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RADIOBIOLOGICAL STUDIES IN THE YOUNG CHICKEN.

The University of Michigan, Ph.D., 1966
Biophysics

University Microfilms, Inc., Ann Arbor, Michigan

RADIOBIOLOGICAL STUDIES IN THE YOUNG CHICKEN

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in the
University of Michigan
1966

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ABSTRACT

Early mortality in the young chick was found to fall into two distinct time periods following exposure to X-rays, thermal neutrons or fission neutrons.

A log organ weight-linear dose response was found for lymphatic depression following exposure to X-rays, thermal neutrons or fission neutrons. Aminoethylisothiouronium (AET) was effective in protecting against both early death and lymphatic involution following exposure to these three types of ionizing radiations.

The lymphatic involution following radiation exposure was determined by partial body exposure techniques to be the result of three different types of injury: 1) scopal damage or direct killing of lymphatic lymphocytes by the ionizing radiation, 2) 'chick-type' abscopal damage, that is involution through indirect processes as the result of damage to tissues other than lymphatic tissues, in this case the same injury which at higher doses leads to early mortality, and 3) 'mammalian-type' abscopal damage or stress resultant from injury to the gastrointestinal tract.

To Joan

Whose patience and love have been tested far more

by this work than any scientific principle

ACKNOWLEDGEMENTS

Professor Claire J Shellabarger by example of his own outstanding research and through his tireless direction and assistance made the conception, construction and completion of these works challenging and rewarding events.

Gerald D. Abrams, Billy E. Frye, John A. Jacquez and the late Charles H. Burns gave unselfishly of their time in discussion of this manuscript.

Throughout the training for and writing of this manuscript my fellow students A. P. Jacobson, M. M. Nofal, and R. C. Riley were sources of stimulating thoughts and ideas.

I would like to express my appreciation to Dr. V. P. Bond and the Medical Department of the Brookhaven National Laboratory for providing facilities and assistance for several of these experiments.

The support and encouragement of W. N. Montour, N. A. Montour and F. O. Montour needs to be recognized far more than is possible here. The friendship and devotion of Corky have been most reassuring throughout my academic training.

The widely separated and most original works of Hieronimus Fabricius of Aquapendente, J. Jolly and John A. Jacquez and David A. Karnofsky were alone sufficient background for the experimental work of this volume.

I would like to express my appreciation for the generous assistance of Mrs. Kathleen McDermott in the preparation of this manuscript.

Finally, the friendship and guidance of Professor E. H. Bashor over the past decade have been a joy and inspiration through many trying experiences.

Financial assistance from the American Cancer Society (Fellowship), Phoenix Memorial Fund (Fellowship), National Cancer Institute and the National Aeronautics and Space Administration are gratefully acknowledged.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	viii
I. INTRODUCTION	1
II. GENERAL METHODS AND MATERIALS	6
A. Animals	6
1. Procurement and Care	6
2. Dissection	7
B. Radiation Exposure Conditions	7
1. X-rays	7
a. High dose rate exposure	8
b. Low dose rate exposure	8
2. Thermal Neutron Exposure	9
3. Fission Neutron Exposure	9
4. 2.2 GeV Proton Exposure	9
III. EXPERIMENTS	11
A. X-ray Induced Early Mortality and Chemical Protection in the Young Chicken	11
1. Introduction	11
2. Methods and Materials	11
3. Results and Discussion	12
4. Summary	21

TABLE OF CONTENTS (continued)

		Page
B.	Early Mortality and Chemical Protection Following Exposure to Densely Ionizing Radiations	22
	1. Introduction	22
	2. Methods and Materials	23
	3. Results	24
	a. Thermal Neutrons	24
	b. Fission Neutrons	31
	c. 2.2 GeV Protons	32
	4. Discussion	32
	5. Summary	38
C.	Involution of Chick Lymphatic Tissues Following High and Low Dose Rate X-ray Exposures	39
	1. Introduction	39
	2. Methods and Materials	39
	3. Results	40
	4. Discussion	47
	5. Summary	50
D.	Lymphatic Response to Densely Ionizing Radiations: Chemical Modification With AET	52
	1. Introduction	52
	2. Methods and Materials	52
	3. Results	54
	a. X-ray Exposure	54
	b. Thermal Neutrons	59
	c. Fission Neutrons	68
	d. 2.2 GeV Protons	73
	4. Discussion	73
	5. Summary	78
E.	Lymphatic Depression Following Partial Body X-irradiation of the Chick	80
	1. Introduction	80
	2. Methods and Materials	80
	3. Results	84
	a. Thymic Response	84
	b. Bursal Response	89

TABLE OF CONTENTS (continued)

	Page
4. Discussion	89
a. Scopal Damage	89
b. Abscopal Damage	89
5. Summary	92
F. X-ray Induced Thymic Depression in Surgically Bursectomized Chicks	95
1. Introduction	95
2. Methods and Materials	95
3. Results	96
4. Discussion	96
5. Summary	98
IV. GENERAL DISCUSSION AND SUMMARY	99
REFERENCES	102

LIST OF TABLES

Table		Page
I.	Hourly Tabulation of Deaths Following Exposure to 750 R, 850 R, and 950 R of X-rays, With and Without Prior AET Administration	14
II.	Effects of AET Administration on 48-Hour Mortality in X-irradiated Chicks	16
III.	Hourly Tabulation of Deaths Following Exposure to Thermal Neutrons, With and Without Prior AET Administration	26
IV.	Hourly Tabulation of Deaths Following Exposure to Fission Neutrons, With and Without Prior AET Administration	28
V.	Hourly Tabulation of Deaths Following Exposure to 2.2 GeV Protons	30
VI.	Survival Time and RBE Values for Early Chick Mortality	34
VII.	Response of Chick Lymphatic Tissue to HDR and LDR X-ray Exposures	44
VIII.	The Relative Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in Producing Mortality and Lymphatic Depression in the Chick	61
IX.	A Comparison of the Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in the Chick and the Mouse	63
X.	X-ray Induced Thymic Involution in Bursectomized Chicks	97

LIST OF FIGURES

Figure		Page
1.	AET Protection Against Early Radiation Mortality	19
2.	Lymphatic Tissue Dose Response to Low Dose Rate and High Dose Rate X-irradiation	42
3.	Involution and Recovery Following LDR and HDR X-irradiation	46
4.	Thymic and Bursal Response to X-rays With and Without Prior AET Treatment: 2 days post-exposure	56
5.	Thymic and Bursal Response to X-rays With and Without Prior AET Treatment: 5 days post-exposure	58
6.	Thymic and Bursal Response to Thermal Neutrons With and Without Prior AET Treatment: 2 days post-exposure	65
7.	Thymic and Bursal Response to Thermal Neutrons With and Without Prior AET Treatment: 5 days post-exposure	67
8.	Thymic and Bursal Response to Fission Neutrons With and Without Prior AET Treatment: 2 days post-exposure	70
9.	Thymic and Bursal Response to Fission Neutrons With and Without Prior AET Treatment: 5 days post-exposure	72
10.	Total Lymphatic Tissue Response to 2.2 GeV Protons: 2 days and 5 days post-exposure	75
11.	Partial Body Exposure Design	82
12.	Thymic Involution Following Whole-body, Anterior-body, or Posterior-body X-irradiation	86
13.	Bursal Involution Following Whole-body, Anterior-body, or Posterior-body X-irradiation	88
14.	Proposed Components of Radiation Damage to Chick Lymphatic Tissues	94

I. INTRODUCTION

Mortality in mammals following a single exposure to ionizing radiation usually occurs within 30 days. This Acute Radiation Syndrome has characteristically been divided into three portions which have been termed 1) the Central Nervous System Syndrome, 2) the Gastro-intestinal Syndrome, and 3) the Hematopoetic Syndrome (Bond, et al., 1953). The Central Nervous System Syndrome is known to occur only after extremely large radiation doses, that is on the order of 10,000 or more roentgens. Further, it is known that the Central Nervous System Syndrome can be produced by exposure of only the central nervous system. This syndrome is characterized by death within a few hours of radiation exposure. The Gastro-intestinal Syndrome and the Hematopeotic Syndrome occur following more modest radiation doses, in the order of a few hundreds of roentgens. Death occurs within a few days but no deaths occur before three days post-exposure. The Acute Radiation Syndrome in mammals is only moderately sensitive to changes in dose rates. That is to say, it makes little difference whether the exposure is given in a few seconds or in a few hours. Lea (1947), Thomson and Tourtellotte (1953), Neal (1960), and Bateman, et al. (1962) have demonstrated that as the dose rate decreases, the total radiation dose needed to produce a given level of mortality increases. The lethal processes involved, however, are not different even when the difference in dose rate is a factor of thousands.

If the young chicken is exposed at dose rates below approximately 10 R/min. mortality patterns are exhibited which are comparable to those of mammals. An additional lethal process was discovered by Jacquez and Karnofsky (1950) who found that with dose rates above 15 R/min. the majority of chicks exposed to 1000 R of X-rays died not within days after the exposure but within 48 hours after the exposure. By exposing only portions of the chick body to X-irradiation and by using doses no larger than 1000 R, Stearner, et al. (1954) excluded participation of the central nervous system in this early or 48 hour mortality. Stearner, et al. (1955) attributed this early 48 hour mortality to renal failure, hypotension, and circulatory failure. In addition, Stearner (1954) described widespread minute hemorrhages that were characteristically found in the soft tissues exposed to the radiation beam but not in adjacent shielded tissues following partial body exposure. Thus, when the radiation exposure rate is below 10 R/min. no mortality occurs within 48 hours of exposure, but if the exposure rate exceeds 15 R/min. 100 per cent mortality occurs within 48 hours of exposure to 1000 R; that is, the young chicken exhibits a completely different pattern of mortality when the dose rate is increased from less than 10 R/min. to 15 R/min. or above. Similar findings have been reported for the duck (Stearner, 1951) but not for parakeets or canaries (Stearner, et al. 1962). Apparently, no other Avian species have been examined for dose rate sensitivity. Among all the mammals which have been examined, no similar dose rate phenomenon has been discovered with the possible exception of the rabbit (Prosser, 1947), and even here it has apparently not been possible to produce a 100 per cent mortality within 48 hours of radiation exposure.

Mortality is, of course, only one measure of radiation injury. One of the most studied effects of radiation exposure is the involution of lymphatic organs. In fact, the involution of the thymus and spleen in mammals has been shown to be a consistent dose dependent response to ionizing radiation (Carter, et al. 1950). Evidence of involution of the mammalian thymus and spleen following chronic or acute stress has been well documented (Doughtery, 1952) and is considered part of the stress syndrome of Selye (Selye, 1950). Despite the overall stress involved in whole body irradiation, the subsequent involution of thymus and spleen is principally the result of direct or scopal effects on the irradiated lymphocytes and only secondarily due to indirect or abscopal effects (Corp, et al. 1961) or stress.

The mechanism of the 48 hour mortality of the young chick is incompletely understood. This early mortality is clearly not due to effects on the central nervous system. The gross anatomical lesions and subsequent physiological alterations which are eventually responsible for the 48 hour death in the chicken are thought to be known. It is believed that the lesions are preceded by changes in capillary permeability. The cause of the permeability changes however is not known. For the following reasons it is likely that the primary cause of the permeability changes is not cell death resulting from radiation damage to nucleo-protein and a suppression of mitosis. 1) Histological examination has failed to provide evidence of cell death as a cause of the changes in capillary permeability. 2) Chicks exposed to a large sub-lethal radiation dose are rendered refractory to a second exposure given from 4 to over 24 hours later (Stearner, et al. 1961). When radiation injury is the result of cell death the damage from two exposures separated by 4 to 24 hours would be nearly additive (Elkind

and Sinclair, 1965 and Elkind, et al. 1965). Published data on cell turnover rates suggest that it is unlikely that any compensatory cell proliferation could occur as early as 4 hours following the initial injury (Bond, et al. 1965). 3) In all systems where radiation induced cell death is a primary cause of injury, all dose rate effects are reduced or eliminated when the radiation source is changed from low LET X- or gamma rays to high LET neutrons. In the early mortality of the chicken, the dose rate effect is equally evident whether X-rays or neutrons are used as the radiation source (Vogel and Stearner, 1955). All of the above, when taken together, suggest that the early radiation mortality of the young chick is not due to cell death produced directly by radiation exposure.

Three major lymphatic organs are found in birds: the thymus, the spleen, and the bursa of Fabricius (Adelmann, 1942). The thymus and spleen are homologous to the mammalian thymus and spleen but the bursa is uniquely Avian. Because of similarities in histology and in physiological responses, the bursa was long thought to be an accessory thymic tissue and to have no function different from that of the thymus (Jolly, 1915). Recent evidence suggests, however, that in birds the bursa is responsible for the formation of potential antibody producing cells while the thymus is responsible for the production of homograft immunity (Arvy, 1963), whereas in mammals these both appear to be functions of the thymus. Recent evidence (Sutherland, et al. 1964) has suggested a possible analogy between the immunological capacity of the rabbit appendix and the chick bursa.

The Avian thymus, bursa, and spleen have been shown to respond to stress in a manner similar to the mammalian thymus and spleen (Newcomer, et al. 1960). Although Jolly in 1915 showed that the thymus and bursa

both involute following X-irradiation no quantitative study has been reported dealing with the dose response of chick lymphatic tissues to ionizing radiations.

As detailed above, it is thought that early radiation mortality in the chick is not due to direct radiation induced cell death. It appeared worthwhile to study the response of the chick lymphatic tissue to ionizing radiation since these responses are due to direct radiation produced cell death. The young chick provided the opportunity of studying both a cell death phenomenon and a non-cell death phenomenon in the same animal.

Several areas of current interest in Radiation Biology were examined using the two different endpoints, mortality and lymphatic involution in the young chicken. Wherever possible, information obtained in these experiments was applied toward further understanding of the unique dose-rate response of the chicken. This study considered the following topics: 1) survival and survival time following exposure to various types of ionizing radiations; 2) involution of lymphatic tissue following exposure to various types of ionizing radiations; 3) modification of lymphatic response to ionizing radiations by the use of partial body irradiation techniques; and 4) modification of post-irradiation survival and lymphatic involution by means of a chemical radioprotective agent.

II. GENERAL METHODS AND MATERIALS

Methods and materials generally employed throughout this investigation are described below. Modifications or alterations pertinent only to a single experiment are described in the appropriate chapter.

A. Animals

1. Procurement and Care

White Leghorn cockerels obtained from a single hatchery* were used in all of these experiments. Birds selected to weigh between 37 and 39 grams at hatching were housed in a standard chick brooder and allowed food (Purine Starter Mash) and water ad libitum . Prior to each experiment, the average weight was determined and only those chicks within 10% of this weight were used for mortality studies and those within 5% for lymphatic studies. In order to eliminate inaccuracies in the recording of body weights due to filled crops, the animals were weighed in the morning before feeding had begun. Food found in the crop during dissection was removed before the body weight was measured.

A post-hatch mortality of 1% to 2% was not uncommon even in non-irradiated animals. These deaths most often occurred between 5 and 8 days after hatching, apparently from starvation although food was available. For this reason, any bird which weighed less than 30 grams

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* Klager Hatchery, Saline, Michigan

at the termination of an experiment was eliminated regardless of its treatment. Several of the mortality studies utilized newly hatched, unfed chicks. The response of these did not differ noticeably from that of fed chicks of the same age when observations were limited to the ensuing 48 hour period.

2. Dissection

Birds were killed and dissected at appropriate daily intervals from the first through the eighth days post-irradiation for thymus, bursa, and spleen weight determinations. Once the pattern of response was determined measurements were usually limited to the second, fifth, and eighth days post-irradiation. The second day was chosen because it most commonly represented maximum involution, the fifth day was chosen in order to compare chick data with published mammalian data usually given on the fifth post-irradiation day, and the eighth day because the degree of recovery was evident in the weights of the lymphatic organs at that time. On the day of dissection the chicks were weighed to the nearest gram, killed with ether and their thymus, bursa and spleen removed and weighed to the nearest 0.1 mg.

B. Radiation Exposure Conditions

1. X-rays

The source of X-rays was a conventional therapy machine operated at 250 kilovolt peak (kvp) and 15 milliamperes(ma) with 0.5 mm Al and 1.0 mm Cu added filtration. The first half of value layer measured 55 cm from the X-ray target was 1.6 mm of Cu (Trout, 1960). Exposure doses were measured with a commercially calibrated Victoreen Condenser R meter. For comparison with other types of irradiation 1 R (exposure dose) of X-rays was estimated to be approximately equivalent to 1 rad

(absorbed dose). Since all dose rates above 10 R/min to 15 R/min. were capable of producing early mortality in the chicken while those below 10 R/min. were not, the former were categorically termed "high dose rate" (HDR) and the latter "low dose rate" (LDR).

a. High Dose Rate Exposure

Groups of 20 or fewer animals were exposed in a cylindrical polyethylene chamber 9 3/4 inches in diameter and 1 3/4 inches in height which was rotated in the radiation field at approximately 2/3 RPM. The dose rate measured at the midpoint of the rotating chamber was approximately 100 R/min. A decrease in dose of less than 5% was found at the perimeter of the chamber and a difference of less than 5% was found between the top and bottom of the chamber. Various doses were obtained by altering the exposure time.

b. Low Dose Rate Exposure

A maximum of 40 chicks per group were exposed in a cardboard cylinder 12.5 inches in diameter and 3.0 inches in height which was rotated in the radiation field at approximately 2/3 RPM. At a distance of 185.4 cm from the X-ray target the dose rate was approximately 8.8 R/min. A difference of less than 1% was found between the dose measured at the periphery and at the midpoint of the exposure chamber. At this distance from the target, a difference of approximately 1% was found between the dose measured at the top and at the bottom of the exposure chamber. Various doses were obtained by altering the exposure time. When groups of 20 or less were exposed the diameter of the exposure chamber was reduced to 10 inches by means of a foam rubber ring in order to maintain constant radiation scattering conditions among the exposed birds.

2. Thermal Neutron Exposure

Thermal neutron exposures were conducted in the biology facility of the Brookhaven National Laboratory Graphite Reactor (Curtis, et al. 1956). The Brookhaven National Laboratory reactor was chosen over the University of Michigan reactor because it possessed an easily accessible, well calibrated thermal column suitable for biological studies with small animals. No such biological facility was available at the University of Michigan reactor. Eighteen or fewer animals per group were exposed in an 8 inch square lucite chamber 1.5 inches in height to a thermal neutron flux of 3.0×10^9 neutrons per cm^2 per second as determined by the activation of gold foil (Curtis, et al. 1956). An additional 0.21 rad/sec. of gamma radiation was present (Handloser, et al. 1955). Various doses were obtained by altering the exposure time.

3. Fission Neutron Exposure

Fission neutron exposures also were conducted in the biology facility of the Brookhaven National Laboratory Graphite Reactor for the reasons mentioned above. By placing a 0.1 cm thick U^{235} fission plate below the exposure chamber described above 29.5 rad/min. of neutrons having a degraded fission spectrum of energies was obtained (Handloser, et al. 1955). The birds were exposed in groups of 16 or less in a 7.5 inch square lucite chamber 1.5 inches in height placed above the fission plate. Gamma contamination was reduced to approximately 10% of the neutron dose by a 1 inch thick bismuth plate interposed between the fission plate and the exposure chamber.

4. 2.2 GeV (2.2×10^9 eV) Proton Exposure*

Proton exposures were performed in the Brookhaven National Laboratory Proton Synchrotron or "Cosmotron" (Lapp and Andrews, 1963).

* The prefix giga (G) indicates a multiple of 10^9

Exposure procedures for both chicks and mice have been described in detail by Jesseph, et al. (1964) and by Bond (1965). Briefly these were as follows. The animals were exposed in groups of 5 to 8 in either a 2.5 cm diameter tube (mice) or in a 4.4 cm diameter tube (chicks) 43.2 cm in length. The proton beam was essentially parallel to the axis of the tube. The exposure tube was rotated in the beam approximately 20 RPM in order to compensate for any variation in particle distribution over the presenting area of the tube. Monitoring of the incident flux was accomplished by measuring the C^{11} induced radioactivity in polyethylene wafers placed over the cross sectional area of the exposure tube. The proton beam was pulsed every 2 seconds with a pulse duration of 1 millisecond giving an average dose rate of approximately 500 rad/min. and an instantaneous dose rate of approximately 10^4 rad/sec. Although accurate dosimetric measurements were made in terms of C^{11} activation and measurement, the estimates of absorbed dose obtained from these measurements, while internally consistent, remain at present only approximations. In addition, responses in other biological systems were not available for comparison. In addition to providing further information concerning radiation responses in the chicken, these experiments added considerably to the knowledge of the biological effectiveness of particles of this energy. The study of particles whose energy is in the GeV range is of interest for at least two reasons. First these particles are very sparsely ionizing but do react with atomic nuclei to cause the formation of nuclear fragments which in turn may be extremely ionizing in nature. Second, these particles are representative of the energies of particles found in solar flares and intergalactic space. The understanding of the biological interactions of these particles is a prime factor in determining the radiation hazard in space. (Saksonov, et al. 1963)

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III. EXPERIMENTS

A. X-ray Induced Early Mortality and Chemical Protection in the Young Chicken

1. Introduction

The objective of this experiment was to examine the dose survival response and the survival time of chicks exposed to doses of X-rays in the lethal range. In addition, the effectiveness of 2-aminoethylisothiouronium bromide (AET) in modifying the response of the chick to lethal amounts of X-radiation was tested. AET is one of a group of sulfhydryl compounds which has been found effective in reducing radiation damage in a wide variety of biological systems (Bacq, 1965). Early radiation mortality in the young chick has been reported not to be reduced by several of these compounds (Beaumariage, 1957 and 1958).

2. Methods and Materials

Day-old white Leghorn cockerels were exposed to 0 R, 750 R, 850 R, or 950 R of X-rays under high dose rate conditions (103 R/min.) with or without prior AET administration. Fifteen minutes before exposure, half of the animals were injected subcutaneously with 10.5 mg (approximately 300 mg/kg of body weight) of AET neutralized in 0.1 cc of 0.1 N NaOH. These animals were then combined for exposure with a similar number of saline injected chicks so that in all cases the AET injected chicks and their controls were irradiated under identical conditions.

Food and water were withheld throughout the 48 hour experimental period.

3. Results and Discussion

The early or 48 hour radiation mortality of the chick has been reported previously (Jacquez and Karnofsky, 1950 and Stearner, 1951). In these publications, survival time was reported only in terms of days after exposure. In the present experiment, continuous observation of the irradiated birds allowed an hourly tabulation of mortality (Montour and Shellabarger, 1963). When this was done, it became apparent that the chicks died within two distinct time periods and with very little overlap between the periods (Table I). Of a total of 95 early deaths in all groups, 33 occurred between 3 and 10 hours post-irradiation, 60 occurred between 17 and 30 hours post-irradiation, and only one in each of the intervals 10 through 17 and 30 through 48 hours post-irradiation (Table II). The average survival time decreased as the dose increased but the individual animals died either 3 to 10 hours or 17 to 30 hours post-exposure regardless of the radiation dose.

As the radiation dose was increased, the number of animals dying in the 3-10 hour post-irradiation period increased. The maximum 3 to 10 hour mortality level in this experiment was 53% following 950 R. In other experiments 100% mortality was obtained in the 3-10 hour period with doses of 1050 R to 1100 R.

The dose response in the 17-30 hour period was considerably obscured by the appearance of deaths in the 3-10 hour period. Approximately equal numbers of animals died in the 17-30 hour period at each of the 3 radiation doses. When analyzed in terms of 10 hour survivors, an increase in 17-30 hour mortality approaching 100% was seen.

Pretreatment with AET effectively protected the acutely irradiated young chicken from death in the period 0 to 48 hours post-exposure at all radiation doses employed in this experiment. There did not appear

TABLE I

Hourly Tabulation of Deaths Following Exposure to 750 R, 850 R, or 950 R of X-rays, With and Without Prior AET Administration

Chicks were exposed to 250 kvp X-rays at a dose rate of 103 R/ minute.

95 of the 200 irradiated chicks died in the early period (48 hours). All of the deaths except 2 occurred in the time periods 3 to 10 and 17 to 30 post-exposure regardless of the dose. Mortality was reduced by the pre-irradiation injection of 300 mg/kg of AET but the pattern of deaths was not altered.

TABLE I

Hourly Tabulation of Deaths Following Exposure to 750R, 850 R, or 950R of X-rays, With and Without Prior AET Administration

Time of deaths in 48-hour mortality period of acutely irradiated chick (Hrs. post-irradiation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
a			1	2	2	7	3	1						1			1	1	2	1	2	2							2	1
a*				1		1	1		1								1		1	2		2	1			2	1			1
b			1	2	2	2			2								3	3	4			1		1		1				
b*																	1	1					1							
c					2				1										5	8	2	1		1	1			1		
c*							1		1															1			1			
d																														
d*																														

* treated with AET

Group a: 950R, N=30
b: 850 30
c: 750 40
d: 0 10

TABLE II

Effects of AET Administration on 48-Hour Mortality in X-irradiated Chicks

In the 17 to 30 hour post-irradiation period the mortality level at 750 R (48%) was not exceeded at higher doses. The number of deaths in the 3 to 10 hour post-irradiation period increased with increasing doses. Pre-irradiation injection of AET reduced mortality in both the 3 to 10 hour period (850 R and 950 R) and the 17 to 30 hour period (750 R and 850 R). The $LD_{50}/2$ day* for 250 kvp X-rays was approximately 750 R.

* The 50% lethal dose (LD_{50}) is the dose required to kill half of a given population within a specified time period.

TABLE II

Effects of AET Administration on 48-hour Mortality in X-irradiated Chicks

(Per cent mortality of irradiated animals given in parentheses)

Treatment	Dose	N	3-9 hr	10-16 hr	17-30 hr	31-48 hr	Total 48 hr
Saline	0 R	10	0	0	0	0	0
AET	0 R	10	0	0	0	0	0
Saline	750 R	40	3 (8%)	0	19 (48%)	0	22 (55%)
AET	750 R	40	2 (5%)	0	2 (5%)	0	4 (10%)
Saline	850 R	30	8 (27%)	0	13 (43%)	1 (3%)	22 (73%)
AET	850 R	30	0	0	3 (10%)	0	3 (10%)
Saline	950 R	30	16 (53%)	1 (3%)	12 (40%)	0	29 (97%)
AET	950 R	30	4 (13%)	0	11 (37%)	0	15 (50%)
Total		220	33	1	60	1	95

to be preferential protection in either the 3 to 10 or the 17 to 30 hour periods, but instead the AET acted to produce an effective dose reduction of 200 R. For example, the number and time of death following 950 R plus AET or 750 R alone are quite similar (Fig. 1). The total mortality in each case was approximately 50% with 10% in the 3 to 10 hour period and 40% in the 17 to 30 hour period.

In the AET treated chicks as in the control chicks, death within 48 hours of irradiation occurred in the intervals of 3 to 10 and 17 to 30 hours post-exposure. From this it was deduced that AET was acting to negate a portion of the primary injury, but once the injury level was sufficiently high, subsequent steps leading to death followed as in the untreated, irradiated chicks.

Beaumariage (1957, 1958) had reported that cystamine, cysteamine, cysteine, and cyanide were ineffective in reducing early radiation mortality in the chick. If the early mortality of the chick could not be modified to any extent by any of the sulfhydryl radioprotective agents then one or more of the following would be suggested. 1) In the chicken the protective chemicals do not reach the critical or radiosensitive site in sufficient concentration to be effective. 2) The effect of these chemicals is specifically on intracellular components whose damage is not involved in early chick mortality. 3) The protective compounds act only on repair processes and the initial injury leading to early chick death is not subject to repair.

The data reported here strongly discourage such a general interpretation of Beaumariage's results. The negative findings of that author might easily have been due to the compound or the dose of the compound used. The effects of AET were described in the present study because it was the most effective compound of those tested in reducing

FIGURE 1

AET Protection Against Early Radiation Mortality

Significantly greater survival was obtained at each dose level in the groups pretreated with AET. The pattern of deaths seen following 950 R + AET was very similar to that seen following 750 R, indicating a dose reduction with AET of approximately 200 R. Of 95 deaths in all groups only one fell in each of the periods 10-16 hours and 31-48 hours post-exposure.

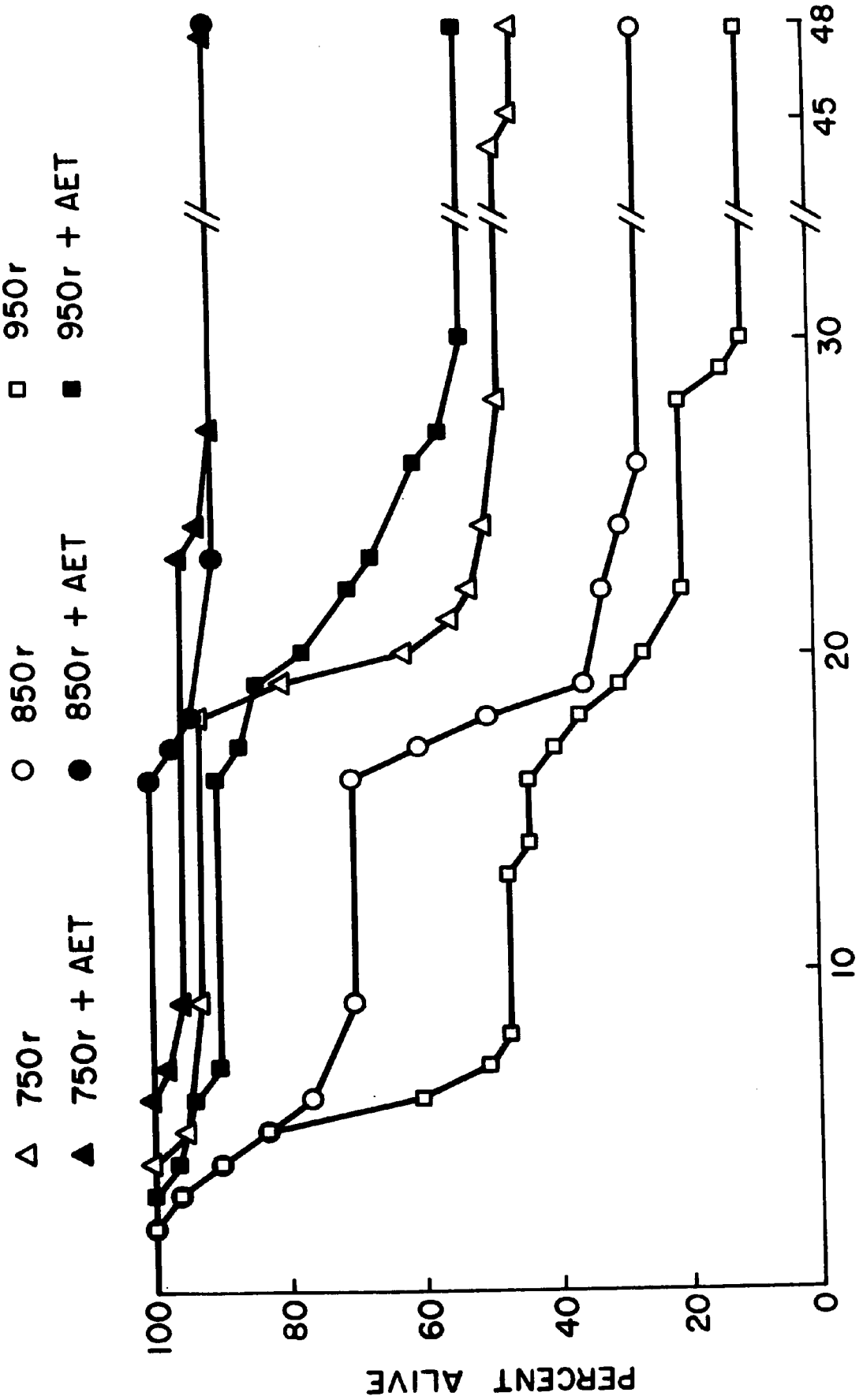

△ 750r
▲ 750r + AET
○ 850r
● 850r + AET
□ 950r
■ 950r + AET
PERCENT ALIVE
100
80
60
40
20
0
10
20
30
45
48
HOURS AFTER EXPOSURE

FIGURE 1

early mortality. Cysteamine and glutathione were also found to be protective but to a considerably lesser degree than AET.

The dose of the compound was also an important factor in producing increased survival. In other experiments, not reported here, a dose of 350 mg/kg killed some birds with no radiation and with this dose of AET all birds died following 650 to 750 R of X-rays. On the other hand, doses of AET below 250 mg/kg afforded little or no protection. These findings are similar to those in mammals, in which near toxic levels of compounds are necessary to give protection (Bacq, 1965).

Stearner, et al. (1954) have demonstrated that epinephrine and low environmental oxygen tension, particularly when used in combination, protected the young chicken from death in the 48 hour post-irradiation period. Since the effective dose reduction noted in the present study with AET was of the same order of magnitude as that found with exposure in 7% oxygen, hypoxia may be involved in the chemical protection of the chick. In mammals, however, even though chemical protection is seldom more effective than hypoxia in reducing radiation effect, chemical protective agents do not appear to exert their entire effect through hypoxia (Bacq, 1965).

Since nuclear damage does not appear to be involved in early chick death the results of this experiment strongly suggest that radioprotective compounds, such as AET, are capable of protecting against radiation damage to cellular components in addition to the nucleus. These findings are consistent with the hypothesis of chemical protection at the level of free radical formation and interaction.

The presence of two distinct time periods of death suggest that two lethal processes were initiated in the irradiated chick. It does not, however, necessarily imply that two different or separate initial

initial lesions were involved. The alternate interpretation of a single injury acting at two levels is consistent with the findings of this experiment. First, as the radiation dose increased, the number of animals that died 3 to 10 hours post-irradiation increased but the number of animals that died 17 to 30 hours post-irradiation decreased. This suggests that those animals that had received sufficient radiation damage to die 17 to 30 hours post-exposure were among the first to obtain the injury level necessary to die in the 3 to 10 hour period. Second, AET was equally effective in reducing radiation-induced mortality in the two periods. The pattern of deaths was unaltered by AET; for example, 950 R with AET produced a pattern of death almost identical to that seen with 750 R. This type of modification in mortality pattern would occur with two initial injuries only if they were almost identically sensitive to the radioprotective chemical.

4. Summary

Early (48 hour) deaths following acute exposure to X-radiation fell into two distinct and separate time periods, 3 to 10 hours post-exposure and 17 to 30 hours post-exposure. It was suggested that the primary or initial injury is similar for all animals which die within 48 hours of exposure but that subsequent steps leading to death are dependent upon the severity of the initial injury.

B. Early Mortality and Chemical Protection Following Exposure to Densely Ionizing Radiations

1. Introduction

The response of mammals to densely ionizing radiations such as neutrons may differ from the response to X-rays. Because of the large amount of energy deposited along the track of these particles, lethal events at the cellular level which may have required the passage of 2 or more gamma or X-ray photons within a short distance of the same sensitive volume are produced by a single, densely ionizing particle. Consequently, while repair is possible between the arrival of the two or more gamma or X-ray photons, repair does not generally occur following exposure to densely ionizing particles. As a result, densely ionizing radiations have generally been found to be more effective per rad than X-rays in producing radiation damage. In the majority of the experiments reported to date, the relative biological effectiveness (RBE) of densely ionizing particles was determined in systems which either directly or indirectly measured nuclear damage and cell death (Storer, et al. 1957). Because early mortality in the chick is very likely not the result of direct radiation induced cell death, it presented the opportunity of studying the RBE's of densely ionizing, particulate radiations using a different endpoint of radiation damage.

Pre-exposure administration of AET or related compounds has been demonstrated to be effective in reducing a number of radiation effects

including mortality in mammals following exposure to sparsely ionizing X- or gamma-rays but generally not following exposure to densely ionizing radiations. The lack of repair and the relatively large ratio of direct to indirect or water radical mediated damage associated with radiations of high linear energy transfer (LET) values are likely involved in this difference (Bacq, 1965). Since early radiation mortality in the chick does not appear to be the result of cell death but is presumably the result of the accumulation of injury within cells at a sublethal level, AET was used in an attempt to modify the lethal response of the young chick to densely ionizing radiations.

In this experiment, chicks were exposed to three types of ionizing radiations: thermal neutrons, fission neutrons, 2.2 GeV protons and their response compared to that following X-irradiation. The thermal neutrons and fission neutrons are both densely ionizing particles but differ in their energy and means of physical interaction (Lapp and Andrews, 1963). The 2.2 GeV protons are primarily very sparsely ionizing particles with a small densely ionizing component.

2. Methods and Materials

Day-old chicks were exposed to graded doses of thermal neutrons, fission neutrons, or 2.2 GeV protons under conditions described previously (pages 8-10). Fifteen minutes prior to exposure, 300 mg/kg of AET was injected into half of the animals at each fission neutron dose and to half of the animals at four of the thermal neutron doses. A control, X-ray series was also included. Because Cosmotron time was limited, an AET series was not added to the proton exposures. The radiation doses and the number of animals exposed to each dose are included in Tables III, IV, and V.

Deaths were recorded at hourly intervals for 48 hours subsequent to radiation exposure. Food and water were withheld throughout the experimental period.

Relative biological effectiveness (RBE) values presented in this and other chapters have all been calculated in terms of doses to produce equal effects, either 50% lethality (LD_{50}/2 day) or 50% lymphatic involution (50% effect dose of ED_{50}). The formula employed was:

$$RBE = \frac{\text{dose of X-rays to produce 50\% effect}}{\text{dose of test radiation to produce 50\% effect}}$$

3. Results

In the control, X-ray series the LD_{50} 48 hours was found to be 850 R for the chicks used in this experiment. The hourly mortality following exposure to thermal neutrons, fission neutrons, or 2.2 GeV protons are presented in Tables III, IV, and V. The results of exposure to these 3 types of radiations are described separately below.

a. Thermal Neutrons

The long exposure times necessary for the thermal neutron exposures resulted in a large dose contribution from the 4 MeV gamma contamination in the thermal column. From Table III, the LD_{50} 48 hours for the thermal neutrons was found to be approximately 6.4×10^{12} neutrons/cm^2 plus 444 rad of gamma rays.

In order to calculate the RBE of thermal neutrons for 2 day chick mortality two assumptions were made: 1) the neutrons and the gamma rays were additive in producing early death; 2) the RBE of the 4 MeV gamma rays was in the same range for producing chick mortality as for mouse mortality where the values of 0.4 to 0.8 have been reported (Storer, et al. 1957). At the 50% mortality level, 0.95 to 1.3×10^{10}

TABLE III

Hourly Tabulation of Deaths Following Exposure to Thermal Neutrons, With and Without Prior AET Administration

Chicks were exposed to thermal neutrons at a dose rate of 3.0×10^9 $n/cm^2/sec.$ with an additional 0.21 rad/sec. of gamma contamination. Total neutron fluxes (n/cm^2) are given in this Table. The deaths at all doses fell into two distinct time periods although the post-irradiation time of these periods was not the same at all dose levels. Total mortality was reduced by pre-irradiation injection of AET. The $LD_{50}/2$ day for thermal neutrons was approximately 6.4×10^{12} n/cm^2 (388 rad) + 434 rad of gamma rays.

TABLE III

Hourly Tabulation of Deaths Following Exposure to Thermal Neutrons, With and Without Prior AET Administration

Time of deaths in 48-hour mortality period of acutely irradiated chick (Hrs. post-irradiation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1a																														
1b										1																				
1c					1	1										1														
1d						2	2		2								1							1						
2e																														
2e*																														2
2f					1		2							1				1												2
2f*																1														
2g				1	1								1		2				1		1		2							
2g*				1	1														1											
2h			2		1							4												1						
2h*																1		1												
2i				1	4								2																	
2i*					1		2						2										1				2			

* treated with AET Nos. 1 and 2 are 2 different experiments.

Group a : 0 (n/cm^2) x 10^{12}, N = 12, 0 Deaths
b : 5.2 12 1
c : 6.4 12 3
d : 7.2 12 8
e : 0 9 0
e*: " " 0
f : 6.6 9 7
f*: " " 3

Group g : 8.2 (n/cm^2) x 10^{12}, N=9, 9 Deaths
g*: " " 3
h : 9.1 9 8
h*: " " 2
i : 10.0 9 7
i*: " " 8

TABLE IV

Hourly Tabulation of Deaths Following Exposure to
Fission Neutrons With and Without
Prior AET Administration

Chicks were exposed to fission neutrons at a dose rate of 29.5 rad/minute. Ten per cent of the absorbed dose was from gamma contamination. Two distinct time periods of death were observed. The post-irradiation time of these periods was not the same at all dose levels. Prior injection of AET significantly reduced total mortality in the 48 hour post-irradiation period. The $LD_{50}/2$ days for fission neutrons was approximately 198 rad of neutrons + 22 rad of gamma rays.

TABLE IV

Hourly Tabulation of Deaths Following Exposure to Fission Neutrons
With and Without Prior AET Administration

Time of deaths in 48-hour mortality period of acutely irradiated chick (Hrs. post-irradiation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
a																														
a*																														
b																	2										1			
b*																														
c																														
c*																														
d				1		1							1		2			1			1	1	1							
d*																		2	1											
e			1		1							3		1		1		1			1						1			
e*																1		1												
f				1				4			2							2								1			2	
f*								1										2												

* treated with AET

Group a: 0 rad, N=9, 0 Dead
b: 136 rad, N=9, 0 Dead
c: 194 rad, N=9, 3 Dead
d 219 rad, N=9, 9 Dead
d* " " " ", 3 Dead

Group e 243 rad, N=9, 9 Dead
e* " " " ", 3 Dead
f 267 rad, N=10,7 Dead
f* " " " " 8 Dead

TABLE V

Hourly Tabulation of Deaths Following
Exposure to 2.2 GeV Protons

The instantaneous proton dose-rate was not known. Total proton flux (p/cm^2) in units of 10^{10} are given in this Table. The deaths following exposure to 2.2 GeV protons did not fall clearly into two time periods. The majority of the deaths, even at the lower doses occurred between 3 and 12 hours post-exposure. The $LD_{50}/2$ day for 2.2 GeV protons was approximately 2.1×10^{10} p/cm^2 (863 rad).

TABLE V

Hourly Tabulation of Deaths Following Exposure to 2.2 GeV Protons

Time of deaths in 48-hour mortality period of acutely irradiated chick (Hrs. post-irradiation)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
a																												1		
b				1																								1		
c							1																							
d															1											3				
e							1																							
f							5	4			1																			
g						5	5			3																				
h			1		6		3	2																						
i				1	12		2																							
j					6		9				1																			
k					5		8				1																			
l			3	1		1	1		2																					

Group a: 0 x 10^{10} p/cm^2, N=16, 0 Dead

Group b: 1.71, 16, 2

Group c: 1.82, 8, 2

Group d: 1.92, 16, 0

Group e: 2.05, 10, 5

Group f: 2.21, 16, 10

Group g: 2.36 x 10^{10} p/cm^2, N=16, 13 Dead

Group h: 2.50, 16, 12

Group i: 2.71, 16, 15

Group j: 2.86, 16, 16

Group k: 3.21, 16, 14

Group l: 3.58, 8, 8

neutrons/cm^2 were found to be equivalent to 1 R of X-rays. Using an average RBE of 0.6 for the 4 MeV gamma rays 1.09 x 10^{10} neutrons/cm^2 was equivalent to 1 R of X-rays and the RBE of thermal neutrons for early chick mortality was calculated to 1.9 compared to 250 kvp X-rays.

Two differences were found between the time pattern of death following thermal neutron irradiation and that found previously, following X-irradiation. As many as seven of nine animals (78%) in an exposed group (8.2 x 10^{12} neutrons/cm^2) died in the second mortality period compared to a maximum of 48% in the same period in the X-irradiated animals. The onset of death in the second period was earlier than with X-rays with deaths occurring as soon as 12 hours post-exposure. Despite this, the two periods of death still appeared to be distinct and separate.

Survival was significantly higher in the groups which were injected with AET prior to irradiation than in the non-injected, irradiated controls. The highest radiation dose used in this experiment (10 x 10^{12} neutrons/cm^2) caused 8 of the 9 AET injected birds to die, but in this group the majority of the deaths were in the second more radiosensitive period, suggesting a dose reduction even at this level.

b. Fission Neutrons

From the fission neutron data in Table IV, an LD_{50} 48 hours of 220 rad was obtained of which only 22 rad was from the gamma contamination. The RBE for 2-day chick mortality was calculated to be 4.2 compared to 250 kvp X-rays.

The time distribution of death was more like that seen with thermal neutrons than that seen with X-rays, but as with both of these radiations two periods of death still appeared to be present.

The percentage survival of the chicks pretreated with AET was significantly higher than in the irradiated controls. At the highest fission neutron dose 8 of 9 animals were dead but as with the highest thermal neutron dose, the deaths occurred primarily in the second more radiosensitive period.

c. 2.2 GeV Protons

A two day LD_{50} of 2.1×10^{10} protons/cm^2 was determined for chick mortality from the data listed in Table V. Assuming that 2.43×10^7 protons/cm^2 deliver 1 rad (Bond, 1965), then 2.1×10^{10} protons/cm^2 would deliver 865 rad. From this information, an RBE of 0.98 compared to 250 kvp X-rays was obtained. By a similar analysis, Jesseph, et al. (1964) found an RBE of 0.85 for mouse mortality. Comparing the LD_{50} 48 hours of 2.1×10^{10} protons/cm^2 with the X-ray LD_{50} 48 hours of 850 R, 2.44×10^7 protons/cm^2 was found to be the equivalent of 1 R of X-rays.

Of a total of 97 deaths in all groups combined only 12 animals died later than 10 hours post-exposure. At only one exposure level (2.05×10^{10} protons/cm^2) was the mortality pattern similar to that seen with the other radiations. At this dose one early death and 4 late deaths were equal to 50% mortality.

4. Discussion

Both the thermal neutrons and fission neutrons were considerably more effective per rad than X-rays in producing early mortality while the protons were as effective as X-rays. It would appear from this that the direct relationship between RBE and LET reported for nuclear damage and cell death also pertains to other types of radiation injury. These results suggest that the radiation injury in the chick is cumulative and subject to some reversal or recovery even at high dose rates of X-irradiation.

The results of the neutron exposures also provide additional information concerning the two periods of lethality seen within 48 hours of acute X-irradiation. The 17-30 hour mortality period was more dose sensitive than the 3-10 hour period but the two periods of mortality greatly overlapped in dose response following X-ray exposure. No more than 50% of the animals died in the second period at doses below which deaths began to occur in the first period and this level of mortality was not exceeded in the second period regardless of the mortality level in the first period. The second period appeared to be more sensitive to high LET radiation than the first period resulting in a separation of the dose responses. With both thermal neutrons and fission neutrons it was possible to kill a majority of the animals in the second period at doses below which significant mortality appeared in the first period. It would thus appear possible to obtain a mortality level of 100% in either period depending upon the type of radiation and dose employed.

Modal survival time in the first period following neutron irradiation was similar to that seen with X-irradiation (Table VI). As with X-rays, the number of animals dead in the first period increased with dose, but the modal survival time in this period did not change with increasing doses. The modal survival time in the second period decreased from 20 hours with X-rays to 17 hours with both thermal and fission neutrons. The time between the two periods of mortality was also shorter and less well defined with the high LET radiation than with the low LET X-rays. As with X-rays, however, the time between the two mortality periods did not appear to be altered in any consistent manner by changes in the radiation dose or by AET treatment.

TABLE VI

Survival Time and RBE Values for Early Chick Mortality

Radiations	kev/μ LET	RBE	MODAL SURVIVAL TIME FIRST PERIOD	SECOND PERIOD
X-rays	3	1	6 hrs.	20 hrs.
2.2 GeV protons	?[a]	0.98	7	7-13 or 26
thermal neutrons	64% = 65 36% = 0.3[b]	1.9	5	17
fission neutrons	43-48	4.2	8	17

a mean LET not known but expected to be below that of 250 kvp X-rays.

b protons with a mean LET of 65 and gamma rays with a mean LET of 0.3 produced by thermal neutron interactions.

The relative biological effectiveness (RBE) of the minimally ionizing protons was very nearly unity, indicating that these particles were approximately as effective per rad as X-rays in producing early mortality. The densely ionizing thermal neutrons and fission neutrons were approximately 2 and 4 times as effective as X-rays in producing early mortality. No systematic change in survival time in either of the two mortality periods within 48 hours of exposure was noted with the four types of radiations.

Because of the great current interest in GeV protons and the sparsity of information concerning their biological effect, the results and implications of this portion of the experiment have been discussed in detail.

Both high LET and low LET components are apparently produced by the interaction of 2.2 GeV protons with biological material and the RBE for any endpoint represents only the average of all interactions. The relative importance of the various components in producing biological damage has not yet been determined but evidence for a measurable contribution from the high LET component has been reported. Jesseph, et al. (1964) found that mice irradiated with proton doses even in the LD_{50} 30 day range died within a week of exposure. Such a short survival time resulting from gut damage is characteristic of exposure to high LET radiations. Following X-irradiation in the midlethal range, mean survival time is approximately 12 days, death being associated with hematopoietic failure. Shellabarger, et al. (1965) found the 2.2 GeV protons to be 1.6 times as effective in producing mammary neoplasia in the rat as in producing death in the mouse. An RBE of approximately 1.36 was calculated for this endpoint, again suggesting some high LET component.

In this experiment, only 6 animals can clearly be distinguished as having died in the second period following proton irradiation (Table V). The modal survival time of these animals was 26 hours, longer than with the least ionizing of the other radiations (X-rays). If survival time in the second period was related directly to LET then these findings would be consistent with the observed RBE of less than 1. On the other hand, if no other deaths can be considered to correspond to second period death then as few as 10 of 16 (62.5%) of a group were

killed with no deaths in the second period. This response is dissimilar to that seen with either the high LET neutrons or the low LET X-rays.

Entertaining the possibility that the second period deaths occurred so soon after the exposure that they overlapped in time with first period deaths, it was possible to estimate the modal survival time for the second period. First, by taking only animals which survived longer than 9 hours the modal survival time was 13 hours. Second, by allowing a maximum of 40% of the deaths in each group which had some 48 hour survivors, a modal survival time of 7 hours was obtained.

Whether or not any of the above analyses describes the true response of the young chick to 2.2 GeV proton irradiation, the results obtained in this experiment do indicate that the biological effects of the minimally ionizing GeV protons are somewhat different from those of the low LET X-rays.

Both the total survival and the pattern of death in the AET treated groups indicate a protective effect of AET against both thermal neutron and fission neutron exposures. At the lower doses, a significantly greater number of AET animals survived than in the control irradiated series. At the highest dose of both thermal neutrons and fission neutrons where the mortality levels were high in both the AET and control groups, AET deaths occurred primarily in the second, more radiosensitive period. The protective effect of AET was present against deaths in both mortality periods. As with the X-ray exposures this could have been the result of protection against two initial lesions or against a single lesion acting at two levels.

The radioprotective effect of AET against mortality emphasizes the difference between the chick death and the mammalian death. Chemical protection is greatly reduced or absent in mammals when high LET radiations are employed, but the protectiveness of AET against chick mortality was about equal for X-rays or neutrons.

The cell death leading to organism death in mammals appears to be the result of a finite number of events within a finite volume. When more than one event is necessary to produce cell death, recovery is possible during exposure to sparsely ionizing radiations such as X-rays. Because of the densely ionizing nature of particulate radiations such as neutrons, the multiple events are often caused by the passage of a single particle and no recovery occurs. If, as suggested, the lethal damage in the chick is the result of the accumulation of injuries at doses not lethal to the sensitive cells, then recovery is possible even with densely ionizing radiations and protection even in terms of recovery is possible.

Because of the large amount of gamma contamination occurring in the thermal neutron exposures, as much as half of the protection against the thermal neutrons could be the result of protection against the low LET gamma radiation. To obtain the degree of protection found in this experiment, however, would require at least some degree of protection against the thermal neutrons. An even smaller portion of the protection against the high LET fission neutrons could be the result of protection against gamma contamination. Only 10% of the absorbed dose was from gamma rays which because of their low RBE contributed even less than 10% to the radiation effects. In this case, complete elimination of the dose contribution from the gamma contamination would account for an insignificant portion of the AET protection.

These findings have been interpreted to indicate that the early (48 hour) radiation mortality in the chick can be reduced by AET whether the radiation dose is delivered with low LET X-rays or high LET thermal neutrons or fission neutrons.

5. Summary

1. The high LET thermal and fission neutrons were more effective per rad than X-rays in producing early mortality in the chick. The low LET protons were about as effective as X-rays in producing early mortality.

2. Two periods of death were evident following both thermal neutron and fission neutron exposures. These periods were less separated in time than with X-rays, but still distinguishable. No relationship between survival time within either mortality period and dose was noted.

3. The two periods were separated in dose sensitivity so that mortality levels approaching 100% were seen in the second mortality period and at higher doses, in the first mortality period.

4. AET was approximately as effective in protecting against early mortality following high LET neutrons exposures as it was against low LET X-ray exposures.

C. Involution of Chick Lymphatic Tissues Following High and Low Dose Rate X-ray Exposure

1. Introduction

Since no information was available previously concerning the radiosensitivity of chick lymphatic tissues, this experiment was designed to determine both the dose sensitivity of the thymus, bursa and spleen of the chick and the pattern of the radiation-induced involution with time following X-irradiation. Both a high dose rate and low dose rate series of exposures were performed in order to examine the dose rate sensitivity of the chick lymphatic tissues.

2. Methods and Materials

Ten-day old chicks were exposed to 0 R, 50 R, 100 R, 200 R, or 400 R of 250 kvp X-rays under either high dose rate (96.5 R/min.) or low dose rate (8.8 R/min.) conditions. Daily, for five days following radiation exposure, a minimum of 8 chicks per exposure group were killed and their thymus, bursa and spleen removed. The organs were immediately cleaned of fat and connective tissue and their wet weights determined to the nearest 0.1 mg.

Because the lymphatic tissues of the chick, like those of mammals, involute due to adrenal corticoid release following stress (Garren, et al (1956, Newcomer and Connally, 1960), all the chicks in this experiment were subjected to both exposure conditions for a period of time equal to the longest actual exposure in each series. Preliminary

studies indicated that at two days a depression of 5% or less in lymphatic organ weights below the weights of organs from the cage controls was obtained by both sham radiation exposures.

3. Results

Unless otherwise noted, all values for organ weights in this and subsequent experiments have been expressed in the following manner.

$$\frac{\dfrac{\text{Organ weight (mg) of treated chicks}}{\text{Body weight (gm) of treated chicks}}}{\dfrac{\text{Organ weight (mg) of untreated chicks}}{\text{Body weight (gm) of untreated chicks}}} \times 100$$

This commonly used conversion allows values for organ weights to be compared with those obtained from animals of different sizes and treatments. The only assumptions involved in this manner of representing the organ weights are that a linear relationship exists between lymphatic organ weights and body weights in the immature chick and that a line through the points on a body weight vs. organ weight scale pass through the origin. Comparison of body weights and organ weights of several hundred control animals indicates that this is a valid assumption within the range of weights employed in these experiments.

The values obtained by this procedure have been plotted in Fig. 2. These values represent the total chick lymphatic tissue (thymus + bursa + spleen). The lines in these figures have been fit by the method of least squares through 1) the average of the points for all days (1-5), 2) the points representing maximum depression at each dose, or 3) the points representing maximum depression within two days of exposure. In the case of the HDR exposures, the latter two lines are the same.

FIGURE 2

Lymphatic Tissue Dose Response
Low Dose Rate and High Dose Rate X-irradiation

The three lines in both the low dose rate (LDR) and high dose rate (HDR) portions of this figure were drawn through (a) the points of maximum depression at each dose, (b) the points of maximum 1-2 day depression and (c) the average of all points at each dose. In the high dose rate (HDR) graph, the first two (a and b) are the same line. The lines in each case indicate that the HDR exposures were more effective than the LDR exposures in producing lymphatic involution.

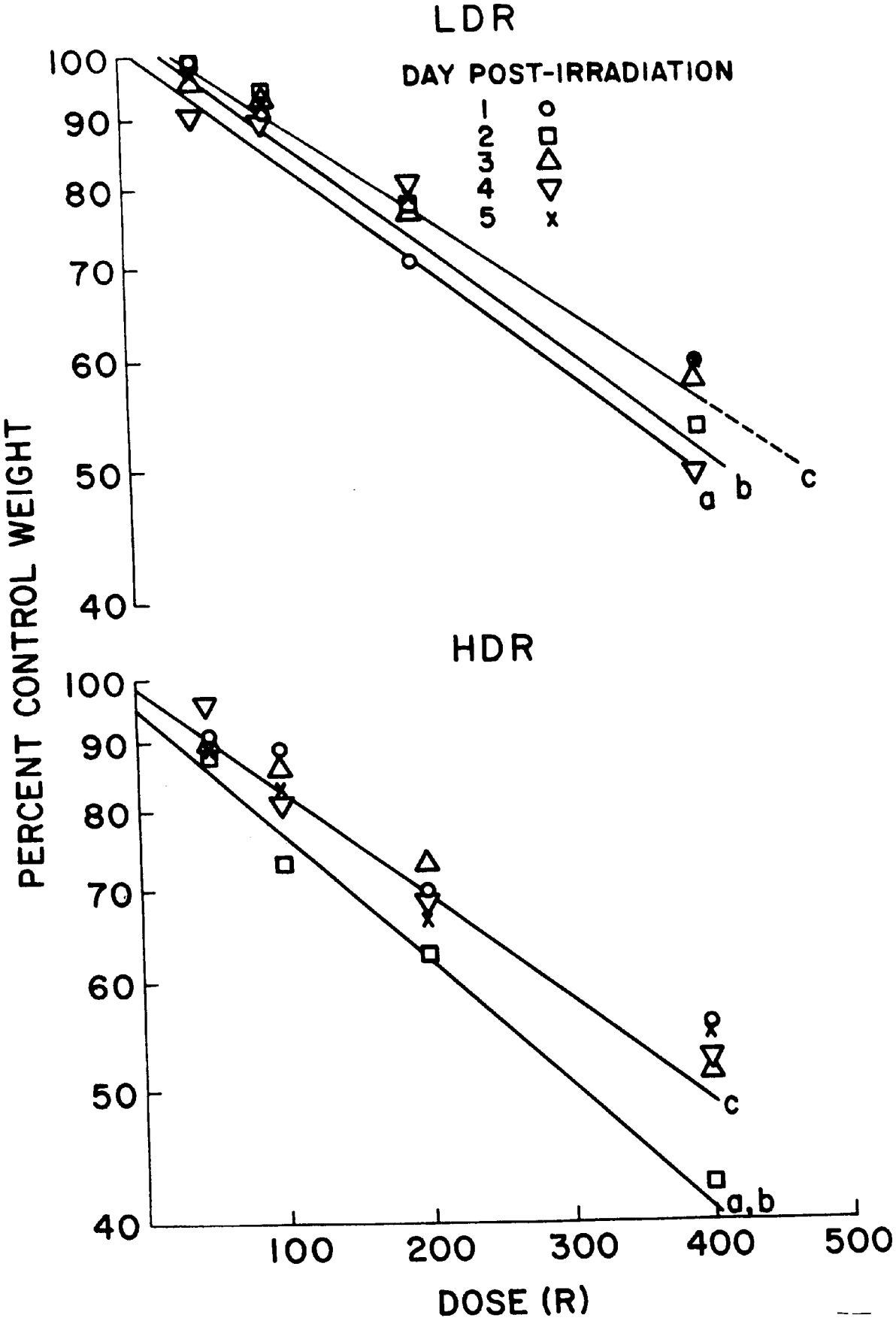

LDR
DAY POST-IRRADIATION
1
2
3
4
5
100
90
80
70
60
50
40
a
b
c
HDR
PERCENT CONTROL WEIGHT
100
90
80
70
60
50
40
a,b
c
100
200
300
400
500
DOSE (R)

FIGURE 2

The slopes, y-intercepts and ED_{50} values obtained graphically from the lines in Fig. 2 have been plotted in Table VII. Although extrapolations were necessary in some cases to obtain ED_{50} values, the concept nonetheless remains useful. A dose of 410 R produced 50% depression within two days at the low dose rate (8.8 R/min.) whereas 305 R at the high dose rate (96.5 R/min.) produced a similar effect. Using the maximum depression for all points in the 1-5 day period, essentially the same results were obtained. Comparing the lines through the average of all points, 50% depression was produced by 475 R at the low dose rate and 395 R at the high dose rate. These differences have been interpreted as being indicative of a dose rate sensitivity of chick lymphatic tissue to whole-body irradiation. The negative slopes were also greater and the y-intercepts lower for the high dose rate lines than for the low dose rate lines (Table VII).

The time-pattern of involution can be seen for each dose and dose rate in Fig. 3. The difference in dose sensitivity at the two dose rates is evident in Fig. 3 as it was in Fig. 2 but, in addition, a difference can be seen in Fig. 3 between the time-patterns of involution at the two dose rates. Without exception, the HDR exposures are characterized by maximum depression on the second day post-exposure following a large depression on the first day post-exposure.

The pattern is somewhat different following low dose rate exposures. Except in the 50 R group where no lymphatic depression was evident until at least the third day, involution on the first day post-exposure (LDR) was nearly equal to that of the comparable radiation dose at the high dose rate. On the second day, however, unlike the HDR exposures, little or no additional involution was detected.

TABLE VII

Response of Chick Lymphatic Tissue to HDR and LDR X-ray Exposures
Values derived from Fig. 2

	ED_{50}		- Slope		y-intercept		$\frac{\text{SLOPE LDR}}{\text{SLOPE HDR}}$
	LDR	HDR	LDR	HDR	LDR	HDR	
All points	475 R	395 R	0.113	0.125	106%	98%	0.85
Max. involution (1-2 d)	410 R	305 R	0.128	0.140	105%	95%	0.91
Max. involution (1-5 d)	400 R	305 R	0.128	0.140	102%	95%	0.91

Comparison of the slopes of the dose response lines indicates that exposure at a low dose rate (LDR) was only 85% to 91% as effective per roentgen in producing lymphatic involution as was exposure at a high dose rate (HDR). In addition, the y-intercepts of the HDR lines were below those of the comparable LDR lines.

FIGURE 3

Involution and Recovery Following LDR and HDR X-irradiation

The pairs of lines in this figure indicate that at each dose employed the high dose rate (HDR) exposures were more effective than the low dose rate (LDR) exposures in producing lymphatic involution. The additional depression at two days following HDR exposure beyond the first day LDR depression was interpreted as being the result of dose rate sensitive 'chick-type' abscopal damage.

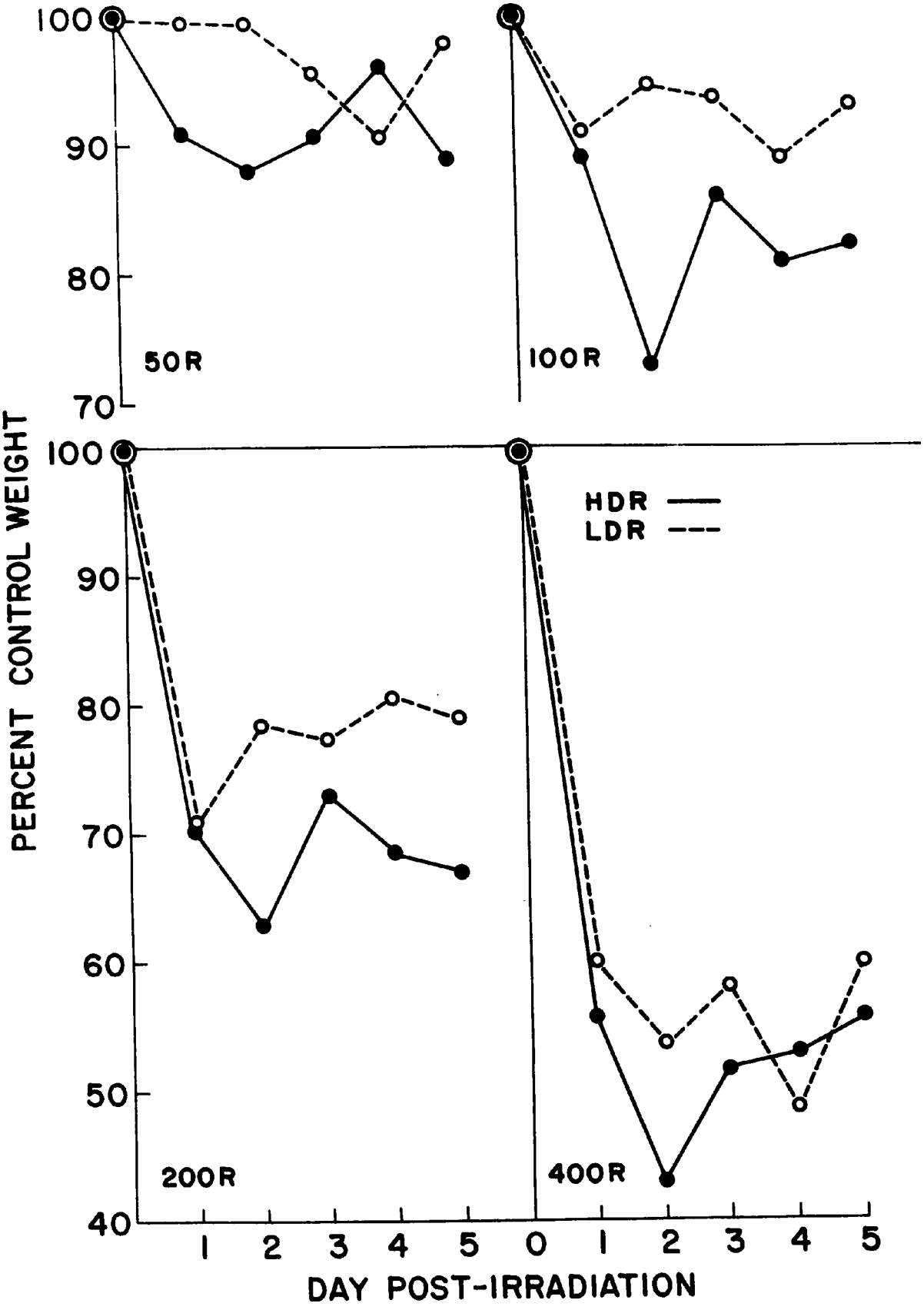

100
90
80
70
50R
100R
100
90
80
70
60
50
40
HDR
LDR
PERCENT CONTROL WEIGHT
200R
400R
1 2 3 4 5 0 1 2 3 4 5
DAY POST-IRRADIATION

FIGURE 3

4. Discussion

Acute involution of lymphatic tissue, whether the result of radiation exposure, a single acute stress or the administration of a lymphocytolytic chemical is maximal within 2 days of treatment (Dougherty, 1952, Kallman and Kohn, 1955). Repair processes apparently begin even before maximal depression is reached. Further involution beyond two days or failure of repair to appear beyond two days as measured by an increase in lymphatic organ weight may be taken as an indication of continuing or chronic stress in the test animal. For these reasons, the maximum two-day depression in this experiment has been considered to be the result of the scopal effects of radiation on the lymphatic tissues and abscopal events (Mole, 1953) occurring during or shortly after radiation exposure.

No evidence is available concerning an immediate abscopal effect on mammalian lymphatic tissues but at doses considerably above 1000 R abscopal damage develops probably as a result of gut damage and anorexia and an adrenal mediated stress response (Bond, et al. 1953, Raventos, 1954, and Corp, et al. 1961). If the 'mammalian-type' abscopal damage in the young chicken is truly comparable to the abscopal effects reported for mammals then the involution of lymphatic tissues caused by this damage would not be expected to be apparent until 3 and more likely 4 to 5 days following radiation exposure (Bond, et al. 1953). Since both mammals and the chicken develop the gut damage leading to this effect whether irradiated at high or low dose rates, 'mammalian-type' abscopal damage should not be expected to be, and has not been reported to be detectably sensitive to dose rate.

A difference between the effectiveness of the high and low dose rate exposures was evident from both the dose responses presented in

Fig. 2 and the time patterns of these responses shown in Fig. 3.

The dose responses differ in two important respects. First, the slopes of the lines differ so that 1 R of X-rays at the low dose rate was only 0.85 (average 5-day depression line) to 0.91 (maximum depression line) as effective as 1 R of X-rays at the high dose rate (Table VII). This difference in slope alone was sufficient to account for a difference of 40-60 R at the ED_{50} level. It is conceivable that a portion of this apparent dose rate effect was at the scopal level, that is, on the direct killing of lymphocytes by radiant energy deposited within the killed cells. Bateman, et al. (1962) have presented good evidence to suggest the existence of a dose-rate effect on mortality in mammals that is detectable even within the range of dose rates employed in this experiment. No evidence has been reported suggesting a dose-rate effect on the direct killing of lymphocytes or lymphatic involution in vivo. Even a dose rate effect as great as that found for survival in mammals would account for less than 1/3 of the observed difference in the response of the chick lymphatic tissues to high dose rate and low dose rate. The difference in the response of the chick lymphatic tissues to high dose rate and low dose rate can be explained in terms of an abscopal effect peculiar to the chicken.

If such a 'chick-type' abscopal effect is occurring, it is different from the 'mammalian-type' abscopal effect in at least two ways. First, the presumed humoural substance involved in producing lymphatic tissue depression is produced during or shortly after exposure as suggested by its effect only two days post-exposure. Second, the 'chick-type' abscopal effect appears to be dose rate sensitive, perhaps related to the dose rate sensitive lethal injury in the chick.

The second important difference between the two sets of lines in Fig. 2 can be seen by a comparison of the y-intercepts. At this level (zero radiation dose) a difference already of approximately 7% was found between the comparable lines in Fig. 2 (LDR vs. HDR). Such a difference in y-intercepts might be expected in a mammalian system or even in a cell suspension by the administration of a lymphocytotoxic agent which acts independently of radiation prior to radiation exposure. The differences between the LDR and HDR lines in Fig. 2 with their different intercepts and slopes are similar to those seen when irradiation is preceded by the administration of a radiosensitizing chemical which both kills cells independently of the radiation and also renders the remaining cells more sensitive to radiation (Kaplan, 1965). In this analogy, the high dose rate group would be comparable to that treated with a radiosensitizer.

Analysis of the pairs of lines in Fig. 3 provides additional information concerning the high and low dose rate responses. Involution on the first day post-exposure is about the same for the two groups except at 50 R, but on the second day, repair is already evident at the low dose rate while an additional decrease in the organ weights of the high dose rate animals has occurred. This difference has been interpreted to be the result of damage to tissues other than the lymphatic tissues which act through some humoural process to produce lymphatic involution additional to that produced by the direct action of the radiation on the lymphatic lymphocytes. Maximal early depression at the low dose rate indicates that involution in response to a single acute insult occurs within less than two days. The later maximal depression at the high dose rate suggests that the humoural substance

continues to act for some time, perhaps hours, after the original radiation injury.

Additional depression or failure to show repair on days 4 and 5 post-exposure have been taken as an indication of the development of damage, most likely to the gastrointestinal tract, which lead indirectly to lymphatic damage. This effect, which required some time to develop, has here been termed 'mammalian-type' abscopal damage because of the similarity to the abscopal damage to mammalian lymphatic tissues. At 50 R (LDR) no direct damage is indicated by the 1 and 2 day weights but a definite indication of abscopal damage is found in the reduced 4 day weights.

5. Summary

Whole body X-irradiation caused the thymus, bursa and spleen of the young chicken to be reduced in size or weight to a degree approximately proportional to the radiation dose at least to 400 R. Exposure at 96.5 R/min. was more effective than exposure at 8.8 R/min. in producing lymphatic organ involution and the time pattern of response at the two dose rates was also different.

The dose response lines and the time pattern of response has been interpreted to suggest the following:

1. A linear relationship between whole-body exposure dose and reduction in the logarithm of lymphatic organ weight.

2. A direct or scopal radiation effect on the lymphatic organs little if at all sensitive to dose rate.

3. A dose rate sensitive abscopal effect acting during and(or) for a few hours after radiation exposure, here termed 'chick-type' abscopal effect.

4. Repair processes beginning before maximum depression and being detectable within 24 hours of maximum depression.

5. Decreased repair or further involution 3-5 days post-exposure as the result of a dose rate insensitive 'mammalian-type' abscopal effect.

D. Lymphatic Response to Densely Ionizing Radiations: Chemical Modification with AET

1. Introduction

In this experiment day-old chicks were exposed to sub-lethal doses of X-rays, thermal neutrons, fission neutrons or 2.2 GeV protons to determine the relative effectiveness of these radiations in producing involution of the chick lymphatic tissues. Except in the proton groups animals pre-injected with AET were also irradiated to explore the possibility of chemically modifying the radiation-induced lymphatic involution. The effectiveness of these radiations in producing lymphatic involution was compared to their effectiveness in producing early mortality in the chick and also with available values for lymphatic involution and mortality in the mouse. Since no information had been previously reported relating to the effect of 2.2 GeV protons on mouse spleen-thymus weights, mice were exposed to this radiation and spleen-thymus weights determined.

2. Methods and Materials

Two-day old chicks were exposed to 250 kvp X-rays (high dose-rate conditions), thermal neutrons, fission neutrons, or 2.2 GeV protons as described in General Methods and Materials. X-ray doses of 200 R, 400 R, and 600 R were employed. Doses of comparable effectiveness for the neutron exposures were estimated from the mortality studies. Since no data were available concerning the response of either Avian or mammalian

lymphatic tissues to 2.2 GeV protons, the sublethal range was more thoroughly covered in that series (5 doses at 2 days and 13 doses at 5 days). In order to obtain a reference point for the proton exposures, eight mice were exposed to each of two sublethal doses and their spleen and thymus weights, five days post-exposure were compared to those obtained from 13 non-irradiated mice. No X-ray series was run, but because of the reproducibility of the spleen-thymus dose response to X-rays, a previously published ED_{50} value was used in making RBE calculations (Storer, et al. 1957).

The chick thymic and bursal weights were determined (5-8 chicks per treatment) either two days or five days post-irradiation. These times were chosen because at high dose rates maximal depression occurred on the second post-irradiation day at low doses and 'mammalian-type' abscopal damage could possibly be detected on the fifth post-irradiation day. Also, the mouse values used as reference were obtained on the fifth day post-irradiation.

In the X-ray, thermal neutron and fission neutron groups half of the chicks were injected prior to exposure with 250 mg/kg of AET. (Montour, 1965).

To determine the effect of AET on the radiation induced depression of the chick lymphatic tissue it was necessary to maintain two zero dose groups. These groups were similarly treated except that one group was injected with AET and the other was not. The AET injected irradiated birds were compared with AET non-irradiated birds and the control or non-injected irradiated birds with similar zero dose animals. This was done in an attempt to eliminate the effect of AET alone on the lymphatic tissues. AET injection alone had a variable effect but generally resulted in a decrease in organ weight of 5% or

less. All unirradiated and irradiated animals were similarly handled and retained in an exposure chamber as long as the longest exposure in order to minimize differences in organ size due to stress.

3. Results

In all groups in which AET injected animals were included the thymic and bursal organ weights have been presented separately rather than as total lymphatic tissue. The resultant presentation was helpful in subsequent experiments involving partial body exposure and also made it possible to determine whether AET affected the radiation response of only one particular organ or of lymphatic tissue in general. Spleen sizes were obtained but were not included in the graphic presentation of the data because of the large variation in the size of this organ in newly hatched chicks. The splenic response to radiation was generally between that of the thymus and bursa.

a. X-ray Exposure

With the exception of the 200 R group 5 days post-exposure the thymus was in all cases depressed more than was the bursa at the same dose. At both 400 R and 600 R the thymic and bursal weights were significantly higher (p 0.01) in the AET injected birds than in the untreated birds (Fig. 4 and Fig. 5). The reduced involution with AET was evident on both the second and fifth post-irradiation days. The amount of lymphatic tissue in the 200 R and 400 R groups was little different on the fifth day compared to the second day post-exposure. At 600 R, however, the weight of lymphatic tissue was less at five days than at 2 days. The lymphatic organs of the AET chicks was similarly less at 5 days following 600 R but the amount of tissue was still significantly greater than in the uninjected 600 R group.

FIGURE 4

Thymic and Bursal Response to X-rays With and Without Prior AET Treatment: 2 Days Post-exposure

The log of the thymic and bursal weights were reduced approximately proportionally to the radiation dose. Prior injection of 300 mg/kg of AET reduced the extent of involution of both the thymus and bursa following 400 R and 600 R of X-rays. A dose equal to one-half of the X-ray $LD_{50}/2$ day is given as a point of reference for comparison with other radiations.

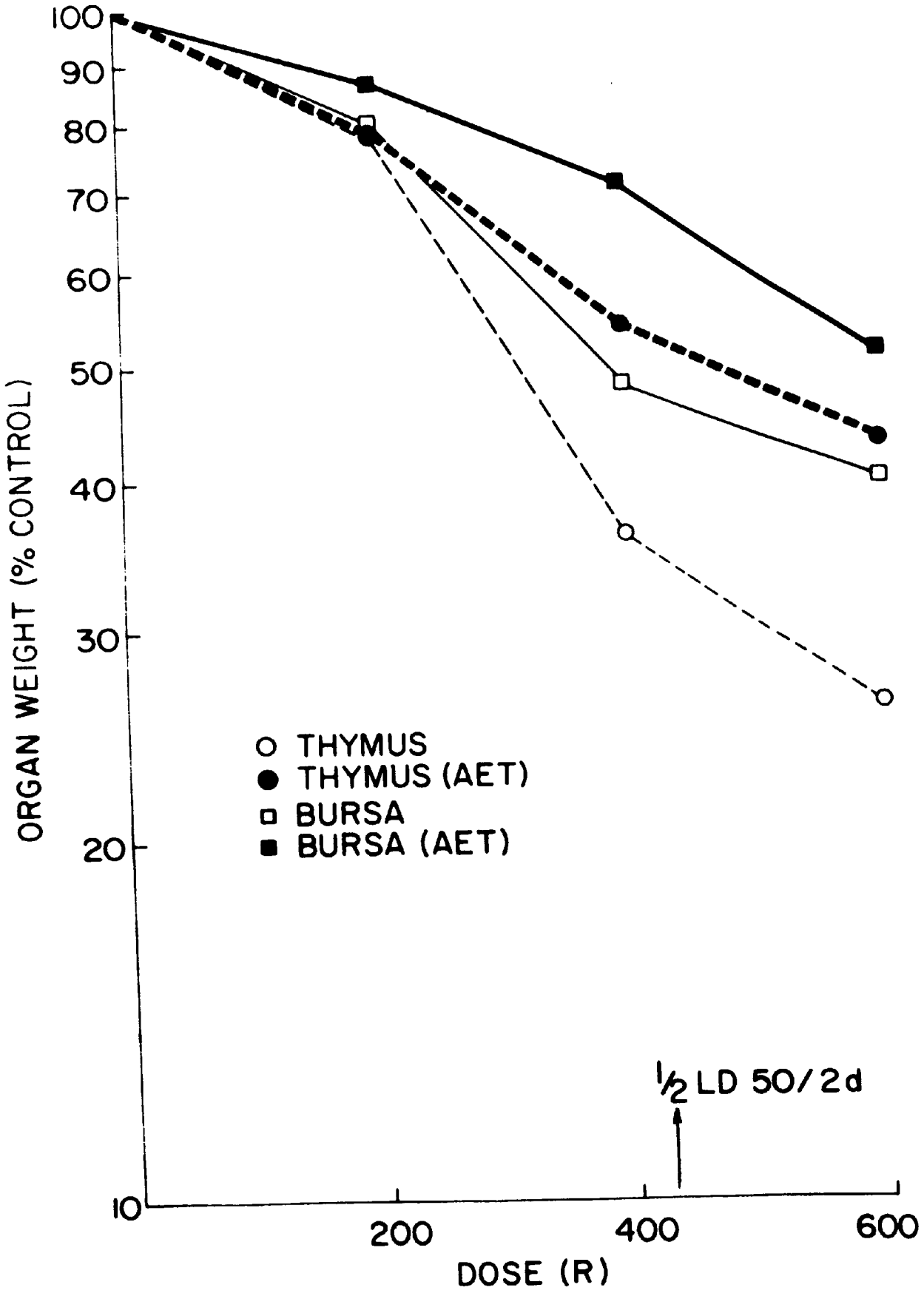

100
90
80
70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
THYMUS
THYMUS (AET)
BURSA
BURSA (AET)
½ LD 50/2d
200
400
600
DOSE (R)

FIGURE 4

FIGURE 5

Thymic and Bursal Response to X-rays With and Without Prior AET Treatment: 5 Days Post-exposure

The difference between the 400 R + AET and 600 R + AET and the similarly irradiated controls which was evident 2 days post-exposure was again detectable 5 days post-exposure.

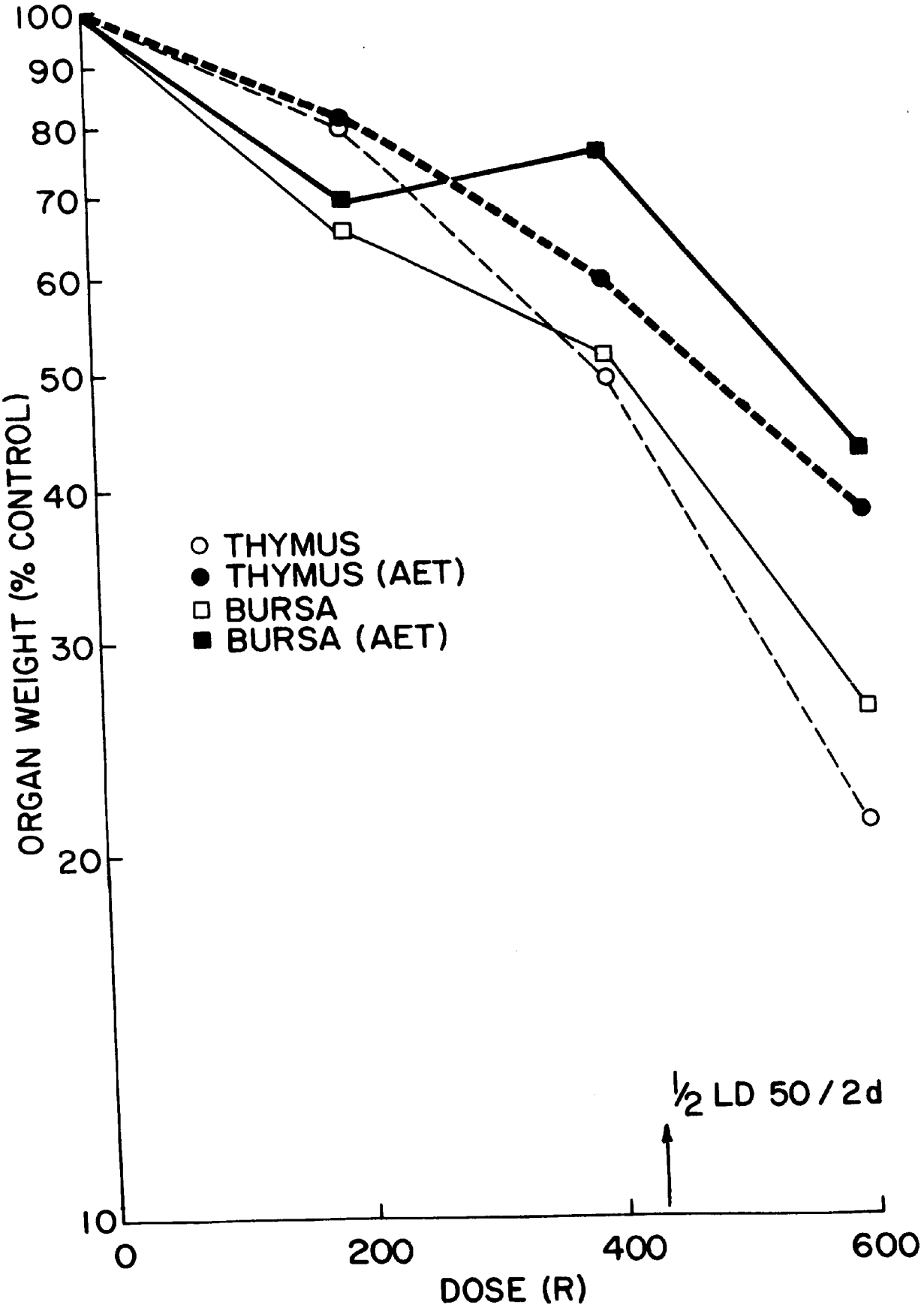

100
90
80
70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
THYMUS
THYMUS (AET)
BURSA
BURSA (AET)
½ LD 50 / 2d
0
200
400
600
DOSE (R)

FIGURE 5

RBE calculations in Table VIII and Table IX were based on ED_{50} values of 350 R (2 days) and 425 R (5 days) for total lymphatic tissue.

b. Thermal Neutrons

The response of the thymus and bursa to three sublethal doses of thermal neutrons, with and without prior AET injection, is presented in Fig. 6 and Fig. 7. As with the X-ray exposures, the thymus was reduced in size more at each dose than the bursa with the difference being greater at the higher doses. From these data it was calculated that on the second day post-irradiation the effect of 1 R of X-rays was produced by 0.75×10^{10} n/cm^2 measured at the ED_{50} level. Assuming that 1.6×10^{10} n/cm^2 is equal to 1 rad of X-rays, RBE values of 2.1 (2 days) and 2.7 (5 days) were found. The RBE of thermal neutrons for early death was found earlier to be 1.4. The thermal neutrons by this measure were from 50% to 100% more efficient in producing lymphatic depression than in producing mortality.

It was necessary in arriving at the above values for thermal neutron effectiveness to assume that the gamma contamination had an RBE of 0.6 for both mortality and lymphatic depression and that the effects of the gamma rays and thermal neutrons were additive for these endpoints.

The thymic and bursal weights of all groups pretreated with AET were significantly larger than those of similarly irradiated non-injected groups ($p < 0.1$ to $p < 0.01$). At the highest dose both the thymus and the bursa were reduced in size on the fifth day compared to the second day. The AET chicks showed a similar decrease in thymic and bursal size from the second to the fifth day but these organs were still significantly larger than those from the similarly irradiated non-AET group.

TABLE VIII

The Relative Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in Producing Mortality and Lymphatic Depression in the Chick

The decrease in RBE for lymphatic involution seen from the second to the fifth day following proton irradiation indicates that recovery was greater during this time than during the comparable period following X-irradiation. The increase in RBE for lymphatic involution seen from the second to the fifth day following thermal neutron or fission neutron exposure indicates that recovery was less during this time than during the comparable period following X-irradiation.

TABLE VIII

The Relative Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in Producing Mortality and Lymphatic Depression in the Chick

Radiation	Lymphatic Depression				Mortality			
	ED_{50}		RBE		$LD_{50}/2$ d	RBE	Lymphatic RBE / Mortality RBE	
	2 d	5 d	2 d	5 d			2 d	5 d
X-rays	350 R	425 R	1.0	1.0	850 R	1.0	1.0	1.0
Protons	1.0×10^{10} p/cm^2 (410 rad)	1.37×10^{10} p/cm^2 (536 rad)	0.85	0.75	2.1×10^{10} p/cm^2 (863 rad)	0.98	0.87	0.77
Thermal neutrons	2.0×10^{12} n/cm^2 (125 rad) + 140 rad gamma	2.0×10^{12} n/cm^2 (125 rad) + 140 rad gamma	2.12	2.72	6.2×10^{12} n/cm^2 (388 rad) + 434 rad gamma	1.51	1.40	1.80
Fission neutrons	263 rad + 26 rad gamma	130 rad + 14 rad gamma	1.41	3.08	198 rad + 22 rad gamma	4.22	0.33	0.73

TABLE IX

A Comparison of the Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in the Chick and the Mouse

The RBE of the minimally ionizing protons was not found to be greater than unity for either lymphatic depression or mortality in either the chick or the mouse. The RBE values for lymphatic depression and for mortality in both the chick and the mouse were greater than unity for both thermal neutrons and fission neutrons. RBE values in the mouse showed less response to changes in LET than did the comparable values in the chick. From the least densely ionizing radiation to most densely ionizing radiation the RBE values for the mouse varied only by factors of 2.7 (mortality) and 2.1 (5 day lymphatic depression) whereas the RBE values for both mortality and 5 day lymphatic depression in the chick varied by factors of approximately 4.

TABLE IX

A Comparison of the Effectiveness of X-rays, 2.2 GeV Protons, Thermal Neutrons and Fission Neutrons in the Chick and the Mouse

Radiation	LET (kev/micron)	Relative Biological Effectiveness				
		Chick			Mouse	
		Lymphatic Depression		Mortality	Lymphatic Depression	Mortality
		2 day	5 day	2 days	5 day	30 day
250 kvp X-rays	3	1	1	1	1[c]	1[c]
2.2 GeV protons	?[a]	0.85	0.75	0.99	0.90	0.85[d]
Thermal neutrons	64% = 65[b] 36% = 0.3	2.1	2.7	1.5	1.4[c]	1.9[c]
Fission neutrons	43-48	1.4	3.0	4.2	1.9[c]	2.3[c]

a Mean LET not known but expected to be below that of X-rays.
b Protons with a mean LET of 65 and gamma rays with a mean LET of 0.3 produced by thermal neutron interactions.
c Values from Storer et al. (1957).
d Value from Jesseph et al. (1964) and Bond (1965).

FIGURE 6

Thymic and Bursal Response to Thermal Neutrons
With and Without Prior AET Treatment:
2 days post-exposure

Thermal neutron dose is given in terms of exposure time. The dose rate was 3.0×10^9 n/cm^2/sec. + 0.21 rad/sec. of gamma contamination. The 2 day ED_{50} for lymphatic depression was 2.0×10^{12} n/cm^2 + 140 rad of gamma radiation. The reference mark of ½ LD_{50}/2 day was equal to 3.1×10^{12} n/cm^2 + 217 rad of gamma radiation.

At each of the three doses employed, AET prior to exposure protected against involution of both the thymus and the bursa.

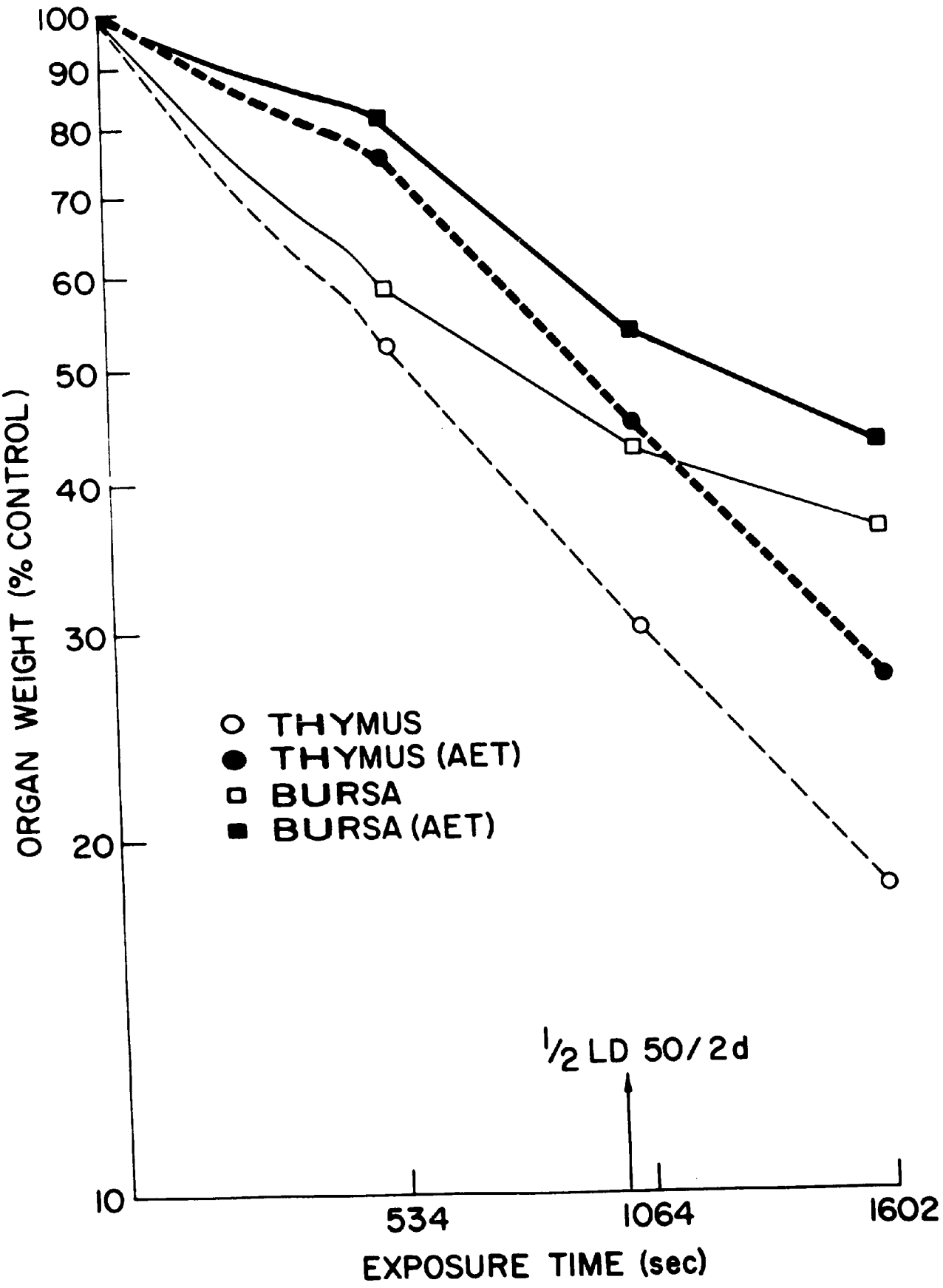

100
90
80
70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
THYMUS
THYMUS (AET)
BURSA
BURSA (AET)
1/2 LD 50/2d
534
1064
1602
EXPOSURE TIME (sec)

FIGURE 6

FIGURE 7

Thymic and Bursal Response to Thermal Neutrons
With and Without Prior AET Treatment:
5 days post-exposure

Thermal neutron dose is given in terms of exposure time. The dose rate was 3.0×10^9 n/cm^2/sec. + 0.21 rad/sec. of gamma radiation. The 5 day ED_{50} for lymphatic depression was 2.0×10^{12} n/cm^2 + 140 rad of gamma radiation. The reference mark of $\frac{1}{2}$ $LD_{50}/2$ day was equal to 3.1×10^{12} n/cm^2 + 217 rad of gamma radiation.

At each of the two higher doses the thymus and bursa were reduced in size below the 2 day values. This was true also at the highest dose in the AET-treated group. At each dose the thymi and bursae of the AET treated groups were larger than those from the similarly irradiated control birds.

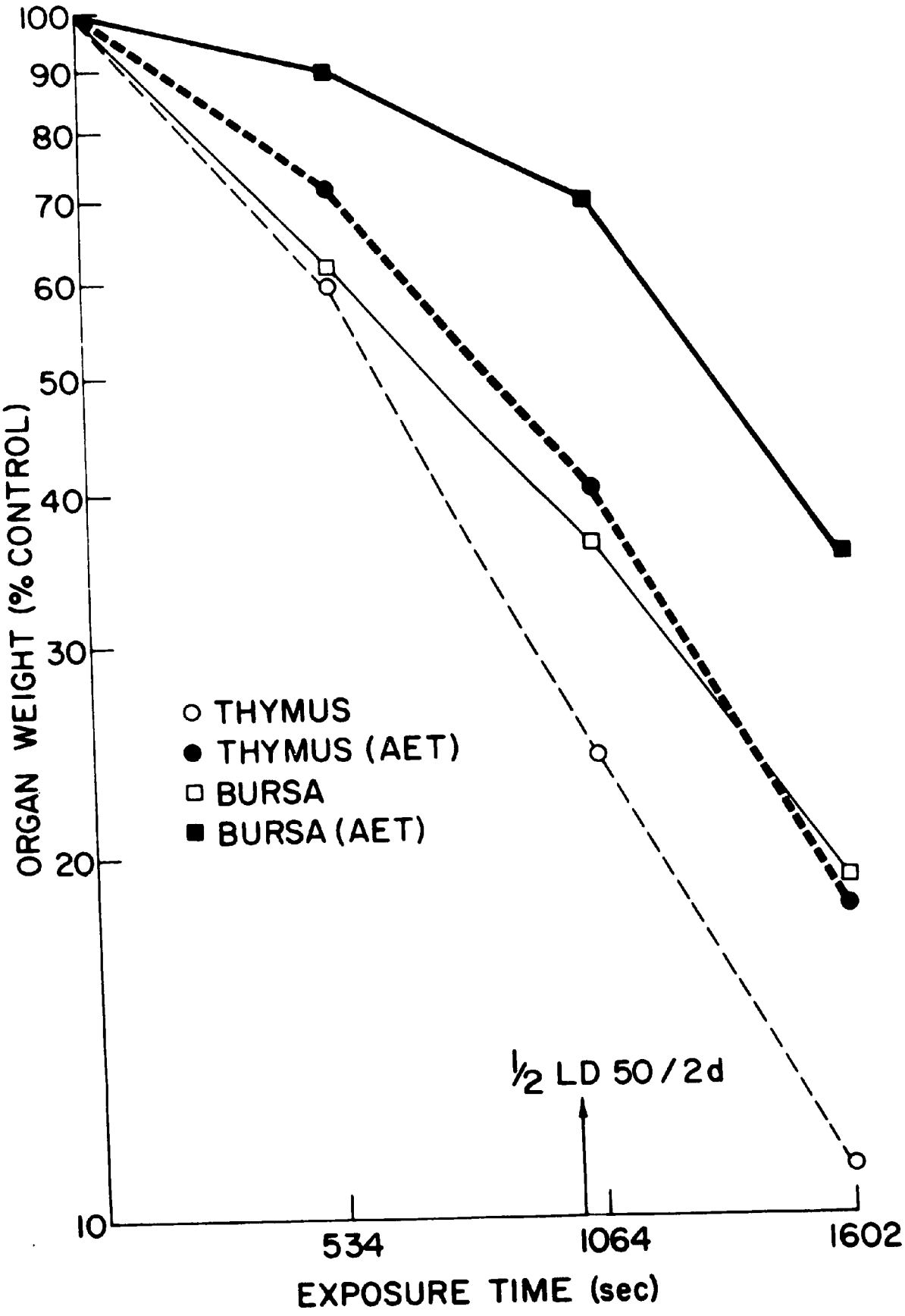

100
90
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70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
THYMUS
THYMUS (AET)
BURSA
BURSA (AET)
½ LD 50 / 2d
534
1064
1602
EXPOSURE TIME (sec)

FIGURE 7

c. Fission Neutrons

The results of exposure to fission neutrons, with and without prior AET injection are presented in Fig. 8 and Fig. 9. The ED_{50} values for total lymphatic tissue were found by extrapolation of the information in Fig. 8 and Fig. 9 to be 236 rad neutrons + 26 rad of gamma rays (2 days) and 130 rad of neutrons + 14 rad of gamma rays (5 days). The RBE values for fission neutrons were found in these two cases to be 1.4 and 3.1 respectively, compared to 4.2 for early mortality. On this basis, the fission neutrons were only 30% to 75% as effective per rad in producing lymphatic depression as in producing early mortality. As with the thermal neutron exposures, the gamma contamination was assumed to have an RBE of 0.6 and the effects of the gamma rays and neutrons were assumed to be additive for the two endpoints.

As with the thermal neutron and the X-ray exposures, the thymus was reduced more at each dose level than the bursa.

Two days post-exposure, the total lymphatic tissue from chicks injected with AET was greater than that from similarly irradiated non-injected chicks.

Five days post-irradiation, the thymus and bursa were reduced more in size compared to the control organs at the two higher doses than were the two day thymic and bursal weights. It was not possible to discern a consistent effect of AET 5 days post-exposure. Complicating the interpretation of the 5 day results was a high level of morbidity and a number of 3-5 day deaths.

FIGURE 8

Thymic and Bursal Response to Fission Neutrons
With and Without Prior AET Treatment:
2 days post-exposure

The fission neutron dose is given in terms of exposure time. The dose rate was 29.5 rad/min. of which 10% was low LET gamma radiation. The dose at the reference point $\frac{1}{2}$ $LD_{50}/2$ day was 99 rad of fission neutrons + 22 rad of gamma radiation. Even at the highest dose employed, which was approaching the lethal range, only a 30% reduction was caused in the size of the thymus and a 20% reduction in the size of the bursa. Extrapolating the lines through the three points an average ED_{50} value for the thymus and bursa was estimated to be 263 rad of fission neutrons + 26 rad of gamma radiation. This value is even greater than the $LD_{50}/2$ day of 198 rad of fission neutrons + 22 rad of gamma radiation. The thymus and bursa were larger at each dose level in the AET treated groups than in the untreated groups. In no group treated with AET was the bursa significantly reduced below the unirradiated level.

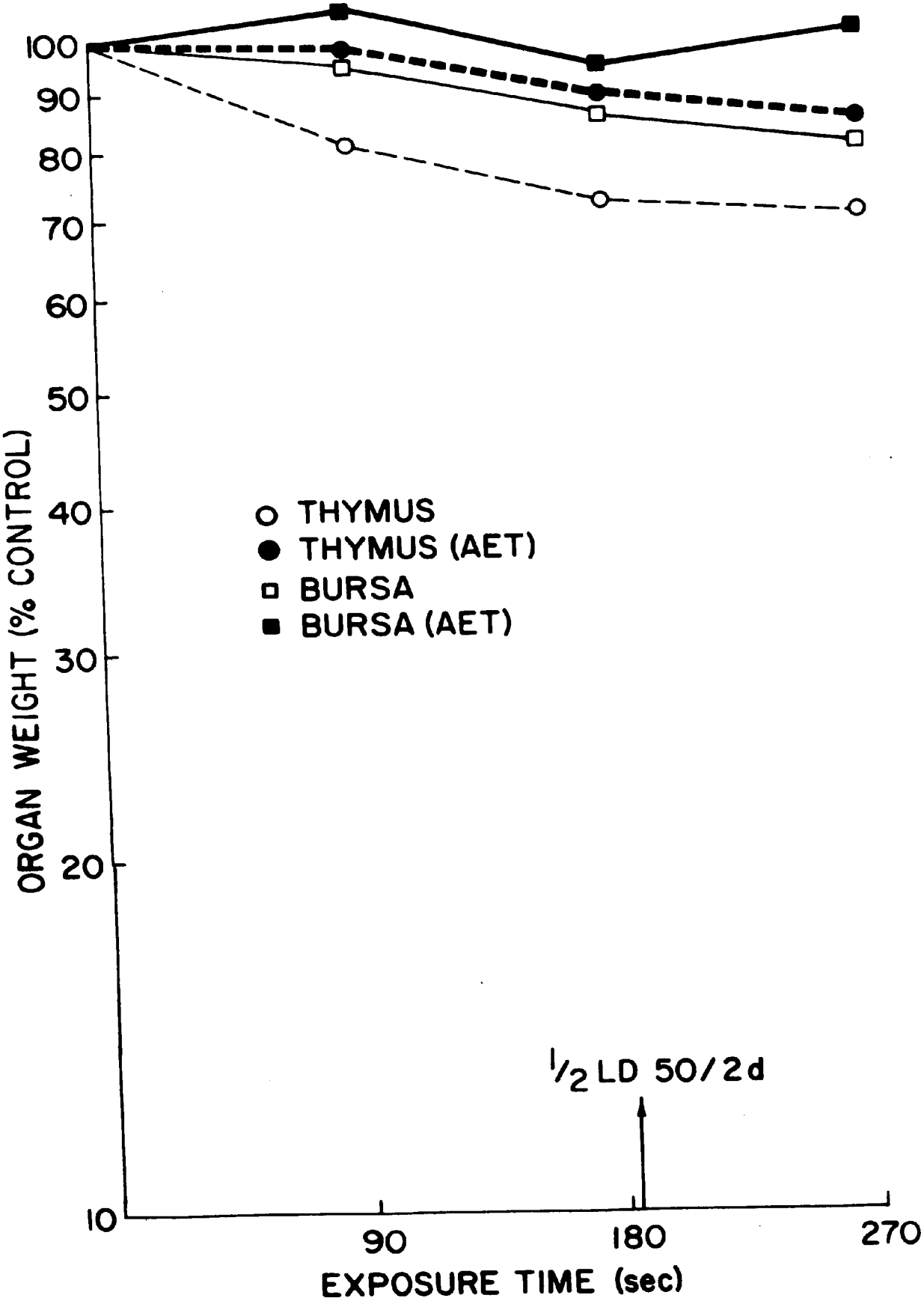

100
90
80
70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
THYMUS
THYMUS (AET)
BURSA
BURSA (AET)
1/2 LD 50/2d
90
180
270
EXPOSURE TIME (sec)

FIGURE 8

FIGURE 9

Thymic and Bursal Response to Fission Neutrons
With and Without Prior AET Treatment:
5 days post-exposure

The fission neutron dose is given in terms of exposure time. The dose rate was 29.5 rad/min. of which 10% was low LET gamma radiation. The dose at reference point ½ LD_{50}/2 day was 99 rad of fission neutrons + 22 rad of gamma radiation. The 5 day involution of both the thymus and bursa was found to be considerably greater than the two day level with an average ED_{50} of 130 rad of fission neutrons + 14 rad of gamma radiation. Only at the highest dose was any indication of a protective effect of AET still apparent and at no point were the organ weights of the AET treated chicks significantly greater than those from the similarly irradiated uninjected chicks.

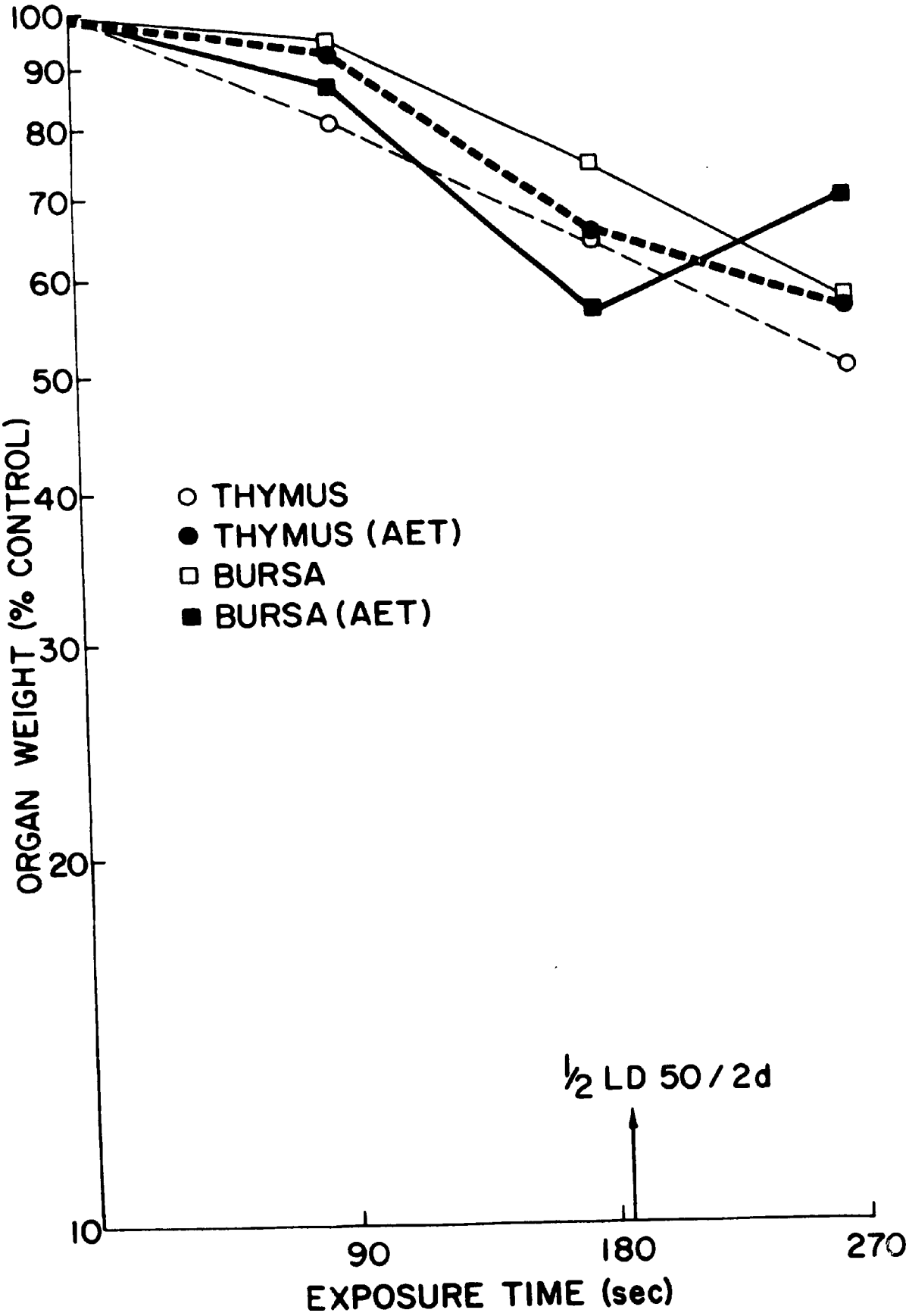

100
90
80
70
60
50
40
30
20
10
ORGAN WEIGHT (% CONTROL)
○ THYMUS
● THYMUS (AET)
□ BURSA
■ BURSA (AET)
½ LD 50 / 2d
90
180
270
EXPOSURE TIME (sec)

FIGURE 9

d. 2.2 GeV Protons

The two and five day lymphatic tissue weights as per cent of unirradiated control values are presented in Fig. 10. The ED_{50} values obtained from this figure were 1.0×10^{10} p/cm^2 (411 rad) two days post-exposure and 1.37×10^{10} p/cm^2 (563 rad) five days post-exposure. The RBE values calculated at the ED_{50} were 0.85 (two days) and 0.75 (five days) compared to 0.98 for early mortality.

The RBE for thirty day lethality in mice exposed under similar conditions was 0.85 (Jesseph, et al. 1964, Bond, 1965) and for five day spleen-thymus depression the RBE was 0.90 measured at the ED_{50} value (Table IX).

4. Discussion

The LET values for the radiations employed in this experiment and the RBE values for chick mortality and lymphatic involution are summarized in Table VIII. RBE values for similar endpoints in the mouse are also included in Table IX for comparison (Storer, et al. 1957, Bond, 1965). Despite the presence of densely ionizing nuclear fragments from the 2.2 GeV protons the mean LET of the interacting particles and protons is low, probably below that of the 250 kvp X-rays, (Bond, 1965). The average LET of the radiations employed in this experiment likely increase from protons, X-rays, thermal neutrons to fission neutrons.

The RBE values for both mortality and five day spleen-thymus depression in the mouse (Table IX) increase with increasing LET. The same relationship between LET and RBE was found in these experiments for early mortality and for five day lymphatic depression (Table IX). From the values in Table IX it appears that the mouse lymphatic tissue involution is somewhat less sensitive than mortality to changes in LET

FIGURE 10

Total Lymphatic Tissue Response to 2.2 GeV Protons:
2 days and 5 days post-exposure

By extrapolation of the two day values an $ED_{50}/2$ day was estimated to be 1.0×10^{10} p/cm^2 or approximately 410 rad. The 5 day ED_{50} was found to be 1.37×10^{10} p/cm^2 or approximately 536 rad.

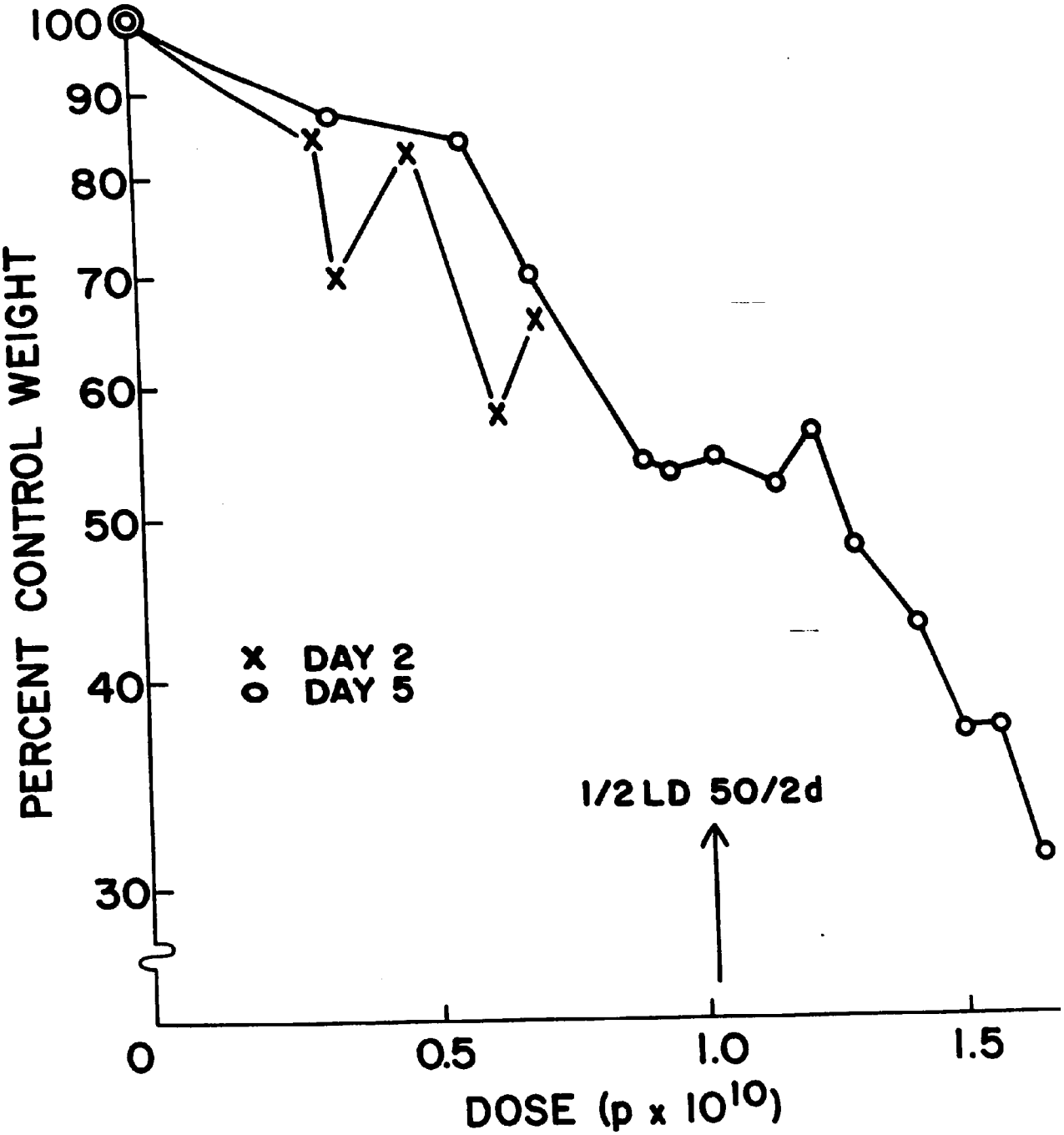

100
90
80
70
60
50
40
30
0
0.5
1.0
1.5
PERCENT CONTROL WEIGHT
DOSE (p x 10^10)
X DAY 2
O DAY 5
1/2 LD 50/2d

FIGURE 10

since the RBE values for lymphatic depression are consistently nearer to unity than those for mortality. In the chick the five day lymphatic depression and mortality were found to be about equally sensitive to changes in RBE, each showing the same fourfold difference in RBE from the least to the most densely ionizing radiation.

In the preceding chapter the maximum X-ray induced lymphatic involution in the chick was described as occurring on the second post-irradiation day and recovery, complicated by 'mammalian-type' abscopal damage occurring beyond that time. A similar pattern is seen in Fig. 4 and Fig. 5. Little change is seen between the thymic and bursal sizes on the second to the fifth day following 200 R and 400 R of X-rays, but at 600 R a decrease in organ weights from day 2 to day 5 has occurred. It was concluded from this that the 'mammalian-type' abscopal damage increased with increasing X-ray dose and at higher doses was sufficiently great to cause a net decrease in lymphatic organ weight. Although only the lower range of doses was covered on day 2 in the proton exposures the response was similar to that following X-ray exposure, that is recovery and increased organ size at low doses from the second to the fifth day post-exposure.

In the thermal neutron exposures, a large decrease in organ size occurred from the second to the fifth post-exposure day at the intermediate and high doses whereas no further depression or a slight increase in organ size was found at the lowest dose. Because the effectiveness of thermal neutrons compared to X-rays was greater for producing lymphatic depression than for mortality, it was possible to give exposures which reduced organ weights to only 10% to 20% of the unirradiated size without producing any early deaths. The difference between the two day and the five day values suggests that the higher

the effective dose the greater the 5 day or 'mammalian-type' abscopal effect. It is also possible that the densely ionizing particles caused irreparable damage to the dividing cells in the lymphatic tissue preventing repair and allowing the abscopal damage to be observed uncomplicated by repair or cell repopulation.

The fission neutron RBE for two day mortality was found to be about three times that found for lymphatic depression. The doses employed in the sublethal range for thymus-bursa studies were consequently lower than those in the thermal neutron sutdies and, as measured by effect, lower than those in the X-ray studies. A large abscopal effect was nonetheless observed at both the intermediate and high doses, even though the total depression in lymphatic tissue weight was not greater than 50% in any instance. The five day or 'mammalian-type' abscopal effect would thus appear to be greater not only at higher doses but also more detectable and possibly greater with densely ionizing radiations even at low doses. It would not be surprising to find both the lymphatic tissue and the gut, which is apparently responsible for the 'mammalian-type' abscopal depression of lymphatic tissue to be sensitive to changes in LET, since both lymphatic tissue damage and gut damage in mammals have been reported to be sensitive to changes in LET (Storer, et al. 1957).

Pretreatment with AET appears to have effectively reduced the radiation induced lymphatic involution following exposure to X-rays, thermal neutrons and fission neutrons. In the X-ray series no protection was found with AET following 200 R either 2 days or 5 days postexposure, but significant protection was seen with AET at both times following 400 R and 600 R. In the thermal neutron exposures, the

lymphatic organs were significantly larger in the AET treated groups at all doses employed.

In the fission neutron series the bursal weights of the AET-injected chicks 2 days post-exposure were not different from the non-irradiated values at any of the doses employed. On the other hand, the logarithm of the bursal weights in the control irradiation series decreased approximately linearly with increasing dose. The thymus was also larger in the AET-injected groups at all doses 2 days post-exposure. From the fission neutron and thermal neutron data it would appear that if any dose threshold exists for AET protection against lymphatic involution from high LET radiation it is very low compared to the lethal range. With X-rays, little or no protection was seen at 200 R but protection was seen at 400 R and 600 R.

5. Summary

The thymus was found to be depressed more by exposure to a given dose of either low LET or high LET radiations than was the bursa. Despite this apparent difference in radiosensitivity, the thymus and bursa both appeared to be responsive to both scopal and abscopal damage. The RBE for both 2 day and 5 day lymphatic depression as with mortality was greater at higher doses and with the high LET radiations than with the low LET radiations, suggesting that scopal + 'chick-type' abscopal damage (day 2 depression) and 'mammalian-type' abscopal damage (day 5) were sensitive to changes in LET.

AET injection prior to either high LET or low LET radiation exposure was effective in reducing lymphatic involution on both the second and fifth post-irradiation days. Since the injuries which lead to early mortality have been shown to be reduced by AET and since these same injuries have been implicated in producing a significant portion

of the two day thymic-bursal involution it is quite likely that modification of these injuries was responsible for at least a portion of the protection of AET measured 2 days post-exposure. On the other hand the degree of protection was so large in some instances that protection against the direct killing of lymphocytes under the radiation beam must have been occurring as well.

E. Lymphatic Depression Following Partial Body X-irradiation of the Chick

1. Introduction

It was suggested in the preceding two experiments that a portion of the thymic and bursal involution following exposure to ionizing radiations occurred through indirect processes as the result of damage to tissues other than lymphatic tissues. To gain direct information on this question chicks were exposed to partial-body X-irradiation so that either the thymus or the bursa was shielded during radiation exposure. The chick was ideally suited to the study of abscopal damage to lymphatic organs because of the extreme anterior position of the thymus along either jugular vein and the posterior position of the bursa dorsal to the cloaca. It was therefore possible to expose a large volume of the chick to X-rays without delivering a measurable dose to either the thymus in one case or the bursa in the other and determine what portion of lymphatic involution following total body X-irradiation was the result of scopal damage and what portion was the result of abscopal damage.

2. Methods and Materials

White Leghorn cockerels, two weeks of age, were exposed at 60 R/min. to 0 R, 200 R, 400 R or 600 R of 250 kvp X-rays delivered to the anterior half of the body, the posterior half of the body or the whole body. The exposure setup is illustrated in Fig. 11. In this

FIGURE 11

Partial Body Exposure Design

X-rays were delivered to either the whole body (lower figure) or the anterior or posterior half of the body (upper figure). At the midpoint of each of the four quadrants of the 8 inch square exposure area the dose varied by less than 1%. Either the bursa and(or) the thymus of each chick was centered at the midpoint of the quadrants. From exposure to exposure the positions of the chicks were systematically changed to cover each possible exposure situation.

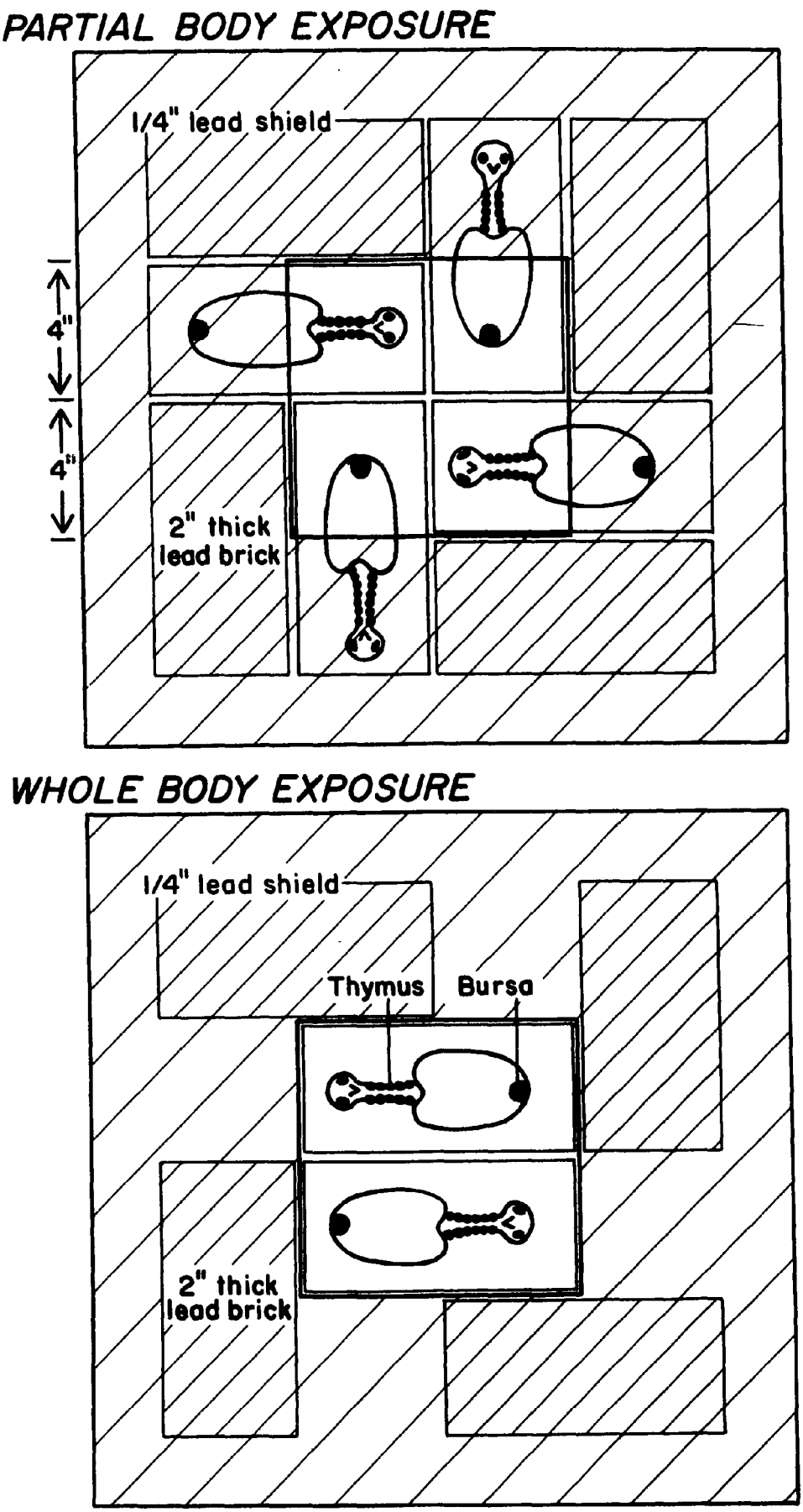

PARTIAL BODY EXPOSURE
1/4" lead shield
4"
4"
2" thick
lead brick
WHOLE BODY EXPOSURE
1/4" lead shield
Thymus
Bursa
2" thick
lead brick

FIGURE 11

arrangement the thymus or bursa was in approximately the same position within its quadrant of the exposure area regardless of which of the three exposures was being performed. Exposure positions were systematically altered throughout, although the measured dose varied by less than 4% from the midpoint of the 8" exposure square to the edge of the shield in any direction. The variation in dose at the midpoints of the four quadrants was less than 1%. The shielded lymphatic organs were from 8-10 cm from the edge of the lead shield. Under the shield the dose was reduced to less than 10% at 1 cm, less than 5% at 3 cm and less than 1% at 8 cm.

The chicks were restrained for exposure by means of pliable vinyl tubes 2" in length slid over the body. The restraining tubes allowed free breathing but maintained the legs in a slightly extended position and prevented wing movement. The animals were then secured to paperboard slabs measuring 8" by 4" by 3/4" by means of cloth backed foam rubber strips pinned over the legs and neck. Although no stress was detected by this procedure alone as measured by reduction in lymphatic organ weight, all control or unirradiated animals were similarly restrained for a period of time equal to the longest exposure time. A total of 24 animals was utilized with each treatment. Eight animals per treatment group were dissected on the second, fifth, and eighth days post-exposure.

Food and water were supplied ad libitum from the day of hatch through the termination of the experiment.

3. Results

The 2, 5, and 8 day thymus and bursa weights as per cent of control weights following 200 R, 400 R, and 600 R of partial-body or whole-body X-irradiation are presented in Fig. 12 and Fig. 13.

a. Thymic Response

Posterior body exposure resulted in a decrease in thymic weight compared to control weights at all dose levels measured 2 days post-exposure (Fig. 12). At 400 R and 600 R the decrease in thymic weight was maximum on the fifth day post-exposure while weight loss was similar on each day after 200 R.

The two day thymus weight-dose responses were approximately linear for both whole-body and anterior-body irradiation with ED_{50} values of 450-500 R. The 200 R and 400 R weights were not different in the two groups but at 600 R the average weight of the whole-body group was significantly less than that of the anterior-body group ($p < 0.05$). Five days post-exposure, the weights were similarly different at 600 R ($p < 0.05$) and at 400 R as well ($p < 0.05$). The five day ED_{50} values were 425 (whole-body exposure) and 575 R (anterior-body exposure).

Eight days post-exposure two differences were observable between the whole-body and anterior-body groups. The average thymic weight of the whole-body group was larger at 200 R ($p < 0.05$) and the average thymic weight of the anterior-body group was considerably larger at 600 R ($p < 0.01$) than that of the whole-body group. The difference noted at 200 R was contradictory to the generally observed equal or greater depression of lymphatic tissue due to whole-body irradiation compared to partial-body irradiation. This discrepancy was not found in any other of four similar experiments.

FIGURE 12

Thymic Involution Following Whole-body,
Anterior-body, or Posterior-body X-irradiation

Anterior body irradiation caused a linear decrease in thymic weight 2 days post-exposure. This response was considered to be most representative of scopal radiation injury. Recovery was apparent 5 and 8 days post-exposure. Additional decreases in thymic weight below these values were considered to be the result of abscopal processes. Any decrease in thymic weight due to posterior-body irradiation was also considered to be the result of abscopal processes.

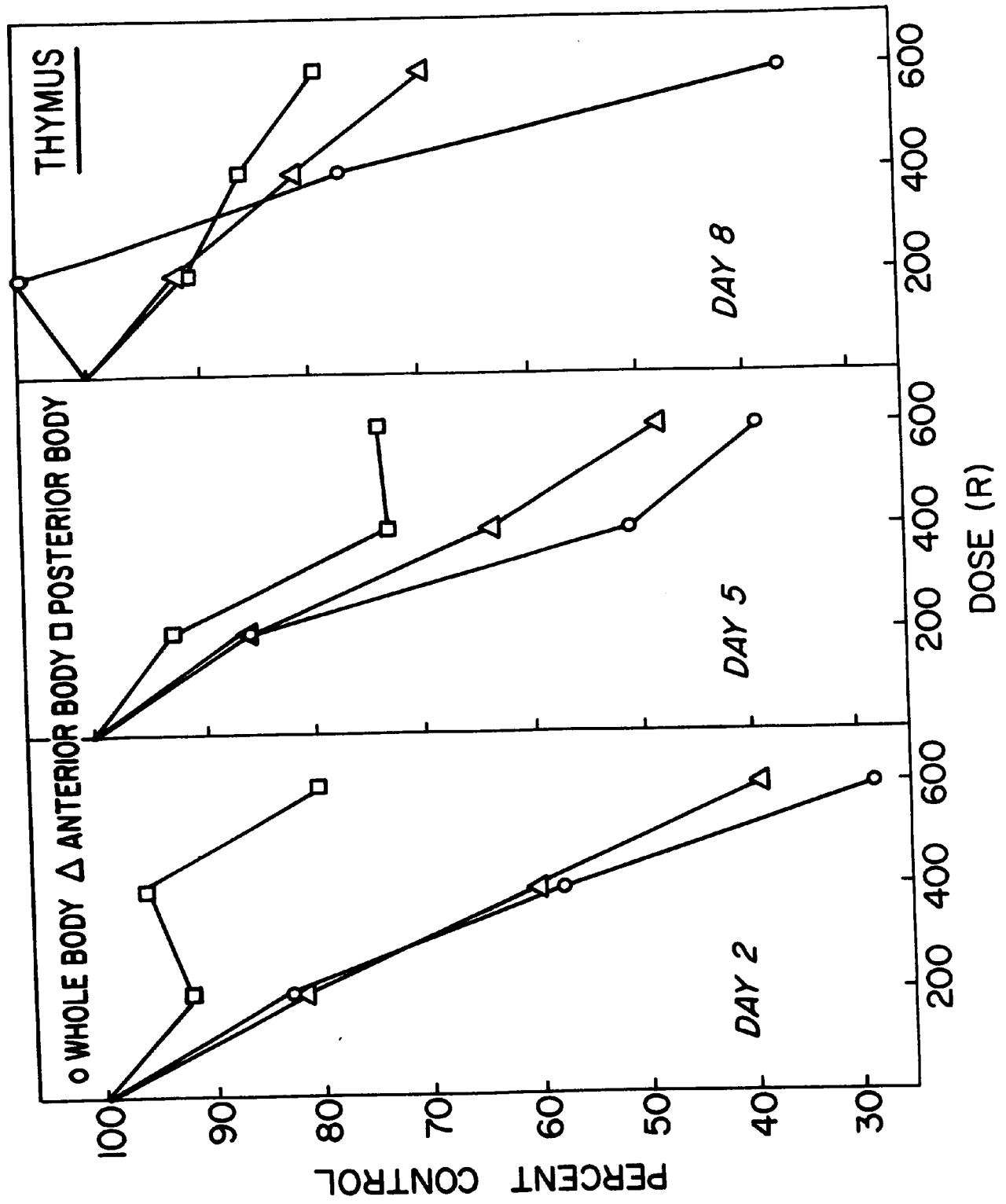

THYMUS
o WHOLE BODY Δ ANTERIOR BODY □ POSTERIOR BODY
DAY 2
DAY 5
DAY 8
PERCENT CONTROL
100
90
80
70
60
50
40
30
200
400
600
DOSE (R)

FIGURE 12

FIGURE 13

Bursal Involution Following Whole-body,
Anterior-body or Posterior-body X-irradiation

No difference was found between the involution due to posterior-body or whole-body irradiation. The more extensive recovery in the posterior-body exposure group compared to the whole body exposure group suggests the presence of abscopal damage in the whole-body exposure group in addition to that present in the posterior-body exposure group. Anterior-body exposure resulted in bursal involution by two days post-exposure, followed by recovery 5 days and 8 days post-exposure indicating 'chick-type' abscopal damage due to anterior-body exposure alone.

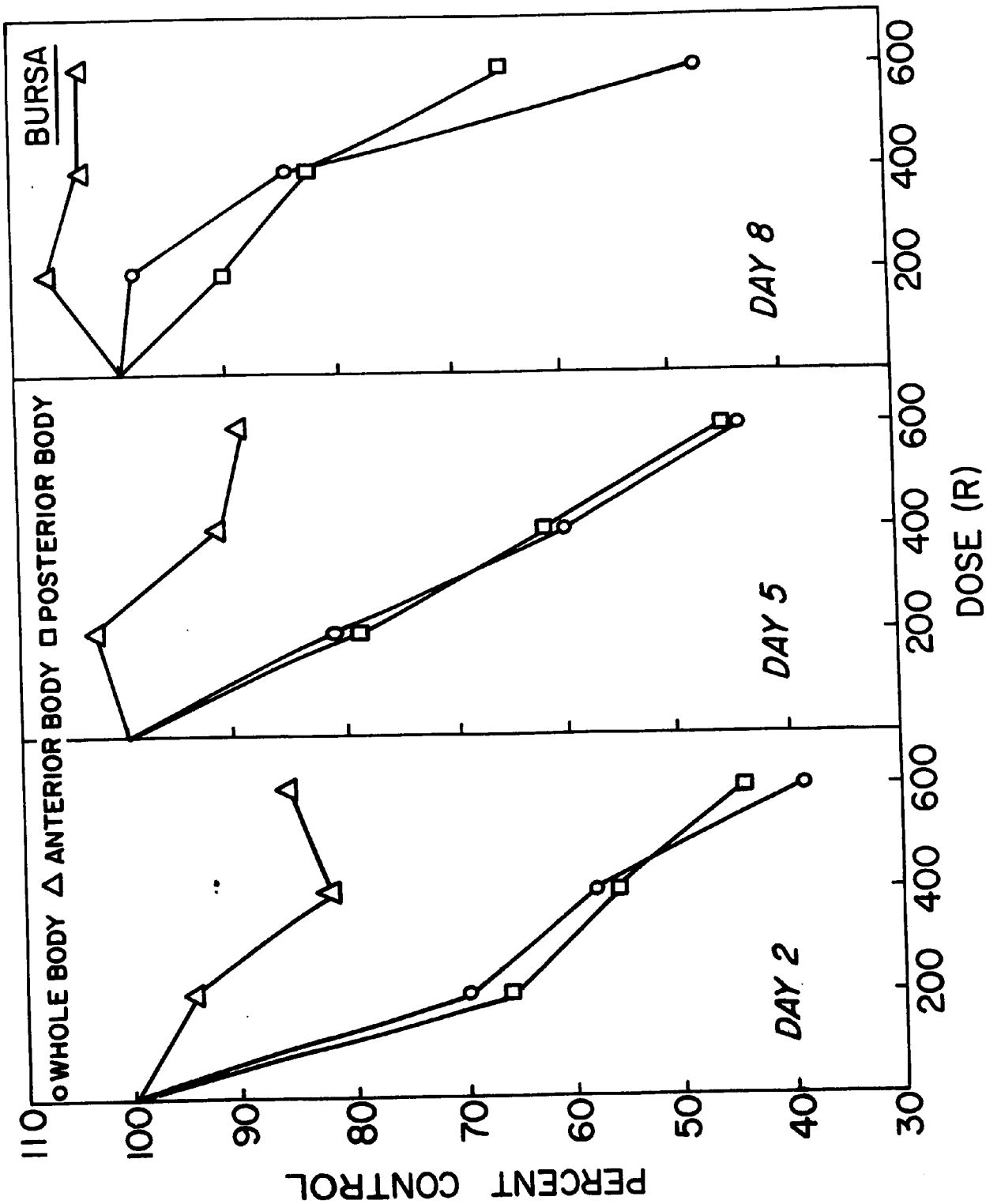

BURSA
WHOLE BODY △ ANTERIOR BODY □ POSTERIOR BODY
PERCENT CONTROL
110
100
90
80
70
60
50
40
30
DAY 2
DAY 5
DAY 8
200
400
600
DOSE (R)

FIGURE 13

b. Bursal Response

Two days after anterior-body irradiation, the bursa was reduced in size compared to the control size at all dose levels and was significantly less at 400 R and at 600 R ($p < 0.05$) (Fig. 13). Five days post-exposure the 400 R and 600 R bursal weights were still below control weights and eight days post-exposure the bursal weights at all dose levels were slightly above those in the control group.

No difference was seen between whole-body exposure and posterior-body exposure except at 600 R on the eighth day when the bursa of the whole-body group was significantly smaller ($p < 0.05$). For whole-body and posterior-body exposure the 2-day ED_{50} was 475 R to 500 R and the 5-day ED_{50} was 500 R to 550 R.

4. Discussion

a. Scopal Damage

The best estimate of the direct or scopal radiation damage and subsequent recovery to the chick lymphatic tissue was obtained from the thymic response to anterior-irradiation. In these animals the thymus along with few other radiosensitive tissues and approximately 1/3 of the total chick volume was exposed, resulting in only a small amount of abscopal damage. The log thymus weight-dose response was approximately linear at maximum depression on the second day after irradiation. The ED_{50} value at this time was 500 R. On the fifth day and eighth day recovery was evident at each dose and the log thymus weight-dose relationship remained essentially linear.

b. Abscopal Damage

Two analyses of the included data were used to determine the presence and extent of abscopal damage.

1. Any difference between the thymic weights of the group whose posterior half was irradiated and the thymic weights of the control animals was assumed to be the result of abscopal damage. Similarly any decrease in bursal weights below control values as the result of anterior-body irradiation was considered to represent abscopal damage.

Two days after anterior-body exposure the bursa was reduced in weight (Fig. 13) and progressive recovery was evident on the fifth and eighth days. These results were interpreted as indicating 'chick-type' or 2-day abscopal damage without any 'mammalian-type' or late abscopal damage due to gastrointestinal injury.

The thymus was reduced below control levels on the second day following posterior-body irradiation and showed about the same extent of 'chick-type' damage as the bursa in the anterior-body exposures (Fig. 12). While the bursa showed progressive recovery on the fifth and eighth days, the thymus showed an additional weight loss on the fifth day and only slight recovery on the eighth day. This later depression and lack of recovery in the group whose gastrointestinal tract was included in the radiation field was interpreted to be indicative of 'mammalian-type' abscopal damage.

2. Any additional decrease in organ size due to whole-body irradiation as compared to partial-body irradiation was also considered to be the result of abscopal damage. This interpretation was valid only when the organ considered was under the beam during the partial body exposure as with the thymus during anterior-body exposure and the bursa during posterior-body exposure. If the scopal and abscopal damages were strictly additive, the whole-body values would be expected to be reduced below the partial-body values by an amount proportional to the degree of depression below the control level seen in the irradiation of

the anterior body in the case of the bursa or the posterior body in the case of the thymus as was described above.

Only on the eighth day and at 600 R was the bursal weight less following whole-body irradiation than following posterior body irradiation. From the 2-day and 5-day lines in Fig. 13 this can be seen to be primarily the result of recovery in the group whose posterior half was exposed.

Two days following exposure to 600 R the thymus was smaller in the whole-body irradiation group than in the anterior-body irradiation group indicating the presence of 'chick-type' abscopal damage. On the fifth day the same relationship was again seen at 600 R and also at 400 R suggesting, at least, the 'mammalian-type' abscopal damage. On the eighth day the thymus only of the 600 R group was smaller following whole-body irradiation than following anterior-body irradiation. The difference, however, was greater than at any other time, suggesting that the 'mammalian-type' abscopal damage continued and possibly increased at this high dose to the whole body, while recovery was occurring from the scopal damage as seen by the increase in size of the thymi of the animals whose anterior half was exposed.

The abscopal depression of chick lymphatic tissue was not nearly as apparent by this second analysis as it was by the first. In the thymus, however, both types of abscopal damage could be seen to occur although only at the higher doses. Although the whole-body exposures were more effective than the partial-body exposures in some instances in reducing the weight of lymphatic organ under the beam, the scopal and abscopal effects appeared to be less than additive.

From the information in this experiment and those previously described idealized dose-response patterns and recovery patterns of the types of damage incurred by the lymphatic tissues of irradiated chicks have been constructed (Fig. 14). The important aspects of these are summarized as follows.

1. Following whole-body irradiation of the chick, the lymphatic organs were caused to be reduced in size by direct damage to the cellular components of the lymphatic organs (scopal effect) and indirectly by damage to tissues other than the exposed lymphatic tissues.

2. Scopal or direct damage (log organ weight) was proportional to dose and maximal at two days with recovery occurring at all doses beyond two days.

3. Two day or 'chick-type' abscopal damage was roughly proportional to dose and possibly related to the amount but apparently not the type of tissue irradiated. The occurrence of 'chick-type' abscopal damage following exposure of either the anterior-half or the posterior half of the chick does not rule out the presence of a sensitive organ or tissue for 'chick-type' abscopal damage but merely the inability of such an organ or tissue to be distinguished by this technique.

4. Five day or 'mammalian-type' damage appeared to depend upon the nature of the irradiated tissue. Irradiation of the lower body, which included most of the gastrointestinal tract, resulted in 'mammalian-type' abscopal damage while irradiation of approximately the same amount of tissue in the anterior half of the chick had little or no effect.

FIGURE 14

Proposed Components of Radiation Damage
to Chick Lymphatic Tissues

The dose scale in these figures is only approximate and a difference of 100 R in either direction would be possible. Although these models have been drawn for X-irradiation, some of the information used was from other types of radiation exposures further emphasizing the more relative than absolute nature of the abcissa.

Scopal Damage - Log-linear depression with dose, maximum two days post-exposure followed by recovery at all doses.

'Chick-type Abscopal Damage - The threshold indicated could instead be depression with a lesser slope than the second component of the lines. Maximum depression two days post-exposure followed by recovery.

'Mammalian-type' Abscopal Damage - No depression 2 days post-exposure and log-linear depression with dose 5 days post-exposure. Occurrence dependent upon type of tissue exposed - primarily associated with gastrointestinal damage.

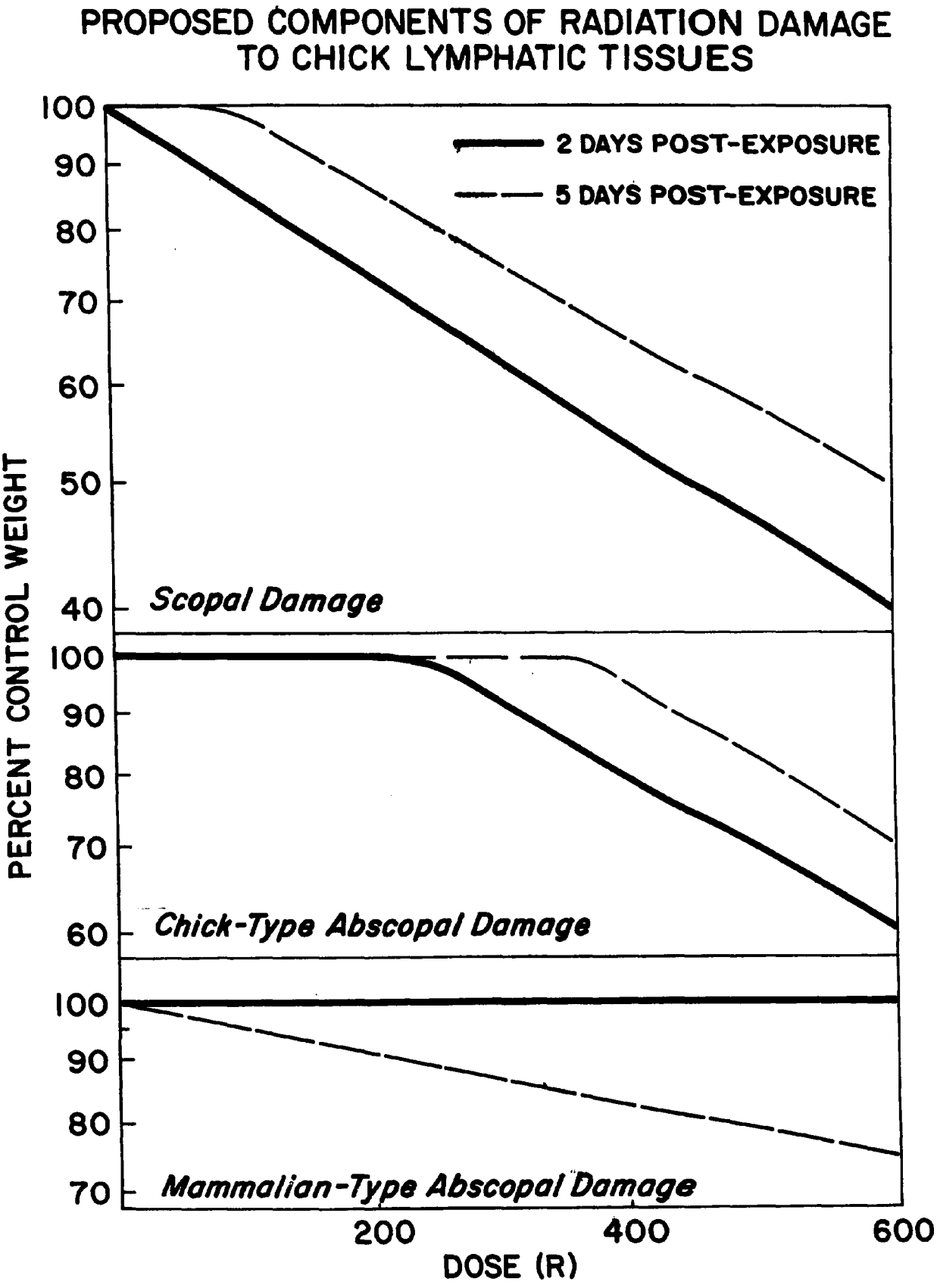

PROPOSED COMPONENTS OF RADIATION DAMAGE
TO CHICK LYMPHATIC TISSUES
2 DAYS POST-EXPOSURE
5 DAYS POST-EXPOSURE
PERCENT CONTROL WEIGHT
100
90
80
70
60
50
40
Scopal Damage
100
90
80
70
60
Chick-Type Abscopal Damage
100
90
80
70
Mammalian-Type Abscopal Damage
200
400
600
DOSE (R)

FIGURE 14

F. X-ray Induced Thymic Depression in Surgically Bursectomized Chicks

1. Introduction

It was concluded in the preceding chapter that irradiation of tissues other than lymphatic tissues could result in both two day and five day lymphatic depression. Since the thymus and bursa are composed of similar cell types, it seemed possible that irradiation of one of these organs during partial body irradiation could cause a depletion in cell number and size of the other by inducing a migration of cells from the unexposed to the exposed organ. If this were the case, the two day or 'chick-type' abscopal damage could not be related directly to the lethal radiation processes in the chick, but would be merely the net effect of cellular redistribution due to damage of a similar lymphatic organ.

This experiment was designed to determine if the two day abscopal effect still existed when no major lymphatic organ was present under the radiation beam.

2. Methods and Materials

Thirty-two White Leghorn cockerels were surgically bursectomized at 14 to 16 days of age. On the eighteenth day of age the animals were divided into four groups. For this distribution the animals were matched according to day of bursectomy, body weight at bursectomy, bursa weight and body weight at eighteen days of age. The animals were then exposed to 600 R of X-rays delivered to the whole body, the

anterior half of the body or the posterior half of the body. One group was maintained as unirradiated controls.

Exposure conditions were identical to those previously described for partial body exposure.

Two days post-exposure the animals were killed and their thymi removed and weighed. Ten chicks which were not bursectomized were also dissected as cage controls.

3. Results

The thymic weights obtained in this experiment have been listed in Table X. The thymic weights of the bursectomized controls were approximately 5% below those of the cage controls. Exposure of the posterior half of the chick resulted in a depression of 11% in the average weight of the thymus below the average control weights (difference, $p < 0.05$). Whole body exposure was significantly (< 0.05) more effective than anterior body exposure in reducing the thymic weight in the bursectomized chicks.

4. Discussion

Both the reduction in thymic weights below the control (bursectomized) thymic weights and the greater effectiveness of whole-body irradiation over anterior-body irradiation in producing thymic depression suggest that a true abscopal effect and not merely cellular redistribution was occurring. The involution seen following both whole-body and anterior-body exposure was approximately equal to that seen two days following similar exposures of the intact animal. The involution following posterior-body exposure was not as great as that seen in the intact animal. Two possible explanations for this are suggested. First, the involution due to the stress of surgery and the

TABLE X

X-ray Induced Thymic Involution In Bursectomized Chicks

Treatment*	N	Thymic Weight (mg/100 g body weight)**	Std. Error
Cage controls	10	451 (105%)	12
0 R	8	432 (100%)	20
600 R Posterior half	8	385 (89%)	20
600 R Whole Body	8	123 (28%)	6
600 R Anterior Body	8	165 (38%)	5

* All groups except cage controls were surgically bursectomized 2-4 days pre-irradiation.

** Figures in parentheses are per cent of 0 R bursectomized weights.

The average thymic weight of the group which received 600 R to the posterior half of the body was lower than the average thymic weight of the unirradiated bursectomized group ($p < 0.05$). The average thymic weight of the group which received whole body irradiation was lower than the average weight of the group which received 600 R to the anterior half of the body.

abscopal damage from posterior-body irradiation were less than additive. Second, the presence of one major lymphatic organ under the radiation beam might enhance the contribution of the abscopal processes perhaps, as was suggested previously, by inducing cellular migration to the injured lymphatic organ.

5. Summary

Partial-body exposure techniques were able to demonstrate the presence of abscopal damage in the bursectomized chick. This was taken as evidence that the abscopal depression in the chick was not merely the result of cellular re-distribution.

IV. GENERAL DISCUSSION AND SUMMARY

Following exposure to X-rays, thermal neutrons or fission neutrons, early (48 hour) deaths were found to fall into two distinct and separate time periods. At high doses of X-rays 100% mortality was produced in the first of these periods (3-9 hours post-exposure) but at no dose was the mortality in the second period (17-30 hours post-exposure) greater than 50%. Exposure to high LET thermal or fission neutrons caused a reduction in the time between the two periods of mortality. The second period of mortality was more sensitive to high LET radiations than the first period so that nearly 100% mortality was obtained in the second at doses below which significant mortality appeared in the first period.

Exposure to 2.2 GeV protons resulted in early chick mortality but the time pattern of deaths was different from that seen with either the low LET X-rays or the high LET thermal neutrons and fission neutrons. The first of the two mortality periods was represented at each dose in which significant mortality was observed, but few additional deaths were observed even at low doses, so that it was not possible to distinguish a second mortality period within 48 hours of exposure to 2.2 GeV protons.

Whole body exposure of the chick at both low dose rates (below 10 R/min.) and high dose rates (above 15 R/min.) caused acute involution of the thymus, bursa of Fabricius and spleen. At all doses studied, the high dose rate was more effective than the low dose rate in producing

lymphatic involution. The difference was characterized by an additional depression two days post-exposure at the high dose rate while recovery was taking place at the same time following low dose rate exposure. The dose rate effect on the chick lymphatic tissues was small compared to the dose rate effect on mortality, but was suggested to be related to the dose rate sensitive lethal processes since there was no reason to believe that the lymphatic lymphocytes were themselves sensitive to changes in dose rate.

Pre-irradiation injection of AET was found to protect the young chick against death or lymphatic involution whether the radiation source was X-rays, thermal neutrons or fission neutrons. Two conclusions were drawn from the protectiveness found with AET against early chick mortality. First, since nuclear damage and cell death are likely not involved in the early chick mortality, it was concluded that protective compounds are capable of acting against damage to cellular components other than the nucleus. Second, it was concluded that the original lesions responsible for the alterations in capillary permeability, eventually responsible for early chick death, are cumulative at sublethal levels and are subject to repair or reversal even when densely ionizing particles are employed as the radiation source.

A large portion of the acute radiation-induced involution of lymphatic tissues is the result of direct or scopal killing of lymphatic lymphocytes. This type of damage has not been reported to be modified by radioprotective chemicals in mammals following X-irradiation and would be expected to be no more sensitive to modification following neutron irradiation. Since the scopal damage involved appears to be very similar in the chick, the protection against lymphatic involution

following both X-irradiation and neutron irradiation in the chick was attributed in large part to protection against abscopal damage and particularly against the dose rate sensitive damage capable of producing death at higher doses. By exposing the whole body, the anterior half of the body or the posterior half of the body to X-rays it was concluded that three types of injury resulted in involution of chick lymphatic tissues: 1) scopal damage or direct radiation killing of lymphatic lymphocytes under the radiation beam, 2) 'chick-type' abscopal damage, that is, involution through indirect processes as the result of damage to tissues other than lymphatic tissues, in this case the same injury which at higher doses leads to early mortality, and 3) 'mammalian-type' abscopal damage or stress resultant from injury to the gastrointestinal tract.

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