AN ANALYSIS OF CELL GROWTH AND DIVISION IN GERMINATING AND MATURE ROOT MERISTEMS OF *VICIA FABA*

By

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ANALYSIS OF CELL GROWTH AND DIVISION IN MERISTEMS OF *V. FABA*

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ABSTRACT

The mature root meristem of the broad bean, *Vicia faba*, consists of a steady-state, asynchronous population of cells in which rates of cell growth and of division are constant, on average, from one time to the next. Measurements of individual cells for any growth parameter, however, show considerable variation about the mean of that parameter. This study was done in order to 1) determine the pattern of cell growth and division in the root meristem during seed germination as it develops into a mature root; 2) determine how this pattern changes in different environments, and 3) determine if events that occur during germination contribute to variation in cell growth and division seen in the mature root.

Cell growth and division were manipulated by 1) changing the amount of water available for growth of germinating and mature roots and 2) treating roots with 5-amino uracil (5-AU), a drug which temporarily arrests cells at the S to G_2 transition point of the cell cycle (Socher and Davidson, 1971). Cell area, nuclear volume and nuclear protein content were used to monitor cell

growth: mitotic index (M.I.) and cell cycle kinetics determined 1) from colchicine accumulation of cells in mitosis and 2) from changes in the frequency of labelled mitoses after pulse labelling cells synthesizing DNA with ^{3}H-thymidine, were used to monitor cell division.

The results show that cells in the mature root meristem reach different steady-states that are influenced by the ambient water level present during germination. The characteristics of each steady-state are unique in the M.I., the size ratio, nucleus: cytoplasm and the relative proportion of fast, slow and non-cycling cells.

Mature roots subjected to a change in the ambient water level appear only to modify the initial steady state established during germination; ie., roots grown initially in different amounts of water and then transferred to the same ambient water level do not attain a steady-state condition with identical mean values for different cell parameters. Treatment of germinating and mature roots with 5-AU allows cells and nuclei to grow to sizes greater than those seen in untreated roots but does not appear to interfere with normal relationships between most growth parameters. Neither the relationship between cell and nuclear size nor nuclear protein content is altered in 5-AU treated roots to produce values not found in untreated roots.

From these results it is proposed that a specific programming of cell growth and division is established during germination and is largely determined by environmental conditions present in the early stages of seedling growth. Modifications of this programmed control of cell growth can be induced by changing the external growth conditions. In addition, there are internal fluctuations, within a meristem, in the environment of identical cells; these fluctuations are thought to contribute to the variation in cell parameters that are seen in mature roots.

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CHAPTER I

INTRODUCTION

Growth of proliferating cells is characterized by an increase in: 1) cell mass and 2) cell number. These increases are usually integrated and each doubling in cell number is accompanied by a doubling in cell mass. In terms of an individual cell this means that in each cell cycle the nucleus doubles its DNA content and this is accompanied by a doubling in cell mass and cell volume. Experimental evidence indicates that the cell cycle can be divided into a "growth cycle" during which synthesis and growth of cellular constituents takes place and a "DNA-division cycle" during which the nuclear DNA replicates and divides (Mitchison, 1971).

A cell cycle consists of mitosis and interphase. DNA synthesis occurs during interphase in a period called the S phase. The pre-S and post-S phases are called G_1 and G_2 (Howard and Pelc, 1953).

Since in typical cell cultures both DNA and cell mass double on average within one cell cycle, the cells are said to show "balanced growth". This is seen in cultured yeast, amoeba and mouse fibroblasts (Mitchison, 1971; Prescott, 1955; Zetterberg and Killander, 1965).

Doublings over one cell cycle have also been reported for total protein, RNA, cell volume, nuclear volume, cytoplasmic and nuclear dry mass (Prescott, 1955; Zetterberg and Killander, 1965; Mitchison, 1971).

Cells whose doubling time is increased after some experimental modification often show large increases in mass and volume: when increases in cell mass occur without a parallel increase in DNA content the cells are said to show "unbalanced growth". Typically this occurs when: 1) the DNA-division cycle is blocked, 2) cell cycle duration is increased and 3) cell growth continues throughout the extended cycle duration. The consequences of blocking the DNA-division cycle are illustrated in the first report of unbalanced growth. A thymine requiring mutant of *Escherichia coli* was deprived of thymine. DNA synthesis and cell division were stopped but cell size and RNA content continued to increase (Cohen and Barner, 1954). Cells with mutations that block the initiation or maintenance of DNA synthesis in the budding yeast, *Saccharomyces cerevisiae*, also grow to sizes much greater than those seen in a normal population (Hartwell *et al.*, 1974). Similarly, giant cells are produced by both yeast and animal cells after treatment with drugs which inhibit DNA synthesis (Slater, 1973; Cohen and Studzinski, 1969; Rueckert and Mueller, 1960).

Most cells experience and withstand changes in their environment; their response to an environmental change is often to change their growth rate. This can result in a change in the DNA to mass ratio. When the fission yeast, *Schizosaccharomyces pombe*, is grown at increasing temperatures, the DNA to mass ratio measured at division increased; cell volume at division and mean cell doubling time decreased (Mitchison, Kinghorn and Hawkins, 1963). However, yeast grown in progressively better nutrient media increased in both mean growth rate and cell size (Nurse and Thuriaux, 1977). Thus, even though DNA content per cell remains constant, when cells with higher growth rates have completed a growth cycle and are about to divide, cell size at division varies directly with the environment.

Cells attain a new constant growth rate when placed in a new stable environment indicating that a new stable state has been achieved. Once this new state is established growth is again balanced. This means that for any cell there is an infinite number of possible balanced growth states and that balanced growth can be defined only for cells that have achieved a stable state within a given environment (Anderson, Peterson and Tobey, 1967).

Studies of single cells have shown that cell size, cell mass and many other parameters undergo a doubling during one growth cycle. In populations of cells, however, most cell parameters show more than a two-fold

spread of values, even when the cells are kept under constant environmental conditions; it is not uncommon to find three- to six-fold ranges of values. Cell cycle duration, cell size, dry mass and RNA content have all been shown to be highly variable (Tables 1-3). This variability is found in cells at early G_1 and at mitosis and is maintained throughout interphase. Even nuclei show a wide range in size and protein contents during the cell cycle (Table 4).

Because individual cells double in both size and cellular constituents during one cell cycle, the high degree of variability seen in cell parameters within a population must arise either by differences in growth rate or by asymmetrical mitoses. Both situations occur: in the root meristem of corn 76 to 79 percent of all pairs of sister cells have different lengths in early G_1 (Ivanov, 1971). In *Vicia faba* each sister nucleus from a pair in G_1 can have a different volume (Stallwood and Davidson, 1977; Davidson and Golding, 1978). Sister cells also differ in cell cycle duration (Kubitschek, 1962; Prescott, 1959; Ivanov, 1971). These differences between sister cells contribute to the variation in growth seen within populations of cells.

The results from studies of cell growth kinetics of unicellular organisms in culture have provided data on different patterns of cell growth and have also been

TABLE 1

VARIATION IN CELL CYCLE DURATION

Mean, range and variation given as the maximum/minimum value of cycle times for individual cells of bacteria, animals and plants. Data are cited directly or have been estimated from the published data. Some values for multicellular organisms are based on average responses obtained from percent labelled mitoses or labelling index curves for a population of cells.

Organism or cell type	Parameter measured	Sample size	Mean ± S.D.	Range	Variation max/min	Reference
Escherichia coli	cycle time	189	26.5 min	13.9 → 53.5 min	3.85x	Kubitschek, 1962
Mouse leukemia	cycle time	115	22.4 hr ± 4.2	14.5 → 35.5 hr	2.45x	Killander, 1965
Tetrahymena geleii	cycle time	766	111 min ± 10	82 → 149 min	1.82x	Prescott, 1959
Amoeba proteus	cycle time	70	~25 hr	21 → 33 hr	1.6x	Prescott, 1956
Amoeba proteus	cycle time		~25 hr	23.5 → 40 hr	1.7x	Prescott, 1955
Human leukemia	cycle time	From PLM & LI's	25.3 hr	~21.9 → >120 hr	>5.5x	Yen *et al.*, 1975(a)
Vicia faba (long lateral root meristem)	cycle time	From PLM & LI's		~14 → ≥30 hr	>2.0x	Webster and Davidson, 1968
Vicia faba (old primary root meristem)	cycle	From PLM & LI's		~19.5 → ≥60 hr	>3.0x	Miller *et al.*, 1978

TABLE 2

VARIATION IN CELLULAR DRY MASS AND CELL SIZE

Mean, range and variation given as the maximum/minimum value of cellular dry mass or cell size during interphase, early G_1 and mitosis. All values are cited directly or estimated from data in the literature.

Organism or cell type	Parameter measured	Sample size	Mean ± S.D.	Range	Variation max/min	Reference
Amoeba proteus	Dry mass in early G_1	70	10.1 mμg ± 1.89	6.2 → 14.4 mμg	2.32x	Prescott, 1956
Amoeba proteus	Dry mass at division	35	20.1 mμg ± 0.7	18.9 → 22 mμg	1.16x	Prescott, 1956
Mouse leukemia	Dry mass in interphase	335	49.1 arbitrary units (A.U.) (some G_0 cells)	48 → 60 A.U.	3.33x	Killander, 1965
Mouse leukemia	Dry mass in early G_1	36	23.8 A.U. ± 9.6	~20.5 → 27 A.U.	1.32x	Killander, 1965
Mouse leukemia	Dry mass at division	25	53.9 A.U. ± 18	~46 → 62 A.U.	1.35x	Killander, 1965
Tetrahymena pyriformis	Cell volume in interphase	600	25,119 μm³ (mode)	~12,589 → 44,668 μm³	3.55x	Scherbaum and Rasch, 1957
Schizosaccharomyces pombe	Cell length at cell plate formation		13.6 μm (median)	9 → 17 μm	1.9x	Mitchison and Wilbur, 1962
Chinese hamster lung V79	Cell volume in interphase	700	705 μm³ (median)	273 → 1977 μm³	7.2x	Sinclair and Ross, 1969
Human leukemia SK-L7	Cell area in interphase	400	162 μm² (median)	50 → 475 μm²	9.5x	Yen *et al.*, 1975(b)
Human leukemia SK-L7	Cell area at division	100	400 μm² (median)	137.5 → 687.5 μm²	5 x	Yen *et al.*, 1975(a)
Vicia faba (lateral root meristem)	Cell area in interphase	300	366 μm² ± 152	106 → 1098 μm²	10.4x	Davidson *et al.*, 1978
Vicia faba (lateral root meristem)	Prophase cell area	100	500 μm² ± 142	245 → 1058 μm²	4.3x	Davidson *et al.*, 1978

TABLE 3

VARIATION IN TOTAL CELLULAR RNA

Mean, range and variation given as the maximum/minimum value of the total cellular RNA present during interphase, early G_1 or mitosis. Values are cited directly or estimated from data in the literature.

Organism or cell type	Parameter measured	Sample size	Mean ± S.D..	Range	Variation min/max	Reference
Mouse leukemia	Interphase Total RNA	227	~171 A.U. (including some G_0 cells)	45 → 395 A.U.	8.8x	Killander, 1965
Mouse leukemia	Early G_1 Total RNA	26	73 A.U. ± 36			Killander, 1965
Mouse leukemia	Mitosis Total RNA	13	127 A.U. ± 32			Killander, 1965

TABLE 4

VARIATION IN TOTAL NUCLEAR PROTEINS AND NUCLEAR SIZE

Mean, range and variation given as the maximum/minimum value of total nuclear proteins or nuclear size during interphase and mitosis. Values are cited directly or estimated from data in the literature.

Organism or cell type	Parameter measured	Sample size	Mean ± S.D.	Range	Variation max/min	Reference
Vicia faba (long lateral root meristem)	Nuclear protein per unit area	95	51 A.U. ± 15	26 → 140 A.U.	5.4x	Bansal and Davidson, 1978
Vicia faba (root meristem long lateral)	Interphase nuclear volume	650	700.1 μm^3 ± 391.9	169 → 2326.8 μm^3	13.8x	Bansal and Davidson, 1978
Vicia faba (young primary)	Interphase nuclear volume	650	781.5 μm^3 ± 369.2	184.3 → 2566.3 μm^3	13.9x	*Ibid.*, 1978
Vicia faba (young primary)	Interphase nuclear volume	650	654.7 μm^3 ± 228.9	202.2 → 1696.5 μm^3	8.4x	*Ibid.*, 1978.
Vicia faba (root meristem long lateral)	Prophase nuclear volume	250	1266.1 μm^3 ± 326.9	428.9 → 2247.6 μm^3	5.2x	*Ibid.*, 1978
Vicia faba (young primary)	Prophase nuclear volume	250	1263.4 μm^3 ± 364.9	437.2 → 2432.4 μm^3	5.6x	*Ibid.*, 1978
Vicia faba (old primary)	Prophase nuclear volume	250	1126.5 μm^3 ± 335.6	429.4 → 2347.1 μm^3	5.5x	*Ibid.*, 1978
Human leukemia SK-L7	Interphase nuclear volume	400	112 μm^3 (median)	25 → 250 μm^3	10x	Yen *et al.*, 1975(a)
Amoeba proteus	Early G_1 nuclear volume	184	7750 μm^3 ± 2103			Prescott, 1955

the basis for the formulation of useful concepts such as balanced and unbalanced growth. In studies of cell proliferation in multicellular organisms, however, several new factors must be considered because of the special properties of populations of proliferating cells. For example, meristems of roots or the intestinal epithelium of mice are steady state systems, that is, the population is not increasing exponentially in size since about half the cells leave the population in a period equal to the mean cell doubling time.

Most cells in unicellular cultures can be grouped into one population of cells with cycle times that are narrowly distributed about the mean cycle time (Kubitschek, 1962). Cells in multicellular organisms, however, often appear to be grouped into sub-populations with distinctly different mean cycle times. Cells within these sub-populations are referred to as fast and slow cycling. Fast and slow cycling cells have been identified in *Vicia, Pisum, Triticum* and *Zea* (Table 5). Cells from these organisms also tend to show a broader range in cell and nuclear sizes than do cells in unicellular cultures (Tables 2 and 4).

The added degree of heterogeneity in growth parameters measured in multicellular systems may force us to re-evaluate our concept of balanced and unbalanced growth in multicellular systems as compared with that

TABLE 5

FREQUENCY OF FAST AND SLOW CYCLING CELLS

Proportions of fast and slow cycling cells and the mean cycle time for the fast cycling cells of different plant meristems. Data are cited directly or estimated from data given in the literature.

Organism	Percent fast cycling cells	Percent slow cycling cells	Mean cycle time of fast cycling cells	Reference
Helianthus annus	86.1[1]	13.9[1]	12.5[2] hr	[1] Evans and Van't Hof, 1975
Pisum sativum	73.9[1]	26.1[1]	12[3] hr	[2] Burholt and Van't Hof, 1971
Triticum aestivum	59.6[1]	40.4[1]	13[1] hr	[3] Van't Hof *et al.*, 1960
Vicia faba (old primary)	62.9[1]	37.1[1]	25[4] hr	[4] Millen *et al.*, 1978
Vicia faba (long lateral)	76	24	14 hr	Webster and Davidson, 1968
Zea mays (stele)	76	24	14.3 hr	Clowes, 1971

seen in single cell cultures. The study reported here examines cell growth and proliferation in a multicellular system, the root meristem of the broad bean, *Vicia faba*. Roots were studied both under conditions which allowed for normal growth and under conditions that resulted in unbalanced growth.

The primary root meristem of *Vicia faba* has; 1) steady state growth kinetics; 2) heterogeneity for cell and nuclear size and 3) variability in cell cycle kinetics. Mature roots show a stable distribution of each of these parameters at successive fixation times. The mature, stable root meristem is the product of a series of changes; it does not begin as a stable system at the time when a dormant seed is allowed to imbibe water. The radicle of a dormant seed of *V. faba* is very different from the growing root. Not only are the cells not growing or dividing, but 90 percent of the cells are normally arrested in G_1 (Jakob and Bovey, 1969). When seeds are allowed to imbibe water, the cells and nuclei are activated and proceed through the first cell cycle in a semi-synchronous wave (Murin, 1967).

One of the primary objectives of the present study, therefore, was to monitor the changes that occur as the cells of the dormant root are activated and proceed through the first two to three cell cycles. Results on 1) cell cycle duration, 2) cell areas,

3) nuclear volumes and 4) total nuclear proteins are presented here. It will be shown that they differ significantly from values of the same parameters from mature roots. These differences led to further determinations that defined when in root growth the form of the distributions of the four parameters given above change from those of the newly germinated radicle to those of the mature root. Secondly, experiments were carried out to determine the extent to which changes in these parameters are influenced by the conditions under which the dormant seed is allowed to germinate. The amount of water available from the onset of imbibition has been shown to influence the subsequent growth pattern of barley seedlings (Davidson, Eastman and Thomas, 1976; Davidson, 1979). Seed from *V. faba* also responds to different volumes of water; small differences in the volume of water in which seeds are allowed to begin imbibition result in significant changes in cell cycle duration, mitotic index and both cell areas and nuclear volumes. Data from *V. faba* demonstrating these differences is presented here.

Seeds allowed to initiate imbibition on one volume of water and then transferred to a different volume of water also undergo a change in overall growth pattern. Little is known, however, about this response at a cellular level. Growing seedlings of *V. faba* were

transferred from either 1) high to low or 2) low to high water volumes. Both radicles of germinating seeds and mature roots less than 120 hours old responded to transfers with changes in cell growth: significant changes in the mitotic index and distributions of cell areas and nuclear volumes were observed. These changes, which are reported here, appeared to mimic those observed for cells that show unbalanced growth.

Unbalanced growth, ie., increases in cell mass or cell volume without parallel increases in DNA content, may be induced in cells treated with amethopterin, fluorodeoxyuridine, hydroxyurea or 5-amino uracil (5-AU). These agents cause a delay in the progress of cells to mitosis, yet, do not inhibit cell growth: the cells show unbalanced growth (Rueckert and Mueller, 1960; Cohen and Studzinski, 1969; Davidson, Golding and Armstrong, 1978). In light of the observation that delay in entry into division results in unbalanced growth, root meristems were treated with 5-AU at various stages during germination. The object of these treatments was to determine whether 5-AU directly stimulates cell growth or whether the increases in cell and nuclear sizes result from a lengthened cell cycle. Evidence was found that, at least in part, the increases in cell and nuclear sizes result from a 5-AU induced stimulation of growth.

From these results, we have:

1) defined the changes in cell area, nuclear volume, total nuclear protein and mitotic index that occur during germination and the production of a stable mature root;

2) defined changes in cell area, nuclear volume, mitotic index and cell cycle duration that occur in response to changes in the ambient water supply during germination. The consequences of these changes as they affect growth in the mature root are also outlined, and

3) defined changes in cell area, nuclear volume and mitotic index in response to treatment with 5-AU both during germination and in the mature root.

The results from these experiments show the unique nature of multicellular systems and emphasize both the similarities and differences between growth in unicellular and multicellular systems.

CHAPTER II

MATERIALS AND METHODS

II.1. Germination and Culturing of Beans

Broad bean seeds, *Vicia faba* L., cv. Sutton's Prolific Longpod or Bush were 1) notched above the embryo axis with a file, 2) laid flat between two layers of cheese cloth in flat bottomed trays. Distilled water was added to a depth of about 5 mm and maintained at this level for 24 hours. Seed coats were then removed and the beans were rinsed in distilled water. Growing roots were obtained in one of the following ways: 1) seeds were skewered on stainless steel rods and placed with the embryo axis in aerated distilled water which was changed at least once every 24 hours; 2) seeds were planted in moistened sand (25 ml water/100 ml sand); and 3) seeds were planted in a moistened mixture (1:1, v/v) of vermiculite:perlite (40 ml water/100 ml mix). Containers of either sand or vermiculite-perlite were covered with aluminum foil during seed growth in order to reduce moisture loss by evaporation. If the surface layer dried out, it was moistened with a little additional water. Roots grown under these conditions were fixed after 24 to 175 hours of germination. All roots were grown at 20°C.

In order to determine the response of seeds to different volumes of available water: 1) seeds were placed in water at a depth of 5, 10 and 20 mm for 24 hours and then grown in tanks of water; roots were fixed after 50 to 68 hours of germination; 2) seeds were placed in 5 mm of water for 24 hours, then grown in 25 to 40 ml water/100 ml sand; roots were fixed at 50 to 70 hours; 3) seeds were placed in 5 mm of water for 24 hours, then grown in 20 to 55 ml water/100 ml vermiculite-perlite; roots were fixed at 70 hours; 4) seeds were placed in 5 mm of water for 24 hours, then grown either in 25 ml water/100 ml sand or 40 ml water/100 ml vermiculite-perlite up to 96 hours and transferred to tanks of water; roots were fixed 1 to 48 hours after transfer; and 5) seeds were placed in 5 mm of water for 24 hours, grown in tanks of water up to 144 hours, then transferred to 40 ml water/100 ml vermiculite-perlite; roots were fixed 1 to 48 hours after transfer.

II.2. Treatments with 5-Amino Uracil

Roots of newly germinated (24 - 48 hours) and more mature (124 - 144 hours) seeds were treated with 5-amino uracil (5-AU). Seeds were germinated in 5 mm of water for 24 hours and then transferred to tanks of water. At 24.3 and 50 hours roots were transferred to tanks of

4 mM 5-AU for continuous treatment. Roots were fixed after 3 to 78 hours in 5-AU. Control roots were fixed after 30.3 to 96.3 hours of germination. Usually 2 long, 2 intermediate and 2 short roots were fixed at each time.

In order to obtain mature roots for treatment with 5-AU, roots were grown: 1) in tanks of water; 2) in 25 ml water/100 ml sand; 3) in 40 ml water/100 ml vermiculite-perlite and 4) in sand or vermiculite-perlite, then transferred to tanks of water. Roots were treated with 4 mM 5-AU at 96 to 144 hours of germination. Roots grown in sand or vermiculite-perlite were also treated in sand or vermiculite-perlite, ie., with 25 or 40 ml 5-AU solution/100 ml of mixture. Roots were fixed after 3 to 24 hours continuous treatment with 5-AU.

II.3. Fixation and Staining

Whole root systems were fixed in a chilled mixture of 3 parts ethanol to 1 part acetic acid (v/v). About 0.1 ml of formaldehyde were added to every 40 ml fixative.

Fixed roots were washed for 1 hour and hydrolysed in 1 N HCl (60°C). Short, thick roots were hydrolysed for 10 minutes; roots longer than 30 mm were hydrolysed for 8.5 minutes. All roots were stained in Feulgen's reagent for 1 hour to stain the nuclear DNA.

After staining, root lengths were measured and root caps removed from the meristems. The apical 1 to 2 mm of the root, containing the meristem, was cut from the root and gently macerated in 45% acetic acid with the blunt end of a pair of forceps. A drop of malachite green solution was added to stain cellular proteins; this made cell outlines visible. Permanent preparations were made using the usual techniques (Darlington and LaCour, 1969). When nuclear volumes and cell areas were to be determined cells were not squashed (Bansal and Davidson, 1978).

II.4. Mitotic Index

Mitotic index (M.I.), the percent frequency of mitoses, was determined by scoring 1000 cells per meristem, generally 3 to 5 meristems were scored for each determination.

II.5. Cell Areas and Nuclear Volumes

The length and width of rectangular cells were measured using a Zeiss ocular micrometer at a magnification of 1250X. All measurements were ± 0.3 μm.

Cell area was calculated as follows:

Cell Area = cell length X cell width

Nuclear volumes were calculated as previously described (White and Davidson, 1976). The major and minor axes of nuclei were measured using a Zeiss ocular micrometer at a magnification of 1250X. All measurements were ± 0.3 μm. Nuclei were classified as: 1) spherical; 2) semi-spherical; 3) oval; or 4) elongate. Nuclear volumes were calculated as follows:

1) Spherical Nuclei (v) = $4/3\ \pi r^3$

where r = 1/2 diameter

2) Semi-spherical Nuclei (v) = $4/3\ \pi ab^2$

where a = 1/2 major axis

b = 1/2 minor axis

3) Oval Nuclei (v) = $4/3\ \pi ab\ \frac{(a + b)}{2}$

where a = 1/2 major axis

b = 1/2 minor axis

4) Elongate Nuclei (v) = $4/3\ \pi b^3 + \pi b^2\ (a - b)$

where a = major axis

b = minor axis

Nuclear areas were estimated from nuclear volumes using the following formula:

$$\text{Nuclear Area} = \text{Nuclear Volume}^{2/3}$$

II.6. DNA Determinations by Microspectrophotometry

Fixed roots were washed, hydrolysed for 10 minutes in 1 N HCl at 60°C and stained in freshly made Feulgen's reagent for 1 hour. After measuring the root length, the apical 1 to 2 mm of the meristem was removed from each root, macerated in 45% acetic acid (v/v) and the cells squashed. Coverslips were removed by the dry ice method and the cells were air dried. Slides were then passed through three 10 minute changes of 0.25% $K_2S_2O_5$ in 0.05 N HCl in order to remove any unbound leuco-basic fuchsin and washed for 5 minutes in distilled water. After air-drying, cells were mounted in Permount.

Determinations of total nuclear DNA content were made using the two wavelength method of Patau (1952). Measurements were made with a Leitz microspectrophotometer, model MPV-2, at a magnification of 630X. The two wavelengths used were 565 and 500 nm, chosen as the peak and 50% of the peak absorption for Feulgen's stain bound to DNA. Fifty interphase nuclei were measured in each root; 2C and 4C DNA values were estimated from measurements of 50 telophase and 50 prophase nuclei from a total of 10 roots.

II.7. Protein Determination by Microspectrophotometry

Nuclei were extracted from the apical 1 to 2 mm of the root meristem using a method modified from Van't Hof (1975). At each time 5 root tips were placed in ice-cold 4% formaldehyde (v/v) in 0.132 M phosphate buffer (pH 6.8). After 5 minutes they were transferred to ice-cold 0.132 M phosphate buffer. In order to isolate nuclei, each meristem was placed in 2 drops of fixative on an ice-cold alcohol cleaned slide and macerated. This popped the nuclei out of the cells. Large pieces of cellular debris were removed and the slides were partially flooded with fixative. Nuclei were spread around the slide in order to minimize cytoplasmic background and then left to air dry.

Proteins were stained using a modification of the method of Mitchell(1967). In order to make comparisons between different samples valid, all slides were stained at the same time. Slides were placed in a 1:1 mixture (v/v) of acetone:ethanol for 30 minutes and then in 0.15 ml 2:4 dinitro-1-fluorobenzene (DNFB) in 100 ml ethanol plus 1 ml 1 M sodium bicarbonate and 5 ml distilled water. Nuclei were stained for 12 hours at 65°C. After staining, slides were washed in 70% ethanol (15 minutes) warm distilled water (30°C, 15 minutes) and then dehydrated

in 70% ethanol (5 minutes), 95% ethanol (10 minutes), 100% ethanol (2 changes for 10 minutes each) and xylene (5 minutes). Nuclei were mounted in Permount.

Measurements of total nuclear protein were made using the 2 wavelength method of Patau (1952). The wavelengths used were 415 and 445 nm; the peak and 50% of the peak absorption for the DNFB-protein complex. All measurements were made at a magnification of 630X using a Leitz microspectrophotometer. A total sample of 100 interphase nuclei, 20 nuclei from each of 5 meristems, were measured at each time.

II.8. Cell Doubling Times

Seeds were grown either in tanks of water or vermiculite-perlite (40 ml water/100 ml mix). At 96 and 148 hours whole plants were transferred either to tanks of 0.05% colchicine or to vermiculite-perlite moistened with 0.05% colchicine. Roots were fixed at 1, 2 and 4 hours and the M.I. determined. Mean cell doubling times were calculated using Puck's formula (1972),

$$\log_{10} (1 + N_M) = 0.301 (T_M + t)/T$$

where N_M = fraction of cells in mitosis at any time;

T = mean cell doubling time;

T_M = time required for mitosis;

t = time spent in colchicine.

When the "$\log_{10}(1 + N_M)$" is plotted against time (t), one obtains a linear relationship. The slope of this line equals "0.301/T" where "T" is the mean cell doubling time.

II.9. Cell Cycle Determination

Seeds were grown either in vermiculite-perlite (40 ml water/100 ml mix) or in tanks of water. At 96 and 144 hours roots were treated for 1 hour either in tanks of thymidine methyl-^{3}H - solution (^{3}H-TdR; 1 µc/ml; specific activity = 200 Ci/mmol) or 40 ml ^{3}H-TdR solution/100 ml vermiculite-perlite. After labelling they were washed in distilled water and returned to tanks of water or vermiculite-perlite. Roots grown in water were fixed at 0 to 31 hours after labelling. Roots grown in vermiculite-perlite were fixed at 0 to 30 hours after labelling.

The numbers of labelled mitotic cells were determined and used to calculate both percent labelled mitoses (P.L.M.) and frequency of labelled mitoses (F.L.M.). For each sample 300 to 500 cells were scored; 100 cells per meristem. The fraction of interphase nuclei labelled after 1 hour in ^{3}H-TdR was determined 0 and 3 hours after labelling; 4 to 6 roots were scored at each time, 1000 cells per meristem.

Other roots were continuously labelled in ^{3}H-TdR. Roots grown for 97 hours in vermiculite-perlite were labelled for 35 and 48 hours; roots grown in tanks of water for 144 hours were labelled for 24 hours. The percentage of labelled interphase nuclei was determined from these roots; 1000 cells were scored per meristem. Samples of 5 to 6 roots were fixed at all times.

Autoradiographs were prepared as outlined in Webster and Davidson (1968). In order to compare grain counts from different slides, all slides were prepared and developed at the same time. Mean durations of cell cycles and of G_1, S and G_2 were calculated from P.L.M. and F.L.M. curves (Webster and Davidson, 1968). The intercepts used on the F.L.M. curves were at 50% of the peak values in the labelled mitosis curves.

II.10. Staging Cells in G_1 and G_2

Sister cells are seen as doublets in a monolayer of cells from the meristem; triplets are seen when one of 2 sister cells produced by the previous mitosis has divided (Ivanov, 1971). Cells in G_1 and G_2 can be identified in the following manner: 1) if one cell from a doublet is in mitosis, the sister cell is most likely in G_2; 2) if one cell from a triplet is in mitosis, the two remaining cells are sisters and most likely in G_1.

Seeds were grown in tanks of water for 144 hours. Cell and nuclear sizes were calculated for 50 cells in each of 1) prophase; 2) G_1 and 3) G_2.

II.11. Data Analysis

II.11.1. Frequency Distribution Diagrams (Histograms)

Data was grouped into intervals and the number of data points within an interval expressed as a percentage of the whole. The interval number was plotted against the percent frequency of the data within that interval.

The number of intervals was estimated with Sturges' Rule:

Number of Intervals = Grown Part of $[1 + 3.3 \log_{10} n]$

where n = sample size

Interval size was estimated by:

$$\text{Interval Size} = \frac{\text{Maximum Value - Minimum Value}}{\text{Number of Intervals}}$$

This value was usually rounded off to the nearest 10 or 100 for ease in plotting.

II.11.2. Comparison of Mean Values and Pairs of Data Points

Mean values of 2 samples were compared using the "Student's t Test" for unknown variances (Bailey, 1959).

A 5% level of significance (P = 0.05) was used.

Groups of paired data points were compared by calculating a regression coefficient, slope and "y" intercept of the regression line on a Monroe (Model 1930) calculator (Litton Business Systems Inc., 1974).

II.11.3. Cumulative Percentage Frequency and Probit Analysis

Data was ranked into ascending order and the cumulative percentage frequency of a given data point (P_{x_i}) was calculated as follows:

$$P_{x_i} = \frac{2i - 1}{2N} \times 100$$ (Sokal and Rohlf, 1969)

where i = rank

x_i = data point at rank i

P_{x_i} = cumulative percentage frequency associated with x_i

N = number of observations.

The cumulative percentage frequency (P_{x_i}) for individual data points was plotted on probability graph paper. If the data points form a straight line, the values are thought to approximate a normally distributed population (Sokal and Rohlf, 1969).

CHAPTER III

RESULTS

III.1. The Onset of Cell Growth and Division During Germination

Cells in the radicle are activated soon after the addition of water to the dry seed. The first sign of cell growth is root elongation. This signals the transition of cells from a dormant to an active state. The first part of this study follows cell growth in the root meristem of *V. faba* during germination. Growth was followed by determining 1) the time of onset of DNA synthesis and the first mitoses; 2) changes in cell and nuclear size as cells progress through interphase to the first mitosis and 3) changes in the protein content of growing nuclei. The inter-relationships among the changes in these parameters with time have also been determined. The results allow us to construct a picture of the sequence of events that take place in the meristem during its transition from a quiescent to an active state. Thus, the object of the first part of this study is to define the sequence and nature of the changes that occur during the initiation of root growth.

All data are from seeds grown in tanks of water, ie., they were never planted in sand or vermiculite as in later studies.

III.1.1. DNA Microspectrophotometry

DNA contents of root meristem nuclei were determined at 24,36 and 70 hours. Measurements made in arbitrary units (A.U.) on 50 telophase and 50 prophase nuclei show that cells in G_1,with 2C DNA, had 119 ± 32 A.U. of DNA while cells in G_2 with 4C DNA, had 234 ± 75 A.U. of DNA (Fig. 4). These values fit the expected 1:2 ratio of DNA contents per cell in G_1 and G_2.

At 24 hours ∿78% of the nuclei had 2C DNA, ∿16% had 4C DNA and ∿6% had intermediate values (Fig. 1). These results show that the sample of *V. faba* seed used in this study is similar to those previously studied, ie. 10 to 27% of the cells in the dormant radicle are arrested in G_2 while 73 to 90% of the cells are arrested in G_1 (Jakob and Bovey, 1969; Brunori, Avanzi and D'Amato, 1966). By 36 hours many cells had moved into S and even G_2 (Fig. 2) showing that DNA synthesis begins between 24 and 36 hours. This agrees with previous observations, using ^{3}H-TdR incorporation, that S began between 28 and 40 hours (Davidson, 1966).

At 70 hours there was a bimodal distribution of 2C and 4C DNA contents (Fig. 3). By this time the G_1

Figures 1 - 4

Percent Frequency Histograms of Nuclear DNA Contents

Values of DNA contents are given in arbitrary units. Each histogram is based on 100 nuclei, 50 from each of two roots. Telophase and prophase values are each based on 50 nuclei.

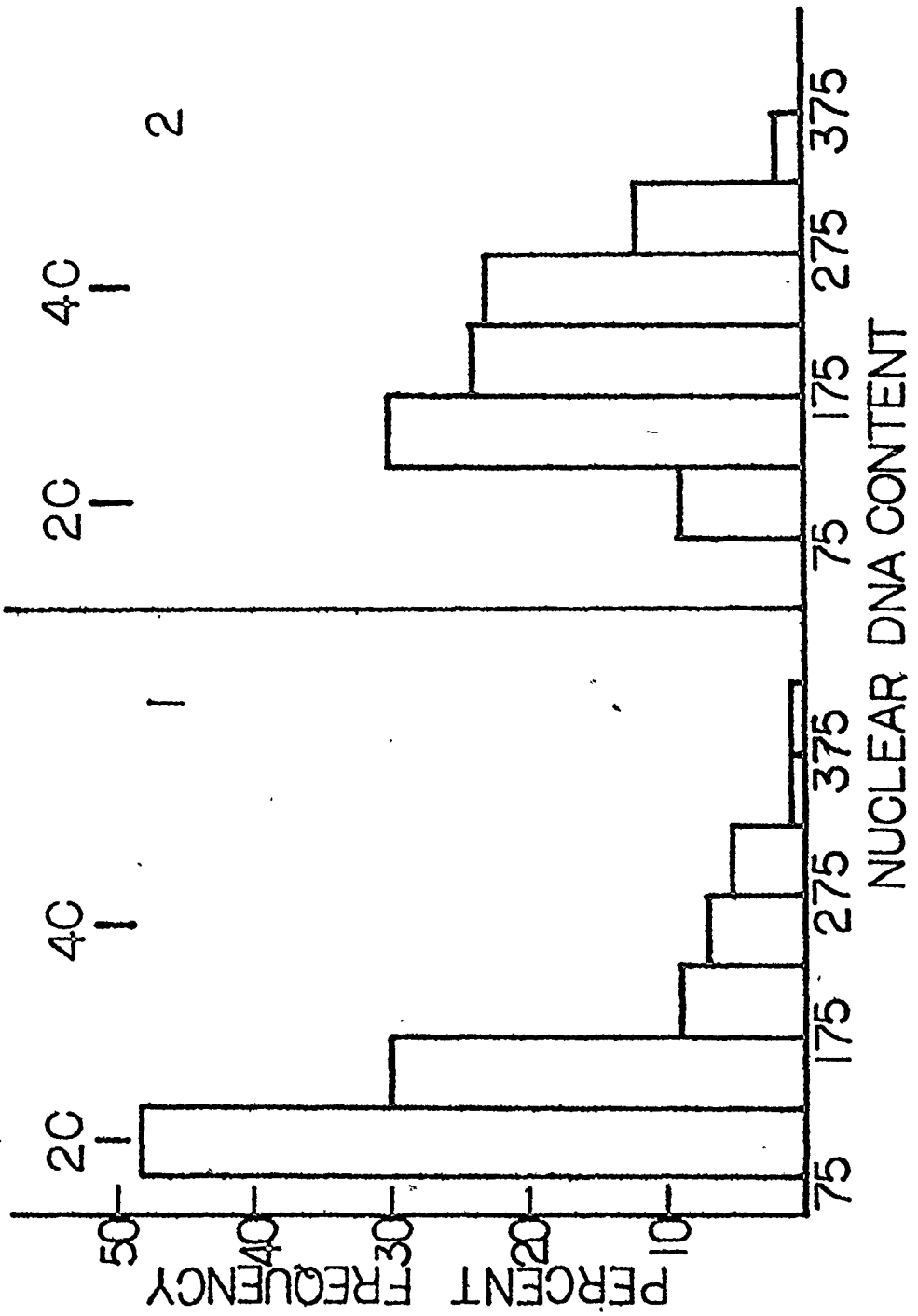
Figure 1: 24 hours

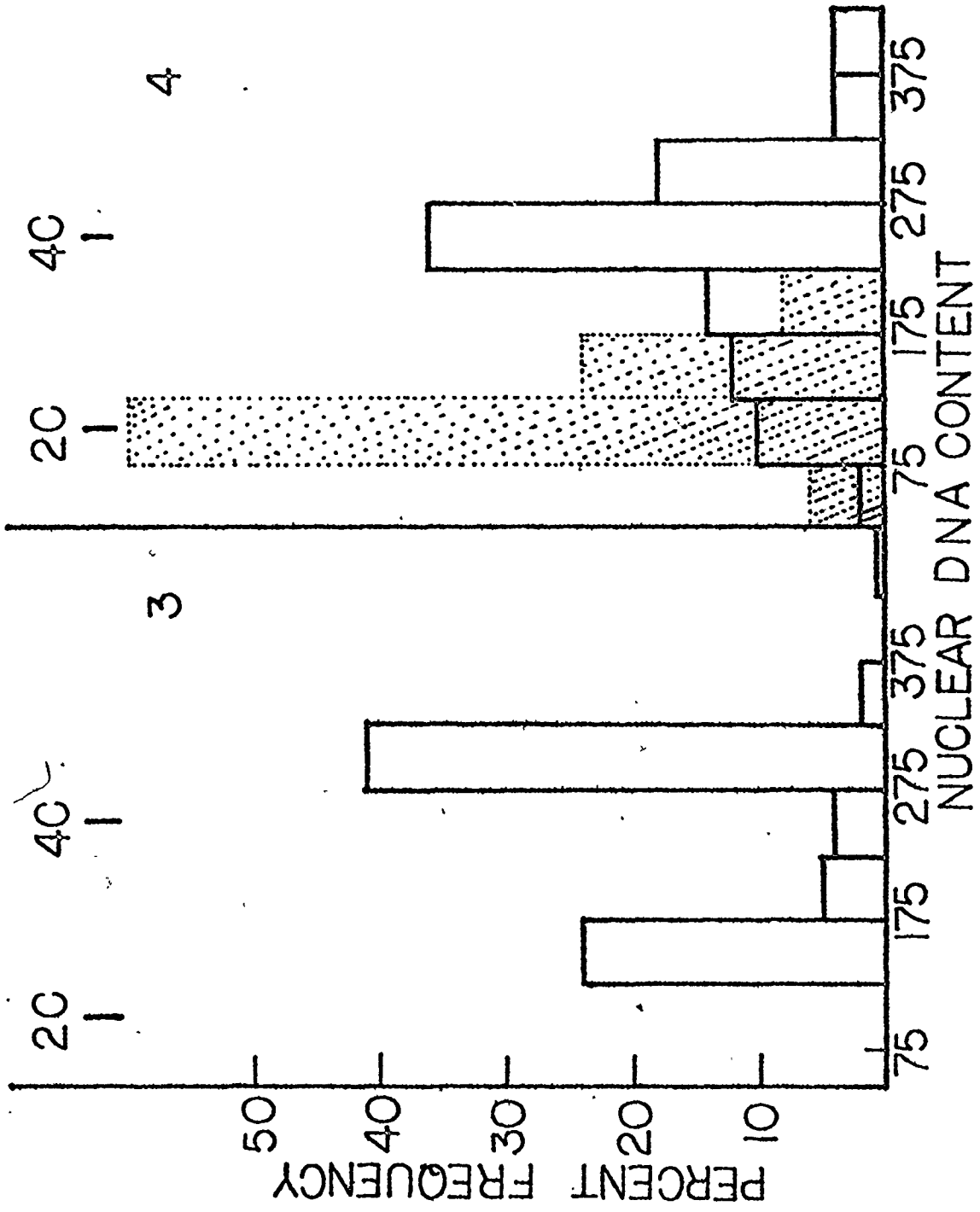
Figure 2: 36 hours

Figure 3: 70 hours

Figure 4: Prophase □

Telophase ▨


PERCENT FREQUENCY
50
40
30
20
10
2C
4C
1
2
75
175
275
375
NUCLEAR DNA CONTENT


PERCENT FREQUENCY
50
40
30
20
10
2C
1
4C
1
3
2C
1
4C
1
4
75
175
275
375
75
175
275
375
NUCLEAR DNA CONTENT

population of cells was being added to by mitoses which occur sporadically at 30 to 40 hours and gradually increase in frequency at later hours (section III.1.2.).

III.1.2. Mitotic Index

The number of cells in mitosis was low up to 36 hours. Between 36 and 110 hours mean mitotic index (M.I.) each based on samples of 4 to 11 roots, increased from 0.4 ± 1.0 to 5.7 ± 3.8, but not in a steady fashion (Fig. 5). The M.I. in individual roots, 36 to 70 hours old, varied from 0.0 to 18.5 (Table 6). By 96 to 140 hours, however, cells in most roots were dividing and from 110 hours on mean M.I. was about 6 to 7 (Fig. 5).

Assuming that the duration of mitosis was 2.5 hours (Section III.1.8) it was calculated that, on average, about 18% of the cells in the meristem had divided by 50 hours. Since estimates of DNA values indicated that 16% of the cells were already in G_2 by 24 hours, it appears that the first cells to divide are ones that were initially arrested in G_2 in the dry seed. These cells took 36 to 50 hours to complete G_2 and enter mitosis. The remaining cells in the meristem took more than 50 hours to complete G_1, S and G_2.

Although some mitotic synchrony is apparent when root meristems undergo their first mitoses, it is not

Figure 5

Mean Mitotic Index in Roots Grown Continuously in Water

Mean M.I. ± S.E. based on 164 roots from seeds imbibed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water for 20 to 152 hours. Sample sizes ranged from 3 roots at 20 hours to 46 roots at 50 hours. The mean number of roots used for each point was 14.

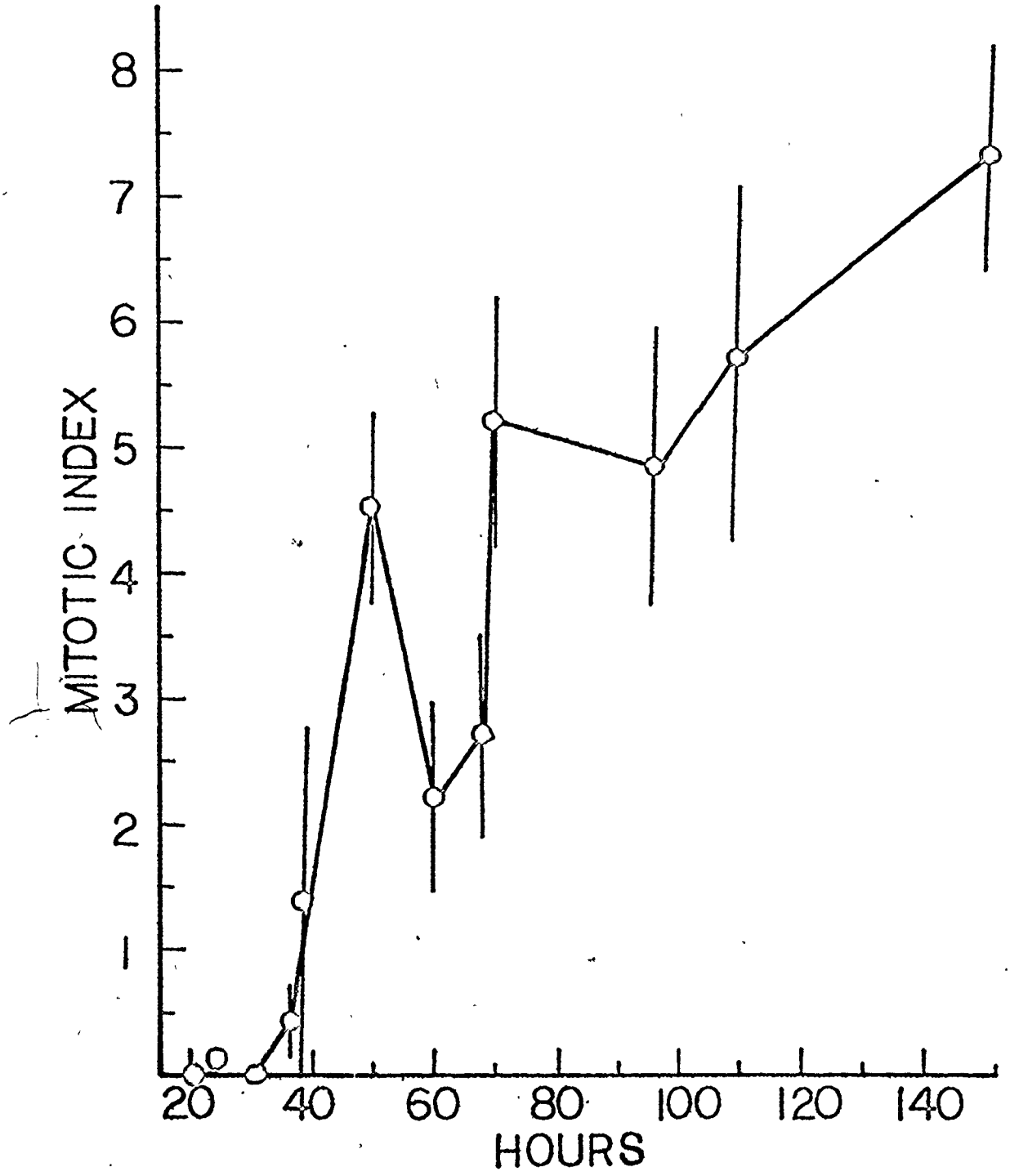

MITOTIC INDEX
0
1
2
3
4
5
6
7
8
20
40
60
80
100
120
140
HOURS

Table 6

Changes in Mitotic Index and Root Length During Germination

The time from addition of water, number of roots, mean root length, mean M.I. and range in M.I. for four different samples of roots in the first 70 hours of growth and at maturity. Each M.I. is based on 1000 cells per root. The variation in root length and mitotic index at any time is expressed as the coefficient of variation (C.V.).

Time from the addition of water (hours)	Number of roots	Root length (mm) mean ± S.D.	Root length (mm) C.V.	Mitotic index mean ± S.D.	Mitotic index C.V.	Range in mitotic index
			Sample I			
20	3	3.0 ± 0	0	0	0	
24	4	3.9 ± 1.0	25.6	0.1 ± 0.3	192.3	0 - 0.5
36	6	5.0 ± 0.7	14.0	0.8 ± 1.3	167.0	0 - 3.4
38	5	5.2 ± 1.1	21.2	4.8 ± 8.0	165.9	0 - 18.5
50	4	7.5 ± 1.7	22.7	2.8 ± 2.6	93.8	0 - 5.8
70	4	14.0 ± 8.7	62.1	5.5 ± 3.9	70.4	0 - 8.7
			Sample II			
30.3	6	3.8 ± 0.4	10.5	0	0	
36.3	6	4.3 ± 0.6	14.0	0 ± 0.0	244.9	0 - 0.1
50.3	5	4.8 ± 1.2	25.0	5.5 ± 3.0	55.0	1.6 - 8.8
60.3	5	6.4 ± 1.1	17.2	2.6 ± 2.6	100.3	0 - 6.8
70.3	6	7.5 ± 2.2	29.3	5.5 ± 4.9	90.2	0.2 - 11.3
			Sample III			
50	10	4.8 ± 1.1	22.9	2.4 ± 3.8	160.7	0 - 12.3
60	11	5.5 ± 1.6	29.1	2.9 ± 4.9	169.1	0 - 16.3
68	11	7.7 ± 2.5	32.5	4.0 ± 4.1	101.7	0 - 13.0
			Sample IV (Mature Roots)			
144 - 175	47	33.4 ± 10.8	32.3	7.2 ± 2.5	34.0	3.0 - 13.9

mitotic synchrony that is their most striking feature but rather the variation in mitotic activity from root to root. Parallel with this variation in onset of mitotic activity there is variation in root growth.

III.1.3. Root Length *versus* Mitotic Index

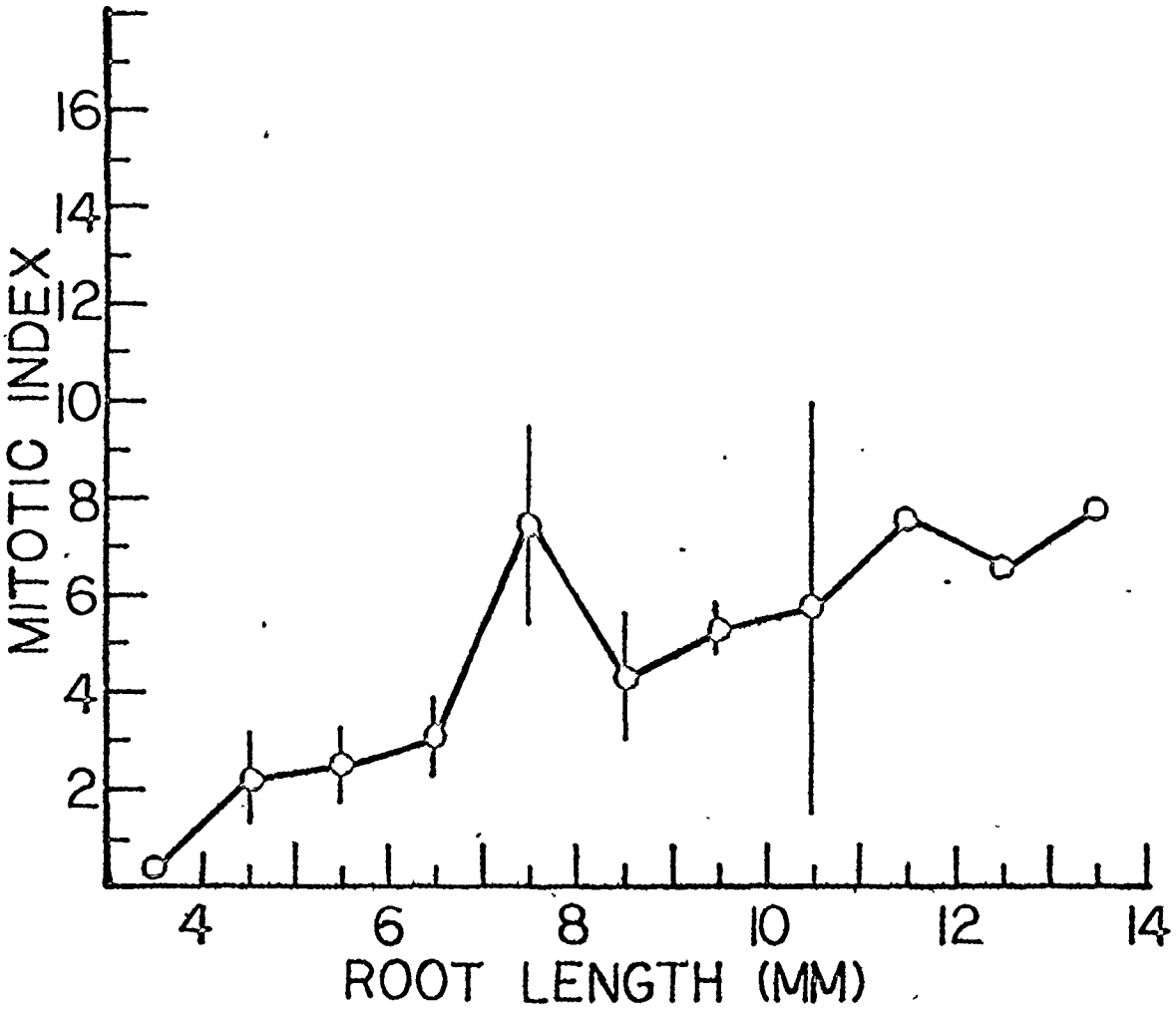
In the early stages of germination, ie., 24 to 70 hours, root lengths were highly variable (Table 6). In a sample of 4 roots, 70 hours old (Table 6, sample I), 2 roots were short, 5 to 6 mm long and had a mean M.I. of 2.9 while 2 roots were long, 21 to 22 mm and had a mean M.I. of 8.2. This observation led to an examination of the relationship between root length and M.I. (Fig. 6).

In roots 50 to 70 hours old mean M.I. increased more or less linearly from 0.3 to 7.8 in roots 3 to 13.5 mm long. The only point well outside of this increase occurred in roots 7 to 8 mm long; this was due to a wave of mitotic activity. Even including the point from 7 to 8 mm roots, the correlation coefficient between root length and mean M.I. was 0.88; the slope of the line was 0.67 with a "y" intercept of -0.86. This shows that in the first 50 to 70 hours of root growth, root length was a fairly reliable indicator of M.I. This agrees with the observations of Bryant (1969) on germinating seeds of *Allium cepa*.

Figure 6

Mean Mitotic Index Plotted Against Mean Root Length

Data from 99 water grown roots, 50 to 70 hours old was used to calculate mean M.I. ± S.E. for roots 3 to 13.5 mm long. Values at 10.5 to 12.5 mm are based on 2 roots per point; the value at 13.5 mm is based on 1 root. No standard error is given for roots longer than 9.5 mm.


MITOTIC INDEX
16
14
12
10
8
6
4
2
4
6
8
10
12
14
ROOT LENGTH (MM)

In roots 18 to 60 mm long and 144 to 175 hours old, the mean M.I. was 7.2 (Table 6, Sample IV). This value is similar to that in roots 11 to 14 mm long (Fig. 6) and it suggests that once roots reach a length of 11 to 14 mm the M.I. no longer fluctuates to the extent seen in roots 3 to 10 mm long; mitotic activity settles into a more or less uniform pattern and the meristem shows the steady state kinetics of a mature, growing root.

The onset of a stable pattern of cell division in the meristem indicates that cells have also attained a stable growth pattern. Changes in cell growth during germination were monitored by following changes in cell area (section III.1.4.).

III.1.4. Cell Area

It has been shown that up to 30 hours most cells are still in G_1 (Fig. 1, cf. Fig. 2), M.I. is 0 (Tables 6 and 7) and mean root length is 3.8 ± 0.4 mm (Table 7). In 30 hour old roots mean cell area was 574 ± 281 μm^2 (Table 7). Mean cell area was almost the same at 70.3 hours, 558 μm^2 but had decreased significantly ($p > 0.05$) to 482 ± 198 μm^2 by 96.3 hours (Table 8). Since this occurs in a period when M.I. is increasing (Fig. 5), it suggests that this period is one in which important changes take place in the meristem; ie., as cell proliferation increases

Table 7

Cell Growth During Germination: Cell Areas

Mean root length, mean M.I., mean cell area and range in cell areas for both interphase and prophase cells at different times during germination. All roots were imbibed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water.

Time from from addition of water (hours)	Root length		Mitotic index		Interphase cell area			Prophase cell area		
	Number of roots	mean ± S.D. (mm)	Number of roots	mean ± S.D.	Number of cells	mean ± S.D. (μm^2)	range (μm^2)	Number of cells	mean ± S.D. (μm^2)	range (μm^2)
30.3	6	3.8 ± 0.4	6	0	140	574 ± 281	169 - 1611			
36.3	6	4.3 ± 0.6	6	0.02 ± 0.04	140	562 ± 243	163 - 1302			
50.3	5	4.8 ± 1.2	5	5.5 ± 3.0	100	601 ± 253	129 - 1319	50	907 ± 331	358 - 1861
60.3	5	6.4 ± 1.1	5	2.6 ± 2.6	100	582 ± 280	155 - 1519	50	768 ± 308	225 - 1514
70.3	6	7.5 ± 2.2	6	5.5 ± 4.9	100	558 ± 266	195 - 1904	80	650 ± 207	296 - 1302
96.3	6	11.0 ± 5.4	6	4.7 ± 5.0	120	482 ± 198	125 - 1058	80	520 ± 182	217 - 1139

overall cell growth decreases. In other words, the size reached by cells as they completed the first mitosis was not reached by cells at the second mitosis. Also, during the period from 70 to 96 hours the meristem forms a steady state, asynchronous population.

The decrease in mean cell area between 70.3 and 96.3 hours could result largely from a change in the relative proportions of cells in G_1 and G_2. This, however, was not entirely responsible for the observed decrease. The size of cells in prophase between 50.3 and 96.3 hours shows a steady downward trend; mean prophase cell area decreased from 907 ± 331 μm^2 at 50.3 hours to 520 ± 182 μm^2 at 96.3 hours. This decrease was significant even between 50.3 and 60.3 hours ($p > 0.05$). There was also a parallel reduction in 1) the range of cell areas and 2) the size of the largest cells seen in prophase between 50.3 and 96.3 hours. Furthermore, of the prophase cells at 50.3 hours, 18% of them had larger areas, 1171 to 1861 μm^2, than the largest prophase cell at 96.3 hours, ie., 1139 μm^2.

The data from the prophase cells confirms that as the daughter cells produced by the first wave of mitoses progressed through interphase they did not double in size. Thus, within the first two cell cycles in the newly emerged radicle, there was a reduction in cell cycle duration and a parallel reduction in the area of

both interphase and prophase cells. The gradual reduction in prophase cell area also shows that there is not a constant cell size that must be achieved before a cell can enter prophase. This conclusion does not support the concept derived from studies of micro-organisms of a critical size control over cell cycle events. Such studies also suggest that nuclear size may be important in controlling cell cycle events (Yen and Pardee, 1979). Consequently, a parallel study of nuclear size during germination was performed.

III.1.5. Nuclear Volumes

Nuclear volumes were determined between 24 and 96.3 hours; a total of 70 roots from 3 samples were scored. Between 24 and 36.3 hours mean nuclear volume was remarkably constant, 751 to 794 μm^3 (Table 8, Samples I and II). Since these values are based on 3500 nuclei the consistency in mean nuclear volume is not likely to result from a sampling error. This implies that there is little or no growth of nuclei between 24 and 36 hours; ie., up to the time when they enter S phase (Section III.1.1.).

From 36 to 70 hours mean nuclear volume increased, though there was some variation between samples, ie., at 60 hours mean nuclear volume decreased (Table 8). Mean volume of prophase nuclei also varied from sample to sample

Table 8

Changes in Nuclear Volume During Germination

Mean root length, mean M.I., mean volume and range in volumes of interphase and prophase nuclei measured for three samples of roots at various times during germination. All roots were placed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water.

Time from addition of water (hours)	Root length		Mitotic index		Interphase nuclear volume			Prophase nuclear volume		
	number of roots	mean (mm)	number of roots	mean	number of nuclei	mean ± S.D. (μm^3)	range (μm^3)	number of nuclei	mean ± S.D. (μm^3)	range (μm^3)
					Sample I					
30.3	6	3.8	6	0	500	772 ± 373	118 - 2196			
36.3	6	4.3	6	0.02	500	794 ± 335	151 - 2409			
50.3	5	4.8	5	5.1	500	776 ± 258	124 - 2521	112	1247 ± 315	559 - 2820
60.3	5	6.4	5	2.6	500	608 ± 211	149 - 2109	112	999 ± 249	385 - 1663
70.3	6	7.5	6	5.5	500	869 ± 389	212 - 2715	112	1361 ± 377	630 - 2506
96.3	7	11.0	7	4.7	600	661 ± 251	99 - 1781	125	1039 ± 332	474 - 2409
					Sample II					
24	4	3.9	4	0	1000	751 ± 481	88 - 3507			
36	6	5.0	6	0.79	1500	760 ± 352	97 - 2645	50	1679 ± 322	883 - 2630
50	4	7.5	4	2.83	1000	921 ± 388	142 - 2986	50	1695 ± 651	559 - 3242
70	4	14.0	4	5.5	1000	1186 ± 685	177 - 5788	50	1660 ± 993	779 - 5788
					Sample III					
50	5	7.4	5	8.3	560	783 ± 263	161 - 1940	56	1038 ± 240	627 - 1689
70	5	12.6	5	4.7	560	843 ± 304	175 - 2723	112	1386 ± 433	544 - 2847
96	7	19.1	7	5.0	756	1010 ± 408	218 - 3401	112	1329 ± 457	434 - 2861

(Table 8; Sample II, cf. Sample III at 50 hours). One factor contributing to this variation was the time at which cells began to divide. In samples with a high M.I. mean volumes of both interphase and prophase nuclei were lower than in samples with a low M.I. (Table 8; Sample II, cf. Samples I and III). It appears that when entry into mitosis is delayed nuclei continue to grow.

Additional factors contributing to variation in nuclear volume are 1) the rate of nuclear growth once cells enter G_2 and 2) the duration of S and G_2 once cells enter their second and third cell cycles. A comparison of values for individual roots 96 and 96.3 hours old shows that even in roots of similar lengths mean nuclear volumes may be strikingly different (Table 9; cf. roots 6,7 and 15 mm long from Samples I and III).

Both internal and external factors probably contribute to the observed variation in root, cell and nuclear growth. Although the internal makeup of a cell is determined genetically, the combined effects of minor fluctuations in the environment during germination seem to give rise to a population of roots with distinct physiological differences. These differences are the consequences of sampling roots with slightly different histories. Evidence that minor changes in the environment exert effects that contribute to the observed variation is considered in

Table 9

Nuclear Volumes in Individual Roots

Root length, mitotic index and mean nuclear volume were determined for individual roots at 96 and 96.3 hours of germination. All beans were placed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water. Mitotic indices are based on 1000 cells per meristem. Nuclear volumes at 96 hours are from 126 cells per meristem and at 96.3 hours from 100 cells. The data is from the same roots used in Table 8.

Root length (mm)	Mitotic index	Nuclear volume mean ± S.D. (μm^3)
	Sample I	
6	0.	526.0 ± 207
7	2.1	811.7 ± 271
9	5.4	683.0 ± 238
14	5.4	619.0 ± 248
15	5.8	588.0 ± 222
20	14.4	740.0 ± 312
	Sample II	
6	0.4	1249.0 ± 446
7.5	0.2	1252.0 ± 593
16	5.2	970.0 ± 360
15	7.8	880.0 ± 283
26	8.6	931.0 ± 398
33	5.2	779.0 ± 293

section III.2. Differences in nuclear growth as they relate to cell growth during germination are examined next.

III.1.5.1. Nucleo-cytoplasmic ratios

Another way of examining cellular changes in the meristem during germination is to follow changes in the relationship between nuclear and cell size. During germination the ratio, mean nuclear area:mean cell area, increased in prophase cells (Table 10). In interphase cells the ratio also increased but fluctuated somewhat (Table 10), reflecting in part variation in the developmental stage reached by different roots at different times. When roots were grouped on the basis of increasing length, ie., 3 to 20 mm, then two trends became obvious: 1) mean cell area decreased while mean nuclear volume remained more or less constant (Table 11), and 2) the ratio mean nuclear area:mean cell area increased. The ratio was 0.157 in 3 mm roots and 0.222 in 20 mm roots (Table 11).

Increases in the nucleo-cytoplasmic ratio were paralleled by increases in the slope of a best fit line drawn through a plot of cell area against nuclear volume (Table 11). The slope value, "m", increased from 0.87 in 3 mm roots to 1.67 in 20 mm roots. When cell and

Table 10

Nucleo-cytoplasmic Ratios in Germinating Roots

The mean M.I. and mean nuclear area to mean cell area ratio of interphase and prophase cells were calculated for roots imbibed in 5 to 10 mm of water for 24 hours and then grown for various lengths of time in tanks of aerated distilled water. Nuclear areas were estimated as the 2/3 root of the nuclear volume ($NV^{2/3}$). Original nuclear volumes are given in Table 8 (Sample I), cell areas are given in Table 7.

Time (hours)	Mean M.I.	Nuclear area/Cell area	
		Interphase	Prophase
30.3	0.0	0.147	
36.3	0.0	0.153	
50.3	5.1	0.141	0.128
60.3	2.5	0.123	0.130
70.3	5.5	0.163	0.189
96.3	4.7	0.157	0.197

Table 11

Nucleo-cytoplasmic Ratios in Roots of Different Lengths

Samples of 100 cells were chosen from roots 30.3 to 102.3 hours old; mean M.I., mean nuclear volume and mean cell area were determined for each sample and the mean nuclear area to mean cell area ratio calculated. Cell area was plotted against nuclear volume and the slope of a best fit line was calculated; the correlation coefficient for cell area *versus* nuclear volume was also calculated.

Root length (mm)	Number of roots	Mean M.I.	Nuclear volume (μm^3) mean ± S.D.	Cell area (μm^2) mean ± S.D.	Nuclear area / Cell area	Slope "M"	Correlation coefficient "R"
3	4	0.8	742 ± 331	523 ± 266	0.157	0.87	0.70
4	5	1.8	853 ± 310	571 ± 259	0.157	0.86	0.71
5	4	2.7	851 ± 330	600 ± 257	0.150	0.88	0.68
6	5	1.7	710 ± 241	619 ± 307	0.129	0.41	0.52
7	4	4.9	870 ± 275	536 ± 203	0.170	0.90	0.66
8	2	6.1	763 ± 351	474 ± 231	0.176	1.25	0.82
9	2	5.9	796 ± 359	486 ± 214	0.177	1.21	0.72
10	2	8.6	818 ± 272	525 ± 178	0.167	1.04	0.68
11	5	4.8	941 ± 439	469 ± 208	0.205	1.68	0.80
13	1	8.2	704 ± 270	454 ± 199	0.174	1.07	0.79
14	3	5.8	891 ± 368	477 ± 211	0.194	1.27	0.73
15	2	5.2	731 ± 341	400 ± 184	0.203	1.43	0.77
17	1	9.8	752 ± 272	420 ± 169	0.197	1.36	0.85
20	1	14.4	723 ± 283	363 ± 142	0.222	1.67	0.84

nuclear size were compared in the 14 samples of roots 3 to 20 mm long, the mean correlation coefficient ± S.D. was 0.73 ± 0.09. This indicates that there was a reasonably good correlation between these two parameters within each sample even though the relative proportions of cells in G_1 and G_2 differed in different roots. In general, these results show that the relative size of cells and nuclei changes in a progressive fashion as roots become longer and older.

The most marked deviations from the general trends shown in Table 11 occurred in roots 6 mm long. In these roots, the nucleo-cytoplasmic ratio, the slope "m" value and the correlation coefficient are all lower than in other roots. The fall in these values appears to coincide with the onset of mitotic activity and, therefore, with a change in the relative proportions of cells in G_1, S and G_2. This suggests that the nucleo-cytoplasmic relationship is related, in part, to position in the cell cycle.

In root meristems individual cells cannot be studied over a cell cycle but mean values for G_1, G_2 and prophase cells can be determined (Materials and Methods, section 10). A comparison of values from these three stages shows that the ratio, mean nuclear area:mean cell area, was high in G_1, decreased in G_2 and rose again in prophase. A plot of cell area versus nuclear volume for each group of cells

(Fig. 7a) also shows that points from G_1 and prophase tend to fall on the same line while cells in G_2 produce an intermediate band of points whose slope is less than that of either cells in G_1 or prophase (Table 12). The combined distribution of points in G_1 and G_2 exactly overlap a random sample of cell area plotted against nuclear volume for the same root (Fig. 7b). This suggests that changes in the nucleo-cytoplasmic ratio occur throughout the cell cycle and that, in turn, these changes result from differences in the relative rates of growth of nuclei and cells at different phases of the cycle.

In samples of G_1, G_2 and prophase cells taken from two roots (Table 12) both nuclear volume and cell area increased, as expected, from G_1 to prophase. However, since the cells scored as G_1 are identified because they have already begun their cycle of growth and prophase cells have not yet completed their cycle, the period of growth that separates the G_1 from the prophase cells scored in these samples must be slightly less than one complete cell cycle. Mean area of prophase cells was 1.7 to 1.8 times larger than that of G_1 cells (Table 12) suggesting that the interval separating the two stages represented in these samples was only 0.7 to 0.8 of a cell cycle.

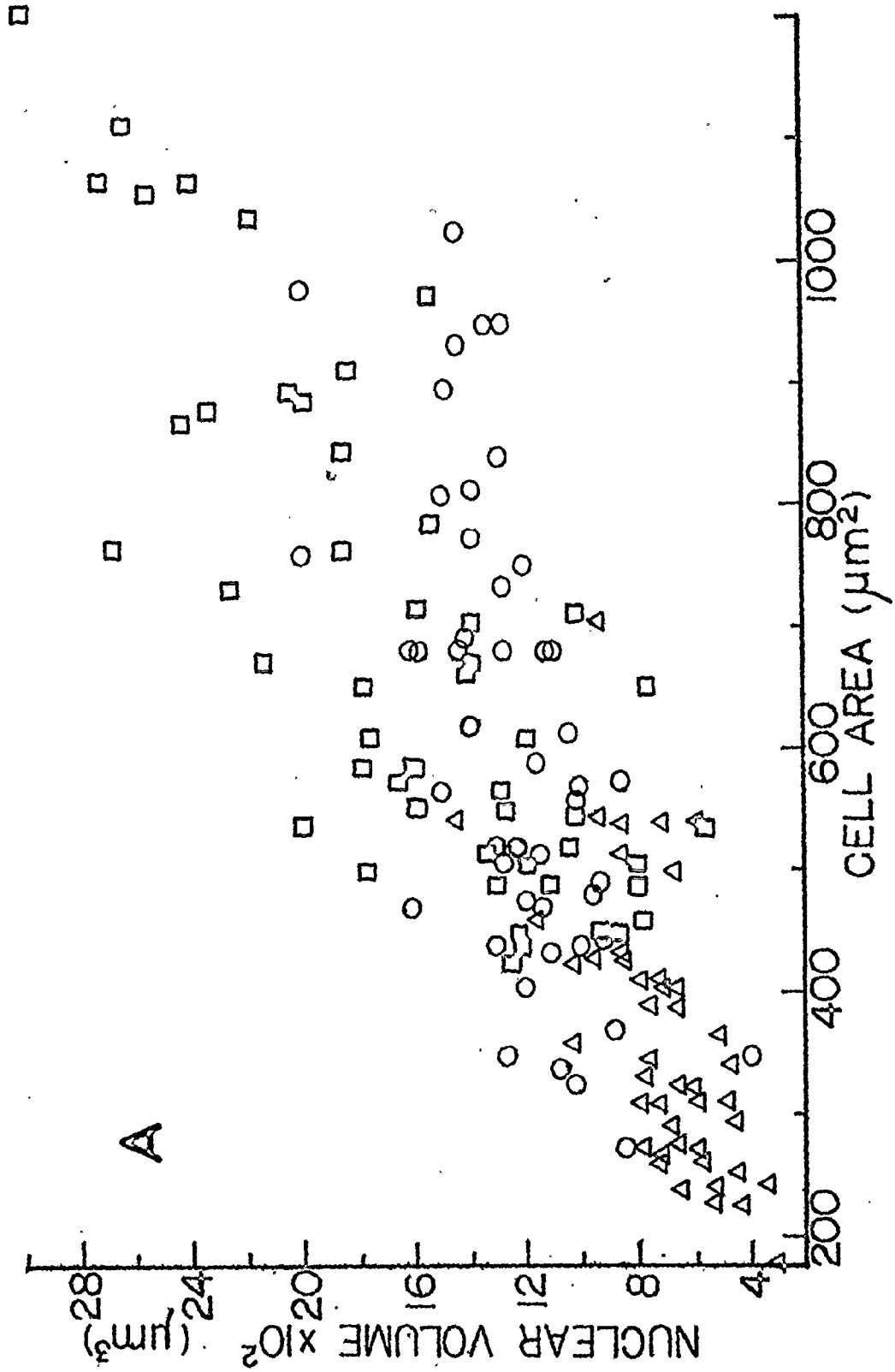
The G_1 cells scored should be daughters of cells similar to those seen in prophase and should be approximately

Figure 7

Cell Area Plotted Against Nuclear Volume for Cells in G_1, G_2, Prophase and Interphase

A. Nuclear volumes and cell areas for 50 cells in each of G_1, Δ, G_2, o, and prophase, ◘ . All values are from 1 root, 38 mm long and 144 hours old.

B. Nuclear volumes and cell areas from 100 randomly chosen interphase cells from the same root as scored in A.


A
NUCLEAR VOLUME x10² (μm³)
28
24
20
16
12
8
4
CELL AREA (μm²)
200
400
600
800
1000

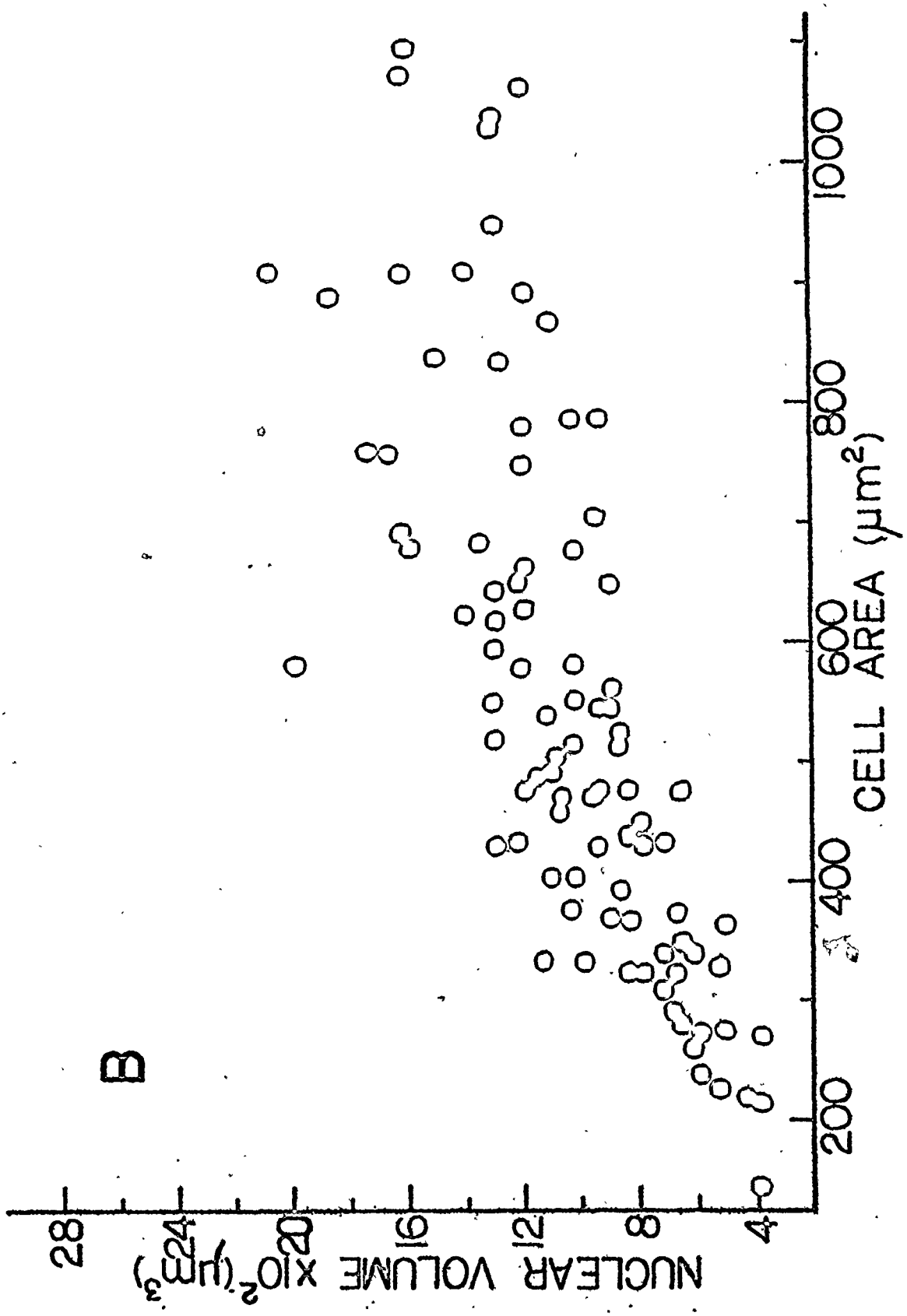

B
NUCLEAR VOLUME x10² (μm³)
28
24
20
16
12
8
4
CELL AREA (μm²)
200
400
600
800
1000

Table 12

Nucleo-cytoplasmic Ratios During the Cell Cycle

Mean nuclear volume, range in nuclear volume, mean cell area and range in cell area were determined in two roots 37 and 38 mm long. For each root 50 cells in G_1, G_2 and prophase and 100 cells randomly distributed throughout interphase of the cell cycle (Materials and Methods, section 10) were scored. The mean nuclear area:mean cell area ratio, slope of a best fit line drawn through a plot of cell area *versus* nuclear volume and the correlation coefficient for cell area *versus* nuclear volume were calculated for each root.

Position in cell cycle	Nuclear volume (μm^3) mean ± S.D.	Nuclear volume (μm^3) range	Cell area (μm^2) mean ± S.D.	Cell area (μm^2) range	Nuclear area / cell area	Slope "H"	Correlation coefficient "R"
			Root I				
G_1	710 ± 211	302 - 1435	371 ± 110	179 - 705	0.214	1.21	0.63
G_2	1254 ± 288	857 - 1993	608 ± 189	324 - 1025	0.191	0.93	0.61
Prophase	1611 ± 598	559 - 3071	684 ± 207	439 - 1201	0.201	2.29	0.79
Random interphase sample	1036 ± 358	377 - 1984	549 ± 225	217 - 1101	0.186	1.22	0.76
			Root II				
G_1	599 ± 151	333 - 1020	400 ± 123	182 - 638	0.178	0.90	0.74
G_2	975 ± 182	649 - 1497	681 ± 203	324 - 1182	0.144	0.40	0.45
Prophase	1251 ± 314	714 - 1984	688 ± 198	342 - 1204	0.169	0.78	0.49
Random interphase sample	782 ± 187	430 - 1212	559 ± 163	195 - 1003	0.152	0.79	0.65

half the size of the prophase cells. When the minimum and maximum values of prophase cells were halved the values obtained were close to the minimum and maximum values seen in G_1 cells. This confirms that both mean cell area and the spread of values for G_1 cells are close to half those seen in prophase cells.

Mean nuclear volumes in G_2 cells were 1.63 to 1.77 times larger than those in G_1 cells but the mean prophase nuclear volumes were 2.1 to 2.3 times larger (Table 12). In both samples nuclei showed a 22% increase in volume between G_2 and prophase. It appears, therefore, that there is a burst of nuclear growth towards the end of G_2 and in prophase but it is not accompanied by a parallel increase in the rate of cell growth. The results from this analysis show that nuclear and cell growth in meristematic cells, as in *Amoeba* (Prescott, 1955) are not synchronous throughout the cell cycle. In addition, as seeds germinate, the nucleo-cytoplasmic ratio increases until the meristem becomes an asynchronous steady state system.

III.1.6. Nuclear Proteins

We now turn to consider whether changes in nuclear size during germination result solely from hydration or whether they reflect parallel changes in macromolecular constituents. For this study, protein contents of individual

nuclei were determined. All values are expressed in arbitrary units (A.U.).

From 20 to 50 hours of germination the mean nuclear protein content increased from 54.6 ± 17.6 to 68.5 ± 22.8 A.U. (Table 13). The modal value also increased from 45 to 70 A.U. (Figs. 8 and 10) and there was a striking increase in the range of values. The range was 32 to 104 A.U. at 20 hours and 30 to 158 A.U. at 50 hours (Table 13).

In most proliferating cells there is a positive correlation between nuclear size and protein content (section III.5.2.) but during the early stages of root growth this correlation is not very strong. The mean protein content of nuclei increased by 24% from 20 to 50 hours while mean nuclear area increased by only 4%. Within one specific size class of nuclei, e.g., 82 μm^2, mean protein content was 50.0 ± 7.7 A.U. at 20 hours but had increased to 64.6 ± 14.0 A.U. at 50 hours (Table 14); thus protein content changed without a parallel change in nuclear size.

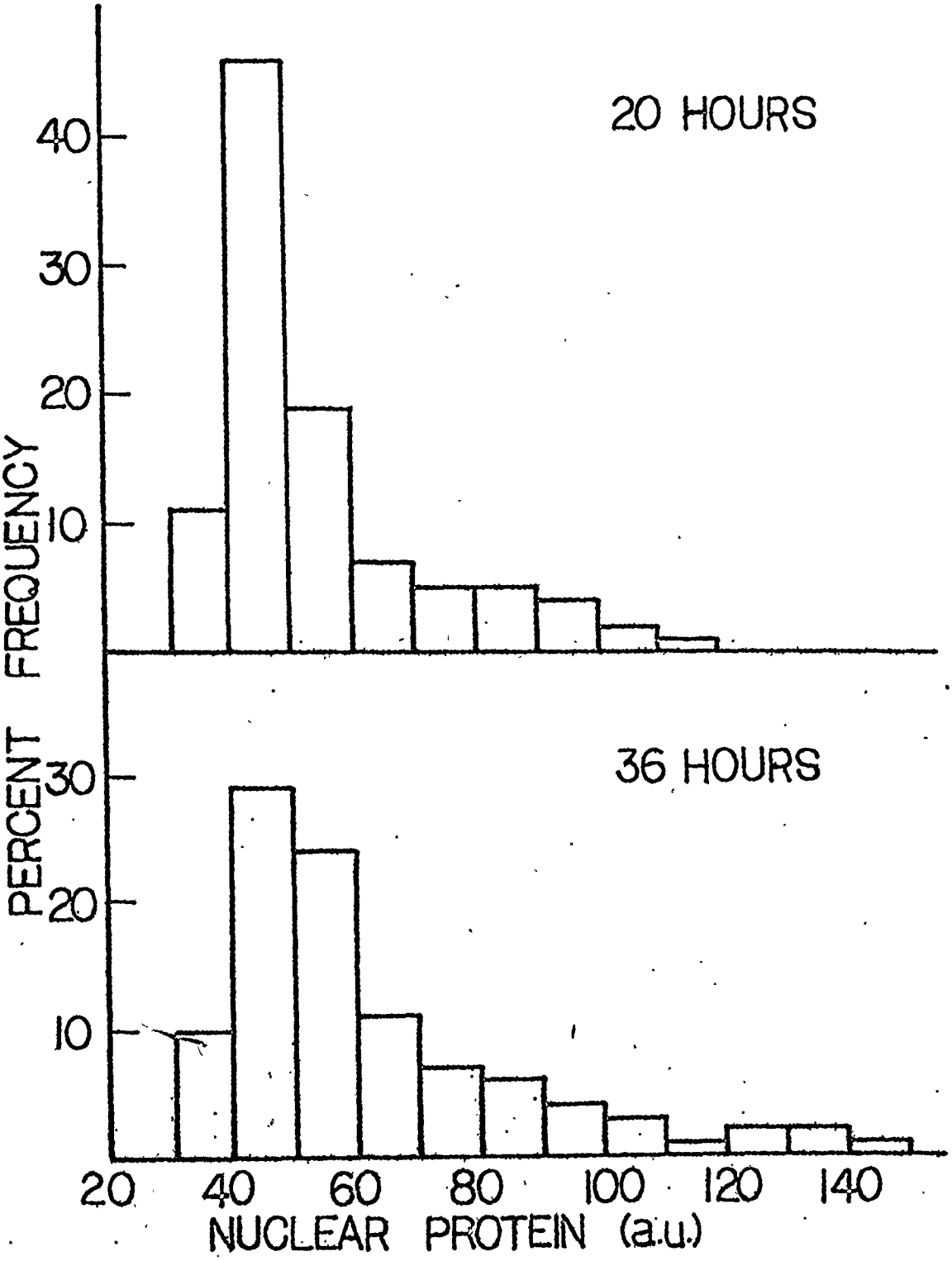
Protein content of nuclei also changed between 50 and 144 hours (Tables 13 and 14; Figs. 10,11 and 12), but in this case nuclear protein contents decreased without a parallel decrease in nuclear size. The change in mean protein content of nuclei from 50 to 144 hours, ie., from 68 to 38 A.U., suggests, at first glance, merely a change in the relative proportions of G_1 and G_2 nuclei. This,

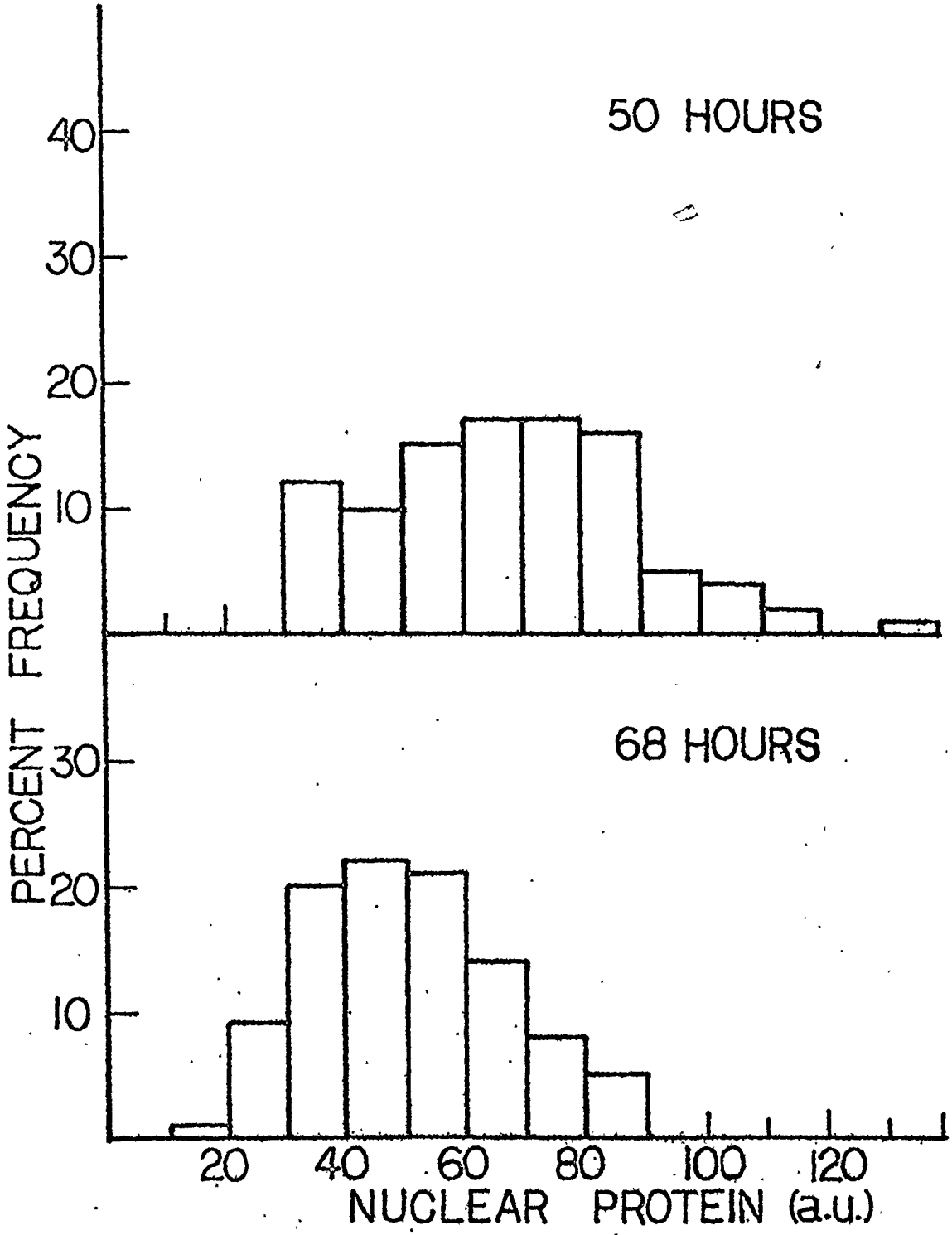
Figures 8 - 13

Percent Frequency Histograms of Nuclear Protein Contents at Various Times During Germination

All seeds were imbibed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water. Each sample consists of 100 nuclei isolated from 5 root meristems. Nuclear protein was measured in arbitrary units (A.U.)

Figure	Time from addition of water (hours)
8	20
9	36
10	50
11	68
12	96
13	144


20 HOURS
36 HOURS
PERCENT FREQUENCY
40
30
20
10
30
20
10
20
40
60
80
100
120
140
NUCLEAR PROTEIN (a.u.)


50 HOURS
68 HOURS
PERCENT FREQUENCY
40
30
20
10
30
20
10
20
40
60
80
100
120
NUCLEAR PROTEIN (a.u.)

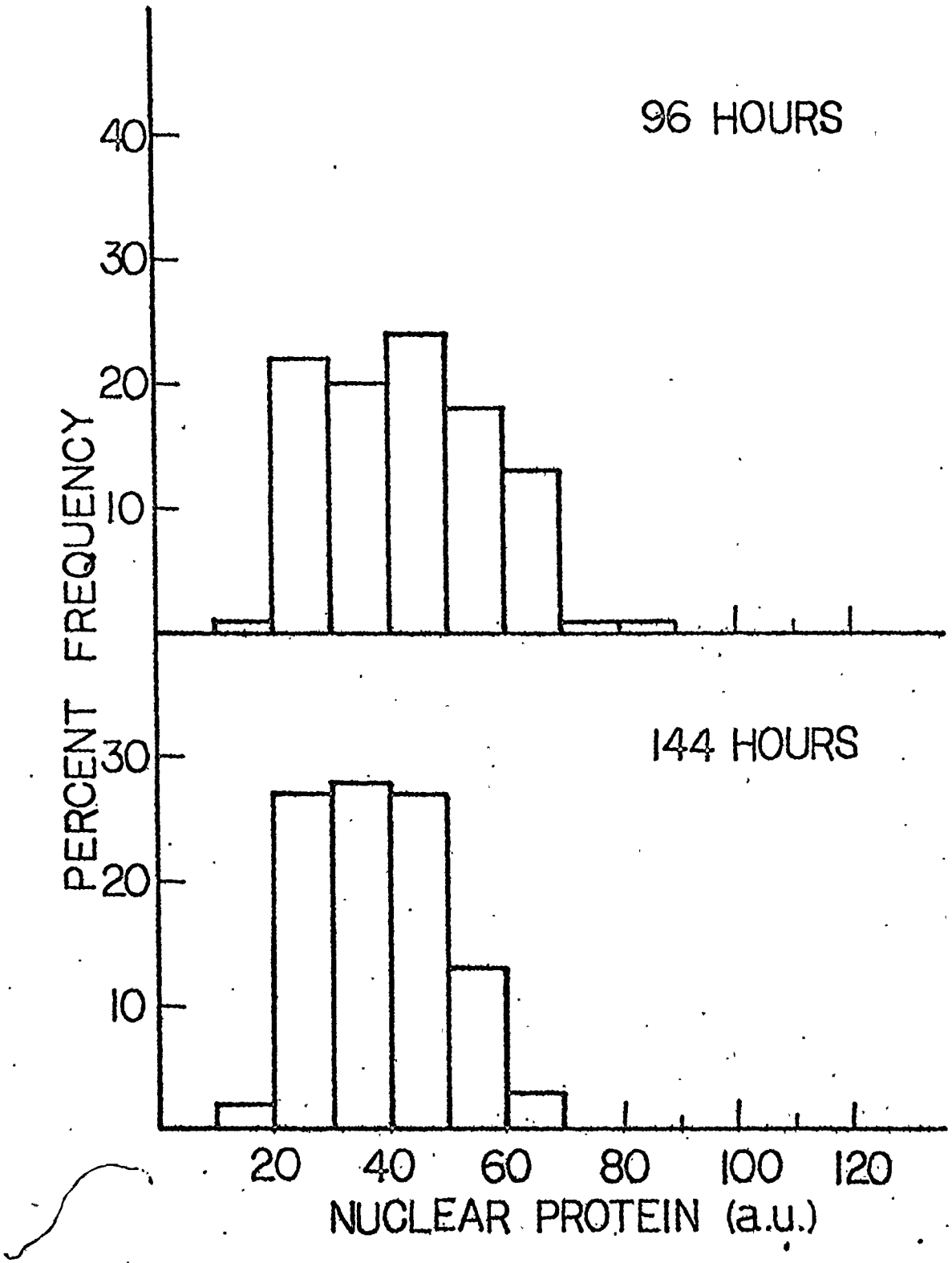

96 HOURS
144 HOURS
PERCENT FREQUENCY
40
30
20
10
30
20
10
20
40
60
80
100
120
NUCLEAR PROTEIN (a.u.)

Table 13

Nuclear Protein Contents in Germinating and Mature Roots

Mean nuclear area, range in nuclear area, mean nuclear protein and range in nuclear protein contents were determined at various times during germination. Each sample was of 100 nuclei. All seeds were imbibed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water.

Time from addition of water (hours)	Nuclear area (μm^2)		Nuclear protein (A.U.)	
	mean ± S.D.	range	mean ± S.D.	range
20	97 ± 28	60 - 172	55 ± 18	32 - 104
36	97 ± 27	52 - 156	62 ± 25	34 - 150
50	101 ± 24	62 - 177	68 ± 23	30 - 158
68	102 ± 24	62 - 169	50 ± 17	19 - 89
96	96 ± 18	62 - 145	43 ± 14	19 - 83
144	89 ± 21	52 - 171	38 ± 11	18 - 61

Table 14

Nuclear Protein Contents within Individual Nuclear Size Classes

The sample size, mean, range and increase in nuclear protein contents per cell from the minimum to the maximum value were determined for nuclei 71, 82, 93, and 110 μm^2 in size at various times during germination. All values are selected from data in Table 13.

Time from the addition of water (hours)	Nuclear area (μm^2)	Sample size	Nuclear Protein (A.U.) mean ± S.D.	Range in nuclear protein (A.U.)	Increase in nuclear protein from minimum to maximum
20	71	17	44.9 ± 9.8	31.5 - 69.4	2.20
	82	16	50.0 ± 7.7	38.4 - 66.2	1.72
	93	12	50.4 ± 13.7	40.8 - 91.1	2.23
	110	12	65.1 ± 25.6	34.0 - 112.3	3.30
36	71	16	52.4 ± 13.6	33.6 - 82.2	2.45
	82	21	52.4 ± 10.9	37.4 - 78.4	2.10
	93	13	56.9 ± 14.2	37.4 - 80.8	2.16
	110	13	72.0 ± 21.0	49.1 - 114.2	2.33
50	71	16	48.4 ± 15.0	30.0 - 82.3	2.74
	82	15	64.6 ± 14.0	43.5 - 89.0	2.05
	93	19	70.0 ± 12.7	43.2 - 92.3	2.14
	110	19	76.6 ± 21.5	30.6 - 115.9	3.79
68	71	7	35.5 ± 10.3	22.0 - 49.1	2.23
	82	27	47.6 ± 13.4	22.0 - 69.0	3.14
	93	13	54.7 ± 14.5	39.4 - 78.2	1.98
	110	15	51.8 ± 16.0	24.9 - 86.1	3.46
96	71	12	29.6 ± 5.6	23.3 - 41.2	1.77
	82	25	36.8 ± 11.4	18.7 - 63.5	3.40
	93	18	50.0 ± 12.6	23.7 - 72.2	3.05
	110	20	52.0 ± 10.5	22.0 - 68.0	3.09
144	71	24	33.5 ± 9.2	22.1 - 54.8	2.48
	82	29	38.5 ± 9.3	24.4 - 58.9	2.41
	93	16	43.3 ± 7.6	28.7 - 60.7	2.11
	110	8	41.7 ± 10.4	27.9 - 54.0	1.94

however, cannot be the only factor contributing to changes in protein contents. If nuclear protein content was related to phase of the cell cycle, then the range of protein contents should be the same at all times in an actively growing meristem. The data shows, however, that between 50 and 144 hours the range of protein contents undergoes a marked contraction: it is 30 to 158 A.U. at 50 hours and only 18 to 61 A.U. at 144 hours (Table 13). Similarly, for each individual size class of nuclei mean protein content shows a progressive decrease from 50 to 144 hours (Table 14). It must be concluded from this observation that the average concentration of proteins, per unit volume of nucleus, decreases as the population of cells develops into an asynchronous, steady-state system. These changes suggest that the process of germination includes a reorganization in nuclear physiology. In part, these changes may be related to changes in cell cycle kinetics. Additional evidence bearing on this question is considered in the next two sections which present data on cell cycle changes in the mature root.

III.1.7. Cell Doubling Times in Mature Root Meristems

In germinating beans, root meristem cells take 36 to 70 hours to complete their cycle and then undergo mitosis (Fig. 5; Table 6). In order to understand the changes in

cell and nuclear sizes that occur between 50 and 144 hours, it was necessary to obtain estimates of cell cycle durations in the second and third cell cycles.

The first parameter to be estimated was mean cell doubling time in the meristem. This can be done by determining, in the presence of colchicine, the rate of accumulation of cells in mitosis. If all cells divide, the mean cell doubling time is equivalent to the mean cycle time for the population. As the proportion of proliferating cells within a population, ie., the growth fraction (G.F.) decreases, the mean cell doubling time increases and exceeds the actual mean cycle time of the proliferating cells. Where G.F. <1 the mean doubling time is not a true estimate of the average cycle time of proliferating cells.

Roots grown in water for 148 hours were placed in 0.05% colchicine for 1, 2 and 4 hours. An estimate of the average population doubling time was made using Puck's accumulation function (Puck, 1972). The mean doubling time for all roots examined was 38.8 hours. Thus even if non-cycling cells were present, the mean cell doubling time of roots 144 hours old was less than the period most meristematic cells needed to complete their first cell cycle. This means that cells must complete their second or third cell cycles faster than they complete the first cell cycle. This decrease results in a reduction

in the time available for cell growth and it is interesting that it is paralleled by decreases in cell area (section III.1.4), nuclear volume (section III.1.5) and nuclear protein contents (section III.1.6) seen during germination.

Roots grown under the same conditions elongate at different rates (section III.1.3). This may reflect differences in the rate of cell production in different root meristems. Roots 14 to 41 mm and 45 to 72 mm long were treated with 0.05% colchicine and the mean cell doubling time determined. In short roots cell number doubled in 54.7 hours while in long roots the mean doubling time was 30.6 hours. This shows that the rate of cell production in the meristem was related to root length. The rate of cell production in long roots is clearly greater than in short roots.

III.1.8. Cell Cycle Duration in the Mature Root Meristem

Some cells in the root meristem of *V. faba* are non-cycling (Webster and Davidson, 1968). When growth fraction (G.F.) is <1, ^{3}H-TdR labelling provides a more accurate estimate of cell cycle duration than cell doubling times obtained with colchicine. Roots grown in water for 144 hours were placed in ^{3}H-TdR for 1 hour, washed and then returned to aerated distilled water. Roots were

sampled 0 to 31 hours after treatment with ^{3}H-TdR.

During the experiment the mean M.I. at the time of sampling fluctuated between 5.1 ± 1.2 and 9.8 ± 2.8; the mean M.I. averaged over the entire experiment was 7.2 ± 2.5 based on 47 roots. Fluctuations in the M.I. distorted estimates of the percent labelled mitoses (P.L.M.) at any time, making it difficult to estimate cycle duration with the degree of accuracy usually achieved with ^{3}H-TdR labelling. For this reason the analysis was performed on a frequency of labelled mitoses (F.L.M.) curve (Fig. 14). This curve follows the frequency of labelled mitoses for those cells which were labelled in S during the 1 hour pulse of ^{3}H-TdR and is less sensitive to variation in M.I. than the P.L.M. curve.

The mean cycle time (T_c) was estimated from the F.L.M. curve to be 23.4 hours. The mean duration of S (T_S) plus the labelling time (T_L) was 10.6 hours and since T_L was 1 hour, T_S was 9.6 hours.

An estimate of the proportion of cells in S phase at any time is made by determining labelling index (L.I.); ie., the percentage of cells labelled after a pulse exposure to ^{3}H-TdR. L.I. was determined for individual roots at 0 and 3 hours after ^{3}H-TdR. The mean L.I. based on 1000 cells from each of 9 roots was 31 ± 9%; ie., 31% of the cells were in S at the time of labelling.

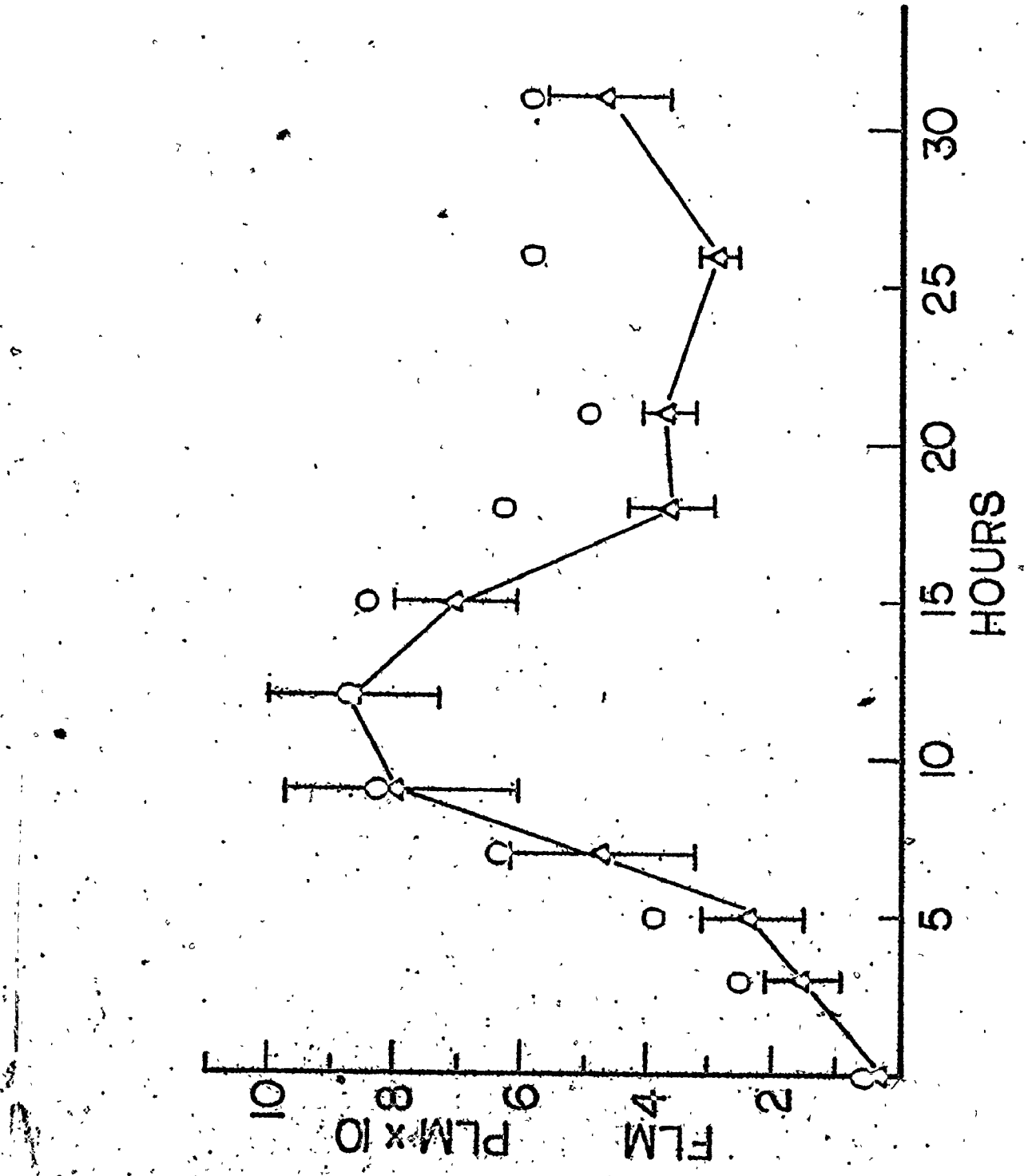
Figure 14

Percent Labelled Mitoses and Frequency Labelled Mitoses at Various Times after Treatment with ^{3}H-TdR

Seeds were imbibed in 5 to 10 mm of water for 24 hours and then grown in tanks of aerated distilled water. Roots 144 hours old were placed in ^{3}H-TdR for 1 hour and fixed 0 to 31 hours after the pulse. Each value is based on 100 mitotic cells from each of 3 to 5 roots. Standard errors are given for the frequency labelled mitoses data.

Percent Labelled Mitoses (PLM) o,

Frequency Labelled Mitoses (FLM) ▲.


HOURS
5
10
15
20
25
30
FLM
PLM x 10
2
4
6
8
10

An estimate of the mean L.I. can also be obtained from the equation:

$$L.I. = \ln 2 \frac{(T_S + T_L)}{T_C} \qquad (1)$$

(modified from Clowes, 1971)

From the F.L.M. curve we know that:

$$T_S + T_L = 10.6 \text{ hours}$$

$$T_C = 23.4 \text{ hours}$$

substituting in equation (1), the mean L.I. is estimated to be 31%. This value is identical to that obtained from the labelled cell method.

An estimate of G.F. can be obtained by determining the number of cells that pass through DNA synthesis over an extended labelling period. Cells were labelled in ^{3}H-TdR for 24 hours and the fraction of labelled cells calculated. By this method the G.F. was estimated to be 0.76 ± 0.03, based on 1,000 cells from each of 7 roots. The fraction of labelled cells ranged from 0.72 to 0.80 in roots 21 to 57 millimeters long.

Growth fraction can also be estimated from the formula:

$$G.F. = \frac{\text{the weight of the area under the M.I. curve over 1 cell cycle}}{\text{the weight of the area under the F.L.M. curve over 1 cell cycle}} \times \frac{L.I.}{\ln 2} \qquad (2)$$

(modified from Clowes, 1971)

where the area under the M.I. curve weighed 1.264 grams; the area under the F.L.M. curve weighed 0.8223 grams, and L.I. = 31%.

Substituting these values in equation (2),

$$G.F. = 0.70$$

This value is in good agreement with the value obtained after continuous labelling.

If we assume that the G.F. was 0.76 as estimated by continuous labelling then the duration of mitosis (T_m) can be estimated by:

$$T_m = M.I. \times \frac{T_C\ (G.F.)}{\ln\ (1 + G.F.)} \qquad (3)$$

(Clowes, 1961)

where G.F. = 0.76

M.I. = 7.2

T_C = 23.4 hours

Substituting these values in equation (3),

$$T_m = 2.27 \text{ hours}$$

Knowing T_m we can find the mean duration of G_2 (T_{G_2}). From the F.L.M. curve (Fig. 14) we obtain:

$$T_{G_2} + \frac{T_m}{2} = 6.6 \text{ hours} \qquad (4)$$

Substituting for $\frac{T_m}{2}$ we obtain, T_{G_2} = 5.5 hours.

The first labelled cells seen in mitosis were present at the end of the 1 hour pulse with ^{3}H-TdR. They were lightly labelled but they had progressed from S to mitosis in 1 hour; this period is taken to be the minimum duration of G_2.

We have seen that the mean population doubling time (T_{DT}) was estimated to be 30.6 hours by colchicine-induced accumulation of mitoses (section III.1.7). We can estimate the mean cycle time for the population of cycling cells (T_{cyc}) by:

$$T_{cyc} = G.F. \times T_{DT} \qquad (5)$$

(Clowes, 1975)

where: G.F. = 0.76

T_{DT} = 30.6 hours

substituting in equation (5), we obtain

$$T_{cyc} = 23.1 \text{ hours}$$

The F.L.M. estimate of cycle time was 23.4 hours. This suggests that the data from the F.L.M. curve represents an average of the entire population of cells.

It is known that *V. faba* meristems show cell cycle heterogeneity: they contain what have been defined as fast and slow cycling cells (Webster and Davidson, 1968). An attempt was made to determine the contribution of these various cell types to the meristem at 148 hours. An

estimate of the mean cycle time of fast cycling cells (T_{FC}) can be obtained from:

$$T_{FC} = \frac{\text{the weight of the area under the M.I. curve over 1 cell cycle}}{\text{the weight of the area under the F.L.M. curve over 1 cell cycle}} \times (T_S + T_L) \quad (6)$$

(Clowes, 1971; 1975)

where the area under the M.I. curve weighed 1.264 grams, the area under the F.L.M. curve weighed 0.8223 grams,

$$T_S + T_L = 10.6 \text{ hours}$$

substituting in equation (6), we obtain:

$$T_{FC} = 16.3 \text{ hours}$$

An estimate of the proportion of all cycling cells that were fast cycling (P_{FC}) is given by:

$$P_{FC} = \frac{T_{FC}}{T_c} \quad (7)$$

(modified from Clowes, 1971; 1975)

where T_{FC} = 16.3 hours

T_C = 23.4 hours

substituting in equation (7), we obtain

$$P_{FC} = 0.70$$

This means that 70% of the cycling cells were fast cycling while 30% of the cycling cells were slow cycling. Because the actual G.F. was 75.6%, as estimated from the continuous

labelling method, then 24.4% of the cells in the root were non-cycling; 22.7% of the cells in the root were slow cycling, and 52.9% of the cells in the root were fast cycling.

Since 1) the time estimates obtained from the F.L.M. curve appear to be for the entire population of cycling cells, and 2) the majority of the cycling cells were fast cycling and should have contributed most to the F.L.M. curve, this implies that the distributions of the duration of S + G_2 for both fast and slow cycling cells overlap in water-grown roots. This prevents an interpretation of the F.L.M. curve in terms only of contributions from fast cycling cells.

The fluctuations in the mean M.I. during the experiment may have contributed to some error in the estimates given above. This error is assumed to be minimal because of the internal agreement in the estimates of G.F. and L.I. with the values obtained from the labelling experiments.

These results show that cells in the root meristem not only vary in cell area, nuclear volume and total nuclear protein content (sections III.1.4, 1.5 and 1.6) but also in cell cycle duration, ie., the mature meristem contains fast, slow and non-cycling cells. This agrees with previous observations of root meristems (Webster and

Davidson, 1968; MacLeod, 1971; Evans and Van't Hof, 1975). Actual estimates of G.F., L.I., and cell cycle duration, however, do vary from those given in the literature (MacLeod, 1971; Miller, Brulfert and Kaufman, 1978; Howard and Pelc, 1953). The following section shows that some of these differences can result from differences in the ambient water supply during germination and root growth.

III.2. Changes in the Amount of Available Water During Germination

The amount of water available during germination has been shown to influence seedling growth (Davidson, Eastman and Thomas, 1976; Davidson, 1979). The following experiments were performed in order to determine how changes in the ambient water available during germination influences M.I., cell area, nuclear volume, nuclear protein content and the rate of cell division in the meristem.

III.2.1. Effect of the Ambient Water Level During the First 24 Hours of Germination

Seeds of *V. faba* were placed in dishes in which the water was 5, 10 and 20 mm deep. After 24 hours they were transferred to tanks of aerated distilled water. Root lengths and M.I. were measured at 50, 60 and 68 hours

of germination. At all times examined (Table 15) both the mean root length and mean M.I. were higher in seeds originally placed in 5 or 10 mm of water than in seeds sown in 20 mm of water. In fact, apart from the M.I. at 50 hours, seeds sown in 5 mm of water had longer roots and a higher M.I. than seeds sown in 10 mm of water (Table 15). This means that more cells had divided by 68 hours when seeds were imbibed in 5 mm of water than in 20 mm of water; ie., the time taken to complete the first cell cycle is much longer in seeds sown on 20 mm than on 5 mm of water. This suggests that the response to different volumes of water is initiated at the very onset of germination.

III.2.2. The Effects of Changes in Water Levels After 24 Hours of Germination

III.2.2.1. Root lengths and mitotic index

Seeds were imbibed in 5 mm of water for 24 hours and then grown in sand or in a 1:1 mixture of vermiculite and perlite to which 20 to 55 ml of water were added per 100 ml of substrate. As with material grown continuously in water (section III.1), seeds grown in moistened sand show an increase in both mean M.I. (Figure 17, cf. Figure 5) and mean root length (Figure 18, cf. Table 6) with time during the initial stages of germination. Similarly, there

Table 15

Effects of Different Water Levels During the First 24 Hours of Imbibition

Mean root length, mean M.I. and the percent roots with a measurable M.I. are given for samples of 10 or 11 seeds placed in 5,10 or 20 mm of water for the first 24 hours of imbibition and then grown in tanks of distilled water for 50,60 and 68 hours.

Duration of growth in water	Water depth in mm during the 1st 24 hr imbibition	$\overline{R.L.}$ ± S.E.	$\overline{M.I.}$ ± S.E.	% roots with a measurable M.I.	Sample size
50 hours	5	4.8 ± 0.36	2.38 ± 1.21	70	10
	10	4.3 ± 0.24	2.62 ± 1.50	60	10
	20	4.0 ± 0.25	0.43 ± 0.32	27.3	11
60 hours	5	5.6 ± 0.47	2.89 ± 1.47	81.8	11
	10	4.3 ± 0.40	1.24 ± 0.98	60	10
	20	4.1 ± 0.52	0.94 ± 0.63	20	10
68 hours	5	7.7 ± 0.76	4.02 ± 1.23	90.9	11
	10	6.2 ± 0.53	1.22 ± 0.88	40	10
	20	5.1 ± 0.35	1.23 ± 0.71	40	10

was a close relationship between mean M.I. and root length (Figure 19, cf. Figure 6).

Both mean root length (Figure 15) and mean M.I. (Figure 16) were affected by the amount of available water and both values were highest with the lower volumes of water tested. At 70 hours of germination a maximum mean root length of 9.9 ± 5.8 mm was obtained in 25 ml of water/100 ml of sand and 12.1 ± 6.2 mm in 40 ml water/100 ml vermiculite-perlite (Figure 15). Similarly, a maximum mean M.I. of 9.9 ± 6.4 was obtained in 30 ml water/100 ml sand and 9.2 ± 5.9 in 40 ml water/100 ml vermiculite-perlite (Figure 16). These results show that high water levels decrease both the rate at which cells enter mitosis and the rate of root growth. This means that cell cycle times for these cells were lengthened.

The mean M.I. for roots grown either in 25 ml water/100 ml sand or in tanks of water reached a maximum in roots 7 mm long and then fluctuated about a plateau level in longer roots (Figure 19, cf. Figure 6). Roots longer than about 4.5 mm, however, had a higher mean M.I. when grown in sand than when grown continuously in water. The sand-grown roots also reached a plateau M.I. about 1.4 X higher than that reached by roots grown in tanks of water. These results show that both the height of the first peak and the plateau level of the M.I. are influenced

Figure 15

Root Length of Beans Grown in Sand or Vermiculite-perlite Containing Various Volumes of Water

Seeds were placed in 5 mm water for 24 hours and then grown either in sand, ▲ or vermiculite-perlite (1:1), ● moistened with various amounts of water per 100 ml dry substrate. At 70 hours mean root length (mm) ± S.E. was determined for 77 to 109 roots at each water volume.

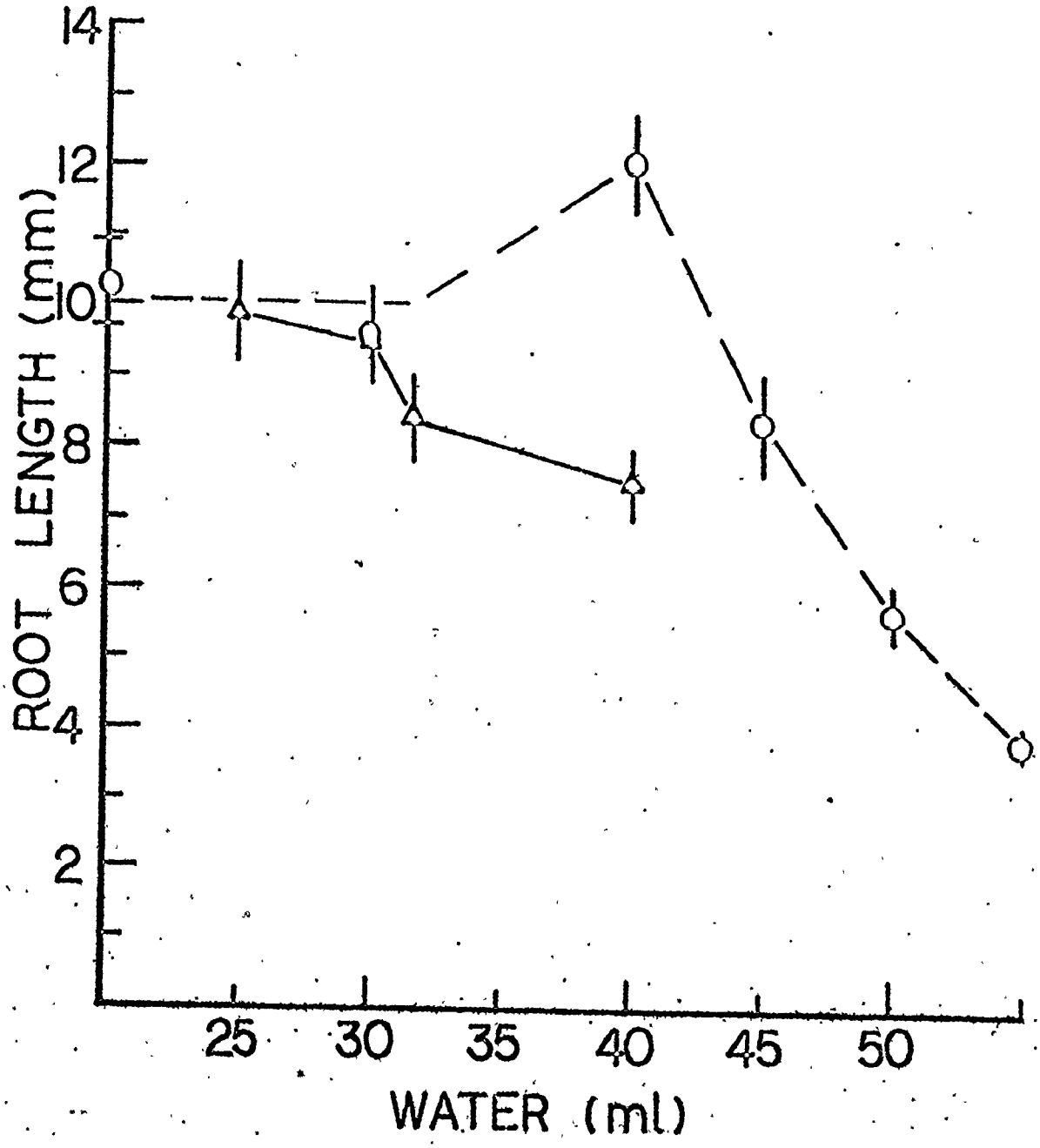

14
12
10
8
6
4
2
ROOT LENGTH (mm)
25
30
35
40
45
50
WATER (ml)

Figure 16

Mitotic Index of Beans Grown in Sand or Vermiculite-perlite Containing Various Volumes of Water

Seeds were placed in 5 mm water for 24 hours and then grown either in sand, ▲ or vermiculite-perlite (1:1), o moistened with various amounts of water per 100 ml dry substrate. At 70 hours mean M.I. ± S.E. was determined for 10 roots at each water level.

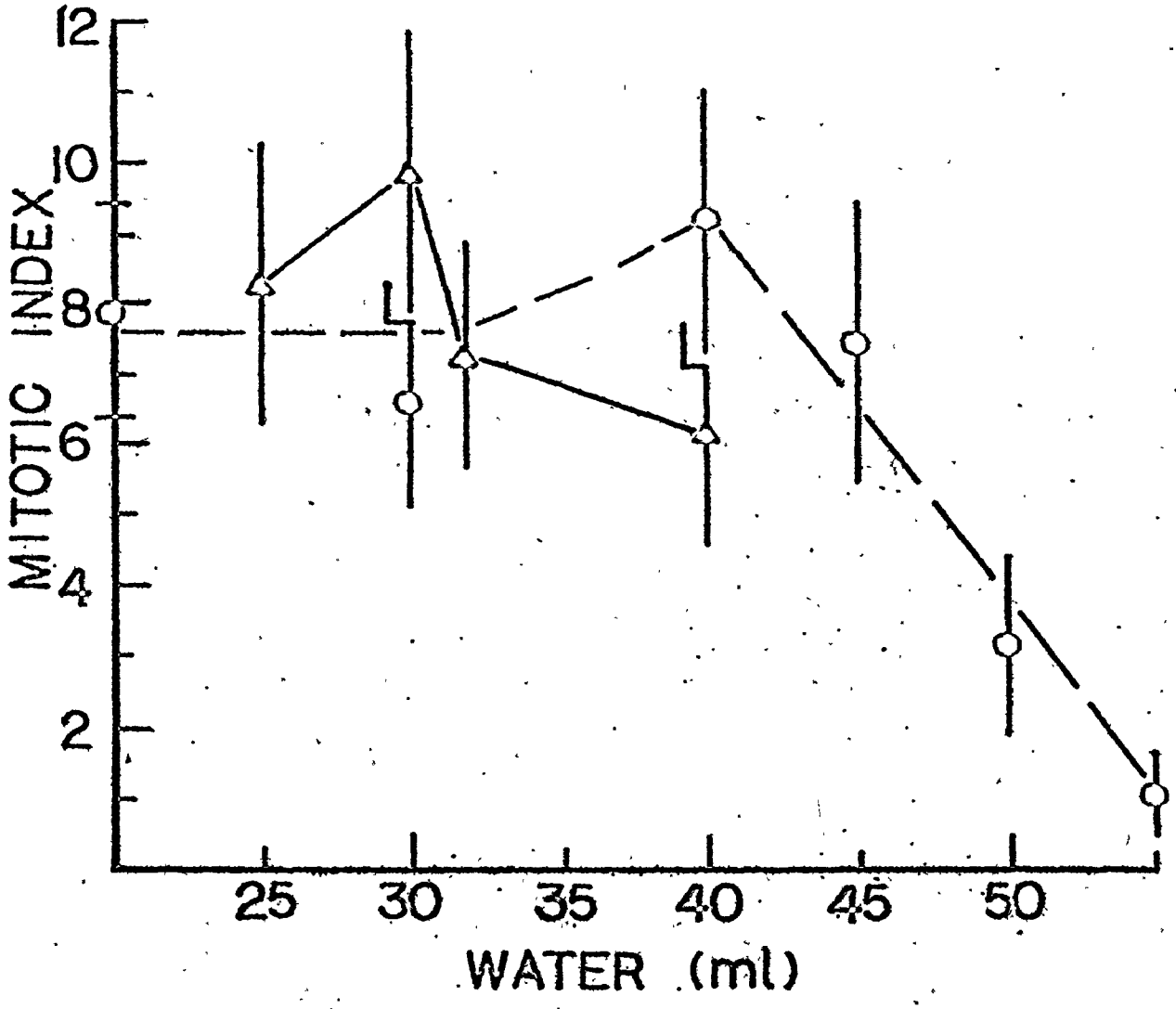

MITOTIC INDEX
12
10
8
6
4
2
25
30
35
40
45
50
WATER (ml)

Figure 17

Increase in Mean Mitotic Index with Time

Mean M.I. ± S.E. was determined for seeds placed in 5 mm of water for 24 hours and then grown in sand (25 ml water/100 ml sand) for 50 to 70 hours. Ten roots were sampled at each time.

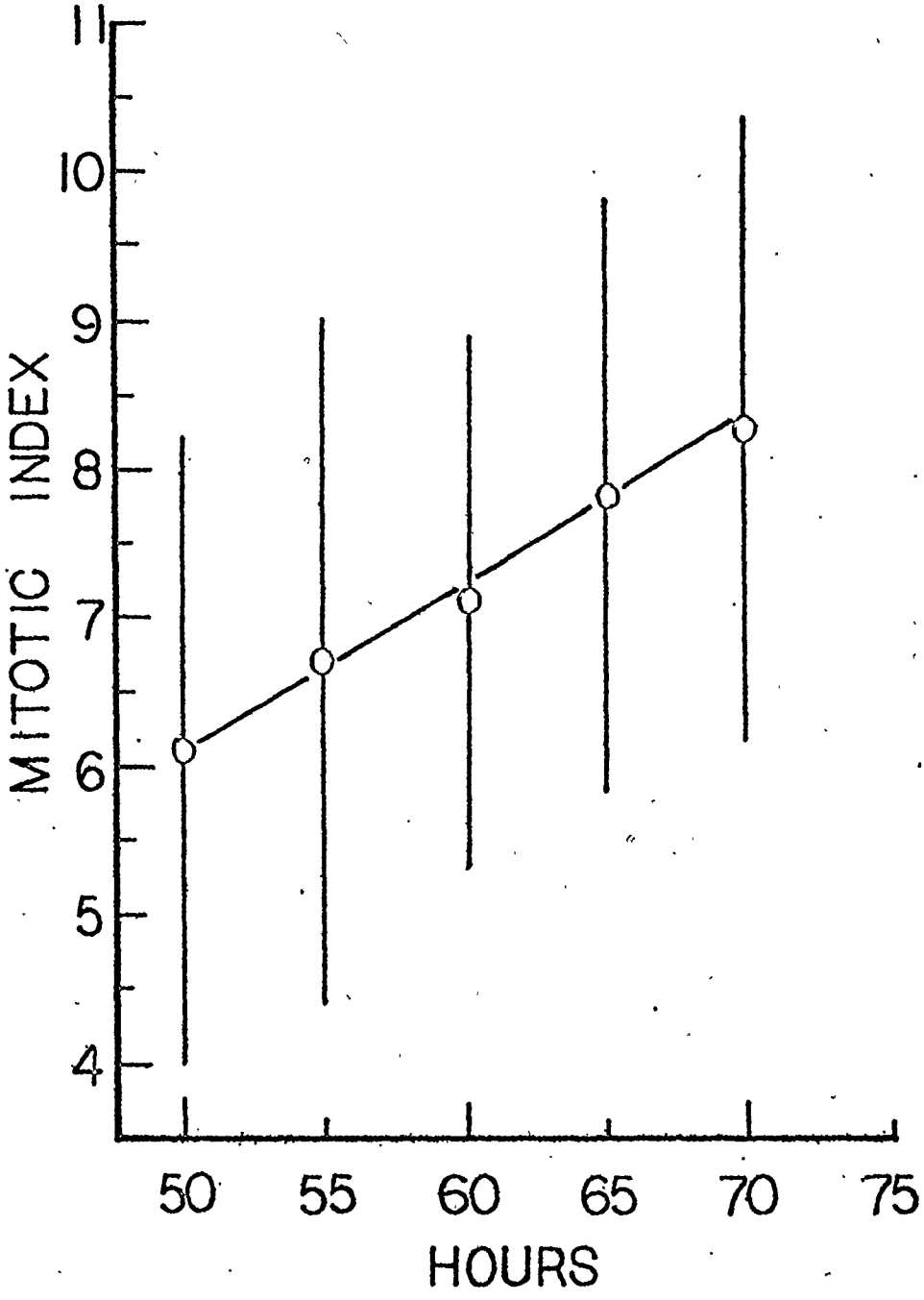

MITOTIC INDEX
11
10
9
8
7
6
5
4
50
55
60
65
70
75
HOURS

Figure 18

Increase in Mean Root Length with Time

Mean root length ± S.E. was determined for seeds placed in 5 mm of water for 24 hours and then grown in sand (25 ml water/100 ml sand) for 50 to 70 hours. Each point is based on 77 to 111 roots.

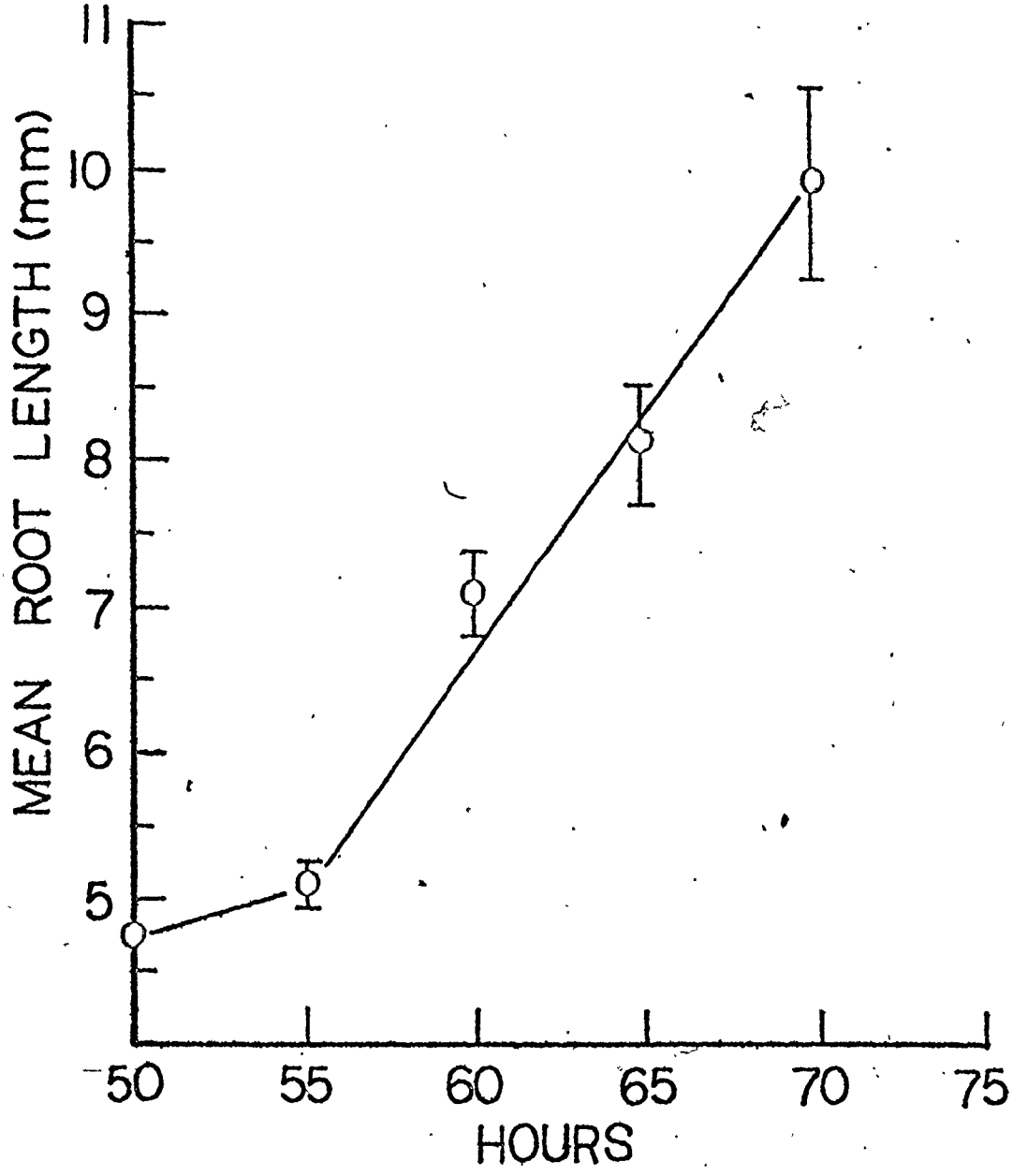
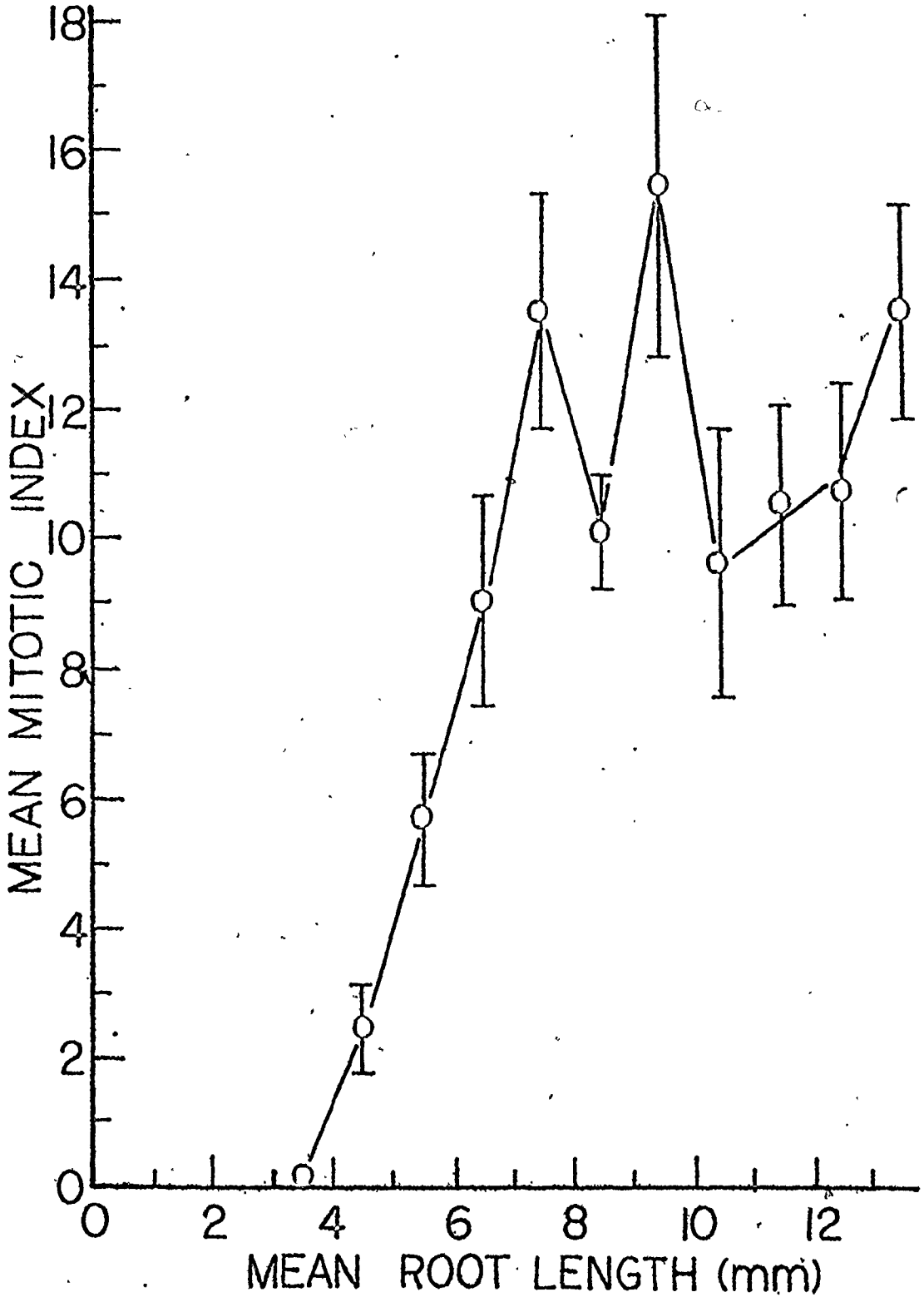

MEAN ROOT LENGTH (mm)
11
10
9
8
7
6
5
50
55
60
65
70
75
HOURS

Figure 19

Mean Mitotic Index Plotted Against Mean Root Length

Mean root length and mean M.I. ± S.E. were determined for seeds placed in 5 mm of water for 24 hours and then grown in moistened sand (25 ml water/ 100 ml sand) for 50 to 70 hours. Each M.I. is based on 1000 cells per root. The average sample size was 13 roots per point.


MEAN MITOTIC INDEX
0
2
4
6
8
10
12
14
16
18
0
2
4
6
8
10
12
MEAN ROOT LENGTH (mm)

by the amount of water available during the initial stages of root growth. The following section shows how these changes affect cell and nuclear growth in the meristem.

III.2.2.2. Nuclear volumes and cell areas

In order to determine the extent of the effect of water availability on cell and nuclear growth the following study was carried out. Beans were sown in water, 5 mm deep, for 24 hours and then grown in either 1) tanks of aerated distilled water; 2) moistened sand, 25 ml of water/ 100 ml of sand, or 3) vermiculite-perlite, 40 ml of water/ 100 ml of mixture. The effects of these growth conditions on nuclear volume, cell area, nuclear protein contents and cell cycle kinetics are given in sections III.2.2.2, to III.2.2.5.

Between 36 and 70 hours of germination nuclei from sand-grown roots show an increase in size similar to that seen in roots grown continuously in water (Sample II, Table 16, cf., Sample I, Table 8). By 96 hours mean nuclear volume reached a plateau level, ie., at 96 and 132 hours the mean nuclear volume in sand-grown roots was 1148 and 1125 μm^3, respectively (Sample III, Table 20). Mean cell area was also constant during this time (Sample III, Table 20). This lack of variation both in nuclear and cell size suggests that root meristem cells, 96 hours old, are both asynchronous and steady state.

Table 16

Comparison of Roots Grown in High and Low Water Levels

Mean M.I., mean nuclear volume and mean cell area are given for seeds placed in 5 mm water for 24 hours and then grown in either 1) tanks of aerated distilled water[a], 2) 25 ml water/100 ml sand[b], or 3) 40 ml water/100 ml vermiculite-perlite[c] up to 144 hours.

Treatment	Time (hours)	Number of roots	Mitotic Index mean ± S.D.	Interphase				Prophase			
				Number of nuclei	Nuclear Volume mean ± S.D.	Number of cells	Cell Area mean ± S.D.	Number of nuclei	Nuclear Volume mean ± S.D.	Number of cells	Cell Area mean ± S.D.
					Sample I						
Sand[a]	36	5	1.6 ± 2.7	560	701 ± 291			56	1495 ± 379		
	50	5	17.8 ± 10.5	560	795 ± 252			112	1161 ± 327		
	70	5	11.7 ± 2.7	500	926 ± 346			56	1512 ± 439		
	96	8	12.5 ± 2.6	1000	971 ± 410			168	1623 ± 496		
					Sample II						
Water[b]	50	6	7.0 ± 5.8	500	885 ± 264	140	642 ± 287	50	1234 ± 280	50	981 ± 326
Sand[a]	50	5	12.7 ± 5.3	500	893 ± 288	140	548 ± 196	50	1521 ± 507	50	778 ± 213
					Sample III						
Water[a]	144	5	9.0 ± 1.9	140	1029 ± 368	140	645 ± 275	50	1895 ± 861	50	868 ± 288
Vermiculite-perlite[c]	144	5	12.7 ± 1.5	140	1381 ± 744	140	538 ± 206	50	2287 ± 985	50	715 ± 182

The mean nuclear volume for sand-grown material obtained at 96 hours ranged from 971 to 1148 μm^3 (Sample I, Table 16 and Sample III, Table 20). These values overlap those of roots grown continuously in water (Table 8). This overlap suggests that the amount of water available to roots is not the only factor controlling nuclear growth. Also, because the mean nuclear volume in mature roots grown under similar conditions was variable, a comparison of absolute values from one sample to the next may not always be meaningful.

It has already been shown that the mean M.I. of roots grown in tanks of water tends to be lower than that of roots grown with less available water (section III.2.1). Both mean interphase and prophase nuclear volumes were also lower in roots grown continuously in water than in roots grown with less water (Samples II and III, Table 16); at 144 hours the mean volumes of interphase nuclei in vermiculite-perlite and water grown roots, ie., 1381 ± 744 and 1029 ± 368 μm^3, were significantly different ($P > 0.001$). In contrast, mean cell areas from roots grown continuously in water were larger than those of roots grown in sand or vermiculite-perlite (Samples II and III, Table 16). This means that the response of cells and nuclei to a change in available water is differential, ie., whereas nuclei are larger, cells are smaller in roots

grown in low amounts of water than they are when roots are grown continuously in water.

III.2.2.3. Nucleo-cytoplasmic ratios

It has been shown that both cells and nuclei grow at different rates with different amounts of available water (section III.2.2.2). In addition to a change in the size of cells and nuclei grown under different environmental conditions the relative size of cells and nuclei also changed. At 50 and 144 hours of germination the ratio, mean nuclear area:mean cell area was lower in roots grown in tanks of water than in roots grown either in sand or vermiculite-perlite (Samples I and II, Table 17). All other sand and vermiculite-perlite grown roots (Samples III and IV, Table 17) also had higher nucleo-cytoplasmic ratios than water-grown roots. This means that nuclei from roots grown in low volumes of water tend to occupy more of the cell than nuclei from roots grown in tanks of water.

The nucleo-cytoplasmic ratio changes during the cell cycle (Table 12) and so if cell cycle kinetics are altered by different growing conditions, the nucleo-cytoplasmic ratio might also change when the proportion of cells in any phase of the cycle changes. Prophase occurs at a fixed point in the cell cycle. Consequently, because

Table 17

Cell and Nuclear Growth in Roots Grown in Water, in Sand or in Vermiculite-perlite

Nuclear area:cell area ratios are given for seeds placed in 5 mm water for 24 hours and then grown in either 1) tanks of aerated distilled water, 2) 25 ml water/100 ml sand or 3) 40 ml water/100 ml vermiculite-perlite for various periods of time. Values are given for both interphase and prophase cells. Nuclear areas were calculated as nuclear volume$^{2/3}$.

Roots grown in	Time (hours)	Nuclear area:Cell area	
		Interphase	Prophase
	Sample I		
Water	50	0.144	0.117
Sand	50	0.169	0.170
	Sample II		
Water	144	0.158	0.177
Vermiculite-perlite	144	0.230	0.243
	Sample III		
Vermiculite-perlite	96	0.193	0.203
Vermiculite perlite	120	0.191	0.207
	Sample IV		
Sand	96	0.203	0.210
Sand	132	0.198	0.209

the nucleo-cytoplasmic ratio of cells in prophase differs with different amounts of available water (Table 17), changes in this ratio must, at least in part, be due to a direct change in the physiological state of cycling cells. It should be noted that roots grown in sand and vermiculite-perlite were longer and had higher M.I.'s than those grown in tanks of water for the same period (section III.2.2.1).

The nucleo-cytoplasmic ratio in mature sand (Sample IV, Table 17) or vermiculite-perlite (Sample III, Table 17) grown roots remained constant in both interphase and prophase from 96 to 132 hours, ie., the ratios for interphase cells from sand grown roots were 0.203 and 0.198. However, it should be noted that both the interphase and prophase ratios may vary somewhat in different samples of mature roots, ie., the interphase nucleo-cytoplasmic ratio in roots grown in vermiculite-perlite was 0.23 in Sample II (Table 17) but only 0.19 in Sample III (Table 17).

The differences in nuclear and cell size seen with different amounts of available water may reflect differences in cellular constitutents. With this in mind, nuclear protein contents in germinating and mature sand grown roots were examined next.

III.2.2.4. Nuclear protein contents

At 36, 50 and 96 hours of germination there were no significant differences in mean nuclear protein content between roots grown either in tanks of water or in sand (Table 13, cf. Table 18). At 68 hours, however, the mean nuclear protein content of roots grown in tanks of water was 50 A.U. (Table 13) while in sand it was 46 A.U. (Table 18); these values are significantly different ($P = 0.05$) Also, at all times examined the range in nuclear protein contents was smaller in sand-grown roots than it was in water-grown roots (Table 18, cf. Table 13).

All differences in nuclear protein contents between water and sand grown roots can be attributed to differences in the rate of cell proliferation in the two systems. Cells in sand-grown roots (section III.2.2.1); 1) complete their first cell cycle faster and 2) have a higher mean M.I. than cells from roots grown continuously in water. This higher rate of cell proliferation results in the production of more G_1 nuclei, which on average are the smallest nuclei in the meristem, in sand-grown roots than in roots grown continuously in water; the result is that mean nuclear area at 68 hours of germination is significantly smaller in sand-grown roots ($P = 0.05$) than in water-grown roots (Table 18, cf. Table 13). It has already been shown (section III.1.6) that 1) small nuclei

Table 18

Changes in Nuclear Protein Content for Roots Grown in Low Water

Mean nuclear area, range in nuclear area, mean nuclear protein and range in nuclear protein are given for seeds placed in 5 to 10 mm water for 24 hours and then grown in about 25 ml water/100 ml sand up to 36 to 96 hours. All samples were of 100 nuclei.

Time from addition of water (hours)	Nuclear area (μm^2)		Nuclear protein (A.U.)	
	mean ± S.D.	range	mean ± S.D.	range
36	94 ± 26	52 - 172	60 ± 21	33 - 113
50	104 ± 22	62 - 173	66 ± 20	24 - 116
68	96 ± 17	62 - 143	46 ± 13	19 - 77
96	93 ± 21	43 - 156	44 ± 13	15 - 76

tend to have lower nuclear protein contents than large nuclei and 2) nuclei with high nuclear protein contents divide before nuclei with lower nuclear protein contents. As a result, sand-grown roots, 68 hours old, have both a lower mean nuclear area and a lower mean nuclear protein content than roots grown in tanks of water. Also, because of the high division rates found in sand-grown roots at all times examined (section III.2.2.1 and III.2.2.5) the range in nuclear protein contents in these roots was smaller than that seen in roots grown in tanks of water (Table 18, cf. Table 13). No decrease in the range in nuclear area was observed in sand-grown roots as compared to water-grown roots (Table 18, cf. Table 13).

These results indicate that changes in the amount of available water during root growth do not affect the final nuclear protein content of mature meristematic cells. Another factor in the control of cell and nuclear size is cell cycle duration. This is examined in the next section.

III.2.2.5. Cell cycle kinetics in mature root meristems

Estimates of 1) growth fraction (G.F.) and 2) cell cycle duration were obtained for mature roots grown in vermiculite-perlite. At 96 hours of germination roots 20 to 89 mm long were placed either in 1) 40 ml 0.05%

colchicine/100 ml vermiculite-perlite for 1, 2 and 4 hours, 2) 40 ml ^{3}H-TdR solution/100 ml vermiculite-perlite for up to 48 hours continuous label or 3) 40 ml ^{3}H-TdR solution/100 ml vermiculite-perlite for a 1 hour pulse, then washed and returned to 40 ml water/100 ml vermiculite-perlite. Note, all treatments were performed in vermiculite-perlite in order to avoid a change in the amount of available water during treatment.

It has already been shown that cells from mature roots grown in tanks of water double in number every 38.8 hours (section III.1.7). The mean cell doubling time for roots grown in vermiculite-perlite was 22.8 hours (Table 19); estimated by colchicine accumulation of mitoses (Puck, 1972). This means that cells from roots grown in low amounts of available water double in number faster than cells from roots grown in tanks of water.

Cell doubling times were also determined for roots 96 hours old, 20 to 44 mm and 45 to 81 mm long; they were 24.0 and 20.8 hours, respectively (Table 19). This parallels observations from roots grown in tanks of water where short roots had longer doubling times than long roots (section III.1.7).

The difference in cell doubling times between water and vermiculite-perlite-grown roots could result from a difference in the number of actively dividing cells in the

two systems; ie., G.F. may be lower in roots grown continuously in water than in roots grown in vermiculite-perlite. After 35 to 48 hours continuous treatment with ^{3}H-TdR beginning at 96 hours of germination, G.F. was 0.95 ± 0.06, based on 1000 cells from each of 12 roots (Table 19). In individual roots 62 to 85 mm long, G.F. ranged from 0.82 to 0.99. This means that roots grown in vermiculite-perlite have about 20% more actively dividing cells than roots grown in tanks of water.

Estimates of cell cycle parameters in roots 96 hours old were made as described in section III.1.8. Roots given a 1 hour pulse of ^{3}H-TdR were 39 to 89 mm long and were sampled at 0 to 30 hours after treatment. Mean root length was 63.5 ± 11.3 mm. Mean M.I. was 10.0 ± 2.6, based on 42 roots (Table 19).

Mean cycle time (T_c) estimated from the F.L.M. curve was 17.0 hours (Figure 20). Mean duration of S (T_S) plus labelling time (T_L) was 6 hours (Figure 20); since T_L was 1 hour, T_S was 5 hours. Both T_S and T_c were shorter for roots grown in vermiculite-perlite than for roots grown in tanks of water (Table 19); ie., T_S was 4.6 hours shorter in vermiculite-perlite grown roots than in roots grown in water while T_c was 6.4 hours shorter. Consequently, about 72% of the difference in T_c between vermiculite-perlite and water-grown roots was the result

Table 19

Cell Cycle Parameters for Roots Grown Continuously in Water or in Vermiculite-perlite Mixtures

All seeds were placed in 5 mm water for 24 hours and then transferred to either 1) tanks of aerated distilled water (High Water) or 2) 40 ml water/100 ml vermiculite-perlite (Low Water). Mature roots 144 and 96 hours old respectively were either labelled with ^{3}H-TdR or treated with 0.05% colchicine. Estimates of T_c, T_m, T_S, T_{G_2}, and T_{G_1} were made using F.L.H. curves (Figures 14 and 20). L.I. was determined after a 1 hour pulse of ^{3}H-TdR and G.F. after 24-48 hours continuous ^{3}H-TdR. Mean M.I. for the ^{3}H-TdR-treated roots is given (N = 47 and 42). Estimates of L.I., G.F., T_{cyc}, T_{FC}, and P_{FC} were determined using equations 1-7 from section III.1.8. Estimates of % fast, % slow and % non-cycling cells are also given. An estimate of T_{DT} in long, short and a combined sample of roots was made using colchicine accumulation of mitoses for 1, 2 and 4 hours (Puck, 1972).

Roots grown in	T_c	T_m	T_S	T_{G_2}	T_{G_1} (hours)	L.I. (labelled cell method) mean ± S.D.	L.I. (equation 1)	M.I. mean ± S.D.	G.F. (continuous labelling method) mean ± S.D.	G.F. (equation 2)
Tanks of distilled water	23.4	2.3	9.6	5.5	6.1	31.0 ± 8.6	31.0	7.2 ± 2.5	0.76 ± 0.03	0.70
40 ml water/ 100 ml vermiculite-perlite	17.0	2.4	5.0	4.0	5.6	25.9 ± 6.5	24.5	10.0 ± 2.6	0.95 ± 0.06	0.93

	T_{cyc} (equation 5) (hours)	T_{FC} (equation 6) (hours)	P_{FC} (equation 7)	% fast cycling cells	% slow cycling cells	% non-cycling cells	T_{DT} (hours) all roots	T_{DT} (hours) root length 14-44 mm	T_{DT} (hours) root length 45-81 mm
Tanks of distilled water	23.1	16.3	0.70	52.9	22.7	24.4	38.8	54.7	30.6
40 ml water/ 100 ml vermiculite-perlite	19.7	15.0	0.88	83.2	11.4	5.4	22.8	24.0	20.8

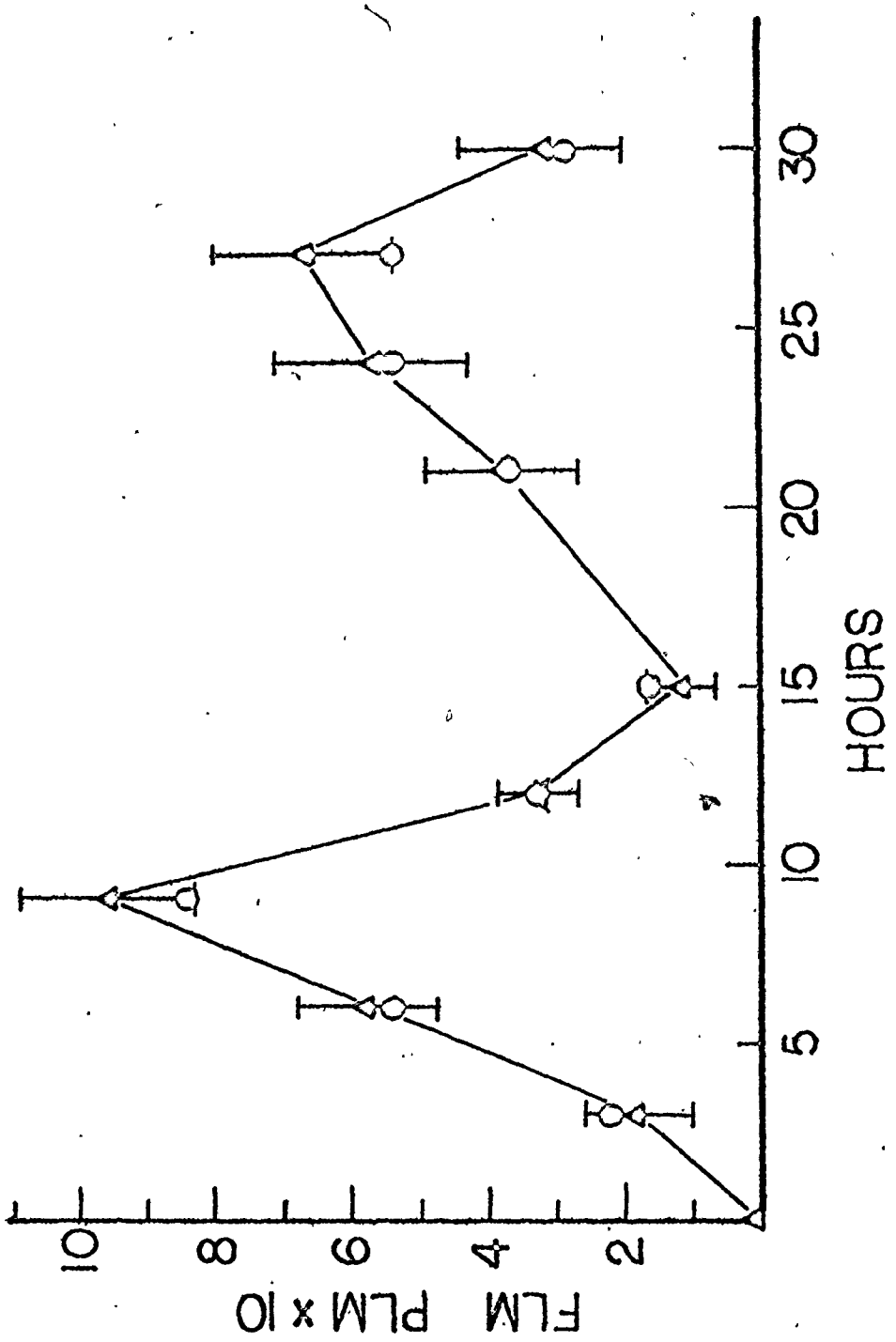
Figure 20

Percent Labelled Mitoses and Frequency Labelled Mitoses at Various Times after Treatment with ^{3}H-TdR

Seeds were imbibed in 5 to 10 mm of water for 24 hours and then grown in vermiculite-perlite (40 ml water/ 100 ml substrate). Roots 96 hours old were placed in ^{3}H-TdR for 1 hour and fixed 0 to 30 hours after the pulse. Each value is based on 100 mitotic cells from each of 3 to 5 roots. Standard errors are given for the frequency of labelled mitoses data.

Percent Labelled Mitoses (PLM) ●;

Frequency Labelled Mitoses (FLM) ▲.


FLM PLM x 10
2
4
6
8
10
5
10
15
20
25
30
HOURS

of variation in the duration of S. This means that DNA synthesis in root meristem cells is very sensitive to environmental changes.

Assuming G.F. was 0.95 as estimated by continuous labelling, T_m was estimated to be 2.4 hours (Table 19). This is almost identical to the estimate of T_m for roots grown in tanks of water (Table 19) indicating that changes in the amount of available water do not affect the duration of mitosis. The durations both of G_2, 4 hours, and of G_1, 5.6 hours, were shorter for roots grown in vermiculite-perlite than for roots grown in tanks of water (Table 19). The relative duration of G_2, however, remained constant at 23.5% of T_c under both types of growth conditions. In contrast, G_1 occupied 26.1% of T_c in water-grown roots and 32.9% of T_c in vermiculite-perlite-grown roots while S occupied 41% of T_c in water-grown roots but only 29.4% of T_c in vermiculite-perlite-grown roots. Consequently, not only were the mean durations of G_1, S and G_2 all longer in roots grown in tanks of water than in vermiculite-perlite (Table 19), but the relative duration of each phase within the cell cycle changed disproportionately with respect to one another under different growth conditions.

Because the number of cells within any phase of the cell cycle is proportional to the duration of that phase, one consequence of a difference in the duration of

S between water and vermiculite-perlite-grown roots should be a change in the proportion of cells found in S at any time; ie., the L.I. should decrease when T_S decreases. After a 1 hour pulse of ^{3}H-TdR mean L.I. was 25.9 ± 6.5% for roots grown in vermiculite-perlite; this represents a 5% decrease from the value obtained for roots grown in tanks of water (Table 19). Mean L.I. was based on 1000 cells from each of 10 roots, 0 and 3 hours after ^{3}H-TdR.

It has been shown that the cell doubling time in vermiculite-perlite for roots longer than 44 mm, was 20.8 hours and G.F. was 0.95 (Table 19). This means that the average cycle time of all cycling cells (T_{cyc}) in vermiculite-perlite was 19.7 hours; the mean cycle time of fast cycling cells (T_{FC}) was 15 hours. Consequently, all estimates of cycle time obtained in vermiculite-perlite, ie., T_{DT}, T_c, T_{cyc} and T_{FC}, were shorter than estimates obtained for roots grown continuously in water. This means that most cells from roots grown in vermiculite-perlite had shorter cycles than cells from roots grown continuously in water.

In vermiculite-perlite the proportion of all cycling cells that were fast cycling was 0.88, therefore, 83.2% of all cells were fast cycling, 11.4% were slow cycling and 5.4% were non-cycling (Table 19). This means that about 30% more fast cycling cells were found in vermiculite-perlite-grown roots than in roots grown in

tanks of water; about 11% fewer slow cycling cells and 19% fewer non-cycling cells were found in vermiculite-perlite than in water-grown roots.

These results indicate that the cell cycle duration in mature meristems is directly related to cell size, ie., cells grown in vermiculite-perlite were smaller (Table 16) and had shorter cell cycles (Table 19) than cells from roots grown in tanks of water. This implies that cell size in these roots is governed by the rate of cell proliferation in the meristem rather than by a critical size control over either rate of growth or cell division.

There also appears to be an inverse relationship between nuclear size and cell cycle duration. Cells grown in vermiculite-perlite had larger nuclei (Table 16) but a shorter cell cycle (Table 19) than those grown in water. This suggests that mean nuclear size is related to the physiological state of the meristem, ie., in addition to large nuclei, roots grown in vermiculite-perlite had a large G.F. and many fast cycling cells. Roots grown in tanks of water had small nuclei coupled with a low G.F. and few fast cycling cells.

These observations show both similarities and differences between roots grown in two different environments. They, however, do not allow one to predict the response of cells to a change in the environment. This

question is approached in the next two sections. Mature and germinating roots were manipulated either by 1) changing the amount of water available for root growth; this altered the physical environment in which cells were grown or 2) treating cells with 5-AU in order to chemically interfere with the cell cycle. The response of cells was measured by the M.I., cell area, nuclear volume, and nuclear protein contents.

III.3. Effects of a Change in the Amount of Water Available for Growth in Mature Roots

It has been shown that the mature root meristem consists of a steady state, asynchronous population of cells; ie., mean M.I., mean nuclear volume, and mean cell area are constant for a particular set of environmental conditions (sections III.1 and III.2). The stability of these parameters in response to a change in the amount of water available for growth of mature roots is examined in this section.

III.3.1. Effects on Mitosis

Roots grown in tanks of water generally have lower M.I.'s than roots grown either in sand or vermiculite-perlite (section III.2.1). Roots grown continuously in tanks of aerated distilled water for 100 hours were

transferred to 40 ml water/100 ml vermiculite-perlite. By 24 hours after transfer there was a significant increase in mean M.I. ($P = 0.05$), ie., mean M.I. was 8.5 ± 1.7 in water and 11.2 ± 1.5 in vermiculite-perlite. M.I. remained high for a further 24 hours; 48 hours after transfer to vermiculite-perlite, mean M.I. remained constant at 11.3 ± 1.9. In contrast, mean M.I. decreased in only 1 out of 3 samples of roots transferred from sand or vermiculite-perlite to tanks of water at 96 hours (Table 20).

Consequently, it appears that roots transferred from tanks of water to vermiculite-perlite underwent a change in cell cycle kinetics as they attained a new balance characteristic of the environment that they were placed in. Roots transferred from vermiculite-perlite to tanks of water, however, were resistant to change. It has already been shown that roots grown in vermiculite-perlite have a higher G.F. and more rapidly dividing cells than roots grown in tanks of water (Table 19). These results imply that physiologically more active cells grown in sand or vermiculite-perlite contain factors, possibly in the form of reserve nutrients, that impart an ability on the cells to resist a deterioration in environmental conditions. Roots grown in tanks of water, however, respond immediately to an improvement in the environment by becoming more metabolically active; ie., M.I. increases in these roots after transfer to vermiculite-perlite.

Table 20

Cell and Nuclear Size Before and After Transfer from Low to High Water

Mean root length, mean M.I., mean nuclear volume and range in nuclear volume, mean cell area and range in cell area, and the nuclear area/cell area ratio are given for 3 samples of interphase and prophase cells. Nuclear areas were calculated as nuclear volume$^{2/3}$. All seeds were placed in 5 to 10 mm of water for 24 hours and then grown in either 25 ml water/100 ml sand or 40 ml water/100 ml vermiculite-perlite for up to 108 hours. Some samples were transferred to tanks of aerated distilled water after 96 hours growth.

Treatment	Number of roots	Root length (mm) mean ± S.D.	Mitotic Index mean ± S.D.	Number of nuclei	Nuclear Volume (μm^3) mean ± S.D.	Nuclear Volume (μm^3) range	Number of cells	Cell Area (μm^2) mean ± S.D.	Cell Area (μm^2) range	NA/CA
				Interphase - Sample I						
A	5	36.2 ± 11.2	8.9 ± 2.3	140	1547 ± 748	406 - 5432	140	692 ± 294	221 - 2002	0.193
B	5	62.4 ± 9.9	10.6 ± 2.1	140	1540 ± 684	501 - 4476	140	699 ± 265	247 - 1480	0.191
C	5	59.8 ± 15.8	11.0 ± 0.9	140	1595 ± 808	444 - 4676	140	663 ± 273	195 - 1519	0.206
				Interphase - Sample II						
D	5	46.6 ± 7.0	13.1 ± 2.8	625	1010 ± 307	216 - 2630	140	402 ± 114	169 - 732	0.251
E	5	51.4 ± 15.6	5.6 ± 3.2	625	691 ± 303	151 - 2347	140	382 ± 168	124 - 1007	0.205
				Interphase - Sample III						
F	5	28.2 ± 7.2	13.1 ± 0.8	140	1148 ± 472	378 - 2630	140	541 ± 212	213 - 1063	0.203
G	5	31.2 ± 5.3	10.6 ± 2.2	140	1125 ± 482	333 - 2901	140	548 ± 212	184 - 1177	0.198
H	5	54.0 ± 14.4	10.8 ± 3.3	140	997 ± 454	319 - 2744	140	485 ± 222	119 - 1111	0.206
				Prophase - Sample I						
I	5	36.2 ± 11.2	8.9 ± 2.3	50	2269 ± 821	1013 - 5356	50	850 ± 264	456 - 1611	0.203
J	5	62.4 ± 9.9	10.6 ± 2.1	50	2347 ± 870	1124 - 4617	50	853 ± 236	469 - 1528	0.207
K	5	59.8 ± 15.8	11.0 ± 0.9	50	2752 ± 1001	984 - 4590	50	844 ± 263	425 - 1563	0.233

Prophase - Sample II									
L	5	46.6 ± 7.0	13.1 ± 2.8	84	1731 ± 544	643 - 3572	50	558 ± 147	287 - 879	0.258
M	5	51.4 ± 15.6	5.6 ± 3.2	140	1140 ± 326	519 - 2792	50	444 ± 112	217 - 729	0.246
Prophase - Sample III									
N	5	28.2 ± 7.2	13.1 ± 0.8	50	1855 ± 590	724 - 3534	50	719 ± 261	293 - 1824	0.210
O	5	31.2 ± 5.3	10.6 ± 2.2	50	1980 ± 885	649 - 4723	50	755 ± 320	358 - 2100	0.209
P	5	54.0 ± 14.4	10.8 ± 3.3	50	1567 ± 603	507 - 3401	50	613 ± 218	313 - 1155	0.220

A = 24 hours water + 72 hours vermiculite-perlite.
B = 24 hours water + 96 hours vermiculite-perlite.
C = 24 hours water + 72 hours vermiculite-perlite + 24 hours water.
D = 24 hours water + 72 hours sand.
E = 24 hours water + 72 hours sand + 48 hours water.
F = 24 hours water + 72 hours sand.
G = 24 hours water + 108 hours sand.
H = 24 hours water + 72 hours sand + 36 hours water.
I = 24 hours water + 72 hours vermiculite-perlite.
J = 24 hours water + 96 hours vermiculite-perlite.
K = 24 hours water + 72 hours vermiculite-perlite + 24 hours water.
L = 24 hours water + 72 hours sand.
M = 24 hours water + 72 hours sand + 48 hours water.
N = 24 hours water + 72 hours sand.
O = 24 hours water + 108 hours sand.
P = 24 hours water + 72 hours sand + 36 hours water.

III.3.2. Effects on Cell Area, Nuclear Volume and Nucleo-cytoplasmic Ratios

Both mean interphase and mean prophase cell areas decreased by 24 to 48 hours after transfer of roots either from sand or vermiculite-perlite to tanks of water (Table 20). This decrease in cell area appears to occur independently of any change in M.I., ie., mean M.I. decreased in sample II, but remained constant in samples I and III (Table 20). Mean nuclear volume, however, decreased in only 2 out of 3 samples examined (Samples II and III, cf. Sample I, Table 20). When there was a drastic reduction in mean M.I., as in sample II, mean nuclear volume also decreased but when mean M.I. was constant, as in samples I and III, the difference from control values, in mean nuclear volume was less marked.

The interphase nucleo-cytoplastic ratio in roots transferred to water was similar in all samples (Table 20); ie., 0.205 to 0.206. The prophase nucleo-cytoplasmic ratio, although more variable than the interphase ratio, was less variable than the ratio for roots grown continuously in sand or vermiculite-perlite; ie., the nucleo-cytoplasmic ratio in low water ranged from 0.207 to 0.258 while that in samples transferred to water ranged from 0.220 to 0.246. It has already been shown that mature roots grown in sand and vermiculite-perlite appear to

attain slightly different physiological states (section III.2). The increase in uniformity of the nucleo-cytoplasmic ratio for roots transferred to water indicates that roots placed under similar growth conditions tend to establish the same balance of nuclear to cell size. This balance is different from that seen in roots grown continuously in tanks of water (Table 16).

These results show that the response of cells in the root meristem to a change in the amount of available water is dependent on the physiological state of the cells being examined. The following section outlines the response of germinating and mature roots to 5-AU, a drug which interferes with the progress of cells through the cell cycle.

III.4. 5-Amino Uracil Treatments

III.4.1. Treatment During Germination

Treatment of meristems with 5-amino uracil (5-AU) delays the progress of cells to mitosis and during the prolonged interphase both cells and nuclei increase in size (Davidson, Golding and Armstrong, 1978). With roots of germinating seeds the response to 5-AU can be studied in cells in G_1, S and G_2 more specifically than is possible in asynchronous cell populations of growing roots. Therefore, the effects of 5-AU on cell and nuclear growth in roots of germinating beans was examined.

Roots grown in tanks of water for 24.3 and 50 hours were placed in 4 mM 5-AU for continuous treatment; ie., cells were treated with 5-AU prior to their first mitosis. At 24.3 hours most cells were in G_1 while at 50 hours cells were in late S or G_2 (section III.1.1). In each case roots were treated with 5-AU continuously up to 96 hours of germination.

III.4.1.1 Mitosis

The mean M.I. of roots grown in tanks of water increased between 36 and 50 hours of germination (section III.1.2). Mean M.I. in roots treated with 5-AU beginning at 24.3 hours also increased by 36 and 50 hours, but not to the extent seen in control roots (Sample I, Table 21, cf. Table 7). This shows that; 1) the onset of mitosis in germinating roots is not delayed by 5-AU but 2) the level of mitotic activity in the meristem is depressed by 5-AU.

Assuming that 5-AU stops cells at the S-G_2 transition point (Socher and Davidson, 1971), cells seen in division between 24.3 + 26 and 24.3 + 46 5-AU (Sample I, Table 21) must already have been in G_2 at the beginning of treatment. The progress of these cells into mitosis is not delayed by 5-AU.

Roots treated with 5-AU beginning either at 24.3 or 50 hours had a mean M.I. < 0.5 by 70 hours of

Table 21

5-Amino Uracil Treatments During Germination

Mean root length ± S.D., mean M.I. ± S.D., number of nuclei scored, mean nuclear volume ± S.D., and range in nuclear volume number of cells scored, mean cell area ± S.D., and range in cell area, and the nucleo-cytoplasmic ratio are given for interphase and prophase cells; seeds were placed in 5 mm water for 24.3 hours, transferred to tanks of water for 0 and 26 hours, then placed in 4 mM 5-AU for continuous treatment.

Treatment	Number of roots	Root length (mm) mean ± S.D.	M.I. mean ± S.D.	Nuclear volume (μm^3) Number of nuclei	mean ± S.D.	range	Cell area (μm^2) Number of cells	mean ± S.D.	range	NA/CA
Sample I (24.3 Hours Water + 5-AU)										
24.3 Water + 6	6	4.3 ± 0.8	0	500	905 ± 405	207 - 2861	140	578 ± 235	152 - 1254	0.162
24.3 Water + 12	6	4.7 ± 0.8	0	500	898 ± 393	151 - 2409	140	666 ± 282	221 - 1389	0.140
24.3 Water + 26	6	5.3 ± 0.8	2.1 ± 1.3	500	851 ± 304	156 - 2148	100	609 ± 270	206 - 1411	0.147
24.3 Water + 36	6	6.7 ± 1.5	0.6 ± 0.6	500	774 ± 271	184 - 1983				
24.3 Water + 46	8	8.3 ± 3.0	0.1 ± 0.3	500	1983 ± 919	394 - 7065	100	926 ± 494	375 - 3143	0.170
24.3 Water + 72	6	10.3 ± 3.4	0	500	1190 ± 385	129 - 2891	140	701 ± 259	228 - 1777	0.160
Sample II (50 Hours Water + 5-AU)										
50.0 Water + 3	6	5.3 ± 1.2	3.3 ± 2.2	600	1098 ± 452	85 - 4131				
50.0 Water + 6	6	6.0 ± 1.1	1.7 ± 2.2	500	849 ± 286	203 - 2141	140	713 ± 330	163 - 1709	0.126

50.0 Water + 10	6	6.4 ± 1.1	2.3 ± 1.2	500	1380 ± 618	294 - 4563	100	639 ± 307	174 - 1606	0.194
50.0 Water + 20	6	9.4 ± 3.1	0.3 ± 0.4	500	1030 ± 368	117 - 3191	100	667 ± 290	176 - 1497	0.153
50.0 Water + 34	6	10.0 ± 4.1	0.6 ± 0.7	500	1177 ± 439	302 - 3141	140	717 ± 255	266 - 1289	0.155
50.0 Water + 46	6	12.5 ± 3.8	1.9 ± 3.2	500	2043 ± 1039	293 - 9166	100	842 ± 330	338 - 1717	0.191
					Prophase					
					Sample I (24.3 Hours Water + 5-AU)					
24.3 Water + 26	6	5.3 ± 0.8	2.1 ± 1.3	50	1750 ± 483	450 - 2789	50	1023 ± 231	629 - 1693	0.142
					Sample II (50.0 Hours Water + 5-AU)					
50.0 Water + 3	6	5.3 ± 1.2	3.3 ± 2.2	112	2065 ± 623	602 - 4060				
50.0 Water + 6	6	6.0 ± 1.1	1.7 ± 2.2	50	1287 ± 336	683 - 2125	50	925 ± 306	474 - 2146	0.128
50.0 Water + 10	6	6.4 ± 1.0	2.3 ± 2.2	112	2382 ± 757	919 - 5113	50	963 ± 316	441 - 1709	0.185
50.0 Water + 46	6	12.5 ± 3.8	1.9 ± 3.2	40	3647 ± 1039	1678 - 6161	40	1386 ± 360	666 - 2076	0.170

Table 21a (Insert)

5-Amino Uracil Treatments During Germination (Controls)

Mean root length ± S.D., mean M.I. ± S.D., number of nuclei scored, mean nuclear volume ± S.D., and range in nuclear volume, number of cells scored, mean cell area ± S.D., and range in cell area, and the nucleo-cytoplasmic ratio are given for interphase and prophase cells; seeds were placed in 5 mm of water for 24 hours and transferred to tanks of water for subsequent growth. Values are taken from Tables 6,7,8 and 10.

Treatment	Number of roots	Root length (mm) mean ± S.D.	M.I. mean ± S.D.	Nuclear volume (μm³) Number of nuclei	mean ± S.D.	range	Cell area (μm²) Number of cells	mean ± S.D.	range	NA/CA
				Interphase Sample I (Controls)						
30.3 hours water	6	3.8 ± 0.4	0	500	772 ± 373	118 - 2196	140	574 ± 281	169 - 1611	0.147
36.3 hours water	6	4.3 ± 0.6	0.2 ± 0.04	500	794 ± 335	151 - 2409	140	562 ± 243	163 - 1307	0.153
50.3 hours water	5	4.8 ± 1.2	5.13 ± 2.9	500	776 ± 258	124 - 2521	100	601 ± 253	129 - 1319	0.141
60.3 hours water	5	6.4 ± 1.1	2.6 ± 2.3	500	608 ± 211	149 - 2109	100	587 ± 280	155 - 1519	0.123
70.3 hours water	6	7.5 ± 2.2	5.5 ± 4.9	500	869 ± 389	212 - 2715	100	558 ± 266	195 - 1904	0.163
96.3 hours water	7	11.0 ± 5.4	4.7 ± 5.0	600	661 ± 251	99 - 1781	120	487 ± 198	125 - 1058	0.157
				Prophase (Controls)						
50.3 hours water	5	4.8 ± 1.2	5.1 ± 2.9	112	1247 ± 315	559 - 2820	50	907 ± 331	358 - 1861	0.128
60.3 hours water	5	6.4 ± 1.1	2.6 ± 2.3	112	299 ± 249	385 - 1663	50	768 ± 308	225 - 1514	0.130
70.3 hours water	6	7.5 ± 2.2	5.5 ± 4.9	112	1361 ± 377	630 - 2506	80	650 ± 207	296 - 1302	0.189
96.3 hours water	7	11.0 ± 5.4	4.7 ± 5.0	125	1039 ± 332	474 - 2409	80	520 ± 182	217 - 1179	0.197

germination, ie., at 24.3 + 46 and 50 + 20 5-AU mean M.I. was 0.1 and 0.3 (Sample I and Sample II, Table 21). Assuming the mean duration of mitosis is 2.5 hours (Table 19) then 7.9% of the cells in the meristem divide between 50 and 70 hours after treatment with 5-AU beginning at 24.3 hours while 19.5% of the cells in the meristem divide if 5-AU is given at 50 hours. The additional cells that are able to divide when 5-AU treatment does not begin until 50 hours must have reached the S-G_2 transition point before 50 hours. This suggests that cells in G_1 at the start of germination either 1) spend less time in G_2 than cells already in G_2 in dry seeds or 2) initiation of cell cycle events in cells originally arrested in G_1 takes less time than initiation of cycle events in cells arrested in G_2 . In either case, some control over initiation of cycling events in G_1 and G_2 arrested cells during germination must exist.

Next, we consider changes in cell and nuclear size after 5-AU treatment of roots of germinating seeds.

III.4.1.2. Nuclear volumes and cell areas

In roots treated with 5-AU beginning at 24.3 hours of germination nuclear volumes were 905 ± 405 μm^3 at 24.3 + 6 5-AU; volumes in untreated roots at 30.3 hours were 772 ± 373 μm^3 (Sample I, Table 8, cf. Sample I,

Table 21). Histograms of the size distribution of nuclei at 24.3 + 6 5-AU and 30.3 hours of germination indicate that all nuclei treated with 5-AU increase in size (Fig. 21a, cf. Fig. 21b), ie., the modal nuclear volume after 24.3 + 6 5-AU was 700 μm^3 while the control value was only 500 μm^3. Similarly, by 24.3 + 12 hours 5-AU mean cell area had increased to 666 ± 282 μm^2 cf. 562 ± 243 μm^2 at 36.3 hours in control roots (Sample I, Table 21, cf. Table 7); these values are significantly different ($P > 0.001$). A comparison of the distribution of cell areas at these times indicates that all cells in the 5-AU treated material had increased in size (Fig. 22a, cf. Fig. 22b). This means that between 24.3 + 6 and 24.3 + 12 hours 5-AU both cell and nuclear growth were greater in 5-AU treated roots than in controls. Since most cells are in G_1 at 24.3 hours of germination (section III.1.1), ie., at the beginning of the 5-AU treatment, these results suggest that 5-AU stimulates nuclear growth during G_1.

Cells and nuclei treated with 5-AU beginning at 50 hours also increased in size; by 50 + 10 hours 5-AU mean size both of interphase and prophase cells and nuclei was larger than in 60.3 hour controls (Sample II, Table 21, cf. Sample I, Table 8 and Table 7). Although most cells are in late S or G_2 at 50 hours, because of the sporadic level of mitotic activity seen in the meristem

Figure 21

A. Frequency Histogram of Nuclei After 24.3 Hours Germination + 6 Hour 5-AU

Seeds were placed in 5 mm of water for 24.3 hours and transferred to tanks of 4 mM 5-AU for 6 hours; 100 nuclei from each of 5 roots were sampled, their nuclear volumes divided into size classes and plotted.

B. Frequency Histogram of Nuclei at 30.3 Hours of Germination

Seeds were placed in 5 mm of water for 24 hours and transferred to tanks of water for 6.3 hours; 100 nuclei from each of 5 roots were sampled, their nuclear volumes divided into size classes and plotted.

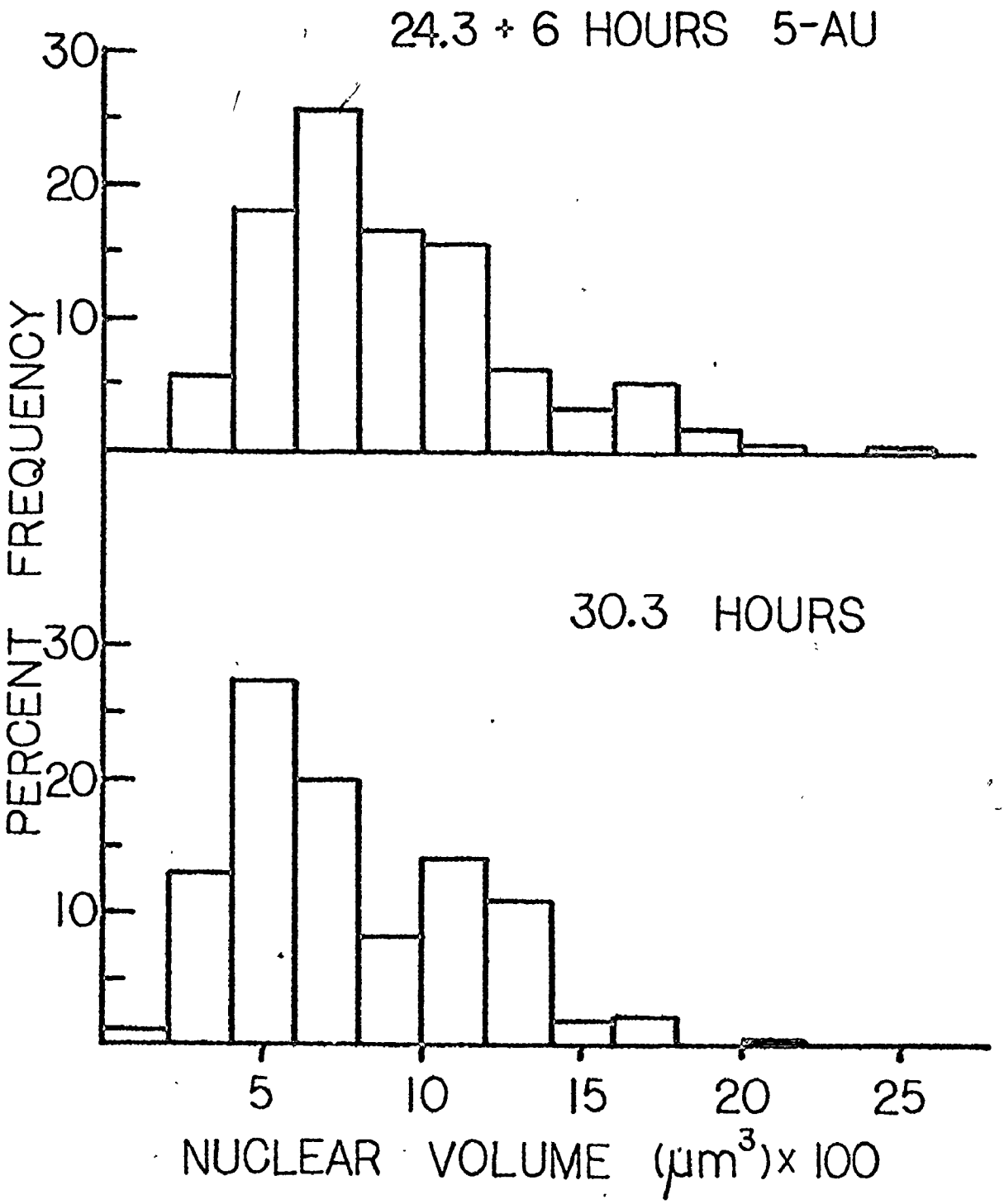

24.3 + 6 HOURS 5-AU
30.3 HOURS
PERCENT FREQUENCY
30
20
10
30
20
10
5
10
15
20
25
NUCLEAR VOLUME (μm³) x 100

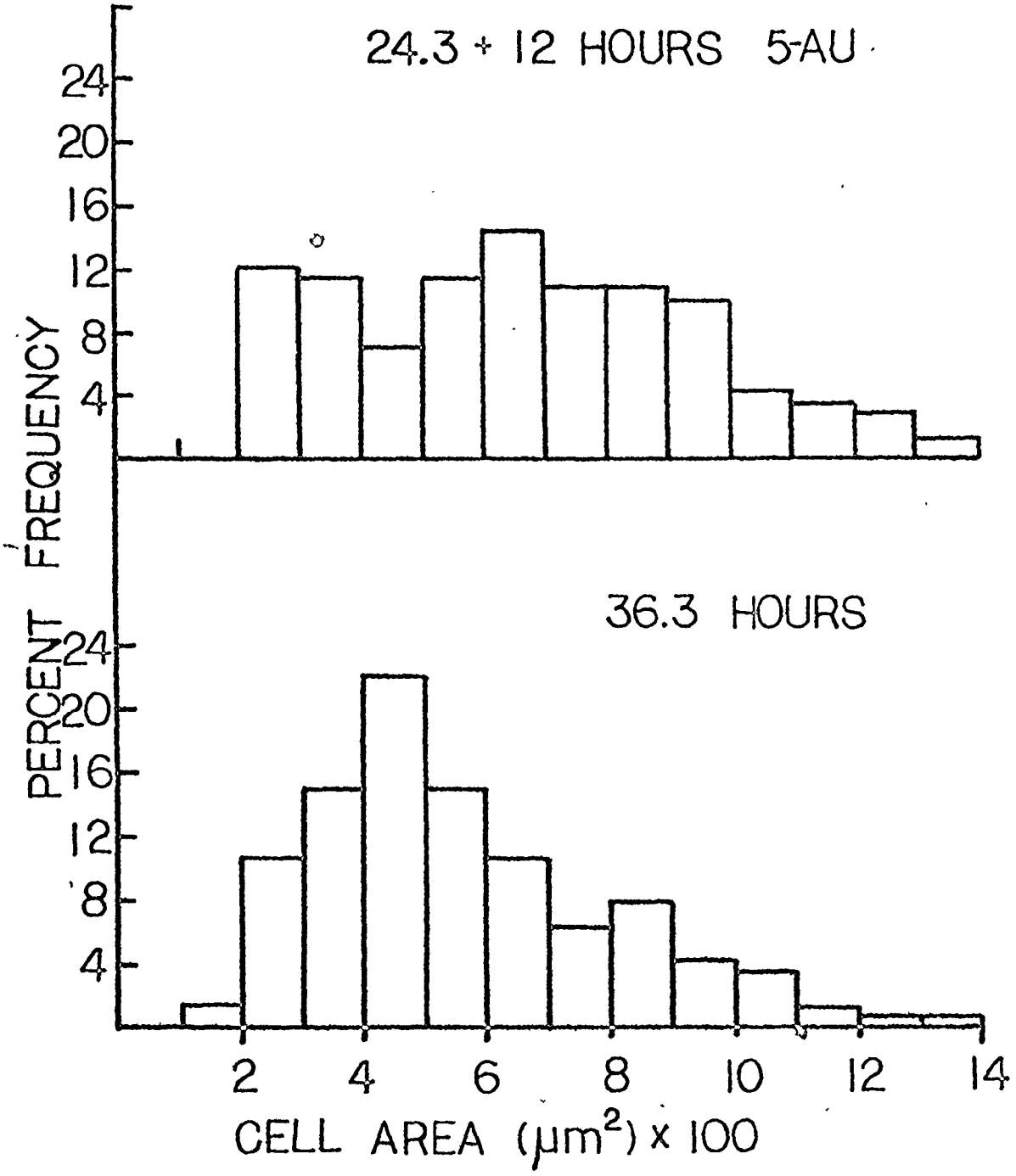
Figure 22

A. Frequency Histogram of Cells After 24.3 Hours of Germination + 12 Hours 5-AU

Seeds were placed in 5 mm of water for 24.3 hours and transferred to tanks of 4 mM 5-AU for 12 hours; 28 cells from each of 5 roots were sampled, their cell areas divided into classes and plotted.

B. Frequency Histogram of Cells After 36.6 Hours of Germination

Seeds were placed in 5 mm of water for 24 hours and transferred to tanks of water for 12.3 hours; 28 cells from each of 5 roots were sampled, their cell areas divided into classes and plotted.


24.3 + 12 HOURS 5-AU
36.3 HOURS
PERCENT FREQUENCY
24
20
16
12
8
4
2
4
6
8
10
12
14
CELL AREA (μm²) x 100

(section III.1.2) it is impossible to be sure if 5-AU 1) stimulates growth of G_2 cells or 2) allows cells to grow larger than normal by slowing the progress of cells through G_2.

Whether 5-AU was added in G_1 at 24.3 hours or in G_2 at 50 hours of germination, maximum cell and nuclear sizes were obtained after 46 hours treatment; ie., at 24.3 + 46 and 50 + 46 5-AU mean interphase nuclear volumes were 1983 and 2043 μm^3 (Table 21). These values are not significantly different ($P = 0.05$). Cell size after 46 hours 5-AU was also similar in both samples ($P = 0.05$). This means that cells and nuclei from germinating roots reach a similar size after 46 hours in 5-AU even though cells at the beginning of treatment 1) were at a different stage in the cell cycle and 2) had a different growth history. This suggests that during germination some limitations exist on cell and nuclear size in the meristem.

Between 50 + 34 and 50 + 46 hours 5-AU mean M.I. increased from 0.6 to 1.9 (Sample II, Table 21), evidence that cells were beginning to recover from the 5-AU treatment. Also, between 24.3 + 46 and 24.3 + 72 hours 5-AU both mean cell area and mean nuclear volume decreased (Sample I, Table 21).

III.4.1.3. Nucleo-cytoplasmic ratios

We have already seen that the nucleo-cytoplasmic ratio, mean nuclear area:mean cell area, changes during the cell cycle, ie., it is high in G_1, decreases through to G_2 and then increases in prophase (section III.1.5.1). By 70.3 hours of germination cells from roots grown in tanks of water are near the end of the cell cycle (section III.1.1) and have a high nucleo-cytoplasmic ratio (Table 10). After 46 hours of continuous treatment with 5-AU, cells from germinating roots are also approaching the end of G_2 (see previous section). At 24.3 + 46 5-AU the interphase nucleo-cytoplasmic ratio was similar to that seen in 70.3 hour controls; ie., 0.170, cf. 0.163 (Table 10, and Sample I, Table 21). By 50 + 46 5-AU, however, the interphase nucleo-cytoplasmic ratio had increased to 0.191 (Sample II, Table 21); this ratio is similar to that seen in prophase controls, 70.3 to 96.3 hours old, ie., 0.189 to 0.197 (Table 10).

These results suggest that the nucleo-cytoplasmic ratio of interphase cells increases during continuous treatment with 5-AU. This increase, with increasing duration of interphase, exactly parallels that seen in meristems of germinating seeds not treated with 5-AU. Nuclei and cells continue to grow throughout the extended interphase but their relative sizes change with time.

An increase in the nucleo-cytoplasmic ratio was also seen in prophase cells treated with 5-AU; ie., from 50 + 6 to 50 + 10 5-AU the prophase ratio increased from 0.128 to 0.185 parallelling an increase in the control ratio from 0.128 to 0.189 at 50.3 to 70.3 hours of germination (Sample II, Table 21 and Table 10).

With longer treatments with 5-AU the first cells that recover and enter mitosis had lower nucleo-cytoplasmic ratios than expected; at 50 + 46 5-AU the prophase ratio was 0.171 while those of controls 70.3 and 96.3 hours old were 0.189 and 0.197 (Sample II, Table 21 and Table 10). This may be a similar phenomenon to that seen during germination where prophase cells with low nucleo-cytoplasmic ratios divided before cells with a higher ratio, ie., between 50.3 and 70.3 hours the nucleo-cytoplasmic ratio rose from 0.128 to 0.189 (Table 10). This is evidence that the first cells to enter prophase have relatively larger areas and smaller nuclei than cells that enter mitosis at later times; as interphase is prolonged nuclei increase in size faster than cells and the nucleo-cytoplasmic ratio increases. But though 5-AU prolongs cell cycle duration the nucleo-cytoplasmic ratio achieved by the cells is, overall, similar to that seen in untreated mature roots.

III.4.2. Mature Roots - Mitotic Index

The response to 5-AU treatments was next tested in mature roots. Seeds were grown either in tanks of water, sand, vermiculite-perlite or transferred to tanks of water from sand or vermiculite-perlite at 96 to 144 hours and then treated continuously with 4 mM 5-AU.

Within 2 hours of the start of treatment M.I. began to fall and reached 1 or less within 8 to 9 hours (Table 22). From a plot of the fall in M.I. it is possible to estimate mean durations of $G_2 + \frac{M}{2}$ and of G_2 (Socher and Davidson, 1971). The values obtained for T_{G_2} ranged from 1.6 to 2.7 hours (Table 22) and they show that the duration of G_2 is slightly longer in roots grown in water than in sand or vermiculite-perlite. Thus, data from 5-AU-treated roots agrees with that from F.L.M. curves (Table 19); ie., growth in water prolongs the duration of G_2.

III.4.2.1. Nuclear volume and cell area

Roots were grown in sand up to 96 hours, transferred to tanks of water for 24 hours and then treated with 4 mM 5-AU. Mean interphase nuclear volumes fluctuated somewhat during the first few hours of treatment,

Table 22

Changes of Mitotic Index During 5-Amino Uracil Treatment and Estimates of the Mean Duration of G_2

Changes in mean M.I. ± S.D. after 0 to 12 hours 5-AU for roots grown with different amounts of water; seeds were placed in 5 mm of water for 24 hours, then grown in 1) tanks of water for 144 hours (Sample I); 2) 25 ml water/100 ml sand for 120 hours (Sample IIa); 3) 40 ml water/100 ml vermiculite-perlite for 120 hours (Sample IIIa); or 4) sand or vermiculite-perlite up to 96 hours, then transferred to tanks of water for 24 hours (Samples IIb and IIIb). Samples IIa and IIb, IIIa and IIIb were grown at the same time. The mean duration of G_2 + M/2 was estimated as the time at which mean M.I. fell to half its initial value in the presence of 5-AU; G_2 was estimated assuming M was 2.35 hours long.

Hours 5-AU	Sample I		Sample IIa		Sample IIIa		Sample IIb		Sample IIIb	
	Number of roots	M.I. mean ± S.D.	Number of roots	M.I. mean ± S.D.	Number of roots	M.I. mean ± S.D.	Number of roots	M.I. mean ± S.D.	Number of roots	M.I. mean ± S.D.
0	5	9.0 ± 1.9	4	11.3 ± 1.2	6	10.6 ± 2.1	5	8.9 ± 1.2	5	11.0 ± 0.9
2			5	8.2 ± 1.8	5	7.5 ± 2.0	5	7.3 ± 0.9	5	7.1 ± 1.5
3	5	5.3 ± 1.6								
4			5	3.9 ± 0.6	5	1.9 ± 0.2	6	4.4 ± 0.9	5	3.5 ± 2.7
6	5	2.6 ± 1.5	5	1.7 ± 0.5	5	1.2 ± 1.2	6	3.7 ± 2.8	6	2.7 ± 0.9
8			5	0.9 ± 0.7	5	0.3 ± 0.2	6	1.0 ± 0.4	5	1.0 ± 0.9
9	5	0.8 ± 0.8								
10			5	0.4 ± 0.3			5	2.3 ± 1.6		
12	5	0.6 ± 0.4	5	0.8 ± 0.9	5	0.2 ± 0.3	5	1.9 ± 1.7	5	0.5 ± 0.3
G_2 + M/2 (hours)		3.8		3.3		2.8		3.9		2.9
G_2 (hours)		2.6		2.1		1.6		2.7		1.7

but from 6 to 24 hours of continuous exposure to 5-AU they increased from 804 ± 336 to 1358 ± 655 μm^3 (Table 23). This increase coincided with a decrease in M.I.: nuclei prevented by 5-AU from progressing into mitosis continued to grow. Mean prophase nuclear volumes also fluctuated initially during treatment with 5-AU, finally increasing between 6 and 24 hours of treatment in parallel with increases seen in interphase nuclei (Table 23).

When cell area is plotted against nuclear volume a wedge-shaped distribution of points is obtained (Fig. 7a) indicating that these two parameters increase more or less in parallel with each other. The same relationship appears to hold following treatment with 5-AU. Mature roots grown in vermiculite-perlite for 120 hours were treated with 4 mM 5-AU in vermiculite-perlite for 12 hours. Values from 5-AU-treated roots exactly overlapped those of controls showing that even as cells and nuclei become larger than normal, the relationship of cell to nuclear growth is not altered (Fig. 23, cf. Fig. 24).

Continued enlargement of cells and nuclei in 5-AU-treated roots could result solely from hydration. In order to determine whether changes in macromolecular contents occur as nuclei grow, nuclear protein contents were determined in 5-AU-treated roots.

Table 23

5-Amino Uracil Treatments of Mature Roots

Mean root length ± S.D., mean M.I. ± S.D., number of nuclei scored, mean nuclear volume ± S.D., and range in nuclear volume for interphase and prophase nuclei treated with 5-AU. Seeds were placed in ~5 mm of water for 24 hours, then grown in sand (25 ml water/100 ml sand) for 72 hours, transferred to tanks of distilled water for 24 hours and then treated with 4 mM 5-AU for 3 to 24 hours.

Hours 5-AU	Number of roots	Root length (mm) mean ± S.D.	Mitotic index mean ± S.D.	Interphase nuclear volume (μm³)			Prophase nuclear volume (μm³)		
				Number of nuclei	mean ± S.D.	range	Number of nuclei	mean ± S.D.	range
0	6	60 ± 15	10.3 ± 2.3	948	791 ± 332	197 - 2901	112	1525 ± 557	495 - 3141
3	4	70 ± 11	5.8 ± 1.1	500	979 ± 506	207 - 3556	56	1310 ± 715	479 - 5083
6	4	65 ± 12	1.5 ± 1.1	500	804 ± 336	195 - 2196	56	1647 ± 558	447 - 3100
12	2	79 ± 4	0.3	500	916 ± 357	218 - 2409			
18	2	81 ± 2	0.1	500	982 ± 467	196 - 3534			
24	6	86 ± 16	0.9 ± 1.2	1000	1358 ± 655	266 - 5484	56	2342 ± 402	1587 - 3714

Figure 23

Cell Area Plotted Against Nuclear Volume for Roots Grown in Vermiculite-perlite

Seeds were placed in 5 mm of water for 24 hours and then grown in vermiculite-perlite (40 ml water/100 ml substrate) for 96 hours. Cell areas and nuclear volumes were determined for 140 interphase cells, 28 cells from each of 5 roots and 50 prophase cells, 10 cells from each of 5 roots. Some points overlap. (Interphase, ●; Prophase, ▲.)

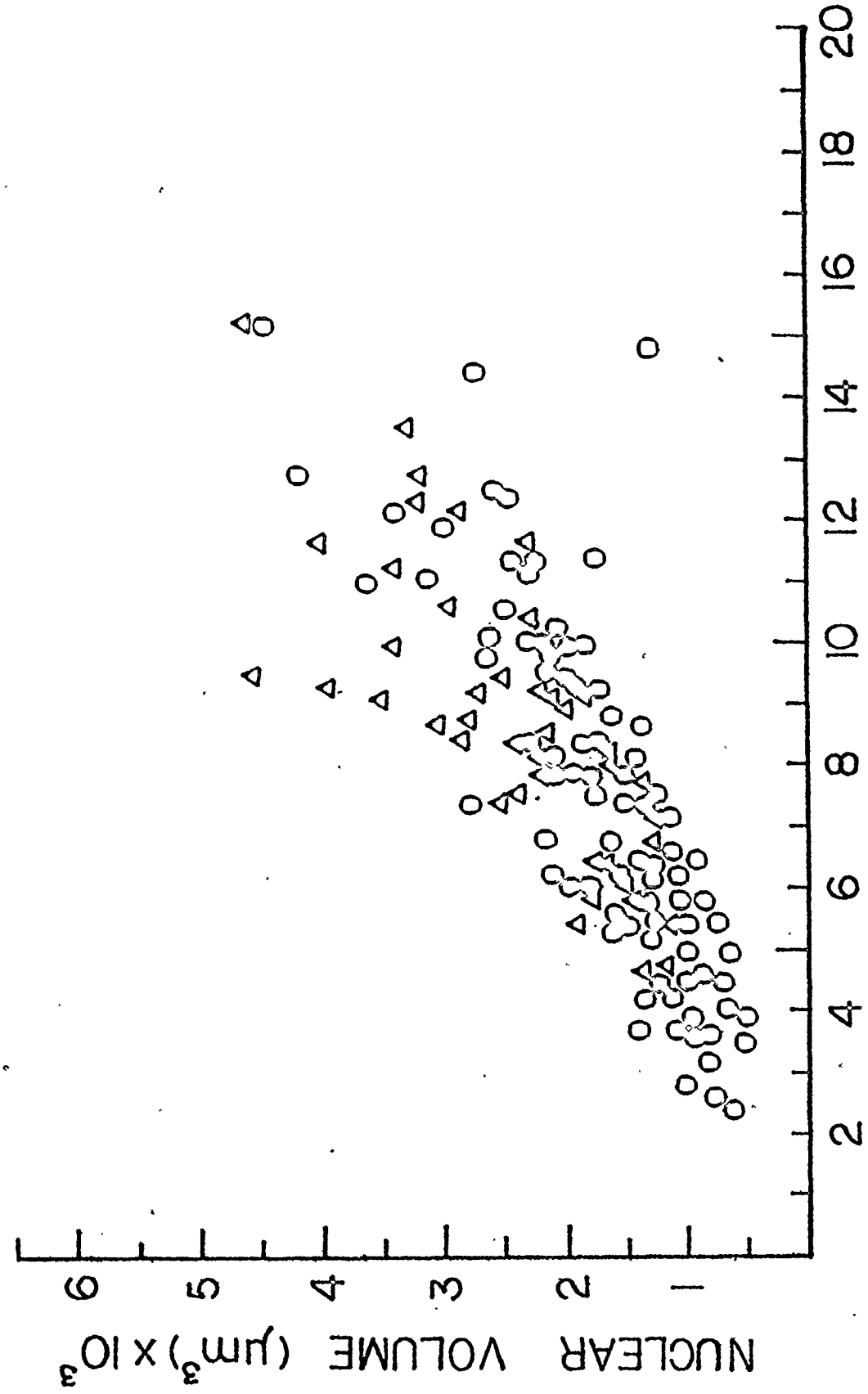
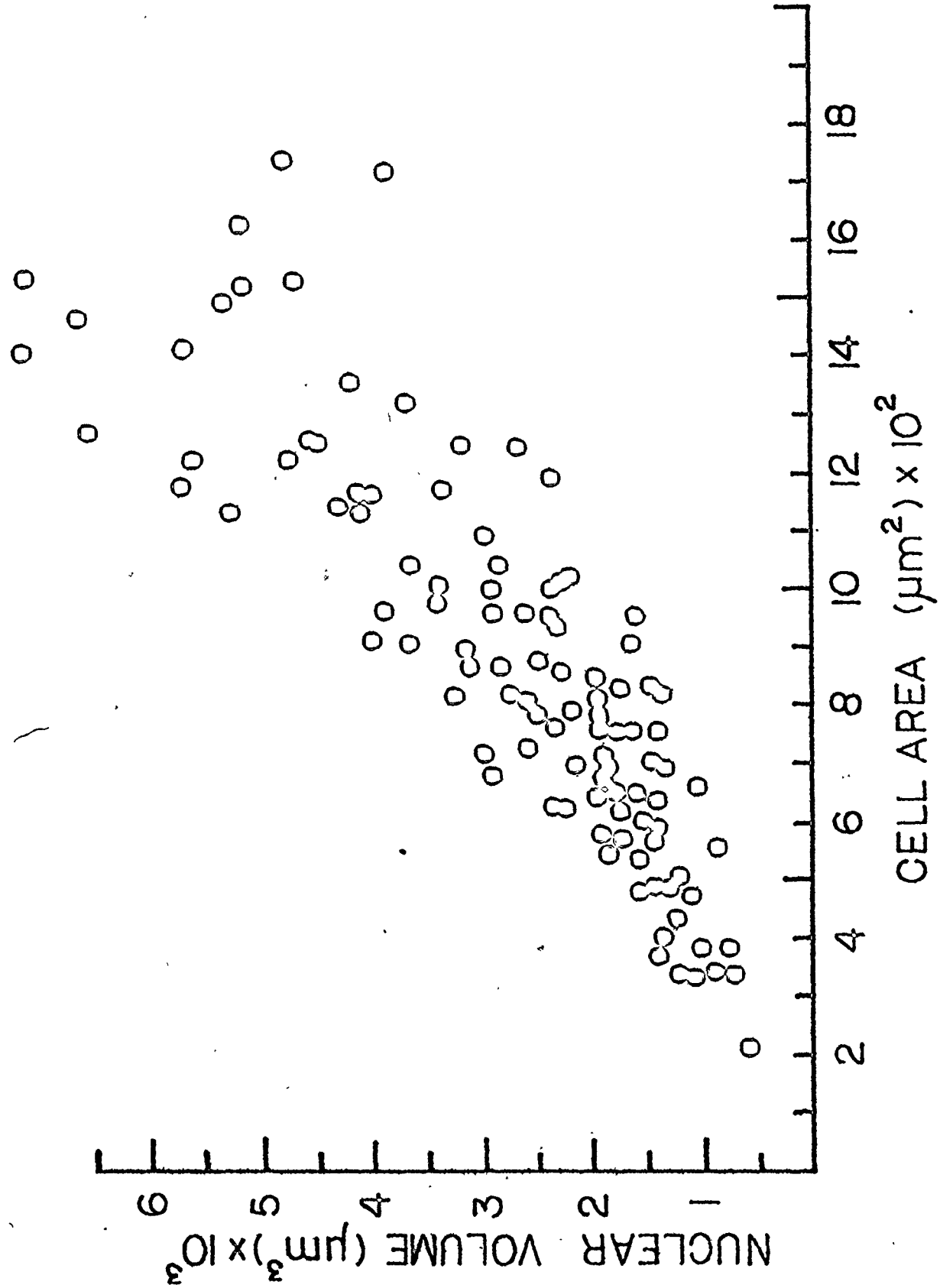

NUCLEAR VOLUME (μm^3) x 10^3
1
2
3
4
5
6
CELL AREA (μm^2) x 100
2
4
6
8
10
12
14
16
18
20

Figure 24

Cell Area Plotted Against Nuclear Volume for Roots Grown in Vermiculite-perlite and then Treated with 5-AU

Roots from the same sample as in Fig. 23 were treated with 4 mM 5-AU for 12 hours. Cell areas and nuclear volumes were determined for 140 interphase cells, 28 cells from each of 5 roots.


NUCLEAR VOLUME (μm^3) x 10^3
1
2
3
4
5
6
CELL AREA (μm^2) x 10^2
2
4
6
8
10
12
14
16
18

III.4.2.2. Nuclear proteins

Mature roots grown in sand up to 96 hours and then transferred to tanks of water for 24 hours were treated with 4 mM 5-AU for 6, 14 and 24 hours. Between 6 and 24 hours mean nuclear protein contents in control roots remained constant at about 41.5 A.U. (Table 24). Treated material, however, decreased in mean nuclear protein content to 38.2 A.U. by 6 hours in 5-AU. This decrease was probably due to the division of nuclei already in G_2, ie., nuclei that would be expected to have the highest content of proteins. Between 6 and 14 hours in 5-AU nuclei do not divide but they gradually increase in size; they also increase in mean protein content, from 38.2 to 46.6 A.U. This increase is significant ($P = 0.05$). Only slight changes in nuclear protein content occurred between 14 and 24 hours 5-AU. Consequently, as the duration of the period in which cells were held in G_2 increased, mean nuclear protein content increased. This implies that increases in nuclear size in the presence of 5-AU are at least, in part, the result of increases in the number of protein molecules per nucleus and not just the result of hydration. This is an important contribution to our understanding of the response of meristematic cells to the block imposed by 5-AU.

Table 24

Changes in Nuclear Protein Contents During 5-AU Treatment

Mean nuclear area ± S.D., mean nuclear protein content ± S.D. and range in nuclear protein contents are given for seeds placed in 5 mm water for 24 hours, then grown in 25 ml water/100 ml sand up to 96 hours and then transferred to tanks of water. At 24 hours after transfer, roots were placed in 4 mM 5-AU for 6, 14 and 24 hours continuous treatment. Samples are of 100 nuclei at each time; nuclear protein contents are given in arbitrary units (A.U.).

Hours in			Nuclear area (μm^2)	Nuclear protein (A.U.)	
sand	water	5-AU	mean ± S.D.	mean ± S.D.	range
96	30	-	89.1 ± 19.1	41.7 ± 15.7	20.4 - 88.9
96	48	-	92.6 ± 29.4	41.2 ± 14.9	15.6 - 84.0
96	24	6	97.6 ± 24.2	38.2 ± 14.4	19.4 - 87.1
96	24	14	100.5 ± 30.2	46.6 ± 15.0	17.1 - 83.6
96	24	24	106.1 ± 28.4	43.2 ± 14.7	12.2 - 86.8

III.5. Relationships Between Growth Parameters

The results presented in previous sections have revealed differences in growth of roots maintained in various water regimes. They have also shown that roots of newly germinated seeds and mature roots exhibit heterogeneity for every growth parameter that has been studied. In this section the relationships between different growth parameters are examined. Specifically, an analysis will be made of the extent to which variation in one parameter parallels variation in a second parameter. First we consider the relationship between nuclear size and DNA content.

III.5.1. The Relationship Between Nuclear Size and DNA Content

Many studies have shown that each species has a fixed amount of nuclear DNA (Mirsky and Ris, 1949; Swift, 1950; Bennett and Smith, 1976) and that this amount is exactly doubled during interphase in each cell cycle (Walker and Yates, 1952; Swift, 1953; Zetterberg, 1966). This doubling occurs during the S phase (Howard and Pelc, 1953; Zetterberg, 1966). DNA content, therefore, is one parameter that is constant during the G_1 and G_2 phases of the cell cycle. It is

also known that nuclei change in size throughout a cell cycle; this has been demonstrated for steady-state systems (Bansal and Davidson, 1978b; Prescott, 1955) and for roots of germinating seeds as they develop into a steady-state system (section III.1.5). Thus, in both G_1 and G_2, nuclei grow without any increase in DNA content.

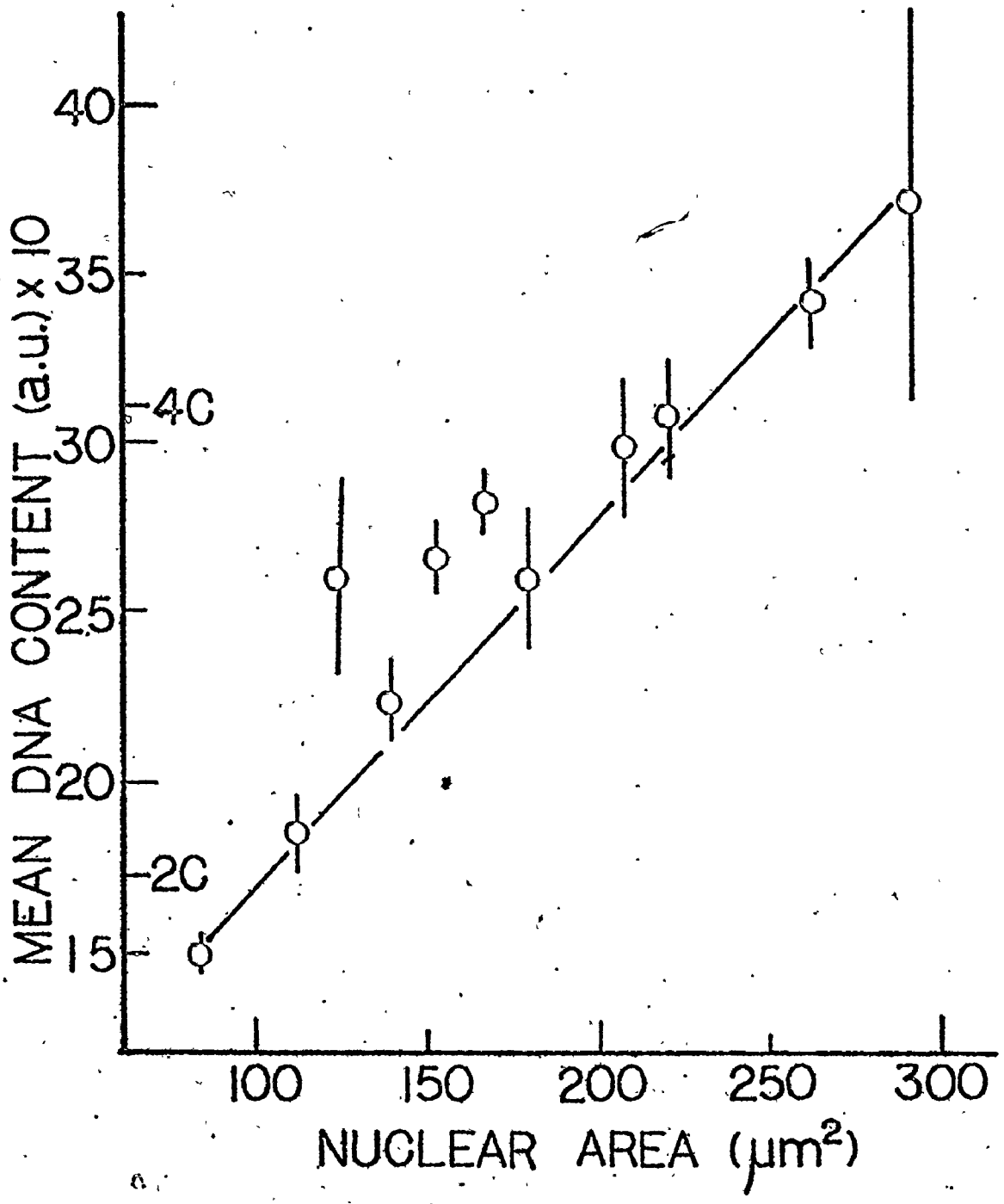
The situation in root meristems is further complicated by the fact that at any phase of the cell cycle nuclei show considerable variation in size. This occurs in G_1 and G_2 and in prophase (Table 12). This suggests that the volume of a nucleus cannot be used to estimate its phase in the cell cycle.

DNA contents and nuclear areas were determined for 200 interphase nuclei. 2C and 4C DNA contents were also determined from telophase and prophase nuclei. Nuclear areas ranged from 82 to 288 μm^2 and they were divided into classes at intervals of 14 μm^2. Of 36 nuclei whose mean area was 137 μm^2, 36% had a 2C DNA content, ie., were in G_1, and 19% were in G_2. The remaining 45% of the nuclei had DNA contents between 2C and 4C. Of 15 nuclei whose mean area was 206 μm^2, 13% were in G_1 and 60% were in G_2. This shows that position in the cell cycle is independent of nuclear size; ie., small and large nuclei may be either in G_1 or G_2.

Figure 25

Plot of Mean DNA Content Against Nuclear Area

Seeds were placed in ∿5 mm of water for 24 hours and then grown in tanks of water for 46 hours. A total of 200 cells were scored; 50 cells from each of 4 roots and divided into nuclear area classes at intervals of 14 μm^2. Mean DNA content ± S.E. was plotted for each interval; the sample size varied from 6 to 36 nuclei/interval. Mean 2C (174 ± 73 A.U.) and 4C (309 ± 88 A.U.) DNA contents were estimated from 10 telophase and 8 prophase nuclei.


MEAN DNA CONTENT (a.u.) x 10
40
35
30
25
20
15
100
150
200
250
300
NUCLEAR AREA (μm²)

Consequently, the size of a nucleus, selected at random, cannot be used absolutely to estimate the position of that nucleus in the cell cycle.

A correlation between "mean" nuclear area and "mean" DNA content does, however, exist. If "mean" values of nuclear area and of DNA content are plotted against each other for a number of samples then the overall conclusion is that nuclear area increases as nuclear DNA content increases (Fig. 25).

III.5.2. Relationship Between Nuclear Size and Nuclear Protein Content

Seeds were grown either in tanks of water or in sand, 25 ml water/100 ml sand for 20 to 144 hours. Nuclear protein contents were determined for 1202 nuclei; the data was then grouped over 5.5 μm^2 intervals of nuclear area which ranged from 49 to 181 μm^2 (Table 25). Overall, the range in nuclear protein contents was from 15 A.U. in nuclei 49 μm^2 in size to 158 A.U. in nuclei 170 μm^2 in size. This far exceeds the 2-fold range in nuclear protein content that would be expected if all nuclei began G_1 with the same amount of protein and if this doubled during a cell cycle. It appears that even at the start of G_1, different nuclei have different protein contents.

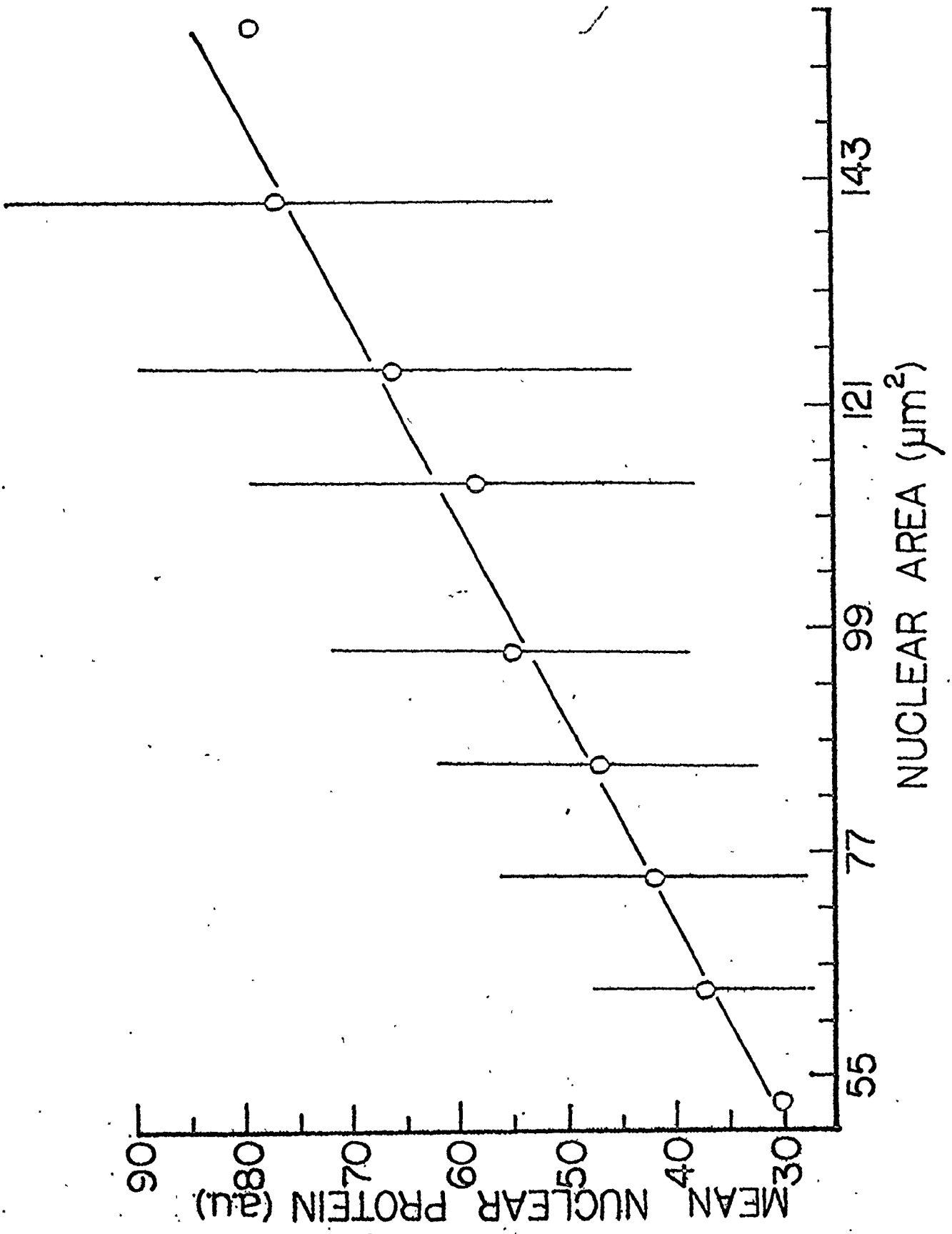
The absolute range in nuclear protein contents within a nuclear area size class tended to increase with nuclear size; e.g., the minimum and maximum nuclear protein contents of nuclei 60 μm^2 in area were 18 and 65 A.U. while in 137 μm^2 nuclei, the minimum and maximum values were 39 and 150 A.U. The absolute difference increased in these two nuclear size classes from 47 to 111 A.U. of protein. On average, however, the range in nuclear protein contents from one class to the next remained about constant: the mean ratio, maximum to minimum protein content was 3.2 ± 1.4. Because this variation is seen in samples from the smallest to the largest nuclei, variation for nuclear protein contents must occur at all stages of the cell cycle; ie., the variation produced early in G_1 is maintained throughout the cell cycle.

The large variation seen in nuclear protein contents resulted in considerable fluctuation in estimates of mean nuclear protein contents in samples of less than 40 nuclei. However, a clear linear relationship between nuclear area and mean nuclear protein content was seen when data was plotted from samples of 40 or more nuclei (Fig. 26). This linear relationship indicates that, on average, nuclear protein contents increase in a regular fashion as nuclei increase in size.

Figure 26

Mean Nuclear Protein Content Plotted Against Nuclear Area

Cells selected from those described in Table 25 were grouped into nuclear area classes at intervals of 5.5 μm^2. Mean nuclear protein ± S.D. was plotted for intervals with 40 or more nuclei. Points at 49 and 154 μm^2 had 20 and 17 nuclei, respectively.


0
MEAN NUCLEAR PROTEIN (a.u.)
30
40
50
60
70
80
90
55
77
99
121
143
NUCLEAR AREA (μm²)

The data in Fig. 26 is best described by a line with a slope of 0.274 and a "y" intercept of 6.51 A.U. By extrapolation, it can be seen that nuclear protein contents increase by about 1.8 to 1.9 fold for one doubling in nuclear area.

Variation in nuclear protein content is also shown by comparing nuclei 60, 82 and 110 μm^2 in area. The minimum nuclear protein content was 18 to 22 A.U. in all three nuclear size classes, while their maximum protein content increased from 65 to 136 A.U. (Table 25). For some nuclei, therefore, nuclear area could increase by ~90% without any increase in their protein content.

Also, nuclear protein contents of nuclei 60, 82 and 110 μm^2 in area were not normally distributed (Fig. 27); ie., based on probit analysis of nuclear protein contents, there appears to be more than one distinct population of nuclei. These results suggest that for individual nuclei, nuclear area varies, to a large extent, independently of the amount of nuclear protein present.

Nuclear area, therefore, is broadly correlated with DNA content and with nuclear protein content. However, the extent of the variation in nuclear protein content is evidence that variation in this parameter does not result in a close parallel change in nuclear size.

Table 25

Change in Nuclear Protein Content with Nuclear Size

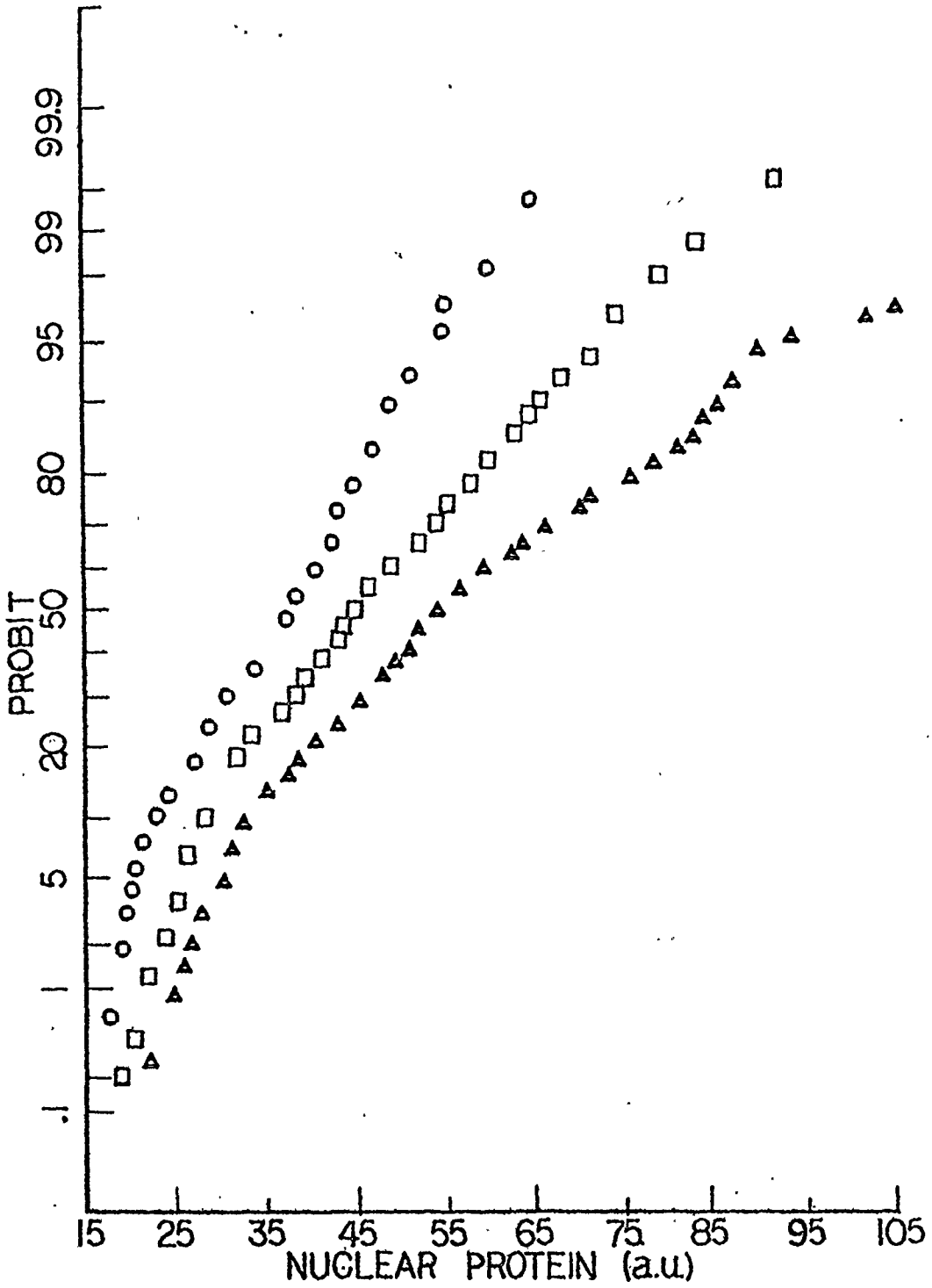
Roots were grown either in tanks of water or sand, ~ 25 ml water/100 ml sand and fixed at 20 to 144 hours of germination; 1202 nuclei were scored and divided on the basis of nuclear size into 5.5 μm^2 intervals. The number of nuclei within each interval, mean nuclear protein content ± S.D., minimum and maximum nuclear protein contents and the ratio maximum/minimum nuclear protein contents are given.

Nuclear area (μm^2)	Number of nuclei	Nuclear protein (A.U.)		Range
		mean ± S.D.	minimum - maximum	(maximum/minimum)
49	20	30 ± 9	15 - 48	3.2
55	1	34	-	-
60	83	37 ± 10	18 - 65	3.6
66	2	43 ± 11	36 - 51	1.4
71	171	42 ± 14	22 - 82	3.7
77	21	37 ± 11	19 - 54	2.8
82	251	47 ± 15	19 - 98	5.2
88	25	41 ± 12	22 - 71	3.2
93	196	55 ± 17	22 - 110	5.0
99	24	45 ± 9	26 - 59	2.3
104	27	51 ± 16	31 - 92	3.0
110	181	58 ± 21	22 - 136	6.2
115	8	44 ± 13	45 - 55	1.2
121	90	66 ± 23	23 - 132	5.7
126	11	50 ± 16	24 - 84	3.5
132	5	60 ± 13	42 - 77	1.8
137	43	77 ± 26	39 - 150	3.8
143	4	57 ± 20	40 - 80	2.0
148	9	71 ± 28	42 - 128	3.0
154	17	79 ± 25	45 - 130	2.9
159	-	-	-	-
165	3	53 ± 16	36 - 68	1.9
170	7	85 ± 38	48 - 158	3.3
176	2	68 ± 22	53 - 84	1.6
181	1	40	-	-

Figure 27

Probit Plot of Nuclear Protein Contents

Nuclear protein contents from nuclei 60, 82 and 110 μm^2 in size were selected from the data given in Table 25, ranked into ascending order and plotted on probit paper. Samples were of 83, 251 and 181 nuclei (60 μm^2 - ●; 82 μm^2 - □ ; 110 μm^2 - ▲).


PROBIT
.1
1
5
20
50
80
95
99
99.9
15
25
35
45
55
65
75
85
95
105
NUCLEAR PROTEIN (a.u.)

III.5.3. The Relationship Between Nuclear Volume and Cell Area

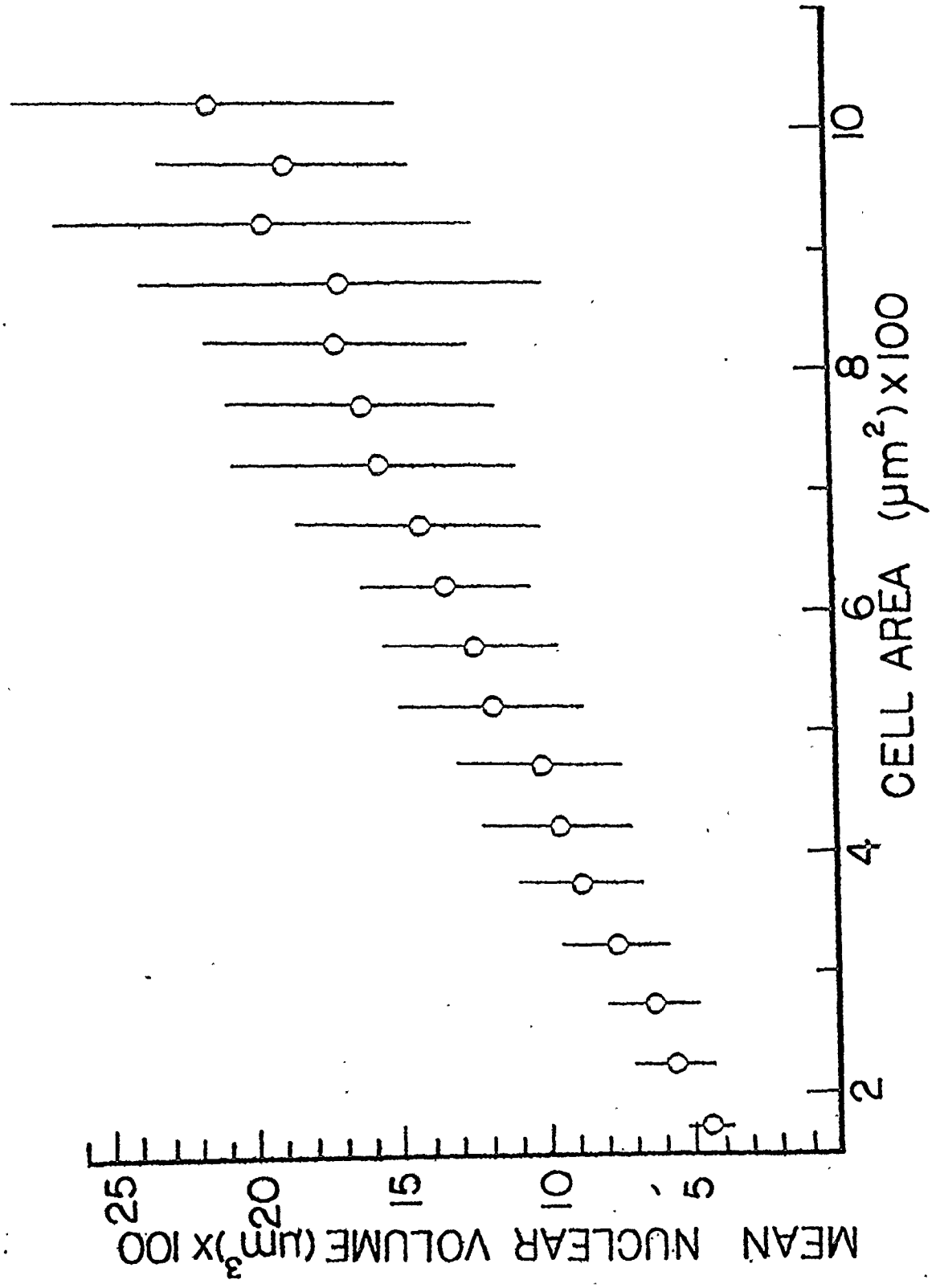
For an analysis of this relationship nuclear volumes and cell areas from 1057 cells of mature roots grown in various water regimes were pooled. Cell areas were grouped in intervals of 50 μm^2 and the mean nuclear volume ± S.D. for all nuclei in each cell area size class was determined. Thus nuclear volumes were studied over a range of cell areas from 175 to 1025 μm^2 (Fig. 28). The results reveal that, on average, nuclei show a 1.8 fold increase in volume for each doubling in cell area. This means that cells double in size at a faster rate than nuclei; it also means that while a cell could double in size over one cell cycle, the average increase in nuclear volume may only be about 1.8 fold, not the expected 2 fold increase.

The relationship between nuclear volume and cell size, however, is not a simple one. First, it has been shown that a sample of nuclei of almost any size class includes nuclei with 2C and 4C DNA contents; ie., almost every size class of nuclei includes G_1, S and G_2 nuclei. Secondly, nuclei show greater increases in volume at the end of the cell cycle than at the beginning (section III.1.5.1) and therefore mixtures

Figure 28

Mean Nuclear Volume Plotted Against Cell Area

Seeds were placed in 5 mm of water for 24 hours and then grown in 1) tanks of water for 120 hours, 2) sand, 25 ml water/100 ml sand, for 72 and 108 hours, 3) vermiculite-perlite, 40 ml water/100 ml substrate, for 72, 96 and 120 hours and 4) sand or vermiculite-perlite for 72 hours and transferred to tanks of water for 24 to 36 hours. A total of 140 cells were scored from each sample, the data combined and then sorted into cell area classes over intervals of 50 μm^2. The mean nuclear volume ± S.D. for each class ranging in size from 175 to 1025 μm^2 was plotted. Samples were of 11 to 93 nuclei/class.


MEAN NUCLEAR VOLUME (μm^3) x 100
25
20
15
10
5
2
4
6
8
10
CELL AREA (μm^2) x 100

of G_1, S and G_2 nuclei will have a mean volume somewhat lower than would be obtained if nuclei grew at a constant rate throughout interphase.

Both minimum and maximum nuclear volumes increased as cell size increased (Table 26). The range increased, in absolute terms, from 293 μm^3 in nuclei from cells 175 μm^2 in area to 3014 μm^3 in nuclei from cells 925 μm^2 in area (Table 26). The relative sizes of the smallest and largest nucleus in each cell size class ranged from 1:1.86 to 1:4.67 and, on average, there was a 3.7 ± 0.5 fold spread of values (Table 26). Mean nuclear volumes show a 4.7 fold increase from the smallest to the largest cells, ie., 450 ± 87 to 2134 ± 650 μm^3 and overall there is a 10.9 fold spread in nuclear volumes, from 340 to 3714 μm^3 (Table 26).

The range in nuclear volume for each cell size class is similar to that seen for protein contents within individual nuclear size classes, ie., 3.7 ± 0.5 cf 3.2 ± 1.4. This suggests that there may be some correlation between nuclear protein content and cell size.

These results establish that neither cell area nor nuclear volume are confined to the two fold spread of values that would be expected if the values for nuclear volume and cell size were: 1) constant at early

Table 26

Changes in Nuclear Volume with Cell Area

Tabulation of the data shown in Figure 28. Mean nuclear volume ± S.D., minimum and maximum nuclear volume and range in nuclear volume are given for groups of cells sorted into cell area classes over intervals of 50 μm^2.

Cell area (μm^2)	Sample size	Nuclear volume (μm^3)		Range
		mean ± S.D.	minimum - maximum	(maximum/ minimum)
175	11	450 ± 87	340 - 632	1.86
225	33	575 ± 141	319 - 1013	3.17
275	61	640 ± 163	333 - 1013	3.04
325	74	770 ± 194	393 - 1612	4.11
375	93	889 ± 221	501 - 1612	3.22
425	93	971 ± 265	427 - 1620	3.80
475	90	1026 ± 294	501 - 2141	4.27
525	74	1193 ± 324	586 - 2381	4.06
575	81	1259 ± 302	590 - 1993	3.38
625	76	1344 ± 290	757 - 2110	2.79
675	86	1429 422	615 - 2504	4.07
725	72	1575 ± 489	730 - 3071	4.21
775	59	1621 ± 465	718 - 3023	4.21
825	47	1705 ± 454	786 - 2721	3.46
875	26	1687 ± 695	786 - 3555	4.52
925	35	1951 ± 714	822 - 3836	4.67
975	28	1876 ± 437	1105 - 2645	2.39
1025	18	2134 ± 650	1361 - 3714	2.73

G_1, and 2) doubled over a cell cycle. Though the latter may occur it is clear that neither mitotic cells nor cells early in G_1 show a fixed size: mitotic cells vary considerably in cell area (e.g., prophase cells, Table 12) and their daughters are often of unequal sizes due to asymmetry of division (Davidson, Pertens and Eastman, 1978).

Parallel with this heterogeneity of cell and nuclear sizes there is heterogeneity for protein contents of nuclei (section III.5.2). It can be concluded then that increases in mean cell size, nuclear volume and protein content of nuclei show a roughly linear relationship. It must also be concluded, however, that individual values for any one of these parameters may vary considerably when one or both of the other two parameters are held constant.

III.5.4. Dry to Fresh Weight Ratio

The dry weight to fresh weight ratio was compared for meristems taken from roots grown for 70 hours in either tanks of water or 25 ml water/100 ml sand. Although the sand grown roots were about 1.8 times longer than water grown roots, the dry to fresh weight ratio was similar in meristems from the two samples of roots (Table 27). This means that differences in cell

Table 27

Comparison of Dry Weight and Fresh Weight of Meristems from Roots Grown in Different Media

Mean root length ± S.D., and the ratio, dry weight/fresh weight are given for 98 to 101 roots grown for 70 hours either in tanks of water or sand, 25 ml water/100 ml sand.

Medium	Root length (mm) mean ± S.D.	Dry weight/ fresh weight	Number of roots
Water	7.0 ± 2.7	0.162	98
Sand	12.9 ± 6.3	0.167	101

size found in root meristems grown in various amounts of water are directly related to differences in total dry mass. Thus, the differences in cell size discussed throughout the Results section must be the consequence of changes in macromolecular constituents within cells.

CHAPTER IV

DISCUSSION

IV.1. Transition from Quiescence to Active Growth and Proliferation

The mature root meristem consists of a steady-state, asynchronous population of cells for which mean M.I., mean nuclear volume and mean cell area remain constant from one time to the next (Table 20). The characteristics of the stable state achieved by roots when they reach maturity differ in roots grown under different conditions, in particular they are somehow related to the ambient water level during germination (section III.2). Mature roots transferred from sand or vermiculite-perlite into tanks of water, however, attain a new stable state that is different from the stable state observed when roots are germinated and grown continuously in tanks of water. This new stable state appears to be a modification of the initial stable state achieved by those roots during germination (section III.3).

Based on these observations, it is proposed that relationships among growth parameters are programmed into

cells during germination; this program is passed from one generation of cells to the next at mitosis. Consequently, actively growing cells from mature roots transferred from one environment to another can only modify the initial stable state achieved in those cells during germination. Also, variation within a meristem in the local environment of cells during germination must result in different programs for relationships among growth parameters within those cells. This is expressed as variation in cell growth in the mature root.

The uniqueness of the transition from quiescence to active growth and proliferation seen during germination is supported by observations of nuclear protein contents in germinating and mature roots (sections III.1.6 and III.2.2.4). Mean nuclear protein contents are high throughout the first cell cycle in germinating roots and then decrease significantly to lower levels in mature roots. This decrease is seen in all sizes of nuclei between 50 and 144 hours (Table 14). Mature roots grown either in tanks of water or in sand have similar mean nuclear protein contents (section III.2.2.4) and transfer of mature roots grown in sand to tanks of water at 96 hours does not significantly ($P \approx 0.05$) alter mean nuclear protein contents in roots examined 30 and 48 hours after the transfer (Table 18, cf. Table 24).

These results suggest that high nuclear protein contents found in germinating roots may be involved in regulation of germination and the establishment of a stable state in the mature root. In contrast, nuclear protein contents in mature roots may be involved in maintenance of stable relationships between growth parameters.

This form of programmed control over cell cycle events has also been suggested by Liskay, Leonard and Prescott (1979). Hybridization of various Chinese hamster cell lines, each with a characteristic G_1 duration, results in G_1^- cells with more rapid cell cycle times. From this it is suggested that different cell lines derived from an organism may have different critical events which control the presence of the G_1 phase and the rate of progression of cells through the cell cycle. Such differences occur even during embryogenesis, e.g., blastomeres of many animals are G_1^- and during embryogenesis there is a normal transition from the G_1^- state to the G_1^+ state found in most cells capable of proliferation. Similarly, in plants events seen during germination are the culmination of events initiated during embryogenesis in the seed.

A close analogy can also be made between events occurring during germination and those seen during the transition of yeast or animal cells in culture from

stationary to log phase growth. The radicle in dry seed of *V. faba* has about 78% of its cells arrested in G_1 (section III.1.1). Dry seed of other genera such as *Allium, Helianthus, Triticum* and *Lactuca* also have large numbers of cells arrested in G_1 (Van't Hof, 1974; Feinbrun and Klein, 1962; Bryant, 1969b). Also, under nutritionally deficient conditions yeast (Fantes and Nurse, 1977) and most normal mammalian cells in culture (Pardee, 1974; Yen, Friend and Clarkson, 1977) arrest in G_1.

During the transition from quiescence to active growth and proliferation both plant and animal cells show increases in protein and RNA synthesis (Jakob and Bovey, 1969; Jakob, 1972; Stanners and Becker, 1971; Becker, Stanners and Kudlow, 1971; Johnson, Abelson, Green and Penman, 1974). These increases are followed by the onset of DNA synthesis (Jakob and Bovey, 1969; Padilla, Creanor and Fraser, 1974; Stanners and Becker, 1971). In *V. faba* (sections III.1 and III.2; Davidson, 1966), yeast (Nasmyth, 1979) and animal cells in culture (Ross and Sinclair, 1972) the first transition cycle is longer than cell cycles of actively proliferating cells. In spite of the length of the first cell cycle, however, a large number of cells begin their first mitosis together, ie., a semi-synchronous wave of mitoses is observed (sections III.1.2 and III.2.2.1; Ross and Sinclair, 1972).

Other similarities between the response of yeast and *Tetrahymena* to a change in available nutrients and the response of *V. faba* to a change in the amount of water available for growth are discussed in the next section.

IV.2. Changes in Growth Conditions

Mean cell size decreases in *Tetrahymena* (Zalkinder, 1979) and yeast (Fantes and Nurse, 1977) when cultures are transferred from good nutrient conditions, e.g., high N or PO_4, to poor nutrient conditions, e.g., low N or PO_4. Cultured mammalian cells may also decrease in size during exponential growth as they deplete available nutrients (Kimball, Perdue, Chu and Ortiz, 1971; Ross and Sinclair, 1972). A similar decrease in mean cell area is seen after transfer of mature roots of *V. faba* from sand or vermiculite-perlite into tanks of water (section III.3.2). In contrast, mean nuclear volume and mean M.I. decrease in some roots after a transfer, but remain more or less constant in others. This shows that the new stable state achieved in roots after a transfer is influenced by the initial stable state achieved in those roots during germination. This means that factors capable of influencing cell growth in future generations of cells must be passed from mother to daughter cells at mitosis.

Transfer of cells from good to poor growth conditions results in a longer cell cycle both in *Tetrahymena* (Zalkinder, 1979) and in yeast (Fantes and Nurse, 1977). In *V. faba* cell cycle duration may also increase after transfer of roots from sand or vermiculite-perlite into tanks of water. Most estimates of mean cycle duration, ie., T_c, published for primary roots of *V. faba* are for roots transferred from sand or vermiculite into tanks of water (Table 28). In all cases, published values of T_c are longer in duration than the T_c estimate obtained in these studies for roots grown in vermiculite-perlite, e.g., Miller, Brulfert and Kaufman (1978) obtained a T_c of 25 hours for roots grown in vermiculite and transfered to tanks of aerated nutrient medium while the results reported here show that roots grown continuously in vermiculite-perlite have a T_c of 17 hours. Estimates of G_1, S and G_2 given in the literature (Table 28) are also different from one another. This means that differences observed in T_c are not just the result of differences in one phase of the cell cycle.

Consequently, it appears that mature roots respond to a change in the amount of available water in the same way as yeast and *Tetrahymena* respond to a change in nutrient conditions. This response is different from that obtained when roots are germinated and grown in different amounts of water.

Table 28

Cell Cycle Kinetics in the Primary Root of *Vicia faba*

The growth history, growth fraction and mean durations of G_1, S, G_2, and M and mean cycle duration (T_c) obtained from percent labelled mitoses or frequency labelled mitoses curves are given for mature primary roots of *V. faba*. Data is taken from the literature and Table 19 of the Results.

Growth history*	Mean duration (hours)					Growth fraction	Reference
	G_1	S	G_2	M	T_c		
1	12	6	8	4	30	0.69	Howard and Pelc, 1953
2	4.5	13.4	4.5	2.5	25		Miller, Brulfert and Kaufman, 1978
3	4.9	7.5	4.9	2	19.3		Evans and Scott, 1964
4	3.45	9.2	2.4	2.95	18	0.60	MacLeod, 1971
5	6.1	9.6	5.5	2.3	23.4	0.76	Thomas, Table 19
6	5.6	5.0	4.0	2.4	17.0	0.95	Thomas, Table 19

*1 = Imbibe 3 days in 4" of water; place between moistened filter paper until ~ 2 cm long; place in tanks of circulating water (19°C).

2 = Imbibe 24 hours; plant in vermiculite; after 7 days place in tanks of aerated nutrient medium (19°C).

3 = Imbibe 2.5 days in running tap water; plant in vermiculite; after 4 days place in tanks of aerated tap water (19°C).

4 = Imbibe 24 hours; plant in sand; after 3 days place in tanks of aerated nutrient medium (20°C).

5 = Imbibe in 5 mm water for 24 hours; place in tanks of aerated water for 5 days (20°C).

6 = Imbibe in 5 mm water for 24 hours; plant in 40 ml water/100 ml vermiculite-perlite for 3 days (20°C).

IV.3. 5-Amino Uracil

In germinating roots of *V. faba* the first cell cycle is very long (sections III.1 and III.2). During this cycle cells and nuclei grow larger than they normally would in a mature root. Treatment with 5-AU stops cells at the S to G_2 transition point (Socher and Davidson, 1971). This prolongs the cell cycle and also results in continued cell and nuclear growth (section III.4; Davidson, Golding and Armstrong, 1978). Consequently, the response of cells to 5-AU treatment appears to mimic events seen during germination.

When germinating seeds are treated with 5-AU beginning either at 24.3 or 50 hours mean cell area and mean nuclear volume increase and maximum values are reached after 46 hours of continuous treatment (section III.4.1.2). In both cases the nucleo-cytoplasmic ratio after 46 hours of treatment approaches that seen in control roots. This is surprising since 1) the position of cells in the cell cycle at the beginning of each treatment was different, ie., most cells treated beginning at 24.3 hours were in G_1 while most cells treated beginning at 50 hours were in late S or G_2 (section III.4.1.3), and 2) 5-AU alters normal cell and nuclear growth; ie., 5-AU stimulates both cell and nuclear growth during G_1 (section III.4.1.2).

These results suggest, 1) that 5-AU does not interfere with the ability of cells to regulate growth in relation to the environment. The relationship between cell and nuclear growth was similar both in 5-AU treated and control roots, 2) that stable relationships between cell and nuclear growth can be attained independent of the ability of a cell to pass through the cell cycle. The nucleo-cytoplasmic ratio was similar both in 5-AU treated and in control roots even though 5-AU stops cells at the S to G_2 transition point (Socher and Davidson, 1971), and 3) that during germination cells have the ability to limit cell and nuclear growth when the DNA division cycle is interferred with. This conclusion agrees with that of Davidson, Golding and Armstrong (1978) who concluded that unbalanced growth due to 5-AU treatment of lateral roots of *V. faba* cannot occur to the same extent as that seen for bacteria or animal cells in culture, ie., in the presence of agents that block the DNA-division cycle, both bacteria and animal cells continue to grow until they burst (Cohen and Barner, 1954; Rueckert and Mueller, 1960).

In mature roots treated with 5-AU, cells and nuclei also increase in size to values greater than those reached by control cells (section III.4.2.1; Davidson, Golding and Armstrong, 1978). The relationship between cell and

nuclear size in 5-AU treated roots, however, is not different from that seen in control roots. This means that 5-AU treatment does not affect changes in the nucleo-cytoplasmic ratio that would normally be seen in untreated roots during the cell cycle.

Measurement of nuclear protein contents during 5-AU treatment shows that, on average, nuclear protein contents increase in parallel with increases in nuclear size (section III.4.2.3). Mean nuclear protein contents, however, appear to plateau after 14 hours continuous 5-AU treatment even though mean nuclear volumes continue to increase (section III.4.2.1). Also, maximum nuclear protein contents within any size class of nuclei are smaller relative to those obtained for similar sized nuclei during germination (Table 24, cf. Table 13). This suggests that increases in nuclear protein contents may only occur during part of the cell cycle, ie., before the end of G_2.

These results agree with those obtained from mammalian cells treated with fluorodeoxyuridine (FUdR), a drug which inhibits DNA synthesis and stops cells at the G_1 to S transition point (Rueckert and Mueller, 1960). During treatment with FUdR cells continue to grow both in size (Cohen and Studzinski, 1969) and in cellular dry mass (Auer, Zetterberg and Foley, 1970). Mean nuclear

dry mass, however, remains more or less constant (*Ibid.*, 1970). After release of cells from the FUdR block nuclear dry mass increases after initiation of DNA synthesis. Nuclear dry mass then increases in parallel with DNA synthesis.

Consequently, it appears that both in mammalian cells in culture and in roots of *V. faba*, nuclear protein contents may increase only during DNA synthesis and early G_2. Further increases in nuclear size seen late in G_2 may be the result of accumulation of RNA in the nucleus (McLeish, 1969). This means that even though cell and nuclear growth during 5-AU treatment mimics that seen during germination, changes in nuclear protein contents in the presence of 5-AU are similar to those expected in untreated roots. Consequently, changes in cell growth resulting from 5-AU treatment are the result of cellular events that are different from events that occur during germination.

IV.4. Variability and Regulation of Cell Growth and Proliferation

Heterogeneity for cell area, nuclear volume, nuclear protein contents and cell cycle kinetics has been shown throughout the results presented here, confirming and

extending previous reports of heterogeneity (Rasch, Rasch and Woodward, 1967; Webster and Davidson, 1968; White and Davidson, 1976; Bansal and Davidson, 1978a,b). Variation in cell and nuclear size can result from asymmetry of mitosis (Davidson and Pertens, 1978; Ivanov, 1971) or variation in cell and nuclear growth rates during the cell cycle (Davidson, Pertens and Eastman, 1978). The model presented at the beginning of the discussion suggests that average relationships between growth parameters are programmed during germination. Because of variation in cell growth, this programming must be flexible. Some indication, however, of the degree of restraint imposed on growth parameters can be obtained from an examination of relationships among growth parameters.

In *V. faba* roots, linear relationships among DNA contents, nuclear volumes and cell areas can be seen (section III.5). Although individual cells clearly diverge from the average relationship seen between any two parameters compared, it appears that either they or their progeny must eventually compensate for this variation in a manner which allows for these linear relationships to be maintained; e.g., when nuclear volume was plotted against cell area (section III.5.3) the relative size of the smallest and largest nuclei within any cell size class

ranged from 1:1.86 to 1:4.67 and on average there was a 3.7 ± 0.5 fold spread of values. This average spread of values was seen in cells whose area ranged from 325 to 925 μm^2. Consequently, even though the absolute difference in volume between the smallest and largest nucleus within a cell size class increases as mean cell area increases, the relative range in nuclear volumes does not. This suggests that there are restrictions on nuclear growth within any cell size class. Similar restrictions can also be shown for the relationship between nuclear protein contents and nuclear area (section III.5.2).

Restrictions on variation in cell cycle duration in *V. faba* also exist. Both fast and slow cycling cells are found in the root meristem (Webster and Davidson, 1968; Table 19). When roots are grown either in tanks of water or in vermiculite-perlite the mean cycle time of the fast cycling cells in the two environments is similar (Table 19). Consequently, differences in the mean doubling time of cells from these roots are primarily the result of differences in growth fraction and in proportions of fast and slow cycling cells. In order to maintain constant proportions of slow and non-cycling cells in the meristem some fast cycling cells must give rise either to slow or non-cycling cells. Conversely, cells in the quiescent centre which lies at the tip of the root meristem are very

slow or non-cycling. They give rise to fast and slow cycling daughters which populate the meristem (Clowes, 1971). Consequently, maintenance of steady state cell cycle kinetics in the root meristem of *V. faba* must depend on regulation of cell cycle duration.

Regulation of cell cycle times has been demonstrated for *Tetrahymena*. Jauker and Cleffmann (1970) have shown that for *Tetrahymena* generation times vary from 170 to 290 minutes and form a skewed normal distribution. Individual generation times, however, alternate, on average, from fast to slow about the mean generation time of the population. These alternations in generation time are non-random.

Regulation of variation in generation rate, ie., 1/generation time, of *Escherichia coli* has also been described. Kubitschek (1966) has shown that the correlation between mother and daughter generation rates is less than that found between generation rates of cousins. An analysis of variance for generation rates also showed that variation in generation rate was restricted after three or more generations. From this he concluded that latent factors influencing the duration of the cell cycle are passed from one generation of cells to the next. These factors might not be expressed for several generations. This conclusion is similar to that outlined at the beginning of the discussion, ie., factors involved in programming

of cells and nuclear growth during germination must be passed from mother to daughter cells in the mature root.

It has been shown that cell cycle kinetics, mean cell area and mean nuclear volume in the root meristem are related to the conditions under which the cells are grown, ie., the metabolic history of those cells, in particular, during germination (section III.2). Consequently, variation in growth parameters might be the result of 1) variation in control of growth and proliferation, e.g., variation in cell growth rate or cell size at mitosis, and 2) variation in the program for cell growth due to cumulative fluctuations in the environment during germination.

The results reported here suggest that the mechanism involved in control of cell growth and proliferation in plant and animal cells is similar. In the model presented at the beginning of the Discussion an attempt is made to account for observations in both systems and at the same time to account for some of the variation observed for growth parameters in these systems.

CHAPTER V

CONCLUSIONS

1. During germination the cell area, nuclear volume and nuclear protein content of individual cells increases until they undergo their first mitosis. After division nuclear protein content and cell area decrease while nuclear volume either increases or decreases, depending upon environmental conditions, to the values normally seen in the mature root.

2. In the mature root values for mean cell area, mean nuclear volume and mean cell cycle duration are influenced by the ambient water level present during germination. The mean nuclear protein content of cells in a mature root does not appear to change when the ambient water supply changes either during germination or at maturity.

3. When the ambient water level is increased for mature roots, mean cell size decreases; mean M.I. and mean nuclear volume either remain constant or decrease.

4. Treatment of roots with 5-AU during germination stimulates cell and nuclear growth during G_1 of the first cell cycle.

5. In both germinating and mature roots treated with 5-AU, mean cell area and mean nuclear volume increase. 5-AU does not interfere with normal relationships between cell and nuclear size.

6. Although mean nuclear protein contents increase during 5-AU treatment of mature roots, individual nuclear protein contents do not increase beyond levels normally seen in untreated roots.

7. Linear relationships exist among DNA contents, nuclear volumes, nuclear protein contents and cell areas. Although heterogeneity exists for all growth parameters examined, evidence of restrictions on variation in growth parameters was found.

8. It is proposed that relationships among cell growth parameters are programmed into cells during germination and that changes in the environment of mature roots only modify the initial programme established during germination. Programming of this kind may contribute to the overall similarity in relationships seen between cell growth parameters of different roots.

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