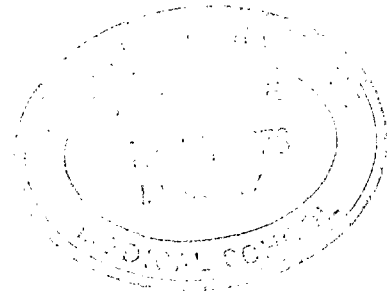
STUDIES TO ELUCIDATE THE RELATIVE ROLE OF SINGLE AND MULTIPLE-EVENT TYPES OF RADIATION DAMAGE IN VICIA FABA(L).

BY

MUHAMMAD TARIQ KHOKHAR B.Sc.,M.Sc.



Thesis submitted for the degree of Doctor of Philosophy at the University of London, 1977.

Department of Medical Physics
Royal Postgraduate Medical School.

To.

Professor Raymond Oliver my mentor, the most inspiring and wonderful teacher I have met, to my parents and family who waited patiently for the completion of this work.

Summary of the thesis.

The response of living organisms to small doses of radiation is of particular importance, both in radiotheraphy, where radiation is given in a number of small fractions, and in the study of environmental hazards, where the doses involved are even smaller. The response to a small dose may be too small to measure accurately, but this difficulty can be overcome by observing the response to multiple small doses separated by sufficient time to allow recovery from sub-lethal damage.

This thesis reports the results of a number of studies on the Vicia faba root system. In the first part the effect of 250 kV X-ray, Co^{60} gamma-ray and 8 MeV electrons were compared. With all these radiations the total dose required to produce a given effect tends to a constant value as the number of fractions is increased, implying that for all of these the survival curve has a non-zero initial slope. The results fit a model to the type:

$$S = \exp(-D/D_1)\left[1 - \{1 - \exp(-D/D_n)\}^n\right]$$

and values for the parameters have been found. However, the model $S = \exp(-\alpha D - \beta D^2)$ is equally consistant with the data. More significant perhaps is the finding that RBE varies with dose per fraction in a manner consistant with the higher LET radiation producing a more pronounced initial slope to the survival curve i.e. effectiveness of the higher LET radiation becomes relatively greater at smaller doses. In the second part sensitisation by the drug Ro-07-0582 has been investigated using 250 kV X-rays.

No variation of enhancement ratio with dose per fraction has been detected, implying, for instance, that single-hit and multi-hit always are equally enhanced. The enhancement ratio for bean irradiation at 3.5°C, in 5mM is 1.6. At 19°C the ratio is 2.2, a significantly higher value.

These studies give additional suport to the view that, even with low LET radiation, there is an initial slope to the survival curve, and they have enhanced the knowledge of sensitisation by the drug 0582. Further, the general resemblance of the results reported here to those obtained with mammalian cells increases confidence in the relevance of studies with the convenient bean root system to problems of radiation response in man.

Contents. Page.

Contents. Page.

Contents. Page.

List of Illustrations.

List of Tables. Page.

All the tables are at the end of their corresponding chapters.

Chapter 4.

Chapter 5.

Chapter 1.

Introduction

CHAPTER 1.

1 - I INTRODUCTION.

Since the early days of radiotheraphy more than six decades ago a considerable body of clinical and biological data has been amassed concerning the reduced effectiveness of a given dose when given in a protracted form. In general, treatments given at low dose rate or by fractionated doses at daily intervals have been shown to cause more damage to tumours for a given level to normal tissue than do single dose treatments. Differences in response of normal tissue and tumour to ionizing radiation, which are necessary requirements in the treatment of malignant diseases have thus been optimised.

The increase of dose with fractionation can be accounted for, at least qualitatively, by the shape of the survival curves for mammalian cells irradiated with single doses of X-rays. (Puck and Marcus, 1956). Later similar responses to radiation were demonstrated both invivo and invitro, in normal and malignant cells. (Hewitt and Wilson, 1959; Elkind and Sutton, 1960; ~~Barendsen et al., 1960~~). A unifying feature of these curves was the shape of a log linear plot Fig.1-1 i.e., the survival curve shows an initial shoulder where the radiation is relatively ineffective followed by an exponential response at high doses. If a second dose is given after a suitable interval a further shoulder occurs, and the effect of a given dose is virtually diminished. This type of curve is called 'type C' by Gunter and Kohn,(1956) and curves of this general shape are often explained by multi-target or multi-hit theories.(Fowler, 1964; Oliver and Shepstone, 1964; Fowler, 1966).

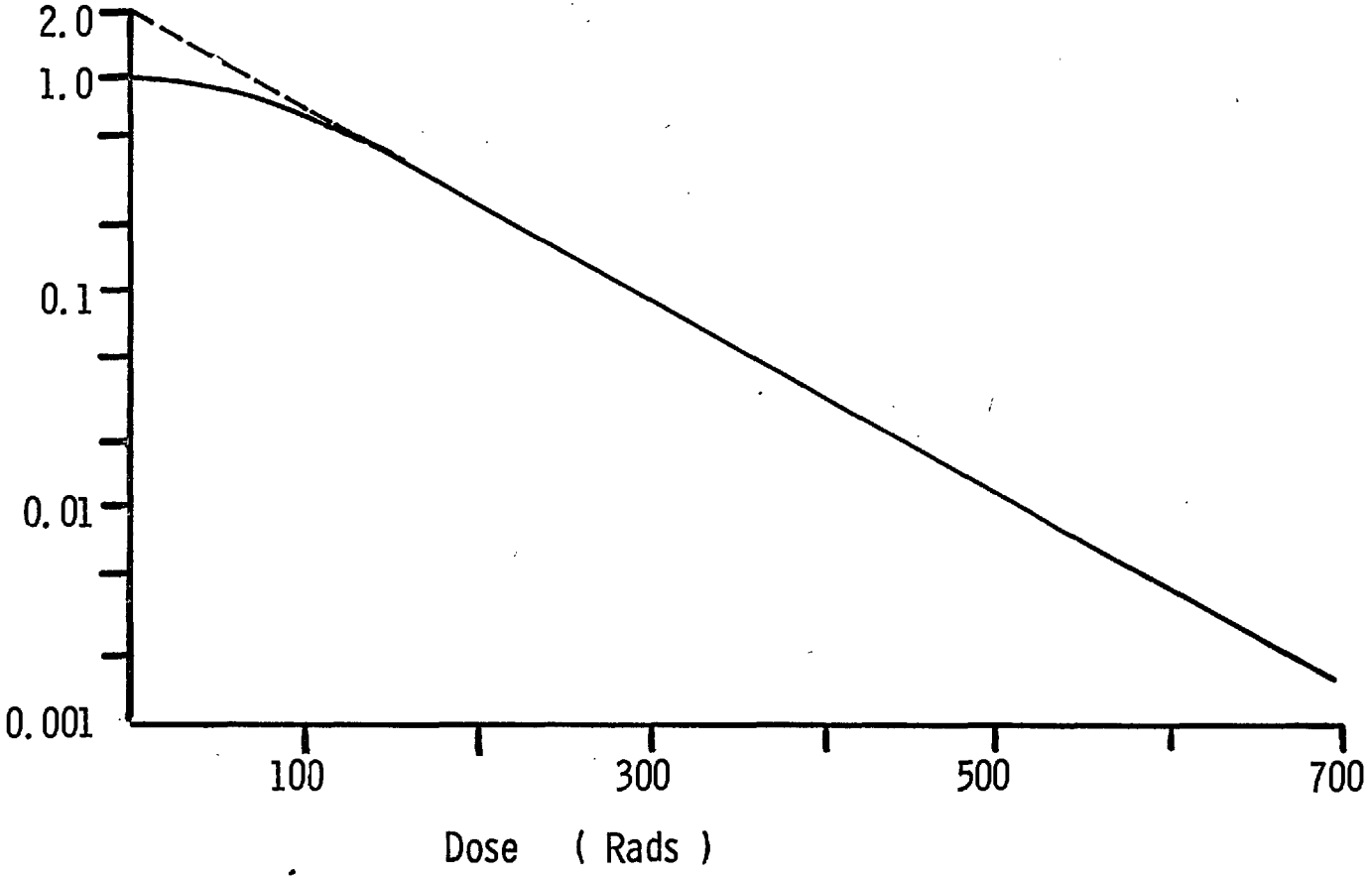


Fig.1-1 Survival of reproductive capacity in HeLa cell as a function of x-ray dose. The points fit the equation $S=1-(1-e^{-D/96})^2$. Puck and Marcus (1956)

According to multi-target theory there is a finite number of independent sites in a cell, the integrity of any one of which is sufficient to ensure the reproductive capacity of the cell. If there are n sites a cell is killed only when all n sites are inactivated. In the course of irradiation the inactivation of sites is random and the probability of survival of any given site will be exponential on the assumption that the sites have the same sensitivity the probability of the survival of the cell S, is given by:

$$S = \left\{1 - (1 - e^{-D/D_o})\right\}^n \qquad \ldots \text{(i)}$$

where D is the absorbed dose, D_o is the mean dose required to inactivate any given site and n is the total number of sites. Applying the binomial expansion to equation (i) we have:

$$S = ne^{-D/D_o} - \frac{n(n-1)}{2!} e^{-2D/D_o} \ldots \frac{(-1)^k \, n! \, e^{-kD/D_o}}{(n-k)! \, k!} \qquad \ldots \text{(ii)}$$

As the dose D is increased, all terms except the first term become negligible, so that for large doses the survival can be very closely approximated by:

$$S = ne^{-D/D_o} \qquad \ldots \text{(iii)}$$

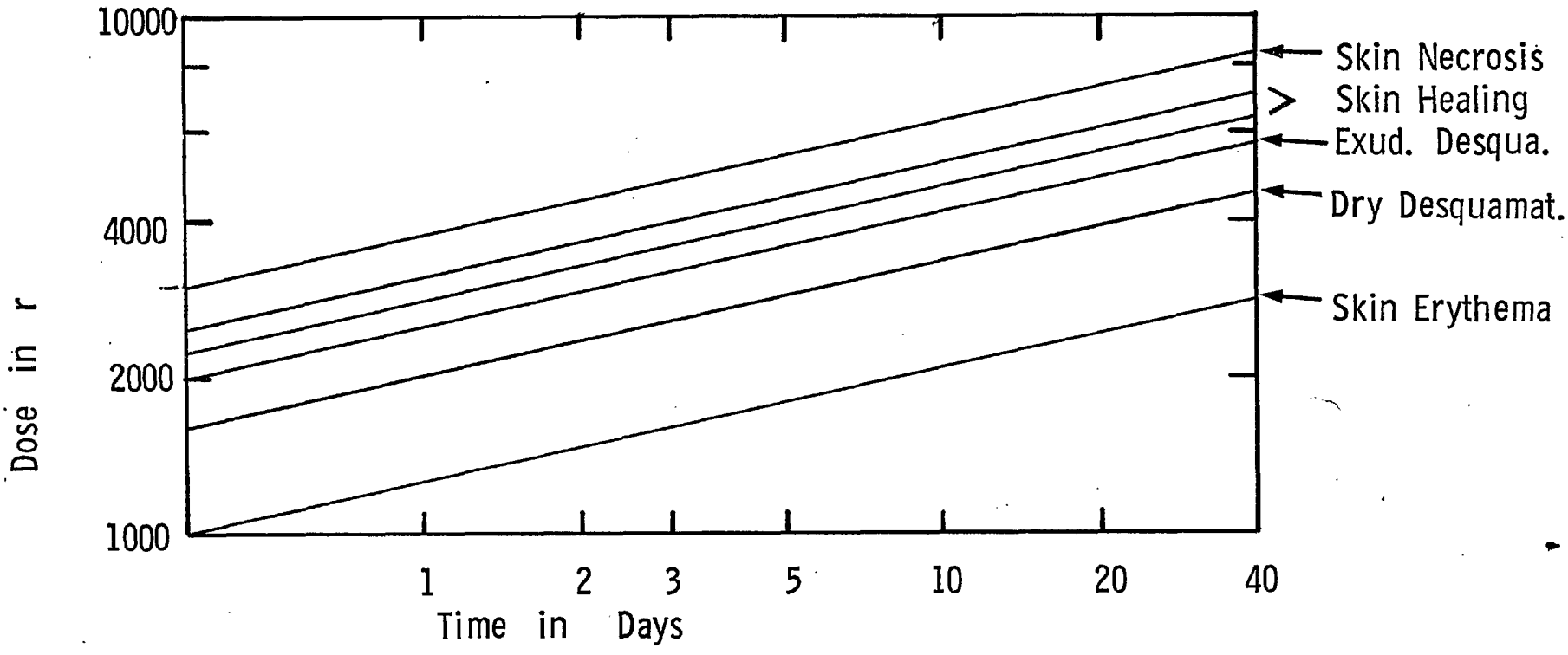
The exponential relationship at high dose results from the fact that virtually all the surviving sites need only one more hit to inactivate them. Extrapolation of the exponential curve (which plots as a straight line on semi-logarithmic co-ordinates) to zero dose gives an intercept on the survival axis at n. This means that for those survival curves which asymptomatically approach a straight line on a semi-logarithmic plot, D_o and n can be determined from

the terminal portion of the curve. D_0 values for various mammalian curves have been found to be between 100-200 rads and n is often between 2-10 for sparsely ionizing radiations. (Elkind and Sutton, 1960; Dewey, 1963; Oliver and Shepstone, 1964; Fowler, 1964; Sinclair, 1966; and Barendsen, 1967). Equation (iii) contains two parameters that could be referred to in describing the general characteristics of a survival curve; n, the 'hitness' number (Alper et. al., 1960) and D_0 which determines the final slope (Alper et. al., 1962). As an alternative to n one can specify a quasi-threshold dose, D_Q, at which the exponential part of the curves extrapolate to 100% survival

$$D_Q = D_0 \cdot \ln N \qquad \ldots (iv)$$

Dose response curves have been used to account for root growth inhibition of Vicia faba (Hall, Lajtha and Oliver, 1962; Hall, 1962; Shepstone and Oliver, 1963). The D_0 and n values obtained for this system are D_0 = 50-90 rads and n = 2-4. The fact that the slope is steeper than for mammalian cells is attributed to the larger DNA content in meristematic cells of Vicia. (Puck, 1956).

The initial shoulder of such a curve is characteristic of the requirement for accumulation of sub-lethal damage before cells become lethally affected. Evidence of this effect was demonstrated by Elkind and Sutton (1959). In their elegant experiments on Chinese hamster cells irradiated with split doses of X-rays the damage of the first dose was repaired within a period of 12 hours. Hall and Lajtha (1963) demonstrated similar type of recovery in Vicia at a temperature of 19°C and also at 3.5°C although more slowly at 3.5°C.



Fig; 1-2 Iso-effect curves relating total dose to overal treatment time for various skin reactions Strandquist (1944)

An early attempt to systematise the effect of fractionation is due to Strandquist (1944). He produced iso-effect curves(Fig1-2) from clinical data for various levels of skin damage. The data when plotted as logarithm of total dose to produce a given effect against logarithm of overall time give approximately straight lines. The overall time is correlated with the number of fractions, since all treatments were given as five fractions per week. The lines are parallel with a common slope of 0.22, implying an empirical function,

$$D/d = (T/t)^{0.22} \qquad \ldots (v)$$

where D and d are total doses required for T and t times respectively.

Equation (v) gives a numerical expression of the significance of fractionation of total dose. Strandquist expected this law to be applicable to various systems, the constants varying but the form of the equation remaining the same. Most of the clinical and biological data that appeared later have been surveyed by Fowler and Stern, (1960) and found to be in accordance with Strandquist. It has been well established that for a given tissue response, the total physical dose must be increased as the overall time is increased.

Steeper iso-effect curves showed that there was more increase in total dose with the increase of overall time. Fowler and Stern, (1963) plotted these lines as total dose against number of fractions rather than overall time and found the spread in the data was reduced. Fowler et. al., (1963) found that in experiments on pig skin the number of fractions was more important than the overall time in

determining the increase in dose. In these and clinical cases the number of fractions N and time T are related. Fowler and Stern, (1963) concluded that as long as the time between fractions is sufficient for recovery of sub-lethal damage (6-8 hours), time, as distinct from number of fractions has no influence on the total dose required unless it exceeds 10-12 cell cycles when presumably cell repopulation and tissue recovery begins to have an effect. In radiotherapeutic applications both N and T may be varied and the total dose required will vary accordingly. Ellis, (1968-1969) proposed a formula expressing the effectiveness of a total dose D after an N fractions over a time T days in terms of a 'nominal single dose' (NSD):

$$TD = (NSD). \quad N^{0.24}. \quad T^{0.11}. \qquad \ldots . \text{(vi)}$$

Clinical and experimental data shows that this law holds but only for a limited range of number of fractions. (Fowler, 1971; Ellis, 1971 and Liversage, 1971)

A pure multi-hit curve has a zero initial slope at low doses, however, Barendsen, (1962) has demonstrated a definite initial slope to the survival curve for human kidney cells irradiated with 250 kVp X-rays. Hall, (1963) has demonstrated that beyond a certain value further reduction of dose rate does not reduce the effectiveness of Co^{60} gamma rays in growth inhibition of bean roots of Vicia. This is consistent with a single event process operating at low doses and low dose rates such as is used in clinical work. Barendsen (1964) suggested that radiation induced damage to the reproductive capacity of the cells may be produced in two ways namely a 'single event' and by a multiple-event type of action. Single event type of damage becomes apparent at sufficiently small doses

or at a lower dose rate.

Oliver, (1964) proposed an equation for the dose response curve for Vicia faba bean roots growth inhibition for gamma irradiation which contains both single event and multiple

$$S = \exp(-D/190)\left[1 - \left\{1 - \exp(-D/70)\right\}^2\right] \quad \ldots \text{(vii)}$$

event components. The single event part, which is predominant at low doses, explains the initial slope of the X-ray survival curve. Therefore repair in this part of the survival curve would not be expected. At higher doses the second part of damage becomes apparent i.e., an appreciable part of the damage is produced by a cumulative type of action assumed to result from the passage of two or more electrons, with this type of action repair of sub-lethal damage might occur, leading to an increase in the dose required to produce a given cell survival if the dose is divided into two or more fractions with sufficient interval between them. If this interpretation of the survival curve is accepted we would expect that by fractionation of a dose of X, γ -rays or electrons into many small doses separated by sufficiently long intervals the cumulative type of damage produced would be negligible; only the single event part of the damage will be effective.

The slope of the survival curve after a given dose of radiation is a measure of the sensitivity of the cells to further radiation and it will be seen that in the shoulder region of the curve the sensitivity increases with the dose delivered. This effect can be explained on the assumption that the cells suffer sub-lethal damage (Elkind and Sutton, 1959) which by itself is

insufficient to kill the cells but which renders them more susceptible to subsequent irradiation. Elkind and Sutton (1960) presented an ideal experiment (Fig. 1 - 3) in which each dose D_1, D_2, D_3 and D_4 was delivered in a number of equal fractions of size D_1 with a time interval between the fractions for full recovery to take place. The shoulder of the survival curve is repeated with each fraction and if only single points were determined corresponding to equal dose increments the overall survival curve that would be observed is shown by broken line (curve C) and would appear to be of an exponential type.

In radiobiological studies an oxygen effect in various systems including bacteria, plants and mammalian cells has been demonstrated (Elkind and Whitmore, 1967; Hall and Cavanagh, 1967, 1969), and also an RBE of x-radiation as compared to gamma-rays and fast electrons has been demonstrated for various systems. (Hall, 1961; Sinclair, 1962; Sinclair and Kohn, 1964; Humphrey and Sinclair, 1963; Hendry, 1972; Malone, Porter and Hendry, 1974).

The initial slope on a survival curve is likely to be the single event type damage associated with the tails of secondary electron tracks where the LET is relatively high, overlapping that of α - particles. (Rossi, 1964). When only a small dose is given within the time for which sub-lethal damage persists i.e., irradiation is given in small fractions or at low dose rate, cell killing would result entirely from single event process, an increase would not be expected in total dose required to produce a given effect when the dose rate or dose per fraction is reduced.

Fig. 1-3

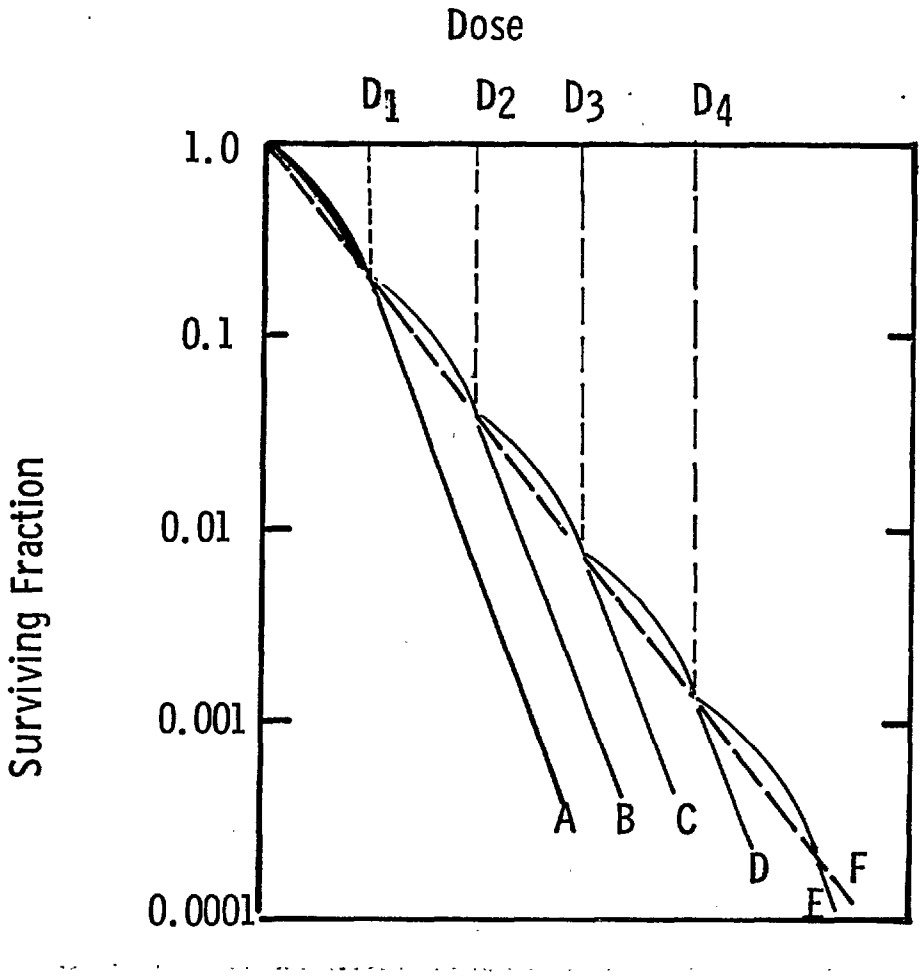


Fig: 1-3 An idealised experiment to demonstrate repeated recovery. Curve A is the survival curve for single dose x-rays. Curves B, C, D. and E the survival of cells which survive the total doses D_1, D_2, D_3 and D_4 repectively and which recover between dose fractions. Curve F would be observed if only single points corresponding to equal dose fractions were determined. (From Elkind and Whitmore 1967)

With radiations of lower energy, event sizes tend to be larger and the ratio of initial slope to final slope of the survival curve may be expected to increase. The biological effectiveness of X-rays relative to gamma-rays may then be expected to start with a value equal to the ratio of the initial slopes of the survival curves but when the shoulder is reached it decreases towards a value equal to the ratio of the final slopes.

The Co^{60} gamma-rays (Chapt.1A) should show an RBE determined for this initial region to be intermediate between the 250 kVp X-rays and 8 MeV electrons. To elucidate the exact shape of the dose response curve in the shoulder region and for the fractionated radiotherapy this is the most important question (Fowler, 1976).

The experiments presented in this thesis were made to study the factors affecting response at low dosesi.e., in the shoulder region of the dose response curve. This topic was also the subject of the 6th L.H. Gray Conference which took place during the period of this study.

1 - II USE OF BEAN ROOT SYSTEM.

It is difficult to obtain satisfactory and statistically reliable results at low doses. One possibility is to obtain an effect large enough to be measureable but related to low dose response by the use of multiple small fractions. In general the response in such circumstances is complicated by cell division taking place between fractions. (Elkind and Sinclair,1964).

However, with the Vicia faba bean root system in which

one observes the effect of radiation in retarding growth, irradiation of long durations can be studied. If the beans are kept at 3.5°C there is negligible cell division. Recovery of sub-lethal damage still takes place but more slowly than at normal temperature (Hall and Lajtha, 1963).

The bean root system is well suited to radiobiological studies in a physics department because of its simplicity, which allows large enough groups to be handled to gain statistically significant results. It, has moreover, been well studied (Read, 1959; Hall et. al., 1962) and yields information that has a useful similarity to that obtained with mammalian systems (Hall, 1963).

In this system cell survival cannot be measured directly, but the efficiency of different irradiation regimes can be compared in terms of the doses required to produce a given retardation of growth.

It was proposed to investigate effects of dose fractionation and to use these results to indicate dose response on shoulder of the survival curve by matching the observed response with those predicted from theoretical dose response curves involving single event and multiple event contribution as Oliver, (1964) has done for low dose rates.

1 - II,a <u>The bean root and radiation.</u>

The primary root of Vicia faba can be divided into four anatomical regions. (Read, 1959; Hall, Lajtha and Oliver, 1962).

Starting from the tip upwards:

1. The first ½ mm is known as root cap and appears to consist of relatively inert cells.

2. The meristem occupies the next 2 mm of the root and is composed of potentially dividing cells which in a normal control root have an average inter-mitotic interval of 25 - 30 hours at an ambient temperature of 19°C. (Howard and Pelc, 1953; Neary, Evans and Tomkinson, 1957). This interval varies with wide limits for individual cells in the meristem (Clowes and Hall, 1962). The existence of a 'quiescent centre' in the meristem consisting of some 1,000 cells has been demonstrated by Clowes (1959). The cells of the quiescent centre divide rarely if at all.

3. The next centimeter or so is called the 'elongating zone' because the cells make no more divisions in this zone but only differentiate and elongate. Cells from the dividing zone pass into the elongating zone and by their elongation add to the length of the root.

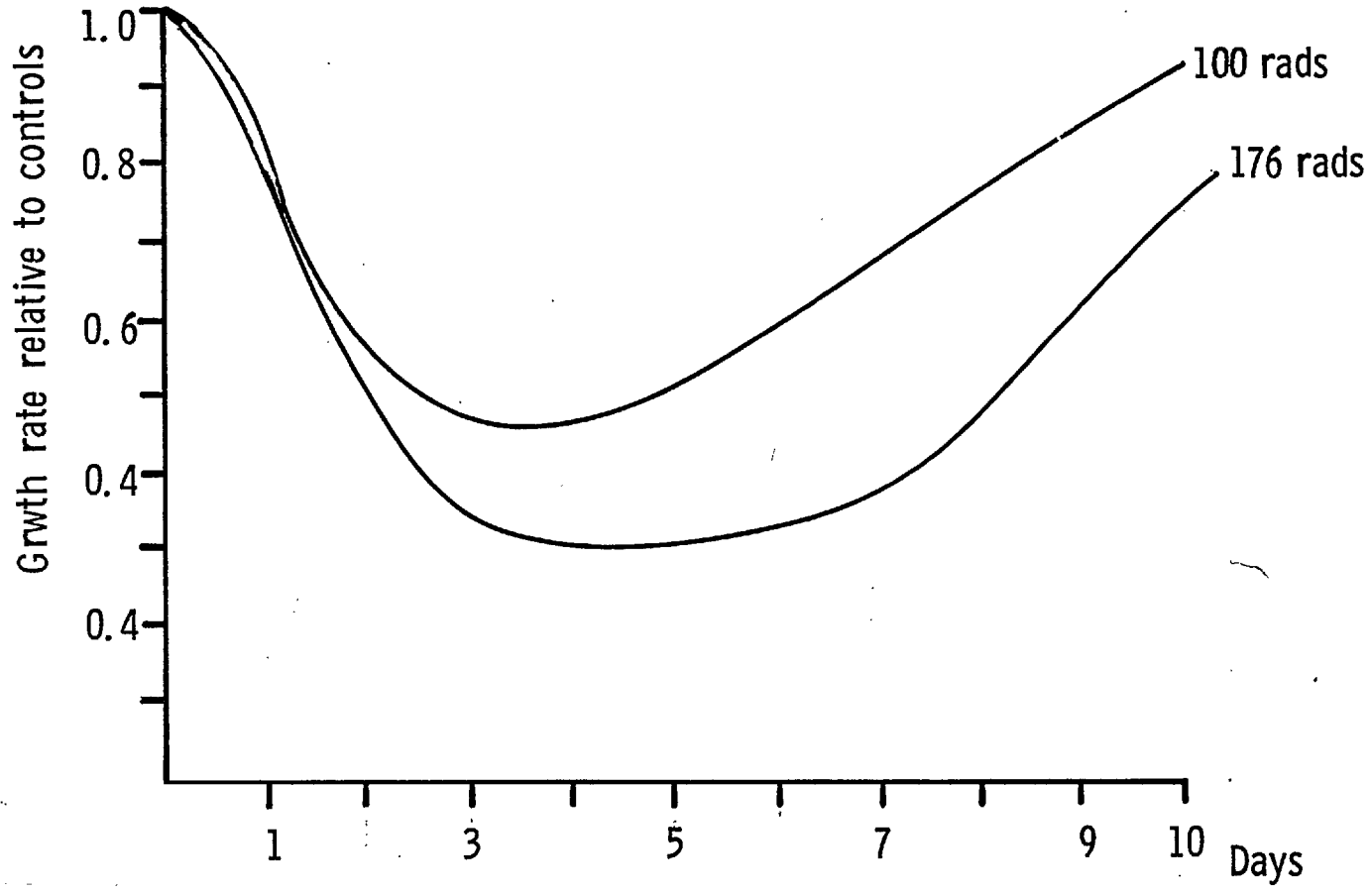
4. The remainder of the root consists of mature cells which are fully elongated.

In an un-irradiated control root the rate at which the cells are produced by divisions of the meristematic cell is balanced by the rate at which cells differentiate and elongate. In this way the number of cells in the meristem remains constant and a steady supply is available to differentiate, elongate and hence contribute to the root growth. When the root is irradiated with a single dose of ionizing radiation the growth is retarded or halted depending on the size of the dose. This effect might conceivably

result from cell sterilization, lengthening of the mitotic cycle, interference with the process of cell elongation or any combination of these factors. However, Hornsey (1956) has shown that three days after irradiation the mitotic cycle is the same as in un-irradiated roots, therefore the reduction in root length which occurs at times later than this cannot be regarded as due to the lengthening of the mitotic cycle. Further, root growth is virtually unaffected by irradiation of the elongation zone itself provided the meristem is shielded (Gray and Scholes, 1951). The observed reduction of growth which follows irradiation must therefore be attributed to the loss of reproductive integrity by a proportion of the dividing cells in the meristem, a fact also recognized many years ago by Lea (1955). Single doses up to about 250 rads cause only temporary inhibition of growth, to be followed by complete recovery in two to three weeks.

1 - II,b Dose Response curve for Vicia.

An explanation of this recovery pattern (Fig. 1-4) can be given by considering the kinetics of the meristem population. When the meristem is depopulated and reduced in size as a result of irradiation, the proportion of cells allowed to differentiate (and therefore the growth rate of the root) is reduced below the equilibrium value. Consequently, the rate of production of cells by division exceeds the rate of loss by differentiation, and the meristem repopulates with viable cells. As recovery progresses the meristem approaches its equilibrium size, Oliver (1964) has assumed that the proportion of meristem cells surviving a



Fig; 1-4 Growth rate curves after single doses of 250kV x-rays at $3.5^{0}C$

dose may be represented by:

$$S = \exp\left(-\frac{D}{D_1}\right)\left[1-\left\{1-\exp\left(-\frac{D}{D_n}\right)\right\}^2\right] \quad \ldots \text{(viii)}$$

where D_1 and D_n refer to the single and multi-event components of the sterilization process respectively. By applying mathematical models (Hall, Lajtha and Oliver, 1962; Hall, 1962; Shepstone and Oliver, 1963; Shepstone, 1963; Hall, 1963), Oliver (1964) deduced the following best values for the parameter; D_1 = 190 rads, D_n = 70 rads, n = 2 - 4. The values refer to irradiations with Co^{60} gamma-rays.

Lajtha and Oliver (1961) and Oliver (1964) gave a theory for the reduction of effect of lower dose rate in terms of an exponential recovery of sub-lethal damage proceeding with half life of 1.5 hours at 3.5°C and 0.7 hours at 19°C.

1 - III PROBLEMS INVESTIGATED IN THIS THESIS.

(i) Demonstration of the effect of dose fractionation at 3.5°C (i.e. with no cell division) and at 19°C (i.e. with cell division) for 250 kVp X-rays and Co^{60} gamma-rays.

(ii) Comparison of RBE of Co^{60} gamma radiation with that of 250 kVp X-rays for high and low dose levels i.e. on the exponential and the shoulder part of the dose response curve and therefore related to single and multiple-event processes respectively.

(iii) Comparison of RBE of X-radiation relative to 8MeV electrons at dose rate of 35 rads/min for both high and low dose levels as for Co^{60} gamma-radiation.

(iv) Comparison of response at high dose rate (i.e. 35 rads/min) to that at low dose rate (120 rads/min) for single doses of X-radiation and RBE of X-radiation relative to Co^{60} gamma-radiation at low dose rate ($\sim$120 rads/hr) [These responses were of clinical concern in the department because of interests of clinical trials of Pierquin technique of beam therapy at $\sim$ 100 rads/hr]. (Pierquin and Bellel, 1972)

(v) Measurement of the effect of radiosensitization on X-ray response at high and low dose levels (i.e. on the shoulder and the exponential parts of the dose response curve and related to single event and multiple event processes respectively.

(vi) Determination of parameters of these dose response curves for meristem cells consistant with theoretical models, and so demonstrating X-rays, Co^{60} gamma-rays, 8 MeV electrons and X-ray response in anoxic and anoxic + Ro - 07 - 0582. The effect of dose fraction under anoxic conditions with 0582 and without have been dealt with in Part II Chapter 5 of the thesis.

Chapter 1A.

A Different Introduction

Physical Considerations of the radiations employed in these studies.

CHAPTER 1A.

A DIFFERENT INTRODUCTION.

Ionizing radiations dissipate their energy through charged particle collisions resulting in excitations and ionizations. The energy of the charged particle traversing matter gradually decreases and at the end of its path the rate of loss of energy increases rapidly. Usually the energy transferred in a single event is only sufficient to produce an ion cluster of a few ion pairs. Occasionally the energy may be large enough to produce a separate track which is called a delta ray. The effects of radiation especially the ratio of effects produced by single events to those produced by the combined effects of several events may be expected to depend on some sort of average of the rate of energy loss along the charged particle track. Such an average is a specification of radiation quality. Lea (1955) produced tables for the primary ionization densities, stopping powers and the spectra of the secondary or delta tracks. Zirkle (1940) named the linear density of all forms of energy transfer including excitations and ionizations as linear energy transfer (LET). Various attempts have been made to describe LET more thoroughly to specify the radiation quality. (Gray, 1947; Cormack and Johns, 1952; Burch, 1957 a & b; Barendsen, 1968). The radiations considered in this thesis are 250 kVp X-ray, Co-60 gamma rays and 8MeV electrons.

An average LET for these radiations can be made in various ways, for instance the track average LET, LET_T, and the absorbed dose average LET, LET_D. The LET of X-rays, gamma rays and electrons used in this study range from the minimum

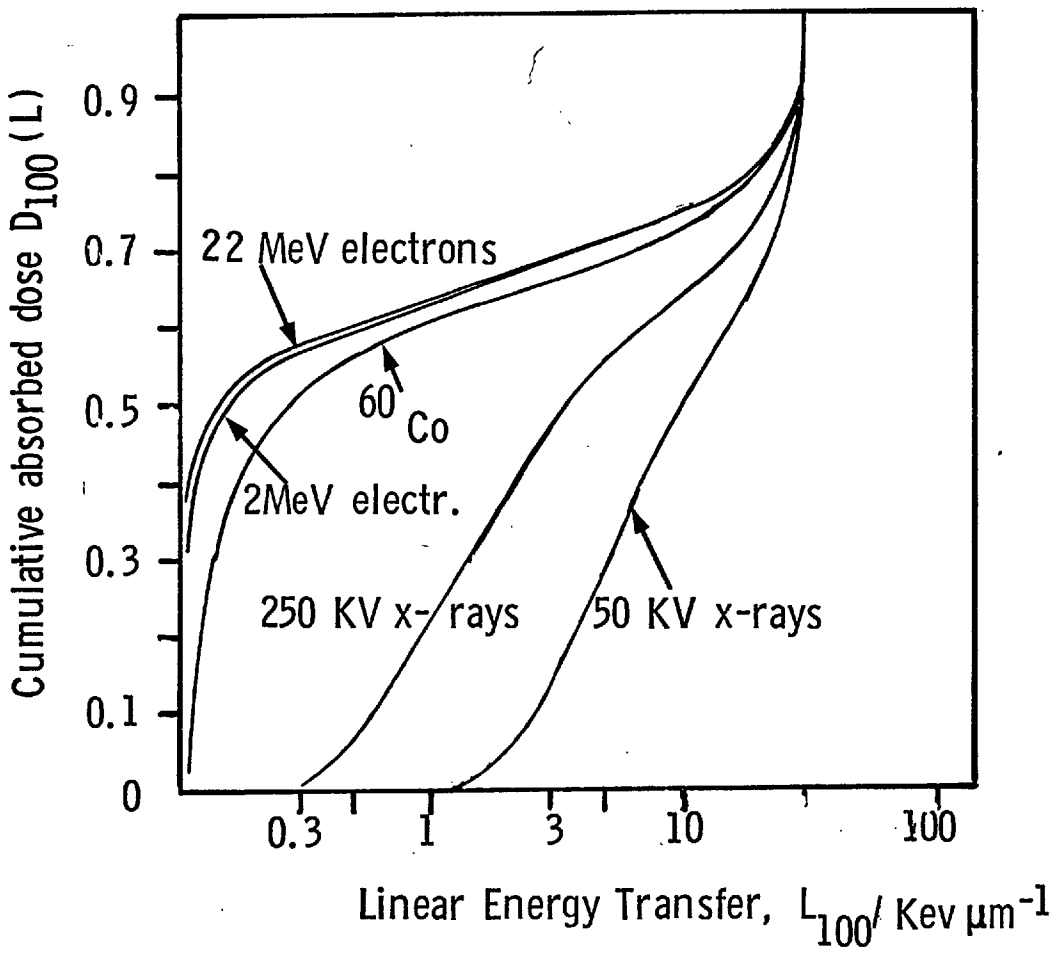


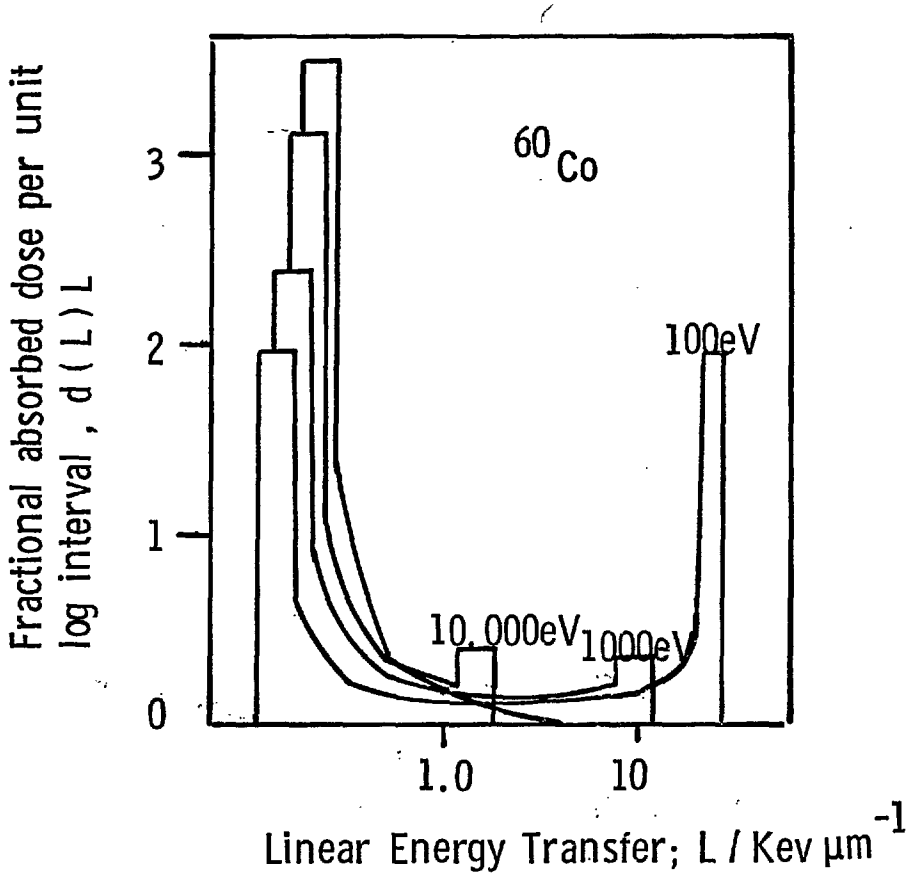
Fig: 1A-1 Cumulative absorbed dose distribution of LET in water for for various radiations for an energy cut-off of 100 eV

(ICRU , 1970)

of about 0.2 KeV/μm to a maximum at the end of the electron paths of about 50 KeV/μm (Cole, 1962; Barendsen, 1968). A better representation of the real physical situation is given by a 'two group model' in which the collisions are divided into two groups by an energy cut-off, Δ , above which the recoil electron is regarded as having a separate track. An LET_{100} (with cut-off energy Δ = 100 eV) would be an LET obtained when energy transfers of 100 eV or more are considered as separate tracks (Lea, 1952; Burch, 1957). The dose distribution of LET of these radiations can be calculated by means of a continuous slowing down approximation (csda), the charged particles are assumed to lose energy continuously along their tracks. The LET thus obtained is the LET (with Δ=100eV) LET's for 200 kV X-rays and Co^{60} gamma-rays and 2MeV electrons and 22 MeV X-rays are given in table 1A - 1.

It is expected that the 8MeV electron beam used in these investigations provide electrons of about 4MeV at the root tips due to absorption in 1.5 mm of perspex and water before they reach to the root tips. 4MeV electrons should lie on fig. 1A - 1 between the 2MeV electrons and 22MeV X-rays (for which the mean initial energy of secondary electrons is approximately 19MeV). The Co^{60} gamma-ray values in Fig. 1A - 2 illustrate the fact that LET depends strongly on the cut-off energy selected. Moreover the value is also greatly influenced by the type of average employed. Nevertheless it can be accepted that Co^{60} gamma-rays are intermediate in 'quality' between 250 kVp X-rays and 4MeV electrons.

In considering 'single hit' damage that would cause an



Fig; 1A-2 Distribution of absorbed dose in LET for water for electrons set in motion by Co-60 gamma rays (ICRU, 1970)

initial slope on a survival curve, it may be important to know not an average LET, but the amount of dose or track length delivered with LET greater than some critical value. This point of view is more readily expressed by the microdosimetric approach of Rossi (1964) in which he describes the distribution of energy deposition in small volumes. Here again it is not possible to ascribe definite values to the various radiations employed without knowledge of the appropriate critical volumes, [Fig. 1A - 3] however, which would not change excessively with a reasonable change of critical volumes, justifies the assumption that Co^{60} gamma-rays are intermediate between the X-rays and the fast electrons. Moreover, the mean specific energy produced by individual charged particles is about twice as large for X-rays as for gamma-rays. (Kellerer and Rossi, 1972). Also it is interesting to note that Rossi's values show that there is a large overlap between the event size distribution for Co^{60} gamma-rays and α - particles, whose effect is almost entirely of single event type.

Fig: 1A-3

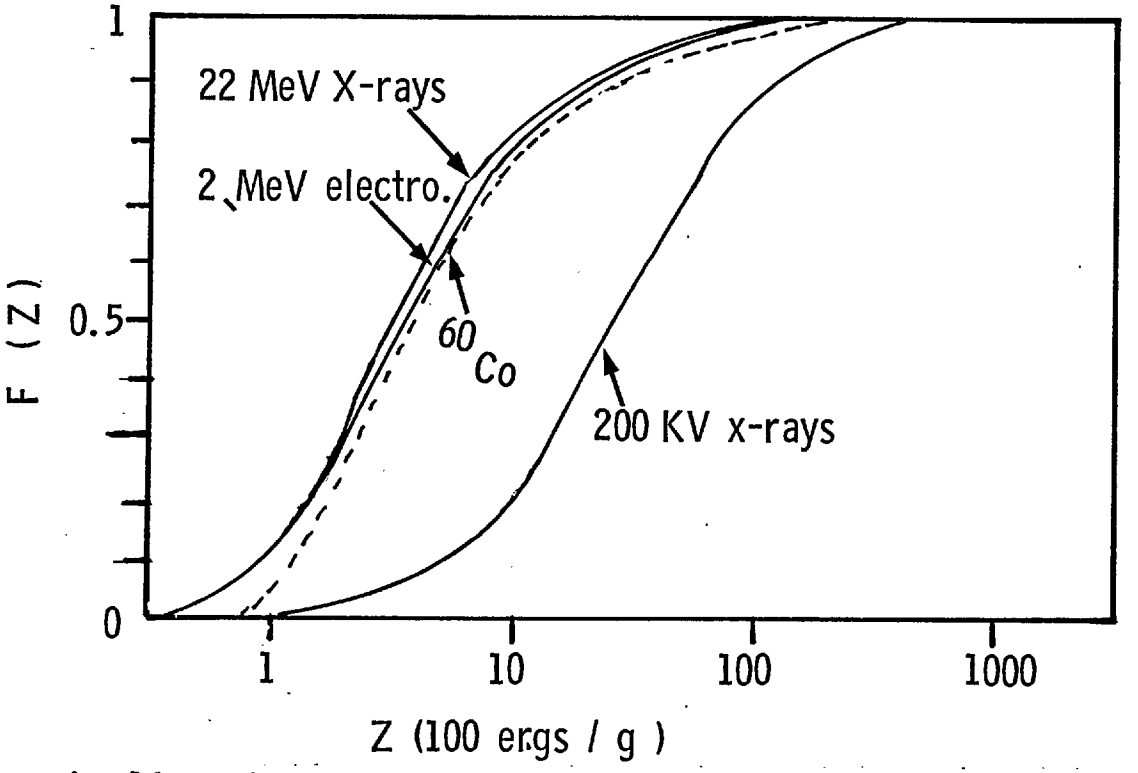


Fig: 1A-3 Some absorpion spectra (Z) plotted as a function of "distribution of the local" energy density Z by a "single event absorption" in a spherical tissue of 1 µm diameter volume (From: Kellerer, 1966)

TABLE NO. 1A - I.

Track-average and Absorbed dose-average values of LET in water irradiated with various radiations. (ICRU,1970).

Radiation	Cut Off Energy eV	LET ,T KeV/mm	LET ,D KeV/mm
200Kv X-rays	100	1.7	9.4
Co^{60} γ-rays	100	0.22	6.9
	1,000	0.23_0	2.8
	10,000	0.23_2	0.48
		0.23_9	0.31
2MeV Electrons	100	0.20	6.1

Chapter 2.

Materials and Methods.

CHAPTER 2.

MATERIALS AND METHODS.

For most of the experiments Sutton's "prolific" long pod broad beans were used. For the last few experiments at low dose rate Aquadulce broad beans, also obtained from Sutton's were used as the prolific long pod variety became unavailable. The methods of culture followed closely those used by Hall (1961).

2.I. GROWTH OF BEANS:

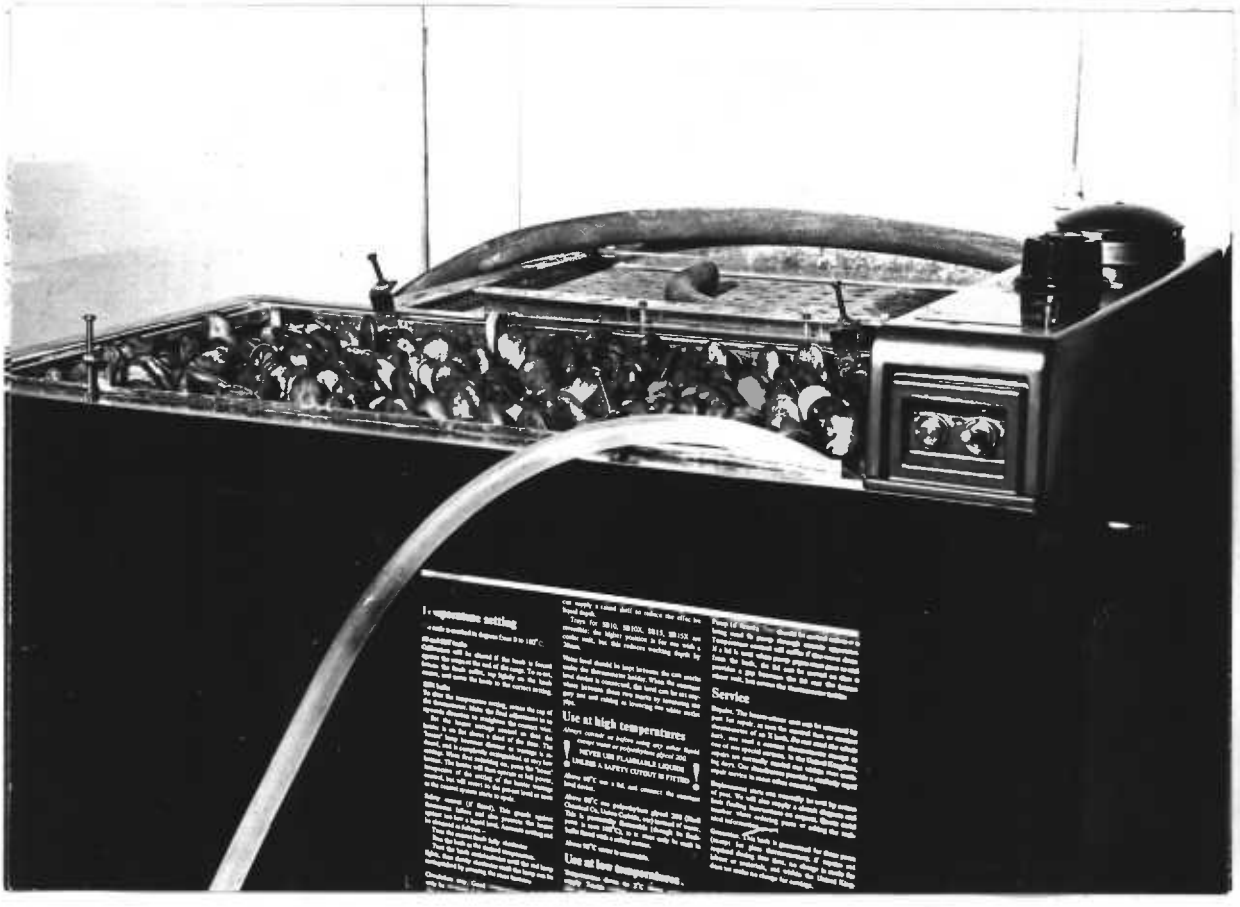
2.I.(a) GERMINATION AND SELECTION:

For each experiment, depending on the individual need, approximately 200-300 beans were soaked in a plastic garden tray (Fig.2-1) under constant water flow from the outlet of a growth tank maintained at 19°C ± 0.2°C. The approximate rate of flow of fresh tap water into the growth tank and hence in the soaking tray was about two litres per minute. After three days the beans that had germinated, as seen by the appearance of a radicle, were planted in moist vermiculite contained in an aluminium tub, 61 X 36cm and 26cm deep. The vermiculite was reused for 5-6 times and then replaced by fresh material. During one period difficulties were experienced with the beans becoming infected with fungus. However, this was overcome by careful steam sterilization of the vermiculite supply and the tub used for planting. The tub was covered with a plastic sheet to maintain culture temperature and the beans left for four days, by which time the majority had developed a primary root 4 to 8cm long. Beans were then removed from the vermiculite washed in water, and testa and

Figure 2-1

Photograph of seeds soaked in running tap water.

Figure 2-2



Photograph of a growth (culture of beans at $19^{0}C$) tank.

plumule removed. Ones shorter than 4cm or longer than 8cm, noticeably thickish or showing signs of severe fungus infection were discarded. The beans with only minor fungus infection were found to grow at the same rate as fully healthy roots. The development of fungus infection was further inhibited by skinning the beans, and in later experiments all the beans were skinned whenever a majority showed such symptoms. All acceptable beans were then transferred to the culture tank.

2.I.(b) GROWTH AT 19°C.

The beans were placed on the aluminium or perspex lid of a growth tank, with their roots passing through holes into the water. The growth tank was rectangular in shape 64 X 34cm and 35cm deep, with a steady flow of tap water passing through. This is shown in a photograph in Fig.2-2. A vigorous stirrer and thermostatically controlled heater (Grant Ltd.) kept the temperature of the tank steady at 19°C ± 0.2°C and also effectively aerated the water. The bean roots were left to grow for 24 hours, before being marked or measured. Next day indelible pencil was used to mark each of the beans with a number on one of the cotyledons, and a reference point on the hypocotyle. Each bean was placed on a wooden cm scale and the root stretched gently down the scale. The length from the reference point to the growing tip was measured and recorded. The bean roots were remeasured after further 24 hours and the growth increment for each bean calculated. Providing the roots were handled carefully such measurements have no harmful effect on the beans even if performed daily (Read 1959). Bean roots with increments much larger or

smaller than average were discarded and the remainder were randomised according to length into 7 or 9 groups, depending on the individual requirement of the experiment. In the earlier experiments 10 to 12 beans were taken in a group. In later experiments 12 to 15 beans were used to provide better statistics and allow for the possibility of one or two roots being broken during an experiment. One group served as control and the remainder were allocated for irradiation according to the plan of the experiment.

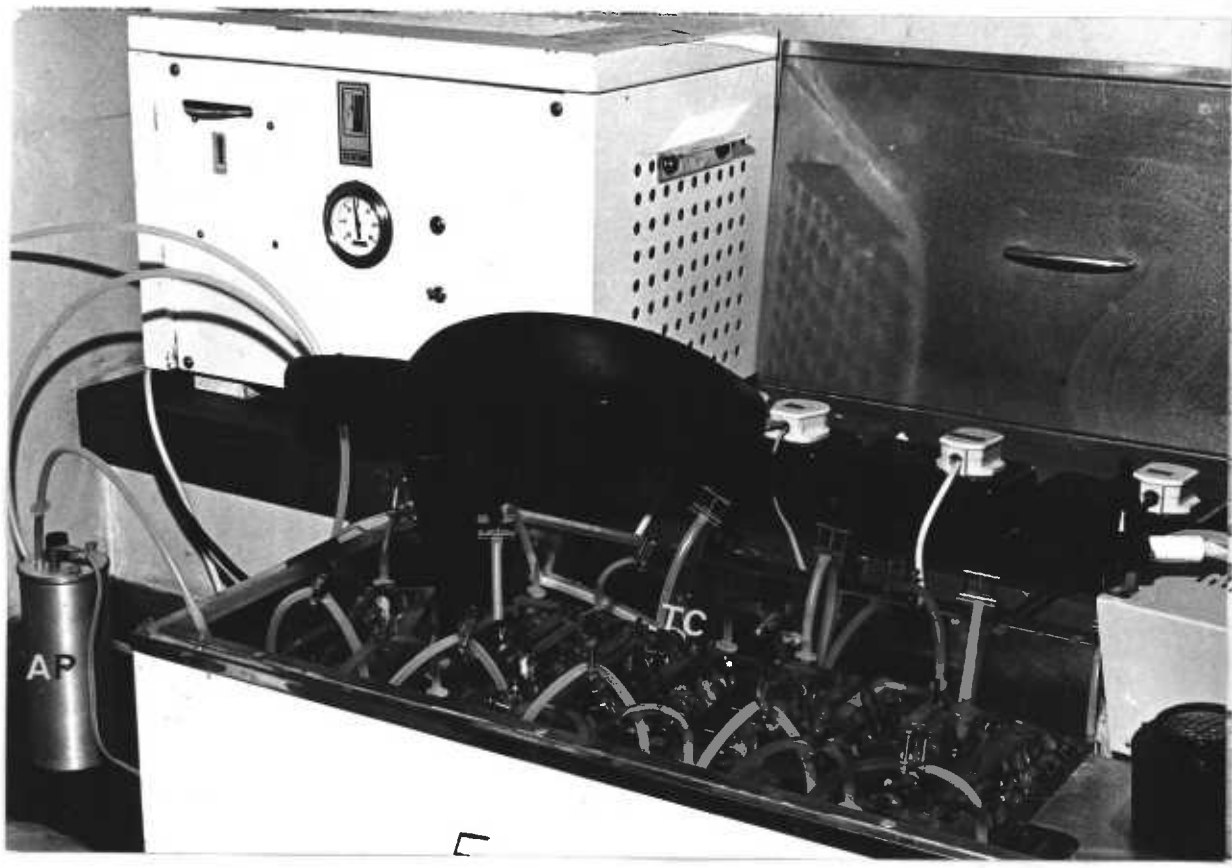
2.I.(c) STORAGE DURING PROTRACTED IRRADIATION at 3.5°C.

For fractionated X, gamma or electron treatments at 3.5°C the beans, in between individual exposures, were stored in a cold water bath. They were placed in special perspex jigs which allowed water and air to be circulated through the compartment containing the roots (Fig.2-3). The thickness of the root compartment of this jig was 3mm. Each group of beans was placed in a separately marked jig and immersed in water in the cold water bath. This was made of stainless steel 83 X 73cm and 30cm deep and was surrounded with expanded polystyrene thermal insulation to assist in the maintenance of the low temperature (Fig.2-4). The temperature was maintained with a Statim Unit, consisting of a refrigeration coil placed under the false bottom of the tank in combination with a heater/stirrer unit (Grant Ltd). The refrigeration was left on all the time and temperature maintained by the heater. A water pump was fitted to circulate the cold water through the jigs by means of polystyrene tubes. Similarly two aquarium pumps were used to circulate air through the jigs, the air being cooled by submerging the air tubes

Figure 2-3

Photograph of a bean jig showing the position of the root tips at the time of irradiations.

Figure 2-4



Photograph of the cold water bath for bean storage between fractionation period.
Abreviations :

AP Air pump
TC Tube connections for air and water circulation

in the cold water bath. To avoid any sudden shock of change in temperature, the jigs were placed in the bath with the water at 19°C temperature and then the refrigerator was switched on. Thus the beans were cooled over a period of a few hours. Similarly at end of a period of storage at 3.5°C the refrigerator was switched off and the beans allowed to warm up slowly before being restored in the growth tank at 19°C.

During transfer to the cold irradiation tank in the irradiation room the jig was placed in a plastic carrier insulated with expanded polystyrene blocks. At end of irradiation it was brought back into the cold water bath and the air and water circulations reconnected. This procedure was repeated for each dose level and the control group of beans was similarly sham irradiated.

The maximum period of storage of beans in the cold water bath was 14 days, but all beans, even for single dose experiments, were kept at 3.5°C for at least 7 days. This is to allow 24 hours before and at the end of irradiation regimes and also in an attempt to equalise the cold storage effect in various fractionation regimes.

2.I.(d) STORAGE DURING PROTRACTED IRRADIATION at 19°C.

For fractionated and single dose experiments at 19°C the same jig was used (Fig.2-3) for the irradiations. The group of beans allocated to certain dose level was transferred into the jig and carried to the irradiation room. The irradiation tank was maintained at 19°C and air was circulated through the bean jig during irradiation. At the

end of each irradiation the beans were transferred back into the growth tank. Similar procedure was repeated for each of the various dose level groups in an experiment and the control group was sham irradiated. The bean roots were measured before each exposure and at the end of last irradiation.

2.I.(e) STORAGE DURING LOW DOSE RATE IRRADIATION.

In order to allow an adequate rate of recovery of sub-lethal damage (Oliver, 1964) low dose rate exposures were carried out only with the beans at 19°C during irradiations. The beans were kept and irradiated at 19°C. They were measured before being placed in the irradiation jig and at the end of all the different dose level exposures. After which they were left in the growth tank for ten days.

2.II. IRRADIATION FACILITIES AND PROCEDURES:

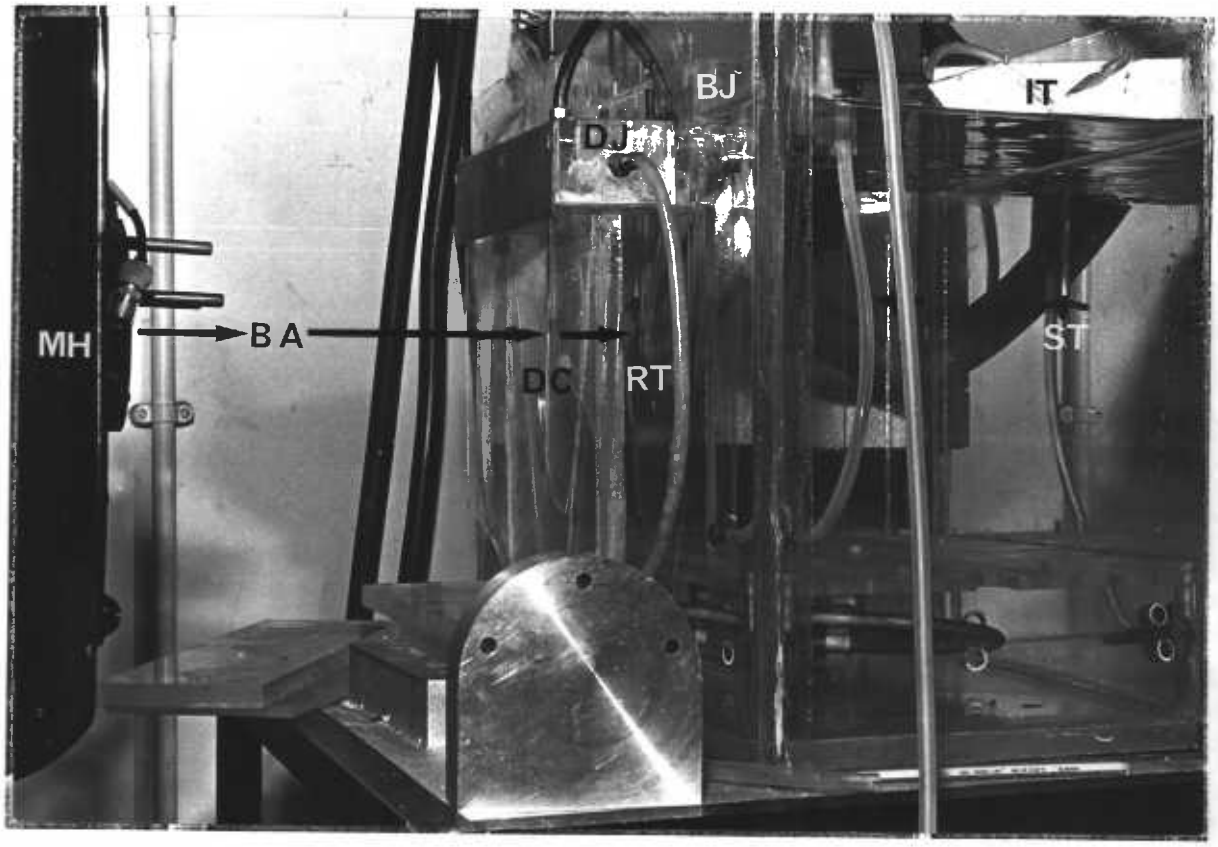
2.II.A. X-IRRADIATION:

2.II.A.(i) Irradiation at high dose rate:

A Maximar Radiotherapy Unit installed in the department of Medical Physics for experimental work was used for X-irradiations. It was operated at 250 kVp and 15 mA. Added filters of 0.5 mm Cu and 1 mm Al resulted in a beam of radiation with HVL of 1.4 mm in Cu. The radiation beam was directed horizontally into the side wall of the irradiation tank, the outer wall of which was at 50 cm focal distance and the surface dose rate was about 62 rad/min. in water.

The water filled, perspex irradiation tank had dimensions at 25.5 X 46.5 cm and 34 cm deep and was fitted with a refrigeration unit and a thermostatically controlled

Figure: 2-5



Photograph of the experimental set up for 250 KV x-irradtiatins.
Abreviations:

MH Maximar head
BA Beam alliance
DC Dosimeter chamber
RT Root tips
DJ Dosimeter jig
BJ Beans jig
IT Irradiation tank
ST Stirrer

heater/stirrer. For irradiations at 3.5°C the refrigeration was kept on all the time and the temperature maintained by the heater, an aquarium pump being used to bubble air through the jig during irradiations the arrangement is shown in Fig.2-5. For irradiations at 19°C the temperature was maintained by thermostatic control.

During irradiation the jig was placed flush to the side wall of the tank facing the beam the root tips were thus at a depth of 3.4 cm from the tank surface. They were brought in the centre of the jig and their alignment along with the centre of the beam was carefully maintained. (See Fig.2-5).

2.II.A.(ii) Irradiation at low dose rate:

To obtain the required low dose rate the Maximar set was operated at 210 KVp 4.5 mA with an additional 3.4 cm Al filter (held in the beam), thus providing a dose rate about 3.8 rads/min at the tank wall and that at root tips being 2.08 rads/min. The HVL was 1.5 mm of Cu at 50 cm FSD. The same irradiation tank was used as described in Section (2.II.A.(i)).

The jig was similar to that used at high dose rate but with a larger (longer) compartment sufficient to hold some 60 beans at a time. All the beans randomised between the different exposure groups were placed in the jig simultaneously with the shorter roots centred and the others arranged to lie within 2 cm of the centre of the field. This jig was placed flush to the irradiation tank wall facing the beam, the root tips still being at a depth of 3.4 cm from the tank surface. As each pre-selected total dose level was reached, the appropriate group was removed and these beans transferred

to the growth tank. In this way the total time needed for the experiment was much reduced. The control group was sham irradiated for about the equivalent time taken by a middle dose level group.

2.II.A.(iii) DOSIMETRY.

HIGH DOSE RATE DOSIMETRY:

Exposure was controlled by the timer or beam monitor, but the actual dose delivered in each fraction was measured with a Baldwin Farmer dose meter compared against a standard instrument held at the hospital which has been previously calibrated at National Physical Laboratory. The chamber of the dose meter was held in a perspex jig similar to that used for beans but with a perspex spacer replacing the water (Fig.2-5). This was placed outside the irradiation tank in the centre of the beam immediately against the tank wall. In order to measure the dose rate at the position of the root tips, the water filled bean jig and the chamber jig were interchanged. The dose rate inside the irradiation tank at the position of the root tips thus measured was 34 rads/min in water. Hence the ratio of the doses monitored to the actual dose received by the root tips was 1.84. These doses were derived for the instrument correction factor of 1.013, a roentgen to rad conversion factor of 0.95 and for appropriate corrections for temperature and pressure.

LOW DOSE RATE DOSIMETRY:

The dosimetry was similar to that at high dose rate (see above section). The ratio of the doses monitored in this case to actual dose received by the root tips was 1.82.

The area occupied by the root tips ~4 cm^2 was adequately covered by the X-ray beam (dimensions of about 10 X 12 cm) as seen by exposing an X-ray film at the position of the root tips. The variations of dose at ± 5 cm across the beam from the centre was less than 5%.

2.II.B. GAMMA IRRADIATION:

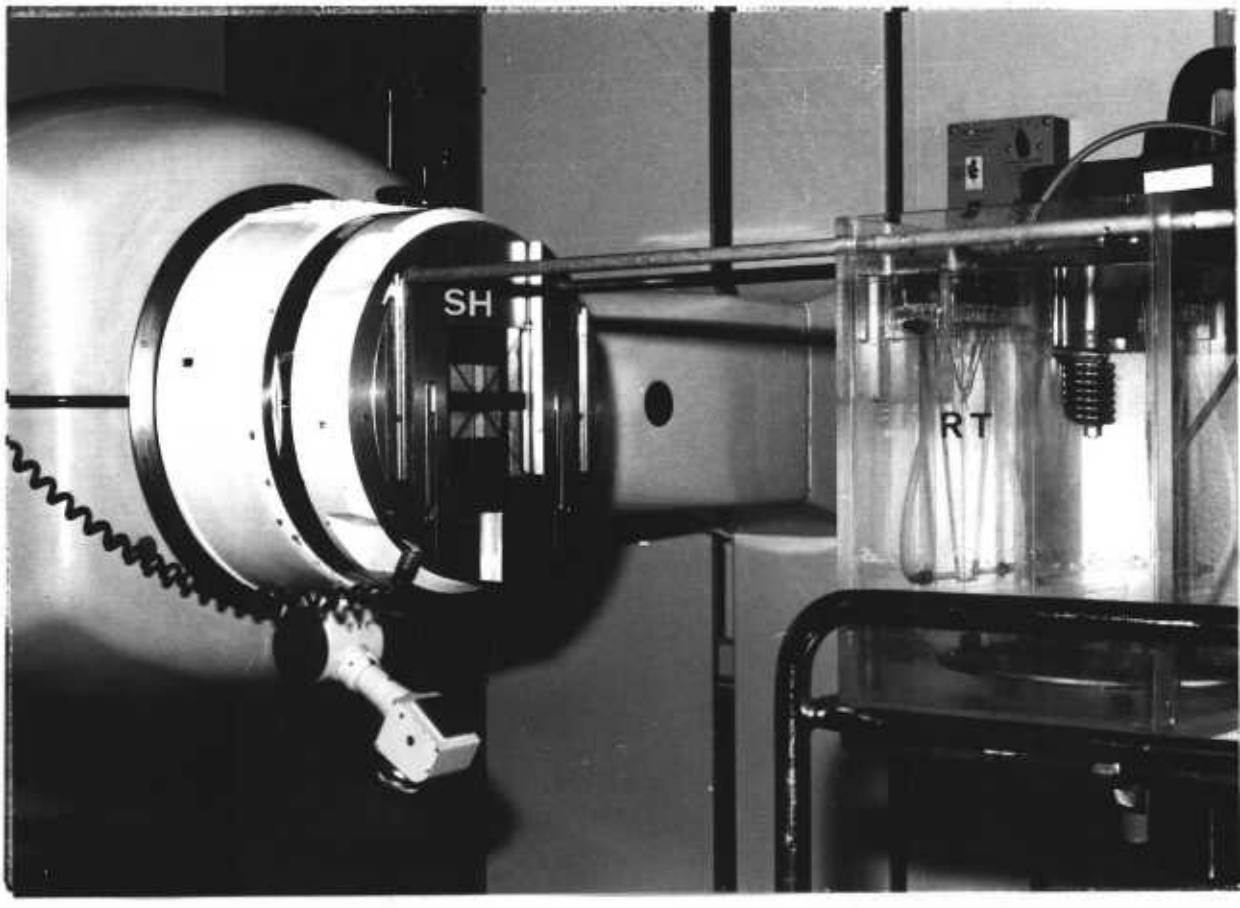
2.II.B.(i) HIGH DOSE RATE GAMMA IRRADIATION:

A TEM Stabilitron Co-60 unit in the department of Radiotherapy was used for gamma irradiations. The field size used was 2.5 cm X 8 cm at 90 cm Source Distance. A horizontal beam was used, the dose rate being about 34 rads/min in water at the position of the root tips. The irradiation tank was similar to that used for X-irradiation (See Section 2-11. A(i)). Two pointers were fixed on the irradiation tank to check that it was at the same geometrical distance from the source whenever the trolley carrying it was brought in for various irradiation procedures. (Fig.2-6). Bean jig to be irradiated either at 3.5°C or at 19°C was placed flush to the wall of the irradiation tank facing the beam. In this case the alignment of the mean area of about 2 cm radius occupied by the root tips was checked with a light beam.

2.II.B.(ii) LOW DOSE RATE GAMMA IRRADIATION:

To obtain the low dose rate, a 4 cm lead filter secured in the applicator was held in the beam. A dose rate of 2.07 rad per minute in water at the root tips position was obtained and otherwise the irradiation procedure and jig was similar to those used for low dose rate X-irradiation.

Figure 2-6



Photograph of the experimental set for ^{60}Co gamma-irradiations from stabilatron.

Abreviations:

SH Stabilatron head
RT Root tips

2.II.B.(iii) DOSIMETRY OF GAMMA IRRADIATION:

The exposures were monitored by a timer. The dose rate was monitored once at the start of the experimental regime using the same Baldwin Farmer Chamber, placed in the position of the root tips at the centre of the beam. In the later experiments the chamber measurements were done only once a month in order to check consistency in the experimental set up and allow for physical decay of the source. The roentgen to rad conversion factor used was 0.97. Dosimetry for low dose rate was the same as that in the case of high dose rate.

2.II.C. ELECTRON IRRADIATIONS:

8MeV electrons from MRC linear accelerator in a horizontal beam were used. The irradiation tank was flush to the 8 cm aluminium applicator with a field size of 5 cm X 5 cm. The doses were measured once by Baldwin Farmer Chamber placed at the position of the bean roots and a computed calibration factor obtained was 2.375 rads per division of the main dosimeter on the linear accelerator consol. The dose rate used was about 34 rads/min. Individual exposures were monitored by readings on the consol counter. The bean root tips were at a depth of ~ 1.5 cm from the surface of the tank.

2.III. CHOICE OF DOSES AND FRACTIONATION PROCEDURES:

2.III.A. HIGH DOSE RATE:

Oliver (1964) deduced a dose response curve for Vicia faba meristematic cells based on various mathematical

and experimental results for root growth inhibition following irradiations. (Hall, Lajtha and Oliver, 1962; Hall, 1962; Hall, 1963; Shepstone and Oliver, 1963; Shepstone, 1963; Hall and Bedford, 1964). This model consisted of two components of radiation interaction i.e., 'single event' and 'multiple event' types.

$$S = \exp\ (-D/190)\ \left[1 - \left\{1 - \exp\ (-D/70)\right\}^{2}\right] \quad \ldots (1X)$$

where S is survival, D_1 = 190 rads; the 37% dose slope for single event, D_n = 70 rads; the 37% dose slope for multiple event type of interaction and n the extrapolation number = 2. These cell parameters for meristematic cells of Vicia faba roots were the most suitably available for predicting various iso-effect doses of gamma radiation to produce a matched effect in terms of root growth inhibitions. Assuming that the shape of the survival curve is unaffected by the previous dose fractions and hence repeats exactly with each fraction (provided the interval between fractions is sufficient for sub-lethal damage recovery), the dose required in N fractions of d rads to be equivalent to single dose D rads is given by:

$$\exp(-D/190)\left[1 - \left\{1 - \exp(-D/70)\right\}^{2}\right] = \exp(-d/190)\left[1 - \left\{1 - \exp(-d/70)\right\}^{2}\right]^{N} \quad \ldots (X)$$

Various fractionated doses were computed to produce the same G_{10} as a single dose of 100, 150, 200 or 250 rads from the above equation. This range of doses provides a straight line on a linear log graph with log dose against

G_{10} values on a linear scale. (Neary, 1957; Hall et.al., 1962; Hall, 1963). Various fractionation regimes of 1, 3, 6, 9 and 12 fractions were used such that the lowest doses of 17 rads/fraction in multi-fraction regime and the highest single doses (250 rad) spanned the whole range of the dose response curve from shoulder to the final exponential regions.

For gamma ray and electron irradiation doses used were 15% greater than for x-rays due to an RBE of 0.85. (Hall, 1961 ; Sinclair and Kohn, 1964).

The fractionation at 19°C was performed by giving similar doses in various regimes to those used at 3.5°C. In the case of fractionation at 19°C the maximum possible number of fractions was six. The reason being for a larger number of fractions the roots grow too long and break easily. This also causes spread in the experimental data. Therefore the experiments at 19°C were carried out only for 1, 3 and 6 fractions.

Fractions were spaced 24 hours apart to allow sufficient time for recovery from sub-lethal damage at both 3.5°C and 19°C. It has been shown that although there is no cell division going on when roots are maintained and irradiated at 3.5°C, recovery of sub-lethal damage still takes place after acute doses but more slowly than at 19°C. (Hall and Lajtha, 1963). The half life of decay of the dose equivalent of the sub-lethal damage is about 2 hours at 3.5°C, and 0.7 hours at 19°C. (Oliver, 1964). Therefore by allowing 24 hours gives roots sufficient time to recover from sub-lethal damage when at a lower temperature of 3.5°C.

2.III.B. LOW DOSE RATE:

A protracted dose response curve was computed utilizing the equation; (Oliver, 1964).

$$D_E = d/\mu \; 1 - \exp(-\mu t) \qquad \ldots (X1)$$

where D_E is the dose equivalent of sub-lethal damage, μ a constant, d is the dose in rads/hr and t the time. The dose response curve calculated for 120 rads/hr was then compared to acute dose response curve computed from equation (i) to select the equivalent doses for low dose rate irradiation regime. The doses used for Co-60 gamma irradiation exposures were 15% greater than those for x-rays taking into account the RBE difference between the two.

RBE of high dose rate X-irradiation against low dose rate X-irradiation was measured for single doses. Also RBE of X and gamma irradiations was measured at low dose rate for single doses.

2.IV. 10 day Growth at 19°C. (G_{10})

To assess radiation damage, the ten day growth parameter (G_{10}) was used. (Read, 1959; Neary, 1959; Hall, Lajtha and Oliver, 1962; Oliver, 1964). At the end of last exposure the bean roots were kept in the growth tank at 19°C ± 0.2°C along with the corresponding control group for ten days to grow. All the beans were measured at the beginning of the last exposure when they were irradiated at 19°C or at the time they were restored into the growth tank when they were irradiated at 3.5°C. No beans were restored to the growth

tank before a period of 24 hours after the end of the last irradiation exposure at 3.5°C. A 'Grant Unit' consisting of a thermostatically controlled heater and a vigorous stirrer kept the temperature constant throughout the growth tank during the ten day period of growth. The constant flow of tap water through the growth tank and the stirrer effectively kept the water aerated during this whole period. Gray and Scholes (1951) and Neary (1957) reported that a change of 1°C resulted in 20 per cent change in the growth of the roots; therefore the temperature of the tank was frequently checked to maintain at 19°C ± 0.2°C. Also for each individual experiment all the groups of beans were randomised within the same growth tank so that all groups were equally affected by any variations of tank temperature.

Each of the bean roots was taken out every day for a period of about 15 seconds to remove the side roots and shoots. The removal of the side roots enhanced the growth of roots lengthwise (Hall, 1961). And the removal of shoots and their prevention from development made the regulation of light and dark condition unimportant. (Shepstone, 1964). The beans were remeasured on the tenth day and the average growth increment in this period was calculated for various groups including the control. The radiation damage is assessed by expressing the ten day growth increment of the irradiated group as a fraction of the ten day growth increment of the corresponding control group.

During this ten day growth period at 19°C roots in the lowest dose level group did not show any marked change in appearance, but the severity of change in appearance

i.e. they become discoloured, brownish and brownish red, increases towards higher dose level group. In the highest dose level group on a few occasions it has been noticed that one or two roots turn brownish black. Such beans have been regarded as dead and were discarded from the experimental data.

DAILY MEASUREMENTS.

In some experiments daily measurements were made to confirm the pattern of recovery at various dose levels in more detail. Also a similar comparison was made between the recovery pattern when the irradiation was given either at 19°C or at 3.5°C. These results were also used to deduce a dose response curve for meristematic cells through the mathematical model of Hall, Lajtha and Oliver (1962).

Chapter 3.

Results.

Chapter 3.

RESULTS.

The ten day growth of irradiated group expressed as a fraction of the control group (G_{10}) for a given dose level determined for various irradiation regimes have been given in tables 3-I to 3-XV on pages 83-98 for all the experiments. Irradiations at high dose rate of 34 rads per minute were given of 250Kv X-rays and Co-60 gamma-rays. For 8MeV electrons the dose rate was about the same i.e. 34 rads/minute. The G_{10} was plotted as ordinate on linear scale against dose as abscissa on a log scale.

Regression lines fitted to pooled data of various independent experiments are shown plotted in figures 3-1 to 3-20. This analysis follows the finding of Read (1959), Neary (1957) and Hall et al (1962) that the total growth in ten days (G_{10}) can be related approximately linearly to log dose. The various modifying treatments give regression lines of about the same slope and from the horizontal separation, an estimation of relative potency of a pair of treatments may be obtained.

3 - I Fractionation Effect for 250 kVp X-rays at 3.5°C.

The fractionation effect for 250KvP X-rays at 3.5°C was determined in two to seven independent experiments giving one, three, six, nine and twelve fraction exposures spaced twenty four hours apart. The data are registered in tables 3-I to 3-IV. All the data is pooled to obtain regression lines, shown in Fig.3-1. For clarity, only the experimental data for single and 12 fraction regimes have been shown on the lines.

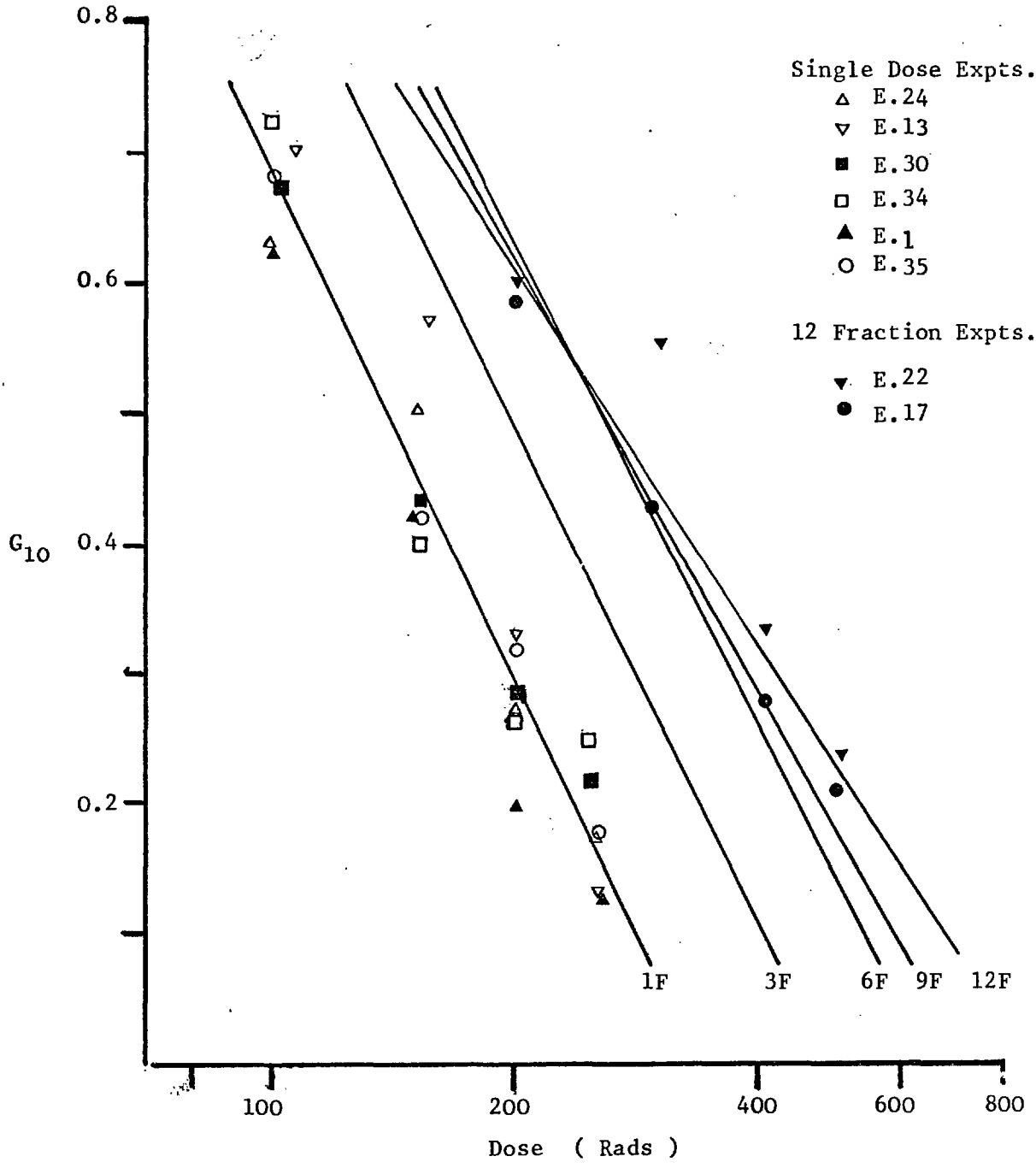


Fig. 3-1 250KVp X-irradiations at 3.5°C. Regression lines 1F, 3F, 6F, 9F and 12F represent one, three, six, nine and twelve fractions respectively given twenty four hours apart.

It is clear that the slope of the regression lines decreases gradually as the number of fraction is increased, and, the curve for three fractions and six fractions are progressively shifted towards the right. Increase of dose with number of fractions is consistent with the view that there is a shoulder on the survival curve such as would result from a significant 'multiple-event' type of interaction. Beyond six fractions there is much less effect (less than 5% increase in dose is required in going from 6 to 12 fractions), suggesting that at the small individual dose, the effect is mainly of 'single-hit' type.

In Fig.3- 2, iso-effect curves have been plotted as logarithms of total dose (log D) against log number of fractions (log N) given for a certain (G_{10}) damage level, following Strandquist (1944). These iso-effect curves bend towards a plateau after six fractions as might be expected due to predominant 'single-event' component. The plateau is more pronounced at higher values of G_{10} i.e. where lower individual doses were given.

The three iso-effect curves upto six fractions can be represented approximately by straight lines, giving exponents in Ellis's (Ellis'68,'69), formula of 0.26 ± 0.01. This is in close agreement with Ellis's value (0.24). The slope of these lines change significantly beyond six fractions giving N exponents of 0.06, 0.09 and 0.12 for $G_{10} = 0.65$, 0.50 and 0.35 respectively.

As beans were kept at $3.5^{o}C$ with cell proliferation virtually halted, there has been no time factor involved in the increase of total dose for a bigger number of fractions therefore the slope of these iso-effect curves represents

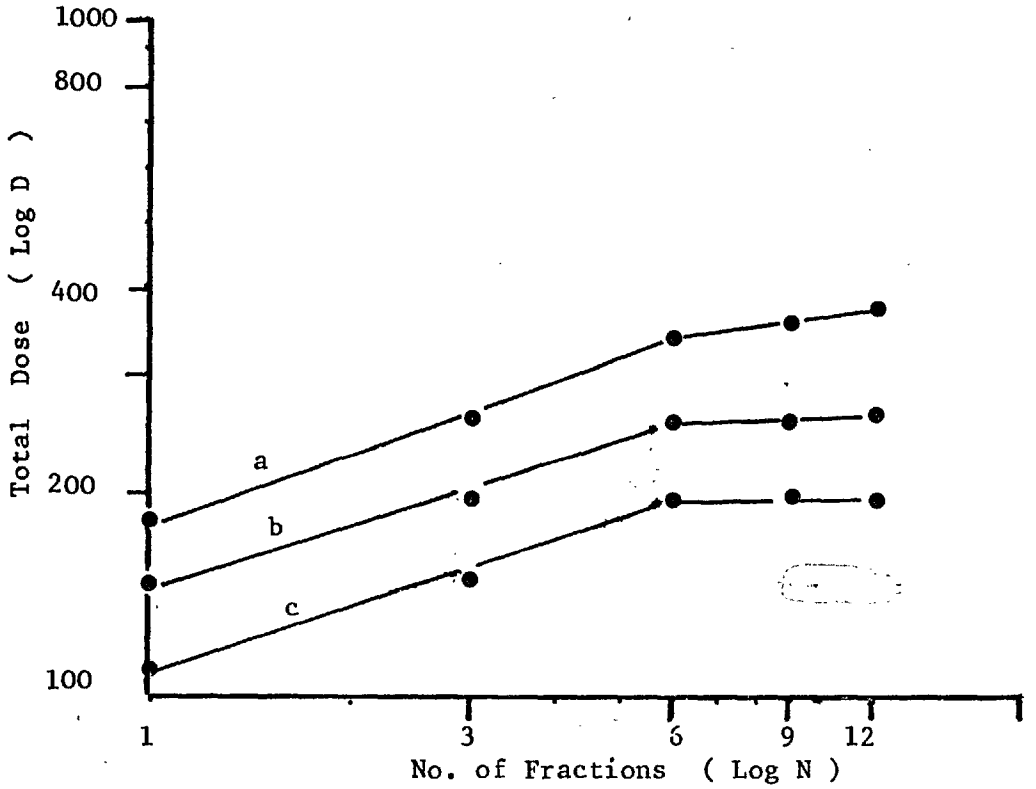


Fig. 3-2 Iso-effect curves for irradiations with 250 KVp X-rays given at 3.5°C temperature. Curves a,b and c represent for a given G_{10} of .65 , .50 and .35 respectively.

a pure N exponent in Ellis's formula.

3 - II Fractionation Effect for Co^{60} gamma rays at 3.5°C.

As with X-rays, two to three independent experimental data for 1,3,6,9 and 12 fraction regimes was pooled. The regression lines have been shown in Fig.3 - 3. To avoid confusion only representative data for 1, 6 and 12 fractions have been plotted on the lines. As with X-rays the curves for higher numbers of fractions are displaced to the right and have progressively shallower slopes.

Iso-effect curves of the Strandquist type have been plotted in Fig.3 - 4, for G_{10} values of 0.65, 0.50 and 0.35. Unlike the X-ray results they can be approximated by straight lines even upto twelve fractions. The lines are about parallel and can be characterised by the single exponent, 0.30 ± 0.04.

3 - III RBE of 250 kVp X-rays Vs Co^{60} gamma rays at 3.5°C.

The Biological Effectiveness of 250KvP X-rays relative to Co-60 gamma-rays was determined for 1,3,6, and 12 fractions given at 3.5°C, spaced 24 hours apart in each regime, this time being sufficient for sub-lethal damage recovery between the exposures. These fractionation regimes provided a dose range from 17 rads to 250 rads per fraction. This range may be expected to cover the whole of the shoulder of the survival curve. It has been pointed out by Zimmer (1961) and Hall (1974) that shapes of the dose response curve should be determined or compared under identical physiological and environmental conditions. Therefore each fractionation regime both for X-rays and gamma-rays was given in parallel

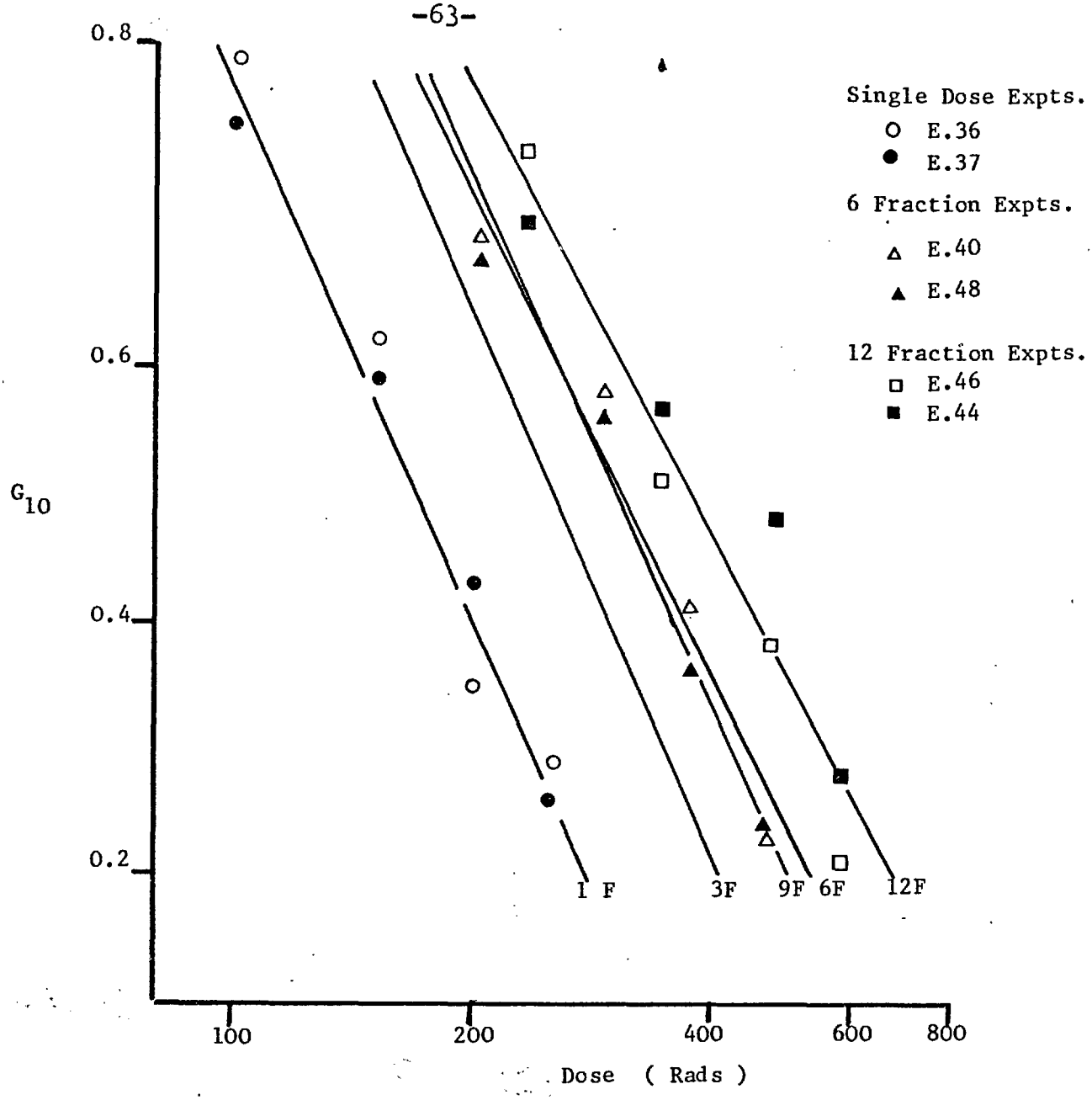


Fig. 3-3 Co-60 gamma irradiations at 3.5°C. Regression lines 1F, 3F, 6F, 9F and 12F represent one ,three, six, nine and twelve fractions respectively given twenty-four hours apart.

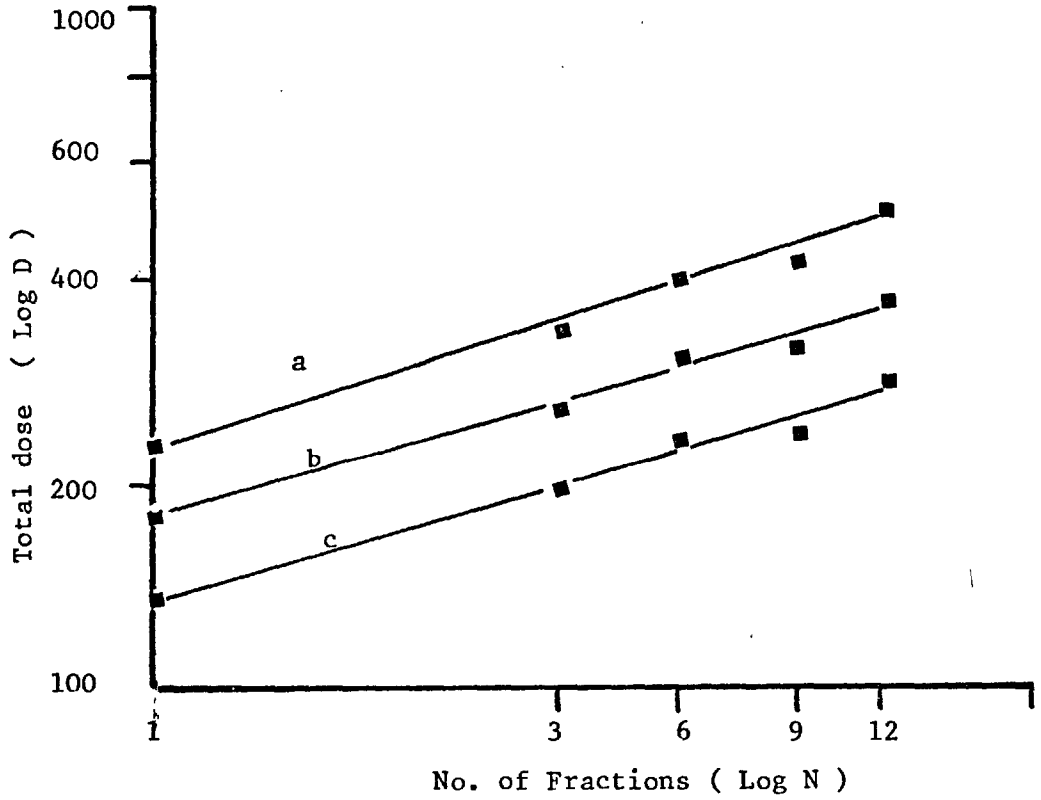
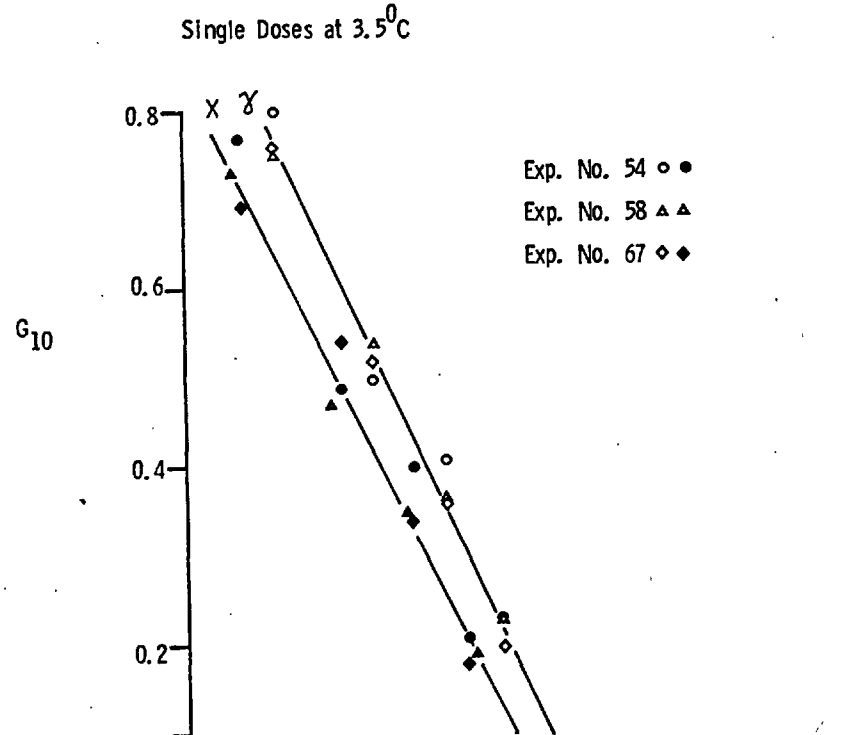
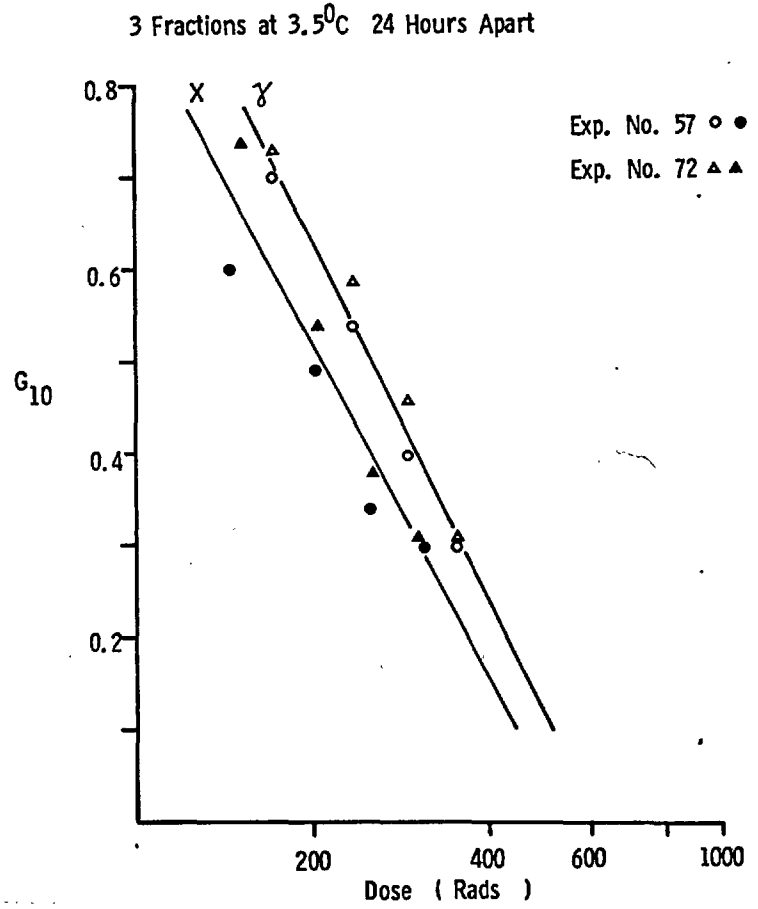


Fig. 3-4 Iso-effect curves for irradiations with Co-60 gamma rays given at 3.5°C temperature. Curves a,b and c represent for a given G_{10} of .65,.50 and .35 respectively.

3 - 5

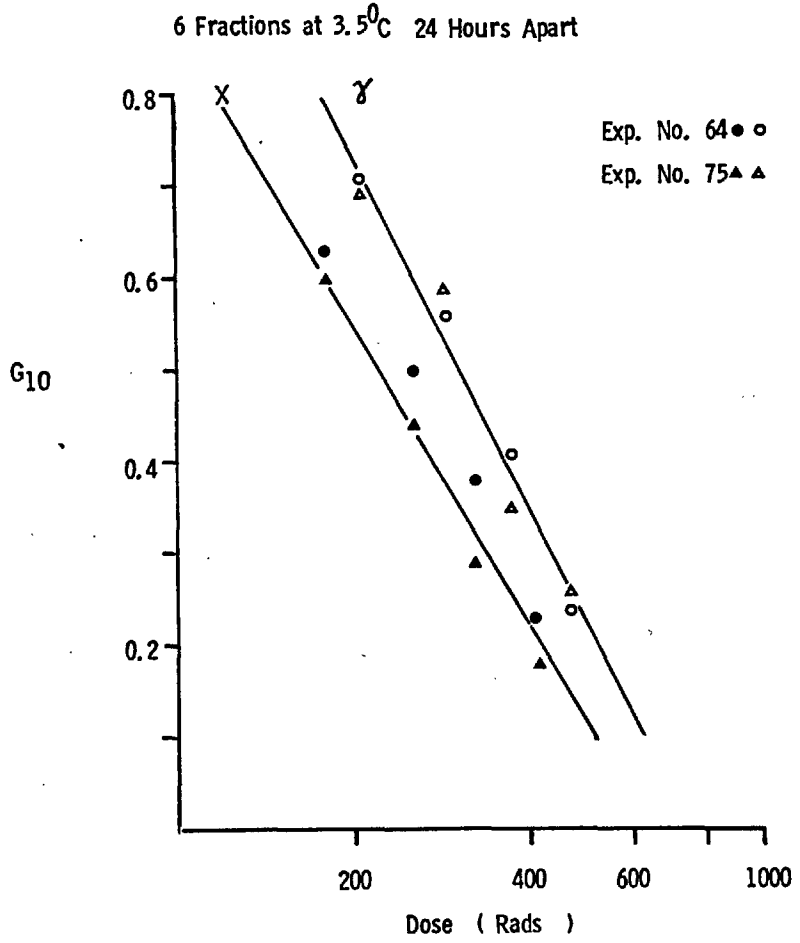


3 - 6

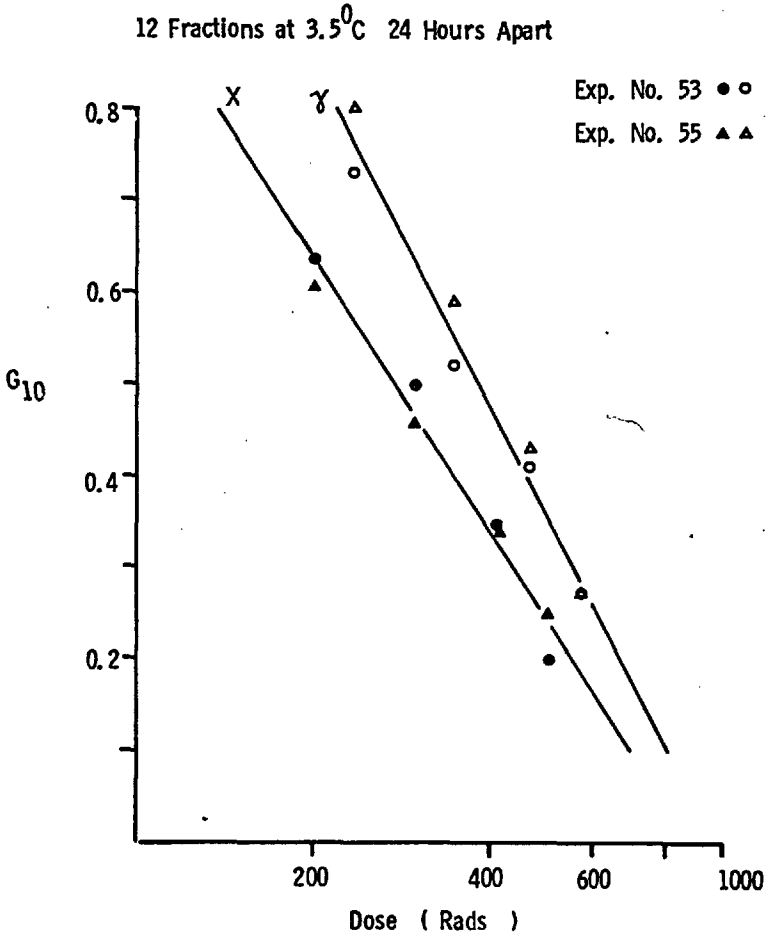


Figs. 3-5 & 3-6 Single and three fractions of 250KVp X-rays (X) and Co-60 gamma rays given in parallel exposures.

3-7



3-8



Figs. 3-7 & 3-8 Six and twelve fractions of 250KVp X-rays (X) and Co-60 gamma rays (γ) given in parallel exposures.

exposures. Two to three independent experiments were carried out for each regime. The results are shown in Fig. 3-5 to 3-8.

At any effect level the horizontal separation of the curves is the logarithm of the RBE. The slope of the regression lines for the two radiations are very much the same in lower number of fractions (1 & 3) but differ significantly for higher numbers of fractions (6 & 12). RBE is relatively constant at about 1.2 for dose fractions greater than 100 rads but increases as the dose per fraction is reduced and reaches 1.5 at 17 rads. There is no evidence of a large effect of fraction number as opposed to fraction size, but if the partial curves for each fraction number were matched where they overlap the apparent change in RBE with fraction size would be enhanced. Increase of RBE at such low dose per fraction, i.e. 17 rads, is compatable with a greater single event contribution relative to multi-hit contribution with X-rays than with Co-60 gamma-rays i.e. the ratio of initial to final slope of the survival curve is greater with X-rays.

3 - IV Fractionation Effect for 8 MeV electrons at 3.5°C.

To demonstrate the fractionation effect for electrons data presented in Fig. 3-16 to 3-19 have been replotted in Fig. 3 - 9. It is clear from the figure that there is separation of the G_{10} curve as the number of fractions is increased. A marked increase in dose of about 40% is required in going from a single dose to three fractions and from three to six fractions an increase of about 30% in dose is required.

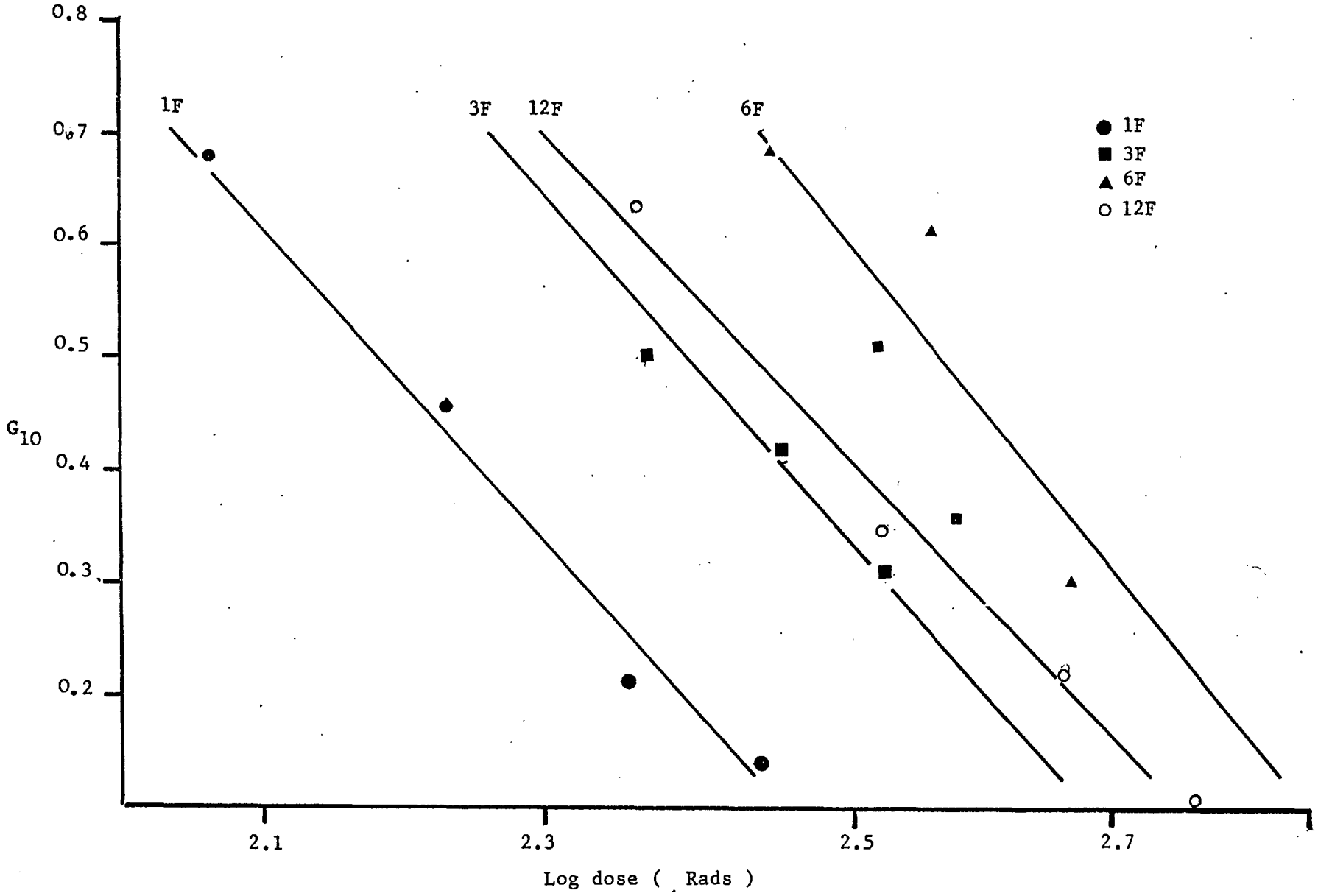


Fig. 3-9 8Mev Electron irradiations given in single (1F), three fractions (3F), six fractions (6F) and twelve fractions 24Hours apart at 3.5°C temperature.

This data has been plotted in Strandquist's plot, i.e. log N Vs log D, in Fig.3-10 for given G_{10} levels of 0.65, 0.50 and 0.35. The slope of these lines represents the N exponent, has been calculated only upto six fractions and has the value 0.35 ± 0.05. Since the lines on Figure 3-10 are parallel the same value applies to all G_{10} levels. The anomalous 12 fraction point (see also figure 3-9) has been regarded as probably due to experimental error.

3 - V RBE of 250 kVp X-rays Vs 8 MeV electrons at 19°C.

Two independent single dose experiments were done to measure the RBE of 250kV X-rays relative to 8MeV electrons at 19°C, in order to check that RBE was the same as the lower temperature at 3.5°C for single doses. The results have been plotted in Fig.3-11.

At 19°C the RBE was 0.81 (95% confidence limits 0.73-0.91) when at 3.5°C the RBE was 0.81 (95% confidence limits 0.74-0.89)

3 - VI Fractionation Effect with X-rays at 19°C.

One to five independent experiments were done to determine fractionation effect of X-rays for each of single, three and six fraction regimes. The three curves i.e. 1, 2 and 3 representing 1, 3 and 6 fractions respectively (Fig. 3-12) are displaced to the right or towards higher doses as we go from the single dose curve to the six fraction curve. To produce a given effect about 50% greater total dose is required in three fractions than in a single irradiation. Similarly a further 50% greater total dose is required in producing a G_{10} in six fraction equal to that in three fractions.

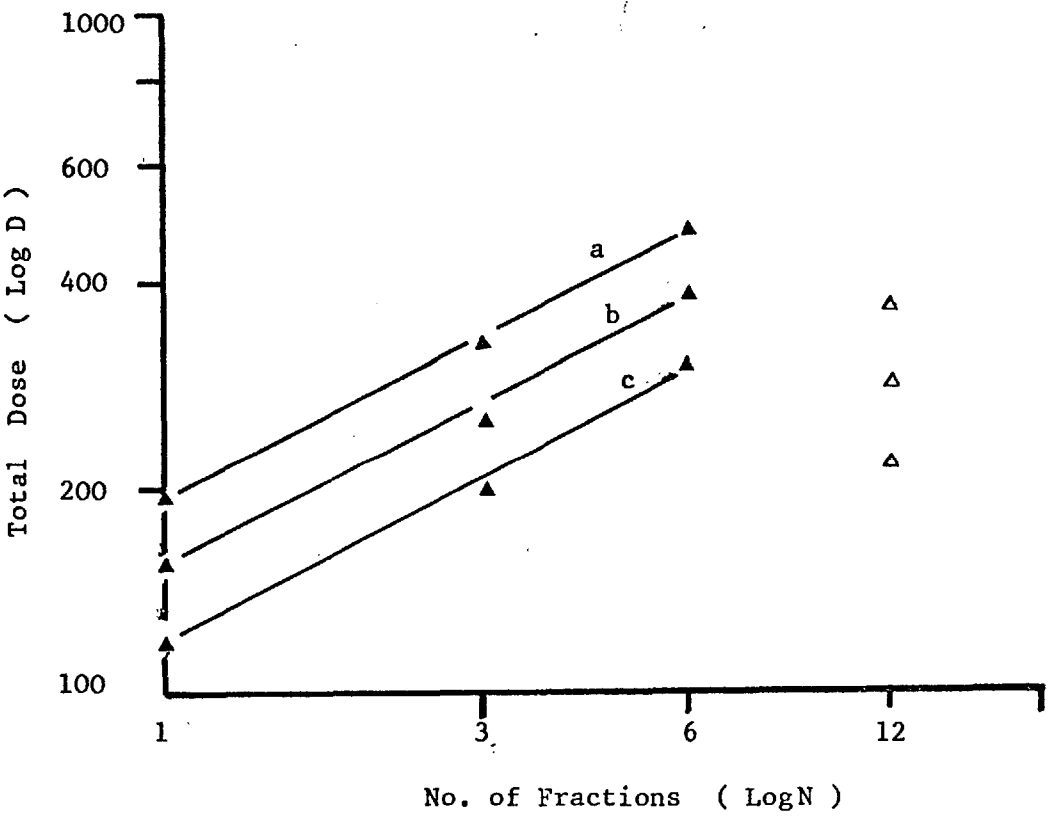


Fig. 3-10 Total log dose (LogD) Vs log number of Fractions (Log N) for 8Mev Electron irradiations given at 3.5°C temperature.Curves a,b,c, represent for a given G_{10} of .65, .50 and .35 respectively.

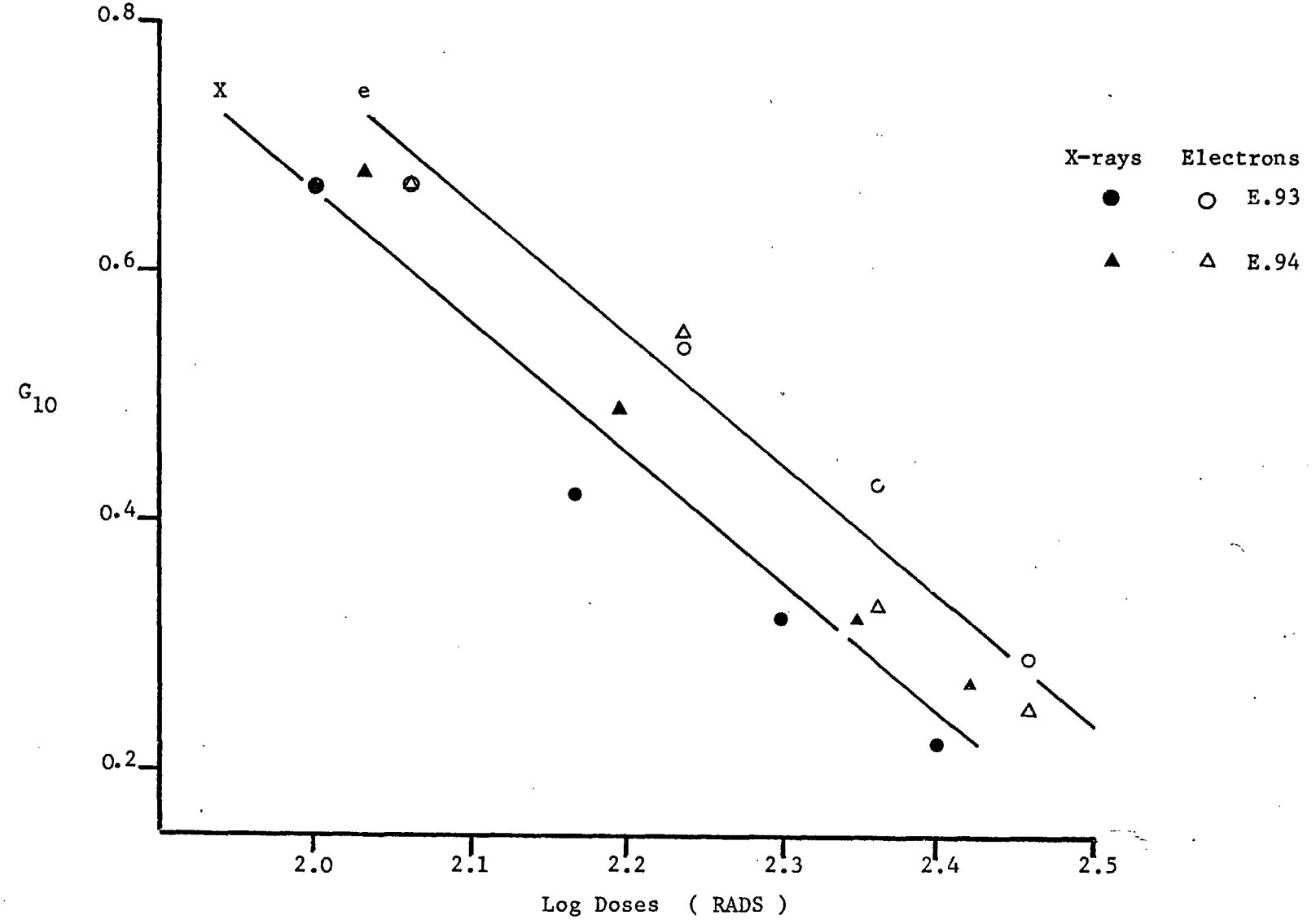


Fig. 3-11 Single doses of 250KVp X-rays and 8Mev Electrons given at 19°C

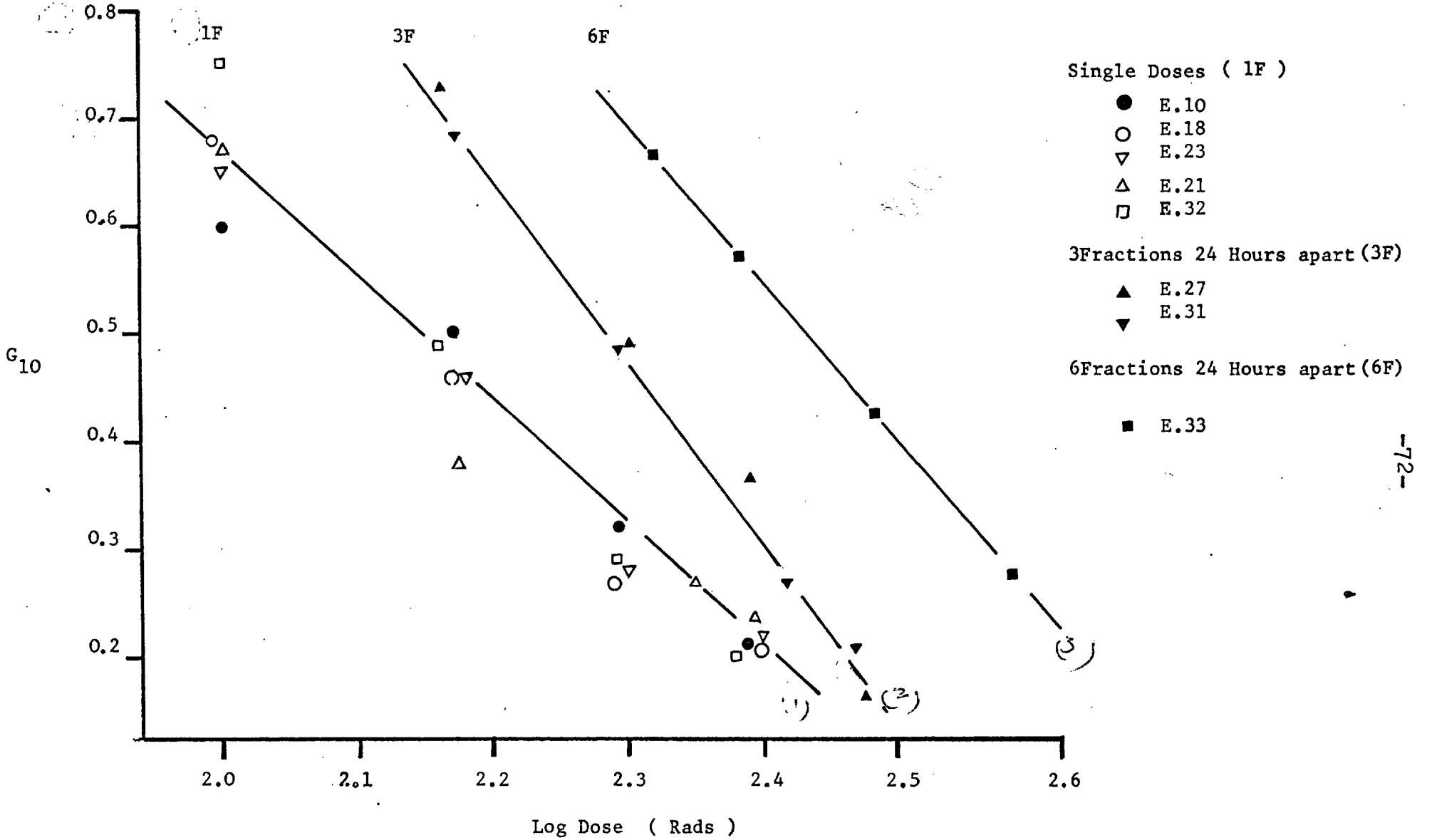


Fig. 3-12 250 KVp X-irradiations given at 19°C temperature

At higher doses the three fraction curve has a higher slope. This may be a consequence of cell synchrony playing a part at higher dose per fractions.

3 - VII Fractionation Effect for gamma rays at 19°C.

Data from two to six independent experiments has been presented in Fig.3-13 for 1, 3 and 6 fractions of Co-60 gamma irradiation at 19°C. There is a consistant shift of the curves towards the right i.e. towards the higher number of fractions. An increase of about 54% total dose is required to produce a G_{10} of 0.50 in three fractions as compared to single dose and similarly about 50% more than three fractions when given in six fractions. The three fractions curve has a markedly high slope similar to that of X-rays at 19°C the reason for this is not known. One possibility is that cell synchrony may be playing a part at high dose per fractions.

3 - VIII Biological Effectiveness of X-rays relative to Co^{60} gamma rays at low dose rate.

The effect of radiation at low dose rate is of great interest in radiotherapy (Perquin,1972). Some irradiations have been carried out at 120 rads/hour. It was not practicable to investigate change of RBE with number of fractions. Two independent single dose experiments were performed at 19°C. The horizontal separation of the two lines gives an RBE of 0.81 (95% confidence limits 0.89-0.74). This value is very similar to that measured at high dose rate (34 rads/minute) for both x and gamma irradiations. Fig.3-14 suggests that if a dose of much less than 50 rads

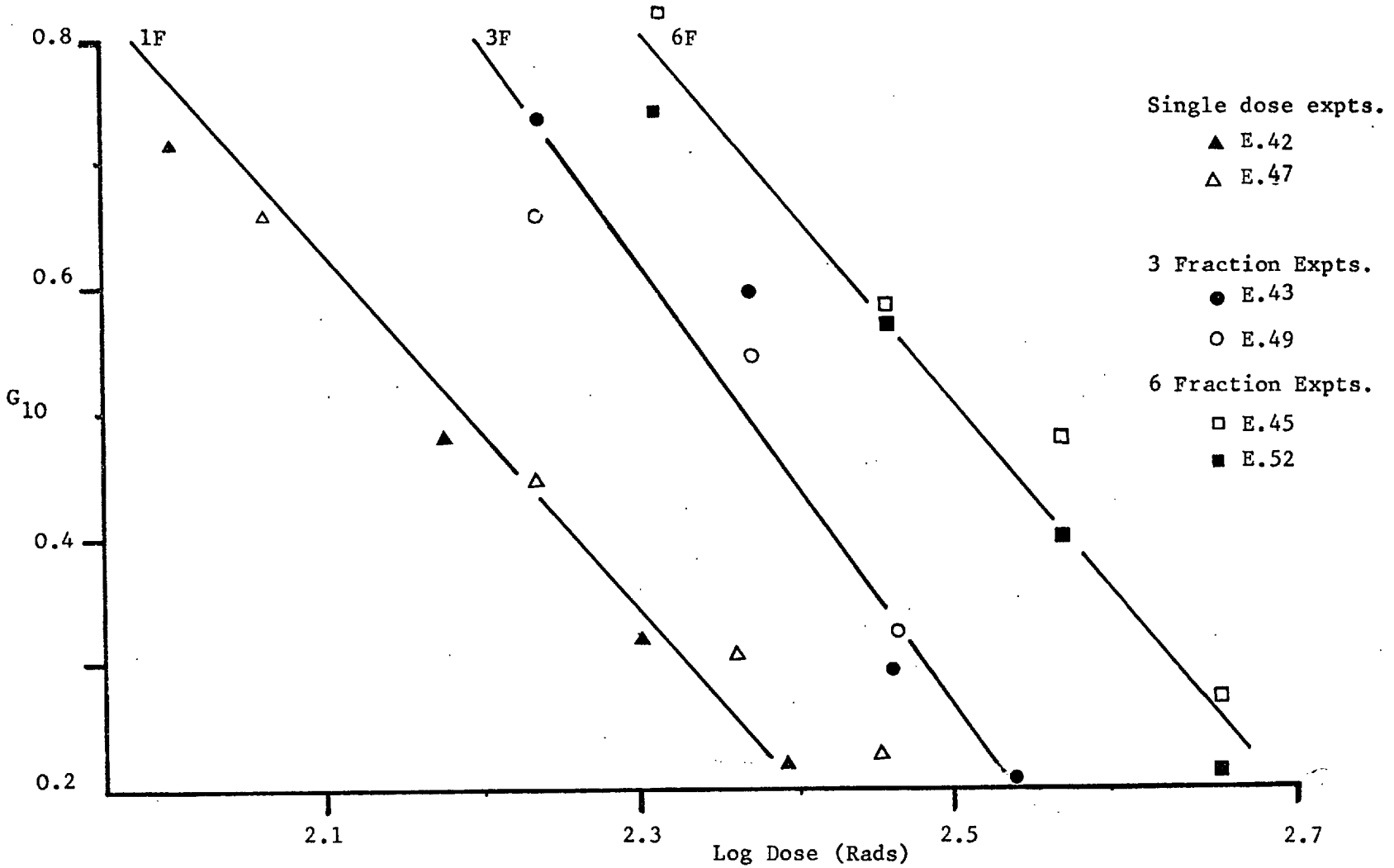


Fig. 3-13 Fractionated irradiations with Co-60 gamma rays given in one, (1F) three (3F) and six (6F) fractions each 24hours apart at 19°C temperature.

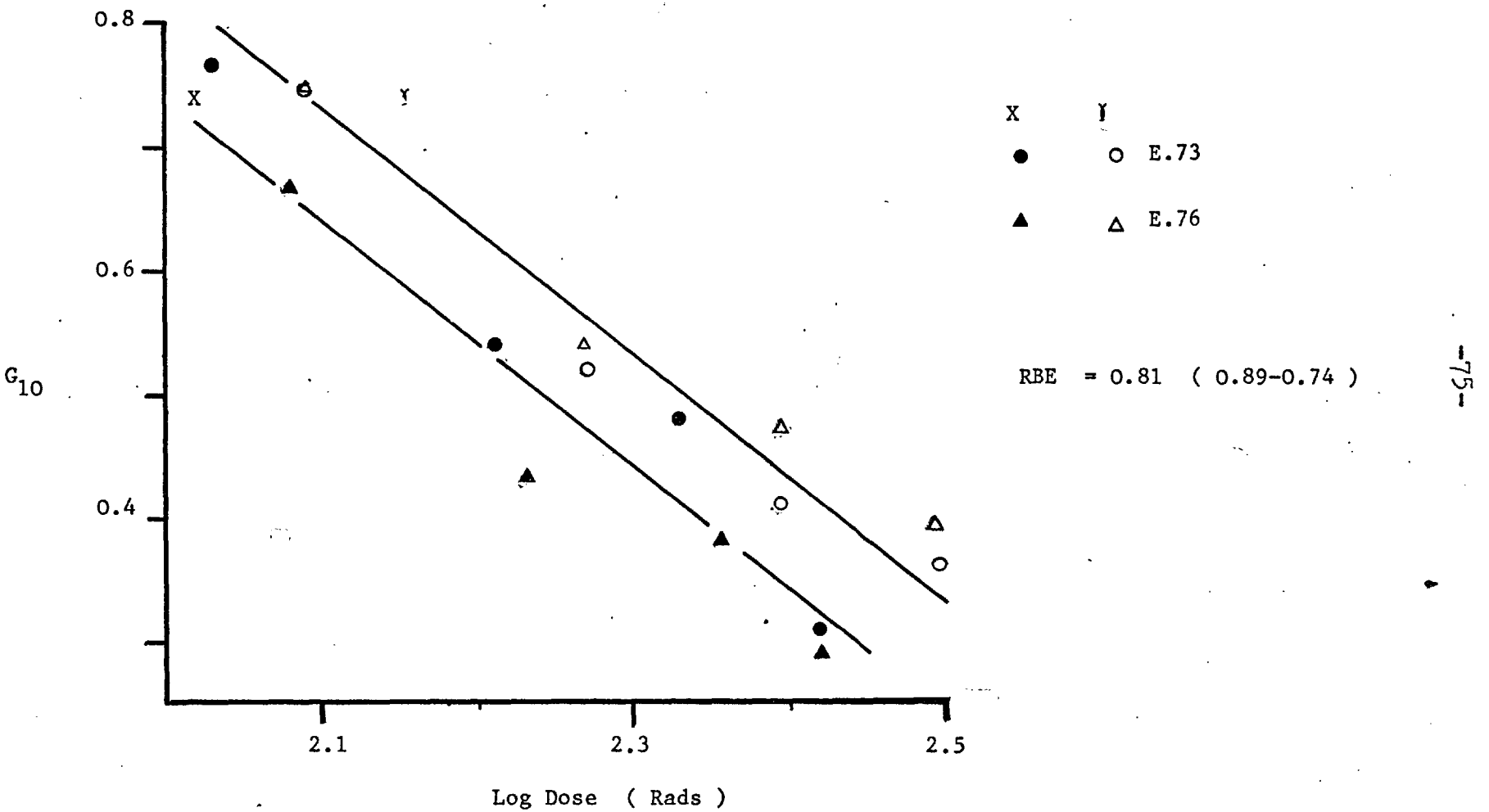


Fig. 3-14 Single doses of 250KVp X-rays and Co-60 gamma rays given at a dose rate of 120 rads per minute at 19°C temperature.

has been received within the time for which sub-lethal damage persists a decrease in RBE would have been seen. Since 50 rads are given in about 25 mins, sub-lethal damage persists for at least this time.

3 - IX Biological Effectiveness of X-irradiation at high dose rate relative to low dose rate.

Pursuing further this interest in low dose rate, three single dose independent experiments were performed to measure effectivenss of X-rays at low dose rate relative to high dose rate at 19°C. The results are presented in Fig.3-15 , at low dose rate the RBE is 0.76 with 95% confidence limits of 0.85-0.68 which is indicative of loss of effectiveness of X-radiation at lower dose rate due to sub-lethal damage recovery during the time of irradiation (2-4 hours). Within the margin of experimental error there is not necessarily a contradiction between this finding and that of the previous section.

3 - X RBE of 250 kVp X-rays Vs 8 MeV electrons at 3.5°C.

RBE of 250 kVp X-rays and 8 MeV electrons was determined giving 1, 3, 6 and 12 fractions in parallel exposures. The data has been plotted in Fig. 3-16to 3-19 . Upto six fractions the RBE of 250kVp X-rays does increase although there is wide overlap of 95% confidence limits. Fig. 3-20. As the 12 fraction experimental point is regarded as probably due to an experimental error therefore RBE cannot be assessed. As has been seen in Fig. 3- 20 that 12 fractions experiments shows a fractionation effect comparable to three fractions.

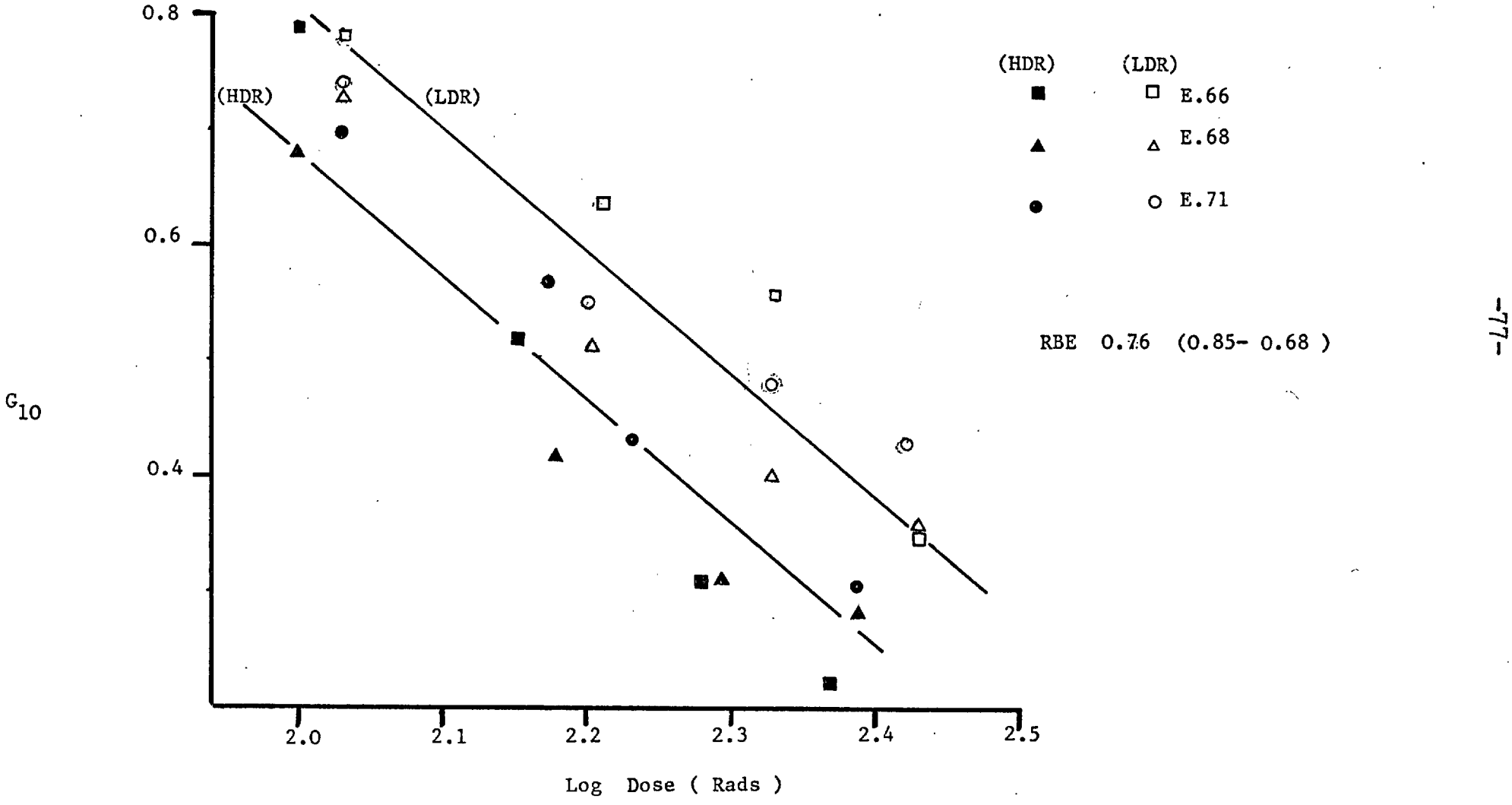


Fig. 3-15 250KVp X-irradiations given at high (HDR) and low dose rate (LDR) in sigle doses at 19°C

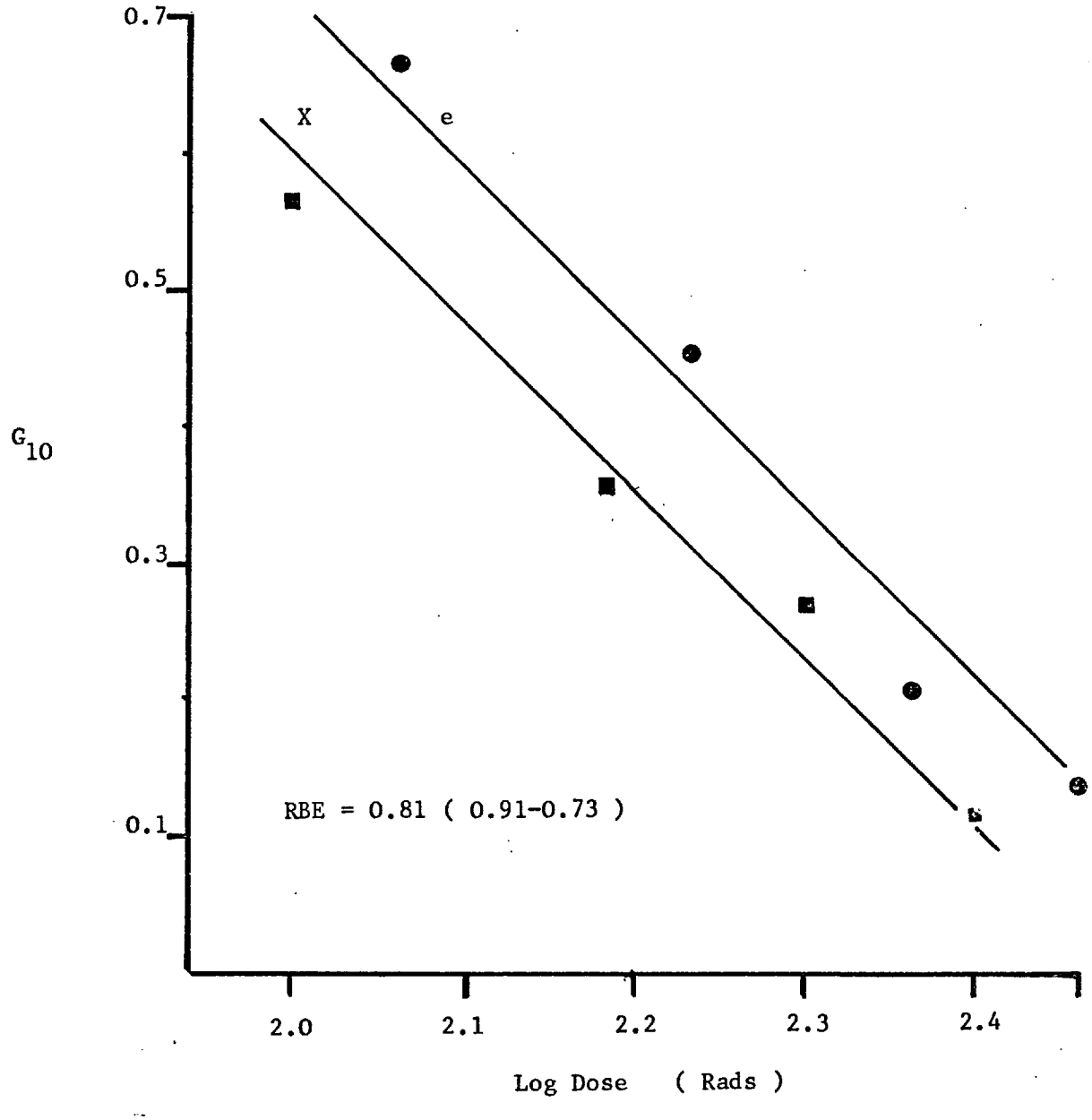


Fig. 3-16 250KVp X-rays and 8Mev Electron irradiations given in single dose at 3.5°C temperature.

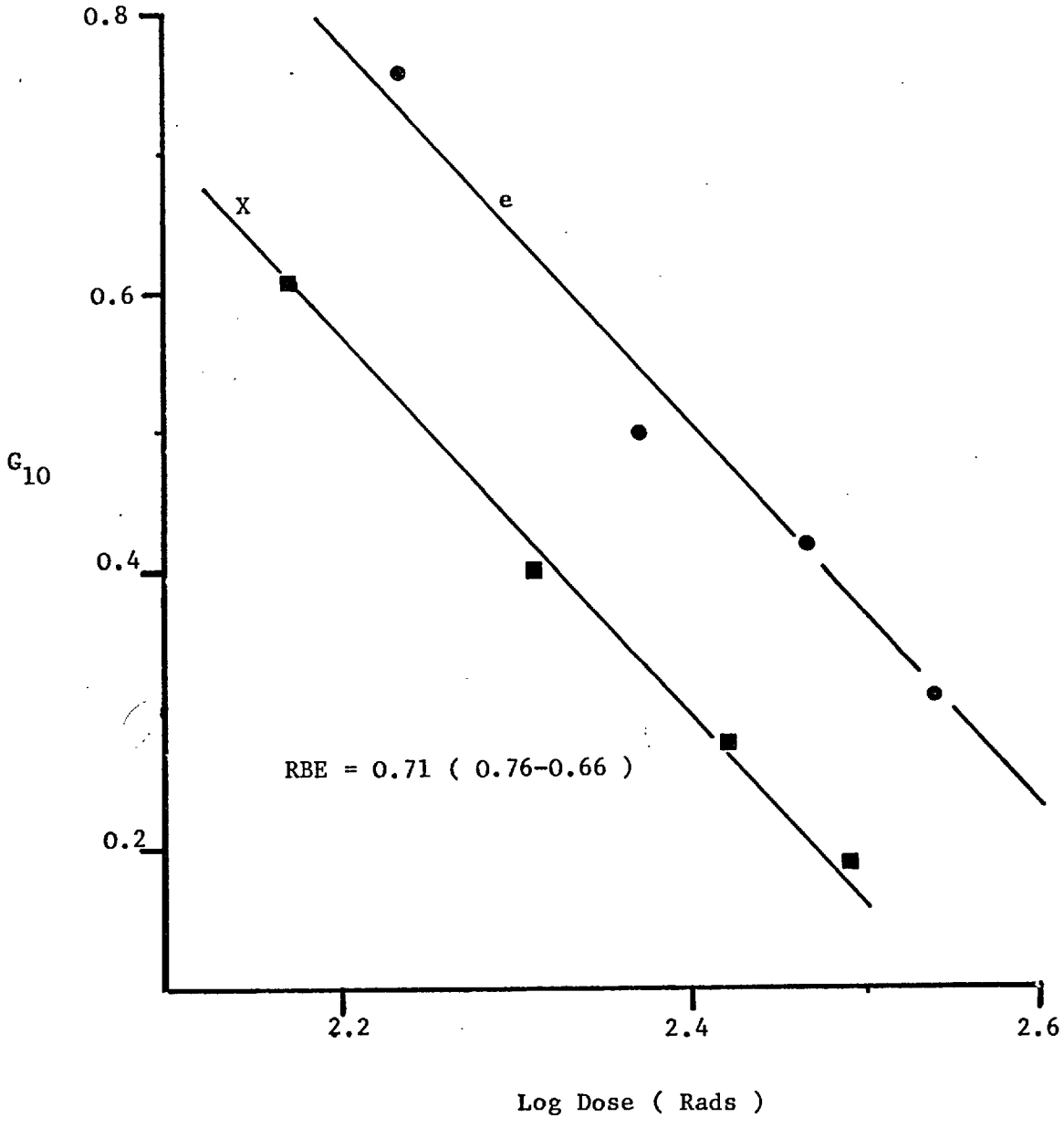


Fig. 3-17 250KVp X-rays (X) and 8Mev Eletron irradiations given in three fractions 24hours apart at 3.5°c.

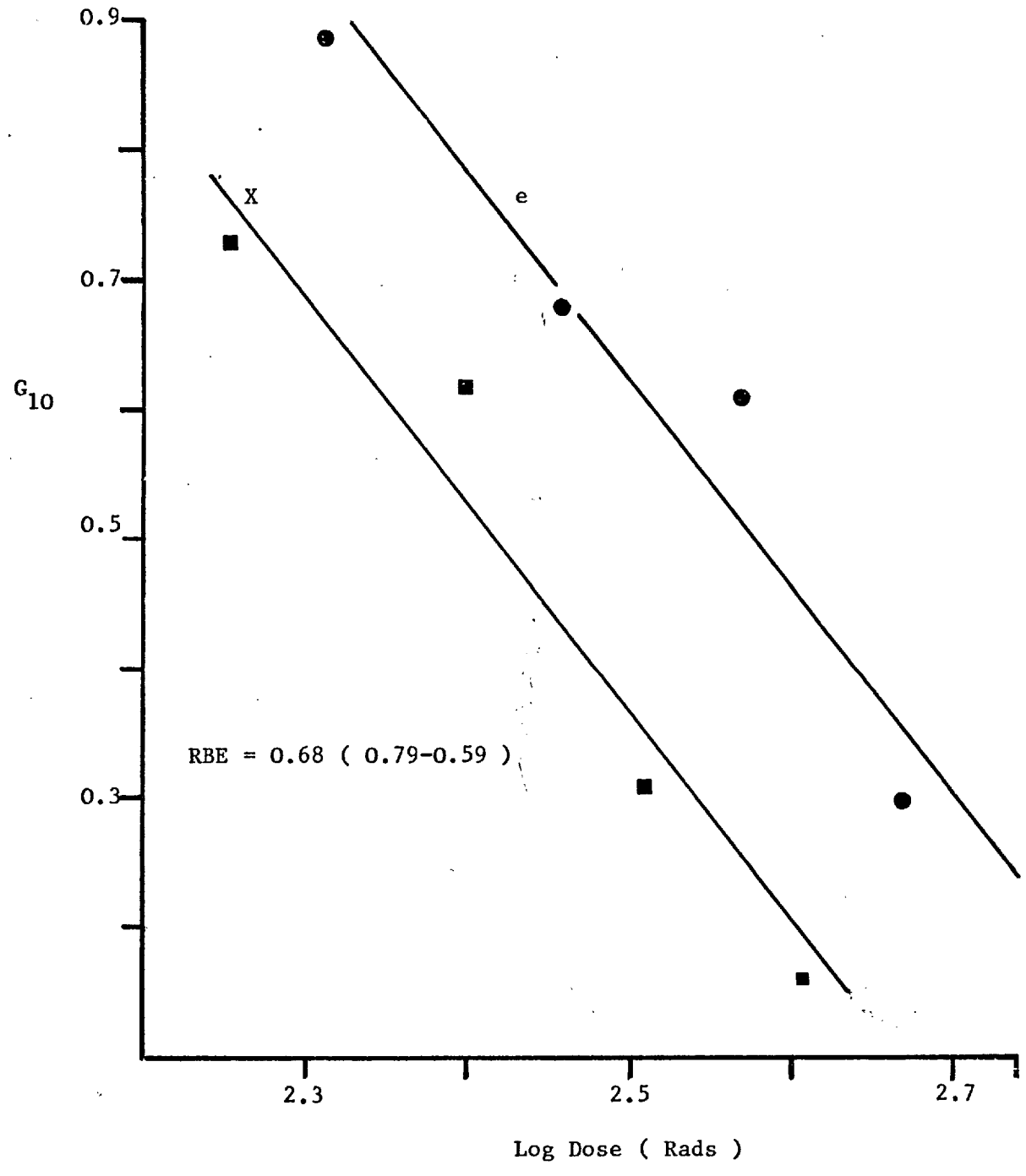


Fig. 3-18 250KVp X-rays (X) and 8Mev Electron (e) irradiations given in six fractions 24hours apart at 3.5°C.

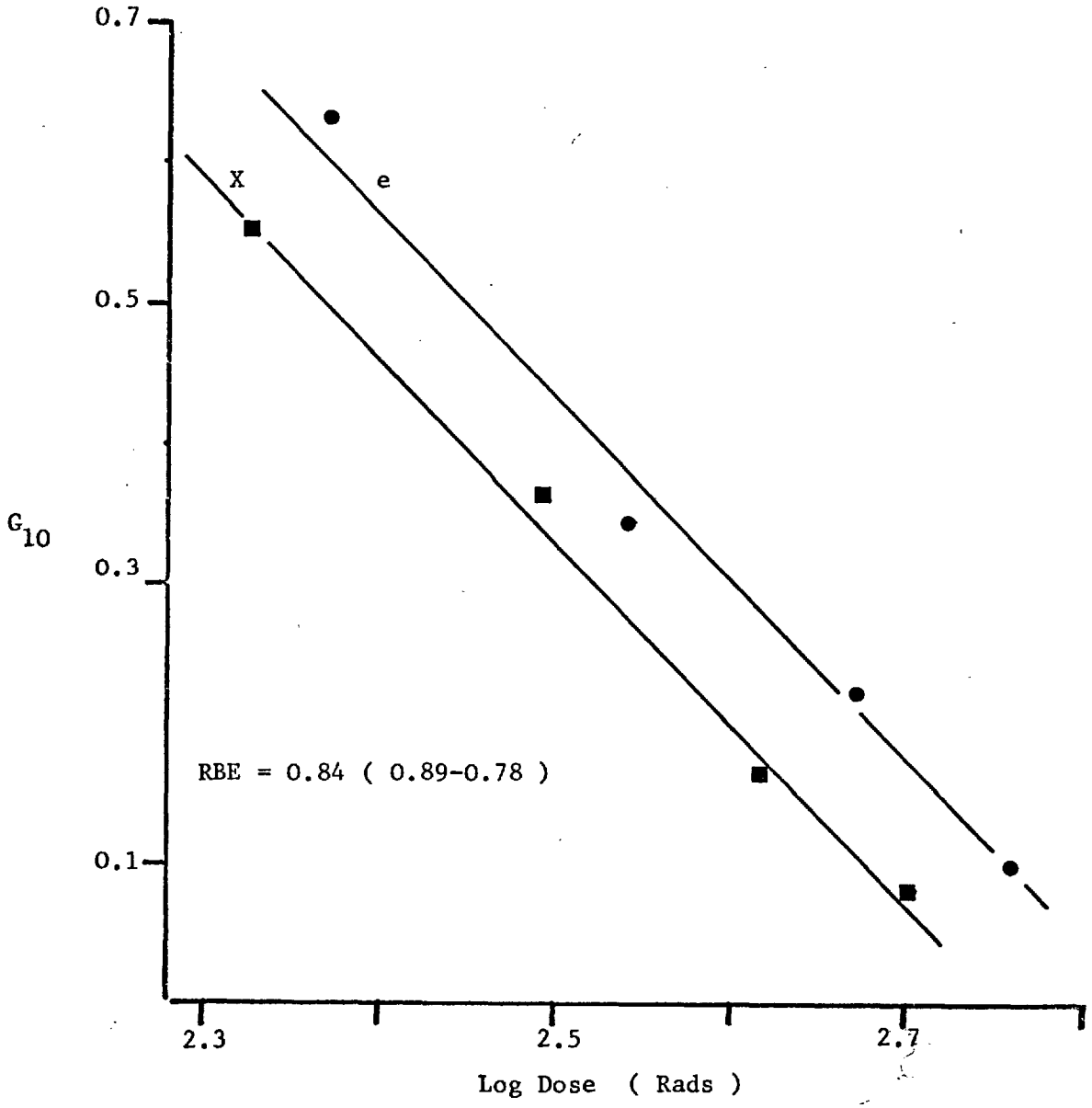
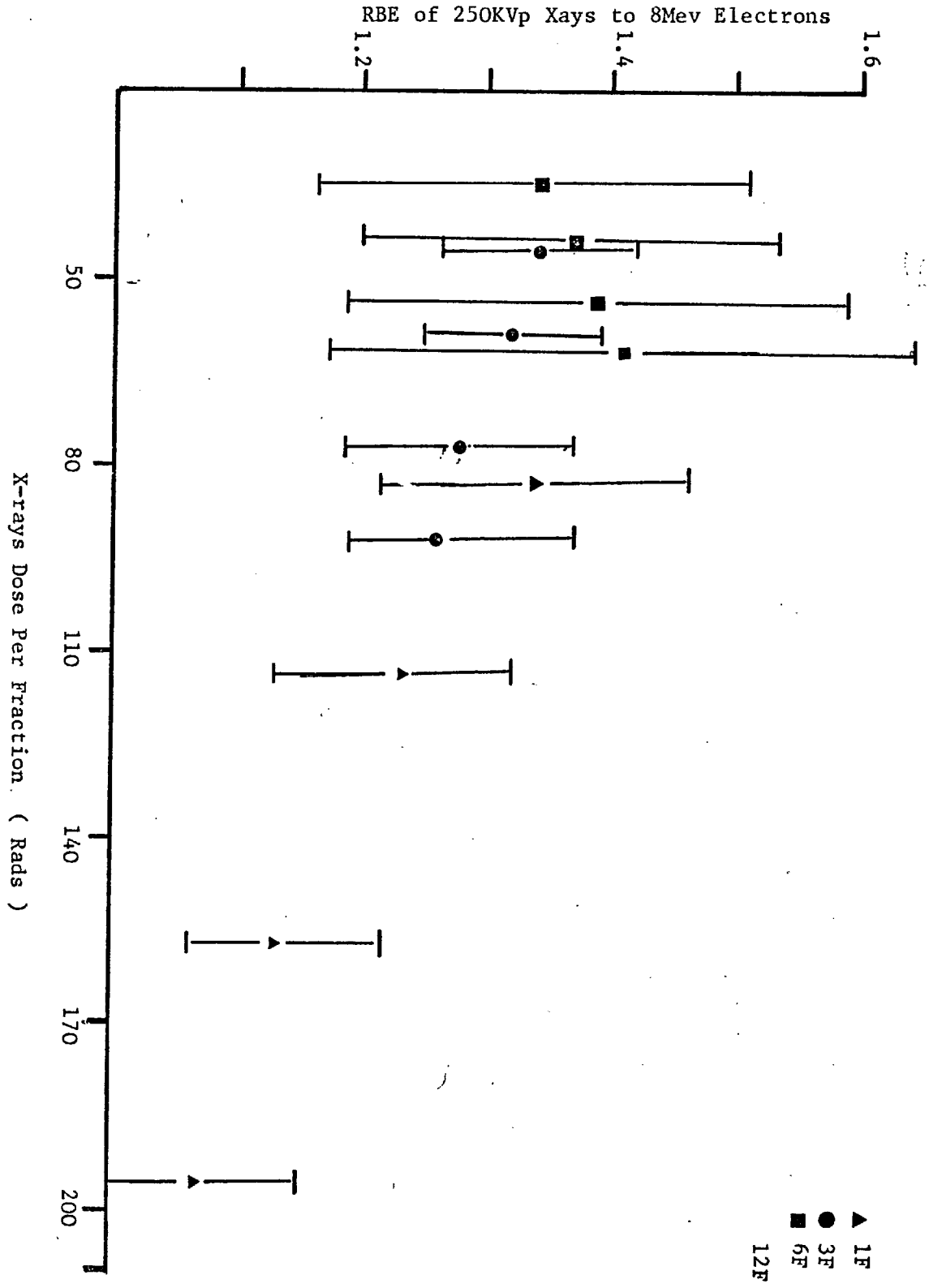


Fig. 3-19 250KVp X-rays and 8Mev Electron irradiations given in twelve fractions 24hours apart at 3.5°C.

Fig. 3-20 RBE of 250KVp X-rays and 8Mev Electrons determined in fractionated irradiations given at 3.5°C.



RESULTS OF X-IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - I

Single Dose at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.1	100	2.000	0.62	± 0.03
	150	2.176	0.42	± 0.02
	200	2.301	0.19	± 0.01
	250	2.397	0.13	± 0.007
E.13	107	2.029	0.71	± 0.07
	160	2.204	0.57	± 0.05
	198	2.296	0.33	± 0.03
	249	2.396	0.22	± 0.02
E.24	100	2.000	0.63	± 0.24
	151	2.179	0.50	± 0.07
	201	2.303	0.27	± 0.10
	252	2.401	0.18	± 0.07
E.30	103	2.012	0.68	± 0.07
	153	2.184	0.43	± 0.13
	200	2.302	0.24	± 0.05
	252	2.401	0.13	± 0.04
E.34	102	2.008	0.72	± 0.09
	153	2.184	0.40	± 0.08
	200	2.301	0.26	± 0.10
	247	2.392	0.25	± 0.07

RESULTS OF X-IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - II

Single Dose at 3.5°C.

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.35	102	2.008	0.69	± 0.07
	151	2.179	0.42	± 0.11
	201	2.303	0.32	± 0.12
	253	2.403	0.17	± 0.08
E.54	101	2.004	0.77	± 0.06
	151	2.179	0.49	± 0.12
	202	2.305	0.40	± 0.07
	249	2.397	0.21	± 0.06

$\bar{x}$ = 2.224
$\bar{y}$ = 0.39
slope = -1.302
S.E. = 0.0079

3 Fractions 24 hours apart at 3.5°C

E.25	147	2.167	0.68	± 0.067
	203	2.307	0.54	± 0.07
	254	2.404	0.37	± 0.09
	306	2.485	0.29	± 0.04
E.26	151	2.179	0.63	± 0.15
	202	2.305	0.46	± 0.08
	265	2.423	0.33	± 0.07
	299	2.475	0.26	± 0.09

$\bar{x}$ = 2.331
$\bar{y}$ = 0.45
slope = -1.252
S.E. = 0.016

RESULTS OF X-IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - III

6 Fractions 24 hours apart at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.14	180	2.255	0.71	± 0.08
	252	2.401	0.55	± 0.06
	324	2.510	0.37	± 0.13
	409	2.612	0.23	± 0.08
E.28	179	2.252	0.63	± 0.09
	249	2.397	0.54	± 0.06
	327	2.515	0.40	± 0.06
	407	2.610	0.23	± 0.05

$\bar{x}$ = 2.444
$\bar{y}$ = 0.46
slope = -1.230
S.E. = 0.018

9 Fractions 24 hours apart at 3.5°C

E.15	191	2.282	0.69	± 0.10
	292	2.465	0.45	± 0.10
	386	2.586	0.28	± 0.07
	446	2.649	0.26	± 0.05
E.19	190	2.278	0.62	± 0.09
	288	2.459	0.40	± 0.12
	385	2.586	0.33	± 0.10
	450	2.653	0.25	± 0.10

$\bar{x}$ = 2.495
$\bar{y}$ = 0.41
slope = -1.085
S.E. = 0.015

RESULTS OF X-IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - IV

12 Fractions 24 hours apart at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.17	202	2.305	0.58	± 0.11
	294	2.469	0.43	± 0.07
	404	2.606	0.28	± 0.10
	493	2.693	0.21	± 0.07
E.22	202	2.306	0.60	± 0.10
	301	2.478	0.53	± 0.09
	413	2.616	0.34	± 0.08
	503	2.702	0.24	± 0.08

$\bar{x}$ = 2.521
$\bar{y}$ = 0.40
slope = -0.95
S.E. = 0.02

RESULTS OF GAMMA IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - V

Single Doses 24 hours apart at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.54	115	2.060	0.80	± 0.08
	172	2.236	0.50	± 0.07
	230	2.361	0.41	± 0.09
	287	2.458	0.23	± 0.08
E.58	115	2.060	0.76	± 0.17
	172	2.236	0.52	± 0.09
	230	2.361	0.36	± 0.10
	287	2.458	0.20	± 0.07
E.67	115	2.060	0.75	± 0.08
	172	2.236	0.54	± 0.10
	230	2.361	0.37	± 0.12
	287	2.458	0.23	± 0.11

$\bar{x}$ = 2.219
$\bar{y}$ = 0.45
slope = -1.295
S.E. = 0.0092

3 Fractions 24 hours apart at 3.5°C

E.72	172	2.236	0.70	± 0.11
	234	2.370	0.54	± 0.10
	293	2.467	0.40	± 0.06
	352	2.546	0.30	± 0.04
E.57	172	2.236	0.73	± 0.14
	234	2.370	0.56	± 0.12
	293	2.467	0.46	± 0.07
	352	2.546	0.31	± 0.12

$\bar{x}$ = 2.465
$\bar{y}$ = 0.50
slope = -1.32
S.E. = 0.05

RESULTS OF GAMMA IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - VI

6 Fractions 24 hours apart at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.40	207	2.316	0.71	± 0.10
	290	2.462	0.56	± 0.10
	372	2.571	0.41	± 0.09
	469	2.671	0.23	± 0.04
E.48	207	2.316	0.68	± 0.10
	290	2.462	0.58	± 0.12
	372	2.571	0.36	± 0.15
	469	2.671	0.24	± 0.11

$\bar{x}$ = 2.505
$\bar{y}$ = 0.47
slope = -1.301
S.E. = 0.017

9 Fractions 24 hours apart at 3.5°C

E.51	216	2.334	0.65	± 0.07
	333	2.522	0.42	± 0.06
	445	2.648	0.36	± 0.08
	516	2.713	0.22	± 0.07
E.41	216	2.334	0.71	± 0.06
	333	2.522	0.55	± 0.09
	445	2.648	0.36	± 0.04
	516	2.713	0.22	± 0.08

$\bar{x}$ = 2.554
$\bar{y}$ = 0.42
slope = -1.231
S.E. = 0.022

RESULTS OF GAMMA IRRADIATIONS AT 3.5°C.

TABLE NO. 3 - VII

12 Fractions 24 hours apart at 3.5°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.44	234	2.369	0.71	± 0.12
	347	2.541	0.57	± 0.15
	468	2.670	0.42	± 0.16
	575	2.760	0.25	± 0.11
E.46	234	2.369	0.77	± 0.19
	347	2.541	0.51	± 0.19
	468	2.670	0.35	± 0.15
	575	2.760	0.21	± 0.12

$\bar{x}$ = 2.585
$\bar{y}$ = 0.49
slope = -1.204
S.E. = 0.023

TABLE NO. 3 - VIII

Results of RBE measurements at 3.5°C.

250kV X-rays. Co-60 gamma-rays.

RBE in Single Doses

	Dose in rads	G_{10}	STANDARD ERROR		Dose in rads	G_{10}	STANDARD ERROR
E.58	101	0.69	± 0.08	E.58	115	0.76	± 0.17
	151	0.54	± 0.08		172	0.52	± 0.09
	200	0.34	± 0.07		230	0.36	± 0.10
	251	0.18	± 0.06		287	0.20	± 0.07
E.67	98	0.73	± 0.17	E.67	115	0.75	± 0.08
	146	0.47	± 0.09		172	0.54	± 0.10
	196	0.35	± 0.09		230	0.37	± 0.12
	258	0.19	± 0.09		287	0.23	± 0.11

RBE in 3 Fractions 24 hours apart.

E.57	153	0.74	± 0.07	E.57	172	0.73	± 0.14
	206	0.54	± 0.10		234	0.56	± 0.12
	256	0.38	± 0.12		293	0.46	± 0.07
	310	0.30	± 0.06		352	0.31	± 0.12
E.72	148	0.60	± 0.12	E.72	172	0.70	± 0.11
	202	0.49	± 0.11		234	0.54	± 0.10
	252	0.34	± 0.09		293	0.40	± 0.06
	304	0.31	± 0.07		352	0.30	± 0.04

TABLE NO. 3 - IX

Results of RBE measurements at 3.5°C.

250kV X-rays. Co-60 gamma-rays.

RBE in 6 Fractions 24 hours apart.

	Dose in rads	G_{10}	STANDARD ERROR		Dose in rads	G_{10}	STANDARD ERROR
E.56	179	0.61	± 0.06	E.56	207	0.52	± 0.10
	251	0.44	± 0.05		290	0.47	± 0.09
	321	0.32	± 0.06		372	0.40	± 0.12
	403	0.20	± 0.04		469	0.35	± 0.13
E.64	179	0.63	± 0.11	E.64	207	0.71	± 0.13
	255	0.50	± 0.07		290	0.56	± 0.11
	324	0.38	± 0.10		372	0.41	± 0.11
	408	0.23	± 0.05		469	0.24	± 0.07
E.75	180	0.60	± 0.13	E.75	207	0.69	± 0.15
	256	0.44	± 0.06		290	0.59	± 0.12
	330	0.29	± 0.03		372	0.35	± 0.13
	415	0.18	± 0.03		469	0.26	± 0.11

RBE in 12 Fractions 24 hours apart.

E.53	203	0.61	± 0.09	E.53	234	0.80	± 0.07
	300	0.46	± 0.10		347	0.59	± 0.15
	415	0.34	± 0.05		468	0.43	± 0.08
	502	0.25	± 0.07		575	0.27	± 0.08
E.55	202	0.64	± 0.09	E.55	234	0.73	± 0.08
	299	0.50	± 0.09		347	0.52	± 0.08
	410	0.35	± 0.10		468	0.41	± 0.05
	502	0.20	± 0.05		575	0.27	± 0.08

TABLE NO. 3 - X

REGRESSION LINES OF RBE RESULTS FOR VARIOUS FRACTIONATION AT 3.5°C.

	X-rays	Gamma-rays
1 Fraction		
	$\bar{x}$ = 2.219	$\bar{x}$ = 2.279
	$\bar{y}$ = 0.45	$\bar{y}$ = 0.47
	slope = -1.295	slope = -1.354
	S.E. = 0.021	S.E. = $\pm$ 0.01
3 Fractions		
	$\bar{x}$ = 2.354	$\bar{x}$ = 2.405
	$\bar{y}$ = 0.46	$\bar{y}$ = 0.50
	slope = -1.214	slope = -1.317
	S.E. = $\pm$ 0.06	S.E. = 0.03
6 Fractions		
	$\bar{x}$ = 2.438	$\bar{x}$ = 2.505
	$\bar{y}$ = 0.41	$\bar{y}$ = 0.48
	slope = -1.14	slope = -1.301
	S.E. = 0.02	S.E. = 0.04
12 Fractions		
	$\bar{x}$ = 2.524	$\bar{x}$ = 2.585
	$\bar{y}$ = 0.42	$\bar{y}$ = 0.50
	slope = -0.954	slope = -1.234
	S.E. = 0.02	S.E. = 0.03

RESULTS OF X-RADIATIONS AT 19°C

TABLE NO. 3 - XI

Single Doses at 19°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.10	100	2.00	0.60	± 0.08
	150	2.172	0.40	± 0.05
	197	2.295	0.32	± 0.08
	243	2.385	0.21	± 0.05
E.18	98	1.993	0.68	± 0.07
	147	2.168	0.41	± 0.04
	196	2.292	0.27	± 0.06
	246	2.391	0.21	± 0.07
E.20	101	2.004	0.65	± 0.06
	152	2.181	0.46	± 0.04
	201	2.303	0.28	± 0.02
	251	2.399	0.22	± 0.02
E.21	102	2.008	0.62	± 0.08
	148	2.169	0.38	± 0.10
	224	2.351	0.27	± 0.07
	247	2.392	0.21	± 0.03
E.32	97	1.989	0.75	± 0.06
	144	2.159	0.49	± 0.04
	194	2.289	0.29	± 0.02
	241	2.382	0.20	± 0.02

$\bar{x}$ = 2.213
$\bar{y}$ = 0.51
slope = -1.138
S.E. = 0.008

RESULTS OF X-RADIATIONS AT 19°C.

TABLE NO. 3 - XII

3 Fractions 24 hours apart at 19°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.27	145	2.162	0.73	± 0.19
	199	2.300	0.49	± 0.07
	249	2.396	0.33	± 0.11
	298	2.474	0.16	± 0.11
E.31	146	2.165	0.68	± 0.12
	196	2.292	0.49	± 0.05
	262	2.418	0.27	± 0.10
	296	2.471	0.21	± 0.06

$\bar{x}$ = 2.347
$\bar{y}$ = 0.43
slope = -1.68
S.E. = 0.03

6 Fractions 24 hours apart at 19°C

E.33	209	2.321	0.67	± 0.07
	243	2.386	0.57	± 0.14
	312	2.495	0.43	± 0.19
	388	2.589	0.27	± 0.17

$\bar{x}$ = 2.462
$\bar{y}$ = 0.51
slope = -1.73
S.E. = 0.13

RESULTS OF GAMMA IRRADIATIONS AT 19°C

TABLE NO. 3 - XIII

Single Dose at 19°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.47	115	2.060	0.66	± 0.08
	172	2.236	0.45	± 0.06
	230	2.361	0.31	± 0.14
	287	2.458	0.23	± 0.08
E.42		2.000	0.71	± 0.05
		2.176	0.48	± 0.06
		2.301	0.32	± 0.08
		2.397	0.22	± 0.05

$\bar{x}$ = 2.286
$\bar{y}$ = 0.47
slope = -0.833
S.E. = 0.02

3 Fractions 24 hours apart at 19°C

E.43	172	2.2368	0.74	± 0.10
	235	2.371	0.60	± 0.20
	294	2.466	0.31	± 0.16
	352	2.546	0.21	± 0.09
E.49	172	2.236	0.66	± 0.08
	235	2.371	0.54	± 0.08
	294	2.466	0.32	± 0.05
	352	2.546	0.11	± 0.07

$\bar{x}$ = 2.347
$\bar{y}$ = 0.43
slope = -1.682
S.E. = 0.03

RESULTS OF GAMMA IRRADIATIONS AT 19°C

TABLE NO. 3 - XIV

6 Fractions 24 hours apart at 19°C

	Total Dose in rads	Log Dose	G_{10}	STANDARD ERROR
E.45	207	2.316	0.82	± 0.13
	290	2.462	0.59	± 0.15
	372	2.571	0.47	± 0.07
	469	2.671	0.27	± 0.12
E.52	207	2.316	0.74	± 0.13
	290	2.462	0.57	± 0.12
	372	2.571	0.39	± 0.09
	469	2.671	0.21	± 0.08

$\bar{x}$ = 2.505
$\bar{y}$ = 0.51
slope = -1.496
S.E. = 0.045

TABLE NO. 3 - X V.

Results of the Measurement of Biological Effectiveness of High Dose Rate X-rays relative to Low Dose Rate X-rays in Single Doses at 19°C.

	High Dose Rate		Low Dose Rate	
Exp. No. 71	2.026	0.70	2.027	0.74
	2.171	0.57	2.200	0.55
	2.230	0.43	2.327	0.48
	2.387	0.32	2.423	0.43
Exp. No. 68	1.979	0.68	2.026	0.73
	2.177	0.42	2.202	0.51
	2.294	0.31	2.326	0.40
	2.384	0.29	2.426	0.36
Exp. No. 66	1.996	0.79	2.032	0.78
	2.153	0.52	2.209	0.64
	2.278	0.31	2.328	0.56
	2.365	0.22	2.424	0.35

Chapter 4.

Discussion and Conclusion.

for Part I.

CHAPTER 4.

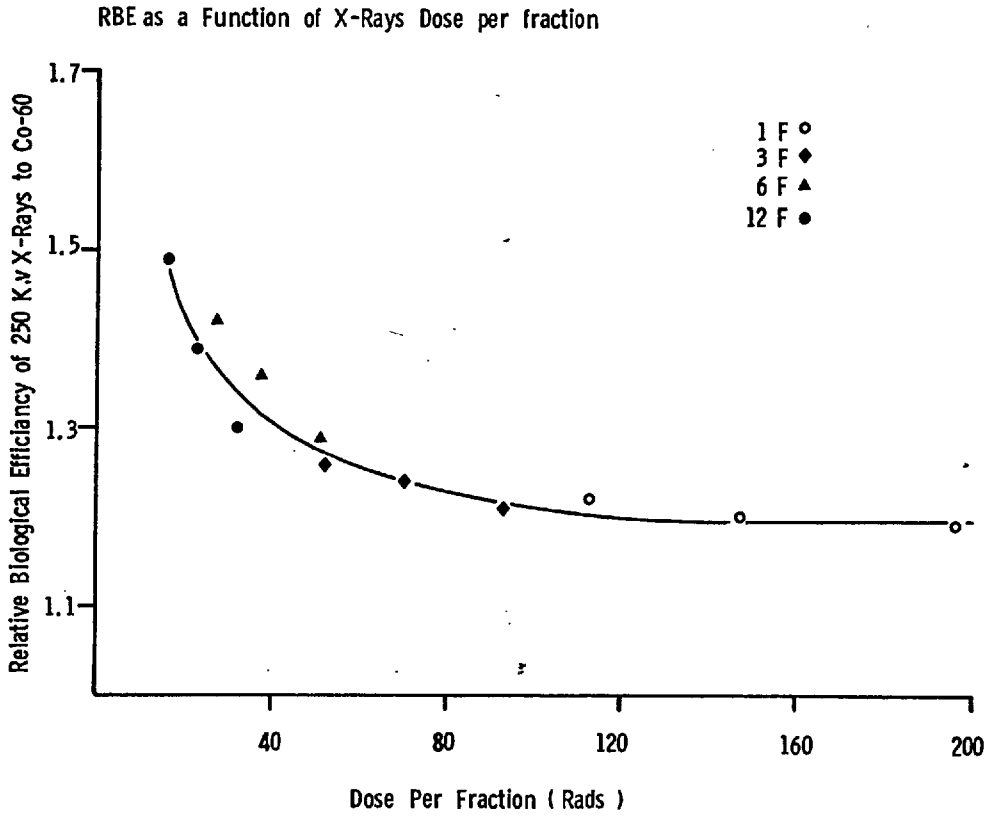
DISCUSSIONS AND CONCLUSIONS.

4-1 RBE of X-rays, Co-60 gamma-rays and 8MeV Electrons.

Early experimental evidence showed that the dose effect curves both for Vicia and mammalian cells are due to with a combination of single-hit and multi-hit effects. In this consideration the RBE of X, gamma and electrons may be expected to vary with dose.

The relative biological effectiveness for root growth inhibition (G_{10}) of 250 kV X-rays and Co^{60} gamma-rays has been plotted against dose per fraction of X-rays (Figure 4-1). The RBE is virtually constant at 1.2 as the dose per fraction is reduced from 200 to 80 rads, and thereafter increases to 1.5 at 17 rads per fraction. This accords with the hypothesis that X-rays, the more densely ionizing radiation, would produce the greater initial slope to the survival curve, and so show an RBE that increases as the dose given is reduced. A similar trend of RBE was expected, but not found, in the single series of experiments conducted in this study comparing 8 MeV electrons with 250 kVp X-rays in fractionated irradiations. It would be desirable to repeat these experiments. On the basis of biophysical characteristics of X-rays, Co^{60} gamma-rays and 8 MeV electons (Chapter 1A) Co^{60} gamma-rays should be intermediate in relative biological effectiveness between 250 kVp X-rays and 8 MeV electrons.

Fig; 4-1



RBE of 250 KVp x-rays and Co-60 gamma-rays determined in fractionated irradiations given in parallel exposures at 3.5^{0}C.

There have been few reports of RBE of X and gamma-rays differing greatly from unity. However, Underbrink et al., (1976) showed that for irradiations of stamen hair of Tradescantia the RBE was 1.7 with doses as low as 2 rads. Schmid et al., (1974) found X-rays to be twice as effective as fast electrons in the production of dicentric chromosomes in lymphocytes. Jamieson and Read (1962), studying Vicia root growth inhibition with 250 kVp X-rays and 24 MeV X-rays, found that the RBE of the X-rays increased from 1.17 to 1.32 as the dose was reduced from 250 to 50 rads.

Kellerer and Rossi (1972) suggested on the basis of their theory of Dual Radiation Action, that the single hit effectiveness of 250 kV X-rays should be twice that of Co^{60} gamma-rays, whereas the dose square effectiveness should be approximately equal.

4-11 Fractionation Effect and the Dose Response Curve for Vicia.

4-11a General Theory.

It is known that growth rates of roots of the bean, Vicia faba is a direct consequence of the proliferative activity of the meristematic cells. (Gray and Scholes, 1951). Survival of the meristem cells cannot be measured directly, but it is a reasonable assumption that dose fractionation regimes given to produce the same value of G_{10} have brought about the same reduction of cell proliferation. A test of the validity of a survival curve is that it should give parameters that lead to iso-effect relationships in accordance with experimental values (Elkind, 1960; Fowler and Stern, 1963). In figure 4-2 iso-effect curves obtained

for various G_{10} levels have been plotted as log total dose against log number of fractions. These iso-effect lines have been computed from the two component models for meristematic cells deduced by Oliver (1964), except curve 5 which represents only the multi-target single hit model (Hall, Lajtha and Oliver, 1962). The parameter values for the two component models were n = 2 - 4, D_1 = 133, D = 64 rads, the values of initial and final slope adopted were derived from the gamma-ray dose response values by use of the experimentally determined RBE's at high and low doses. Hall (1962) and Hall, Lajtha and Oliver (1962) concluded that multi-target single hit equation:

$$f = 1 - (1 - e^{-D/D_n})^m \quad \ldots (X11)$$

where f is the surviving fraction, D_n the reciprocal of the slope of the final straight portion of the survival curve and m the extrapolation number, is adequate to explain the dose effect relation for Vicia for doses higher than about 100 rads. The same is true of Puck and Marcus (1956) observations on the dose response for mammalian cells in culture. Curve 5. which assumes this model, predicts that the dose to produce a given effect increases without limit as the number of fractions is increased. However, when a single hit type of component is added, the result is as in curves 1 to 4, a less steep rise in dose, tending towards a constant value with higher number of fraction. Eventually with six or more fractions, where the dose is about 35 rads or less per fraction, a horizontal line is obtained indicating no further increase in total dose. The dose per fraction at which this plateau is reached

depends somewhat on the G_{10} values and n selected. A small dependence of total dose on number of fraction is known to be the characteristic of high LET radiations, and suggests that damage is mainly of single hit type with such low doses.

4-11b EXPERIMENTAL ISO-EFFECT AND DOSE RESPONSE FOR X-RAYS.

The experimental results for fractionated irradiations with 250 kVp X-rays at 3.5°C have been plotted on the theoretical iso-effect lines in (Fig. 4-2). They show no agreement with curve 5, assuming only multi-target single hit type of interaction, but agree fairly well with iso-effect curves derived from the two component models at all G_{10} levels. The closest agreement is with curve 4 assuming n = 3.5. In fitting the iso-effect curves by eye it appeared that the values of 133 rads and 64 rads for D_1 and D_n respectively could not be changed by more than 5% without significantly worsening the fit. The curve obtained from the two component model tends to an exponential where slope is given by the equation:

$$1/D_o = 1/D_1 + 1/D_n \quad \ldots\ldots \quad (X111)$$

Accepting the values of 133 and 64 rads for D_1 and D_n respectively for X-rays, the D_o of this curve would be 43 rads. The ratio of the initial slope D_1 and the final slope D_o is $D_1/D_o = 133/43 = 3.09$. This value is in quite good agreement with that inferred by Hall (1963) from a comparison of high and low dose rate experiments with bean roots, where a similar type of response was observed at a low dose rate of 2.6 rads per hour. The presence of an

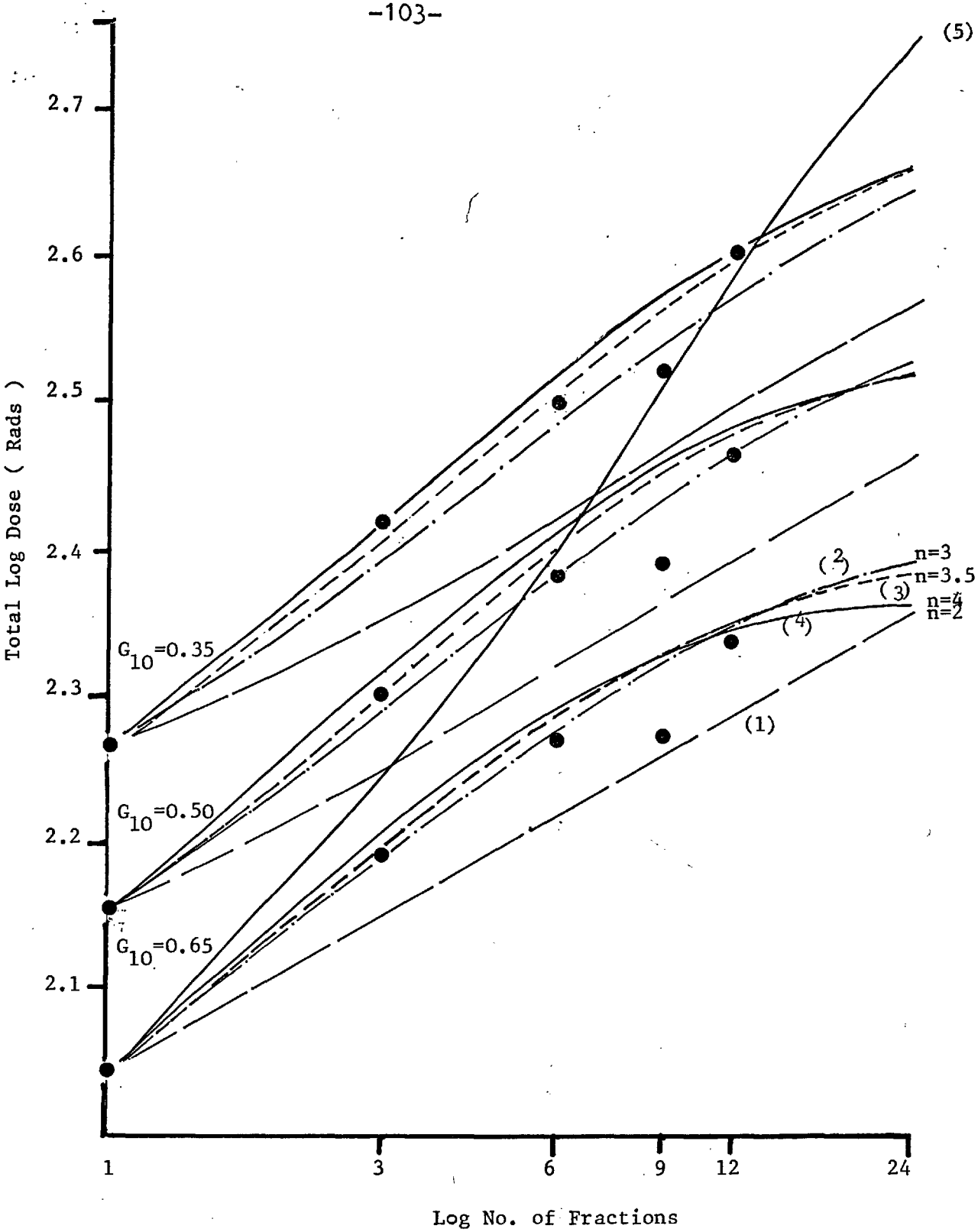


Fig. 4-2 Iso-effect curves cumputed with the equation ;

$$S = e^{-D/133}\left[1-\left\{1-\left(e^{-D/64}\right)\right\}^{n}\right]$$

different values of n has been applied as given on the curves (1), (2), (3), and (4) similar curves has been cumputed for given G_{10} levels of o.65, .0.50 and 0.35. Curve (5) have been computed with only multiple-event part of the equation with n=2. Experimental points for 250KVp X-irradiation regimes have been plotted on these curves.

initial slope to the survival curves for kidney cells was pointed out by Barendsen (1962) and was accepted to be a real feature in later studies (Fowler, 1966; Sinclair, 1966 and Barendsen, 1967). Bender and Gooch (1964) found with mammalian cells that a satisfactory fit was obtained with the two component model. More recent studies in which the shape of the shoulder region of the single dose survival curve has been derived from fractionated irradiations have revealed an initial slope to be present for almost all biological systems studied, although the slope ratio varies very widely (Elkind, 1975). Table 4-I shows initial to final slope ratios deduced from fractionation studies with various mammalian systems. A ratio of 3.09 for Vicia is in general agreement with the values of 2.0 - 5.0 for mammalian cells.

4-11-c ISO-EFFECT AND DOSE RESPONSE FOR GAMMA-RAYS.

Theoretical iso-effect curves were computed assuming a two component model (Oliver, 1964) for dose response of gamma-rays with parameters D_1 = 190 rads and D_n = 70 rads and choosing values 2, 3, 3.5 and 4 for n (Fig. 4-3). A curve for multi-target single-hit model above has also been plotted. (curve 5). The experimental values have been plotted in the figure. As with X-rays the results disagree with the pure multiple event model, and give best agreement with curve computed for n = 3.5, D_o for the composite curve is 51 rads. The initial to final slope ratio thus obtained is D_1/D_o = 190/51 = 3.72. Compared with the ratio of 3.09 for X-rays this indicates that the contribution of single hit damage is less with gamma-rays. These values are in

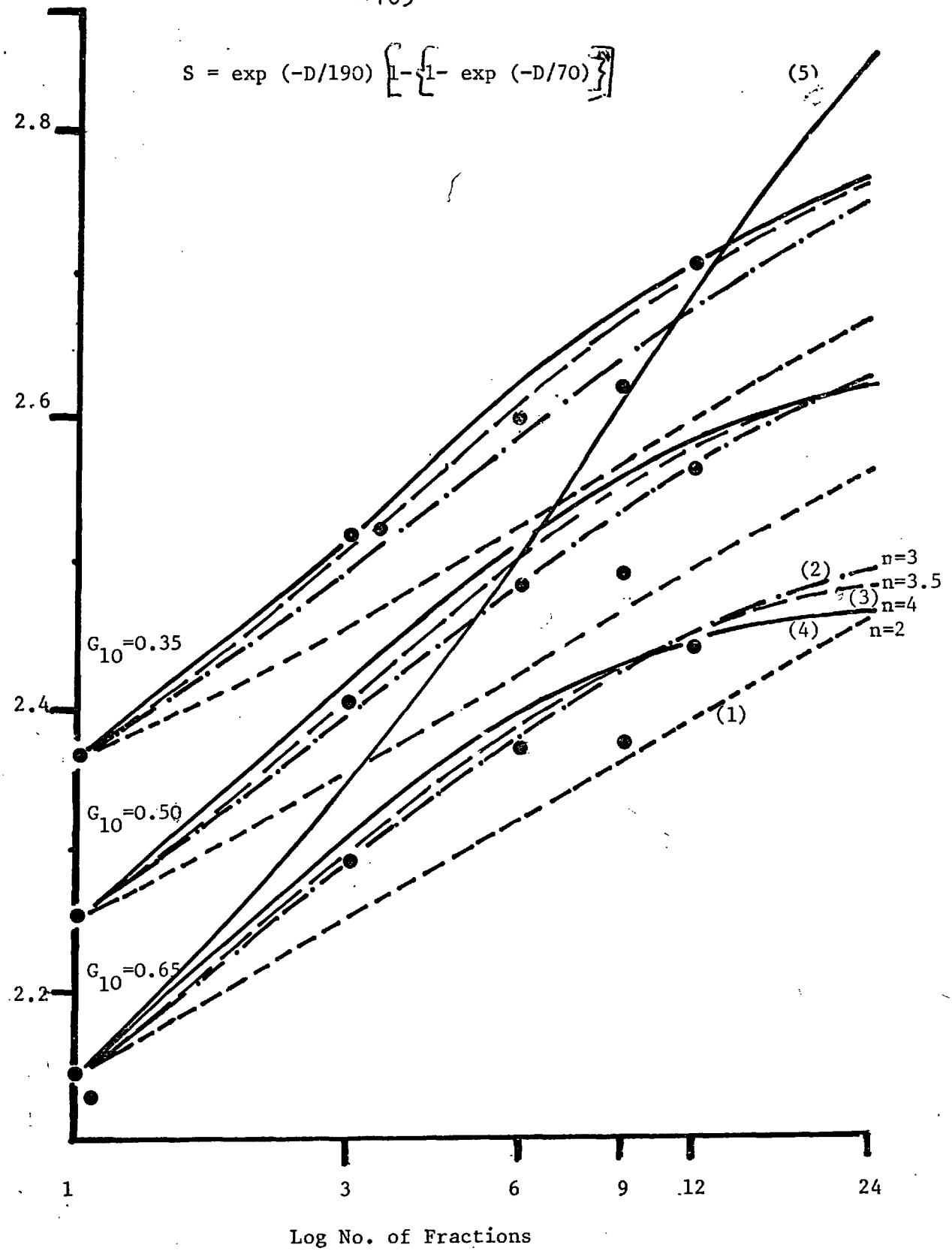


Fig. 4- 3 Iso-effect curves cumputed with the above eqation different values of n have been applied as given on the curves from 1 to 4, similar curves have been cumputed for given G_{10} levels of 0.65, 0.50 and 0.35. Curve (5) have been cumputed with pure multiple-event part of the equation with n=2. Experimental points for Co-60 gamma irradiation regimes have been plotted on these curves.

general agreement with values deduced for mammalian systems (Elkind, 1975).

From the above iso-effect curve fitting for both X and gamma radiation regimes it appears that dose response curves for both radiations could only be described by including a single-hit component. Field et al., (1975) and Elkind (1975) came to similar conclusions for mouse skin reaction studies and various other mammalian system results presented in the 6th L.H. Gray conference. The initial to final slope ratio of both radiations for Vicia root growth inhibitions also being similar to that of mammalian systems. Lower slope ratio in case of X-rays than Co^{60} gamma-rays explains the substantial increase in RBE for the single hit type of damage.

4-111 DOSE RESPONSE CURVES BY FOWLER'S METHOD.

By plotting the reciprocal of the number of fractions against the dose per fraction required to a particular level of survival (and hence a particular G_{10}), a curve is obtained in which the ratio of initial and final slopes is the same as the ratio of initial to final slopes of the logarithmic survival curve, and the intercept on the dose axis is D_Q, the quasi-threshold dose (Fowler et al., 1974). The curves for various values of G_{10}, for X-rays and Co^{60} gamma-rays at 3.5°C are shown in figures 4-4 and 4-5. The values read from the graph vary a little with the values of G_{10} adopted. For G_{10} = 0.65, 0.50 and 0.35 the values for X-rays are 3.0, 2.3 and 2.3 respectively while for Co^{60} gamma-rays they are 3.0, 2.9 and 2.8 respectively. A possible reason for the discrepancies is that there are too few

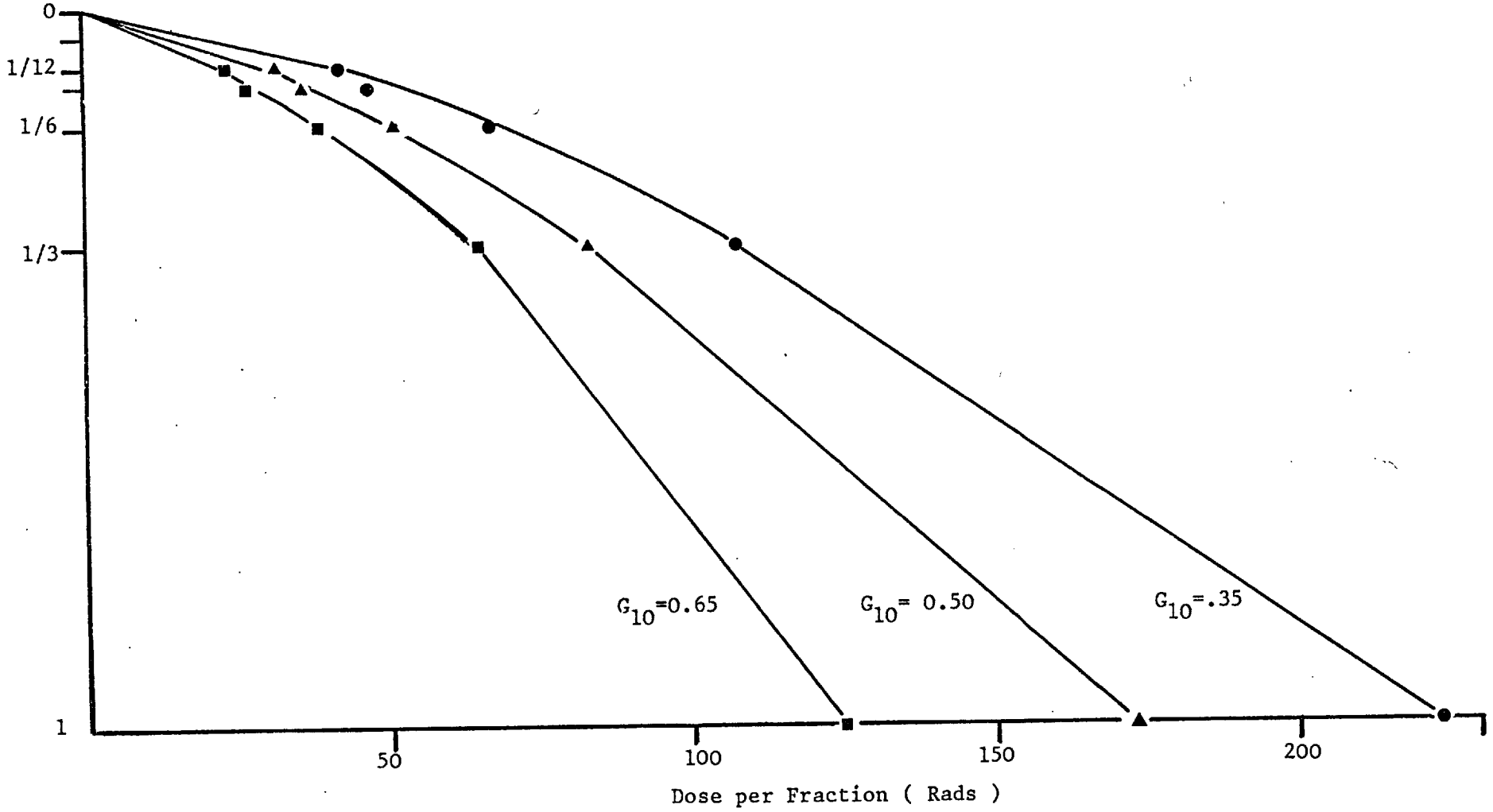


Fig. 4-5 Dose Response Curves by Fowler's Method for Co-60 gamma radiation.

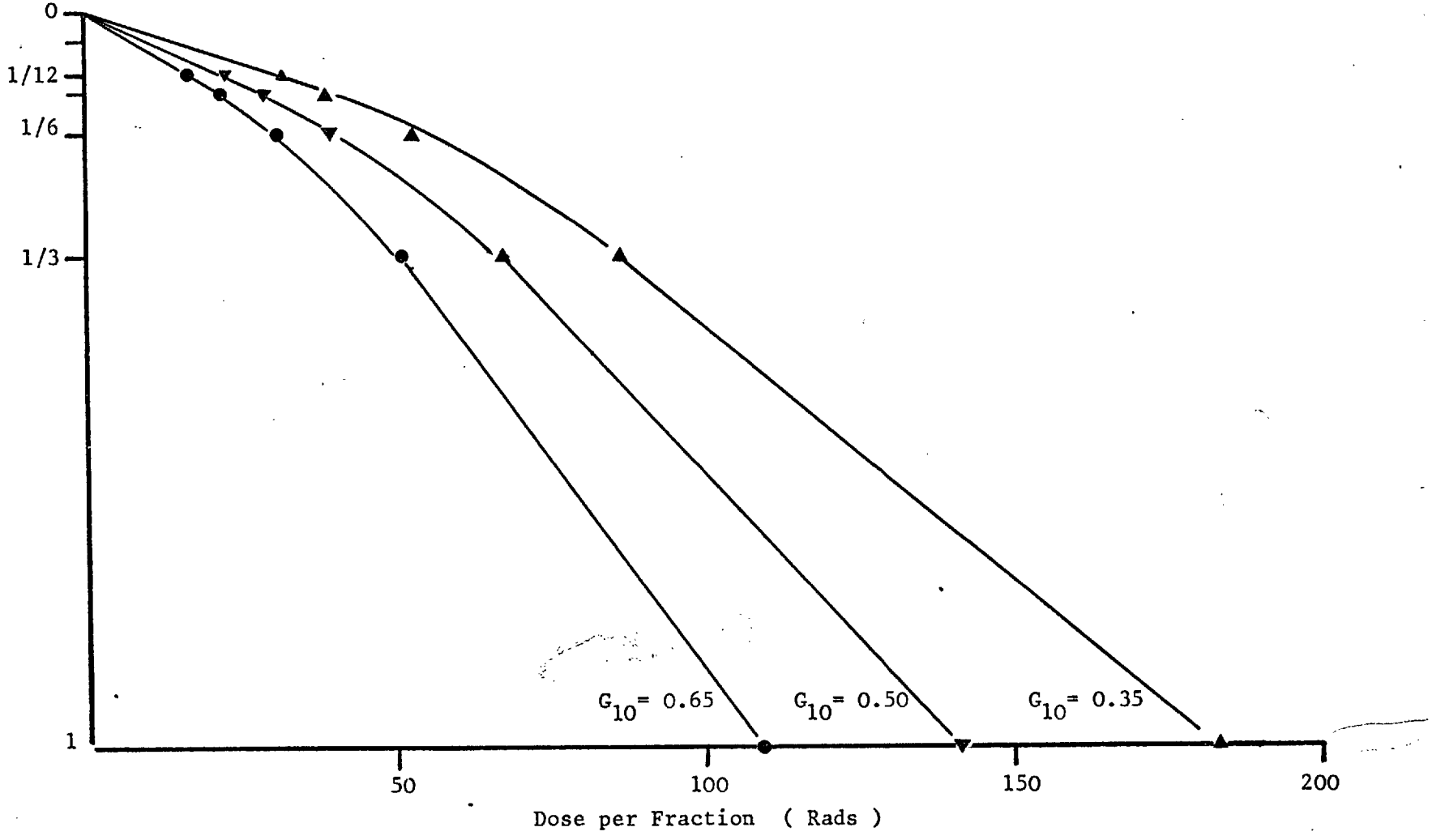


Fig. 4-4 Dose response Curve by Fowler's method for 250KVp x-rays.

experimental points to yield accurate values. The values for similar G_{10}'s i.e. derived for higher doses are more likely to be accurate.

4-IV Ellis's Formula.

It is of interest to consider how well the bean root results can be represented by expressions of the type given by Ellis. (1968).

$$TD = NSD \times N^{0.24} \times T^{0.11}$$

Irradiations given at 3.5°C, a temperature at which no cell division takes place, should depend only on the N exponents

4-IV-a X-rays at 3.5°C.

The iso-effect curves for 250 kV X-rays plotted as log D against log N are not straight lines throughout the range of fractions (Fig. 3-2) and therefore cannot be expressed over their full range in terms of Ellis's power law. However, two straight lines of different slopes can be fitted to the data, one for 1-6 fractions and the other for 6 to 12 fractions (Table 4-IIA). In the later range, where the dose per fraction is small, the N exponent is very small. This result in Vicia is in close agreement with the observations on radiotherapy patients of Deutreix and Wambersie (1973). They found exponents of 0.15 over a range 200 to 500 rads dose per fraction and 0.30 for 500 to 1000rads dose per fraction. Hornsey and Field (1975) also found a similar trend for skin reactions in mice.

4-IV-b X-rays at 19°C.

The iso-effect curves for fractionation effect with

250 kV X-rays at 19°C have N exponents given in Table 4-IIIA. It is possible that time might become a factor in these iso-effect doses due to cell proliferation at a temperature of 19°C.

4-IV-c Co^{60} gamma-rays at 3.5°C.

The N exponent for Co^{60} gamma-ray iso-effect lines are given in Table 4-IIB. Since the lines at different values of G_{10} are parallel they are represented by the same exponent and unlike X-rays a single value applies for 1 to 12 fractions. This is presumably due to the lower effectiveness of single hit component.

4-IV-d Co^{60} gamma-rays at 19°C.

For iso-effect curves with Co^{60} gamma-rays at 19°C temperature the N exponents are given in Table 4-IIIB. They are not significantly different from those of gamma rays at 3.5°C temperature with no influence of cell proliferation on the iso-effect doses.

4 - V. Attempt to fit a Quadratic Exponent.

It is of interest that the quadratic equation of Chadwick and Leenhouts (1973, 1974) has been found to give better description of the survival curve than two-component models, especially when there is no constant final slope. (Douglas and Fowler, 1976). This equation is:

$$S = \exp -(\alpha D + \beta D^2)$$

where S is the survival fraction and the two terms α and β refer to single hit damage and the two hit repairable damage respectively.

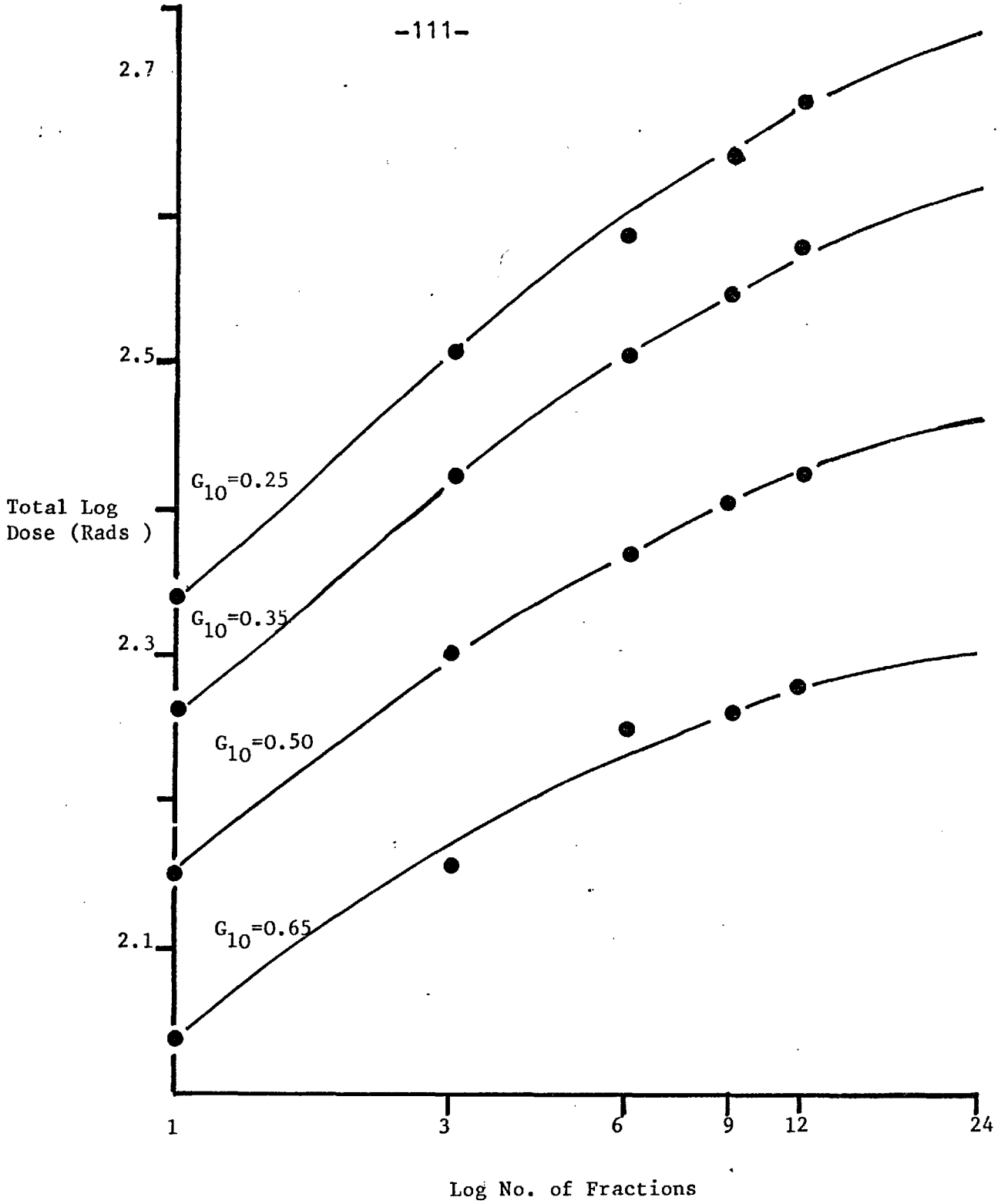


Fig. 4- 6 Iso-effect curves cumputed with the equation ;

$$S = e^{-\alpha D - \beta D^2}$$

with values for α as 0.0054 and that of β as 0.000050. The experimental points of 250KVp X-irradiations obtained in all the fractionation experiments performed at 3.5°C have been plotted on the lines.

For a given G_{10} iso-effect curves were computed assuming α and β values of 0.0054 and 0.000050 respectively. (Fig 4-6). The experimental points for X-rays at 3.5°C have been plotted on these lines. A rather better fit with the theoretical curves is apparent.

Implication for Radiotherapy.

The above results are relevant to fractionation in radiotherapy with supervoltage radiation (Fowler, 1976). The results presented in this section of the thesis confirm that in Vicia roots the effectiveness of X-rays relative to lower LET radiations increases as the dose diminishes. This tendency appears to be present in mammalian systems also. In radiotherapy RBE itself is of no direct importance once an adjustment of dosage has been made. The therapeutic advantage of a treatment regime lies in the differential response of malignant and normal tissues, and more information is required before studies such as these can influence radiotherapeutic practice. However, Barendsen, (1975) and Elkind (1975) have suggested that substantial improvement could be made, if we have adequate knowledge of fractionation effect in human tumours and their supporting tissue.

TABLE NO. 4 - I.

INITIAL TO FINAL SLOPE RATIO'S, deduced from fractionation experiments with various mammalian systems with those for Vicia faba of the present study.

SYSTEM	RADIATION	D_1/D_o	AUTHORS
Human Skin	Co^{60} gamma-rays	3	WAMBERSIE et.al. (1974)
Mouse Intestine	Co^{60} gamma-rays	3	WAMBERSIE et.al. (1974)
Mouse Skin	Co^{60} gamma-rays	3	WAMBERSIE et.al. (1974)
Mouse Skin	240Kv X-rays	5,3	DOUGLAS et.al. (1975)
Mouse Skin	240Kv X-rays	3(0.18-0.50)	DENEKEMP and HARRIS (1975)
Mouse Lung	250Kv X-rays	3,7	FIELD and HOUNSEY(1975)
Mouse Skin	250Kv X-rays	3,7	FIELD and HOUNSEY(1975)
Mouse Testis	Co^{60} gamma-rays	2.5	WITHERS (1975)
Mouse Colon	Co^{60} gamma-rays	2.6	WITHERS (1975)
Mouse Jejunum	Co^{60} gamma-rays	3.3	WITHERS (1975)
Mouse E.S.C.	Co^{60} gamma-rays	3.3	WITHERS (1975)
Mouse Skin	250Kv X-rays	2.5	FOWLER et.al. (1974)
Mouse Skin	250Kv X-rays	5-10	FIELD et.al. (1975)
Vicia faba	250Kv X-rays	3.09(2.3-3.5)	PRESENT RESULTS
	Co^{60} gamma-rays	3.72	PRESENT RESULTS

E.S.C. Endogenous spleen colony forming cells.

REFERENCES IN PROC 6th L.H. GRAY CONF.

TABLE NO. 4 - II.

A 250 kV X-rays at 3.5°C.

1 - 6 Fractions

G_{10}	N EXPONENT	95% CONFIDENCE LIMITS
0.65	0.25	± 0.01
0.50	0.26	± 0.03
0.35	0.27	± 0.02
6 - 12 Fractions		
0.65	0.06	± 0.02
0.50	0.09	± 0.02
0.35	0.12	± 0.03

B Co^{60} gamma-rays at 3.5°C.

1 - 12 Fractions

G_{10}	N EXPONENT	95% CONFIDENCE LIMITS
0.65	0.28	± 0.04
0.50	0.30	± 0.04
0.35	0.28	± 0.06

TABLE NO. 4 - III.

A X-radiation at 19°C.

1 - 6 Fractions.

G_{10}	N EXPONENTS	95% CONFIDENCE LIMITS
0.65	0.40	± 0.04
0.50	0.35	± 0.11
0.35	0.56	± 0.06

B Co^{60} gamma-radiation at 19°C.

1 - 6 Fractions.

G_{10}	N EXPONENTS	95% CONFIDENCE LIMITS
0.65	0.37	± 0.04
0.50	0.33	± 0.11
0.35	0.29	± 0.17

Part II.

Chapter 5

Radiosensitization Effect of Misonidazole (Ro-07-0582) with fractionated irradiations of 250 kVp X-rays in *Vicia faba* roots.

5-1 INTRODUCTION.

It is still an open question whether oxygen exerts its sensitizing effect as a simple dose modifier or whether the degree of sensitization is a function of dose (Moore, 1975). It is established that effect of oxygen decreases with increasing LET of the radiation. The presence of a definite initial slope on a dose response curve suggests that some depositions of energy are large enough to produce these observed radiation effects in a single event. Even in a low LET radiation there is an effective high LET component (Rossi, 1965). One might therefore expect a low oxygen enhancement ratio (OER) with doses on the initial region of a survival curve or at low dose rates. (Neary, 1956; Porter, 1965). Wideroe uses the term α - component of radiation damage and postulates that this component is independent of oxygen. Under extreme hypoxic conditions it has been found that only the α - effect component of radiation damage occurs. (Wideroe, 1976). Lower OER has been observed for low dose rates in Hela cells by Hall, Bedford and Oliver (1966) and low dose rate limit is similar to anoxic acute responses. (Oliver, 1967; Hall et. al., 1966). OER has been measured directly on both the initial region and on the terminal slope of a dose response curve and also in irradiations given as repeated small doses. Contradictory results have been found (Moore,1975).

It is likely that the presence of hypoxic cells in tumours present a problem in radiotherapy (Fowler, 1972) and the use of hypoxic cell sensitizers offers an interesting possibility for overcoming this difficulty. (Fowler and Adams, 1973). Among these Flagyl (May and Baker) and Ro 07-0582 (Roche) have proved to have most suitable pharmacological

properties for clinical use. (Asquith et. al., 1974; Denekemp Michael and Harris, 1974). Ro 07-0582 is more effective than Flagyl (Denekemp and Harris, 1976; Sheldon et. al., 1976) and has found its way through to clinical trials (Adams, 1976).

As a test system for the early development of hypoxic radiosensitizers Vicia faba bean roots have been used as well as bacteria and mammalian cells (Fowler, 1972). The effect of Flagyl on the irradiation of anoxic roots at 19°C temperature has been investigated by Foster and Wilson, (1973); Asquith et. al., (1974); Hall and Fairchild, (1975).

5-1A Questions Investigated.

The importance of the shape of the shoulder region of a survival curve has radiotherapeutic as well as radiobiological significance. Shoulder modifiers could lead to improved radiotherapy as well as help elucidate the kinetics of radiation damage. Therefore meausrements were made of the enhancement ratio of the drug under anoxic conditions with single doses of 250 kVp X-rays and with fractionated doses. The beans were held at low temperature to avoid cell proliferation. To confirm that drug 0582 is a selectively hypoxic radiosensitizer a single dose experiment was done with irradiations given under aerobic conditions with and without added drug.

It is known that many hypoxic radiosensitizers interfere with cellular metabolism (Biaglow et. al., 1974) therefore enhancement ratio of the drug was also measured at 19°C (an optimum temperature for cell proliferation) for single doses of 250 kVp X-rays.

5-11 MATERIALS AND METHODS.

Materials and methods adopted were almost the same as described in chapter 2 (Part I).

Eight groups of beans were prepared for each experiment. Three groups were allocated to each of the pair of treatment schemes i.e. anoxic irradiations and anoxic with 5mM 0582 treated irradiations. Two control groups were allocated to each of the irradiation schemes and these were sham irradiated. One, three, six and twelve fraction experiments were done by giving all the exposures at 3.5°C and fractions were similarly spaced 24 hours apart to allow sub-lethal damage recovery.

5-11A Drug Ro 07-0582 Treatment And Production Of Hypoxia.

The drug solution was prepared at a concentration of 5mM fresh before use. This concentration of drug was within the non toxic concentration range (clinically) and was also expected to produce a substantial effect considering its reaction with mammalian cells (Hall and Chapman, 1975; Hall et. al., 1975; Denekemp and Harris, 1976). The drug solution was stored in the dark in a refrigerator to cool the solution temperature to 3.5°C before treatment to the beans. In the jigs allocated to anoxia + 0582 irradiation the drug solution was replaced with water about 35 minutes before irradiation. Flagyl penetrated within 10 minutes in the bean roots at 19°C therefore 35 minutes was thought to be sufficient at a lower temperature of 3.5°C for drug penetration. During this treatment period white spot nitrogen was bubbled through all the jigs at a rate of $\sim$ 2 litres/minute.

The efluent gas from the jigs was monitored by a 'thermox O_2' analyser and contained 8ppm of O_2 after 35 min. (Dr. Noel Evans; M.R.C., Cyclotron, efforts for this are gratefully acknowledged.) This period of N_2 introduction at this purity is sufficient to create a biologically significant hypoxia, (Hall and Cavanagh, 1967) and had no untoward effects on bean root growth.

5-11B Irradiations.

For irradiations, the N_2 tube connections were removed and plugs were placed on the openings, thus the jigs were air tight during transfer to and back from the irradiation room. In the irradiation room N_2 bubbling was resumed until the end of an exposure. Nylon tubing was used as much as possible for N_2 connection. At the end of irradiation each of the drug treated bean roots group was washed with cold water at a temperature of 3.5°C three times before these jigs were immersed in the cold water bath.

5-111 RESULTS.

5-111A Measurement Of OER At 3.5°C.

First the OER for single doses of X-rays at 3.5°C for Vicia faba root growth was determined. The results are plotted in figure: 5 -1. The data have been plotted as G_{10} on linear scale versus log dose. A pair of parallel lines was drawn through the experimental points. The OER is given by the horizontal separation of the lines drawn for aerated and nitrogen (anoxic) responses. A value thus obtained is OER 2.65 with 95% confidence limits of (2.19 - 3.21) which is quite in agreement with that measured at Oxford. (Hall and Cavanagh, 1967).

5-111B Drug Effect At Different Fraction Regimes At 3.5°C.

Two independent experiments were done to measure the enhancement ratio of the drug for single doses. One experiment was done for each scheme of three, six and twelve fractions given in the absence and presence of 5mM concentration of Ro 07-0582. The results are given in table 5 - I and II and plotted in figures 5 - (2 - 5). The sensitivity enhancement ratios are given in table 5 - II for one, three, six and twelve fractions respectively. The 95% confidence limits given in brackets. These values closely overlap on each other showing a constant enhancement ratio at all dose levels in various regimes.

5-111C Drug Effect Under Aerated Condition.

One pilot experiment as control was done for single doses given under aerated conditions with and without the drug at the same concentration. The data ~~given in table 5 - II and~~ have been

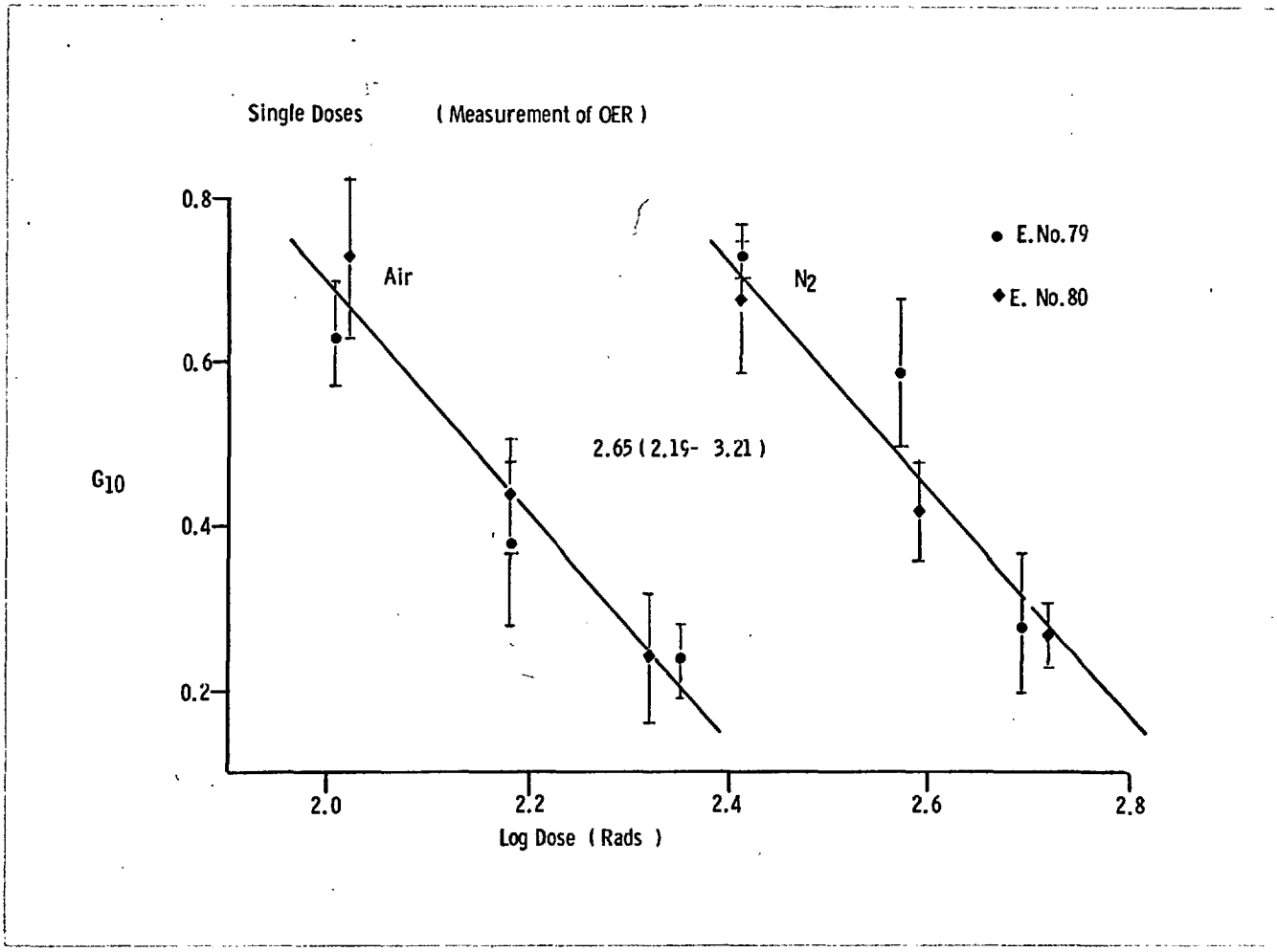
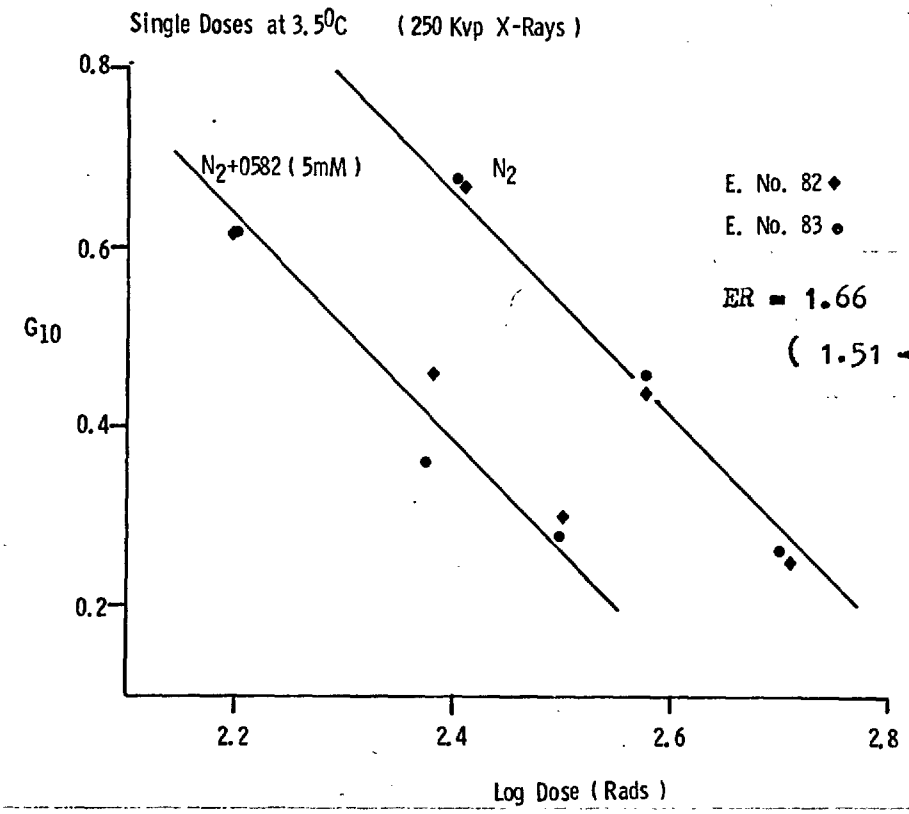
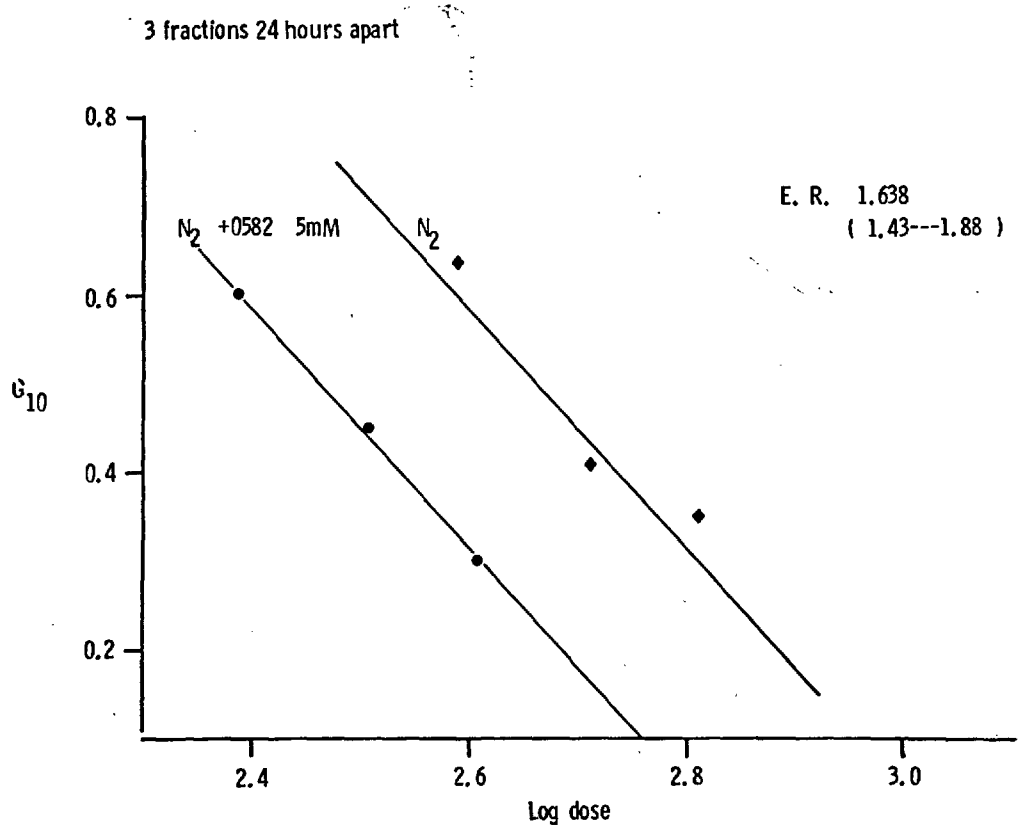


Fig.: 5-1 Single doses of 250 KV x-rays given under anoxic (N_2) and aerated (Air) conditions at 3.5^0C.

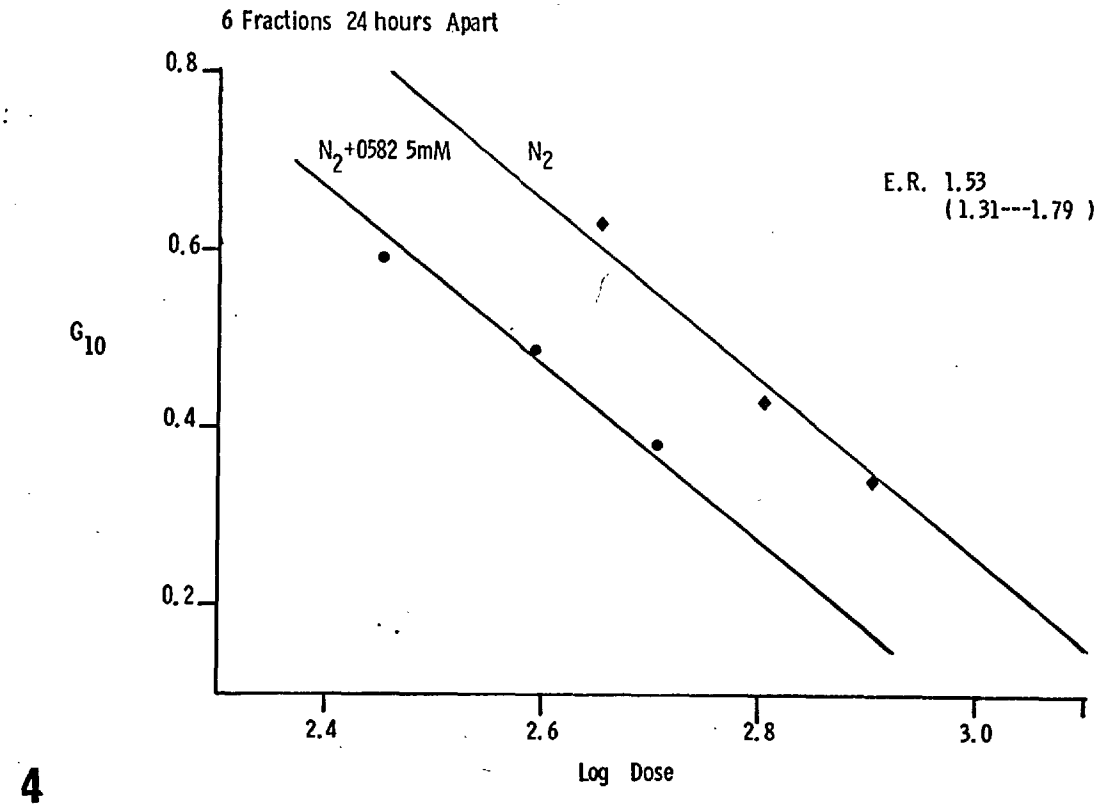


2

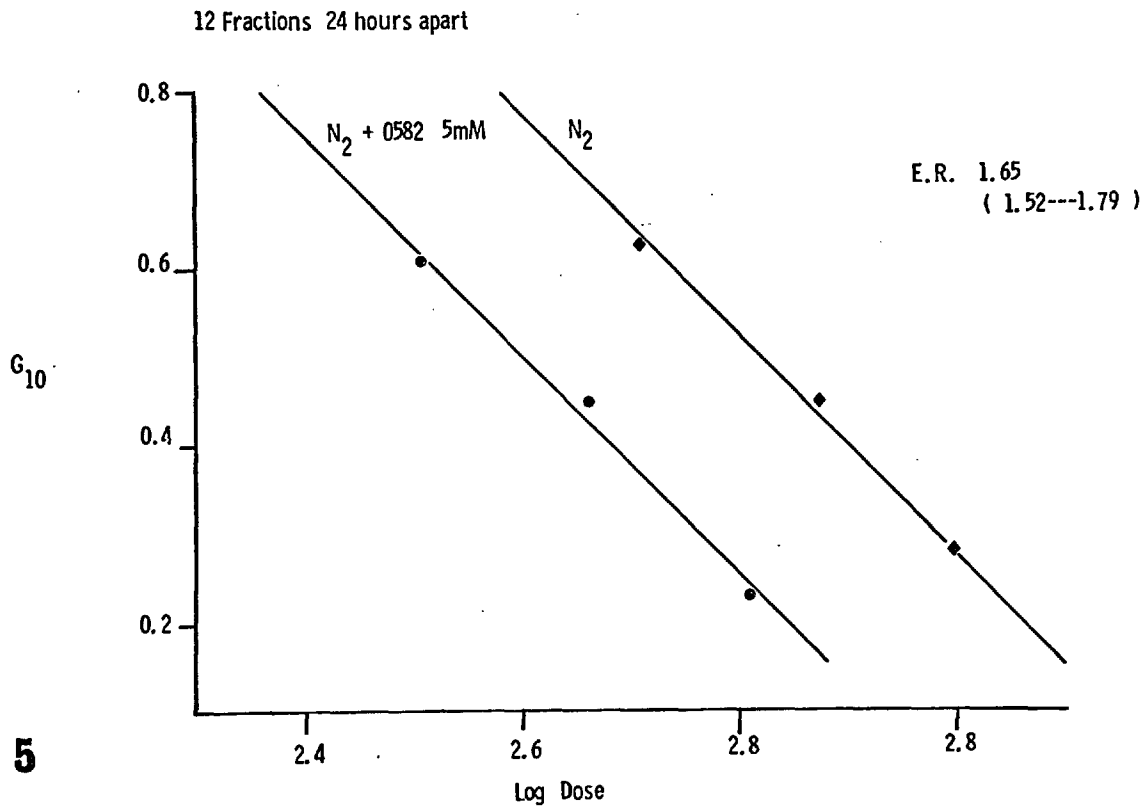


3

Figs. 5, 2-3 Single doses and three fractions of 250 KV x-rays given under anoxic condition with the drug (N_2+0582) and without (N_2) at 3.5°C



4



5

Figs. 5, 4-5 Six and 12 fractions of x-rays given under anoxic condition with the drug and without at 3.5^0C.

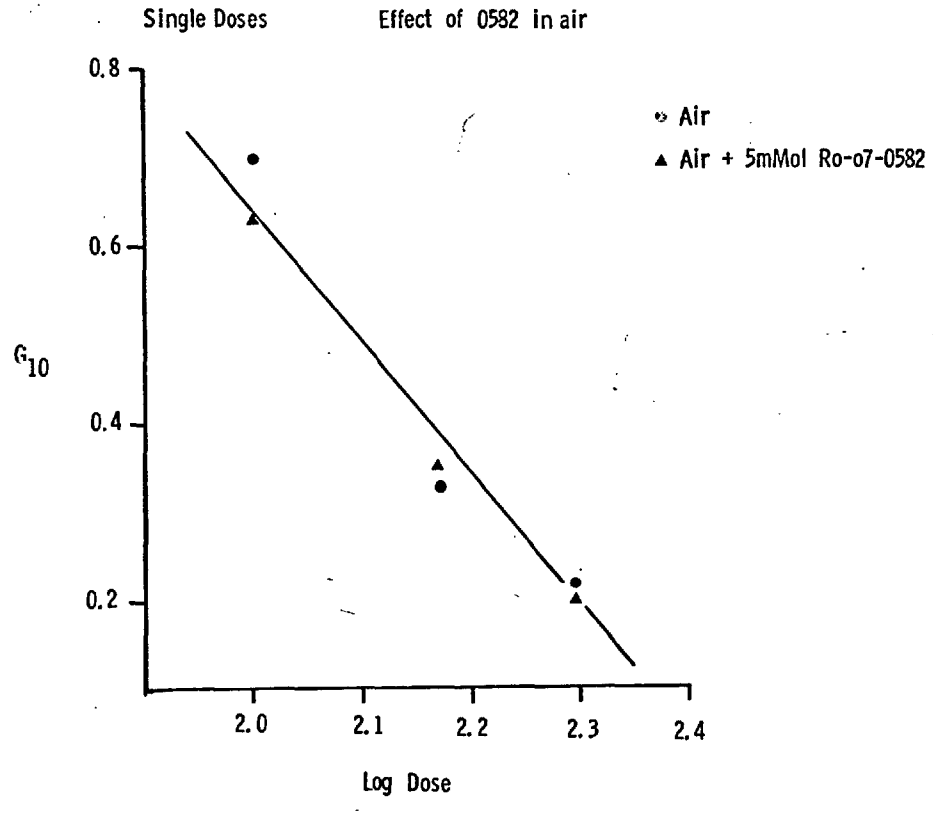


Fig. 5-6 Single doses of x-rays given under aerated condition with the drug and without at 3.5^{0}C.

plotted in Fig.5-6 shows that the drug 0582 has no detectable effect under aerated conditions. All the points lie essentially on a same straight line for both conditions.

5-111D Drug Effect At 19°C Under Anoxia.

A single dose experiment was done to measure the enhancement ratio at 19°C with same concentration i.e. 5 mM. Fig. 5-7 show the results. The enhancement ratio at 19°C has been increased to 2.23 (2.02 - 2.45 95% confidence limits) and is significantly different from the values determined for enhancement ratios measured with fractionation regimes at 3.5°C.

Bean roots in this experiment were measured daily, to check whether the pattern of recovery was seriously influenced by the drug. The results have been plotted in Fig.5-8. In view of the experimental enhancement ratio of 2.23 one would expect the results with 157 rads and 235 rads with sensitizers to be similar to doses of 375 rads and 500 rads respectively. This is apparently the case.

TOXICITY.

To estimate the possible toxicity of the drug, the ten day growth of the control in the drug treated group was compared with that of non-treated to give G_{10} of the drug toxicity. The number of exposures of the drug was the same as the number of fractions in the corresponding irradiated group. The results are plotted in Fig.5-9 and give no evidence of amplified toxicity.

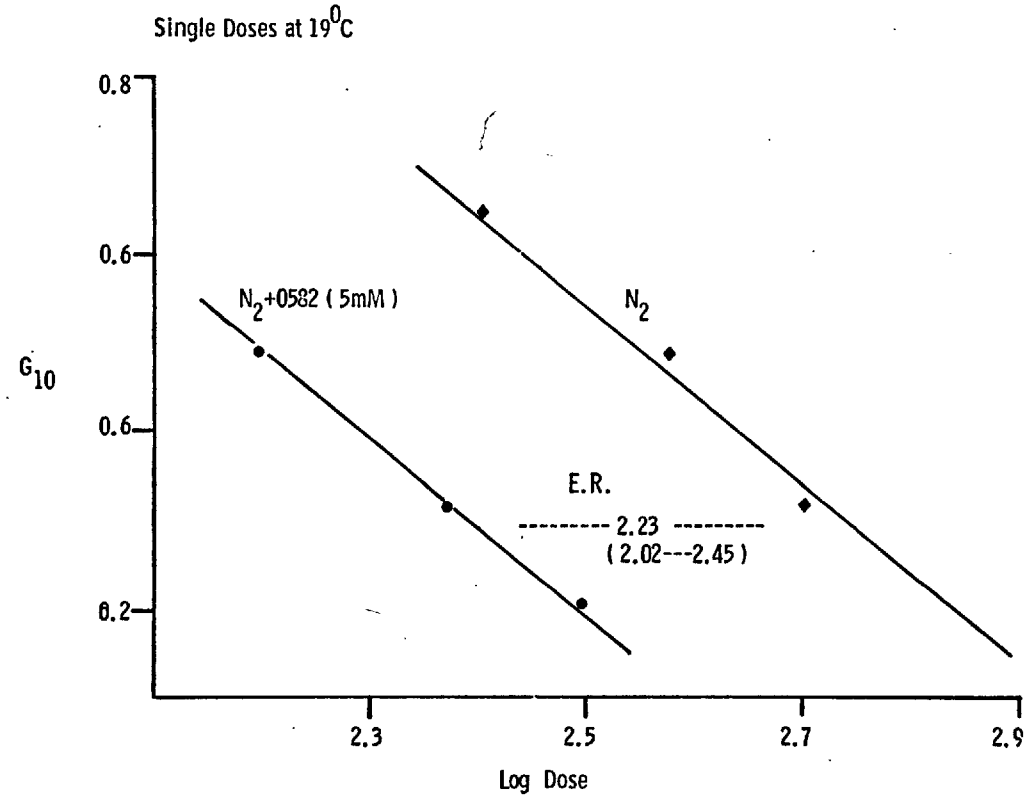


Fig.: 5-7 Single doses of x-rays given under anoxic condition with the drug (N_2+0582) and without at normal temp. of 19^0C for root growth.

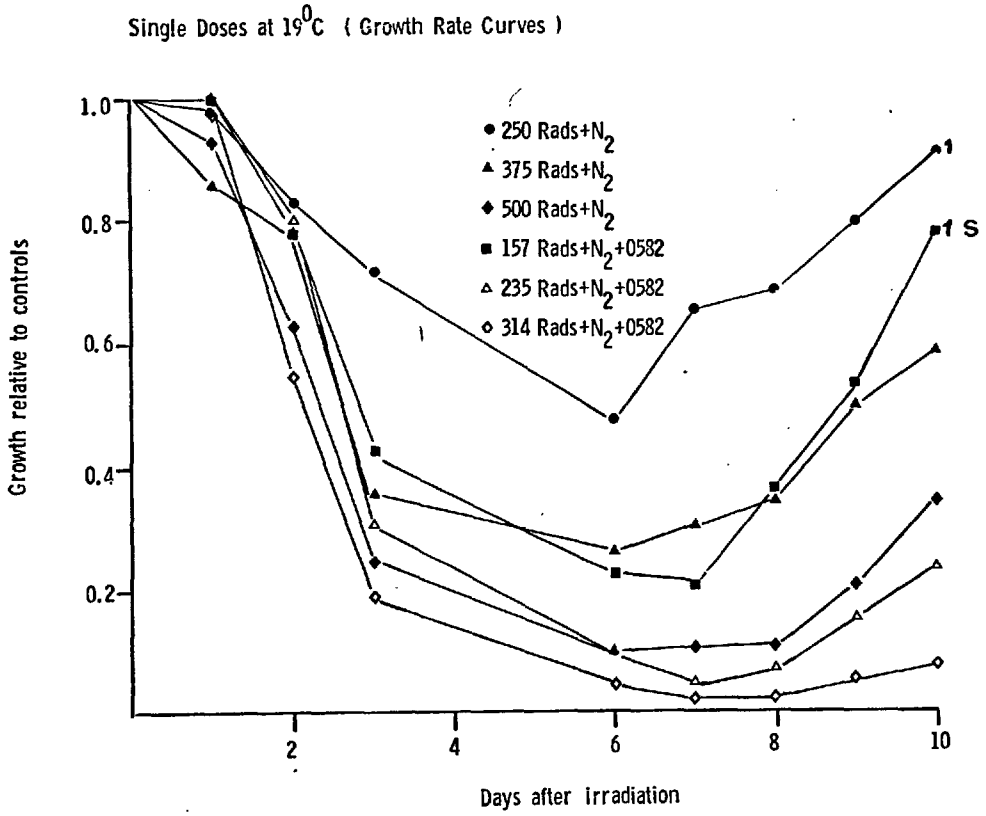


Fig.: 5-8 Growth rate after various single doses of x-rays given under anoxic condition with the drug (N_2+0582) and without (N_2) .

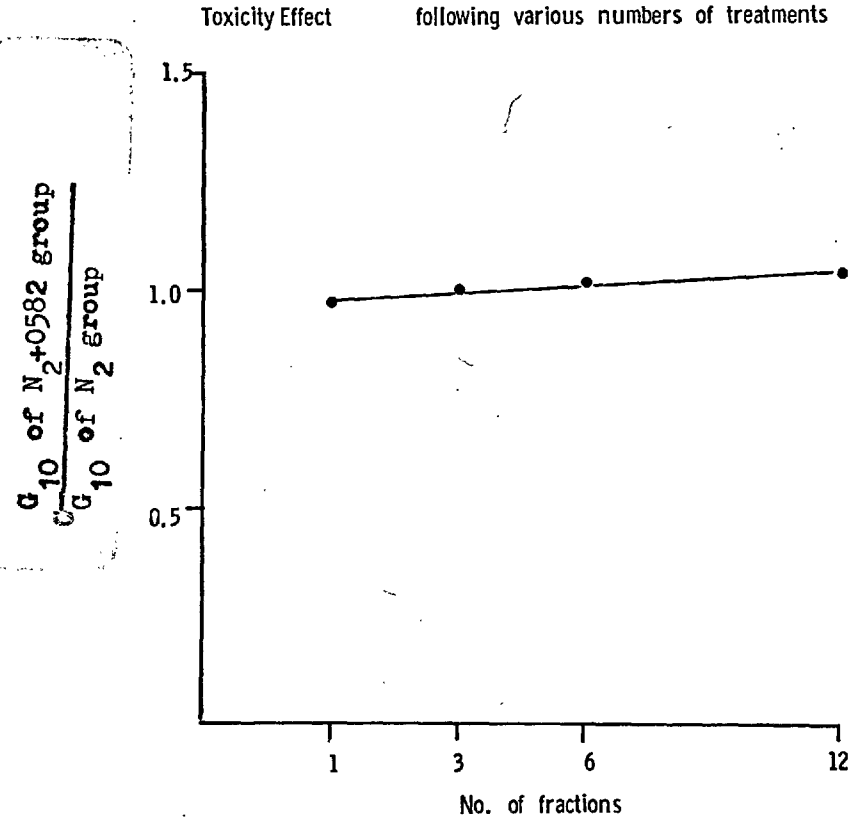


Fig.: 5-9 The effect of the drug 0582 in the absence of irradiation on G_{10} at 3.5^0C

5-1V DISCUSSION AND CONCLUSION.

All the regression lines for 1, 3, 6 and 12 fraction regimes at 3.5°C under anoxic conditions with Ro 07-0582 and without are replotted in Fig. 5-10&5-11. It is evident from these figures that when the dose was delivered in three fractions or six fractions the dose response curves have shifted to the right and a substantial increment of dose is required to produce an equivalent effect to the single dose. However, when the dose was delivered in twelve fractions no further shift in the dose response curve is evident. It would appear that for doses less than 50 rads per fraction (the lowest dose in the 6 fraction irradiation) the effect is predominantly due to single event interaction, from which there is no recovery. Similar response has been demonstrated in the first part of this thesis when 250 kVp X-ray fractions were given under aerated conditions at 3.5°C temp rature. (Fig.3-1 Page. 59). The fractionation effect at higher doses per fraction shows that recovery of sub-lethal damage does take place under such a level of hypoxia and that it is not influenced by the additions of drug . (Hall et. al., 1975; Hall, 1972; Revesz and Littbrand, 1975).

A constant enhancement ratio has been observed throughout the range of doses given in various fractionated regimes. Table 5 - II, Figs:5-12&5-13 show the results for single and twelve fractions under anoxic conditions with and without addition of the sensitizer 0582 compared with the results under aerobic conditions. The results strongly support the conclusion that the drug has equally affected both the multiple and single event types of damage. The drug 0582

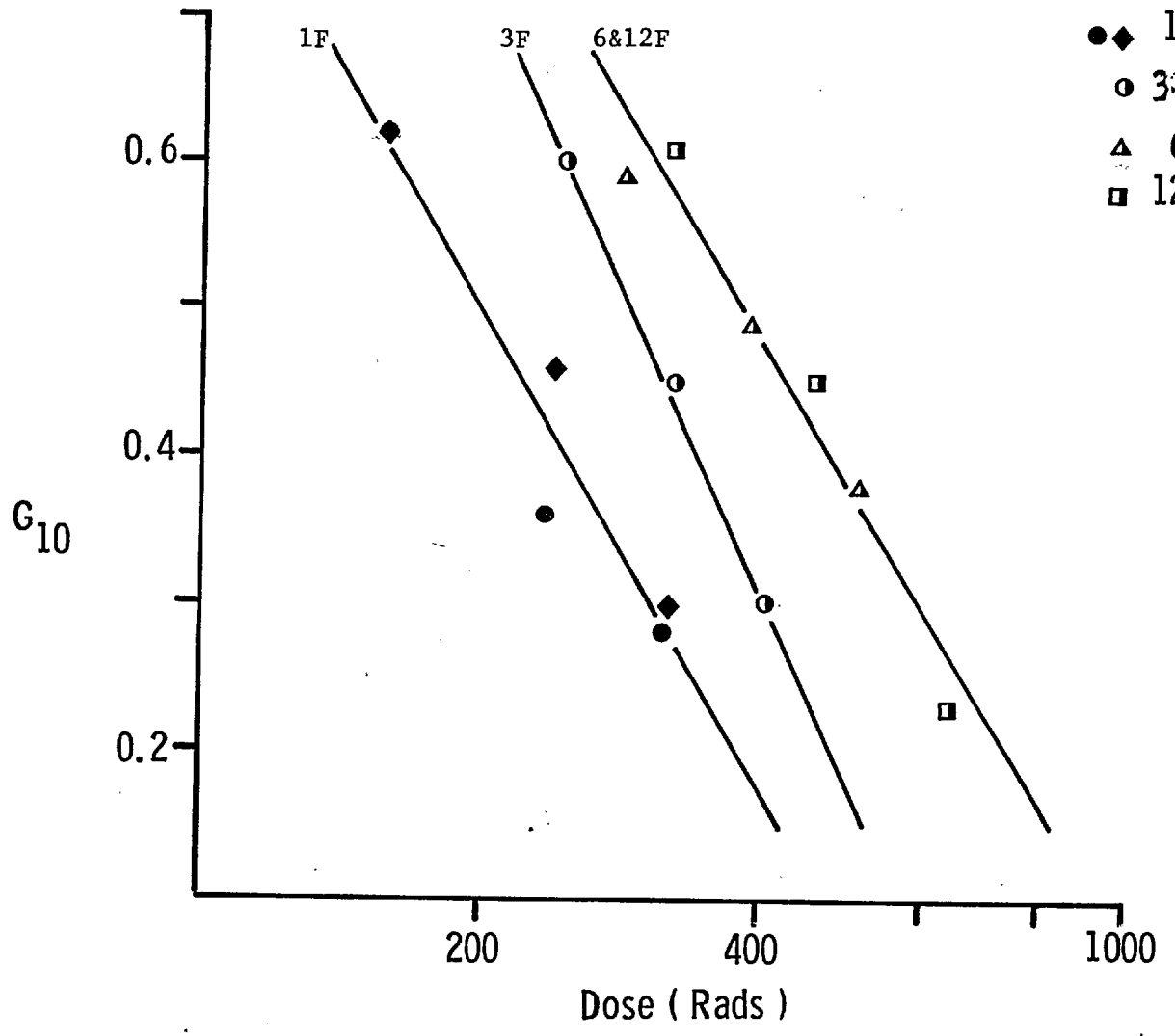


Fig. 5-10 Fractionated irradiations with 250KVp X-rays at 3.5°C under anoxia with Ro-o7- 0582.
(A single regression line have been fitted to the entire data of 6 and 12 fractions.

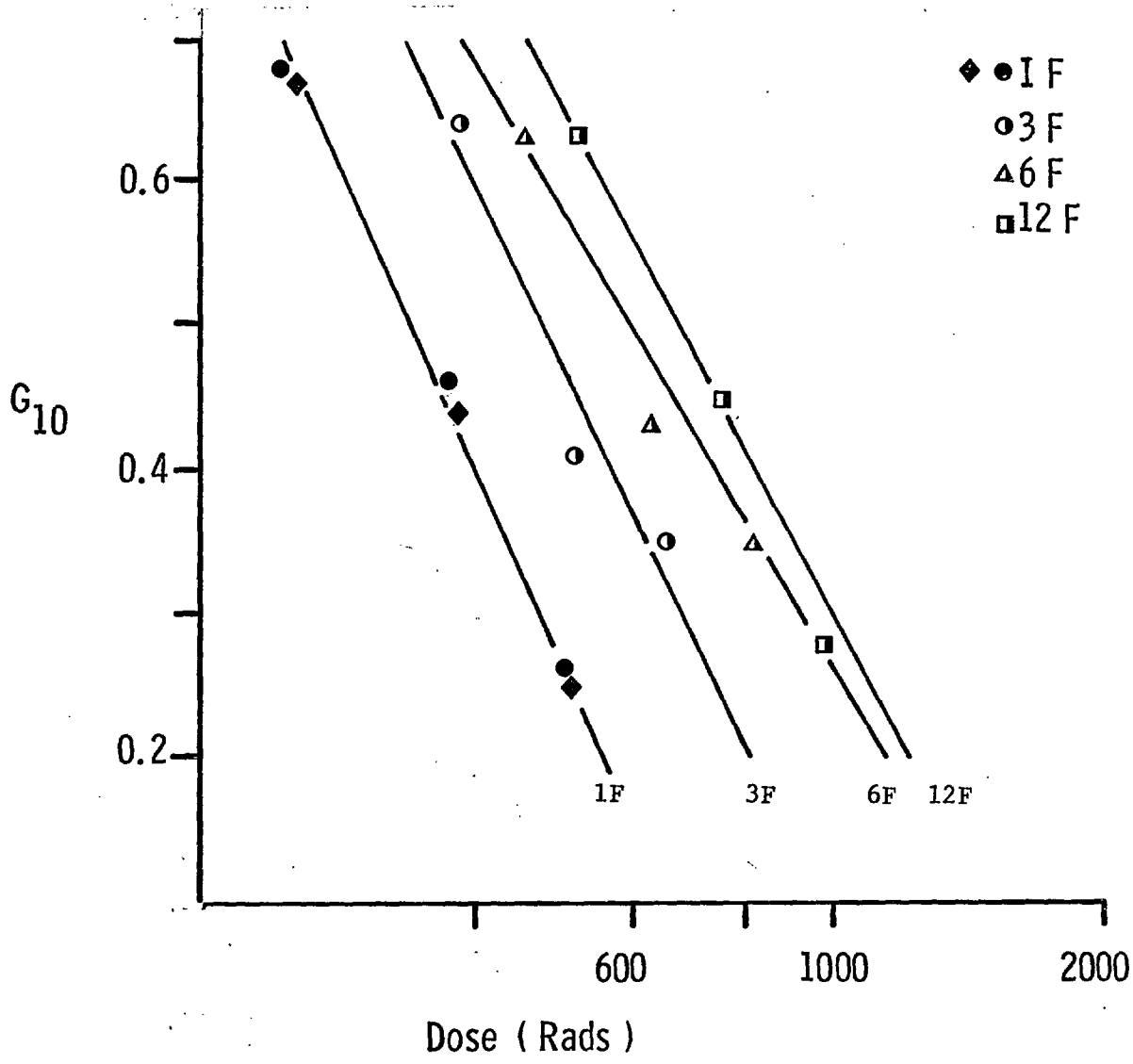


Fig. 5-11 Fractionated irradiations with 250KVp X-rays at 3.5°C under anoxia.

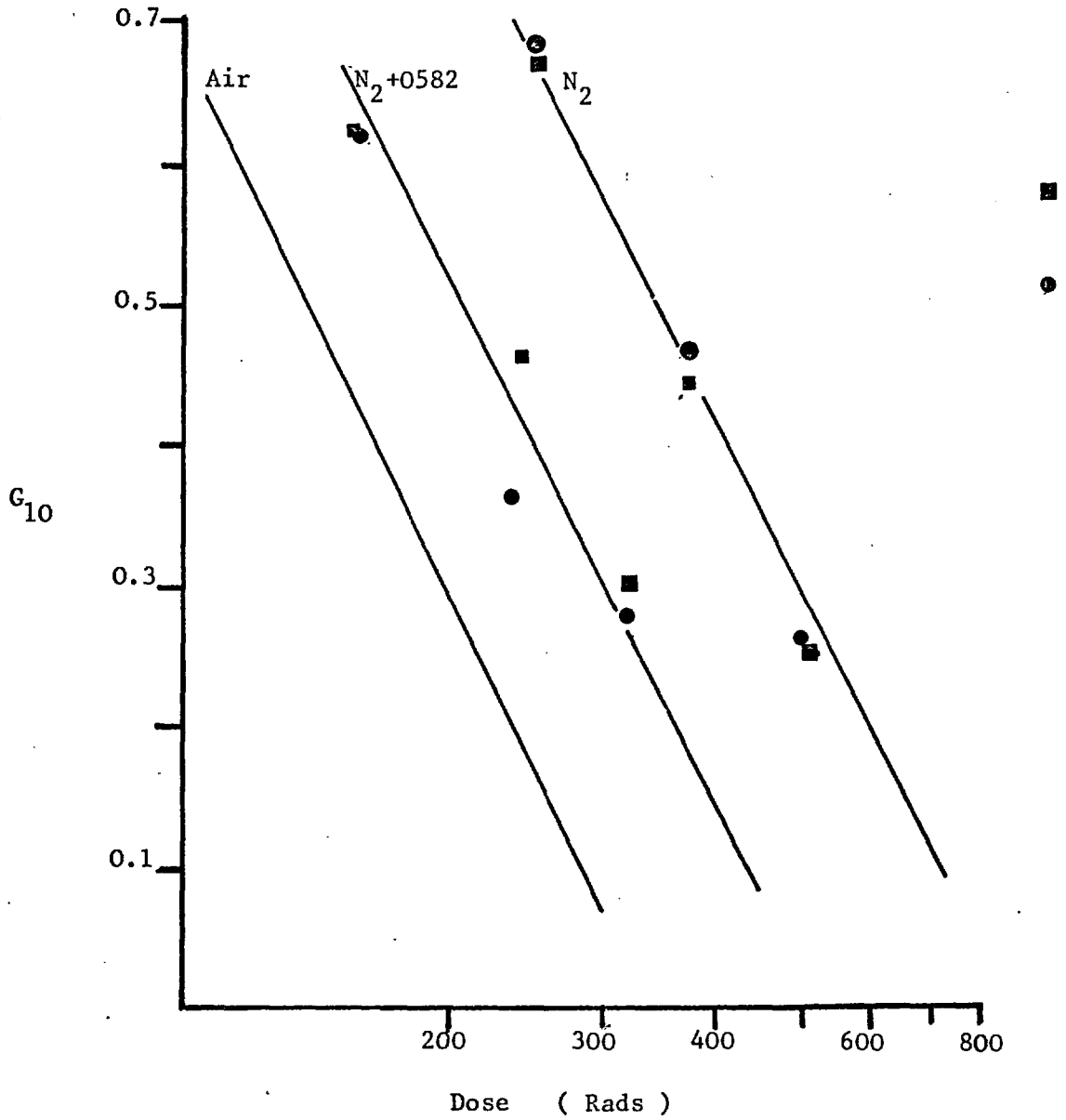


Fig. 5-12 Single doses of 250KVp X-rays given under aeration (Air), anoxia with 0582 (N_2+0582) and anoxia (N_2) at 3.5^{0}C (Lines fitted on assumption that OER and ER are same at all dose levels.)

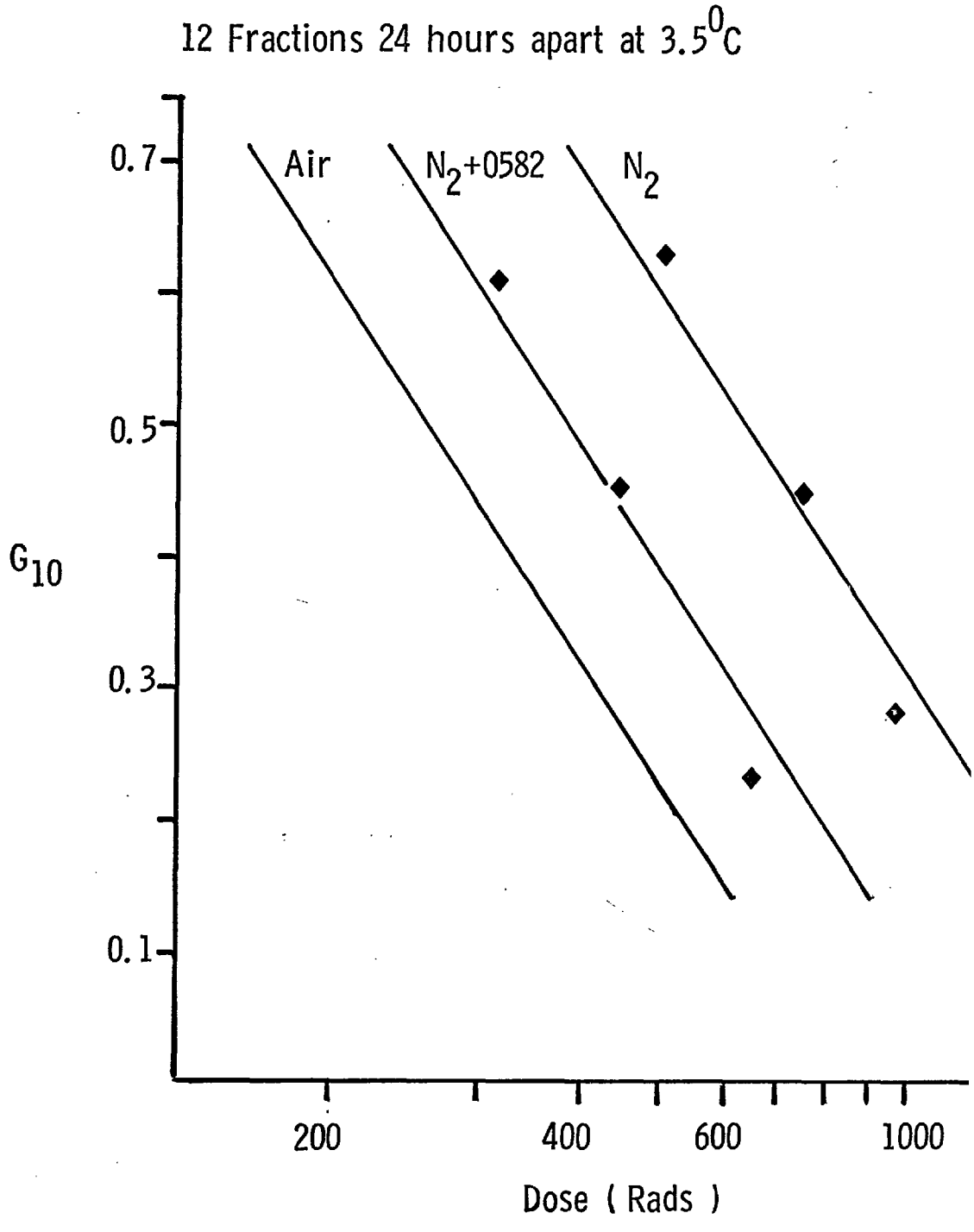


Fig. 5-13 Fractionated irradiations with 250KVp X-rays under aeration (Air) , anoxia with 0582 and anoxia only (N_2).
(Lines fitted on assumption that OER and ER are the same at all dose levels)

appears to act similarly to oxygen, as a simple dose modifying agent.

These results contradict the hypothesis of Wideroe (1975) that alpha and beta types of interactions are affected differentially. The hypothesis might, however, hold good under extreme hypoxic conditions (Revesz and Littbrand, 1975). It has been shown with Chinese hamster cells the drug 0582 enhanced the quadratic component ' ' (Chapman, Gillespie, Reuvers and Dugle, 1975) while present results demonstrate that both linear and quadratic or alpha and beta components respectively are equally influenced by the drug when added under anoxic conditions,

Comparison of the Results with Oliver's (1964) Dose Response Curve.

Theoretical iso-effect curves assuming a constant enhancement ratio of 1.6 for irradiation under anoxia with and without 0582 were computed. They were based on the values of D_0 = 43 rads, D_1 = 133 rads and n = 3.5 as under aerobic irradiations (Chapter 4 Section 4-11B). Assuming the enhancement ratios of 1.6 with drug and 2.5 under anoxia without drug. Experimental values for both conditions i.e. anoxia with 0582 and without were compared. There appeared to be a reasonable agreement between the experimental values and the theoretical predictions. Thus the initial to final slope ratio (0.3) remains constant under both anoxic with drug and without drug.

Radiosensitization with 0582.

At a concentration of 5mM, 0582 gave an enhancement ratio

(ER) of 1.6 which implies that the use of 0582 would be as effective in overcoming the resistance of hypoxic cells as the use of neutrons. (Hall, 1969). The ER increased to 2.3 at 19°C which is very much the same as OER (Fig.5-7 Page. 121), for this system. Therefore at a temperature of 19°C the optimum for cell metabolism, the drug mimics the effect of oxygen. This finding is common with those in various mammalian systems. (Hill and Fowler, (1977) ER = (2.0); Peters, (1976) ER = (2.1); Sheldon et. al., (1976) ER = (1.8); Sheldon, (1975) ER = (2.0); Brown, (1975) ER = (2.3); Stone and Withers, (1975) ER = (2.4)). Hall and Roizive-Towell (1975) found for Chinese hamster cells that the effect of 5mM concentration of 0582 was 40 - 80% of that of oxygen effect. An increased enhancement ratio of 2.3 at 19°C compared to 1.6 at 3.5°C suggests that metabolism is playing a significant part in producing an increased effectiveness. It may be that the drug interacts in two different ways: direct interaction which is purely electron affinic in nature and temperature independent like O_2 (Neary, 1957) and an indirect action is a multitude of biochemical reaction dependent on metabolism and therefore on temperature.

Toxicity.

No toxicity has been apparent at the lower temperature employed in the fractionated x-irradiations study (Fig. 5-9 Page. 128). The toxicity of the drug 0582 is being studied in some detail (Moore et. al., 1976) it has been demonstrated for Chinese hamster cells that the drug is selectively toxic to the hypoxic cell (Hall and Roizive-Towell, 1975), this may be another point in favour of the clinical use of 0582.

Toxicity of 0582 on the roots at 19°C has not been investigated. From the Growth Rate curves (Fig.5-8) it is apparent that the drug treated groups show a delayed recovery from the radiation damage, due to interference with the repair of potentially lethal damage producing a delay in recovery.

TABLE 5-I

Data for ER measurements for different Fractionation regimes of 250kVp X-ray.

Single Dose at 3.5°C

With 0582			Without 0582		
Log Dose	Dose in rads	G_{10}	Log Dose	Dose in rads	G_{10}
2.202	159	0.62	2.403	253	0.68
2.374	236	0.36	2.577	377	0.46
2.498	315	0.28	2.703	504	0.26
2.197	157	0.62	2.408	256	0.67
2.380	240	0.46	2.578	378	0.44
2.506	320	0.30	2.710	513	0.25

3 Fractions 24 hours apart at 3.5°C

2.386	243	0.60	2.586	385	0.64
2.508	322	0.45	2.710	513	0.41
2.605	402	0.30	2.809	644	0.35

6 Fractions 24 hours apart at 3.5°C

2.452	283	0.59	2.655	452	0.63
2.593	392	0.49	2.804	637	0.43
2.706	508	0.38	2.906	805	0.34

12 Fractions 24 hours apart at 3.5°C

2.506	320	0.61	2.707	509	0.63
2.661	458	0.45	2.875	750	0.45
2.808	642	0.23	2.991	979	0.28

TABLE 5-II

ER with 5mM drug RO-07-0582 for 1,3, 6 and 12 fractions at 3.5°C and 1 fraction at 19°C.

Fraction at 3.5°C	ER at G_{10} = 0.50	95% limit
1	1.66	(1.51 - 1.82)
3	1.64	(1.43 - 1.88)
6	1.53	(1.31 - 1.78)
12	1.65	(1.52 - 1.79)
at 19°C		
1	2.23	(2.02 - 2.45)

ACKNOWLEDGEMENT.

It is my great pleasure to express my gratitude to my supervisor the late Professor Raymond Oliver whose continuous encouragement, knowledgable discussions and useful criticism were essential assests to complete this study. And not the least to Dr. E.W. Emery who took charge after his death, for the completion of this study.

I am grateful to Dr. S. Leeman who took painful interest to help build up my physics background in this subject and his encouragement.

I am thankful to Dr. Morrison for allowing me to use the stabilatron machine for Co-60 gamma irradiation.

Dr. Alf Linney and Miss Aviva Petrie for solving my statistical problems.

I am grateful to all the members of Medical Physics Department who helped me through the various difficulties in carrying out experiments especially Miss R. Lager, Principal Physicist and Mr. W. Coupland, Chief Technician.

I am obliged to the following eminent scientists for discussions: Prof. E.J. Hall, Dr. T. Alper, Dr. S.B. Field, Dr. S. Hornsey, Dr. J. Denekamp, Dr. W. Liversage, Mr. J.L. Foster and Dr. R. L. Willson.

Thanks to the following bodies who provided grants for maintenance, apparatus and thesis preparation: Medical Research Council, Royal Postgraduate Medical School, Wali Mohammad Award Foundation, Lord Mountbatten Foundation and Cancer Research Fund, Churchill Hosptial, Oxford.

Finally, I would like to thank Mrs. S. Wainwright for so ably typing this thesis.

BIBLIOGRAPHY.

Adams, G.E., 1976, Seminar at MRC Cyclotron in 1976 on 0582.

Alper, T., Gilles, N.E. and Elkind, M.M., 1960. The Sigmoid Survival Curve in Radiobiology, Nature, 186:1062 - 1063 (1960).

Alper, T., Fowler, J.F., Morgan, R.L., Vonberg, D.D., Ellis, F. and Oliver, R., 1962. The Characterisation of the 'Type C' Survival Curve, Brit. J. Radiol., 35:722 - 723 (1962).

Asquith, J.C., Foster, J.L., Willson, R.L., Ings, R. and McFadzean, J.A., 1974. Metronidazole ('Flagyl'). A radiosensitizer of hypoxic cells, Brit. J. Radiol., 47:474 - 481 Aug. 1974.

Barendsen, G.W., Beusker, T.L.J., Vergrosen, A.J. and Budke, L., 1960. Effects of Different Ionizing Radiations on Human Cells in Tissue Culture II Biological Experiments, Radiation Research 13: 841-849 (1960).

Barendsen, G.W., 1962. Dose - Survival Curves of Human Cells in Tissue Culture irradiated with alpha, beta, 20Kv, X and 200Kv X-radiation. Nature, 193, (4821): 1153 - 1155, 24 March 1962.

Barendsen, G.W., Walter, H.M.D., Fowler, J.F. and Bewley, D.K., 1963. Effects of Different Ionizing Radiations on Human Cells in Tissue Culture. Radiation Research 18: 108 - 119 (1963).

Barendsen, G.W., 1964: Modification of Radiation Damage by Fractionation of the Dose, Anoxia and Chemical Protectors in relation to LET. Ann. N.Y. Acad. Sci., 114: 96 - 114 (1964).

Barendsen, G.W. and Walter, H.M.D., 1964: Effects of Different Radiations on Human Cells in Tissue Culture. Radiation Research 21 : 314 - 329 (1964).

Barendsen, G.W., 1966. Dose-Rate and Survival curves. Brit. J. Radiol., 39, (458) : 155 - 156, Feb. 1966.

Barendsen, G.W., 1967. Mechanism of Action of Difficient Ionizing Radiations on the Proliferative Capacity of Mammalian Cells. In Theoretical and Experimental Biophysics 1 : 167 - 231 (Ed. Arthur Cole). Edward Arnold (Publishers) Ltd., London, 1967.

Barendsen, G.W., 1968. Responses of cultured cells, tumours and normal tissues to radiations of different linear energy transfer. In Current Topics of Radiation Research (Ed. Ebert M and Howard A.), North-Holland Publ. Co., Amsterdam, 4 : 293 - 356 (1969)

Barendsen, G.W., 1975. Characteristics of cell survial curves for different radiations in relation to iso-effect curves for fractionated treatments of a rat rhabdomyosarcoma. In cell survival of low doses of radiation (Ed. T. Alper) Institute of Physics, John Wiley and Sons Ltd, London, 1975.

Bender, M.A. and Gooch P.C., 1962. The kinetics of X-ray Survival of Mammalian Cells in Vitro. Int. J. Radiat. Biol. 5, (2) : 133 - 145 (1962).

Biaglow, J.E., Nygaard, O.F. and Greenstock, C.L. Radox reactions of anoxic radiosensitizers. Radiation Res., 59 : 158 (1974). (Abstract).

Brown, Martin J. Selective Radiosensitization of the Hypoxic Cells of Mouse Tumours with Nitroimidazoles, Metronidazole and Ro-07-0582. Radiat. Res. 64 : 633 - 647 (1975).

Burch, P.R.J., 1957a. Calculations of Energy Dissipation Characteristics for Water for Various Radiations. Brit. J. Radiol. 6 : 289 - 301, March 1957.

Burch, P.R.J., 1957b. Some Physical Aspects of Relative Biological Effeciency. Brit. J. Radiology, 30 : 524 - 529, October 1957.

Chadwick, K.H. and Leenhouts, H.P., 1973. A Molecular Theory of Cell Survival. Phys. Med. Biol. 18, (1) : 78 - 87 (1973).

Chapman, J.D., Gillespie, C.J., Reuvers, A.P. and Dugle, D.L., 1975. The Inactivation of Chinese Hamster Cells by X-rays. The effects of Chemical Modifiers on Single and Double Events. Radiat. Res. 64 : 365 - 375, 1975.

Clowes, F.A.L. and Hall, E.J., 1962. The Quiescent Centre in Root Meristems of Vicia faba and its Behaviour after acute X-radiation and chronic Gamma Irradiations. Radiat. Bot. 3 :45 - 53, 1962.

Clowes, F.A.L., 1961. Duration of the mitotic cycle in a meristem. J. Exp. Biol., 12 : 283 - 293.

Cole, A., 1962. Some physical properties of Low-Voltage Electrons (Abstract). Radiat. Res., 16 : 597 - 598 (1962).

Cormack, D.V. and Johns, H.E., 1952. Electron Energies and Ion Densities in Water Irradiated with 200KeV, 1 MeV and 25 MeV Radiations. Brit. J. Radiol. 25 : 369 - 381, July 1952.

Denekamp, J., Michael, B.D. and Harris, S.R., 1974. Hypoxic Cell Radiosensitizers: Comparitive Tests of some Electron Affinic Compounds using Epidermal Cell Survival in Vivo. Radiat. Res. 60 : 119 - 132 (1974).

Denekamp, J. and Harris, S.R., 1975. Tests of two Electron Affinic Radiosensitizers in Vivo using Regrowth of an Experimental Carcinoma. Radiat. Res. 61 : 191 - 203 (1975).

Denekamp, J. and Harris, S.R., 1976. The Response of a Trans-plantable Tumour to Fractionated Irradiation. I. X-rays and the Hypoxic Cell Radiosensitizer Ro-07-0582. Radiat. Res. 66 : 66 - 75 (1976).

Douglas, B.G. and Fowler, J.F., 1976. The Effects of Multiple Small Doses of X-rays on Skin Reactions in the Mouse and a Basic Interpretation. Radiat. Res. 66 ; 401 - 426, (1976).

Dutreix, J. and Wambersie, A.,1973. Relationship between Iso-effect, Total Dose and Fraction number, for Human Skin Reactions. Radiol. 2, Proc. XIII CONG. OF RADIOL. MADRID, 15 - 20 Oct. 1973. EXPERTA MED. AMSTERDAM.

Dewey, W.C., 1963. The X-ray sensitivity of Serratia marscescens. Radiat. Res. 19 : 64 - 87 (1963).

Elkind, M.M. and Sutton, H., 1959. X-ray Damage and Recovery in Mammalian Cells Grown in Culture. Nature, London, 184 : 1293 - 1295, Oct. 24, 1959.

Elkind, M.M., 1960. Cellular Aspects of Tumour Therapy. Radiology 74, (4) :529 - 541, April 1960.

Elkind, M.M. and Sutton, H., 1960. Radiation Response of Mammalian Cells Grown in Culture. I. Repair of X-ray Damage in Surviving Chinese Hamster Cells. Radiat. Res. 13 : 556 - 593 (1960).

Elkind, M.M. and Sinclair, W.K., 1965. Recovery of Sub-lethal damage. In Curr. Topics of Radiat. Res. 1, Chapter 5 (Ed. Ebert and Howard). North-Holland Pub. Co. 1964.

Elkind, M.M. and Whitmore, G.F., 1967. The Radiobiology of Cultured Mammalian Cells, Gordon and Breach, New York, London and Paris, 1967.

Elkind, M.M., 1975. A Summary and Review of the 6th L.H. Gray Conference On Cell Survival after Low Doses of Radiation (Ed. T. Alper). The Institute of Physics, John Wiley and Sons, London, 1975.

Ellis, F., 1942. Tolerance dosage in radiotherapy with 200 kv X-rays. Brit. J. Radiol. 15 : 348 - 350. Dec. 1942.

Ellis, F., 1968. In Current Topics in Radiation Research (Ed. Ebert M and Howard A), 4, Amsterdam North-Holland Publication Co. 1968.

Ellis, F., 1969. Dose, time and fractionation: a clinical hypothesis. Clin. Radiol. 20 : 1 - 7, Jan. 1969.

Ellis, F., 1971. Nominal Standard Dose and Ref. Brit. J. Radiol. 44 : 101 - 108, Feb. 1971.

Field, S.B. and Howsey, S., 1975. The response of mouse skin and lung to fractionated X-rays. In Cell Survival after low doses of radiation. Institute of Physics, John Wiley and Sons, London, 1975. P. 362.

Field, S.B., Morris, C., Denekamp, J. and Fowler, J.F., 1975. The response of mouse skin to fractionated X-rays. Europ. J. Cancer II : 219 - 229 (1975).

Foster, J.L. and Willson, R.L., 1973. Radiosensitization of Anoxic Cells by Metroinidazole. Brit. J. Radiol. 46 : 234 - 235, March 1973.

Fowler, J.F. and Stern, B., 1960. Dose-Rate Effects. Some Theoretical and Practical considerations. Brit. J. Radiol. 33, (390), 389 - 395. June 1960.

Fowler, J.F. and Stern, B., 1963. Dose-Time relationship in radiotherapy and the validity of cell survival curve models. Brit. J. Radiol. 36, (423) : 163 - 173. March, 1963.

Fowler, J.F., Morgan, R.L., Silvester, J.A., Bewley, D.K. and Turner, B.A., 1963. Experiments with fractionated X-ray treatment of the skin of pigs. Brit. J. Radiol. 36, (423) : 188 - 196, March 1963.

Fowler, J.F., 1964. Differences in Survival Curve Shapes for Formal Multi-target and Multi-hit Models. Phys. Med. Biol. 9 (2) : 177 - 188, 1964.

Fowler, J.F., 1965. The estimation of total dose for different numbers of fractions in radiotherapy. Brit. J. Radiol. 38 : 365 - 368, May 1965.

Fowler, J.F., 1966. Distributions of Hit-Numbers in Single Targets. In Biophysical Aspects of Radiation Quality. 63 - 80, IAEA, Vienna 1966.

Fowler, J.F., 1971. Experimental animal results relating to time-dose relationships in radiotherapy and the 'ret' concept. Brit. J. Radiol. 44 : 81 - 90, February 1971.

Fowler, J.F., 1972. Current Aspects of Radiology as applied in Radiotherapy. Clin. Radiol. 23 : 257 - 262 (1972).

Fowler, J.F., Denekamp, J., Delapeyre, C., Harris, S.R. and Sheldon, P.W., 1974. Skin reactions in mice after multifractionation X-irradiation. Int. J. Radiat. Biol. 25 : 213 - 223, (1974).

Fowler, J.F. and Adams, G.E., 1975. Radiosensitization of hypoxic cells in solid tumours in mice. Brit. J. Radiol. 48, 77 - 78, (abstract) Jan. 2975.

Fowler, J.F., 1976. High Energy Photons and Electrons: Clinical Applications in Cancer Management. Ed. Simon Kramer, Nagalingam Santharalingam and George F. Zinninger. John Wiley and Sons pp 78. (Discussion).

Gray, L.H. and Scholes, M.E., 1951. The effect of ionizing radiations on the broad bean root. Brit. J. Radiol. 24, 285 - 291. Brit. J. Radiol. 24 : 285 - 295.

Greene, D., Hendry, J.H. and Massey, J.B. RBE values for Megavoltage X-rays. Brit. J. Radiol. 48 : 875, Oct. 1975. (Letter).

Gunter, Shirley E. and Kohn, Henry I., 1956. The Effect of X-rays on the Survival of Bacteria and Yeast. A Comparative Study of Dose-Survival Curves. J. Bacteriol. 71 : 571 - 581 (1956).

Hall, E.J., 1961. The Relative Bilogoical Efficiency of X-rays at 220 kVp and Gamma Radiation from a Co^{60} Therapy Unit. Brit. J. Radiol. 34 (401) : 313 - 317, May 1961.

Hall, E.J., 1962. A method of deducing a dose-response relationship for reproductive integrity of cells exposed to radiation by means of fractionation experiments. Brit. J. Radiol. 35, (414) : 398 - 402, June 1962.

Hall, E.J., Lajtha, L.G. and Oliver, R., 1962. X-ray dose-response relationship for reproductive integrity of Vicia faba. Brit. J. Radiol. 35 (414) : 388 - 397, June 1962.

Hall, E.J., 1963. Dose-Response Relationship for Reproductive Integrity of Vicia faba deduced from Protracted Irradiation Experiments. Radiat. Res., 20 (2) : 195 - 202, Oct. 1963.

Hall, Eric J. and Lajtha, Laszolo G., 1963. The Recovery of Vicia faba Meristem cells from X-Radiation. Radiation Res. 20, (2) : 187 - 194, Oct. 1962.

Hall, Eric J. and Bedford, Joel S., 1964. A comparison of the effects of acute and protracted gamma-radiation on the growth of seedlings of Vicia faba. Part I Experimental Observations. Int. J. Radiat. Biol. 8 , (5) : 467 - 474, 1964.

Hall, Eric J., Bedford, Joel S. and Oliver, R., 1966. Extreme hypoxia, its effect on the survival of mammalian cells irradiated at high and low dose-rates. Brit. J. Radiol. 39 : 302 - 307, April 1966.

Hall, E.J., Bedford, J.S. and Porter, E.H., 1966. The Oxygen Effect at Low Dose-Rate. Brit. J. Radiol. 39 , (469) : 958 - 959, December 1966. (Letter).

Hall, Eric J. and Cavanagh, Josephine., 1967. The Oxygen Effect for acute and protracted radiation exposures measured with seedlings of Vicia faba. Brit. J. Radiol. 40 : 128 - 133, Feb. 1967.

Hall, Eric J. and Cavanagh, Josephine., 1969. The Effect of Hypoxia on recovery of sub-lethal radiation damage in Vicia seedlings. Brit. J. Radiol. 42 : 270 - 277, April 1969.

Hall, Eric J., 1969. Radiobiological measurements with 14MeV neutrons. Brit. J. Radiol. 42 : 805 - 813, Nov.1969.

Hall, Eric J., 1972a. Radiation dose-rate: a factor of importance in radiobiology and radiotherapy. Brit. J. Radiol. 45 : 81 - 97, Feb. 1972. (Review Article).

Hall, Eric J., 1972b. The Effect of Hypoxia on the Repair of Sub-lethal Radiation Damage in Cultured Mammalian Cells. Radiat. Res. 49 : 405 - 415,(1972).

Hall, Eric J., 1974. September. Personal Communication.

Hall, Eric J., Lenhart, Shirley and Roizin-Towle, Laurie., 1974. Split-Dose Experiments with Hypoxic Cells. Radiology, 112 : 425 - 430, August 1974.

Hall, E.J. and Chapman, J.D., 1974. Radiosensitization of hypoxic cells with metronidazole. Brit. J. Radiol. 47 : 513 - 514, Aug. 1974.

Hall, Eric J. and Roizin-Towle, Laurie., 1975. Hypoxic Sensitizers : Radiobiological Studies at the Cellular Level. Radiology 117 : 453 - 457, November 1975.

Hendry, J.H., 1972. The response of haemopoietic colony-forming units and lymphoma cell irradiated in soft tissue (spleen) or a bone cavity (femur) with single doses of X-rays, gamma-rays or D.T. neutrons. Brit. J. Radiol. 26, (4) : 355 - 362, Dec. 1972.

Hethcote, H.W., McLarty, J.W. and Thames, J.W.Jr., 1976. Comparison of Mathematical Models for Radiation Fractionations. Radiat. Res. 67 : 387 - 407 (1976).

Hewitt, H.B. and Wilson, C.W., 1959. A Survival Curve for Mammalian Cells Irradiated in vivo. Nature, 183 : 1060 - 1061, (1959).

Hill, S.A. and Fowler, J.F., 1977. Radiosensitizing and Cytocidal Effects on Hypoxic Cells of Ro-07-0582 and Repair of X-ray Injury, in an Experimental Mouse Tumour. Brit. J. Cancer (1977) 35, 461.

Hornsey, S., 1956. The effect of X-irradiation on the length of the mitotic cycle in Vicia faba roots. Exptl. Cell Res., 11 : 340 - 345, (1956).

Hornsey, Shirley, Kutsutani Yuzuru and Field, Stanley B., 1975. Damage to Mouse Lung with Fractionated Neutrons and X-rays. Radiology, 116 : 171 - 174, July 1975.

Howard, A. and Pelc, S.R., 1953. Synthesis of Deoxyritonucleic Acid in Normal and Irradiated Cells and its Relation to Chromosome Breakage. Herdity, 6 : 261 - 273, (1953).

Humphrey, R.M. and Sinclair, W.K., 1963. The Relative Biological Effectiveness of 22 MeV X-rays, Cobalt-60 Gamma-Rays, and 200 kVp X-rays. Radiat. Res. 20, (4) : 593 - 599, Dec. 1963.

Jamieson, H.D, and Read, John., 1962. The relative biological efficiency of 24 MeV and 200 kV X-rays measured by the reduction in growth of Vicia faba. Int. J. Radiat. Biol., 4, (5) : 487 - 493, Feb. 1962.

Keller, A., 1966. Analysis of Dose-Effect Relations. Biophys. Aspects of Radiat. Quality. IAEA pp-95-114, (1966).

Kellerer, A.M. and Rossi, H.H., 1972. The theory of dual radiation action. Curr. Top. Radiat. Res. Quat. 8 : 85 - 158 (1972).

Lajtha, L.G. and Oliver, R., 1961. Some Radiobiological Considerations in Radiotherapy. Brit. J. Radiol. 34 : 252 - 257 (1961).

Lea, D.E., 1955. Actions of Radiations on Living Cells. Cambridge University Press. London and New York, 1955.

Liversage, W.E., 1971. A critical look at the ret. Brit. J. Radiol. 44 : 91 - 100, Feb. 1971.

Leenhouts, H.P. and Chadwick, K.H., 1974. Radiation Induced DNA Double Strand Breaks and Chromosome Aberrations. Theoset. and Applied Genetics. 44 : 167 - 172 (1974).

Malone, J.F., Porter, D. and Hendry, J.H., 1972. The r.b.e. of Co^{60} gamma-rays with respect to 300 kVp X-rays for the survival of Hela Cells, irradiated in different states of proliferation. Int. J. Radiat. Biol. 26 , (4) : 355 - 362, (1974).

McNally, Nicholas J., 1976a. The effect of a change in radiation quality on the ability of electron affinic sensitizers to sensitize hypoxic cells. Int. J. Radiat. Biol. 29, (2) : 191 - 196 (1976).

McNally, Nicholas J. and DeRonde, Jennifer, 1976. The effect of repeated small doses of radiation recovery from sub-lethal damage by Chinese Hamster cells irradiated in the plateau phase of growth. Int. J. Radiat. Biol. 29, (3) : 221 - 234, (1976).

Moore, J.L., 1975. Discussion of the session on Modifications of the Initial Regions by Oxygen and Chemical sensitizers in the 6th L.H. Gray Conference.

Moore, B.A., Palcic, B. and Skarsgård, L.D., 1976. Radiosensitizing and Toxic Effects of the 2 Nitroimidazole Ro-07-0582 in Hypoxic Mammalian Cells. Radiation Research. 67 : 459 - 473, (1976).

Neary, G.J., 1956. Progress in Radiobiology, (Oliver and Boyd, London, 1956), 355 Coded by Lajtha and Oliver, 1961.

Neary, G.J., 1957. Dependence on Oxygen and Temperature of the Sensitivity of Broad Bean Roots to gamma-radiation. Nature, 180, (4679) : 248 - 249, Aug 3, 1957.

Neary, G.J., Evans, H.J. and Tomkinson, S.M., 1959. A Quantitative Determination of the Mitotic Delay Induced by Gamma Radiation in Broad Bean Root Meristems. Journal of Genetics. 56, (3) : 363 - 394, Dec. 1959.

Neary, G.J., 1965. Chromosome aberrations and the theory of RBE. 1. General Considerations Int. J. Radiat. Biol. 9, (5) : 477 - 502, 1965.

Oliver, R., 1964. A Comparison of the effects of acute and protracted gamma-radiation on the growth of seedlings of Vicia faba. Part II. Theoretical Calculations. Int. J. Radiat. Biol. 8, (5) : 475 - 488, (1964).

Oliver, R. and Shepstone, B.J., 1964. Some Practical Considerations in determining the Parameters for Multi-target and Multi-hit Survival Curves. Phys. Med. Biol. 9, (2) : 167 - 175, April 1964.

Oliver, R. and Shepstone, B.J., 1965. Theoretical growth-rate curves for Vicia faba roots under continuous radiation exposures at low dose-rate. A comparison with experimental results. Int. J. Radiat. Biol. 9, (4) : 333 - 347, 1965.

Oliver, R., 1967. The Influence of Anoxia on the Recovery of Sub-lethal Radiation Damage - Some Possible Implications. Brit. J. Radiol. 40 : 476 - 477, June 1967.

Peters, L.J., 1976. Modification of the radiocurability of a syngenic invasive squamous carcinoma by its site of growth, by electron affinic drugs, and by ICRF 159. Brit. J. Radiol. 49 : 708 - 715, Aug. 1976.

Porter, E.H., 1965. Dose-rate and survival curves. Brit. J. Radiol. 38 : 607 - 612, August 1965.

Pierquin, B. and Bailler, F. The differential effect of continuous irradiation at low dose-rate. Brit. J. Radiol. 44 : 236 - 232, March 1971.

Puck. T.T. and Marcus, P.I., 1956. Action of X-rays on Mammalian Cells. J. Exptl. Med., 103 : 653 - 666, (1965).

Puck, T.T., 1959. Quantitative Studies on Mammalian Cells in Vitro. Reviews of Modern Physics., 31, (2) : 433 - 448, April 1959.

Read, J., 1959. Radiation Biology of Vicia faba (Blackwall Scientific Publication, Oxford, 1959).

Revesz, L., Littbrand, B., Midander, J. and Scott, O.C.A., 1975. Oxygen effects in the shoulder region of cell survival curves. In cell survival after low doses of radiation (The Institute of Physics, John Wiley and Sons Ltd, London, 1975) P. 141 - 149.

Rossi, H.H., 1964. Correlation of Radiation Quality and Biological Effect. Ann. N.Y. Acad. Sci., 114 : 4 - 15 (1964).

Rossi, H.H., 1965. Biophysical considerations concerning the existance of a threshold dose for radiation effects. Ann. J. Roentgenol. 93 : 196 - 199, Jan.1965.

Schmid, E., Rimpl, G. and Banchinger, M., 1974. Dose-Response Relation of Chromosome Aberrations in Human Lymphocytes after in Vitro Irradiation with 3 MeV Electrons. Radiat. Res., 57 : 228 - 238, (1974).

Sheldon, P.W., Foster, J.L. and Fowler, J.F., 1974. Radiosensitization of C_3H Mouse Mammary Tumours by a 2-Nitroimidazole Drug. Brit. J.Cancer., 30 : 560 - 565, (1974).

Sheldon, P.W., Hill, S.A., Foster, J.L. and Fowler, J.F., 1976. Radiosensitization of C_3H mouse mammary tumours using fractionated doses of X-rays with the drug Ro-07-0582. Brit. J. Radiol., 49 : 76 - 80, Jan. 1976.

Shepstone, B.J. and Oliver, R., 1963. Theoretical Cell Population Kinetics following Irradiation of the Root Meristem of Vicia faba. S.A. Nat. Conf. on Nuclear Energy, Pretoria., P. 526 - 535.

Shepstone, B.J., 1963. Analysis of fractionation experiments using high-speed digital computer. Brit. J. Radiol., 36, (430) : 755 - 757, Oct. 1963.

Shepstone, B.J., 1964. Ph.D. thesis of Oxford University, Oxford.

Sinclair, W.K., 1962. The Relative Biological Effectiveness of 22MeV X-rays, Co^{60} gamma-rays and 200 kVp X-rays. Radiation Research, 16 : 394 - 398, (1962).

Sinclair, W.K. and Morton, Richard A., 1963. Variation in X-ray Response during the Division Cycle of partially Synchronised Chinese Hamster Cells in Culture. Nature, (London), 199 : 1158 - 1160, Sept. 21, 1963.

Sinclair, W.K. and Kohn, Henry J., 1964. The Relative Biological Effectiveness of High-Energy Photons and Electrons. Radiolgy, 82, (5) : 800 - 815, May 1964.

Sinclair, W.K., 1966. The Shape of Radiation Survival Curves of Mammalian Cells cultured in Vitro. Biophys. Aspects of Radiat. Qualit. IAEA, Vienna, 1966 p.21 - 43.

Stone, Helen B. and Withers, H. Rodney, 1975. Enhancement of Radiation Response of a Murine Mammary Carcinoma by two Nitrofuran Derivatives. Radiat. Res., 64 : 443 - 451, (1975).

Strandquist, M., 1944. Studien uher die kumulative Wirkung der Rontgenstrahlen bei Fraktionierung. Acta Radiologica Stockholm Supp lemen, 55.

Underbrink, A.G., Kellerer, A.M., Mills, R.E. and Sparrow, A.H., 1976. Comparison of X-ray and Gamma-ray Dose-Response Curves for Pink Somatic Mutations in Tradescantia clone 02. Rad. and Environm Biophys. 13 : 395 - 303, (1976).

Wambersie, A. Dutriex, J. and Gueulette, J., 1973. Determination of the shape of mammalian cell survival curves by the evaluation of cellular recovery as the function of the size of the fraction. Comparison with theoretical modes. In Proc. IVth Symp. on Micro-dosimetry, Verhamia-Pallanza, Italy, 1973, Comm. of the Europ. Communities IX p. 24 - 28.

Wambersie, A. and Dutreix, J., 1974. Cellular Repairs after X or Gamma Irradiations determined for several 'Gross' Criteria on Animals and Patients. Europ. J. Cancer, 10 : 235 - 239, (1973).

Wideroe, R., 1975. Remarks on Oxygen Effects. Radiat. and Environm Biophys., 12 : 233 - 240, (1975).

Wideroe, R., 1975. Cellular survival and iso-effect formulae. A study in applied radiobiology. Forschr. Rontgenstr., 123, 5 (1975) 475 - 481, Nov. 1975.

Winston, B.M., Berry, R.J. and Oliver, R., 1974. Hypoxic protection at low X-ray doses : A fractionated dose using Vicia faba. Int. J. Radiat. Biol., 26, (3) : 251 - 258, (1974).

Zimmer, K.G., 1961. Studies on Quantitative Radiation Biology, Oliver and Boyd, London, 1961.

Zickle, R.E., 1940. Killing of bacteria mould spores and yeast by X-rays. J. Cell. Comp. Physiol., 16 : 221.

Appendex 1

Last copy - (corrections page 381)

INT. J. RADIAT. BIOL., 1975, VOL. 28, NO. 4, 373-383

An investigation of chromosome damage in *Vicia faba* root tips after exposure to 1·5 MHz ultrasonic radiation

M. T. KHOKHAR and R. OLIVER

Department of Medical Physics, The Royal Postgraduate Medical School, Du Cane Road, London W12 0HS

(*Received* 23 *June* 1975; *accepted* 23 *September* 1975)

Roots of seedlings of *Vicia faba* were exposed to 1·5 MHz continuous-wave ultrasound at approximately 5 W cm^{-2} intensity, averaged over the dividing region of the meristem, thus producing considerable non-thermal cell damage and severe inhibition of root growth. No significant increase in the number of conventional chromosome aberrations was observed during the 26 hours after exposure, although a significant number of cells did show bridged, agglomerated or clumped chromosomes. It is concluded that after exposure to ultrasound, genetic damage in surviving cells is unlikely.

1. Introduction

The continuing increase in the use of ultrasound in diagnostic medicine, particularly in obstetric examinations, emphasizes concern over any possible genetic hazard to the patient, such as is associated with exposure to even low doses of ionizing radiation. This has led to a search for evidence of chromosome aberrations after ultrasonic irradiation. Macintosh and Davey (1972) did report a significant and dose-dependent increase in the number of chromosome aberrations in human lymphocytes exposed *in vitro* to ultrasonic radiation at power levels of only 10–50 mW cm^{-2}, and a rather similar report has been made by Bugnon, Gottin, Kraehenbuhl and Weill (1972). However, these particular results do not seem to have been repeatable (Macintosh, Brown and Coakley 1975), and many other groups have found *no* significant increase in chromosome aberrations in human blood even after long exposures at much higher power levels (e.g. Bobrow, Blackwell, Unrau and Bleaney 1971, Coakley, Slade, Braeman and Moore 1972, Mermut, Katayama, Del Castillo and Jones 1973). Thus, it seems probable that genetic changes do not occur in cells which survive ultrasonic irradiation, and in a very wide review of the subject Thacker (1973) has concluded that ' present evidence does not point to a high risk of genetic effects from the medical applications of ultrasound '. But as the subject is so important, further studies appear to be worth while in any suitable biological system that is available.

As a test system for damage induced by ionizing radiations, inhibition of root growth in seedlings of *Vicia faba* has been used for many years (e.g. Read 1959, Hall, Lajtha and Oliver 1962). More recently Bleaney and Oliver (1972) have obtained dose–response relationships for non-thermal damage in this system after ultrasonic irradiation at 1·5 MHz in the power range 1·5–5 W cm^{-2}, results which have been confirmed by Leeman, Khokhar and Oliver (1975) in this laboratory. Further, the six pairs of large chromosomes in *Vicia faba* have been used extensively by cytogeneticists for the analysis of chromosome aberrations after exposure to ionizing radiation. These techniques have therefore been applied in this study to the investigation of possible genetic

effects after ultrasonic irradiation previously shown to produce non-thermal cell damage with severe inhibition of root growth (Bleaney and Oliver 1972).

2. Materials and methods

The beans were germinated and grown as described in detail by Hall (1961), and the roots of the seedlings exposed in degassed water to 1·5 MHz continuous-wave ultrasound for 30 min, at a power level of approximately 5 W cm^{-2}, averaged over the dividing region of the meristem. Eight beans at a time were mounted on a Perspex jig so that the root tips were brought together on the beam axis at 10 cm from the 2·5 cm diameter transducer. The walls of the tank were lined with rubber absorber to minimize reflections and ensure that exposure was to a simple travelling wave field, the power level at the position of the jig being calibrated with a pressure balance (Leeman *et al.* 1975). Control roots were also kept in the tank for similar periods, but without exposure to ultrasonic radiation.

After 30 min, the beans were returned to the growth tank at 19°C. Savage (1971) has stressed the importance of assessment of damage at different times after irradiation. Groups of beans were therefore fixed after growth periods varying in steps of two hours from 2–26 hours after sonication, that is 13 groups covering a complete mitotic cycle for the cells of the meristem at 19 °C. Sham-irradiated groups were grown for 8, 16 and 24 hours, respectively.

The cytological techniques adopted were those described by Savage (1967). For two hours before fixation the beans were treated with aerated 0·05 per cent colchicine solution at 19 °C, after which the roots were washed and excised at 4–5 mm from the tip. These specimens were immediately transferred to freshly prepared osmic fixative solution under vacuum for 2–3 hours (Evans 1961). The root tips were then washed several times and transferred to the bleaching solution, three changes of solution over a period of 10–30 min being used to ensure that the chromosomes were well bleached without undue softening of the tissues. These were rewashed (two to three washes) and stained in Feulgen reagent and permanent squash preparations of the meristem prepared by the dry-ice method (Conger and Fairchild 1953). A single meristem was squashed on each slide, four to eight slides being prepared from each group of beans.

Two or more well-stained slides were selected from each irradiated and control group. These were coded before scoring, 50 scorable metaphases on each slide being first located under dry ×45 objective and then studied under ×100 oil-immersion.

The following conventional classification of chromosome aberrations was adopted (Savage 1975):

(i) Gaps and isolocus gaps.

(ii) Chromatid breaks and isochromatid breaks, the latter divided into four categories, viz. sister unions (SU), non-proximal unions (NUp), non-distal unions (NUd), and non-proximal and non-distal unions (NUpd).

(iii) Chromosome breaks in the secondary construction region of the two largest chromosomes (N.O. fractures), breaks occurring at the centromere.

(iv) Exchanges and rings with or without fragments.

Fixation post-irrad.	Slide No.	Gaps	Isolocus gaps	Chromatid breaks	Iso-chromatid breaks				N.O. fractures	Centro-meric breaks	Rings		Total
					SU	NUp	NUd	NUpd			Frag-mented	Without fragment	
2 hours	A		2						1	1			4
	B			1					2	2			5
4 hours	A									2			2
	B									1			1
6 hours	A	1	2	1				1	4	1			10
	B		1	1					2	1			5
8 hours	A	1		1					2				4
	B		2						2	3			7
10 hours	A†		7	10				1	17	5			40
	B									3			3
	C				1				3				4
12 hours	A								2				2
	B			2	1	1		2	7	6			19
14 hours	A		1						3	1			5
	B		1									1	2
16 hours	A								1	1		1	3
	B									3			3
18 hours	A								1				1
	B		2							5			7
20 hours	A		4	1					2	2			9
	B	1		1		1			2	1			6
22 hours	A			1					8	3			12
	B			5					5	3			13
24 hours	A		2	4					2	1			9
	B								3	1			4
26 hours	A								2	3			5
	B								1	3			4
												Average	7·0

† See text.

Table 1. Irradiated roots: number of chromosome aberrations per slide observed in 50 metaphase nuclei.

Fixation post sham-irrad.	Slide No.	Gaps	Isolocus gaps	Chromatid breaks	Iso-chromatid breaks				N.O. fractures	Centro-meric breaks	Rings		Total
					SU	NUp	NUd	NUpd			Frag-mented	Without fragment	
8 hours	A			1					3	3			7
	B			2					1	3			6
16 hours	A		1		1				1	4			7
	B		1	2					1				4
24 hours	A				1								1
	B			1						1			2
8 hours	C			1									1
	D		1	1					4	5			11
	E					1			2	1			4
	F									4			4
16 hours	C								5	5			10
	D								1				1
24 hours	C	1	2					1	7	2			13
	D									1			1
16 hours	E												0
	F			2					1				3
												Average	4·7

Table 2. Control roots: number of chromosome aberrations per slide observed in 50 metaphase nuclei.

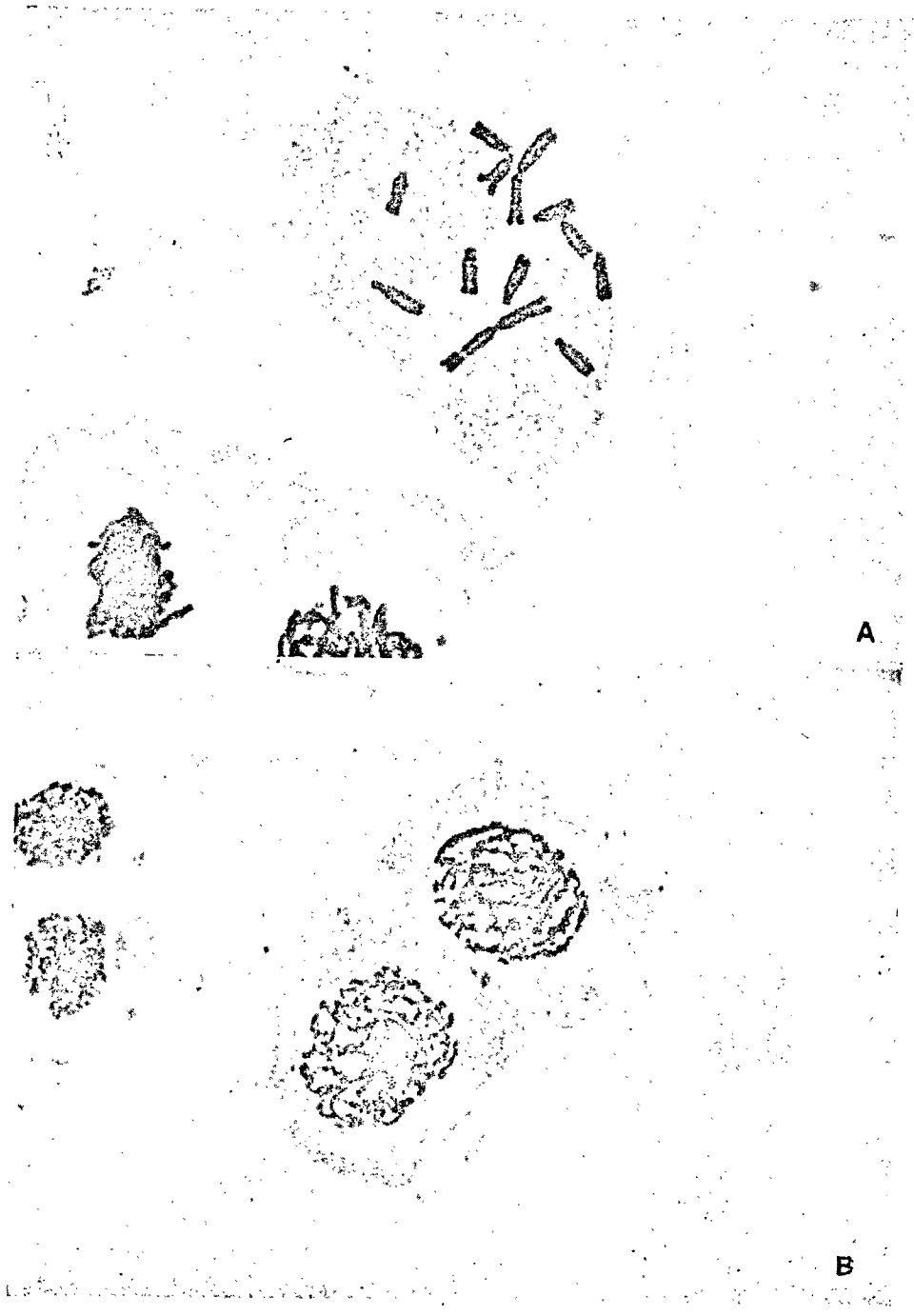


Figure 1. Sonicated but normal cells, (A) metaphase, (B) prometaphase stages.

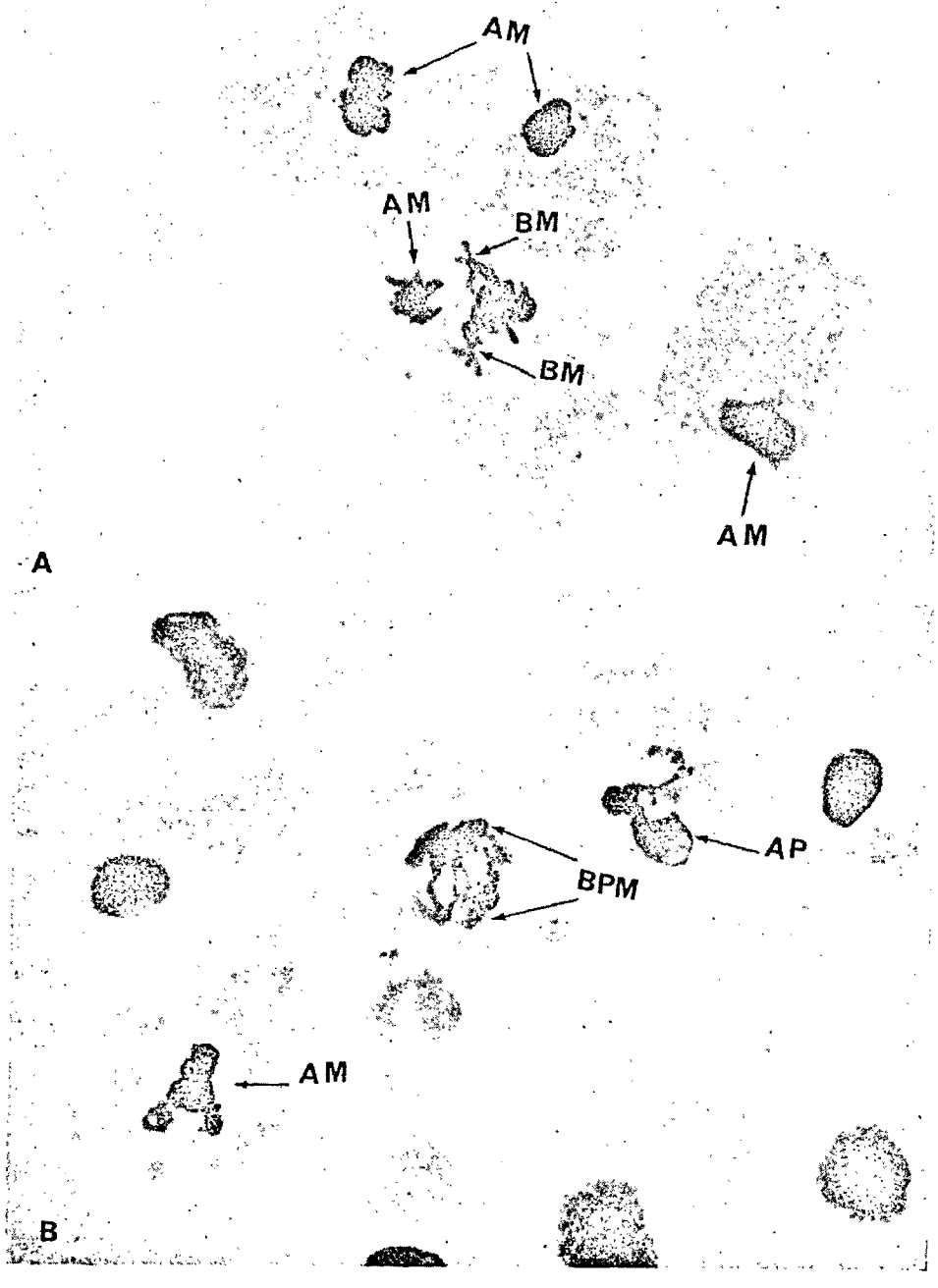


Figure 2. Sonicated but damaged nuclei, (A) showing examples of (AM) agglomerated mitotics and (BM) bridged metaphases, (B) showing (BPM) bridged prometaphase and (AP) agglomerated prophase.

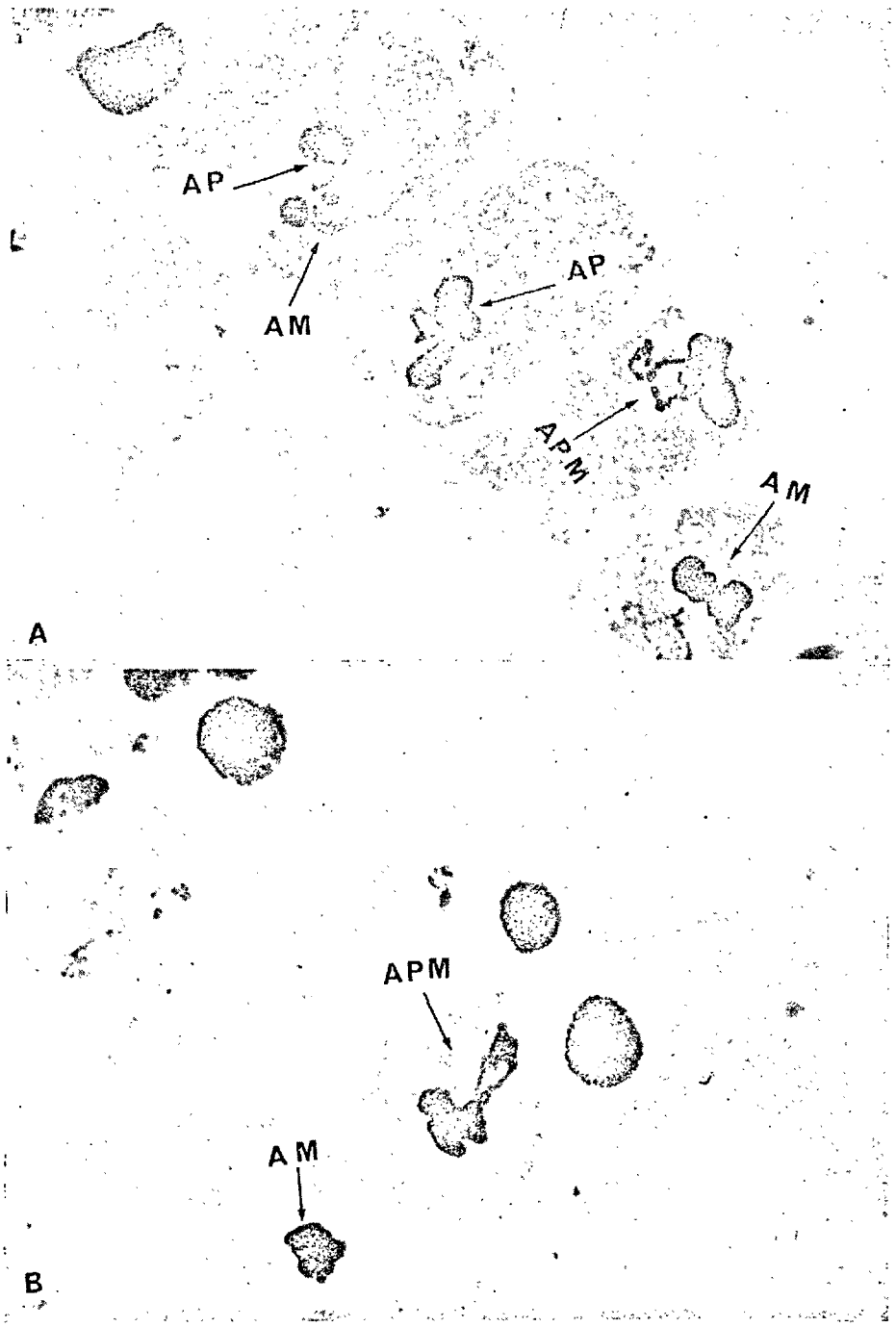


Figure 3. Sonicated but damaged nuclei, (A and B) showing (APM) agglomerated prometaphase together with other types of damage (cf. figure 2).

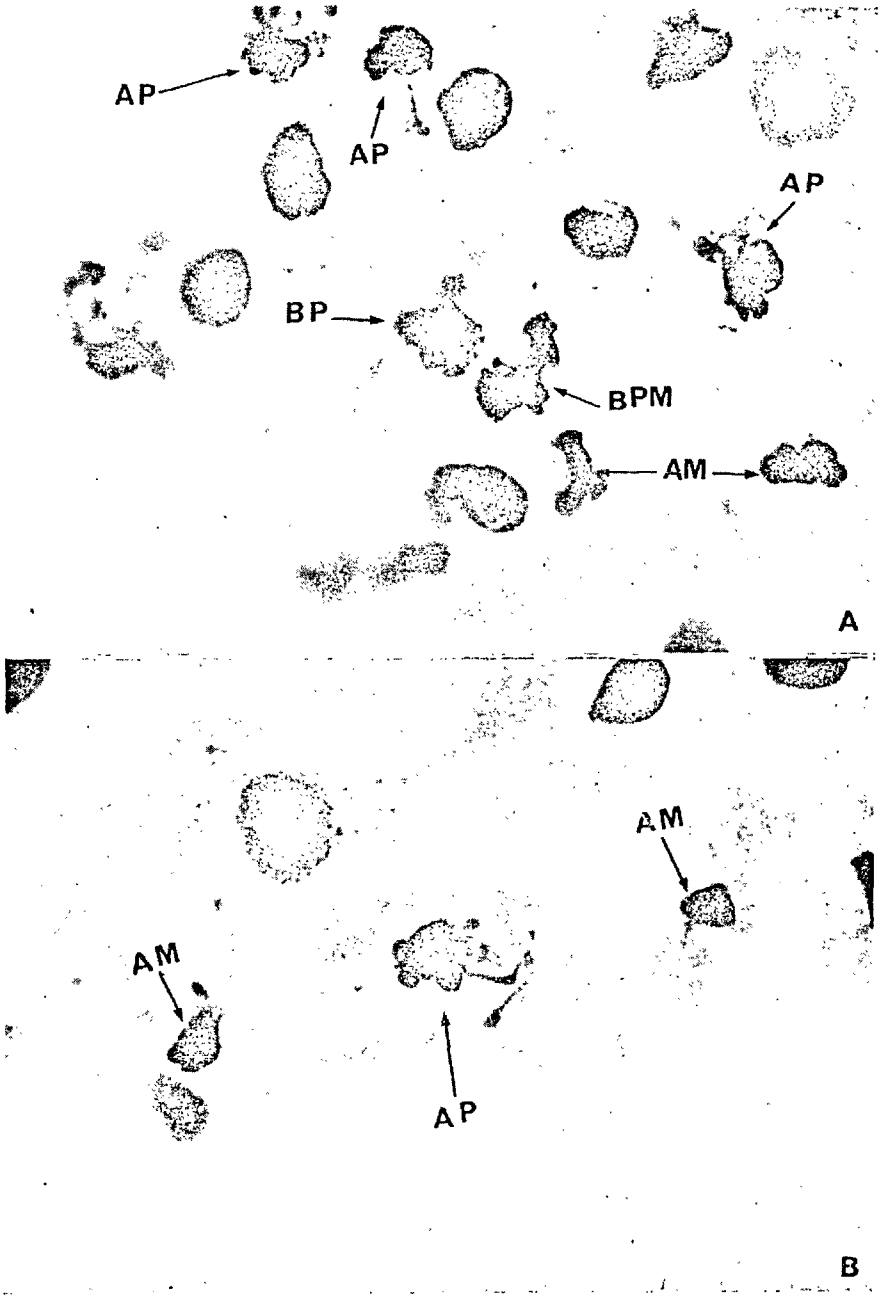


Figure 4. Sonicated but damaged nuclei, (A and B) showing similar sort of damage as seen in figures 2 and 3.

Subsequently, the slides were rescanned to score 100 cells in prophase, prometaphase or metaphase stages and to evaluate the number showing nuclear damage of the types recently referred to by Cataldo, Miller, Gregory and Carstensen (1973), classified as (BM) bridged mitosis, (BPM) bridged prometaphases, (AM) agglomerated mitotics, (AP) agglomerated prophases, (APM) agglomerated prometaphases. This type of damage was also reported by Lehmann (1965).

3. Results

The number and types of aberrations observed in 50 metaphase cells from each slide are recorded in table 1 for roots fixed at various times after sonication. Results for control roots are given in table 2. Table 3 lists the corresponding number of cells showing nuclear damage (see above) in 100 prophase, prometaphase or metaphase nuclei from the same slides scanned for conventional aberrations.

The average number of aberrations for irradiated slides (table 1) is 7·0 and for the control slides (table 2) 4·7. This difference arises mainly from one result, that of 40 aberrations recorded in slide A, table 1. This high value is not supported by the results for two other slides fixed at the same time after irradiation (three and four aberrations respectively), but even when this individual root is included a chi-square test indicates that there is no significant difference between the results for the two series ($P \sim 0{\cdot}3$).

On the other hand in table 3 the average number of cells with ' damaged ' nuclei (see figures 2, 3 and 4) on the slides from the irradiated series is 15·0

Irradiated series

Fixation post-irrad. (hours)		2	4	6	8	10	12	14	16	18	20	22	24	26	Average
Number of damaged cells per 100	Slide A	40	19	10	4	12	8	23	10	14	36	12	9	11	15·0
	Slide B	15	17	41	16	6	18	3	11	13	2	8	12	21	

Control series

Fixation post sham-irrad. (hours)	8						16						24				Average
Number damaged cells per 100	11	16	15	3	5	6	9	6	5	3	5	6	6	2	5	2	6·56
Slide	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	

Table 3. Number of cells with ' damaged ~~chromosomes~~ Nuclei '† observed in 100 prophase, prometaphase or metaphase nuclei per slide.

† Of types recently referred to by Cataldo *et al.* (1973) and including bridged prophases, bridged metaphases, agglomerated mitoses and clumped chromosomes (see text).

compared with 6·56 for the control slides. The difference between these two series is highly significant ($P \sim 0{\cdot}05$).

4. Discussion

Inhibition of root growth as a result of non-thermal damage after exposure to ultrasonic radiation at power levels in the range 0·5–10 W cm^{-2} has been demonstrated previously by Bleaney and Oliver (1972) and Leeman *et al.* (1975).

After the relatively high exposures used here, roots continue to grow at a reduced rate for several days, but few, if any, recover. However, although gross nuclear damage was observed, this study provides no evidence of any significant increase in the number of conventional chromosome aberrations over controls associated with this severe inhibition of root growth. This is in agreement with the report by Cataldo *et al.* (1973) that, after exposure of *Vicia faba* roots to 1 MHz ultrasound at power levels of 1–20 W cm^{-2}, they observed no ' classical chromosome aberrations ' but significant numbers of the ' chromosomal abnormalities ' referred to above, with a peak effect occurring for fixation at some 4–6 hours after exposure, as also suggested by the results in table 3. These damaged nuclear forms persisted until 20 hours and 26 hours (table 3) after sonication and their occurrence lacked any systematic trend with time. This suggests that the cells were in division at the time of sonication, and are just stuck, frozen, fixed or agglomerated into a deformed shape, and degenerate slowly over a long time. This view is supported by the investigation of Lehmann (1965), who first observed this type of damage and ascribed it to the effect of ultrasound cavitation. Further investigation is needed to clarify matters.

This study supports the conclusion that gross genetic effects are not a feature of damage due to ultrasonic radiation at a frequency of a few megahertz. Even when non-thermal damage occurs after exposure at relatively high intensity chromosome aberrations are not observed within a period of 26 hours. Genetic effects in surviving cells therefore appear to be very unlikely under the conditions used in medical applications.

Acknowledgments

Helpful advice on cytogenetic procedures provided by Dr. J. R. K. Savage of the Medical Research Council's Radiobiology Unit, Harwell, is gratefully acknowledged. This work formed part of a study of the biological effects of ultrasound supported by a grant from the Medical Research Council.

Les racines des jeunes plants de *Vicia faba*, qui ont été exposées à des ondes continuelles ultrasoniques de 1,5 MHz, à l'intensité d'environ 5 W/cm^2 en moyenne sur la région divisée du meristème, ont montré que leurs cellules avaient souffert des dommages non-thermiques considérables et que la croissance des racines était sévèrement inhibée.

Pourtant aucune augmentation significative de l'aberration conventionelle des chromosomes n'était observe à la suite de l'exposition de 26 heures effectuée à des intervalles de deux heures, bien que des cellules en nombre significatif aient présenté des chromosomes agglomérés, groupés ou joints.

Donc on conclut que les cellules survivantes exposées à des ondes ultrasoniques ne souffrent vraisemblablement pas de dommages génétiques.

Die Wurzeln von *Vicia faba* Sprösslingen wurden kontinuierlich mit 1,5 MHz Ultraschallwellen bei einer mittleren Intensität von 5 W/cm² über dem Meristem bestrahlt, was eine beträchtliche nichtthermale Zellschädigung und schwere Wachstumsstörungen der Wurzel zur Folge hatte. Bei zweistündlicher Kontrolle, über einen Zeitabschnitt von insgesamt 26 Stunden, konnten keine signifikanten Chromosomenaberrationen beobachtet werden, obwohl sich bei einer signifikanten Anzahl von Zellen Brückenbildungen und Zusammenballungen zeigten. Daraus wurde gefolgert, dass die Entstehung eines genetischen Schadens bei Zellen, die eine Ultraschallbestrahlung überleben, als unwahrscheinlich angesehen werden kann.

References

Bobrow, M., Blackwell, N., Unrau, A. E., and Bleaney, B., 1971, *J. Obstet. Gynaec. Br. Commonw.*, **78**, 730.

Bugnon, C., Gottin, Y., Kraehenbuhl, I., and Weill, F., 1972, *J. Radiol.*, **53**, 750.

Bleaney, B., and Oliver, R., 1972, *Br. J. Radiol.*, **45**, 358.

Cataldo, F. L., Miller, M. W., Gregory, W. D., and Carstensen, E. L., 1973, *Radiat. Bot.*, **13**, 211.

Coakley, W. T., Slade, J. S., Braeman, J. M., and Moore, J. L., 1972, *Br. J. Radiol.*, **43**, 328.

Conger, A. D., and Fairchild, L. M., 1953, *Stain Technol.*, **28**, 281.

Evans, H. J., 1962, *Genetics*, **46**, 257.

Hall, E. J., 1961, *Br. J. Radiol.*, **34**, 313.

Hall, E. J., Lajtha, L. G., and Oliver, R., 1962, *Br. J. Radiol.*, **35**, 388.

Leeman, S., Khokhar, M. T., and Oliver, R., 1975, *Br. J. Radiol.* (in the press).

Lehmann, J. F., 1965, *Therapeutic Heat and Cold* (Maryland: Waverley Press Incorporated, Baltimore), p. 321.

Macintosh, I. J. C., Brown, R. C., and Coakley, W. T., 1975, *Br. J. Radiol.*, **48**, 231.

Macintosh, I. J. C., and Davey, D. A., 1972, *Br. J. Radiol.*, **45**, 320

Mermut, S., Katayama, K. P., Del Castillo, R., and Jones, H. W., 1973, *Obstet. Gynec., N.Y.*, **41**, 4.

Read, J., 1959, *Radiation Biology of* Vicia faba *in Relation to General Problems* (Oxford: Blackwell Scientific Publications).

Savage, J. R. K., 1967, *Sch. Sci. Rev.*, **48**, 771; 1971, *Int. J. Envir. Stud.*, **1**, 233; 1975, *Radiat. Bot.*, **15**, 87.

Thacker, J., 1973, *Curr. Topics Radiat. Res. Q.*, **8**, 235.

1977, *British Journal of Radiology*, *50*, 237–238 MARCH 1977

Proceedings of The British Institute of Radiology

Abstracts of papers read at the Radiobiology Work-in-Progress meeting held in the Reid Knox Hall, Institute House, 32 Welbeck Street, London W1 on Friday, November 19, 1976

SOME RADIOBIOLOGICAL CONSEQUENCES OF MYCOPLASMA CONTAMINATION IN TISSUE CULTURE

By Beulah M. Cullen and Luisa Manjil

M.R.C. Cyclotron Unit, Hammersmith Hospital
Du Cane Road, London W12 0HS

Cells of the mouse Ehrlich ascites carcinoma grown *in vitro* became contaminated with an arginine-splitting mycoplasma. The first sign of trouble appeared when the slopes of the radiation survival curves of the cells assayed by cell colony forming ability became extremely variable; eventually it became impossible to grow colonies at all. Experiments on the feeder cell requirement showed that, whereas for clean cells the maximum plating efficiency was obtained within a range of 5×10^4 to 3×10^5 feeder cells in a 5 cm dish, contaminated cells would only produce colonies in the presence of between 10^4 to 2×10^4 feeder cells. Doubling the concentration of arginine in the medium allowed contaminated cells to grow with maximum plating efficiency within an increased range of 10^4 to 4×10^5 feeder cells. The mycoplasmas were apparently behaving as feeders, reducing the requirement for added feeder cells, but also depleting the medium of essential arginine. It seems likely that the variability in slope of the survival curves was also caused by arginine deficiency for the following reason. The number of cells added to each Petri dish was increased with the dose of radiation to be given, to allow similar numbers of cells to survive in each dish; there would be a concomitant increase in mycoplasma concentration. Cells surviving the radiation would therefore have a probability of succumbing to arginine deficiency, which would increase with dose, so producing an artificially steep survival curve. The cells were eventually decontaminated by passaging them through a mouse as an ascites tumour.

RELATIVE BIOLOGICAL EFFECTS OF X AND GAMMA RAYS WITH FRACTIONATED IRRADIATIONS OF *VICIA FABA* ROOTS

By M. T. Khokhar, R. Oliver and E. W. Emery

Royal Postgraduate Medical School
London W12 0HS

he investigation set out to examine how the biological ectiveness of 250 kV X rays relative to ^{60}Co γ rays in ducing the growth of bean roots varies with the size of the se. The effect observed was the elongation of the roots a period of ten days relative to the unirradiated controls 10). In order to make observable the effect of small doses, mparable total doses were given in varying numbers of ual fractions, separated by 24 hours to allow recovery m sub-lethal damage, and with the beans maintained 3·5°C to prevent cell proliferation.

It is generally accepted that reduction of cell growth is an expression of cell-sterilization in the meristem (Gray and Scholes, 1951) and it is probable that the cell survival curve has a definite initial slope, such as might be expected to result from cell killing by a single radiation event. On this view it is likely that X rays, the more densely ionizing radiation, would produce the greater initial slope to the survival curve, and so show an RBE that increases as the dose given is reduced. Experiments were conducted with irradiations in 1, 3, 6 and 12 fractions, producing G10's ranging from 0·65 to 0·15. The smallest dose fractions were only 17 rad. The observed RBE was virtually constant at 1·2 from 200 to 80 rad/fraction, and thereafter increased to 1·5 at 17 rad per fraction, in accordance with the above hypothesis.

REFERENCE

GRAY, L. H., and SCHOLES, M. E., 1951. The effect of ionizing radiations on the broad bean root. *British Journal of Radiology*, *24*, 285–291.

MAST CELL RESPONSE IN IRRADIATED LUNG

By Elizabeth L. Travis

Cancer Research Campaign, Gray Laboratory
Mount Vernon Hospital, Northwood
Middlesex HA6 2RN

Mast cells have been implicated in the pathogenesis of oedema and fibrosis after many types of trauma, including irradiation. The aim of this study was to determine qualitative and quantitative changes in mast cell distribution after pulmonary irradiation.

The right thorax of Sprague-Dawley rats was exposed to 0·5, 10, 20 or 40 gray of X rays. Animals from each group were sacrificed at 2, 4, 6, 8, 12 and 72 weeks post-exposure and tissues from both lungs studied by light and electron microscopy. Tissues for light microscopy were stained with haematoxylin and eosin, Azure A at pH 1·5 for mast cells, Masson's trichrome for collagen, and Gordon-Sweet's reticulin method. Tissues for electron microscopy were fixed by standard glutaraldehyde-osmium techniques and thin sections stained with uranyl acetate-lead citrate.

Lungs exposed to 5 and 10 gray exhibited no pathological changes; mast cells were located in pleural, peribronchial, and perivascular sites, consistent with controls. An early pneumonitis progressing to fibrosis was observed in lungs exposed to 20 and 40 gray and an interstitial mast cell infiltrate was present in the alveolar walls. The time of appearance of this infiltrate and the cell numbers involved were dose-dependent. Mast cell degranulation was evident and increased mast cell numbers were present in fibrotic areas. Electron microscopy revealed mast cells in close spatial approximation to fibroblasts with membrane alterations indicative of pinocytosis.

1975, *British Journal of Radiology*, *48*, 954

Correspondence

(The Editors do not hold themselves responsible for opinions expressed by correspondents.)

THE EDITOR—SIR,

ULTRASONIC IRRADIATION OF BEAN ROOTS AND THE INFLUENCE OF EXPOSURE PARAMETERS

Bleaney and Oliver (1972) have demonstrated dose response curves for inhibition of root growth in *Vicia faba* seedlings following irradiation with continuous wave 1·5 MHz ultrasound at intensities up to about 3 W/cm², averaged over the dividing region of the meristem. It appears therefore that this system is one in which the biological effects of different irradiation parameters may be studied and further experiments have been carried out in this laboratory, using essentially the same techniques of culture and irradiation, in an attempt to elucidate the interaction mechanisms involved. These results will be reported in detail elsewhere but some points form an immediate extension of their earlier studies.

A useful indicator of the biological effect of ultrasound radiation is G_1, the average root growth in the first day following sonication for an irradiated group of about 12 beans expressed as a fraction of the corresponding average growth for a group of sham-irradiated control beans. In Fig. 1 this parameter has been plotted against exposure, calculated as the product of the power in W/cm², averaged over the meristem and elongation region of the root tip, and the period of irradiation in minutes. The effect (reduction in G_1) continues to increase with exposure even for times up to 180 minutes. For a given frequency (1·5 MHz, curves A and B), the effect for a given exposure increased with the intensity used as previously demonstrated by Bleaney and Oliver (1972), and if allowance is made for differences in averaging procedure the two sets of results are in reasonable accord. However, for a given exposure and power level the damage is *less* at higher frequency over the range 0·75–3·0 MHz (curves C,D and A,B). Thus it appears that the radiation effect is not simply related to energy absorbed, which in this range *increases* with frequency.

In order to maximize any radiation effect at low intensity, the results in Fig. 2 relate to G_1 following long exposures of 120–180 minutes at 1·5 MHz or 90 minutes at 0·75 MHz for various levels of ultrasonic power. These indicate a clear and relatively sharp threshold for effect at both frequencies, occurring in the range 0·4–0·7 W/cm² average intensity, and the response curves in Fig. 1 for different intensities at 1·5 MHz (curves A and B) can be brought together by plotting exposure in both cases as the product of irradiation time and the *excess* of average intensity above a threshold of 0·5 W/cm². Demonstration of a threshold response is consistent with mechanical effects of ultrasonic irradiation and suggests that such damage may be avoided by the use of very low average power levels as apply at a depth in tissue during clinical applications.

Yours, etc.,
S. LEEMAN,
M. T. KHOKHAR,
R. OLIVER.

Department of Medical Physics,
Royal Postgraduate Medical School,
Du Cane Road,
London W12 0HS.

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council, and one of us (S.L.) is now in receipt of a Fellowship from the Wellcome Foundation.

REFERENCE

BLEANEY, B., and OLIVER, R., 1972. The effect of irradiation of *Vicia faba* roots with 1·5 MHz ultrasound. *British Journal of Radiology*, *45*, 358–361.

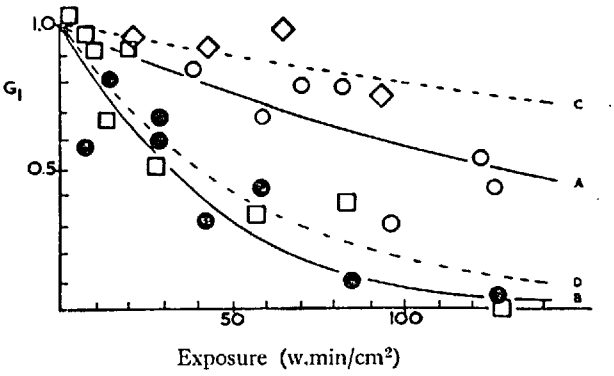


FIG. 1.

G_1, the average root growth in the first day following sonication for an irradiated group of beans as a fraction of the corresponding average growth for a group of sham-irradiated control beans, plotted against exposure, expressed as the product of the period of irradiation in minutes and the ultrasonic intensity in W/cm² averaged over the meristem and elongation region of the root tip. The curves are fitted by eye to the data points.

—○— Curve A. 1·5 MHz continuous radiation, average power 0·65 W/cm².
—●— Curve B. 1·5 MHz continuous radiation, average power 2·85 W/cm².
- -◇- - Curve C. 3 MHz continuous radiation, average power 1·40 W/cm².
- -□- - Curve D. 0·75 MHz continuous radiation, average power 1·45 W/cm².

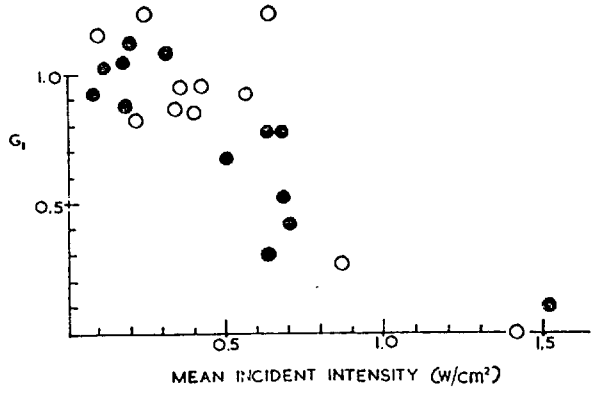


FIG. 2.

G_1, following long exposures plotted against incident ultrasonic intensity averaged over the meristem and elongation region of the root tip.

● 1·5 MHz continuous irradiation, exposure time 120–180 minutes.
⊙ 0·75 MHz continuous irradiation, exposure time 90 minutes.

Correspondence

THE EDITOR—SIR,

ACTIVE BONE MARROW DISTRIBUTION IN THE ADULT

Since publication of the above article in THE BRITISH JOURNAL OF RADIOLOGY, 39, 735–739 (1966), we have had a number of requests for more detailed information about the study described therein.

The technical report series of this institute is available for limited internal distribution. It includes many useful details and more data than would be appropriate for journal publications. We believe that a notice to the effect that we are willing to send copies of the technical report on which the journal article was based would be appreciated by your readers. We will then send the technical report promptly to those requesting them.

We hope you will approve of this and will be anxious to hear from you. Thank you for your continued assistance.

Yours etc.,

W. J. RUSSELL.

Radiation Effects Research Foundation,
5–2 Hijiyama Park, Hiroshima 730, Japan

EDITORS NOTE:
A copy of this report has been deposited in the British Institute of Radiology Library.

THE EDITOR—SIR,

GAMMA CAMERA FIELD UNIFORMITY CORRECTION

The recent letter by Hannan and Bessent (1974) conflicts with results obtained in Aberdeen.

We have a Nuclear Enterprises Mark III camera (updated to Mark IV standard), and a new Mark V. Both of these can be connected to a Nuclear Data 50/50 display unit, which is on-line to a PDP-8/I computer.

The display unit produces a 64×64 matrix of image points, and profiles in the x or y direction may be displayed on the oscilloscope or printed out on a teletype via the computer. The element size is approximately 5 mm on the old camera and 4 mm on the Mark V.

For one year we have been using a solid ^{57}Co flood source obtained from Amersham to correct our $^{99}Tc^{m}$ scans. Provided the count rate of both the flood field and the scan is below 10,000 counts per second we have found that correction with the ^{57}Co source gives excellent results. Above this count-rate baseline shifting occurs and we have found discrepancies in camera correction similar to those described by Hannan and Bessent.

As an example the Mark III camera was irradiated with firstly the ^{57}Co source giving a count rate of 5,500 counts per second, and then with a liquid plane source of $^{99}Tc^{m}$ giving a count rate of 4,000 counts per second. About 4,000 counts per channel were collected in each case, this being the limit of the storage capacity on the 50/50. An energy window of 30 per cent set symmetrically about the photopeak was used and the setting was peaked carefully for each isotope. The central x profile was plotted in each case, and the profiles were normalized at the centre channel.

The discrepancies between the profiles were explicable by statistical fluctuations, all but two or three pairs of channels lying within one standard deviation of each other. The correlation coefficient between the results for the two isotopes was 0·989 showing an excellent correlation despite statistics.

It has been found that the presence of scattering material has a more important effect on camera correction than the use of ^{57}Co. Correcting a flood field produced with 15 cm of scattering material interposed between source and camera, with a field produced with no scattering material produces a dip in the centre count rate of 1–2 per cent compared with the edge count rate.

When using ^{57}Co one has to take care that the level of contaminants, *i.e.* ^{56}Co, ^{58}Co and ^{60}Co is low. It is possible that a 1 per cent level of these isotopes in the source used by Hannan and Bessent could explain the discrepancy between their results and ours. The Amersham source has impurities of $< 0{\cdot}2$ per cent.

On a system which restricts the total counts collected in a channel to 4,095 it has been found that an undesirable increase in statistical fluctuations is produced by camera correction. This can be improved by smoothing the camera correct frame.

Yours, etc.,

D. A. CAUSER.

Department of Medical Physics,
University of Aberdeen,
Foresterhill, Aberdeen AB9 2ZD.

REFERENCE

HANNAN, W. J., and BESSENT, R. G., 1974. Pitfalls in gamma camera field uniformity correction. *British Journal of Radiology*, *47*, 820–821.