A Study of the Response of the Root Meristem of Maize to Acute Doses of Ionizing Radiations

by

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CONTENTS

CHAPTER I

The Choice of a System

At the commencement of any studies in radiobiology it is necessary to decide on a suitable system for investigation, either animal or plant.

As long ago as 1913, Mottram reported the effect of ionising radiations on seedlings of the broad bean, *Vicia faba*. In the late 1930's, Read and Gray tried tissue cultures, tadpoles, paramecia and colloids before settling for *Vicia faba*, and investigating the effects of ionising radiation on its root system. References to a vast number of plant systems other than *Vicia faba* may be found in the radiobiological literature. In particular, species such as *Tradescantia*, *Alliumcepa*, *Pasum sativum*, and *Mordeum vulgare* have been extensively studied. In this respect the extensive work in the radiation botany of nearly all the common plant species, both large and small, done by Sparrow and his colleagues at the Brookhaven National Laboratory should not be forgotten. In deciding also to use a plant system, namely *Zea mays*, for the present investigation a number of factors were considered.

It was realised that there was a danger that information derived from the study of a plant root system might have no relevance to the human problem.

Therefore some regard was paid to the literature which described the responses of human malignant tissue, and of mammalian tissue in general, to ionising radiations. It was noted with some satisfaction that numerous close parallels had appeared from time to time with the results obtained with plant systems such as Vicia faba.

Of importance in the present investigation was the fact that nearly all animal systems require the collaboration of other workers. Plant systems, however, other than very ambitious projects, have the advantages of cheapness of material and equipment, simplicity, and the ability to handle large enough numbers to reduce random errors to a reasonable level. The avoidance of the suffering which animals must experience in many radiation experiments played a not inconsiderable part in the choice of a system. Furthermore, it is possible to irradiate root systems under conditions which would often be impossible with animals.

The reasons given for choosing a plant system in the first place clearly apply in the case of Vicia faba. Since the early pioneer days an ever-increasing number of workers have used Vicia faba for explaining the basic problems of radiobiology.

One of the reasons for this is the small number of large chromosomes contained in the root meristem cells, which are particularly conducive to simple cytological analysis. The seedlings also have other attractive features. The fact that the meristematic differentiating and fully differentiated tissues are spatially separated, enables each to be separately irradiated, and since, in an untreated root, a cell progresses at a rate which is now fairly accurately known from one state to another, the consequences of injury to the dividing cell population may be quantitatively evaluated.

Just before the second World War, Read began a detailed investigation of the gross effects shown by Vicia roots after irradiation, namely reduction or cessation of growth. Using these criteria to assess radiation damage he performed his classical experiments to demonstrate the influence of oxygen on the response of living cells to ionising radiation. Together with Gray he also investigated the relative efficiency of different types of radiation in producing biological damage.

A great deal of the early work on the population kinetics of the root meristem was done by botanists using orthodox methods, but at a later stage they employed radiation as a tool in their studies. The work of Clowes (1959) on the quiescent centre is notable in this respect. Hall, Lajtha and Oliver (1962) on the other hand began to adopt the complimentary point of view, and use the population kinetics of the root to deduce a dose response relation with respect to reproductive integrity of the meristematic cells.

Clowes (1963) regards the choice of Vicia faba for work on the irradiation of root meristems as an unfortunate one, because its meristem is not very clearly divided into regions. On the other hand this greater homogeneity in the cells of the root tip of the broad bean has enabled simplified models of its meristem to be constructed.

By comparison, Zea mays, does have a meristem consisting of well-defined regions. Nevertheless it is very similar to Vicia faba (provided the growth of the primary root is encouraged), in that it can be studied using similar well established culture techniques, and the same criteria of radiation damage may be applied. Consequently it also becomes obvious that the mathematical complexities involved will also be similar, due allowance having been made for the regional differences mentioned above.

Of particular interest in Zea mays root meristems is the large and clearly defined quiescent centre. From autoradiographs made from sections of apices fed with labelled adenine at various intervals after irradiation, Clowes (1959) has convincingly demonstrated that in Zea the quiescent centre often disappears after irradiation - its cells synthesize DNA and subsequently divide. Many of the normally meristematic cells, on the other hand, stop synthesizing DNA and cease dividing. There is thus "a reversal of roles in the apex", and from this Clowes concluded that the quiescent centre is a reservoir of cells which will become meristematic after the normally meristematic cells have suffered genetic death.

It was therefore decided to study the radiation kinetics of the root meristem of Zea mays to see to what extent the simple meristematic models, deduced in the case of the bean root, could be used to explain the population kinetics in Zea root tips, and how much account has to be taken of the quiescent centre in these determinations.

Of additional interest was the hypothesis that the cells in the quiescent centre might be insensitive to radiation as a result of anoxia. Hall, Lajtha and Oliver (1962) had found that this is not the situation in the case of Vicia faba, but it was considered to be of interest to find how the dose response curve of the meristem of Zea with respect to reproductive integrity could be modified under anoxia.

A tentative investigation carried out by Shepstone (1964) using Zea seems to indicate that much can be deduced from its use. However, the variety used, Canada Gold, proved to be particularly fragile, and statistics consequently unreliable. Of a group of 30 or more irradiated only 4 or 5 would survive. As will be seen, Chapter VI, the variety, Kalahari Blitz, chosen for the present investigation was extremely hardy and statistics consequently good.

CHAPTER II

Relevant General Radiobiology

Before proceeding to a detailed discussion of the morphology of Zea mays and its root, it will be necessary to examine a few concepts related to the investigation.

Phases of the Cell cycle.

The cells of a tissue with cell renewal pass through four principal phases.

1. Cell formation is by mitosis in most tissues of fairly undifferentiated cells (comprising the generative cell fraction), whereas most differentiated cell forms (the maturing cell fraction) apparently lost the ability to divide under normal circumstances. This phase is also known as the M (mitotic) phase. The duration of morphologic mitosis differs between cell types; it seems as though most mitotic durations fall within a range of 40 to 70 minutes, although durations of several hours were reported for some tissues.
2. A phase of differentiation when cells elaborate secretions, evolve cilia or striated border or undergo keratinisation. It is followed by the metabolic or functional phase associated with cellular activities, such as secretion and absorption, or physical protection. The phases of differentiation and metabolic activity are combined as the G_1 phase, or first long (post-mitotic) gap. Different cell

populations manifest a great range in the duration of the G_1 phase. While it may be very brief with the cells of intestinal crypts, the G_1 phase occupies several weeks with those of mesothelia and urinary tract epithelia, for instance. Moreover, the cells of non-proliferating populations can be conceived as being sustained in a perpetual G_1 phase.

3. Cells of renewing populations invariably pass sooner or later into the S phase, or phase of DNA synthesis. In cells destined to divide, DNA synthesis ensues in an augmentation of the DNA content from the diploid to a teraphoid amount of DNA. Recent investigations have disclosed the duration of the S phase to be fairly constant, lasting from 6 to 8 hours with many mammalian cell populations.

4. The S phase is followed by a brief period, the second premitotic gap or G_2 phase, intervening between completion of DNA synthesis and onset of morphologically discernible mitosis. The G_2 phase ranges from less than 1 hour to about 4 hours in different cell populations, and only exceptionally is somewhat longer. From the G_2 phase, cells pass gradually into the first stage of the M phase, that is into morphologic prophase.

In renewing cell populations, the cells responsible for supplying new cells by division (the generative cell fraction) pass unceasing through the M, G_1, S and G_2 phases. Some of the daughter cells remain undifferentiated and recommence the cycle; others differentiate while at the beginning of the G_1 phase (the maturing cell fraction) and subsequently execute the functional activities peculiar to a particular cell type. They become eventually lost, and are replaced by new cells arising by mitoses of cells comprising the generative fraction of the cell population.

The great latitude of the duration of the G_1 phase in particular is responsible for the dissimilar lifespan of various cell types, and thus for the different turnover or renewal times of cell populations. Turnover times appear to be associated with adaptions of the various cell populations to physiological and environmental factors. On the other hand, the velocity of cell renewal of one and the same cell population is fairly constant but may be subject to species differences, and variability with age, and modifiable by a variety of morbid conditions.

The renewal velocity of cell populations has been investigated extensively. Data of turnover times of almost all significant renewing cell populations were ascertained at least on animal material. Two techniques, application of tritiated thymidine and colchicine, contributed greatly to the disclosure of such information.

Effects of radiation on integrated functions.

When irradiated in comparable conditions, different cellular populations react in similar patterns. With increasing doses, effects often become experimentally measurable in the following order: modifications of growth rate, mitotic delay, inhibition of mitoses, delayed or reproductive death and interphase death.

Growth rate Under chronic irradiation, the total mass of cell cultures first increases and then decreases. The initial increase of the total cell mass of the culture accompanies the emergence of giant cells, the volume and usually the ploidy of which increase without division. This phenomenon has been observed among bacteria yeasts and mammalian cells, and seems therefore to be fairly general. As dose accumulates, the total weight of the culture diminishes and becomes lower than that of controls. In general, radiation reduces growth rate, it increases generation time; however, under certain metabolic conditions, the generation time can be shorter than in control cultures once irradiation is discontinued. Interference with growth rate has been detected in isolated cells, even with doses as low as, and of the order of, 0.001R.

Mitotic delay When a cell has been irradiated before prophase, division is delayed. This delay can be modified by dose rate and by oxygen concentration; this may mean that metabolic processes are involved. During mitosis there is a critical stage coinciding with the condensation of chromosomes into visible filaments and with the disappearance of the nuclear membrane and nucleolus. If a dose as low as 1R is given to the cell before that critical stage, development of mitosis is delayed. However, this delay does not occur when the same or an even slightly higher dose is given later. In this latter case subsequent mitoses are delayed. More recent experiments have shown that the critical stage may be somewhat earlier in the mitotic cycle i.e. in mid-prophase. The critical sensitive period and the duration of the various phases of mitosis may differ in different types of cells. In consequence, precise comparisons are difficult.

The main characteristic of mitotic delay is its temporary nature. The mechanism of mitotic delay is still not understood. Since DNA metabolism is known to be effected by radiation, it is tempting to attribute mitotic delay to inhibition of DNA synthesis. This explanation is speculative and it may well be that reduction in DNA synthesis, when observed, is the consequence rather than the cause of mitotic delay. In particular, the radiosensitive period for producing mitotic delay usually occurs when DNA synthesis is already complete. In some instances, DNA metabolism is apparently normal despite inhibition of cellular division e.g. mammalian cells in culture. This suggests that delay in division may be a consequence of injury to an unknown mechanism controlling the onset of division and that there is no direct involvement of DNA synthesis. Yamada and Puck (1961) have proposed that reversible mitotic lag, like irreversible reproductive death, is due to chromosome damage, and that the lag may reflect interference with chromosomal condensation just before and perhaps in, the early stages of mitosis. Other hypotheses have also been advanced: interference of radiation with oxido-reduction of sulphydryl compounds produced during cellular division, and inhibition of the division mechanism of the cytoplasm or of the formation of the spindle. Production of anti-metabolites may also be responsible.

Inhibition of mitosis and cellular death: reproductive and interphase death With increased doses, cellular death usually occurs. Cells can be killed immediately (interphase death) or after a few divisions (delayed or reproductive death). In general, the doses required to achieve interphase death are higher, although there are cells which undergo interphase death even if irradiated by relatively small doses e.g. small lymphocytes, primary öocytes, neuroblasts, insect ganglia cells. Reproductive death occurs in bone marrow, intestinal crypt cells, lymphomas and spermatogonia. It should be noted that the latter group consist of cells with a high mitotic index; with these interphase death would probably require a higher dose.

The processes leading to reproductive or interphase death are still unknown; it is likely that more than one mechanism is involved. In delay death, chromosome breaks and mutations have been involved as possible mechanisms. The mechanisms resulting in cellular death may be better understood when the role of repair processes in irradiated cells have been studied, since the ultimate expression of a radiation effect depends not only on initial injury but also on the ability of the cell to repair the injury. Most chromosome breaks rejoin, metabolic and synthetic processes take part in healing, energy from ATP being required. Recent experiments by Elkind and Sutton (1959) have made it clear that repair operates in mammalian cells and influences the ultimate expression of late effects.

A clear distinction should be made between biochemical processes leading to delayed death and those leading to interphase death. In the former, synthesis of nucleic acids and proteins continues. Radiation-induced interphase death is sudden and marked by an arrest of metabolic behaviour e.g. cells not dividing (lymphocytes), cells dividing infrequently (öocytes) and cells continually dividing (B spermatogonia).

The biochemical causes of interphase death are not understood but may be due to inhibition of nuclear phosphorylation, which is an extremely radiosensitive process. As yet, this process has been detected in nuclei of so-called radio-sensitive tissues only; it has, therefore, been suggested that cells dependent upon this source of energy are those which undergo interphase death at small doses. Creasy and Stocken (1959) remark, however, that the failure to show nuclear phosphorylation in radioresistant cells may be due to an increased activity of degradative enzymes rather than to absence of this metabolic process.

Nuclear phosphorylation could also be involved in reproductive death if the energy necessary to heal chromosomes was provided by this phosphorylation.

A role of mitochondrial oxidative phosphorylation in interphase and reproductive death cannot be excluded. X-irradiation in vivo, in fact, damages mitochondria in liver cells even at doses as low as 25 R.

It is difficult to draw a coherent picture of the biochemical basis of cellular death at this time. The possible role of nucleic acids and protein synthesis has been discussed, but much more information is needed on the cytological alterations of sub-cellular structures produced immediately after irradiation. Nor can other biochemical processes affecting permeability, the maintenance of ionic balance, or the disruption of nuclear and cytoplasmic membranes, be ignored in the mechanism of cellular death.

Gross effects of radiation on root growth.

When seedlings of *Zea mays* are exposed to a dose of X-rays in the range 200 to 2,000 rads, the growth rate of the primary root follows a characteristic pattern (Figure 2.1). For the first day it is virtually unaffected, but thereafter falls progressively until a minimum is reached some 4 to 6 days later, to be followed by a gradual recovery. The extent to which the growth rate is decreased, and also the time taken for recovery, depend upon the size of the dose. For the dose range already mentioned, recovery is complete by 10 to 15 days after exposure, by which time the irradiated roots are growing as quickly as unirradiated control roots of the same age. Sometimes the growth rate as a fraction of controls becomes greater than unity and this enhanced growth rate may be due to the suppression of lateral roots by the radiation. A larger dose may cause a progressive reduction of the growth rate to zero, and the root, which turns brown, may not grow again. This will be the fate of a small proportion of roots exposed to 3,000 rads. In roots that survive, some of the cells die and are crushed by the growth of others, and Read (1959) believes that cell mortality is high after 130 rads in *Vicia faba*. If the root recovers from the irradiation, its growing point is often thinner than in normal roots of the same age. All types of ionizing radiation produce the same gross effects as those described for X-rays, but the relationship to dose may be very different.

Individual roots vary considerably, in both growth rate and response to radiation, and consequently most workers have exposed a group of between 9 and 20 roots to each dose level, and treated the results by statistical methods. In general three parameters have been used by various workers to score the effect of radiation on root growth:-

(a) The 'mean lethal dose' - defined to be the dose which results in cessation of growth for four or more days by half the roots of the group (Gray and Read, 1942; Spalding, Langham and Anderson, 1956, 1958).

(b) The 'minimum growth rate' of the roots, reached 6 to 8 days after irradiation and expressed as a fraction of control roots of the same length (Gray and Scholes, 1951), or as a fraction of roots of the same age (Lajtha,Hall and Oliver 1962). The quantity is marked G_{min} in Figure 2.1.

(c) The 'growth in ten days' - defined to be the mean increment in length of the irradiated roots in the ten days following irradiation, expressed as a fraction of control roots in the same period (Read, 1952). It is, in effect, the area under the curve up to the tenth day in Figure 2.1.

Parallel experiments in the literature in which radiation damage has been assessed, on the one hand by reduction in root growth as determined by the above parameters, and by the appearance of chromosome aberrations, lead to essentially similar results. Based on this evidence, Read (1959) has drawn the far-reaching conclusion that since inhibition of root growth reflects the loss of reproductive integrity by a proportion of meristem cells, this loss arises mainly from structural damage to the genetic material.

Mathematical models describing the cell proliferation pattern of the root meristem have been postulated to explain the above gross effects of radiation. These models, in so far as they are applicable to the present situation, will be introduced in Chapter VI and discussed in detail in the Appendix.

Special attention is now given to the so-called 'multihit' curve which has been quoted so frequently by radiobiologists in recent years without further investigations into its derivation.

The conceptions of Hit Theory and Target Theory

The basic idea of hit theory (Dessauer, 1922) can be stated thus:

The reaction to be studied (e.g. lethality, loss of reproductive integrity, appearance of chromosome aberrations) occurs to a particular one out of a great number of irradiated individuals (e.g. cell population) if a determinable number (hit number) of hits occurs in that single individual. Since the region in which the hit must occur need not be identical with the volume of the individual, each individual can have one or more targets ascribed to it.

According to this view, the form of the observed dose-response curve is due to the fact that absorption or radiation is not a continuous but a quantized process which follows a Poisson distribution.

Target theory (Crowther, 1924) on the other hand, concerns itself with the concept of a 'hit' or, more accurately, a 'hit event', since the most varied types of physical processes can be visualized as such 'events' is so far as they transfer energy from radiation to matter. The significant part of this work is that it offers the possibility of calculating from the dose-response curve a volume, the target, within which the required number of these absorption events must occur during irradiation, with given probability. Basically, all sorts of physical processes which give rise to absorption processes have equal claim, and the choice of one of them can and must be made according to the aim of the experiment and the biological data. Only in researches with visible and ultra-violet light is the ascription rigidly determined by physical principles.

These two theories will now be more closely examined, commencing with Hit Theory, and it will be shown how the formulae in current radiobiological use have been derived.

The Multi-hit Curve

Suppose that a number N_o of biological individuals (e.g. cells), identical in all respects, are irradiated in a macroscopically uniform field of radiation and that consequently some 'hits' (microphysical 'hit events' such as excitations or ionizations) occur. If the dose is E hits/cm^3, VE hits should occur in a volume of V cm^3. Since radiation is not microphysically homogeneous, the hits will be distributed independently of each other according to Poisson's law.

Thus the probability that exactly n hits occur in a volume V, when the expected number of hits is VE, is given by

$$P_n = \frac{(VE)^n e^{-VE}}{n!} \qquad (2.1)$$

Therefore the probability that there should be no hit (n = 0) in one individual is

$$P_o = \frac{(VE)^o e^{-VE}}{o!} = e^{-VE} \qquad (2.2)$$

or (converting the probability to numbers) the number N of the N_o individuals irradiated, which after a dose E still have received no hit is

$$N = N_o e^{-VE} \tag{2.3}$$

In other words, the proportion N^*/N_o which has received one or more hits is given by

$$\frac{N^*}{N_o} = 1 - \frac{N}{N_o} = 1 - e^{-VE} \tag{2.4}$$

and the proportion surviving any hit is e^{-VE}.

The proportion of individuals which have received one hit is given by

$$P_1 = VE\,e^{-VE} \tag{2.5}$$

and so the proportion of individuals which have received not more than one hit is given by

$$e^{-VE} + VE\,e^{-VE} \tag{2.6}$$

Similarly the proportion of individuals which have received two hits is given by

$$P_2 = \frac{(VE)^2 e^{-VE}}{2!} \tag{2.7}$$

and so the proportion of individuals which have received not more than two hits (i.e. the sum of the proportions receiving 0, 1 and 2 hits) is given by

$$e^{-VE} + VE\,e^{-VE} + \frac{(VE)^2 e^{-VE}}{2} \tag{2.8}$$

Therefore, in general, the number of individuals which have received not more than n - 1 hits is

$$N = N_o e^{-VE}\left(1 + VE + \frac{(VE)^2}{2!} + \text{-----} + \frac{(VE)^{n-1}}{(n-1)!}\right) \tag{2.9}$$

$$\text{or } \frac{N}{N_o} = e^{-VE} \sum_{k=0}^{k=n-1} \frac{(VE)^k}{k!}$$

The fraction of those which after dose E have received at least n hits is thus

$$\frac{N^*}{N_0} = 1 - e^{-VE} \sum_{k=0}^{k=n-1} \frac{(VE)^k}{k!} \tag{2.10}$$

Curves calculated from Equation (2.10) show close similarity in shape to curves which give the dependence of quantitatively measurable biological actions of radiation on dosage. For hit number n = 1, as Equation (2.3) shows, the curve is a pure exponential, but for larger hit numbers the curve is sigmoid. On a semilogarithmic plot, the single-hit curve is a straight line whilst the multiple-hit graph shows a downward curvature. Use will be made of these curves in Chapter VI and so further discussion of their computation is reserved until then.

Experiment yields N^*/N_0 for a particular dose, but hit number n and volume V must be deduced from the shape of the curve. V need not be identical with the volume of the irradiated biological entity, and so it must be replaced by v, the <u>formal target volume</u>. In this case it is assumed that of the hits occurring in the volume V of the irradiated individual, only those happening in the volume v are effective. Thus one can write

$$\frac{N^*}{N_0} = 1 - e^{-vE} \sum_{k=0}^{n-1} \frac{(vE)^k}{k!} \tag{2.11}$$

The task now remains of determining n and v from a series of pairs of observations according to Equation (2.11). Possibly the most convenient method of finding n and v is the graphical one, by which the experimental points can be entered on a graph on which the theoretical hit curves have been drawn. This process is greatly simplified if the abscissa is logarithmic, under which circumstances the curves can be moved along this axis for matching purposes without changing their shape.

Single- and multiple-hit phenomena in a divided target

In the above analysis it was assumed that each of the irradiated biological units exhibited only one target, impact on which of one or more hits induced the required reaction. Other assumptions are obviously also possible.

Taking Equation (2.11) again,

$$\frac{N^*}{N_0} = 1 - e^{-vE} \sum_{k=0}^{k=n-1} \frac{(vE)^k}{k!}$$

one can set $e^{-vE} \sum_{k=0}^{k=n-1} \frac{(vE)^k}{k!} = B$, so that the equation becomes

$$\frac{N^*}{N_0} = 1 - B \qquad (2.11a)$$

If one assumes that each biological unit presents m targets, each of which has the formal volume v, and that each of these m targets must receive n hits to cause the unit to react, one has

$$\frac{N^*}{N_0} = (1 - B)^m \qquad (2.12)$$

after delivery of a dose E.

Because of inevitable variation in the effectiveness of the hit process, not all the biological units will have the required number m of individual targets (e.g. yeast colonies consisting of individual yeast cells) and prescribed formal target volumes, v. If grounds therefore exist for the assumption of m different targets of volume $v_1, v_2, \ldots v_i \ldots v_m$, Equation (2.12) can be modified as below:

$$\frac{N^*}{N_0} = \prod_{i=1}^{m} (1 - B_i) \tag{2.13}$$

In practice, however, because of biological variability, it is scarcely possible to distinguish between curves derived from Equations (2.11), (2.12) and (2.13). Zimmer (1961) quotes several examples of ambiguity arising from this fact.

There are also cases in which it is permissible to assume that each biological unit exhibits m targets and that to give the reaction it suffices for <u>one</u> of them to receive n hits. If B represents the proportion of the targets which after dose E have not received n hits (i.e. have not received more than n - 1 hits), then B^m is the proportion of the units in none of whose m targets n hits have occurred. Therefore

$$\frac{N^*}{N_0} = 1 - B^m \tag{2.14}$$

is the fraction of the units irradiated with dose E,in which at least one of the m targets of a unit has received at least n hits,and which shows the reaction.

<u>The Multi-target Curve</u>

A method of analyzing curves derived from Equation (2.12) has been given by Atwood and Normal (1949), Kimball (1953) and Atwood (1959) which, at least for hit number n = 1, permits rapid determination of the target number m. For n = 1, Equation (2.12) simplifies to

$$\frac{N^*}{N_0} = (1 - e^{-vE})^m \tag{2.15}$$

and the fraction surviving dose E is

$$\frac{N}{N_0} = 1-\left(1-e^{-vE}\right)^{m} \qquad (2.16)$$

By expansion in series

$$\frac{N}{N_0} = 1-\left(1-me^{-vE}+\cdots\cdots \pm e^{-mvE}\right) \qquad (2.16a)$$

it is seen that with high doses the terms which follow me^{-vE} are neglible and that for high doses, therefore

$$\ln\frac{N}{N_0} = \ln m - vE \qquad (2.17)$$

For $E = 0$, $\frac{N}{N_0} = m$, and so on a semilogarithmic plot, extrapolation enables the target number to be read off directly from the ordinate.

Lea (1947) derives this equation in a different, although basically similar, way. He suggested that the shoulder observed in many bacterial survival curves might be explained by clump formation of cells that basically are exponentially inactivated. Each organism has a probability of surviving a dose which is expressed as

$$\text{Fraction surviving} = e^{-x}$$

where x is proportional to dose. Therefore, the Probability of killing an organism = $1 - e^{-x}$

If the organisms are distributed in clumps of m individuals, then the probability of killing all organisms in the clump (so that no colony appears) is

$$\left(1-e^{-x}\right)^{m}$$

and the survival curve then will be written as

$$\text{Fraction surviving} = 1 - (1 - e^{-x})^{m} \qquad (2.18)$$

As remarked by Powers (1962), this expression describes also the survival of a single cell in which the probability of inactivating a site within the cell necessary for the continued existence of the cell is also $(1 - e^{-x})^m$, and in which m is the number of such sites in the cell. Not unusually this expression has been interpreted as revealing the number of 'hits' necessary to bring about the death of the cell containing the mean number of replicated sensitive sites. There has occurred an increased awareness that this interpretation without further experiment is not warranted and the non-interpretative term 'extrapolation number' is sometimes used (Alper, Gillies and Elkind, 1960).

The exponent of e includes the measure of radiation sensitivity and may be written as

$$x = kD \qquad (2.19)$$

with radiation sensitivity being expressed numerically as k, the slope of the response curve, and D as the dose. If D is expressed in rads, k will be in reciprocal rads. If the slope is measured by the reciprocal of the increment in dose D_o necessary on the straight part of the log survival curve to reduce survival to e^{-1} or 37 per cent, (which dose has therefore been called the 37 per cent dose slope (Lajtha and Oliver, 1961) and obviously corresponds to an average of one hit per target), then k is replaced by the symbol λ and thus Equation (2.18) becomes

$$S = 1 - (1 - e^{-\lambda D})^m \qquad (2.20)$$

where S is the fraction surviving. This form has been called a Type C survival curve by Gunter and Kohn (1956).

All that now remains is to show the relationship between λD in Equation (2.20) and vE in Equation (2.15). This can be done more simply after a brief discussion of the nature of a 'hit' or 'hit event', which is the basic point of conjecture in so-called 'target theory'. (Zimmer, 1961).

As long as $m \gg 1$, and as long as the high dose portion is exponential in nature, the two numbers m and λ give a complete description of the response curve without further qualification. The value of this complete description is self-evident. The two constants can be evaluated graphically or, more accurately, by an iterative process which seeks those values of λ and m which give the minimum variance residue. Since the two constants have to be estimated simultaneously and a number of trials have to be made for the best values, manual methods of estimating them and their errors are very time consuming. Therefore several high speed computer programmes have been produced to yield the best estimates of m and λ together with estimates of their errors (e.g. Tyler and Dipert, 1962;)

The simplified Equation (2.20) has its limitations, however, and the more general equations must always be considered. However, Equation (2.20) has been found to approximate, over a wide range of dose, the dose-response curves obtained by a large number of workers, e.g. Puck and Marcus (1956); Hewitt and Wilson (1959); Elkind and Sutton (1959); Barendsen, Beusker, Vergroesen and Budke (1960); Berry and Andrews (1963).

The nature of the Hit Event

It is known from many physical researches that light-energy is absorbed in discrete quanta (photons), and that by absorption the atom or molecule aquires the whole energy of the photon. The absorbing atom is put into an excited state or perhaps (although seldomly) ionized, and thus has a special capacity for reaction. For all similar molecules irradiated with monochromatic light, the probability for absorption is the same and so the distribution of absorption events is a purely statistical one.

Thus in the case of light a 'hit' must be regarded as the absorption of a photon and 'dose' must be in 'absorbed photons per cm^3'. Due attention must of course be paid to the uncertainty principle.

In researches with ionizing radiation many possibilities lie open for designation of physical processes or 'hits'. The choice must be made according to the purpose in view and also with consideration of the characteristics of the biological objects involved. The identification of the absorption of a photon as a 'hit' is not valid here as the greater part of the energy of the photon is not taken up by the absorbing atom (or molecule) but is converted to kinetic energy of electrons which distributes it over a certain volume.

The identification 'passage of a fast electron' with a 'hit' has also been suggested. In this case it is not the mere passage of a particle which produces the effect, but ionizations produced by the passage. The process in its entirety constitutes a unit event of energy transfer. In this case, the dose is specified in 'number of particles crossing per cm^2' and the evaluation of dose-response curves expresses not the spatial target, but a target area or action cross-section. This specification, however, can only be applied to particles randomly distributed in space.

The ascription 'ionization = hit event' is the one most frequently used and is applicable to all ionizing radiation. The dose is then given in 'number of ionizations per cm^3', but various complications arise in its usage, for ionizations seldom occur individually but in small groups called 'primary ionizations'. Lea (1946) therefore prefers to take the ion cluster rather than the individual ionization as the effective unit. A further complication is the presence of an associated excitation.

The nature of the target

It has already been mentioned in the earlier sections that the volume v, in which the n hits which are necessary to initiate the reaction under study must occur, need not be identical with the volume V of the irradiated unit. The frequent cases in which $v < V$ gave rise originally to the target 'theory' in which the target is treated as an important control centre within the unit. Zimmer (1961), however, considers this idea not to be sufficiently general since it embodies the unstated assumption that all hits on the target are effective. He therefore favours the concept of action probability which assumes that there is in each individual a volume w within which the action probability $P = \text{constant} = p$ and outside which $P = 0$. The target v computable from dose-effect curves is then related to w by the relation

$$v = p w \tag{2.21}$$

Lea (1946) assumes that p decreases from the value 1 in the centre of the target outwards according to a distribution function.

Therefore v is not a real volume, but being the product of a real volume w with the probability p, it defines a quantity analagous to a reaction cross section. The target so defined is designated the formal target or reaction volume. The real volume w, the true target, cannot be found directly from dose-response curves: a knowledge of the action probability p would be necessary for its determination. A method of decreasing the uncertainty is to work with such densely ionizing particles that as a result of the massive energy transfer to the structure concerned, a reaction probability p = 1 can be assumed.

Using the same technique, attempts have been made to get further information by carrying out experiments with particles of different linear energy transfer (Fano, 1938; Zimmer, 1943; Timofeéff-Ressovsky and Zimmer, 1947; Pollard, 1953; Pollard, Guild, Hutchinson and Setlow, 1955). These researches have shown that the dependence of action cross-section on ionization density often agrees with that computed under the assumption of a constant action cross-section, p.

The relation between λ and v

It has been shown that λ is the reciprocal of D_o, the dose which corresponds to an average of one hit per target of volume v. If ϵ 'hit events' are produced per cm^3 per rad, a dose of D_o rads will correspond to $D_o\epsilon$ events per cm^3 or $D_o\epsilon v$ events per volume v, and this must equal unity by the above definition.

Therefore $D_o \epsilon v = 1$

$$\text{or} \quad \frac{1}{D_o} = v\epsilon \qquad (2.22)$$

and ϵD = number of 'hit events' per cm^3 = E.

$$\text{Therefore} \quad \lambda D = v\epsilon D = vE \qquad (2.23)$$

and Equations (2.16) and (2.20) are identical.

The 'single-event component' of the Multi-target Curve

Experiments by several authors (Lea, 1946; Gray and Scholes, 1951; Barendsen, Beusker, Vergroesen and Budke, 1961; Barendsen, 1961, 1962; Berry and Andrews, 1963) have shown that the survival curves obtained with densely ionizing α-radiation having high average linear energy transfer are as a rule purely exponential, and therefore represent a single ionization event in a single target. This shape suggests that the deposition of a sufficiently large amount of energy in a small volume anywhere in a relatively large part of the nucleus will be effective (Lea, 1946).

Consideration of the spatial distribution of energy deposition by sparsely ionizing x-irradiation makes it plausible that at least a small part of the damage caused by ionizing radiations of low average linear energy transfer will be due to the same type of locally concentrated energy deposition, especially in the tails of the electron tracks i.e. part of the X-ray damage may be considered to be caused by a single event type of action. The greater part of the energy, which is deposited in less concentrated form, may be assumed not to be as effective. The damage caused by this part of the deposited energy might be reparable.

For this reason a 'single-event component' is sometimes included in Equation (2.20), which then reads

$$S = e^{-\mu D}\left\{1-(1-e^{-\lambda D})^{n}\right\} \tag{2.24}$$

where μ is the reciprocal of the 37 per cent dose slope for the single-event component. Unfortunately, in the majority of experiments, the data is insufficient to provide an accurate estimate of this component.

Oxygen effect

The influence of oxygen tension on the response of biological systems to radiation is one of the fundamental phenomena of radiobiology. This influence is generally called the 'oxygen effect'. It has been observed in a great variety of biological systems and may be described as follows:

(a) In the absence of oxygen, or at reduced oxygen tension, the effects of radiation are diminished, but not eliminated; oxygen acts as a dose multiplying agent. In the case of *Shigella flexneri* for example, survival is exponentially related to dose at all oxygen tensions, and the slope of the curve may be used as a measure of radiosensitivity. It has been found that, when a sufficiently dilute suspension of bacteria is vigorously bubbled throughout the period of irradiation with gases containing different percentages of oxygen, the relation between radiosensitivity, S, and the concentration of oxygen $[O_2]$ in the medium is given by

$$\frac{S - S_N}{S_N} = (R - 1)\frac{[O_2]}{[O_2] + K} \qquad (2.25)$$

where S_N is the sensitivity under anaerobic conditions, obtained by bubbling oxygen-free nitrogen through the solution, and R and K are constants. In general, R is the ratio between the effectiveness of a given dose when oxygen is freely available, and the effectiveness when oxygen is absent. Thus (R - 1) may be considered as the ratio of the oxygen-dependent to the oxygen-independent components of radiosensitivity. The constant K is the concentration of oxygen at which the sensitivity is exactly midway between anaerobic and fully aerobic values. The ratio R varies around 3 for a wide range of cell types and effects. The similarity between values of K (in the range 4.5 - 5.0 μM/1) for irradiation of bacteria, yeast, ascites tumour cells, and plant root cells may be fortuitous, since a somewhat higher value of K (10 ± 2.8 μM/1) has been reported for Tradescantia pollen tube chromosomes.

(b) In wet metabolizing systems, the presence of oxygen during irradiation appears to be essential, since no effect has been seen in bacteria irradiated under anoxic conditions when oxygen is introduced only 20 milliseconds later.

Even stronger evidence is supplied by studies of the inactivation of Serratia marcescens by very short pulses of high intensity electron beams. Cell suspensions were irradiated with 1.5 MeV electrons delivered either as a single pulse of 2 μseconds duration (10 - 20 krad total dose) or for five minutes at a dose rate of 1000 rad/min.; both treatments were applied either in hydrogen or in a 1% oxygen and 99% nitrogen mixture. When irradiation was very short, the radiosensitivity of the bacteria was the same as under anoxic conditions, whereas with the longer irradiation, oxygen enhanced the sensitivity by a factor of 2.5.

However, in dry bacterial spores two actions of oxygen, one realised only if oxygen is present during irradiation, the other at appreciable times after irradiation, have been shown.

(c) An important aspect of the oxygen effect is that the enhancement ratio, R, varies with the type of radiation, being highest with radiation of lowest LET. The effect is less marked when cells are exposed to high LET.

The nature of radiochemical reactions in the oxygen effect, especially the role of O_2H^o radicals, has been widely discussed. Proof has been cited that oxygen-free radical interaction takes place in bacterial spores to bring about biological damage by X-rays. However, these spores are semi-dry, and the role of water in these interactions has been studied as yet only in a preliminary fashion.

Thus a generalization involving the metabolizing cell cannot be made now.

The belief that the oxygen effect depends on cellular aerobic metabolism is challenged by experiments in micro-organisms with normal and defective cytochrome systems in which the oxygen effect is the same. However, oxygen effect varies with the cells' physiological state. Freshly harvested yeast cells before starvation, have a considerably higher oxygen enhancement ratio (R = 3.6) than cells which have been starved. The ratio R decreases as the starvation period is prolonged, reaching a minimum value of R = 2 after two days starvation.

The observation that <u>oxygen alone</u> causes chromosome aberrations when in high concentrations complicates interpretation.

The oxygen effect must not be confused with the effect of oxygen given in the post-irradiation period. Since the development of radiation injury depends on metabolism, it is likely that there are systems in which the magnitude of radiation lesions can be altered by changes in oxygen tension after irradiation.

Papers have also dealt with the effect of anoxia; these have shown that anoxic conditions in metabolizing cells after irradiation reduce damage in others enhance it.

<u>Fractionation</u>.

We have shown that the dose response curves for individual biological units irradiated with ionizing radiations obey an equation of the form Equation (2.20)

i.e. $S = 1 - (1 - e^{-\lambda D})^m$

If a cell population with this type of dose-response curve is exposed to two doses of radiation each of D_f rads, separated by a time interval, the surviving fraction will be given by

$$S = \{1 - (1 - e^{-\lambda D_f})^m\}^2 \qquad (2.26)$$

This will be true provided the interval between the doses is sufficient for any 'sublethal' damage due to the first exposure to be fully repaired before the second i.e. the effects of the two doses are independent. Elkind and Sutton (1959) have reported that for Chinese hamster cells grown in vitro 'sublethal' damage is fully repaired in about 10 hours. For bean root cells recovery takes place more slowly and is not complete until about 15 hours (Hall, Lajtha and Oliver, 1962), in fact a time between 17 and 24 hours was used by Hall (1962) and a similar figure was assumed to hold for <u>Zea mays</u>.

If now an experiment is performed to find an equivalent single dose (D_s) i.e. a dose which given in one exposure produces the same radiation effect as the two doses of D_f rads separated by sufficient time for complete repair of sublethal damage then:-

$$1 - (1 - e^{-\lambda D_s})^m = \{1 - (1 - e^{-\lambda D_f})^m\}^2 \qquad (2.27)$$

This will be true for any biological system in which sub-lethal damage repairs completely.

If this experiment is repeated for different values of the single and fractionated doses

(D'_s and D'_f) respectively then it is also true that

$$1-(1-e^{-\lambda D_s'})^m = \{1-(1-e^{-\lambda D_f'})^m\}^2 \qquad (2.28)$$

Equations (2.27) and (2.28) are simultaneous equations in which two quantities are unknown, namely m and λ. Values for the two unknowns may be deduced by several methods; either graphically, or by a method of successive approximations starting with estimated values of m and λ, or by enlisting the aid of a computer. The method used was that attributed to Professor C.A. Coulson (Hall 1962).

If the Doses D_f and D_s are large enough to fall on the straight portion of the dose-response curve, equation (2.27) is indistinguishable from

$$ne^{-\lambda D_s} = (ne^{-\lambda D_f})^2 \qquad (2.29)$$

because when D is large high order terms in the binomial expansion of equation (2.27) became experimentally undetectable. Rearranging this becomes:-

$$(2D_f - D_s) = \frac{\ln m}{\lambda} = \text{constant.} \qquad (2.30)$$

Therefore in this case, matching the effect of a single dose with two dose fractions will give a value for the ratio of $\log_e$ m and λ: but repeating the experiment at a different dose level will not provide a second simultaneous equation yielding individual values for m and λ.

For some cell populations it is technically not possible to observe the effects of doses small enough to be on the initial shoulder of the curve, and then the matchings of single and fractionated doses can only provide a ratio of ln m and λ. A range of values for m could be assumed and the

corresponding range of values for λ calculated. The ratio itself may be of some interest because it is the dose at which the straight portion of the dose response curve, extrapolated backwards reaches a fractional survival of unity.
It gives some indication therefore of the width of the initial shoulder of the dose response curve. With many cell populations, however, including for example the meristem of Vicia faba, the effects of smaller doses can be compared, and values for both m and λ can be compared.

The method described may be applied to any cell population where the effect of single and fractionated doses can be matched by a parameter which depends on reproductive integrity, i.e. if two treatment regimes produce the same value of this parameter, they result in the same proportion of cells remaining integer. It is not necessary to know the relationship between this parameter and reproductive integrity, but it is necessary to assume that the dose response relationship in respect of reproductive integrity is of the form described by equation (2.27). The conditions under which the method applies are summarised below:-

1. Complete recovery of sublethal damage is assumed possible and the fractionated doses must be spaced by a sufficient time interval for this to take place.
2. At least one value of the dose fraction, (D_f), must be small enough to be on the initial shoulder

of the dose response curve to obtain values for both the extrapolation number and the 37 per cent dose slope. If this is not possible and the dose fraction falls on the straight portion of the dose response curve, only the ratio ln m and λ can be determined.

3. The second exposure of the fractionated doses should be timed to coincide with the single doses in order that the subsequent effects should be comparable.

4. For steady state populations, it has to be assumed that during the time interval between the two fractions, the population kinetic pattern (i.e. proportion of cells differentiating) does not change.

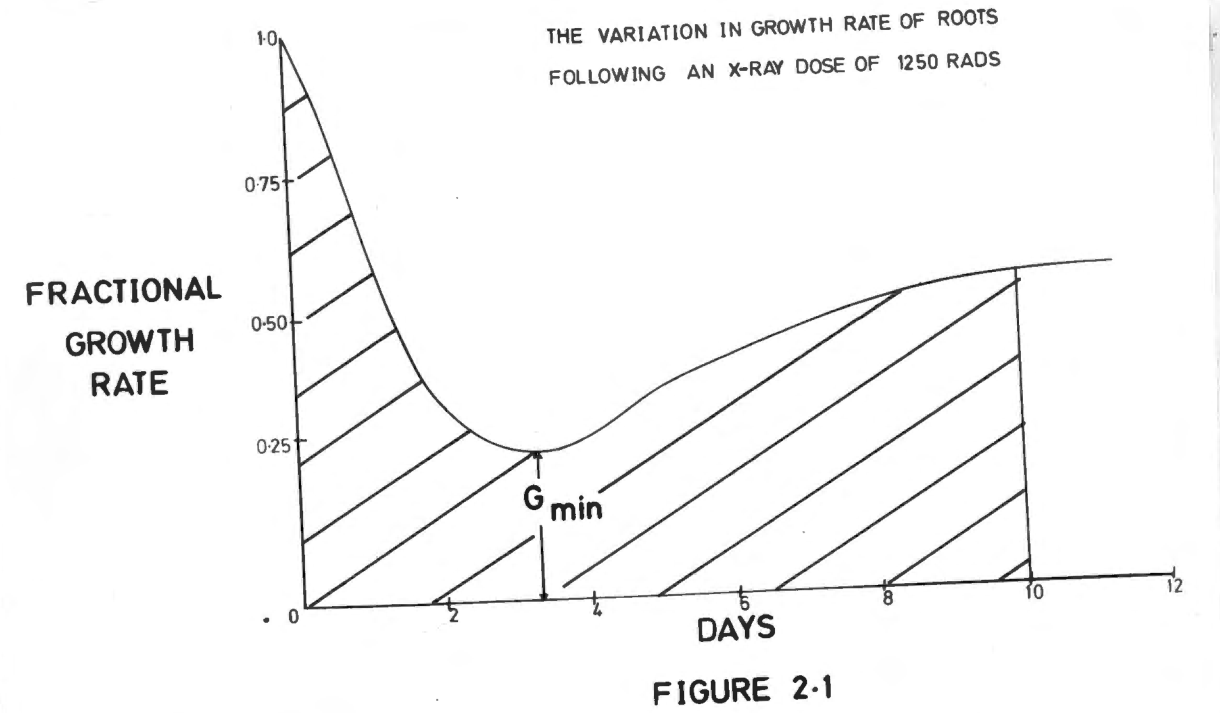


FIGURE 2.1

The variation in growth rate of roots following on x-ray dose of 1250 rads. G_{min} is the "minimum growth rate" and the area under the curve up to the 10th day is the growth in 10 days.

CHAPTER III

The Morphology and Microscopic

Anatomy of the Root of Zea Mays

Zea mays (from the Indian 'mahiz') is a Monocotyledenous plant belonging to the family Graminae, a subclass of the Angiospermae (or Flowering Plants), which form a class within the phyllum, or division, of the Tracheophytes, the Plant Group comprising land inhabiting plants with strong stems, good conducting systems and broad leaves.

Gross Morphology and Germination.

In the dry grain one end, the former point of attachment to the cob is more or less tapering whilst the other is broad and rounded, two sides of the grain being generally somewhat flattened. One one of these flat faces a whitish oval depression marks the position of the embryo, the remaining yellow part of the contents of the grain consisting of endosperm. Above that end of the oval depression which lies remote from the scar of attachment of the grain,careful examination will reveal a second scar-like projection, the remains of the stigma. This shows that the grain is still enclosed in the ovary-wall, and therefore really represents a fruit containing a single seed. The seedcoat is joined up with the surrounding ovary wall so that the two cannot be readily distinguished from one another, and as a consequence the micropile cannot be seen. A little above the middle of the pale patch caused by the embryo, at the end nearest the stigma-scar, a shallow and slightly darker depression marking the position of the plumule, is frequently recognised.

After removal of the envelope from a soaked grain, the white embryo and yellow endosperm are readily distinguished. A faint line runs vertically up the face of the exposed embryo. A slit along this line will reveal two peg-like projections, one directed toward the original point of attachment of the grain and representing the radicle, the other placed in the opposite direction and constituting the plumule. These will form the primary root and primary shoot respectively. The remainder of the white embryo comprises the single large cotyledon which is wrapped round both plumule and radicle.

The embryo occupies the whole of the base of the seed, but tapers off to one side above, whilst the cotyledon lies in close contact with the endosperm. The short blunt radicle (terminated by a darker root cap) is enclosed in a special cap-like sheath, the root-sheath, whilst the plumule consists of the central meristem encased in a succession of sheathing leaves. From one side of the axis of the embryo, where plumule and radicle join, the large shield shaped cotyledon arises on a short thick stalk. The whole margin of the cotyledon is drawn out, so as to surround the rest of the embryo, leaving a slit-like aperture opposite the plumule. The root sheath and the outermost sheath of the plumule both arise near the point of attachment of the cotyledon.

Around the former point of attachment of the grain is some whitish chaff (representing remains of the Maize flower), which probably facilitates the absorption of water, but the soaked grains of Maize exhibit but a slight increase in size as compared with the dry ones. The radicle is again the first part of the embryo to receive water and the first to emerge, bursting through the skin of the grain and growing down into the soil. The emergence of the radicle is very soon followed by the outgrowth of the plumule, which pierces the skin by its own active growth, and pushes its way to the surface. The radicle does not grow into a sturdy main root as in Vicia faba, but is almost immediately followed by a number of similar roots, developing near the base of the radicle, these adventitious roots are sometimes recognisable already before germination, and often grow more vigorously than the primary one which is soon indistinguishable from them.

The plumule, unlike that of the Dicotyledenous seedlings (Vicia faba), remains straight during its passage through the soil, but the soft meristem, and the young leaves encasing it, are nevertheless amply protected by the outermost sheath (the coleoptile), the tip of which is hard and pointed. It is thus a structure already matured within the seed that has to endure friction with the particles of the soil. On reaching the surface of the ground the sheath becomes exposed to the light and the inner leaves of the plumule burst through.

During the whole process of germination the cotyledon remains underground within the seed, and is actively absorbing nourishment for the growing embryo from the adjacent endosperm. Two regions are distinguishable in the latter, the portion nearest the embryo being white and relatively soft whilst the remainder is yellow and harder. The white portion alone will give the starch-reaction with iodine; the remainder of the endosperm consisting of thick-walled tissue.

The Root System

The two chief functions of any root system are attachment to the soil, and absorption of water and dissolved nutrients. In the root of a young seedling can be distinguished in general a root cap, root hairs and lateral roots.

The root cap serves the purpose of protecting the delicate cells of the meristem at the tip of the root from injury whilst the latter is forcing a passage through the soil. In a longitudinal section the cap is found to consist of a number of concentric layers of thin-walled parenchymatous cells whose arrangement becomes less regular toward the outside. During elongation of the root the outermost cells of the rootcap gradually become mucilaginous and break down so that the root tip glides easily between the particles of the soil. The substance of the cap is constantly renewed from the underlying meristem.

There is a varying extent of bare root between the apex and the region occupied by the root hairs.

It is in this region and here only, that increase in length is taking place in the new units formed by division of the cells of the meristem.

In order to pierce its way through the earth the growing root must obviously be capable of exerting considerable force. This force is the result of the increase in size of the cells in the zone of elongation and in nature where the older part of the root is firmly fixed by root hairs and lateral roots it serves to drive the apex downwards. Owing to the restriction of growth in length to a short region, the driving power is exerted close to the apex and therefore the direction of application of the force and the moving tip tend to remain in the same straight line; thus a maximum effect is secured.

The root hairs arise above the elongating portion of the root and the advantage of this is clear when it is remembered that their purpose is to absorb water from the soil, a function which could not be fulfilled if their position were continually changing with the growth of the root. Under such conditions the delicate root hairs would become torn and useless. They develop from that part of the root which has just concluded its growth in length. The root hairs insinuate themselves between the small particles of soil and come into close contact with the thin film of moisture thereon. This moisture will pass through the walls of the root hairs and into the main root by means of osmosis.

The type of root commonly found among the Dicotyledons of which <u>Vicia faba</u> is an example, is characterized by the possession of a prominent main root (or tap root) growing vertically downward into the soil, and bearing numerous branches (or lateral roots) which become progressively smaller.

In Monocotyledons on the other hand (e.g. Zea mays) the root system consists of a number of members in which we cannot distinguish any single one as specially prominent: such a root system is termed fibrous. The radicle which usually gives rise to the tap root in all fibrous root systems fails to develop to any considerable extent. The tuft of roots originates either by outgrowth from the base of the stem or from the hypocotyl. Owing to the fact that they do not arise from roots, these laterals are described as adventitious. Figure 3.1 shows a Zea seedling with these roots before their removal. In Zea mays after the plant has reached a certain height additional adventitious roots arise from the stem. These prop roots serve the purpose of augmenting the somewhat feeble primary root system, which is insufficient to maintain the tall maize plant in the erect position.

Structure of the root tip.

In the root tip of Zea mays shown diagrammatically on Figure 3.2, there are three tiers of initials (permanently meristematic cells) in the initial zone. One gives rise to the stele or central cyliner, the second to the cortex and the third to the root cap. The epidermis differentiates from the outermost layer of the cortex and arises from the same initials. The swollen wall substance originates through the gelatinization of the wall between the root cap and the protoderm a phenomenon associated with the sloughing of the root cap. The stele is separated from the root cap by a single layer of cells at the pole. This layer is part of the cortex-epidermis complex mentioned above and its cells form the distal surface of the quiescent centre (see next section). The elongating zone extends to about 4 mm from the end of the root and after this there is no sign of division.

Figure 3.3 represents a median section of the normal root apex according to Clowes (1963) and the shaded area represents the quiescent centre.

Figure 3.4 represents a section through a primary root. Starting from the tip upwards it may be divided approximately into the following sections.

(1) The root cap, the cells of which appear to be relatively inert.

(2) The meristem composed of dividing cells which in a normal unirradiated root have an approximate intermitotic interval of 24 hours. This value, estimated for a temperature of 25°C, was based on the results of Clowes (1963) and its variation with temperature (Shepstone 1964). The corresponding value for Vicia faba is 30 hours, though these values vary within wide limits for individual cells. In particular Clowes (1959) has demonstrated the existence in the meristem of a quiescent centre consisting of about 1000 cells which divide very infrequently if at all.

(3) The next centimeter or so of the root is termed the elongating zone because cells here make no more divisions, but only differentiate and elongate. Cells from the dividing zone pass into the elongating zone, and by their elongation cause an increase in the length of the root.

(4) The remainder of the root is composed of mature cells which are fully elongated.

There is naturally some overlap between the meristematic and elongating zones, although the transition is quite rapid. In the first 1.5 mm of the meristem, the proportion of cells showing signs of differentiation is only 10 per cent, and so this proportion of the meristem may be regarded as a population of freely dividing cells.

The development of roots and the concept of initial cells

Ever since a single 'apical cell' was discovered in the root meristem of ferns, attempts have been made to interpret the cell pattern in seed plants such as *Vicia faba* as if there were also a single totipotent cell. Over the years it has become progressively simpler to understand the construction of the apex, but there is still some doubt about the number of initial cells - the permanently meristematic cells or promeristem - and their status.

Clowes (1959) suggested that the 'initials' were situated around the surface of the 'quiescent centre' - the cells of which divide very infrequently under normal circumstances. These studies were a direct result of researches based on the so-called Körper-Kappe theory, which describes the planes of cell division by an analysis of the pattern of cells. This theory is an improvement on the histogen theory of Hanstein (1868), which divided the meristem into three regions, according to whether they produce stele, cortex or epidermis. The difficulty of the latter theory is that it cannot explain how the histogens are maintained and it merely divides the meristem into regions based on the assumed differentiation of their cells. Recent investigations have shown that a combination of the Körper-Kappe theory with a modified histogen theory is adequate for explaining the pattern of cells in root meristems. Cells from various geometrical parts of the root would thus constitute the initials of the several anatomical regions of the root e.g. the meristematic cells above the quiescent centre initiate the stele and cortex, while those below initiate the root cap.

Guttenberg (1947) analyzed the cell pattern in several species of dicotyledons and came to the conclusion that there was a single central cell from which all tissues could be derived. Guttenberg maintained that the 'initials' of the histogens were renewed from the central cell and that those of the cortex and the cap were removed more frequently than were those of the stele.

Guttenberg's views were supported by Brumfield (1943) who induced chromosome aberrations in young radicles of <u>Vicia faba</u> by x-irradiation. After a period sufficiently long to eliminate cells not derived from the initials existing at the time of irradiation, he examined sections of the root for the position of aberrant cells. He discovered that some of the roots were sectorial chimeras, in which particular aberrations existed in sections of the root extending from the stele to the cap, and for about one-third of the circumference. He concluded that there were three initial cells for the whole root, each of which had produced a sector of tissue.

Popham (1955) criticized this paper on the grounds that in Vicia the classical hypothesis of a large transverse promeristem could also account for the presence of sectorial chimeras, because the transverse promeristem develops from a smaller promeristem in the young radicale. In other words, Brumfield's conclusions should apply to embryos rather than to mature plants.

Surgical experiments were designed by Clowes (1953, 1954) to discriminate between hypotheses which require a large promeristem and those which require a small promeristem. The experiments consisted of excising an oblique segment from the apex. The depth of the cut was varied so that in some roots the knife cut through the pole of the stele, in others the cut was more superficial. After the excisions the roots were allowed to grow a further 10-20 cm. If the promeristem consists of a small number of cells as required by the hypotheses of Guttenberg or Brumfield, the root should regenerate completely or not at all. If the promeristem is large there ought to be an additional class of experiments where part of the root is normal, regenerated from the intact part of the promeristem, and part abnormal, regenerated from the cut surface. Clowes found the latter to be the case.

Davidson (1960) used X-rays to produce chromosome aberrations in Vicia faba and has followed these in the production of lateral branches of the irradiated mother 3 - 4 weeks after irradiation. He found that one of his lateral roots had five different chromosome complements as well as normal cells and therefore deduced that at least six different kinds of cells contributed to the formation of the lateral root primordium. In fact, the actual number of cells involved was likely to be greater than six. This result implies that six different kinds of cell exist together within a very small region of the mother root - a very different result from that of Brumfield.

More recently, Davidson (1960) has used aberrant chromosome complements as cell markers to deduce the number of initial cells from which a root irradiated with 600R of X-rays is able to regenerate. After three weeks post-irradiation growth he was able to recognise an average of eight abnormal cell types and a ratio of about three normal to one atypical cell. Assuming that no two cells would suffer identical aberrations, and assuming that the ratio of normal to atypical cells ramained unchanged, he calculated the average number of meristem initial cells to be 32 - 8 abnormal and 24 normal cells. Davidson (1961) later raised the number to between 40 and 50.

The quiescent centre

Following a study of the pattern of division in apical meristems, Clowes (1954) postulated the existence in Zea mays apices of a quiescent centre, a region in which cells can divide rarely, if at all, under normal growth conditions.

Clowes reasoned that the pattern of cells in the root apex of Zea mays is such that unambiguous conclusions can be drawn about the planes of division, and the relative rates of division. The central rows in the caps do not divide longitudinally and therefore do not grow transversely. This means that the contiguous cells at the pole of the cortex-epidermis complex also do not divide longitudinally, and it is known that they do not divide transversely because, on the axis, there is only one layer of cells between the clearly defined boundaries of the stele and the cap. Thus Clowes was able to conclude that the cells at the pole of the stele and cortex-epidermis complex do not divide at all.

The quiescent centre refers to root apices only. Clowes' work (1959) and the work of others (Partanan and Gifford 1958) and Cutter (1959) have shown that there is no region in shoot apices at all similar to the quiescent centre, nor is there any geometrical reason why there should be one. Clowes stresses that the quiescent centre should not be likened to the French méristème d'attente which has been completely discredited.

The constituent cells of the quiescent centre are carried forward passively by the growth of the surrounding meristem and contribute few cells to the root. They are quiescent only because of their position within the apex, and not because of any inherent disability. The pattern of growth in a root meristem can change both spontaneously and when stimulated, and when this happens, cells in the quiescent centre can become meristematic. Clowes (1961).

Whilst the geometrical approach does not give unambiguous results when applied to root apices of the other species of plant, with poorly defined tissue boundaries, other methods show that there is a quiescent centre in all roots except for those with a single apical cell. Clowes (1961, 1958).

The degree of quiescence varies considerably and in general the bigger the meristem the more prominent the quiescent centre, but there could be exceptions imposed by special conditions.

Since a quiescent centre exists in a root apex that has no clear tissue boundaries, such as Zea has (Clowes, 1956), this makes it difficult to believe that the tissues have common initial cells in the normal growth of the root, and so cell patterns that suggest a common initial cell, or a small group of initial cells, do so because they reflect behaviour in the embryo or lateral root primordium.

Clowes (1958) showed that the origin and development of the quiescent centre can actually be followed * in autoradiographs of embryos or lateral primordia fed with radioactive precursors of DNA. In the primary roots of Sinapis the origin occurs a few days after germination for at first all the cells are meristematic in the radicle. Then a few cells become quiescent at the pole of the stele and cortex, followed by more of their neighbours until 500 to 600 cells form a distinct quiescent centre in the growing root. In lateral roots of Pistia and Eichhornia the quiescent centre is created similarly and is fully developed before the root emerges from the cortex of the mother root.

Clowes (1959) has also demonstrated the presence of the quiescent centre in Vicia faba, by feeding the roots with tritium labelled thymidine which is incorporated into the DNA of the cells prior to their division. In autoradiographs of prepared root sections, the quiescent centre was clearly demarcated from the remainder of the meristem because the labelled DNA precursors were incorporated at a much slower rate, indicating infrequent cell division. This method can also be used to measure rates of mitosis. Roots are fed with tritiated thymidine for various periods, and the time at which the maximum number of nuclei become labelled gives an estimate for T, the average duration of the mitotic cycle. However the average rate of mitosis in the quiescent centre is too low for this method to be useful in this region. Radiation damage from the tritium becomes apparent in the meristematic cells after 2 or 3 days of feeding and this affects the rates of mitosis in the meristem. Nevertheless after 32 hours of feeding only 7% of the *quiescent centre cells are labelled compared with about 90% in the meristematic region of the apex, Clowes (1961). This means that the average duration of the mitotic cycle, T, is longer than anywhere else in the meristem.

The other method used to measure T in the different regions of root meristem is that of metaphase accumulation devised by Evans, Neary and Tonkinson (1957) which involves using colchicine for various periods and finding the rate at which metaphases increase. This method gives values for T which agree closely with those found by the labelling method applied to the meristematic regions of the apex. Values for T are given in the Table of Figure 3.5. These are average values based on the assumption that all the cells in each region do divide. The absolute values are of no special importance here, but the relative values do demonstrate what is meant by quiescence.

It is possible that the maintenance of a quiescent centre depends to some extent upon competition for substrates of synthesis, enhanced by the consequent development of differences in the gene-enzyme systems so the partial synchrony observable in <u>Sinapis</u> may explain the prominence of its quiescent centre.

The quiescent centre in <u>Vicia</u> consists of about 1000 cells in a hemispherical volume as shown in Figure 3.3

Clowes (1959) has shown that the roots of the meristem may be so badly damaged by X-rays that they stop synthesizing DNA and dividing. When this happens the root may continue to grow by forming a new meristem which arises in the quiescent centre. These therefore form 'a reservoir of cells which are less vulnerable because of their quiescence', but are able to restart DNA synthesis and division when the normally meristematic cells stop. Autoradiographs in fact demonstrate that there is a reversal of distributed label between the normally dividing meristems and the quiescent centre.

It is possible to see what these changes in rates of mitosis involve in anatomical reorganisation in Zea because the original boundary between the cap and the rest of the root remains visibly distinct in spite of the internal disruption. Clowes (1963).

It is not possible to follow reorganisation in this way in other kinds of roots with no permanently discernible marker in the apex, but the evidence from autoradiographs shows that the same kind of reorganisation can occur in Vicia, although in this case the new meristem does not invariably arise from an axial group of cells Clowes (1959), but occasionally from a lateral group of cells near the outer margin of the pole of the stele. In Zea the new meristem always seems to arise from the quiescent centre.

In these early experiments by Clowes with Sinapis, Pistia and Eichhornia, the roots investigated showed a sharply delimited boundary between the quiescent centre and the contiguous cap initials. The proximal boundary of the quiescent centre is not always so clear. One can only speculate about what it is that maintains such a big difference in the rates of cell division in contiguous cells, but with differences of the order of 15 fold one would expect to find also other differences in the cells of the regions of the meristem. The quiescent centre must have lower rates of synthesis than the rest of the meristem. This is known to be true for DNA and protein, Clowes (1958), and the cells of the quiescent centre are known to have less RNA and protein and, on the average, less DNA than other parts of the apex Jensen (1958). They have smaller nuclei (90μ^3 versus 150μ^3 in the cap initials in *Zea*), smaller nucleoli (4.6μ^3 versus 12.2μ^3) smaller Golgi bodies, few mitochondria per cell and less endoplasmic reticulum. All these features change abruptly in passing from the quiescent centre to the cap initials, and all of them can be related to the difference in the rate of mitosis.

Clowes explains Brumfield's root chimeras induced by X-rays (Brumfield 1943) by considering that a very small group of viable cells in the quiescent centre will be in a position to populate the whole of the apex, and if one of these cells carries a nonlethal genetic mutation, the root will be chimerical. The chimeras may not be very stable compared with shoot chimeras, but they could be sufficiently stable to remain in existence for several weeks.

Similarly it can be seen how it is possible for a root to remain alive and growing after suffering heavy radiation damage from the incorporation of radioactive substrates.

Clowes (1961) showed that roots survived after feeding with tritiated thymidine of high activity, and the reason that they do so is that the cells of the quiescent centre do not incorporate the tritium during their exposure and so remain viable and are able to continue the growth of the root when removed from the radioactive solution by forming a virtually new meristem by their proliferation.

There has been a great deal of thought concerning the reason why the quiescent cells are 'less vulnerable because of their quiescence'. Davidson (1959) has suggested that the quiescent cells may be less vulnerable because of their slow rate of division. Differences in water content may account for differences in sensitivity between dry and wet tissue since the ionization products of water can be toxic. This is, however, an unlikely source of explanation in the root apex, though there must be chemical differences between the quiescent and the active cells. One mechanism that could explain the difference in sensitivity is based on the view that the quiescent centre is anoxic. Hall, Lajtha and Clowes (1962) have, however, shown that the cells from which root recovery takes place are further protected by anoxic conditions in normal roots i.e. cells which are already anoxic could not be further protected by making the root anoxic. They also found that the progeny of these cells have the same radiosensitivity as ordinary cells. Calculations were also presented to show that the number of cells in the meristem surviving irradiation is enough to promote recovery without attributing any special degree of protection to any of the cells; but if recovery can also take place from a small region of the root, the possibility cannot be excluded that cells in this region have reduced radiosensitivity.

Clowes himself (1963), after observations on the micronuclei produced after irradiation, has suggested that most of the nuclei of the quiescent centre are in G_1, which (he states) implies that a greater than normal proportion of them are in the least sensitive phase of the mitotic cycle. His conclusions are based on Jensen's evidence (1958) that the cells of the quiescent centre have the diploid amount of DNA, whereas the rest of the meristem have amounts ranging from diploid to tetraploid.

Finally, it may be mentioned that the dose used by Clowes (1959) in his own work on the quiescent centre in Vicia faba was much larger (360R) than those used in most root-growth experiments (50-200R), and the quiescent centre is unlikely to be important at these lower dose levels. The region is of much greater importance in the case of Zea mays.

The control of differentiation.

Bunning (1952) cut off the apical 2 mm of roots and replaced the tips on the stumps after rotating them through an angle about the axis. He found that the vascular tissue in the tip differentiated out of line with that in the stump. This means that differentiation is not influenced by the existing differentiated tissue. Torrey (1955, 1957) reached a similar conclusion after a study of the changes in vascular patterns which occur during the culture of the excised roots of Pisum.

Gray and Scholes (1951) found that although the expression of the radiation effect is in the elongating zone, this inhibition is achieved by irradiating the meristem; irradiation of the elongating cells themselves having no effect. Also, Hornsey (1956) found that mitoses was present every day from 1 to 10 following 140r of X-rays.

From these two studies Davidson (1961) has argued that the reduction or cessation of root growth which follows irradiation cannot be attributed to inhibition of mitosis but must be largely due to a reduction in the number of cells elongating. Thus it seems that the pattern of differentiation, and consequently root growth, is governed by events within the meristem. Use will be made of this conclusion when the kinetics of the meristem are discussed.

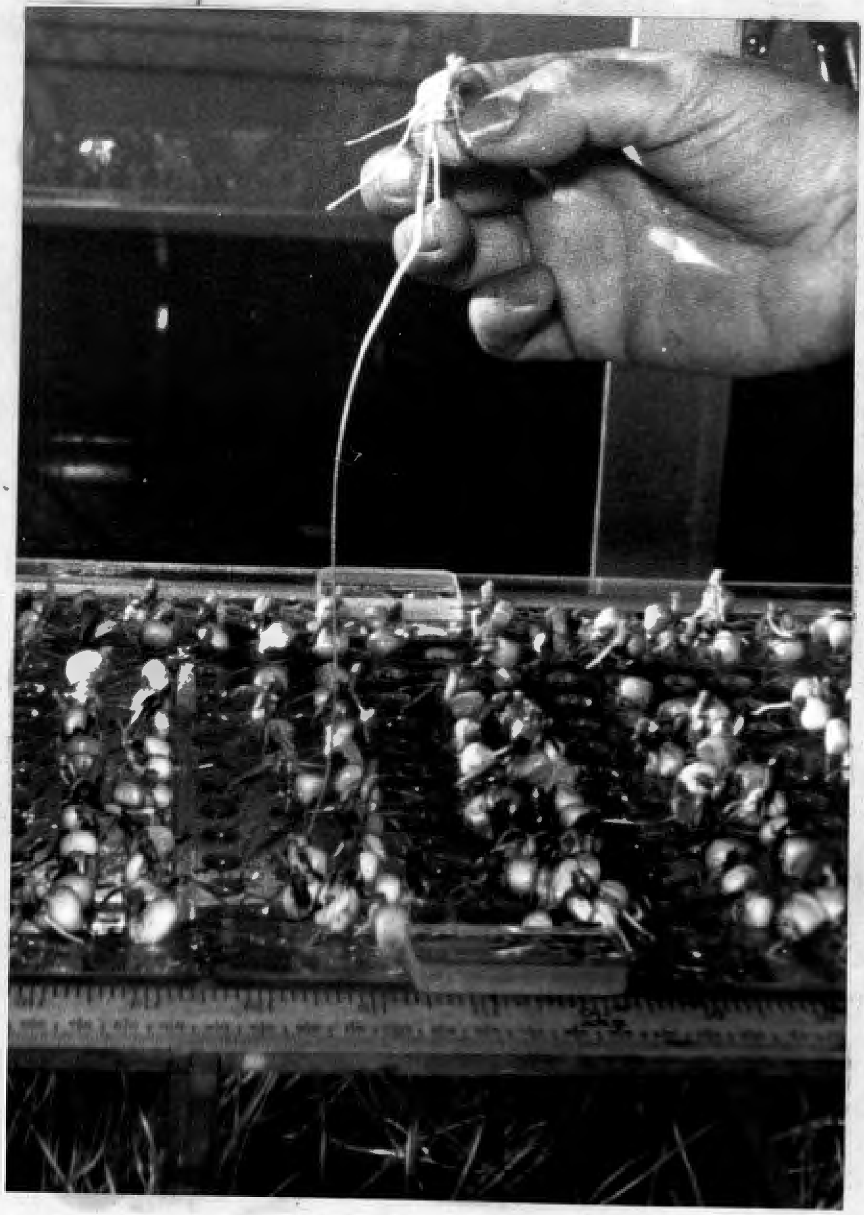


FIGURE 3.1

This shows the adventitious roots growing from above the join of the primary root and hypocotyl.

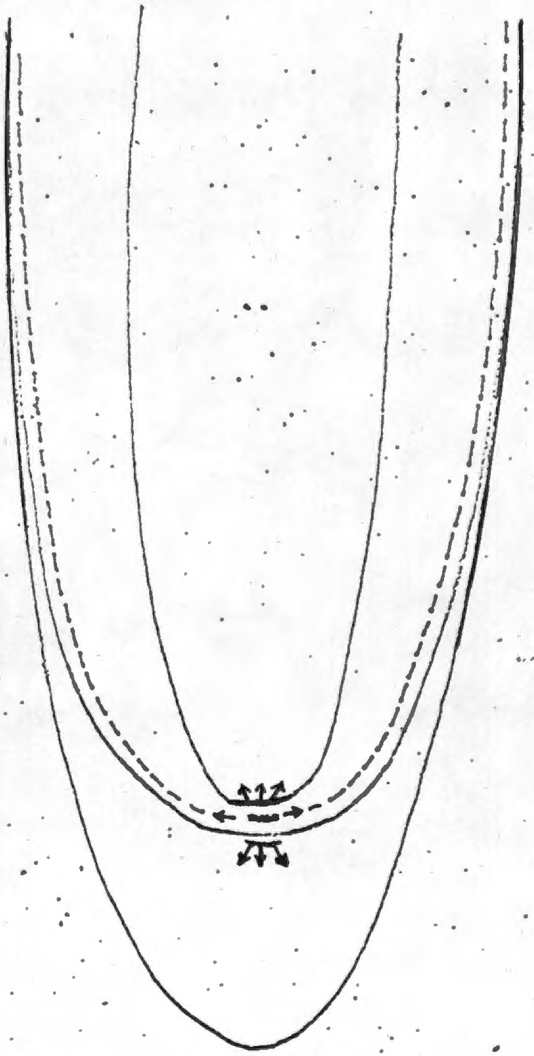


FIGURE 3.2

Diagram of the root tip of Zea, demonstrating the three tiers of initials in the initial zone. One gives rise to the central cylinder; the second to the cortex; the third to the rootcap. The epidermis differentiates from the outer layer of the cortex.

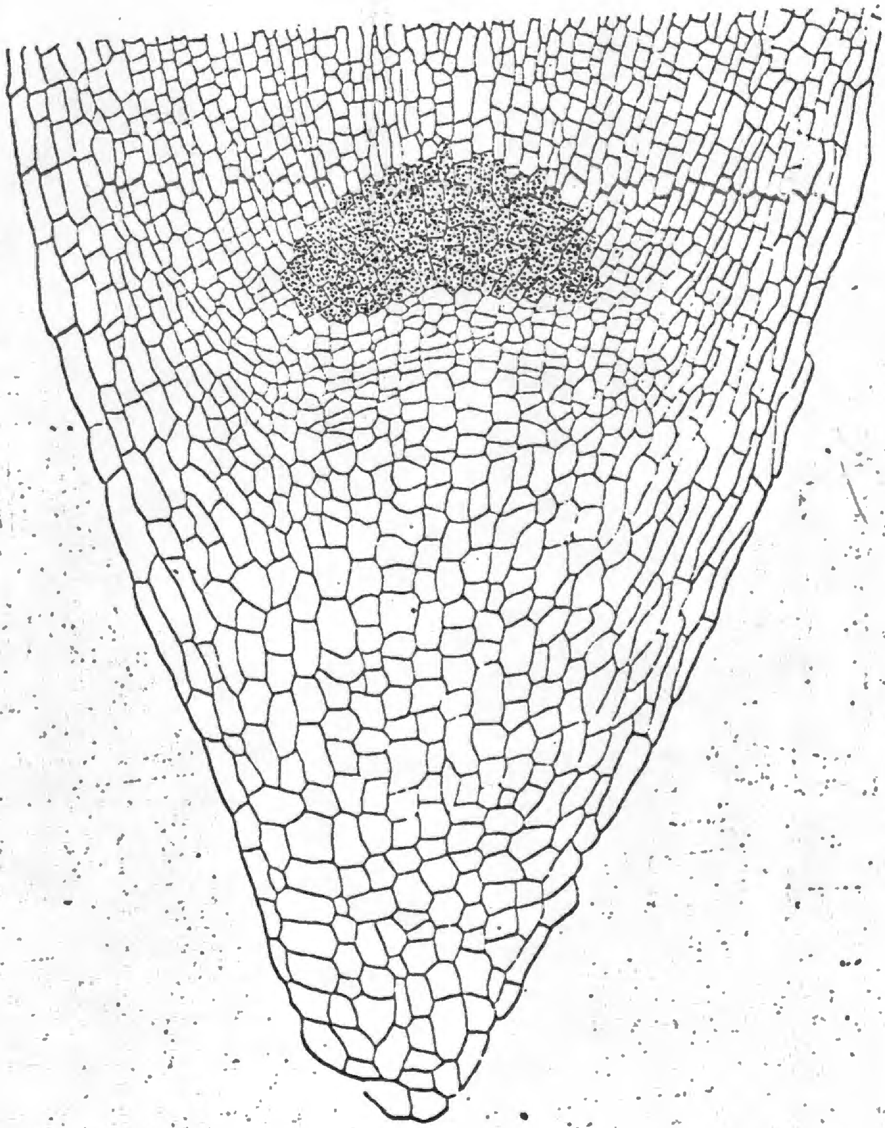


FIGURE 3.3

Median section of root apex of Vicia faba showing the position of the quiescent centre (shaded).

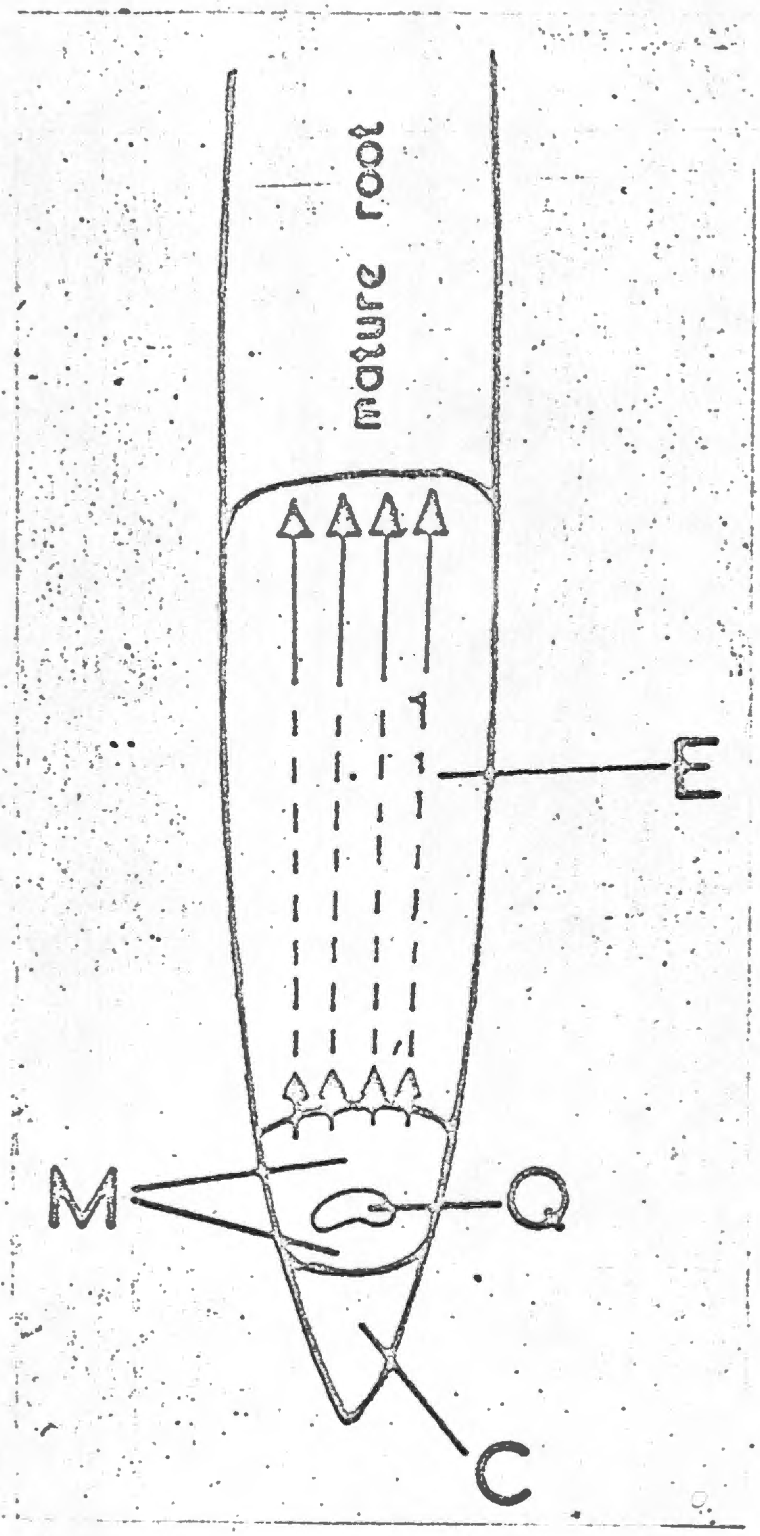


FIGURE 3.4

The structure of the root. C represents the root cap, Q the quiescent centre, M the dividing meristematic cells surrounding the quiescent centre, and E the elongating zone.

TABLE 3.5

Average Durations of the Mitotic Cycle (in hours) in Three Regions of Root Meristems, the Quiescent Centre and the Immediately Adjacent Parts of the Cap and Stele.

System	Quiescent Centre	Cap Initials	Stele
Zea mays	174	12	28
Vicia faba	292	44	37
Sinapis alba	520	35	32

(From The Quiescent Center in Meristems and Its Behaviour After Irradiation)

F.A.L. CLOWES.

CHAPTER IV

The Problem Stated

In the foregoing chapters relating to the choice of Zea mays as a radiobiological system worthy of attention, the general radiobiology relevant to the study, and a consideration of the morphology of the plant, with particular reference to its root, the stage has been set for an investigation of the response of the root meristem of Zea to acute doses of ionizing radiation.

It would therefore be appropriate at this stage to set out the primary aims of the study, and what conclusions might be drawn from the results.

The work falls into five categories:-

(1) A culture method, very similar to that used in the past for Vicia has been used for the cultivation of the maize roots and it will be demonstrated that similar growth parameters to those employed for Vicia can be used to assess radiation damage.

(2) Hall, Lajtha and Oliver (1962) have considered the growth pattern of the bean root in terms of the proportion of cells maintaining reproductive integrity, and two theoretical kinetic models allowing recovery have been formulated. These two models (termed Models A and B) are used to derive the dose response curve with respect to reproductive integrity of the cells of the root meristem of Zea mays to acute doses of X-radiation under both aerobic and anaerobic conditions.

It is assumed that the curve has the same form as that reported for mammalian cells viz. Equation (2.20)

$$S = 1 - (1 - e^{-\lambda D})^m \qquad (2.20)$$

where S is the fraction of cells surviving a dose of D rads, λ is the reciprocal of the 37 per cent dose slope, and m is the extrapolation number.

From these results it will be possible to deduce values of m and λ for this system under aerobic and anaerobic conditions.

(3) If the characteristics of the dose-response curve could be deduced by a different method to that based on a model of the meristem, it would provide a further check on these values. Using simple fractionated doses, and equating these with the equivalent single dose at two dose levels, Hall (1962) has derived m and λ graphically in the case of <u>Vicia faba</u>. This method has been adapted in the present instance with <u>Zea mays</u>, and a numberical method of analysis employing the Wang desk calculator used to verify the characteristics of the aerobic dose response curve obtained by the theoretical models.

(4) From the dose response curves derived with respect to the criteria used for assessing radiation damage, it is possible to derive a value for the oxygen enhancement ratio in the case of this radiological system. The value obtained will be considered with reference to other systems.

(5) Finally the characteristic of the dose response curve will be discussed in relation to other known parameters of the root meristem of <u>Zea mays</u>. Particular attention will then be paid to any conclusions which pertain to the quiescent centre.

CHAPTER V

Materials and Methods

On the advice of the Agricultural Department of the University of Stellenbosch the variety of Zea mays used was that known as Kalahari Blitz. This was the only variety that could be obtained in bulk, uncontaminated by other varieties, and the continued supply of which could be guaranteed. In the past other workers have found great difficulty in obtaining a hardy variety, but in the present case great success in this respect has been achieved. The method of culture employed was that used by Hall, Lajtha and Oliver (1962).

For each experiment approximately 400 seeds were placed in a Polythese bowl to which fresh water at ± 25°C was continuously added. After three to four days the seedlings which had germinated were planted in moist horticultural Vermiculite contained in a large brass tank. This Vermiculite had previously been autoclaved up to 126°C. This growing tank was maintained at a relatively constant temperature in the neighbourhood of 25°C.

The method of planting was to press the seed into the Vermiculite until the broad upper surface of the cotyledon just showed. In this way about 300 seeds could be accommodated (Figure 5.1). When subsequent root growth caused the cotyledon to lift, more Vermiculite was added and the covered seedling pressed gently down. After growing in this way for four days with frequent moistening of the surface, the seedlings were carefully lifted from the Vermiculite and washed. All damaged and malformed roots were discarded and, from those remaining, enough were chosen, with roots of 5 cm. length and over, to fill all the holes in the Perspex lid of the culture tank (Figure 5.2).

The culture tank consisted of a rectangular Perspex tank 2 ft x 1 ft x 1 ft through which a continuous flow of tap water was passed at the rate of about 1 litre per minute. The temperature of the water in the culture tank was maintained at $25^{\circ} \pm 0.1^{\circ}C$ by a Braun thermostatically controlled heater, which also incorporated a vigorous stirrer. Gray and Scholes (1951) reported that a change of $1^{\circ}C$ resulted in a 20 per cent change in the growth of the roots, so it was felt that meticulous control of the temperature was necessary.

The seedlings were placed on the Perspex lid of the tank with their roots passing down through the holes into the water. All shoots and adventitious roots were nipped off close to the scutellum taking care not to damage the latter. This was done to ensure that growth was confined to the primary root only. The scutella were covered at all times during the experiment with moist surgical gauze to prevent their dehydration.

The selected seedlings were allowed to grow for 24 hours in the culture tank. They were then examined, and all those whose roots showed malformation or stunted growth were discarded. The remainder were divided at random into one group of 28 (there were 14 holes across the tank hence this was a convenient number) to serve as controls, and as many groups of about 20 as were required for irradiation. The roots were selected for each group in such a way that a fair range of lengths appeared in each group. It was impossible to carry out rigorous randomisation due to labelling difficulties.

Method of Irradiation for Acute Exposure.

The radiation source was a Philips 250/25 X-ray Therapy Unit operated at 250 KVp 15 mA with an added filter of 0.8 mm Tin, 0.25 mm Copper and 1.0 mm Aluminium. The Half Value Layer of the beam was 3.5 mm Copper. By not using an applicator, but retaining the cone shaped adaptor (which in daily therapy enabled the same applicators to be used on all machines), an output of 110 rad/min was obtained. In view of this high dose rate it was thought unnecessary to replace the composite filter by a copper one which would have raised the output at the end of the applicator. This arrangement provided perfectly reproducible geometry. A picture of the experimental arrangement used is appended (Figure 5.3).

A Perspex jig was constructed to hold about 22 Zea mays seedlings at one time, and in such a way that their tips were congregated near each other. This was accomplished by a funnel-shaped cavity in the jig (Figure 5.4) some 3 mm. deep in the direction of the irradiating beam and situated 2 cm. from the outside wall of the tank into which it fitted. The jig could be slid into slots at one end of the 1 ft. 6 in. x 1 ft. x 1 ft. Perspex tank and had a projecting tube at the bottom through which air or Nitrogen could be passed.

For the purposes of dosimetry this jig could be removed and replaced by another containing a cavity shaped to accommodate the chamber from a Baldwin Farmer Substandard Dosemeter, which could then be placed in the exact position occupied by the root tips during irradiation.

Although the whole of the root is irradiated in this arrangement the root tip is the sensitive volume and irradiation of the remainder of the root does not affect the growth of the primary root Read (1959). The cotyledons were shielded during all the radiation experiments in order to confine radiation effects to the growing root.

Scoring Radiation Damage.

In all experiments where Zea mays roots were exposed to single doses of ionising radiation, the length of the primary root was measured immediately following irradiation, and before replacing the root in the hole in the lid of the culture tank. The measurement was carried out by placing the hypocotyl firmly against the end of the Boxwood rule clamped to the long side of the culture tank, and gently smoothing the root along the scale. A stroking action was used to avoid undue pressure on the humps which could fracture the root, and to prevent stretching (Figure 5.5).

As the scutella are too small to number individually, the length on day 0 was recorded against a number which was allocated according to the position of the root in the tank and which was maintained throughout the experiment. Controls were also measured on day 0.

In the experiment where fractionated doses were given, measurement of root lengths was made after the second dose and the controls were also measured at this time.

All roots were measured at approximately the same time (± 1 hour) on alternate days for 10 days and then finally on the eleventh day, for reasons to be described later.

The lifting of the seedlings for measurement of the root, and removal of shoots and adventitious roots, was done with care to avoid damaging them. Gray and Scholes (1951) reported that this handling did not affect the growth of the root.

At this point it must be mentioned that no attempt was made to influence the amount of light falling on the growing _Zea mays_ seedlings. Mottram (1913) had suggested that roots should be grown in the dark to avoid development of a rhythm of cell divisions as he thought that dividing cells were more sensitive to radiation. Read (1959) failed to reproduce these findings in _Vicia Faba_, and Evans, Neary and Tonkinson (1957) have shown that, provided the shoots are removed and prevented from developing it is not necessary to grow roots in the dark. Hence in these experiments no attempt was made to regularise the light conditions, but shoots and adventitious roots were removed daily.

After each set of measurements the average growth increment for two days was evaluated for each dose and expressed as a fraction of control growth for the same period. Each increment was regarded as that pertaining to a time halfway between the times at which the two measurements were made. It was found that the growth rate of the controls was not constant, but decreased steadily during the course of the experiment. It was for this reason that growth increments were expressed in terms of control growth increments over the same period i.e. with respect to controls of the same age. A possible explanation for this is the inhibiting action of applied auxin (a root hormone considered to be identical with indole-3-acetic acid) over a wide range of concentrations. Pilet (1961) found that the destruction of auxin is greater in old root tissues than in young tissues, hence we would expect the rate of growth to fall off at an increasing rate with age.

Corresponding to each dose a curve was then drawn, showing the variation of growth rate as a fraction of controls of the same age with time. The minimum value of this curve, referred to in Chapter II as G_{min}, was one of the parameters used to assess radiation damage. The other parameter, G_{10}, or "growth in 10 days" was evaluated as follows. The average difference between root lengths on day 10 and day 0 for each dose was expressed as a fraction of controls over the same period.

Four experiments were carried out, the procedure being similar in each case.

For the first experiment - the irradiation of Zea mays by acute doses under aerobic conditions - the tank was set up at 25 cm. from the tube focus with the long axis of the tank along the beam axis (as shown in Figure 5.6). The dose rate was measured with the chamber of the Baldwin Farmer substandard instrument in position in the special jig. The time taken to deliver 50 R was measured three times and the mean noted. The dose rate in rads per minute was computed using corrections for temperature, pressure, and quality and using the appropriate Roentgen to rad conversion factor. The quality factor for this instrument was obtained from the Council for Scientific and Industrial Research standards laboratory by comparison with their Free Air chamber in 1967. The Roentgen to rad conversion factor was that recommended by the International Commission for Radiological Units (1962). From the jig and field dimensions the variation in dose across the root tips was estimated to be within 2 per cent of the measured dose.

The times to deliver the doses to the groups involved in this experiment were computed, the chamber removed and the jig exchanged for the one designed to hold the *Zea mays* seedlings for irradiation. In reviewing the doses used in the past by Clowes (1959, 1963? these ranged from about 300R to 1,800R. It was therefore decided to give doses in the range 250 rads to 2,500 rads so as fully to cover these. Both jig and tank were filled with clean fresh tap water, and the outlet from an air cylinder attached to the jig. The group of seedlings to be irradiated were arranged in the jig with their roots sloping toward the middle of the funnel the longer roots being placed towards the outside. The tube head was adjusted to ensure that all roots were in the uniform part of the field. A steady stream of air was bubbled through the jib. Each group was left for 15 minutes to equilibrate before irradiation. Read (1959) maintains that this is a sufficient time for equilibrium to be reached between the oxygen tension of the water and the tissues of the root.

Before commencing irradiation the water level in the jig was checked and topped up if necessary as the bubbling of the air through the small volume increased the rate of evaporation and also splashed water out leaving part of the roots uncovered by water.

During the irradiation of any group the correct tube voltage and tube current were maintained using the manual controls.

Following irradiation the roots in each group were measured as described and placed in the holes in the Perspex cover of the culture tank. A row of empty holes was maintained between groups and a diagram kept of the positions of the groups and doses employed. These initial measurements were recorded as those appropriate to Day 0.

A check was made on the dose rate after the groups had all been irradiated. A variation of less than 1 per cent from the dose rate was found.

A second experiment was then performed in order to fill in the gaps between the curves derived from the first experiment, and to extend the range of doses used. It was felt that a dose of 3,000 rads would kill the root and so this was made the upper limit of the dose range. The lower limit of 200 rads was made out of interest as little damage was expected to result. However, a value at this end of the range does enable the shoulder of the response curve (see Chapter VI) to be obtained.

Excellent agreement was found between the values estimated as a result of the first experiment and the values subsequently obtained from the second experiment.

A third experiment - the Irradiation of the Root Meristem of Zea Mays under Anaerobic/Anoxic Conditions - was then performed.

The seedlings were cultivated in exactly the same manner as described, and the experimental procedure during irradiation was the same except (i) for the use of Nitrogen in place of Air and (ii) the doses ranged from 250 rads to 5,000 rads. These doses were used in anticipation of there being an oxygen effect present in this radiobiological system.

After irradiation the roots were returned to the culture tank and treated in exactly the same way as in the case of the aerobic experiment.

From the growth increments per 48 hours obtained for the controls in each of the three experiments, a normal growth curve was obtained. This curve, the minimum growth rate, the 10 day growth rate, and dose response curves, are given at the end of Chapter VI

In a fourth experiment - the Derivation of the Dose-response Curve Parameters using a Fractionation method - the roots were given two fractions of ionizing radiation separated by an interval of time.

The groups of Zea mays seedlings were irradiated in the manner described for the first two experiments, but measurements of the root lengths were not made following the first irradiation. Instead, the roots were placed in the holes in the Perspex cover of the culture tank in the groups, but no cognisance was taken of the position of that hole within the group.

In one case two doses of 500 rads were given at intervals of 18.5, 20 and 24 hours. In the other, two doses of 1,000 rads were given at the same time intervals. The lengths of the roots were measured immediately following the second dose and their position in the tank noted. Thereafter measurements were made on alternate days at approximately the same time of day (± 1 hour) as described earlier.

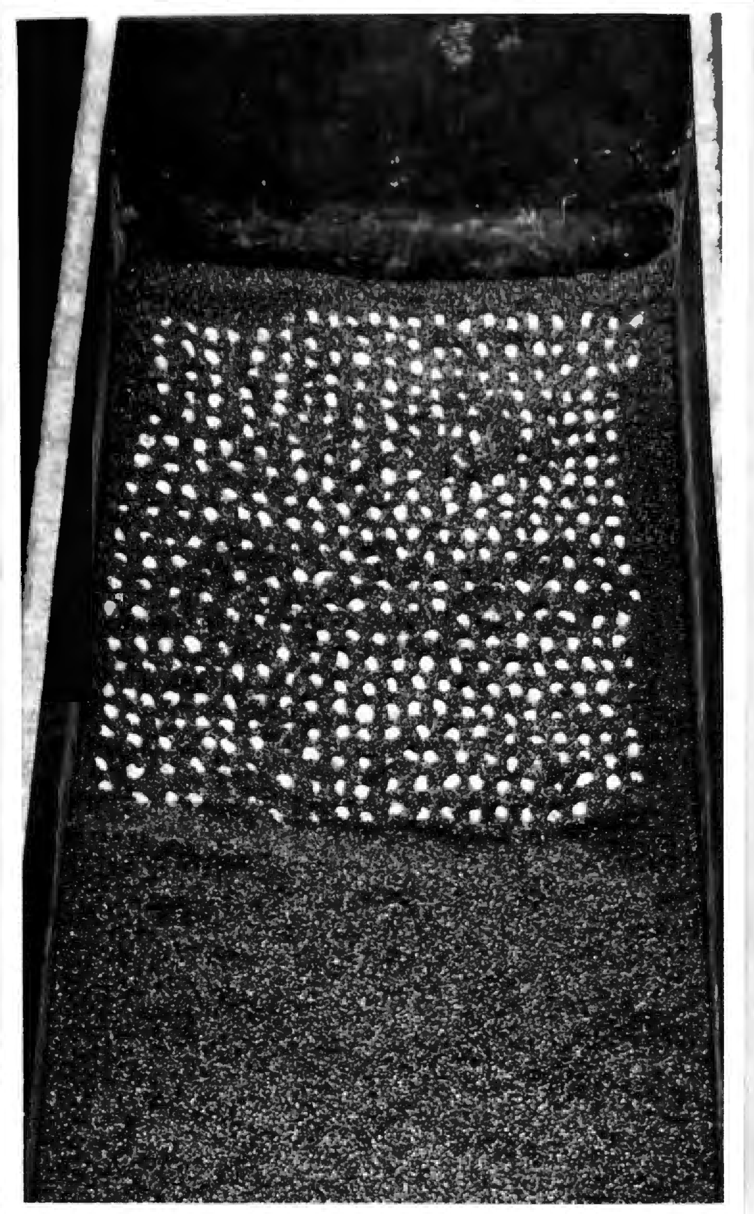


FIGURE 5.1

View of tank showing seeds embedded in Vermiculite. Half of the seeds have already been covered over.

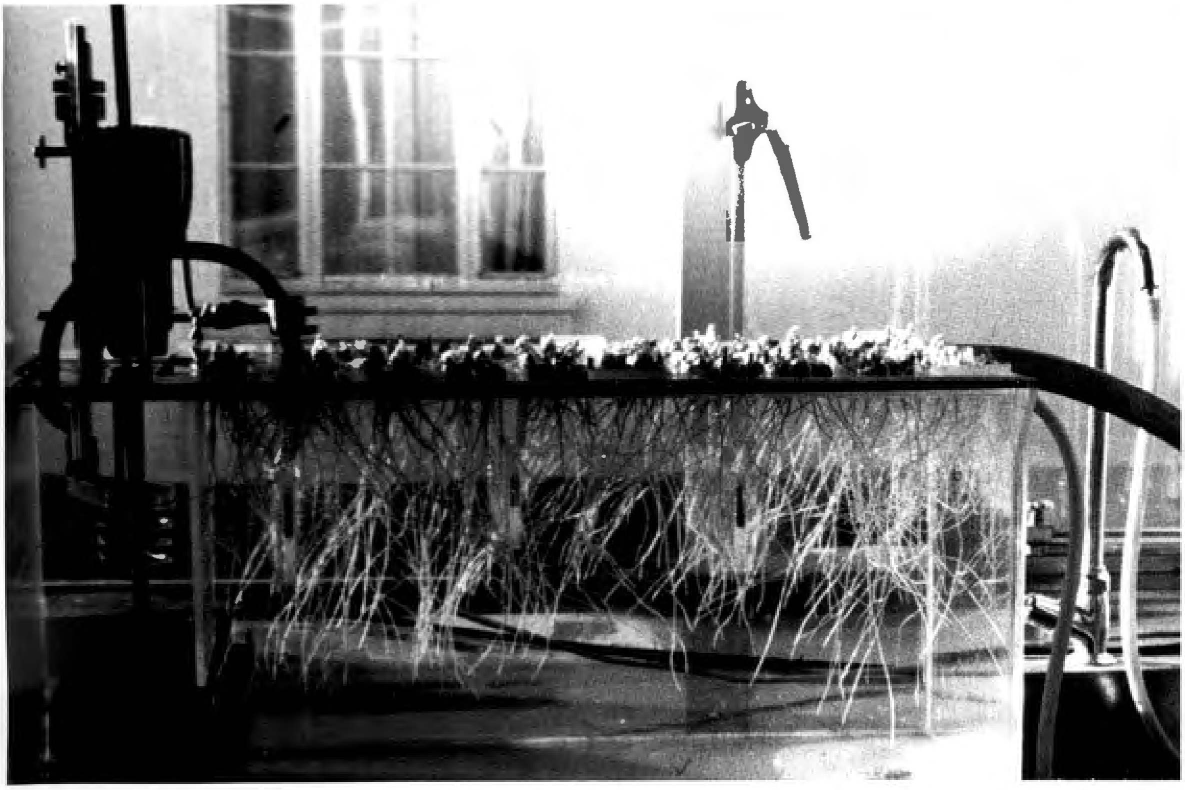


FIGURE 5.2

View of culture tank showing heater, compartments and inlet outlet points.

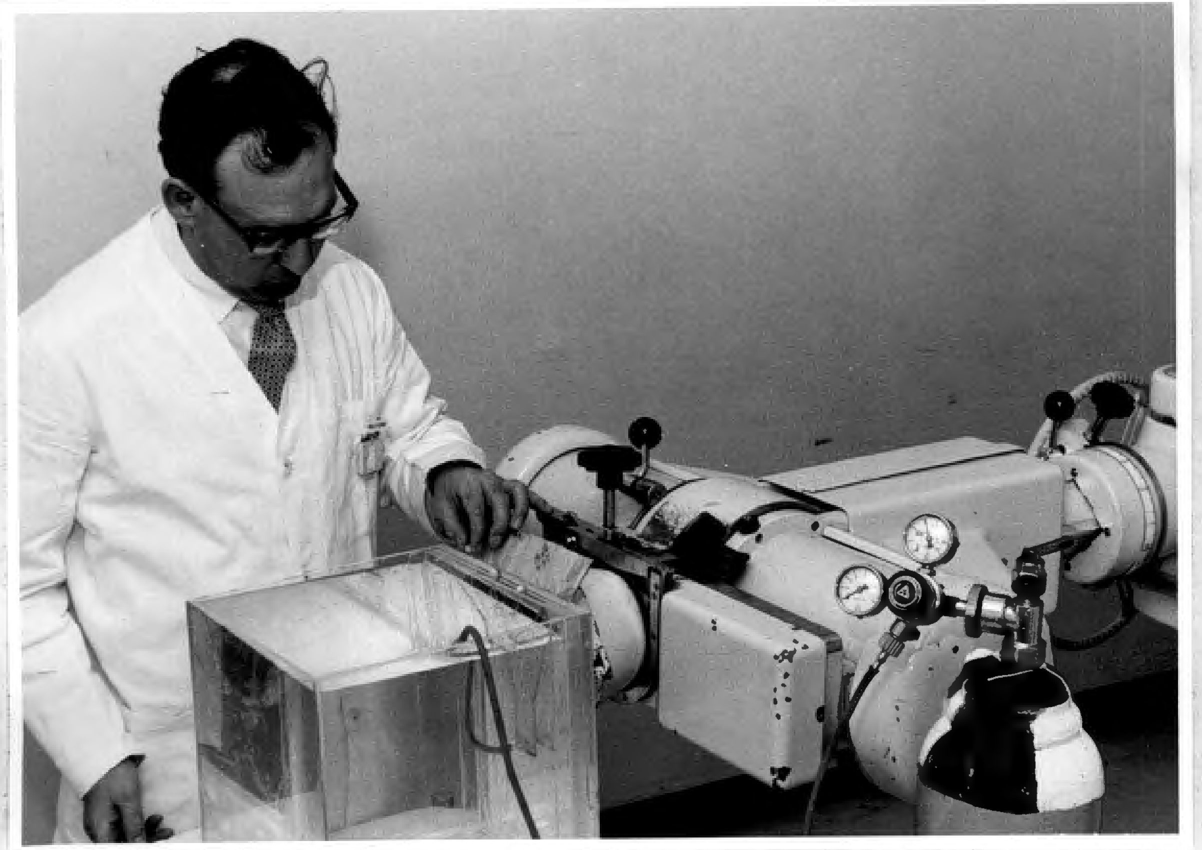


FIGURE 5.3

View of experimental arrangement.

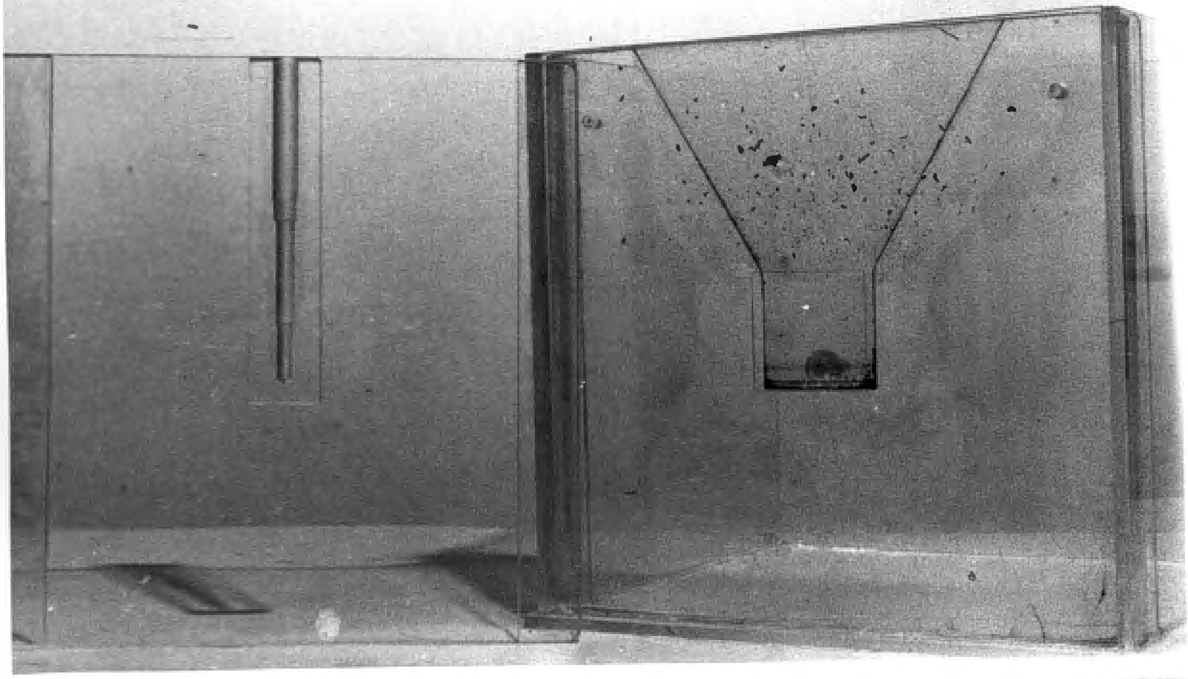
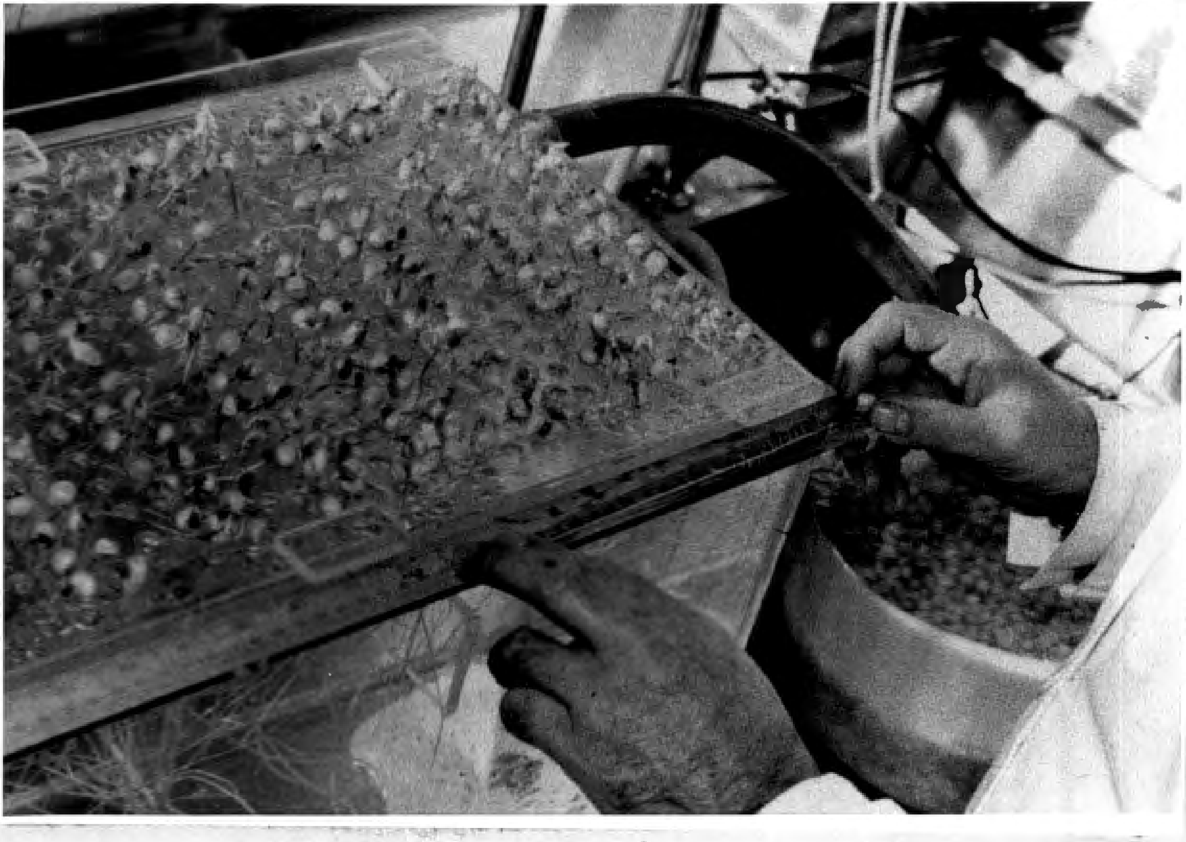


FIGURE 5.4

Radiation jig showing the plate which replaces that with a funnel-shaped cavity for dosimetry purposes.

FIGURE 5.5

Measurement of the length of a root.



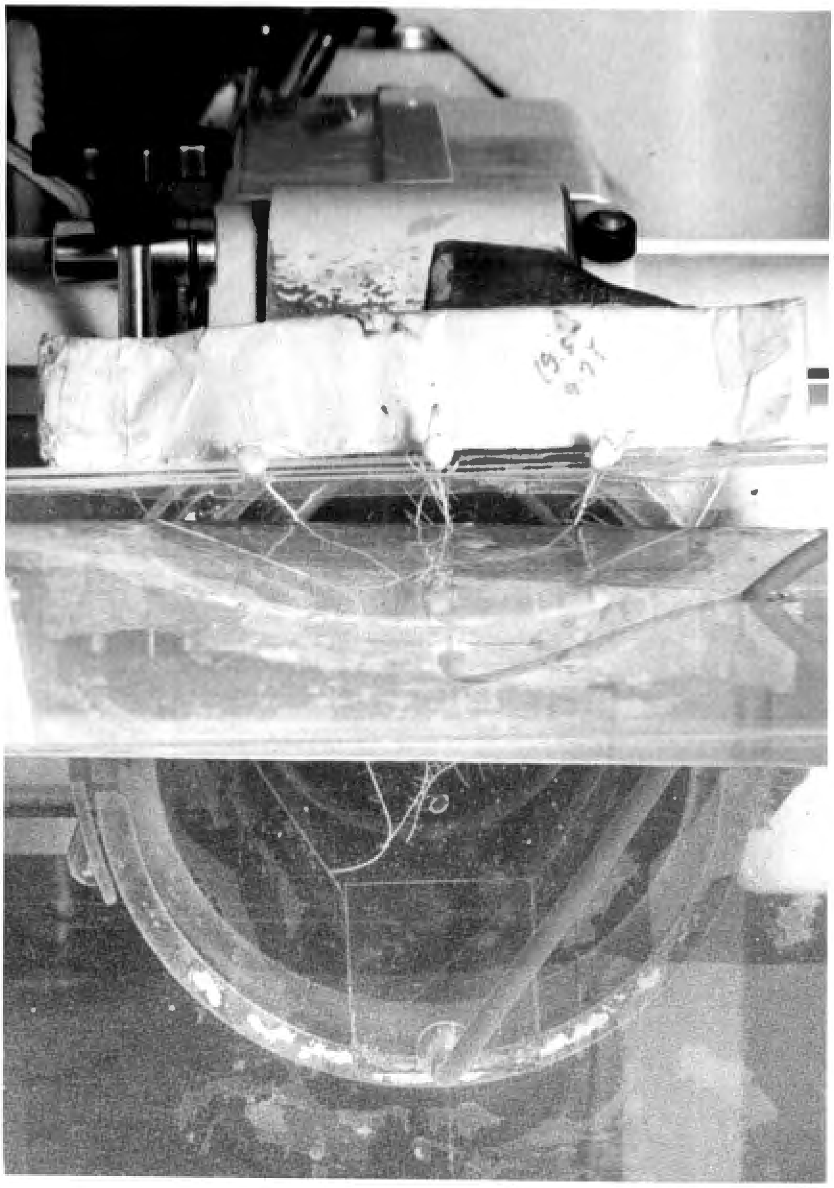


FIGURE 5.6

View of experimental arrangement along the axis of the beam.

CHAPTER VI

Results

Figure 6.1 depicts the daily growth of control roots as a fraction of time. It will be seen that this curve gradually falls off, and for this reason the growth rates of all irradiated roots were expressed as a fraction of the growth rate of control roots of the same age. The growth curves for Zea roots irradiated in air are given in Figures 6.2 and 6.3.

The values of the minimum growth rate, G_{min}, ten day growth rate, G_{10}, and the growth increment in ten days are recorded against dose in Table 6.1. The values of G_{min} were read from the growth curves. The values of G_{10} were obtained by subtracting the average length of the roots on day 0 from that at day 10 for each group, and expressing this as a fraction of the corresponding value of the control roots.

The growth curves for the roots irradiated in nitrogen are given in Figures 6.4(a) and (b). The corresponding values of G_{min}, G_{10} and growth increment in ten days are listed against dose in Table 6.2.

The percentage standard deviation at the G_{min} was of the order of 8 per cent, and this improved at points above the minimum. Error bars were not included on the curves in order to preserve clarity of presentation.

The following method for arriving at a value for the oxygen enhancement ratio (O.E.R.) is due to

Hall, Brown and Cavanagh (1968). The values of G_{10} and dose used in Figure 6.6. are plotted with values of G_{10} as ordinate and $\log_{10}$ (Dose) as abscissae. The straight line regressions fitted to the data by the method of least squares are described by the equations:

$$Y_o = - 0.8517\, X_o + 3.0797 \qquad (6.1)$$

$$Y_n = - 0.8971\, X_n + 3.5848 \qquad (6.2)$$

where the subscripts o and n refer to irradiation under aerobic and anaerobic conditions.

No significance can be attached to the small difference in slopes of the two lines and the pair of parallel lines which fit the data most closely are described by the equations

$$Y_o = - 0.8744\, X_o + 3.1463 \qquad (6.3)$$

$$Y_n = - 0.8744\, X_n + 3.5075 \qquad (6.4)$$

This pair of parallel lines, together with the data are shown in Figure 6.9. The oxygen enhancement ratio (O.E.R.) is the ratio of the doses given under anaerobic and aerobic conditions which produce the same biological effect. Consequently the O.E.R. is the separation between the lines in the direction of the dose axis. The lines are parallel with dose on the log scale which implies that the O.E.R. is a constant factor for all dose levels. The value of the O.E.R. obtained was 2.61 and this is given in Table 6.6.

From the growth curves Figures 6.2, 6.3, 6.4(a) and (b) the fraction of the number of meristematic cells surviving irradiation was found for each dose. In order to calculate the surviving fraction two models of the root meristem mechanism were used as originally devised and described by Hall, Lajtha and Oliver (1962). These models will be described with brief reference only to the relevant mathematics here. A full mathematical treatment will be given in the Appendix.

In formulating the two models it was assumed that the pattern of differentiation in the growing root must be determined within the meristem, and that it is not influenced by the existing differentiated tissue. The primary effect of radiation is the loss of reproductive integrity by a proportion of cells in the cell population. Hence the sterilization of meristematic cells must ultimately account for the reduction in growth (Lea 1946), and it would appear that the intermediate mechanism is the reduction in the number of cells presenting themselves for elongation.

In a control root growing at a constant rate, it is assumed that when a cell differentiates and leaves the meristem to elongate, another meristematic cell divides to maintain the total dividing population at constant level. In effect, during the course of one cycle, half of the cells in the meristem differentiate, while the other half divide and double in number. The cell population of such a meristem is thus maintained in homeostatic equilibrium while providing a continuous and constant supply of elongating cells.

There are three possible ways in which this meristematic compartment may be expected to behave after being subjected to a dose of radiation.

(1) The pattern of differentiation within the meristem may be unaltered i.e. although damaged cells depopulate the compartment 50 per cent per cell cycle may still elongate, and the rest divide. If this were so, the growth rate of irradiated roots would fall to a value characteristic of the proportion of cells sterilized, and would remain at this level. Therefore the recovery normally observed in practice is not explained by this first postulate, which is consequently rejected out of hand.

(2) The "size" of the integer meristem may be the dominant factor. Production of elongating cells is stopped as soon as the meristem is depopulated and is not restored until the meristem regains its normal size. Once this has been accomplished elongation would recommence. Such a system, however, suggests a temporary cessation of growth, followed by a sudden recovery to the pre irradiation level for any dose. Once more, this model is inconsistent with experimental facts which never demonstrate a cessation of growth rate (only a slowing down) and the recovery is gradual and never sudden.

(3) The third possiblity is that when a number of meristematic cells is less than normal, as a result of radiation induced cell death, then the <u>proportion</u> of cells which differentiate in a given time interval is also less than normal. Hall, Lajtha and Oliver

have considered this postulate in great detail, and have suggested two possible meristematic models, Model A and Model B, which will now be discussed in some detail.

Model A.

This assumes that the meristem population is in exponential growth, this growth being balanced by a removal mechanism that ensures that the proportion of cells differentiating is proportional to the fractional size of the meristem. This would lead to an exponential distribution of cells within the cell cycle. Following radiation damage, since the intermitotic cycle is unaltered, (Hornsey 1956), the proportion of cells dividing in a given time interval is unimpaired and the meristem is gradually repopulated. As recovery progresses and the compartment approaches its normal size, rate of differentiation, and hence root growth returns to its steady state value. This model satisfies the observation that the minimum growth rate observed decreases with increase in the dose, and that the subsequent recovery is gradual over a period of several days. Also the pattern of differentiation is determined within the apex which is in accord with the conclusions of Clowes (1959).

Hall, Lajtha and Oliver (1962) derive the expression

$$\frac{dD}{dt} = \frac{y\, I_s}{\left\{1 + \left(\frac{I_s}{I_o} - 1\right) e^{-yt} \right\}^2} \tag{6.5}$$

for the rate of differentiation, D, at any time in terms of the number I_o of integer cells in the model meristem immediately after irradiation, the number I_s, of cells in the meristem under steady state growth conditions, and the time constant y defined as:-

$$y = \frac{\ln 2}{\text{intermitotic period}} \tag{6.6}$$

In order to simplify the derivation of the above expression, the authors have assumed that fatally damaged cells are removed immediately following irradiation. Therefore the curve derived from the above equation cannot be simply matched to experimental growth rate curves to derive the initial population. Hall and his co-workers avoid this difficulty by introducing a step by step calculation, tracing the attempts of the integer cells in the meristem to repopulate the compartment by division, while assuming that the fraction of cells which differentiate (and are lost from the compartment) depends on the total number of cells present -integer or sterile, I_t.

The basis of the calculation is the curve for daily growth rate as a fraction, G, of that for control roots of equal age. See Figure 6.2, 6.3 and 6.4 (a) and (b) as mentioned previously. The method will be described for a single dose of 1000 rads as an example, but must be repeated for each dose level. A step by step calculation of the kind to be described must inevitably be an approximation, but an increase in the number of steps, whilst increasing the accuracy, adds complication. As a compromise the interval of a quarter of a day was chosen.

The growth rate as a fraction of that of controls, G, is read from the curve for each time interval, and recorded as in Table 6.1(a). From equation A.1 derived in the Appendix, the rate of differentiation in an irradiated, and therefore depopulated, meristem equals $y\left(\frac{I_t^2}{I_s}\right)$, and the corresponding quantity for a control root is $y \cdot I_s$. Expressed as a fraction of control roots, the rate of differentiation and, therefore, the growth rate G becomes

$$G = \left(\frac{I_t}{I_s}\right)^2 \qquad (6.7)$$

This equation illustrates the basic postulate of the model, namely that the rate of differentiation at any time is determined by the fractional size of the meristem defined as:-

$$P = \frac{I_t}{I_s} \qquad (6.8)$$

In Hall's work (1962) at 19°C, the relevant cell cycle was about 30 hours, so a fifth of a cell cycle would be equivalent to a quarter of a day in hours. He assumes that a fifth of the total population will divide during a time interval equal to one fifth of a cell cycle and so during this time the number of cells in the meristem will be increased by the factor F, where

$$F = 1 + \frac{1-P}{5} \qquad (6.9)$$

I_t is the total number of cells present at the beginning of that interval. The value of I_t, and therefore of P, is changing continuously but its value at the beginning of each time interval is assumed to apply throughout that short interval.

The product of all the numbers in the third column of Table 6.1(a) is the factor by which the number of integer cells on day 0 must be multiplied to give the number present on day 10¼. Hall, Lajtha and Oliver (1962) consider this to be a suitable end point for the calculation, because by the tenth day it may be assumed that the growth rate is almost exclusively due to cells which are descendants of those which retained their reproductive integrity at the time of irradiation.

If f is the initial fraction surviving the radiation, then $f.I_s$ is the number of integer cells in the meristem at day 0, and 8.415 fI_s is the number at day 10¼ (See Table 6.1(a). From column 2 it is known that on day 10¼, $P^2 = \left(\frac{I_t}{I_s}\right)^2 = 0.636$.

Therefore $\frac{I_t}{I_s} = 0{\cdot}80$ and $I_t = 0{\cdot}80\, I_s$

These two quantities can now be equated and the fraction, f has the value f = 0.80/8.415 = 0.095. This calculation was then repeated for each dose.

In the present investigation the cycle time of <u>Zea mays</u> cultivated at 25°C can be taken as approximately 24 hours. This value is derived from a consideration of the work of Evans and Savage (1959) on the cycle time of <u>Vicia</u> roots at different temperatures and of Clowes (1963) who deduced the cycle time in different regions of the meristem of Zea at 19°C. As a general approximation it is assumed that the cycle time derived for cells of the stele just above the meristem is representative of the meristem as a whole. The value for these cells in Zea is about 30 hours, which is the same as for squash preparations of Vicia at this temperature. Now, as the value for the cycle time in the case of Vicia rises to about 24 hours at 25°C, according to Evans and Savage, it is assumed that the cells of the stele above the quiescent centre in Zea follow suit.

In addition a slight modification should be made to equation (6.9). It will be appreciated that this equation assumes a linear distribution of cells within the cell cycle, which is not valid for the present model in which the meristem is assumed to be in exponential growth. Although the results obtained by Hall et al are not appreciably different from the corrected results it was thought that the discrepancy should be remedied.

If an exponential distribution of cells within the cycle obtains, the fraction of the total population which will divide during a time interval equal to one fifth of a cell cycle must be derived by integration over the interval. It should, however, be remembered that only those cells in the time stage immediately before mitoses will divide. Others further away from mitosis will be lost by differentiation before reaching division. If cells less than one hour from mitosis are assumed to divide, then the fraction of the total population which will divide during a time interval equal to one fifth of a cell cycle is given by:-

$$\frac{\frac{T}{5}\int_0^1 e^{\frac{0.693t}{T}}\,dt}{\int_0^T e^{\frac{0.693t}{T}}\,dt} \qquad (6.10)$$

where T is the cycle time.

If now the intermitotic cycle time is 24 hours, a quarter of a day corresponds to a quarter of a cycle and not one fifth. Equation (6.10) now becomes

$$\frac{\frac{T}{4}\int_0^1 e^{\frac{0.693t}{T}}\,dt}{e^{\frac{0.693t}{T}}\,dt} \qquad (6.11)$$

The fraction of the total population which now divide during this time interval of a quarter of a cell cycle, by substitution in equation (6.11) for T=24 is $\frac{1}{7.1098} = \frac{1}{7.11}$

The fractions surviving irradiation, f, were re-evaulated for each dose, using the equation

$$F = 1 + \frac{1-P}{7.11} \qquad (6.12)$$

to obtain the factors at each step.

The values of f obtained using equations (6.9) and (6.12) are presented together with the corresponding doses in the upper half of Tables 6.3 and 6.4. The reason why the calculations were also done using Equation (6.9), the constants of which are not pertinent to the temperature used for the experimental work in this investigation, will be clarified in the following chapter.

Model B.

Although the above model appears to give a reasonable explanation of the experimental curves, Hall, Lajtha and Oliver (1962) have considered it to be more satisfactory if a mechanism could be offered for the observed growth pattern.

An alternative model was therefore proposed in which it is assumed that all meristematic cells are preparing for division, but that the proportion of cells maintaining their reproductive integrity is proportional to the concentration of a specific substance, i.e. the fraction of those preparing for division is proportional to this concentration. It is also assumed that the maintenance of the reproductive integrity implies utilisation of this substance, so that the fall in concentration of this substance in a given region or layer of cells is proportional to the number of cells present which retain their reproductive integrity. Cells which have lost their reproductive integrity due to lack of the substance differentiate.

For this model, the growth rate as a fraction of that for a steady state population is given by

$$G = \frac{1{\cdot}595P + e^{-1{\cdot}595P} - 1}{0{\cdot}7975} \qquad (6.13)$$

where P is the proportion of the total population. The increase in the fractional size of the meristem in a time equal to $^1/_T$th of the cell cycle is

$$F = 1 + \frac{1}{1{\cdot}595TP}\left(2 - 2e^{-1{\cdot}595P} - 1{\cdot}595P\right) \qquad (6.14)$$

which is

$$F = 1 + \frac{1 - 1{\cdot}595P}{T} \qquad (6.15)$$

if two terms of the exponential series are taken. The equations are deduced in the Appendix. It will be seen that this model leads to a linear distribution of cells within the cell cycle, and hence the simple fractions of the cell cycle pertain. The step by step calculation to compute the initial surviving fraction, f, is essentially the same as for Model A, but the computer programme is more involved because there is no simple relationship between G and P. The value of P can be obtained from the observed growth rate using graphical methods, but this procedure is tedious and inaccurate, and in the programme a simple iterative procedure was used to solve for P.

Using this model with both T=30 hours and T=24 hours the values of f listed against dose in the lower halves of Tables 6.3 and 6.4 were obtained.

The values of fraction of meristematic cells surviving irradiation obtained for Models A and B using the four time-constants discussed above were plotted on semilogarithmic paper against dose. Figure 6.7.

Dose response curves using Model B values only and T=24 hours for irradiation under aerobic and anaerobic conditions were then plotted as Figure 6.8. From these, values of m and 37 per cent dose slope were obtained. These are given in Table 6.6.

The values of m given represent the upper and lower limits estimated by backward extrapolation of the straight portion of the dose-response curves to intercept the ordinate axis. The figure quoted for the 37 per cent dose slope is that for the straight portion of the dose response curves as required by the theory set out in Chapter II.

The growth curves obtained in the fractionation experiments are given in Figure 6.10. The percentage standard deviation pertaining to these experimental values was of the order of 10 per cent. The relevant values of G_{min} and G_{10} are given in Table 6.5. These values are then used in turn to calculate values of m and the 37 per cent dose slope according to the theory outlined in Chapter II. The final values are given in Table 6.6.

TABLE 6.1 Single Acute Doses in Air at 25^{o}C

Experiment No:	Dose in rads	Increment in 10 days in cm.	"Growth in 10 days," G_{10}	"Minimum Growth Rate" G_{min}
1	Controls	39.1	-	-
	250	37.4	0.952	0.909
	500	31.8	0.812	0.657
	1000	21.2	0.541	0.314
	1250	17.6	0.448	0.251
	1500	14.5	0.370	0.170
	2000	7.8	0.198	0.060
	2500	7.1	0.180	0.025
2	Controls	41.55		
	200	40.2	0.968	0.838
	350	38.2	0.918	0.825
	700	30.4	0.728	0.515
	750	28.7	0.689	0.480
	1750	11.2	0.270	0.114
	3000	5.8	0.138	0.010

TABLE 6.1(a) Data for 1000 rads using Model A

Day	Growth Rate as a fraction of Controls $G = {\frac{I_t}{I_s}}^{2}$	Factor, F, by which number of cells in meristem increased during ¼ day $F = 1 + \frac{1 - \frac{I_t}{I_s}}{5}$
0	1.000	1.000
¼	0.934	1.007
½	0.861	1.014
¾	0.780	1.023
1	0.690	1.034
¼	0.604	1.045
½	0.520	1.056
¾	0.446	1.066
2	0.390	1.075
¼	0.351	1.082
½	0.330	1.087
¾	0.316	1.088
3	0.314	1.088
¼	0.319	1.087
½	0.330	1.085
¾	0.348	1.082
4	0.371	1.078
¼	0.399	1.074
½	0.425	1.070
¾	0.446	1.066
5	0.473	1.063
¼	0.491	1.060
½	0.509	1.057
¾	0.524	1.055
6	0.540	1.053
¼	0.555	1.051
½	0.570	1.049
¾	0.580	1.048
7	0.593	1.046
¼	0.601	1.045
½	0.609	1.044
¾	0.615	1.043
8	0.618	1.043
¼	0.620	1.043
½	0.624	1.042
¾	0.625	1.042
9	0.625	1.042
¼	0.625	1.042
½	0.626	1.042
¾	0.629	1.041
10	0.631	1.041
¼	0.636	Product = 8.415

TABLE 6.2 Single Acute Doses in Nitrogen at 25°C

Experiment No.	Dose in rads	Increment in 10 days in cm.	"Growth in 10 days" G_{10}	"Minimum Growth Rate" G_{min}
3	Controls	43.3	-	-
	250	39.1	0.904	0.734
	500	36.6	0.845	0.725
	1000	35.7	0.825	0.710
	1500	34.7	0.802	0.620
	2000	28.0	0.648	0.498
	2500	20.8	0.482	0.356
	3000	19.7	0.455	0.298
	3500	21.2	0.490	0.298
	4000	14.8	0.342	0.183
	4500	11.7	0.271	0.140
	5000	11.7	0.271	0.132

TABLE 6.3 Fraction Surviving Single Acute Doses of Ionising Radiation in Air at 25°C using Two Models

MODEL A.

Dose in rads	Fraction Surviving, f T=7.11	 T=5
200	0.744	0.669
250	0.813	0.752
350	0.670	0.589
500	0.574	0.456
700	0.376	0.264
750	0.302	0.201
1000	0.176	0.095
1250	0.117	0.055
1500	0.079	0.032
1750	0.033	0.011
2000	0.018	0.005
2500	0.010	0.002
3000	0.003	0.000

MODEL B.

Dose in rads	Fraction Surviving, f T=4	 T=5
200	0.536	0.572
250	0.607	0.635
350	0.478	0.518
500	0.370	0.429
700	0.212	0.268
750	0.160	0.210
1000	0.071	0.109
1250	0.039	0.066
1500	0.021	0.039
1750	0.006	0.013
2000	0.002	0.006
2500	0.001	0.003
3000	0.000	0.001

TABLE 6.4 Fraction Surviving Single Acute Doses of Ionising Radiation in Nitrogen at 25°C using Two Models

MODEL A

Dose in rads	Fraction Surviving, f	
	T=7.11	T=5
250	0.732	0.649
500	0.511	0.420
1000	0.602	0.490
1500	0.557	0.440
2000	0.250	0.155
2500	0.137	0.069
3000	0.103	0.047
3500	0.127	0.062
4000	0.059	0.023
4500	0.029	0.009
5000	0.028	0.009

MODEL B

Dose in rads	Fraction Surviving, f	
	T=4	T=5
250	0.526	0.566
500	0.340	0.387
1000	0.398	0.454
1500	0.353	0.413
2000	0.123	0.170
2500	0.051	0.082
3000	0.033	0.057
3500	0.045	0.074
4000	0.014	0.028
4500	0.005	0.011
5000	0.005	0.011

TABLE 6.5

Treatment	Fractional Growth in 10 days G_{10}		Minimum growth rate as a fraction of controls G_{min}	
Single doses				
500	0.812		0.657	
1000	0.541		0.314	
1500	0.370		0.170	
2000	0.198		0.060	
2500	0.180		0.025	
3000	0.138		0.010	
Fractionated doses D_f		Equivalent single dose (rads) D_s		Equivalent single dose rads D_s
2 x 500 rads interval of 24 hours	0.562	1020	0.360	980
2 x 500 rads interval of 21.5 hours	0.439	1330	0.345	1000
2 x 500 rads interval of 18.5 hours	0.634	870	0.430	830
2 x 1000 rads interval of 24 hours	0.137	2800	0.055	2130
2 x 1000 rads interval of 21.5 hours	0.177	2500	0.070	2000
2 x 1000 rads interval of 18.5 hours	0.176	2500	0.060	2100

TABLE 6.6

A Summary of Results

Origin	Extrapolation Number m		37% Dose slope D_o rads	
	In Air	In Nitrogen	In Air	In Nitrogen
Final dose response curves Figure 6.8*	1.4 to 2.4	1.5 to 1.85	410	1050
Split dose experiments (However see comment Chapter VIII)	2.0	-	350	-

Origin	Oxygen Enhancement Ratio O.E.R.
Growth rate curves as plotted Figure 6.9	2.61
37% Dose slopes (air and nitrogen) Figure 6.8.	2.56

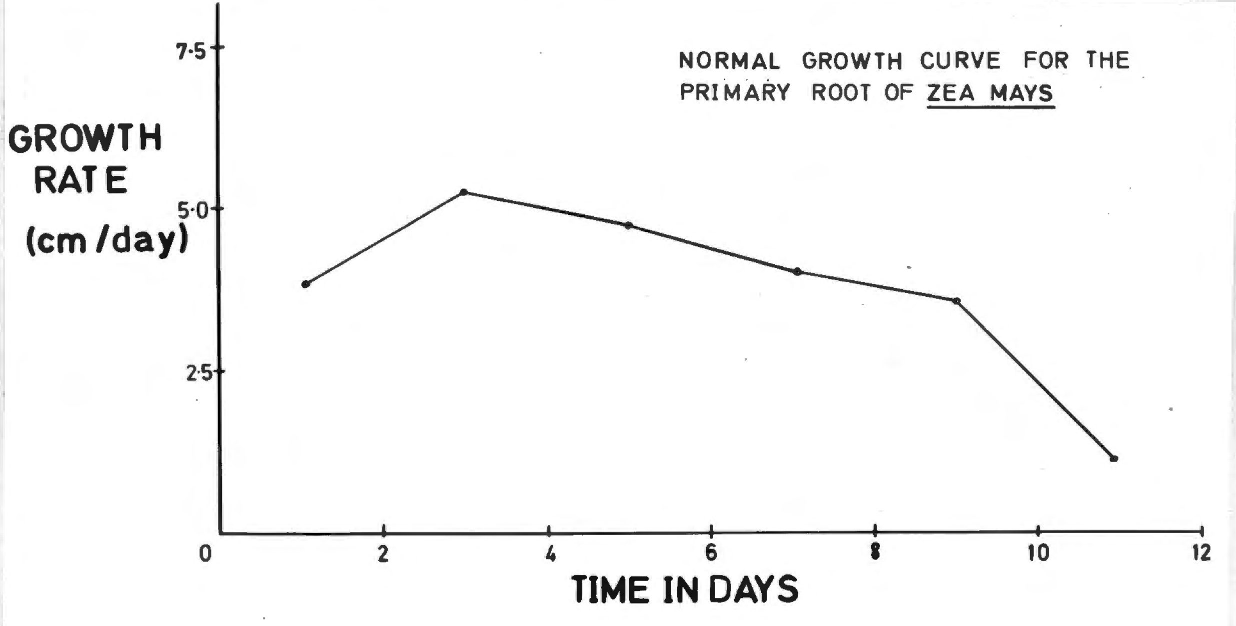


FIGURE 6·1

FIGURE 6.1

Normal growth rate curve for the primary root of *Zea mays*.

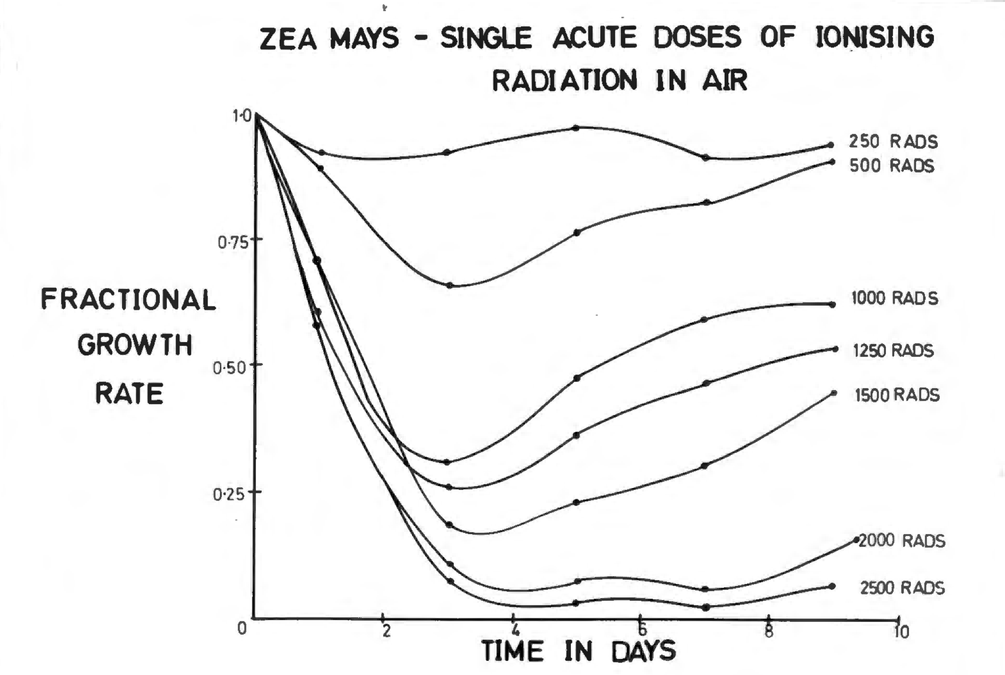


FIGURE 6·2

FIGURES 6.2 and 6.3

Growth rate curves for *Zea mays* irradiated in air.

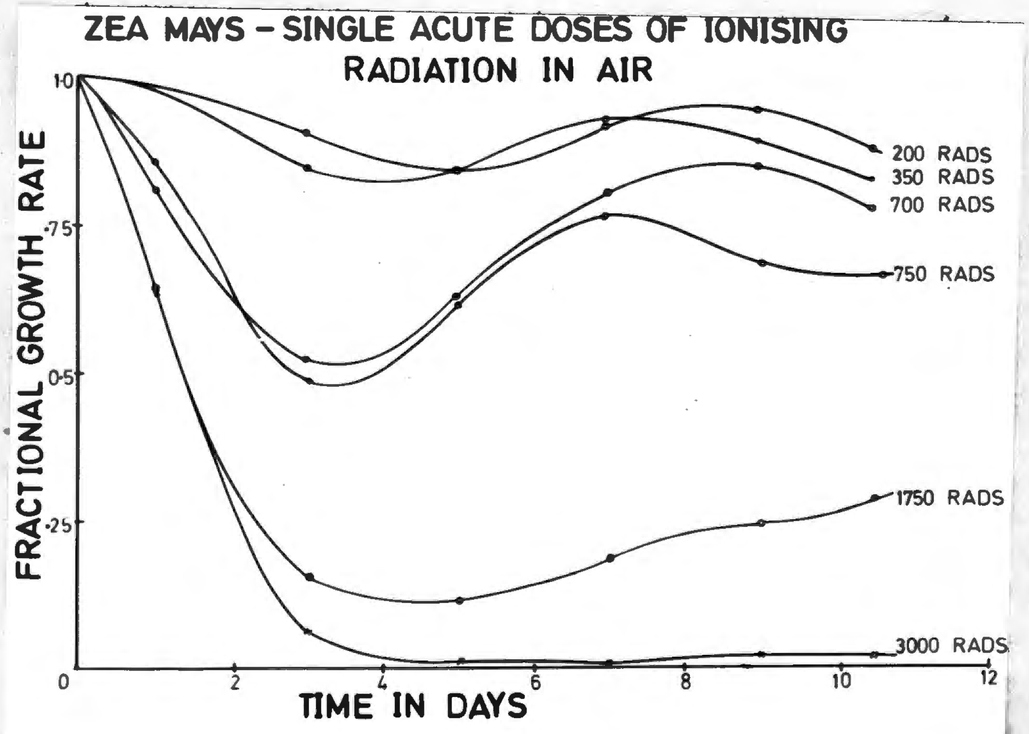


FIGURE 6·3

FIGURES 6.4(a)
and 6.4(b)

Growth rate curves for Zea mays roots irradiated in Nitrogen.

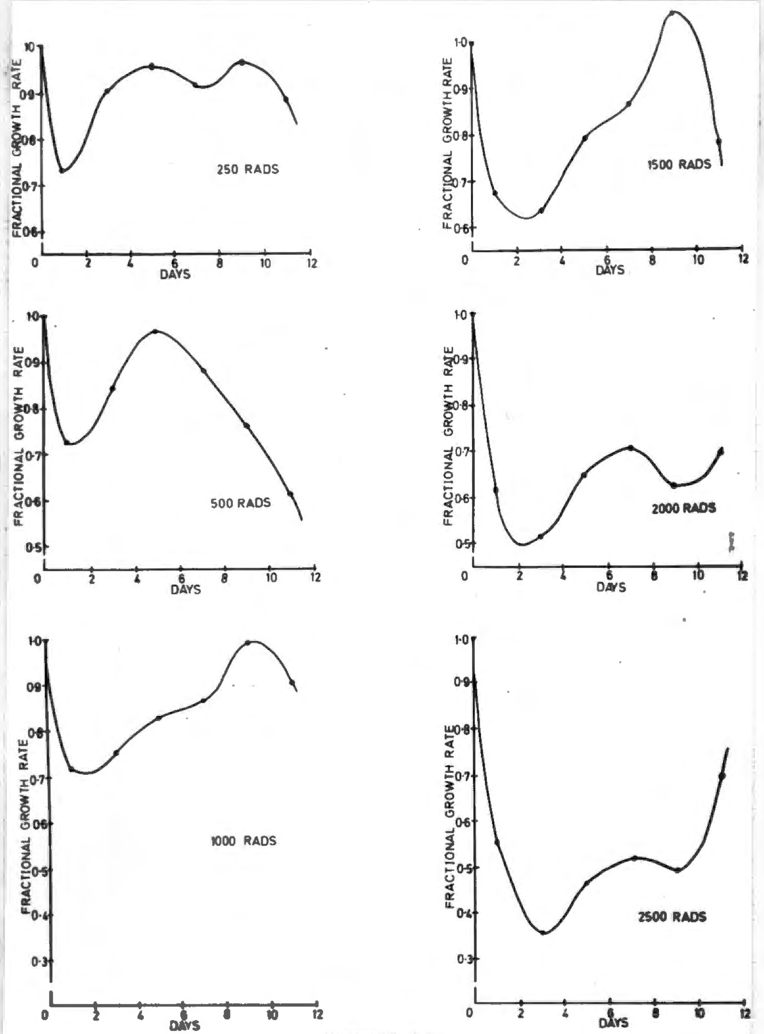


FIGURE 6·4a

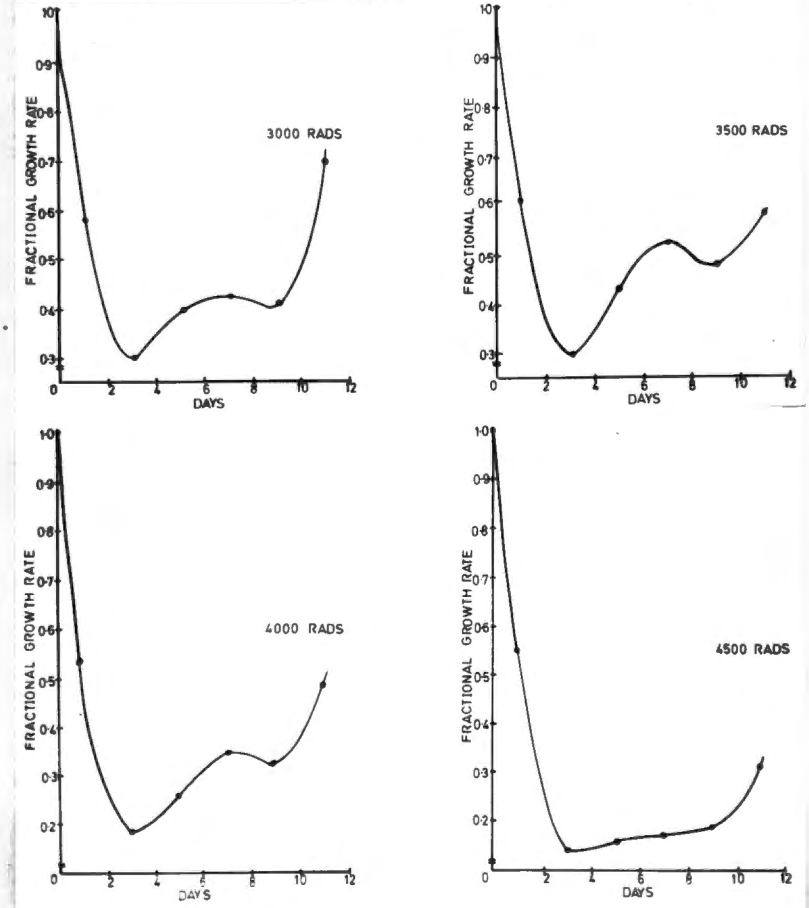


FIGURE 6·4b

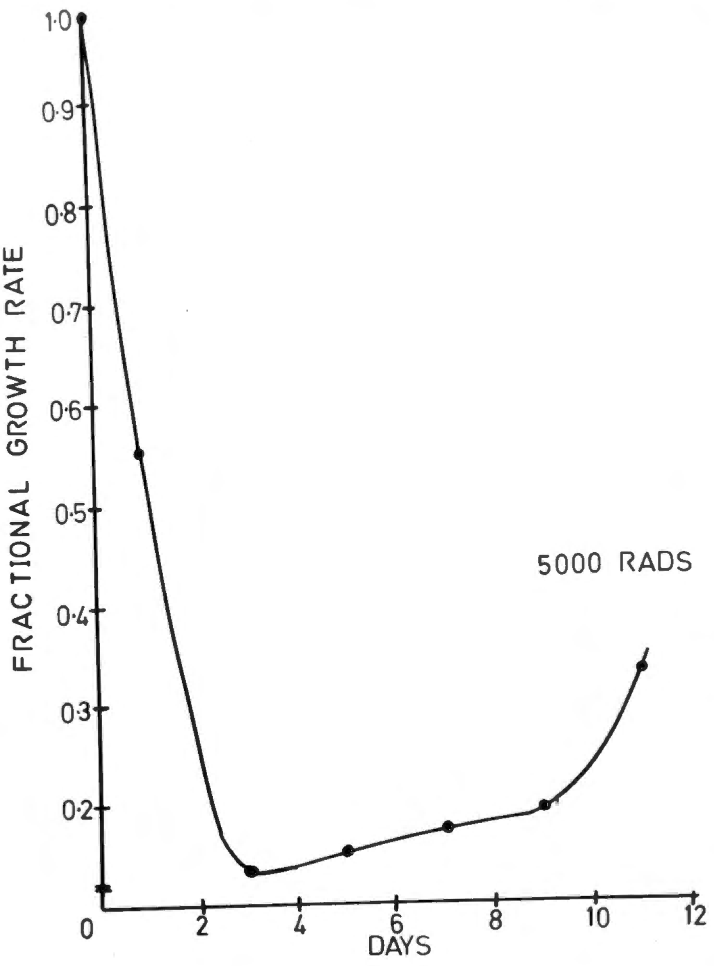


FIGURE 6·4b continued

FIGURE 6.4(b) continued.

Growth rate curves for *Zea mays* roots irradiated in nitrogen.

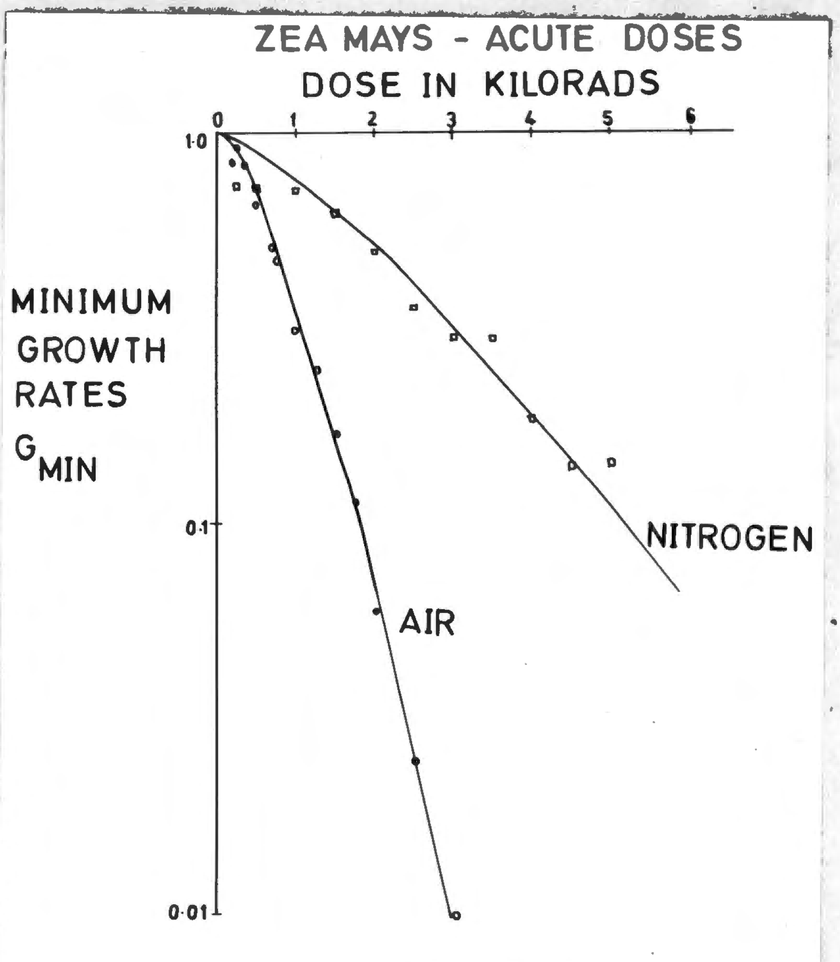


FIGURE 6·5

FIGURE 6.5

"Minimum growth rate" as a function of dose for roots of *Zea* irradiated in air and in nitrogen.

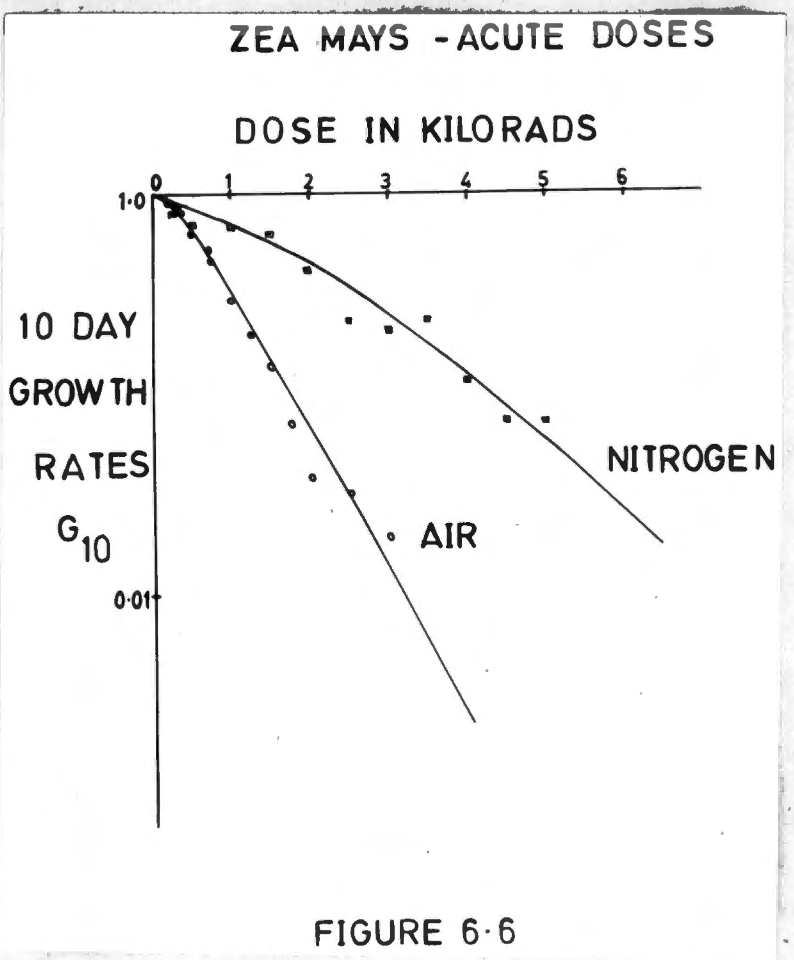


FIGURE 6.6

Growth in 10 days as a function of dose for roots of Zea mays irradiated in air and nitrogen.

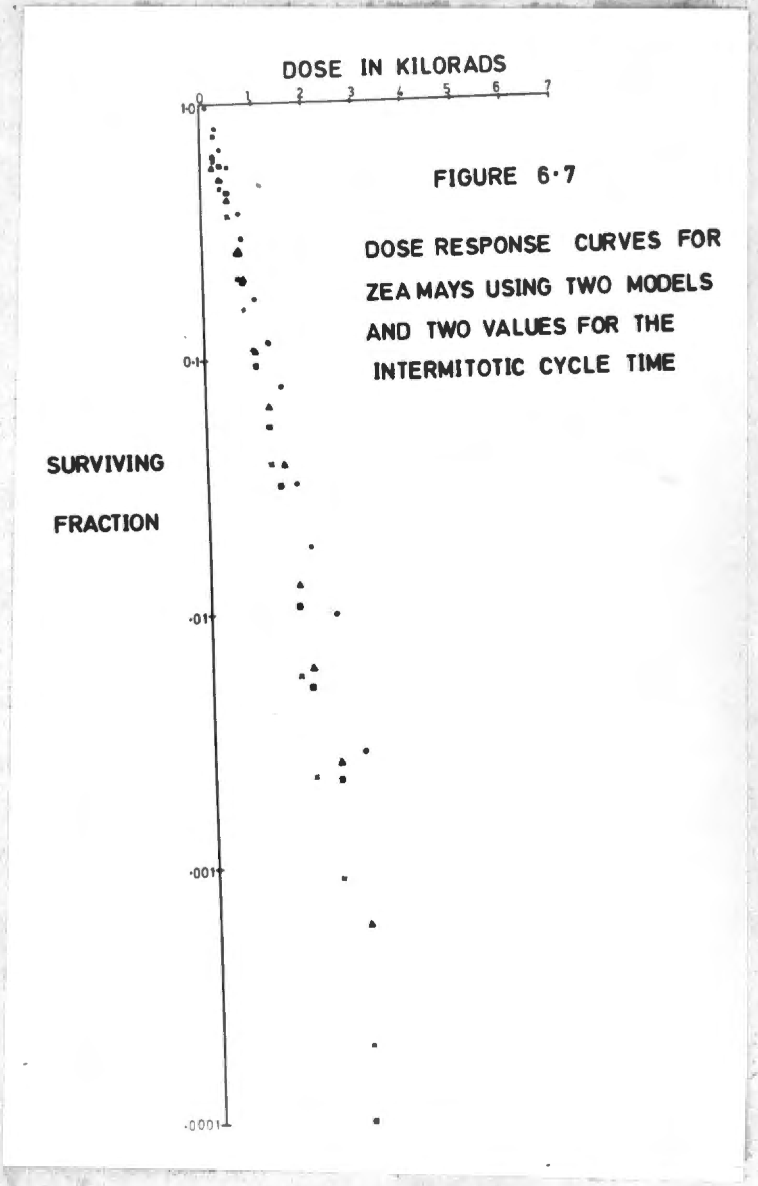


FIGURE 6.7

The variation of surviving fraction with dose for *Zea* roots irradiated in air, using two models and two cycle times.

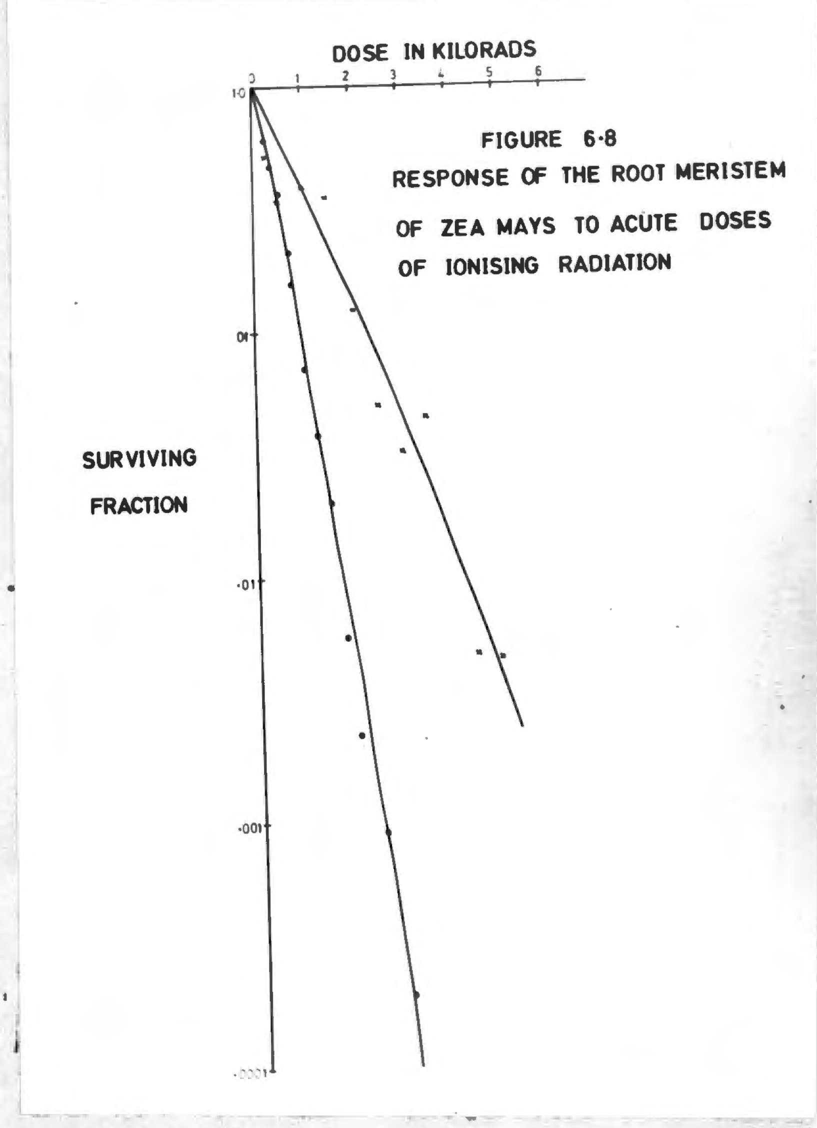


FIGURE 6.8

Dose response curves with respect to reproductive integrity for Zea mays roots irradiated in air using Model B only and a cycle time of 24 hours.

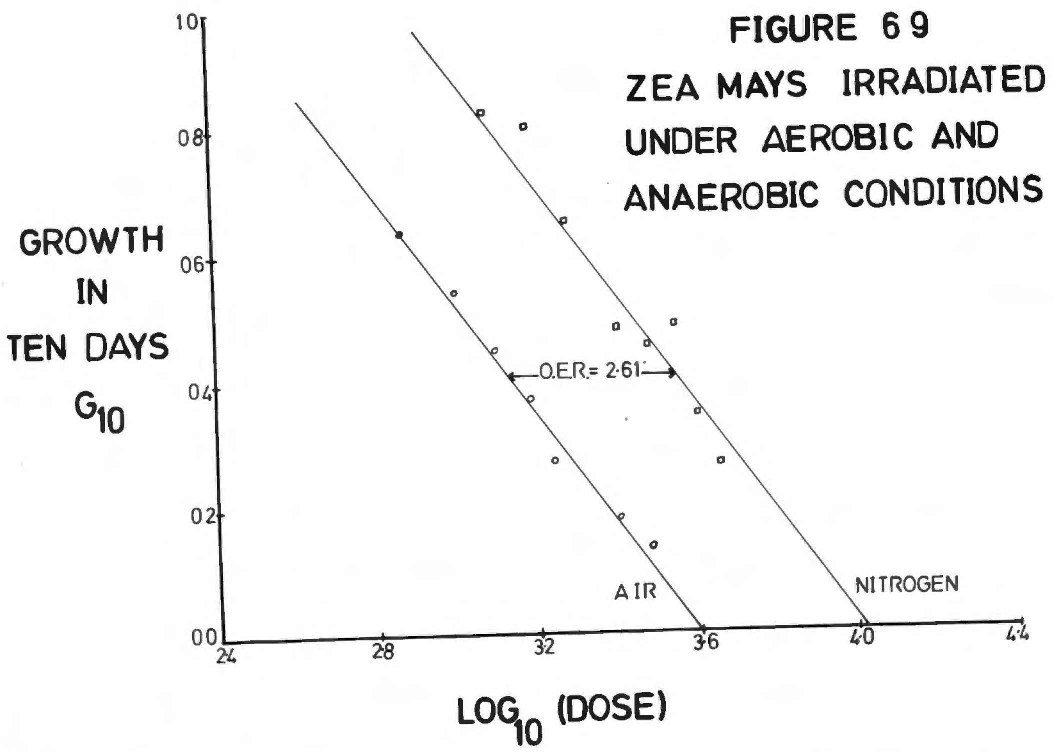


FIGURE 6.9.

Variation of growth in ten days of Zea mays roots irradiated in air, with logarithm of dose to base 10, to obtain a value of oxygen enhancement ratio.

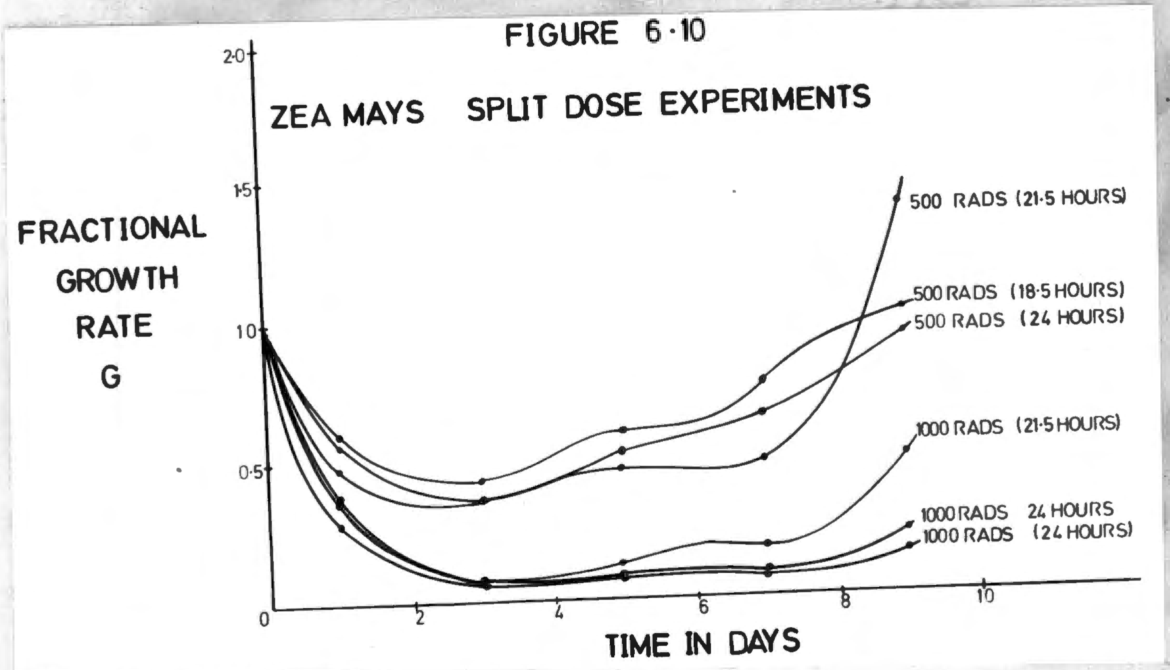


FIGURE 6.10

Growth rate curves for Zea mays roots irradiated in air using split doses and varying intervals between doses.

CHAPTER VII

Discussion

The Control Roots

As can be seen from Figure 6.1 the growth rate of control roots decreases steadily throughout the course of an experiment. This may be because of a failure in nutrition, but may also have to do with hormone balance. As was pointed out earlier in the thesis, roots are inhibited in their growth by applied auxin over a wide range of concentrations, and Pilet (1961) has shown that the destruction of auxin is greater in old root tissues than in young tissues. This seems to contradict other evidence which shows that the auxin content increases with increasing age. It is therefore supposed that the two processes operate simultaneously i.e. the tissues greatly increase their ability to destroy mature auxins in proportion to the age of the roots, at the same time as the accumulation of auxin is increasing. Even if the destruction is greater, the final auxin content rises because auxins are produced faster than they are destroyed (Figure 7.1).

In any event it is difficult to decide whether the length or age of the root is responsible for the decline in growth rate, since in a control root the two factors are interdependent. Gray and Scholes (1951) preferred to express the daily growth rate of irradiated _Vicia_ roots as a fraction of controls of the same _length_. However, Hall Lajtha and Oliver (1962) have shown that relating growth rate to controls of equal age is the more satisfactory especially where an open culture system is used. This procedure has been followed in the present studies, and Figure 6.1 shows that the growth of the controls is maintained fairly satisfactorily over the period of the experiment as long as adventitious roots are continually removed.

The Growth Curves

The growth curves for roots of Zea mays exposed to ionizing radiation in air are shown in Figures 6.2 and 6.3, and the general shape of these compare comparatively well with those found in the case of Vicia, an example of which is given in Figure 7.2. There is an initial decrease in the growth rate as a fraction of controls of equal age. The curve then passes through a minimum before returning to pre-irradiation levels, and in some cases, even over-shooting. At lower doses there are marked oscillations in the curves, which are very reminiscent of the response of a second-order system with a low damping ratio to a step-function input signal, and gives a hint that time-dependent phenomena play a very important part in the radiation response. Contrasted with Vicia, the minima in the case of Zea are much flatter and sometimes even show a suspicion of having two minima.

In order to explain the shape of the growth-rate curve over the first few days, it is necessary to take into account the fact that damaged cells do not die immediately after doses of the order of a few hundred rads in mammalian cell systems, (Puck and Marcus, 1956), and after even higher doses in some plant systems (Oliver and Shepstone, 1965). Some succeed in completing two or even more divisions, and it is assumed that all are capable of differentiating if called upon to do so. These cells, therefore, make a significant, although continuously decreasing, contribution to the growth rate of the root in the first few days following irradiation (Figure 7.3). At the same time, the meristematic cells which retained their reproductive integrity make an increasing contribution to the growth rate. This contribution from the integer cells, is represented by the chained line in Figure 7.3, and can be derived from a consideration of the mathematical models. It is because of these two processes that the growth-rate curve has a minimum value corresponding to the point where the two contributions are approximately equal, and are about to interchange their order of importance.

The initial depopulation produced by the radiation cannot be obtained simply by matching the theoretical and experimental growth-rate curves, because the theoretical curves ignore the presence of the sterile cells. It can, however, be obtained by the step by step calculation explained in Chapter VI.

The growth curves for roots irradiated in nitrogen show the tendency towards a second minimum very clearly, and it is even possible that we are dealing with two separate populations, one of which can survive more divisions after the radiation dose, and therefore makes a contribution to the growth rate which decreases even more slowly than that of the more sensitive population. It is, of course, tempting to think that the second minimum is a consequence of the cells of the quiescent centre, especially as it seems to be more prominent at the higher doses, where the quiescent centre is assumed to play a more dominant role (Clowes, 1963). An important point, however, is that when plotted as a function of dose, the second minimum yields a curve with a similar slope to that produced by the first minimum.

The bizarre oscillations, which occur at the end of some of the growth curves for roots irradiated in nitrogen, are probably artefacts as they show no consistency throughout the series, or on repetition. They are probably related to the state of the particular batch of seeds towards the end of the experiments as they are not evident in the others.

The Dose Response Curves

The curves depicting the two radiation parameters, G_{min} and G_{10}, as a function of dose when plotted on semi-log paper, are very similar to those obtained for Vicia, with the same suggestion of an initial shoulder followed by a fairly linear portion as the dose increases. The pattern is the same for irradiations in air and in nitrogen and in both instances the G_{10} and the G_{min} curves have similar slopes, but probably very different extrapolation numbers.

The curves depicting "Growth in ten days" as a function of dose, in air, (i.e. under conditions when cells exhibit full oxygen sensitivity) and in nitrogen, have been used to derive an Oxygen Enhancement Ratio, O.E.R., of 2.61, for the meristematic cells of Zea mays. As stated in Chapter II the O.E.R. is the ratio required to produce equal degrees of damage in anoxic and deoxygenated cells.

The Oxygen Enhancement Ratio.

The enhancing action of oxygen is a universal feature of cellular radiobiology, applying not only to all forms of cell but to all tests of damage. For a while there was a tendency to believe in some sort of invariant factor, about 3, by which oxygen reduced the dose necessary to achieve a given effect. This could be expected if the oxygen effect were to operate by modifying the decomposition products of water. This mechanism for the oxygen effect is now not favoured. In any event, substantial variations in oxygen enhancement ratios by no means attributable to inadequacies in technique are undoubtedly observed (Alper, 1964):-

1. Differences, for the same test of damage, in closely related strains.
2. Differences, for different tests of damage, in the same organism.
3. Differences occurring by virtue of differences in the physiological or nutritional state of the organism at the time of irradiation.
4. Differences observed for cells irradiated together, but subjected to different culture conditions after irradiation.

Whatever mechanism may be postulated for the cell-killing effect of ionizing radiation, and for the involvement of oxygen in this effect, it would have to be very elaborate to account for these facts, if all deaths were attributed to *one* mode of action on *one* type of target. If, however, one adopts an hypothesis that cell death may occur by virtue of more than one type of damage, and that these types are differently modifiable by oxygen, variability in an over-all modifying action may be accounted for.

In view of this then, it is probably more of a coincidence than experimental accuracy which makes the O.E.R. of 2.61 for *Zea* (See Figure 6.9) so close to that of 2.67 obtained by Read (1952) in his classical experiments on *Vicia* exposed to acute doses of X-rays. As in the present studies, the reduction in the rate of elongation of the primary root of *Vicia* was used as the index of radiosensitivity. Since then the effect of oxygen has been confirmed in many different systems, including mammalian cells *in vitro*, and *in vivo*, and for acute doses the O.E.R. has been always found to be between 2 and 3.5. Hall and Cavanagh, (1967), obtain a value of 2.67 at 3°C and of 2.58 at 19°C for exposure of *Vicia faba* to X-rays, and in this system Hall, Brown and Cavanagh (1968) found that the ratio does not change significantly with the phase of the cell cycle. For exposure to γ-rays Neary (1957) obtains a value of 2.4 in *Vicia*. Čerček, Ebert and

Greene, (1968), obtain a value for barley roots of 3.4 and Evans and Neary, (1959) find a similar value for Tradescantia. However, using a different variety of barley, ('Earl'), Ebert and Barber, (1961), obtain a value of 2.8.

The O.E.R. is usually lower for protracted exposures (2.06 for Vicia at 19°C) and, of course, the extent to which oxygen enhances radiation damage is known to depend on the type of radiation used. High values of O.E.R. are usually observed for radiation of low mean linear energy transfer, L.E.T., (e.g. X-rays or γ-rays), while with radiation of high mean L.E.T., lower values of O.E.R. are measured (Barendsen and Broerse, 1966). For example, the value for the O.E.R. in Vicia using neutrons is 1.4 at 22°C. Révèsz and Littbrand, (1964), suggest that a genetic injury may prevail in connection with anoxic x-irradiation, and a non-genetic mechanism may be in operation with aerobic treatment. This is in agreement with the postulate (Alper, 1962) that DNA is more heavily involved in killing by x-irradiation under anoxic as compared with aerobic conditions.

Final Dose Response Curves with respect to Reproductive Integrity.

We now consider the dose response curves in air and in nitrogen with respect to reproductive integrity, deduced as described in Chapter VI and the Appendix, on the basis of Models A and B. These models have been described in previous papers by Hall, Lajtha and Oliver, (1962), and by Shepstone and Oliver (1963). They refer to the feedback-control relationships which are postulated to determine the reduction in the proportion of cells differentiating when the meristem is reduced below its normal size, thus providing an excess of cells produced and undergoing division over those lost by differentiation so that repopulation of the meristem can take place.

Model A considers the cells in the meristem to be in exponential growth with a uniform cell-cycle time. Under normal equilibrium, production of new cells by division is assumed to be balanced by removal of an equal number of cells for differentiation. To provide for repopulation of a depleted meristem it is postulated that the proportion of cells removed from the population for differentiation per unit time is itself proportional to the ratio of the meristem population at that time to the normal equilibrium population. This results in a corresponding increase in the proportion of cells dividing and a gradual increase in the total population back to normal level.

Although Model A provides for a theoretical growth pattern similar to the pattern observed experimentally, it is difficult to put forward a mechanistic explanation for the proposed relationship.

Model B (described in the appendix of the paper by Hall <u>et al</u>, 1962), is an attempt to provide the right kind of feedback control on the basis of a possible biological response to a population change. Here it is suggested that in the normal meristem all the cells present themselves for division but that equilibrium is maintained, because for the meristem as a whole, only half of these cells are able to divide. The other half, failing to divide, differentiate. The proportion of cells able to divide varies throughout the meristem from virtually 100 per cent to zero at the edge of this region, due possibly to a variation in concentration of some substance which must be utilized for maintenance of reproductive integrity. In the depleted meristem, the given supply of the substance provides for more than half the cells reaching division to retain their reproductive integrity, and so divide. The proportion increases as the size of the meristem is reduced, thus enabling repopulation to occur.

One is tempted to speculate how any regulation mechanism is operated in the root. It may be possible that auxins, e.g. indole-acetic acid, are involved in these processes, because their biosynthesis is greatly impaired by the same doses which reduce root growth (Gordon 1955), or because the pattern of restoration after single doses of X-rays may be altered by giving auxins in physiological concentrations (Kiefer, 1965).

Although the mathematical relationships are somewhat different, it has not proved possible to eliminate either of these models by consideration of the growth patterns after acute radiation exposure, as the theoretical growth-curves and derived cell-survival curves are very similar in the two cases (Shepstone and Oliver, 1963). In the present case it can also be seen from Figure 6.7 that for the same assumed cell cycle time, there is little to choose between the survival curves in air derived from the two models. Obviously, the exact theoretical model for the meristem control is not critical. Therefore, although all calculations were in fact carried out for both models, in the final dose response curve shown in Figure 6.8 only the curve for Model B is depicted, the choice in favour of this particular model being only that it seems biologically the more feasible of the two.

In similar vein it is also demonstrated in Figure 6.7 that the particular cell cycle time (as represented by its appropriate constants in the formulae) chosen for the calculations is also not very critical. This is comforting in view of the tremendous variation in the measurement of this parameter, both within the meristem, and under different experimental conditions. The values for the cycle time pertinent to Zea mays at 25°C, as described in Chapter VI is used in the final dose-response curve.

Ionizing radiation causes reproductive death of cells. The mechanism is not well understood, so that any quantitative examination must start from an inevitably oversimplified model. The model, as set out in Chapter II, yields a mathematical enterpretation which in its turn may be capable of representing the experimental data. One pictures the cell as containing a number (on average m) of sensitive sites, or 'targets', with the assumption that if any one of these targets remains intact the cell will not be sterilized. The resulting equation for the surviving fraction in a population of cells is the familiar one deduced in Equation (2.20).

$$S = 1 - (1 - e^{-D})^{m} \qquad (7.1,\ 2.20)$$

Another model pictures a cell with but one target, and assumes that more than one energetic event ('hit') is required to sterilize the cell. If n hits are required the resulting equation is

$$S = e^{-\lambda D}\left\{1 + \sum_{k=1}^{n-1} \frac{(\lambda D)^{k}}{k!}\right\} \qquad (7.2)$$

In both equations is a parameter with dimensions rad -1, and may be thought of as the average number of hits produced per rad in a volume of the same size as the target. It is the inverse of the 37 per cent dose slope (D_o). Rossi's work (1964) has shown that on a microscale radiation causes events with a continuous distribution of energies. In the models, this continuium is split into two classes, one causing irreparable one-hit damage, and the other reparable n-hit damage.

Unfortunately research workers measure, not survival 'curves' but a limited number of discrete points. These data, even if free from artefacts which would render them meaningless, have such limited statistical significance that it is often possible to fit more than one mutually exclusive theoretical curve through them.

If survival curves are plotted on a logarithmic scale, as dose on a linear scale, the multi-target model leads one to expect a dose-response curve, which, after an initial shoulder, becomes straight; the multihit model would predict a curve which bends continuously and never straightens, although the curvature may be very small over the range of survival used in experimental investigations. Before the shape of experimentally determined curves is regarded as evidence for or against any theoretical model, it is of importance to ensure that all artefacts are removed from the experimental technique. Berry (1964) has pointed out one such artefact which caused an apparent bending of his He La survival curve: failure to change the medium at 7 days in plates containing a large number of cells resulted in spuriously low values of survival at high doses. Bedford and Hall (1966) describe how over crowding during low survival estimates could also cause this effect. In the present studies such artefacts are, of course, also possible.

For instance Gray and Scholes (1951) reported that elongated cells in irradiated roots were slightly shorter than in control roots. Also, there is a delay of several hours in the first mitotic cycle after irradiation (Howard and Pelc, 1953, Neary, Evans and Tonkinson, 1959). Neither of these factors have been taken into account. However, their omission will not effect the basic model, and since they are second order effects, the error introduced into the final dose-response curve should be small.

Statistical theory (Porter, 1963, quoting from Snedecor's book) gives the variance of estimate of log m as

$$V(\log m) = \frac{S^2}{N}\left(\frac{r+1}{r-1}\right)^2 \qquad (7.3)$$

Here s^2 is the error variance per observation and N is the number of observations. r is the factor by which the high dose of the experiment is greater than the low dose. For this equation to be valid the survival must be known exactly.

Reducing s^2 will certainly reduce V (log m) and so improve precision, but there is a minimum value (about 0.03 in the case of in vivo mammalian systems), below which this value cannot be reduced. Increasing N, i.e. increasing the size of the whole experiment, will steadily reduce the variance of log m, and it is also clear that the larger r, the better. Porter, (1963), suggests that r should be at least 5.

This optimum' design is highly specialized for the estimate of log m, but it will also yield a fairly accurate estimate of D_o. In the present experiments N has been made as large as possible, with larger groups than have hitherto been used in the Zea system, and in both the aerobic and anoxic cases r is greater than 5.

In the calculation of the surviving fraction, the result of an assay of irradiated cells is divided by the result of a control assay of unirradiated cells. If the same control figure is used for all the observations, they are linked together by this. Random error in the control figure used will affect all the observations shifting them (and the estimate of log m) up or down by an equal amount. Bias in the controls will of course always bias the estimate of log m, but will not affect the estimate of D_o. In the present case, therefore the greatest confidence may be attached to the estimate of the 37 per cent dose slope.

Because the final dose response curves have the characteristics described by Equation(7.1) a tentative value for m and D_o may be read off under aerobic and anoxic conditions. These have been summarised in the previous chapter.

The extrapolation number in air varies between estimates of 1.4 and 2.4, and the equivalent value for D_o is 410 rads. The respective values in nitrogen are 1.5 - 1.85 and 1050 rads.*

The results of the fractionation experiments, with the exception of three cases, unfortunately do not provide sufficient data to confirm these values rigorously. as was intended. The reason for this is that it would appear that at the dose levels chosen, the repair of sublethal damage is not sufficient for the equations to be applied with any accuracy. This fact has been largely confirmed by Hering (personal communication), who finds the degree of sublethal damage repair in <u>Zea</u> at the dose levels used very low and subject to a great deal of statistical variation.

In the cases where the bias is towards the correct side, i.e. the single equivalent dose is less than the sum of the divided doses, a solution of the equations is provided by m = 2 and D_o = 350 rads.

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* Giving a further estimate of 2.6 for the O.E.R. derived from the 37% dose slopes in air and nitrogen.

The extrapolation number in air is therefore rather lower than the values of 3 to 4 obtained by Hall, Lajtha and Oliver (1962), in the case of Vicia faba and by Hall (1962), who obtained values from 2 to 3.5 using the method of dose fractionation in the same system. It is also lower than the range between 3 and 4 obtained by Shepstone (1964) for Zea. A fraction of a target is not really possible, so the extrapolation number is probably really 2, and so on the whole one expects that an equivalent number of sites have to be inactivated in both cases, although a case for the dominance of single-hit events in Zea can possibly be presented. A similar argument probably holds for the anoxic case.

Révèsz and Littbrand (1964) found that the survival curves for asynchronous cultures of several cell times irradiated in oxygen-free argon with X-rays exhibited a reduced extrapolation number that was indistinguishable from unity, and they deduced that the dose-effect relationship after anoxic treatment with sparsely ionizing X-rays is similar to that after treatment with densely ionizing radiations. A similar reduction in the extrapolation number of the survival curve of cells treated by X-rays in an anoxic medium equilibriated with air has been noted by other authors (e.g. Humphrey, W.C. Dewey and Cork, 1953, Legrys and Hall, 1969), and it would seem that the present results also agree with this.

However, the opposite result has been reported by D.L. Dewey, and the whole question awaits confirmation.

The value of the 37 per cent dose slope for irradiation in air obtained in the case of Zea is estimated to be 410 rads. This is appreciably greater than the value obtained by Hall et. al. for Vicia, which ranged between 35 and 90 rads on various estimatis. The present value agrees with that of 430 rads obtained by Shepstone (1964).

It is clear that Zea will survive much higher doses of radiation then Vicia, a conclusion in keeping with that of Clowes (1959, 1963), who found that the root of Zea reorganized its internal meristematic organization after doses up to 1800 rads of X-rays. In Zea, 300 R produced no anatomical deviations in the root meristem and there is a clear quiescent centre as in normal roots. After 1200 R, some, but not all of the root meristems became disorganized. In Vicia irradiated with 1200r, the roots grow for about 3 days, and then stop. After 360r, most meristems become disorganized, but the roots usually survive.

The difference between the values of the 37 per cent dose slope for Vicia faba and Zea mays, may be explained on the basis of the extensive requirements of Sparrow and his colleagues at Brookhaven. Sparrow, Underbrink and Sparrow (1967) have plotted the D_o values of 79 organisms (mostly viruses and lower plants). against their respective chromosome volumes (equal to the nuclear volume divided by the chromosome number). This procedure has resulted in eight regression lines, with slopes of -1. For organisms within each regression group (so-called radio taxon), D_o is inversely related to the chromosome volume and therefore the size of chromosome appears to be a highly significant index of cellular radiosensitivity (loss of reproductive integrity).

Although <u>Vicia</u> and <u>Zea</u> have not yet been isolated into radio taxons, it is well established that their interphase chromosome volumes (and DNA content per chromosome of the root meristem) are inversely proportional to D_o. The values are tabulated below, (Sparrow and Miksche, 1961; Sparrow, 1964)

	Vol. of nuclei (3)	Chromosome No.	DNA ( ) per chromosome	Interphase chromosome Vol. (3)	D_o (rads)
<u>Vicia</u>	490	12	3.2	41	35-90
<u>Zea</u>	280	20	0.7	14	400

The basis for the relationship between interphase chromosome volume and sensitivity is difficult to understand. However, from accumulated evidence that this relationship exists for other effects e.g. the degree of chromosome breakage, the somatic mutation rate, and the ease with which all division is inhibited, one suspects that the basis for all these effects is injury or impairment of genetic material.

The question of the quiescent centre remains. Many eminent authors have speculated on the reason for the enhanced resistance with respect to reproductive integrity of the constituent cells of the quiescent centre and some of these arguments have been presented in Chapter III.

One of the original thoughts was that the cells of the quiescent centre might be resistent to radiation because of anoxia. This idea was dispelled by Hall, Lajtha and Clowes (1962) who subjected Vicia roots to mean lethal doses of ionizing radiation. They found that the cells which promote recovery are not protected by anoxia and that their progeny have normal radiosensitivity. They presented calculations to show that the number of cells in the meristem surviving irradiation is enough to promote recovery without attributing any special degree of protection to any of the cells; but if recovery can only take place from a small region of the root, the possibility could not be excluded that cells in this region have reduced radiosensitivity.

The dose-response curve for Zea deduced here still shows an oxygen enhancement at high dose levels when the quiescent centre cells might presumably be operational, and so this must afford additional proof that the quiescent centre is not further protected by anoxia, thus confirming the conclusion of the above authors.

A possible explanation for the enhanced resistance of the quiescent centre may be deduced from the principle established emperically by Sparrow, and stated above, that the interphase chromosome volume is proportional to the radioresistance. It is known that the cells of the quiescent centre have on the average less

DNA than the other parts of the apex (2C amount instead of 2C to 4C elsewhere, Jensen 1958), as well as smaller nuclei (90 μ^3 versus 150 μ^3 in the cap initials in Zea). Chromosome number is not pertinent, of course when comparing different cells in the same plant, so it may be assumed that on the basis of nuclear volume and DNA content per chromosome alone, the cells of the quiescent centre aught to be more radio resistent than at least the cap initials and most probably the rest of the apex.

The failure to find evidence to support a contention that the cells of the quiescent centre are atypically radio resistant saves one from a radiobiological dilema: one need not entertain the disturbing notion that bean and maize roots have built-in mechanisms for repairing damage done by radiobiologists.

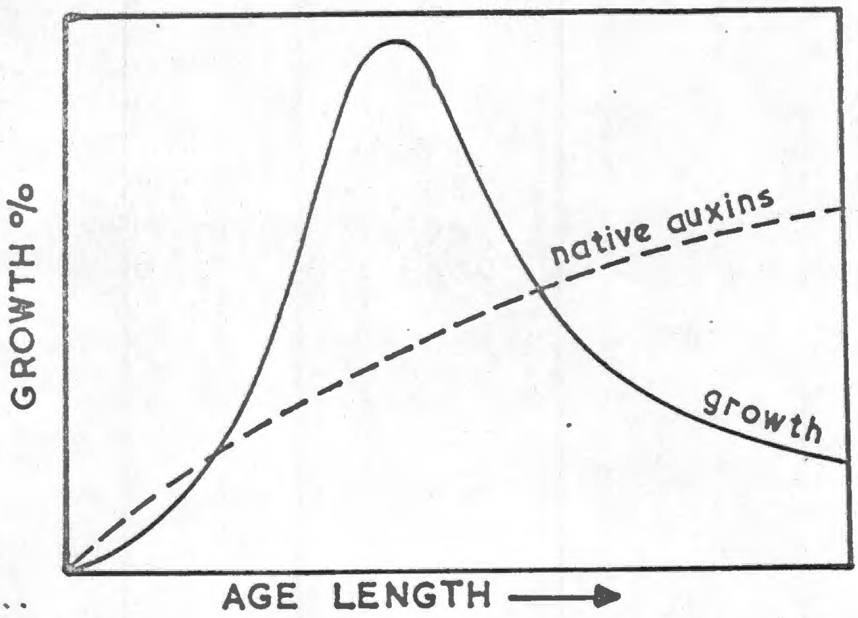

FIGURE 7.1

Roots, unlike stems and hypocotyls, are inhibited in their growth by applied auxin (the root growth hormone considered to be identical with indole-3-acetic acid) over a wide range of concentrations. The results of Pilet (1961) indicate that the destruction of auxin is greater in old root tissues than in young tissues. This seems to contradict other evidence which shows that the auxin content increases with increasing age. It is therefore supposed that the two processes operate simultaneously i.e. the tissues greatly increase their ability to destroy native auxins in proportion to the age of the roots at the same time as the accumulation of auxins is increasing. Even if the destruction is greater, the final auxin content rises because auxins are produced faster than they are destroyed. This is shown diagramatically above.

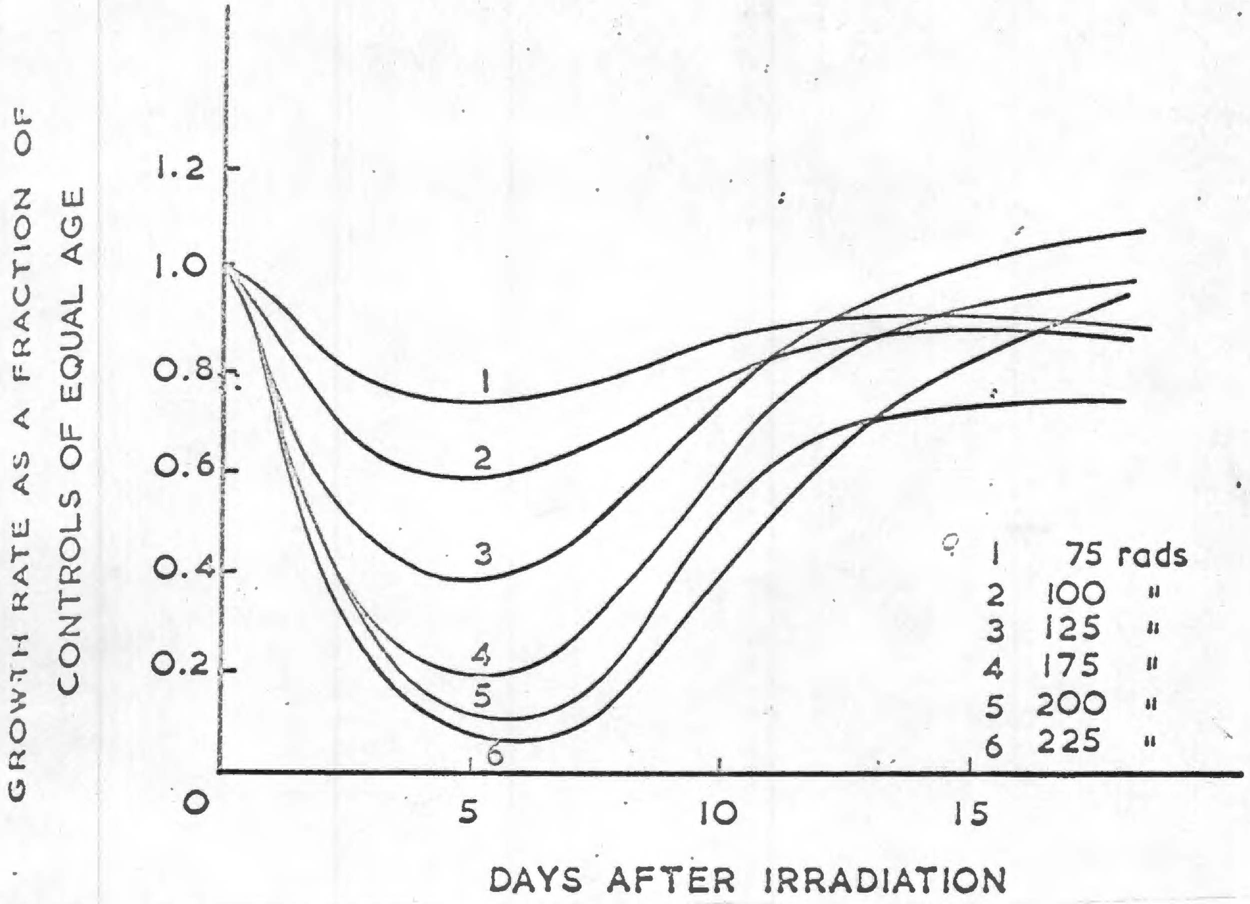

FIGURE 7.2

Patterns of root growth after single acute doses in Vicia faba.

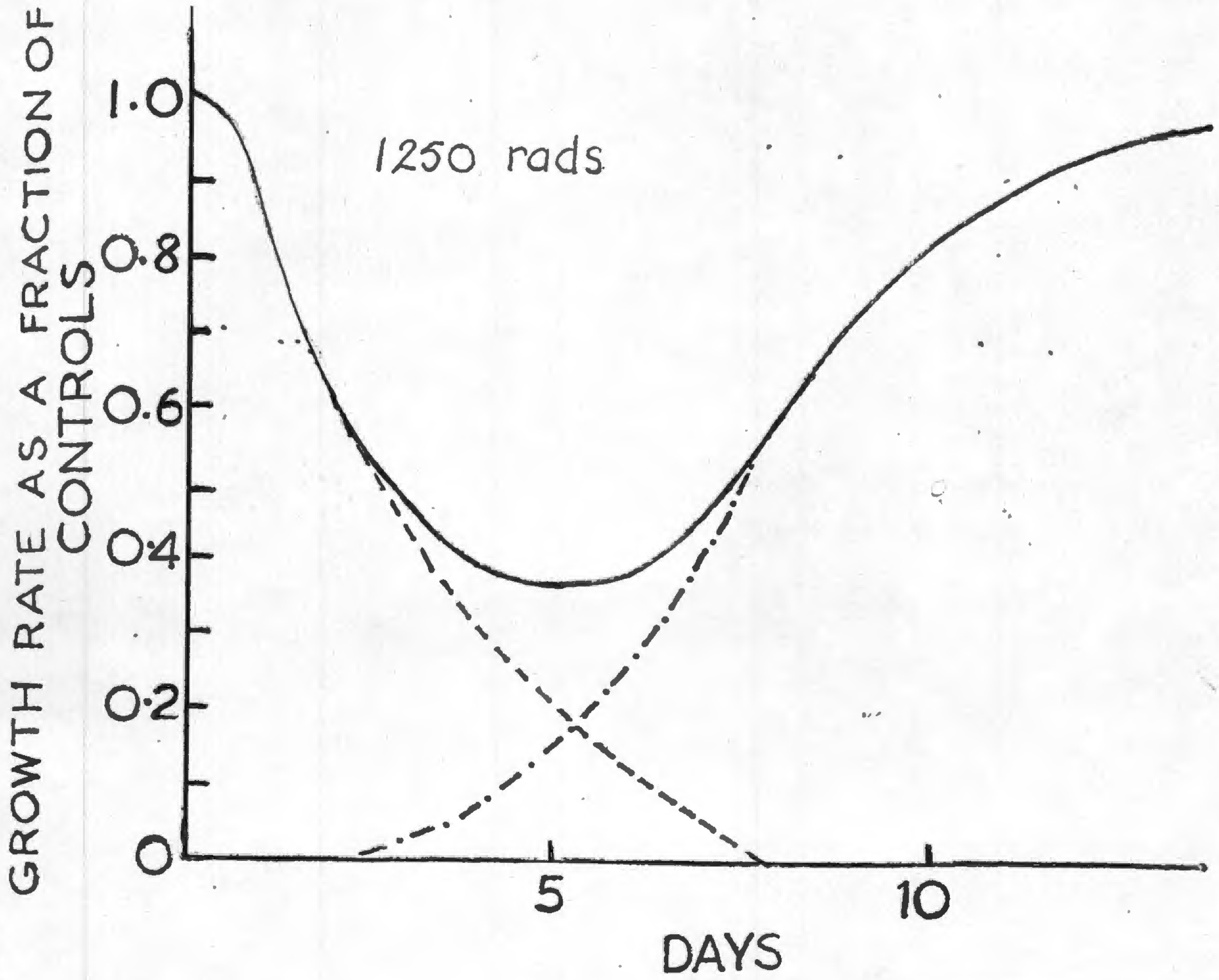

FIGURE 7.3

The curve for the growth rate of roots exposed to 1250 rad as a fraction of that of controls (full line) is built up of two components. The broken line represents the decreasing contribution from cells which have lost their reproductive integrity; the chained line represents the increasing contribution from integer cells.

CHAPTER VIII

Summary

Using a very similar culture method to that used in the past for Vicia faba, for the cultivation of the maize roots, it has been possible to assess radiation damage by means of the minimum growth rate, G_{min}, and growth in ten days, G_{10}, concepts. The values of G_{min} and G_{10} plotted against dose on semilogarithmic paper gave similar curves to those published in the literature for Vicia faba.

From the values of G_{10} and dose, using a graphical method, a value of 2.61 was obtained for the oxygen enhancement ratio for this system. A further value of 2.6 was obtained from a consideration of the values obtained for the 37 per cent dose slope under aerobic and anaerobic conditions given below.

By means of the two meristematic models devised by Hall et al (1962), designated Model A and Model B, the growth pattern of roots, in terms of the proportional of cells retaining their reproductive integrity, was assessed. It was found that there is little to choose between the survival curves in air using the two models: it has also been demonstrated (Figure 6.7) that the particular cell cycle time chosen for the calculations is not critical. Using Model B and a time of 24 hours the values of extrapolation number, m, and 37 per cent dose slope, D_o, under aerobic and anaerobic conditions found were in the ranges m = 1.4 to 2.4, D_o= 410 rads, and

and m = 1.5 to 1.85, D_o 1050 rads respectively. Thus m is probably 2 in both cases.

The fractionation experiments yielded only one satisfactory pair of values of m and D_o of 2 and 350 rads respectively. (See Chapter VII P125 for further comments in this respect).

A possible explanation for the enhanced resistance of the quiescent centre in the root meristem has been advanced, based on the principle established by Sparrow that the interphase chromosome volume is proportional to the radioresistance.

Appendix

Mathematical Derivation of Model A.

Suppose I_s is the number of cells in the model meristem under steady growth rate conditions. If these are assumed to be all in uniform cell cycle, the number of cells dividing per unit time is yI_s where

$$y = \frac{\ln 2}{\text{intermitotic period}} \qquad \text{(A.1)}$$

In order to maintain equilibrium an equal number of cells must differentiate per unit time.

Suppose that after a dose of radiation, the number of integer cells in the meristem is reduced to I, and that fatally damaged cells are removed immediately. The number of integer cells dividing per unit time is then yI. Further, suppose that the number of cells which differentiate per unit time is no longer equal to the number which divides, but is reduced in the ratio $\frac{I}{Is}$, i.e. the number is given by $\frac{I}{Is}$, yI or $y.\frac{I^2}{Is}$. As a result, I will increase as the integer cells divide.

The characteristic of the meristem which governs the rate of differentiation is thus postulated to be its fractional size, the ratio of the actual meristem population to the equilibrium value. The rate of change with time of the total number of cells, I, in the meristematic department, is then the difference between the increase in the number of cells resulting from division, and the loss due to differentiation.

1.e

$$\frac{dI}{dt} = yI - y\frac{I^2}{I_s} \qquad \text{(A.2)}$$

It is evident from this model that the rate of differentiation is small when I is small and increases with I. When I equals I_s, the rate of change of the number of cells on the meristem becomes zero, i.e. steady growth rate conditions prevail. The expression in equation (A.2) can be integrated by standard methods and results in the following expression:

$$I = \frac{I_s}{1 + \left(\frac{I_s}{I_o} - 1\right)e^{-yt}} \qquad \text{(A.3)}$$

where I_o is the value of I just after irradiation. The rate of differentiation at any time is given by:-

$$\frac{dD}{dt} = y\left(\frac{I^2}{I_s}\right) \qquad \text{(A.4)}$$

Substituting the expression for I deduced in equation (A.3) we have:-

$$\frac{dD}{dt} = \frac{y\,I_s}{\left[1 + \left(\frac{I_s}{I_o} - 1\right)e^{-yt}\right]^2} \qquad \text{(A.5)}$$

The area under this curve over a time interval of one day represents the total number of cells differentiating during that period. The corresponding quantity for a control root is the area under the curve:-

$$\frac{dD}{dt} = y\,I_s \qquad \text{(A.6)}$$

It is therefore possible to evaluate the total amount of differentiation and, therefore, the growth of irradiated roots, as a fraction of controls for each successive day after the initial depopulation. The growth rate is small during the early days after irradiation or depopulation, while the meristem is being repopulated, but then increases to a steady value as equilibrium is restored.

The initial depopulation produced by the radiation cannot be obtained simply by matching theoretical and experimental growth rate curves because the theoretical curves ignore the presence of sterile cells. It can be obtained, however, by means of the step-by-step calculation described in Chapter VI.

Mathematical Derivation of Model B.

It is assumed that all meristematic cells are preparing for division, but that the proportion of cells maintaining their reproductive integrity is proportional to the concentration of a specific substance - in other words, the fraction of the population dividing per unit time is proportional to this concentration. It is also assumed that the maintenance of reproductive integrity implies a 'consumption' of this substance - so that the fall in concentration of this substance in a given region or layer of cells is proportional to the number of cells present which retain their reproductive integrity. Cells having lost their reproductive integrity differentiate; for the purposes of simplification of the mathematical calculations it is assumed here that such cells are unable to divide even once.

Let N be the number of cells expected to divide per unit time in a population with 100 per cent reproductive integrity (no cells differentiating) corresponding to the concentration, C_o, of the postulated specific substance. As the substance diffuses through such a region, its concentration would fall from C_o to C_n.

From the above assumptions:

$$C_N = C_0 e^{-KN} \quad \text{(where K is a constant)} \qquad (A.6)$$

The proportion of cells with reproductive integrity at a concentration level of C_n is $\frac{C_N}{C_o}$, and from equation (A.6)

$$\frac{C_N}{C_0} = e^{-KN} \qquad (A.7)$$

In any infinitesimal part of the region containing dN cells ,(the number expected to divide per unit time if 100 per cent reproductive integrity is maintained), e^{-KN} dN cells will in fact divide. In the whole region, therefore, instead of the possible N cells, the total number of cells dividing per unit time will be

$$\int_0^N e^{-KN} dN = \frac{1}{K}\left(1 - e^{-KN}\right) \qquad (A.8)$$

This corresponds to a proportion $\frac{1}{KN}$.$(1-e^{-KN})$ For steady state to be achieved this proportion must be 0.5 : whence KN = 1.595.

Considering now the total population reduced to proportion P (following, for example, radiation damage) one is concerned with PN instead of N in the above formulae. Therefore the proportion of the possible PN cells to divide per unit time will be

$$x = \frac{1}{KPN}\left(1 - e^{-KPN}\right) \qquad (A.9)$$

and the proportion differentiating (1-x).

The number differentiating, D, will be PN(1-x), that is:-

$$D = \frac{1}{K}\left(KPN + e^{-KPN} - 1\right) \qquad (A.10)$$

This is a measure of growth rate (the corresponding value for steady state equilibrium being 0.5N). Consequently, G, the growth rate as a fraction of that for a steady state population is given by:-

$$G = \frac{KPN + e^{-KPN} - 1}{0\cdot5N}$$

$$\text{i.e } G = \frac{1\cdot595P + e^{-1\cdot595P} - 1}{0\cdot7975} \qquad \text{(A.11)}$$

An approximate solution, taking the first three terms only of the exponential term will be

$$G = 1\cdot595\,P^2 \qquad \text{(A.12)}$$

From equation (A.9), the number of cells dividing per unit time is

$$\frac{PN}{KPN}\left(1 - e^{-KPN}\right) = \frac{1}{K}\left(1 - e^{-KPN}\right) \qquad \text{(A.13)}$$

The net increase in the total population per unit time is given by the difference between the number dividing and the number differentiating. Given T units of time per cell cycle, the total population will be PNT, and the factor of increase, F, may then be calculated from equations (A.10) and (A.13):

$$F = 1 + \frac{1}{KPNT}\left(1 - e^{-KPN} - KPN - e^{-KPN} + 1\right)$$

$$= 1 + \frac{1}{KPNT}\left(2 - 2e^{-KPN} - KPN\right)$$

$$\text{i.e } F = 1 + \frac{1}{1\cdot595TP}\left(2 - 2e^{-1\cdot595P} - 1\cdot595P\right) \qquad \text{(A.14)}$$

$$\text{as } KN = 1\cdot595$$

Expanding this expression to the first two terms of the exponential series gives:-

$F = 1 + \frac{1 - 1\cdot595P}{T}$ which is $F = 1 + \frac{1 - 1\cdot595P}{5}$ for an intermitotic time of 30 hours, and $F = 1 + \frac{1 - 1\cdot595P}{4}$ for an intermitotic time of 24 hours.

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