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FINE STRUCTURE OF CELL DIVISION IN  
PSILOTUM NUDUM.

Iowa State University of Science and Technology  
Ph.D., 1964  
Botany

University Microfilms, Inc., Ann Arbor, Michigan

FINE STRUCTURE OF CELL DIVISION IN PSILOTUM NUDUM

by

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A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Major Subject: Cell Biology

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1964

## TABLE OF CONTENTS

|                       | Page |
|-----------------------|------|
| INTRODUCTION          | 1    |
| LITERATURE REVIEW     | 3    |
| MATERIALS AND METHODS | 31   |
| OBSERVATIONS          | 34   |
| DISCUSSION            | 49   |
| SUMMARY               | 65   |
| LITERATURE CITED      | 68   |
| ACKNOWLEDGMENTS       | 78   |
| APPENDIX              | 79   |

## INTRODUCTION

The concept that all cells originate from pre-existing cells through the process of cell division is a foundation stone of modern biology. Upon this aphorism rests the principle of genetic cellular continuity wherein is embodied the major principle of heredity and the very existence and perpetuation of life.

Since the first observations of cell division in the 1740's (cf. Hughes, c1959), a great deal of light microscope work concerning cell division has been undertaken. However, the early investigators were limited by the resolution of their microscopes. With the development of the electron microscope, attainable resolution changed from a maximum of  $.2 \mu$ , with the light microscope, to 10 - 20 A. The advantages of this improvement in resolution for the study of cellular fine structure are obvious.

This research is directed toward an overall look at cell division in one organism as well as supplying information on the following specific areas: (1) the assembly of the filamentous structure in the mitotic apparatus, (2) the behavior of nucleoli through mitosis, and (3) the fine structure of mitotic chromosomes.

Cell division as used in this paper will refer only to the actual processes of mitosis and cytokinesis, during which the nucleus and the cytoplasm are divided. Information about the interphase cell will be used to help clarify aspects of this partition, e.g., descriptions of cellular components in interphase for purposes of comparison with similar components during the division process.

Psilotum nudum (L.) Beauv. was chosen as the organism for this study. This plant is considered by most botanists as being phylogenetically close to the oldest fossil vascular plants and, consequently, it has been called a "living fossil". The sporophyte generation is composed of a rhizome with aerial branches on which are borne sporangia and small bract-like appendages which lack vascular tissue. The most interesting external features of the plant are its lack of true roots and true leaves and its dichotomous branching.

This organism is well adapted to the present study for several reasons. The plant is easily grown in the greenhouse where, under the proper conditions, it grows vigorously the year round; thus it provides dividing cells whenever they are needed. The primary sporogenous tissue in the developing sporangia undergoes several mitotic divisions in the formation of sporocytes which then undergo meiosis, giving rise to spores. During the mitotic divisions some synchrony of division occurs resulting in packets of cells in the same stage of division. Another advantage of this plant is its very high chromosome number, reported to be  $2n = \text{ca. } 104$  for diploids and  $2n = \text{ca. } 208$  for tetraploid forms (Ninan, 1956). In studying the fine structure of the chromosome, this high number permits the observation of a larger number of chromosomes at one time in the electron microscope than would have been possible with plants having lower numbers, and the probability of observing profiles of chromosomes cut in such a way as to reveal details of structure is much higher. Finally, these cells have very thin walls, making the tissue easier to section.

## LITERATURE REVIEW

The Cytology of Psilotum nudum

A very large number of studies of cell division have been published during the past century. Most of these employed the light microscope as the main tool of investigation. Reviews of this literature may be found in Wilson (1928), Hughes (1952, c1959) and Schrader (1953). Mazia (1961) has written a more recent review incorporating such work as the modern techniques of ultracentrifugation, autoradiography and electron microscopy.

Psilotum nudum (L.) Beauv. has been the object of several cytological studies (cf. Chiarugi, 1960; Fabbri, 1960, 1963). Diploid forms ( $2n = \text{ca. } 104$ ), triploid forms ( $2n = \text{ca. } 156$ ) and tetraploid forms ( $2n = \text{ca. } 208$ ) have been reported. Under favorable environmental conditions, meiosis in the diploid and tetraploid forms is regular while that in the triploids is quite irregular (Ninan, 1956; Bierhorst, 1958). The basic haploid number appears to be 52, which may have been derived from lower numbers such as 26 and 13. This number, as Ninan has pointed out, may be significant since most of the ancient genera of ferns possess chromosome numbers which are multiples of 13. That Psilotum may comprise a natural autopolyploid series has been suggested by Manton (1950) and Ninan (1956). In comparing this plant with the large group of pteridophytes she has investigated, Manton has stated that Psilotum is the only clear case where there are positive grounds for suspecting autopolyploidy. This statement was based on the observation of multivalent

pairing within triploids and tetraploids undergoing meiosis.

The size of the chromosomes is fairly large when compared with other ancient vascular plants (Ninan, 1956). This is particularly significant considering the large number of chromosomes found in this plant.

#### Fine Structure of the Meristematic Plant Cell

##### Cytoplasmic components

Three recent papers (Whaley et al., 1960a; Manton, 1961a; Buvat, 1963) review electron microscope work dealing with plant material.

The meristematic cell differs from most differentiated plant cells in three major ways. First, the meristematic cell has a very large nucleus in relation to its size (Whaley et al., 1960a); secondly, it has a group of small vacuoles, whereas the differentiated cell usually has one very large vacuole occupying the greatest portion of the cell volume; finally, the cell wall is composed of a primary wall only, with no thickening.

The cytoplasm contains the usual components, mitochondria, endoplasmic reticulum and Golgi bodies, that are common to all nucleated higher plant and animal cells, as well as the proplastids and vacuoles that are typical of higher plant cells. The mitochondria of plant cells do not have the elaborated lamellar cristae (Whaley et al., 1960a; Chardard, 1962) described in animal cells (Palade, 1952b) but the cristae, formed by infolding of the internal membrane of the mitochondrial wall, appear more frequently as tubules with a more sinuous, irregular orien-

tation (cf. Buvat, 1963). The vacuoles stain differently depending on the procedures of fixation and staining used. In potassium permanganate fixed material, treated in the usual way, they appear more electron dense than the ground substance (Whaley et al., 1960a; Manton, 1961a), while in osmium fixed material, vacuoles appear electron transparent.

The endoplasmic reticulum (Porter, 1953, 1956; Palade, 1955b) is composed of a membrane-bound, vesicular system extending throughout the cytoplasm. Continuities between the endoplasmic reticulum and the nuclear envelope (Mollenhauer, 1959; Porter and Machado, 1960; Marinos, 1960; Whaley et al., 1959, 1960b) have been shown frequently, whereas, obvious continuities with the plasma membrane (electron micrograph by N. Poux in Buvat, 1963) are less frequent. Because of the continuities with the endoplasmic reticulum, the nuclear envelope has been considered a component of this vesicular system (Watson, 1955, 1959; Whaley et al., 1960b). The endoplasmic reticulum has also been shown to extend to the cell surface and pass through the wall into neighboring cells through plasmodesmata (Whaley et al., 1960a).

Associated with the outer surface of the endoplasmic reticulum are ribosomes, ribonucleoprotein granules of about 150 A diameter (Palade, 1955a, b, 1956 and many others). Elements of the endoplasmic reticulum containing these particles are referred to as rough endoplasmic reticulum while regions free of particles are called smooth endoplasmic reticulum. Ribosomes are also scattered throughout the ground substance of the cytoplasm (Buvat, 1958; Caporali, 1959; Porter and Machado, 1960). Ribosomes appear to group together into whorls (Palade, 1955a; Watson,

1959 and many others) and helical arrangements (Behnke, 1963; Waddington and Perry, 1963). These latter arrangements are probably larger groups than the "polyribosomes" (Waddington and Perry, 1963) and their significance has not yet been determined. Fabbri (1960) has shown that the interphase cell in Psilotum is particularly rich in ribonucleic acid (RNA).

The plasma membrane is a unit membrane. It shows variations in its appearance at different stages of activity (Whaley et al., 1960a; Chardard, 1962). Usually it has a smooth, regular appearance but at other times it develops numerous invaginations suggesting pinocytotic activity (Chardard, 1962).

The nuclear envelope, on the other hand, consists of two unit membranes, each 75 - 80 A thick, separated by a perinuclear space of 50 - 110 A (Chardard, 1962; Whaley et al., 1960b). The outer membrane, as already stated, is continuous with the endoplasmic reticulum and has ribosomes associated with it. The membranes of the nuclear envelope differ from other membranes only by the presence of pores (De, 1957). These pores are described as circular openings from 200 - 600 A in diameter (Whaley et al., 1960a; Marinos, 1960) and, after potassium permanganate fixation, show no annulus or structure within the pore. Watson (1959) described pores in the nuclear envelope of animal cells after osmium tetroxide fixation as "pore complexes". Each complex is composed in part of a low density channel in the nucleoplasm. This channel surrounds the inside of a nuclear pore through which the contents of the channel pass to form a small extrusion in the cytoplasm.

The channel material, the cortex of the cytoplasmic extrusion and the pore margin are organized into a radially symmetrical formation. Thus any passage of material through the pore complex would appear to be controlled by structural organization within the pore.

The nuclear envelope goes through a sequence of events during the division process which has been described by Moses (1960) in the crayfish, Cambarus clarkii, as follows: 1) localized irregularities appear in the profiles of both membranes and uneven separations occur between the membranes; 2) interruptions in the nuclear envelope arise in areas where the chromatin is not attached; 3) finally pinching off of small (100 - 150  $\mu$ m) vesicles from the edges of the segments of the nuclear envelope takes place.

These vesicles are indistinguishable from other vesicles normally found in the cytoplasm. Whole sheets of double membranes may also be detached from the envelope; these may or may not break up into small vesicles later. Porter (1956) has also shown that the endoplasmic reticulum seems to go from a continuous to a discontinuous phase during prophase.

The segments of the nuclear membrane still contain pores (Moses, 1960), however, these do not appear to persist through division (Afzelius, 1955).

At telophase, vesicles, similar to those of the endoplasmic reticulum, surround the groups of chromosomes and fuse into flattened cisternae, starting at the poleward edge of the chromatin masses and eventually surrounding the chromatin. This has been shown by Moses

(1960), not only for animal cells but also for meristematic cells of the root tips of Allium and Vicia and also for orchids by Chardard (1960, 1962).

The nuclear membrane, according to Buvat (1963), does not have pores when it is first formed.

#### Nuclear components

The nucleus at interphase contains from one to several nucleoli, chromatin masses and a ground substance, the nucleoplasm, and these are surrounded by an envelope which has already been described. Membranous structures, found occasionally within the nucleoplasm, are probably due to accidental inclusion when the nuclear envelope was reformed during the preceding telophase.

The nucleoplasm appears, in electron micrographs, to be composed of granules and fibrous material embedded in an electron transparent substance. The nucleoplasm often appears denser than the cytoplasm (Whaley et al., 1960a; Buvat, 1963). Swift (1959), using osmium tetroxide fixation at acid pH and lead subacetate stain, described a granular component of the interchromatic areas of nuclei of acinar cells of the newt, Triturus. These particles, not obviously related to either nucleolus or cytoplasm, measured 300 A in diameter. Lafontaine and Chouinard (1963) have described similar dense granules measuring 150 to 300 A in the nucleoplasm of plant material.

In cells of the rat liver Swift (1959) found clusters of smaller particles in the interchromatic areas using acrolein as the fixative and uranyl acetate stain. These particles were roughly comparable in size

to those found on the endoplasmic reticulum. After ribonuclease pretreatment he found that both the particles in the nucleus and in the cytoplasm stained much less intensely while the staining of chromatin was similar to that in controls. He concluded that these granules in both the cytoplasm and the interchromatic space contain ribonucleic acid (RNA). Smetana et al. (1963) have found further evidence for RNA localization within the interchromatic granules using toluidine blue dye to stain for ribonucleoprotein (RNP) and Feulgen staining for deoxyribonucleic acid (DNA). Using tumor cells and rat liver cells, these workers further found that the ribonucleoprotein of the cytoplasm differs from that of the ribonucleoprotein of the nucleus, the former is soluble in 0.14 M NaCl while the latter is not. In the light microscope Smetana et al. describe the ribonucleoprotein in the nucleus as forming a network of dense fibers which seems to radiate from the nucleolus to the nuclear envelope. They believe that this network, as viewed in the light microscope, may correspond to patches of dark particles distributed throughout the nucleus in ultrathin sections. These patches of dark particles have dimensions such that they may be profiles of the strands of this network.

Chromatin is found in masses distributed throughout the interphase cell. Many of these masses are associated with the internal membrane of the nuclear envelope except in the channel regions of the pore complex. Moses and Lafontaine (1961) have described two stages of interphase nuclei in Vicia which differ in the size and configuration of the chromosome strands. The interphase nuclei in which they have shown by

autoradiographic techniques that the chromatin has not yet duplicated have tortuous, apparently anastomosing strands .2 - .3  $\mu$  wide which occasionally appear double, especially near the nuclear envelope. The smallest strands observed were .15  $\mu$  wide. Several clumps of "heterochromatin" (1.5 - 2.5  $\mu$ ) were also evident. A large portion of the nucleus appeared to be interchromatin space. Nuclei in which the chromatin had already duplicated did not show the large, heterochromatin masses and had a smaller proportion of interchromatin space. The strands, .4 - .5  $\mu$ , appeared to be distributed equidistantly more often in these nuclei than in the other type. In thin sections the strands could be followed for longer distances indicating less twisting than earlier in interphase. They did not seem to be double except near the nuclear envelope where the individual elements of the pair appeared as .25  $\mu$  strands. Short segments of single .15  $\mu$  strands were occasionally visible.

Chromatin could be seen to contact and even penetrate the nucleolus (Moses and Lafontaine, 1961; Buvat, 1963) in favorable sections.

#### Mitotic Apparatus

The first sign of the spindle in dividing cells of many plants is the formation of a "clear zone" around the nucleus. This has been shown in the light microscope using fixed material (Fabbri, 1960) and, in a striking manner, in the microcinematography studies of Bajer (1954, 1957) viewing living endosperm cells with the phase-contrast microscope. This "clear zone" was later shown by Inoué and Bajer (1961), using a

polarizing microscope, to exhibit positive birefringence. This zone is composed of two or more pointed arms extending away from the nucleus in opposite directions. These are the equivalent of the "polar caps" of classical cytologists. These polar caps extend completely around the nucleus. The contents of the entire zone display a birefringence and shows a fibrous texture running in the direction of the protrusions and, therefore, tangential to the nuclear envelope in the area of the equator. The larger cytoplasmic organelles, which appear as granules in the light microscope, are excluded from this region. Fabbri (1960) says that these polar caps are rich in RNA and appear to contain spindle elements. This intense staining for RNA continues in the spindle region throughout mitosis reaching a maximum in early telophase.

During late prophase the polar caps increase rapidly in size (Bajer, 1957). This did not seem to occur as the result of a loss in volume of the nucleus, thus Bajer (1957) suggests that the material in this zone appears to be the cytoplasmic contribution to the mitotic spindle, while the establishment of the polar caps may determine the polarity of the spindle. In a later study Richards and Bajer (1961) have shown a sharp drop in the total mass of the nucleus during prophase before the breakdown of the nuclear membrane. Richards (1960) attributes this loss to proteins. Others (Becker, 1938; Wada, 1950; Satô, 1960) feel that the spindle is of nuclear origin while Roth and Daniels (1962) have suggested that spindle formation takes place only after the nucleoplasm and cytoplasm have mixed to form a "mixoplasm". The latter authors feel that chemical substances from both the cytoplasm and nucleus are

necessary for spindle formation but, for these to get together, the nuclear membrane must break down, at least in part, since they do not generally pass through the pores of the nuclear envelope. Inoué and Bajer (1961) detected no birefringence in the nucleus before the breakdown of the nuclear envelope, but as soon as this occurred, birefringence extended into the nuclear area.

The "contraction stage" of mitosis, in which the group of chromosomes appears to come together, (Bajer, 1958a) is initiated by the breakdown of the nuclear membrane. The spindle fibrils or fibers are formed out of the positively birefringent material of the polar caps. Some of these apparently terminate on the chromosomes; the birefringence becomes the strongest next to the chromosomes (Inoué and Bajer, 1961). The chromosomes, after undergoing complex movements (Bajer, 1958b), finally become aligned on the metaphase plate; the association of the spindle fibers with the primary constriction of the chromosomes can now be seen. These represent the chromosomal spindle fibers (cf. Schrader, 1953) and are observed to be more compact and birefringent adjacent to the constriction.

The chromosome fibers, as seen in electron micrographs, are made up of several unit elements (Satô, 1958; Bowen, 1959; Harris, 1962). Satô has estimated that in Lilium there are 15 - 20 fibrils in a bundle comprising these chromosome fibers and that the fiber at its compact end near the kinetochore is about 0.4  $\mu$  wide. In the sea urchin Harris estimates the number of fibrils in a fiber to be ten.

The birefringence of the spindle reaches a maximum at the onset of anaphase. The chromosomal fibers shorten as the chromosomes move apart in anaphase but the greatest birefringence is always adjacent to the kinetochore which precedes the rest of the chromosome to the poles (Inoué and Bajer, 1961).

Continuous fibers are difficult to demonstrate during early anaphase (Inoué, 1953) but by late anaphase and telophase they again appear where they condense toward the equatorial plate to form the phragmoplast fibers.

Inoué and Bajer (1961) concluded from their study that the polar cap substance is a spindle precursor material which is organized into spindle fibers shortly after the breakdown of the nuclear membrane. This organization of chromosomal fibers is facilitated by the kinetochore. This organizational ability is first evident at early prometaphase.

Early electron microscope observations of the mitotic apparatus of higher plants have been made by Sedar and Wilson (1951), Porter (1956), Satô (1958, 1960) and Wada and Satô (1958). Bowen (1959) reported chromosomal fibers in electron micrographs of Psilotum sporogenous cells. More recently Ledbetter and Porter (1963, 1964) have shown high resolution micrographs of spindle "microtubules" in several kinds of plants. They show that a great many of these tubules pack the interzonal region between the chromosome masses and are oriented parallel to the long axis of the spindle intermingling with the pectin vesicles and endoplasmic reticulum at the level of the forming cell plate. Some

tubules pass directly through the plate zone. These tubules in dividing cells occur only in the spindle, none being found in the cortex subjacent to the plasma membrane as Ledbetter and Porter have shown in interphase cells. These authors estimate there are more than 500 tubules in a spindle.

The spindle microtubules measure approximately 200 A in diameter and are of undetermined length. The dense wall of these elements is about 70 A thick and the lighter area within about 100 A in diameter. The wall does not show the three-layered structure of the unit membrane and, indeed, is not as uniformly dense as the lines in this membrane. Cross sections of microtubules present in the cortices of plant cells have been shown (Ledbetter and Porter, 1964) to have 13 subunits in their walls or the spacing between the units is at an average of  $1/13$  of the circumference with vacant spaces between some of the subunits. The center-to-center spacing between the subunits was reported to be about 45 A. The subunits, appearing circular in cross section, may represent the macromolecular elements of which the microtubules are made. These authors feel that the spindle elements have a similar morphology to the microtubules in the cortex, a morphology which may be common to both plants and animals since the flagellar fibrils of rat and human sperm tails have similar longitudinal filaments with 55 to 60 A center-to-center spacing (Pease, 1963; André and Thiéry, 1963). The main morphological difference seems to be in the number of elements, flagellar microtubules reportedly having only 10 or 11, however, different techniques were used by these latter authors.

Finally Ledbetter and Porter (1963) conclude that these microtubules are responsible for the birefringence of the spindle in the area of the phragmoplast. As to their permanence in the cell, Ledbetter and Porter think the microtubules may migrate from their position in the spindle and phragmoplast at the end of telophase to the vicinity of the walls or they may form anew in the cortex as they appear to do in the formation of the mitotic spindle.

Inoué (1960), discussing the physical properties of the spindle, believes that the elements are composed of aligned spindle micelles which are bathed in a pool of randomly oriented, slightly more hydrated micelles. The oriented micelles may be slightly crosslinked but this is sensitive to pH and ionic strength.

Recent results of studies on the mitotic apparatus using animal material have been reported by Roth and Daniels (1962), Harris (1961, 1962), Harris and Mazia (1962), Kane (1962), Roth and Shigenaka (1964) and Daniels and Roth (1964) and are reviewed by Roth (1964).

In animals the spindle elements have the same dense wall with electron transparent center suggesting a tube but they are usually smaller than plant material, most reports showing them to be 15  $\mu$  in diameter. Material adhering to the surface of the filaments up to anaphase has been reported by Roth and Daniels (1962) and Roth and Shigenaka (1964) but questioned by Harris (1962).

Both chromosomal and continuous spindle filaments, whose structures are similar, have been observed. These may or may not be grouped into bundles forming fibers during telophase after they have become dis-

sociated from the kinetochore. The diameter of the filament does not change from one stage to the next during division, however, the length of the filament may change. This is also true of the larger spindle fibers (Inoué, 1964). Ribosome-like particles are usually scattered between the filaments but are probably not structurally associated with the filaments.

Inoué (1964) in a review article on spindles has stressed the dynamic aspects of the spindle. This fact has also been emphasized by Roth (1964) and Roth and Shigenaka (1964). The spindles are in a state of flux. According to Inoué (1964) they may readily be built up, broken down or reorganized. The same material can in this way be used in the formation of several types of fibers at different times, depending on which "orienting center" happens to be active at that time.

The idea of orienting centers has been supported recently by U.V. microbeam irradiation (Inoué, 1964). The orienting center orients and organizes the fibers of the spindle. By irradiating different parts of the mitotic apparatus it was found that the kinetochore and cell plate act as centers of organization. Inoué sums up his observations by stating that birefringent fibers are present, they are organized by centers and they exist in a highly dynamic state.

#### Plant Nucleoli

The phenomenon of nucleoli persisting through at least part, if not all, of mitosis has been shown in plants to be a common occurrence (Bajer, 1953). Tischler (1951) has tabulated the older literature on

this subject while Fabbri (1960) lists those published after 1942. No published electron microscopic studies of this phenomenon have been found.

In Psilotum Fabbri (1960) has described persistent nucleoli in detail. The nuclei of the meristematic cells of this plant contain, on the average, 4 to 6 nucleoli. The nucleoli are more or less spherical with diameters of 1.5  $\mu$  to about 4  $\mu$ . A clear zone called the perinucleolar sphere has been described surrounding the nucleolus. The nucleoli are very rich in RNA. Internally they may contain irregular transparent vacuoles or a filamentous network resembling the "nucleolonema" described by Estable and Sotelo (1956).

With the onset of prometaphase some of the nucleoli disintegrate immediately while others persist in the cytoplasm throughout the succeeding stages of nuclear division. These persistent nucleoli may appear to be "normal" up to late telophase.

Often one or two of the nucleoli leave the metaphase plate and move out to the poles of the spindle body, taking up the position that would normally be occupied by the centrosome in the animal cell. Other nucleoli may be found near the periphery of the equatorial plate; these often show signs of disintegration.

By midtelophase newly formed nucleoli begin to appear in the daughter nuclei. During the formation of the nuclear envelopes around the two masses of daughter chromosomes, any persistent nucleoli still remaining are left in the cytoplasm. Only fragments of these nucleoli remain by the time the daughter nuclei have taken on the characteristic

form of interphase.

Plant nucleoli most thoroughly studied in the electron microscope have been those of Vicia faba and Allium cepa by Lafontaine (1958a, b) and Lafontaine and Chouinard (1963) and of certain orchids by Chardard (1962). A recent review (Sirlin, 1962) has been published on the nucleolus.

The nucleolus, according to Estable and Sotelo (1956), is constituted of a "nucleolonema", which appears as a dense filament taking the shape, in interphase, of a more or less tight glomerulus, included within a homogeneous material, the "pars amorpha". The nucleolonema show tight helicoidal turns and irregular curves. These authors believe that this part of the nucleolus is a permanent component found in all nucleated cells. It undergoes changes in morphological structure during the mitotic cycle but is never lost. Nucleolonemata are visible before the appearance of the corresponding pars amorpha. The nucleolonema is described as unfolding from the tightly woven tuft, becoming thinner and spreading out close to the chromosomes in prophase. It becomes longitudinally associated with the chromosomes in metaphase and divides along with the chromosomes in anaphase. It then becomes thicker and shorter during telophase, separates from the chromosomes, and assumes its typical interphase appearance.

Such a structure has not been well demonstrated in the electron microscope in plant material. Lafontaine (1958a, b) has described three components of nucleoli in Allium cepa and Vicia faba using osmium tetroxide fixation. The main constituent appears similar to the chromatin

strands and is seen as closely packed, round or elliptical profiles, each 100 - 150 A in diameter. It was suggested that these are profiles of tubules or possibly paired fibrils of smaller diameter. A dense, particulate component, usually having a doughnut shape in profile, with an average diameter of 140 A is also present. These particulates are not visible after formalin fixation. The third component in some interphase nucleoli consists of dispersed granules, 100 - 150 A in diameter, which resemble the first component but are less closely packed.

Two types of nucleoli were found. The most common were homogeneous compact structures consisting of the tubular component first described. Many small internal areas of low electron density appear in these nucleoli and larger electron-transparent areas are not uncommon. The 140 A granules appear in about half of these nucleoli. The second type of nucleoli has two distinct zones, a light core separated by a sharp boundary from a surrounding denser cortex. The cortex is composed of the tubular component. There are no dense 140 A granules in this type of nucleolus, however, the central portion seems to consist only of the dispersed granular component previously described. Many of the nucleoli found by Lafontaine appeared to be intermediates between these two types.

The type of nucleolus in a given cell appears to depend on either some sort of nuclear differentiation or variations in the physiological state of the cell since the two nucleoli in one nucleus were always of the same type.

Nucleoli are often associated with regions of intermediate electron density identifiable as chromatin material by their texture and density.

This nucleolar "organizer" chromatin is often continuous with other chromatin strands in the nucleus. Rattenbury and Serra (1952) claim that nucleolar organizers are heterochromatic, are not necessary for the formation of nucleoli, and, therefore, suggest the more neutral name of "nucleolar zones".

Chardard (1962) describes a dense, homogeneous type of nucleolus in orchids consisting of fine, very tightly coiled fibrils and "foamy particles". No distinction could be made between the nucleolonema and the pars amorpha.

No nucleolar membrane has been demonstrated by the electron microscope. Porter (1960) has suggested that one of the elements of which the nucleolus is composed is similar in character to the RNP particles of the cytoplasm and that this might be the source of cytoplasmic RNA which gets into the cytoplasm through the breakdown of the nuclear envelope or by way of the pores.

In a later study of the nucleoli of Vicia faba, Lafontaine and Chouinard (1963) have described nucleolar changes in detail during mitosis. They found that the preprophase nucleolus contained a number of unstained vacuole-like structures, varying in size and shape. No perinucleolar halo around the nucleolus (Chayen et al., 1953) was seen (Lafontaine and Chouinard, 1963). Two components were found in the denser portion of the nucleoli. The peripheral portion of the nucleolus proper, the surface of the larger vacuoles and a number of zones of varying width extending more or less radially in between, were composed predominantly of densely packed granules of 150 A diameter. These

granules, on closer observation, seemed to be organized into thread-like arrangements in the granular zone. The other, more homogeneous-appearing zone, consists of small tightly packed convoluted fibrils, 60 - 100 A in diameter. During prophase the nucleolus was described as becoming irregular in outline, losing its vacuoles and disintegrating into the surrounding nucleoplasm. Nucleolar material could be identified, using phase-contrast microscopy, among the chromosomes in the peripheral portion of the nucleus. By the time of nuclear envelope breakdown, the nucleolus was no longer present as an organized body.

In electron microscope studies made by the same authors of cells at this stage the nucleolus appeared to undergo a general "loosening-up" resulting in the loss of the thread-like arrangement of the granules, eventually progressing to a blending of the granular and fibrillar zones with the other granules and fibrils already in the nucleoplasm. This mixture of granules showed an increased density, but the individual elements were still less electron-dense than the free ribosomes in the cytoplasm. As the nuclear envelope broke down the nucleoplasm containing the disintegrated nucleolar material became indistinguishable from that of the forming spindle. No evidence of nucleolar structures was seen during prometaphase, metaphase or anaphase nor was there any evidence of any coating of material over the exterior of the chromosome.

During early telophase they detected a thin coating of material on the surface of the chromosomes and in the spaces between the chromonemal gyres. This material was described as increasing until at midtelophase small masses of this material could be recognized as corresponding to

the forming nucleoli. These nucleoli enlarge at the same time the material coating the chromosomes disappears. Under the electron microscope the material coating the surface of the chromosomes appeared denser than that of the spindle and consisted of loosely arranged fibrillar elements, 60 to 100 A in diameter, intermingled with dense 150 A granules. The segments of the chromosome identifiable as secondary constrictions were not coated with this fibrillogranular material. The nucleoli, as they formed, resembled closely the nucleoli of preprophase and prophase. Even in the youngest nucleoli the granular and fibrillar material was segregated into zones.

In Allium cepa the dense 140 A granules were described as disappearing in prophase and not reappearing until late anaphase when they were seen as loose clusters between the chromosome arms. These then agglomerated into dense areas during telophase and finally formed one or two large masses by late telophase (Lafontaine, 1958b).

### Chromosome Fine Structure

#### The elementary fibril

The fine structure of chromatin and chromosomes in plant cells has been examined by many investigators, including Ris (1956b), Kaufmann and De (1956), Shigenaga (1957), Bopp-Hassenkamp (1959) and Shinke (1959). All of these authors report the presence of fibrils of about 100 A diameter in the chromosomes or chromatin regions of the nucleus. This fibril has been reported in such a wide variety of organisms and in so many different cellular types (for reviews see Ris, 1961; Grell, 1962;

Swift, 1962) that Ris (1961) has called it the "elementary chromosome fibril". Higher magnification and better techniques demonstrate that this 100 A fibril is composed of two 35 - 40 A fibrils (Bopp-Hassenkamp, 1959; Shinke, 1959; Ris, 1961; Brinkley, 1964). This is about as much as the electron microscope has been able to conclusively demonstrate about chromosome fine structure. The organization of these elementary fibrils into the chromosome has been a matter of controversy for many years.

### The interphase chromatin

The interphase nucleus in most plants studied in the electron microscope shows electron dense granular masses with ill-defined contours scattered throughout the nucleus. Many of these masses are in close contact with the inside surface of the nuclear envelope. Originally these dense granular masses were believed to consist of microfibrils of 200 to 250 A diameter twisted into spirals with a tight pitch (Ris, 1956a, b; Lafontaine and Ris, 1958). These have since been described as consisting of two fibrils, each of 100 A diameter, wound around each other (Ris, 1960).

Moses and Lafontaine (1961) describe two types of interphase nuclei in Vicia faba. The first type, shown to be that of early interphase before DNA synthesis, contains strands of .2 - .3  $\mu$  width, in tortuous array, and apparently anastomosing. In profile there is a suggestion that they are coiled or kinked. In nuclei which have already synthesized DNA for the next division the masses of chromatin are smaller and more evenly spaced. The strands, still appearing to anastomose, are now .4 -

.5  $\mu$  wide and are less twisted. Occasionally doubleness of these strands is evident, each unit being about .25  $\mu$  in width.

By spreading red blood cells on a water surface and then picking up the chromosomes on carbon-coated grids, Gall (1963) has shown long fibers in the interphase nuclei of the newt. These are 400 - 600 A in diameter. Human and grasshopper chromosomes, treated in the same way, show similar fibers. When stained with uranyl acetate these fibers show a dense core which is approximately 150 A.

Recently Hay and Revel (1963) have interpreted their electron microscope studies of interphase nuclei of the cells of regenerating salamander limbs as showing a meshwork of deoxyribonucleoprotein (DNP). The filaments of this meshwork are 50 - 75 A in diameter while the interstices are less than 200 A wide. Within the interphase nucleus two components were described. The one had a finely textured appearance and was shown to synthesize DNP, the second was composed of dense "granules" embedded in a material of low electron density. The latter was never shown to synthesize DNP. The first of these two components is composed of the meshwork of 50 - 75 A filaments. The net-like arrangement is thought to result from branching or anastomosing of individual filaments.

Clumps of electron-dense chromatin may be found in interphase. Hay and Revel think that these clumps may form from the less dense, loosely organized chromatin. Because they do not synthesize DNP and because they appear to be similar to darkly staining chromatin masses observed with the light microscope in interphase cells of various

organisms (cf. Swanson, c1957), they are considered by these authors to be "heterochromatin". These clumps may represent centers around which the chromosomes organize.

#### The chromosome

During cell division the chromosomes contract into manageable units which divide and are then distributed to the two daughter nuclei. Lafontaine and Chouinard (1963) have studied this phenomenon in dividing cells of Vicia faba. In preprophase nuclei of Vicia the diameter of the chromatin clumps is less constant than in subsequent stages. Within these clumps tightly packed convoluted fibrils of approximately 100 A in diameter can be seen associated with dense granules which reach 150 A in diameter. During early prophase portions of convoluted chromonemata can be seen between which irregular light spaces occur. As prophase progresses these light spaces decrease, both in size and number, which is probably due, in part, to a closer and closer approximation of the successive chromonemal coils in each chromatid. Only a very few light spaces are observed by late prophase. Densely packed convoluted fibrils and a number of granules can still be identified within the chromosomal material. No matrix material was found coating the outer surface of the chromosomes. If one was present, its fine structure must have been indistinguishable from that of the chromosome itself.

During prometaphase, metaphase and anaphase the chromatids were found to be compact masses much denser than the surrounding spindle material. Again the only structural materials resolved within the chromosome mass were the convoluted fibrils and granules. A chromosomal

core was occasionally seen in anaphase. Chromosomal gyres could not be distinguished in the electron microscope. Only the wavy contours of the chromosomes suggested their presence. Chromatid segments, identified as nucleolar secondary constrictions, were shown to consist of a low density fibrillar material devoid of any granular elements. During early telophase the chromosomes were described as being coated with prenucleolar material. The only part of the chromosome not coated at this time was the nucleolar organizer region.

In studies on orchids Chardard (1962) concluded that chromosomes were not surrounded by a membrane and no matrix material could be observed. He described 100 A fibrils which contain DNA and make up the chromosome.

Hay and Revel (1963) think that the chromosome becomes organized by the aggregation of the "chromatin meshwork" and not by a coiling of threads. Two things occur when this happens, the chromatin increases in density and the synthesis of DNA stops. A fine filamentous meshwork of 50 - 75 A filaments, similar to that of interphase, was reportedly seen in the chromosome.

#### The kinetochore

The kinetochore after osmium tetroxide fixation appears to have a greater electron density than the body of the chromosome (Nebel, 1959; Harris, 1961, 1962; Nebel and Coulon, 1962; Mota, 1962). Mota (1962) believes this is due to a closer packing of the 100 A fibrils which are present in this region. No sharp transition zone is evident between the kinetochore and the euchromatin. Nebel and Coulon (1962) have described

a rather elaborate acorn-shaped structure, in the chromosomes of the pigeon, to which the spindle tubules are attached. In the sea urchin Harris (1962) describes the kinetochores as electron opaque "plates" roughly 50  $\mu$  thick and 150  $\mu$  in diameter, located on the surface of the chromosome.

#### The perichromatin granule

Watson (1962), working with rat tissue, described a granule found at or near the surface of chromatin masses, which he called the "perichromatin granule". Its size is approximately 30  $\mu$  and it is separated from the surrounding chromatin by a clear zone or mantle of 25  $\mu$  thickness. Thus the overall diameter of both parts was reported to be 75  $\mu$ . Its density is slightly greater than that of neighboring chromatin. From 5 to 20 of these granules may be seen in an average section of a nucleus. They did not seem to be preferentially located except next to the chromosome. Identification of these bodies was possible only when the chromatin was clumped. After fixation with aldehyde the perichromatin granules were still evident (Watson and Aldridge, 1964). Extraction with cold perchloric acid (PCA) to remove RNA did not result in much, if any, decrease in staining. However, upon extraction with PCA at 20 - 25° C for 24 hours, the staining was weaker, paralleling the decrease in staining of the chromatin. These authors thus concluded that these granules may contain DNA and possibly a small amount of RNA. Watson (1962) has suggested that these granules might be viral particles.

### Chromosome models

Many models have been proposed for the organization of DNA and its associated proteins into a typical chromosome as seen in the light microscope in dividing cells. Swift (1962) classifies these into three categories.

1. The "protein backbone" theory. DNA is attached to a structural protein or DNA alternates with protein segments arranged end to end (Taylor, 1957; Schwartz, 1958; Nebel and Coulon, 1962).
2. Multistranded or "rope" theory. The chromosome is considered to be a bundle of coiled DNA-protein filaments running the length of the chromosome (Ris, 1957; Steffensen, 1959; Kaufmann et al., 1960).
3. "Differential coiling". The chromosome consists of one or two strands running the length of the chromosome with certain regions tightly coiled and others not (Callan, 1956; Gall, 1958).

Ris (1961, 1962) has proposed a model of the "elementary chromosomal fibril". This model consists of two 40 A fibrils twisted together to form the 100 A fibril. The 40 A fibril is composed of DNA-histone complexes attached end to end by non-histone protein to form a long thread. The histone is thought to be bound in the grooves of the DNA double helix (Wilkins, 1956). The histone is then involved somehow in cross-linking the two nucleohistone units in the 100 A fibrils.

### Cytokinesis

Between the two daughter sets of chromosomes which have passed to the poles during anaphase, a fibrous barrel-shaped structure (Sató, 1959; Inoué and Bajer, 1961) known as the phragmoplast appears. It has been suggested that it forms from a substance released by the spindle as the

chromosomes move toward the poles (Becker, 1938; Buvat, 1963). It contains numerous dense granules, identical to the ribosomes of the cytoplasm, filaments (Satô, 1958; Inoué and Bajer, 1961; Ledbetter and Porter, 1963) and extensions of the endoplasmic reticulum which are usually oriented parallel to the long axis of the spindle (Porter and Machado, 1960). It is a fairly rigid body from which the larger organelles of the cytoplasm of the mother cell are excluded. Eventually it loses its shape and texture becoming indistinguishable from the rest of the cytoplasm.

Vesicles, 25 - 500  $\mu$  (Porter and Caulfield, 1960) appear at the center of the phragmoplast. These vesicles, starting at the center of the old equatorial plane, coalesce to form the cell plate (Porter and Caulfield, 1960). The same sequence of events was suggested by Becker in 1938. The fusion of vesicles continues at the margins of the expanding cell plate until it is connected to the side walls of the parent cell.

Dense bodies, of approximately .2  $\mu$  in diameter, have been described in connection with the phragmoplast. They were called "phragmosomes" by Porter and Caulfield (1960), but their importance, if any, is not understood since they persist during interphase in at least some cell types (Manton, 1961b).

The origin of the vesicles at the equatorial plate was described by Porter and Machado (1960) as coming from the ends of sheets of endoplasmic reticulum. These sheets moved down between the chromosomes as the chromosomes made their way to the poles. Chardard (1962) also con-

cluded that the cell plate was derived from vesicles of endoplasmic reticulum. Whaley and Mollenhauer (1963) have more recently implicated the Golgi apparatus in this function. They suggest that dictyosomes located at the cell plate may constitute the phragmoplast as viewed in the light microscope. These Golgi bodies produce vesicles containing an excretion product. Thus as these vesicles fuse to form the cell plate they contribute part of the substance of the cell plate as well as the new plasma membrane material.

Small discontinuities, called plasmodesmata, appear within the forming cell plate. Endoplasmic reticulum may also be found extending across the phragmoplast region between the two daughter protoplasts (Buvat and Puissant, 1958; Porter and Machado, 1960). Porter and Machado have suggested that the plasmodesmata may actually be determined by these extensions. Evidence for this lies in the size of the tubules of the endoplasmic reticulum which are 30  $\mu$  in diameter (Porter and Caulfield, 1960) which is close to the size of plasmodesmata (Buvat, 1963). Also these canals are often in close contact with extensions of the endoplasmic reticulum (Buvat, 1957; Strugger, 1957; Porter and Machado, 1960). Whaley et al. (1960a) has shown micrographs of the actual continuity of tubules of endoplasmic reticulum across the cell wall. The inside of these intercellular canals is closely lined with a cell membrane. Thus the cell membrane is continuous from one cell to the next (Buvat, 1957).

## MATERIALS AND METHODS

## Electron Microscopy

Glutaraldehyde post-osmium tetroxide fixation

The Psilotum plants used in this study have been growing in the Horticulture and Botany greenhouses at Iowa State University, Ames, Iowa, for many years and are of unknown origin. Vigorously growing shoots were harvested around midday and placed between wet blotters in a covered dish until they could be fixed. Since the third and fourth sporangia distal to the tips of the shoots usually contain many cells in mitosis, they were cut into 1 mm. slices and immediately placed in fixative. The usual fixation procedure essentially followed that of Sabatini et al. (1963). Glutaraldehyde with a final concentration of 3.4 percent in 0.1 M Sorensen phosphate buffer at pH 7.4 was used as the fixative. Fixation extended for 1 to 2 hours at 4° C.

The tissue was then washed for 5 minutes in a 0.2 M sucrose solution buffered with 0.1 M phosphate, as in the fixation, followed by a postfixation in a 1 percent solution of osmium tetroxide buffered by Veronal acetate (Palade, 1952a) at pH 7.4 for 45 minutes in an ice bath.

Dehydration was routinely accomplished in increasing concentrations of ethanol. Epon 812 was used as the embedding medium according to Luft (1961). Sections were cut using a DuPont diamond knife and an LKB ultramicrotome. The sections (40 - 90  $\mu$  thick) were picked up either on clean 400 mesh copper grids or on formvar coated 75 mesh copper grids which had been lightly coated with carbon. Uranyl acetate (Watson,

1958a) and lead salts (Millonig, 1961; Karnovsky, 1961; Watson, 1958b; Normann, 1964) were used to stain the sections.

An RCA EMU 3F electron microscope was operated at 50 KV using an objective aperature 30 - 40  $\mu$  in diameter. The micrographs were taken at machine magnifications of 2,000 to 24,000 X on Kodak contrast plates. Development was carried out in Kodak D-19 developer for 4 minutes, and negatives were routinely enlarged 2.8 to 4.2 times on Kodak F-5 Kodabromide paper.

#### Potassium permanganate fixation

Sections of sporangia were fixed in 4 percent potassium permanganate (Luft, 1956; Mollenhauer, 1959) which was either unbuffered or buffered at pH 7.2 with Veronal acetate (Palade, 1952a). Part of the tissue from each of these two groups was then fixed for 10 minutes and part was fixed for one hour. After the fixation the tissue was briefly rinsed in distilled water and then dehydrated and embedded in the manner previously described. Best results were obtained using the buffered fixative and the shorter fixation time.

#### Light and Electron Microscopy of the Same Cell

Since complete synchrony of division was never observed throughout the whole sporangium, it was necessary to devise a means of locating cells in division, recording the stage of division and orienting the cells so they could be cut in known planes for thin sectioning and electron microscopic observation. This would eliminate a great deal of useless trimming and sectioning to find cells in the proper stage of

division and orientation.

A simple method brought about the desired results. After the tissue was fixed, dehydrated and embedded in the usual way for electron microscopy, the blocks were trimmed down to a small face. Using a sharp razor blade the block was hand-sectioned as thinly as possible, parallel to the face. These sections were then placed on clean slides in a drop of 50 percent glycerin and a cover slip was affixed. The tissue was then observed by means of a phase-contrast microscope. In this way a large number of cells could be surveyed in a short time. Sections with dividing cells in the orientation and stage desired were then selected and a rough map of the section was drawn showing the location of the cell in the section. In many cases a light micrograph was also taken at this time for a permanent record of the cell, its phase of division and orientation. The cover slip was then removed and the sections picked up and glued with epoxy glue to the face of a pretrimmed dummy block. A small amount of 50 percent glycerin did not seem to affect the binding of the section to the dummy block. The blocks were placed in an oven at 60° C for one hour to allow the glue to harden.

After this time interval the material was sectioned for electron microscopy. By referring to the map of cell locations, the block can be trimmed to a very small face around the cell that was selected to be sectioned. The block is then oriented in the ultramicrotome so that its face is parallel to the knife edge. Sections were then cut and later observed in the electron microscope.

## OBSERVATIONS

## Light Microscope Observations

Chromosome counts were made at metaphase I of meiosis. Because of the size and large number of chromosomes in Psilotum, it was very difficult to spread the synapsed chromosomes. For this reason and because of the possibility of mistaking multivalents for bivalents, results were only approximate. However, since counts obtained from several cells all exceeded the triploid number of 156 and were all lower than the reported tetraploid number of 208 (Ninan, 1956), it was concluded that the plants used in this study were tetraploids.

Mitosis appeared regular, only once was a tripolar spindle observed, and lagging chromosomes were rarely noted at anaphase.

## Electron Microscope Observations

Interphase cell

The cytoplasm of sporogenous cells of Psilotum at interphase contains the usual organelles found in meristematic tissue of higher plants. These organelles are arranged in a definite pattern about the nucleus after glutaraldehyde-osmium fixation (Figure 1). The mitochondria and proplastids can, for the most part, be found in a zone adjacent to the nucleus. Between this zone and the cell wall may be found a very compact zone of vacuoles of varying sizes. A few small homogeneous dense bodies, presumably lipoidal, are also found. One receives the impression that the cytoplasm is packed with organelles and that very little

ground substance and endoplasmic reticulum along with ribosomes can occupy the small space between these larger components. This does seem to be the case and the endoplasmic reticulum is only scantily present (Figure 1). Ribosomes appear both free in the cytoplasm and also in conjunction with the endoplasmic reticulum forming the rough endoplasmic reticulum. Profiles of ribosomes, from 15 to 24  $\mu$  in diameter, were usually found in groups, sometimes appearing as whorls or spirals. Tubular elements similar to spindle microtubules or microtubules of the cortex were not apparent in sporogenous interphase cells; however they were observed in cells of the sporangial wall (Figure 10).

After potassium permanganate fixation the cytoplasm did not appear as full as after glutaraldehyde-osmium fixation (Figure 2), the organelles of the cytoplasm being interspersed with a larger amount of apparently unorganized material. The endoplasmic reticulum was much more evident, was occasionally seen to be continuous with the nuclear envelope, and had an interesting association with the proplastids. Each proplastid is partially encircled by a segment of this membrane system (Figure 2). Many electron-dense masses may be scattered throughout the cytoplasm particularly in the area close to the cell wall (Figure 2). These are thought to be the vacuoles which are transparent to electrons after glutaraldehyde-osmium fixation. They, however, appear much smaller, which may be related to the increased material between the organelles seen after potassium permanganate fixation; at least, the cristae were more obvious (Figure 2). Ribosomes were not visible after this fixation.

Nuclei are relatively homogeneous and granular in permanganate-fixed cells (Figure 2). Denser regions of the granular material, corresponding to chromatin clumps (Figure 2), are scattered throughout the nucleus but the contents of the nucleus seem to be poorly preserved. At higher magnifications potassium permanganate-fixed cells have a very granular appearance; thus this fixation is unsuitable for high resolution electron microscopy of the nucleus and its components.

After glutaraldehyde-osmium fixation the nucleus at interphase contains masses of chromatin dispersed throughout the nucleoplasm (Figures 1, 3, 4 and 7). The nucleoplasm is composed of granules and fibrous material (Figures 3, 4 and 7) in a homogeneous background that does not stain for electron microscopy with any of the techniques used in this research. The nucleoplasmic granules are generally larger than the ribosomes of the cytoplasm, ranging from 22 to 45  $\mu$  in diameter but averaging approximately 33  $\mu$ . They are found in clumps and may be connected with each other by fibrous material (Figures 3 and 4). These nucleoplasmic granules are often found in association with chromatin masses (Figure 7) and may be connected to this material by the same fibrous substance (Figures 3 and 4).

Nucleoli vary somewhat in organization. Most of the nucleoli observed were composed of small granules and fibrils (Figure 5). The small nucleolar granules, approximately 17  $\mu$  in diameter, tend to be most abundant around the periphery of the nucleolus; the central portion appears to be composed of tightly packed fibrils. Around the nucleolus

is an electron-transparent perinucleolar sphere separating it from the granular nucleoplasm and the masses of chromatin (Figures 4 and 5) except where the nucleolus was seen to be attached to, or surround, a clump of chromatin (Figure 5). There is always a lighter staining material between the nucleolus and the larger mass of electron-dense chromatin at the point of attachment (Figure 5). The fibrils in the nucleolus seem to be more tightly packed than in the chromatin. Electron-transparent areas often occur in the nucleoli. The larger of these clear areas are usually associated with regions of attachment of the nucleolus to chromatin material (Figure 5), whereas, smaller vacuole-like areas appear at the outer edge of the nucleolus where the nucleolar material seems less tightly held together and thus more irregular in outline (Figures 4 and 5).

Other types of nucleoli have also been observed in interphase. One is composed of a very dense mass of fibrils with small vacuoles scattered in an orderly manner throughout its body (Figure 8). Associated with the periphery of this nucleolus are granular profiles, which will be referred to as "large" nucleolar granules, of approximately 40  $\mu$  diameter. These profiles may be aggregations of smaller granules or cross-sections of coiled fibrils. Similar structures were also observed within the vacuoles (Figure 8). This type of nucleolus does not have a perinucleolar space surrounding it and appears to be attached at an edge to a mass of chromatin. A possible third type of nucleolus was observed in one interphase cell and in telophase nuclei. It seems to be composed of a very loose mass of fibers surrounded by a clear perinucleolar sphere

(Figure 9).

Chromatin in interphase is found in small clumps throughout the nucleus as well as in a finely dispersed state throughout the nucleoplasm (Figure 3). There is a close association of chromatin clumps with the inside of the nuclear envelope except immediately inside each nuclear pore (Figures 1, 2 and 11). The chromatin is basically composed of a mass of fibrils, each 100 - 140 A in diameter. This can be seen best during the various stages of division (Figures 24, 26 and 31) but is also clearly evident in favorable sections during interphase (Figure 3). The fibril itself is composed of a series of dark bands, 30 - 40 A thick, separated by less dense regions (Figure 3). The distance from the center of one dark band to the next dark band is approximately 65 A. This banding of the 100 - 140 A fibril appears to be the result of one or two tightly coiled 30 - 40 A fibrils making up the larger fibril. Short segments of the 100 - 140 A fibrils which generally appear to be unorganized can be found, particularly in the chromosomes, to lie parallel to each other.

A granule, usually associated with chromatin, was found in the nucleus during interphase (Figure 13). It will be referred to as granule "A" to distinguish it from others already described. This granule is 33  $\mu$ m in diameter. At its center, as seen in cross section, is a dense particle of approximately 50 A and around its outer edge are 7 or 8 dense particles similar to the one in the center. The substance between the center and periphery is of medium electron density compared with the dense particles and the less dense nucleoplasmic ground sub-

stance. Profiles of these granules were not seen frequently, but, when found, are located close to chromatin material but separated from it by a zone of low electron density. It is not yet clear whether these are short cylinders or spheres. No elongated cylindrical profiles of this size have been observed.

The nucleus is surrounded by an envelope consisting of two membranes in which nuclear pores are quite regularly spaced. In sections tangential to the nuclear envelope (Figure 11), the pores appear circular and approximately 110 m $\mu$  in diameter. Their structure is essentially the same as that of the pore complex described by Watson (1959). In longitudinal sections the pore complex is composed of a cylinder consisting of the pore, a low density channel in the nucleoplasm, whose base encircles the pore and extrusions of the channel contents into the cytoplasm (Figure 12). In Psilotum the channels in the nucleoplasm seem to anastomose to form a reticular network of low density just inside the nuclear envelope (Figure 11). In glutaraldehyde-osmium fixed material the pores never appear to be simple openings but have a dense material of complex structure within them (Figures 11 and 12).

#### Prophase cell

The first suggestion of prophase in the electron microscope is the observation of cytoplasmic zones at opposite ends of the nucleus essentially free of the larger cytoplasmic organelles. These zones, or polar caps of classical cytologists, encircle the nucleus and meet at the equator. Around the equator this zone of the cytoplasm is very narrow but at the poles it becomes large (Figures 14 and 18), occupying a

major part of the cytoplasm.

This region contains many ribosomes (Figures 14 and 18), a large number of irregular sacs (Figure 19) which seem to be part of the endoplasmic reticulum and many microtubules (Figures 15, 19, 20 and 21). The ribosomes are grouped into whorls (Figure 17) and spirals (Figure 16) as in interphase and are either free (Figures 16 and 19) or attached to the endoplasmic reticulum (Figures 18 and 19) and the outer membrane of the nuclear envelope (Figures 16 and 17).

Probably the most striking feature of prophase cytoplasm is the presence of a large number of microtubules, especially in the polar cap regions (Figures 14, 18, 19, 20 and 21). These microtubules appear in two sizes; the smaller (Figures 19 and 20) are approximately 18  $\mu$  and seem to be more abundant while the larger (Figures 15, 19 and 20) are 27 to 30  $\mu$ , the same size as the microtubules of the metaphase and anaphase spindle. These microtubules seem to be randomly oriented except at the periphery of the future equatorial plane where they tend to be oriented perpendicular to this plane. The length attained by these microtubules can not be determined but measurements as long as 1.6  $\mu$  have been obtained. A few of these microtubules are scattered between the large organelles in the cytoplasm outside the polar caps. A medium dense area containing some fibrous material surrounding individual microtubules (Figures 15, 19 and 20) can be observed in favorable sections. Ribosomes, for the most part, are excluded from such regions. Similar tubules also occur within the occasional cytoplasmic arm observed extending into the nucleus (Figure 15) but these tubules

have never been found in the nucleus at this stage.

Other components of the polar cap areas are spherical vesicles (Figures 18 and 19) which are somewhat larger than spindle elements, 60 - 120  $\mu$ , but smaller than the irregular sacs previously described, and electron-dense bodies (Figures 14 and 18) composed of fibrous or tubular elements between which dense granules are scattered. These latter bodies are surrounded by a double membrane and vary in width from about .2 - .5  $\mu$ . They generally have an elliptical profile.

The nucleus at this stage is surrounded by an intact envelope (Figure 14) which, like that of interphase, contains typical nuclear pores (Figure 14). Whorls of ribosomes may be found attached to the outer surface of the nuclear envelope (Figure 17). Some of the nucleoli may be in a degenerating condition as suggested by the greater number of transparent areas appearing in their outer regions.

Another sign of the onset of prophase is the beginning of chromosomal spiralization; however, this is very difficult to detect by electron microscopy. In later prophase this is accomplished by a pulling-away of the chromatin (Figure 14) from the inside of the nuclear envelope. No chromatin is appressed to the nuclear envelope by the end of prophase. As far as the fine structure of the chromatin is concerned (Figures 14 and 15), it looks little different in prophase from that in nuclei not undergoing division. The clumps of chromatin are, however, larger than they were during interphase.

### Prometaphase

Prometaphase is marked by the breakdown of the nuclear envelope (Figure 23) which appears to take place initially in localized regions, large portions of the porous, double envelope remaining intact. Microtubules and typical ribosomes of the cytoplasm may be found between the chromosomes before the envelope has completely disintegrated. Vesicles and irregular sacs, presumably remnants of the nuclear envelope and the endoplasmic reticulum, appear between the chromosomes during prometaphase.

The nucleoli are detached from the chromosomes, the granular material of some is interspersed with many clear areas while others maintain their normal compact appearance. One favorable section (Figure 22) shows a spindle element associated with a chromosome which is surrounded by a nucleolus except for a small break where the spindle element is located.

In prometaphase the chromosomes (Figure 23) are more condensed by the time they begin to align onto the equatorial plate. In tangential sections through chromosomes, bands of chromatin appear to radiate out from electron transparent areas (Figure 24). In longitudinal sections the margins of the chromosomes appear as regular waves, the distance from the peak of one wave to the next is of the order of .2 - .3  $\mu$ . The fine structure of the chromosome appears less granular than during interphase or prophase and seems to consist of the elementary 100 - 140 A fibrils (Figure 24) already described.

## Metaphase

Metaphase covers a relatively short period of time when the chromosomes are aligned on the equatorial plate. The large cytoplasmic organelles are excluded from the region of the mitotic apparatus but many ribosomes, nucleoplasmic granules, and irregular sacs are present in this region. Within the spindle bundles of spindle microtubules, 27 - 30  $\mu$  in diameter (Figures 25 and 27), with approximately 10 microtubules in a group, are associated with the individual chromosomes from which they extend toward the poles. Psilotum has a convergent spindle with distinct polar regions. Despite careful study of serial sections of many nuclei fixed with glutaraldehyde-osmium tetroxide, no centrioles were ever observed at the poles.

At metaphase both apparently well organized (Figure 30) and disintegrating nucleoli have been seen at the periphery of the equatorial plate and at the poles of the mitotic apparatus. The position assumed by the nucleoli at the poles is similar to the position normally occupied by the centrosomes in animal cells.

The chromosomes at this stage in the division cycle are compact units. The profile of a sectioned chromosome is very regular (Figure 28) and does not exhibit the lateral extensions (Figures 23, 31 and 32) of chromatin usually observed at other stages. The regions of the kinetochore, euchromatin and heterochromatin (Figure 25) can be identified. The only obvious structural difference in the region of the kinetochore, identified by its association with spindle elements, is

that it exhibits a lower electron density (Figure 25) than euchromatic regions after glutaraldehyde-osmium fixation and lead hydroxide staining. Heterochromatic regions observed at other stages exhibit a similar reaction (Figures 5 and 23). There is a special local organization of euchromatin near the kinetochore. Within this region curved segments of 100 - 140 A fibrils, approximately 40  $\mu$  long, lie parallel to each other (Figure 26). The distance between individual segments is about 37  $\mu$ . Several rows of these curved parallelly arranged segments appear to be stacked side by side. These appear to represent several large coils lying next to each other.

Remnants of the nuclear envelope and elements which may be segments of the endoplasmic reticulum are interspersed between the chromosomes at metaphase (Figure 25). No pores are evident in these segments.

### Anaphase

The first evidence of anaphase is the appearance of the two daughter chromosomes in the region of the kinetochores as the chromosomes separate and move toward the poles. Each group of chromosomes exhibits a kidney-shaped profile by late anaphase. The polar regions contain a great many ribosomes, nucleoplasmic granules and small vesicles, some with ribosomes attached to their outer surface, and endoplasmic reticulum sacs but no large organelles. As in metaphase, many sacs probably formed from the endoplasmic reticulum are found between the chromosomes (Figures 32 and 33). Often persistent nucleoli of "normal" appearance (Figure 31) are found at the poles.

In early anaphase microtubules are only sparsely present between the groups of daughter chromosomes (Figure 32). On the polar sides of these groups spindle microtubules may be seen oriented toward the poles (Figure 31).

Cross sections of spindle microtubules (Figure 33), lying between the anaphase chromosomes, show a distinct substructure. They are circular in cross section with an overall diameter of 27 - 30  $\mu$ m and an inside diameter of 15  $\mu$ m. The wall which appears to be composed of approximately 12 to 13 units (inset Figure 33) lying side by side is 75 A thick. In many of these microtubules threads of electron dense material can be observed which seem to be linking the subunits of the wall together (inset Figure 33). These cross linking threads are of the order of 15 - 30 A thick.

Once anaphase has started chromosomes begin to lose their smooth contour and longitudinal profiles exhibit more or less regular chromatin extensions (Figure 32). In cross section chromosomes exhibit small electron-transparent areas from which bands of chromatin often appear to radiate (Figure 33).

Anaphase chromosomes at the poles are uniformly spaced from each other as if there were a mutual repulsion (Figure 32). However, mechanical factors such as the presence of vesicles or chromatin extensions might account for this spacing.

## Telophase

During telophase the cell reorganizes into the typical interphase form. This period may be quite easily divided into early, middle and late telophase, with cells in late telophase changing gradually into the interphase cell.

Because of the orientation of the typical kidney-shaped chromosome masses in telophase, the daughter cells produced by the division of one sporogenous cell can easily be recognized. Cytoplasmic arms extend into the daughter nuclei from the polar sides of the nuclei (Figure 36). Thus the continuous sides, lacking the cytoplasmic protuberances, of the two daughter nuclei will face the newly formed cell plate and each other. This configuration and the anaphase orientation of chromosomes persists through interphase and is carried over into prophase.

An early indication of telophase is the formation of the nuclear envelope around each of the chromosome masses (Figure 34). This is the result of the close orientation of vesicles and irregular sacs around the chromosome mass where they coalesce to form the nuclear envelope. Nuclear pores form very early and, indeed, are found in the envelope before it is completely formed (Figure 34). As early as they can be seen, these pores resemble the pores of interphase and prophase. It is interesting to note the small amount of material other than chromatin that is included in the newly formed nucleus, large organelles and most of the ribosomes and vesicles being excluded (Figure 34).

The polar region at early telophase is characterized by segments of microtubules, many vesicles, ribosomes and clumps of disintegrating nucleoli (Figure 34) all randomly scattered.

Some nucleoli persist in the cytoplasm as late as telophase but are excluded from the daughter nuclei as these are formed. Most of these nucleoli show signs of disintegration and all have disappeared by the end of telophase.

The chromatin continues to slowly despiralize throughout telophase. This first appears as a loosening of the compact chromosome and an overlapping of chromatin of one chromosome with that of another (Figure 34). By late telophase, therefore, the nucleus has a striated appearance which can be observed both in the light microscope and in electron micrographs (Figure 37). This is due to a characteristic organization of the denser chromatin strands into parallel rows separated by the less dense nucleoplasm.

By midtelophase the new nuclei have changed considerably. Already a large number of nucleoplasmic granules are scattered throughout the clumps of chromatin which are no longer recognizable as individual chromosomes (Figures 35 and 36). Fully formed nucleoli, with their perinucleolar spheres (Figure 36), and masses of loosely formed fibrous material differing from the chromatin clumps and surrounded by clear zones, appear in the nucleus (Figure 35).

The function and fate of the nucleoplasmic granules, which appear larger and less dense than the ribosomes in the cytoplasm, is an interesting problem. Clumps of these granules can be observed occasionally in the cytoplasm in mid and late telophase (Figures 37 and 39).

The process of cytokinesis occurs in telophase. In early telophase irregular sacs, presumably segments of the endoplasmic reticulum, small vesicles, and microtubules are evident at the site of the previous equatorial plate where they form the phragmoplast (Figure 38). The small vesicles are lined up along the equatorial plate in the center of the phragmoplast. The microtubules and segments of the endoplasmic reticulum lie perpendicular to the plane formed by these small vesicles. Many microtubules and a few segments of the endoplasmic reticulum extend through this plane. After the cell plate is almost completely formed, spindle elements can still be seen to pass through it. Later these microtubules appear as dense linear arrays of unorganized material (Figure 39). The plasmodesmata formed in the cell plates of Psilotum are approximately 30  $\mu$  in diameter and usually do not appear to have a complex internal structure.

## DISCUSSION

## General Aspects of Meristematic Cells

The cells of sporogenous tissue of Psilotum nudum are quite similar to the meristematic plant cells of other species that have been studied with the electron microscope (Porter, 1956; Buvat, 1958; Porter and Machado, 1960; Whaley et al., 1960a; Chardard, 1962; Lafontaine and Chouinard, 1963 and others). The cytoplasm contains the usual organelles including mitochondria, Golgi bodies, proplastids, and the endoplasmic reticulum (Figures 1 and 2). Ribosomes appear both free in the cytoplasm and connected to the endoplasmic reticulum and outer membrane of the nuclear envelope (Figures 14, 16, 17 and 18). The organelles of the interphase cytoplasm appear to be preferentially oriented (Figure 1). The fact that many small vacuoles tend to lie close to the cell wall with most of the proplastids and mitochondria lying between the vacuoles and the nuclear envelope may relate to some specific metabolic activity requiring this configuration of organelles. At the least it seems probable that the environment of the nucleus is partially controlled by this orientation.

A number of organelles all having a size around  $.2 - 1 \mu$ , bounded by a single membrane and with various internal organizations, have been reported in the literature. Multivesicular bodies (Sotelo and Porter, 1959), spherosomes (Perner, 1953), microbodies (Rouiller and Bernhard, 1956) and phragmosomes (Porter and Caulfield, 1960) fall within this group. The dense body prominent in the polar cap regions of Psilotum

is within this size range but differs from these other bodies by having a double membrane. Because of this characteristic it appears to resemble more closely the first stage of proplastid development as described by von Wettstein (1958).

During prophase the environment of the nucleus is altered by the formation of the polar caps or "clear zone" (Inoué and Bajer, 1961) which completely surrounds the nucleus (Figure 14). At this stage the larger organelles are excluded from the region adjacent to the nucleus and the zone shows a marked increase in ribosomes which correlates with Fabbri's (1960) observation that this zone is rich in RNA. This may influence in some way the succeeding steps in the breakdown of the nuclear envelope and the process of mitosis.

With the completion of the nuclear envelope around the kidney-shaped nucleus an extension of cytoplasm into the nucleus becomes noticeable (Figure 36). This remains throughout telophase and may persist through interphase and prophase of the next division. It probably has no significance to the cell, remaining only as a relic of the previous telophase; however, it may have utility for the experimenter in serving as a marker indicating the orientation of the previous spindle pole. This could be of value in studies of spindle formation and orientation and the relative role of cytoplasm and nucleus in these processes. Any information regarding the polar regions of plant cells, typically lacking centrioles and asters, would add to the meager knowledge of this subject.

## Microtubules in the Mitotic Apparatus and Cytokinesis

The establishment of the polar caps in early prophase apparently determines the position of the poles of the spindle in Psilotum nudum. Fibrous material was apparent within the polar cap regions before any breaks were observed in the nuclear envelope (Figure 14). This has been reported in light microscope studies of other plant cells (Fabbri, 1960; Inoué and Bajer, 1961) and also by Sedar and Wilson (1951) in early electron microscope investigations. The orientation of the microtubules in Psilotum, which correspond to the fibrous material of previous studies, is not always parallel to the long axis of the spindle, especially in the clear zone adjacent to the poles of the spindle. However, in the thinner regions of this zone next to the site of the future equatorial plane their orientation in this direction is more constant.

An interesting finding is the presence of two distinct sizes of microtubules in this area, with no intermediate sizes being detected (Figures 19 and 20). The significance of this is not easy to establish but it is possible that development of the spindle microtubules involves an increase in size. One observation can be given in favor of this hypothesis. After the breakdown of the nuclear envelope and the organization of the spindle only the larger, 27 - 30  $\mu$ , microtubules were seen making up the fibers of the spindle (Figures 25 and 27). The smaller elements were no longer visible. If this is true the accepted idea that spindle filaments or microtubules do not change their diameter throughout cell division (cf. Harris, 1962; Roth and Daniels, 1962) will need to be reconsidered. Thus, if a change in size occurs in prophase,

it may also occur in anaphase along with the movement of the chromosomes. However, no such change in diameter at this stage of division was ever seen in Psilotum.

Occasionally a linear array of poorly organized material (Figure 21) can be seen in the polar cap region. This may represent an early stage in the organization of microtubules. Similar disorganized dense lines have been seen traversing the newly formed cell plate (Figure 39) and are interpreted here as microtubules undergoing disorganization.

Where does the raw material come from for the formation of these microtubules? The cytoplasm, nucleoplasm and mixtures of the two have all been suggested as the source. Microtubules can form outside intact nuclear envelopes, e.g., asters of animal cells and in polar cap regions of certain plants, inside intact envelopes, e.g., protozoans (Roth and Shigenaka, 1964) and fungi (Berlin, 1964) or they may not form until the cytoplasm and nucleoplasm mix (Roth and Daniels, 1962). Thus the raw material must be able to freely cross the nuclear envelope or else it can be produced both within the nucleus and cytoplasm in the same cell or at least at different sites in different cells. However, it seems reasonable to assume that the site of production of this protein is more localized than this. Considering Inoué's (1959, 1960, 1964) hypothesis of the dynamic characteristic of spindle fibers and evidence presented by Richards (1960) and Richards and Bajer (1961) of protein movement from the nucleus to the cytoplasm, it seems reasonable that the raw material may move freely between the cytoplasm and the nucleoplasm and be organized into microtubules when the environmental condi-

tions such as pH or ionic strength are in proper balance, or when the raw material comes under the influence of an organizer region. This may occur within the nucleus in some cells, in the cytoplasm in others, or after the two have mixed in yet other cell types.

Attention is also directed toward the large number of ribosomes found in the polar cap zones (Figures 14 and 18). Ribosomal function is reportedly that of protein synthesis and spindle microtubules are composed of a high proportion of proteins (cf. Mazia, 1961). It seems unlikely that the high proportion of ribosomes and the many microtubules found together in this zone is purely a coincidence, and yet no structural connections have been seen between ribosomes and microtubules in Psilotum. However, these ribosomes may still be functioning to produce protein which will later be organized into microtubules.

Inoué (1959, 1960) has described the spindle fibrils as being composed of oriented micelles. He has postulated a pool of micelles from which this oriented material is derived. If this is true, one might find zones of micellar material around the microtubules that are not oriented to as great an extent as that in the microtubules themselves. Roth and Daniels (1962) and Roth and Shiganaka (1964) report wisps of material in the area around the filaments of the spindle in various protozoans which tend to support this idea. There is some indication of a similar unorganized material surrounding the microtubules in Psilotum (Figures 15, 19 and 20). A medium dense area in which very fine fibrous material may be observed typically surrounds these elements.

Ribosomes and other material are usually excluded from this area which is thought to be present even in anaphase (Figure 33).

When the nuclear envelope breaks down, the cytoplasm of the polar cap regions and the nucleoplasm seem to mix freely and rapidly. The spindle elements can soon be observed in close proximity to the chromosomes, presumably at the kinetochores (Figure 22). Several individual microtubules, all having similar diameters, associate with one kinetochore region (Figure 25). These make up a chromosomal fiber which appears to be particularly well organized near the chromosome. The kinetochore may control the orientation and organization of the spindle microtubules in this area. Spindle microtubule termination at the poles has not been observed or at least not recognized under the electron microscope in Psilotum. Spindle microtubules do not seem to be associated in pairs as Porter (1956) reported in early electron microscope work on Allium cepa.

In the early stages of separation of the daughter chromosomes in anaphase "continuous" or pole-to-pole microtubules are not evident in the region between these two groups of chromosomes (Figure 32). However, there is a sharp increase in the number of microtubules in the area of the equatorial plane by early telophase. These microtubules are apparently not of the pole-to-pole type since most of them seem to terminate at the borders of the phragmoplast (Figure 38). This no doubt accounts for the birefringence reported by Inoué and Bajer (1961) in this region. Fibrils in this region have previously been reported by Satō (1959) and Ledbetter and Porter (1963). By the time the cell plate

has completely formed in Psilotum the typical microtubules are no longer visible in electron micrographs (Figure 36).

It may be that the microtubules are not organized in the region between the early anaphase chromosomes in plant cells since this area seems to be devoid of them. Possibly there is a difference in pH and/or ionic strength in this region or there may be no "organizer" under whose influence the spindle material in this region lies. Later, however, the forming cell plate appears to act as a spindle organizer (Inoué, 1964), thus it would seem possible that the raw material for the formation of spindles is present in this region as soon as the chromosomes separate and is not organized into microtubules until the cell plate becomes active as a spindle organizer. The raw material for the formation of the microtubules may come from a breakdown of the chromosomal fibers as the chromosomes move toward the poles. Evidence from the polarizing microscope studies of Inoué and Bajer (1961) could be used as an argument in favor of this idea.

Porter and Machado (1960) have suggested that extensions of the endoplasmic reticulum across the region of the cell plate were responsible for the formation of plasmodesmata. In addition to this, it also appears that plasmodesmata may result from the formation of the cell plate around microtubules. In fact, in Psilotum, it appears likely that this is the manner in which the majority of these connections form. Evidence for this may be listed as follows: 1) microtubules are evident in the region of the forming cell plate in sufficient number to account for the numerous plasmodesmata formed (Figure 38) whereas the number of

endoplasmic reticulum extensions across this zone appears to be too few to account for all plasmodesmata, 2) the size of the microtubules and the plasmodesmata, approximately 30  $\mu$  each, agree quite closely, 3) finally dense disorganized material extending through regions of the developing cell plate where plasmodesmata will later be (Figure 39) apparently represents microtubules undergoing disorganization. Association of elements of the endoplasmic reticulum with plasmodesmata (Whaley *et al.*, 1960a) may occur after the formation of the latter.

#### Nucleolar Behavior

Many higher plants have nucleoli which do not break down during late prophase or prometaphase of the mitotic cycle and thus may be found either at the spindle poles or at the periphery of the equatorial plate during metaphase, anaphase and even as late as telophase (Bajer, 1953; Fabbri, 1960).

The interphase nucleolus (Figure 5) typically consists of an electron-dense mass containing scattered electron-transparent areas of different sizes. A closely packed fibrous material makes up the bulk of the dense zone, and electron-dense granules (small nucleolar granules) surround the outside of the nucleolus and line some of the electron-transparent regions, particularly the larger ones. Material of lower density may also be seen in association with the nucleolus, and can be identified as chromatin when the sections are cut in such a way as to show their continuity with chromatin masses outside the nucleolus. A perinucleolar sphere free of chromatin and nucleoplasmic granules is

generally found around interphase nucleoli (Figures 4 and 5).

Other types of nucleoli (Figures 8 and 9) have occasionally been observed in the interphase nucleus. A possible function for one of these types (Figure 9) will be discussed later.

Nucleoli typically retain their interphase appearance until the disappearance of the nuclear envelope (Lafontaine, 1958b; Lafontaine and Chouinard, 1963), at which time they disintegrate very rapidly. The granules and fibrils of the nucleolus become dispersed throughout the nucleoplasm and can no longer be distinguished.

In Psilotum this is true of only some of the approximately 6 nucleoli present in a single nucleus. Nucleoli that do not lose their integrity become detached from the chromosome as the chromosomes move toward the equatorial plane. From here they may move out to the periphery of the equatorial plate or they are often seen to pass out to the polar regions of the spindle, taking up a position typically occupied by the centrosome in animal cells (Figure 31).

Three unanswered questions arise at this point. Why do these nucleoli not break down as their more typical counterparts do, how and why do they move out of the region of the spindle, and what significance, if any, can be attributed to the frequent positioning of a nucleolus at the polar regions? The lack of breakdown may result from differences in salts and/or a variation in their concentration in the area of the nucleolus. Heath (1954) showed that low concentrations of cobalt salts in animal embryo cells resulted in a suppression of nucleolar disintegration. Fabbri (1960) feels that the nucleoli may persist in Psilotum

due to a large accumulation of proteins in these bodies as a result of an excessively long interphase period. He attributes this long interphase period to the machinery which is responsible for the preparations in the cell for the mitotic movement in the next division. He postulates that because of the phylogenetic position of this plant this machinery may not work as efficiently as in higher plants and the cell thus takes longer to get ready for division. The movement of nucleoli out to the periphery of the spindle may be due to movements associated with the formation of spindle fibers or may be a simple matter of displacement. Fabbri (1960) has speculated on the significance of the nucleolus in the polar regions of Psilotum nudum. He supposes that the nucleolus might contain two different ribonucleic entities analogous to genes. These then supervise the principal vital activities of the cell and also may be responsible for the initiation and primary control of mitosis. No evidence has been found in the present study that suggests answers to any of these questions. It would seem, however, very possible that the position of the persistent nucleoli is more a matter of interest to the observer than one of importance to the cell.

Persistent nucleoli may be seen at the poles in both metaphase (Figure 30) and anaphase (Figure 31). They have also been observed in telophase, but by this time their irregular outlines suggest that they may be disintegrating. No nucleoli were found in the cytoplasm of late telophase cells.

The origin of the new nucleoli of the daughter cells in Vicia has been studied by Lafontaine and Chouinard (1963) who described the ap-

pearance of granules along the chromosomes during early telophase. According to these workers this material supposedly forms a coat over the chromosomes; this coat then moves toward the nucleolar organizer region where a typical nucleolus is formed. The very first indication of nucleolar formation in Psilotum appears to be small tufts of fibrous material (Figures 9 and 35) which have been found in nuclei from mid-telophase until early interphase. Thus it seems that not all nucleoli develop at the same time. These tufts have not been observed in association with any clumps of chromatin, however, such connections may be out of the plane of the sections studied to date. They are considered to be nucleolar because of their appearance and since they are surrounded by a region of low density which is interpreted as the perinucleolar sphere. Another micrograph (Figure 6) shows a clump of dense homogeneous nucleolar material attached to chromatin. This may be a subsequent stage in nucleolar development in which nucleolar material may have accumulated around a tuft similar to those just described, obscuring it from detection in electron micrographs. If this be true, the tuft may correspond to the nucleolonema of Estable and Sotelo (1956).

The nucleolus apparently develops very rapidly since fully formed nucleoli may be evident as early as midtelophase within the daughter nuclei. However, the source of the nucleolar material is not known since no "pre-nucleolar" material (Lafontaine and Chouinard, 1963) could be found coating the chromosomes, and no very dense 140 A granules with doughnut-shaped profiles were ever observed in the nucleolar material. These granules were used by Lafontaine (1958b) to follow the sequence of nucleolar formation in Allium cepa.

## Chromatin and Chromosome Fine Structure

One must use caution interpreting structure, particularly chromosome structure, in electron micrographs of the nucleus. Errors of interpretation may result from specimen damage during sectioning, astigmatism in the microscope, slightly out of focus micrographs, the texture of the embedding medium, or patterns in the photographic emulsion that may form during development or fixation. Figure 41 shows a section of Epon 812 outside the area of the tissue. Definite patterns are visible which might be misleading when superimposed on such seemingly unordered material as chromatin masses or chromosomes. The photographic plate itself after exposure only to electrons and then development in the usual way also shows faint patterns (Figure 40).

Glutaraldehyde-osmium fixation seems to preserve the chromosomes as well, if not better, than any other fixative used by electron microscopists. Uranyl acetate was routinely used as an electron stain. During interphase clumps of chromatin are scattered at random throughout the nucleus. The fine structure of the chromatin is not usually well defined at this stage. It may be that optimum fixation at this stage has not been obtained, that chromosomes are truly in a different state, or finally that some component is mixed with and masks the elementary 100 - 140 A fibrils seen in the mitotic chromosome. Usually only larger fibers, approximately 30  $\mu$ , are apparent, however in favorable material the 100 - 140 A fibrils are clearly evident (Figure 3). The only chromatin immediately apparent in electron micrographs is found in clumps but other evidence (Hay and Revel, 1963; Brinkley, 1964) shows that

interphase nuclei have a "meshwork" of chromatin which may spread throughout the entire nucleus. This may be in such a diffuse state that it is not usually apparent in the electron microscope. Portions of the nucleus (Figure 3) suggest such a "meshwork". The dense clumps of chromatin may be the "heterochromatin" of classical cytologists.

During prophase the chromatin material becomes organized into visible chromosomes. This is generally thought to be due to a complex process of coiling of the units of the chromatin. Hay and Revel (1963) have recently suggested that the chromatin "meshwork" of diffuse, interconnected strands of chromatin simply aggregates to form the chromosomes.

In Psilotum the chromatin of prophase appears similar to that of interphase. The fine fibrils still seem to be masked by an extra component within the chromosome. However, at some time during the process of chromosome organization, the appearance of the chromosome changes and by prometaphase the elementary 100 - 140 A fibrils are seen scattered throughout the chromosome. In these sections and with ideal conditions of fixation and staining these fibrils appear to be composed of one or more basic units of approximately 30 - 40 A (Figure 24) which are in a tight coil forming the larger fibril. Indication of coiling of a higher order, particularly in the region of the kinetochore (Figure 26), is often seen. However, generally the 100 - 140 A fibrils seem to be randomly arrayed.

On a larger scale cross sections or tangential sections of chromosomes display a very large order of coiling (Figure 24). This can be seen in prometaphase, metaphase and anaphase cells. Only rarely are

there profiles that could be interpreted as the two chromatids of a chromosome, probably because of their close contact with each other. Electron-transparent regions can often be seen in anaphase chromosomes (Figure 33). This is thought to be an indication of coiling, the axis of the coils being filled with nucleoplasm since the texture of the cores resembles that of the nucleoplasm.

It seems apparent from these observations that the unit structure of the chromosome is a 100 - 140 A fibril. This, in turn, may be composed of one or more 30 - 40 A units coiled together. Higher orders of coiling can also be seen. The 100 - 140 A elementary unit is in accord with that hypothesized by Ris (1961, 1962) and many others. Strictly on the basis of electron microscopy, a protein backbone model of the chromosome, such as that proposed by Taylor (1957) or Nebel and Coulon (1962) does not seem to fit the image viewed in the micrographs. How models such as those of Schwartz (1958) and Freeze (1958) would appear in the electron microscope is not known. A model such as that proposed by Steffensen (1959) would seem to fit the electron micrographs of Psilotum chromosomes. Steffensen has shown how such a multistranded rope model might divide, however, he does not answer all of the questions of geneticists. At least it can be positively stated, contrary to the ideas of Hay and Revel (1963), that some coiling does take place in the organization of chromosomes.

Differentiated regions of the chromosomes are also evident. Heterochromatin, as used by electron microscopists, appears to be less tightly packed (Figure 29) and is less electron-dense than the bulk of the chro-

mosome which is composed of euchromatin. This can be observed in regions of association between chromatin and nucleoli (Figure 5). Kinetochores, which can be identified by association with spindle microtubules, are areas of about the same density as the heterochromatin (Figure 25). Thus the regions of the kinetochore and those of the nucleolar organizers seem to be similar in texture and density, both are heterochromatic. No other structural component is evident in the area of the kinetochore. The spindle microtubules often appear to extend deep into the chromosome material. The chromosome itself seems to be more highly organized in the region of the kinetochore where large coils may be detected lying parallel to each other (Figure 26). Thus it appears that the units of chromatin are attached to the kinetochore in an orderly fashion.

Many granules, varying in size and location with respect to chromatin, are seen in the nucleus. Larger ones, referred to as granule "A", associated with chromatin and demonstrating a highly organized substructure (Figure 13) may contain DNA and be the perichromatin bodies described by Watson (1962) and Watson and Aldridge (1964). The nucleoplasmic granules (Figures 3, 4 and 7) presumably contain RNA. Their fate upon the breakdown of the nuclear envelope and the mixing of the nucleoplasm and cytoplasm is not clear. However, clumps of these granules (Figures 37 and 39) may be seen in the cytoplasm during mitelophase.

Other techniques will need to be developed before the complete story of chromosome fine structure is known, but it does not appear to

be an impossible problem and will eventually yield to inquiring minds.

## SUMMARY

1. The sporogenous cells of Psilotum nudum were studied by means of electron microscopy. Most of the tissue was fixed in glutaraldehyde, post-fixed in osmium tetroxide and stained on the grid with uranyl acetate. Embedding was in Epon. The fine structure of these cells appears similar, in most respects, to meristematic cells of other plants.
2. An electron dense body, .2 - .5  $\mu$  in diameter, with an elliptical profile was apparent, especially in the prophase cytoplasm. It can be distinguished from similar organelles described by many other authors by its double membrane. It may represent a stage in the development of proplastids.
3. The nucleoplasm contains a large number of granular profiles. A distinction was made between one special granule, referred to as granule "A", and other particles called nucleoplasmic granules. Granule "A", in cross section, consists of a central dense particle with 7 or 8 similar dense particles around its periphery, the material between is of medium density. This granule is compared with the perichromatin granule of Watson (1962). At least some of the nucleoplasmic granules may be profiles of fibrous elements containing RNA.
4. A "clear zone" surrounds the nucleus during prophase. It contains no large organelles but many ribosomes, segments of the endoplasmic reticulum and microtubules. These microtubules appear in two distinct sizes, 18  $\mu$  and 27 - 30  $\mu$ . They are probably precursors

of spindle microtubules.

5. The spindle microtubules are 27 - 30  $\mu$  in diameter. The dense outer wall is 75 A thick and seems to be composed of 12 or 13 subunits aligned in such a way as to form a cylinder. In favorable sections electron-dense strands, 15 - 30 A thick, appear within the electron-transparent central region of the microtubule which may link the subunits of the wall together.
6. Spindle microtubules usually appear to be better organized in the polar regions of the spindle than in the zone between the chromosome masses in early anaphase. Continuous or pole-to-pole fibers were not clearly demonstrated. However, during cytokinesis many microtubules appear in the area of the phragmoplast.
7. Plasmodesmata in Psilotum appear to result from the formation of the cell plate around microtubules. Evidence for this lies in the correlation of the number of microtubules present in this area with the large number of plasmodesmata formed, the similar size of microtubules and plasmodesmata, and the appearance of dense material crossing the cell plate through developing plasmodesmata. This material appears to be microtubules in a state of disintegration.
8. Typical interphase nucleoli are composed of a dense mass of granules, fibrils, and electron-transparent areas of different sizes. They are usually associated with chromatin. The fibrils appear to make up the body of the dense mass; this is surrounded by the granular material.

9. Not all nucleoli in Psilotum break down during late prophase or early prometaphase. Nucleoli have been observed in metaphase, anaphase and even in the cytoplasm of telophase daughter cells. They are not included within the new nuclei and all appear to disintegrate by late telophase. Often these nucleoli occupy a position at the spindle poles during division.
10. The first indication of nucleolar formation is a loose tuft of fibrous material found in midtelophase nuclei. Nucleolar development is very rapid since fully formed nucleoli have also been observed at this stage. No "prenucleolar" material could be seen coating the chromosomes during late anaphase or early telophase, therefore the site of formation of nucleolar material has not been determined.
11. Chromatin appears in two states during interphase, in relatively electron-dense clumps as well as in a diffuse state throughout most of the nucleus. In the best electron micrographs the elementary chromatin unit seems to be a fibril of 100 - 140 A diameter. This fibril itself appears to be composed of one or two threads of 30 - 40 A coiled together into a tight helix. This 100 - 140 A elementary unit is also observable in the chromosomes of other stages.
12. Large order coiling can also be observed in the chromosome. Thus the electron microscope evidence seems to indicate that coiling is responsible for the organization of the chromatin into the body of the chromosome. These observations are considered in light of presently existing chromosome models.

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## ACKNOWLEDGMENTS

The helpful suggestions, advice and constructive criticism of Dr. C. C. Bowen, the author's major advisor, are greatly appreciated. His suggestion of the problem and material on which to perform this study is also acknowledged. Thanks are also expressed to Dr. L. E. Roth for his helpful suggestions and encouragement.

The author is deeply indebted to his wife, Marilyn, for typing the original draft of this thesis and for her patience and understanding throughout the years of graduate work, climaxed by this study.

This investigation was supported, in part, by a grant, C-3982, of the National Cancer Institute, U. S. Public Health Service to Dr. C. C. Bowen and, in part, by a Public Health Service Predoctoral Fellowship, Number GPM-18,665, to the author.

## APPENDIX

## Explanation of Figures

## Key to all Labels

|     |   |  |
|-----|---|--|
| A   | - | Granule "A"                                  |
| C   | - | Chromatin Clump                              |
| CH  | - | Chromosome                                   |
| CM  | - | Chromatin "matrix" in the interphase nucleus |
| CP  | - | Cytoplasmic protuberance into the nucleus    |
| CPL | - | Cell plate                                   |
| CW  | - | Cell wall                                    |
| DB  | - | Dense body found in the cytoplasm            |
| ER  | - | Endoplasmic reticulum                        |
| ET  | - | Electron-transparent region of nucleolus     |
| G   | - | Golgi body                                   |
| HC  | - | Heterochromatin                              |
| K   | - | Kinetochores                                 |
| L   | - | Lipid inclusions                             |
| LG  | - | Large nucleolar granules                     |
| LT  | - | Large microtubule                            |
| M   | - | Mitochondrion                                |
| N   | - | Nucleoplasm                                  |
| NC  | - | Nucleolus                                    |
| NE  | - | Nuclear envelope                             |
| NF  | - | Nucleolar fibrillar material                 |
| NG  | - | Nucleoplasmic granules                       |
| NP  | - | Nuclear pore                                 |
| NU  | - | Nucleus                                      |
| P   | - | Proplastid                                   |
| PC  | - | Low density channel of pore complex          |
| PH  | - | Phragmoplast                                 |
| PL  | - | Plasmodesmata                                |
| PS  | - | Perinucleolar sphere                         |
| R   | - | Ribosome                                     |
| SG  | - | Small nucleolar granules                     |
| SP  | - | Polar region of the spindle                  |
| ST  | - | Small microtubule                            |
| T   | - | Microtubule                                  |
| V   | - | Vacuole                                      |
| VE  | - | Vesicle                                      |

Figure 1. Portion of a sporogenous cell of Psilotum nudum in interphase. The cell wall (CW), vacuoles (V), mitochondria (M), proplastids (P), lipid inclusions (L) and endoplasmic reticulum (ER) are apparent outside the nucleus. The nucleus (NU) contains chromatin clumps (C) scattered throughout the nucleoplasm (N) and granular and fibrous material making up part of the nucleoplasm. The organelles of the cytoplasm show a characteristic zoning with the vacuoles lying inside the cell wall and the proplastids and mitochondria occupying a region between the vacuoles and the nucleus. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X8,400

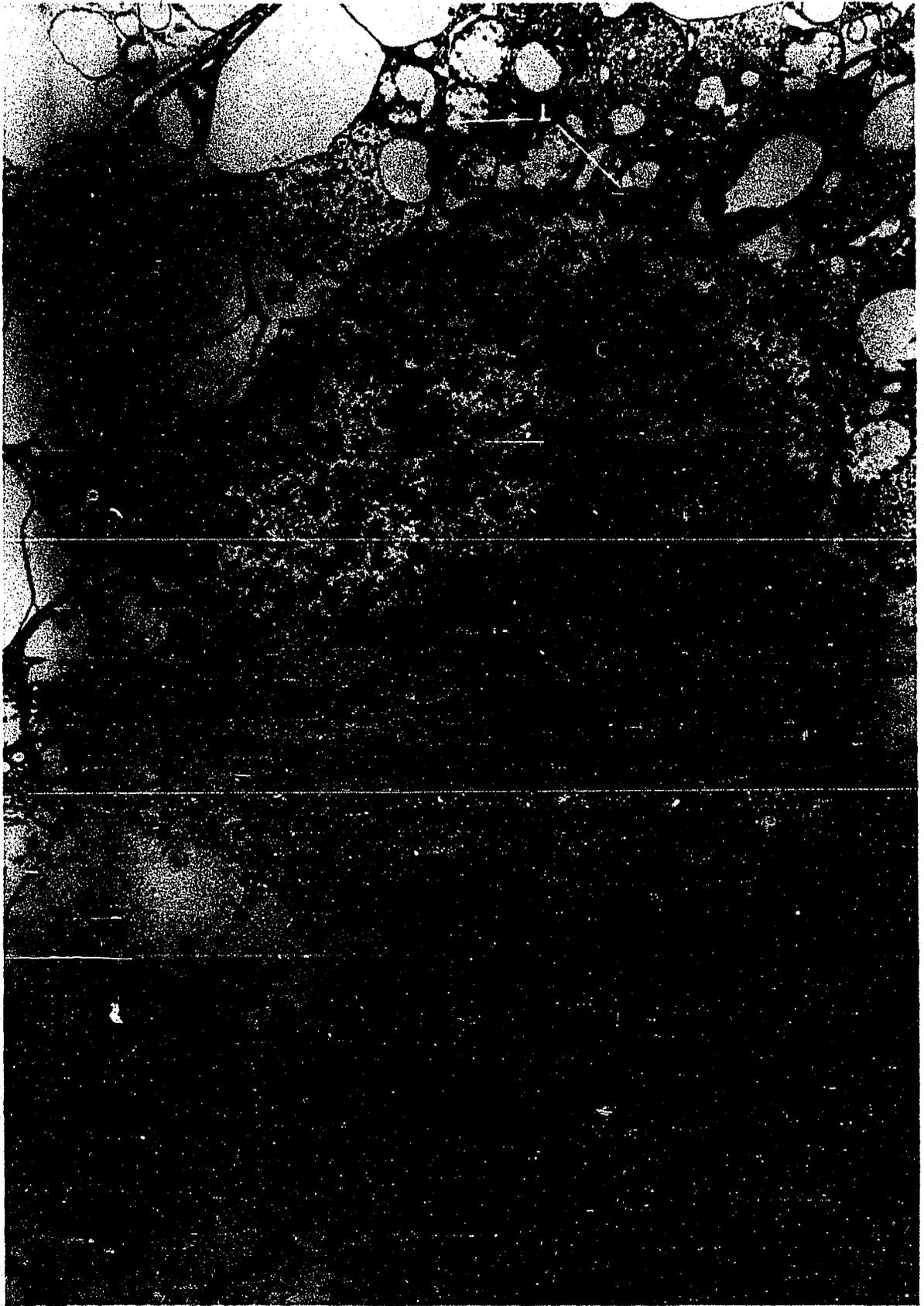


Figure 2. Portions of four sporogenous cells showing the large size of the nucleus of these cells. The cell wall (CW), endoplasmic reticulum (ER), mitochondria (M), proplastids (P), Golgi bodies (G), plasmodesmata (PL), the nuclear envelope (NE) and nuclear pores (NP) are shown. The cytoplasm appears to have more ground substance with this fixation. Proplastids are partially surrounded by segments of the endoplasmic reticulum. Fixation in potassium permanganate; no stain. Approximately X8,400

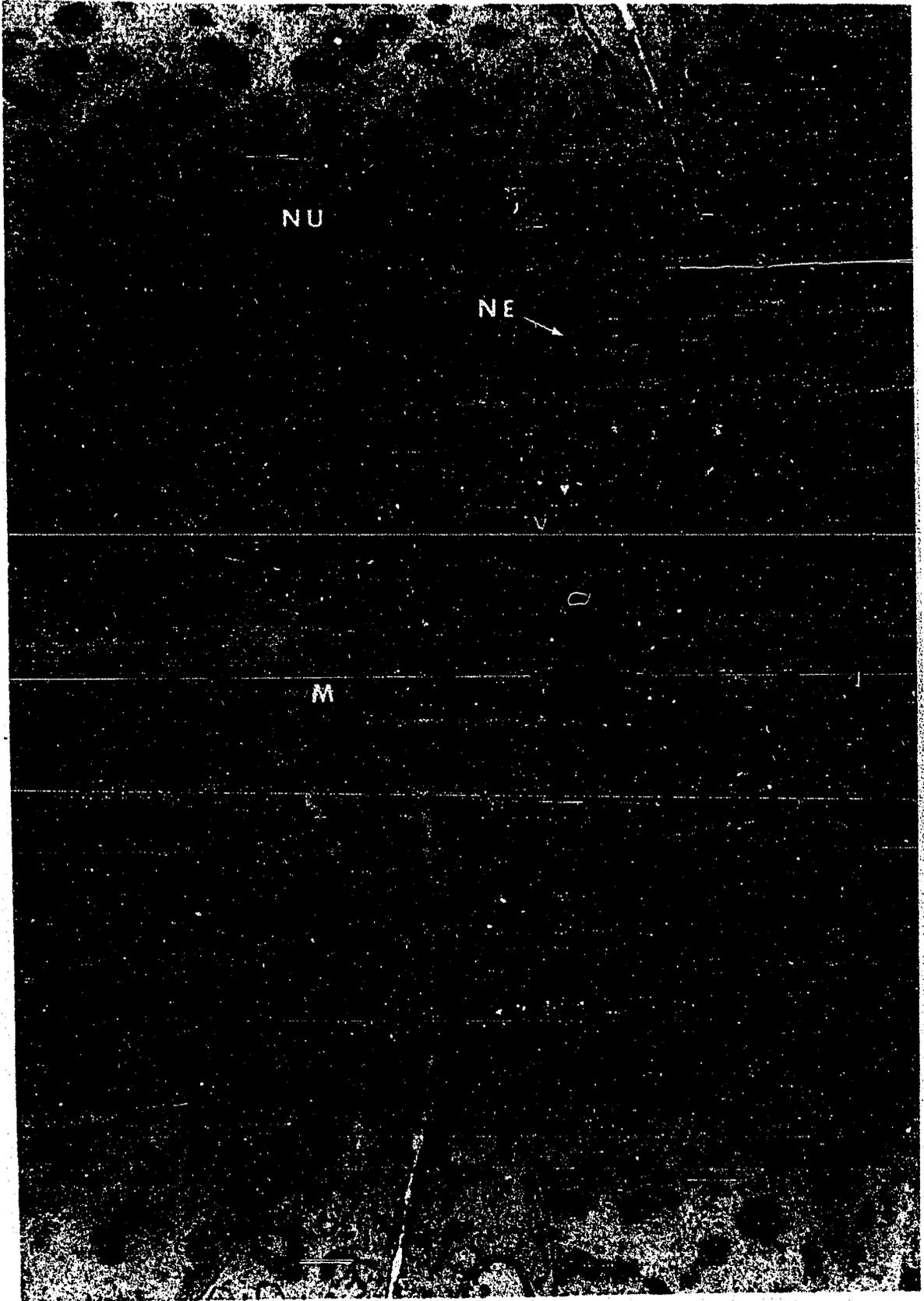


Figure 3. Chromatin is present in two phases in the interphase nucleus in clumps (C) and in a finely dispersed phase (CM) which spreads throughout most of the nucleus. The area in the lower right corner seems to be devoid of this dispersed phase. Darker clumps of chromatin (C) are thought to be euchromatin while lighter more loosely packed masses may be heterochromatin (HC). Many granular profiles are found within the nucleoplasm. Nucleoplasmic granules (NG) may be connected to each other by fibrous material (single wedges) or may be connected to chromatin (double wedge).

Figure 3a is an enlargement of the chromatin clump enclosed in white in Figure 3. Arrows point to short segments of fibrils, 100 - 140 Å in diameter which appear to be composed of smaller strands, 30 - 40 Å in diameter, coiled into a tight helix. Glutaraldehyde fixation, post-fixed in OsO<sub>4</sub> and stained with uranyl acetate. Figure 3 approximately X45,000. Figure 3a approximately X134,000

Figure 4. Interphase nucleus showing nucleolus (NC), chromatin (C) and nucleoplasmic granules (NG). Fibrous material seems to connect clumps of chromatin as well as chromatin and nucleoplasmic granules (double wedges). Glutaraldehyde fixation, post-fixed in OsO<sub>4</sub> and stained with uranyl acetate. Approximately X32,000

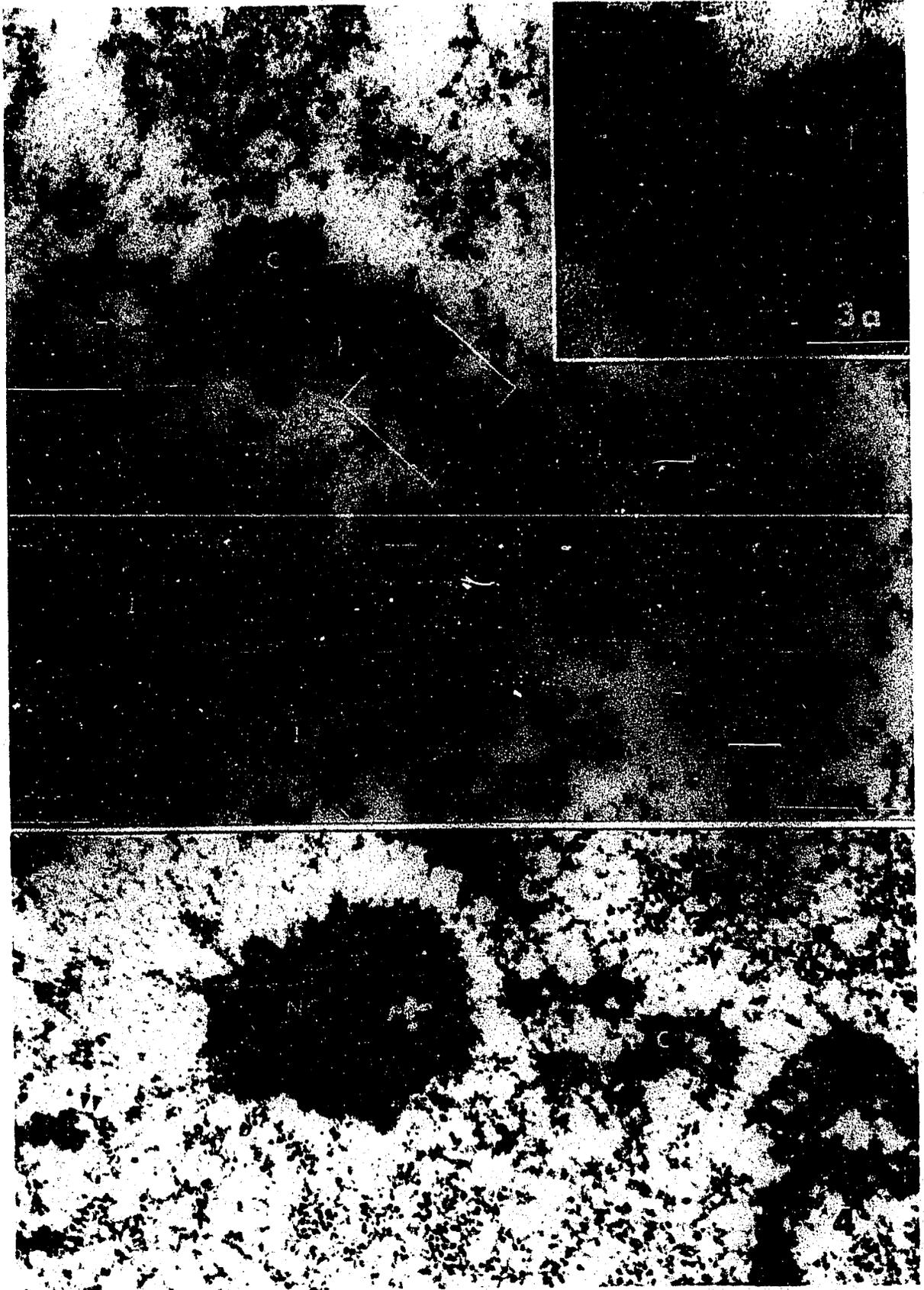


Figure 5. Interphase nucleolus showing electron-transparent zones of different sizes (ET) and a dense zone composed of fibrillar (NF) and granular material. The granules have been referred to as small nucleoplasmic granules (SG) to distinguish them from larger granules of other nucleoli. The nucleolus is attached to regions of less-dense material (HC), presumably heterochromatin. A region of typical euchromatin (C) is also apparent in association with the heterochromatin. The larger transparent zones are associated with the attachment of the nucleolus to the chromatin while the smaller transparent zones are typically found within the granular material at the outer edge of the nucleolus. A perinucleolar sphere (PS) surrounds the nucleolus. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 59,000$

Figure 6. Small nucleoli (NC) attached to a clump of chromatin (C). These may represent an early stage in nucleolar development. Perinucleolar spheres (PS) are apparent around the nucleoli. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 45,000$

Figure 7. Nucleoplasmic granules (NG) are often found in association with chromatin (C) during interphase. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 41,000$

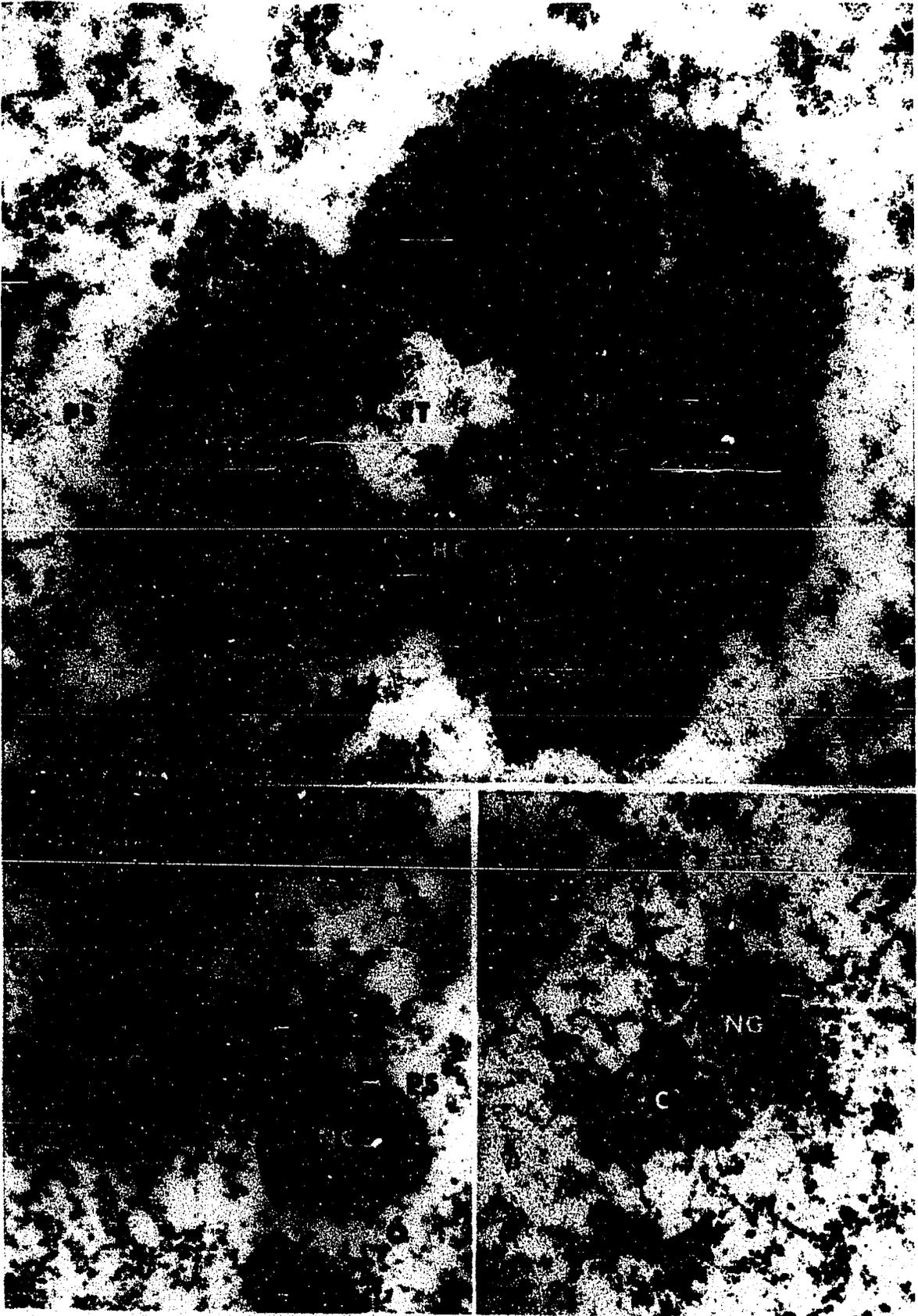
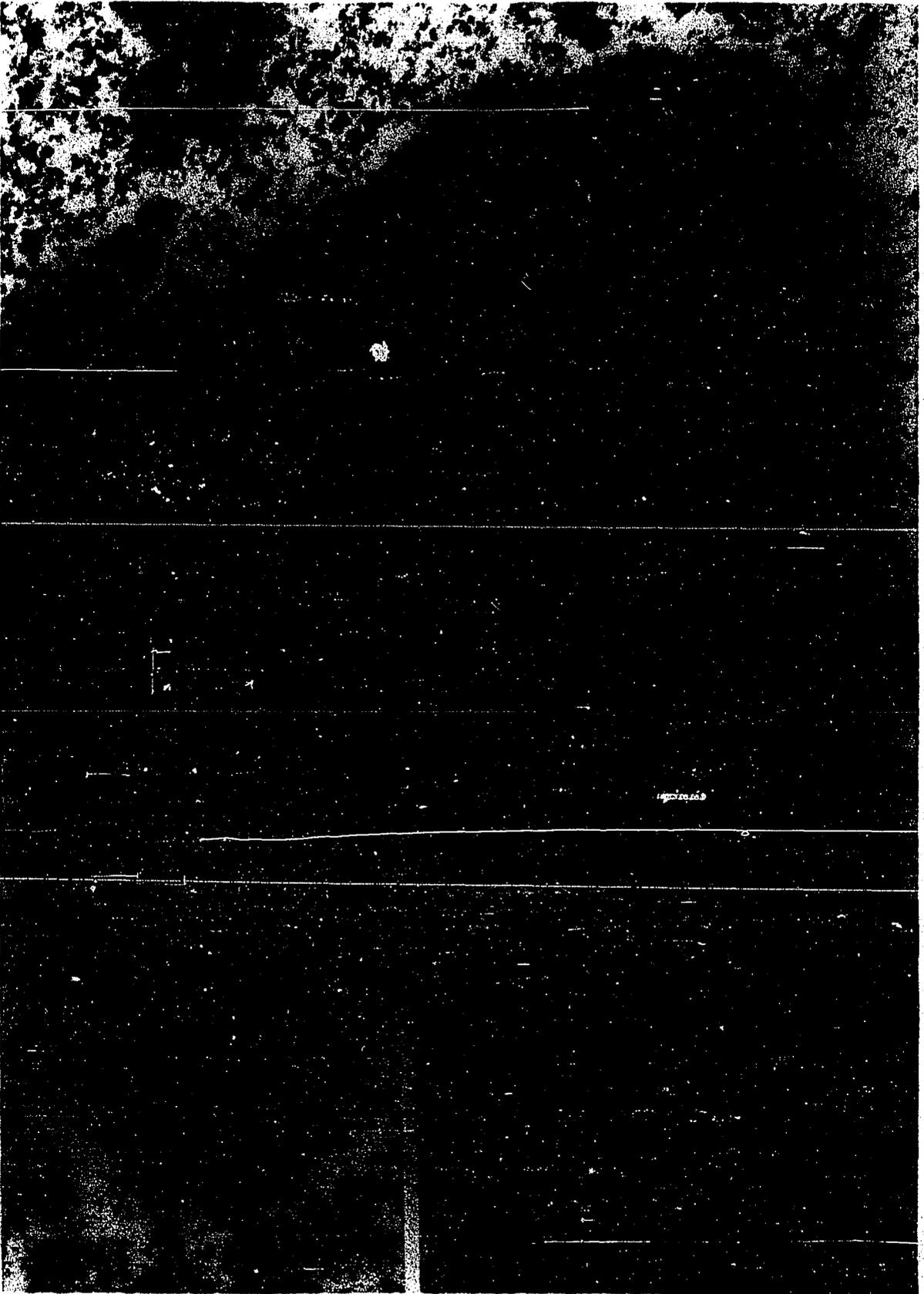


Figure 8. Dense homogeneous nucleolus found in an interphase nucleus. It appears to be composed of "large" granules (LG) which may be seen lining the periphery of the nucleolus and within the vacuole-like regions (ET) that are scattered throughout the body of the nucleolus. The attachment of the nucleolus to chromatin (C) may be seen in the lower left corner of the electron-micrograph. No perinucleolar sphere is present. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 41,000$

Figure 9. A tuft of loosely woven fibrous material found in an early interphase nucleus. It is surrounded by a clear area which is presumably a perinucleolar sphere (PS). This may be the first stage in nucleolar reformation; the tuft may correspond to the nucleolonema of other authors. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 59,000$

Figure 10. Microtubules (arrow) found in the cortex of a sporangial wall cell. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately  $\times 47,000$



- Figure 11. A section tangential to the nuclear envelope showing cross sections at different levels through the pore complexes (NP). At the level of the nuclear envelope the pore appears very dense. Material extends into the nucleoplasm from the pore where it is surrounded by an electron-transparent channel (PC). This channel seems to anastomose with channels of other pore complexes just inside the nuclear envelope. Chromatin (C) is closely associated with the inside of the nuclear envelope between the pores. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X59,000
- Figure 12. A section normal to the nuclear envelope showing the pore complex in longitudinal view (arrows). Fibrous material may be seen to extend into the clear nucleoplasmic channel from the pore at the level of the bottom arrow. Ribosomes are attached to the outer membrane of the nuclear envelope. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X67,000
- Figure 13. A granule (A), referred to in the text as granule "A", is found in close association with the chromatin (C). A small zone of lower density surrounds the granule. Figure 13a shows an enlargement of the same granule. A central dense particle of approximately 50 A and 7 or 8 similar peripheral particles are located within this body. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Figure 13 approximately X67,000. Figure 13a approximately X294,000

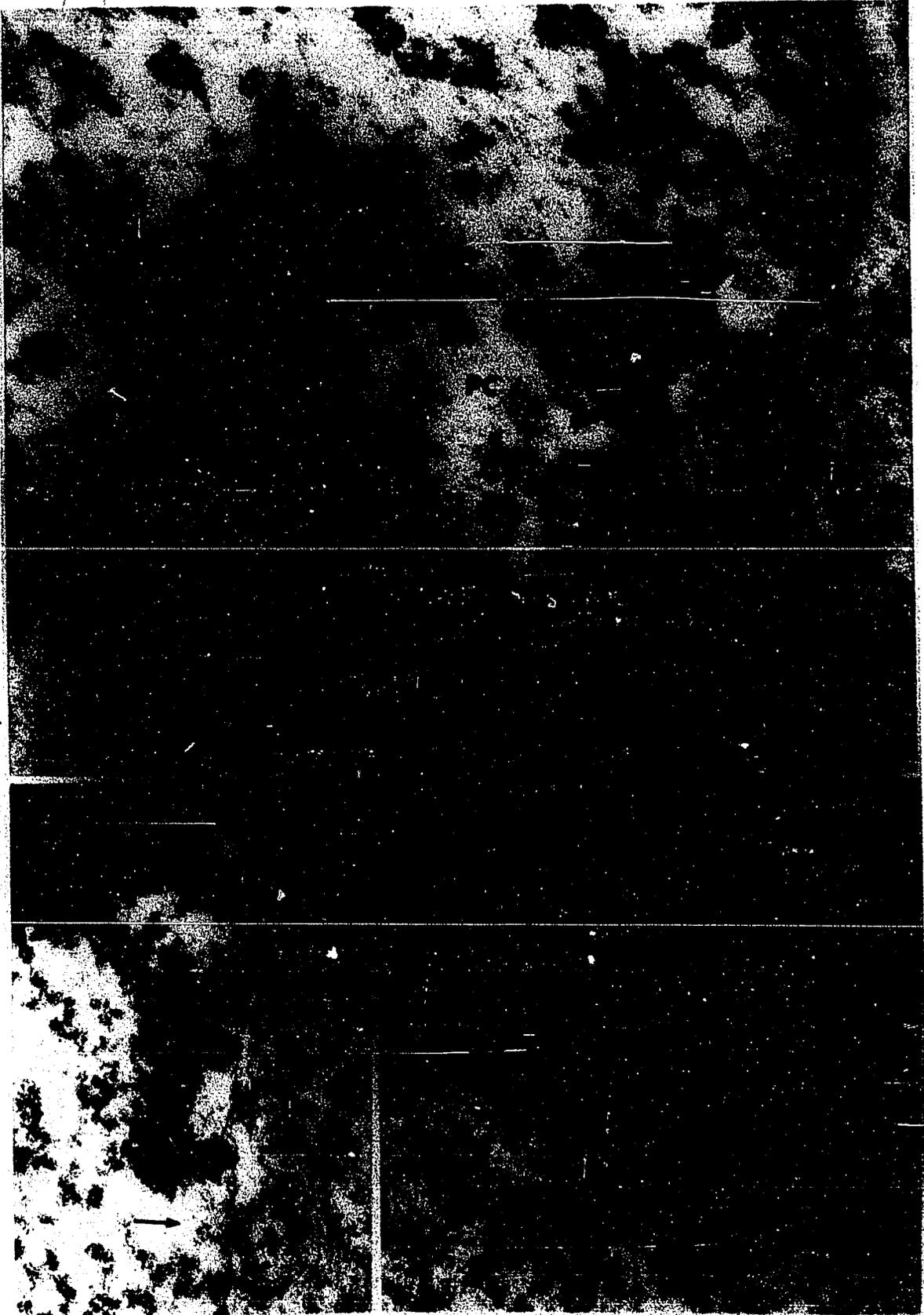
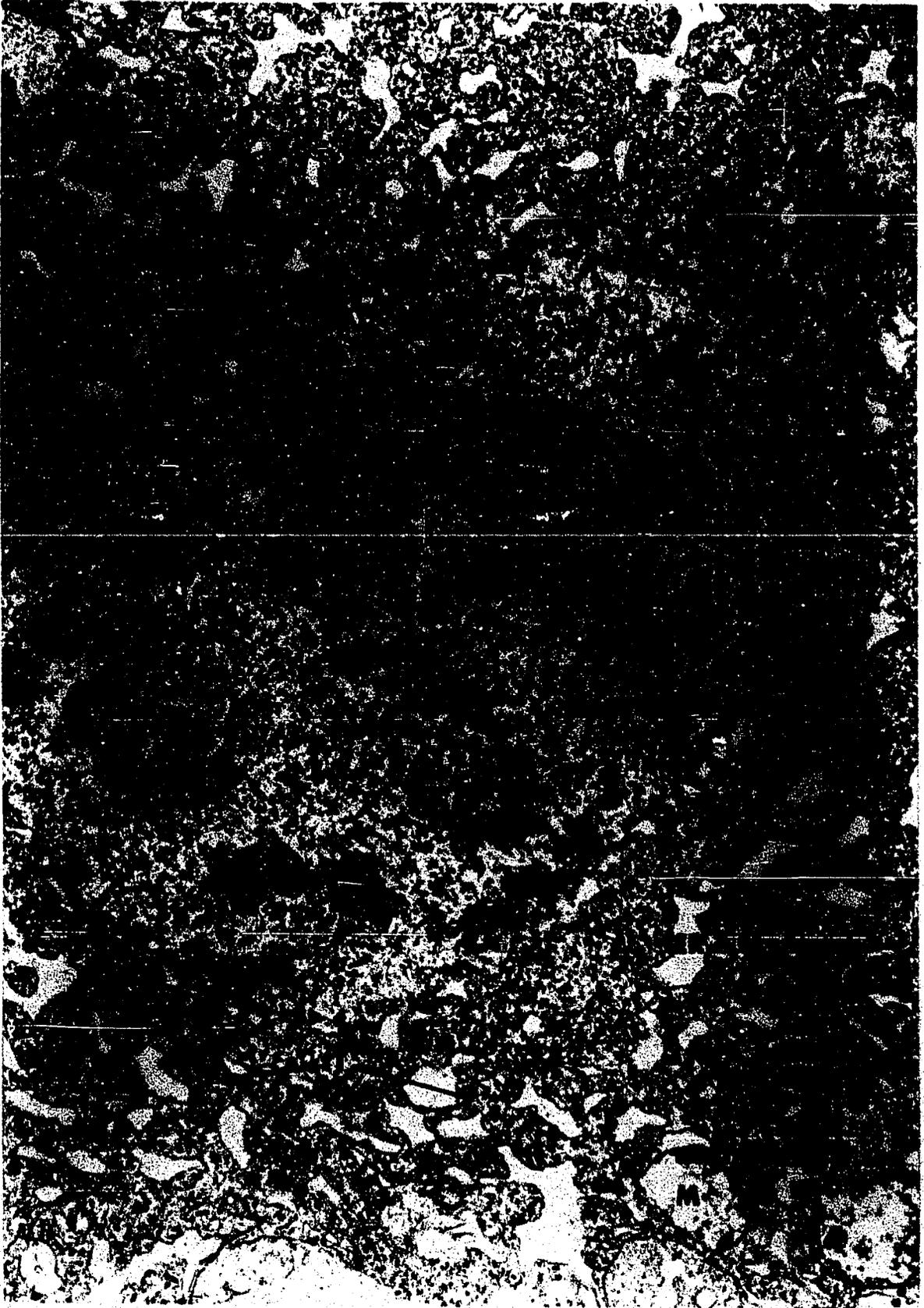


Figure 14. Tip of a prophase nucleus in the polar cap region. The nuclear envelope (NE) is intact, nuclear pores (NP) are evident due to the tangential sectioning. The larger organelles are excluded from the polar cap zone. Endoplasmic reticulum (ER), many ribosomes (dark spots in cytoplasm), microtubules (T) and an occasional dense body (DB) may be found in this region. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X14,000



- Figure 15. A prophase nucleus often surrounds a cytoplasmic protuberance (CP) due to the configuration assumed by the previous telophase nucleus. This extension is shown to contain microtubules before the nuclear membrane (NE) has broken down. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X58,000
- Figure 16. Ribosomes (R) are sometimes organized into spiral configurations, this is particularly true in the polar cap zone as shown here. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X55,000
- Figure 17. Whorls of ribosomes (R) are often found in the polar cap zone in contact with the outer membrane of the nuclear envelope (NE). Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X55,000

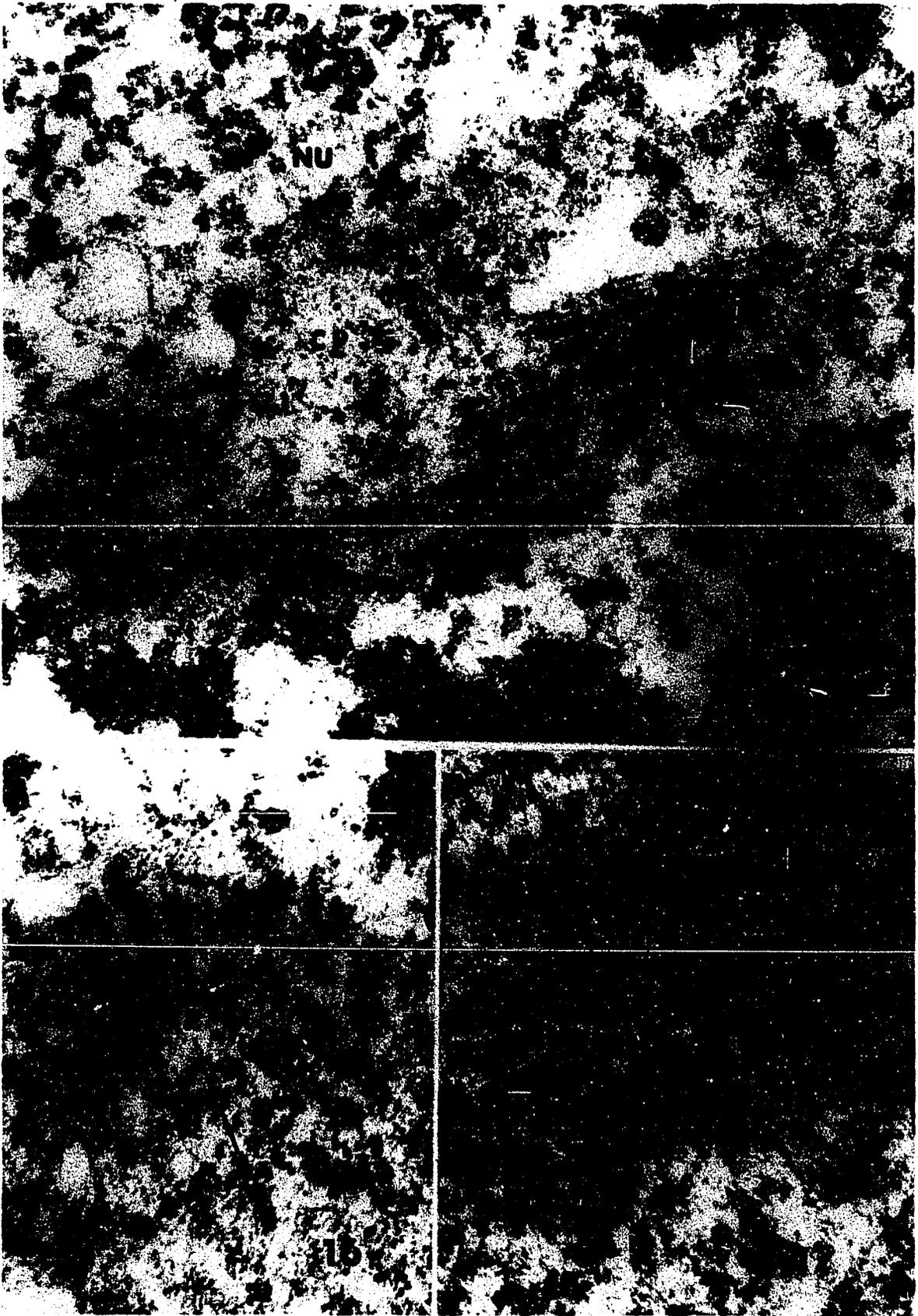


Figure 18. A section through the polar cap zone beyond the tip of the nucleus. Microtubules, endoplasmic reticulum (ER) and dense bodies (DB) are present. Many ribosomes are also present in this zone. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X14,000

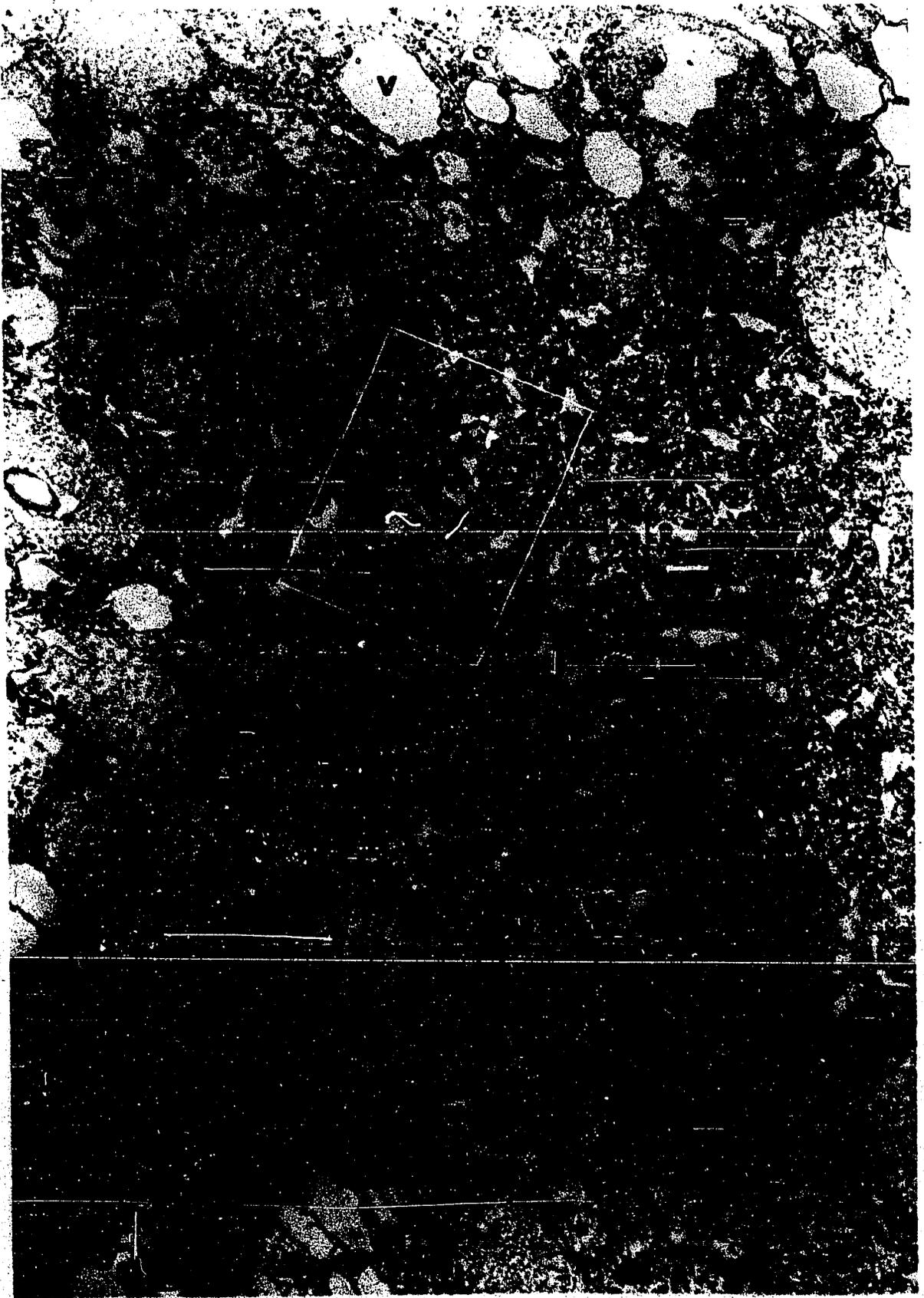


Figure 19. A higher magnification of the portion enclosed in white in Figure 18. Two sizes of microtubules may be seen. The larger (LT), 27 - 30  $\mu$ , are the same size as spindle microtubules, the smaller (ST), 18  $\mu$ , may represent a stage in the development of spindle microtubules. Many vesicles (VE) are scattered throughout the polar cap zones. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X45,000

Figure 20. Cross sections of small microtubules (ST) and large microtubules (LT) in the polar cap zone. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X46,000

Figure 21. A dense mass of material (arrow) arranged in linear order. This may be an early stage in the development of microtubules before they have attained their typical tubular organization. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X59,000

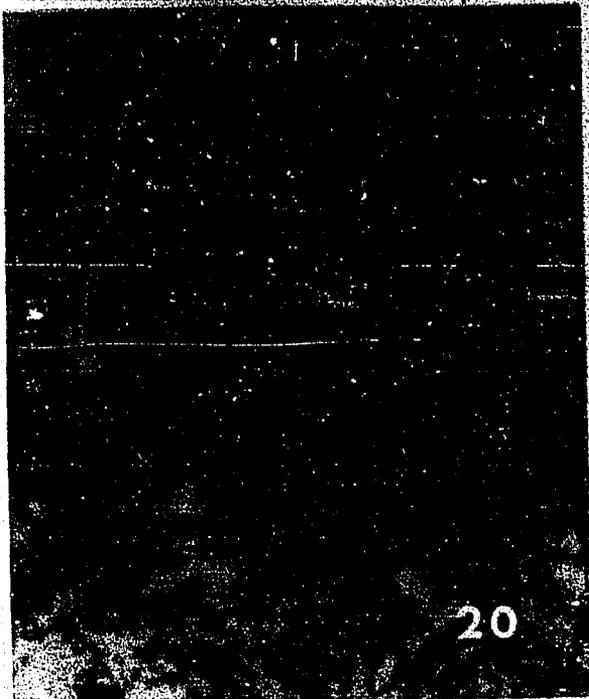
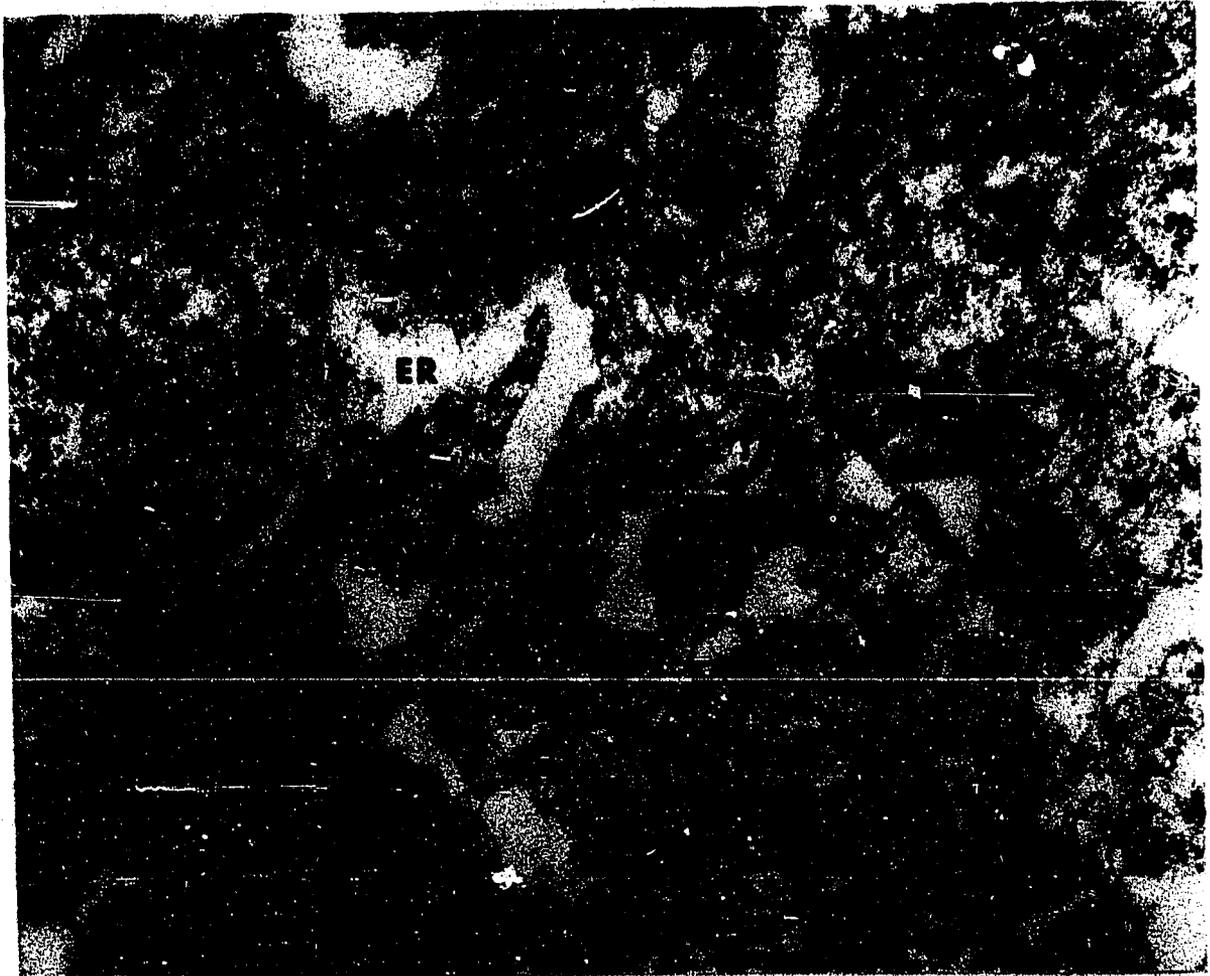


Figure 22. Nucleolus at prometaphase showing signs of disintegration. The fibrillar component (NF) still appears normal but the granular material (SG) is loosely held together. Many clear spaces (ET) appear within this peripheral zone of granules (SG). The nucleolus encircles a mass of heterochromatin (HC) except for a small break in which a spindle microtubule (arrow) appears. Association of microtubules with chromatin after the breakdown of the nuclear envelope appears to be very rapid. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X76,000

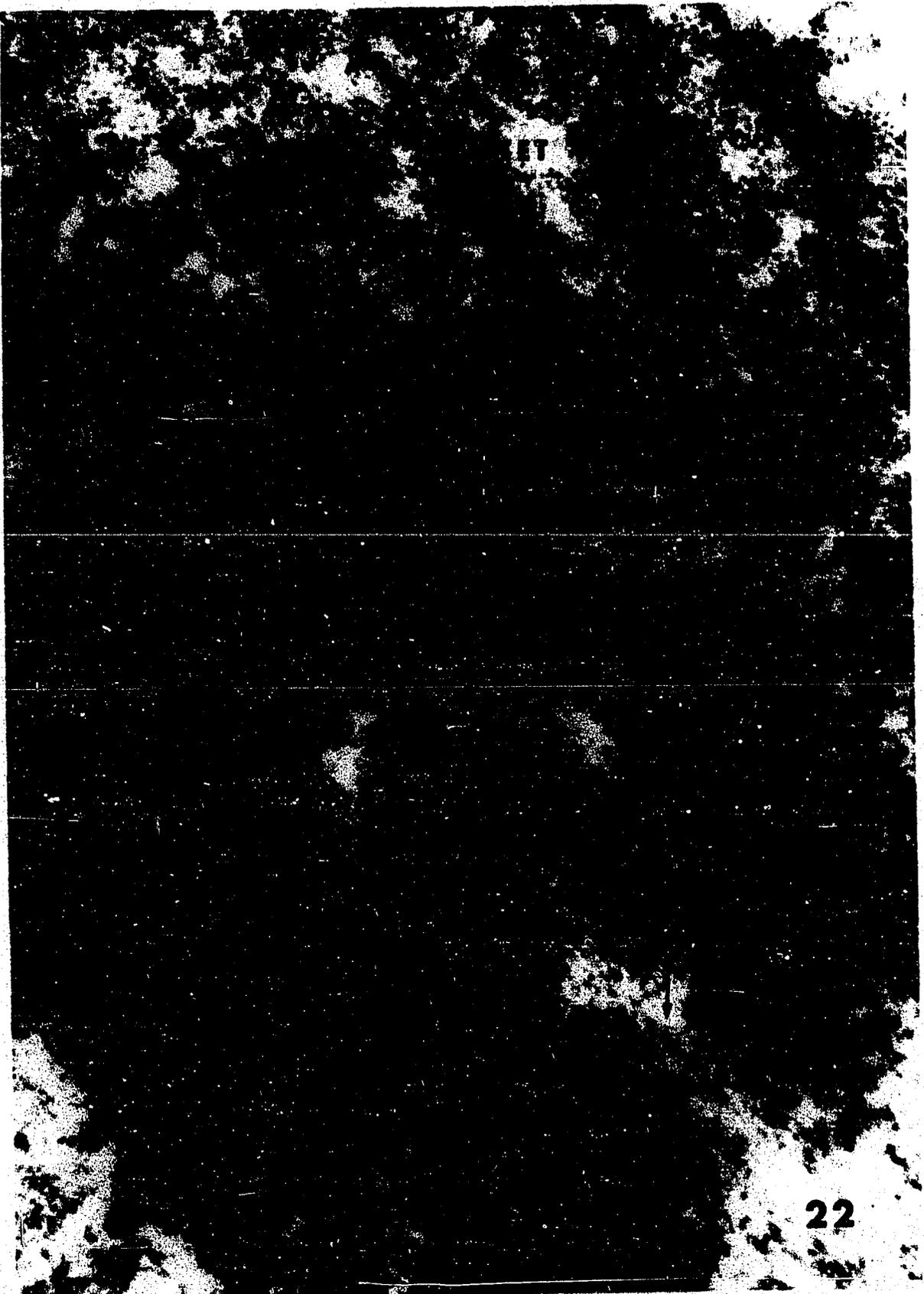


Figure 23. Prometaphase cell showing the condensed chromosomes (CH) and segments of the nuclear envelope (NE). The nuclear envelope apparently has just broken down. Heterochromatic regions (HC) appear lighter than the more darkly staining euchromatin. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X10,700

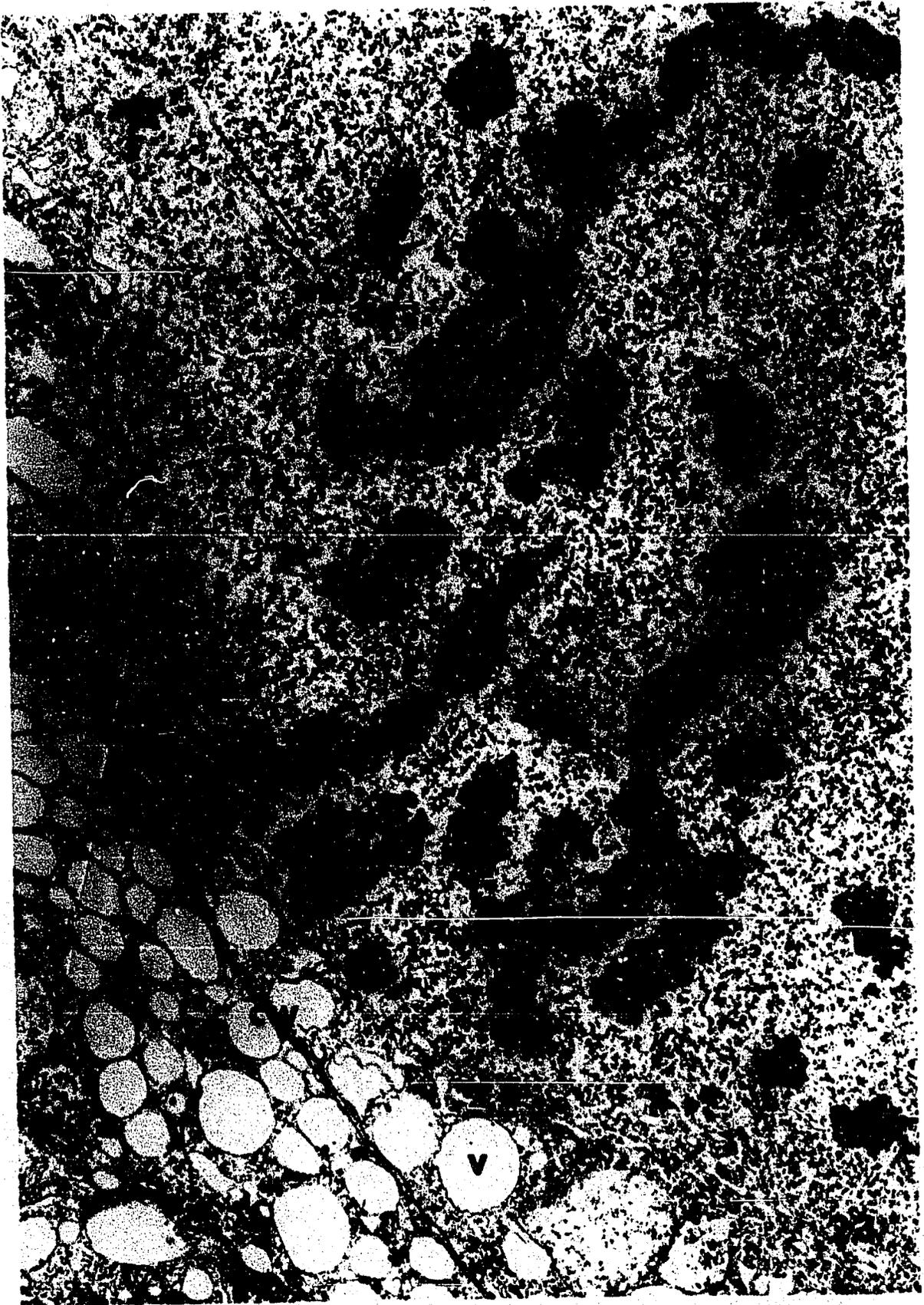


Figure 24. Tangential section through a prometaphase chromosome. The circular profile appears to be a large coil surrounding an electron-transparent core. Chromatin fibers seem to radiate out from this core. The chromatin material appears to be coiled within the body of the chromosome. Figure 24a is an enlargement of the area enclosed in white to show elementary 100 - 140 A fibrils (arrows) which make up the dense chromosome body. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Figure 24 approximately X59,000. Figure 24a approximately X134,000

Figure 25. Kinetochore regions (K) of three chromosomes. These regions are identified by their association with spindle microtubules (T). Kinetochore regions of chromosomes usually appear lighter, suggesting that they are composed of heterochromatin and are of a composition similar to the nucleolar organizer regions. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with lead hydroxide. Approximately X28,000

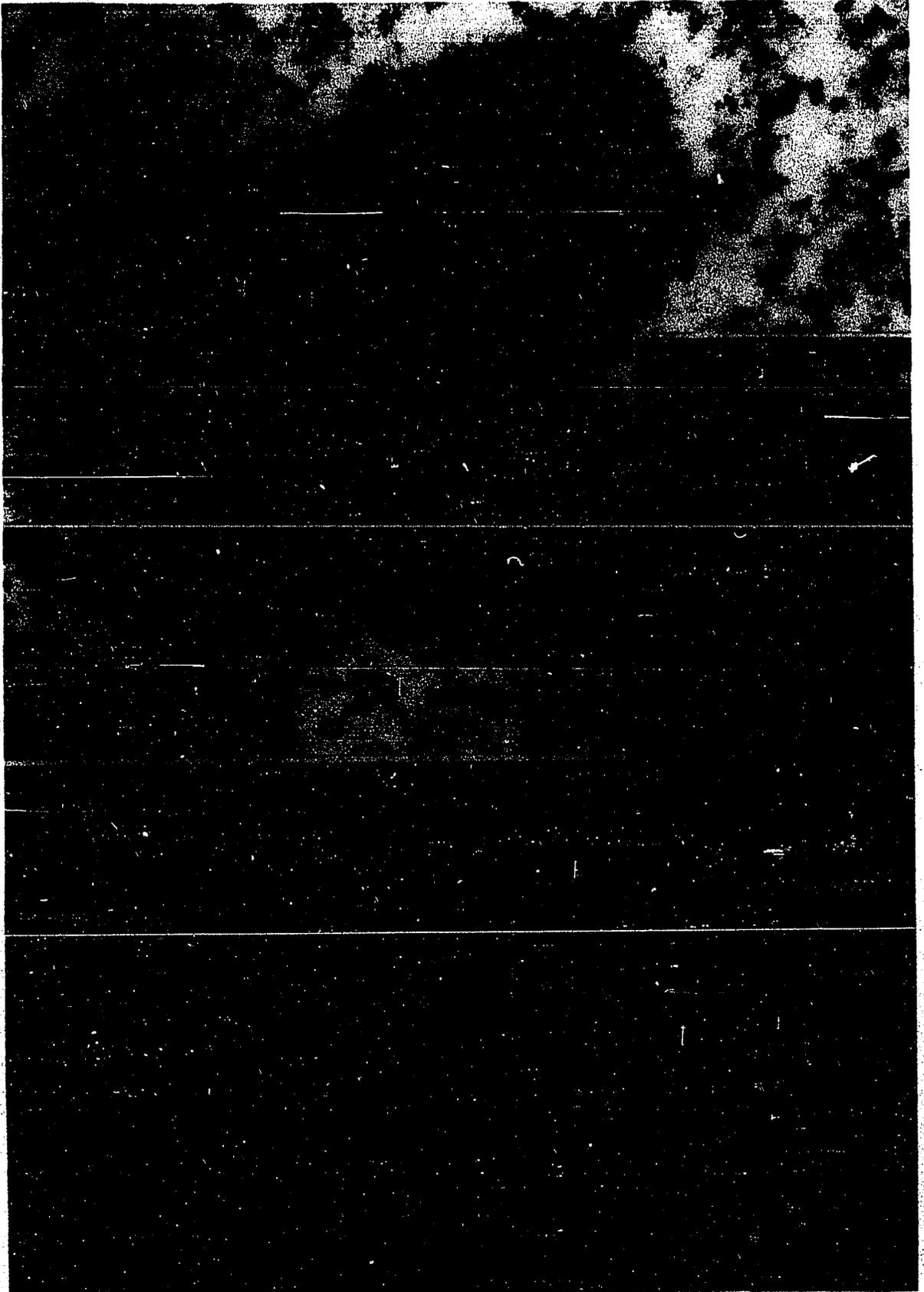


Figure 26. This is an enlarged view of the region enclosed in white in Figure 25. Some large coils (between brackets) can be seen in the region of the kinetochore (K). Several curved fibrils may be seen lying parallel to each other. This appears to represent a coil. Several of these large coils may be aligned side by side in this electron micrograph. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with lead hydroxide. Approximately X80,000

Figure 27. Longitudinal sections of spindle microtubules at metaphase. This group of microtubules seen here probably represent a chromosomal fiber. Such a fiber consists of approximately 10 spindle microtubules in close association. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with lead hydroxide. Approximately X41,000

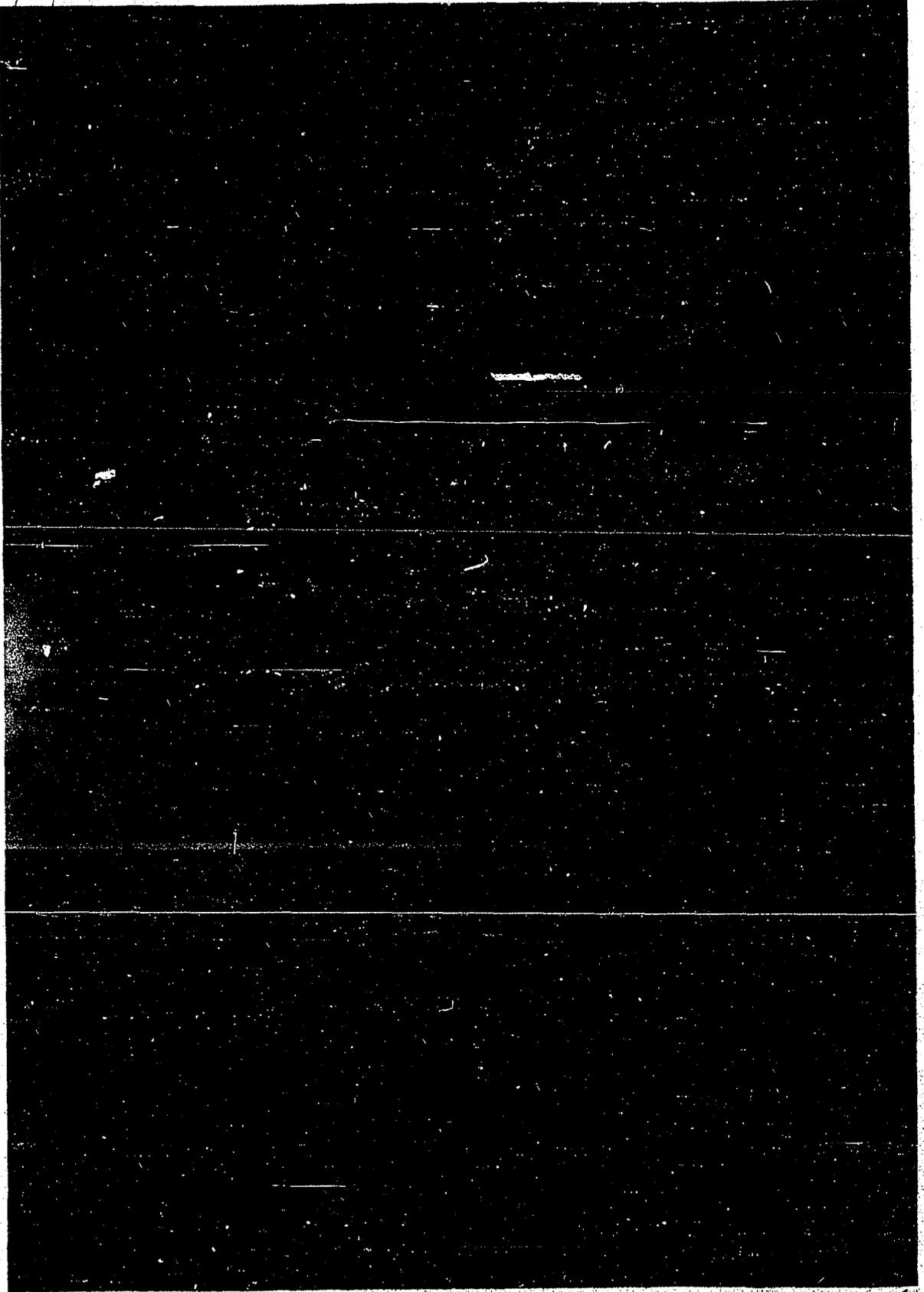


Figure 28. Chromosomes (CH) in late metaphase. These chromosomes show a very regular outline which indicates that maximum condensation has been achieved. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X41,000

Figure 29. Chromosomes at early metaphase displaying an irregular outline. The coarser, less tightly-held-together material to the right of the electron micrograph may be heterochromatin (HC). The other chromosome sections (CH) are through regions of euchromatin. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X59,000

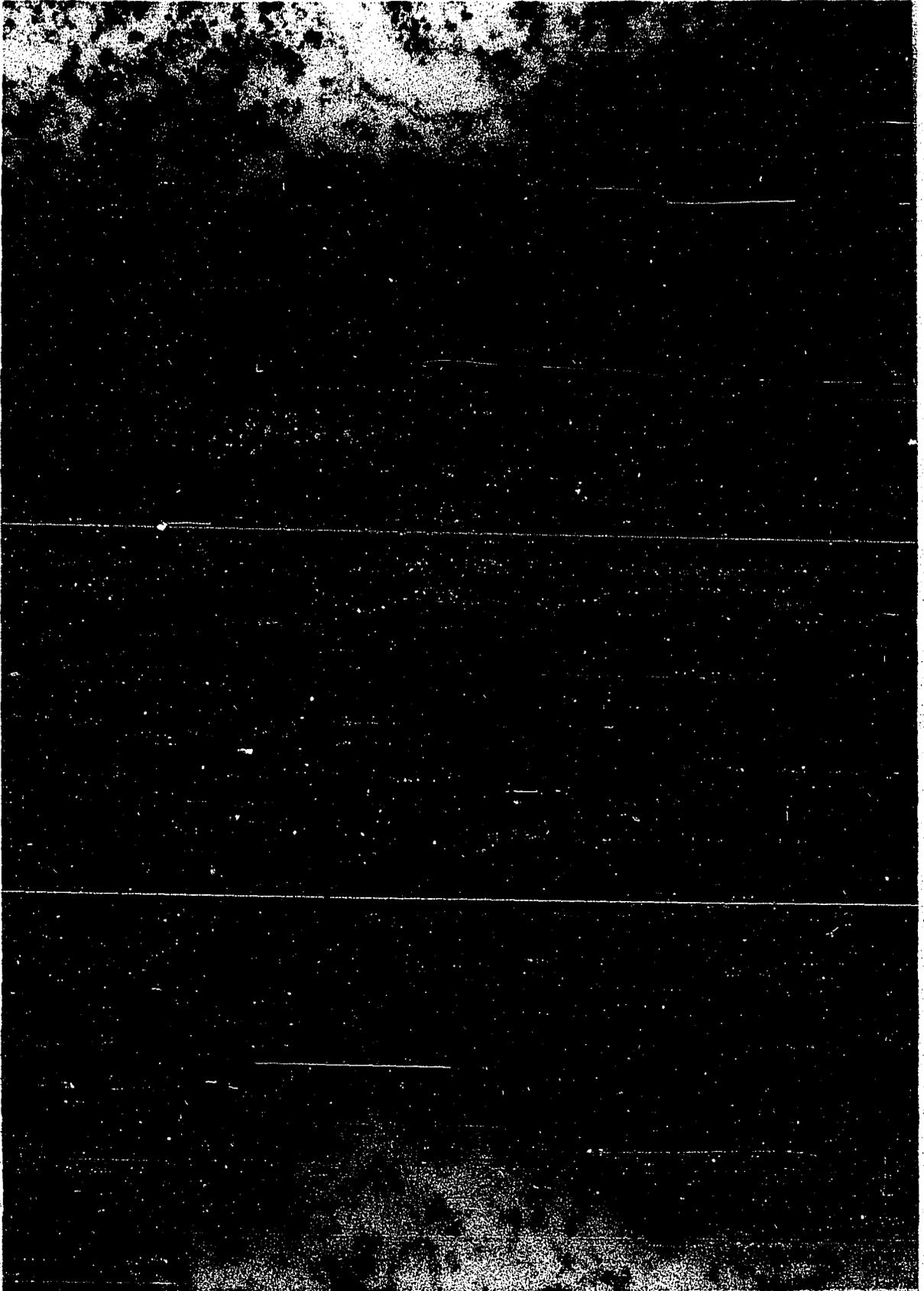


Figure 30. A nucleolus persisting in a metaphase cell. It still appears to be well organized at this stage. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X59,000

Figure 31. A nucleolus (NC) persisting at the spindle pole (SP) in an anaphase cell. Again the nucleolus appears well organized with no apparent signs of disorganization. The spindle microtubules (T) are directed toward the pole. Many ribosomes, irregular sacs of the endoplasmic reticulum (ER), smaller vesicles and microtubules (T) are found in the polar regions of the spindle. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X10,100

Figure 32. A section through an anaphase cell. The two groups of daughter chromosomes (CH) have separated from each other and the interzonal region between these groups is filled with ribosomes, segments of the endoplasmic reticulum (ER) and other vesicles. Only a few microtubules are seen in this zone. Many sacs and vesicles may be found between the chromosomes. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X8,400

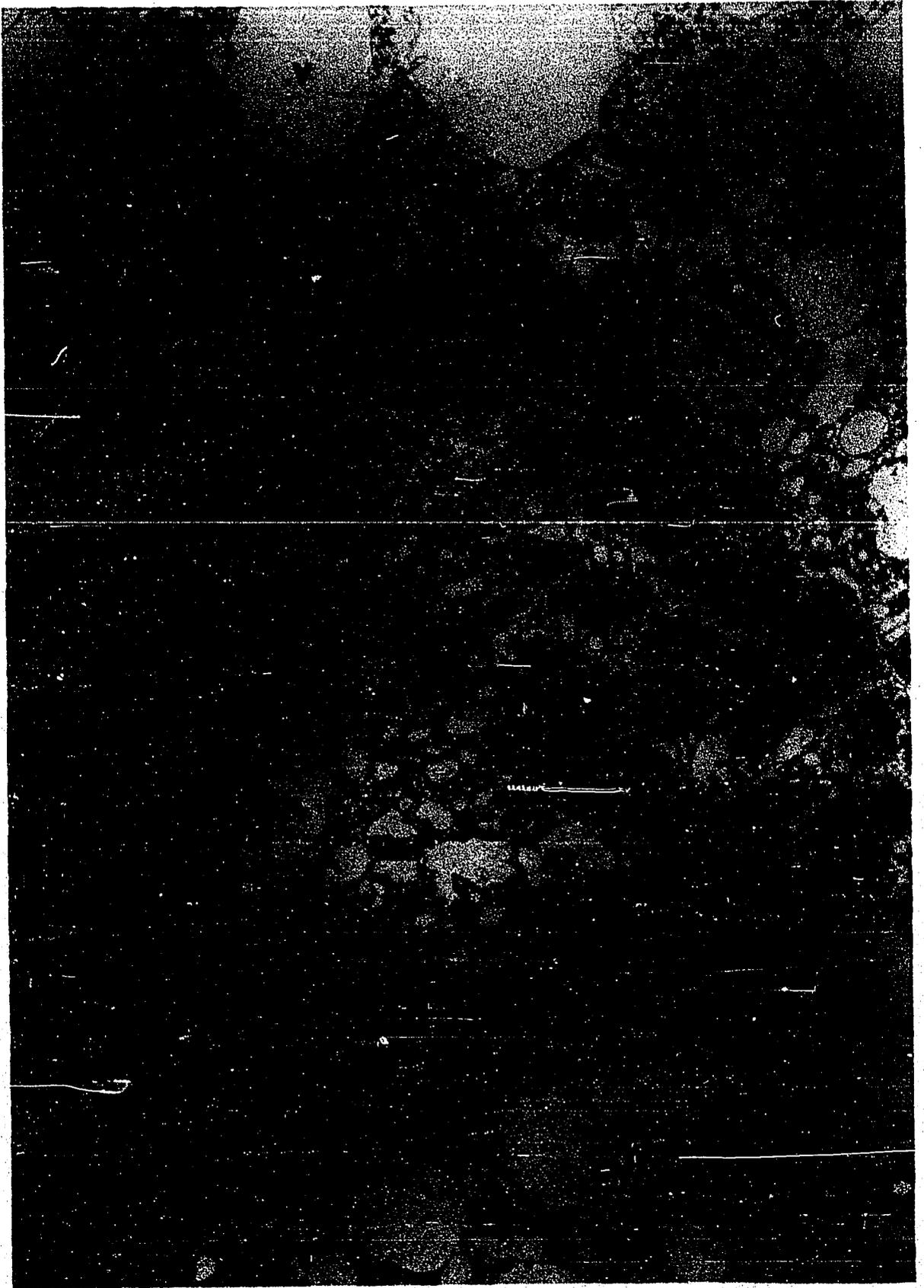


Figure 33. Cross sections of anaphase chromosomes (CH). Many cross sections of spindle fibrils are also present, some seemingly well preserved. The chromosomes have lost their smooth contours and extensions of chromatin trail off from the sides of the chromosome. Many short segments of fibrils can be seen in the chromosome along with some irregular electron-transparent areas. Irregular sacs of the endoplasmic reticulum or old nuclear envelope lie between the chromosomes. Figure 33a shows an enlarged view of the spindle microtubule indicated by the arrow in Figure 33. It appears to be cylindrical with 12 or 13 subunits making up its dense wall. Fine strands of material within the cylinder seem to connect the sides of the microtubule together. These strands are from 15 - 30 A thick. The overall size of the microtubule is 30  $\mu$ m, the wall is 75 A thick and the internal diameter is 150 A. Glutaraldehyde fixation, post-fixed in OsO<sub>4</sub> and stained with uranyl acetate. Figure 33 approximately X39,000. Figure 33a approximately X294,000



Figure 34. Nuclear reformation during early telophase. The chromosomes (C) have lost their compactness and have undergone some despiralization. The nuclear envelope (NE) has almost completely reformed. Nuclear pores (NP) are evident. The cytoplasmic protuberance (CP) has been established due to the configuration of the anaphase chromosomes. The polar region of the spindle (SP) is filled with vesicles of various sizes, disintegrating nucleoli (SG), microtubules and ribosomes. Very little spindle material other than the chromosomes is incorporated into the newly formed nucleus. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X15,000

Figure 35. A tuft of loosely knit fibrous material surrounded by a clear area which may be a perinucleolar sphere (PS). This tuft was found in a midtelophase cell and may represent the first stage in nucleolar development. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X60,000

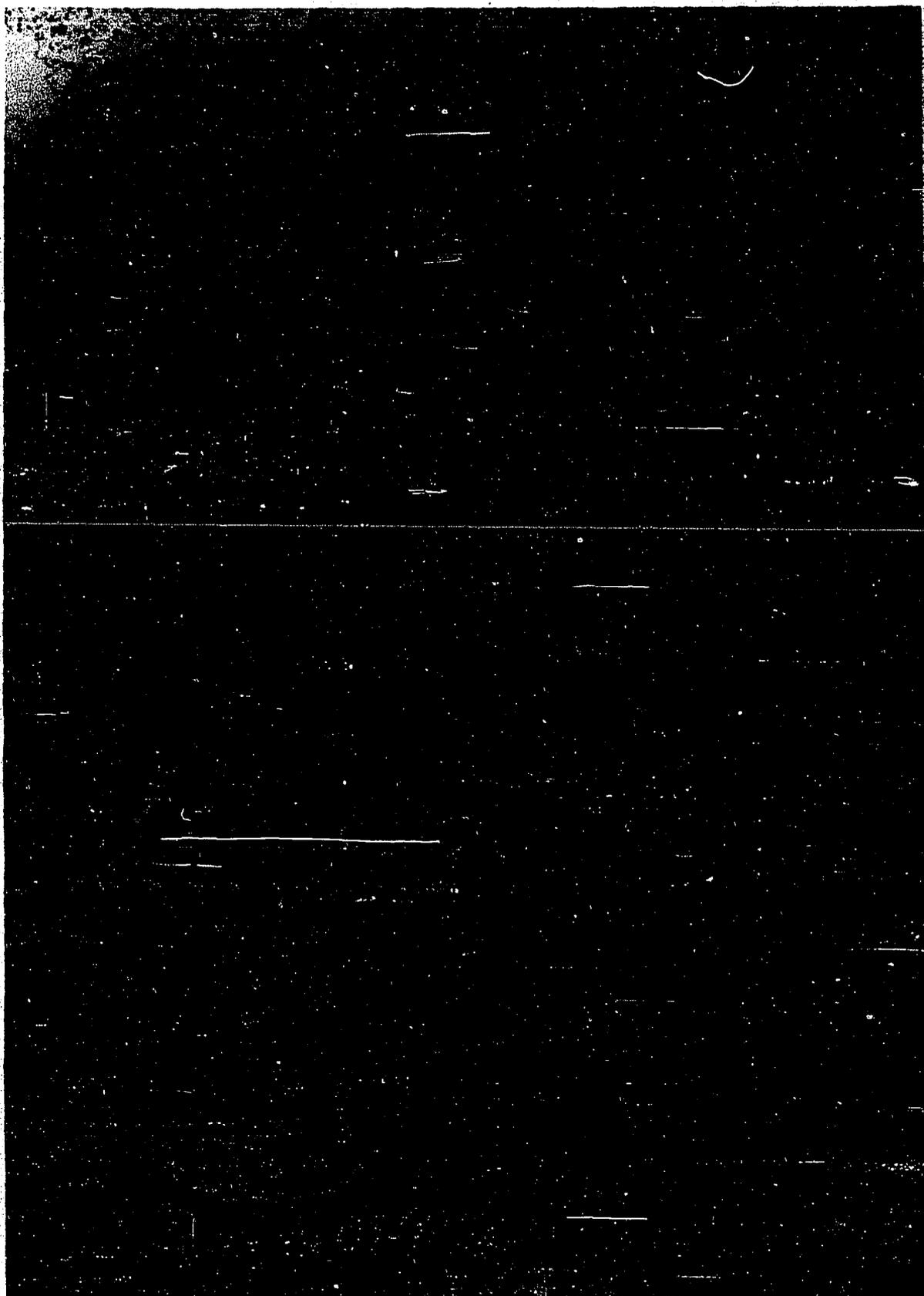


Figure 36. A midtelophase nucleus. The cytoplasmic protuberance (CP) entering the nucleus from the side opposite the cell plate (CPl) is evident. The cell plate still contains a small discontinuity (arrow). Large nucleoli (NC) are already present in these nuclei. The chromosomes have despiralized further and have been widely separated by a large quantity of nucleoplasm containing many nucleoplasmic granules (NG). Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X4,700

Figure 37. A portion of a late telophase cell showing the characteristic striated appearing nucleus (NU). This is due to the parallel organization of the chromatin strands (C) separated by nucleoplasm. Clumps of nucleoplasmic granules (NG) may still remain in the cytoplasm. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X6,000

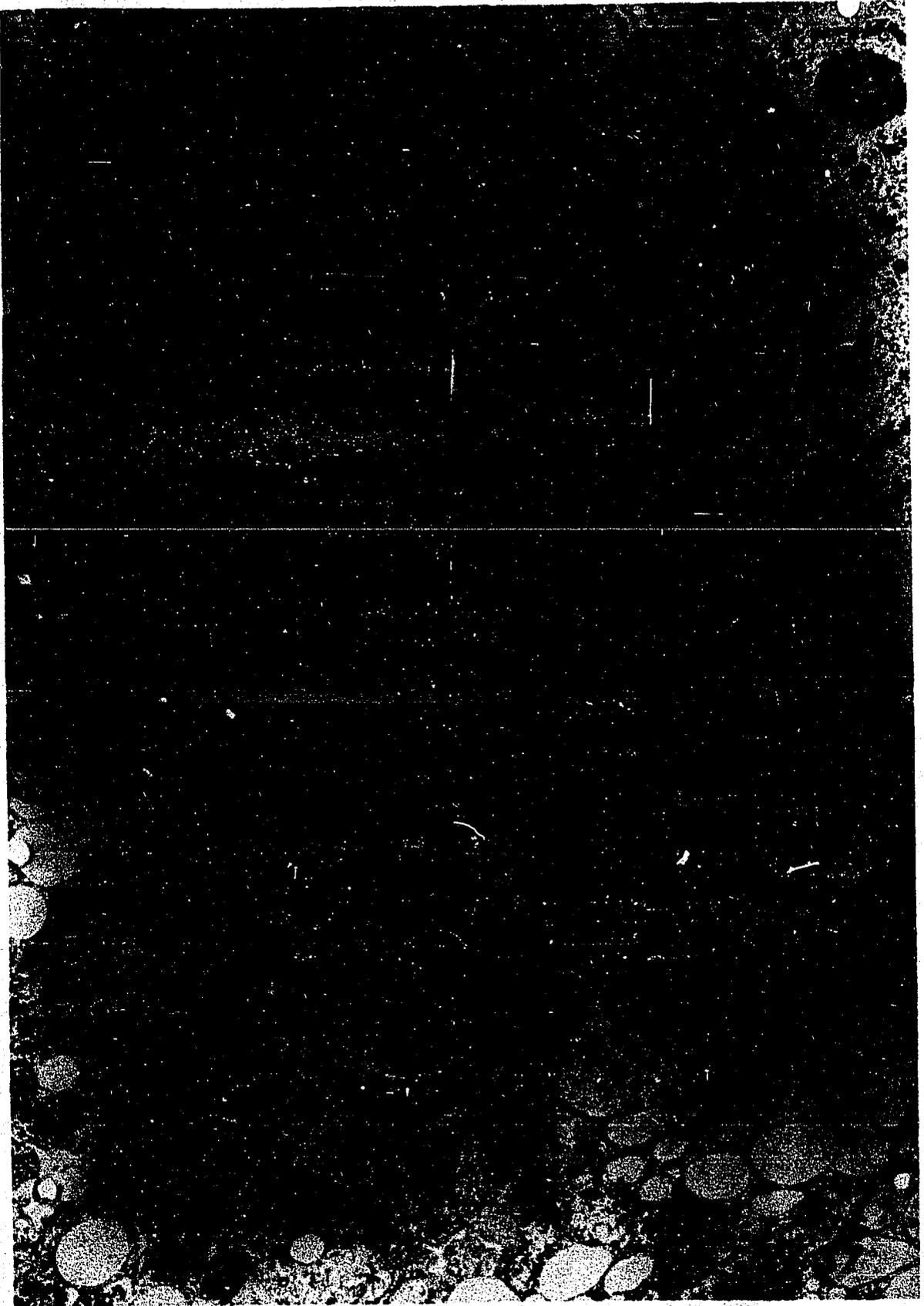


Figure 38. Early stages of cell plate (CPL) formation show many microtubules (T), segments of endoplasmic reticulum (ER) and smaller vesicles (VE) at the site of the former equatorial plate. These all combine to form the phragmoplast (PH). This zone is also rich in ribosomes (R). The microtubules and segments of the endoplasmic reticulum are oriented parallel to the spindle axis. The microtubules do not extend far on either side of the forming cell plate. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X19,000

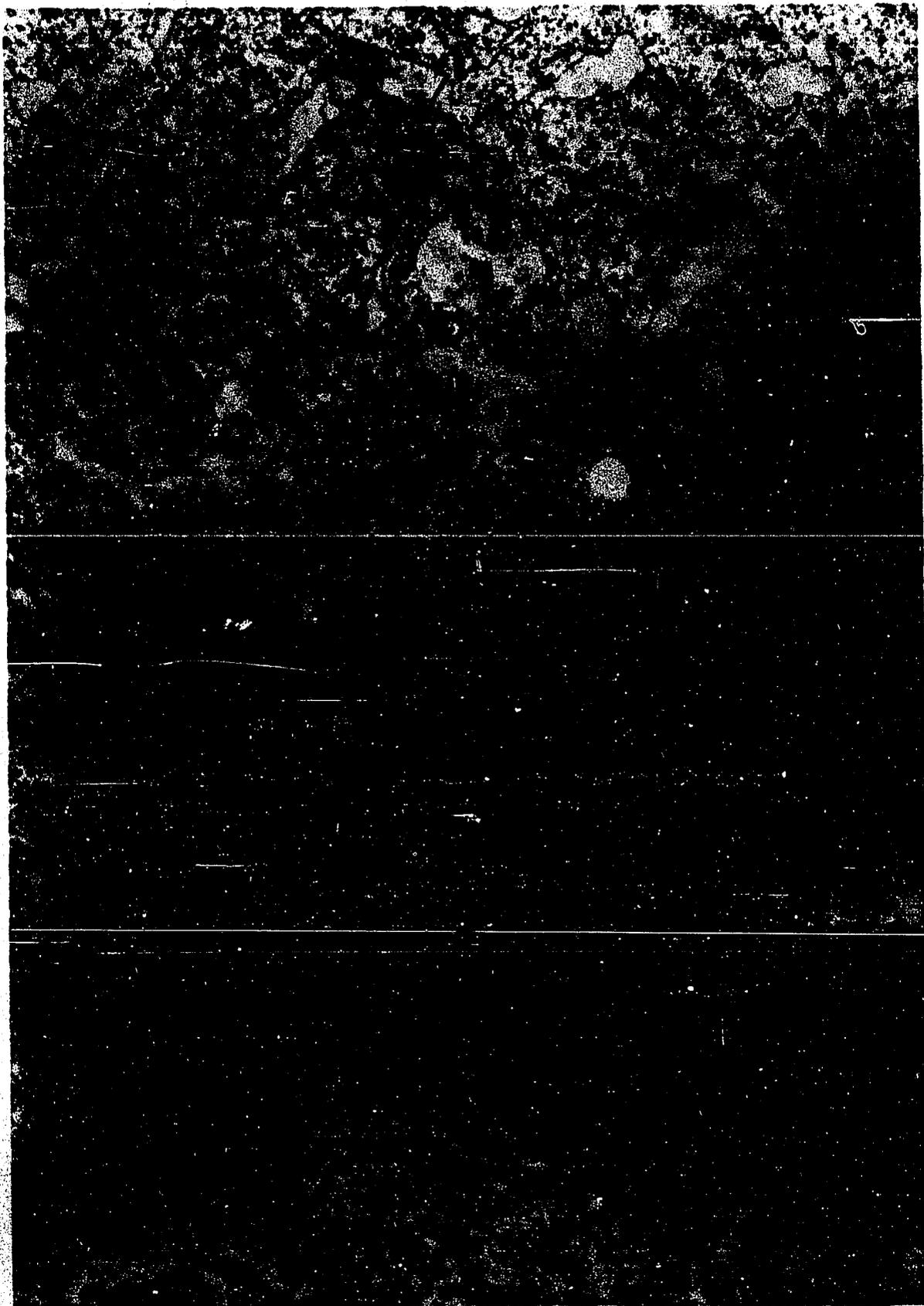


Figure 39. Dense material (T) may be found extending across the newly formed cell plate (CPl). This material may represent microtubules in a state of disorientation. Plasmodesmata (PL) probably result, in part, from the cell plate forming around spindle microtubules. The phragmoplast (PH) region still contains segments of intact microtubules (T). An organelle having a myelin sheath arrangement (?) is present but may only be an artefact. A clump of nucleoplasmic granules (NG) outside the daughter nucleus is also evident. Glutaraldehyde fixation, post-fixed in  $\text{OsO}_4$  and stained with uranyl acetate. Approximately X12,500

Figure 40. A picture of photographic emulsion after exposure to electrons and development in the usual manner. Some faint patterns (arrows) can be recognized. Enlarged X8 photographically

Figure 41. An electron micrograph of Epon 812 in a region outside the tissue containing region. The Epon appears granular, definite patterns (arrows) are evident. This section was stained with uranyl acetate. Approximately X134,000. Negative was enlarged X8

