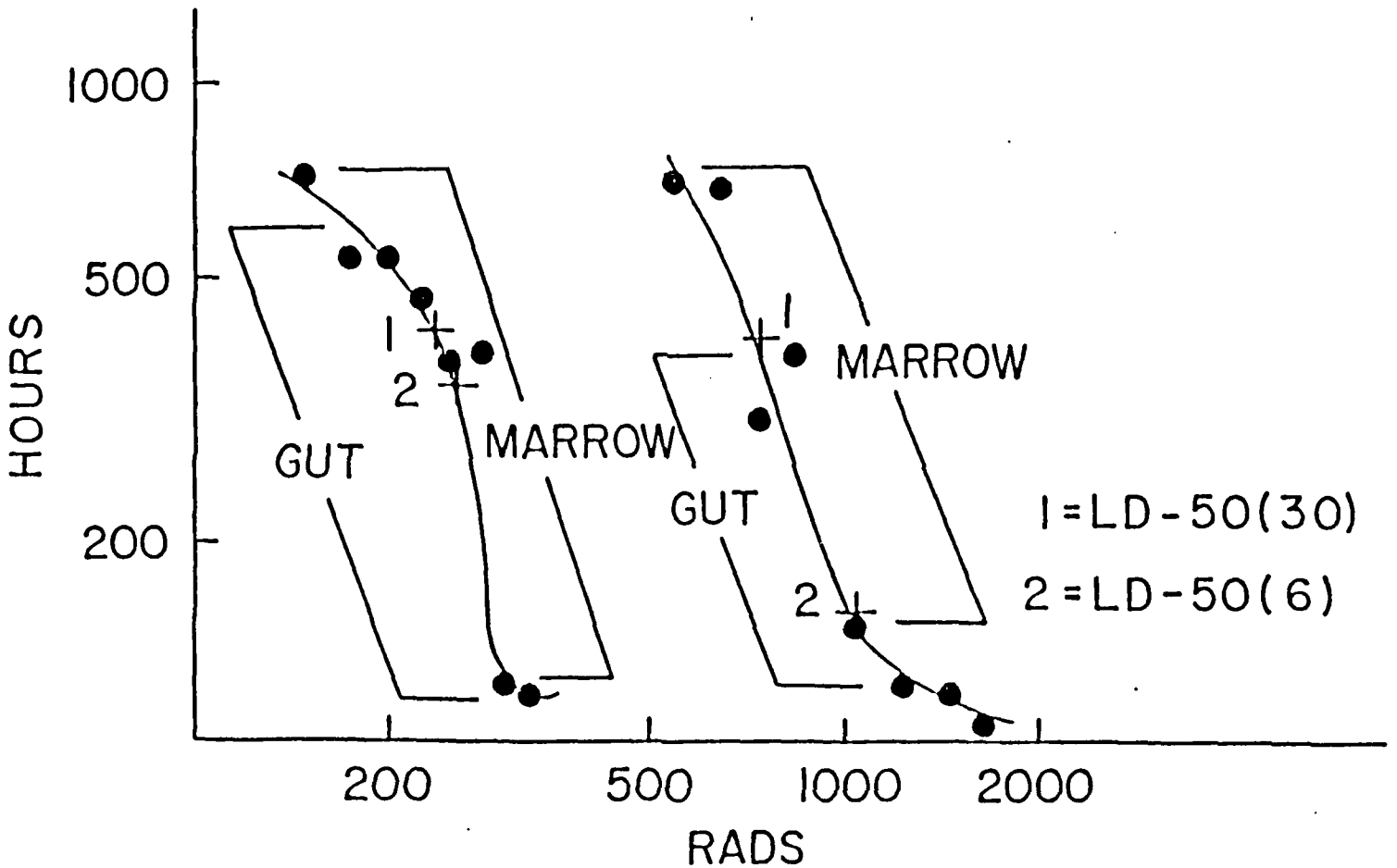
FIGURE 3

FIGURE 4

Survival Time After Low or High LET Radiation

Survival time (hours) of mice irradiated with either Cobalt-60 gamma or fission spectrum neutrons. Points designated as 1 and 2 indicate the LD50(30) and the LD50(6) values obtained for the two radiation modalities. The brackets on each curve indicate the data used in the probit analysis for determining the median lethal dose.

FIGURE 4


1000
500
200
HOURS
GUT
MARROW
1
2
MARROW
GUT
1
2
1=LD-50(30)
2=LD-50(6)
200
500
1000
2000
RADS

especially with fission neutrons. This phenomenon and the proximity of the LD50(6)'s for gastrointestinal and hematopoietic death again illustrate the points discussed above with regard to differences in lethality analysis found in comparisons of the effects of high LET and low LET radiation. This examination of survival time was extended with two experiments performed in the reactor pulse mode. These experiments were intended to elucidate mechanisms of lethality at higher radiation doses in order to determine if radioprotective drugs could be useful over a wide range of radiation doses and against different radiation lethality syndromes. Figure 5 shows the survival time curve when fission neutron doses of up to 5000 rads were used. The striking thing about these results is that even at the highest doses, there is no indication of entry into the central nervous system (CNS) syndrome. Fission neutron radiation showed an RBE on the order of three to four for hematopoietic and gastrointestinal death. Previous studies indicate that the LD50 for CNS death in the mouse is approximately 25,000 rads. Applying an RBE of three to four suggests that the LD50 for CNS lethality with fission neutrons should be in the range of 4000 to 6000 rads. The data shown in Figure 5 clearly indicate that this is not the case and that the RBE for CNS death following fission neutron irradiation is considerably lower than that observed for other radiation syndromes.

Radiation lethality experiments were performed with the following drugs in order to determine the protective effects of the compounds against the lethal response of either the gastrointestinal (six day) or hematopoietic (thirty day) radiation syndrome: WR-1065, WR-2529, WR-2721, WR-3689, WR-44923, WR-109342, WR-151327, and WR-168643. All pertinent results of the radiation lethality experiments may be found in Table 2.

B. WR-1065

WR-1065 is the dephosphorylated metabolite of WR-2721. As such, the deprivation of the phosphate group renders this compound much more toxic than WR-2721. The toxic LD50 for WR-1065 following i.p. injection was determined to be 375 mg/kg in our studies, versus 1108 mg/kg for WR-2721. This small structural change apparently has no implications which affect the radioprotective properties of the drug as related to gastrointestinal radioprotection. The LD50(6) for mice injected with WR-1065 prior to whole-body fission neutron irradiation was 358 rads.

FIGURE 5

Survival Time After Fission Neutron Irradiation

The natural log of survival time in hours plotted as a function of the log of the neutron dose. The low dose portion of the curve represents the survival time in the bone marrow region while the horizontal portion represents the dose independent survival in the gastrointestinal range.

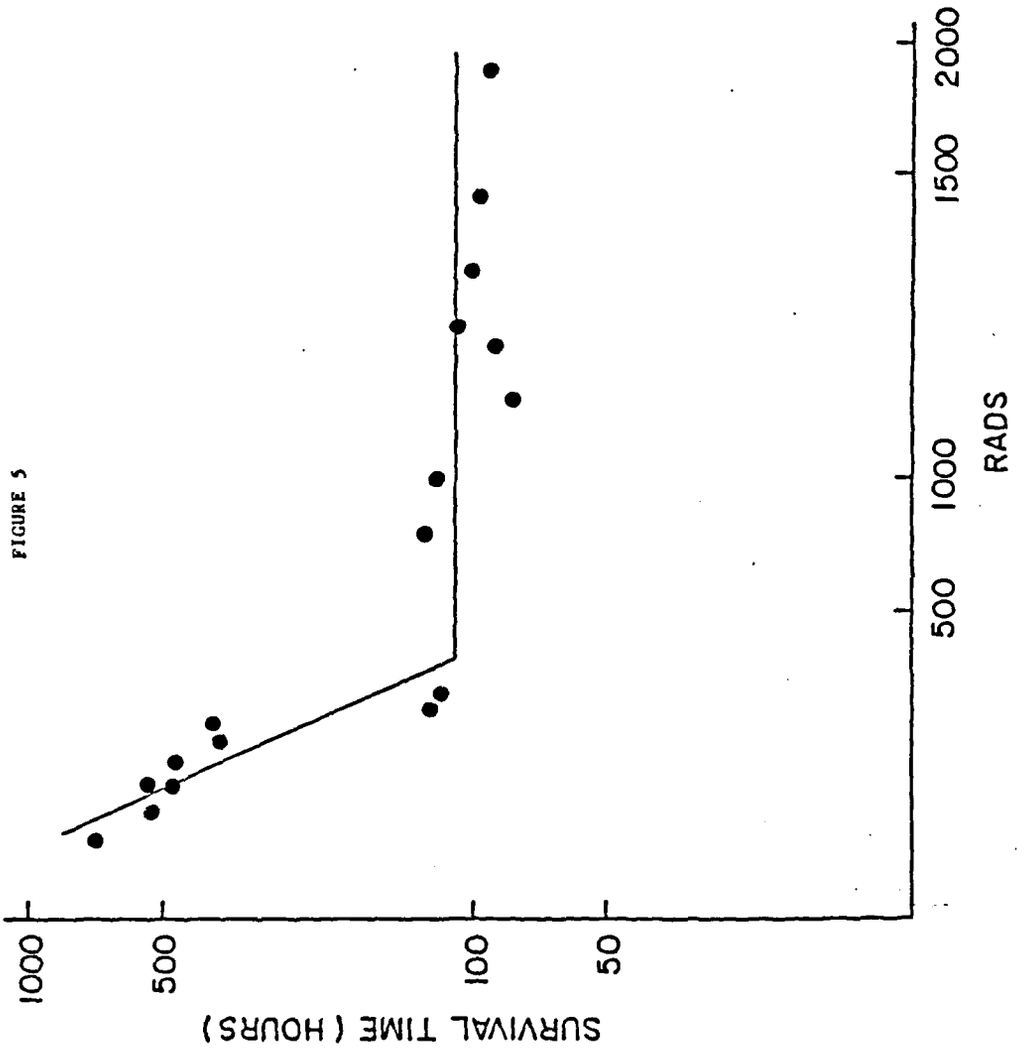


FIGURE 5

Compared to the control value of 252 rads, this gives a dose modification factor (DMF) of 1.42 as contrasted to the DMF of 1.39 for WR-2721 (vide infra) found for protection against gastrointestinal lethality. However, the LD50(6) value found for WR-1065 is subject to some question due to the fact that the experiment designed to determine the LD50(6) and LD50(30) for this drug was flawed due to premature reactor shutdown ("scram") for several of the radiation doses designed to be tested. Therefore, the Probit Analysis performed on the lethality results of WR-1065 contained only the four highest doses, ie., LD50(6) and the three lowest doses; LD50(30). Because of the lack of weighing of very low response data or very high response data in the Probit method of lethality analysis, the inherent error in the LD50 determination is very large in this particular case. WR-1065 did not protect very well against lethality due to the hematopoietic syndrome, the LD50(30) being 250 rads as compared to the previously determined control value of 220 rads, giving a DMF of 1.14. However, the same caveat must apply to the LD50(30) results as applies to the LD50(6) results due to the unexpected reactor "scram".

C. WR-2529

The neutron LD50(6) for WR-2529 following an i.p. injection of 1944 mg/kg (two-thirds of the toxic LD50) was determined to be 370 rads. This value was calculated by Probit Analysis and the 95% confidence limits were determined to be 336 rads to 403 rads. From the studies performed in the previous year of this contract, the LD50(6) for mice receiving no radioprotective compound was shown to be 252 rads with 95% confidence limits of 239 to 266 rads. Therefore, the dose modification factor (DMF) for this compound was 1.47, thus making it one of the better drugs which we have assayed for protective efficacy against the neutron-induced gastrointestinal syndrome. The LD50(30) for mice injected with WR-2529 prior to whole-body fission neutron irradiation was found to be 327 rads (95% confidence limits = 272-381 rads). When this value is compared to the control value for hematopoietic lethality from the earlier study, the DMF is calculated as 1.49, again showing this WR-2529 one of the better compounds which we have found to protect against neutron radiation.

D. WR-2721

This drug is generally considered to be the best radioprotective compound yet discovered. Because of the relatively low protective effects found with WR-2721 in the first year of this study, the gastrointestinal and hematopoietic lethality studies were repeated with a wider range of doses. In all experiments, the i.p. dose of WR-2721 injected was 741 mg/kg, two-thirds of the toxic LD50 of 1108 mg/kg. The results of several experiments resulted in a redefining of the WR-2721 LD50(6) as 351 rads (95% confidence limits = 343 - 358 rads), compared to the previously reported value of 318 rads. The resultant DMF was 1.39, thus substantiating WR-2721 as one of the better radioprotectors. Similar reassessment of results occurred when the LD50(30) for WR-2721 was repeated. The DMF for hematopoietic death was 1.3, as calculated from a LD50(30) of 287 rads (95% confidence limits = 280 - 293 rads), an increase of 20 rads over the aforedetermined value of 267 rads.

E. WR-3689

The toxic LD50 for WR-3689 following per os (p.o.) administration was found to be 1815 mg/kg. Accordingly, two-thirds of this dosage, or 1216 mg/kg, was used in the radiation lethality experiments. The LD50 for gastrointestinal death was 343 rads (95% confidence limits = 246 - 439 rads, DMF = 1.36), a value not significantly different from the LD50(6) reported for i.p. administration of the same drug (337 rads). However, the p.o. LD50 value displays a rather large confidence interval. This is due to the fact that there were unexpected drug toxicities encountered during the fission neutron irradiation experiment with resultant decremental effect on the numerical composition of the radiation dosage groups. The same effect obtained in the LD50(30) results, where the LD50 was computed to be 250 rads (DMF = 1.04) with 95% confidence limits of 180 to 314 rads, which may, however, be statistically flawed.

F. WR-44923

Radiation LD50 values for WR-44923 were previously reported, but due to paucity of data, these experiments were repeated in the present series. Using an i.p. dose of 517 mg/kg, an LD50(6) of 447 rads (95% confidence limits = 422 -472 rads) was

obtained. This value gave a DMF of 1.77, by far one of the better protection factors conferred by any of the drugs being tested in the current protocols. Reconfirmation of this result was being planned at the termination of the study. However, the LD50(3) was found to be 293 rads (95% confidence limits = 247 - 339 rads), which gave a DMF of 1.32. This result is not unexpected however, as there does not seem to be a correlation between gastrointestinal and hematopoietic protective effects conferred by these compounds following fission neutron irradiation. This may be due to the phenomenon discussed above, where the differing radiosensitivities found for the two modes of lethality are not as great for fission neutron radiation as they are for the low LET (gamma) radiation, therefore resulting in either a lowered resistance to gastrointestinal death or a greater resistance to hematopoietic death.

G. WR-109342

WR-109342 is an extremely toxic drug, the i.p. LD50 being 37.1 mg/kg and the p.o. LD50 being 58.0 mg/kg. Attempts to assay the gastrointestinal and hematopoietic protective effects of this drug following i.p. injection were unsuccessful due to toxicity problems which may have been exacerbated by use of the formerly used nylon mouse tubes which allowed little ventilation and therefore, build-up of physiological exhalants. The p.o. assay of WR-109342 following administration of a dose of 38 mg/kg was more successful, resulting in an LD50(6) of 371 rads (95% confidence limits = 323 - 420 rads, DMF = 1.47) and an LD50(30) of 290 rads (95% confidence limits = 264 - 315 rads, DMF = 1.32). It was recommended to the funding agency that this agent be removed from the screening procedure due to its toxicity.

H. WR-151327

Toxicity data indicated an i.p. LD50 of 1011 mg/kg, thus making WR-151327 one of the less toxic of the compounds tested in this series. Two-thirds of the LD50 or 677 mg/kg was the dosage used in all radiation evaluations of WR-151327. Fission neutron radiation lethality experiments were performed in order to determine the radioprotective efficacy of this drug. The results for protection against the gastrointestinal radiation syndrome resulted in a LD50(6) of 562 rads with a 95% confidence limits of 554-571 rads. These results indicate a DMF of 2.23 which is extraordinarily high. Therefore, it is

recommended that data on WR-151327 be followed up and expanded. A LD50(30) for WR-151327 was obtained and determined to be 321 rads (95% confidence limits = 300 - 343 rads). This provides a DMF of 1.46 for protection against hematopoietic death, thus again showing no correlation between protection against gastrointestinal death and hematopoietic death. The unexpected high DMF using this agent stimulated repeated experiments all of which gave similar results. Studies, in progress with other neutron facilities have confirmed that this compound should be high on the list for indepth studies.

I. WR-168643

The toxic LD50 for this compound following i.p. injection was 1272 mg/kg and following p.o. administration was 1142 mg/kg. Therefore, the respective dosages used for i.p. and p.o. administration were 852 mg/kg and 765 mg/kg. The i.p. LD50(6) for WR-168643 was 310 rads (95% confidence limits = 274 - 346 rads, DMF = 1.23). The LD50(30) for whole-body irradiation following i.p. injection of the drug was 300 rads with 95% confidence limits showing no significance from the control LD50(30) of 240 rads. Protection by WR-168643 against gut death following oral administration was limited, showing a LD50(6) of 330 rads (95% confidence limits = 303 - 374 rads) and a DMF of 1.31. The p.o. LD50(30) was 332 rads (DMF = 1.51). The radioprotective effect demonstrated by WR-168643 was remarkable inasmuch as the oral administration was found to improve the protective response to fission neutron radiation.

J. WR-176542

This radioprotector was delivered to this contractor in such small quantities that only limited experiments could be performed. Its toxicity was tested and found to have a LD50 of 649 mg/kg after i.p. injection. Its 95% confidence limits were 629 to 671 mg/kg. Initial experiments were performed to test the compounds ability to protect against fission neutrons; however, reportable data was not obtained prior to depletion of the existing inventory of the drug.

2. INTESTINAL MICROCOLONY SURVIVAL EXPERIMENTS

The following drugs were assayed for their protective efficacy in the intestine utilizing microcolony survival: WR-347, WR-1065, WR-2529, WR-2721, WR-3689, WR-44923, WR-151327 and WR-168643. All of the drugs tested showed some protection as seen by this method, protection which was commensurate with that seen in the lethality assays. Table 3 presents the salient data for the protective compounds tested.

A. Control

Animals were irradiated without the benefit of drug protection in separate experiments using both Cobalt-60 gamma rays and fission neutrons. The resulting survival curves are shown in Figure 6 and the parameters describing these curves may be found in Table 3. The differences between the low LET gamma ray survival curve and the high LET fission neutron survival curve were as expected. When plotted on the same axis, the respective curves demonstrate the usual response of neutrons vis a vis gamma rays. Compared to the gamma ray response curve, the neutron response curve is shifted to the left (D_q for neutrons = 205 rads, D_q for gamma rays = 907 rads) and displays a steeper slope ($m = -0.0198$ vs. $m = -0.0093$). The resultant Do's were for neutrons 50.5 rads and for gamma rays 108 rads. When the RBE is calculated as the ratio of Do's a value of 2.14 is recorded, which is substantially lower than the RBE for gastrointestinal death calculated as the ratio of LD50(6)'s (RBE = 4.15). However, when the RBE is calculated as the ratio of radiation doses at a known level of survival, viz., the dose needed to reduce the number of surviving cells per circumference to 10, the RBE is calculated as 3.28, nearer the value obtained from the radiation lethality experiments. It should be noted that any discrepancy in the RBE's calculated from cell survival curves and lethality experiments likely arises from the fact that in the whole-body radiation used to induce the gastrointestinal lethality response, other factors than strictly intestinal cell death are probably components of the entire syndrome of symptoms and reactions which contribute to the ultimate gastrointestinal lethality. The measured parameter of intestinal cell survival as derived from microcolony formation is only one part of this entire complex of systemic and local physiological and histological responses.

TABLE 3

INTESTINAL STEM CELL SURVIVIAL

DRUG	i.p. DOSE (mg/kg)	SLOPE	CORRELATION COEFFICIENT	D_0 (RADS)	D (RADS)	D-10 (RADS)	DMF
None	Cobalt-60	-0.0093	-0.9916	108	907	1359	
None	Neutrons	-0.0198	-0.9824	50.5	205	417	
WR-347	220	-0.0194	-0.9282	51.5	221	436	1.05
WR-1065	251	-0.0166	-0.9494	60.2	236	426	1.02
WR-2529	1944	-0.0198	-0.9410	50.5	251	451	1.08
WR-2721	741	-0.0226	-0.9826	44.4	297	481	1.15
WR-3689	970	-0.0177	-0.9664	56.6	280	517	1.24
WR-44923	517	-0.0200	-0.9466	49.9	266	475	1.14
WR-151327	677	-0.0189	-0.9533	52.9	305	476	1.15
WR-168643	852	-0.0258	-0.9005	38.7	247	422	1.01

FIGURE 6

Intestinal Stem Cell Survival: Unprotected

A plot of intestinal stem cell survival (cell/circumference) as a function of absorbed dose of either Cobalt-60 or fission neutrons. This curve is designated control because the animals were not pre-treated with any of the anti-radiation compounds involved in this study. From this curve a RBE of 2.14 was obtained from a ratio of the survival curve slopes.

FIGURE 12

Intestinal Stem Cell Survival: Neutrons - WR-44923

A plot of intestinal stem cell survival (cells/curcumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-44923 (517 mg/kg). From this curve a DMF of 1.14 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 11

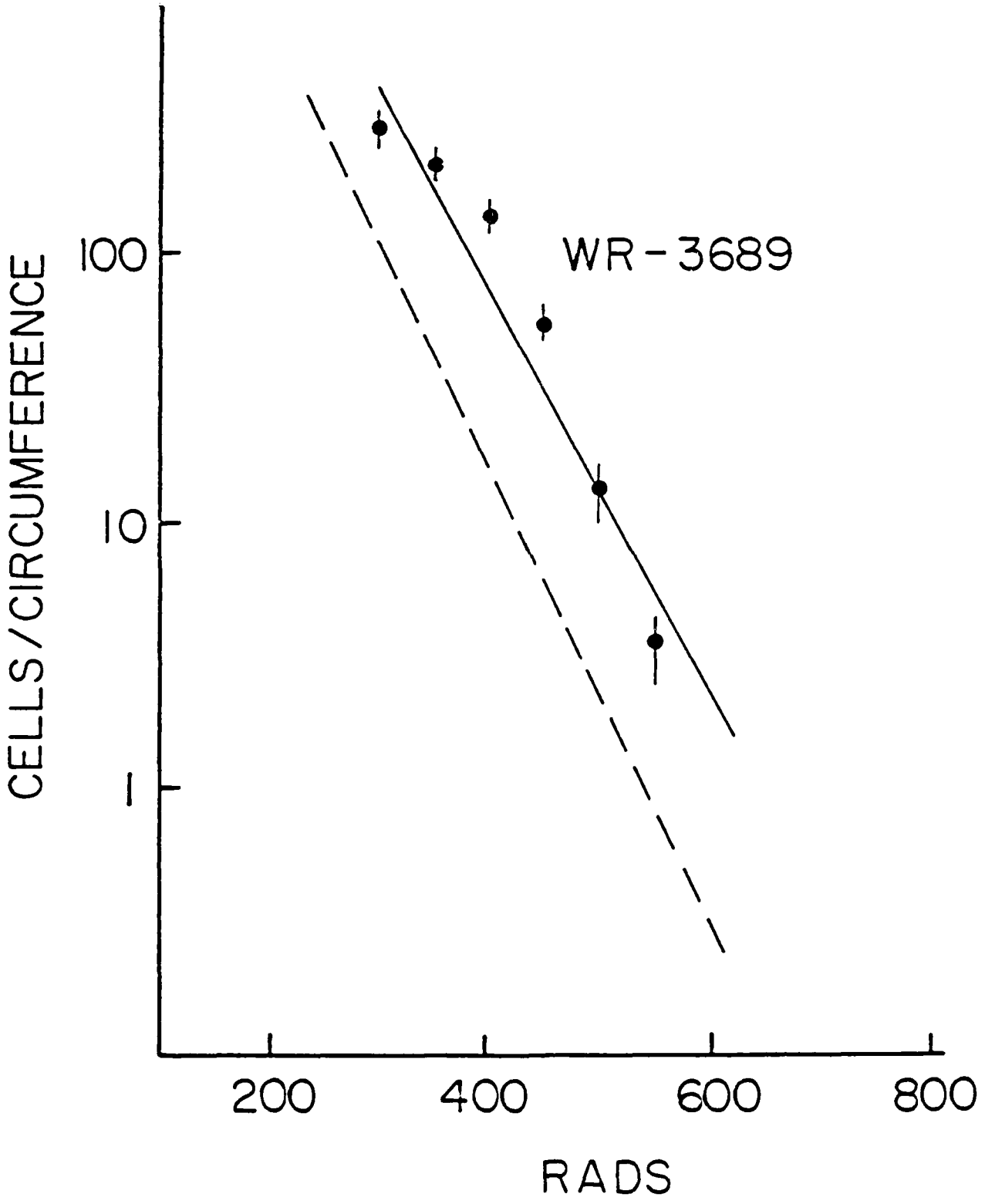


FIGURE 11

Intestinal Stem Cell Survival: Neutrons - WR-3689

A plot of intestinal stem cell survival (cells/curcumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-3689. From this curve a DMF of 1.24 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

F. WR-3689

This compound exhibited moderate protection against gastrointestinal radiation damage, showing a DMF of 1.24 as determined by the ratio of the D-10's. The slope was -0.0177 (non-significantly different from controls), the D_o was 56.6 rads, and the quasi-threshold dose (D_q) was 280 rads. The correlation coefficient (r) was -0.9664, indicating a significant degree of correlation between surviving cells per circumference and neutron dose. The D-10 used to calculate the dose modification factor was 517 rads.

G. WR-44923

WR-44923 demonstrated a degree of protection in this assay system which was unremarkable in its difference from the other compounds tested in the current series. The DMF was 1.14 with a D-10 of 475 rads. The slope was -0.0200, not different from controls, the D_o was 49.9 rads, the correlation coefficient was -0.9466, and the D_q was 266 rads.

H. WR-151327

Using the rad difference at the D-10 level, the DMF for WR-151327 as determined by the microcolony assay system was 1.15, as good as WR-2721. The slope (m = -0.0189) was not significantly different from the control slope (m = -0.0198) and demonstrated significant linearity (r = -0.9533). The D_o was 52.9 rads, and the D-10 was 476 rads. It was of interest to note that the very high DMF for lethality using this agent was not reflected in the stem cell response. This may indicate that there are other targets of neutron damage which are better protected by this compound than is the intestinal stem cells.

I. WR-168643

This drug exhibited a DMF of 1.19. The correlation between decremental survival and dose was -0.970, the slope of the survival curve was -0.028, the D_o was 35.7 rads and the D-10 was 311 rads.

FIGURE 10

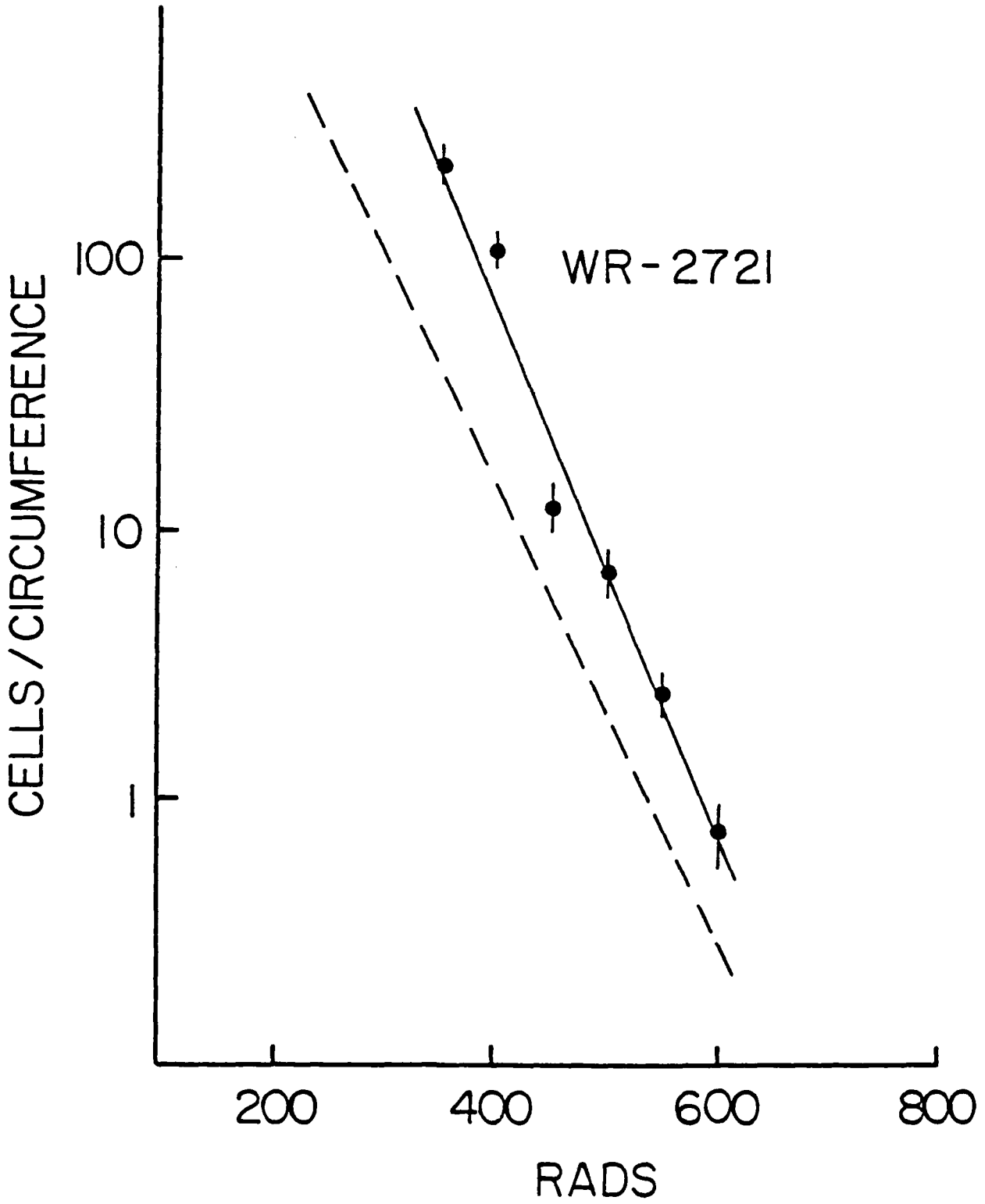


FIGURE 10

Intestinal Stem Cell Survival: Neutrons - WR-2721

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-2721. From this curve a DMF of 1.15 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 9

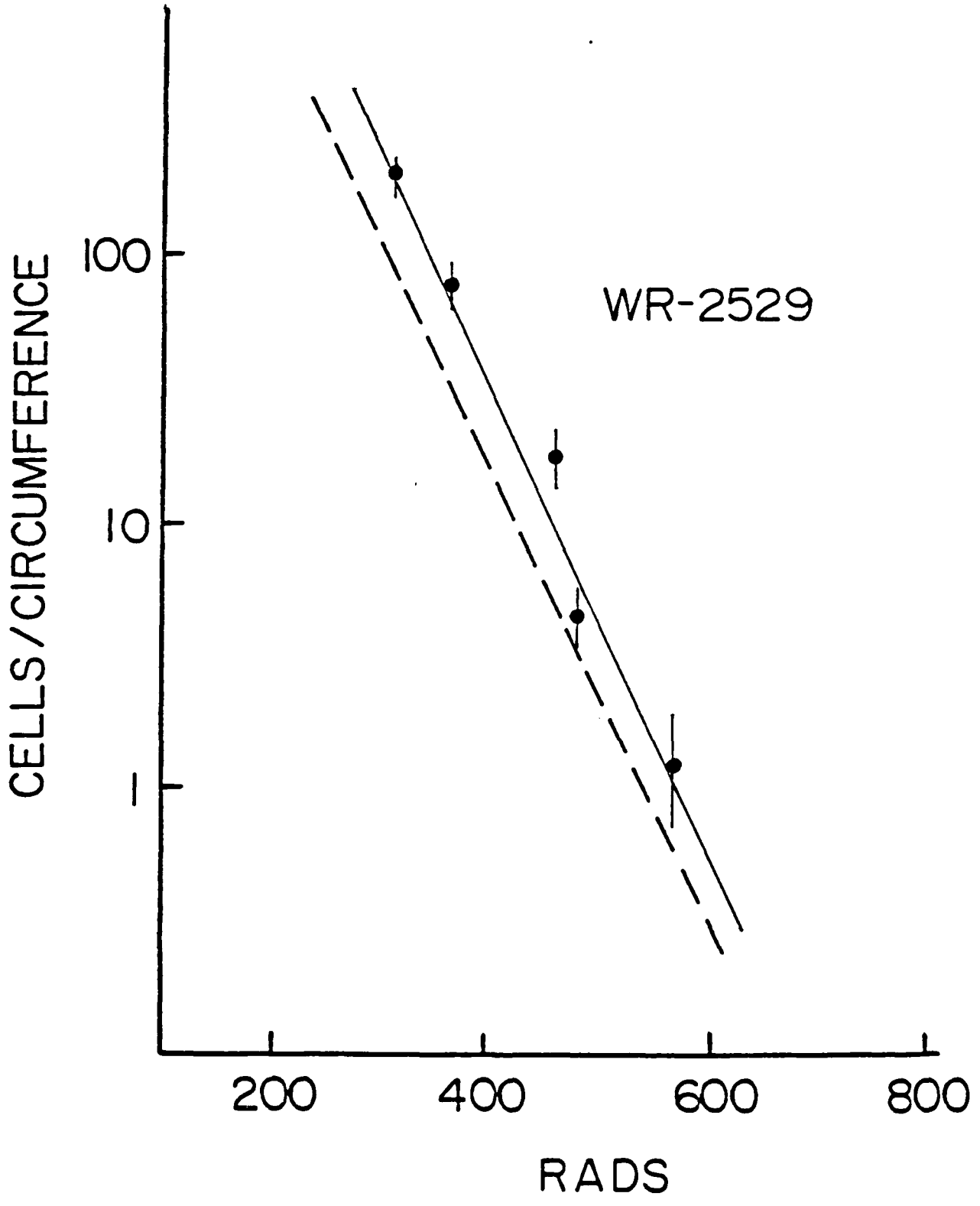


FIGURE 9

Intestinal Stem Cell Survival: Neutrons - WR-2529

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-2529. From this curve a DMF of 1.08 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 8

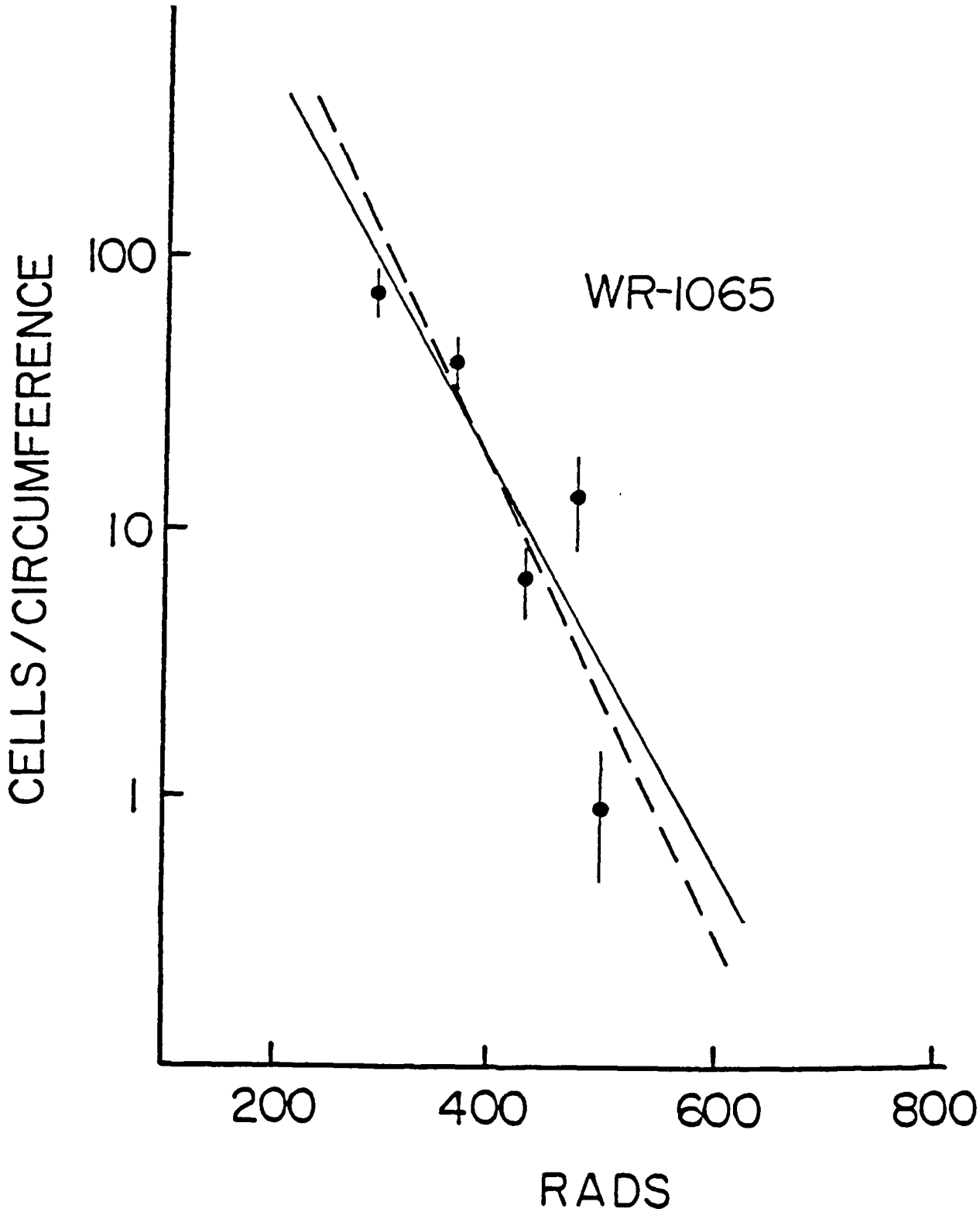


FIGURE 8

Intestinal Stem Cell Survival: Neutrons - WR-1065

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-1065. From this curve a DMF of 1.02 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 7

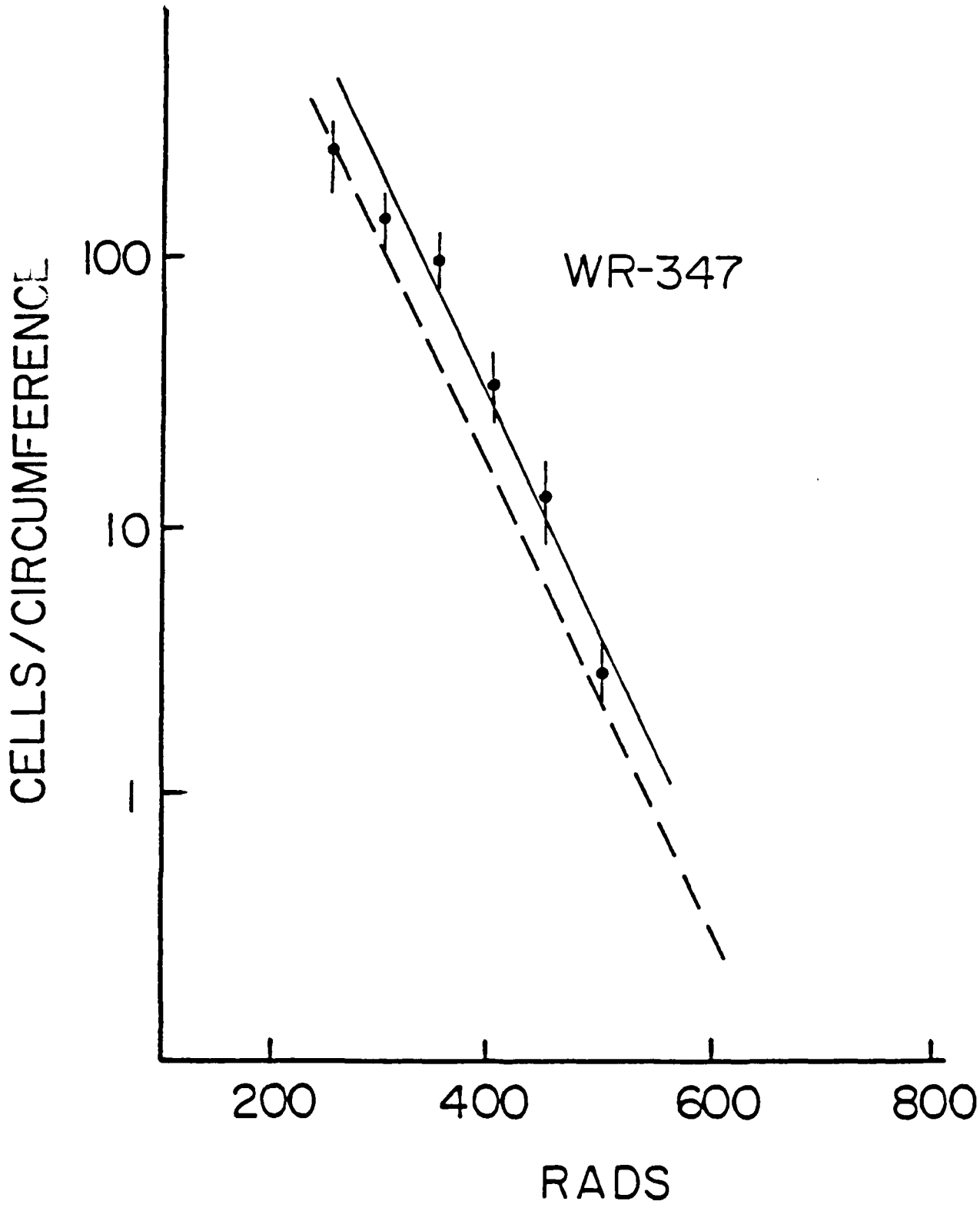


FIGURE 7

Intestinal Stem Cell Survival: Neutrons - WR-347

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-347. From this curve a DMF of 1.05 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

B. WR-347

This compound showed the least radioprotection in this particular cellular assay system than any of the drugs tested in this series. The ratio of the radiation doses needed to reduce the number of surviving cells per circumference to 10 (treated:untreated, hereafter termed the D-10) was 1.05, significantly lower than the DMF of 1.39 obtained through lethality assays, although this difference may be accounted for by the reasons stated above. The slope for the WR-347 microcolony survival curve ($m = -0.0194$) is not significantly different from that obtained from the untreated control animals, thereby exhibiting parallelism, indicating a single mechanism of protection. For WR-347, the correlation coefficient (r) was -0.9282, the D_o was 51.5 rads, the D_q (quasi-threshold dose) was 221 rads, and the D-10 was 436 rads.

C. WR-1065

Using the rad difference at the D-10 level, the DMF for WR-1065 as determined by the microcolony assay system was 1.03. The slope ($m = -0.0166$) was not significantly different from the control slope ($m = -0.0198$) and demonstrated significant linearity ($r = -0.9494$). The D_o was 60.2 rads, and the D-10 was 426 rads.

D. WR-2529

Using the rad difference at the D-10 level, the DMF for WR-2529 as determined by the microcolony assay system was 1.09. The slope ($m = -0.0198$) was identical to the control slope ($m = -0.0198$) and demonstrated significant linearity ($r = -0.9410$). The D_o was 50.5 rads, and the D-10 was 451 rads.

E. WR-2721

Using the rad difference at the D-10 level, the DMF for WR-2721 as determined by the microcolony assay system was 1.15, again not as great as the DMF determined by lethality (DMF=1.39), but within the bounds of error as imposed by the respective assay systems and discussed above. The slope ($m = -0.0226$) was not significantly different from the control slope ($m = -0.0198$) and demonstrated significant linearity ($r = -0.9826$). The Do was 44.4 rads, the D_q was 297 rads, and the D-10 was 481 rads.

FIGURE 6

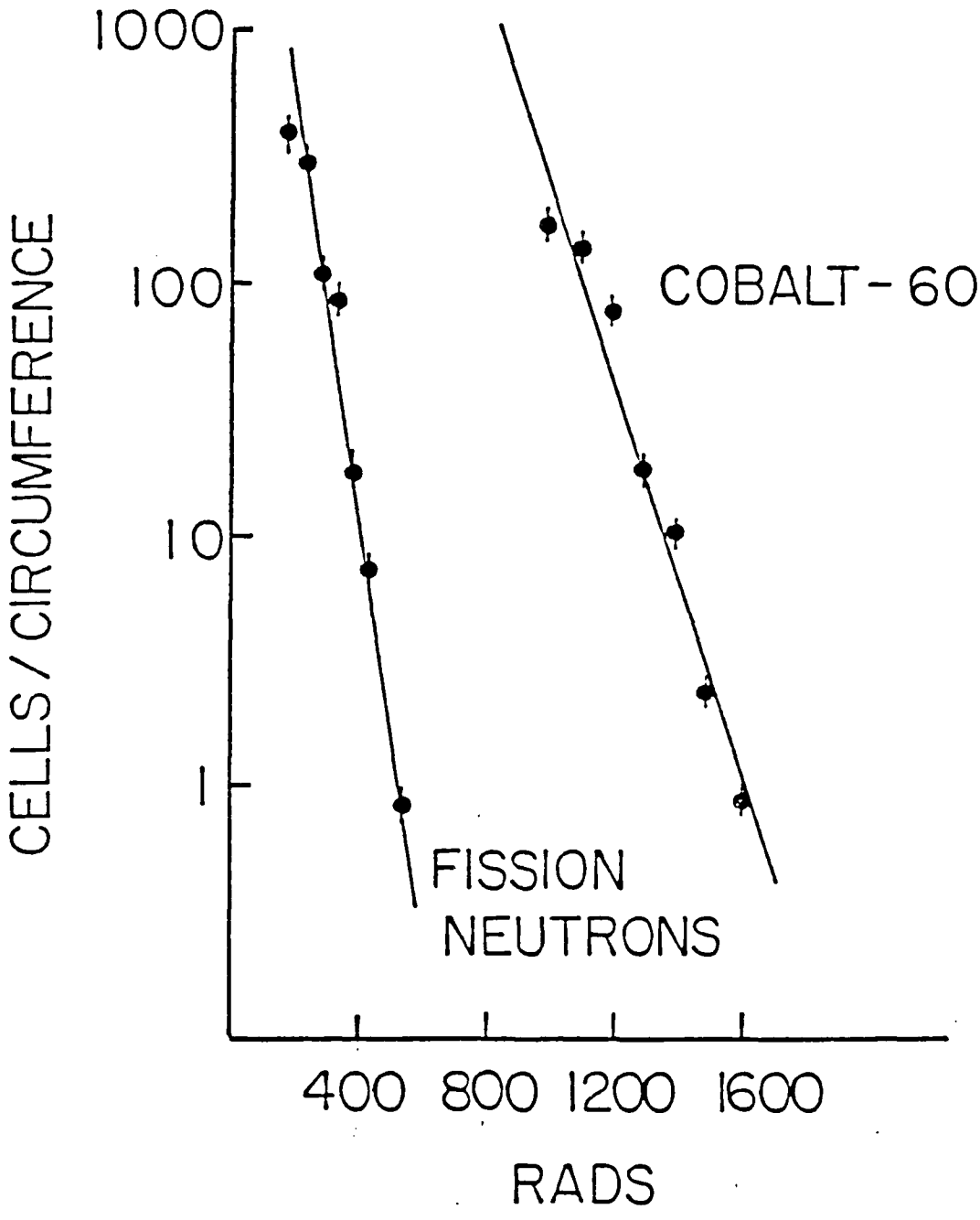

1000
100
10
1
CELLS / CIRCUMFERENCE
COBALT-60
FISSION
NEUTRONS
400
800
1200
1600
RADS

FIGURE 12

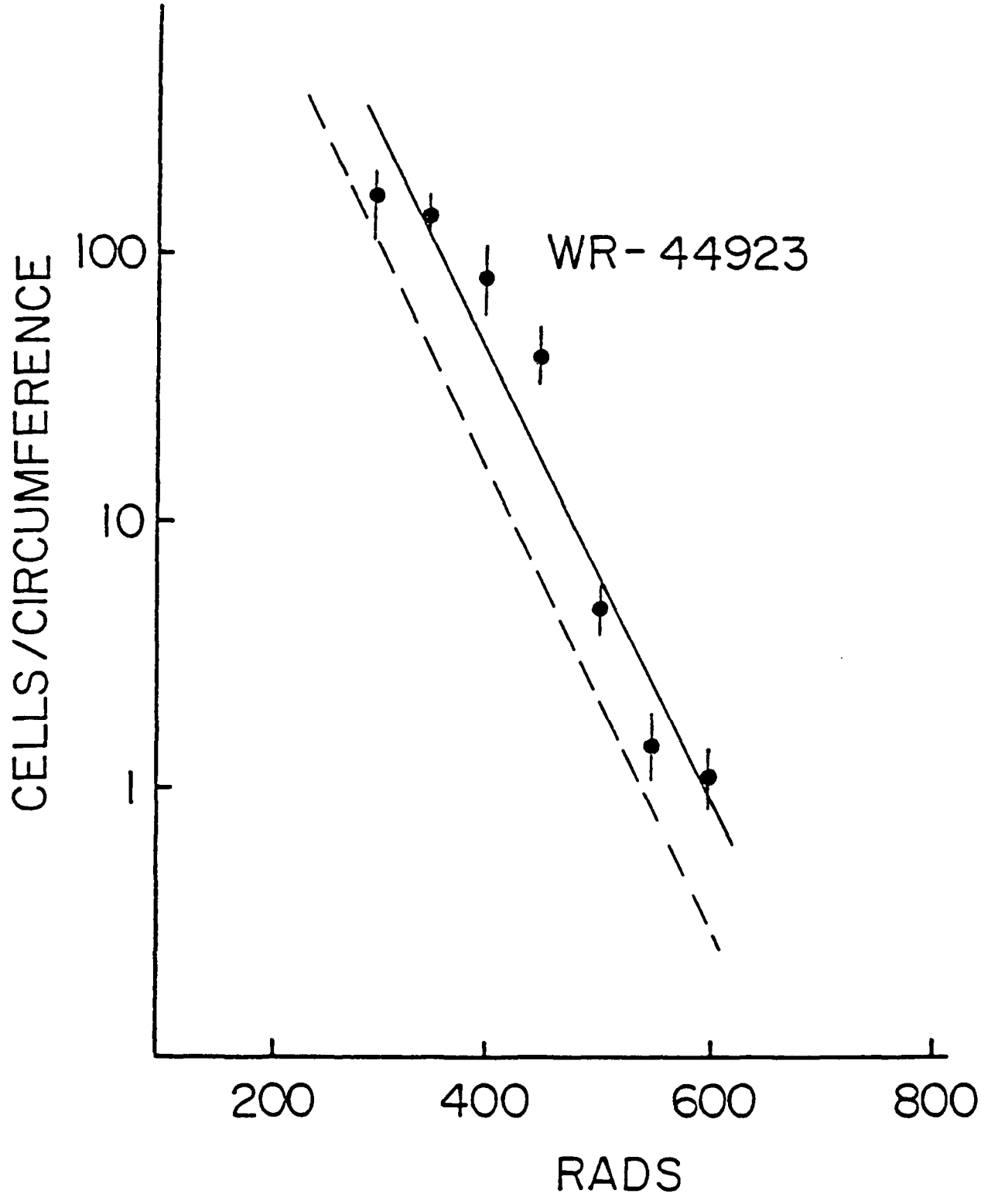


FIGURE 13

Intestinal Stem Cell Survival: Neutrons - WR-151327

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-151327 (677 mg/kg). From this curve a DMF of 1.15 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 13

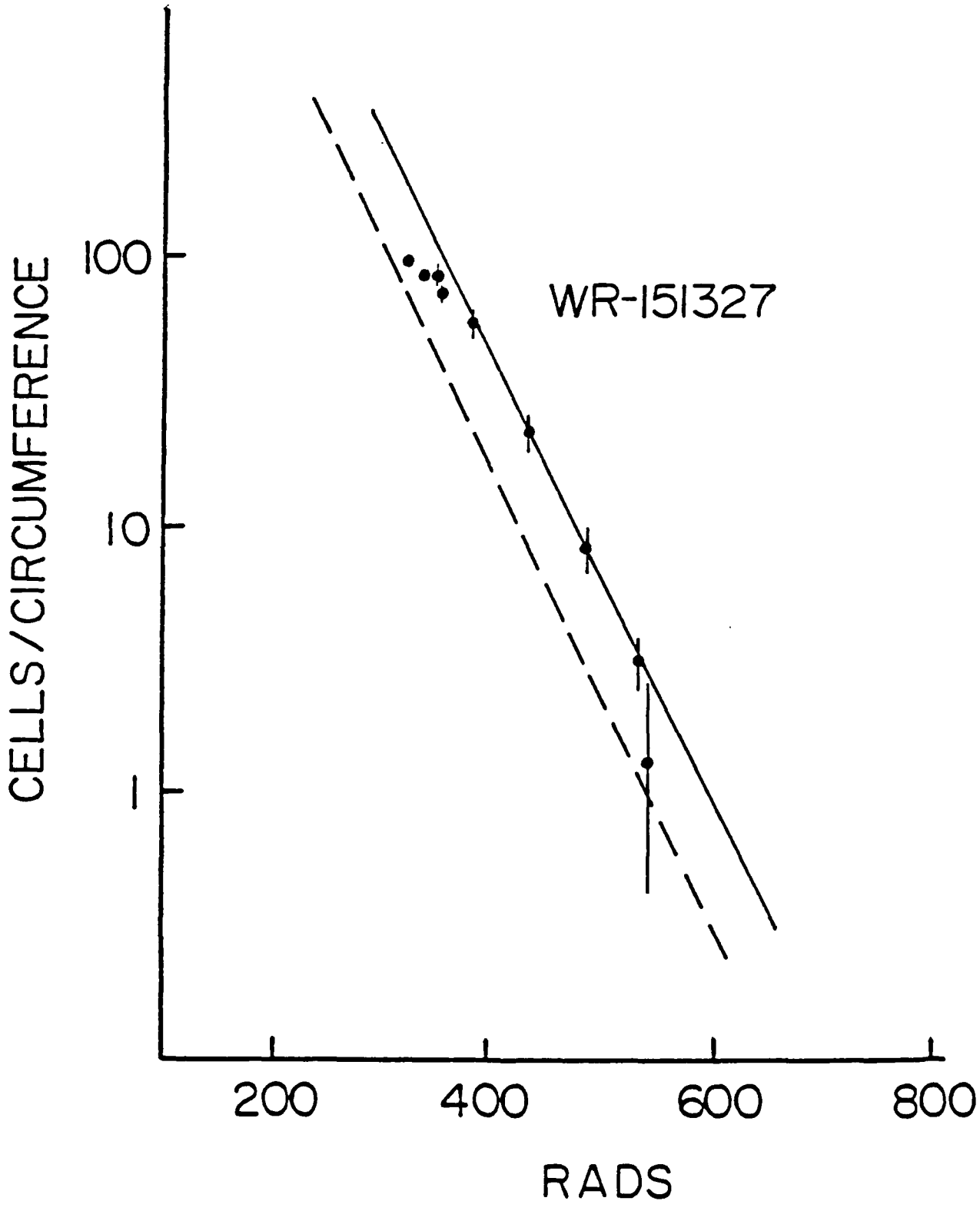
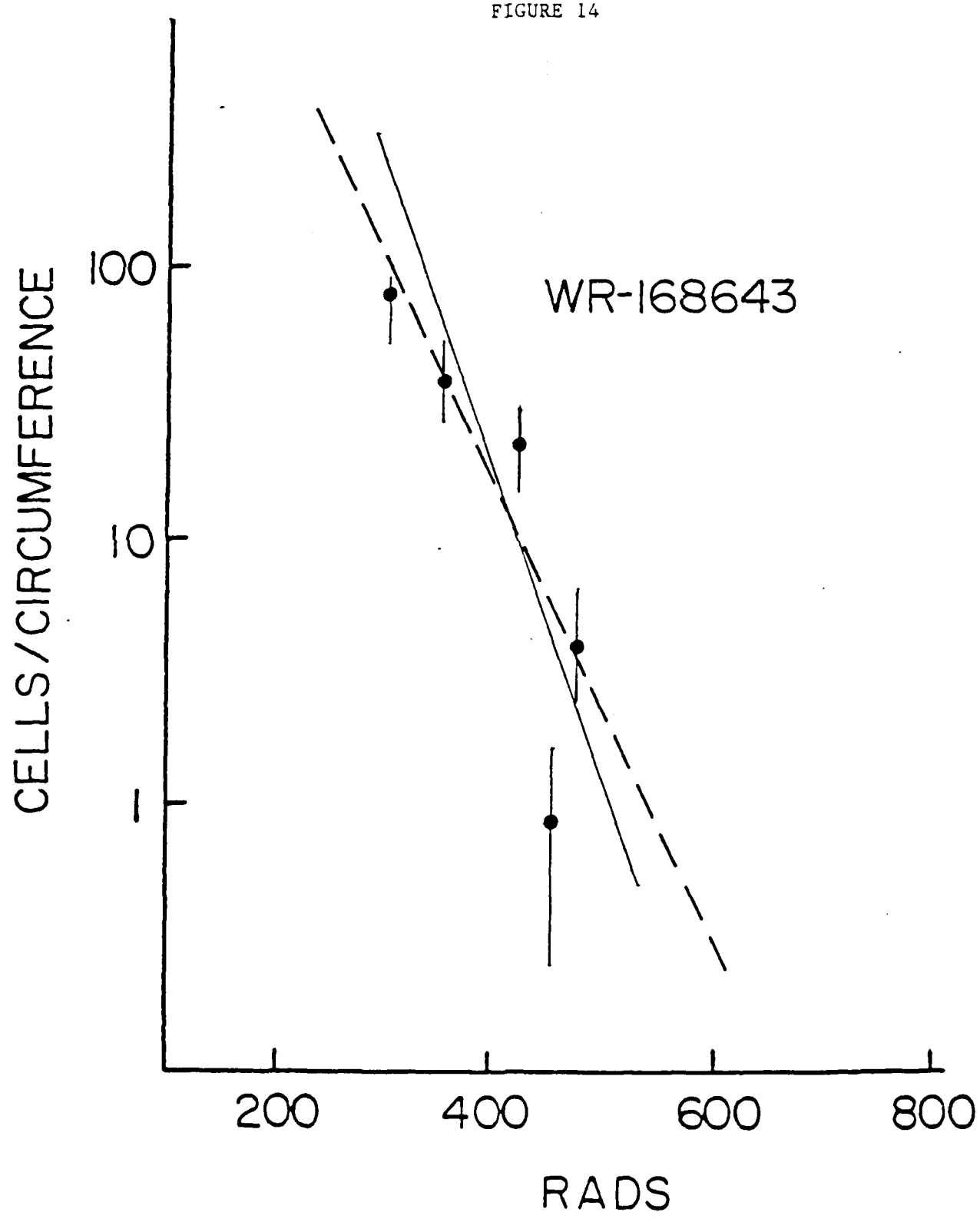


FIGURE 14

Intestinal Stem Cell Survival: Neutrons - WR-168643

A plot of intestinal stem cell survival (cells/circumference) as a function of absorbed dose of fission neutrons. This curve graphically shows the protective ability of the compound designated WR-168643 (852 mg/kg). From this curve a DMF of 1.01 was obtained from a ratio of the D-10 dose (dose sufficient to reduce the stem cell population to 10 cells/circumference) in the protected and control groups.

FIGURE 14



J. Split-Dose Survival Experiments

1) Low LET Radiation

Following a priming dose of 600 rads of Cobalt-60 gamma rays, a second dose of 600 rads 2 hours later showed a demonstrable increase in crypt survival toward that elicited with a single comparable dose. An initial 600+600 (1200 rad total dose at zero time interval between doses) rad dose decreased the number of microcolonies to 57 as compared to the 600 rad single dose value of 112. Two hours separation of doses increased this value to 95 microcolonies. At 4 hours separation between doses, microcolony survival had attained single dose values and it remained at this level at the largest separation used between doses, 24 hours. The data is presented in Figure 15.

2) High LET Radiation

A single dose of 300 rads (0 hours separation) of fission neutrons provides a survival level of 73 microcolonies per circumference, a number not significantly different from that seen with a single Cobalt-60 dose of 1200 rads. However, when a fission neutron dose of 150 rads is followed by other neutron doses at increasing intervals of time, no recovery toward 150 rad single dose levels is observed. Instead, there is a decrease in survival, as denoted by counts of microcolonies/circumference, until a nadir of survival is reached at 3 to 6 hours dose separation. After this time, an increase in crypt survival is noted, although at 24 hours separation between doses, single dose levels still have not been attained. This apparent sensitization of repair capabilities to fission neutron radiation effects was in contrast to the repair abilities of the intestinal epithelium seen with the low LET Cobalt-60 radiation.

FIGURE 15

Sublethal Damage Repair

This graph shows the results of experiments designed to test the ability of the gastrointestinal track to repair sublethal damage after either Cobalt-60 gamma or fission neutron irradiation. surviving crypts is plotted against time separating the split-dose. Data presented as squares is from gamma experiments while the closed circles represents neutron radiation. The zero time data describes the experimental results when the two doses were given simultaneously.

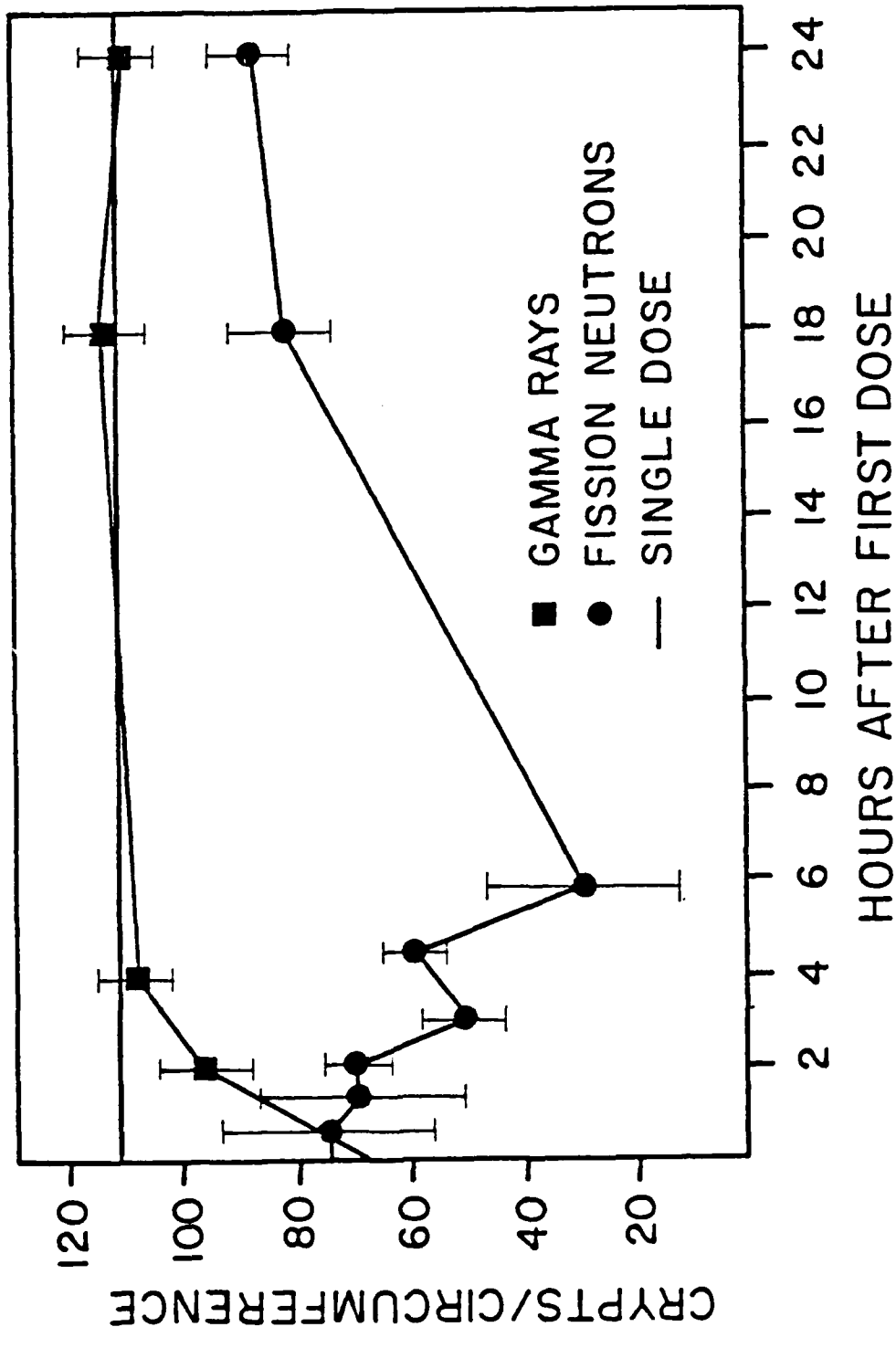

CRYPTS/CIRCUMFERENCE
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
20
22
24
HOURS AFTER FIRST DOSE
GAMMA RAYS
FISSION NEUTRONS
SINGLE DOSE

FIGURE 15

FIGURE 16

Sublethal Damage Repair (Cell Survival)

This graph shows the results of experiments designed to test the ability of the gastrointestinal track to repair sublethal damage after fission neutron irradiation. Surviving crypts is plotted against radiation dose of the second fraction. All mice received 150 rads conditioning dose 6 hours prior the the second exposure. These results indicate the ability of the gut to repair approximately 50 rads of damage in the time separating the two doses.

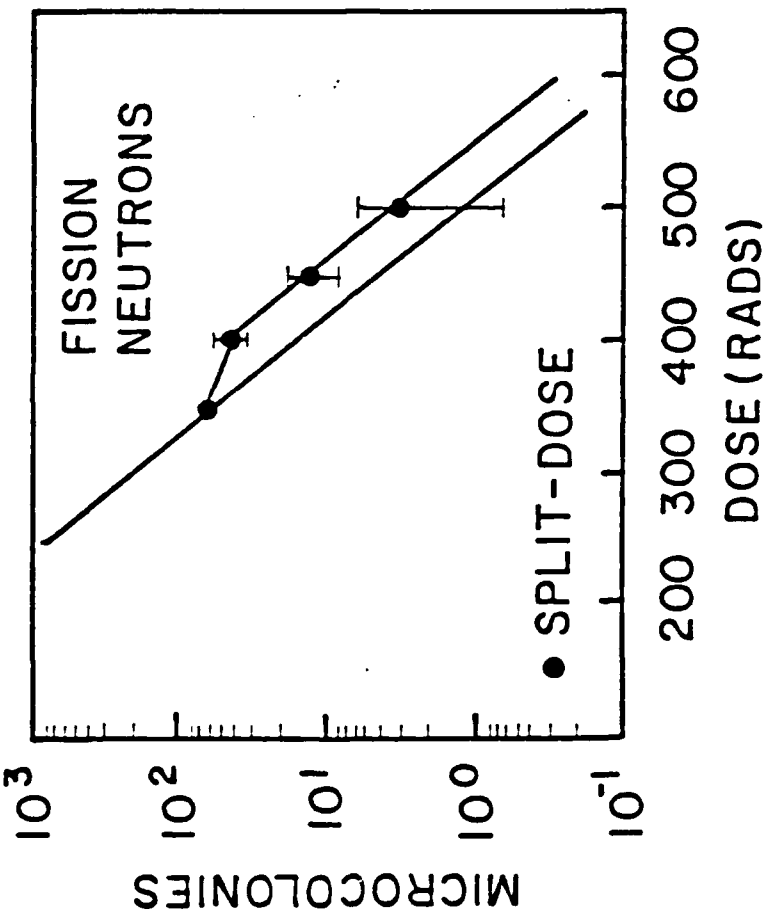

FISSION
NEUTRONS
SPLIT-DOSE
MICROCOLONIES
10^3
10^2
10^1
10^0
10^{-1}
200 300 400 500 600
DOSE (RADS)

FIGURE 16

3. ENDOGENOUS SPLEEN COLONY ASSAYS

Experiments to determine the degree of neutron radiation damage to the blood-forming organs were performed utilizing the endogenous spleen colony assay. These studies were done on untreated (control) animals following either fission neutron or Cobalt-60 gamma radiation and with the following drugs after fission neutron radiation: WR-347, WR-1065, WR-2529, WR-2721, WR-3689, WR-44923, WR-151327 and WR-168643.

A. Control

Untreated mice were irradiated with doses of Cobalt-60 gamma radiation ranging from 450 to 1000 rads. This resulted in a decremental response over three decades of transformed spleen colony counts, from approximately 20 to 0.2 colonies per spleen. There was a high degree of correlation between colonies/spleen and radiation dose, with the correlation coefficient equivalent to -0.9874. The slope of the survival curve was -0.008, and the D_o was 130 rads. The D-1.0 (the radiation dose needed to reduce the colony survival to an average of one per spleen), was 825 rads. Following fission neutron irradiation, the endogenous spleen colony assay used on untreated mice produced a survival curve with the following characteristics: correlation coefficient = -0.9974, slope = -0.029, D_o = 34.8, D-1.0 = 262 rads. As expected, when plotted on the same axis as the low LET survival curve, the neutron curve is found shifted to the left and with a significantly steeper slope. The RBE for fission neutrons, calculated as the ratio of D-1.0(gamma rays):D-1.0(neutrons) was 3.15, nearly the same as the RBE calculated for lethality due to bone marrow death. This similarity in RBE's for the two modes of response (marrow death versus spleen colony survival) indicates that the endogenous spleen colony assay is a good predictor of whole-body radiation lethality in the hematopoietic radiation dose range at the D-1.0 level.

B. WR-347

WR-347, a sulphydryl compound without a covering phosphate, provided essentially no protection using the endogenous spleen colony assay. The dose modification factor (DMF), calculated from the ratio of the D-1.0(treated):D-1.0(untreated) was 0.939. The slope was -0.025, the D_o was 40.1, and the correlation coefficient was -0.869.

TABLE 4

SPLEEN STEM CELL SURVIVIAL

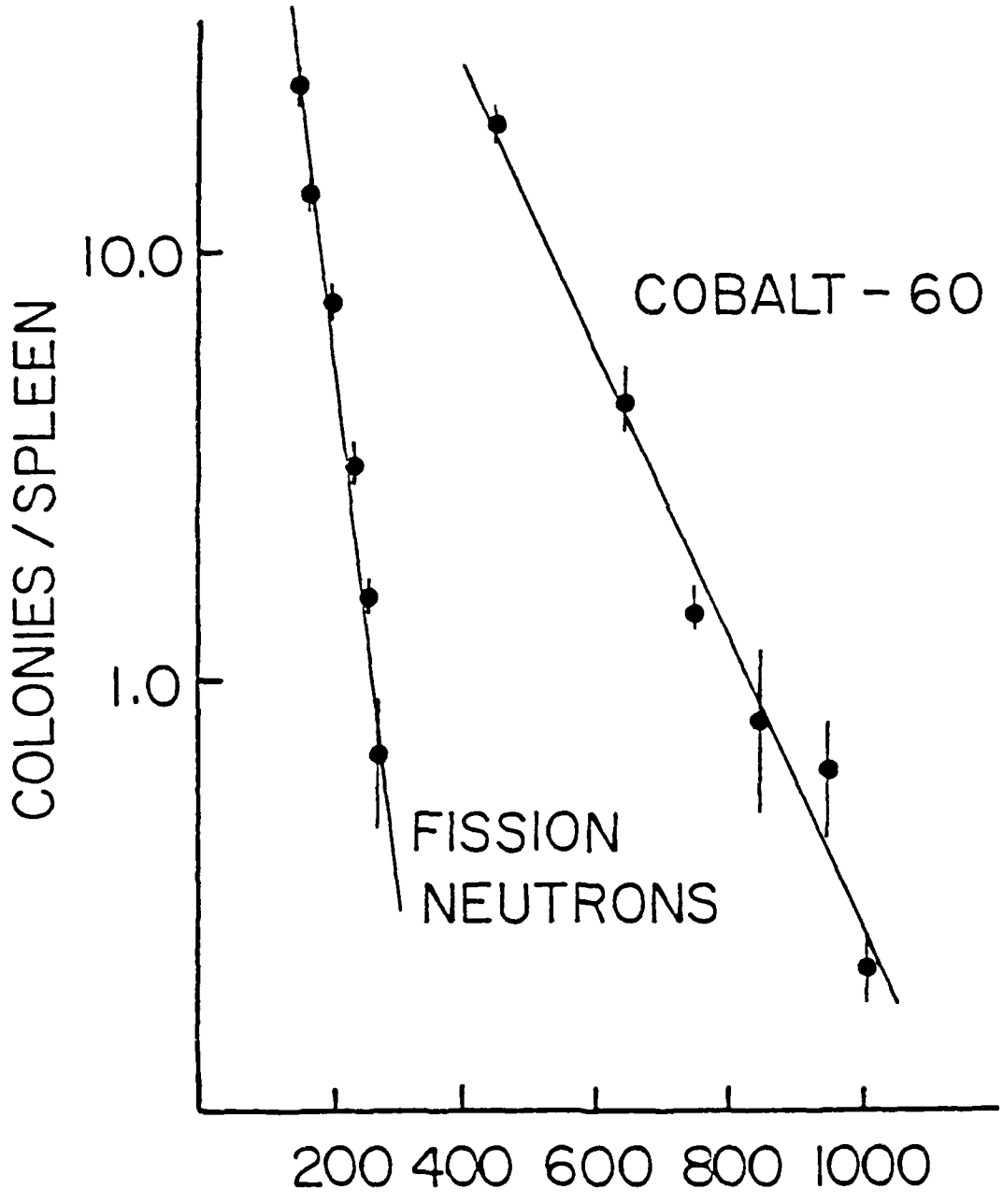
DRUG	i.p. DOSE (mg/kg)	SLOPE	CORRELATION COEFFICIENT	D_0 (RADS)	D-1.0 (RADS)	DMF
None	Cobalt-60	-0.0080	-0.9874	130	825	
None	Neutrons	-0.0290	-0.9974	34.8	262	
WR-347	220	-0.0250	-0.8691	40.1	246	0.94
WR-1065	251	-0.0200	-0.9330	41.6	298	1.14
WR-2529	1302	-0.0250	-0.9864	40.7	302	1.15
WR-2721	741	-0.0240	-0.9092	41.6	287	1.10
WR-3689	970	-0.0270	-0.9865	37.3	308	1.18
WR-44923	517	-0.0180	-0.9337	56.8	267	1.02
WR-151327	677	-0.0280	-0.9820	35.7	281	1.07
WR-168643	872	-0.0280	-0.9700	35.7	311	1.19

FIGURE 17

Spleen Colony Survival: Unprotected

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using either Cobalt-60 gamma or fission spectrum neutrons. The animals were not benefited with an anti-radiation drug prior to irradiation. These results were used from comparison purposes in the screening procedure.

FIGURE 17


COLONIES / SPLEEN
10.0
1.0
COBALT - 60
FISSION
NEUTRONS
200 400 600 800 1000

H. WR-151327

This drug exhibited a DMF of 1.07. The correlation between decremental survival and dose was -0.982, the slope of the survival curve was -0.028, the D_o was 35.7 rads and the D-1.0 was 281 rads.

I. WR-168643

This drug exhibited a DMF of 1.19. The correlation between decremental survival and dose was -0.970, the slope of the survival curve was -0.028, the D_o was 35.7 rads and the D-1.0 was 311 rads.

FIGURE 23

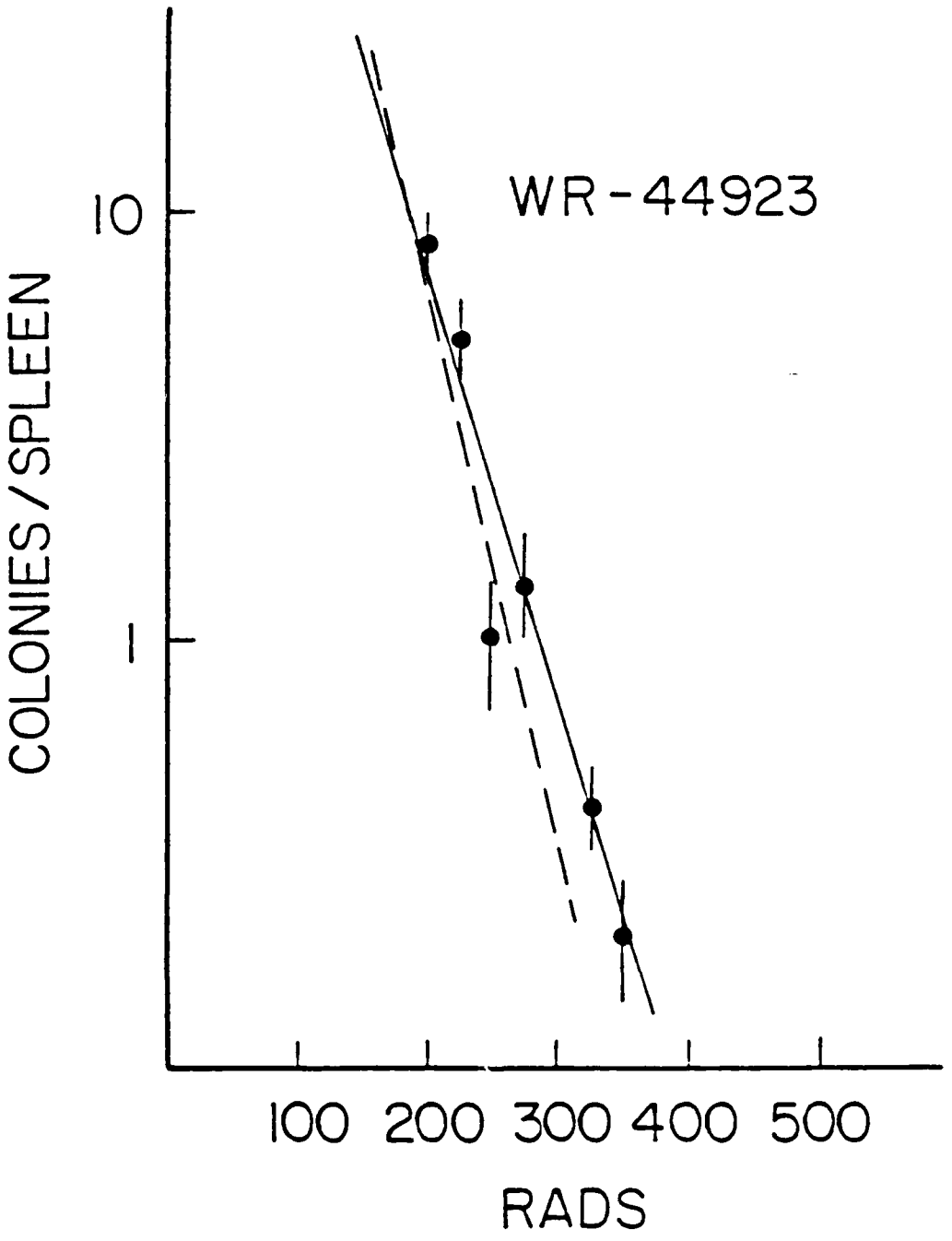


FIGURE 23

Spleen Colony Survival: Neutrons - WR-44923

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-44923 (517 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.02 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 22

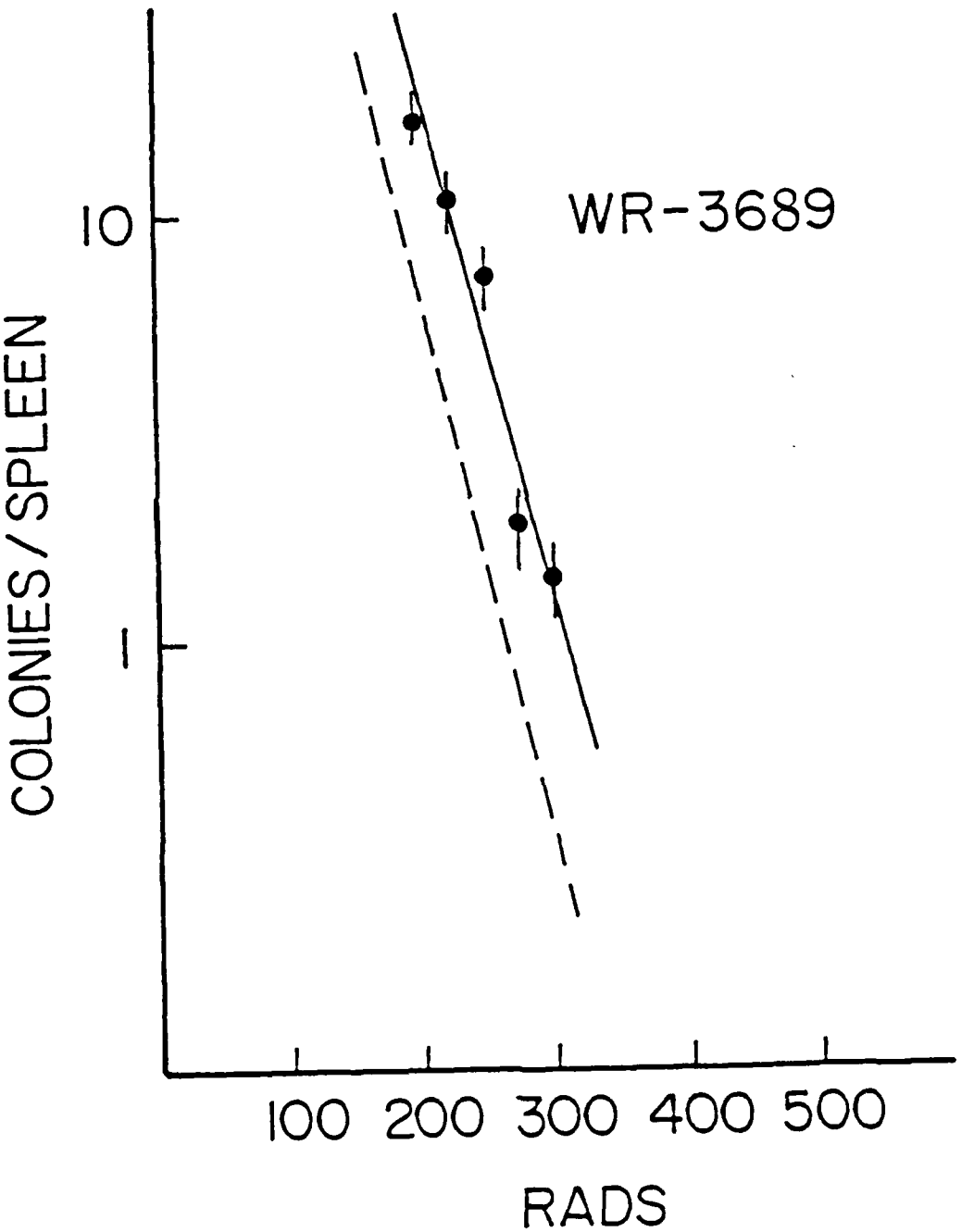


FIGURE 22

Spleen Colony Survival: Neutrons - WR-3689

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-3689 (970 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.18 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 21

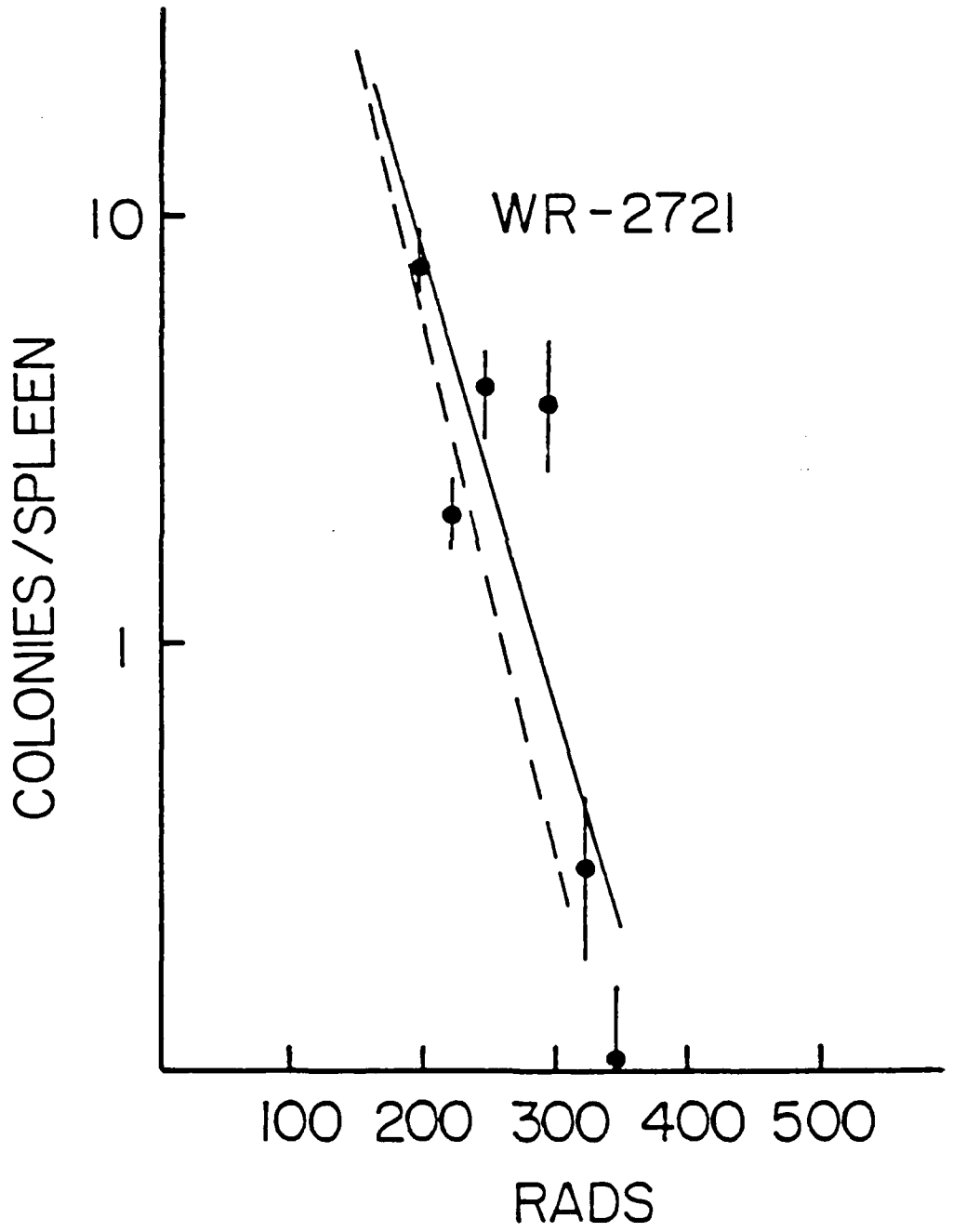


FIGURE 21

Spleen Colony Survival: Neutrons - WR-2721

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-2721 (741 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.10 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 20

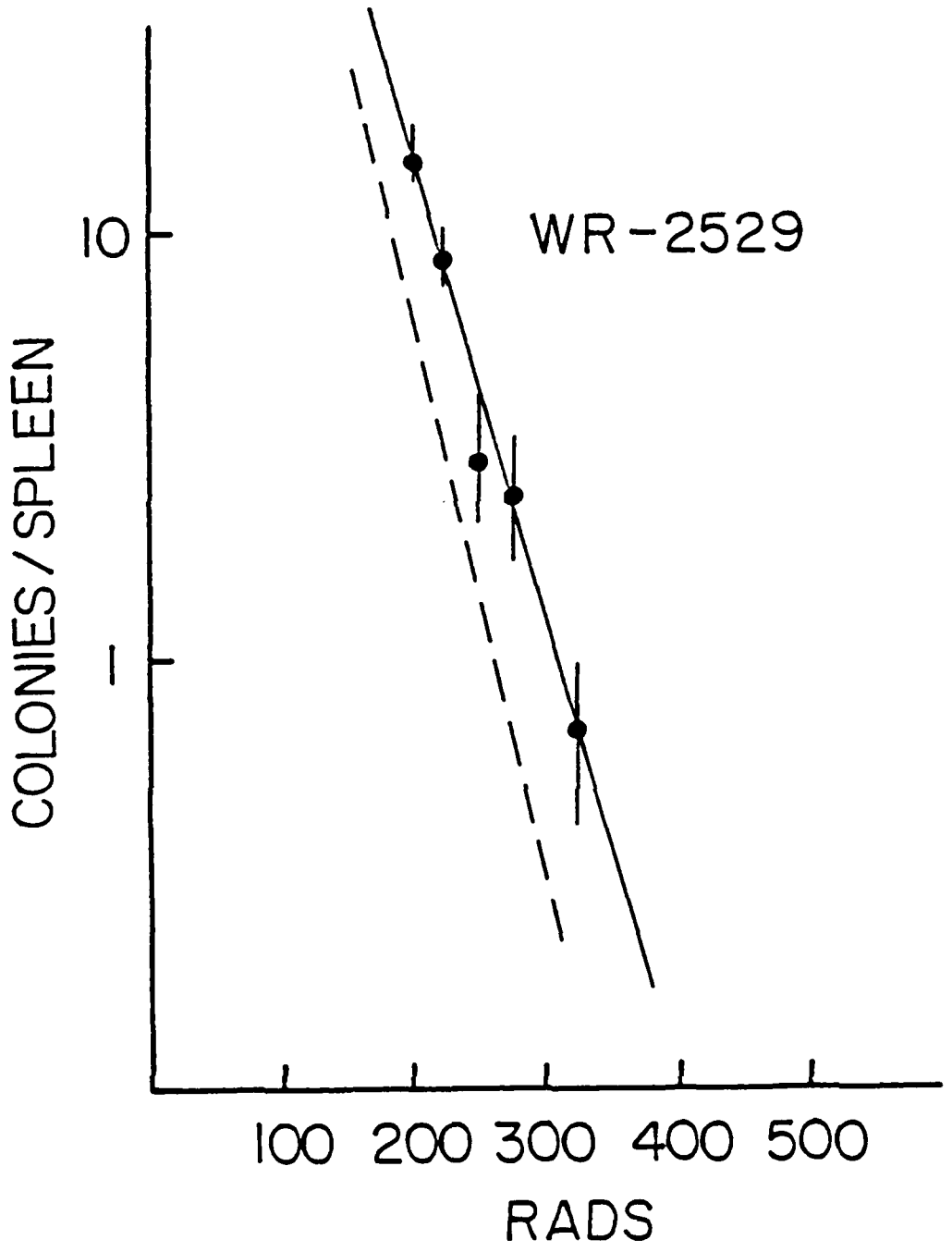


FIGURE 20

Spleen Colony Survival: Neutrons - WR-2529

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-2529 (1302 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.15 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 19

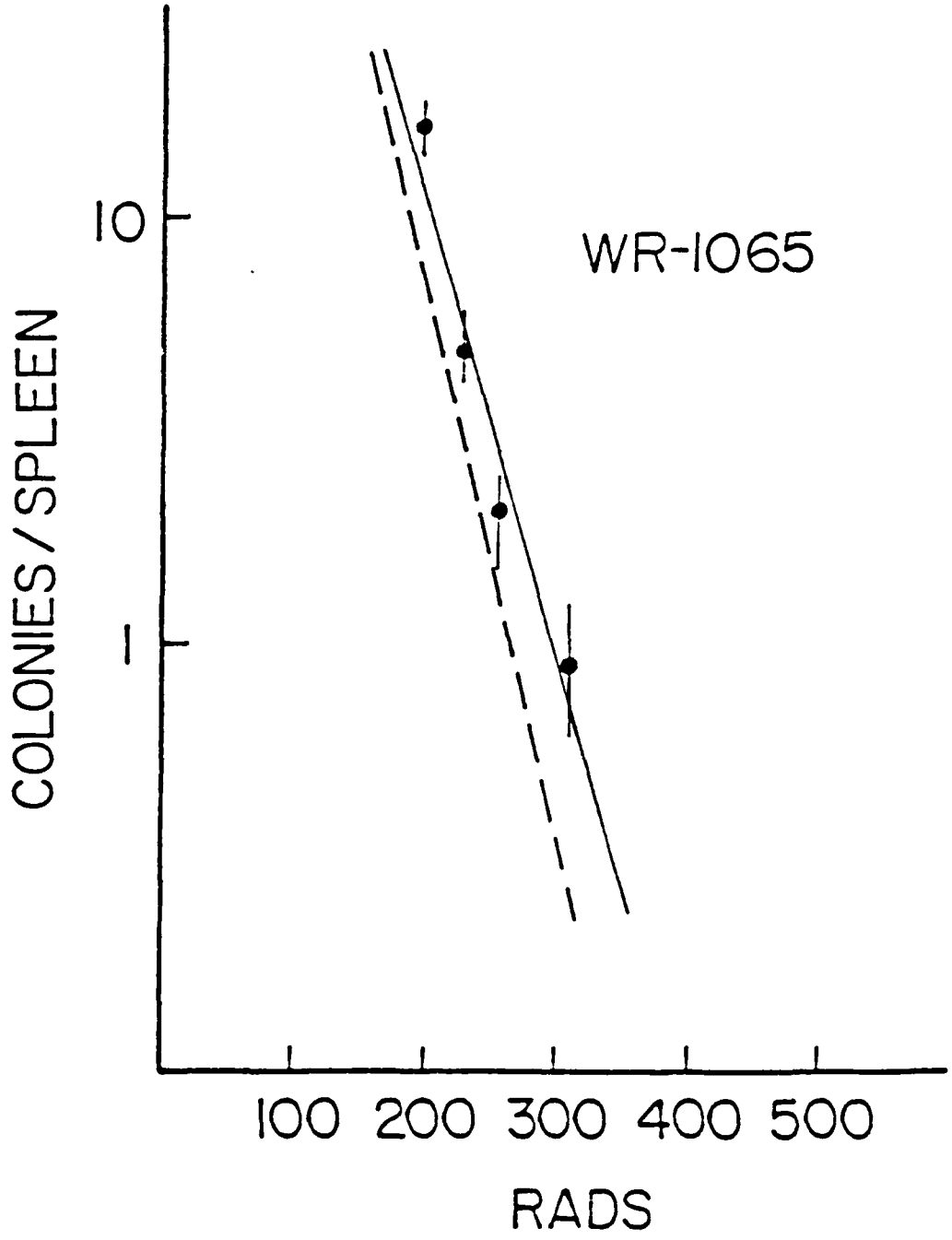


FIGURE 19

Spleen Colony Survival: Neutrons - WR-1065

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-1065 (251 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.14 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

C. WR-1065

This drug exhibited a DMF of 1.14. The correlation between decremental survival and dose was -0.933, the slope of the survival curve was -0.024, the D_o was 41.6 rads and the D-1.0 was 298 rads.

D. WR-2529

This drug exhibited a DMF of 1.15. The correlation between decremental survival and dose was -0.986, the slope of the survival curve was -0.025, the D_o was 40.7 rads and the D-1.0 was 302 rads.

E. WR-2721

WR-2721 was found to have a DMF of 1.10 using this assay. Apparently for spleen colony survival following irradiation, there is little correlation between the protective effect against lethality and the protective effect against blood-forming structure damage in the spleen. The correlation coefficient for the survival curve was -0.909, the slope was -0.024, the D_o was 41.6 rads, and the D-1.0 was 287 rads.

F. WR-3689

The DMF for WR-3689 was 1.18. The parameters for the survival curve were as follows: correlation coefficient = -0.986, slope = -0.027, D_o = 37.3 rads, and D-1.0 = 308 rads. This drug showed the best protection of those tested with the endogenous spleen colony assay.

G. WR-44923

Using the D-1.0 ratio to calculate the DMF resulted in a value of 1.02 for WR-44923. The slope of the transformed spleen colony survival curve was -0.0176, the D_o was 56.8 rads, the correlation coefficient was -0.9337, and the D-1.0 was 267 rads.

FIGURE 18

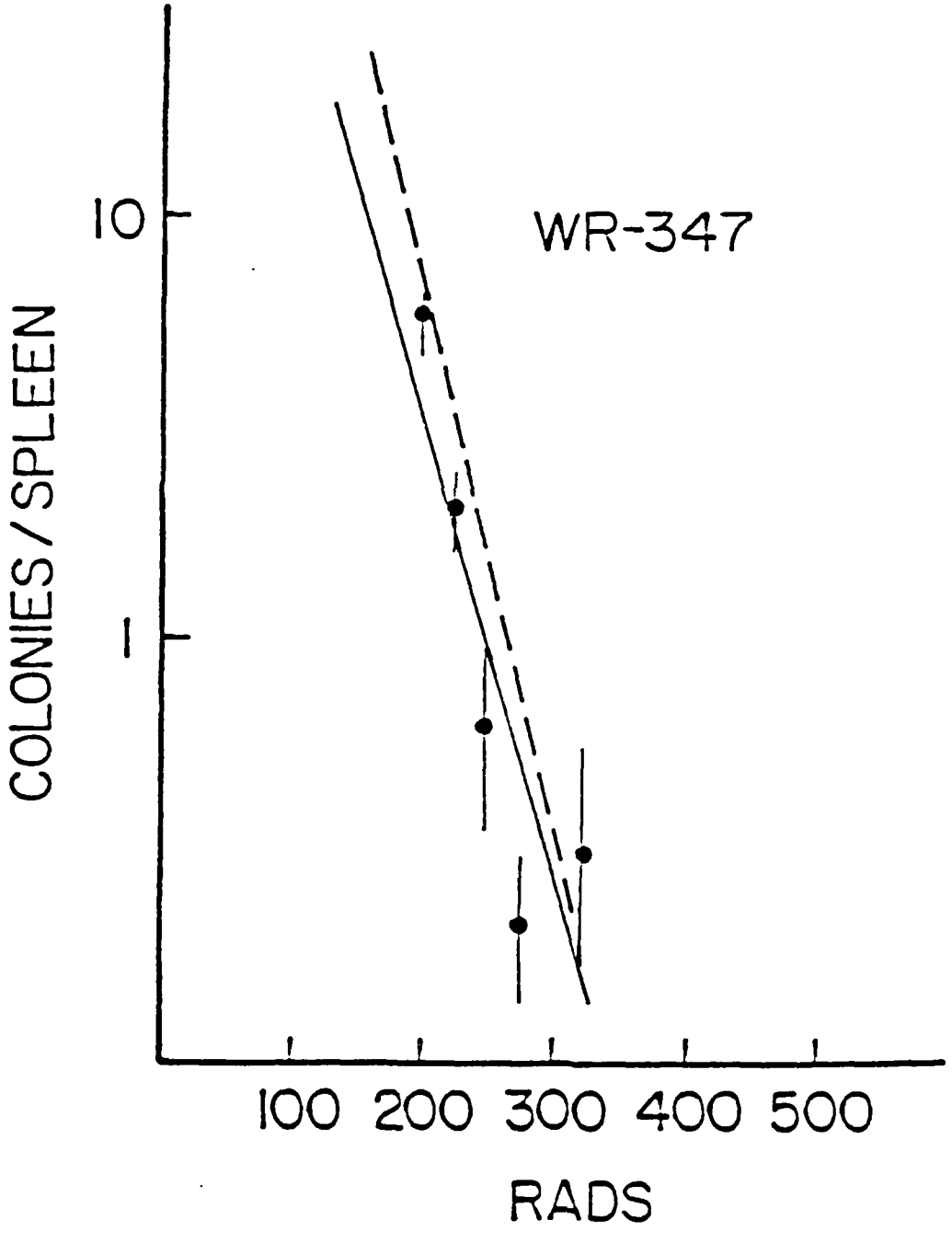


FIGURE 18

Spleen Colony Survival: Neutrons - WR-347

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-347 (220 mg/kg) prior to irradiation. The results obtained gave a DMF of 0.94 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 24

Spleen Colony Survival: Neutrons - WR-151327

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-151327 (677 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.07 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 24

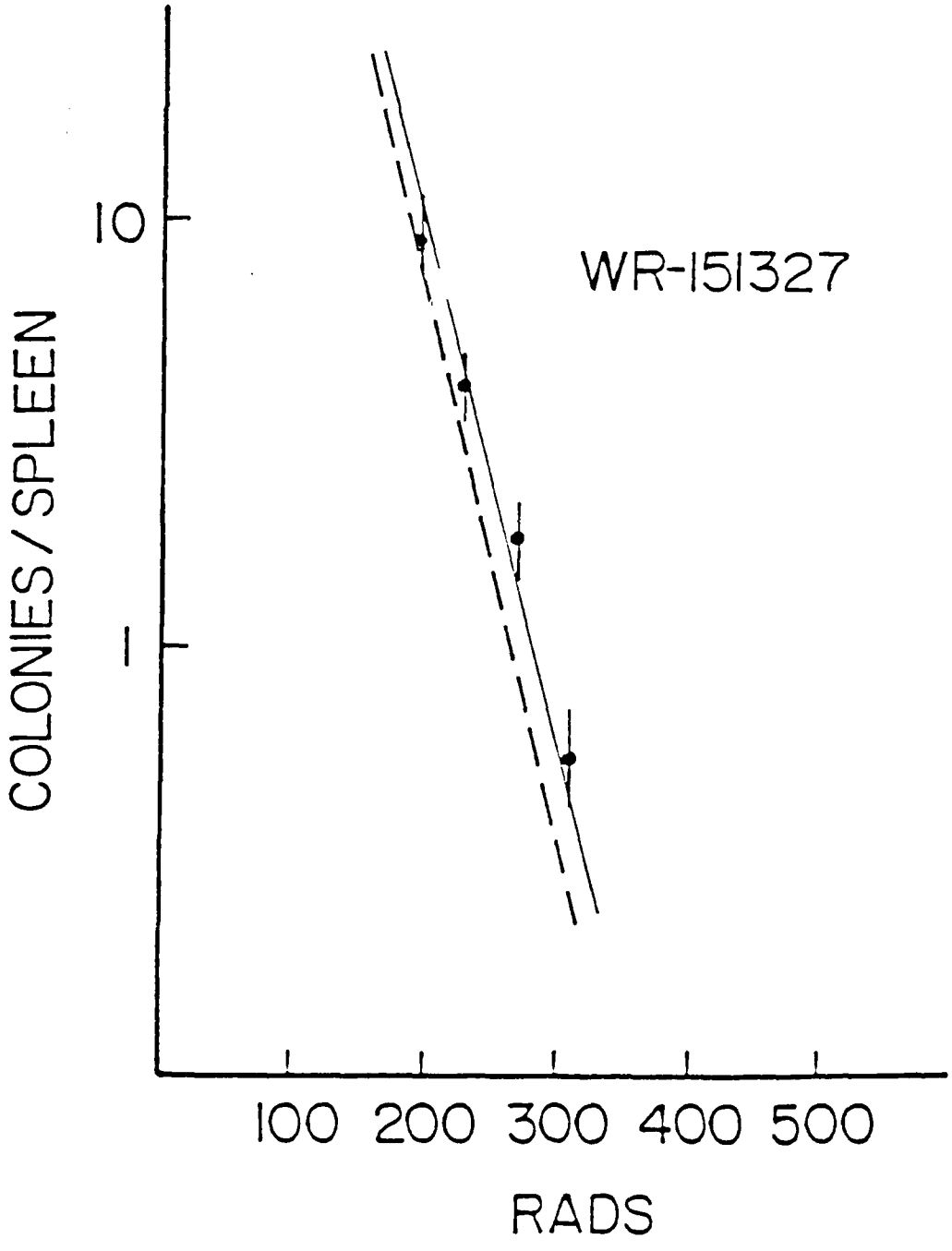
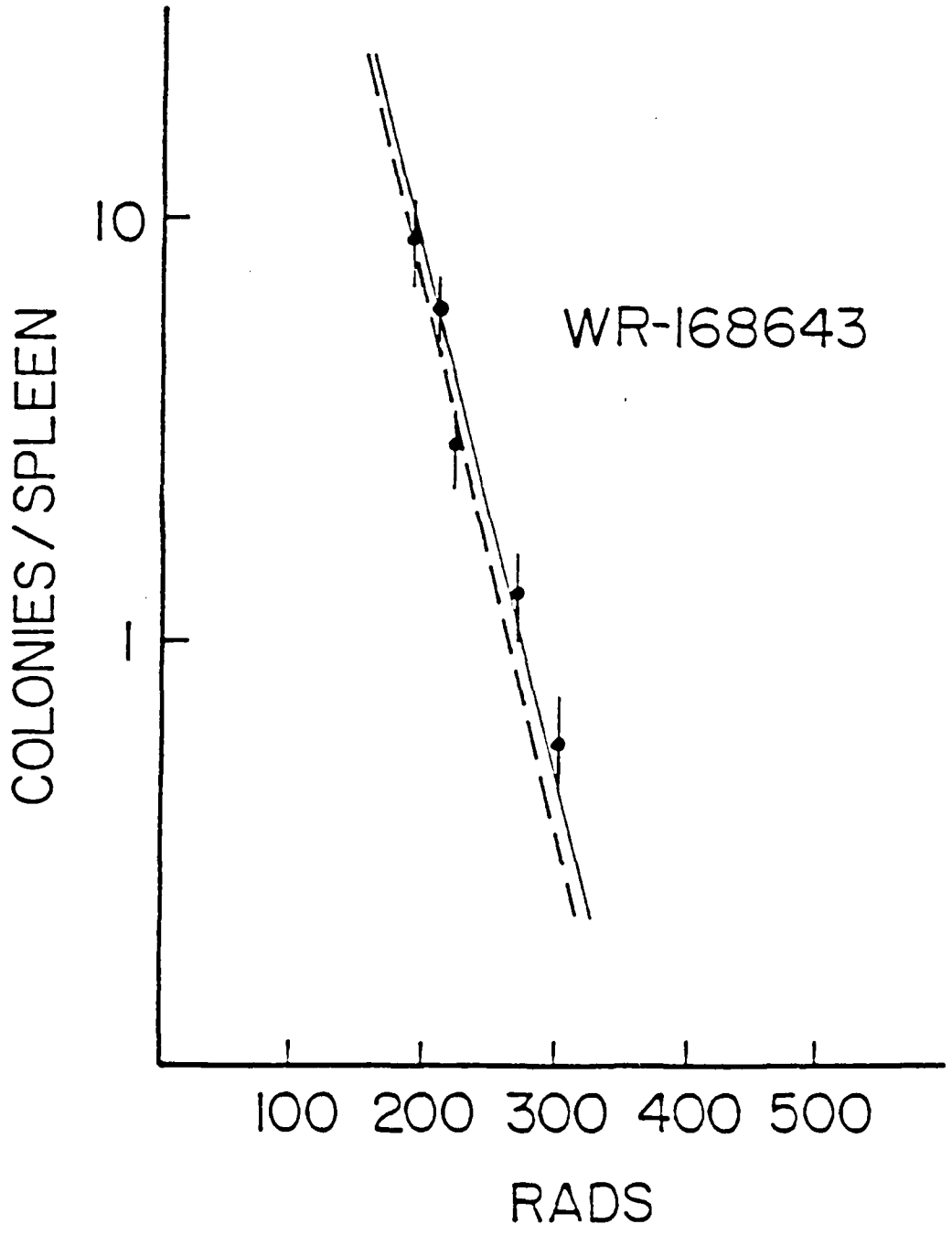


FIGURE 25

Spleen Colony Survival: Neutrons - WR-168643

A plot of endogenous spleen colony survival (colonies/spleen) as a function of radiation dose in rads. Data is presented from experiments using fission spectrum neutrons. The animals were pre-treated with WR-168543 (872 mg/kg) prior to irradiation. The results obtained gave a DMF of 1.19 using the ratio of the D-1.0 doses (dose sufficient to reduce the spleen cell population to 1 CFU/spleen) for protected and control groups.

FIGURE 25



LIST OF PUBLICATIONS

Peer Reviewed:

A.M. Connor and C.P. Sigdestad. Chemical Protection Against Gastrointestinal Radiation Injury in Mice by WR-2822, WR-2823 or WR-109342 After 4 MeV X-rays or Fission Neutron Irradiation. In CHEMICAL MODIFICATION: RADIATION AND CYTOTOXIC DRUGS Robert M. Sutherland, Editor. Pergamon Press (1981) pp 547-554.

A.M. Connor and C.P. Sigdestad. Intestinal Crypt Survival in Mice Irradiated with Either 4 MeV X-rays or Fission Neutrons: A Chemical Protection Study. International J. Radiat. Oncol. Biol. & Physics. 8:547-551 (1982).

Manuscripts in Preparation:

C.P. Sigdestad, D.J. Grdina, W.R. Hanson and A.M. Connor. Comparative Radioprotection From Three Neutron Sources with WR-2721 and WR-151327.

C.P. Sigdestad and A.M. Connor. Radiation Protection from Fission Neutron Irradiation: A Chemical Protection Study.

C.P. Sigdestad, A.M. Connor and K.P. McConnel. Assessment of the Ability of Dietary Seino-Methionine to Protect Against Ionizing Radiation.

Abstracts and Papers Presented:

C.P. Sigdestad and A.M. Connor. Chemical Protection Against Gastrointestinal Radiation Injury in Mice by WR-2822, WR-2823 or WR-109342 After 4 MeV X-ray or Fission Neutron Irradiation. Inter. CROS Conference on Chemical Modification. Key Biscayne, FL, (September 1981).

A.M. Connor and C.P. Sigdestad. Assessment of New Thiophosphate Compounds as Radioprotectors Against Either Fission Neutron or Gamma Radiation. Annual Radiation Res Soc Meeting, Salt Lake City, UT, (April 1982).

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