The Effects of Low Dose X-Irradiation on Hematopoiesis in the Bone Marrow of the Laboratory Mouse

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Submitted for approval on April 22, 1992 to the Honors Committee as partial fulfillment of an Honors Project

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Abstract

The frequency of micronucleated cells among the red blood cell's in bone marrow of thirty Swiss Barrier female mice was determined after exposure to varying doses of X-irradiaton. The average number of white blood cell's per field of view was also calculated. Results showed a possible linear dose-response curve where an increase in exposure to X-irradiation resulted in a decrease in microbody frequency in the red blood cells but no significant difference in white blood cell frequency. This contradicts several other similar studies conducted with gamma-irradiation.

Introduction

Numerous studies have investigated the in vitro effects of low-dose radiation (Almassy et al., 1986; Countryman and Heddle, 1976; Fenech and Morely, 1985; Jagetia, 1990), however, reports involving dose-response curves in vivo are few (Jagetia, 1990). The following investigation attempts to explore the potentially damaging effects of varying doses of low-dose X-irradiation by measuring the frequency of micronuclei formation and the number of leukocytes in the bone marrow of mice. The research hypothesis was that an increase in microbody formation in the red blood cells and a rise in white blood cell frequency would result from an increase in radiation dose.

Previous studies have shown that cells derived from the central cavity of the mouse femur exhibit radiation sensitivity (Allalunis-Turner and Chapman, 1985; Senn and McCulloch, 1970; Testa et al.,

1973) Micronuclei, also called Howell-Jolly Bodies, are fragments of chromosomes or chromatids that are not incorporated into daughter nuclei at mitosis (Jagetia, 1990). These spherical nuclear fragments of DNA may be observed in erythrocytes on a blood stain or bone marrow smear and usually only one per cell is found (Diggs et al., 1985). A possible source of confusion in identifying these erythrocyte inclusions is that they stain the same as the normal reticulocytes. However, their large spherical form makes confusion with reticulum unlikely (Spivak, 1980).

It is believed that the high energy X-rays in low doses have the ability to break bonds in the DNA of the cell. When the hematopoietic stem cells that differentiate into the red blood cells spew out their nucleus, these broken off fragments are left behind and may be readily observed. By studying the presence of microbodies in the red blood cells it is assumed that the trends seen there are representative of all marrow cells.

Dillehay (1990) explains that there are three major processes thought to be responsible for the dependency of the rate at which cell killing occurs on dose rate for low-dose radiation. These are: repair of damage, redistribution of cells in the cell cycle during irradiation and cell division during irradiation. In this study the repair of damage and effects of cell division in the hematopoietic stem cells will be addressed.

Methods and Materials

Thirty Swiss Barrier female mice approximately six weeks old were randomly assigned into three groups. After an initial weight was taken they were exposed to varying doses of X-irradiation over a nine week period. Group 1 was exposed to 30Kvp for 1/2 min (approximately 10 rads) five days per week and given the weekend to recover. At the end of the experiment the total body dose received by each Group 1 mouse was calculated to be 450 rads (for a total of 45 exposures).

Group 2 was exposed to the same daily dose as Group 1, but only on Tuesdays and Thursdays. However, they were transported to and from the radiation lab while group 1 was being radiated on the other days to insure that all mice were treated equally except for frequency of exposure. Group 2 mice started on a Thursday and received a total body dose of 190 rads at the end of the nine week period (for a total of 19 exposures).

Group 3 was the control group and received no direct radiation. They were also transported to and from the radiation lab each day the other mice were radiated.

The mice were radiated individually with the Hewlett Packart Faxitron X-ray system model number 43804N. The mice were placed in a plastic restrainer and put in the center of the middle tray at a distance of 63cm from the radiation source during each X-ray exposure.

After the nine week period a final weight of each mouse was obtained and they were then sacrificed by cervical dislocation. Two bone marrow smears were made from each mouse.

Studies have shown that the frequency of micronuclei formation increases from 8 hrs to 24 hrs post-irradiation and decreases there after (Jagetia and Ganapathi, 1990). Hofer et al. (1987) who also monitored the total number of nucleated cells, observed that regeneration of bone marrow tissue may begin at early intervals of the first days following its damage. Thus to maximize microbody formation and minimize repair to the cells, the mice were sacrificed as close to one day post-irradiation as possible.

Preparation of Slides

Bone marrow was obtained by cutting off the femur, inserting a 1cc syringe, containing approximately 0.5cc's of a phosphate buffered saline solution, into the femur and suctioning out the marrow. The bone marrow was then released onto the slide, dried on a histological slide tray, and stained by the LeukoStat Staining Process (Fisher Diagnostics, 1985).

Staining Process

LeukoStat is a modification of the Wright's Stain technique which uses a water soluble stain (Fisher Diagnostics, 1985). This process reduces the 4 minute staining process to only 15 seconds. The standard procedure for this process involves 5 dips of the slide into

each of three solutions. The first is a fixative solution made of Malachite green in methanol which stabilizes the cellular components. The second solution is a buffered solution of Eosin Y, formaldehyde, sodium phosphate and potassium phosphate. The third solution is a cationic dye consisting of thyazine dyes.

After preliminary tests with the preparation of the bone marrow smears, the staining process was modified by using only 3 dips in each solution instead of the standard five dips to achieve a lighter stain thereby enabling better viewing of microbody formation. This change in procedure was employed after having great difficulty in reading some of the slides. The modified procedure used in this experiment proved to be a simple and reliable technique showing fairly consistent differential staining of the bone marrow smears.

Cell Counting

The 60 slides were then coverslipped and coded so as to avoid bias in cell counting. Approximately one hundred random red blood cells were counted per slide; among these red blood cells, any one with a fragment of nuclear material in it was counted as a microbody containing cell. Cells containing a nucleus and a microbody were counted as microbody containing cells and those containing large spherical bodies clumped together were not counted as they were probably at the development stage just prior to nuclear expulsion. An average frequency of microbodies per hundred red blood cells was then calculated for each animal. Treatment group means and standard deviations could then be computed.

A white blood cell count was also done for each slide. In each field of view that red blood cells were counted, a total number of white blood cells was noted. These numbers were summed up and averaged over the observed number of fields so that an average white blood cell concentration could be recorded for each mouse.

Thirty seven slides were counted in this manner: thirteen from Group 1, eleven from Group 2, and thirteen from the control group. There was at least one slide representative from each mouse. If two slides were read from the same mouse, their results were averaged. There were some smears which were not analyzed due to the difficulty in reading some of the slides.

Percentages of microbody containing cells per 100 red blood cells and an average number of white blood cells per field of view were calculated for each group of radiated mice. T-tests for significant differences between groups were performed on each set of data using a 0.05 significance level. A comparison of initial and final weights was also done.

Results

Results shown in Table 1, 2 and 3 in the appendix contradict the initial research hypothesis that an increase in microbody formation would be seen in the RBC's with an increase in total body X-irradiation. It was also hypothesized that there would be an increase in the number of WBC's per field of view as X-ray dose increased. However, for the latter, there was no significant increase or decrease

in the number of white blood cells (see graph 1 and table 3 in appendix). The control group averaged 29.820 WBC's per field of view while Group 2 averaged 29.111 cells and Group 1, which received the highest radiation dose, averaged 27.919 WBC's per field of view. T-tests showed no significant difference between any of the three groups, so the null hypothesis of no difference was accepted.

There also appeared to be a decrease in the micronuclei formation in the red blood cells of the bone marrow with an increase in radiation dose (see graph 2 and table 3 in appendix). The control group averaged 10.779 microbodies per 100 RBC's while Group 2 averaged 9.502 and Group 1 averaged only 7.711 microbodies. T-tests show a significant difference ($p<0.05$) between Group 1, which received a total body dose of 450 rads, and Group 2, which received a total body dose of 190 rads. There was also a significant difference ($p<0.05$) between Group 1 and Group 3. There was no significant difference between Group 2 and Group 3.

All of the mice showed a significant weight gain over the 9 week radiation period most likely due to growth and maturation of the mice who were six weeks old at the start of the experiment. There was no significant difference in weight gains between the treatment groups and the control.

Discussion

Jagetia (1990) conducted a study similar to this one on the micronuclei formation in bone marrow cells after exposure to varying doses of gamma irradiation. The dose response curve

observed in this study was linear, and micronucleated cells increased with the increase in exposure dose. Jagetia's study is in agreement with similar studies performed by Jenssen and Famel (1976, 1978) and Cole et al. (1981), who also found a linear dose-response curve with increased levels of gamma irradiation.

Contrary to their results, in the present study a negatively linear dose-response curve was observed in which an increase in dose of X-irradiation corresponded to a decrease in microbody frequency in the red blood cells in the bone marrow.

One possible explanation for the decrease in the number of microbodies in the red blood cells could be that the X-irradiation is causing damage or death to the hematopoietic stem cells thus reducing the production of the differentiated daughter cells such as red blood cells. A study conducted by Hofer et al., (1990) found similar results. In this experiment the researchers subjected mice to small doses of gamma radiation (0.2 Gy) three times weekly and at the end of the first six months they saw a decrease in the number of leukocytes and an overall decrease in the number of bone marrow cells in the radiated mice compared to the control group.

This evidence could be used to support the theory that due to the damage sustained by the hematopoietic stem cells a decrease in number of differentiated daughter cells could result thus explaining the decrease in micronuclei formation in the RBC's of the bone marrow. If the radiation is killing stem cells that will eventually differentiate into microbody containing RBC's, then there would be a decrease in the number of microbodies per 100 RBC's as seen in the Hofer et al. study. To further evaluate this theory a complete

differential cell count must be done on the bone marrow smears and a comparison made between treatment groups and the control.

There was no significant difference between the average number of microbody containing cells per 100 RBC's in the control group and in Group 2. This is consistent with previous studies (Jagetia and Ganapathi,1990; Hofer et al.,1987) which concluded that repair and regeneration of cells damaged by radiation begins one day post-irradiation. Since group 2 was only radiated 2 days per week, the five days rest may have been enough time for significant regeneration and repair of damaged cells to occur.

While this study does not enable us to draw a definite conclusion, it opens up doors for future research. One possibility suggested earlier is to do a complete differential marrow count on the slides prepared. This would indicate whether or not there is a decrease in the hematopoietic stem cells which could be causing a decrease in microbody formation in the RBC's.

One possible means for improving this experiment would be to have several more groups of mice and to give larger variety of X-ray doses to these groups. This would allow for more data points and more reliable results.

Microbodies per 100 Red Blood Cells

Animal	Group1 (450 rads)	# slides analyzed	Group2 (190 rads)	# slides analyzed	Group 3 (0 rads)	# slides analyzed
1	0.000	1	12.360	1	8.400	2
2	11.429	1	13.830	1	6.931	1
3	7.455	2	7.762	2	18.478	1
4	5.680	2	9.615	1	9.534	2
5	8.499	2	16.304	1	17.391	1
6	11.215	1	13.160	1	10.780	1
7	5.714	1	6.522	1	11.980	2
8	7.143	1	3.920	1	12.766	1
9	14.655	1	5.155	1	5.172	1
10	5.319	1	6.931	1	6.360	1

Table 1: Average number of microbodies per 100 RBC's

Average Number of White Blood Cells per Field of View

Animal	Group1 (450 rads)	# slides analyzed	Group 2 (190rads)	# slides analyzed	Group 3 (0 rads)	# slides analyzed
1	31.000	1	19.067	1	27.629	2
2	33.000	1	24.667	1	31.900	1
3	22.000	2	35.190	2	38.667	1
4	28.145	2	29.333	1	27.455	2
5	21.081	2	17.833	1	15.000	1
6	24.375	1	46.500	1	36.250	1
7	27.556	1	39.500	1	18.965	2
8	32.286	1	25.800	1	28.667	1
9	25.000	1	30.889	1	28.000	1
10	34.750	1	22.333	1	45.670	1

Table 2: Average Number of White Blood Cells per field of view.

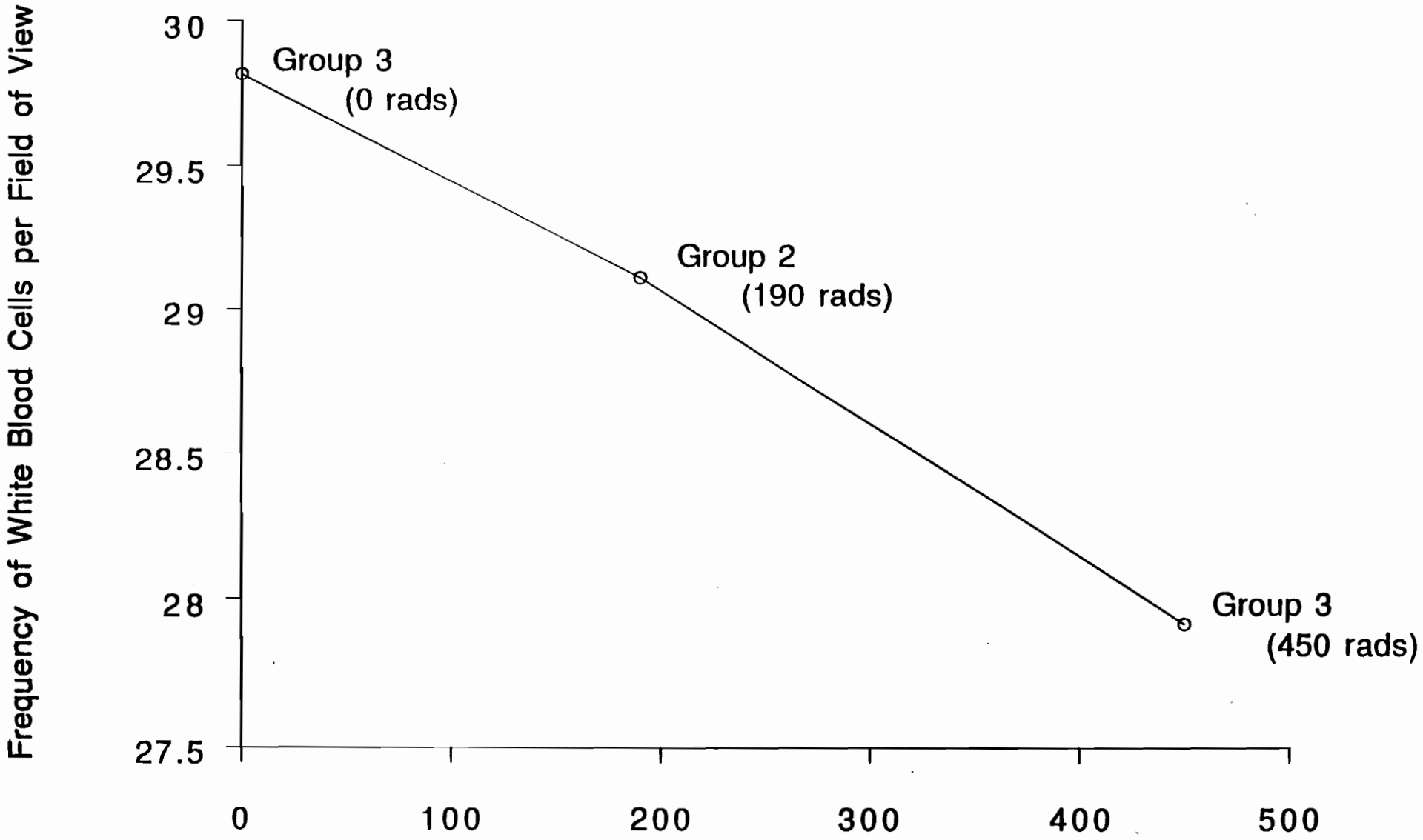
Summary of Data Results

	Group 1 (450 rads)	Group 2 (190 rads)	Group 3 (0 rads)
Avg. Initial Body Weights	26.10g	26.55g	26.65g
Avg. Final Body Weights	32.75g	33.20g	32.05g
* Avg. Microbody Frequency per 100 Red Blood Cells	7.711	9.502	10.779
+/- standard dev.	+/- 4.064	+/- 4.193	+/- 4.489
Avg. Frequency of White Blood Cells per field of view	27.919	29.111	29.820
+/- standard dev.	+/- 4.762	+/- 9.160	+/- 8.992

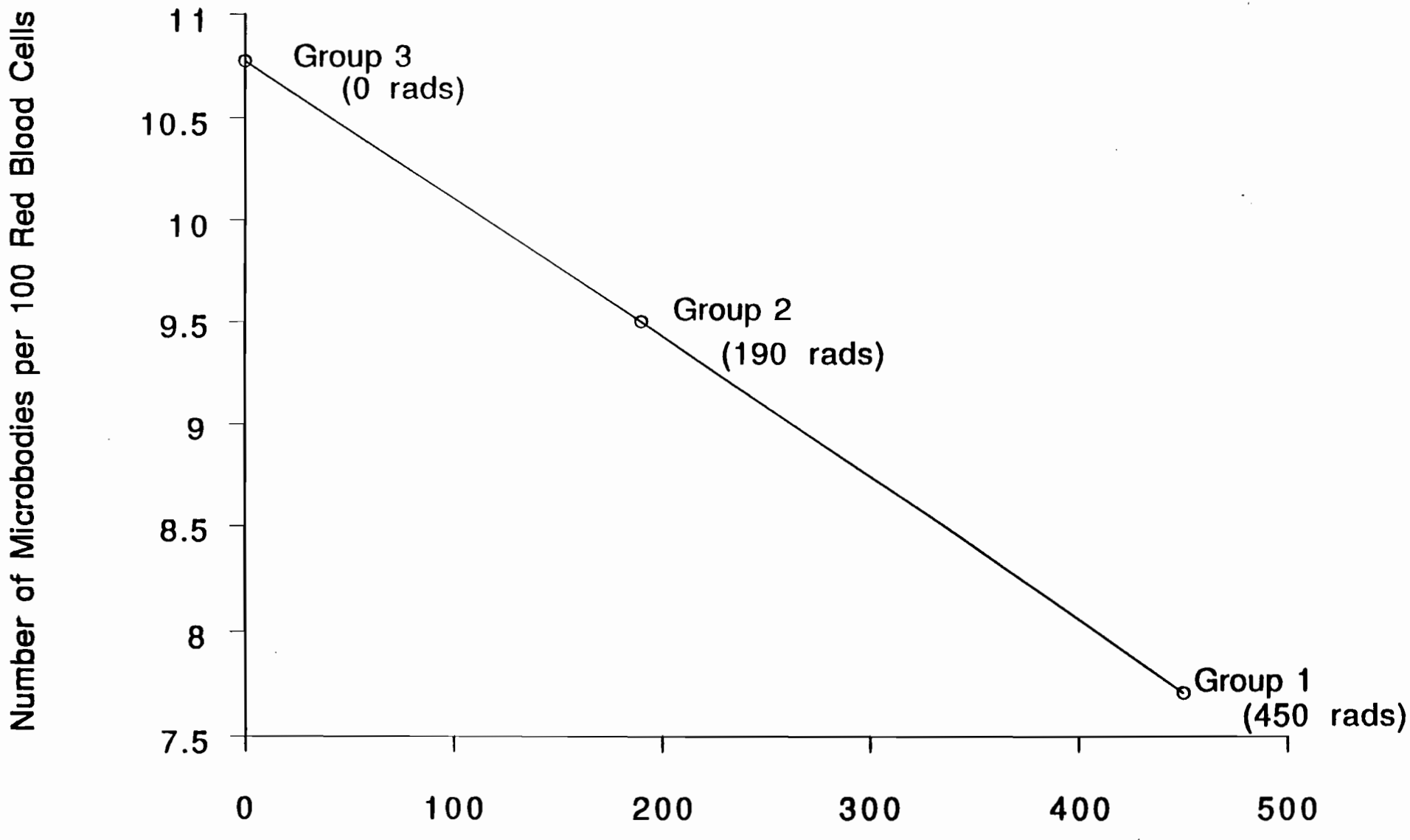
*Significant differences ($p<0.05$) occurred between Groups 1 and 2 and between Groups 1 and 3.

Table 3: Summary of data results.

Graph 1: Frequency of WBC's per Field of View vs Total Body Radiation Dose



Graph 2: Number of Microbodies per 100 RBC's vs Total Body Radiation Dose



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