A STUDY COMPARING THE EFFECTS OF VINCRISTINE WITH THOSE OF IONIZING RADIATION ON THE ROOT MERISTEM OF *VICIA FABA*

by

Dawne Haddad, B.Sc. (Hons.) (Cape Town).

A thesis submitted in fulfilment of the requirements for the degree of Master of Science in the Faculty of Science of the University of Cape Town.

Department of Bio-Engineering
and Medical Physics,
Groote Schuur Hospital,
Cape Town.

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CHAPTER 1.

THE CHOICE OF A SYSTEM.

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The Choice of a System.

Vincristine and vinblastine, two closely related alkaloids are extracted from the Madagaskar Periwinkle, Vinca rosea Linn. They have been shown to be effective oncolytic agents and cause arrest of mitosis at the metaphase stage, similar to that of colchicine and colcimid.

Clinically, vincristine has been found to produce a complete remission in patients with acute leukaemia. Tumour regression in patients with Hodgkin's disease, lymphosarcoma and certain childhood solid tumours have also been reported (Palmer, 1960; Armstrong, et al., 1961.)

Clinical studies with vincristine and vinblastine have been carried out in the Radiotherapy Department of the Groote Schuur Hospital. For example, with the aid of these alkaloids, the potential doubling times of human squamous carcinomas have been determined, (Sealy, and Greenstein, 1971).

Vincristine was found to posess antitumour, anti-mitotic, stathmokinetic and toxic properties. The mechanism of the stathmokinetic property remains obscure. Although the morphological effects are indistinguishable from that produced by colchicine, the overall biological effects of colchicine are dissimilar to those of the alkaloids.

Research programmes in the Department of Bio-Engineering and Medical Physics are being conducted on various biological systems, to determine the mode of action of the alkaloids. These systems are: the cheek pouch of the Syrian hamster, Hela and Elhrich-ascites cells in culture, and the root meristem of Vicia faba.

The reason for choosing Vicia faba as a tool for the study of radiomimetic properties of drugs, is that the cell parameters of the bean root are known with great accuracy. In the past, seedlings of Vicia faba have been used to investigate biological effects of ionizing radiation. As early as 1913, Mottram, then Director of Research at the Mount Vernon Hospital, reported effects of radiation on Vicia seedlings.

Just before the Second World War, Read began a detailed investigation of the gross effects shown by roots after irradiation, namely reduction or cessation of growth. (Read, 1952) .

Since then the root meristem of Vicia faba has been used by a widening circle of workers, and it is probable that today more is known about its response to radiation than about any other biological system. Pioneering work on Vicia includes investigations by Kumoro (1922-31), Jüngling (1923-32), and Ingber (1931-32).

A great deal of work has been done by botanists on the cell population kinetics of the root meristem of Vicia faba. Radiation has often been a tool for their

studies. The work of Clowes (1959) on the quiescent centre is notable in this respect. Hall, Lajtha and Oliver (1962) have, on the other hand, used the population kinetics of the root and deduced the dose response relations with respect to the reproductive integrity of the meristematic cells.

As important consideration in choosing Vicia as a system to work on is the cost involved. Plant systems have the advantage of cheapness of materials, and the ability to handle large enough numbers to reduce experimental errors to a reasonable level. Also, plant systems are favourable above animal systems because of the suffering which animals experience. Irradiation of root systems is also much simpler than the procedures taken to irradiate animals.

Vicia has an added advantage above most other systems; the effect of x-rays and radiomimetic drugs can be assessed conveniently by the growth reduction of the primary root. Also, the meristem of Vicia contains a small number of large chromosomes, and simple cytological analysis can easily be made. Many studies have been made of the histology and cytology of the root tip, so that the ability of radiation to produce mitotic delay/and chromosome aberration have been investigated in detail.

Read (1952) has demonstrated the influence of oxygen on the response of living cells to ionizing radiation. Read and Gray, (1959) have shown that close parallels

exist between the responses of the bean root and those of other organisms and human tissue. Vincristine has been found to be a radiomimetic drug, it is thus believed that an extrapolation of results with vincristine on Vicia faba to the human situation is possible.

CHAPTER 2.

THE QUANTITATIVE BASIS OF RADIOBIOLOGY

CHAPTER 2

"THE QUANTITATIVE BASIS OF RADIOBIOLOGY"

Before discussing the morphology of *Vicia faba* and its root, it is necessary to examine some of the radiobiological background.

Dose Response Curves.

The effect of radiation on biological functions is usually studied by determining the variation in that function with increase in dose, yielding what is known as a dose-response curve.

In 1956 Puck and Marcus described a technique of colony culture *in vitro* for mammalian cells, which permitted the determination of the x-ray dose-response curve of these cells with respect to their reproductive integrity. Later Hewitt and Wilson (1959) described a technique for determination of such a dose response curve for mouse leukaemia cells *in vivo*. Since then both these methods have been applied by other workers (e.g. Elkind and Sutton, 1959; Barensen *et al*, 1960; Berry and Andrews, 1963; Berry, 1969).

A typical dose-response curve showing the surviving fraction of hamster fibroblast cells plotted against dose in rads is shown in Fig. 2.1. The mathematical derivation and meaning of the symbols used will be discussed later in this chapter.

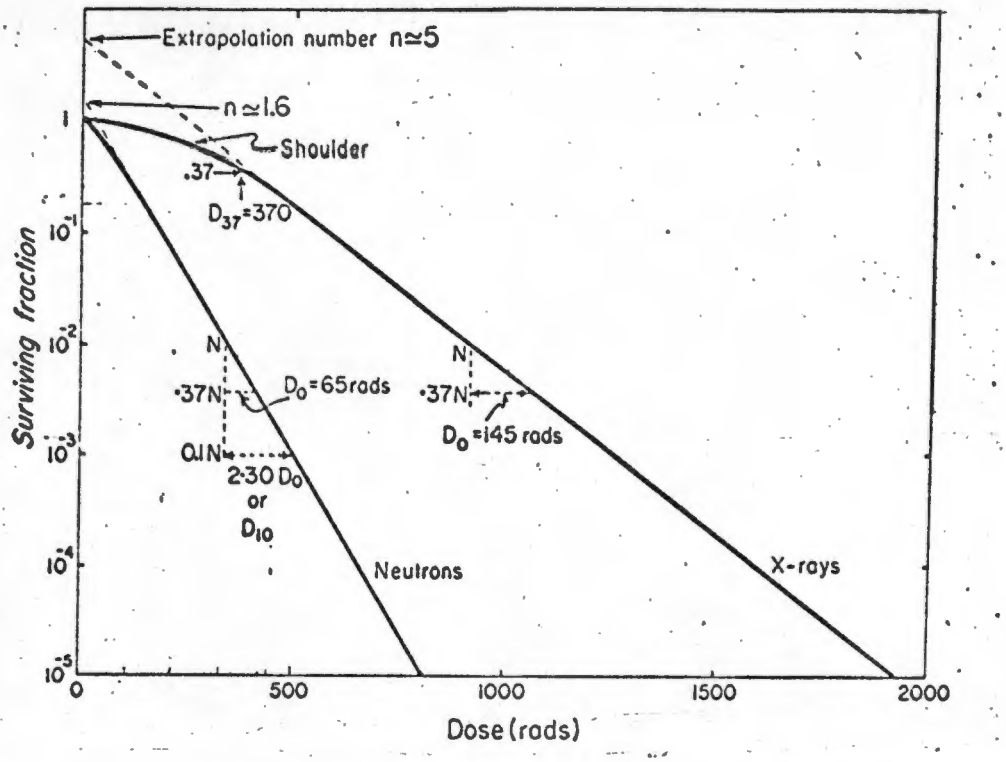

Fig. 2.1 A typical dose - response curve showing the surviving fraction of hamster fibroblast cells plotted against dose in rads. (Whitmore, 1964).

The Shape of Survival Curves.

To explain the shape of survival curves, it is convenient to speak of the region in which ionization has to be produced to obtain mutation, killing or other effect studies as the target. The passage of the ionizing particle may be spoken of as a hit.

Consider the type of action caused by a single hit: it is evident that the number of hits is simply proportional to the dose of radiation given. If the dose given is such that only a small proportion of the targets are hit, no distinction need be made between the total number of hits and the number of targets hit. The number of targets hit is then proportional to the dose, and a straight line is obtained by plotting the yield of the reaction against the dose.

If the dose is larger, so that the number of targets hit is a considerable proportion of the whole number, cases will occur of several hits being obtained in a single target. The number of targets hit will then be less than the number of hits. Although the total number of hits increases in strict proportionality to the dose, the number of targets hit increases more slowly, so that the yield plotted against the dose gives a curve which is convex upwards tending asymptotically to 100%.

If one is following, say, the killing of bacteria or single-celled organisms, the numbers killed by successive increments of dose is not equal, but each increment of dose kills the same proportion of the numbers of organisms which have survived until then. The number of viable organisms falls off in a geometrical progression, i.e. the survival curve is sigmoid, as in Fig. 2.1.

The Hit and Target Theories.

Theories, explaining the shape of survival curves, will be discussed in this and following sections.

The basic idea of the hit theory by Dessauer (1922) can be stated as follows: the reaction to be studies (e.g. lethality, loss of reproductive integrity, appearance of chromosomes aberations), occurs to a particular one out of a great number of irradiated individuals (e.g. cell populations) if a determinable number (hit number) of hits occur in that single individual. Since the region

in which the hit number must occur need not be identical with the volume of the individual each individual can have one or more targets ascribed to it.

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

Target theory (Crowther, 1924) concerns itself with concept of a "hit" or a "hit event", since the most varied types of chemical processes can be visualised as such "events" in so far as they transfer energy from radiation to matter. This concept offers the possibility of calculating from the dose-response a volume, i.e., the target, within which the required number of these absorption events must occur during irradiation with given probability.

Exponential Inactivation

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 in proportion to the number of entities present. This statement may be expressed mathematically by

$$dN = -\frac{1}{D_o} N \, dD \qquad \ldots\ldots\ldots 2.1$$

where $\frac{1}{D_o}$ is a constant of proportionality. D_o represents a dose, because dN and N have the same dimensions.

Rearranging, e.g. 2.1 becomes

$$dD = - \frac{dN}{N} \quad D_o \ldots\ldots\ldots\ldots 2.2$$

If dN is made equal to N, then D_o 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_o 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.

Equations 2.1 and 2.2 only apply to very small increments in dose dD.. They may be integrated to give

$$N = N_o e^{- D/D_o} \quad \ldots\ldots\ldots 2.3$$

where N is the number of unaffected biological entities present, after dose D, and N_o is the initial number present.

If $D = D_o$, e.g. 2.3 becomes

$$N = N_o e^{- 1} \quad \text{or} \quad N = 0.37\ N_o$$

Thus, the mean lethal dose is the dose required to reduce the population of entities to 37 per cent of its initial value, and thus destroy 63 per cent of the population.

Typical survival curves for a line of hamster cells exposed to x-rays and neutrons are given in Fig. 2.1. From equation 2.3, a straight line is expected if N/N_o

(the surviving fraction) is plotted against dose on semilogarithmic paper. This is nearly the case for neutrons, but low x-ray doses show a pronounced "shoulder". The curves become straight at large doses, so that equation 2.3 applies to large doses.

D_o is obtained from the straight portion and is the dose required to reduce the number of surviving cells from any value N to 0.37N as indicated in Fig. 2.1.

Multi-Target Survival Curves.

It can be shown (e.g. Zirkle, 1952; Fowler, 1964) that the general form of the survival curve for identical individuals having m targets, each requiring a minimum of n hits before the individual is inactivated, is given by:

$$S = 1 - (1-B)^m \quad \ldots\ldots\ldots\ldots (2.4)$$

where $$B = e^{-x}\left(1 + x + \frac{x^2}{2!} + \ldots\ldots \frac{x^{(n-1)}}{(n-1)!}\right) \ldots\ldots (2.5)$$

and $$x = D/D_o$$

S is the surviving fraction after dose D (rads) and D_o is the dose (in rads) to give an average of one "hit" in each formal target volume.

The multi-target model is derived by assuming that only one hit is required in each of m targets, i.e. n = 1 and therefore $B = \exp(-D/D_o)$

The resulting survival curve is of the form:

$$S = 1 - \{1 - \exp(D/D_o)\}^m \quad \ldots\ldots\ldots\ldots 2.6$$

Expansion of equation 2.6 indicates that for large doses, the higher terms are negligible and under these conditions the relationship approximates to:

$$S = m \exp(-D/D_o)$$

so that $\ln S = \ln m - D/D_o$

Thus, if the log of the surviving fraction is plotted against dose on a linear scale, after an initial shoulder, a straight-line graph is obtained with a slope determined by $- D/D_o$ and extrapolating back to intercept the ordinate scale at m. This form has been called a Type C survival curve by Gunter and Kohn (1956). (Oliver & Shepstone, 1964)

CHAPTER 3.

THE MORPHOLOGY OF THE ROOT

VICIA FABA

CHAPTER 3.

The Morphology of the Root of *Vicia faba*.

The species *Vicia faba* belongs to the genus of *Viciaceae*, of the *Leguminosae* family. General features of the fruit and seed of species belonging to this family are: the pistil is monocarpellary. The ovary is one-celled, and bears several ovules, arranged in one or two rows along the ventral suture. The fruit is termed a legume and is strictly so when it is dry along the dorsal suture. The embryo is large, the cotelydons are flat or plano-convex, and the radicle is superior and incurved.

The Development of the Seed.

The seed itself is a very complex structure, composed of a plant embryo, a seed coat, and a supply of stored food. . . . .

The mature embryo of *Vicia faba* consists of an axis bearing two cotelydons, or seed leaves. At the summit of the axis, above the cotyledonary node, is the plumule. This is the apex of the embryonic shoot, and is composed of the apical meristem together with embryonic leaves.

At germination, the plumule gives rise to that portion of the shoot above the cotelydons. The tapering end of the embryo, called the radicle, develops into the primary root when the seed germinates.

The Structure of the Root Tip.

In the root tip of Vicia faba there are three tiers of initials (permanently meristematic cells) in the initial zone. One gives rise to the stele or central cylinder, the second to the cortex and the third to the root cap. The epidermis differentiates from the outermost layer of the cortex and arises from the same initials.

The stele is separated from the root cap by a single layer of cells at the pole. This layer is part of the cortex-epidermis complex and its cells form the distal surface of the quiescent centre (see next section). The elongating zone extends to about 4 mm from the tip of the root. Fig. 3.1. represents a section through the primary root of Vicia, showing the position of the quiescent centre in relation to other parts of the root. Fig. 3.2. represents a median section of the normal root apex according to Clowes (1963) and Hall (1962). The shaded area represents the quiescent centre.

Fig. 3.3. represents a section through the primary root. Starting from the tip upwards, it may be divided approximately in the following sections:

1. The root cap, which occupies the first $\frac{1}{3}$ mm of the root tip, the cells of which are relatively inert. As the root pushes forward between the soil particles by growth in the zone of elongation, the root tip is protected from mechanical injury by the root cap. The cells

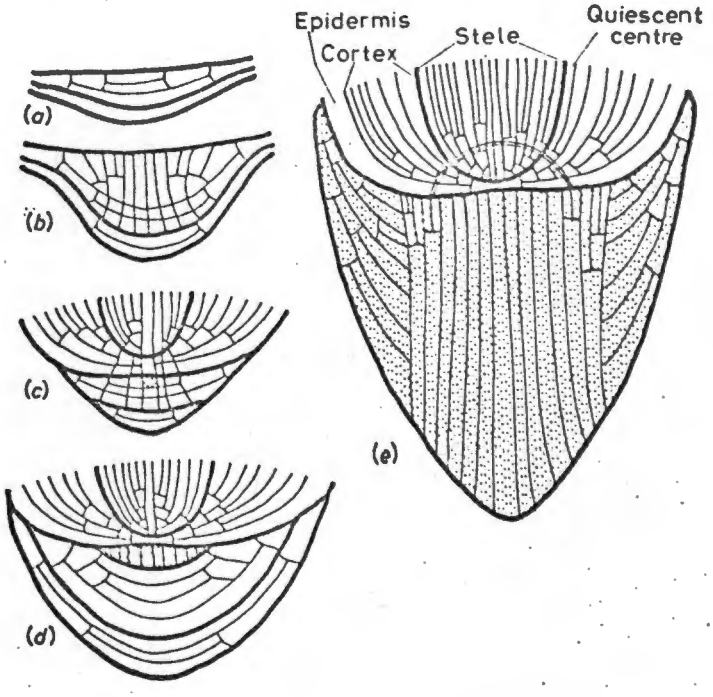

Fig. 3.1. A section through the primary root of Zea mays, showing the position of the quiescent centre in relation to other parts of the root. (Clowes, 1959).

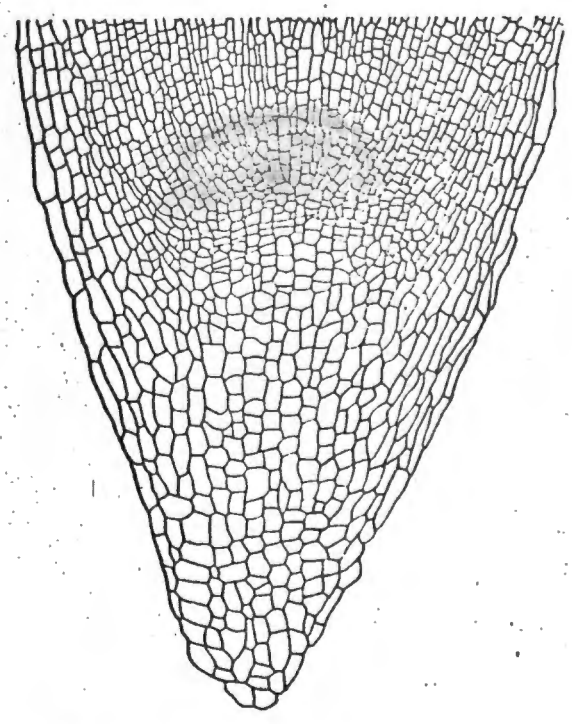

Fig. 3.2. Median section of the root apex of Vicia faba showing the position of the quiescent centre. (Clowes, 1962)

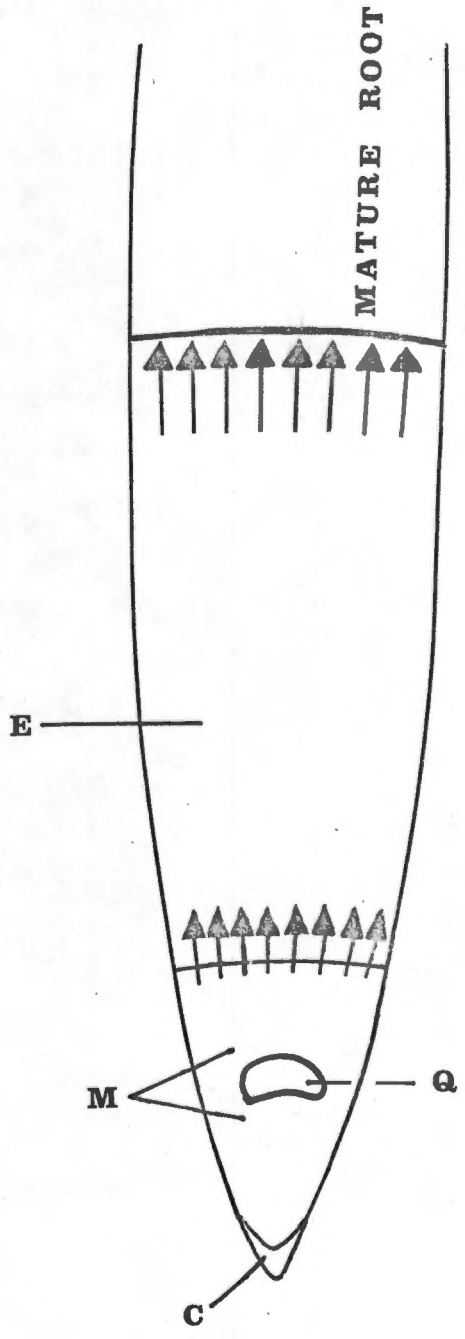

Fig. 3.3. A section through the primary root of Vicia faba.
M = meristematic zone. C = cap.
E = elongating zone.
Q = quiescent centre. (Clowes, 1959).

of the root cap are renewed from within in various ways, depending on the species.

2. The meristem occupies the next 3 mm of the root. The cells of this zone, like those in the apex of the stem, are small, thin-walled and filled with protoplasm. Vacuoles are small and numerous. Cell division of the apical meristematic cells adds little to the length of the root. For a normal, unirradiated root, the dividing cells have an intermitotic interval of 24 hours at 25°C.
3. Some of the daughter cells from division in the meristematic zone become part of the region directly behind it, the zone of elongation. Cell division may proceed for a time in the latter zone but cell enlargement, chiefly by elongation, predominates. The elongation of cells causes an increase in the length of the root.
4. The remainder of the root is build up of mature cells which are fully elongated. Maturation involves the development of undifferentiated, nonspecialised cells into specialised cells that play various roles in the activities of the root.

The Concept of Initial Cells.

Ever since a single 'apical cell' was discovered in the root meristem of ferns, attempts have been made to interpret the cell pattern in seed plants such as Vicia faba as if there were also a single totipotent cell.

Clowes (1959) suggested that the 'initials' were situated around the surface of the 'quiescent centre' - the cells of which divide very infrequently under normal circumstances.

These studies were a direct result of research based on the so-called "Körper-Kappe" theory, which describes the planes of cell division by an analysis of the pattern of cells (Clowes, 1968). This theory is an improvement on the histogen theory of Hanstein (1868), which divided the meristem into three regions, according to whether they produce stele, cortex or epidermis. The difficulty of the latter theory is that it cannot explain how the histogens are maintained and it merely divides the meristem into regions based on the assumed differentiation of their cells. Recent investigations have shown that a combination of the Körper-Kappe theory with a modified histogen theory adequately explains the pattern of cells in root meristems. Cells from various geometrical parts of the root would thus constitute the initials of the several anatomical regions of the root e.g. the meristematic cells above the quiescent centre initiate the stele and cortex, while those below initiate the root cap. (Guttenberg, 1955; Popham, 1955; Clowes, 1954).

The Quiescent Centre.

From a geometrical analysis of the pattern of division in apical meristems, Clowes (1954) postulated the existence in root apices of Zea of a quiescent centre - a region in which the cells divide rarely, if at all, in the normal growth of the root.

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

The constituent cells of the quiescent centre are carried forward passively by the growth of the surrounding meristem and contribute few cells to the root. They are quiescent only because of their position within the apex, and not because of any inherent disability. The pattern of growth in a root meristem can change both spontaneously and when stimulated, and when this happens, cells in the quiescent centre can become meristematic (Clowes, 1962).

Whilst the geometrical approach does not give unambiguous results when applied to root apices of the other species of plants with poorly defined tissue boundaries, other methods show that there is a quiescent centre in all roots except for those with a single apical cell. (Clowes, 1962, 1959).

The existence of the quiescent centre was proved by feeding Vicia faba roots with radioactive D N A precursors. In autoradiographs of prepared root sections, the quiescent centre was clearly demarcated from the remainder of the meristem because the labelled D N A precursors were incorporated at a much slower rate, indicating infrequent cell division. This method has also been used to measure rates of mitosis (Clowes, 1968).

In Vicia faba, the quiescent centre cells are grouped together in a hemispherically shaped volume. About 1000 out of 250000 actively dividing cells occupy the quiescent centre. Zea mays has roughly 600 cells compared to 125000 actively dividing cells in the quiescent centre.

The table below shows the average duration of the mitotic cycle (in hours) in three regions of root meristems for Zea mays and Vicia faba.

	Quiescent Centre	Cap Initials	Stele
Zea mays	174	12	28
Vicia faba	292	44	37

(Clowes, 1968)

Sinapsis, Pistia and Eichhornia roots showed a sharply delineated boundary between the quiescent centre and the contiguous cap initials. The proximal boundary is not always as clear. At present one can only speculate about what it is that maintains such a big difference in rates of cell division in contiguous cells, but with differences of the order of 15-fold one would expect to find also other differences in the cells of the regions of the meristem. The quiescent centre must have lower rates of synthesis than the rest of the meristem. This has been proved true for D N A and protein (Clowes, 1959), and the cells of the quiescent centre are known to have less D N A and protein and, on the average, less D N A than other parts of the apex (Jensen, 1958). They have smaller nuclei, smaller nucleoli, smaller Golgi bodies, fewer mitochondria per cell, and less endoplasmic reticulum. All these features change abruptly in passing from the quiescent centre to the cap initials, and all of them can be related to the difference in rate of mitosis (Clowes, 1963).

Behaviour of the Quiescent Centre after Irradiation.

Generally, after irradiation, the growth of a root slows down, reaches a minimum after a few days, and then recovers if the dose is not too high. In earlier explanations of how the changes in rates of root growth occurred, it was assumed that all the cells of the meristem were equally meristematic. To investigate

the behaviour of meristems after irradiation by autoradiographic methods, roots were fed with precursors of D N A at various time intervals after irradiation (Clowes, 1959). From this work it became clear that the quiescent centre behaved differently from the rest of the meristem. D N A synthesis stopped in many of the normally meristematic cells and started in the previously quiescent cells. There was thus a reversal in the roles of the two parts of the apex. This observation has been further investigated by measuring the rates of mitosis in the same way as in the normal meristems. The cells in the quiescent centre therefore form a 'reservoir' of cells which are less vulnerable because of their quiescence, but are able to restart D N A synthesis and division when the normally meristematic cells stop (Clowes, 1959).

The results are summarized in the following table for Vicia faba subjected to acute irradiation. (Clowes, 1963).

Average Duration of the Mitotic Cycle (in hours) After Acute X-Irradiation.

Dose (rads)	Days After Irradiation	Quiescent Centre	Cap Initials	Stele
0	-	292	44	37
360	3	65	95	95
360	7	38	55	55
360	10	46	51	58
360	14	162	74	41

(Clowes, 1963).

Population Kinetics in the Root Meristem of *Vicia faba*

It has been shown that, after three days of irradiation of Vicia faba roots, the mitotic cycle is the same as unirradiated roots (Hornsey, 1956). Consequently, the reduction in growth-rate which is observed at times later than three days after irradiation, does not result from a lengthening of the mitotic cycle. Further, root growth is unaffected by irradiation of the elongating zone itself, even to a very high dose, provided the meristem is shielded (Gray and Boag, unpublished; Cit. Read, 1959). Consequently, the pattern of differentiation must be determined within the meristem, and is not influenced by the existing differentiated tissue. This conclusion is supported by the work of Bünning (1952), Torrey (1955, 1957) and Ball (1948, 1951), and is discussed in detail by Clowes (1959).

The fundamental effect of radiation is the loss of reproductive integrity by a proportion of cells in the cell population. Hence the sterilizing of meristematic cells must ultimately account for the reduction in growth (Lea, 1946). It is therefore logical to conclude that the intermediate mechanism is the reduction in the number of cells that have differentiated and are presenting themselves for differentiation.

Theoretical Patterns.

Four different theoretical models of the meristem, which could explain the normal growth of the root to a fair degree of satisfaction, have been proposed by different authors. The first two of these were by Hall, Lajtha and

Oliver (1962), the third by Oliver and Shepstone (1963), and the fourth by Dewey and Howard (1963).

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

It is possible to postulate three ways in which the meristematic compartment may be expected to behave after being subjected to a dose of radiation:

(1) The pattern of differentiation within the meristem may be unaltered by the radiation; i.e. in spite of its compartment becoming depopulated as damaged cells die, 50 per cent per cell cycle may still elongate and the remainder divide. If this were true, the growth rate of irradiated roots would fall to a value characteristic of the proportion of cells sterilized, and would remain at this level (see Fig. 3.4.).

This system cannot explain the recovery that is observed in practice.

(2) The second possibility is that the "size" of the meristem may be the all-important factor, and that, once depopulated, production of elongating cells stops until cell proliferation in the meristem restores it to

its original size. Once this has been accomplished, elongation would re-commence. However, such a system would result in a temporary cessation of growth, followed by a sudden recovery to the pre-irradiation level (see Fig. 3.5). This is not consistent with the observed facts, since, even after 200 rads, the growth-rate slows but never becomes zero, and recovery takes place gradually over a period of several days.

(3) The third possibility is that when the number of meristematic cells is less than normal as a result of radiation-induced cell death, then the proportion of cells which differentiate in a given time interval is also less than normal. Hall, Lajtha and Oliver (1962) have considered this postulate in great detail and have suggested two possible meristematic models, Model A and Model B. These models will now be discussed, with brief reference only to the relevant mathematics. A full mathematical treatment will be given in the appendix.

Model A.

This model assumes that the meristem population is in exponential growth, this growth being balanced by a removal mechanism that ensures that the proportion of cells differentiating is proportional to the fractional size of the meristem. This would lead to an exponential distribution of cells within the cell cycle. Following

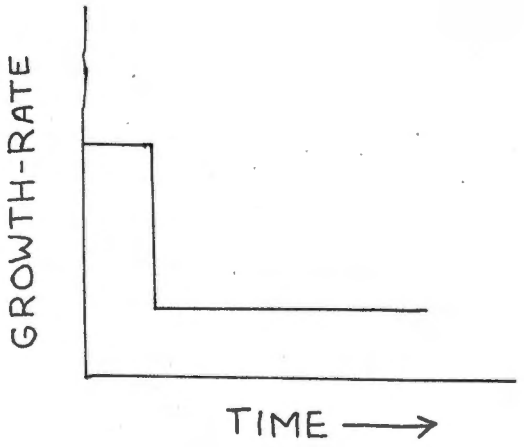

Fig. 3.4.

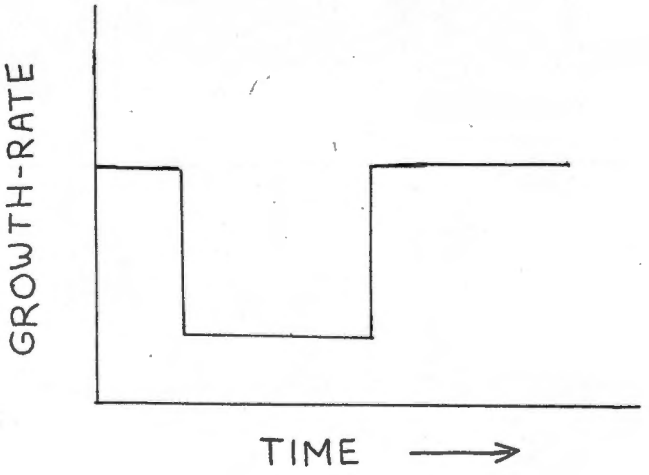

Fig. 3.5.

The postulated behaviour of meristematic compartments after being subjected to radiation dosages.

radiation damage, since the intermitotic cycle is unaltered (Hornsey, 1956), the proportion of cells dividing in a given time interval is unimpaired and the meristem is gradually repopulated.

As recovery progresses, the compartment approaches its normal size, rate of differentiation, and hence root growth returns to its steady state value. This model satisfies the observation that the miminum growth rate decreases with increase in dose, and that the subsequent recovery is gradual over a period of several days. Also, the pattern of differentiation is determined within the apex which is in accord with the conclusions of Clowes (1959).

Hall, Lajtha and Oliver (1962) derive the expression

$$\frac{dD}{dt} = \frac{y\, I_s}{\left\{1 + \left(\frac{Is}{Io} - 1\right) e^{-yt}\right\} 2} \qquad (3.1)$$

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

$$y = \frac{\ln 2}{\text{intermitotic period}} \qquad (3.2)$$

In order to simplify the derivation of the above expression, the authors have assumed that fatally damaged cells are removed immediately after irradiation. Therefore, the curve derived from the above equation cannot simply be matched to experimental growth rate curves to derive the initial population. Hall and his co-workers avoid this

difficulty by introducing a step-by-step calculation, to trace the attempts by the integer cells in the meristem to repopulate the compartment by division, while assuming that the fraction of cells which differentiate (and are lost from the compartment) depends on the total number of cells present, i.e. integer or sterile, I_t.

The term "integer" when applied to an irradiated cell implies that it has been left reproductively intact and has therefore retained its reproductive integrity.

The basis of calculation is the curve for daily growth rate,"G", as a fraction of that for control roots of equal age. A step-by-step calculation for time intervals of a quarter day has to be made. This type of calculation is inevitably an approximation, but an increase in the number of steps, whilst increasing the accuracy, adds complication. The interval of a quarter day was chosen as a compromise. G is read off from the daily growth curve for each time interval. The rate of differentiation in an irradiated, and therefore depopulated meristem equals $\left(y \frac{I_t^2}{I_s}\right)$ and the corresponding quantity for a control root is yI_s. Expressed as a fraction of control roots, the rate of differentiation, and therefore the growth G becomes

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

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

$$P = \frac{I_t}{I_s}$$

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

$$F = 1 + \frac{1 - P}{5}$$

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

The product of all the values of F is the factor by which the number of integer cells on day 0 must be multiplied to give the number present on day $10\frac{1}{4}$. Hall, Lajtha and Oliver (1962) consider this a suitable end-point for the calculation, because by the 10th day it may be assumed that the growth rate is almost exclusively due to the cells which are descendants of those which retained their reproductive integrity at the time of irradiation.

A computer programme was written to find the fraction of cells surviving each dose (Hall, Lajtha and Oliver, 1962). This programme was modified and written for use on a Wang computor. A flow diagram for this programme appears in the Appendix B.

Model B.

An alternative model was proposed by Hall, Lajtha and Oliver (1962), in which it is assumed that all meristematic cells are preparing for division, but that the proportion of cells maintaining their reproductive integrity is proportional to the concentration of a specific substance, i.e. the fraction of those preparing for division is proportional to this concentration. It is also assumed that the maintenance of the reproductive integrity implies utilization of this substance in a given region or layer of cells and is proportional to the number of cells present which retain their reproductive integrity. Cells which have lost their reprodictive integrity due to lack of substance differentiate.

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

$$G = \frac{1.595P + e^{-1.595P} - 1}{0.7975}$$

P is the proportion of the total population. The increase in the fractional size of the meristem in a time equal to $\frac{1}{T}$th of the cell cycle is

$$F = 1 + \frac{1}{1.595TP}\left[2 - 2e^{-1.595P} - 1.595P\right]$$

Expanding this expression to the first two terms of the exponential series gives (see Appendix C)

$$F = 1 + \frac{1 - 1.595P}{T}$$

This may be compared with the simple assumption

$$F = 1 + \frac{1 - P}{T}$$ derived in Model A.

This model leads to a linear distribution of cells within the cell cycle, and hence the simple fractions of the cell cycle pertain. The step-by-step calculation to compute the initial surviving fraction, f, is essentially the same as in Model A. The computor programme in Model B is more involved, however, because there is no simple relationship between G and P. In the programme an iterative procedure was used to solve for P, (Hall, et al, 1962). This programme was modified and written for use on a Wang computor. A flow diagram for this programme appears in the Appendix.

Model C.

In this model, proposed by Oliver and Shepstone (1963), the meristem is assumed fixed so that, as division takes place, the excess cells are "squeezed out" of the compartment and differentiate.

It was previously suggested (Hall, et al, 1962) that this model would imply no differentiation during recovery but if sterile cells remain in the compartment and count towards the total until themselves extruded, the pattern

of reduced growth rate and slow recovery is to be expected. The population in this instance is assumed to be in exponential growth.

Model D.

In this model, brought forward by Dewey and Howard (1963), an attempt is made to explain the dynamics of the cells in the distal 2 mm of the root under standard conditions. The presence of differentiating cells is taken into account throughout the meristematic region. A method to evaluate time parameters related to the mitotic cycle of the cells is given in this model.

CHAPTER 4.

THE VINCA ALKALOIDS AND OTHER CYTOTOXIC AGENTS

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Introduction.

A phytochemical investigation of the Periwinkle Vinca rosea Linn. has demonstrated that a number of alkaloidal substances with antitumour activity can be obtained from it. Over 30 alkaloids have been extracted, of which four - vinblastine, vinleurosine, vincristine and vinrosidene are known definitely to be active.

The two genera, Vinca and Catharanthus comprise the group of plants referred to as the Periwinkle, however, much confusion exists concerning the proper nomenclature within these genera.

The Periwinkles are members of the alkaloid-rich Apocynaceae. A paper by Bisset(1958) represents a comprehensive review of the Apocynaceae.

Botanical Considerations.

Pichon (1951) consider the genus Catharanthus to comprise six species of small shrubs and herbs which are predominently indigenous to Madagaskar. It spread to India, Indochina, Australia, South Africa and to other countries. It is a fast growing shrub, woody at the base, 40 - 80 cms high, with erect branches.

The plant enjoyed a popular reputation in indigenous medicine in various parts of the world. Peckholt (1910) described the use in Brazil of an infusion of the leaves to control scurvy and haemorrhage, as a mouth-wash for toothaches, and for the healing of chronic wounds.

The folklore reputation which the plant enjoyed, independently stimulated its phytochemical investigation in two different laboratories, unknown to each other. One of the groups included Nobel, Beer and Cutts at the Collis Laboratories in Ontario. The other group included Svoboda, Johnson, Neuss and Gorman in the Lilly Research Laboratories. (Johnson, et al, 1963.)

Extractions from Vinca rosea Linn.

The Canadian group under Noble, Beer and Cutts (1958) observed bone marrow depression in rats associated with certain fractions when treated with extracts of Vinca rosea. Continued investigations led to their preparation of vincaleukoblastine, (V.L.B.) an alkaloid capable of producing severe leukopenia in rats . (Johnson et al, 1963). An extensive phytochemical investigation resulted in the obtaining of leurosine, an alkaloid closely related chemically to V.L.B., as well as V.L.B. sulphate.(Svoboda, 1956).

It was shown that the activity of the alkaloids of the leaves of Vinca rosea Linn were more active than those contained in either the stem or roots. (Johnson et al, 1963; Svoboda, 1956). The leaf material was therefore used for the preparation of different compounds and a procedure of differential extractions was developed which separated the alkaloids according to their basicities.

The structures of vinblastine, vincristine, and, to a lesser extent, vinleurosine, are reasonably well established. Modification of these large alkaloidal molecules is difficult. After making minor changes in the configuration of these alkaloids, marked differences in activity and side-effects have been observed, when treating mice with P1534 leukaemia.

Properties and Mode of Actions of a Few Drugs Extracted from Vinca rosea Linn.

Of all the alkaloids extracted from Vinca rosea Linn., vinleurosine, vincristine, vinblastine and vinrosidene have been found to be the most "active". Studies in vivo and in vitro have shown that these alkaloids have antitumour, antimitotic, stathmokinetic and toxic effects. These four properties are closely related and makes distinction between them difficult.

1. The Antimitotic Effects.

Vinblastine and vincristine, two closely related alkaloids, have been shown to be effective oncolytic agents. They cause an arrest of mitosis at the metaphase stage in a manner which may be similar to that of other mitotic poisons, such as colchicine and colcimid. They have been shown to cause dissolution of the mitotic spindle of Pectinaria oöcytes. (Malawista, 1968). Polarized light and electron-microscopy was used to study the effects of various mitotic poisons on mitotic spindles.

2. Stathmokinetic Properties.

Studies of the stathmokinetic effects and other biological effects of vincristine on rats were performed by Frei et al. (1964). After a single dose of vincristine, an increase in the mitotic index in the marrow, duodenum and hair follicle of the rat was observed. In the bone marrow, this increase occured after 12 hours. Thereafter, the mitotic counts decreased rapidly. The increase in mitosis was found to be entirely due to the increase in the number of cells in metaphase.

The effects of vincristine on the bone marrow of five patients with malignant neoplastic diseases were studies. (Frei et al, 1964). In all five patients the M.I. increased sharply after single intravenous vincristine administrations, and a peak in M.I. occured after 12 hours. After 12 hours the M.I. decreased to normal.

Vincristine and vinblastine have been found to produce metaphase increase in vivo or in vitro in every mammalian system in which it has been studied. The mechanism of this stathmokinetic effect remains obscure. Although the morphological effects have been found to be indistinguishable from those produced by colchicine, the over-all biological effects of colchicine are dissimilar to those of the periwinkle alkaloids.

3. Antitumour Studies.

The most striking experimental biological effect of the four vinca alkaloids, vinblastine, vincristine, vinrosidene and vinleurosine, is their effectiveness in prolonging life or, in some cases "curing" DBA/2 mice given implants of the P-1534 leukaemia. A comparison of the anti-P-1534 activity of the four compounds yielded the following results: vinblastine, vincristine and vinrosidene resulted in 100 per cent prolongation of leukaemia, whereas a 50 - 100 per cent prolongation resulted after vinleurosine treatment. A comparison of the activities of the compounds on solid tumours has been made as well. (Johnson, et al. 1963).

Vincristine was found to produce a complete remission in 50 per cent patients with acute leukaemia, (Karon, et al, 1962); objective tumour regression in patients with Hodgkin's disease, lymphosarcoma, and certain childhood solid tumours (Carobone et al. 1963; Palmer, 1960; Armstrong, 1962). Vincristine has produced definite, though limited benefit in patients with carcinoma of the breast. (Armstrong, 1962).

4. Toxic Effects.

It was found that multiple intravenous doses of vincristine and vinblastine caused marked leukopenia in rats and dogs, causing death in some instances. Toxicity studies with vincristine have also been conducted in rabbits, monkeys, and cats. The acute

intravenous lethal dose for vincristine in mice is approximately 2.0 mg/kg. Multiple doses of vincristine of 0.1 mg/kg. caused death in some instances. (Johnson, et al. 1963).

The major clinical toxic manifestations of vincristine relate to the neuromuscular system, the gastro-intestinal tract and the skin. (Frei et al. 1964).

The relation between antitumour stathmokinetic and toxic action of the vinca alkaloids remains obscure. The stathmokinetic effects of vincristine in vivo obtained by Cardinali (1963) are in good agreement with the results obtained in vitro by Palmer and Warren (1962). While vinblastine and vincristine posess the same type of antimitotic activity, they seem to differ quite markedly in their antitumour effect. For instance, vincristine sems to be active against acute lymphatic leukaemia, (Costa, et al.1962; Karon, et al 1962; Rohn and Hodes, 1962; Palmer et al, 1962). Vinblastine, on the other hand, seems to be of little use as an anti-leukaemic agent. At present, the question of the relationship between antitumour and antimitotic effect of the two alkaloids mentioned above has no definite answer. It is possible that the anti-tumour effect is completely independent of the antimitotic effect, but it is also possible that the two phenomena are more or less correlated.

A Comparison of the Effects of X-Rays and 8-Ethoxycaffeine.

Considerable work has been done on the effects of chemicals described as "radiomimetic" or "nucleotoxic" on the cells of broad bean meristems. Read and Gray (1959) have noted that a close parallel exists between the production of chromosome aberrations caused by ionizing x-rays and the gross effect on the growth of roots giving G_{min} and G_{10}. Meristematic cells of Vicia have been examined after ionizing radiation. It was found that the chromosomes appeared to be sticky and characteristic errors in spiralization have been observed (Darlington and La Cour, 1945). After the effects of stickiness had disappeared, the chromosomes appeared to be broken and out of line with each other.

Experiments on 8-Ethoxycaffeine (to be represented by E.O.C.) arose from the discovery by Kihlman and Levan (1949) that various purine derivatives could induct chromosome changes in root tips of the onion, Allium cepa. These changes appeared to be of the same kind as those produced by x-rays. In addition, mitosis was temporarily inhibited. Further similarities were that the production of chromosome aberrations by E.O.C. in Allium and in Vicia cells were influenced by

the concentration of dissolved oxygen in the E.O.C. solutions.

After these discoveries were made by Kihlman and Levan (1949), experiments on Vicia seedlings were conducted by Read and Gray (1959).

The effect of E.O.C. and x-rays on the root meristem of Vicia faba was shown to bear a quantitative as well as a qualitative similarity. Qualitatively, a comparison could be made of the reduction of growth of seedlings after E.O.C. treatment with the reduction in growth after ionizing x-ray dosage. It was thus possible to correlate E.O.C. treatment with x-ray treatment. Quantitatively, by reducing the growth of two groups of roots by the same degree, by an E.O.C. treatment on the one hand, and by an x-ray treatment on the other, it was found that the two groups carried equal proportions of cells with damaged chromosomes. (Read and Gray, 1959).

CHAPTER 5.

THE MODE OF ACTION OF DRUGS ON RECEPTORS.

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INTRODUCTION.

The classical theories used to describe the relationship between the dose of a drug given and the response of a tissue observed are all based on the law of mass action. Approaches of this nature have been developed by Clark (1926), Gaddum (1957), and Stephenson (1956). A "kinetic" theory of drug action has been developed by Paton (1961, 1964). This theory is based on the rate of drug-receptor combination.

A further theory, giving an analysis of the actions of drugs and the relations between structure and action has been developed (Ariëns 1964; Drill 1958). This theory of dose-response relations for single drugs will be discussed in this chapter.

THEORY.

When the effect of a drug is studied on a simple isolated organ suspended in a bath fluid, the influence of drug transference, transport, chemical transformation, excretion, etc., is reduced to a minimum.

As a rule, the number of molecules added to the bath fluid will be very large in comparison with the number of molecules bound by the receptors. The specific receptors are the counterpart of the drug molecules as far as the specific interaction required for the induction of effect is concerned.

The receptors may be molecules, parts of molecules or molecule complexes. A single receptor can be occupied by only one molecule of the drug at one time.

The formation of a drug-receptor complex may imply a very temporary interaction between drug-molecule and receptor: it may be just a slight contact between the drug molecule and the receptor. On the other hand, there may be a definite, possibly a prolonged, chemical binding between them.

The relation between a drug, A, and the receptor, R can be represented by:-

$$[R] + [A] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [RA] \qquad \ldots.(5.1)$$

where [R] is the concentration of free receptors,

[A] is the drug concentration in the biophase, and

[RA] is the concentration of the drug-receptor complex, that is, the quantity of drug bound to the specific receptors. The total concentration of receptors, [r], is [R] + [RA] .

k_1 is the association rate constant,

k_2 is the dissociation rate constant .

Equation 5.1 represents a <u>reversible</u> interaction of drug molecules with receptors.

An increase in the concentration of the drug will result in an increase of the quantity of the drug-receptor complexes. Since the number of specific receptors in the biological object is limited, the maximal amount of drug-receptor complex has a limit, too. Increase of the concentration of the drug causes a saturation of the receptors. (Ariëns, 1964).

The arguments given below pertain as long as a gradual increase of the dose of the drug results in a gradual saturation of the receptor system.

In the case where the concentration of the drug is high compared with the number of specific receptors, it can be assumed to remain constant. Then the fraction of the total number of receptors, occupied by drug A, is represented by equation 5.2:

E
|
R
↕
A

$$\frac{[RA]}{[r]} = \frac{1}{1 + (K_A / [A])} \qquad \ldots.5.2$$

where $K_A = \frac{k_2}{k_1}$

$\frac{[RA]}{[r]}$ increases with concentration of the drug, $[A]$, and decreases with the dissociation constant K_A of the drug-receptor complex RA. Thus, the "affinity" of the drug to the receptors is proportional to the reciprocal of K_A.
E represents the effector cells.

In order to induce an effect, a drug must interact with the receptors, that is, it must have an "affinity" for the receptors. It must also interact with the receptors in an "effective" way; the drug must have an "intrinsic activity".

The effect of the drug will be proportional to the quantity of drug-receptor complexes formed. The proportionality constant, or the intrinsic activity of the drug is a measure of its ability to contribute to the stimulus, and thus, to its effect.

Let S_A be the stimulus, and S_M the maximum stimulus obtainable.

Thus,

$$\frac{S_A}{S_M} = \frac{\alpha}{(1 + (K_A/[A]))} \qquad \ldots.5.3$$

where α represents the intrinsic activity of the drug.

If a linear proportionality between stimulus and effect is assumed, then the effect, E_A, induced in the effector, E, by a certain concentration of the drug, [A], as a fraction of the maximal effect, E_m, obtainable with a drug, is represented by:

$$\frac{E_A}{E_m} = \frac{[RA]\ \alpha}{[R]} = \frac{\alpha}{1 + (K_A/[A])} \qquad \ldots..5.4$$

The effect obtained with a certain dose of A, that is, the activity of A in a general sense, increases with the intrinsic activity, α, and with the affinity, $\frac{1}{K_A}$. With high doses of A, the maximal effect for A, E_m, is reached and

$$\frac{E_A}{E_m} = \alpha$$

Further, $K_A = [A]$ if $E_A = \frac{1}{2} E_m$ (α taken as unity). . . .5.5

This is thus a mathematical method for determining the values of the affinity and the intrinsic activity of the drug. The affinity is proportional to the reciprocal of that concentration of the drug, which gives a response equal to half of the maximal response obtainable with that drug. For a particular biological object and particular type of drug the intrinsic activity is proportional to the maximal effect obtainable with the drug in question. The intrinsic activity of the drug that gives the highest response is taken as unity; then, relative values for the intrinsic activity of other drugs can be given.

The dose-response curves as calculated from equation 5.4 are hyperbolas. On a log-dose scale they take the character of sigmoid shaped curves. Fig. 5.1 represents the theoretical dose-response curves calculated from equation 5.4. The affinity as well as the intrinsic activity are varied.

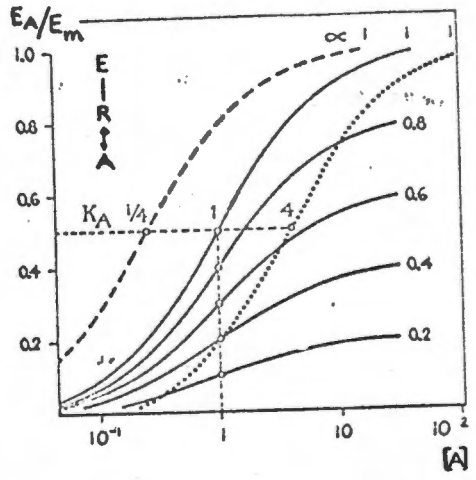

Fig. 5.1 Theoretical log concentration-response curves for compounds with varying values for the affinity ($1/K_A$) and the intrinsic activity (α). Note the parallel shift in the curves with a variation in K_A, and the decrease in the maximal height and in the slope with a decrease in α. (Concentration in M^{-1}).
(From Ariëns, E.J., 1964)

CHAPTER 6.

THE PROBLEM STATED

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THE PROBLEM STATED.

Some effects of vincristine on the broad bean root will be investigated.

(i) Vincristine is a known mitotic inhibitor and by exposing seedlings to various doses of the drug, growth patterns, similar to those resulting from x-irradiation studies, ought to result. As with radiation, survival curves could then be obtained, using models, such as Models A and B. Such survival curves could then be compared to those obtained by x-irradiation of Vicia faba. In this way the dose of drug could be equated with x-ray dose.

(ii) Further, it ought to be possible to interpret the action of vincristine on the root meristem of Vicia faba in terms of existing theories on the action of drugs on tissue. The survival curves obtained by using Models A and B could again be compared to theoretical patterns deduced from the existing drug laws. In this way the validity of the mathematical models could be further tested, this time to a mode of action different to that of radiation.

CHAPTER 7.

MATERIALS AND METHODS

CHAPTER 7.

MATERIALS and METHODS

The beans used for all experiments to be described were of the hardy "Primo" variety, which stood up to the radiation and vincristine treatments very well. In experiments which involved the bean roots to be exposed to aerobic and hypoxic solutions, the roots remained unaffected by gas bubbling through the containers in which they were emmersed.

Preparation of Seedlings for Experiments.

For each individual experiment, about 300 seedlings of *Vicia faba* were used. The method of culture of the beans was based on that used at the Medical Research Council Radiobiological Research Unit, Harwell, (Evans, Neary and Tonkinson, 1957; Read, 1959). This culture method was used by Hall, Lajtha and Oliver (1962) as well.

Vicia seedlings were soaked in wet cottonwool in a specially prepared lucite tray. They were moistened daily for four days, or until the radicle just started to appear.

The seedlings which had germinated were then carefully planted, with the radicle pointing downwards, in moist horticultural vermiculite, which was contained in a large brass tank. The tank was kept at room temperature. The vermiculite was autoclaved monthly at 126°C to remove all fungi.

After four days the radicle had grown about 4 cms in length. The seedlings were then carefully removed

from the vermiculite, washed, and their seed-coat peeled off. Those that did not germinate, and all damaged, abnormal or fungus infected beans were discarded.

A cotyledon of each bean was numbered, and a fudicial mark on the hypocotyl was made with permanent black ink. This served as a reference point from which the length of the bean root was measured.

The seedlings were then placed in the main culture tank in fresh tap water for two days before the actual experiment commenced. Two full cell cycles could thus be completed under these conditions and the cell turn-over in the meristem could thus reach equilibrium.

The culture tank was made from Perspex, and measured 70cm x 30cm x 30cm. A continuous flow of clear tap water was passed into the tank at a rate of half a litre per day. No nutrients were added to the water. With the aid of a "Grant" temperature controller, the temperature in the tank was kept constant at $25^{\circ}C \pm 0.5^{\circ}C$. A propeller stirred the water continuously to maintain circulation and ensured that the temperature was kept uniform throughout the tank. Gray and Scholes (1951) reported that a change of temperature of $1^{\circ}C$ resulted in a 20 per cent change in the growth of the roots, therefore meticulous control of the temperature was needed.

A perspex tray with rows of holes, so that the seedlings could pass through them easily, covered the tank. The roots, suspended by the cotelydons, could dangle freely in the water.

The plumules of the seedlings were removed as soon as they appeared. Observations of previous experiments have shown that there is a tendency for diurnal rythm in mitotic index and root elongation throughout a twenty-four hour period (Mottram 1913; Jüngling, etal 1930). In order to eliminate this diurnal fluctuation, many investigators remove the plumules and culture bean seedlings in darkness. Evans (1964) found that, by removing the plumules, the light effect of the growth is eliminated.

After two days of growing in the main culture tank, the seedlings were examined again. Those that showed any signs of malformation were discarded - the rest were arranged in groups of ten to twelve, depending on the nature of the experiment. The groups consisted of beans of varying lengths. Lateral roots were removed as soon as they appeared.

Preparation of Vincristine Solutions.

Vincristine (oncovin) ampoules No. 649, containing 1 mg. oncovin and 10 mg. lactose, were obtained from Eli Lilly and Company, Indianapolis, U.S.A. (An accompanying ampoule of diluting solution was not used for the experiments to be described. This solution contained 90 mg. sodium chloride, with 0.9 per cent benzyl alcohol as a preservative).

A series of experiments was conducted using different concentrations of vincristine for varying lengths of time. Solutions of vincristine were made by dissolving the vincristine powder in appropriate amounts of distilled water, depending on the concentration required. The solution was then transferred to small, flat, perspex jigs, which could hold up to 30 seedlings comfortably. These jigs had gas inlets, so that nitrogen or medical air could pass through the solutions to create hypoxic or aerobic conditions. A steady flow of gas was maintained throughout the treatment. To keep the temperature of the solution constant during treatment, these perspex jigs were suspended in the main culture tank, (see Fig. 7.1).

After remaining in the drug for the desired length of time, the seedlings were washed, measured and returned to the culture tank.

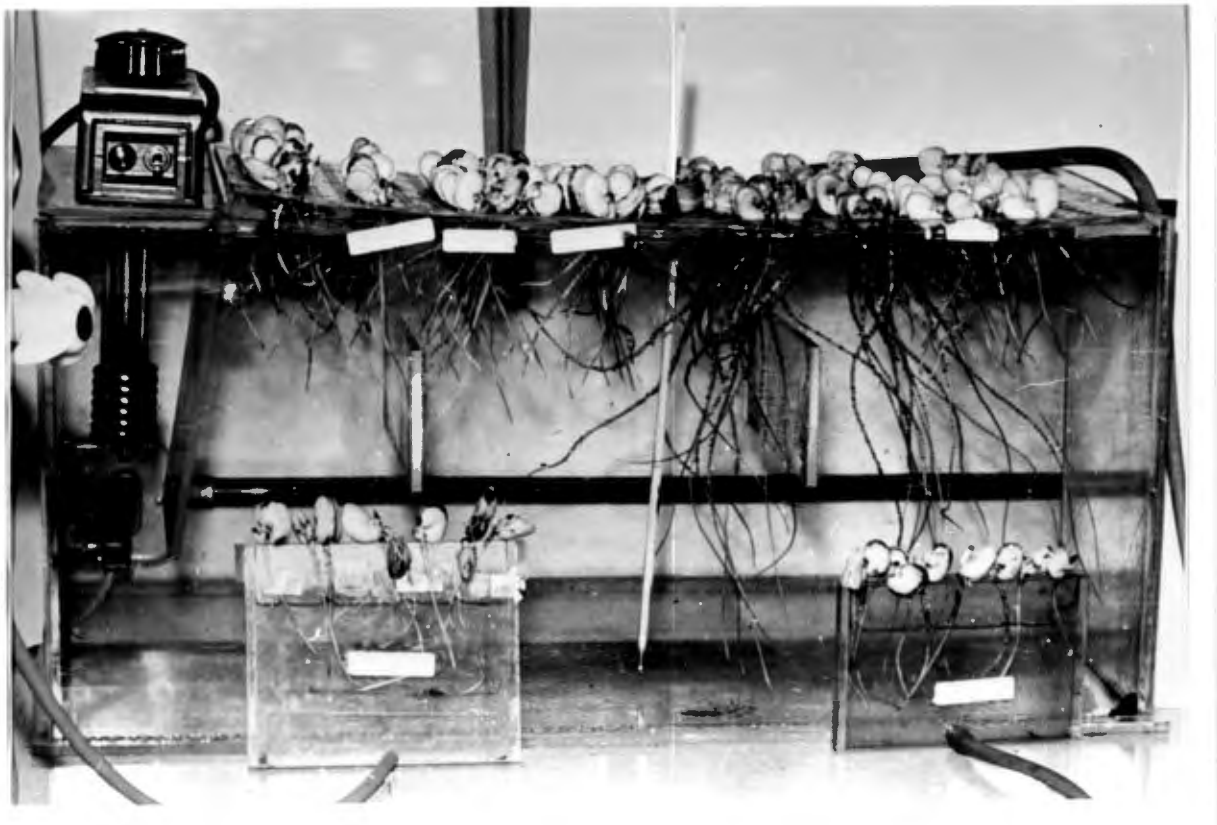

Fig. 7.1. The culture tank with the "Grant" temperature controller. Seedlings to be treated were emersed in flat perspex jigs which contained vincristine solutions.

Method of Irradiating.

Seedlings to be irradiated were placed in a flat perspex container, designed to hold about 30 seedlings at a time. It was narrower at the bottom than the top, so that the roots were congregated together and thus were exposed to the same amount of radiation. This jig measured 3 mm. deep in the direction of the beam of radiation and 7 cm x 7 cm in cross section. It has a gas inlet, through which medical air could be passed to create aerobic conditions. This small container could be slit into slots at one end of a 30 cm x 30 cm x 30 cm perspex tank which was filled with water.

This tank was set up at 25 cm from the tube focus with the long axis of the tank along the beam axis.

To measure the exact dose to the roots, a Baldwin Farmer dosemeter was placed in a perspex holder and slid into the treatment tank so that its centre coincided with the centre of the volume occupied by the tips of the roots during irradiation, (see Fig. 7.2).

The time taken to deliver 50 R was measured and the dose rate in rads per minute was computed using corrections for temperature and pressure, and using the appropriate Roentgen to rad conversion factor. (This factor was that recommended by the International Commission for Radiological Units, 1962). After the times to deliver the doses to the seedlings had been computed, the chamber was removed, and its holder was exchanged for the one to hold the Vicia seedlings.

Fig. 7.2 Perspex tank which was used for irradiating purposes. On the left is the perspex holder to accomodate a Baldwin Farmer dosemeter.

Both jig and tank were filled with tap water. Each group of beans to be irradiated was transferred in turn to the jig, and medical air was passed through the water during exposure and for fifteen minutes beforehand. This is sufficient time for equilibrium to be reached between oxygen tension of the water and the tissue of the root (Read 1952). Precautions were taken to ensure that the water level in the tank and jig remained constant.

The x-ray machine used to irradiate the seedlings was a Philips 250/25 Therapy Unit, operated at 250 k.Vp and 15 mA. The beam was filtered through 0.25 mm copper, 0.8 mm Sn and 1.0 mm Al filters. A 20 cm x 20 cm applicator was used to cover the volume occupied by the root tips adequately. During the irradiation of any group, the tube voltage and tube current were maintained by using the manual controls.

The group of seedlings to be irradiated were arranged in the jig with their roots sloping towards the centre of the funnel, the longer roots being placed towards the outside. The tube head was adjusted to ensure that all roots were in the uniform part of the field.

Gray and Scholes (1951) found that the root tip was sensitive to radiation, and that irradiation of the remainder of the root does not affect subsequent growth of the primary root. The cotelydons were, however, shielded from radiation with the aid of lead strips (see Fig. 7.3.).

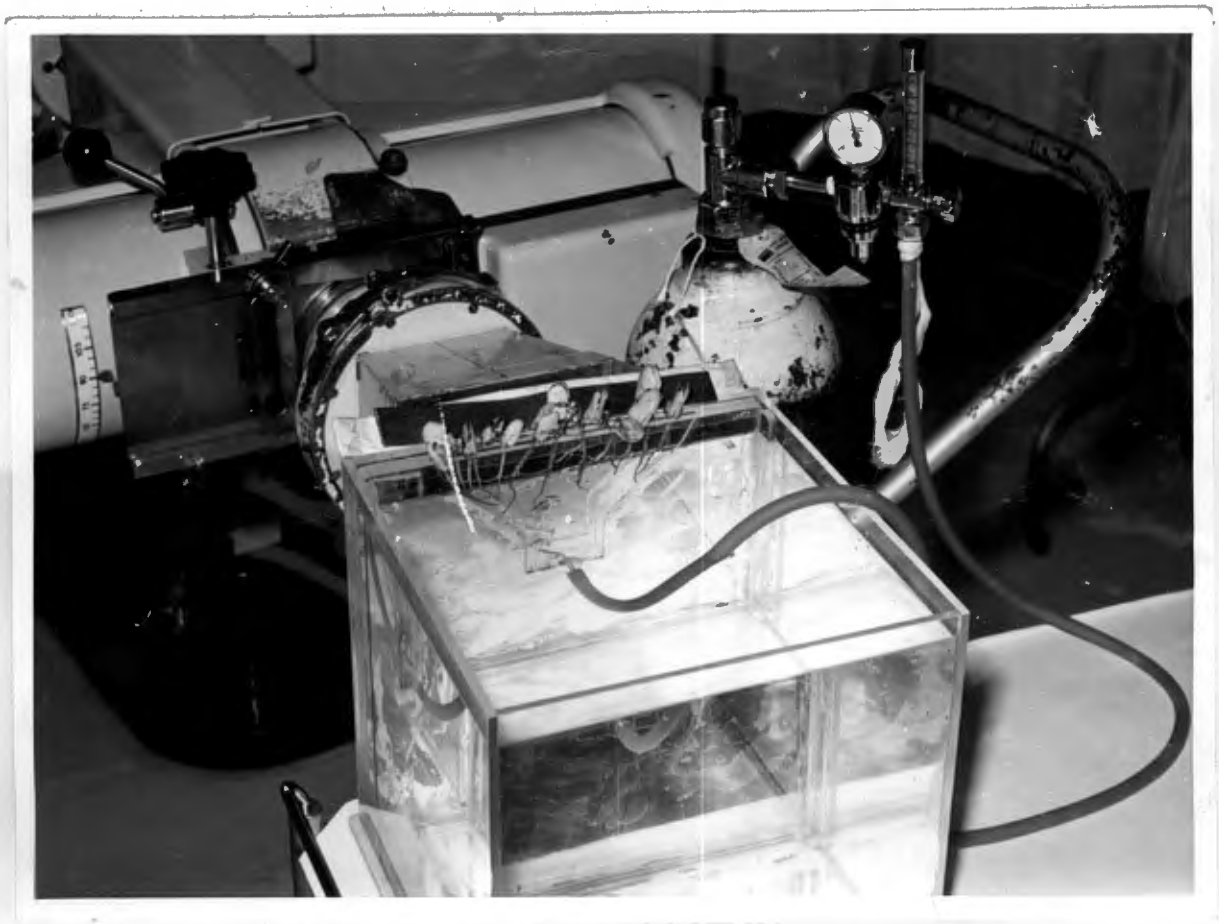

Fig. 7.3. Perspex jig set up in front of x-ray machine. Seedlings were aerated by passing medical air through the tank.

Macroscopic Method for Assessing Damage by Radiation and Vincristine.

Individual roots vary considerably in both growth rate and response to radiation and drugs. Consequently, groups of between nine and fifteen roots were exposed to each treatment and the results were treated by statistical methods. The statistical methods are described in the appendix A.

After an exposure to ionizing radiation, the growth rate of the roots is reduced to a degree which depends on the size of the dose. A large dose may cause a progressive reduction to zero, and the root, which turns brown, may not grow again. In some cases a white cone appears at the tip, and growth is resumed as a thin thread. After smaller doses the growth rate may not reach zero. There is a minimum growth rate after four days, followed by a recovery. The growth rate may eventually exceed that of the controlls, but the roots are thinner.

The roots of seedlings treated with vincristine react in a similar way. The growth rate is reduced, depending on the concentration of, and length of exposure to the drug. Again, a large dose may cause a progressive reduction in the growth rate to zero. After smaller doses, a minimum growth rate after four days, followed by a recovery is observed. Some of the treated roots, however, become hard and stubby, whereas, after very severe doses, roots tend to become very soft, jellyish and difficult to handle without damaging them. Some root tips had a

tendency to corkscrew - this made the measuring of them rather inaccurate.

The control groups, as well as the treated beans were measured on day 0 as well as each day following experimentation for eleven days. Reasons for making these measurements will be given later in this section. Measurements of the groups took place at roughly the same time each day.

The measurements of the beans were made as follows:- a meter rule was placed along the length of the culture tank. The beanroot to be measured was carefully removed from the tank and placed along the rule. The reference mark just below the cotelydon was placed opposite the "0" mark on the rule, the root was carefully straightened, and its length to the tip measured.

Lateral roots were removed, the plumules cut off, and after measurement, the seedling was placed into its position in the culture tank.

Gray and Scholes (1951) have reported that the lifting and measuring of the bean root did not affect the growth of the root.

It was found that the growth increment of the control group was not contant, and that it declined towards the eigth day. Thus the beans had to be measured daily, and their growth increment calculated as a fraction of the growth increment of the control group measured on the same day. Pilet (1961) offers a possible explanation for this decrease in control growth: a root hormone,

auxin, inhibits growth. It is thought that auxin inhibits growth more in older tissue than in younger. Thus it can be expected that growth rate falls as the root ages.

From the daily growth increments of the treated beans, curves were drawn, correcponding to each dose, to show the "daily growth" as a function of the control group. This increment in growth was regarded as that pertaining to a time halfway between the times at which the measurements were made.

In general, three parameters have been used by various workers to score the effect of radiation on root growth:

a) The 'mean lethal dose' - defined to be the dose which results in cessation of growth for four or more days by half the roots of the group. (Gray and Scholes, 1951; Spalding, Langham and Anderson, 1956, 1958).

b) The 'minimum growth rate' of the root, reached four to five days after irradiation and expressed as a fraction of the control roots of the same length (Gray and Scholes, 1951) or as a fraction of roots of the same age (Lajtha, Hall and Oliver, 1962). This quantity is marked G min in Fig. 7.4.

c) The 'growth in ten days' - defined to be the mean increment in lengths of the irradiated roots in ten days following irradiation, expressed as a

fraction of control roots in the same period (Read, 1952). It is, in effect, the area under the curve up to the tenth day in Fig. 7.4

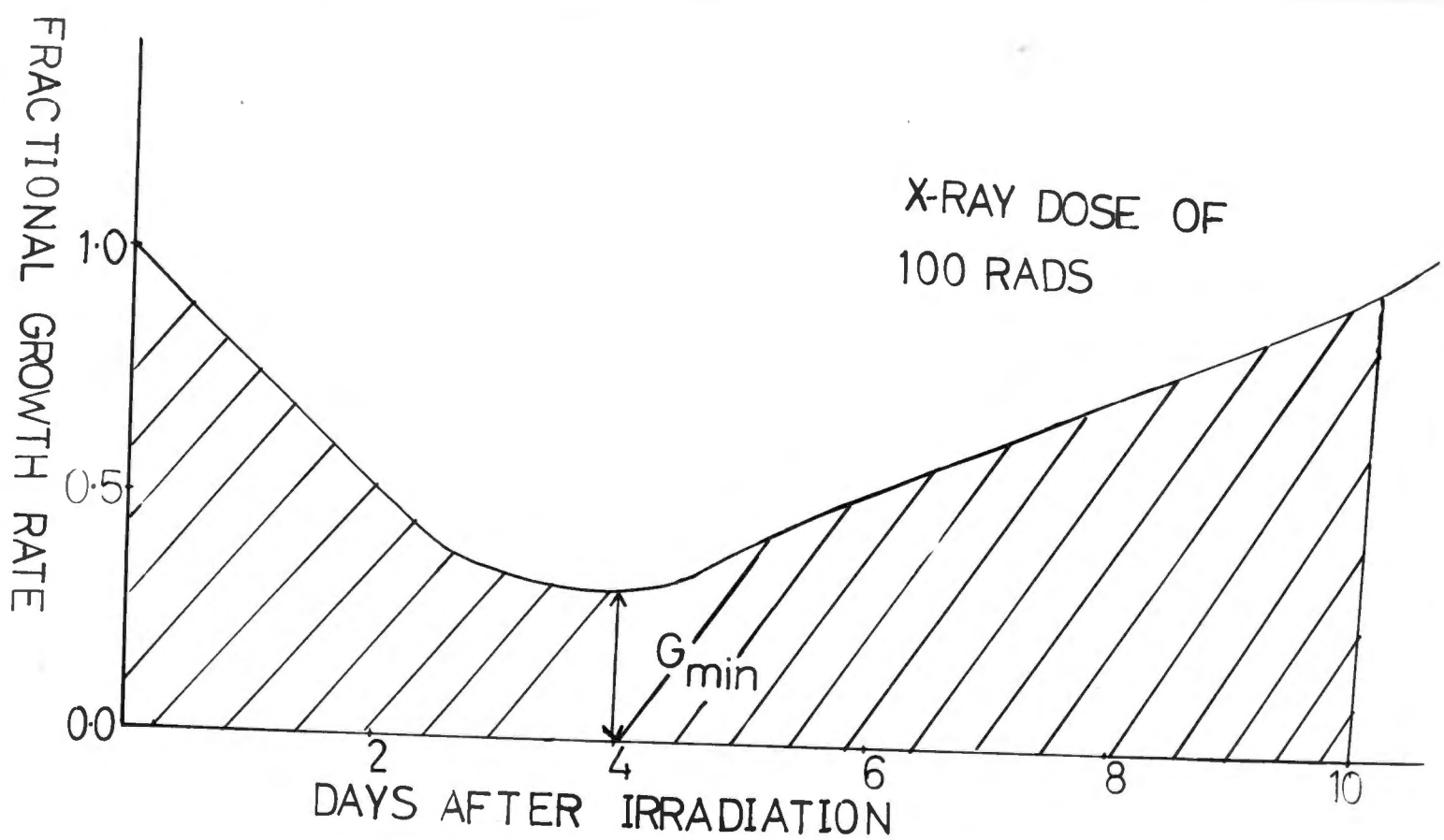

Fig.7.4. Fractional growth rate of seedlings after 100 rads x-irradiation. G_{10} is the area under the curve up to the tenth day (shaded).

CHAPTER 8.

RESULTS

CHAPTER 8.

RESULTS.

Experiment 1.

The response of Vicia faba seedlings to x-irradiation.

Following the method of irradiation and measurements described in Chapter 7, doses of 50, 100, 150, 200 and 250 rads were given respectively to five groups of fifteen roots per group. On surveying the literature on irradiation doses to Vicia seedlings, it was found that doses in the range 50 - 250 rads caused adequate reduction in the growth rate of the roots. Doses greater than 250 rads proved to be too severe for recovery to take place. (Gray and Scholes 1951; Hornsey, S. 1956; Hall and Lajtha, 1963; Hall, 1961).

The relevant growth curves are given in Fig. 8.1. The values of G_{10} and G_{min} are given in Table 8.1.

Using Model A and Model B separately, the fraction of cells surviving radiation damage was calculated. The factor F, by which the number of cells in the meristem increases has been calculated for each $\frac{1}{4}$ day (corresponding to one-fifth of the cell cycle). Using this factor, the surviving fraction F has been calculated. As an example, the data for 50 rads using the two models is given in Table 8.2 and 8.3. This calculation was repeated for each dose. Table 8.4 gives the fraction surviving

the single acute doses of radiation, using Models A and B. Fig. 8.2 shows the dose response curve for Vicia using the two models.

The standard deviation on the fraction of cells surviving 50 rads using Model A was calculated by reading the maximum values of the fractional growth and finding the corresponding fraction of cells surviving. This was repeated using the lowest figures of fractional growth. A maximum deviation of 0.14 was found. It is shown in Fig. 8.2.

Table 8.1.

Dose (rads)	G_{min}	G_{10}
50	0.579±0.11	0.775±0.12
100	0.35 ±0.04	0.58 ±0.14
150	0.178±0.02	0.376±0.12
200	0.148±0.02	0.228±0.12
250	0.11 ±0.02	0.173±0.12

TABLE 8.2

Data for 50 rads, using Model A.

Time (days)	$\sqrt{G}=P=\frac{I_t}{I_s}$	Factor F**	Product
.00	1.0000	1.0000	1.0000
.25	0.9273	1.0145	1.0145
.50	0.8246	1.0350	1.0501
.75	0.8000	1.0400	1.0921
1.00	0.7874	1.0425	1.1385
1.25	0.7745	1.0450	1.1898
1.50	0.7681	1.0463	1.2480
1.75	0.8246	1.0350	1.2887
2.00	0.8000	1.0400	1.3402
2.25	0.7874	1.0425	1.3972
2.50	0.7615	1.0476	1.4639
2.75	0.8306	1.0338	1.5134
3.00	0.8366	1.0326	1.5629
3.25	0.8660	1.0267	1.6048
3.50	0.9000	1.0200	1.6368
3.75	0.8831	1.0233	1.6751
4.00	0.8602	1.0279	1.7219
4.25	0.8366	1.0326	1.7782
4.50	0.8246	1.0350	1.8405
4.75	0.8366	1.0326	1.9007
5.00	0.8602	1.0279	1.9538
5.25	0.8831	1.0233	1.9995
5.50	0.8944	1.0211	2.0417
5.75	0.8888	1.0222	2.0871
6.00	0.8831	1.0233	2.1358
6.25	0.8717	1.0256	2.1906
6.50	0.8717	1.0256	2.2468
6.75	0.8944	1.0211	2.2942
7.00	0.9219	1.0156	2.3300
7.25	0.9486	1.0102	2.3540
7.50	0.9539	1.0092	2.3756
7.75	0.9273	1.0145	2.4102
8.00	0.9165	1.0166	2.4504
8.25	0.8888	1.0222	2.5049
8.50	0.8660	1.0267	2.5720
8.75	0.8955	1.0211	2.6263
9.00	0.9327	1.0134	2.6616
9.25	0.9539	1.0092	2.6862
9.50	0.9746	1.0050	2.6998
9.75	0.9746	1.0050	2.7134
10.00	0.9746	1.0050	2.7272
10.25	0.9746	1.0050	2.7410

** Factor F, by which number of cells in meristem is increased during $\frac{1}{4}$ day. $F = 1 + \frac{1 - P}{5}$

If f is the initial fraction surviving radiation, then $f\ I_s$ is the number of integer cells in the meristem on day 0, and $2.7410\ fI_s$ is the number on day 10.25. From the second column it is known that on day 10.25, $\frac{I_t}{I_s} = 0.9746$. Therefore the total number of cells present is $0.9746\ I_s$.

These two qualities can now be equated:

$$2.7410\ fI_s = 0.9746\ I_s$$

$$\therefore\ f = 0.36$$

TABLE 8.3:DATA FOR 50 RADS, USING MODEL B.

Time (Days)	P'	F	Product of F's
0.00	1.000000	0.9998	0.9998
0.25	.910617	1.0109	1.0108
0.50	.788641	1.0276	1.0387
0.75	.760987	1.0316	1.0716
1.00	.745925	1.0338	1.1079
1.25	.732098	1.0359	1.1378
1.50	.724567	1.0371	1.1904
1.75	.788641	1.0276	1.2233
2.00	.760987	1.0316	1.2620
2.25	.745925	1.0338	1.3048
2.50	.717037	1.0383	1.3548
2.75	.796172	1.0265	1.3907
3.00	.802469	1.0256	1.4263
3.25	.836419	1.0208	1.4561
3.75	.857777	1.0179	1.5050
4.00	.830123	1.0217	1.5377
4.25	.802469	1.0256	1.5771
4.50	.788641	1.0276	1.6206
4.75	.802469	1.0256	1.6622
5.00	.830123	1.0217	1.6983
5.25	.857777	1.0179	1.7287
5.50	.870370	1.0162	1.7568
5.75	.874074	1.0170	1.7868
6.00	.857777	1.0179	1.8189
6.25	.843950	1.0198	1.8549
6.50	.843950	1.0198	1.8917
6.75	.870370	1.0162	1.9224
7.00	.903086	1.0119	1.9454
7.25	.935802	1.0077	1.9604
7.50	.942098	1.0069	1.9741
7.75	.910617	1.0109	1.9957
8.00	.896790	1.0127	2.0211

Cont. overleaf.

Table 8.3 (Cont.)

Time (Days)	P'	F	Product of F's
8.25	.864074	1.0170	2.0557
8.50	.836419	1.0208	2.0985
8.75	.870370	1.0162	2.1326
9.00	.916913	1.0101	2.1543
9.25	.942098	1.0069	2.1693
9.50	.968518	1.0036	2.1773
9.75	.968518	1.0036	2.1853
10.00	.968518	1.0036	2.1934
10.25	.968518	1.0036	2.1934

From equation C6 in Appendix C,

$$G = 1.595\ P^2,$$

G has been calculated and plotted in terms of P.

Taking experimentally observed values of G for the meristem, the corresponding values of P and hence of F, have been read off the graph to give the factors of increase in the meristem population.

From Table 8.3, the product of all the F factors is 2.1934. From the graph, the population as a fraction of the control population at $10\frac{1}{4}$ days corresponding to a growth factor (G) of 0.94, is 0.8. Thus the initial fraction (f) of the population surviving a dose of 100 rads is given by :

$$f = \frac{0.94}{2.1934}$$

$$f = 0.43$$

Table 8.4.

Comparison of the fraction of cells surviving radiation dosage, using Model A and Model B.

Dose (rads)	Model A	Model B
50	0.3556 ± 0.14	0.43
100	0.1350	0.198
150	0.0458	0.09
200	0.0078	0.0121
250	0.001	0.001

Straight line regressions were fitted to the data (on the exponential portion of the graph) by the method of least squares. This was done for both models. The slopes of the graphs, as well as the intercepts on the Y-axis, could then be calculated. The results appear in Table 8.5. The straight line regression on Model A values is shown in Fig. 8.2.

Table 8.5.

Dosage	Fraction Surviving	
(rads)	Model A	Model B
50	0.416	0.552
100	0.121	0.173
150	0.035	0.051
200	0.010	0.017
Intercept on Y-axis	1.4	1.8
Slope of line	-0.25	-0.02

37 per cent dose slope : 40 rads.

The 37 per cent dose slope was read off the graph (see Fig. 8.2) and was found to be 40 rads. This corresponds to the dose required to reduce the surviving fraction to e^{-1} of its original value.

As in chapter 5, let E_A = the effect induced by the effector, E, i.e. the number of cells "hit".

Let E_m = maximal effect obtainable.

Then : Fraction of cells hit $= \frac{E_A}{E_m}$

And : Fraction of cells surviving $= \frac{E_m - E_A}{E_m}$

$$= 1 - \frac{E_A}{E_m}$$

$\therefore$ Fraction of cells "hit" = 1 - Fraction of cells surviving.

Table 8.6 gives the fraction of cells hit for various radiation dosages.

TABLE 8.6

Dose (Rads)	Fraction surviving.	(1 - Fraction surviving)	
		Model A	Model B
10	1.0	0	0
50	0.416	0.584	0.57
100	0.121	0.879	0.802
150	0.035	0.965	0.91
200	0.010	0.990	0.999

Fig 8.3 depicts the effect of radiation, E_A, as a fraction of the maximal effect, E_m , against $\log_{10}$ of the radiation dose. The "relative affinity" of the radiation to Vicia seedlings , i.e. $1/K_A$ was found to be 1/40 $(\text{rads})^{-1}$.

Experiment 2.

The response of Vicia faba seedlings to vincristine.

Preliminary experiments with vincristine were carried out to obtain suitable methods of application of the drug to Vicia seedlings and to determine which concentration - time relations would apply to radio-biological studies. Treatments using 1 mg vincristine/ 10 ml water proved to be too severe for recovery of the seedlings to take place.

Dose-response curves were obtained using 1 mg vincristine dissolved in 40 ml water for 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 hours. For each treatment time 15 seedlings were used. A group of 20 untreated seedlings served as controls. The method of drug application has been described in Chapter 7. After treatment with vincristine, the seedlings were returned to the culture tank (kept at 25°C) and were measured daily for 11 days.

The daily growths of the seedlings appears in Fig. 8.4. The fraction of cells surviving each dose was calculated by using Model A. The standard deviation on the fraction of cells surviving 1mg/40 ml for 0.75 hours was calculated, and found to be 0.1. (The method of this calculation has been discussed in Experiment 1, on the standard deviation on the fraction of cells surviving 50 rads).

The experiment was repeated, using the same concentration-time relations as in the previous experiment. The fraction of cells surviving each dose was calculated using Model A. The two experiments will be referred to as Exp. 2(a) and 2(b) respectively. The results of these experiments appear in Table 8.7, and Fig. 8.5

Table 8.7

Fraction of cells surviving vincristine (1 mg/40 ml) using Model A.

Time (hours)	Percent conc. x time x 10^{-3}	Fraction surviving	
		Exp. 2(a)	Exp. 2(b)
0.50	1.25	0.7200	0.3800
0.75	1.85	0.25±0.1	0.1500
1.00	2.50	0.005	0.0049
2.00	5.00	0.0040	0.003
3.00	7.50	0.0025	0.0025
Intercept on Y-axis:		30.20	41.41
Slope:		-0.70	-0.87

The unit of "dose" used turns out to be the product of the percentage concentration and the time in hours. This product is expressed in the results as a decimal fraction without units.

The 37 per cent dose slope on the initial exponential portion of Fig. 8.5 was found to be 0.2 x 10^{-3}. This corresponds to a period of 4.8 minutes of exposure to the drug.

Experiment 3:-

A comparative study, using 1 mg/80 ml was carried out for 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hours. In this case, both Models A and B were used to compute the fraction of cells surviving each dose. The values appear in Table 8.8.

Fig. 8.5 shows the dose-response curves obtained when seedlings were exposed to 1 mg/40 ml as well as 1 mg/80 ml for varying lengths of time. Straight line regressions were fitted to the exponential portions of the survival curves by the method of least squares. From these, the intercepts on the Y-axis as well as the 37 per cent dose slope could be calculated. The values are given in Tables 8.7 and 8.8.

Fig. 8.6 shows the fraction of cells surviving doses of 1 mg/80 ml, using Models A and B.

Table 8.8

Fraction of cells surviving doses of vincristine (1mg/80 ml) using Models A and B.

Time (hours)	Per cent conc. x time ($\times 10^{-3}$)	Fraction surviving	
		Model A	Model B
1	1.25	0.310	0.110
1.5	1.87	0.025	0.030
2	2.50	0.005	0.005
4	5.00	0.0051	0.0051
6	7.50	0.0033	0.0030
8	10.0	0.0035	0.0030
10	12.50	0.0025	0.0020
Slope:		-0.80	-0.82
Intercept on Y-axis:		35.60	36.0

The 37 per cent dose slope on the initial exponential portion of the graph (Fig. 8.6) was found to be 0.2×10^{-3}, which corresponds to a period of 9 minutes of exposure to the drug.

Experiment 4:-

Vicia seedlings were exposed to various concentrations of vincristine for a fixed period of time.
The concentrations that were applied were:

1 mg / 80 ml for 1 hour

1 mg / 40 ml for 1 hour

1 mg / 20 ml for 1 hour

1 mg / 10 ml for 1 hour.

The daily growth curves for these groups of seedlings appear in Fig. 8.7

Table 8.9 gives the fraction of cells surviving the doses (using Model A):-

Table 8.9

Drug concentration	per cent conc. x time	Fraction surviving
1 mg/80 ml	1.25×10^{-3}	0.42
1 mg/40 ml	2.5×10^{-3}	0.10
1 mg/20 ml	5.0×10^{-3}	0.04
1 mg/10 ml	10.0×10^{-3}	0.01

Drug Effect.

As in Chapter 5, let E_A represent the effect of the drug of concentration [A]. Let E_m represent the maximum effect obtainable with the drug. i.e. all the cells occupied.

Then: Fraction of cells "hit" $= \frac{E_A}{E_m}$

and Fraction of cells surviving $= \frac{E_m - E_A}{E_m}$

∴ Fraction of cells "hit" = 1 - Fraction of cells surviving.

Table 8.10 shows the variation of E_A / E_m with different dosages of vincristine. (The concentration was kept constant, i.e. 1 mg/40 ml, but the time of exposure to the drug was varied. Data from experiment 2(b)).

Table 8.10

per cent conc. x time x 10^{-3}	Fraction surviving (Model A)	1 - Fraction surviving
1.20	0.3800	0.6200
1.85	0.1500	0.8500
2.50	0.0049	0.9951
5.00	0.0030	0.9970
7.50	0.0025	0.9975

Table 8.11 shows the variation of E_A/E_m with different dosages of vincristine (The concentration was kept constant, i.e. 1 mg/80 ml, but the time of exposure to the drug was varied. Data from Experiment 3).

Table 8.11

per cent conc. x time x 10^{-3}	Fraction surviving (Model A)	1 - Fraction surviving
1.25	0.310	0.690
1.87	0.025	0.975
2.50	0.005	0.995
5.00	0.0051	0.995
7.50	0.0033	0.9967
10.00	0.0035	0.9965
12.50	0.0025	0.9975

Table 8.12 gives the variation of E_A/E_m with different dosages. (Here the time of exposure to the drug was kept constant, i.e. for one hour, while the concentration of the vincristine was varied. Data from Exp. 4)

Table 8.12

per cent conc. x time x 10^{-3}	Fraction surviving (Model A)	1 - Fraction surviving
1.25	0.42	0.58
2.50	0.10	0.90
5.0	0.04	0.96
10.0	0.010	0.99

Figure 8.8 shows the log dose-response curve. The data has been taken from Tables 8.10, 8.11 and 8.12.

The "affinity" of the drug to the receptors, i.e. $\frac{1}{K_A}$ was found to correspond to a vincristine dose of $\frac{1}{10^{-3}}$ i.e. 10^{+3}.

Because drug dosage is expressed as a decimal fraction without units, the "affinity" of the drug is also expressed without units. (The affinity is proportional to the reciprocal of that concentration of the drug that gives a response equal to half the maximal response obtainable with the drug).

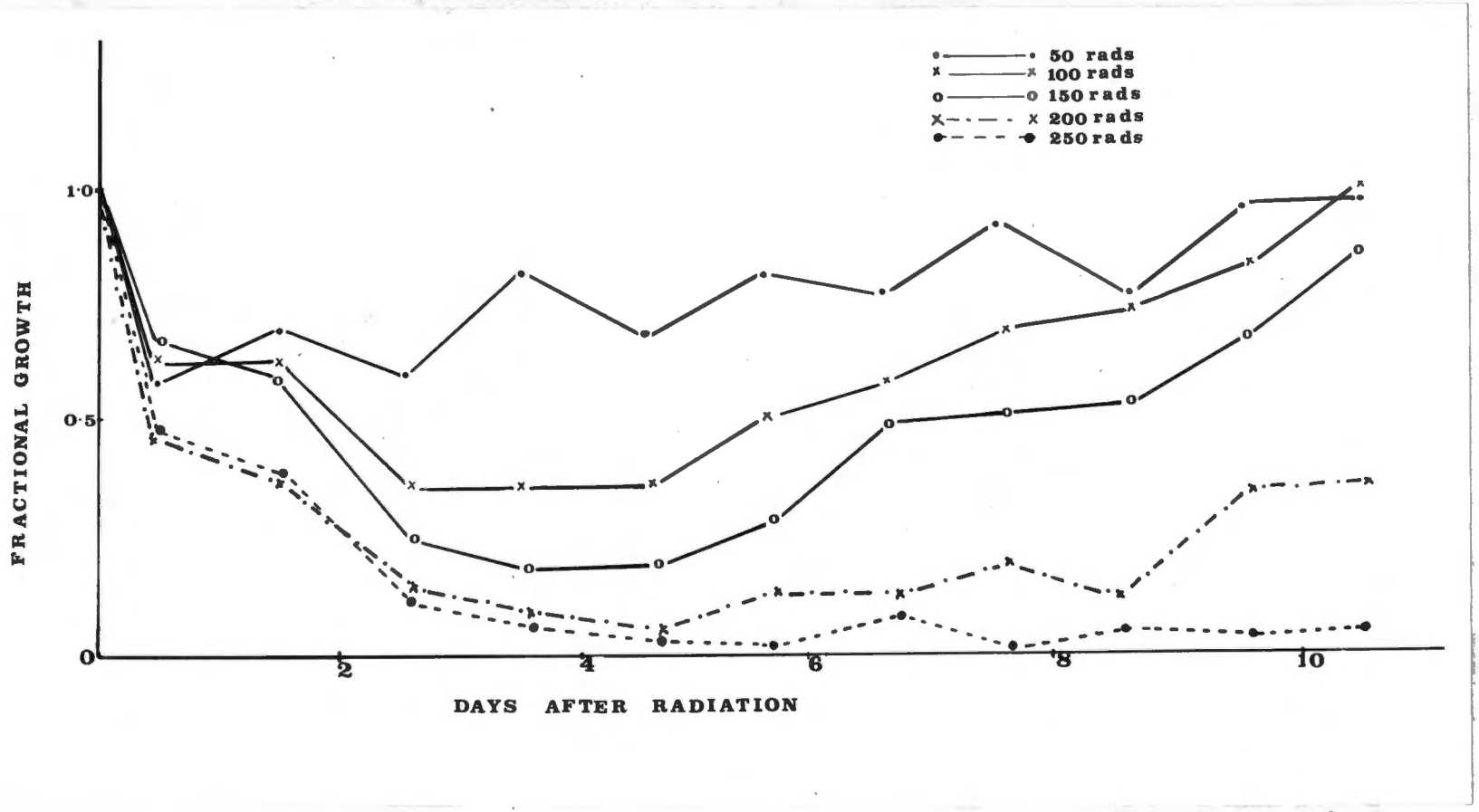

Fig. 8.1. Ordinate: Growth rate, after various doses of x-irradiation, expressed as a fraction of that for controls of equal age. Abscissa: days after irradiation. (Exp.1)

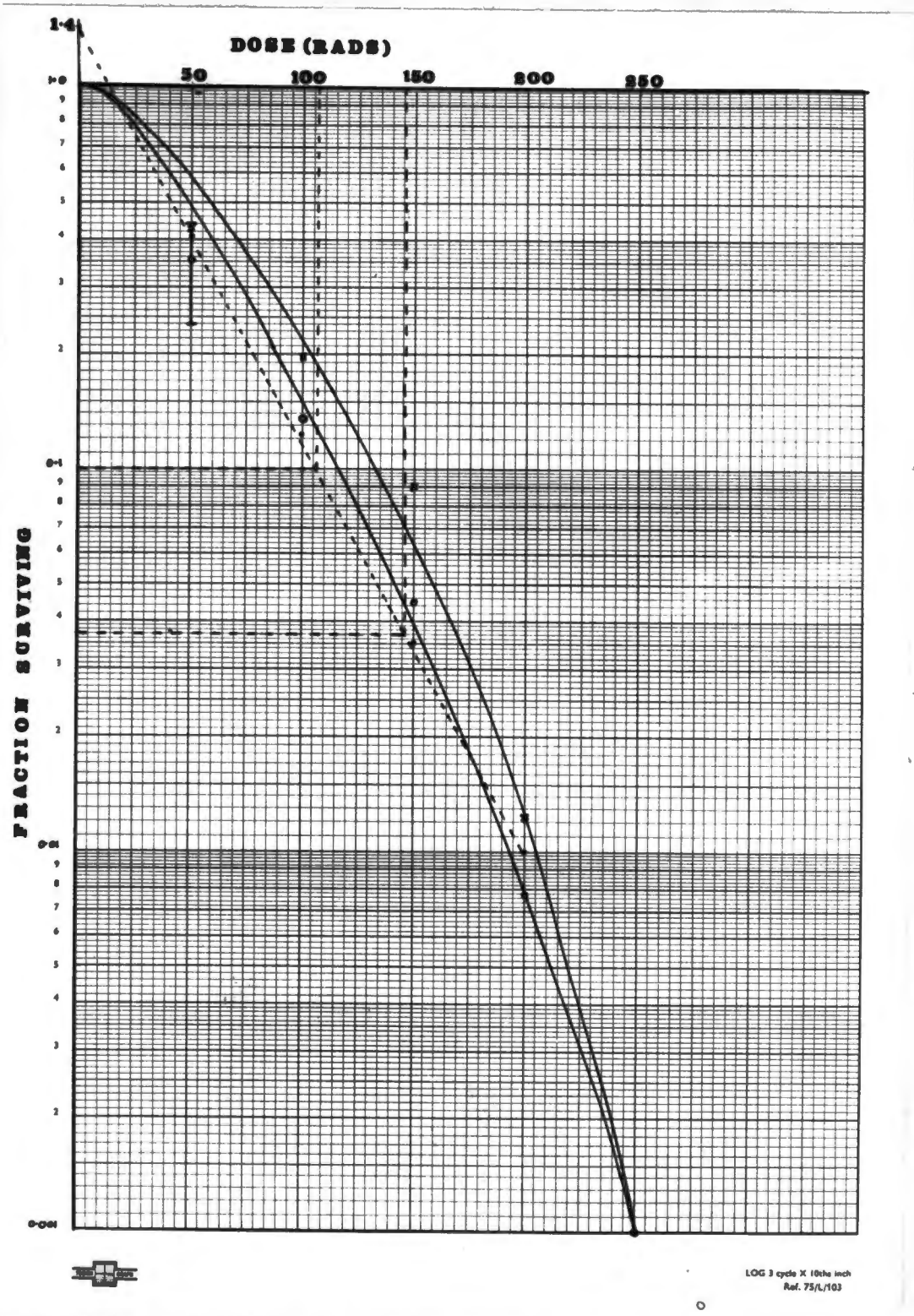

Fig. 8.2 Fraction of cells surviving radiation dosages.(Exp.1).

•———• (Model A)

x———x (Model B)

Dotted line: least squares fit to Model A.

The 37% dose slope yields a dose of 40 rads.

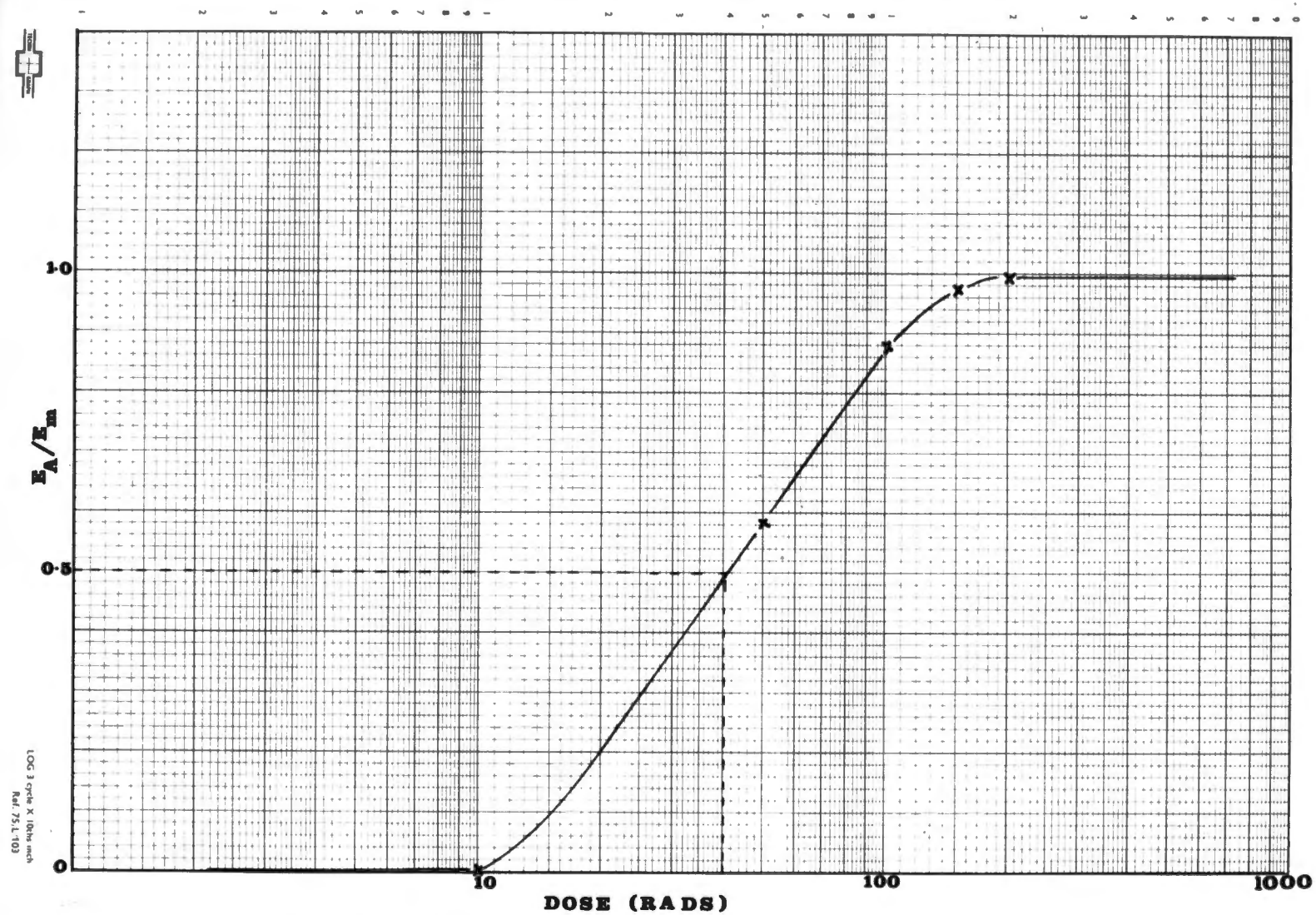

Fig. 8.3. Log-concentration response of Vicia seedlings to radiation. The "relative affinity", $1/K_A = \frac{1}{40}(\text{rads})^{-1}$

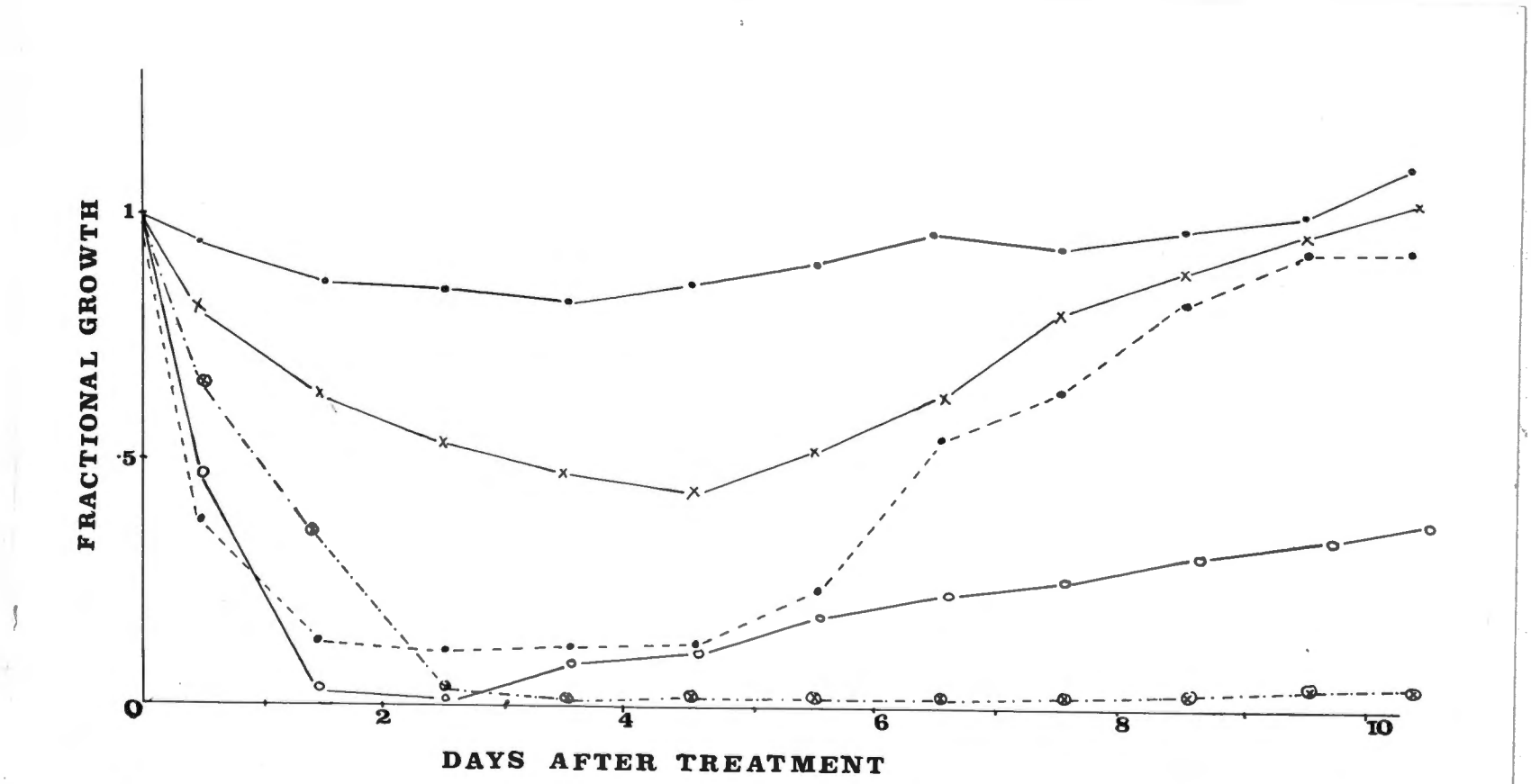

Fig. 8.4: Ordinate: Fractional growth of Vicia seedlings after exposure to vincristine (1 mg/40 ml) for various lengths of time. Abscissa: Days after treatment.(Exp.2).

- 0.5 (hours)
- 0.75 (hours)
- 1.0 (hours)
- 1.5 (hours)
- 2.0 (hours)

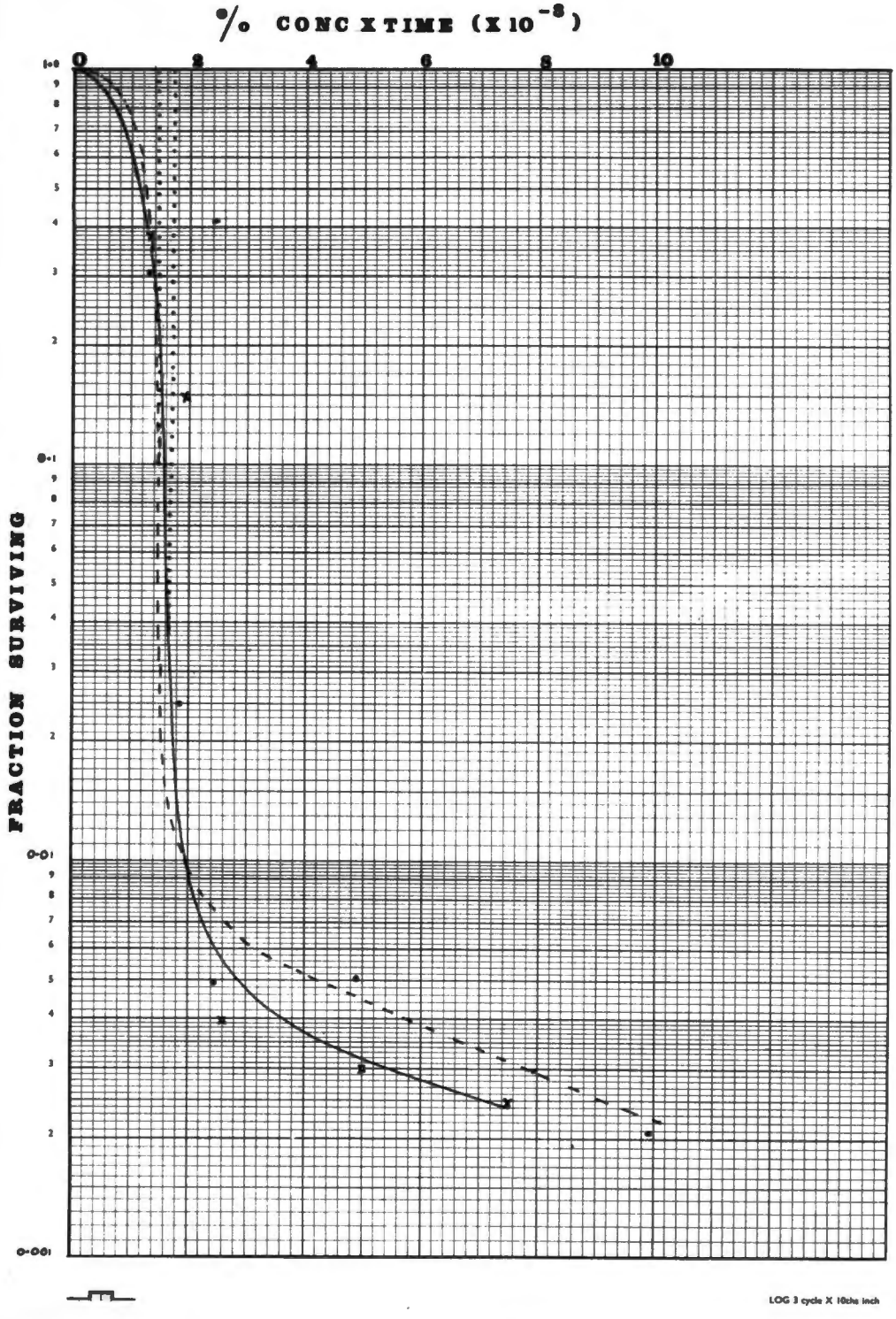

Fig. 8.5: Fraction of cells surviving vincristine doses (Using Model A.)

×——× 1 mg/40 ml (Exp. 2(b))
•-----• 1 mg/80 ml (Exp. 3)

The 37% dose slope: 0.2×10^{-3}, i.e.4.5 minutes of exposure to the drug (1 mg/40 ml) and 9 minutes of exposure (1 mg/80 ml).

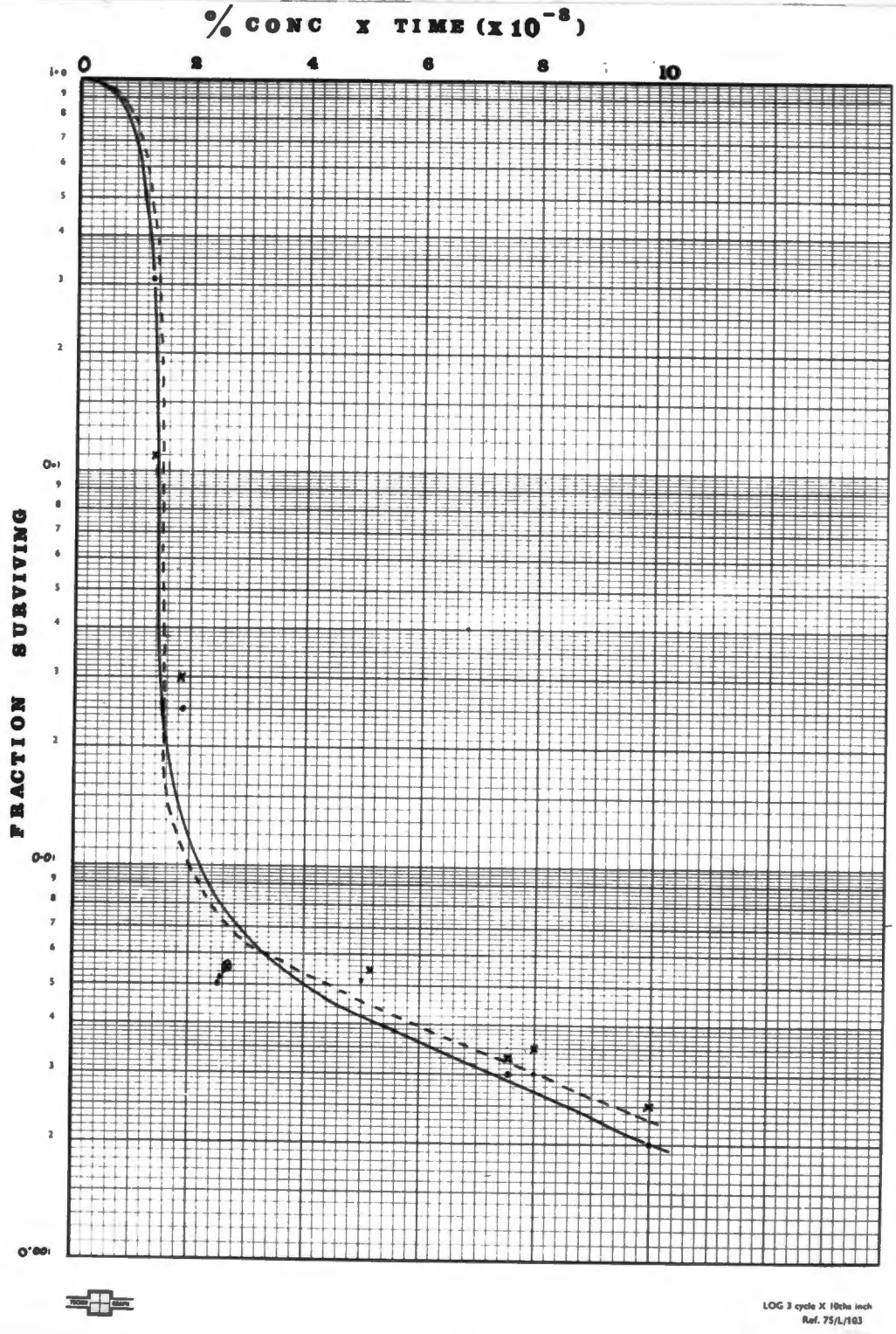

Fig. 8.6: Fraction of cells surviving vincristine doses of 1 mg/80 ml.(Exp.3).

•----• Model A.

x——x Model B.

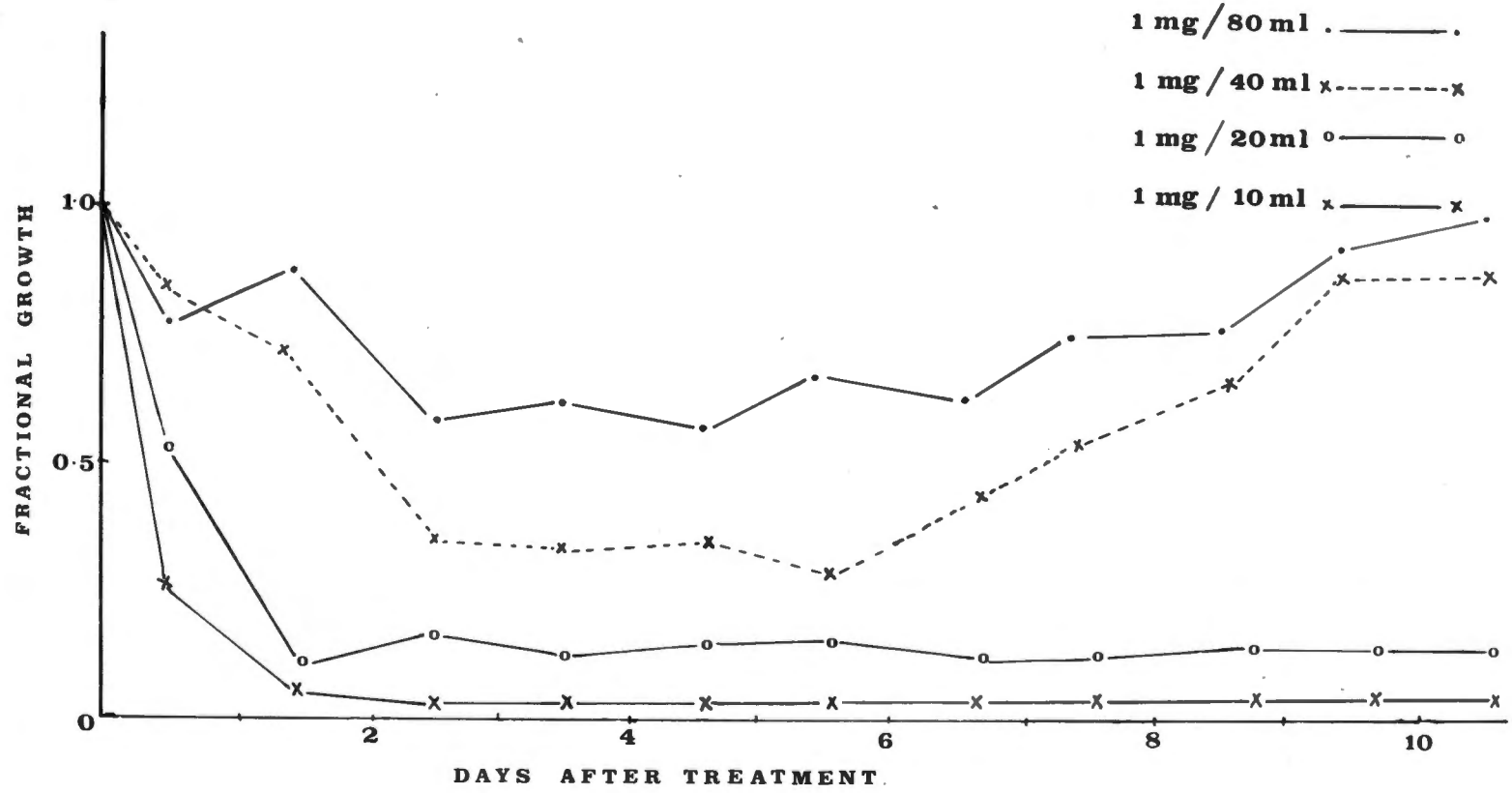

Fig. 8.7: Fractional growth rate of Vicia seedlings after various dosages of vincristine against days after treatment. Various concentrations of the drug were used for a fixed period of time (1 hour) (Exp. 4).

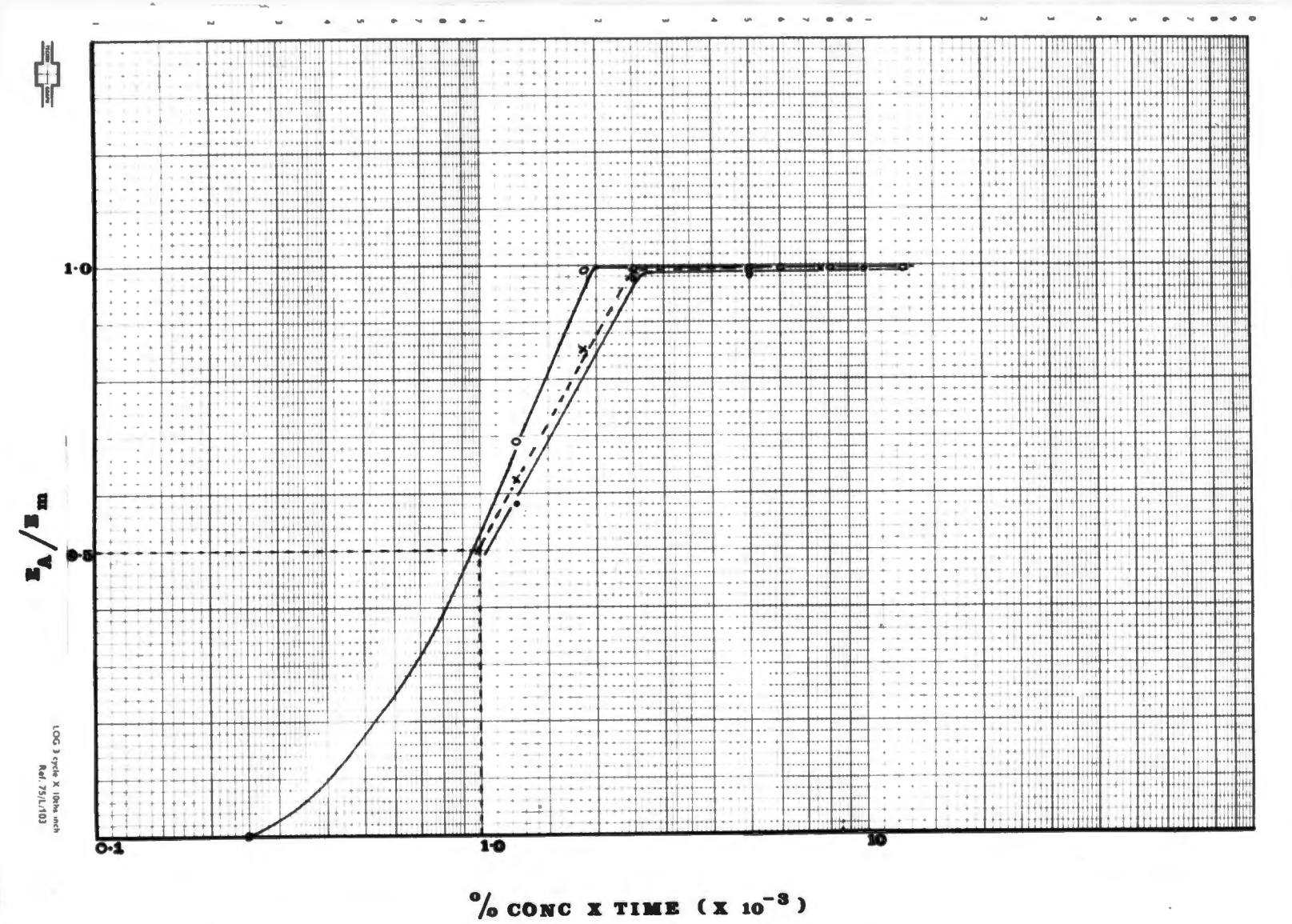

Fig. 8.8 Log-concentration response curves.

o——o 1 mg/80 ml (Exp.2)

x------x 1 mg/40 ml (Exp. 3)

•——• One hour exposure to the drug, using different concentrations (Exp. 4). $\frac{1}{K_A} = 10^{+3}$

CHAPTER 9.

DISCUSSION

CHAPTER 9.

DISCUSSION.

The Growth Curves.

The growth curves for *Vicia* exposed to ionizing radiation in air are shown in Fig. 8.1. There is an initial decrease in the growth rate as a fraction of controls of equal age. The curve then passes through a minimum before returning to pre-irradiation levels, and in some cases, even over-shooting. The minimum value of the growth rate, and the time taken for recovery depends on the size of the dose.

In order to explain the shape of the growth curve over the first three 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). Some succeed in completing two, or even more divisions, and it is assumed that they are all capable of differentiating if called upon to do so. These cells thus make a continuing, though decreasing contribution to the growth-rate of the root in the first few days following irradiation. At the same time the meristematic cells which retained their reproductive integrity make an increasing contribution to the growth-rate. This contribution from the integer cells is

represented by the chained line in Fig. 9.1, and can be derived from a consideration of the mathematical models. It is because of these two processes that the growth-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.

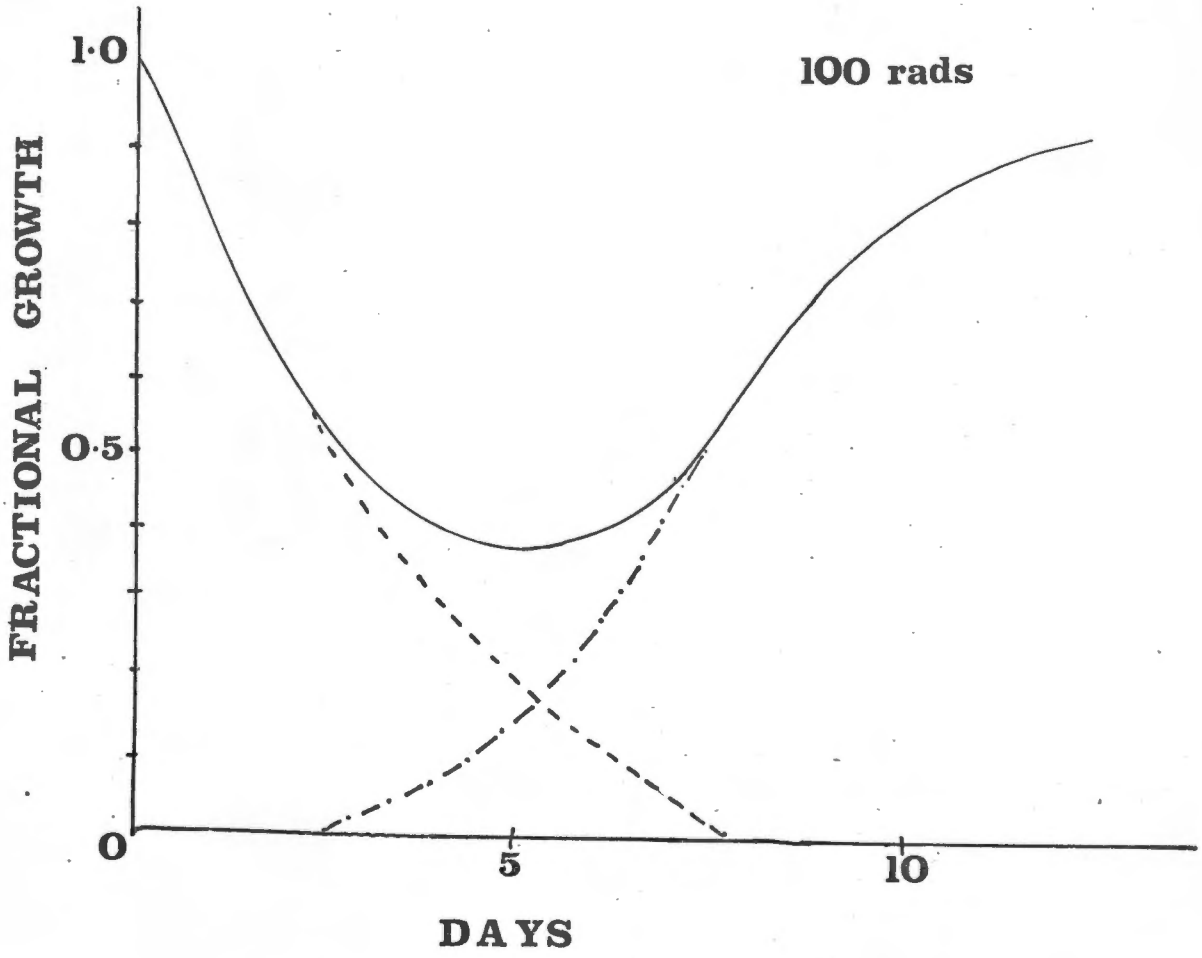

Fig. 9.1. The growth curve of roots exposed to 100 rads as a fraction of that of controls (full line) is built up of two components. The broken line represents the decreasing contribution of cells which have lost their reproductive integrity; the chained line represents the increasing contribution of integer cells.

Dose response curves with respect to reproductive integrity.

The dose response curves of the Vicia seedlings irradiated in air were deduced as described in Chapter 3 and Appendix B and C, using Models A and B. The basis for these models is that, when the number of meristematic cells is less than normal as a result of radiation-induced cell death, then the proportion of cells which differentiate in a time interval is also less than normal. Thus, an excess of cells undergoing division is provided, over those lost by differentiation, so that repopulation of the meristem can take place.

The curve obtained when the fraction of cells surviving radiation dosage is plotted against the dose, is of the sigmoid or "type C" form, in that the curve is composed of an initial pseudothreshold, or shoulder region, followed by a region of exponential decline. (Fig. 8.2).

The shape of the dose response curve obtained is similar to one which has been reported to fit the observed data on the x-ray dose response curve for reproductive integrity of mammalian cells (Puck and Marcus, 1956; Berry, 1969).

The mathematical form of the curve is the following:

$$S = 1 - \left\{ 1 - \exp\left(-D/D_o\right) \right\}^m \quad \ldots\ldots\ldots 9.1.$$

and $\ln S = \ln m - D/D_o$ (see Chapter 2)

The intercept, m, on the Y-axis was found to be 1.5 and 1.8 using Models A and B respectively. Here each biological unit presents m targets, and each of these m targets must receive n hits to cause the unit to react. Assuming that only one hit is required in each of m targets, the fraction of cells surviving can be represented by equation 9.1. This equation is of the form corresponding to a multi-target model (each target requiring one hit for inactivation).

The 37 per cent dose slope was found to be 40 rads. This is in agreement with results obtained by Hall (1962), where intercept m was found to be either 3 or 4, with a corresponding dose slope of 40-43 rads. G_{10} and G_{min} values (Table 8.1) are also in close agreement with those obtained by Hall (1962).

The Mode of action of vincristine.

When Vicia seedlings were exposed to a fixed concentration of vincristine for varying lengths of time, and also, when, for a fixed period of time seedlings were exposed to varying concentrations of the drug, growth curves were obtained. (Figs. 8.4 and 8.7). These curves were found to be similar to those when Vicia seedlings were exposed to increasing doses of ionizing radiation. The growth rates decreased initially, and passed through a minimum value, before recovering to the pre-treatment levels. In the cases where the drug doses were too severe for recovery to take place, (e.g. 1 mg vincristine/40 ml water for three hours), the growth rate decreased and remained at a low level without any subsequent recovery. The same effect was observed when Vicia seedlings were exposed to doses of ionizing x-irradiation exceeding 200 rads.

As the drug-dose to the bean root was increased, G_{min} decreased. This decrease in G_{min} was also observed when seedlings were exposed to increasing doses of ionizing radiation.

Because the growth curves following drug administration are similar to those following radiation dosages, one is justified in using similar parameters in drug studies, i.e. G_{10} and G_{min}.

Postulates, similar to those of Models A and B which describe the response of the meristem to radiation, will therefore also describe the response of the meristem to drugs

Following drug administration, the number of meristematic cells would be less than normal as a result of drug-induced cell death. Therefore the proportion of cells which differentiate in a given time is also less than normal. The proportion of cells dividing in a given time-interval is unimpaired, and the meristem gradually becomes re-populated. As recovery progresses, the compartment approaches its normal size and the rate of differentiation and hence root growth, returns to its steady state values.

Using Models A and B, survival curves, depicting the fraction of cells surviving drug-administration have been derived. (Figs. 8.5 and 8.6).

The Shape of the Survival Curves.

Sigmoid survival curves were obtained when meristematic Vicia cells were exposed to varying doses of vincristine. The curve is composed of an initial shoulder followed by a region of steep exponential decline. A second, slower, exponential decrease is observed at higher doses.

The term "fraction surviving" represents that fraction of meristematic Vicia cells which have not combined with the vincristine molecules. The term "dose" used in the drug studies is a product of the concentration of the dose (in mg/ml) and the time of exposure of the seedlings to the drug. As in drug-receptor theory (Chapter 5) the vincristine molecules are assumed to be in excess, and occupation with receptor molecules does not alter the concentration of the drug.

The survival curves obtained through radiation studies may be superficially compared with those obtained through drug studies:-

The initial exponential portion of the drug curves could be matched with the radiation survival curves. It should therefore be possible to read off the extrapolation number of the drug curve and compare it with the extrapolation number obtained from the radiation dose-response curve.

Straight-line regressions were fitted to the data of Tables 8.7 and 8.8. In the case of 1 mg/80 ml, the extrapolation number was found to be 35.6. This correspond closely to the extrapolation number obtained when 1 mg/40 ml was used for half the periods of time, of 41.4. For radiation, the intercept on the Y-axis was found to be 1.5 and 1.8 using Models A and B respectively. The fact that the extrapolation number is higher for drugs than it is for radiation, implies that, in the case of cytotoxic agents on cells, there are many more "receptor sites" per cell than in the case of radiation on cells.

The 37% dose slope, i.e. the mean lethal dose for radiation was found to be 40 rads. In the case of the drug, for a concentration of 1 mg/40 ml, this value was found to be 4.8 minutes, and for half the drug concentration, i.e. 1 mg/80 ml, the mean lethal dose was found to be 9 minutes.

The shoulder obtained on the radiation survival curve is an indication of the sublethal repair that takes place immediately after cells have been "hit". For drugs, the magnitude of the width of the shoulder could be an indication

of the time necessary to penetrate the cells and to inactivate them. Therefore a comparison of the width of the shoulders of the two types of survival curves can not be made, because of the two different mechanisms involved.

The second, slower decrease of the drug-survival curve could possibly be due to the deeply situated cells in the Quiescent Centre. These cells would become inactivated, provided that the time of exposure to the drug is long enough. In the case of radiation on meristematic Vicia cells, it has been shown (Clowes, 1959) that cells in the Q.C. form a "reservoir" of cells which are less vulnerable because of their quiescence, but are able to restart DNA synthesis and division when normal meristematic cells stop. Therefore, it is thought that, as in the case of radiation, cells in the Q.C. probably are relatively insensitive to the action of vincristine due to their situation within the meristem, or due to the fact that they divide more slowly than other meristematic cells.

Another possible explanation for the slower decrease in the survival curve is that a second type of receptor exists which is less sensitive to vincristine. Once these receptors have been saturated by the drug molecules, the subsequent slow decline in survival follows.

Pharmacological Dose-Response Curves:

By using Models A and B, the fraction of cells surviving drug dosages have been calculated. As in Chapters 5 and 8, let E_m represent the maximum effect of the drug, i.e. total number of cells occupied.
Let E_A represent the "effect" induced by a certain concentration of the drug [A].

Fig. 8.8 depicts the fraction of cells occupied against log vincristine dosage, i.e. E_A/E_m. The dose response-curves thus obtained are similar to those in Fig 5.1 which were deduced from equation 5.5.
On a log-dose scale, S-shaped curves were obtained (Fig. 8.8). These curves are of similar shape to the theoretical log concentration-response curves.

From the dose-response curves obtained experimentally, the "affinity", $\frac{1}{K_A}$, of the vincristine molecules to the Vicia receptors could be found. The affinity of a drug to receptors is constant for a specific drug. $\frac{1}{K_A}$ should therefore be independent on the mode of drug-administration.
$\frac{1}{K_A}$ was found to be of constant value in both the experiments in which a constant concentration was used for varying lengths of time, (Experiments 2 and 3) as well as in Experiment 4 where, for a fixed time, different concentrations of the drug was used. From Fig. 8.8, K_A was found

to correspond to a vincristine dosage of 1×10^{-3}

The "affinity" of vincristine to the receptors, i.e. $\frac{1}{K_A}$, is thus $\frac{1}{10^{-3}} = 10^{+3}$.

(The affinity of the drug to the cells is expressed as a fraction without units.) It can be assumed that vincristine acts on meristematic Vicia cells under equilibrium conditions, and therefore equation 5.5 is valid for this system.

Because only one drug was used, the "intrinsic" activity of vincristine relative to another drug could not be obtained.

For comparative purposes, a similar dose-response curve was drawn for radiation-induced cell mutation. (Fig. 8.3). Here, the dosage corresponding to a response equal to half the maximum response, was found to be 40 rads. The "affinity" of radiation to vincristine molecules is therefore $\frac{1}{40}(\text{rads})^{-1}$.

Oliver (1962) had found that both Models A and B approximate the true kinetics of the meristem after irradiation.

On the action of drugs on receptors, both Models A and B (which were used to determine the fraction of cells occupied by the vincristine molecules), because they accurately represent the mode of action of drugs on receptors, are therefore reliable models of the cell kinetics pertaining in the meristem.

From Fig. 8.8 the following deductions can be made:-

i) An increase in the drug dose results in an increase of the quantity of cells inactivated by the drug. This obeys the pharmacological law, where an increase in the concentration of the drug results in an increase of the fraction of drug-receptor complexes. Hence equation 5.5 is obeyed in this respect.

ii) Since the number of specific receptors in the meristem is limited, the maximum amount of cells that is able to become inactivated, is limited as well. From Fig. 8.8, a saturation of the receptors is observed after a certain drug dosage has been reached. This again obeys the pharmacological laws laid down in Chapter 5, where, the maximal amount of drug-receptor complexes is limited. An increase of the concentration of the drug causes a saturation of the receptors.

iii) Equation 5.5, which gives the relation between the fraction of cells occupied, and the concentration of the drug, is valid as long as a gradual increase of the dose of the drug results in a gradual saturation of the receptor system. In the experiments on Vicia faba, the time taken for the cells to be saturated, varied with the different concentrations. For a concentration of 1 mg/80 ml, the saturation time was about 1 hour (Table 8.8), while for 1 mg/40 ml, the time for saturation was between 0.5 and 1 hour. (Table 8.7 and Fig. 8.8).

These deductions are further proof that the pharmacological laws were obeyed when Models A and B were used to determine the fraction of cells surviving vincristine dosages.

Comparison of cell-death induced by drugs with that induced by ionizing radiation:-

i) The drug acts via the occupation of specific receptors. It is assumed that receptor sites are of the same order of magnitude as the drug molecules, whereby, in the simplest case, a reversible bi-molecular reaction occurs between drugs and receptors. In radiation studies, the mode of action is assumed to be irreversible, although sub-lethal recovery occurs immediately after the cells have been exposed to radiation.

ii) For drug studies, each cell is assumed to have a number of receptor sites allied to it. Before a cell is completely inactivated, each receptor site has to be occupied by a drug molecule. It was found that the number of receptor sites per cell is of the order of 45 for vincristine on meristematic Vicia cells.
Similarly, with radiation studies, each cell has m targets. Before a cell is sterilised, it must receive at least n hits in each of its m targets.

iii) One molecule of drug is assumed to react with 1 receptor molecule. Similarly, for radiation, in the multi-target single-hit theory, one hit is required to inactivate one target. (n = 1)

iv) It is assumed that all the individual receptors in question have the same "affinity" to the drug molecules, and that occupation of some receptors does not interfere in any way with the chance of interaction with the unoccupied ones.

In radiation theory, the x-rays are very penetrative, and the same condition holds as with drug theory.

v) The term "dose" for vincristine acting on meristematic Vicia cells is given as a product of the concentration of the drug (in mg/ml) and the time that the cells are exposed to the drug. "Dose" in radiation theory is given in terms of rads, i.e. the dosage absorbed by the tissue. The time that the cells are exposed to radiation is negligible compared to the time that cells are exposed to the drug.

SUMMARY

SUMMARY.

Models A and B are two mathematical models which have been successfully used in the past to find the fraction of meristematic Vicia cells surviving radiation dosages.

Vicia seedlings, exposed to varying doses of vincristine, yielded growth curves similar to those obtained when exposed to ionizing x-irradiation.

Because of the similarity of the growth curves, it was felt that similar parameters, i.e. G_{10} and G_{min}, and eventually Models A and B, could be used to explain the response of the meristem to drugs.

Dose-response curves were thus obtained, but these were different from those obtained through x-irradiation studies. In both cases, an initial shoulder to the curves were observed, followed by a region of exponential decline. In the case of drugs, however, a second, slower, exponentially declining component was observed.

The radiation-and drug-survival curves could be superficially compared with each other: the extrapolation number, $\underline{m}$ (i.e. the number of targets per cell), as well as the mean lethal dose of the two functions, could be determined. For radiation, $\underline{m} = 1.4$, whereas for the drug, $\underline{m} = 35.6$ and $\underline{m} = 41.4$ (using 1 mg/80 ml and 1 mg/40 ml respectively). The fact that the extrapolation number is higher for drugs than it is for radiation, implies that, in the case of cytotoxic agents on cells, there are many more "receptor sites" per cell than in the

case of radiation on cells. The 37% dose slope (i.e. the mean lethal dose) for radiation was found to be 40 rads. For a vincristine dose of 1 mg/40 ml, this value corresponded to an exposure time of 4.5 minutes, and for 1 mg/80 ml, the time of exposure of the drug to the cells was 9 minutes. The magnitude of the width of the shoulders of the survival curves could not be compared, because of the different modes of action of radiation and drugs.

The dose-response curves based on Models A and B were found to be similar to the curves based on the action of drugs on receptors. The drug-receptor theory was adapted specifically to explain the action of vincristine on Vicia meristematic cells. A value for the "affinity" of the drug to Vicia cells was deduced according to the drug-receptor laws. A comparative value for the "affinity" of radiation to Vicia cells was also deduced. The "affinity" was found to be 10^{+3} and $\frac{1}{40}(\text{rads})^{-1}$ for vincristine and radiation respectively.

Analogies were drawn between the theoretical dose-response curves and those obtained experimentally. These suggest that the cell-kinetic assumptions made in Models A and B accurately represent the cell population kinetics of meristematic Vicia cells, thus substantiating Olivers' work on radiation.

APPENDIX A.

APPENDIX A.

Statistics and Computor Programmes for the Calculations Involved.

The standard deviation σ was calculated for each group of Vicia faba seedlings measured:

$$\sigma = \sqrt{\frac{\Sigma (X - \bar{X})^2}{n}}$$

where $\bar{X}$ is the arithmetic mean of the group of n values. The results of measurements were always given as $\bar{X} \pm \Delta X$ is $\frac{\sigma}{n}$.

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

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

To find the standard error of the mean for a large number of groups of roots, a programme was written for the WANG 370 Desk Calculator. The flow diagrams for the calculation are shown in Fig. A.1. and A.2. for the calculation of $(\Delta X, \Delta y)$ and ΔG respectively.

Programme print-outs to calculate these values are given as well.

TO CALCULATE STANDARD DEVIATION σ AND STANDARD ERROR OF THE MEAN: $\sigma/\sqrt{n}$

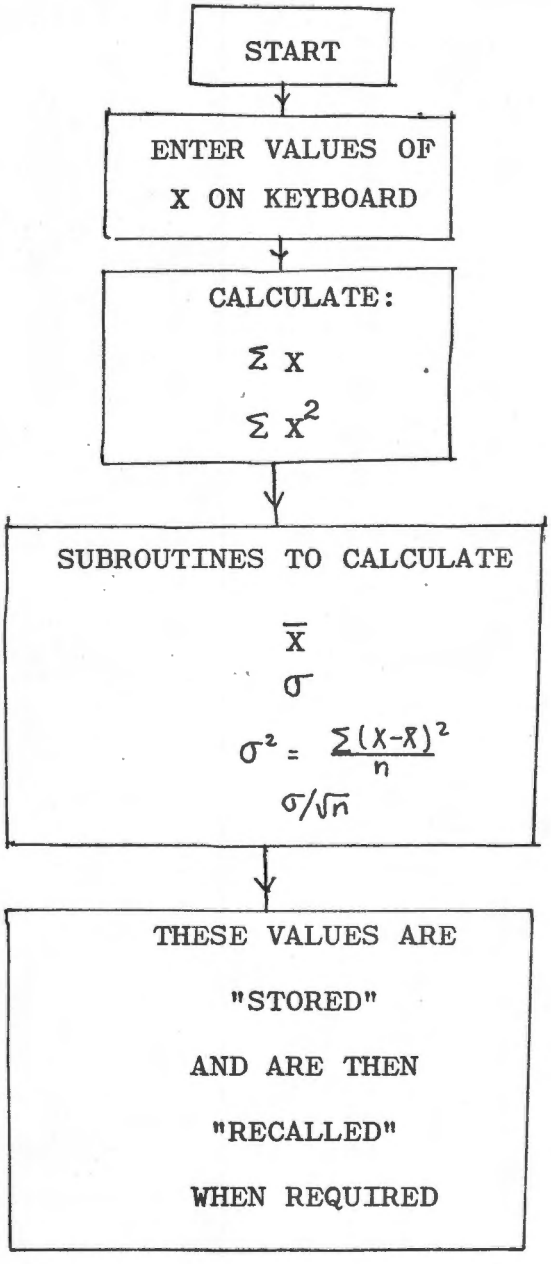

Fig. A.1.

TO CALCULATE STANDARD DEVIATION ΔG

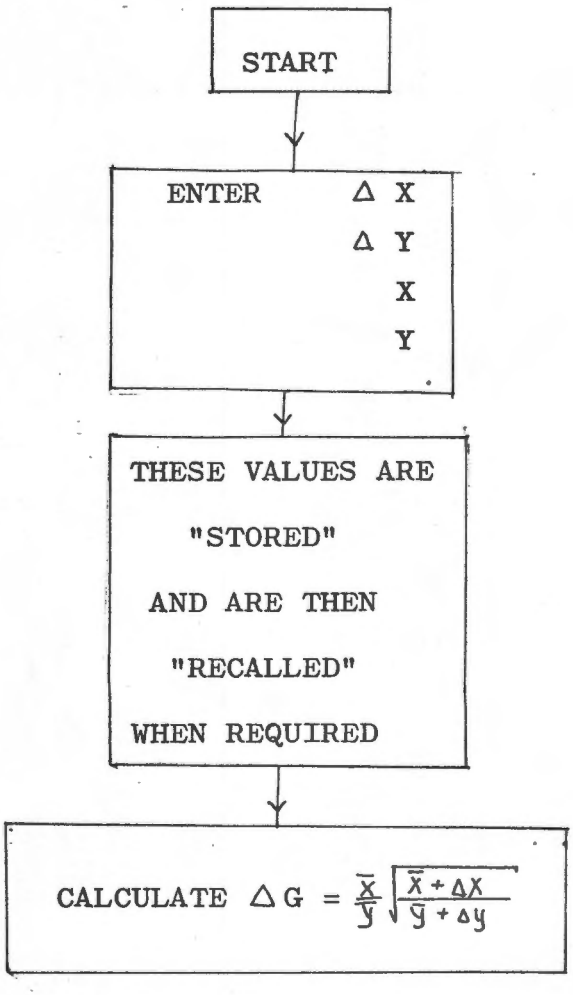

Fig. A.2.

TO CALCULATE σ AND $\sigma/\sqrt{n}$:

GSH.86

WANG PROGRAM NO BY ON

No.	Cmd	Code	COMMENT
00	Mark	07	
01	J	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	Mark	07	
11	2	62	
12	STOP	02	index X
13	SF	13	
14	9	69	$\bar{X}$ in SF 9
15	AF	12	
16	0	60	$\sum X$ in 0
17	RF	17	
18	9	69	
19	X^2	45	X^2
20	AF	12	
21	1	61	$\sum X^2$ in 1
22	1	61	Counter
23	AF	12	
24	2	62	n in 2
25	Search	02	
26	2	62	
27	Mark	07	
28	3	63	
29	RF	17	
30	0	60	$\sum X$
31	Enter	41	
32	RF	17	
33	2	62	n
34	÷	47	$\bar{X}$
35	SF	13	
36	4	64	$\bar{X}$
37	X^2	45	$\bar{X}^2$
38	Enter	41	
39	RF	17	

No.	Cmd	Code	COMMENT
40	2	62	n
41	X=	46	$n\left(\frac{\sum X}{n}\right)^2$
42	ClAl	54	
43	-Al	57	$-n(\bar{X})^2$
44	RF	17	
45	1	61	$\sum X^2$
46	+Al	56	$\sum X^2 - n(\bar{X})^2$
47	Enter	41	
48	RF	41	
49	2	62	n
50	ClAl	50	
51	+AR	50	
52	1	61	
53	-AR	53	n-1
54	÷	47	σ^2
55	SF	13	
56	5	65	
57	$\sqrt{X}$	44	σ
58	SF	13	
59	6	66	
60	X^2	45	σ^2
61	Enter	41	
62	RF	17	
63	2	62	n
64	÷	47	σ^2/n
65	$\sqrt{X}$	44	$\sigma/\sqrt{n}$
66	SF	13	
67	7	67	
68	RF	17	
69	4	64	$\bar{X}$
70	STOP	01	
71	Mark	07	
72	4	64	
73	RF	17	
74	4	64	$\bar{X}$
75	ENTER	41	
76	RF	17	
77	8	70	Control $\bar{Y}$
78	÷	47	$\bar{x}/\bar{y}$
79	STOP	01	

VERIFY PROGRAM:

3174

KEYBOARD INSTRUCTIONS:

PRIME

CONTINUE

ENTER X_1

CONTINUE

ENTER X_2

CONTINUE

.....

ENTER X_n

CONTINUE

SEARCH 3

$\bar{X}$ will show in display

RF 5 displays σ^2

RF 6 diaplays σ

RF 7 displays σ/n

If Control $\bar{Y}$ is in SF 8, on pressing "CONT", $\bar{X}/\bar{Y}$ will be computed.

TO CALCULATE Δ G:

GSH.

WANG PROGRAM Nº

BY

ON

No.	Cmd	Code	COMMENT	No.	Cmd	Code	
00	Mark	07		40			
01	1	61		41			
02	STOP	01	Enter ΔX	42			
03	SF	13		43			
04	0	60		44			
05	STOP	01	Enter ΔY	45			
06	Sf	13	Enter Y	46			
07	1	61		47			
08	STOP	01	Enter X	48			
09	SF	13		49			
10	2	62		50			
11	STOP	01	Enter Y	51			
12	SF	13		52			
13	3	63		53			
14	÷	47	1/Y	54			
15	ENTER	41		55			
16	RF	17		56			
17	1	61	Δ Y	57			
18	X=	46	ΔY/Y	58			
19	X^2	45		59			
20	ClA1	54		60			
21	+A1	56	$(\Delta Y/Y)^2$	61			
22	RF	17		62			
23	0	60	ΔX	63			
24	Enter	41		64			
25	RF	17		65			
26	2	62		66			
27	÷	47	ΔX/X	67			
28	X^2	45		68			
29	+A1	56	$(\Delta X/X)^2+(\Delta Y/Y)^2$	69			
30	$\sqrt{X}$	44	$\sqrt{(\Delta x/x)^2+(\Delta y/y)^2}$	70			
31	Enter	41		71			
32	RF	17		72			
33	2	62	X	73			
34	X=	46		74			
35	Enter	41		75			
36	RF	17		76			
37	3	63	Y	77			
38	÷	47	$\frac{x}{y}\sqrt{(\Delta x/x)^2+(\Delta y/y)^2}$	78			
39	STOP	01		79			

VERIFY PROGRAM;
1461

KEYBOARD INSTRUCTIONS:

PRIME

SEARCH 1

ENTER ΔX

CONTINUE

ENTER ΔY

CONTINUE

ENTER X

CONTINUE

ENTER Y

CONTINUE

The value of ΔG is shown on display.

APPENDIX B.

APPENDIX B.

Mathematical Derivation of Model A.

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

$$\text{where } y = \frac{\log_e 2}{\text{intermitotic period.}}$$

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

Suppose that after a dose of radiation the number of integer cells in the meristem is reduced to I, and that fatally damaged cells are removed immediately, the number of integer cells dividing per unit time is then yI. Further, suppose that the number of cells which differentiate per unit time is no longer equal to the number which divides, but is reduced in the ratio I/I_s, i.e., the number is given by

$$\left(\frac{I}{I_s}\right)\left(yI\right) \quad \text{or} \quad y\frac{I^2}{I_s}$$

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 compartment, is then the difference between the increase in the number of cells resulting from division and the loss due to differentiation, i.e.,

$$\frac{dI}{dt} = yI - y\frac{I^2}{I_s} \qquad \text{.....(B.1.)}$$

It is evident from this model that the rate of differentiation is small when I is small and increases with I. When $I = I_s$, the rate of change of the number of cells in the meristem becomes zero, i.e., steady state growth rate conditions prevail. The expression in equation B.1 can be integrated to give

$$I = \frac{I_s}{1+\left(\frac{I_s}{I_o} - 1\right)e^{-yt}} \qquad \text{.....(B.2.)}$$

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

$$\frac{dD}{dt} = y\left(\frac{I^2}{I_s}\right) \qquad \text{.....(B.3.)}$$

Substituting the expression for I deduced in B.2,

$$\frac{dD}{dt} = \frac{yI_s}{\left\{1 + \left(\frac{I_s}{I_o} - 1\right)e^{-yt}\right\}^2} \qquad \text{.....(B.4.)}$$

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 the control roots is the area under the curve

$$\frac{dD}{dt} = yI_s \qquad \text{.....(B.5)}$$

Therefore it is 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 depopulation, while the meristem is being repopulated, then then increased to a steady value as equilibrium is restored.

The initial depopulation produced by radiation cannot be obtained by simply matching theoretical and experimental growth rate curves, because the theoretical curves ignore the presence of sterile cells. It can be obtained, however, by means of the step-by-step calculation described in Chapters 3 and 8.

A computer programme was written for use on a WANG 270 Desk Calculator. The $\frac{1}{4}$ day growths for each treatment was found from the daily growth curves and this data was punched on tape and fed into the Teletype Unit. A flow diagram of the programme is shown in Fig. B.1. The programme print-out is given as well.

GSH.8

WANG PROGRAM Nº BY ON

No.	Cmd	Code		No.	Cmd	Code	
00	Mark	07		40	RF	17	
01	.1	61		41	0	60	
02	1	61		42	X=	46	
03	Sf	13		43	Write	24	
04	0	60		44	12	12	
05	0	60		45	SF	13	
06	Sf	13		46	0	60	
07	1	61		47	Search	02	
08	Mark	07		48	2	62	
09	2	62		49			
10	Write	24		50			
11	CR/LE	71		51			
12	RF	17		52			
13	1	61		53			
14	Write	24		54			
15	11	11		55			
16	.	75		56			
17	2	62		57			
18	5	65		58			
19	AF	12		59			
20	1	61		60			
21	Read	22		61			
22	X	44		62			
23	Write	24		63			
24	12	12		64			
25	ClAl	54		65			
26	-Al	57		66			
27	1	61		67			
28	+Al	56		68			
29	Enter	41		69			
30	RF	17		70			
31	2	62		71			
32	-	47		72			
33	ClAl	54		73			
34	+Al	56		74			
35	1	61		75			
36	+Al	56		76			
37	Write	24		77			
38	12	12		78			
39	Enter	41		79			

Verify Programme:
1985
Speacial Instruction:
Put T in SF 2
Prime
Search 1

MODEL A

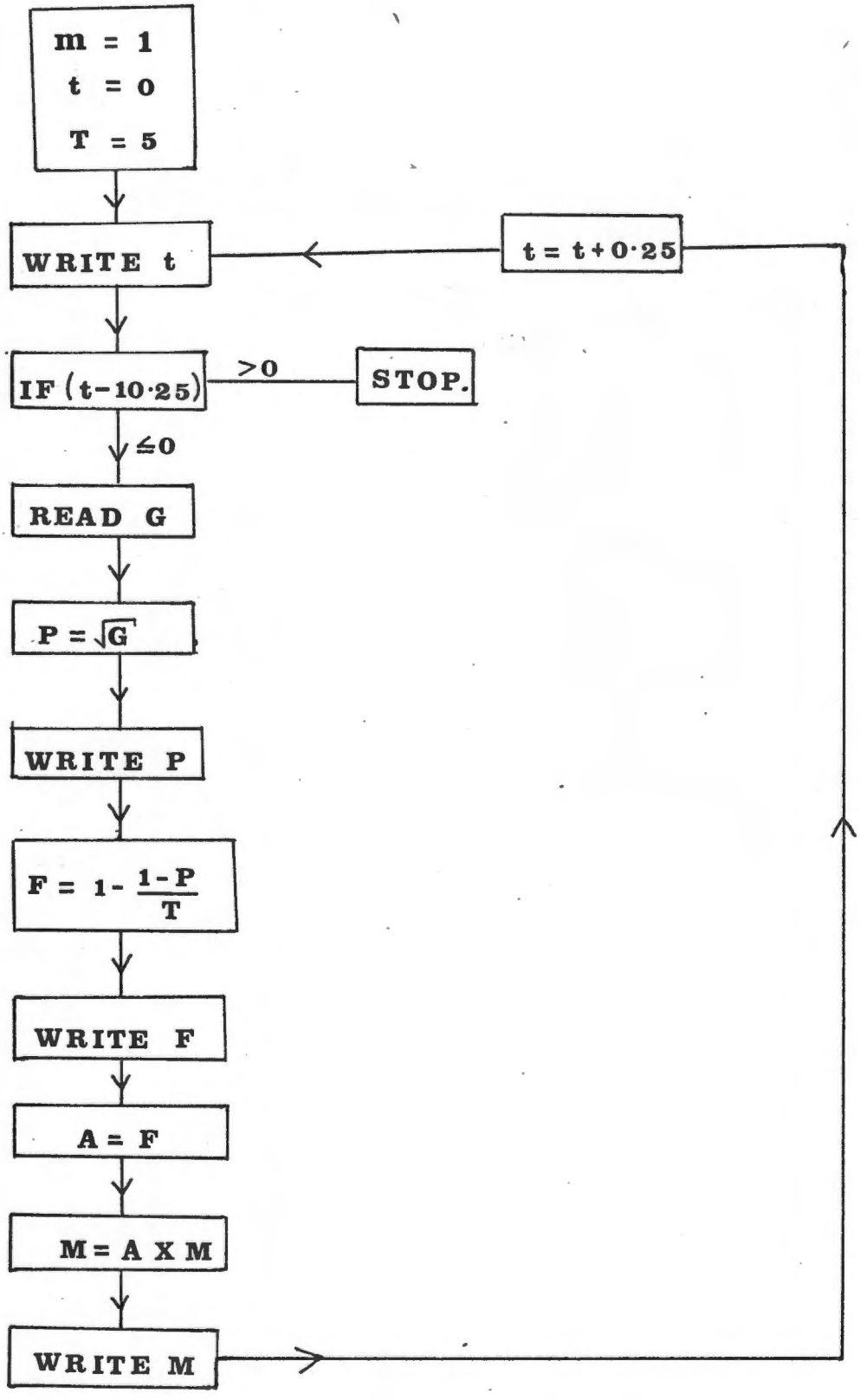

Fig. B1.

APPENDIX C.

APPENDIX C.

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 the 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 purpose of simplification of the mathematical calculations it is assumed here that such cells are unable to divide even once.

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

assumptions:

$$C_N = C_o e^{-KN} \quad \text{(where K is a constant)} \ldots\ldots C.1.$$

The proportion of cells with reproductive integrity at a concentration level of C_N is $\frac{C_N}{C_o}$ and

from equation C.1,

$$\frac{C_N}{C_o} = e^{-KN} \quad \ldots\ldots\ldots\ldots\ldots\ldots (C.2)$$

In any infinitesmal 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:

$$\int_0^N e^{-KN} = \frac{1}{K}\left(1-e^{-KN}\right) \ldots\ldots\ldots\ldots (C3)$$

This corresponds to the 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 a proportion P (following e.g. radiation damage) one is concerned with PN instead of N in the above formulae. Therefore the proportion x of the possible PN cells to divide per unit time will be:

$$x = \frac{1}{KPN}(1-e^{-KPN}) \quad \ldots\ldots\ldots\ldots\ldots\ldots (C.4).$$

And the proportion differentiating equals (1-x). The number differentiating (D) will be PN (1-x), that is:

$$D = \frac{1}{K}\left(KPN + e^{-KPN} - 1\right) \quad \text{.....(C.5.)}$$

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

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

$$= \frac{1.595\ P + e^{-1.595P} - 1}{0.7975}$$

$$= 1.595P^2 \quad \text{.....(C.6)}$$

From equation (C.4) the number of cells dividing per unit time is

$$\frac{PN}{KPN}\left(1 - e^{KPN}\right) = 1/K\left(1 - e^{-KPN}\right) \text{....(C.7}$$

The net increase in the total population per unit time is given by the difference between the number dividing and the number differentiating. Given T units of time per cell cycle, the total population will be PNT, and the factor of increase, F, may be calculated from equations (C.5) and (C.7).

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

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

$$= 1 + \frac{1}{1.595TP}\left(2 - 2e^{-1.595P} - 1.595P\right) \quad \ldots.(C.8)$$

As KN = 1.595

An iterative computer programme was written to find suitable values of P. (In the programme P = P'). These values were used to calculate F, and hence f, the initial fraction of the population surviving doses could be calculated. Data on the $\frac{1}{4}$ day growths was punched on tape and fed into the WANG Teletype unit. A flow diagram of the programme used appears in Fig. C.1. The programme print out is given as well.

MODEL B

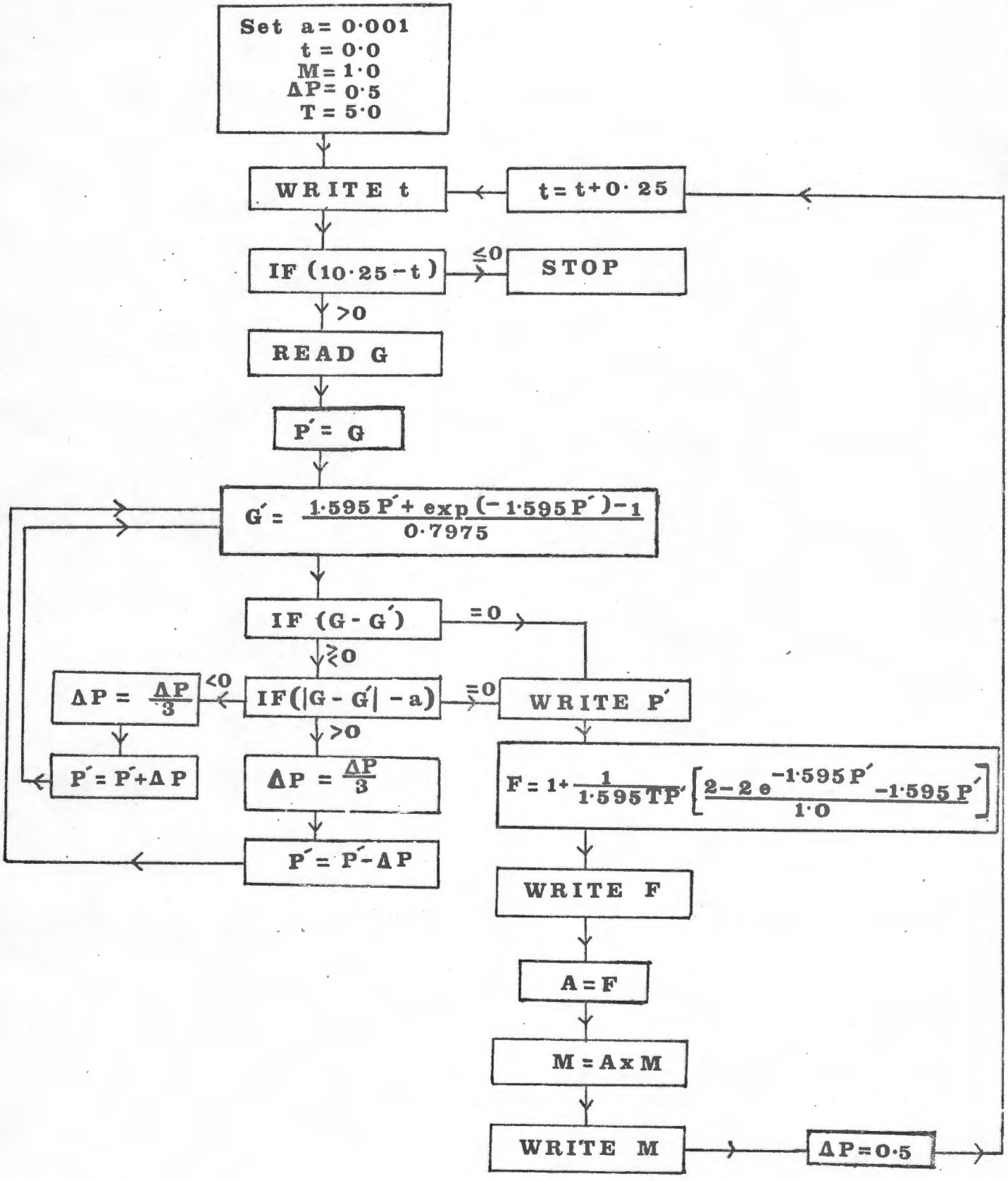

FIG C1.

MODEL B

GSH.86

WANG PROGRAM Nº BY ON

No.	Cmd	Code	COMMENT	No.	Cmd	Code	COMMENT
00	Mark	07		40	.	75	
01	1	61		41	7	67	
02	0	60		42	9	69	
03	SF	13		43	7	67	
04	0	60		44	5	65	
05	1	61		45	÷	47	
06	SF	13		46	ClAl	54	
07	4	64		47	-Al	57	
08	Mark	07		48	Rf	17	
09	2	62		49	1	61	
10	.	75		50	+Al	56	
11	1	61		51	SH^{+}	05	
12	Sf	13		52	Sch	02	
13	2	62		53	4	64	
14	RF	17		54	Rf	17	
15	0	60		55	5	65	
16	Write	24		56	-Al	57	
17	CR/LF	71		57	SH^{+}	05	(G-G') -e
18	WRITE	24		58	Sch	02	
19	11	11		59	5	65	
20	Read	22		60	Mark	07	
21	SF	13		61	8	68	
22	1	61		62	Rf	17	
23	ClAl	54		63	2	62	
24	SF	13		64	AF	12	
25	3	63		65	3	63	
26	Mark	07		66	Search	02	
27	3	63		67	3	63	
28	Enter	41		68	Mark	07	
29	RF	17		69	4	64	
30	7	67		70	RF	17	
31	X=	46	1.595P	71	5	65	
32	ClAl	54		72	+Al	65	
33	+Al	56		73	SH^{+}	05	
34	CHS	77		74	SCH	02	
35	e^{x}	43	$e^{-1.595P}$	75	7	67	
36	+Al	56	1.595P	76	SCH	02	
37	1	61		77	5	65	
38	-Al	57		78	Mark	07	
39	ENTE$_{R}$	41		79	7	67	

VERIFY PROGRAM:

6671

KEYBOARD INSTRUCTIONS:

PUT e in SF 5

1.595 in SF 7

T in SF 6

PRIME

SEARCH 1

GSH.86

WANG PROGRAM NO BY ON

No.	Cmd	Code		No.	Cmd	Code	
00	RF	17		40	ENT	41	
01	2 •	62		41	R1A1	55	
02	Sub F	16		42	X=	46	
03	3	63		43	C1A1	54	
04	RF	17		44	+A1	56	
05	2	62		45	1	61	
06	ENT	41		46	+A1	56	
07	3	63		47	WI	24	
08	÷	47		48	12	12	
09	SF	13		49	ENT	41	
10	2	62		50	RF	17	
11	Sch	02		51	4	64	
12	8	68		52	X=	46	
13	Mark	07		53	SF	13	
14	5	65		54	4	64	
15	Rf	17		55	4	64	
16	3	63		56	÷	47	
17	WI	24		57	AF	12	
18	13	13		58	0	60	
19	ENT	41		59	C1A1	54	
20	RF	17		60	-A1	57	
21	7	67		61	1	61	
22	X=	46		62	0	60	
23	C1A1	54		63	.	75	
24	C1Ar	50		64	1	61	
25	+Ar	52		65	+A1	56	
26	-A1	57		66	SH^{+}	05	
27	e^{x}	43		67	STOP	01	
28	ENT	41		68	RF	17	
29	2	62		69	4	64	
30	X=	46		70	WI	24	
31	-A1	57		71	CR/LF	71	
32	2	62		72	W1	24	
33	+A1	56		73	22	22	
34	RF	17		74	SCH	02	
35	6	66		75	2	62	
36	ENT	41		76			
37	RAr	51		77			
38	X=	46		78			
39	÷	47		79			

VERIFY PROGRAM:
3377

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