

A STUDY OF THE RESPONSE OF THE ROOT MERISTEM

OF MAIZE TO FRACTIONATED AND PROTRACTED

DOSES OF IONIZING RADIATION.

by

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Vicia faba, and investigating the effects of ionizing radiation on its root system. In particular, species such as Tradescantia, Pisum, Allium and Mordeum vulgare have been extensively studied.

Nevertheless an ever-increasing number of workers have used Vicia faba for explaining the basic problems of radiobiology. One of the reasons 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. Clowes (1963) regards this as an unfortunate choice of species for examining organization because its meristem is not very clearly divided into regions. On the other hand this greater heterogeneity in the cells of the root tip of the broad bean has enabled simplified models of its meristem to be constructed, a procedure which might not afford reliable results in meristems which can be clearly delineated into regions.

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 for radiation damage may be applied. Consequently the mathematical

complexities will also be similar. Of particular interest in Zea mays root meristems is the large and clearly defined quiescent centre. Clowes (1959) has shown that the quiescent centre is a reservoir of cells which will become meristematic after the normally meristematic cells have suffered genetic death.

The majority of experiments indicating a stimulatory effect of protracted radiation on cells, have been done on plant systems. Most of the early work was done on field crops in an attempt to increase the yield from such plants. Later, more sophisticated experiments were carried out by Sparrow and others on a large variety of species of plants.

Vicia faba was also used by Hall, Oliver, Shepstone and others in an attempt to explain a possible stimulatory effect of gamma-rays.

Elkind and Sutton (1960) were the first to use fractionated doses of radiation to demonstrate the mechanism of sublethal damage and recovery in Chinese hamster cells.

Experiments by Hall (1963) on Vicia faba showed evidence of similar patterns of sublethal recovery of the cells to the radiation. A repetition of the experiments by Hall, using a plant system (such as Zea mays) with cells having a different intermitotic cycle time and different radiation parameters, was thus contemplated to confirm these patterns of sublethal recovery for plant cells.

Although much of the work on the effect of radiation on cells was done on mammalian cell systems, numerous close parallels had appeared from time to time with results obtained with plant systems.

Of importance in the present investigations was the fact that nearly all animal systems require the collaboration of other workers. Plant systems, however, 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. Furthermore, it is possible to irradiate root systems under conditions which would often be impossible with animals.

The first radiobiological experiments on Zea to determine the dose survival curve with respect to reproductive integrity (Shepstone, 1964), were hampered by the fact that the variety used, "Canada Gold", was not a hardy variety.

Later experiments (Fenner, 1970) proved that the local variety known as "Kalahari Blitz" was hardy enough to obtain results which were, as far as statistics is concerned, superior to those obtained using "Canada Gold".

It was therefore decided to study the effect of fractionated and protracted doses of ionizing radiation on the kinetics of the root meristem of Zea mays and to see to what extent the meristematic models, deduced in the case of the bean root, could be used to explain the population kinetics in Zea root tips.

CHAPTER IIGENERAL RADIOBIOLOGY.Introduction.

Radiation biology had its start in the year 1895, with the discovery of X-rays by W.C. Roentgen. Many diverse and apparently contradictory reports of a radiobiological nature appeared in the early literature following this discovery. For example, radiation was reported to shorten the life of some types of protozoa, but to lengthen the life of others; to increase the number of blood cells in some animals but to decrease the count markedly in others.

Ionizing radiation acts on a biological system by altering the molecules of which it is composed. The initial chemical change is rarely detected directly and may be repaired almost immediately. Some of the molecular changes may, however, occur within important structures, and a small initial change may eventually result in alterations which are readily recognizable. The transition between a chemical change in a system and the biological manifestation of this change is complicated and often obscure.

Radiation results in such widely varied effects as increased permeability of membranes, gross structural chromosome changes, and subtle chemical changes in the structure of the DNA molecules.

The bulk of both direct and inferential evidence suggests that cell nuclei are a major site of radiation damage leading to cell death. For example Sparrow (1965) has found marked correlations in plants between cell sensitivity and many nuclear parameters such as chromosome number, nuclear volume,

and DNA content.

Effects of radiation on the function of cells.

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

Growth rate.

Under continuous 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 of which increases without division. This phenomenon has been observed among bacteria, yeasts and also among higher organisms e.g. mice (Lorenz et al, 1947), and seems therefore to be fairly general. As the dose accumulates, the total weight of the culture decreases and becomes lower than that of the controls. In general, radiation reduces growth rate and 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 0.001R.

Mitotic delay.

When a cell has been irradiated before prophase, division is delayed. The main characteristic of mitotic delay is its temporary nature, but the mechanism of mitotic delay is still not understood. The delay of mitosis can be modified by dose rate and by oxygen concentration, and this may mean that metabolic processes are involved.

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. Reproductive death occurs for example in intestinal crypt cells, a group of cells with a high mitotic index (i.e. a large number of cells in this group are in mitosis). With these cells interphase death would probably require a higher dose.

The processes leading to reproductive or interphase death are not completely understood.

The Target Theory.

Early in the history of radiation biology it was noted that there was a direct relationship between the number of microorganisms which were killed by a radiation exposure and the radiation dose they had received. In order to put this relationship into mathematical terms, the target theory was formulated by Crowther (1924). The theory states that the production of ionization in or very near to some particular molecule or structure (target) is responsible for the measured effect. The production of an effective event in the target is often called a hit. The target may be a whole cell, part of a cell, or a critical molecule. Generally the system studied is a cell population in which the measured effect may be cell death or inability to grow or divide.

In the simplest form of the target theory one hit is sufficient

to produce the measured effect in the associated organism.

Exponential Inactivation, (i.e. "single hit" cell kinetics).

Suppose a biological sample, which contains N biological entities (these entities may be enzyme molecules, viruses, tumour cells etc.) is given a small dose of radiation dD . It is required to calculate the number of entities dN which are inactivated by this dose dD . The number of inactivations produced should be proportional to the dose and proportional to the number of entities present. This statement may be expressed mathematically by:

$$dN = -\frac{1}{D_0} N dD \quad (2.1)$$

where $\frac{1}{D_0}$ is a constant of proportionality.

Rearranging Equation (2.1), one obtains:

$$dD = -\frac{dN}{N} D_0 \quad (2.1a)$$

When dN is made equal to N in the above equation, then it is clear that D_0 is the dose that would be required to inactivate all the entities if they continued to be inactivated at the initial rate of inactivation. The quantity D_0 is called the mean lethal dose and is the dose that is required on the average to place one inactivating event ("hit") in each of the biological entities.

Equation (2.1a) can be integrated to yield:

$$\frac{N}{N_0} = e^{-D/D_0} \quad (2.1b)$$

where N_0 = number of biological entities present initially.

N = number of entities surviving after a dose D .

The slope of the straight line obtained when plotting $\log \left(\frac{N}{N_0} \right)$ vs. D is equal to $-\frac{1}{D_0}$ ($\frac{1}{D_0}$ is sometimes denoted by λ).

This fact is used when the effect of radiation on different cell systems is being compared.

When a dose D has been given such that $D/D_0 = 1$ then since $e^{-1} = 0.37$, D_0 is also called the 37% dose slope (Lajtha and Oliver, 1961). Thus when there has been an average of one hit per target, 37% of the original number of organisms will still survive.

Many assumptions in the simple target theory are oversimplifications. For example, it assumes that the degree of effect is not influenced by the dose rate, and that experimental conditions during irradiation or immediately after will be unimportant. In order to explain survival curves of non-exponential form, the following extension of the target theory has been suggested.

Multitarget theory.

According to the multitarget theory, certain organisms contain more than one target, In order to inactivate the entire unit, each of the targets must receive a hit.

This can be expressed mathematically by the equation:

$$\frac{N}{N_0} = 1 - (1 - e^{-\lambda D})^m \quad (2.2)$$

where m represents the number of targets in this model and is called the "hitness" or extrapolation number. The other symbols are as before.

The curve described by equation (2.2) is sigmoid. If $\log (\frac{N}{N_0})$ is plotted vs. the dose D , then the survival curve consists of an initial "shoulder" and a straight portion as depicted in Figure (2.1) for Zea mays. The slope of the straight portion of the curve is equal to $-\lambda$. The extrapolation number is the intercept on the ordinate of the exponential part of the curve (Alper, Gillies and Elkind, 1960).

At values of $D \gg D_0$ equation 2.2 becomes:

$$\frac{N}{N_0} = m e^{-\lambda m} \quad (2.2a)$$

i.e. for large doses the relation between N and D is exponential.

The two numbers, m and λ , give a complete description of the response curve without further qualification. These 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 (Tyler and Dipert, 1962; Porter, 1964).

The "single event component" of the multitarget curve. (where an event is defined as a hit on a target).

Experiments by several authors (Lea, 1946; Gray and Scholes, 1951; Berry and Andrews, 1963) have shown that the survival curves obtained with densely ionizing alpha-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 (2.2) which then reads:

$$\frac{N}{N_0} = e^{-\mu D} \{ 1 - (1 - e^{-\lambda D})^m \} \quad (2.3)$$

where μ is the reciprocal of the 37 per cent dose slope (D_1) for the single event component. Unfortunately, in the majority of experiments the data is insufficient to provide an accurate estimate of this component.

The recovery of sublethal radiation damage.

If the shape of the survival curve for a cell population is sigmoid (Equation 2.2) it implies a threshold type of response, in which damage must be accumulated before its effect becomes apparent.

Elkind and Sutton (1959) have shown that for Chinese hamster cells grown in vitro, sublethal damage is fully repaired in about 10 hours. Fractionated dose results of Dewey and Humphrey (1965) and Sinclair and Morton (1964, 1965) with synchronized Chinese hamster cells, and those of Whitmore et al (1965) with synchronized mouse L-cells confirm that repair of sublethal damage is prompt (i.e. within a few hours).

Hall and Lajtha (1963) have also demonstrated the effect in the root meristems of Vicia faba where the effect of two doses of 100 rads of X-rays each, separated by a variable time interval, is described. They used the results to infer the rate of recovery of radiation damage, and this rate is shown to vary with the temperature at which the roots are

stored between doses. As has been previously stated by Lea (1946), macroscopic changes in root growth are related to the proportion of cells sterilized in the meristem. It may therefore be concluded that the recovery of the organized population in the root meristem of Vicia faba follows the same pattern as reported for mammalian cells in culture.

The curve in Figure 2.2 illustrates the way in which the "growth in 10 days" (which is a function of cell survival and is defined in Chapter V), varies with the time interval between the two dose fractions. The smooth curve has an exponential form and has the empirical equation designated by:

$$19^{\circ}\text{C}: \text{"Growth in ten days"} = 0.49 - 0.15 e^{-\frac{0.693t}{1}} \quad (2.4)$$

If there had been no recovery of sublethal damage between the fractions, the two doses of 100 rads would have been completely additive, and the "growth in ten days" identical with that after a single dose of 200 rads. Alternatively if there had been complete recovery of sublethal damage between the doses, the reduction in ten day growth due to both doses of 100 rads would be the square of that due to the single 100 rads dose. The experimental data are consistent with the second possibility, provided the two doses are separated by 12 hours or more. For small separations there is only partial recovery. Thus on the plateau region of the smooth curve in Figure 2.2 the recovery is at a maximum. There is an initial rapid rise in the curve corresponding to an increase in recovery with an increase in the time between the doses. The smooth curve assumes the recovery process to be an exponential function of time with a corresponding half period as given by Equation 2.4.

Of special interest in such experiments, especially in those concerned with cells in culture, is the fact that the recovery curve is not smooth, but contains a secondary maximum and minimum. The first demonstration of this effect was given by Elkind and Sutton (1959,1960) using Chinese hamster cells. These cells were exposed to fixed doses separated by varying intervals of time and the resulting curve is colloquially referred to as the "Elkind recovery curve". As an example of this type of curve, a plot of the percentage survival vs. time between doses for the intestinal crypt cells is given in Figure (2.3).

Fractionation results indicate that the structure in the 2-dose fractionation curve during the recovery period is due to the combined effects of repair plus the cell proceeding through the cell cycle (and thus its sensitivity is changed by the time the second dose is given). Partial synchrony is produced by the conditioning dose and since surviving cells may progress through the cell cycle or repair damage or both at different rates it is possible that fluctuations in the survival parameters (m and λ) will be observed in time after a conditioning dose (Elkind et al,1961). This explains the "kink" in the curve depicted in Figure 2.3.

Elkind and Whitmore (1967) point out that the observed value of m may in any case be a poor indication of the individual m -values in a heterogeneous population, as for example the different populations of cells in the root meristem of Zea mays. According to the authors the extrapolation number can often be approximated by:

$$m = u_i m_i \quad (2.5)$$

where u_i is the fraction of the population which is least

sensitive and has an extrapolation number m_1 . In the case of Zea this will correspond to the m -value for the cells in the quiescent centre.

Hall and Lajtha (1963) found evidence of such a "kink" in the recovery curve for Vicia at 19°C (dotted curve in Figure 2.2), but it was not possible to resolve such a fine structure with statistical certainty from the results. There was no evidence of any "kink" at 3.5°C.

It is interesting to note, as suggested by Hall and Lajtha (1963), that there is a correspondence between the rate of recovery of radiation damage and the repair of chromatid breaks. Savage, Neary and Evans (1960) obtained a value of one hour for the average time for which breaks were available for union at 19°C, and the corresponding quantity at 3°C, calculated from Lea's G-function was about 12 hours, with 95 per cent confidence limits of 6 and 24 hours. The 12°C results, giving a time constant of 5.5 hours, lie between these two extremes.

The constants of the survival curve (i.e. λ and m) deduced from the response to unequal divided doses of X-rays.

As the fractional survival of cells after two split doses of 100 rads each is greater than in the case of a single dose of 200 rads, there is a dose "loss" due to fractionation.

This "lost" dose is referred to as the "quasi threshold dose", D_Q (Alper, Fowler, Morgan, Vanberg, Ellis and Oliver, 1962). This value, D_Q , is used by some authors in addition to the two constants, m and λ , which are used to describe the multitarget curve. D_Q is defined as the intercept on the abscissa, drawn through 100 per cent survival, of the extrapolated exponential part of the curve (Fig. 2.4). If the phenomenon described by Elkind and Sutton (1959, 1960) is generally applicable, then the value of D_Q for a given cell population may be determined by comparing, at an interval which gives full recovery, the single dose required to produce a defined effect with the sum of two doses, separated in time, needed to give the same effect. The difference between the single dose and the sum of the split doses is a measure of D_Q , as illustrated in Fig. 2.4. If cells which have been exposed to a dose of D_3 rads (Fig. 2.4) i.e. exposed to a dose just large enough for sublethal damage to be a maximum (D_3 lies at the start of the straight portion of the survival curve), are irradiated again at a time along the plateau region of the 2-dose fractionation curve (Fig. 2.3), then the resulting fractionation survival curve normalized to the survival resulting from the initial D_3 rad exposure, lies well above the survival curve resulting from single, acute exposures. In any period without full recovery, a smaller value, say D'_Q is obtained.

Hornsey and Vatistas (1963) have used a method of divided doses to determine the size of the "shoulder" on the sigmoid survival curve for the crypt cells of Lieberkuehn.

Their results have shown that when two doses of radiation were separated by a variable time interval (Fig. 2.3) it

was also found that the survival was possibly greater if the doses were separated by six hours than if they were separated by nine hours. When the doses were separated by intervals larger than nine hours the survival level was greater and approached a plateau between 12 and 14 hours.

Their data verify that the sum of two doses, separated in time, is greater than the single dose required to produce a postulated end-point, which is taken by the authors as the total dose Q to reduce a population of mice by 50%. From theoretical considerations it would be expected that when the first dose is small, so that the surviving fraction of a population of cells falls on the shoulder of the survival curve, only a small increase in Q would be necessary to reach the postulated end-point. As the first dose is increased, the total dose needed would increase until the first dose would be large enough to fall on the exponential part of the survival curve. (When the survival curve becomes a straight line, sublethal damage is maximal). The value of Q should then remain fairly constant until the first dose is sufficiently near to Q for a single dose of irradiation, that only the part of the "shoulder" of the curve of the second irradiation would be needed.

Hornsey and Vatistas's results fit this theoretical pattern, as shown in Figure 2.5, in which the total dose required to kill 50 per cent of the population of mice is plotted against the first dose for an interval between doses of 6 hours. The total dose required rises to a maximum and remains constant until it falls. The difference between the maximum value of Q for the split doses and the value of Q for a single dose

should be equivalent to D_Q . This figure, which was found to be more than 400 rads for the crypt cells in the mice, also offered further proof of a definite minimum in the curve of survival as a function of the interval between two separate doses.

After the irradiation the cells suffer mitotic delay and it is therefore unlikely that repopulation occurred before the second dose of radiation was given. D_0 , D_Q and m are related by the expression (Alper et al, 1962) :

$$D_Q = D_0 \ln(m) \quad (2.6)$$

Hornsey and Vatistas calculated D_0 to be equal to 580 rads for $D_Q = 400$ rads and $m = 2$ for the population of crypt cells.

Some factors influencing sublethal damage and recovery.

Research on sublethal damage and recovery over the past few years has lead to the following conclusions:

1. It has been found that although cell growth and division is strongly dependent on temperature, repair of sublethal damage is not so dependent (Elkind and Alescio, 1963; Elkind et al, 1965; Elkind, 1965).
2. As demonstrated by Whitmore et al (1965) as well as Sinclair and Morton (1964), repair occurs in cells in all parts of the mitotic cycle. This is true for all temperatures.
3. With regard to the degree of sublethal damage, the results both with mouse L cells (Whitmore et al, 1965) and with Chinese hamster cells (Sinclair and Morton, 1964) are consistent with essentially complete repair before division.

4. 2-dose results obtained with synchronized Chinese hamster cells have shown to be consistent with the 2-dose survival data observed with asynchronous cells (Sinclair and Morton 1964,1965; Elkind and Sinclair,1965).

The behaviour of apical root meristems under continuous radiation exposure at low dose rates.

Growth inhibition resulting from chronic exposure is a well-known radiobiological effect. Early investigators, however, have also found a stimulatory effect of gamma rays on plants of agricultural importance. For example, among early investigations, Sparrow and Singleton (1953) reported an earlier flowering of tobacco plants exposed to 100-350r of gamma-rays daily and Breslavets (1958) increased the green weight of corn by 36%, using 1.6r/day from a 1Ci Cobalt-60 source. These and many more reports on the stimulatory effects of radiation on plant growth are summarized by Sax (1963).

Chronic irradiation presents a different problem to the organization of the meristem as in the case of acute irradiation. The radiation dose is now applied throughout the mitotic cycle or until the cycle itself is stopped by failure of the cell to divide. Differences between different regions as a result of the predominance of a phase of the cycle cannot effect the relative sensitivity of cells in the same way as in acute irradiation. The slower the rate of mitosis the greater will be the accumulated dose to a particular cell under chronic irradiation. Thus Van't Hof and Sparrow (1963) plotted damage against dose per mitotic cycle in roots of Pisum and found a linear relation up to about 450 Roentgen per cell cycle. As a measure of damage they used the percentage of aberrant anaphases in squashes of whole root tips, and as a measure of the rate of mitosis they used the time necessary to produce 1% of dividing

tetraploid nuclei after colchicine treatment. They produced differences in cycle duration solely by varying the temperature, and considered that the irradiation itself did not generally produce a significant change in cycle time over the three days of the irradiation, provided exposure was below the critical level of about 450 Roentgen per cycle. They believe that the temperature itself is unimportant in determining the amount of damage suffered because, when plotted against dose per cycle, all their results fell on the same curve. Thus the effect of temperature is merely that of varying the duration of the cycle and hence the total dose received by the average cell. Following a study on the population kinetics of the root meristem of Vicia faba exposed to continuous irradiation, Hall, Oliver, Shepstone and Bedford (1966) support the hypothesis that it is the dose per cell cycle that determines the degree of damage produced in a population of dividing cells exposed continuously to radiation.

Wimber (1960) has shown that chronic irradiation changes the relative lengths of the phases of the mitotic cycle considerably. Clowes (1965) therefore feels that the radiation effect will depend initially on the duration of the mitotic cycle, though radiation may then change the relative durations of the phases. It is for this reason that at first the quiescent centre of both Vicia and Zea, by virtue of the long cell cycle applicable here, will show least damage and in Zea the cap initials can be expected to be especially sensitive. The accumulation of dose during the mitotic cycle will initially tend to lengthen

the mitotic cycle, but whether it will continue to do so or not depends upon whether the accumulated damage inflicted during one mitotic cycle can be considered as being passed on to the next or if, as Van't Hof and Sparrow's view seems to imply, there is no effect to pass on to the next cycle.

Clowes and Hall (1966) measured the change in the duration of the mitotic cycle of the cells in the different regions of the meristem in Vicia and Zea roots exposed to continuous irradiation from a gamma - source at different dose rates. The percentage of cells with micronuclei was used as a measure of radiation damage (Clowes 1963b; 1964a). The metaphase - accumulation technique was used (Clowes 1961a) to measure the entry of cells into mitosis. The average duration of the mitotic cycle (T) and the percentage of cells containing micronuclei are given in Table 2.1 for the four regions of the meristem investigated. In Zea all regions of the meristem other than the quiescent centre lower their rates of mitosis. The most spectacular change is in the cap initials which, in normal roots, have the highest rate of mitosis and which are the most sensitive to acute radiation. In Zea the quiescent centre seems to be least affected. In Vicia the chronic irradiation increases the rate of mitosis in the quiescent centre, but has no marked consistent effect on the other regions except at 12°C when there is a slight general stimulation of mitosis. According to Clowes the variations between the results at the different dose rates are the consequence of considerable reorganization within the meristem. As meristematic cells

cease to divide under the radiation whole tracts of new cells invade the regions that would normally be occupied by the progeny of the sterilized cells. In spite of this reorganization, the meristem maintains some resemblance to a normal meristem. This disruption of cell lineages is more noticeable in the stele and cortex of Vicia than in Zea, though the cap initials of Zea are frequently disorganized and replaced from the quiescent centre (Clowes 1963b).

It is not known if a steady state is reached in the regions of the meristem but Evans and Sparrow (1961) have shown that there is, for the meristem as a whole in Vicia, a steady state in the frequency of micronuclei which is reached in three to seven days of chronic irradiation. The same is also true for mammalian cells. For example, Lamerton and Lord (1963) have shown that the small intestinal epithelium of young rats can maintain a steady population under 400 rads per day, but the animal dies from failure to maintain a population of stem cells in the blood-forming organs.

Mathematical models for the behaviour of the meristem under conditions of protracted irradiation.

In the case of roots continuously exposed to radiation, the population of actively dividing meristematic cells is continually being depleted as a result of radiation sterilization as well as differentiation. Under these circumstances, the population kinetics of the meristem is described by Hall (1963) as follows:

$$\begin{array}{l} \text{Rate of change of} \\ \text{viable cells in} \\ \text{meristem} \end{array} = \begin{array}{l} \text{Gain due to} \\ \text{division} \end{array} - \begin{array}{l} \text{Loss due to} \\ \text{sterilization} \end{array} - \begin{array}{l} \text{Loss due to} \\ \text{differentiation} \end{array} \quad (2.7a)$$

To make the above conceptual equation quantitative, Hall put forward two assumptions:

1. The viable cells of the meristem are in exponential growth and have an intermitotic cycle time of T hours.
2. The dose response relationship for reproductive integrity of the meristem cells exposed to low dose rates is an exponential function of dose i.e. the inactivation of the cells as a result of protracted irradiation is assumed to be a "single event" process.

Thus,

$$F = e^{-\lambda_1 D} \quad (2.7 b)$$

where F is the fraction of cells surviving a dose D and $\frac{1}{\lambda_1}$ is the slope of the survival curve when $\ln F$ is plotted against the dose D.

Thus equation (2.7a) now becomes:

$$\frac{dI}{dt} = \mu I - r \lambda_1 I - \text{loss of cells due to differentiation.} \quad (2.8)$$

where $\mu = 0.693/T$

I = number of viable cells in the meristem at a time t.

r = dose rate, in rads per hour

It is possible to postulate various models to account for the "loss of cells due to differentiation" similar to those described by Hall, Lajtha and Oliver (1962) for roots exposed to single doses of radiation at a high dose rate. However Hall (1963) considers that at the limiting dose rate for which the root just stops growing, differentiation is zero and it is not necessary to assume any model. At the same time, the

production of cells by division is just balanced by the loss due to radiation sterilization, consequently, $\frac{dI}{dt}$, the rate of change of the number of cells in the meristem, is also zero. For this limiting case, therefore, equation (2.8)

$$\text{simplifies to } \frac{0.693}{T} = r_c \lambda, \quad (2.9)$$

where r_c is the critical dose rate, defined to be the dose rate at which cell division just compensates for the loss of cells due to radiation sterilization.

If we accept Model A (to be described below) to account for the "loss of cells due to differentiation", then, for a small interval of time we would have:

$$\begin{array}{l} \text{The net increase} \\ \text{in number of} \\ \text{meristem cells.} \end{array} = dI = \mu I - r \lambda_1 I - \frac{\mu I^2}{I_0} \quad (2.10)$$

where, as mentioned previously, the radiation damage can be taken as to be entirely due to a "single hit" process.

The symbols in the above equation are as used previously.

It is assumed that recovery of latent damage during irradiation reduces the possibility of a higher target number to negligible proportions, an assumption which has in fact been confirmed by experiments by Hall (see Shepstone, 1964).

Because of the fact that the relevant cell population kinetics are so complicated, only conditions on the plateau can be considered. This consideration leads to the following argument.

Worded as above the formulation seems to suggest that all cells coming up to division divide successfully, that independently a certain number of cells is removed for differentiation (this number being smaller than the number dividing

if the meristem population is reduced from the normal equilibrium value, so allowing recovery) and that, again independantly, a number of cells is removed from the population by radiation damage, this number being proportional to dose rate. There is no apparent connection between this number of cells removed by irradiation and the number of divisions, i.e. it would seem (without further explanation) to imply an interphase death.

Actually a more detailed consideration leads to a very similar equation, but these steps should be clarified.

Accepting Model A, on the assumptions of which Equation (2.10) is based, the first and third terms of the right-hand side are correct. However, the radiation damage is usually assumed to lead to a mitotic death i.e. cells will be removed from the population in this time interval only out of those coming to division. The significant dose is at present usually assumed to be the total dose collected over the cell cycle. (This may be the whole answer if, for instance, radiation sensivity varies over the cell cycle and different parts of the cycle are affected differently).

Thus of the μI cells coming to division a proportion die in division - not only do these cells not divide and so add to the population as assumed by the first term, but in fact the parent cell itself is lost from the meristem population. The actual fate of the cell is referred to later, but all that matters here is that neither does it produce a daughter nor afterwards itself count in the meristem population I .

Therefore the second term should be $-2\mu I(1 - e^{-\alpha D})$

Now $D = rT$, where r is the dose rate in rads per hour and T is the cell cycle time in hours. If $\lambda_1 D$ is small, this term approximates to

$$-2\mu I \left(\frac{0.6931}{\mu} \right) r \lambda_1 = -1.386 \lambda_1 r I \quad (2.11)$$

It is to be noticed that, as in the original equation, this term does not include μ , i.e. the number of cells being removed from the population per hour is independent of the cycle time. An increase of the cycle time reduces the number of cells coming to division per hour, but at a given dose rate, the dose per cell cycle and (so long as $\lambda_1 D$ is small) the proportion of cells coming to division which die is itself proportional to dose per cell cycle. The proportion of cells dying in division therefore increases by the same factor by which the number of cells coming to division is reduced, and the overall number remains constant depending only on dose rate and not on the cell cycle.

In fact, the approximation used above for the proportion of dividing cells which dies is not very accurate under these conditions. The values are $r = 2.5$ rads per hour and $T = 46$ hours, so that the dose per cell cycle, $D = 115$ rads.

This is not small compared with λ_1 , so that the approximation using the first term only of the exponential expansion is not fully justified under these conditions -

$$(1 - e^{-115/230}) = 1 - e^{-0.5} = 1 - 0.6931 = 0.307$$

compared with the approximation used of $115/230 = 0.5$.

Considering the expression in detail, under the limiting conditions

$$\mu I = 2\mu I (1 - e^{-\lambda_1 D})$$

$$\text{or } e^{-\lambda_1 D} = 0.5$$

$$\text{i.e. } \lambda_1 D = 0.6931$$

$$\text{and } \frac{1}{\lambda_1} = \frac{D}{0.693} = \frac{T_c}{\mu}$$

where T_c is the critical dose rate as given by Hall.

Thus for $D = 2.5 \times 46 = 115$ rads, $\frac{1}{\lambda_1} = 115/0.693 = 166$ rads. To sum up, Hall's equation as stated would apply if the radiation death as an interphase process is proportional to dose rate. This could be postulated, but it is rather unlikely. Considering the death as affecting only cells in division and depending on the dose received per cell cycle, Hall's $r \lambda_1$ should be replaced by $2\mu(1 - e^{-\lambda_1 \frac{0.6931}{\mu} \tau})$. This gives, in fact, without approximation, a value of $\frac{1}{\lambda_1}$ for the parameters stated of 166 rads, identical with his value, although the derivation appears to be different.

The above theory applies only to the critical dose rate at which growth is just prevented, but to reproduce the full growth curve after the start of the irradiation, the kinetics of the meristem cells must be considered in detail. The decrease in the number of cells produced per unit time could be the result of either an increase in the duration of the average cell-cycle or a reduction in the number of cells proliferating, or both. Oliver and Shepstone (1965, 1963) have considered the root-growth of Vicia faba under protracted irradiation (at low dose rates, 1-10 rads/hour) in terms of adaptations of existing theoretical models (Model A and Model B as described below) for explaining the recovery of the meristem from single exposures at high dose rates (Hall, 1962). They demonstrated that the reduced growth rate is in fact, largely the result of a reduction in the number of cells proliferating and that any contribution from an increase in cell cycle is only of second order importance. The latter aspect was investigated as a result of the increases in cell cycle times reported by Clowes and Hall (1962, 1965).

They predicted that if the cell cycle time is varied similar growth-rate curves should be observed for the same dose received per cell cycle, (rather than for the same dose rate). They have also demonstrated that it is imperative to consider the role of sterilized cells in contributing to the observed growth rate, and how this may be the cause of certain reports on the stimulation of growth rate under conditions of chronic irradiation.

The adapted models for the behaviour of the meristem (hereafter referred to as Model A and Model B) under continuous irradiation as described by Oliver and Shepstone (1965) 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 by division over those lost by differentiation so that repopulation of the meristem can take place.

Model A.

This model 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 the normal level.

Model B.

Here an attempt is made to provide the right type of feedback control on the basis of possible biological response to a population change. It is suggested here that in the normal meristem all the cells present themselves for division but an 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 through 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 a depleted meristem, the given supply of this substance provides for more than half the cells reaching division to retain their reproductive integrity and so divide. This proportion increases as the meristem is reduced, thus enabling repopulation to occur.

The mathematical derivations of Model A and Model B are given in Appendix A.

Calculation of the model kinetics under protracted irradiation.

The method of calculation adopted is similar to that which has been used for theoretical consideration of the kinetics of the bone-marrow stem cell system. (Lajtha, Oliver and Gurney, 1962)

It is assumed that the intermitotic cycle time is divided into 10 equal sections or compartments (to be denoted by the subscript (n) in any instance) and the appropriate proportion of the cell population occurring in each of these cell cycle compartments is calculated for the particular model which is assumed to apply. The progress of the group of cells in each cell cycle compartment to the next compartment at each time step can be calculated, considering the necessary changes in numbers as they are transferred from one compartment to the other.

In the case of the unirradiated meristem the change in the number of cells is due to the removal of cells from the population for differentiation.

In Model A, the population is regarded as being in exponential growth, a sufficient proportion of the cells throughout the cell cycle being removed for differentiation per hour to maintain a constant total population, and as a result the number of cells per compartment must vary exponentially through the cell cycle. (Oliver, 1963).

Then if there are say ten cells in the compartment before mitosis, the distribution of cells in each compartment will be initially as set out in Table 22 for $t=0$. The value of 'Q' in the abovementioned table is found from the relation $Q^{10} = \frac{1}{2}$ (i.e. $Q=0.933$). It is the time constant for the exponential distribution of the cells throughout the cell cycle in accordance with the requirements of the model outlined earlier.

Thus a constant proportion, Q, of cells are being lost due to differentiation in each period of one-tenth of the cycle time, corresponding to transfer from one compartment

to the next, to leave a total of say 10 in the compartment prior to mitosis. A number 10 in this compartment will be doubled at division, to give 20 cells. This will be the state of affairs in the meristem at the onset radiation.

When radiation has commenced we proceed to find the number of cells being transferred from each compartment $(n-1)$ to the next compartment (n) at the end of each interval of time (t) equalling one tenth of the cell cycle time T . For the irradiated meristem it is also assumed that a proportion, P , of the cells are damaged per one tenth of a cell cycle. But the sterilized cells only 'die' (and are removed from the population) at division. Under low dose rate conditions it is further assumed that these sterilized cells do not die until their second division after receiving radiation damage. P will then be proportional to the dose rate. For the calculations, therefore, the cells in each compartment must be considered in three groups or categories:

Category A: Those cells that are reproductively intact. (integer)

Category B: Those that are sterilized as a result of radiation damage, but which will be able to undergo one division to produce two 'sterile' cells; and

Category C: These sterile daughter cells which are destined to 'die' and be removed from the population at the next division.

The principle of calculation is then to set out a table as shown in Table 2.2 listing the number of cells in each of the ten cell cycle compartments. In each compartment the cells are to

be listed in three categories, viz. $(A_n)_t$, $(B_n)_t$ and $(C_n)_t$, where n denotes the compartment number and t the time (in steps of one tenth of the cell cycle time). During each one tenth of a cell cycle, the cells in each cell cycle compartment will progress through this compartment to the next (adjacent) compartment.

$(1-Q)$ is the fraction of the cells of the compartment which are removed by differentiation per one tenth of a cell cycle and this will be reduced in proportion to $(F)_t$, the total meristem population as a fraction of the steady state value at the time t after the start of irradiation. The value of F is changing continuously, but its value at the beginning of each time interval is assumed to apply throughout that short interval. Thus the fraction of cells differentiating per one tenth of the cell cycle will be $(1-Q)F$. Therefore in the case of the cells in category A at the time t , the number of cells proceeding from compartment $(n-1)$ to compartment n is given by:

$$(A_n)_t = X_t (A_{n-1})_{t-1} \quad (2.12)$$

where $X_t = 1 - (1-Q)F_t$

There is, however, a further loss due to radiation damage at a rate P per one tenth of a cell cycle, and the number of cells actually reaching A is:

$$(A_n)_t = X_t (1-P) (A_{n-1})_{t-1} \quad (2.13)$$

Transfer of cells from compartment number 10 at the time t to the next i.e. compartment number 1 at the time $t=t+1$ involves the special change at division. Cells must be removed for differentiation and sterilization as before and the

remainder is doubled to correspond to division.

Thus the number of cells proceeding from compartment number 1 at the time t to compartment number 2 at the time $t=t+1$ is given by:

$$(A_1)_{t+1} = X_{t+1} (1-P) (A_{10})_t \quad 2 \quad (2.14)$$

In the case of the cells in category B the damaged cells at the time t viz. $X_t P(A_m)_t$ are transferred from $(A_m)_t$ to $(B_{m+1})_{t+1}$. No cell death takes place in this category because of the fact that in the present instance only death in second mitosis is being considered. Also, the damaged cells in this compartment are considered to be immune from further radiation damage. The appropriate formulae for this compartment are:

$$(B_m)_t = X_t (B_{m-1})_{t-1} + X_t P (A_{m-1})_{t-1} \quad (2.15)$$

$$(B_{10})_{t-1} = 0 \quad \text{for the compartment after mitosis.}$$

$$(B_1)_t = X_t P \cdot 2 (A_{10})_{t-1} \quad (2.16)$$

Equation 2.16 holds because twice the number of cells formerly in B_{10} , are now at the beginning of their second post irradiation cycle and must therefore by definition be in compartment C. Thus the equations for this compartment are:

$$(C_m)_t = X_t (C_{m-1})_{t-1} \quad (2.17)$$

$$(C_1)_t = 2 (B_{10})_{t-1} X_t \quad (2.18)$$

Thus during the first cell-cycle after the start of irradiation (i.e. during the first 10 time steps), there will be no cells in category C in the compartment immediately prior to mitosis (C_{10}).

The fractional size of the meristem at any time t would be:

$$F_t = \frac{\sum_{m=1}^{10} (A_m)_t + \sum_{m=1}^{10} (B_m)_t + \sum_{m=1}^{10} (C_m)_t}{20(Q + Q^2 + \dots + Q^{10})} \quad (2.19)$$

where $\sum_{m=1}^{10} (A_m)_t$; $\sum_{m=1}^{10} (B_m)_t$ and $\sum_{m=1}^{10} (C_m)_t$ represent the sum^o of all the cells in the respective categories at the time t.

The growth rate G (at the time t) as a fraction of controls is therefore:

$$G_t = F_t^2 = \left\{ \frac{\sum_{m=1}^{10} (A_m)_t + \sum_{m=1}^{10} (B_m)_t + \sum_{m=1}^{10} (C_m)_t}{20(Q + Q^2 + \dots + Q^{10})} \right\}^2 \quad (2.20)$$

This would be the growth rate if the cells died at their second mitosis after receiving the necessary lethal radiation effect, and were removed from the population. If, however, such cells were not removed from the population (as may well be envisaged at these exposure levels) but, unable to divide, differentiated and contributed to the growth rate, the growth rate G' as a fraction of controls would be then:

$$G'_t = G_t + \frac{(C_{10})_t}{10} \quad (2.21)$$

It might even be possible that these sterile cells were able to divide, both daughters being able to differentiate and contribute to the growth rate.

In this case equation 2.21 would become:

$$G''_t = G_t + \frac{2(C_{10})_t}{10} \quad (2.22)$$

A Computer program (Appendix D) has been written in FORTRAN IV to calculate G, G', and G'' for a large number of values of P on the IBM 1130 Computer. The flow diagram for the calculation is also outlined in Appendix D.

For Model B, exactly similar considerations can be applied.

In this case the cells are assumed to come up to division,

when a certain proportion retain their reproductive integrity and divide; the remainder, unable to divide, differentiate. The dividing proportion is 0.5 in the normal equilibrium and higher in a meristem of reduced size, thus permitting recovery. Under continuous irradiation a further proportion of the cells, which would be expected to divide, fail to do so successfully, dying a mitotic death.

As before, this means that not only do these cells not produce new cells by division, but they themselves are also removed from the population. It is again assumed that the intermitotic cycle time is divided into ten equal compartments and that there are ten cells in the last stage. In the case of this model there will also be ten cells in each of the other compartments. The same division into integer (A) and two sterile categories (B and C) as described in the previous case are considered and for this model the initial situation will be as shown in Table 2.3 for $t=0$. This arrangement will then account for the fact that in this model there is a linear distribution of cells within the cell cycle.

If F is the fractional size of the meristem, Model B states that only a fraction X_t of the possible $2(A_{10})_t$ (in the case of the integer compartment) will divide per unit time, where

$$X_t = \frac{1 - \exp(-1.595F_t)}{1.595F_t} \quad (2.23)$$

It is again assumed that when irradiation has commenced a proportion P of the cells are damaged per one tenth of a cell cycle. Also, the cells are again considered to die at their second division after receiving the lethal radiation dose.

The equations describing the population kinetics of the meristem under conditions of protracted irradiation on the basis of Model B will therefore be as follows:

$$(A_m)_t = (1-P) (A_{m-1})_{t-1} \quad (2.24)$$

$$(A_1)_t = 2 X_t (A_{10})_{t-1} (1-P) \quad (2.25)$$

$$(B_m)_t = (B_{m-1})_{t-1} + P(A_{m-1})_{t-1} \quad (2.26)$$

$$(B_1)_t = 0 + 2P(A_{10})_{t-1} X_t \quad (2.27)$$

$$(C_m)_t = (C_{m-1})_{t-1} \quad (2.28)$$

$$(C_1)_t = 2 X_t (B_{10})_{t-1} \quad (2.29)$$

The fractional size of the meristem at any time t will be given by:

$$F_t = \frac{\sum_{m=1}^{10} (A_m)_t + \sum_{m=1}^{10} (B_m)_t + \sum_{m=1}^{10} (C_m)_t}{100} \quad (2.30)$$

where $\sum_{m=1}^{10} (A_m)_t$, $\sum_{m=1}^{10} (B_m)_t$ and $\sum_{m=1}^{10} (C_m)_t$ are the sums of all the cells in the respective compartments. The growth rate G , as a fraction of controls is again derived from F :

$$G_t = \frac{1.595F_t + \exp(-1.595F_t) - 1}{0.7975} \quad (2.31)$$

Alternatively it can be shown that

$$G_t = \frac{\{(A_{10})_t + (B_{10})_t + (C_{10})_t\} \{1 - X_t\}}{5} \quad (2.32)$$

Again if we include the cells dying at division with those differentiating, we get an increase in the growth rate given by the following equation:

$$G'_t = G_t + \frac{X_t (C_{10})_t}{5} \quad (2.33)$$

If these cells divide before differentiating we have:

$$G''_t = G_t + \frac{2X_t (C_{10})_t}{5} \quad (2.34)$$

A Computer program (Appendix D) has been written in FORTRAN IV to calculate G , G' , and G'' for a large number of values of P on the IBM 1130 Computer. The flow diagram for the calculation is also outlined in Appendix D.

The fate of dying cells.

To explain the stimulatory effect observed for roots under continuous irradiation (Hall, 1963), as shown in Figure 2.6 formulae were derived for which it was assumed that the cells sterilized by radiation 'die' at their second mitosis after receiving the radiation damage. These cells are then no longer considered to count in the population total and to simply 'vanish'. After large radiation doses, damaged cells can be seen, and these probably collapse and are removed. However under the conditions of low dose-rate exposure another hypothesis was considered - that these sterilized cells, being unable to divide at their second attempted mitosis, did not vanish but differentiated, thus contributing to the observed growth rate. Further it was considered to be conceivable that the sterilized cells were able to carry out a final division to produce two daughter cells, both of which differentiated and contributed to the growth rate. The experimental results by Hall actually show growth rates greater than the normal equilibrium value during the first day after the onset of irradiation. This implies that the stimulation effect is virtually immediate. The formulae above, however, simulate a stimulatory effect only after the first

cell cycle. The calculations were consequently repeated, this time without any cells in category C. This implies that the cells 'die' at their first mitosis after being sterilized by the radiation. The effect on the computed growth rate due to the sterilized cells not dying but differentiating or dividing and differentiating was also considered.

The appropriate changes made to the computer programs, designated to calculate G , G' and G'' with cell death occurring at the second mitosis after sterilization, are discussed in Appendix D.

The effect of the cell cycle time.

In the above formulae, no account has been taken of any effect of the radiation on the length of the cell cycle. However, the experimental results of Clowes (1965) have shown that under continuous irradiation there is a gradual increase in this time for Zea mays, at any rate at the higher and intermediate dose rates.

The computer programs (Appendix D) for the calculation of G , G' and G'' on the assumption that the cell cycle (T) is constant (and that cell death occurs after the first or second mitosis after sterilization) were thus modified to account for a gradual lengthening of the cell cycle during irradiation. These calculations take into account the corresponding increase in radiation damage per one tenth of a cell cycle i.e. the corresponding increase in P . The value of P for the m th cell cycle is then given by:

$$P_m = P_0 + (T_m - 1)P_0 = P_0 T_m$$

where P_0 is the value of P for T constant.

$$T_m = T_{m-1} + \frac{\Delta T}{T_0 + \Delta T(m-1)} \quad \text{with } T_1 = \frac{T_0 + \Delta T}{T_0}$$

ΔT = fractional increase in cell cycle time

= $\frac{\text{Total increase in cycle time}}{\text{Number of cell cycles over which the increase takes place}}$

T_0 = cycle time at equilibrium
(i.e. when no radiation is present)

TABLE 1-1

The duration of the mitotic cycle (T) and the percentage of cells with micronuclei (per cent) after continuous irradiation of the root meristems of Vicia faba and Zea mays for 14 days and 7 days respectively.

Stele 1 is the stele just above the quiescent centre and stele 2 is the stele at 250 μ (for Vicia) or 200 μ (for Zea) from the quiescent centre.										
Dose-rate (rads/hr)	Temp. (°C)	Cap initials		Quiescent centre		Stele 1		Stele 2		
		T	per cent	T	per cent	T	per cent	T	per cent	
0	19	50	..	<u>Vicia</u> 246	..	38	..	32	..	
0.59	"	45	6.6	106	7.7	25	5.1	29	5.2	
1.14	"	71	8.9	77	9.3	31	8.5	28	8.7	
1.52	"	25	9.2	29	7.6	26	8.4	31	8.6	
2.28	"	99	16.0	64	12.3	45	8.6	37	9.1	
0	12	71	..	252	..	62	..	149	..	
0.89	"	107	4.8	75	5.3	41	5.4	64	7.9	
1.52	"	67	19.6	221	16.4	163	14.3	229	13.5	Irradiated 22 days
2.44	"	40	28.7	65	20.7	45	26.6	69	20.6	
0	19	14	..	<u>Zea</u> 174	..	22	..	23	..	Data from Clowes (1965)
2.44	"	209	7.0	144	3.4	48	3.7	47	6.4	
7.57	"	99	6.5	176	4.3	40	7.7	58	6.8	
12.60	"	299	6.3	178	1.5	45	10.9	41	11.1	
16.51	"	3580	8.5	132	3.9	53	8.1	73	7.4	

TABLE 2.2.: SCHEME FOR THE CALCULATION OF G, G' and G".

MODEL-A

Time $t=0$ $Q=0.933$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_0 = 20Q$	$(A_2)_0 = 20Q^2$	$(A_{10})_0 = 20Q^{10}$
Cells in category B	$(B_1)_0 = 0$	$(B_2)_0 = 0$	$(B_{10})_0 = 0$
Cells in category C	$(C_1)_0 = 0$	$(C_2)_0 = 0$	$(C_{10})_0 = 0$

$$F_0 = \frac{\sum_1^{10} (A_m)_0 + \sum_1^{10} (B_m)_0 + \sum_1^{10} (C_m)_0}{20(Q + Q^2 + \dots + Q^{10})}$$

$$G_0 = F_0^2$$

$$G'_0 = G_0 + \frac{(C_{10})_0}{10}$$

$$G''_0 = G_0 + \frac{(C_{10})_0}{5}$$

Time $t=1$ (= 1/10 of the cell cycle)

$$X_1 = 1 - (1-Q)F_0 \quad Y_1 = X_1(1-P) \quad Z_1 = X_1P$$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_1 = 2Y_1(A_{10})_0$	$(A_2)_1 = Y_1(A_{10})_0$	$(A_{10})_1 = Y_1(A_{10})_0$
Cells in category B	$(B_1)_1 = 2Z_1(A_{10})_0$	$(B_2)_1 = X_1(B_{10})_0 + Z_1(A_{10})_0$	$(B_{10})_1 = X_1(B_{10})_0 + Z_1(A_{10})_0$
Cells in category C	$(C_1)_1 = 0$	$(C_2)_1 = 0$	$(C_{10})_1 = 0$

$$F_1 = \frac{\sum_1^{10} (A_m)_1 + \sum_1^{10} (B_m)_1 + \sum_1^{10} (C_m)_1}{20(Q + Q^2 + \dots + Q^{10})}$$

$$G_1 = F_1^2$$

$$G'_1 = G_1 + \frac{(C_{10})_1}{10}$$

$$G''_1 = G_1 + \frac{(C_{10})_1}{5}$$

Time $t=2$ (= 2/10 of the cell cycle)

$$X_2 = 1 - (1-Q)F_1 \quad Y_2 = X_2(1-P) \quad Z_2 = X_2P$$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_2 = 2Y_2(A_{10})_1$	$(A_2)_2 = Y_2(A_{10})_1$	$(A_{10})_2 = Y_2(A_{10})_1$
Cells in category B	$(B_1)_2 = 2Z_2(A_{10})_1$	$(B_2)_2 = X_2(B_{10})_1 + Z_2(A_{10})_1$	$(B_{10})_2 = X_2(B_{10})_1 + Z_2(A_{10})_1$
Cells in category C	$(C_1)_2 = 2X_2(B_{10})_1$	$(C_2)_2 = 0$	$(C_{10})_2 = 0$

$$F_2 = \frac{\sum_1^{10} (A_m)_2 + \sum_1^{10} (B_m)_2 + \sum_1^{10} (C_m)_2}{20(Q + Q^2 + \dots + Q^{10})}$$

$$G_2 = C$$

$$G'_2 = G_2 + \frac{(C_{10})_2}{10}$$

$$G''_2 = G_2 + \frac{(C_{10})_2}{5}$$

TABLE 2.3: SCHEME FOR THE CALCULATION OF G, G' and G". MODEL B

Time $t=0$

$$X_0 = \frac{1 - \exp(-1.595F_0)}{1.595F_0} = \frac{1}{2}$$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_0 = 10$	$(A_2)_0 = 10$	$(A_{10})_0 = 10$
Cells in category B	$(B_1)_0 = 0$	$(B_2)_0 = 0$	$(B_{10})_0 = 0$
Cells in category C	$(C_1)_0 = 0$	$(C_2)_0 = 0$	$(C_{10})_0 = 0$

$$F_0 = \frac{\sum_{n=1}^{10} (A_n)_0 + \sum_{n=1}^{10} (B_n)_0 + \sum_{n=1}^{10} (C_n)_0}{100}$$

$$G_0 = \frac{\{(A_{10})_0 + (B_{10})_0 + (C_{10})_0\} \{1 - X_0\}}{5}$$

$$G'_0 = G_0 + \frac{X_0 (C_{10})_0}{5}$$

$$G''_0 = G_0 + \frac{2X_0 (C_{10})_0}{5}$$

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Time $t=1$ (= 1/10 of the cell cycle)

$$Y = 1 - P$$

$$X_1 = \frac{1 - \exp(-1.595F_0)}{1.595F_0}$$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_1 = 2YX_1(A_{10})_0$	$(A_2)_1 = Y(A_1)_0$	$(A_{10})_1 = Y(A_9)_0$
Cells in category B	$(B_1)_1 = 2X_1P(A_{10})_0$	$(B_2)_1 = (B_1)_0 + P(A_1)_0$	$(B_{10})_1 = (B_9)_0 + P(A_9)_0$
Cells in category C	$(C_1)_1 = 2X_1(B_{10})_0$	$(C_2)_1 = (C_1)_0$	$(C_{10})_1 = (C_9)_0$

$$F_1 = \frac{\sum_{n=1}^{10} (A_n)_1 + \sum_{n=1}^{10} (B_n)_1 + \sum_{n=1}^{10} (C_n)_1}{100}$$

$$G_1 = \frac{\{(A_{10})_1 + (B_{10})_1 + (C_{10})_1\} \{1 - X_1\}}{5}$$

$$G'_1 = G_1 + \frac{X_1 (C_{10})_1}{5}$$

$$G''_1 = G_1 + \frac{2X_1 (C_{10})_1}{5}$$

Time $t=2$ (= 2/10 of the cell cycle)

$$Y = 1 - P$$

$$X_2 = \frac{1 - \exp(-1.595F_1)}{1.595F_1}$$

Cell-cycle compartment-n	1	2	3	4	5	6	7	8	9	10
Cells in category A	$(A_1)_2 = 2YX_2(A_{10})_1$	$(A_2)_2 = Y(A_1)_1$	$(A_{10})_2 = Y(A_9)_1$
Cells in category B	$(B_1)_2 = 2X_2P(A_{10})_1$	$(B_2)_2 = (B_1)_1 + P(A_1)_1$	$(B_{10})_2 = (B_9)_1 + P(A_9)_1$
Cells in category C	$(C_1)_2 = 2X_2(B_{10})_1$	$(C_2)_2 = (C_1)_1$	$(C_{10})_2 = (C_9)_1$

$$F_2 = \frac{\sum_{n=1}^{10} (A_n)_2 + \sum_{n=1}^{10} (B_n)_2 + \sum_{n=1}^{10} (C_n)_2}{100}$$

$$G_2 = \frac{\{(A_{10})_2 + (B_{10})_2 + (C_{10})_2\} \{1 - X_2\}}{5}$$

$$G'_2 = G_2 + \frac{X_2 (C_{10})_2}{5}$$

$$G''_2 = G_2 + \frac{2X_2 (C_{10})_2}{5}$$

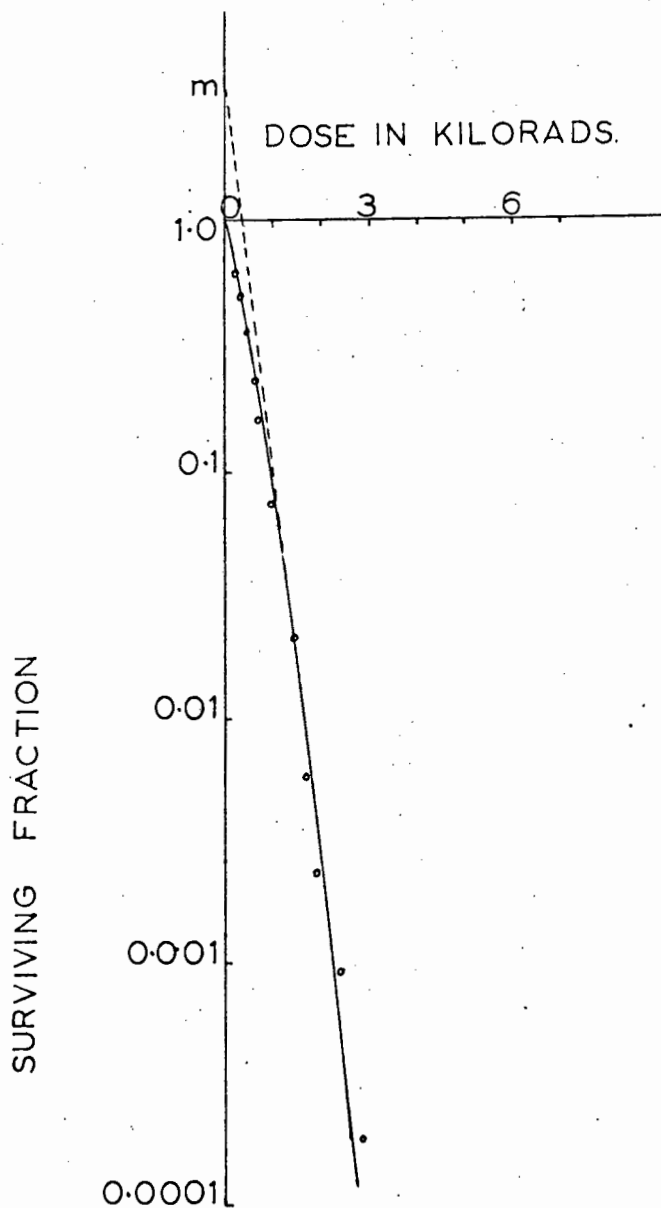


FIGURE 2.1

RESPONSE OF THE ROOT MERISTEM OF ZEA MAYS
TO ACUTE DOSES OF IONIZING RADIATION.
(FROM FENNER, 1970)

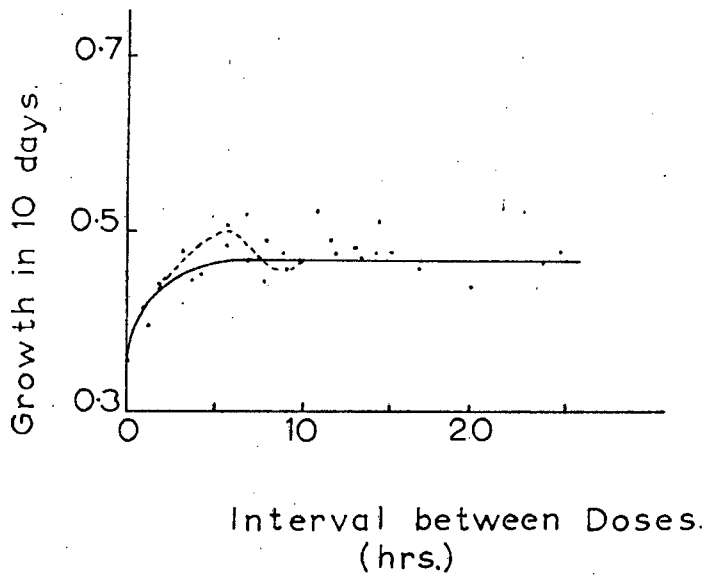


FIGURE 2.2

THE "GROWTH IN TEN DAYS" FOR ROOTS OF VICIA FABA
EXPOSED TO TWO DOSES OF 100 RADS SEPARATED BY
VARIOUS TIME INTERVALS.
(FROM HALL AND LAJTHA, 1963)

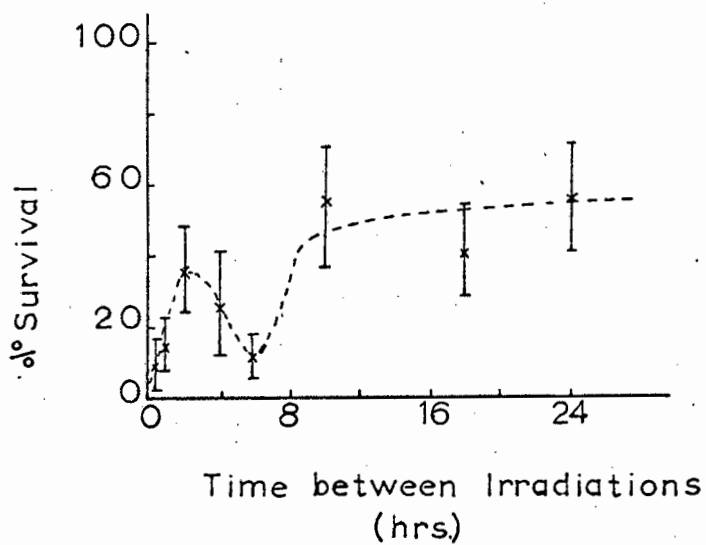


FIGURE 2.3

THE PERCENTAGE OF SURVIVING MICE FOUR DAYS AFTER X-IRRADIATION. THE IRRADIATIONS WERE SEPARATED BY A VARIABLE TIME INTERVAL.
(FROM HORNSEY AND VATISTAS, 1963)

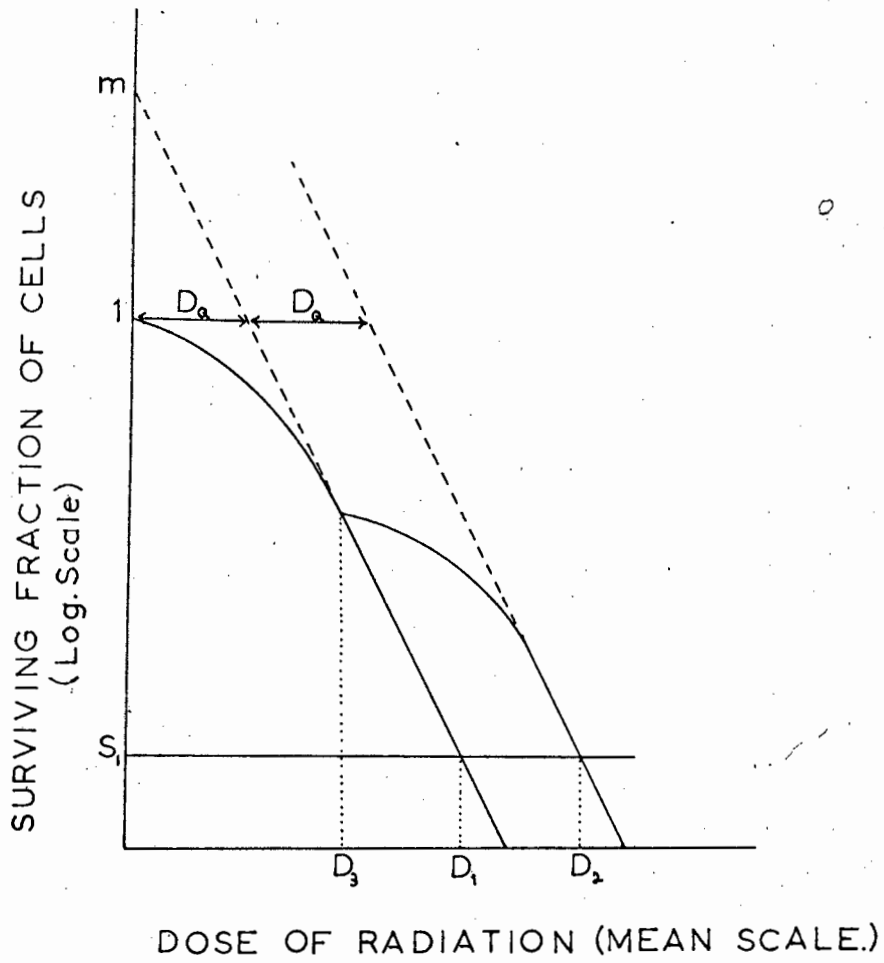


FIGURE 2.4

THE RELATIONSHIP OF D_0 TO THE INCREASE IN TOTAL DOSE NECESSARY TO PRODUCE A GIVEN EFFECT WHEN THE RADIATION IS GIVEN IN TWO DOSES COMPARED WITH A SINGLE DOSE.

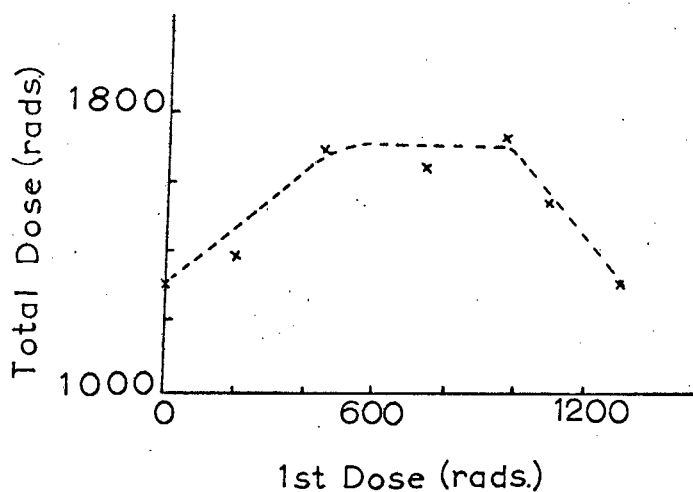


FIGURE 2.5

THE TOTAL DOSE OF RADIATION NECESSARY TO KILL 50 PER CENT OF ANIMALS WHEN THE RADIATION WAS GIVEN IN TWO DOSES SEPARATED BY AN INTERVAL OF SIX HOURS PLOTTED AGAINST THE SIZE OF THE FIRST DOSE.

(FROM HORNSEY AND VATISTAS, 1963)

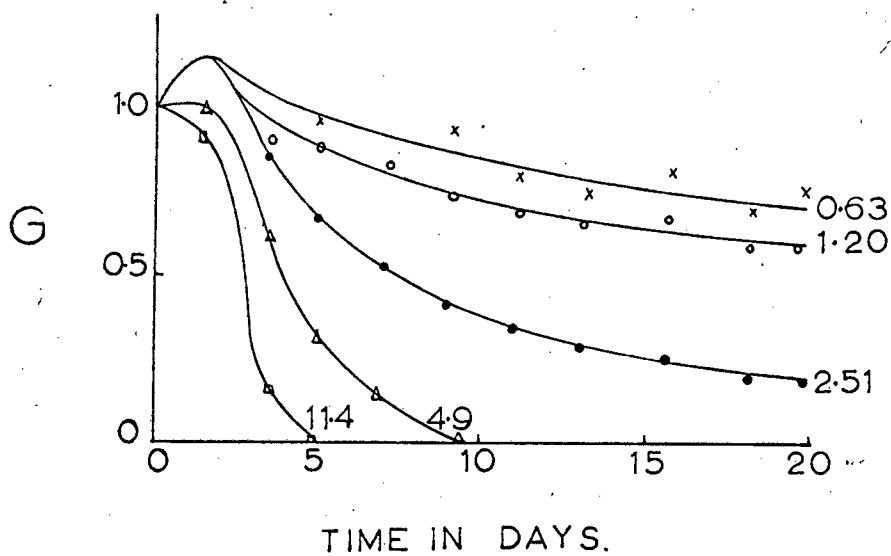


FIGURE 2.6

PATTERNS OF THE DAILY GROWTH RATE AS A FRACTION OF CONTROLS OF EQUAL AGE (G) OF ROOTS EXPOSED TO PROTRACTED IRRADIATION AT DOSE RATES IN THE RANGE 0.63 TO 11.4 RADS/HOUR; THE DOSE RATES ARE INDICATED ON THE CURVES.

CHAPTER IIITHE MORPHOLOGY AND MICROSCOPIC ANATOMY
OF THE ROOT OF ZEA MAYS.

Maize, an annual cereal now known only in cultivation, is almost certainly of Tropical American origin. Bright sunshine, warm days and nights, abundant rainfall and good drainage are all conducive to its welfare. One of the most unfavourable conditions that can be visited upon the plant is to compel it to stand through cold cloudy weather with its roots in a water-soaked soil. The agricultural conquest of the colder latitudes by maize has been accomplished both by a shortening of the growing season and by development of a hardiness to withstand temperatures near the freezing point. But these advantages have been gained at a sacrifice of the size of the individual.

It is a markedly variable plant and can best be described under the comprehensive name given it by Linnaeus, Zea mays.

Maize, a flowering seed-bearing plant (Angiosperm), belongs to the family of monocotyledonous plants technically designated as Gramineae and commonly known as grasses. It is a plant of the tribe Maydeae and is classed as an endogenous plant for the reason that it increases in height and diameter of stem by internal growth.

Morphology.

The corn plant (Fig.1) is a tall, annual grass with a stout, erect solid stem, a fibrous root system, large narrow leaves with wavy margins, spaced alternately on opposite sides of the stem, and male and female flowers are borne on separate inflorescences. In size, shape, colour and external form, the grain of corn is variable, but it constantly possesses three fundamental parts: a tough, dry, membranous covering; an embryonic corn plant; and a reserve food supply.

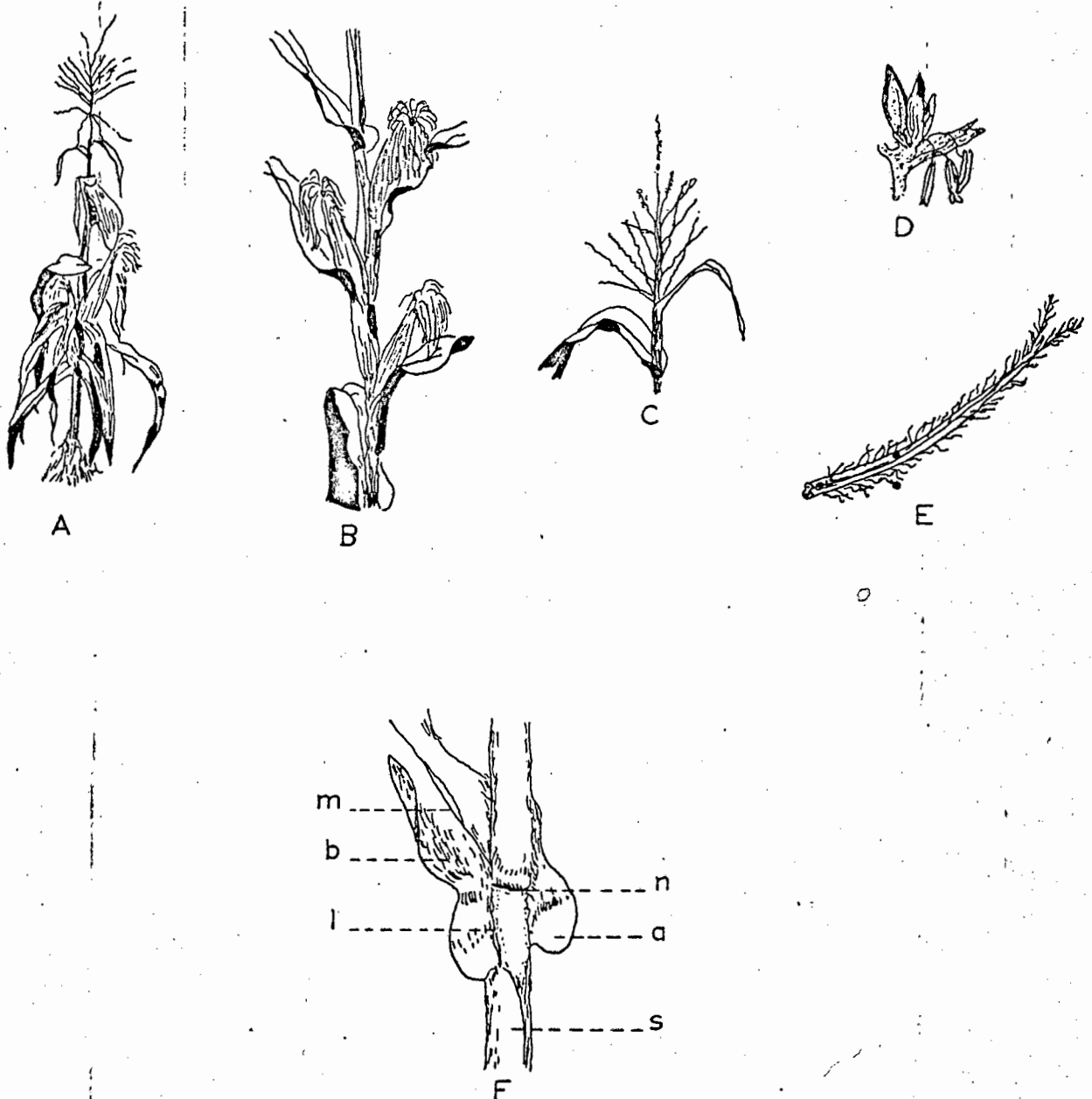


FIGURE 1

- A: Entire maize plant.
 B: Stalk showing female or pistillate inflorescences.
 C: The male or staminate inflorescence.
 D: Staminate spikelet, showing anthers.
 E: Portion of style or silk magnified showing pollen grains caught in hairs.
 F: Parts of the leaf.
 Auricle (a); Blade (b); Ligule (l); Midrib (m);
 Node (n); Sheath (s);

From: Enc.Brit. vol.6 p.449 A.

P.Weatherwax: "The story of the maize plant." p.47

Membraneous covering:

The outer wall of the fruit (Fig. 2C) is called the pericarp (p). At one end of the grain the pericarp is marked by the minute, beaklike base of the silk that was attached during development. At the other end it merges into the chitinous pedicel by which the grain was attached to the cob. The seed inside is invested by a thin yellowish or brownish membrane, constituting the seed coat and is called the testa. Pericarp and testa are both thin and fused together to form a single envelope. An aleurone layer containing protein granules is positioned directly under the testa in the part covering the endosperm.

The embryonic corn plant.

On one of the flat faces of the grain (Fig. 2A) a whitish oval depression marks the position of the embryo (e), the remaining yellow part of the contents of the grain consisting of endosperm (Fig. 2C, en). Two regions are distinguishable in the endosperm, a portion nearest the embryo being white and relatively soft, whilst the remainder is yellow and harder, the "corneous" endosperm.

The embryo (Fig. 2) consists of two peg-like projections, one directed towards the original point of attachment of the grain and presenting the radicle (r), the other placed in the opposite direction and constituting the plumula (pl). The remainder of the white embryo comprises the single large cotyledon (c), the portion in contact with the endosperm being called the scutellum. When viewed under the microscope the plumula and radicle can be seen to be covered by a sheath, the coleoptile (ps) and the coleorhiza (rs) respectively.

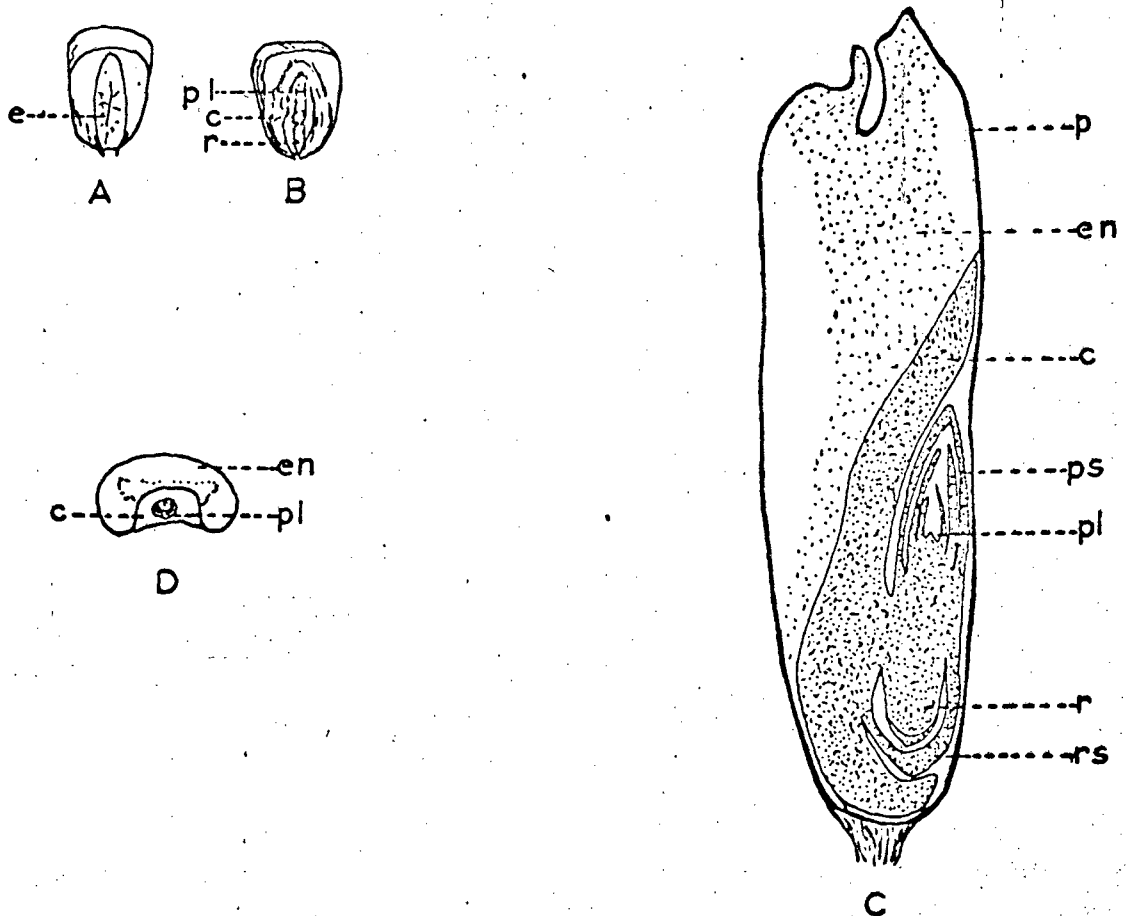


FIGURE 2

Structure of the grain of the maize.

- A: Entire grain showing the outline of the embryo.
 B: Grain with envelope removed and edges of cotyledon folded back to show the plumule and radicle.
 C: Longitudinal section of the complete grain (much enlarged).
 D: Cross-section of grain with envelope removed.
- r= radicle; pl= plumule; c= cotyledon; p= pericarp;
 ps= coleoptile; rs= coleorhiza; en= endosperm; e= embryo.

From: F.E. Fritsch & E.T. Salisbury:
 "Botany for students of medicine and Pharmacology." p.32

P. Weatherwax:
 "The story of the maize plant." p. 32 °

The Germination of the Seed.

The seeds of maize are capable of germination as soon as mature, no after-ripening process or other period of dormancy being necessary. Most grains of corn retain their viability for two or three years.

When a viable seed is surrounded with proper conditions of moisture, temperature and air, germination proceeds by an orderly succession of definite steps.

The first change after the grains are placed under conditions suitable for germination is the imbibition of water by all the cells until they become turgid. As the cells become turgid, the groove over the embryo widens. The coleorhiza enlarges and in some 20 hours breaks the grain coat and protrudes beyond the surface for about 2 mm (Fig. 3A). The radicle at the same time swells and bursts the coleorhiza a few hours later (Fig. 3B). Cell division of its meristem also begins about the time it breaks through. When the radicle is about 2 cm long (Fig. 3C), the plumule pierces the grain coats by its own active growth. All growth of the seedling results from the elongation of the radicle and the plumule. The hypocotyl (Fig. 3E, h) does not elongate. About the same time when the plumule appears, the lateral rootlets begin to develop. The first to appear are the initials which lie right and left of the median plane. Owing to their position, they tend at first to grow upwards between the axis of the embryo and the scutellum. After their emergence they respond to gravity and start downward. Later a third rootlet appears on the face of the seedling above the point of attachment of the scutellum (Fig. 3D). These roots give rise to the main root system of the plant. Adventitious roots are also later formed from

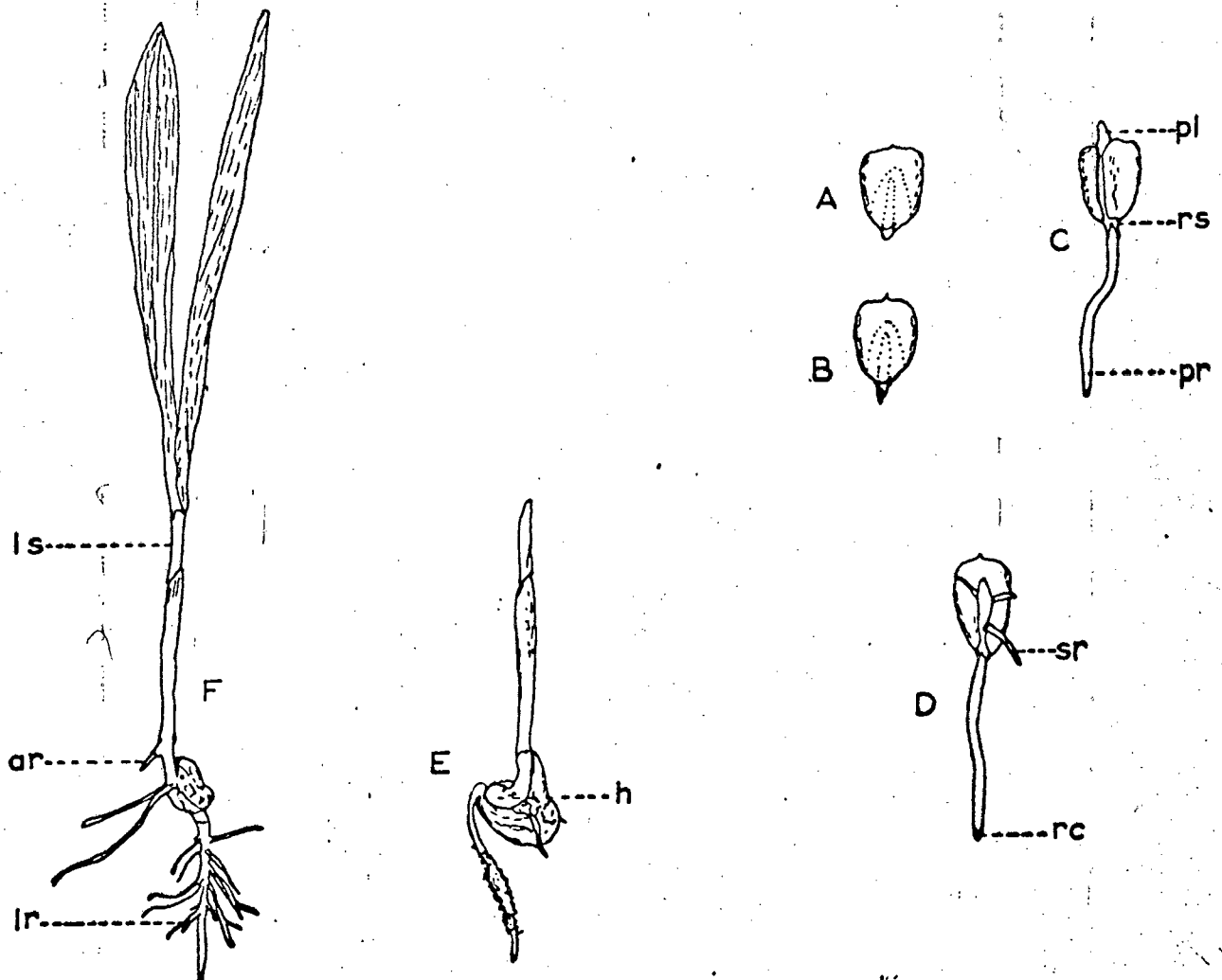


FIGURE 3

The germination of the maize.

A, B, C & D: The different stages during germination of the seed.

E; Late stage in germination showing the escape of foliage leaves from the sheath.

F: Mature seedling.

h= hypocotyl; pr= primary root; lr= lateral root; pl= plumule;
ar= adventitious root; sr= secondary root; ls= leaf sheath;
rs= coleorhiza; rc= root cap.

From: F.E. Fritsch & E.T. Salisbury:

"Botany for students of medicine and Pharmacology." p.32

P. Weatherwax:

"The story of the maize plant." p.35

the succession of closely spaced nodes at the base of the shoot (Fig. 3F). The primary root may give rise to lateral roots but it is usually of short duration. About the time of the first elongation of the radicle, the cells of the epithelium begin to swell and elongate. The surface is also increased by fissures. Later the whole scutellum advances into the endosperm by an enlargement of its individual cells.

When the plumule reaches the surface its inner leaves begin to grow rapidly and, bursting the outermost sheath, become exposed to the light. 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.

Roots.

The roots are fibrous and of three types: Seminal roots, Adventitious roots and Brace or Prop roots.

1. Seminal roots.

The primary root develops from the radicle of the embryo. It appears early and after growing to a considerable length, branches. The secondary seminal roots are typically 3 in number - a pair which develop right and left of the median plane at a point slightly above the insertion of the scutellum; and a third which develops opposite the scutellum. Owing to their position, the median pair first grow upward between the scutellum and the axis of the embryo, thereafter turning sharply downwards. Variation is frequent. An extra root may form above the insertion of the median pair, and also above the third root. Smith and Walworth (1926) found the number of secondary seminal roots to vary between 0 and 10. Except for the last few inches, these roots are always profusely branched.

2. Adventitious Roots.

The adventitious roots develop from the nodes near ground level. They are said to form about an inch below the surface no matter how deep the planting. Not more than four develop in whorls at the succeeding nodes which at the base of the stem are crowded together. (Weaver 1926)

The early-formed adventitious roots (in plants up to 5 weeks old) are 10 to 15 in number. They run almost parallel to the soil surface or at a slight angle and are confined almost entirely to the surface foot of the soil. They vary in length from 0.1 to 2.6 feet, and are branched profusely to near the apices. In older plants they spread laterally for a distance of 2 to 4 feet; they then generally turn sharply downwards for up to 3 feet. The younger roots are more bunched, the spread being only some 10 to 20 inches. They grow almost vertically downwards, the maximum depth reached being 4 to 7 feet.

The colour is either brown or white depending on the age.

In general, maize appears to have an average lateral spread of 3.5 feet, and a penetration of 5 to a maximum of 8 feet.

3. Brace or Prop roots.

At the time of rapid elongation of the stem several of the nodes above the ground level may send out whorls of brace roots. These roots are thicker than the normal and are often deeply pigmented. Their surface is somewhat mucilaginous with the epidermis silicified and the sclerenchyma well developed. On entering the soil they behave as ordinary roots.

Anatomy of the root.

In a longitudinal section of the apex of the root (Fig.4), we

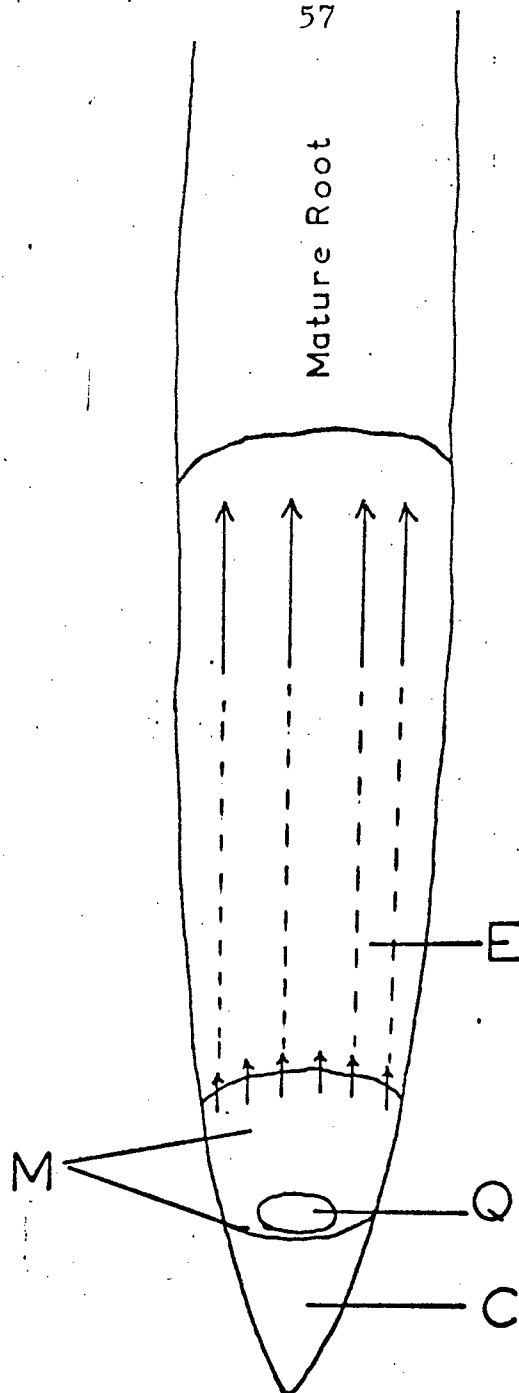


FIGURE 4

The structure of the root.

C = root - cap.

Q = quiescent centre.

M = dividing meristematic cells surrounding the quiescent centre.

E = elongating zone.

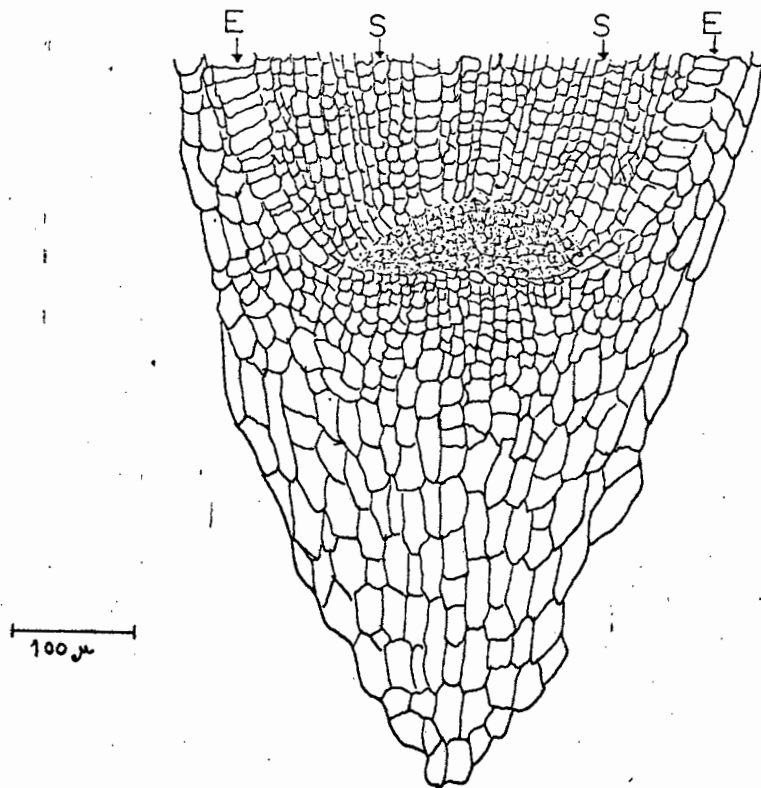


FIGURE 5

Median section of the root apex of *Zea mays* showing the position of the quiescent centre (shaded).

E = epidermis

S = outer layer of stele

(From Clowes, 1959)

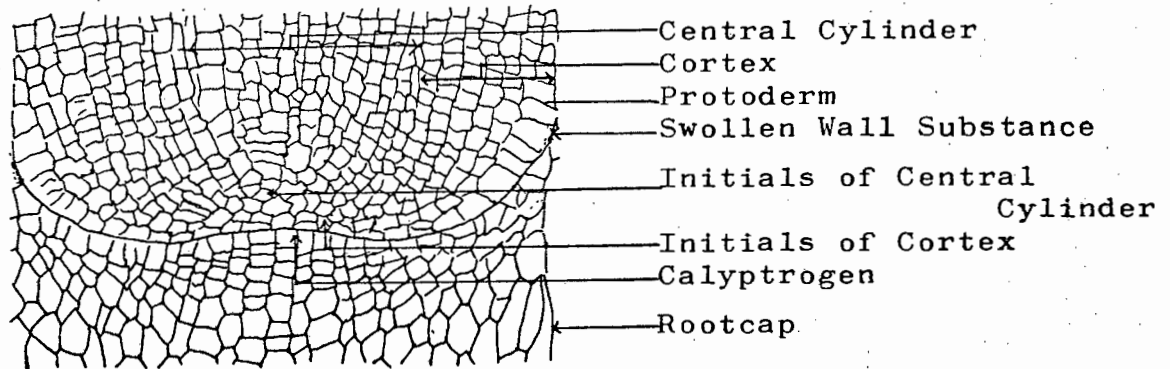


FIGURE 6

Root tip of Zea mays in longitudinal section. The epidermis arises from the same initials as the cortex.

The swollen wall substance originates through the gelatinization of the wall between the rootcap and the protoderm, a phenomenon associated with the sloughing of the root cap.

From: Esau: "Plant Anatomy." p.117

find on the outside the root cap, a covering layer of somewhat elongated parenchymatous cells, whose surface is constantly being worn away, but whose mass is kept constant by additions on the inner face from the meristem, the latter being the principal seat of formation of new cells as the root grows.

Clowes(1954) has demonstrated the existence in the meristem of a quiescent centre(Fig.5), a region consisting of about 600 cells which divide very infrequently, if at all.

Passing upwards the meristem differentiates into periblem and plerome.(Fig.7) The periblem resolves itself into:

(1) the piliferous layer on the outside, (2) a cortex of several layers and (3) the endodermis, the innermost layer of the cortex.

The plerome becomes the central cylinder or stele. Its outermost layer in contact with the endodermis is the pericycle. Some distance within 6 to 8 cells are recognizable owing to their size. As they pass upwards they differentiate into 6 to 8 primary vessels. Between and behind these cells, smaller vessels are also differentiated. Between each of these is the scanty phloem. This region of the root is termed the "elongating zone", because the cells here 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. The elongating zone extends to about 4 mm. from the end of the root and after this there is no sign of division. The remainder of the root is composed of mature cells which are fully elongated.

A section, a few centimetres from the apex, (Fig.8), shows the same tissues; (1) the cortex bounded by the piliferous layer to the exterior and the endodermis to the interior. This endodermis is thickened on its inner and radial walls. (2) The central cylinder. The outermost layer is the pericycle, a continuous band of

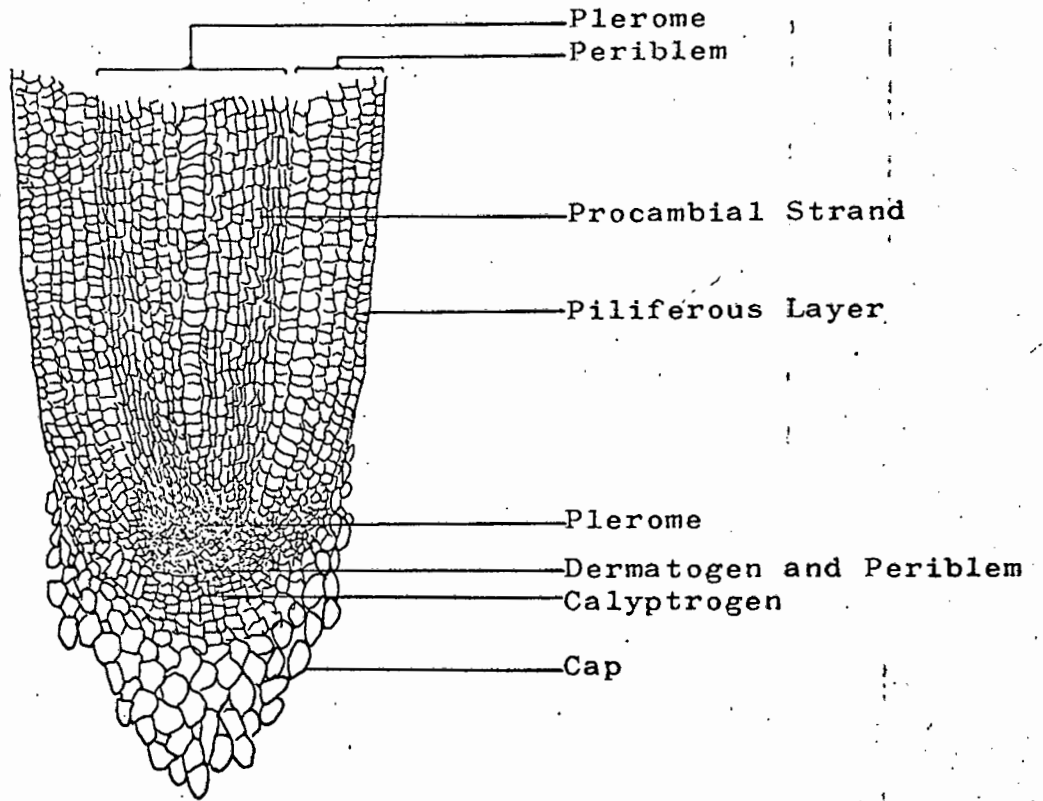


FIGURE 7

The root apex of a monocotyledon.

From: Lawson, 'Textbook of Botany.'

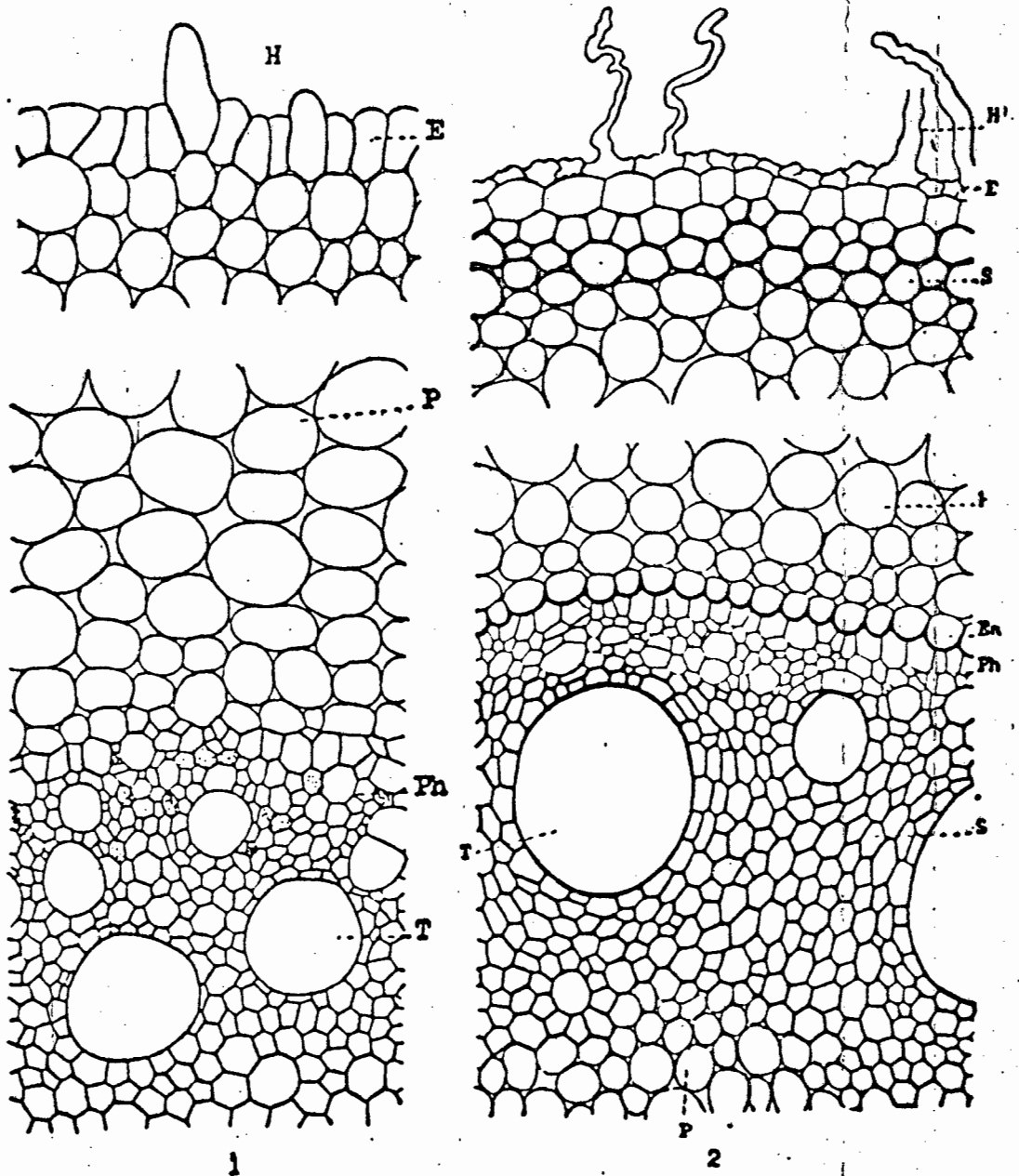


FIGURE 8

1. Portions of a cross-section of a young root.

H= root hair; E= epidermis; P= parenchyma; Ph= phloem;

T= trachea.

2. Portions of a cross-section of an old root.

H= root hair; E= epidermis; S= sclerenchyma; P= parenchyma;

En= endodermis; Ph= phloem; T= trachea.

From: P. Weatherwax: "The story of the maize plant."

small cells adjoining the endodermis. Beyond this are alternate bands of xylem and phloem, the number varying somewhat. The phloem consists of 3 to 4 sieve tubes; the xylem of small vessels, with annular or spiral thickening; and the centripetally-formed vessels which develop later and are generally wider and pitted. The interior is filled with conjunctive tissue. In this tissue, however, the 6 to 8 large vessels already referred to occur. They lie opposite certain of the xylem strands. The large vessels are primary and are formed almost simultaneously with the protoxylem. When elongation has ceased the root hairs appear. A fully developed root hair is a cylindrical elongation of a single epidermal cell, rendered more or less irregular by the pressure of particles of soil around it. (Fig. 8) They are generally short-lived, but as the older ones die, fresh ones are continually being produced near the apex as long as it is actively growing.

Development of lateral roots: Normal root-branches are developed entirely from the pericycle. The cortical tissue of the parent-root takes no part in the formation of the tissues of the lateral branch. The development begins some little distance behind the elongating zone of the parent-root. When development begins, two or more cells of the pericycle divide i.e. become meristematic and produce a growing point, which soon shows a distinction into root-cap, periblem and plerome. The young branch-root gradually elongates, stretching and then breaking the endodermis and overlying cortical tissue till it reaches the surface of the parent-root. The structure of the lateral root is identical with that of the parent-root and the conducting tissues of the former make contact with those of the latter. Adventitious roots are developed similarly.

Transition from root to stem:

The vascular system is continuous in root and stem. It is evident that transition from the arrangement characteristic of the root to that characteristic of the stem is effected in that region of the axis which lies between typical stem and typical root. This region is the hypocotyl.

The development of roots and the concept of initial cells.

In root apices the cells are arranged in patterns which reflect their origin. Attempts have been made to interpret these patterns to show how a root is constructed in its ontogeny. The most important of the limitations of such analyses in Angiosperms is the identification of initials, the cells which initiate new tissues. In many ferns a single "apical cell", which initiates all tissues, has been discovered in the root meristem.

In Angiosperms it was often thought that there were several initials constituting the permanently meristematic cells or promeristem; but the size and shape of the promeristem was controversial. By following the pattern of cells in Zea mays, Clowes (1954) derived a so-called minimal constructional centre.

(The minimal constructional centre is the minimum number of cells needed to maintain the constructional pattern within the apex.)

Clowes (1959) suggested that in the case of Zea the "initials" were situated around the surface of the "quiescent centre" - the cells of which divide very infrequently, if at all, under normal circumstances. These studies were the direct result of researches based on the so-called Koerper-Kappe theory (Schuepp, 1917), which describes the planes of cell division by an analysis of the pattern of the 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. Investigations have shown that a combination of the Koerper-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 the cortex, while those below initiate the root-cap.

It was generally assumed at first that the minimal constructional centre was identical with the promeristem. Guttenberg and his associates believed this assumption to be correct and reduced the size of the promeristem to a single "central cell" or to a few initials (Guttenberg, 1947; Schade and Guttenberg, 1951) from which cell 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. Thus the central cell can function, like the apical cell of a fern, throughout the growth of the root, or it can function only in the embryo or primordium and give rise later to a multicellular promeristem in the root. Guttenberg originally seemed to favour the former interpretation but later stressed the latter one.

(Guttenberg, Heydel and Pankow, 1954; Guttenberg, Burmeister and Brossel, 1955).

Guttenberg's views were supported by Brumfield (1943) who induced chromosome aberrations in young radicles of Vicia faba 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 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 radicle. In other words Brumfield's conclusions should apply to embryos rather than to mature plants.

Surgical experiments were designed by Clowes (1953, 1954) on Zea 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.

The large promeristem was also confirmed by determination of the relative rates of nucleic acid and protein synthesis, (Clowes, 1956 a, b; 1958 b) the rates of mitosis of the cells in the apex both from continuous and pulse labelling of nuclei with tritiated thymidine (Clowes 1965) and also from the accumulation of phases of mitosis blocked by inhibitors (Clowes, 1961 a).

From this it emerged that the cap initials of Zea consist of a disc of about 150 cells lying next to the quiescent centre, and that they maintain the highest rate of division in the root. (Table 1). There is also evidence that they maintain a higher rate of D.N.A. synthesis than is necessary even for their high rate of mitosis (Clowes, 1968 c). This high rate of mitosis of cap initials does not occur in species with differently organized meristems, as in the case of Allium sativum (Thompson and Clowes, 1968) and Vicia faba (Clowes and Hall, 1962).

The cap initials give rise to the differentiated cells of the cap. There may be up to 2000 dividing cells in the cap (Clowes, 1968, a). The rest of the meristem in Zea adds about 100000 cells a day to the length of the root. The concept of initials in these other parts of the root is more nebulous because of the problem of the quiescent centre occupying the poles of the stele and cortex.

A diagram of the root tip of Zea, demonstrating the three tiers of initials in the initial zone, is given in Fig. 6 and 9. One gives rise to the central cylinder; the second to the cortex; the third to the root cap. The epidermis differentiates from the outer layer of the cortex.

Davidson (1960, a) 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 cell contributed to the formation of the lateral root primordium. In fact, the actual number involved was likely to be greater than six. This result implies that six different kinds of cell

TABLE 1

Rates of mitosis in regions of the root meristems of four species.

The figures are average durations of a mitotic cycle in hours.

From: Clowes,

"Anatomical Aspects of Structure and Development." 1968

	Quiescent centre	Cap initials	Stele just above QC	Stele 200-250 μ m above QC	Method	References
Zea mays	174	12	28	29	Metaphase accumulation Pulse labelling	Clowes, 1961c Clowes, 1965
	167	14	22	23		
Vicia faba	292	44	37	26	Metaphase accumulation	Clowes & Hall, 1962
Sinapis alba	520	35	32	25	Metaphase accumulation	Clowes, 1962
Allium sativum	173	33	35	33	Metaphase accumulation	Thompson and Clowes, 1968
	173	27	26	26	Pulse labelling	Thompson and Clowes, 1968

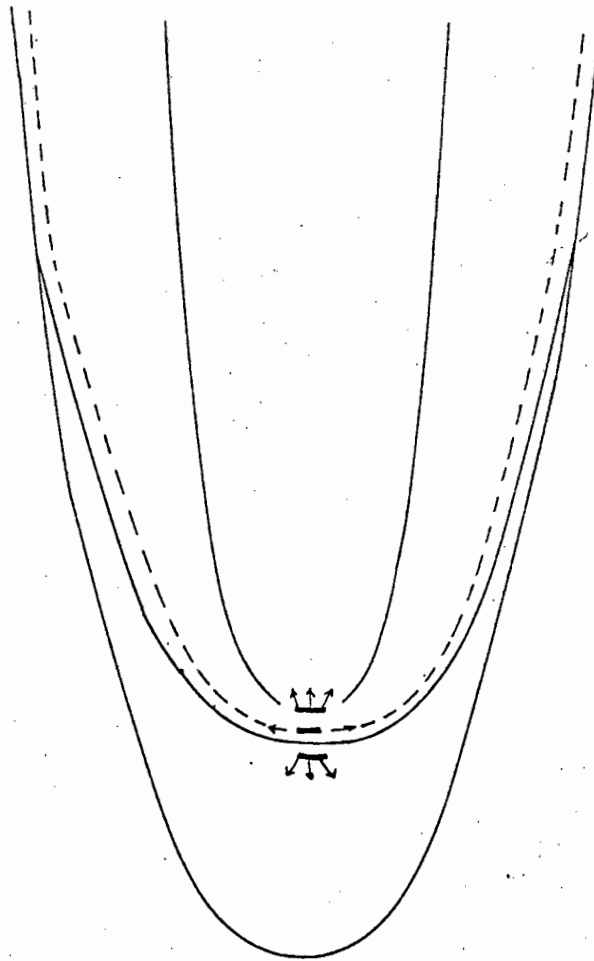


FIGURE 9

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.

exist together within a very small region of the mother root - a very different result from that of Brumfield. Davidson (1960,b) 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. 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 control of differentiation.

Bunning(1952) cut off the apical 2mm 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 mitosis was present every day from 1 to 10 following 140 r 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 of elongating cells 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.

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, 1956 a, b). The constituent cells of the quiescent centre are carried forward passively by the growth of the surrounding meristem. They are quiescent only because of their position within the apex and not because of any inherent disability.

The presence of a quiescent centre to the root meristem of Zea mays has been demonstrated experimentally by Clowes (1956 a, b; 1958 b). The method involves a comparison of the rates of nucleic acid synthesis among the cells of the apex. Comparative rates of synthesis of deoxyribose nucleic acid (D.N.A.) can be determined by autoradiography of nuclei fed with a radioactive precursor of the nucleic acids. In the original experiments on Zea, phosphate labelled with P-32 or adenine labelled with carbon-14, was supplied to the roots. It is now usual to use tritiated thymidine for this purpose (Clowes, 1968 a) because the synthesis of DNA is directly coupled with mitosis.

In the autoradiographs prepared from 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. In the case of Zea, the boundary in the autoradiographs between the quiescent centre and the distal cells of the meristem is very sharp (Fig.5).

On the proximal side, in the stele and cortex, the quiescent centre gradually merges into the meristem. The quiescent centre consists of about 600 cells in a hemispherical volume surrounded by the meristem of app. 125000 actively dividing cells. (Clowes, 1968 b)

The distal neighbours of the quiescent centre cells are the initials of the cap, and, as the cap is discrete from the rest of the root, the sharp boundary between its initials and the quiescent centre was as expected. But this sharp boundary is also found in roots whose cap cannot be considered to be discrete as in the case of Vicia faba (Clowes, 1959). Here the quiescent centre consists of about 1000 cells in a hemispherical volume (Fig. 10) surrounded by app. 250000 meristematic cells. (Clowes, 1968 b). The root apices of Vicia show no clear anatomical boundaries to the histogens. The cell patterns have been interpreted in two ways in this case (Reeve, 1948; Neumann, 1939). There is quantitative data about the rates of mitosis of the cells in the apex of Zea, both from continuous and pulse labelling of nuclei with thymidine (Clowes, 1965) and from the accumulation of phases of mitosis blocked by inhibitors (Clowes, 1961 a). These show that the cells in the quiescent centre divide some ten times slower than the adjacent cells on either side. (Table 1).

Not only is the mitotic cycle especially long in the quiescent centre and especially short in the adjacent cap initials (Clowes, 1967), but these anomalies are due to extending the G_1 period (the part of the mitotic cycle between mitosis and the following DNA synthetic period) in the quiescent centre and eliminating G_1 in the cap initials. (Clowes, 1965, 1963 c).

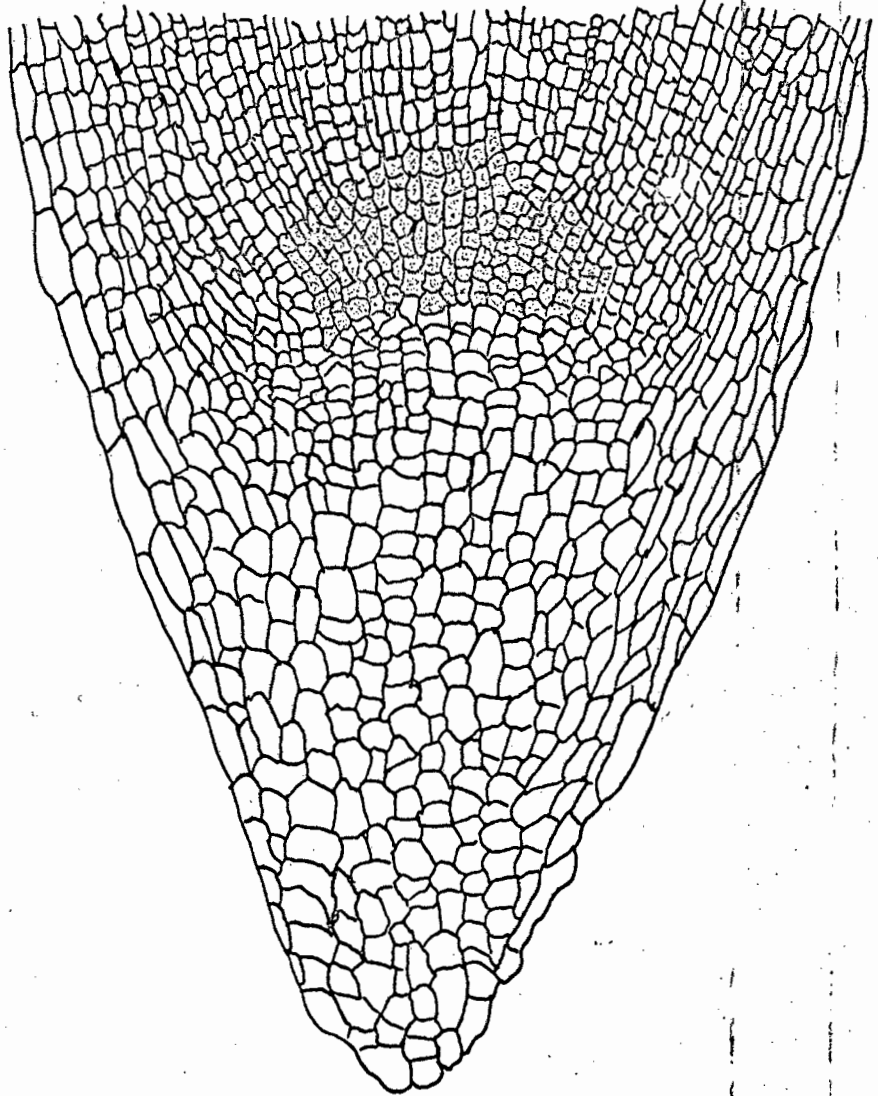


FIGURE 10

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

(From Clowes, 1959)

The actual durations of the phases in hours are listed in Table 2.

The negative value of G_1 for the cap initials of Zea indicates that DNA synthesis starts before the end of the previous mitosis in some of the cells. The difference in the rate of mitosis of the cap initials and cells of the quiescent centre is not as marked in other species e.g. Vicia (Table 1).

As well as having the lowest DNA content, the cells of the quiescent centre have the lowest content of both cytoplasmic and nucleolar RNA, the lowest rate of protein synthesis, the smallest Golgi bodies, the smallest nucleoli, the fewest ribosomes and the fewest mitochondria (Barlow, 1968; Clowes, 1956a; 1958a; Clowes and Juniper, 1964; Hyde, 1967; Pilet and Lance-Nougarede, 1965).

The microdensitometer shows that in Zea the mean DNA content in the quiescent centre is 7.7 units and in the cap initials is 12.6 units (Clowes, 1968c).

Clowes (1959, a) has shown that the cells of the meristem of Zea mays 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. The cells of the quiescent centre thus 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 meristem and the quiescent centre. Clowes (1970) actually observed that if the cells of the quiescent centre are given 1800 rads of X-rays respond at once by coming into mitosis although most are at G_1 before irradiation.

TABLE 2

Durations in hours of the phases of the mitotic cycle determined by pulse labelling in different regions of root meristems.

S is the period spent in synthesizing DNA,

G₁ and G₂ are the periods of interphase before and after S.

M is the period spent in mitosis.

From: Clowes,

"Anatomical Aspects of Structure and Development." 1968

		G ₁	S	G ₂	M
Zea mays	Quiescent centre	151	9	11	3
	Cap initials	-1	8	5	2
	Peripheral cap meristem	7	8	4	2
	Stele just above quiescent centre	2	11	7	2
	Stele 200 μ m above quiescent centre	4	9	6	4
	Stele 400 μ m above quiescent centre	12	5	3	3
	Allium sativum	Quiescent centre	142	17	8
Cap initials		3	13	6	5
Stele just above quiescent centre		4	12	4	6
Stele 200 μ m above quiescent centre		4	12	4	6

He also found that there is a stimulation of DNA synthesis in the quiescent centre, but not elsewhere immediately after irradiation.

Because of this behaviour of the normal root meristems, Clowes (1959) maintained that it is not legitimate to argue about the behaviour of normal root meristems from chimeras induced by irradiation. 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 may 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 differences in sensitivity is based on the view that the quiescent centre is anoxic.

Hall, Lajtha and Clowes (1962) have, however, shown in the case of Vicia faba 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.

The difference of radiosensitivity of the cells in the meristem was further confirmed by a different way of irradiating the meristem, this time by using the soft beta - rays from the disintegration of tritium-atoms to irradiate the dividing cells, but not the quiescent centre cells (Clowes, 1961, b).

The fact that a dose of X-rays to all cells throughout the apex had the same effect as the beta - particles confined to the meristem outside the quiescent centre led to the investigation of relative radio-sensitivity in the different regions of the meristem. Clowes found that the quiescent centre was less sensitive and the cap-initials more sensitive than the meristematic cells of the stele. (Clowes 1963, a, b, c; 1964, a; Clowes and Hall, 1966).

This, combined with the determination of the time parameters of the mitotic cycle, leads to a general explanation of the empirical fact that cells that divide rapidly suffer more radiation damage than cells that divide slowly. Clowes feels that these differences are likely to be due to the balance in length between G_1 and the rest of the mitotic cycle since this balance controls the average size of the target presented to the radiation. It could be that for this reason that the cap initials (which are mostly at S or G_2) in Zea are hypersensitive and that the quiescent centre in all roots is relatively resistant enabling it to provide the most probable site of regeneration when the meristem is injured by radiation.

A somewhat similar point has been made by Van't Hof and Sparrow (1965) comparing the overall radiosensitivity of dormant and active roots of Tradescantia. Here the average nuclear volume in dormant roots is $491 \mu^3$ compared with $733 \mu^3$ in active roots. They found that the X-ray dose required to reduce root growth

to 37 per cent of the former rates was 305 rads in dormancy and 184 rads in activity. Bearing in mind the difference in average nuclear size, they calculated that the energy absorbed per nucleus is very similar for the two doses that reduce growth by similar percentages.

The possession by the root apex of a population of cells in G_1 and a population in G_2 resembles the situation postulated by Gelfant (1962,1963) for mammalian systems.

The original observation here was that in mouse ear epidermis the cells appear to stay in G_1 until some event restarts the mitotic cycle and yet some cells reach mitosis very quickly after a stimulus suggesting that they had rested at G_2 and could proceed immediately to mitosis.

Gelfant considered that there are two genetically discrete populations one with G_2 of less than six hours and the other with G_2 longer than two days.

CHAPTER IVTHE PROBLEM STATED.

The problems studied in the present series of investigations in cell population kinetics can be classified according to whether the radiation is applied continuously over a period of time or given in fractions of acute doses.

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 Model A and Model B) were adapted by Oliver and Shepstone (1965) to explain the behaviour of the root meristem under conditions of protracted irradiation as observed by Hall (1963).

Computer programs will be written to calculate these theoretical growth curves for conditions of protracted irradiation. Chronic irradiation of Zea roots at 25°C will be carried out in order to provide experimental curves for comparison with the computer calculated results. Of importance in the analysis of these results is the fact that the intermitotic cycle time of the meristematic cells increases under conditions of chronic irradiation (Clowes and Hall, 1962, 1965). The influence of this effect on the theoretical response of the models will be considered. Another important factor is whether sterilized cells are removed immediately after irradiation, or whether they remain and contribute to the differentiated population and thus account for a possible

stimulatory effect of the radiation on the cells. This possibility will also be included in the theoretical calculations and the latter compared with the experimental results.

From these comparisons a value of D_1 , which is the 37 per cent survival dose for the exponential curve corresponding to the "single hit" process which is believed to apply under these low dose rate conditions (Barendsen 1962, Hall 1963, Hall and Bedford 1964, Oliver 1964), will be found.

The characteristic shape of the survival curve for the cells in the root meristem of Zea mays exposed to X-rays is sigmoid (Shepstone, 1964; Fenner, 1970); this implies a threshold type of response, in which damage must be accumulated before its effect becomes apparent. Elkind and Sutton (1959) found that sublethal damage in mammalian cells was repaired within a period of about 12 hours. Many workers (Hornsey and Vatisas, 1963; Hall and Lajtha 1963), inspired by this report, have sought to repeat with organized systems what has been demonstrated so conclusively with cells in culture. Such an attempt will be described in the present experiments with a relatively simple organized system, the root meristem of the maize plant, Zea mays.

(Elkind and Sutton 1960, Berry and Oliver, 1964) have demonstrated the presence of a "kink" in the graph of survival as a function of the interval separating the divided doses in experiments on mammalian cells in culture. Hall and Lajtha (1963) have found a suggestion of this "kink" in the recovery curves for bean roots.

A search will be made for it in the present experiments on

Zea.

From the split dose experiments the value of the quasi - threshold dose (D_Q) will be found and used to calculate a value of D_0 using the formula:

$$D_Q = D_0 \ln (m) \quad (4.1)$$

where m is the extrapolation number and D_0 the 37 per cent dose slope for the cell population under consideration (in this case the meristematic cells in the root tip of Zea mays), found by irradiating the cells to single acute doses (Shepstone, 1964; Fenner, 1970).

CHAPTER VMATERIALS AND METHODS USED IN THE STUDY
OF THE ROOT MERISTEM OF ZEA MAYS.

In the past, research workers have found great difficulty in obtaining a hardy variety of maize suitable for their radiobiological experiments. On the advice of the Agricultural Department of the University of Stellenbosch the variety known as 'Kalahari Blitz' was tried and great success has been achieved in this respect. This was also the only variety that could be obtained in bulk, uncontaminated by other varieties, and the continued supply of which could be guaranteed.

Culture methods

The method of culture employed was that used by Hall, Lajtha and Oliver (1962) for Vicia faba.

For each individual experiment, approximately 500 seeds were placed in a polythene bowl, to which water at $\pm 25^{\circ}\text{C}$ was continuously added. After two or three days the seedlings which had germinated were planted in moist horticultural Vermiculite contained in a large brass tank. This Vermiculite had previously been autoclaved at $\pm 126^{\circ}\text{C}$. This growing tank was maintained at a relatively constant temperature in the neighbourhood of 25°C .

The seeds were pressed into the Vermiculite with the broad upper part of the cotyledon facing the surface of the Vermiculite, to a depth of approximately 1 cm. In this way about 200 seeds could be accommodated. When the subsequent

root growth caused the cotyledon to lift, more Vermiculite was added and the covered seedling pressed down gently. The seedlings were grown in this way for four days with frequent moistening of the surface. After this period, the majority of the seedlings had developed a primary root between 4 and 8 cm. long and in many cases adventitious roots had also developed. (See Fig. 5.1)

The seedlings were then removed from the Vermiculite, washed in water and the plumule and adventitious roots 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. All damaged and malformed roots were discarded and, from those remaining, enough were chosen, with roots of 4 cm length and over, to be placed in the Perspex lid of the tank, with their roots passing downwards through the holes into the water (Fig. 5.2).

The Perspex culture tank was rectangular in shape, 3 ft. x 1 ft. and 1 ft. deep, with a steady flow of tap water passing through at the rate of about 1 litre per minute. The temperature of the water in the culture tank was maintained at $25^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ by a Braun thermostatically controlled heater, which also incorporated a vigorous stirrer and thus also effectively aerated water. Gray and Scholes (1951) reported that a change of 1°C in the temperature of the water resulted in a 20 per cent change in the growth of the roots, so it was felt that meticulous control of temperature was necessary.

The selected seedlings were allowed to grow for 24 hours in the culture tank. The cotyledons were covered at all times

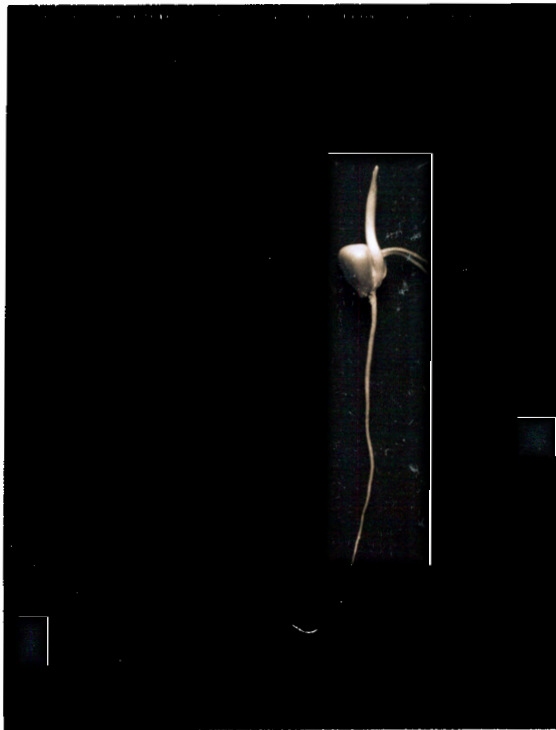


FIGURE 5.1

THIS SHOWS A SEEDLING OF ZEA MAYS WITH SOME
ADVENTITIOUS ROOTS GROWING FROM ABOVE THE
JOIN OF THE PRIMARY ROOT AND HYPOCOTYL.

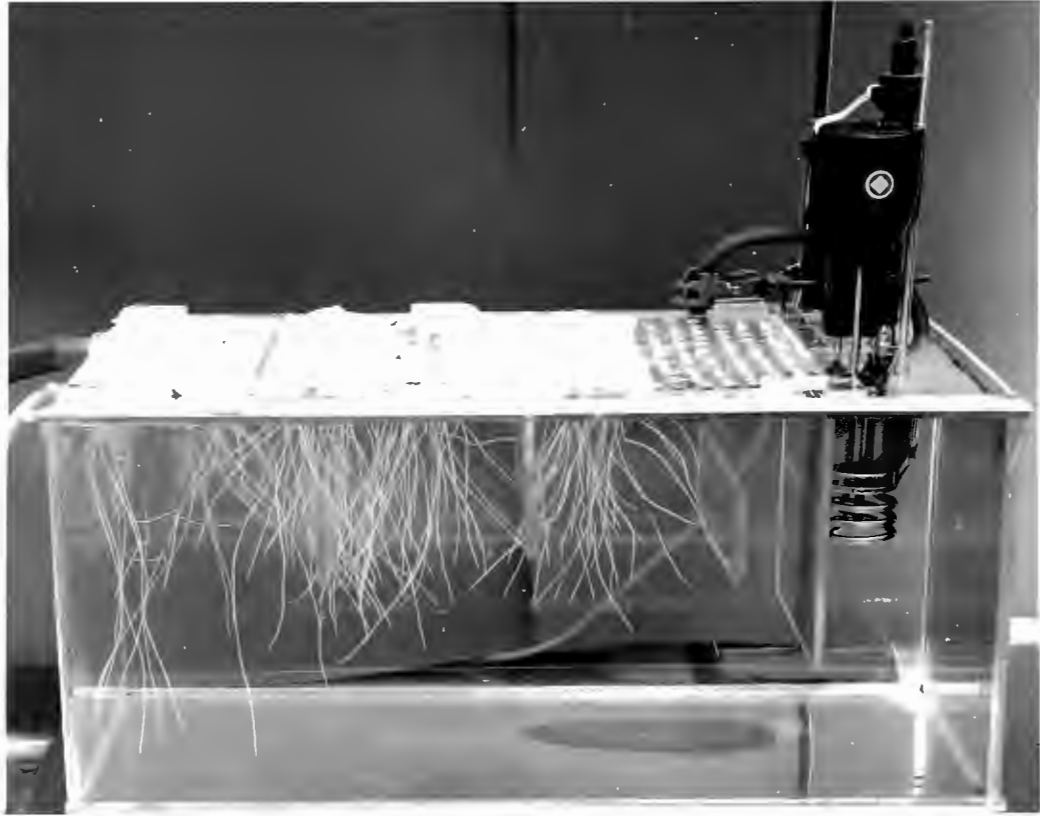


FIGURE 5.2

VIEW OF THE MAIN CULTURE TANK SHOWING HEATER, INLET-OUTLET POINTS AND THE PERSPEX LID HOLDING SEEDLINGS OF ZEa, SOME OF WHICH HAVE BEEN UNCOVERED (SEE TEXT) TO SHOW THEIR POSITIONS.

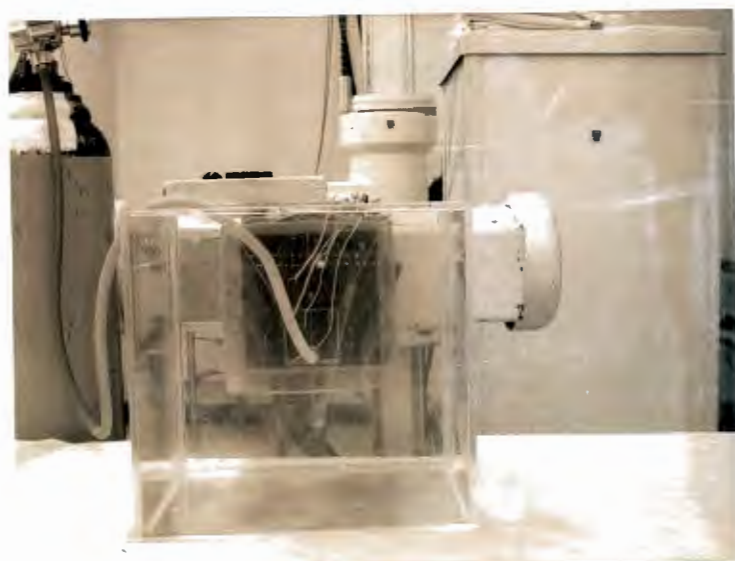
during the experiment with moist surgical gauze to prevent their dehydration. The seedlings were then examined and all those with malformed or stunted growth were discarded.

The remainder were divided at random into one group of 20 to serve as controls, and as many groups of 14 (there were 14 holes across the tank, hence this was a convenient number) 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. The position of the seedlings was marked by numbering of the holes in the Perspex lid.

There has been some controversy in the literature as to whether it is necessary to culture the maize in the dark to avoid the establishment of a rhythm of cell division. In the case of Vicia faba, Mottram (1913) regarded this as particularly important, because he thought dividing cells were more sensitive to radiation than the remainder of the root, and claimed that more damage was done to roots irradiated during the night when mitotic activity was at a maximum. Read (1959) failed to reproduce these findings 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. The equivalent investigations have not been performed in the case of Zea, but it is assumed that its behaviour will resemble that of Vicia. Consequently in all the experiments to be described, no attempt was made to regulate light conditions, but shoots were removed daily at the same time as the adventitious roots.



A



B

FIGURE 5.3

A: VIEW OF EXPERIMENTAL ARRANGEMENT.
B: VIEW OF EXPERIMENTAL ARRANGEMENT
ALONG THE AXIS OF THE BEAM.
THE LEAD SHIELD FOR THE COTYLEDONS
HAS BEEN MOVED ASIDE TO SHOW THE
POSITION OF THE SEEDLINGS IN THE JIG.

Method of irradiation for acute exposures in the
fractionation experiments.

The radiation source was a Philips 250/25 X-ray Therapy Unit operated at 250 KVP 15mA with an added filter of only 0.075mm Copper to achieve a high dose rate in the region of 170 rads/min.

A Perspex jig was constructed to hold about 25 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 (Fig. 5.4) some 3mm deep in the direction of the irradiation beam and situated 2cm from the outside wall of the tank into which it fitted. The jig could be slid into slots at one end of the 1ft.6in. x 1ft. x 1ft. Perspex tank and had a projecting tube at the bottom through which air could be passed.

For the purposes of dosimetry this jig could be removed and replaced by another (Fig. 5.4) 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 effect 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.

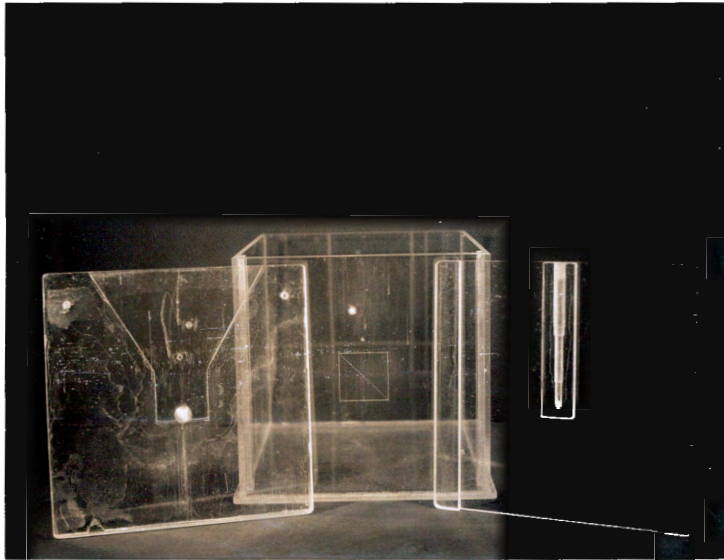


FIGURE 5.4

RADIATION JIG SHOWING THE PLATE WHICH
REPLACES THAT WITH A FUNNEL - SHAPED
CAVITY FOR DOSIMETRY PURPOSES.

Scoring radiation damage.

In all experiments in which the roots were exposed to fractionated doses of radiation, the length of the primary root was measured immediately following the second radiation dose. The roots of the control group were also measured at this time. 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. The length on Day 0 was recorded against a number which was allocated to the root. All roots were measured at approximately the same time (\pm 1 hour) on every second day following the irradiation for 10 days and then finally on the eleventh day.

The lifting of the seedlings for measurement of the root, and removal of shoot and adventitious roots, was done with care to avoid damaging them. Gray and Scholes (1951) reported that in the case of Vicia this handling did not affect the growth of the root, and the same seems to be the case with Zea.

After each set of measurements the average growth increment for two days was evaluated for each total 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 different time interval between the two doses a curve was 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 as G_{min} , was one of the parameters used to assess radiation damage (Gray and Scholes, 1951). The other parameter, G_{10} , or 'growth in ten days' (Read, 1952) was evaluated as follows.

The average difference between root length on Day 10 and on Day 0 for each dose was expressed as a fraction of controls over the same period.

For the study of the behaviour of the roots of Zea mays exposed to fractionated doses of radiation, the following experiments were carried out.

For the first experiment- the irradiation of Zea mays to two doses of 1000 rads each, separated by a time interval which was varied from 0 to 24 hours in steps of one hour- the tank was set up with its surface at 50cm from the tube focus with the long axis of the tank along the beam axis (as shown in Fig. 5.3).

A square field of 20 x 20cm was used to cover adequately the volume occupied by the root tips.

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 variations in dose across the root tips was estimated to be within 5 per cent of the measured dose. This measurement was checked at regular intervals and never differed by more than two per cent from the initial value corresponding to a dose rate of 165.5 rads per minute. The times to deliver the dose of 1000 rads 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.

Both jig and tank were filled with 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 towards the middle of the funnel, the longer roots being placed towards the outside. A steady stream of air was bubbled through the jig. 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 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 the first irradiation dose, the roots were transferred back to the culture tank and were then again irradiated after a set time using the same procedure as above. For the second irradiation the roots were transferred to the irradiation jig about 20 minutes before the required time of irradiation to allow them to equilibrate as described.

Following the second irradiation, the roots in each group were measured as described and placed in holes in the Perspex cover of the culture tank and their positions noted. The initial measurements were recorded as those appropriate to Day 0. Thereafter measurements were made on alternate days at approximately the same time of day (± 1 day) as described earlier.

A pilot experiment in which two doses of 2000 rads were used separated by the same time intervals as above, showed that a total dose of 4000 rads was too large as all the roots died after receiving the radiation.

A second series of experiments was performed in which the time between the two doses was kept constant at 7 hours, but the first and second doses of irradiation were varied over a large range. For a fixed first dose, the second dose of irradiation was varied from a dose equalling the first dose and then increasing in steps of 200 rads until a total dose of about 3000 rads was reached. The first dose of irradiation was varied in steps of 200 rads from 0 rads to 1800 rads.

For each experiment the seedlings were cultivated in exactly the same manner as described. The experimental procedure for the irradiation was also the same as for the previous experiment except that the roots were left for 48 hours in the culture tank before irradiating them. G_{10} was found for each set of doses as described before.

It was found necessary to autoclave the Vermiculite used for the germination at regular intervals. This was done once a month.

Method of irradiation for chronic exposures.

The method followed is that of Hall (1963).

A Perspex jig was constructed to hold the seedlings of Zea during irradiation (Fig. 5.5). The latter consisted of five compartments (30cm x 20cm x 0.4cm) 10cm apart and at different distances from two Ra-226 sources of equal activity. Each compartment could hold about 20 seedlings at any one time. A projecting tube at the bottom and top of each compartment was used to circulate fresh tap water through the latter.

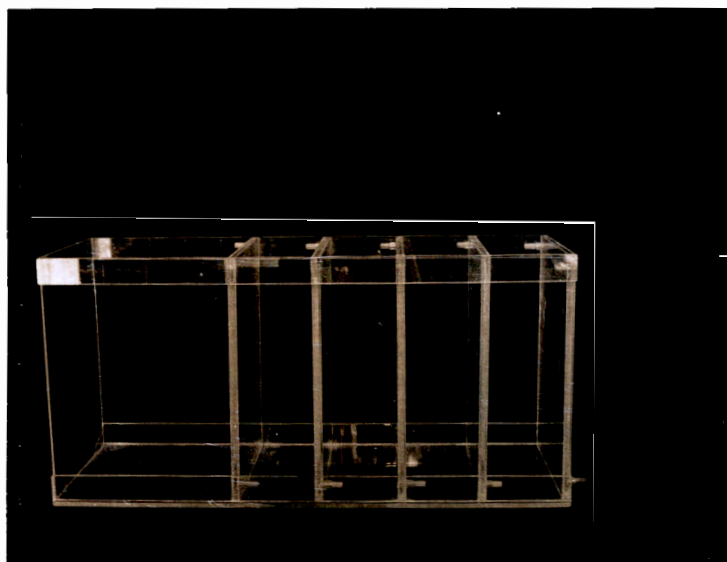


FIGURE 5.5

PERSPEX JIG FOR CONTINUOUS IRRADIATION STUDIES.

The Ra-226 was contained in a hollow iridioplatinum needle (42mm long) provided with a point and an eyelet. (These needles are used in the Hospital for Radium Therapy).

The encapsulation of the Radium by the abovementioned corrosion resistant material implies a filtration of the emitted radiation by 0.6mm of Platinum.

A Radium needle was inserted into each hole drilled into one end of each of the two Perspex rods (Fig. 5.6).

The needle was kept from slipping out when positioning the rod on the jig by blocking the entrance of the hole using a match. The height of the sources as well as their distance from the compartments could be adjusted by shifting the Perspex rods. For the very low dose rates a facility was provided to shield the roots from the Radium sources with lead as shown in Fig. 5.6. The position of the rods themselves on the jig is depicted in Fig. 5.7.

The entire jig was immersed in a Perspex tank (26in. x 10in. and 12in. deep), filled with water and provided with an outlet.

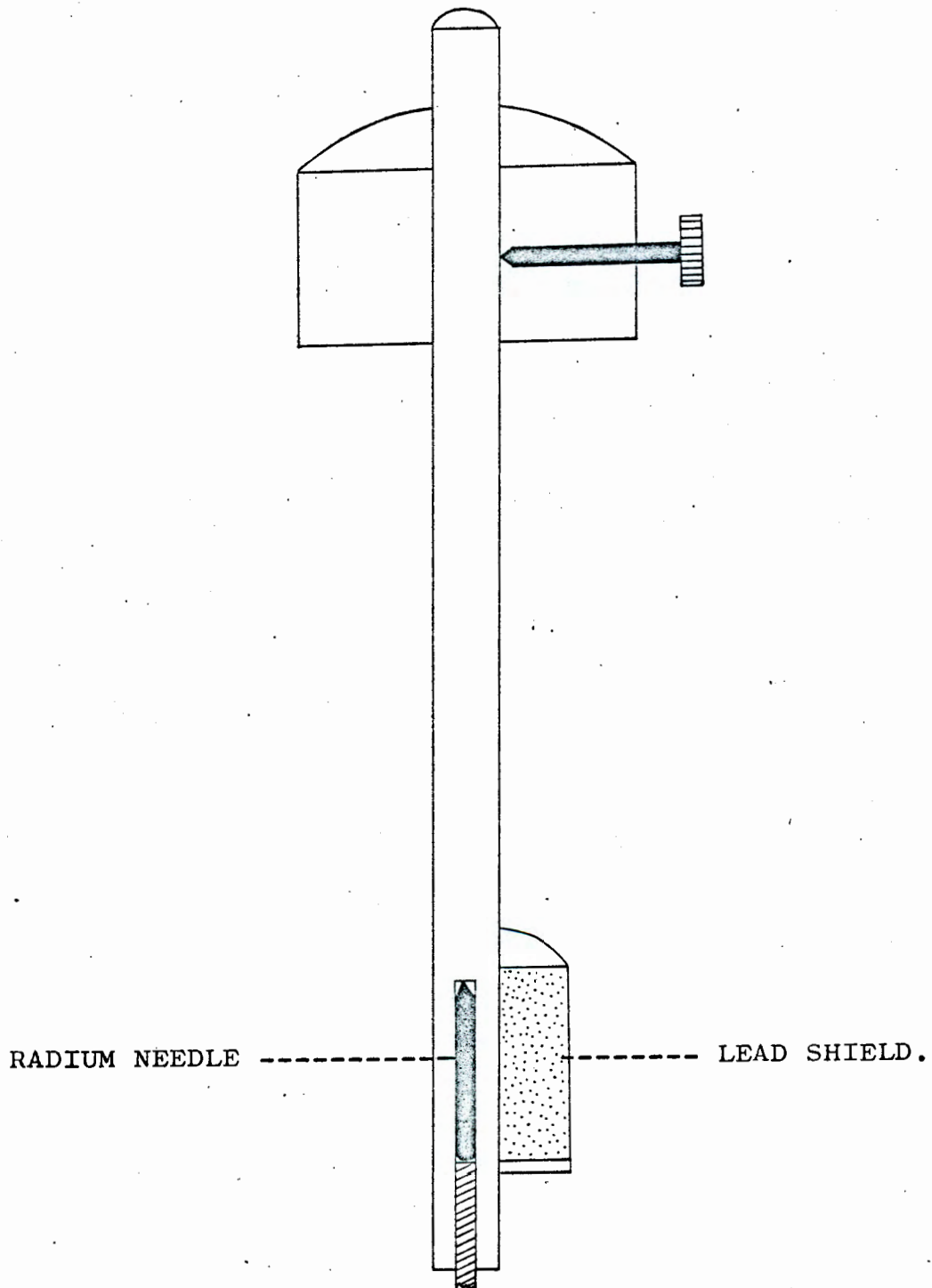
The temperature of the water in the tank was maintained at $25 \pm 0.2^{\circ}\text{C}$ by a Braun thermostatically controlled heater.

Tap water was passed through each compartment, entering the compartment at the bottom and overflowing into the tank. The flow of water through each compartment was adjusted, to ± 50 cc. per minute using a series of 'taps' as depicted in Figure 5.7.

For the study of the behaviour of the roots of Zea mays exposed to continuous irradiation, the experiments described on the next pages were carried out, the procedure being similar in each case.

FIGURE 5.6

CROSS-SECTION THROUGH THE PERSPEX ROD HOLDING THE RADIUM NEEDLE.



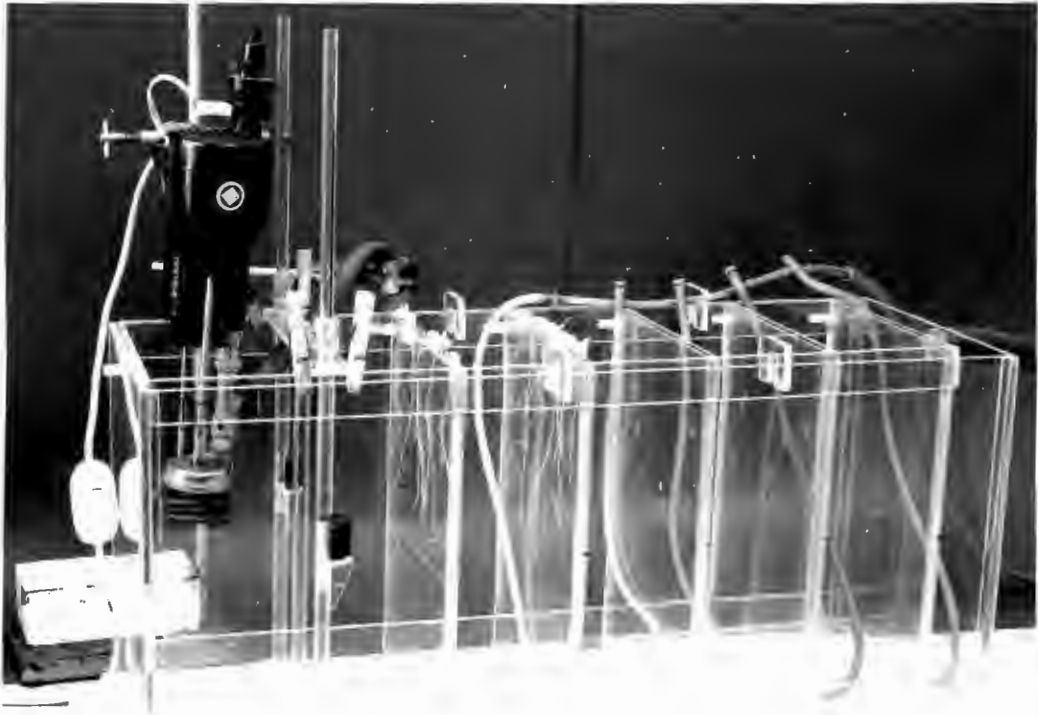


FIGURE 5.7

VIEW OF THE CULTURE TANK USED FOR THE CONTINUOUS
IRRADIATION EXPERIMENTS.

In the first experiment the roots of Zea were exposed to the radiation from 1mg of Ra-226 continuously for 15 days.

The method of culturing the seedlings was similar to the one described previously. The cotyledons were, however, not covered with moist surgical gauze. This prevented the fungus infection which tends to occur if the scutella are kept moist for long periods.

The 20 control roots and the 15 roots in each of the five compartments were measured just before the radium sources were put into position. These initial measurements on Day 0 were recorded against a number which was allocated according to the position of the root in the tank and which was maintained throughout the experiment.

The Radium sources of 0.5mg Ra-226 each were then positioned at a distance of 10cm from the first compartment, one source at each side. The height of the sources was adjusted to a level corresponding to half of the depth of the tank. No lead shielding was used in this experiment.

The dose to the roots was measured using Thermoluminescent Dosimetry as described in Appendix B, as the dose rate was too small to be detected suitably by a Baldwin Farmer dosimeter. The polythene bags containing the aliquots of the thermoluminescent powder, were attached to the outside of each compartment on the same level with the sources.

The roots were measured every second or third day (\pm 1 hr), the sources being removed from the jig during the measurement.

In a second, similar experiment the dose rate to the roots was increased, using two Radium sources of 1mg each.

A third experiment was performed in which the dose rate to the roots was decreased to a very low level. This was achieved by using two Radium needles of 1mg each (the lowest activity available) and reducing the amount of radiation to the roots further by placing the Radium sources behind a lead screen as shown in Fig. 5.7. The thickness of the lead used was equal to one Half Value Layer for Radium gamma rays.

In these experiments the control roots were kept in the culture tank used for the fractionation experiments. Since the nature of this experiment requires the control roots to be kept in a separate tank, it was also thought advisable to compare the growth of roots grown in the continuous irradiation jig without any radiation with their growth in the other culture tank.

CHAPTER VIRESULTS.1. Results of the continuous irradiation experiments.

The pattern of growth rate observed for roots exposed to protracted irradiation for several low dose-rate values are illustrated in Figures 6.1, 6.2 and 6.3.

In these curves the growth rate (G), as a fraction of controls of equal age, is plotted against the time after onset of the radiation. The dose rates are included in the figures. Error bars were not included on the curves in Figure 6.2 in order to preserve clarity of presentation.

For the same reason error bars have been omitted on some of the other graphs mentioned in this chapter, but are always of the same order as those depicted.

The comparison of the growth of unirradiated roots grown in the continuous irradiation jig with their growth in the main culture tank, as described in the previous chapter, is shown in Figure 6.4.

2. Results of the fractionation experiment using equal divided doses.

Figure 6.5 depicts the daily growth of control roots as a function of time. 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 (Gray and Scholes, 1951; Read, 1952).

Growth curves for Zea roots irradiated with two doses (the 'conditioning dose' and 'test dose' respectively) of 1000 rads each, separated by various time intervals, are given in Figures 6.6 and 6.7a,b, where the growth rate as a fraction of controls (G) is plotted against time.

The 'growth in ten days' (G_{10}) and the minimum growth rate (G_{\min}) for the different time intervals between the conditioning and test doses ranging from 0 to 24 hours in steps of one hour are listed in Table 6.1.

The method for obtaining the values of G_{10} and G_{\min} has already been discussed (Chapter V). The values of G_{10} and G_{\min} thus obtained were plotted against the interval between the doses as shown in Figure 6.8 and 6.9 respectively.

3. Results of the fractionation experiments using unequal divided doses.

For a conditioning dose of 200 rads, with test doses varying from 200 rads to a total dose of 2200 rads in steps of 200 rads, the value of the 'growth in ten days' was plotted against the total dose as shown in Figure 6.10. Growth curves were also drawn from the measurements of the length of the roots taken on every alternate day and these curves are shown in Figures 6.11 and 6.12.

Similar graphs of G_{10} vs. Total Dose were drawn for various other conditioning doses viz. 0, 400, 600, 800, 1000, 1200, 1400, 1600, and 1800 rads. These graphs are depicted in Figures 6.13 to 6.18. Some of these results are also

tabulated in Table 6.2. For the higher first doses the second doses were increased in steps of 400 rads.

In Figures 6.19 to 6.23, growth curves are presented for various values of the total dose.

From the graphs of G_{10} vs. Total Dose the total dose necessary to reduce the 'growth in ten days' to 0.25 was obtained and plotted against the corresponding value of the first dose (Figure 6.24). These values are also tabulated in Table 6.3 together with the corresponding values for the first dose.

The error bars for these experimental results were calculated as described in Appendix C.

FIGURE 6.1

PATTERN OF DAILY GROWTH RATE OF ROOTS OF ZEA MAYS
EXPOSED TO PROTRACTED IRRADIATION AT A DOSE RATE
OF 2.2 MRADS/HOUR.

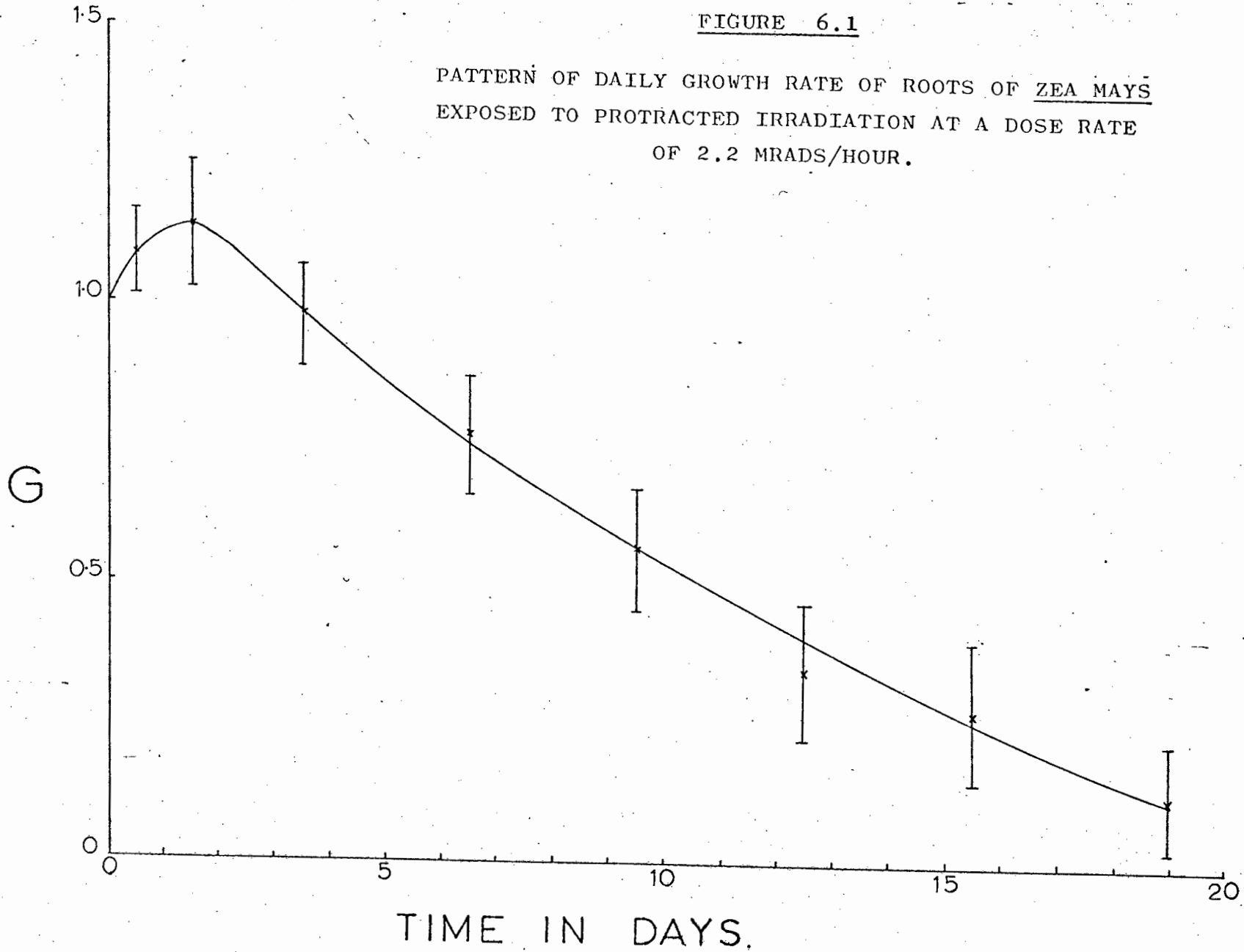


FIGURE 6.2

PATTERNS OF DAILY GROWTH RATE OF ROOTS OF ZEA MAYS
EXPOSED TO PROTRACTED IRRADIATION AT DOSE RATES
IN THE RANGE 0.4 TO 42.8 MRADS/HOUR.

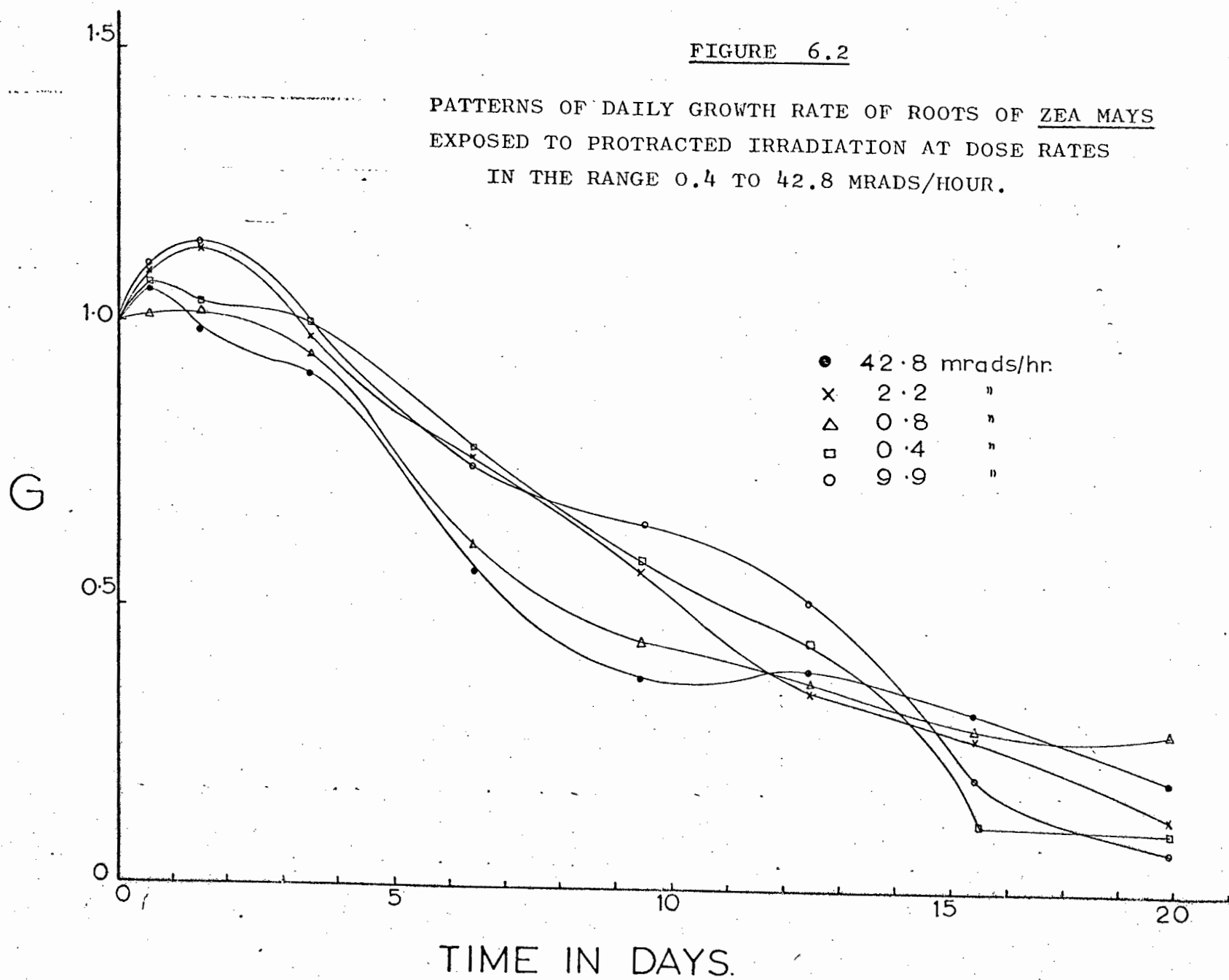


FIGURE 6.3

PATTERNS OF DAILY GROWTH RATE OF ROOTS OF ZEA MAYS EXPOSED TO PROTRACTED IRRADIATION AT DOSE RATES OF 85.6 AND 171.2 MRADS/HOUR.

Δ 85.6 mrads/hr.
● 171.2 mrads/hr.

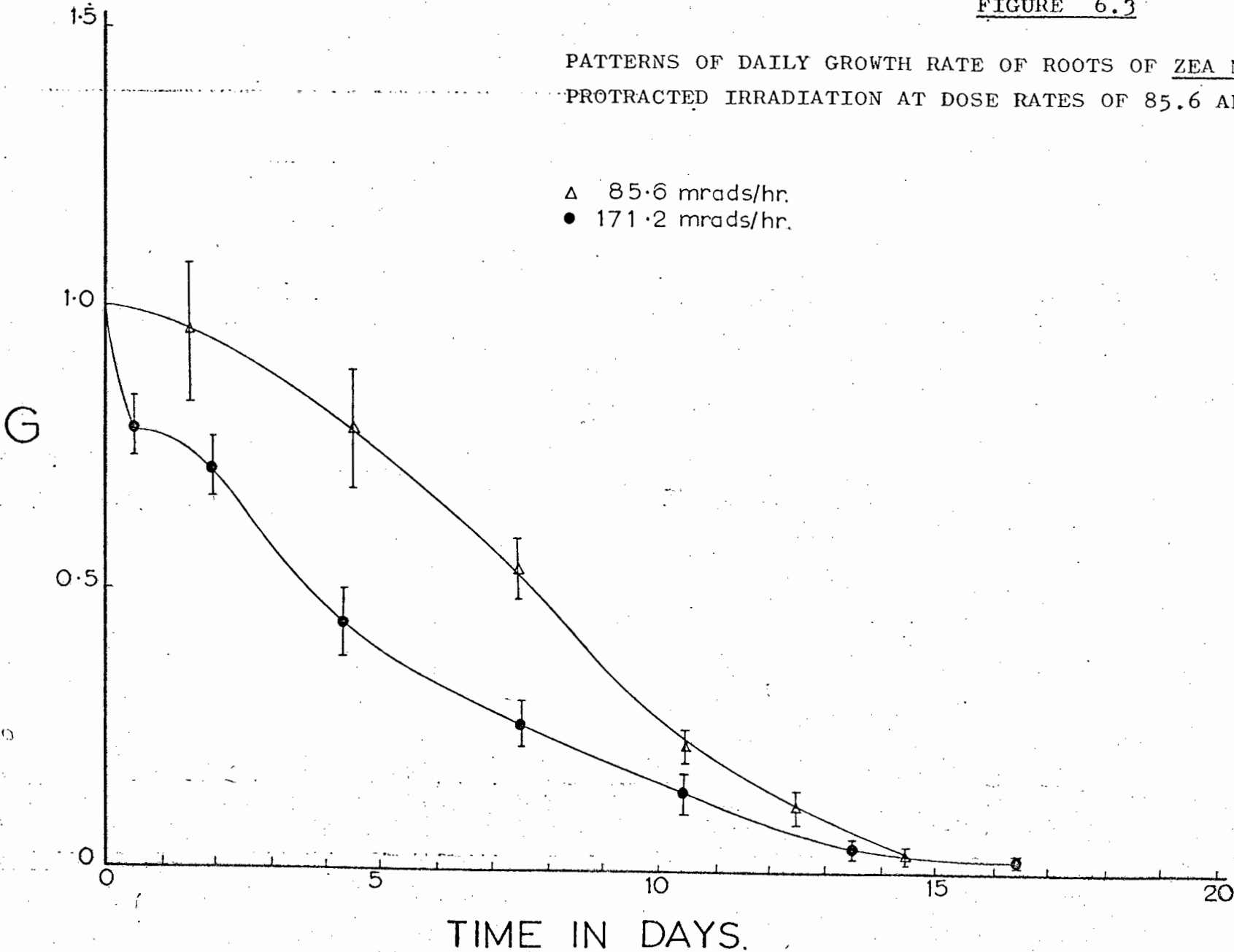


FIGURE 6.4

COMPARISON OF ROOT GROWTH IN THE CONTINUOUS IRRADIATION JIG WITH THEIR GROWTH IN THE MAIN CULTURE TANK.

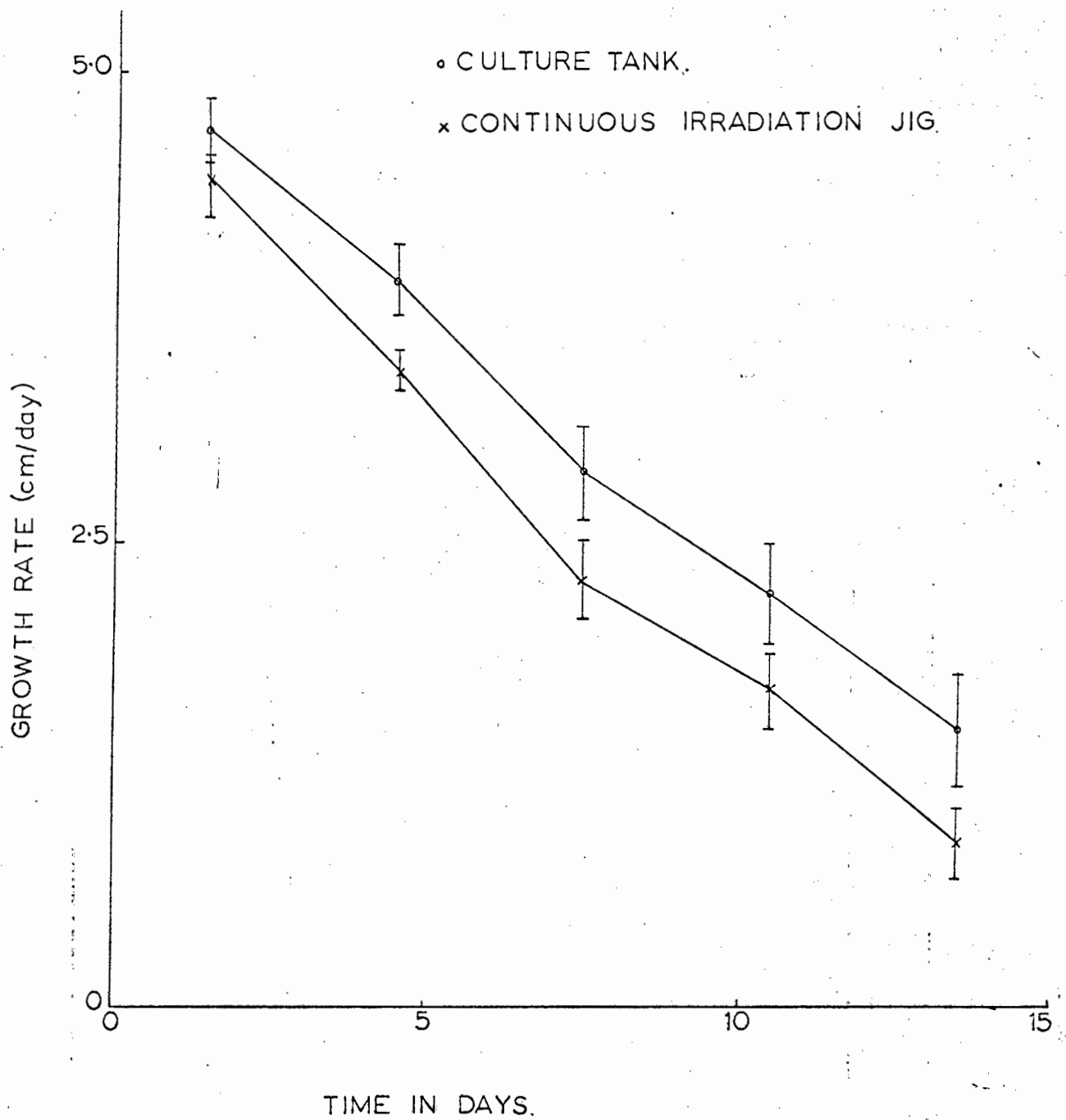


FIGURE 6.5

NORMAL GROWTH CURVE FOR THE PRIMARY ROOT OF ZEA MAYS.

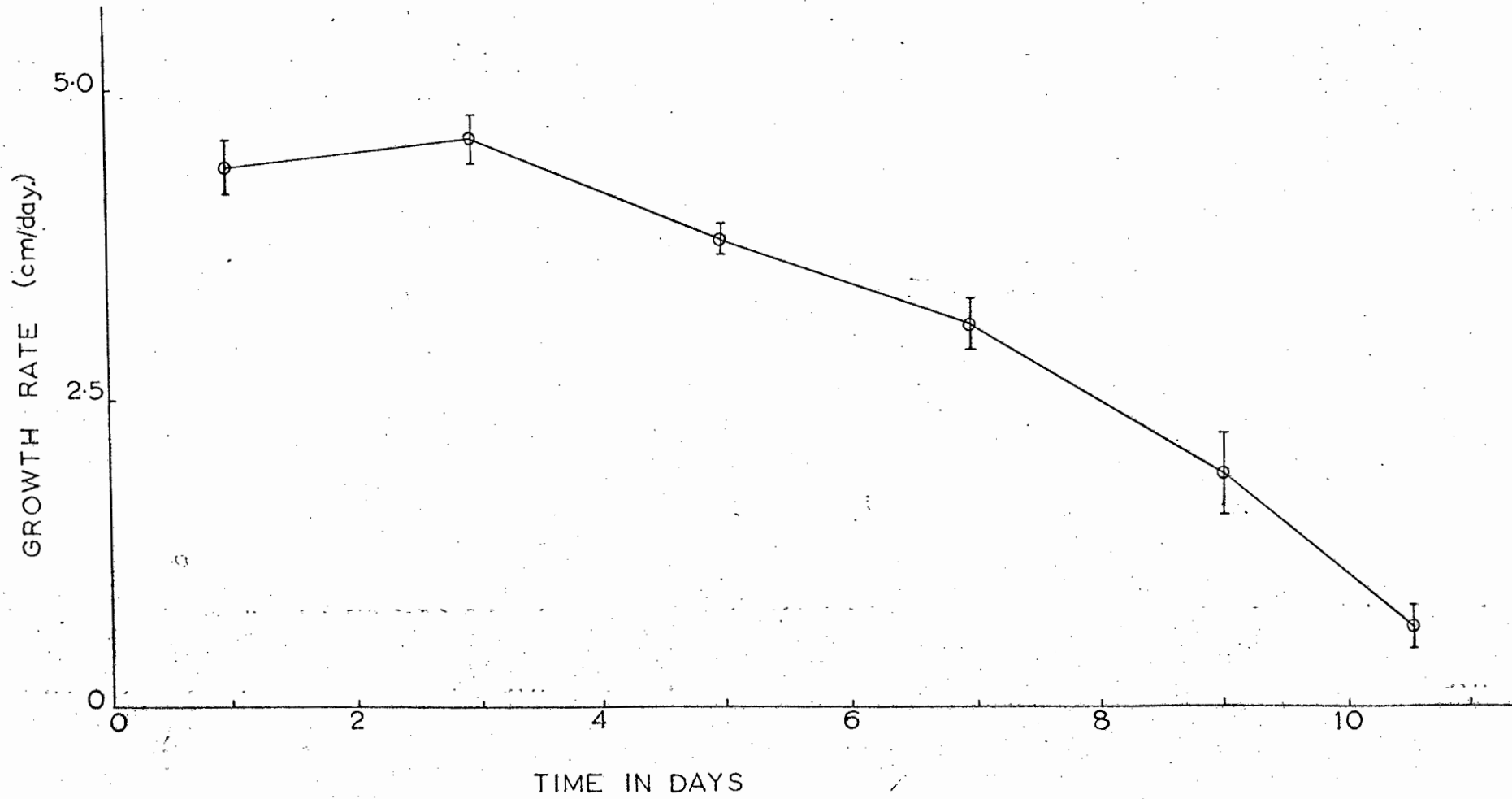


FIGURE 6.6

THE VARIATION IN GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS OF EQUAL AGE (G) FOLLOWING TWO X-RAY DOSES OF 1000 RADS EACH, SEPARATED BY A TIME INTERVAL OF SEVEN HOURS.

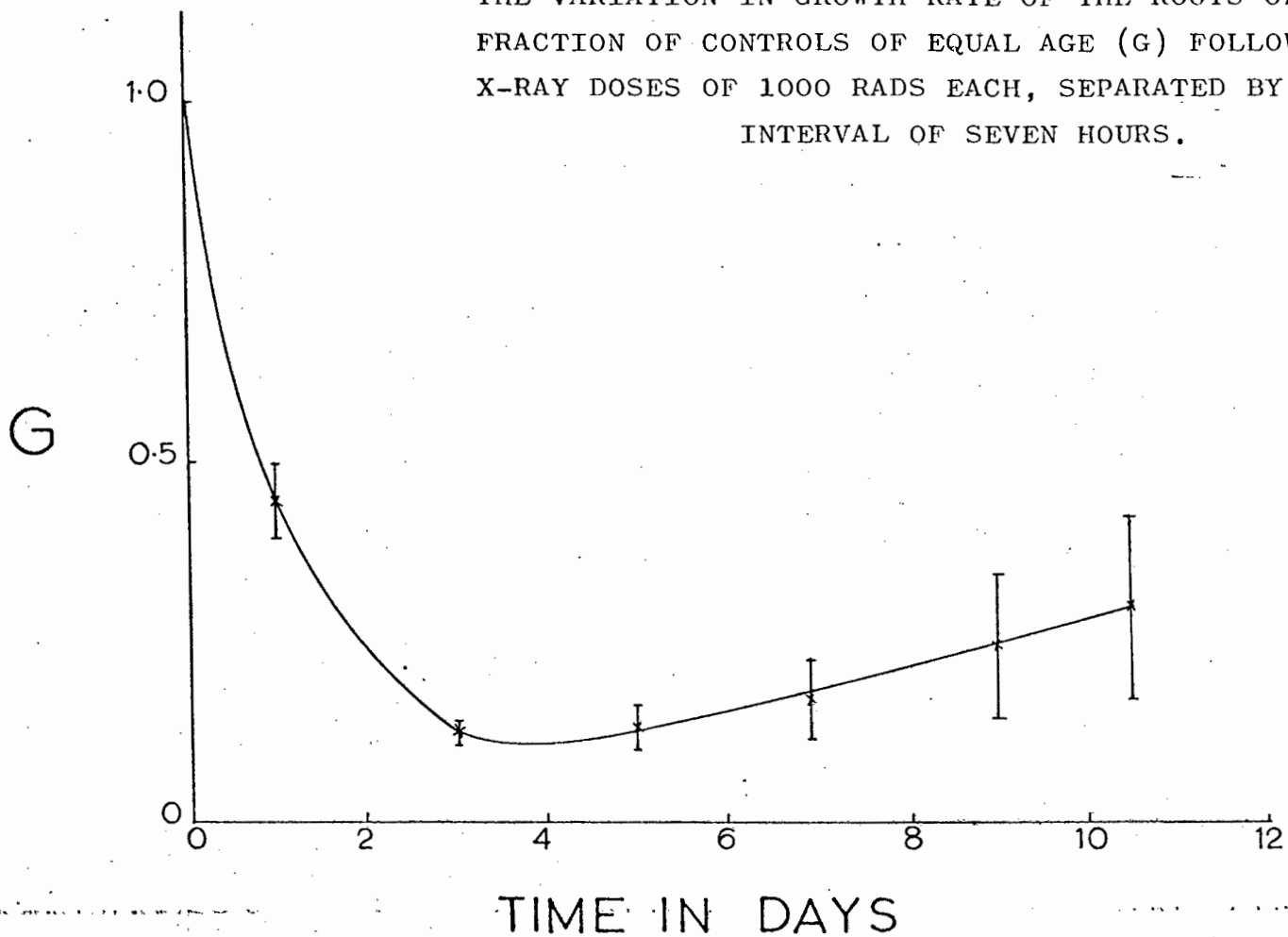


FIGURE 6.7a

PATTERNS OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) FOLLOWING TWO X-RAY DOSES OF 1000 RADS EACH, SEPARATED BY TIME INTERVALS IN THE RANGE 0 TO 6 HOURS.

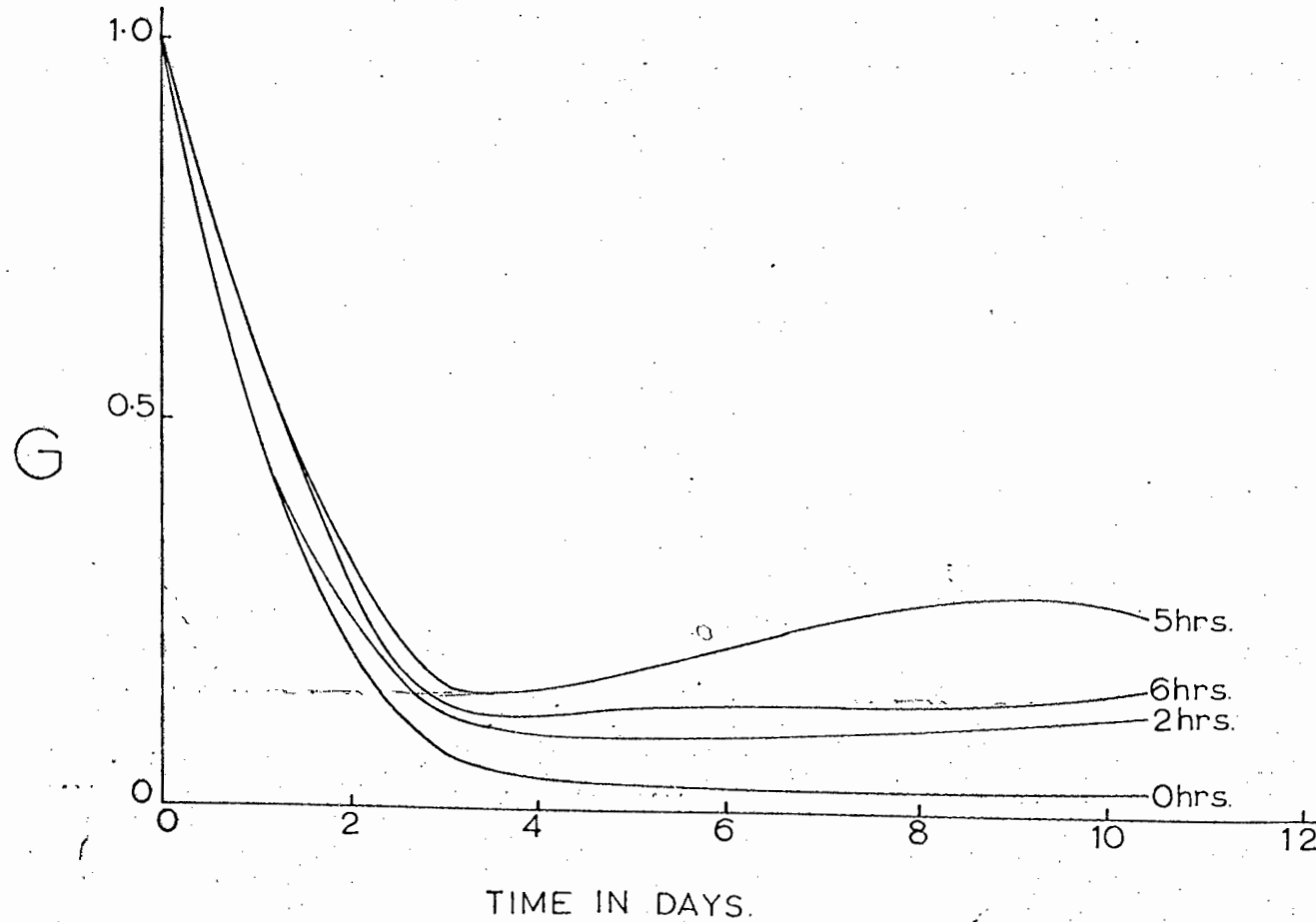


FIGURE 6.7b

PATTERNS OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) FOLLOWING TWO X-RAY DOSES OF 1000 RADS EACH, SEPARATED BY TIME INTERVALS IN THE RANGE 9 TO 24 HOURS..

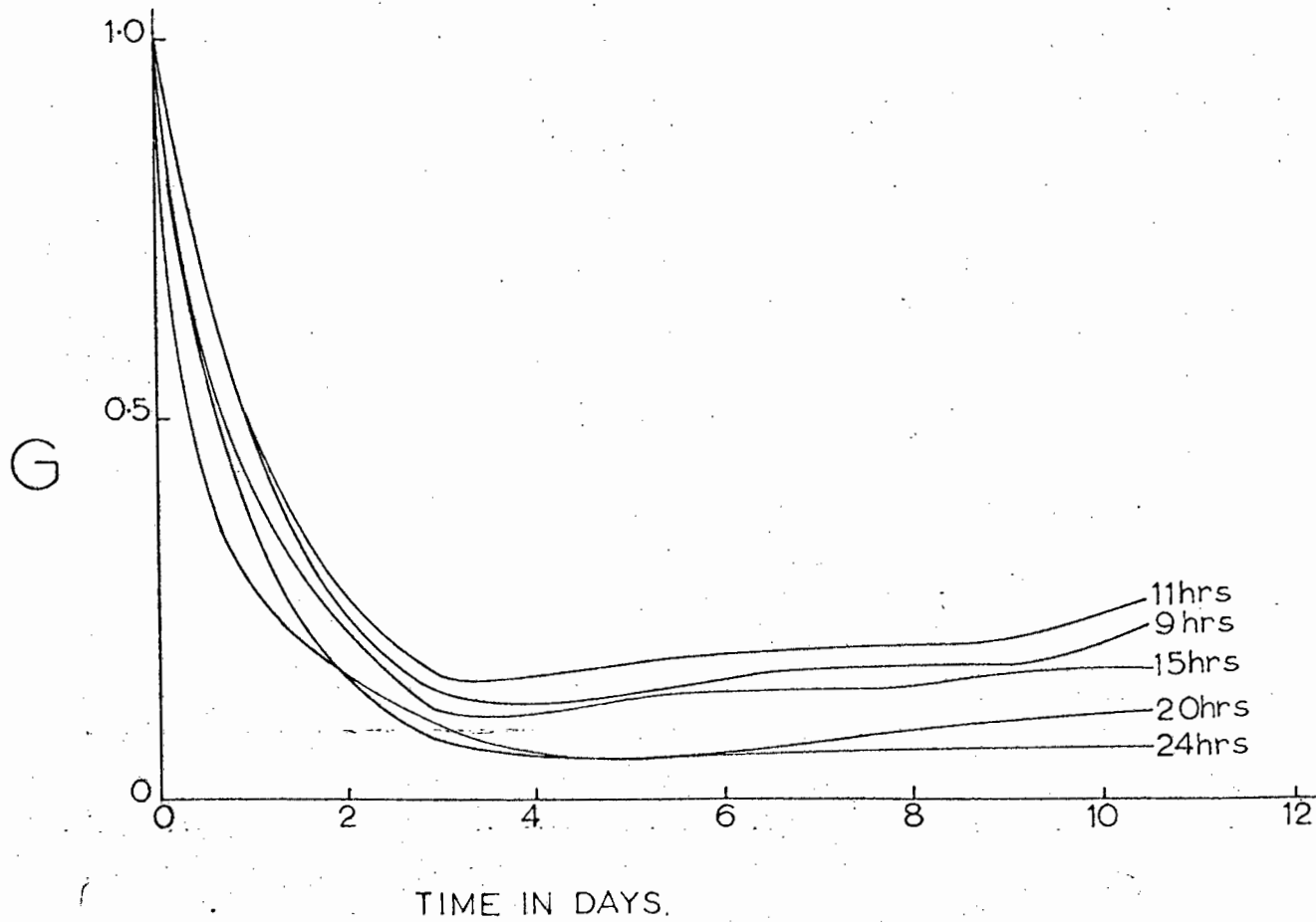


FIGURE 6.8

THE VARIATION OF THE MINIMUM GROWTH RATE (G_{\min}) FOR ROOTS EXPOSED TO TWO DOSES OF X-RAYS SEPARATED BY VARIOUS TIME INTERVALS.

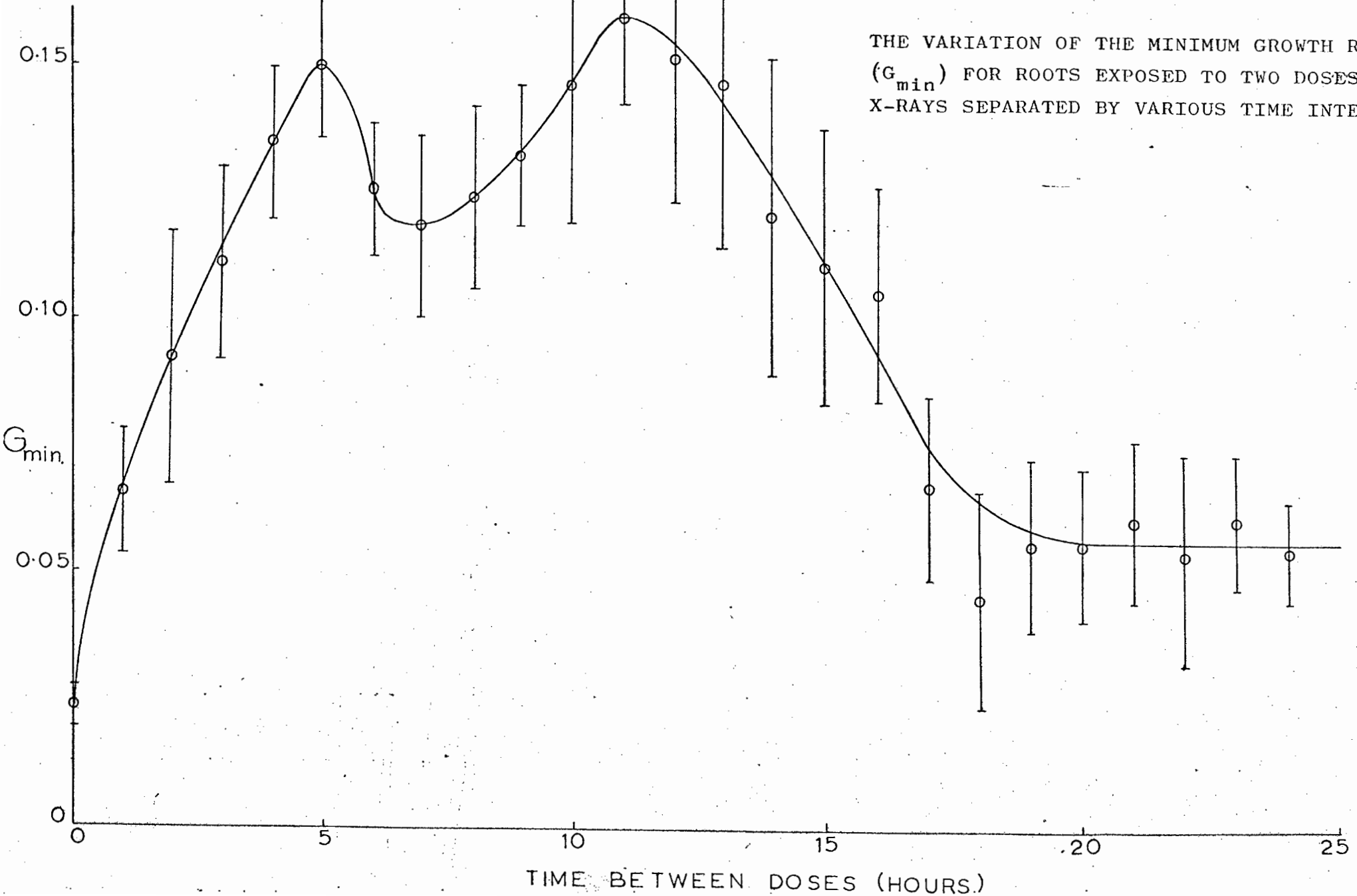


FIGURE 6.9

THE VARIATION OF THE "GROWTH IN TEN DAYS" (G_{10})
FOR ROOTS EXPOSED TO TWO DOSES OF X-RAYS
SEPARATED BY TIME INTERVALS IN THE RANGE
0 TO 24 HOURS.

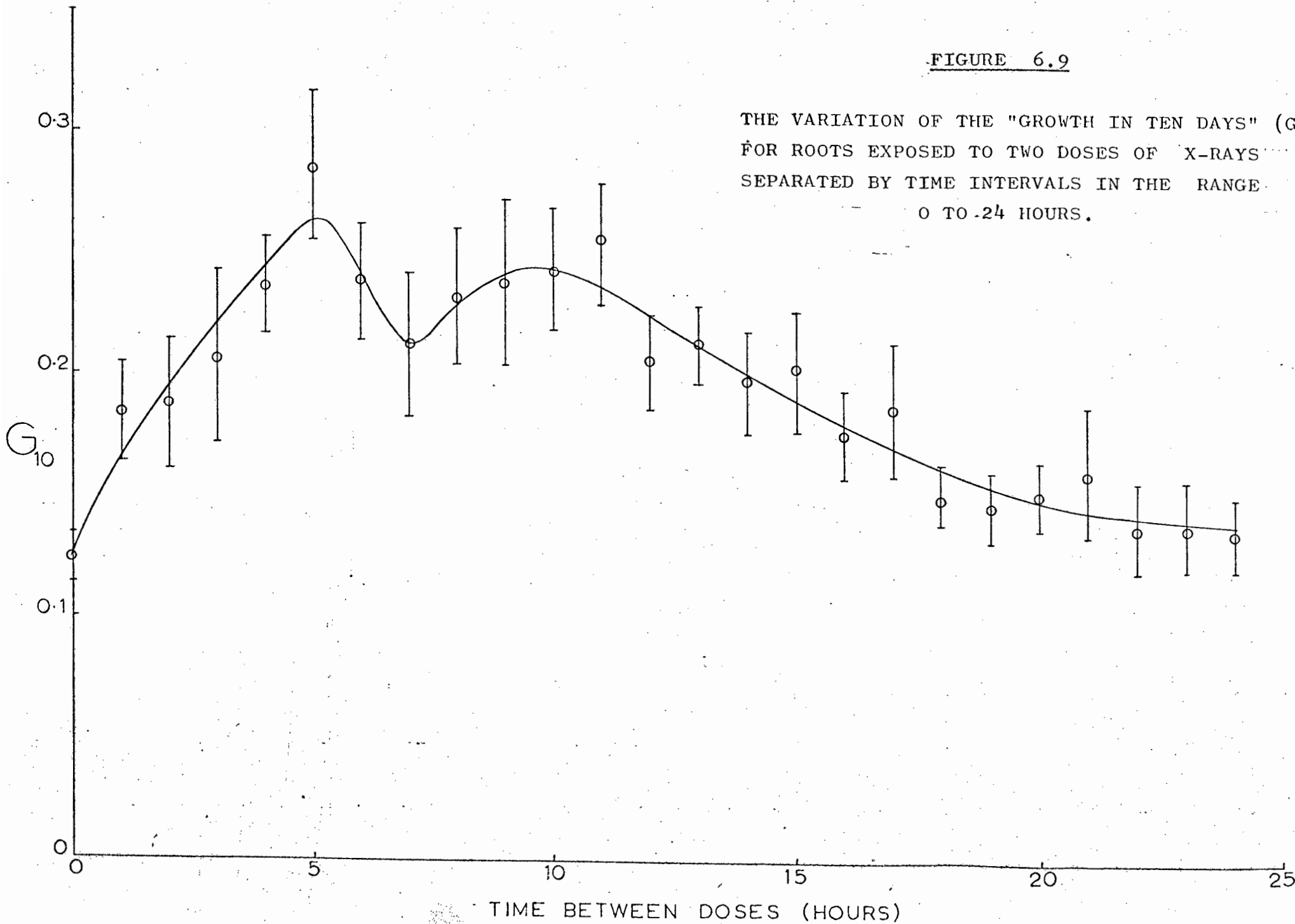


FIGURE 6.10

THE VARIATION OF THE "GROWTH IN TEN DAYS" (G_{10}) FOR THE ROOTS OF ZEA EXPOSED TO TWO UNEQUAL DOSES OF X-RAYS SEPARATED BY A TIME INTERVAL OF SEVEN HOURS.

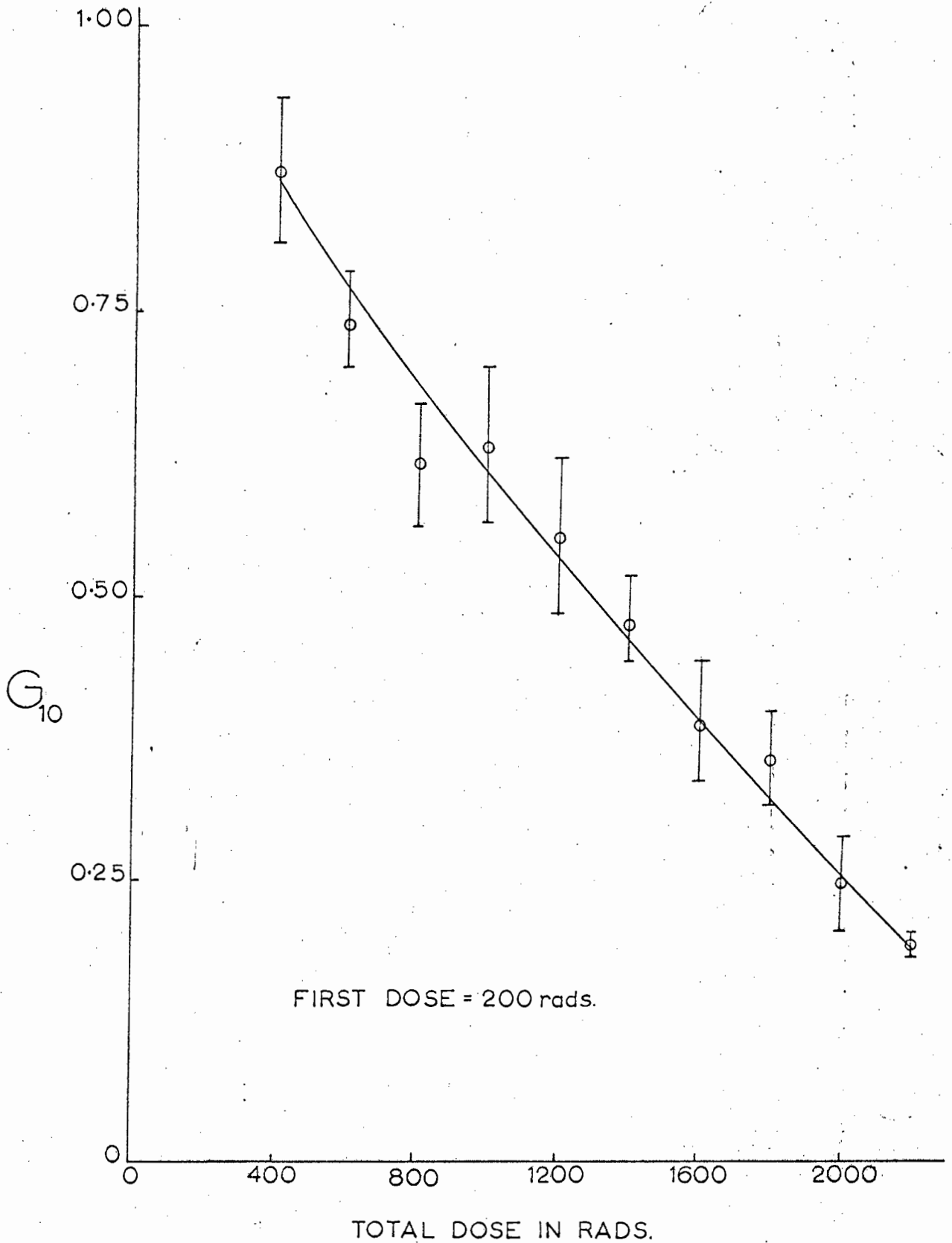


FIGURE 6.11

PATTERN OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) FOLLOWING TWO DOSES OF 200 AND 1400 RADS OF X-RAYS SEPARATED BY A TIME INTERVAL OF SEVEN HOURS.

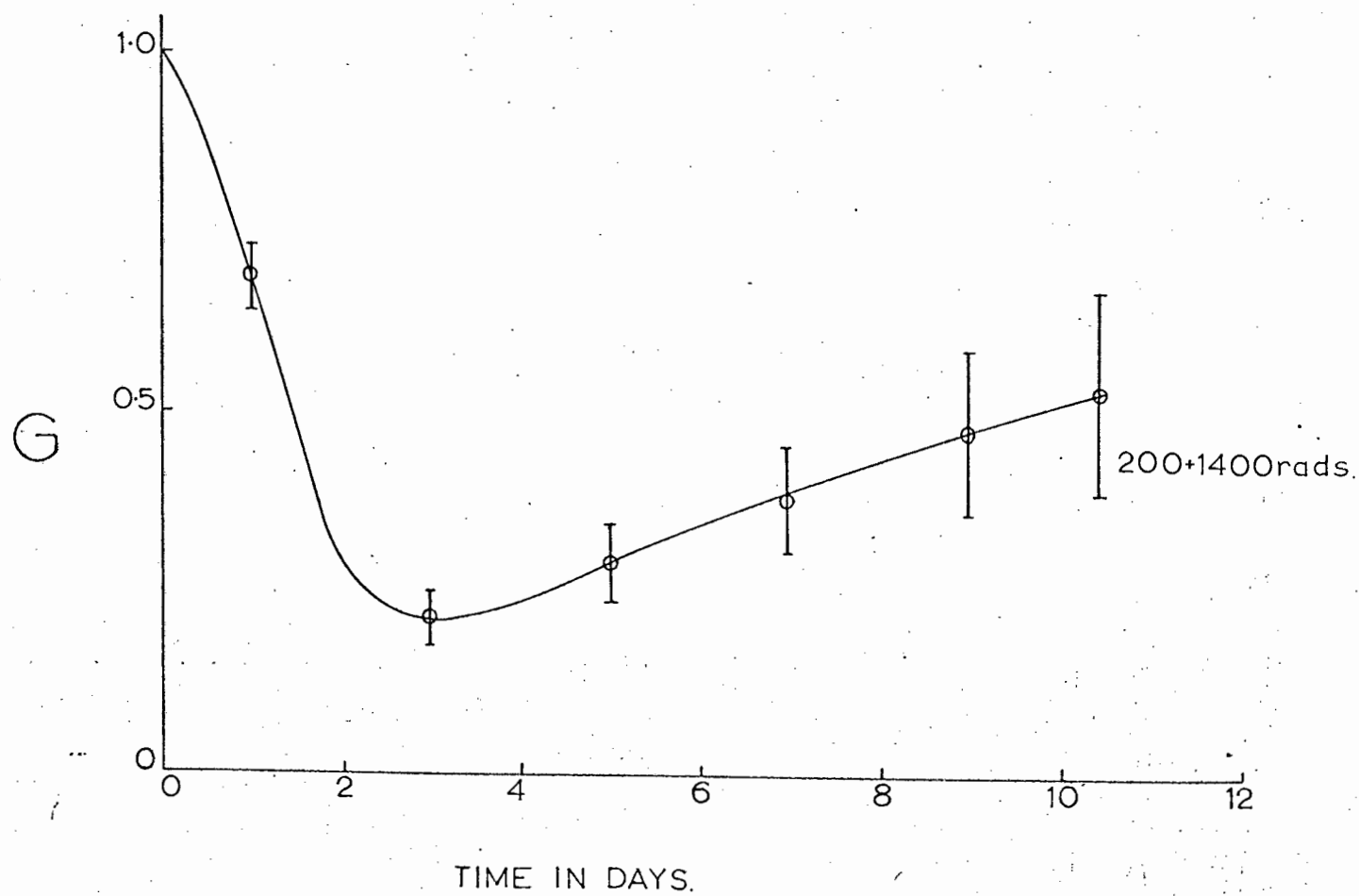


FIGURE 6.12

PATTERNS OF THE GROWTH RATE OF THE ROOTS OF ZEA AS A FRACTION OF CONTROLS (G) FOLLOWING A CONDITIONING DOSE OF 200 RADS AND TEST DOSES IN THE RANGE 200 TO 2000 RADS.

THE TWO DOSES ARE SEPARATED BY SEVEN HOURS.

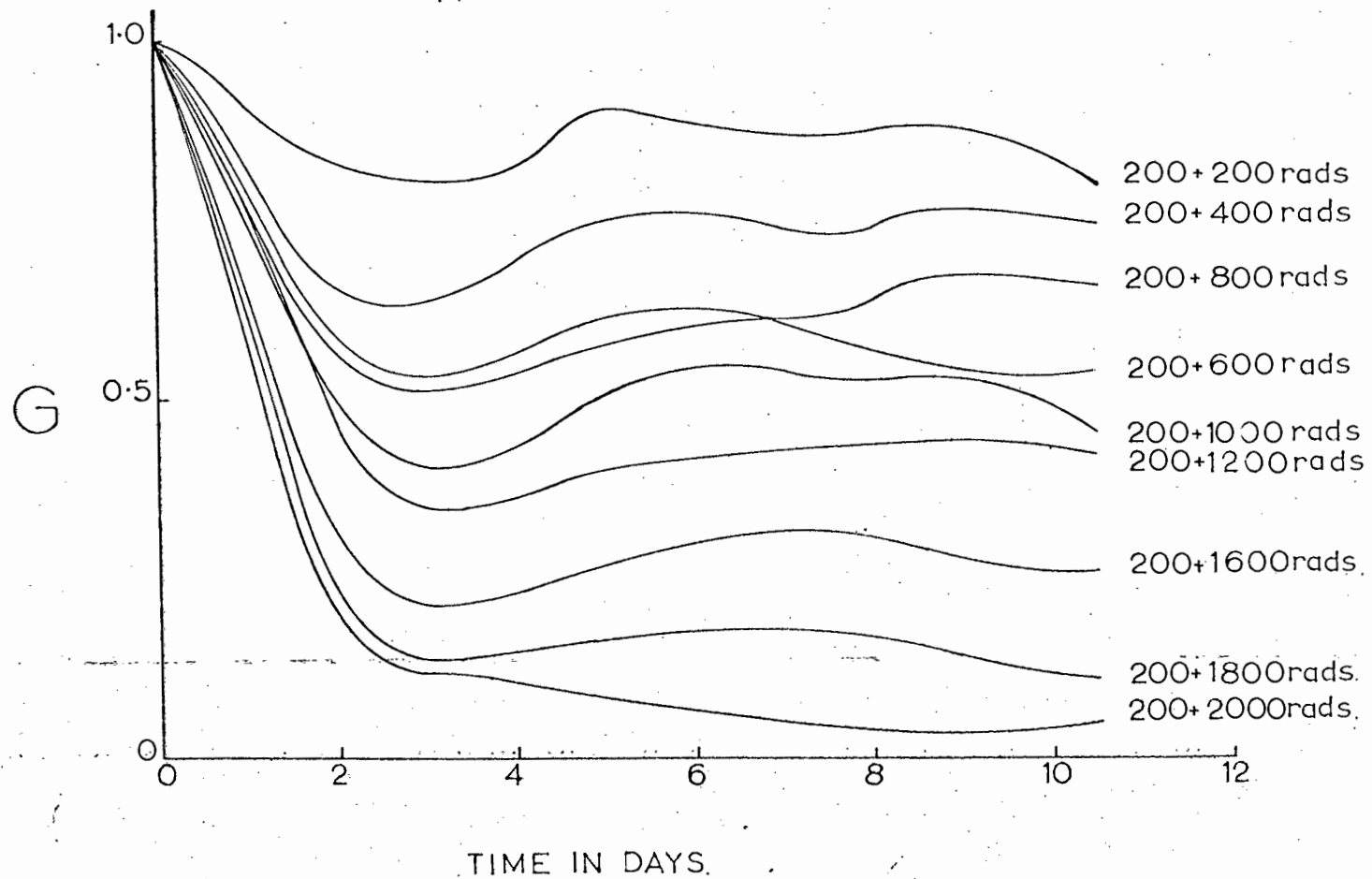


FIGURE 6.13

AS FOR FIGURE 6.10, BUT WITH A FIRST DOSE OF 0 RADS.

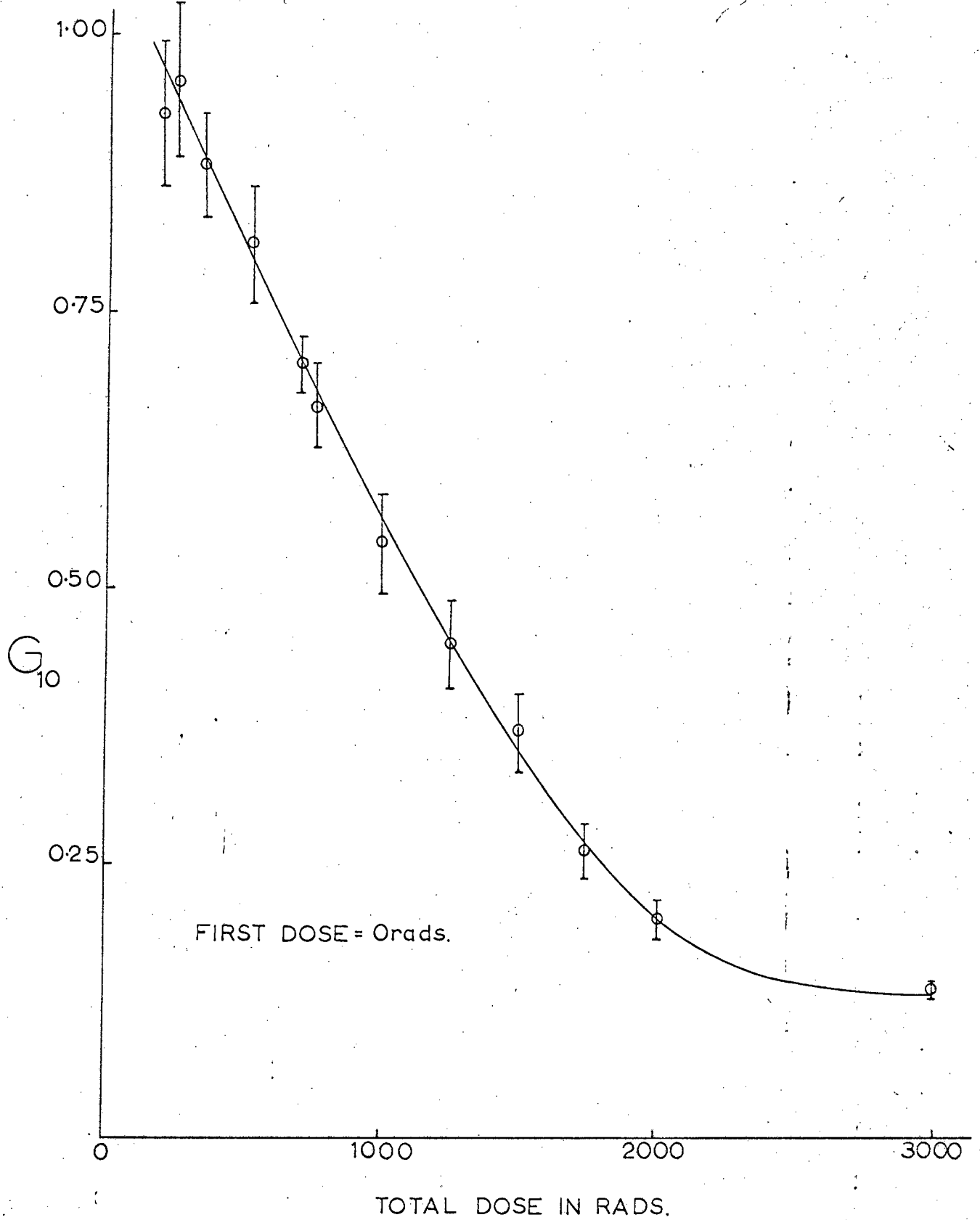


FIGURE 6.14

AS FOR FIGURE 6.10, BUT WITH A FIRST DOSE OF 400 RADS.

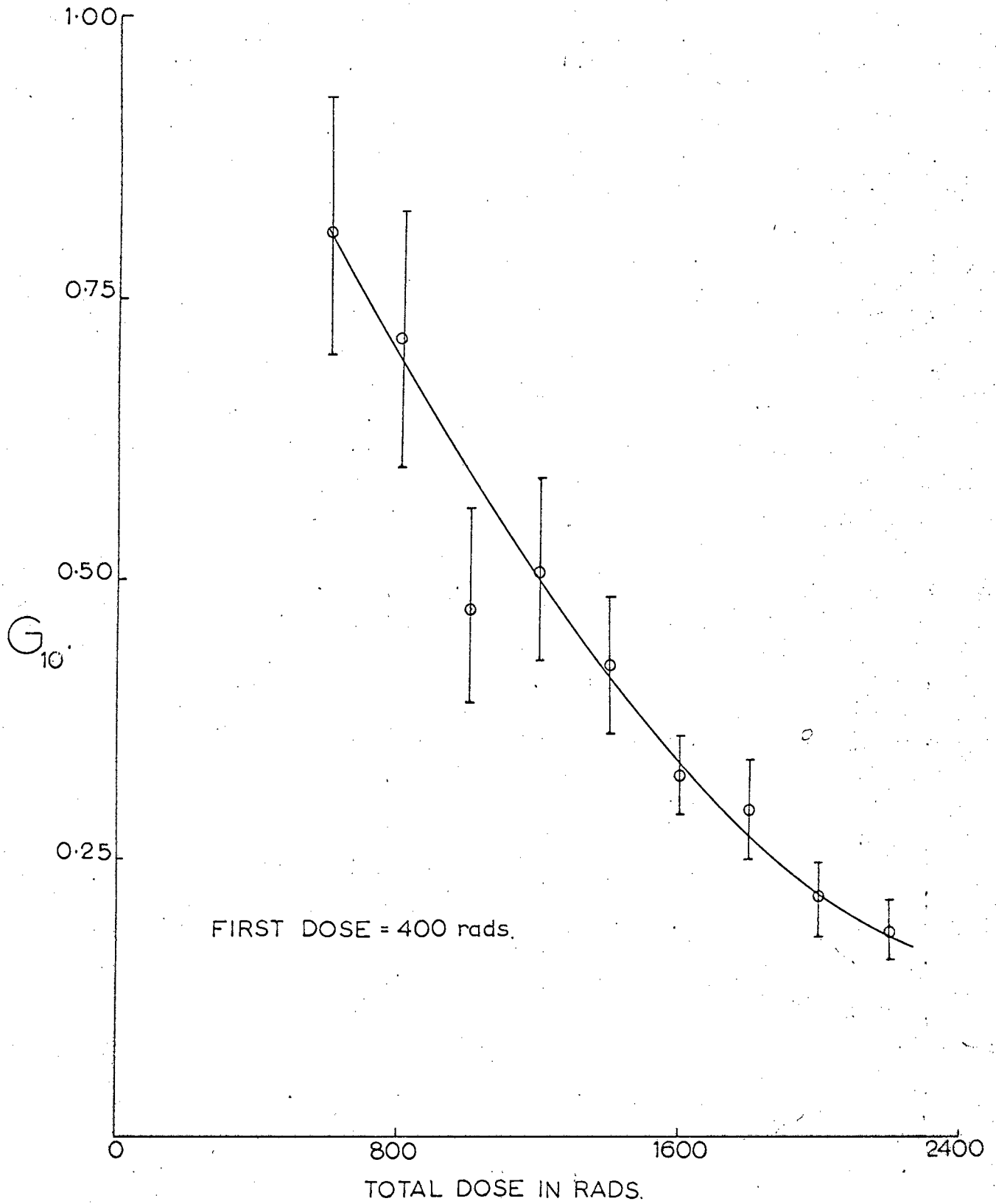


FIGURE 6.15

AS FOR FIGURE 6.10, BUT WITH A FIRST DOSE OF 600 RADS.

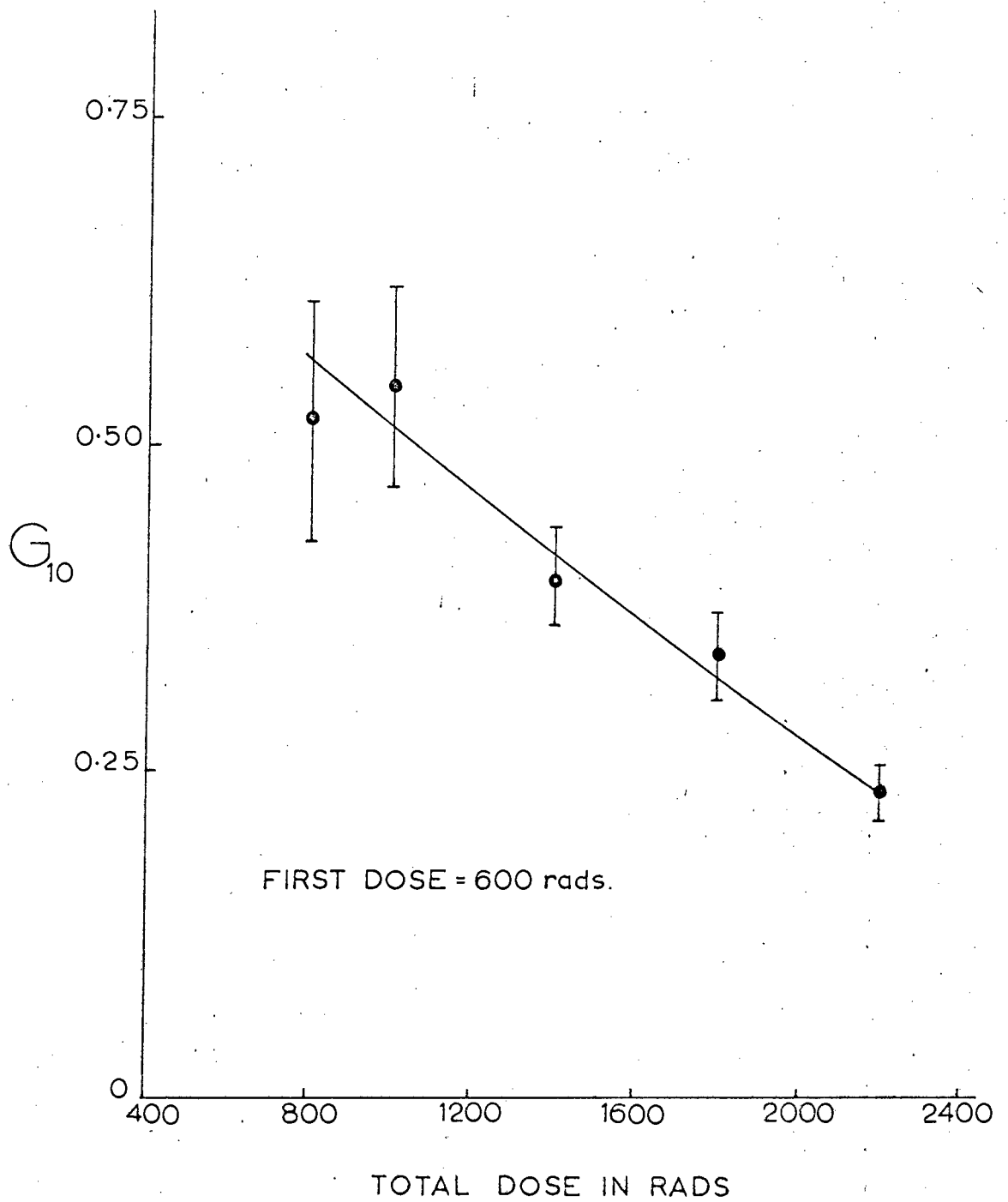


FIGURE 6.16

AS FOR FIGURE 6.10, BUT WITH A FIRST DOSE OF 800 RADS.

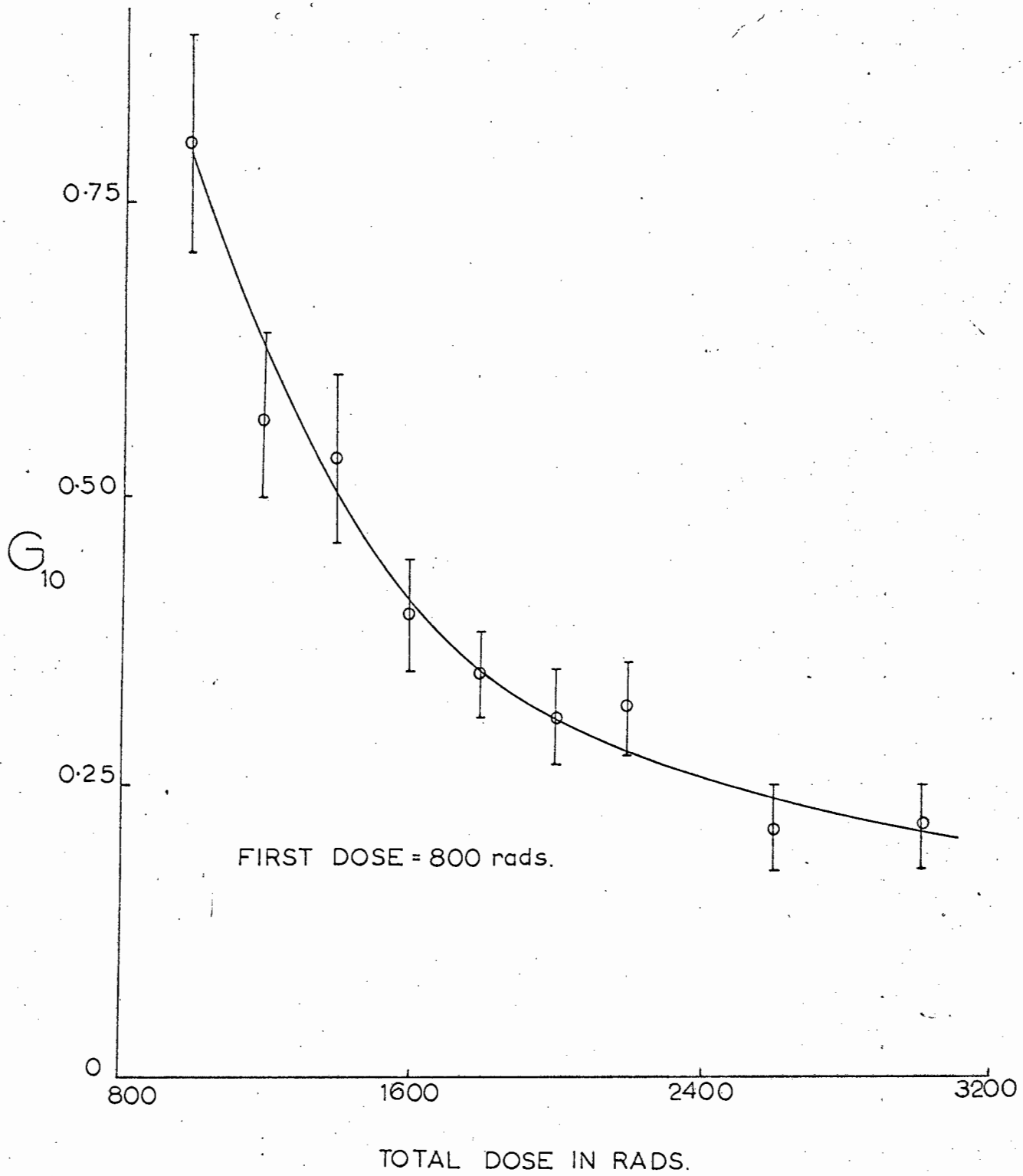


FIGURE 6.17

AS FOR FIGURE 6.10, BUT WITH FIRST DOSES OF 1000, 1200 AND 1400 RADS.

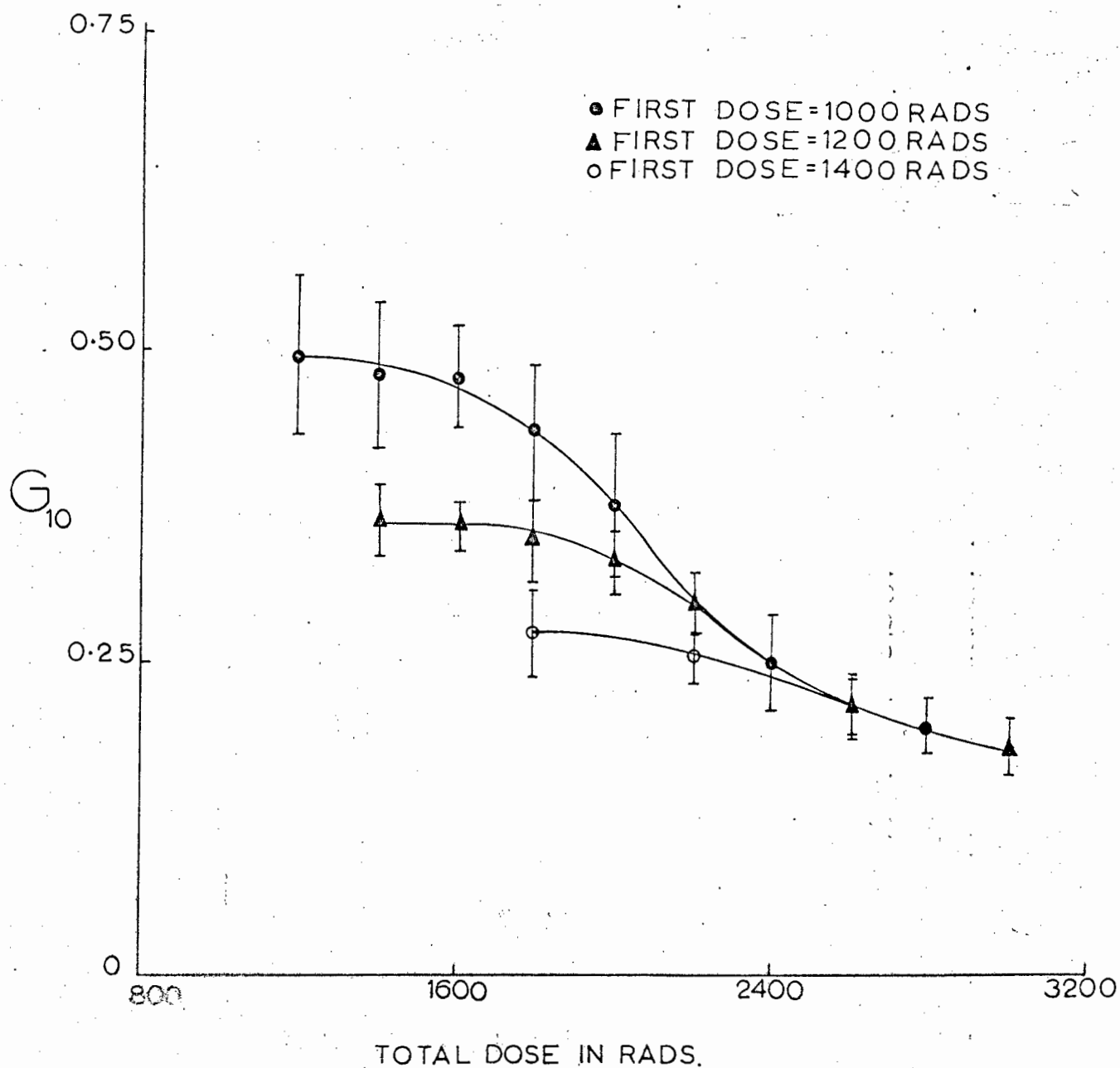


FIGURE 6.18

AS FOR FIGURE 6.10, BUT WITH FIRST DOSES OF 1800
AND 1600 RADS.

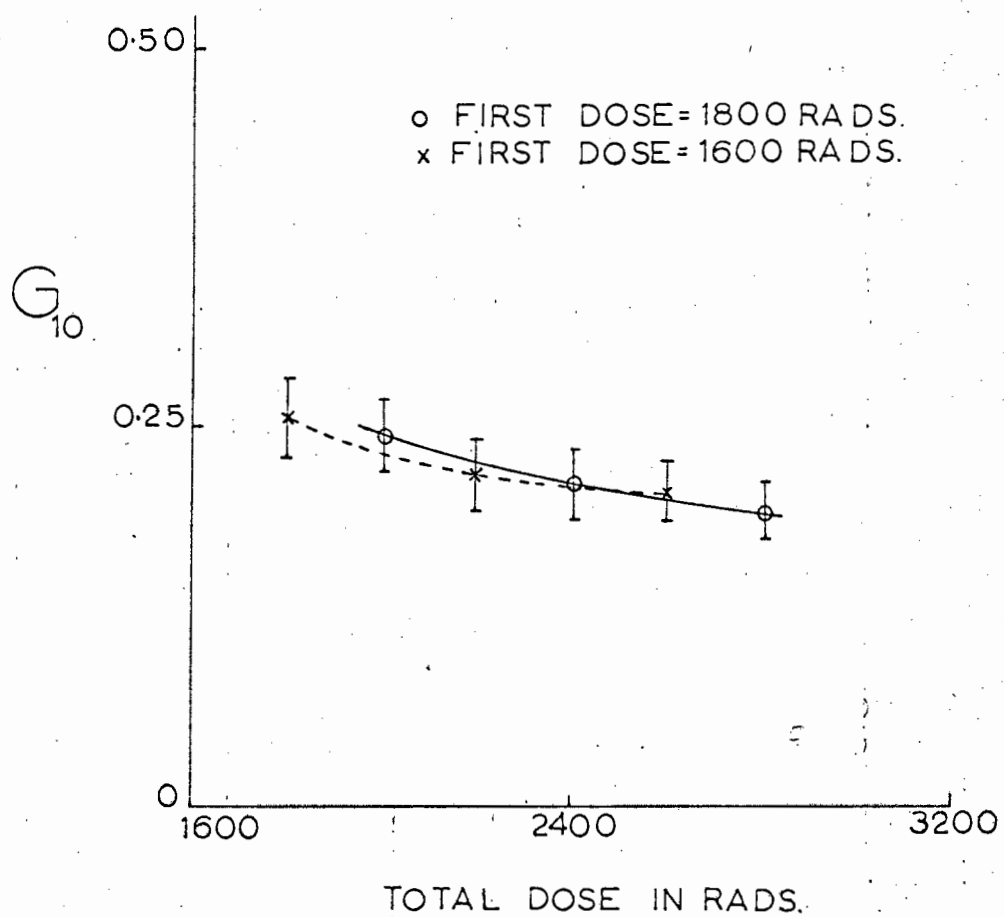


FIGURE 6.19

AS FOR FIGURE 6.12, BUT WITH A FIRST DOSE OF 0 RADS
AND VARIOUS SECOND DOSES RANGING FROM 350 TO 3000 RADS.

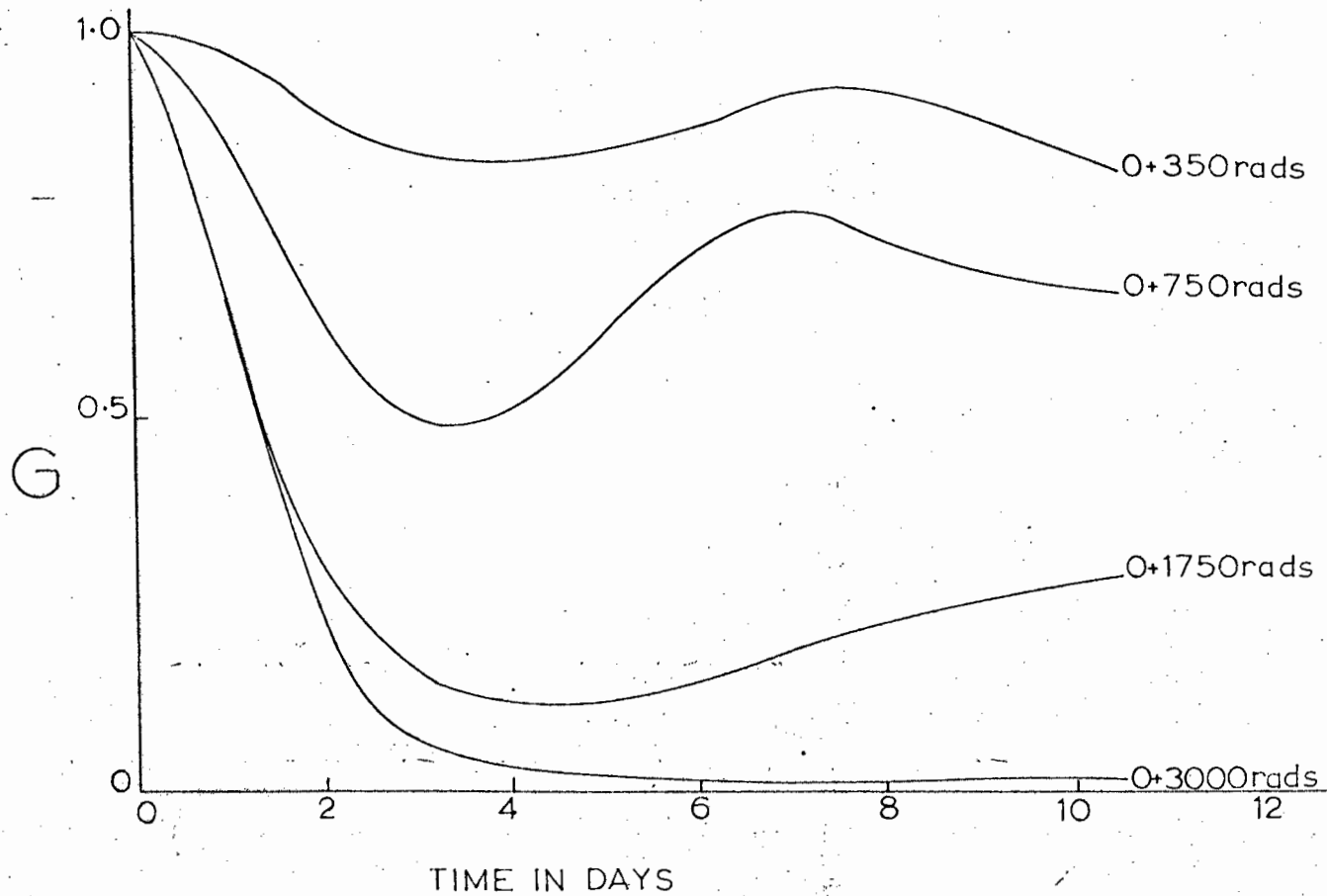


FIGURE 6.20

AS FOR FIGURE 6.12, BUT WITH FIRST DOSES OF 400 AND 600 RADS AND WITH SECOND DOSES OF 200, 1000, AND 1800, AND 400 AND 1600 RADS RESPECTIVELY.

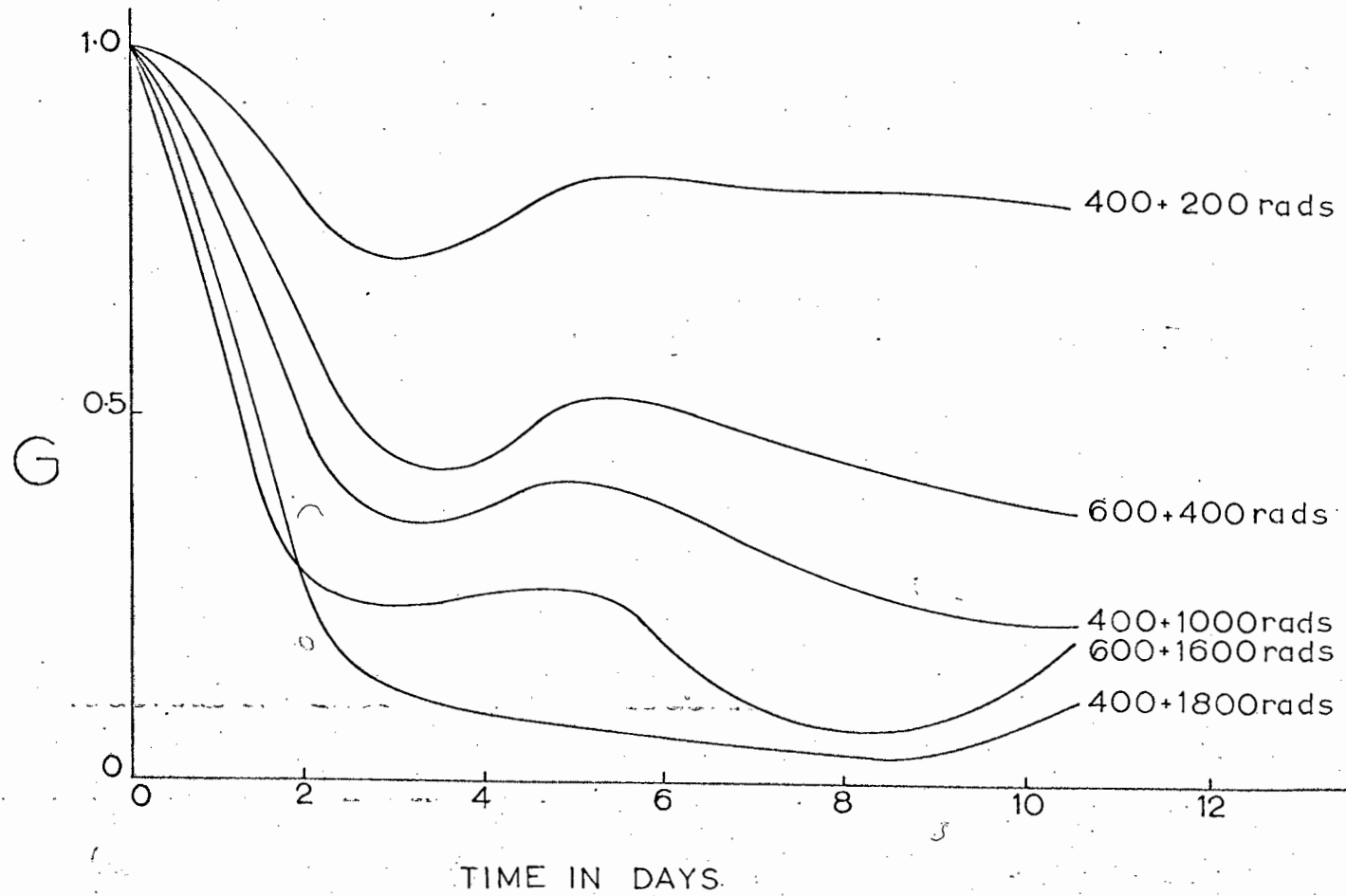


FIGURE 6.21

AS FOR FIGURE 6.12, BUT WITH FIRST DOSES OF 800 AND 1000 RADS AND WITH SECOND DOSES RANGING FROM 400 TO 1400 RADS, AND 200 TO 1800 RADS RESPECTIVELY.

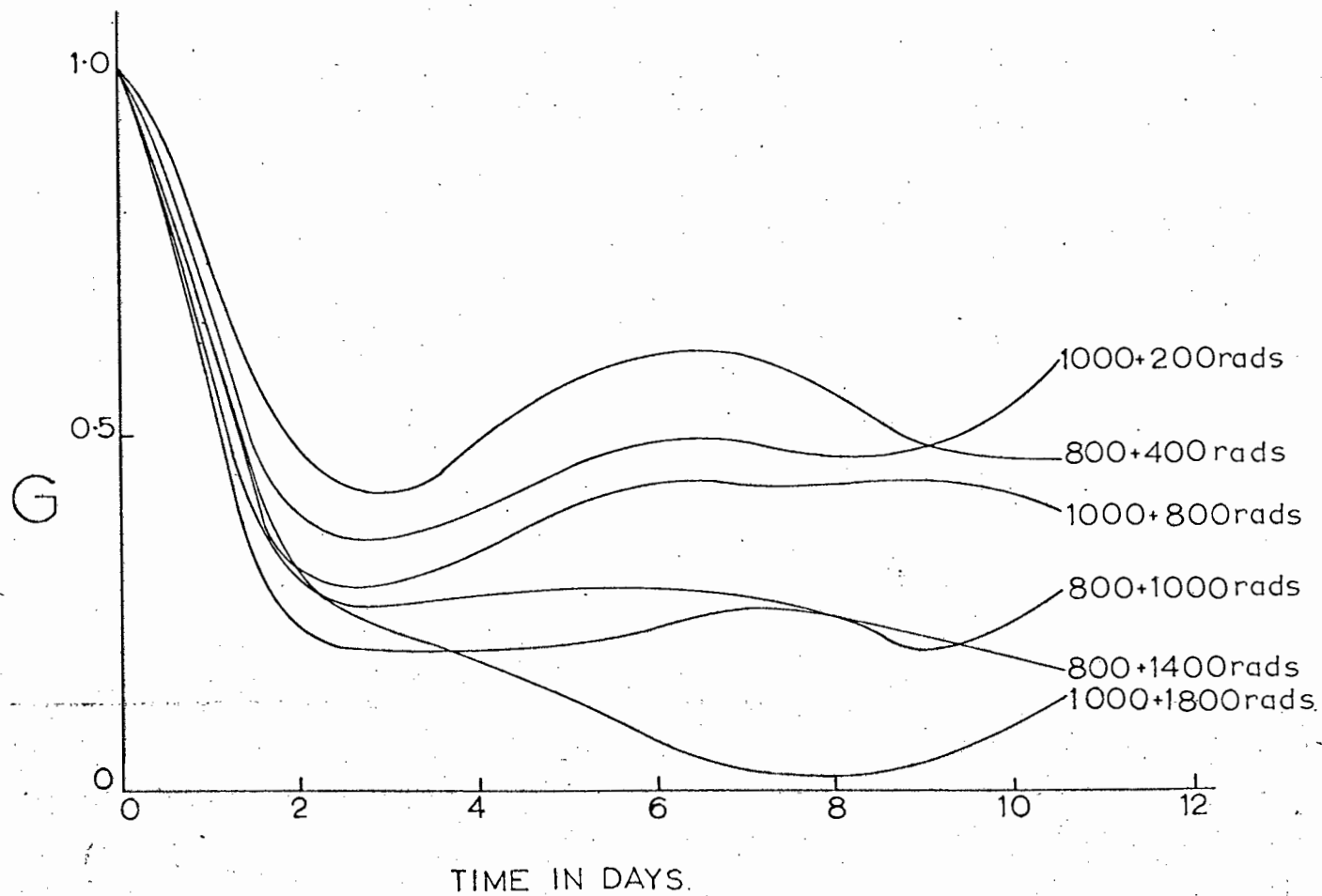


FIGURE 6.22

AS FOR FIGURE 6.12, BUT WITH FIRST DOSES OF 1200 AND 1400 RADS AND WITH SECOND DOSES OF 400 AND 800 RADS, AND 800 AND 1200 RADS RESPECTIVELY.

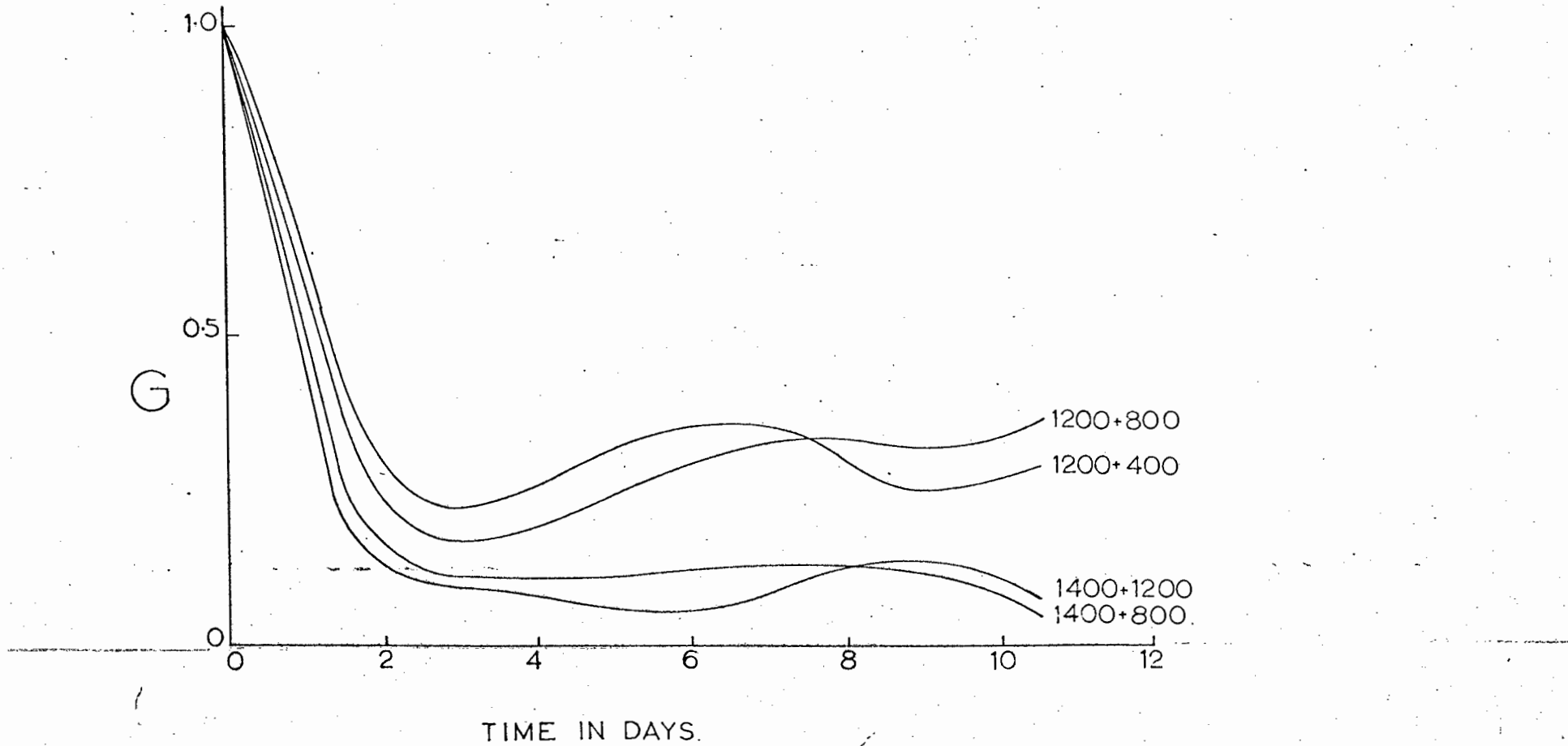


FIGURE 6.23

AS FOR FIGURE 6.12, BUT WITH FIRST DOSES OF 1600 AND 1800 RADS AND WITH SECOND DOSES OF 200 AND 1000 RADS, AND 1000 RADS RESPECTIVELY.

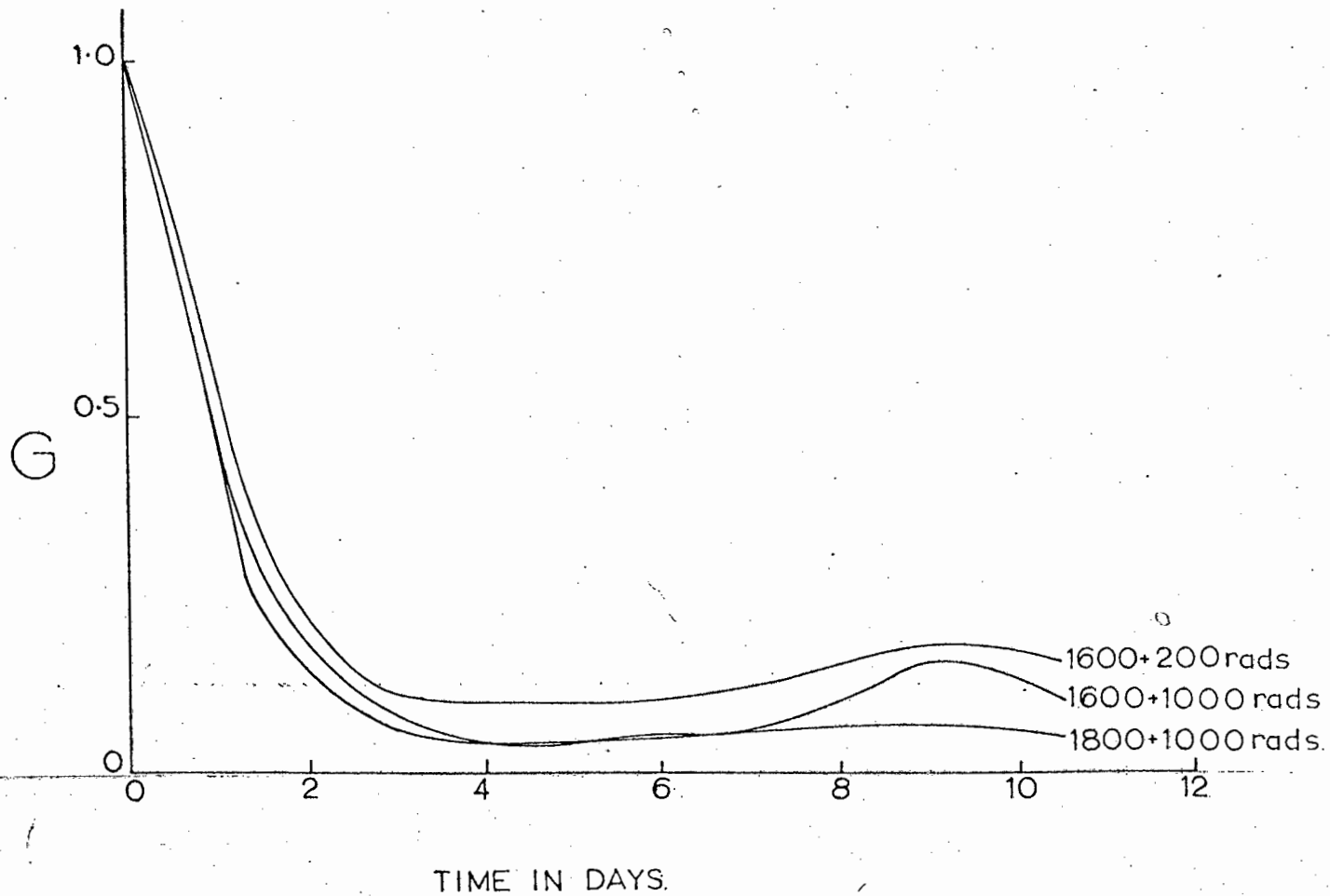


FIGURE 6.24

THE TOTAL DOSE OF RADIATION NECESSARY TO REDUCE THE "GROWTH IN TEN DAYS" TO 0.25, WHEN THE RADIATION IS GIVEN IN TWO DOSES SEPARATED BY AN INTERVAL OF SEVEN HOURS, PLOTTED AGAINST THE SIZE OF THE FIRST DOSE.

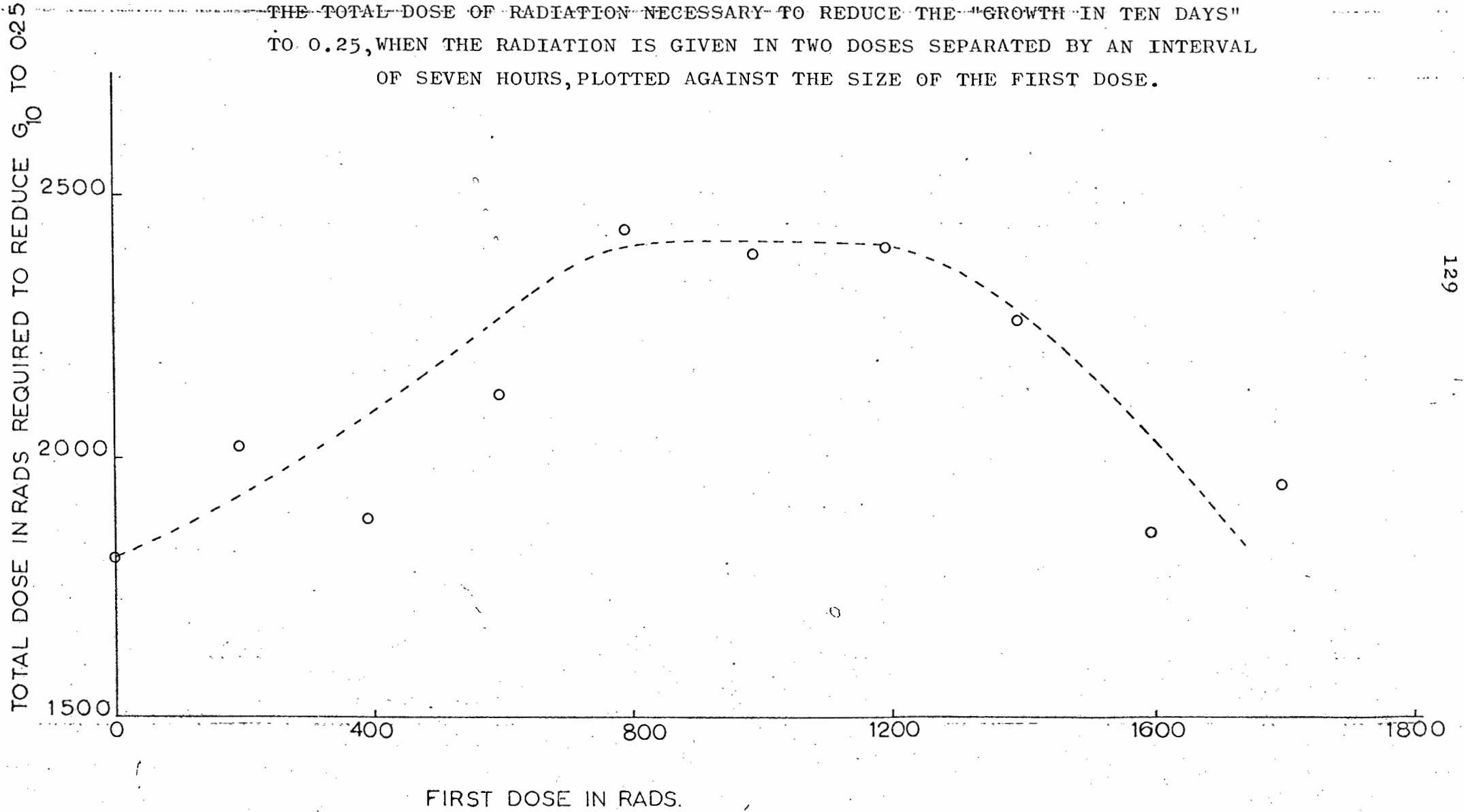


TABLE 6.1.

Two equal doses of 1000 rads, 25°C.

Interval between doses (hrs.)	'Growth in 10 days' (G ₁₀)	'Minimum growth rate' (G _{min})
0	0.124 ± 0.010	0.024 ± 0.004
1	0.184 ± 0.021	0.066 ± 0.012
2	0.187 ± 0.027	0.092 ± 0.025
3	0.207 ± 0.035	0.110 ± 0.018
4	0.236 ± 0.020	0.135 ± 0.015
5	0.285 ± 0.031	0.150 ± 0.014
6	0.238 ± 0.024	0.125 ± 0.013
7	0.212 ± 0.030	0.118 ± 0.018
8	0.232 ± 0.028	0.125 ± 0.018
9	0.238 ± 0.034	0.132 ± 0.014
10	0.243 ± 0.025	0.147 ± 0.027
11	0.254 ± 0.025	0.160 ± 0.017
12	0.206 ± 0.019	0.152 ± 0.028
13	0.213 ± 0.016	0.146 ± 0.032
14	0.197 ± 0.021	0.120 ± 0.031
15	0.202 ± 0.025	0.110 ± 0.027
16	0.176 ± 0.018	0.105 ± 0.021
17	0.186 ± 0.028	0.067 ± 0.018
18	0.151 ± 0.012	0.045 ± 0.022
19	0.146 ± 0.014	0.055 ± 0.0.7
20	0.151 ± 0.014	0.056 ± 0.015
21	0.161 ± 0.027	0.060 ± 0.016
22	0.137 ± 0.019	0.053 ± 0.021
23	0.138 ± 0.018	0.060 ± 0.013
24	0.135 ± 0.015	0.054 ± 0.010

TABLE 6.2.

Interval of 7 hours between doses, 25°C.

First dose (rads)	Second dose (rads)	Mean growth increment in ten days (cm)	"Growth in ten days."
Controls 400	-	33.67 ± 3.76	1.000
	200	27.39 ± 2.31	0.813 ± 0.114
	400	23.99 ± 2.77	0.713 ± 0.114
	600	15.96 ± 2.33	0.474 ± 0.087
	800	17.02 ± 1.94	0.506 ± 0.081
	1000	14.25 ± 1.37	0.423 ± 0.062
	1200	10.91 ± 0.59	0.324 ± 0.035
	1400	9.82 ± 1.04	0.292 ± 0.045
	1600	7.20 ± 0.76	0.214 ± 0.033
	1800	6.22 ± 0.60	0.185 ± 0.027
Controls 800	-	31.62 ± 2.62	1.000
	200	25.20 ± 2.12	0.797 ± 0.094
	400	17.89 ± 1.67	0.566 ± 0.071
	600	16.83 ± 1.82	0.532 ± 0.073
	800	12.51 ± 1.14	0.396 ± 0.049
	1000	10.90 ± 0.73	0.345 ± 0.037
	1200	9.70 ± 0.96	0.307 ± 0.040
	1400	9.95 ± 1.06	0.315 ± 0.042
	1800	7.69 ± 1.42	0.210 ± 0.040
	2200	7.85 ± 1.24	0.214 ± 0.035
Controls 1200	-	35.40 ± 0.97	1.000
	200	12.93 ± 0.98	0.365 ± 0.029
	400	12.77 ± 0.79	0.361 ± 0.024
	600	13.24 ± 1.06	0.354 ± 0.032
	800	11.60 ± 0.83	0.328 ± 0.025
	1000	10.59 ± 0.82	0.299 ± 0.025
	1400	7.75 ± 0.92	0.211 ± 0.027
1800	6.63 ± 0.69	0.181 ± 0.020	

TABLE 6.3.

The total dose in rads required to reduce the "growth in ten days" to 0.25 when X-rays are given in two doses separated by seven hours.

First dose (rads)	Total dose range (rads)	Total dose in rads to reduce the "growth in ten days" to 0.25.
0	200 - 3000	1800
200	400 - 2200	2016
400	600 - 2200	1872
600	800 - 2200	2112
800	1000 - 3000	2432
1000	1200 - 2800	2384
1200	1400 - 3000	2400
1400	1800 - 2600	2256
1600	1800 - 2600	1840
1800	2000 - 2800	1958

CHAPTER VII.DISCUSSION.Control roots.

The growth rate (cm/day) of the control group of roots is not constant but decreases steadily throughout the course of an experiment as shown in Figure 6.5. This may be due to a failure in nutrition, but may also have to do with the inhibition of growth due to the change in the concentration of the root hormones present.

As was pointed out earlier in this thesis (Chapter V), 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 the 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 more satisfactory especially where an open culture system is used. This latter procedure has been followed in the present studies, and Figure 6.5 shows that the growth of the controls is maintained fairly satisfactorily over the period of the experiment.

The comparison of the growth of the roots in the continuous irradiation jig with their growth in the main culture tank revealed a slightly larger growth rate for the roots in the latter (Figure 6.4).

Gray and Scholes (1951) circulated water through their growing tank in a closed system, aerating and controlling the temperature of the water, but not changing it during an experiment. This is a basic difference in technique to the one used for culturing the roots in the main culture tank in the present experiments.

This difference in technique is reflected in the growth rate of the roots (Hall, Lajtha and Oliver, 1962). They observed that the roots grown in the closed system showed a much more rapid fall-off of the growth rate as the length of the root increased. This has been attributed to the possible shortage of trace elements which are gradually exhausted in the closed system; when growing seedlings of Vicia in distilled water, Davidson (1960, a) found it necessary to add Hoagland's solution daily to supply the essential minerals.

When roots are grown in the compartments of the continuous irradiation jig which contain a volume of water much less than that of the main culture tank, the possibility of a

depletion of trace elements arises here as well. The flow rate of water through the compartments can not be increased to any level as a large volume of water passing through will make a proper temperature control impossible.

A correction factor to allow for this discrepancy was introduced when the curves were plotted for comparison with the theoretical growth curves. Such comparisons also necessitate a change in the time base for the experimental curves from "days" to "cell cycles", since the theoretical curves show the variation of growth rate as a fraction of controls of equal age with the number of cell cycles after the onset of the radiation. Here it is assumed that a constant cycle time of 19 hours (which will be derived later in the chapter) applies for Zea cultured at 25°C.

Continuous irradiation experiments.

Experimental growth rate curves.

Figures 6.1, 6.2 and 6.3 indicate the pattern of growth rate for roots of Zea exposed continuously to gamma radiation for a period of 15 to 20 days. At dose rates of between 0.4 and 42.8 mrads/hour, the growth rate increases initially and then after a day or two falls steadily until the majority of the roots cease to grow, and the plants die.

There is little indication of the growth rate attaining a steady but reduced value, as was observed for Vicia faba by Hall (1963).

A qualitative formula can be put forward to indicate the processes of recovery and loss of cells in an irradiated meristem.

Thus:

Growth rate = f (recovery of cells from sublethal radiation damage + recovery of cells due to the homeostatic system - loss of cells due to radiation sterilization - loss of cells due to "natural" causes),

where f stands for "function of".

Thus the reduction in growth rate which results directly or indirectly from the killing of some of the dividing cells in the meristem (Lea, 1946), is due to radiation sterilization of meristematic cells as well as death as a result of the natural ageing of the root. There is unfortunately no way of distinguishing the latter from cell death due to radiation sterilization.

The decrease in the growth rate of unirradiated roots of Zea with time (Figure 6.5) is very much more rapid than in the case of Vicia (Shepstone, 1964). This rapid death of the roots of Zea can account for the growth rate of the irradiated roots not reaching a marked steady value, the recovery of the system not being able to compensate adequately for a "natural" death in addition to a radiation death.

When corrections are made to the curves in Figures 6.1, 6.2 and 6.3 to account for the lengthening of the cell cycle time for the cells in the irradiated meristem as well as for the difference in growth rate observed for the control and irradiated groups of roots, as will be described later in this chapter, curves are obtained (Fig. 7.9 to 7.19), some of which show plateau regions. This means that for the continuously irradiated roots of Zea a steady state can be

reached (if a correction is made for the accelerated natural death of the roots grown in the continuous irradiation jig) at which the number of cells differentiating remains constant and thus a better correspondence is obtained (as will be described later) between experimental and theoretical growth curves, the latter being utilized to suggest possible modes of action of radiation on cells in the meristem.

Hall (1963) also exposed the roots of Vicia to gamma-radiation at dose rates low enough to produce only a slight decrease in growth rate. This dose rate (0.32 rads/hour) is higher than the largest dose rate (0.17 rads/hour) used on Zea in the present experiments. To reduce the growth rate of the roots of Zea in a similar way, a very low dose rate would be needed which was found to be not feasible, considering the lowest activity per Radium needle available and the limited sensitivity of the thermoluminescent powder used for the measurement of the radiation dose.

For the higher dose rates, viz. 85.6 and 171.2 mrad/hour, no initial rise in growth rate was observed. At the dose rates of the order of 171.2 mrad/hour the decrease in growth rate is much more rapid than in the case of the lower dose rates, since the proportion of cells sterilized per cell cycle will be much larger in the case of the high dose rates. A higher dose rate also implies that the lengthening of the cycle time will be even further enhanced (Clowes and Hall, 1962, 1966), which in turn means a larger dose per cell cycle and a correspondingly larger proportion of dying cells.

Theoretical growth rate curves:

Figures 7.2, 7.3 and 7.4 show theoretical growth rate curves computed for various values of P (the proportion of cells sterilized in one tenth of a cell cycle) and the results using hypotheses of Model A and Model B are compared.

Here it is assumed that cell death occurs at the second mitosis after radiation damage and that the cell cycle time (T) is constant.

The theoretical curves on the basis of these assumptions have been considered first since cell death at low dose rates is believed to occur only after two or more post-irradiation mitoses (Puck and Marcus, 1956). These authors studied the effect of X-rays on HeLa cells in tissue culture. They observed that the cells were able to divide two or more times after receiving single doses of X-rays of the order of 50 rads.

The lengthening of the cell cycle for the low dose rates used in the present experiments is not believed to be appreciable when compared with the change in cycle time for the very much higher dose rates dealt with by Clowes and Hall (1962, 1966). The effect of an increase in the cell cycle time on the theoretical growth curves will, however, be considered later in this chapter.

The abovementioned figures represent the theoretical curves of daily growth rate as a fraction of controls with:

- (a) the dying cells not contributing to root growth (G vs. Cell Cycles in Figure 7.2)
- (b) the dying cells differentiating and thus contributing to root growth (G' vs. Cell Cycles in Figure 7.3) and

(c) the dying cells dividing and each thus contributing two cells to root growth (G'' vs. Cell Cycles in Figure 7.4).

From the above comparisons it follows that Model A tends to produce initial peaks which are lower than those produced by Model B, but the plateau regions for the curves using Model B are only at a slightly higher level than those for Model A. Model B however, seems to be more plausible since in this model an attempt is made to provide the right type of feedback control for the repopulation of the meristem after radiation sterilization of some of its cells, on the basis of a possible biological response to the population change.

For the calculation of the growth curves as shown in Figures 7.2, 7.3 and 7.4 for Model A and Model B, it is assumed that all sterilized cells "die" at the second mitosis after receiving radiation damage. Therefore the growth rate remains constant for the first cell cycle after the start of exposure, after which the value falls more or less rapidly according to the damage level assumed.

In Figures 7.5 and 7.6 these theoretical curves for Model B are compared with growth-curves computed assuming that the sterile cells "die" at their first division subsequent to radiation damage.

This possibility was considered since the experimental growth curves (Figure 6.1 and 6.2) showed an increase in growth rate above that of the control group as soon as the first day after onset of the radiation. For the higher dose rates the decrease in growth rate (Figure 6.3) was observed from the first day onwards, indicating an immediate response of the root to the radiation.

The growth rate for the theoretical growth rate curves assuming cell death at the first mitosis after radiation damage, is seen to increase (Figure 7.5) or decrease (Figure 7.6) from the beginning of the exposure to the radiation, as would be expected since the differentiating cells are contributing towards root growth at an earlier stage and the dying cells are removed sooner than would be the case if cell "death" occurred at the second mitosis. The general form of the computed curves is identical in both cases.

It has been pointed out (Clowes and Hall, 1962;1966) that there is experimental evidence of an increase of the cell cycle time (T) under conditions of continuous irradiation at any but the lowest dose rates (Table 2.1, Chapter II).

In Figures 7.7 and 7.8, therefore, curves are shown which have been computed for two values of P , assuming a gradual increase in cell-cycle time during irradiation of the order of that discussed below. These calculations take into account the corresponding increase in radiation damage per tenth of a cell-cycle and the change in time scale relative to the earlier calculations (which assumed a constant cell cycle time), which latter curves are repeated in Figures 7.7 and 7.8 for comparison.

These curves were calculated for an irradiation period of 12 cell cycles, as the data on the increases in cell cycle time for Zea is only available for an irradiation period of seven days.

As would be expected, the correction for an increase in cycle time leads to continuously falling growth-rate curves, since

the lengthening of the cell cycle time implies a larger dose per cell cycle, which in turn means an increase in damage per cell cycle. The difference between the curves for constant T and for variable T , decreases as P increases. For $P = 0.09$ no difference between the two curves is observed. At the higher dose rates the number of cells dying is much larger than for the lower dose rates and therefore the effects of an increase in cycle time will be masked in the case of the high dose rates.

For the unirradiated meristem the cycle time of these cells in the case of Zea cultivated at 25°C can be taken as approximately 19 hours. This value is derived from a consideration of the work of Evans and Savage (1959) on the cycle time of Vicia roots at different temperatures and of Clowes (1965) who deduced the cycle time in different regions of the meristem (Table 2.1, Chapter II).

Here it is assumed that the cycle time of Zea shows the same percentage decrease as in the case of Vicia for a given increase in temperature (19°C to 25°C). This assumption is considered reasonable since the internal structure of the root tip of Zea closely resembles that for Vicia as is described in Chapter III, and thus their behaviour at different temperatures can be expected to be similar.

Also 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, because they constitute the major proportion of the meristem.

Assuming that the above reasoning also applies for cells under continuous irradiation, we can derive a cycle time of

42 hours for those cells subjected to 2.44 rads/hour for 7 days at 25°C.

The maximum dose rate in the present experiments on Zea was found to be about 0.17 rads/hour and assuming that the percentage change in cycle time is similar to the case of Vicia it was decided that for Zea the cycle time should change by 1.6 hours in 7 days at 25°C.

Comparison of experimental and theoretical growth curves:

In Figures 7.9 to 7.15, the curves computed on the basis of both models (with cell death occurring at the first and second mitosis respectively, and a constant cell cycle time) are compared with some of the experimental results.

Although the fit to the experimental curves is by no means absolute, the computed curves (on the basis of the assumption that cell death occurs at the second mitosis) have nearly the correct shape with respect to the observed results.

Thus it would seem that under these conditions of irradiation the majority of cells in the meristem are able to undergo at least one successful division after sterilization.

Any remaining discrepancy could be ascribed to the fact that some of the cells could also die in the first mitosis following the lethal radiation event, to variation in cell cycle and to the possibility of cell cycle delay.

At the higher dose rates, namely 85.6 and 171.2 mrad/hour, it seems that sterilized cells do not contribute to the growth rate, but are removed from the population.

At intermediate and low dose rates, however, the mathematical models demonstrate that the initial "hump" observed in the experimental results can possibly be explained if the damaged cells remain and contribute to the growth rate. The case of damaged cells also being able to divide yields initial peaks on the basis of the models which fit the observations in some cases. Thus both hypotheses, that 'dead' cells themselves may differentiate, or divide and differentiate and thus contribute to root-growth, provide theoretical growth-curves of the correct general form.

It is possible that a large number of reports on the stimulatory effect of chronic irradiation outlined in Chapter II may well be due to the continued presence of damaged cells which have been impaired with respect to their capacity for division. However, the effect of radiation on plant hormones must also be considered in this respect. As Skoog (1951) and Kiefer (1965) have pointed out, relatively small doses of X-rays reduce or inactivate auxin production, and a development of lateral buds are promoted by irradiating root tips. However, as a reduction in auxin concentration will stimulate shoot meristematic growth, it is believed to have the opposite effect in roots (Meyer and Anderson, 1949), and therefore it seems as if this possibility may be eliminated.

The effect is also known in mammalian systems. Lorenz and his colleagues (1947) have studied the effect of long - continued gamma irradiation on mice, guinea pigs and rabbits, and found an increase in weight of all irradiated animals at the lower exposure levels (1.1 and 2.2 r per day),

amounting in male mice exposed to 1.1 r per day to 50 per cent of that of control animals. It is possible that the same factors are operative here as in the case of plants.

Another possible explanation of the stimulatory effect of radiation could be put forward on the basis of the presence of "giant" cells which have been observed in irradiated mammalian tissues. In these cells the chromosomes have divided but no physical division into two daughter cells has taken place and a single large cell which has lost its reproductive integrity is produced. Puck and Marcus (1956) have, however, found that only 6.7% of a colony of cells irradiated to 50 r had transformed into giant cells.

No production of giant cells has been observed for the meristematic cells of Vicia for a few days after the onset of continuously applied radiation (Shepstone, 1964).

Squashes prepared from the meristematic cells of Zea irradiated to the dose rates at which a stimulatory effect has been observed, have not revealed the presence of any giant cells, (Figure 7.16).

When cells are irradiated, the cell membrane will inevitably be effected. Changes in the amount of charge on the cell membranes have been observed only for doses of radiation of more than 600 rads, thus indicating a change in the chemical composition of the membrane (Repacholi, 1970). For the membranes to be damaged to such an extent as to allow the imbibition of water and thus resulting in an increase of the size of the cell, much higher doses will most probably be required. At the low doses of radiation applied to the roots at which stimulation of growth was observed the

possibility of an increase in the size of the cells due to the imbibition of water can thus be excluded.

The possibility of a change in the response of the cells to the radiation with time after the onset of the radiation was considered in matching theoretical and experimental growth curves as shown in Figure 7.17, where the experimental growth curve (full line) for an applied dose rate of 0.4 mrads per hour is compared with the theoretical results of Model A, with the assumption of a constant cell cycle time T and cell death occurring at the first mitosis after radiation sterilization.

Of importance here is the fact that if the radiation changes the response of the cells to the radiation after a couple of cell cycles it would imply that accumulated damage inflicted during one mitotic cycle can be considered as being passed on to the next. This would be in contradiction to Van't Hof and Sparrow's view that there is no effect to pass on to the next cycle.

If radiation damage can be "transferred" from one cycle to the next the reproductive integrity of the cell would be "reduced". Thus it was found reasonable for the comparison in Figure 7.17 to 'construct' the theoretical curve as follows. The initial growth rate larger than unity was taken from the curve calculated on the assumption that the "dying" cells divide and differentiate (dotted part of the theoretical curve in Figure 7.17). The dashed part of the curve in Figure 7.17 was obtained from the theoretical curve calculated on the basis that the "dying" cells would differentiate only.

For a given dose rate the value of P has to remain the same for both parts of the curve (the damage per cell cycle will remain the same for a constant dose rate and a constant cell cycle time - Hall, Oliver, Shepstone and Bedford 1966).

This method of comparing experimental and theoretical growth curves did not result in a better agreement between these curves.

In Figures 7.18, 7.19 and 7.20, the curves computed on the basis of both models, corrected for an increase in T, are compared with some of the experimental results. For a reason mentioned earlier in this chapter, this comparison can be made considering the first 12 cell cycles after the onset of the radiation only.

The effect of this modification is to change the gradients of the theoretical curves, but there is no appreciable change in the particular P-value of the theoretical curve which most closely approximates to a given experimental curve, and at the high dose rates (where the biggest variation in T occurs) the change is non-existent. It may therefore be deduced that the reduced growth rate is essentially the result of a reduction in the number of cells differentiating and the contribution from an increase in cell cycle time is not significant by comparison. This conclusion is in keeping with the findings of Van't Hof and Sparrow (1963), who found reduced growth rates in chronically irradiated root meristems of Pisum without any evidence of change in the minimum cycle time of the meristematic cells.

It is of some interest (Oliver and Shepstone, 1965) to consider the corresponding values of dose rate and the

parameter P for matching experimental and theoretical growth rate curves shown in Figures 7.9 to 7.15, in relation to the survival curves predicted by the calculations.

At such low dose rates it is to be expected that the relevant dose-response curve will be of the "single-hit" type, the rapid recovery of sublethal damage making any multi-hit damage process unlikely (Barendsen 1962, Hall 1963, Hall and Bedford 1964, Oliver 1964). The surviving fraction S following a dose D rads will therefore be given by;

$$S = \exp\left(-\frac{D}{D_1}\right) \quad (7.1)$$

where D_1 is the 37 per cent survival dose for the exponential curve corresponding to the "single-hit" process applying under these conditions.

In the calculations for the theoretical growth curves, P is the fraction of cells sterilized in one tenth of a cell cycle. For a dose rate d rads/hour and a cell cycle time of T hours the relationship therefore becomes:

$$P = 1 - \exp\left(-\frac{d \cdot T}{10 D_1}\right) \quad (7.2)$$

From the comparison of experimental and theoretical growth curves discussed earlier, a value of P was found for each value of the dose rate. These values of P together with the corresponding dose rates and the values of D_1 calculated from Equation 7.2 are tabulated in Table 7.1. The cycle time (T) was taken as 19 hours.

The values of D_1 found for Zea (Table 7.1) are very much less than those derived for Vicia, viz. 165 rads (Hall, 1963),

190 rads (Oliver, 1964) and 225 rads (Oliver and Shepstone, 1965). This means that the value of $1/D_1$, i.e. the dose to reduce the irradiated population of cells to 37% of their original number for the "single-hit" inactivation which is assumed to apply at the low dose rates used in the present experiments, is very much larger in the case of Zea as compared to Vicia. The fact that Zea will survive much higher doses of radiation than Vicia will be discussed later in this chapter.

Thus we have, in the circumstances, a reasonable agreement between experimental results and the application of a simple model system.

The hypothesis is put forward above that cells unable to divide as a result of radiation damage may, in fact, differentiate or even divide and differentiate and so contribute to root elongation. Theoretical curves of growth rate which take this into account are in better agreement with the experimental results and show the initial increase in growth rate relative to controls which is observed at low dose rates.

Fractionation Experiments.The growth curves.

Some of the growth curves for the root of Zea exposed to two equal doses of X-rays (1000 rads each) separated by time intervals in the range 0 to 24 hours are depicted in Figures 6.6, 6.7a, and 6.7b.

The graphs show the variation of the growth rate as a fraction of controls of equal age (G) with time. The growth rate is seen to fall progressively until a minimum is reached some 3 to 5 days later, to be followed by a gradual recovery.

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 (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 (dashed line in Figure 7.21). At the same time, the meristematic cells make an increasing contribution to the growth-rate (dotted line in Figure 7.21). 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.

In general, two parameters have been used by various workers to score radiation damage, viz. the "minimum growth rate" (G_{\min}) and the "growth in ten days" (G_{10}), as described in Chapter V. The exact relationship between these parameters and the proportion of meristematic cells which retain their reproductive integrity is not known (Hall, 1962); all that can be said is that the reduced growth of irradiated roots results directly or indirectly from the killing of some of the dividing cells in the meristem (Lea, 1946) and thus the above parameters can be used as a measure of radiation damage.

From the growth curves in Figures 6.6, 6.7a and 6.7b it follows that the minimum growth rate (G_{\min}) varies with the time interval between the doses, although the total dose given in each instance is the same.

Since G_{\min} is a measure of radiation damage, it follows that two doses of 1000 rads each separated by a time interval of 0 hours (which corresponds to a single dose of 2000 rads), produce more damage than when the irradiation is given in two fractions of 1000 rads each separated by a time interval of a couple of hours. This is attributed to the recovery of sublethal radiation damage as has been demonstrated for mammalian cells in culture (Elkind and Sutton, 1959) and also in the meristem of Vicia faba (Hall and Lajtha, 1963; Shepstone, 1964).

The variation of G_{\min} (for the roots of Zea exposed to two doses of X-rays of 1000 rads each) with the time interval between the doses, is shown in Figure 6.8. This curve shows an initial rapid rise corresponding to an increase in

recovery with an increase in the time between the doses. This is in keeping with the results of Elkind and Sutton (1959), Dewey and Humphrey (1965), Sinclair and Morton (1964, 1965) and Whitmore et al (1965), who have demonstrated that the repair of sublethal damage is prompt (i.e. within a few hours).

The recovery curves for equal split doses of radiation on mammalian cells (Elkind and Sutton 1959; Hornsey and Vattistas 1963) as well as those for Vicia (Shepstone 1964; Hall and Lajtha 1963) also show an initial rapid rise (Figure 2.2, Chapter II) as was observed in the case of Zea mentioned above (Figure 6.8), indicating partial recovery for small separations in time between the doses.

The curve in Figure 2.2 does, however, level off when the doses are separated by an interval of about 12 hours, indicating that the recovery of sublethal damage is at a maximum. The curve for Zea (Figure 6.8), instead of levelling off at a time of about 12 hours between the doses, decreases from this time onwards until a plateau region is reached when the time interval between the doses is about twenty hours. This plateau region is, however, at a level above the value for G_{min} corresponding to a single dose of 2000 rads (2 doses of 1000 rads each, separated by 0 hours). Thus a certain amount of recovery (on the plateau region of the recovery curve this will be due to the proliferation of the least sensitive cells in the meristem since the recovery of sublethal damage is complete at this stage - Johns and Cunningham 1969) does still take place if the doses are separated by a time of 20 hours and above.

This discrepancy could possibly be explained on the basis of the rapid ageing of the roots of Zea as compared to Vicia (Shepstone 1964) and the mammalian cells used by Elkind and Sutton (1959). 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).

The curve of G_{\min} vs. time between doses discussed above is not smooth, but contains a secondary maximum and minimum. This effect has also been demonstrated by Elkind and Sutton (1959, 1960) using Chinese hamster cells and by Shepstone (1964) and Hall and Lajtha (1963) for the meristematic cells of Vicia. Partial synchrony is produced by the conditioning dose and since surviving cells may progress through the cell cycle or repair damage or both at different rates, it is possible that fluctuations in the survival parameters (m and λ) will be observed in time after a conditioning dose (Elkind et al, 1961). This explains the "kink" in the curve depicted in Figure 6.8.

The variation of the 'growth in ten days' (G_{10}) for the roots of Zea exposed to two doses of X-rays separated by time intervals in the range 0 to 24 hours (Figure 6.9) shows a curve similar to the one of G_{\min} vs. time between doses as depicted in Figure 6.8. This correspondence between the two curves is to be expected since both G_{\min} and G_{10} are a measure of radiation damage.

The above results also verify that the sum of two doses, separated in time, must be greater than the single dose required to produce a postulated end-point. From theoretical considerations (Hornsey and Vatistas, 1963) it would be expected that when the first dose is small, so that the surviving fraction of a population of cells falls on the shoulder of the survival curve, only a small increase in the total dose Q would be necessary to reach a postulated end-point. As the first dose is increased, the total dose needed would increase until the first dose would be large enough to fall on the exponential part of the survival curve. The value of the total dose (Q) should then remain fairly constant until the first dose is sufficiently near to Q for a single dose of irradiation, that only part of the "shoulder" of the curve of the second irradiation would be needed. Shepstone's (1964) results on Vicia faba fit this theoretical pattern which has been introduced in Chapter II. In order to investigate the validity of this theory for Zea, the roots of the latter were exposed to a large range of conditioning and test doses.

Some of the growth curves for the roots of Zea exposed to two unequal doses of X-rays, separated by a time interval of 7 hours, are shown in Figures 6.11, 6.12 and 6.19 to 6.23. Here again there is an initial decrease in the growth rate as a fraction of controls of equal age. The curves then pass through a minimum and in the case of the lower total doses return nearly to pre-irradiation levels. At the lower total doses there are also 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 dependant phenomena play a very important part in the radiation response.

Hornsey and Vatistas (1963) have shown recovery curves for different values of the conditioning and test doses. All of these revealed a minimum in recovery at a time interval of 6 hours between the doses. In some cases, however, the plateau value was not yet reached at a time interval of 24 hours. Thus in the present experiments, Hornsey's example was followed and the time between the split doses was chosen to be 7 hours, corresponding to the minimum in recovery on the curves in Figures 6.8 and 6.9.

In Figures 6.10 and 6.13 to 6.18, the variation with the total dose of the "growth in ten days" (G_{10}) for the roots of Zea exposed to two unequal doses of X-rays separated by a time interval of 7 hours, is shown for various values of the conditioning dose ranging from 0 to 1800 rads in steps of 200 rads. From these graphs the total dose Q required to reduce the "growth in ten days" to 0.25, was read off. These values are tabulated in Table 6.3. The particular choice of the end-point does not effect the final results as long as it is on the exponential part of the dose - response curve.

Figure 6.24 shows the variation with the first dose of the total dose of radiation necessary to reduce the "growth in ten days" to 0.25, when the radiation is given in two doses separated by an interval of seven hours. This graph indicates that the behaviour of the meristematic cells of Zea

fit the abovementioned theoretical pattern as the total dose required to reduce G_{10} to 0.25 rises to a maximum and remains constant until it falls after a first dose of about 1200 rads.

The difference between the maximum value of Q for the split doses and the value of Q for the single dose of 2000 rads should be equivalent to D_Q , the quasi-threshold dose. From Figure 6.24 the value of D_Q was found to be about 620 rads for the meristematic cells of Zea.

Using values of 2 and 3.5 for the extrapolation number (m), as determined experimentally by Fenner (1970) and Shepstone (1964) on irradiating the roots of Zea to single acute doses of X-rays, values for the 37 per cent dose slope (D_0) equalling 895 and 495 rads respectively, were found using the formula $D_Q = D_0 \ln(m)$ as introduced in Chapter II. These values compare reasonably well with the values of 410 and 430 rads for D_0 respectively, found by the above authors.

However, it should be pointed out that at seven hours the increase in G_{min} is at a minimum and therefore the estimate of D_Q for Zea is a minimum value. If D_Q were measured at 5 hours or 10 hours, a higher value would be obtained, probably by 100 to 200 rads.

The values of D_0 for Zea are appreciably higher than those obtained by Hall and others (e.g. Hall, Lajtha and Oliver, 1962) for Vicia, which ranged between 35 and 90 rads on various estimates.

It is thus clear that Zea will survive much higher doses of radiation than Vicia, a conclusion in keeping with that

of Sparrow and Miksche (1961) who have found that the daily exposure required to produce severe growth inhibition in the case of Zea is 500 r, and in the case of Vicia is 270 r. According to Sparrow (1964) the mean DNA content per cell in Zea is about $1.8 \times 10^{-5} \mu\text{g}$, with a calculated nuclear volume of $230 \mu^3$. The equivalent values for Vicia are $4 \times 10^{-5} \mu\text{g}$ DNA per cell and $500 \mu^3$ respectively.

The difference in sensitivity between Zea and Vicia may therefore to a certain extent be explained on the basis of differences in nuclear volume and DNA content, the values in Zea being down by a factor of about two on those of Vicia. Sparrow has proved conclusively that plant species with big nuclei are very sensitive; species with small nuclei are relatively resistant.

The length of the mitotic cycle for the dividing cells in the unirradiated meristem has been estimated to be approximately 19 hours at 25°C , the temperature at which the experiments were carried out. The above equation relating D_Q and D_0 is based on the assumption that a repopulation of the meristem has not occurred before the second dose of radiation was given (Alper et al, 1962). After irradiation, the actively dividing cells in the meristem suffer a mitotic delay (Hornsey, 1956) and it is unlikely that repopulation will have occurred due to the division of these cells.

At higher doses the quiescent centre, which is less radiosensitive than the dividing cells in the meristem (Clowes, 1963 a,b,c; 1964 a) is assumed to play a more dominant role (Clowes, 1963 a,b) and may account for a certain amount of repopulation of the meristem as Clowes (1970)

has recently found that the cells of the quiescent centre of Zea respond at once by coming into mitosis when irradiated to a dose of 1800 rads.

According to Elkind and Whitmore (1967) the value of the extrapolation number found by irradiating a population of cells to single acute doses may in any case be a poor indication of the individual m-values in a heterogeneous population, as found in the root tip of Zea. These authors maintain that the extrapolation number can sometimes be approximated by the product of the fraction of the least sensitive group of cells in the population and the corresponding m-value for this group of cells. In the case of Zea this will correspond to the m-value for the cells in the quiescent centre which has not been determined because of the experimental complexity that would be involved in such an attempt.

TABLE 7.1

Fraction of cells sterilized in one tenth of a cell cycle. P	Dose rate (mrads/hour) d	37% survival dose (rads) D ₁
0.02	0.3	0.028
0.01	0.4	0.076
0.015	2.2	0.165
0.015	9.7	0.728
0.02	42.8	4.025
0.02	85.6	8.05
0.03	171.2	10.68

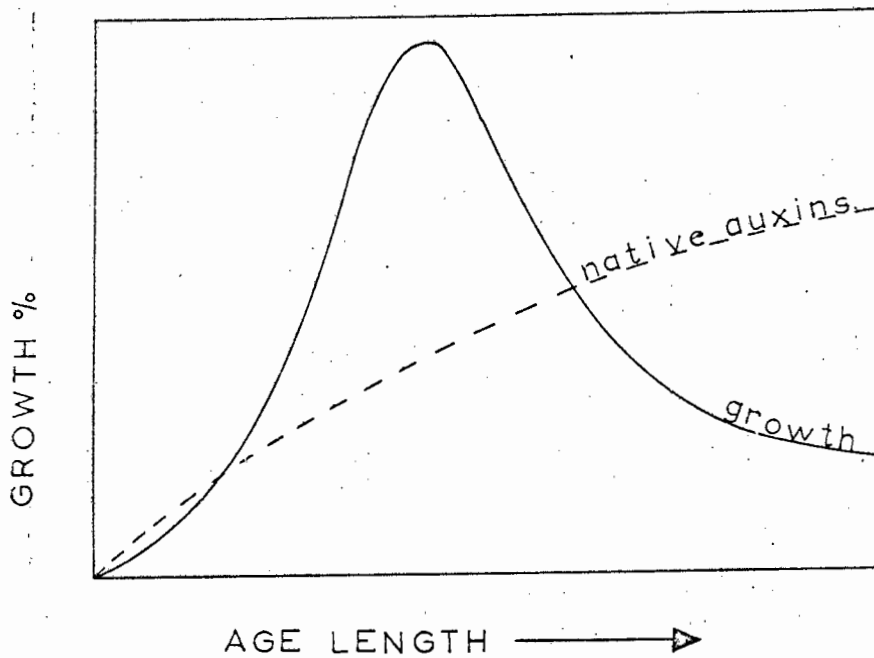


FIGURE 7.1

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 diagrammatically above.

FIGURE 7.2

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

----- MODEL A

———— MODEL B

ASSUMPTIONS: CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE, CONSTANT CELL CYCLE TIME, DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

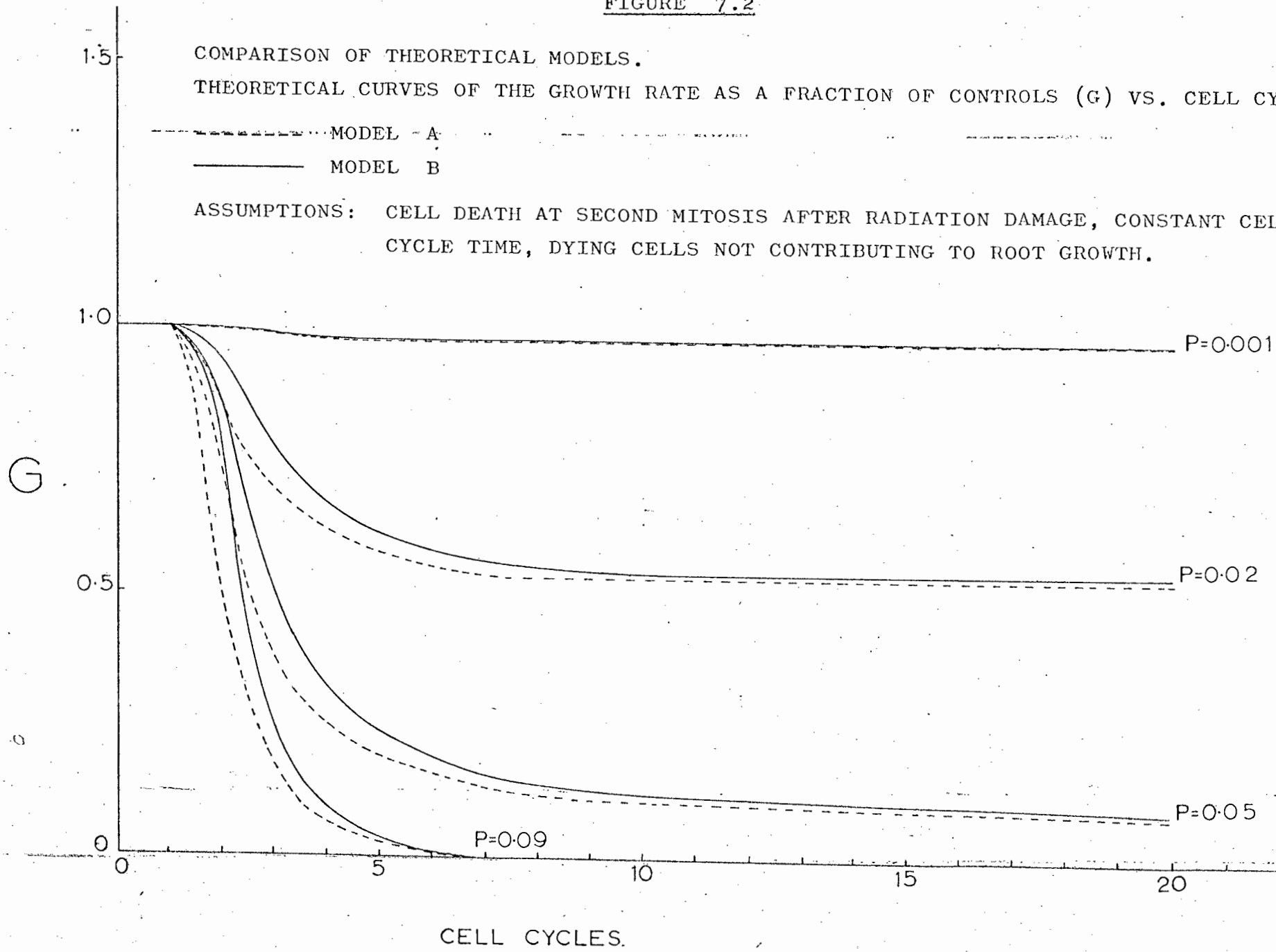


FIGURE 7.3

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G') VS. CELL CYCLES.

----- MODEL A

———— MODEL B

ASSUMPTIONS: CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE, CONSTANT CELL CYCLE TIME, DYING CELLS DIFFERENTIATING AND THUS CONTRIBUTING TO ROOT GROWTH.

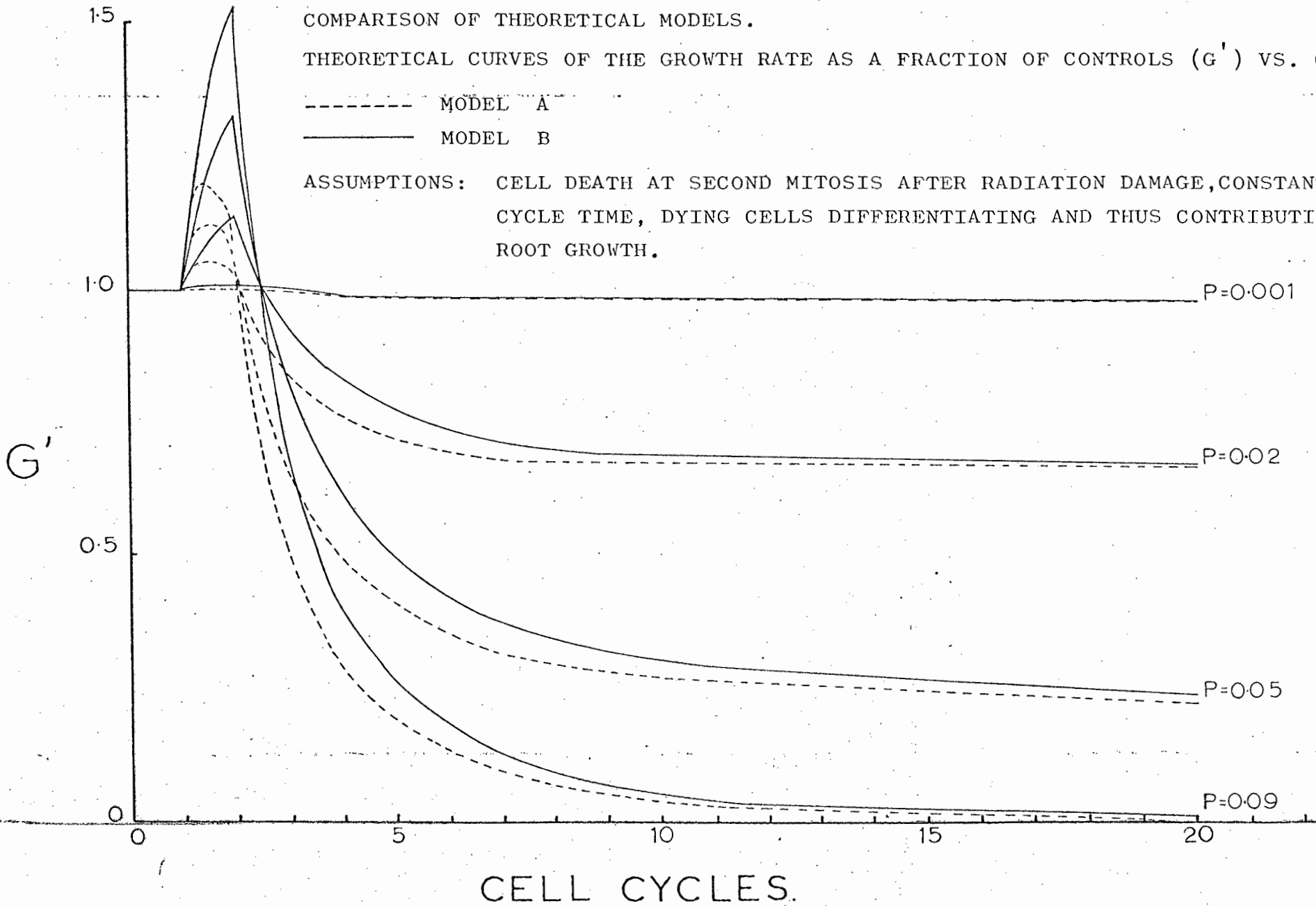


FIGURE 7.4

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G'') VS. CELL CYCLES.

----- MODEL A

———— MODEL B

ASSUMPTIONS: CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE, CONSTANT CELL CYCLE TIME, DYING CELLS DIVIDING AND BOTH DAUGHTER CELLS CONTRIBUTING TO ROOT GROWTH.

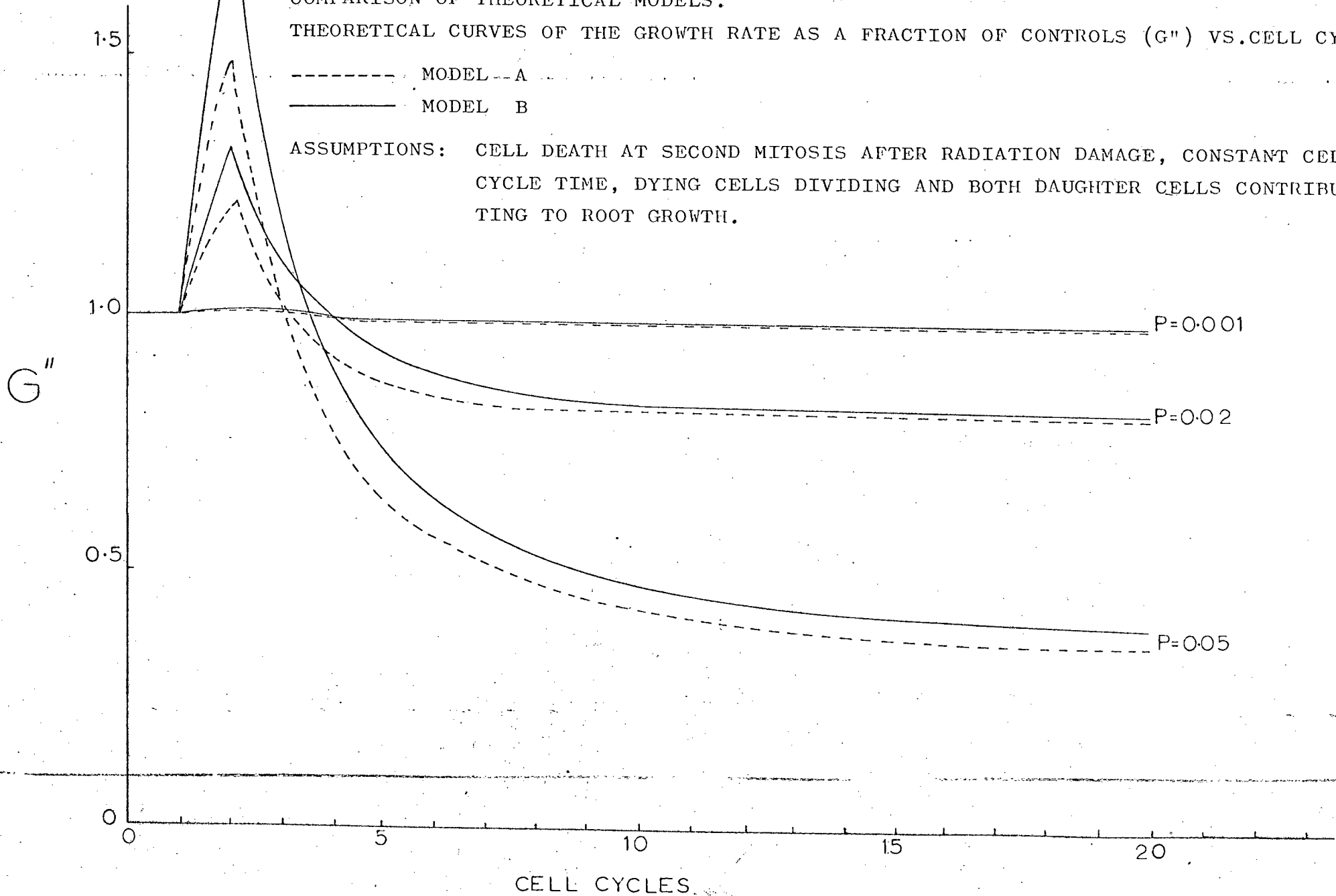


FIGURE 7.5

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G') VS. CELL CYCLES.

----- CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE.

———— CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL B, CONSTANT CELL CYCLE TIME, DYING CELLS DIFFERENTIATING AND THUS CONTRIBUTING TO ROOT GROWTH.

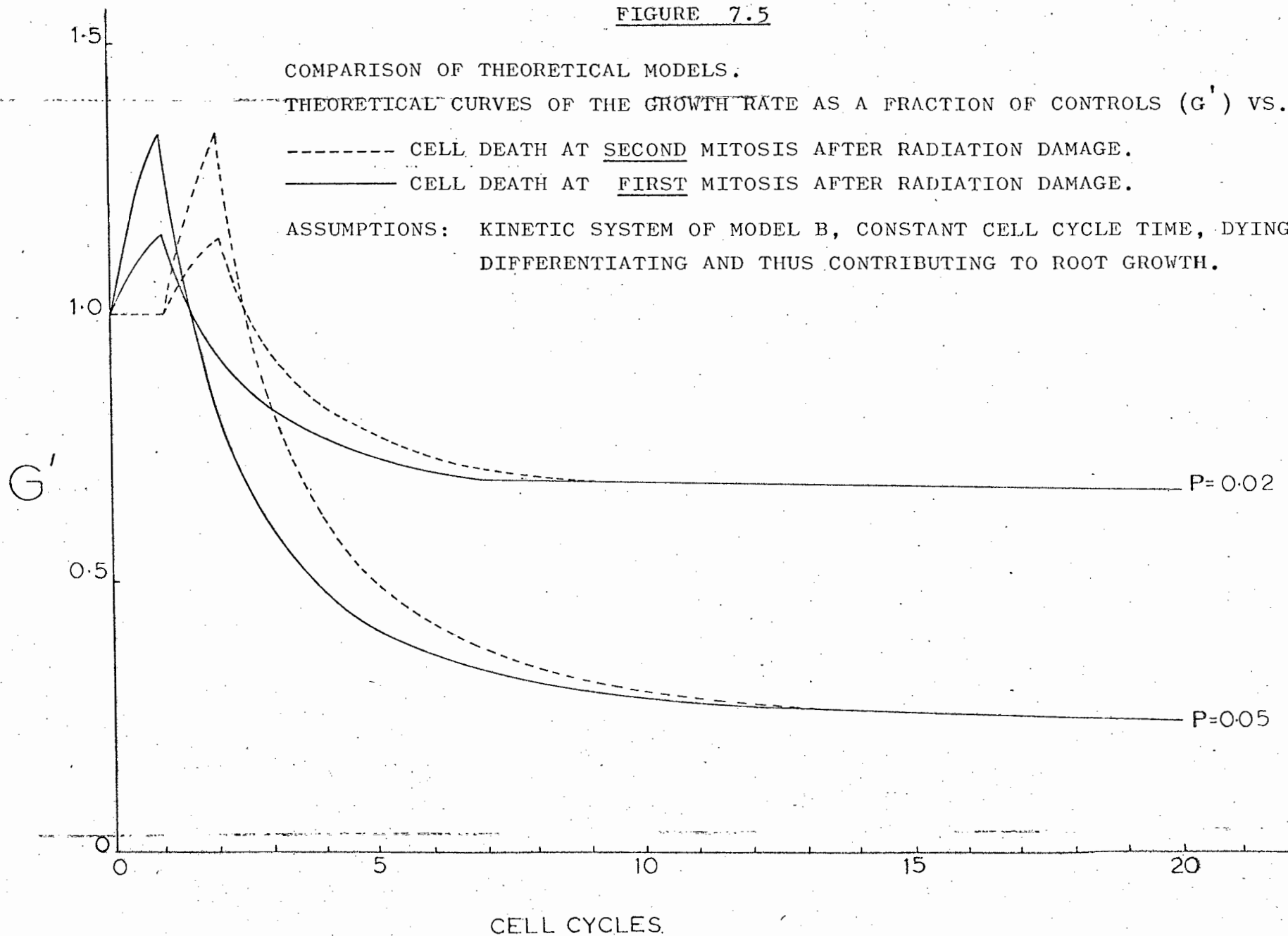


FIGURE 7.6

COMPARISON OF THEORETICAL MODELS.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

----- CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE.

———— CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL B, CONSTANT CELL CYCLE TIME, DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

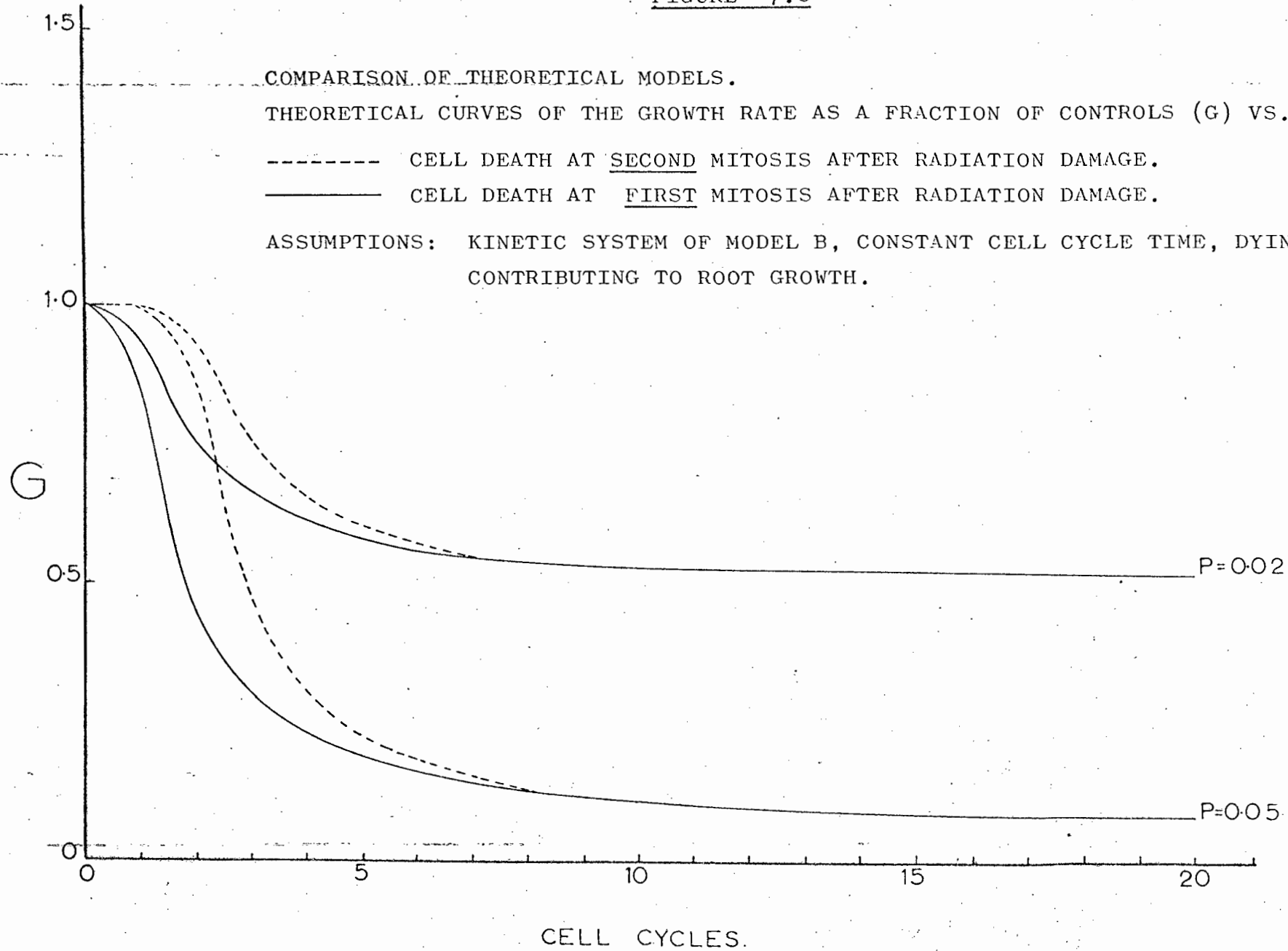


FIGURE 7.7

EFFECT OF INCREASE OF CELL CYCLE TIME UNDER IRRADIATION.

THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G') VS. CELL-CYCLES.

----- CONSTANT CELL CYCLE TIME

————— INCREASING CELL CYCLE TIME

P_0 = PROPORTION OF CELLS STERILIZED PER TENTH OF A CELL CYCLE AT THE ONSET OF THE RADIATION.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE, DYING CELLS CONTRIBUTING TO ROOT GROWTH.

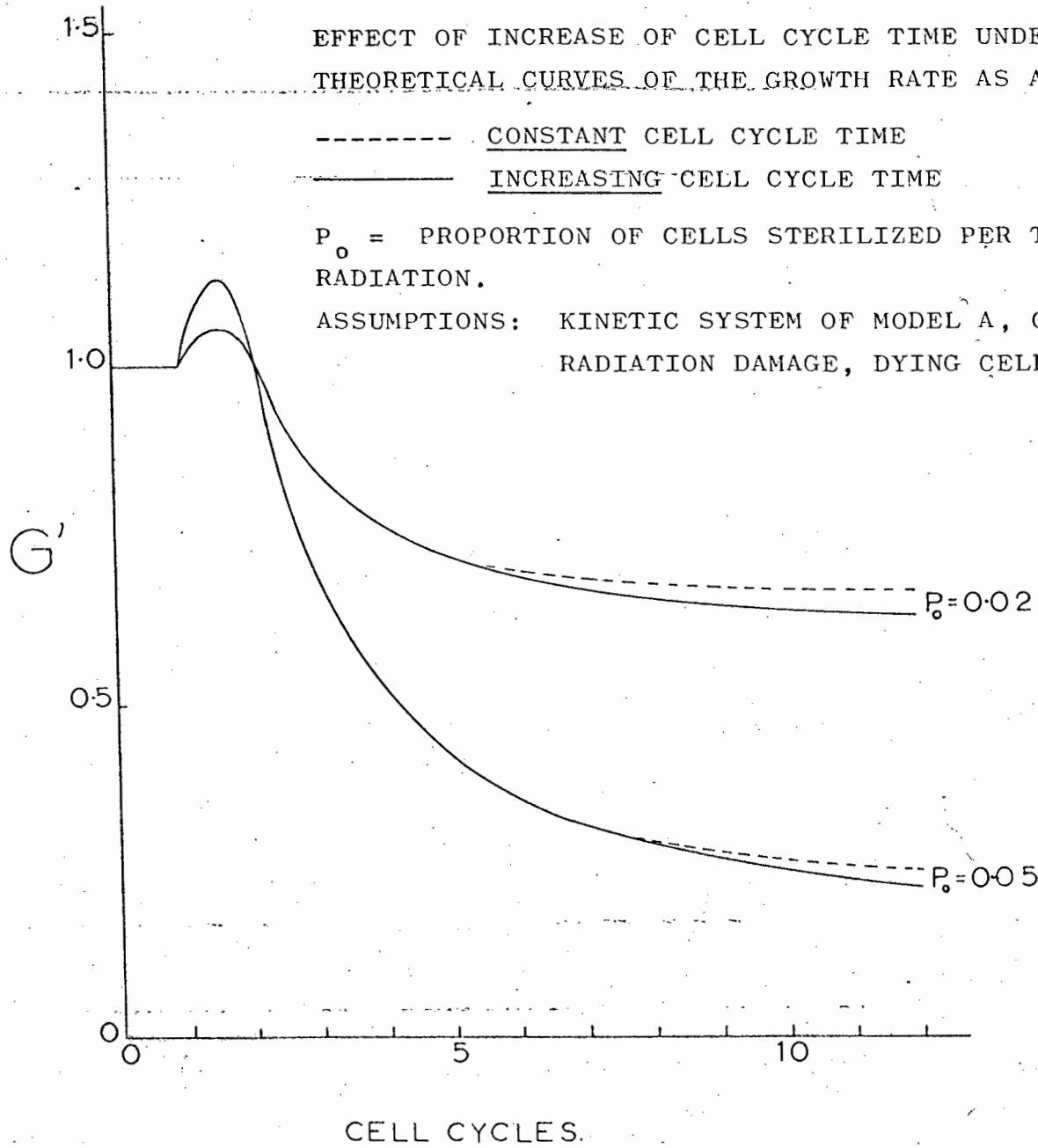


FIGURE 7.8

EFFECT OF INCREASE OF CELL CYCLE TIME UNDER IRRADIATION.
THEORETICAL CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

----- CONSTANT CELL CYCLE TIME
———— INCREASING CELL CYCLE TIME

P_0 = PROPORTION OF CELLS STERILIZED PER TENTH OF A CELL CYCLE AT THE ONSET OF THE RADIATION.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE, DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

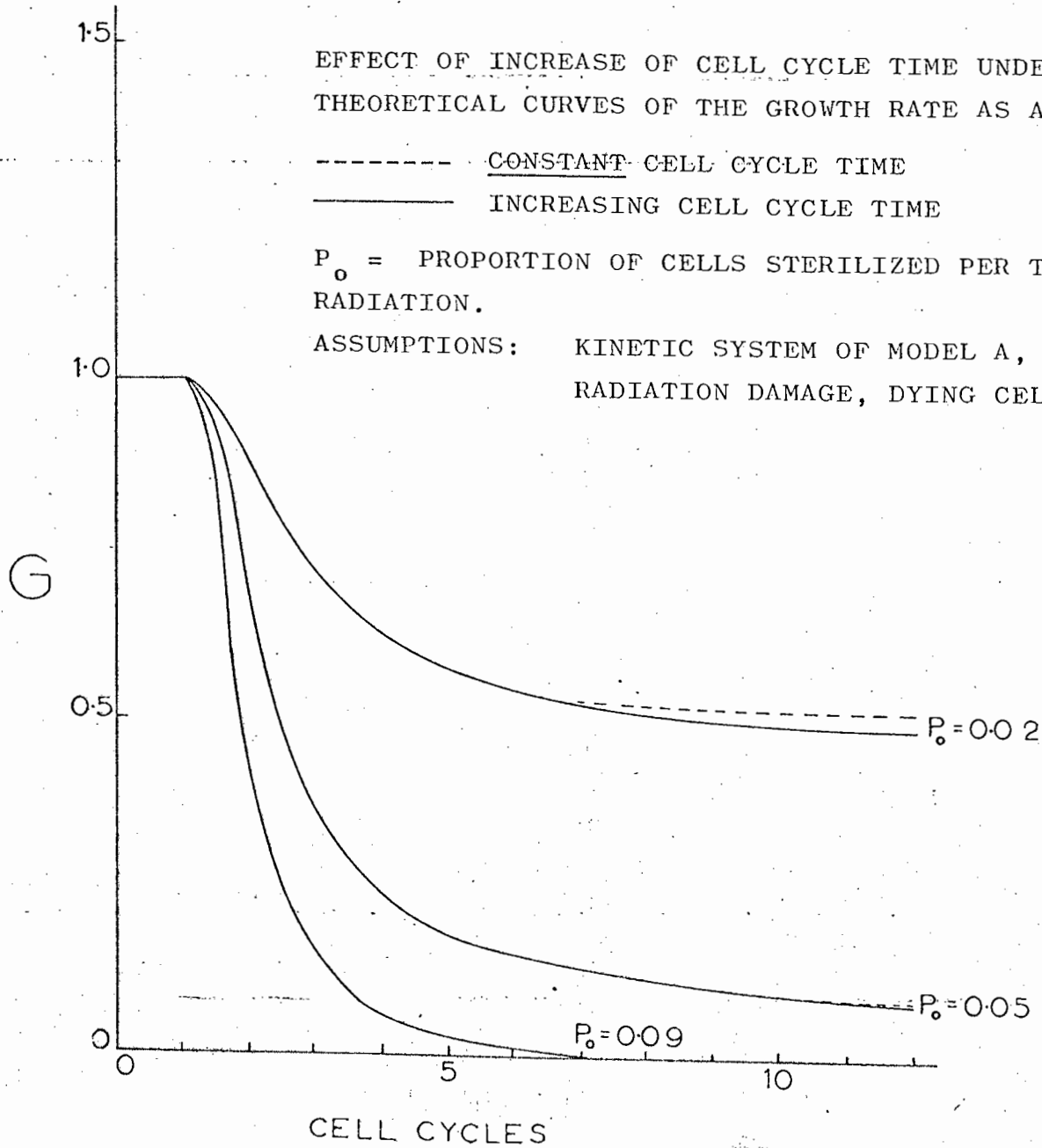


FIGURE 7.9

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

- EXPERIMENTAL RESULTS
- - - CALCULATED, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE
- CALCULATED, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CONSTANT CELL CYCLE TIME,
DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

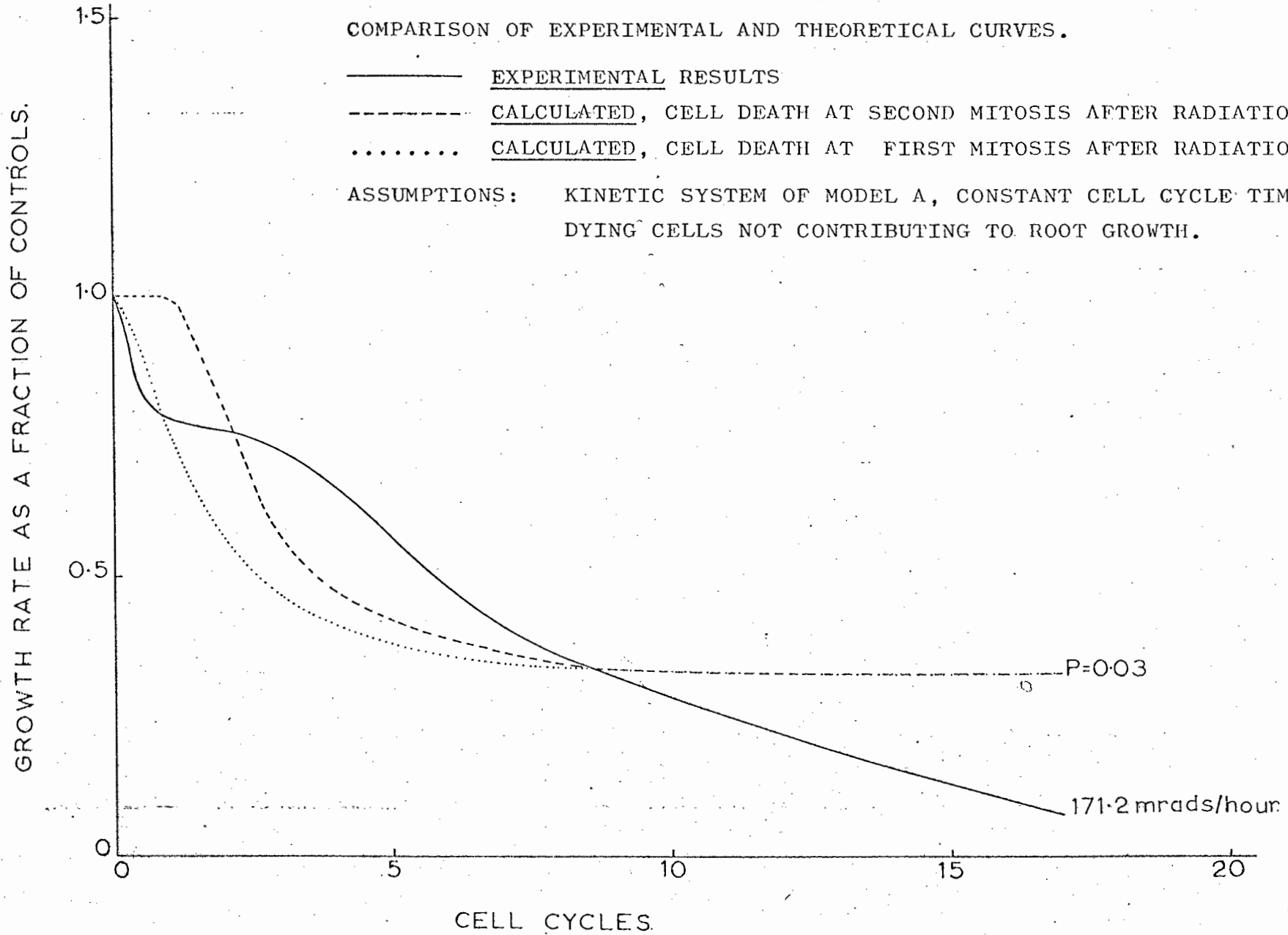


FIGURE 7.10

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

- EXPERIMENTAL RESULTS
- - - CALCULATED, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE
- CALCULATED, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE

ASSUMPTIONS: KINETIC SYSTEM OF MODEL B, CONSTANT CELL CYCLE TIME,
DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

GROWTH RATE AS A FRACTION OF CONTROLS.

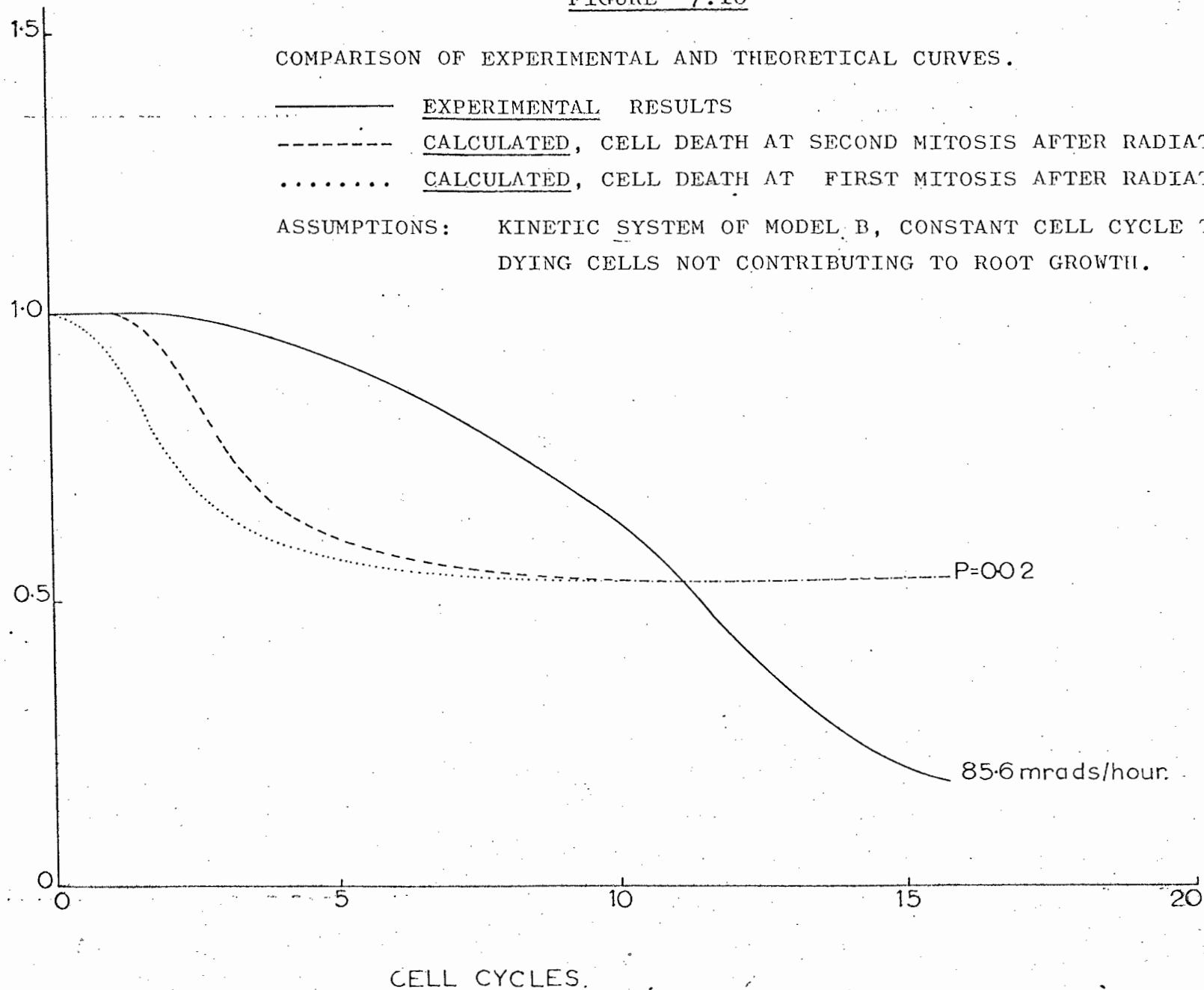


FIGURE 7.11

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

- EXPERIMENTAL RESULTS
- - - - - CALCULATED, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE
- CALCULATED, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE

ASSUMPTIONS: KINETIC SYSTEM OF MODEL B, CONSTANT CELL CYCLE TIME, DYING CELLS DIFFERENTIATING AND THUS CONTRIBUTING TO ROOT GROWTH.

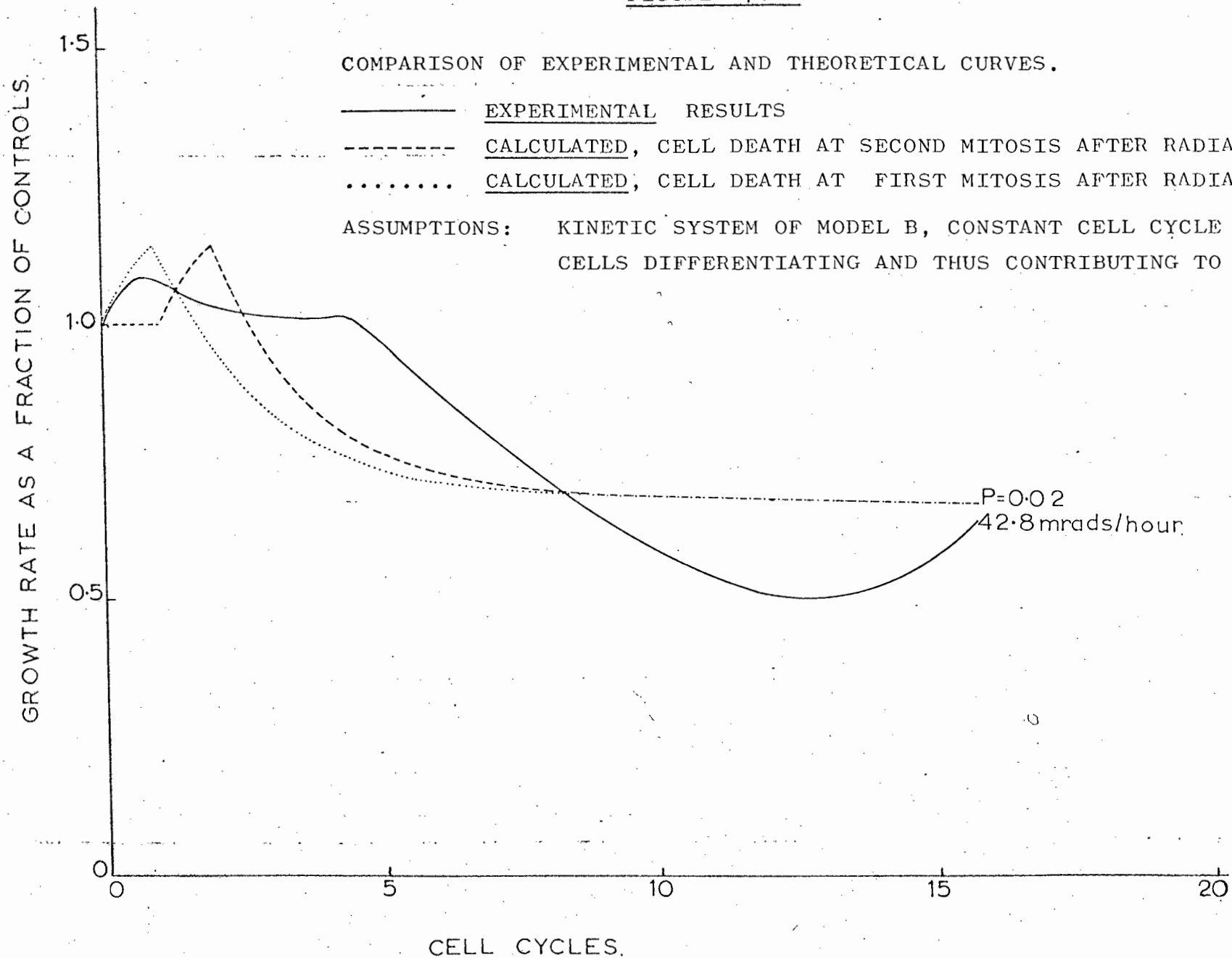


FIGURE 7.12

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

- EXPERIMENTAL RESULTS
- - - CALCULATED, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE
- CALCULATED, CELL-DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE

ASSUMPTIONS: KINETIC SYSTEM OF MODEL B, CONSTANT CELL CYCLE TIME, DYING CELLS DIVIDE AND BOTH DAUGHTER CELLS CONTRIBUTE TO ROOT GROWTH.

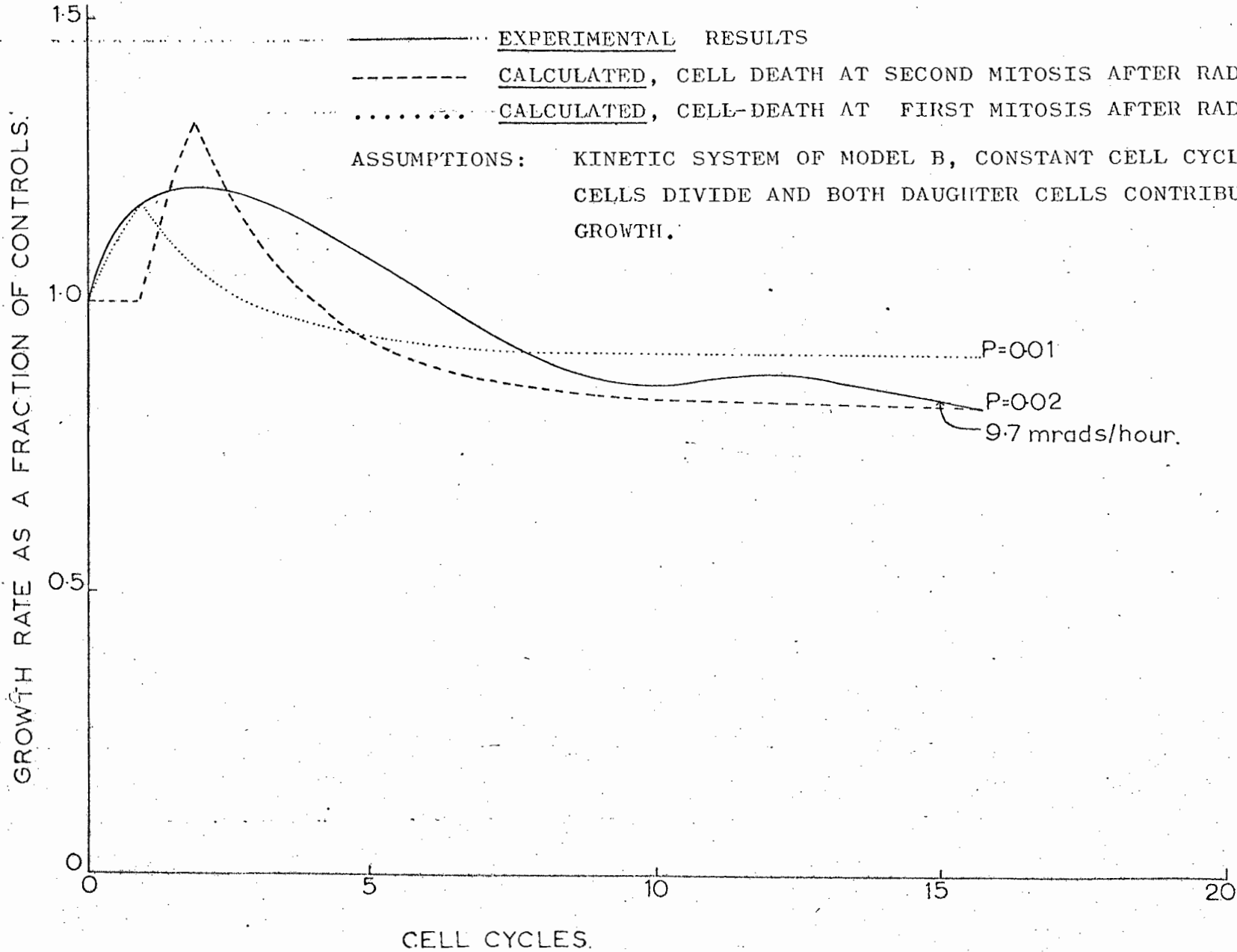


FIGURE 7.13

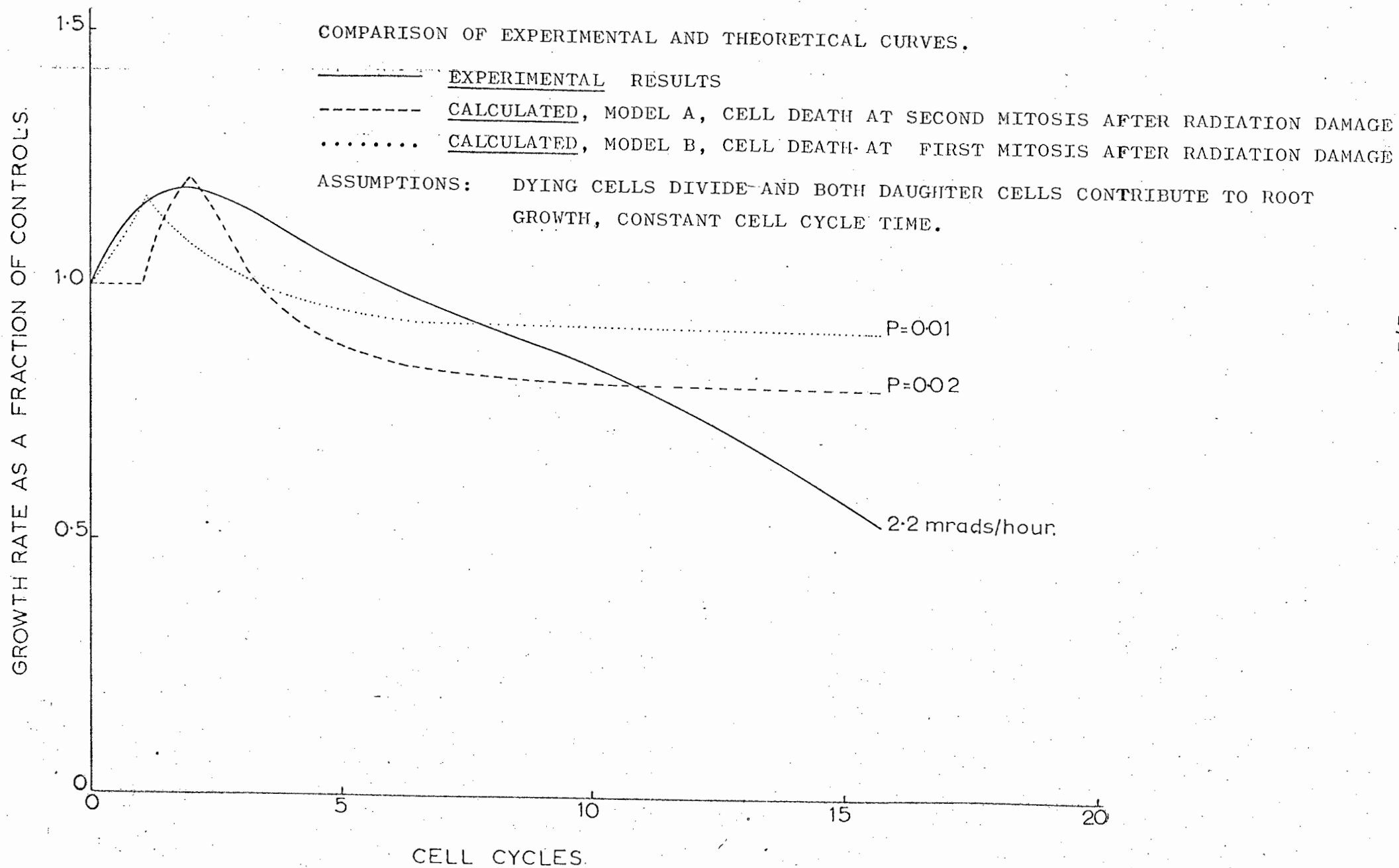


FIGURE 7.14

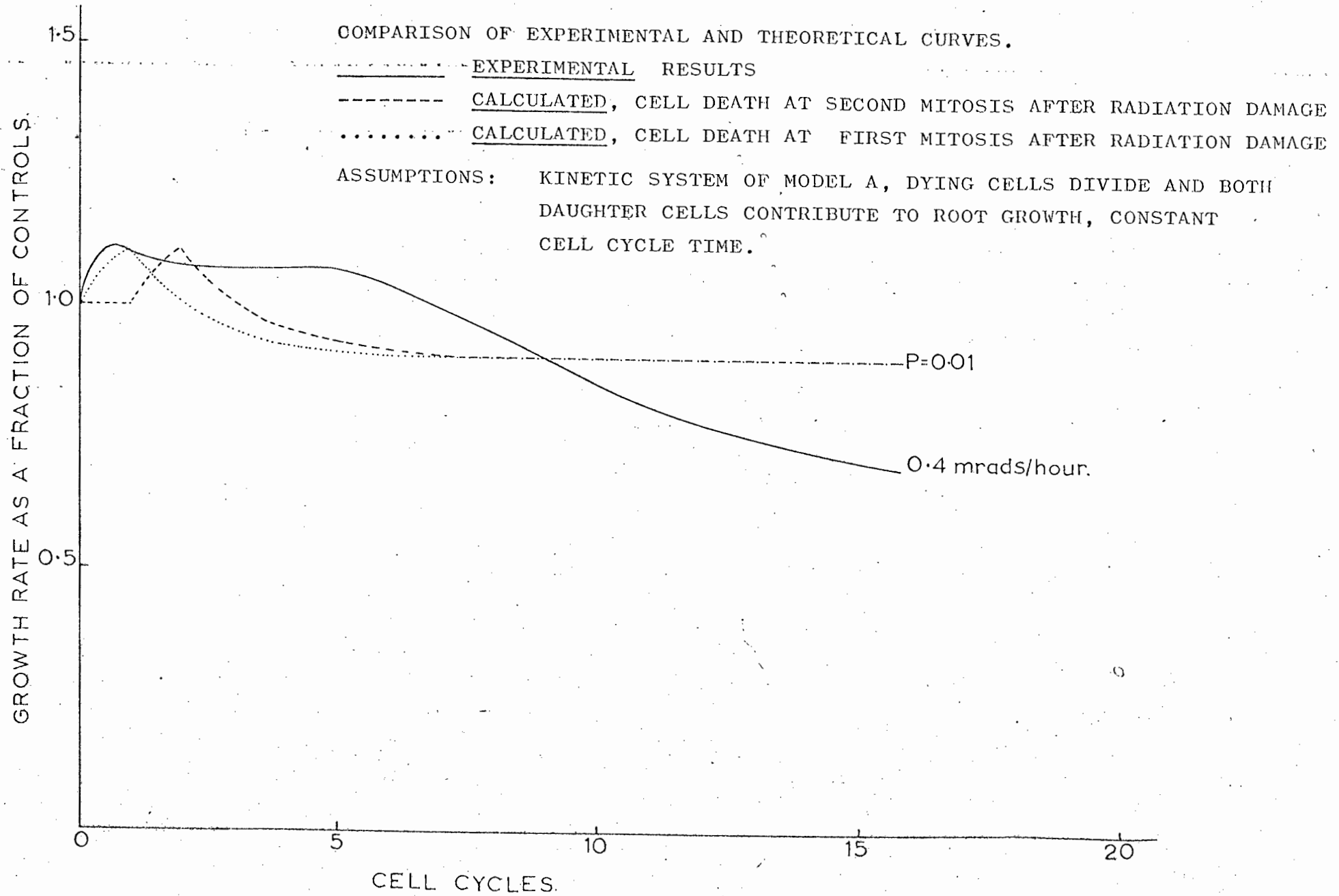
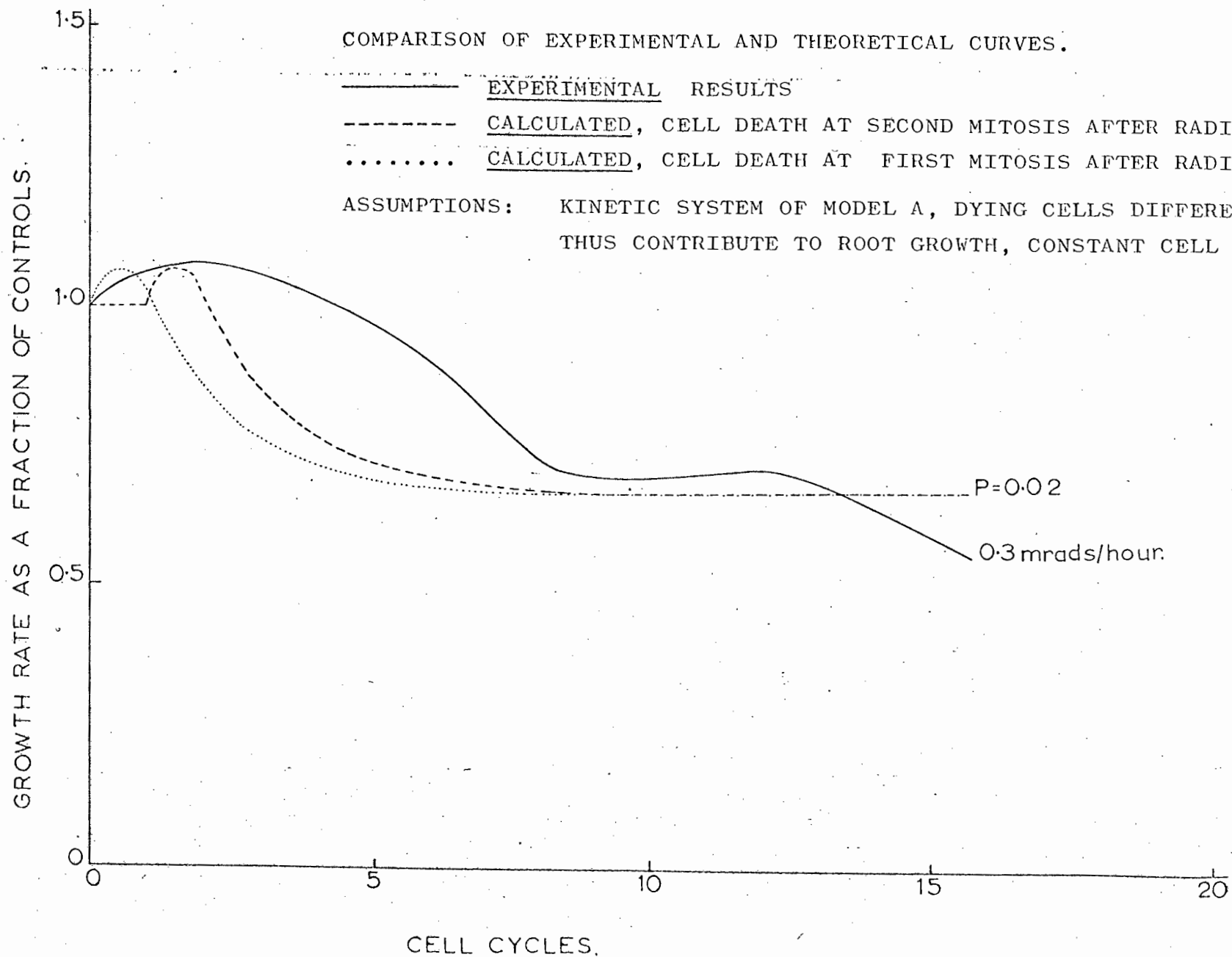


FIGURE 7.15

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———— EXPERIMENTAL RESULTS
----- CALCULATED, CELL DEATH AT SECOND MITOSIS AFTER RADIATION DAMAGE
..... CALCULATED, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, DYING CELLS DIFFERENTIATE AND
THUS CONTRIBUTE TO ROOT GROWTH, CONSTANT CELL CYCLE TIME.



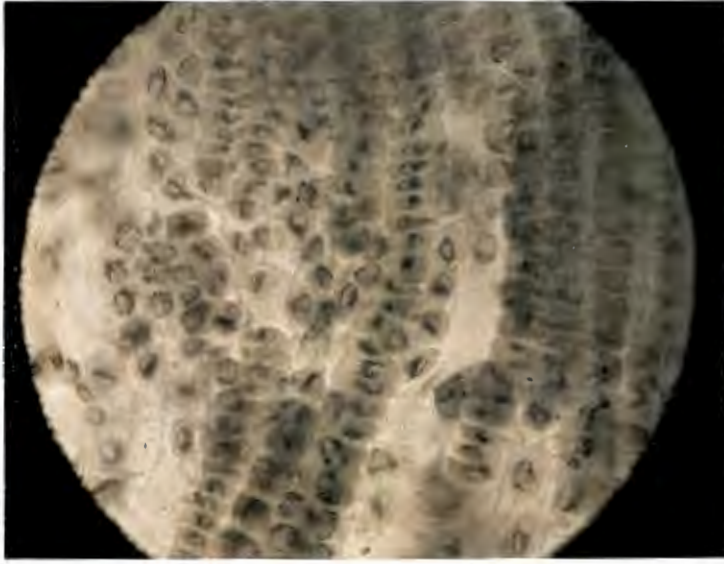


FIGURE 7.16

SQUASHES PREPARED FROM IRRADIATED ROOT
TIPS OF ZEA MAYS. (BOTH X400)

FIGURE 7.17

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

CURVES OF THE GROWTH RATE AS A FRACTION OF CONTROLS (G) VS. CELL CYCLES.

———— EXPERIMENTAL RESULTS

----- CALCULATED, DYING CELLS DIFFERENTIATING AND THUS CONTRIBUTING TO ROOT GROWTH

..... CALCULATED, DYING CELLS DIVIDE AND BOTH DAUGHTERS CONTRIBUTE TO ROOT GROWTH

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CONSTANT CELL CYCLE TIME, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE.

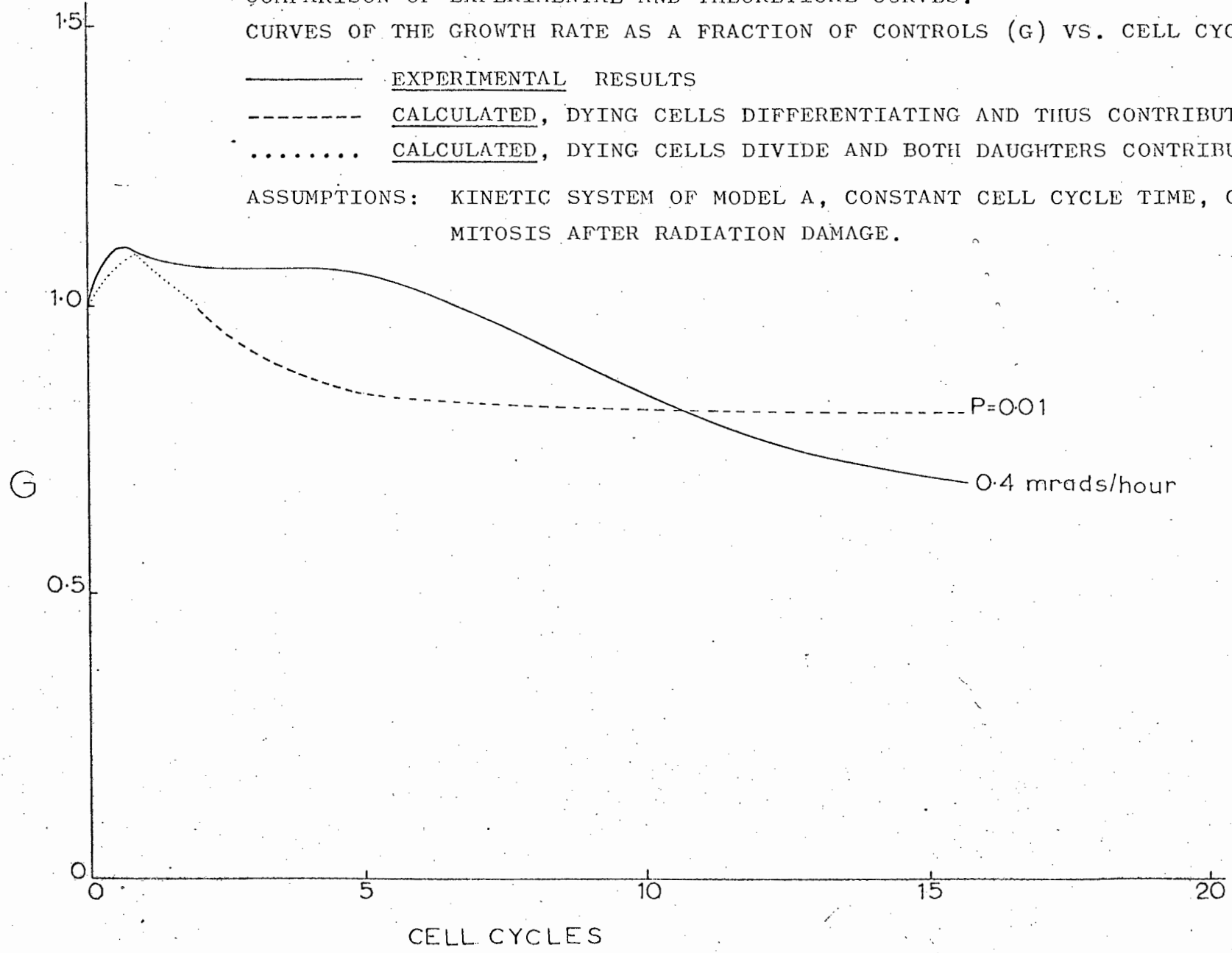


FIGURE 7.18

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

———— EXPERIMENTAL RESULTS

----- CALCULATED, CONSTANT CELL CYCLE TIME

..... CALCULATED, INCREASING CELL CYCLE TIME

P_0 = PROPORTION OF CELLS STERILIZED PER TENTH OF A CELL CYCLE AT THE ONSET OF THE RADIATION.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE, DYING CELLS DIFFERENTIATING AND THUS CONTRIBUTING TO ROOT GROWTH.

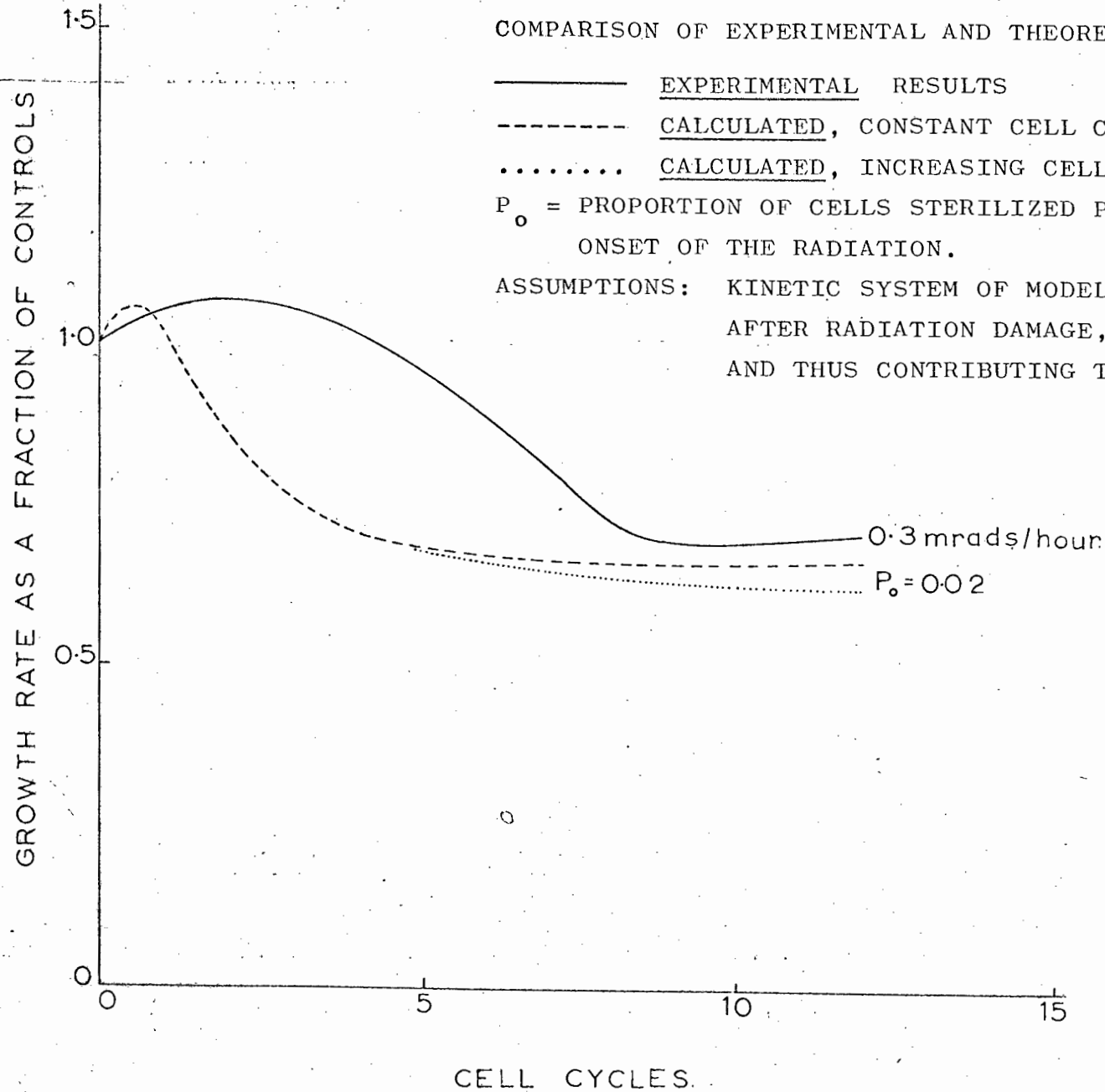


FIGURE 7.19

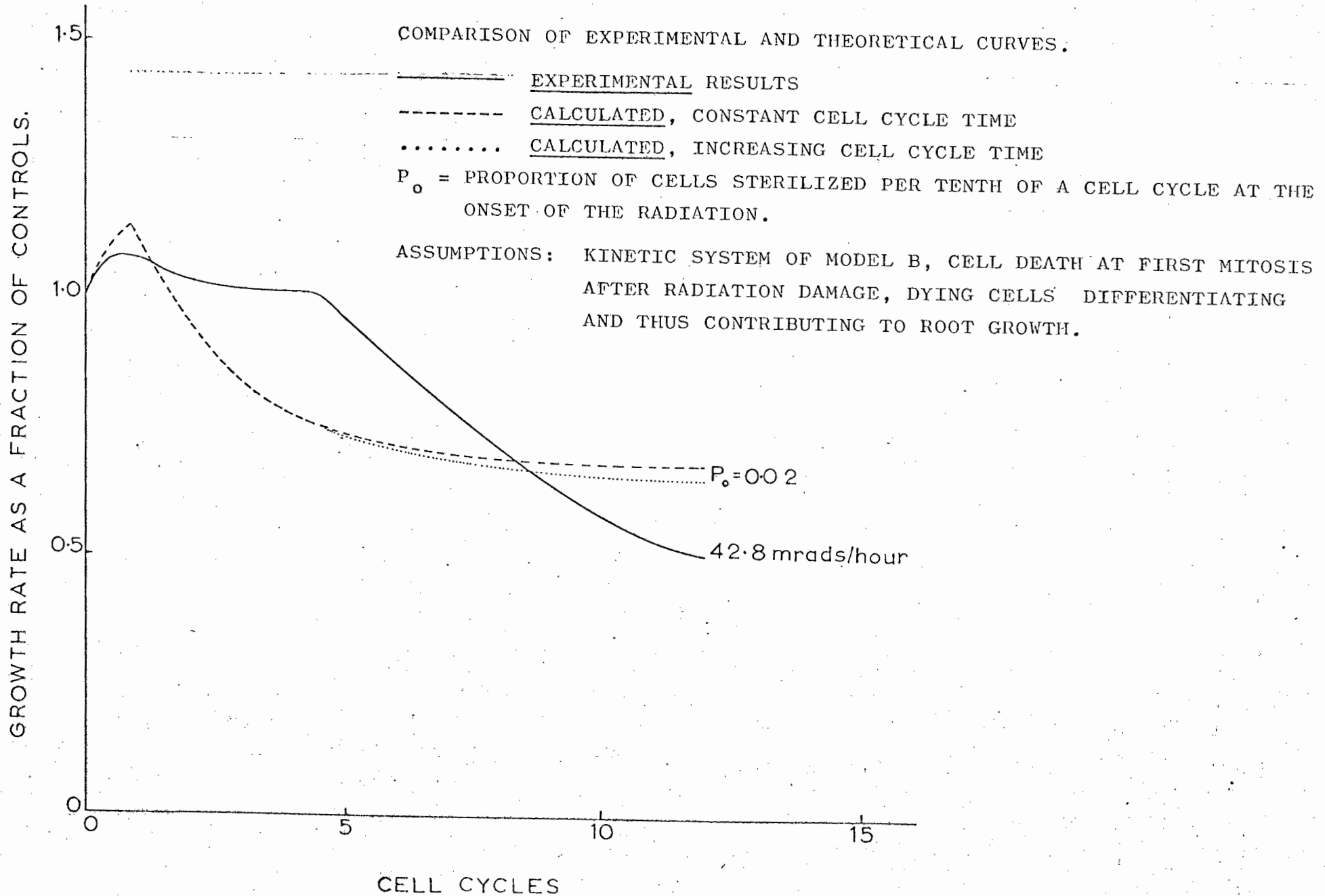


FIGURE 7.20

COMPARISON OF EXPERIMENTAL AND THEORETICAL CURVES.

- EXPERIMENTAL RESULTS
- - - CALCULATED, CONSTANT CELL CYCLE TIME
- CALCULATED, INCREASING CELL CYCLE TIME

P_0 = PROPORTION OF CELLS STERILIZED PER TENTH OF A CELL CYCLE AT THE ONSET OF THE RADIATION.

ASSUMPTIONS: KINETIC SYSTEM OF MODEL A, CELL DEATH AT FIRST MITOSIS AFTER RADIATION DAMAGE, DYING CELLS NOT CONTRIBUTING TO ROOT GROWTH.

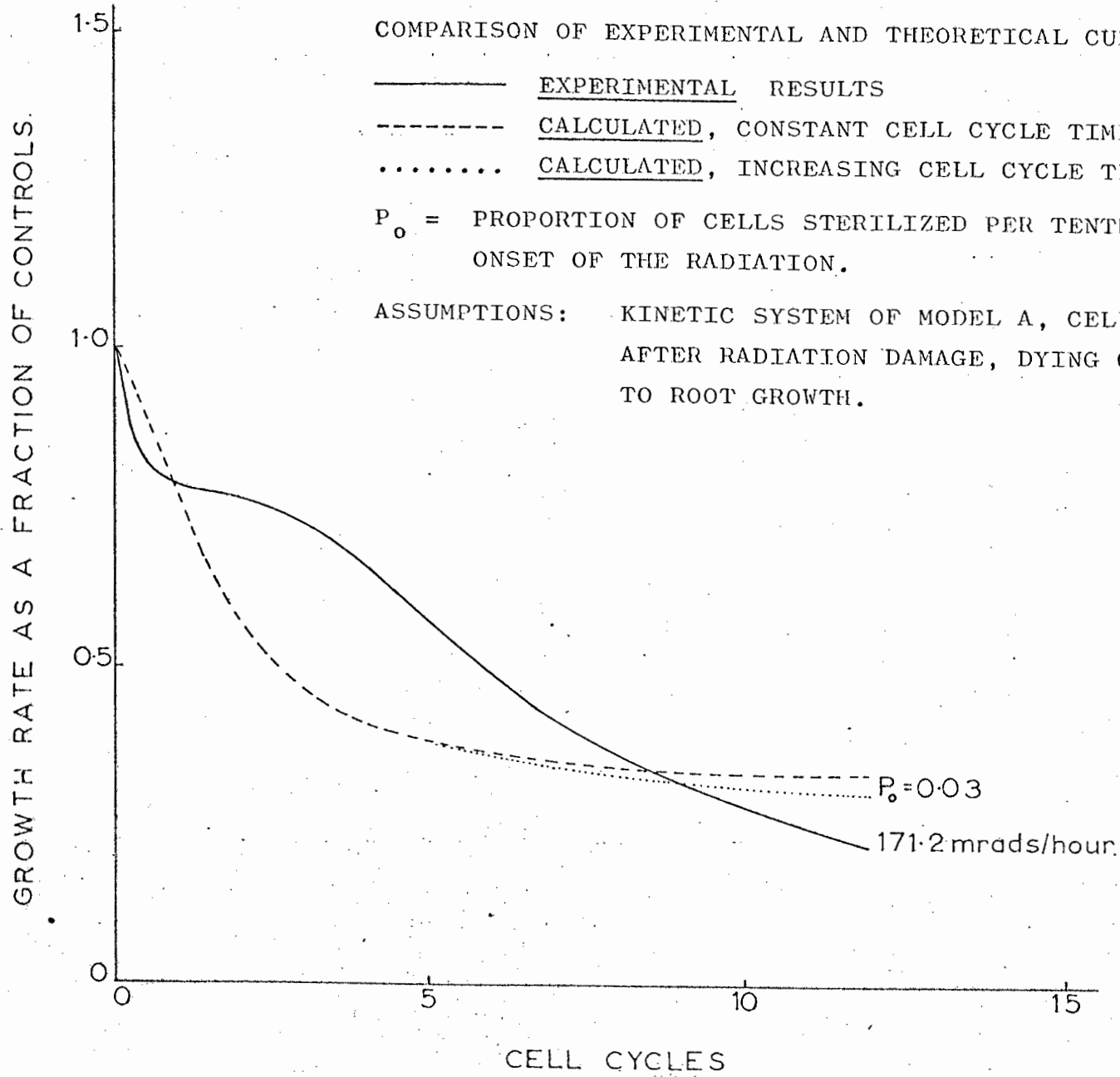
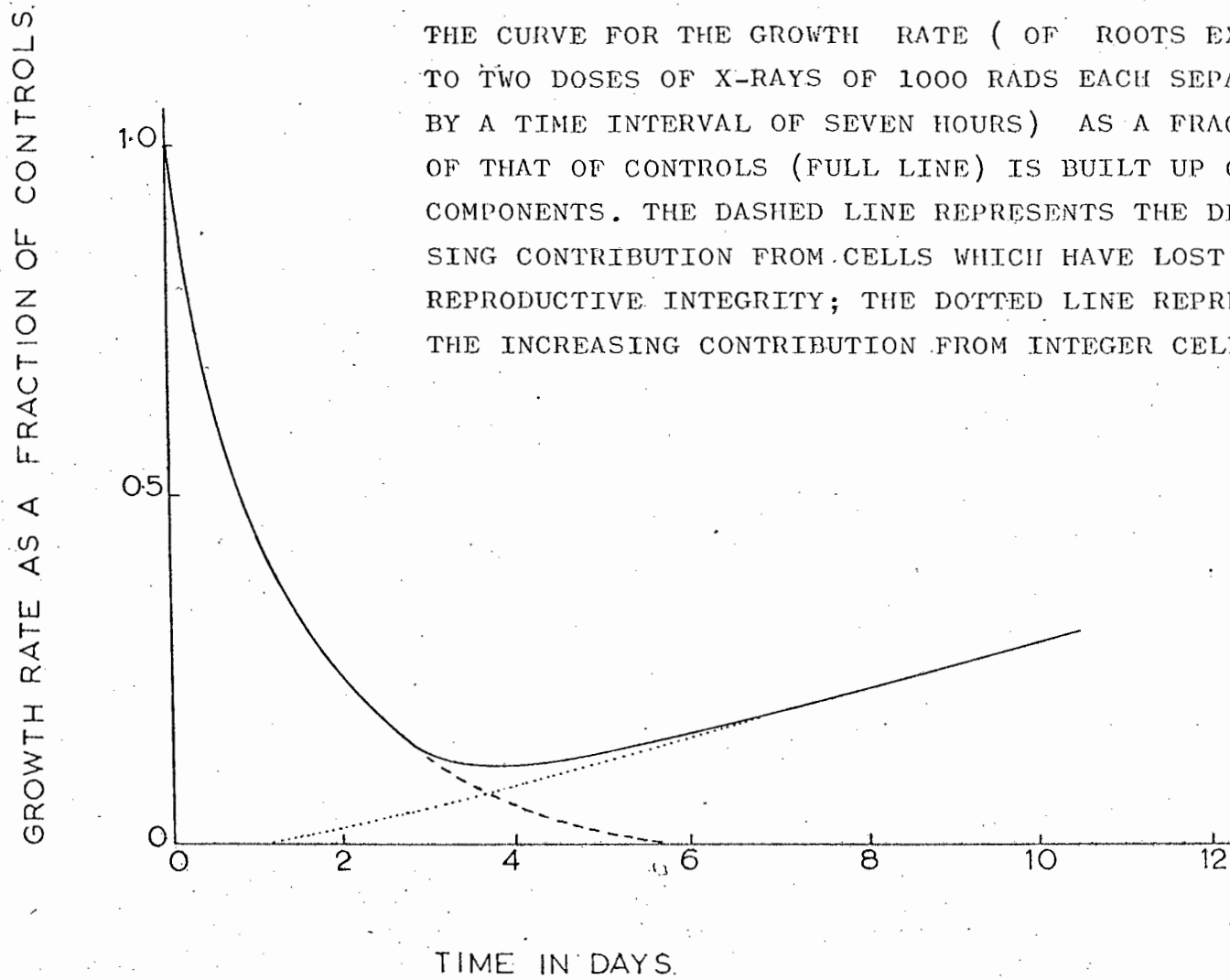


FIGURE 7.21

THE CURVE FOR THE GROWTH RATE (OF ROOTS EXPOSED TO TWO DOSES OF X-RAYS OF 1000 RADS EACH SEPARATED BY A TIME INTERVAL OF SEVEN HOURS) AS A FRACTION OF THAT OF CONTROLS (FULL LINE) IS BUILT UP OF TWO COMPONENTS. THE DASHED LINE REPRESENTS THE DECREASING CONTRIBUTION FROM CELLS WHICH HAVE LOST THEIR REPRODUCTIVE INTEGRITY; THE DOTTED LINE REPRESENTS THE INCREASING CONTRIBUTION FROM INTEGER CELLS.



CHAPTER VIII.SUMMARY.Continuous irradiation experiments.

Chronic irradiation of the roots of Zea to dose rates in the range 0.3 to 171.2 mrads/hour, has revealed a stimulation of the growth rate of the irradiated roots for dose rates up to 42.8 mrads/hour. For the higher dose rates of 85.6 and 171.2 mrads/hour, no stimulatory effect has been observed.

Using models for the cell kinetics in the irradiated root meristem of the broad bean as proposed by Oliver and Shepstone (1965), theoretical curves of growth-rate have been computed for conditions of continuous radiation exposure at the low dose rates mentioned above. These theoretical growth curves have been compared with the experimental results for the roots of Zea.

As well as having nearly the correct shape, the curves generated on the assumption that sterilized cells differentiate or even divide and both daughter cells differentiate and thus contribute to root growth (with cell "death" occurring at the first or second mitosis after radiation damage), showed a similar increase in growth rate to that observed for the roots of Zea during the first few days after the onset of the radiation.

For the higher dose rates (i.e. 85.6 and 171.2 mrads/hour) the theoretical curves based on the assumption that sterilized cells are removed from the population of meristematic cells, provided growth curves of the correct general form.

No better agreement between theoretical and experimental curves was obtained when some allowance was made for a gradual increase in cell cycle time during irradiation.

The match between theoretical and experimental curves has suggested a value of D_1 in the range 0.028 to 10.68 rads for the "single-hit" type of inactivation assumed to apply at these dose rates.

Dose fractionation experiments.

The recovery curve obtained for the roots of Zea exposed to two equal doses of X-rays separated by various time intervals (Figure 6.8) has indicated that the recovery of sublethal damage for the meristematic cells of Zea is prompt (i.e. within a few hours).

The above curve has also revealed the presence of a "kink" (see Chapter VII for further comments).

From the split dose experiments using two unequal divided doses a value for the quasi-threshold dose (D_Q) was found and using the values of 2 and 3.5, corresponding to the minimum and maximum values for the extrapolation number (m) found by various authors, values for the 37 per cent dose slope (D_0) equalling 895 and 495 rads respectively were determined.

APPENDIX AMATHEMATICAL 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 μI_s where

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

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 μI . 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 $\frac{I}{I_s}$, i.e. the number is given by $\frac{I}{I_s}$,

$$\mu I \text{ or } \mu \frac{I^2}{I_s}$$

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.

$$\text{i.e.} \quad \frac{dI}{dt} = \mu I - \mu \frac{I^2}{I_s} \quad (\text{A.2})$$

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:

$$I = \frac{I_s}{1 + \left(\frac{I_s}{I_0} - 1\right) e^{-\mu t}} \quad (\text{A.3})$$

where I_0 is the value of I just after irradiation.

The rate of differentiation at any time is given by: -

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

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

$$\frac{dD}{dt} = \frac{\mu I_s}{\left[1 + \left(\frac{I_s}{I_0} - 1\right) e^{-\mu t}\right]^2} \quad (\text{A.5})$$

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: -

$$\frac{dD}{dt} = \mu I_s \quad (\text{A.6})$$

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.

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_0 , of the postulated specific substance. As the substance diffuses through such a region, its concentration would fall from C_0 to C_N .

From the above assumptions:

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

The proportion of cells with reproductive integrity at a concentration level of C_N is $\frac{C_N}{C_0}$, and from equation (A.6)

$$\frac{C_N}{C_0} = e^{-kN} \quad (\text{A.7})$$

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

$$\int_0^N e^{-kN} dN = \frac{1}{k} (1 - e^{-kN}) \quad (\text{A.8})$$

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 F (following, for example, radiation damage) one is concerned with FN instead of N in the above formulae. Therefore the proportion of the possible FN cells to divide per unit time will be

$$X = \frac{1}{kFN} (1 - e^{-kFN}) \quad (\text{A.9})$$

and the proportion differentiating $(1-X)$.

The number differentiating, D , will be $FN(1-X)$, that is:-

$$D = \frac{1}{k} (kFN + e^{-kFN} - 1) \quad (\text{A.10})$$

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:-

$$G = \frac{kFN + e^{-kFN} - 1}{0.5N}$$

i.e.
$$G = \frac{1.595F + e^{-1.595F} - 1}{0.7975}$$

(A.11)

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

$$G = 1.595 F^2$$

(A.12)

APPENDIX BMEASUREMENT OF RADIATION DOSE FOR THE CONTINUOUS IRRADIATION
EXPERIMENTS USING THERMOLUMINESCENT DOSIMETRY (T.L.D.).

Thermoluminescent dosimetry is based upon the fact that light, which is emitted when a phosphor is heated, is a function of the number of trapped electrons which in turn is determined by the dose of radiation to which the dosimeter (i.e. the aliquot of the phosphor used for the measurement of a radiation dose) has been exposed.

The ISOTOPES/CON-RAD TLD readout instrument was used for the present measurements. Fig. B1 depicts the apparatus as well as the annealing oven.

In this readout instrument the dosimeter is heated and the amount of light emitted within a set period of time is integrated and the integral is presented digitally.

Manganese-activated lithium tetraborate thermoluminescent powder was chosen to be a suitable dosimeter for the following reasons:

- (a) It exhibits a linear dose response at the dose levels used in the present experiment.
- (b) The integrated thermoluminescence per unit dose is independent of photon energy.
- (c) The 'tissue equivalence' is good, i.e. the ratio between the mass energy absorption coefficients of the powder and the medium in which the dose is measured varies little with photon energy.
- (d) The glow curve characteristics do not change on thermal treatment.

Before use, the powder was annealed for three hours at 300°C in the annealing oven in order to remove any possible residual thermoluminescence. Aliquots of this powder, weighing between 20 and 60 mg, were individually sealed in small polythene bags to protect the powder from contamination when measuring doses in a liquid medium.

TLD measurements are relative measurements, based upon the comparison of the response to an unknown dose to the values obtained with known doses. Thus a set of dosimeters, mounted between 2 slabs of wax (0.5 cm thick) to provide the necessary electron build-up, were exposed to Co-60 gamma rays for a set time (Picker Cobalt Unit; 50 SSD; field size = 10x10 cm²). The output of the Cobalt Unit was determined accurately using a Baldwin Farmer Substandard dose meter as described in Chapter V. To convert the dose rate measured on this instrument from Roentgen/min to rads/min a factor of 0.9505 was used as defined by Johns & Cunningham (1969). Thus the dose received by the dosimeters is given by:

$$\text{Dose to dosimeters (in rads)} = \text{time exposed to radiation} \times 1.036 \times 0.9505 \times \text{output (in Roentgen/min)}.$$

where 1.036 is the 'Back Scatter Factor' for the particular field size and quality of the radiation used (Johns & Cunningham, 1969).

These dosimeters were exposed to the gamma-rays at the time of removal of the other dosimeters from the continuous irradiation jig (described in Chapter V), to allow for simultaneous fading of thermoluminescence after the irradiation. The dosimeters were 'read out' on the TLD apparatus 20 days after the date of irradiation or in the case of the dosimeters on the continuous

irradiation jig, 20 days after the last day of the irradiation period. By this time virtually no more fading of the thermoluminescence occurs. The weight of each sample of powder was determined accurately and normalized to a standard weight (taken as 30 mg). For the weight correction, dosimeters of known weight were exposed to the same dose and a graph was plotted of Thermoluminescent Output vs. Weight of powder as depicted in Fig. B2.

For all measurements the phosphor sample was surrounded by a nitrogen atmosphere (by passing 7 litres/min. of nitrogen through the heating chamber), to reduce the spurious thermoluminescence due to chemothermoluminescence on heating of the sample.

The average background reading, measured on unirradiated powder samples, was found to be 54.3 ± 3.1 counts.

From these measurements the sensitivity of the powder (in rads/count) was determined and this, multiplied by the number of counts for the dosimeter exposed to continuous irradiation, gives the actual dose received by the latter. Some of the actual measurements are listed in Fig. B3.

For all the above measurements a 'heater current' of 0.53 Amps was used. The E.H.T. was adjusted using a standard light source and the approximate sensitivity as supplied by the manufacturers for the particular batch of powder used.

The accuracy of the measurements mentioned in this Appendix is expressed by the standard deviation (σ) evaluated as described in Appendix C.

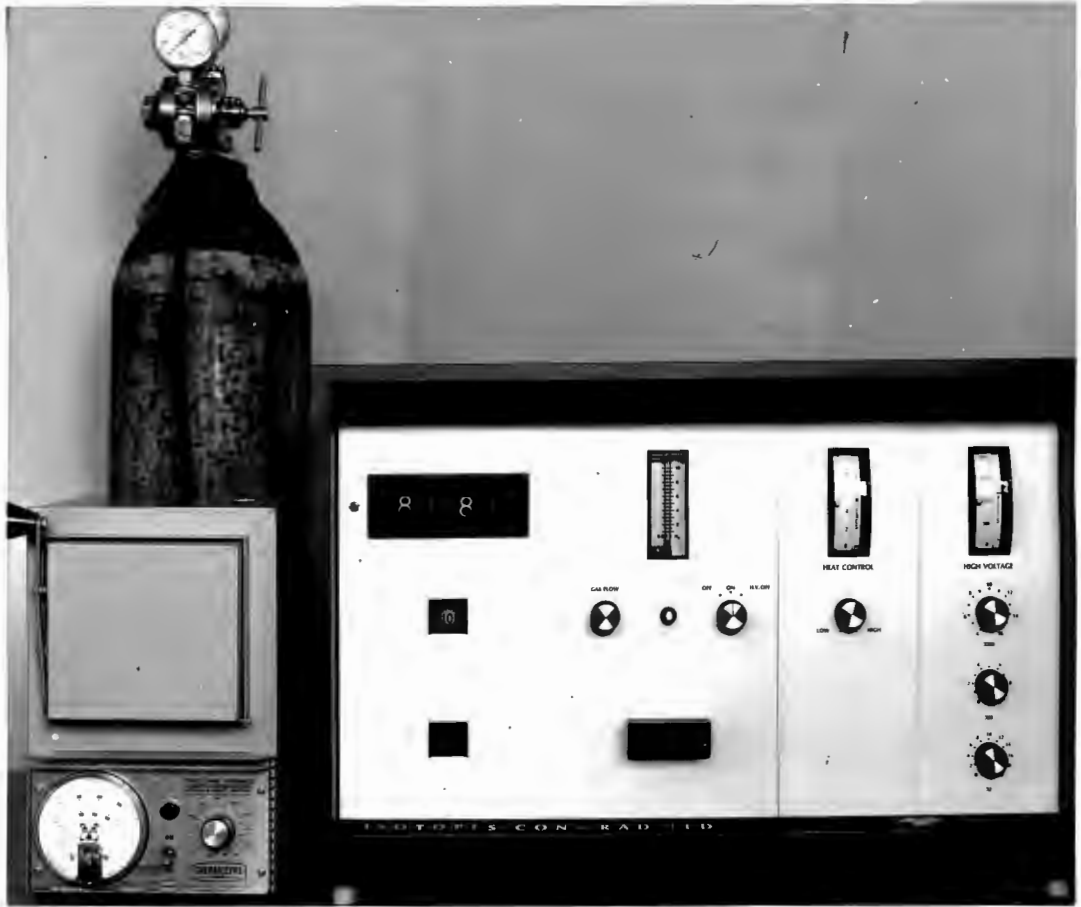


FIGURE B1

THE ISOTOPES/CON-RAD TLD READOUT INSTRUMENT.

FIGURE B2

THERMOLUMINESCENT OUTPUT FOR DIFFERENT WEIGHTS
OF POWDER IRRADIATED TO AN EQUAL DOSE.

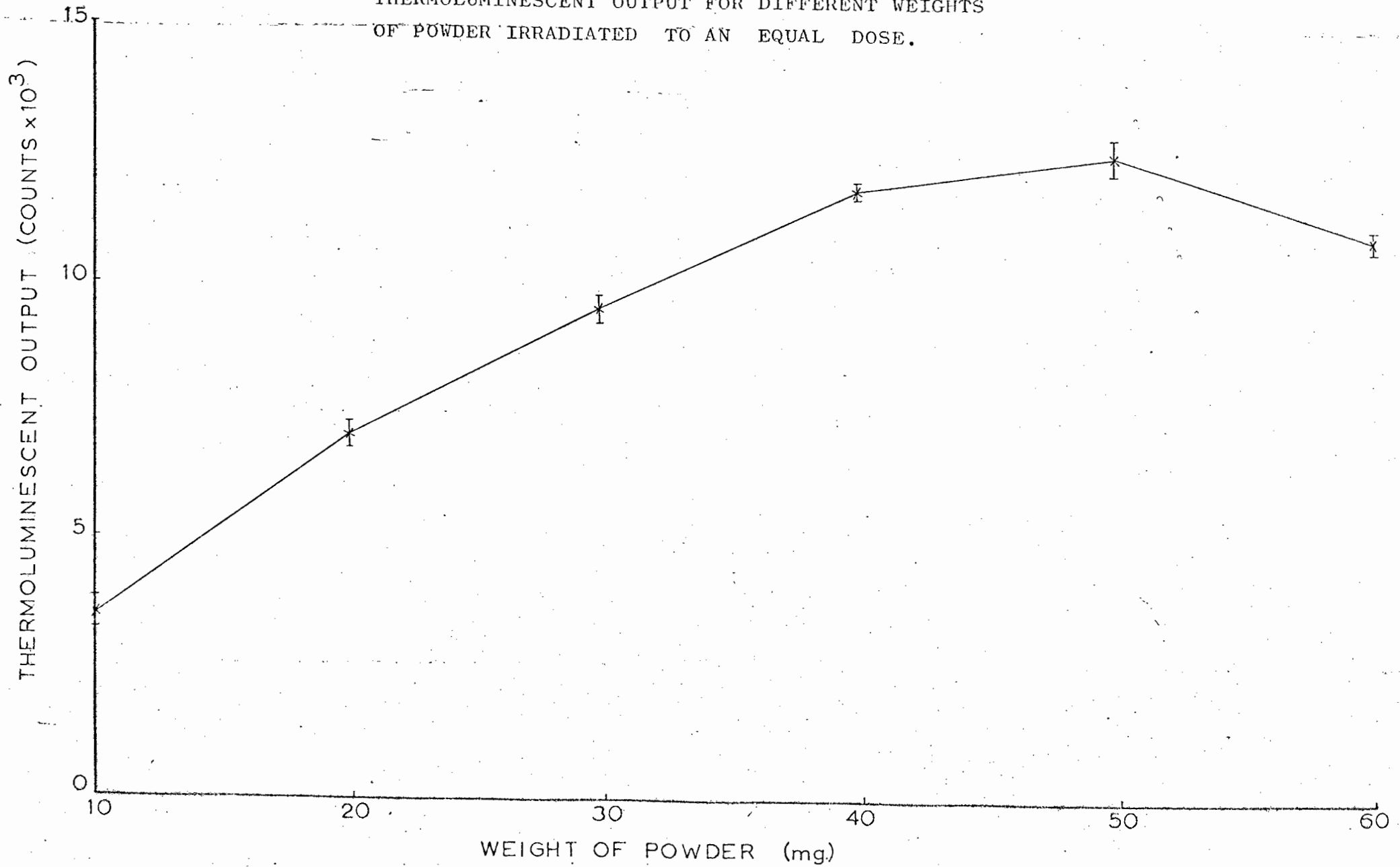


FIGURE B 3.

Dosimeters 30 cm from the sources totalling 2mg Ra-226.

Duration of irradiation: 15 days.

Type of dosimeter: Lithium tetraborate (manganese activated).

Heater current: 0.53 A.

Nitrogen flow: 7 litres/min.

Light source B: 1386 counts at 1236 volts.

Background: 54.3 ± 3.1 digits.

Sensitivity: 0.00175 rads/digit.

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Dosimeter No.	Weight of powder (mg)	Thermoluminescence (counts)	Counts for 30mg	Mean counts -Background	Dose (rads)
1	58.31	1036	889		
2	40.40	1253	1011		
3	42.90	1074	860		
4	43.55	1563	1240	898	1.572
5	49.32	991	763	± 184	± 0.322

APPENDIX CRELEVANT STATISTICS AND COMPUTER PROGRAMSFOR THE CALCULATIONS INVOLVED.

For the measurements on the maize roots as well as the measurements for the thermoluminescent dosimetry the standard deviation σ was found using the formula:

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n}}$$

where \bar{X} is the arithmetic mean of the group of n values. The results in the previous chapters are always given (unless stated otherwise) as follows:

$$\bar{X} \pm \frac{\sigma}{\sqrt{n}}$$

where $\frac{\sigma}{\sqrt{n}}$ is the standard error of the mean.

The growth of the roots \bar{X} is always expressed as a fraction of the growth of the control group, \bar{Y} . For this ratio (i.e. $\frac{\bar{X}}{\bar{Y}} = G$), the standard error of the mean ΔG was found from the following relationship:

$$G \pm \Delta G = \frac{\bar{X} \pm \Delta X}{\bar{Y} \pm \Delta Y} = \frac{\bar{X}}{\bar{Y}} \pm \frac{\bar{X}}{\bar{Y}} \sqrt{\left(\frac{\Delta X}{\bar{X}}\right)^2 + \left(\frac{\Delta Y}{\bar{Y}}\right)^2}$$

where ΔX and ΔY are the standard errors of the mean for the irradiated and control groups respectively, which have been found using the above formula for the standard deviation σ .

To find the standard error of the mean for a large number of groups of roots, a program was written to perform the calculation on the WANG 370 Desk Calculator. The flow diagrams for the calculation are shown in Figure C1 and C2 for the evaluation of $(\Delta X, \Delta Y)$ and ΔG respectively. The programs themselves are outlined in Fig. C3 and Fig. C4, together with the appropriate instructions for the keyboard.

FIGURE C1

FLOW DIAGRAM FOR THE CALCULATION OF THE STANDARD DEVIATION σ
 AND THE STANDARD ERROR OF THE MEAN $\frac{\sigma}{\sqrt{n}}$

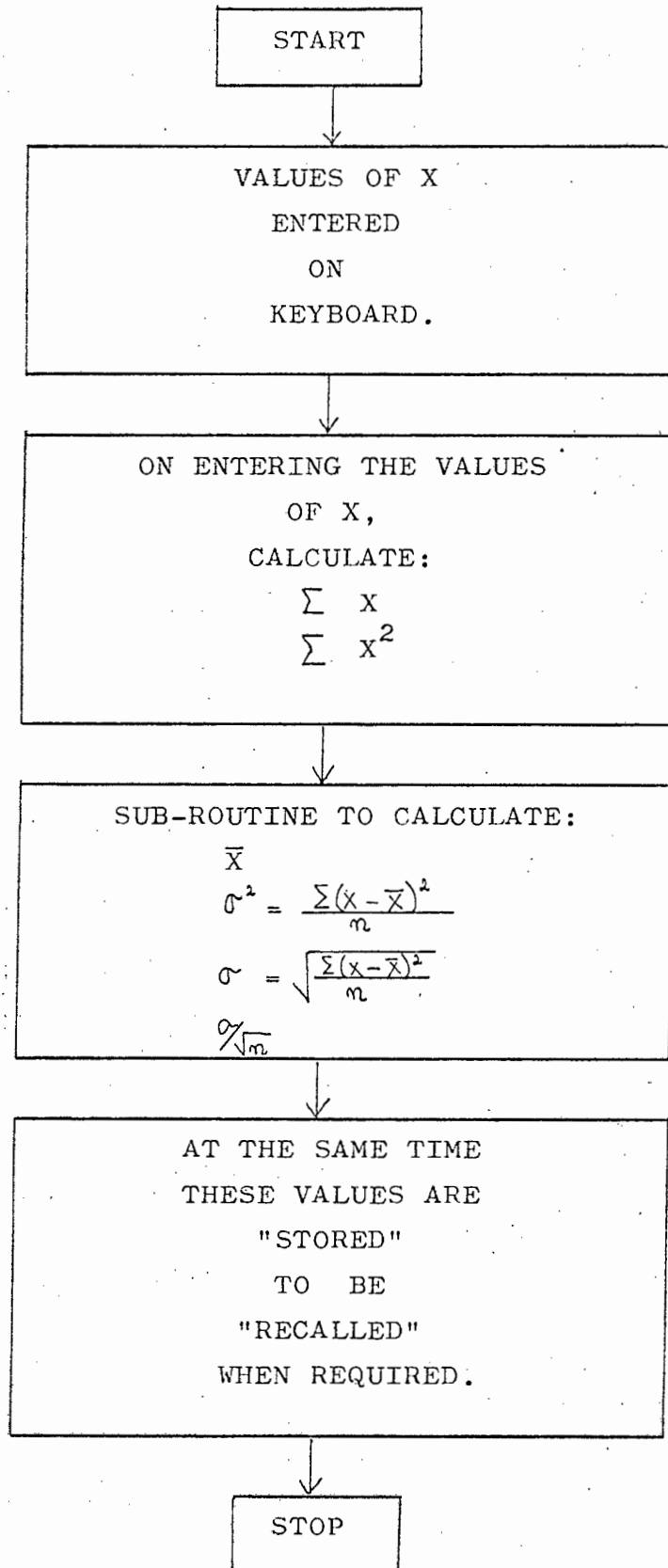


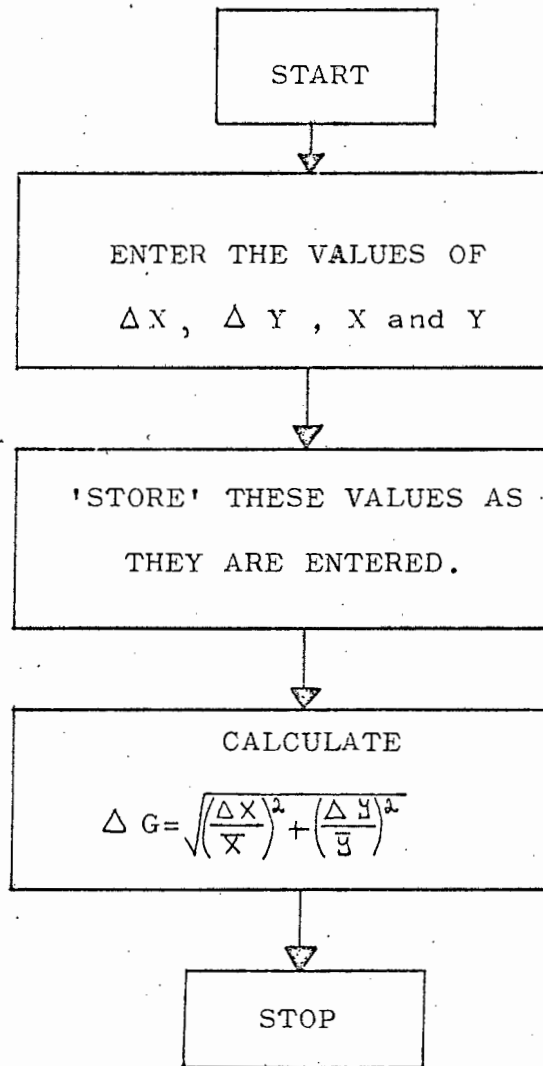
FIGURE C2FLOW DIAGRAM FOR THE CALCULATION OF THE STANDARD DEVIATION ΔG .

FIGURE C3

PROGRAM FOR THE WANG 370 DESK CALCULATOR TO EVALUATE THE
STANDARD DEVIATION σ AND THE STANDARD ERROR OF THE MEAN $\frac{\sigma}{\sqrt{n}}$

Keyboard instructions for the entering of the data and to
initiate the calculation:

PRIME
CONTINUE
ENTER X_1
CONTINUE
ENTER X_2
CONTINUE
.....
ENTER X_n
CONTINUE
SEARCH 3.

CARD: V.P. 3174

<u>NUMBER</u>	<u>COMMAND</u>	<u>CODE</u>
00	Mk	07
01	1	61
02	SF	13
03	0	60
04	SF	13
05	1	61
06	SF	13
07	2	62
08	SF	13
09	3	63
10	Mk	07
11	2	62
12	STOP	01
13	SF	13
14	9	71
15	AF	12
16	0	60
17	RF	17
18	9 ²	71
19	X ²	45
20	AF	12
21	1	61
22	1	61
23	AF	12
24	2	62
25	Sch	02
26	2	62
27	Mk	07
28	3	63
29	RF	17
30	0	60
31	ENT	41
32	RF	17
33	2	62
34	\div	47
35	SF	13

FIGURE C3 Continued

<u>NUMBER</u>	<u>COMMAND</u>	<u>CODE</u>
36	4	64
37	X ²	45
38	ENT	41
39	RF	17
40	2	62
41	X=	46
42	C _L A _L	54
43	- A _L	57
44	RF	17
45	1	61
46	+ A _L	56
47	ENT	41
48	RF	17
49	2	62
50	C _L A _R	50
51	+ A _R	52
52	1	61
53	- A _R	53
54	÷	47
55	SF	13
56	5	65
57	√X	44
58	SF	13
59	6 ₂	66
60	X	45
61	ENT	41
62	RF	17
63	2	62
64	÷	47
65	√X	44
66	SF	13
67	7	67
68	RF	17
69	4	64
70	STOP	01

When the calculation has been completed, the value of \bar{X} is shown in the display.

The keyboard instructions for the display of σ^2 , σ and $\frac{\sigma}{\sqrt{n}}$ are as follows:

"RECALL FULL 5" will display σ^2 .

"RECALL FULL 6" will display σ .

"RECALL FULL 7" will display $\frac{\sigma}{\sqrt{n}}$.

FIGURE C4

PROGRAM FOR THE WANG 370 DESK CALCULATOR TO EVALUATE ΔG .

Keyboard instructions for the entering of data and to initiate the calculation:

PRIME
SEARCH 1
ENTER ΔX
CONTINUE
ENTER ΔY
CONTINUE
ENTER \bar{X}
CONTINUE
ENTER \bar{Y}
CONTINUE

CARD: V.P. 1461

<u>NUMBER</u>	<u>COMMAND</u>	<u>CODE</u>
00	Mk	07
01	1	61
02	STOP	01
03	SF	13
04	0	60
05	STOP	01
06	SF	13
07	1	61
08	STOP	01
09	SF	13
10	2	62
11	STOP	01
12	SF	13
13	3	63
14	\div	47
15	ENT	41
16	RF	17
17	1	61
18	X=	46
19	X ²	45
20	C _L A _L	54
21	+ A _L	56
22	RF	17
23	0	60
24	ENT	41
25	RF	17
26	2	62
27	\div	47
28	X ²	45
29	+ A _L	56
30	\sqrt{X}	44
31	ENT	41
32	RF	17
33	2	62
34	X=	46
35	ENT	41

FIGURE C4 Continued

<u>NUMBER</u>	<u>COMMAND</u>	<u>CODE</u>
36	RF	17
37	3	63
38	÷	47
39	STOP	01

When the calculation has been completed, the value of ΔG is shown in the display.

APPENDIX DFLOW DIAGRAM AND COMPUTER PROGRAMS FOR THE ANALYSIS
OF THE CONTINUOUS IRRADIATION EXPERIMENTS.

The computer programs written for Model A and Model B (with the assumption that the cell cycle time (T) is constant and that cell death occurs after radiation sterilization at the second mitosis) are given in Fig.(D1,2) and Fig.(D4,5,6) respectively. The values of G, G' and G'' (defined in Chapter II) for the various times after the onset of radiation are printed by the computer as shown in Fig.D3 and Fig.D7 for Model A and Model B respectively. For the initial period i.e. for the time equalling 3 times the cycle time after the onset of radiation the values of G, G' and G'' are printed out for each increment of time equalling one tenth of the cycle time in order to determine the exact shape of the peak representing the stimulatory effect of protracted irradiation on the roots of Zea. Thereafter the values of G, G' and G'' are given only at intervals of time equalling the cycle time. On the computer print-out $G(C10 \text{ DIE}) = G$, $G(C10 \text{ DIF.}) = G'$ and $G(C10 \text{ DIVIDE +DIF.}) = G''$.

The flow diagram for the computation is given in Fig. D8. This diagram is applicable to both models under consideration. For a set value of P, the number of cells in each compartment of category A,B and C are calculated at the time $t=0$, using the initial conditions for the particular model under consideration. The number of cells in each compartment at the end of each successive time interval equalling

one tenth of the cycle time is evaluated using the appropriate formulae as given in Chapter II. The corresponding values of G , G' and G'' are printed as described above. This process is repeated until a time equalling $20T$ is reached.

The computer program for Model A and B with the assumption that cell death occurs at the first mitosis after receiving radiation damage is similar to the one discussed above except that this time there are no cells in category C.

Thus $\sum_{n=1}^{10} (C_n)_t = 0$ for all values of t and

$$F_t = \frac{\sum_{n=1}^{10} (A_n)_t + \sum_{n=1}^{10} (B_n)_t}{139.38} \quad \text{for Model A}$$

$$F_t = \frac{\sum_{n=1}^{10} (A_n)_t + \sum_{n=1}^{10} (B_n)_t}{100} \quad \text{for Model B}$$

The formulae for $(C_n)_t$ will now fall away in the case of both models.

In order to account for the lengthening of the cell cycle time all programs were modified to allow for the corresponding change of P . The formulae used for the computation are discussed in Chapter II and the appropriate values for the cycle time at equilibrium and at the end of an irradiation period of 7 days are derived in Chapter VII.

COMPUTER PROGRAM FOR MODEL AFIGURE D 1

// JOB

// FOR

*IOCS (2501 READER, 1403 PRINTER,DISK)

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

```

DIMENSION A(100),B(100),C(100),F(100),GNCD(100),GW1CD(100),GW2CD(1
100)
  Q=0.933
  DO 1 K=1,100,10
    IF(K-1)32,31,32
31 P=K
  P=P/1000.0
  GO TO 33
32 K13=K-1
  P=K13
  P=P/1000.0
33 WRITE(5,100)
100 FORMAT(1H1,2X,'FRACTIONAL GROWTH RATE=G (ROOTS UNDER CONTINUOUS IR
IRADIATION)-MODEL A')
  WRITE(5,101)P
101 FORMAT(1H ,2X,'P=',1X,F5.3,/)
  WRITE(5,106)
106 FORMAT(1H ,20X,'G-VALUES AT TIME INTERVALS=INTERMITOTIC CYCLE TIME
1',////,'TIME(IN CELL CYCLES)',4X,'G(C10 DIE)',4X,'G(C10 DIF.)',4X,
2'G(C10 DIVIDE+DIF.)')
  A(1)=20.0*0.933
  B(1)=0.0
  C(1)=0.0
  DO 2 K1=2,10,1
    L=K1-1
    A(K1)=A(L)*0.933
    B(K1)=B(L)*0.0
  2 C(K1)=C(L)*0.0
  F(1)=1.0
  DO 8 K8=1,20,1
    M=11
    N=20
    DO 3 K2=1,10,1
      IF(M-11)50,51,50
51 DO 4 K3=M,N,1
    L2=K3-11
    L1=K3-1
    X=1.0-((1.0-Q)*F(K2))
    Y=X*(1.0-P)
    IF(K3-M)10,11,10
11 A(K3)=2.0*A(L1)*Y
    B(K3)=X*P*2.0*A(L1)
    C(K3)=X*2.0*B(L1)
    SUMA=A(K3)
    SUMB=B(K3)
    SUMC=C(K3)
    GO TO 4
10 A(K3)=Y*A(L2)

```

```

B(K3)=(X*B(L2))+(X*P*A(L2))
C(K3)=X*C(L2)
SUMA=SUMA+A(K3)
SUMB=SUMB+B(K3)
SUMC=SUMC+C(K3)
4 CONTINUE
K3=N
K5=K3
M=M-10
N=N-10
GO TO 53
50 DO 5 K3=M,N,1
L2=K3+9
L1=K3+19
X=1.0-((1.0-Q)*F(K2))
Y=X*(1.0-P)
IF(K3-M)54,55,54
55 A(K3)=2.0*A(L1)*Y
B(K3)=X*P*2.0*A(L1)
C(K3)=X*2.0*B(L1)
SUMA=A(K3)
SUMB=B(K3)
SUMC=C(K3)
GO TO 5
54 A(K3)=Y*A(L2)
B(K3)=(X*B(L2))+(X*P*A(L2))
C(K3)=X*C(L2)
SUMA=SUMA+A(K3)
SUMB=SUMB+B(K3)
SUMC=SUMC+C(K3)
5 CONTINUE
K3=N
M=M+10
N=N+10
K5=K3
53 F(K2)=(SUMA+SUMB+SUMC)/139.38
GNCD(K2)=F(K2)*F(K2)
GW1CD(K2)=GNCD(K2)+(C(K5)/10.0)
GW2CD(K2)=GNCD(K2)+(C(K5)/5.0)
IF(K8-3)70,70,71
70 K20=K8-1
E=K2
E=E/10.0
H=K20
E=E+H
WRITE(5,111)E
111 FORMAT(1H,8X,F3.1)
WRITE(5,110)GNCD(K2),GW1CD(K2),GW2CD(K2)
110 FORMAT(1H+,25X,F5.3,10X,F5.3,13X,F5.3)
71 K10=K2+1
3 F(K10)=F(K2)
K2=10
IF(K8-3)80,80,81
81 WRITE(5,103)K8
103 FORMAT(1H,8X,I3)
WRITE(5,102)GNCD(K2),GW1CD(K2),GW2CD(K2)
102 FORMAT(1H+,25X,F5.3,10X,F5.3,13X,F5.3)
80 F(1)=F(K10)
8 CONTINUE
1 CONTINUE
CALL EXIT
END

```

FIGURE D 3

FRACTIONAL GROWTH RATE=G (ROOTS UNDER CONTINUOUS IRRADIATION)-MODEL A
 P= 0.050

G-VALUES AT TIME INTERVALS=INTERMITOTIC CYCLE TIME

TIME(IN CELL CYCLES)	G(C10 DIE)	G(C10 DIF.)	G(C10 DIVIDE+DIF.)
0.1	0.998	0.998	0.998
0.2	0.998	0.998	0.998
0.3	0.998	0.998	0.998
0.4	0.998	0.998	0.998
0.5	0.998	0.998	0.998
0.6	0.998	0.998	0.998
0.7	0.998	0.998	0.998
0.8	0.998	0.998	0.998
0.9	0.998	0.998	0.998
1.0	0.998	0.998	0.998
1.1	0.998	1.048	1.098
1.2	0.985	1.083	1.180
1.3	0.961	1.104	1.246
1.4	0.929	1.115	1.301
1.5	0.891	1.118	1.345
1.6	0.848	1.116	1.383
1.7	0.803	1.109	1.415
1.8	0.756	1.100	1.443
1.9	0.708	1.089	1.470
2.0	0.659	1.078	1.496
2.1	0.611	1.014	1.417
2.2	0.569	0.957	1.346
2.3	0.532	0.907	1.283
2.4	0.498	0.862	1.226
2.5	0.469	0.822	1.175
2.6	0.443	0.785	1.128
2.7	0.419	0.752	1.086
2.8	0.397	0.722	1.047
2.9	0.378	0.695	1.011
3.0	0.360	0.669	0.978
4	0.244	0.499	0.754
5	0.186	0.408	0.631
6	0.152	0.354	0.555
7	0.131	0.317	0.504
8	0.116	0.292	0.468
9	0.106	0.274	0.442
10	0.098	0.260	0.422
11	0.093	0.250	0.407
12	0.088	0.242	0.395
13	0.085	0.235	0.386
14	0.082	0.230	0.378
15	0.080	0.226	0.372
16	0.078	0.223	0.367
17	0.077	0.220	0.363
18	0.076	0.218	0.360
19	0.075	0.216	0.358
20	0.074	0.215	0.355

COMPUTER PROGRAM FOR MODEL B

// JOB

// FOR

*IOCS (2501 READER, 1403 PRINTER,DISK)

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

```

    DIMENSION A(100),B(100),C(100),F(100),GNCD(100),GW1CD(100),GW2CD(1
    100)
    DO 1 K=1,100,10
    IF(K-1)32,31,32
31 P=K
    P=P/1000.0
    GO TO 33
32 K13=K-1
    P=K13
    P=P/1000.0
33 WRITE(5,100)
100 FORMAT(1H1,2X,'FRACTIONAL GROWTH RATE=G (ROOTS UNDER CONTINUOUS IR
    IRADIATION)-MODEL B')
    WRITE(5,101)P
101 FORMAT(1H ,2X,'P=',1X,F5.3,/)
    WRITE(5,106)
106 FORMAT(1H ,20X,'G-VALUES AT TIME INTERVALS=INTERMITOTIC CYCLE TIME
    1',////,'TIME(IN CELL CYCLES)',4X,'G(C10 DIE)',4X,'G(C10 DIF.)',4X,
    2'G(C10 DIVIDE+DIF.)')
    DO 2 K1=1,10,1
    A(K1)=10.0
    B(K1)=0.0
    2 C(K1)=0.0
    F(1)=1.0
    DO 8 K8=1,,20,1
    M=11
    N=20
    DO 3 K2=1,10,1
    IF(M-11)50,51,50
51 DO 4 K3=M,N,1
    L2=K3-11
    L1=K3-1
    X=1.0-(EXP(-1.595*F(K2)))
    X=X/1.595
    X=X/F(K2)
    Y=1.0-P
    IF(K3-M)10,11,10
11 A(K3)=2.0*X*Y*A(L1)
    B(K3)=X*P*2.0*A(L1)
    C(K3)=X*2.0*B(L1)
    SUMA=A(K3)
    SUMB=B(K3)
    SUMC=C(K3)
    GO TO 4
10 A(K3)=Y*A(L2)
    B(K3)=(A(L2)*P)+B(L2)
    C(K3)=C(L2)
    SUMA=SUMA+A(K3)

```


FIGURE D 5

```

SUMB=SUMB+B(K3)
SUMC=SUMC+C(K3)
4 CONTINUE
K3=N
K5=K3
M=M-10
N=N-10
GO TO 53
50 DO 5 K3=M,N,1
L2=K3+9
L1=K3+19
X=1.0-(EXP(-1.595*F(K2)))
X=X/1.595
X=X/F(K2)
Y=1.0-P
IF(K3-M)54,55,54
55 A(K3)=2.0*X*Y*A(L1)
B(K3)=X*P*2.0*A(L1)
C(K3)=X*2.0*B(L1)
SUMA=A(K3)
SUMB=B(K3)
SUMC=C(K3)
GO TO 5
54 A(K3)=Y*A(L2)
B(K3)=(A(L2)*P)+B(L2)
C(K3)=C(L2)
SUMA=SUMA+A(K3)
SUMB=SUMB+B(K3)
SUMC=SUMC+C(K3)
5 CONTINUE
K3=N
M=M+10
N=N+10
K5=K3
53 F(K2)=(SUMA+SUMB+SUMC)/100.0
X=1.0-(EXP(-1.595*F(K2)))
X=X/1.595
X=X/F(K2)
GNCD(K2)=((A(K5)+B(K5)+C(K5))*(1.0-X))/(5.0)
GW1CD(K2)=GNCD(K2)+((X*C(K5))/5.0)
GW2CD(K2)=GNCD(K2)+(2.0*((X*C(K5))/5.0))
IF(K8-3)70,70,71
70 K20=K8-1
E=K2
E=E/10.0
H=K20
E=E+H
WRITE(5,111)E
111 FORMAT(1H,8X,F3.1)
WRITE(5,110)GNCD(K2),GW1CD(K2),GW2CD(K2)
110 FORMAT(1H+,25X,F5.3,10X,F5.3,13X,F5.3)
71 K10=K2+1
3 F(K10)=F(K2)
K2=10
IF(K8-3)80,80,81
81 WRITE(5,103)K8
103 FORMAT(1H,8X,I3)
WRITE(5,102)GNCD(K2),GW1CD(K2),GW2CD(K2)

```

FIGURE D 6

```
102 FORMAT(1H+,25X,F5.3,10X,F5.3,13X,F5.3)
80 F(i)=F(K10)
8 CONTINUE
1 CONTINUE
CALL EXIT
END
```

```
// XEQ
```

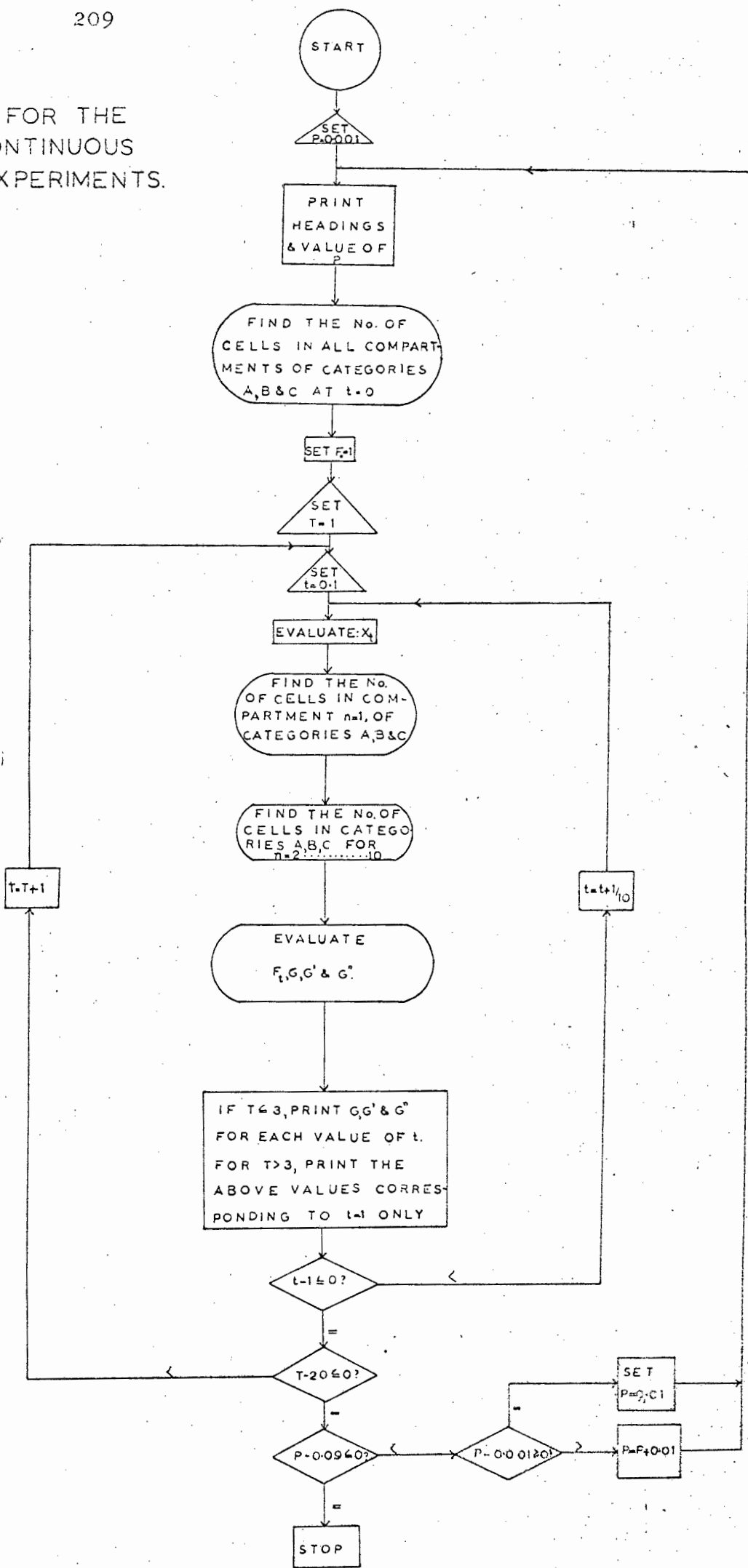
FIGURE D 7

FRACTIONAL GROWTH RATE=G (ROOTS UNDER CONTINUOUS IRRADIATION)-MODEL B
 P= 0.050

G-VALUES AT TIME INTERVALS=INTERMITOTIC CYCLE TIME

TIME(IN CELL CYCLES)	G(C10 DIE)	G(C10 DIF.)	G(C10 DIVIDE+DIF.)
0.1	1.000	1.000	1.000
0.2	1.000	1.000	1.000
0.3	1.000	1.000	1.000
0.4	1.000	1.000	1.000
0.5	1.000	1.000	1.000
0.6	1.000	1.000	1.000
0.7	1.000	1.000	1.000
0.8	1.000	1.000	1.000
0.9	1.000	1.000	1.000
1.0	0.999	0.999	0.999
1.1	0.999	1.049	1.099
1.2	0.996	1.094	1.192
1.3	0.991	1.134	1.278
1.4	0.982	1.171	1.359
1.5	0.972	1.204	1.437
1.6	0.959	1.235	1.510
1.7	0.944	1.262	1.580
1.8	0.927	1.288	1.649
1.9	0.908	1.311	1.715
2.0	0.886	1.332	1.778
2.1	0.819	1.252	1.685
2.2	0.760	1.181	1.602
2.3	0.708	1.118	1.527
2.4	0.662	1.061	1.459
2.5	0.621	1.009	1.397
2.6	0.584	0.963	1.342
2.7	0.551	0.921	1.291
2.8	0.521	0.882	1.244
2.9	0.494	0.847	1.201
3.0	0.469	0.815	1.161
4	0.309	0.599	0.889
5	0.229	0.485	0.740
6	0.184	0.416	0.648
7	0.155	0.371	0.586
8	0.136	0.339	0.542
9	0.123	0.316	0.510
10	0.113	0.299	0.486
11	0.105	0.286	0.467
12	0.100	0.276	0.453
13	0.095	0.268	0.441
14	0.092	0.262	0.432
15	0.089	0.257	0.424
16	0.087	0.252	0.418
17	0.085	0.249	0.413
18	0.083	0.246	0.409
19	0.082	0.244	0.406
20	0.081	0.242	0.403

FIGURE D8.
 FLOW DIAGRAM FOR THE
 ANALYSIS OF CONTINUOUS
 IRRADIATION EXPERIMENTS.



B I B L I O G R A P H Y
=====

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