ORGANISATION OF APICAL MERISTEMS

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Abstract

The shoot apex of rice, Oryza sativa CV Balilla was studied with a view to obtaining information about the meristematic activity of the different regions. The vegetative state only was studied and the rice was grown for 4-5 days at 30°C or 35°C under 4cm water in darkness, intermittent white light or continuous white, red, far-red or blue light. The part of the shoot apex studied was that above the level of the youngest leaf primordium and it was divided into six regions which took into account the tunica and corpus and regions of leaf initiation.

The mitotic index was found every 2hrs. throughout 24hrs. in plants growing in continuous darkness, in a 12hr. day with white light of 10,000 lux and in continuous white light of 2,400 lux. The mitotic index varied little and no pronounced synchrony was evident under any of these conditions. The mitotic index of the flank and corpus regions was higher than that of the summit and sub-summit regions.

The meristematic activity of the regions was investigated further using colchicine to accumulate metaphases. Colchicine blocks mitosis at metaphase, but does not affect the rate of entry of the cells into mitosis. The gradient of the increase in metaphases over 3-4 hours was used to calculate the cell-doubling time in each region and the effects of different environments on this were found. Cells of the summit region were found to divide at least 7 times more slowly than cells of the flanks and corpus and intermediate rates occurred in the sub-summit regions. In 10,000 lux white light and at 30°C. the cell-doubling time for flanks and corpus averaged 13 hours which is

shorter than any reported for equivalent regions in other plants. Cell-doubling times for the summit were over 6hrs. In experiments where the intensity of white light varied during the growth of the seedlings a different relationship between the regions was found. The cells of the flanks and corpus divided more slowly and only about twice as fast as in the summit.

The effect on cell-doubling times of excision on the shoot was studied and the immediate result was to speed up cell cycles in the leaf primordium and corpus. Excision has often been used to facilitate entry of a chemical into the shoot apex and it may have given a misleading picture of the situation in undisturbed shoot apices.

Nuclear DNA synthesis in the regions was studied using tritiated-thymidine. In 1,000 lux continuous white light the flanks and corpus showed a gradual increase in labelling throughout the feeding which lasted for 72hrs. while the sub-summit and summit regions showed a negligible or low level of labelling in the same period of time. The level of DNA synthesis taking place in the regions thus paralleled that of meristematic activity found by using colchicine. The supply of tritiated thymidine to plants grown in continuous white light at 10,000 lux produced a non-linear low labelling pattern which could not be easily explained. Many attempts were made to overcome this lack of success involving excision, and surgery of various kinds, use of (6- ^{3}H) thymidine as well as (methyl-^{3}H) thymidine and experimentation with red, far-red and blue light. However, none of these succeeded and so a pulse-labelling experiment was not attempted.

The effect of an acute dose of X-rays was studied by finding the mitotic index immediately afterwards, $\frac{1}{2}$hr. afterwards and 24hrs.

afterwards and by studying the level of metaphases after colchicine in control and irradiated plants. A dose of 1, 2,4 and 8 k.rad had a differential effect on the regions which is interpreted as a reflection of their different levels of meristematic activity. No stimulation of division of the summit was found which is the reverse of what has been found in the quiescent centre of roots after acute X-irradiation, although this region is often equated to the summit of the shoot.

A study of cell pattern throughout a plastochron suggested, on the basis that evidence of cell division and cell size are related, that meristematic activity in the tunica is highest in the flanks and that, as the activity progresses round the circumference of the apex in the formation of the leaf primordium, which sheaths the stem, cell division also takes place for a short distance towards the summit. In this way, preparation for formation of a leaf primordium occurs sometime before it appears as a bump. The summit is considered to be carried forward passively and not to contribute a significant number of cells to the rest of the meristem. This interpretation supports the view that the summit is not the site of apical initials in higher plants as considered by the orthodox view.

A study of the effect on cell kinetics of red and far-red light by using accumulation of metaphases with colchicine revealed a change in response to far-red light with the age of the seedling. Forty hrs. red and far-red light given to dark-grown $3\frac{1}{2}$ day-old seedlings caused increased meristematic activity to take place in all regions but 16hrs far-red light reduced activity and 16hrs. red light was stimulating. Forty hrs. far-red light after $2\frac{1}{2}$ days dark gave high rates similar to $4\frac{1}{2}$ day-old plants given 16hrs. far-red. Comparison of these rates

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A study of the effect on cell kinetics of red and far-red light by using accumulation of metaphases with colchicine revealed a change in response to far-red light with the age of the seedling. Forty hrs. red and far-red light given to dark-grown 3½ day-old seedlings caused increased meristematic activity to take place in all regions but 16hrs. far-red light reduced activity and 16hrs. red light was stimulating. Forty hrs. far-red light after 2½ days dark gave high rates similar to 4½ day-old plants given 16hrs. far-red. Comparison of these rates

on the basis of the age of the seedling at the end of the treatment revealed that plants which are 2.5 days old had similar rates after 16 or 40hrs. far-red, but plants which were 4 days old had higher rates after 40hrs. far-red than after 16hrs. far-red. Variation of response to far-red with age of the seedling has been found before and the link between phytochrome and leaf initiation is discussed. All regions of the apex, including the summit respond to light by a speeding-up of mitotic cycles but the summit is still at least seven times slower than the flanks and corpus.

Finally, consideration is given to the significance of the results in rice and the **méristème** d'attente controversy. Cell divisions in the summit may vary in importance in different apices but in the absence of the possibility of proving presence or absence of apical initials the controversy is put aside. The shift of growth balance in apices of plants subjected to different conditions is brought out and the importance of the summit to growth of the shoot apical meristem and the nature of its influence is discussed.

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I. INTRODUCTION

The whole shoot system of the plant is produced by the activity of its apical meristems. This capacity of the meristem for continuous addition of organs throughout life is a fundamental characteristic of plants and is in direct contrast to the situation in animals where, in most cases, all the organs are formed at the beginning of the life of the separate individual.

By the activity of the shoot apical meristem, new stem tissue is produced and leaves are initiated in a regular and orderly fashion. However, we understand little of how this process occurs. Many observations have been made, but what is especially needed now is quantitative data and it is this that is difficult to acquire. This is mainly because of the relative inaccessibility of the shoot apex and its continual state of morphological flux throughout a plastochron. In the present anxiety about world food supplies it has become more than ever desirable to understand the processes which take place in the shoot apex since this increases the possibility of breeding and growing more efficient food-producing plants.

The subject of this work is some aspects of the organisation of the shoot apical meristem and is restricted to the angiosperms.

The Story So Far

The pattern of cells which appears when the shoot apex is examined in longitudinal section led Hanstein (1868) to propose three histogens to be responsible for the production of cells forming the epidermis, cortex and stele. However, many shoot apices could

not be interpreted in this way and a different concept was proposed by Schmidt (1924). On the basis of the orientation of cell division he described the apex as consisting of an outer tunica, which could be one or several cells thick, in which the plane of cell division was predominantly anticlinal, (at right angles to the surface) and so resulted in an increase in surface area, and an inner corpus whose cells divided in all planes thus increasing in volume. These regions were not assigned any histogenic role so that, for example, leaves could arise from the tunica, or from both the tunica and part of the corpus. So Schimdts's theory had some similarities to the germ layer concept of animal embryology and moved away from the tissue-specific histogens idea in Hanstein's theory. The tunica-corpus theory was accepted by anatomists as a useful way of describing shoot apical organisation and is still used.

However, when dynamic aspects of development of the shoot apex are considered it is not sufficient since it does not deal with how fast the cells are multiplying or whether they are being produced at the same rate throughout the apex. From a study of foliar helices Plantefol (1947) considered that the source of growth of the shoot apex was at the side of the dome in a ring at the level of leaf initiation which he named the 'anneau initial'. Bersillon (1951) developed this theory, and proposed subdividing the ring into three. These comprised a localised area involved in leaf initiation, an area which has just produced a leaf and was reconstituting the anneau initial and a third area which was resting, prior to leaf initiation. The apex above this ring he suggested calling the méristème d'attente since mitoses were rare until flower initiation. The cells of this region in Papaver, before flowering, stained less strongly, were

larger and contrasted with the cells of the anneau initial in other ways. The concept of the méristème d'attente went against the accepted view of the time that this part of the apex was the site of the apical initials. The work of Buvat (1952) and Lance (1952) studying histochemistry and mitotic index followed soon after, in support of the méristème d'attente.

The contributions of the French school stimulated a great deal of work on the shoot apex, but agreement has not been reached on the nature of the summit. The French hold that the summit has no apical initial role (Nougarède 1967, page 251), while others (Gifford 1954, Wardlaw 1957, Gifford and Corson 1971) still support the orthodox view. It is generally agreed now that the summit, during the vegetative phase, is less mitotically active than the flanks, but most workers outside France maintain that this does not preclude any organogenetic function for the summit (Gifford and Tepper 1962).

The Present Situation

Work relating to this controversy will be dealt with by taking the methods of investigation in turn and selecting examples to illustrate the kind of evidence each furnishes.

Mitotic Index

Many workers besides Bersillon have based their conclusion about the activity of the different regions on the number of mitotic figures seen in these regions in a longitudinal section of the apex (eg. Popham 1958, Lance 1952). They fixed samples at regular intervals for 24hrs. Lance found that the summit had fewer mitotic figures than the flanks in Vicia faba while Popham found the region with

fewest mitotic figures in Chrysanthemum to be sub-terminal. However, Popham did not count the total number of cells and so the variation in cell size of the different regions was not taken into account. A more fundamental criticism is concerned with the principles of the method itself. Comparison of mitotic index between regions for the purpose of assessing rates of mitosis is only valid if the proportion of the cell cycle taken up by mitosis is the same. The mitotic index depends on the ratio M:(M+I), where M is the duration of mitosis and I, that of interphase (Brown, 1951). Two regions could have the same index but different rates of mitosis if the proportion of time taken up by mitosis in their respective cycles is different. The caution with which the index should be treated is demonstrated very clearly by Clowes (1961b,1972a). He showed that the cap initials of root meristems have the highest rate of division, but not the highest mitotic index. However, the region of the root apex with the lowest index is the quiescent centre and this divides about ten times more slowly than the cap initials. So mitotic index can be taken as an indication of mitotic rate, but there must also be investigation by some other method. If the mitotic index is found frequently enough over a long enough period of time and almost no divisions are seen, it is a fair indication that the mitotic activity of a region is low.

Metaphase Accumulation with Colchicine

Corson (1969) combined his study of the mitotic index of Datura with colchicine experiments. Colchicine arrests mitosis at metaphase by preventing the formation of the mitotic spindle. The rate of accumulation of metaphase is a measure of the rate of mitosis of the

cells. Corson measured the accumulation between 4 and 8 hrs. and found that the summit cells divided at half the rate of the flank cells, ie. the average durations of cycles were 76hrs. and 36hrs. respectively. He found that these reflected more or less the relative mitotic indices and so he decided that the length of mitosis could be taken to be more or less constant in all regions in this particular instance.

There are many reports of experiments with colchicine and most give the same relative rates as Corson (Denne1966a, Lyndon 1970a, Leshem and Clowes 1972). Difficulty is experienced by all with regard to the application of colchicine to the shoot apex. Denne (1966a) applied a drop between the two halves of the sheathing leaf of Trifolium which she slit down the middle but does not mention renewing the application although the experiment lasted for 12 hrs. Corson used a cotton wool plug soaked in 0.5% colchicine solution and renewed the solution hourly. The solution included Tween, which is a wetting agent and he also did controls with Tween alone. Leshem and Clowes excised the apical 3cm of potato. All of these methods involved subjecting the apex to abnormal conditions, viz. surgery, enclosing the apex etc. and this has to be remembered when interpreting results.

Labelling Experiments

The same caution has to be applied to experiments in which labelled precursors of nucleic acid (usually tritiated thymidine) are applied to the shoot apex. Cells synthesise DNA usually only in preparation for division and so those cells which are labelled are assumed to be meristematic. However, it has to be remembered that

cells may increase their DNA and become polytene or polyploid instead of dividing.

The methods employed to accomplish penetration by the solution of the labelled chemical are various. Steeves, Hicks, Naylor and Rennie (1969) excised the apex as did also Raju and Ho (1973). Most other methods have involved drops applied to the bud, eg. Gifford (1960) sometimes after partial defoliation, Gifford, Kupila and Yamaguchi (1963) or complete defoliation, Bernier and Bronchart (1963). Bernier and Bronchart added a glass tube to surround the apex and sealed it with lanolin at the base. Occasionally the solution has been injected, Clowes (1959a) Lance - Nougarède (1961). Dath (1963) separated the leaves and inserted a plug of wadding to which she added the solution.

The low mitotic index in shoot apices has led workers to use long labelling times of several days to obtain sufficient labelled cells to count (Clowes 1959a, Gifford 1960), but labelling periods of six hours and less have been found adequate (Brown, Hiksche and Smith 1964, Besnard-Wibaut 1970).

Nearly all workers reported more labelled nuclei in the flanks than the summit. (Brown 1970, Evans 1971, Lance-Nougarede 1961). Besnard-Wibaut (1970) found 3.6% labelled in the central zone and 12.3% in the peripheral zone after 4hours with Arabidopsis and similar results were found by Bernier (1964) and Saint-Come (1966).

Jacqmard (1970) who has carried out the only pulse-labelling experiment on a higher plant shoot apex gave Rudbeckia bicolor plants a half-hour pulse and sampled up to 40 hours afterwards. She found the results indicated a cell cycle time of 30 hours in the flanks while that in the summit was too long to be measured. A

pulse-labelling experiment is particularly valuable since it can be used to give details of all the phases of the cell cycle. The summit was found to have a longer DNA synthesis phase (S) than the flanks and the time between S to half-way through mitosis (G_2 + M/2) was also longer in the summit.

Of those who reported a lack of unequal distribution in labelling most have used long labelling times (Clowes 1959a,Gifford 1960) except Berg (1970) who appeared to leave out the leaf primondia in his investigation and Lyndon who attributes the relatively uniform labelling to a variation in S in the different regions so that the proportion of the cell cycle occupied by S is similar. Long labelling times would be expected to produce some labelling even in more slowly multiplying regions although this should not obscure a difference between two regions which are multiplying at different rates because the faster region should have proportionally more nuclei labelled. Gifford later, with Kupila and Yamaguchi (1963) did report more labelled nuclei in the peripheral zone than the summit.

The same results are used by different workers to support opposing arguments. Labelling, in the summit is used to substantiate the presence of apical initials (Gifford 1954) but the same density of labelling leads Nougarede (1967 p. 251) to say that the level of activity indicated is not enough to produce the cells necessary for leaf initiation and she criticises Gifford for placing too much weight on two weakly labelled nuclei./ (1965). Gifford (1954) seems to be alone in being able to pinpoint the essentials of the controversy. He considers that Buvat argues against a view not taken by anyone, as no-one has said that the summit cells were responsible for producing all the cells but the accepted view is that organ initiation is in cells

derived from the summit cells. The difficulty would appear to rest in the definition of what an initial cell is. The same position obtains in vascular cambial activity where the progeny of the cambial cells also undergo division. Obviously, what is needed here is measurement of absolute values rather than semi-quantitative comparisons only.

Sussex and Rosenthal (1973) have used labelling techniques to "tag" cells rather than to measure lengths of cell cycle. They grew quarterised apices in culture containing label and after 16 days when new apices were fully reorganised transferred them to non-radioactive medium and found after a further 16 days period of growth that the flanks became unlabelled while the summit remained labelled. The medium used for culture contained kinetin but they maintained that this did not affect the growth pattern but only the vigour of the growth and they concluded that the summit made no cellular contribution to the flanks.

Chimeras

Gifford (1954) finds support for apical initials in the summit from work on chimeras. Chimeral plants are composed of genetically different cells. The most stable chimeras in shoots are periclinal in which the genetically different cells form a complete layer, like a skin, throughout the whole plant. The maintenance of the periclinal chimera is explained very satisfactorily by the tunica corpus theory and has been used as evidence for the discreteness of the different germ layers. Evidence in favour of apical initials has been put forward by Stewart and Dermen (1970) from study of the occasional breakdown of the periclinal chimera. They have made much use of the variegated

plants that are periclinal chimeras in which a whole tunica layer is genetically white. The cells of this layer have, for one reason or another, lost the capacity to develop chlorophyll. Occasionally the pattern of variegation changes and a sector of the shoot appears white or green where it would be expected to be green or white. The explanation put forward for this is that the strict tunica corpus arrangement has broken down and either replacement or displacement has taken place. Replacement is the intrusion of a cell from one tunica layer into the next layer underneath as a result of a periclinal division. Displacement referes to the reverse, when a cell from an inner layer is displaced into a layer over it. Thus, a sector of a tunica layer becomes genetically different and the chimera is called mericlinal. Stewart and Dermen observed that the wider sectors lasted for more modes than the narrower ones. Since these sectors spanned 1/3 or $\frac{1}{2}$ of the circumference of the plant and reasoning that a sector is produced as a result of just one periclinal division, there must be one to three apical initials for any layer, and these must be situated in the summit. They further calculate from their observations that these initals divide, on average, once every four plastochrons but are nevertheless "the source of all growth". Stewart and Dermen have however, concentrated their study on what Nougarède described as special cases (Nougarède 1967 p. 228), which would not be true for normal apices. Howard (1971), using the same kind of evidence, has proposed six initial cells for just the second tunica layer of potato. The normality of behaviour of chimeral meristems has been questioned by Thielke and others (Clowes 1961ap.80), but Stewart and Burk (1970) in their discussion of this, point out that a leaf which is half white and half green is

symmetrical indicating that growth in this case is normal. However, in others, abnormal behaviour is evident (Clowes, 1957).

Development During a Plastochron

Some work has been carried out comparing sections of apices at different stages of development and attempting to deduce growth patterns by comparison of the successive stages. Lyndon (1970b) concentrated on the orientation of cell divisions in different parts of the apex during the course of a plastochron and found in the dome above the primordium a difference in the first half of the plastochron from the second half, resulting in the direction of growth being more in the horizontal plane, lateral to the primordium in the first half and more vertical and outward in the second half. He also (1971) studied cell displacement between the regions and concluded that a leaf primordium arises from displacement of cells from the axis and dome with localised cell division of the epidermal cells while the rate of division through the plastochron does not change. His results, however, are difficult to compare with those of other workers because he subdivides the apex in a different way. Also, the regions are rigid throughout the plastochron, although the apex is changing, and so he is really comparing different regions within a plastochron. There is an interesting contribution from Lance-Nougarède, Bronchart, Bernier and Rondet (1964), especially in view of the fact that they support the méristème d'attente, for they state here that as the Perilla apex is so small the summit must participate in plastochronic functioning. Catesson (1953) came to a similar conclusion after working on Luzula and thought all small apices would have an active summit.

Histochemistry

A great deal of significance is placed by some, especially in France, on the histochemistry of the apex. Buvat found support for his theory from the lighter staining of the summit cells. The view held is that the activity of the cells in a region can be revealed by their chemistry, so they compared the DNA and RNA content and concluded that the summit cells are inactive because they have less densely staining nuclei and a lower RNA content. These facts have been confirmed by most workers: Corson and Gifford (1969) for Datura; Knox and Evans (1966) for Lolium; Heslop-Harrison and Heslop-Harrison (1970) for Plumbago, although they do not necessarily agree with the French school.

The size of the nucleolus has been found to vary in the different regions, but here, there is conflicting evidence. While it is generally reported that the summit cells have larger nucleoli eg. Ho and Raju (1972), Gifford and Nitsch (1970), this is not always so and the French have concentrated on the examples of summit cells with smaller nucleoli to support their case, (Lance 1957). The situation was very adequately discussed by Gifford and Tepper (1962). They pointed out that Lance (1957) found only slightly less RNA in summit cells in plants grown at high light intensities when leaves are being produced rapidly. The nucleoli were only slightly smaller than in the flanks and stained intensely. They went on to point out the lack of uniformity in the method of measurement of nucleoli, as some take the diameter of the largest, and some the range of diameters. Nougarède (1965) has contributed useful detailed information on nucleoli and has studied the variation through a plastochron and found that sometimes the difference between zones was insignificant. She

criticised Gifford and Tepper (1962) on the basis that their material was in the intermediate phase phase intermédiare. The existence of this phase in the development of the apex was proposed by Lance in 1957. and has since been supported by others (Heslop-Harrison and Heslop-Harrison, 1970) and has contributed greatly to our understanding of the shoot apex. After the vegetative stage of plant growth, the apex can continue to produce leaves although the apex itself has changed and has taken on some of the characteristics of a flowering apex eg. increased mitotic activity especially in the summit cells, a change in shape and an increase in size. It is important to remember this, since studies of apparently vegetative apices, ie. producing leaves, could be of intermediate apices. Nougarède maintains that some of the conflicting evidence is due to a lack of understanding of this and she thinks the material Gifford and Tepper used was probably in the intermediate phase and that this would explain why they found large nucleoli in the summit, unlike the French school. Total nucleolar volume is not usually found, though in a later paper produced by a joint effort of the opposing schools, ie. Nougarède, Gifford and Rondet (1965), total nucleolar volume per cell was measured for Amaranthus. They reported that the average nucleolar volume is greater in the flanks in the minimum area phase (just after a leaf primordium has been formed), but greater in the summit in the maximum area phase (just before a leaf primordium is formed) although the zonation of the meristem is still maintained because the cytoplasmic RNA level in the summit remains low. It is not possible to draw any conclusions about the meristematic activity of the regions from this, for, as Gifford and Tepper pointed out

before (1962), we need first to understand the significance of nucleolar form.

DNA Content

Some interesting information has come from measurements of the DNA content of nuclei, although, here again, the significance is not fully understood. Naylor (1958) found that summit cells of a dormant lateral bud were at the 2C level which, after release from apical dominance by removal of the terminal bud, changed to the 4C level. From comparisons with root apical meristems (Clowes 1968), this would tend to suggest that the summit of a dormant bud and the quiescent centre are similar in having a high proportion of cells in the 2C condition. In the root this correlates with a very long G_1 phase (the phase after mitosis and before DNA synthesis) and a slow rate of division. However, as the level goes up to 4C in a proportion of nuclei at the end of dormancy when the bud grows out, the position is confusing. Jacqmard and Miksche (1971) found more cells in shoot apex squashes at the 4C level before floral induction than afterwards, indicating that slower dividing cells in the shoot apex spend more time in G_2 than G_1. Rembur (1972) compared the content of summit and flanks cells in Xanthium and found 86% at 2C in the summit and 73% at 2C in the flank, though the flank varied through the plastochron, there being more flank cells in G_1 at the maximal area phase, which is the regenerative phase when the flanks are producing a primordium. However, the difference is not great, and the difference between regions is certainly not as great as in the root, so it is hard to draw conclusions from these results although Rembur believes they support the méristème d'attente theory. Lyndon (1973) found no

difference in the proportion of cells in G_1 and G_2 in the summit and flanks. Since he knew the cell cycle time was longer in the summit he concluded that the summit cells had a protracted G_1 and G_2 period compared with the flank cell population. Microdensitometry on sections is difficult because individual nuclei are not easy to isolate as they overlap and some are cut.

Ultrastructure

Ultrastructural studies of summit and flank cells reveal differences which are attributable to a less active state in the summit. Nougarède (1967) reported that in Perilla, summit cells compared with flank cells had larger nuclei and nucleoli (which would appear to contradict evidence presented in a different part of the paper, as mentioned on page 11), fewer ribosomes and more vacuoles. Gifford and Stewart (1965) found in Chenopodium that the endoplasmic reticulum in the summit cells formed only a single layer near the wall and there were few lobed plastids, whereas in the flank cells they were more developed.

Ionising Radiations

While a great deal has been elucidated from studies of the reaction of the root meristem to radiation (Clowes 1965), this is unfortunately not the case in the shoot. One piece of work by Sagawa and Mehlquist (1957) indicated that summit cells were more sensitive to X-rays than subjacent cells, since they become necrotic four days after a dose of 5,000 roentgens, and this resulted in the colour of the carnation, which was a chimera, changing, as corpus cells divided

to replenish the damaged tunica layers. There is an interesting piece of work furnished by Foard (1971). He found that at the level of γ-irradiation which prevents cell division in wheat grains, protrusions in the position of the expected leaf primordia still took place. This is an interesting comment on the fundamental nature of cell growth and differentiation and its independence from division. Langenauer and Davis (1973) obtained interesting results from a very thorough analysis of the responses of Helianthus annuus to acute X-irradiation. The shoot apex could sustain damage, which could be estimated by anatomical examination but continue to produce normal leaves, apparently by a process of "self-healing". After producing normal leaves the same apex produced a damaged flower. They concluded that the summit's main contribution was to flowering and so found support for Buvat's "revised" (their quotation marks) méristème d'attente theory (Buvat 1955).

Living Apices

There is one approach which criticises most of the work so far discussed on the basis that the experimental procedure involves killing the plant and so only one stage of the plant can be studied. Those who take this approach work on living apices which they have isolated from the plant and grown in sterile nutritive medium. Ball (1960) photographed the surface of the apex of Lupinus, Vicia and Asparagus every few minutes and found that the summit cells did divide, though less than the cells of the foliar primordia. While some summit cells divided to produce a group of cells, others did not divide at all and the direction of growth was away from the site of initiation of the new leaf where new cells were being

produced and expanding. Ball concluded that there were no permanent initials and no quiescent zone, the summit dividing as fast as the flanks. It would seem that here there was more disagreement as to interpretation rather than in the evidence produced, because Plantefol's initial ring was divided into segments, only one of which was very meristematic and involved in leaf initiation. It really rests on what is included in the initial ring; Plantefol included the leaf initiation zone, and Ball left it out. Such a difference of view rather than of fact was pointed out by Newman (1956) who carried out similar work on Tropaeolum and Coleus but made camera lucida drawings instead of taking photographs. Newman, who found divisions in the summit favoured a view of the apex where cells act as initials by virtue of their position. He considered that such initials need not be very meristematic to be responsible for initiation. Once they pass out of the initiating region they cease to be initials. This is much the view taken by Clowes for his analyses of the root. Nougarède (1967) pointed out that in the work by Ball in 1960, the apices were excised and grown in medium which contained gibberellic acid which probably affected cell division. However, the experiments of Soma and Ball (1964) were on entire plants with no hormone added. Their results were similar to those of Ball 1960. Loiseau (1962) found similar cell division patterns in Impatiens raylei which he followed using coloured spots on the surface of the summit. Here again, similar results are used to support opposing ideas for Loiseau supported the méristème dâttente theory and described the divisions that did occur as feeble and not sufficient to indicate the presence of initials.

Surgery

Experiments in which parts of the apex have been severed or damaged have yielded interesting results but the difficulty with interpretation is always that of assessing the extent to which what is found is true of the undisturbed apex and what is due to wound reaction. However, the shoot apex can survive quite extensive damage which in itself is an indication of its robustness. Many early experiments arose from an interest in phyllotaxis and related problems (M and R. Snow, 1933). Results for higher plants were often conflicting but did give information about the capacity of the summit to grow normally after isolation from the rest of the apex (Cutter 1965). Experiments also indicated that the vascular system was the main pathway for control by the apical meristem since incisions which severed the vascular connections caused lateral shoots to grow but more shallow incisions resulted in production of leaves by the flanks and not buds. Destruction of the summit still allows leaf formation, though phyllotaxis and leaf form may be affected. This result is similar to that of Langenauer and Davis on X-ray damage of the shoot apex. Experiments by Cutter on Nuphar (Cutter 1958) in which the site of the next leaf initial was isolated by a deep incision indicated that determination to produce a leaf or a bud took place before this since a leaf was still formed and not a bud.

Interpretation of surgical experiments in which the results of two surgical experiments are compared rather than the result from surgery being compared with a control without surgery are less likely to be confused with wound reaction (Clowes 1953). Wetmore (1956) excised the summit and flanks of Helianthus annuus separately and

found that the former did not grow while the latter did which is surprising if the summit does contain the apical initials.

The Purpose of this Investigation

The evidence upon which much of the controversy about the contribution of the summit to the growth of the shoot apex rests, is mainly indirect viz. mitotic index, chimeras, cell patterns and histochemistry. Where the direct approach of measuring the rates of cell multiplication of the different regions with colchicine or level of DNA synthesis has been used the evidence has pointed to a less active summit, but the techniques of applying solutions by injections below the apex, or excision of the apex or by direct application after cutting away, or parting young leaves, do introduce unknown factors into the system and can therefore not be relied upon to reflect the situation in undisturbed shoot apices.

Measurement of the rates of cell multiplication has been used with great success in root meristem studies by Clowes and it was this work that inspired the attempt to investigate the shoot in a similar way. The problem of application of solution to the shoot apex during experimental procedures was surmounted by using rice since it normally grows submerged in its early stages. Rice has additional advantages in being easily obtainable in large, standard quantities because of its economic importance and large numbers of apices are readily produced within a few days of sowing.

Mitotic index has been studied through 24hours and the cell-doubling time measured in the different regions using colchicine-induced metaphase accumulation. Experiments have been carried out

with 6-^{3}H- thymidine and methyl -^{3}H- thymidine and the immediate effect on mitotic index in the apex after X-irradiation followed. Consideration has been given to the pattern of cells seen in longitudinal sections throughout the plastochron. The effect of red and far-red light on the rates of cell division has been used to study the early effects of this treatment on rates of leaf initiation. All these studies point to a very low activity of the summit cells and the question of their function as initials is dealt with in the final discussion.

2. EXPERIMENTAL MATERIAL AND METHODS

The material and general methods will be described in this section and details of particular experiments recorded later, in the relevant sections.

Material

Rice (*Oryza sativa*) var. Balilla was used for the majority of the experiments. Balilla rice was kindly given by Dr. Grancini of the Instituto Sperimentale per la Cerealicoltura, Italy. The seeds were sown under 4cm of water at 30 or 35°C. When grown for four days in the dark, the shoots were about 30mm long and the coleoptiles closed. When grown for four days in the light the shoots were about 20mm and the coleoptile had split open revealing the first true leaf. (fig. 2.1). At this stage, the total number of leaves is four. The ripe seed embryo has three leaves plus the coleoptile and so the first plastochron during germination is about four days.

The shoot apex of Balilla rice is about 40-50 μm high and 40-50 μm wide at the base at the level of insertion of the youngest leaf primordium (fig. 2.2). The mode of leaf formation is similar in all grasses (Sharman 1945). A short distance from the crest of the apex (about 6 cells in Balilla rice) the primordium begins as a periclinal division in a cell immediately below the tunica. As cell division continues, a swelling occurs. This swelling then grows in three directions ie. upwards and laterally on either side so that eventually a collar is formed with a spur (fig. 2.3). The collar



$1\frac{1}{2}$ times life size.

Fig. 2.1 Four-day old rice seedlings grown in 10,000 lux at 30°C under 4cm water. Coleoptiles are open and the first and second true leaves can be seen.

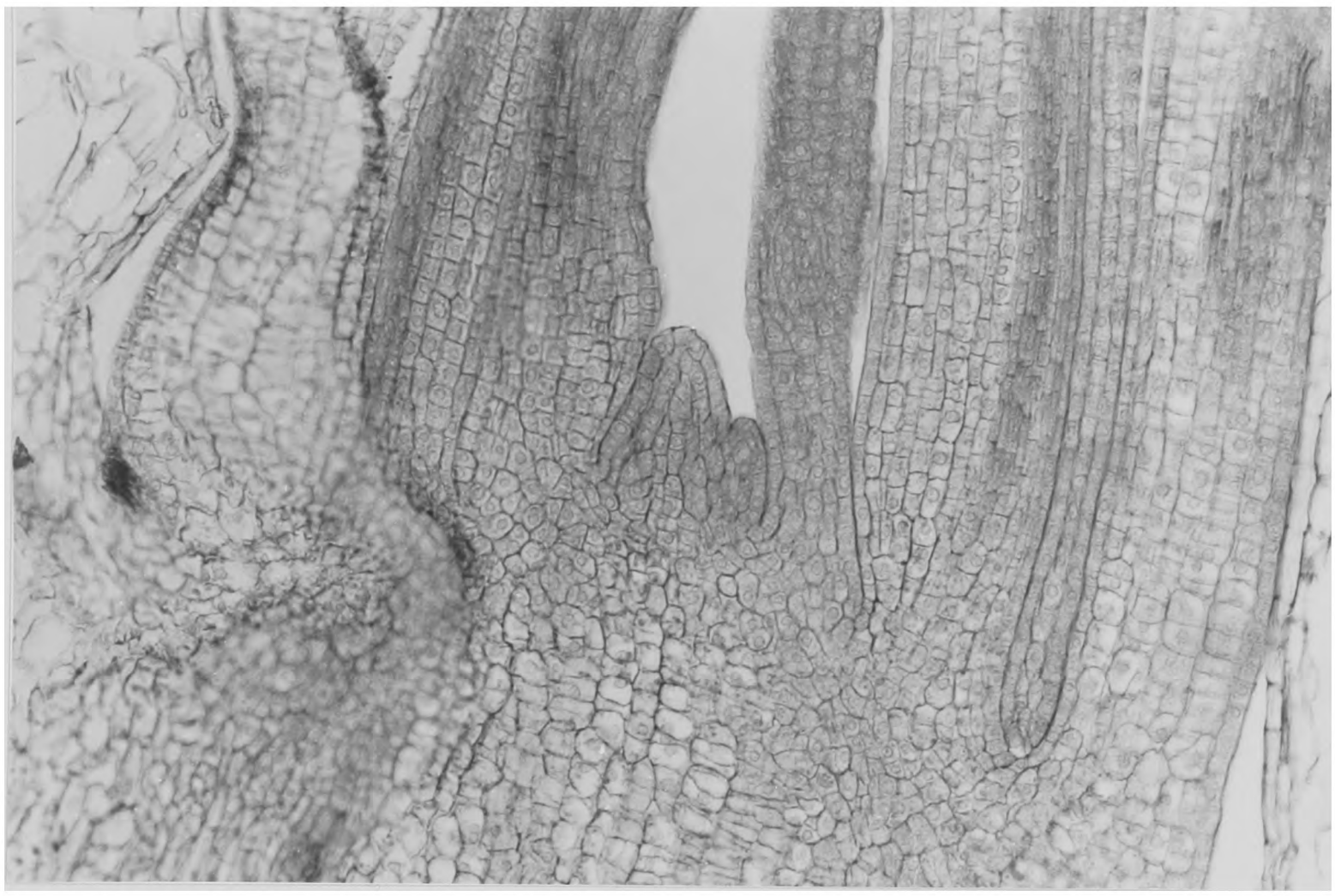


Fig. 2.2 L.S. through Balilla rice shoot apex. Sectioned at 8µm; Sharman's stain.

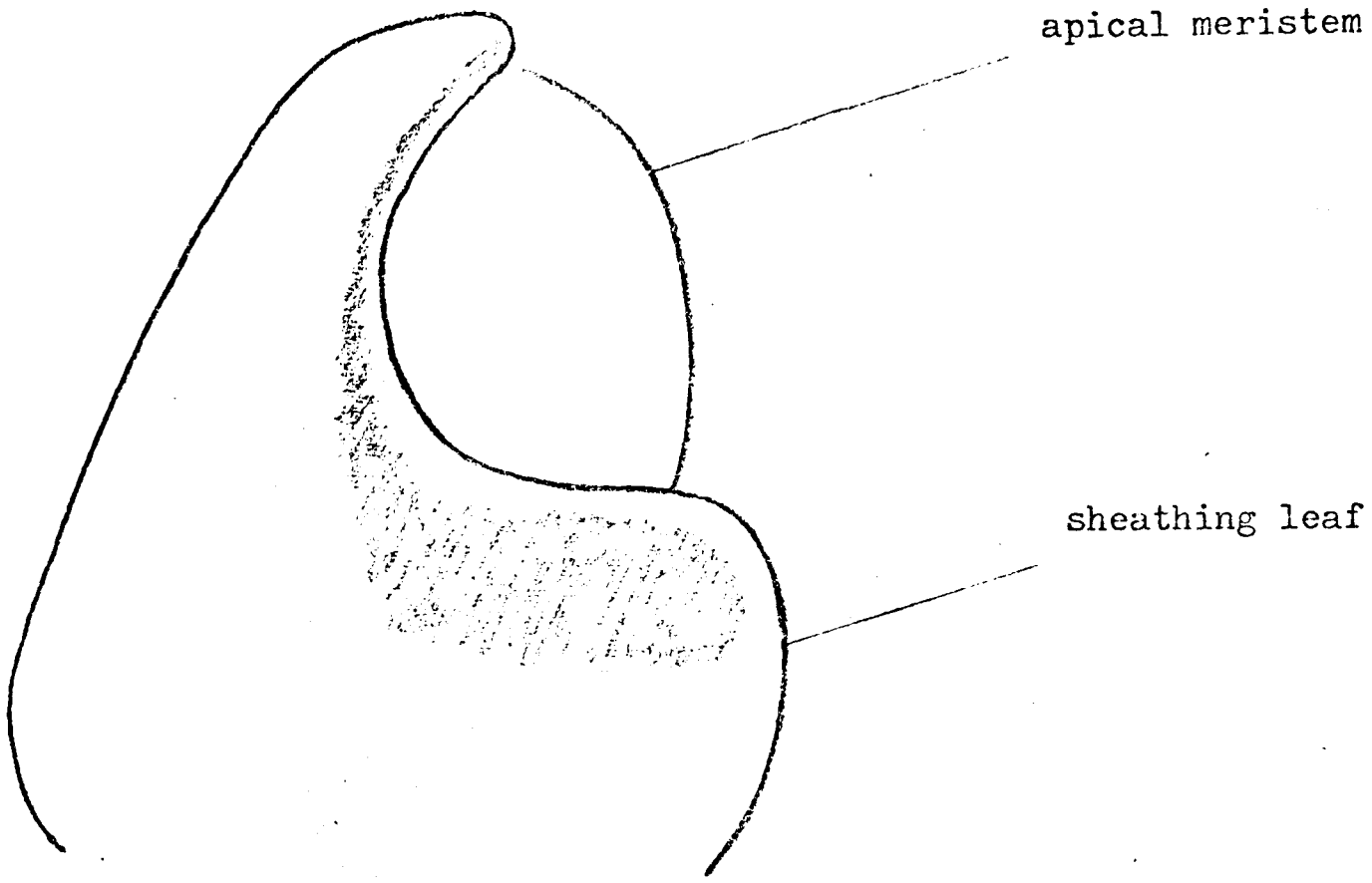


Fig. 2.3 Plan diagram of the shoot apex of the type found in grasses to show an early stage in leaf development.

extends upwards and eventually the apex is enclosed. The leaf continues to elongate and the next leaf primordium arises at the base of the apex, just above the previous leaf and on the opposite side of the stem from where the previous leaf began ie. the phyllotaxis is distichous. The leaves thus form around the apex in concentric circles, the margins of the leaves overlapping. (fig. 2.4).

The regions of the apex

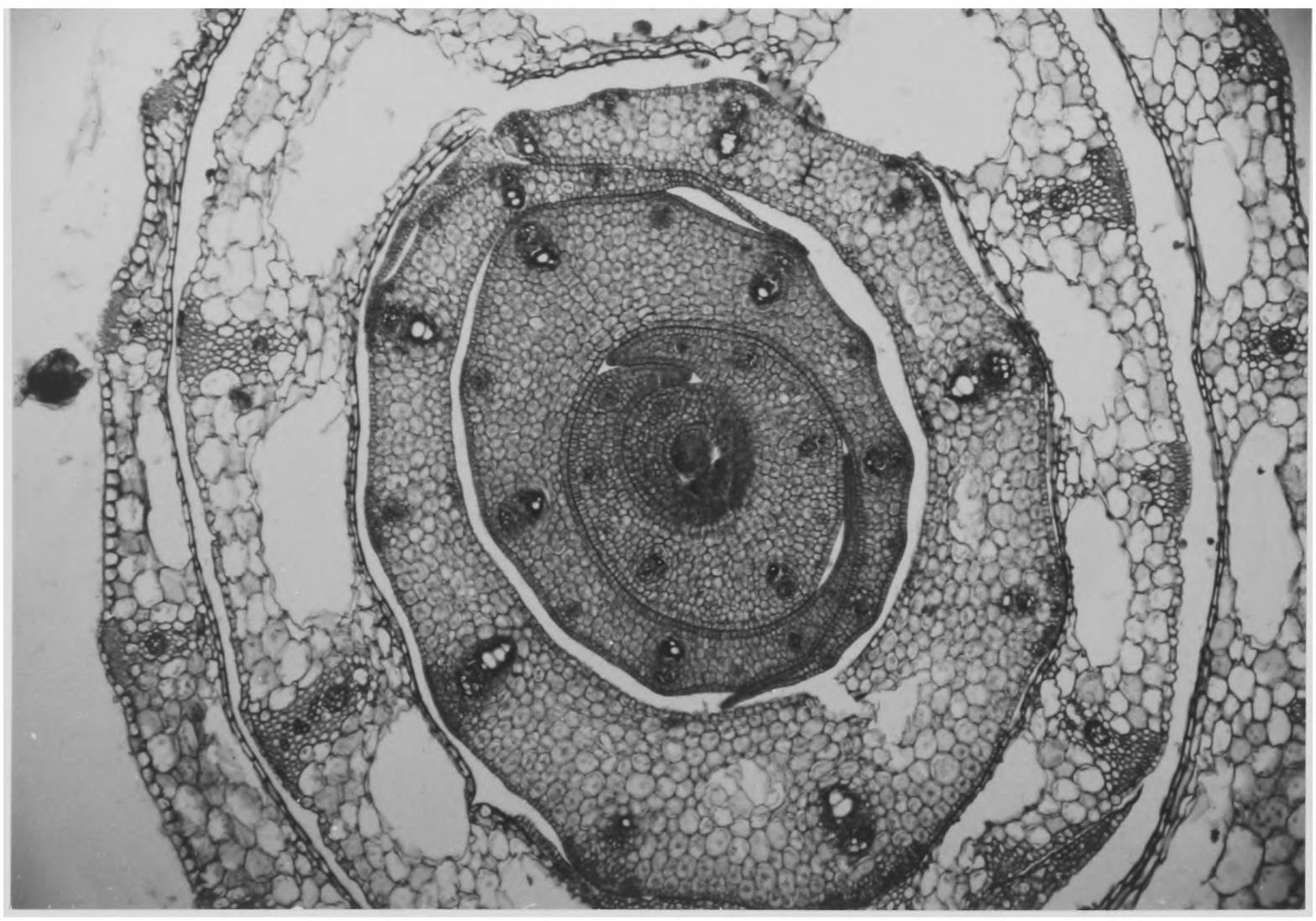
Six regions were chosen for study in the shoot apex paying attention to tunica and corpus and the area of the leaf primordium and initiation (fig. 2.5). The side of the apex nearest to the middle of the leaf which appears first is called the leading flank and the opposite side, the trailing flank.

Methods

Experiments were carried out on plants four to five days old Plants were easily transferred from water to the aqueous solution of colchicine or tritiated thymidine, thus avoiding much disturbance to the plant.

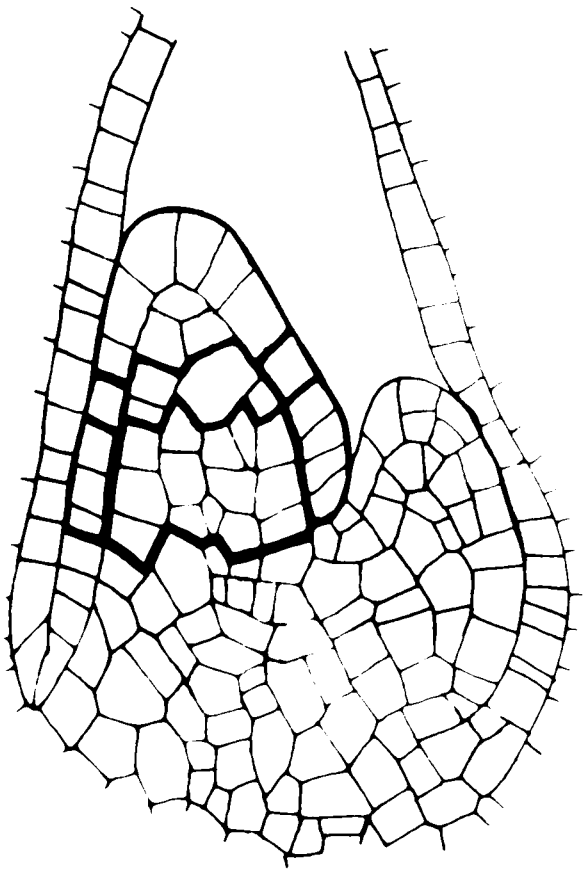
Plants were grown at 30°C or 35°C either in the dark, in a daily light/dark cycle or in continuous light. The white light source was from fluorescent tubing and intensity of illumination was either 1,000 lux, 2,400 lux or 10,000 lux. Details of the blue, red and far-red sources are tabulated below.

Light	Wavelength (nm)	Energy at level of plants ($\mu W\ cm^{-2}$)
Red	660nm	50.4
Blue	440nm	57.0
Far-red	700-800nm	33.0

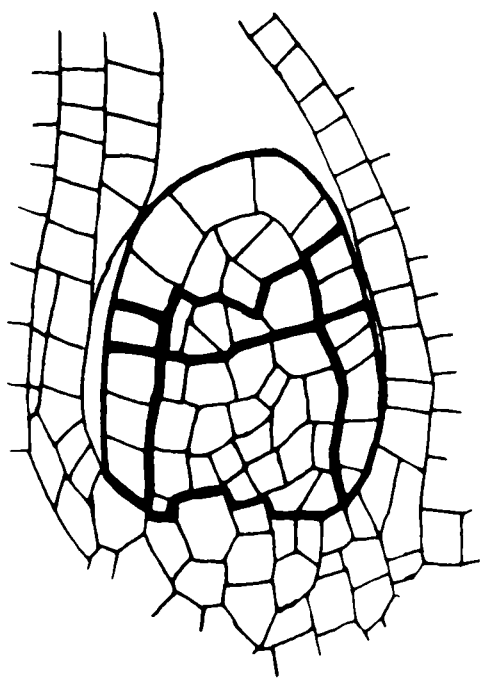


0.5 mm.

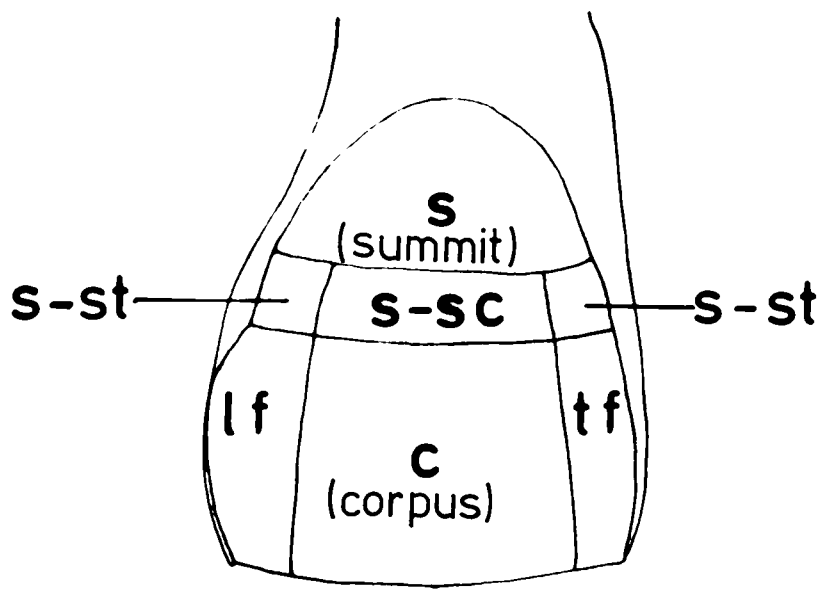
Fig. 2.4 T.S. through Balilla rice plant at level of shoot apex to show leaf arrangement. The rice was grown in pots at 25°C in 2,700 lux for 4 weeks. Sectioned at 8 μm; Sharman's stain.



I

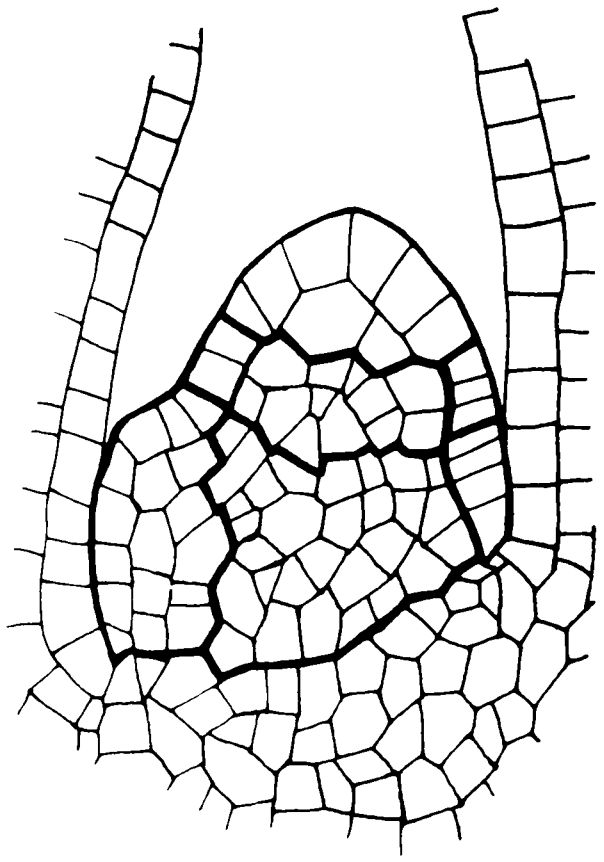


II



KEY

s-st	sub-summit tunica
s-sc	sub-summit corpus
lf	leading flank
tf	trailing flank



III

50 μm

Fig. 2.5 The six regions of the rice shoot apex throughout a plastochron.

The apices were examined by excising the stem and cutting off the leaves about 50mm above the apex. The tissue was fixed in a mixture of three parts ethanol to one part acetic acid. Reduced pressure was applied to remove air bubbles trapped between the leaf bases. After 24 hours at 4°C, the tissue was dehydrated, embedded in paraffin wax, sectioned longitudinally at 8μm (or 6μm in the case of tritiated thymidine experiments) and stained in Feulgen after 6 minutes hydrolysis in 1 Normal hydrochloric acid at 60°C.

The Phases of Mitosis

The numbers of cells in each region in the three median sections were counted and those in mitosis were recorded as being in prophase, metaphase, anaphase or telophase using the following criteria for distinguishing between the phases, in addition to the commonly accepted characteristics.

Prophase: chromosomes swollen.

Metaphase: nuclear membrane absent.

Anaphase: chromosomes still fat.

Telophase: remnant of mitotic spindle still present and when two nuclei had dense chromatin and were obvious twins.

3. THE MITOTIC INDEX THROUGH TWENTY-FOUR HOURS

Introduction

The mitotic index of the shoot apex was studied over 24 hours in order to test for diurnal rhythm in cell division. Presence of synchrony in division would severely restrict the methods which could be used to measure the rate of cell multiplication. Mitotic index also gives a tentative pointer to the meristematic activity of a region.

Method

Samples of ten apices were taken every two hrs for 24 hours and the three most median longitudinal sections examined. In each region, the number of cells in mitosis and interphase was counted and the percentage of cells in mitosis calculated to obtain the mitotic index. Four investigations were carried out in the conditions set out below

Experiment	Light (Lux)	Temperature (°C)	Rice Variety
1	Absent	33/4	Unknown
2	Absent	33/4	Balilla
3	10,000 for 12 hours daily	28/9 in light 25/6 in dark	" "
4	2,400 for 24hrs.	33/4	"

Table 3.1. Experiments carried out to investigate synchrony.

Results

The results are shown in Figs. 3.1 - 3.6 in which the results are grouped together with the same regions from each of the four experiments shown on the same page. Each value was from ten apices. The average cell number counted in each region from ten apices is recorded. The standard errors are calculated from the following formula

$$S.E. = \pm\sqrt{\frac{\frac{r}{N}\left(1 - \frac{r}{N}\right)}{N}}$$

where r is the number of mitoses

where N is the number of cells

This formula was used because the more usual formula

$$\left(\pm\sqrt{\frac{\Sigma(X - \bar{X})^2}{N \quad (N - 1)}}\right)$$

is not valid when there are more than one or two zero values, which is the case here, several apices having zero mitotic index in some regions. It can be used for populations which have a normal variation, and this was found using the χ^2 contingency test.

Discussion

The general level of mitotic index was low compared with root apices. Average mitotic indices for each region through 24 hours are given in Table 3.2. The range is 0.3 to 2.5, whereas in maize roots it is 1.6 in the quiescent centre to 14.5 in the stele first above it (Clowes 1961b). The summit of the rice shoot apex had a lower index than all regions and often no mitoses were seen at all. The corpus and flanks always had much higher indices than the regions

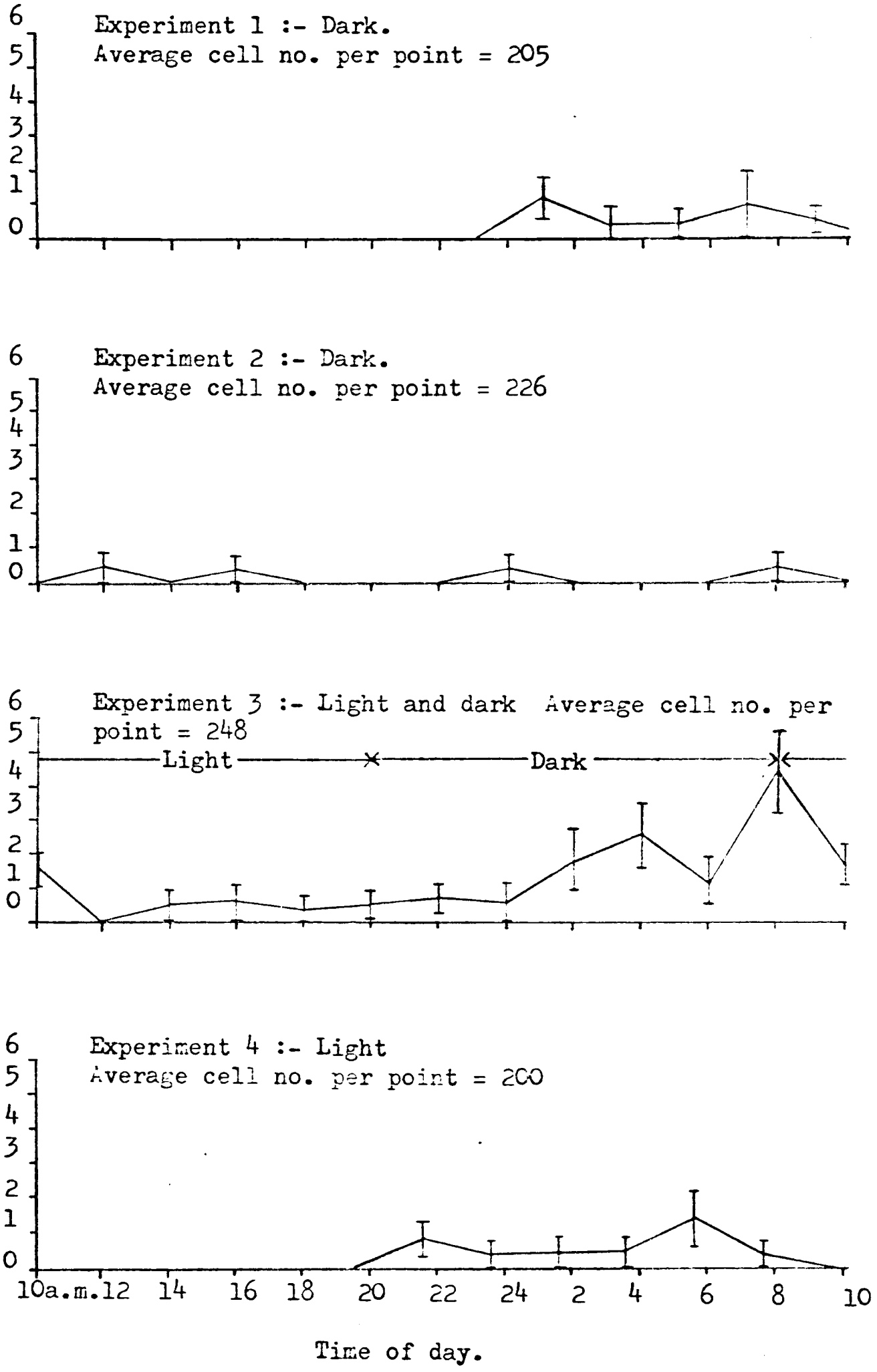


Fig. 3.1. Variation of mitotic index through 24hrs in the summit

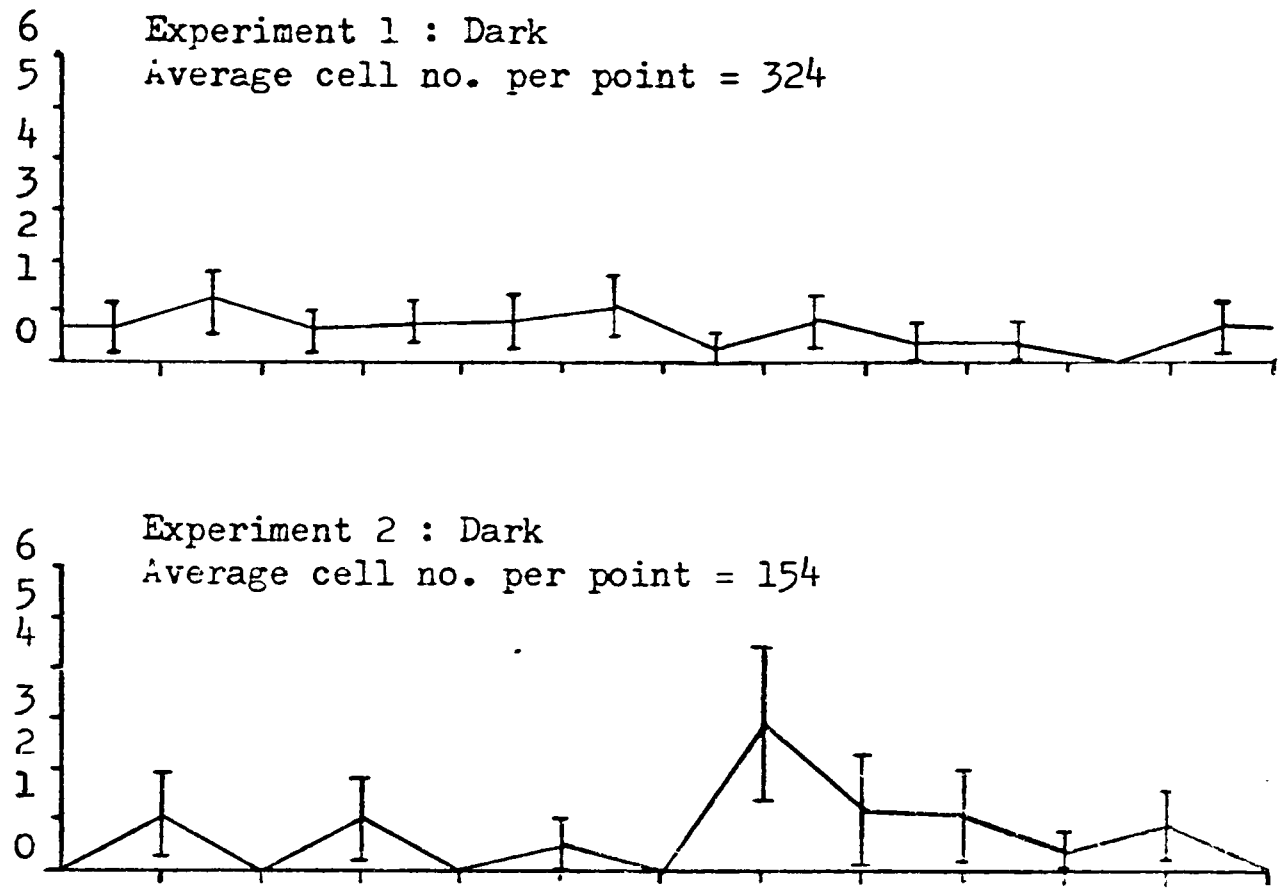


Fig. 3.2 As in Fig. 3.1: sub-summit tunica.

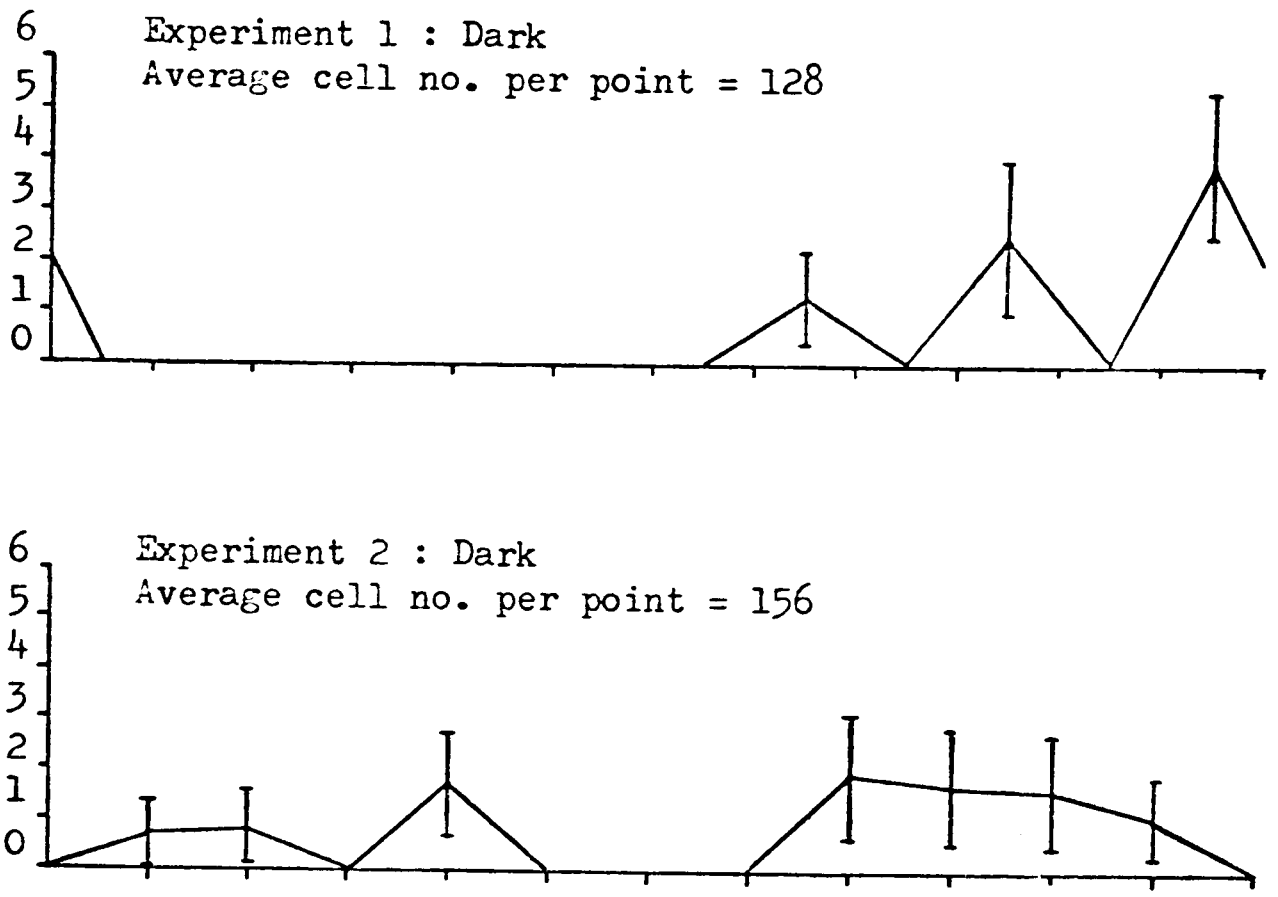


Fig. 3.3. As in Fig. 3.1. Sub-summit corpus

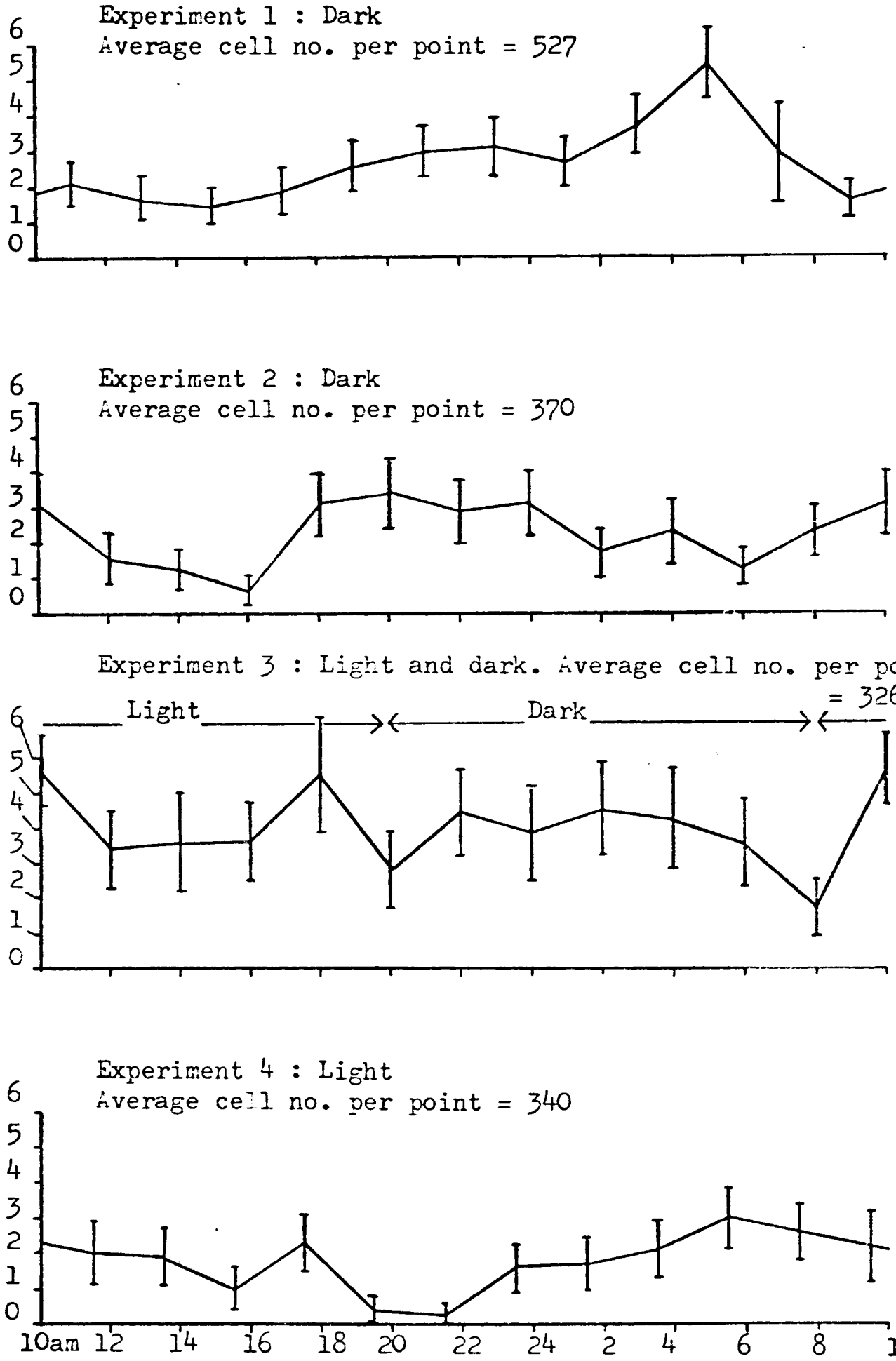


Fig. 3.4. As in Fig. 3.1 : leading flank

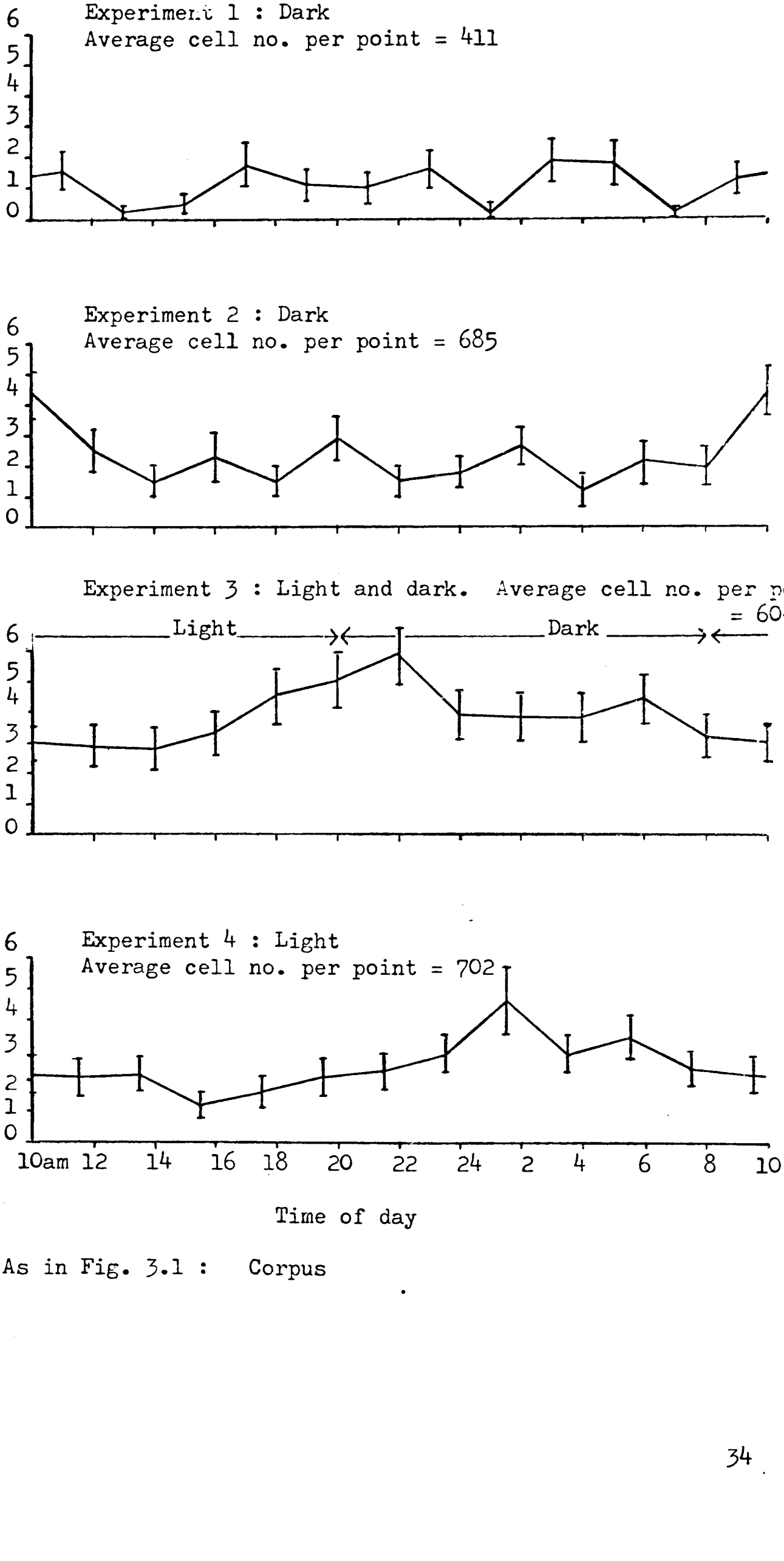


Fig. 3.5 As in Fig. 3.1 : Corpus

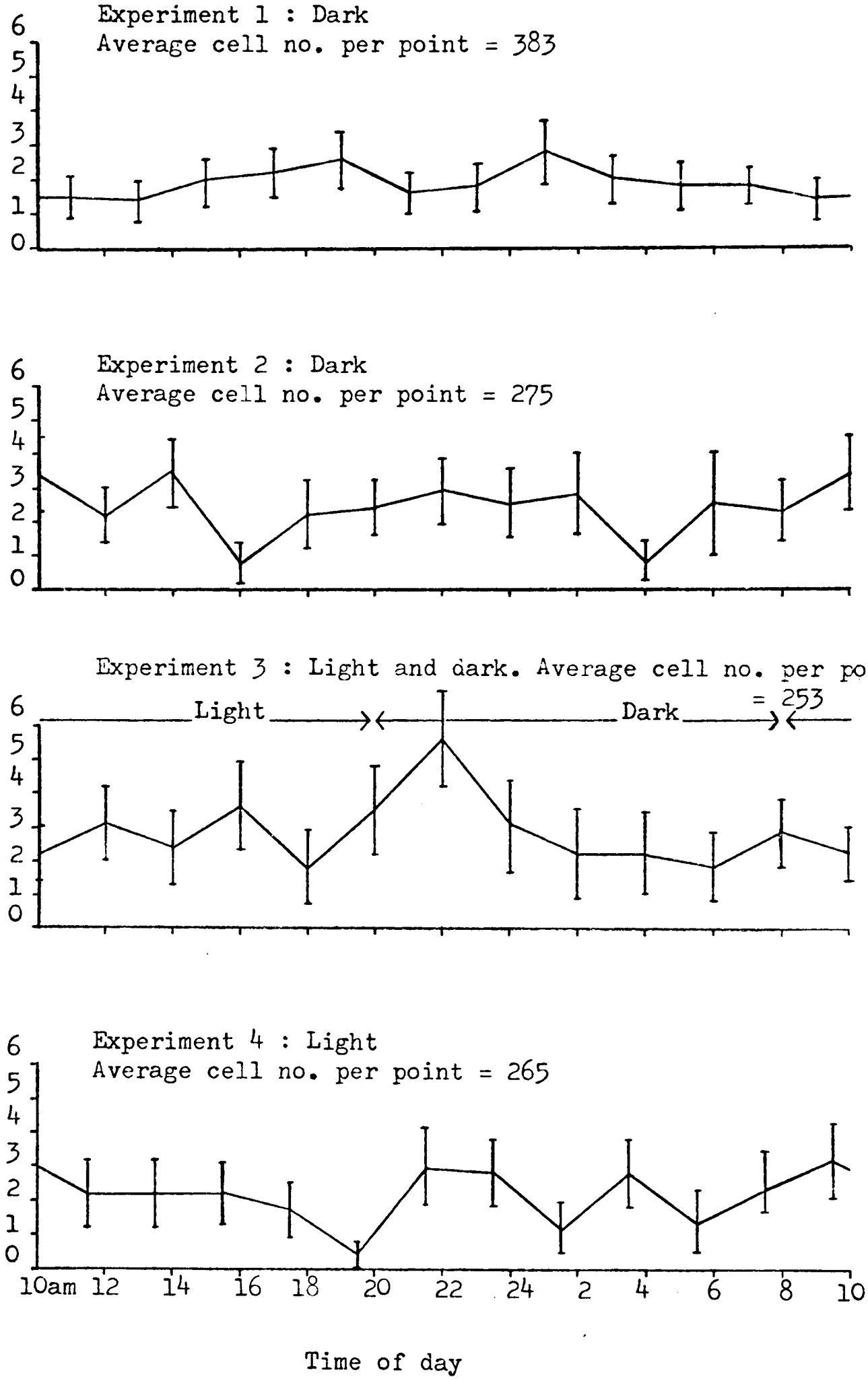


Fig. 3.6. As in Fig. 3.1. trailing flank

Region	Dark grown, 33-34°C		12 hours light 28-29°C 12 hours dark 25-26°C	Constant light 33-34°C
Summit	0.3	0.1	1.3	0.3
Sub-summit tunica	0.6	0.8	2.4	0.4
Sub-summit corpus	0.6	0.8	2.5	1.1
Leading flank	2.7	2.2	3.9	1.8
Corpus	1.1	2.2	3.9	2.5
Trailing flank	1.8	2.3	3.0	2.1

Table 3.2 Average Mitotic Indices in the Regions over 24 hours.

distal to them. These are the areas where rate of cell production would be expected to be high since these are the sites of leaf and stem formation.

The higher values in the plants grown in a light/dark régime as opposed to constant dark or constant light may be due to greater activity in this more natural régime or the higher light intensity. However, it would require further examination to rule out the alternative explanation that the relative length of mitosis compared with interphase is prolonged.

There seems to be no pronounced diurnal rhythm in the conditions used here even in those plants subjected to a diurnal light régime. It could be said that the variability of the material combined with the low numbers of cells and low mitotic indix could hide any small diurnal rhythm that existed. The most pronounced peak occurred in the leading flank in the first dark experiment at 5pm. An analysis of variance test (see Appendix 1) indicated that the peak would occur rarely (1 in 1,000) if the cells were completely independent. So there is the possibility of synchrony present in this case. However, the increase in mitotic index is only 2% and could be explained by a small amount of local synchrony as a result of first and perhaps even second generation daughter cells tending to divide in phase. This was the only pronounced peak, and was present in the first rice used and not in the Balilla variety which was used for all the succeeding experiments.

Synchrony has been variously reported absent (Popham 1958, Denne 1966b)and present (Lance 1952, Edgar 1961, Denne 1966b)and there does not seem to be any great significance attached to it.

The few divisions through 24hrs seen in the summit in each of

the four experiments (10, 4 38, 9 out of an average total number of 2,700 cells) does indicate very low meristematic activity compared with the leaf primordium values of 168, 99, 194 and 91 out of 4-6,000 cells. The reality of these observations is tested in the next section, where rates of cell multiplication are found.

4. THE LENGTH OF THE MITOTIC CYCLE MEASURED BY COLCHICINE-INDUCED METAPHASE ACCUMULATION

The use of colchicine to measure meristematic activity is based on its action in stopping dividing cells at metaphase. The faster the level of metaphases pile up with time, the higher must be the rate of division.

Methods

Four-day old rice plants grown in darkness or continuous light, at 30°C or 35°C were transferred to 0.05% colchicine solution. Samples of at least ten plants were made at the beginning (0hrs) and at various times afterwards, up to 18hrs. The nuclei in each of the four stages of mitosis and in interphase were counted and the percentage in metaphase calculated and represented graphically.

Long experimental times were used initially in the assumption that the expected low rates of cell multiplication would necessitate many hours in order to accumulate a measurable increase in the percentage of metaphases. However, in order to find accurately the pattern of accumulation, progressively shorter times were used. As can be seen from the graphs, (4.1 - 4.4) this finally led to experimental times of just a few hours.

Values in the first experiments were calculated from 30 apices per sample. Later experiments were from 10 apices per sample since a high degree of accuracy was not required in preliminary experiments in which the experimental times were the main question. (See also paragraph under 'Sample size'). Experimental conditions were also changed in later experiments by growing the plants in the light, in

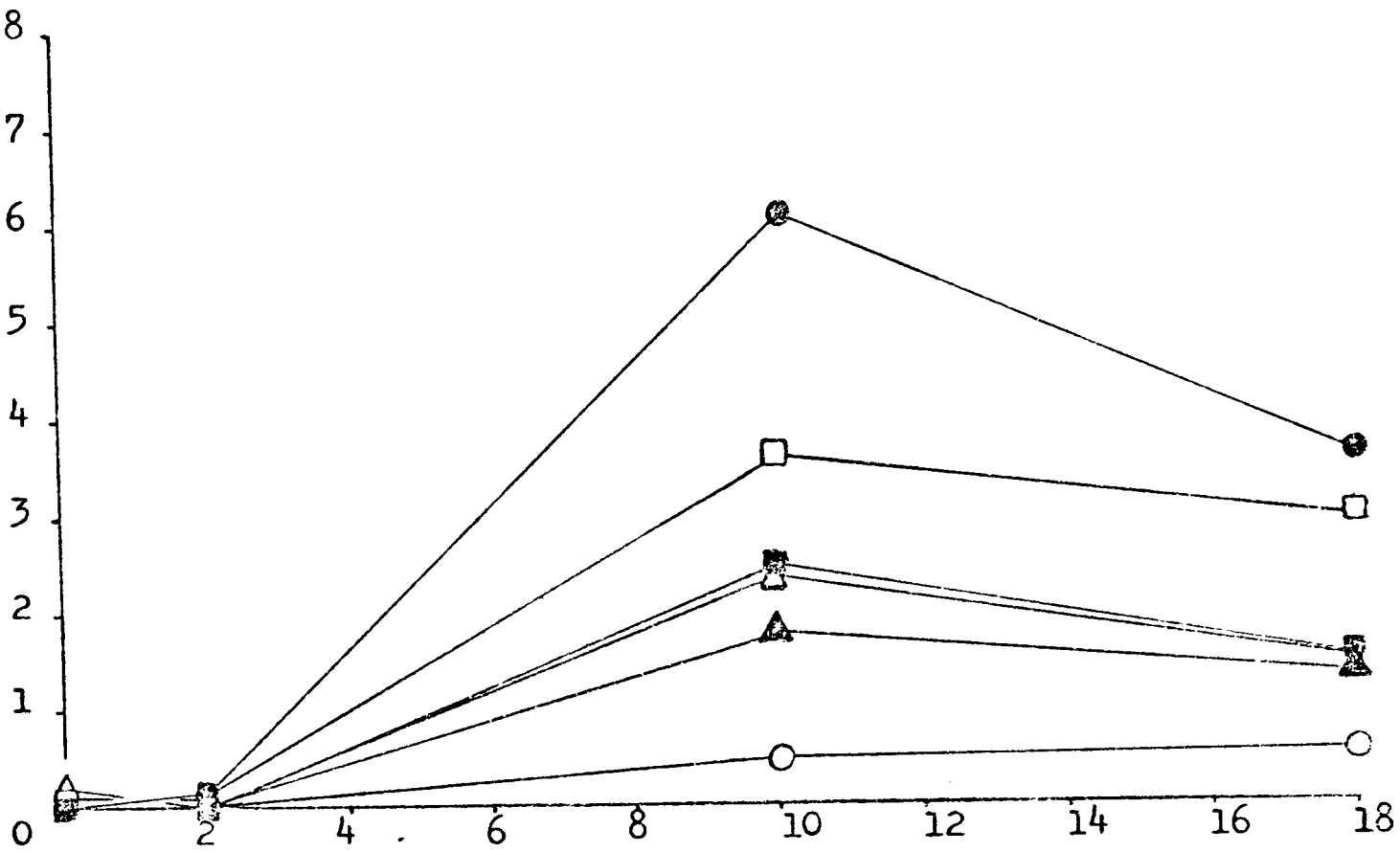


Fig. 4.1 Each point was from 30 apices. The rice plants of the unknown variety were grown in darkness at 35°C for 5 days.

Explanation of symbols

summit
sub-summit corpus
corpus
sub-summit tunica
leading flank
trailing flank

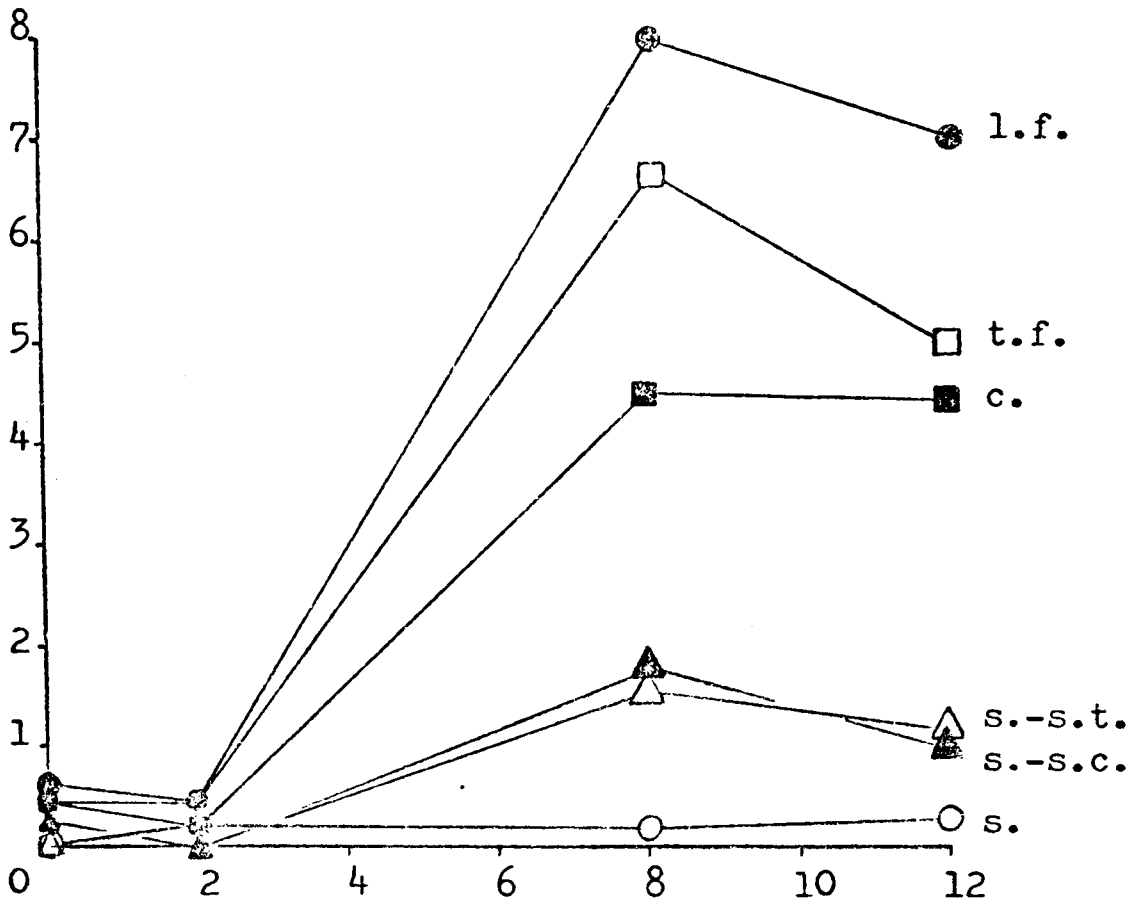


Fig. 4.2 Each point was from 30 apices. Rice plants, var Balilla were grown in the dark at 35°C. for 5 days.

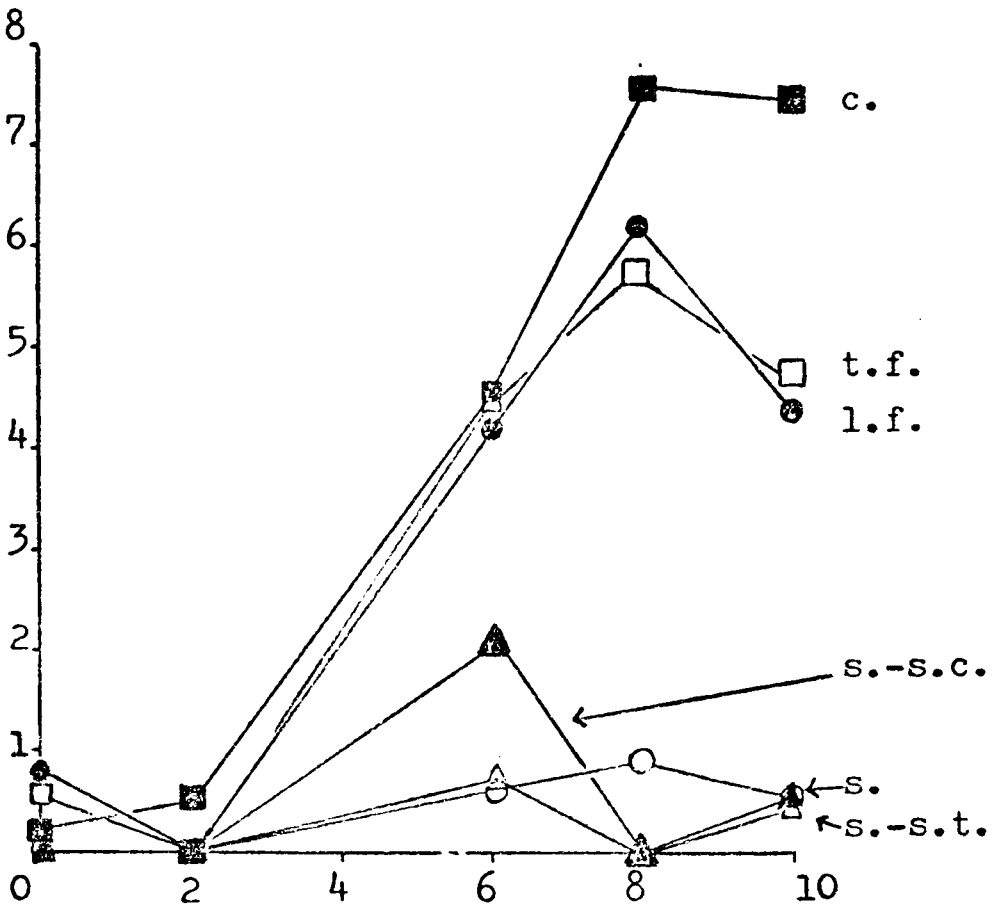


Fig. 4.3 Each point from 10 apices. Plants grown in 2,400 lux fluorescent light at 35°C for 4 days.

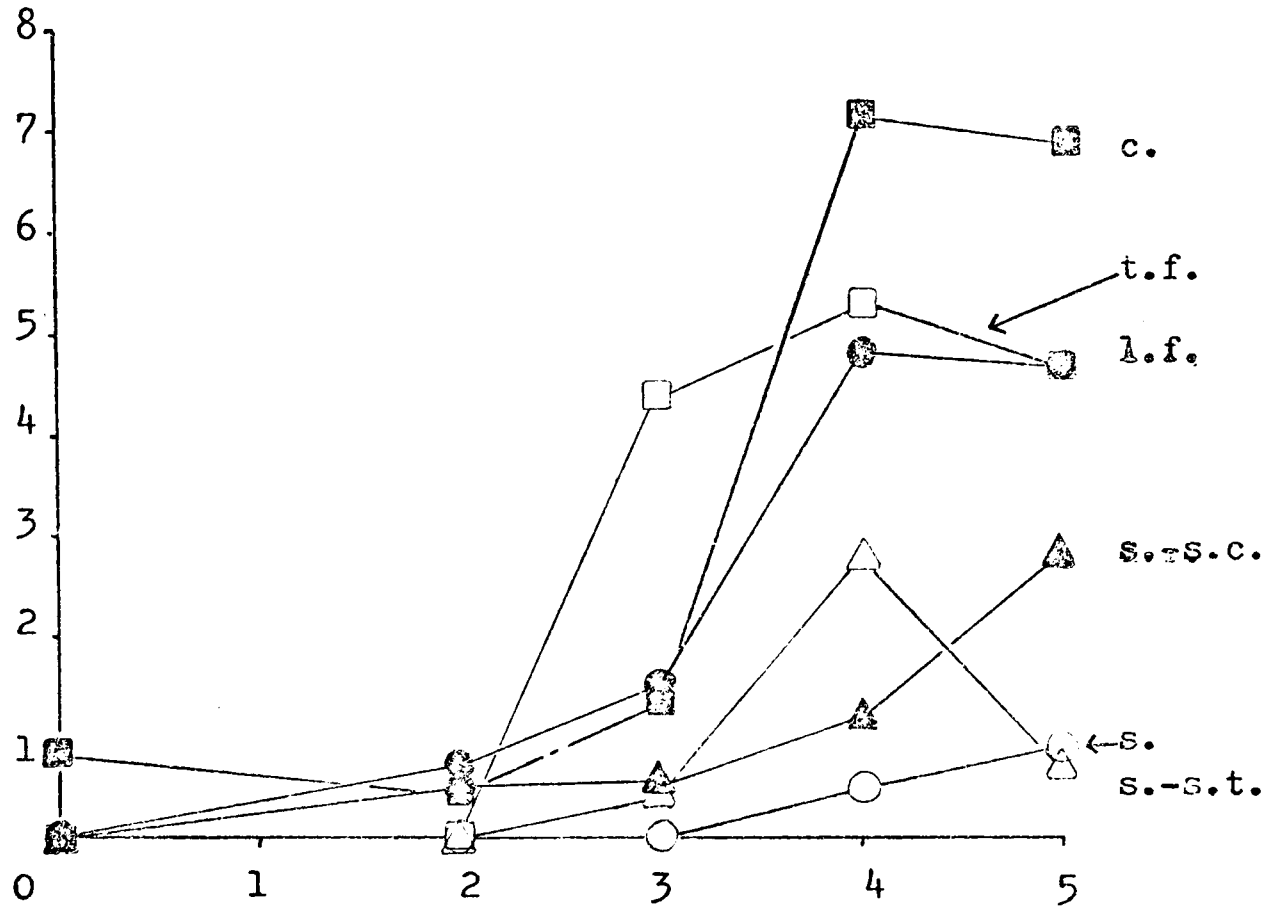


Fig. 4.4 Each point from 10 apices. Plants grown in 2,400 lux fluorescent light at 35°C. for 4 days.

order to obtain maximum growth rates.

Results

The four graphs depicted in Figs. 4.1 - 4.4 show an initial lag phase of at least two hours in which metaphases do not accumulate. This is due to the slow permeation of the apex by the colchicine and can readily be confirmed when sections are viewed under the microscope since there are no characteristic colchicine-type metaphases ie. no mitotic-spindle and with the chromosomes clumped together instead of aligned round the spindle equator, until after 2hrs.

After an increase in percentage metaphase the levels dropped due to the formation of restitution nuclei in which the cells pass into interphase without separation of the chromosomes and the nucleus becomes tetraploid.

The calculation of T and its significance

The gradient of the increase in percentage metaphase is related to the average rate of cell multiplication and a value for the duration of the cell cycle can be obtained using the formula of Evans, Neary and Tonkinson which was adapted by Clowes to give cell-doubling times in cases when the growth fraction is not known. (Evans, Neary and Tonkinson 1957, Clowes 1961b). This is because a population of dividing cells may have a proportion of cells which are not cycling and where this proportion is unknown, the actual rate of mitosis is not revealed in this method. The fraction of

cells in a region which are cycling ie. the growth fraction, can only be found readily by pulse-labelling techniques. The use of colchicine however, does enable the average rates of cell multiplication of the areas to be found and thus gives absolute values which allow useful comparisons to be made.

The formula used to calculate cell-doubling time was as follows:-

$$T = \frac{\log_e 2}{\lambda}$$

where T is the complete mitotic cycle time

and λ is a constant which is obtained from the gradient of the slope of metaphase increase given by

$$e^{\lambda s} . \lambda (1 + \lambda \bar{x})$$

where s is a constant related to the number of cells in metaphase, anaphase and telophase in an untreated meristem by the following formula

$e^{\lambda s} - 1$ = fraction of cells in metaphase, anaphase and telophase and $\bar{x}$ is half the time interval over which the gradient of increase in metaphase is taken

Percentage prophase

The formula of Evans, Neary and Tonkinson for calculation of cell-doubling time assumes that the rate of entry into mitosis is unaltered by the colchicine treatment and since there are some reports (MacLeod and Davidson 1968) that colchicine may sometimes affect other parts of the mitotic cycle beside metaphase, this had to be checked. MacLeod et al working on Vicia faba roots, found that 0.025% colchicine had no effect on prophase until 7 hours but 0.5% colchicine depressed

prophase slightly in the first few hours. An example of prophase indices during the course of an experiment is given in Fig. 4.5. These indicate a slight depression in most of the regions after 2 hours which may be due to colchicine or may be within the normal fluctuation of the system. The cell-doubling times calculated could, therefore, be slightly less than actual, but the effect is not great enough to discount the method entirely so long as a count of the percentage prophase in each experiment is made.

Sample size

The value for each point was calculated from ten, twenty or thirty plants. Where ten plants were used the standard errors could be considered rather large (Table 4.1) and so an examination of the reliability of the results was carried out. This is set out in the appendix on sample size. It showed that useful preliminary results could be obtained with ten plants in spite of the small cell numbers involved.

Experiments were carried out using the shorter treatment times of 0 - 4,5 or 6 hours. Conditions of growth were varied in order to find those which produced fastest growth ie. rate of cell production since these are necessary for investigation of the cell cycle by pulse-labelling techniques in which a short pulse must be used but sufficient nuclei must be labelled to produce high enough numbers labelled. Percentage metaphase levels are shown in Figs. 4.4,4.6 &4.7 and cell-doubling times found in the different regions with the growth conditions used are summarised in Table 4.2. It will be seen here that the higher illumination, coupled with a lower temperature, produced extremely fast rates. This experiment was initially carried out with 10 apices

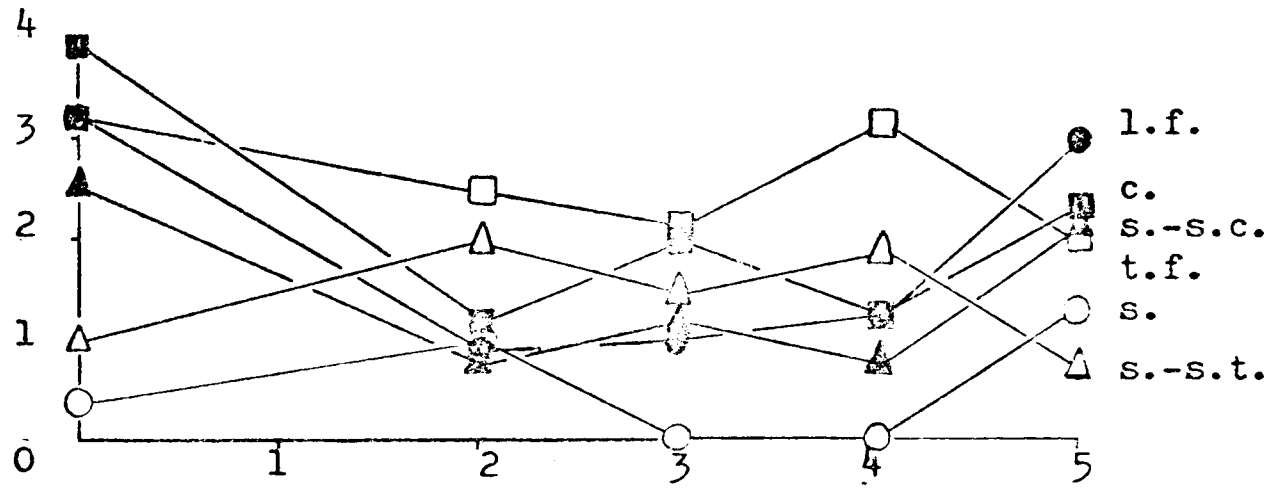


Fig. 4.5 Percentage prophase of plants grown in 10,000 lux. Samples at 0, 3, 4 & 5 hrs. were of 20 plants; the 2 hr. sample was 10 plants.

Region	Hours in colchicine				
	0	2	3	4	5
s	0	0	0	0.46±0.46	0.91±0.64
s-s.t.	0	0	0.41±0.41	2.78±1.20	0.70±0.70
s-s.c.	0	0.50±0.50	0.49±0.49	1.18±0.83	2.70±1.33
l.f.	0	0.60±0.42	1.52±0.60	4.67±1.20	4.58±1.24
c.	0.81±0.36	0.35±0.24	1.28±0.45	7.14±1.10	6.75±1.15
t.f.	0	0	4.26±1.20	5.18±1.40	4.63±1.43

Table 4.1 Percentage metaphase values in plants grown at 2,400 lux continuous light, 35°C. Samples were of 10 plants and standard errors are given

Illuminance (Continuous Light in Lux)	Number of Apices per Sample	Temperature °C	Cell Doubling Time (hrs.) Region					
			s.	s-s.t.	s-s.c.	l.f.	c.	t.f.
2,400	10	35	302	50	208	35	21	27
10,000	10	30	∞	∞	12	7	7	6
10,000	20	30	86	50	28	11	12	16

Table 4.2 Cell-doubling times of the six regions in the apex under different growth conditions

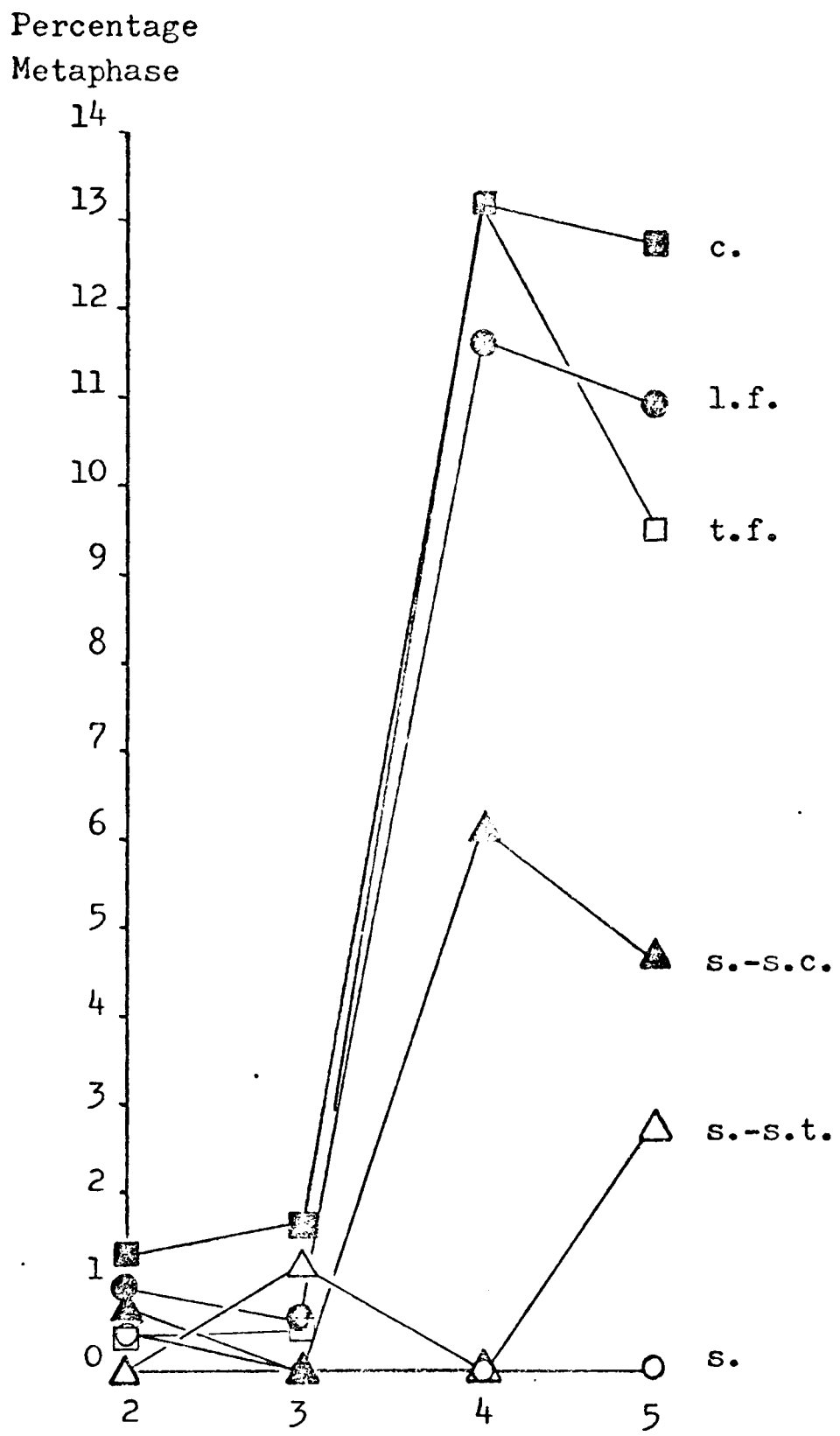


Fig. 4.6 Metaphase accumulation on rice grown in 10,000 lux continuous light at 30°C. for 4 days. Each point from 10 apices.

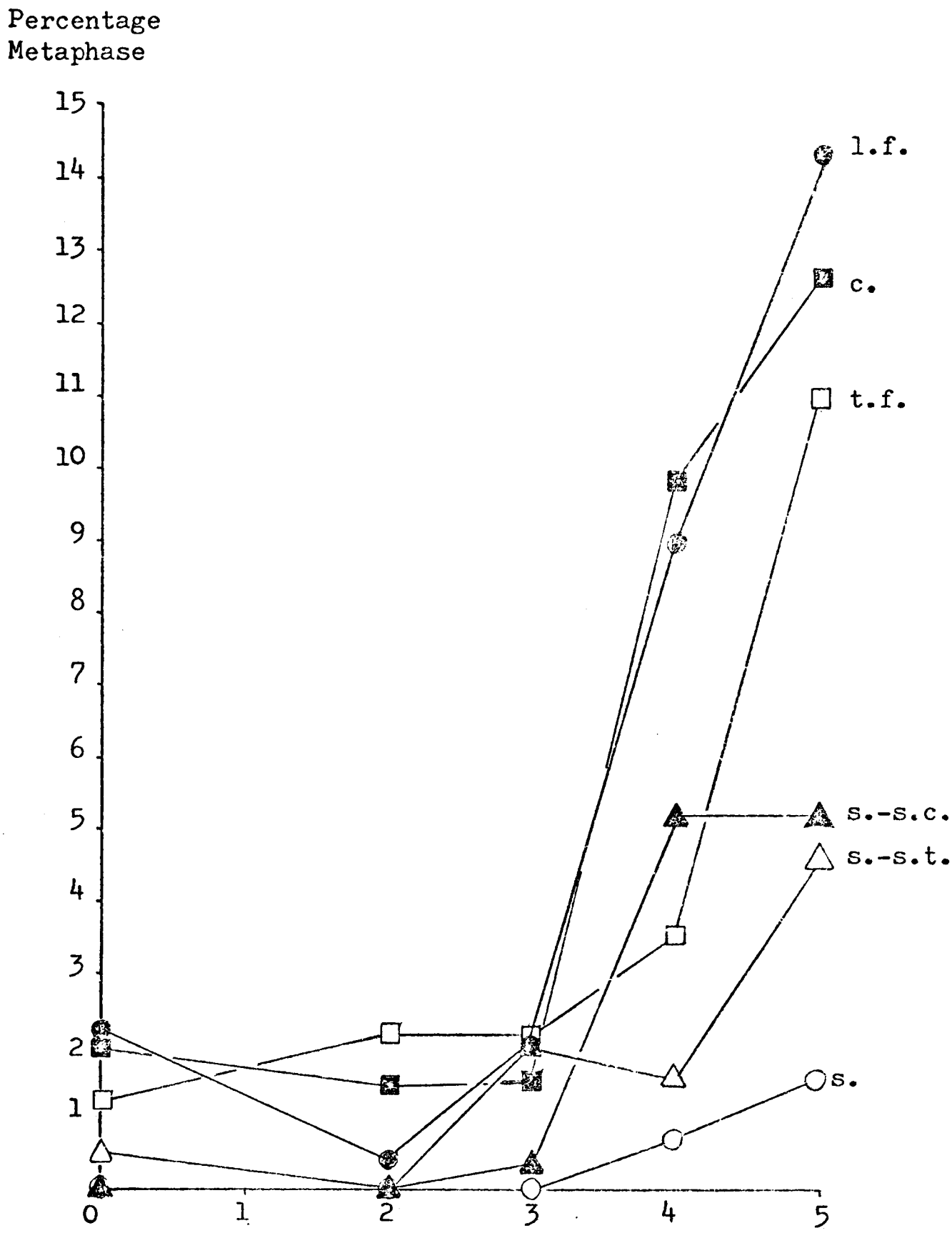


Fig. 4.7 Metaphase accumulation on rice grown in 10,000 lux continuous light at 30°C. for $4\frac{1}{2}$ days. Each point from 20 apices apart from 2 hour sample which was from 10 apices.

per sample, but was repeated using 20 per sample.

Discussion

The major result which is borne out by all these experiments is the extremely low level of metaphase accumulation and long cell-doubling times found in the summit. Where the rate of cell production was measurable, the value was at least eight times slower than in the leaf primordium.

Absolute Values

The times measured at 2,400 lux continuous illumination are comparable with those reported elsewhere. (Corson 1969, Lyndon 1970a). At an illumination of 10,000 lux at 30°C, the cell-doubling times found in the corpus and flank regions were shorter than any reported in shoot apices. It is impossible to compare the times for rice with those of most other workers, since the latter do not give the light intensities used. Corson (1969) using Datura gave 8hrs of 1,600 - 2,000 ft. candles (about 16,000-20,000 lux) at 25°C and found cell multiplication times of 36hrs for the flanks and 76hrs for the summit. He measured accumulation of metaphases between 4hrs and 8hrs and divided the apex into only three regions and so the summit region was larger. The much higher rates of mitosis found in rice could be explained by the higher temperatures used or the shorter accumulation times which record the rise in metaphases more accurately. Bodson (1975), when comparing the results from different authors, thought shorter cycling times were due to higher temperatures and younger plants.

The values given for the sub-summit regions are less reliable

than for other regions. They are the smallest regions in the apex in terms of cell number and overall size and so the counts of cells in metaphase will necessarily be subject to larger fluctuations. However, they do represent a different ontogenetic part of the apex in that they are not at the summit, and the outer region, the sub-summit tunica is where the leaf primordium will arise. Because of this they should be considered separately. The cell-doubling times found vary widely, but are always longer than in the regions below, and shorter than in the summit.

Delayed penetration

The delay of penetration of the colchicine into the shoot apex compared with the root, which is penetrated in less than 15 minutes, is interesting. The root, of course, is an organ especially adapted for uptake from the surrounding medium, while the shoot is not. The path of penetration of colchicine seems to be through the leaves. This highlights the difficulty of work with shoot apices because of their inaccessibility. However, the advantage of using rice is demonstrated by the fact that the colchicine does, at least, reach the apex without subjecting the plant to the disturbances employed by other methods.

The Effects of Excision

While the use of rice did provide a means of measuring rates of cell multiplication using colchicine without interference with the plant by excision or other such means, rice did not lend itself immediately to work with ^{3}H - thymidine since this also was slow to be taken up (see next section). Since many workers were excising apices

in their investigations, it was thought worthwhile to assess the effect this has on the organisation of the apical meristem in rice and also to see if excision of rice apices could be used to give meaningful results with ^{3}H - thymidine. With these two aims in view, an experiment was designed and carried out as follows.

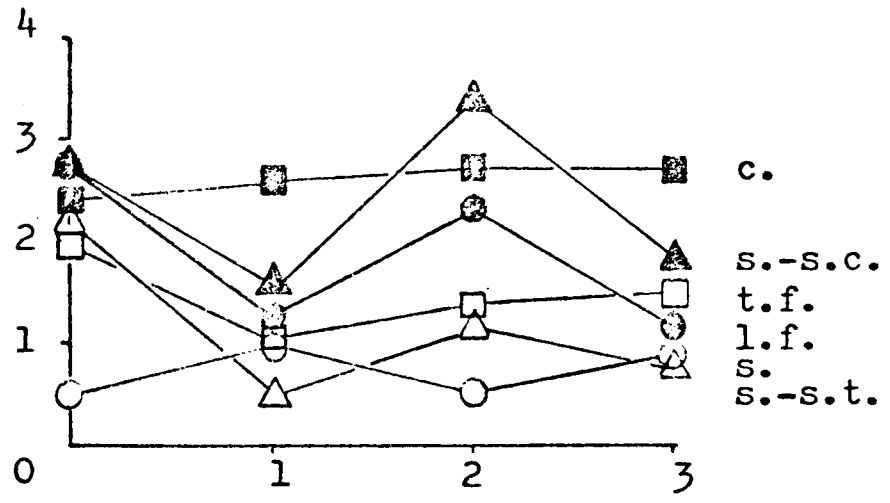
Experiment

The shoots of 4-day old seedlings grown in continuous light were excised at the level of the epicotyl. The coleoptile was stripped off as well to free the rest of the shoot completely. The shoots were placed in 0.05% colchicine at the same time as the control group of uncut plants. Samples of 25 plants were taken hourly for five hours. Since it was well established that colchicine did not penetrate until after two hours in normal circumstances, the control plants were not sampled until 2 hrs. The results are shown in Figs. 4.8 and 4.9. Values for cell-doubling times are shown in Table 4.3. Calculations were made from 3 and 5 hour values in the control and 1 and 2 hour values in the excised plants. Colchicine was taken up more quickly in the excised plants, so that metaphase accumulation was taking place at two hours.

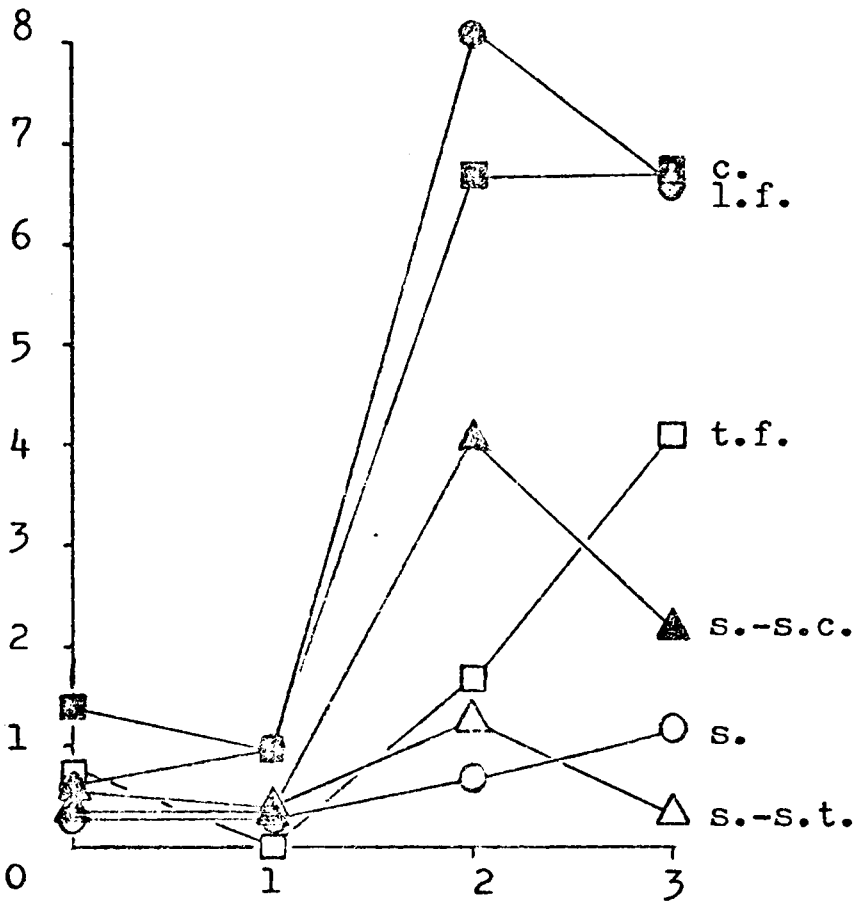
Result

The shorter cell-doubling times in the sub-summit corpus, leading flank and corpus in the excised plants indicate that the cells were stimulated. The trailing flank does not appear to be stimulated. This is surprising since it would be expected to react in the same way as the leading flank because they both are involved in formation of the same leaf. The summit and sub-summit tunica regions do not appear to

Percentage
Prophase

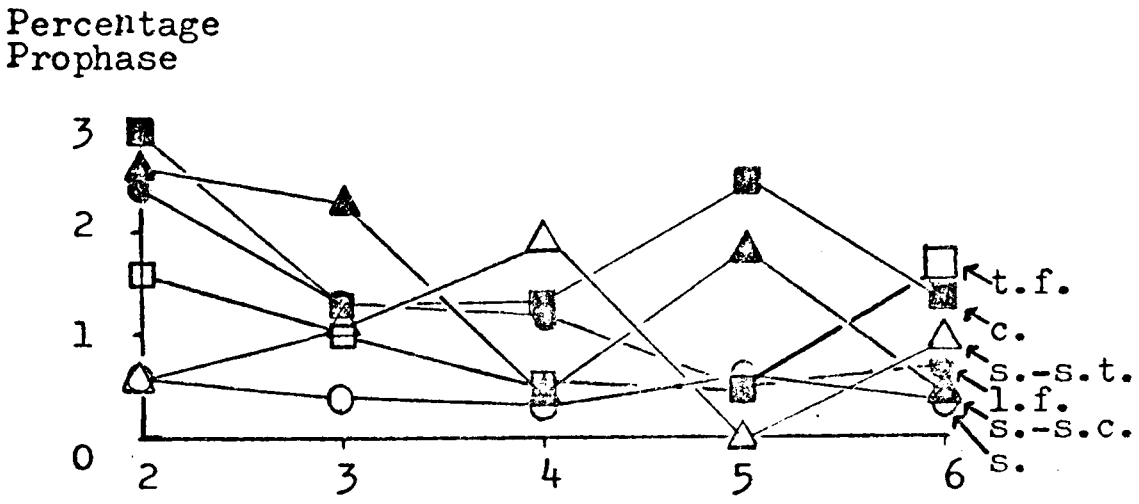


Percentage
Metaphase



Hours in Colchicine

Fig. 4.8 Prophase and metaphase levels in excised plants.



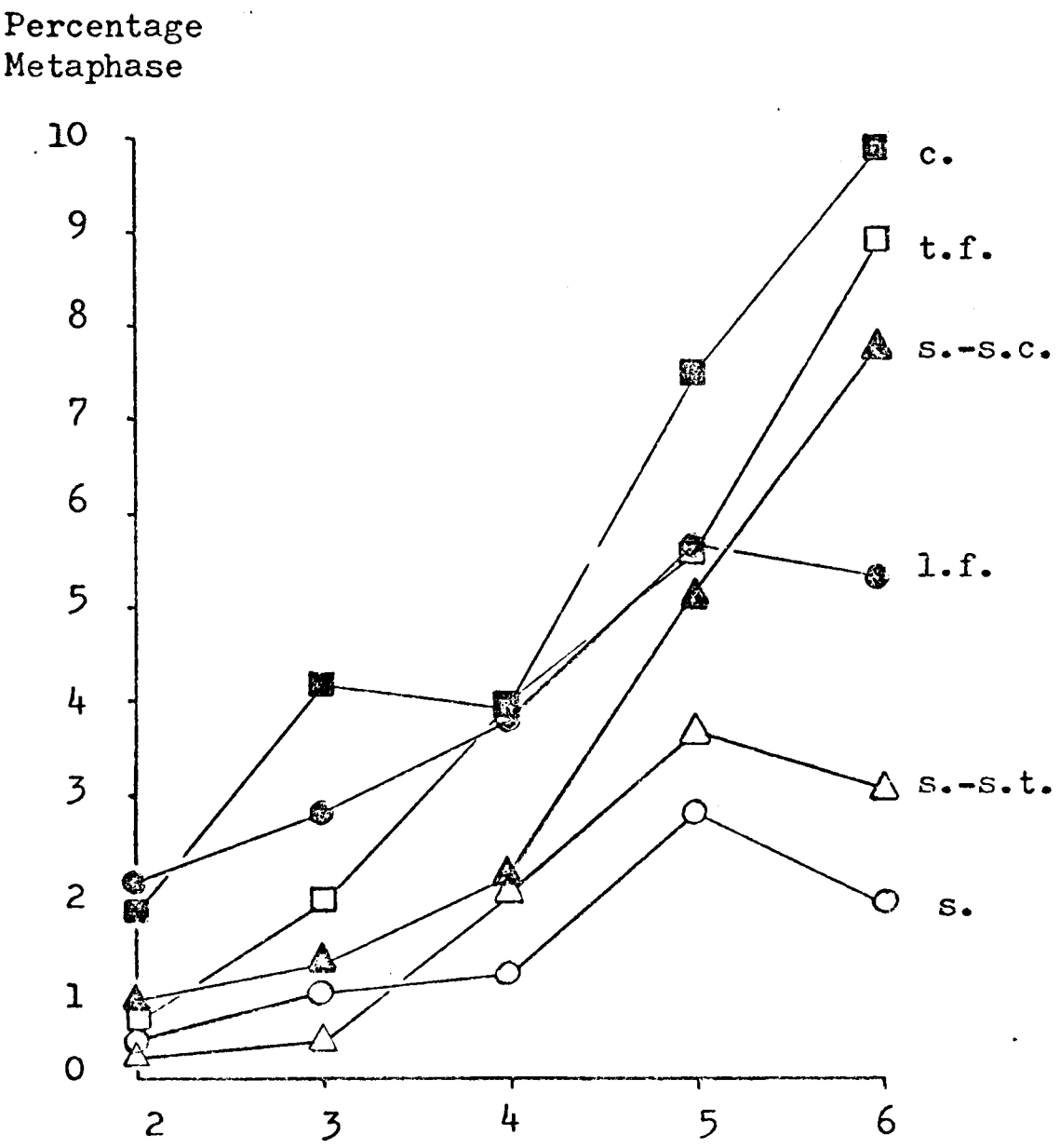


Fig. 4.9 Prophase and metaphase levels in control plants.

Apices per Sample	Treatment	Cell-Doubling Time (hrs.) Region s.	s-s.t.	s-s.c.	l.f.	c.	t.f.
20<	Control	73	41	37	51	44	37
20<	Excised	139	70	18	10	12	41

Table 4.3 Cell-doubling times in the six regions in plants which had been excised with control values for comparison. Plants had been grown in 2,400 lux continuous light at 34°C for the first 3 days and at 10,000 lux continuous light and 32°C for a further 2.5 days.

be stimulated.

Discussion

These results are interesting since they show that, in the conditions used here, the organisation of the shoot apex is altered by excision, at least in the first few hours after the cut. They make an interesting comparison with the work of Evans, Knox and Rijven (1970) who found that excision stimulated uptake of ^{3}H - uridine into RNA in the summit and the regions between the leaf primordia of Lolium in the same way that an inductive long day stimulated the uptake of ^{3}H - orotic acid.

The control values here show a different relationship between the regions, compared with results shown earlier. The rates of cell multiplication do not differ so much between the summit and leaf primordium. The latter has a slower rate than in the other experiments and the sub-summit corpus's rate is unusually high. The plants used for this experiment were grown at two light intensities which may have altered the organisation. Also, these plants were $5\frac{1}{2}$ days old, and could have been approaching the end of their food reserves. It is interesting to find this result and it would be useful to know the cause. A similar result was found in the control experiment in the investigation of the effect of X-irradiation (Section 6). The plants for the control in this case were 4 days old. The growth conditions were the same. These results will be discussed in Section 9.

Conclusions

The main contribution to our knowledge of the shoot apex in the work reported here is the markedly long cell-doubling times in the

summit compared with the flanks, a difference of at least eight times. All other reports show only a two-fold difference. We do not know whether this is a feature of grasses, or rice in particular. The big difference found in rice may, however, be a more representative picture of shoot apical meristem organisation and the results recorded for other species may be affected by the various techniques employed to surmount the obstacles inherent in the work. If the reason for the slow cell multiplication of the summit and the influence different conditions have on it, could be found, this would give a pointer to our general understanding of the subject.

The observation of a cell-doubling time in the summit eight times longer than the flanks in rice is particularly interesting in the light of the French view. As early as 1953, Catesson working on Luzula thought that an inactive apical region would only exist when the apex was large enough and the proliferative activity of the anneau initial slow enough not to involve the summit. In the work produced here, rice which has a small apex has a higher meristematic activity in the flanks than any ever reported so, ostensibly these results are not in line with Catesson's views. However, Catesson was making her observations in the climate of opinion that a summit never had any mitoses and so her idea of a 'active' summit could be accomodated by the present idea of quiescence.

The effect of excision in stimulating the areas of the meristem most involved with leaf initiation is an interesting one. It may be similar to a wound reaction though the site of reaction is some distance from the cut, or it may be part of the general reaction of the plant to the sudden loss of the root and endosperm. The organisation of the shoot meristem is changed with this alteration, though this is probably

only shortlived and one imagines that once the normal balance of the plant is restored, by growth of the adventitious roots, that of the meristem is also. If the summit cells were responsible for leaf initiation one might expect them to be stimulated too, but this is not the case, at least within the first few hours of excision.

It may be concluded from this work that the summit cells are multiplying at a very much slower rate than the flanks and that they respond in a different way to excision of the shoot.

5. THE UPTAKE OF LABELLED THYMIDINE

Experiments with labelled precursors of DNA fed to shoot apices have shown, in an overwhelming number of cases, a greater proportion of labelled nuclei in the flanks, compared with the summit. They have, however, been on material which has had to be subjected to abnormal conditions in order to apply the solution. Since this could be avoided in rice the possibility of studying the incorporation of DNA in the apex of an undisturbed plant was explored.

A second aim was to obtain measurements of the rate of cell multiplication and compare these with cell-doubling times from metaphase accumulation techniques.

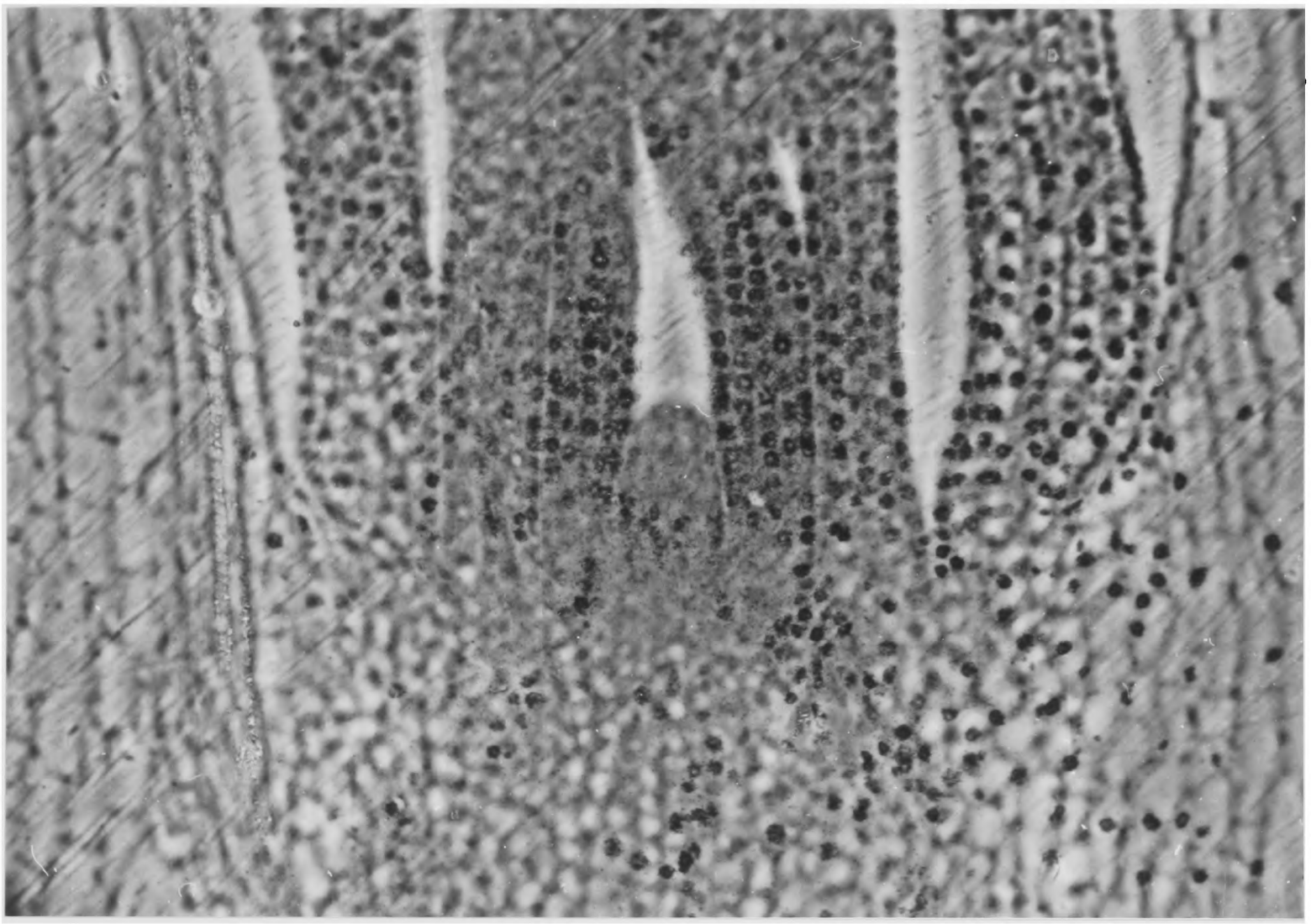
Method

Seeds were sown under 4cm of water and grown at a low illumination of about 1,000 lux or high illumination of 10,000 lux at 30 or 35°C. Labelled thymidine was fed to the rice plants at 0.5 or 1 μci per ml activity (specific activity of thymidine was 23.3 ci/m M). At intervals afterwards, samples were taken and the incorporation of the label investigated by preparing autoradiographs from 6 μm sections using Kodak AR 10 stripping film. The percentage of nuclei labelled in each of the six regions after the various feeding periods was recorded.

Experiments at 1,000 lux continuous illumination

Results

Figure 5.1 shows a longitudinal section of a shoot apex grown at 500-1,000lux and 35°C with silver grains grouped over nuclei.



100 µm.

Fig. 5.1 Rice shoot apex showing labelled nuclei in flanks after 72 hrs. in ^{3}H - thymidine. Plants grown at 35°C for $3\frac{1}{2}$ days in 500 - 1,000 lux.

Sections were Feulgen stained, covered with AR 10 stripping film and photographed with the aid of phase-contrast microscopy.

Two nuclei in the flanks are heavily labelled while the other regions show almost no label. This photograph also shows a high percentage of labelled nuclei in the lower regions of developing leaves and in the procambial strands.

The pattern of labelling with time is rather interesting (Fig. 5.2) since it shows a general increase with time in the lower regions (corpus and flanks) but a fluctuation with time in the upper regions (summit and sub-summit). This fluctuation does show a decrease after the first sample at 24hrs to 0% and then a slight increase again, but this may not have any significance in itself and would require further investigation to assess whether this was a real phenomenon. Remacle-Dath (1965) does report a lack of proportionality with time for labelled cells for experimental times longer than 24 hours and attributes this to the effect of tritium in a nucleus. However, since there is so little tritium in the regions here, this explanation would not at first sight apply here.

Discussion

This was the only experiment in which any of the nuclei in the summit were labelled but this was only to the level of 1% of the cells ie. 1 cell in 128 after 72 hours. Similar results were obtained when the experiment was repeated at 30°C. At this time, 30% of the cells in the leaf primordium were labelled so that thirty times more cells had gone through the DNA synthetic period in the leaf primordium compared with the summit.

The rate of increase in the labelling in the lower regions would produce a level of 100% after 8 days in region 4, if all the cells were cycling. This seems rather long, even allowing for a

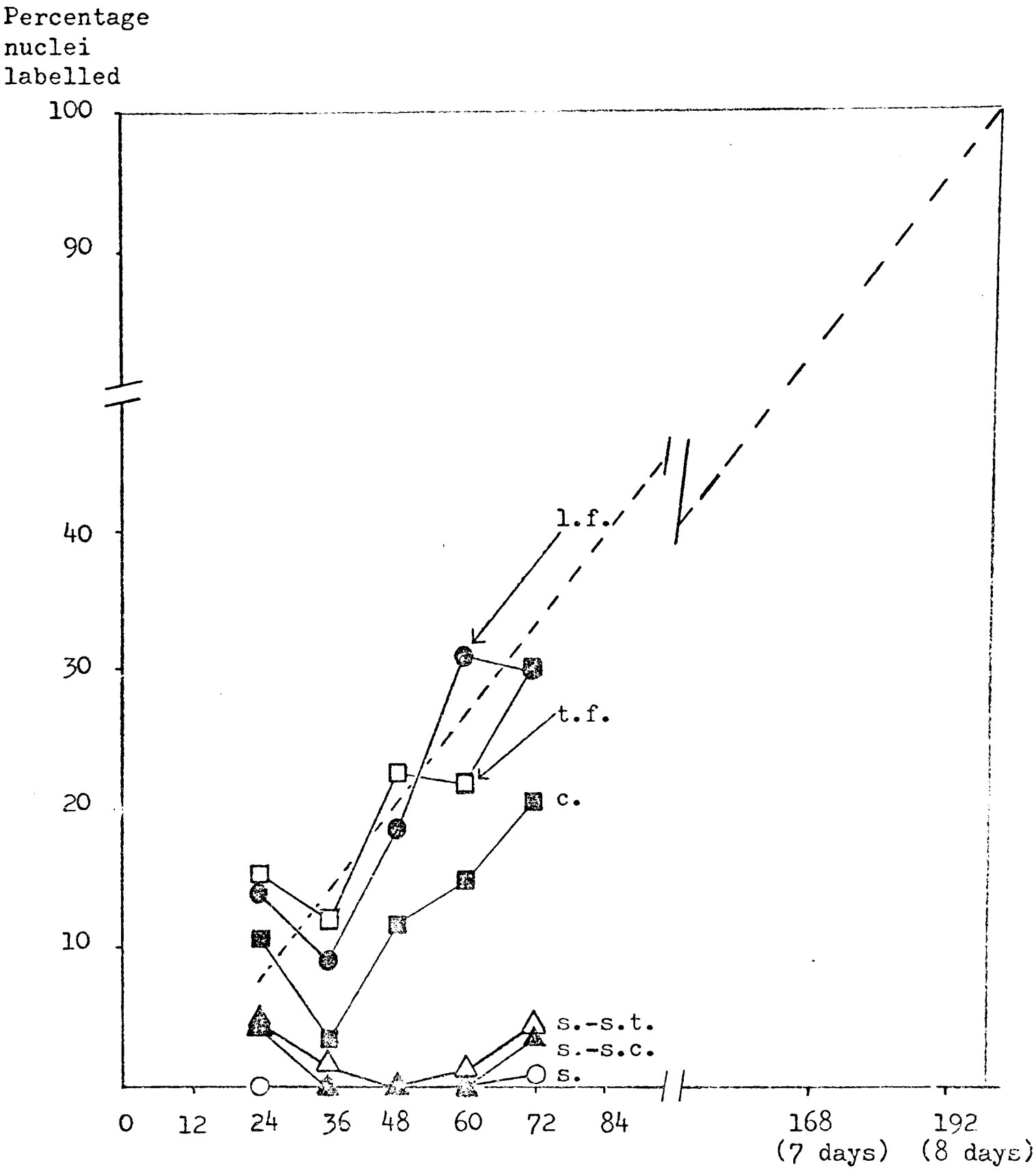


Fig. 5.2 Uptake of ^{3}H-6-thymidine in the shoot apex of rice growing at 500 - 1,000 lux.

delay in uptake of about 12hrs, when compared with cell-doubling times values for the region of 35hrs at 2,400 lux, and 35°C and at least 58hrs in the dark and 35°C.

Whatever the reason for the low rate of increase in the flanks and corpus and the fluctuation in the summit and sub-summit regions there is a marked difference in the pattern of labelling with time, between these two areas. The higher % labelling in the flanks and corpus indicates that the population of cells in the summit region is different from that in the flank regions and the synthesis of DNA is at a much lower level.

Experiments at 10,000 lux continuous illumination

Results

Experiments with plants grown at higher illuminance (which, from metaphase accumulation experiments, produced high rates of cell multiplication) resulted, surprisingly, in no labelling at all.

This could be due to lack of penetration by the chemical into a plant which was less etiolated and therefore more compact and might offer greater resistance to exchange with the surrounding medium, or it could be due to a different physiological balance produced in the plants by a different environment. This balance might be nutritional so that plants at lower light intensities have less opportunity to synthesise their own metabolites and so are more likely to take up and use the added thymidine in the solution. The roots of the plants grown at high light intensities were examined to check that techniques were being carried out correctly and were found to be labelled as early as one hour and in the expected pattern ie. dense labelling in the cap initials and stele but no labelling in the quiescent centre.

Since the high rates of cell multiplication found at high light intensities made the possibility of carrying out a pulse-labelling experiment more feasible, and since a pulse-labelling experiment would yield so much information about the cell kinetics of the different regions, many attempts were made to label the apex at the high intensities (Table 5.1). Some attempts involved mutilation of the plant which, as has been stated, are undesirable, but as any effects on cell kinetics could be checked with metaphase accumulation experiments and compared with control, undisturbed plants it was felt a worthwhile compromise. Other attempts were the use of $^{3}H - CH_3$ - thymidine instead of ^{3}H - 6 - thymidine and growth of plants in red, far-red and blue light since far-red light produces growth similar in many respects to dark-grown plants (see section 8).

Discussion

Where surgery of the plant did produce labelling (and this was not always the case) the chronological pattern was very different from the experiments with whole plants. The meristems became labelled much earlier (about 3 hours), but labelling did not increase with time. In addition the grain density was much less. (Fig. 5.3). The roots of these plants were sampled at the same time and exhibited normal labelling patterns. No increase in labelling of the shoot apex was obtained by using double the concentration ie. 2 Ci/ml.

The failure of the apex to accumulate label with time prevented measurement of the rate of cell turnover by this method, which was disappointing. The pulse-labelling technique was also inapplicable, particularly as the label appeared not only to be not accumulated but actually lost.

Results

a) Surgical experiments with ^{3}H - 6 - thymidine. Plants grown under 10,000 lux.

Treatment	Labelling* successful or not	Stem apex regions labelled*	Chronological labelling* pattern
Coleoptile removed	✓	Flanks and corpus	Maximum at 5 hours
Coleoptile removed 14 hours before experiment	✓	Flanks and corpus	Maximum at 8 hours
Seed removed	X		
Seed and coleoptile removed	X		
Seed and root removed ie. whole shoot excised	✓	Flanks and corpus	Maximum at 5 hours
Shoot excised and coleoptile removed	✓	Trailing flank and corpus	Maximum at 5 hours
Leaves cut	Slight	Flanks and corpus	
Roots cut and warm air blown over to encourage strong transpiration stream	X		
Injection to area just above apex.	X		

Table 5.1 Tritiated thymidine experiments on plants grown at high illumination or different light qualities.

* "Labelling" in this case meant a grain density above the background level but which never reached levels obtained in low illuminance experiments.

b) Experiment with $^{3}H - CH_3$ - thymidine. Plants grown under 10,000 lux.

Treatment	Labelling* successful or not	Stem apex regions labelled*	Chronological pattern of labelling*
Shoot excised	✓	Leading flank	More at 3 hours than 7 hours
Decoleoptiled	X		
Whole plant	X		

c) Experiments with different light quality and ^{3}H - 6 - thymidine

Treatment	Labelling* successful or not		
Red (λ = 660nm) ($50\ \mu W\ cm^{-2}$)	Slight		
Blue (λ = 440nm) ($57\ \mu W\ cm^{-2}$) (Blue light has similar effects on growth as far-red light)	X		
Far-red (λ = 700-800nm) ($33\ \mu W\ cm^{-2}$)	Slight		

Table 5.1 Tritiated thymidine experiments on plants grown at high illumination or different light qualities.

* "Labelling" in this case meant a grain density above the background level but which never reached levels obtained in low illuminance experiments.

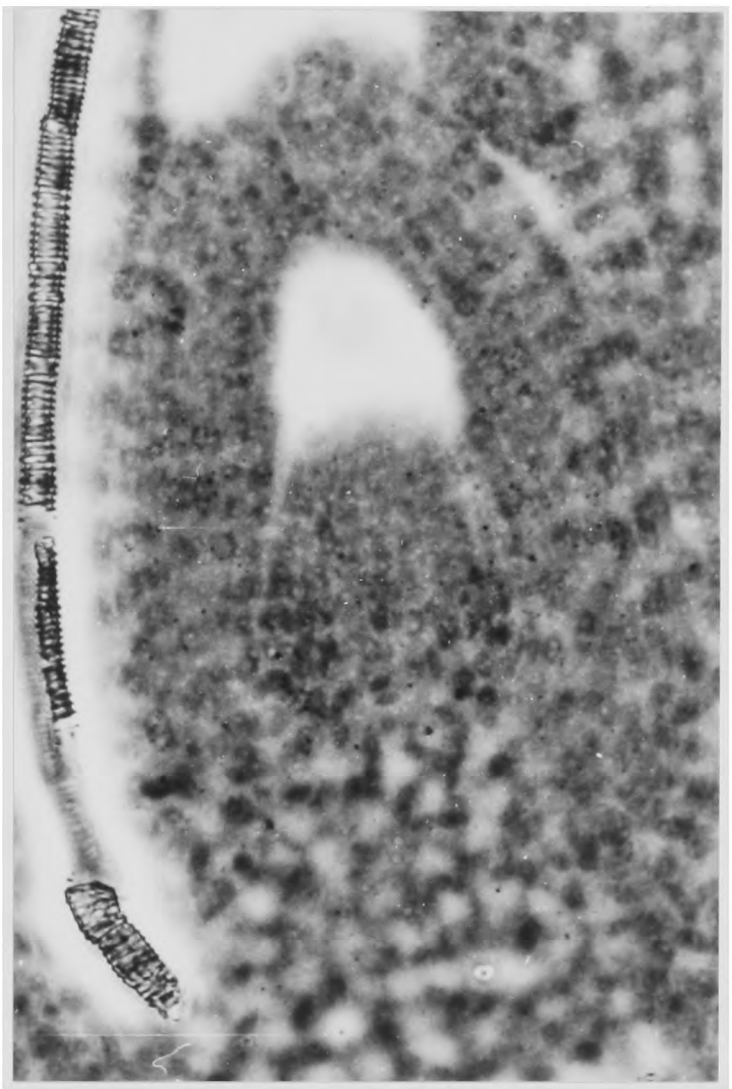


Fig. 5.3 Rice shoot apex showing sparse labelling characteristic of results in plants grown at high levels of illumination and which had been subjected to surgery of one kind or another.

Sections were Feulgen stained, covered with AR 10 stripping film and photographed using phase-contrast microscopy.

These results indicate that thymidine was not taken up by the plant at high illumination presumably for both structural and physiological reasons, since removal of the coleoptile or complete excision of the shoot did produce labelling where none had taken place before, but, as labelling was only slight and transitory, this indicated a biochemical barrier of some kind. The pattern was the same in the nuclei of the stem and leaves except that they were labelled earlier and for longer.

Conclusion

Experiments with labelled thymidine did not provide any extra information about the cell kinetics of the shoot apex, but they did follow the expected pattern indicated by the results from other methods used here ie. that the flank cells are more meristematically active than the summit.

The results of other workers in which the level of labelling in the flanks has been found to be greater than that in the summit have been found in rice also, and this has occurred in both intact and mutilated plants.

The rate of labelling in rice was unexpectedly low, if it is assumed that all the cells are cycling, since a plastochron is four to five days in plants grown in the dark at 35°C. In view of the anomalous patterns of labelling found we must conclude that the system is complicated and not immediately subject to absolute measurements of rates of cell multiplication. The results do, however, support the view that the summit is much less active than the flanks and possibly that this level is low enough to be comparable with the quiescent centre in the root.

6. THE EFFECT OF X-IRRADIATION

A great deal of information has come out of work on the early effects of ionising radiations, notably the different reaction of the quiescent centre compared with the meristematic regions on its borders. (Clowes 1959b, 1970, 1972b). An acute dose of X-rays caused the cells of the quiescent centre to divide, but the meristematic activity of the cap initials decreased.

Differential sensitivity to radiation is believed to be due to differences in the rates of cell division. The law of Bergonié and Tribondeau (1906) states that X-rays are more effective on cells that divide actively than on cells that divide slowly. The reason for this was not obvious and it was possible that the effect on the more slowly dividing cells was the same, but not seen because the damage did not appear until mitosis. Clowes found that quiescent centre cells did show less damage. A measure of the damage sustained by a cell is the production of micronuclei. A micronucleus is produced when a chromosome is broken and the fragment is left out of the daughter nuclei at telophase. Quiescent centre cells have a lower percentage of cells with micronuclei after an acute dose of X-rays than the neighbouring regions which multiply faster ie. the cap initials and stele. The reason for the lower sensitivity in terms of chromosome breakage of the slower multiplying regions is thought to be that so few of the cells are in S (the DNA-synthetic stage), G_2 or mitosis. It has been postulated that a nucleus in G_1 is less likely to be damaged by an acute dose of X-rays because it provides a smaller target. After an acute dose of X-rays, the root meristem was found to reorganise. In Zea, three days after receiving 360 rads, cell multiplication in the cap initials and

stele slowed down and the quiescent centre was stimulated.

In addition to these longer term effects of X-irradiation, there is an immediate response of the meristem. In experiments on Zea the mitotic index of the quiescent centre rises and that of the stele and cap initials falls within the first few hours. Experiments on cell kinetics with colchicine and tritiated thymidine showed that the rates of multiplication in these regions changed so that the quiescent centre showed an increase immediately after the radiation followed by a decrease and the cap initials and stele showed a decrease.

Since there is some similarity between the quiescent centre of the root and the summit of the shoot of rice ie. low mitotic index and low rate of cell multiplication, and because it is difficult to get information about the shoot apex, the effect of X-irradiation was investigated. It might uncover a further similarity between the summit of the shoot and the quiescent centre of the root and hence contribute more evidence about the summit's kinetics and also, since there is no difficulty with penetration of X-rays as there is with chemical solutions, it could afford a useful technique for studying shoot apices.

Methods

The plants, grown at 35°C and 2,400 lux for 3 days followed by 10,000 lux for 2 days were subjected to X-rays and sampled at various intervals afterwards ie. immediately after the dose, half an hour and twenty-four hours later. Four dose levels were used: 1,2,4, and 8 k.rad. X-rays were delivered from a machine working at 100 KV giving 120 röentgens per minute. The effective radiation dose on living material from 100 röentgens assuming that cells are 90% water, is 93 rads. These levels were chosen in order to cover a wide

range of doses at a 'high' level, ie. high when compared with doses used for most experiments of X-irradiation on plants, which are on roots eg., Hall, Lajtha and Clowes 1962. This was because the small amount of work on X-irradiation of shoots indicates that they can withstand much higher doses than roots eg Nagawa and Kehlquist 1957. A wide range was chosen because there was no information as to the levels which could usefully be used with rice. The sensitivity of a species is related to the amount of DNA per nucleus. Preliminary measurements of the amount of DNA in rice indicated about 1.1 picogram (pg) per 2c nucleus, which is very low when compared with other plants eg. Zea has 5pg and Allium cepa has 33.5pg. The value for rice was obtained by comparison with herring sperm using an integrating micro-densitometer.

The mitotic index of each region in each sample was recorded (Fig. 6.1 - 6.4).

Results

The most interesting result from the data is the differential response of the different regions. After 1 k.rad, the summit stands out from the rest in that its mitotic index is not affected whereas the other regions show an immediate increase in mitotic index. After 2k.rad, the summit and sub-summit regions have a much lower index at 0 hrs. than the corpus and flanks. After 4 k.rad all the regions but the leading flank show a significant increase in mitotic index at 0 hrs. and after 8 k.rad, the only region to show an increased index is the sub-summit tunica.

In those cases where mitotic index increased, the increase was not sustained and all regions after all doses returned to normal, or

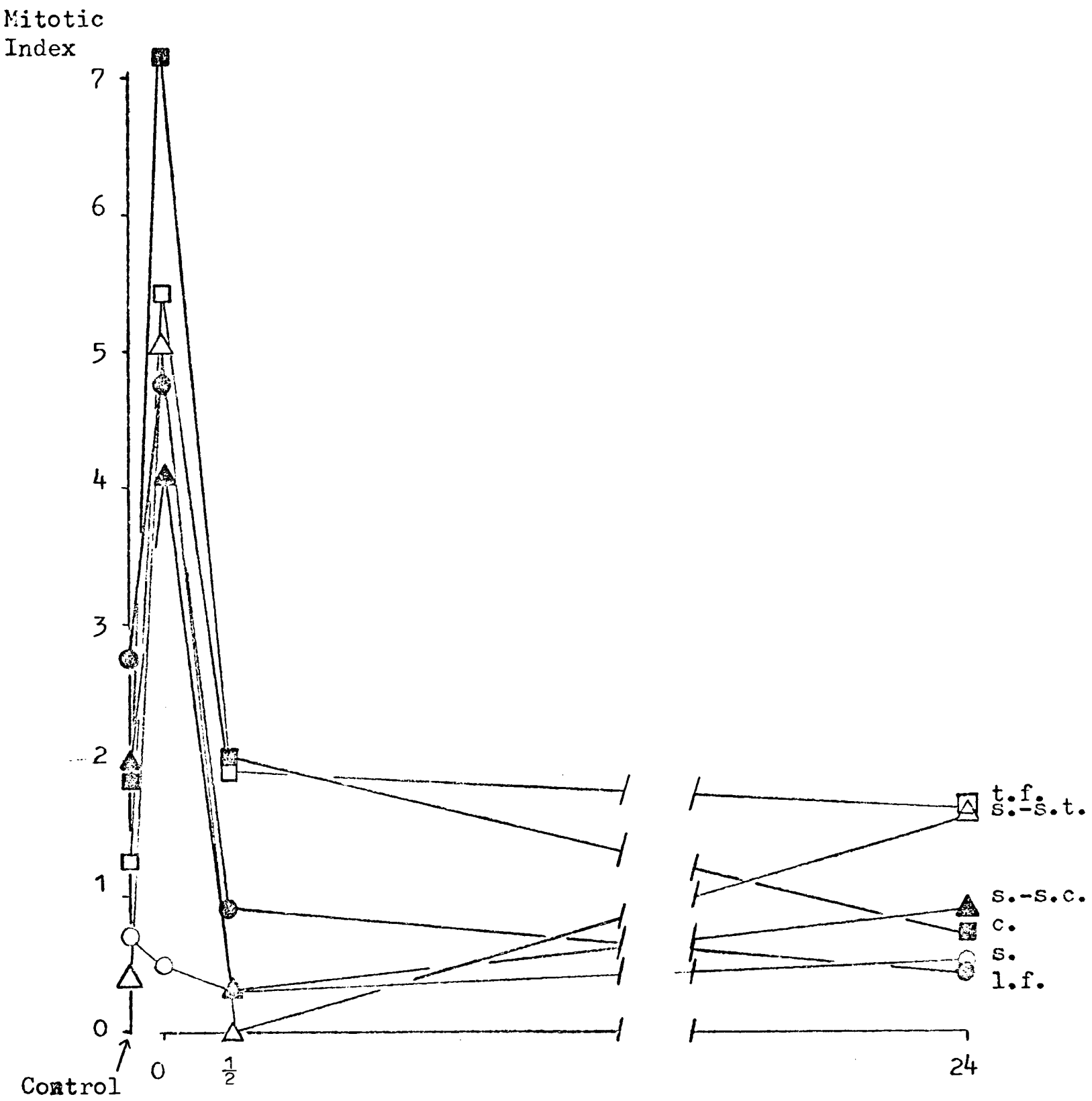


Fig. 6.1 Mitotic index in the different regions after 1 k.rad of X - irradiation.

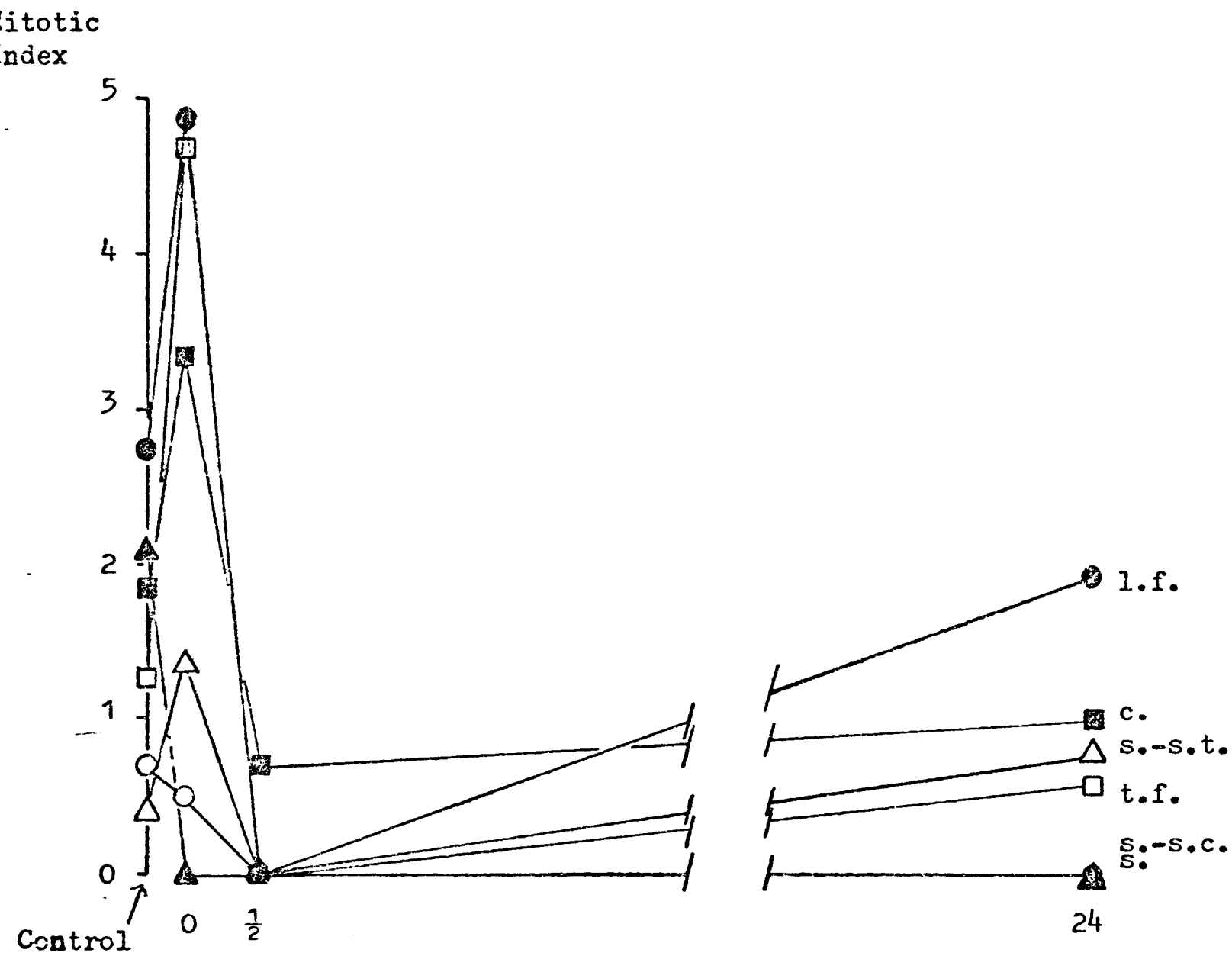


Fig. 6.2 As in 6.1 , after 2 k.rad. dose.

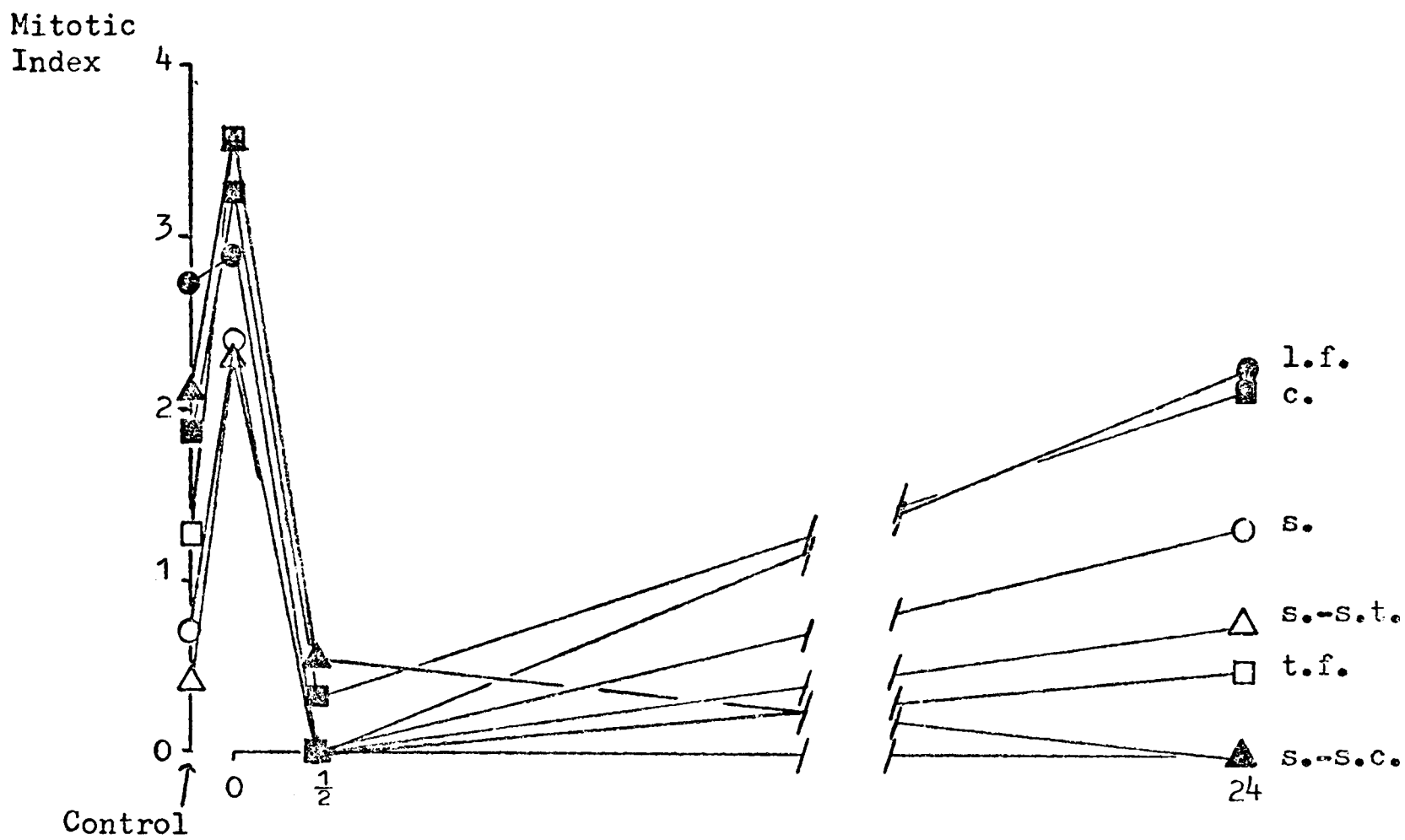


Fig. 6.3 As in 6.1 , after 4 k.rad. dose.

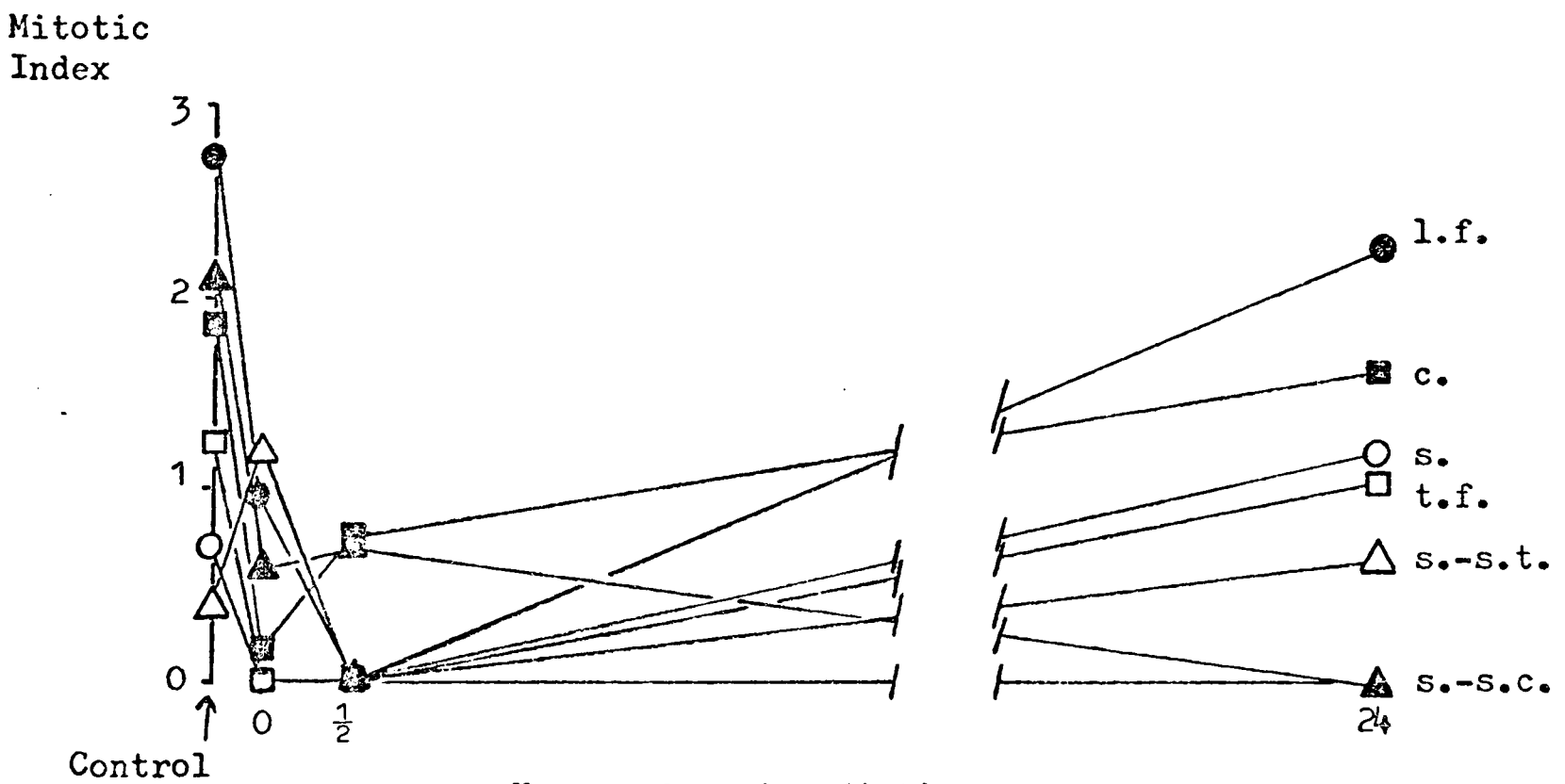


Fig. 6.4 As in 6.1 , after 8 k.rad dose.

lower than normal levels soon afterwards. The 24 hour values indicated that the meristems were returning to normal, except for the leading flank after 1 k.rad whose mitotic index was still depressed and the raised values in the summit after 4 and 8 k.rad.

Prophase values (Figs. 6.5 - 6.8) follow closely changes in mitotic index except for the leading flank which, after 1 and 2 k.rad are at the same level at 0 hours and are depressed at 0 hours after 4 and 8 k.rad. This is an interesting difference and has similarities with the reactions of the stele and cap initials in Zea which are also the faster multiplying regions in the meristem. After a dose of X-rays, prophase levels fell and this was found to be due to inhibition of meristematic activity.

Metaphase and anaphase values in the shoot showed no pronounced change but telophase appeared to be stimulated at 0 hours, mainly in the more rapidly dividing regions, and more after the lower dosage levels. (Full results are given in Appendix III).

Discussion

The summit shows the least reaction in all the experiments. It may be that, in its response to X-rays, it is basically quite different from the quiescent centre of the root and is not stimulated by X-rays, or that the dose of radiation which would affect it was not one of those used. It is not unreasonable to postulate that a higher dose than 8 k.rad might stimulate it, since the level of radiation which will affect a shoot apex is much higher than that which will affect a root.

While the immediate response of the regions apart from the summit

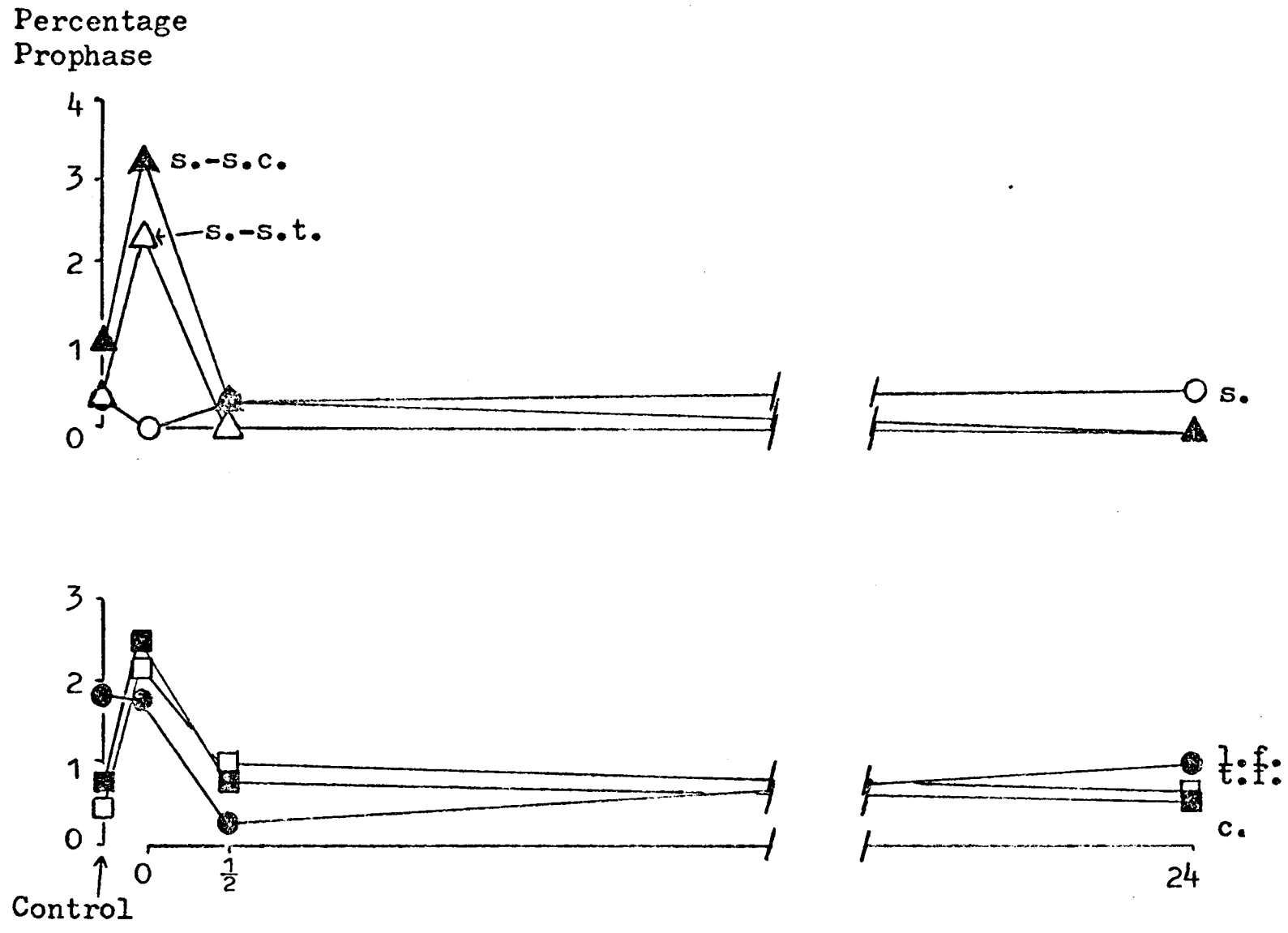


Fig. 6.5 Effect of 1 k.rad.

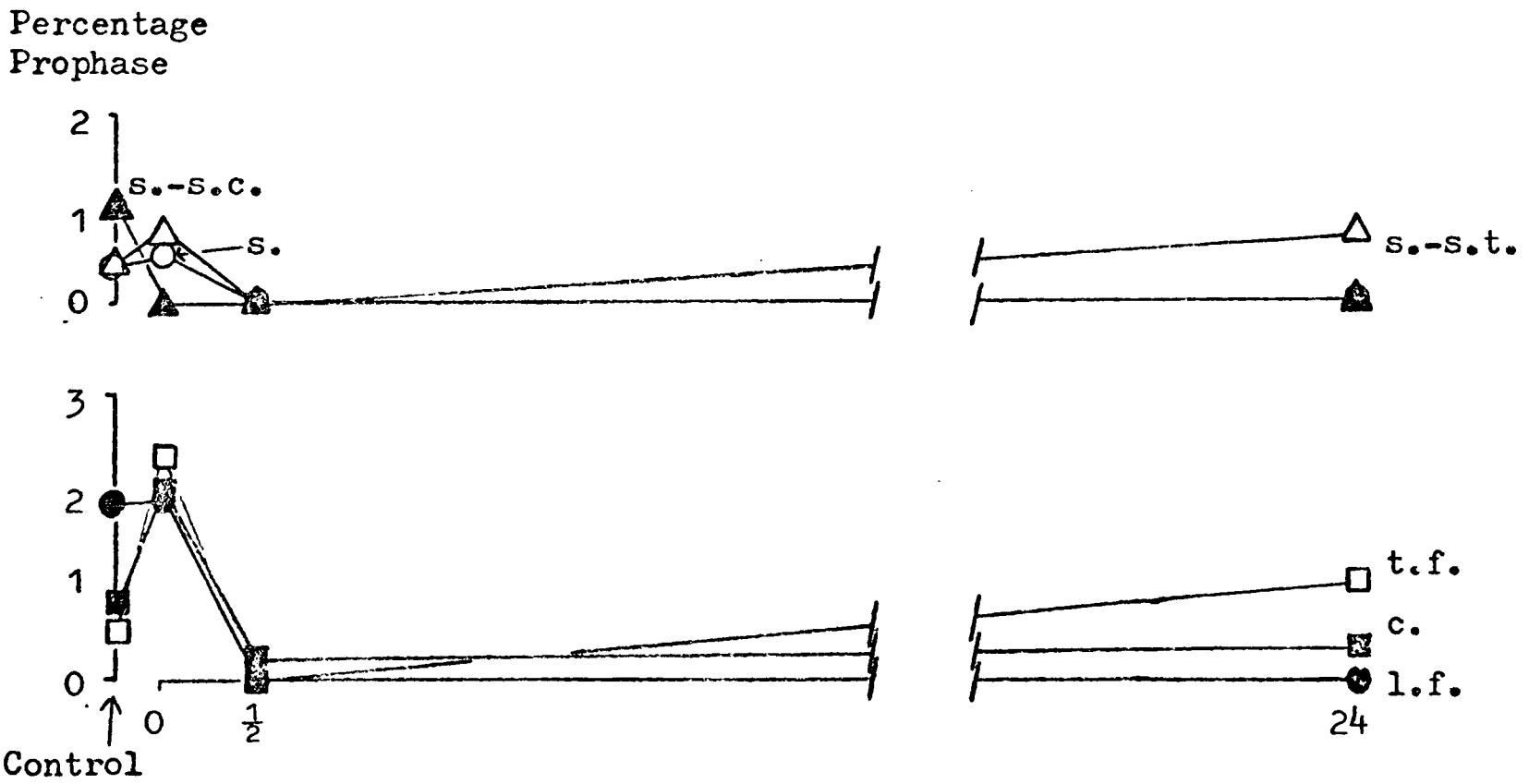


Fig. 6.6 Effect of 2 k.rad.

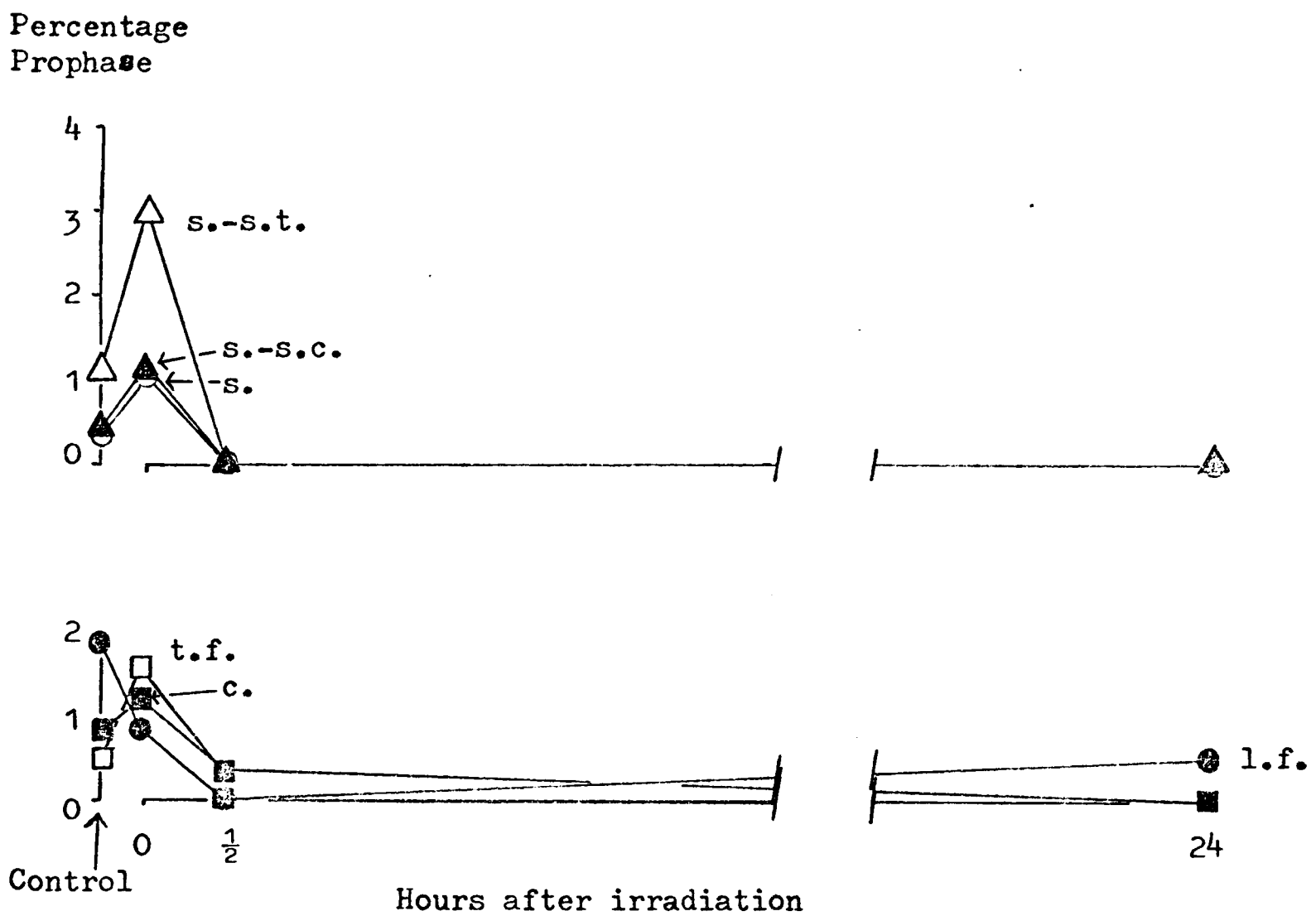


Fig. 6.7 Effect of 4 k.rad.

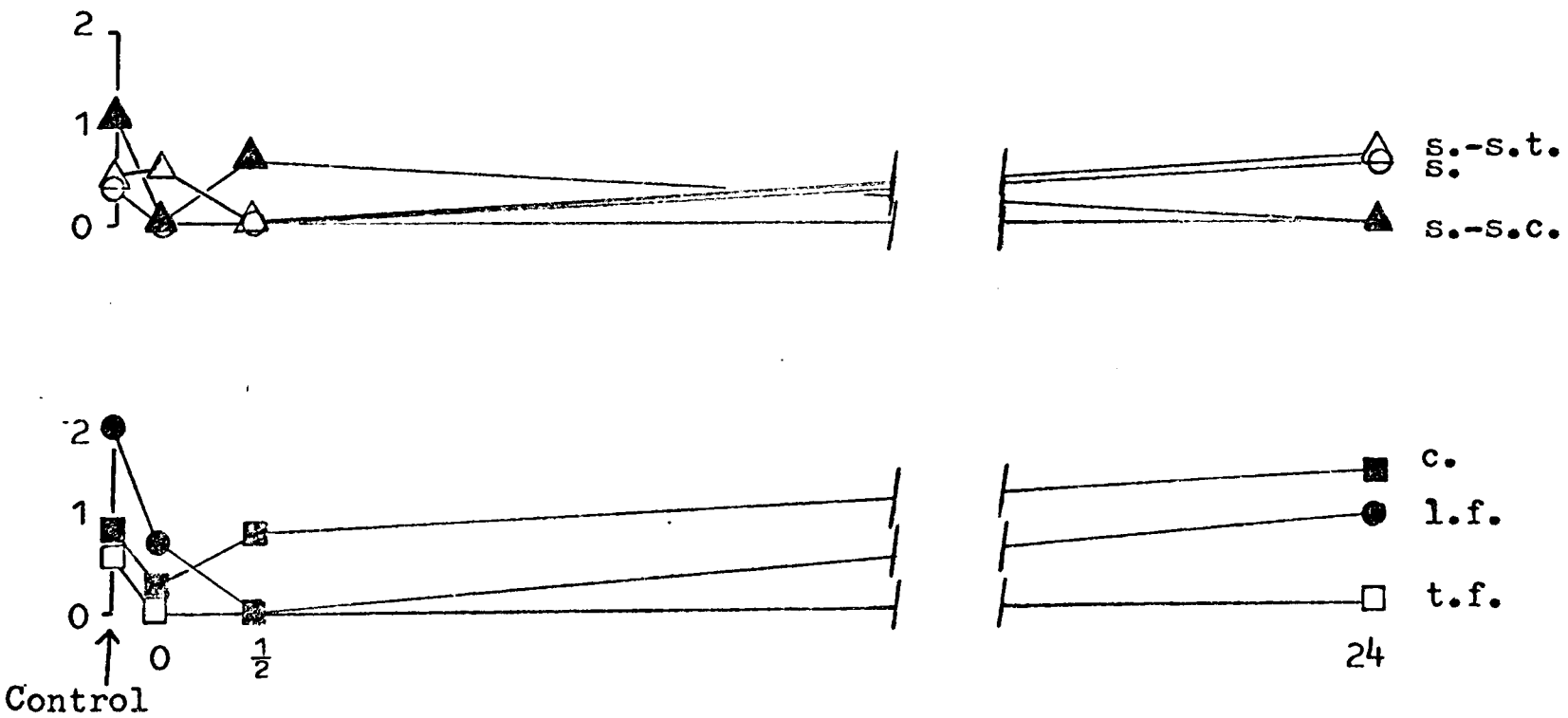


Fig. 6.8 Effect of 8 k.rad.

is a rise in mitotic index except after 8 k.rad, the index drops below control levels soon afterwards, suggesting a drop in mitotic activity. It was decided to test this by measuring cell-doubling times after a dose of 2 k.rad.

Metaphase accumulation after X-irradiation

Plants were grown at 35°C in four cms of aerated water. The water was aerated because the effect of X-rays on rice seeds was reported to be much higher in the presence of oxygen. Illuminance was 2,400 lux for three days and 10,000 lux for the fourth day.

The effect of the dose was studied after one hour and after four hours. For the one hour measurement, the plants were irradiated after they had been in colchicine for two hours to allow for penetration of the colchicine. A third group of plants which had been grown with the others was treated with colchicine and not X-rayed.

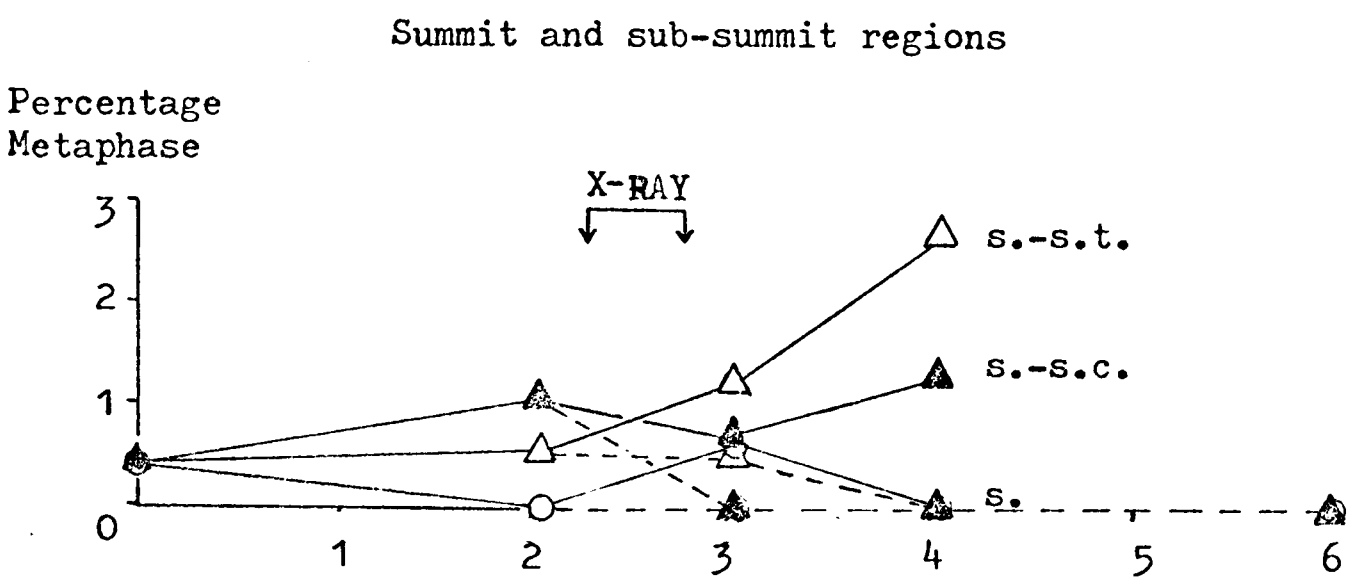
Results

After one hour the metaphase index was reduced to zero but after four hours a few metaphases were observed, indicating some recovery of meristematic activity (Fig. 6.9).

Discussion

The low percentage of metaphases after one hour implied that the immediate rise in the mitotic index of most of the regions did not indicate a rise in mitotic activity.

The rise in mitotic index after 2 k.rad could be due to a condensation of chromosomes caused by the shock. It is interesting that this does not happen to all the regions and suggests that the nuclei



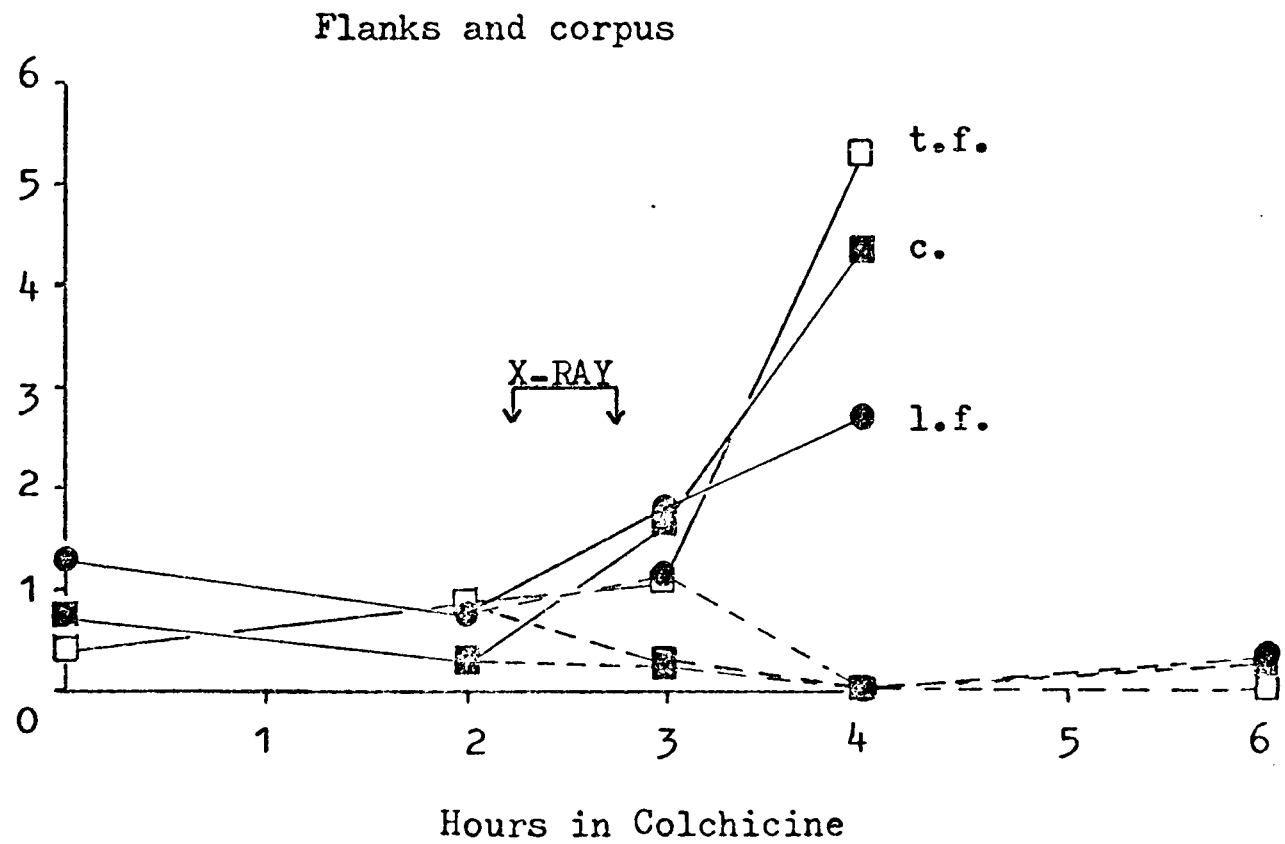


Fig. 6.9 Effect of 2 k.rad. X - ray dose on metaphase accumulation by colchicine. ——— control, - - - - - after X - ray. 10 apices per point.

in the summit and sub-summit tunica are in a different state from the cells in the rest of the meristem.

The control experiment carried out here may be compared with the control experiment carried out in similar conditions in the investigation on the effects of surgery reported in Section 4. The leading flank is not dividing as fast as in experiments in which the illumination was high all the time. This will be discussed further in Section 9.

Immediate stimulation of mitotic index levels has only previously been reported by Darlington and La Cour (1944) and Clowes (1959b). This phenomenon appears to be seen in cells with relatively long cell-doubling times.

Reports on the morphological effects of ionising radiations on plants indicate that heavy and medium X-ray doses cause injury or retardation of growth. Low doses have been variously reported as stimulatory and not. (Gunckel and Sparrow 1953). The difficulty with interpretation of morphological effects is that many factors are involved. Expression of injury sustained by plants is considered to be due to physiological as well as mitotic effects. Auxin levels are probably affected, and treatment by various chemicals can produce similar effects to X-rays. (Gunckel and Sparrow 1953).

Analyses of the reaction of plant tissue in the first few hours after receiving the dose can give more precise information about the mechanism of the plant's reaction and the facts supplied in this work on rice contribute to this understanding.

Differential sensitivity has been found in other shoot apices but the interpretations have varied. Sagawa and Mehlquist (1957) found the tunica layer in the summit of carnations to which they gave 5,000

röentgens of X-rays showed damage first and concluded that the tunica here was most sensitive. However, Langenauer and Davis (1973) found that 500 rads caused 50% of their sunflower plants to develop abnormal inflorescences although leaf production was normal. They thought this supported the méristème d'attente theory because the sub-lethal damage in the summit was expressed only on flowering. Gunckel and Sparrow (1953) found damage from chronic ϒ-radiation given to tomato plants appeared first in the region just below the shoot meristem, of vegetative meristems but floral meristems were highly sensitive. The effects from chronic ϒ-radiation are more difficult to interpret than those from acute irradiation but they thought that this did point out that histological evidence of radiation damage did not always appear first in the meristems, which is contrary to the situation described by Bergonié and Tribondeau.

The immediate reactions of rice shoot apices to the X-ray doses suggest that the summit is less sensitive than the flanks. Although Gunckel and Sparrow (1953) think that it is not possible to compare reactions of different plants or even the same plant and different stages of development when considering the morphological changes, the results found on the differential sensitivity of the different shoot apex regions do seem to have a common basis. The meristems react according to the law of Bergonié and Tribondeau if it is accepted that flowering apices are more meristematically active than vegetative apices, and that the flanks of vegetative apices are more active than the summit. Sagawa and Mehlquist's work on carnations does not fall in line with this and a possible explanation might be the chimeral nature of their carnation varieties. The tunica layer, which is responsible for flower colour, and is genetically different from the corpus, is

likely to have a different sensitivity to X-irradiation from the cells beneath.

7. CELL ARRANGEMENT

Introduction

Work on the shoot apex before the 1950's consisted to a large extent of observation of the size, shape and staining pattern of the cells and on the effects of surgery on the morphology and development of the apex. Assumptions were made about the activity of the cells at a time when the dynamics of meristem cell populations were little understood. The traditional view that the summit was the prime site of cell production went unchallenged for decades and descriptions of the apex were interpreted in support of this view. Although the emphasis of work today has been away from this approach the function of the summit was first questioned before this swing (Plantefol 1947) and did not occur as a result of it. The value of these studies is therefore obvious, if, at the same time, enough caution is exercised. With this in mind the following studies are presented.

Methods

Longitudinal sections of the rice shoot apex at all stages of the plastochron are shown in the following drawings which pick out the variations in cell size in the apex. The tunica, which is where the leaf is initiated, receives most of the attention, for it is here that the relationships between cells are easiest to trace and it is here also, that the changes which take place during a plastochron are most marked.

Results

In Fig. 7.1 the outer leaf, L - 1, shown is thicker on the left side where the primordium began, L. The primordium, L, on the right,

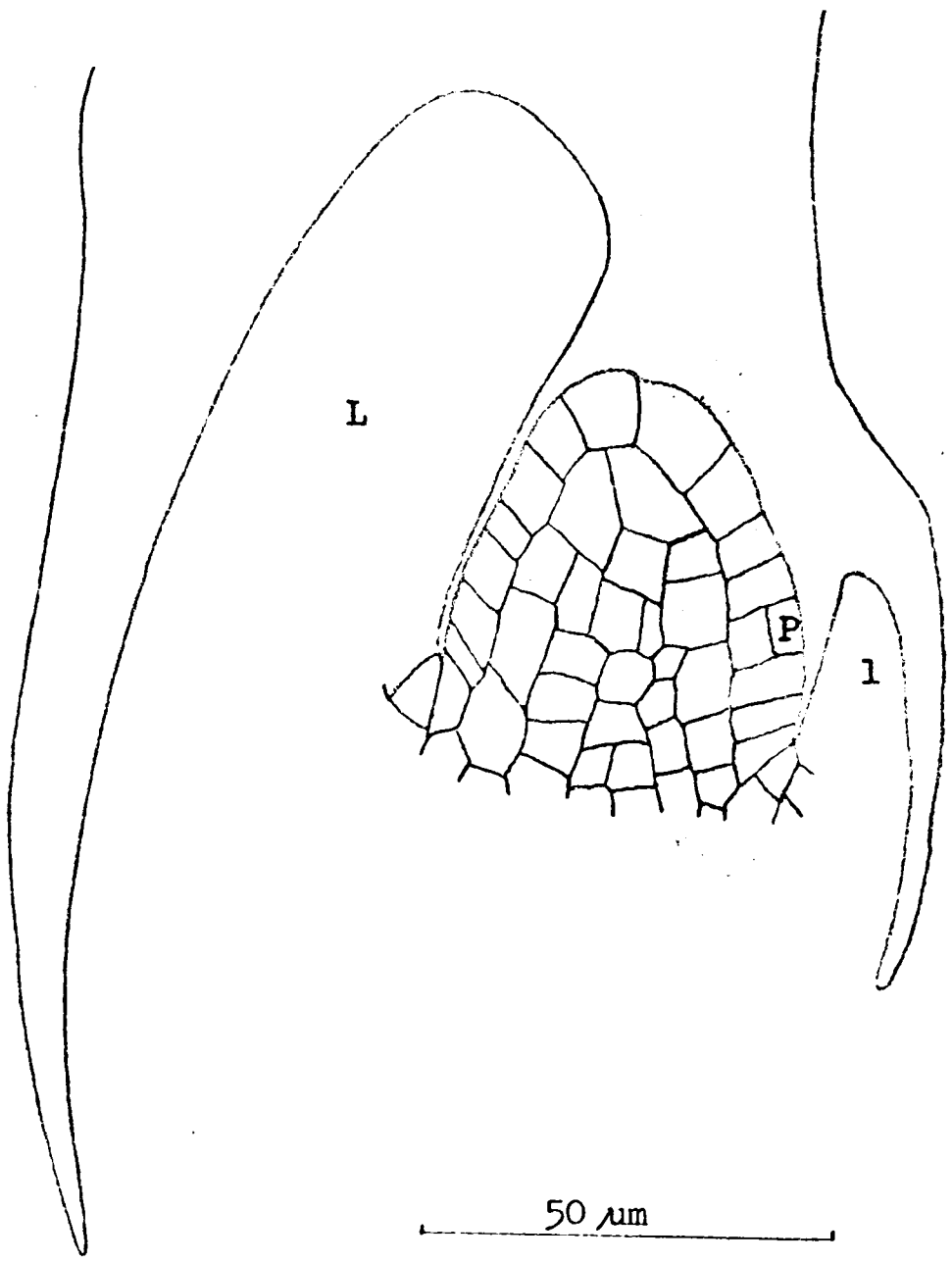


Fig. 7.1 Stages in leaf formation - 1.
L - 1, youngest leaf; P, primordium.
Drawn with camera lucida.

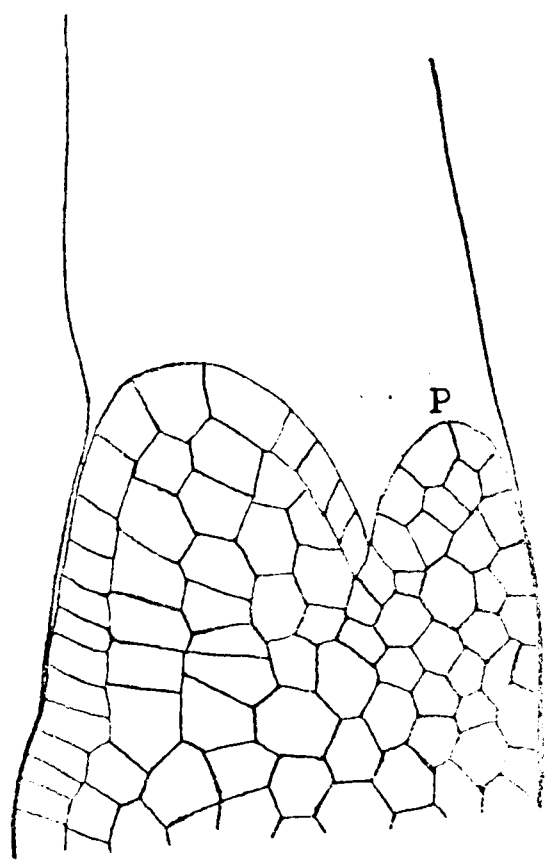


Fig. 7.2 Stages in leaf formation - 2.
P, primordium.
Drawn with the help of a camera lucida.

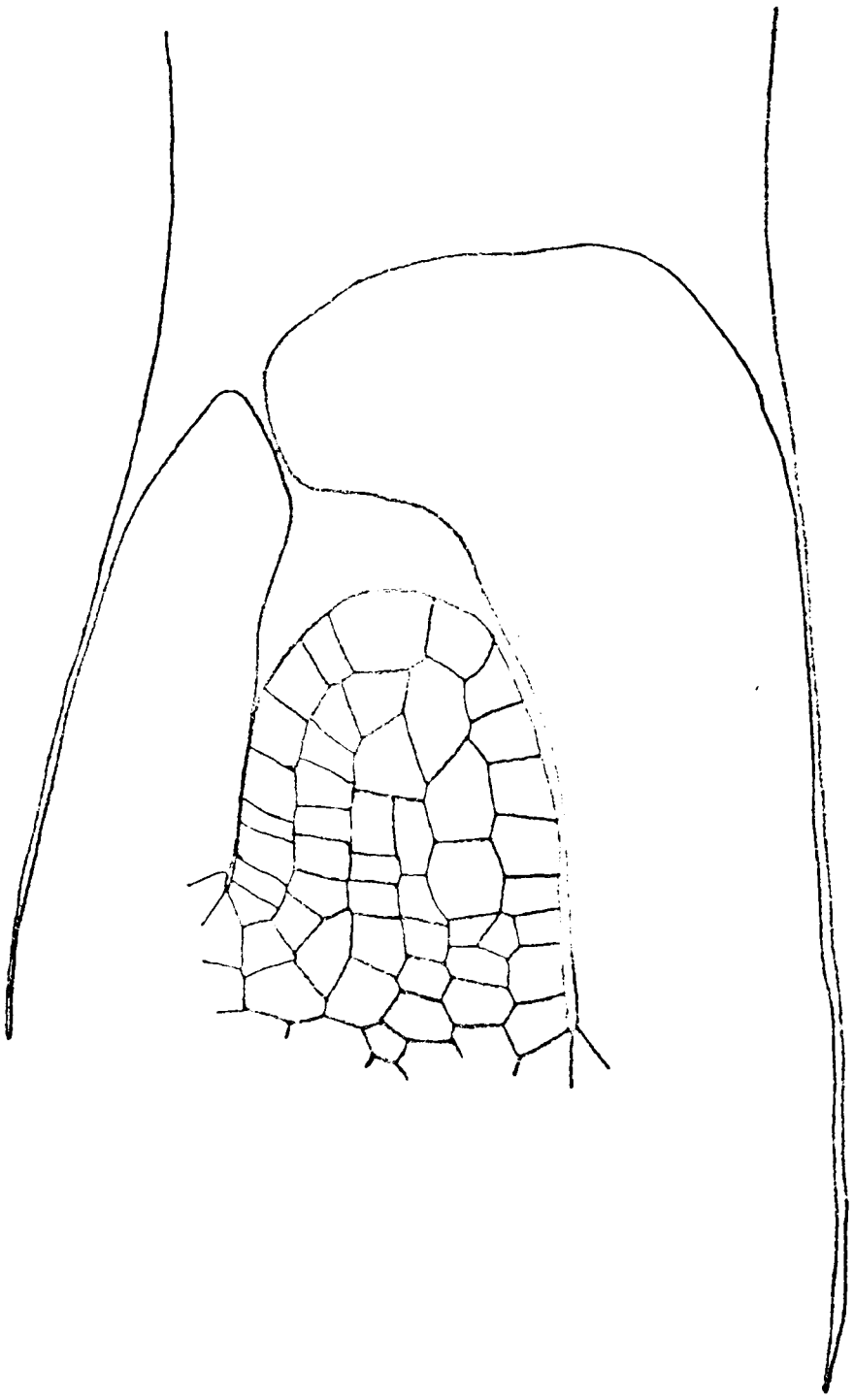


Fig. 7.3 Stages in leaf formation - 3.
Drawn with camera lucida.

is only just visible. The periclinal division in the tunica need not conflict with Sharman's report that the leaf is initiated by a periclinal division in a cell below the tunica. The first cell to divide could be in a different plane from that of the section. Fig. 7.2 shows a later stage when the collar of the primordium has encircled the apex. The cells on the opposite side of the apex to P are now smaller than before indicating that they have undergone division. Fig. 7.3 shows a later stage in which the primordium has grown to overarch the summit and the collar has grown in length also, so that the whole leaf surrounds the apex. The cells in the axil of this leaf (which were above P) are larger, particularly in the axial direction, while the cells opposite are smaller. This indicates multiplication in the latter position and enlargement in the former, though this may not be an 'all or nothing' situation. ie. where multiplication is indicated, the cells may also have enlarged, but the appearance indicates that multiplication has predominated; similarly where enlargement is indicated, multiplication is not precluded. The whole process repeats itself on the opposite side of the apex.

While the changes in the tunica at the base of the apex are occurring the summit cells appear to have more or less constant dimensions. The summit tunica cells have thicker cell walls (Fig. 7.4) suggesting absence of high cell flux here.

Discussion

If size and shape of cells is indicative of their meristematic state in the examples presented, a fairly simple pattern of cell multiplication through a plastochron can be formulated. (Fig. 7.5).

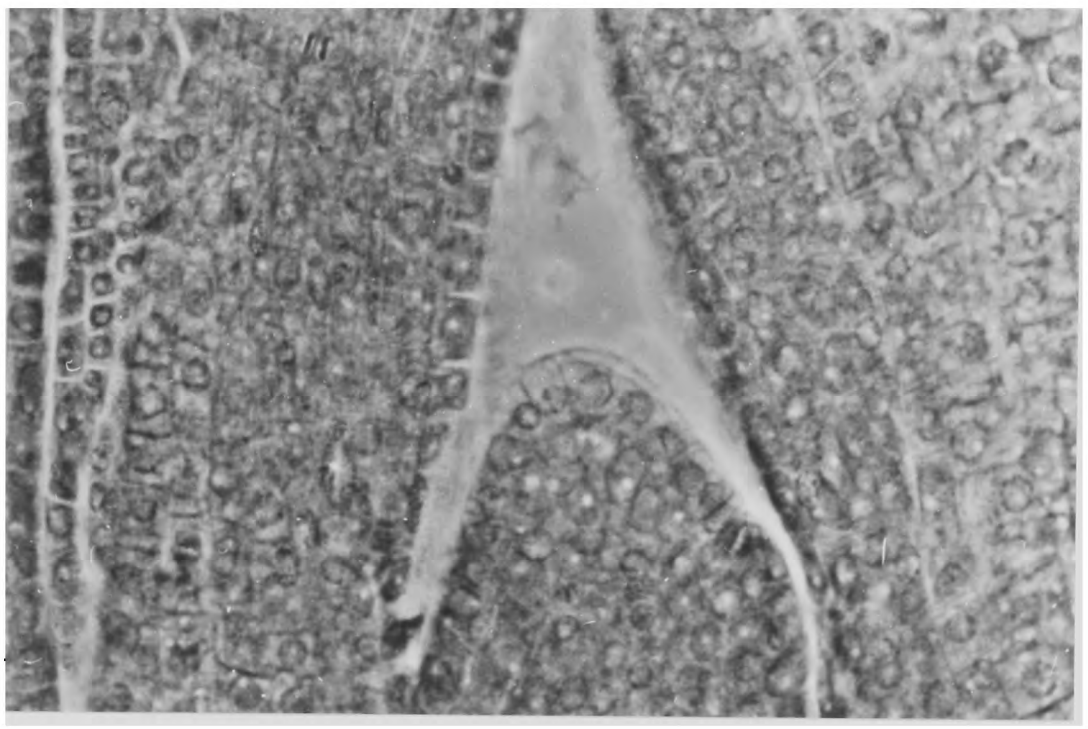
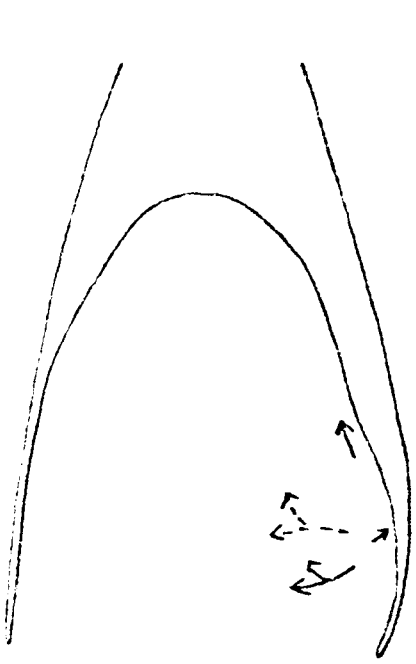
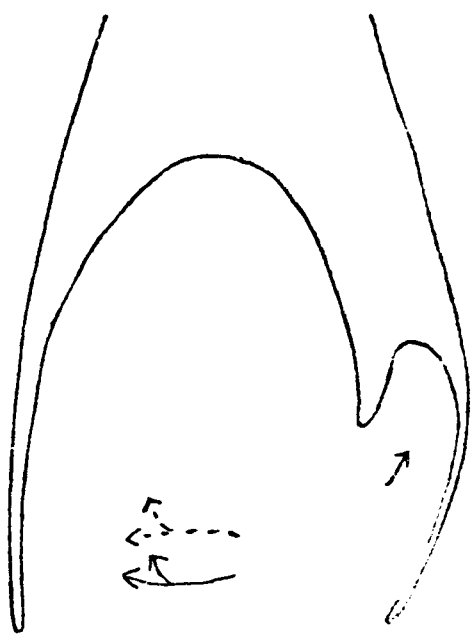


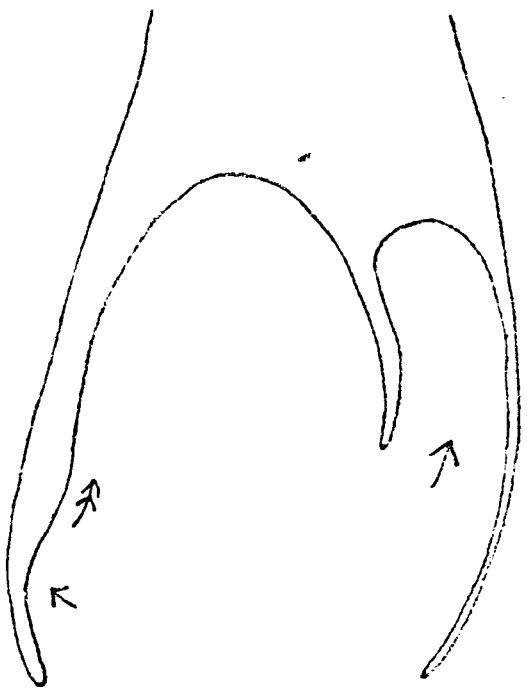
Fig. 7.4 Phase-contrast picture of rice shoot apex showing thick cell wall of summit tunica cells.



A. Cell multiplication, indicated by arrows, spreads round the apex in two directions from the primordium and also acropetally for a small distance.



B. Cell multiplication continues in the primordium and the spread round the circumference has reached the opposite side.



C. The next primordium has appeared and the cells above have received a 'double dose' of cell multiplication.

Fig. 7.5 Possible mode of growth of the rice shoot apex through a plastochron.

Multiplication occurs in the formation of a leaf primordium and this spreads around the circumference of the apex, forming the collar, and at the same time it spreads acropetally for a short distance. This acropetal spread follows the collar round the apex until the opposite side is reached. In this way, the apex above the primordium has grown in cell number but the summit has been somewhat impassive, being merely lifted up by the activity of the cells beneath.

At the point where the collar is completed, cell multiplication, and presumably the stimulus which causes it, has arrived from two directions. It is tempting to suggest at this point that if the stimulus for a cell to multiply was a measurable quantity, the point where the collar was completed would receive a 'double dose', so that in addition to the acropetal spread of cell multiplication these cells divide further and form a new primordium. This pattern of organisation could only occur, at least in this simple form, in plants which have an alternate pattern of leaf production. However, only rice has been studied in this investigation.

The corpus has not been discussed, mainly because events here are less striking, and cell relations less easy to follow. In the system outlined above, the general pattern of growth usually attributed to this area would fit quite acceptably ie. a pith-rib meristem which would produce growth predominantly in the axial direction. It is possible in most sections to trace vertical files of cells, although there is a less clear demarkation between the files than often occurs in dicotyledons, where the stem is more developed.

A striking feature in the cell pattern in the tunica of rice shoot apices is the small size of the cells above a developing leaf

primordium. The cells here will not be involved in the development of a leaf primordium until the next plastochron. Concomitant with this is the shape of the dome above the leaf. Inspection of the dome itself would often lead one to predict that the next leaf primordium would appear on the opposite side from that on which it does. In Fig. 7.3 for example, the dome is larger on the side next to P, but, with an distichous phyllotaxis, the next primordium will arise on the opposite side. This preparation for leaf formation is in line with several observations that determination of a leaf in angiosperms is thought to occur quite early, (Cutter 1965). Denne (1966c) found the mitotic index of the flank producing the next primordium declined during the plastochron while that on the opposite side increased in Tradescantia. Studies on another plant with an alternate phyllotaxis, in this case, a dicotyledon Tropaeolum led Nougarède (1967) to propose a similar idea also, namely, that the leaf buttress is formed in association with the primordium immediately below it (buttress ébauche meaning the swelling of the primordium at its inception).

If cells which eventually form a leaf primordium are produced in association with the previous leaf, then the bulging out of the primordium would be a second stage in leaf production. Recalling Foard's - plantlet experiments referred to in Section 1 which indicate that leaf production can take place without cell division, then cell enlargement could take place at this 'second stage', with cell division following, 'to keep up'. Unfortunately we are nowhere nearer understanding what controls leaf production and the expression of the various phyllotaxes.

Regeneration of the apex from the anneau initial has been proposed for another monocotyledon, Luzula, by Catesson (1963) which has a two-thirds phyllotaxis. Catesson thought that small apices would differ from large apices in that the summit would be encroached on by meristematic activity spreading from the anneau initial. This view resembles that taken for Perilla by Lance-Nougarède et al (1964), already mentioned in the introduction.

Conclusion

The pattern of growth outlined here gives a method of shoot apex organisation for rice which does not involve apical initials at the summit. The interpretation supports Plantefol's anneau initial theory that the source of growth is at the side of the apex and not at the summit.

8. THE EFFECT OF RED AND FAR-RED LIGHT ON THE SHOOT APEX

The phytochrome system

Since the opposing effects of red and far-red light on seed germination were found by Flint and McAlister in 1935, many effects of this photoconvertible pigment system have been reported e.g. anthocyanin production, tuber development, the opening of the plumular hook and enlargement of cotyledons (Smith 1975). The responses require only low energies of irradiation and are mediated by the pigment phytochrome which can exist in two states. The P_{fr} state is receptive to far-red light (λ = 730nm) and is generated by red light (λ = 660nm). The P_r state is receptive to red light and is generated by far-red light. This antagonistic effect of red and far-red light was shown by Borthwick and Hendricks in 1952. Photoconversion of phytochrome from one form to the other can take place in a matter of seconds.

A further phenomenon associated with the phytochrome pigment is the 'high irradiance res onse'. Prolonged far-red irradiation was found to be more effective than a short red irradiation in many cases (Mohr, 1972) e.g. cotyledon expansion of Sinapis alba.

Phytochrome and leaf production

The effect of red and far-red light on the rate of leaf initiation was studied by Mohr and Pinnig (1962) in Sinapis alba. Production of new leaf primordia ceased almost entirely in the dark and was stimulated by light. This phenomenon was shown to be red/far-red reversible which indicates phytochrome dependency.

With the combined interest of investigating further the link between the rate of leaf initiation and the phytochrome system

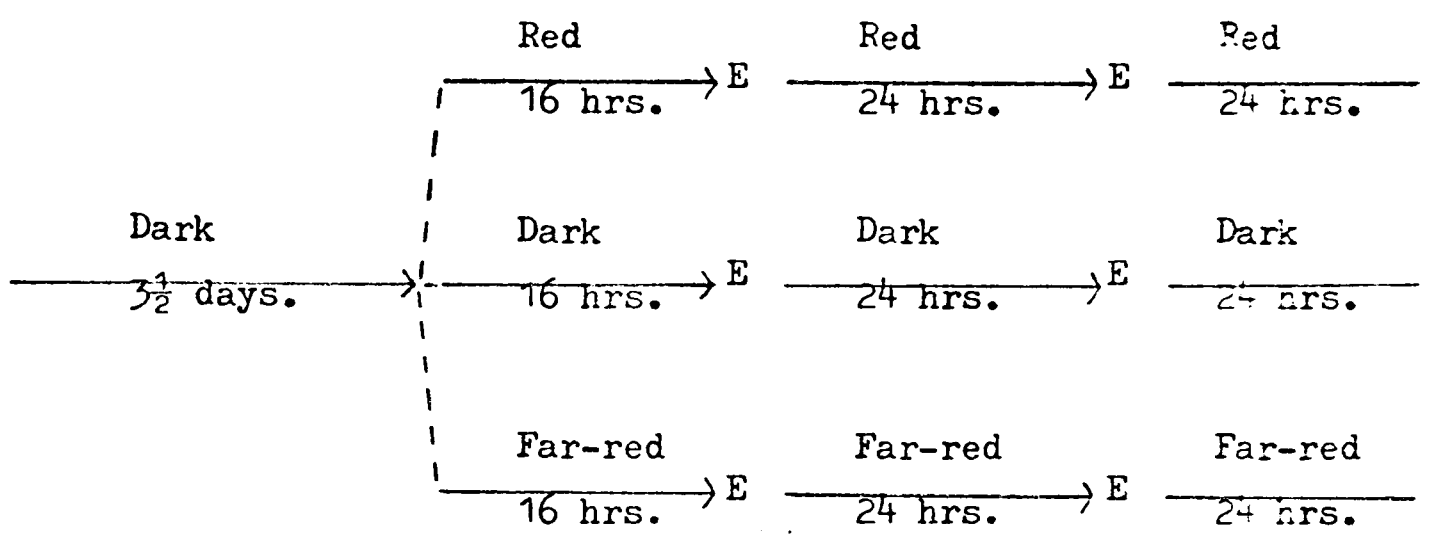
and the effect of different stimuli on the organisation of the shoot apical meristem, the effect of red and far-red light on rice shoot apex cell kinetics was studied.

Methods

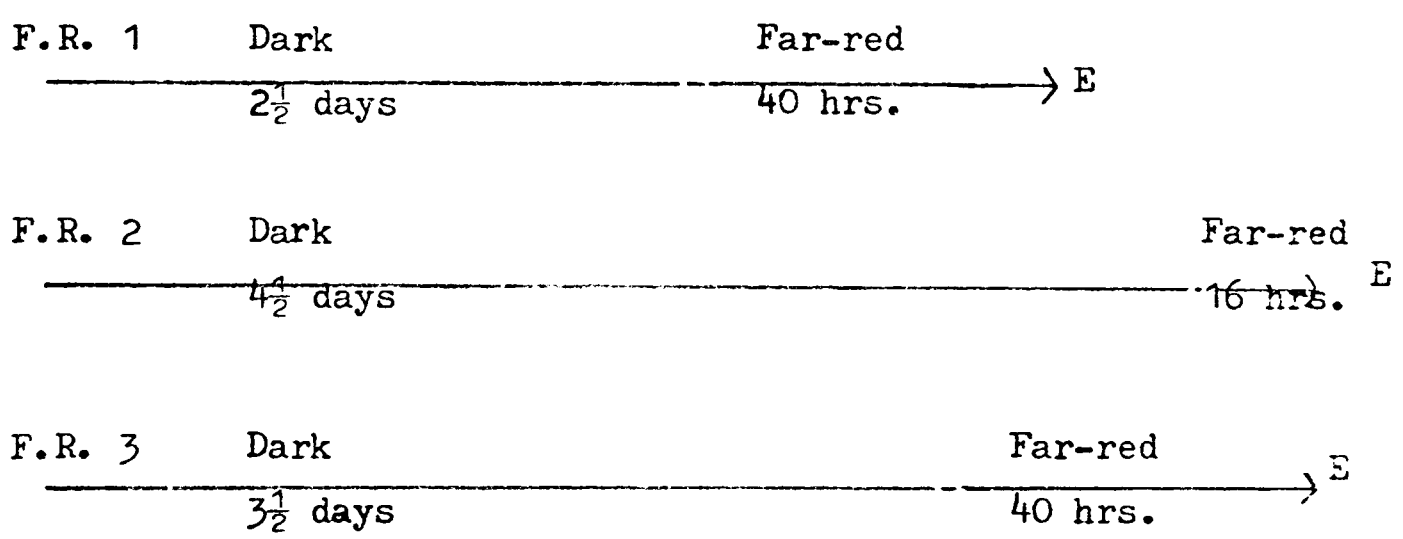
The rice plants were grown under 3 to 4 cm of aerated water at 30°C for 3 to 5 days. In the first set of experiments, the plants were grown in the dark for 3.5 days and then one third were transferred to far-red irradiation and one third to red irradiation, the temperature of the water being maintained all the while at 30°C. Experiments to find the rate of cell multiplication in the different regions of the shoot apex were carried out after 16 hrs. and after 40 hrs. in the three sets of plants. Samples of 10-15 plants were made at 0, 4 and 5 hrs. Previous experience (see Section 4) showed this to be the time at which metaphases accumulate under the standard conditions used here. For the initial experiments into the effect of red and far-red it was decided that three samples i.e., at 0, 4 and 5 hrs. would be sufficient to show major differences in behaviour.

In the second set of experiments, which were designed to look more closely at the effect of far-red irradiation, plants were grown in the dark for 2.5 days after which a third were transferred to far-red for 40 hrs., a third were left a further two days in the dark and then transferred to far-red irradiation for 16 hrs. and the remaining third were transferred to far-red irradiation after only one additional day in darkness, and given 40 hrs. far-red irradiation. The rates of cell multiplication were found as

I First group of experiments.



II Second group of experiments.



E = colchicine mataphase-accumulation experiment.

Fig. 8.1. Plan of experiments.

before, except that more samples were taken i.e. at 0, 2, 3, 4, 5 and 6 hrs. It will be seen that the third batch is a repeat of one of the treatments given in the first set of experiments.

The treatments and points at which cell-doubling times were measured are set out in fig. 8.1. Experiments in group I were grown on for a further day to investigate any differences in external morphology or internal anatomy between the red, far-red and dark grown plants.

Results

Percentage metaphase levels are shown in Figures 8.2 to 8.8. Graphs in the group I experiments which did not have 3 hr. samples are drawn to account for metaphase accumulation beginning at 3 hrs. Cell-doubling times are shown in Table 8.1. Inspection of the graphs shows that in some cases the values for the sub-summit regions are subject to downward fluctuations. This is presumed to be due to the low numbers of cells involved in these regions which are the smallest of all the regions. To obtain estimates of values for their cell-doubling times the following methods were employed. In some cases the values have been averaged and the result treated as a 2-hr. accumulation from the 3 hr. point which is when the colchicine begins to act. In other cases the accumulation values have been taken from slightly different parts of the graph more representative of the general trend. The leading flank in one group II experiment shows an odd fluctuation which may be spurious or may be due to synchronisation by the light treatment. The possibility of synchrony in cell-division as a result of moving the plants into red or far-red cannot be

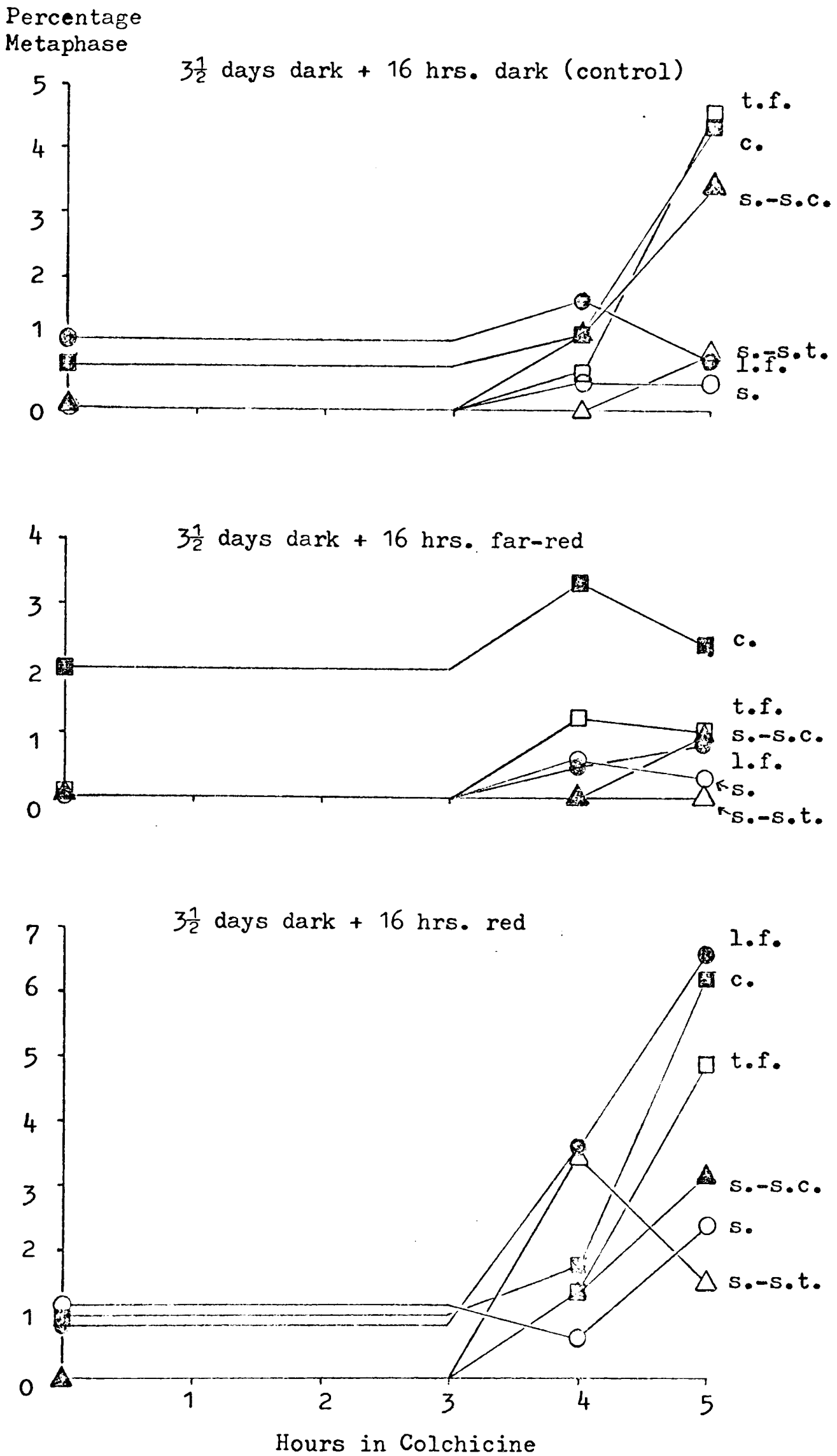


Fig. 8.2 Percentage metaphase levels in group I ;
$3\frac{1}{2}$ days dark + 16 hrs. treatment.

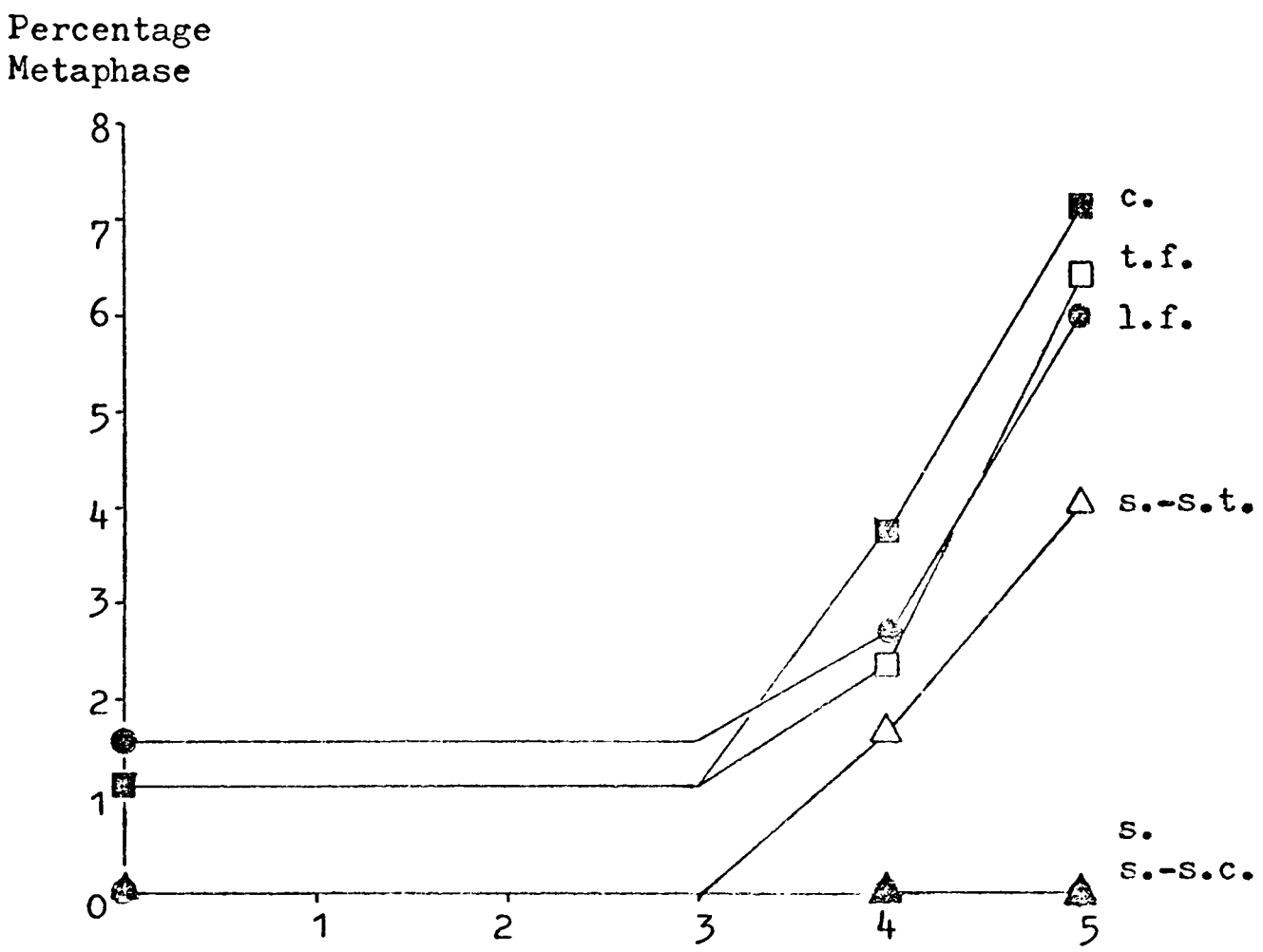


Fig. 8.3 Percentage metaphase levels in group I ;
$3\frac{1}{2}$ days dark + 40 hrs. dark (control).

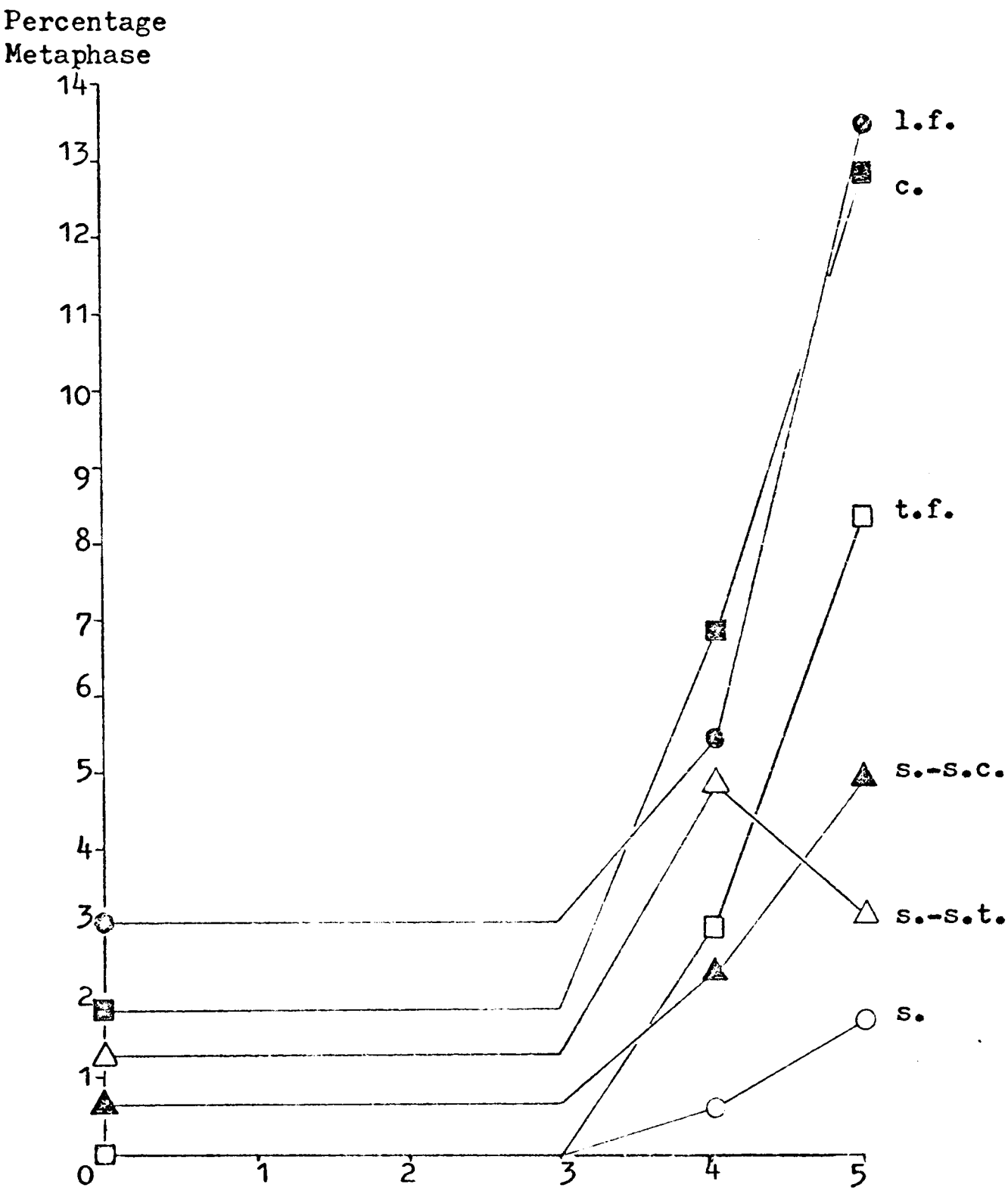


Fig. 8.4 Percentage metaphase levels in group I ; .
$3\frac{1}{2}$ days dark + 40 hrs. far-red.

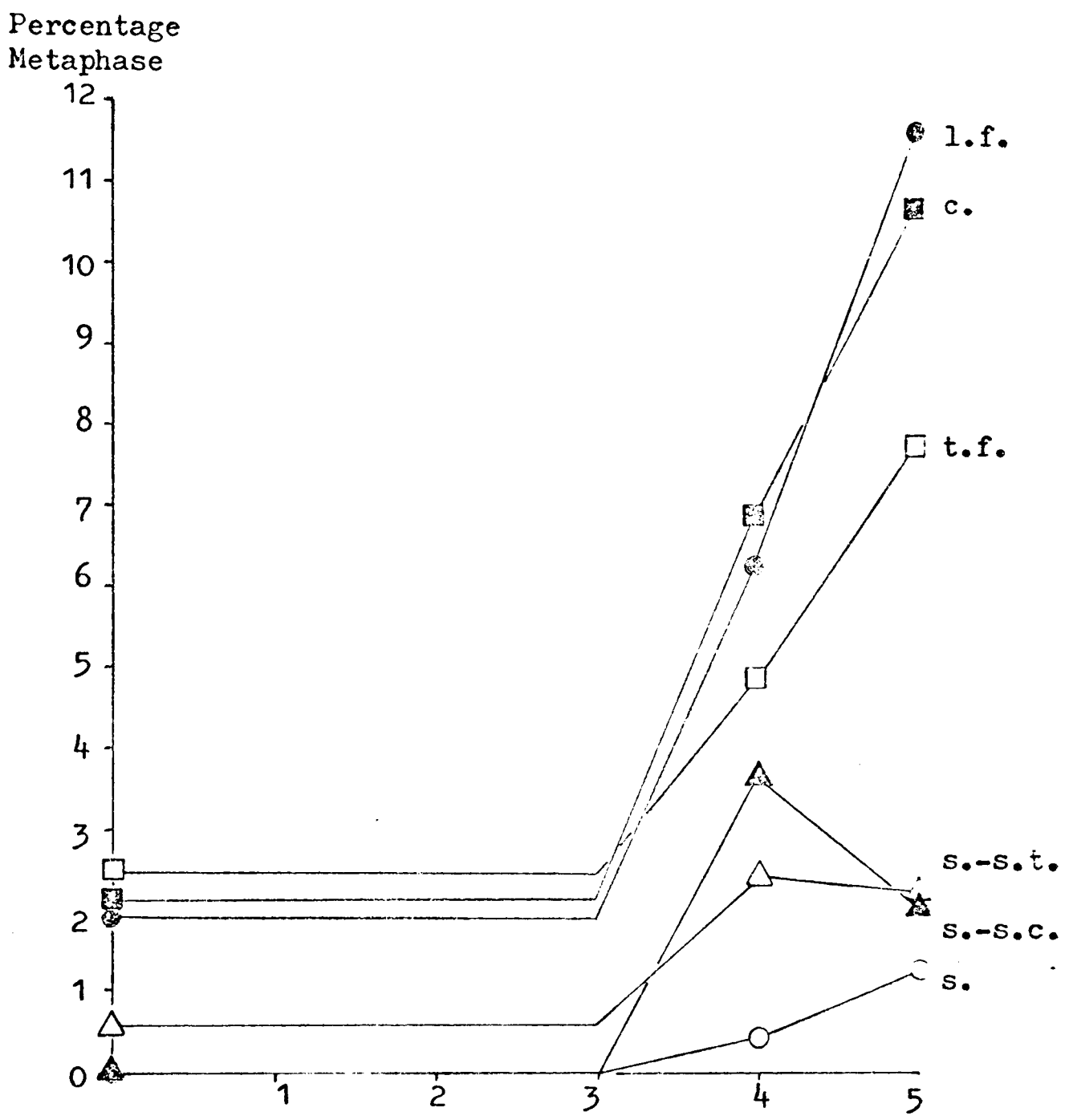


Fig. 8.5 Percentage metaphase levels in group I ;
$3\frac{1}{2}$ days dark + 40 hrs. red.

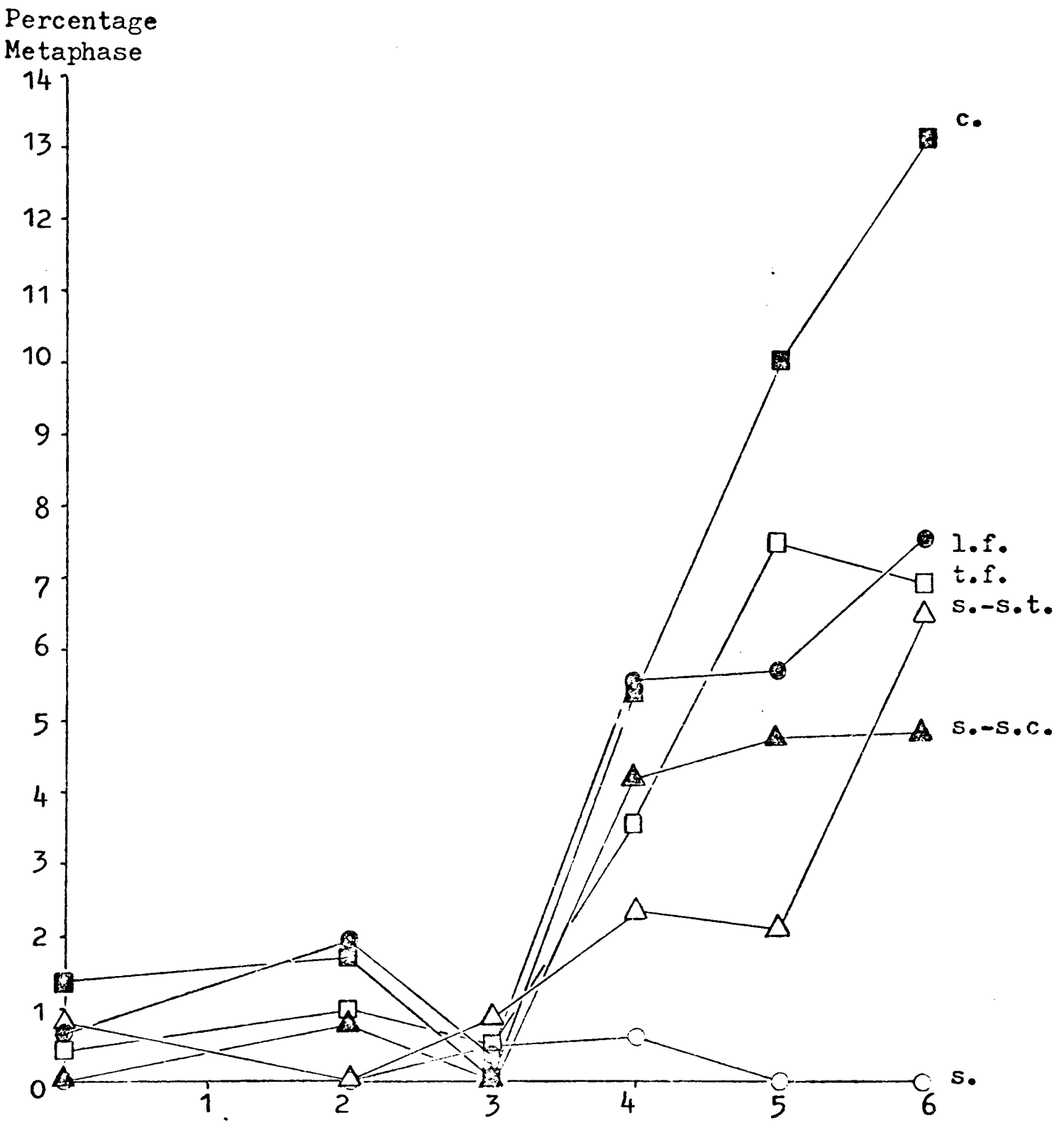


Fig. 8.6 Percentage metaphase levels in group II ;
$2\frac{1}{2}$ days dark + 40 hrs. far-red.

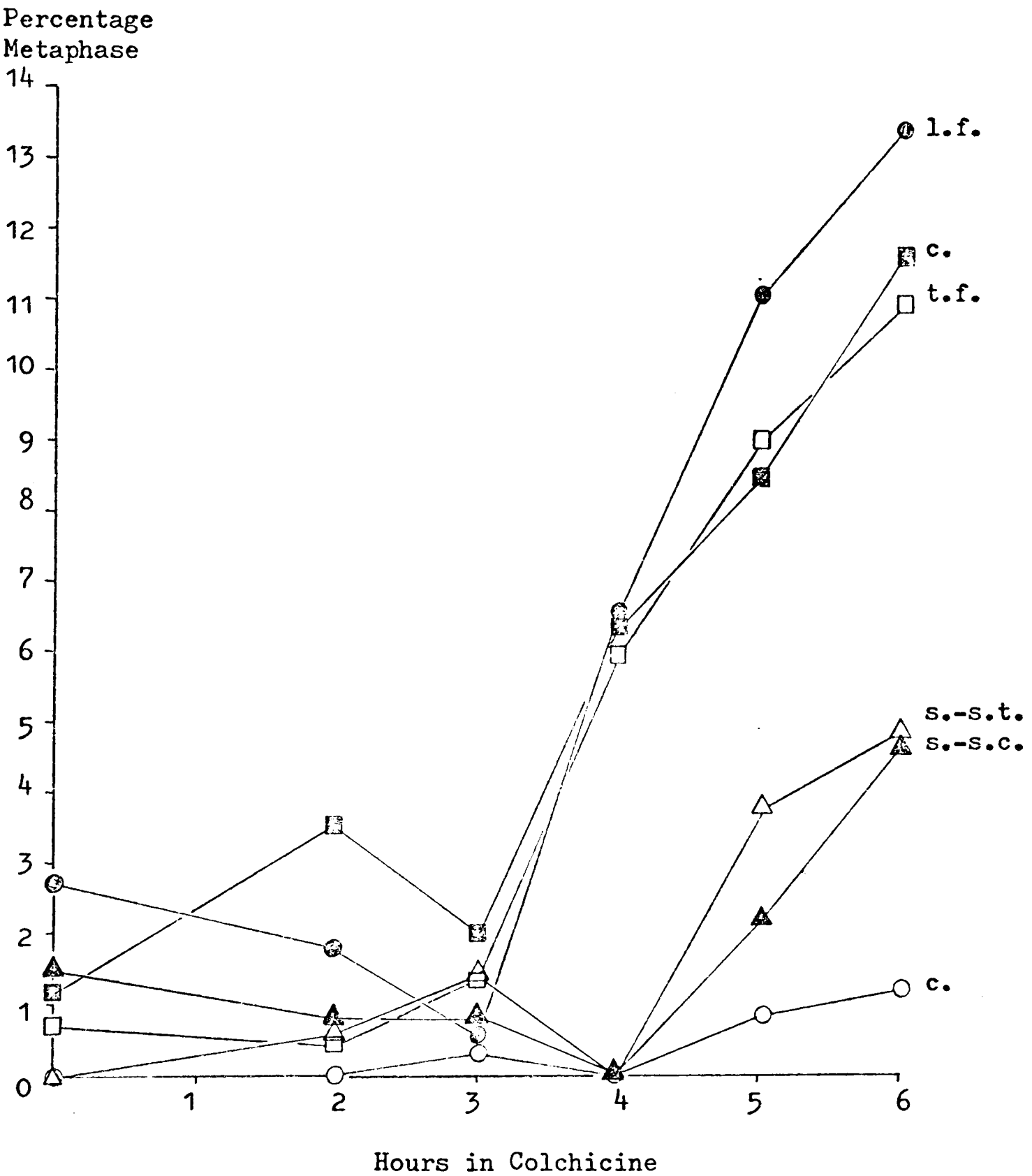


Fig. 8.7 Percentage metaphase levels in group II ; $4\frac{1}{2}$ days dark + 16 hrs. far-red.

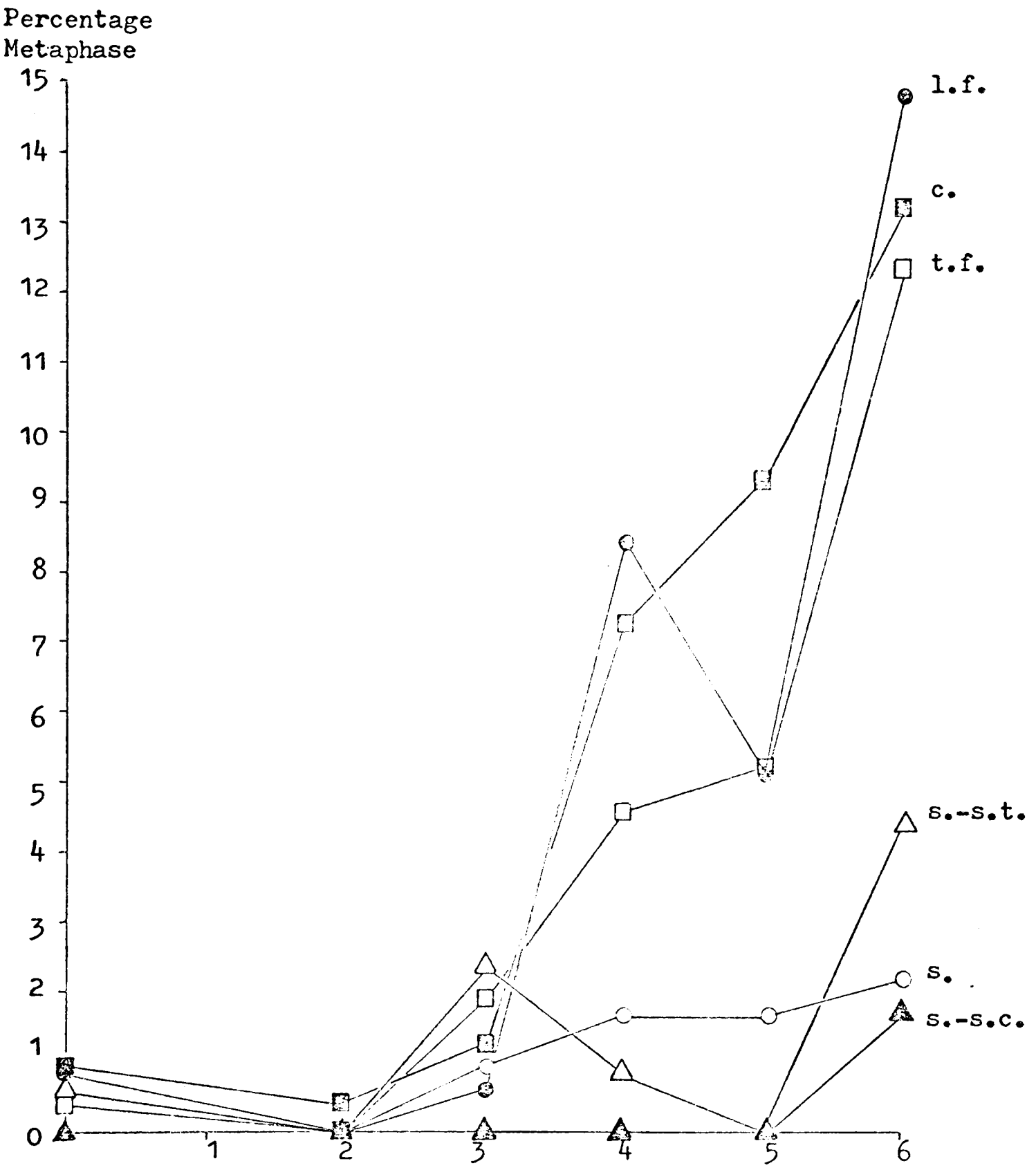


Fig. 8.8 Percentage metaphase levels in group II ;
$3\frac{1}{2}$ days dark + 40 hrs. far-red.

	I						II		
	$3\frac{1}{2}$ days dark						$2\frac{1}{2}$ days dark	$4\frac{1}{2}$ days dark	$3\frac{1}{2}$ days dark
Region	plus 16 hrs. dark	plus 16 hrs. far-red	plus 16 hrs. red	plus 40 hrs. dark	plus 40 hrs. far-red ⊛	plus 40 hrs. red	plus 40 hrs. far-red	plus 16 hrs. far-red	plus 40 hrs. far-red ⊛
	(Calculated from 4 & 5 hr. values)						(Calculated from 3 and 5 hr. values)		
Summit	∞	∞	41	∞	63	86	∞	271	166
Sub-summit tunica	86	∞	58	30	52*	62*	121	57	40**
Sub-summit corpus	30	∞	36	∞	27	49*	29	102	77**
Leading flank	∞	∞	23	21	9	13	26	13	17***
Corpus	21	∞	15	20	12	19	14	22	17
Trailing flank	17	∞	19	17	13	25	18	19	19

⊛ These two experiments have identical light treatments
* Estimated from 4 and 5 hr. values averaged and accumulation over 2 hrs. from 3 hrs.
** From 4 and 6 hr. values
*** Treating 4 hr. value as 5 hr. value

Table 8.1 Cell-doubling times calculated in hours for group I and II experiment. Treatments as indicated.

avoided. The cell-doubling times calculated will only be true for short periods since the absolute values may change within the calculated cell-doubling time.

With three exceptions the levels of prophase did not alter during the experiments indicating that colchicine was not affecting the rate of entry of cells into mitosis. The three exceptions all involved far-red treatments. After the 16 hrs. far-red treatment in group I experiments the sub-summit corpus had no prophases at 0 and 5 hrs. but 6% at 4 hrs. The percentage prophase in the sub-summit tunica after 40 hrs. far-red changed from zero at 0 hrs. to 3.7% at 4 hrs. and 0.6% at 5 hrs. The experiment in the group II set which is a repeat of the longer far-red treatment in group I i.e. 3.5 days dark followed by 40 hrs. far-red showed several peaks of prophase. The sub-summit corpus had a peak at 5 hrs. of 4.3%, the corpus at 4 hrs. of 3.9%, the leading flank at 3 hrs. of 3.3% and the sub-summit tunica at 4 hrs. of 2.8%. Since prophase levels in other experiments carried on at the same time in exactly the same conditions apart from the light treatment showed no marked change it may be assumed that the rate of entry into mitosis is not affected by colchicine, but by the disturbing effects of far-red. The odd fluctuation in the level of metaphases in the leading flank may also be due to this.

Plants grown on for a day in the different light treatments in group I appeared as expected i.e. dark and far-red grown plants were colourless and red-grown plants were green. All three sets had coleoptiles well open but red- and far-red- treated plants had a more developed secondary root system than those grown in the dark.

Discussion

Comparison of cell-doubling times is more valuable if the graphs of metaphase accumulation are taken into account at the same time. This is because fluctuations in the graphs due to variability of the population are carried into the calculations particularly in the small sub-summit regions. Large differences in cell-doubling times are more reliable indications of treatment effects.

Group I

Rates of cell multiplication after 40 hrs. red and far-red illumination are faster compared with dark controls. This increase in rate is already taking place after 16 hrs. red light, but 16 hrs. far-red light appears to be inhibitory. There may be some synchrony as already discussed, so that true rates of cell multiplication would not be reflected here. The experiment in Group II which was given 16 hrs. far-red light had a normal prophase pattern. However, the plants had been moved into red at the same time and so the difference in metaphase-accumulation patterns must be due to different effects of the treatment. The higher rates of division caused by 40 hrs. far-red compared with 16 hrs. far-red may be due to the longer irradiance or the age of the seedling. Response to red light may take place earlier than response to far-red light or more far-red may be needed to produce the same effect as red light.

Group II

This effect of far-red was investigated more closely in the three experiments in group II. They were designed so that short irradiations were given to older plants and longer ones to younger plants. The third set was a check on the high rate found after 40 hrs. far-red in the first set of experiments and gave similar results.

Similar rates were obtained in plants that had received 40 hrs. far-red and 16 hrs. far-red when the seedlings were the same age at the end of the treatment (3.5 days dark followed by 40 hrs. far-red, and 4.5 days dark followed by 16 hrs. far-red). In this case 16 hrs. far-red was not inhibitory and no synchrony was suggested in the prophase levels. At the same time, 40 hrs. far-red on 2.5 day-old seedlings gave higher rates than 16 hrs. far-red on 3.5 day-old seedlings. In this instance plants of the same age at the end of the treatment which had received different amounts of far-red were reacting in a different way. From this investigation then, variation in the duration of far-red illumination produced different effects on the younger plants but not in plants one day older.

Conclusions

1. Physiological

The physiological implication of the effects of red and far-red irradiation on the rates of cell division in the shoot apex regions are interesting. Changes in sensitivity to far-red with stage of growth are known. The competence for anthocyanin

formation in Sinapis increases and decreases with age, with a maximum at 36 hrs. from sowing, (Wagner and Mohr 1966). For further information on the link between leaf initiation rates and phytochrome the effects of red light should be investigated in more detail. It would be interesting to see if a short red pulse had the same effect as a long red pulse, and whether this was reversed by far-red. If so, this would confirm the participation of phytochrome as a photosensor. Long red irradiation was used in these experiments because some responses are known for which a short red exposure is ineffective e.g. inhibition of radish elongation (Jose 1975). Likewise long far-red irradiation was used because some responses are known where red has little effect but prolonged far-red is effective e.g. hypocotyl elongation inhibition in lettuce (Turner and Vince 1969).

2. Shoot apical organisation

All regions of the shoot apex are affected by light. Apart from the effect of 16 hrs. far-red on 3½ day-old dark-grown seedlings (group I) the rates of cell-multiplication were increased. With one exception (16 hrs. red on 3½ day-old dark-grown seedlings) the summit is at least seven times slower than the flank and corpus regions. The fairly high rate of cell multiplication in the summit of plants given 16 hrs. far-red after 3½ days dark growth may indicate a slightly deeper involvement of the summit in growth at the beginning of leaf initiation and it would be interesting to carry out a more thorough investigation of this.

Comparison of the dark controls at different ages shows that there is a switch in activity from the sub-summit corpus to the

sub-summit tunica and leading flank. This may indicate the start of leaf production in the dark. This link is also suggested in the four day old plants which had received 40 hrs. far-red. The flank cells were noticably slower than the corpus cells while the sub-summit corpus had a consistently high level of metaphases.

Comparison of cell-doubling times with those reported in section 4 indicate that, in most cases, red and far-red light have a similar effect to white light though experiments in white light were carried out in unaerated water.

The effect of red and white light on the pea shoot apex was investigated by Thomson and Miller (1962). They found no size difference between apices grown in the dark or in a 16 hr. light period of red or white. The plants were grown for 8 days in white light and 10 days in the dark. Butler and Lane (1959) compared apices from etiolated and normal Vicia seedlings and found no difference in external form, dimensions or distribution of mitotic index. But they did find more mitoses in the light grown plants. They assumed that interphase is longer in the etiolated plants grown in the dark since it is unlikely that darkness would speed up the mitotic phase itself. They concluded that the higher mitotic frequency in the light accounted for the greater cell production and increase in rate of leaf formation. This is certainly borne out in the work on rice.

9. GENERAL DISCUSSION

The most important contribution to knowledge of the shoot apex provided here is the measurement of rates of cell multiplication in the summit and flanks. The difference between them by a factor of at least seven is the first evidence for support of a quiescent summit which can be related in any sense to the quiescent centre of the root.

The absolute rates found are much faster than any reported for other shoot apices. The link between cell cycle times and temperature suggested by Bodson has already been mentioned. The short cell cycle times are also consistent with the view of Van't Hof and Sparrow (1963) that there is a direct relationship between the DNA content of a nucleus and the minimal cycle duration since rice has a very low nuclear DNA level of 1.1pg. (Section 6).

In addition to several measurements of cell-doubling times of rice in different conditions, the characteristics of the summit of infrequent mitosis throughout 24hrs. and a negligible number of nuclei labelled after feeding with tritiated thymidine give further support based upon entirely different kinds of evidence for a quiescent summit. The difference between the labelling in the summit and flanks in rice was greater than any reported for equivalent regions in other higher plants. The diversity of the summit and the flanks in their reaction to X-irradiation is an additional piece of evidence for considering them as quite different meristematically and the appearance of the cells throughout a plastochron suggests a method of growth of the apex in which the summit plays a relatively passive role.

Some consideration should be given to the nature of the controversy which surrounds the summit. The views of the 'Franco-

Belgian School' in support of the méristème d'attente and those of the 'Anglo-Saxonnes' to quote Nougarède (1967) against the méristème d'attente concern the difference of opinion as to the nature of an initial cell. Both schools now agree that the summit is meristematically less active than the flanks, but differ concerning the importance of the few divisions that do occur in the summit for growth of the apical meristem. As both schools originally took more extreme views on the activity of the summit viz. that the méristème d'attente was totally inactive (Buvat 1952) or that there was absolutely no difference between it and the flanks (Gifford 1960) the situation at present does represent a convergence of originally opposed views. This is underlined by the joint work of Gifford with Nougarède (Nougarède, Gifford & Mondet 1965) in which the authors support the initial ring theory of Plantefol and by the occasional references in Nougarède's work to the contribution of the summit to the flanks in certain species eg. Impatiens which has a small apex (Nougarède 1967). However, the central issue of the importance of the summit to apical ontogeny and the presence or absence of apical initials is not resolved. It would appear not worthwhile pursuing the controversy until some definition of an 'initial' can be agreed upon, and further, that it should be something identifiable with the techniques we have available. However, the difference in rate of cell multiplication between the summit and the flanks in rice gives more substantial support for quiescence in the summit than existed before. This is support for a quiescent summit not a méristème d'attente which has idealogical overtones about the function of the summit.

The experiments of Sussex and Rosenthal mentioned in the introduction (Section 1) in which they "tagged" cells and those of

Langenauer and Davis on the response to X-irradiation, also mentioned in the introduction are good evidence that the cell-divisions in the summit are not essential for growth of the shoot apex. However, since cell divisions do occur in the summit, they must be explained. Occasional divisions may occur merely as a function of the summit being alive and so are part of a general "ticking-over". The apex increases in size with age and so some divisions will contribute to this process. There may be an inconsequential "leakage" of cells to areas below or more active involvement in cell production which Nougaréde envisages for small shoot apices.

In addition to the question of the role of the summit in the shoot apical meristem there is a general need to understand more about the vegetative shoot apex both for its own sake and for related studies, particularly the events in the summit need to be interpreted in relation to the state of the apex before flowering.

Results from the control experiment for excision in Section 4, and the control experiment for X-irradiation effects in Section 6, in which plants were grown in two light intensities showed a smaller ratio between the rates of cell cycling in the flanks and corpus regions and the summit and sub-summit regions than in plants which had been grown in constant illumination or constant darkness. The cells of the leading flank in particular are slower. Other cases where the cells of the leading flank are noticeably slow are the dark grown control for 16 hrs. light treatments in group I in Section 8, the 16hr. far-red treatment in the same group and the 40hr. far-red-treated plants in group II which had been growing in the dark for 2½ days. In all these the sub-summit corpus cells and/or the corpus cells are noticeably faster

than usual. This reflects a change in the balance of growth of the shoot apical meristem to varying external conditions.

The measurements on rice have all been on seedlings. It would be interesting to follow the activity of the regions especially the summit with the age of the plant but in rice the shoot apex becomes increasingly inaccessable by the growth of more leaves and their silicification making investigation more and more difficult.

The emphasis of this work has been on the meristematic activity of the shoot apical regions. While it seems reasonable to conclude that the summit does not play a great part in contributing cells, surgical experiments indicate that it does play an important part in the regulation of the vegetative apex perhaps by chemical means, Wardlaw 1957, 1965.) Unfortunately, this can only be in the realm of speculation since we do not know about the relationships between the regions at this level and we do not know what causes a cell to prepare for division. The chemical characteristics of a cell and their relationship to development and differentiation and the part they play at the intercellular level will probably provide the next stage in our understanding of the development and organisation of the shoot apical meristem.

APPENDIX I

STATISTICS

The variation of mitotic index in the leading flank through 24 hours of the unknown rice variety in the dark (experiment 1) was subjected to a χ^2 contingency test. Cells in each sample of ten plants were treated as if they were from one population. The χ^2 factor was calculated for the null hypothesis that the frequency did not vary through 24 hours.

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

$$\text{Expected frequency} = \frac{\text{Mean mitotic cells}}{\text{Mean total cells}} \times \text{Total cells.}$$

TIME OF DAY

	11.00	13.00	15.00	17.00	19.00	21.00	23.00	01.00	03.00
Mitotic cells	11	9	9	10	13	15	14	13	12
Total cells	526	523	591	542	506	542	448	483	595

continued

	05.00	07.00	09.00	Total
Mitotic cells	29	15	8	168
Total cells	522	512	538	6328

Expected frequency for 11hrs is therefore:-

$$\frac{168}{6328} \times 526 = 13.96$$

and for 13hrs is

$$\frac{168}{6328} \times 523 = 13.9$$

The values for all the readings are as calculated in the same way.

So, χ^2 is given by:-

$$\frac{(11 - 13.96)^2}{13.9} + \frac{(9 - 13.90)^2}{13.9} \quad \text{... etc.}$$

By calculation, this is 28.59

For 11 degrees of freedom, this value is at the 0.1% level of probability. There is therefore a 0.1 chance of so large a value of χ^2 occurring when the mitotic index is not affected by the time of day and so there may be some synchrony.

χ^2 values for the summit and sub-summit tunica in the same species gave values of 5.18 and 4.98 respectively (which are within 99% probability) indicating that variation was significantly close to expected frequencies.

APPENDIX II

THE NUMBER OF APICES IN A SAMPLE

The low numbers of cells in mitosis and the small number of cells gave rather large standard errors and so it was necessary to establish the number of apices in a sample which would give useful results.

A colchicine experiment on dark grown rice in which samples of 30 apices for each point were taken was analysed by dividing the samples randomly into three groups of 10 apices each and the length of mitosis for each group calculated separately. This was repeated with data from 20 apices per sample obtained from combining the groups of 10 in three different ways ie. with three groups of 10 apices forming groups A, B & C, then the three groups of 20 apices were formed from A + B, B + C & A + C.

As can be seen from Table A.2.1 and Fig. A.2.1 10 apices gives values which vary over 11hrs. for region 4 (the largest region) and over 168hrs. for region 2 but the pattern of rates between the regions however is basically similar. The range is cut down considerably using 20 apices. The three different sets of 20 apices gave a variation of 4hrs. for region 4 and 71hrs. for region 2. (Table A.2.2 & Fig. A.2.2).

It seems reasonable to conclude from these results that a sample of 10 apices gives a useful guide to the effect of a treatment while 20 apices gives more reliable results, especially for the upper regions.

REGION	CELL-DOUBLING TIME (T) IN HOURS		
	GROUP A	GROUP B	GROUP C
Summit	2102	∞	∞
Sub-summit tunica	263	426	213
Sub-summit corpus	104	180	206
Leading flank	54	53	64
Corpus	107	72	158
Trailing flank	72	52	93

Table A.2.1 Cell-doubling times calculated from three different sets of 10 apices in the same experiment.

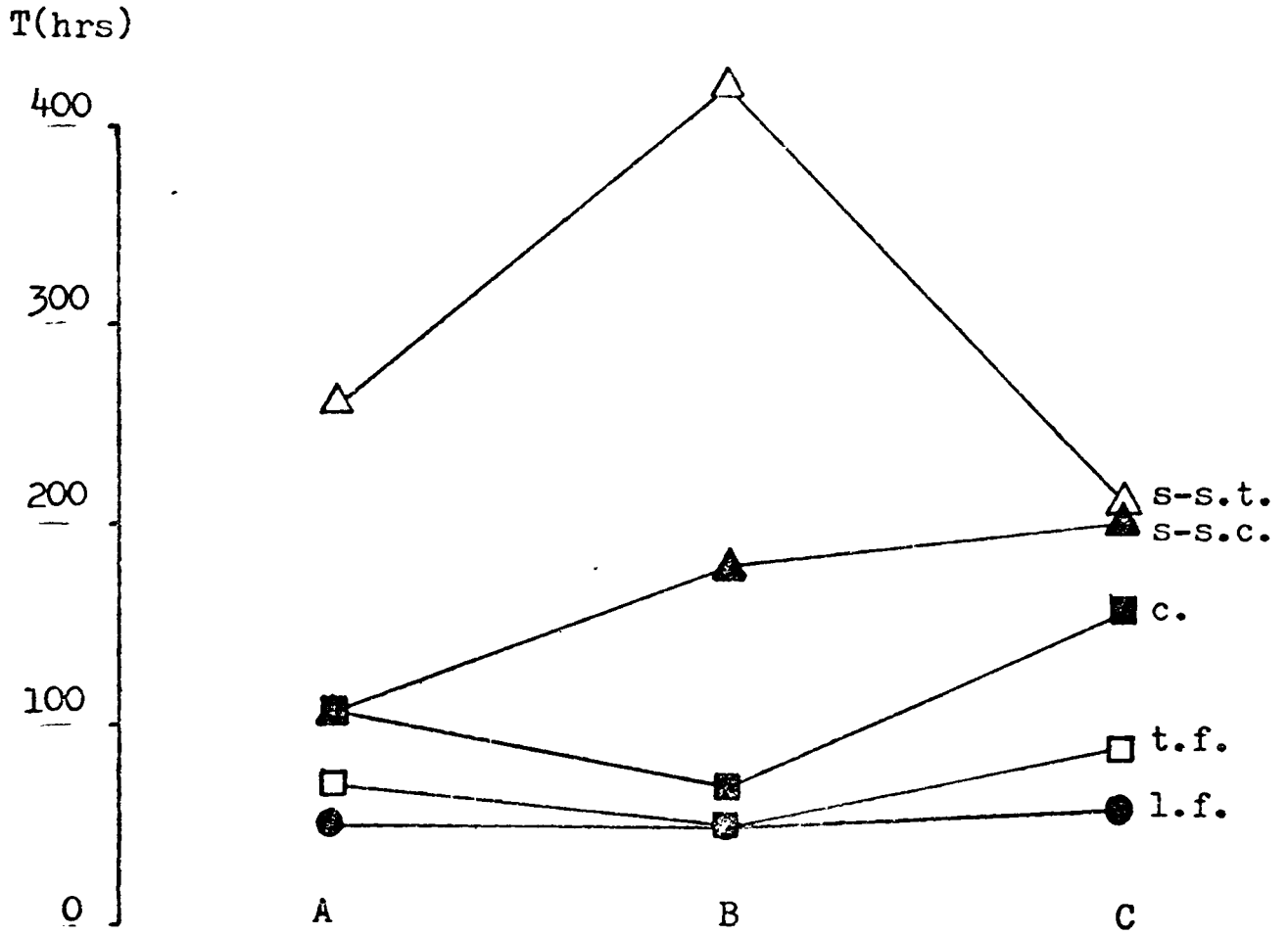


Fig. A.2.1 Graphic presentation of results tabulated in Table A.2.1 omitting summit.

REGION	CELL-DOUBLING TIME (T) IN HOURS		
	GROUPS (A+B)	GROUPS (B+C)	GROUPS (A+C)
s.	4158	∞	4125
s.s.t.	305	275	234
s.s.c.	135	193	141
l.f.	53	59	60
c.	85	101	130
t.f.	61	68	83

Table A.2.2 Cell-doubling times calculated from three different sets of 20 apices (see text) in the same experiment.

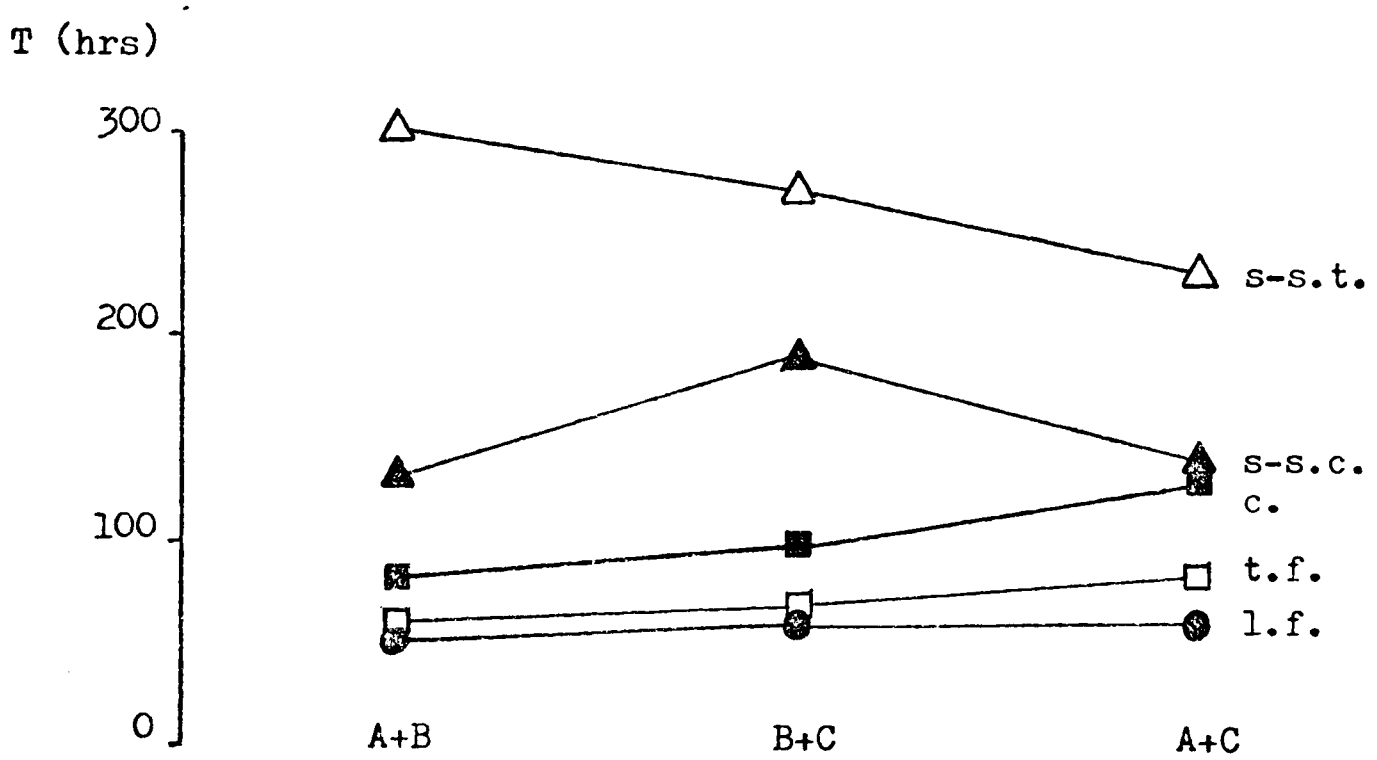


Fig. A.2.2 Graphic representation of results tabulated in Table A.2.2 omitting summit.

APPENDIX III

MITOTIC INDEX AND PERCENTAGE OF THE DIFFERENT PHASES OF MITOSIS AFTER X-IRRADIATION

Full figures are given here. The values for mitotic index and percentage prophase have been represented graphically in the Section 6.

Mitotic Index

After 1 k.rad.

Region	Control	½ hour	1 hour	24 hour
summit	0.7	0.5	0.3	0.5
sub-summit tunica	0.4	5.1	0	1.6
sub-summit corpus	2.1	4.2	0.3	0.9
leading flank	2.8	4.7	0.9	1.5
corpus	1.[illegible]	7.1	2.0	0.7
trailing flank	1.3	5.4	1.9	1.6

After 2 k.rad.

s.	0.7	0.5	0	0
s-s.t.	0.4	1.4	0	0.8
s-s.c.	2.1	0	0	0
l.f.	2.8	4.9	0	2.0
c.	1.8	3.4	0.7	1.0
t.f.	1.3	4.7	0	1.6

Mitotic index continued.

After 4 k.rad.

Region	Control	0 hour	½ hour	24 hour
s.	0.7	2.4	0	1.4
s-s.t.	0.4	2.3	0	0.8
s-s.c.	2.1	3.6	0.6	0
l.f.	2.8	2.9	0	2.3
c.	1.8	3.2	0.3	2.1
t.f.	1.3	3.5	0	0.5

After 8 k.rad.

s.	0.7	0	0	1.2
s-s.t.	0.4	1.2	0	0.7
s-s.c.	2.1	0.6	0.7	0
l.f.	2.8	1.0	0	2.3
c.	1.8	0.2	0.7	1.7
t.f.	1.3	0	0	1.1

Percentage Prophase

After 1 k.rad.

Region	Control	0 hour	½ hour	24 hour
s.	0.4	0	0.3	0.5
s-s.t.	0.4	2.3	0	0
s-s.c.	1.0	3.2	0.3	0
l.f.	1.8	1.8	0.2	0.5
c.	0.8	2.5	0.8	0.4
t.f.	0.5	2.2	1.0	0.5

After 2 k.rad.

s.	0.4	0.5	0	0
s-s.t.	0.4	0.7	0	0.8
s-s.c.	1.0	0	0	0
l.f.	1.8	1.9	0	0
c.	0.8	2.0	0.2	0.3
t.f.	0.5	2.4	0	1.1

Percentage prophase continued

After 4 k.rad.

Region	Control	0 hour	½ hour	24 hour
s.	0.4	0	0	0
s-s.t.	0.4	1.1	0	0
s-s.c.	1.0	3.0	0	0
l.f.	1.8	0.8	0	0.5
c.	0.8	1.1	0.3	0
t.f.	0.5	1.5	0	0

After 8 k.rad.

s.	0.4	0	0	0.6
s-s.t.	0.4	0.6	0	0.7
s-s.c.	1.0	0	0.7	0
l.f.	1.8	0.6	0	0.9
c.	0.8	0.2	0.7	1.2
t.f.	0.5	0	0	0

Percentage metaphase

After 1 k.rad.

Region	Control	0 hours	½ hour	24 hour
s.	0	0.5	0	0
s-s.t.	0	0.5	0	0.8
s-s.c.	0	0	0	0
l.f.	0.5	0.6	0.2	0.5
c.	0.9	1.1	0.4	0
t.f.	0.3	0.7	0.4	1.1

After 2 k.rad.

s.	0	0	0	0
s-s.t.	0	0	0	0
s-s.c.	0	0	0	0
l.f.	0.5	0.8	0	1.5
c.	0.9	0.2	0	0
t.f.	0.3	0.4	0	0.5

Percentage metaphase continued

After 4 k.rad.

Region	Control	0 hours	½ hour	24 hours
s.	0	1.0	0	0
s-s.t.	0	0	0	0
s-s.c.	0	0.6	0	0
l.f.	0.5	0	0	0.5
c.	0.9	0.4	0	0.7
t.f.	0.3	0	0	0

After 8 k.rad.

s.	0	0	0	0
s-s.t.	0	0	0	0
s-s.c.	0	0	0	0
l.f.	0.5	0	0	0.5
c.	0.9	0	0	0.4
t.f.	0.3	0	0	0

Percentage Anaphase

After 1 k.rad.

Region	Control	0 hour	½ hour	24 hours
s.	0	0	0	0
s-s.t.	0	0	0	0
s-s.c.	0.5	0	0	0
l.f.	0	0.3	0	0
c.	0	0.4	0.2	0
t.f.	0.3	0.7	0.2	0

After 2 k.rad.

s.	0	0	0	0
s-s.t.	0	0	0	0
s-s.c.	0.5	0	0	0
l.f.	0	0.6	0	0
c.	0	0.2	0	0
t.f.	0.3	0	0	0

Percentage anaphase continued

After 4 k.rad.

Region	Control	0 hour	½ hour	24 hours
s.	0	0	0	0.7
s-s.t.	0	0.6	0	0
s-s.c.	0.5	0	0	0
l.f.	0	0.4	0	0.9
c.	0	0.2	0	0
t.f.	0.3	0.5	0	0

After 8 k.rad.

s.	0	0	0	0
s-s.t.	0	0	0	0
s-s.c.	0.5	0.6	0	0
l.f.	0	0.3	0	0
c.	0	0	0	0
t.f.	0.3	0	0	0

Percentage Telophase

After 1 k.rad.

Region	Control	0 hour	½ hour	24 hour
s.	0.4	0	0	0
s-s.t.	0	2.3	0	0.8
s-s.c.	0.5	0.9	0	0.9
l.f.	0.5	2.1	0.4	0.5
c.	0.2	3.2	0.6	0.4
t.f.	0.3	1.8	0.2	0

After 2 k.rad.

s.	0.4	0	0	0
s-s.t.	0	0.7	0	0
s-s.c.	0.5	0	0	0
l.f.	0.5	1.5	0	0.5
c.	0.2	1.0	0.5	0.7
t.f.	0.3	2.0	0	0

Percentage telophase continued

After 4 k.rad

Region	Control	0 hour	½ hour	24 hours
s.	0.4	0.5	0	0.7
s-s.t.	0	0.6	0	0.8
s-s.c.	0.5	0	0.6	0
l.f.	0.5	1.7	0	0.5
c.	0.2	1.5	0	1.4
t.f.	0.3	1.5	0	0.5

After 8 k.rad

s.	0.4	0	0	0.6
s-s.t.	0	0.6	0	0
s-s.c.	0.5	0	0	0
l.f.	0.5	0	0	0.9
c.	0.2	0	0	0
t.f.	0.3	0	0	1.1

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