AN ELECTRON MICROSCOPIC ANALYSIS OF THE FINE STRUCTURE OF MERISTEMATIC CELLS DURING THE MITOTIC CYCLE IN *PISUM SATIVUM*

Thesis for the Degree of Ph. D.

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Lee Virn Leak

1962

THESIS

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thesis entitled

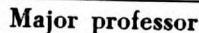
AN ELECTRON MICROSCOPIC ANALYSIS OF THE FINE STRUCTURE OF MERISTEMATIC CELLS DURING THE MITOTIC CYCLE IN PISUM SATIVUM

presented by

Lee Virn Leak

has been accepted towards fulfillment of the requirements for

Ph.D degree in Botany



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AN ELECTRON MICROSCOPIC ANALYSIS OF THE FINE STRUCTURE OF MERISTEMATIC CELLS DURING THE MITOTIC CYCLE IN *PISUM SATIVUM*

By

Lee Virn Leak

A THESIS

Submitted to
Michigan State University
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ABSTRACT

AN ELECTRON MICROSCOPIC ANALYSIS
OF THE FINE STRUCTURE OF MERISTEMATIC CELLS
DURING THE MITOTIC CYCLE IN
PISUM SATIVUM

by Lee Virn Leak

This study was undertaken to investigate fixatives for electron microscopic studies that would reveal the fine structure of the spindle element along with the preservation of other cellular components. Observations revealed that the fixatives containing metallic salts as $CdCl_2$, $PbCl_2$, $K_2Cr_2O_7$ and CrO_3 in a 7% to 10% formalin solution preserve the fine structure of the spindle, however, the fine structure of the cytoplasmic organelles were often distorted. A formalin and modified OsO_4 fixative gave detail spindle structure along with the preservation of other cytoplasmic components. By using the above fixatives the sequence of spindle development could be studied in the meristematic cells of *Pisum sativum* during active mitosis.

The chromosomal and interzonal fibers emanate from the chromosomes with the chromosomal fibers migrating toward the polar regions and the interzonal fibers occupying the mid-region of the cell. The continuous fibers extend from

one polar region to the other and are attached to the chromosomes by fine fibrils.

The fibers are composed of fibrils with granules along their length.

The phragmoplast is formed from the interzonal fibers and is separated in two units by the cell plate which is formed by a process of granular fusion or polymerization across the mid-region of the cell.

The fibrillar orientation is disrupted when cells are treated with a threshold concentration of colchicine. As a result of this disruption the chromosomal fibers, interzonal fibers, continuous fibers, phragmoplast, and cell plate are not formed. Hence, cytokinesis does not occur, and the resulting cell is either a polyploid, binucleate or multinucleate one.

Cytoplasmic components migrate or are pushed by the action of the spindle body (during mitosis) in equal proportions to the ends of the cell for redistribution in the daughter cells.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to his colleagues in the cytology group at Michigan State University for their aid during this investigation.

To Dr. G. B. Wilson, special thanks for the continued guidance and inspiration that were always present throughout the course of this study.

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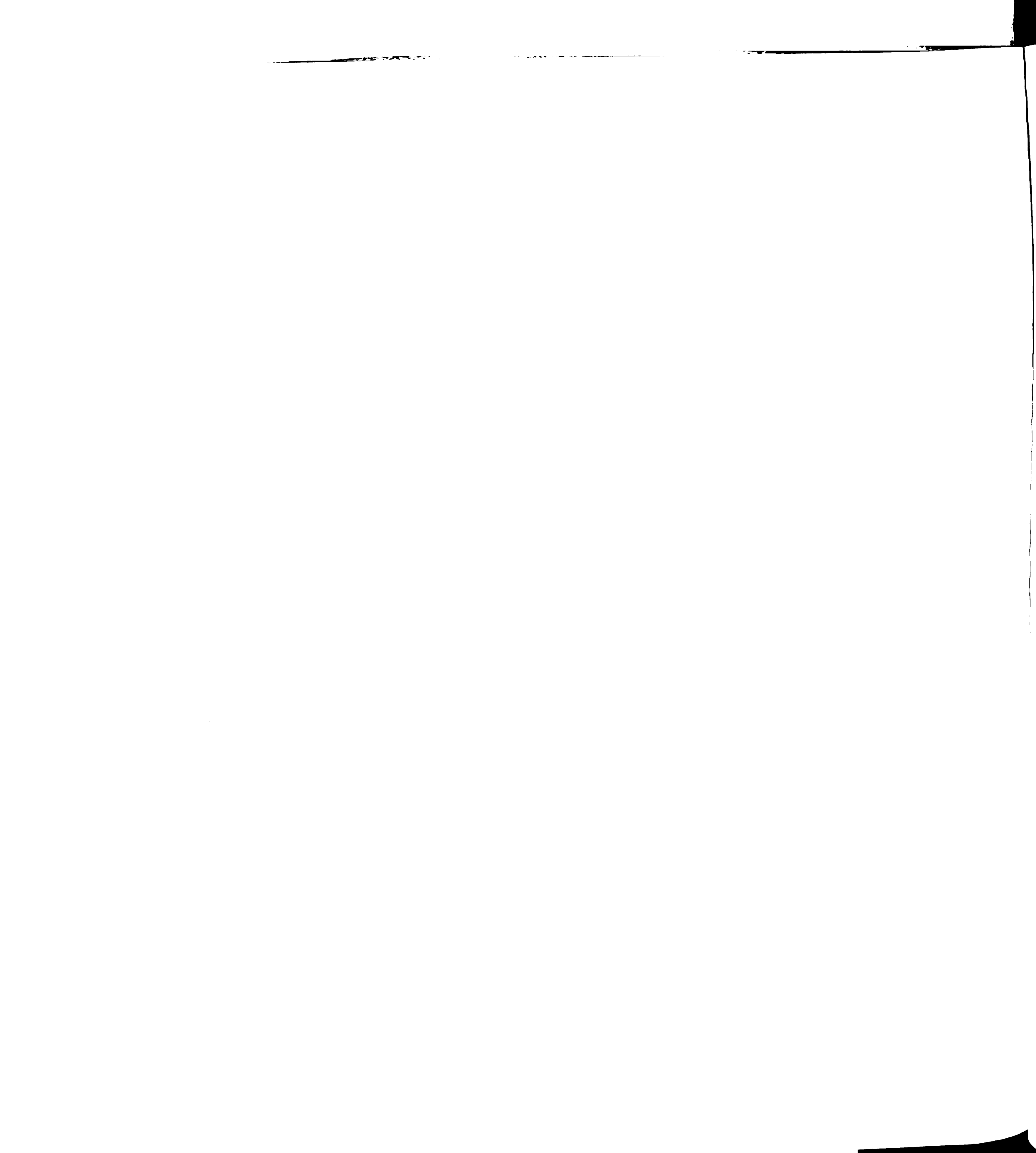

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INTRODUCTION

In his never-ending search for an explanation of the many complex phenomena occurring during embryogenesis and continuing throughout the life span of the living organism, the biologist is constantly prompted to investigate the numerous processes occurring within the living organism under diverse experimental conditions. Such investigations led Robert Hooke to the discovery of the cell, which was later proposed by M. Schleiden, the botanist, and T. Schwann, the zoologist, to be a basic unit of plants and animals. Later this theory was confirmed by R. Virchow as he showed that all cells come from pre-existing cells (OMNIS CELLULA E CELLULA).

In his attempt to resolve the complex phenomena of the living organism, the biologist must continue to probe the micro and ultra-fine structures of the cell as well as to seek correlation with function. The application of the electron microscope in the area of biological research has made it possible to begin bridging the chasm between the micron and angstrom levels.

The mechanism of cell division has been a subject of constant analysis since its early observation by Fleming, O. Hertwig, Van Beneden and others in the late 1800's. In

all higher plant and animal cells, in protozoa, in most algae and fungi, cell replication involves the method of mitosis. With the aid of phase microscopy and cine-micrography, we are able to observe this dynamic process in the living cell, especially by utilizing tissue culture methods.

Mitosis is but one phase of the mitotic cycle that one can readily identify by well-defined morphological features as: (1) a condensation of genetic material in the form of chromosomes, (2) nuclear disruption which involves the breakdown of the nuclear membrane, (3) the separation and movement of sister chromatids to opposite poles, and (4) reconstitution of daughter nuclei. The second part of the mitotic cycle has been termed the synthetic phase, in which the synthesis of DNA, RNA and proteins occur.

The movement phenomena of mitosis have been analyzed by numerous investigators, and the mechanisms explained by various hypotheses, in which a great deal of importance is placed on the spindle element and its relation to chromosome movement.

Early observers generally considered the spindle fibers to be a fixation artifact. However, as techniques in histology and cytology were improved, these structures were shown to be definite parts of the mitotic apparatus.

Recent investigations utilizing electron microscopy have revealed that the spindle element is composed of a definite fibrillar structure. Therefore, the present investigation was undertaken to determine: (1) the spindle formation in the meristematic cells of *Pisum sativum*, (2) its fibrillar nature as revealed by electron microscopic studies, (3) cell plate formation, (4) cytokinesis, and (5) the effect of colchicine on the fibrillar structure of the spindle fiber and formation of the cell plate.

LITERATURE REVIEW

The early information on the mitotic spindle as a fibrous structure was obtained from observations of fixed cells. Observations of living, untreated dividing cells, however, frequently revealed the mitotic spindle to be optically homogeneous (Cooper, 1941). Chambers (1924) was unable to demonstrate fibers in the spindle by using microdissection techniques. Since the fibrous structure was observed mainly in the fixed cell, this led to the belief that the spindle fibers have no morphological reality as such. Because of this apparent lack of structure in the living cell, the spindle was regarded as being the result of faulty fixation (Martens, 1929; Robyns, 1929; and Bleier, 1931a and b). Lewis (1923) was able to show the spindle fibers in chick tissue culture cells only when the medium was brought down to pH 4.6. Because of the association with pH change, she concluded that the fibers were artifacts. During this time the use of vital stains had added very little to the demonstration of the spindle in the living cell, although these dyes stained the nuclear components and some cytoplasmic organelles, there was always the possibility of damage to the living cell (Ries, 1938).

<u>Reality of the Spindle</u>: Numerous experiments by various investigators have been carried out in an effort to settle the question of the reality of the spindle element. Bělăr (1929a and b), Schrader (1934) and Schmidt (1936a, b and c) showed optical anisotropy in the spindle of living sea urchin eggs. Cooper's (1941) observations of dividing blastomeres of the living, untreated eggs of the mite, *Pediculopsis graminum*, with the light microscope, strongly suggested that fibrous structures comprise the spindle elements in these dividing cells. Carlson (1952), contrary to the results of Chambers' investigations, showed that the metaphase spindle in the living cells of *Chortophage*, including chromosomes and asters, constitutes a physical entity that can be moved about with the aid of a micro-needle. In the spermatocytes of some *Coccidae*, Hughes-Schrader and Ris (1941) were able to observe the spindle as a unit before the nuclear membrane had broken down.

In testing for the reality of the spindle fibers, Lillie (1909), Morgan (1910), Spooner (1911) and Andrews (1915) subjected living cells to centrifugation and found that the fibers could be bent or torn. Later studies of this nature were conducted by Schrader (1934), Beams and King (1936) and Shimamura (1940) with similar results.

In addition to the demonstrations of Cooper (1941) and Cleveland (1938a and b) of the chromosomal fibers in the living spindle with the light microscope, further evidence for the reality of the spindle was provided by Schmidt (1936a and b, 1939) with the aid of polarized light. Under such optical conditions, the living spindle shows positive birefringence in its long axis. Since protein molecules are known to behave in this fashion, it was concluded that there existed a longitudinal structure made up of protein molecules with parallel orientation. Confirmation of these conclusions may be found in the studies of Runnstrom (1936), Pfeiffer (1940), Hughes and Swann (1948) and Inoue (1953). X-ray diffraction experiments by Schmitt (1940) also further substantiate these conclusions. These observations together with the demonstration of birefringence in fixed spindles identical to that produced by the living spindle, led Schmitt to the belief that fixation induces no fundamental changes in it, and that the permanent preparations probably give a true picture of the actual conditions.

With his modification of the polarizing microscope, Inoue (1951, 1953) confirmed the evidence for birefringence in the spindle and demonstrated a definite fibrous structure

in the living mitotic spindle similar to that which had already been observed in the fixed cell.

The observations of Bajer (1951, 1957), utilizing cinemicrographic methods, and those of Taylor (1959) also provide evidence that the spindle is an organized fibrillar region.

Origin of the Spindle Element: The studies of Bleier (1930a and b), Koerperich (1930), Wada (1935) and Becker (1938) suggested that the spindle fibers of the metaphase configuration are located in a substance that is quite distinct from the cytoplasm. These investigators maintained that the spindle is probably derived entirely or in a greater part from the extra-chromosomal content of the prophase nucleus. Wada (1941) concluded that, in the case of plants, the spindle body appears to be entirely intranuclear in origin. On the other hand, observations of Mazia (1956) and Bajer (1957) suggested that the spindle body is at the outset, of cytoplasmic origin. Bajer reported that the first sign of the spindle rays are those fibers that extend from the centrioles to the equatorial plane (Painter, 1916).

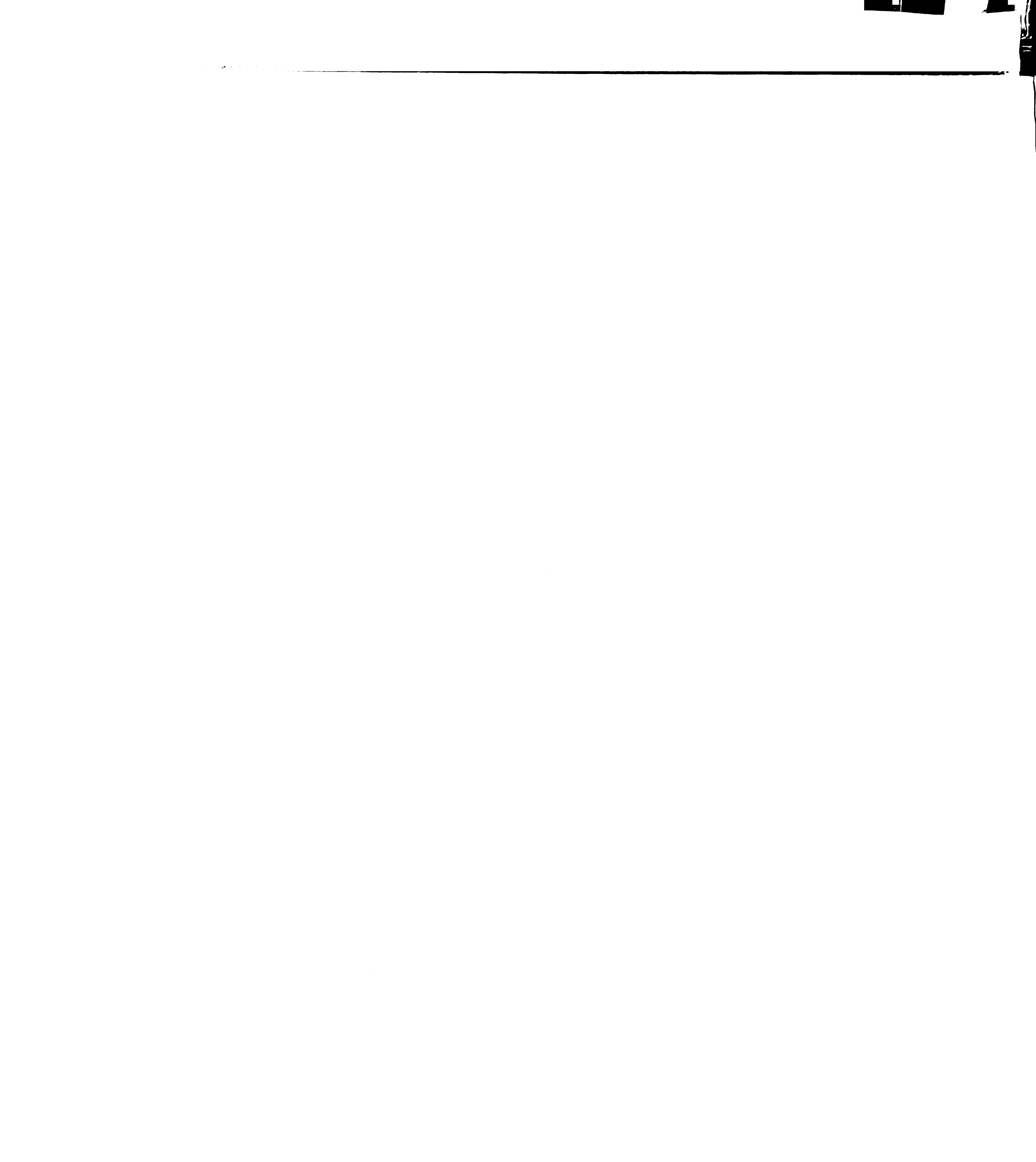
The interzonal connections extending only between the separating chromatids make up the interzonal fibers (Schmidt, 1939).

In the plant cell the interzonal region is linked with the development of the phragmoplast and the subsequent formation of cell plate or cell wall that is associated with cytokinesis.

Esau (1960) defines the phragmoplast as a fibrous structure that appears at telophase of a mitosis between the two daughter nuclei, and plays a role in the formation of the initial partition, dividing the mother cell in two units. It is a barrel shaped region between the daughter nuclei which becomes flattened and ring-like as cytokinesis begins.

Cytokinesis: The division or separation of the extranuclear material into two units is referred to as cytokinesis or cell division. In plant cells the new cell wall is also formed at this time. In somatic divisions, which characterize growth as a result of meristematic mitotic activity, there is a close correlation between nuclear and cellular division. However, these operations are separate in the formation of pollen and endosperm in many angiosperms, and in the development of the female gametophyte and the proembryo in gymnosperms (Sharp, 1934).

The inception of cytokinesis is marked by the appearance of a membrane-like line between the daughter nuclei. This

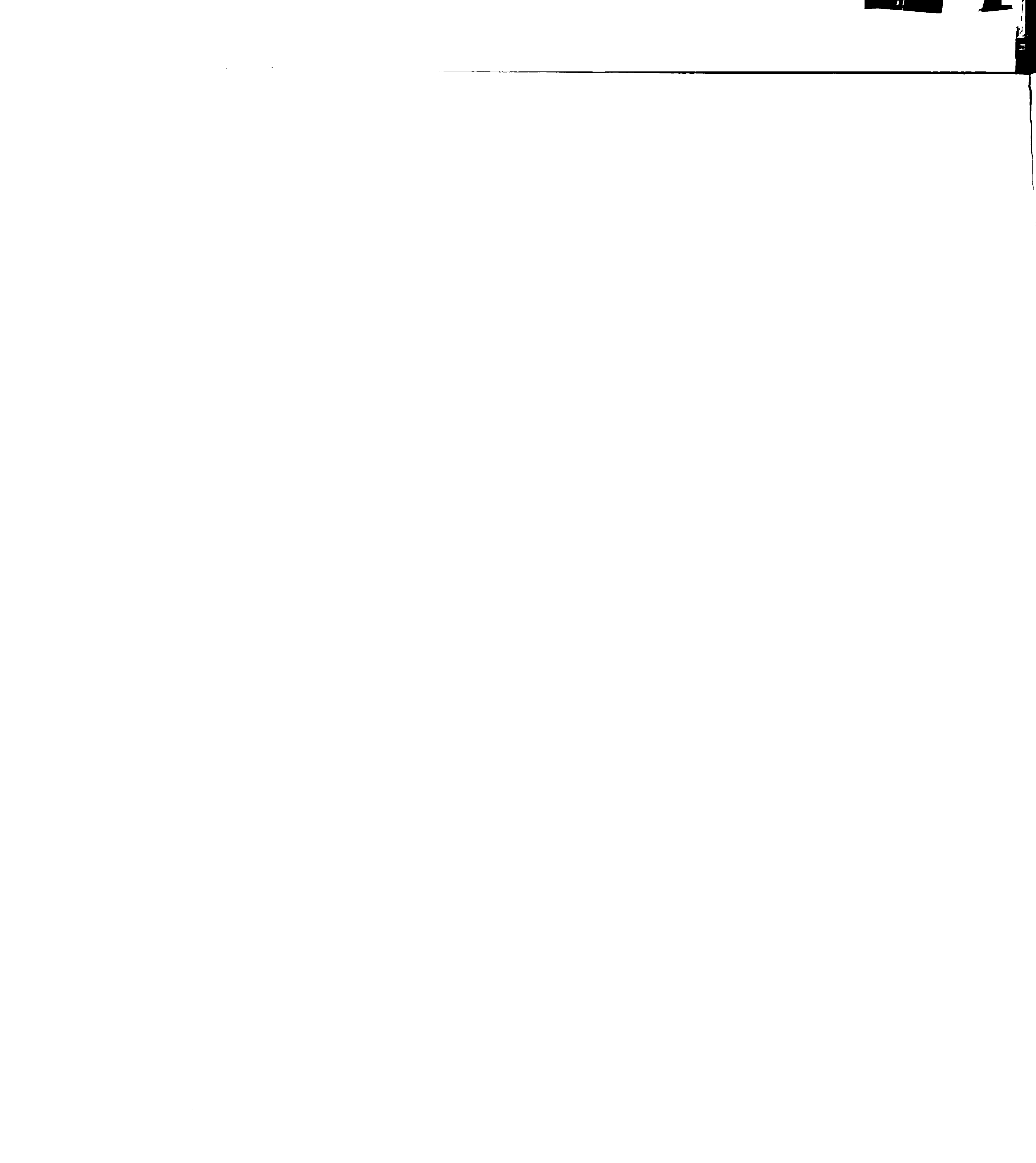


line which arises in the equatorial plane of the fibrous spindle (the phragmoplast) is referred to as the cell plate (Esau, 1953).

According to Esau (1953), in some cases during the early prophase of nuclear division, before the inception of cytokinesis, a cytoplasmic plate, the phragmosome, is formed across the cell in the plane of the division. It is derived from strands of parietal cytoplasm, and thus forms a medium in which the phragmoplast and the cell plate will develop.

In his studies, Yasui (1939) observed the appearance of fine granules at the equatorial region of the phragmoplast, noting that they increased in number and fused together, resulting in the formation of the cell plate. Yasui divided the process of cytokinesis into two phases: (1), the bipartition of the cytoplasm by the lateral growth of the phragmoplast, and (2) the bipartition of the phragmoplast by the formation of the cell plate in its equatorial plane.

More recently, Porter and Machado (1960) observed the occurrence of numerous circular and oval bodies in the region of the developing cell plate that appeared to be associated with the phragmoplast, and to represent an early



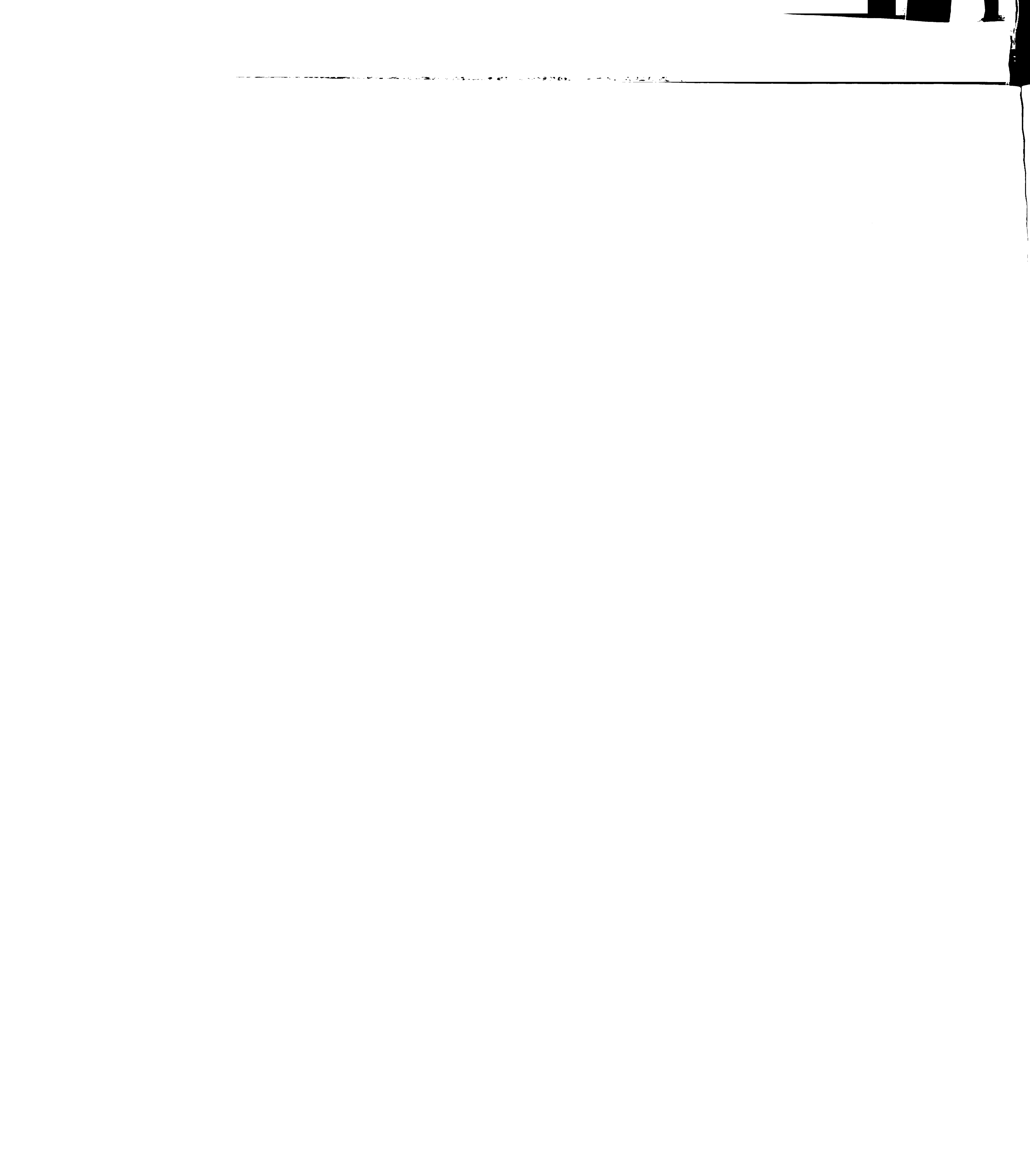
stage in the formation of the cell plate. They disappeared with the completion of separation of the daughter cells, as though they were used up in the process of formation of the middle lamella. Because of their association with the development of the cell plate, Porter and Machado referred to these bodies as phragmosomes.

The Structure of the Spindle Element: Studies of the spindle element in the living or fixed cell with the light microscope, the polarizing microscope, and by x-ray diffraction methods give evidence that its structure is fibrillar in nature (Inoue, 1953; Schmitt, 1940).

Recent investigations with the electron microscope have revealed both the astral and spindle fibers as being composed of fine fibrils 150-200 Å thick and several microns in length (Beams *et al.*, 1950, Amano, 1957; Bernhard and deHarven, 1958; Bessis *et al.*, 1958). Sedar and Wilson (1951) and Sato (1958, 1959 and 1960) showed that the spindle fiber is composed of submicroscopic fibril units. Sato's studies revealed the fine structure of the phragmoplast as consisting of a large number of parallel fibrils lying vertical to the equatorial plane, and he also noted that a fluid substance of low electron density fills up the interfibrillar space.

Spindle Disruption by Colchicine Treatment: Chemicals that inhibit mitosis in metaphase have been referred to as spindle poisons or "C-Mitotic" agents. Cytological studies by Ludford (1936), Nebel and Ruttle (1938), Eigsti (1938) and Levan (1938) indicated that colchicine stops mitosis in the living cell, while those of Hadder and Wilson (1957) and Molè-Bajer (1958) showed that colchicine disrupts specific phases of the mitotic cycle. Treatment of the isolated mitotic apparatus of sea urchin eggs with colchicine produced an amorphous gel, indicating that colchicine could affect the secondary bonding assumed to contribute to the integrity of the spindle structure (Mazia, 1956). It seems that in colchicine-treated eggs (sea urchin), the protein fibrils that constitute the normal spindle form an irregular network instead of lying parallel. It is probable that the protein molecules which build up the spindle fibers are altered in their composition or structure by colchicine. Thus they would still form protein fibers through oxidation of -SH- groups but these fibers would be incapable of parallel orientation by hydrogen bonding (Mazia, 1956).

The observations of Inoue (1952) and Swann (1951) with the polarizing microscope showed that the orientation of



the protein fibers is changed after colchicine treatment. The inhibition of the development of the spindle in colchicine-treated cells is determined by the concentration of the colchicine used and the duration of exposure. Gaulden and Carlson (1951), Hadder and Wilson (1957) and Van't Hof (1961) showed that the greater the concentration and time of exposure, the greater will be the degree of disorganization of spindle or interference with its development.

By using short exposures with near threshold concentrations (2.5×10^{-4} M and 2.5×10^{-3} M) of colchicine, Sedar and Wilson (1951) showed a progressive swelling and solubilization of fibrous material as revealed by the electron microscope.

MATERIALS AND METHODS

The observations reported in this investigation were made from the first 2-3 mm of the distal portion of the meristematic region of the root tips of pea seedlings, Pisum sativum var. Alaska, which were furnished by the Ferry-Morse Seed Company.

The peas were soaked for six hours in distilled water at 25° C, then rolled in paper toweling and placed vertically in 250 ml beakers that were one-third filled with distilled water. Wax paper was placed around the rolled toweling to prevent the peas from drying out by evaporation of the water. The peas were allowed to germinate in an incubator at 25° C for 48 hours, at which time the primary root had reached a length of 3-5 cms.

Seedlings with the desired root length (2.5 - 3.5 cms) were suspended on waxed, one-quarter inch wire mesh over 250 ml beakers which contained one-quarter strength Hoagland's nutrient (balanced salt) solution. The one-quarter strength Hoagland's solution consists of the following in grams per liter:

$Ca(NO_3)_2$ -------------------- 0.0246

NH_4NO_3 -------------------- 0.033

$MgSO_4 \cdot 7H_2O$ --------------- 0.0462

KH_2PO_4 ------------------------ 0.0342

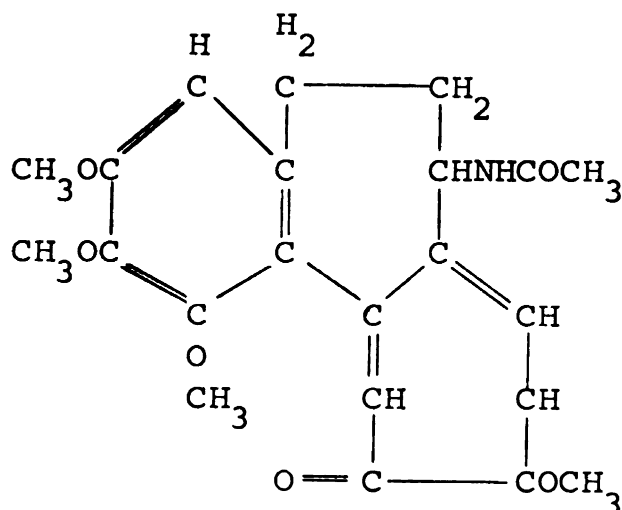
K_2HPO_4 ------------------------ 0.00179

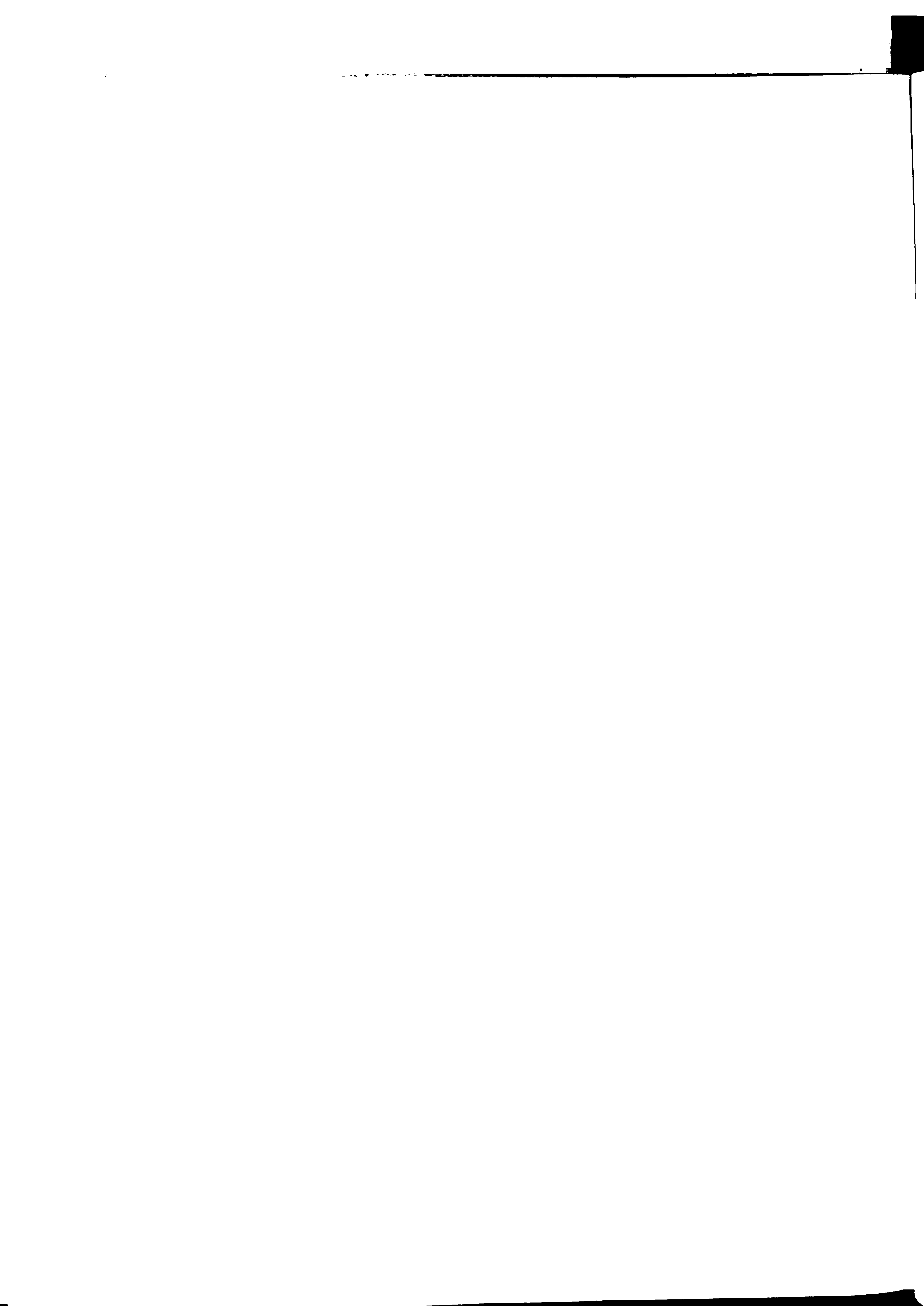
Seedlings to be used as controls were placed in the nutrient solution and samples taken at zero hrs., 4 hrs., 8 hrs., 12 hrs., and 24 hrs.

Seedlings to be treated were placed in beakers containing the colchicine and the nutrient solution. After treatment was completed, the seedlings with the wire mesh were removed from the solution of colchicine, washed and placed in a beaker containing only the nutrient solution.

The solution used had a pH range of 5.5 - 5.7. One M HCl and a 3.57 M KOH solution were used when pH adjustments were necessary.

The colchicine used in the experiment was purchased from Light's Organic Chemical Ltd., and its structural formula (Muldoon, 1950) has been determined to be:





The colchicine solution was prepared shortly (5-10 minutes) before use in order to obviate chemical change. Seedlings were treated for 30 minutes with a 4.5×10^{-4} M concentration of colchicine.

Tips 1 to 2 mm in length were harvested with a sharp razor blade, with some tips being split in half longitudinally and immediately placed in a fixative.

Of the cellular components, the spindle element has been the most difficult to demonstrate consistently while still preserving other cellular organelles.

Presently, the electron microscopist does not have at his disposal the "Universal Fixative" which will preserve simultaneously the chromosomes, spindle element, and the major cytoplasmic organelles. In experimenting with a range of fixatives it was found that the heavy metallic salts as: $CdCl_2$, $CoNO_3$, $CrSO_4$, $K_2Cr_2O_7$, $PtCl_6$, $SnCl_4$ and $HgCl_2$ gave detailed spindle and fair chromosome preservation while the cytoplasm and its components were highly disrupted.

By using the following fixatives (A and B) in a 1:1 ratio the spindle element along with other cytoplasmic and nuclear components were preserved:

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Solution A: 4% CrO_3 ----------------- 10 ml

4% $K_2Cr_2O_7$ -------------- 10 ml

10% formaldehyde -------- 10 ml

1% NaCl ----------------- 10 ml

Saturated picric acid --- 5 ml

The above salts with the exception of NaCl were made up in Hoagland's nutrient solution, and had a final pH of 3.5.

Solution B: 2% OsO_4 made up in the nutrient solution. The spindle element along with cytoplasmic components could be demonstrated when solution B was adjusted to pH 5 - 5.6. By adding $CdCl_2$ to solution A to make a 2% solution and adjusting the pH to 5 - 5.6 the spindle could also be demonstrated. Tips were also fixed in formalin and post fixed in buffered OsO_4 at pH 7.2 and pH 5.6.

Tips were also fixed in an ethanol, formaldehyde and propionic acid fixative (Leak and Wilson, 1960) for routine light microscopic analysis.

Fixation time ranged from 15 minutes to 12 hours. After fixation, the tips were washed, rapidly dehydrated in increasing concentrations of ethanol with 0.01% uranyl nitrate to cut down on local explosion. Tips were embedded in normal butyl methacrylate or 90% butyl methacrylate and 10% methyl methacrylate and catalyst with 0.01% uranyl

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nitrate added to reduce explosion due to uneven polymerization as recommended by Ward (1958). Polymerization was allowed to occur in an oven starting at 37° C for 24 hours, and then increased to 60° C for an additional 24 hours.

Tips fixed in $KMnO_4$ plus OsO_4, and OsO_4 were embedded in Epon 812 according to Luft (1959).

Sections were obtained with a diamond knife (Fernandez-Moran, 1954, 1956) and glass knives on a Leitz ultra microtome. Thick sections 5-10 microns were cut adjacent to thin ones and examined with the phase contrast microscope in order to determine the exact position of the cells in the root tip (Ornstein and Pollister, 1952). Methacrylate sections were spread by holding a wooden stick moistened with xylene over the sections in the knife trough. Grey (approximately 30-60 mμ thick) and silver (approximately 60-90 mμ thick) sections (Peachey, 1958) were placed on copper grids covered with a parlodion film and coated with a thin film of carbon (Pease, 1960). Sections were observed with a Philips 100B electron microscope.

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OBSERVATIONS

The observations described in this investigation were obtained from studies of numerous electron micrographs of sections from the meristematic region of root tips of *Pisum sativum*.

In the meristematic cells of *Pisum*, as in most mitotic cells of plants and animals the condensation of the genetic material in the form of filamentous or rope-like chromosomes along with a spherical or ovoid nucleolus marks the prophase stage, i.e., the inception of active mitosis or nuclear division (fig. 1). With the progression of prophase, the chromosomes become more pronounced as rods or condensed filamentous bodies (fig. 2) and the nucleolus disappears. A subsequent breakdown of the nuclear envelope characterizes the termination of the prophase stage (fig. 3). After the nuclear envelope breaks down, the chromosomes are arranged in the central region of the cell with no specific orientation; this marks the prometaphase stage. At this stage, chromosome fibers are observed associated with the chromosome (figs. 4, 5 and 6). Since fibers were not observed in the polar region of the cells at this stage, but were only associated with or attached to chromosomes, it appears that

the chromosome fibers emanate from some region along the chromosome. Light microscopic studies reveal that the fibers are attached at the kinetochore region of the chromosome. Observations of the living or fixed dividing cell show that the kinetochore region precedes the other regions of the chromosome in its movement toward the pole (Bajer, 1951).

The migration of the chromosomes toward the center of the cell and their alignment along the equatorial plane of the cell marks the metaphase stage. At this stage, the chromosome fibers are observed attached to the chromosome and extending toward the polar region of the cell (figs. 7 and 8). In this investigation, the term chromosomal fibers will be used to designate those fibers emanating from the chromosome and extending toward the polar region. Those fibers extending from one polar region to the other will be termed the continuous fibers, and those fibers situated between the separated chromatids, and which are attached to them at either end, will be referred to as the interzonal fibers.

What appears to be a single fiber when viewed with the light microscope is, at the electron microscopic level, actually a bundle of fibers, each with an average diameter

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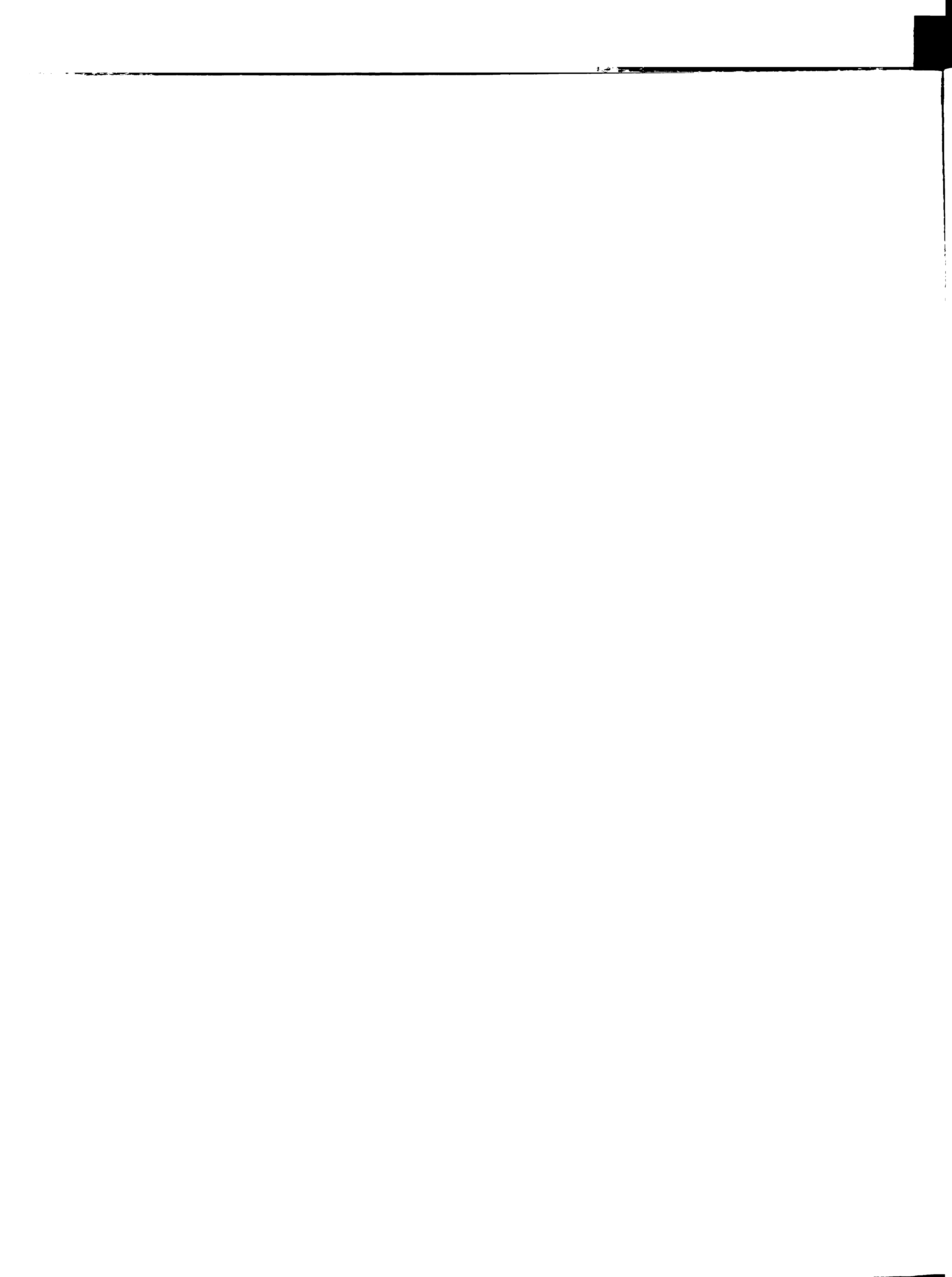
of 800 Å. Each fiber is made up of several fibrils with each fibril measuring 125-150 Å in diameter. The chromosomal fibrils observed by Sato (1958) ranged from 200 to 700 Å in diameter.

That the chromosomal fibers emanate from the chromosome and are attached to the chromosome is suggested in figs. 8 and 9. The bundle of fibers emerging from the chromosomes in fig. 8 appears to be very compact at the point of attachment to the chromosome and less compact as they extend toward the polar regions.

Continuous fibers are indicated in fig. 10, where they appear to occupy a position tangential to certain portions of the chromosomes observed in the section of this cell. These continuous fibers are of the same dimensions as the chromosomal fibers.

In the cells fixed with $KMnO_4$ there is an indication of a fibrous structure associated with the metaphase chromosome as suggested in figs. 11, 12 and 13. However, these fibers are not as pronounced as those of the metaphase cells fixed in the OsO_4 or the metallic salts described earlier. The granules can be detected in the homogeneous area that surrounds the less dense metaphase chromosomes. Their low electron density is due to the fixation used.



In the cells fixed in OsO_4 and metallic salts, well-defined chromosomal fibers were observed in this area. The endoplasmic reticulum and other cytoplasmic components are better preserved with the $KMnO_4$ fixation as also indicated in figs. 11, 12 and 13. These cytoplasmic components appear either to be migrating, or being pushed from the metaphase plate toward the opposite ends of the cell for future distribution in the daughter cells. That the location of these components might be due to pushing is suggested by their compactness around the periphery of the homogeneous area of the metaphase plate. This compact condition of the cytoplasmic components does not exist in the interphase or prophase stages (figs. 14 and 15).

The separation of the sister chromatids characterizes the beginning of anaphase (figs. 16 and 17). With this separation the interzonal fibers are formed between them and occupy the mid-region of the cell. With the progression of anaphase, the chromatids move toward the polar regions, toward which the chromosome fibers are oriented and in which they end (figs. 18, 19 and 20). At this stage the dimensions of the chromosome fibers are the same as those in the metaphase stage. The anaphase cell in fig. 19 was fixed in a formalin and metallic salt solution at a

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pH 7.2. These anaphase chromosomes appear to be attached to the clear lines, which probably represent the areas occupied by the spindle fibers. The fixative used at this pH does not reveal the fibers as well defined, electron dense lines. In figs. 21 and 22, the interzonal fibers are preserved along with the chromosomes and other cytoplasmic organelles. This fixative (modified OsO_4) also shows the fibrillar nature of the spindle (fig. 23). The interzonal region is revealed with granules along the length of the interzonal fibers after fixation in formalin, CrO_3 and propionic acid as shown in fig. 24. The average dimensions of the fibers here are the same as those fixed in the OsO_4 fixative. Close analysis of the anaphase cells fixed in $KMnO_4$ reveals interzonal fibers between the separated chromatids as indicated in figs. 25 and 26. However, the fibers here are not as pronounced as those revealed by the metallic salts or the OsO_4 fixations. Here the cytoplasmic organelles are packed in the opposite ends of the cell.

The grouping of the daughter chromosomes and the reconstitution of the nuclear envelope marks the beginning of telophase. Along with nuclear reorganization, marked changes occur in the central region of the cell. The midregion of the interzonal fibers becomes highly granular,

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and these granules become aligned along the mid-region in two definite rows (fig. 27), fusing to form a double membrane system which marks the formation of the cell plate, which starts in the center of the cell and continues toward the periphery of the cell wall. The granulation and subsequent fusion in the mid-region of the cell separates the interzonal fibers into two units. At this stage the interzonal fibers become detached from the chromatids and form a body that is barrel-shaped in contour and is termed the phragmoplast (fig. 28). The granular fibers of the phragmoplast appear to be oriented or migrating toward the developing cell plate (figs. 29, 30 and 31).

As the granular fibers of the phragmoplast continue to decrease in the mid-region of the interzonal area, the double line of fused granules becomes more pronounced as a double membranous system to form the cell plate of the daughter cells (fig. 32). As cell plate development progresses, the space between the two membranes is increased as shown in figs. 33 and 34. Each of the membranes measures about 400 Å in diameter, and the less dense area between them measures about 800-1000 Å across its width. This clear area between the two membranes contains dense strands extending across its width; these probably mark

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the intercellular connections, the plasmodesmata. In some cases (fig. 33), the two membranes of the cell plate are continuous with each other, along their length, forming a pore or opening across its width, which provides a cytoplasmic connection between the two protoplasts.

In figs. 34 and 35, there are small spherical bodies and granules associated with the developing cell plate. These are reminiscent of the phragmosomes described by Porter and Machado (1960).

The completion of the nuclear envelope formation around the regrouped daughter chromosomes (fig. 36), and the extension of the membranous cell plate across the width of the cell, with the disappearance of the granular fibrils in the interzonal region, mark the end of the telophase stage, and the separation of the daughter nuclei into two cells.

Many observations of colchicine-treated animal and plant cells indicate that this chemical affects the spindle fibers. Whether this is a partial or complete disruption of the spindle depends on the concentration of the chemical used.

In the examination of colchicine-treated seedlings, two major types of configurations are observed in light microscopic studies: (1) "scattered metaphase," where the

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chromosomes are spread about the cell, and (2) "clumped pro-metaphase" where the chromosomes are arranged in a ball-shape in the central region of the cell. These configurations were previously described by Levan (1938), Bowen (1953), and Hadder and Wilson (1957). It is assumed that the "scatters" arise as a partial effect of the chemical action on the spindle fibers and are produced by a less concentrated dose of colchicine, while a greater concentration completely destroys or disrupts the spindle fibers thus producing a "pro-metephase clump."

Observations from light microscopic studies also indicate that the "scatter" configurations appear first, with the "clumps" appearing later, provided that the concentration of colchicine is high enough to produce them. Hadder and Wilson (1957) maintained that the "scatters" do not give rise to "clumps" because, at low concentrations, only the "scatters" are noted and the "clumps" never appear, regardless of the time of exposure of the seedlings to the colchicine.

Electron microscopic observations of cells from seedlings treated with 4.5×10^{-4} M of colchicine for 30 minutes also reveal that "scatters" and "clumps" occur (figs. 37 and 38). The "clumped pro-metaphase" shows very little if any evidence

of spindle fibers while in the "scattered" condition definite fibers are revealed attached to the chromosomes (fig. 37), but not oriented as are the fibers in the control condition.

As the "scatters" and "clumps" continue in the mitotic process, the chromosomes become telemorphic (like the telephase chromosomes in appearance and structure) and a nuclear envelope is organized around them. At this stage the restitution nucleus (fig. 39) contains four sets of chromosomes (28 chromosomes, the diploid being 14). As a result of spindle disruption, there is no organization of the interzonal fibers; therefore, no phragmoplast and cell plate formation occurs, and cytokinesis does not take place. No observable difference was detected in the cytoplasm of the colchicine-treated cells from that of the control cells.

DISCUSSION

Until recently, the major studies of the spindle element were made by means of the light and polarizing microscopes and from these, conclusive evidence was obtained for the reality of its fibrillar nature (Inoue, 1952). Thus far, studies of the spindle, utilizing electron microscopic techniques, have been relatively few, when one considers the numerous studies on some of the other cellular components such as mitochondria or plastids. The main reason for the paucity of observation of the fine structure of the spindle probably lies in the difficulty of cell preservation during the fixation and embedding process for electron microscopic analysis. Although spindle preservation still presents a problem, one can demonstrate spindle fibers by using special fixation procedures as described earlier. The micrographs described above present additional evidence that the structural unit of the spindle fiber consists of fibril units. The probability that the spindle elements revealed here are complete artifacts seems remote, since similar or identical spindle structures are observed in various cells subjected to different fixation procedures.

In this investigation, observations reveal that the spindle element consists of chromosomal fibers, interzonal

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fibers, a phragmoplast, and apparent continuous fibers.

The notion that the spindle fibers, i.e., the chromosomal, continuous and interzonal fibers, are of nuclear origin and are derived specifically from the kinetochore region of the chromosomes, has received added support from the studies of Inoue (1953), who utilized the polarizing light microscope to study spindle development in dividing cells. The birefringence produced by the spindle fibers was greatest near the chromosome and decreased toward the polar regions. At metaphase and anaphase the fibers are birefringent all the way to the poles. Since the observations in this study with the light microscope and the electron microscope revealed that the spindle first appears to be associated with the chromosomes and apparently grows toward the polar region, and since there was no indication that the spindle is formed in the polar region and extends toward the chromosomes, the notion that the spindle fibers originate from the kinetochore region of the chromosome seems a reasonable one (Sato, 1958).

If the fibrous materials emanate from the entire area of the kinetochore region, then as the chromosome is aligned along the metaphase plate, the fibers perpendicular to the equatorial plane would be oriented toward the polar region.

On the other hand, the fibers that extended into the horizontal plane, i.e., parallel to the equatorial plane, would have to undergo a reorientation in order to be directed toward the polar region. If, however, the spindle element is formed as a fluid or granular substance (fig. 4) and this is oriented in a poleward direction during fibrillar organization or polymerization, then this could probably account for the continuous fibers that extend from one polar region to the other which would then be tangential to the lateral side of the chromosome.

That the spindle element could also emanate from regions of the kinetochore that are perpendicular to the polar regions should also be considered. In this condition, whether the spindle is produced as ready-formed fibrils or in a fluid or granular state, with fibrillar polymerization occurring afterwards, there would still be polar orientation.

Whether the spindle element is produced as fibrils, as a fluid, or in the form of granules with the subsequent polymerization is not certain at this point. However, the notion that the spindle fibers are produced in the form of granules with a subsequent polymerization into fibrils seems reasonable. This is indicated by the fact that, on close examination of the point of fibril attachment to the

chromosome, granules appear along the length of the fibrils that are embedded well into the chromosome area. Granules are also arranged along the length of the chromosomal, interzonal, and continuous fibers. Small, dense granular-like fibrils were also described by Beams *et al.* (1950), Sedar and Wilson (1951), and more recently by Sato (1960), as making up the spindle body.

The spindle fibers attached to the chromosomes at prometaphase and persisting until late anaphase are referred to as the chromosomal fibers. The combined light and electron microscopic studies give evidence that these fibers are attached to the chromosome at the kinetochore region and extend to the polar regions (Sato, 1958). That the fibers are directly attached to the chromosome is indicated in fig. 7, and on close examination are seen embedded in the chromosome (fig. 9).

With the poleward orientation of the chromosomal fibers, the cytoplasmic organelles are concentrated in the opposite ends of the cell and are pushed away from the area near the nuclear boundary that they occupied at late prophase before the nuclear envelope broke down. That the cytoplasmic components are pushed by the spindle body is suggested by the progression of the chromosomal fibers from the equatorial

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plate to the polar region. During this poleward development the spindle body takes up areas that were once occupied by some of the cytoplasmic components. This apparent pushing of the cytoplasmic organelles into opposite ends of the cell could serve to distribute them within the daughter cells.

It seems apparent that the chromosomal fibers play a role in the movement of chromosomes to the polar regions. Mota (1956) suggested that the release of spindle material from the kinetochore possibly could cause a poleward reaction which would push the chromosome and cause the anaphase movement. Whether this poleward migration of the chromosome is a push or pull action by the chromosomal fibers is still uncertain.

Although the existence of continuous fibers is apparent, it is still difficult to account for their formation. Their apparent extension from one polar region to the other indicates a possible formation at the polar region. This, however, seems remote, since there are no observable structures in the polar region that give the appearance of producing such fibers (Sato, 1958). Because these fibers are also associated with the chromosomes and appear to be attached or connected to them in a tangential fashion

(lateral side of the chromosome), it leaves one to speculate that the spindle substance released from the lateral region of the kinetochore might possibly account for the continuous fibers. The substance would be released more or less along a common region, and on polymerization would be oriented toward both polar regions (see fig. 10), thus forming the fibers that extend from one pole to the other.

On separation of the sister chromatids, fibers are produced between them which occupy the mid-region and form the interzonal fibers. Here, as in the formation of the chromosomal fibers, there is a polymerization of fibrous substance with a subsequent orientation parallel to the longitudinal axis of the cell, making up the interzonal fibers. These extend the length of the interzonal region between the chromatids and are attached to them (fig. 24). As anaphase progresses, the fibers are extended, increasing the width and length of the interzonal region. As the interzonal fibers increase in length, the chromosomal fibers decrease in length, and the chromosomes are moved toward the polar regions. Whether this increase in the interzonal fibers is brought about at the expense of the chromosomal fibers is not quite clear at this point. However,

the chromosomal fibers decrease and finally disappear as the development of the interzonal fibers continues.

The separation of the interzonal fibrils into two units by a horizontal orientation of granules across the mid-region transforms this region into a barrel-shaped fibrillar region, the phragmoplast, from which the cell plate will develop (Sato, 1960). Along with these changes in the mid-region of the cell, striking changes also take place in the reforming nuclear regions, where the chromosomes become regrouped and the nuclear envelope is organized around them to form the daughter nuclei. As restitution of the nuclei occurs, cytokinesis is also completed at about the same time.

Wada (1955) maintained that the phragmoplast substance is induced from the atractoplasm (spindle body) by hydration, and that during this change from atractoplasm into the phragmoplast substance, the fibrils swell in this area. The observations here revealed no appreciable difference in the dimensions of fibrils of the phragmoplast from those of the interzonal or chromosomal fibrils. However, there appears to be an increase in the number of granules in the general interzonal area (fig. 28) and an ordered arrangement of these granules along the mid-region of the phragmoplast.

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From these observations it seems probable that the phragmoplast and the interzonal fibers are one and the same, with the former resulting from changes in the fibrillar arrangement of the interzonal fibers to give a barrel-shaped zone reduced in fibrillar structure. The phragmoplast is located in the interzonal region between the reforming daughter nuclei.

The occurrence of the granules across the mid-region of the phragmoplast marks the early inception of cell plate formation. In considering the origin of these granules, one is tempted to speculate that they may be formed by a depolymerization of the interzonal or chromosomal fibrils. This is inferred from the disappearance of the chromosomal fibrils with the progression of late anaphase and the inception of telophase. There is an apparent increase in granularity throughout the length of the interzonal fibers along with these processes. The orientation of these granules into two lines along the mid-region of the phragmoplast and their extension in a lateral direction indicate the operation of some type of central force, since the apparent diminishing or depolymerizing granular fibrils seem to be directed or oriented toward this central region (see fig. 32).

As telophase progresses, the two lines of granules along the mid-region of the phragmoplast fuse to form a double membranous cell plate (see fig. 34). This condition was also reported by Rozsa and Wyckoff (1950). These two membranes appear to form simultaneously and appear as double units from their inception. This development of the cell plate starts at the central part of the phragmoplast and proceeds laterally toward the peripheral region of the cell. As formation of the cell plate nears completion, the unit fibrils of the phragmoplast gradually disappear. From the observations in this study it is apparent that the cell plate, which is a double membranous system separated by a clear area, is a product of: (1) a granulation process of the interzonal fibrils, (2) a granular orientation at the mid-region, and (3) a granular fusion to form a membranous system, the cell plate. On completion of karyokinesis (nuclear division) and the separation of the two protoplasts by cytokinesis, two daughter cells are formed, completing active mitosis.

It is apparent from the observations of short-term colchicine treatment of cells in mitosis, that the effect is not an inhibition of mitosis, but rather an inhibition of the spindle development and orientation or a disruption

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of the formed spindle fibrils. These observations agree with those of Sedar and Wilson (1951) and also those reported by Mazia (1956) on the isolated mitotic apparatus from colchicine-treated eggs of the sea urchin. The material that would normally form the spindle is present; however, it does not acquire the typical fibrillar orientation that is characteristic of the spindle element. It seems probable that the protein molecules that build up the spindle are altered in their composition or structure by colchicine treatment. Mazia (1956) suggested that protein molecules could still possibly form fibers through oxidation of -SH- groups, but that these fibers would not be capable of parallel orientation by hydrogen bonds.

It seems evident from the numerous studies of the effect of colchicine on the dividing cell, that its ability to alter the mitotic cycle lies in its direct action on the spindle fibrils. That it affects the fibrils directly is inferred from the observations of living dividing cells treated with colchicine. When strong concentrations (50 and 25 X 10^{-6} M) of colchicine were added to mid and late anaphase, the spindle fibers were destroyed, and the chromosomes stopped in their movement to the poles, and the two groups of sister chromatids

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intermingled, forming a single telophasic nucleus. When concentrations of 2.5×10^{-6} M of colchicine were applied at the same stage, no detectable results were observed (Eigsti and Dustin, 1955). Eigsti and Dustin also observed that reduction of the metaphase spindle could be accomplished with lesser concentrations than those required for anaphasic spindle reduction. From their observations of dividing cells treated with colchicine, Gaulden and Carlson (1951) concluded that the effectiveness of colchicine in spindle destruction or interference with its further development depends upon the concentration used, and that a greater concentration is necessary to destroy the more advanced spindle, i.e., at anaphase than at pro-metaphase; and the form of a particular spindle is directly related to the characteristic type of metaphasic pattern that will develop after treatment, i.e., "scatters" or "clumps."

Observations of dividing cells treated with varying concentrations of colchicine indicate that low doses cause a "scattering" effect, while the high doses produce complete spindle destruction, and therefore "clumps" are produced (Eigsti and Dustin, 1955; Hadder and Wilson, 1957). In the "clumps" observed in this study, there were no well-defined spindle fibers detected, while in the case of "scatters," definite fibrils were observed.

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The destruction of spindle fibers prevents the formation of the metaphase plate and the subsequent movement of chromosomes to the polar regions. This failure of the poleward movement in the absence of an oriented spindle element indicates the important role played by the spindle fibers in the movement of chromosomes to the poles. As a result of this fibrillar disorganization, the chromosomes continue their morphological cycle *in situ*. Although the typical telophase stage is not formed, the chromosomes become telomorphic (become like telophase chromosomes) and an interphase nucleus is eventually reconstituted which is a tetraploid. While the molecules of the spindle fibrils are disrupted and the fibrillar orientation affected, chromosome replication is not affected, and the chromatid separation is not impaired. Therefore, the chromosome complements for the two daughter cells are present in the one cell in which cytokinesis failed to occur because of spindle fibrillar destruction by colchicine treatment.

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SUMMARY

1. The fine structure of the spindle element is revealed by the use of certain metallic salts as $CdCl_2$, $K_2Cr_2O_7$, and $PbCl_2$. The spindle fibers were also demonstrated by using OsO_4 at various pH ranges while still preserving other cellular organelles.

2. In Pisum meristematic cells the spindle element consists of (1) chromosomal fibers, (2) interzonal fibers, (3) continuous fibers, and (4) a phragmoplast which gives rise to the cell plate. The fibers measure about 800 Å and are composed of unit fibrils which measure 125 - 150 Å in diameter. The fibers are grouped together to form bundles which in the light microscope appear as a single unit.

3. The fibers originate from the kinetochore region of the chromosome. The phragmoplast develops from the interzonal fibers and gives rise to the new cell plate.

4. The cell plate is formed as two lines of granules along the mid-region of the phragmoplast. These granules fuse to form a double membranous system which separates the two protoplasts into the two daughter cells at cytokinesis.

5. On treating the dividing cell with a threshold concentration of colchicine the fibrillar orientation of the fibers is disrupted, thereby destroying the spindle

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fibers as an organized system. Because of spindle disruption the developing chromosomal and interzonal fibers are not formed, neither do the formed fibers maintain their orientation when treated with such concentrations of colchicine. Therefore, the cell plate does not develop and cytokinesis fails to occur.

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BIBLIOGRAPHY

Amano, S. 1957. The structure of the centriole and spindle body as observed under the electron and phase microscopes. Cytologia, 22:2-193.

Andrews, F. M. 1915. Die wirkung der Zentrifugalkraft auf Pflanzen. Fahrt. Wiss. Bot., 56:221-253.

Bajer, A. 1951. Studies on spindle and chromosome movement. Acta. Soc. Bot. Pol., 21:95-111.

______ 1957. Cine-micrographic studies on mitosis in endosperm. III. The origin of the mitotic spindle. Exp. Cell Res., 13:493-502.

Beams, H. W. and R. L. King. 1936. The effect of ultracentrifugation upon chick embryonic cells, with special reference to the resting nucleus and the mitotic spindle. Biol. Bull., 71:188-198.

Beams, H. W., T. C. Evans, V. V. Breeman and W. W. Baker. 1950. Electron microscopic studies on structure of the mitotic figure. Proc. Soc. Exp. Biol. and Med., 74:717-720.

Becker, W. A. 1938. Recent investigations *in vivo* on the division of the plant cell. Bot. Rev., 4:446-472.

Bělǎr, K. 1929a. Beiträge zur Kausalanalyse der Mitose. II. Arch. Entwmk., 118:359-480.

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Bernhard, W. and E. deHarven. 1958. L'ultrastructure du centriole et d' autre éléments de l'appareil achromatique. IV. Internal. Conf. Elect. Microsc. Berlin.

Bessis, K., J. Breton-Gorius and J. P. Thiery. 1958. Centriole, corps de Golgi et aster des leucocytes, étude au microscope électronique. Rev. l'Hemat., 13:363-386.

Bleier, H. 1930a. Experimentell-Zytologische Untersuchungen. Zellf., 11:218-236.

______ 1930b. Untersuchungen über das Verhalten der verschiedenen Komponenten bei der Reduktionsteilung von Bastarden. La Cellule, 40:85-144.

______ 1931. Zur Kausalanalyse der Kernteilung. Genetica, 13:27-76.

Bowen, C. C. 1953. A comparative study of the effects of several antimitotics (Ph.D. Thesis, Mich. State Univ.).

Carlson, J. G. 1952. Microdissection studies of the dividing neuroblast of the grasshopper, Chartophaga viridifasciata (DeGeer). Chromosoma, 5:200-220.

Chambers, R. 1924. The physical structure of protoplasm as determined by microdissection and injection. In General Cytology, 237-309. Chicago, Univ. Chicago Press.

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Cleveland, L. R. 1938a. Longitudinal and transverse division in two closely related flagellates. Biol. Bull., 74:1-41.

______ 1938b. Origin and development of the achromatic figure. Biol. Bull., 74:41-55.

Cooper, K. W. 1941. Visibility of the primary spindle fibers and the cause of mitosis in the living blastomeres of the mite, Pediculopsis graminun. Proc. Nat. Acad. Sci., 27:480-483.

Eigsti, O. J. 1938. A cytological study of colchicine on polyploidy in plants. Proc. Nat. Acad. Sci., 24:56.

______ and P. Dustin Jr. 1955. Colchicine in Agriculture, Medicine, Biology, and Chemistry. The Iowa State College Press, Ames, Iowa.

Esau, K. 1953. Plant Anatomy. New York. John Wiley & Sons.

______ 1960. Anatomy of Seed Plants. New York. John Wiley & Sons.

Fernandez-Moran, H. 1954. A diamond knife for ultra-thin sectioning. Exp. Cell. Res., 5:255.

______ 1956. A new microtome with diamond knife. Industr. Diamond Rev., 16:128.

Gaulden, M. E. and J. G. Carlson. 1951. Cytological effects of colchicine on the grasshopper neuroblast in vitro, with special reference to the origin of the spindle. Exp. Cell Res., 2:416-433.

Hadder, J. C. and G. B. Wilson. 1957. Cytological assay of C-mitotic and prophase poison actions. Chromosoma, 9:91-104.

Hughes, A. F. and M. M. Swann. 1948. Anaphase movements in the living cell. J. Exp. Biol., 25-45-70.

Hughes-Schrader, S. and H. Ris. 1941. The diffuse spindle attachment of Coccida verified by mitotic behavior of induced chromosome fragments. J. Exp. Zool., 87:429-456.

Inoue, S. 1951. A method for measuring small retardations of structures in living cells. Exp. Cell Res., 2:513-517.

______ 1952. The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. Exp. Cell Res. Suppl., 2:305-318.

______ 1953. Polarization optical studies. Chromosoma, 5:487-500.

Koerperich, J. 1930. Étude comparative du noyau. Chromosomes et leurs rélations avec le cytoplasme. La Cellule, 39:306-401.

Leak, L. V. and G. B. Wilson, 1960. Relative volume changes of the nucleolus, in relation to cell and nucleus. Trans. Amer. Microsc. Soc., 79:154-160.

Levan, A. 1938. The effect of colchicine on root mitosis in Allium. Hereditas (Lund), 24:471-486.

Lewis, M. R. 1923. Reversible gelation in living cells. Johns Hopkins Hosp. Bull., 34:373-379.

Lillie, F. R. 1909. Karyokinetic figures of centrifuged eggs: an experimental test of the center forces hypothesis. Biol. Bull., 17:101-120.

Ludford, R. J. 1936. The action of toxic substances upon division of normal malignant cells _in vitro_ and _in vivo_. Arch. Exptl. Biol. Zellforsch. Genebezucht., 18:411.

Luft, J. H. 1956. A new fixative for electron microscopy. J. Biophy. Biochem. Cytol., 2:799.

______ 1959. (Correspondence.)

Martens, P. 1929. Nouvelles recherches éxperimentales sur la cinèse dans la cellule vivante. La Cellule, 38:67-174.

Mazia, D. and K. Dan. 1952. The isolation and biochemical characterization of the mitotic apparatus of dividing cells. Proc. Nat. Acad. Sci., 38:826-838.

Mazia, D. 1956. The life history of the cell. Am. Scientist, 44:1.

Molè-Bajer, J. 1958. Cine-micrographic analysis of "C-mitosis" in endosperm. Chromosoma, 9:352-358.

Morgan, T. H. 1910. Cytological studies of centrifuged eggs. J. Expl. Zool., 9:593-657.

Nebel, B. R. and M. Ruttle. 1938. The cytological and genetical significance of colchicine. J. Hered., 29:3-9.

Mota, M. 1956. A new hypothesis of the anaphase movement. Proc. Int. Genetics Symposium. 113-116.

Muldoon, H. C. 1950. Organic Chemistry. 3rd edition. Blakiston, Philadelphia.

Ornstein, L. and A. W. Pollister. 1952. Application of phase microscopy in cytology and electron microscopy. Trans. N. Y. Acad. Sci., 14:194.

Painter, T. S. 1916. Contributions to the study of cell mechanics. J. Exp. Zool., 20:509-529.

Palade, G. E. 1952. The fixation of tissues for electron microscopy. J. Exp. Med., 95:285.

Peachey, L. D. 1958. Thin sections. I. Study of section thickness and physical distortion produced during microtomy. J. Biophysics Biochem. Cytol., 4:233.

Pease, D. C. 1960. Histological Techniques for Electron Microscopy. Academic Press, N. Y.

Pfeiffer, H. H. 1940. Experimentelle Cytologie. Leyden, Chronica Batanica Co.

Porter, K. R. and R. D. Machado. 1960. Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. J. Biophysics and Biochem. Cytol., 7:167-180.

Ries, E. 1938. Grandriss der histolophysiologie; Allgemeine methoden und problems. Akad. Verlagag.

Robyns, W. 1929. La figure achromatique, sur materiel frais, dans les divisions somatiques des phanerogames. La Cellule, 39:83-121.

Rozsa, G. and R. W. G. Wyckoff. 1950. The electron microscopy of dividing cells. Biochem. Biophy. Acta, 6:334-339.

Runnstrom, J. 1936. Uber die Veränderung der Plasmakolloide bie der Entwicklungserregung des Seeigels. Protoplasma, 5:201-310.

Sato, S. 1958. Electron microscope studies on the mitotic figure. I. Fine structure of the metaphase spindle. Cytologia, 23:383-394.

______ 1959. Electron microscopic studies on the mitotic figure. II. Phragmoplast and cell plate. Cytologia, 24:98-106.

______ 1960. Electron microscopic studies on the mitotic figure. III. Process of the spindle formation, Cytologia, 25:119-131.

Schmidt, W. J. 1936a. Keinspindel und Chromosomen im lebenden sich furchenden Ei von Psammechinus miliaris (Mull). Ber. Oberhess. Ges. Nat. Heilk., 17:140.

______ 1936b. Doppelbrechung von Chromosomen und Kernspindel in der lebenden zelle. Naturw., 24:463.

______ 1936c. Die Doppelbrechung de kernspindel und ihre Feinstruktur, ein Argument fur die Zugfasertheorie und die fadenmolikültheorie, Biodynamics, 22.

______ 1939. Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. Chromosoma, 1:253-164.

Schmitt, F. O. 1940. The molecular organization of protoplasmic constituents. Collecting Net., 15:145.

Schrader, F. 1934. On the reality of spindle fibers. Biol. Bull., 67:519-534.

Sedar, A. M. and D. F. Wilson. 1951. Electron microscope studies on the normal and colchicinized figures of the onion root tip (Allium cepa). Biol. Bull., 100:107-115.

Shimamura, T. 1940. Studies on the effect of the centrifugal force upon nuclear division. Cytologia, 11:186-216.

Sharp, L. W. 1934. An Introduction to Cytology, 3rd ed. New York, McGraw-Hill Book Company.

Spooner, G. B. 1911. Embryological studies with the centrifuge. J. Exp. Zool., 10:23-50.

Swann, M. M. 1951. The birefringence of the metaphase spindle and asters of living sea urchin egg. J. Exp. Biol., 28:417-443.

Taylor, E. W. 1959. Dynamics of spindle formation and its inhibition by chemicals. J. Biophys. and Biochem. Cytol., 6:193.

Van't Hof, J. 1961. Rate change of the mitotic cycle. (Doctoral Thesis, Mich. State Univ., East Lansing, Mich.).

Wada, B. 1935. Mikrurgische Untersuchungen lebender Zellen in der Teilung. II. Cytologia, 6:318-406.

______ 1941. Uber die Spindelfigur bei der somatischen mitose der prothalliumzellen von _Osmunda japonica_ Thunb., _in vivo._ Cytologia, 11:353-369.

______ 1955. Some problems on the mitotic figures studied in the living cell. Caryologia, 7:389-401.

Ward, R. T. 1958. Prevention of polymerization damage in methacrylate embedding. J. Histol. Cytochem., 6:348.

Whaley, W. G., H. H. Mollenhauer and J. H. Leech. 1960. The ultrastructure of the meristematic cell. Amer. J. Bot., 47:401-449.

Wilson, E. B. 1925. The Cell in Development and Heredity. 3rd. Ed. New York, Macmillan Co.

Yasui, K. 1939. On the cytokinesis in some Angiosperms, with special reference to the middle lamella formation and the phragmoplast. Cytologia, 9:557.

EXPLANATION OF FIGURES

KEY TO ABBREVIATIONS

Extra Cellular Components

w - cell wall

pd - plasmodesmata

pm - plasma membrane

Cytoplasmic Components

er - endoplasmic reticulum

d - dictyosome

m - mitochondria

pp - proplastid

i - cytoplasmic inclusion body

v - vacuole

t - tonoplast

pr - polar region

Nuclear Components

ne - nuclear envelope

n - nucleus

ch - chromatin material

nu - nucleolus

Spindle Element

chf - chromosomal fiber

if - interzonal fiber

cf - continuous fiber

cp - cell plate

Fig. 1. A low-power image of a longitudinal section through the meristematic region of a root tip, showing the interphase and prophase stages. The clear areas between the cells represent the cell wall (w), while the dense line that forms a boundary around the cytoplasm is the plasma membrane (pm). The mitochondria and proplastids are shown as oval or elongated dense bodies (see arrows). Because of low magnification it is difficult to differentiate between these two organelles. The endoplasmic reticulum (er) is shown as a dense membranous system distributed throughout the cytoplasm. Small dense particles are also distributed through the cytoplasm forming a granular ground substance. The nuclear envelope (ne) is shown as a dense double membrane, the chromatin material (ch) in the nucleus (n) is shown as dense bodies and the nucleolus (nu) as a dense oval body. Fixation in 1% OsO_4. Approximately X 8,000.

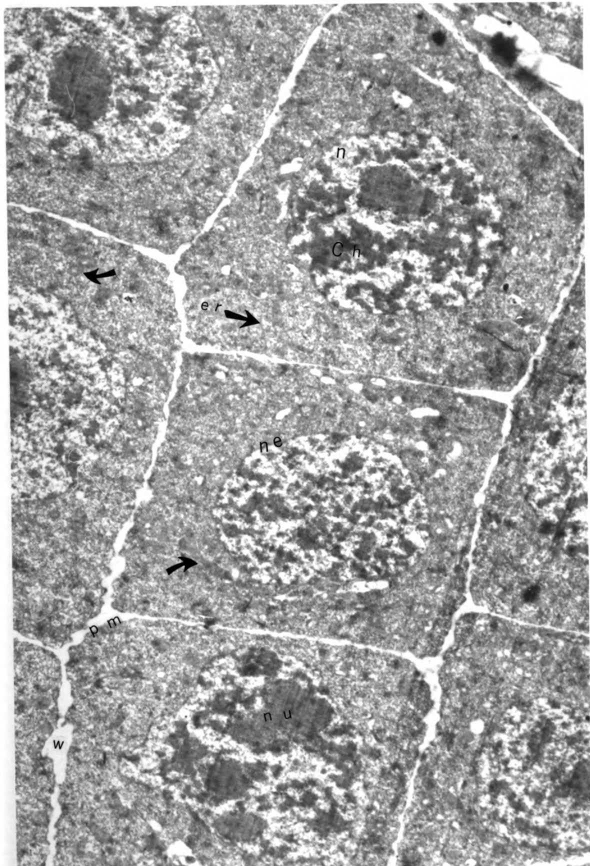


Figure 1

Fig. 2. A late prophase with some of the chromosomes (ch) located at the periphery of the nucleus and adjacent to the nuclear envelope (ne). Fixation in 1% OsO_4. Approximately X 20,800.

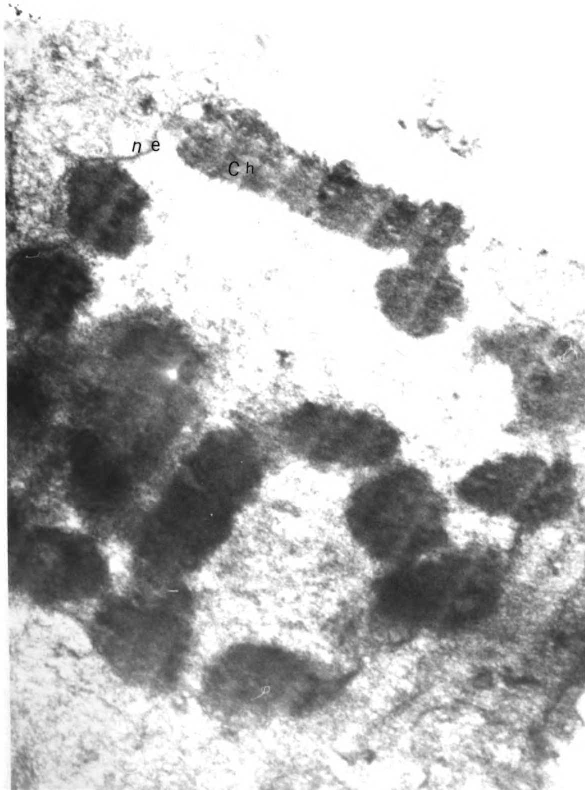


Figure 2

Fig. 3. Longitudinal section through interphase and late prophase cells. The nucleolus (nu) of the interphase cell is shown as a spherical dense body with a less dense central area (x). Chromatin (ch) material appears in both cells as dense bodies. The nuclear envelope (ne) of the late prophase cell appears to be in the process of breaking down (see arrows). Fixation in 10% formalin and post fixed in 1% OsO_4. Approximately X 10,800.

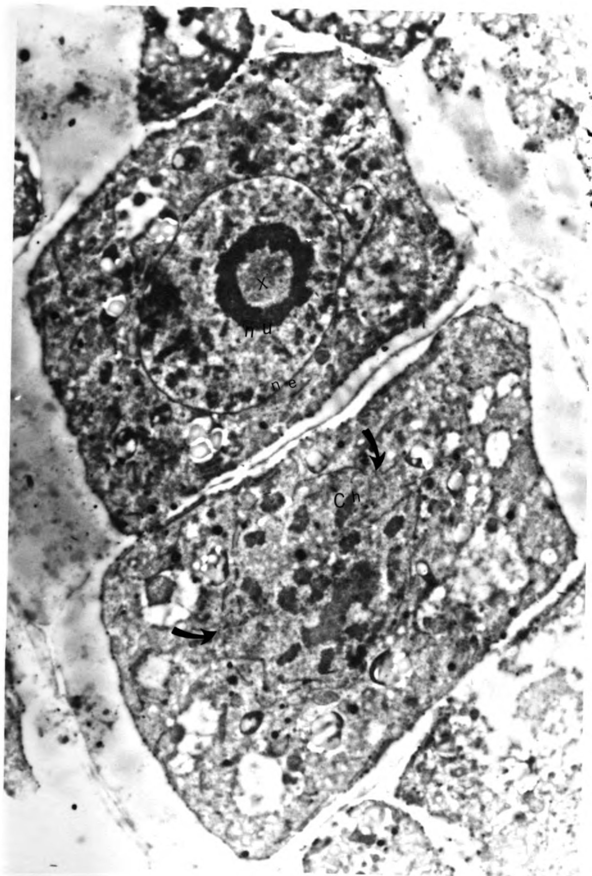


Figure 3

Fig. 4. A diagram depicting a probable method of spindle fiber production by the kinetochore region of the chromosome. A, the spindle substance is produced along the kinetochore region as granules, B, polymerization of granules into fibrils, C, orientation of fibrils toward polar regions, to give chromosomal fibers (chf), D, X.S. of kinetochore region depicting polymerization and orientation of granules into fibrils, with the fibrils at Cf probably giving rise to the continuous fibers, E, formation of interzonal fibers (if) at the separation of the sister chromatids during early anaphase, F, late anaphase, where the chromatids have moved closer to the polar regions, G, early telophase with phragmoplast (see arrows), and two rows of granules across the mid-region marking the inception of cell plate development (cp), H, late telophase with diminishing phragmoplast and membranous cell plate, and I, completion of karyokinesis and cytokinesis with the production of two daughter cells.

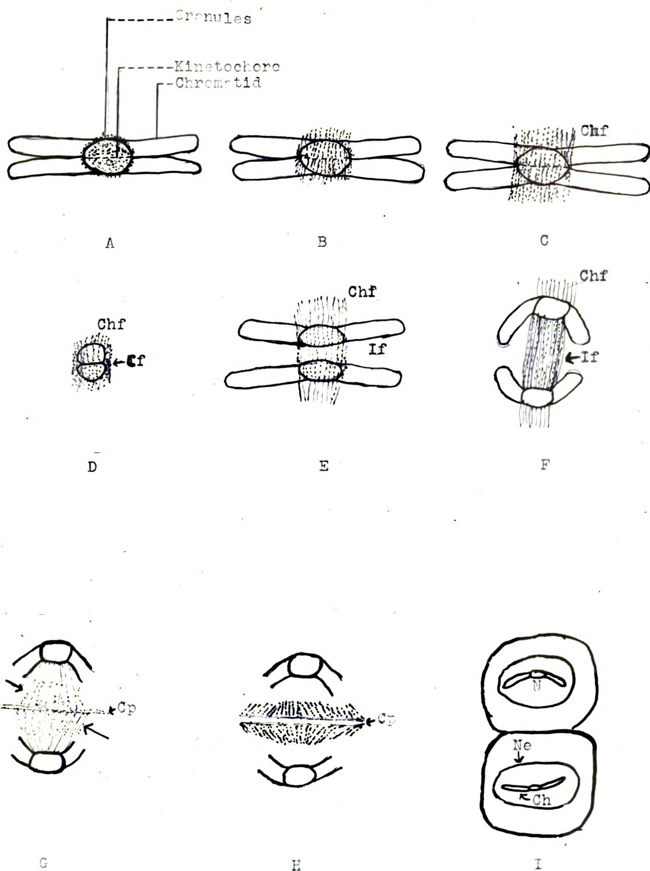
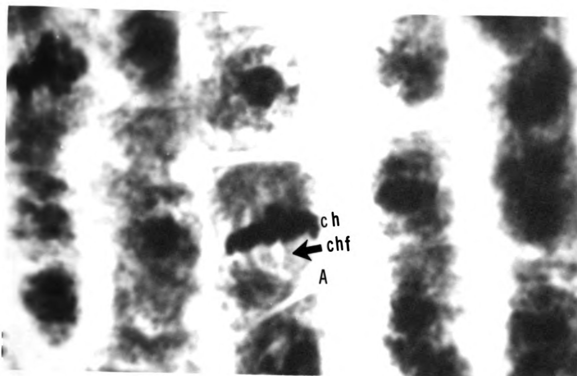


Figure 4

Fig. 5. (A) A light micrograph showing a metaphase with chromosomal fibers (chf) extending from the chromosome (ch) to the poles. (B) shows a telophase with the interzonal fibers (if) and a clear line across the mid-region indicating the formation of the cell plate (see arrows). Fixation in Bouin's fixative. X 3,500.



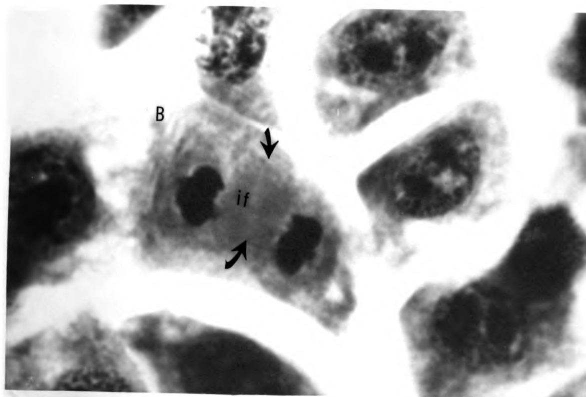


Figure 5

Fig. 6. Section showing chromosomal fibers (chf) emanating from pro-metaphse chromosomes (ch), and cell wall (w). Fixation in 2% $K_2Cr_2O_7$. Approximately X 13,200.

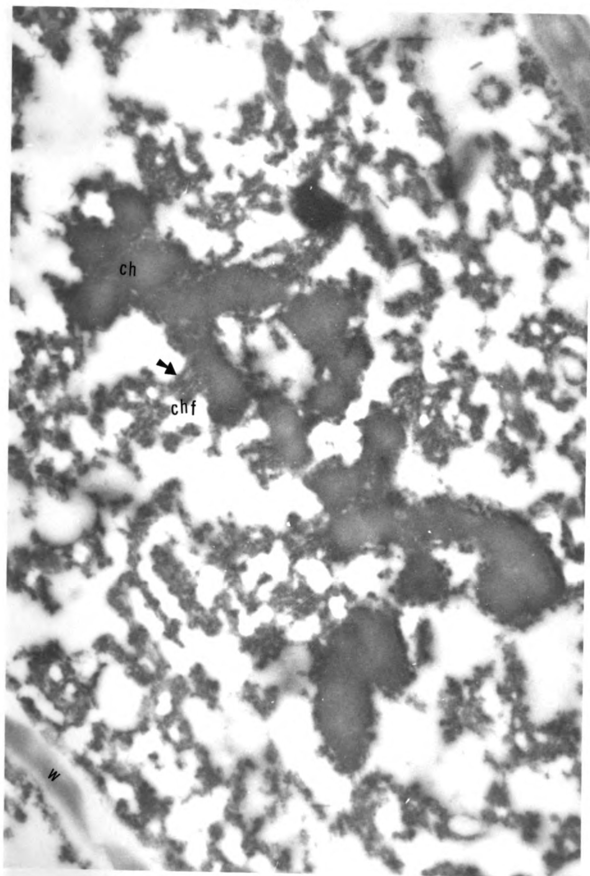


Figure 6

Fig. 7. Portion of meristem cell showing metaphase plate with chromosomal fibers (chf) attached to chromosomes (ch), and extending to polar regions (pr), plasma membrane (pm) and cell wall (w). Fixation 1% $PbCl_2$ and 10% formalin. Approximately X 13,200.

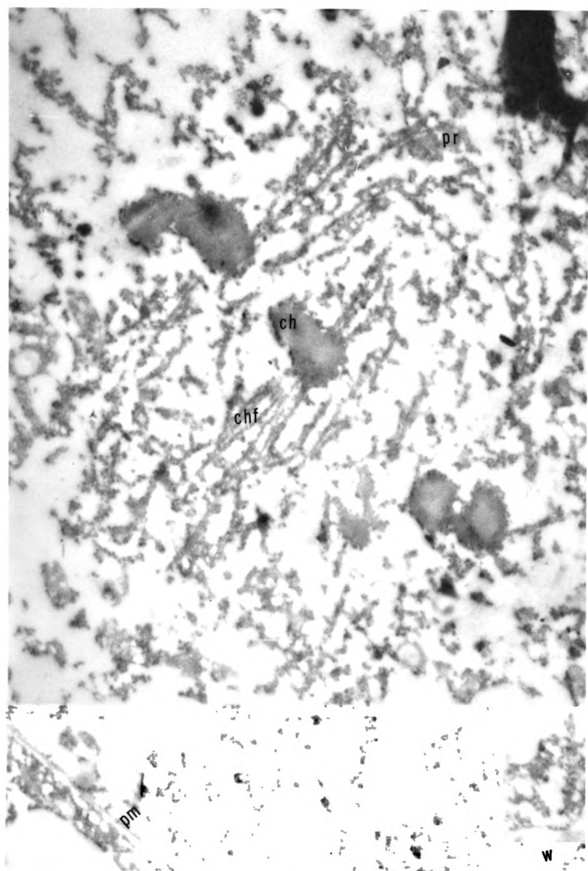


Figure 7

Fig. 8. Section through metaphase plate showing bundle of chromosomal fibers (chf), see arrows. Fixation in 2% CrO_3. Approximately X 13,200.

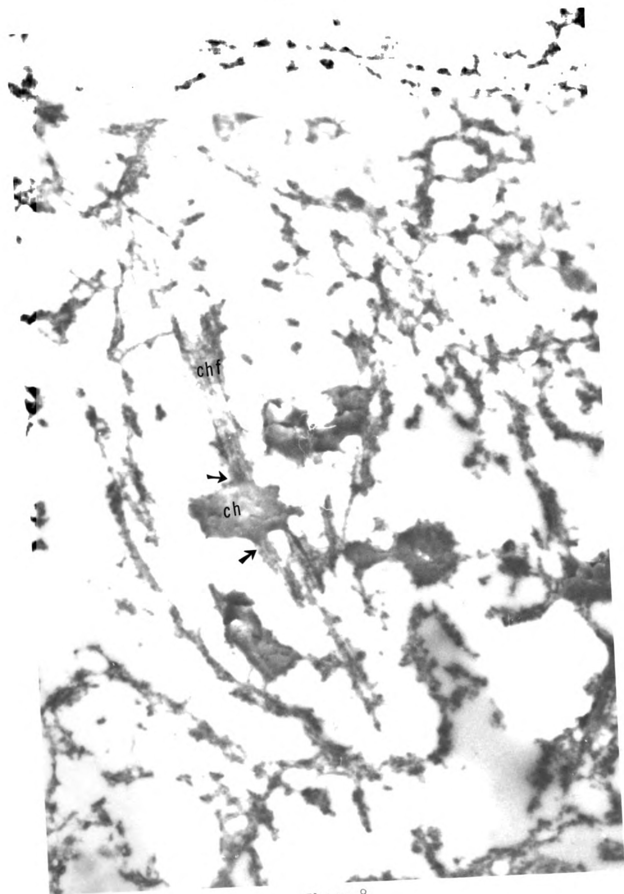


Figure 8

Fig. 9. Portion of fig. 7 enlarged to show attachment of fibers (chf) to chromosome (ch) and the granular appearance of the fibers (see arrows). Approximately X 84,000.

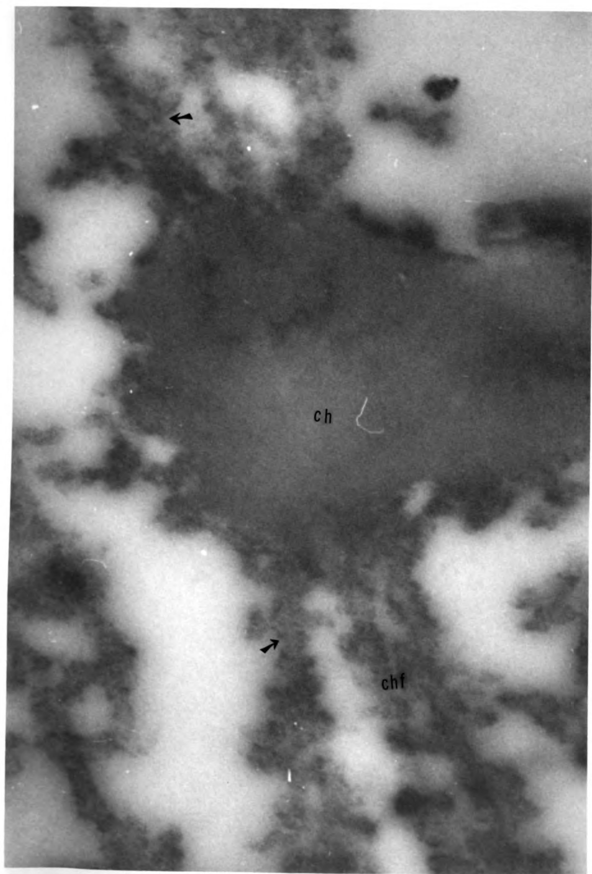


Figure 9

Fig. 10. Portion of a metaphase plate showing what appear to be continuous fibers (cf) along with chromosomal fibers (chf). Fixation in 2% $CdCl_2$. Approximately X 17,600.

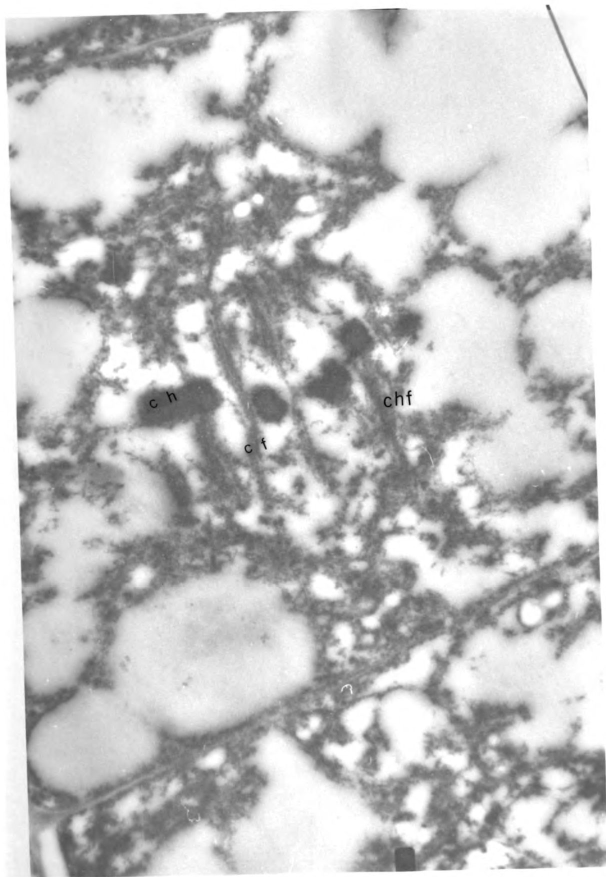


Figure 10

Fig. 11. A tangential section across metaphase plate of meristem cell, in which the chromosomes (ch) appear as low-electron dense areas across the cell, granules are shown in the spindle area (see arrows), mitochondria (m), endoplasmic reticulum (er), cytoplasmic inclusion bodies (i), proplastids (pp), plasma membrane (pm), plasmodesmata (pd), cell wall (w). Fixation in 2% $KMnO_4$. Approximately X 13,200.

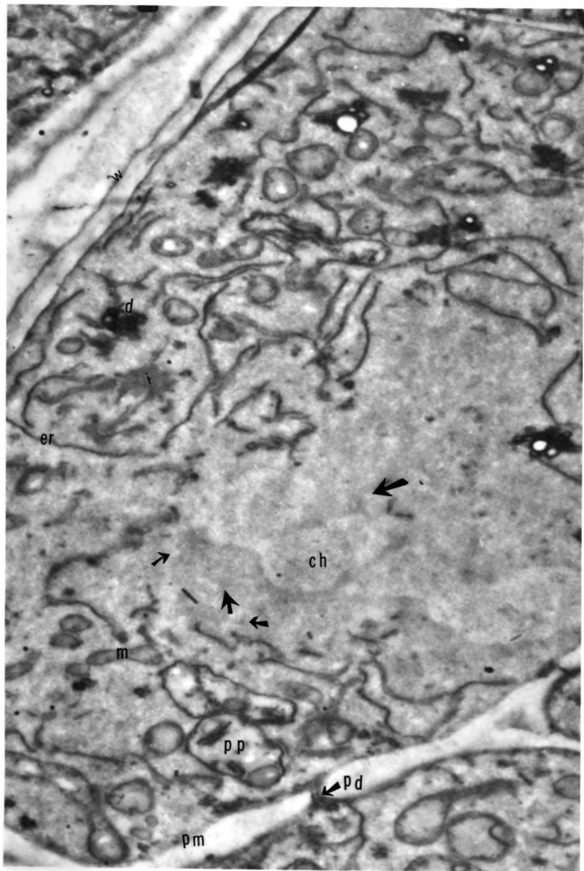


Figure 11

Fig. 12. This section shows a metaphase plate with bits of broken-down nuclear envelope (ne) and endoplasmic reticulum (er) surrounding the chromosomes (ch) that are in an area containing granules (see arrows). Dictyosomes (d), mitochondria (m), and other cytoplasmic inclusions are also shown. 2% $KMnO_4$ fixation. Approximately X 13,200.

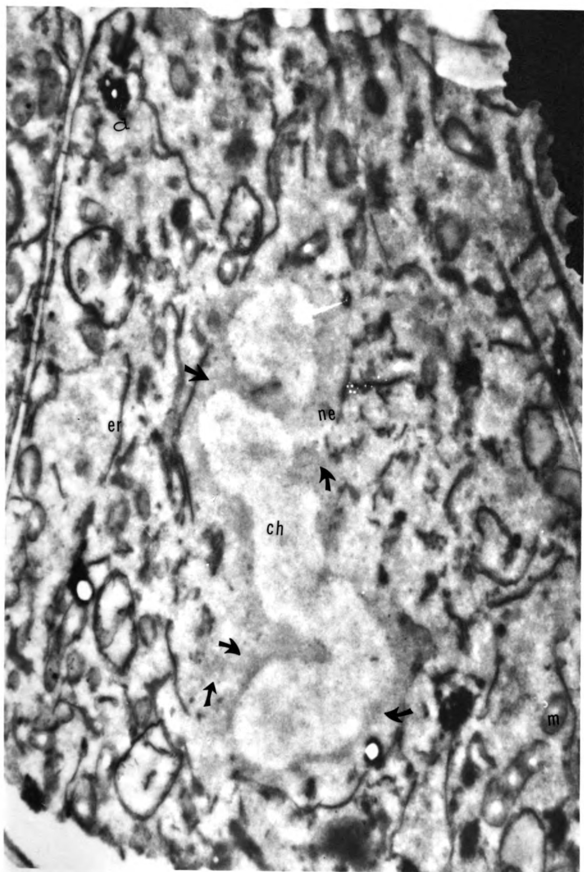


Figure 12

Fig. 13. Section through the central part of a meristem cell that shows chromosomes (ch), aligned along the equatorial plane that are surrounded by spindle area (see arrows), bits of nuclear envelope (ne), endoplasmic reticulum (er), proplastids (pp), cytoplasmic inclusions (i), dictyosomes (d), and other unidentified particles are also shown, along with the plasma membrane (pm), plasmodesmata (pd) and cell wall (w). Fixation is in a 2% $KMnO_4$ solution. Approximately X 10,800.

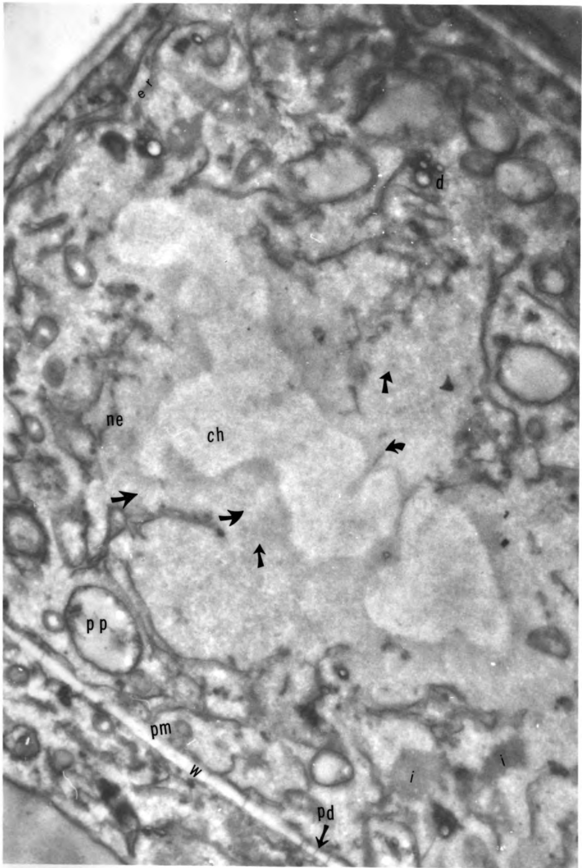


Figure 13

Fig. 14. Longitudinal section of interphase cell which shows the distribution of cytoplasmic components. Note the loose distribution of components here, in contrast to the compactness of components in the opposite ends of the cell as in the case of the metaphase stage. The plasmodesmata (pd), plasma membrane (pm), along with the dictyosomes (d), proplastids (pp), mitochondria (m), and endoplasmic reticulum (er) are shown. The nuclear envelope (ne) surrounds the homogeneous nucleus (n) which has a wavy appearance due to knife marks from sectioning. The endoplasmic reticulum (er) appears to be connected to the nuclear envelope (see arrow). Fixation in a 2% $KMnO_4$ and a 2% PTA solution. Approximately X 13,200.

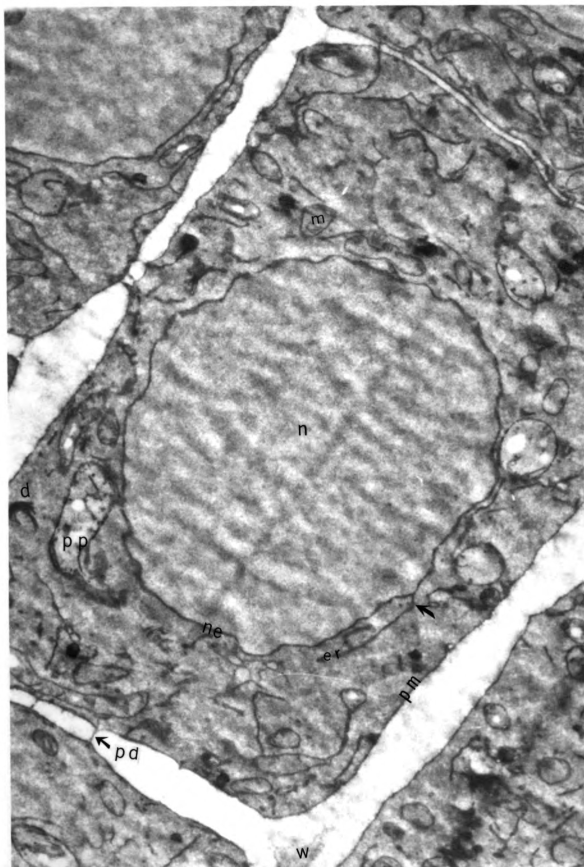


Figure 14

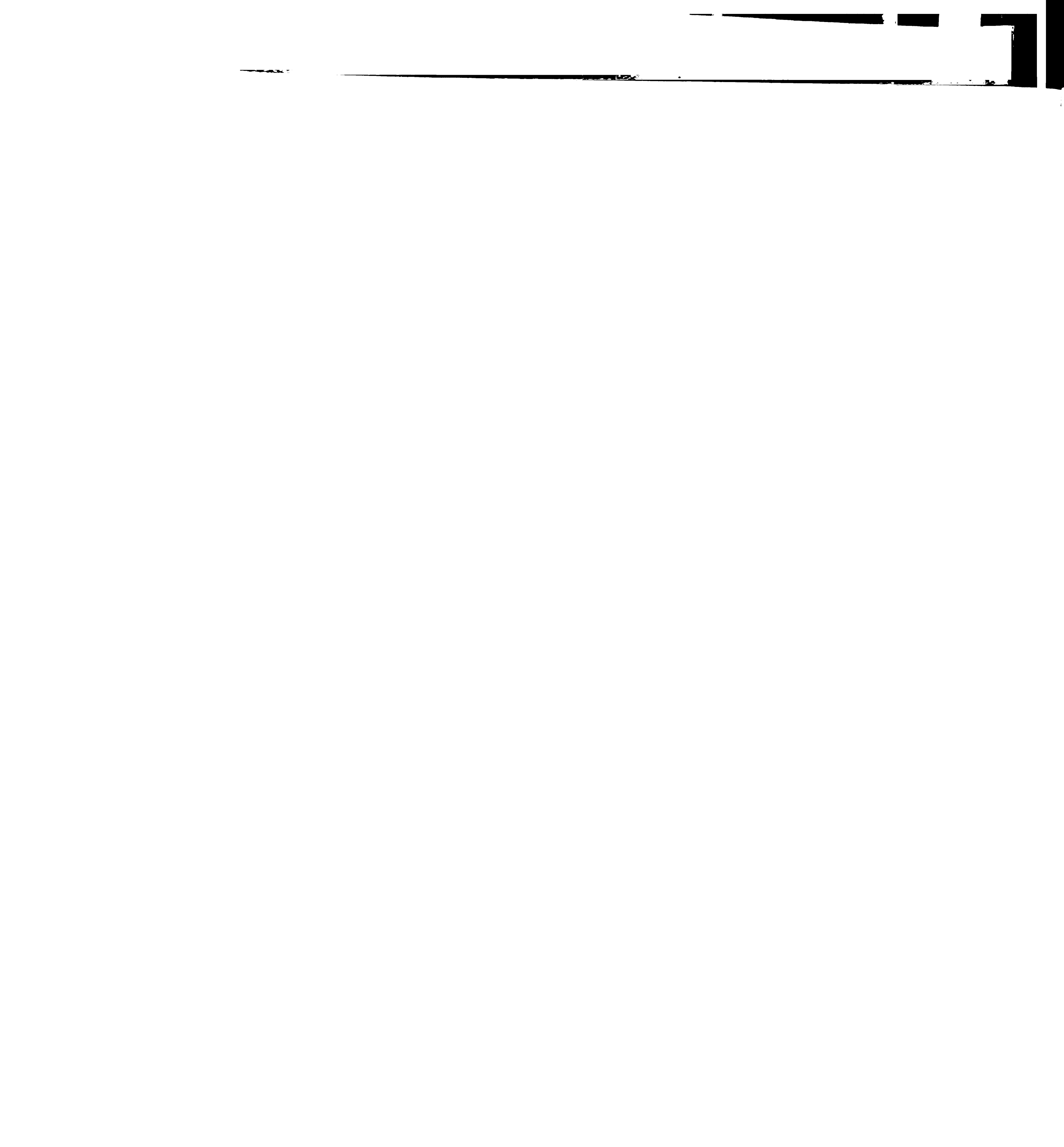


Fig. 15. This micrograph shows a longitudinal section of an interphase cell that reveals the distribution of cytoplasmic components after OsO_4 fixation in contrast to fig. 13, which is fixed in $KMnO_4$. Note that the distribution of components is similar to that of fig. 13; however, the nuclear components appear as dense granular or rod-like bodies. Vesicles are also distributed throughout the cytoplasm (arrows). The cellular components are marked as before. Approximately X 13,200.

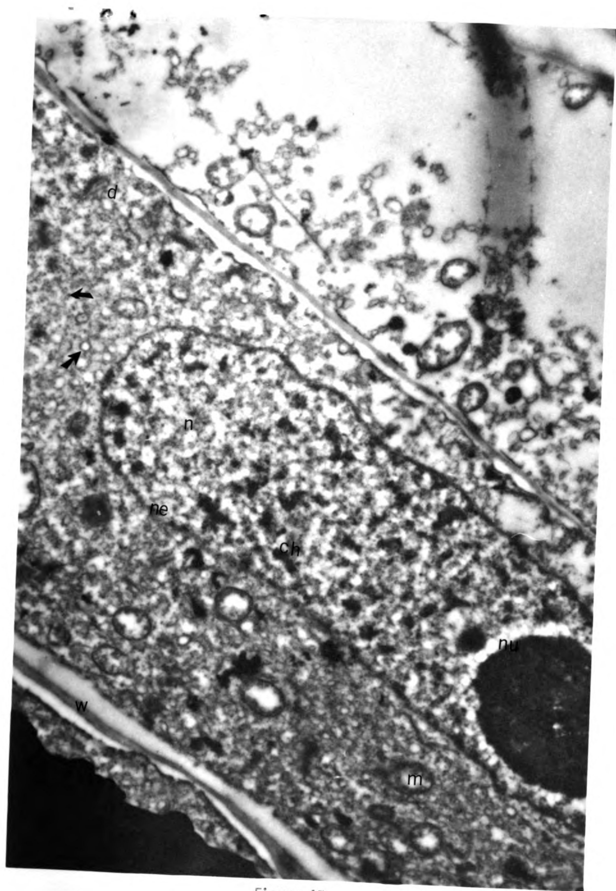


Figure 15

Fig. 16. This section shows the association of chromosomal fibers (chf) and the interzonal fibers (if) with the separating chromatids (ch). Vacuoles (v) with a dense membrane surrounding them, the tonoplast (t). Dense inclusion bodies (i) and the plasma membrane (pm) are well preserved. Fixation in modified OsO_4. Approximately X 5,600.

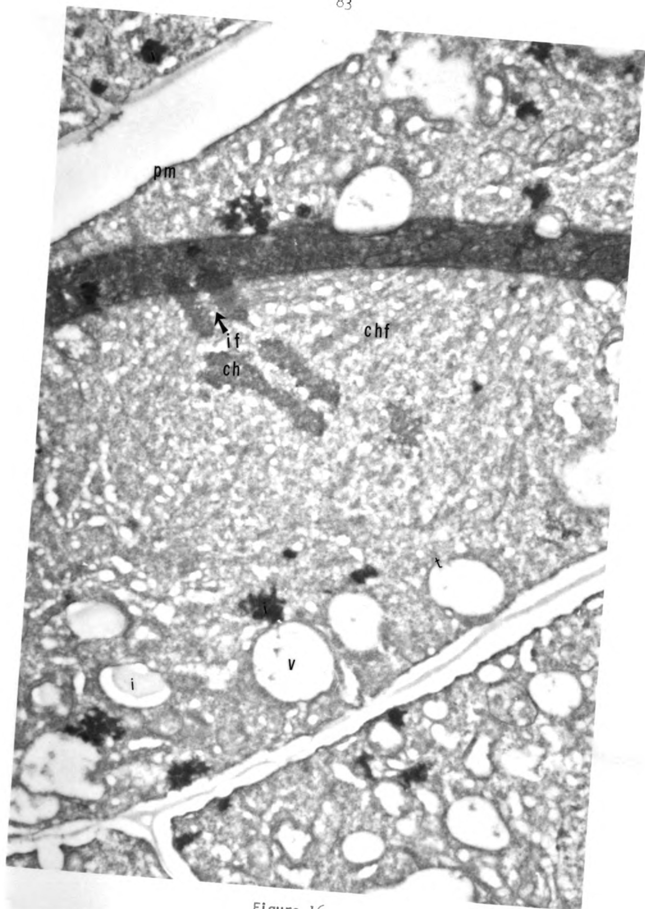


Figure 16

Fig. 17. A portion of a meristematic cell which shows a section through an early anaphase with chromosomal fibers (chf), interzonal fibers (if), and dense chromosomes (ch). Fixation in 1% OsO_4. Approximately X 20,800.

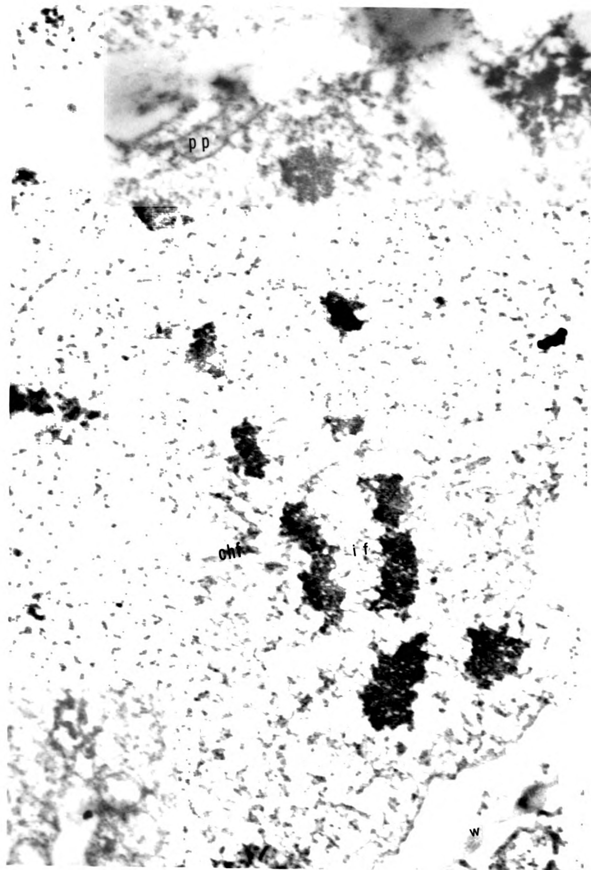


Figure 17

Fig. 18. This section shows the relation of chromosome (ch) to spindle (see arrows) and to the polar regions (pr), toward which the chromosomal fibers (chf) are oriented during anaphase. Fixation 2% CrO_3. Approximately X 13,200.

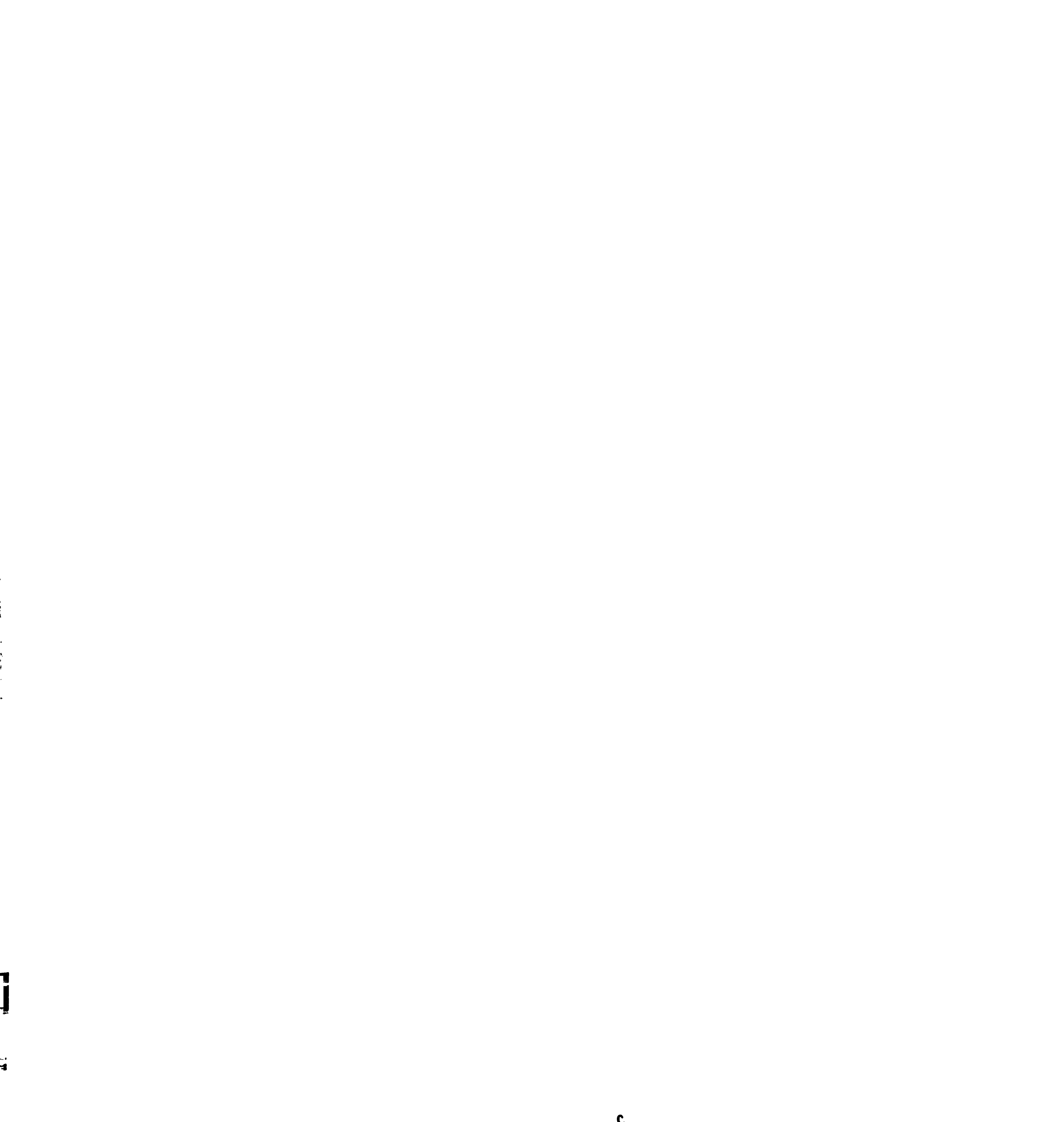


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Fig. 19. Micrograph showing a section through the central part of a meristematic cell in which the anaphase chromosomes are associated with clear lines. These probably represent the areas occupied by the spindle fibers, but with the fixative used, the fibers are not revealed as well defined electron dense lines. These clear lines (see arrows) occupy areas similar to the fibers demonstrated in fig. 18. The clear lines extend to the polar regions which contain a dense body (X). Whether these dense bodies at the polar regions are portions of chromosomes or inclusion bodies is not clear at this point. Vacuoles (v) occur in the cytoplasmic area surrounding the spindle body. Fixation in 2% $K_2Cr_2O_7$ and 10% formalin at pH 7.2. Approximately X 13,200.

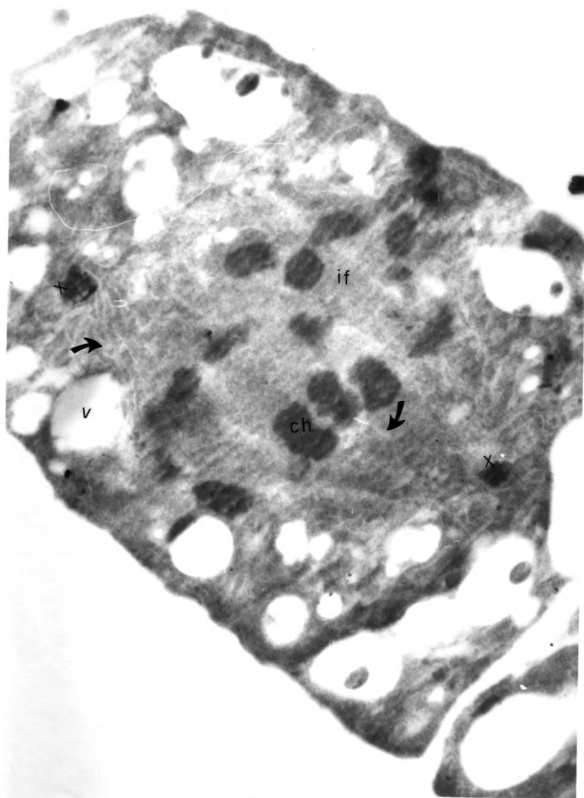


Figure 19

Fig. 20. This micrograph shows a section through a cell at mid-anaphase in which the chromosomal fibers (chf) are oriented toward and ending in the polar region (pr). The chromosomes (ch) are shown with lagging arms (X) and with kinetochores directed toward the poles. The interzonal fibers (if) are located between the separated chromatids. Fixation in 2% CrO_3. Approximately X 13,200.

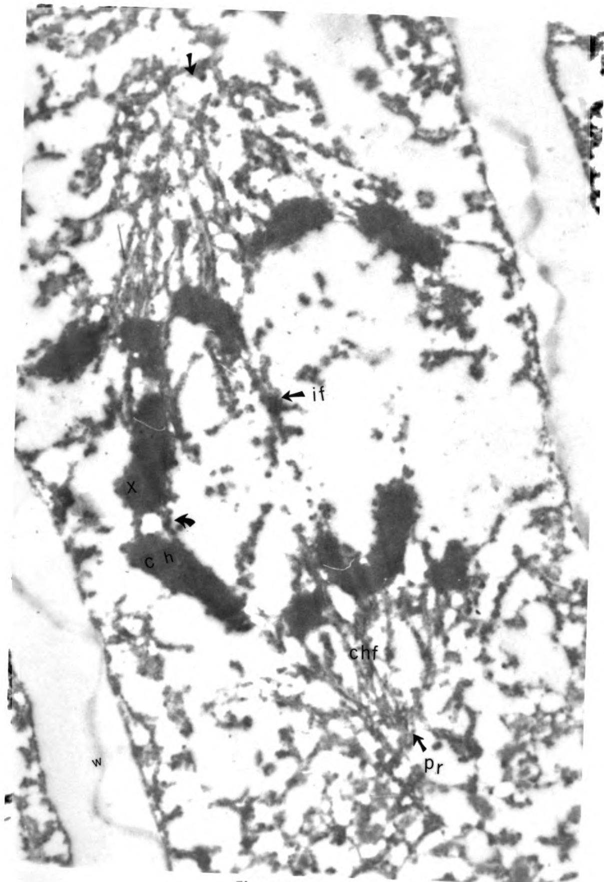


Figure 20

Fig. 21. Portion of an anaphase cell showing cytoplasmic organelles being pushed to both ends of the cell (see arrows), chromosomes (ch), interzonal fibers (if), proplastids (pp), and mitochondria (m). Dense granules appear throughout the cytoplasm. Fixation in modified OsO_4 at pH 5.6. Approximately X 13,200.

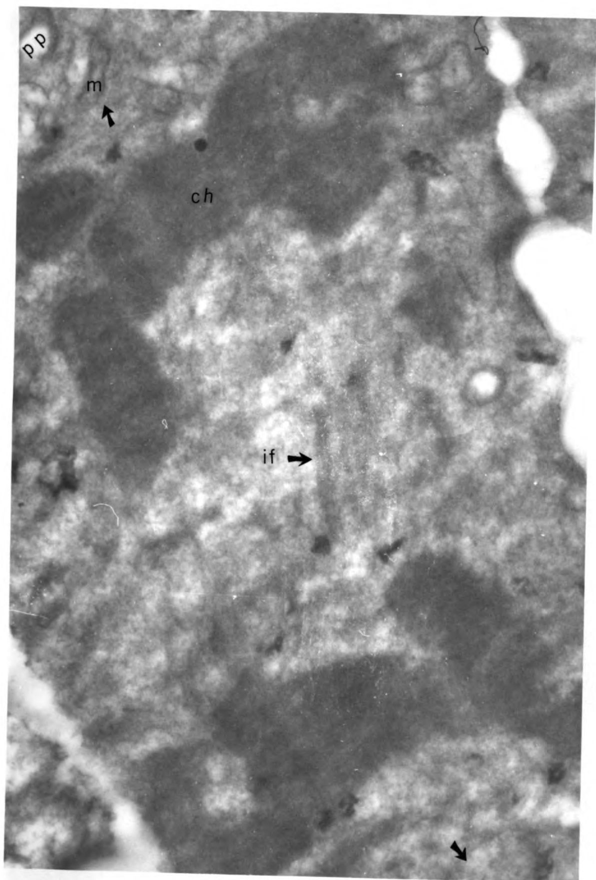


Figure 21

Fig. 22. This micrograph shows a longitudinal section through a late anaphase stage, from the meristematic region of the root tip. The vacuoles (v) contain dense granular inclusion bodies (i). The chromosomes (ch) appear as dense bodies with the chromosomal fibers (chf) and interzonal fibers (if) attached to them (see arrows). Fixation in 1% OsO_4 and 1% $CdCl_2$. Approximately X 20,800.

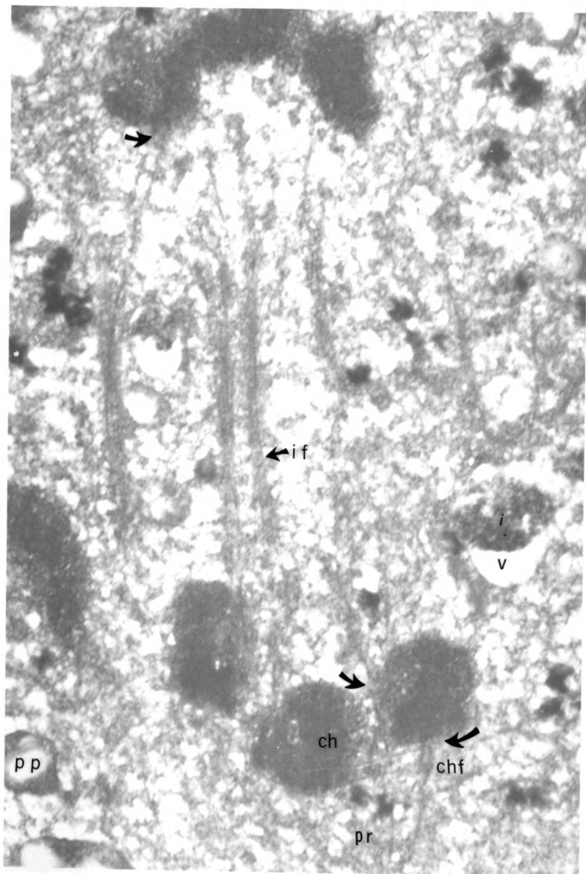
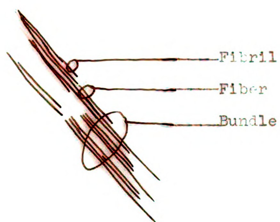


Figure 22

Fig. 23. An enlarged view of fig. 21 which shows the fibrillar nature of the interzonal fibers (if). A bundle consists of several fibers (see overlay), while the fibrils make up the fibers (overlay). Fixation in OsO_4. Approximately X 63,600.


Fibril
Fiber
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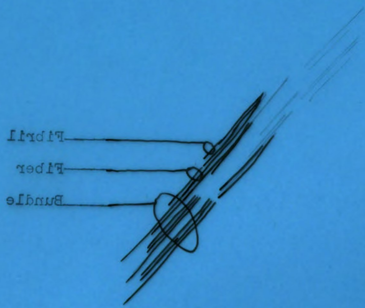
Fibril
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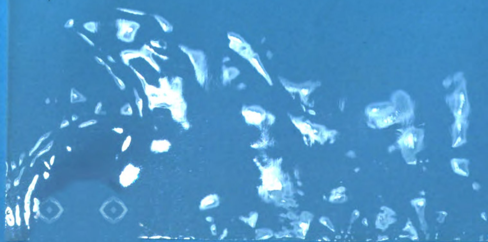

Figure 23

Fig. 24. A section through an anaphase cell which shows chromosomes (ch) with lagging arms (see arrows), and granules along the length of the interzonal fibers (if). Fixation in $K_2Cr_2O_7$ and 10% formalin. Approximately X 25,600.

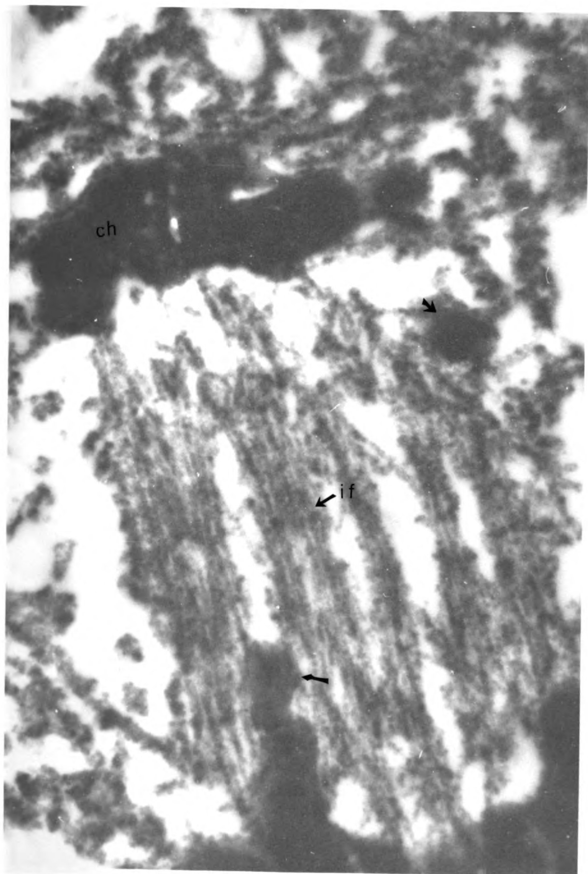

Figure 24

Fig. 25. This micrograph shows an anaphase cell after fixation in $KMnO_4$. The chromosomes (ch) appear as non-electron dense areas. The interzonal region (area between the separated chromatids) contains granules (see arrows). This fixative (a 2% $KMnO_4$) does not preserve the spindle fibers as very dense lines as does the OsO_4 and the CrO_3. Note the compactness of the cytoplasmic components in the opposite ends of the cell (X_1 and X_2). Approximately X 10,800.

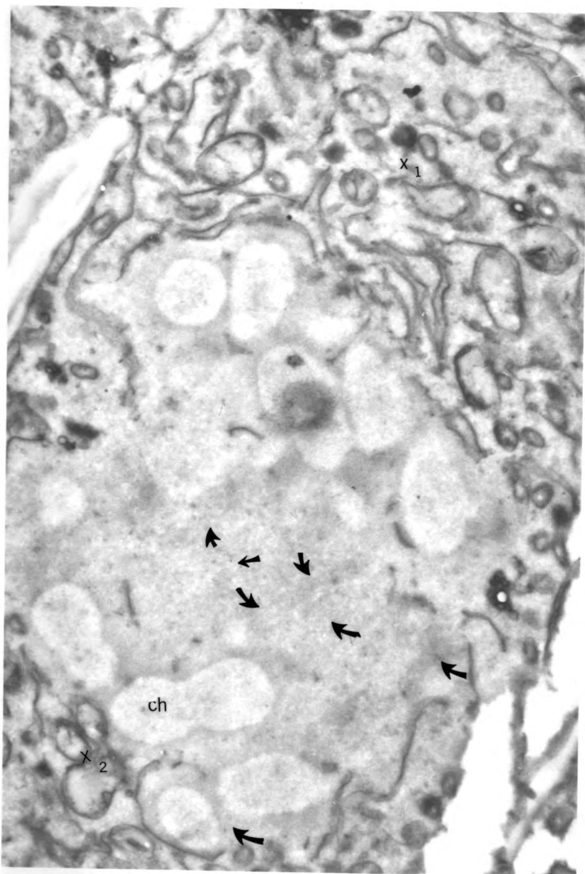

Figure 25

Fig. 26. Section through an anaphase cell that shows bits of either the broken-down nuclear envelope (ne) or the endoplasmic reticulum (er) around the area surrounding the anaphase chromosomes (ch), the dense streak across the cell is a fold in the plastic film on which the section is being supported. Note granules in the interzonal region (arrows). Fixation as in fig. 25. Approximately X 10,800.

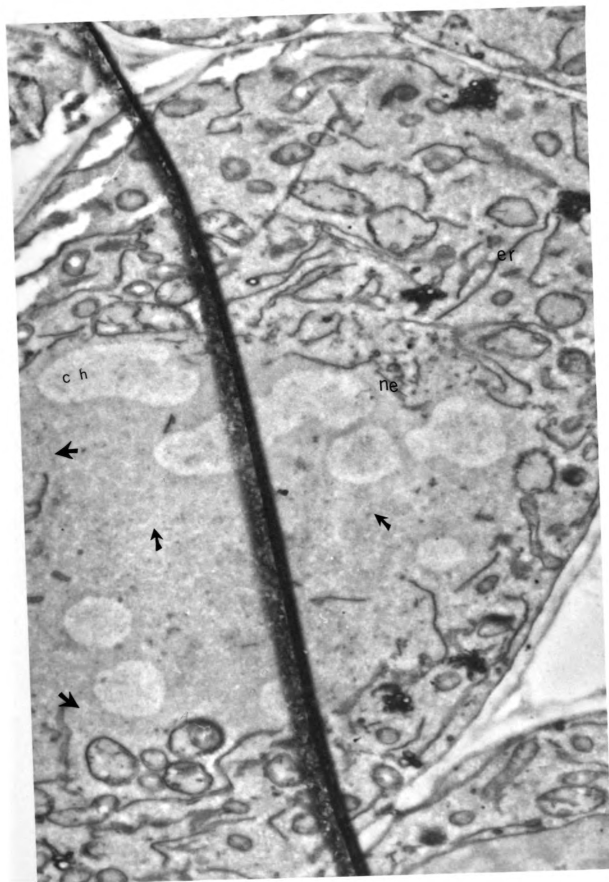

Figure 26

Fig. 27. A portion of an early telophase with two rows of granules across the mid-region (see arrows) of the cell. This marks the inception of cell plate formation. Chromosomes (ch) are in the process of regrouping for daughter nuclear formation. Fixation is in a modified OsO_4. Approximately X 20,800.

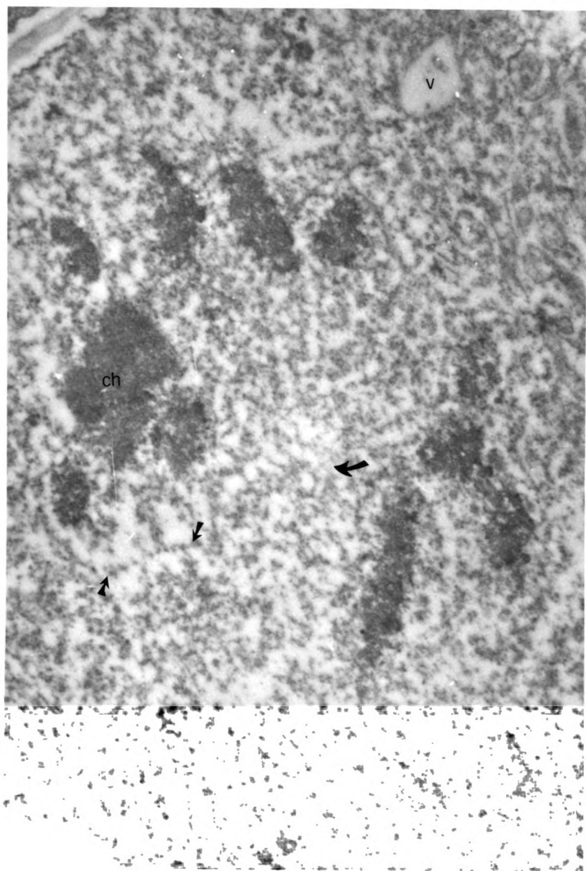

Figure 27

Fig. 28. This section through the cortical region of the meristem shows a telophase cell with regrouping of chromatids (ch) to form the daughter nuclei (the dense particle near the left side of the lower regrouping nucleus is a piece of debris on the section). The mid-region contains a "barrel-shaped" phragmoplast (see arrows) with two apparent rows of granules across its mid-region marking the cell plate formation (cp). The mitochondria (m) proplastids (pp), vacuoles (v), plasma membrane (pm) and cell wall (w) are also preserved. Fixation in 1% OsO_4. Approximately X 13,200.

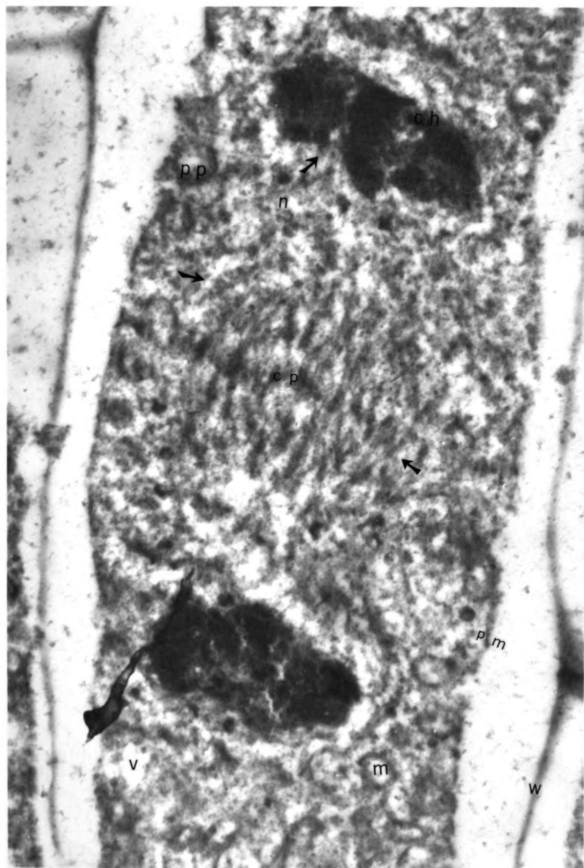

Figure 28

Fig. 29. Section showing barrel-shaped phragmoplast (see arrows) regrouped chromosomes (ch) developing cell plate (cp). Note the adjacent cell to left where the cell plate (cp) has extended to the cell wall (w). Fixation in 2% $CdCl_2$. Approximately X 8,000.

Figure 29

Fig. 30. A longitudinal section through a telophase which shows the diminishing fibrils of the phragmoplast (see arrows), and the cell plate (cp). Fixation in 10% formalin and 1% OsO_4. Approximately X 8,000.

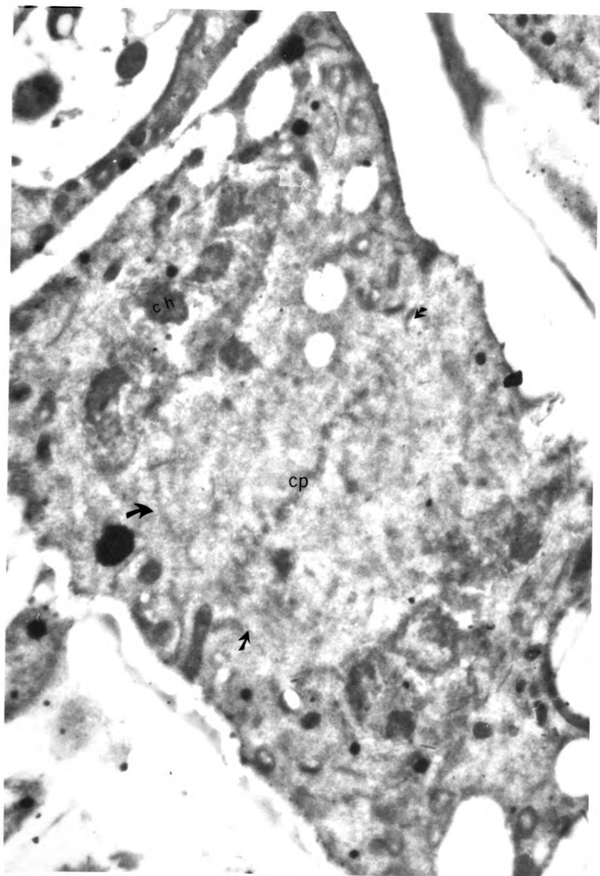

Figure 30

Fig. 31. Portion of telophase cell where the fibrils of the phragmoplast are increasing in granularity (see arrows). Fixation in 2% $K_2Cr_2O_7$ and 10% formalin. Approximately X 10,800.

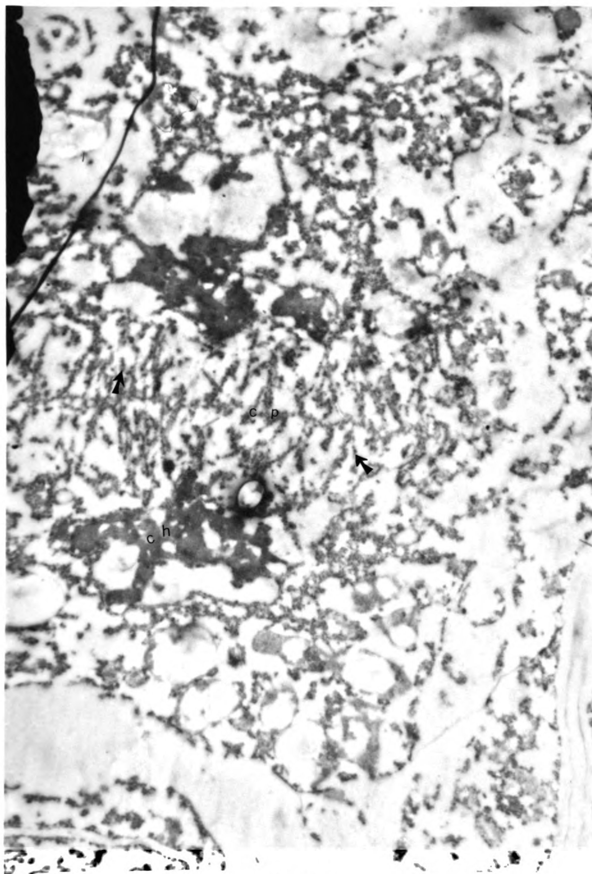

Figure 31

Fig. 32. This micrograph shows a section through a telophase cell where the cell plate (cp) extends the entire width of the cell (see arrows) separating the daughter nuclei (n). Fixation in 2% $K_2Cr_2O_7$ and 10% formalin. Approximately X 13,200.

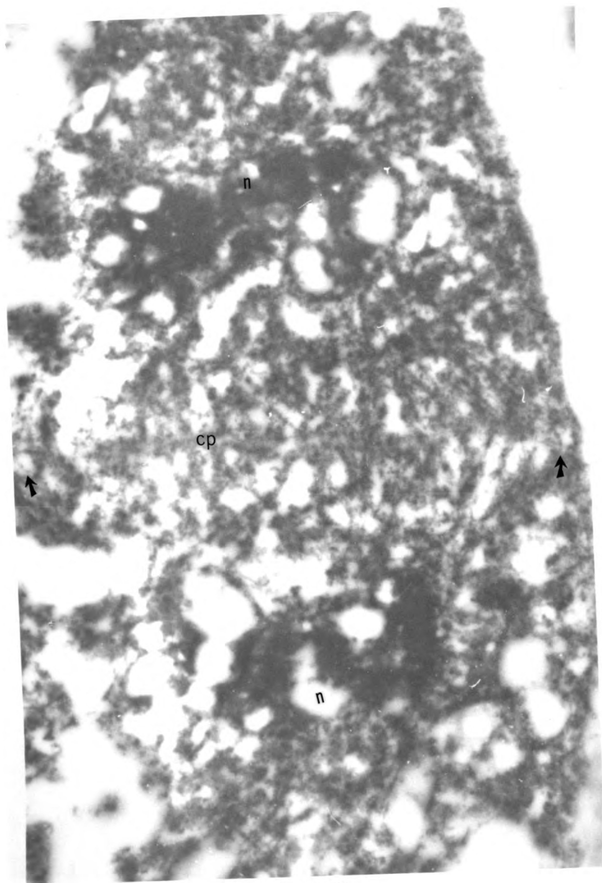

Figure 32

Fig. 33. Portion of late telophase to show diminishing fibers of phragmoplast (see arrows) and extension of cell plate (cp) across width of the cell. At X the two membranes of the developing cell plate are continuous with each other forming an opening across its width, which provides a cytoplasmic connection between the two protoplasts. Fixation as in fig. 32. Approximately X 20,800.

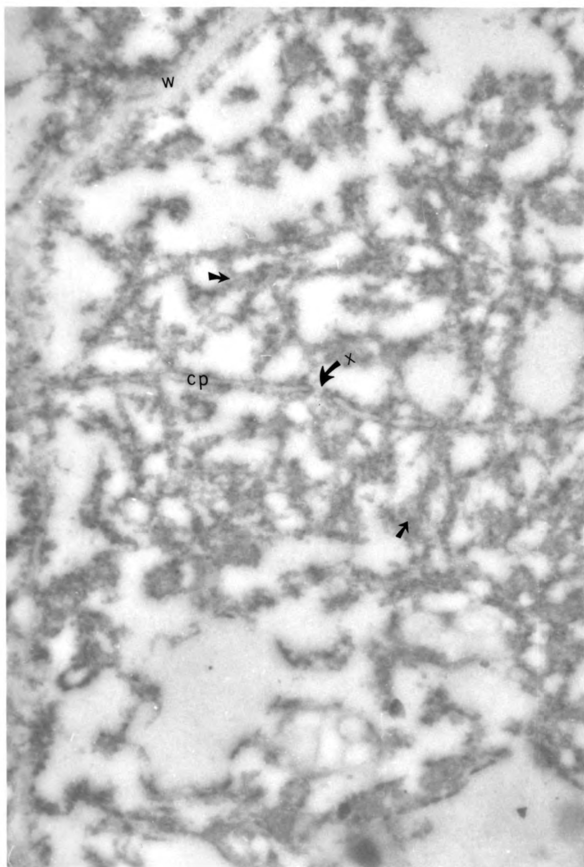

Figure 33

Fig. 34. Longitudinal section through the peripheral region of a telophase cell (missing daughter nuclei altogether), cell plate (cp), plasmodesmata (pd), inclusion bodies (i) and other cytoplasmic components are shown as described before. Fixation in 2% $KMnO_4$. Approximately X 13,200.

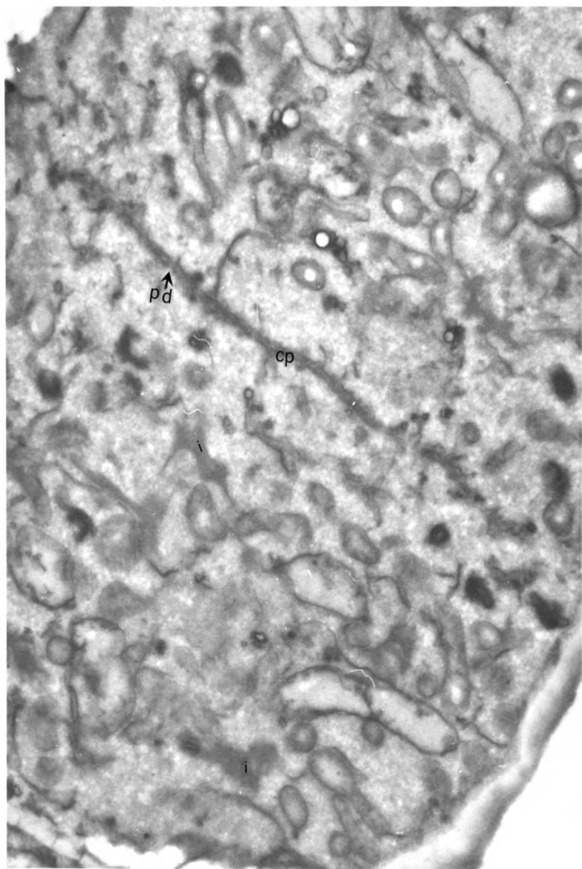

Figure 34

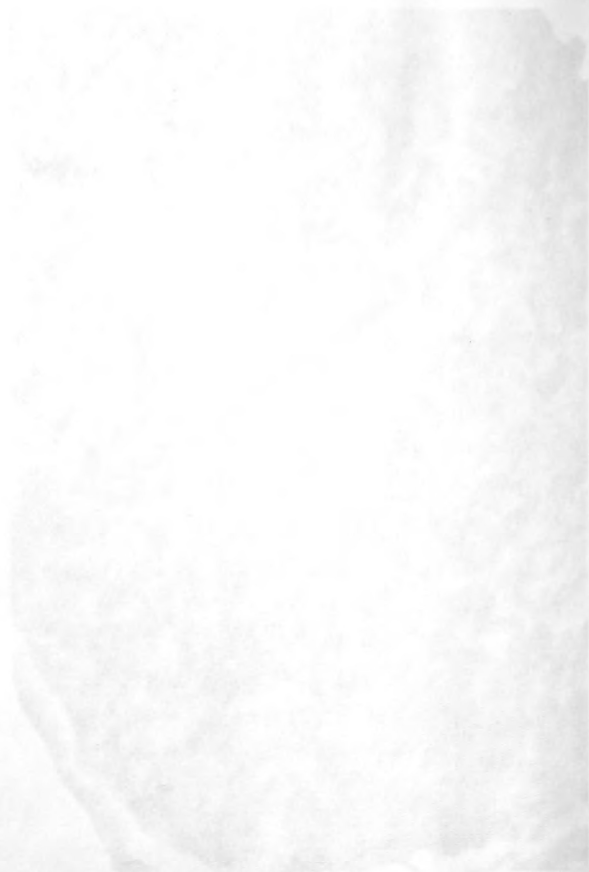

Fig. 35. Section through one end of a telophase cell which shows the reorganization of the nuclear envelope (ne), inclusion bodies (i), mitochondria (m), proplastids (pp) and small dense granules (see arrows) associated with the developing cell plate (cp). Fixation in 2% $KMnO_4$. Approximately X 13,200.

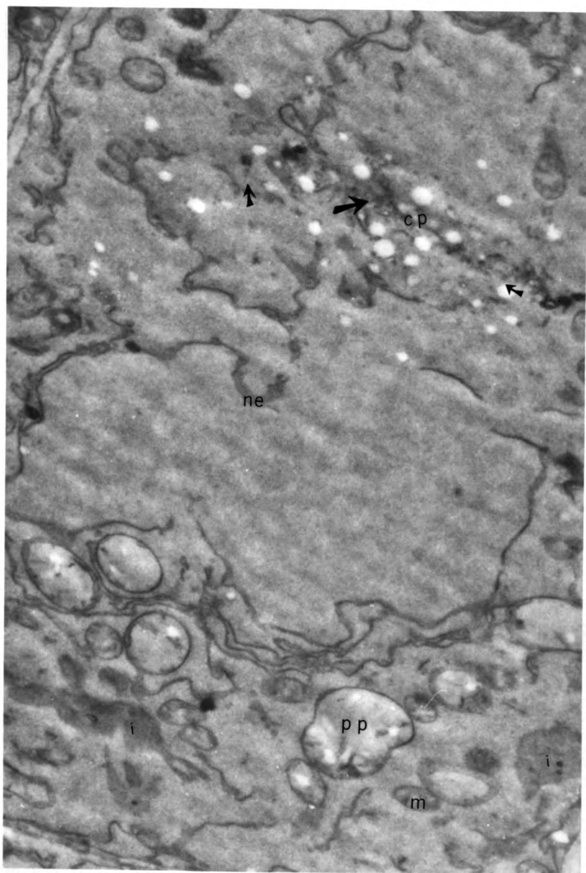

Figure 35

Fig. 36. Longitudinal section through telophase cell showing regrouped chromosomes (ch) within the newly re-organizing daughter nuclei (n). Note nuclear envelope (ne) reformation, cell plate (ce) in center of cell with mitochondria (m), proplastids (pp), endoplasmic reticulum (er) and other cytoplasmic organelles appearing in the central region (see arrows) of the cell. $KMnO_4$ fixation. Approximately X 10,800.

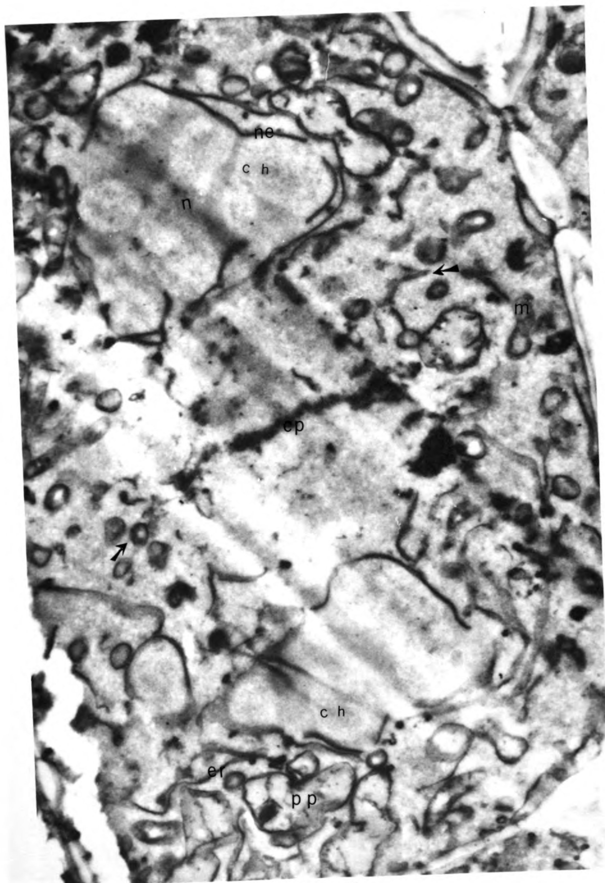

Figure 36

Fig. 37. Portion of dividing cell showing "scattered" arrangement of chromosomes (ch) with bits of attached chromosomal fibers (chf, see arrows) after treatment with a 4.5 X 10^{-4} M of colchicine for 30 minutes and recovered for 6 hours. Fixation in OsO_4. Approximately X 20,800.

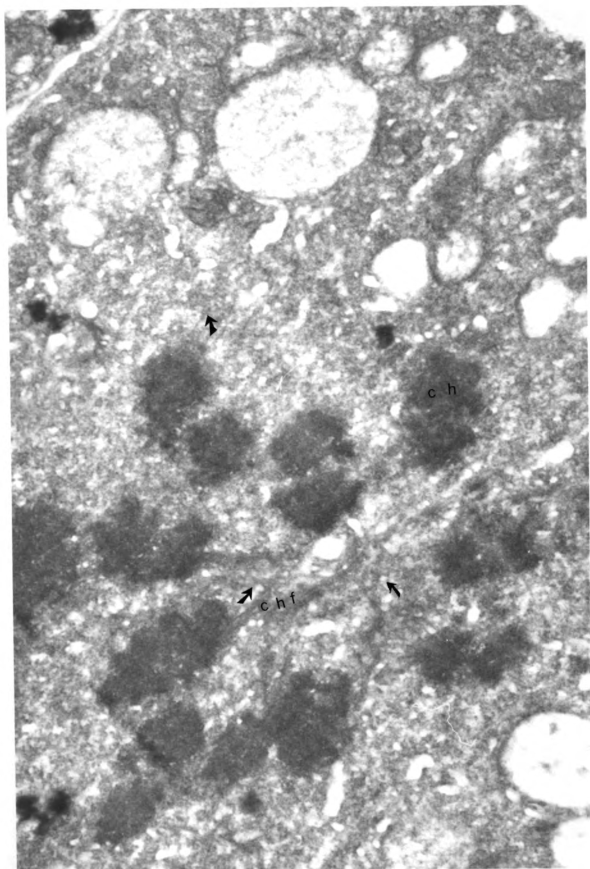

Figure 37

FIGURE 33

Fig. 38. This micrograph shows a section through a prometaphase "clump." As a result of treatment with 4.5 X 10^{-4} M of colchicine for 30 minutes and a recovery of 6 hours the usual metaphase plate is not established (as in figs. 4, 6 and 12), because the chromosomal fibers are destroyed, preventing this arrangement from taking place. Here the chromosomes (ch) appear as dense bodies. Fixation in 2% $CdCl_2$ and 1% OsO_4. Approximately X 20,800.

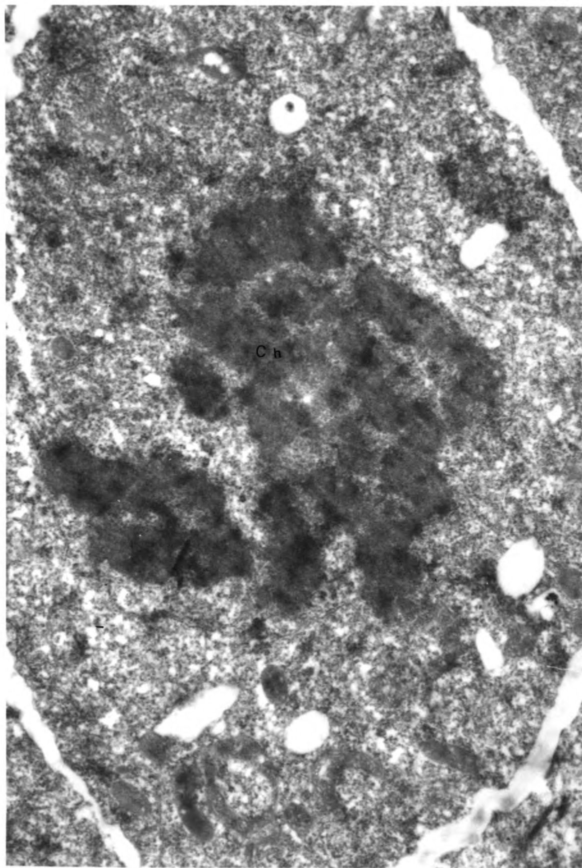

Figure 38

Fig. 39. Section through a tetraploid interphase cell. As a result of fibrillar disorganization by colchicine treatment, the chromosomes continue their morphological cycle *in situ*, becoming telomorphic and the interphase nucleus is eventually reconstituted which is a tetraploid. Here the chromatin (ch) and nucleoli (nu) appear as dense bodies within the nuclear area (n). Fixation in 2% CrO_3 and formalin at pH 7.2. Approximately X 13,200.

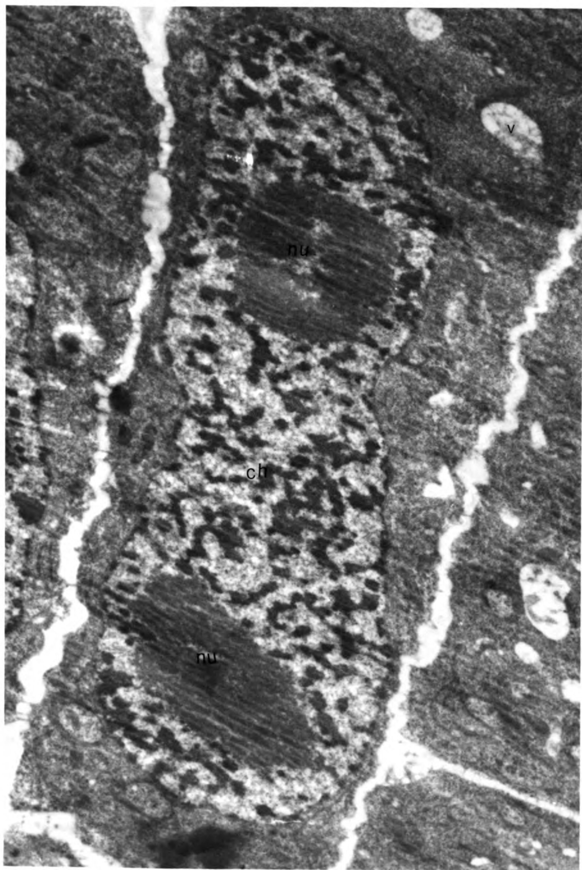

Figure 39

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