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HEMATOLOGICAL RESPONSE IN SHEEP GIVEN PROTRACTED EXPOSURES TO ^{60}CO GAMMA RADIATION

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ABSTRACT

The changes in the total leukocytes in sheep irradiated with ^{60}Co gamma at a rate of 1.9 R/hr have been determined. After 50 R total exposure, maximum depression occurred 24 hours later. After 100 R or 175 R, maximum depression occurred within one hour. Recovery to control values was seen by 8 - 9 days for all three exposure groups. The recovery pattern for the circulating leukocytes was qualitatively the same for each of the three groups. An early lymphopenia was seen in all the groups, with recovery to the normal by 5 - 8 days. The percentage of neutrophils was greatly increased during the first 5 days. The data indicate that total leukocyte counts may not be a reliable means of predicting the exposure an animal sustains under protracted irradiation conditions.

SUMMARY

Problem:

Deeply penetrating space probes have conclusively shown that one of the significant hazards facing the space traveler is ionizing radiation. Best estimates to date indicate that the rate of exposure will probably be on the order of a few R/hr for relatively low total doses. In order to evaluate the hazards to personnel of this low level irradiation, it is essential to establish well-founded guidelines concerning the biological effects of protracted irradiation on humans as related to injury, recovery, and lethality. Such data are virtually nonexistent or not well documented.

Radiation studies with rodents have greatly contributed to the understanding of radiation effect in general, but the extent to which these data may be generally applied to larger animals or to humans is uncertain. Previous work in this laboratory has shown that large animals differ from rodents in their radiation sensitivity and in terms of recovery from radiation injury. Therefore, studies with large animals whose size, radiosensitivity, and recovery processes may be more comparable to man are necessary. In the work reported here, we have investigated the response of a radiation-sensitive organ system, the hematopoietic system, to different exposures of ^{60}Co gamma radiation at a protracted exposure rate.

Findings:

In sheep exposed to 50 R of ^{60}Co gamma radiation at an exposure rate of 1.9 R/hr, maximum depression of total leukocytes was observed within 24 hours, whereas, with 100 and 175 R exposures, maximum leukocyte depression was observed within one hour after the exposures. Associated with the early leukocyte drop was a lymphopenia and a neutrophilia. Return to control values was observed by 8 - 9 days in each of the groups. A wave-like pattern of depression and recovery was seen during the sampling period of approximately 75 days for each group. The changes observed in each of the groups were not significantly different, although they appeared to be exposure related.

INTRODUCTION

One of the significant hazards that a de_p space traveler will encounter is ionizing radiation in the form of electromagnetic waves, electrons, protons, and ionized nuclei (1 - 3). Of these, protons are the most serious biological threat because of their numbers and energies (4). However, current estimates suggest that the traveler will receive a relatively low total exposure accumulated over a relatively long time span. For planning and design purposes, it is necesssary to know the effect of the radiation received under these conditions on the individual, and, particularly, the effect on a very sensitive organ system, such as the hematopoietic system.

Because of the difficulties encountered in exposing large animals to protons for a long time period at a protracted rate, no reports are yet available concerning the response of the hematopoietic system to protons under these conditions. However, it has been established (5, 6) that over the energy range of most concern, protons have an RBE of approximately 1 as compared to ^{60}Co. Studies with large animals exposed to ^{60}Co should give a good approximation of what might be expected from proton exposures. Therefore, this experiment was designed to measure the changes in the peripheral white blood cells of sheep after exposures to ^{60}Co at a protracted rate.

Animals and Experimental Design:

Yearling, castrate male Columbia-Rambouillet sheep were used for the study. The animals were obtained in a single shipment and were maintained on pasture until selected for the exposures. Weights ranged between 90 and 120 pounds. Prior to the start of the exposures, a large group was moved into small holding pens, the wool over the jugular areas was removed by clipping and a depilatory cream, and three basline blood samples were taken. Animals that were more than one standard deviation from the mean white blood cell count were eliminated. From the remaining animals, the control group and exposure groups were randomly selected. Throughout the exposure and sampling periods, animals were fed commercial feed and allowed free access to water.

The irradiations were done at two time periods. Initially, 16 animals were exposed to 100 R; seven weeks later, two groups of 15 were exposed to 50 and 175 R, respectively. Eight animals served as the controls for the three groups.

Blood samples were taken aseptically from the external jugular vein into a heparinized syringe. The total white blood cell (WBC) counts were made on a Coulter Electronic Particle Counter (Model B). Slides for the differential count were prepared by routine clinical methods.

exposure technique have been previously reported (7 - 9). Briefly, for this experiment, 36 portable pens of galvanized wire and pipe construction, 4 x 8' in size, were set along an isodose contour on a sloping hillside. Two multi-curie ^{60}Co sources were positioned together as a point source such that an exposure rate of 1.9 R/hr was measured in air at the center of each portable pen. The exposure rate was determined by Lithium Fluoride powder and Landsverk ionization chambers. Previous exposures (10) have shown that because of the random movements of the sheep in the pens, the animals receive an approximately equal exposure to both sides during the total exposure period, although at any given time, the exposure is unilateral.

Data Analysis:

An NRDL computer program was used to determine the mean values, the 95% confidence intervals, standard errors, and standard deviations for the total WBC's and the granulocytes and the mononuclear cells. Because of the time variable of seven weeks between exposures and the sampling period extended over several months, it was deemed desirable to express the data as a percent of the values for the control animals at the corresponding sample times. This method tends to minimize the influence of any external factors that may have been present. Additionally, all the samples for the control animals were pooled and the numerical counts for the total WBC's are related to the mean of all the controls.

RESULTS AND DISCUSSION

The changes that are seen in the peripheral white blood cell counts are a fairly reliable and sensitive index of the injury to the hematopoietic tissue in animals that have been exposed to whole-body gamma radiation. The change seen in the total WBC pattern of sheep exposed to 50, 100, and 175 R whole-body ^{60}Co gamma radiation are compared in Figures 1 and 2. In Figure 1, values are plotted as percent of the control for each exposure group; in Figure 2, the total control samples were pooled and the total counts for each exposure group are plotted in relation to the mean of all the controls.

White blood cell depression was apparent within an hour after termination of the exposures in the 100 and 175 R groups and by the first day in the 50 R group. Recovery from this initial depression had begun within the first 7 days and the 50 and 175 R groups were practically at control levels by 8 days postexposure. However, the 100 R animals showed a return to control levels by 11 days postexposure. After this initial depression and recovery, a cyclic pattern of depression and recovery continued in each group for the remainder of the sampling period. The maximum depression occurred in the 175 R-exposed animals and the minimum depression in the 50 R-exposed animals. Conversely, the animals exposed to 175 R showed the least recovery and the animals exposed to 50 R the earliest recovery. Although the counts for each group could be reasonably correlated with the total exposure, there were no significant differences between the counts of the control animals and the corresponding test animals. By pooling and averaging the control values, a much narrower confidence interval is obtained. The early depression observed at the end of the exposure in the 100 and 175 R animals and the depression noted in the 50 R animals at day 1 is significant when compared to the pooled value. Using this method, the 100 and 175 R animals remained significantly depressed for most of the observation period. The numerical counts and 95% confidence limits are summarized in column A of Tables I, II, and III.

The total neutrophils, basophils, and eosinophils were grouped as granulocyte cells and are presented in Figure 3 as a percentage of the control values for each of the exposure groups. The total lymphocytes and monocytes were grouped as mononuclear cells and are presented in the same manner in Figure 4. The pattern of changes observed were similar for each of the exposure groups. Absolute counts and 95% confidence limits are listed in columns B and C of Tables I, II, and III.

In the granulocytic series there was an early (within 24 hours) slight decrease from the control values. This was reversed during the period of 2 through 4 days, and the exposed animals were as much as 220% of the control animals by day 4. This was due to an increase in the percentage of neutrophils seen on the differential counts. By days 6 and 7, the percentages were again less than control values and were greater than control values by days 8 and 9. These wave-like patterns were seen throughout the sampling schedule. Animals in the

50 R group showed the least variation and the least decrease from the controls and returned to normal ranges the earliest. The 100 R animals showed the earliest decrease, 80% of control values at the end of the exposures, and appeared to lag behind the 175 R animals in the early overshoot, but showed the highest increase of approximately 220% of the controls at day 4. The early changes seen in the granulocytic cells of the 175 R animals were hardly distinguishable from the 50 R animals, but there was a clear separation after about 16 days.

A decrease in the total monunuclear cells was seen within the first hour in the 100 and 175 R groups and by the end of the first day in the 50 R group. The maximum decrease (50%) was seen in the 100 R animals on day 4 postexposure. The decrease seen in the first 4 days was due to a lymphopenia and a shift in the differential count wherein there were fewer lymphocytes and more neutrophils. There was a return toward the normal in all the groups during the period of 5 to 8 days, followed by these wave-like patterns throughout the sampling periods. The greatest variation was noted in the 100 R animals, especially during the first 10 to 12 days. However, after 25 days, the 100 R animals appeared more stable than the 50 or 175 R animals.

Although the postirradiation changes in the peripheral blood elements of most laboratory animals acutely exposed to gamma radiation are well known (11, 12), there are few reports concerning the changes that occur in large animals exposed under protracted conditions. Changes are known for the goat, rhesus monkey, and sheep.

Female Spanish goats exposed to 300 R of ^{60}Co gamma radiation at the rate of 15 R/20-hour irradiation day by Hupp et al. (13) show a low value of WBC counts at 10 days postirradiation and recovery to only 75% of control values by 14 days. In Spanish goats exposed to ^{60}Co until death at rates of 20 or 40 rad/20-hour irradiation day, Leidy et al. (14) have found significant decreases in the WBC count in females by the end of the first week and in males by the end of the second week of exposure.

Holland and Spalding (15) exposed rhesus monkeys to ^{60}Co at rates of 2.13, 3.19, and 4.26 rad/hr for total doses of 500, 750, and 1000 rad. They have found WBC depression by day 2 of the exposure period for animals exposed at the rate of 2.13 (after 100 rad) or 4.26 rad (after 200 rad)/hr, but not in animals exposed at 3.19 rad (150 rad)/hr. They noted the lowest counts at 8 days postexposure, but there was no difference in the animals exposed at 3.19 or 4.26 rad/hr. Significant recovery occurred by 14 days postexposure with recovery progressing at similar rates in each group. A return to preexposure values was found by approximately 21 days postexposure.

In sheep we found immediate depression in animals exposed to 100 or 175 R and depression by day 1 after exposure to 50 R. Recovery to normal values occurred by 8 to 11 days, and as with the monkeys, recovery in each group progressed similarly.

These data that are available for animals exposed to ^{60}Co gamma radiation at protracted rates do not show a consistent pattern as to values occurred by 8 to 11 days, and, as with the monkeys, recovery in each group progressed similarly.

These data that are available for animals exposed to ^{60}Co gamma radiation at protracted rates do not show a consistent pattern as to the time of the maximum depression of the white blood cells or as to the time of recovery. The results obtained from the sheep exposures indicate that changes in total leukocyte counts may not be a reliable means of predicting the exposure an animal sustains under protracted rates. They do indicate that as little as 50 R given over a period of approximately 26 hours (1.9 R/hr) causes a detectable decrease in the total leukocytes. This is a factor that will have to be considered by those involved in the planning of space missions where ionizing radiation may be present.

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TABLE I - A

Day	A. Leukocytes X10^{-3} Controls	50 R	B. Granulocytes X10^{-3} Controls	50 R
Pre-Exposure				
9	7.69 (6.50-8.87)*	8.50 (7.57-9.43)	2.01 (1.48-2.54)	1.59 (1.06
5	7.39 (6.10-8.69)	8.22 (7.71-8.73)	1.49 (0.67-2.30)	1.31 (1.01
2	8.05 (6.72-9.37)	8.65 (7.42-9.89)	0.78 (0.25-1.29)	0.69 (0.34
Post-Exposure				
0	7.83 (6.52-9.15)	7.86 (7.03-8.68)	1.88 (1.14-2.62)	1.94 (1.44
1	7.82 (6.65-9.00)	6.79 (5.99-7.60)	2.08 (0.80-3.35)	1.96 (1.50
2	7.96 (6.80-9.11)	7.18 (6.16-8.19)	1.50 (0.45-2.56)	1.49 (0.96
3	8.38 (6.51-10.26)	7.18 (6.30-8.06)	0.80 (0.36-1.26)	1.16 (0.47
6	7.22 (5.64-8.79)	7.04 (5.95-8.12)	0.91 (0.57-1.24)	0.78 (0.32
8	8.15 (5.60-10.7)	8.10 (6.68-9.52)	0.64 (0.18-1.10)	1.15 (0.53
10	7.69 (4.93-10.45)	7.34 (5.40-9.29)	1.08 (0.40-2.20)	1.16 (0.22
16	8.49 (6.33-10.64)	7.18 (6.52-7.83)	0.60 (0.42-0.76)	1.33 (0.87
23	7.00 (5.31-8.69)	7.21 (6.29-8.14)	0.71 (0.17-1.24)	0.87 (0.28
30	8.43 (5.58-11.2[illegible])	6.45 (5.79-7.11)	1.81 (1.14-2.48)	1.61 (1.23
56	7.00 (5.49-8.53)	7.40 (6.59-8.21)	1.34 (0.52-2.19)	1.42 (0.60
72	8.61 (6.62-10.61)	8.62 (7.85-9.39)	1.62 (1.09-2.16)	2.10 (1.38

*Figures in parentheses are the 95% Confidence Interval Values

TABLE 1-B

...es $X10^{-3}$		C. Mononuclear Cells $X10^{-3}$	
	50 R	Controls	50 R
...5'.	1.59 (1.06 - 2.12)	5.67 (4.61-6.73)	6.91 (5.94 - 7.80)
...30)	1.31 (1.01-1.62)	5.91 (4.71-7.10)	6.91 (6.45-7.36)
...29)	0.69 (0.34-1.03)	7.27 (6.07-8.48)	7.97 (6.77-9.16)
...62)	1.94 (1.44-2.44)	5.96 (5.23-6.68)	5.92 (5.25-6.58)
...35)	1.96 (1.50-2.42)	5.75 (4.46-7.04)	4.83 (4.19-5.47)
...56)	1.49 (0.96-2.02)	6.45 (5.46-7.45)	5.68 (4.95-6.42)
...26)	1.16 (0.47-1.86)	7.58 (5.67-9.49)	6.02 (5.30-6.74)
...24)	0.78 (0.32-1.23)	6.31 (4.98-7.64)	6.26 (5.57-6.96)
...10)	1.15 (0.53-1.78)	7.51 (5.05-9.98)	6.95 (5.66-8.24)
...20)	1.16 (0.22-2.11)	6.61 (4.79-8.42)	6.18 (4.77-7.59)
...76)	1.33 (0.87-1.80)	7.89 (5.77-10.02)	5.84 (5.27-6.42)
...24)	0.87 (0.28-1.46)	6.29 (4.97-7.62)	6.35 (5.38-7.32)
...48)	1.61 (1.23-1.99)	6.63 (4.13-9.12)	4.84 (4.26-5.42)
...19)	1.42 (0.60-2.23)	5.65 (4.88-6.42)	5.99 (5.18-6.79)
...16)	2.10 (1.38-2.82)	6.99 (5.41-8.57)	6.52 (5.68-7.36)

TABLE II - A

Day	A. Leukocytes X10^{-3}		B. Granulocytes X10^{-3}	
Pre-Exposure	Controls	100 R	Controls	100 R
30	6.65 (5.46-7 83)*	6.86 (5.85-7.87)	1.39 (1.11-1.66)	1.54 (1.23-
20	7.17 (6.11-8.22)	8.10 (7.10-9.09)	1.78 (1.30-2.27)	2.06 (1.65-
14	8.88 (7.21-10.55)	8.18 (7.32-9.04)	3.24 (2.33-4.15)	2.79 (2.42-
Post-Exposure				
0	8.19 (6.94-9.45)	6.07 (5.33-6.80)	2.47 (1.83-3.11)	1.97 (1.69-
2	7.91 (5.88-9.95)	6.54 (5.77-7.31)	2.31 (1.66-2.97)	2.22 (1.83-
3	8.14 (5.85-10.44)	6.58 (5.71-7.46)	2.73 (1.86-3.60)	2.86 (2.32-
4	8.39 (6.41-10.38)	7.25 (6.13-8.37)	2.76 (1.74-3.78)	4.37 (3.53-
7	7.17 (5.75-8.58)	6.15 (4.77-7.52)	1.59 (1.12-2.06)	1.22 (0.51-
9	8.92 (7.56-10.28)	7.25 (6.37-8.12)	1.84 (1.08-2.61)	2.25 (1.75-
11	6.46 (5.56-7.35)	6.49 (5.37-7.62)	1.93 (1.11-2.75)	1.69 (1.14-
17	8.06 (6.69-9.44)	6.84 (6.11-7.58)	1.49 (1.21-1.77)	2.05 (1.70-
24	6.69 (5.71-7.66)	5.54 (4.50-6.58)	0.80 (0.34-1.27)	0.44 (0.22-
31	7.79 (5.79-9.79)	6.76 (6.10-7.42)	1.53 (0.37-2.68)	1.06 (0.67-
52	7.39 (6.10-8.69)	6.63 (5.65-7.62)	0.71 (0.32-1.11)	0.91 (0.57-
112	7.01 (5.49-8.53)	6.60 (5.65-7.56)	1.41 (0.62-2.19)	0.45 (0.25-
195	8.61 (6.62-10.60)	6.93 (3.7-10.12)	1.62 (1.09-2.16)	1.68 (1.10-

*Figures in parentheses are the 95% Confidence Interval Values

Table II - B

3	C. Mononuclear Cells $X10^{-3}$	
100 R	Controls	100 R
1.54 (1.23-1.85)	5.26 (3.95-6.57)	5.32 (4.49-6.15)
2.06 (1.65-2.47)	5.38 (4.37-6.40)	6.03 (5.26-6.80)
2.79 (2.42-3.15)	5.64 (4.64-6.64)	5.39 (4.86-5.92)
1.97 (1.69-2.25)	5.72 (4.91-6.54)	4.10 (3.57-4.62)
2.22 (1.83-2.61)	5.60 (4.19-7.01)	4.32 (3.81-4.84)
2.86 (2.32-3.40)	5.41 (3.75-7.08)	3.73 (3.20-4.26)
4.37 (3.53-5.21)	5.64 (4.13-7.14)	2.88 (2.22-3.54)
1.22 (0.51-1.93)	5.58 (4.52-6.63)	4.93 (3.69-6.17)
2.25 (1.75-2.74)	7.08 (5.23-8.93)	5.00 (4.32-5.67)
1.69 (1.14-2.24)	4.53 (3.11-5.95)	4.80 (3.91-5.70)
2.05 (1.70-2.39)	6.57 (5.33-7.82)	4.80 (4.21-5.38)
0.44 (0.22-0.66)	5.88 (4.83-6.94)	5.10 (4.15-6.05)
1.06 (0.67-1.45)	6.26 (4.89-7.63)	5.70 (5.19-6.21)
0.91 (0.57-1.25)	6.68 (5.50-7.86)	5.72 (4.81-6.64)
0.45 (0.25-0.66)	5.60 (4.82-6.38)	6.14 (5.29-7.00)
1.68 (1.10-3.25)	6.99 (5.41-8.57)	5.26 (2.87-7.65)

TABLE III - A

Day Pre-Exposure	A. Leukocytes X10^{-3} Controls	 175 R	B. Granulocytes X10^{-3} Controls	
7	7.69 (6.50-8.87)*	8.22 (7.28-9.16)	2.01 (1.48-2.54)	2.04
3	7.39 (6.10-8.69)	8.10 (7.23-8.97)	1.49 (0.67-2.30)	1.89
0	8.05 (6.72-9.37)	8.04 (7.13-8.95)	0.78 (0.25-1.29)	0.82
Post-Exposure				
0	7.83 (6.52-9.15)	6.61 (5.77-7.46)	1.88 (1.14-2.62)	2.07
1	7.82 (6.65-9.00)	5.84 (4.79-6.90)	2.08 (0.80-3.35)	1.84
2	7.96 (6.80-9.11)	6.14 (5.33-6.94)	1.50 (0.45-2.56)	1.70
3	8.38 (6.51-10.26)	6.64 (5.79-7.49)	0.80 (0.36-1.26)	1.21
6	7.22 (5.64-8.79)	6.35 (5.32-7.39)	0.91 (0.57-1.24)	0.86
8	8.15 (5.60-10.7)	7.67 (6.06-9.29)	0.64 (0.18-1.10)	1.19
10	7.69 (4.93-10.45)	6.02 (5.20-6.83)	1.08 (0.40-2.20)	1.12
16	8.49 (6.33-10.64)	5.84 (4.84-6.83)	0.60 (0.42-0.76)	1.24
23	7.00 (5.31-8.69)	5.55 (4.71-6.39)	0.71 (0.17-1.24)	0.30
30	8.43 (5.58-11.29)	5.66 (4.86-6.45)	1.81 (1.14-2.48)	1.95
56	7.00 (5.49-8.53)	5.47 (4.73-6.21)	1.34 (0.52-2.19)	0.84
72	8.61 (6.62-10.61)	7.44 (6.25-8.62)	1.62 (1.09-2.16)	1.67

*Figures in parentheses are the 95% Confidence Interval Values.

TABLE III-3

...es X10-3		C. Mononuclear Cells X10-3	
	175 R	Controls	175 R
54)	2.04 (1.50-2.58)	5.67 (4.[illegible]1-6.73)	6.18 (5.32-7.03)
30)	1.89 (1.45-2.33)	5.91 (4.71-7.10)	6.21 (5.34-7.08)
29)	0.82 (0.44-1.19)	7.27 (6.07-8.48)	7.22 (6.49-7.96)
62)	2.07 (1.67-2.46)	5.95 (5.23-6.68)	4.54 (3.99-5.10)
35)	1.84 (1.60-2.07)	5.75 (4.46-7.04)	4.00 (3.07-4.94)
56)	1.70 (1.08-2.32)	6.45 (5.46-7.45)	4.44 (3.57-5.31)
26)	1.21 (0.92-1.51)	7.58 (5.67-9.49)	5.42 (4.62-6.22)
24)	0.86 (0.54-1.17)	6.31 (4.98-7.64)	5.50 (4.60-6.39)
10)	1.19 (0.50-1.88)	7.51 (5.05-9.98)	6.48 (5.36-7.60)
20)	1.12 (0.70-1.69)	6.61 (4.79-8.42)	4.82 (4.13-5.52)
76)	1.24 (0.84-1.64)	7.89 (5.77-10.02)	4.60 (3.83-5.37)
24)	0.30 (0.17-0.44)	6.29 (4.97-7.62)	5.25 (4.45-6.05)
48)	1.95 (1.45-2.45)	6.63 (4.13-9.12)	3.71 (3.25-4.16)
19)	0.84 (0.56-1.13)	5.65 (4.88-6.42)	4.63 (3.91-5.35)
16)	1.67 (1.32-2.01)	6.99 (5.41-8.57)	5.77 (4.75-6.74)

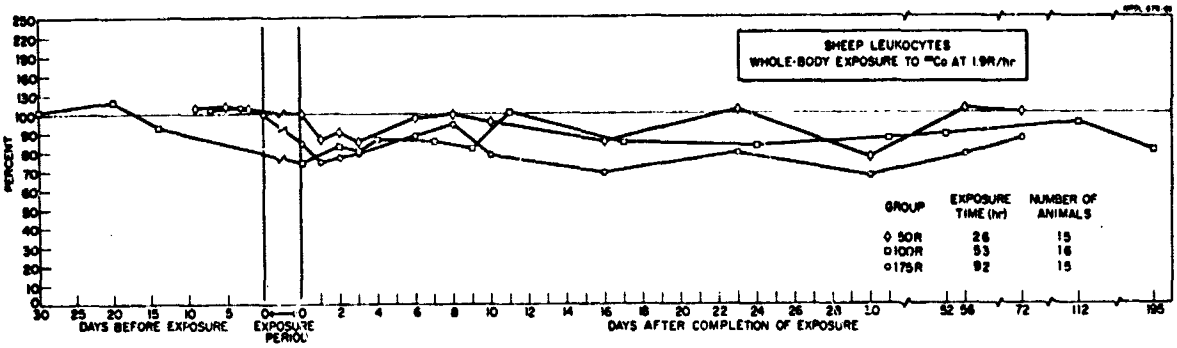

Figure 1. Leukocyte counts in sheep given exposures of 50, 100, or 175 R at 1.9 R/hr as percent of control.

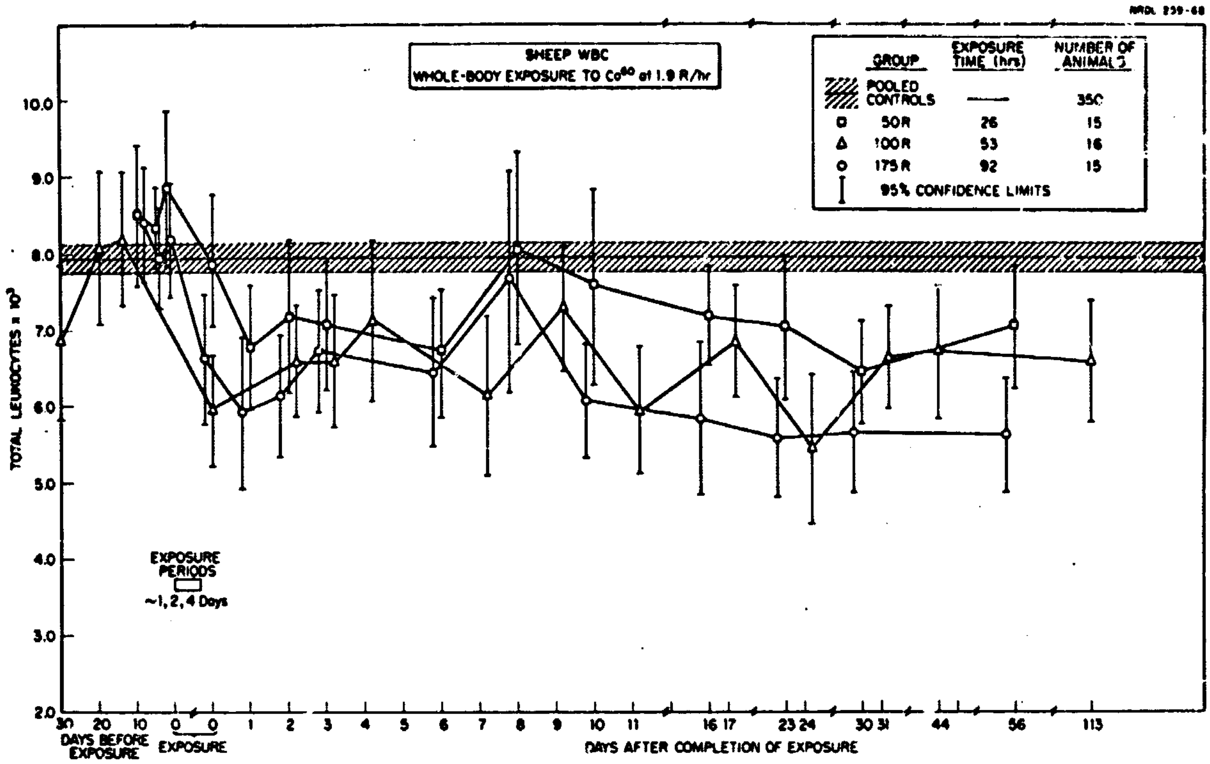

Figure 2. Total leukocyte counts in sheep given exposures of 50, 100, or 175 R at 1.9 R/hr.

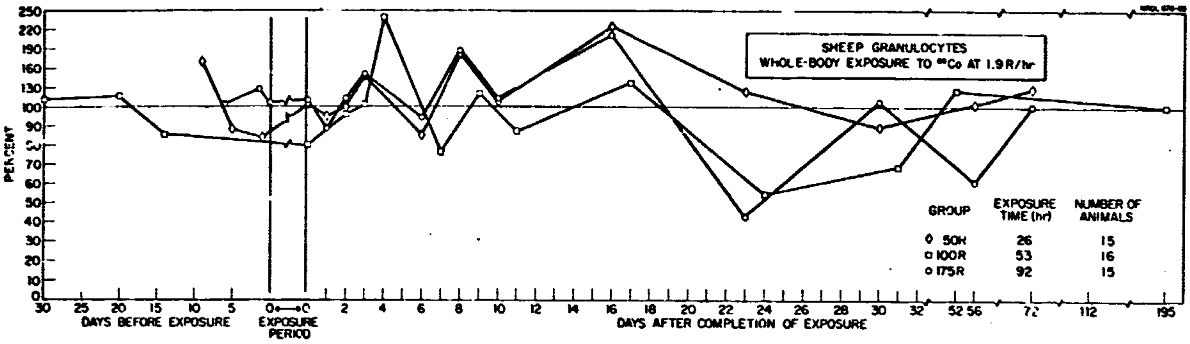

Figure 3. Granulocyte counts in sheep given exposures of 50, 100, or 175 R at 1.9 R/hr as percent of control.

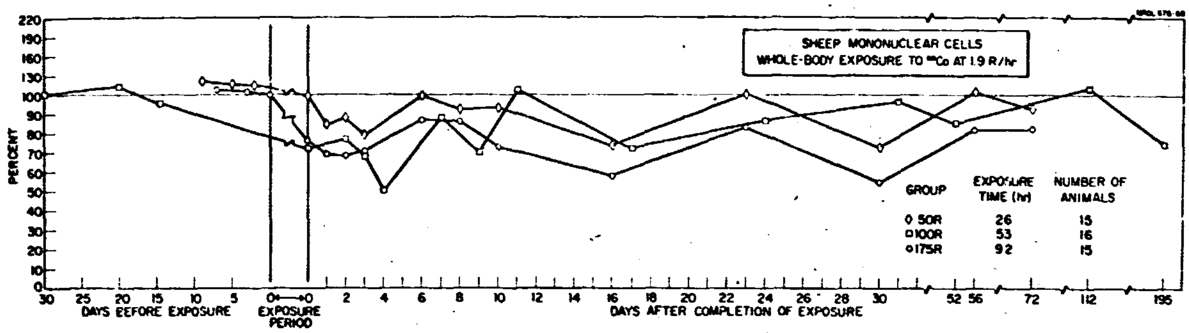

Figure 4. Mononuclear counts in sheep given exposures of 50, 100, or 175 R at 1.9 R/hr as percent of control.