RELATIVE VOLUME CHANGES OF THE NUCLEOLUS IN RELATION TO CELL AND NUCLEUS IN PISUM SATIVUM AND TRADESCANTIA PALUDOSA

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Lee Virn Leak

1959

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RELATIVE VOLUME CHANGES OF THE NUCLEOLUS IN RELATION TO CELL AND NUCLEUS IN *PISUM SATIVUM* AND *TRADESCANTIA PALUDOSA*

By

Lee Virn Leak

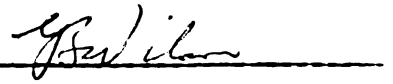
AN ABSTRACT

Submitted to the College of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1959

Approved By 

A knowledge of the volume changes of the nucleolus in relation to that of the cell and nucleus is of prime importance to the cytologist in considering the mitotic cycle. The phases or points of volume increase and decrease of the cell components must also be considered.

Meristematic cells of Pisum root tips and microspore cells of Tradescantia were used as merterials for obtaining measurements of nucleolus, nucleus and cell. The nucleolus, nucleus and cell are clearly differentiated after specific fixing and staining procedures.

The nucleolus shows a definite volume increase at the inception of active mitosis (interphase to early prophase) irregardless of nucleolar fusion. Thereafter volume increases cease and during late prophase the nucleolar volume is again the same or less than that in interphase. Along with nucleolar volume increase there is also a volume increase in cell and nucleus.

The number of nucleoli vary from one to three in Pisum sativum and from one to four in Tradescantia paludosa.

Fusion of nucleoli was observed from interphase to late prophase in Pisum, however, there was no indication of fusion in stages 1, 2, 3 and 4 in the microspore cells of Tradescantia.

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INTRODUCTION

The nucleolus is a spherical or oval-shaped intranuclear body, which exhibits variable degrees of affinity for acid and basic dyes. Nucleolar formation is generally regarded as taking place during telophase in association with a special region of one or more chromosomes of the haploid complement (Gates, 1942; Kaufmann, 1948; Hankansson and Levan, 1952). Rattenbury and Serra, (1952) observed that the number of nucleoli per cell varies with different organisms. It may be observed as a conspicuous component of the nucleus during interphase, early prophase, late prophase and telophase.

The nucleolus has been the subject of extensive cytological study since its discovery by Fontana in 1781. Little, however, is known at the present time of its exact function or the morphological changes which it undergoes in either the dividing or nondividing cell.

Biochemical evidence indicates that the nucleolus contains ribonucleo-protein, and is an active site of incorporation of labelled nucleic acid and protein precursors in certain cell types (Brachet,1957).

It is generally assumed that there is a progressive diminution in nucleolar volume from interphase to its dissolution before the onset of metaphase (Swanson, 1957). Preliminary experiments in this laboratory have indicated, however, that the inception of mitosis is accompanied by a corresponding nucleolar volume increase (Wilson and Morrison, 1958).

In view of the little known concerning the function of the nucleo-

lus during cell division, it is essential that more precise cytological information be available in order to understand more thoroughly the role played by this cellular organelle in the mitotic process. The present investigation, therefore, was carried out to determine: (1) the changes in nucleolar volume from interphase through late prophase in meristematic cells of Pisum sativum, (2) changes in the nucleolus relative to cell and nuclear volume change, (3) nucleolar fusion in meristematic cells of Pisum, and (4) changes in the nucleolus from interphase to prophase in microspore cells of Tradescantia paludosa.

LITERATURE REVIEW

Since the discovery of the cell by Robert Hook in 1665 and the formulation of the cell-theory by Schleiden, (1938) and Schwann, (1839), many studies have been made on the cell as a unit as well as on its structural components. There remain however, many questions as to the function of many cellular components.

Early References To The Nucleolus: The nucleolus is an organ of the cell which is almost universally present. It is absent or at least not detectable by present techniques in lower Cyanophyceae (Oscillatoria) as shown by Spearing, (1937). According to Montgomery, (1898) Fontana in 1781 was the first to observe the nucleolus in the epithelial cells of the eel, and described it as " a rounded or oval body with a spot in the middle". Valentin describes the nucleolus in conjunctiva as a rounded corpuscle which appeared to form "a kind of second nucleus" within the nucleus (Gates, 1942). The nucleolus was first discovered in plants by Schleiden (Wilson, 1898).

Origin Of The Nucleolus: It is generally recognized by cytologists that the nucleolus is formed during telophase of reproductive and somatic cells in both plants and animals. Some maintain that the origin of nucleolar substance is the chromatin (McClintock, 1934; Caspersson, 1950), while others claim it to be a derivative of the periplasm (see Dermen, 1933; Tischler, 1942). According to Heitz (1931) and Matsuura (1938) every chromosome of the complement can be regarded

as a "nucleolar chromosome" in the sense that they can all produce nucleoli under certain specified conditions. Rattenbury and Serra (1952) consider that the two types of nucleolar formation are possible at telophase, namely: (1) the production of layers of pronucleolar substance over the chromosome surface and in the lacunae between them (layer type), and (2) the formation of pronucleolar droplets (droplet type).

During the mitotic process the nucleolus gradually diminishes, and is usually dissolved or dissipated by metaphase. In some organisms, however, the nucleoli may persist throughout the mitotic period, dividing at anaphase or passing undivided to one or the other of the poles (Swanson, 1957).

According to Rattenbury and Serra (1952) the number of nucleoli formed at telophase varies considerably. The number of nucleolar droplets formed may exceed the number of nucleolar zones present, the former ranging from one to one or two hundred in some species. These droplets fuse, thus bringing about a decrease in number. A mutation has been produced by Elsdale, Frischberg and Smith (1958) that reduces the nucleolar number in Xenopus laevis. An expected Mendelian ratio (1:2:1) is obtained among the offspring from a cross of two heterozygotes, i.e., one quarter with cells containing two nucleoli, one half having but a single nucleolus in each cell, and one quarter having no nucleoli at all.

Composition Of The Nucleolus: By using a preparation of ribonuclease, Brachet (1941) was able to show that the basophillia of the nucleolus was due to its content of ribonucleic acid (RNA). With the

ultra-violet absorption spectrum Caspersson and Schultz (1940) observed that although the nucleolus had a nucleic acid absorption spectrum, it did not give a positive Feulgen reaction. They concluded, on this basis that the nucleolus contained ribonucleic acid. That protein is present in considerable quantities has been reported by Pollister and Ris (1948); Caspersson (1950); Nurnberger et al (1952); Vincent and Huxley (1954); and Vincent (1955). The presence of Feulgen positive material in nucleoli has also been reported many times in the literature (Duryee and Doherty, 1954). Brachet (1957) suggested this could result from penetration of heterochromatic segments of chromosomes into portions of the nucleolus. The nucleoli of certain cells have been reported to contain phosphatase (Danielli, 1933; Bradfield, 1951), the DPN-synthesizing enzyme (Baltus, 1954), and minerals (Vincent, 1952; Immers, 1954). The presence of lipoidal materials has been suggested from the action of certain lipid solvents on nucleolar structure (Gates, 1942).

Observations on fusion in living and fixed material (Montgomery 1898; Strangeway, 1923; Gates, 1942; and Hughes, 1952) suggest the nucleolus to be a more or less fluid body. That it exists as a coacervate is suggested by Ehrenberg (1946). Others, however, maintain that it contains variable quantities of proteins with high densities and appears as a semi-solid body (Pollister and Ris, 1947; Vincent, 1955).

Nucleolar Function: Numerous hypotheses of nucleolar function have been suggested. Vincent (1955) gives the following list: (1) to shield the chromosomes from the cytoplasm during mitosis; (2) to serve as a transfer of chromosomal influence to the cytoplasm; (3) to func-

tion as a storage point for the materials produced at a restricted rate by the chromosomes; (4) to operate as a site for limiting rates of reaction necessary for maintenance of cytoplasmic synthesis; (5) to serve as a site of accumulation of chromosomal or intranuclear products; (6) to serve as a site of accumulation of unutilized and/or unusable materials of cytoplasmic origin which enter the nucleus but cannot return to the cytoplasm; and (7) to function as a reservior of energy source for nuclear activity. None of these suggested functions has much experimental basis.

There is very little information on the relationship of the nucleolus to the cell or nucleus, or on its volume change from interphase to its dissolution or to the state where it is no longer recognizable.

MATERIAL AND PROCEDURE

For cytological investigation of the cell, nucleus and nucleolus, a fixative and staining procedure must be used that will differentiate these cellular components with a minimum amount of physical distortion. A method which met these criteria would allow one to make accurate measurements of the cell components in question.

Several fixatives were tested in order to obtain preparations in which chromatin, nucleolus, and cytoplasm could be observed. The "alcohol-formal-acetic" fixative (Battenbury, 1951) and numerous combinations of methanol, chloroform and propionic acid were tested. These did not give satisfactory results for accurate measurements of nucleolus, nucleus and cell volumes. By using 15 parts of ETOH (ethyl alcohol), 1 part 40 per cent formaldehyde and 3 parts propionic acid the desired results were obtained (Morrison, Leak and Wilson, 1959).

The meristems of young root tips (2-3 cms. basipetally from the root cap) of Pisum sativum var. Alaska, and microspore cells of Tradescantia paludosa were used as materials for measurements. Neither the Pisum nor Tradescantia was treated with chemicals prior to fixation, so measurements were made of "normal" tissue.

Procedure For Handling Meristematic Tissue Of Pisum: The peas were soaked overnight in glass distilled water at 25°C. They were then rolled in paper toweling and the rolls were placed vertically in beakers one third filled with glass distilled water. The peas were allowed to ger-

minate in an incubator at 25°C for 48 hours. The root tips were excised (one centimeter basipetally from the root cap) and fixed in 15 parts 95 per cent ETOH, 1 part 40 per cent formaldehyde and 3 parts propionic acid. While in the fixative the root tips were placed in a vacuum and evacuated from 1-2 hours, after which they were removed from the vacuum and allowed to fix for 12 hours under refrigeration.

Squash Preparations: The tissue was hydrolyzed in 1 normal HCl for 15 minutes at 60°C, stained with Schiff's Reagent for 30 minutes, then stained in aceto-carmine from 10-15 minutes. After staining with aceto-carmine the meristmes were removed from the excised root tips and squashed in a drop of .05 per cent fast green in 45 per cent acetic acid. A cover glass was set over the squashed material and the underside of the slide was gently heated. The slide was then placed between several layers of filter paper and pressure applied. After squashing the slides were placed in 90 per cent TBA for 24 hours, removed and made permanent with diaphane.

Sectional Preparations: The root tips were removed from the fixative, dehydrated and embedded in paraffin (as cited by Mericle, 1957). Longitudinal sections were cut with the microtome at 16 microns, the sections then mounted on slides and allowed to dry, after which the paraffin was removed with xylene. The tissue was hydrolyzed in 1 normal HCl for 18 minutes at 60°C, and stained with Schiff's Reagent for 30 minutes. This was followed by additional staining with aceto-carmine for 3-5 minutes, and counter staining in .05 per cent fast green in 45 per cent acetic acid for 2 minutes. The tissue was transferred to a staining jar containing equal volumes of alcohol and xylene for 1-2 mimutes, and then

cleared in xylene and mounted in clarite.

Procedure For Handling Microspore Cells: Buds were selected in series from flowering heads of Tradescantia plants with stages ranging from the tetrad through the first microspore division. The fixative used for the root tips did not give the differentiation necessary for accurate examination of the cell components. After various proportions of 95 per cent ETOH, 40 per cent formaldehyde and propionic acid were tested it was found that 15 parts of 95 per cent ETOH, 5 parts of 40 per cent formaldehyde and 3 parts of propionic acid gave the desired results (clearly defined boundaries) for accurate measurements of cell, nucleus and nucleolus.

Microspore Smear Preparations: The six anthers were dissected from each bud and placed on separate slides. By gently teasing the anthers with the flattened end of a glass rod, the microspore cells were extracted with very little damage to the cell walls, and with little contamination from the anther wall. The microspore cells readily adhered to the surface of the dry slides, which were quickly placed in a staining jar containing the fixative and allowed to fix from 3-5 minutes. After fixation the tissue was washed in distilled water for 3 minutes, hydrolyzed in 1 normal HCl for 3 mimutes at room temperature and stained with Schiff's Reagent for 3-5 minutes. The slides were rinsed with 1 normal HCl, stained in aceto-carmine for 2 minutes, counter stained with .05 per cent fast green in 45 per cent acetic acid for 1 minute. Cover glasses were placed on the slides and the measurements were made from temporary mounts. The mounts were placed in a glass chamber that was moistened with 45 per cent acetic acid and stored under

refrigeration to prevent drying.

In root tips of Pisum and the microspore cell of Tradescantia the nucleolus is stained a bluish green. The aceto-carmine is used to intensify the staining of the chromosomes. With the above technique the boundaries of the nucleus and nucleolus are clearly defined in both the meristematic cells of Pisum and the microspore cells of Tradescantia.

CYTOLOGICAL EXAMINATION

The preparations were examined with a 97X oil objective and 25X oculars. A 25X ocular containing a micrometer scale was used for measuring the width and length of cell, nucleus and nucleolus. The index of volume of cell, nucleus and nucleolus from squash preparations and longitudinal sections of Pisum, and microspore cells of Tradescantia were calculated by using the formulae $(\frac{l+w}{2})^3$, (Wilson, 1948) and $\frac{4\pi r^3}{3}$. Assuming the microspore cells of Tradescantia to be oblate spheroids the formula $\frac{4\pi a^2 b}{3}$ was also used to calculate the index of cellular volume, (Tables 1-9).

Volume indices of the meristematic cells in root tips of Pisum were calculated from diameter measurements of a randomly chosen sample of 50 cells per slide in the interphase, early prophase and late prophase stages. Four slides for a total of 200 cells for each of the three stages were examined from the sectioned material. From the squash preparations a total of 100 cells were measured for each of the three stages.

Measurements and statistical analysis of data obtained for both squash preparations and longitudinal sections were made and compared in order to detect any changes ascribable to difference in methodology.

One hundred cells were used for measurements of nucleoli, nuclei and cells for each of the four stages from the microspore cells of Tradescantia.

Nucleoli of three hundred cells were chosen at random from preparations of meristematic and microspore cells to determine whether fusion occurs, and if so, to what degree. The data obtained were analyzed statistically to test the significance of observed differences between stages.

Table 1. Index of cell volume in squash preparations in interphase, early prophase and late prophase in Pisum.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		$\frac{(1+w)^3}{2}$	
INTERPHASE	100	4.07	±.009	7.76	±.009
E. PROPHASE	100	5.75*	±.009	10.94*	±.009
L. PROPHASE	100	5.90*	±.009	11.24*	±.009

* Significant volume increase from interphase.

Table 2. Index of nuclear volume in squash preparations in interphase, early prophase and late prophase in Pisum.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		$\frac{(1+w)^3}{2}$	
INTERPHASE	100	.69	±.003	1.30	±.003
E. PROPHASE	100	1.10*	±.005	2.10*	±.005
L. PROPHASE	100	0**		0**	

*Significant volume increase from interphase.
**Nuclear membrane has disappeared.

Table 3. Index of nucleolar volume in squash preparations in interphase, early prophase and late prophase in Pisum.

STAGE	No. OF CELLS MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		$\frac{(1+w)^3}{2}$	
INTERPHASE	100	.013	±.001	.024	±.001
E. PROPHASE	100	.029*	±.002	.055*	±.002
L. PROPHASE	100	.021*	±.002	.039*	±.002

* Significant volume increase from interphase.

Table 4. Index of cell volume in longitudinal sections in interphase, early prophase and late prophase in Pisum.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		INDEX OF VOLUME $\frac{(l+w)^3}{2}$	
INTERPHASE	200	1.32	±.005	2.46	±.005
E. PROPHASE	200	1.77*	±.005	3.31*	±.005
L. PROPHASE	200	2.08*	±.006	3.87*	±.006

* Significant volume increase from interphase.

Table 5. Index of nuclear volume in longitudinal sections in interphase, early prophase and late prophase in Pisum.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		INDEX OF VOLUME $\frac{(l+w)^3}{2}$	
INTERPHASE	200	.25	±.002	.46	±.002
E. PROPHASE	200	.41*	±.002	.78*	±.002
L. PROPHASE	200	0**		0**	

* Significant volume increase from interphase.
** Nuclear membrane has disappeared.

Table 6. Index of nucleolar volume in longitudinal sections in interphase, early prophase and late prophase in Pisum.

STAGE	No. OF CELLS MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$		INDEX OF VOLUME $\frac{(l+w)^3}{2}$	
INTERPHASE	200	.013	±.002	.020	±.002
E. PROPHASE	200	.025*	±.002	.039*	±.002
L. PROPHASE	200	.021*	±.001	.030*	±.001

* Significant volume increase from interphase.

Table 7. Index of cell volume in stage 1, 2, 3 and 4 in microspore cells of Tradescantia.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$	$\frac{(l+w)^3}{2}$	$\frac{4\pi a^2 b}{3}$
1	100	5.59 ±.004	10.65 ±.004	5.75 ±.004
2	100	5.59 ±.004	10.65 ±.004	6.09 ±.004
3	100	9.23* ±.004	15.63* ±.004	9.53* ±.004
4	100	11.53* ±.004	19.68* ±.004	12.00* ±.004

* Significant volume increase from stage 1.

Table 8. Index of nuclear volume in stage 1, 2, 3 and 4 in microspore cells of Tradescantia.

STAGE	No. MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$	$\frac{(l+w)^3}{2}$
1	100	.53 ±.009	1.00 ±.009
2	100	.53 ±.009	1.00 ±.002
3	100	1.44* ±.003	2.74* ±.003
4	100	3.06* ±.003	5.83* ±.003

* Significant volume increase from stage 1.

Table 9. Index of nucleolar volume in stages 1, 2, 3 and 4 in microspore cells of Tradescantia.

STAGE	No. OF CELLS MEASURED	INDEX OF VOLUME $\frac{4\pi r^3}{3}$	$\frac{(l+w)^3}{2}$
1	100	.025 ±.002	.047 ±.002
2	100	.050* ±.003	.091* ±.003
3	100	.214* ±.003	.389* ±.003
4	100	.290* ±.005	.551* ±.005

* Significant volume increase from stage 1.

OBSERVATIONS

Measurements of nucleoli in meristematic cells of Pisum sativum indicate quite clearly that there is a significant increase in volume from interphase to early prophase (Test figure 1). This increase is then followed by a decrease from early prophase with dissolution normally occuring by metaphase.

Meristematic Cells Of Pisum sativum: The interphase cells in meristematic tissue of Pisum sativum are mostly isodiametric with a spherical nucleolus centrally located. The cell volume increases from interphase to early prophase by 41 per cent and then remains constant or increases very slightly from early prophase to late prophase (Text figure 2).

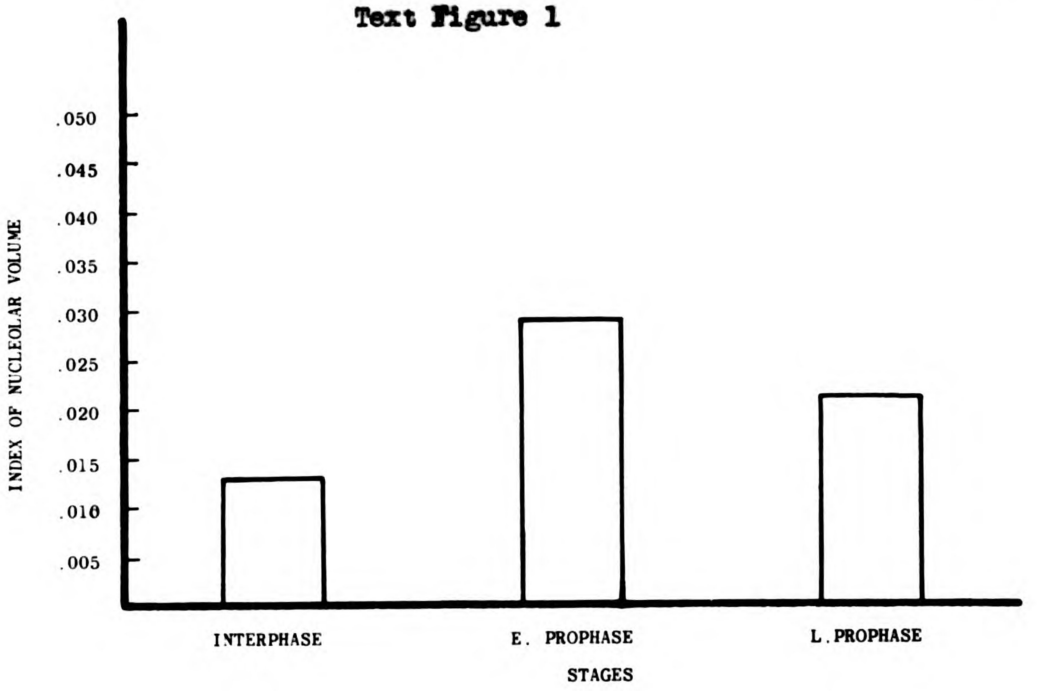
The interphase nucleus is characterized by the presence of a chromatin reticulum and one to four nucleoli (Plate I - fig. 1). There is a 60 per cent volume increase in the nucleus from interphase to early prophase (Text fig. 2). By late prophase the nuclear membrane has dissolved leaving the chromosomes and nucleolus or nucleoli centrally orientated in the cell.

There is a 123 per cent nucleolar volume increase from interphase to early prophase, but thereafter a decrease to late prophase, when the volume is again about the same as or slightly less than that in interphase (Text fig. 2). There were one to four nucleoli observed in the interphase stage, and one to three in early and late prophase stages.

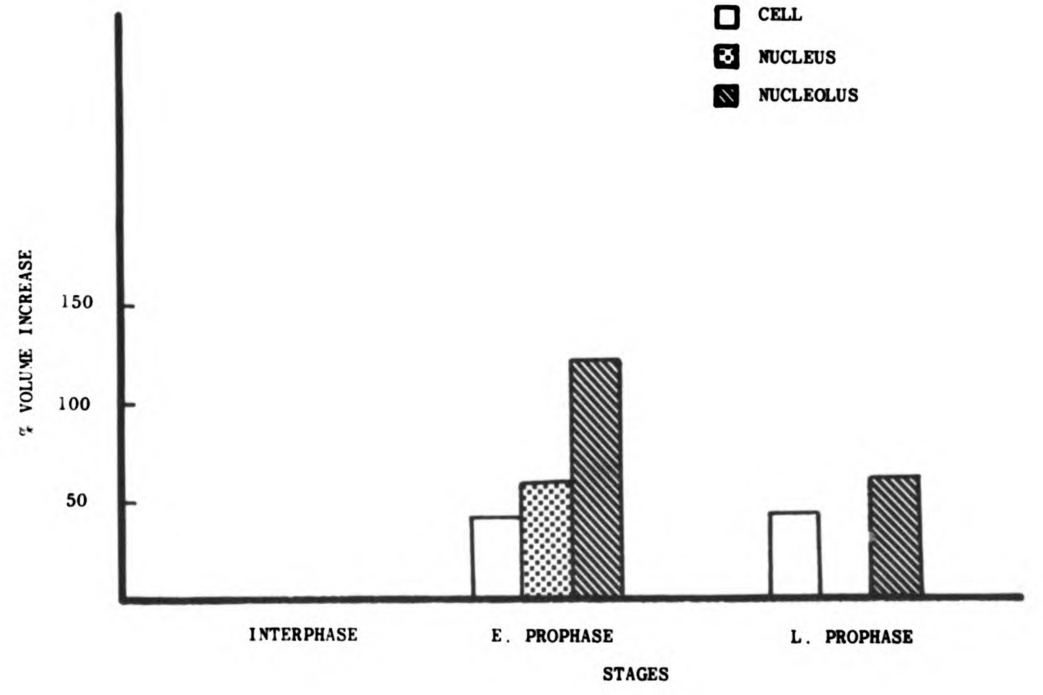
Text fig. 1 Mean volume of nucleolus during interphase, early prophase and late prophase in meristematic cells of Pisum from squash preparations.

Text fig. 2 Percentage volume increase of cell, nucleus and nucleolus based on per cent increase from interphase to early prophase and interphase to late prophase in squash preparations from Pisum root tips.

Text Figure 1



Text Figure 2



DESCRIPTION OF PLATE

PLATE I

Fig. 1 Interphase cell with one nucleolus centrally located from squash preparations of Pisum root tips.

Fig. 2 Early prophase cell with nucleus containing two nucleoli from squash preparations of Pisum root tips.

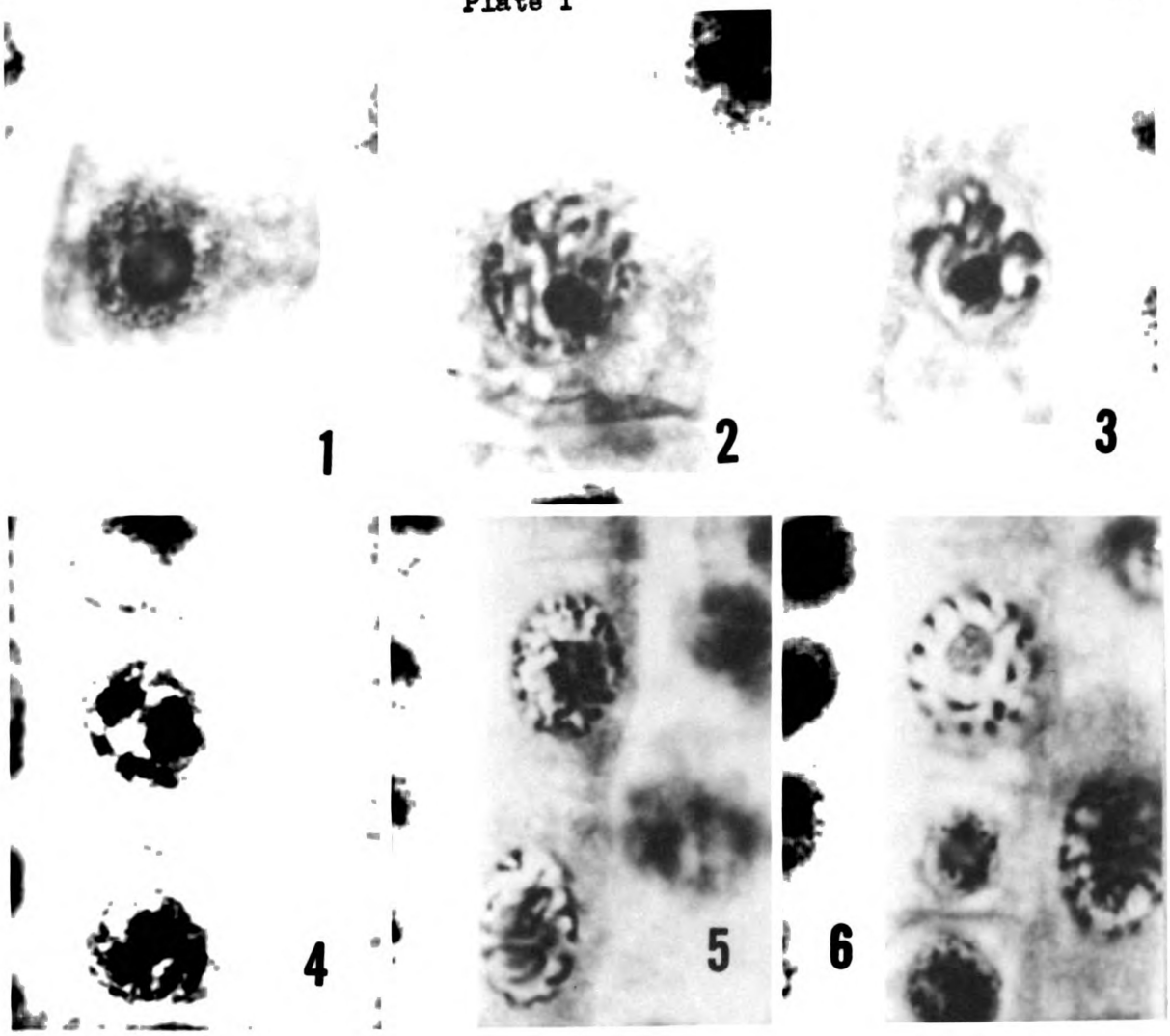
Fig. 3 Late prophase cell containing one nucleolus with nuclear membrane showing signs of dissolution in squash preparations of Pisum root tips.

Fig. 4 Interphase cell with nucleus containing multinucleoli from longitudinal section of Pisum root tip.

Fig. 5 Early prophase cell showing nucleus with nucleolus centrally located in longitudinal section of Pisum root tip.

Fig. 6 Late prophase cell with one nucleolus in longitudinal section of Pisum root tip.

Plate I



In nuclei which contained a single nucleolus the latter is more or less centrally located with a pronounced vacuole. When more than one nucleolus is present they may be of equal or unequal volumes.

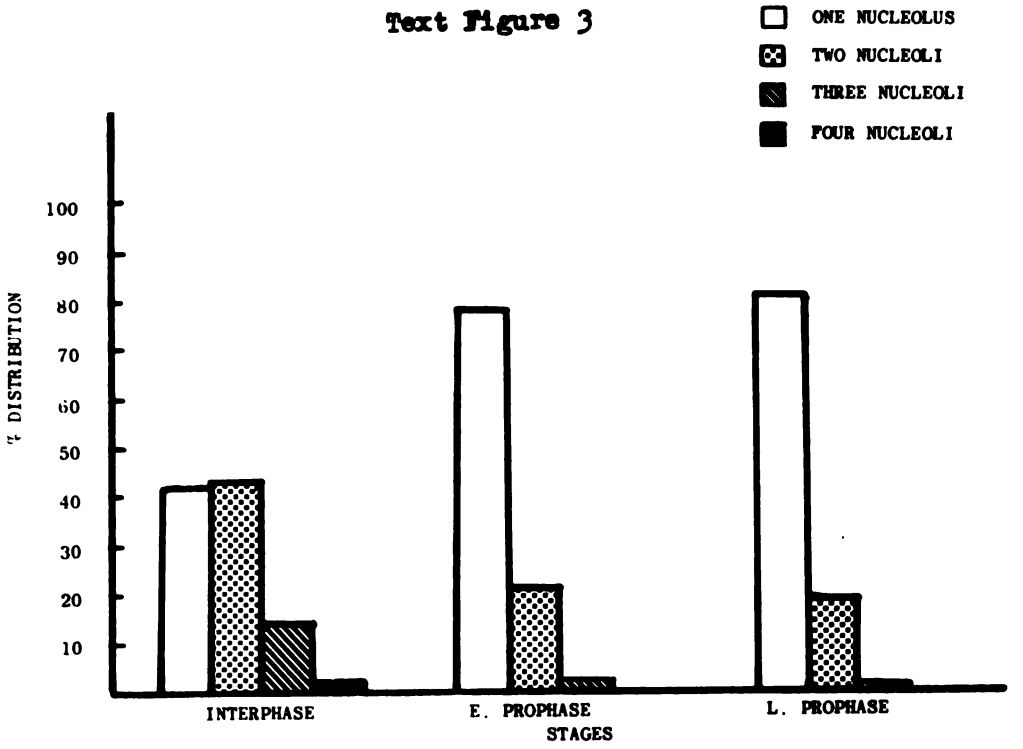
Out of 300 cells counted in the interphase stage, 42 per cent contained a single nucleolus, 43 per cent two nucleoli, 14 per cent three and 1 per cent four (Text fig. 3). In early prophase 78 per cent of the cells contained one nucleolus, 21 per cent two nucleoli and 1 per cent had three nucleoli, with no cells containing four (Text fig. 3). The number of nucleoli per cell in late prophase was not significantly different from the number in early prophase, there being 80.7 per cent containing one nucleolus, 19 per cent with two nucleoli and .3 per cent containing three, with no cells being observed with four (Text fig. 3). The increase in the number of cells with one nucleolus from interphase to early prophase indicates fusion in early prophase. The volume of the compound (fused) nucleolus is greater than the sum of the nucleolar volumes when there are two or more nucleoli in interphase, early prophase and late prophase (Text fig. 4).

Cells, nuclei and nucleoli all show a significant increase in volume from interphase to early prophase. The increase in cell volume from early prophase to late prophase is not as great as its volume increase from interphase to early prophase (Table 1). The nuclear membrane has disappeared by late prophase (Table 2) and the nucleolus has decreased in volume in transition from early to late prophase, indicating that dissolution has begun (Table 3).

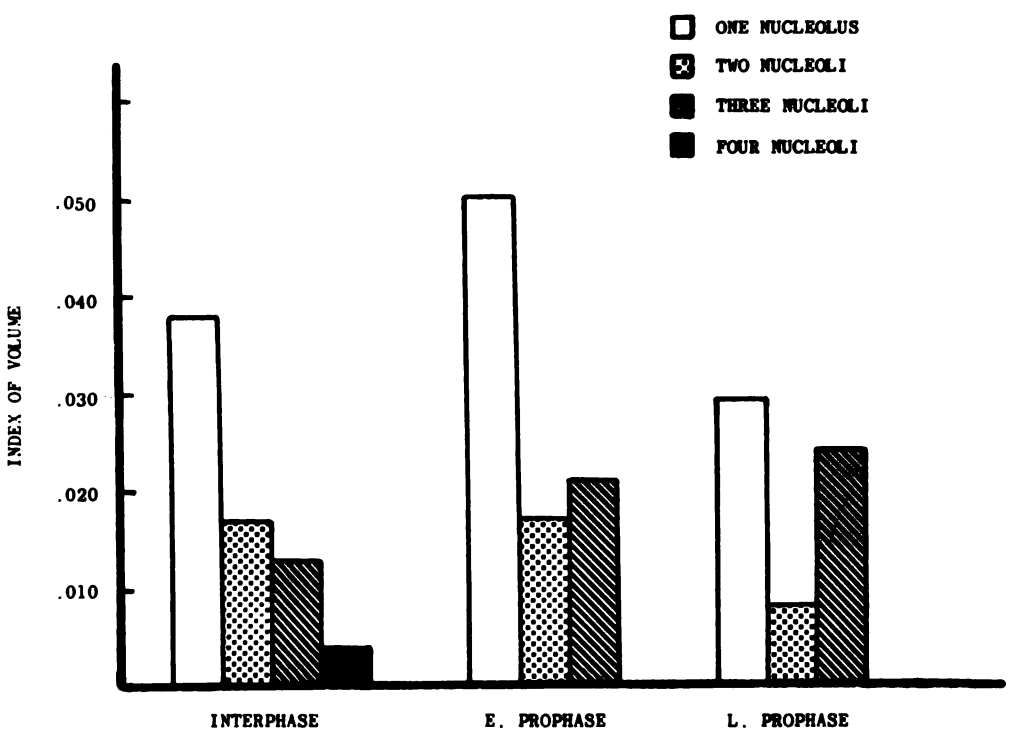
Text fig. 3 Frequency of cells containing one, two, three and four nucleoli in interphase, early prophase and late prophase from squash preparations in Pisum.

Text fig. 4 Mean volume of one, two, three and four nucleoli per stage in meristematic cells of Pisum.

Text Figure 3



Text Figure 4



In order to check possible distortion produced by the squash technique, similar measurements were made of sectioned material. Upon examination of data from the sectioned material it was observed that the cell, nucleus and nucleolus showed a volume increase from interphase to early prophase and late prophase as was observed in the squash preparations (Text fig. 5). Both techniques showed similar trends in the index of volume increase for cell, nucleus and nucleolus (Tables 1-6. Text figs. 2 and 3).

Microspore Cells Of Tradescantia: Measurements of cells, nuclei and nucleoli were made from post meiotic interphases to prophase of the first microspore division. During this period four stages can be distinguished on morphological grounds, and have been designated as stages 1, 2, 3 and 4 (Plate II, figs. 1-4).

Stage 1: This is the stage which follows the formation of the tetrad. Stage 1 is characterized by a spheriodal cell with an oval shaped nucleus which is centrally located. In the nucleus there are 1 to 4 nucleoli of variable sizes randomly distributed.

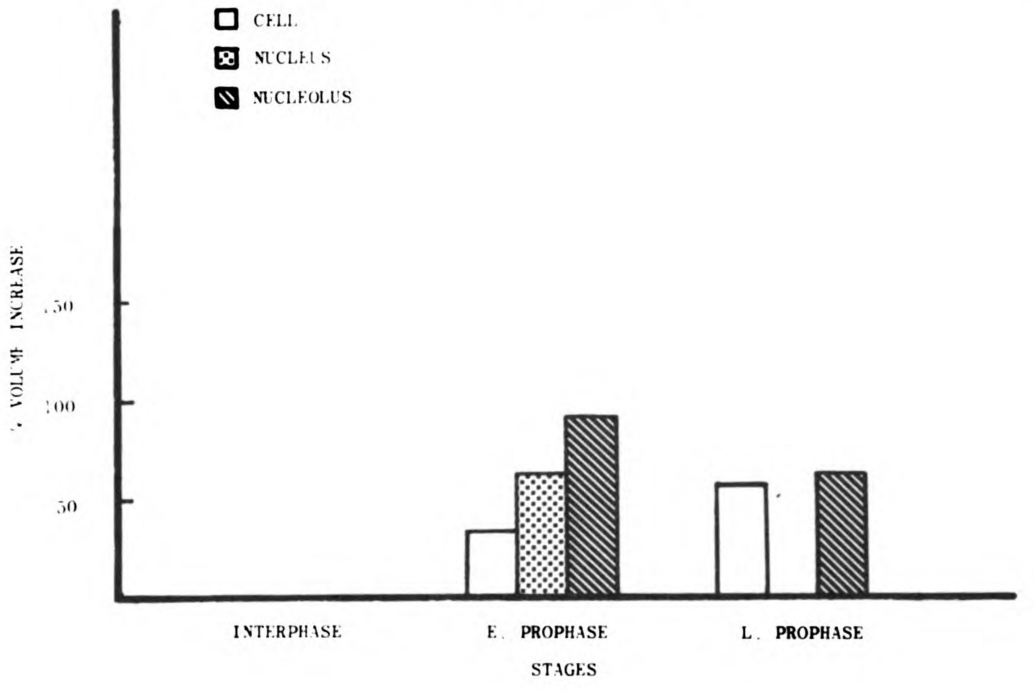
Stage 2: The second stage is recognizable by an elongation of the cell, and movement of the nucleus to one end. While there is no significant change in volume of the cell and nucleus over that of stage 1, there is a significant change in nucleolar volume (Tables 7, 8 and 9). The number of nucleoli per nucleus remains the same as in stage 1.

Stage 3: This stage is characterized by a significant volume increase in both cell and nucleus, as well as in the nucleolus (Tables 7, 8 and 9). The number of nucleoli remain constant as in the two preceding stages.

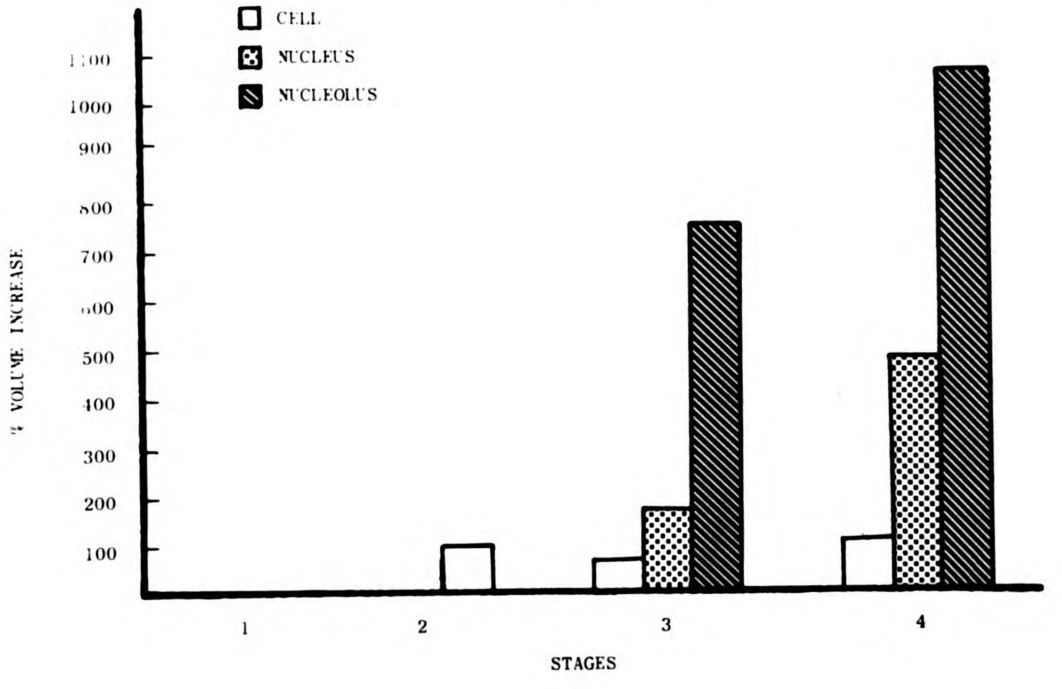
Text fig. 5 Percentage volume increase of cell, nucleus and nucleolus based on per cent increase from interphase to early prophase and from interphase to late prophase in longitudinal sections of Pisum.

Text fig. 6 Percentage volume increase of cell, nucleus and nucleolus based on per cent volume increase from stage 1 for stages 2, 3 and 4 in microspore cells of Tradescantia paludosa.

Text Figure 5



Text Figure 6



DESCRIPTION OF PLATE

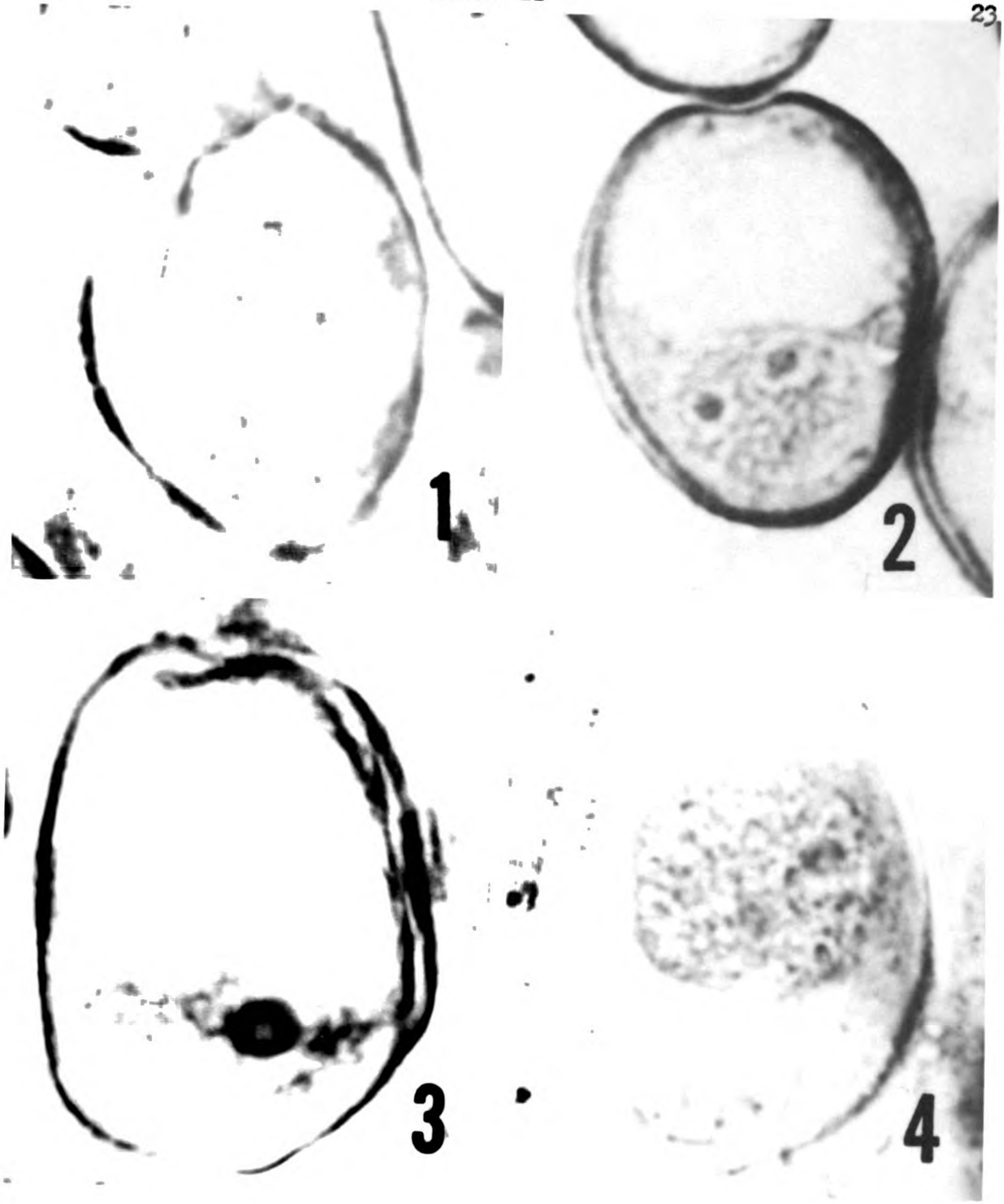
PLATE II

Fig. 1 Stage one with nucleus containing three nucleoli from smear preparations of microspore cells of Tradescantia paludosa.

Fig. 2 Stage two showing movement of nucleus to one end of cell with the nucleus containing two nucleoli in microspore cells of Tradescantia.

Fig. 3 Stage three showing nucleus containing one nucleolus, which contains a vacuole. The cell, nucleus and nucleolus showing a marked increase in volume from the preceding stage in microspore cells of Tradescantia.

Fig. 4 Stage four showing movement of nucleus back to the center of cell, with the nucleus containing two nucleoli in microspore cells of Tradescantia.


1
2
3
4

Stage 4: The volume of cell, nucleus and nucleoli continue to show a significant increase over that of the previous stage. This stage is also characterized by the movement of the nucleus back to the center of the cell, where it is embedded in a mass of cytoplasm which extends the width of the cell (Plate II, fig. 4). The average number of nucleoli per nucleus has not changed from that found in the preceding stages.

The cell volume remains relatively constant from stages 1 to 2, but by stage 3 it has increased by 65 per cent (Text fig. 6) of its original size (stage 1). This increase continues through stage 4, resulting in a 108 per cent increase in volume over that of stage 1 (Text fig. 6).

There is no significant volume increase in the nucleus from stage 1 to 2. By stage 3 the nucleus has increased by 171 per cent from stage 1, and by stage 4 to 477 per cent of its original volume (Text fig. 6).

Nucleoli of microspore cells are visible as deep bluish-green spherical bodies which are more or less centrally located when there is only a single nucleolus per cell and randomly distributed in nuclei with more than one nucleolus.

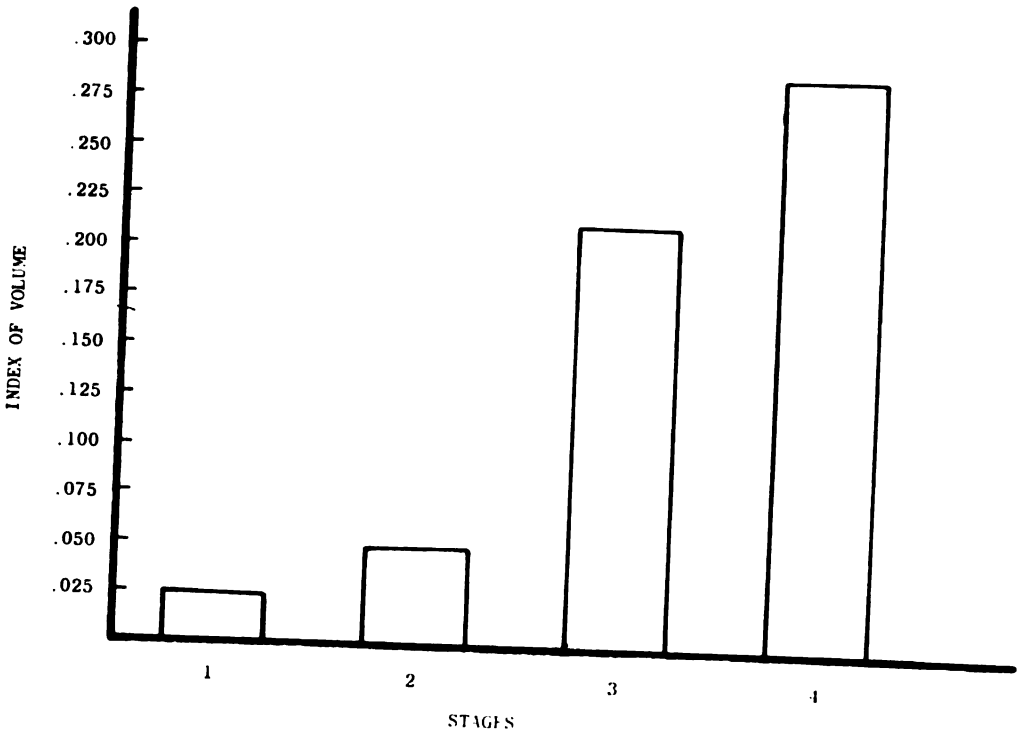
The nucleolus shows a continuous volume increase from stages 1 through 4 in microspore cells of Tradescantia (Text fig. 7). The per cent increase from stage 1 to stages 2, 3 and 4 is 100 per cent, 756 per cent and 1060 per cent respectively (Text fig. 6).

In stages 1 through 4 the volume of the single nucleolus is greater than the sum of the nucleolar volumes when there are two or more nucleoli per nucleus (Text fig. 8).

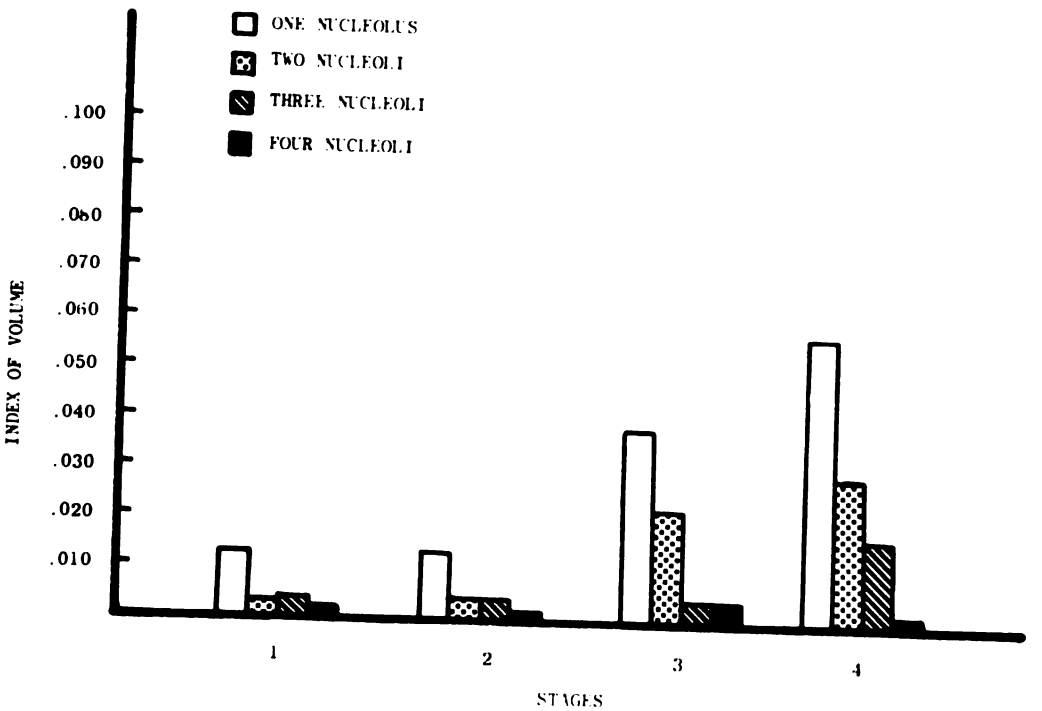
Text fig. 7 Mean volume of nucleolus during stages 1, 2, 3 and 4 in microspore cells of Tradescantia.

Text fig. 8 Mean volume of one, two, three and four nucleoli per stage in microspore cells of Tradescantia.

Text Figure 7



Text Figure 8



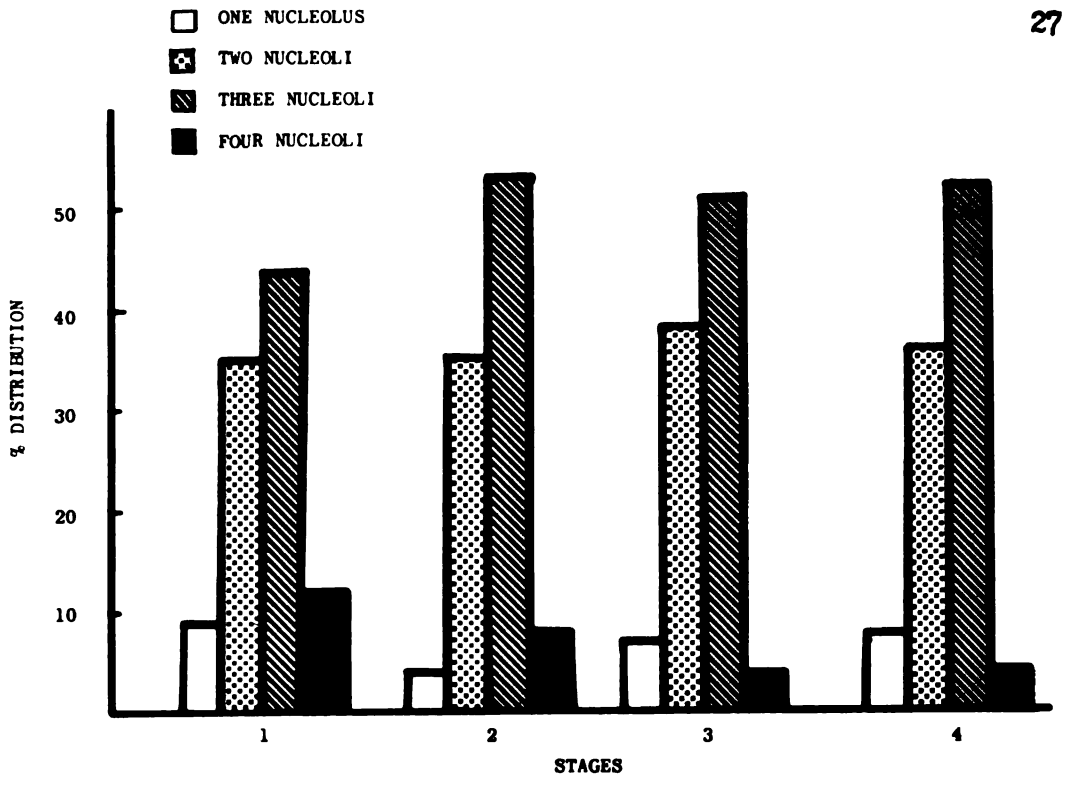
In microspore cells of Tradescantia, neither cell nor nucleus show a significant volume increase from stage 1 to stage 2, but both show an increase in volume by stages 3 and 4. The nucleolus, however, shows a continuous volume increase from stages 1 through 4.

Three hundred cells were counted from the four stages to determine whether or not there is significant fusion of multiple nucleoli. Examination of the histogram as shown in Text figure 9 indicates that the number remains relatively constant throughout stages one to four. There is, therefore, no evidence that nucleolar fusion occurs during interphase and prophase in microspore cells of Tradescantia.

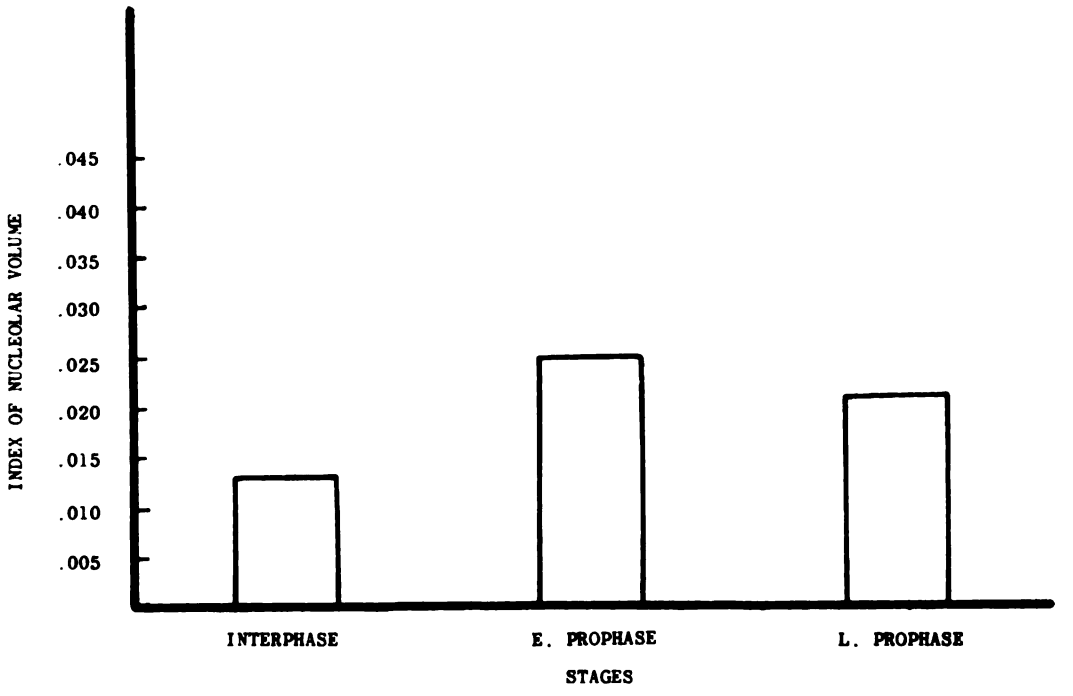
Text fig. 9 Frequency of cells containing one, two, three and four nucleoli in microspore cells of Tradescantia.

Text fig. 10 Mean volume of nucleolus during interphase, early prophase and late prophase in meristematic cells of Pisum sativum from longitudinal sections.

Text Figure 9



Text Figure 10



DISCUSSION

The onset of cell division in both meristematic cells of Pisum and microspore cells of Tradescantia, is characterized by an overall increase in volume of cell, nucleus and nucleoli. This volume increase suggests several questions with regard to the function and relationship of cell components, e.g., (1) Is cell volume increase necessary for cells to divide? (2) Is the volume increase restricted to the cell or must there also be an increase in nucleus and nucleolus? (3) Do volume increases of components coincide? (4) What is the relationship of nucleolar volume to that of the cell and nucleus? One approach to the elucidation of the above, is an examination of cell, nuclear and nucleolar volumes at the stage prior to division (interphase) and the first stages of division (early prophase and late prophase). The stage at which no morphological activity may be observed was termed the "Resting Stage" by the early cytologists. It is now recognized that the so-called interphase cell is in a metabolically active state. It seems to be well established, for example, that DNA-synthesis occurs during interphase (Howard and Pelc, 1951; Vincent, 1952 and Brachet, 1957).

Meristematic Cells Of Pisum sativum: There is a significant increase in cell volume from interphase through late prophase. It is apparent that the major proportion of this increase is occuring during the onset of division; i.e. from interphase to early prophase, with relatively little increase from early to late prophase. This volume in-

crease may be due to either an increase in membrane permeability of cell and nucleus, which allows an increase in uptake of water and solutes, or a synthetic process with the incorporation of proteinaceous materials into the nucleus, or a combination of the two (Sirlin and Waddington, 1956 and Brachet, 1957).

Some workers maintain that the relationship between nucleus and cell is always constant, while others hold that no such constancy exists, but rather, that there is a wide variability in the relative size increase of cells and nuclei (see Trombetta, 1939). In the meristematic cells of Pisum there appears to be a reasonably constant relationship, between relative rates of volume change in cell and nucleus, with the ratio being 6:1 in interphase and 5:1 in early prophase (Table 10).

Observations in the present study indicate a significant increase in nucleolar volume in meristematic cells of Pisum from interphase to early prophase. The increase ceases during early prophase, and the volume begins to decrease. By metaphase the nucleolus has dissolved. An examination of the sectioned material also showed a nucleolar volume increase from interphase to early prophase (Text fig. 10). These findings are not in agreement with those of Hankansson and Levan (1942), who found that the size of the nucleolus in Pisum is the same during resting (interphase) stage and the early prophase stages.

Nucleolar Fusion In Pisum: The fusion of nucleoli has been frequently reported to occur in a variety of cell types (Gates, 1942). The fusion of nucleoli commences in late telophase (Gates, 1942; Hankansson and Levan, 1942; Duryee and Doherty, 1954). As shown by Heitz (1930),

Table 10. Showing ratios of cell/nucleus, cell/nucleolus and nucleus/nucleolus in Pisum.

STAGE	CELL / NUCLEUS	CELL / NUCLEOLUS	NUCLEUS / NUCLEOLUS
INTERPHASE	6	313	53
E. PROPHASE	5	198	37
L. PROPHASE	-	281	-

Table 11. Showing ratios of cell/nucleus, cell/nucleolus and nucleus/nucleolus in Tradescantia.

STAGE	CELL / NUCLEUS	CELL / NUCLEOLUS	NUCLEUS / NUCLEOLUS
1	11	223	21
2	11	111	10
3	6	43	7
4	4	39	10

when the nucleolus organizing portions of two chromosomes lie close together in the telophase nucleus, the chance of fusion between the two developing nucleoli is greatly enhanced. Woods (1937) suggested, on the basis of volumetric studies of root tip nucleoli in Tulipa, that the relative amount of plastin generated in telophase may play a part in the fusion of nucleoli, with large amounts having a tendency to favor fusion, thus giving a reduction in nucleolar number. However, in the meristematic cells of Pisum fusion continues through interphase and early prophase. The degree of nucleolar fusion was determined by counting the number of nucleoli in three hundred cells of each stage (Text fig. 3). There was no indication of budding or fragmentation of nucleoli in the meristematic cells of Pisum sativum.

Microspore Cells Of Tradescantia paludosa: A volume increase in cell components preceded microspore division as was found in meristematic cells of Pisum. The relative volumes of cell, nucleus and nucleolus of microspore cells were examined from four stages (1, 2, 3 and 4) as explained earlier.

There is no significant increase in cell volume from stage 1 to stage 2. However, by the end of stage 3 the cell volume has increased by almost half its original value (Stage 1), and this increase continues through stage 4 (Text fig. 6).

There is also no volume increase in the nucleus from stages 1 to 2. It is apparent that the cell and nucleus are synchronized with regard to time of volume increase, in that neither increases in stages 1 and 2, while both increase through stages 3 and 4. The magnitude of nuclear volume increase, however, is much greater than that of the cell

(Table 11). In stage 3 and 4 the nuclear volume is increasing at a greater rate than that of the cell. This greater increase of nuclear volume gives a decreasing cell/nucleus ratio in stages 3 and 4 respectively (Table 11). The cell/nucleus ratio of the microspore cells of Tradescantia differ from the ratio in meristematic cells of Pisum, in that the latter it remains relatively constant (Table 10).

Bryan (1951) observed the microspore cells of Tradescantia that: (1) the DNA content of developing microspores increases during the long interphase period (Stages 1-3); and (2) this increase is rather slow during early interphase (Stage 1 and 2) and extremely rapid just prior to microspore prophase (Stage 3). The nuclear volume increases as observed in the present study correlates well with the rate of increase in DNA as observed by Bryan. These findings suggest that the nuclear DNA synthesis is associated with nuclear volume increase. This volume increase also may be associated with an increase in permeability of the nuclear membrane. Bahr and Beerman (1954) observed in giant nuclei that the rate of transport through the membrane per unit area is higher than that in nuclei and cells of normal size.

Nucleolar volume increase begins in stage 1 and continues through stage 4, while cell and nuclear volume increases commence in or just before stage 3.

The nucleolar volume increase in stage 1 through stage 4 is not due to fusion, as the number per nucleus remains relatively constant in each of the four stages (Text fig. 9). The foregoing observation suggests that the nucleolar volume increase is due to either synthesis or the incorporation of nuclear substances into the nucleolus, this uptake

being influenced by the metabolic and nutritive state of the nucleus and cell (Gates, 1942). Gates observed that nucleolar size was found to be greatly influenced by nutrition. By growing pieces of leaves in sugar solutions he found that the nucleoli were larger, and contained more carbohydrates, than nucleoli of leaves kept in darkness. These results indicated that the nucleolus is an important storage organ in leaf cells. Bretschnider and Hirsch (1937) observed that in eggs of certain teleosts (Lamelli branch) the nucleus and nucleolus at first grow coincidently; then the latter stops while the former continues its growth. The present study indicates that similar volume relationships exists in meristematic cells of Pisum, while this condition was not observed in microspore cells of Tradescantia.

It is apparent that the nucleolar volume is increasing at a relatively faster rate than that of the cell or nucleus, the order of magnitude being nucleolus, nucleus and cell (Text fig. 6).

In regards to nucleolar volume per nucleus it was observed that in meristematic cells of Pisum and the microspore cells of Tradescantia, the volume of the compound nucleolus (fused nucleoli) was consistently larger than the sum of the nucleolar volumes when there were two or more nucleoli (Text fig. 8). This condition was also reported by deMole (1928) and Bhatia (1938). Similarly Parthasarathy (1938) found in the pollen mother cells of rice at diakenesis that the volume of the single fusion nucleolus was greater than the sum of the volumes when there were two nucleoli. It was found in a polyploid series of Musae that nucleolar size increases with increase in degree of ploidy (Wilson, 1948).

There was no indication of fusion in the four stages examined in the microspore cells of Tradescantia, in contrast to that which was found in the meristematic cells of Pisum sativum.

SUMMARY

1. The problem of nucleolar volume increase, fusion, its relation to nuclear and cell volume increase from interphase to late prophase in meristematic cells of Pisum and from interphase to prophase during post meiotic interphase of Tradescantia was studied.

2. Methods for fixation and staining of cells for cytological study of nucleoli, nuclei and cells were developed.

3. The relative volumes of cell, nucleus and nucleolus were calculated for the interphase and prophase stages in the meristematic cells of Pisum sativum, and the microspore cells of Tradescantia paludosa. The data were statistically analyzed to ascertain the size relationship existing between cell components prior to and during the onset of division.

4. Measurements were made using squash preparations and longitudinal sections from meristematic tissue of Pisum to determine if there were significant differences in the relative volumes of cell components in the preparations of the two methods. There was no appreciable difference in the ratio of volume increase exhibited by nucleus, nucleolus and cell in the two types of preparations.

5. The question of volume increase in cell, nucleus and nucleolus is discussed. There is a rapid increase in nuclear, nucleolar and cell volumes from interphase to early prophase. Howard and Pelc (1951) reported that this phase of division is characterized by a high degree of

protein synthesis.

6. The ratios of cell/nucleus, cell/nucleolus and nucleus/nucleolus were considered. The results indicate that the nucleolar volume increases at a much faster rate than that of cell or nucleus in the meristematic cells of Pisum and the microspore cells of Tradescantia. The magnitude of increase is in the order of nucleolus, nucleus and cell.

7. It was observed that nucleolar size (volume) increases from interphase through early prophase. Following early prophase the volume of the nucleolus decreases in late prophase and has disapppeared by metaphase in meristematic cells of Pisum. This observation of nucleolar volume increase from interphase to early prophase is not in agreement with the findings of Hankansson and Levan (1942).

8. Three hundred nucleoli per stage were counted to determine the degree of fusion in both Pisum and Tradescantia. It was found that the per cent of nuclei containing one nucleolus is significantly increased from interphase to early prophase in Pisum indicating nucleolar fusion. In Tradescantia, the percentage of cells with 1, 2, 3 and 4 nucleoli remained constant through the stages measured. It is apparent that fusion does not occur in microspore cells of Tradescantia, from the interphase through prophase.

9. An attempt was made to correlate the observed morphological changes occuring in Pisum and Tradescantia, with reported biochemical information on cellular components.

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