1988

Ultrastructural And Associated Biochemical Changes Related To The Endogenous Rhythm And Flowering Response In Chenopodium Rubrum

Isabel Joy Dickson-Clark

I dedicate my thesis to my mother

Alice Isabel Dickson

whose continuous academic support and encouragement has continued throughout my life

and to my daughter

Dr. Carolynn Joy Clark

whose steadfast support is faithfully given.

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui sont déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

ULTRASTRUCTURAL AND ASSOCIATED BIOCHEMICAL CHANGES RELATED TO THE ENDOGENOUS RHYTHM AND FLOWERING RESPONSE IN CHENOPODIUM RUBRUM

by

I. Joy Dickson-Clark

Department of Biochemistry

Submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Ontario
August, 1987

ISBN 0-315-40764-6

I dedicate my thesis to my mother

Alice Isabel Dickson

whose continuous academic support and encouragement has continued throughout my life

and to my daughter

Dr. Carolynn Joy Clark

whose steadfast support is faithfully given.

ABSTRACT

Experimental work is carried out using seedlings of the early flowering Chenopodium rubrum system. Flowering can be controlled photoperiodically. Experimental series of seedlings are exposed to either continuous light, continuous dark or to a selected period of dark followed by light. Growth and development in terms of plastochron stage, ontogenetic state, at the apex within the experimental time are examined, as also are the effects of the light and dark treatments. It is found that the plastochron age differs among seedlings of the same chronological age under differing treatments. The effect of the dark is to produce a delay or interruption effect in normal growth and development compared with continuous light; that this is concommitant with flower induction; that the ontogenetic stage of induced apices in the light following the dark consequently is dependent only on the length of the light following the dark and is independent of the length of the dark; that the first observation on examining the apices of both induced and non-induced series is that of a growth spurt and much increased plastochron rate. The same processes and same hormones are required for both apices; that the first observation on examination of the cells within both the induced and non-induced apices is of cell

enlargement followed by cell division. This involves a "grow and divide" message and with the same content - for both apices. The implications for flowering theory are discussed and the concepts of induction and evocation. It is deduced that flowering is conceptually an apical event.

Changes in biochemical activity are consistent with changes in ultrastructure and this was examined in apical cells, primordial leaf and cotyledons. The metabolic status correlates with the phase of the endogenous rhythm of which flowering acts as a marker. The thesis shows that at opposite phases of the rhythm the metabolic status does differ. At those phases of the rhythm when flowering can be induced the apices are metabolically poised. The sensitivity of the seedlings to floral induction, changes with changing phase of the rhythm and parallels the endogenous rhythm in ultrastructural and metabolic status. The implications for flowering theory and the concepts of induction and evocation are again discussed.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. B.G. Cumming who supported the early work and initiated me into the mysteries of endogenous rhythms and the Chenopodium rubrum early flowering system.

I especially would like to express very great appreciation to Dr. W. Chefurka and Dr. P. Fitz-James for their continuous support, help and great patience through many vicissitudes. Their advice and encouragement have been invaluable.

Thanks are due to Dr. R.B. Van Huystee and Dr. R. Greyson for their discussions and suggestions.

I am indebted to Mrs. Leah Mitchell, Mrs. Doryth Lowey and Mr. A. Noon for their reliable, competent and willing technical assistance. I especially want to thank Mrs. Maureen Coleman and Mrs. Ruth Webber for their endless patience and encouragement. Without them, there would be no thesis. My special thanks goes to my daughter, Carolynn, without whose encouragement and support my thesis would never have been collated.

LIST OF FIGURES

CHAPTER 3 CONT'D

CHAPTER 4 CONT'D

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ULTRASTRUCTURAL ABBREVIATIONS

cch	=	condensed chromatin
D	=	dictyosome
F	=	fibrillar appearance
FF	=	forming face
G	=	granular appearance
HC	=	heterochromatin
K	=	karyosome
KC	=	knotty character
L	=	lacuna
m-b	=	membrane-bound
MNu	=	micronucleolus
NE	=	nuclear envelope
Nu	=	nucleolus
OG	=	osmiophilic globules
PD	=	plasmodesmata
Pf	=	puff
PG	=	perichromatin granules
PM	=	plasma membrane
RER	=	rough endoplasmic reticulum
SER	=	smooth endoplasmic reticulum
VE	=	vesicle
VL	=	vacuole

EXPERIMENTAL ABBREVIATIONS

D	=	continuous Dark series
DEP	=	diethyl pyrocarbonate
x g	=	x acceleration of gravity
H-L	=	high light 3000 ft candle
3-IAA	=	3 indole acetic acid
L/D	=	Light-after-Dark series
L-L	=	low light 1200 ft candle
NAA	=	napthalene acetic acid
PTA	=	phosphotungstic acid
RNase	=	polyribonucleotide 2-oligonucleotido-transferase
RNP	=	ribonucleoprotein
S_{OBS}	=	observed sedimentation coefficient

CHAPTER 1

INTRODUCTION

Starvation is a worldwide problem with severe social and political consequences. Control of flowering means control of fruiting and of food crop yields. More efficient production of food and fibre crops, and the development of plants more vigorous and more resistant to diseases and environmental problems is an urgent need.

Flowering systems provide the biologist with excellent experimental systems which can be manipulated to induce flowering or leave vegetative. The methods can be non-invasive such as change in the light-dark regime for photoperiodically inducible systems, or change in the temperature regime such as vernalisation. This factor is important for any later biochemical work. Investigations can be made of growth, development and morphogenesis both of the whole organism, and at the cellular or ultrastructural level. Although a great deal of work has been done, over the last half a century the control of flowering is still poorly understood despite its importance as a physiological problem.

In 1937 Chailakhyan proposed and defined the concept of a floral hormone florigen (Chailakhyan, 1937). It was hypothesized to be induced and synthesized in the leaves under favourable conditions (especially favourable photoperiods) and then transported to the apex. This would result in evocation at the apex and be regarded as "switching on" a new floral programme. Accompanying this the vegetative apex would undergo morphological and biochemical changes as the first steps in the formation of the new floral apex. Bernier wrote "after more than 40 years of extensive ~~and careful work~~ no isolation of the presumptive universal hormone or florigen has yet been made" (Bernier, Kinet, Sachs, 1981). This still holds today, yet it remains ~~the~~ most widely held theory of flowering. Bernier comments further upon this situation, "The physiology of flowering is thus .... perhaps unique in biology that it is still dominated by a concept proposed more than 40 (50) years ago. It is recognized more and more that this concept puts severe constraints on further developments in both fundamental and applied research programmes in which the onset of flowering is the key process." Furthermore, "no matter how well established the theory may be, (it) requires consideration of alternate Hypotheses ... we suggest that a reconsideration of accepted notions ... is absolutely and urgently required" (Bernier, Kinet, Sachs, 1981). Although the

florigen theory is the dominant theory, another theory in 1949-1950 was proposed by Lona, von Denffer et al. This was the theory of floral inhibitors. Here flower initiation occurs under conditions preventing production of the inhibitors (Lona, 1948) (von Denffer, 1950). Workers have even proposed a mechanism which operates as a balance between the inductive and the inhibitor substances.

With the dogma above firmly established in the flowering field it was decided that any biochemical approach selected would be futile without a careful preliminary examination of the nature of the Chenopodium rubrum system chosen. Further, this initial investigation needed to address the seedling's behaviour, its reaction to stimuli in terms of morphological, physiological and biochemical parameters. Only after these factors were addressed with an open mind and depending upon what results were found, could any detailed biochemical work be planned and carefully focussed. Many studies have been undertaken on the premise that the classical florigen theory is correct. Indeed such studies comprise much of the flowering literature. It follows that if the wrong research question is asked of the system, then either the wrong answer or no valid answer at all will be forthcoming. The initial questions asked of the system here are evident in the following chapters. Flowering is an

apical issue. A grasp of the concept of apex is essential to understand flowering and will now be discussed.

Of necessity only related physiological and morphological aspects of apices can be considered here. The detailed evidence and arguments of fossil studies, evolutionary evidence, taxonomic evidence, and such theories as the Telome Theory etc. would also need to be considered in any rigorous discussion of questions such as "What is a root?" "What is a shoot?" and "What is a flower?" These are inevitably involved in considering and contrasting root, shoot and floral apices.

To contrast the apices of shoot and root, and the floral apex, it is necessary to understand the meaning of apex and the significance of the apex with respect to the whole plant body. It is essential to have a concept of apex. What is an apex? The apex is a meristem and hence it is necessary to understand the properties of growing cells and their behaviour.

Cell Potential

If a single cell capable of growth and division is supplied with all the nutrients required in a symmetrical environment it will divide into two and if the daughter cells remain equally capable of division, a continually growing globular mass of tissue will result. For brief periods some embryos exhibit this

potential. However, indefinite continuation of unrestrained growth in all directions rapidly encounters limitations, as discussed by (Steward, 1968, 1969). In fact the extent to which this type of growth is or is not achieved lies at the centre of the problem of differentiation and morphogenesis.

While it might be expected that such cells would have an equal chance to divide, this is not the case -

- In tissue culture in a growing globular mass the rate of division of cells on the surface is promoted over those within the mass (and is also more rapid than for completely free cells) (Steward, 1958b) (Blakely and Steward, 1964). A type of division of labour results, both in space and time. Together with the inherited ability of the cells to adapt under this environment, this division of labour appears to be an intrinsic property of cell masses and not of the cells themselves.

- Examination of intact plant tissues shows a similar type of division of labour among cells both in space and time. Notice the difference in pattern of the division of labour between root cells and those found in

the floral apex. This is also seen to be an intrinsic property of growing plant tissue masses.

Thus, one expects to find a pattern of cells, some dividing more actively than others in both the shoot and root apices, and in the floral apex.

Cellular Organisation

On examining the apices, differences in cellular pattern are observed. These have been variously interpreted (Sinnott, 1960; Esau, 1965; Eames, McDaniels, 1947; Clowes, 1961; Gemmell, 1969; Nougarede, 1967; Lang, 1965; Evans, 1969; Hillman, 1962) as shown by the various classical descriptions illustrated in some of the diagrams and in the combined physiological/morphological approach of Steward (1968).

Discussion

Several basic questions arise when comparing the various classical and modern interpretations of stem and root apical patterns and then considering the problems of the floral apex relative to these vegetative apices. These questions will be examined, along with work which throws some light on these problems.

In apices the cells all have the same heredity - the same DNA complement, being all derived from the same cell; they have similar potential, as non-dividing cells are capable of further division and under suitable conditions can revert to a very active meristematic status. It has been shown (Steward, 1968) (Steward, Mapes, Mears, 1958) with carrot-root tissue, that phloem cells in tissue culture can give rise to embryonic structures which can develop root and shoot apices and grow on into normal plants. Thus, each individual cell of the root or shoot or floral apex has the same heredity and similar potential. Nevertheless, among these same cells in the apex, some divide actively and some not. Why not? What stops them? Furthermore, the apical cell pattern of dividing cells in the root is consistently different from the shoot, and also differs from the floral apex.

QUESTION 1

On examining the mature vegetative apices - the mature shoot and root apices - one can ask only, "What is an apex?". (The differing results of the activities of these apices is not under consideration here.) This involves the concept of cell pattern, its consistent maintenance and the relationship between cells within this set pattern. The question, "Why don't all the cells divide?" again involves the concept of pattern of cells.

"What maintains the pattern?" This is the type of problem considered in Question 1. This is the problem of the mature shoot and root apex and involves the concept of apex.

QUESTION 2

. . Why do the root and shoot apices differ in pattern? becomes the question of what is a root? What is a shoot?

This is resolvable only if asked at the zygote-embryo level, because the root/shoot apex question is determined and set at this level. Here is the origin of the cellular pattern recognised as apex, and also of the two basically different apical patterns called root and shoot. This is the problem of the embryo, and is another of the basic questions.

The comparison of root and shoot apices is concerned with the concept of pattern of cells, how this is maintained and what factors set this up (Brown, 1958; S.E.B. Symposia, 1963; Stebbins, 1964; Steward, 1968). The plant embryo does not inherit a root and it does not inherit a shoot. Rather it consists of growing cells with a predisposition or capability of involvement, within the cell mass, to form certain distinctive cell-patterns. The zygote-embryo level is strictly concerned with the cell itself - its properties and relationships with other individual cells. The two differing patterns set up at this cellular level give rise to differently

behaving groups (or tissues) of cells. These function at the mature organised apex level in a set cellular pattern recognised as shoot and root.

QUESTION 3

Flowering is an apical issue. A clear concept of what an apex is, and a grasp of the fundamental properties and behaviour of the cellular mass termed apex, is fundamental to an understanding of flowering whether it be floral morphology and homology, floral physiology and the variety of stimuli, etc! Any interpretation of flowering must be related back to and be interpreted in terms of a concept of apex.

If shoot and root apex behaviour depends on the maintenance of cell pattern, then to change the behaviour of the apex as witnessed at the floral apex, there must be a change of cell pattern. Therefore, the floral apex represents the resultant effect of a process of alteration of the cellular pattern of an apex - the shoot apex.

3a - This concept raises questions as to whether the floral apex can be regarded as -

(i) a basic change of cell pattern implying a complete change in the type of apex. Hence the floral apex would be regarded in the

light of a different concept from the vegetative shoot apex and would be a valid third type of apex.

(ii) a modification of shoot cell pattern implying that the floral shoot or apex is basically similar or homologous with the vegetative shoot. An extreme question could be asked as to whether the modification is even more superficial with no basic pattern change. In this situation only redistribution of the dividing cell pattern on the apical flanks occurs with a change in activity of the quiescent cell group providing special nutrients. At this point one questions whether cell-pattern is a legitimate concept in this context or whether it is no longer an apical question but rather a lateral organ, and lateral organ derivation question.

In view of the modification of the shoot apical cell-pattern (as under ii), in whatever form that may take, the question resolves into the existence of

two apices - the shoot plus root,

plus one modified apex, (of the stem, which is called the floral apex).

(iii) or whether the floral apex can be regarded as a continually altering apex.

Perhaps the predominant characteristic of the floral apex is its failure to reach equilibrium or achieve stability of new apical cell pattern. Here sepals and petals would arise from an intermediate apical pattern (and there is evidence for this) (Evans, 1969) (Hillman, 1962) (Nougarede, 1967) with the sporophylls as terminal forms.

It is to be noted that a number of interesting issues are inherent in this type of concept. Namely, determinant floral growth for all plants; self-destruction for monocarpic plants; also that senescence of monocarpic plants is inherent in this apical system; could the problem of senescence in all plants be an apical problem? is there fundamentally an inherent instability in the shoot apex? (not fourd in the root). The

embyronic characteristic bestowed, of growth in volume (compared with root growth in length) is of significance.

3b - The principles involved with factors which maintain apical cell pattern are as applicable to the floral apex as to the root and shoot apices. The question then arises, what type of factors or forces would be required, not to maintain, but to cause an alteration in the pattern for the floral apex? What, where and how, do the great variety of stimuli infringe upon the cell milieu of the apex? What is the message the apex retrieves from the varied stimuli?

The message retrieved by the apex is a vegetative message to a vegetative apex whose cells are in a state susceptible to disorientation. This message is implicit in a concept in which a careful balance or equilibrium in the apex maintains the set cell-pattern. Outside stimuli or causative factors are required to change this status quo pattern before the floral apex can emerge. Even if details of stimuli are not known it is reasonable to deduce that they are a required concept within such a system.

Several basic questions implicit in the concept of apex as outlined above have been raised. The behaviour of cells and the concept of apex will be discussed. This is the essence of Question 1 and is fundamental to the other questions also.

As stated the concept of apex is concerned with cell pattern, its consistent maintenance and the relationship between cells within this set pattern.

This raises the following questions. What maintains consistent root or shoot behaviour? What determines which of the cells at the apex actively divide and which ones not and thereby maintains a set pattern of cells at the apex which is constant within and exclusive to the shoot or the root? Is this behaviour - division of labour or type of "differentiation" a basic inevitable property of cells? Of individual cells? Or groups of cells (i.e. a multicellular phenomenon)? Considerations of this type underlie an understanding of factors involved in the maintenance of distinctive vegetative apices (Q1) and also of those factors involved in any apical alteration such as that in the floral apex (Q3). The work of Steward is significant here (Steward, Mapes, Smith, 1958) (Steward, Mapes, Mears, 1958) (Steward, 1958a) (Steward, 1958b) (Blakely, Steward, 1964) (Steward, 1969).

Steward attempts methods of culturing cells and tissues to determine what makes cells grow at their maximum rates and to attempt to understand how they then differ in their most actively growing state from their counterparts, alive but quiescent in the intact plant body. Thus he examines free cell growth and compares it with the growth of cell clusters or colonies. The explants used are of secondary phloem of carrot-root tissue (2.5 mg or approximately 25,000 cells) isolated at a distance of (1-2 mm) from the cambium. The stock cultures are grown in 250 mls liquid in rotated (1 rpm) 1 litre flasks with 10 "nipples" each 3 cm long blown around the "equator" of the flask, or in Erlenmeyer flasks gently shaken on a platform-shaker under controlled light and temperature. Cultures are maintained by serial monthly sub-culturing in a liquid nutrient medium (modified White's - using 1/10 the concentration of vitamin stock, and iron tartrate 5 mg/litre for ferrous sulphate) with added 10% cocoanut milk and added 200 ppm of casein hydrolysate. (Whenever Haplopappus with slightly different requirements is used 2% cocoanut milk with 0.5 ppm naphthaleneacetic acid and 200 ppm casein hydrolysate is added to the liquid nutrient medium). Very gentle abrasion of cultured tissues as they grow in slowly rotating (1 rpm) culture tubes or flasks results in some of the actively growing surface cells of explants floating off into the ambient

liquid. Hence free cells are obtained and can be grown and subcultured either in liquid media or plated out onto agar media.

Steward reports (Steward, Mapes, Smith, 1958) that freely suspended cells under suitable culture conditions exhibit a wide range of size and form and their varied behaviour cannot be directly interpreted according to cell behaviour in plant tissues. These free cells are all capable of division and growth (under suitable culture conditions). Each cell behaves independently and grows in various unsymmetrical and distinctive ways. They display an unexpected range of morphogenetic behaviour. The free cell has potential properties not apparent when it is growing within a plant tissue. On examining cell clusters or colonies (Steward, Mapes, Mears, 1958), (Steward, 1958) it is found that cells in the centre of the cluster behave differently from those on the surface. There is rapid division on the surface but not internally. All cells do not divide independently and freely nor do they show a range of behaviour comparable to each free cell above. Steward states that "cell multiplication is largely confined to the surface of the explant and for the growing cluster the outermost cells grow more rapidly than those within". Thus a morphological division of labour arises naturally in any cell mass. This is not a property of the individual cells per se (as seen above). This is an

intrinsic property of all cell masses - a multicellular phenomenon. (Question 1). This division of labour between dividing and non-dividing cells is not peculiar to the concept of apex. It is also seen that cells in a group or mass exert influence and control on each other.

On further examination of cell groups Steward reports that the rate of division of peripheral cells is proportional to and greater than the rate for the mass of cells within (Steward, 1958). The greatest rate of cell division is found in the case of the relatively large tissue explant; the rate of cell division is less when the cells are present in smaller callus-like aggregates, and is even less for separate cells freely suspended in the medium. A similar result (in concept) is obtained on examination of cell survival in relation to unit size (Blakely, Steward, 1964). The frequency with which units grow to form colonies rises sharply with an increase in their initial size - cells attached to a group had a better chance of growing than did single free cells. So both division rates and survival of daughter cells are higher when cells exist in a colony along with non-dividing cells which appear to be stimulating those in a position to divide. Therefore, the degree of growth of each cell within a cell cluster is not determined by the intrinsic properties of the individual cell at this stage but rather is under the control of the cell milieu. It is to be noted that

this is of fundamental significance to an understanding of the apex especially with respect to maintenance of the cell pattern (Question 1).

Steward in further work (Blakely, Steward, 1964) reports that the larger non-dividing cells which promote division of cells attached to them at the surface of the cell cluster do release stimuli. These promote the division of surface cells, although at the same time greatly reduce the range of morphogenetic responses of these cells. The larger non-dividing cells also secrete into the medium substances which promote the viability of free cells in their proximity. Steward also presents several other pieces of evidence in support of this same point, e.g. the effect of "washing" his suspensions. Steward concludes that "the type of stimulus which is responsible for greater growth of cells around a core of explanted tissue may be communicated to the ambient liquid ... also may be responsible for the reason why cells grow better in clusters than in complete isolation ... also may be the reason why free cells appear to have more stringent growth media requirements than do cells in a group. Thus Steward indicates a physiological division of labour within a group of cells. This is the case of the more infrequently dividing cells deep within a cell colony and has also been considered in the case of the organised apex. There is evidence that the cells of the quiescent centre (Clowes, 1961) are not inert but

contribute to the efficiency of the whole multicellular organisation by acting as a source of very potent stimuli. Hence a physiological division of labour, can arise spontaneously in any cell mass. It is helpful to reiterate here the conclusions reached earlier on morphological cell behaviour or cell pattern of colonies, in terms of physiological factors. This is not a property of individual cells per se this is also an intrinsic property of all cell masses - a multicellular phenomenon (Question 1). This physiological division of labour among cells is not a property peculiar to the concept of apex either. Therefore here is the probable type of mechanism which enables cells in a group or cell mass to exert influence and control over each other; the type of control exerted by the cell milieu which enables it to maintain a set cell pattern (Question 1).

Thus considering the work of Steward (Steward, Mapes, Smith, 1958) (Steward, Mapes, Mears, 1958) (Steward, 1958a) (Steward, 1958b) (Blakely, Steward, 1964) (Steward, 1969) (Steward, 1968) on cell behaviour and the concept of apex implicit in Question 1 and its ramifications in Questions 2 and 3, it is concluded that which of the cells divide in the shoot and root apices and which do not, although all are potentially able, is determined or controlled by the cell environment - by the cell milieu of the apex - a consequence of the place the cells occupy in the organisation of the apex. This

cell division in the shoot and root is not controlled by intrinsic genetically determined factors of the cells themselves. Control over regions of dividing cells and non-dividing cells results in an organised cell pattern. Indeed this is a multicellular phenomenon. It is conceivable that the shoot or root cell pattern is involved with a pattern of physiological factors (a "patterned environment") which controls and maintains the integrity of the existing cell pattern of either shoot or root apex. It has been seen that cells do influence and control the growth and behaviour of each other although the detailed molecular mechanisms are not at all clear. Within the organised apex also there is evidence that quiescent cell groups influence adjacent cells to divide (Clowes, 1961); (Steward, 1968). Furthermore, on examination of the difficulties of tissue culturing sections of the shoot apex it is found that the dividing cells are interdependent on older cells or tissues beneath to provide needed nutrients or stimuli (Steward, 1968). The origin of the pattern of the apex, as of the two basically different apical patterns of the root and shoot, is at the zygote-embryo level. Following floral induction/evocation there is a serious disturbance in the balance or equilibrium of this "patterned environment" with a consequent change of cell pattern of the stem apex (Evans, 1969). As Steward states, "the sequence of development is determined by

both the hereditary "nature" of the cells and the special environmental nurture" they receive. The cells are permitted to express only part of the potential information stored in their genetic material depending on their position within the patterned environment of the apex. These controls must be interpreted in chemical terms.

Thus, factors and interactions and questions of the type considered in the section "Discussion" above, concerning growing cells and their behaviour, must be asked about the apex, although in greater depth than can be pursued here. The concept of apex involves not only a spatial or morphological problem but also a physiological and metabolic problem. Many workers (S.E.B. Symposia, 1963) concur with Steward's comments (Steward, 1968) that the problem resolves itself into a closer association than has yet been possible of the environment, of the chemical and physical milieu within the organisation of the apex, and for floral induction of the shoot apex in particular ... The parameters that define the milieu are neither single nor simple! An apex is a meristem. To understand a meristem requires an understanding of the properties of growing cells and their behaviour. Hence a more profound understanding of the growth processes of cells, of the morphological,

physiological and metabolic behaviour of growing cells, is fundamental to further advances in the understanding of the apex, and to flowering at the apex.

Clonal analysis provides a powerful tool enabling many of the above questions to be explored. Epigenetic variation (of cultured plant cells) and the gradual process of habituation (Meins and Binns, 1978) undoubtedly have direct relevance to the apical system, as have concepts such as pattern regulation and cell commitment (Bryant, 1978).

The experimental flowering system used was of seedlings of Chenopodium rubrum (Evans, 1969), an obligate short day plant. The flowering response is under photoperiodic control (Hendricks and Borthwick, 1965) (Cumming, Hendricks and Borthwick, 1965) (Cumming, 1963) (Cumming, 1969). It is also rhythmic and the period of the free running endogenous rhythm is approximately 30 hours. This is normally entrained to a 24 hour circadian rhythm by the normal night/day cycle. The Chenopodium rubrum system has a number of advantages (Cumming, 1966). Firstly, it is an early flowering system. Seedlings can be induced to flower when one week old and consequently, many experiments can rapidly be completed. This compares with the three to four months wait for full grown flowering plants. The C. rubrum seeds can be sown in petri dishes grown in controlled environment conditions in large numbers and the week old

10 mm seedlings can be counted statistically. They exhibit photoperiodic induction of flowering and show all of the properties of the flowering system. The second advantage is that induction of flowering requires only one long night. This is important for biochemical experiments. Changes in biochemical status can be shown to occur and also changes in metabolism and changes in membrane permeability at certain phases of the rhythm. No addition of chemicals is required. Thirdly, six ecotypes are available. The exact time of flowering is set for each ecotype and is in strict order according to their latitudes of origin over the range from Alaska in the North to California in the South. The ecotypes show adaptation to extremes of temperature and to differing day lengths, longer in the North and shorter in the South. These ecotypes would be very useful for membrane research. A fourth advantage of the C. rubrum system is that most species of the Chenopodiaceae ($P_4A_4\underline{G}(2)$) are halophytes. This is interesting with regard to their membranes and for membrane transport. Fifthly and finally, Chenopodium rubrum has well defined endogenous rhythms. Many investigations of these rhythms have been carried out. The periods of some of these rhythms are:

Chenopodium rubrum:

Rhythm in --	F-R Period
Chlorophyll Content	15 hours
Betacyanin Synthesis	30 hours
NADP GPD (Chloroplasts)	15 hours
NAD - GPD (Cytoplasm)	15 hours
Adenylate Kinase	30 hours
Photosynthesis	15 hours
(cf chlorophyll content and NADP-GPD)	

(Cumming, 1967) (Cumming 1969b) (Cumming, 1969c) (Cumming, 1971) (Andrae and Hopkins, 1973) (Chia-Loo i, 1971) (Cumming and Warner, 1968) (Frosch and Wagner, 1973) (King, 1971) (King and Cumming, 1972) (Wagner and Cumming, 1970) (Schwall, Wendorf and Wagner, 1985) (Lecharny, Schwall and Wagner, 1985) (Tsala, Frosch, Bonzon, Bergfeld, Greppin, Wagner, 1984) (King, 1979) (Cumming and Seabrook, 1985). The well defined endogenous rhythm of flowering makes it possible to use experimentally the flowering data on photoperiodic induction as a marker for the phase of the endogenous rhythm. My hypothesis is that the biochemical status of the system differs at opposite phases of the rhythm and that parallel to this the sensitivity of the system to floral induction changes with changing phase of the

rhythm. These results should also yield data on flowering and photoperiodism. This hypothesis will be explored in the following chapters.

CHAPTER 2

EXPERIMENTAL

Seeds from one ecotype of Chenopodium rubrum (Selection #374/70) originating from 60° 47'N 137° 32'W and harvested in 1970 at U.W.O. were used in all experiments. Seeds of Chenopodium rubrum are subject to tegumentary dormancy and do not germinate without pretreatment and some form of induction of germination (Cumming, 1967). Seeds were pretreated for 30 minutes with 1% aerosol solution (Fisher Scientific Co.) to hasten water inbibition and rupture of seed coat, and then for 30 minutes, with 10% sodium hypochlorite solution to surface sterilize them before use on sterile media. When thoroughly washed with distilled water and completely dried they could be stored at 5° C.

Maximum and uniform germination can be induced by alternating temperatures of 32°/10° C using low intensity fluorescent light (Figure 1A). There is a light requirement also for germination. Greatest stimulation occurs with light of high red/far red ratio. Hence fluorescent light (Figure 1B) was used which also minimizes hypocotyl elongation in the small petri dishes. In each 6 cm petri dish approximately 100 seeds were sown on sterile agar made with 0.8% agar, in

Hoagland's No. 1 nutrient solution (Hoagland and Arnon, 1939) containing 4 ml/litre of saturated sequestrene solution (sodium ferric ethylenediamine tetraacetate) as an iron source (Cumming, 1967). The dishes were immediately incubated in growth cabinets of constant humidity; constant fluorescent light of 600 foot-candles (20° C), (700 ft. c/32° C and 475 ft. c/10° C), (of source G.E.T10J cool white fluorescent lamp (Figure 1B) and calibrated with a selenium photovoltaic cell (Weston illumination meter, model 756, Weston Electrical Instrument Corporation); alternating 12 hour/12 hour temperatures of 32° C/10° C for 4 1/2 days (Figure 1A). All growth cabinet temperatures and timing were charted continuously and automatically by recorder (Honeywell). Beyond 4 1/2 days the temperature remained constantly at 20° C ± 0.5° C for a further 24 hours when after 5 1/2 days from seeding the seedlings had acquired full photoperiodic sensitivity and were ready to be used experimentally (Figure 1A).

All experimental work reported throughout this thesis used these 5 1/2 day old seedlings and time 0 hr for all experimental treatments commenced at this point. Experimental times in hours quoted are all times beyond this chosen 0 hr point, as noted in each footnote of legends for the plates. The experimental treatments consisted of providing some seedlings with continuous Light (L) others with continuous Dark (D) and yet others

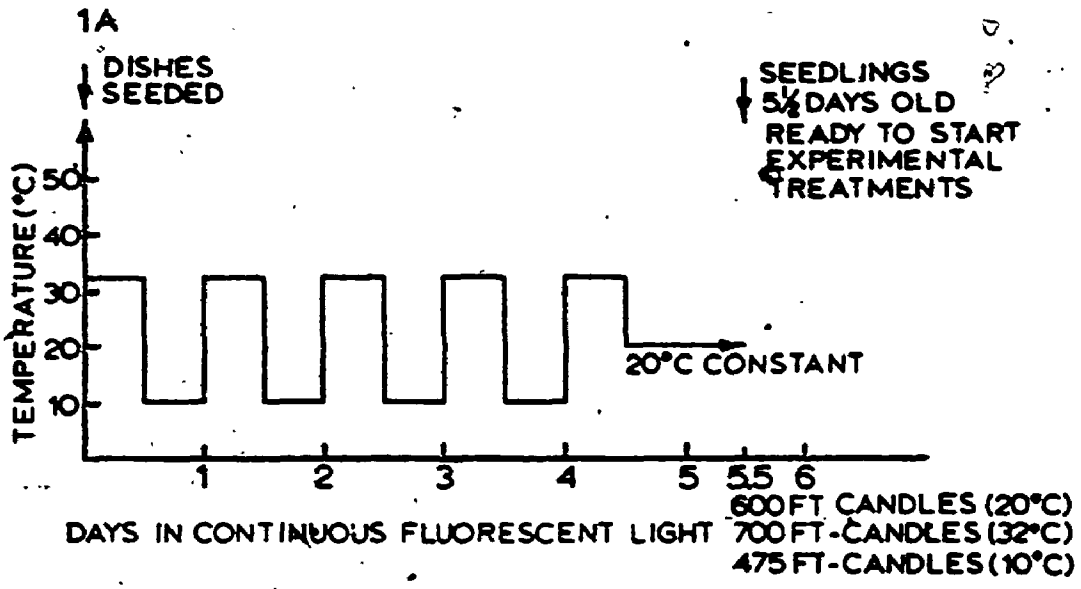
FIGURE 1.

Experimental conditions for induction of germination and seedling growth.

1A Temperature alternation of 32° C/10° C for 12 hours/12 hours for 4 1/2 days followed thereafter by constant temperature of 20° C. Continuous fluorescent light at 600 ft. c (20° C) (700 ft. c (32° C); 475 ft. c (10° C)).

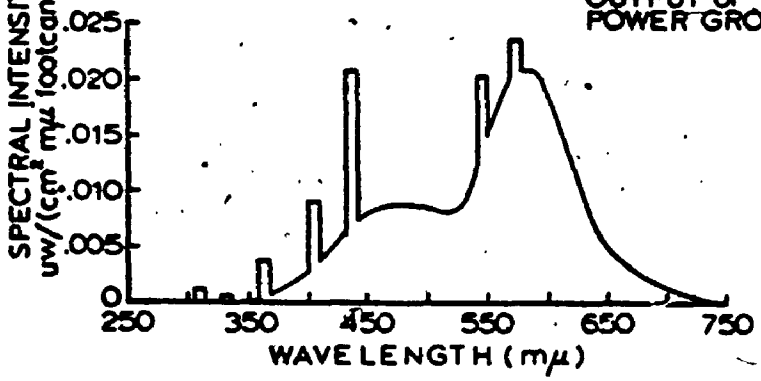
1B Spectrum of G.E. cool white fluorescent T10J lamps used in growth cabinets for germination and seedling growth.

1A



1B

LAMP TYPE	WATT.	LUM.EFF.	LUM.WATT.
STANDARD	40	79.0	
HIGH OUTPUT	60	66.0	
VERY HIGH OUTPUT or POWER GROVE	110	62.5	



with varying periods of dark followed by varying periods of light (L/D). Certain combinations of treatment in the L/D series resulted in those seedlings being induced with subsequent flower development, while all of the remainder of the seedlings were non-induced and remained vegetative. Each of the detailed experimental treatments are described in later chapters and presented diagrammatically in Figure 2.

Assessment of flower induction was achieved by observing the percent of plants sampled that showed floral initiation within a given time (not less than 2 weeks) following seeding and synchronized germination. A random group of ten seedlings in a petri dish was observed and counted under a dissecting microsocope (magnification 25x). This was repeated 3 or 4 times in dishes of the same treatment. On floral initiation, the low undifferentiated dome of cells with leaf primordia of the vegetative apex was found to have become high dome shaped and to bear floral primordia (K4A4) and a distinct upper peripheral ring with an inner protubèrance was seen - - the primordial development of the gynoecium (G(2)). The latter distinctive feature was taken as the first positive proof of flower initiation. The flowering percentage in all experimental petri dishes was recorded. A minimum of 2 dishes untouched, together with sampled dishes was used in pilot experiments. However a minimum of 4 dishes was used in

FIGURE 2.

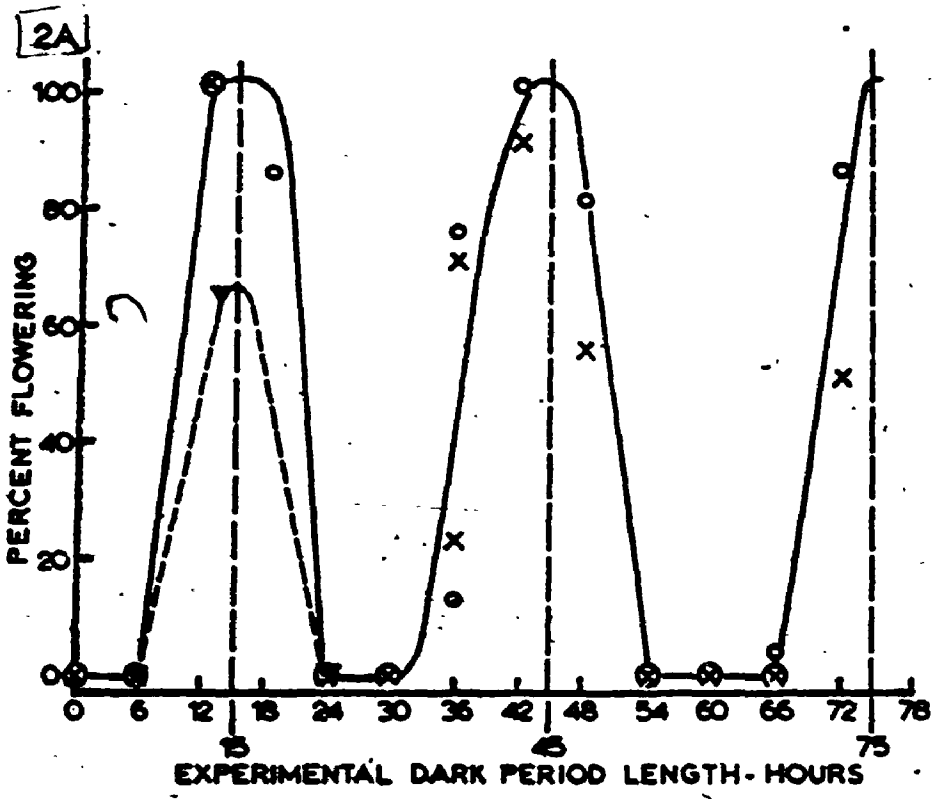
The endogenous rhythm of flowering — the free-running dark rhythm in C. rubrum, and the experimental sampling schedule.

2A The flowering response of C. rubrum resulting from a single long dark period with sampling at regular intervals commencing after completion of 5 1/2 days of induction of germination and seedling growth.

Pilot seedling experiment (b) (curve denoted --- ---) with lowest light intensity of 600 ft. c (20° C).

Seedling experiments (c) (curve denoted —⊗—) with high light intensity of 3,000 ft. c (20° C) which is the highlight series H-L (—o—); and also lower light intensity of 1,200 ft. c (20° C) which is the lower-light series L-L (—x—):

These experiments demonstrate the endogenous rhythm of flowering - - the free-running dark rhythm - - in C. rubrum and include points from the sampling schedule below (2B). Period length is marked with vertical lines.


2A
PERCENT FLOWERING
0
20
40
60
80
100
0 6 12 18 24 30 36 42 48 54 60 66 72 78
15
45
75
EXPERIMENTAL DARK PERIOD LENGTH - HOURS

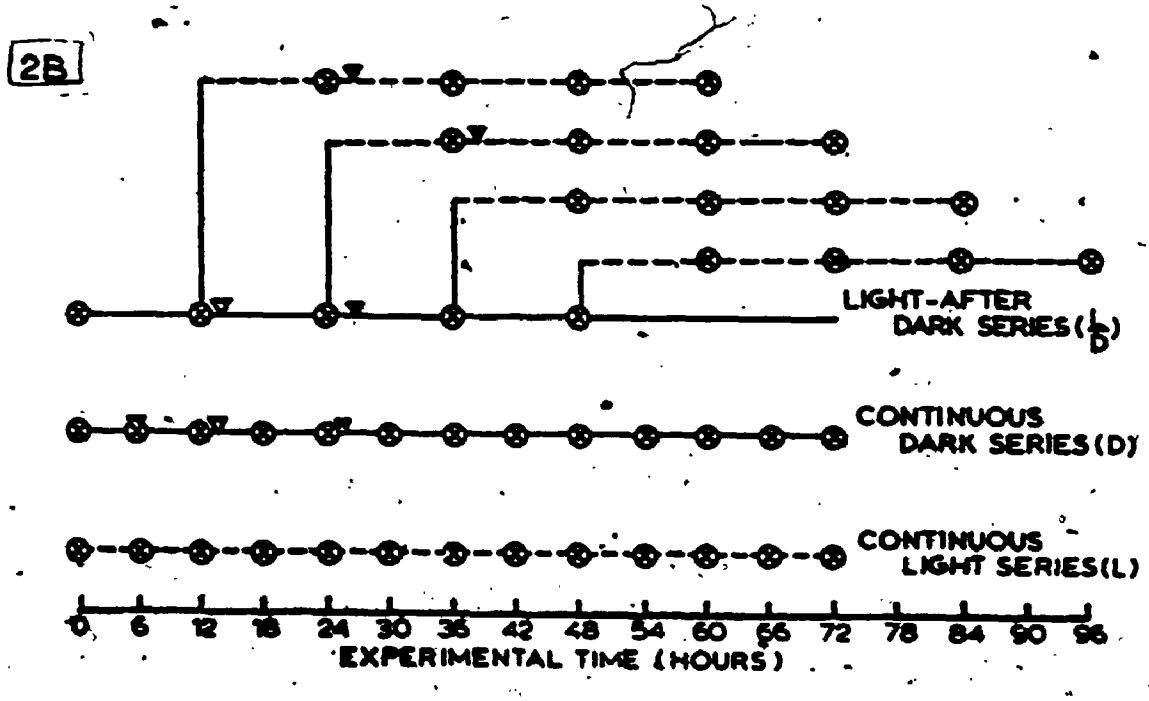

2B
LIGHT-AFTER DARK SERIES ($\frac{L}{D}$)
CONTINUOUS DARK SERIES (D)
CONTINUOUS LIGHT SERIES (L)
0 6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96
EXPERIMENTAL TIME (HOURS)

FIGURE 2.

2B The experimental sampling schedule giving the pattern followed for all experiments reported. Sampling was made during dark treatment (⊢—⊗—⊣) and during light treatment (⊢--⊗--⊣).

Pilot seedling experiment (b) sampling points are denoted (▽). Seedling experiments (c) sampling points H-L (⊢—○—⊣) and L-L (⊢—×—⊣).

major seedling experiments with some series up to as many as 10 dishes. For ultrastructural work sampling points yielding 0% or 100% flowering were usually chosen. On all individual specimens sampled for light microscopy and for electron microscopy a further check was possible. This microscopic examination of apical sections confirmed whether the individual specimen was induced and would flower, or was non-induced and would remain vegetative.

A supply of ecotype seed (#372 (62° 46'N 136° 36'W), + #374 (60° 47'N, 137° 32'W), + #184 (50° 10' N, 105° 35' W), + #194 (34° 20' N, 119° 50' W)) was obtained by seeding in sterile agar petri dishes and inducing germination as above. The 5 1/2 day old seedlings continued to grow under the same conditions for a further week until large enough to be replanted into a standard potting mixture in 3" pots (200 pots with 3 seedlings per 3" pot). The plants then grew in the greenhouse under continuous fluorescent light until several feet tall. Ecotype #372 flowered and seeded under these conditions in 10 weeks, #374 in 17 weeks, #184 in 20 weeks, and #194 in 22 weeks. In order to induce flowering both ecotypes #184 and #194 required long dark nights. This selection of 4 ecotypes proved to be reproductively isolated -- one set seed before the next started flowering. This progressed neatly in order of latitude with the most northerly first. Plants were

harvested and when dried out the seed was rubbed by hand from the pods. These seeds were then sieved, and only large seeds so selected had enough reserves for prolonged dark experiments. These large seeds were then cleaned of remaining seed pods and chaff by grinding with a pestle and mortar. they were washed and pretreated (as above) prior to storage at 5^{o} C or use in experimental work.

CHAPTER 3

ONTOGENY, GROWTH, DEVELOPMENT AND MORPHOGENESIS

A: INTRODUCTION

Throughout this dissertation, flowering is regarded as an apical event. The shoot apex is defined "as that portion of the terminal meristem above the axil of the youngest leaf primordium. A foliar organ with height above its axil is a primordium. The nascent foliar organ is a leaf buttress. Subsequently division and enlargement of cells in a buttress cause it to grow vertically and become by definition a leaf primordium" (Gifford and Tepper, 1962). In order to explore this issue it is necessary to examine very carefully the apices of vegetative seedlings and the behaviour at the apices, throughout all series. In order to compare seedlings of various ages and rate of growth the plastochron rates were compared. A plastochron is defined as "the interval required for the apex to produce one leaf primordium and the internode that subtends it" (Richards and Schwabe, 1969). Without such a comparison it is difficult to assess what processes

the apices are exposed when they are placed in the light after different periods of dark. Some sets of these L/D seedlings were induced to flower while others were not.

Since here the apex structure and apical behaviour are regarded as pivotal for flowering, induction must be redefined. It is a different concept. Evans in 1971 states "the processes occurring in the leaves, under photoperiodic control, which gives rise to a floral stimulus, constitutes photoperiodic induction". Salisbury in 1987 has proposed the definition of induction as "a phenomenon in which some response, e.g. flowering can be caused (induced) in an organism by some treatment (e.g. an environmental condition such as cold or short days) and the response continues, or typically first appears, after the treatment has been discontinued". This is compatible with the system studied here. Evocation follows the phenomenon of induction in a flowering system and Evans (1971) states, "the initial events at the shoot apex in response to the arrival of the photoperiodic stimulus, which commit the plant to the subsequent formation of flower primordia, are referred to as floral evocation". Salisbury (1987) offers three evocation definitions for consideration:

evocation, floral - Process or processes in the apex required for irreversible commitment to initiate flower primordia; the first stage of morphological change to floral organ production following inductive treatment. (Must they always be irreversible?)

evocation - Early responses of receptor tissue following environmental triggering or other form of induction; usually related to the flowering responses that occur at the shoot apex after arrival of floral stimuli and prior to flower differentation; defined by Evans (1969) to distinguish from induction. (This version most closely agrees with Evans' original definition, which was based on a term used by Waddington for the avocation of any new development pathway, not just flowering. This version has my preference).

evocation - First state of morphological change to floral organ production following upon inductive treatment.

None are entirely suitable. Here evocation shall be used to denote the process(es) at the apex following induction, that first commence(s) the expression of the potential to flower. This is essential within a given time limit otherwise the induction effect and potential to flower will pass.

In this chapter the ontogeny, growth, development and morphogenesis of the seedlings will be examined. Questions such as the involvements of the dark period, light effects, the message - its contents and the status of the apex, induction and evocation will be explored.

B. MATERIALS AND METHODS

a) Tissue cultures of cotyledon tissues of 2 ecotypes #184 and #374 of Chenopodium rubrum were available (Chai-Looi). These were grown under continuous light in culture media of pH 5.5 (based on previous nutrient media (Hoagland's solution, etc. as noted for seedling petri dishes) plus thiamine hydrochloride, i-inositol (Meso), sucrose, casein hydrolysate, kinetin, 3-indole acetic acid (3-IAA) or napthalene acetic acid (NAA) with nicotinic acid + pyridoxin hydrochloride). Specimens of each culture were fixed and embedded for microscopy (as described later) for preliminary examination of cultured cotyledon cells and for assessment of fixation and embedding techniques suitable for the cells.

b) A pilot seedling experiment was carried out using 5 1/2 day old seedlings of Chenopodium rubrum 374 as described previously (Chapter 2 and Figures 1 and 2). Trays of petri dishes ready for experimental work were sealed with aluminum foil and placed in the dark in growth cabinets at 20° C constant temperature and constant humidity. Samplings were made as illustrated in the sampling schedule of Figure 2A and 2B at 13 1/2 hours dark when (1) 2 dishes of seedlings were removed and 10 seedlings from each were fixed for micfoscopy in

the dark, with the aid of a green safelight. A sampling also was made after 6 hours dark as seen in Figure 2A and 2B. In addition, (2) 4 dishes more were removed and placed together with sampled dishes in growth cabinets with continuous fluorescent light of 600 ft. candles (20° C), temperature constant 20° C and constant humidity. A sampling of 2 dishes was made from the latter 4 dishes after 12 hours of continuous Light. Ten seedling specimens per dish were fixed for microscopy while the other 2 dishes remained untouched together with the remainder of each set of sampled dishes until floral evocation was observed and flowering percentage could be assessed. The same procedure was repeated at 25 1/2 hours dark on the remaining dishes in the dark growth cabinet.

All sampled seedlings were fixed and embedded for microscopy (as described later), for improving of EM techniques of fixing and embedding on whole seedlings, for improving of the sectioning technique for the very small apices, and for initial examination of the cells of cotyledon, primordial leaf and apex. After a variety of procedures, it was found that whole seedlings were fixed best in the cold (4° C) for 3 hours with 6% glutaraldehyde in 0.1 M phosphate buffer (pH 5.9) followed by triple rinsing in the buffer and they were stored in the 0.1 M phosphate buffer at 5° C. Fixation was then completed in cold 2% (w/v) osmium tetroxide in

0.1 M phosphate buffer for 1 hour. After fixation the seedlings were rinsed in cold (4° C) buffer and were dehydrated through a cold graded ethanol series to cold 100% ethanol. They were brought to room temperature with another rinse in 100% ethanol at 20° C and finally were rinsed twice in propylene oxide. The seedlings were embedded in Epon 812 which was found more satisfactory than Vestopal for whole seedlings.

c) A major seedling experiment was carried out using Chenopodium rubrum 374 and the type of techniques outlined under (b) above and illustrated in Figures 2A and 2B. As described previously (Chapter 2 and Figures 1 and 2), 5 1/2 day old seedlings were placed in

i) continuous Light and sampled at 6 hour intervals for 72 hours. This is termed the "continuous Light series" (L) which provides light controls for the continuous Dark series, and provides non-flowering controls for the flowering series.

ii) continuous Dark and sampled at 6 hour intervals for 72 hours. This is termed the "continuous Dark series" (D) and is one of the main experimental series. In this continuous Dark series, the endogenous rhythm of flowering is demonstrable and the seedlings can be induced to flower at certain set points in time but cannot be induced to flower at

other set points in time (as seen in Figure 2A). At opposite points it is hypothesized that the ultrastructural and biochemical status of the seedlings differs.

iii) a series termed the "Light-after-Dark series" (L/D) in which batches of dishes removed at 12 hour intervals from the continous Dark series (i.e. removed after 12, 24, 36, 48 hours of darkness) and placed in continuous Light were then sampled at 12 hour intervals for 48 hours in the light following the end of the dark period (i.e. sampled after 12, 24, 36, 48 hours of this light following darkness) (Figure 2B). This is also an important experimental series. Here some series of seedlings are committed to flower and can be followed ultrastructurally and biochemically. Concomitant series, in which flowering is inhibited act as controls both for the flowering series and for the effect of light and dark treatments compared with the light only or dark only series. For further experimental detail see sampling schedule Figure 2B.

In this experiment (c) much increased light intensities were used and were found to result in photoperiodic induction producing 100% flowering. The

amplitude of flowering rhythm peaks for experiment (b) and those for this experiment (c) can be seen in Figure 2A. This was a necessity as statistical samples could not be entertained due to EM technique and the large number of sampling points already involved. Whenever light treatments for experiments under (c) were carried out, two parallel series were used. The first used high light intensity, 3,000 ft. candles (high-light series H-L) and the second used 1,200 ft. candles (lower-light series L-L) (cf low light of 600 ft. candles was used for experiments (b)). The 4 1/2 day germination and growth schedule for seedlings in experiment (c) was carried out as described in Chapter 2, and for the final 24 hours (from 4 1/2 to 5 1/2 days), the temperature was set constant at 20° C $\pm$ 0.5 C. However at this point the intensity of the fluorescent light was increased either to the 3,000 ft. candles for the high-light series (H-L) or to the 1,200 ft. candles for the lower-light (L-L) series. Hence, increased light intensity was used for 24 hours immediately preceding the dark period experimental treatment (during the time from 4 1/2 days to 5 1/2 days). Also, these increased light intensities were used for all light treatments following the dark period, both during experimental sampling and until flowering was assessed. A total of 82 samplings of 10 seedlings each was made.

All seedlings were fixed in glutaraldehyde and osmium tetroxide and embedded in Epon (as above). Eight hundred and twenty embeddings were prepared. Sections of apices were cut using a Porter-Blum ultramicrotome MT-1 and for light microscopy 1 μM sections were prepared, transferred to glass slides by the method of Marinozzi (Marinozzi, 1964), and stained with 0.2% azure B in 1% sodium bicarbonate solution (pH 9) (Hoefert, 1968). Criteria for identification of the median sections of apices were worked out -- the basal vascular trace positions in this case being the most reliable of the criteria used. The light microscope serial sections (thick azure B stained) provided the proof and were used also as visible controls for the region of sectioning of the thin electron microscope sections.

Microphotographs of serial sections of a selected vegetative apex and of a corresponding flowering apex were prepared using a Zeiss microscope and camera, the light intensity being adjusted with the aid of a Leitz-Microsix L light meter. Kodak 35 mm Panatomic-X film was used and developed in Kodak Microdol-X for 9 minutes at 20° C. Also, microphotographs of median sections of all apices through all series ((i) (L) (ii) (D) (iii) (L/D)) were prepared. These provided definite evidence as to whether the individual apex sectioned was vegetative or flowering. They provided comparative data on the status of apices being studied in terms of growth and

development - - the ontogenetic stages and the effect of the various light and dark treatments. These data will now be presented in the following section.

C: RESULTS

VEGETATIVE APICES - [NON-INDUCED APICES]

Continuous Dark Series - Sections through the shoot tip (Gifford and Tepper, 1967) of seedlings from all sampling points show the first pair of primordial (Gifford and Tepper, 1967) leaves well developed; no axillary buds present; the apical dome smooth; no second set of primordial leaves yet formed. There is a detectable difference in the apices (Gifford and Tepper, 1967) of those seedlings exposed to (H-L) high light treatment (3000 ft. c) prior to dark treatment compared with those exposed to corresponding (L-L) low light treatment (1200 ft. c) prior to dark treatment. For L-L, throughout the whole set, including 72 hrs of darkness, classic smooth dome apices occur. For H-L, as early as 12 hrs. of darkness the smooth dome of the apex has a faint hint of lopsidedness due to increased mitotic activity of some peripheral zone cells - the very early signs of where a leaf buttress (Gifford and Tepper, 1967) is forming. Throughout the whole (H-L) series no primordial leaves develop from these buttresses - not even to the stage of the 30 hr. H-L seedlings of the light series.

PLATE 1

MAGNIFICATION*

Differing stages in development in apices all of the same chronological age (60 hours experimental treatment).**

1A 60 hours continuous Dark.

1B 60 hours continuous Light.

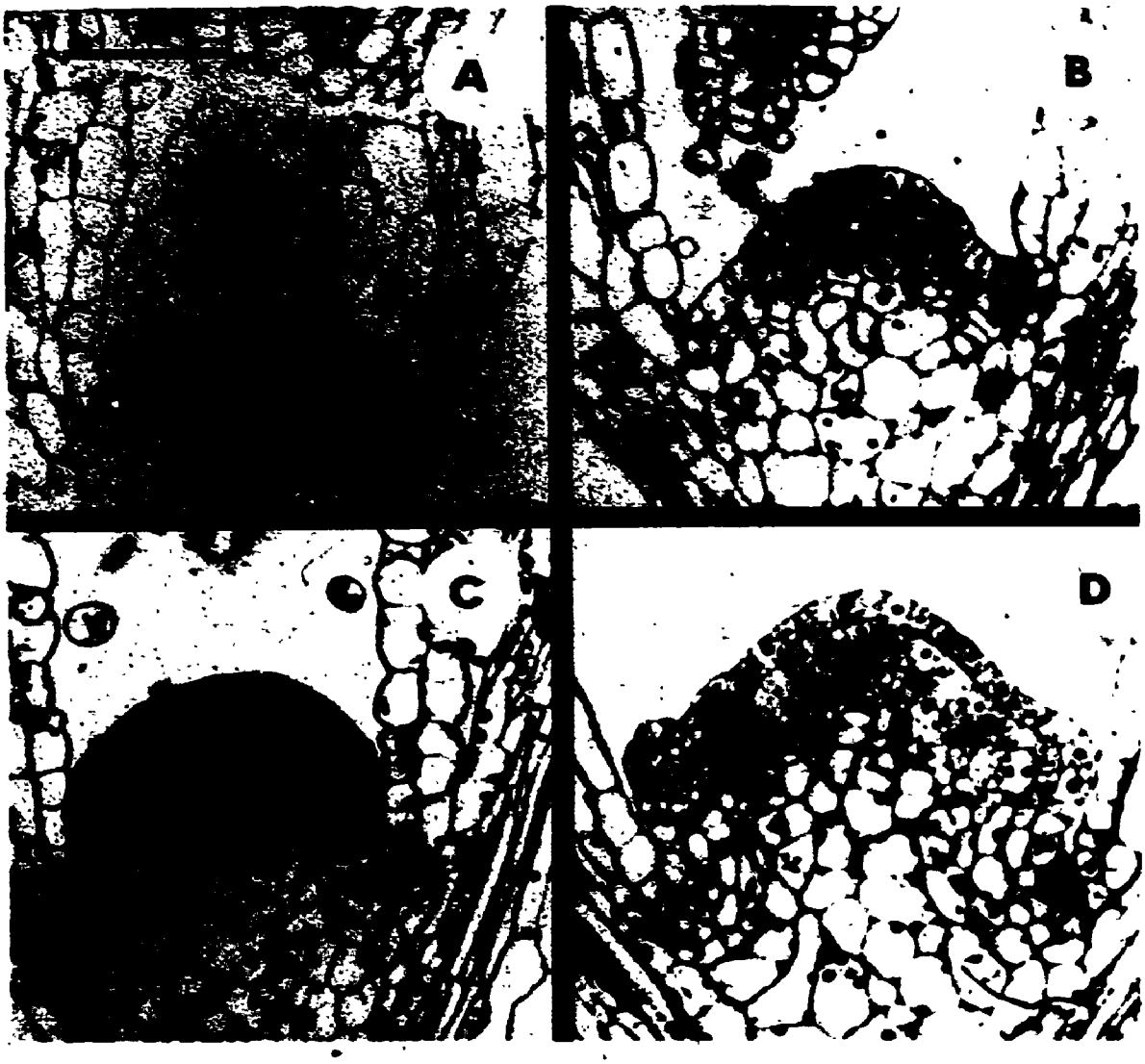
1C 60 hours consisting of 24 hours in Dark followed by 36 hours Light.

1D 60 hours consisting 12 hours in Dark followed by 48 hours Light.

---

* Magnification for the plate is indicated by a fifty micrometer (50 μM) bar in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.


A
B
C
D

Therefore, in the continuous Dark series no second set of primordial leaves is formed, within the period and under the conditions of the experiments (Plate 1A and Plate 2A).

Continuous Light Series - Sections through the shoot tip from all sampling points show the first pair of primordial leaves well developed; no axillary buds present; the apical dome becomes 'mishappen' showing the early development of the second set of leaf primordia, as seen in L-L sets as early as 30 hours. By (L-L) 54 hours development is pronounced.

Early in the continuous Light series leaf buttresses are formed and leaf primordia develop rapidly on the apices of seedlings of corresponding chronological ages to those of the above continuous Dark series. At 30 hours, L-L set shows morphologically 'ears' (primordia) taking shape and by 54 hours well developed leaf primordia are formed. Some variation is seen in the farther development of primordia among later specimens but all beyond 30 hours have primordia present. In earlier specimens (e.g. 18 hours H-L) the first signs of leaf buttress formation are seen - comparable to those seen and discussed in the H-L continuous Dark series.

PLATE 2

MAGNIFICATION*

Differing stages of development of the vegetative [non-induced] apices under different experimental treatments.

2A 72 hours** continuous Dark.

2B 42 hours continuous Light.

2C 72 hours continuous Light.

2D 48 hours consisting of 24 hours Dark followed by 24 hours Light.

2E 72 hours consisting of 24 hours Dark followed by 48 hours Light.

---

* Magnification for the plate is indicated by a fifty micrometer (50 μM) bar in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.


A
B
C
D
E

Therefore, in the continuous Light series the second set of primordial leaves develops within the period and under the conditions of the experiments (Plate 2B and 2C).

Light-After-Dark Series - The series used is that of 12, 24, 36, 48 hrs light following 24 hrs dark treatment. Chronological age - (from the start of the experimental treatment):

36 hrs consisting of 24 hrs Dark followed by 12 hrs Light [both H-L & L-L sets]

48 hrs consisting of 24 hrs Dark followed by 24 hrs Light [both H-L & L-L sets]

60 hrs consisting of 24 hrs Dark followed by 36 hrs Light [both H-L & L-L sets].

72 hrs consisting of 24 hrs Dark followed by 48 hrs Light [both H-L & L-L sets].

Using the sampling points above, sections through the shoot tips of these seedlings show the first pair of primordial leaves well developed and no axillary buds. Furthermore, early in the series at 12L/24D (both L-L & H-L) and 24L/24D (both L-L & H-L) sampling points the cells of the apical dome have become very large and expanded (Plate 2D) - they appear as big cells compared with those in corresponding apices in the continuous Light or continuous Dark series. Finally, early in the series, the apical dome shows the very early signs of

where the leaf buttresses are forming for the second set of leaf primordia. The developed primordia are seen in apices from the 48L/24D (both L-L & H-L) sampling point (Plate 2E).

Typical vegetative apical development is seen throughout this series (except for 1 seedling - a 10% flowering, H-L, 36L/24D hour specimen) and is proof that seedlings actually used are all vegetative - in spite of 10% - 20% flowering, statistically in the H-L set. The L-L set are all 0% flowering. The exception is a seedling, with a heavy bacterial infection, and with 36L/24D hour H-L treatment, which is flowering. The flowering percentage is 10% and this is the 1 in 10!

In the Light-After-Dark series therefore leaf buttresses form early at the stage when the apical cells have become enlarged. Leaf primordia do develop but only at the end of the series.

FLOWERING APICES [INDUCED APICES]

Light-After-Dark Series - Two experimental sets were examined.

- 12,24,36,48 hrs Light following 12 hrs Dark treatment.
- 36, 48 hrs Light following 48 hrs Dark treatment.

- also the exception from the non-inductive L/D series was examined, the seedling with a heavy bacterial infection, with 36 hrs Light following 24 hrs Dark (H-L) treatment as previously noted.

Chronological age was (from start of experimental treatment):

24 hrs consisting of 12 hrs Dark followed by 12 hrs Light

36 hrs consisting of 12 hrs Dark followed by 24 hrs Light

48 hrs consisting of 12 hrs Dark followed by 36 hrs Light

60 hrs consisting of 12 hrs Dark followed by 48 hrs Light

84 hrs consisting of 48 hrs Dark followed by 36 hrs Light

96 hrs consisting of 48 hrs Dark followed by 48 hrs Light

60 hrs consisting of 24 hrs Dark followed by 36 hrs Light.

Using the sampling points above sections through the shoot tips of these seedlings show the first pair of primordial leaves well developed; precocious development of axillary buds; the second set of primordial leaves well developed in all induced seedlings before modification of the apex has proceeded to the point where perianth primordia arise. The modification of the

PLATE 3

MAGNIFICATION*

Maximum development observed of an induced apex within the experimental period. Experimental treatment** - 60 hours, consisting of 12 hours dark followed by 48 hours light.

3A Primordial leaf, inserted at right angles to the cotyledons, of the first set of primordial leaves of the seedling.

3B Sectioned beyond the point of insertion of the primordial leaf as in 3A, and now revealing the side of the apex with a primordial leaf of the second set of primordial leaves.

3C A median apical section showing growth of perianth primordia on the sides of the enlarged apex.

3D Continued on next page.

---

* Magnification for the plate is indicated by a fifty micrometer (50 μM) bar in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.

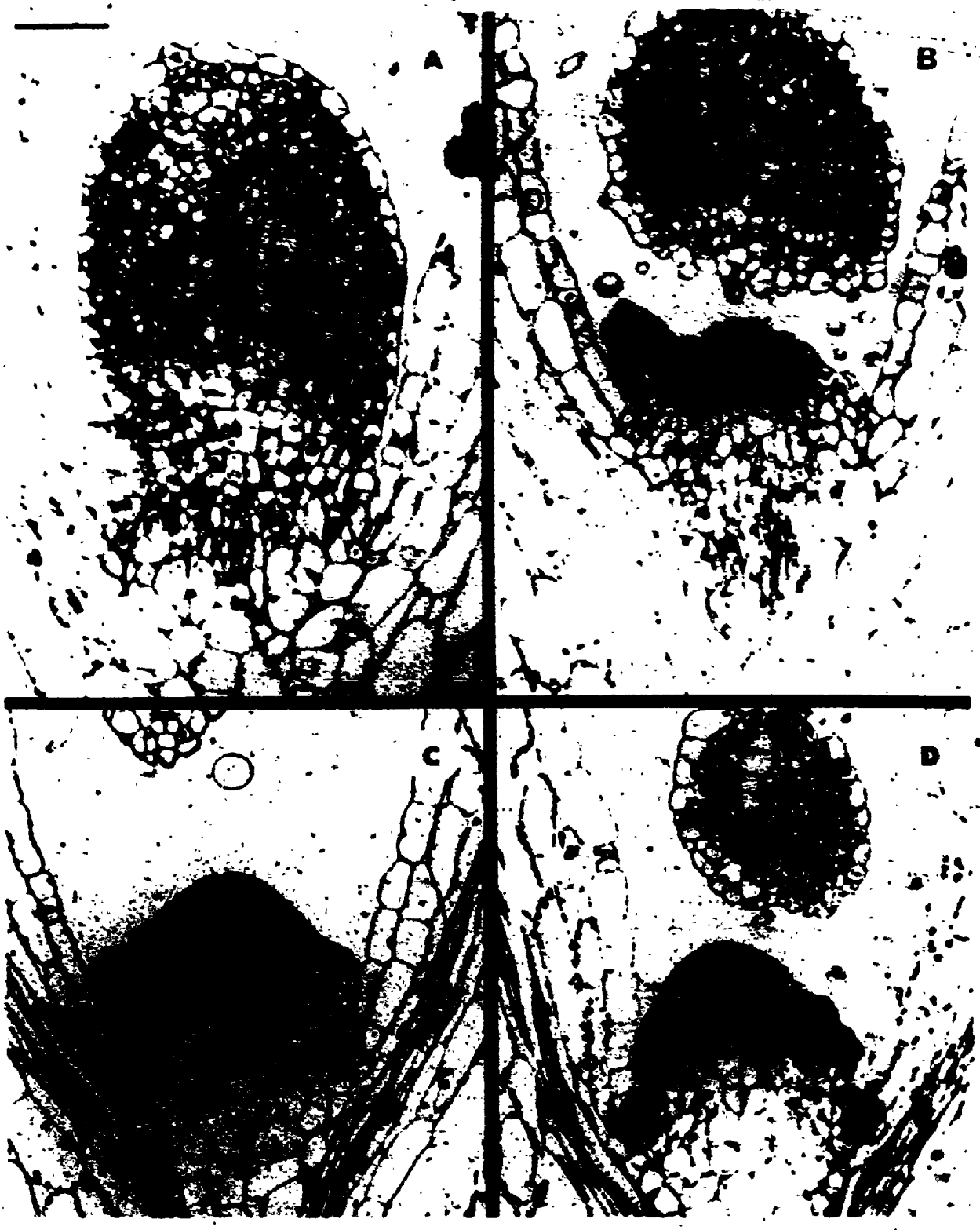

A
B
C
D

PLATE 3

MAGNIFICATION

3D Sectioned beyond 3C showing axillary bud development at the base of the apex and on the right side of the apex immediately above the perianth primordium commencement of stamen primordium development is seen. The tip of the second primordial leaf of the first set of primordial leaves is now visible above the apex.

apex is seen by changes in the set vegetative shoot apical cell pattern, and the production of perianth primordia. The first indications of initiation of stamen primordia are observed at the end of the series (Plate 3).

Early in the series the first observable change is that of cell size - a rapid cell enlargement is seen at the earlier sampling points (12L/12D and 24L/12D) especially in the tunica. In these apices this enlargement is accompanied by progressive decrease in regularity of layering of cells (Table 1). The tunica and corpus become rapidly less distinct and less layered in appearance. The enlarged domed apices have a large - and irregular-celled appearance especially when compared with the small-celled, tight-three-layered, small sized apices found throughout both the continuous Light and the continuous Dark series (Plate 5). The cell patterning of the shoot apex is changing. The first indications of cell division occur early and cell division proceeds rapidly. This contributes to the "doming up" of the apex and the increase in the cell layers (~ 4 to 5 cells deep in specimens sampled at 24L/12D, and by the end of the series (48L/12D) the apex is ~ 5 to 6 cells deep). In the later specimens (36L/12D, 48L/12D, 36L/48D, 48L/48D, 36L/24D) these cells become dense, have prominent nucleoli and due to the presence of a lot of dividing cells tend to appear

small again at the end of the series (Plate 3C and 3D). In these later specimens (36L/12D, 48L/12D, 36L/48D, 48L/48D, 36L/24D) the burst of growth and development is seen to have resulted in precocious axillary bud development; in very rapid development of the second set of primordial leaves which are advanced both in time and stage; in the initiation of perianth primordia seen at sampling points 36L/12D, 36L/48D, 36L/24D and at the end of each series (48L/12D, 48L/48D) of perianth primordia and the first signs of stamen primordia (Plate 3D).

In the second experimental set of 36 hrs, 48 hrs light following 48 hrs dark treatment the ontogenetic stages are found to correspond exactly to those of the set 36 hrs, 48 hrs light following 12 hrs dark treatment (Plate 4). The single 36L/24D specimen also parallels the ontogenetic stage of specimens sampled at 36L/12D and 36L/48L. However growth has been very rapid in this specimen as the second set of primordial leaves are very large and the perianth primordia well developed. The effects of high light and also of bacterial infection are considered in Section D: Discussion. Apical development in these seedlings is NOT dependent upon the length of time spent in the dark (as above) (Plate 4), nor on chronological age (60D, 60L, 60(24D + 36L), 60(12D + 48L)(Plate 1) but only on the LENGTH of LIGHT exposure following the inductive dark (see Table 1 and Figure 3).

PLATE 4

MAGNIFICATION*

Induced apices showing parallel development dependent upon identical lengths of the light period following the dark, but independent of the differing lengths of dark periods.**

4A 48 hours consisting of 36 hours Light following 12 hours Dark.

4B 84 hours consisting of 36 hours Light following 48 hours Dark.

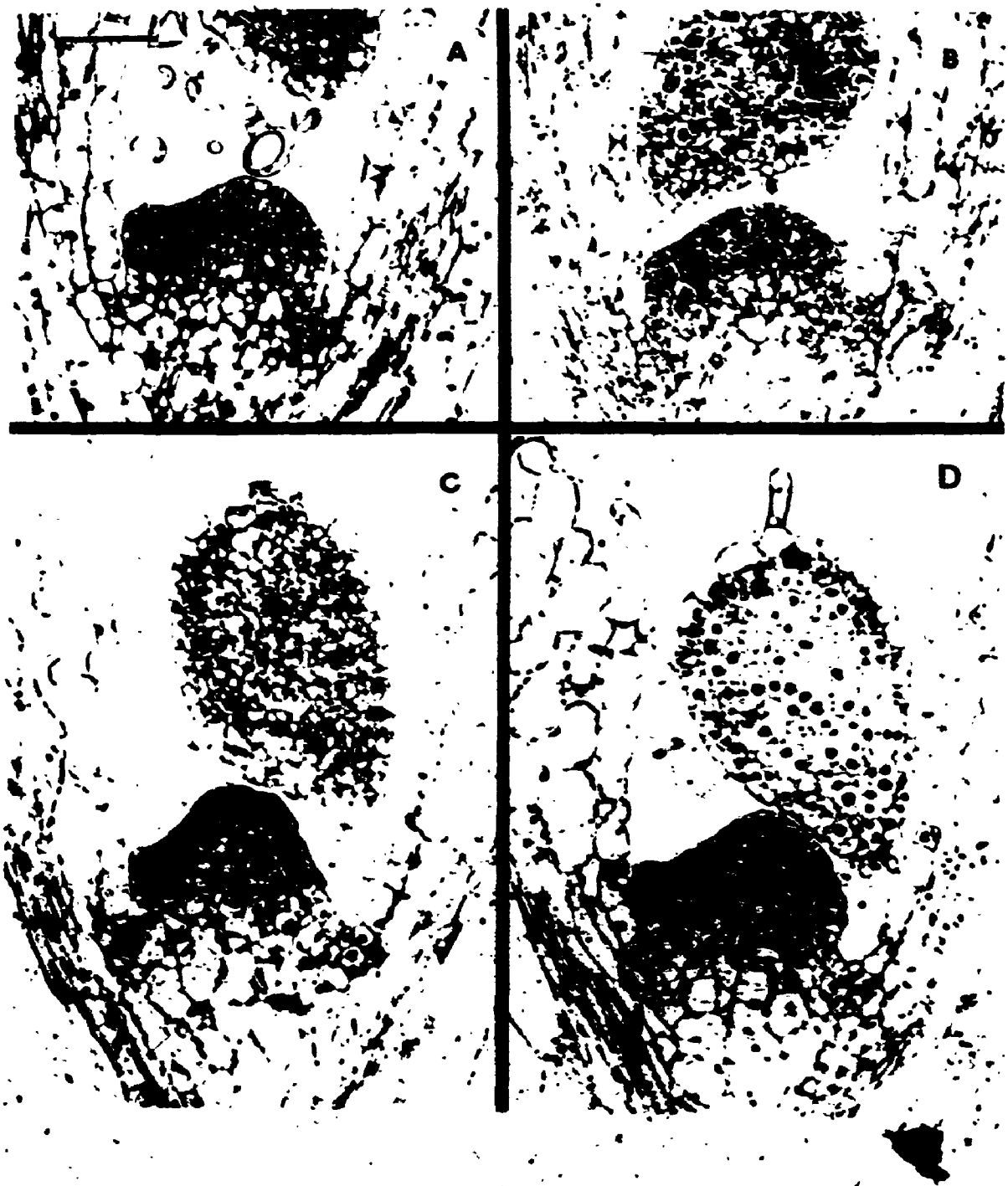
4C 60 hours consisting of 48 hours Light following 12 hours Dark.

4D 96 hours consisting of 48 hours Light following 48 hours Dark.

---

* Magnification for the plate is indicated by a fifty micrometer (50 μM) bar, in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.


A
B
C
D

Therefore in the LIGHT-AFTER-DARK SERIES [INDUCTIVE] floral evocation is observed in all apices of all seedlings sampled. Where the seedlings are exposed to the light following the inductive dark a burst of growth and development is first observed in the apices. This is seen as cell enlargement followed by very active cell division (Table 1 and Figure 3). This results in precocious axillary bud development and development of the second set of primordial leaves advanced both in time and stage. Later in the series the set vegetative shoot apical cell pattern of dividing and non-dividing cells has been modified to the stage where perianth primordia arise and at the end of the series, within the period and under the experimental conditions, initiation of stamen primordia is seen (Plate 3). The series described are all of low light (L-L - 1200 ft. c) treatments, with the apical changes being more gradual and the steps easier to discern.

Within the Light-after-Dark inductive series the sets show parallel development. Such development is not related to the length of the dark period whether it be 12 hrs D, 48 hrs D, (24 hrs D), nor is it related to the considerable difference in (experimental) chronological age (for such young seedlings) whether it be 48 hrs, 60 hrs (60 hrs), 84 hrs, 96 hrs. Furthermore, <u>all</u> sets

Table I

Change in number of cells (inversely related to the cell size) within a given area, including the uniseriate tunica of the central zone, of the longitudinal sections of apices of Chenopodium rubrum after exposure to light following dark treatment.

1) Numbers based on all cells counted within the given area.

2) Numbers based on only the first two cell layers (including uniseriate tunica) counted within the above given area.

3) Numbers are for overall averages for a given length of light period irrespective of length of previous dark treatment, and are derived from (1) above.

Table I.

Treatment[3]	# cells (1) Range	# cells (1) Average	# cells (2) Range	# cells (2) Average	Treatment[3]	# cells (3) Average
Continuous light[1] across 72 hours (14-10)[4]	11-13	11.9	8-9	8.2		
					Continuous treatment (32-22)[4]	11.9
Continuous dark[1] across 72 hours (18-12)[4]	11-12	11.9	7-9	8.0		
Light following 12 hours dark[2]					12 hours light following dark (9-4)[4]	8.4
-12 (4-2)[4]	8	8.0	6-7	6.2		
-24 (5-3)[4]	5-9	7.5	4-7	5.6		
-36 (5-2)[4]	9-10	9.2	6-7	6.8	24 hours light	
-48 (19-4)[4]	11-13	12.2	7-10	8.0	following dark (11-6)[4]	8.0
Light following 24 hours dark[1]						
-12 (5-2)[4]	8-9	8.8	6-7	6.4	36 hours light	
-24 (6-3)[4]	8-9	8.6	6-7	6.1	following dark (18-7)[4]	8.6
-36 (5-3)[4]	6-8	7.8	5-7	6.0		
-48 (5-3)[4]	12-13	12.2	7-8	7.7		
					48 hours light	
Light following 48 hours dark[2]					following dark (26-9)[4]	11.8
-36 (3-2)[4]	8-10	8.8	6-7	6.2		
-48 (2-2)[4]	11	11.0	8	8.0		

1 Seedlings non-induced and will remain vegetative.

2 Seedlings induced and will flower.

3 All hours quoted refer to time following the start of experimental treatment only.

4 (14-10), 14 = # of sections counted and averaged, 10 = # nd apices from which 14 sections derived.

FIGURE 3.

Effect of the light after the dark treatment, both with induced apices (those with 12 hour dark or 48 hour dark treatments) and non-induced apices (those with 24 hour dark treatments), compared with continuous Light or continuous Dark only treatments:

Parallel change in number of cells (inversely related to the cell size) within a given area including the uniseriate tunica, of the central zone of longitudinal sections of apices of Chenopodium rubrum, after exposure to light following dark treatment.

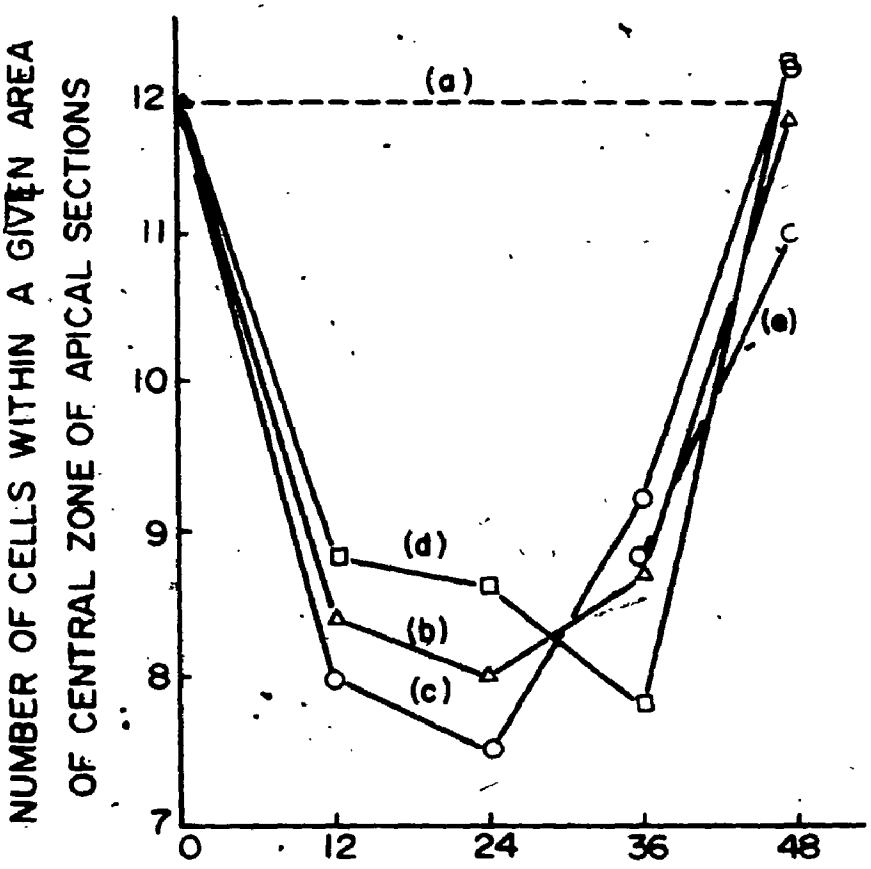
a) (——) Control - - continuous Light [L] or continuous Dark [D].

b) (Δ) Overall average of all points for total area (1) (See column (3) of Table I).

c) (○) Induced seedlings - - after 12 hours dark treatment [L / 12D]

d) (□) Non-induced seedlings - - after 24 hours dark treatment [L / 24D]

e) (○) Induced seedlings - - after 48 hours dark treatment [L / 48D]



L(a) and D(a) $P < 0.50$

$\frac{12L}{12D}$(a) and $\frac{12L}{24D}$(d) $P < 0.01$

$\frac{24L}{12D}$(c) and $\frac{24L}{24D}$(d) $P < 0.20$

$\frac{12L}{}$(b) and $\frac{24L}{}$(b) $P < 0.30$

$\frac{12L}{}$(b) and $\frac{36L}{}$(b) $P < 0.50$

[12L - 24L - 36L] and 48L $P < 0.001$

L and 48L $P < 0.50$

HENCE

[12L - 24L - 36L] and 48L $P < 0.001$

[12L - 24L - 36L] and L and D

sampled having the same LIGHT period following the inductive dark period are at the SAME ontogenetic stage (Plate 4, Table 1 and Figure 3).

D: DISCUSSION

I ONTOGENETIC CONSIDERATIONS

HENCE [I], on comparison of Dark series with Light series sets (both non-induced-vegetative apices - (Plate 2, 2A, 2B, 2C) the pairs of sampling points which correspond in chronological age are found to differ in plastochron age. The Light series set is much more advanced than the Dark series set.

HENCE [11], in the Light-after-Dark series (non-induced-vegetative apices - Plate 2, 2D and 2E) leaf primordia do develop and the plastochron age of seedlings of this series is more advanced than that of the corresponding chronological aged seedlings of the Dark series where no leaf primordia develop. However in comparison with the Light series, those seedlings of the Light-after-Dark series which had been out of the dark for only short periods of time, are delayed. There then follows in the light a stimulus to rapid growth and development, so that these early specimens show cell enlargement and early signs of leaf buttress formation, while in late specimens leaf primordia have developed equivalent to those of the Light series.


2
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
MICROCOPY

HENCE [111], in the Light-after-Dark series (apices induced to flower - Plate 3 and Plate 4), precocious bud formation is seen and primordial leaves are well-developed much earlier - the plastochron age is much advanced over that of any other series. There has been a great increase in plastochron rate.

In comparison with the Light-after-Dark (non-induced-vegetative apices) series it is observed that in the Light-after-Dark (induced) series there also follows in the light a stimulus to rapid growth and development. Here also it is first observed an apical cell enlargement followed by rapid cell division (Table 1, Figure 3 and Plate 6). It is to be noted that this is the same fundamental process in both - both non-induced and induced seedling apices.

The development is found to correlate with the period of light following the dark and is independent of the length of the dark period. There is a consistency in development or in the ontogenetic stages in the sets of light following 12 hrs dark treatment, and light following 48 hrs dark treatment, (also even in the odd heavily infected apex 24 dark). This is dependent on the hours of light only following the inductive dark and not on the hours of the dark period. The dark effects a delay in the processes of growth and development which are seen under light conditions.

PLATE 5

MAGNIFICATION*

The effect of continuous Dark** or continuous Light treatment is seen in the resultant non-induced apices which remain smaller with smaller and fewer cells.

5A 42 hours continuous Dark treatment.

5B 60 hours continuous Dark treatment.

5C 42 hours continuous Light treatment.

5D 60 hours continuous Light treatment.

---

* Magnification for the plate is indicated by a fifty micrometer (50 μM) bar in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.

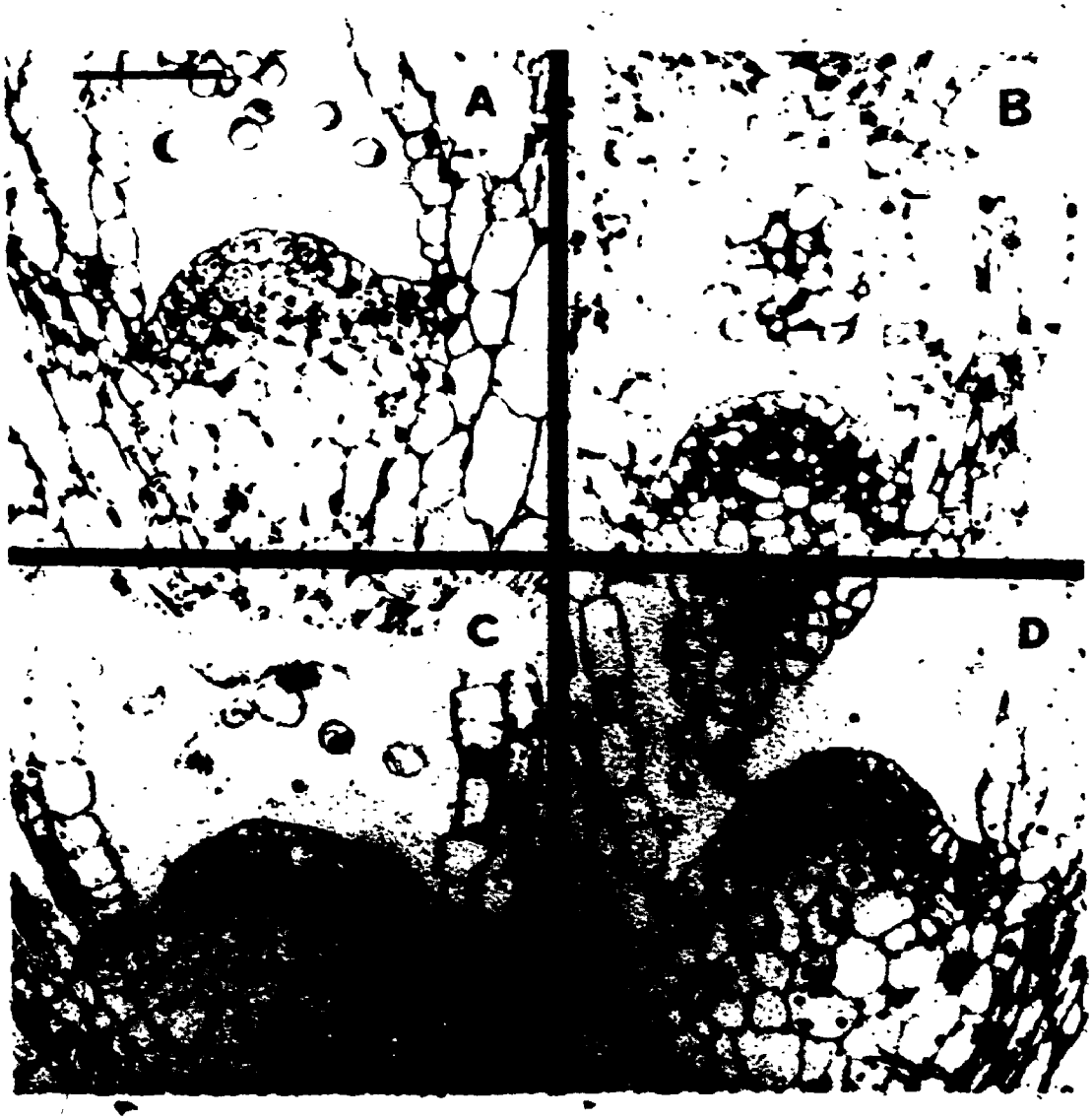

A
B
C
D

PLATE 6

MAGNIFICATION*

The effect of light following dark,** or dark interruption of light, in BOTH induced and non-induced apices is seen in the formation of larger apices arising from early rapid growth by cell enlargement and then by an ensuing burst of cell division as readily seen at the later stages.

Induced Apices

6A 24 hours consisting of 12 hours Dark followed by 12 hours Light.

6B 36 hours consisting of 12 hours Dark followed by 24 hours Light.

6C 60 hours consisting of 12 hours Dark followed by 48 hours Light.

---

* Magnification for the plate is indicated by a fifty micrometer (50 µM) bar in the first photo of the plate.

** All hours quoted refer to time following the start of experimental treatment only.

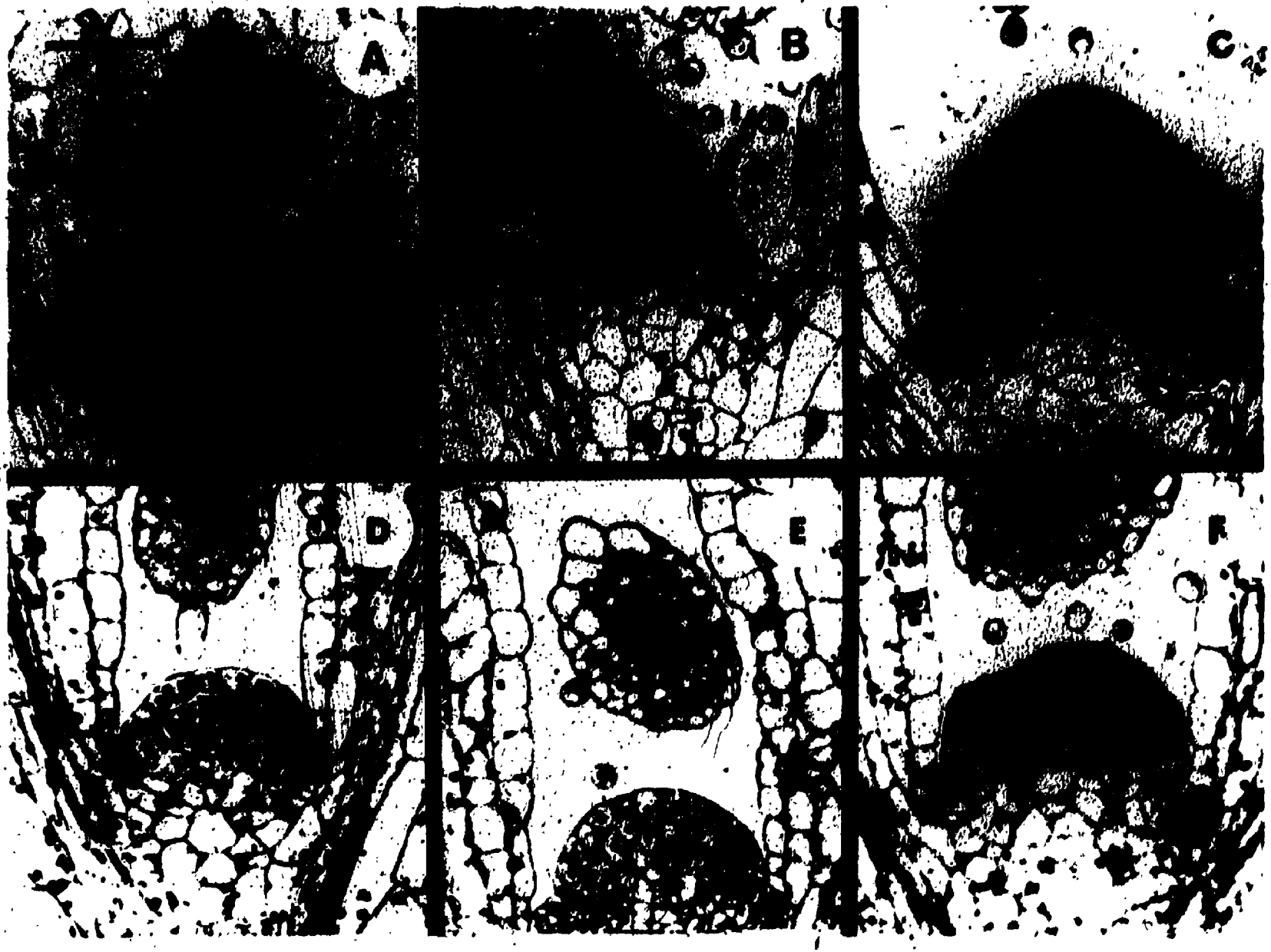

A
B
C
D
E
F

PLATE 6

MAGNIFICATION

Non-Induced Apices

6D 36 hours consisting of 24 hours Dark followed by 12 hours Light.

6E 48 hours consisting of 24 hours Dark followed by 24 hours Light.

6F 72 hours consisting of 24 hours Dark followed by 48 hours Light.

II GROWTH, DEVELOPMENT AND MORPHOGENETIC CONSIDERATIONS

(a) THE DARK-PERIOD: GROWTH RATE AND FLOWER INDUCTION

Within the period and under these experimental conditions, the effects of the various light and dark treatments on the apex are seen in the data which show that in continuous Dark compared with continuous Light the pattern of development is unchanged. However, in continuous Dark experimental treatment the rate of growth and development is slowed down or delayed or interrupted compared with that of continuous Light (HENCE [I] Chapter 3: D. Discussion: I Ontogenetic Considerations).

In those seedlings held in the dark from the start of the experimental treatment and later exposed to the light (so called Light-after-Dark series) but not induced to flower, the dark again effects a delay or interruption in development (HENCE [II] Chapter 3: D. Discussion: I Ontogenetic Considerations). The light following the dark stimulates a burst of growth and development. This results in growth which rapidly catches up to that of the corresponding continuous Light specimens.

Those seedlings held for different dark periods from the start of the experimental treatments and later exposed to light (so called Light-after-Dark series) are consequently induced to flower. These show that the dark

period again effects a delay or interruption and that the subsequent development at the apex in the light following the dark within these induced sets is independent of the length of the dark period, and entirely dependent on the length of the subsequent light period (HENCE [III] Chapter 3: D. Discussion: I Ontogenetic Considerations).

HENCE [IV], in photoperiodic induction of flowering the dark interruption of light can be regarded as causing at the apex a major slowing, a delaying or interruption in both rapid growth, and in development, and finally in the physiological and biochemical factors controlling this. This is to be compared with that seen in bright light.

The data also clearly indicate that growth and development proceeds optimally under normal environmental alternation of light and dark and that either continuous Light or continuous Dark (the extremes of the light/dark ratio) represent a disturbance in the balance of normal healthy rhythm and equilibrium of metabolism, hormone action etc. As above, it is to be noted that any changes in the length of the light period relative to the dark period between these extremes would also constitute a change in this balance.

It could be submitted that a halt or interruption or some form of decline in rapid growth and development and in the implied biochemical factors controlling this

is concommitant with flower induction (i.e. is a probable fundamental prerequisite of flowering in all plants). It is also shown in this system that this phenomenon at the apex occurs rhythmically, (Figure 2) very rapidly and very early and is an aspect of the method of flower induction, namely photoperiodic induction, used in this short-day system. It is to be noted that other methods of flower induction in mature plants, such as stress - due to drought, or injury, or transplanting, etc. - are associated with a halt in vegetative growth as a prerequisite for flowering. Vernalisation is consistent with this concept. It is also known that in some short-day plants, long dark nights can replace low temperatures, resulting in flowering.

Rapid growth is consistent with tight control and maintenance of the status quo of the apex - in other words, of the set stem apical pattern of cell division. When growth slows or is interrupted and then followed by reinstatement of rapid growth, the data will support that at the point of reinstatement the possibility opens for a change in growth pattern of cells at the apex. In other words, a change in the pattern of which cells do the dividing and which do not, arising from a change in the influence of extracellular factors. This appears rhythmically in this system. As above it is possible that a slowing, interruption, or interference with the

cell cycle of dividing cells is required so that all cells of the apex are of 'non-dividing status' or 'slowed status' before it is possible to change the pattern of apical cell division. Perhaps the dividing cells themselves are actually involved, influencing the maintenance of the set apical cell pattern producing signals in the microenvirnoment which inhibit the non-dividing cells. If that were so, then interference with the cell cycle division behaviour of these cells may be necessary before it is possible to get a change in the pattern of apical cell division. Herein lies the importance of the dark period at certain points in the rhythm of this system. It is fundamental to flower induction. This effect can be achieved in a large number of ways, as outlined above in the variety of growth stresses. Also consistent is the case of large apices, especially in plants where ageing over many years is necessary before they can be induced to flower - consider the Bamboos. Apices will become large and the control within the apex weakens with increase in size. A certain critical apical size would be required before control of the cell pattern is sufficiently weakened in certain apices to enable a change in cell pattern to occur, either automatically or for the apex to become sensitive to external induction stimuli, with resultant flowering.

(b) LIGHT: DEVELOPMENT AND MORPHOGENESIS

From the results of high light (3,000 ft. c) (H-L) treatments compared with low light (1200 ft. c) (L-L) treatments it appears that H-L produces a maximal continuous Light effect and speeds up development at the apex. In comparison the L-L results in development intermediate between that resulting under continuous Light (H-L) and that under continuous Dark experimental treatments. (HENCE [1] Chapter 3: D. Discussion: Ontogenetic Considerations). This effect is also shown in the continuous Dark Series (Chapter 3: C. Results: Vegetative Apices - Dark Series), where the differential rate of development is dependent upon whether light treatment immediately prior to continuous Dark was H-L (3,000 ft. c) or L-L (1200 ft. c). With L-L, growth and development at the apex is slowed relative to H-L, whereas in the continuous Dark experimental treatment the effect of the dark on growth and development is that of delay or interruption (as discussed in section (a) above).

HENCE [V] developmentally at the apex, it is seen that H-L is more effective than L-L. Furthermore it appears that in H-L all the parameters consistent with rapid vegetative growth and development at the apex are not consistent with those required for susceptibility to flowering (to apical flower induction/evocation or morphogenesis). These latter processes require an

FIGURE 4.

Flowering response in Chenopodium rubrum of the high-light series (3,000 foot-candles) compared with the low-light series (1,200 foot-candles). All hours quoted refer to time following the start of experimental treatment only.

4A Data from Light-after-Dark series.

4B Data from continuous Dark series.

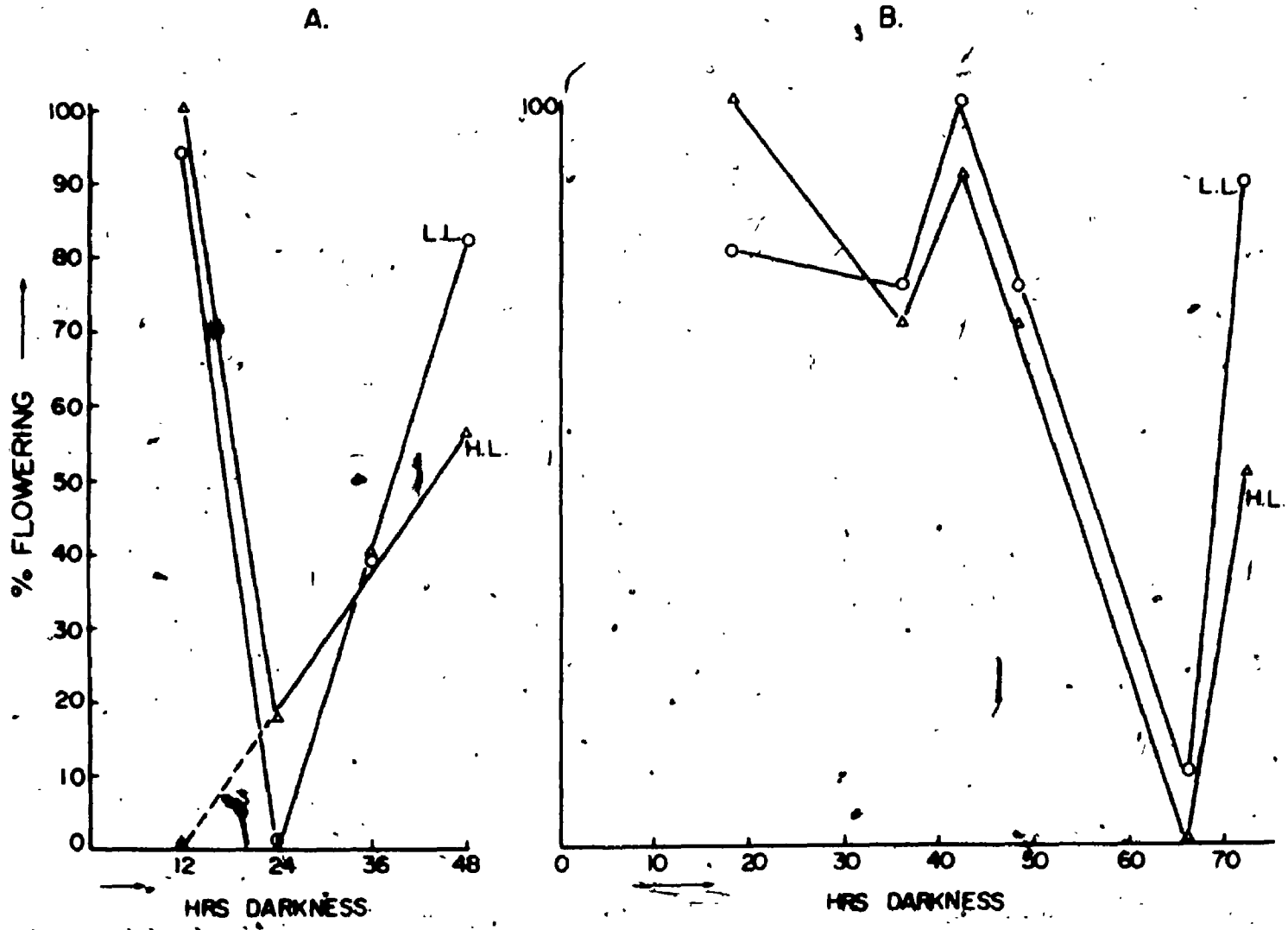
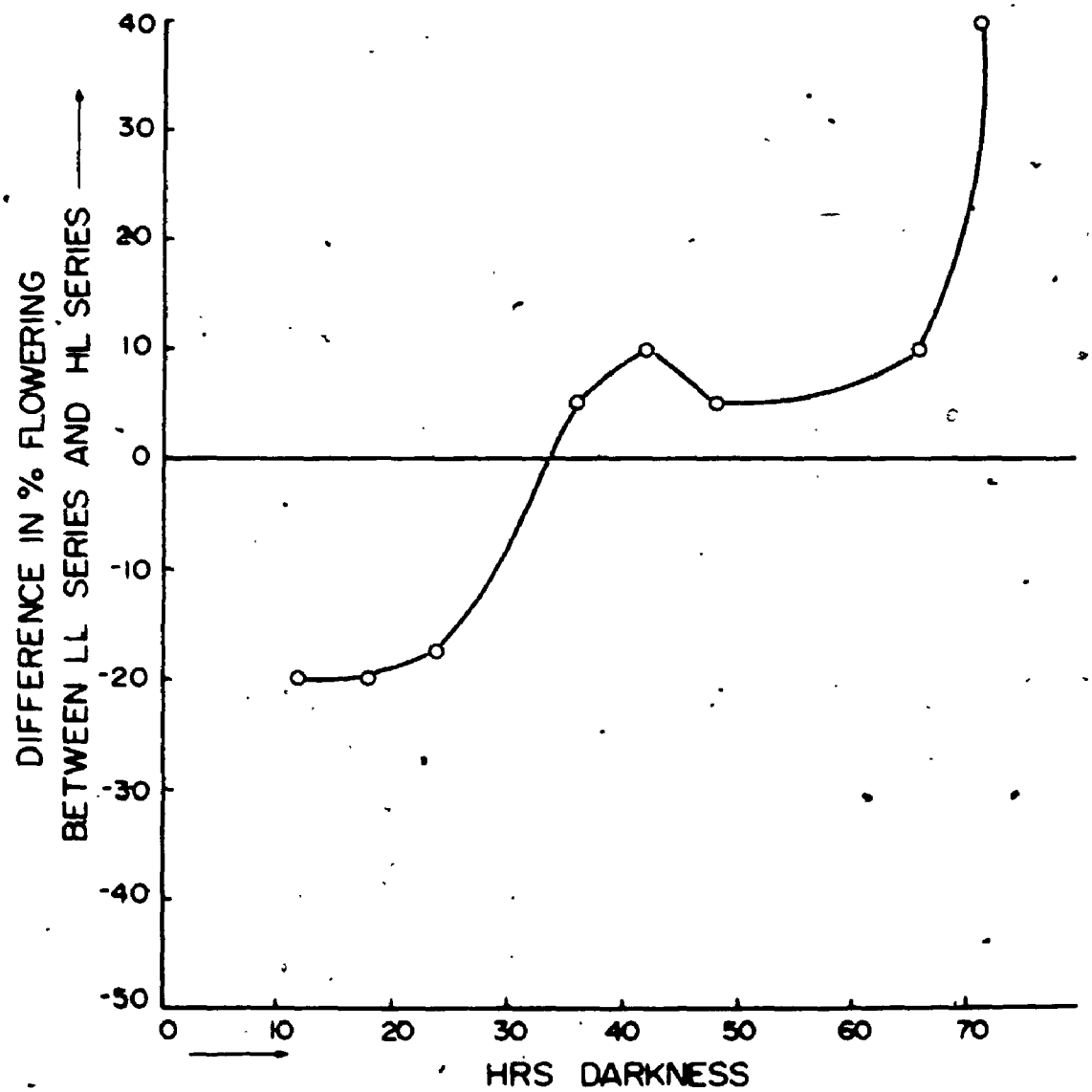

A.
B.
% FLOWERING
100
90
80
70
60
50
40
30
20
10
0
L.L.
H.L.
12
24
36
48
HRS DARKNESS
SETS FROM LIGHT-AFTER-DARK SERIES
0
10
20
30
40
50
60
70
HRS DARKNESS
SETS FROM DARK SERIES

FIGURE 5.

Difference in sensitivity to flower induction of high-light series compared with low-light series as measured by the differential in flowering percentage of samples taken over increasing periods of dark treatment.


DIFFERENCE IN % FLOWERING
BETWEEN LL SERIES AND HL SERIES
40
30
20
10
0
-10
-20
-30
-40
-50
0
10
20
30
40
50
60
70
HRS DARKNESS

interruption or halt in rapid vegetative growth processes at the apex. In this system the impetus is supplied by the dark interruption of the light. Light is associated with a development of the "status quo", and the dark interruption can effect a change in this "status quo", or morphogenesis.

Using the data presented under Chapter 3: Results the effect of H-L compared morphogenetically with L-L at the apex, is found to be more complex than the developmental situation discussed above. This is seen on analysis of the effect of H-L compared with L-L on flowering percentages and the sensitivity to flower induction (Figures 4 and 5) in these experiments. From the graphs, under L-L treatments, susceptibility to flower induction rises, with increasing length of time in the experimental dark period. Susceptibility with H-L treatments decreases in contrast to L-L treatments. The H-L results and rapid growth may be predicted, particularly in cases where one of the contributions of H-L treatment prior to the dark interruption is that of photosynthates. Considering the developmental results above, perhaps the L-L case may be expected to be lower and parallel to this. In fact this is not so - while H-L prior to the experimental dark period at first produces maximum susceptibility to flower inductions (including "breakthrough" in non-inductive phases of the rhythm) the effect rapidly falls and quickly (within the order

of 30 - 40 hours of experimental dark) a cross-over occurs (Figures 4 and 5). With increasing length of time in the dark L-L is seen to be superior in predisposing susceptibility to flower induction, in this system.

HENCE [VI] morphogenetically more complex light factors, including phytochrome status, are involved. This is highly significant to flower induction. It is known that the effect of light prior to the experimental dark period not only serves a prime function in providing photosynthates but in fact is also a complex, integral part of the light/dark - rapid growth/halt or interruption - phytochrome pattern prerequisite for flower induction or morphogenesis in this system. Developmental results show H-L to have an optimum effect on rapid development of the apex when H-L is given both prior to the experimental dark period (HENCE [V] as above) and when H-L is given following the experimental dark period. This produces maximum floral expression and is undoubtedly a major parameter in floral evocation (defined here as early development of the already induced apex). Here it is seen to play another role with respect to morphogenesis and photoperiodism proper and more factors than photosynthesis are involved.

(c) THE MESSAGE - GROWTH AND DEVELOPMENT

On examining both the induced and non-induced apices in the light following the experimental dark interruption, the second set of primordial leaves are seen to undergo a growth spurt by the end of the experimental period. The plastochron rate for both has increased beyond that of either the continuous Light series or the continous Dark series (HENCE [II] and [III], Chapter 3: D. Discussion: I Ontogenetic Considerations). Furthermore, in the case of the induced apices there is an even greater increase in plastochron rate than that in the non-induced apices. Precocious axillary bud development has also occurred in the induced apices (HENCE [III], Chapter 3: D. Discussion: I Ontogenetic Considerations). It is to be noted that during light/dark treatments all other experimental conditions are maintained identical for all series compared in order that variables known to affect the length of the plastochron (eg. temperature) remain the same and constant.

HENCE [VII], early in the sequence, rapid development occurs at the APEX in BOTH induced and non-induced apices as above and it is of the SAME ORGANS in the SAME SEQUENCE in BOTH non-induced and induced series. Therefore the same processes (and hormones etc.) required in the vegetative case are still available and effective at the induced apex after so called floral

induction and floral evocation. Perhaps the situation at the apex in the induced series is such that greater quantitites of required growth substances are available to the growing cells of the shoot tip, thus, the very marked increase in plastochron rate. While the quantities would be expected to differ the kind must be the same, in both non-induced and induced cases. - in other words, this INVOLVES FUNDAMENTALLY and CONCEPTUALLY the SAME (VEGETATIVE, DEVELOPMENTAL) PROCESS SUBSEQUENT to the EVENT(s) of INDUCTION//EVOCATION.

While the quantities of growth hormones available for development of the second set of primordial leaves are initially high in the induced apex this situation alters as changes in the set cell-pattern of the APEX progress. Indeed, while non-induced primordial leaves continue to grow in the induced case, despite the early rapid growth, the rate of growth in these primordial leaves soon falls off and they remain small. The SAME development occurs initially of the SAME organs of the vegetative apex as usual, and ALSO of the INDUCED apex whose cell pattern is in the process of modification but is not yet sufficiently modified to cause a modification in the form of the primordia. This soon follows, however.

At this early stage it is seen that the SAME "GROW AND DIVIDE" message or the VEGETATIVE MESSAGE received by the leaf primordia/buttresses/apex when the cells belong to a non-induced apex occurs in the induced apex. This apex still develops leaves normally and requires the usual vegetative message to do so (usual hormone requirements etc.) in the light following the dark treatment. In order for the same development to proceed, it must be the same message for both the early non-induced and the early induced apex. It CANNOT be caused by a SPECIAL, UNIQUE, FLORAL HORMONE with the properties of causing morphogenesis of a unique new organ - a flower.

This same vegetative stimulus is a DEVELOPMENTAL stimulus acting on the particular apical cell pattern at that point in time. While the set pattern persists, that which is consistent with the normal vegetative apical pattern, the leaf primordia develop. As the cell pattern of the apex slowly changes, or the apex is in a transitory or continually modifying or changing pattern state, this developmental stimulus will develop modified primordia determined by and consistent with the stages of the modified apical cell pattern. This is well seen in the floral primordia development even within the experimental period here. (HENCE III, Chapter 3: D. Discussion: I Ontogenetic Considerations).

(d) APICAL CELL STATUS AND THE MESSAGE

Examination of the effect of dark interruption of light (Light-after-Dark series - HENCE [II] and [III]) shows that the light following the dark treatment immediately results in the SAME process occurring in the cells of BOTH the non-induced (vegetative) apices and the induced (flowering) apices. The light following the dark firstly

(i) causes rapid cell enlargement in both types of apices and secondly

(ii) this is followed by rapid cell division in both types of apices. (HENCE [II] and [III], Chapter 3: D. Discussion: I Ontogenetic Considerations). (Table 1, Figure 3 and Plates 5 and 6).

HENCE [VIII], this is the SAME PROCESS for both, requiring the SAME MESSAGE - SAME HORMONES etc. This message affects the cells in the apices, irrespective of whether induced or non-induced, in the SAME manner. These data also indicate that it is NOT a different process unique to induced apices and inferring a unique floral hormone operative. The process is the same and the stimulus itself is fundamentally a VEGETATIVE MESSAGE consistent with vegetative development, and the contents of which must be "GROW AND DIVIDE" and notably nothing more.

The data indicate that at this stage it is not a different process. The real difference lies in which cells of the apex do the dividing in (ii) above, and a changing pattern of dividing cells is seen at the induced apex (Chapter 3: C. Results and Plate 3). It is this CHANGING CELL PATTERNING at the APEX which portends modification in appendages and subsequent so-called floral development. It is this change in apical cell patterning which is the essence of the photomorphogenesis. Those factors involved in the initial change of cell patterning at the apex are those factors involved in the process of induction/evocation. The effect of light after the dark interruption appears to be analogous to the "developing" of a photographic negative, the exact imprint of which is previously determined. Similarly, here in the induced apex the rigid set apical cell patterning of the vegetative apex has the elements of change, together with an inherent instability of apical cell patterning ALREADY set upon it and determined, AT, or NOT LATER THAN the DAWN SIGNAL (at the close of the experimental dark interruption). This is shown by the data herein and the process of 'development' in the light following the dark is the same for both non-induced (vegetative) and induced (potentially flowering) apices.

The essence of the flowering problem lies in which of the cells in the apex will divide, which in turn portends all later development. This has already been determined by the status of the seedling at that point in time when the dawn signal occurs. The data indicate that differential control of whether floral development in the apex will proceed or not lies back in the status of the apex in the dark which from the data (Figure 2 and Figure 8) must change with different points in time.

(e) INDUCTION AND EVOCATION

The data show that at the dawn signal the induction point or induction status of the apex in this system is unstable, transient, ephemeral. In addition, there is an absolute requirement that rapid growth and development occur within a set time-period if flowering is to proceed. This is seen by the following:

(1) Examining the effect of prolonging the dark period in the continuous Dark series reveals that a seedling held in the dark for 12 hours then immediately exposed to a long light period will certainly flower. In contrast, if that seedling in 12 hours dark was held a further 12 hours (total 24 hours dark treatment) and then exposed to a long light period it would certainly no longer flower. The induction status is in fact seen to appear rhythmically (Chapter 2, Figure 2).

(2) In this system the same effect as extended dark, (1) above, would be the expected result from severe interference with normal growth and development factors over the critical developmental period following on the induction point or the evocation period eg. by defoliation - in these seedlings removal of the cotyledons. This in fact is seen in the data of King (King, 1972), page 698. Immediate interference by removal of cotyledons prior to the induction point, at this point, the dawn signal, or subsequent to it within a period of 15 - 20 hours of light following the induction dark period (page 699) is observed to abolish flowering. Beyond that period there is an increase in flowering percentage.

(3) With regard to commitment to flower, the data on Chenopodium rubrum seedlings in the light following the inductive dark indicate that very early in the light period (at least within the first 12 hours). manipulations can readily abort flowering. This is so despite the fulfillment of induction/evocation requirements. However, in the time beyond this point the seedlings have already a certain commitment to flower. The data of Bernier (Bernier, 1971), page 816 are also consistent with this.

(4) Bernier (page 814), states, "All available evidence indicates that the mitotic phase is an essential component of the flowering process at the

meristem". Also that "examination of the problem of essentiality of the mitotic phase (in the induced meristem - in Sinapis) led to the discovery that ... it was possible to produce the mitotic phase in meristems remaining in the vegetative condition." In addition Bernier (1967, page 322), found that not only the mitotic index of the meristem proper but also stimulation of the mitotic index of the pith rib meristem occurred and that the mitotic stimulaton was a very general effect. He comments (page 814) that "what is remarkable is the fact that the activation begins in non-target tissues as early as in target tissues" and that (page 815), this may be viewed as related to the growth of cells. Since it relates later events at the apex to those early ones described in the results herein, it is important to note that Bernier states in his review (page 813), that "on the basis of its characteristic the morphogenesis phase (as defined in classical flowering theory) appears as identical with the prefloral phase" described in earlier studies on the ontogenetic development of a variety of apical meristems. The present analysis shows that these changes are only late consequences of more essential events that occur earlier ie. the induction and evocation events.

HENCE [IX], despite the fulfillment of induction/evocation requirements, NO flowering will occur unless the time limit regarding ESSENTIAL

DEVELOPMENT - IMMEDIATE DEVELOPMENT is fulfilled. (HENCE [VII], Chapter 3: D. Discussion (c) Growth Development and HENCE [VIII], Chapter 3: D. Discussion (d) Apical Cell Status and The Message). This is evidenced in the extended dark (1) above - and in excision of cotyledons (2) above. This is what King's data (page 698) (2) above are <u>really</u> saying and it is submitted that it is not caused by a failure in supply of a special, unique, foral stimulus arriving from the leaves or cotyledons. This stimulus is a vegetative stimulus promoting normal growth and development. No flowering can follow induction in a seedling unless either enough cotyledon is left, or the cotyledons remain long enough in the light following the dark treatment that the supply of components is adequate for the developmental spurt of evocation up to completion of a commitment to the first mitotic increase (HENCE [II] and [III], Chapter 3: D. Discussion: I Ontogenetic Considerations). For <u>Chenopodium rubrum</u>, King's data (page 699), indicate this to be a period of 15 - 20 hours of light after the dark treatment. Data in (3) indicate not less than 12 hours light. As above in (3) and (4), this is essential, since the early mitotic cycles following induction/evocation represent the <u>accomplishment</u> of the first changes in the cell patterning at the apex. It represents the <u>first commitment</u> of the induction status

of the apex to apical modification (i.e. evocation) and without this initial commitment the transient induction status will pass.

In this system, this is why the light following the dark is essential, why H-L here is more effective than L-L (HENCE [V], Chapter 3: D. Discussion (b) Light: Development and Morphogenisis) and why the presence of cotyledons is required. This is the real significance of the rapid, growth and development in the light following the dark (Chapter 3: D. Discussion (c) The Message, (d) Apical Cell Status). Herein is the importance of the essential cell enlargement and cell division activity and the essential requirement that this proceed immediately following the induction point, the dawn signal, or the induction status. Otherwise modification or change of the cell patterning of the apex will not occur and consequently flowering will not occur. This is the essence of floral evocation.

The data support the contention that in evocation, as defined here, in the light following the dark the essence of the problem is a growth and development and differentiation one, and not a morphogenetic one.

The essence of the morphogenetic problem lies back in the status of the apex in the dark at a point not later than the dawn signal (HENCE [VIII], Chapter 3: D. Discussion (d) Apical Cell Status). This is the essence of floral induction. It is to be compared with the

hypothesis of the classical flowering theory which holds that arrival at the apex (evocation) from the induced leaves of a floral stimulus is concommitant with commencement of the morphogenetic problem within the light period following the dark.

E: CONCLUSION

The data presented here indicate that flowering is conceptually an apical event. Careful examination of apical status during the dark period (the experimental dark interruption of the light) and particularly of those factors which control or can cause a change in the apical cell patterning; of ultrastructural and biochemical changes associated with the endogenous rhythm and flowering; of those factors not uniquely supposed to affect certain 'floral' cells but common to all, or rather of apical rhythms producing changing status of cells - all cells - at particular points in time; - should yield new data very significant for the questions raised by this whole system.

CHAPTER 4

ULTRASTRUCTURE AND ASSOCIATED BIOCHEMICAL STATUS

A: INTRODUCTION

In Chenopodium rubrum seedlings, flowering is a photoperiodically controlled response (Doorenbos and Wellensiek, 1959) (Cumming, 1963). Flower induction and inhibition have been used as markers for the phase of the endogenous rhythm (Cumming, 1969) (Cumming, 1971) (Cumming and Wagner, 1968) (King and Cumming, 1972). This enables examination of the ultrastructural and associated biochemical status of seedling tissues (cotyledon, primordial leaf and apex) at these known phases of the rhythm, and also of those factors which may be involved in the control of flower induction and evocation.

The structure of the vegetative apex has long been debated. Schmidt introduced the concept of a "tunica" of superficial layers covering an underlying "corpus" of dividing cells (Schmidt, 1924). Grégoire developed a different concept for the structured vegetative apex compared with the reproductive meristem which has a "meristematic mantle" overlying a core of parenchyma cells he called the "fronte-meristem" (Grégoire, 1938).

Foster examined size and staining of cells and showed that the apex is organised into zones - the "central (axial) zone", the "peripheral zone" (a flank meristem) and the "pith zone" (or file meristem) (Foster, 1938). In the 1950's Buvet and Lance introduced the concept of the "meristem d'attente" - the inactive central zone waiting to become active at the start of flowering (Buvet, 1952) (Lance, 1957) (Nougarede, 1967) (Gifford and Corson, 1971). Clowes contributed to the vast meristem concept by describing a corresponding quieseent zone - a centre with very low cell division rate (Clowes, 1958, 1963a, b). Stimulated by early reports of a flowering hormone florigen (Chailakhyan, 1936) various studies have been done on apical cell structural changes associated with induction and evocation. However, there have been a limited number of ultrastructural studies (Gifford and Tepper, 1962) (Gifford, 1963) (Healy, 1964) (Gifford and Stewart, 1965) (Bernier, 1967) (Bernier, Kinet and Bronchart, 1967) (Havelange, Bernier and Jacquard, 1974) (Havelange and Bernier, 1974) (Auderset and Greppin, 1977) (Lin and Gifford, 1976) (Havelange, 1980).

In this study a search was made for ultrastructural changes in the apex throughout the continuous Dark period and at regular sampling points corresponding to opposite phases in the endogenous rhythm of flowering. In addition, the status of the seedling apices was

followed for forty-eight hours, beginning from selected dark sampling point and progressing out into the L/D. This Light-after-Dark series consisted of both induced (flowering) and non-induced (vegetative) apices and an apical comparison was made. A continuous Light series was also sampled in the same manner as the continuous Dark series above. In addition to the ultrastructural study above, information about biochemical changes and metabolic status was also sought. The following questions were raised. Is the apex metabolically poised at induction? What factors at the apex are involved in, or permit flower induction? Is generalized growth the process occurring at both induced and non-induced apices, regardless of the cells involved, or is this a specialized flowering phenomenon unique to the induced apex? (Chapter 3: D. Discussion, E. Conclusion).

The following ultrastructural and biochemical observations were made:

1) genetic activity involving the nuclear chromatin region and the nucleolus.

2) protein synthetic activity involving the ribosomes free in the cytoplasm and membrane-bound ribosomes

3) membrane activity of nuclear membrane, endoplasmic reticulum, Golgi and plasma membrane.

B: MATERIALS AND METHODS

General experimental materials and methods as described in Chapter 2: B and Chapter 3: B and in Figures 1A, 1B, 2A and 2B were used here. The ecotypes of Chenopodium rubrum used were those described in Chapter 3: B. a) b) and c). The experimental regime was that described in Chapter 3: B. a) b) and c) and, as in c), three series of seedling specimens were produced, namely, "continuous Light series" (L), "continuous Dark series" (D), and "Light-after-Dark series" (L/D). After sampling, fixing, and embedding as described in detail in Chapter 3: B, eight hundred and twenty Epon embeddings of seedlings were prepared.

For each embedding sectioned, three sets of sections (cotyledon, primordial leaf, and apex) of the seedling were made. For each of these sets of sections both thick sections (1 μM mounted on glass slides) stained with 0.2% azure B, and thin sections (600 Å mounted on grids) were made. The embedding was cut and trimmed and the cotyledon was sectioned and then recut and retrimmed for the primordial leaf sections. However, the small apex(~80 μM across) had to be approached with microtome thick sectioning and then thin serial sections made to ensure that the median section through the apex was obtained oriented in the plane of the cotyledons.

Original manual microtoming with a glass knife had to be replaced by diamond knife automatic ultramicrotoming (LKB 8800A Ultratome III) to obtain these critical thin median sections. For criteria see Chapter 3. The thin sections were mounted on Formvar-carbon coated grids and were double stained with 1% uranyl acetate solution for 1 hour and 0.1% lead citrate solution (Reynolds, 1963) for 3 minutes.

The thin sections of cotyledon, primordial leaf and apex of seedlings from the various sampling points of experiment c) were examined using a Philips 200 electron microscope at 60 KV. Kodak fine grain positive 35 mm film was used for photography, and developed in Kodak D-19 for 7 minutes at 20° C. Low magnification (4,900 x) composite micrographs of apices were prepared enabling exact position and identification to be made of the individual cells in the high magnification (26,600 x to 180,000 x) micrographs. The order of 2,500 electron micrographs have been prepared.

C: RESULTS

ULTRASTRUCTURAL AND BIOCHEMICAL OBSERVATIONS

(1) GENETIC ACTIVITY

(a) Electron Microscopy of the Nuclear Chromatin Region

The micrographs presented show only the main structures which are relevant to this particular study and to the various experimental series.

Chromocentric interphase nuclei (LaFontaine & Lord 1976, 1974), or the prochromosomal nuclei of earlier workers (Manton, 1935 & LaFontaine, 1968) are observed in Chenopodium rubrum seedlings (Plates 7 and 8). The dense black condensed chromatin masses (heterochromatin of Heitz, 1929, Manton, 1935, LaFontaine, 1968), corresponding to the centromeric regions of the chromosomes, are found closely associated with the inside of the nuclear envelope or associated with the nucleolus (Plate 10 Upper). Puffs, in the chromocentric nuclei of this material are difficult to distinguish (Plate 10 Lower). They represent a phenomenon associated with dispersion of the condensed chromatin masses on the rim and are difficult to separate from the activity of the diffuse chromatin described below. Perhaps there are certain aspects of the activity in common and therefore

not readily distinguishable. Perhaps both are involved in an allied type of dispersion with accumulation of products producing a similar diffuse appearance. This would make it difficult for the eye to make the distinction at the level examined here. The nuclei contain one or very rarely two nucleoli (Plate 9 Upper). Micronucleoli (Plate 9 Lower) are present in many sections and absent in many others. No consistent pattern is evident in this material in either continuous Light or continuous Dark series. For a definitive statement, serial sections through all nucleoli examined would be necessary to ensure that no small micronucleoli are missed from the field of the sections examined. The nuclear cavity is pervaded by fine loose fibrillar material, the so called diffuse chromatin of the nucleus. Perichromatin granules appear as discrete granules in the nucleoplasm. However, as diffuse chromatin becomes flocculent difficulty arises in determining whether the granules observed are associated with this chromatin or whether they are actually discrete perichromatin granules. Thus, this appearance is described herein as "knotty" character of the threads and this includes any very closely allied dark staining lumpy or granular material. Distinct, absolutely separate granules cannot be consistently distinguished with certainty (Plate 12 Upper). Karyosomes are fibrillar bodies, homogeneous in texture and 0.3-0.5 μm

ELECTRON MICROGRAPHS SHOWING NUCLEAR STRUCTURES AND STATES OBSERVED.

PLATES 7 to 13 INCLUSIVE.

PLATE 7

Electron micrograph showing a chromocentric interphase nucleus, elongate and aligned parallel to the cell wall, typical of mesophyll cells of the cotyledon of Chenopodium rubrum seedlings. The dark condensed chromatin (cch) is seen here closely and exclusively associated with the inside of the nuclear envelope. A micronucleolus (MNu) is also present and is associated with the nucleolus (Nu). Magnification x 24,500.

PLATE 8

Electron micrograph showing a chromocentric interphase nucleus, spherical in shape and occupying a central position, typical of cells of the central zone of the apex and of mature cells of the first primordial leaves of Chenopodium rubrum seedlings. The dark condensed chromatin (cch) is seen associated with the inside of the nuclear envelope (NE) and another dense mass is seen near the nucleolus. Magnification x 27,000.

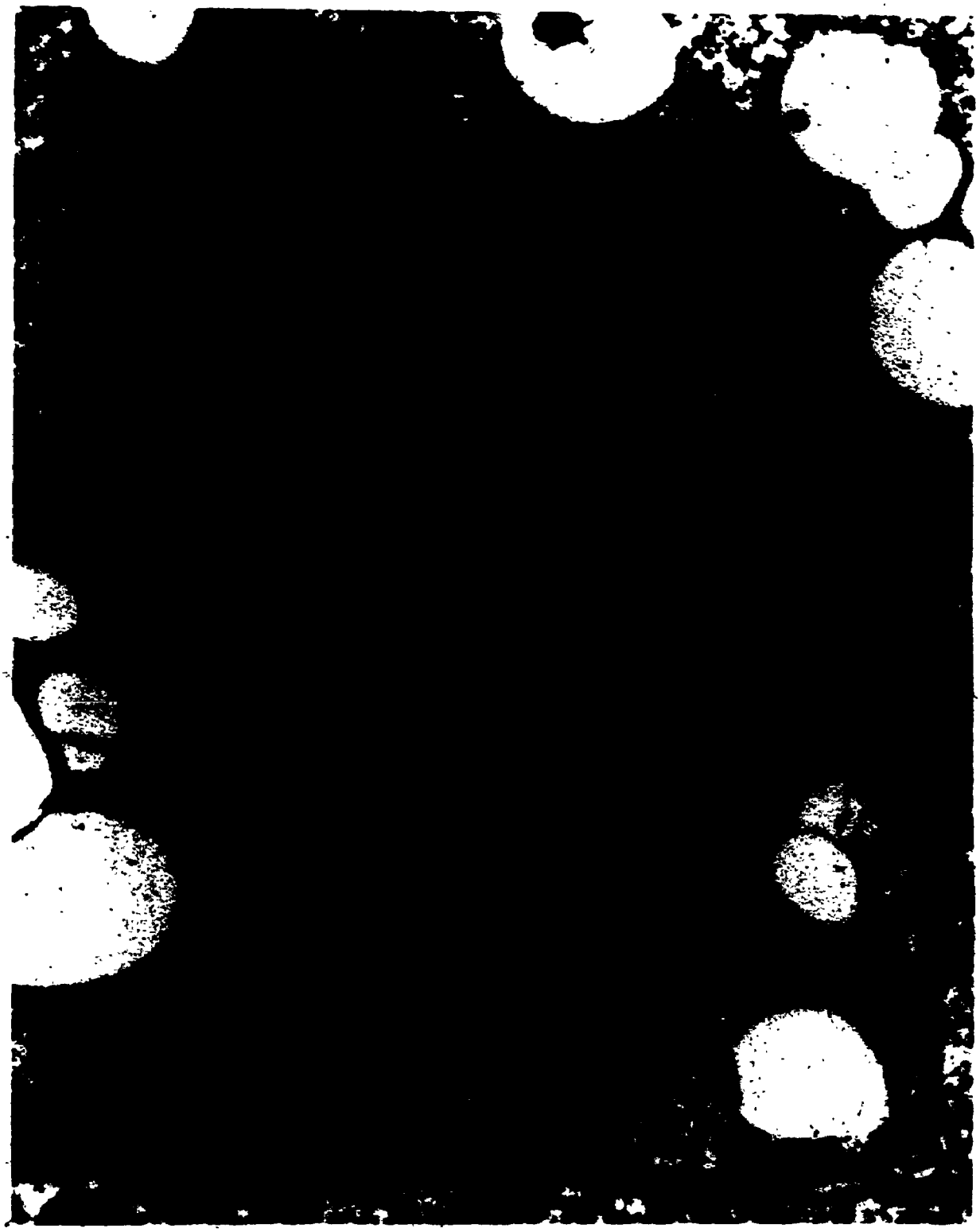


PLATE 9

Electron micrograph showing nuclear structures observed.

9 Upper — The nuclei observed in the Chenopodium rubrum seedling material usually contain one nucleolus and only rarely two as seen here. Magnification x 27,000.

9 Lower — Micronucleoli are present in many nuclei, and one may be observed here associated with a large nucleolus. Magnification x 24,500.

PLATE 10

Electron micrograph showing nuclear structures observed.

10 Upper — Cytoplasmic diverticulum which include an organelle, is observed making a deep incursion into the nucleus. The chromocentric distribution of condensed chromatin along the diverticular region of the nuclear envelope also can be seen. Magnification x 45,000.

10 Lower — An occasional karyosome (K) is seen here free in the diffuse chromatin region of the nucleus. It is intermediate in electron density compared with the nucleolus and the surrounding condensed chromatin and shows a consistent fibrillar texture. Magnification x 27,000.

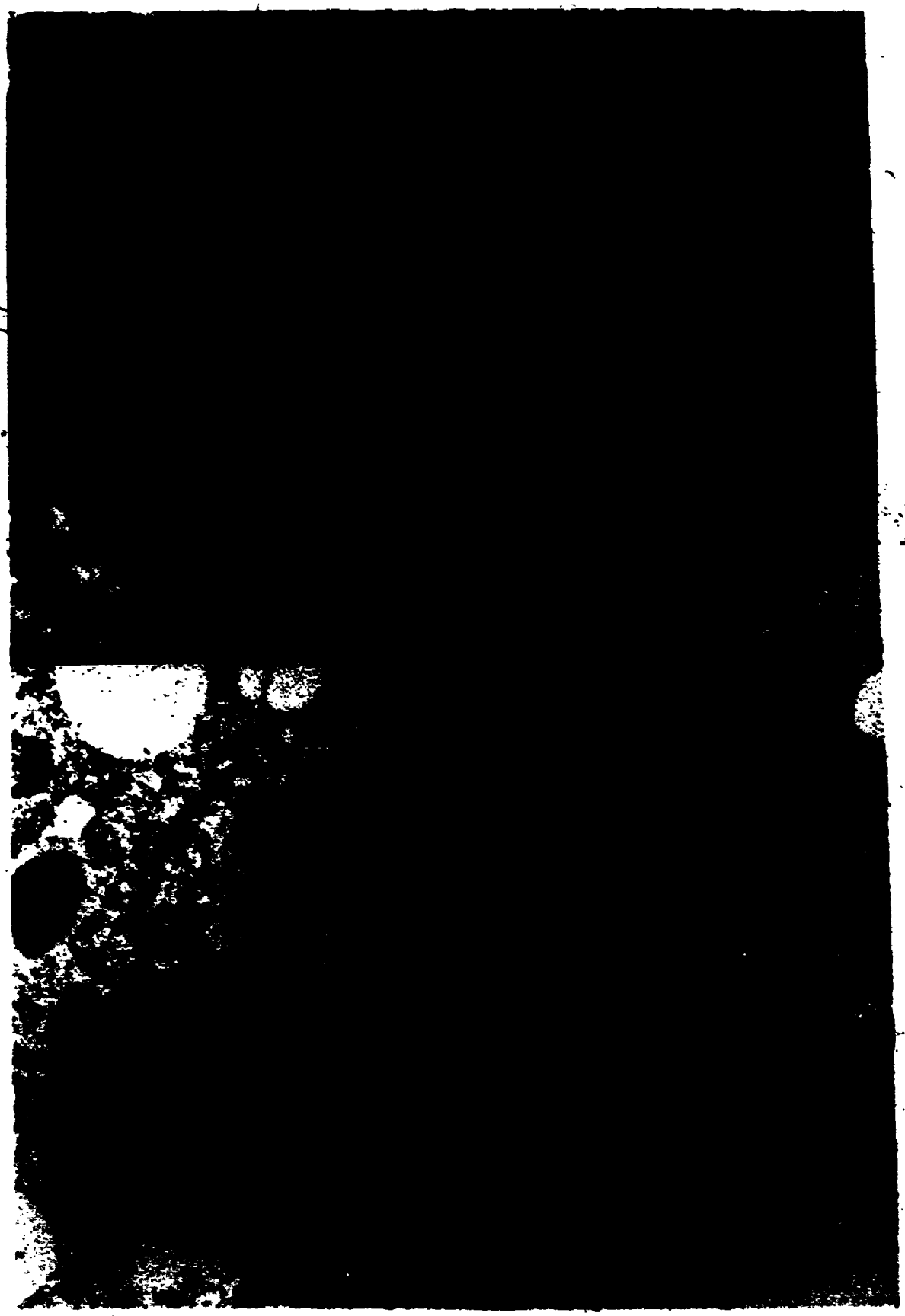


PLATE 11

Electron micrographs showing nuclear structures and states observed.

The two micrographs taken from a chronological series allow a comparison of the appearance presented by differing states of the heterochromatin.

11 Left — 12 Hours continuous Dark Treatment. The heterochromatin lining the nuclear envelope has become less electron-dense, less compact and somewhat dispersed and scattered along the nuclear envelope and somewhat into the nucleoplasm. Magnification x 27,000.

11 Right — 42 Hours continuous Dark Treatment. Heterochromatin masses are well defined and again line the rim of the nucleus. Magnification x 27,000

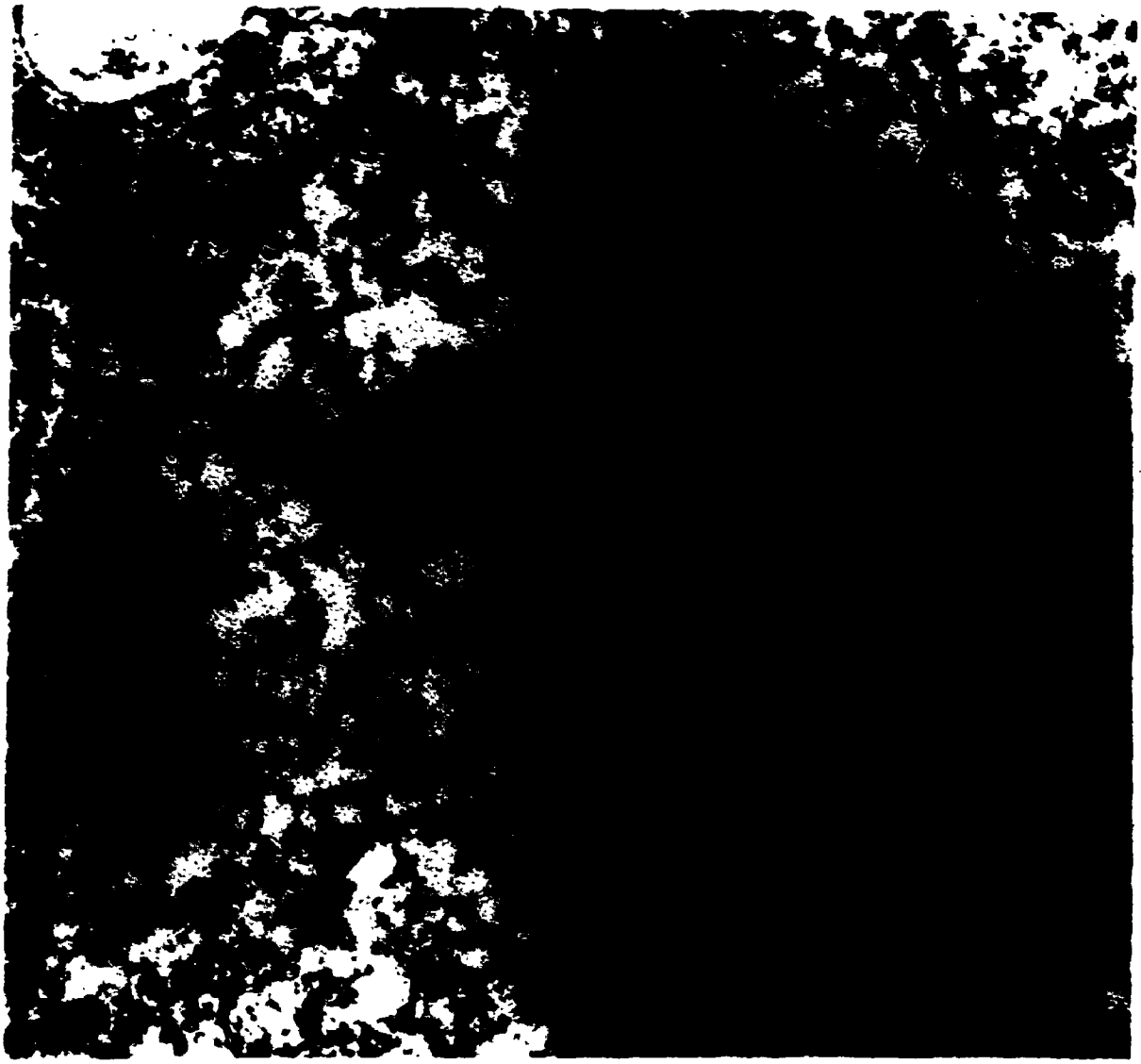


PLATE 12

Electron micrographs showing nuclear structures observed.

12 Upper — Perichromatin granules (PG) are seen surrounding the nucleolus and the diffuse chromatin of the nuclear cavity exhibits the flocculent or "knotty character" (KC) described under C (1), "Electron Microscopy of the Nuclear Chromatin Region". Magnification x 78,000.

12 Lower — A possible puff (Pf) is seen associated with the condensed chromatin lining the nuclear envelope. Magnification x 27,000.

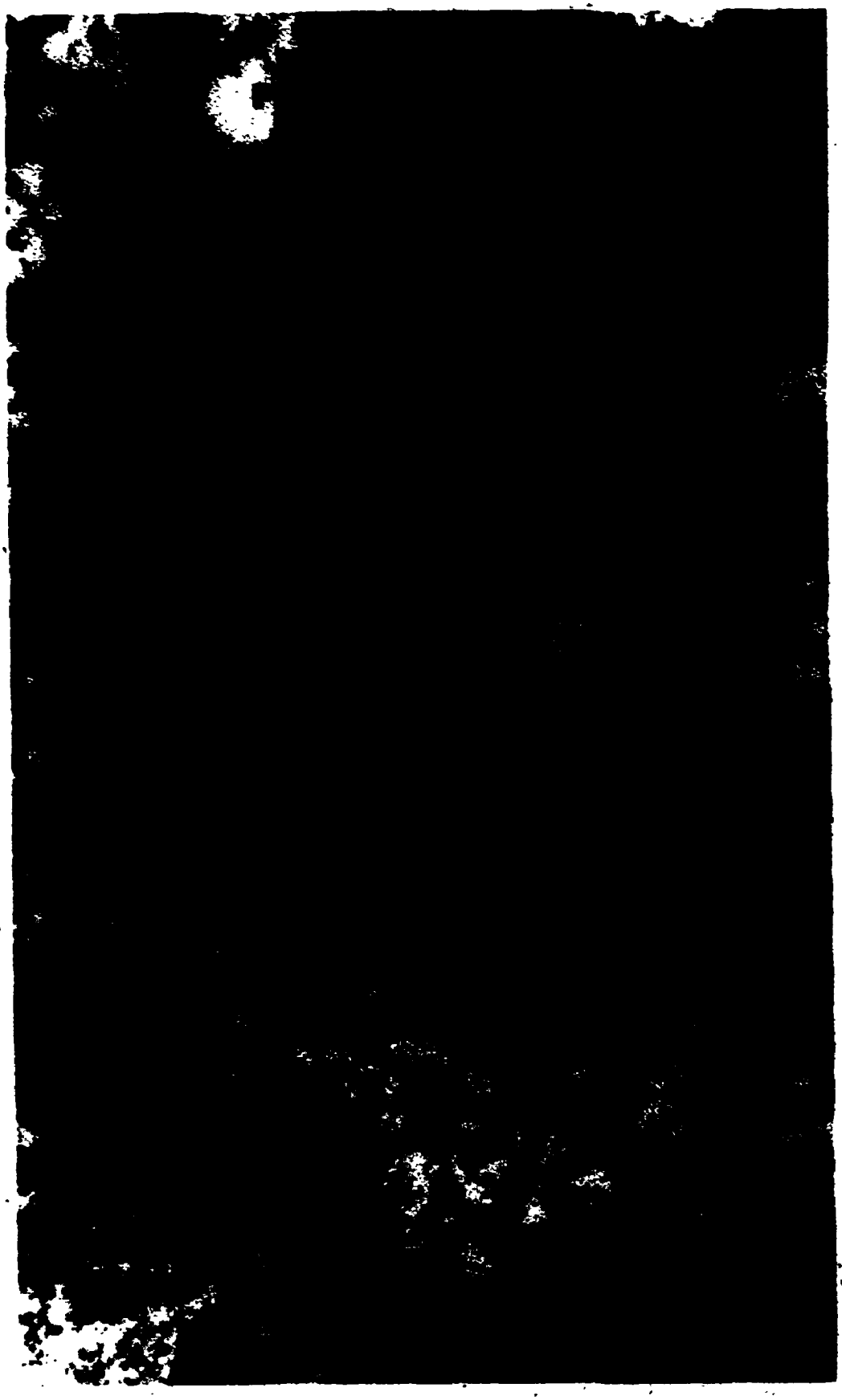


PLATE 13

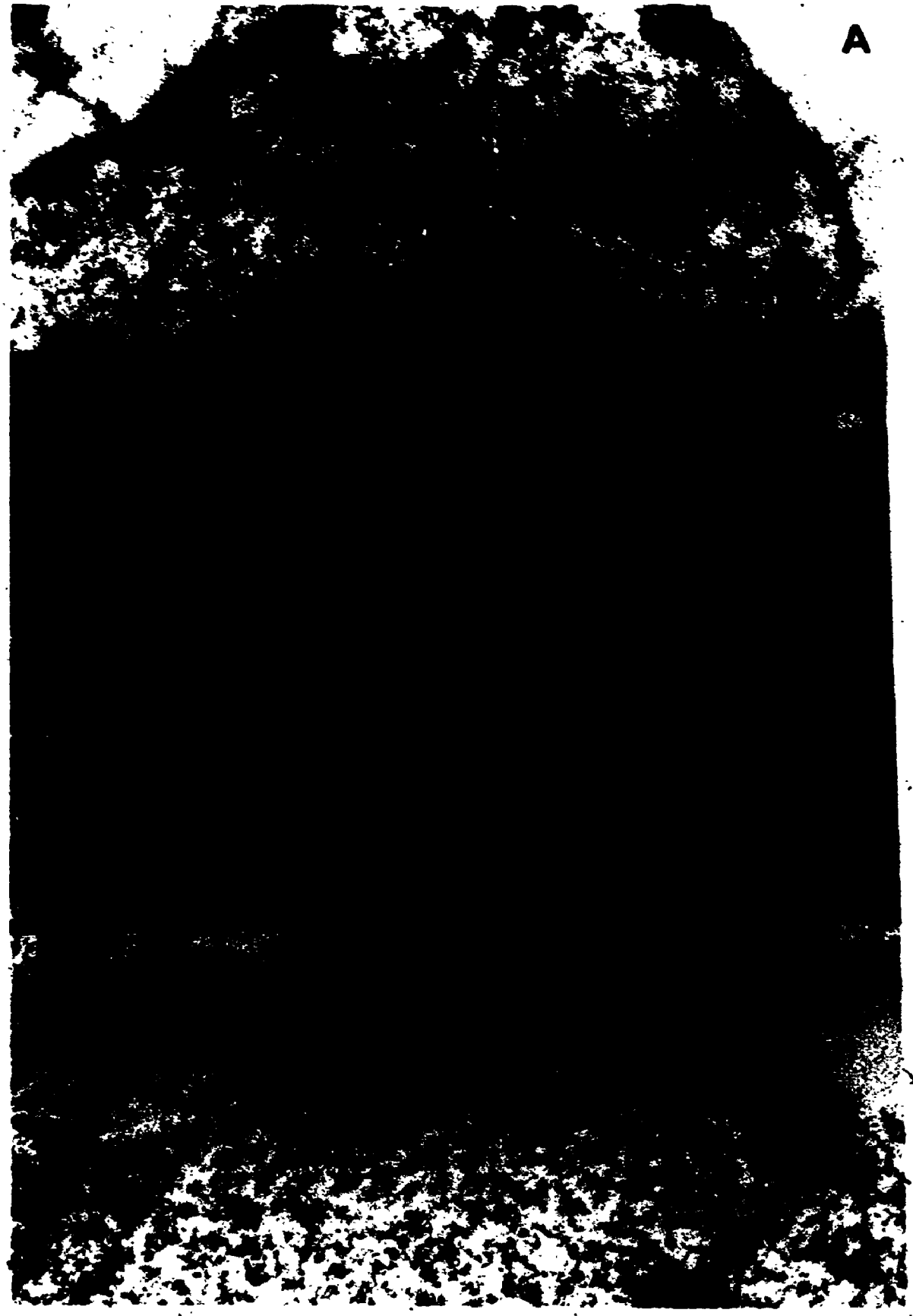
Electron micrographs showing nuclear states and structure:

The three micrographs allow a comparison of the appearance presented by the diffuse chromatin of the nucleus when the nucleus is in an apparent active state (flocculent or knotty character or in an apparent less active or non-active state (absence of intense flocculent or knotty character).

13 A Relatively Inactive. Maganification x 27,000.

13 B Active. Magnification x 47,600.

13 C Inactive. Magnification x 27,000.


A

in diameter in the nucleus. They are scattered and are of intermediate electron density compared with the condensed chromatin and the nucleolus, (Hyde, 1967). Very occasionally they are observed free in the diffuse chromatin region (Plate 10 Lower). Insufficient numbers are observed in this material to discern any pattern of distribution.

The nuclei in the cells of the central zone of the apex are spherical in shape and occupy a central position. This also applies to the mature cells of the first primordial leaves. The nuclei in the mature cells of the cotyledon (Plate 7) are elongated and aligned along the cell wall. Despite these differences in nuclear shape and position all of these nuclei at interphase present a typical consistent chromocentric conformation. Furthermore, under the given experimental conditions they respond to changes in these conditions in a similar manner, except as noted.

CONTINUOUS DARK SERIES:

In the continuous Dark series the heterochromatin and also that chromatin associated with the nucleolus undergo clear changes as early as 6 hours dark. The heterochromatin masses become less electron-dense and appear paler. The masses become sparse along the inner periphery of the nuclear envelope and that associated with the nucleolus disappears. By 12-18 hours dark the

smaller, paler masses are less compact and smaller patches become evident sometimes scattered across an area of nucleoplasm. By 24 hours dark the heterochromatin masses again line the rim of the nucleus and are now larger and well defined. The dark, well defined masses of chromatin associated with the nucleoli are again prominent. Similar changes to those described above also occur towards the end of the continuous Dark series - the first indication appearing around 60 hours and by 66-72 hours dark the changes appear similar to those of 6-12 hours dark. No clear cut pattern of changes could be discerned across the middle period of the continuous Dark series. Hence the heterochromatin does show clear changes in size, position and stainability following a change from light to dark and again toward the end of the series (Plate 11). However, at this point, these changes do not appear to parallel or correlate with other nuclear or nucleolar changes (Figure 6).

In the continuous Dark series the decondensed chromatin in the nucleoplasm appears uniform, paler staining, diffuse, regular and uniformly threadlike. This is the non-active form. At certain times during this series the active state is seen and this appears much denser and darker staining, with the nucleoplasm being non-uniform and chromatin appearing uneven, denser, flocculent and knotty. This is the active form

FIGURE 6.

Diagram to illustrate the structural changes with time observed in the nucleus and nucleolus within the continuous Dark series in C. rubrum, apex, first primordial leaf, and cotyledon tissues as explained in the text.

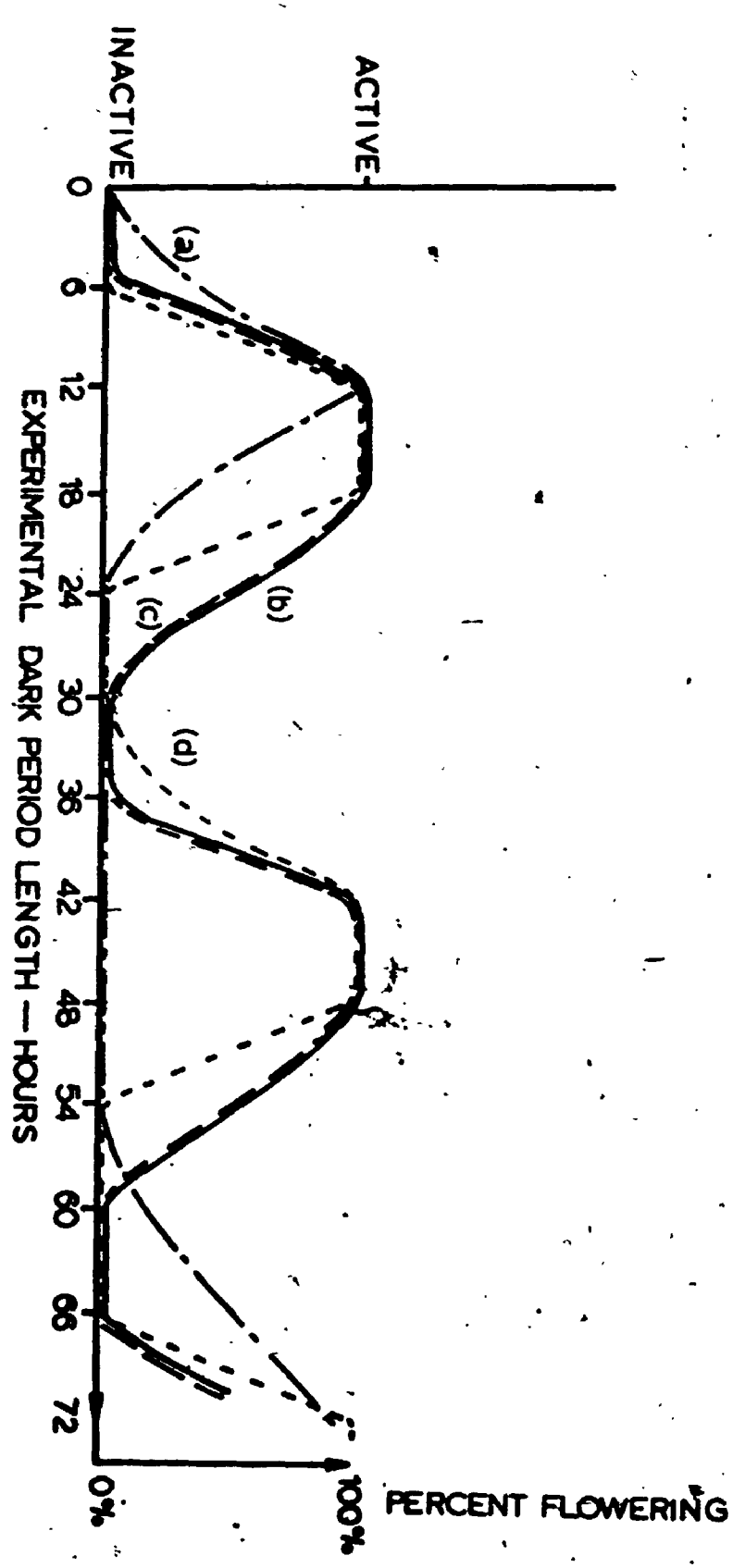
a) (----→ heterochromatin.

b) (——) diffuse chromatin of the nuclear cavity.

c) (— — -) nucleolus.

The endogenous rhythm of flowering is shown for comparison (see also Figure 2).

d) / (-----) flowering response in the continuous Dark series.


INACTIVE
ACTIVE
0
6
12
18
24
30
36
42
48
54
60
66
72
(a)
(b)
(c)
(d)
EXPERIMENTAL DARK PERIOD LENGTH — HOURS
0%
100%
PERCENT FLOWERING

when transcription is occurring with acculumations of ribonucleoprotein products giving the nucleoplasm a "knotty" character.

This state is seen clearly in sampling points of 12-18 hours and 42-48 hours and starts to reappear at 72 hours (Figure 6). The 24 hour and 54 hour points show evidence of the remains of this activity, but the nucleoplasm is much less dense and presumably at a stage when transport is still active. By the following points 30 hours and 60 hours respectively no evidence of this activity is seen and the non-active state of the diffuse chromatin is apparent. Hence, marked rhythmic changes are found in the diffuse chromatin of nuclei of the continuous Dark series, as seen in Figure 6 and Plates 13, and 16 to 19 inclusive).

CONTINUOUS LIGHT SERIES:

In the continuous Light series the heterochromatin shows some variation in degree of compactness, position of heterochromatin masses against the rim of the nucleus (some appear to "wander" a little from the rim in certain cases) and consistency of the heterochromation associated with the nucleolus (i.e. these tend to become relaxed). While some variation is evident in this continuous Light series there is no clearly emerging pattern of heterochromatin changes. Nuclei at some phases are less compact, but not the whole series as a

pattern. Knowing the times (rhythm) of cell division (King, 1971, Mitotic Index Results) and rechecking at these pertinent points confirms this result. Cell divisions are rarely observed - none appear in the random EM micrographs.

From sampling points in the continuous Light series, the diffuse chromatin in the majority of nuclei has a relatively inactive conformation. That is, the chromatin appears uniform and pale with the thin thread-like appearance as described above. However, in a small proportion of nuclei some activity is observed in the diffuse chromatin which becomes uneven in appearance, rather more flocculent and acquires a certain degree of 'knottyness'. In these nuclei this occurs over the 12-18 hour light period and again in the middle of the series at 42-48-54 hours light. Thus, it seems there are two populations of cells, the larger group of which has relatively inactive nuclei, while the remainder appear rather more active. This activity does not appear as great nor as clear cut as that found in the continuous Dark series. However, when it does occur it would appear to cycle or be rhythmic when compared with the continuous Dark series, (Figure 7 and Plates 14 and 15).

FIGURE 7.

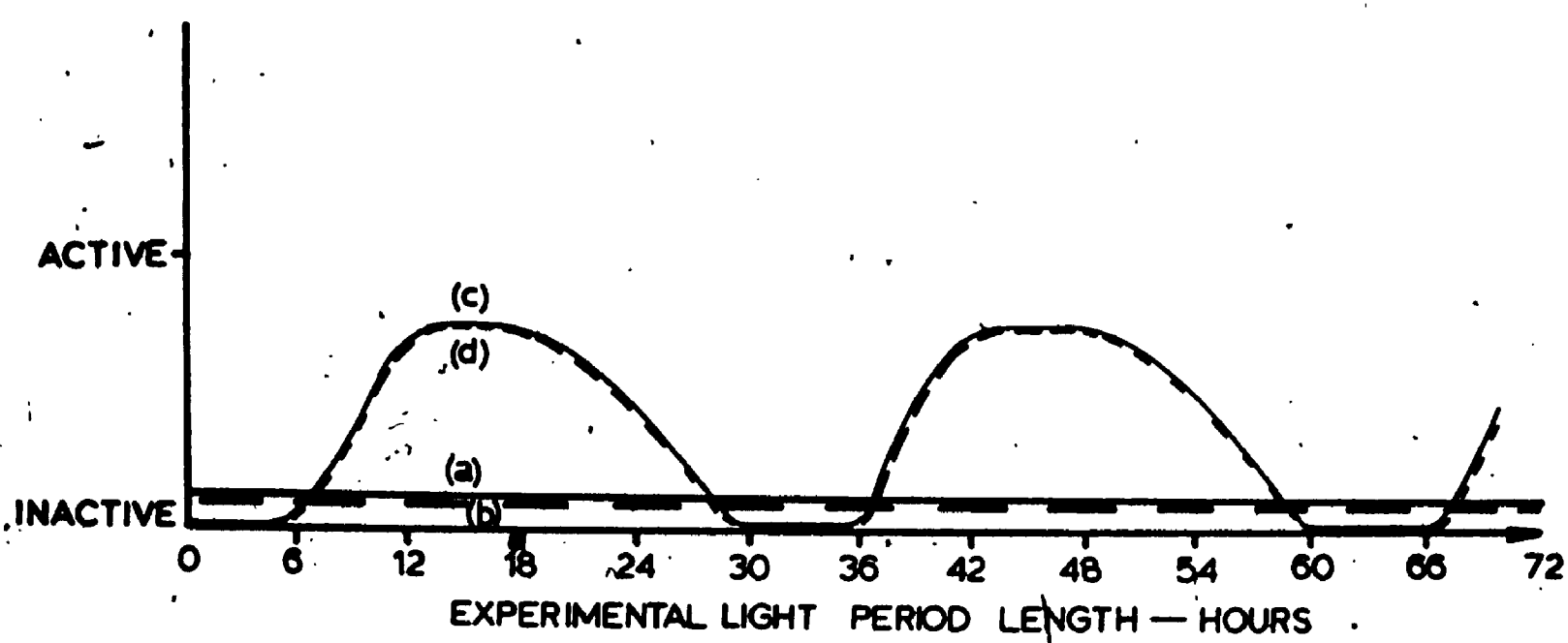
Diagram to illustrate the structural changes with time observed in the nucleus and nucleolus within the continuous Light series, in C. rubrum, apex, first primordial leaf, and cotyledon tissues as explained in the text.

a) (——) diffuse chromatin of the nuclear cavity as seen in the majority of cells.

b) (— —) nucleolus as seen in the majority of cells.

c) (——) diffuse chromatin of the nuclear cavity as seen in a small minority of cells.

d) (– – –) nucleolus as seen in a small minority of cells.


ACTIVE
INACTIVE
(c)
(d)
(a)
(b)
0
6
12
18
24
30
36
42
48
54
60
66
72
EXPERIMENTAL LIGHT PERIOD LENGTH — HOURS

LIGHT-AFTER-DARK (INDUCED) SERIES:

For all sampling points in the Light-after-Dark (induced) series (L/D), the condensed chromatin (heterochromatin) masses show dispersion - they appear much smaller, paler and distributed in a sparser manner. Furthermore, for all sampling points in this series the diffuse chromatin across the nucleoplasm is 'knotty' and the chromatin threads 'flocculent' (Plates 21 and 22).

Thus all sampling points in the L/D series have active nuclei, with 48L/12D showing even more marked activity than at 36L/12D. Those samplings in the L/48D series show the same consistent form as that above and confirm the results of the L/12D series (Figure 8). Active cell division is apparent particularly at 36L/12D,36L/48D and 48L/12D,48L/48D.

LIGHT-AFTER-DARK (NON-INDUCED) SERIES:

Throughout the Light-after-Dark (non-induced) series the heterochromatin is seen as small dark peripheral masses. There is variation, some being less compact especially later in the series. These are associated with the nuclear envelope and with the nucleolus, especially early in the series.

Early in the Light-after-Dark (non-induced) series (12L/24D, 24L/24D) the diffuse chromatin has an even, uniform, thread-like character. However, by the end of the series (48L/24D) the diffuse chromatin has become

FIGURE 8.

Diagram to illustrate the structural changes with time observed in the nucleus and nucleolus within the three Light-after-Dark series, in C. rubrum, apex; first primordial leaf, and cotyledon tissues as explained in the text.

In the Light-after-Dark series (induced - 100% flowering) and in the Light-after-48 hours Dark series (induced - 100% flowering):

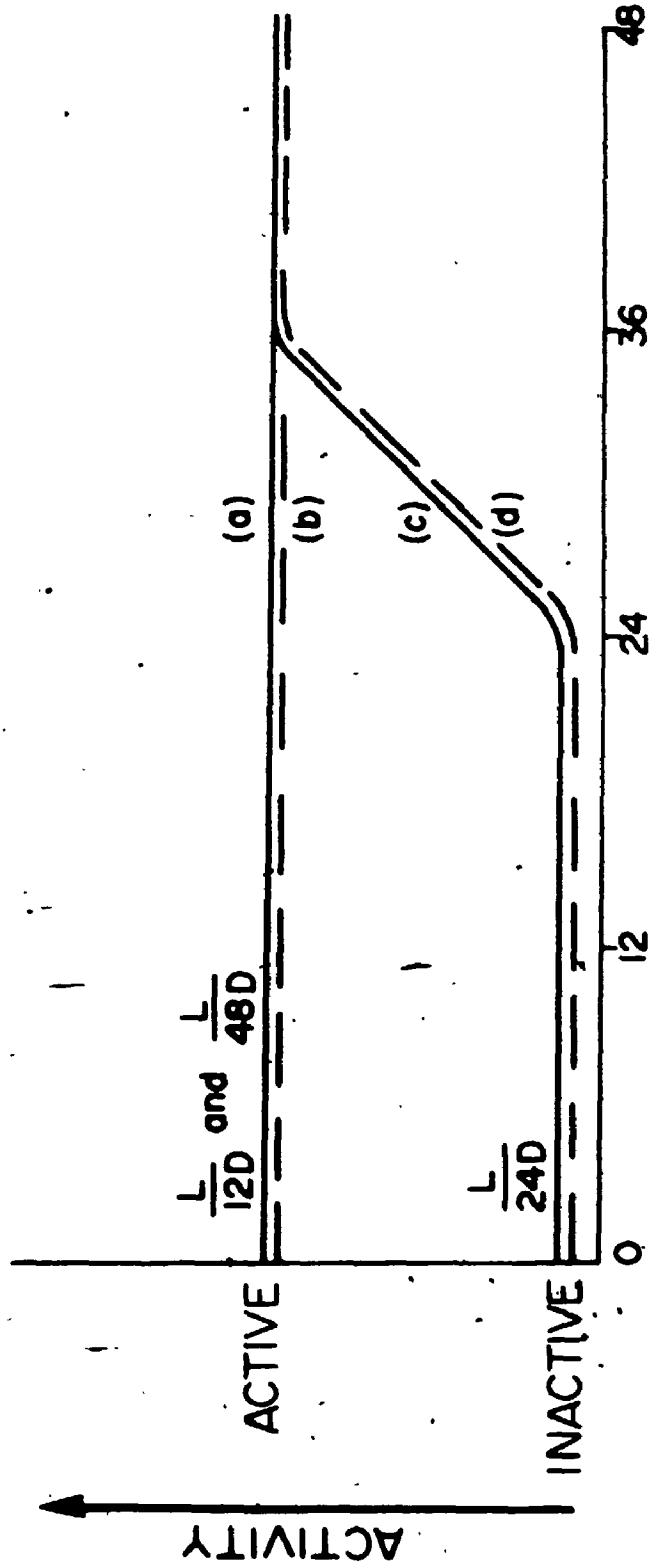
a) (——) diffuse chromatin of the nuclear cavity.

b) (— —) nucleolus.

In the Light-after-24 hours Dark series (non-induced - 0% flowering):

c) (——) diffuse chromatin of the nuclear cavity.

d) (— —) nucleolus.


ACTIVITY
ACTIVE
INACTIVE
L/12D and L/48D
L/24D
(a)
(b)
(c)
(d)
0
12
24
36
48
EXPERIMENTAL LIGHT PERIOD LENGTH-
FOLLOWING DARK PERIOD - HOURS

ELECTRON MICROGRAPHS SHOWING NUCLEI FROM SEEDLINGS WHICH HAVE UNDERGONE THE VARIOUS EXPERIMENTAL TREATMENTS DESCRIBED IN CHAPTER 2 AND CHAPTER 3: B. MATERIALS AND METHODS c) i).- iii).

PLATES 14 to 22 INCLUSIVE.

PLATE 14

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Light Series.

Both seedlings have been exposed to 12 hours continuous Light (L Series) experimental treatment.

14 Upper Non-Active State. Magnification x 27,000.

14 Lower Active State. Magnification x 27,000.

These states are discussed under Chapter 4: C 1(1) Results Nucleus: continuous Light Series.

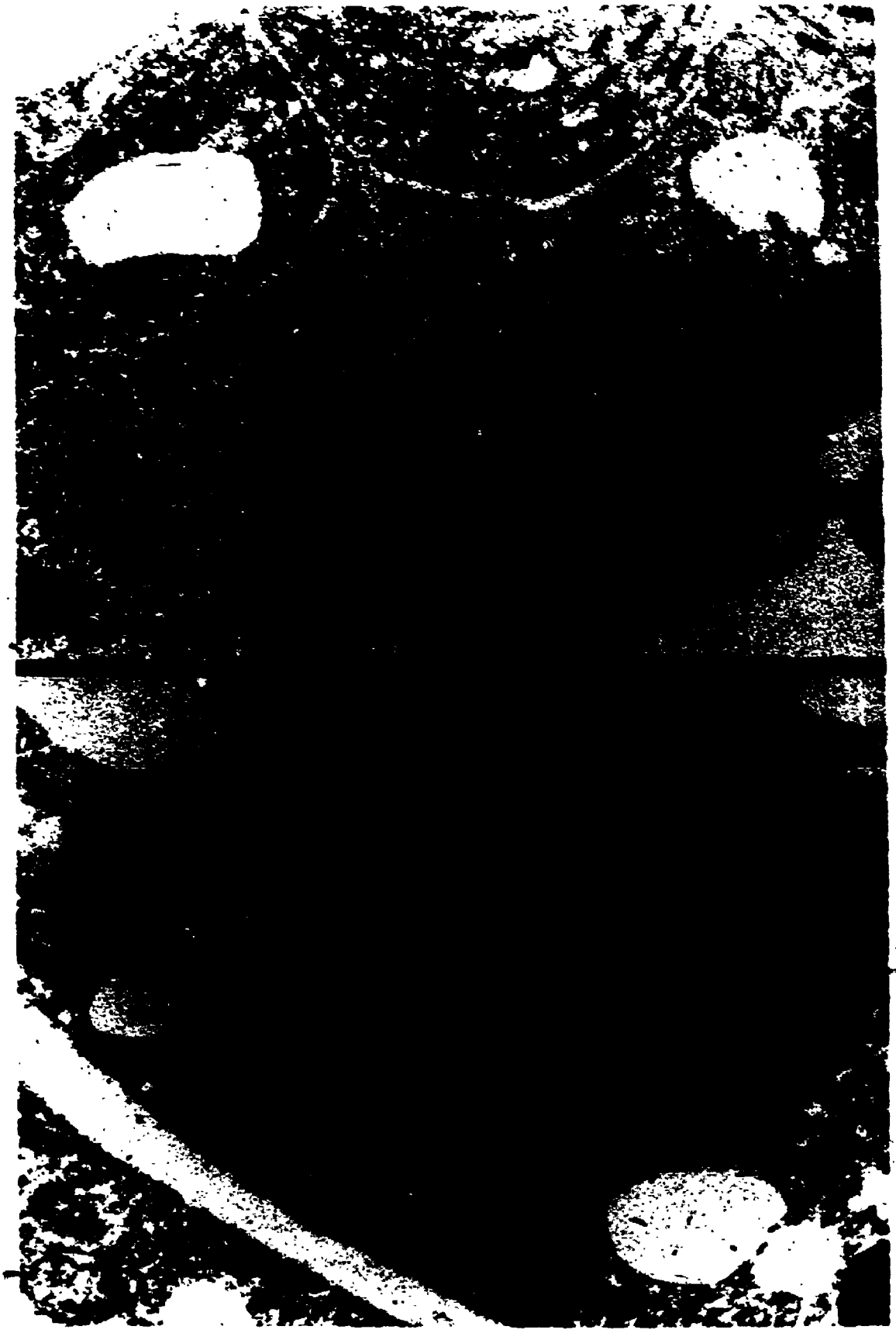


PLATE 15

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Light Series.

15 Upper — 30 hours continuous Light treatment. - Non-Active. Magnification x 45,000.

15 Lower — 0 hours treatment (i.e. point at start of treatments). - Non-Active. Magnification x 16,000.

PLATE 16

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

16 Upper 12 hours continuous Dark treatment. - Active. Magnification x 27,000.

16 Lower 24 hours continuous Dark treatment. - (Active). Magnification x 24,500.

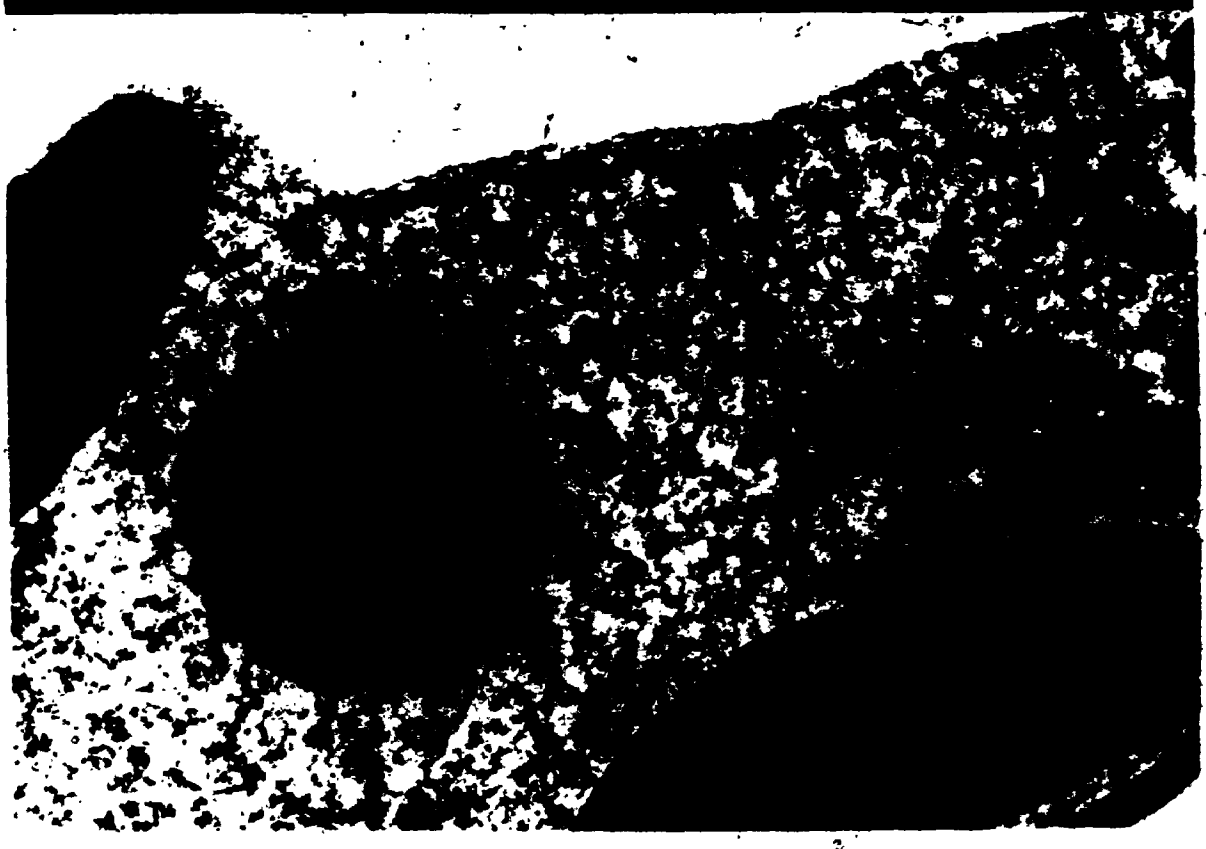


PLATE 17

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

17 Upper — 30 hours continuous Dark treatment. - Non-Active. Magnification x 27,000.

17 Lower — 42 hours continuous Dark treatment. - Active. Magnification x 27,000.

PLATE 18

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

18 Upper — 54 hours continuous Dark treatment. - (Active). Magnification x 47,600.

18 Lower — 66 hours continuous Dark treatment. - Non-Active. Magnification x 27,000.

PLATE 19

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

19 72 hours continuous Dark treatment, the last of this series.
- Active. Magnification x 45,000.

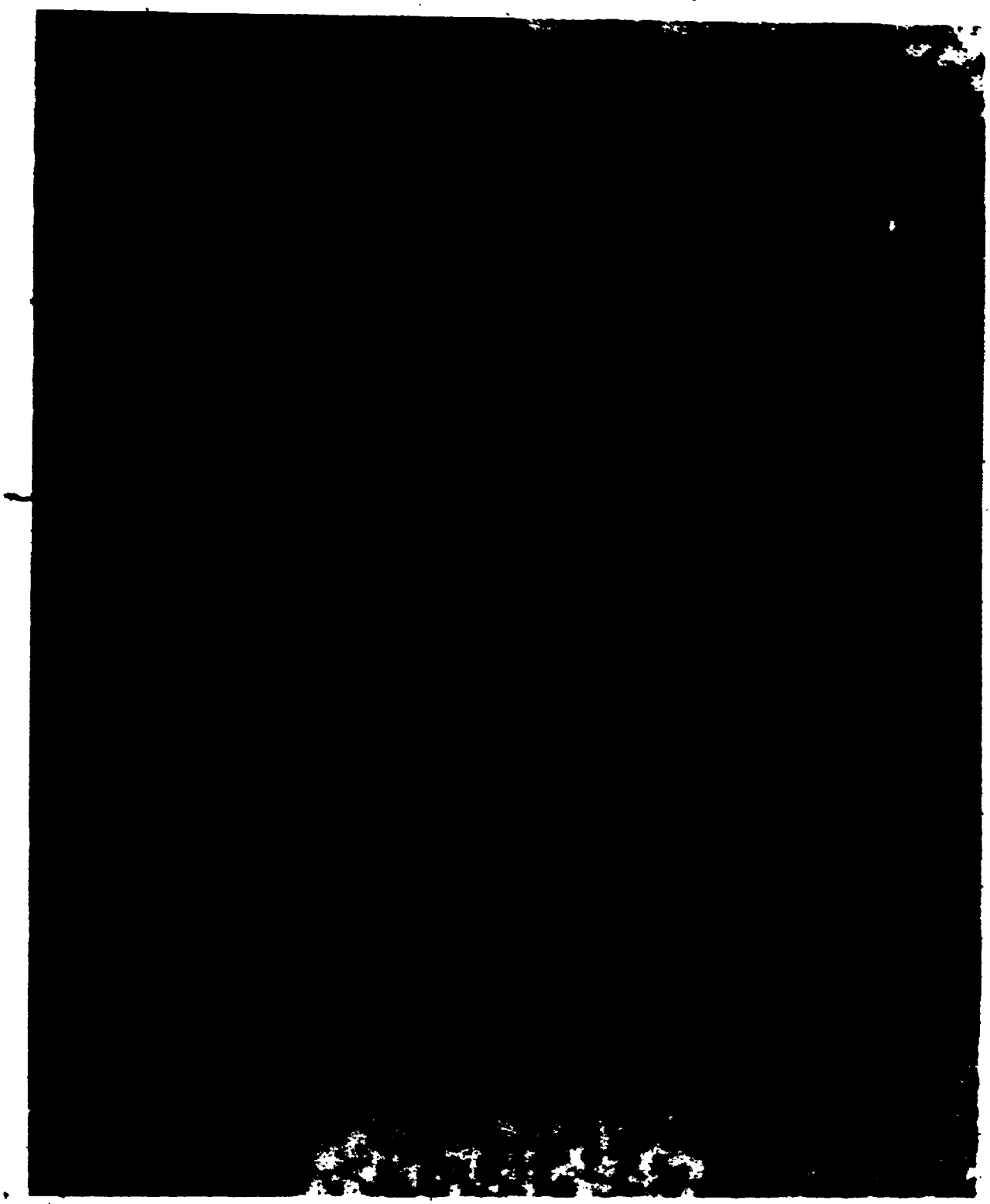


PLATE 20

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: Materials and Methods c) i) - iii).

Light-After-Dark Non-Induced (Vegetative) Series.

20 Upper — 12 hours Light following 24 hours Dark.
- Non-Active. Magnification x 27,000.

20 Lower — 48 hours Light following 24 hours Dark.
- Active. Magnification x 27,000.

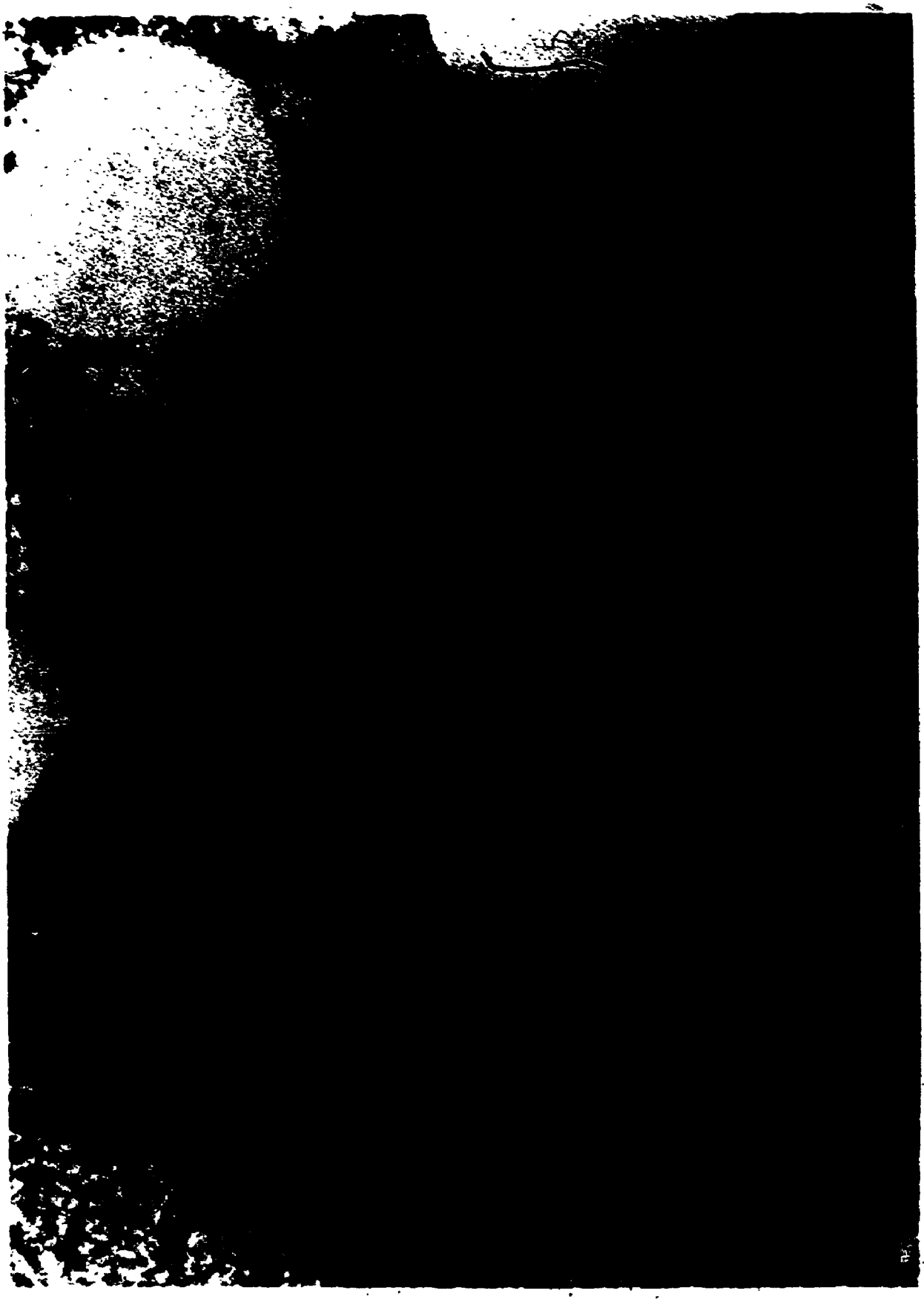


PLATE 21

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: Materials and Methods c) i) - iii).

Light-After-Dark Induced (To Flower) Series.

21 Upper — 12 hours Light following 12 hours Dark.
- Active. Magnification x 27,000.

21 Lower — 36 hours Light following 24 hour Dark..

This is the seedling with the bacterial infection. The apex has been induced to flower in spite of the fact it has been exposed to the non-inductive 24 hours Dark.
- Active. Magnification x 27,000.

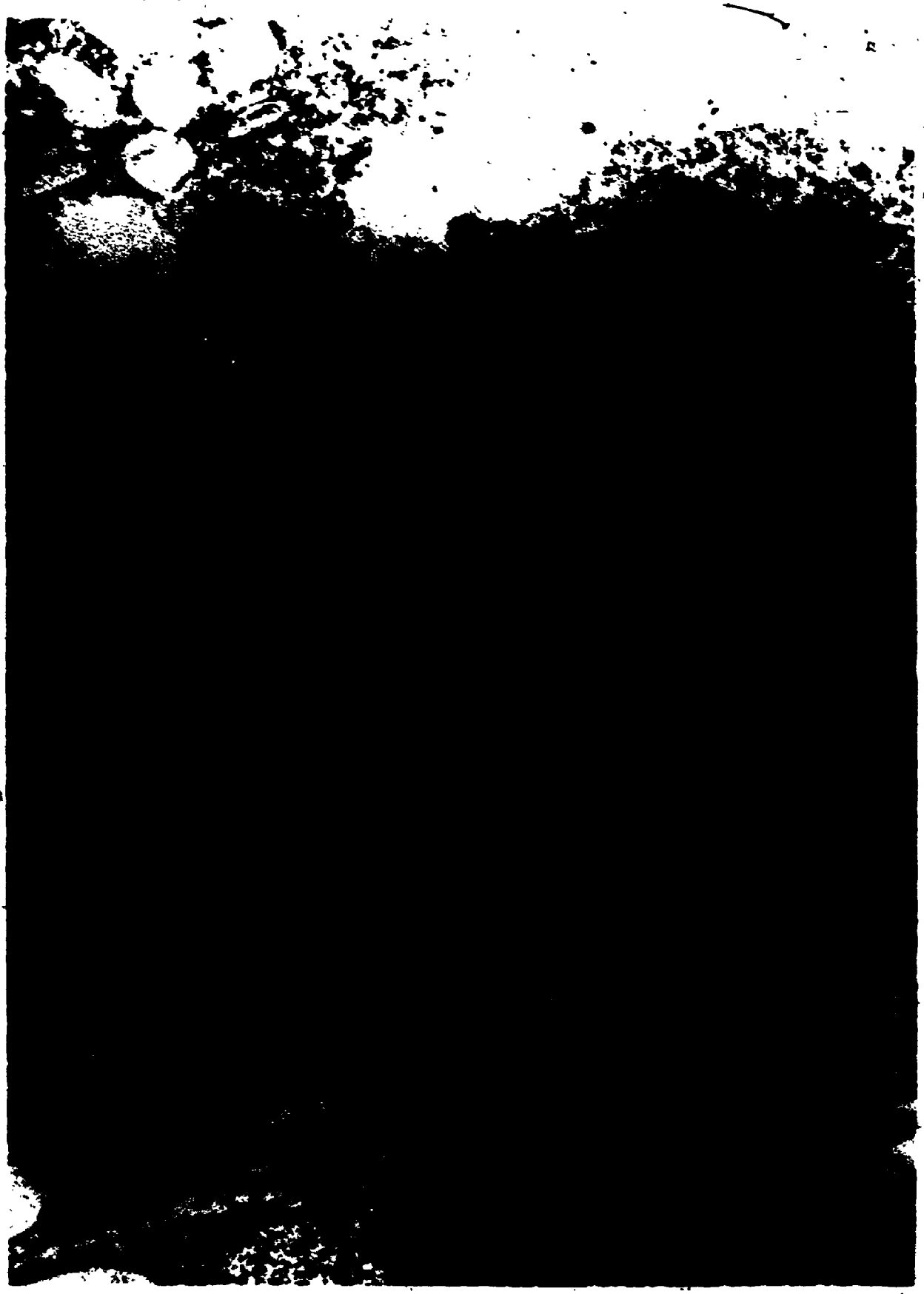
PLATE 22

Electron micrographs showing nuclei from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: Materials and Methods c) i) - iii).

Light-After-Dark Induced (To Flower) Series.

22 Upper — 36 hours Light following 48 hours Dark.
- Active. Magnification x 27,000.

22 Lower — 48 hours Light following 12 hours Dark.
- Active. Magnification x 27,000.



flocculent, denser, and 'knotty'. Hence, in the first half of the Light-after-Dark (non-induced) series, (12L/24D, 24L/24D) the nuclei appear relatively inactive while later in the series, by 48L/24D, the nuclei have become active (Figure 8 and Plate 20). Active cell division in the buttresses is apparent by the end of the series (36L/24D, 48L/24D).

(b) Electron Microscopy of the Nucleolus

The nuclei of Chenopodium rubrum seedlings, contain one nucleolus and only very rarely are two found. This is the case regardless of whether the central zone of the apex is examined, whether the mature cells of the primordial leaf or the mature cells of cotyledons are examined. All of these nucleoli at interphase present a consistent conformation dependent upon the experimental conditions and the sampling time.

The structure of the nucleolus (Plate 23) shows marked changes. In some samples it is a small, smooth round body of tightly packed fibrillar material with a narrow scattered dark rim of granular particles. There are few or no distinguishable lacunae present and no vacuoles. This is the dormant or non-active form. In other samples, the nucleolus is a large irregular edged round form in which the fibrillar chromatic material is somewhat obscured by granular particles. These particles also form a dense halo on the outside of the nucleolus.

ELECTRON MICROGRAPHS SHOWING NUCLEOLAR STRUCTURES AND STATES OBSERVED.

PLATES 23 and 24

PLATE 23

Electron micrographs showing nucleolar structures observed.

23 Upper — Electron micrograph showing an active nucleolus containing two major regions. One is predominantly granular (G) in appearance and consists of the accumulation of ribonucleoprotein particles. The other is fibrillar (F) in appearance and is composed mainly of closely packed fine fibrils. Seen within the fibrillar zone are lacunae (L) which enclose fine chromatin threads. Very small vacuoles (VL) are also in evidence. Magnification x 27,000.

23 Lower — Electron micrograph showing a nucleolus with two very large vacuoles (VL) within which scattered ribonucleoprotein granules can be seen. Magnification x 45,000.

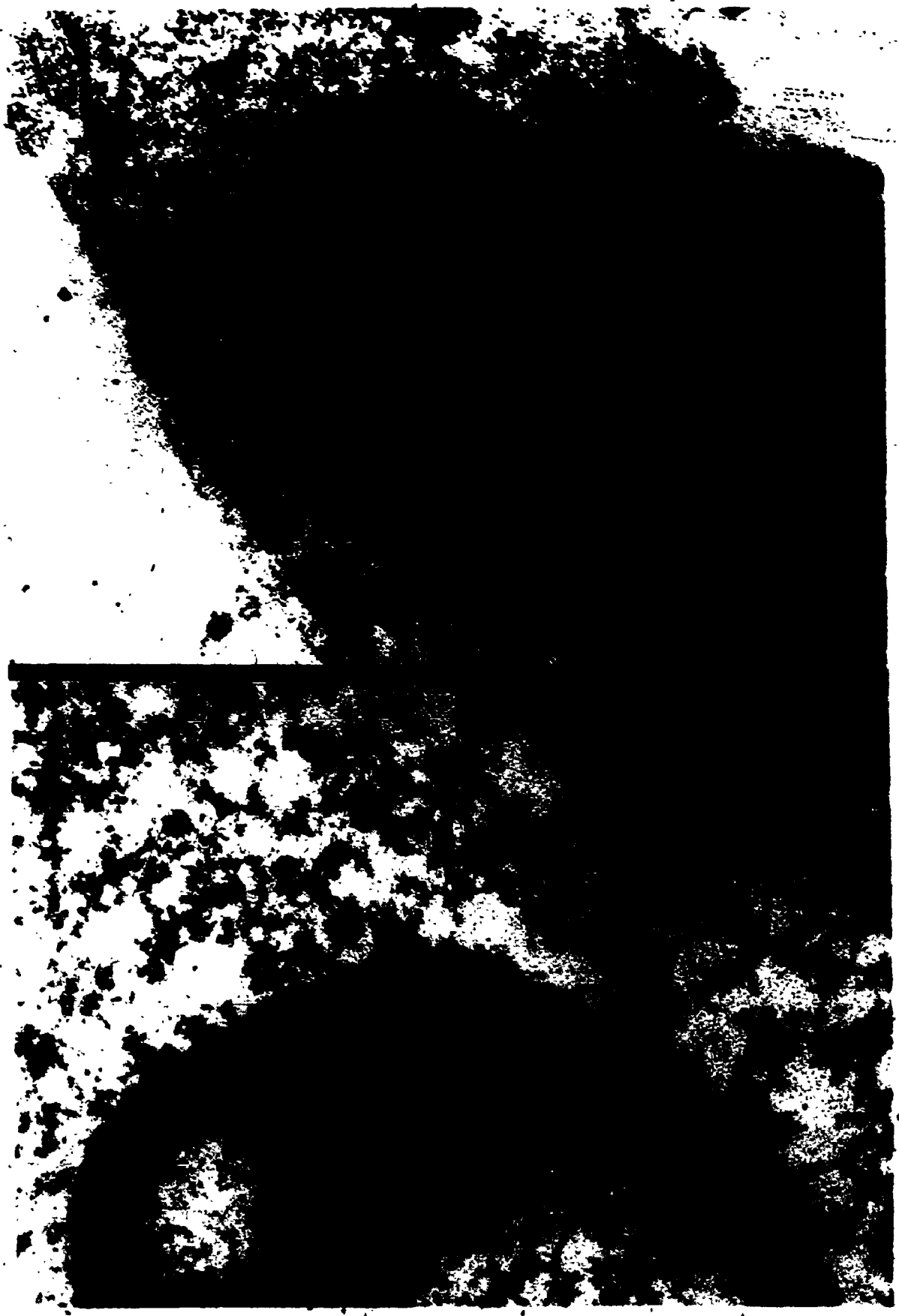


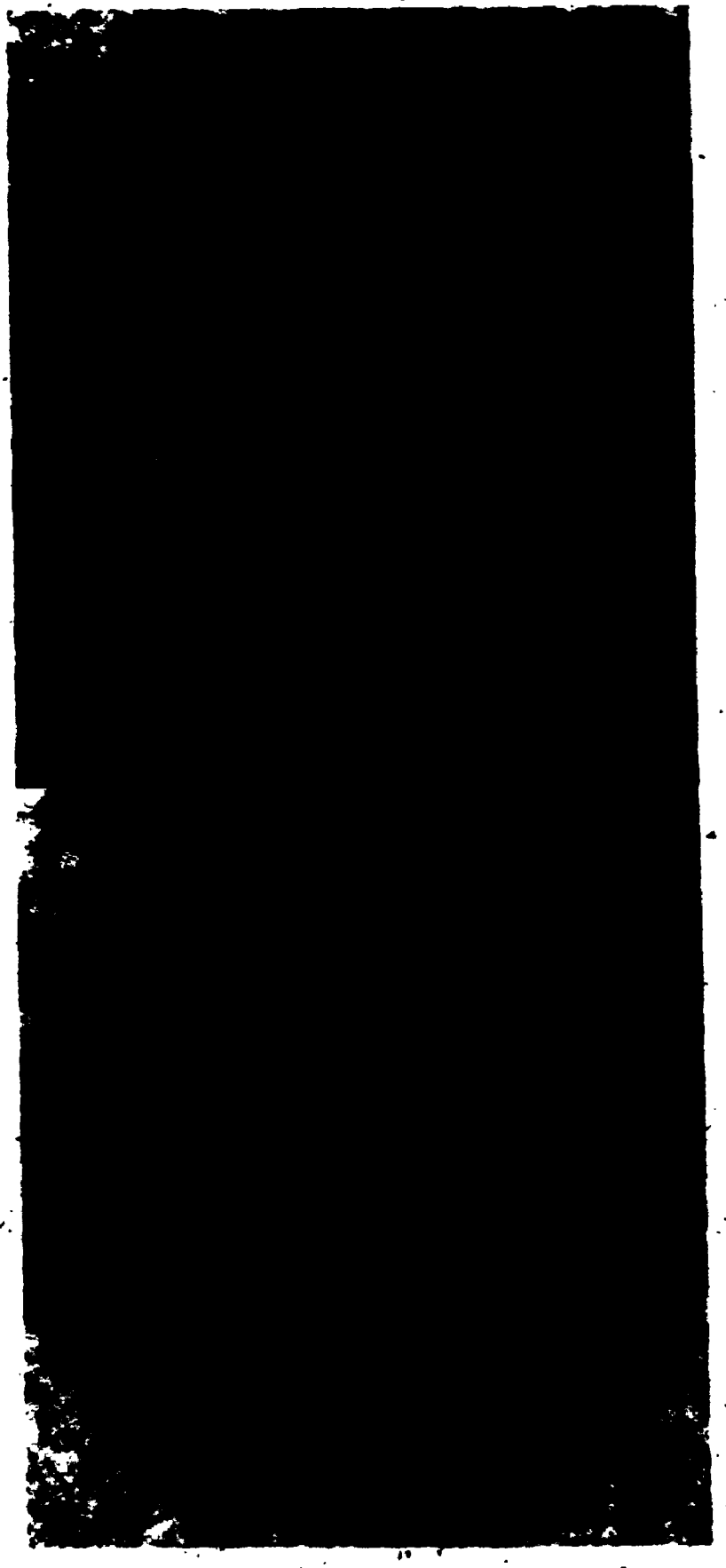
PLATE 24

Electron micrographs showing nucleolar structures and states observed.

The two micrographs allow a comparison of the appearance presented by the nucleolus when the nucleolus is in an apparent active state. They show an extensive dark dense granular region with an irregular margin; many small lacunae present; vacuoles present. In comparison the nucleolus is in an apparent less active or non-active state, showing a smooth round body of tightly packed fibrillar material with at most a narrow scattered dark rim of granular particles; with few or no lacunae present; none or small vacuoles present.

24 Upper Active. Maghification x 27,000.

24 Lower Relatively Inactive. Magnification x 27,000.



Early in this activity many small lacunae appear which frequently enclose fine chromatin threads. Small vacuoles start to appear, although at this stage they can be difficult to identify accurately. It is not easy to distinguish the vacuoles embedded in the granular grooves from the lacunae bordered by an accumulation of granular particles and embedded in fibrillar regions. These many small lacunae and presumably some small vacuoles appear as "moth-holes" in the dense granular nucleolus and lend a characteristic "motheaten" appearance to this stage. This is regarded as the active form when rRNA transcription is occurring along with the appearance of chromatin containing lacunae and the accumulation of dense ribonucleoprotein granules (Plate 24).

CONTINUOUS DARK SERIES:

In the continuous Dark series the above active state is observed at sampling points of 12-18 hours and 42-48 hours and is starting to reappear at 72 hours (Figure 6). The succeeding points of 24 hours and 54 hours show variable remains of this activity designated (active) in plate legends. In these, more of the tightly packed fibrillar zone is visible along with some residual RNP granules. Large vacuoles containing mixtures of fibrils and granules may be seen. Alternatively the remains of vacuoles may be observed

aligned with the periphery of the nucleoli. The transcription activity is followed by a stage of active transport during which products are moved out of the nucleolus. The data here indicate this is a time of increase in size and activity of vacuoles. By 30 hours and 60 hours dark, and similar to 0 hours dark, the non-active state of the nucleolus returns. It again becomes a compact predominantly fibrillar body with loss of the RNP granules and loss of active lacunae and vacuoles. Hence, marked and rhythmic changes are found in the nucleolus of nuclei of the continuous Dark series, as seen in Figure 6 and Plates 27 to 30.

CONTINUOUS LIGHT SERIES:

Throughout all sampling points of the continuous Light series the nuclei have nucleoli which are predominantly non-active or show a low degree of transcriptional activity. The body of the nucleolus is compacted and fibrillar with a few scattered RNP granules on the periphery and no active lacunae or vacuoles in evidence. However, a low percentage of nucleoli are observed to be somewhat more active. These have a narrow granular rim with some granules scattered in the interior, and also many small lacunae, some containing definite chromatin threads. This is observed at 12 hours and to a lesser degree at 18 hours continuous Light. There is also evidence of cellular

activity. The active state occurs across the 42 hour period with vacuoles remaining in the nucleoli by 54 hours. This activity is of a lesser degree and much less clear cut than that of the continuous Dark series. Hence, again there appears to be two populations of cells; the majority with nucleoli relatively inactive while those of the remainder are more active. This activity appears to cyclic or rhythmic when compared with the continuous Dark series (Figure 7 and Plates 25 and 26).

LIGHT-AFTER-DARK (INDUCED) SERIES:

For all sampling points in the Light-after-Dark (induced) series the cells have nuclei with active nucleoli. The compacted fibrillar zone is surrounded by and interspersed with large areas of ribonucleoprotein particles. The granular zone is dense around the rim of the nucleolus as well as extending through the interior. Within the fibrillar zone and often surrounded by granules are many lacunae of various sizes, containing fine fibrils. Small vacuoles are also observed in the granular zone although, in some regions, these are difficult to distinguish with certainty from lacunae.

All sampling points in the L/D series have active nucleoli of the above type with 48L/12D showing even greater activity than at 36L/12D. Those samplings in the

L/48D series show the same consistent form as that above and confirm the results of the L/12D (Figure 8) (see Plates 32 and 33).

LIGHT-AFTER-DARK (NON-INDUCED) SERIES:

For early sampling points - 12L/24D, 24L/24D in the Light-after-Dark (non-induced) series the nuclei have nucleoli which consist of fibrillar material with few RNP granules at the rim. A few small lacunae, some containing chromatin threads are visible. However by the end of the series (48L/24D) the nucleolus appears dense and dark from a heavy accumulation of RNP granules. The remains of a few large lacunae or vacuoles are visible while in others many small lacunae/vacuoles are evident.

Hence the early part of the series (12L/24D) (24L/24D) shows nucleoli of the inactive type but by the end of the series (48L/24D) the nucleoli are involved in active transcription (Figure 8 and Plate 31).

ELECTRON MICROGRAPHS SHOWING NUCLEOLI FROM SEEDLINGS WHICH HAVE UNDERGONE THE VARIOUS EXPERIMENTAL TREATMENTS DESCRIBED IN CHAPTER 2 and CHAPTER 3: B. MATERIALS AND METHODS c) i) - iii).

PLATES 25 to 33 INCLUSIVE.

PLATE 25

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii) .

Continuous Light Series.

25 Upper 0 hours Light treatment (point at start of treatments).
- Non-Active. Magnification x 27,000.

25 Lower 12 hours Light treatment.
- Active. Magnification x 45,000.

This state is discussed under Chapter 4: C(1) Results Nucleolus: Continuous Light Series.

PLATE 26

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Light Series.

26 Upper — 12 hours continuous Light treatment. This state is discussed under Chapter 4: C(1) Results Nucleolus: Continuous Light Series. Magnification x 27,000.

26 Lower — 42 hours continuous Light treatment. - Non-Active. Magnification x 27,000.

PLATE 27

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

27 Upper 12 hours continuous Dark treatment. - Active. Magnification x 78,000.

27 Lower 24 hours continuous Dark treatment. - (Active). Magnification x 27,000.


3
1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8
MICROCOPY

PLATE 28

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

28 Upper 30 hours continuous Dark treatment.
- Non-Active. Magnification x 27,000.

28 Lower 42 hours continuous Dark treatment.
- Active. Magnification x 27,000.

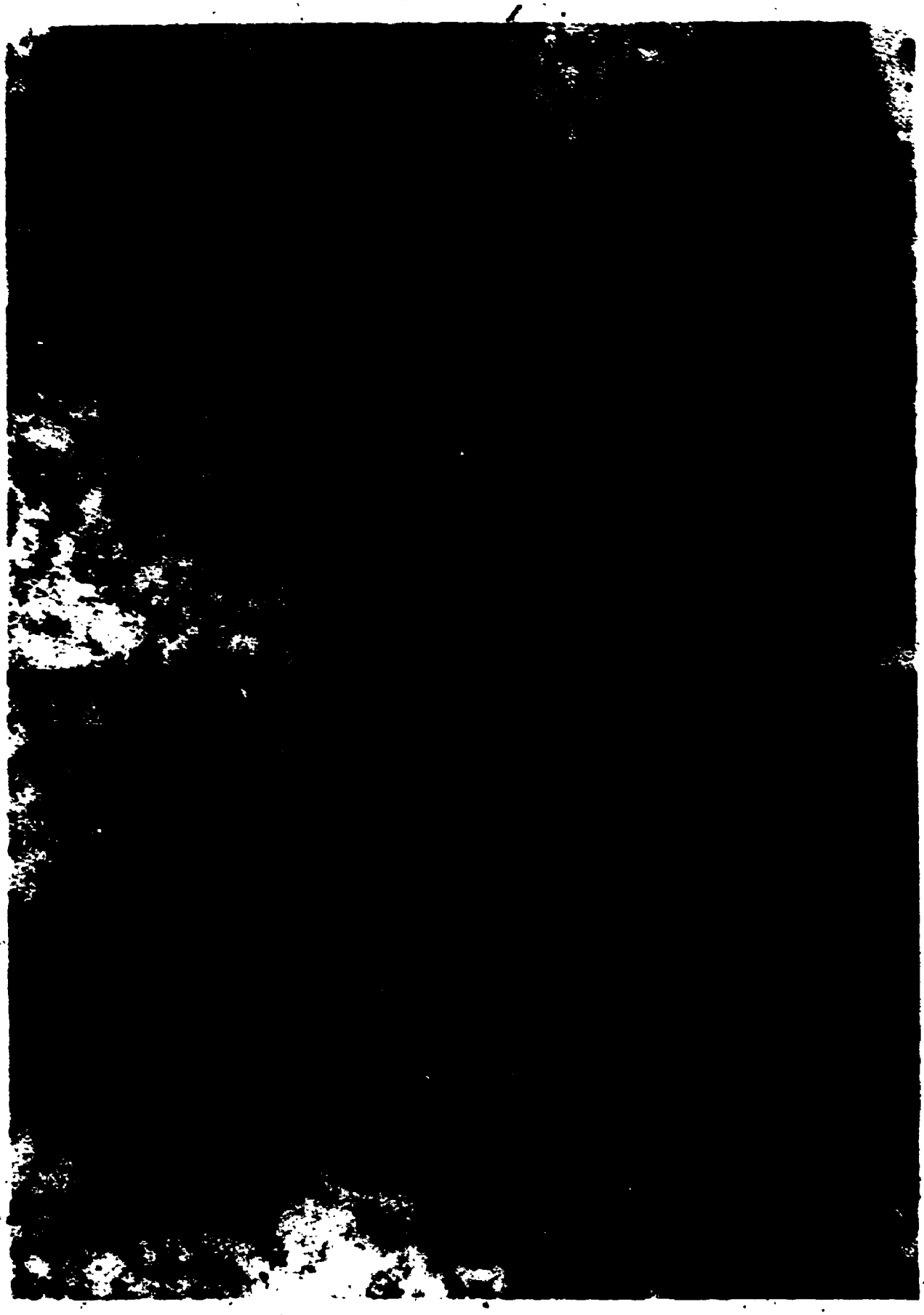


PLATE 29

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

29 Upper 54 hours continuous Dark treatment. - (Active). Magnification x 45,000.

29 Lower 66 hours continuous Dark treatment. - Non-Active. Magnification x 45,000.

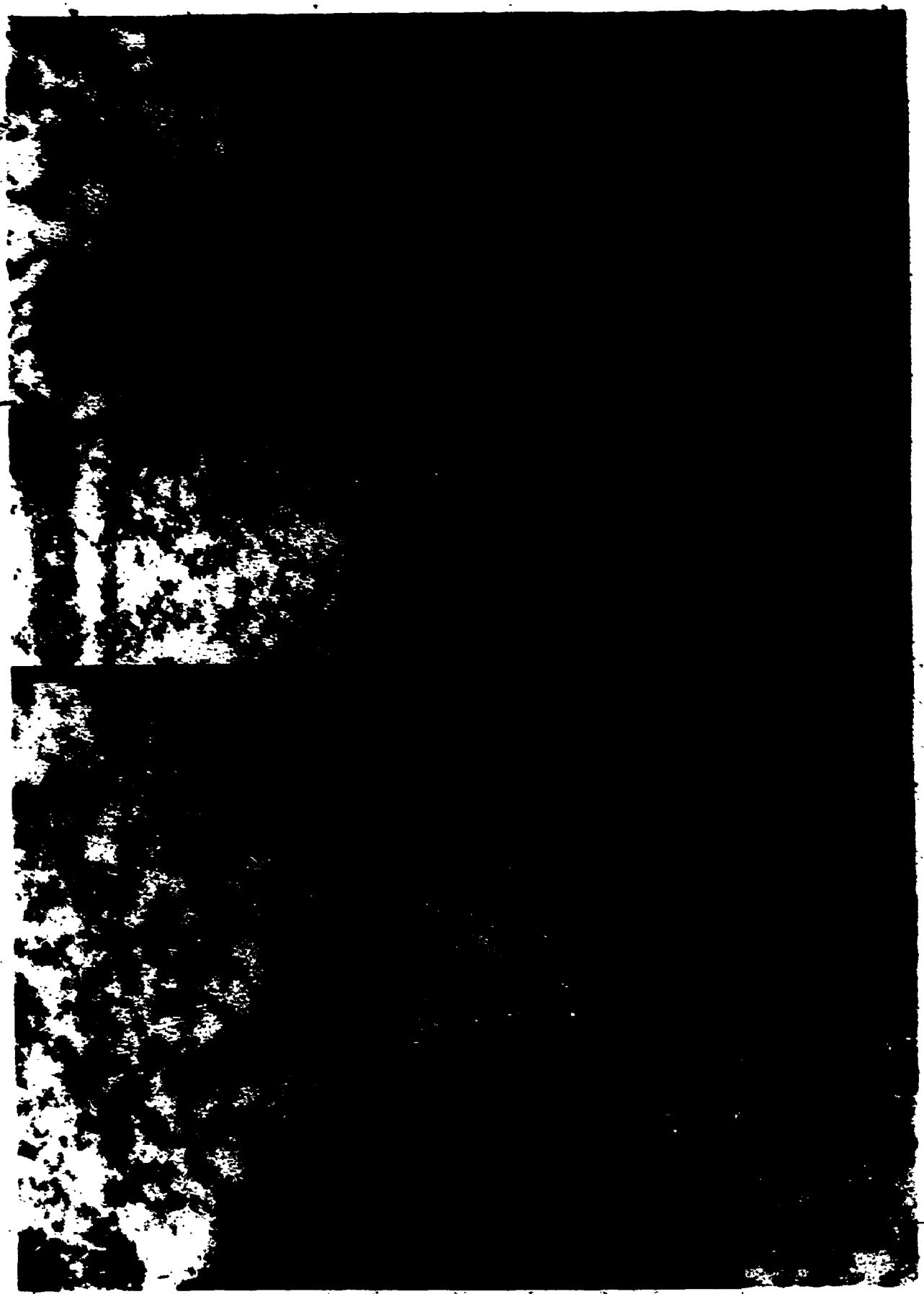


PLATE 30

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Continuous Dark Series.

72 hours continuous Dark treatment, the last of this series.

- Active. Magnification x 45,000.

PLATE 31

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Light-After-Dark Non-Induced (Vegetative) Series.

31 Upper 48 hours Light following 24 hours Dark treatment.
- Active. Magnification x 27,000.

31 Lower 12 hours Light following 24 hours Dark treatment.
- Non-Active. Magnification x 27,000.

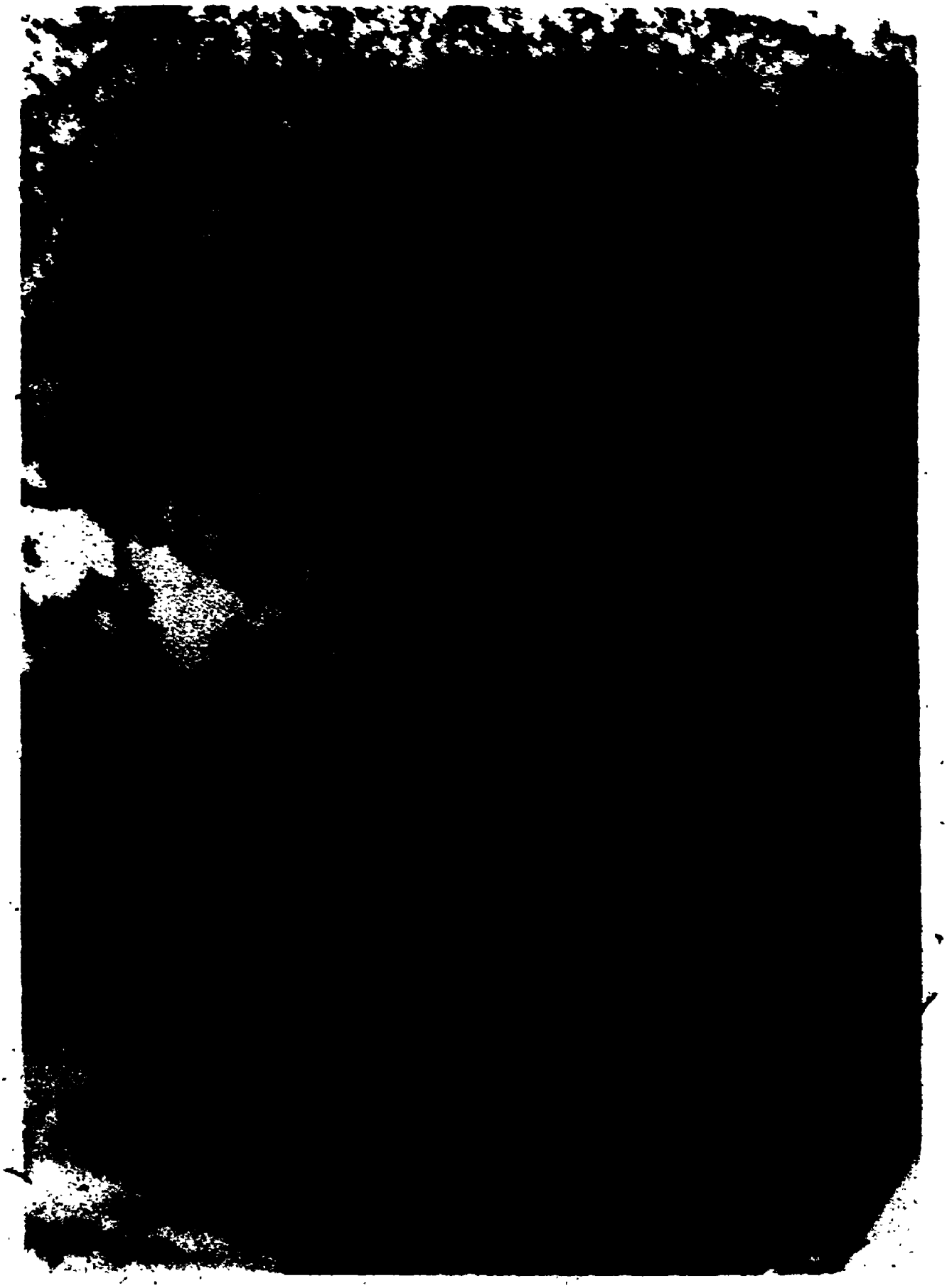


PLATE 32

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Light-After-Dark Induced (To Flower) Series.

32 Upper — 12 hours Light following 12 hours Dark treatment.
- Active. Magnification x 27,000.

32 Lower — 36 hours Light following 24 hours Dark treatment.

This is the seedling with the bacterial infection. The apex has been induced to flower in spite of the fact it has been exposed to the non-inductive 24 hours Dark.
- Active. Magnification x 27,000.

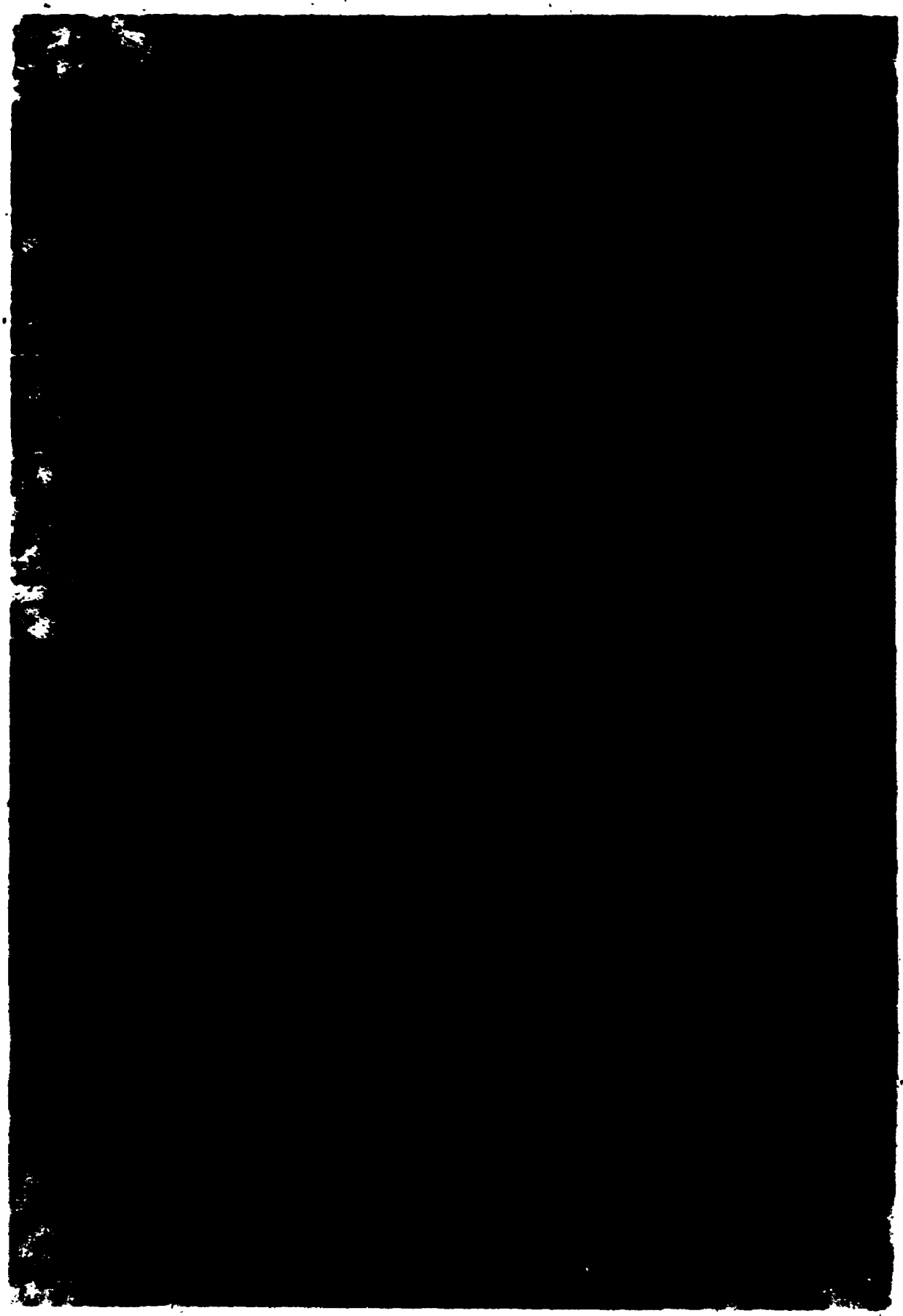


PLATE 33

Electron micrographs showing nucleoli from seedlings which have undergone the various experimental treatments described in Chapter 2 and Chapter 3: B. Materials and Methods c) i) - iii).

Light-After-Dark Induced (To Flower) Series.

33 Upper 36 hours Light following 48 hours Dark treatment.
- Active. Magnification x 27,000.

33 Lower 48 hours Light following 12 hours Dark treatment.
- Active. Magnifitation x 27,000.

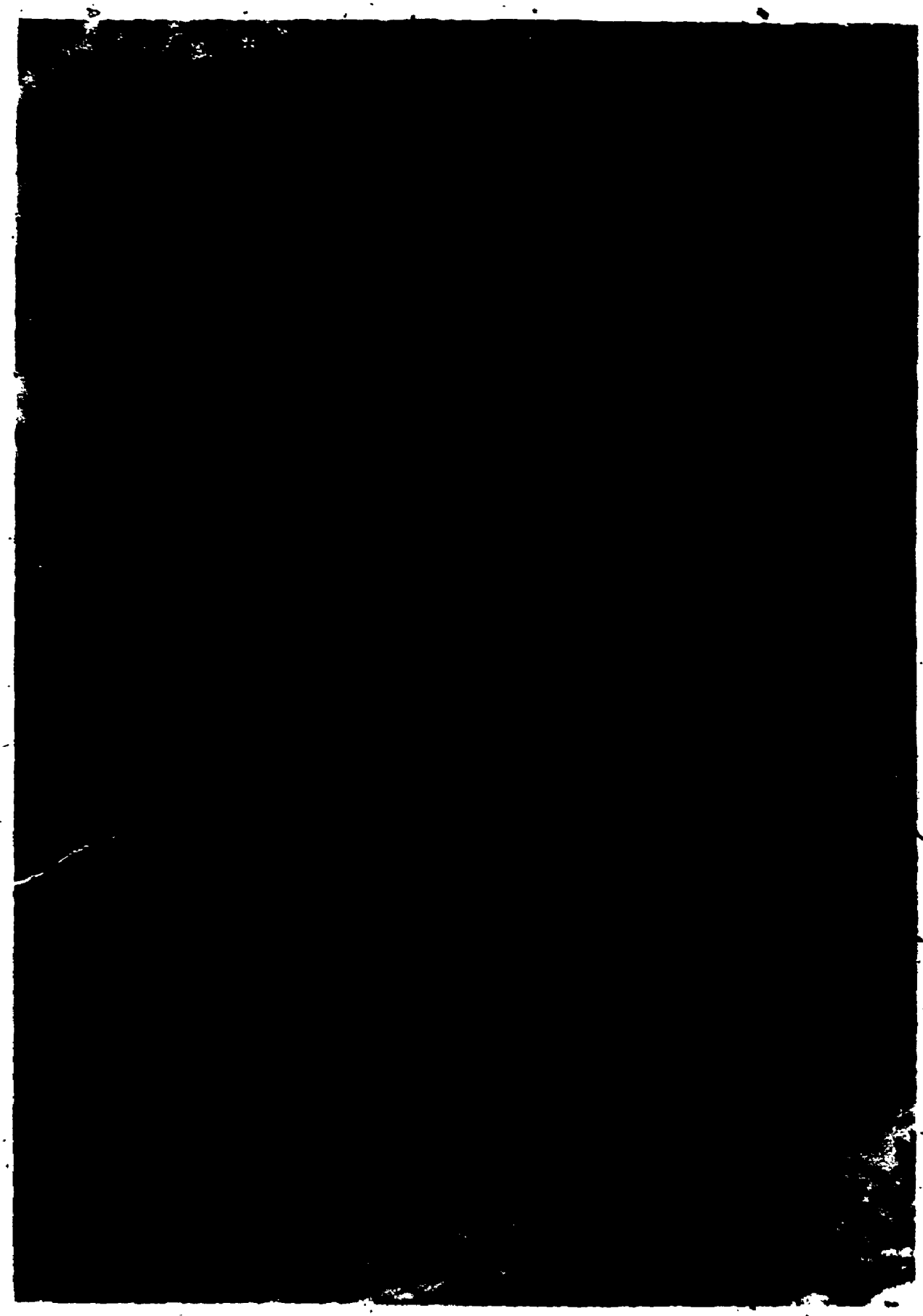


PLATE 34

An electron micrograph showing a nucleus from a lysed cell suffering overwhelming bacterial infection. The non-active nucleoplasm and nucleolus are completely depleted structures. Magnification x 12,600.

FIGURE 9.

Diagram to illustrate the changes in genetic activity with time, in C. rubrum, apex, first primordial leaf, and cotyledon tissues as explained in the text. Shown is the rhythm in the continuous Dark series and the relationship with that rhythm of the three Light-after-Dark series, the lines drawn here representing a third dimension horizontally back into the paper.

a) (——) continuous Dark series.

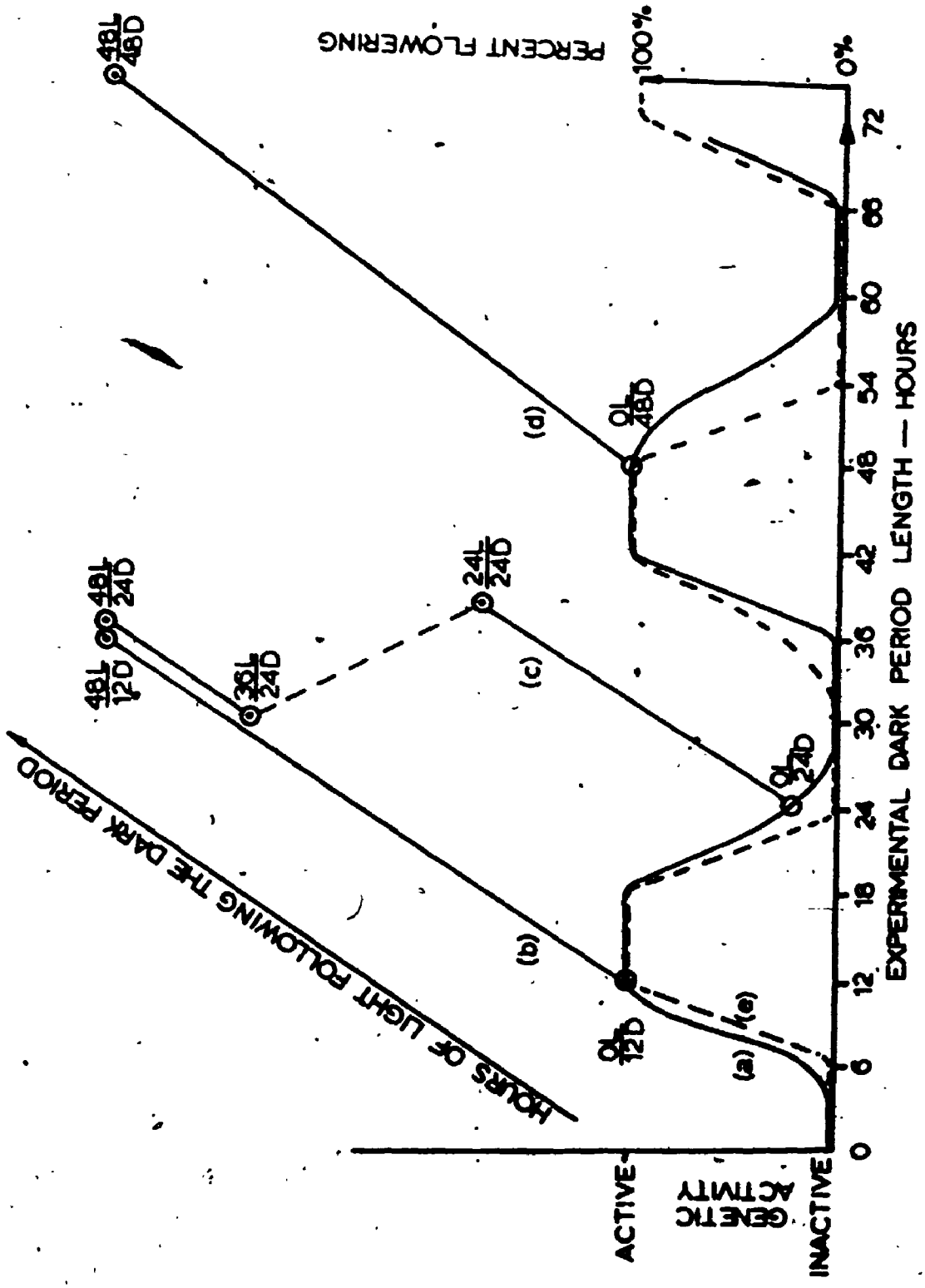
b) (——) Light-after-12 hours Dark series (induced - 100% flowering).

c) (— - -) Light-after-24 hours Dark series (non-induced - 0% flowering).

d) (—) Light-after-48 hours Dark series (induced - 100% flowering).

The endogenous rhythm of flowering is shown for comparison (see also Figure 2).

e) (- - -) Flowering response in the continuous Dark series.


PERCENT FLOWERING
100%
0%
72
68
60
54
48
42
36
30
24
18
12
6
0
EXPERIMENTAL DARK PERIOD LENGTH — HOURS
GENETIC ACTIVITY
ACTIVE
INACTIVE
HOURS OF LIGHT FOLLOWING THE DARK PERIOD
(a)
(b)
(c)
(d)
0L/12D
48L/12D
36L/24D
48L/24D
24L/24D
0L/24D
0L/48D
48L/48D

(2) PROTEIN SYNTHETIC ACTIVITY

(a) Electron Microscopy of Ribosomes, Polysomes, Membrane-bound Polysomes

Ribosomes are observed in all specimens as monosomes and as polyribosomes (polysomes) (Plate 35). The polysomes are found free in the cytoplasm or membrane-bound (m-b polysomes) to the endoplasmic reticulum (RER, rough endoplasmic reticulum) (Plate 36 and 37), or the nuclear envelope (NE) (Plate 39).

Specimens in which much cytoplasmic activity is apparent show the cytoplasm as dense and darkly staining, probably due to an accumulation of products. There is considerable density of monosomes and polysomes (Plate 40). It then becomes difficult to assess the proportion of monosomes to polysomes as only the end of a polysome chain might be seen. It is also virtually impossible to define all configurations. However, it is very clear that the ribosomes are not distributed in the cytoplasm at random. At best, in some specimens the section appears "clumpy" due to groupings of ribosomes possibly representing sections through helical polysomes or rosettes of four ribosomes in transverse section (Plate 41). In contrast some specimens appear "stringy", often with parallel strings (Plate 42). Frequently short strings or chains (4-8 ribosomes) appear to predominate. These configurations confer on the specimens either an overall "clumpy" (Plates 41, 43 and 45) or a "stringy"

ELECTRON MICROGRAPHS SHOWING RIBOSOME, FREE POLYSOME AND MEMBRANE-BOUND POLYSOME STRUCTURES OBSERVED.

PLATES 35 to 39 INCLUSIVE.

PLATE 35

Electron micrograph showing conformations of ribosomes observed in the cytoplasms. These are indicated by arrows. Starting with the uppermost, a helical polysome, then a monosome, a polysome string and lowest a polysome rosette of four visible ribosomes - possibly a transverse section through a helical polysome. An osmiophilic globule can also be seen on the edge of a colourless vacuole. Magnification x 78,000.

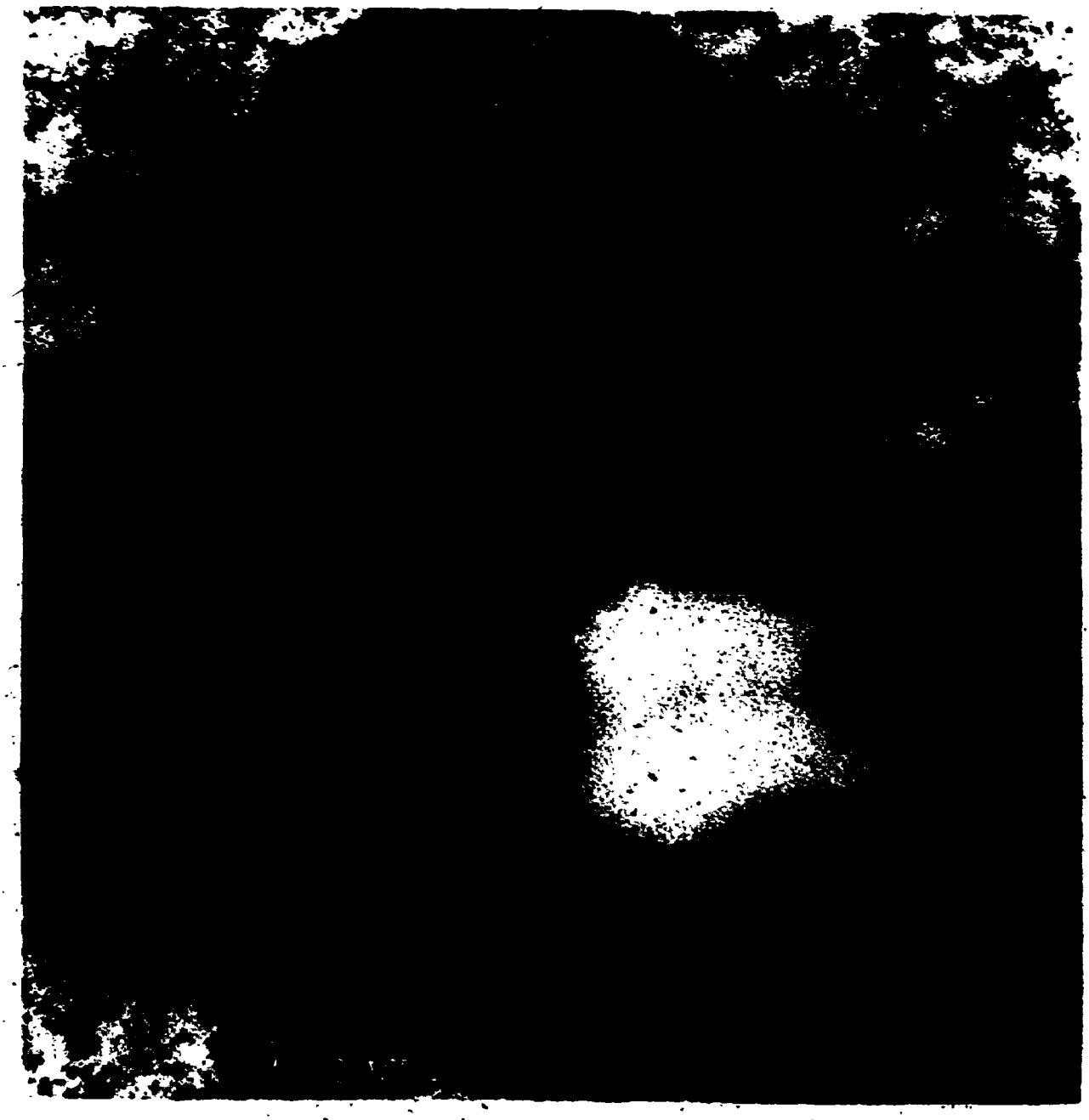


PLATE 36

Electron micrograph showing clearly membrane-bound polysomes on the endoplasmic reticulum. Stretches of rough endoplasmic reticulum (RER) are seen here parallel with the nuclear membrane and more sheets parallel with the plasma membrane. Magnification x 78,000.

PLATE 37

Electron micrograph showing a knot of rough endoplasmic reticulum (RER) resembling a "tangle of spaghetti". Polysomes are dense in this area. A neighbouring dictyosome is seen with three distinct cisternae and many vesicles at the forming face. Magnification x 181,000.

PLATE 38

Electron micrograph showing a rarely observed semi-tangential section of the rough endoplasmic reticulum bearing spiral polysomes. The cytoplasm is dense and darkly staining, probably with an accumulation of products, and with a considerable density of monosomes and polysomes. Magnification x 27,000.

PLATE 39

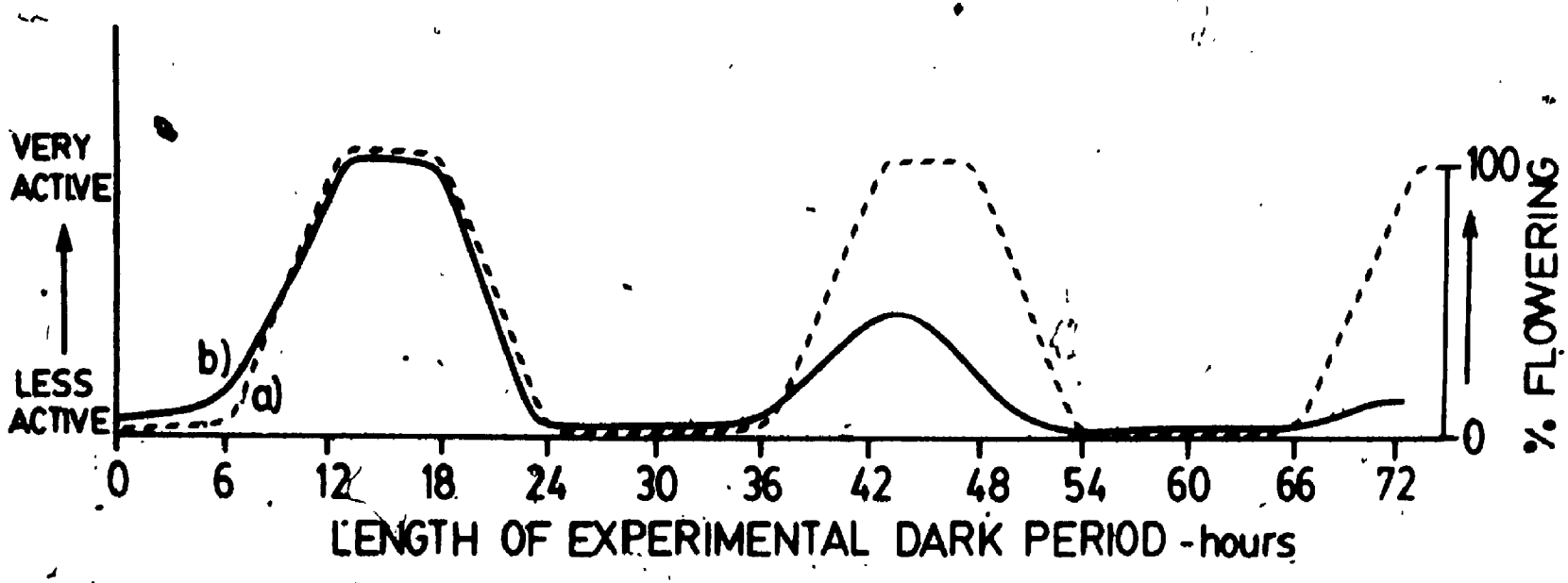
Electron micrograph showing nuclear membrane (NE) with many attached membrane-bound polysomes. Magnification x 27,000.

FIGURE 10.

Diagram to illustrate changes over time within the cytoplasm and the monosome and polysome compliment, during the continuous Dark period in c. rubrum, apex, first primordial leaf, and cotyledon tissues as explained in the text.

10 A The endogenous rhythm of flowering is shown for comparison (Figure 2). (---) Flowering response in the continuous Dark series.

10 B (——) Cytoplasm and monosome and polysome compliment in the continuous Dark period.


VERY ACTIVE
LESS ACTIVE
b)
a)
100
0
% FLOWERING
0 6 12 18 24 30 36 42 48 54 60 66 72
LENGTH OF EXPERIMENTAL DARK PERIOD - hours

appearance (Plates 42, 44 and 46). In some sections such appearances may be very dense, while in others much less so. Nevertheless, all sections have considerable populations of monosomes and polysomes, presumably in support of basic metabolism and at certain points in time the pattern becomes much more complex. Perhaps because of the consistent plane of sectioning of the apices (median longitudinal) it is very rare to obtain tangential sections across membrane in which attached polysomes may be seen and which give any hint of the conformation of the m-b polysomes (Plate 38).

Monosomes and polysomes were extracted from seedlings ground in buffer and the pellet obtained was examined a) by electron microscopy, b) in the Analytical Ultracentrifuge where sedimentation coefficients were calculated for the particles and finally c) polysome profiles were obtained by sucrose density gradient centrifugation. (Addendum II).

(b) Series

CONTINUOUS DARK SERIES:

Examination of ribosome configurations show two states - the less active state and a more active one. Considering first the less active state in the continuous Dark series, the difficulties in assessing the monosomes and polysomes are discussed above.

Monosomes are present in the cells of all specimens. Chains or strings are the predominant distinguishable form of polysomes, consistent to a greater or lesser degree in the cytoplasm of all cells of this series. Early in the series, small, tight clusters of ribosomes are seen, the more prominent being compact rosettes of four ribosomes. The overall impression is of associated ribosomes in clusters and strings and not at random. The cytoplasm and ribosomes of less active cells appear less dense and have an overall "stringy" appearance (Plates 42, 44 and 46). Polysomes are seen attached to the nuclear envelope. Short pieces of RER are mostly parallel to the plasma membrane but some are adjacent and parallel to the nuclear envelope. This is the picture of the less active state (Plates 42, 44, 46).

When the tissue becomes very active the cytoplasm itself appears to become much denser and is darker staining. Some monosomes are still present. There are many short polysome strings, some of which lie in parallel pairs. However, polysomes now exhibit a lot of varied clumps of the type seen above, including helical forms, "bunches of grapes" and other conformations free in the cytoplasm. Thus a very dense and complex population of ribosome configurations are observed. The overall picture is cytoplasm with ribosomes in a very "clumpy", very dense and very "busy" state - a lot of activity is evident (Plates 41, 43 and 45). Membrane-

bound polysomes appear on the RER which is present as many short lengths again distributed as above. In the very active cells are knots of RER resembling a "tangle of spaghetti" and these specific regions occur close to the plasma membrane or near the nuclear envelope (Plate 37). Polysomes can be very dense in these regions.

The cells of the cotyledons, primordial leaves and apex can be compared. The cytoplasm of the cotyledons of the less active state appear somewhat denser than those of the corresponding primordial leaf and the apex. However, the cytoplasm of active state cotyledon cells is extremely dense and markedly more so than that of the corresponding primordial leaves and apices. This is likely related to the fact that the cotyledon cytoplasm is highly vacuolate with the reduced, condensed cytoplasmic volume being spread around the periphery of the cell and bordering the huge central vacuole. Yet a large volume of this thin band of cytoplasm is also occupied by numerous, large chloroplasts. Although the cotyledon cytoplasm is denser than that of the corresponding primordial leaf and apex, the change in activity or status runs parallel and consistent between all three types of tissue.

The very active state is well seen at the sampling point 12 hours dark and is in contrast to less-active states at sampling points 6 hours and 18 hours on either side. A similar rise in activity is again seen at 42

hours in contrast to less active states at 36 hours and 48 hours. However, the rise in activity here is much less marked than that seen at 12 hours and is only clearly detectable when compared with adjacent sampling points of even lower activity. Similarly, a rise in activity also occurs at 72 hours, but with the stringy conformation making an increasing contribution. The less active state is evident at 24 hours and 30 hours, at 54 hours and 60 hours. (Figure 10).

CONTINUOUS LIGHT SERIES:

Throughout this series examination of the endoplasmic reticulum shows many membrane-bound polysomes. There are, however, many empty stretches of endoplasmic reticulum. The cytoplasm, the monosomes and the conformations (as discussed above) of free polysomes reveal a less active state. No clear pattern of relative change in activity is detectable.

LIGHT-AFTER-DARK (INDUCED) SERIES:

In the series having 12 hours dark followed by sampling after 12 hours, 24 hours, 36 hours and 48 hours of subsequent light very active conformations are seen in the cells at all sampling points. There are similarities with the preceding 12 hours dark (as described above). The cytoplasm is dense and there are large numbers of intricate ribosome conformations as

well as ribosome strings. Throughout this series the sections have a very busy, active "clumpy" appearance. The parallel induced series L/48D has a similar appearance at all sampling points and confirms the above results. As previously noted, non-infected seedlings of the L/24D series are vegetative, while the L/24D bacterially infected seedling is induced to flower (Addendum VI). It is of note that the cells of this seedling are similar in appearance to the corresponding cells of the L/12D and L/48D flowering series above.

LIGHT-AFTER-DARK (NON-INDUCED) SERIES:

In this series seedlings have been exposed to 24 hours dark followed by light and sampled at 12 hours, 24 hours, 36 hours and 48 hours light. The cells of the first sampling at 12L/24D are packed with ribosomes, many of which are monosomes. Strings of polysomes predominate giving the sections an overall stringy and busy appearance. While general cell conformations show similarities with the preceding 24 hour dark samples there is a lot more activity with tightly packed polysome strings. At 24L/24D, strings of polysomes are much in evidence and now more complex polysome configurations are mixed among the strings. By 36L/24D and 48L/24D the cytoplasm has a "clumpy", very active

appearance and is indistinguishable in appearance from comparable cells of the above Light-after-Dark (induced) series.

ELECTRON MICROGRAPHS SHOWING RELATIVE MONOSOME, POLYSOME MEMBRANE-BOUND POLYSOME AND CYTOPLASMIC STATES OBSERVED

PLATES 40 to 45 INCLUSIVE.

PLATE 40

Electron micrograph showing the cytoplasmic status referred to as "clumpy" in the text. This status is labelled very active. Different polysome conformations are indicated by three arrows. Magnification x 45,000. This Plate is to be compared with Plate 41.

PLATE 41

Electron micrograph showing the cytoplasmic status referred to as "stringy" in the text. This status is labelled less active. Three arrows indicate a monosome and two polysome strings. Magnification x 45,000. This Plate is to be compared with Plate 40.

PLATE 42

Electron micrograph showing low power appearance of very active status of cytoplasm. Many complex clumpy polysome conformations are seen. Magnification x 27,000. This Plate is to be compared with Plate 43.

PLATE 43

Electron micrograph showing low power appearance of less active status of the cytoplasm. Monosome and polysome strings predominate. Magnification x 27,000. This Plate is to be compared with Plate 42.

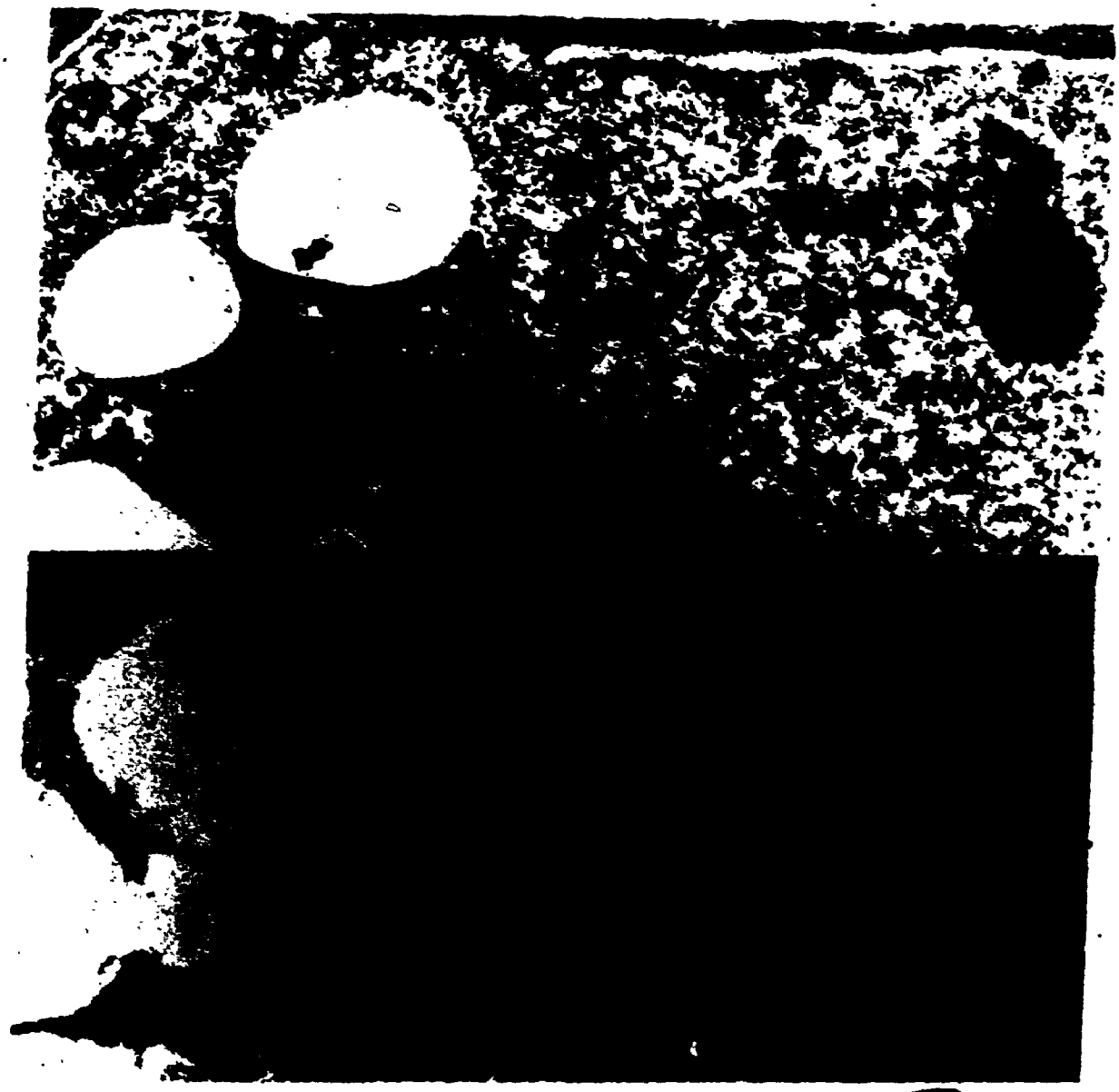


PLATE 44

Electron micrograph showing high power detail of a very active status of the cytoplasm. Note the dense staining and the complex conformations of the polysomes as marked by two arrows. Magnification x 181,000. This Plate is to be compared with Plate 45.

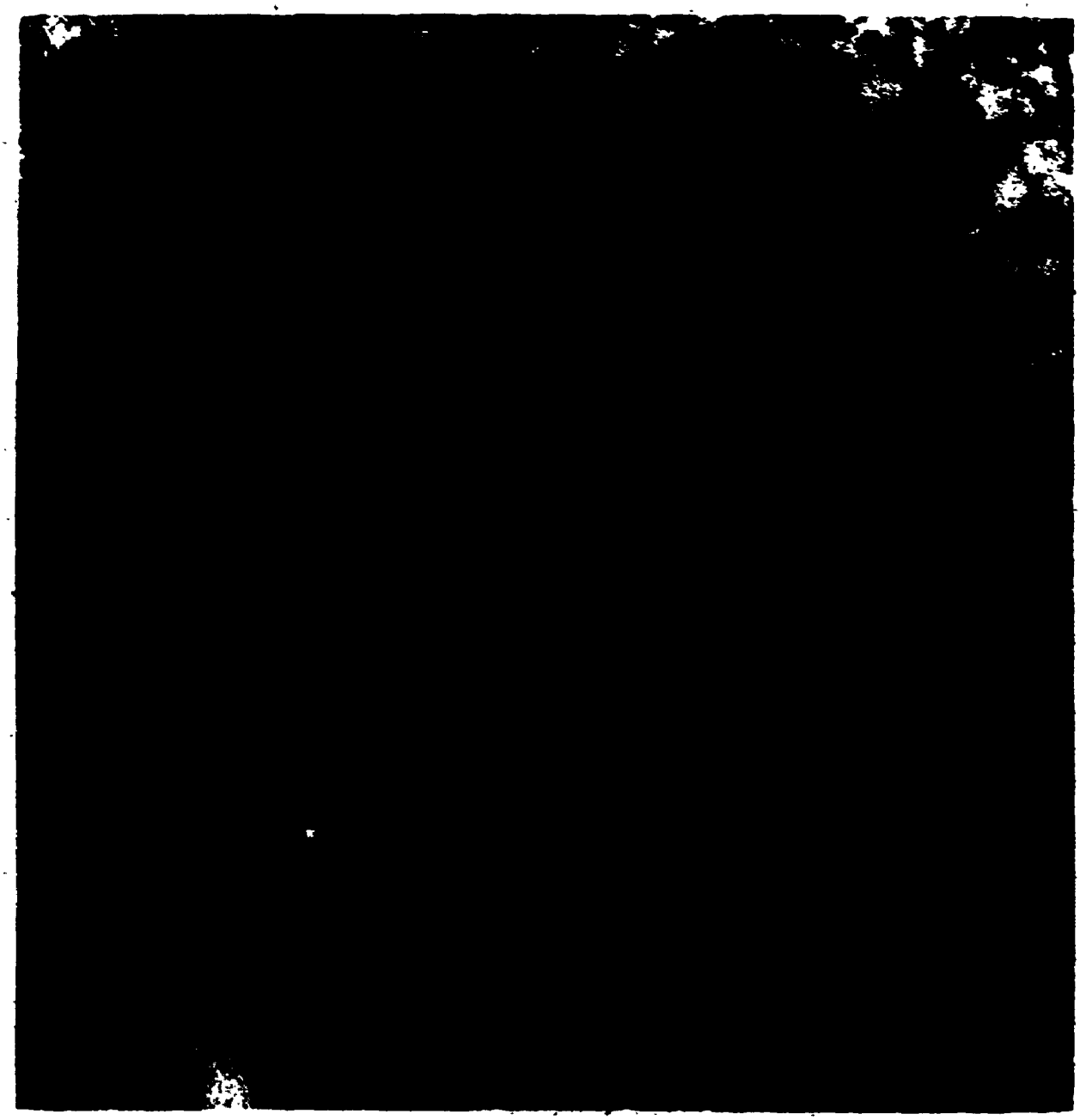


PLATE 45

Electron micrograph showing high power detail of a less active status of the cytoplasm. Note the much paler staining and less dense material seen compared with Plate 44 even although the dense blackness of the cell walls along one edge of plate is similar. Monosome and polysome strings predominate (as marked by three arrows) and the appearance presented is much simpler, less busy and less active. Magnification x 181,000. This Plate is to be compared with Plate 44.

(3) MEMBRANE ACTIVITY

(a) Electron Microscopy of the Nuclear Membrane, Endoplasmic Reticulum, Golgi Bodies and Plasma Membrane

All seedling specimens show evidence of some membrane activity. The nuclear envelope (NE) clearly surrounds all nuclei. Nuclear pores are found and the membrane of the envelope often bears ribosomes.

In some sections, the rough endoplasmic reticulum (RER) appears in the vicinity of the nuclear membrane or near the plasma membrane as short segments (Plate 46). In other specimens long sheets are observed in similar regions, more frequently parallel to the plasma membrane (Plate 36). Smooth endoplasmic reticulum (SER) is never in evidence.

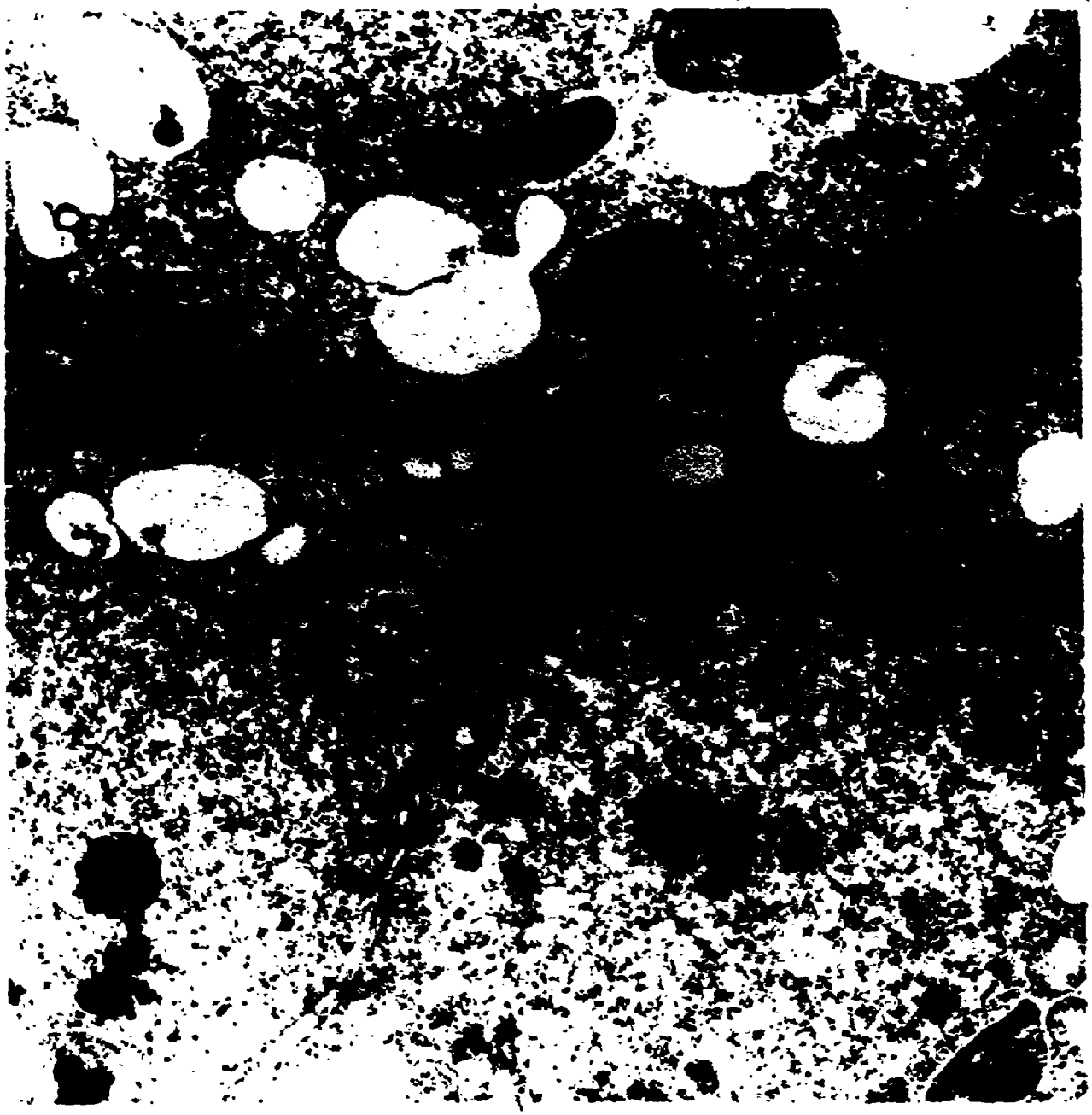
Golgi apparatus is visible in many cells and dictyosomes most frequently consist of three distinct cisternae in addition to the forming face (Plate 50). Sometimes the vesicles budding off the cisternae stain darkly and are full of an osmiophilic secretion (Plate 49). They are observed in transit to and at the plasma membrane (Plate 49, 50). The plasma membrane in turn incorporates additional membrane (Plate 46, 49) and by exocytosis expels the vesicle's contents between the plasma membrane and the cell wall.

ELECTRON MICROGRAPHS SHOWING MEMBRANE STRUCTURES OBSERVED.

PLATES 46 to 52 INCLUSIVE.

PLATE 46

An electron micrograph showing membrane structures observed and their relative positions and relationships within the cell. The membranes of the nuclear envelope (NE) are visible. Short lengths of rough endoplasmic reticulum (RER) are present beside the nuclear envelope and alongside the plasma membrane. Dictyosomes (D) are present and the vesicles lie between that and the plasma membrane. Two vesicles are seen to have arrived at the membrane. Several plasmodesmatta (Pd) cross the cell wall. Magnification x 16,000.



6)

PLATE 47

An electron micrograph showing a rare continuity of the endoplasmic reticulum (RER) with the plasma membrane (PM) seen between the two labels. Magnification x 16,000.

PLATE 48

An electron micrograph showing membrane.

Left: Seen are lomasomes as indicated by arrows. Vesicles are seen stradling the plasma membrane or the outside in the space between the plasma membrane and the cell wall.

Right: Two dictyosomes (D) are seen and so is a "spaghetti tangle" in the endoplasmic reticulum (RER).

Magnification x 45,000.

PLATE 49

The electron micrograph shows -

Upper - a dictyosome (D) with the vesicles filled with a densely staining material and ready to bud off.

Lower - a dictyosome (D) with the vesicles scattered across to the plasma membrane, until they become associated with the plasma membrane.

Magnification x 45,000.

PLATE 50

The electron micrograph shows high power detail of the membrane and cisternae of a dictyosome (D). The vesicles (Ve) associated with the dictyosome are seen between it and the plasma membrane. Further vesicles of a different origin are seen at the forming face (FF). Magnification x 181,000.

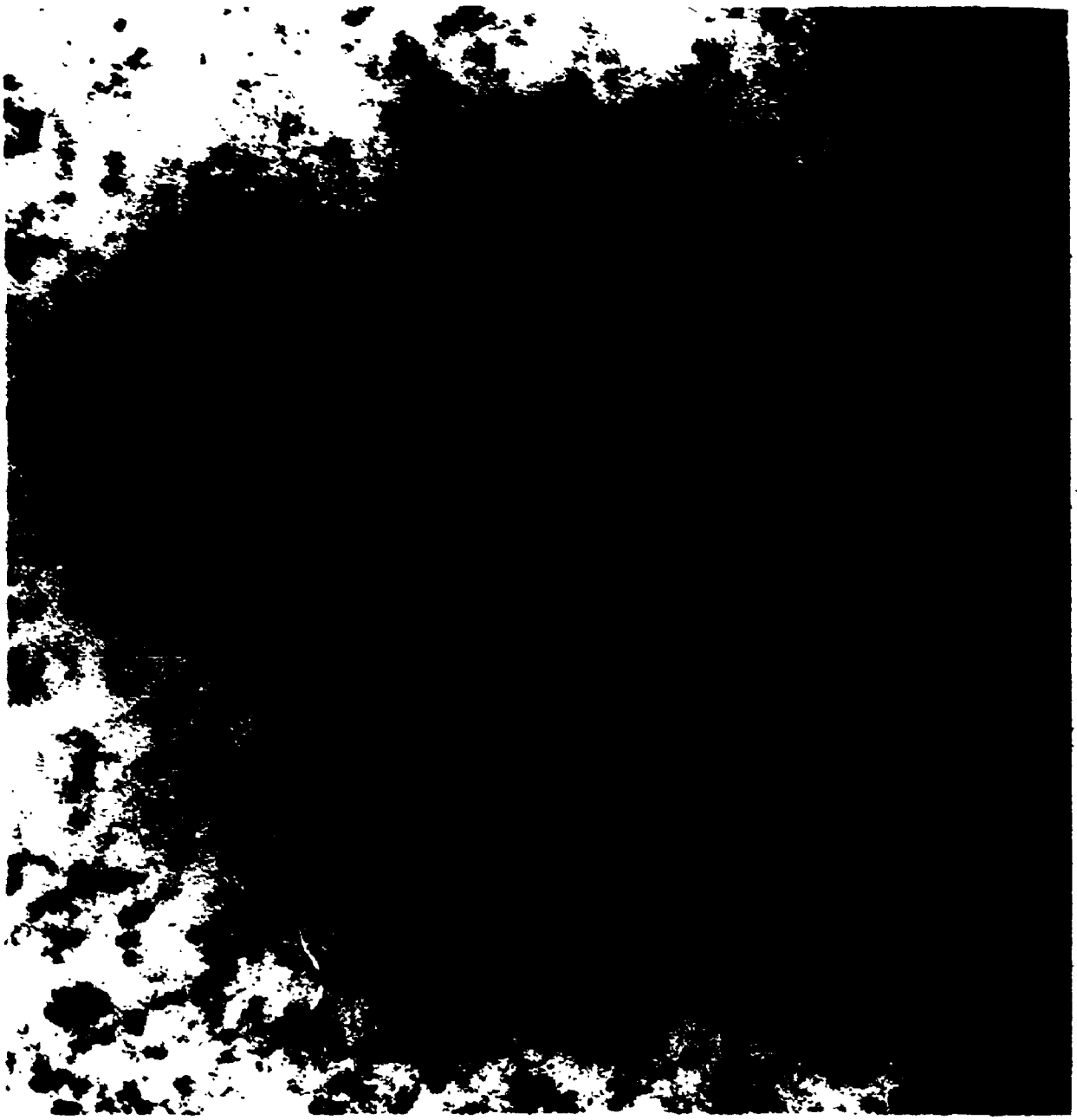


PLATE 51

The electron micrograph shows extensive endoplasmic reticulum (RER) and some areas are distended and filled with an osmiophilic substance. These areas are seen on both sides of the cell wall. Magnification x 27,000.

PLATE 52

The electron micrograph shows large osmiophilic globules (OG), two between the plasma membrane and the cell wall and one on the edge of a vacuole. The swollen empty cisternae of the endoplasmic reticulum are to be noted. Magnification x 45,000.

Distinct from these small vesicle contents, large osmiophilic globules are sometimes observed in this same region but are more frequently seen extruded into the vacuoles (Plate 52). They are membrane-bound and until the membrane breaks they retain their spheroid shape. There are no indications that these osmiophilic globules arise from the Golgi bodies. However, they are seen associated with large empty cisternae of the endoplasmic reticulum (Plate 51).

(b) Series

In view of the cellular activity of these seedlings, the nuclear membrane is undoubtedly active. Nevertheless, no direct nuclear envelope membrane activity is observable (For nuclear envelope membrane-bound polysomes, see previous section (2) Protein Synthetic Activity).

As described above, the RER is frequently seen as short sections in the region of the plasma membrane or the nuclear envelope in all series. A most unusual connection with the plasma membrane is observed in Plate 47. Lengths of SER are not seen in these cells. In very active cells, the RER is frequently noted to have proliferated and "spaghetti like" tangles of membrane result (Plate 37 and 48). This is observed clearly in 12 hours Dark, to a lesser extent in 42-48 hours Dark

specimens but seem to be very vigorous in all of the Light-after-Dark series. Specimens taken close to the end of all series show longer stretches of RER distributed as above,

The Golgi apparatus is active throughout all series and frequently several dictyosomes are seen in each section of the cells. In the Light-After-Dark series many more are observed. However, these are too few in number to be able to compare or conclude any quantitative changes in the continuous Dark or the continuous Light series. The dictyosomes are active. Sometimes dense osmiophilic contents fill the cisternae and the budding off process is visible (Plate 49). Occasionally small membrane-bound vesicles bud off and other similar ones are observed in transit to the plasma membrane. There is an increase in the amount of plasma membrane as a result of membrane contributions from vesicles. Vesicle contents are observed outside the plasma membrane in the space between that and the cellulose cell wall (Plate 48). These small vesicle contents now qualify as lomasomes and presumably they are involved in cell wall synthesis. Hence, in all the series the dictyosomes show secretory activity. Sections through the exact regions with this activity are relatively rare and hence cannot be used to compare the activity of specimens of different series.

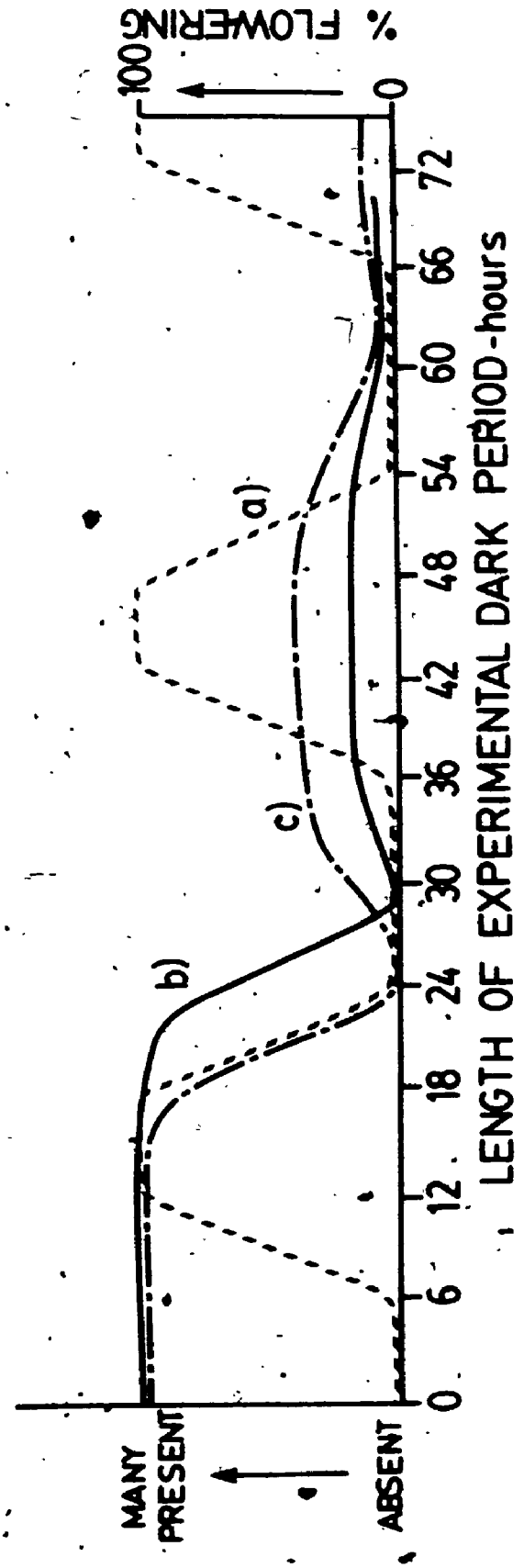
FIGURE 11

Diagram to illustrate the changes over time of osmiophilic globules and osmiophilic material in the distended cisternae of endoplasmic reticulum. These changes were observed in the cytoplasm of c. rubrum during the continuous Dark and the continuous Light period.

11 A The endogenous rhythm of flowering is shown for comparison (Figure 2). (---) The flowering response in the continuous Dark series.

11 B (——) Presence or absence of osmiophilic material during the continuous Dark series.

11 C (—·—) Presence or absence of osmiophilic material during the continuous Light series.


% FLOWERING
100
0
72
66
60
54
48
42
36
30
24
18
12
6
0
LENGTH OF EXPERIMENTAL DARK PERIOD-hours
a)
b)
c)
MANY PRESENT
ABSENT

CONTINUOUS DARK SERIES:

On examining sampling points many osmiophilic globules are seen and some cisternae of the endoplasmic reticulum are distended with osmiophilic material early in the series from 6 hours, 12 hours, 18 hours and 24 hours. However, by 30 hours all globules are gone and there are no osmiophilic, swollen cisternae. Thereafter the ER is often distended and full of the osmiophilic material but few globules are seen. Indeed, by 60 hours there are no globules in evidence and no osmiophilic material in the ER. From 60 hours through to the final 72 hour sample only very little osmiophilic material is observed in the ER and virtually no osmiophilic globules (only one) are seen (Figure 11).

CONTINUOUS LIGHT SERIES:

Sampling points show a similar pattern of events to that described above. However, all globules are gone and by the 24 hour sampling point there are no cisternae swollen with osmiophilic material. The 60 hour point again has cells with all osmiophilic material absent. In sections sampled between 24 and 60 hours globules are still seen and a lot of osmiophilic material fills in the swollen sections of ER. Points after 60 hours have this ER activity again, but no globules are seen (Figure 11).

LIGHT-AFTER-DARK SERIES:

Both of these series show much membrane activity in the proliferation of the ER, particularly in the "spaghetti tangle" forms described above. There is also much Golgi activity near the end of the series since there are more dictyosomes present. There are many osmiophilic globules and a lot of osmiophilic material in the cisternae. There is more of this osmiophilic material with time. The L/12D (induced) series shows more vigour, with greater accumulations of osmiophilic material than the L/24D (non-induced) series. However, the same processes occur and the same materials accumulate in both series.

D: DISCUSSION

Ultrastructure of plant shoot apices has been examined mostly to elucidate the structure of the vegetative apex or to elucidate the structure of the induced apex. Only a few investigations have also studied changes occurring from induction of the vegetative apex through to evocation events. None have examined the ultrastructural changes throughout the dark period of photoperiodically induced apices. No effort has been made to correlate ultrastructural changes with the changing phases of the endogenous rhythm of flowering. Neither has work been done on the relative metabolic state of apices capable of induction nor on the factors controlling the ability to induce or not induce those apices. In this study examination of the ultrastructural and associated biochemical changes correlated with the endogenous rhythm, of which flowering acts as a marker for the phase of that rhythm.

Ultrastructure of apical cells, primordial leaf cells and cotyledons have been examined. These have been assessed in terms of whether they are active, relatively inactive or just less active. In comparing seedling cell structures throughout the series, disappearance of a structure indicates decrease or cessation of that activity. Alternatively, if products are still present

and processing then the cell may be categorized as inactive. Elements or particular structures may become sparse, or the overall picture may become one of simplification. This is seen when cytoplasm monosome-polysome patterns are assessed. Complex polysome configurations which lend a complex appearance to the cytoplasm, resolve to monosomes with predominant polysomes present as strings. Thus, the polysome pattern shows considerable simplification. Evident activity changes from a multiform complexity to a simple uniform activity and this new activity is classified as "less active". Considerable activity remains, but the level of complexity of functions is much reduced and thus "less active". The activity in the nucleoplasm also further illustrates a marked change in activity and is regarded as relatively "inactive". The categories "less active" or "relatively inactive", do not refer to absolute activity insofar as it is recognized that the tissue is still functioning and basal metabolism will continue. It is the change in this state that is discussed and the changes are all relative ones.

The results obtained are also seen in the diagrams of Figures 6, 7, 8, 9, 10 and 11. During the dark period the transcriptional activity (Figure 6) of the diffuse chromatin in the nucleus parallels the genetic activity of the nucleolus, both of which approximate the curve of the flowering rhythm. At those sampling points where

seedlings can be induced to flower the cells are genetically active and active transcription is occurring in both the diffuse chromatin in the nucleoplasm and also in the nucleolus. A lag in the two curves relative to the flowering rhythm curve is seen between sampling points of 24 hours and 30 hours, 54 hours and 60 hours. During these periods the nucleolus has decreasing numbers of ribonucleoprotein granules, although quantities still remain, and many active nuclear vacuoles are seen. There is a decrease in the number of lacunae observed and an increase in the fibrillar area seen. In fact this is a period during which transcription has ceased and active transport of granules is occurring as seen by the presence of active vacuoles and a decreasing pattern of granules. Here the results have been presented strictly according to the data. Ribonucleoprotein granules are still present and the vacuoles of the nucleolus still "active". Hence the nucleolus is classified as still being "active" and the diagram drawn accordingly. If genetic activity in the nucleolus is defined in terms of transcription activity with active production of r-RNA and ribonucleoprotein granules, then the curve of genetic activity would approach the flowering curve. At 24 hours both would approach zero.

The diffuse chromatin in the nucleoplasm follows a similar pattern of activity. Perichromatin granules and other material are being transported and there is a reduction in the complexity of the flocculent knotty character of the chromatin threads. This indicates that active transcription has ceased here also.

The transcriptional activity shown in the continuous Light series (Figure 7) is seen for two populations of apices as described under Results. One is not at all active and the other shows a rhythm similar to that seen in the continuous Dark, although the absolute activity is not as great. It is interesting that other aspects of this study indicate that of all the experimental treatments imposed on the seedlings the continuous Light disturbs their normal metabolic functioning the most. The ultrastructural results here support this.

The transcriptional activity in the Light-after-Dark series - induced (Figure 8) is seen to be very active throughout and correlates with the active (poised) status when it is removed from the dark. Throughout the series, this status persists into the light. In the Light-after-Dark - non-induced series the apical status correlate with the non-active status when it was removed from the dark. This status persists into the early part of the series in light but rapidly acquires active status by the end of the series. This is

reminiscent of ontogenetic results given in Chapter 3 for these seedlings. The activity of the Dark series and the interrelationship with the three Light-after-Dark series is given in Figure 9.

A rhythm of protein synthetic activity (Figure 10) is seen. The peaks correlate with the endogenous rhythm of flowering and with the previous nuclear activity. This would be expected since the rhythm of nucleoprotein granules are dependent upon a supply of protein translated in the cytoplasm. Polysome activity decreases with time in the dark as illustrated by the decreasing peak height. Complex polysomes are very dependent upon the level of energy - ATP levels - and in the dark this declines and the amount of polysomes decreases (Clark, Matthews and Ralph, 1964). Polysome profiles were prepared in order to examine the sizes of polysomes present and to discover whether this method would be sufficiently sensitive to compare differences in amounts of polysomes present in tissues from different sampling points (Addendum II: Results). No attempt was made to count monosomes and polysomes as many difficulties are reported in the literature. It is often difficult to assess polysome configurations when they are closely packed and the cytoplasm dense (Chaly and Setterfield, 1974). Literature sources warn about counting organelles without knowledge of their particle shape (Havelange, Bernier, Jacquard, 1974). There are considerable

difficulties in accurately assessing the density of ribosomes - per area? - per volume? per cell? Each results in a different distribution pattern. Which of these is significant biochemically - total number of ribosomes or concentration? (Lin and Gifford, 1976). In this study a change in polysome conformation was used such that many complex comformations of polysomes represented an increase in translation of different types of protein. Additional activity was evident.

A rhythm in membrane secretory material synthesis, the characteristic pink-red β-cyanin, was seen. This endogenous rhythm appeared to have a period of 30 hours. However those sample points close to 30 hours may be masked because of the quantity of betacyanin present in the endoplasm reticulum or out into the wall space or in the vacuoles. Active synthesis may have ceased prior to this and the products present masked this. This pink pigment can be extracted into surrounding media only at certain points in time and the rhythm in cell membrane permeability has been studied. This important rhythm is discussed in the conclusion.

CHAPTER 5

CONCLUSION

It was hypothesized that the ultrastructural and hence the biochemical status differs at opposite phases of the endogenous rhythm (e.g. 12 hours sampling point compared with 24 hours). This thesis shows that at opposite phases of the rhythm the status does differ. At those phases of the endogenous rhythm when flowering can be induced the apices are biochemically poised (Chapter 4) and are metabolically active.

The cells also show genetic activity, the diffuse chromatin in the nucleus is transcribing messenger RNA precursors plus tRNA precursors. The 5 S RNA of the large ribosomal subunit is synthesized in this region also. The nucleolus transcribes the rRNA precursors. Transport of mRNA is also dependent on the nucleolus. That protein synthesis is occurring, is also seen as the newly transcribed rRNA accumulates as ribonucleoprotein (RNP) granules in quantities that obscure the face of the nucleolus. Formation of these RNP particles is dependent on the supply of special ribosomal proteins (which are translated in the cytoplasm and transported into the nucleus). The nucleolus is essential to protein synthesis upon which cell growth and metabolism depends.

Thus the nucleolus is a window on the degree of activity of the cell. At this point the nucleolus and hence the cell are very active.

The cells show active protein synthesis. This is seen both as above in the formation of RNP granules in the nucleolus and in the changes of RNP structures ie: membrane-bound polysomes, complex polysomes, and strings of polysomes (Chapter 4; C. Results (2)). These large numbers and varied conformations of polysomes are indicative of translation of a wide variety of proteins. These polysome conformations remain only so long as active mRNA is supplied to the cytoplasm and so long as proteins are being translated. On completion of translation the ribosomes become free and separate into subunits. Thus in the absence of protein synthesis the micrographs will show a high monosome population.

The cells show membrane activity. The endoplasmic reticulum is seen to proliferate and form spaghetti-like tangles. This RER is associated with dense polysomes which would indicate active protein synthesis and presumably resultant secretion formation in the cisternae, although nothing is discernable within these areas. Golgi appear very active and vesicles budded off the dictyosome cisternae are seen in transit to the plasma membrane. There they discharge their contents outside the plasma membrane. The vesicle membrane contributes to the plasma membrane. Thus, the Golgi

secrete substances which contribute to cell wall formation and also produce plasma membrane. Since anthocyanin stains similarly, it is presumed that this darkly staining substance in the Chenopodium rubrum seedlings is betacyanin, the pink-red substance responsible for the red coloration in the tissues of this species. The endogenous rhythm in synthesis has a period of 30 hours (Figure 11). A rhythm in permeability of the membranes has been shown with this pink β-cyanin extraction (Wagner and Frosch, 1971) (Cumming and Wagner, 1970) (Berlin, Sieg, Strack, Bokern and Harms, 1986).

At the other phase of the endogenous rhythm when the seedling apices cannot be induced and remain vegetative, they are ultrastructurally and biochemically, markedly less active (Chapter 4). The metabolic status of these apices differs from the active status of those at the opposite phase of the rhythm.

The sensitivity of the seedlings to floral induction changes with the changing phase of the rhythm and parallels the endogenous rhythm in ultrastructural and hence biochemical status as described above. Those induced seedlings exposed to light following the inductive dark period are already 'poised' and active, and maintain this active status out into the light period. The corresponding non-induced seedlings of markedly less active status when transferred into the

light, show by 12 hours some increased activity but still retain a lot of features in common with the original status. By 24 hours these seedlings become much more active until later in the series when the status parallels that of the induced apices. This ultrastructure correlates with and mirrors the results from Chapter 3 when ontogenetically in the dark these seedlings are delayed. Then there follows in the light a stimulus to rapid growth and development. The same fundamental process is observed for both induced and non-induced apices (Chapter 3: D. Discussion I Ontogenetic Considerations). Both of these sets of apices undergo a wave of mitotic divisions. For the induced seedlings, accomplishment of the first wave of mitotic divisions and the start of evocation indicates that the potential for floral induction at the apex has been realised. This is essential, otherwise the induction status will pass. With this wave of mitotic division throughout the apex, the first stage of change is upon the set cell pattern of the shoot apex. The biochemical - ultrastructural status is that of very active cells set for rapid growth and development. Indeed by 48 hours in the light, these apices show floral primordia established.

For a change of pattern at the apex, it is important which of the cells divide. In the normal course of events metabolism will be active and may be

observed ultrastructurally. The change in activity of these cells will epigenetically impress a change upon the microenvironment and send messages to other cells of the cell mass (Chapter 1: Introduction). The balance and equilibrium of the cell mass (the apex) must undergo biochemical change. This is seen, and well documented as activity in the central core of the induced apex at evocation and beyond. This is later followed by the expression of changing pattern, with the modification of primordia on the flanks.

Which of the cells divide, determines the set cell pattern of the apex, as discussed before. In the induced apex the set cell pattern of dividing cells is changing and the question becomes, why do the less active cells in the apex, many of them reported to be in G_2, start dividing? This becomes the question of induction. There are a wide variety of ways in which an apex can become induced, such as light regimes (photoperiodic induction), temperature regimes (vernalization), age factors, etc.

Perhaps it is more effective to ask, "How does the apex become induced?" This involves the question of how the less active cells held inactive by epigenetic factors within the organisation of the cell mass (Steward, Chapter 1: Introduction) are released to start dividing. These are the type of factors maintaining the set cell pattern of the organized shoot apex. It is

axiomatic that some stimulus or condition foreign to the stable set cell pattern of the apex must be introduced. It may come from outside the system (various theories of floral stimulus) or be present within the system constantly or perhaps appear rhythmically. In the Chenopodium rubrum system, rhythm is the key - metabolically and biochemically and hence ultrastructurally. The seedling apex is metabolically poised at points on the endogenous flowering rhythm where the apex can be induced to flower. This poised state is absent at phases of the rhythm where seedlings cannot be photoperiodically induced. Thus, there are apical factors, those from within the apex, contributing or predisposing the apex to induction. This partially answers the question of why the cells divide in the induced apex but not in the non-induced apex at set times.

The next question, "What is needed by the cells to release them to divide?" They need cell division factors, the normal "grow and divide" hormones. These are either inactive within the cell or must be supplied externally to these cells. That these are already accessible to apex cells is demonstrated by the other cells which do regularly divide. If either access of hormone to change the balance or access of factors to release inactive hormones within the cell, is a problem for these less active cells, then the status of the

plasma membrane may be crucial. This study has shown the presence of betacyanin in the cells and a rhythm of betacyanin synthesis (Chapter 4: D. Discussion). Betacyanin, acts as an indicator and is released across the cell membrane at certain times. A number of studies (Wagner, et al., as above) have shown this release to be rhythmic in plasma membrane permeability which could here provide the means of access, enabling cell division factors to become active.

The question now becomes one of normal cell division and how hormones could be involved. The results of Chapter 3 describe normal plant growth and cell division hormones - the same ones currently required for the other cells which regularly divide within the set apical cell division pattern of the shoot apex. The only change now is that less active cells in the central zone of the apex start dividing. The molecular events of induction and floral evocation are still largely unknown. From the ultrastructural study it is not possible to reliably deduce the activity of specific plant hormones.

The literature is full of contradictions. The different tissues used probably further confuse the issue. For instance Short, Tepper and Fosket, 1974 found stimulation of polysome formation by cytokinins. However, Chaly, 1973 in searching for this found no stimulation of polysome formation by cytokinin.

Nevertheless, cytokinin is well documented as the hormone required to stimulate cell division and is undoubtedly required by the less active cells that enter cell division (Chapter 3: D. Discussion). It would be expected that all parts of the apex and the primordia would respond and this is seen in this system (Chapter 3: C. Results and D. Discussion). Cytokinin cell division activity is also well known to be promoted by auxin and there is a high probability, given the burst of cell division and subsequent growth of the induced apex, that auxin could also be a requirement. The concentration ratio of these two hormones could be critical. The requirement for gibberellin is less easy to hypothesize. The spectacular cases of rosette plants that bolt with subsequent flowering have now been shown to have a gibberellin requirement for stem elongation and this requirement has now been experimentally separated from the subsequent flowering. There are many other contradictory reports in the literature, each on different flowering systems. The requirement for gibberellins is less clear at this initial stage, although it is possible that they act in concert with the other hormones. From published reports the gibberellin activity may be more evident immediately following the initial cell division stage. They are known to be involved in increased plastochron rate, precocious axillary bud development modification of leaf

shape and change in phyllotaxis. All of these are observed as in this system (Chapter 3: C. Results, D. Discussion). Optimum levels of gibberellin concentration would also be expected to be critical.

Hence in the Chenopodium rubrum system it is here considered that no special specific floral hormone is required to induce flowering. Induction in this system requires an apex status which arises rhythmically during the Dark period. This apex status predisposes the cell's (all apical cells) accessibility to normal plant hormones and metabolites required for dividing cells and an increased number of rapidly growing cells. The metabolism of the apex is already poised. If the requirements of growth hormones and metabolites are not supplied from the leaf within a set period of time, in the light following the dark period so that evocation can proceed, this status passes and flower induction is lost until, in the Chenopodium rubrum system the same phase of the rhythm recurs.

ADDENDUM I

BACTERIAL INFECTION

A: INTRODUCTION

Bacteria were occasionally found in the seedling tissues. This infestation appeared to be a single bacterial species. The effect of this bacterium on the tissues was of considerable interest in the continuous Dark series sampling point of 24 hours. Here it was found that those seedlings with no infection were 100% non-induced and remained vegetative (Figure 2 and Chapter 3: C. Results). In contrast, the one infected seedling at this same sampling point was induced and flowered vigorously. Do these bacteria play a role in the floral induction of the normally non-induced seedling? A preliminary investigation was undertaken.

B: MATERIALS AND METHODS

(i) The micrographs which were prepared for examination of ultrastructure as in Chapter 4: B. Materials and Methods were examined for evidence of infection in the seedling tissues and to ascertain whether infection was associated with any particular experimental condition (continuous Light (L) or continuous Dark (D) or Light-after-Dark (L/D) treatments).

Thin sections of cotyledon, primordial leaf, and apex prepared as in Chapter 4: B. Materials and Methods were used to examine the nature of the infection and electron micrographs were prepared to locate the bacterial site of infection, the nature of any damage, and any host-parasite interaction observable.

(ii) Chenopodium rubrum (selection #374/70) of origin 60° 47'N 137° 32'W included in all other experiments reported was used here together with three other ecotypes, namely #372, #194 and #184. Seedlings of 5 1/2 days were used as described in Chapter 2: B. Materials and Methods and Figures 1A and 1B. As the greatest infection was found to occur with light treatment, 5 1/2 day old seedlings of each of the ecotypes were exposed to 12 hour continuous light and then harvested. With sterile scissors the uppermost parts of the seedlings (including cotyledons) were finely chopped onto the surface of nutrient agar (Difco) plates and spread with a sterile wire. Seedlings were also taken and ground to a mush with a sterile pestle and mortar (sterilized in alcohol) and again spread over nutrient agar with a sterile wire. This was done for each of the ecotypes of Chenopodium rubrum (#374, #372, #194, #184).

Although the plates were made up with Difco standard nutrient agar, added Hoagland's solution was added instead of water. Bacterial colonies were obtained

from two of the ecotypes (374, 194). These colonies were subcultured onto the same nutrient agar. The new growth of these subcultures was examined two to three days later under the phase contrast microscope and found to resemble the bacteria seen in EM photographs of the seedling tissue.

Gram staining (Hucker modification) was undertaken as part of the attempt to identify the bacteria. Fermentation tests were carried out using Bacto-Purple Broth Base (DIFCO Manual) in the agar. At a pH of 6.8 this agar mixture is purple (bromcresol purple), becoming yellow at pH 5.2 and below indicating fermentation. The sugars glucose and dextrose were used as well as cell wall pectin and cellulose.

C: RESULTS

(i) While a few seedlings in all of the series were infested, those of the continuous Light series were the most heavily infested. In these seedlings the tissues were extensively invaded. Micrographs reveal collections of bacteria ~~in~~ the intercellular spaces (Plate 53). It appears that bacteria invade the cell wall (Plate 54) with the pectic middle lamella staining darkly in regions ahead of the bacteria. Ultimately the cell wall splits down the middle lamella (Plate 55). In the host pathogen interaction, the plant cell releases membrane-bound vesicles into the intercellular spaces

and in regions of ruptured wall, where they are found in association with bacteria (Plate 56). Cells adjacent to the invading bacteria contain extensive membrane figures (Plate 57) which can also be released into the vacuoles sometimes accompanying the osmiophilic globules (Plate 58). The infection has a very localised effect, the seedlings as a whole appear quite healthy. However, under certain treatments, the bacteria are able to overwhelm seedling defense mechanisms and cause total wall breakdown and lysis of neighbouring cells.

(ii) These bacteria were found to be gram-positive while very young, becoming gram-negative with age. A micrograph was prepared of an isolated bacterium (Plate 59) and the gram(-)^{ve} wall structure is clearly seen. A definitive species identity of this organism remains uncertain. It did not ferment the sugars dextrose and glucose, nor pectin and cellulose.

D: DISCUSSION

The results show that the infective organism in the Chenopodium rubrum seedlings was a gram(-)^{ve} bacterium. This is consistent with symbiotic bacteria and bacterial pathogens of plants. On examining the electron micrographs the nature of the infection is seen. This shows a progressive onslaught of bacteria on the cell wall. First the middle lamella dissolves and the removal of the pectin cementing the cell walls results in

disintegration of the wall and collapse of the cellulose fibres. This behaviour is typical of pectolytic bacteria. However, the bacteria failed to digest the pectin when plated onto fermentation media with pectin. Other workers have encountered this problem (Starr, Chatterjee, Starr and Buchanan, 1977). Commercial grades of pectin have been found to be by no means homogeneous (Aspinall, 1970) (Bateman and Bashaw, 1976). Sigma and ICN brands of pectin are not satisfactory for this test and the Fisher brand used above must join them. Further work has shown the brand of pectin suitable for this procedure to be very critical (Starr et al., 1977). This could account for the discrepancy in the pectin digestion test here.

With most experimental treatments an equilibrium is established between the host seedling and the bacterial pathogen. The dark period, whether continuous or followed by light (L/D series) is needed to maintain this balance. A maverick L/24D infected seedling which flowered was a very healthy, vigorous seedling (Chapter 3: C. Results). Continuous light disturbed the metabolic equilibrium between host and pathogen and frequently the host could not contain the bacterial infection. This can be dependent on the physiological-biochemical status of the host which can in turn be determined by the day length (i.e. length of light period) (Sackston and Sheppard, 1973). Oligogalacturonides; the products

released by pectin-degrading enzymes, may possibly play a role in disease resistance in the plant host. Oligogalacturonides derived from pectic polysaccharides of plant cell walls can serve as regulatory molecules that induce phytoalexin accumulation. This has been well documented in soybean (Davis, Darvil, Albersheim and Dell, 1986). However, a different infecting organism with bean tissue showed a lack of correlation between pathogenicity and pectolytic enzyme activity (Lumsden, 1976).

Could the bacteria here alter the metabolic equilibrium? Agrobacterium tumefaciens is well known for causing rapid and unorganized growth leading to galls or tumors in plant tissue. Infected callus in culture mimics cytokinic addition (Nester, Gordon, Amasino, Yanofsky, 1984). The Rhizobium genus causes rapid cell growth forming nitrogenous nodules on leguminous plant roots. Bacterial pathogens which liberate peroxidases can change H^+ and K^+ efflux across membranes and so damage lipids (Atkinson and Baker, 1987). Pseudomonas syringae emits a toxin damaging the host cells (Zang and Takemoto, 1987). Xanthomonas campestris has been shown to produce an auxin-IAA (Fett, 1987). Knowledge of whether the bacterium present in the tissues of the maverick L/24D flowering seedling can disturb the membrane equilibrium, produce special metabolites or even hormones (Morris, 1986), and whether a special

metabolic status of the host cells is involved would be of considerable interest for the understanding of why and how that seedling in a non-inductive environment succeeded in flowering vigorously.

PLATE 53

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing the location of bacteria in Chenopodium rubrum tissues. A number of bacteria are seen congregated in an intercellular space. Two plant cell walls (CW) can be seen. Magnification x 45,000.


CW

PLATE 54

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing two bacteria apparently lysing the cell wall (CW). The middle lamella stains ahead of them. It has been digested by pectinase enzymes and consequently split allowing entry to the bacteria. Magnification x 45,000.

PLATE 55

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing extensive splitting of the wall along the middle lamella, resulting from digestion of the pectin. Magnification x 27,000.

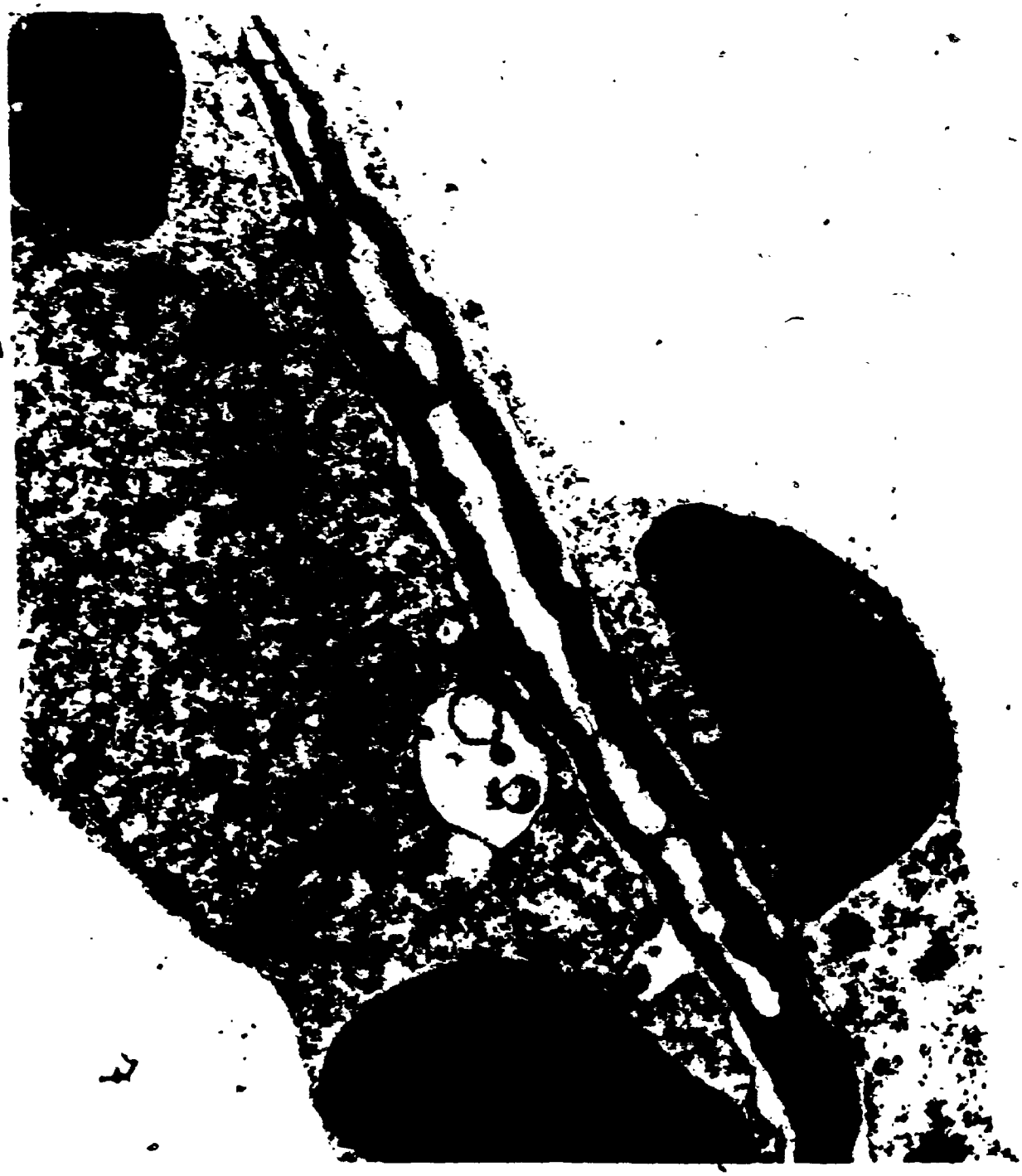


PLATE 56

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing complete dissolution of the cell wall with release of cellulose fibres.

Magnification x 78,000.

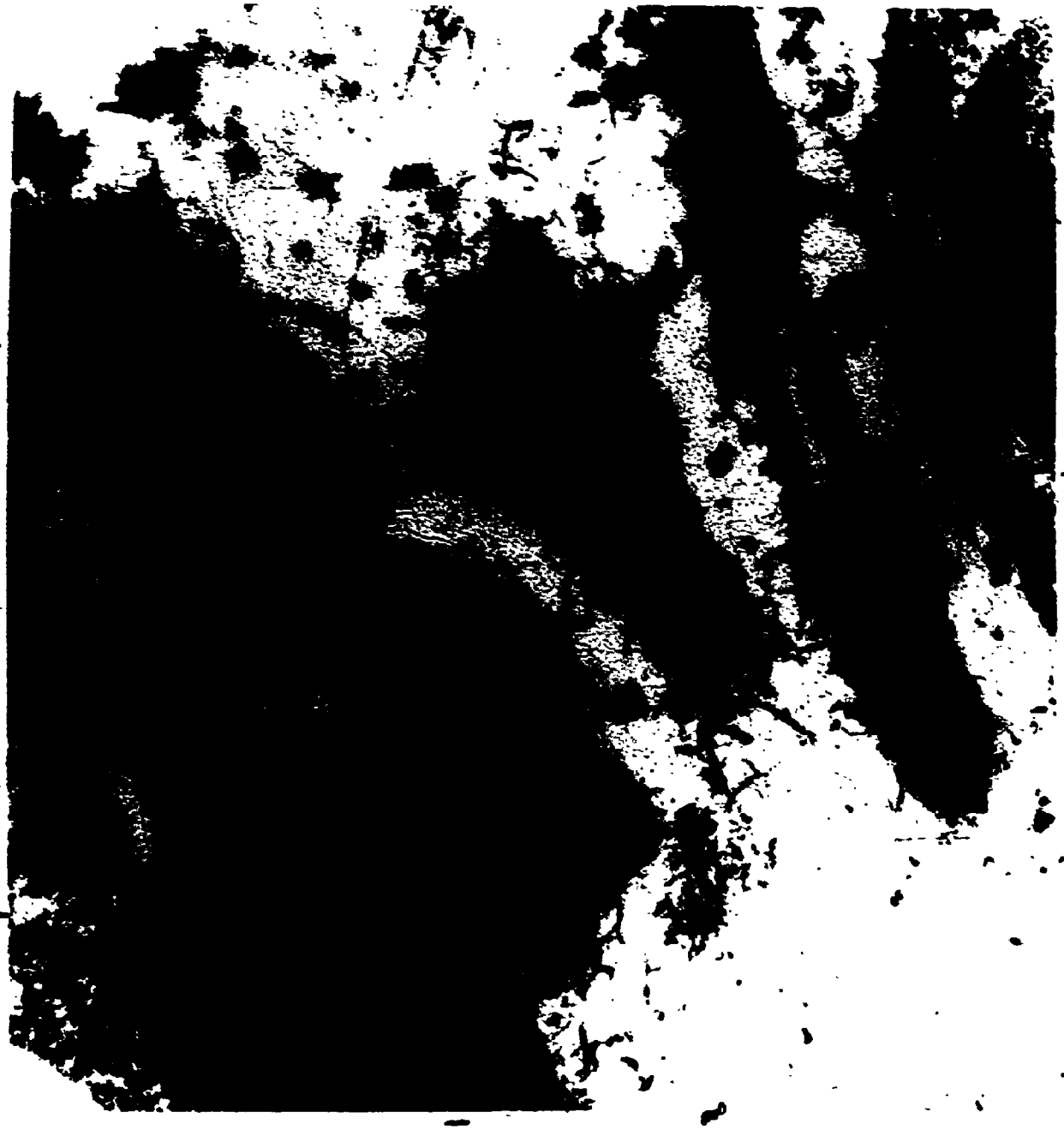


PLATE 57

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing host response to presence of bacteria in the intercellular space. Membrane-bound vesicles are seen discharging their contents in the vicinity of the bacteria. Magnification x 103,000.

PLATE 58

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing another host response to the bacterial presence in the tissues. Large convoluted membrane formations are seen in vacuoles. Magnification x 78,000.

PLATE 59

ADDENDUM I: BACTERIAL INFECTION

Here out in the vacuole, excess membrane is found associated with the osmiophilic globules. Magnification x 45,000.

PLATE 60

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing the isolated cultured bacterium. The three-layered gram-negative wall structure with the peptidoglycan layer between the outer and inner membrane can be clearly seen. Magnification x 181,000.

ADDENDUM II

MONORIBOSOME AND POLYRIBOSOME ANALYSIS

A: INTRODUCTION

Examination of ribosomes in the electron micrographs of seedling sections from selected sampling points shows different polysome conformations and varying proportions of monosomes (Chapter 4: (2) Protein Synthetic Activity, Figure 10). Distinctly different conformations of polysomes, including membrane-bound polysomes on the RER, indicate synthesis of different types of protein (Bollini and Chrispeels, 1979). The length of the polysome approximates the length of the messenger RNA strand being translated. This gives an indication of the size (molecular weight) of the protein being synthesized (Gunning and Steer, 1975). Few polysomes and an abundant monosome population indicate low protein synthetic activity (Lin and Key, 1967). Hence, an assessment of monosomes and polysomes gives information on the protein synthetic activity of cells. Maintenance of polysome structure requires energy expenditure (Marks, Burka, Conconi, Perl and Rifkind, 1965). Thus, examination of polysomes present is also an indicator of the cellular energy status. Monosome and polysome isolation and analysis were undertaken.

B: MATERIALS AND METHODS

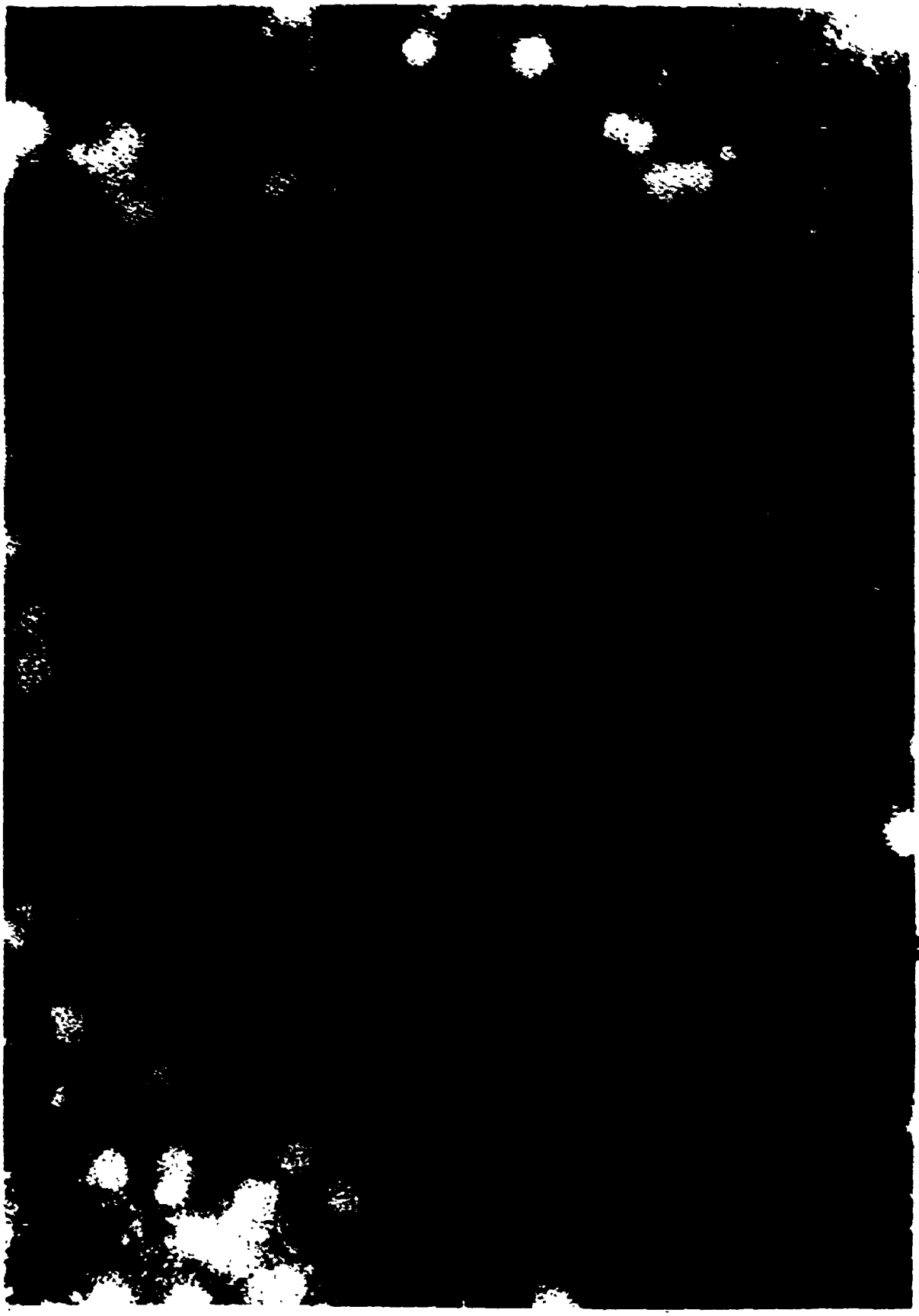
Six experiments were run on seedlings grown (Chapter 2B) and treated as under (Chapter 3B) the Light-after-Dark series. Batches of 5 1/2 day old seedlings were all exposed to 13 1/2 hours dark followed by 12 hours light or multiples of 12 hours light. These seedlings were committed to flower and in the electron micrographs the cells are packed with clustered polysomes. These were used in an attempt to refine the technique as applied to seedling tissues prior to examination of the continuous Dark series. At some point in this series the polysomes in the electron micrographs appear linear and sparser.

Seedlings were harvested into ice-cold water, weighed and filtered, then 2 gms lightly ground for 3 minutes at 5° C using a pestle and mortar with 0.5 gm sand and 5 mls of pelleting buffer (0.25 M sucrose, 0.05 M Tris-HCl, 0.002 M $MgCl_2$, 0.1 mM Clelands Reagent) of pH 7.8 at 5° C. The ground tissue was strained through cheese cloth/miracloth and centrifuged in pelleting buffer (above) for 10 minutes at 2,000 x g then for 10 minutes at 30,000 x g. To the post-mitochondrial supernatant was added 1/9 by volume of 5% deoxycholate in pelleting buffer and the solution was centrifuged for 30 minutes at 250,000 x g. The ribosome/polysome pellet thus obtained was resuspended in gradient buffer (0.05 M

PLATE 61

ADDENDUM II: MONORIBOSOME AND POLYRIBOSOME ANALYSIS

This negative-stained electron micrograph shows that the isolated ribosome pellet (Addendum II - Method) does not contain much cell debri and especially no membranes. It does contain monosomes and some polysomes as indicated by the arrow. The large white areas are dried buffer crystals from the suspension medium.



Tris-HCl, 0.002 M $MgCl_2$, 0.1 mM Clelands Reagent) of pH 7.8 at 5° C and used for a) electron microscopy, b) ultracentrifugal analysis, c) sucrose gradient analysis.

a) A small drop of resuspended pellet material was mixed with a small drop (1 loopful) of 2% sodium molybdate or 1% PTA and dried down with filter paper mopping on a carbon-formvar grid and examined in the electron microscopy.

b) The resuspended pellet was examined in the analytical ultracentrifuge and plates of the schlieren patterns measured for the isolated ribosomes and polysomes (Chervenka, 1969) (Schachman, 1957). Sedimentation coefficients were calculated. These were obtained for sample alone, sample plus RNase, and sample plus DEP plus RNase, to ascertain the degree of protection afforded by the DEP.

c) 0.4 ml samples of resuspended pellet (as above) were layered over 12 ml 10%-34% sucrose gradients for linear density gradient fractionation, and centrifuged at 1° C for 1 hour at 200,000 x g. Prior to layering on the gradient parallel samples of resuspended pellet were treated with 1.0 µg of RNase/1 ml sample for 7 minutes at 30° C and then centrifuged simultaneously. The fractions were collected through a needle piercing the bottom of the tube and continuously monitored (254 mµ) with an automatic density gradient fractionator,

Instrumentation Specialities Company, Lincoln Nebraska or ISCO (Brakke, 1963). For resultant ribosome-polysome profiles see Section (c) Results c).

The ordered fractions from the ISCO were collected and all U.V. spectra examined with the Beckman DB spectrophotometer and the Cary as proof of the presence of nucleoprotein in the peak fractions and absence from the blanks, buffers, etc. The ribosome-polysome profiles (Figures 15 and 16) indicate polysome degradation. It was also found that the pellet had to be used immediately for both b) and c) otherwise progressive rapid degradation occurred and the presence of very high levels of RNAase in the pellet was inferred, as has been shown to be the case with other seedling cotyledon tissue. In addition to the usual technique precautions (low temperature, adequate Mg^{2+} ion concentration (Mehta, Hadziyev and Zalik, 1969), grinding procedures, etc.) diethyl.pyrocarbonate (DEP) was considered to be the most suitable nuclease inhibitor available. Thus, DEP 0.1 ml in 10 ml of pelleting buffer was added immediately prior to grinding the seedlings, and the above isolation and fractionation procedures repeated.

C: RESULTS

a) The electron micrograph (Plate 61) shows that the material from the pellet is relatively pure, containing primarily ribosome and polysome material. It

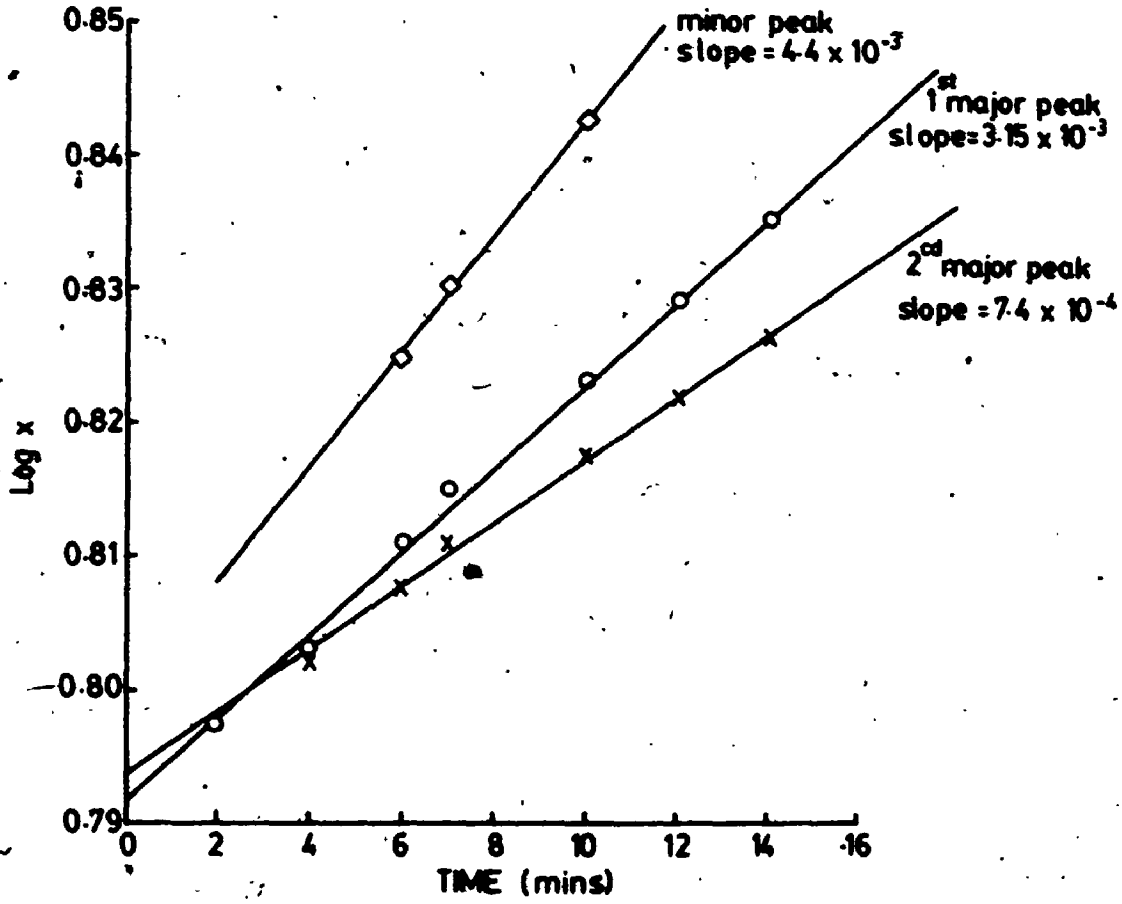
TABLE 2

Ribosomal preparations were examined in the Spinco Model E centrifuge with Schlieren optics. Table shows measurements (Inner Reference Edge to Centre of Peak) from the Schlieren plates, and calculation of Log_{10} x for the first run.

Time (mins)		(Inner Reference edge to centre of peak) Meas. Dist. (cm) (x)		Magnif. Factor Correction (2.1) (x/2.1)	Cubic Correction [illegible]	
2'	1st MAJOR PEAK	1.1760 1.1687 1.1768 1.1706 1.1756		0.5599	[illegible]	
4'	1st MAJOR PEAK	1.6009 1.6003 1.6062 1.6064	(Meas. from Shadow Edge of Inner Reference Edge on are all rest of measurements)	[illegible]	[illegible]	
	2nd MAJOR PEAK	0.8656 0.8680 0.8671 0.8704 0.8668	SMALL PEAK beyond 1st MAJOR - 2.0635	[illegible]	[illegible]	
		0.1965 0.1986 0.2007 0.1986	WIDTH of Inner Ref. edge GAP			
6'	1st MAJOR PEAK	1.3522 1.3506 1.3510 1.3551	SMALL PEAK beyond 1st MAJOR 1.6221	[illegible]	[illegible]	
	2nd MAJOR PEAK	0.9165 0.9187 0.9137 0.9132 0.9164		[illegible]	[illegible]	
7'	1st MAJOR PEAK	1.7257 1.7262 1.7244 1.7264	SMALL PEAK beyond 1st MAJOR - 2.2165	[illegible]	[illegible]	
	2nd MAJOR PEAK	0.9938 0.9953 0.9936 0.9902		[illegible]	[illegible]	
		0.1977 0.1978 0.1976 0.1978	WIDTH of Inner Ref. edge GAP			
10'	1st MAJOR PEAK	1.9916 1.9878 1.9858 1.9911	SMALL PEAK beyond 1st MAJOR - 2.6366	[illegible]	[illegible]	
	2nd MAJOR PEAK	1.0556 1.0571 1.0521 1.0538		[illegible]	[illegible]	
		0.1960 0.1926 0.1937 0.1956 0.1939 0.1906	WIDTH of Inner Ref. edge GAP			
14'	1st MAJOR PEAK	2.1910 2.1913 2.1900 2.1900 2.1932 2.1908	SMALL PEAK Tear of end	1.0433	[illegible]	[illegible]
	2nd MAJOR PEAK	1.0939 1.0932 1.0940 1.0916 1.0949 1.0914 1.0965 1.0932		0.5209	[illegible]	[illegible]
		0.2009 0.1973 0.1992 0.1955 0.1959	WIDTH of Inner Ref. edge GAP			
45'	1st MAJOR PEAK	2.3876 2.3891 2.3880 2.3896 2.3816		1.1369	[illegible]	[illegible]
	2nd MAJOR PEAK	1.1339 1.1373 1.1362 1.1362 1.1335 1.1336		0.5400	0.2509	[illegible]
		0.1979 0.1983 0.1988 0.1968 0.1982	WIDTH of Inner Ref. edge GAP			

FIGURE 12

Determination of the sedimentation coefficient from a plot of Log x (x = distance of boundary to axis of rotation) versus t (t = time in minutes).


minor peak
slope = 4·4 x 10⁻³
1st major peak
slope = 3·15 x 10⁻³
2nd major peak
slope = 7·4 x 10⁻⁴
0·85
0·84
0·83
0·82
0·81
0·80
0·79
Log x
0
2
4
6
8
10
12
14
16
TIME (mins)

SEDIMENTATION COEFFICIENT CALCULATIONS

$$S_{OBS} = \frac{1}{w^2 r} \cdot \frac{dr}{dt}$$

S = 42,040 RPM

from Table 6, pg. 28

$w^2 = 1.937 \times 10^7$

$$= \frac{2.303}{60.w^2} \cdot \frac{d \, Log_{10} x}{dt'}$$

$\frac{2.303}{60.w^2} = 1.982 \times 10^{-9}$

$S_{OBS} = (1.982 \times 10^{-9}) \cdot$ (slope from graph)

SAMPLE ONLY

1st MAJOR PEAK Slope = 3.15×10^{-3}

$S_{OBS} = 62.43 \times 10^{-13}$

2nd MAJOR PEAK Slope = 7.4×10^{-4}

$S_{OBS} = 14.67 \times 10^{-13}$

MINOR PEAK Slope = 4.4×10^{-3}

$S_{OBS} = 87.21 \times 10^{-13}$

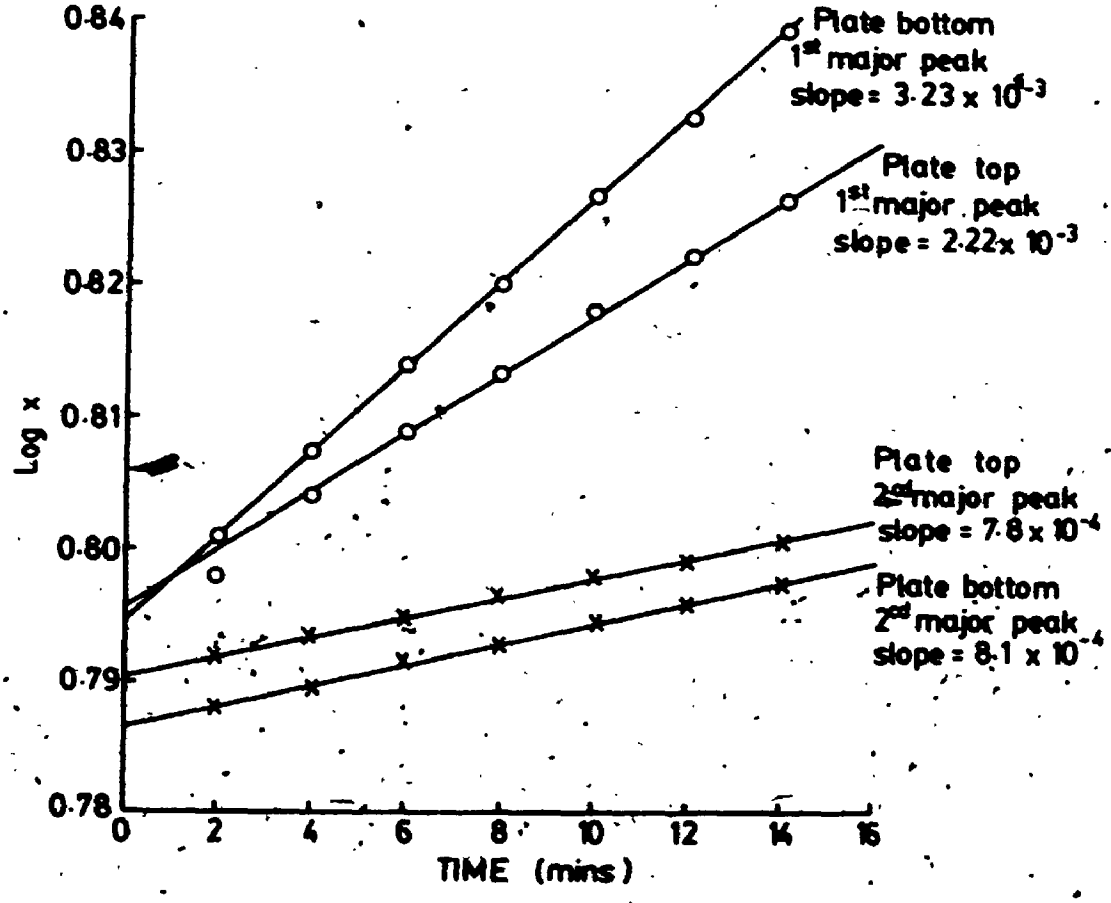
TABLE 3

Ribosomal preparations were examined in the Spinco Model E centrifuge with Schlieren optics. Table shows measurements (Inner Reference Edge to Centre of Peak) from the Schlieren plates, and calculation of Log_{10} x for the second run.

TIME (mins)		Measured from Top of Cell to Centre of Peak (Plug left in so as Inner Reference edge visible. (y')) (cm) PLATE TOP	BOTTOM	y = 0.1962 - y Correction (for Inner Ref. edge CAP.) y		Magnif. Factor Correction (2.1) (y/2.1)		Rotor Centre (5.71) y/2.1 + 5.71 = x cm		$\log_{10} x$	
	1st MAJOR PEAK	1.0816 1.0846 1.0811 1.0843 1.0802	1.0867 1.0890 1.0857 1.0863 1.0857	1.1978	1.2829	0.5303	0.6109	6.2803	6.3209	0.7980	0.8008
	2nd MAJOR PEAK	0.8240 0.8273 0.8250 0.8166	0.7039 0.7047 0.7032 0.7040	1.0160	0.9001	0.4838	0.4286	6.1938	6.1386	0.7920	0.7881
	1st MAJOR PEAK	1.1872 1.1877 1.1862 1.1900	1.2865 1.2862 1.2836 1.2862	1.1834	1.4827	0.6587	0.7060	6.3687	6.4160	0.8061	0.8073
	2nd MAJOR PEAK	0.8622 0.8626 0.8609 0.8612	0.7475 0.7457 0.7482 0.7460	1.0984	0.9437	0.5040	0.4493	6.2140	6.1593	0.7934	0.7895
	1st MAJOR PEAK	1.3323 1.3301 1.3312 1.3300	1.4939 1.4941 1.4932 1.4923	1.5285	1.6900	0.7278	0.8047	6.4378	6.5147	0.8088	0.8139
	2nd MAJOR PEAK	0.9131 0.9151 0.9133 0.9137	0.8176 0.8061 0.8062 0.8127	1.1099	1.0038	0.5284	0.4780	6.2383	6.1880	0.7951	0.7916
	1st MAJOR PEAK	1.4723 1.4781 1.4707 1.4732	1.6913 [illegible] 1.6918 1.6909	1.6685	1.8879	0.7945	0.8990	6.5045	6.6090	0.8133	0.8201
	2nd MAJOR PEAK	0.9622 0.9600 0.9617 0.9602	0.8404 0.8497 0.8509 0.8496	1.1584	1.0450	0.5516	0.4980	6.2616	6.2080	0.7967	0.7930
	1st MAJOR PEAK	1.6261 1.6236 1.6257 1.6292	1.9092 1.9060 1.9096 1.9040	1.8223	2.1016	0.8657	1.0006	6.5777	6.7106	0.8181	0.8268
	2nd MAJOR PEAK	1.0091 1.0076 1.0102 1.0095	0.8975 0.8996 0.8961 0.8960	1.2053	1.0937	0.5739	0.5208	6.2839	6.2308	0.7982	0.7946
	1st MAJOR PEAK	1.7649 1.7670 1.7630 1.7635	2.1096 2.1078 2.1102 2.1106	1.9607	2.3036	0.9336	1.0979	6.6436	6.8079	0.8224	0.8330
	2nd MAJOR PEAK	1.0413 1.0418 1.0416 1.0407	0.9412 0.9401 0.9425 0.9421	1.2375	1.1376	0.5892	0.5418	6.2992	6.2516	0.7993	0.7960
	1st MAJOR PEAK	1.9044 1.9038 1.9002 1.9032	2.3252 2.3203 2.3200 2.3220	2.1006	2.5336	1.0002	1.2016	6.7102	6.9110	0.8267	0.8396
	2nd MAJOR PEAK	[illegible] [illegible] 1.0783 1.0816	0.9878 0.9829 0.9800 0.9821	1.2761	1.1801	0.6076	0.5615	6.3176	6.2719	[illegible]	0.7936

FIGURE 13

Determination of the sedimentation coefficient from a plot of Log x (x = distance of boundary to axis of rotation) versus t (t = time in minutes).


0·84
0·83
0·82
0·81
0·80
0·79
0·78
Log x
0
2
4
6
8
10
12
14
16
TIME (mins)
Plate bottom
1st major peak
slope = 3·23 x 10-3
Plate top
1st major peak
slope = 2·22x 10-3
Plate top
2nd major peak
slope = 7·8 x 10-4
Plate bottom
2nd major peak
slope = 8·1 x 10-4

SEDIMENTATION COEFFICIENT CALCULATIONS

$$S_{OBS} = \frac{1}{w^2 r} \cdot \frac{dr}{dt}$$

S = 42,040 RPM

from Table 6, pg. 28

$w^2 = 1.937 \times 10^7$

$$= \frac{2.303}{60.w^2} \cdot \frac{d \log_{10} x}{dt'} \qquad \frac{2.303}{60.w^2} = 1.982 \times 10^{-9}$$

$S_{OBS} = (1.982 \times 10^{-9}) \cdot$ (slope from graph)

PLATE - TOP - SAMPLE + RNase

1st MAJOR PEAK Slope = 2.22×10^{-3}

$S_{OBS} = 44.00 \times 10^{-13}$

2nd MAJOR PEAK Slope = 7.8×10^{-4}

$S_{OBS} = 15.46 \times 10^{-13}$

PLATE - BOTTOM - SAMPLE ONLY

1st MAJOR PEAK Slope = 3.23×10^{-3}

$S_{OBS} = 64.02 \times 10^{-13}$

2nd MAJOR PEAK Slope = 8.1×10^{-4}

$S_{OBS} = 16.05 \times 10^{-13}$

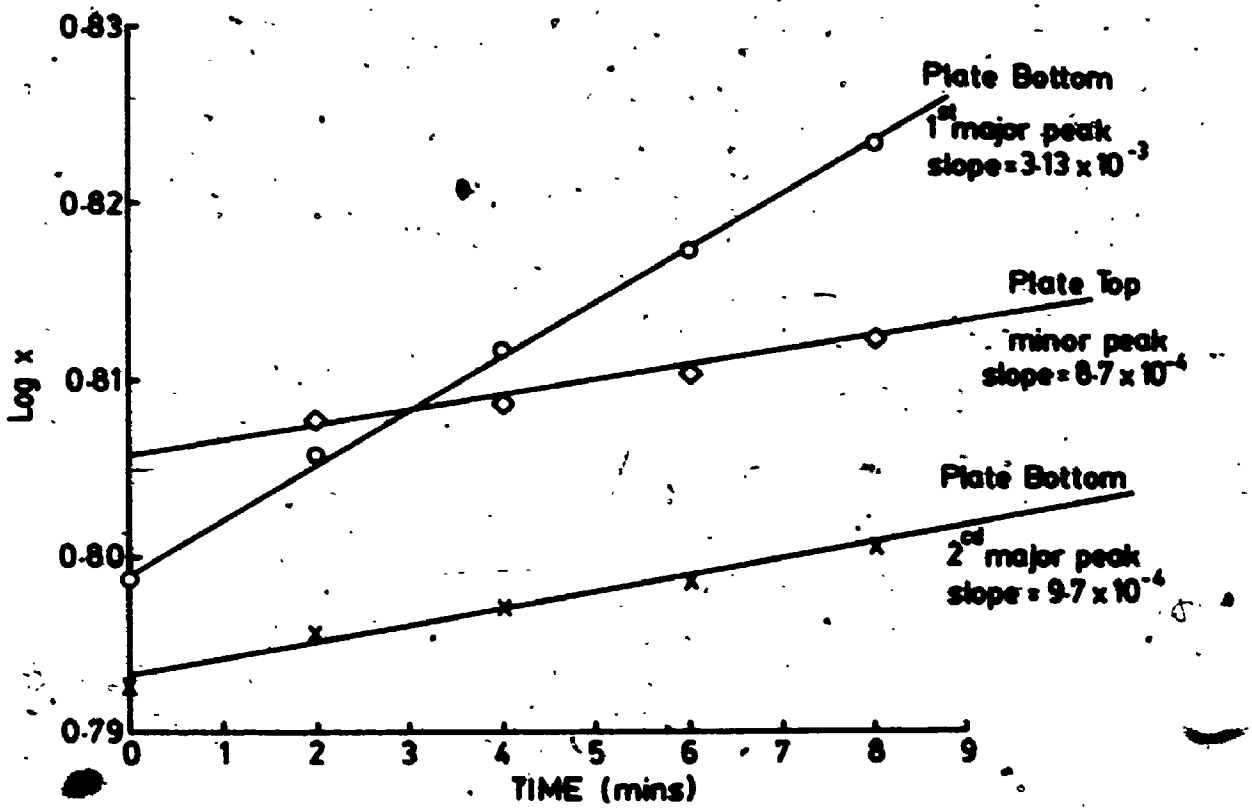
TABLE 4

Ribosomal preparations were examined in the Spinco Model E centrifuge with Schlieren optics. Table shows measurements (Inner Reference Edge to Centre of Peak) from Schlieren plates, and calculation of Log_{10} x for the third run.

Time (hours)	Peak	[illegible] Plate [illegible]	[illegible]	Corrected for Inner Reference [illegible]		[illegible] Ratio [illegible]		[illegible] Centre Ratio Correction [illegible]		[illegible]	
[illegible]	1st MAJOR PEAK	[illegible]		[illegible]		[illegible]		6.[illegible]		[illegible]	
			[illegible]		[illegible]		[illegible]		6.42[illegible]		[illegible]
	2nd MAJOR PEAK	[illegible]		[illegible]		0.5[illegible]		6.2[illegible]		0.79[illegible]	
6'	1st MAJOR PEAK	[illegible] 1.4291 [illegible] [illegible]		1.6216		[illegible]		6.4831		[illegible].8118	
			1.3337 1.3269 1.3452 1.3516		1.5299		[illegible].7285		6.4385		0.8088
	2nd MAJOR PEAK	[illegible] 0.9812 0.9791 0.9796		1.1763		0.5600		6.2700		0.7973	
6'	1st MAJOR PEAK	1.6032 1.6090 1.5984 1.6023		1.7996		0.8568		6.5668		0.8176	
			1.3868 1.3807 1.3848 1.3950		1.5830		0.7538		6.4638		0.8103
	2nd MAJOR PEAK	1.0210 1.0191 1.0222 1.0218		1.2172		0.5796		6.2896		0.7987	
8'	1st MAJOR PEAK	1.8066 1.8050 1.8118 1.8036		2.0030		0.9538		6.6638		0.8237	
			1.4478 1.4510 1.4386 1.4539		1.6440		0.7828		6.4928		0.8125
	2nd MAJOR PEAK	1.0807 1.0796 1.0784 1.0841		1.2769		0.6080		6.3180		0.8006	
0'	1st MAJOR PEAK	1.0239 1.0280 1.0243 1.0213		1.2201		0.5810		6.2910		0.7987	
	2nd MAJOR PEAK	0.8406 0.8420 0.8364 0.8429		1.0366		0.4936		6.2036		0.7927	

FIGURE 14

Determination of the sedimentation coefficient from a plot of Log x^c (x = distance of boundary to axis of rotation) versus t (t = time in minutes).


0·83
0·82
0·81
0·80
0·79
Log x
0
1
2
3
4
5
6
7
8
9
TIME (mins)
Plate Bottom
1st major peak
slope = 3·13 x 10⁻³
Plate Top
minor peak
slope = 8·7 x 10⁻⁴
Plate Bottom
2nd major peak
slope = 9·7 x 10⁻⁴

SEDIMENTATION COEFFICIENT CALCULATIONS

$$S_{OBS} = \frac{1}{w^2 r} \cdot \frac{dr}{dt}$$

S = 42,040 RPM

from Table 6, pg. 28

$w^2 = 1.937 \times 10^7$

$$= \frac{2.303}{60.w^2} \cdot \frac{d \text{ Log}_{10} x}{dt}$$

$$\frac{2.303}{60.w^2} = 1.982 \times 10^{-9}$$

$S_{OBS} = (1.982 \times 10^{-9}) \cdot$ (slope from graph)

PLATE TOP - SAMPLE + RNase + DEP

THIN PEAK Slope = 8.7×10^{-4}

$S_{OBS} = 17.24 \times 10^{-13}$

PLATE BOTTOM - SAMPLE + RNase

1st MAJOR PEAK Slope = 3.13×10^{-3}

$S_{OBS} = 62.04 \times 10^{-13}$

2nd MAJOR PEAK Slope = 9.7×10^{-4}

$S_{OBS} = 19.23 \times 10^{-13}$

CALCULATION OF S_{OBS} FROM ANALYTICAL ULTRACENTRIFUGATION WITH SCHLIEREN OPTICS

The Values are Averaged from Five Experiments.

Average Value	Probable Particle
$S_{OBS} = 87.21 \times 10^{-13}$	Monosome (~ 80S)
	Sub-units
$S_{OBS} = 62.67 \times 10^{-13}$	(~ 60S)
$S_{OBS} = 42.61 \times 10^{-13}$	(~ 40S)
$S_{OBS} = 16.11 \times 10^{-13}$	rRNA (~ 18S)

The pellet of ribosomes is pure. There is no contamination by membrane, or a population of 70S ribosomes or sub-units of these arising from bacteria, chloroplasts or mitochondria.

is free of any membrane fraction. The 5% deoxycholate in the pelleting buffer successfully removed the membrane-bound polysomes from the rough endoplasmic reticulum. Proof of the presence of polysomes in the fresh pellet is important for further work as in b) and c) below.

b) The results of the analytical ultracentrifugation of the resuspended pellet are given (Tables 2, 3 and 4) as are the sedimentation coefficients (Figures 12, 13, 14 and respective calculations).

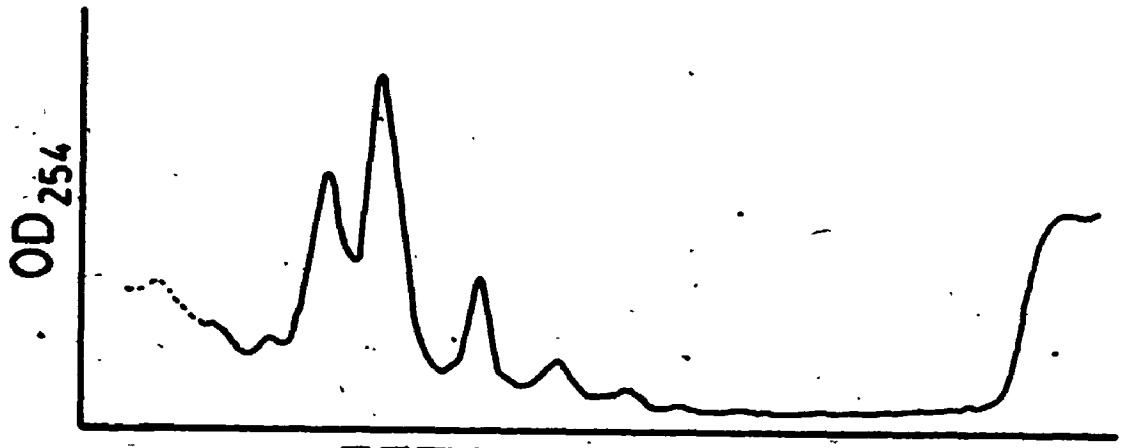
c) The resultant polysome profiles are shown in Figure 15. Looking at the first experiment discussed, the uppermost trace of sample only shows (reading from the left) the peaks of ribosome subunits and monosomes, and then evidence of 8-9 peaks of polysomes rapidly decreasing in size. The lower trace of sample plus RNase lacks the polysome peaks as predicted, leaving a large subunit peak and a prominent monosome peak. The second experiment discussed is shown in Figure 16. The first and uppermost trace of sample only, resembles that of the first experiment above. The second trace is of sample plus DEP, a nuclease inhibitor. Instead of DEP protecting the polysomes the trace shows them to be completely degraded. This leaves sharp subunit peaks and a small monosome peak. The third trace of sample plus DEP plus RNase shows a prominent subunit peak and a monosome peak. The DEP has not protected the polysomes

FIGURE 15

Sucrose gradient profiles of ribosomes from Chenopodium rubrum seedlings grown for 13 1/2 hours in the dark followed by 12 hours light and sampled (as in the text, Addendum II: B. Method). The first experiment discussed -

Upper: Seedling tissue sample only.

Lower: Seedling tissues sample + RNase (10 µg of RNase/ml incubated for 7 minutes at 30° C).


OD254
EFFLUENT FRACTIONS

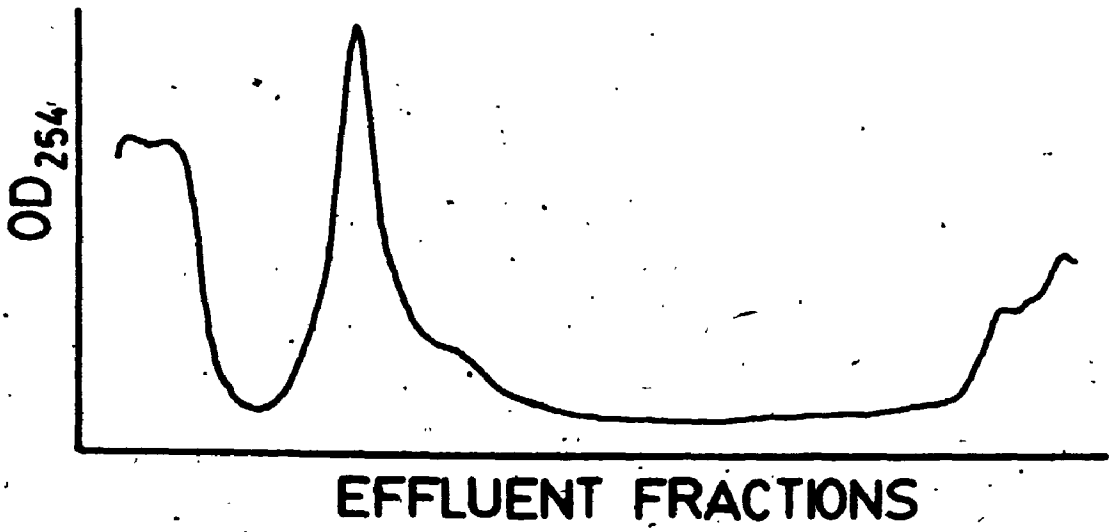

OD254
EFFLUENT FRACTIONS

FIGURE 16

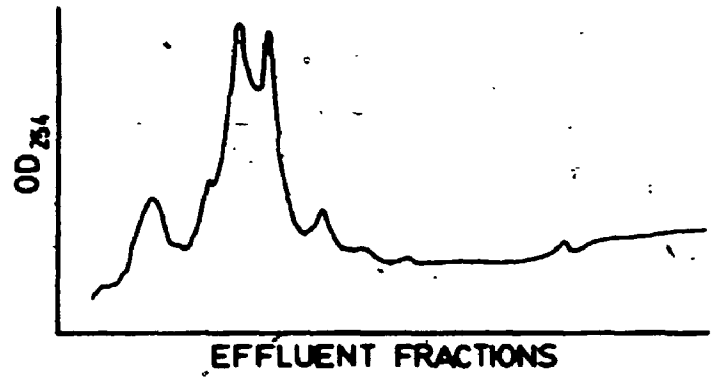
Sucrose gradient profiles of ribosomes from Chenopodium rubrum seedlings grown for 13 1/2 hours in the dark followed by 12 hours light and sampled (as in the text, Addendum II: B. Method. The second experiment discussed -

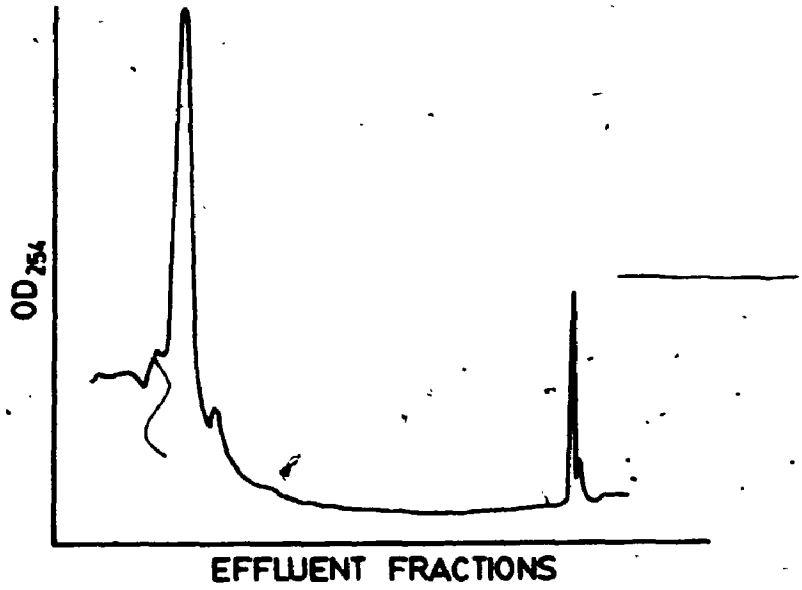
Uppermost: Seedling tissue sample only.

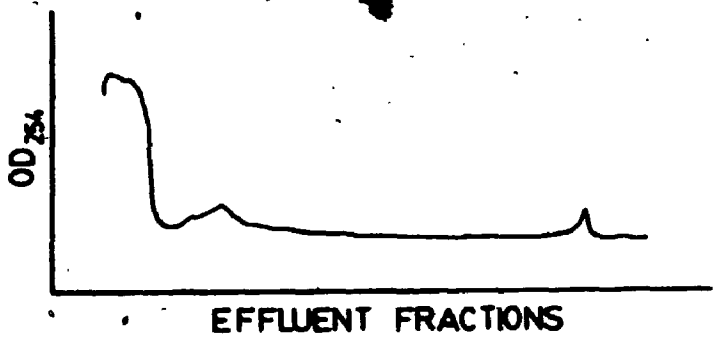
Next: Seedling tissue sample + DEP.
Lower (0.01 mls DEP/ ml).

Next: Seedling tissue sample + DEP +
Lower RNase.

Lowermost: Seedling tissue sample + RNase.


OD_{254}
EFFLUENT FRACTIONS


OD_{254}
EFFLUENT FRACTIONS


OD_{254}
EFFLUENT FRACTIONS

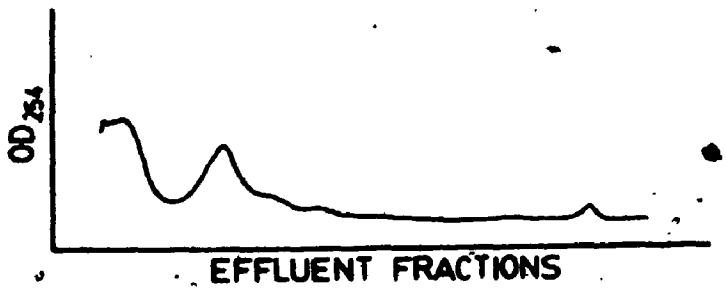
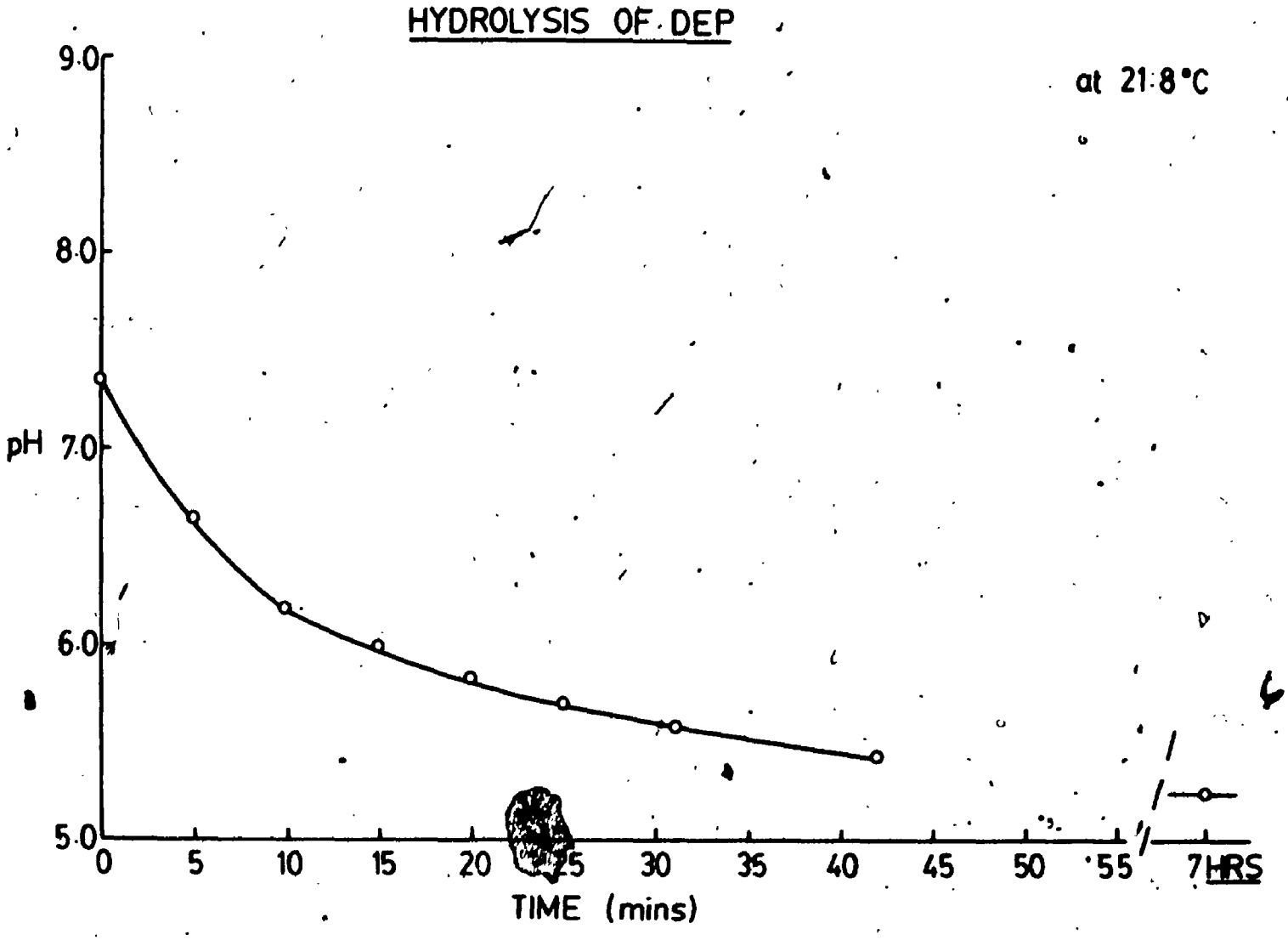

OD_{254}
EFFLUENT FRACTIONS

FIGURE 17

0.1 ml of DEP was added to 9.9 mls of 0.05 M Tris-HCl buffer solution and the pH monitored. The plot of pH against time shows rapid hydrolysis of DEP accompanied by an unacceptable drop in pH. This hydrolysis accounts for the lack of protection of the polysomes. The active DEP itself is rapidly destroyed and the pH change is equally damaging. Considerable increase in the Tris-HCl buffer concentration could not correct this adequately and the increased concentration would itself produce more problems.


HYDROLYSIS OF DEP
at 21·8 °C
9·0
8·0
pH 7·0
6·0
5·0
0
5
10
15
20
25
30
35
40
45
50
55
7 HRS
TIME (mins)

from the RNase. The lowermost reference trace of sample plus RNase shows loss of polysome peaks. This leaves a medium size subunit peak and a monosome peak which is not as badly degraded as that when DEP was present with the sample and RNase.

D: DISCUSSION

A sensitive, accurate method of polysome analysis is necessary for two reasons. Firstly, not only are polysome levels to be measured in this tissue, but also subtle quantitative differenees in the levels of polysome types and monosomes present. Secondly, the total ribosome population decreases with time to a very low level in some series (e.g. the continuous Dark series, Figure 10). Tissues with a maximum level of polysomes (as shown, by the electron micrographs) were selected for these experiments in an effort to refine the technique.

The high level of RNase present is the principle problem in the extraction and analysis of polysomes in certain tissues. The RNase (polyribonucleotide 2-oligonucleotido-transferase (cyclizing, EC 2.7.7.16) extensively attacks the mRNA (Lin, Key and Bracker, 1966). This RNase is particularly active in some plant tissues and demonstratably high in Chenopodium rubrum seedlings.

Plate 61 is an electron micrograph of the fresh pellet and indicates that polysomes are present in the pellet. During the grinding and extraction the RNase remains sufficiently compartmentalized that the integrity of the polysomes is generally preserved. Once in the pellet form however, the combination of highly concentrated free monosomes and polysome and the now high concentration of free Rnase leads to polysome destruction. If this material is stored in the cold overnight the RNase quickly digests polysomes with a resultant total loss of these polysomes.

Extraction media and buffers used for isolation of polysomes from bacteria or animal tissues are unsuitable for many plant tissues. The Chenopodiaceae are a halophytic family. Ribosomes from halophytes are not as stable in commonly used buffers. Consequently, buffers and solutions were reviewed (Good, Winget, Winter, Connolly, Izawa and Sing[illegible] 1966) (Boulter, 1970). In addition, ribonuclease inhibitors were reviewed and all in common use were noted to have severe limitations (Eilam, Butler, Simon, 1970). The most promising inhibitor was diethyl pyrocarbonate (DEP) (Weeks and Marcus, 1969). Results of experiments herein show that no amount of DEP protects the polysomes. This is seen in polysome profiles (Figure 16) and is confirmed by the sedimentation coefficients calculated from analytical ultracentrifugation results. Although

ultracentrifugation is an insensitive method, no polysomes were detected; only cytoplasmic monosomes, and ribosomal subunits. These results, together with examination of DEP properties, show that DEP also has severe disadvantages (Figure 17) (Solymosy, Huvös, Guylás, Kovits, Gaál, Bagi and Farkas, 1970) (Venkatesan and Steele, 1972).

It is futile to pursue this experimental method unless two criteria are met. Firstly, the RNase problem must be resolved. Secondly, recovery of a sufficiently accurate, representative sample of the total ribosome population is necessary. The sedimentation analysis of the type needed for this study is also fraught with difficulties. Induction of dissociation in the course of sedimentation is reported (Spirin, Belitsina and Lishnevskaya, 1972). The results (b) giving values of the sedimentation coefficients above are of note. Discussing the methods of analysis normally employed, Leaver and Dyer point out that "a substantial proportion of the total ribosome population is not recovered. Further, a selective recovery of polyribosomes is obtained, which may result in a misleading representation of the ribosome distribution and cellular capacity for protein synthesis of the tissue under study" (Leaver and Dyer, 1974). In view of the level of experimental error inherent in this method and the difficulties in comparing subtle differences between low

populations of ribosomes, this present method does not yield the results required. However, qualitatively, the range of polysome sizes, shown in the polysome profiles (Figure 15 and 16), are obtained.

BIBLIOGRAPHY

ANDREAE, S. and W.G. HOPKINS (1973) Interaction of abscissic acid and red light during induction of flowering of Chenopodium rubrum. Plant Physiol. 51, suppl. 158, pg. 29.

ASPINALL, G.O. (1970) Pectins, Plant Gums and Other Plant Polysaccharides, p. 515-536. In W. Pigman and D. Horton (ed.). The Carbohydrates, Chemistry and Biochemistry. Second Edition, Vol. IIB. New York and London: Academic Press Inc.

AUDERSET, G. and H. GREPPIN (1977) Effet de l'induction florale sur l'evolution ultrastructurale de l'apex caulinaire de Spinacia oleracea, Nobel Protoplasma. 91, 281-301.

BATEMAN, D.F. and H.G. BASHAW (1976) Degradation of Plant Cell Walls and Membranes, p. 316-355. In R. Hertefuss and P.H. Williams (ed.). Physiological Plant Pathology, Berlin, Heidelberg and New York: Springer-Verlag.

BERLIN, J., S. SIEG, D. STRACK, M. BOKERN and H. HARMS (1986) Production of betalains by suspension cultures of Chenopodium rubrum. L. Plant Cell Tissue Organ Culture 5, 163-174.

BERNIER, G. (ed.) (1970) Cellular and molecular aspects of floral induction. Proceedings of a Symposium Liege, Belgium, 4-8 September, 1967. London: Longman Group Ltd.

BERNIER, G. (1971) Structural and metabolic changes in the shoot apex in transition to flowering. Can. J. Bot. 49: 803-819.

BERNIER, G., J.M. KINET and R. BRONCHART (1967) Cellular events at the meristem during floral induction in Sinapis alba. Physiol. Veg. 5, 311-324.

BERNIER, G., J.M. KINET and R.M. SACHS (1981) The Physiology of Flowering. Vol. II, Boca Raton, Florida: CRC Press Inc.

BLAKELY, C.M. and F.C. STEWARD (1964) Growth and Organized Development of Cultured Cells vs. the Growth of Colonies From Free Cells in Nutrient Agar. Am. J. Botany. 51, 780-791.

BOLLINI, R. and M.J. CHRISPEELS (1979) The Rough Endoplasmic Reticulum is the Site of Reserve-Protein Synthesis in Developing Phaeolus vulgaris cotyledons. Planta. 145, 487.

BOULTER, D. (1970) Protein Synthesis in Plants. Ann. Rev. Plant Physiology. 21, 91-114.

BRAKKE, M.K. (1963) Photometric Scanning of Centrifuged Density Gradient Columns. Analytical Biochemistry. 5, 271-283.

BROWN, R. (1958) Cellular Basis for Induction of Morphological Structures. Nature. 181, 1546-7.

BRYANT, S.V. (1977) Pattern Regulation and cell commitment in Amphibian Limbs. In The Clonal Basis of Development. 36th Symposium of the Society for Developmental Biology. New York: Academic Press, Inc.

BUVAT, R. (1952) Structure, evolution et fonctionnement du meristeme apical de quelques dicotyledones. Ann. Sci. Nat. Bot. Biol. Veg. 11é ser, 13, 199-300.

BUVAT, R. (1958) Recherches sur les infrastructures du cytoplasme dans les cellules du meristeme apical des ebauches foliarires et des feuilles developpees d'Elodea canadensis. Annu. Des. Cr. Nat. Bot. XI. 19, 121-161.

CHAILAKHYAN, M.H. (1936) On the mechanism of photoperiodic reaction. C.R. (Doklady) Acad. Sci. U.R.S.S. 10, 89-93.

CHAI-LOOI, and AH SING (1971) Ph.D. Thesis, University of New Brunswick, Fredricton, N.B.

CHALY, N. (1973) Cytological studies of growing cells in the root. M.Sc. Thesis, Carleton University, Ottawa, Canada.

CHALY, N.M. and G. SETTERFIELD (1975) Organization of the Nucleus, Nucleolus, and Protein Synthesizing Apparatus in Relation to Cell Development in Roots of Pisum Sativum. Can. J. Bot. 53, 200-218.

CHERVENKA, C.H. (1969) A Manual of Methods for the Analytical Ultracentrifuge. Palo Alto, California: Spinco Division of Beckman Instruments Inc.

CHOUINARD, L.A. (1974) An Electron-Microscope Study of the Intranucleolar Chromatin in Root Meristematic Cells of Allium Cepa. J. Cell Sci. 15, 645-657.

CLARK, M.F., R.E.F. MATTHEWS and R.K. RALPH (1964) Ribosomes and Polyribosomes in Brassica Pekinensis, Biochimica et Biophysica Acta, 91, 289-304.

CLOWES, F.A. (1958) Development of quiescent centres in root meristems. New Phytol. 57, 85-88.

CLOWES, F.A.L. (1961) Apical Meristems. Oxford: Blackwell Scientific Publications.

CLOWES, F.A. (1963a) X-irradiation of root meristems. Annu. Bot. 27, 343-352.

CLOWES, F.A. (1963b) The quiescent center in meristems and its behaviour after irradiation. In Meristems and Differentiation. Brookhaven Symposia in Biology. 16, 46-58. Brookhaven Nat. Labs., Assoc. Univ. Inc.

CUMMING, B.G. and JANET E.A. SEABROOK (1985) Chenopodium In C.R.C. Handbook of Flowering. Vol. II, Halevy, Abraham M. (ed.) C.R.C. Press, Boca Raton, FLA.

CUMMING, B.G. (1963) Evidence of a Requirement for phytochrome P_{FR} in the Floral Initiation of Chenopodium rubrum. Can. J. Bot. 41, 901-926.

CUMMING, B.G. (1966) Early-Flowering Plants. In F.H. Wilt and N.K. Wessells (ed.). Methods in Developmental Biology. Thomas Y. Crowell Co.

CUMMING, B.G. (1967) Circadian rhythmic flowering responses in Chenopodium rubrum: effects of glucose and sucrose. Can. J. Bot. 45, 2173-2193.

CUMMING, B.G. (1969) Circadian Rhythms of Flower Induction and their Significance in Photoperiodic Response. Can. J. Bot. 47, 309-324.

CUMMING, B.G. (1969b) Chenopodium rubrum L. and related species. In L.T. Evans (ed.). The Induction of Flowering, pp. 156-189. Ithaca, New York: Cornell University Pres.

CUMMING, B.G. (1969c) Photoperiodism and rhythmic flower induction: complete substitution of inductive darkness by Light. Can. J. Bot. 47, 1241-1250.

CUMMING, B.G. (1971) The Role of Circadian Rhythmicity in Photoperiodic Induction in Plants. Proc. Int. Symp. of Ciroadian Rhythmicity (Wageningen, 1971), 30-82.

CUMMING, B.G., S.B. HENDRICKS and H.A. BORTHWICK (1965) Rhythmic flowering responses and phytochrome changes in a selection of Chenopodium rubrum. Can. J. Bot. 43, 825-853.

CUMMING, B.G. and E. WAGNER (1968) Rhythmic Processes in Plants. Annu. Rev. Plant Physiol. 19, 381-415.

DAVIS, K.R., A.G. DARVIL, P. ALBERSHEIM, and A. DELL (1986) Host-Pathogen Interactions. Plant Physiology. 80, 568-577.

DE BARSY, TH., R. DELTOUR and R. BRONCHART (1974) Study of Nucleolar Vacuolation and RNA Synthesis in Embryonic Root Cells of Zea Mays. J. Cell Sci. 16, 95-112.

DOORENBOS, J. and S.J. WELLENSIEK (1959) Photoperiodic Control of Floral Induction. Annu. Rev. Plant Physiol. 10, 147-184.

EAMES, A.J., L.H. MCDANIELS (1947) Plant Anatomy. New York, McGraw-Hill Book Co. Inc.

EILAM, Y. R.D. BUTLER and E.W. SIMON (1971) Ribosomes and Polysomes in Cucumber Leaves during Growth and Senescence. Plant Physiol. 47, 317-232.

ELGIN, S.C. and H. WEINTRAUB (1975) Chromosomal Proteins and Chromatin Structure. Annu. Rev. Biochem. 44, 725-774.

ERICKSON, R.O. and F.J. MICHELINI (1957) The Plastochron Index. Am. J. Bot. 44, 297-304.

ESAU, K. (1965) Plant Anatomy. Second Edition. New York: J. Wiley and Sons.

EVANS, L.T. (ed.) (1969) The Induction of Flowering. Ithaca, New York: Cornell University Press.

EVANS, L.T. (1971) Flower Induction and the Florigen Concept. Annu. Rev. Plant Physiol. 22, 365-394.

FOSTER, A.S. (1938) Structure and Growth of the short apex in Gingko bilboa. Bull. Torrey Bot. Club. 65, 531-556.

FROSCH, S. and E. WAGNER (1973) Endogenous rhythmicity and energy transduction. II Phytochrome action and the conditioning of rhythmicity of adenylate kinase, NAD- and NADP-linked glyceraldehyde-30-phosphate dehydrogenase in Chenopodium rubrum by temperature and light intensity cycles during germination. Can. J. Bot. 51, 1521-28.

GEMMELL, A.R. (1969) Developmental Plant Anatomy. London: Edward Arnold Ltd.

GIFFORD, E.M. (1963) Developmental studies of vegetative and floral meristems. In Meristems and Differentiation. Brookhaven Symposia in Biology, 16, 126-137. Brookhaven Nat. Labs. Assoc. Univ. Inc.

GIFFORD, E.M. Jr. and G.E. CORSON (1971) The Shoot Apex in Seed Plants. Bot. Rev. 37, 143-217.

GIFFORD, E.M. Jr. and K.D. STEWART (1965) Ultrastructure of Vegetative and Reproductive Apices of Chenopodium album. Science. 149, 75-76.

GIFFORD, E.M. and H.B. TEPPER (1962) Histochemical and autoradiographic studies of floral induction in Chenopodium album. Am. J. Bot. 49, 706-714.

GIFFORD, E.M. and H.B. TEPPER (1962) Ontogenetic and Histochemical Changes in the Vegetative Shoot Tip of Chenopodium album. Am. J. Bot. 49, 902-911.

GIFFORD, E.M. and H.B. Tepper (1967) 1961 Ontogeny of inflorescence in Chenopodium album. Am. J. Bot. 48, 657-667.

GOOD, N.E., G.D. WINGET, W. WINTER, T.N. CONNOLLY, S. IZAWA and R.M.M. SINGH (1966) Hydrogen Ion Buffers for Biological Research. Biochemistry. 5, 467-477.

GREGOIRE, V. (1938) La Morphogenese et l'autonomie morphologique de l'appareil floral. I. Le Carpelle Cellule. 47, 287.

GUNNING, E.E.S. and M.W. STEER (1975) Plant Cell Biology an Ultrastructural Approach, Plate 20. London: Edward Arnold (Pub) Ltd.

HAVELANGE, A. (1980) The Quantitative Ultrastructure of the Meristematic Cells of xanthium strumarium During the Transition to Flowering. Am. J. Bot. 67, 1171-1178.

HAVELANGE, A. and G. BERNIER (1974) Descriptive and Quantitative Study of Ultrastructural Changes in the Apical Meristem of Mustard in Transition to Flowering. I. the Cell and Nucleus. J. Cell Sci. 15, 633-644.

HAVELANGE, A. G. BERNIER and A. JACQUARD (1974) Descriptive and Quantitative Study of Ultrastructural Changes in the Apical Meristem of Mustard in Transition to Flowering. II. the Cytoplasm. Mitochondria and Proplastids. J. Cell Sci. 16, 421-432.

HEALY, P.L. (1964) Histochemistry and ultrastructure in the shoot of Pharbites before and after floral induction. Ph.D. Dissertation, University of California, Berkeley, California.

HEITZ, E. (1929) Heterochromatin, Chromozentren, Chromomeren. Ber. dt. bot. Ges. 47, 274-284.

HENDRICKS, S.B. and H.A. BORTHWICK (1965) The physiological functions of phytochrome, pp. 405-436. In Goodwin (ed.), Plant Pigments, New York: Academic Press.

HILLMAN, W.S. (1962) The Physiology of Flowering. New York: Holt, Rinehart and Winston.

HOAGLAND, D.R. and D.I. ARNON (1939) The Water culture method for growing plants without soil. Cuc. Calif. Agr. Exptl. Sta. 327, 1-39.

HOEFERT, L.L. (1968) Polychromatic stains for thin sections of Beta-embedded in Epoxy Resin. Stain Technol. 43, 145-151.

HYDE, B.B. (1967) Changes in Nucleolar Ultrastructure Associated with Differentiation in the Root Tip. J. Ultrastruct. Res. 18, 25-54.

KING, R.W. (1971) Time Measurement in Photoperiod control of flowering. Ph.D. Thesis, Department of Plant Sciences, University of Western Ontario, London, Ontario.

KING, R.W. (1972) Timing in Chenopodium rubrum of export of the floral stimulus from cotyledons and its action at the shoot apex. Can. J. Bot. 50, 697-702.

KING, R.W. (1979) Photoperiodic Time Measurement and Effects of Temperature on Flowering in Chenopodium rubrum. Aust. J. Plant Physiol. 6, 417-422.

KING, R.W. and CUMMING, B.G. (1972) The Role of Phytochrome in Photoperiodic Time Measurement and its Relation to Rhythmic Time Keeping in the Control of Flowering in Chenopodium rubrum. Planta 108, 39-57.

LAFONTAINE, J.G. (1968) Structural components of the nucleus in mitotic plant cells, pp. 151-196. In A.J. Dalton and F. Haguenau (ed.). In Ultrastructure in Biological Systems: The Nucleus, Vol. 3, New York and London: Academic Press.

LAFONTAINE, J.G. and A. LORD (1974a) An ultrastructural and radioautographic study of the evolution of the interphase nucleus in plant meristematic (Allium porrum). J. Cell Sci. 14, 263-287.

LAFONTAINE, J.G. and A. LORD (1974b) A correlated light- and electron-microscopic investigation of the structural evolution of the nucleolus during the cell cycle in plant meristematic cells (Allium porrum). J. Cell Sci. 16, 63-93.

LANCE, A. (1957) Recherches cytologiques sur l'evolution de quelques méristemes apicaux et sur ses variations provoquées par des traitment photopériodiques. Ann. Sci. Nat. Bot. Biol. Veg. ser. 11, 18, 91-421.

LANG, A. (1965) "Physiology of Flower Initiation". Encyclopedia of Plant Physiology Edt. Ruhland, W. Volume VV/1 Springer-Verlag.

LEAVER, C.J. and J.A. DYER (1974) Caution in the Interpretation of Plant Ribosome Studies. Biochem. J. 144, 165-167.

LECHARNY, A., M. SCHWALL and E. WAGNER (1985) Stem Extension Rate in Light-grown Plants Effects of Photoperiodic and Thermoperiodic Treatments on the Endogenous Circadian Rhythm in Chenopodium rubrum. Plant Physiol. (Bethesda) 79, 625-629.

LIN, J. and E.M. GIFFORD (1976) The distribution of ribosomes in the vegetative and floral apices of Adonis aestualis. Can. J. Bot. 54, 2478-2483.

LIN, C.Y., J.L. KEY and C.E. BRAKER (1966) Association of d-RNA With Polyribosomes in Soybean Root. Plant Physiology. 41 (6), 976-982.

LIN, C.Y. and J.L. KEY (1967) Dissociation and Reassembly of Polyribosomes in Relation to Protein Synthesis in the Soybean Root. J. Mol. Biol. 26, 237-247.

LONA, F. (1948) La fioritura della bevidiurna Chenopodium amaranticoror coste et Rein coltivata in soluzione nutritizia con saccarosio, in assenza di stimolo fotoperiodico euflorigeno. Nuova Giorn. Botan. Ital. 55, 55-59.

LORD, A. and J.G. LAFONTAINE (1976) An ultrastructural and radioautographic study of the chromocentric interphase nucleus in plant meristematic cells (Raphanus Sativus). J. Cell Sci. 21, 193-207.

LUMSDEN, R.D. (1976) Pectolytic enzymes of Sclerotinia sclerotiorum and their Localization in Infected Bean. Can. J. Bot. 54, 2530-2641.

MANUAL OF MICROBIOLOGICAL METHOD. Society of American Bacteriologists Committee on Bacteriological Technique. New York: McGraw-Hill Book Co. Inc. (1975).

MANTON, I. (1935) Some new evidence on the physical nature of plant nuclei from intraspecific polyploids. Proc. R. Soc. B 118, 522-547.

MARINOZZI, V. (1964) J. Ultrastructure. Res. 10, 433.

MARKS, P.A., E.R. BURKA, F.M. CONCONI, W. PERL and R.A. RIFKIND (1965) Polyribosome Dissociation and Formation in Intact Reticulocytes with Conservation of Messenger Ribonucleic Acid. Proc. Nat. Acad. Sci. Wash. 53, 1437.

MEHTA, S.L. and S. ZALIK (1969) Amino Acid Incorporation by Cytoplasmic Polysomes from Wheat Leaves. Biochem. Biophys. Acta 195, 515-522.

MEINS, F. and A.M. BINNS (1977) Epigenetic Clonal Variation in the Requirement of Plant Cells for Cytokinins. In The Clonal Basis of Development. 36th Symposium of the Society for Developmental Biology. New York: Academic Press, Inc.

MORRIS, R.O. (1986) Genes Specifying Auxin and Cytokinin Biosynthesis in Phytopathogens. Annu. Rev. Plant Physiol. 37, 509-538.

NESTER, E.W., M.P. GORDON, R.M. AMASINO and M.F. YANOFSKY (1984) Crown Gall: A Molecular and Physiological Analysis. Annu. Rev. Plant Physiol. 35, 387-413.

NOUGAREDE, A. (1967) Experimental cytology of the short apical cells during vegetative growth and flowering. Int. Rev. Cytol. 21, 203-351.

REYNOLDS, E.S. (1963). The use of Lead Citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-212.

ROSE, R.J., G. SETTERFIELD and L.C. FOWKE (1972) Activation of Nucleoli in Tuber Slices and the Function of Nucleolar Vacuoles. Expt. Cell Res. 71, 1-16.

SALKSTON, W.E. and W.W. SHEPPARD (1973) Effect of Day Lengths on Reaction of Sunflowers to Verticillium Wilt. Can. J. Bot. 51, 23-26.

SCHACHMAN, H.K. (1957) Ultracentrifugation, Diffusion and Viscometry. New York: Academic Press Inc.

SCHMIDT, A. (1924) Bot. Arch. 9, 345.

SCHWALL, M., H. WENDORF and E. WAGNER (1985) Diurnal Modulation of Arginine Decarboxylase Activity of Chenopodium rubrum by Temperature and Light. Plant Cell Physiology 26, 1607-1661.

S.E.B. SYMPOSIA. (1963) Cell Differentiation, vol. 17. Cambridge: At The University Press.

SHORT, K.C., D.A. TEPPER and D.E. FOSKET (1974) Regulation of Polyribosome formation and cell division in cultured soybean cells by cytokinin. J. Cell Science 15, 75-87.

SINNOTT, E.W. (1960) Plant Morphogenesis. New York: McGraw-Hill.

SOLYMOSY, F., P. HUROS, A. GUYLAS, I. KAPOVITS, O. GAAL, G. BAGI and G.L. FARKAS (1970) Diethyl Pyrocarbonate, a New Tool in the Chemical Modification of Nucleic Acids? Biochim. Biophys. Acta 238, 406-416.

SPIRIN, A.S., N.V. BELITSINIA and E.B. LISHNEVSKAYA (1972) On Some Artifacts of sucrose gradient sedimentation of ribosomes. FEBS Letters. 24, 219-224.

STARR, M.P., A.K. CHATTERJEE, P.B. STARR and G.E. BUCHANAN (1977) Enzymatic Degradation of Polygalacturonic Acid by Yersinia and Klebsiella Species in Relation to Clinical Laboratory Procedures. J. Clinical Microbiology. 6, 379-386.

STEBBINS, G.L.V. (1964) Four Basic Questions of Plant Biology. Am. J. Botany 51, 220-230.

STEWARD, F.C. (1958a) Interpretations of the Growth from New Cell to Carrot Plant. Am. J. Botany 45, 709-713.

STEWARD, F.C. (1958b) Totipotency and Variation in Cultured Cells: Some Metabolic and Morphogenetic Manifestations. Plant Tissue and Organ Culture .. a Symposium 1961. Ed. P. Maheshwari and N.S. Ranga Swamy. Publ. Int. Soc. Plant Morphologists. Delhi, 1963.

STEWARD, F.C. (1968) Growth and Organisation in Plants. Addison-Wesley.

STEWARD, F.C. (1969) Plant Physiology: Analysis of Growth - The Responses of Cells and Tissues in Culture. Vol. VB. Academic Press.

STEWARD, F.C., M.O. MAPES, J. SMITH (1958) Growth and Divisions of Freely Suspended Cells. Am. J. Botany 45, 693-703.

STEWARD, F.C., M.O. MAPES, K. MEARS (1958) Organisation in Cultures grown from Freely Suspended Cells. Am. J. Botany 45, 705-708.

TSALA, G., S. FROSCH, M. BONZON, R. BERGFELD, H. GREPPIN and E. WAGNER (1984) Photoperiod and Light Quality Effects on Rate of Dark Respiration and on Photosynthetic Capacity in Developing Seedlings of Chenopodium rubrum. Physiol. Plant 630, 146-152.

VENKATESAN, N. and W.J. STEELE (1972) Effects of Diethyl Pyrocarbonate on the Isolation and Recovery of Bound Polysomes from a Particulate Fraction of Rat Liver. FEBS Letters. 24, 215-218.

VON DENFFER, D. (1950) Naturwissenschaften, 37, 296.

WAGNER, E. and B.G. CUMMING (1970) Betacyanin accumulation, chlorophyll content and flower initiation in Chenopodium rubrum as related to endogenous rhythmicity and phytochrome action. Can. J. Bot. 48, 1-18.

WAGNER, E. and S. FROSCH (1971) Conditioning circadian rhythmic control of Betacyanin content in Chenopodium rubrum by temperature and light intensity cycles during germination. Can. J. Bot. 49, 1981-1985

WAGNER, E., L. HAERTLE and M.G. HOLMES (1980) Photoperiodic control of flowering with and without photosynthesis as related to changes in the pattern of adenylate kinase isozymes in Chenopodium rubrum.

WEEKS, D.P. and A. MARCUS (1969) Polyribosome Isolation in the Presence of Diethyl Pyrocarbonate. Plant Physiology. 44, 1291-1294.

ZANG, L. and J.Y. TAKEMOTO (1987) Effects of Pseudomonas syringae Phytotoxin, Syringomycin on Plasma Membrane Functions of Rhodotorula pilimanae. Phytopath. 77, 297-303.

PLATE 55

ADDENDUM I: BACTERIAL INFECTION

Micrograph showing extensive splitting of the wall along the middle lamella, resulting from digestion of the pectin. Magnification x 27,000.