# LYMPHATIC INVOLUTION AND EARLY MORTALITY IN THE YOUNG CHICKEN PRODUCED BY 2.2 GeV PROTONS[1]

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1. Supported in part by NASA (NsG 214-62)
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4. Brookhaven National Laboratory is supported by the U.S.A.E.C.

Young single-comb white Leghorn cockerels were subjected to single acute doses of either 2.2 GeV protons or 250 kVp X-rays. Since young chickens exposed in the lethal range die within 48 hours of exposure, an hourly tabulation of deaths was recorded for this length of time after exposure. Animals which were exposed to sublethal doses were killed five days after exposure and their major lymphatic organs, (thymus, bursa, and spleen), removed and weighed.

In the lethal range, animals exposed to 2.2 GeV protons died sooner than those receiving similar doses of X-rays, but total mortality was similar in each case at similar dose levels. The 48 hour $LD_{50}$ was determined to be 710 rad. Measured five days after exposure, 50% depression ($ED_{50}$) for lymphatic organs occurred as follows: thymus, 350 rad; bursa 500 rad; spleen 450 rad. In all case R.B.E. values were not different from unity.

The total dose from protons of this energy is comprised of a low LET portion due to dE/dx energy loss and a high LET portion due to nuclear interactions. R.B.E. values considerably below unity would be predicted on the basis of dE/dx energy loss alone. It is suggested, then, that the R.B.E. of that portion of the dose contributed by nuclear interactions is very high. If high LET interactions account for only 5% of the total dose, then these interactions could have an R.B.E. great as 7.

## INTRODUCTION

Exposure of young chickens to electromagnetic radiations at dose rates above 15 R/min. results in death within 48 hours of exposure (ref. 1) with $LD_{50/48\ hour}$ values between 700 R and 1000 R. Vascular leakage, hypotension, circulatory failure, and renal failure have been the major characteristics associated with this early death. (ref. 2). This is quite different from the case in mammals where death occurs 8 - 30 days after exposure to similar doses. Inability of irradiated cells, particularly those involved in blood cell formation, to reproduce is the prime cause of death. Alterations in vascular permeability do occur in mammals, including man, after irradiation but these are generally transitory. Using the young chicken, it was possible to measure the effectiveness of 2.2 GeV protons in producing a physiologic change whose endpoint (death) was easily

determined.

Lymphatic organ weight loss in mice has been shown to be a reproducible measure of radiation dose and has been used as an endpoint for R.B.E. studies (ref. 3,4). This is also the case in the young chicken, where lymphatic organ weight loss has been used as a biological dosimeter and a measure of R.B.E. values for thermal and fission neutrons. (ref. 5).

In the experiments reported here we have used these two endpoints, early mortality and involution of lymphatic organs, to examine the relative biological effectiveness of the 2.2 GeV proton beam of the Brookhaven National Laboratory Cosmotron.

## MATERIALS AND METHODS

Animals - Single comb white leghorn cockerels were obtained from Klager Hatchery, Saline, Michigan, shortly after hatching. Within 24 hours the animals were transported to the Medical Department of Brookhaven National Laboratory and housed, 50 per tier in a standard chick brooder. Purina starter mash and water were available ad libitum. Radiation exposures were performed on the third day of age. The number of animals in each experimental group is indicated in Table I and Table II. In the mortality study, an hourly tabulation of deaths was maintained for thirty hours after exposure. Animals involved in the lymphatic-involution study were killed and dissected five days after exposure. Body weights were recorded to the nearest gram and wet weights of thymus, bursa of Fabricius and spleen recorded to the nearest 0.1 mg. Because the lymphatic organs of the chick, like those of mammals, are sensitive to adrenal

Table I

Acute (30 Hour) Death Following Exposure to 250 KVP X-Rays or 2.2 GEV Protons

| Dose[1] | N | Time Post Exposure In Hours* | | | | | | | | | | | | | | | | | | | | | | | | | | Fraction Killed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X-Rays | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | (12 | 16) | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| 713 | 40 | | | 2 | | | | 1 | | | | | | | 5 | 8 | 2 | 1 | | 1 | 1 | | | 1 | | | | 22/40 |
| 808 | 30 | 1 | 2 | 2 | 2 | | | 2 | | | | | 3 | 3 | 4 | | | 1 | | 1 | | 1 | | | | | | 22/30 |
| 903 | 30 | 1 | 2 | 2 | 7 | 3 | 1 | | | | 1 | | 1 | 1 | 2 | 1 | 2 | 2 | | | | | | | 2 | 1 | | 29/30 |
| Protons | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 575 | 16 | | 1 | | | | | | | | | | | | | | | | | | | | | 1 | | | | 2/16 |
| 613 | 8 | | | | | 1 | | | | | | | | | | | | | | | | | | 1 | | | | 2/8 |
| 647 | 16 | | | | | | | | | | | | | | | | | | | | | | | | | | | 0/16 |
| 690 | 10 | | | | | 1 | | | | | | 1 | | | | | | | | | | 3 | | | | | | 5/10 |
| 744 | 16 | | | | | 5 | 4 | | 1 | | | | | | | | | | | | | | | | | | | 10/16 |
| 795 | 16 | | | | 5 | 5 | | 3 | | | | | | | | | | | | | | | | | | | | 13/16 |
| 842 | 16 | 1 | | 6 | | 3 | 2 | | | | | | | | | | | | | | | | | | | | | 12/16 |
| 913 | 16 | | 1 | 12 | | | 2 | | | | | | | | | | | | | | | | | | | | | 15/16 |
| 963 | 16 | | | 6 | | 9 | | | 1 | | | | | | | | | | | | | | | | | | | 16/16 |
| 1081 | 16 | | | 5 | | 8 | | | 1 | | | | | | | | | | | | | | | | | | | 14/16 |
| 1206 | 8 | 3 | 1 | | 1 | 1 | | 2 | | | | | | | | | | | | | | | | | | | | 8/8 |

1. Dose in rads

*Numbers in these columns indicate the number of animals dead during hourly intervals indicated.

Table II

Lymphatic Organ Weights Following Exposure to 2.2 GeV Protons or 250 KVp X-Rays

| | Dose[1] | N | Body[2] Weight | Thymus[3] | Bursa | Spleen |
|---|---|---|---|---|---|---|
| Control | 0 | 10 | 53 | 309 | 283 | 106 |
| X-Rays | 190 | 10 | 54 | 247 | 232 | 84 |
| | 380 | 10 | 51 | 158 | 170 | 52 |
| | 570 | 10 | 49 | 113 | 124 | 41 |
| Protons | 112 | 9 | 56 | 242 | 242 | 91 |
| | 188 | 6 | 54 | 231 | 267 | 74 |
| | 230 | 5 | 52 | 185 | 237 | 75 |
| | 299 | 5 | 51 | 143 | 176 | 71 |
| | 316 | 5 | 49 | 171 | 175 | 55 |
| | 344 | 5 | 50 | 148 | 196 | 62 |
| | 386 | 7 | 50 | 146 | 168 | 62 |
| | 406 | 5 | 52 | 150 | 196 | 56 |
| | 435 | 5 | 51 | 139 | 151 | 57 |
| | 437 | 5 | 51 | 112 | 151 | 47 |
| | 498 | 5 | 49 | 96 | 139 | 43 |
| | 522 | 5 | 48 | 98 | 142 | 48 |
| | 551 | 6 | 46 | 83 | 124 | 43 |
| | 576 | 6 | 47 | 109 | 140 | 45 |
| | 613 | 7 | 48 | 113 | 152 | 42 |
| | 647 | 5 | 47 | 85 | 123 | 34 |

1. Dose in rads
2. Body weight in grams
3. Organ weights in mg/100 gms. body wight

corticoid release following stress (ref. 6, 7) all animals were maintained in the exposure holders for periods equal to that of the longest exposure. Half of the control animals were sham-irradiated in the proton exposure apparatus and half in the X-ray chamber. No difference was seen between the two control groups, so they were pooled for subsequent analysis. To compensate for variations in body weights between groups, organ weights are presented as mg/100 gm of body weight.

## EXPOSURE CONDITIONS

Protons - Proton exposures were carried out as previously described for mice (ref. 8, 4) and rats (ref. 9), in a lucite tube 4.5 cm in diameter and 128 cm in length. Four chicks were exposed per Cosmotron run. The exposure tube was rotated about its longitudinal axis at 20 rpm. The beam was pulsed 25/min. with a pulse duration of 1 m sec. giving an instantaneous dose rate of 14,000 rads/sec. during pulses. The integrated dose rate was 350 rads/min. from the pulsed Cosmotron beam. Dosimetry of the proton beam has been previously described. (ref. 8, 10). The doses expressed in this paper are based on: $2.82 \times 10^7$ p/cm$^2$ = 1 rad.

X-Rays - The source of X-rays was a standard therapy machine operated under the following conditions: 250 kVp; 15 mA; 0.5 mm Cu, and 1.0 mm Al added filtration. The dose-rate measured 60 cm from the target with a Victoreen condenser R-meter was 118 R/min. (under conditions of maximum backscatter). In order to compare the absorbed X-ray dose with that of protons, 1 R was considered equivalent to 0.95 rads. Eight or ten chicks were exposed at a time in a rotating lucite chamber 23 cm in diameter and 4 cm thick.

## RESULTS

Lethality - The time distribution of deaths following exposure to either X-rays or protons is given in Table I. In the X-ray group, all animals which died, with one exception, did so in the intervals 3 - 9 hours or 17 - 30 hours after exposure. As the dose was increased, a larger percentage of the animals died in the earlier (3 - 9 hours) period. Animals which died following proton exposure did so primarily between 3 and 11 hours after exposure, regardless of dose or 30-hour mortality level. The few animals which did die in the later period were, however, in the lower dose groups. A probit plot of the accumulated 30-hour mortality is presented in Figure 1. A visual fit of the data yields a 50% mortality ($LD_{50/30\ hours}$) dose of 710 rads for both protons and X-rays. The single line in Figure 1 fit both the proton and X-ray points.

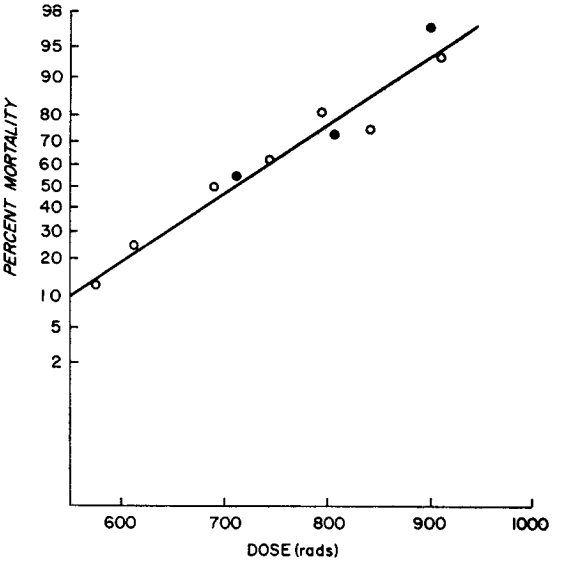


Figure 1. Thirty-hour mortality in the young chicken following proton or X-irradiation.

Lymphatic Organ Involution - Body weights and lymphatic organ weights obtained five days after exposure to sublethal doses of either X-rays or protons are given in Table II. Organ weights, as percentage of unirradiated controls are presented in Figure 2 (thymus), Figure 3 (bursa), and Figure 4 (spleen). From these figures, 50% depression ($ED_{50}$) values of approximately 350 rads (thymus), 500 rads (bursa) and 450 rads (spleen) were obtained for protons and nearly identical values for X-rays.

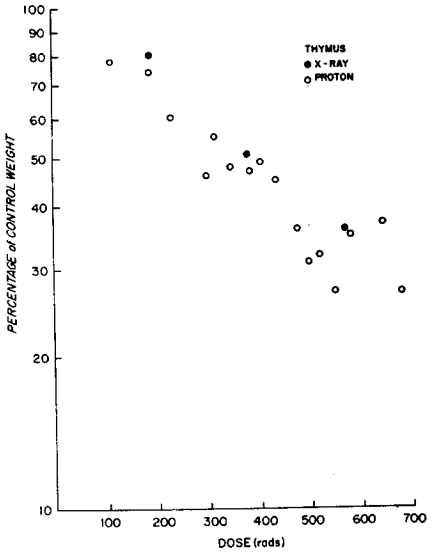


Figure 2. Thymic weights five days after proton or X-irradiation.

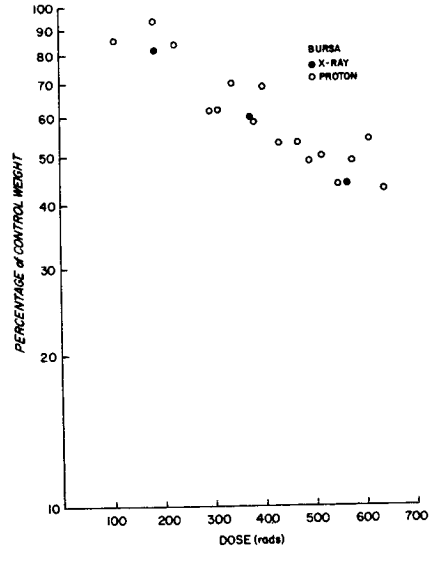


Figure 3. Bursal weights five days after proton or X-irradiation.

## DISCUSSION

From Figure 1 it can readily be seen that both proton irradiated and X-irradiated chicks have $LD_{50/30\text{-hour}}$ values of 710 rads, giving an R.B.E. for early mortality of 1.0. When high LET radiations such as fission neutrons are employed, not only is the R.B.E. considerably above 1 (4.2 for early mortality, ref. 5) but the slope of the mortality curve is much steeper. For 2.2 GeV protons this is not the case. From Figure 1 it can be seen that a single slope is sufficient to describe both the proton and X-ray mortality curves. Both the R.B.E. of 1.0 and the identical slopes of the mortality curves strongly suggest that these protons act primarily as low LET radiations. The only difference seen between the proton and X-ray groups is the tendency for the proton bombarded animals to die in the earlier of the two mortality periods. Although the reasons for this are not clear, it is not suggestive of high LET exposures since our own unpublished observations indicate that neutron exposed animals die fairly uniformly throughout the 3 - 30 hour post-exposure period and do not exhibit the bimodal concentration of deaths seen with X-rays.

The weights of the thymus, bursa, and spleen five days after exposure to various doses are shown as percentages of control weights in Figure 2, Figure 3, Figure 4. These curves reflect decreases in cell populations due to both scopal (direct) and abscopal (indirect) radiation damages and subsequent recovery and repopulation. Five days after irradiation, the net result of these processes gives an approximately straight

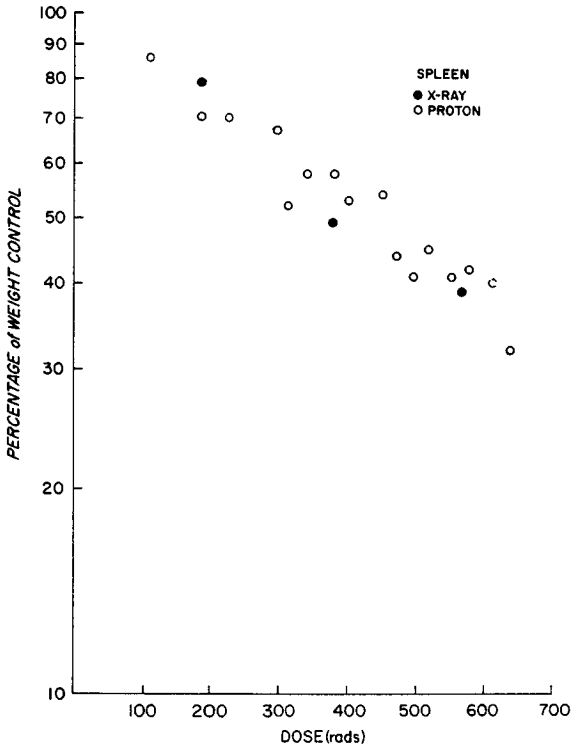


Figure 4. Splenic weights five days after proton or X-irradiation.

line dose-response relationship. The $ED_{50}$ values of 350 rad (thymus), 500 rad (bursa) and 450 rad (spleen) are useful for determining R.B.E. values but are not indicative of cell sensitivity since considerable repair has occurred, especially at the lower doses. In each case, any line fit through the proton points could equally fit the X-ray points indicating that the R.B.E. of 2.2 GeV protons for lymphatic organ involution is indistinguishable from unity.

These experiments strongly suggest that the minimally ionizing 2.2 GeV protons act primarily as low LET radiation, but do not exclude the possibility that a small component of high LET is present.

Ueno and Grigoriev (ref. 11) have suggested that a single R.B.E. of 0.82 ± 0.04 can be assigned to all mammalian endpoints tested for protons between 126 MeV and 730 MeV. This certainly is not the case with the 2.2 GeV protons from the Brookhaven Cosmotron. R.B.E. values from 0.87 to 2.5 have been found under identical exposure and dosimetric conditions and, in the case of mouse experiments, the same strain of animals (ref. 4,8,9,12, and this paper). The R.B.E. of the 2.2 GeV protons would, therefore, appear to be dependent upon the particular biological endpoint under study.

These conclusions have certain practical implication for space flights. Since the protons act as low LET radiation, radioprotective chemicals suitable for man would be effective against high energy protons( ref. 9, 13). The effectiveness of low LET radiations is dose-rate dependent for most endpoints. The establishment of acceptable dose limits for proton exposures in space should, therefore, consider expected dose-rates as well as total doses. In addition, since the R.B.E. of protons differs for different endpoints, the tolerance dose limits must also consider what biological endpoints are critical.

ACKNOWLEDGEMENTS

The authors wish to thank Miss R.F. Straub, Drs. W. H. Moore, E. P. Cronkite, V. P. Bond, and the Cosmotron crew.

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