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IN VIVO RADIOBIOLOGICAL STUDIES OF 910 MeV HELIUM ION BEAM

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(Ph. D. Thesis)

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CONTENTS

Page

LIST OF TABLES 2
LIST OF FIGURES 3
ABSTRACT 4
INTRODUCTION 6
MATERIALS AND METHODS 8
Animals 8
Irradiation 9
General techniques 9
Modes of delivery 10
1. Single dose in air 10
2. Split dose in air 11
3. Single dose under hypoxia 11
Dosimetry 12
A. Bragg peak alpha 13
B. Plateau alpha 12
C. Cobalt-60 gamma 13
Post-irradiation procedures 14
I. 6-Day animal survival 14
II. 30-Day animal survival 15
III. Microscopic intestinal colonies 15
IV. Macroscopic endogenous spleen colonies 16
RESULTS 17
I. Gut death 19
II. Marrow death 23
III. Survival of intestinal crypt cells 27
IV. Survival of spleen colonies 31
V. Miscellaneous 35
ANALYSIS 41
1. Relative biological effect in air 41
2. Recovery pattern in air 41
3. Oxygen enhancement ratio 42
4. Correlation of acute radiation lethalities with cellular radiosensitivities 43
DISCUSSION 46
I. Acute intestinal syndrome 46
II. Acute marrow syndrome 50
III. Radiation response of intestines 55
IV. Radiation response of hemopoietic tissues. 60
V. Miscellaneous 68
ACKNOWLEDGMENTS 75
REFERENCES 76

LIST OF TABLES

Page

I 6-Day Animal Survival Data 19
II 30-Day Animal Survival Data 23
III Microscopic Intestinal Colony Data 27
IV Macroscopic Spleen Colony Data 31
V Body Weight Data after Single Dose in Air ... 38
VI 30-Day Animal Survival Data after Split Dose Cobalt-60 Exposure in Air 40
VII Summary of Results 44
VIII Summary of Analysis 45

LIST OF FIGURES

			Page
I	(1)	6-Day Animal Survival after Single Dose in Air	20
I	(2)	6-Day Animal Survival after Split Dose in Air	21
I	(3)	6-Day Animal Survival after Single Dose under Hypoxia	22
II	(1)	30-Day Animal Survival after Single Dose in Air	24
II	(2)	30-Day Animal Survival after Split Dose in Air	25
II	(3)	30-Day Animal Survival after Single Dose under Hypoxia	26
III	(1)	Intestinal Crypt Stem Cell Survival after Single Dose in Air	28
III	(2)	Intestinal Crypt Stem Cell Survival after Split Dose in Air	29
III	(3)	Intestinal Crypt Stem Cell Survival after Single Dose under Hypoxia	30
IV	(1)	Endogenous Spleen CFU Survival after Single Dose in Air	32
IV	(2)	Endogenous Spleen CFU Survival after Split Dose in Air	33
IV	(3)	Endogenous Spleen CFU Survival after Single Dose under Hypoxia	34
V		Body Weight Changes after Single Dose in Air	39
VI		Recovery Pattern of 30-Day Animal Survival after Split Dose Cobalt-60 Exposure in Air	40

ABSTRACT

Both the modified Bragg peak and the plateau portion of the 910 MeV helium ion beam from the Berkeley 184" synchrocyclotron (henceforth abbreviated as "peak α" and "plateau α" respectively) were compared to cobalt-60 gamma rays (^{60}Co γ) by using 3 modes of total body exposure to evaluate 3 parameters in each of 4 *in vivo* systems in 13 to 14 week old female LAF_1 mice. The 3 modes of exposure were (1) single dose in air, (2) split dose in air, and (3) single dose under hypoxia, and the 3 parameters evaluated included (1) relative biological effectiveness (RBE) in air, (2) recovery pattern in air expressed as percentage of recovery of the first dose (%R), and (3) oxygen enhancement ratio (OER). The 4 test systems consisted of (I) 6-day animal survival, (II) 30-day animal survival, (III) survival of microscopic intestinal colonies, and (IV) survival of macroscopic endogenous spleen colony forming units (CFU).

Because the survival curves in each set were not parallel, the doses to achieve iso-effect were compared at several survival levels in order to elicit a more composite picture. For lethality studies, besides the more reliable median lethal dose (LD_{50}) conventionally used, the 90% and 10% lethal doses (LD_{90} and LD_{10} respectively) were also evaluated. For intestinal colonies, in addition to the slope (inverse of D_0) and the 'extrapolation number' at zero dose (n), the doses resulting in 100 colonies and 10 colonies were computed. For spleen colonies, similar data were obtained except that the 2 levels of survival selected were 10 colonies and 1 colony.

(1) The RBE of peak α was significantly greater than unity for all end points of the 4 systems under consideration. It ranged from 1.17 to 1.39, being greater than 1.25 in most instances. On the other hand, plateau-α RBE was not significantly different from unity, with a mean effectiveness of 1.12.

(2) Recovery at 48 hours after exposure was in general greater in the intestines than in hemapoietic tissues. For gut death, it amounted to 51.4 - 67.4%, depending on the survival level and was almost identical for all 3 types of radiation. For marrow death, peak-α irradiated mice showed significantly less capacity for recuperation than those exposed to plateau α or ^{60}Co γ, especially at LD_{90} level (16.7 vs 33.6 and 47.5%). For intestinal colonies, again, the 3 types of radiation were comparable, and remarkable recovery of 80.0 - 114.6% was observed. Moreover, all 3 recovery regression lines were less steep than their single dose counterparts, the change in D_0 ranging from 50.9 - 66.1%. For spleen colonies, the change in D_0 was least with peak α (15.2%) than with plateau α (44.8%) or ^{60}Co γ (50.9%). At 10 colony level, all 3 radiations resulted in similar recovery (45.6 - 51.6%). However, at 1 colony level, again peak α produced more irreparable damage as evidenced by only 49.6% recovery compared to 64.0 - 65.8% after ^{60}Co γ and plateau α exposure.

(3) The oxygen effect varied according to the end point chosen, even for the same type of radiation. Nonetheless, a comparison of corresponding end points showed that peak α had a lower OER (2.02-2.22) than ^{60}Co γ (2.30 - 2.57) except for intestinal colony survival, and that although plateau α appeared to have slightly less oxygen effect than ^{60}Co γ, the difference was of doubtful statistical importance.

(4) Good correlation was noted between gut death and crypt cell survival and between marrow death and spleen CFU survival.

The motivation of this work is to shed some light on the therapeutic potentials of heavy ions.

INTRODUCTION

Until we find the cause of cancer or find improved methods of treatment and can thereby prevent its occurrence, surgery and radiotherapy will continue to be the major methods in its treatment. Attempts to treat human diseases with ionizing radiation were made soon after discovery of the Roentgen ray and radium before the turn of the last century. The initial development of radiation therapy was essentially based on empiricism. However, through the ensuing years, it has gradually evolved from an art to a partial science with the improvement in physical dosimetry and in our understanding of the effects of radiation on living tissues. The development of clonal culture of mammalian cells was a great stride in quantitating radiation response in vitro. This was soon followed by the establishment of various ingenious techniques for estimating the clonogenic response in vivo. It has become evident that successful radiotherapy depends on our knowledge of the fundamental basis of radiobiology.

A major goal of radiotherapists is to deliver enough radiation to eradicate a tumor without causing intolerable damage to the surrounding normal tissues. In spite of modest improvement in methods for tumor localization, the introduction of megavoltage radiation, careful treatment planning with beam collimation and shaping, and the use of crossfiring techniques with multiports or multiaxial rotations, the presence of relatively radioresistant hypoxic cells in a tumor still apparently constitute a major reason for failure which is all too common. Various methods have been used to circumvent this difficulty. Conventional dose fractionation apparently results in a recovery differential between tumor and normal cells and in the reoxygenation of some hypoxic tumors, though not to the same extent nor at the same rate. At present detailed knowledge of cycle population kinetics and phasic

variation of radiosensitivity of different neoplasms and normal cells is not sufficient to permit manipulation of the timing and size variation of the fractions. The split-course technique is better tolerated by normal tissues but does not clearly prolong patient survival (Holsti, 1969). Treatment schemes of fewer fractions per week are mostly for convenience and probably are justified (Ellis, et al, 1970). Intra-arterial perfusion with hydrogen peroxide has not proved definitely helpful (Germon, et al, 1968). Tourniquet-induced hypoxia of surrounding normal tissues has only limited application (Baker, et al, 1966). Hyperbaric oxygen initially seemed promising from tissue culture data and theoretical prediction (Churchill-Davidson, et al, 1957) but thus far has not shown definite improvement as judged from accumulated clinical experience (Wildermuth, et al, 1969). More cases and longer follow up are necessary. Concomitant chemotherapy and ultra high dose rate methods are also being thoroughly investigated. The pioneering studies of Lawrence, et al (1936) showed a greater biological effect of high LET radiation on neoplastic tissue than on normal mammalian tissue when compared to low LET radiation. In recent years, there has been a rekindled interest in research on the application of fast neutrons. Preliminary data on π meson also appear encouraging. It is apparent that further studies on heavy ions deserve parallel enthusiasm.

There have been many radiobiological studies on heavy ions at both the plateau and the Bragg peak regions. It is commonly accepted that when the Bragg peak is widened by varying-thickness-absorbers, the average LET drops so that there would be less

therapeutic advantage over conventional radiations. However, this concept has not been well substantiated, and further investigations are certainly indicated. Before heavier ions with enough penetration for practical use are available, the helium ion beam of the Berkeley 184" synchrocyclotron must be evaluated for its potential use in cancer therapy and to guide future research on heavier ions. Evaluation of the effect in 2 important normal tissues, the intestinal and the hemopoietic systems, should serve as a useful pilot study.

MATERIALS AND METHODS

Animals

The mice used were all LAF_1 young adult female hybrids between $C_{57}L$ xA/He strains. They were 12 weeks old when they left Jackson Laboratory of Bar Harbor, Maine. To avoid transportation stress complicating the experimental results, they were acclimatized in our own animal facilities for 1 to 2 weeks before use. At this time, their body weights ranged from 19 to 25 grams. The occasional ones outside this range were discarded. Actually, most weighed between 20 and 24 grams. They were assigned to experimental groups so that the weight distribution at each dose was roughly identical, but otherwise completely at random. The animal room was lighted from 8 a.m. to 10 p.m., and maintained at a temperature of 72-74°F. For lethality studies the mice were housed in individual glass jars to avoid cage effect. For intestinal colony assay they were kept 4 to 8 animals per cage and for spleen colony assay 8 to 12 per cage. They were all fed standard "Mouse Feed" from the Feedstuff Company of

San Francisco, California and given chlorinated water ad libitum. Each experimental point was obtained by using 20 mice in most cases except for colony assay where only 4 to 8 mice were used at each dose since 5 sections were cut from each small intestine. The jars and cages as well as the wood shavings, food and water were changed once a week and the former sterilized by autoclaving. Because of extensive experience in this Laboratory this strain of hybrid mice had been found to be relatively more sturdy than others and very easy to maintain without unexpected deaths from extraneous causes. They were also well tamed, and fighting between mates in the same cage was never observed.

Irradiation

General technique. - All exposures were by total body irradiation. There were essentially 12 primary experiments based on three modes of exposure: (1) single dose in air, (2) split dose in air, and (3) single dose under hypoxia; and 4 test systems: (I) gut death, (II) marrow death, (III) intestinal stem cell survival, and (IV) hemopoietic 'stem cell' survival. In each of these 12 experiments 3 types of radiation were employed: (A) modified Bragg peak alpha particles, (B) plateau alpha particles, and (C) cobalt-60 gamma rays. Constant irradiation conditions were maintained within practical limits. For each irradiation run, 2 identical double-walled circular disc-like lucite holders were used alternately to expose 3-4 mice at a time. The inner chamber was donut-shaped and measured 12 cm in its outer diameter and 2 cm x 2 cm in the squared cross-section of its circular tunnel. Mice were introduced through a side

hole with the aid of a funnel. After loading, the side hole was plugged with a lucite cap and the outer chamber closed. The entire mouse holder was rotated continuously at a rate of 10 revolutions a minute by a small motor. Gassing was done through a 3/16"-bore, affluent rubber tube that led into the inner chamber by radial spokes. Gas escaped from the inner chamber through perforated holes into the outer chamber and thence through a 10"-long effluent tube of 3/16"-bore to the outside atmosphere. The gas influx was driven by a pressure head of 1 arbitrary division of the U. S. Gauge Meter and maintained at a constant flow rate of ∿600 cc per minute. With this arrangement, the heat generated by the enclosed mice usually caused a 4-5°F elevation of the inner chamber temperature above the room temperature.

Modes of Delivery

(1) Single Dose in Air. - For the comparison of the relative biological effectiveness of the 3 types of radiation in air, single graded doses were used to cover a dose range which would produce varying degrees of response either observable directly or amenable to assay procedures. For animal lethalities, the doses were chosen so that for each type of radiation, the lowest dose would result in no death and the highest dose in no survival. For intestinal colonies the dose range was adjusted to yield a mean count between 10-80 per section and for spleen colonies between 1-20 colonies per spleen. Using marrow death as an end point, several small control experiments were performed to evaluate the age factor, cage effect, diurnal or seasonal variation in radiosensitivity, and dose rate

effect.

(2) Split Dose in Air. - To study the recovery pattern for each type of radiation a fixed conditioning first dose (D_1) was delivered equivalent to 2/3 of the LD_{50}. Forty-eight hours later, graded challenging second doses (D_2) were given to obtain new sets of survival data. The difference between the total dose (D_1+D_2) and the corresponding single dose (D_S) gave a measure (D_R) of the overall effect of intracellular repair and cellular repopulation as well as mitotic delay, partial cell synchronisation, cyclic variation of radiosensitivity and changes in oxygenation of tissue. The amount of recovery was expressed as percentage of $\frac{D_R}{D_1}$. Initially the plan was to isolate the component of rapid intracellular repair for each type of radiation by studying the response after split dose irradiation with a fixed total dose but varying time intervals between the 2 doses. This was abandoned after a test run on the 30-day mortality from ^{60}Co exposure because of uncertainty in the constancy of the slope of the corresponding survival curves and the expense and time involved for each run.

(3) Single Dose under Hypoxia. - Oxygen effect was studied by carrying out a paired experiment for each of the 4 end points and each of the 3 types of radiation by using a hypoxic mixture of 7% O_2, 0.05% CO_2 and 92.95% N_2 instead of air during exposure and a dose range increment by a factor of 2-2.5. The 7% O_2 was chosen after tolerance tests with lower concentration had shown that more than 10-15% of the mice succumbed to 10 min. of hypoxic treatment alone. Addition of a small amount of CO_2 was found to improve survival. Another modification necessary was to have

only 3 mice instead of 4 in each irradiation batch and to insert perforated partitions between them to prevent suffocation from overcrowding. By monitoring the outflow gas with a Beckman Model E-2 Gas Analyser it was noted that with the pressure and flow rate used it required 40-60 seconds for complete replacement of the air originally enclosed inside the mice holder which had an empty space of approximately 200 cc. Consequently, 90 seconds of gassing time was allowed prior to all hypoxic exposures. To evaluate whether hypoxia itself would affect the animals and bias the results, a control experiment was performed by subjecting a group of mice to hypoxia for 10 minutes and observing any mortality within the 30-day post-irradiation period. Another control experiment consisted of similar hypoxic treatment immediately prior to exposure in air at a selected dose and the response compared with that of mice exposed to an identical dose but without initial hypoxia.

Dosimetry

(B) Plateau α. - The helium ion beam of the 184" synchrocyclotron at Berkeley had an energy of 910 MeV ± 5% and a range of about 33 cm in water or soft tissue. Before each animal run, the Bragg ionization curve was obtained by a pair of parallel plate ionization chambers, one as a monitor in front of absorbers of different thickness, and the other behind to detect changes. These chambers were calibrated against a Faraday cup which was used as a primary standard to determine particle flux. Various combinations of quadrupole focusing settings and scattering absorbers were tried to achieve maximum beam uniformity across

its 6" diameter, circular cross-section. Heterogeneity of less than 5% was noted as measured by a small semi-conductor detector placed at different positions of the beam profile inside a water tank. All plateau irradiations were chosen at a point approximately 20 cm before the Bragg peak, i.e., after the beam passed through a 1 and 1/4 inch copper absorber. The average LET was estimated at around 1.5 KeV/μ. The mice and the lucite holder had a total soft tissue equivalent thickness of about 3 cm. Difference between entrance and exit doses due to scattering was less than 2%.

(A) Peak α. - The Bragg peak of the unmodified beam had an average energy around 80 MeV, a model LET of 10 KeV/μ, a half-value width of only a few mm, and a peak to plateau ratio between 2-3 depending on multiple factors. Ridge filters were used to differentially degrade the particles to widen the peak to 5 cm. The variation of ionization across this distance was less than 3%. The LET spectrum of this extended peak is currently being investigated.

(C) Cobalt-60 Gamma. - A 1500 curie source with a 60° collimator cone was used in all ^{60}Co γ irradiations. Most of the secondary radiations arising from the collimator were absorved by the walls of the lucite chambers, which also eliminated the build-up factor in the mice. Back and side scatters were not estimated because most were accounted for by measuring the air dose with the Victoreen chamber inserted into the inner chamber with 4 dead mice *in situ*. At each of 4 fixed positions, 3, 6, 9 and 12 o'clocks, 3 Victoreen readings were taken at a distance of 40 cm from the cobalt source. No significant difference was found, indicating that the cross-section dose distribution was sufficiently homogeneous. Measurements were

repeated with only 3 dead mice to simulate the condition of hypoxic exposure. Similar readings were obtained. The Victoreen chamber had been standardized against the National Bureau of Standards' instrument. Mid-plane absorbed dose in the mice was calculated by multiplying the air dose with 3 correction factors: 0.930 for the Victoreen cap absorption, 0.965 for Roentgen to rad conversion, and 0.975 for attenuation through 1 cm of mouse tissue. The Inverse Square Law alone would give rise to a 1% dose variation between the 2 surfaces of the mouse. The exact distance for each run was adjusted to account for radioactive decay, so that the dose rate remained constant at 400 r/min. Different desired doses were delivered by varying the cobalt timer calibrated to read hundredths of a minute. Initially, the absorbed dose was checked with thermoluminescent dosimeters. Capsules containing 40 mg of lithium fluoride were sutured longitudinally along the lumbar spines of 8 dead mice and irradiated *in situ*. Readouts were done with a Mark IV Series 1100 TLD Reader made by Radiation Detection Company of Mountain View, California, and the dose evaluated by comparing with a standard curve obtained by exposing LiF capsules from the same batch over a suitable dose range. Close agreement to within 4% of the calculated dose was observed.

Post-irradiation Procedures

I. 6-Day Animal Survival. - During the first 6 days after irradiation, the mice were checked for mortality 3 times a day and weighed daily with a mouse balance accurate to 0.5 gm. Although the weight data did yield qualitative information relating to the mode of death,

they were not accurate enough for quantitative comparison of the 3 types of radiations. The weighing procedure was abandoned after the single dose in air experiment. Initially, all decedents were autopsied and the small intestines salvaged for crypt cell survival study. However, more often than not, too much autolysis had set in to permit meaningful evaluation. Consequently post-mortem examination was discontinued. The survivors after the 6th postirradiation day were kept for observation to be included in 30-day survival experiments. In the split dose study, the day of the challenging dose, rather than that of the conditioning dose, was taken as day 0.

II. 30-Day Animal Survival. - This was similar to the above except that observation was made only once a day and the mice weighed less frequently. Again, the original attempt to salvage the spleens and other organs from deceased mice was not carried through because of the poor yield of useful data.

III. Microscopic Intestinal Colony Technique. - The mice were sacrificed by cervical dislocation 4 days after irradiation and the small intestines dissected out, straightened and measured. Their lengths varied from 44-50 cm. The middle 20-cm segment was removed from each small intestine, pinned on a cork strip and fixed overnight in a modified Teelyesniczky's fluid. Five 1 cm-long fragments were cut out at roughly equal intervals of 4 cm and subjected to routine histologic processing. Staining of the 4-5μ thick transverse sections was done with standard hematoxylin and eosin and the viable crypts were counted under a low power microscope. Selection criteria consisted of 10 or more cells per regenerating group, prominent nucleus

and little cytoplasm with basophilic staining. In contrast, non-viable crypts contained no cell or were sparcely populated by large cells with disintegrated nucleus and eosinophilic cytoplasm. Aggregates of blue staining and smaller lymphocytes were also easily differentiated. A group of 10 unexposed mice were killed to assess the average number of crypts per circumference. This was found to be about 120. Assuming that the crypt stem cells survived independently of one another and that one surviving cell was sufficient to regenerate a crypt, a viable crypt would represent the survival of one or more of its constituent stem cells and a non-viable crypt the survival of none. If after a certain dose of radiation, the observed mean number of colonies per section was x, the fraction of crypts destroyed would be $\frac{120-x}{120}$. Applying Poisson statistics, the number of surviving cells per crypt would be $-\log_e \frac{120-x}{120}$ and the total number of surviving cells per circumference $120(-\log_e \frac{120-x}{120})$.

IV. Macroscopic Endogenous Spleen Colony Technique. - The mice were killed 10 days after exposure and their spleens removed, dissected clean of extraneous tissues and fixed in Bouin solution for 24 hours. With a magnifying lens, all nodules greater than 0.5 mm were counted, care being taken not to double score those at the edges of the spleen. A maximum score of 25 nodules per spleen was arbitrarily taken when they were too numerous to be counted accurately. At the beginning the animals were weighed individually before dissection and their spleens were also weighed to the nearest mg before fixing. A group of unexposed mice were killed to serve as control for body and spleen weights.

Preliminary data showed no significant correlation between animal weights and spleen weights nor between spleen weights and numbers of nodules. In addition, there was such a wide variation in the spleen weight of mice receiving the same dose that correlation of splenic weight loss with dose was not possible. Therefore the weighing procedure was not followed through in subsequent experiments.

RESULTS

Experimental data from the 12 primary experiments were summarized in TABLES I through IV and plotted in FIGURES I_1 through IV_3.

For lethality studies, the sigmoid survival curves on linear plot were hand-drawn while the least square regression lines from probit transformation were fitted by computer which also gave the equation for each line for calculation of the LD_{90}, LD_{50} and LD_{10}. Each point was given equal significance without assigning a weighting coefficient. Attempt to use log dose instead of linear dose on the abcissa did not yield better-fit lines. Approximate standard errors (SE) were represented by bars at the data points. They were estimated by using the formula $\sqrt{\frac{p \times q}{N}}$ where p and q were probabilities of survival and mortality and N the number of animals used for each point.

For intestinal and spleen colony survival, regression lines were drawn in a similar way except log values of mean counts were used on the ordinate. A modification of the computer program was used to give a direct print-out of D_0 (dose to reduce survival to 37%) and of n ('extrapolation number' at zero dose).

Doses at selected survival levels (100 and 10 intestinal colonies and 10 and 1 spleen colonies) were calculated from the respective equations for correlation of whole animal radiation response with radiosensitivity at cellular level. The coefficient of correlation was better than 0.95 for most regressions.

TABLE I

6-Day Animal Survival Data

Mode of Exp.	Type of Rad. / Dose (rad)	(A) Peak α				(B) Plateau α				(C) ^{60}Co γ			
		No. mice alive	No. mice used	%S	SE	No. mice alive	No. mice used	%S	SE	No. mice alive	No. mice used	%S	SE
(1) Single Dose in Air	900	20	20	100	....								
	1000	18	20	90	6.7	20	20	100	....				
	1100	9	20	45	11.1	19	20	95	4.9	20	20	100	....
	1200	1	20	5	4.9	14	20	70	10.2	19	20	95	4.9
	1300	0	20	0	....	8	20	40	11.0	18	20	90	6.7
	1400	0	20	0	....	2	20	10	6.7	14	20	70	10.2
	1500					1	20	5	4.9	7	20	35	10.7
	1600									4	20	20	8.9
	1700									1	20	5	4.9
	1800									0	20	0	....
(2) Split Dose in Air	1400	16	20	80	8.9								
	1500	9	20	45	11.1	20	20	100	....				
	1600	3	20	15	8.0	17	20	85	8.0	20	20	100	....
	1700	0	20	0	....	15	20	75	9.7	18	20	90	6.7
	1800	0	20	0	....	10	20	50	11.2	17	20	85	8.0
	1900					4	20	20	8.9	16	20	80	8.9
	2000					1	20	5	4.9	10	20	50	11.2
	2100									5	20	25	9.7
	2200									2	20	10	6.7
	2300									3	20	15	8.0
(3) Single Dose under Hypoxia	1800	12	12	100	....								
	2000	11	12	91.5	8.1	12	12	100	....				
	2200	13	16	81.5	9.7	12	12	100	....				
	2400	6	20	30.0	10.2	19	20	95.0	4.9	12	12	100	....
	2600	1	17	5.9	5.7	16	19	84.2	8.5	16	16	100	....
	2800	1	11	9.1	8.7	10	18	55.5	11.7	15	16	93.8	6.0
	3000					8	18	45.5	11.9	9	10	90.0	9.5
	3200					1	10	10.0	9.5	12	16	75.0	10.8
	3400									7	14	50.0	13.3
	3600									4	17	23.2	10.2
	3800									3	13	23.1	11.7
	4000									0	9	0	....
	4200									1	11	9.1	8.7

FIG. I-1

6-Day Animal Survival After Single Dose in Air

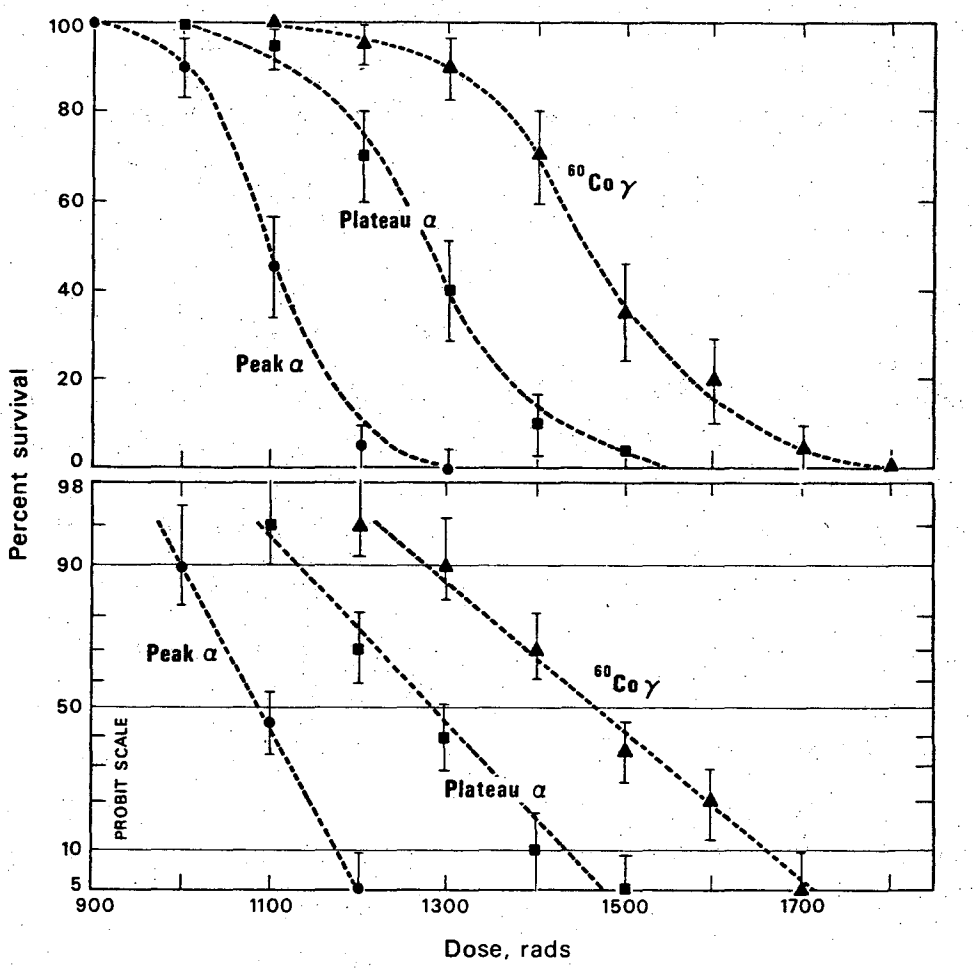


FIG. I-2

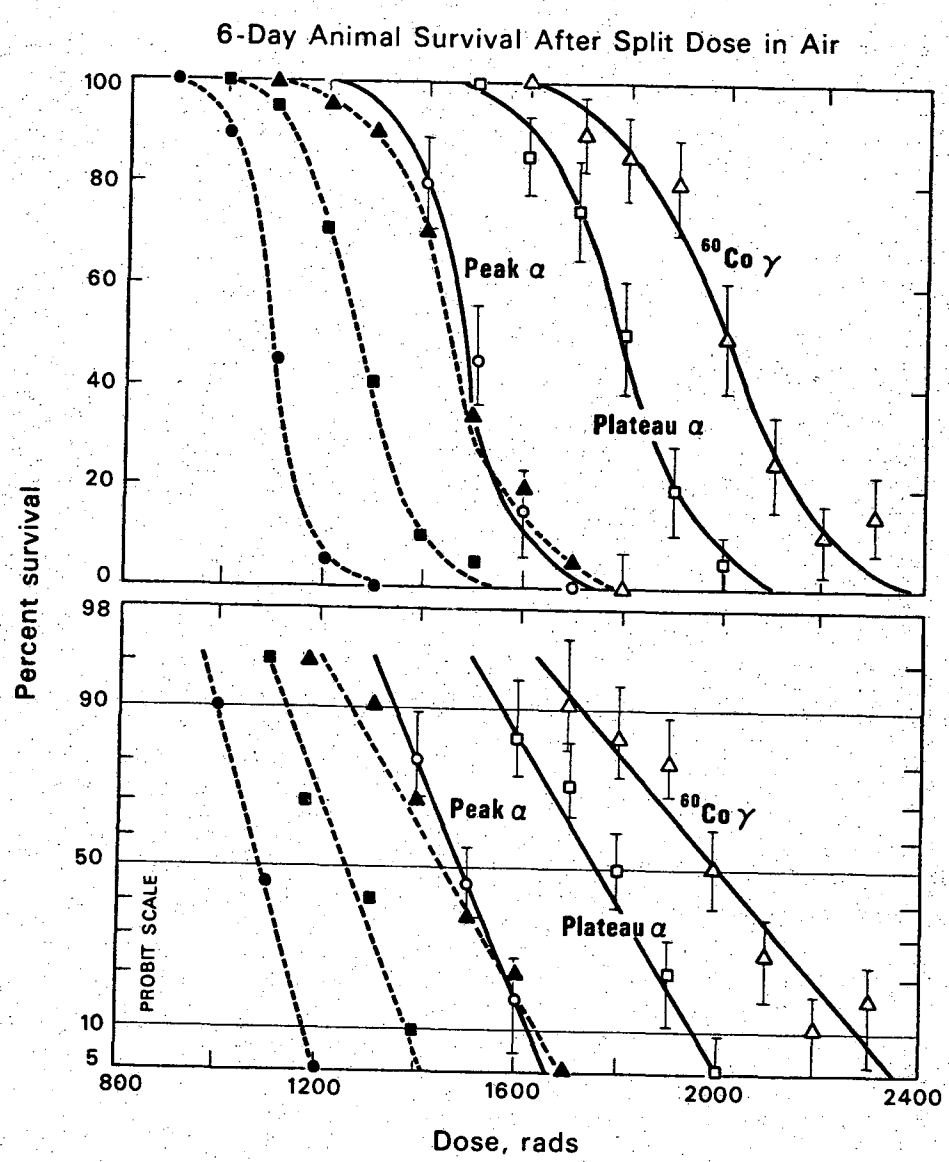

6-Day Animal Survival After Split Dose in Air
Percent survival
100
80
60
40
20
0
98
90
50
10
5
PROBIT SCALE
Peak α
Plateau α
60Co γ
800
1200
1600
2000
2400
Dose, rads

FIG. I-3

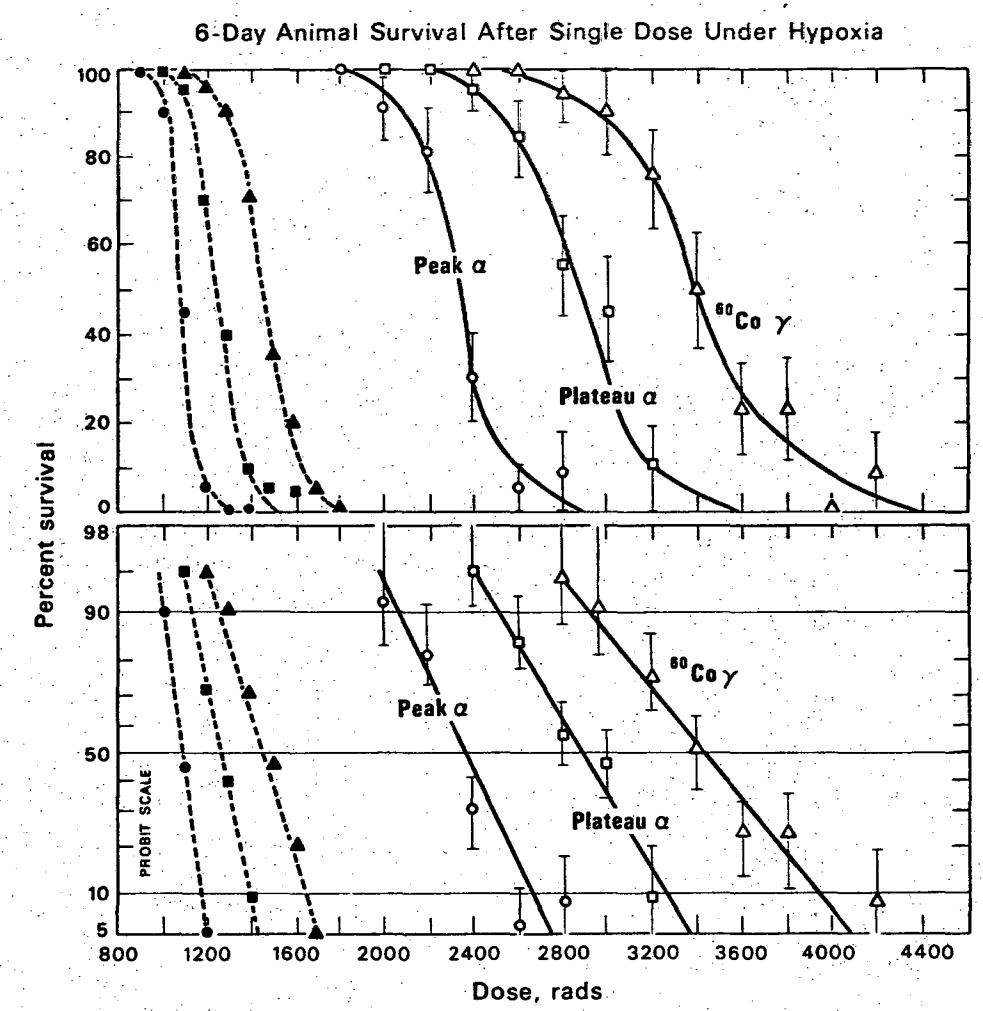

6-Day Animal Survival After Single Dose Under Hypoxia
Percent survival
100
80
60
40
20
0
98
90
50
10
5
PROBIT SCALE
Peak α
Plateau α
60Co γ
800
1200
1600
2000
2400
2800
3200
3600
4000
4400
Dose, rads

TABLE II

30-Day Animal Survival Data

Mode of Exp.	Type of Rad. / Dose (rad)	(A) Peak α No. mice alive	No. mice used	%S	SE	(B) Plateau α No. mice alive	No. mice used	%S	SE	(C) ^{60}Co γ No. mice alive	No. mice used	%S	SE
(1)	650	20	20	100	....								
Single	700	18	20	90	6.7	20	20	100	....	20	20	100	....
Dose	750	10	20	50	11.2	18	20	90	6.7	20	20	100	....
in Air	800	1	20	5	4.9	14	20	70	10.2	19	20	95	4.9
	850	0	20	0	....	7	20	35	10.7	17	20	85	8.0
	900	0	20	0	....	1	20	5	4.9	10	20	50	11.2
	950					1	20	5	4.9	6	20	30	10.2
	1000					0	20	0	....	2	20	10	6.7
	1100									0	20	0	....
(2)	700	20	20	100	....								
Split	800	18	20	90	6.7	20	20	100	....	20	20	100	....
Dose	900	14	20	70	10.2	18	20	90	6.7	20	20	100	....
in Air	1000	10	20	50	11.2	17	20	85	8.0	19	20	95	4.9
	1100	4	20	20	8.9	14	20	70	10.2	19	20	95	4.9
	1200					10	20	50	11.2	16	20	80	8.9
	1300	1	20	5	4.9					11	20	55	11.1
	1400									10	20	50	11.2
	1500					0	20	0	....	4	20	20	8.9
(3)	1200	12	12	100	....								
Single	1400	15	16	93.8	6.0								
Dose	1500	9	18	50.0	11.8	12	12	100	....				
under	1600	6	20	30.0	10.2					12	12	100	....
Hypoxia	1700	1	21	4.8	4.7	14	16	87.5	8.3				
	1800					16	22	76.2	9.1	15	16	93.8	6.0
	1900					8	17	47.1	12.1				
	2000									14	16	87.5	8.3
	2100					2	15	13.3	8.8	11	14	78.6	11.0
	2200									8	15	53.5	12.9
	2400									2	9	22.2	13.9
	2600									1	8	12.5	11.7

FIG. II-1

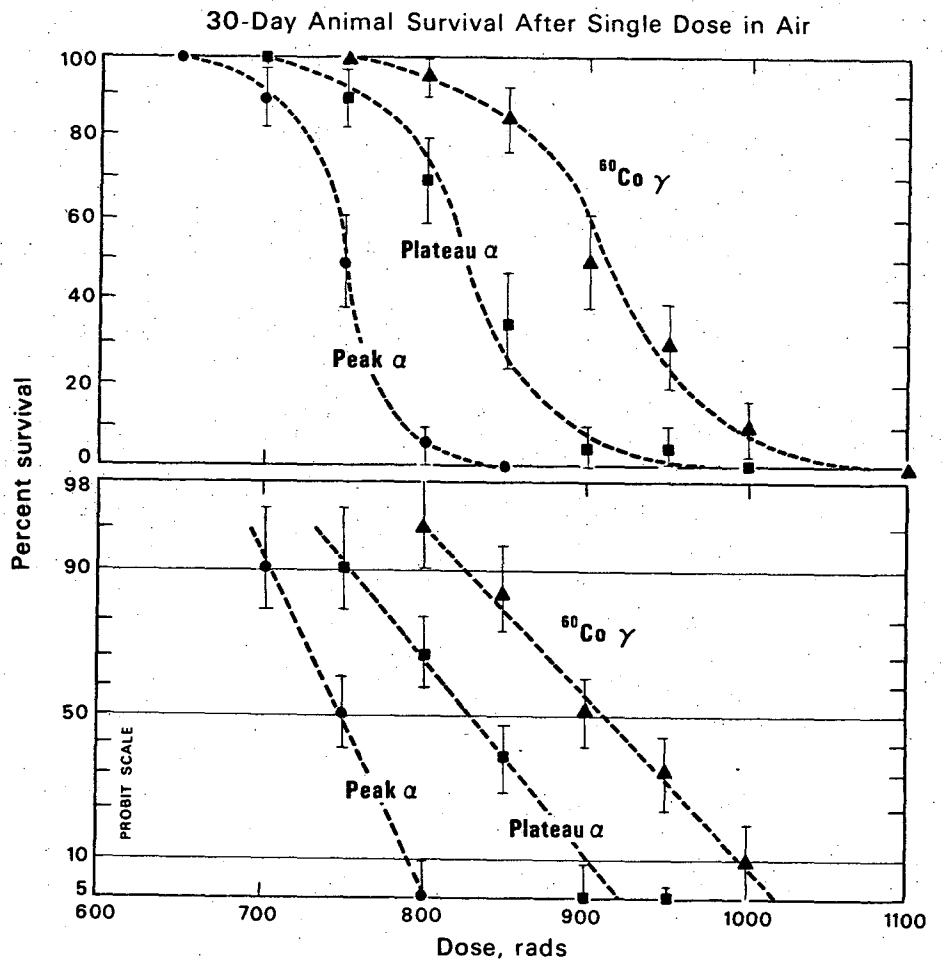


FIG. II-2

30-Day Animal Survival After Split Dose in Air

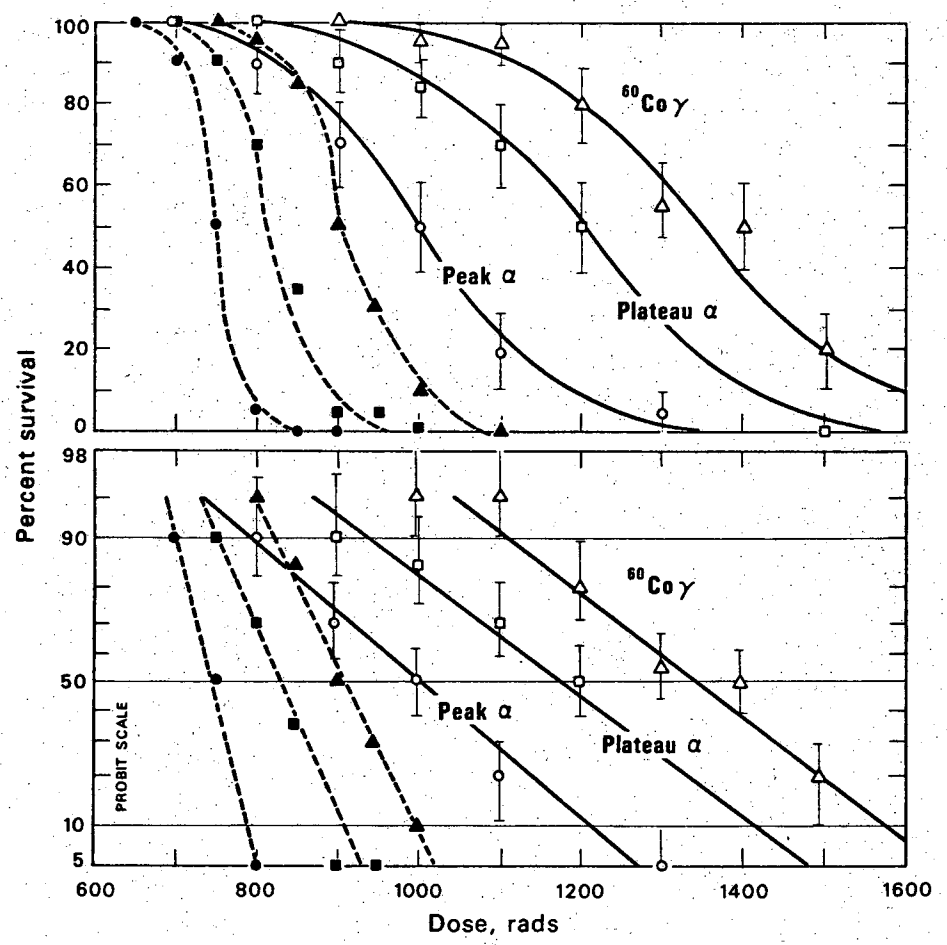


FIG. II-3

30-Day Animal Survival After Single Dose Under Hypoxia

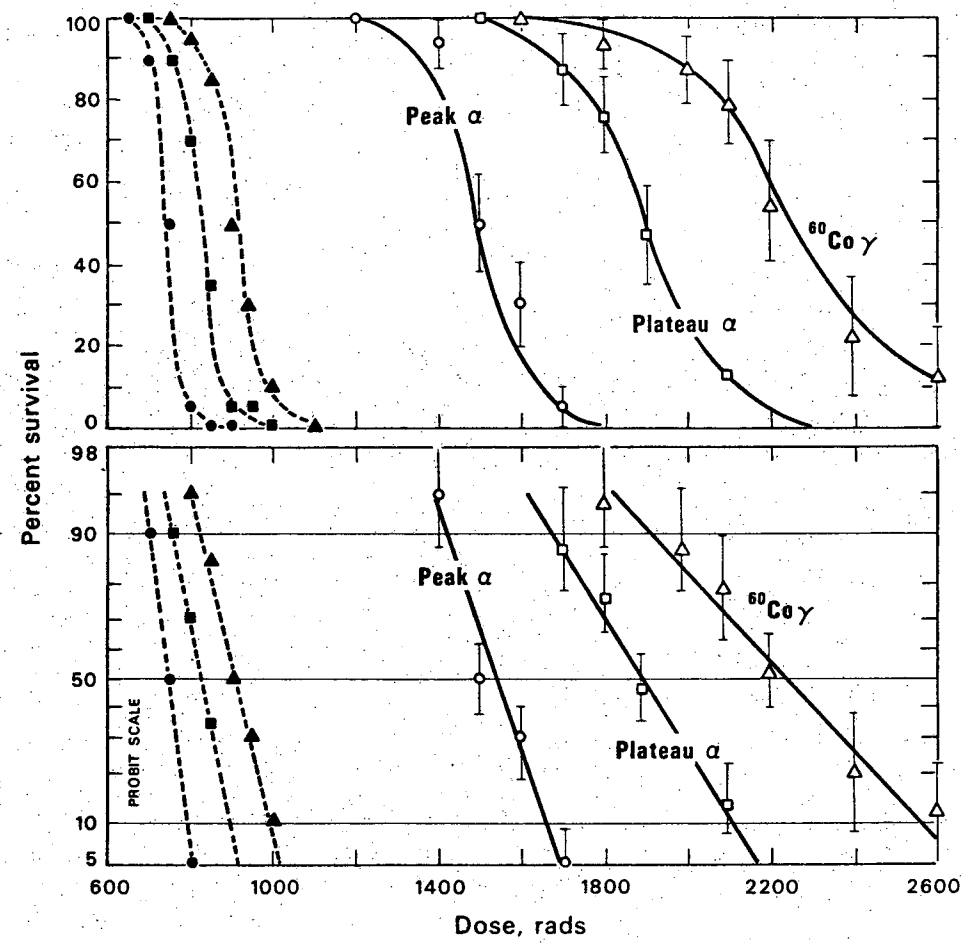


TABLE III

Microscopic Intestinal Colony Data

Type of Rad.	(A) Peak α					(B) Plateau α					(C) ^{60}Co γ				
Mode of Exposure	Dose in rad	Mean count per section	Adjusted mean count per section	S.D.	S.E.	Dose in rad	Mean count per section	Adjusted mean count per section	S.D.	S.E.	Dose in rad	Mean count per section	Adjusted mean count per section	S.D.	S.E.
(1) Single Dose in Air	800	68.9	102.4	23.9	5.4	900	77.7	125.0	28.0	6.3	1000	91.9	194.3	25.8	5.7
	900	34.2	40.3	19.0	2.9	1000	51.5	67.2	24.0	3.8	1100	67.7	99.6	11.3	1.8
	1000	18.9	20.5	13.8	2.2	1100	28.7	32.8	18.3	2.9	1200	51.1	66.6	16.3	2.6
	1100	8.6	9.0	6.7	1.0	1200	15.5	16.5	12.2	1.9	1300	26.7	30.1	12.6	2.0
	1200	3.0	3.0	2.9	0.5	1300	9.8	10.3	5.3	0.8	1400	14.3	15.2	7.1	1.2
	1300	1.5	1.5	1.6	0.4	1400	3.3	3.3	3.4	0.5	1500	8.2	8.5	6.9	1.1
											1600	4.6	4.7	4.1	0.9
(2) Split Dose in Air	1300	90.7	169.3	20.7	4.6	1500	82.3	139.0	32.8	7.3	1700	92.1	194.8	22.6	5.1
	1450	63.9	91.1	17.0	2.7	1650	61.4	86.1	25.2	4.0	1850	64.0	91.8	29.1	4.6
	1600	37.3	44.7	19.2	2.8	1800	43.9	54.7	20.6	3.2	2000	49.6	63.9	23.9	3.1
	1750	15.5	16.5	8.6	1.4	1950	25.9	29.2	15.8	2.5	2150	29.7	34.0	17.5	2.5
	1900	8.9	9.4	6.2	1.4	2100	8.2	8.5	5.9	1.5	2300	13.1	13.8	7.2	1.6
(3) Single Dose under Hypoxia	1700	81.1	133.2	27.4	4.9	2300	78.2	128.4	22.5	4.1	2500	83.4	142.5	23.9	4.4
	1900	45.7	57.6	19.8	3.7	2500	47.7	60.7	19.9	3.7	2700	52.5	68.8	22.8	4.1
	2000	38.0	45.8	15.2	2.8	2600	30.0	34.5	17.1	3.1	2800	36.6	43.7	18.1	3.3
	2100	22.5	24.9	12.0	2.2	2700	24.4	27.2	11.7	2.1	2900	35.1	41.4	20.8	3.8
	2200	19.7	21.5	11.7	2.2	2800	22.1	24.3	8.2	1.5	3000	29.1	33.3	12.2	2.4
	2400	7.4	7.7	4.9	1.1	3000	11.4	12.0	4.8	0.9	3200	15.3	16.3	8.0	1.8

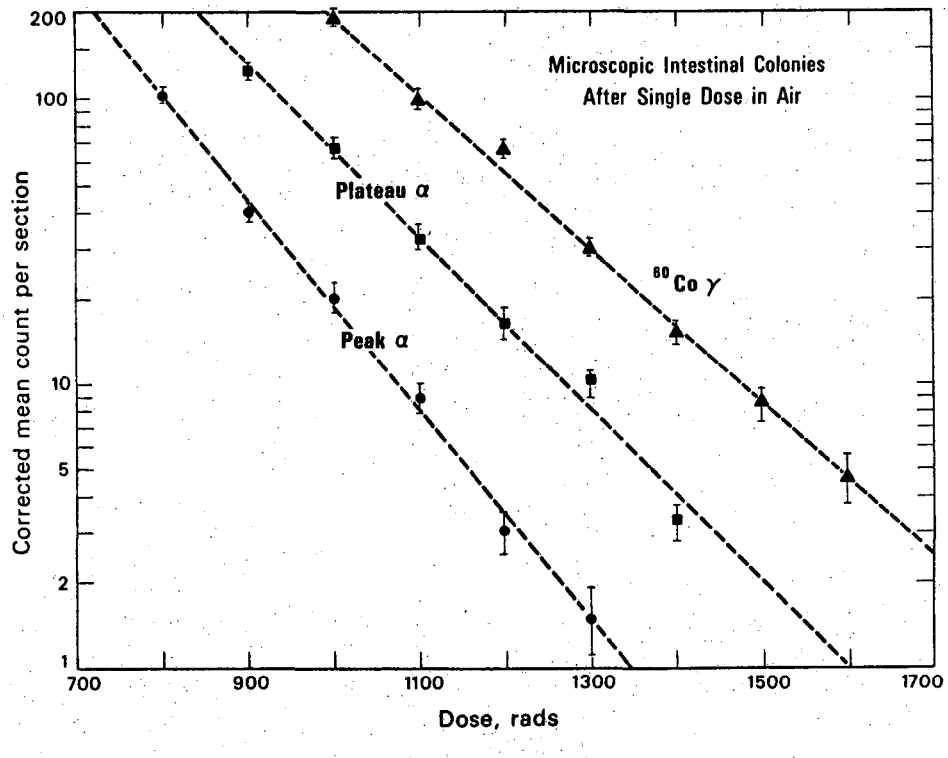


FIG. III-1

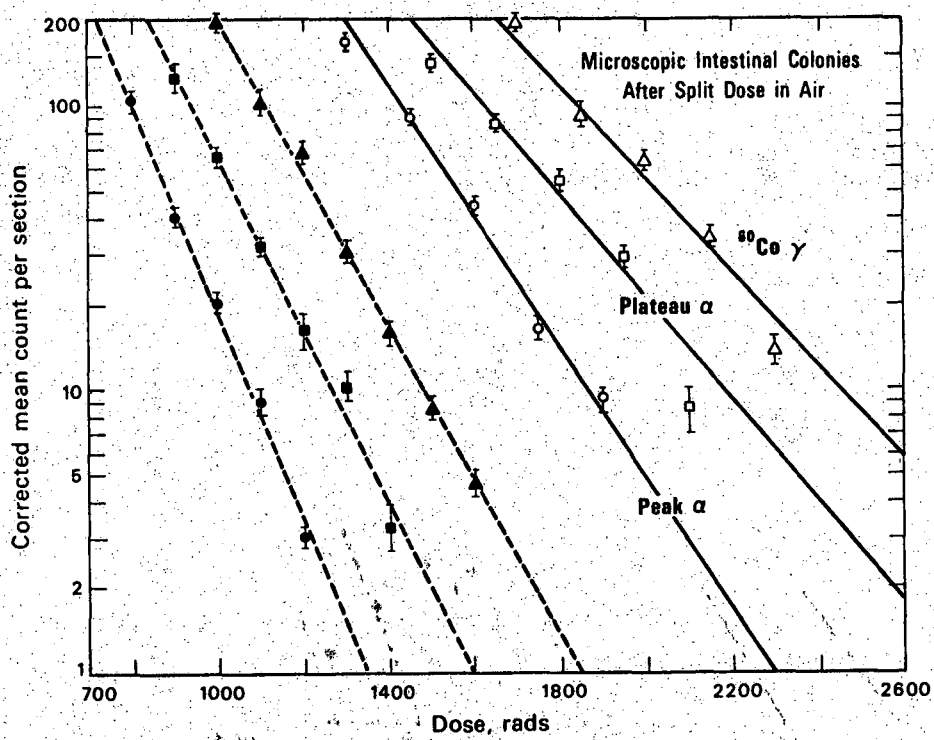


FIG. III-2

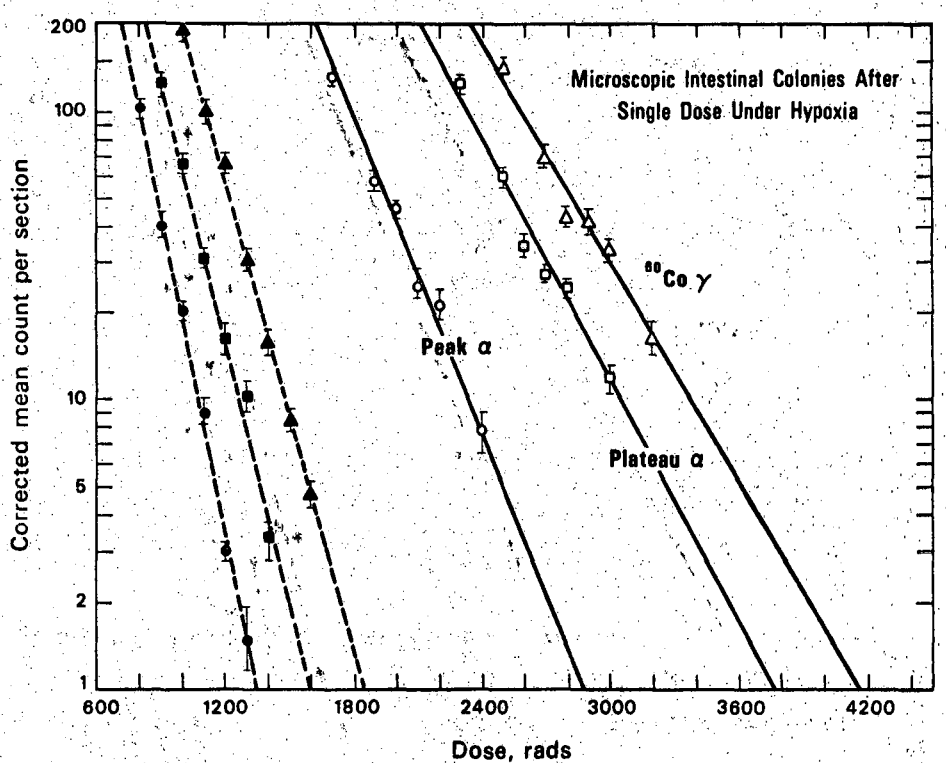


FIG. III-3

TABLE IV

Macroscopic Endogenous Spleen Colony Data

Type of Rad.	(A) Peak α				(B) Plateau α				(C) ^{60}Co γ			
Mode of Exp.	Dose in rad	Mean count per spleen	S.D.	S.E.	Dose in rad	Mean count per spleen	S.D.	S.E.	Dose in rad	Mean count per spleen	S.D.	S.E.
(1) Single Dose in Air	500	14.8	5.5	1.2	550	15.2	4.0	0.9	600	18.6	4.0	0.9
	550	7.7	3.2	0.7	600	8.3	5.0	1.1	650	10.3	4.6	1.0
	600	3.7	2.8	0.6	650	5.2	4.7	1.0	700	7.7	3.3	0.7
	650	3.6	2.0	0.4	700	5.0	4.2	0.9	750	4.4	4.3	1.0
	700	1.4	0.8	0.2	750	1.8	1.5	0.4	800	2.2	2.2	0.5
	800	0.3	0.6	0.1	850	0.9	1.2	0.3	850	2.1	2.2	0.5
									900	1.0	1.3	0.3
(2) Split Dose in Air	700	23.0	6.5	1.5	800	18.9	3.9	0.9	850	18.2	3.3	0.7
	750	10.4	4.4	1.0	900	7.1	3.1	0.7	950	11.7	2.6	0.6
	800	5.7	3.2	0.7	1000	3.9	3.5	0.8	1050	3.3	1.6	0.4
	850	4.7	1.5	0.3	1100	2.4	2.3	0.5	1150	2.3	1.3	0.3
	900	2.2	2.2	0.5	1200	0.9	1.0	0.2	1250	1.8	1.6	0.4
(3) Single Dose under Hypoxia	1000	16.0	3.2	0.7	1200	19.4	3.9	0.9	1400	19.5	4.2	0.9
	1100	9.3	2.0	0.5	1300	13.0	3.0	0.7	1500	12.1	3.9	0.9
	1200	4.7	1.6	0.4	1400	7.0	3.3	0.7	1600	11.1	4.7	1.0
	1300	2.0	1.2	0.3	1500	5.0	2.3	0.5	1700	9.0	5.0	1.2
	1400	1.0	1.0	0.2	1600	4.4	2.0	0.5	1800	4.1	2.5	0.6
	1500	1.6	1.9	0.4	1700	1.7	1.4	0.3	1900	3.7	2.2	0.5

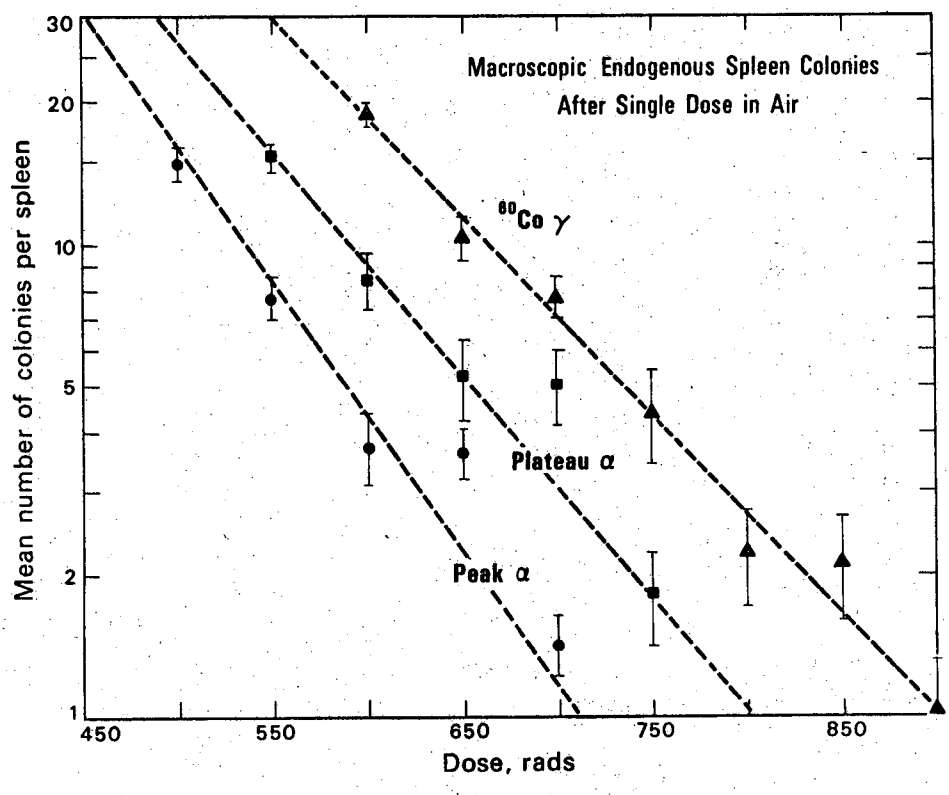


FIG. IV-1

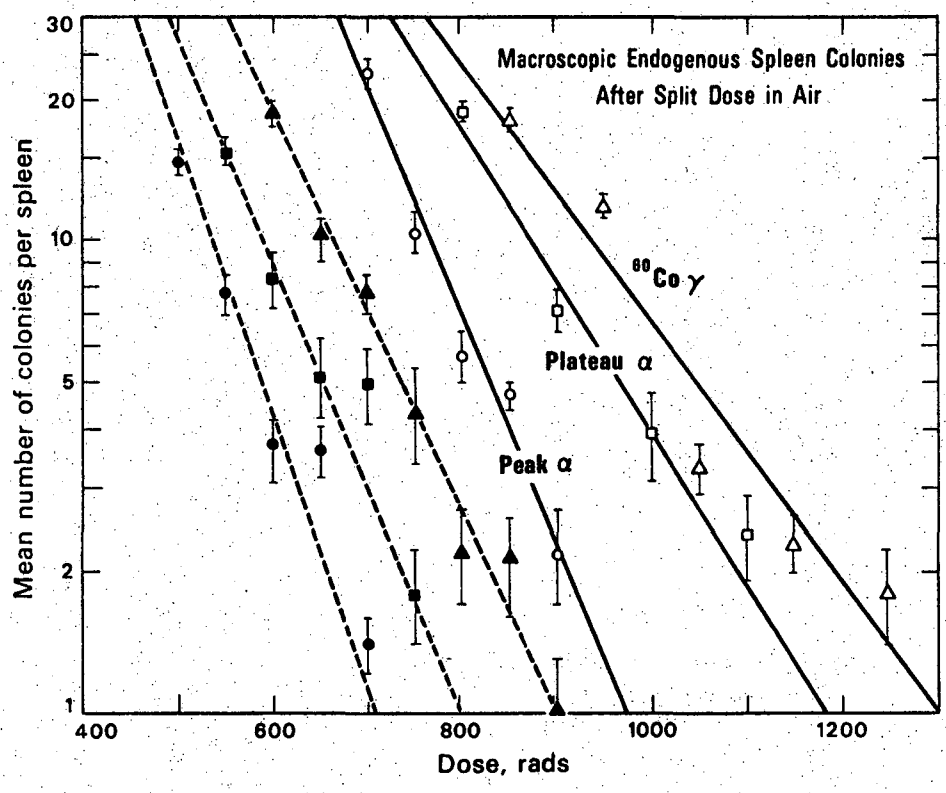


FIG. IV-2

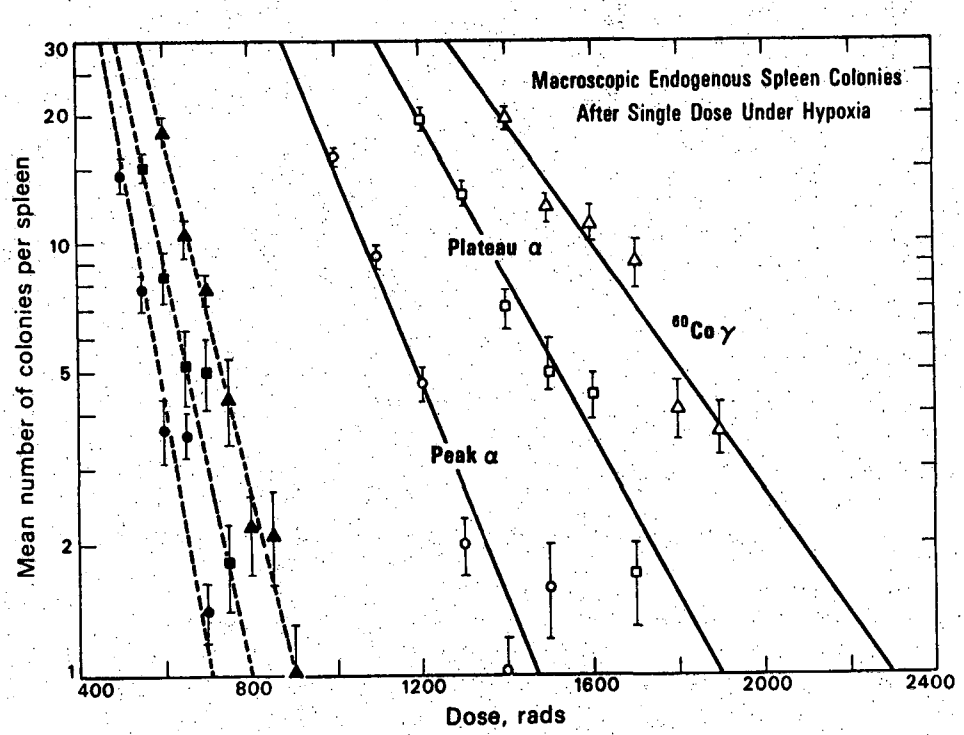


FIG. IV-3

V. Miscellaneous

(i) Animal Survival Time. - For gut death, even at supralethal doses, no mortality occurred before day 5 and roughly equal numbers of mice died on day 5 and day 6 regardless of the type of radiation used.

For marrow death, most mortalities after lethal exposure occurred within the second postirradiation week with a mean survival time of about 11 days for all 3 types of radiation employed. At minimal lethal doses the small number of deaths did not permit an accurate evaluation of the mean survival time. In addition, there was a possible error of ±1 day for all doses because of the uncertainty in the exact time of death so that a plot of the data points looked like a scatter diagram regardless of whether linear, semilog or double log paper was used. However, although they failed to provide quantitative comparison of the dose response relation, they did show a qualitative distinction in the temporal pattern between the 2 modes of death.

(ii) Animal Weight. - Body weight data after single exposure in air were given in TABLE V, expressed as absolute weight, weight change and percentage of weight change compared to day 0 weight. The latter were plotted in FIG. V. Because of appreciable animal to animal variation and possible error in the weighing procedure, they were not sufficiently accurate to permit quantitation of radiation response. Nonetheless, they did confirm a qualitative separation between the 2 modes of death. Unirradiated control mice gained an average of 4.3% in weight every week. Those having received a maximal sublethal marrow dose

experienced a peak weight reduction of 10% around day 10 but regained their day 0 weight by day 22 and were not too much underweight compared to the control by day 30. Mice exposed to minimal lethal marrow dose (or maximal sublethal gut dose) showed a gradual decrease in weight amounting to 38.7% by day 13 beyond which no survivor remained. In contrast, animals irradiated with lethal gut dose suffered a more rapid decline in weight although the total weight loss of 32.6% at death was comparable as this occurred about a week earlier.

(iii) Supplementary Split Dose Study. - Seventeen to eighteen week old mice were given two ^{60}Co exposures of 500 rad each, separated by different time intervals between them. The findings were illustrated in TABLE VI and FIG. VI. A survival peak was observed at 2 hr and a trough at 3 hr. From 5 to 48 hours there was a very gradual upward trend. The minor fluctuations in between were probably due to statistical variation.

(iv) Age Factor. - Marrow death after single dose in air was used to assess the radiosensitivity of 17-18 week old mice. The survival percentages after 850, 900, 950, 1000 and 1050 rad were 100, 85, 50, 25 and 10 respectively. The $LD_{50(30)}$ was 950 rad compared to 910 rad for 13-14 week old mice.

(v) Cage Effect. - Animals were kept 10 to a cage instead of individually in separate jars. The 30-day survival rates of 13-14 week old mice were 100, 100, 70, 80 and 0% after 800, 850, 900, 950 and 1000 rad respectively. Besides very poor fitting of the survival curve, it was noted that the time of death was bunched up within a day or so instead of being

scattered over a much longer period.

(vi) Diurnal and Seasonal Variation. - A rough estimate was done on 17-18 week old mice left over from primary experiments by using only one selected dose of 1000 rad on 3 groups of animals irradiated at different times. Survivals of 20, 25 and 30% for exposures at 9 a.m. and 5 p.m., July 1970, and 9 a.m., January 1971, respectively, were not significantly different from one another.

(vii) Dose Rate Effect. - Similar check was carried out using two dose rates of 300 rad/min and 900 rad/min. Values of 30% and 20% survival were considered similar.

(viii) Hypoxic Control. - A group of 46 mice, age 17-18 weeks were subjected to 10 minutes of hypoxic treatment. Five could not tolerate the procedure. Of the 41 survivors, 20 were given 1000 rad of ^{60}Co irradiation immediately after the hypoxic atmosphere was replaced by air. Along with the remaining 21 unexposed animals, they were observed for lethalities, 14 of the 20 irradiated and none of the 21 unirradiated had died at the end of 30 days after exposure. It was concluded that a brief period of hypoxia per se did not cause any bias in the result of hypoxic radiation response.

TABLE V

Body Weight Data After Single Dose In Air

Equiv. ^{60}Co Dose (rad)	Post-irrad. Body Day / Weight	0	1	2	3	4	5	6	7	8	9	10	11	12	13	15	20	25	30
0 (Control)	Absolute (gm)	21.7	22.1	22.2	21.8	22.2	22.2	22.5	22.5	22.4	22.4	23.0	23.0	23.1	23.4	23.6	24.0	24.1	24.5
	Change (gm)	....	+0.4	+0.5	+0.1	+0.5	+0.5	+0.8	+0.8	+0.7	+0.7	+1.3	+1.3	+1.4	+1.7	+1.9	+2.3	+2.4	+2.8
	% Change	....	+1.9	+2.3	+0.5	+2.3	+2.3	+3.8	+3.8	+3.8	+3.2	+5.9	+5.9	+6.5	+7.8	+8.8	+10.6	+11.1	+12.9
800 [Max $LD_{100(30)}$]	Absolute (gm)	21.5		21.0		20.7		20.4		19.6		19.4		19.7		20.0	21.0	22.2	23.5
	Change (gm)	....		-0.5		-0.8		-1.1		-1.9		-2.1		-1.8		-1.5	-0.5	+0.7	+2.0
	% Change	....		-2.3		-3.7		-5.1		-8.8		-9.7		-8.4		-7.0	-2.3	+3.2	+9.3
1100 [Min $LD_0(30)$ or Max $LD_{100}(6)$]	Absolute (gm)	22.2	21.8	21.1	21.1	20.4	19.9	19.5	18.9	18.3	17.6	16.5	15.6	14.6	13.6				
	Change (gm)	....	-0.4	-1.1	-1.2	-1.8	-2.3	-2.7	-3.3	-3.9	-4.6	-5.7	-6.6	-7.6	-8.6				
	% Change	....	-1.8	-4.9	-5.4	-7.6	-10.4	-12.1	-14.9	-17.6	-20.7	-25.7	-29.7	-34.2	-38.7				
1700 [Min $LD_0(6)$]	Absolute (gm)	21.8	21.1	20.3	19.3	17.7	15.9	14.7											
	Change (gm)	....	-0.7	-1.5	-2.5	-4.1	-5.9	-7.1											
	% Change	....	-3.2	-6.9	-11.5	-18.8	-26.6	-32.6											

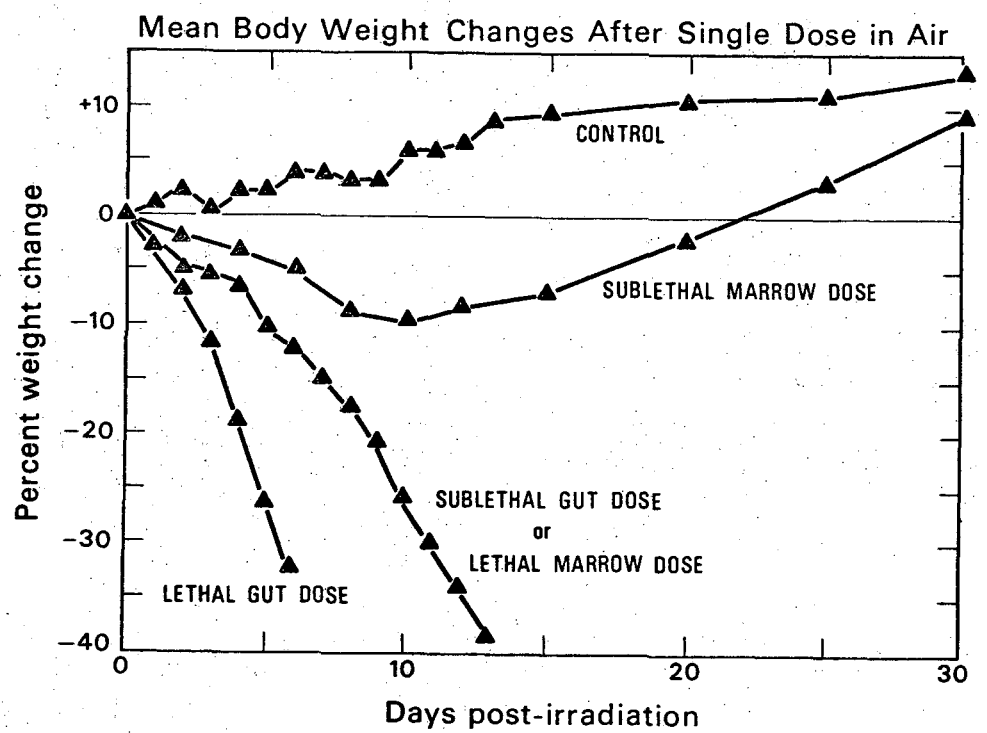
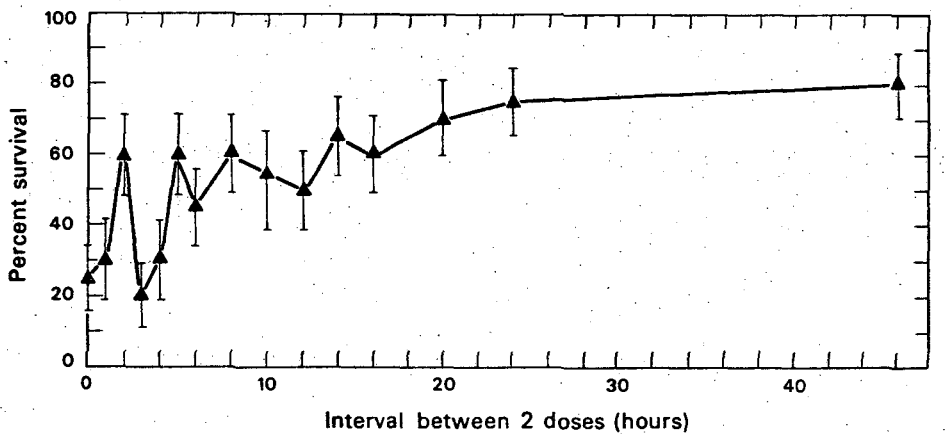


FIG. V

TABLE VI and FIGURE VI

Recovery Pattern of 30-Day Animal Survival after Split Dose ^{60}Co γ Exposure in Air (500 rad + 500 rad)

Interval (hours)	0	1	2	3	4	5	6	8	10	12	14	16	20	24	48
% S	25	30	60	20	30	60	45	60	55	50	65	60	70	75	80
S E	9.7	10.2	11.0	8.9	10.2	11.0	11.1	11.0	11.1	11.2	10.7	11.0	10.2	9.7	8.9



ANALYSIS

The pertinent results of the primary experiments were summarized in TABLE VII and the 3 parameters evaluated from them in TABLE VIII. The standard errors of estimate were calculated for the LD_{50}'s and D_0's. Values without overlapping of the 95% confidence limits were considered significantly different.

(1) Relative Biological Effectiveness (RBE) in Air. - ^{60}Co γ was used as a reference radiation against which peak and plateau α particles were compared.

For lethality studies, because the 3 types of radiation did not give parallel probit regression lines (Fig. I_1 and Fig. II_1), RBE values varied according to the level of survival chosen for comparison. RBE's based on LD_{90}'s were smaller than those based on LD_{10} with intermediate values when calculated from LD_{50}'s.

For cellular survival studies, the corresponding n values were regarded as similar because of appreciable uncertainty in extrapolation. RBE's were obtained by comparing the slopes of the corresponding regression lines as well as the doses which produced an iso-effect at 2 selected survival levels.

All peak α RBE's were significantly greater than unity. Some plateau α RBE's were probably also larger than one but to a lesser extent while most were not much different from unity. For both types of α radiation, the RBE's for gut death and intestinal colonies were greater than those for marrow death and spleen colonies.

(2) Recovery Pattern in Air. - Since most of the regression lines in split-dose experiments were not parallel to their correspondent single dose counterparts, the amount of recovery in terms of percent of the first dose depended on the level of survival chosen. Therefore, again multiple iso-effects were used to compare the 3 types of radiation. For 6-day animal survival similar amount of recovery was noted regardless of the level of survival chosen or the type of radiation used. For 30-day animal survival, it was much less at LD_{90} level than at LD_{10} level for each type of radiation, and significantly less at all levels for peak α than for plateau α or ^{60}Co γ. For intestinal colonies, there was more recovery when 100 colony or 10 colony survival rather than D_0 was considered. However, at corresponding end point, the 3 types of radiation were similar. For spleen colonies, recovery at 1 colony was less than that at 10 colonies, and peak α irradiated mice had significantly less recovery than those exposed to plateau α or ^{60}Co except at the 10 colony level. The 'extrapolation number' did not change appreciably except for the spleen colonies after peak α exposure where a 6-fold increase was noted.

(3) Oxygen Enhancement Ratio (OER). - For each of the three types of radiation there was a general tendency for the OER to increase from LD_{90} through LD_{50} to LD_{10} for both types of animal lethalities and from D_0 through D_{10} col. to D_{100} col. for intestinal colonies and from D_{10} col. through D_1 col. to D_0 for spleen colonies. Peak α had significantly lower OER than ^{60}Co for all end points except intestinal colony survival, while plateau α was similar to ^{60}Co except probably for spleen colony survival at the 1 colony level. For all 3 types of radiation n remained unchanged for

crypt cell survival and was slightly lower than after air exposure for spleen colony survival.

(4) Correlation of Acute Radiation Lethalities with Cellular Radiosensitivities. - For all 3 types of radiation there was a close correspondence of within 5% between the respective $LD_{50}(6)$ and D_{10} int. col. and between $LD_{50}(30)$ and D_1 spl. col. for all 3 modes of exposure except for split-dose exposure on gut death and intestinal colonies where the $LD_{50}(6)$ was about 25% lower than the corresponding D_{10} int. col. and about 10% higher than the D_{100} int. col.

TABLE VII

Summary of Results

System	Type of Radiation / Mode of Exposure \ End Point	(A) Peak α			(B) Plateau α			(C) ^{60}Co γ		
		LD_{90}	LD_{50} (±SE)	LD_{10}	LD_{90}	LD_{50} (±SE)	LD_{10}	LD_{90}	LD_{50} (±SE)	LD_{10}
I. 6-Day Animal Survival	1. Single dose in air	1010	1090 (±24)	1170	1141	1278 (±37)	1415	1291	1462 (±40)	1633
	2. Split dose in air	1370	1490 (±37)	1610	1614	1779 (±42)	1944	1768	2004 (±50)	2240
	3. Single dose under hypoxia	2091	2352 (±82)	2613	2566	2862 (±110)	3160	2975	3465 (±116)	3955
II. 30-Day Animal Survival	1. Single dose in air	706	746 (±15)	786	755	829 (±19)	903	825	910 (±20)	1000
	2. Split dose in air	798	999 (±46)	1190	939	1190 (±60)	1441	1110	1355 (±61)	1600
	3. Single dose under hypoxia	1428	1536 (±38)	1644	1709	1898 (±65)	2111	1950	2243 (±107)	2536

System	Mode of Exposure \ End Point	D_{100} col	D_{10} col	D_0 (±SE)	n	D_{100} col	D_{10} col	D_0 (±SE)	n	D_{100} col	D_{10} col	D_0 (±SE)	n
III. Intestinal Micro-colonies	1. Single dose in air	804	1074	118 (±3)	91850	944	1269	143 (±8)	92250	1110	1476	159 (±4)	105550
	2. Split dose in air	1421	1876	196 (±10)	119050	1624	2115	225 (±30)	131200	1865	2403	239 (±19)	243700
	3. Single dose under hypoxia	1783	2347	251 (±14)	119100	2347	3028	301 (±24)	236250	2589	3369	339 (±27)	207000

System	Mode of Exposure \ End Point	D_{10} col	D_1 col	D_0 (±SE)	n	D_{10} col	D_1 col	D_0 (±SE)	n	D_{10} col	D_1 col	D_0 (±SE)	n
IV. Splenic Macro-colonies	1. Single dose in air	537	721	79 (±7)	7674	539	831	96 (±10)	5495	664	903	106 (±6)	5443
	2. Split dose in air	765	969	91 (±9)	44005	877	1193	139 (±10)	5322	945	1287	160 (±21)	4504
	3. Single dose under hypoxia	1084	1460	162 (±25)	3267	1353	1844	252 (±26)	1844	1603	2244	274 (±39)	2161

TABLE VIII

Summary of Analysis

Parameter	Type of Radiation / End Point / System	(A) Peak α			(B) Plateau α			(C) ^{60}Co γ		
1. RBE in Air	I 6-Day Animal Survival	LD_{50} 1.28	LD_{50} 1.34	LD_{10} 1.39	LD_{50} 1.13	LD_{50} 1.14	LD_{10} 1.15			
	II 30-Day Animal Survival	LD_{50} 1.17	LD_{50} 1.22	LD_{10} 1.27	LD_{50} 1.09	LD_{50} 1.10	LD_{10} 1.10			
	III Intestinal Micro-colonies	$D_{1\,col}$ 1.38	$D_{1\,col}$ 1.37	D_0 1.35	$D_{1\,col}$ 1.17	$D_{1\,col}$ 1.16	D_0 1.12			
	IV Splenic Macro-colonies	$D_{1\,col}$ 1.24	$D_{1\,col}$ 1.25	D_0 1.34	$D_{1\,col}$ 1.14	$D_{1\,col}$ 1.08	D_0 1.10			
2. % Recovery (or change in D_0) in Air	I 6-Day Animal Survival	LD_{50} 51.4	LD_{50} 57.1	LD_{10} 62.9	LD_{50} 55.7	LD_{50} 58.9	LD_{10} 62.2	LD_{50} 53.0	LD_{50} 60.2	LD_{10} 67.4
	II 30-Day Animal Survival	LD_{50} 16.7	LD_{50} 50.6	LD_{10} 60.8	LD_{50} 33.6	LD_{50} 65.6	LD_{10} 97.8	LD_{50} 47.5	LD_{50} 74.1	LD_{10} 100.0
	III Intestinal Micro-colonies	$D_{1\,col}$ 88.1	$D_{1\,col}$ 114.6	D_0 66.01	$D_{1\,col}$ 80.0	$D_{1\,col}$ 99.5	D_0 57.3	$D_{1\,col}$ 83.8	$D_{1\,col}$ 104.1	D_0 50.3
	IV Splenic Macro-colonies	$D_{1\,col}$ 45.6	$D_{1\,col}$ 49.6	D_0 15.2	$D_{1\,col}$ 51.6	$D_{1\,col}$ 65.8	D_0 44.8	$D_{1\,col}$ 46.8	$D_{1\,col}$ 64.0	D_0 50.9
3. OER	I 6-Day Animal Survival	LD_{50} 2.07	LD_{50} 2.16	LD_{10} 2.22	LD_{50} 2.24	LD_{50} 2.24	LD_{10} 2.23	LD_{50} 2.30	LD_{50} 2.37	LD_{10} 2.42
	II 30-Day Animal Survival	LD_{50} 2.02	LD_{50} 2.06	LD_{10} 2.09	LD_{50} 2.27	LD_{50} 2.30	LD_{10} 2.31	LD_{50} 2.36	LD_{50} 2.46	LD_{10} 2.54
	III Intestinal Micro-colonies	$D_{1\,col}$ 2.21	$D_{1\,col}$ 2.19	D_0 2.13	$D_{1\,col}$ 2.49	$D_{1\,col}$ 2.39	D_0 2.11	$D_{1\,col}$ 2.33	$D_{1\,col}$ 2.28	D_0 2.13
	IV Splenic Macro-colonies	$D_{1\,col}$ 2.02	$D_{1\,col}$ 2.04	D_0 2.05	$D_{1\,col}$ 2.25	$D_{1\,col}$ 2.22	D_0 2.61	$D_{1\,col}$ 2.41	$D_{1\,col}$ 2.47	D_0 2.57

DISCUSSION

In view of the enormous amount of literature on subjects related to the current experiments the following discussion is essentially confined to those findings in mice unless otherwise stated.

I. Acute Intestinal Syndrome

This syndrome occurring after whole body irradiation is characterized by an associated dose range of 1000 to 10,000 rad; a survival time of 3-6 days, depending on species and other conditions but almost invariant with dose; and damage to the small intestine with denudation of the lining (Quastler, 1956). At the dose levels used in the present experiment, overlapping of this syndrome with the hyper-acute syndrome caused by damage to the central nervous system is unlikely, although transition between it and acute marrow syndrome is possible. In fact, it has been suggested that sometimes another mode of death, probably from 'abdominal injury', may occur between 5-8 days in some strains (Austin, et al, 1956). A detailed discussion of acute radiation syndromes and their underlying mechanisms was presented by Bond, et al (1965).

Post-irradiation gut death has been used to compare the relative effectiveness of different ionizing radiations, to evaluate the recovery phenomena and to study the oxygen effect in vivo. To facilitate interpretation of results, probit transformation of dose-lethality data can be performed (Finney, 1952). The main advantage of this system lies in its simplicity and relative expediency in scoring results, and the main inherent disadvantages are that relatively high doses are required and that the range of sensitivity is

rather narrow.

(1) Previous RBE studies on heavy ions compared to 250 kVp x rays and based on 6-day median lethal dose showed values of 0.96-1.20 for 730 MeV protons (Sondhaus, et al, 1964; Ashikawa, 1963), 0.98 for 200 MeV protons (Ashikawa, 1967), and 1.10-1.20 for 138 MeV protons (Dalrymple, et al, 1966). My plateau α RBE of 1.14 compares favorably with the above but my peak α RBE of 1.34 is slightly but significantly higher although still far below the RBE of 1.90-2.60 for fast neutrons (Rothermel, 1956; Silverman, et al, 1958; Hornsey, et al, 1965). Another unexplained difference is that although a smaller RBE is noted at the 90% than at the 10% survival level for my data, the reverse was observed for fast neutrons.

(2) The capacity for recovery after x irradiation was studied by various split-dose methods from which a dose of 400 r to give maximum recovery and an extrapolation number of 28 were deduced at 6 hours dose separation (Hornsey and Vatistas, 1963). The shape of the survival curve as a function of time interval between the paired doses was very similar to that obtained by Elkind and Sutton with cell cultures (1961). After fast neutron irradiation the degree of recovery is only about 2/3 of that after exposure to x rays (Hornsey, et al, 1965). 730 MeV protons or 910 MeV alphas also caused more permanent damage than x rays (Ashikawa, 1967). The current results show no significant difference between alpha particles and ^{60}Co γ, although a small difference could have been masked because of appreciable recuperation after a longer interval of 48 hours. The finding that continuous irradiation of mice with ^{137}Cs γ at 325 rad/day up to 3 days did not

change the $LD_{50(s)}$ from acute exposure also attests to the remarkable power of recovery of the intestine (Cairnie, 1969).

(3) The oxygen effect has been investigated by using a brief period of less than 60 sec of pure nitrogen vs pure oxygen breathing under general anesthesia and megavoltage electrons delivered at sufficiently fast dose rates in the order of 10^5 rad/min. A dose reduction factor of 2.5-2.7 was observed (Alper and Hornsey, 1968; Hornsey, 1971). Pure oxygen at 3 atmospheres can enhance post-irradiation gut death by 12% compared to air exposure (Christensen, et al, 1969). My data using 5-10 min gassing with 7% oxygen vs air show OER values of 2.07-2.22, 2.23-2.24 and 2.30-2.42 for peak α, plateau α and ^{60}Co γ respectively, each depending on the survival level under consideration. Peak α is associated with a lower OER for every corresponding end point compared to ^{60}Co γ except at 10% survival where a difference of only 0.15 is noted. No other report on the in vivo oxygen effect of heavy particle irradiation is available for comparison. However, in vitro exposure of mouse lymphoma cells to the same helium ion beam demonstrated that the unmodified Bragg peak gave a significantly lower OER than the plateau, 1.8-2.1 compared to 3.2-3.5 (Feola, et al, 1969). Supporting evidence also comes from an experiment performed with almost identical modification of the Bragg peak on T-1 human kidney cells in culture (Raju, et al, 1970). The plateau α OER of 2.5 was similar to the x-ray value but the modified peak α OER of only 1.9 compared favorably with the value of 1.6 for 14 MeV neutrons on the same system (Barendsen and Broerse, 1966).

It ought to be pointed out that the in vivo induction of hypoxia may be associated with complex

changes in the mice such as alteration in pH, electrolyte distribution and different metabolic processes, so that other unknown factors may also play a role in modifying the radiosensitivity of the animals.

II. Acute Marrow Syndrome

Marrow death after whole-body irradiation has been used most extensively in the evaluation of *in vivo* radiation responses of mammals. It has been demonstrated that, providing meticulous care is taken in controlling undesirable physical and biological variables, highly reproducible results can be achieved (Ellinger, *et al*, 1955). The advantages and disadvantages of this system are similar to those of intestinal death discussed previously except that a longer observation period of 28 to 30 days is necessary.

(1) Numerous RBE studies have been done in mice by comparing the marrow death rates after different types of ionizing radiations. The potency of megavoltage x or γ rays is usually accepted as 0.85-0.90 compared to 100-250 kVp x rays (Kohn and Kallman, 1956; Hall, 1961; Sinclair and Kohn, 1964). Since ^{60}Co γ is used as the reference radiation in the present experiment, all the following RBE values quoted from the literature have been converted accordingly wherever 100-250 kVp x ray was employed as the standard. In the case of heavy charged particles, RBE's of 2.00 and 3.20 were noted for the $^{10}B(n,\alpha)^{7}L_1$ and $^{6}L(n,\alpha)\ ^{3}H$ reactions respectively (Bond and Easterday, 1956, 1959); $\sim$1.00 for 138 MeV protons (Dalrymple, 1966); $\sim$0.85 for 440 MeV protons (Oldfield, *et al*, 1963; Bradley, *et al*, 1964); $\sim$1.15 for 592 MeV protons (Baarli and Bonet-Maurey, 1965); $\sim$1.19 for 660 MeV protons (Wang, *et al*, 1962; Kurlyanskaya, 1962); $\sim$0.88 for 730 MeV protons; $\sim$0.9 for 730 MeV protons whether unmodified or degraded to 200 MeV with carbon or copper absorbers (Ashikawa, *et al*, 1967); and about $\sim$1.00 for 2.2 BeV (Jesseph, *et al*, 1968). The

value of 1.13-1.15 obtained currently for plateau α agrees with the above but that of 1.28-1.39 for peak α is significantly higher though still much lower than most reported values for fast neutrons. These were 1.75, 5.88, 2.0 and 1.82 for cyclotron produced neutrons (Lawrence, et al, 1937; Hager, 1950; Upton, 1956; Strike, 1970) and 4.6, 2.3, 2.0, 2.0, 2.65, 2.69, 3.18 and 2.22 for fission neutrons (Henshaw and Zirkle, 1947; Storer, et al, 1957; Vogel, 1957; Delihas and Curtis, 1958; Ainsworth, 1964; Gambino, et al, 1968; Davids, 1970).

(2) Various aspects of recovery of mice after total body exposure have been studied, most commonly by different split-dose methods with variations in the conditioning dose, challenging dose or the time interval between them. Multiple fractionation has also been practiced to similate the clinical treatment scheme. Different models of recuperation kinetics have been proposed using linear, exponential or polynomial equation to fit the presented data (Blair, 1952; Spalding, 1961; Storer, 1961; Dalrymple, 1963; Stearner, et al, 1963, 1964; Kallman, 1964; Krebs and Brauer, 1965; Kallman, et al, 1966; Corp and Mole, 1966; Taenzer and Krobowski, 1968). Summarily, the early repair pattern consists of a period of maximum refractoriness occurring several hours after the priming dose, followed by a period of maximum sensitivity several hours later. The cyclic fluctuations gradually decrease in amplitude and are almost completely dampened by 48 hours. This picture bears a rather striking similarity to the recovery pattern of hemopoietic stem cell survival (Till and McCulloch, 1963) and that of mammalian cells in tissue culture in general (Elkind,

1961). The phenomenon is best explained by the effects of rapid intracellular repair of sublethal damage and/or partial synchronization with stage-dependent radiosensitivity. Later recovery appears to occur at a fairly constant rate, apparently due to the logarithmic nature of cell division in the irradiated host, and it is therefore determined by the doubling time and D_0. Another interesting observation which may have some important implications in radiotherapy is that severe hypoxia might impair recovery after fractionated radiation (Phillips, 1968; Phillips and Ainsworth, 1969).

Recovery studies on marrow death after heavy particle bombardment have been rather limited. Vogel in 1959 found that fission neutrons resulted in a longer recovery half-time (10 days) compared to cobalt-60 (6 days). However, by the 30th postirradiation day, there was almost complete recovery. Dalrymple and his coworkers noted slower recuperation after exposure to 55 MeV protons compared to cobalt-60 but attributed the discrepancy to the difference in the priming doses (1966). In contrast, other investigators observed no apparent repair of injury following exposure to 730 MeV protons, 910 MeV alphas or x rays (Ashikawa, et al, 1967). The present data indicate that appreciable recovery (65.6-74.1%) occurs after both plateau α and ^{60}Co γ exposure, but to a lesser degree (50.6%) in peak α irradiated mice.

(3) The oxygen effect was studied as early as 1950 by Dowdy and his colleagues. They tried various concentrations of oxygen and found that their rats could tolerate 5% oxygen but their mice only 7%. An OER of 2.0-2.2 compared to air breathing was reported by them. Van den Brenk and Moore (1959), in elucidating

the mechanism of action of radioprotections in mice obtained a twofold reduction in radiosensitivity using similar partial pressure of oxygen, while Phillips (1968), in evaluating the effect of hypoxia on recovery, also observed a similar dose reduction factor with 5% oxygen which was apparently quite well tolerated by his mice. Using brief period of nitrogen vs oxygen breathing and exposure to fast electrons, OER values of 2.3-2.5 and 2.0 were derived by Wright and Bewley (1960), and Hornsey (1971) respectively. An age dependency was stressed by Lindop and Rotblat (1960). Variation of OER over a range from 1.73-2.58 was seen in the same strain of mice but of different ages. Maximum protection occurred among the 4-week olds while both the 1-day and 82-week old mice were least protected. Hasegawa and Landahl (1965) obtained dose reduction factor of 2.35 after intraperitoneal administration of sodium nitrite and showed by polarographic measurement of tissue oxygen tension and irradiation under various oxygen pressure that the protection was mediated through tissue hypoxia. Evans and his associates (1965) achieved significant radioprotection with nitrous oxide, krypton and xenon gases but thought that it was not related to tissue anoxia. Pure oxygen at 3 atmospheres was found to enhance marrow death by 13% (Christensen, et al, 1969). OER's of 2.30 and 2.46 for plateau α and ^{60}Co γ derived from the present data are higher than expected. However the value of 2.06 for peak α does suggest a slight but significant relative reduction of the oxygen effect.

Marrow death after single or multiple x irradiation has been used extensively to evaluate the effect of chemical radioprotectors (Leitch, 1961; Balner, et al, 1961; Smith, et al, 1963; Wang and Ballantyne, 1964;

Maisin, et al, 1968; Wang and Hasegawa, 1968; Novak, 1969; Vittorio and Amey, 1970; Hasegawa and Landahl, 1970; and Sonka, et al, 1971). The dose reduction factor ranged from 1.5 to 2.8. Using 250 kVp x rays and 150-440 MeV protons, Oldfield and Plzak (1965) noted a similar degree of protection by mercaptoethylamine or p-aminopropiophenone. On the other hand, using a combination of chemical radioprotectors, homologous marrow cells and streptomycin, Vogel and his associates (1969) found that mice exposed to fission neutrons were less protected than those following x irradiation, the DRF being 1.3 and 2.5 respectively. It may be expected that peak α irradiated mice probably exhibit intermediate protection from similar treatment. But this remains to be determined. The last paragraph is inserted to emphasize the feasibility of using marrow death in quantifying differences in radiation response.

III Radiation Response of Intestines

Vulnerability of the gastrointestinal system to ionizing radiations has been established for a long time. Various aspects of the resultant damage have been assessed both qualitatively and quantitatively by means of physical, functional, histologic and cyto-kinetic studies.

Dose dependent weight loss of the small intestine was demonstrated by Conrad in 1954. Early electron microscopic changes of the villi were detected after as low as 200 rad of x irradiation but absorption and transportation were impaired at much higher doses and after a longer interval (Quastler and Hampton, 1962). Using radioautographic technique, Lipkin and his co-workers (1963) noted that DNA synthesis in the crypts was reduced by 50% after a dose of only 100 rad although protein synthesis in general was impaired at much higher dose. Based on mitotic activities, Maisin and Doherty (1963) observed that sulfhydryl compounds appreciably shortened the recovery time. Studies on the generation cycle of duodenal crypt cells by means of tritiated and/or ^{14}C-labelled thymidine showed that a new steady state was reestablished when mice received a daily dose of 200-250 rad (Fry, et al, 1963; Lamerton, et al, 1966; Wimber and Lamerton, 1966; Lesher and Lesher, 1970). Increased cell loss was compensated by a shortening in the cell cycle especially in its G_1 phase and by an increase in the number of proliferative cells. Actually this concept had long been applied clinically in fractionated radiotherapy to minimize damage to the intestines. However, it has been suggested by Wiernik (1966) that the therapeutic ratio might be higher if larger but less frequent fractions

were used. He found that peroral jejunal biopsies from patients thus treated did not indicate any excessive injury. The need for further investigation in order to establish fractionation schemes on a scientific basis was stressed by Tubiana (1970).

Crypt cell survival characteristics were first deduced by Withers and Elkind in 1968. They irradiated an exteriorized 2 cm-long loop of small intestine to a dose ranging from 1675 to 2375 rad, care being taken to sterilize the margins with about 3000 rad to prevent cell migration from the unirradiated segments. The mice were sacrificed 13 days after exposure and the macro-colonies scored. Their data were fitted beautifully by an exponential survival curve which yielded a D_0 of 100 rad.

In 1970, the same authors reported another technique for assaying crypt cell sensitivity. Micro-colonies per transverse section were counted after routine histologic processing of the jejunum, 3 to 4 days after the animals received total body irradiation. With appropriate adjustments, a remarkable correlation with macro-colonies was observed. It was used successfully by Gilette and his associate (1970) to illustrate the age-dependent variation in radiosensitivity through different phases of the crypt cell cycle. In view of the obvious advantages, this method has been adopted in the present experiment although other techniques based on the number of viable cells per crypt or per unit weight of the jejunum from tritiated thymidine labeling also seem appealing (Kononenko and Farafonov, 1969; Devik, 1971; Hagemann, 1971).

(1) RBE studies on crypt cell survival are relatively few. Based on the long-term histopathologic

examination up to one year after local irradiation of the rectum of rats, Stenson (1969) derived an RBE of only 0.6-0.7 for 185 MeV protons compared to 220-220 kvp x rays. Even allowing for the difference between 220 kvp x rays and cobalt-60 γ rays, his result does not agree with the plateau α RBE of 1.08-1.14 from our data possibly because neither the experimental conditions nor the end points are comparable. In any event the peak α RBE of 1.24-1.34 is definitely higher than the plateau value. With respect to studies on fast neutrons, Lawrence and Tennant (1937) reported almost identical histological findings in the intestines of mice receiving either 230 r to 290 r of cyclotron produced neutrons or 1000 r of x rays. Based on cytological damage in the duodenum after fission neutrons and cobalt-60 exposures, Lesher and Vogel (1958) stated a ratio of 1.7. Using microcolony assays, a RBE of 1.75-1.90 compared to x rays was determined for neutrons produced at the Hammersmith cyclotron (Hornsey, 1970). In contrast, a value of only 1.15 was observed for 14 MeV monoenergetic neutrons (Withers, et al, 1970).

(2) Recovery studies by means of the standard paired dose method on intestinal microcolonies after x irradiation showed typical fluctuation of survival when plotted against fractionation intervals (Withers and Elkind, 1969). They also noted that full survival curves obtained at different intervals after the priming dose might not be parallel to the single dose curve. Similar study after exposure to 14 MeV monoenergetic neutrons revealed a smaller degree of recuperation of 29% compared to 48% after x irradiation, (Withers and Elkind, 1970). A smaller quasi-threshold

dose for cyclotron neutrons was confirmed by Hornsey in 1970. With regard to charged particles, Ashikawa and his associates (1967) examined the histopathological changes in the intestines after split dose exposures and found that 730 MeV protons and 910 MeV alphas caused more permanent damage than x rays. But the difference was not quantified. My data show almost complete recovery at 48 hours after α and γ irradiation and suggest possibly an overshoot or overcompensation for peak α and ^{60}Co γ if the 10-colony survival is chosen for comparison.

(3) The effect of oxygen on the in situ radiation response of the intestines has not been thoroughly explored. Using local infusion of nor-epinephrine and sodium nitrite to create a hypoxic state and histologic criteria for evaluation, Stenson (1970) observed approximately equal protection of the rectal mucosa of rats receiving 187 MeV proton or cobalt-60 gamma irradiation locally. When mice breathed pure oxygen instead of air during local body x irradiation, the sensitivity of small intestine was increased by 10% according to both macro- and micro-colony assays (Withers and Elkind, 1970). Using 7% oxygen vs air breathing and comparing the slopes of the crypt cell survival curves comparable OER (2.11-2.55) is noted for all 3 types of radiation used presently. However, when based on iso-survivals, peak α appears to have a slightly lower OER (2.19-2.2) than plateau α or ^{60}Co γ (2.28-2.49). The discrepancy is probably due to a difference in the shoulder width.

(4) Correlation between cytokinetics of the gastrointestinal system and acute intestinal syndrome have been discussed in detail (Patt and Quastler, 1963; Patt, 1968). It has often been assumed that animal

survival depends on a critical number of viable crypt stem cells (Hornsey and Vatistas, 1963; Wilson, 1964). Different mathematical models based on cell population kinetics have been proposed (Gilbert and Lajtha, 1965; Sacher and Irucco, 1966; Robinson, 1968; Lange, 1968, 1970).

Functional importance of the villus cells had been well known. But competence of the Paneth cells was first stressed by Hampton (1966). He noted remarkable similarity between the effects of x irradiation and nitrogen mustard pertaining to the changes in the villus cells (1967). Good correlation between animal survival after abdominal irradiation and macrocolonies in the jejunum was noted by Withers and Elkind (1968). Similar observation was made by Hornsey (1970) in connection with the effect of dose rate. It is not surprising that my data show an agreement of better than 5% between each 6-day median lethal dose and the dose resulting in 10 colonies per circumference for all single dose exposures. On the other hand, the lack of correlation observed in the split dose experiments indicates that a more complex relation exists. It is possible that a requisite level of proliferative cellarity at an earlier stage after exposure is a more decisive factor in determining the outcome of the animal (Hagemann, et al, 1971).

IV. Radiation Response of Hemopoietic Tissues

There are 2 broad categories of spleen colony assays used in quantitating radiation response of mammalian cells. (A) One involves preparing spleen, marrow or liver cell suspensions from a donor mouse and injecting them within several hours into a previously exposed recipient mouse from which the exogenous spleen colonies are scored at the end of 8-10 days after transplantation. (B) The other does not require a donor but consists of simply irradiating mice and counting the endogenous colonies after a similar period.

(A) In exocolony techniques, all recipients receive equal amount of prior whole body irradiation to suppress endocolony formation. (i) Donor mice may be given graded doses of in vivo exposure before cell suspensions are prepared. (ii) Donor cells in suspension may be irradiated in vitro before injection into recipients. (iii) Recipient mice may be exposed in vivo to graded doses of radiation before injection and reciprocally graded doses after injection so that the injected cells receive graded exposure but the recipient mice a constant total exposure (Till and McCulloch, 1961; McCulloch and Till, 1962; Till, 1963; McCulloch and Till, 1964; Siminovitch, et al, 1965.

(B) In endocolony techniques, all irradiations are delivered in vivo. (i) This can be done first to the exteriorized spleen, then to the whole body so that the spleens of different groups of mice receive equal amounts of sterilizing radiation but the rest of the bodies, graded doses. Thus a dose response relation of spleen nodules derived from non-splenic tissues can be established (Boggs, et al, 1968). (ii) Part of a skeleton (e.g., a femur) can be shielded to different

degrees while the rest of the body is exposed. This would isolate the response of femoral marrow alone because the colony formers which have migrated to and settled down in the spleen are scored (Hanks, 1964). (iii) Mice can simply be given graded full body exposures and the spleen nodules which develop in situ counted (Marsh, et al, 1967).

There are advantages and particular applications as well as inherent disadvantages associated with each method. In essence, all exogenous procedures might introduce errors in the preparation and injection of cell suspensions although these would be minimized in experienced hands. However, the in vitro technique (A-ii) has a unique attraction in that a survival curve covering several decades may be obtained by varying the dilution of cell suspensions. In contrast, in all other methods only a narrow dose range of a few hundred rad can be used thus giving rise to appreciable uncertainty in extrapolation. In general, the endogenous procedures should give results more closely related to the radiation response of the whole animal, at least in theory. The in situ technique (B-iii) which is used currently has additional benefits in its simplicity and relative independence of factors affecting migration and distribution of cells in the mouse.

Clonal nature of the exocolonies was suggested by cytologic demonstration (Becker, et al, 1963; Welshons, 1964) and confirmed by karotypic analysis of chromosome marked cells (Chen and Schooley, 1968). It is still moot whether the colony formers truly represent multipotential hemopoietic stem cells. Recent evidence from comparison with erythropoietic sensitivity tests using multiple techniques indicates the presence

of an intermediate population (s) of erythropoietin sensitive cells between the hemopoietic stem cells and the colony-forming cells (Schooley, 1969). There are as expected, 3 histologic varieties of colonies: erythropoietic, granulopoietic, and undifferentiated. Their proportions differ depending on the time of examination and their origin (Jenkins, et al, 1969). The significance of this observation is being investigated. By means of retransplantation technique, it has been shown that the distribution of colony forming cells per colony is extremely heterogenous, which cannot be entirely attributable to variation in the total cell counts per colony (Siminovitch, 1963). This distribution approximates more closely a Gamma rather than a Poisson function. Similar distribution has been noted with respect to gross nodule size (Ainsworth and Larsen, 1969). Thus the slope of the survival curve may vary considerably especially for endocolonies according to which size criterion is chosen. Nonetheless, in spite of some degree of subjectivity, meaningful information can still be obtained provided the same selection is followed throughout. The counting procedure could have been facilitated by a recently developed staining technique (Larsen and Ainsworth, 1969). However, it is not used presently because it has not been found to alter the estimation of radiation sensitivity.

(1) RBE studies based on spleen colonies are rather scarce. In fact, apart from comparison between x and γ rays there has not been any report on particulate radiations to my knowledge. RBE values of 1.24-1.34 for peak α and 1.08-1.14 for plateau α obtained here are in keeping with those of 1.17-1.27 and 1.09-1.10 based on marrow death.

(2) The recovery pattern of the hemopoietic tissues, however, has been investigated quite commonly by spleen colony assays. Early intracellular repair of exo-colony formation was studied by Till (1963) employing 2 doses of 200 rad given at various time intervals and comparing the survival ratio. A peak noted at 5 hours and a trough at 10 hours were similar to Elkind's (1961) observation on mammalian cells in culture. Almost identical results were obtained with endo-colonies (Till and McCulloch, 1963). By means of an alternative split dose technique, i.e., giving graded second doses to reevaluate the full survival curve at different times after a fixed conditioning dose, they noted a transient increase in the D_0 for both exo- and endo-colonies which was maximal around 24 hours returning to single dose value by 48 hours (McCulloch and Till, 1964). Fluctuations in survival of exo-colonies at different times after the transplantation and of endo-colonies at different times after a conditioning dose could be attributed to partial synchrony and differential sensitivity of different phases in the cell cycle (Maruyama, 1968; Frindel, et al, 1966). Another factor that might play a role could be changes in oxygen consumption of the spleen after exposure (Evans, 1968). Attempt to correlate the recovery pattern of animal survival with that of exo-colonies of marrow origin was unsuccessful (Hanks and Ainsworth, 1967). In evaluating the effect of small changes in daily dose in the range commonly employed in fractionated radiotherapy, a discrepancy of the overall effect was noted between exo- and endo-colonies (Chaffey and Hellman, 1968). This was presumably due to a difference in their states of proliferation. Thus endo-colony formation would seem more applicable to the

clinical situation. My data indicate that peak α probably causes slightly more irreparable damage than plateau α or ^{60}Co γ.

(3) The oxygen effect on spleen colony survival has been studied by several investigators. No significant difference was noted in exo-colony development when marrow cells were irradiated in vitro before transplantation either in air or when a mixture of 95% O_2 and 5% CO_2 was bubbled through the suspension (McCulloch and Till, 1962). Similar results were obtained when donor mice were exposed in vivo either breathing air or pure oxygen (Maruyama, 1968). Hasegawa and Landahl (1967) showed that when mice were forced to breath graded hypoxic mixtures, there was a corresponding drop in oxygen tension in the spleen as measured polarographically. Using the endogenous technique, Vacek and Sugahara (1967) irradiated mice either in air or in an atmosphere of 8% O_2 in N_2 and obtained a dose modifying factor of 2-4 depending on the strain used, while Hornsey (1971), exposing 2 other strains of mice to brief periods of pure N_2 vs pure O_2 deduced a value of 2.0. Using the exogenous method, Cole and Davis (1968) derived a DMF of 1.9-2.1 when anoxia was produced by sacrificing the donor mice 3-5 minutes before exposure. Using both exo- and endogenous techniques and a 5% O_2 hypoxic mixture vs air an OER of 2.3-2.6 was obtained in other strains of mice (Phillips and Hanks, 1968; Phillips, 1968). They also noted an apparent absence of recovery after hypoxic exposure. It is interesting to note that previously similar DMF was observed when mice were subjected to 1°C hypothermia and their spleen weight scored after irradiation (Weiss, 1960). The value of 2.22-2.61 for plateau α and ^{60}Co γ I obtained is comparable to

the above while that of 2.02-2.05 for peak α is slightly but significantly lower. In passing, it should be mentioned that there is negligible effect from the brief hypoxic treatment under discussion in contrast to the well known hematologic changes after prolonged hypoxia which is used so frequently in the study of erythropoiesis.

To lend further support to the validity in using endogenous spleen colony formation to quantitate radiation response of mammalian tissues in situ, the following additional works on radioprotectors are cited: difference in dose reduction factor of single or multiple doses of intraperitoneal injection of S. typhosa endotoxin based on the D_0's (Smith, et al, 1966); effect of the interval between intraperitoneal administration of cystamine by comparing the doses which result in the same number of colonies per spleen (Juraskova, 1967); kinetics of stem cell depletion and proliferation as evidenced by the effects of vinblastine and vincristine in control and irradiated mice (Smith, et al, 1968); difference in mechanism of radioprotection afforded by AET and urethrane (Cole and Davis, 1968); influence of separate and combined use of 5-methoxy-troptamine and cystamine (Zaitseva, et al, 1969) and effect of estradiol on the different histologic types of spleen colonies (Jenkins, et al, 1969).

(4) There have been many attempts to correlate acute marrow syndrome with the survival characteristics of the hemopoietic cells since the "salvage experiment" of McCulloch and Till (1960). Differential radiosensitivity of normal and genetically anemic mice can be explained on a cellular basis using peripheral blood counts and marrow cellularity (Russell, et al, 1963).

The radiation effects in cell renewal systems are determined largely by their cellular kinetics, which may account for the selective advantage of clinical dose-fractionation in tumor therapy (Patt and Quastler, 1963; Patt, 1963, 1968). By quantitating the decrease in nucleated cells, it has been shown that the spleen and marrow have similar cell survival curves with respect to reproductive inhibition (Puck, 1966). Based on the assumption that the whole animal survival depends on a critical number of certain sensitive cells, several approaches have been proposed to establish the relation of whole animal mortality and the survival curve of single cells (Munro and Gilbert, 1961; Lange and Gilbert, 1968; Robinson, 1968; Gilbert, 1969). In general, in spite of empiricism and either oversimplification or complicated mathematical formulation, there is good agreement for the quoted illustrations. Both β-mercaptoethylamine and S. typhose endotoxin increase postirradiation animal survival as well as comparable endogenous spleen colony survival (Smith, et al, 1966). Using both exo- and endo-colonies and both Pseudomonas endotoxin and AET as radiomodifiers, Ainsworth and Larsen (1969) have been able to predict the $LD_{50(30)}$'s to within 2-10%. Employing heat-damaged red blood cells as a radioprotector, a fair correlation between marrow death reduction and enhanced endogenous spleen colony survival has been observed (Morley, et al, 1970). Furthermore, 30-day lethality appears to be more closely related to endogenous spleen colonies than exogenous colonies of marrow original as studied with post-irradiation colchicine treatment (Brecher, et al, 1967) or in mice differing markedly in erythropoietic activity (Fogh, 1971). The present finding of 50% animal survival

corresponding to 1 endo-colony per spleen agrees with previous observation of a correlation with 3×10^4 nucleated cells (Kurnick and Nokay, 1965).

(5) Splenic weight loss has been shown to be a sensitive indicator of acute radiation effect (Carter, et al, 1950) and used as a means of comparing different ionizing radiations (Harris and Brennan, 1952; Jordan, et al, 1956; Bateman, et al, 1961) and assessing the radioprotective effect of marrow cells (Wu, et al, 1968; Takada, 1971) or chemicals (Zaitseva, et al, 1969). However, complicating factors of regeneration arise when the weights are scored more than a few days after exposure. Stroud and his coworkers (1955) showed that when the irradiation was fractionated over weeks, the active red pulp instead of losing weight like the white pulp actually increased in mass as a result of extramedullary hematopoiesis. Kallman and Kohn (1955) pointed out that there was appreciable inherent variability unless large numbers of animals were used and that abscopal effects might also play a role in causing reduction in splenic weight. The latter was confirmed by Kurnick and Nokay (1962) by shielding the exteriorized spleen. Attempts to use postirradiation weight changes of spleen and thymus to evaluate the influence of the adrenals on the radiation effects of lymphoid tissue was unsuccessful (Mendelsohn, 1955). Thus, it is not surprising that poor dose response correlation is noted in my preliminary data.

V Miscellaneous

(i) Biological Factors

The influence of mouse strain on acute radiation lethality has long been recognized (Kohn and Kallman, 1956, 1957). Even though the slope function of the probit-transformed dose-effect curves remained surprisingly constant for all the inbred and hybrid strains tested, there was significant variation in the median lethal dose as well as the mean survival time. The recovery half-time examined by the paired dose method also showed a definite strain dependence. On a genetic basis, Grahn (1957) pointed out that relative radioresistance was dominant over sensitivity. However, Frölen, et al,(1961) presented evidence to support the importance of additive or recessive genes. Studies using fast neutrons instead of x rays revealed comparable strain factor (Hightower, et al, 1968). The similarity of response between wild and domestic mice of the same species seemed to indicate that natural selection was of minor significance (Golley, et al, 1965). A relation between the efficiency of chemical radioprotectors and animal strain has also been demonstrated (Yuhas, 1970).

The age factor is another important determinant of radiosensitivity, not only in terms of the 50% lethal dose and time of peak mortality but also pertaining to the capacity to recover and the effect of oxygen (Lindop and Rotblat, 1962; Spalding and Truyillo, 1962; Rugh and Pardo, 1963; Trowell, 1963; Storer, 1965; Fred and Smith, 1967; Yuhas and Storer, 1967; Jones, et al, 1968, 1969; Yuhas, et al, 1969). Despite minor discrepancies, some generalization may be made. In

essence, new-borns are most susceptible to radiation, young adults most resistant, and vulnerability again increases with advancing age. Possibly due to a faster metabolic rate the young tend to recover better than the old and have a higher tolerance to hypoxia and therefore exhibit a greater oxygen effect.

The effect of sex on radiation response is not as marked as that of strain or age (Kohn and Kallman, 1956; Lindop and Rotblat, 1962). In general, the females are slightly more sensitive before 10 weeks and somewhat more resistant at older age, probably because of the influence of estrogen.

From the above discussions, it is apparent why the same strain and sex of mice were used throughout the current investigation and why effort was made to maintain a narrow age range of no more than 2 weeks.

(ii) Environmental Conditions

A definite cage effect on acute radiation lethality has been demonstrated by previous experimenters (Hahn and Howland, 1963; Yuhas, et al, 1966). Singly caged mice survive a higher dose of radiation and longer than those kept in lots of two or more. My small control experiment is not conclusive but certainly suggestive of similar findings. Thus, all the mice used for lethality studies were housed individually. Ideally those for intestinal and spleen colonies should have been similarly kept, but because they were sacrificed before death occurred spontaneously, the effect of crowding was considered negligible.

The influence of diet was examined by Ershoff (1961) and Dymsea, et al (1963). In view of their positive evidence, the mice were fed the same food throughout the

entire experiment. The water was chlorinated and all the containers autoclaved to minimize the chances of infection. It is not certain whether the use of germ-free animals might yield significant additional information as far as the objectives of the present investigation are concerned. The axenic state has been found by many to increase and prolong animal survival both after x irradiation (Wilson, 1963; McLaughlin, et al, 1964; Matsuzawa and Wilson, 1965; Walburg, 1966) and following exposure to fast neutrons (Jervis, et al, 1971; McLaughlin, et al, 1971). The temperature and the periods of light and dark of the animal room were kept constant to reduce any seasonal and diurnal variation in radiosensitivity. No apparent influence was observed in my small control experiments, but a circadian rhythm was determined by some investigators (Nelson, 1966; Vacek, et al, 1968; Ueno, 1948; Pizzarello and Witcofski, 1970). The higher sensitivity at night compared to the day is possibly mediated through a humoral mechanism and/or the fluctuations of the stage-dependent sensitivity through different phases of the cell division cycle.

(iii) Irradiation Conditions

Possible link between metabolic activity and radiosensitivity was presented by Tsuchyia, et al (1963). By administering thyroid or anti-thyroid agents, he observed a definitive difference in sensitivity. He was able to explain some strain-factor purely on the basis of a physiological difference in the thyroid function. Spalding and his associates (1970) also attributed the difference of radiosensitivity between two sublines of the same strain of mice to a corresponding difference

in their voluntary activities. With the container rotating during exposure in my experiments most of the mice moved in the opposite direction. But possible variation in the amount of physical activity cannot be excluded. Hurley and Joslin (1964) found an enhancement of radiation lethality when the irradiation temperature was raised from 22° to 37°C. However, Williams and his colleagues (1968) did not observe a difference over the range from 16° to 32°C. Most of my exposures were delivered at a room temperature of about 20°C, with a mean elevation of about 4-5°C inside the mice holder at the end of the irradiation.

The dose rate effect on radiosensitivity has been studied extensively. A correspondence of lethality to the cube root of the dose rate was proposed by Bateman, et al (1962). For x or α radiation, no significant difference in survival rate was found between 10 and 95 rad/min (Fowler and Lawrey, 1960) nor in survival time between 2.5 to 250 rad/min (Spalding, et al, 1967). However, a dependency on dose rate between about 0.5 to 70 rad/min was noted by Joslin, et al (1967), who also observed that the influence on gut death was more pronounced than on marrow death. A close correlation between gut death and dose rate was confirmed by Krebs and Leong (1970) using a range from 1 to 100 rad/min. Using fast electrons delivered at 6000 rad/min, Hornsey and Alper (1966) showed a decrease of about 20% in the $LD_{50(4)}$ compared to the usual dose rate of about 100 rad/min. However, using 3 MeV pulser x rays at 10^{12} rad/min, Caldwell and Sloan (1967) did not detect any significant change in the $LD_{50(30)}$ compared to the exposure at a dose rate of 300 rad/min. With heavy particles similar discrepancy of a dose rate effect

on gut death but not on marrow death was observed by Ashikawa, et al, (1963) using 730 MeV protons between 100 and 1000 rad/min, and by Dalrymple, et al (1966), using 138 MeV protons between 86 and 550 rad/min. My data on marrow death resulting from α irradiation at 300 and 900 rad/min are in agreement with the above. Negligence to repeat a similar experiment on gut death might have caused a relatively higher RBE compared to marrow death for both peak α and plateau α irradiation because they were generally performed at dose rate around 900 rad/min in contrast to the maximum rate of 300 rad/min available from the cobalt source. In any event, there is a clear biological difference between peak α and plateau α delivered at similar dose rate. With respect to fast neutrons, $LD_{50(30)}$ studies did not reveal any differences between fission neutrons delivered at 40 or 10^6 rad/min (Ainsworth, et al, 1964). On the other hand, using 14 MeV monoenergetic neutrons and scoring by the same endpoint, a significant dose rate effect was demonstrated by Strike (1970) even over a narrow range from 3 to 50 rad/min. The reason for this discrepancy is not apparent. It seems unlikely to be accounted for by a different LET.

(iv) Supplementary Postirradiation Observations

The median survival time of animals exposed to the lethal marrow dose range has been shown to decrease exponentially with increasing dose (Storer, et al, 1957) and found to be shorter after irradiation by higher LET radiations (Upton, et al, 1956; Bond and Easterday, 1959). Within the limitation of accuracy in my data, equivalent doses of all 3 types of radiation appear to result in roughly equal mean survival time.

Changes in animal weight after total body exposure has been found to be correlated with mortality data (Dowdy, 1950; Chapman, et al, 1950, 1955, 1956). However, it is felt here that the association is probably too gross to permit distinction among the radiations used and not sensitive enough for an accurate evaluation of modifications of radiation response.

(v) Linear Energy Transfer

The significance of ionization density in radiobiology and its implication in radiotherapy have long been recognized (Lawrence, et al, 1937). The most widely used criterion is based on the LET concept (Lawrence, et al, 1937; Tobias, 1952; Zirkle, 1954; Bond, 1957; Todd, 1964; Barendsen, 1965; Andrews, 1965; Fowler, 1966; Bewley, 1968). Because of differences in the choice of LET definition and biological test system there are as yet no universally applicable relations between LET and biological effects. Nonetheless, certain general agreements can be summarized as follows. (1) RBE increases with LET from about 1 KeV/μ up to about 50-100 KeV/μ, beyond which it gradually falls again. (2) There is more irreparable damage at higher LET. (3) The influence of modifying agents such as oxygen and chemicals is reduced at high LET. The limits where recovery begins to vanish completely and where the dose modifying factor approaches unity are probably in the order of 300-500 KeV/μ.

Under the above considerations, the data reported herein would seem unexpected because the maximum mean LET at the Bragg peak is only around 10 KeV/μ. Several explanations are offered without verification. Perhaps a small component of very high LET particles is enough to cause the observed differences from the uniformly low LET γ radiation. Furthermore, the energy distribution on a microscopic scale is nonuniform. The proposal by Rossi, et al (1961), of using a probability function to define the true spatial energy distribution could be more important in determining the biological outcome. It is doubtful that the higher instantaneous dose rate in the vicinity of 10^8-10^9 rad/min of α radiation might play any role.

The modified Bragg peak appears to be significantly different from cobalt-60 gamma to warrant further research on other normal tissues and some tumor systems, especially when heavier ions become available in the near future.

In summary, the attractive features of heavy ions lie in the relative ease of beam collimation, the possibility of achieving more ideal 3 dimensional dose distribution, and the ability to irradiate cancer cells with the high-LET peak making use of its biological advantages while the intervening normal cells in the beam paths are exposed to the low LET plateau portion. Even the present helium-ion beam may prove useful in certain situations where a small therapeutic gain is critical in enabling the delivery of a cancerocidal dose without excessive damage to the surrounding normal tissues. Whether the benefits would justify the expense in setting up a heavy-ion treatment center for cancers remains to be ascertained; but at this Laboratory where such beams are available therapeutic studies on patients with inoperable and radiation incurable cancers continue. It is to be hoped that other centers that have these high energy particles available will use them similarly, and that heavier, more densely ionizing and penetrating ions will soon be available such as those produced by the proposed Bevalac now in the planning stages for use in physics, chemistry, biology, nuclear and space research.

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