FACTORS MODIFYING THE RESPONSE OF LARGE ANIMALS
TO LOW-INTENSITY RADIATION EXPOSURE

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In assessing the biological response to space radiation, two of the most important modifying factors are dose protraction and dose distribution to the body. Studies are reported in which sheep and swine were used to compare the hematology and lethality response resulting from radiation exposure encountered in a variety of forms, including acute (high dose-rate), chronic (low dose-rate), combinations of acute and chronic, and whether received as a continuous or as fractionated exposure. While sheep and swine are basically similar in response to acute radiation, their sensitivity to chronic irradiation is markedly different. Sheep remain relatively sensitive as the radiation exposure is protracted while swine are more resistant and capable of surviving extremely large doses of chronic irradiation. This response to chronic irradiation correlated well with changes in radiosensitivity and recovery following an acute, sublethal exposure. Swine recover remarkably fast and develop a large and persistant radioresistance. The change in radiosensitivity of sheep after either acute or chronic sublethal exposure is basically the same, consisting of a triphasic pattern of an initial slow recovery, transient radioresistance and regression into a long-lasting period of relative radiosensitivity. The overall effect of receiving both acute and chronic exposures within a short period of time may depend upon the sequence of the exposures. In addition to protraction, spatial or body distribution is a significant factor in the response of large animals to radiation exposure.

Somatic effects of radiation are generally categorized into the familiar classification of early and late effects as utilized for terrestrial forms of radiation (1). However, space radiation exposure takes on added dimensions and complexities not normally found in conventional earth exposures that makes dose-response estimations most difficult. Certain sources of radiation are predictable with a fair degree of certainty while others are quite unpredictable. The predictable or "expected" sources include the earth's trapped radiation belts, galactic cosmic radiation, and radiation from nuclear power systems. In the category of the unpredictable are such events as solar flares, excessive exposure to the nuclear reactor in emergency repair or during rendezvous procedures, and the inadvertent or uncontrollable orbiting of a spacecraft in the earth's radiation belts. While the probability of an unpredictable exposure may be minimal for a short lunar mission, it nevertheless must be considered possible, and perhaps even probable, if the missions increase in duration and frequency.

Exposure to radiation on a space mission will likely be in the form of a more-or-less constant, low-level background of 30-50 mrads per day from galactic radiation, with moderate to high-intensity exposures occuring during transit through the earth's trapped radiation belts or from periodic, and largely unpredictable, solar-flares. Although solar flares are generally brief, and of low intensity, they may range up

to a few days with peak dose-rates, of 10-20 rads per hour at the average depth of the bone marrow (2). Thus an intense solar flare lasting a day or two or several smaller flares could result in an exposure of several hundred rads to the space crew. In such a case, acute manifestations such as skin desquamation, prodromal responses, hematological depression and perhaps even lethality could result with disasterous consequences.

For space operations, certainly two of the most important factors that can modify the dose-response relationship are dose-protraction and nonuniform dose distribution to the body. The studies that we will report involved the use of large animals to explore these factors, especially as they relate to hematological depression and lethality from radiation exposure. We will employ the term "acute exposure" to refer to exposure at a high dose-rate, e.g., 450 R/hour. "Chronic exposure" will refer only to a continuous exposure at a low dose-rate. "Protraction" of an exposure is the process by which a total radiation dose is given over a longer time period. This could be accomplished by chronic exposure or by fractionation into two or more doses separated in time.

Both methods of dose protraction were used in these studies. Chronic exposure was studied with animals continuously exposed until death or by terminating the exposure after selected doses for LD50 determinations. Using the fractionated method experiments were conducted to

determine the effects of: a) fractionating acute exposures using various time intervals, b) chronic exposure followed immediately or after various time periods by acute exposures, and c) acute exposure followed immediately by chronic exposures. Figure 1 illustrates these various exposure situations.

This program was conducted at the Naval Radiological Defense Laboratory with funding provided by the Defense Atomic Support Agency and Office of Civil Defense. Unfortunately, a couple of the experiments were concluded prematurely due to the unanticipated closure of the laboratory. Since the animal management practices, dosimetry techniques, irradiation procedures and experimental design, have been previously reported (3,4) only highlights of the methodology will be repeated here.
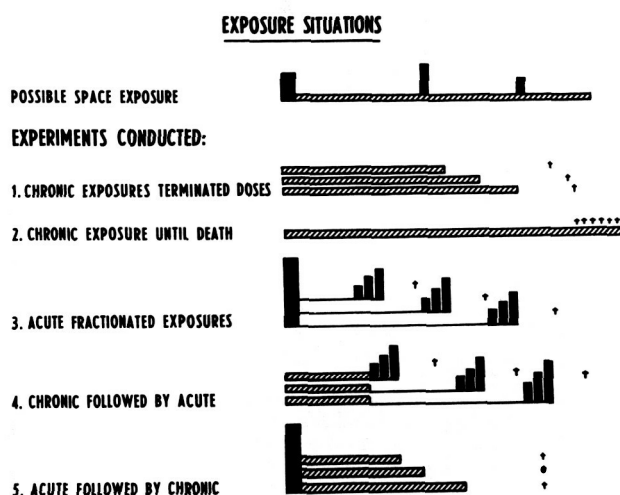
EXPOSURE SITUATIONS

Figure 1. Experimental situations as used in the NRDL large animal experiments and their relationship to possible space exposure.

METHODS AND MATERIALS

ANIMALS

Sheep and swine were the main species used in these studies. They were selected on the basis of their being more like man than rodents in body size, depth-dose distribution, basic (or acute) radiosensitivity, metabolic rate, and lifespan. Another important consideration was our ability to obtain them in large numbers, healthy and uniform in size and age, for the entire period of the study.

The sheep were obtained at one year of age from a single source located in the Sacramento Valley of California. They were castrated-males of Columbia-Rambouillet cross-breed, weighed 35-45 kg, and measured 23-30 cm in width at the abdomen at the time they were placed on experiment.

Except for the final few lots, swine were also obtained from a single local source, with an attempt being made to reduce biological variability by a planned breeding,

selection, and environmental control program. The swine were 8-12 month old, female, pure bred Durocs weighing approximately 90-110 kg at the time of irradiation.

RADIATION SOURCES AND EXPOSURE METHODS

Two types of radiation were used, cobalt-60 gamma and 1 Mvp X-rays. The cobalt-60 exposures were made at the NRDL Radiation Range, Camp Parks, California while the X-ray exposures were made with a GE Resotron, operated at 1000 KVp/3mA, producing X-rays having a HVL of 2.2 mm lead and an effective energy of approximately 300 Kev.

For the acute (high dose-rate) exposures the bilateral method of irradiation was used. For chronic (low dose-rate) irradiation, exposures were continuous for periods of up to 60 days. Since confinement in exposure boxes for such long periods was neither practical nor humane, the sheep were exposed in individual pens, 4 X 8 feet in size, situated on a gently sloping hillside, as schematically illustrated in Figure 2. This facility permitted the exposure of a large group of animals (up to 50) at the same time and same dose rate. In this configuration "uniform" whole-body exposure was dependent upon the animals' random movement in the pens since at any given time the exposure was unidirectional rather than bilateral. Food and water was provided on both sides of the pen to encourage turning of the animals during the exposure. Lithium flouride dosimeters secured to each side of groups of sheep indicated that the animal's movement resulted in equal exposure to both sides during the exposure period. Thus, both methods of exposure were effectively bilateral. Time required for servicing the pens averaged one to two hours every two days.
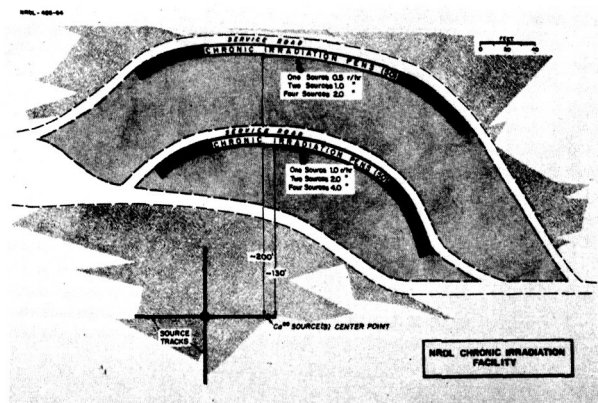

Figure 2. Schematic representation of NRDL cobalt-60 radiation range used for chronic irradiation of large animals.

DATA ANALYSIS

The method used to determine the median lethal doses (LD50's) and other parameters of mortality response was by probit analysis of the percent mortality on the natural logarithm of the radiation exposure. Dosimetric measurements were made with Landsverk, Victoreen and Phillips ionization chambers which were cross-calibrated with a National Bureau of Standards-calibrated Victoreen R-chamber. In addition thermoluminescent dosimetry (LiF) was used. The dose-rate was measured in air at the approximate midline of the exposure box or pen. The midline tissue dose, at maximum body diameter, was about 65% of the midline air dose for cobalt-60 and 60% for 1 Mvp X-ray. The radiation units expressed throughout this paper will be in Roentgens as measured in air.

RESULTS

DOSE PROTRACTION

Protraction of a given radiation dose can be attained mainly by two methods: a) by chronic exposure, i.e., lowering the dose-rate and continuously exposing the animals for a longer period of time, and b) fractionating or dividing the dose into two or more fractions with radiation-free time between exposures. Both methods were used in these studies.

A. CHRONIC EXPOSURES:

1. Terminated Exposures:

Studies to assess the effect of decreasing dose rate on dose-response (lethality) by terminating the exposures after giving predetermined doses were conducted with both sheep and swine. Table I presents the data while Figure 3 shows the correlation between the dose rates used and the LD50's that were obtained.
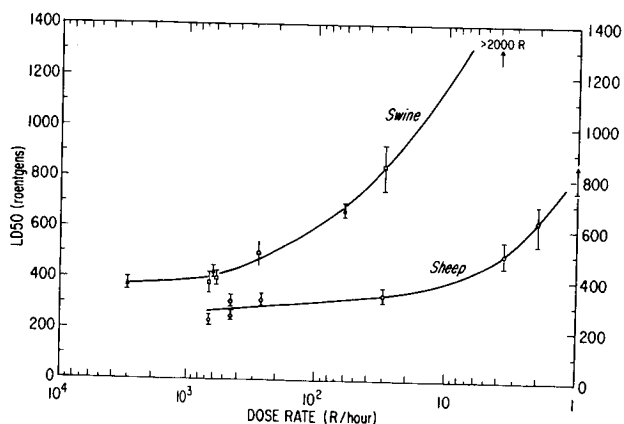
TABLE I
EFFECT OF RADIATION INTENSITY ON LD50 OF SHEEP AND SWINE

SPECIES	DOSE RATE (R/HOUR)	LD50/60 (ROENTGENS)	DURATION OF LD50 EXPOSURE (HOURS)
SHEEP	660	237 (215-257)	0.4
	450*	252 (233-276)	0.6
	450*	316 (297-335)	0.7
	261	318 (291-343)	1.2
	30	338 (313-369)	11.3
	3.6	495 (450-558)	137.5
	2.0	637 (538-698)	318.5
SWINE	651	381 (341-423)	0.6
	570*	399 (371-424)	0.7
	275	500	1.8
	30	849 (752-936)	28.3
	4	3444 (2259->5000)	861.0

Exposures were to cobalt-60 gamma or 1 Mvp X-rays*
Sheep: 35-45KG, yearling, castrated-male, Columbia-Rambouillet
Swine: 100-115 KG, 8-9 month Durocs; (NRDL - ♀; U.T.- ♀ and ♂)
Details of above LD50 studies can be found in references 3-8

In the sheep study the LD50's were determined within a short period of time, using sheep randomly selected from the same lots with the exception of the second LD50 at 450 R/hour, viz., 316 R. The LD50 of 316 R was determined a number of months later using sheep that appeared physically similar but from different lots. They had a slightly higher LD50 than was found previously at the same dose rate.

The results of a swine study conducted by Brown and Cragle (6) at the University of Tennessee Agricultural Research Laboratory are included in Figure 3 for comparative purposes. Those data correlate quite well with the NRDL data. It can be seen that swine show a considerable increase in LD50 as the dose rate is decreased below 600 R/hour. In contrast such a dramatic change in dose effectiveness does not occur with sheep until the dose rate has dropped below 30 R/hour.

When we consider the LD50 as a function of exposure-time (figure 4) the difference between sheep and swine are perhaps even more evident. As the exposure time is increased from 1/2 hour to 12 hours with sheep, the increase in LD50 is slight, perhaps 15-20%. Based upon the curve through the data points, protracting the exposure to 48 and 96 hours results in no more than 50 and 100% increase in the LD50.
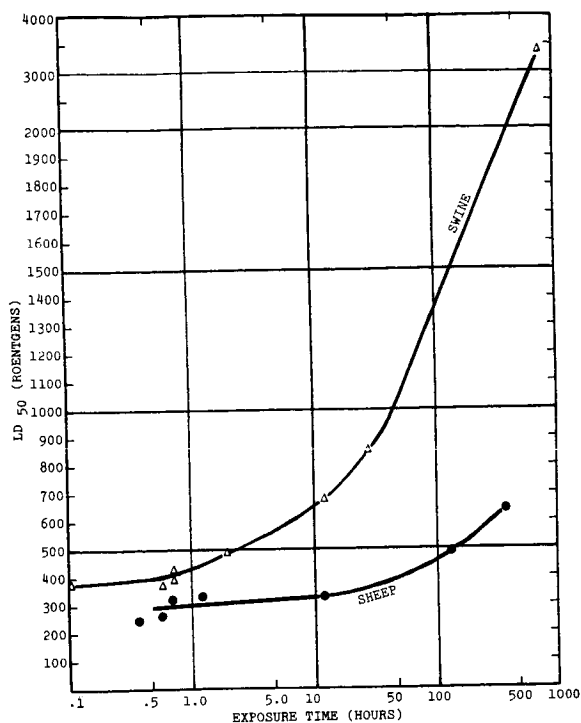
Figure 3. Comparison of radiosensitivity (LD50) of sheep and swine as related to dose-rate.

Figure 4. Comparison of radiosensitivity (LD50) of sheep and swine as related to exposure-time.

Figure 5. Percent effect dose as related to dose-rate of the exposure.

The effect of similar protraction with swine is much greater. When the exposure time is lengthened to 12, 48 and 96 hours, there is an increase in the LD50 of 75%, 100% and about 300%. Protraction of the exposure to 2 weeks results in a 4-5 fold increase in the LD50 of swine compared to 1-2 fold increase in sheep.

In Figure 5 the percent survival has been plotted as a function of the log of the dose rate. For sheep, the difference between 10 and 90% survival doses for dose rates of 30 R/hour and above was relatively constant amounting to no more than about 200 R. At 4 and 2 R/hour, the spread has doubled with a difference of 400 R. For swine the splaying out of effect curves is not obvious at 30 R/hour whereas at 4 R/hour the heterogeneity in response is enormous with well over 1000 R spread between the 10 and 90% effect doses.

2. Continuous Exposure Until Death:

These studies (9) were undertaken to determine the adaptability of a large animal to a continuous exposure at a low dose-rate, as indicated by the survival time and changes in the peripheral blood counts. The median time to death for sheep exposed continuously to cobalt-60 gamma radiation at a rate of 1.96 R/hour was 43 days. The first
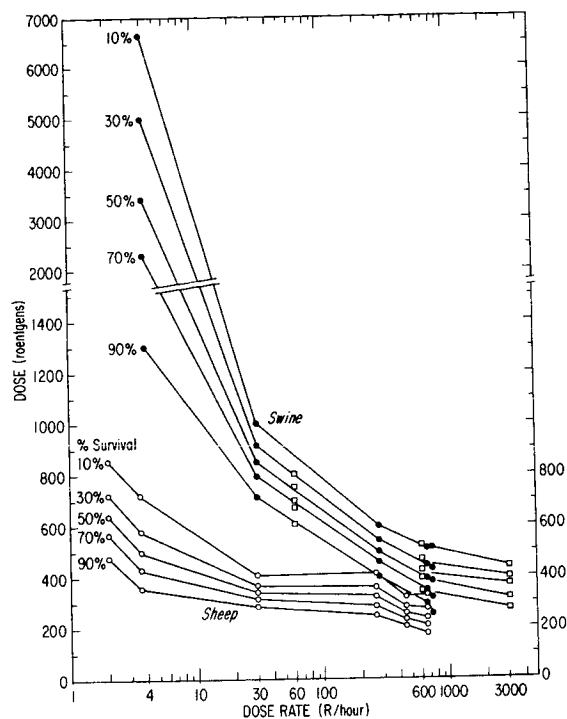
death occurred on day 25 after an accumulation of 1100 R; thereafter the deaths were sporadic in appearance, with no one period where a large number died. The last death was on the 60th day, after an accumulation of about 2760 R. The accumulated mortality is shown in Figure 6. Also included in this figure is the dose-response curve for sheep exposed at 2R/hour to predetermined doses. It is quite evident from the dose response curves that the dose required for a given effect is considerably less for terminated exposures, with a much greater slope to the curve.

The changes that occur in the peripheral blood cell counts are a fairly reliable indication of the injury sustained by the hematopoietic tissue following radiation exposure. In these animals, there was an almost immediate depression of the white cell count, reaching significant proportions by day 4 or after an exposure of 180 R. This early change can be attributed primarily to a decrease in the circulating mononuclear cells, for the granulocyte cells remained within the normal range for about 18 days. There is a suggestion of an abortive rise around 12-14 days. By the 25th day, both the mononuclear and granulocytic cells reached an average of 1000 or less and it was at this point that deaths began to occur. The pattern or
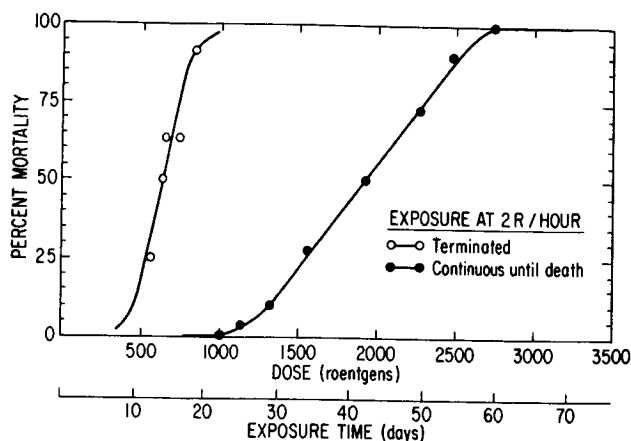
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Figure 6. Dose-response curves for sheep irradiated at 1.96 or 2 R/hour.

B. FRACTIONATED EXPOSURES:

The effect of fractionation on dose response was studied using three different sets of conditions: a) acute exposures separated by various time intervals, b) chronic exposure followed by acute exposures at various time intervals and c) acute exposure followed immediately by a chronic exposure. In keeping with terminology used extensively in the literature we will refer to the first or initial exposure as the conditioning dose.

1. Acute Exposures:

In this series, large groups of sheep and swine were acutely exposed to a conditioning dose of 2/3rds of their acute single-dose LD50, i.e. 177 R for sheep and 265 R for swine. The acute LD50 was determined on subgroups of the conditioned animals at various time intervals thereafter. The results are presented in Table II and Figure 8. Immediately after the conditioning exposure (zero-time) the LD50 plus the initial dose is equivalent to the single-dose LD50. When the conditioned sheep were allowed to wait for 7 or 11 days before re-exposure, there was little change in the LD50 from that found at zero-time. However, by 16 and 20 days the LD50's were greater than that of the controls indicating an induced-radioresistance. This resistance was quite transient, and by the 24th day the animals had reverted to a sensitive stage again, remaining that way at least through the 75th day after conditioning (11).

In contrast to the slow change in radiosensitivity with time found in the sheep study, the change for swine is rapid, such that by the seventh day the LD50 was approximately the same as that of the controls. An even greater radioresistance was found with swine in that by the 16th day, the LD50 was about 165% of controls. The induced-radioresistance was still evident at 61 and 107 days (8).

In a smaller study (5), sheep were conditioned with 100 R at 450 R/hour and LD50's determined at 7 and 16 days. Although this conditioning dose was only about 1/3rd of the acute LD50, a significant amount of the injury was not repaired by one week as the LD50 was still below that of the controls. By 16 days, the LD50 was 180% of controls indicating that a dramatic radioresistance had been induced. The data for this study are included in Table III while Figure 9 contains a curve pertaining to this study. Unfortunately it was not possible to conduct studies at later time periods due to the closure of the laboratory.

changes in the mononuclear and granulocytic cells is shown in Figure 7. It is most evident that sheep are unable to adapt to radiation exposures continuously given at a rate of 2 R/hour.

For comparative purposes, a group of sheep were exposed to 50, 100 or 175 R at a similar dose-rate, 1.9 R/hour, with leukocyte counts made at comparable time periods. Exposure times for these animals were approximately 1, 2 and 4 days. The results obtained again showed an early decrease in the leukocyte counts, reasonably correlated with the total dose. However, since the exposures were terminated before reaching lethal levels of injury a return to nearly normal occurred by the end of the third week. These studies demonstrated the significant effectiveness of low dose-rate exposure on the hematopoietic system of sheep (10).
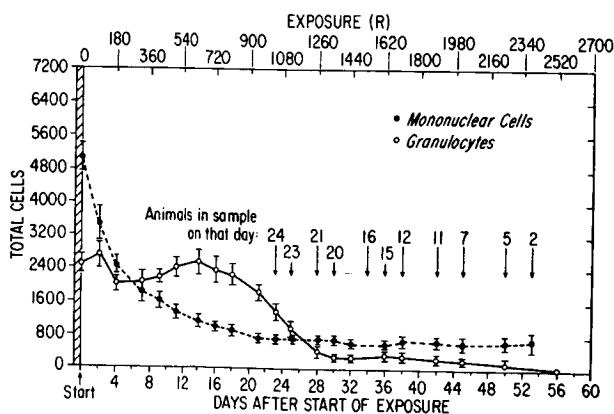

Figure 7. Peripheral leukocyte changes of sheep during continuous exposure at 1.96 R/hour.

TABLE II

RADIOSENSITIVITY (LD50) OF SHEEP AND SWINE AT VARIOUS TIMES AFTER A SUBLETHAL ACUTE EXPOSURE

SPECIES	SINGLE DOSE LD50	INITIAL DOSE	LD50 AT VARIOUS TIMES (DAYS) AFTER INITIAL EXPOSURE									
			0	3	7	11	16	20	24	30	45	75
SWINE[a]	399	265[b]	134	282	306	-	-	654	-	-	-	c
SHEEP	252	177	75	-	86	111	275	324	207	179	193	218
SHEEP	316	100	216	-	256	-	567	-	-	-	-	-

All exposures in Roentgens midline air dose. Radiation source - 1 Mvp X-ray
Dose rates used: Sheep - 450 R/hour; Swine - 540-600 R/hour
a Swine study conducted by Nachtwey (8)
b 240 R used for day 3 study
c LD50 after 61 days was approximately 700R; LD50 after 107 days was greater than 400 R
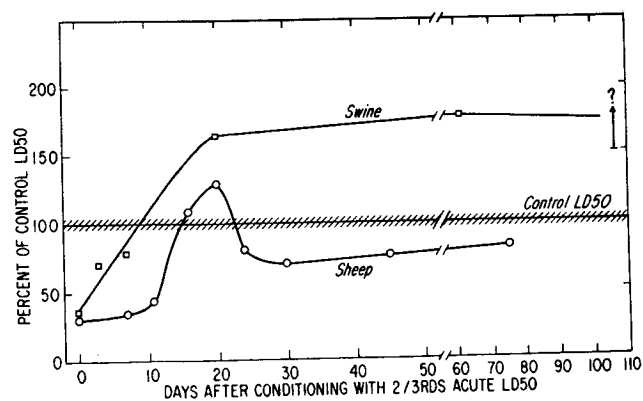   based upon 0/9 mortality from challenge with 399 R.

Figure 9. Temporal changes in radiosensitivity (LD50) of sheep after sublethal acute or chronic radiation exposures.

Figure 8. Changes in radiosensitivity (LD50) of sheep and swine following acute exposure to 2/3rds of their acute LD50.

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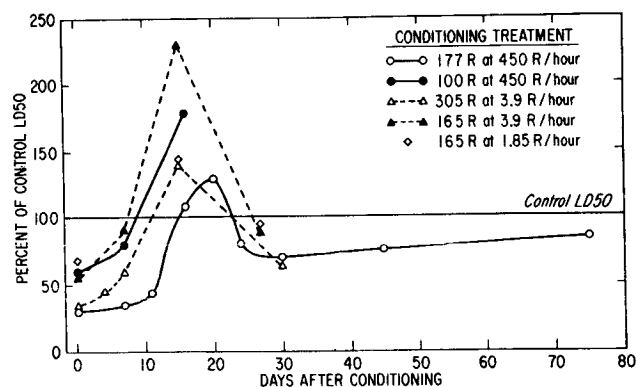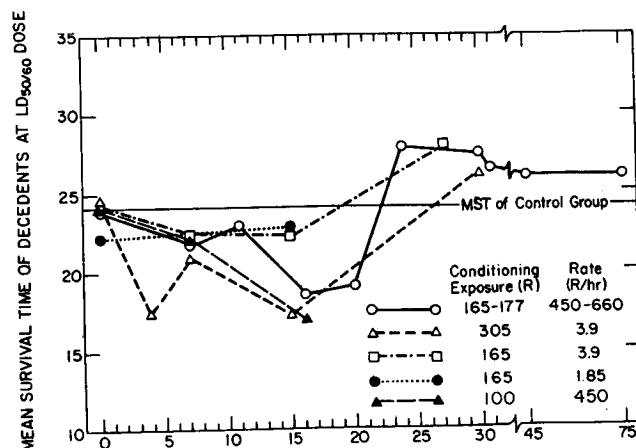Figure 10. The mean survival time of decedents at the LD50 dose as a function of time after sublethal conditioning radiation exposures.

TABLE III
ACUTE LD50 OF SHEEP AFTER A SUBLETHAL CHRONIC RADIATION EXPOSURE

INITIAL DOSE	DOSE RATE (R/HOUR)	SINGLE DOSE ACUTE LD50	ACUTE LD50 AT VARIOUS TIMES (DAYS) AFTER INITIAL EXPOSURE					
			0	4	7	15	27	30
305	3.9	260	86	118	157	361	-	163
165	3.9	237	133	-	218	546	210	-
165	1.85	237	162	-	-	342	225	-

2. <u>Chronic Exposure Followed by Acute Exposure</u>:

In this study, groups of sheep were conditioned by three different radiation regimens, 165 and 305 R at 3.9 R/hour and 165 R at 1.85 R/hour. The relative radiosensitivity with time was determined using the acute single-dose LD50 again as the baseline or reference value. Immediately (zero-time) and at various time intervals following the chronic irradiation, portions of the conditioned group were exposed to graded acute exposures for LD50 determinations. The data for these studies are presented in Table III, while Figure 9 illustrates the time-related changes in radiosensitivity (LD50). The reference LD50 for the 305 R experiment was not the same as that for the 165 R experiments due to different dates, animal lots and a slightly lower dose-rate employed for the acute LD50 determinations. However, the two LD50's are not statistically different at the 95% confidence level.

The acute LD50 at the end of the 165 R exposure at 1.85 R/hour was 162 R or 68% of normal. Theoretically if the two exposures were completely additive, the LD50 would have been 72 R (237 minus 165). Thus 93 of the 165 R (56%) was repaired by the end of the chronic exposure. The other chronic studies also showed non-additivity or repair of a significant portion of the injury produced during the chronic exposure. At the end of the 165 R exposure at 3.9 R/hour, 104 R injury was present or 61 R (37%) had been repaired.

The injury repaired during the 305 R exposure amounted to 131 R. This was a slightly higher percentage recovery (43%) at 305 R than was found after 165 R. The trend toward higher recovery rates with larger doses, continued for doses of 400 and 495 R as recovery rates of 50 and 52%, respectively, where found (3). This might indicate that a specific recovery mechanism lags somewhat at the beginning and gains impetus with the greater exposure times required for the larger doses.

A consistent pattern developed in sheep allowed to rest for various periods before being subjected to acute exposures. All groups were alike in that the change in radiosensitivity (LD50) was somewhat slow for several days and remained below normal at least for the first week. This slow change or recovery phase was followed toward the end of the second week or beginning of the third week by a rapid transition into a dramatic radioresistant condition. The degree of radioresistance varied from 140 to 230 percent of normal. This radio-resistance was quite transient, however, with all groups back to normal or slightly on the radiosensitive side by the 4th week.

The mean survival time (MST) of decedents at the LD50 dose was calculated by linear regression of the MST of each group on the dose received by it through the range of doses used in any particular group. The calculation of MST by this method allows a comparison of survival times normalized to a common biological endpoint, the LD50.

The survival time in the recovery experiments, appeared heavily influenced by the time interval between the sublethal conditioning exposure and the challenge LD50. In Figures 9 - 10, the derived mean survival time (MST) at the LD50 dose is graphed as a function of the time after conditioning. In all cases the MST's for animals re-exposed less than 20 days after conditioning by either an acute or protracted exposure, were considerably shorter than that of the controls. When the recovery period was greater than 20 days, the MST values were somewhat

longer than in controls and in most cases significantly longer than was seen when the recovery period was less than 20 days. The gross pathology and clinical symptomatology were not different for the specific groups. Since detailed physiological or cell kinetic studies were not conducted, one can only offer speculation to explain the nature of such findings. Death likely results from damage to several different tissues although that of the gastrointestinal tract and hematopoietic system are considered the most important in the midlethal range. Each undoubtedly has its own characteristic time course of injury and recovery. The differences observed in the survival time conceivably could reflect an alteration in tissue radiosensitivity, the capability of the stem cell constituents to respond to a second dose of radiation, or perhaps even a change in the relative contribution of various syndromes, e.g., gastrointestinal or hematopoietic, to the lethal injury.

3. Acute Followed by a Chronic Exposure:

Animals in this study (12) were acutely conditioned with 155 R cobalt-60 gamma radiation at a rate of 510 R/hour and immediately exposed to predetermined graded doses at 3.85 R/hour. The LD50 at 3.85 R/hour for the acutely conditioned sheep was 171 R. Thus the combined acute exposure plus the protracted LD50 was 326 R, compared to the single-dose acute LD50 of 314 R. Based upon previous studies, 45 - 50% recovery occurs during chronic exposure at 3.6 R/hour (3). Had a similar recovery occurred in this situation, the LD50 at 3.85 R/hour would have been greater than 300 R, about double that actually found. It can be inferred that the acute exposure has suppressed the recovery mechanisms that operate in the protracted exposure.

DISCUSSION

Of the numerous factors that can modify the dose-response relationship to space radiation, two of the most important are dose protraction and nonuniform dose distribution within the body. It is generally conceded that the reduction in dose-effectiveness observed in protracted exposure is due to recovery mechanisms that act to offset the injury as it is produced. This paper has dealt with the total of the recovery processes and has not attempted to identify the specific mechanisms involved, e.g., intracellular repair or repopulation of vital stem cells.

The two methods of protracting an exposure, i.e., by continuous exposure at lowered dose-rates (chronic exposure) or fractionating the exposure into two or more doses, were compared in these large animal experiments. It is quite apparent

TABLE IV

EFFECT OF ACUTE SUBLETHAL EXPOSURE ON DOSE RESPONSE TO SUBSEQUENT CHRONIC EXPOSURE

Control LD50 at 510 R/Hour	314 R
Conditioning Dose at 510 R/Hour	155 R
Difference	159 R
LD50 of Conditioned Animals at 3.85 R/Hour	171 R
Expected LD50 Based Upon 45-50% Recovery During Exposure at 3.85 R/Hour	>300 R
Recovery Rate at 3.85 R/Hour After an Acute Exposure	7%

CONCLUSION: Acute Exposure Has Suppressed Ability To Recover During Low Dose-Rate Exposure

that the dose-response to both chronic irradiation and fractionation varies greatly between the sheep and swine. The ability of swine to survive large doses of radiation under protracted conditions was also found by Brown et al (6), using daily fractions of 50 or 100 R/day until death. The exposure was at a moderate dose-rate, about 30 R/hour; thus the actual exposure times were about 1 1/2 - 3 hours per day. In those studies swine demonstrated a remarkable ability to outlive cattle and burros. The mean accumulative lethal doses at 100 R/hour averaged 3900 R for swine compared to 3200 R for cattle and 2330 for the burro. The results at 50 R/hour were even more striking. At that rate, the mean lethal dose for swine was over 10,000 R compared to 2250 and 1510 R for cattle and burros, respectively. Thus the LD50 of 3444 R found at NRDL under chronic exposure at 4 R/hour (approximately 100 R/day) and the mean accumulative lethal dose of 3900 R found at the University of Tennessee at 100 R/day fractionated exposures are quite comparable, especially if one discounts a certain portion of the 3900 R as unnecessary or wasted radiation.

In contrast to the remarkable ability of swine to survive at 50 R/day with over half the animals alive after 200 days, none of the sheep chronically exposed at slightly less than 50 R/day survived past 60 days, with the median time of death 43 days. In effect the swine survived about 5 times as long. In going from an acute exposure to 4 R/hour continuous exposure, the ratio of chronic LD50 to acute LD50 for sheep was about 2.5%. For swine, their remarkable recovery ability again was demonstrated as the ratio of chronic:acute was nearly 9:1.

The rapid recovery and large and persistent radioresistance of swine following an acute sublethal exposure again differed from the recovery of sheep. Sheep were

slow to begin recovery and while they also progressed into a resistant state by the end of the second week, it was quite transient and by the 24th day was gone. There are data from the University of Tennessee laboratory that would tend to support the persistent radioresistance of swine. Shively et al (13) found the LD50 for swine exposed 4 months previous was 60% greater than the controls of the earlier study.

It became apparent from the dose-rate and recovery studies that swine were not the preferred animal model for extrapolation purposes. With this in mind the great bulk of the large animal studies at NRDL were conducted with sheep. Recovery or relative radiosensitivity patterns were determined after exposures to radiation at either acute or chronic exposure rates. In addition the influence of size of the exposure on subsequent recovery was assessed.

We were a little surprised with the consistency in recovery patterns of sheep after both high and low levels of injury produced at acute or chronic dose-rates. In all cases the changes in radiosensitivity (or recovery) consisted of a triphasic pattern with an initial slow phase for the first week in which no group had returned to normal by the 7th day. This was followed by an induced-radioresistance toward the end of the second week. The radioresistance phase was transient, however, and the sheep had returned to a slightly sensitive state during the third week.

While neither the dose-rate nor size of the conditioning exposure changed the basic pattern, there were differences in the extent and temporal patterns of some of the fluctuations. It would appear that recovery after acute exposure is slower than after chronic exposure. The greatest radioresistance was induced by a dose equivalent to 1/3 of the LD50, i.e., 100 R at 450 R/hour and 165 R at 4 R/hour, with LD50's 180 and 230% of the controls at 15-16 days. It was financially impossible to describe the curves at more time points as one might desire. It is probable that the 15-16 days do not represent the maximum overshot or resistance stage since no testing was done after the chronic exposures in the period of 16-27 days. In fact the 20th day was the most resistant time after the 177R acute exposure. One might speculate that an optimum dose exists for stimulating marrow cell proliferation which likely accounts for the resistance condition. If the dose is too great, the stock of progenitor cells may be reduced to a level which takes time to repopulate with minimal capability to overshoot. If the dose is too small, the stimulus for repopulation may not be as dynamic.

A significant and unexpected finding was the influence of an acute exposure to negate the usual recovery that takes place during chronic exposure. In such a situation where an acute exposure precedes a chronic exposure the doses are additive. This conceivably could be of considerable importance in assessing the effect of radiation on space travelers. Under certain conditions in which both acute and chronic exposures are received the assigning of 0.5 as a relative injury factor for chronic exposure, as suggested by the Space Radiation Study Panel (1), may not be too appropriate. Due to the potential importance of this point we feel this situation should be explored further using additional large animal species, such as the nonhuman primate and dog.

Table V summarizes the differences that we have observed in additivity of acute and chronic exposures under the various conditions as described in this paper.

TABLE V
DIFFERENCES IN ADDITIVITY OF ACUTE AND CHRONIC EXPOSURES OF SHEEP

1ST EXPOSURE	2ND EXPOSURE	EFFECT	INTERPRETATION
ACUTE (165 R at 660 R/hour)	ACUTE (0-11 days after 1st exposure)	ADDITIVE No immediate recovery 8% recovery at 7 days 20% recovery at 11 days	SLOW RECOVERY during 1st week after acute exposure
CHRONIC (165 R at 3.9 R/hour)	ACUTE (immediately and 7 days after 1st exposure)	NON-ADDITIVE 50% recovery during chronic exposure; 90% recovery at 7 days	FAST RECOVERY during chronic exposure. Nearly complete recovery within one week.
ACUTE (155 R at 510 R hour)	CHRONIC (exposure at 3.9 R/hour immediately after acute exposure)	ADDITIVE 7% recovery during chronic exposure	SLOW RECOVERY during chronic exposure which is preceded by acute exposure

Nonuniform distribution to the body can also be a significant modifier of the dose-response relationship. It is probable that space exposure will be relatively nonuniform due to variations in shielding within the space craft, and the unidirectional aspect of solar and nuclear reactor radiations. Due to body size and thus self-shielding, nonuniform exposure of man and large animals is an important consideration. The observed ratio of midline tissue dose (MLT) to a midline air dose (MLA) is highly dependent upon the size of the animal. The following values for Cobalt-60 or x-irradiation (250-1000 kvp) were presented in a recent survey (14): .82 - .86 for dogs, .6 - .68 for swine, .58 - .65 for sheep, and .40 - .50 for cattle and burros. A factor of .65 is used by Lushbaugh, et al (15) to convert exposure dose to an epigastric rad dose for man.

With a large inhomogeneity of dose distribution, one would expect that the unilateral exposure might be considerably less effective than a bilateral exposure if the damage to bone marrow is the critical determinant for survival. The gamma and X-ray unilateral LD50's for larger animals are generally 20-30% greater than bilateral LD50's while the dog irradiated dorsally has been reported to have an LD50 approximately 50-60% higher than with bilateral irradiation (14). Bond and Robinson (16) concluded that the main factor in the decreased effectiveness of nonuniform (unilateral) exposure versus uniform (bilateral) exposure, is the relatively large fraction of stem cells surviving in areas of bone marrow receiving the lowest dose.

It would appear that shielding of only a minute but select portion of the bone marrow can have a dramatic protective effect. In a study by Cole (17) at NRDL, lead shielding, completely surrounding a single elbow of the dog for a length of 4 - 6 inches, resulted in 50% survival at 1000 R a dose 3 X greater than the whole-body LD50/30. Those animals that did die, succumbed between 6 - 8 days from what appeared to be gastrointestinal involvement. A relatively complete shielding of a single elbow was more effective than the use of a larger total amount of lead placed over all four elbows. This apparent paradox was explained on the basis of the exponential nature of cell-killing by irradiation.

The results described from these large animal studies have demonstrated that dose protraction and spatial distribution are certainly important modifying factors in determining the response to a given radiation exposure. It is quite evident that there are major differences between swine and sheep in their response to chronic exposure, pattern of recovery and relative radiosensitivity following a sublethal exposure. In attempting to extrapolate animal results to assess effects in man, selection of the appropriate animal model becomes of considerable importance. Based upon the results obtained in radiotherapy, we propose that sheep are a better model for man than is swine or small laboratory rodents. While the rhesus monkey would appear to be the choice for performance studies, he suffers greatly from being small and not as comparable in depth-dose and dose distribution to specific organs. In addition the rhesus monkey has an acute LD50 in the range of 500 - 600 R which is greater than man's.

The studies of Lushbaugh, et al (15) demonstrate that man's response to both acute and chronic radiation is reasonably close to that found with the sheep. The acute LD50 for radiotherapy patients is about 250 rads. It appears that man is slow to repair radiation-induced hematopoietic damage and remains relatively radiosensitive as the dose-rate is decreased. Using Bateman's (18) method of indexing radiosensitivity response to changes in intensity by relating the LD50 to the reciprocal cube root of the dose-rate, a slope constant for normal man was found to be quite close to that of sheep (19). Bateman's analysis of mice data in the literature and these sheep and swine data gave results of 0.65 for sheep, 0.95 for mice and 1.6 for swine. Thus, of all species analyzed by that method sheep would appear the closest to man. In addition, Lushbaugh found that fractionated, daily, acute exposures over an 8 day period had a greater effect than the same dose given chronic (continuously) over the same exposure period.

Unfortunately, data on the nonhuman primate, especially the rhesus monkey, are conflicting as to the recovery rate and their response to chronic exposure. We have more confidence in using the data obtained with sheep for assessing hematopoietic and lethality effects in man than that obtained with swine or rodents.

SUMMARY

1. While sheep and swine are basically similar in response to acute radiation, their sensitivity to chronic irradiation is markedly different. Sheep remain relatively sensitive as the radiation exposure is protracted while swine are more resistant and capable of surviving extremely large doses of chronic irradiation.
2. This response to chronic irradiation correlated well with changes in radiosensitivity and recovery following an acute, sublethal exposure. Swine recover remarkably fast and develop a large and persistant radioresistance. The change in radiosensitivity of sheep after either acute or chronic sublethal exposure is basically the same, consisting of a triphasic pattern of an initial slow recovery, transient radioresistance and regression into a long-lasting period of relative radiosensitivity.
3. The overall effect of receiving both acute and chronic exposures within a short period of time may depend upon the sequence of the exposures. At least with sheep an acute exposure appears to reduce the recovery potential for chronic exposures that follow within a short time. In such a situation the individual response to both acute and low chronic exposures are additive. In contrast the response to a chronic exposure before an acute exposure is not additive with significant recovery occurring during the chronic exposure.
4. The spatial or dose distribution within the body of a large animal is a significant factor. Unilateral or partial-body exposure is considerably less effective for a given dose than is a bilateral or total body exposure. A simple lead-cuff around a small but select portion of the bone marrow can result in significant protection.

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