THE MODE OF CHROMOSOME DUPLICATION DURING MEIOSIS AND MITOSIS IN HAPLOPAPPUS GRACILIS

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M.A.; M.Sc.; M. Tech.

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

August 1965

DOCTOR OF PHILOSOPHY (1965) McMASTER UNIVERSITY

(Biology) Hamilton, Ontario

TITLE: The mode of chromosome duplication during meiosis and mitosis in Haplopappus gracilis

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NUMBER OF PAGES: vii, 67

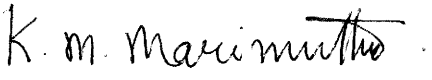
SCOPE AND CONTENTS: The mode of chromosome duplication during meiosis and mitosis in Haplopappus gracilis was investigated. Tritiated thymidine was incorporated into the pollen mother cells during premeiotic interphase, and the cells were allowed to reach the tetrad stage. The autoradiographs prepared from the tetrads showed an unequal distribution of grains over their nuclei, suggesting a conservative mode of chromosome duplication during meiosis. Seedlings were fed with tritiated thymidine for the duration of one cell cycle and also for the duration of several cell cycles. The autoradiographs prepared from the root tip cells, thus treated, showed both labelled and unlabelled chromatids in the anaphases of all the experiments, thus again suggesting a conservative mode of chromosome duplication. A chromosome model to explain the results is discussed.

PREFACE

This thesis describes studies carried out in the Department of Biology, McMaster University, from September 1961 to December 1964. Except where others are specifically mentioned it consists entirely of my own original work. No similar thesis has been submitted at any other university.

My grateful thanks are due to my supervisor, Dr. S. F. H. Threlkeld for advice and never failing encouragement throughout the work.

I am also deeply indebted to the Department of Biology and to the Government of Ontario for the award of scholarships covering the period of the research reported herein.



Biology Department

McMaster University

August 1965

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INTRODUCTION

Plaut and Mazia (1955, 1956) were the first to use autoradiography techniques to investigate the mode of chromosome duplication during mitosis, by making use of the following discoveries: 1) the amount of deoxyribonucleic acid (DNA) per nucleus in different somatic tissues of an organism is constant, and it is specifically localized in the chromosomes (Mirsky and Ris, 1949; Swift, 1953), 2) DNA synthesis is restricted to chromosome duplication (Alfert, 1955; Bloch and Godman, 1955), 3) all or the major part of DNA is synthesized during interphase (Swift, 1950; Howard and Pelc, 1952; Walker and Yates, 1952; Taylor, 1953; Thoday, 1954); 4) thymidine is a specific precursor of DNA and it is not diverted to the synthesis of RNA (Freidkin et al. 1956).

Following the terminology of Delbruck and Stent (1957), Mazia (1961) proposed three possible modes of chromosome duplication: 1) a conservative scheme in which one of the daughter chromosomes is completely new and the other one is parental; 2) a semi-conservative mode in which each of the daughter chromosomes is made up of half of the parental material (DNA) and half of the newly synthesized material; 3) a dispersive mode in which the parental material and the newly synthesized material are distributed randomly between the daughter chromosomes.

Plaut and Mazia (1955, 1956) grew seedlings of Crepis capillaris, with a chromosome number of ($2n = 6$), in a solution of C^{14} labelled thymidine with a specific activity of 0.5 to 0.8 μc/ml in distilled water for twelve hours (a duration estimated to be less than the time for one cell cycle), after which the root tips were fixed in acetic alcohol (1:3).

The root tips were then hydrolysed in IN HCl at 60° C for 3-4 minutes and squashed on a slide in a drop of 45% acetic acid. After removal of the cover slips by a technique involving freezing with dry ice, the slides were passed through absolute alcohol, and washed extensively with water. Autoradiographs were made using the stripping film technique. The estimation of the number of grains over the pairs of anaphase and telophase chromosome sets revealed the distribution of labelled DNA with varying degrees of inequality, as well as cases of near equality. It was concluded that the parental chromosome material (DNA) was not equally distributed between the daughter chromosomes. The authors claimed that their results clearly ruled out the possibility of a semi-conservative mode of duplication and left the possibility of a conservative mode of duplication or a dispersive mode.

In order to obtain autoradiographs with higher resolution in similar experiments, Taylor, et al. (1957) made use of tritium labelled thymidine. Vicia faba seedlings were grown in a nutrient solution containing tritiated thymidine for 8 hours, a time approximately equivalent to one-third of the cell cycle. The seedlings were then transferred to a nutrient solution containing colchicine at a concentration of 0.05% for 10 hours, and for 34 hours. It is known that colchicine prevents the formation of the daughter cells by inhibiting the spindle formation, but it does not prevent the duplication of the chromosomes. Thus the number of duplications following the treatment of the tritiated thymidine can be determined for any individual cell by counting the number of chromosomes. Colchicine blocked mataphases (C-metaphases) with a

normal complement of 12 chromosomes were observed in roots fixed after 18 hours, and C-metaphases with a complement of 12, 24, and 48 chromosomes were observed in roots fixed after 42 hours. The autoradiographs showed that those cells with 12 chromosomes had both sister chromatids of all chromosomes uniformly labelled, and those cells with 24 chromosomes had all the chromosomes labelled but only in one of the sister chromatids. In a few cells with 48 chromosomes approximately one-half of the chromosomes of a complement contained one labelled and one unlabelled chromatid, while the remainder showed no label in either chromatid. In cells with 24 and 48 chromosomes a few chromatids were labelled along only a part of their length, but in those instances the sister chromatids were labelled in complementary positions. This pattern of labelling was attributed to sister chromatid exchanges. From these results it was concluded "that a chromosome is composed of two physical entities probably complementary to each other and that after replication of each to form a chromosome with four entities, the chromosome divides so that each chromatid regularly receives an "original" and a "new" unit." Since these findings were at variance with those of Plaut and Mazia (1956) Taylor suggested "that it was entirely possible that in the experiments of Plaut and Mazia (1956) more than one division occurred between the time of incorporation and the time of anaphase and telophase nuclei analyzed." Taylor (1963) further commented that "at least a clear result in a cell with a few chromosomes in their (Plaut and Mazia) autoradiographs could have distinguished between a conservative scheme versus a semi-conservative or dispersive replication, and so their results did not provide a clear answer, although they were interpreted by the authors to favor a conservative scheme."

LaCour and Pelc (1958) repeated the experiments of Taylor et al. (1957), with and without colchicine. They conducted three experiments: 1) Vicia faba seedlings were grown in a nutrient solution containing tritiated thymidine for 12 hours. The roots were fixed after 24 and 48 hours, 2) seedlings were treated with a 0.05% colchicine solution for 1 hour and transferred to a nutrient solution containing tritiated thymidine for 8 hours and finally fixed after 20 and 24 hours, and 3) a similar experiment to that of Taylor, et al. (1957) described earlier in this thesis. In the first experiment, in which the root tips were fixed after 24 hours, it was found that both chromatids of all chromosomes were labelled, and in those roots fixed after 48 hours, a duration estimated to be two cell cycles, 12% of the chromosomes were labelled in single chromatids and the remaining 88%, were labelled in both chromatids. In the second experiment after fixing at both 20 and 24 hours some chromosomes were labelled along the length of both chromatids and some only along the length of one. Very few C-metaphases showed all the 12 chromosomes with labelling in both chromatids. In the third experiment, the results were similar to those found in the second. These observations conflicted with those of Taylor et al. (1957) and the investigators concluded that "the results obtained by Taylor et al. (1957) were due largely to the extended treatment with colchicine and specially to the fact that the second duplication of the chromosomes took place in the presence of this agent. It is justifiable to assume that colchicine probably influences the segregation of new and old strands, possibly by an effect on the centromere." A multistranded chromosome model was proposed with a conservative mode of duplication.

According to which each chromosome consists of four strands before duplication, and after replication each chromatid is composed of 2 new strands and 2 old strands. In that case, the appearance of equally labelled sister chromatids would be expected in the first and in the second divisions. The experiments of LaCour and Pelc were not sufficiently conclusive to prove that the 88% of the chromosomes observed in the roots fixed after 48 hours, showing that both the chromatids labelled had undergone two divisions; however, it is reasonable to assume that not all of the 88% involved only one division in 48 hours, a duration equivalent to two cell cycles.

Woods and Schairer (1959) replied to the study of LaCour and Pelc (1958) by performing two experiments. 1) They grew a Vicia faba seedling in a nutrient solution containing tritiated thymidine, and colchicine at a concentration of 0.03% for 12 hours and two root tips were fixed. 2) Another Vicia faba seedling was grown in a nutrient solution containing only tritiated thymidine for 8 hours, and the seedling was then transferred to a non-radioactive nutrient solution containing colchicine for 6 hours, and finally 2 root tips were fixed. Autoradiographs were made from both the experiments. Grain counts on 50 pairs of sister chromatids were made from each experiment and it was found that there was no statistically significant difference between the two experiments. It was concluded that colchicine had no effect on DNA replication, and that LaCour and Pelc's (1958) findings were the result of poor squashing technique and consequently faulty contact of the chromosomes with the autoradiographic emulsion.

LaCour and Pelc (1959) answered the criticism of Woods and Schairer (1959) by explaining that they were also aware of the short range of beta particles emitted by tritium and that they took care to avoid the doubtful cases. They pointed out that Woods and Schairer's experiments deviated from their own experiments in the following three ways: 1) Woods and Schairer did not pretreat the plants with colchicine, 2) the colchicine concentration used by Woods and Schairer was also different from that used by LaCour and Pelc. Further, LaCour and Pelc examined the statistical analysis of Woods and Schairer's grain counts of the sister chromatids in the first division and found that the X^2 tests held good for any ratio between 1:1 to 1.75:1. In addition, Woods and Schairer took grain counts for only 50 chromosomes which is less than the complements of 5 complete nuclei. LaCour and Pelc concluded that Woods and Schairer's (1959) results were "neither sufficient nor accurate to detect a change in colchicine or to contradict the previous results by Plaut and Mazia (1956)."

Firket and Verly (1958) labelled chick fibroblast cells with tritiated thymidine and observed that the newly synthesized DNA was equally distributed to the daughter nuclei. They concluded that the results of Plaut and Mazia (1956) might be due to use of C^{14} labelled thymidine and the resulting low resolution autoradiographs.

Plaut (1958) questioned the validity of Firket and Verly's (1958) experiments on three grounds: 1) chick fibroblast cells contain more than 60 chromosomes and it is virtually impossible to arrive at any significant quantitative conclusion with such a system, 2) the average number of grains counted per cell in these experiments was only 47, and 3) the number of cells analysed was only 19.

Sueoka (1960) grew Chlamydomonas reinhardi cells in a medium containing N^{15} for several generations and then transferred them to a medium containing N^{14}. Samples of the cultures were collected after 0, 2, 4, 6, 8, and 10 hours after the transfer, and the distribution of N^{15} label in the DNA was examined by determining the density distribution of the DNA, using the equilibrium density gradient centrifugation technique. Ultraviolet absorption pictures of the DNA bands resulting from the density gradient centrifugation were taken for all the samples. Sueoka's zero time picture showed only one band of N^{15} labelled heavy DNA, his 2 hours picture showed two bands, one representing heavy DNA, and the other representing N^{14} and N^{15} hybrid DNA with intermediate density; and his 8 hours picture showed no heavy DNA band, but showed 2 bands, one of the hybrid DNA, and the other of N^{14} light DNA. Thus, the pictures showed a transition from heavy DNA to intermediate, then to light, indicating a semi-conservative mode of duplication of DNA at a molecular level.

Djordjevic and Szybalski (1960) incorporated 5-bromodeoxyuridine (BUDR) into the DNA of a single cell isolate derived from human sternal marrow and studied the mode of distribution of BUDR in the daughter cells using the density gradient technique. In one experiment, cultures were grown in a medium containing BUDR for a period of 1, 24, 48 and 96 hours, and in another experiment the cultures were grown in BUDR for several generations and then transferred to BUDR free medium containing thymidine, for 13, 24, 65, and 112 hours. The densities were calculated for all samples in both the experiments. It was noticed in both the experiments that DNA banded in 3 layers. When correlated

with the rates of division, the data of the first experiment indicated that during the first DNA duplication BUDR was incorporated into only one strand of each newly synthesized DNA and thus the molecules of intermediate density completely replaced the light parental molecules. In the subsequent replications the heavy DNA molecules appeared with the incorporation of BUDR in both the strands of DNA. In the second experiment the parental, heavy molecules appeared to be replaced by the molecules of intermediate density in the first division, and in the subsequent replications the molecules of intermediate density appeared to be replaced by the light molecules.

To obtain a greater density difference between unlabelled DNA and labelled DNA Simon (1961) labelled the DNA of HeLa cells with 5-bromodeoxyuridine. Samples of the HeLa cells were collected after one replication, and after two replications of DNA in 5-bromodeoxyuridine and the densities were calculated. After one duplication of DNA it was noticed that there was only one band, indicating that all the DNA was half labelled, and after two replications there were two bands, one representing the half labelled DNA, and the other representing the fully labelled DNA. It was concluded that these results were consistent with those of Sueoka (1960) showing a semi-conservative mechanism of DNA replication in HeLa cells.

Prescott and Bender (1963) labelled the chromosomes of several hamster cell cultures and human leucocyte cultures with tritiated thymidine for 30 minutes and fixed them after varying lengths of time. In all the experiments the cultures were treated with colchicine for the last 8-12 hours. They observed that the newly synthesized DNA

in both the hamster cells and the leucocytes was equally distributed to the daughter chromosomes in the first division, and that only one of the sister chromatids was labelled in all the chromosomes of the second division. One of the main purposes of their study was to help to resolve the conflicting reports of LaCour and Pelc (1958), and Plaut and Mazia (1956) with those of Taylor et al. (1957), but the system selected by Prescott and Bender (1963) was entirely different from that of the other studies involved. Prescott and Bender also used colchicine which had previously been shown to effect the segregation of the newly synthesized DNA (LaCour and Pelc, 1958).

Peacock (1963) designed experiments with Vicia faba to discover the mode of duplication of chromosomes as well as the effect of colchicine on the segregation of the newly synthesized DNA as seen in the daughter chromosomes. He found in the first division that both chromatids of all the chromosomes were labelled and in the second division two kinds of labelling occurred: 1) chromosomes showing labelling in only one chromatid and 2) chromosomes labelled in both chromatids. The former occurred at a frequency of 67-68% and the latter with a frequency of 14-33%. Peacock proposed a polyneme chromosome model, in which each chromosome consists of more than one DNA double helix, with each double helix duplicating semi-conservatively and segregating independently, so that two kinds of labelling may be observed in the second division, i.e. one in which both chromatids appear labelled and the other in which only one chromatid of a pair is labelled.

Most of the information concerning the mode of chromosome duplication has been derived from mitotic chromosomes. The investigators present 3 conflicting conclusions: 1) the chromosome is unistranded and it duplicates semi-conservatively (Taylor 1963). 2) the chromosome is multistranded and each strand duplicates semi-conservatively and segregates independently to the daughter chromosomes (Peacock 1963). 3) the chromosome is multistranded and each strand duplicates conservatively and segregates independently (Mazia 1961). Thus it is difficult to come to a definite conclusion concerning the mode of chromosome duplication in somatic cells.

So far little information is available regarding the mode of chromosome duplication during meiosis. Although several workers (Swift, 1950; Sparrow, 1952; Taylor, 1953; Mitra, 1956) have estimated the time of DNA duplication in the pollen mother cells, using various techniques in various organisms, little attempt was made to discover the mode of chromosome duplication during meiosis. The lack of information regarding chromosome duplication at meiosis may be due to the want of suitable techniques, or to the assumption that meiotic chromosomes should also duplicate semi-conservatively to follow the pattern of duplication of the mitotic chromosomes proposed by several workers (Taylor, 1957; Sueoka, 1960; Peacock, 1963). Some workers have suggested that the mode of chromosome duplication during meiosis might be conservative rather than semi-conservative (Pritchard, 1960; Rhoades, 1961; Bernstein, 1963). These suggestions were made largely because of the difficulty in explaining copy-choice, one of the earlier

and strongly postulated recombination mechanisms for both Protokaryots and Eukaryots (Lederberg, 1955; Lindegren, 1951; Mitchell, 1955).

This thesis concerns a study undertaken to discover both the mode of chromosome duplication during meiosis and the mode of chromosome duplication at mitosis in Haplopappus gracilis. Haplopappus gracilis has the lowest chromosome number (n=2) (Jackson, 1957) known among the flowering plants. It was expected that because of the low chromosome number involved, it would be possible to follow the distribution of the labelled chromosomes in individual nuclei of a tetrad and thereby to distinguish between a semi-conservative and a conservative mode of duplication of chromosomes during meiosis. The two pairs of chromosomes are morphologically distinct and thus it should be relatively easy to follow the individual chromosomes during the divisional stages. In somatic cells one pair has a length of 8 μ (Kamra and Kamra, 1962), with a median constriction and non-satellited and the other pair has a length of 6.5 μ with a submedian constriction and satellited.

MATERIAL AND METHODS

Haplopappus gracilis (Nutt) Gray, a member of the family Compositae was used in these studies.

The study of the mode of chromosome duplication during premeiotic interphase in pollen mother cells.

Method of growing the plants:-- The seeds were germinated in soil beds in the green house with a constant illumination of 16 hours (8 a. m. to 12 p. m.). The temperature of the greenhouse was maintained between 25°C - 27°C during the illumination period, and between 15°C - 17°C during the dark period. When the seedlings were 2 weeks old, they were transplanted to pots and grown to maturity. At the time of flowering they were transferred to a growth chamber with a constant illumination of 16H and with a constant temperature of 25°C. The estimation of the developmental stages of the florets in a capitulum was made in the following way:--Suitable sized flower buds were fixed in Navashin's solution (Darlington, 1960), dehydrated, embedded in paraffin, sectioned by a microtome and stained with Haematoxylin in the conventional way (Darlington, 1960). Transverse sections of capitulums revealed that all the outer row of disc florets, numbering 10-12 were in the same stage of development.

The time taken for the pollen mother cells to pass from the premeiotic mitotic prophase to the tetrad stage under the experimental conditions was estimated in the following way:--A minimum amount of

involucre of bracts was carefully removed from one side of a flower bud with a pair of pointed forceps, and 2 florets from the outermost row were placed on a clean glass slide, without injury to the rest of the florets, and squashed in a drop of 1% acetocarmine in 45% acetic acid. The developmental stage of the pollen mother cells was observed. With the help of a very fine, plated sewing needle one end of a wick consisting of seven strands of cotton embroidery threads was inserted through the peduncle, a few millimeters below the base of the head, and the other end of the wick was placed inside a 5 ml. glass tube containing cold thymidine (0.08 mg/ml) in distilled water with the pH adjusted to 6.8. The mouth of the test tube was brought as close as possible to the base of the flower bud without touching. As this adjustment exposed only a minimum length of wick to the air the amount of evaporation of thymidine from the wick was reduced (plate 1). After 12 hours the wick was carefully removed from the peduncle and a few drops of sterile distilled water was poured over the point of injury so that a film of water was allowed to stay over it. Immediately after this the developmental stage of the pollen mother cells was determined by making squashes of 2 outermost florets as described above. Thus, at an interval of every 12 hours the developmental stages of the pollen mother cells were observed so that the time intervals between the various stages from the premeiotic mitotic prophase to the thick walled microspore stage were estimated.

Incorporation of tritiated thymidine into the pollen mother cells at the time of premeiotic interphase was accomplished by the following method:--A suitable sized bud was selected and, as described

above, 2 outermost florets from two different sides of the bud were removed; a squash was prepared and the developmental stage of the pollen mother cells was determined. If the pollen mother cells were in premeiotic interphase, the flower was selected for treatment. Then as described before, one end of a cotton wick was inserted through the peduncle and the other end was placed inside a 5 ml glass test tube containing varying concentrations of tritiated thymidine (12.5 µc to 100 µc/ml) in sterile distilled water with the pH adjusted to 6.8. After varying durations (6 hours to 10 hours) of treatment the wick was carefully removed as previously described. The bud was left to grow for varying lengths of time (2 hours to 40 hours) after treatment, till the outer florets reached the desired stage, then it was cut, and fixed in acetic alcohol (1:3) for a minimum period of 12 hours.

Smear preparation for autoradiography:-- Fixed flowers were washed several times in distilled water for 2-3 hours, then the involucre of bracts was carefully removed, care being taken to keep the flower always moist, hydrolysed in 1N HCl for 5-6 minutes at 60°C, washed well in distilled water for 3-5 minutes, stained in 1% aceto-orcein in 45% acetic acid for 2-3 hours, and then washed well in distilled water for 5-10 minutes. The florets were separated in water in a cavity slide; 3 or 4 flowers were pooled on a clean "subbed" slide (Darlington, 1960); the ovary and the pappus hairs were removed and the rest of the florets were smeared under No. 2, 60 x 22 coverslip. The pollen mother cells were fully flattened by applying pressure over the coverslip under the thumb, dried in air for 3-5 minutes, and the

coverslip was released in 35% acetic acid. Both the slide and the coverslip were brought to distilled water through 20% acetic acid, and washed well in distilled water for 10 to 15 minutes.

Autoradiography technique:--The slides and the coverslips bearing the radioactive tissue were dipped 1 or 2 times in Eastman Kodak NTB 2 photographic emulsion melted in a water bath maintained at 40° to 45°C. The back of the slides and the cover slips were wiped clean with Kleenex tissue paper and the melted emulsion was allowed to dry by standing the slides on ends for 45 minutes to 1 hour. The coverslips and the slides were stored in separate black plastic boxes for exposure for 60-110 days. A small cloth bag containing dririte ($CaSO_4$) was placed in the box to insure dryness. After exposure the preparations were developed for 2 1/2 to 3 minutes in Eastman Kodak Dektol developer, rinsed in tap water and fixed for 5-6 minutes in acid fixer, and then washed in running water for 10-15 minutes, and finally dehydrated in an ethyl alcohol series and mounted in Euparal. The developing rinsing and fixing solutions were maintained at 18° - 20°C.

The study of the mode of chromosome duplication during mitotic interphase in root tip cells

Method of germination of seeds:--The seeds were placed directly on a glass petri dish and covered with two number one filter papers soaked well with buffered tap water. (To 1,000 cc of tap water 15 cc of 0.05 molar phosphate buffer at pH^7 was added.) The seeds were left for germination in an incubator with a constant illumination of 18 hours (8 a. m. - 2 a. m.) at 20°C. The first two leaves emerged from the seed coat on the third or fourth day.

Method of treatment:--After the two cotyledonous leaves unfolded, the seedlings were carefully picked up with a pair of pointed forceps and placed in a 10 ml beaker containing tritiated thymidine at a concentration of 1 µc/ml in buffered tap water. The seedlings floated in the solution. After varying lengths of treatment, the seedlings were removed from the radioactive thymidine solution and washed well in buffered tap water and left in a 10 ml beaker containing buffered tap water for varying lengths of time in the incubator.

Staining and squashing method for autoradiography:--Root tips were fixed in acetic alcohol (1:2) for a minimum period of 12 hours, washed well in distilled water for 10-15 minutes, hydrolysed in 1N HCl for 5-6 minutes at 60°C, washed well in distilled water for 3-5 minutes, stained in 1% aceto-orcein in 45% acetic acid for 2-3 hours, washed in distilled water for 5-6 minutes, differentiated in 45% acetic acid at 60°C for 1-1 1/2 minutes, and washed with distilled

water. The meristematic regions of three or four root tips were cut and pooled on a clean subbed slide, squashed in a drop of 45% acetic acid under a number two, 22 x 60 mm coverslip. The coverslip was released in 35% acetic acid, and the slide and the coverslip were passed through 20% acetic acid to distilled water and washed well for 15-20 minutes.

Autoradiography technique:--Similar to the technique adopted for meiosis preparations.

The estimation of the minimum duration of one mitotic division:--It is known that colchicine, by inhibiting the spindle formation, prevents the anaphase movement and the formation of the daughter nuclei. But colchicine does not prevent the duplication of the chromosomes. Making use of this property of colchicine, 3-5 days old seedlings were treated with 0.03% colchicine for 1/2 hour, washed well and transferred to buffered tap water, after which root tips were fixed in acetic alcohol at hourly intervals. In each experiment 12 root tips were squashed in order to detect the appearance of the tetraploid chromosome sets in early metaphase stages. More than 50 dividing cells were observed in each root tip. None of the slides showed tetraploid cells before 8.5 hours from the start of the experiment. Thus the minimum time required for the cells to pass from one metaphase to another metaphase was 8.5 hours. All experiments were run at a similar time of the day. By using tritiated thymidine and autoradiography techniques, the mitotic cycle time was estimated to be 12-14 hours. (Sparvoli, Unpublished).

The estimation of the minimum time required by the root tip cells to pass from interphase to anaphase:--3-5 day old seedlings were treated with tritiated thymidine for 1/2 hour, washed well and placed in buffered tap water. Every one hour after the treatment, root tips were fixed up to 15 hours. Autoradiographs were made to detect the incorporation of the label into the chromosomes. It was noticed that the labelled chromosomes began to appear in prophase 3 hours after the beginning of the treatment with labelling solution, and in anaphase 4 hours after the beginning of the treatment.

Estimation of the minimum time required to label all the chromosomes fully:--3-5 day old seedlings were treated with tritiated thymidine for 1/2, 1, 1-1/2, 2, 2-1/2, 3 and 3-1/2 hours, washed well and transferred to buffered tap water and fixed four hours after they were removed from the labelling solution. The autoradiographs of the samples treated for one hour and more showed four fully labelled chromosomes.

The method of investigating the mode of chromosome duplication during mitosis:--1) 3-5 day old seedlings were treated with tritiated thymidine solution with a concentration of 1 μc/ml for 3 hours, washed well and transferred to buffered tap water for 3-5 hours and fixed for autoradiography. 2) Seedlings were treated with tritiated thymidine for 7-9 hours, washed and fixed. 3) Seedlings were treated with tritiated thymidine solution for 28 hours, washed and fixed. 4) Seedlings were treated in tritiated thymidine for 28 hours and were then transferred to fresh tritiated thymidine for another 28 hours and fixed for autoradiography.

In order to determine the effectiveness of the tritiated thymidine remaining in the solution, a second set of seedlings was placed in the same tritiated thymidine solution from which the seedlings completing a 56 hour treatment had been removed. The second set of seedlings were fixed after an hour's treatment. The autoradiographs prepared from the root tips of the second set of seedlings showed a heavy amount of labelling in the nuclei.

RESULTS

Meiotic chromosome studies

The time interval between various stages from premeiotic mitosis to the formation of thick walled microspores is provided in Table I.

Good labelling of meiotic chromosomes was obtained from the various concentrations of tritiated thymidine (12.5 µc, 15 µc, 25 µc, 50 µc, 75 µc and 100 µc per ml). The autoradiographs prepared with the 3 lower concentrations required an exposure time of 90-130 days and the autoradiographs prepared with the higher concentrations required 50 to 75 days exposure. Tetrad and diad data were collected from the autoradiographs prepared from the concentrations of 50 µc and 75 µc/ml. The degree of labelling of diad and tetrad nuclei was estimated by counting the number of grains over these nuclei, and subtracting the background count for the appropriate areas. The background count was estimated by counting the number of grains over an unit area at 10 different places chosen randomly from each slide, and dividing the total number of grains by ten. The background count was low and it did not exceed 3 grain counts either for a diad or a tetrad nucleus. Table II and Plate II (E-H), provide information regarding the number of grains over pairs of diad nuclei.

Labelling of prophase and metaphase chromosomes of meiosis I was observed in flower buds that were fixed from 24-28 hours after the initial introduction of tritiated thymidine. Flower buds fixed from

28-32 hours after the initial treatment were used for studying the labelling of diad nuclei. Plate II (A-D) shows the labelled prophase and metaphase chromosomes.

Nuclei of tetrads were grouped in pairs according to the counts of silver grains reflecting the degree of labelling, so that the two pairs of a tetrad would have counts approaching a 1:1 ratio. It is reasonable to assume that the members of a pair of nuclei thus arranged are derived from a single nucleus of a diad. Using this procedure in no case did one pair of nuclei differ significantly in grain counts from the other pair of nuclei in any one tetrad. On this basis the tetrads were classified into three groups: 1) those in which both nuclei of each pair showed a 1:1 ratio; 2) those in which the members of one pair of nuclei differ significantly from each other; and 3) those in which members of both pairs of nuclei differ significantly from each other. The heterogenivity of the labelling of the tetrad nuclei was further checked by subjecting the grain counts of the 4 nuclei of each tetrad to a X^2 test for a 1:1:1:1 ratio.

Table III and Plates III and IV provide information concerning the distribution of grains over the tetrad nuclei. Table IV was constructed from the data provided in Table III. The mean value for the total number of grains over a tetrad was calculated for every slide and the distribution of the equally labelled and unequally labelled tetrads about the mean value was tabulated.

TABLE I THE TIME INTERVAL BETWEEN VARIOUS DEVELOPMENTAL STAGES FROM PREMEIOTIC MITOTIS TO THE FORMATION OF THICK WALLED MICROSPORES

Experiment No.	At 0 Hour	After 12 Hours	After 24 Hours	After 36 Hours	After 48 Hours	After 60 Hours	After 72 Hours	After 84 Hours
1	premeiotic mitotic prophase & metaphase	premeiotic interphase	premeiotic interphase	premeiotic interphase	meiotic late prophase	intact tetrads	Free microspores	Thick walled microspores
2	premeiotic mitotic meta and anaphase	"	"	"	meiotic I meta and I anaphase	intact tetrad and free microspores	"	"
3	premeiotic mitotic inter and prophase	"	"	"	meiotic early prophase	II ana and II telophase	"	"
4	premeiotic interphase	"	early prophase	II ana and II telophase	tetrad	Free microspores	Thick walled microspores	
5	"	"	late prophase	"	"	"	"	
6	premeiotic mitotic prophase	"	premeiotic interphase	premeiotic interphase	early prophase	intact tetrad	Free microspores	Thick walled microspores
7	premeiotic interphase	"	early prophase	I ana and II anaphase	tetrad	Free microspores	Thick walled microspores	
8	"	"	"	"	"	"	"	"
9	"	"	late prophase	II telophase and tetrad	intact tetrad and free microspores	"	"	
10	"	"	early prophase	I ana and II anaphase	tetrad	"	"	

TABLE II DISTRIBUTION OF GRAINS OVER THE PAIR OF DIAD NUCLEI

No.	Number of Grains on a Diad		X^2 Value
	1st nucleus	2nd nucleus	
1	25	29	0.29
2	24	29	0.47
3	12	14	0.15
4	11	13	0.16
5	36	30	0.54
6	26	32	0.62
7	28	35	0.76
8	33	24	1.42
9	26	23	0.18
10	11	8	0.46
11	20	15	0.70
12	15	18	0.26
13	15	11	0.61
14	11	13	0.16
15	21	17	0.42
16	11	8	0.46
17	15	17	0.12
18	10	13	0.38
19	14	16	0.13
20	34	28	0.58
21	15	12	0.32
22	22	18	0.40
23	28	34	0.58
24	27	17	2.27
25	15	12	0.32
26	22	23	0.02
27	54	48	0.35
28	20	18	0.10
29	28	22	0.72
30	22	24	0.08

TABLE II CONTINUED

No.	Number of Grains on a Diad		X^2 Value
	1st nucleus	2nd nucleus	
31	25	23	0.08
32	24	19	0.58
33	17	19	0.11
34	13	16	0.31
35	13	8	1.19
36	20	18	0.10
37	33	35	0.06
38	15	17	0.12
39	30	28	0.07
40	20	18	0.10
41	29	42	2.38
42	30	42	2.00
43	36	40	0.21
44	28	32	0.27
45	34	26	1.07
46	13	18	0.84
47	58	58	0
48	62	75	0.62
49	73	82	0.26
50	50	45	0.13
51	31	27	0.14
52	22	23	0.04
53	21	31	0.96
54	78	72	0.12
55	55	51	0.07
56	44	58	0.96
		Total X^2	26.82

D. F. = 55

p > .98

None of any two nuclei of a diad show a significant difference in grain counts.

TABLE III DISTRIBUTION OF GRAINS OVER THE NUCLEI OF TETRADS

Treatment and slide number	Tetrad number	Number of grains on a tetrad				Total No. of grains on a tetrad	X^2 value for 1:1:1:1 ratio	X^2 value for 1st pair	X^2 value for 2nd pair	Number of pairs of nuclei differ significantly
		No. of grains on 1st pair		No. of grains on 2nd pair						
		1st on nucleus	2nd on nucleus	1st on nucleus	2nd on nucleus					
50 µc/ml	1	25	10	16	18	69	6.64	6.43	0.12	one
Slide No.	2	9	30	17	22	78	11.90	11.31	0.64	one
1	3	7	22	20	14	63	8.70	7.76	1.06	one
	4	20	6	9	17	52	10.00	7.54	1.69	one
	5	18	41	19	34	112	13.75	8.97	4.25	two
	6	41	15	35	28	119	12.58	12.07	7.78	two
	7	39	39	48	21	147	11.02	0	10.57	one
	8	27	14	17	24	82	5.32	4.12	1.20	one
	9	26	26	24	24	100	0.16	0	0	
	10	26	32	28	24	110	1.28	0.62	0.31	
	11	26	32	28	30	116	0.69	0.62	0.07	
	12	24	3	22	10	59	20.30	16.33	3.43	one
	13	15	30	24	9	78	13.40	5.00	6.82	two
	14	46	18	24	28	116	14.99	12.25	0.31	one
	15	29	7	11	29	76	21.45	13.44	8.10	two
50 µc/ml	16	16	4	9	6	35	9.43	7.20	0.60	one
Slide No.	17	24	1	12	6	43	27.41	21.16	2.00	one
2	18	22	11	18	22	73	4.39	3.67	0.40	
	19	8	8	9	10	35	0.31	0	0.05	
75 µc/ml	20	8	0	9	0	17	16.05	8.00	9.00	two
Slide No.	21	17	14	28	11	70	9.42	0.29	7.41	one
3	22	20	18	26	16	80	2.80	0.11	2.38	
	23	15	15	18	19	67	0.76	0.00	0.03	
	24	15	19	22	5	61	10.80	0.47	10.70	one
	25	16	13	26	7	62	12.19	0.31	16.03	one

TABLE III CONTINUED

Treatment and slide number	Tetrad number	Number of grains on a tetrad				Total No. of grains on a tetrad	X^2 value for 1:1:1:1 ratio	X^2 value for 1st pair	X^2 value for 2nd pair	Number of pairs of nuclei differ significantly
		No. of grains on 1st pair		No. of grains on 2nd pair						
		1st on nucleus	2nd on nucleus	1st on nucleus	2nd on nucleus					
	26	26	17	29	13	85	7.94	1.88	6.10	one
	27	27	17	26	27	97	2.91	2.27	0.02	
	28	7	17	14	10	48	4.83	4.17	0.67	one
	29	15	18	16	15	64	3.75	0.27	0.03	
	30	16	15	12	17	60	0.93	0.03	0.86	
	31	19	10	14	13	56	3.00	2.29	0.04	
	32	13	20	20	18	71	1.84	1.49	0.11	
	33	20	23	22	17	82	1.12	0.21	0.64	
	34	6	17	12	16	51	10.12	5.26	0.57	one
	35	28	37	29	33	127	1.59	0.02	0.23	
	36	31	15	27	24	97	5.72	5.57	0.18	one
	37	36	34	44	26	140	4.62	0.06	4.63	one
	38	16	18	17	17	68	1.17	0.12	0.00	
	39	23	30	29	38	120	3.76	0.92	1.21	
75 µc/ml Slide No. 4	40	27	4	12	14	53	12.58	17.06	0.15	one
	41	22	9	15	14	60	1.73	5.45	0.03	one
	42	14	13	13	21	61	2.93	0.04	1.88	
	43	22	5	20	19	66	10.96	10.70	0.03	one
	44	23	8	18	3	52	19.23	7.26	10.71	two
	45	17	34	16	44	111	19.65	10.12	12.07	two
	46	26	11	24	17	78	7.23	6.08	1.20	one
	47	17	20	16	32	85	7.82	0.24	5.33	one
	48	15	30	27	23	95	5.54	5.00	0.32	one
	49	17	14	8	32	71	17.90	0.29	6.47	one
	50	16	2	9	5	32	13.75	10.89	1.14	one
	51	38	14	34	17	103	16.80	11.08	5.67	two

TABLE III CONTINUED

Treatment and slide number	Tetrad number	Number of grains on a tetrad				Total No. of grains on a tetrad	X^2 value for 1:1:1:1 ratio	X^2 value for 1st pair	X^2 value for 2nd pair	Number of pairs of nuclei differ significantly
		No. of grains on 1st pair		No. of grains on 2nd pair						
		1st on nucleus	2nd on nucleus	1st on nucleus	2nd on nucleus					
75 µc/ml	52	21	21	22	24	88	0.27	0.00	0.09	
Slide No.	53	24	35	30	33	122	2.26	2.05	0.05	
5	54	40	13	38	24	115	16.79	2.77	5.77	one
	55	29	35	27	45	136	5.76	2.20	4.50	one
	56	24	37	27	12	100	12.72	2.77	5.77	one
	57	11	18	16	18	63	2.07	1.69	0.12	
	58	21	2	20	2	45	35.55	15.70	14.73	two
	59	36	14	30	14	94	16.12	9.68	5.82	two
	60	18	32	27	20	97	5.05	3.92	1.04	one
75 µc/ml	61	28	5	26	10	67	22.90	16.03	7.11	two
Slide No.	62	27	16	19	19	81	2.56	2.81	0.00	
6	63	34	4	26	14	78	21.10	23.6	2.64	one
	64	33	1	21	8	63	38.20	30.0	5.82	two
	65	25	12	16	17	70	5.08	4.56	0.03	one
	66	20	12	18	10	60	4.53	1.00	2.28	
	67	32	24	28	26	110	1.28	1.14	0.07	
75 µc/ml	68	62	36	60	54	212	7.93	6.90	0.43	one
Slide No.	69	38	63	37	77	215	21.50	6.12	14.04	two
7	70	33	8	16	24	81	17.50	15.24	1.60	one
	71	68	39	59	44	210	10.02	7.86	2.18	one
	72	36	31	31	36	134	0.75	0.37	0.37	
75 µc/ml	73	30	18	22	18	88	4.36	3.00	0.40	
Slide No.	74	14	24	22	22	82	2.87	2.63	0.00	
8	75	9	9	10	3	31	3.96	0.00	3.77	

TABLE III CONTINUED

Treatment and slide number	Tetrad number	Number of grains on a tetrad				Total No. of grains on a tetrad	X^2 value for 1:1:1:1 ratio	X^2 value for 1st pair	X^2 value for 2nd pair	Number of pairs of nuclei differ significantly
		No. of grains on 1st pair		No. of grains on 2nd pair						
		1st on nucleus	2nd on nucleus	1st on nucleus	2nd on nucleus					
	76	28	13	26	10	77	12.81	5.69	7.11	two
	77	24	12	20	12	68	6.35	4.00	2.00	one
	78	48	11	38	24	121	25.92	23.70	3.77	one
	79	15	10	13	12	50	1.64	1.00	0.04	
	80	12	12	6	14	44	3.09	0.00	3.20	
	81	17	30	17	27	91	6.07	3.60	2.27	
	82	6	31	14	21	72	18.77	16.89	1.40	one
	83	10	8	4	14	36	5.77	0.11	5.56	one
	84	17	30	20	28	95	4.95	3.60	1.33	
	85	26	10	11	17	64	10.12	7.11	1.29	one
	86	7	28	23	20	78	12.35	12.60	0.12	one
	87	26	18	21	22	87	1.73	1.23	0.03	
	88	18	18	34	7	77	17.75	0.00	17.78	one
	89	12	10	9	15	46	1.82	0.18	1.50	
	90	27	4	17	8	56	13.85	17.06	3.24	one
	91	26	10	30	9	75	12.58	7.11	11.31	two
	92	34	19	22	33	108	6.43	4.25	2.20	one
	93	21	5	16	15	57	9.46	9.85	0.63	one
	94	15	54	34	42	145	22.2	16.42	1.50	one
75 µc/ml Slide No. 9	95	18	11	18	11	58	3.45	1.69	1.69	
	96	12	12	18	10	52	2.77	0.00	2.29	
	97	26	6	7	15	54	19.04	12.50	2.91	one
	98	9	9	7	15	40	3.60	0.00	2.91	
	99	24	5	14	7	50	17.68	12.48	2.33	one
	100	16	1	9	14	40	13.40	13.24	1.09	one

TABLE III CONTINUED

Treatment and slide number	Tetrad number	Number of grains on a tetrad				Total No. of grains on a tetrad	X^2 value for 1:1:1:1 ratio	X^2 value for 1st pair	X^2 value for 2nd pair	Number of pairs of nuclei differ significantly
		No. of grains on 1st pair		No. of grains on 2nd pair						
		1st on nucleus	2nd on nucleus	1st on nucleus	2nd on nucleus					
	101	12	6	6	8	32	3.00	2.00	0.29	
	102	18	25	17	28	88	3.90	1.14	2.69	
	103	6	14	5	21	46	16.38	3.20	9.87	one
75 µc/ml	104	11	22	14	8	55	7.82	3.67	1.64	
Slide No.	105	10	14	13	14	51	1.83	0.67	0.04	
10	106	9	11	13	12	45	0.70	0.20	0.04	
	107	23	7	22	13	65	10.75	8.53	2.31	one

	Tetrads
Both nuclei of each pair showed 1:1 ratio	41
Members of one pair of nuclei differ significantly	51
Members of both the pair of nuclei differ significantly	15
Total number of tetrads studied	107
Number of tetrads differ significantly from 1:1:1:1 ratio	53

TABLE IV THE DISTRIBUTION OF EQUALLY AND UNEQUALLY LABELLED TETRADS ABOUT THE MEAN VALUE FOR THE TOTAL NUMBER OF GRAINS OVER THE TETRAD NUCLEI

Slide No.	Mean - more than one standard deviation		Mean ± one standard deviation		Mean + more than one standard deviation	
	No. of equally labelled tetrads	No. of unequally labelled tetrads	No. of equally labelled tetrads	No. of unequally labelled tetrads	No. of equally labelled tetrads	No. of unequally labelled tetrads
1	0	3	4	4	1	3
2	1	0	0	2	1	0
3	0	1	11	5	3	0
4	0	1	3	5	1	2
5	2	1	1	2	2	1
6	1	0	2	3	1	0
7	0	1	1	3	0	0
8	3	0	8	8	1	2
9	2	1	2	3	1	0
10	1	0	1	1	0	1
	10	8	33	36	11	9

Slide No.	Mean - more than 1/2 standard deviation		Mean ± 1/2 standard deviation		Mean + more than 1/2 standard deviation	
	No. of equally labelled tetrads	No. of unequally labelled tetrads	No. of equally labelled tetrads	No. of unequally labelled tetrads	No. of equally labelled tetrads	No. of unequally labelled tetrads
1	1	5	2	1	2	4
2	1	1	0	1	1	0
3	3	3	6	3	5	0
4	1	3	2	3	1	2
5	1	1	2	2	2	1
6	1	2	2	1	1	0
7	1	1	0	0	0	3
8	5	2	4	6	3	2
9	2	1	2	3	1	0
10	2	0	0	1	0	1
	18	19	20	21	16	13

Results of the mitotic chromosome studies:--Good grain counts were obtained from the autoradiographs exposed for 5-6 days. Data have been collected from all the four experiments where the anaphase chromosomes were sufficiently separated to distinguish the individual chromosomes. All such anaphases were recorded. Table V and Plates V and VI provide information regarding the number of labelled anaphase chromosomes in all the four experiments. The results of the experiments 1 and 2 were pooled together because in both the experiments the duration of the experiments was less than one divisional cycle.

TABLE V NUMBER OF CHROMATIDS LABELLED IN ANAPHASES

Experiments	Number of cells with 8 chromatids labelled	Number of cells with 7 chromatids labelled	Number of cells with 6 chromatids labelled	Number of cells with 5 chromatids labelled	Number of cells with 4 chromatids labelled	Total number of cells studied
1 and 2	3	2	2	2	33	42
3	6	6	8	5	10	35
4	5	4	5	4	2	20

PLATE I

Method of incorporation of tritiated thymidine into the pollen mother cells through the peduncle by a cotton wick.

PLATE I

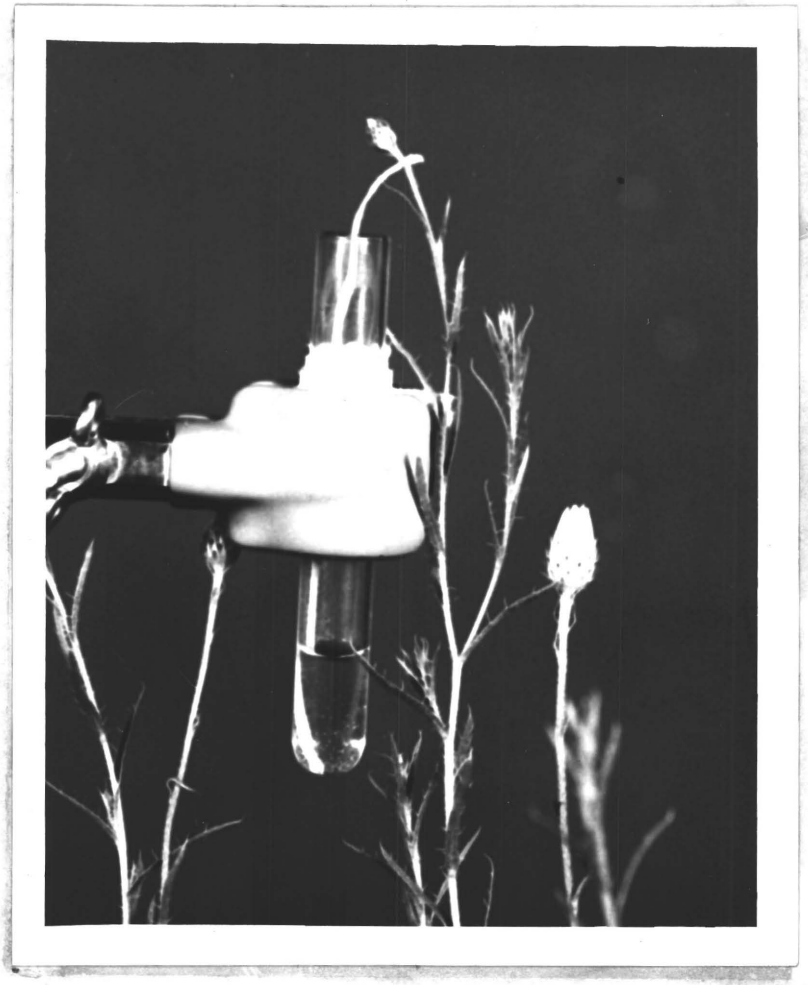


PLATE II

Autoradiographs of the pollen mother cells.

A-B - one pollen mother cell at Prophase I

C-D - one pollen mother cell at Metaphase I

E-H - 2 diads

A, C, E, G - Focal level of chromosomes

B, D, F, H - Focal level of grains

PLATE II

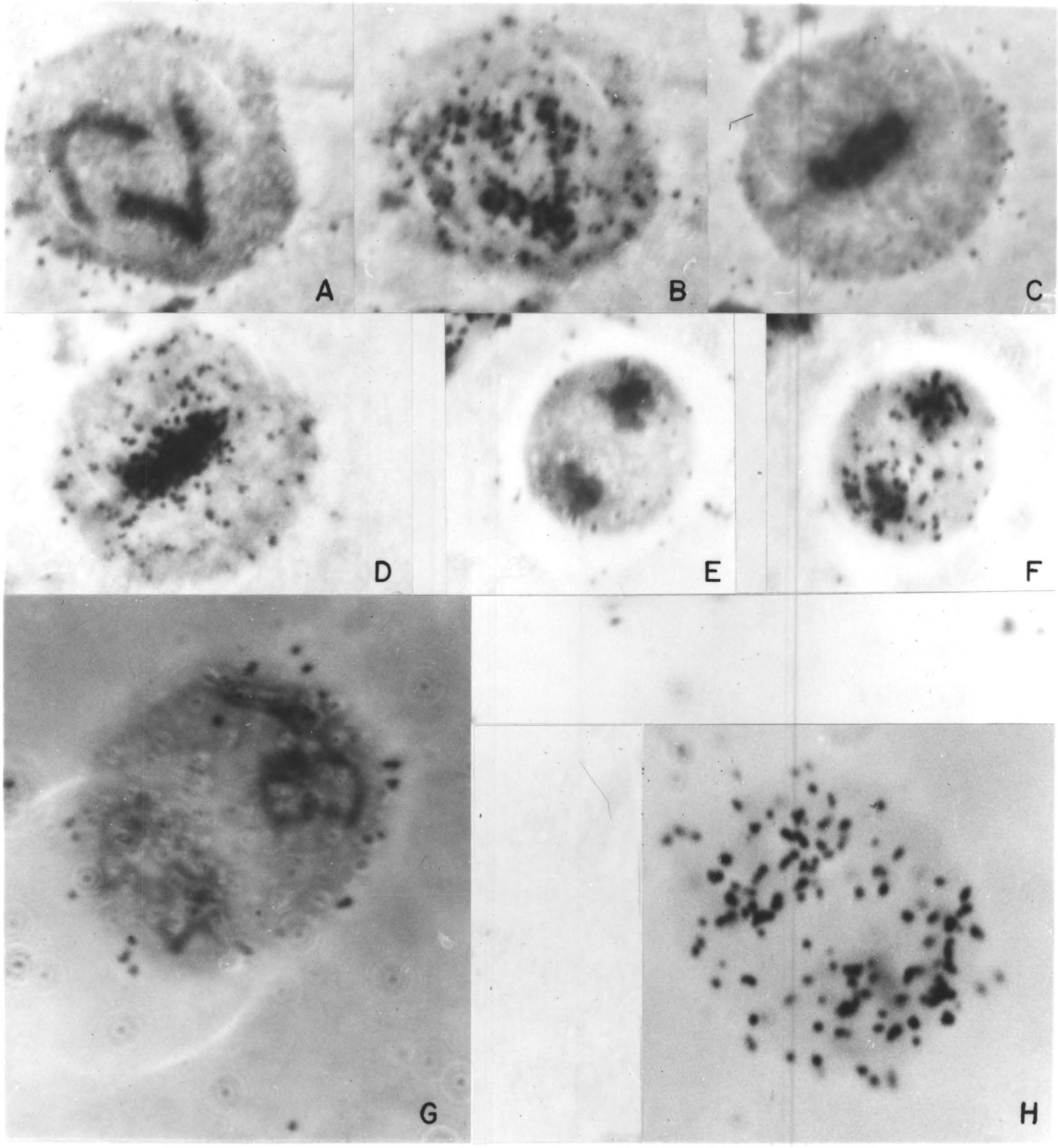


PLATE III

Autoradiographs of the tetrad

A-B - a tetrad showing all the four nuclei equally labelled

C-J - 4 tetrads showing, members of one pair of nuclei are equally labelled, while the members of the other pair are unequally labelled

K-L - a tetrad showing members of both pair of nuclei unequally labelled. (Refer to text page 24.)

A, C, E, Cr, I, L - Focal level of chromosomes

B, D, F, H, J, K - Focal level of grains

PLATE III

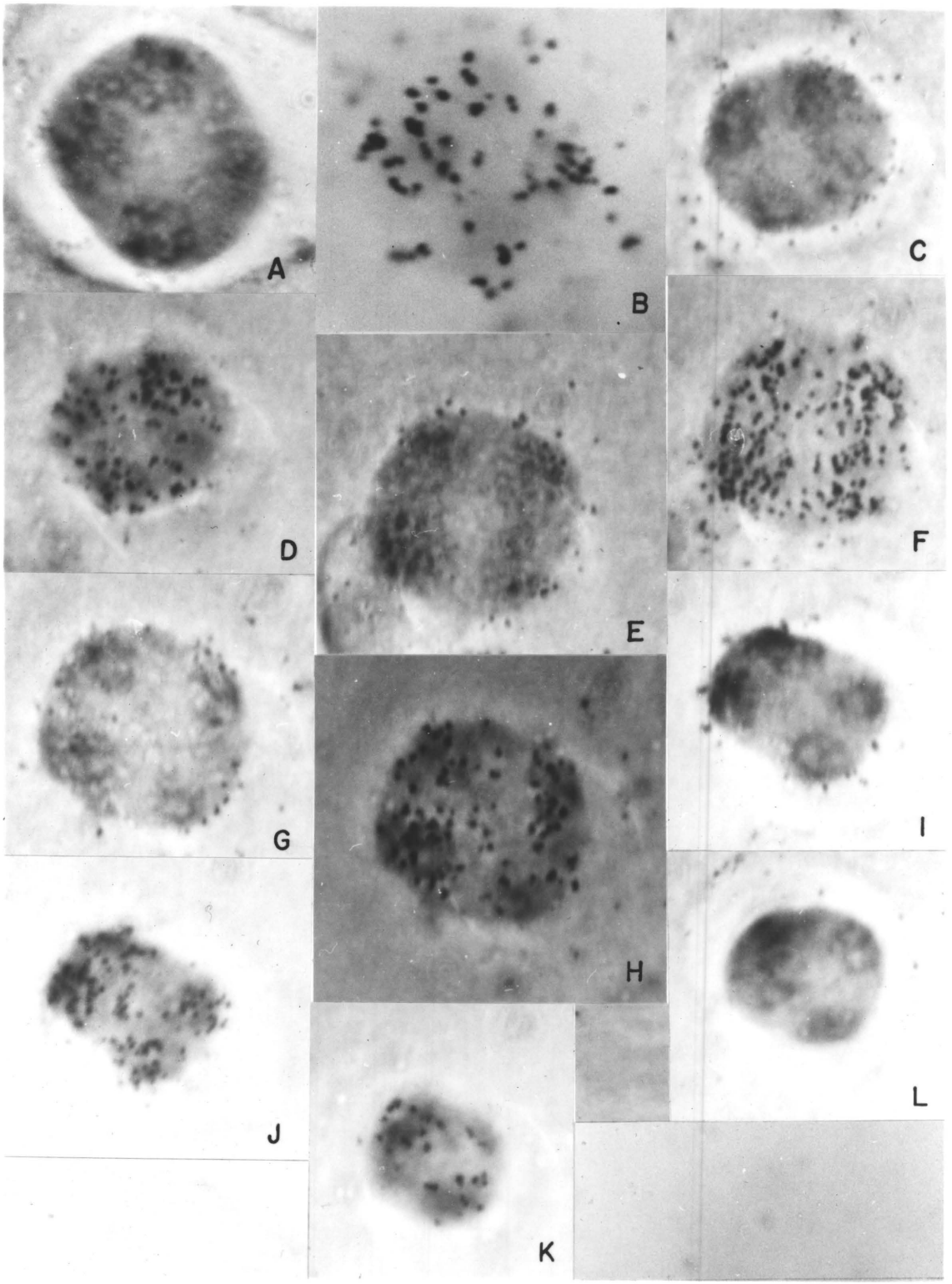


PLATE IV

Autoradiographs of the tetrads and the microspores

A-B - 2 different tetrads. The upper one shows unequal labelling in the members of both pair of nuclei and the lower one shows an unequal labelling in the members of one pair of nuclei.

C-F - Microspores of two different tetrads. Both the tetrads show an unequal labelling in the members of one pair of microspores, while the other pair is equally labelled.

(Refer to text, page 24.)

A, C, E - Focal level of nuclei

B, D, F - Focal level of grains

PLATE IV

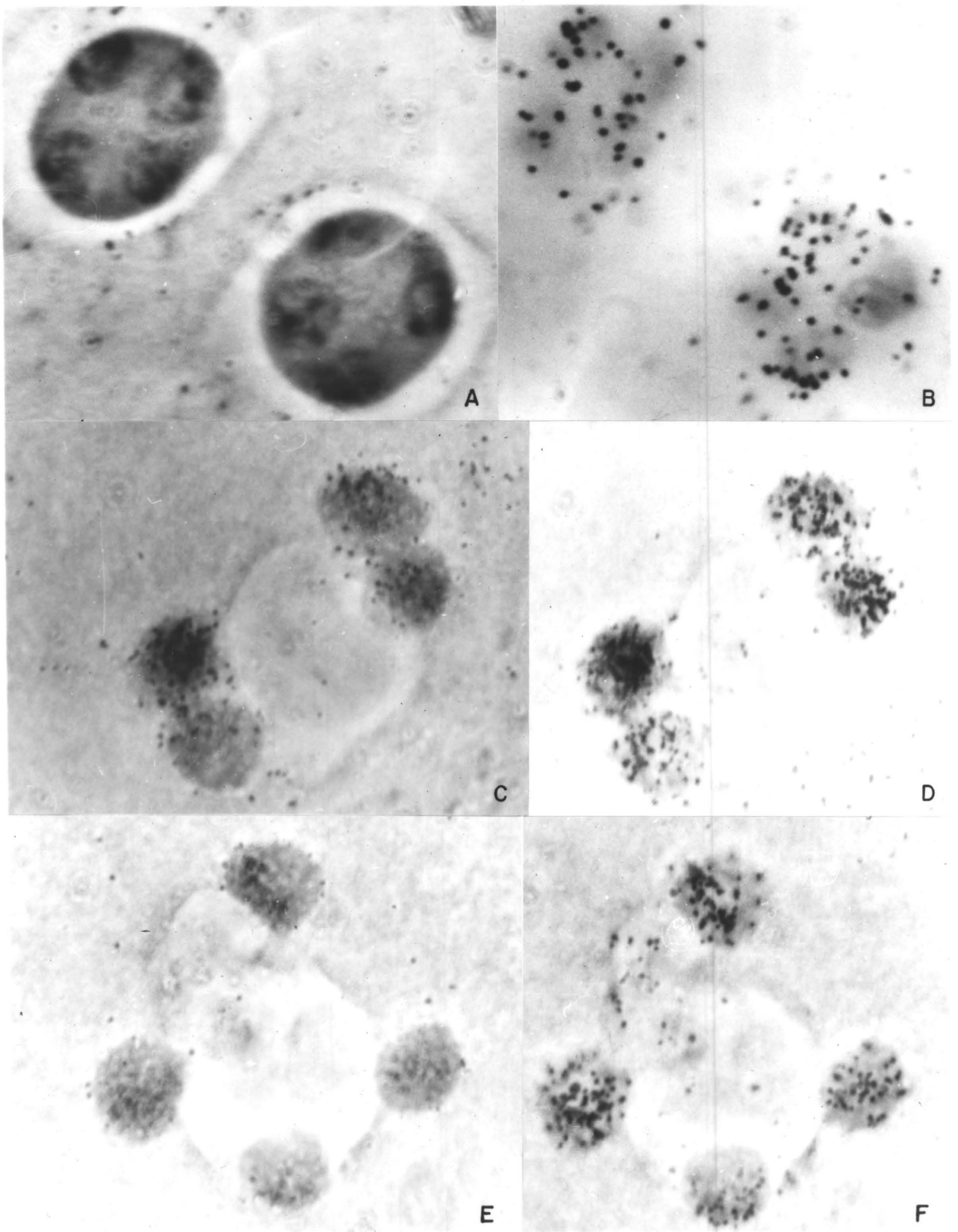


PLATE V

Autoradiographs of the root tip cells

A-F - 3 anaphases resulting after a treatment for 8 hours in tritiated thymidine

G-J - 2 anaphases resulting after a treatment for 28 hours in tritiated thymidine

A, C, E, G, I - Focal level of chromosomes

B, D, F, H, J - Focal level of grains

PLATE V

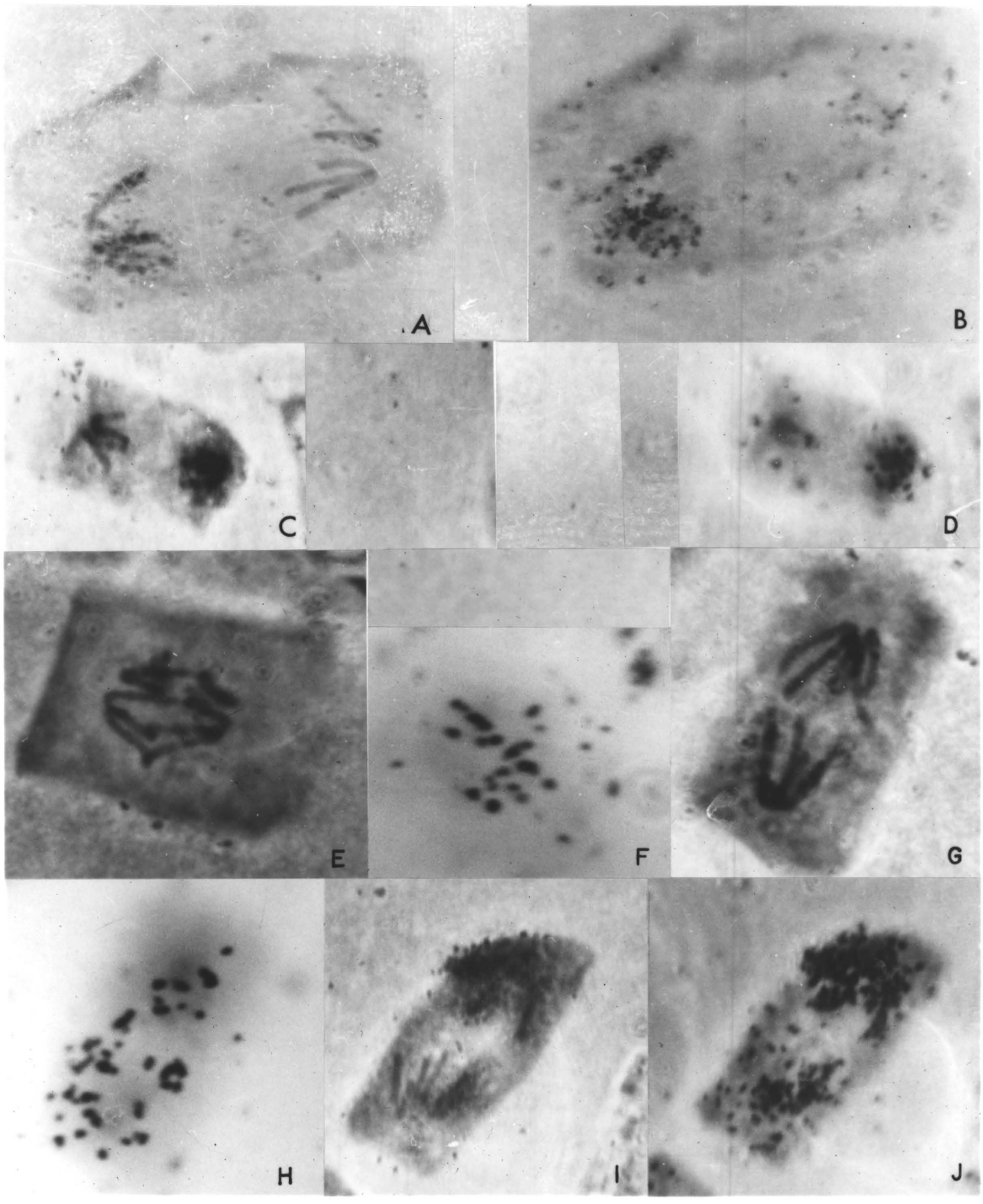


PLATE VI

Autoradiographs of the root tip cells

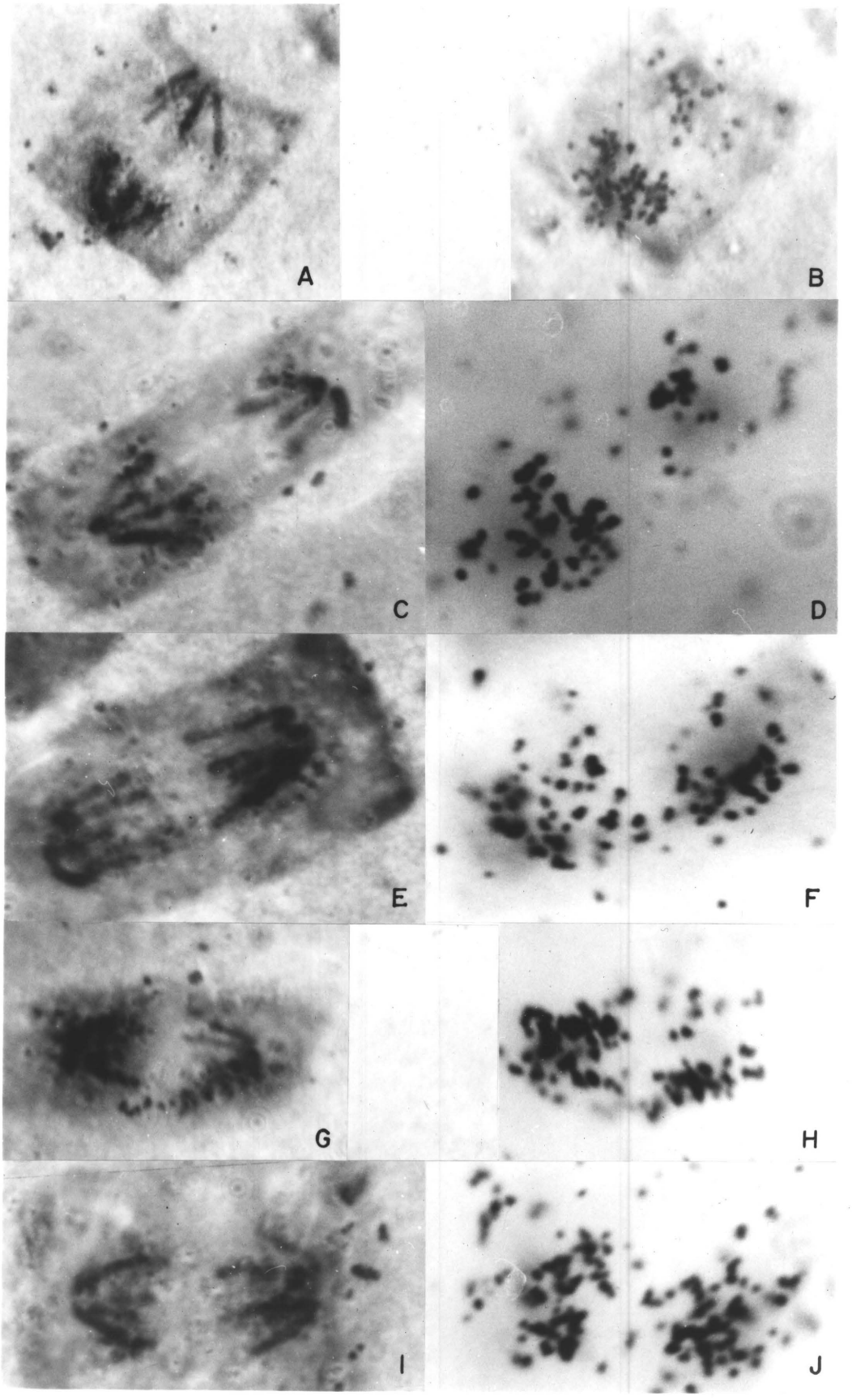
A-J - Five anaphases resulting after a treatment for 56 hours in tritiated thymidine

A-H - Note the varying number of chromatids unlabelled

I-J - All the 8 chromatids are labelled

A, C, E, G, I - Focal level of chromosomes

B, D, F, H, J - Focal level of grains


A
B
C
D
E
F
G
H
I
J

Discussion

The mode of chromosome duplication during meiosis:--Soon after its chromosome number was determined (Jackson, 1957) Haplopappus gracilis became one of the important experimental organisms in the field of biology (Jackson, 1959, 1960, 1962; Blakely and Steward 1961; Mitra and Steward, 1961; Kamra and Kamra, 1962, Kamra, 1963, Ostergreen and Frost, 1962; Mora Urpi, 1963; Marimuthu and Threlkeld, 1964). The plants take 8 to 10 weeks to flower and the flowering continues for more than 2 months with a yield of 100 to 150 flowers per plant. So a continuous production of flowers can be maintained throughout the year by raising new sets of plants at intervals of two months. The characteristic inflorescence, the capitulum, provides unique experimental advantages. As the outer florets, 10-12 in number are at the same stage of development, the estimation of the time interval between the premeiotic mitotic interphase and premeiotic interphase, and between meiotic interphase and formation of tetrads was made possible with the least amount of injury to the flowers as described earlier. The incorporation of tritiated thymidine into 10-12 florets was possible with a single operation. The success of the experiments may mainly be due to the technique by which tritiated thymidine was incorporated into the pollen mother cells. Lima-de-Faria (1962) was able to label the meiotic chromosomes of Agapanthus umbellatus with tritiated thymidine. In this species the whole inflorescence is enclosed in a big "bag" formed by a large bract. The tip of the bag was cut to form a natural cup with the flowers at the base of the cup. The cup was filled with

tritiated thymidine for incorporation into the pollen mother cells. This method can be used only for those plants which possess this special kind of bract. Taylor (1953) incorporated P^{32} into the premeiotic interphase chromosomes of Lilium longifolium by keeping the cut flower buds in a medium containing P^{32} but by this technique it is difficult to grow the buds from the premeiotic interphase to the tetrad stage. (Sparrow, et al. 1955, Vasil, 1957.) It is reasonable to assume that by making use of the technique described in this thesis it should be possible to incorporate tritiated thymidine into the pollen mother cells through the pedicels of both monocots and dicots. The other major factor that may have contributed to the success of the experiments described in this thesis was the relatively short duration of time for the pollen mother cells to pass from premeiotic interphase to the tetrad stage. Taylor (1952) found that Lilium longifolium required 5-6 days for meiosis. Lima-de-Faria (1962) estimated a duration of 12 days for the cells to go from premeiotic interphase to pachytene stage in Agapanthus umbellatus. In Haplopappus gracilis less than 48 hrs. was required for the cells to pass from the premeiotic interphase to the tetrad stage.

It has been established that DNA duplication takes place in the pollen mother cells of Lilium longipolium during interphase (Taylor, 1953) and in Tradescantia DNA duplication extends from interphase to the beginning of prophase (Taylor, 1953), and that there is no DNA duplication in the second interphase after the first meiotic division. (Taylor, 1953; Swift, 1953).

With regard to the results presented in this thesis, if the mode of chromosome duplication is semi-conservative, and the labelled chromosomes, either polyneme or unineme, are labelled during the premeiotic interphase, labelled material will be equally distributed to the four products of meiosis whether or not crossing over takes place (Fig. 1A). On the other hand, if the mode of chromosome duplication is conservative and the incorporation of the labelled material takes place at the premeiotic interphase and if no crossing over takes place then the pattern of distribution of the labelled materials in the tetrad would fall into three distinct classes. (Fig. 1B). 1) Either all of the four nuclei in a tetrad will be equally labelled, 2) among the four nuclei two of them will be equally labelled, third one would have double the amount of label as the two, leaving the fourth one unlabelled, or 3) two of the nuclei will be heavily labelled leaving the other two unlabelled. In other words, the grain distribution over the four nuclei of the tetrad will be 1:1:1:1; 2:0:1:1; or 2:0:2:0 and these classes will be in the ratio of 1:2:1. With the occurrence of cross overs these three classes tend to become less distinct. If the chromosome is multistranded and each strand, after duplication, assorts independently to the daughter chromosomes as described earlier in this thesis (Mazia 1961), then those three classes will be even less distinct.

It is possible to get an unequal distribution of the labelled material in the tetrad by a semi-conservative mode of duplication if the chromosomes were labelled at the last premeiotic mitotic interphase

(Fig. 2). In the experiments described above, this possibility was avoided by a carefully made cytological examination of the developmental stages of the two outermost florets from two different points of the flower, before the bud was treated. The buds were selected for treatment only when the pollen mother cells were in interphase; all other flowers were rejected. It is possible that along with the chromosomes at the premeiotic interphase in the outer florets the chromosomes at the premeiotic mitotic interphase in the inner floret may also be labelled and would reach the tetrad stage. But a duration of at least twenty hours was estimated for the cells to pass from the premeiotic mitotic prophase to the premeiotic interphase. Hence for the cells to pass from the premeiotic mitotic interphase to premeiotic interphase it should take twenty hours plus the hours they would take to pass from mitotic interphase to mitotic prophase. Thus the time difference between the cells of the premeiotic mitotic interphase and the premeiotic interphase was more than twenty hours. Thus it would require at least 68 (20 + 48 hours to go from premeiotic interphase to tetrad stage) hours, for the cells that were in premeiotic mitotic interphase to reach the tetrad stage (Table I). None of the experiments exceeded more than 50 hours from the start of the treatment to the fixing of the flower bud in acetic alcohol. Further, it was estimated that the cells that were in the tetrad stage developed into fully matured thick-walled microspores within 20 hours. Thus when the cells of the inner florets, that were labelled at the premeiotic mitotic interphase reached the tetrad stage, the cells of the outer florets that were labelled at the premeiotic interphase would be at the thick walled

microspores and tetrad stages. Additional evidence against the possibility of the unequally labelled tetrads reflecting incorporation of label at the premeiotic mitotic interphase, stems from the fact that such tetrads would tend to be either more lightly labelled (Fig. 2) if those cells pass through the premeiotic interphase in the absence of H^3 thymidine, or more heavily labelled, if those cells pass through the premiotic interphase in the presence of H^3 thymidine than those tetrads associated with the incorporation of label at the premeiotic interphase. An examination of the data in Table IV shows no indication that unequally labelled tetrads are also more lightly labelled or more heavily labelled. Thus the possibility that the autoradiographic data might have been obtained from the tetrads that were labelled at the premeiotic mitotic interphase chromosomes is unlikely.

The other possibility by which tetrad nuclei may be unequally labelled following a semiconservative mode of chromosome duplication, is if the two members of a homologous chromosome duplicate asynchronously. (Fig. 3A and 3B). However, as can be seen from the figures, such an occurrence would result in unequal labelling of nuclei within a diad.

The results (Table II and Plate II. E-H) give no indication of unequally labelled nuclei within a diad. Results (Table III and Plates III and IV) show a large number of nuclei within a tetrad to be unequally labelled. The simplest interpretation of these data is that the mode of chromosome replication at meiosis in Haplopappus gracilis is conservative.

The mode of chromosome duplication during mitosis:--

The conflict between the conclusions presented in this thesis, and a body of literature favouring a semi-conservative mode of chromosome duplication at mitosis, made a study of mode of chromosome duplication at mitosis in Haplopappus gracilis imperative. However, the results from the study of chromosome duplication during mitosis again support a conservative mode of replication for the chromosomes in Haplopappus gracilis. Haplopappus gracilis is well suited for the study of the distribution of labelled material in the mitotic chromosomes. The percentage of seed germination was more than 90%; the seedlings were ready for treatment from 76 hours after soaking; the mitotic index was quite high, and it was easy to make stained root tip squashes.

If it is assumed that the structure of the chromosome is single stranded, or unineme and duplication at mitosis is conservative, and if the seedlings were grown in a solution containing tritiated thymidine for i) a duration less than one complete divisional cycle, and ii) for a period of two or more than two divisional cycles, then in the first case in the labelled anaphases of the first division, only one chromatid of each chromosome will be labelled. Thus out of eight chromatids only four will show labelling and they will be distributed to the two poles in three possible ways, namely 4:0, 3:1, or 2:2. (Fig. 4). In the second case the anaphases after two or more duplications in tritiated thymidine, will show labelling in 4, 5, 6, 7, or in all of the 8 chromatids (Fig. 4).

If a semi-conservative mode of duplication with a unineme chromosome structure is assumed then both in the first and second experiments the labelled anaphases should show all the eight chromatids labelled.

In the first and second experiments, out of 42 anaphases of the first division studied, 33 showed only four chromatids labelled, (Table 5) and wherever it was possible to identify the sister chromatids in these 33 anaphases, only one of the sister chromatids appeared to be labelled. It is possible for only four to be labelled in the first anaphases with a semi-conservative mode of duplication if it is assumed that out of the two pairs of chromosomes one pair duplicated at one time and the other pair duplicated some time later (Fig. 5). In that case, the distribution of the labelled chromatids to the two poles would always be 2:2, never 3:1 or 4:0, and both the sister chromatids would be labelled in that pair of chromosomes. Such a distribution of labelling was not observed in the experiments. Further, the root tips treated for only one hour in tritiated thymidine and three hours in cold thymidine showed metaphase plates with all four chromosomes fully labelled, thus ruling out the possibility of the labelling of only four chromatids in the first anaphases, being due to duplication of chromosomes at different times, with a semi-conservative mode of duplication. Thus plate (V, A-H) shows an unequal distribution of label at the first anaphase following the treatment. Although the cell cycle time was carefully estimated it may be argued that in the absence of colchicine it is not possible to be

sure of the number of divisions that have elapsed since the termination of the treatment.

However, further evidence in support of a conservative mode of chromosome duplication in the organism under discussion is provided by the results (Plates V 1-J & VI A-J) of the experiments 3 and 4. Plates V 1-J & VI A-J are pictures of anaphase figures derived from root tips whose cells have passed through several mitotic divisions while continuously present in the labelled solution. The unlabelled daughter chromosomes are interpreted as being derived from the ancestral chromosomes present before the time of the incorporation of label. It appears that the ancestral chromosome has maintained its conservative integrity throughout a number of divisions.

In long duration (56 hours) treated materials it is possible for unlabelled chromatids with a semi-conservative mode of duplication to occur if it is assumed that all the tritiated thymidine in the solution has been completely used up in the first duplication, and tritiated thymidine was not available in the solution for the subsequent duplication or duplications. This possibility was avoided by transferring the seedlings to fresh tritiated thymidine solution after 28 hours, and further, the autoradiographs of the roots of the seedlings treated for one hour in the tritiated thymidine solution previously used for 28 hours showed heavy labelling in the nuclei.

However, the fact that 9 of the 42 anaphases of the first division showed more than 4 chromatids labelled, cannot be explained

by an unineme chromosome model. This, and the other results presented in this thesis can be very well explained by Mazia's model (1961). According to Mazia the chromosome is a multistranded structure and each strand duplicates conservatively. After the duplication, the strands, both new and old, assort independently to sister chromosomes (Fig. 6). In that case the newly synthesized material may be present either in only one of the sister chromosomes, or in both the sister chromosomes of the first and subsequent divisions. Further, the possibility of newly synthesized material being distributed to both the sister chromosomes would increase with increasing numbers of strands within the chromosome.

However with a semiconservative mode of duplication in a multistranded structure, if some of the strands duplicate before they were treated with H^3 thymidine, and the rest of the strands duplicate in H^3 thymidine then it is possible to obtain labelled and unlabelled chromatids in the first anaphase after labelling. However after the second duplication all the strands would be duplicated either once or twice in H^3 thymidine, and hence it is unlikely that one or more unlabelled chromatids would be present in the second and subsequent divisions. The results of experiments 3 and 4 where the seedlings were treated with H^3 thymidine continuously for more than 2 divisional cycles (28H and 56 hours), showed one or more unlabelled chromatids in 44 out of 55 cells (Table V). However the root tip cells of those experiments might possibily include cells that have undergone only one division, but it is unlikely that 44

out of 55 cells have undergone only one duplication in the time sequences involved. Thus the possibility of obtaining one or more unlabelled chromatids in the second and subsequent divisions with a semi-conservative mode of duplication in a multistranded structure is unlikely.

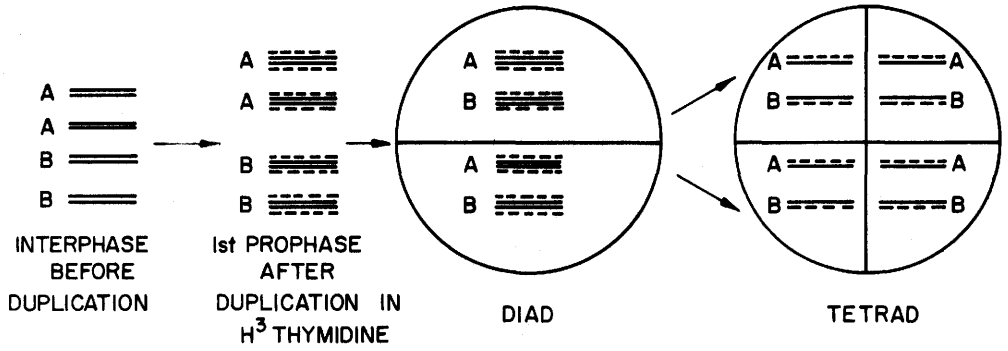


Fig. 1 A. Distribution of labelled material in the tetrad according to a semiconservative mode of duplication.

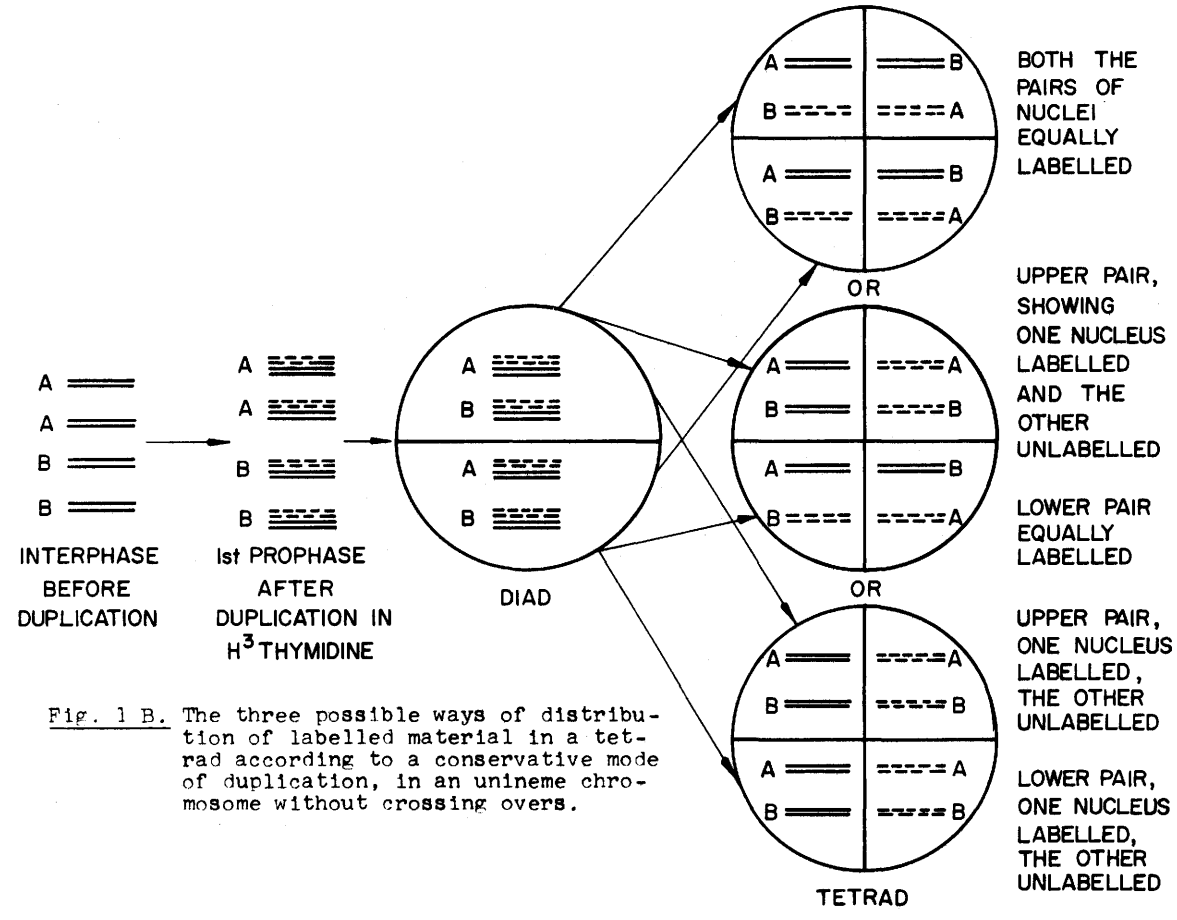


Fig. 1 B. The three possible ways of distribution of labelled material in a tetrad according to a conservative mode of duplication, in an unineme chromosome without crossing overs.

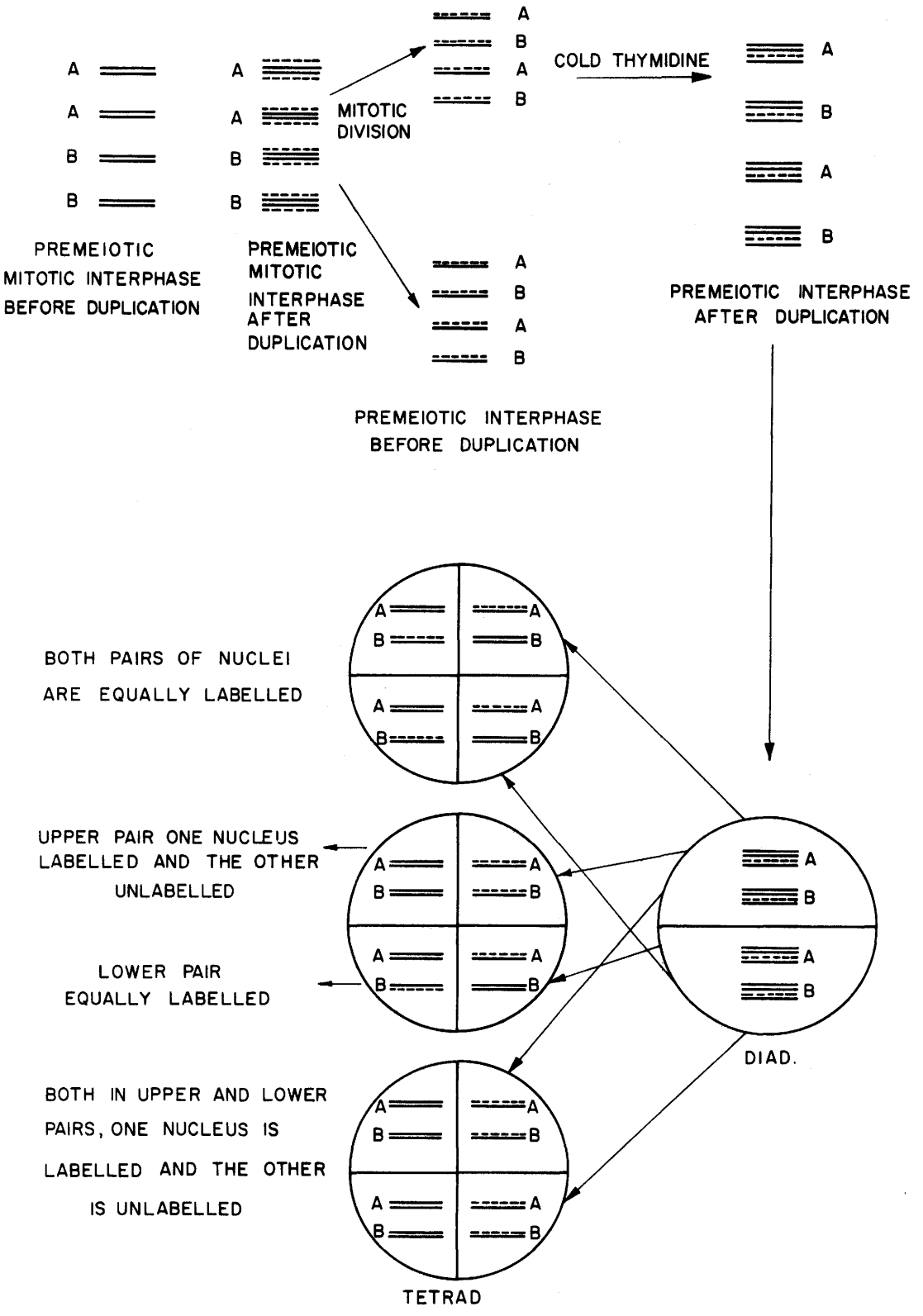


Fig. 2. The 3 possible ways of distribution of the labelled material in a tetrad if the labelling takes place in the premeiotic mitotic interphase and passes through premeiotic interphase in the absence of H^3 thymidine.

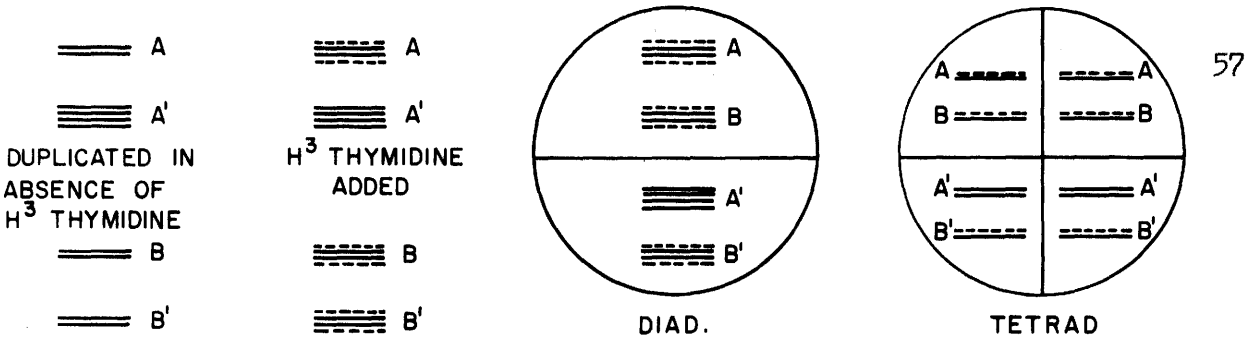


Fig. 3 A. Distribution of the labelled material in the tetrad if the mode of duplication is semi-conservative and if one pair of homologous chromosomes duplicate at different time.

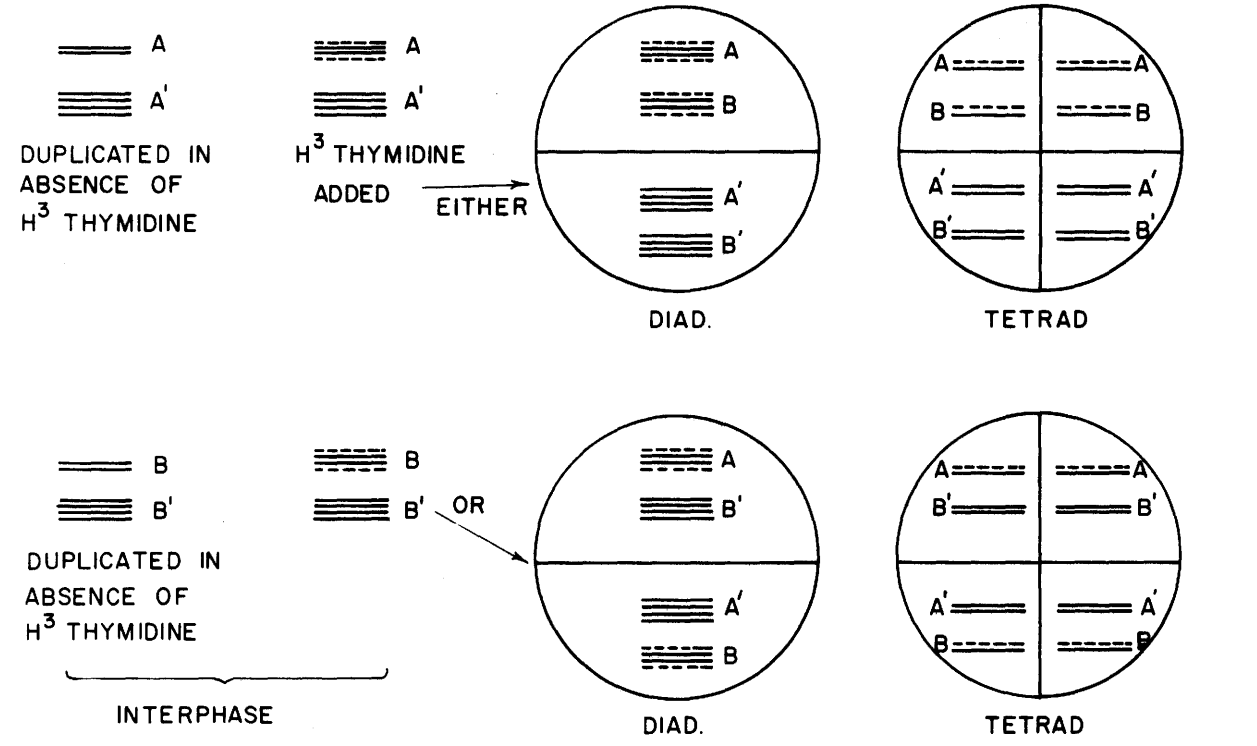


Fig. 3 B. Distribution of labelled material if the members of the homologous chromosomes duplicate at different times.

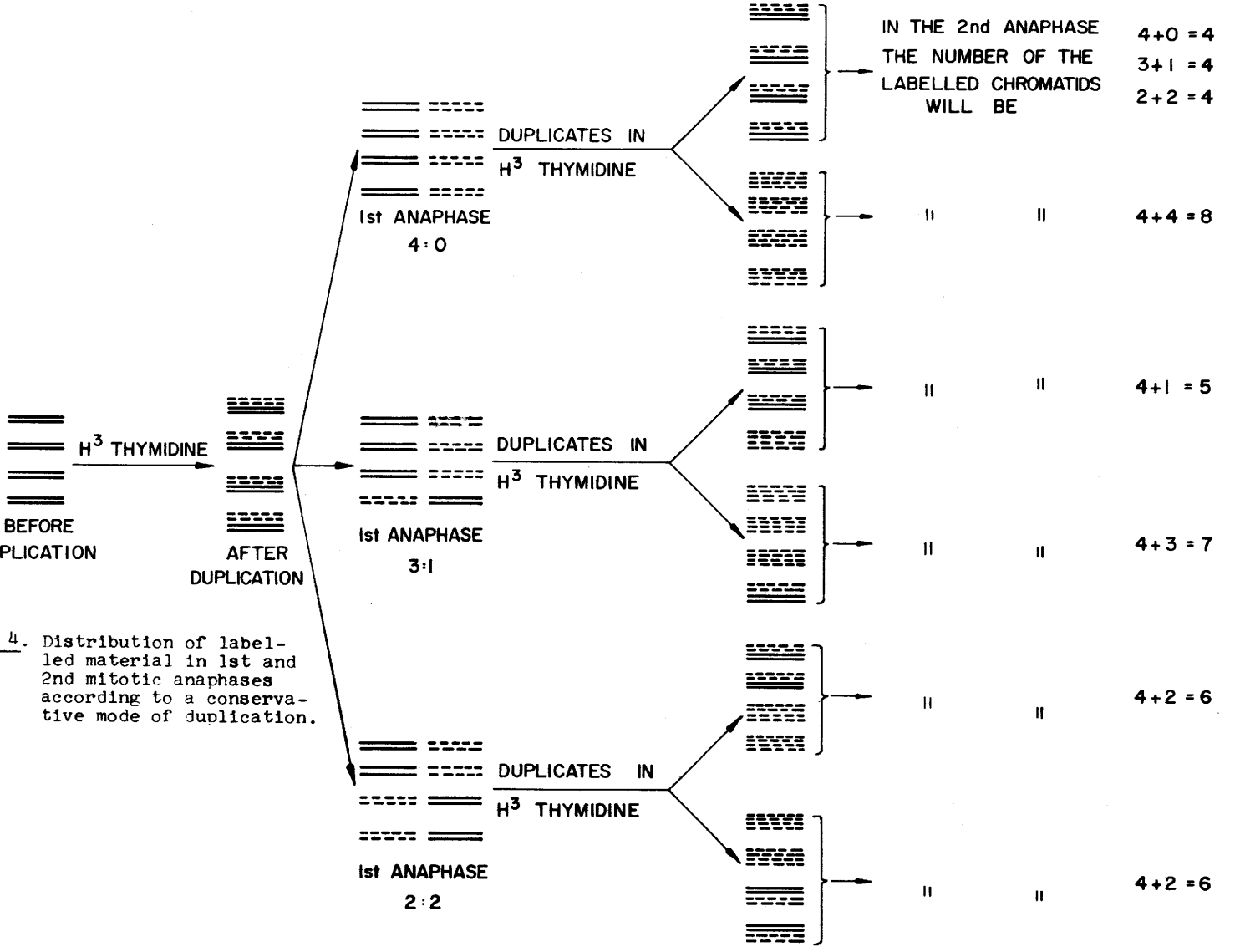


Fig. 4. Distribution of labelled material in 1st and 2nd mitotic anaphases according to a conservative mode of duplication.

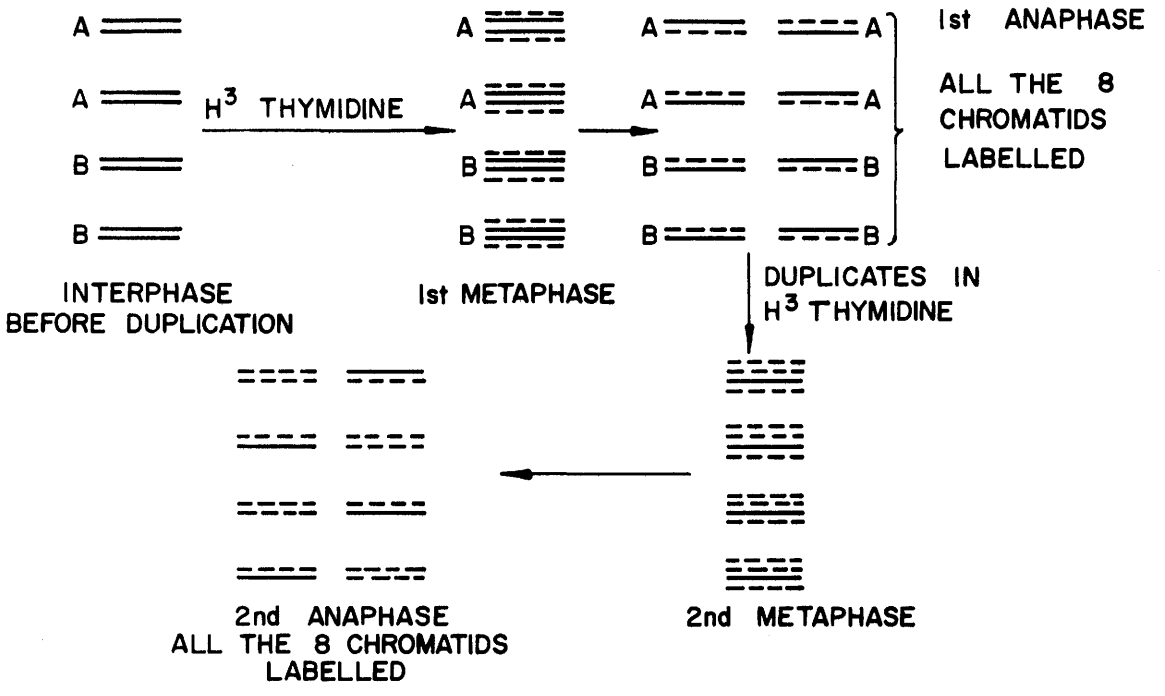


Fig. 5 A. Distribution of the labelled material in the first and second mitotic anaphases according to a semiconservative mode of duplication.

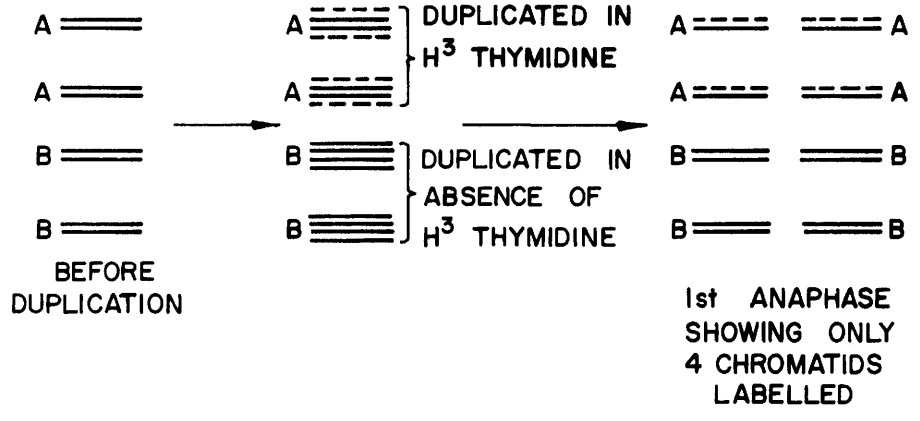


Fig. 5 B. Distribution of the labelled material in the first anaphase if one pair of chromosomes duplicate earlier than the other pair.

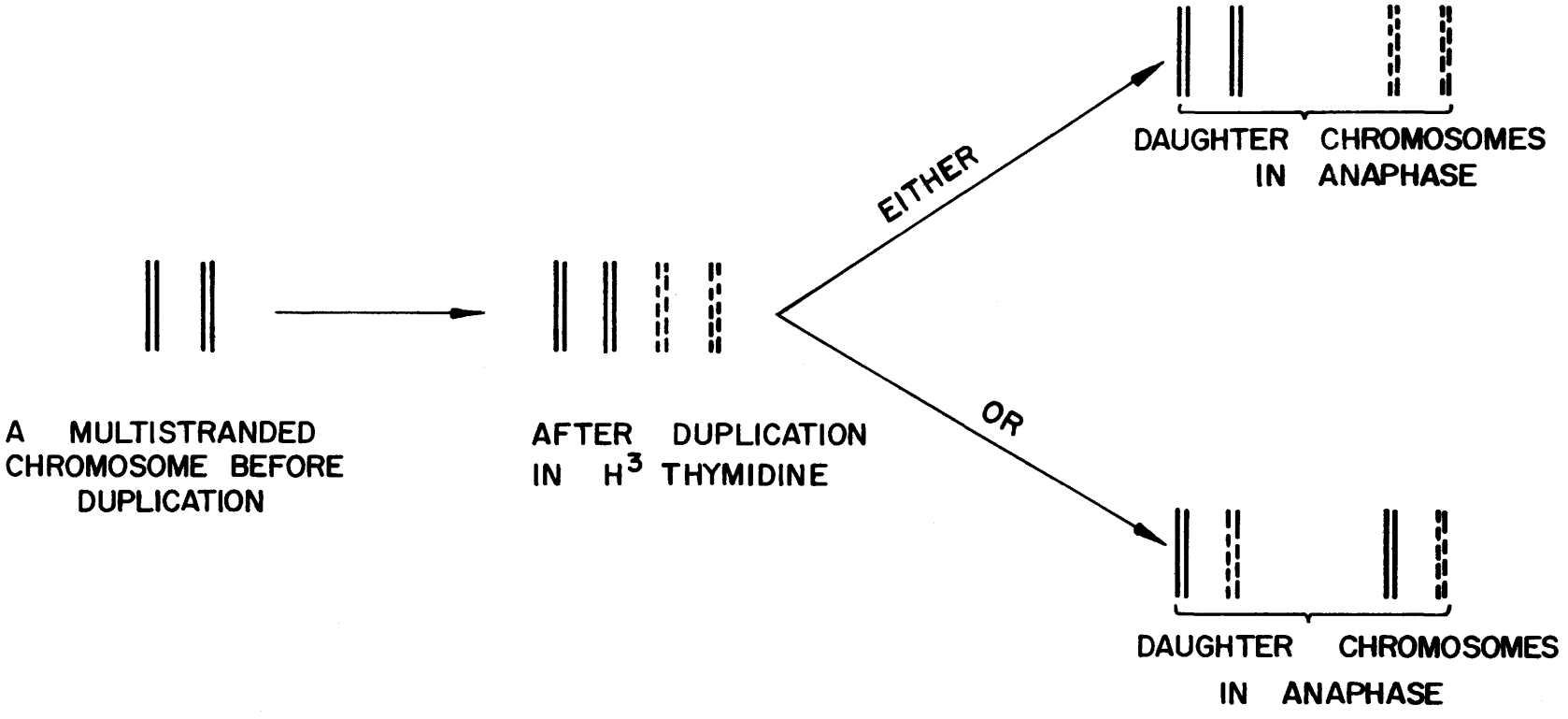


Fig. 6. The distribution of labelled material in daughter chromosomes in the first anaphase if a chromosome is multistranded, and if each strand duplicates conservatively and assorts independently to daughter chromosomes.

SUMMARY

The mode of chromosome duplication during meiosis and mitosis in Haplopappus gracilis was investigated. Tritiated thymidine was incorporated into the pollen mother cells during premeiotic interphase, and the cells were allowed to reach the tetrad stage. The autoradiographs prepared from the tetrads showed an unequal distribution of grains over their nuclei, while grains were equally distributed of the nuclei of diads, suggesting a conservative mode of chromosome duplication during meiosis. Seedlings were fed with tritiated thymidine for the duration of one cell cycle and also for the duration of several cell cycles. The autoradiographs prepared from the root tip cells, thus treated, showed unlabelled together with labelled chromatids in the anaphases of all the experiments, thus again suggesting conservative mode of chromosome duplication. A chromosome model to explain the results is discussed.

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