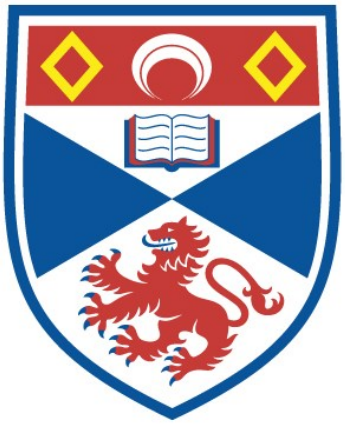
ASPECTS OF THE BEHAVIOUR OF THE ROOT MERISTEM OF ALLIUM SATIVUM

Janet Elizabeth Taylor

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1966

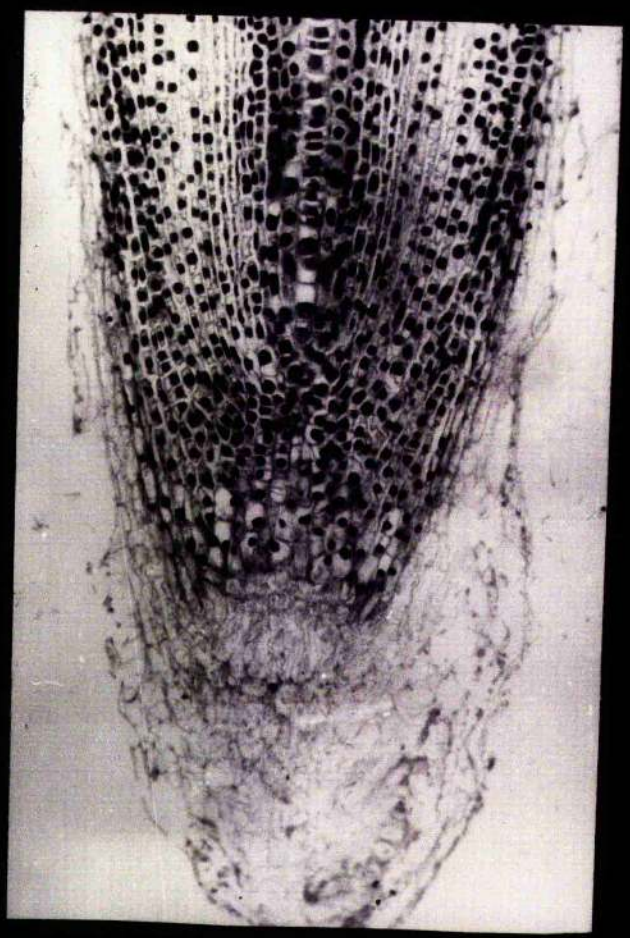


PLATE 5. Median section of a root of Allium sativum nine days after irradiation with 250 rads.

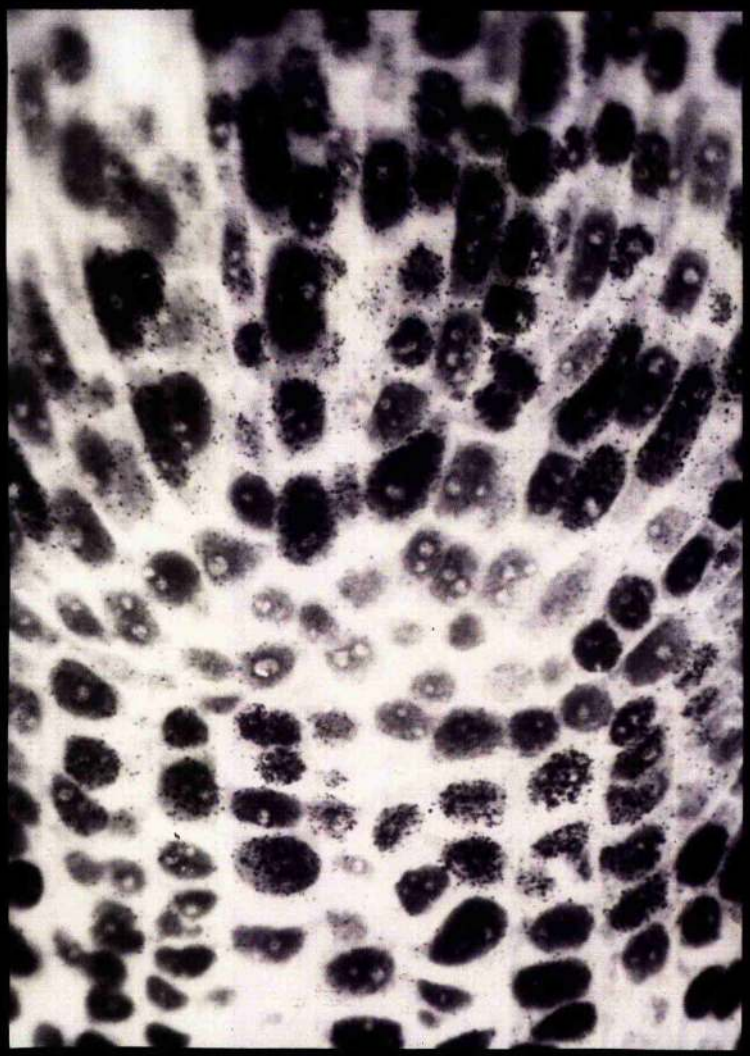


PLATE 4. Autoradiograph of the area of the stele pole of the root apex of Allium sativum.

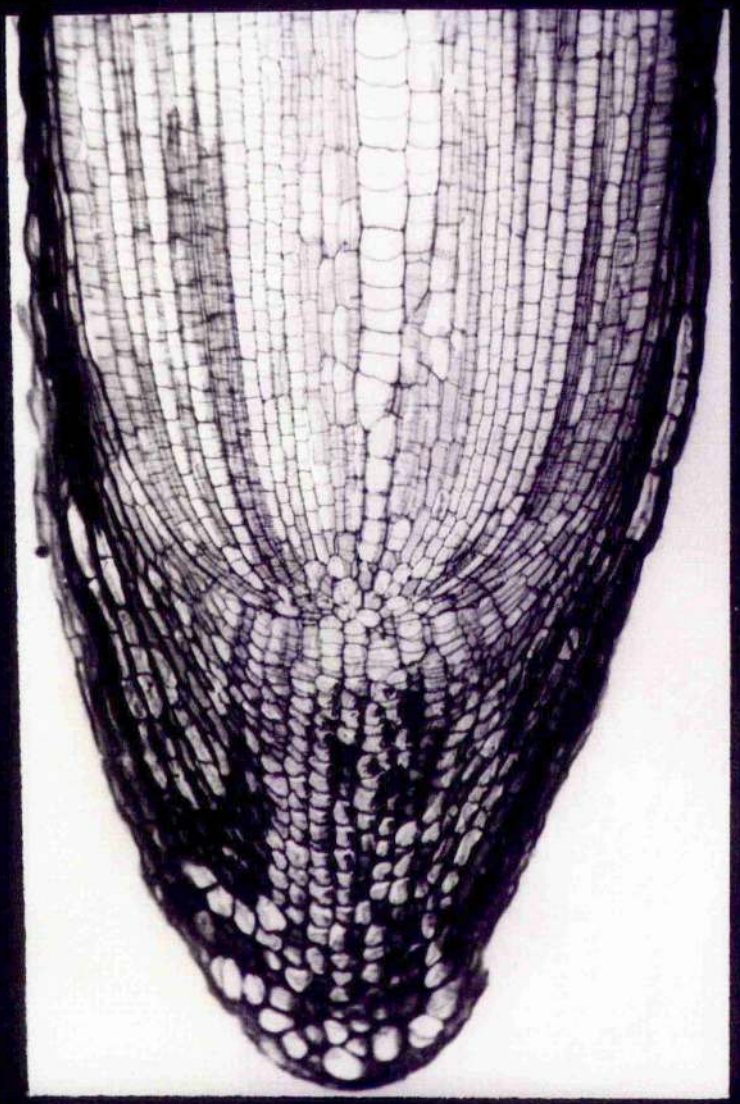
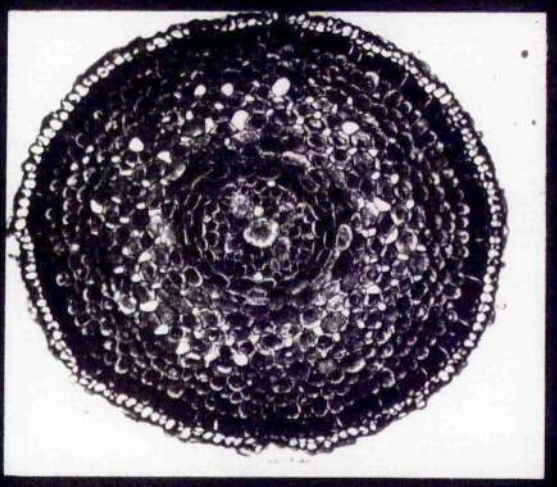
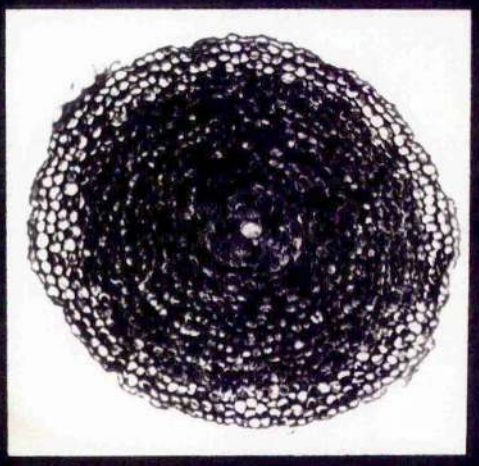


PLATE 3. Median section of the root apex of Allium sativum stained by the P.A.S. reaction.





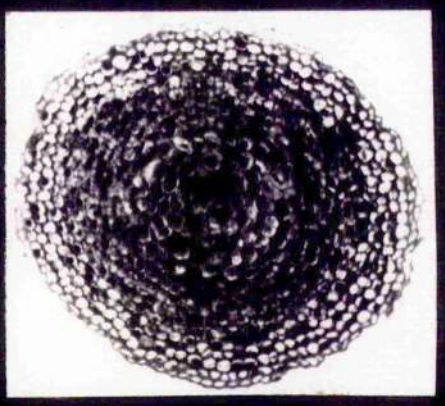


PLATE 2. Transverse sections through the root of Allium sativum 1200 400 and 350 from the apex.

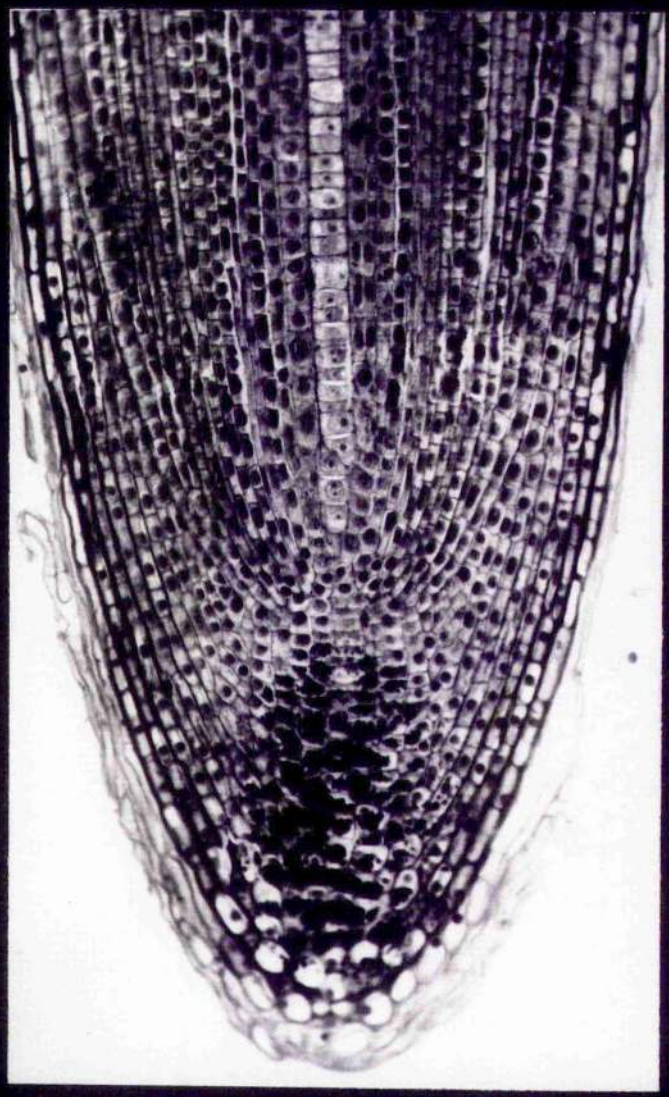


PLATE 1. Median section of the root apex of Allium sativum stained in safranin, tannic acid and orange G.

ASPECTS OF THE BEHAVIOUR OF THE ROOT MERISTEM OF *ALLIUM SATIVUM*.

BY

JANET ELIZABETH TAYLOR

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

Department of Botany

St. Salvator's College

University of St. Andrews

December 1966

ACKNOWLEDGEMENTS.

I should like to thank Professor J. A. Macdonald and Dr. D. Davidson for supervising my work and all those people who have given me encouragement and the benefit of their experience during the past three years, in particular Dr. F.A.L. Clowes.

I acknowledge gratefully the receipt of the Berry Scholarship from the University of St. Andrews during the period 1963 - 1966

DECLARATION.

I hereby declare that the following thesis is based on a record of work done by me, that the thesis is my own composition and has not previously been presented for a Higher Degree.

My research during the past three years has been carried out in the Department of Botany at St. Salvator's College of the University of St. Andrews and the Botany School, Oxford by kind permission of Professors J. A. Macdonald and C. D. Darlington. I was supervised during the first year by Dr. D. Davidson now of the Department of Biology, Western Reserve University, Cleveland, Ohio and thereafter by Professor J. A. Macdonald of the Department of Botany, St. Andrews.

CERTIFICATE

I certify that Janet Elizabeth Taylor has carried out nine terms of research work under the supervision of Dr. D. Davidson and myself at St. Andrews, and of Dr. F.A.L. Clowes at Oxford, that she has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and that she is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

CONTENTS.

INTRODUCTION

A challenging problem in biology today is that of the control of growth and differentiation. Little is known of the physiological and biochemical mechanisms controlling and directing the pattern of cell development. Even less is known of the control of the interaction and integration of cells in a multicellular system. The pattern of organisation within a cell population is assumed to be determined by the genetic make-up and chemical environment of the constituent cells. Yet it is not understood how this organisation, a complex interdependence of cells, is brought about and maintained.

One way in which it is possible to assist in solving this problem is by providing information concerning the various components of growth and differentiation in relation to the organisation of cell populations in intact organisms. Careful analysis of the development of a cell population with a recognisable pattern of organisation should make it easier to see where the pathways of control lie.

A convenient multicellular system for work of this kind is pro provided by the root apices of plants. The developing root is a simple axial structure, lacking lateral appendages and containing a wide variety of cell types. Under normal conditions the root is

heterotrophic. It depends for its energy supply upon carbohydrates supplied from the seed and then the shoot, or under experimental conditions from some external source. Within the root it is possible to distinguish the different components of growth - cell division, elongation and maturation - because the progressive stage of differentiation occur in a linear sequence from apex to base. I is perhaps this feature of root development which has in the past been most important with respect to its choice as experimental material in morphogenetic studies.

Many workers have studied root apices in vivo and in vitro and much data concerning their organisation and behaviour have been accumulated. The lines of research have been varied. Some workers have been interested in physiological aspects of differentiation, others in the growth requirements of roots in tissue culture and others in the kinetics and arrangement of cells in the meristem. I is along this last line of research that I have been working. I ha investigated the growth pattern and organisation of the apical meristem in roots of Allium sativum with a view to gaining information about the root meristem of a species which has a relatively easily defined quiescent centre and whose chromosome complement facilitates analysis of chromosome aberrations induced by experimental treatments. Evidence has been collected to test th thesis that cells of the quiescent centre, in roots with multi-

cellular promeristems, are held in the G1 phase of the mitotic cycle (Clowes, 1956). G_1 is the period of the mitotic cycle between the end of telophase and the onset of DNA synthesis for the next mitosis. Present evidence that quiescent centre cells are held at the G_1 stage comes from measurements of the duration of G_1 in different regions of the meristem of Zea. Using a pulse labelling technique Clowes (1965) has found that G_1 occupies more than seven-eighths of the whole mitotic cycle in the quiescent centre cells of Zea mays. Chemical assays of DNA content by Jensen (1956) have also shown that the DNA content of cells is at a minimum in transverse slices of Vicia faba root taken at the level of the quiescent centre.

The pattern of organisation in the root apex may be resolved by the planes and rates of growth and division of cells in the meristem. With information concerning the growth pattern of the normal root based on both these aspects of organisation it is possible to analyse the response of the root meristem to various experimental treatments. I have examined the effect of two experimental treatments, X-rays and colchicine, on the behaviour of the root meristem of A. sativum and correlated these effects with the time parameters of the mitotic cycle in different regions of the meristem.

It is these studies of the root meristem of Allium sativum that are reported here.

MATERIAL AND METHODS

A good reason for choosing to work with *Allium sativum* is the ease with which large numbers of roots of similar age and size may be grown.

Cloves of *A.sativum* were peeled to expose the root primordia. These cloves were threaded on stainless steel wires and suspended over beakers of tap water at room temperature. A thermograph run over a period of a week showed that with the background heating used during the experiments room temperature was 21 ± 1° C. An electric pump was used to aerate the water continuously. Each day the water was changed to prevent any build up of fungal and bacterial contamination. In the autumn roots emerged within 12 hours and were 1-2 cms long after 2 days growth but in late spring 4 days growth was frequently necessary for the roots to reach a length of 1 to 2 cms. These variations in the time required for root primordia to emerge and their subsequent growth rates are probably due to initial differences in the desiccation of the garlic clove. Using roots of 1-2 cms length for experimental purposes ensures that meristematic cells in the apex are dividing rapidly and that growth is not due

to cell elongation alone. Roots used to measure rates of mitosis were grown in the dark to avoid any possibility of partial synchrony being induced by diurnal changes of light and dark.

Roots for histological studies were fixed in FPA and those for chromosone analysis or autoradiography were fixed in acetic-alcohol. After experimental treatment the terminal ½ cm of thirty to forty straight roots were fixed in about 30 ccs of fixative. Twenty-four hours later the fixative was replaced by 95% alcohol except that if the roots were to be kept for more than a few days before embedding or squashing they were stored in 70% alcohol.

Most of the data reported here comes from longitudinal sections of paraffin embedded roots because in sections it is possible to delimit discrete regions of the meristem.

Roots were embedded in paraffin wax (melting point 56°C) via benzene. To reduce tissue and cytoplasmic shrinkage in the molten wax embedded roots were kept under reduced pressure to evaporate benzene quickly and were not heated above 60°C. Sections were cut on a rotary microtome at 8 μ for histological preparations and 6 μ for autoradiographs. Sections for autoradiographs were spread on water and stuck to subbed slides. Other sections were spread on 3% formalin and stuck

to slides smeared with Haupt's adhesive. Several standard staining techniques were used but the most useful one for counting nuclei in different regions of the meristem was Feulgan and fast green.

Squash preparations were used for chromosome analysis. Roots were stained in leucobasic fuchsin. The terminal 2mm was tapped out in 45% acetic acid covered with a coverslip previously smeared with glycerol albumen and squashed. Slides were made permanent by floating off the coverslip in 45% acetic acid and then dehydrating in alcohol before mounting in Eupanal.

X-irradiations were given using a Newton Victor machine delivering a dose of approximately 120 r/min. at 70 kv and 5 ma. Cloves of roots for irradiation were laid on water-sodden cotton wool around the periphery of a Petri dish which fitted under the cone of the machine.

Sections of tritium labelled roots were prepared as autoradiographs by a stripping film method using Kodak fine grain AR 10 stripping plates. They were exposed for 28 days at 5°C developed in full strength ID 19 for 6 minutes at 20°C and fixed in IF 9. The autoradiagraphs were washed in running water for 30 minutes, dried and examined under oil

immersion. Labelled sections were stained before applying the film with leucobasic fuchsin or stained after exposure and development through the film with Leishman Giemsa.

Sections and squashes were examined microscopically. An eyepiece graticule was used to facilitate counting of nuclei in different regions of the meristem. Drawings of chromosomes were made using a camera lucida and inked in with Indian ink. Cell maps of the median section of meristem were prepared by drawing round the cell walls of a photograph of the meristem with Indian ink and then reducing the photograph with ferrocyanide. Photographs were taken on 35mm film with an eyepiece camera.

The root meristem and quiescent centre of *Allium sativum*.

Introduction

There are two hypotheses which are useful in describing the organisation of the apical meristems of roots. The first, is the classical histogen theory (Hanstein, 1868) which describes the organisation of the meristem in terms of the tissues to which it gives rise. The limitations of this method are that histogens in roots are not as discrete as was once thought and no account is made of how histogens are maintained. The other hypothesis is the Körper-Kappe theory of Schüepp (1917) which describes the arrangement of cells in terms of the planes of cell division within the meristem. Using a combination of a modified histogen theory and analyses of cell complexes based on the Korper-Kappe theory Clowes (1950) described the organisation of the apical root meristem of *Fagus sylwatica*. This investigation and subsequent work on *Vicia* and other roots with broad columellas led Clowes (1952) to suggest that such roots have broad multicellular promeristems. The promeristem, which is here taken to mean the functional initials of the meristem, is cup shaped and contains four to five groups of initial cells which give rise to

tissues sufficiently discrete to be classified as histogens. The promeristem may alter in size and shape during the life of the root and thus the status of particular constituent cells may be changeable.

Further researches into the nature of the promeristem in roots without columellas or with narrow columellas as in Zea or Tritium have led Clowes (1953) to designate a minimal constructional centre within the promeristem, the minimum number of cells required to maintain the pattern of organisation.

The minimal constructional centre consists of the cells at the poles of the stele and cortical complex and those at the head of each file of columella cells in the centre of the cap. As a result of experience gained from surgical experiments on roots Clowes believed that these minimal construction centres played no important part in the actual growth of a root except for those of its cells in the cap since the surgical experiments led him to believe that the actual promeristem was larger than the minimal constructional centre. He believes that the antithesis between the evidence for a minimal construction centre and the evidence for a much bigger promeristem results from the fact that the pattern of cells reflects the past behaviour of the apex in the embryo or root poimordium as well as the current behaviour. The

earlier work on root apices of the so called ''open'' type in which there is no easily discernible boundary between stele and cap, as there is in grasses, leads to ambiguities of interpretation and these had prevented a proper analysis of the situation. The discovery by geometrical analysis of the cell pattern of a quiescent centre to the meristem confirms the distinction between the promeristem and the minimal construction centre. This quiescent centre lies in a more or less hemispherical region around the poles of the stele and cortex and consists of cells that divide rarely or not at all. Clowes has confirmed the presence of a quiescent centre in the meristem of root apices by auto-radiographic techniques, (Clowes, 1956) and by measurements of the average rates of mitosis in different regions of the meristem (Clowes 1961).

Many workers agree with Clowes' concept of the promeristem, but there are two other theories prevalent concerning the nature of the cytogenerative centre in angiosperm roots namely the central cell hypothesis (Guttenberg, 1947) and the initial group hypothesis (Brumfield, 1943) ; Guttenberg and his associates (Schade and Guttenberg, 1951) believe the cytogenerative centre to be a single totipotent cell similar to the apical cell of some Pteridophytes. This central cell occasionally divides and is the centre of

development from which all histogens are derived. Brumfield's view is somewhat similar to that of Guttenberg in that he thinks the cytogenerative centre comprises few cells. Brumfield (1943) tagged meristem cells of Crepis and Vicia with X-ray induced chromosome aberrations and then examined sections of the primary roots after a period considered sufficient to eliminate cells not derived from the initials existing at the time of irradiation. He found that the meristems of some roots were sectional chimeras in which particular aberrations occurred in sections or of the root extending from the stele to the cap and for about a third of the circumference. From this he concluded that there were three initial cells and that each initial produced a third of each of the tissue regions. In interpreting his results in this way Brumfield made two assumptions; that all similar aberrant cells are derived from a single mutant cell and that irradiated cells behave exactly as normal cells in a meristem. Both these assumptions are invalid, the second more seriously so. Many irradiated cells die and those which survive do not necessarily divide at the same rate as before irradiation. However, it is possible to explain Brumfield's results in terms of a large multicellular promeristem without making any tenuous assumptions. Davidson (1961) has also followed cell lineages during recovery

of Vicia roots from irradiation. Regeneration involves the formation of a new root primordium and, from relative frequencies of cells with changed chromosome complements, Davidson has estimated that this primordium is composed of 40-50 cells. The primordium could comprise derivatives of only three cells present at the time of irradiation, but this is different from saying that the promeristem of the regenerated root comprises only three cells.

The limits of the quiescent centre are readily defined in Zea and other members of Gramineae because of the distinct boundary between the root cap and the rest of the meristem. Thus the discreteness and size of the quiescent centre in Zea recommend it for experimental purposes, but unfortunately the chromosomes of Zea are small, numerous and light by staining. This makes chromosome analyses after experimental treatments extremely difficult. In Vicia which has few (12), large, densely staining chromosomes the root has a broad columella and the boundaries of quiescent centre are hard to see. The following investigation was carried out in an attempt to find a species whose roots would provide good experimental material from the point of view of ~~discrete~~ easy recognition of a quiescent centre and accurate

chromosome analysis. Preliminary tests were carried out with several species known to have low chromosome numbers including: Sorghum, Secale, Allium cepa and Allium sativum. Sorghum seeds were difficult to germinate, Allium cepa sets are not available throughout the year and the bulbs are expensive and inconvenient sources of roots and mitotic indices in Secale were low under the test growth conditions. The most promising species seemed to be Allium sativum so further investigation of this root meristem was made and the presence of a quiescent centre determined by autoradiography.

Material and methods

Roots were grown as described in Section 2, thus all the roots investigated were adventitious roots. The apices of some roots, fixed in FPA, were prepared as longitudinal or transverse sections 8μ thick and stained with tannic acid, orange G and safranin according to the recipe of Sharman (1943). Longitudinal sections of other FPA-fixed roots were stained by the PAS technique (Jensen 1962). Autoradiographs were prepared by the stripping film technique using longitudinal sections cut at 6μ of roots fixed in acetic-alcohol after feeding with tritiated thymidine

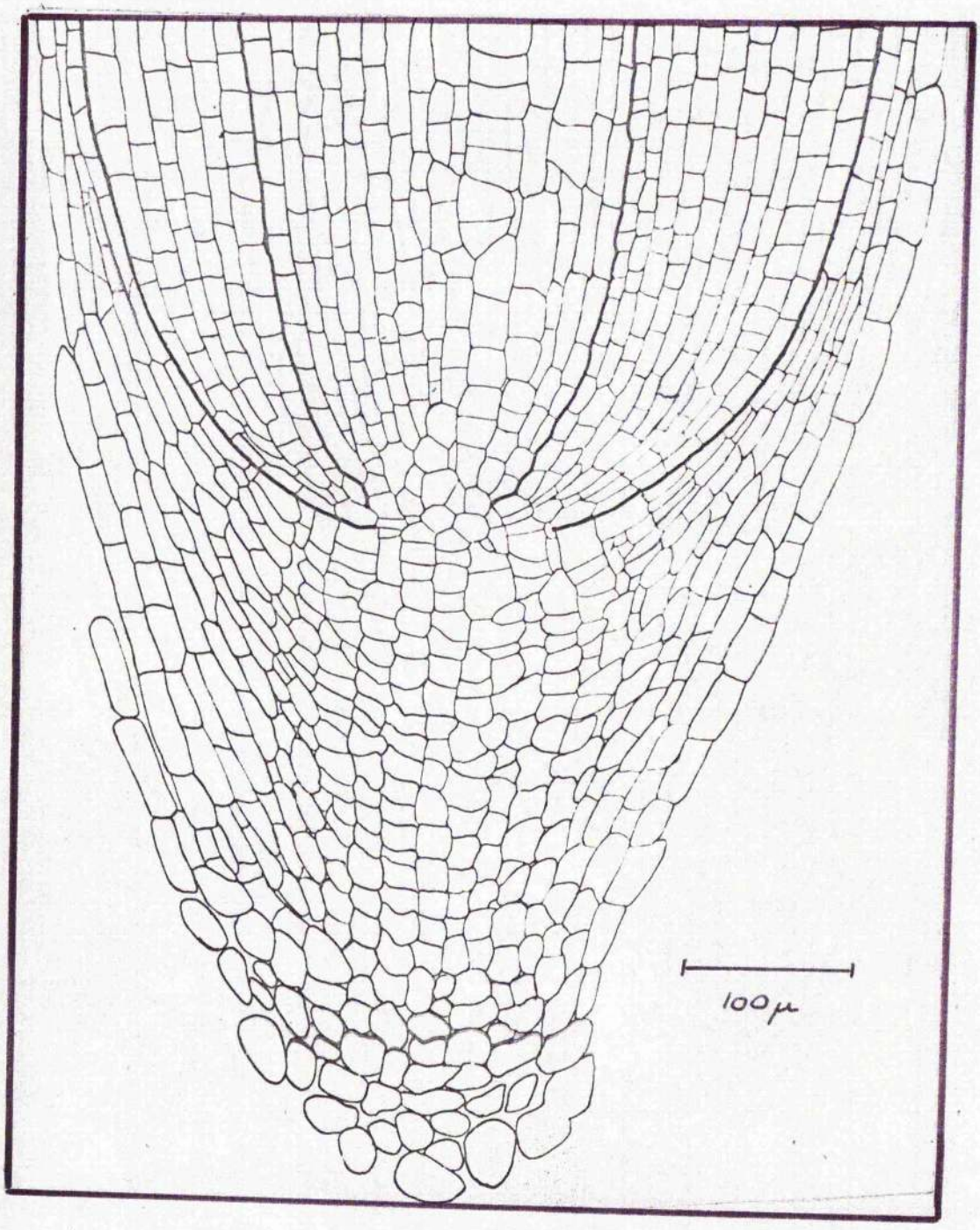
of activity c200μC/L and specific acitivty 1·8 C/mM for 48 hours.

Feulgen stained root tip squashes confirmed a diploid complement of sixteen large darkly staining chromosomes.

Meristem of Allium sativum

The following brief outline of the anatomy of the root apex in Allium sativum is based on examination of ten median and ten sets of transverse sections of roots stained with tannic acid orange G and safranin. A. sativum has a prominent root cap the central portion extending on average 500μ distal to the stele pole and the peripheral cell layers sheathing the root extending for about 800μ proximal to the stele pole. Attempts to remove root caps as in Zea (Juniper et al, 1965) usually resulted in the fracture of this peripheral part of the cap. In the central region of the cap there is least variation in the size of cells there are usually 6-7 vertical rows of cells each with an individual initial cell. Meristematic activity in these cells extends through only 3 or 4 cell rows distal to the stele pole cap cells lose their meristematic activity beyond this region and enlarge. The cells towards the apex of the cap are crammed with numerous large starch gains. Cells in the peripheral layers of the root cap are

Figure 2



Drawing of the median section of the root apical meristem of Allium sativum (see Plate 3)

smaller than those of the central region and less homogeneous. The walls of outer cells of the cap stain darkly, the cells lose their contents and become rounded off.

At the pole of the stele the minimal constructional area comprises 10-13 homogeneous small cells with small rounded nuclei. Under normal conditions less than 1% of these cells are in division. Stained with tannic acid orange G and safranin these nuclei look particularly red whereas most other interphase nuclei are more yellow. The chromatin network has a texture slightly different from that of most other nuclei in the meristem, it looks more fibrous. The numbers of interphase nuclei of this red colour and of the yellowish colour were counted for the different regions of the meristem. The percentage of red interphase cells corresponds with the percentage of cells in G1 in the different regions of the meristem (Section 4). It is not possible from this investigation to decide whether the staining reaction emphasises differences in the condensation of chromatin during the cell cycle - chromosomes are bright red - or whether it reflects changes in the chemistry or structure of the chromatin. Nevertheless it is obvious that quiescent centre cells have a different appearance from normal meristematic cells, though

differences are no doubt enhanced by the homogeneity of the group of cells. But this homogeneity is in itself indicative of differences in the behaviour of these cells compared with the behaviour of other cells in the asynchronous population of the root meristem.

In the roots examined, the stele is a pentarch or tetrach protostele about 12 cells in diameter. The large central file of cells is the first to begin to differentiate and the demarkation of the single layered pericycle soon follows. The central cells differentiate as elements of metaxylem vessels, but the first phloem elements, the protophloem sieve tubes at the periphery of the vascular tissue between the xylem poles, mature before the xylem vessel elements. However maturation of vascular tissue does not occur within the region of the root apex used throughout the investigations reported here. The cortical parenchyma consists of layers of thin walled cells with large intercellular spaces at their interstices. The endodermis differentiates very soon after the pericycle. At a slightly higher level the epidermis becomes differentiated by a different staining reaction from the cortical cells and by the more boxlike shape of its cells. Under the growth conditions of these investigations no root hairs developed.

Figure 2

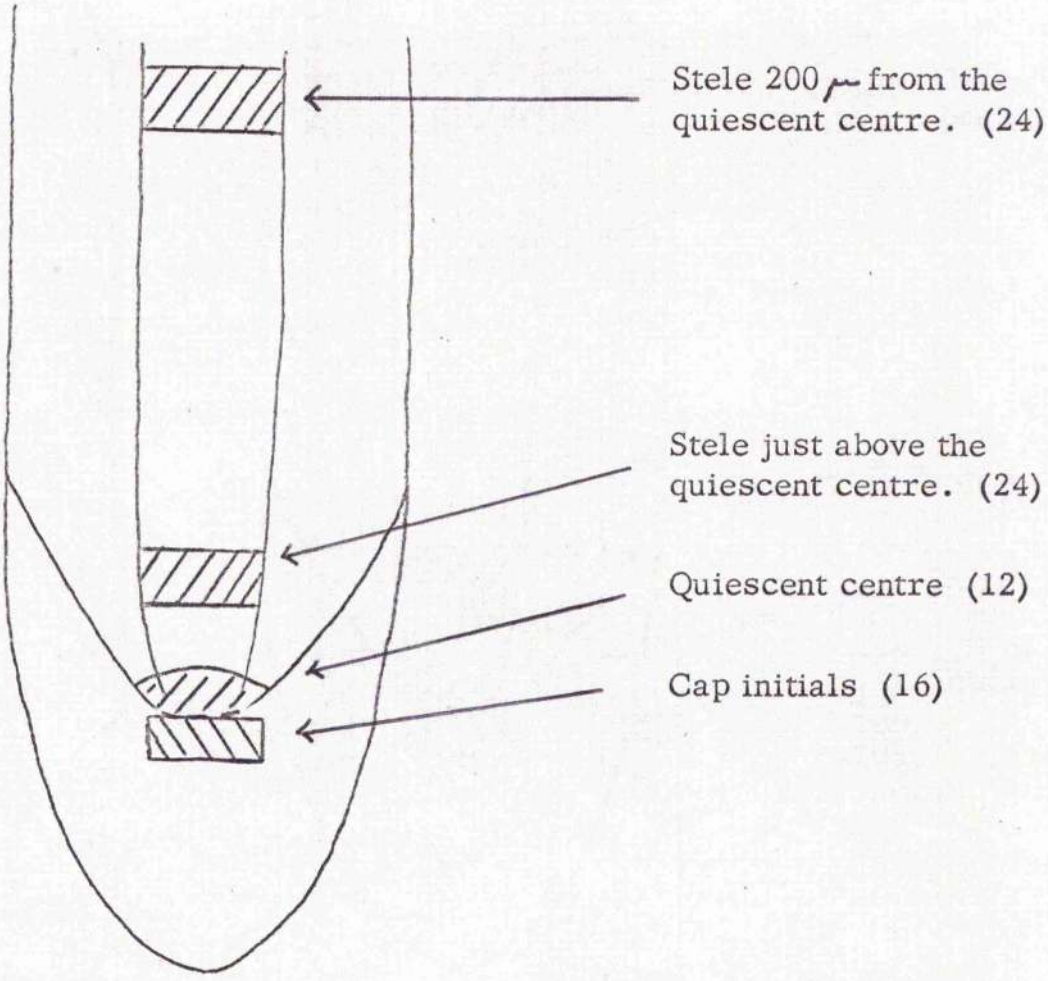


Diagram of the regions of the meristem investigated in the experiments reported in this thesis. Estimates of the number of cells counted per root for each region of the meristem are given in parenthesis.

Fig. 2 is of a diagram illustrating the regions of the meristem which were examined in the following investigations. An average estimate of the cells counted in each region is given.

Körper-Kappe cell patterns.

In median sections of root apices files of cells radiate from a region near the pole of the stele. Where a single file of cells is continuous with a double file the directions of cell division at the junction are reflected in a T configuration of the cell walls. Schüepp (1917) divided the root meristem into Körper and Kappe according to the directions of the T divisions. The central cylinder of the root apex is narrower than the stele to which it will give rise thus, ~~the~~ in the cell pattern of this region, T divisions have the capital of the T facing the root tip. But in the root cap longitudinal cell divisions occur to compensate for the sloughing of outer cells and the capital of the T faces the base of the root. The boundary of the Körper-Kappe regions may be in a constant position in relation to the histogens in some species, but, in others, may vary with the width of the root.

Plate 3 shows a photograph of the median section of a

Figure 3

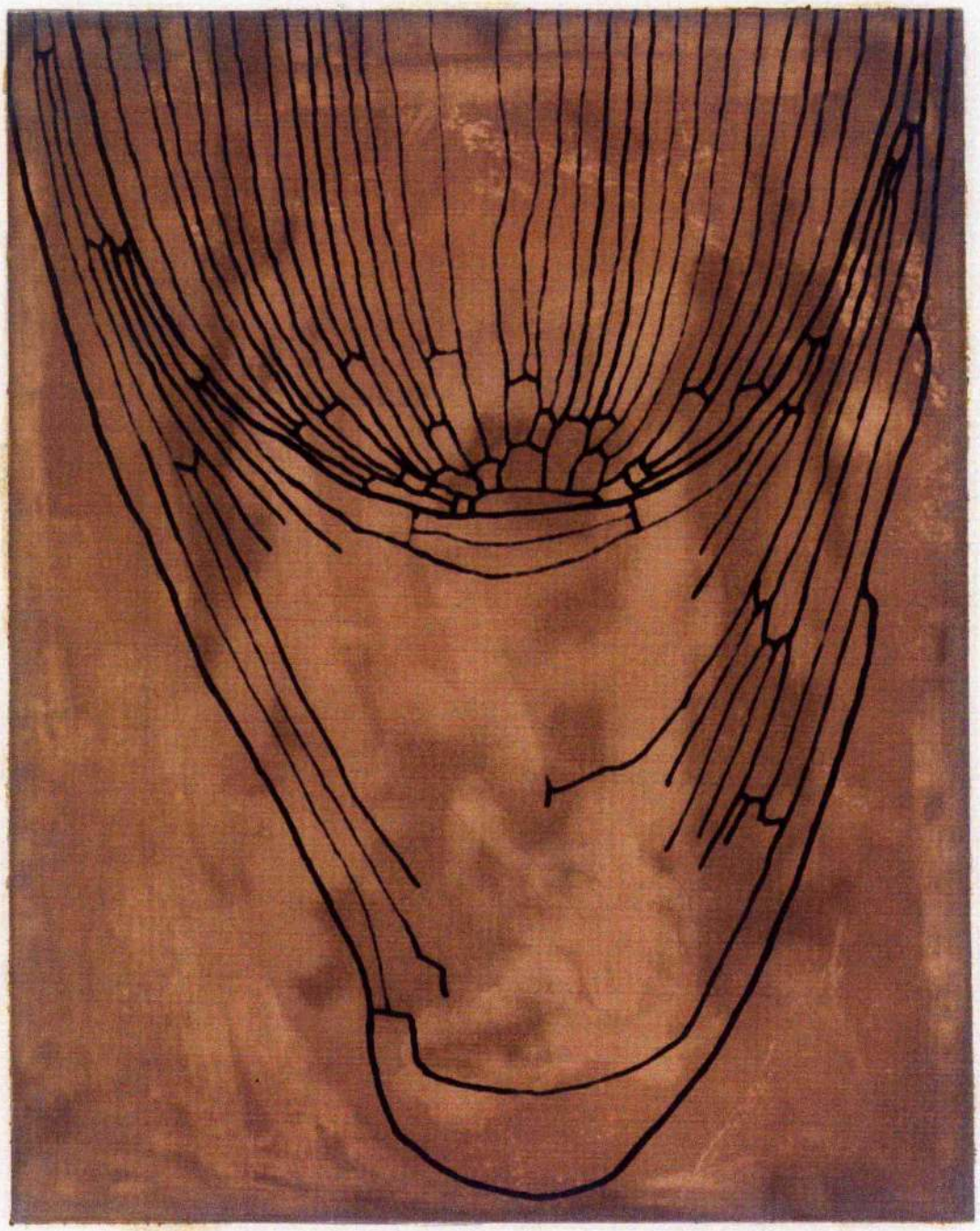


Diagram of the Körper-Kappe cell patterns of the root apical meristem of Allium sativum.

PAS-stained root of Allium sativum and Fig. 3 gives a diagram of the Körper-Kappe cell pattern of the same root. The Kappe complex of the root is confined to the root cap where all the T divisions face the base of the root. Longitudinal divisions are infrequent near the axis of the root cap where the predominant plane of division is transverse, contributing to the length of the cap. Here the pattern which catches the eye is of 5-8 longitudinal rows of cells comprising the columella, but this is because of distortion between adjacent rows of cells, the cap is in fact divided by T divisions over its whole length.

Increase in the girth of the root is achieved in the usual way by increase in the tangential and radial width of cells and by anticlinal and periclinal longitudinal divisions. Transverse sections (Plate 2) show that the cessation of meristematic activity in tissues also follows the usual trend. Cell division ceases first in the central cells of the stele and last in the epidermis. The later divisions in the epidermis and outer cortex are all anticlinal. Anticlinal division occur more frequently in the outer regions of the root to accomodate the increase in internal volume.

Median sections and the Körper-Kappe pattern of cell complexes suggest that the initials of the cortex are distinct from those of the stele and those of the periphenal part of the root cap.

However, it is impossible from the pattern of cells to tell if the cells at the poles of the stele and cortex divide or not because the cortical cells are not sandwiched between the cap and the stele as they are in grasses. Thus from the pattern of cells in A. sativum there is no evidence about the existence of a quiescent centre.

Autoradiographs

In autoradiographs of the median sections of roots fed with tritiated thymidine for 48 hours there is a group of cells corresponding in site to the quiescent centre of other species at the poles of the stele and cortex few of whose nuclei have autoradiographs. This region comprises about 10-13 cells in section (ie. 30-50 in the solid) and has the shape of a plano-convex lens whose diameter extends over the central third of the diameter of the root. The region of unlabelled cells consittutes the quiescent centre. There was no incorporation of thymidine and therefore no DNA synthesis in most of the cells during a 48 hour period (Plate 4).

MEASUREMENT OF THE RATES OF MITOSIS IN THE ROOT MERISTEM OF ALLIUM SATIVUM.

Introduction

Cells are lost continually during root growth from the meristem to the zone of differentiation. Corresponding replacement of these differentiating cells occurs to give the pattern of organisation in the meristem. The rate of cell division in the meristem maintains the pattern imposed by the predetermined planes of division. So it is only with information about both the direction and rate of cell division and growth that it is possible to appreciate the pattern of organisation in the meristem and thus analyse the effect of treatments disrupting the normal pattern of growth and differentiation.

Some workers have drawn conclusions about rates of division from Mitotic Indices (the percentage of cells in division). However it has been pointed out that percentages of cells in division give no real estimate of the frequency of division (Brown, 1951; Evans, Neary and Tonkinson 1959; Clowes, 1961). The Mitotic Index is unchanged by factors affecting all stages of the mitotic cycle equally. At the same

time an increase in Mitotic Index does not necessarily mean that cells are dividing faster. An increase in Mitotic Index may represent an increase in the proportion of the total cell cycle occupied by mitosis while the rate of division remains constant, or a decrease in the proportion of the cycle occupied by interphase while the rate of division becomes slower. Similarly a decrease in the Mitotic Index does not necessarily indicate a decrease in the rate of cell division.

In synchronous cell populations measurement of the length of the mitotic cycle is a simple matter of direct observation. Unfortunately synchronous cell populations occur rarely in nature. In most organisms, as in many root apices cells divide asynchronously or only partially synchronously and here indirect methods must be used to measure rates of mitosis.

Several methods have been used to measure the length of the mitotic cycle in root apices but so far most values obtained for its length have been average values for the whole of the root meristem. Such values take no account of the variations in rates of mitosis between the different regions of the meristem. It is these variations which are of importance in determining the pattern of organisation.

However, Clowes (1961 and 1965) chose three methods for determining rates of mitosis which could be adapted for use with longitinal sections of root apices and measured the length of the mitotic cycle for different regions of the meristem of Zea mays. Using the same three methods I have measured rates of mitosis for different regions of the root meristem of Allium sativum.

Measurement of the duration of the length of the mitotic cycle by accumulation of metaphases by colchicine treatment.

Introduction.

The first method I have used for determining the time parameters of the mitotic cycle in the meristem of *Allium sativum* roots is one using colchicine. This method was used by Evans, Neary and Tonkinson (1957) and Evans and Savage (1959) to measure average values for the length of the mitotic cycle (T) for whole meristems of *Vicia faba* roots and modified by Clowes (1960) to measure values of T for discrete regions of the root apical meristem of Zea mays. It depends upon the fact that colchicine inhibits cells from entry into anaphase by failure of spindle formation (Levan, 1938 and Ostergren, 1950) but does not prevent or delay the entry of cells into prophase (Dustin, 1943). In a population of asynchronously dividing meristematic cells the rate of entry into metaphase equals the rate of entry into mitosis. So during a colchicine treatment the rate of accumulation of metaphases equals the rate of entry of cells into mitosis and hence the length of the mitotic cycle may be calculated. If meristems are exposed to colchicine for long periods of time, nuclei restitute and form polyploid cells. It is necessary therefore to use short treatments with colchicine to avoid losing cells from metaphase to interphase through restitution. Evans, Neary and Tonkinson (1957) presented evidence that in some cells of *Vicia faba* restitution nuclei were formed after 8 hours exposure to colchicine. In the experiment described here the colchicine treatment is not extended beyond 3 hour

Method.

Three day old garlic roots, 1-2 cms. long, were transferred to 0.05% aqueous colchicine solution which was aerated continuously in the dark. Samples of roots were fixed in FPA 0,2,4 and 8 hours after immersion in the colchicine, embedded in paraffin wax and sectioned at 8μ. The sections were stained in leuco basic fuchsin and fast green and the number of nuclei in the various phases of mitosis were counted for the four different regions of the median sections of twenty root meristems per fixation. Percentage values of these counts are tabulated (Table I) and standard errors are given for the metaphases. ~~plotted in Fig.~~

Results.

Throughout the 8-hour colchicine treatment the mitotic index rises. The percentage of prophases over this period is reasonably constant and after 2 hours there are no anaphases or telophases present. The increase in the mitotic index is due to an increase in the number of cells at metaphase. The data also show that the rate of metaphase accumulation between 1 and 8 hours is approximately constant indicating that there is no inhibition of cells from entry into mitosis and that few cells escape from metaphase to polyploid interphases during the colchicine treatment. A few cap initial cells show signs of restituting at 8 hours but these cells were counted in with the metaphases. The length of the mitotic cycle is calculate from the rate of accumulation of metaphases between 2 and 8 hours. The rate of accumulation of metaphases at the beginning of treatment

TABLE I

Percentages of cells in different stages of mitosis after colchicine treatment.

	hours in colchicine	% prophases	% metaphases	% anaphases + telophases	% inter-phases	MI
cap initials	0	6.3	0.9 ± 0.6	1.5	90.2	9.8
	2	4.2	5.2 ± 0.9	0	89.9	9.4
	4	3.3	11.7 ± 1.4	0	85.0	15.0
	8	3.4	18.8 ± 2.0	0	77.8	22.2
quiescent centre	0	0.4	0.4 ± 0.4	0	99.1	0.9
	2	1.3	0.9 ± 0.5	0	97.8	2.2
	4	0.9	1.7 ± 0.9	0	97.4	2.6
	8	2.1	3.4 ± 1.2	0	94.5	5.5

central stele just above quiescent centre	0	8.1	2.2 ± 0.7	4.4	85.3	14.7
	2	6.9	4.9 ± 0.7	0.2	88.0	12.0
	4	6.1	10.6 ± 1.3	0	83.3	16.7
	8	6.6	18.5 ± 1.5	0	74.9	25.1
central stele 200 above quiescent centre	0	5.9	2.0 ± 0.7	4.4	87.7	12.3
	2	7.6	7.4 ± 1.4	0	85.0	15.0
	4	5.5	12.8 ± 1.6	0	81.7	18.3
	8	4.5	21.6 ± 1.7	0.2	74.1	25.9

Average values from 20 roots using the median section of each root. Standard errors for metaphases given.

is avoided because Evans, Neary and Tonkinson (1957) have shown that different concentrations of colchicine disrupt already formed spindles with different efficiencies and Davidson (1965) has shown that cells of the same meristem may have differing sensitivities to colchicine.

In calculating T, I have used the method of Evans, Neary and Tonkinson except that, like Clowes (1960), I have not made a correction for any cells which may escape from metaphase to anaphase or interphase. It is assumed that during the period of the experiment all or very nearly all the cells in the regions considered here are meristematic and dividing asynchronously. Experimental evidence presented elsewhere in this thesis suggests that this is a fair assumption.

If m is the total number of meristematic cells in a population and the proportion of these cells in division at any one time is constant then the increase in cell number is exponential.

$$m = m_0 e^{\lambda t} \qquad (1)$$

where m_0 is the number of meristematic cells at time $t = 0$ and λ is a constant. If T is the duration of the complete mitotic cycle then according to equation (1) m_0 meristematic cells at time $t = 0$ would have multiplied to $2 m_0$ by time $t = T$. Therefore from equation (1) $e^{\lambda T} = 2$ so that

$$\lambda = \frac{\log_e 2}{T} \qquad (2)$$

One dividing cell contributes one extra meristematic cell to the population and so the number of cells dividing in unit time equals the rate of increase of the population so from equation (1)

$$\frac{dm}{dt} = \lambda m_o e^{\lambda t} \qquad (3)$$

It may be deduced from equation (3) that for an increasing population the number of cells in various stages of mitotic cycle at a given instant is not constant. The population is continually increasing by division of cells but the number of cells coming into division is increasing proportionally thus maintaining the mitotic index (M.I., the percentage of cells in division per unit time) constant. At a fixed time t the number of cells in the population which will complete a division after a further interval of time between s and (s + ds) is the rate from equation (3) at time (t + s) multiplied by ds

$$\lambda m_o e^{\lambda(t+s)} ds \qquad (4)$$

From equations (1) and (4) the MI of the meristematic cells may be expressed as

$$MI = \frac{1}{m_o e^{\lambda t}} \int_{s=0}^{s=\tau} \lambda m_o e^{\lambda s} e^{\lambda t} ds$$

where τ is the length of mitosis. If any terms which are independent of s are removed from the integral then we have

$$MI = \frac{1}{m_o e^{\lambda t}} m_o e^{\lambda t} \int_{s=0}^{s=\tau} e^{\lambda s} ds$$

$$= \lambda \int_{s=0}^{s=\tau} e^{\lambda s} ds$$

$$= \lambda \left[\frac{e^{\lambda s}}{\lambda} \right]_{s=0}^{s=\tau}$$

$$MI = e^{\lambda\tau} - 1 \qquad (5)$$

A series expansion of e^x is as follows:

$$e^x = 1 + x - \frac{x^2}{2} + \frac{x^3}{6} + \ldots\ldots\ldots \qquad ①$$

if both sides of this equation are differentiated

$$x = 0 + 1 + x + \tfrac{1}{2}x^2 + \ldots\ldots\ldots\ldots \qquad ②$$

$e^{\lambda\tau}$ can be expanded in similar fashion and if $\lambda\tau$ is small by adding ① and ② and neglecting powers of $\lambda\tau$ greater than 1

$$e^{\lambda\tau} - 1 = \lambda\tau \qquad (6)$$

Therefore from 5 and 6

$$MI = \lambda\tau$$

If the value λ from equation (2) is substituted in equation 6 and if $\frac{\tau}{T}$ is small then

$$MI = \frac{\tau}{T} \log_e 2$$

Now under the action of colchicine the rate of entry into metaphase at time t is equal to the rate at which these cells would have emerged from telophase after an interval s_2 ie. at time $t + s_2$ if they were not prevented from progressing to anaphase by the colchicine. s_2 is a constant marking the boundary between prophase and metaphase in treated and untreated roots. The rate of entry into metaphase dM/dt is therefore from equation (3)

$$\frac{dM}{dt} = m_o e^{\lambda (t+s_2)}$$

where M(t) is the number of cells in metaphase at time t. If t_1 is a time during treatment at which there is an effective barrier to anaphase the number of cells accumulated in metaphase during

a subsequent period up to t_2 is given by

$$\int_{t_1}^{t_2} \frac{dM}{dt}\, dt = \int_{t_1}^{t_2} \lambda m_o e^{\lambda(t+s_2)}\, dt$$

which evaluated gives

$$M(t_2) - M(t_1) = m_o e^{\lambda s_2} \left[e^{\lambda t_2} - e^{\lambda t_1} \right]$$

or as a fraction of the total constant number of meristematic cells $m_o e^{\lambda t_1}$

$$\frac{M(t_2) - M(t_1)}{m_o e^{\lambda t_1}} = e^{\lambda s_2} \left[e^{\lambda (t_2 - t_1)} - 1 \right] \qquad (7)$$

Thus the accumulation of metaphases after the formation of a barrier between metaphase and anaphase is an exponentially increasing function of time and equation (7) can be expanded like equation (5) writing x for $(t_2 - t_1)$

$$\frac{M(t_2) - M(t_1)}{m_o e^{\lambda t_1}} = e^{\lambda s_2} \left[e^{\lambda x} - 1 \right]$$

$$\frac{Mt_2 - Mt_1}{m_o e^{\lambda t_1}} = e^{\lambda s_2} \left(\lambda x + \tfrac{1}{2} \lambda^2 x^2 \right)$$

If the values for the accumulation of metaphases are spaced over a total time interval $2\bar{x}$ then the line fitted by least squares to the data will leave a slope q

$$q = e^{\lambda s_2} (1 + \lambda \bar{x})$$

The boundary between prophase and metaphase in untreated roots and roots treated with colchicine is constant s_2. So the ratio

of the number of cells in metaphase and later stages of mitosis to the total number of cells is corresponding to equation (5)

$$e^{\lambda s_2} - 1 = p$$

This equation and that for the slope provide a quadratic from which may be calculated

$$\frac{p}{q+1} = \bar{x}\lambda^2 + \lambda = r \quad \text{(define)}$$

$$\bar{x}\lambda^2 + \lambda - r = 0$$

$$= \frac{-1 \pm \sqrt{1 + 4\bar{x}r}}{2\bar{x}}$$

By equating the mean rate of entry into metaphase between 2 and 8 hours (ie. $2\bar{x} = 6$) to the mean slope q and equating the percentage of metaphases and ana / telophases in normal roots to p a value for λ could be calculated. T may then be found by subsitution of λ in equation (2) and τ by substitution of λ and the value for the mitotic index of untreated roots in equation (5).

Taking the accumulation of metaphases between 2 and 8 hours the duration of the mitotic cycle T was found to be 33 hours for the cap initials 35 for the stele just above the quiescent centre and 33 hours for the central stele 200 μ from the quiescent centre. The duration of the mitotic cycle for quiescent centre cells was 173 hours. Values of τ based on λ and mitotic indexes at 0 hours are given in Table 2.

TABLE 2

Duration of the mitotic cycle and mitosis calculated from rates of metaphase accumulation (in hours)

	mitotic cycle	mitosis
cap initials	33	4.8
quiescent centre	173	2.3
stele just above quiescent centre	35	7.4
stele 200 from quiescent centre	33	6.1

Discussion.

These results confirm that rates of mitosis in the quiescent centre are very low. The value for the length of the mitotic cycle obtained here is nearly identicial with that of 174 hours for the quiescent centre of Zea mays found by the same method (Clowes 1960). The duration of the mitotic cycle is six times greater in the quiescent centre than in the other regions of the meristem investigated.

It is notable that in the other regions of the meristem, the cap initials and the two stelar regions, there is little variation between the rates of mitosis measured. There is not the sharp distinction, which Clowes found in Zea, between the length of the mitotic cycle in the cap initials and the length of the mitotic cycle in the stele. In Zea, cap initial cells divide once in 12 hours while stele cells divide only once in 28 hours. In Zea, cap initials divide more than twice as fast as cells in other parts of the meristem. For this reason cap initials in Zea are more sensitive to radiation damage than other parts of the meristem (Clowes 1963). In Allium sativum one would expect the cap initials and stele to be equally sensitive to irradiation since there is no marked difference between the rates of mitosis in these regions of the meristem.

These results are discussed more fully at the end of the chapter

Duration of the mitotic cycle from timing of DNA synthesis.

Introduction.

The second independent method I have used to measure the rate of mitosis in *Allium sativum* involves timing DNA synthesis by finding the rate at which nuclei become labelled when the meristem is fed with a radioactive precursor of DNA. Howard and Pelc (1951 and 1953) have used this method to measure average rates of mitosis for whole meristems of *Vicia faba* roots and Clowes has used it to measure rates of mitosis in different regions of the meristem of *Zea mays*. In the original experiment Howard and Pele used P^{32} labelled phosphate as the DNA precursor but Clowes used tritiated thymidine. In this experiment I have also used tritiated thymidine. Not only does tritium give autoradiographs of higher resolution and cause less radiation damage than P^{32} because it emits softer β particles but also by using a labelled substrate which is a specific precursor of DNA the necessity to give an excess dose and then remove RNA and other compounds from the preparation is obviated.

Method.

Allium sativum roots were grown in the usual way until they were 1 - 2 cm. long and then transferred to a solution of tritiated thymidine with an activity of c.250 μc/l. The solution was aerated continuously and maintained at 20°C in the dark. Samples of roots were fixed in acetic alcohol after 2, 4, 8, 16, 32, 48, 60 and 72

hours treatment. The root apices were sectioned longitudinally at 6μ, stained with leuco basic fuchsin, prepared as autoradiographs on high resolution stripping film and exposed for 28 days. The numbers of labelled interphases and mitoses were counted in the four different regions of the meristem for 10 roots per treatment. The percentage values of these counts are given in Table 3 and Fig. 4.

Results.

Tritiated thymidine labels cells in the DNA-synthetic phase (S) of the mitotic cycle. This phase occurs during interphase between G_1 , the period from the end of the previous mitosis to synthesis and G_2 the period from DNA synthesis to the onset of the next mitosis.

Cells which are in the DNA-synthetic phase when they are first exposed to the tritiated thymidine become labelled very quickly. After 2 hours exposure to the labelled thymidine about 40% of cells in the cap initials and stele were labelled and less than 5% of cells in the quiescent centre. The number of labelled interphases rises as cells which are in G_1, mitosis and finally G_2 at the time of initial exposure to the labelled thymidine reach DNA synthesis.

No mitoses become labelled until cells which are in S at the beginning of exposure to label reach mitosis. Labelled mitoses first appear between 4 and 8 hours after exposure. In the cap initials and stele all cells reaching mitosis are labelled before 32 hours.

TABLE 3

Percentages of cells labelled after feeding with tritiated thymidine.

	hours in H^3	mitoses	interphases	MI
cap initials	2	0	35.1	6.6
	4	0	42.1	2.4
	8	87.5	55.7	6.4
	16	92.3	88.4	10.7
	32	100	95.2	9.8
	48	100	96.2	9.8
	60	100	98.2	4.2
	72	100	98.7	3.4
quiescent centre	2	0	3.6	0.8
	4	0	9.6	0
	8	33.3	16.9	2.4
	16	50	29.7	1.7
	32	100	49.5	1.7
	48	75.0	66.0	3.2
	60	100	74.5	6.7
	72	100	77.3	4.3

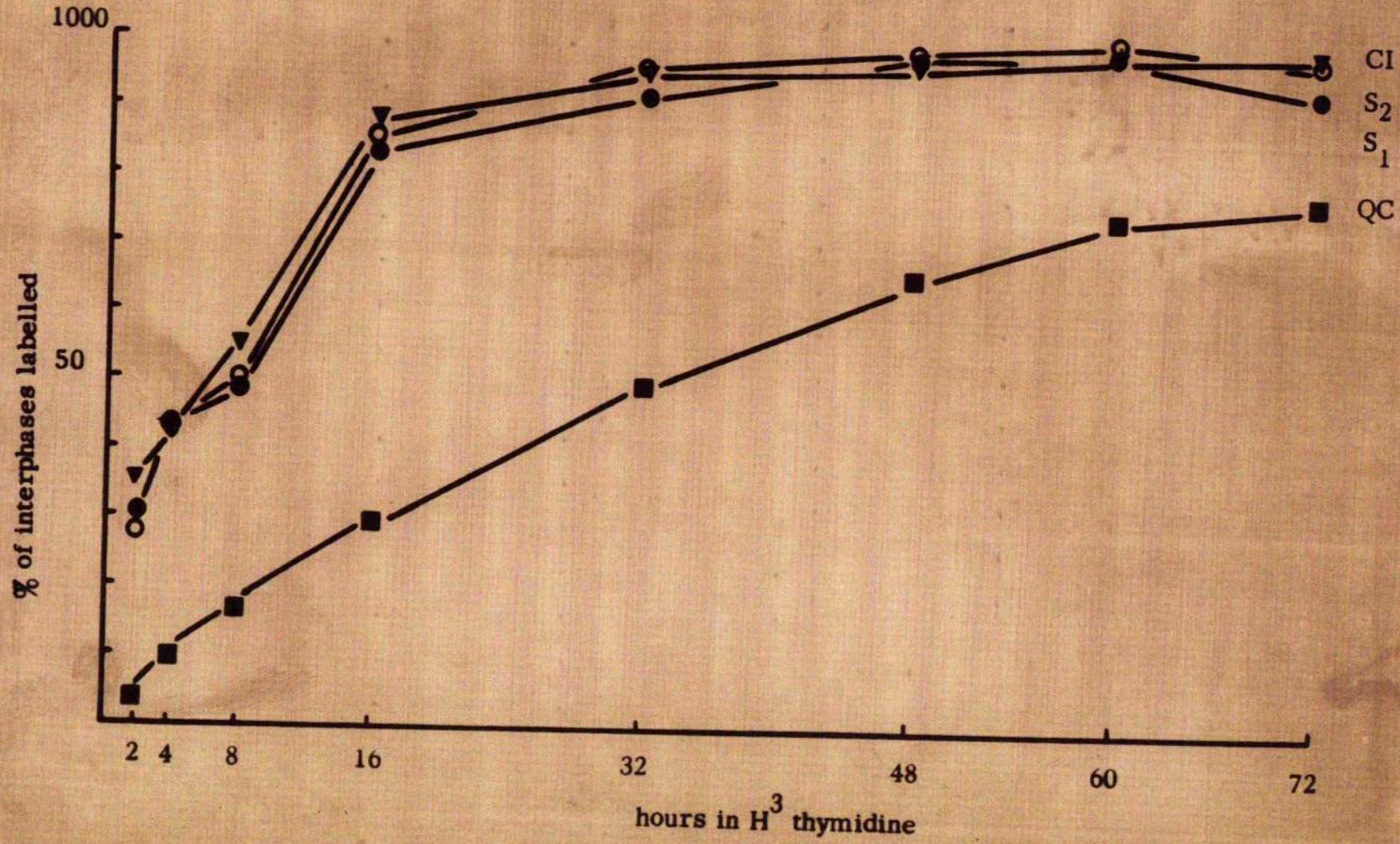
stele just above quiescent centre	2	0	30.6	7.6
	4	0	42.7	6.1
	8	90.5	48.7	8.7
	16	91.7	82.9	10.2
	32	100	91.5	9.8
	48	100	97.5	9.3
	60	100	95.6	7.8
	72	100	92.8	6.3
stele 200 μ above quiescent centre	2	0	27.4	6.3
	4	0	43.3	4.7
	8	90.5	50.5	9.2
	16	94.7	85.4	16.5
	32	100	95.1	5.9
	48	100	98.9	9.3
	60	100	100	10.6
	72	100	98.5	4.8

Average values from median section of ten roots per fixation.

Figure 4

▼ cap initials
■ quiescent centre
● stele just above the quiescent centre
○ stele 200μ above quiescent centre

Percentage of interphases labelled after feeding with H^3 thymidine.



The time required to reach the maximum number of labelled interphase nuclei is equivalent to the duration of the whole mitotic cycle (T). These values of T for the regions of the meristem investigated are given in Table 4. Assuming that increase in cell number in the meristem is an exponential function of time the Mitotic Index is approximately equal to $(\tau/T) \log_e 2$ where τ is the duration of mitosis (Hoffman, 1949). Values of τ based on Mitotic Indices at 2 hours are given in Table 4. Thus it is not possible to obtain a useful value for T for the quiescent centre from this method. Normally the rate of mitosis is so low in the quiescent centre that long before all the cells become labelled, radiation damage to the rest of the meristem induces changes in the behaviour of the quiescent centre cells . Clowes (1961) has found that when Allium cepa roots are exposed to concentrated solutions of tritiated thymidine (20 C/ml) for 15 hours the quiescent centre disappears within 72 hours. radiations from the tritium affect the meristem in a similar way to X-rays in Zea (Clowes 1961). Radiation damage to the cells of the rest of the meristem and the resulting slower rates of mitosis cause quiescent centre cells to synthesise DNA and enter mitosis .

In the present experiment there is evidence that cells of the ground meristem suffer radiation damage. After 32 hours exposure to tritiated thymidine cells in the cap initials and stele contain micronuclei. During the 72 hour treatment the mitotic index falls in the cap initials and stele and rises in the quiescent centre .

TABLE 4

Duration of the mitotic cycle and mitosis from rate of thymidine labelling (in hours) of interphase cells

region of root apex	mitotic cycle	mitosis
cap initials	28	3
quiescent centre	-	-
stele just above the quiescent centre	34	4
stele 200μ above the quiescent centre	29	3

Discussion.

These results again show that cap initials in *Allium sativum* do not divide much faster than cells in the stele, suggesting that these regions of the meristem are about equally radiosensitive. Although no measurement of the duration of the mitotic cycle in the quiescent centre is possible the rate of labelling experiment emphasises the low rate of mitosis in this region.

However this is not such a satisfactory method for measuring rates of mitosis in different regions of the root meristem as metaphase accumulation. It does not allow low rates of mitosis to be measured and causes considerable radiation damage to cells dividing quickly and incorporating relatively large quantities of tritium. Also errors due to scoring are more probable than in metaphase accumulation because of difficulties in deciding whether a heavily labelled nucleus is in interphase or mitosis and whether a nucleus covered by few silver grains is labelled or not. Neverthe less results obtained here based on the rate of labelling DNA are useful in comparison with results obtained by other methods.

Duration of the different phases of the mitotic cycle.

Introduction

The two previous experiments reported here show that the quiescent centre cells of *Allium sativum* divide far more slowly than cells in other regions of the meristem. Values for the duration of mitosis show that it is not the time spent in division which accounts for this discrepancy. It is the duration of interphase which is so markedly prolonged. So it is necessary to establish which part or parts of interphase are those responsible for the extent of the mitotic cycle in quiescent centre cells of *Allium sativum*.

The average length of the component phases of the mitotic cycle may be found by pulse labelling with a radioactive precursor of DNA. This method was first used on mouse intestinal epithelium (Quastler and Sherman, 1959) but has since been applied to roots. Howard and Dewey (1960) used it with squash preparation of whole root apices but the disadvantages of this technique are discussed elsewhere.

Clowes (1965), using sections, determined the duration of component phases of the mitotic cycle in different regions of the meristem of Zea mays.

I have applied the method to Allium sativum roots using tritiated thymidine as the DNA precursor and delimiting specific regions of the meristem in longitudinal sections of the root apices as before.

Method

Allium sativum roots 1-2 cms. long were placed in tritiated thymidine solution of high specific activity and concentration c. 500μC/l for 30 minutes. They were then washed and transferred to aerated tap water, maintained at 20°C in the dark. Root tips were fixed in acetic alcohol at 2 hourly intervals for 36 hours, sectioned at 6μ and stained in leuco-basic fuchsin. Autoradiographs were prepared and exposed for 28 days. Nuclei in mitosis in the four regions of the meristem were scored as labelled or unlabelled and the percentage of labelled nuclei plotted against time from removal from the tritiated thymidine. Because of the difficulties in deciding whether some nuclei were labelled or not and whether some nuclei covered with silver grains were in

interphase or mitosis, two people scored independently all the ten roots for each treatments and the percentages of labelled mitosis given in the graphs Fig. 5 p.48 are the pooled results of these scorings.

Results

Cells in the DNA synthesis phase (S) of the mitotic cycle during exposure to the tritiated thymidine become labelled. The graphs show the double peaks of the first two post labelling mitotic cycles of these cells except for the quiescent centre where the second peak is not reached during the 36 hour period of the experiment. Labelled cells do not come into division immediately after exposure to the tritiated thymidine. The interval between removal from the labelled thymidine and the time when 50% of the cells in mitosis are labelled equals the duration of $G_2 + \frac{1}{2}\tau$ (where G_2 is the phase between S and the following mitosis, when a diploid cell contains the 4c amount of DNA, and τ is mitosis). The percentage of labelled cells in mitosis rises to over 80% in the first peak and stays at this level while all the cells which were

Figure 5

Percentage of mitoses labelled after pulse labelling with H^3 thymidine

in S during labelling reach mitosis and then falls as cells that were in G_1 (the phase of the cycle prior to synthesis) at the time of labelling come into division. So the width of the peak of labelled misoses at the 50% level gives an estimate of the duration of S.

In these graphs there is no evidence of a dip in the middle of the first peak such as reported by Clowes (1964) for Zea and Howard and Dewey (1960 and 1961) for Vicia which it is suggested is due to non-uniform labelling during S. However no stress should be laid on this since percentage values of labelled mitoses 12 hours after removal from the hot thymidine are unfortunately missing here.

The percentage of labelled mitoses continues to fall as cells which were in M or G_2 during labelling come into their second post-labelling mitosis. Then the percentage of labelled mitoses rises again when the daughters of cells that were in S at the time of labelling reach mitosis. The time between the peaks or their leading edges if the second peak is not well defined provide an estimate for the average duration of the complete mitotis cycle T. If the number of meristematic cells is increasing exponentially then the duration of mitosis may be found using the equation $M1 = \frac{\tau}{T} \log_e 2$ The Mitotic Indices at 2 hours, given in Table 5 are used to calculate τ . (Hoffman 1949).

TABLE 5

Duration of the mitotic cycle and its component phases (in hours) from pulse labelling.

	cap initials	quiescent centre	stele just above quiescent centre	stele 200 μ above quiescent centre
T	27	(173)	26	26
G_1	4	143	5	5
S	13	17	12	12
G_2	6	8	4	4
M	5	(6)	6	6
MI	13.0	2.4	15.2	15.8

- the values in parentheses are derived from a value of T obtained from metaphase accumulation.

The lengths of the four phases of the mitotic cycle in the different regions of the meristem obtained from the data in the graphs are given in Table 5.

This experiment does not provide a value for the total length of the mitotic cycle in the quiescent centre but if 173 hours, the value for T obtained by metaphase accumulation, is used M and G_1 may be calculated. The duration of G_1 for the quiescent centre, 143 hours, is about thirty times greater than values of G_1 for the other regions of the meristem. G_1 values for the cap initials and the two stelar regions are very similar being 4 and 5 hours respectively. Values for the duration of the total length of the mitotic cycle in these regions are also very similar. S occupies just slightly less than half the total cycle in these regions. A similar proportion of the mitotic cycle is occupied by S in the stele of Zea (Clowes, 1965) and Tradescantia whole root tips (Wimber and Quastler, 1963) but Howard and Dewey (1960) and Grant (1964) found that S occupies a smaller fraction of the mitotic cycle in whole root tips of Vicia faba.

Comparing the four regions of the meristem S, G_2 and M do not vary considerably from one region to another. This is again similar to the situation in Zea (Clowes 1965).

Discussion.

These results show that it is because of the length of time spent in G_1 that the duration of the mitotic cycle is so long in the quiescent centre. It is the length of time spent in G_1 which distinguishes quiescent centre cells from cells in other parts of the meristem.

Quiescent centre cells contain the 2c amount of DNA for 87% of their mitotic cycle in Allium sativum. So they offer on average a smaller target to incident irradiations than cells which contain the 2c amount of DNA for a smaller proportion of their mitotic cycle. It is expected therefore that quiescent centre cells are far more resistant to radiation damage than other cells in the meristem. Clowes has shown that this is true for Zea (1963) and Vicia (1962).

In the cap initials of Zea mays G_1 is absent or extremely short (Clowes 1965). Clowes has suggested that this is the reason for the extreme sensitivity of the cap initials, compared with cells in other parts of the meristem of Zea to the effects of X-rays (Clowes 1963) β rays (1961) and radio mimetic chemicals such as 5-amino uracil (1965). In the cap initials of Allium sativum G_1 is scarcely different from G_1 in the cells of the stele. So it is expected that cap initials in Allium are no more radiosensitive than stelar cells.

TABLE 6

Duration of the mitotic cycle (T) and mitosis (τ) in hours calculated from rates of labelling and metaphase accumulation.

	pulse labelling		continuous labelling		metaphase accumulation	
	T	τ	T	τ	T	τ
cap initials	27	5	28	3	33	5
quiescent centre	(173)	6	-	-	173	2
stele just above q.c.	26	6	34	4	35	7
stele 200 μ above q.c.	26	6	29	3	33	6

Discussion.

The experiments reported in this section give three independent values for the length of the mitotic cycle (T) for different regions of the root meristem of Allium sativum. The only other estimates for T in discrete regions of the meristem are those of Clowes for roots of Zea mays (1961 and 1964), Sinapis alba (1962) and Vicia faba (Clowes and Hall, 1962). Most of the previous evaluations of T in roots or shoots are averages for whole meristems.

Gray and Scholes (1951) found that for Vicia roots grown at 19°C the value of T is 25 or 19 hours depending upon how much of the root tip is included in the meristem. The method they used involves finding the rate of production of new cells from the number of cells in transverse sections, the size of the cells and the rate of root elongation. From these data and the numbers of cells in mitosis and in interphase T can be calculated. One of the drawbacks to this method is that it depends upon an arbitary delimitation of the meristem. Another error is caused by the necessity to assume that all cell divisions contribute to the length

of the root. Howard and Pelc (1953) obtained a value for T of 30 hours for Vicia roots grown at 19°C by the continuous labelling method. While Evans and Savage (1959) from accumulation of metaphases at 19°C found T equal to 26 hours in Vicia roots.

Brown and Rickless (1949) devised a method for measuring rates of mitosis which involves macerating the root apex in a known volume of fluid and counting a sample of the suspended cells in a haemocytometer. Counts are made for given lengths of root before and after a known growth period. The total increment of cells divided by half the increment of meristematic cells gives the number of divisions which occur during the known period of growth. In this method a cell lacking a vacuole is considered to be meristematic, but, as Clowes (1960) has pointed out, vacuolation does not necessarily distinguish a non-meristematic cell from a meristematic cell. However using this method on the roots of Pisum seedlings Brown (1951) found T equal to 25 hours at 15°C and 14 hours at 30°C.

Average values for the length of mitosis are useful in demonstrating the gross effect of external factors such as temperature on the length of the mitotic cycle (Brown, 1951; Evans and Savage, 1959). But they do not help to solve

morphogenetic problems and no comparison can be made between average values for T in whole meristems and values for T such as those obtained in the present work. In gaining average values for the length of mitosis workers have assumed that all cells in the meristem behave similarly. Clowes (1960) has shown that this need not be so. Development and the maintenance of organisation is in part achieved by differential rates of cell division. For example in tissue cultures it is only when differential rates of mitosis are set up that a group of cells loses its amorphous habit becomes organised and differentiates ( ). Similarly in both plant and animal embryos differential rates of cell division are an integral part of the pattern of development. It is reasonable that differential rates of division which are of such importance in the early development of the plant should be of similar importance in maintaining organisation in the growth zones, the apical meristems of the mature organisms.

Hejnowicz (1959) compared the rates of mitosis at different levels in the meristem of Triticum roots grown at 18°C. He counted mitoses in 11μ transverse sections and used photographs of the root surface to calculate the rate

of growth. In the epidermis he found T to be 25 hours at the level of the stelar pole, approximately 21 hours between 0.15 mm and 0.6 mm from the stelar pole and about 60 hours a further 0.3 mm proximal to the pole. There is a similar pattern of an initial decrease and then increase in the length of mitosis with increasing distance from the stelar pole in the metaxylem and outer cortex. Hejnowicz gave no values for T in the quiescent centre but Clowes (1961) considers that Hejnowicz's results are consistent with the existence of a quiescent centre in Triticum.

For comparison the values for T and τ in Allium found by the three different methods are given in Table 6 p. 53 The values for T found by the labelling methods are very similar, but those found by metaphase accumulation are about 6 hours higher. The roots for the two labelling experiments were grown together and the labelling treatments carried out simultaneously. This could account for the close similarity of the two sets of results from the labelling experiments. The roots used for the metaphase accumulation experiment could have been growing more slowly. However, the three sets of results show clearly that the rates of division in the various regions of the meristem investigated are reasonably similar.

In all cases rates of cell division in the cap initials and in the stele 200 μ from the quiescent centre are similar. Stelar cells just above the quiescent centre divide slightly more slowly than any other cells in the meristem for although the only figure for T given is that derived from ~~M~~ metaphase accumulation the other experiments indicate a high, though undetermined, value.

In comparing the values for T in Allium with those obtained for Zea by Clowes (1961 and 1964) there is one major similarity and two differences. In both Allium and Zea the cells of the quiescent centre divide many times more slowly than cells in the rest of the meristem. In Allium and in Zea the length of the mitotic cycle is due to a prolonged G_1 phase of the mitotic cycle. Quiescent centre cells spend nearly 90% of the mitotic cycle in G_1. However the cap initials in Allium do not divide more quickly than cells in the stele whereas in Zea the cap initials divide at least twice as fast as cells in the stele, cortex and endodermis. In Alliumwith the exception of the quiescent centre there is little or no distinction between the different regions of the meristem with respect to rates of cell division but in Zea there is relatively wide variation in the values of T for the different regions of the meristem.

Clowes (1963) has correlated the radiosensitivity of cells in different regions of the meristem with the length of the mitotic cycle and in particular with the proportion of the cell cycle occupied by G_1. The present results suggest therefore that with the exception of the quiescent centre the different regions of the meristem of Allium should be equally radiosensitive.

The quiescent centre and the effect of colchicine on root meristems

Introduction

Colchicine inhibits spindle formation in mitosis; chromosomes are arrested at metaphase (Levan 1938; Östergren, 1950). The chromatids of arrested chromosomes eventually fall apart, aggregate, despiralise and form a single restitution nucleus. Since these chromatids would normally constitute two diploid nuclei the resulting restitution nucleus is tetraploid containing the 4c amount of DNA. Thus colchicine induces polyploidy in the sensitive dividing cells of a root meristem. While the cells of a root are exposed to concentrations of colchicine above the threshold for spindle inhibition each entry into mitosis results in a doubling of the chromosome number.

Colchicine also has a physiological effect on the growth pattern of roots. There is a temporary inhibition of longitudinal growth associated with the formation of a sub terminal apical swelling, the c-tumour(*Allium cepa*, Levan, 1938; *Vicia faba*, Davidson, 1961). This c-tumour swelling is

caused by the isodiametric expansion of cells, particularly cortical cells, in the region of the root apex which would normally constitute the zone of elongation.

Roots exposed to colchicine for short periods frequently recover. Cell elongation resumes a few days after treatment and the root apex grows away from the c-tumour. The meristem of the regenerating root is mixoploid.

Levan (1938) noted that shortly after treatment the meristem of roots exposed to colchicine for short periods contained many tetraploid cells, some cells of higher ploidy and, towards the apex, some diploid cells. D'Amato and Avanzi (1948) reported that even after prolonged treatments with colchicine not all the cells in the meristem become polyploid. Cells near the root cap are diploid when other cells in the meristem are 16-ploid or more. These diploid cells did not undergo mitosis during the effective period of spindle inhibition.

Davidson (1960) has shown that the mixoploid condition of the regenerating root is temporary. Polyploid cells are replaced in the meristem by diploid cells. Over a period of several days the frequency of polyploid cells falls until all or very nearly all the meristematic cells in the root are

diploid. The lineages of diploid cells which repopulate the meristem during recovery from colchicine treatment arise from cells that do not divide during the period of exposure to colchicine or while it continues to be effective in inhibiting spindle formation.

There are some cells in the root apex which do not divide (very frequently) and whose chromosome complements remain visibly unaffected by experimental treatments which cause changes in the chromosome complement of meristematic cells. These cells comprise the quiescent centre. Quiescent centre cells are capable of division and following exposure to high doses of X-rays divide to provide diploid initial cells. Derivatives of these diploid initials repopulate the meristem (Clowes, 1963)

It is highly probable that quiescent centre cells provide the source of normal diploid cells upon which regeneration of the root after colchicine treatment is dependent (Davidson, 1960; Clowes, 1960). The experiment reported here was carried out to test this hypothesis.

Method

Allium sativum roots were grown in the usual way until they were 1-2 cms long. Half the roots were transferred to 0.025% freshly prepared aqueous colchicine solution for 3 hours. The rest were treated with 0.05% colchicine for 3 hours. The colchicine was aerated and maintained at 20°C. The roots were thoroughly washed after treatment and grown on in tap water at 20°C. Samples of each set of roots were fixed in FPA just before and immediately after treatment and at daily intervals for 5-6 days following treatment. The roots were embedded, sectioned longitudinally at 8 μ and stained in leucobasic fuchsin and fast green. The number of cells in interphase and the phases of mitosis were counted for the four different regions of the meristem in the median sections of ten roots per sample. The percentage values of these counts are given in Tables 7 and 8 and ~~the percentages of cells in mitosis are plotted in fig.~~ and

Results

Roots of *Allium sativum* respond in the usual way to brief colchicine treatments. Longitudinal growth is inhibited and between 12 and 36 hours after treatment a sub-terminal c-tumour swelling is formed. Elongation is resumed and the root apex begins to grow away from the tumour about 5-6 days after treatment. The data given in Tables 7 and 8 show that during the three hour colchicine treatment cells are arrested at metaphase as expected. The percentage of cells in metaphase rises sharply in all regions of the meristem and there is a slight rise in the mitotic index.

Twenty four hours after treatment the root meristem is mixaploid. In roots treated with 0.025% colchicine the majority of cells in division are completing normal mitoses. The mitotic indices though in the cap initials and the stele regions fall to about half those found in untreated roots. But within the following twenty four hours the mitotic indices in the cap initials and the stele just above the quiescent centre rise again to values higher than in the corresponding regions of untreated roots. This rise and fall in mitotic

TABLES. 7

Percentages of cells in interphase and other phases of mitosis during recovery from colchicine treatment with 0.025% for three hours.

	treatment (hours)	interphases %	prophases %	metaphases %	anaphases telophases	MI.
cap initials	0	90.0	7.5	0.6	1.9	10.0
	3	88.5	4.0	7.5	0	11.5
	24	94.0	5.4	0	0.6	5.0
	48	86.9	8.7	1.9	2.5	13.1
	72	94.4	5.0	0	0.6	5.6
	96	96.2	2.5	0	1.3	3.8
	120	94.4	3.1	0.6	1.9	5.6
quiescent centre	0	100	0	0	0	0
	3	97.8	1.3	0.9	0	2.2
	24	97.4	1.7	0	0.9	2.6
	48	96.3	2.8	0.9	0	3.7
	72	90.8	7.5	0	1.7	9.2
	96	97.2	2.8	0	0	2.8
	120	95.4	4.6	0	0	4.6
stele just above q.c.	0	85.7	9.3	0.7	4.1	14.3
	3	83.3	6.9	10.0	0	16.7

TABLE continued. 7

	treatment (hours)	interphases %	prophases %	metaphases %	anaphases telophases	M.I.
stele just above the quiescent centre	24	90.3	7.4	0	2.3	9.7
	48	83.4	15.1	0.5	1.0	16.6
	72	90.0	8.0	0.8	1.2	10.0
	96	91.0	3.1	2.0	3.9	9.0
	120	92.0	3.6	1.2	3.2	8.0
stele 200μ above the quiescent centre	0	81.5	13.2	2.1	3.2	18.5
	3	81.4	7.6	10.7	0	18.6
	24	94.9	2.6	0.5	2.0	5.1
	48	90.3	8.3	0	1.4	9.7
	72	91.8	7.4	0.8	0	8.2
	96	91.3	5.1	1.6	2.0	8.7
	120	93.5	3.3	1.2	2.0	6.5

TABLE. 8

Percentages of cells in interphase and other phases of mitosis during recovery from treatment with 0.05% colchicine for three hours.

	treatment (hours)	interphases %	prophases %	metaphases %	anaphases telophases	M.I.
cap initials	0	90.6	6.3	1.9	1.2	9.4
	3					
	24	93.1	1.3	5.6		6.9
	48	88.4	4.2	4.2	3.2	11.6
	72	93.2	5.2	1.0	0.5	6.8
	96	93.3	2.9	2.5	1.2	6.6
	120	94.6	2.3	1.8	1.3	5.4
	144	93.8	3.1	1.2	1.9	6.3
quiescent centre	0	98.4	0.8		0.8	1.6
	3		~~0.7~~			
	24	96.7	2.5	0.8	2.1	3.3
	48	96.7	0.7	0.7	2.8	3.3
	72	92.4	3.5	1.4	3.3	7.6
	96	92.2	3.3	1.2	3.6	7.8
	120	91.1	4.8	0.5	3.3	8.9
	144	95.8		0.9		4.2

TABLE continued. 8

	treatment (hours)	interphases %	prophases %	metaphases %	anaphases telophases	M.I.
stele just above the quiescent centre	0	87.0	7.1	1.7	4.2	13.0
	3					
	24	88.3	4.6	7.1		11.7
	48	88.7	6.2	2.0	3.1	11.3
	72	91.7	4.2	1.3	2.8	8.3
	96	91.1	4.7	3.3	0.9	8.9
	120	93.2	3.5	1.2	2.1	6.8
	144	90.8	5.8	1.7	1.7	9.3
stele 200 above the quiescent centre.	0	88.8	5.8	1.3	4.2	11.2
	3					
	24	84.6	7.5	7.9		15.4
	48	87.7	5.4	3.2	3.1	12.3
	72	91.7	3.7	2.3	2.8	8.3
	96	93.4	3.5	1.5	0.9	6.6
	120	93.4	2.3	2.9	2.1	6.6
	144	90.8	4.0	1.6	1.7	9.2

index in the first forty eight hours after treatment is probably due to partial synchrony of the cell cycle caused by the colchicine treatment. The mitotic index in the stele 200 μ from the quiescent centre fails to rise to levels found in untreated roots. This is because cells in this region are differentiating. These cells would be several hundred microns removed from the stele pole if the colchicine treatment had not inhibited longitudinal growth.

In roots treated with 0.05% colchicine there are far more cells in which chromosomes are still arrested at metaphase twenty four hours after treatment. Some of these cells are tetraploid. It is because cells are still being arrested at metaphase that the mitotic indices of the cap initials and the stele are rather higher than in roots treated with the lower concentration of colchicine. Forty eight hours after treatment the mitotic indices in these regions are not as high as in roots treated with the lower concentration.

The mitotic index in the quiescent centre during the first two days recovery from either treatment is slightly higher than in untreated roots. But within a further twenty four hours the number of cells dividing in the quiescent

centre rises to several times the number in untreated roots whereas in other regions of the meristem about half the usual number of cells is dividing. Thus three days after exposure to 0.025% colchicine there is a peak in the number of cells dividing in the quiescent centre and the percentage of cells dividing in the quiescent centre is almost as high as elsewhere in the meristem. The period of high mitotic activity in the quiescent centre is longer in roots exposed to the higher concentration of colchicine. In these roots the percentage of cells in division in the quiescent centre does exceed that of the rest of the meristem five days after treatment.

On the sixth day after treatment elongation resumes and as a result the mitotic index in the stele 200 μ from the quiescent centre rises. It is interesting to note that the mitotic index in this region on the sixth day of recovery is equivalent to that of the stele just above the quiescent centre on the previous day. Six days after treatment the mitotic index in the stele just above the quiescent centre is restored to very nearly the original value in untreated roots. With this increase in the mitotic index of the surrounding tissues the mitotic index in the quiescent centre falls.

During recovery from colchicine treatment there is a period when the concentration of residual colchicine is near the threshold for mitotic spindle inhibition. At this level of colchicine concentration some cells complete normal mitoses others are arrested at metaphase. But in many cells there is no all or nothing effect. Partial recovery leads to the formation of abenant mitotic spindles which give rise to abnormal anaphase segregations. Changes in the condensation of the chromosome chromatin may also contribute to abnormal anaphase segregations. These differences in behaviour of the constituent cells of the meristem may reflect inherent differences in their sensitivity to colchicine or they may be due to uneven distribution of physiologically active colchicine within the root.

Twenty four hours after either treatment some interphase cells are multinucleate or carry micro-nuclei. Multinucleate cells are the result of multi-polar spindles or faults in restitution while micro-nuclei are due to 'sticky' anaphase bridges or lagging chromosomes or chromatids. The number of cells carrying abnormal nuclei increases markedly between twenty four and forty eight hours after treatment. But as far as it is possible to judge in sections of roots fixed forty

eight hours after treatment all mitoses are normal. Grossly abnormal nuclei are either lost to the zone of differentiation or become pycnotic and die.

Discussion

Meristematic cells suffer two types of alteration to their chromosome complements after colchicine treatment. Some cells become polyploid, but this is a balanced change in genetic material and does not seem to impair to any extent the reproductive integrity and efficiency of the cell or its ability to differentiate normally (Davidson 1961; Taylor 1963). Other cells become aneuploid to varying degrees and, though slight changes in the genetic complements of cells may allow them to remain viable, aneuploidy frequently leads to cell death or reduced reproductive efficiency. Thus the genetic damage sustained by many cells in the meristem on recovery from exposure to colchicine is similar to the damage caused by irradiation.

On recovery from colchicine treatment the number of cells in the meristem is reduced because cells lost to the zone of differentiation are not replaced in the usual way. Derivatives

of cells killed or damaged by the effect of the colchicine are missing and it is these cells which would normally replace differentiating cells. This investigation shows that the fall in the number of cells in the meristem capable of dividing is associated with a stimulation of division in the cells of the quiescent centre. Increase in the Mitotic Index of the quiescent centre is correlated with a decrease in the Mitotic Index of the rest of the meristem. This finding exactly parallels those for root meristems on recovery from doses of X-rays. Cells in the quiescent centre come into division about three days after irradiation, after genetic and physiological damage to meristematic cells and subsequent reduction in the size of the meristem. (Clowes, 1959).

This investigation demonstrates the role of the quiescent centre in the regeneration of the root after exposure to colchicine. Two factors are of particular importance with respect to the efficiency of the quiescent centre cells in bringing about the regeneration of the root apex. First, the position of the quiescent centre is such that its derivatives can reach all parts of the meristem. Secondly it provides a large enough association of adjoining diploid cells to form a new root primordium. Cells in the quiescent centre are

protected from the action of colchicine because they normally divide so infrequently. Thus when the majority of cells in the meristem have ceased to divide they are capable of proliferating at such a rate as to double or treble their number in two to three days. From the occurrence of cells with a typical chromosome complement in regenerated roots Davidson (1960) has estimated that in *Vicia* a group of 40 to 50 or more cells is necessary to establish a new root primordium. After colchicine treatment the only source of so large a group of normal diploid cells in the root apex is the quiescent centre and its derivatives. Although there may be more than the required number of normal cells dividing in other parts of the meristem they are distributed randomly and the pattern of growth in the root apex prevents their association in any way which would lead to the establishment of a new root primordium.

The importance of the quiescent centre in regeneration of roots after exposure to colchicine and X-rays is now well established. But we still know little of the factors controlling the mitotic activity of cells.

The quiescent centre after X-irradiation and the radio-sensitivity of various regions of the root meristem of *Allium sativum*.

Introduction

Bergonié and Tribondeau (1906) expressed the rationale of radio-therapy in the treatment of cancer as a law which states that X-rays are more effective in damaging cells that divide actively than cells which divide slowly. This law has been the subject of much controversy because no one has been able to explain it satisfactorily. There are some obvious exceptions to the law (Bond 1959) and some workers have been led to dispute any credance in the law. Lajtha (1961) suggests that initial differences in sensitivity seen in different organs may be due solely to the rates at which different cells exhibit radiation damage. However, Clowes has shown that this law may apply to root meristems; in *Zea* (1963) and in *Vicia* (1964) the radio sensitivity of various regions of the meristem is correlated with the respective rates of mitosis in these regions. In particular radio sensitivity is correlated with the proportion of the cell cycle spent in the G_1 period of interphase prior

to DNA synthesis and Clowes has suggested that an explanation of the mechanism of the law of Bergonié and Tribondeau is that slowly dividing cells spend a greater proportion of the cell cycle in G_1 the least radio sensitive stage of the mitotic cycle.

X-irradiation upsets the normal pattern of behaviour in the root meristem in a variety of ways and thus there are several criteria by which it is possible to gauge radio sensitivity eg. inhibition of longitudinal cell growth, mitotic delay, chromosome damage and cell survival. But one of the easiest ways of estimating radiation damage is by the frequency of cells carrying micronuclei and this is a convenient method of measuring the relative radiosensitivity of different regions of the meristem in sections of roots.

It is generally accepted that X-rays induce three types of chromosome aberration and the type of aberration induced is related to the unit of breakage which depends upon the phase of the cell cycle at the time of irradiation (Thoday, 1955; Evans, 1962). Chromosome breaks are induced in early interphase on G_1 cells (Howard and Pelc, 1951) while chromatid breaks are induced in cells which are undergoing or have completed DNA synthesis and sub-chromatid breaks are induced in cells irradiated during

late prophase or premetaphase. Some of these aberrations give rise to acentric fragments which are often excluded from the nuclear membranes of the daughter nuclei at the end of mitosis. The fragment becomes spherical and forms a micronucleus trapped in one of the daughter cells. Aberrations resulting from chromosome breaks and some chromatid breaks are detectable at the first mitosis after irradiation, but those resulting from sub-chromatid and some chromatid breaks are not detectable untill the second mitosis after irradiation. Thus micro nuclei may be formed at the end of the first or second mitosis subsequent to irradiation. A few micronuclei are capable of self replication and some may be absorbed in time, but within a few days of irradiation the number of micro nuclei is an efficient measure of chromosome damage; Evans, Neary and Williamson (1959) have shown that micronuclei reflect about 60% of fragment frequency in *Vicia faba*.

The measurements of rates of mitosis in the various regions of the meristem of Allium sativum reported in Section 4 suggest that the cap initials and the stelar cells should be about equally sensitive to radiation damage. The experiments described here were carried out to test this and to note the behaviour of the quiescent centre after various doses of X-rays.

Method

Allium roots were grown in the usual way until they were 1-2 cms. long. A sample of roots was fixed in FPA and the rest divided into four batches which were exposed to various doses of X-rays 150, 250, 400 and 600 rads as described in Section 2. The roots were returned to tap water and grown on, continuously aerated and maintained at c. 20°C. Samples of roots from each treatment were fixed in FPA at daily intervals for 5-6 days after irradiation and a further fixation was made after 9 days recovery from irradiation. The roots were sectioned longitdinally and stained in leuco basic fuchsin and fast green. Median sections of 10 roots per fixation were examined and the number of cells in different stages of mitosis and the number of cells carrying micronuclei counted in the different regions of the root meristem. The results are given in Table 9 and Figs. 6, 7 and 8.

Results

This investigation indicates that roots of *Allium sativum* are extremely radiosensitive, relatively more sensitive than roots of *Vicia faba*, with the semi-lethal dose for *Allium* roots at about the level of 250 rads. Hence it was not possible to collect

TABLE 9

Percentages of cells in different stages of mitosis in different regions of the root meristem of Allium sativum on recovery from exposure to 150 rads of X-rays.

	days recovery	prophases	metphases	anaphases + telophases	interphases	interphases + micronuclei	MI
cap initials	1	1.9	0.6	0.6	96.9	5.6	3 1
	2	6.3	2.4	4.4	86.9	11.9	13.1
	3	5.0	0	3.1	91.9	12.5	8.1
	4	4.4	0	1.2	94.4	14.4	5.6
	5	3.8	0	0.6	95.6	16.3	4.4
	6	2.5	0.6	0	96.9	23.7	3.1
	9	0.6	0	0.6	98.8	24.4	1.2
	control	5.0	1.6	3.1	90.4	0	9.6
quiescent centre	control	0.9	0	0	99.1	0	0.9
	1	0	0	0	100	1.7	0
	2	0	0	0	100	0	0
	3	5.8	0	2.5	91.7	0.9	8.3
	4	4.1	0	0	95.9	1.7	4.1
	5	4.1	0	1.7	94.2	1.7	5.8
	6	4.2	0	2.5	91.6	4.2	8.4
	9	2.4	0	2.4	95.3	5.8	4.7

stele just above quiescent centre	control	8.4	0.9	3.1	87.6	0	12.4
	1	1.7	0	0	98.3	9.2	1.7
	2	6.7	1.2	1.2	90.8	5.8	9.2
	3	5.8	1.2	1.7	91.3	9.6	8.7
	4	3.1	0.4	0.8	95.9	11.7	4.2
	5	2.5	0.4	0.4	96.7	13.3	3.3
	6	5.4	1.6	0.4	92.6	15.0	7.4
	9	1.2	0.4	0	90.3	10.4	1.7
stele 200 μ above quiescent centre	control	5.2	4.2	5.8	84.3	0	15.2
	1	1.7	0.4	1.7	96.2	5.5	3.8
	2	5.8	0.8	2.1	91.3	8.8	8.7
	3	6.3	0.8	2.1	90.8	13.8	9.2
	4	2.5	0.4	0.4	96.7	10.4	3.3
	5	1.6	0	1.2	97.8	11.7	2.8
	6	2.1	1.2	0.9	95.8	17.5	4.2
	9	0.8	0	0.8	98.3	9.6	1.7

TABLE 9 continued

Percentages of cells in different stages of mitosis in different regions of the root meristem of Allium sativum on recovery from exposure to 250 rads X-rays.

	days recovery	prophases	metaphases	ana/telo phases	interphases	inter phases + micro-nuclei	MI
cap initials	control	5.0	1.6	3.1	90.4	0	9.6
	1	1.3	0.6	0	98.1	11.9	1.9
	2	5.6	1.9	1.3	91.2	14.4	8.8
	3	2.6	0	0	97.4	20.6	2.6
	4	3.8	3.8	2.6	89.8	26.8	10.2
	5	6.6	1.3	0	98.1	18.1	1.9
	9	3.1	0	0	96.9	30.0	3.1
quiescent centre	control	0.9	0	0	99.1	1.7	0.9
	1	0	0	0	100	0	0
	2	0.8	0	0	99.2	1.7	0.8
	3	0	1.7	0	98.3	1.7	1.7
	4	3.3	0.8	6.7	93.3	1.7	6.7
	5	4.2	0.8	2.5	92.5	0.8	7.5
	9	5.9	2.5	2.5	89.2	9.2	10.8

stele just above quiescent centre	control	8.4	0.9	3.1	87.6	0	12.4
	1	0.4	0	0.4	99.2	9.2	0.8
	2	5.0	0.8	1.2	93.6	16.7	7.0
	3	2.1	1.2	0.4	96.3	15.0	3.7
	4	3.1	0.8	0.8	95.1	19.3	4.9
	5	2.1	0.4	0.8	96.7	18.4	3.3
	9	2.5	0.8	0.8	95.8	21.2	4.2
stele 200 μ above quiescent centre	control	5.2	4.2	5.5	84.8	0	15.2
	1	1.2	0	1.2	97.6	10.4	1.4
	2	2.1	1.7	0	96.2	20.8	3.8
	3	2.5	1.2	1.2	95.1	15.4	4.9
	4	3.3	0.4	0.8	95.5	21.6	4.5
	5	2.1	0.8	0.4	96.7	21.6	3.3
	9	0.8	0	0.4	98.8	18.7	1.2

TABLE 9 continued

Percentages of cells in different stages of mitosis in different regions of the root meristem of Allium sativum on recovery from exposure to 400 rads X-rays.

	days recovery	prophases	metaphases	ana/telo phases	interphases	micro-nuclei + interphases	MI
cap initials	control	5.0	1.6	3.1	90.4	0	9.6
	1	0	0.6	0	99.4	1.9	0.6
	2	2.5	0	0.6	96.9	1.9	3.1
	3	3.2	0	0	96.8	10.0	3.2
	4	2.5	0	0	97.5	9.3	2.5
	5	0.6	0	0	99.4	11.3	0.6
quiescent centre	control	0.9	0	0	99.1	0	0.9
	1	0	0	0	100	0	0
	2	0.8	0	0	99.2	0.8	0.8
	3	0.8	0	1.7	99.5	0.8	2.5
	4	0.8	0	0.8	98.3	1.7	1.7
	5	0.8	0	2.5	96.7	3.3	3.3

stele just above quiescent centre	control	8.4	0.9	3.1	87.6	0	12.4
	1	0.4	0	0	99.6	2.1	0.4
	2	1.7	0.4	0.8	97.1	5.0	2.9
	3	1.3	0	0	98.7	5.0	1.3
	4	1.7	0	1.7	96.7	6.3	3.3
	5	0.4	0.4	0.4	98.7	7.5	1.3
stele 200 μ above quiescent centre	control	5.2	4.2	5.8	84.8	0	15.2
	1	1.2	0.4	0.8	97.6	1.7	2.4
	2	5.0	0.8	1.3	92.9	6.3	7.1
	3	1.7	0.4	0	97.9	10.8	2.1
	4	1.7	0.4	0.8	97.1	11.7	2.8
	5	1.3	0	0	98.7	12.5	1.3

Figure 6

Percentages of cells with micronuclei in the root meristem of Allium sativum after exposure to 150 rads of x-rays.

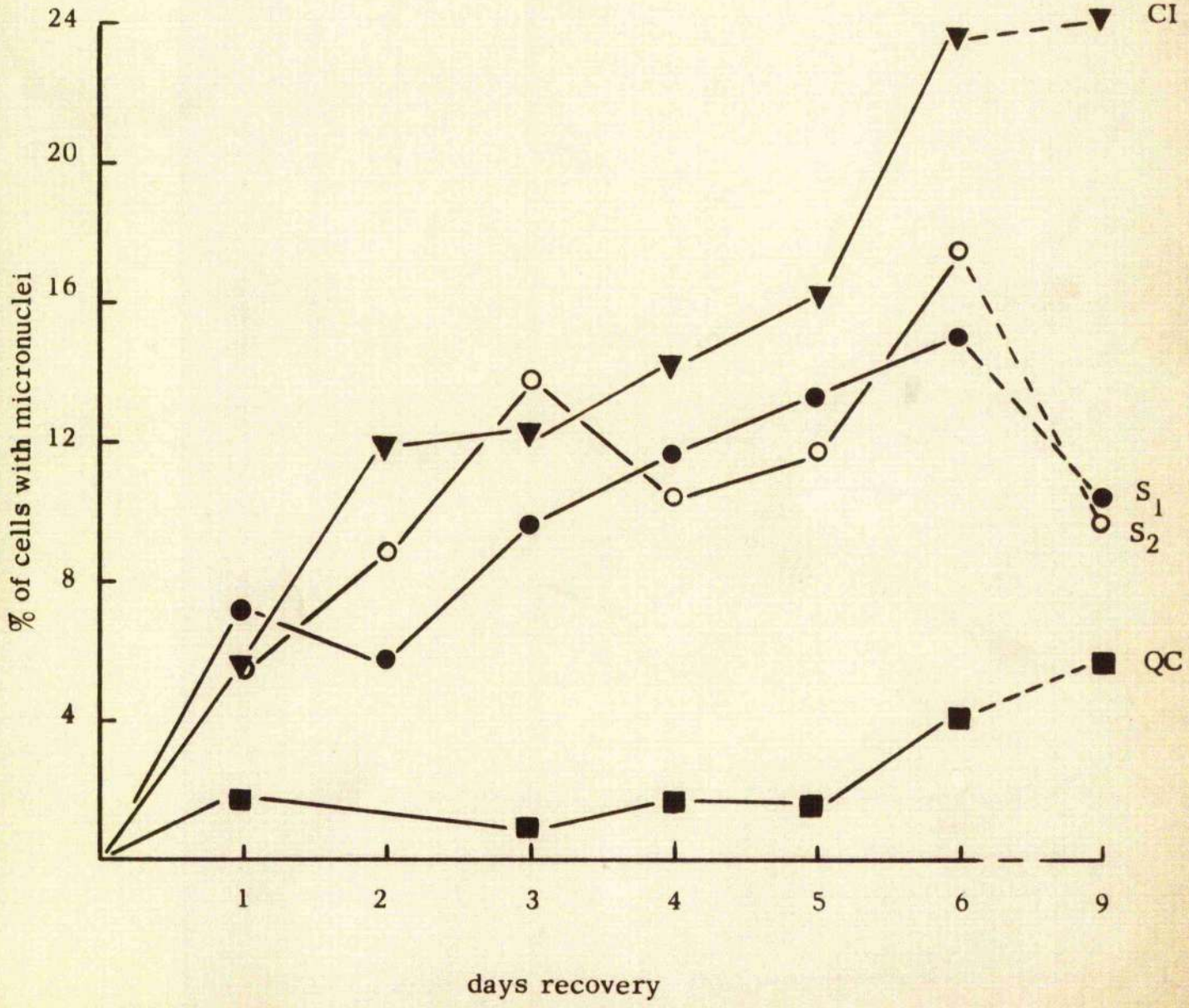


Figure 7

Percentages of cells with micronuclei in the root meristem of Allium sativum after exposure to 250 rads of x rays.

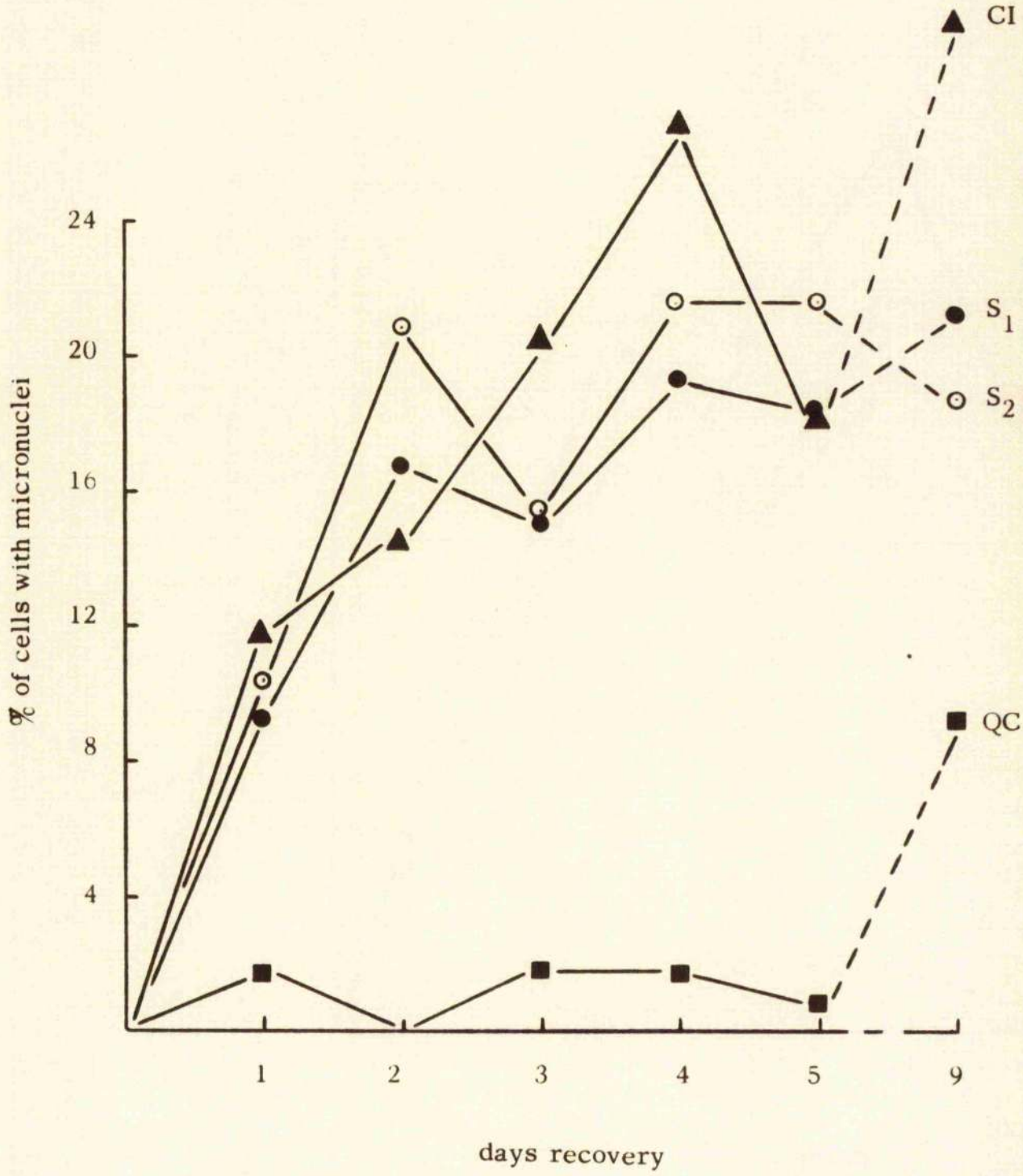
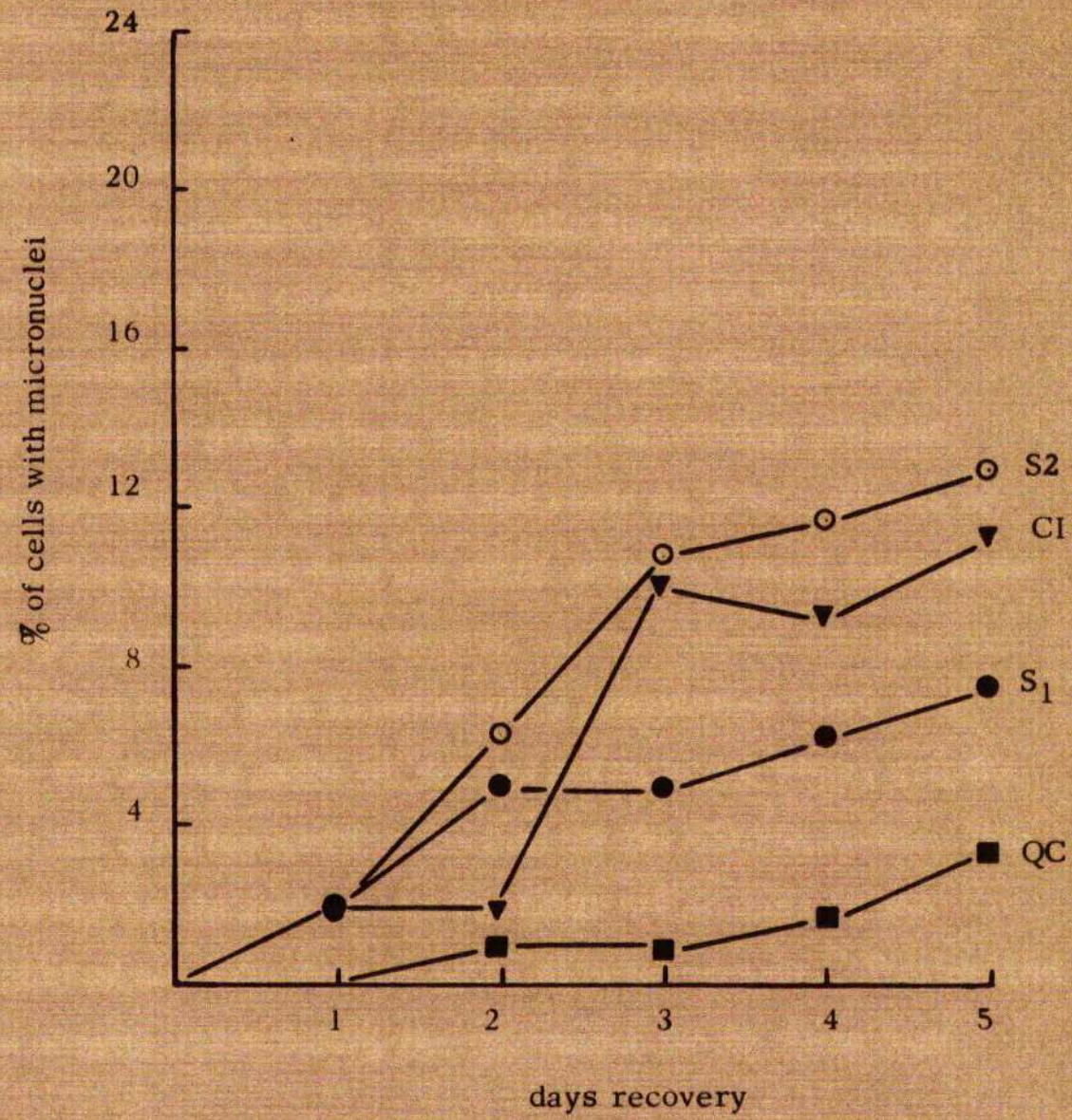


Figure 8

Percentage of cells with micronuclei in the root meristem of Allium sativum after exposure to 400 rads of x-rays.



a complete set of data for the roots irradiated with 600 rads, many meristematic cells died and others differentiated prematurely so that five days after irradiation over 50% of the roots sectioned were dead or no longer contained any meristematic cells. After 150 rads longitudinal growth of Allium roots is initially stimulated but then 2-3 days after irradiation the rate of elongation falls below that of the control roots. Irradiation with higher doeses of X-rays causes root elongation to be partially inhibited almost immediately and nearly to cease 4-5 days after irradiation. After nine days recovery some of the roots exposed to 150 and 250 rads show signs of recovery as cell elongation is resumed and derivative cells of the quiescent centre grow through the region previously occupied by the cap initials. In none of the roots is there evidence of regeneration occurring from any group of cells except derivatives of the quiescent centre.

Table 9 shows that 24 hours after irradiation there is a fall in the mitotic index of the cap initials and stele, and the greater the dose the greater the fall of the mitotic index. This is probably due in part to mitotic delay and in part to the inability of severely damaged cells to enter mitosis. Fluctuations in the mitotic index during the subsequent two or three days

suggest that there is partial synchrony of the cell cycle in the remaining meristematic cells caused by the delay of the first post-irradiation mitosis. In the cap initials 48 hours after irradiation with 150 rads the mitotic index is higher than in the control roots. Again 2 days after 250 rads the mitotic index in the cap initials is 8.8%, similar to that in the control roots, but twenty four hours later it is only 2.6% and yet, within a further twenty four hours, it is 10.2%, higher than in the control roots. In the stele although the fluctuations follow a similar pattern the peak mitotic indices after irradiation do not reach the levels found in control roots. However partial synchrony does not appear to persist beyond the initial few days. Many cells die and others differentiate prematurely; the mitotic index in the cap initials and stele stabilises at a low level about 5-6 days after irradiation suggesting that many cells are capable of completing one or two mitoses subsequently to irradiation but then cease to be meristematic.

In the quiescent centre the mitotic index is very low in the control roots and remains at low levels until there is a sharp rise a few days after irradiation. The delay between irradiation and this rise in the mitotic index increases with increase in X-ray dose. The mitotic index in the quiescent

centre becomes 8.3% three days after irradiation with 150 rads, 6.7% 4 days after 250 rads and 3.3% five days after 400 rads. Nine days after irradiation the mitotic index in the cap initials and stele is very low but in the quiescent centre and its derivatives it is still appreciably higher than in control roots.

The plots in Figs. 6-8 show that, as expected, more micronuclei are produced after exposure to the dose of 250 rads than after the 150 rad dose, but over the period of observation there are fewer cells with micronuclei after the 400 rad dose. This is probably due to the higher dose causing greater mitotic delay and more widespread cell death. The numbers of cells carrying micronuclei in the quiescent centre are consistently low after each dose, reflecting the relative insensitivity of quiescent centre cells to genetic damage after irradiation. The cells in the quiescent centre are relatively protected by the proportion of their cell cycle spent in G_1. That the percentage of cells with micronuclei in the cap initials after 150 and 250 rads is higher for most of the time than in the two regions of the stele suggests that the cap initials are the most sensitive region of the meristem with respect to chromosome aberrations. Similarly the areas under the curves for the stelar regions suggest that the stele cells further from the quiescent centre are on average slightly more sensitive than those closer to it.

This result is extremely interesting since the similarity of the rates of mitosis in the cap initials and stele led me to expect these three regions of the meristem to be equally sensitive to radiation. However the measurements of the length of G_1 obtained from pulse labelling (p. 50) for the cap initials of Allium is 4 hours as opposed to 5 hours for the two regions of the stele. It is probably this difference which accounts for the increased sensitivity of the cap initials to radiation damage. There is evidence (p. 53) that stelar cells near the quiescent centre divide slightly less frequently than cells 200μ from the quiescent centre but since G_1 and S, the next least sensitive stage of the mitotic cycle (Howard and Dewey 1960), are so similar in these regions this would not seem to explain their differences in radiosensitivity.

Thoday (1951) counting the total number of micronuclei produced after irradiation in squash preparations of whole root meristems of Vicia faba has found a single peak of micronuclei 1-2 days after irradiation which falls away as cells multiply without replication of the micronuclei and Hornsey, (1956) counting, as here, the number of cells with micronuclei in the whole meristem of Vicia, has found a similar single peak. But Clowes (1963 and 1964) has found that by examining small regions of the meristem of Zea and Vicia after X-irradiation with near semi-lethal doses

and smaller doses it is possible to indentify a double peak in the number of cells with micronuclei which is masked by examining the whole meristem. Clowes explains the double peak in terms of the differential sensitivity to mitotic delay of cells in G_1 and G_2 at the time of irradiation. Cells in G_2, delayed less than cells in G_1, come into division and form micronuclei at the end of their first post-irradiation mitosis. If these cells proceed to a second mitosis before G_1 cells enter their first (mitosis) there is a fall in the number of micronuclei before the second peak is reached when G_1 cells form micronuclei. The relative heights of the two peaks depend upon the relative lengths of G_1 and G_2 as well as their relative sensitivity.

Clowes' observations were made at daily intervals over a period of 10 days following the irradiations. The present data for Allium are not so extensive, but they do show that the organisation of the meristem of Allium is different from that in Zea and Vicia. Like those of Zea, the quiescent centre cells of Allium are markedly the least radiosensitive and also, as in Zea, the cap initials are the most sensitive though the duration of the mitotic cycle is not distinct from other regions of the meristem and is double that of Zea cap initials. However the stele cells just above the quiescent centre are more comparable

to those of Vicia, excepting the quiescent centre they are the least sensitive and yet they do not have an especially prolonged mitotic cycle time in relation to other regions of the meristem.

In the plot of cells with micronuclei in Allium after 250 rads the percentages correspond quite closely with those in Vicia during the initial 5 days after 360 rads (1964) except that the cap initials take the place of the stele cells 200μ from the quiescent centre. In the cap initials and the two regions of the stele the frequency of cells with micronuclei reaches a peak at 4 days and is again high at 9 days. Hornsey's counts of cells with micronuclei for whole meristems of Vicia are very low nine days after irradiation. So it is possible that after doses of X-rays near the semi-lethal level discrete regions of the meristem of Allium behave in a similar way to those of Zea and Vicia producing a double peak of micronuclei. But unfortunately here there is no indication of the extent of any of the dip in micronuclei frequency between 4 and 9 days after irradiation. This must be remedied. A point, certainly worth following up is, the time of production and frequency of micronuclei resulting from chromated and sub chromated breaks revealed at the second post-irradiation mitosis. I believe such micronuclei

may contribute considerably to the total number of micronuclei produced. Theoretically if these aberrations are as likely to yield micronuclei as chromosome breaks and 10% of cells are in prophase or metaphase at the time of irradiation then 10% of all micronuclei produced would come from these cells, but cells in mitosis are 10-15 times as sensitive to radiation damage as interphase cells. (Deschner and Sparrow, 1955 and Mitra, 1958). If there is no double peak in the occurrence of micronuclei like those in Zea and Vicia this would be due to the second mitosis of cells irradiated in G_2 occurring later in Allium than in Zea or Vicia. It would be due to differences in the behaviour of the micronuclei once formed, they would be absorbed less quickly or divide more frequently.

Discussion

There are intrinsic variations in the radio-sensitivity of cells during the mitotic and meiotic cell cycles. The relative sensitivity of the different phases of the cell cycle differs according to the criteria by which radiation damage is assessed. In plants if sensitivity is measured in terms of mitotic delay then cells in G_1 at the time of irradiation are the most sensitive since they are delayed the longest in reaching the first post-

irradiation mitosis (Neary, Evans and Tonkinson, 1959). However analyses of the frequency of chromosome aberrations in the first two mitoses after irradiation show that if radiation damage is considered in terms of chromosome breakage then cells irradiated in prophase or metaphase are more sensitive than cells irradiated in interphase and cells at the end of interphase (G_2) are considerably more sensitive than cells in early interphase (G_1). (Sparrow, 1951, Mitra, 1958, Bishop, 1950, Davidson, 1958).

The present results show that with respect to genetic damage the quiescent centre cells of Allium are on average less radiosensitive than other cells in the meristem. Cells in the two regions of the stele are equally radiosensitive while the cap initials are the most sensitive. These results, like those of Clowes for Zea (1963) and Vicia (1964), reflect the rates of division and in particular the proportion of the cell cycle spent in G_1 in the respective regions of the meristem. Although the rate of mitosis in the cap initials is similar to the rate of mitosis in cells of the stele the G_1 phase of the cycle is shorter and hence the cap initials are more sensitive.

Thus it has been established that the quiescent centre cells of Zea, Vicia and now Allium sativum are on average less

sensitive to genetic damage caused by X-irradiation than other cells in the meristem. Further this reduced sensitivity is related to the increased proportion of the cell cycle spent in G_1 - but what of the mechanism of protection inferred by the G_1 state? One of the factors influencing the radiosensitivity of the genetic material during the cell cycle is the oxygen tension of the cell at the time of irradiation. In general the dose required to give comparable radiation damage under anoxic conditions is 2 to 3 times the dose required under fully aerobic conditions. However a reduced oxygen tension in quiescent centre cells does not account for their relative insensitivity to radiation damage and their ability to initiate root recovery. Hall, Lajtha and Clowes (1962) have shown that the lethal effect of high doses of X-rays on Vicia faba roots is depressed if the roots are irradiated under anoxic conditions. This would not be possible if quiescent centre cells were already protected by anoxia. The sensitivity of cells may also vary during the cell cycle because of changes in the level of intracellular substances such as thiol proteins which act as radical scavengers (Bacq and Alexander 1961). Stern (1958 and 1959) has shown that in the mitotic and meiotic cells of Trillium and Lilium anthers the

concentration of acid soluble -SH and SS falls markedly as the cell proceeds from interphase through division. Changes in the level of thiol compounds during the cell cycle are such as to render interphase cells less sensitive to irradiation than cells dividing actively at the time of irradiation. But there is no evidence to suggest that G_1 cells are protected more effectively than G_2 cells by the presence of such compounds.

Experiments with fractionated doses of X-rays show that once quiescent centre cells enter mitosis they are as sensitive to a further irradiation as any other cells of the meristem (Hall, Lajtha and Clowes, 1962). This means that the protection afforded quiescent centre cells is a direct consequence of their normal metabolic state rather than any inherent genetical property. It is well known that changes in chromosome structure and the DNA - protein content of the cell not only influence the types of chromosome aberration induced but also influence the resulting frequencies of these aberrations. In tetraploid cells the observed chromatid interchange frequency is doubled relative to diploid cells (Evans, 1961). Similarly in a diploid cell DNA synthesis and chromosome replication lead to a substantial

increase in chromatid interchange (Revell, 1960). Cells are less sensitive to genetic damage before DNA synthesis and this seems to be for two reasons; firstly, 2c nuclei offer smaller targets to impending radiations and secondly and perhaps more important, breaks induced in un-duplicated chromosomes frequently restitute, an occurrence enhanced by mitotic delay (Wolff, 1961). Thus on present evidence these two facts offer the only acceptable explanation of the relative resistance of quiescent centre cells to X-ray induced genetic damage; but is this the whole answer?

Accumulations of metaphases and rates of mitoses after X-irradiation of *Allium sativum* roots.

Introduction.

Exposure of roots to acute doses of X-rays or brief treatments with colchicine causes inhibition of cell elongation and changes in the chromosome complements and rates of mitosis of meristematic cells. Some of these changes affect the pattern of differentiation and some may result in cell death. Thus after X-irradiation or colchicine treatment there is inhibition of longitudinal growth, disruption of organisation and reduction in the size of the apical meristem. Nevertheless roots frequently recover. Davidson (1961) has concluded from experiments with Vicia that during recovery from X-ray or colchicine treatment a new multicellular primordium is formed within the damaged meristem and that elongation of cells proximal to this primordium initiates regeneration by carrying the new group of apical cells forward out of the old meristem. Analysis of cells with changed complements in regenerating roots indicates that cells contributing to the new primordium are derived from stock relatively resistant to and unchanged by the experimental treatment. In *Vicia* and in

Zea quiescent centre cells come into division a few days after X-irradiation (Clowes 1962 and 1963) and Clowes has demonstrated that it is derivatives of quiescent centre cells which contribute the cell population of the new primordium from which root growth is resumed. Investigations reported here show that quiescent centre cells of Allium sativum are also stimulated to enter mitosis after X-irradiation or brief treatment with colchicine. Quiescent centre cells, being less radiosensitive than other cells in the meristem (Clowes 1963) are better able to maintain their reproductive integrity and so, through cell lineages initiated by the new promeristem, provide the regenerating root with a high proportion of normal diploid cells.

Thus the broad outline of the mechanism of root recovery from exposure to colchicine or X-rays involves the establishment of a new primordium and the resumption of cell elongation, but what of the causal sequence and relative importance of events leading to recovery? Davidson (1961) considers that regaining the ability to promote cell elongation is a basic step in the regeneration of roots following irradiation. Treatment of Vicia roots with doses of X-rays or colchicine that lead to the cessation of longitudinal growth do not entirely inhibit mitosis.

This suggests that it is inhibition of cell elongation rather than inhibition of mitosis that is the major factor in preventing longitudinal root growth. However, inhibition of polar growth in the zone of elongation follows irradiation of cells in the meristem, not cells in the zone of elongation; the expression of the effect of irradiation is remote from the site of induction. (Gray and Scholes, 1951; Gray and Boag quoted Howard and Pelc, 1953). Thus cell elongation and meristematic activity may be inter-dependent aspects of cell behaviour. There is evidence that cell elongation in Vicia roots occurs along a concentration gradient of indole acetic acid (IAA) (Audus, 1960) and, under normal conditions, the auxin (IAA) concentration of the root is at a maximum at the root apex (Leopold, 1955) while IAA oxidase activity is at a minimum (Galston and Dalberg, 1954). Branching roots provide evidence that each root has autonomous control of its axis of elongation (Davidson, 1961). For these reasons and because of the effect of IAA on the differentiation of vascular tissue in regenerating decapitated roots Torrey (1957) and Davidson (1960) have suggested that the meristem is the site of IAA synthesis. This suggestion is supported by the work of Gordon on the metabolism of IAA in pea roots after irradiation. Following low doses of X-rays the auxin concentration at the

root apex falls (Gordon, 1955) because irradiation inhibits IAA synthesis, since the enzyme catalysing the conversion IAc (indole acetate) ^ —> IAA is extremely radiosensitive, though IAA itself is relatively stable (Gordon, 1954). The fall in auxin concentration at the root apex following X-irradiation is solely attributable to an increase in the destruction or utilisation of IAA (Gordon, 1957). Davidson (1961) suggests that, in regeneration of roots after irradiation, meristematic activity of the new primordium results in the stimulation of the elongation of cells basal to the primordium.

Clowes has been more interested in the other aspect of root regeneration, the part played by the quiescent centre. Here the important questions are: what ,maintains the normal metabolic inactivity of quiescent centre cells? and what, after irradiation stimulates these cells to meristematic activity? Measuring rates of mitosis after irradiation by accumulation of metaphases Clowes has correlated high rates in the quiescent centre with low rates elsewhere in the meristems of Zea and Vicia. But Hornsey (1956) assessing the length of the mitotic cycle after irradiation by the incidence of micronuclei following a second dose of X-rays obtains results indicating that three days after

irradiation of Vicia roots with 130 rads any post-irradiation delay in the mitotic cycle has disappeared and rates of mitosis are equivalent to those in unirradiated roots. Clowes has suggested that an explanation of the apparent discrepancy in these findings lies in the fact that since Hornsey's estimate is for the whole meristem her result is more nearly comparable with Clowes' value for stele cells 250 μ from the quiescent centre. Clowes finds that rates of mitosis in these cells are least affected by irradiation. However, Neary, Evans and Tonkinson (1958), having modified their method of calculating rates of mitosis from rates of accumulation of metaphases determined radiation-induced mitotic delay in meristem cells of Vicia roots and their results indicate that, 48 hours after irradiation with low doses of gamma rays from a ^{60}Co source, rates of mitosis are approaching levels found in un-irradiated control roots.

The following experiments were conducted to investigate the behaviour of Allium sativum root meristems after X-irradiation.

Method

Allium roots were grown in the dark until they were 1-2 cms. long, three quarters of them were then exposed to 250 rads of

X-rays in the manner described in Section 2 p. 6 . Samples of both the irradiated roots and the control roots were fixed in FPA within 30 mins. of the irradiations. The rest of the control roots and about a third of the irradiated roots were transferred to aerated 0.05% aqueous colchicine solution and samples of roots were fixed at 2 hourly intervals for 8 hours. The other irradiated roots were grown on for 3 days in aerated tap water, on the third day half the roots were treated with colchicine and fixed as before and on the fourth day the rest were treated similarly. The roots were sectioned longitudinally and stained in leuco-basic fuchsin and fast green. The number of cells in different stages of mitosis were counted for the different regions of the meristem in the median sections of ten roots per fixation and the rates of mitosis were calculated from the rates of accumulation of metaphases as in Section 4. The counts are given in Table 10 and the accumulations of metaphases are plotted in Figs 9, 10, 11 and 12

Results

The plot of percentages of metaphases in the unirradiated control roots (Fig. 9) shows that in all regions of the meristem the rate of metaphase accumulation was reasonably consistent

TABLE 10

Percentages of cells in different stages of mitosis in unirradiated control roots after colchicine treatment.

	hours in colchicine	prophases	metaphases	anaphases + telophases	inter-phases	MI
cap initials	0	4.5	1.1 ± 0.6	1.9	92.5	7.5
	2	4.9	3.9 ± 1.1	0.5	90.6	9.4
	4	2.3	10.7 ± 1.7	0	87.0	13.0
	6	6.6	13.1 ± 1.7	0	80.3	19.7
	8	4.9	16.2 ± 3.5	0	78.9	21.1
quiescent centre	0	4.0	0 ± 0	0	96.0	4.0
	2	4.6	2.3 ± 1.1	0	93.1	6.9
	4	0	3.3 ± 1.2	0	96.7	3.3
	6	2.4	4.8 ± 1.7	0	92.7	7.3
	8	1.6	4.9 ± 1.7	0	93.4	6.6

stele just above quiescent centre	0	6.6	1.6 ± 0.5	4.3	87.5	12.5
	2	5.2	6.0 ± 1.4	0.4	88.4	11.6
	4	4.0	8.8 ± 2.2	0	87.1	12.9
	6	3.2	14.4 ± 0.9	0	82.4	17.6
	8	4.8	21.0 ± 2.3	0	74.2	25.8
stele 200 μ above quiescent centre	0	4.0	2.6 ± 0.8	3.5	89.2	10.1
	2	7.2	5.3 ± 1.3	1.0	86.5	13.5
	4	4.3	13.5 ± 1.1	0	82.3	17.7
	6	8.4	15.9 ± 1.3	0	75.7	24.3
	8	5.5	27.7 ± 3.0	0	66.8	33.2

values for median sections of roots per treatment.

TABLE 10 continued

Percentages of cells in different stages of mitosis after colchicine treatment

30 minutes after exposure to 250 rads

	hours in colchicine	prophase	metaphase	anaphase + metaphases	interphase	MI
cap initials	0	13.1	2.5	8.1	76.3	23.7
	2	5.0	5.0	0	90.0	10.0
	4	0.8	3.1	0	96.1	3.9
	6	1.2	1.2	0	97.6	2.4
	8	0	0	0	100	0
quiescent centre	0	9.2	1.7	4.2	84.9	15.1
	2	6.7	0	0.8	92.5	7.5
	4	0	0	0	100	0
	6	2.5	0.8	0	96.7	3.3
	8	0	0	0	100	0

stele just above quiescent centre	0	7.1	2.1	4.2	86.6	13.4
	2	5.0	2.9	0	92.1	7.9
	4	2.1	5.0	0	92.9	7.1
	6	6.4	3.3	0	90.3	9.7
	8	0.8	2.1	0	97.1	2.9
stele 200 above quiescent centre	0	10.0	2.1	2.9	75.0	15.0
	2	5.8	2.5	0	91.7	8.3
	4	2.9	4.2	0	92.9	7.1
	6	2.9	5.8	0	91.3	8.7
	8	1.6	2.9	0	95.5	4.5

TABLE 10 continued

Percentages of cells in different stages of mitosis after colchicine treatment

3 days after exposure to 250 rads X-rays.

	hours in colchicine	prophases	metaphases	anaphases	inter-phases	MI
cap initials	0	5.6	1.3	0.6	92.5	7.5
	2	6.8	1.3	0	91.9	8.1
	4	4.4	10.6	0	85.0	15.0
	6	3.8	11.8	0	84.4	15.6
quiescent centre	0	5.8	1.7	0.8	91.7	8.3
	2	5.0	3.7	1.7	90.0	10
	4	4.2	13.3	0	82.5	17.5
	6	6.6	17.5	0	75.8	24.2

stele just above quiescent centre	0	4.6	2.1	0.4	92.9	7.1
	2	4.1	3.0	0	92.9	7.1
	4	2.5	5.0		92.5	7.5
	6	4.6	12.0	0	83.4	16.6
stele 200 μ above quiescent centre	0	8.8	0.4	2.1	88.7	11.3
	2	3.7	3.7	0	92.5	7.5
	4	1.7	10.8	0	87.5	12.5
	6	5.0	10.4	0	84.6	15.4

(values for median sections often roots per section)

TABLE 10 continued

Percentages of cells in different stages of mitosis after colchicine treatment 4 days after exposure to 250 rads X-rays.

	hours in colchicine	prophases	metaphases	anaphase + telophases	inter-phases	MI
cap initials	0	3.5	1.3 ± 1.1	1.9	92.9	7.1
	2	1.9	2.7 ± 1.4	0	95.4	4.6
	4	4.1	6.8 ± 2.0	0	89.1	10.9
	6	0	6.3	0	93.7	6.3
	8	7.5	7.5	0	75.0	15.0
quiescent centre	0	7.0	1.7 ± 1.0	3.5	87.8	12.2
	2	4.0	4.0 ± 2.4	0	92.0	8.0
	4	2.8	7.3 ± 1.7	0	89.9	10.1
	6	4.2	15.9	0	79.9	20.1
	8	4.1	21.7	0	74.2	25.8

stele just above quiescent centre	0	6.3	3.7 ± 1.0	1.6	88.4	11.6
	2	8.2	6.8 ± 1.8	0	85.0	15.0
	4	3.6	11.7 ± 1.6	0	84.7	15.3
	6	6.0	10.3	0	83.7	16.3
	8	2.0	12.1	0	85.9	14.1
stele 200 μ above quiescent centre	0	7.4	3.4 ± 1.1	0.6	88.6	11.4
	2	7.4	4.9 ± 1.1	0	87.7	12.3
	4	3.1	10.5 ± 1.6	0	86.7	13.3
	6	1.1	15.6	0	83.3	16.7
	8	6.7	11.7	0	81.6	18.4

Figure 9

Accumulation of metaphases in unirradiated control root apices of Allium sativum

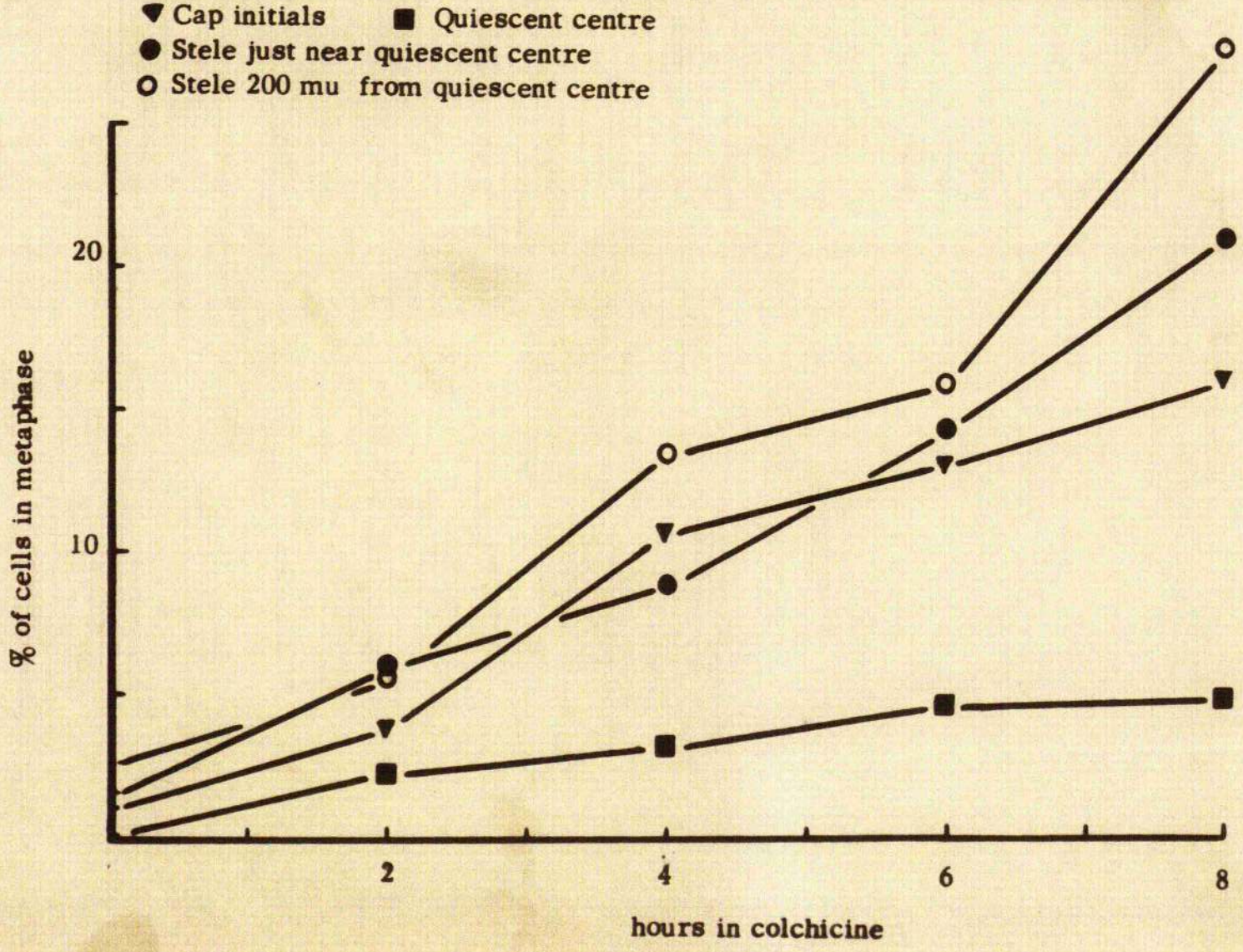


Figure 10

Accumulation of metaphase in the root meristem of Allium sativum 30 minutes after exposure to 250 rads

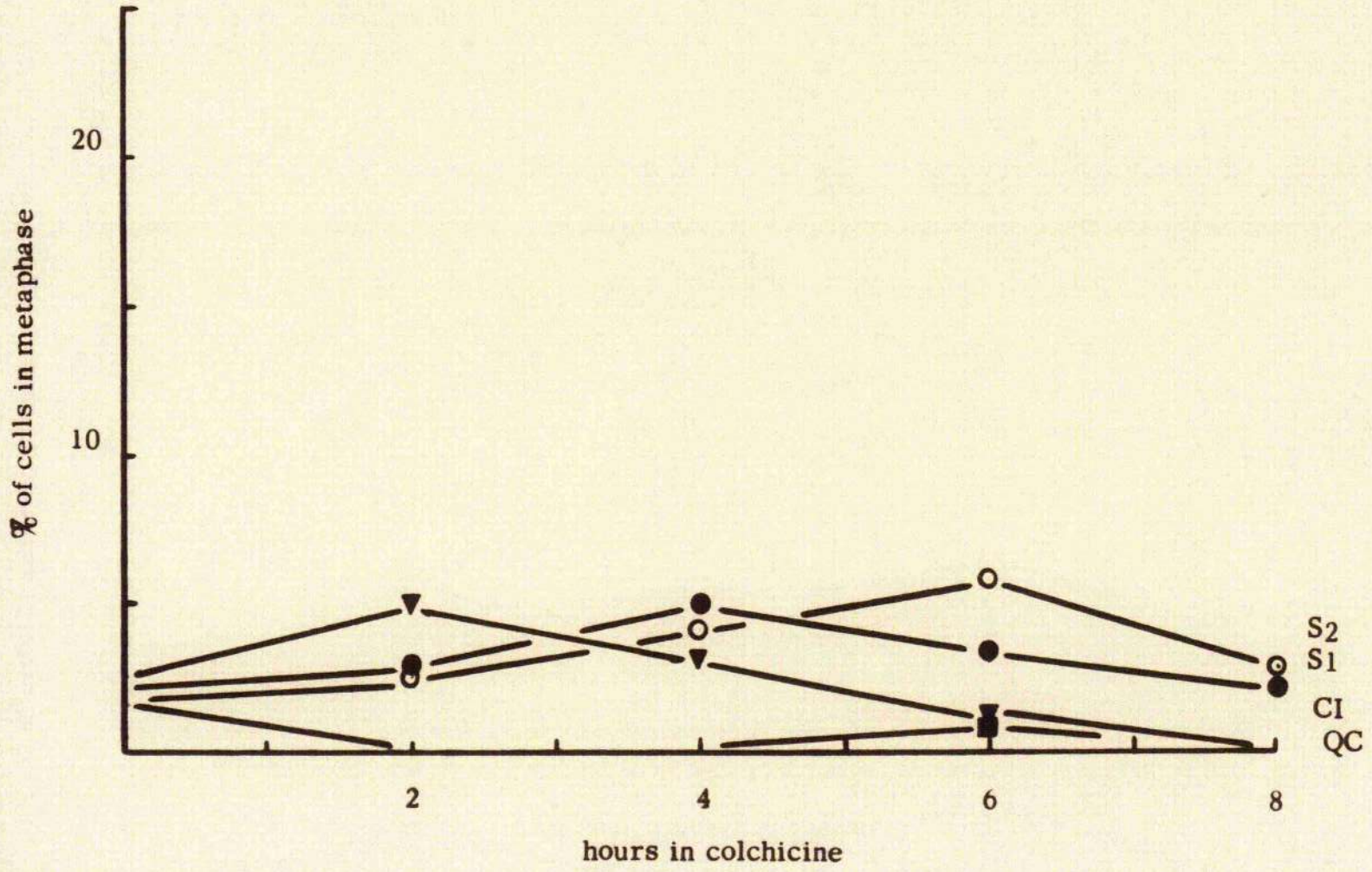


Figure 11

Metaphase accumulation in Allium sativum root apices 3 days after exposure to 250 rads of X-rays.

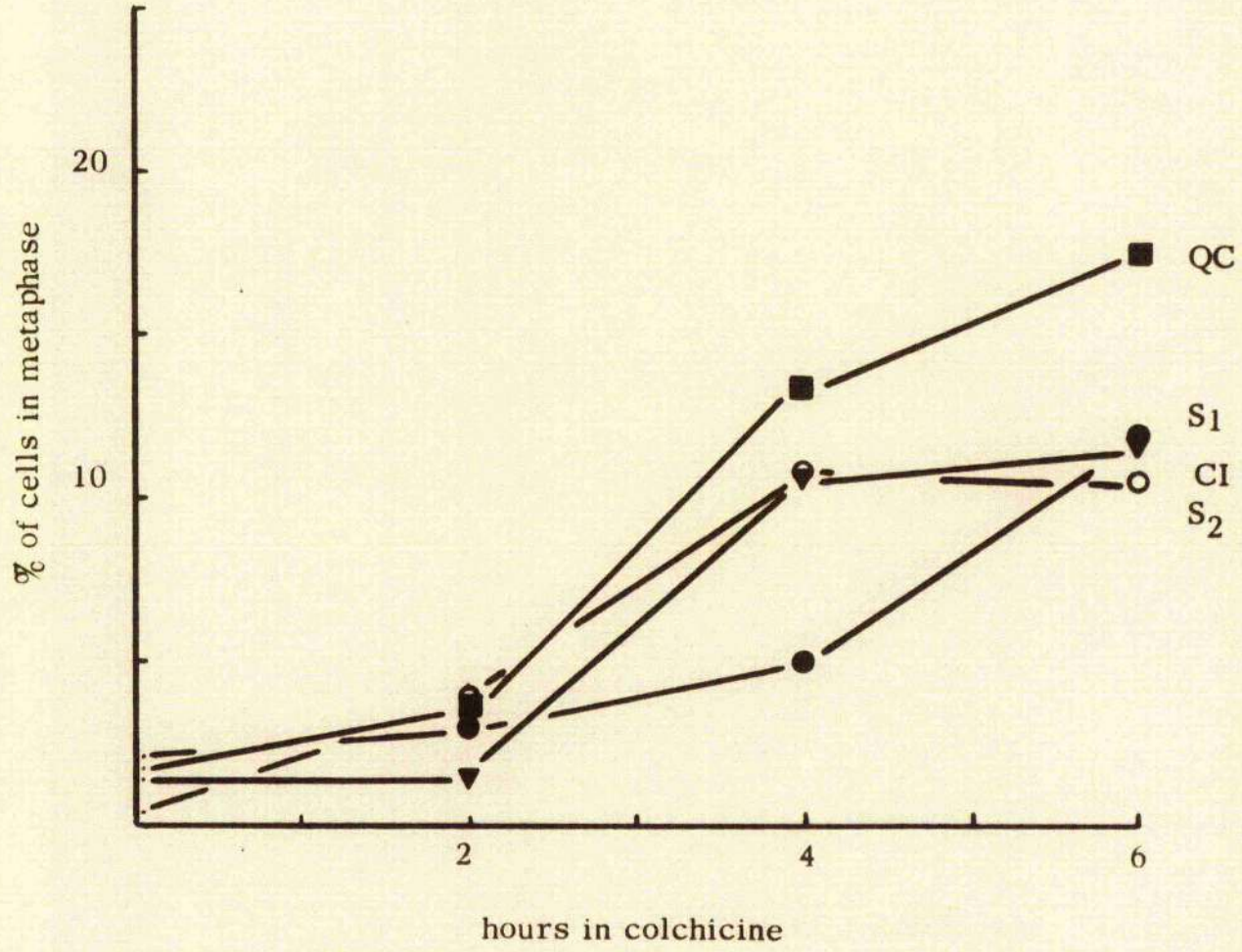


Figure 12

Accumulation of metaphase in Allium sativum roots 4 days after exposure to 250 rads of X-rays

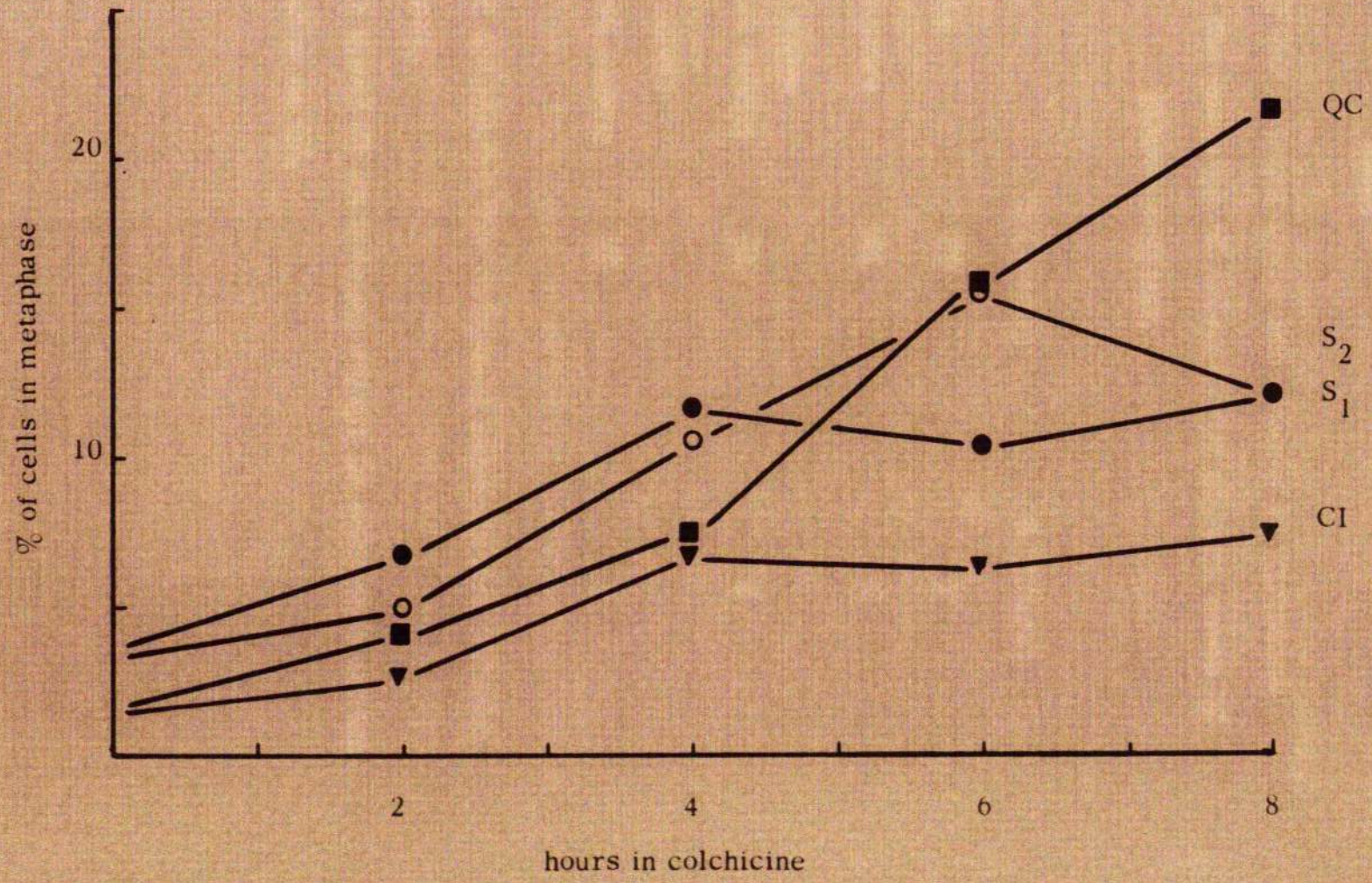


TABLE II

Duration of the mitotic cycle and mitosis in different regions of the meristem of unirradiated control roots of *Allium sativum*.

	T (hours)	t (hours)
cap initials	27	3
quiescent centre	381	6
stele just above quiescent centre	31	6
stele 200 μ above quiescent centre	23	3

Values of T calculated for metaphase accumulation between 2 and 8 hours.

Values of t based MI at 0 hours except in quiescent centre where MI taken as 1%.

TABLE 12

Duration of the mitotic cycle and mitosis in Allium sativum roots 3 and 4 days after irradiation with 250 rads.

	3 days	4 days
	T	T
cap initials	29	a) 176 b) 34
quiescent centre	22	a) 27 b) 43
stele just above quiescent centre	33	a) 68 b) 31
stele 200μ above quiescent centre	43	a) 66 b) 13

Values of T after 3 days based on metaphase accumulation between 2 and 6 hours. Values a) and b) of T after 4 days based on metaphase accumulation between 2 and 8 hours and 2 and 4 hours respectively.

throughout the 8-hour period of exposure to colchicine. The rates of mitosis calculated from these results (Table 11) correspond quite closely with those previously obtained for Allium sativum roots (Table 2) but even though the two sets of roots were grown under similar conditions no special precautions were taken to standardise conditions and the roots came from different cloves. However, the similarity of the two sets of results indicates that the rate of accumulation of metaphases by colchicine treatment provides a reliable method of calculating rates of mitosis in Allium roots.

In normal roots there is equivalent accumulation of metaphases over similar short time periods throughout an 8-hour colchicine treatment indicating that cells enter mitosis at a steady rate and proceed through the cell cycle in sequential order. This means that under normal conditions the meristem comprises an asynchronous cell population since in meristems with partially synchronous cell cycles there would be periods of no metaphase accumulation corresponding with periods of no entry into mitosis.

A totally different picture is presented by roots treated with colchicine half an hour after irradiation with 250 rads. First there are striking changes in the initial mitotic indices of the cap initials and the quiescent centre. These are more than

three times those in the control roots. Values for stelar cells are also higher than those in the control roots, but not as markedly so as in the cap initials and quiescent centre. Other workers have found similar rises in mitotic indices following irradiation of asynchronous cell population; in Vicia faba roots (Deufel, 1961) and in Trillium roots (Darlington and La Cour, 1945). In a more detailed analysis of the cells in mitosis in whole meristems of Vicia roots after irradiation Neary, Evans and Tonkinson (1958) have shown that, as here, the increase in mitotic index is largely attributable to an increase in the occurrence of prophases. However, in their results the peak in mitotic index, exceeding that in control roots, occurs about 2 hours after irradiation, rather later than in Allium after 250 rads. After the 2 hour peak the mitotic index in Vicia falls away to a minimum about 5-6 hours before recovery to near normal values 48 hours after irradiation. In the present results there is a dramatic fall from the initial mitotic index even though the cells are exposed to colchicine. Neary et al. find that their rise in prophases between 1 and 2 hours is reflected in a peak in metaphase accumulation 3¾ hours after irradiation. The present results show no obvious peak in metaphase accumulation at 2 or 4 hours which could be associated with these earlier high numbers

of prophase cells. This is perhaps due to cells in a transitory peak of prophases passing into metaphase before the colchicine took effect in preventing spindle formation: with 0.05% colchicine Vicia root cells are not arrested at metaphase until about 34 minutes after treatment (Evans, Neary and Tonkinson, 1957).

Neary et al. discount the possibility of the initial increase in mitotic index being due to chromosome stickiness since the percentage of prophases as well as of later stages of mitosis is increased. They attribute the increase in prophases to a release of cells blocked by irradiation at the end of interphase for about half an hour and the increase in later stages of mitosis to a wave of stimulation induced in cells irradiated in early prophase causing them to reach metaphase more quickly than usual. Neary et al. based this explanation of their results on measurements of mitotic delay at different times after irradiation calculated from data obtained by comparing metaphase accumulation over discrete 2 hourly periods of exposure to colchicine with accumulation of metaphases in control roots. It is possible that with different time parameters (these must depend on species, dose etc.) a pattern of behaviour after irradiation such as described for Vicia could account for the observed peak in mitosis after X-irradiation of Allium. However, one difficulty in

interpreting the present results that would not be explained by the pattern of behaviour in *Vicia* concerns the quiescent centre cells. Under normal conditions about nine tenths of quiescent centre cells are held at G_1, about half the remainder are in S and the rest in G_2 or M. If the value of 9.2% for prophases in the quiescent centre half an hour after irradiation, an increase of 5.2% over the value in control roots is not anomalous, how do so many cells reach mitosis in 30 mins if irradiation induces a block of entry to prophase from G_2? Do quiescent centre cells irradiated in S and G_2 behave differently from other meristematic cells irradiated at these stages of the mitotic cycle or could they enter mitosis without syntherising DNA and form 1C but diploid nuclei?.

For the doses used in their experiment Neary *et al.* were able to assume that no cell failed by early death or other disorder to complete mitosis or indeed to enter a second mitosis. However, in *Allium* roots there is evidence of cell death, chromosome stickiness, early restitution and other mitotic abnormalities within 4 hours of irradiation with 250 rads. This reduction in the number of cells capable of completing mitosis is reflected in the fall in the number of metaphases in all regions of the meristem during exposure to

colchicine (Fig. 10 p. 114). The results show differences in the behaviour of cells in different parts of the meristem. In the cap initials, on average the most radiosensitive cells in the meristem, the loss of cells from mitosis exceeds entry into metaphase only 2 hours after irradiation despite the presence of colchicine and, eight hours after irradiation, there are no cap initials in mitosis.

The mitotic indices and the number of metaphases in Allium roots 3 and 4 days after irradiation (Tables 10 and 10+) indicate that active meristematic cells are not entering mitosis at a steady rate. The plots of the percentages of metaphases during colchicine treatment 3 and 4 days after irradiation (Fig. 11 and 12) show that in the quiescent centre there is a fairly steady accumulation of metaphases over the 8 hour period of exposure, but, in other regions of the meristem, metaphase accumulation is irregular. The plots for the cap initials and stele are reminiscent of those for the partially synchronous meristem of Sinapis alba (Clowes 1962). In the cap initials and stele just above the quiescent centre there is effectively no metaphase accumulation between 4 and 8 hours exposure to colchicine 4 days after irradiation.

The regularity of metaphase accumulation in the quiescent centre suggests that these cells are dividing asynchronously and

calculated rates of mitosis show that they are dividing once every 20-30 hours (Tables 12 and 12+). The rate of mitosis in quiescent centre cells is about 10 times faster than in control roots. Calculations of the duration of the mitotic cycle in other regions of the meristem based on these metaphase accumulations suggest that there might be partial synchrony of cell cycles (Table 10). If the duration of the mitotic cycle in the cap initials 4 days after irradiation is calculated from metaphase accumulation between 2 and 4 hours a value of 34 hours is obtained, but, if the net# metaphase accumulation between 2 and 8 hours is used the value of T is 176 hours. Here the lack of metaphase accumulation between 4 and 8 hours cannot be entirely due to loss of cells to interphase or an increased sensitivity to toxic effects of colchicine since, in the cap initials 3 days after irradiation, there is no accumulation of metaphases during the first two hours of exposure to colchicine; neither are cells delayed in prophase. Genuine synchrony of the cell cycle is further suggested by the value of 13 hours for T in the stele cells 200 μ from the quiescent centre 4 days after irradiation calculated from metaphase accumulation between 2 and 4 hours. This value is about half that for control roots and provides good evidence that irregular metaphase accumulation in

Allium roots 3 to 4 days after irradiation is not due to the intermittent passage of asynchronous cells through mitosis such as would occur if blocks of cells were killed in particular phases of the cell cycle while the behaviour of the remaining cells was left undisturbed. The results do not show whether irregularity in metaphase accumulation is due to wide variations in the rates of mitosis of individual cells or partial synchrony of the cell cycle.

Discussion

Metaphase accumulation is irregular in samples of the cell populations of ten root meristems of Allium sativum 3 or 4 days after irradiation with 250 rads. In the present experiments insufficient roots have been scored to decide whether this irregularity is due to sampling errors, large variations in the mitotic cycles of individual cells or partial synchrony of cell cycles. It has been shown that accumulation of metaphases in Allium a few days after irradiation over longer periods of exposure to colchicine (2-8 hours) give values for greatly depressed rates of mitosis in all regions of the meristem except the quiescent centre. These values are comparable with those for Vicia (Clowes and Hall 1962) and Zea (Clowes 1963). Rates of mitosis after

irradiation in Zea and Vicia (Clowes and Hall 1962) are based on metaphase accumulations between two fixations and it was assumed that partial synchrony of cell cycles induced by the irradiation had disappeared by the time of colchicine treatment. However, metaphase accumulation figures for Allium over short periods of exposure to colchicine give values of T similar to or even smaller than those found in unirradiated control roots. Thus from the present results it is not possible to measure the average rates of mitosis in different regions of the meristem with any confidence. Not only is there the difficulty involved with possible partial synchrony of the cell cycles but there is the problem of defining the meristem after irradiation. The present method gives a measure of the average rate of mitosis in the cells which constituted the meristem immediately prior to irradiation and their derivatives. However, many of these cells are no longer capable of meristematic activity and no allowance for such cells is made in these calculations or in those for Zea or Vicia since all interphase cells contribute to lowering the percentage of metaphases. There is no empirical data on the reduction in the size of the effective meristem of roots after irradiation but it has been suggested by Gray and Scholes (1959) that about 50% of cells in the meristem of Vicia die after a

dose of about 130 rads, this estimate seems high, but certainly in Allium roots after 250 rads many cells do become meristematicly ineffective either by death or by differentiation. Thus death or differentiation of cells in the meristem of Allium after 250 rads add to the difficulties of assessing rates of mitosis in discrete regions of the meristem other than the quiescent centre by an unmodified metaphase accumulation method.

However, these experiments show that a few days after irradiation, rates of mitosis in the quiescent centre rise to levels similar to those found in other regions of the meristem in unirradiated control roots and while in these regions cell cycles may be partially synchronous, in the quiescent centre cells certainly appear to be dividing asynchronously. This is another aspect of cell behaviour less affected by irradiation in the quiescent centre than elsewhere in the meristem. It is possible, that in cells of other parts of the meristem which survive irradiation to continue active division, the length of the mitotic cycle 3 days after irradiation is similar to that found in unirradiated roots as Hornsey's results for Vicia (1956) suggest. If this is so, although the 3 day peak of mitosis in the quiescent centre is a reflection of the radiation-induced mitotic delay of cells in G_1 at the time of irradiation the continued meristematic

activity of quiescent centre cells may be associated with the reduction in the size of the meristem rather than reduction in the rates of mitosis of cells in the rest of the meristem, but the present results do not allow such distinctions to be made.

SUMMARY.

1. The root meristem of Allium sativum has been described from longitudinal and transverse sections of the root apex. No evidence for the presence of a quiescent centre can be gained from a Korper - Kappe analysis of cell patterns.

2. The presence at the pole of the stele of a quiescent centre comprising 30 - 50 cells has been demonstrated by autoradiographs of roots fed with tritiated thymidine.

3. Rates of mitosis in different regions of the root meristem have been measured by three independent methods:
   a) metaphase accumulation
   b) continuous labelling
   c) pulse labelling
   The rsults of the three methods are consistent and show that the rate of mitosis in the quiescent centre is of the order of ten times that in other parts of the meristem.

4. The duration of the different phases of the mitotic cycle have been measured for various regions of the meristem by a pulse labelling technique. It has been found that the time spent in mitosis and the later stages of interphase is approximately similar in all regions of the meristem. The extent of the

mitotic cycle in quiescent centre cells is due to the length of time spent in G_1 the period of interphase prior to DNA synthesis. Quiescent centre cells contain the 2C amount of DNA during approximately 90% of their cell cycle.

5. It has been found that during recovery from colchicine treatment the mitotic index in the quiescent centre rises while it falls in other regions of the meristem. The cells forming the primordium from which the root regenerates after colchicine treatment are derivatives of quiescent centre cells.

6. Using the number of cells carrying micronuclei as an estimate of radiation damage the radiosensitivity of different regions of the root meristem of Allium Sativum have been investigated after various doses of X-rays. It has been found that the cap initials are the most radiosensitive and quiescent centre cells by far the least sensitive. The ~~derivation~~ duration of G_1 is shorter in the cap initial than elsewhere in the meristem while it is longest in the quiescent centre. Cells from the two regions of the stele are approximately equally seasitive, the average values of G_1 in these two regions are the same.

7. Rates of mitosis in the different regions of the meristem have been measured by metaphase accumulation at various times after irradiation with 250 rads of X-rays. In the quiescent centre three or four days after irradiation the rate of mitosis is greater than in the other regions of the root being ten times that found in the quiescent centre of unirradiated control roots. Average rates of mitosis in the rest of the meristem are considerably reduced.

BIBLIOGRAPHY

Bacq, Z. M. and Alexander, P. (1961) Fundamentals of radiobiology. Pergamon Press, London.

Bergonié, J and Tribondeau, L. (1906) Interpretation de quelques résultats de le radiothérapie et essai de fixation d'une technique rationelle. C. R. Acad. Sci. Paris 143, 983-985

Bishop, C. J. (1950) Differential sensitivity of Tradescantia chromosomes during the mitotic cycle. Genetics 35, 175

Bond, V. P. (1959) Sensitivity of different cells in the same organism. in Radiobiology at the intra- cellular level. p. 55 Pergamon Press, London.

Brown, R. (1951) The effects of temperature on the different stages of cell division in the root-tip. J. exp. Bot. 2, 96-110

Brown, R. and Rickless, P. (1949) A new method for the study of cell division and cell extension with some preliminary observations on the effect of temperature and of nutrients. Proc. roy. Soc. B. 136, 110-125.

Brumfield, R. T. (1943) Cell-lineage studies in the root meristems by means of chromosome rearrangements induced by X-rays. Amer. J. Bot. 30, 101-109.

Clowes, F. A. L .(1950) Root apical meristems of Fagus sylvatica. New Phytol. 49, 248-268.

(1953) The cytogenerative centre in roots with broad columellas. New Phytol. 52, 48-57.

(1956) Localisation of nucleic acid synthesis in root meristems. J. exp. Bot. 7, 307-312.

(1961) Duration of the mitotic cycle in a meristem. J. exp. Bot. 12, 283- 294.

Clowes, F. A. L. (1961) Apical meristems. Blackwell Scientific Publications, Oxford.

(1962) Rates of mitosis in a partially synchronous meristem. New Phytol. 61, 111-118

(1963) The quiescent centre in meristems and its behaviour after irradiation. Brookhaven Symp. Biol. 15, 46

(1965) The duration of the G_1 phase of the mitotic cycle and its relation to radiosensitivity. New Phytol. 64, 355.

(1965) Synchronization within a meristem by 5-amino-uracil J. exp. Bot. 16, 581- 586.

Clowes, F.A. L. and Hall, E. J. (1962) The quiescent centre in root meristems of Vicia faba and its behaviour after acute ~~X~~radiation and chronic gamma irradiation. Rad. Bot. 3, 45-53

D'Amato, F. and Avanzi, M. G. (1948) Reazioni di natura auxinica rizogeni in Allium cepa. Nuovo G. bot. ital. N.S. 56, 161

Darlington, C. D. and La Cour, L. F. (1945) Chromosome breakage and the nucleic acid cycle. J. Genet. 46, 180-267

Davidson, D. (1958) The irradiation of dividing cells. II. Changes in the sensitivity to X-rays during mitosis. Ann. Bot. 22, 183-195

(1960) Meristem initial cells in irradiated roots of Vicia faba. Ann. Bot. 24, 287-295.

(1961) Mechanisms of reorganisation and cell repopulation in meristems in roots of Vicia faba following irradiation and colchicine. Chromosoma 12, 484-504.

Deschner, E. E. and Sparrow, A. H. (1955) Chromosomes rejoining capacity with respect to breakage sensitivity to X-rays and thermal neutrons. Genetics 40, 460-475.

* Gordon, S.A. (1957) The effects of ionising radiations on plants: biochemical and physiological aspects. Quart. Rev. Biol. 32 3-14.

Deufel, J. (1951) Influence of chemicals and X-radiations on mitosis on Vicia faba. Chromosoma 4, 239.

Eigsti, O. J. and Dustin, P. (1955) Colchicine in - Agriculture, Medicine, Biology and Chemistry. Iowa.

Evans, H.J. (1963) possible reasons for variations in chromosome radiosensitivity during mitotic and meiotic cycles. - in Sobels; Repair from Genetic radiation.

Evans, H.J. (1961) Chromatid aberrations induced by gamma radiations. The structure and frequency of chromatid interchanges in dploid and tetraploid cells of Vicia faba. Genetics 46, 257-275.

Evans, H.J. (1962) Chromosome aberrations induced by ionising radiations. Intern. Rev. Cytol. 13, 221-321.

Evans, H.J. (1957) The use of colchine as an indicator of mitotic rate in broad bean root meristem cells of Vicia faba. Exp. Cell Res. 18, 51-61.

Evans, H.J. and J.R.K. Savage (1959) The effect of temperature on mitosis and on the action of colchicine in meristem cells of Vicia faba. Exp. Cell Res. 18, 51-61.

Galston, A.W. and L.V. Dalberg (1954) The adaptive formation and physiological significance of indole acetic acid oxidase. Amer. J. Bot. 41, 373-380.

Gordon, S.A. (1955) Studies on the mechanism of phytohormone damage by ionising radiations.
- Peaceful uses of atomic energy 11 ; Biological effects of radiations.

Gordon, S.A. (1954) Occurrence, formation and inactivation of auxins. Ann. Rev. Plt. Physiol. 5, 341.

Grant, C.J. (1965) Induced chromosome breakage and chromosome cycle. D. Phil. thesis. University of Oxford.

Gray, L.H. and Scholes, M.E. (1951) The effect of ionising radiation

on the broad bean VII. Growth rate studies and histological analyses. Brit. J. Radiol. 24.

Guttenberg, H. von (1947) Studien uber die Entwicklung des Wurzelvegetationspunktes derDikotyledonen. Planta 35 360-396.

Hall, E.J., Lajtha, L.G. and Clowes, F.A.L. (1962) The role of the quiescient centre in the recovery of Vicia faba roots from radiation. Radiation Biology, 2, 189-194.

Heynowicz, Z. (1959) Growth and cell division in the apical meristem of wheat roots. Physiol. Plant. 12, 124-139

Hoffman, J.G. (1958) Theory of the mitotic index and its application to tissue growth measurement. Bull. Math Biophys. 11, 139-144.

Hornsey, S. (1956) the effect of X-radiation on the length of the mitotic cycle in Vicia faba roots. Exp. cell Res. 11, 340.

Howard, A. and Dewey, D.L. (1960) Variations in the period preceding D.N.A. synthesis in bean roots cells. Faraday Soc. The cell nucleus London Butterworth 155-162.

Howard, A. and Dewey, D. (1961) Non uniformity of labelling rate during D.N.A. synthesis. Exptl. Cell Res. 24, 623-624.

Howard, A. and S.R. Pelc (1953) Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosomal breakage. Heredity 6 Suppl. 261-273.

(1951) Synthesis of deoxyribose nucleic acid and nuclear incorporation of ^{35}S as shown by autoradiographs. Isotopes in Biochemistry (C. I. B. A.) 138-148.

Jensen, W. A. (1962) Botanical histochemistry. W. H. Freeman and Company, San Francisco.

Juniper, B. E., Groves, S., Landau-Schachar, B. and Audus L.J. (1965) Root cap and the perception of gravity. Nature 209, 93-94

Lahiri, A. N. and Audus, L. J. (1960) Growth substances in the roots Vicia faba. J. exp. Bot. 11, 341-350.

Lajtha, L.G. (1961) The effect of ionising radiations and tumour chemotherapeutic agents on the bone marrow. Progr. Biophys. 11, 79-109.

Leopold, A. C.(1955) Auxins and plant growth. Berkeley, California.

Levan, A. (1938) The effect of colchicine on root mitoses in Allium. Hereditas, Lund. 24, 471-486

Mitra, S. (1958) Effects of Xrays on chromosomes of Lilium longiflorum during mitosis. Genetics 43, 771-789.

Neary, G. J., Evans, H. J. and Tonkinson, S. M. (1958) A quantitative determination of the mitotic delay induced by gamma radiations in broad bean root meristems. J. Genet. 56, 363-394.

Östergren, G. (1950) Cytological standards for the quantitative estimation of spindle disturbances. Hereditas 36, 371-382

Quastler, H. and Sherman, F. G. (1959) Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res. 17,4

Read, J. (1959) Radiation biology of Vicia faba in relation to the general problem. Blackwell Scientific Publications, Oxford

Revell, S. H. (1960) An attempt at the continuous metaphase estimation of chromatid and chromosome aberration frequenci in broad bean root meristem cells in the period 2-23h. afte 50r. of X-rays. Proc. Symp. on the effects of ionizing radiations on seeds. I. A. E. A. Vienna, 229-242.

Schade, C. and Guttenberg, H. von (1951) Über die Enterwicklung des Wurzelvegetationspunktes der Monokotyledonen. Planta 40, 170-198.

Schüepp, O. (1917) Untersuchungen über Wachstun und Formwechsel von Vegetationspunkten. Jb. wiss. Bot. 57, 17-79.

Sharman, B. C. (1943) Tannic acid and iron alum with safranin and orange G in studies of the shoot apex. Stain Technol. 18, 105-111.

Sparrow, A. H. (1951) Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Anns. N. Y. Acad. Sci. 51, 1508-1540.

Stern, H. (1959) in Sulphur proteins. Ed. Bensch, R. et al. p.391 Acad. Press N. Y.

Taylor, J. E. (1963) Effects of colchicine on the root meristem of Vicia faba. Honours project, Botany Dept. St. Andrews.

Thoday, J. M. (1951) The effect of ionizing radiations on the broad bean root IX. Chromosome breakage and lethality of ionizing radiations to the root meristem. Brit. J. Radiol. 24, 572, 622.

Torrey, J. G. (1957) Auxin control of vascular pattern formation in regenerating pea root meristems grown in vitro. Amer. J. Bot. 44, 859-870.

Wimber, D E? and Quastler H. (1963) A ^{14}C and ^{3}H- thymidine double labelling technique in the study of cell proliferation in Tradescantia root-tips. Exp. Cell.Res. 30, 8.

Wolff, S. (1961) Some post-irradiation phenomena that effect induction of chromosome aberrations. J. Cellular Comp. Physiol. 58, Suppl. 1, 151-162.