

ELECTRON MICROSCOPIC STUDIES OF MITOSIS IN AMEBAE

II. The Giant Ameba *Pelomyxa carolinensis*

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ABSTRACT

Dividing nuclei from the giant ameba *Pelomyxa carolinensis* were fixed in osmium tetroxide solutions buffered with veronal acetate to pH 8.0. If divalent cations (0.002 M calcium, magnesium, or strontium as chlorides) were added to the fixation solution, fibrils that are 14 m μ in diameter and have a dense cortex are observed in the spindle. If the divalent ions were omitted, oriented particles of smaller size are present and fibrils are not obvious. The stages of mitosis were observed and spindle components compared. Fibrils fixed in the presence of calcium ions are not so well defined in early metaphase as later, but otherwise have the same diameter in the late metaphase, anaphase, and early telophase. Fibrils are surrounded by clouds of fine material except in early telophase, when they are formed into tight bundles lying in the cytoplasm unattached to nuclei. Metaphase and anaphase fibrils fixed without calcium ions are less well defined and are not observably different from each other. The observations are consistent with the concept that spindle fibrils are composed of polymerized, oriented protein molecules that are in equilibrium with and bathed in non-oriented molecules of the same protein. Partially formed spindle fibrils and ribosome-like particles were observed in the mixoplasm when the nuclear envelope had only small discontinuities. Remnants of the envelope are visible throughout division and are probably incorporated into the new envelope in the telophase. Ribosome-like particles are numerous in the metaphase and anaphase spindle but are not seen in the telophase nucleus, once the envelope is reestablished, or in the interphase nucleus.

INTRODUCTION

Fibrillar material is a major component of the mitotic spindle. The skepticism that prevailed toward this concept when only fixed cells could be examined has been dispelled by the demonstration of birefringence in the spindle of living cells by numerous investigators (17, 32, 43, 48, 52-54) and finally by the demonstration of spindle fibrils in living cells by Inoué (18). Observations by Cooper (13) on mitosis in a mite, by Cleveland

(*e.g.* 12) on phase microscopy of protozoa, and by Tahmisian (56) on grasshopper spermatocytes have added further evidence.

The isolation of the entire mitotic apparatus from sea urchin eggs, first achieved by Mazia and Dan (30) and later improved (26), has allowed chemical analyses to be performed. Antigen-antibody analysis using Ouchterlony gel diffusion methods (60), microbeam irradiation (66), syn-

chronous division techniques (47), and mitotic inhibition studies (11) including deuterium inhibition (20) are all contributing to a rapid expansion of knowledge of the biophysics and biochemistry of the mitotic apparatus.

Even though few definite answers have been achieved, a physicochemical concept of the spindle is emerging. Precursor protein molecules are probably synthesized in the interphase (60), polymerized by S—S or protein—SH bonds into non-oriented chains, and then formed by secondary urea-soluble bonds into oriented micelles that are surrounded by more highly hydrated, non-oriented micelles (27). Thus an equilibrium that can be altered by slight modifications of the conditions involved is thought to exist, and it has been suggested that the chromosome movements are effected in this way (19, 27).

Electron microscopic studies of mitosis have become numerous in only the past few years and are just beginning to contribute significantly in this area. Spindle structure has been described (1, 7, 15, 24, 34, 36, 42, 45, 46) to the extent that unit fibrils below the limit of resolution of the light microscope have been recognized as the components of the spindle fibers. However, no detailed description or agreement on morphology has been achieved. Several studies of the nuclear envelope in mitosis (2, 6, 7, 31, 33) have established that the nuclear membranes are reduced in the prometaphase to a series of vesicles that enter the cytoplasm and are morphologically indistinguishable from other cytoplasmic vesicles. Re-formation of the envelope involves the close apposition, to the telophase chromosome mass, of membranes (2, 6, 33, 37, 64) that are probably derived from the endoplasmic reticulum (6, 33, 37). Centrioles have been described (1, 7, 8, 63) as consisting of nine fibrils arranged cylindrically with structures occasionally appended peripherally. The primary limitation of such studies has been the difficulty in preparing enough selected nuclei to overcome the uncertainties of fixation and of the orientation of sections.

Few cells have so many favorable attributes for mitotic studies as the giant amoebae. They are large, can be cultured easily, have a characteristic gross appearance during division, and contain several hundred nuclei that divide synchronously (23). Mitotic events are of the anastral type which occurs commonly in Protista and in plants, and is said by Wilson (61) to be the most primitive form of mitosis. The numerous chromosomes

condense in the prophase, aggregate into a metaphase plate as the spindle fibrils develop, and separate in a typical anaphase; the nuclear membranes become discontinuous in the metaphase (although large remnants remain), allowing the usual formation of a mixoplasm.¹ There is no phragmoplast formation or direct involvement of the spindle in cytokinesis or plasmotomy, and centrioles and cilia are lacking. Thus the metaphase spindle is acentric, non-convergent, and anastral.

MATERIALS AND METHODS

The giant amoeba, *Pelomyxa carolinensis* Wilson, used in this study was obtained from the Carolina Biological Supply Company, Elon College, North Carolina, and from the stock isolated by Kudo (22). Cultures were maintained in 4-inch finger bowls at 23°C in double-distilled water and were fed with *Paramecium caudatum* and *Chilomonas paramecium*.

Cultures with a relatively large number of dividing forms were produced by a heavy feeding several hours before organisms were needed. Organisms with the mulberry appearance indicating that nuclear division is in progress (23) were selected under a dissecting microscope and immediately pipetted into fixation solutions. A second selection was made after embedding by cutting a free hand section, several micra thick, with a razor blade, covering it with immersion oil and a coverslip, and examining it by phase microscopy. By the first selection, organisms in division were obtained; by the second, organisms with nuclei at particular stages were chosen.

Osmium tetroxide solutions (1 per cent) buffered with veronal acetate (sodium barbital 0.14 M and sodium acetate 0.14 M) to pH 8.0 were used for fixation at 23°C for 30 to 80 minutes, and the divalent ions calcium, magnesium, or strontium were added when desired as chlorides at 0.002 M. Dehydration was performed by 10- to 20-minute changes of 50 per cent (2 changes), 75 per cent, 95 per cent, and absolute (2 changes) ethanols. Infiltration in methacrylate was performed in three steps (usually 20, 30, and 20 minutes each), after which individual organisms were pipetted into separate gelatin capsules. The methacrylate used was a mixture of 2 parts ethyl and 3 parts *n*-butyl methacrylate with 1 per cent Lupercol GDB, and was polymerized at 60°C in an oven. The methacrylates were used as received

¹ The mixoplasm can be defined as the protoplasm formed when the substance of the clear zone seen typically in prophase cells mixes with the nucleoplasm following rupture of the nuclear envelope. The term was first proposed by Wassermann in 1929 (59).

from the manufacturer with the exception that the above mixture was dried before use by filtering through anhydrous, powdered Na_2SO_4 .

Sections were cut on a Porter-Blum microtome at settings of 25 to 150 μ and were mounted on carbon or methacrylate membranes and usually overlaid

contrast lantern slide plates and printed on Kodabromide F-3 or F-4 paper.

This study utilized 26 dividing and numerous interphase organisms fixed in fifteen different "runs." Since each organism contains a few hundred nuclei randomly oriented to the plane of section, several

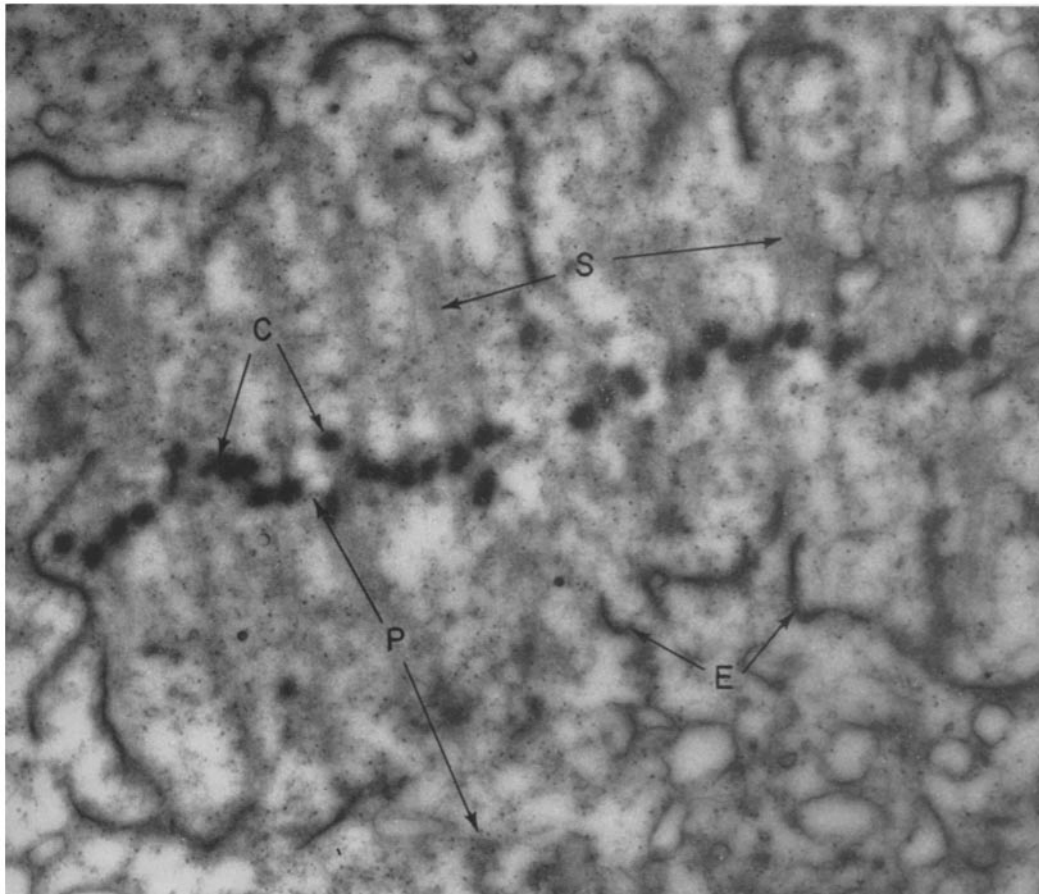


FIGURE 1

Survey section of a metaphase nucleus oriented with the spindle fibrils (*S*) parallel to the plane of section and perpendicular to the chromosome (*C*) plate. Ribosome-like particles (*P*) are scattered throughout the mixoplasm and cytoplasm, and remnants of the nuclear envelope (*E*) are still present. $\times 11,000$.

with a methacrylate membrane (41). Most of the micrographs included here were made using methacrylate membranes both as substrate and as overlay (41). Sections were usually stained with potassium permanganate (25) after being mounted on grids and before the methacrylate overlay was added. An RCA EMU 3E electron microscope operated at 100 kv with a 25 or 50 μ objective aperture was used. Photographs were made on Kodak medium

nuclei were observed in each organism before one was found that was sectioned optimally. Thus it is estimated that about 250 nuclei have been observed, most of which were in division. Of these organisms, 13 contained metaphase-plate formations with well developed spindles, while the remainder represented different times in the division stages.

Giant amoebae are experimentally useful since an organism in mitosis can be divided micrurgically

into several pieces without apparent ill effect. For example, to obtain early anaphase nuclei an amoeba was cut into six pieces, one of which was expended for phase microscopy to determine that it had metaphase nuclei, while the others were fixed thereafter at 3-minute intervals for electron microscopy. The result was one piece with nuclei in the metaphase, three pieces with nuclei in progressive times of the anaphase, and one piece with nuclei in early telophase. Therefore, the nucleus in Fig. 17 is known to be 2 to 3 minutes past the onset of the anaphase, and those in Figs. 16 and 18 about 3 minutes later. Such cutting may be done free hand with a fine glass needle under a dissecting microscope.

OBSERVATIONS

In all the micrographs to be described, sections have been chosen that are oriented either parallel to the fibrils, referred to as longitudinal sections, or perpendicular to the fibrils (parallel to the metaphase plate), referred to as cross-sections. No sections that are oblique by more than a few degrees have been included since they show little of the structure of small fibrils.

The Metaphase Spindle

The general features of a nucleus in the metaphase can be observed in low power survey micrographs. The chromosomes (Fig. 1, *C*) are numerous, small, and similar in shape, and are arranged in a thin metaphase plate. The spindle fibers (Fig. 1, *S*) are non-convergent and nearly parallel, which causes the entire mitotic figure to have the shape of a right circular cylinder and to appear square rather than spindle-shaped in sections that parallel the fibers (40).

In higher resolution micrographs (Figs. 2 to 7), details of spindle structure can be observed. A spindle fibril is seen as a pair of dark lines in longitudinal section (Fig. 2, *S*) and as a dark circle in cross-section (Figs. 3 and 4, *S*). This structure is interpreted as a fibril that has a dense cortex with a less dense center; its diameter measures about 14 $m\mu$. There is a slight indication of

cross-banding at about 6 $m\mu$ intervals. The cortical density is not high in comparison with that of other cellular components, so that permanganate staining and quite thin sections must be used to visualize the fibrils adequately. Chromosomes are denser than the spindle fibrils and are thus more obvious in the micrographs (Figs. 2 to 4, *C*). The fibrillar component of the chromosomes is smaller than the spindle fibril (Figs. 2 to 5, *C*). Connections of spindle fibrils with chromosomes have been observed (Fig. 5, *J*) though no darkening or specialized structure has been seen at their junction. At the polar ends of the spindle fibrils attachment to a structure is less certain. The fragments of the envelope appear often to be placed at the end of the fibrils, but no connection can be demonstrated.

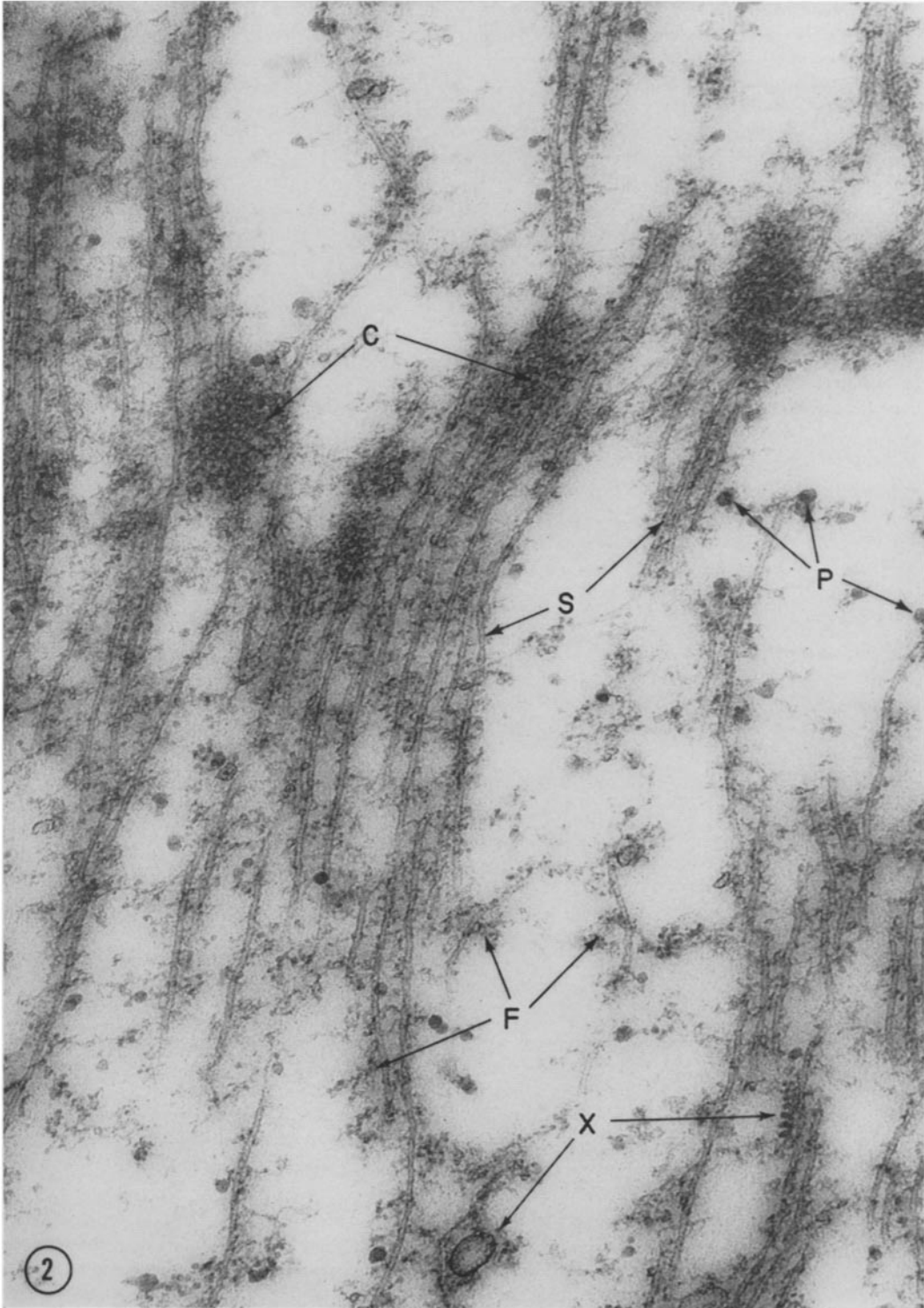
Closely associated with and surrounding the spindle fibrils is a cloud of fine material (Figs. 2 to 5, *F*). It is composed of more than one type of structure in the 2 to 6 $m\mu$ range, is not oriented in any regular way with respect to the fibrils, and is less dense after fixation than the structural elements in the chromosomes.

Many particles that are 30 to 40 $m\mu$ in diameter are seen throughout the spindle. They resemble ribosomes in appearance and are found in large numbers in the cytoplasm and spindle at the metaphase (Figs. 1 and 2, *P*). They are not regularly associated with the spindle fibrils, although it is not difficult to find cases in which a particle seems to be attached to a fibril or embedded in the surrounding fine material. Other structures have been observed, but so rarely that no significance can be attached to them (Fig. 2, *X*).

The above description is appropriate for the metaphase nuclei of amoebae fixed in osmium tetroxide solutions that contain the divalent cations calcium (Figs. 2 to 5), magnesium (Fig. 6), or strontium (Fig. 7). Fixation in osmium tetroxide solutions lacking divalent cations results in a different appearance of the fibrils. A linear orientation of fine elements is still discernible, but obvious fibrils cannot be seen (Fig. 8, *S*); fine

FIGURE 2

Higher magnification of a section oriented like that in Fig. 1. Metaphase spindle fibrils (*S*) are shown to have fine material (*F*) adhering to them and ribosome-like granules (*P*) between them. Chromosomes (*C*) and some rarely observed configurations (*X*) are also included. This organism was fixed in a calcium-containing osmium tetroxide solution. $\times 70,000$.



adhering material is not easily distinguished from formed fibrils. Chromosomes (Fig. 8, *C*) and ribosome-like particles (Fig. 8, *P*) are still present, and the general appearance of the other organelles in the organism is not obviously altered.

Observations in Other Division Stages

The interphase nucleus has an irregular outline, numerous peripherally located nucleoli (23), and a typical double-membraned envelope (Fig. 9). The interphase cytoplasm has many ribosome-like particles that are not associated with cytoplasmic membranes (Fig. 9, *P*) and that are not present in the nucleoplasm (40). The helical structures described by Pappas (35) were observed only rarely in the interphase and not at all in division stages.

Prophase nuclei, in contrast, have a more spherical shape, have nucleoli that are no longer peripherally located (23), and have material layered on the inner surface of their envelopes (Fig. 10, *E*). At higher magnification the inner layer is shown as a feltwork or network of fibrils that measure 10 μ or less in diameter (Fig. 11, *I*). The morphology of these fibrils is not identical with that of spindle fibrils and their significance is not readily apparent. They are present in prophase nuclei that were fixed in osmium tetroxide solutions either containing or not containing divalent ions.

In the earliest stages of fibril formation observed, fibrils extended from the chromosomes (Fig. 12, *C*), which were beginning to form a metaphase plate, about halfway to the envelope (Fig. 12, *S*). Small discontinuities, that have probably already allowed a mixing of the nucleoplasm and cytoplasm, are present in the envelope (Fig. 13, *D*). In both longitudinal (Fig. 14, *S*) and cross-sections (Figs. 15 and 16, *S*), fibrils fixed in calcium-

containing osmium tetroxide solutions are less well defined than in later metaphase (compare Figs. 15 and 16, *S*, with Figs. 3 and 4, *S*). A cloud of fine material is associated with the fibrils which are already arranged into groups (Figs. 15 and 16). Ribosome-like particles (Figs. 13 and 14, *P*) are present in the mixoplasm, and the nucleoli are no longer present. No early metaphase nuclei that were fixed in calcium-free osmium tetroxide solutions have been observed.

Nuclei that were fixed in calcium-containing solutions a few minutes after the onset of the anaphase have well developed spindle fibrils both between the separating chromosome plates and poleward (Figs. 17 to 19, *S*), and a cloud of fine material surrounds all fibrils (Figs. 18, *S*, and 19, inset). Chromosomal and continuous fibrils are not observably different in appearance (Fig. 18, *S*). Cross-sections have been obtained through the chromosomes and slightly poleward at a level indicated on the survey micrograph (Fig. 17, arrows) and show that the spindle is composed of bundles or sheet-like arrays of fibrils (Fig. 19, *S*). Included in the spindle are remnants of the envelope now closely applied to the chromosomes (Figs. 17 to 20, *E*), and vacuoles (Figs. 18 and 19, *V*) that are similar in appearance to some of the many vesicles in ameba cytoplasm. Early anaphase organisms fixed in calcium-free osmium tetroxide solutions have fibrils similar to those seen in calcium-free metaphase nuclei (compare Fig. 20, *S*, with Fig. 8, *S*).

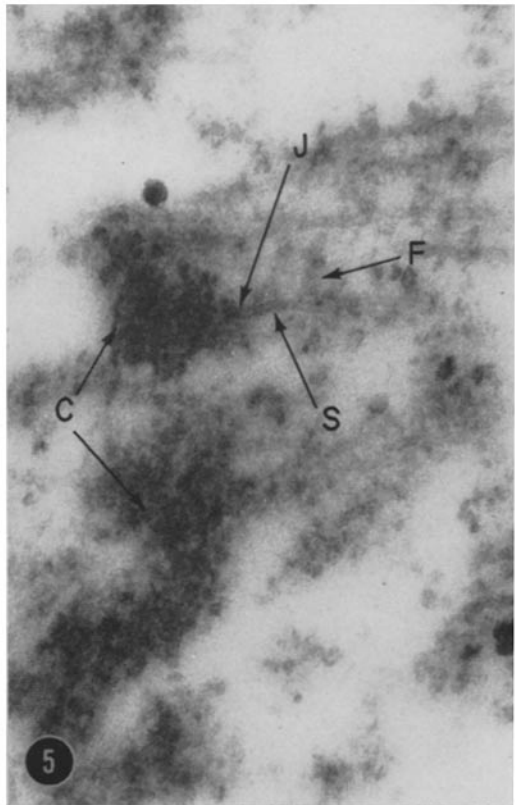
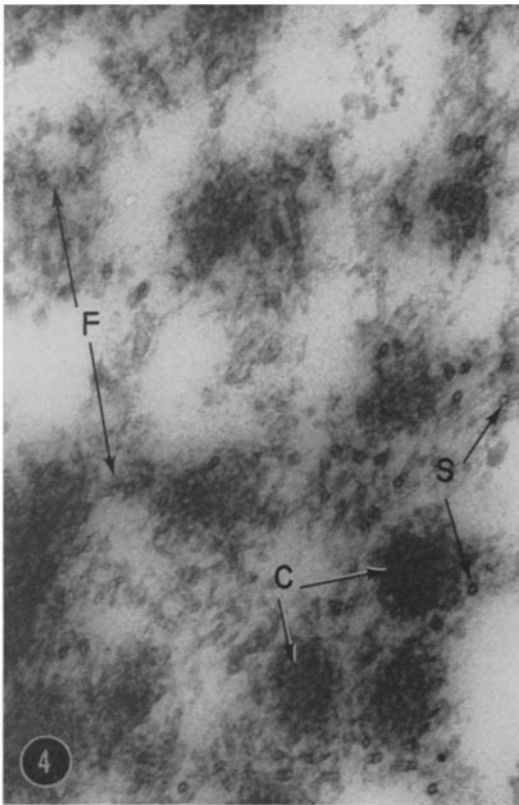
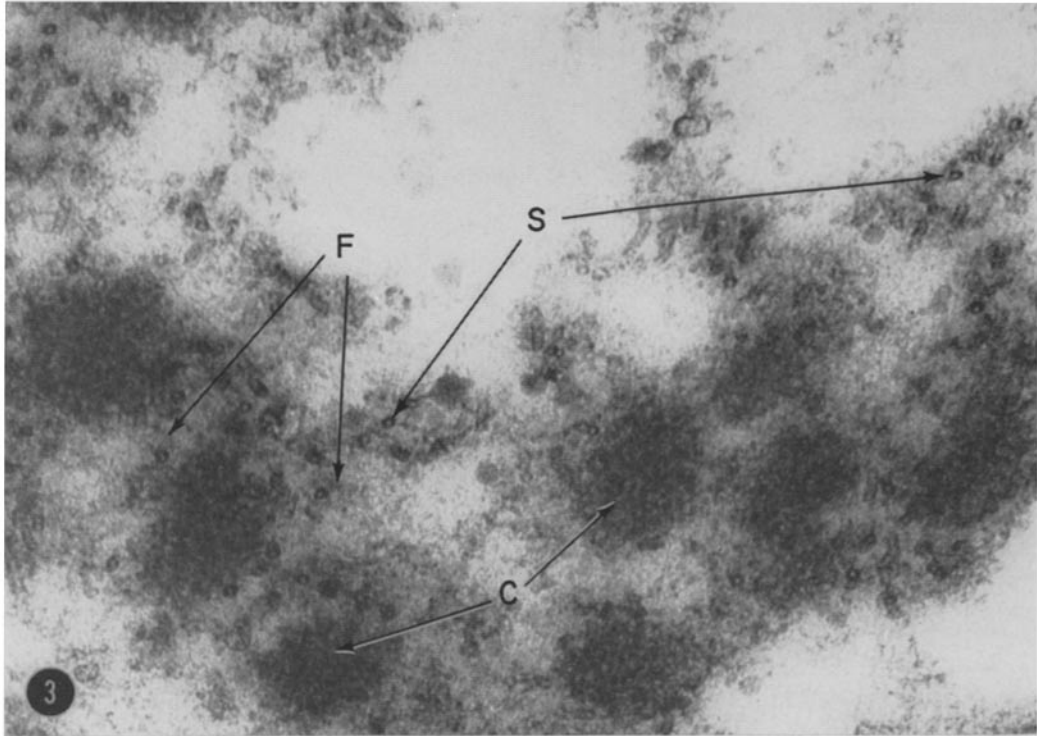
The remnants of the nuclear envelope in the anaphase have a different location from that occupied earlier. In early metaphase they were located at the "polar" ends of the fibrils (Fig. 1, *E*), and in late metaphase they were often found among and parallel to the spindle fibers. At the anaphase, these remnants are located quite close to the chromosomes (Figs. 17 to 20, *E*) except at

FIGURES 3 AND 4

Cross-sections of metaphase spindle fibrils (*S*) at the level of the chromosome (*C*) plate. A cloud of fine material (*F*) is shown closely surrounding the fibrils. Different nuclei but the same organism as in Fig. 2. Fig. 3, $\times 91,000$; Fig. 4, $\times 74,000$.

FIGURE 5

Metaphase chromosomes (*C*) and a spindle fibril (*S*) at their point of junction (*J*); no darkening or specialized structure has been observed at the attachment of spindle fibril to chromosome. Fine material (*F*) surrounding the fibrils is again visible. $\times 77,000$.



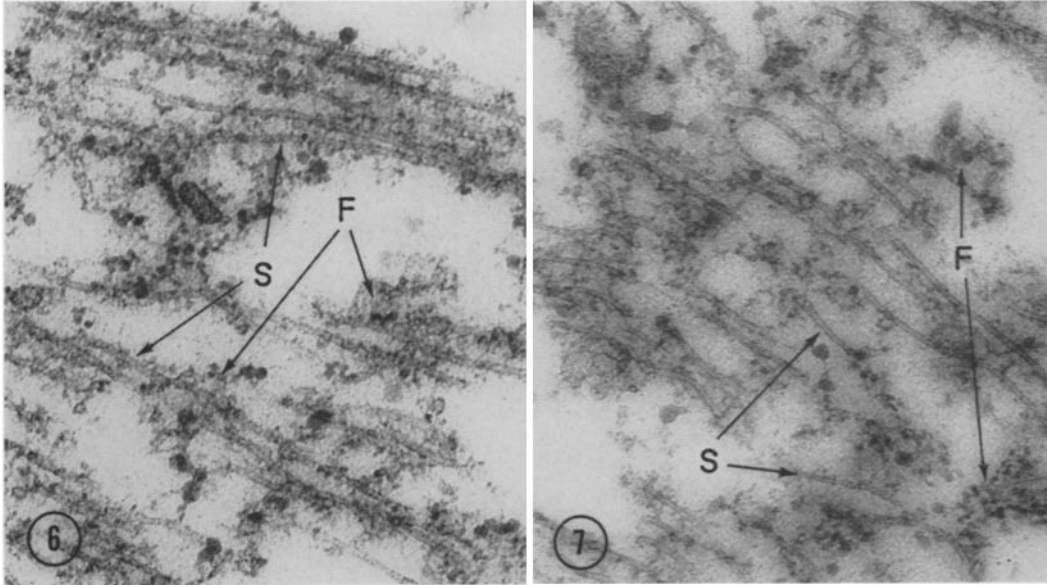


FIGURE 6

Metaphase spindle fibrils (*S*) and adhering fine material (*F*) in an organism fixed with a magnesium-containing osmium tetroxide solution. $\times 66,000$.

FIGURE 7

Metaphase spindle fibrils (*S*) and adhering fine material (*F*) in an organism fixed with a strontium-containing osmium tetroxide solution. $\times 65,000$.

the periphery of the plate, where large remnants characteristically precede the chromosomes poleward (Fig. 17, *E*, right).

In early telophase, the spindle fibrils are freed from their chromosomal attachment and are found as free, compact bundles in the cytoplasm (Fig. 21, *S*). The fibrils themselves are well defined and similar in appearance and diameter to those in early stages, but they are now much more closely packed together. Whereas their distance of separation was usually three or four times their diameter in the metaphase and anaphase, they are now consistently and rather regularly separated by one diameter or less and have little fine material surrounding them (Fig. 22, *S*). The nuclear envelope, which has again become continuous (Fig. 23, *E*), is closely applied to the

chromosomes, which are still arranged in a thin, flat plate (Fig. 23, *C*). Ribosome-like particles are excluded from the newly defined nuclear volume.

DISCUSSION

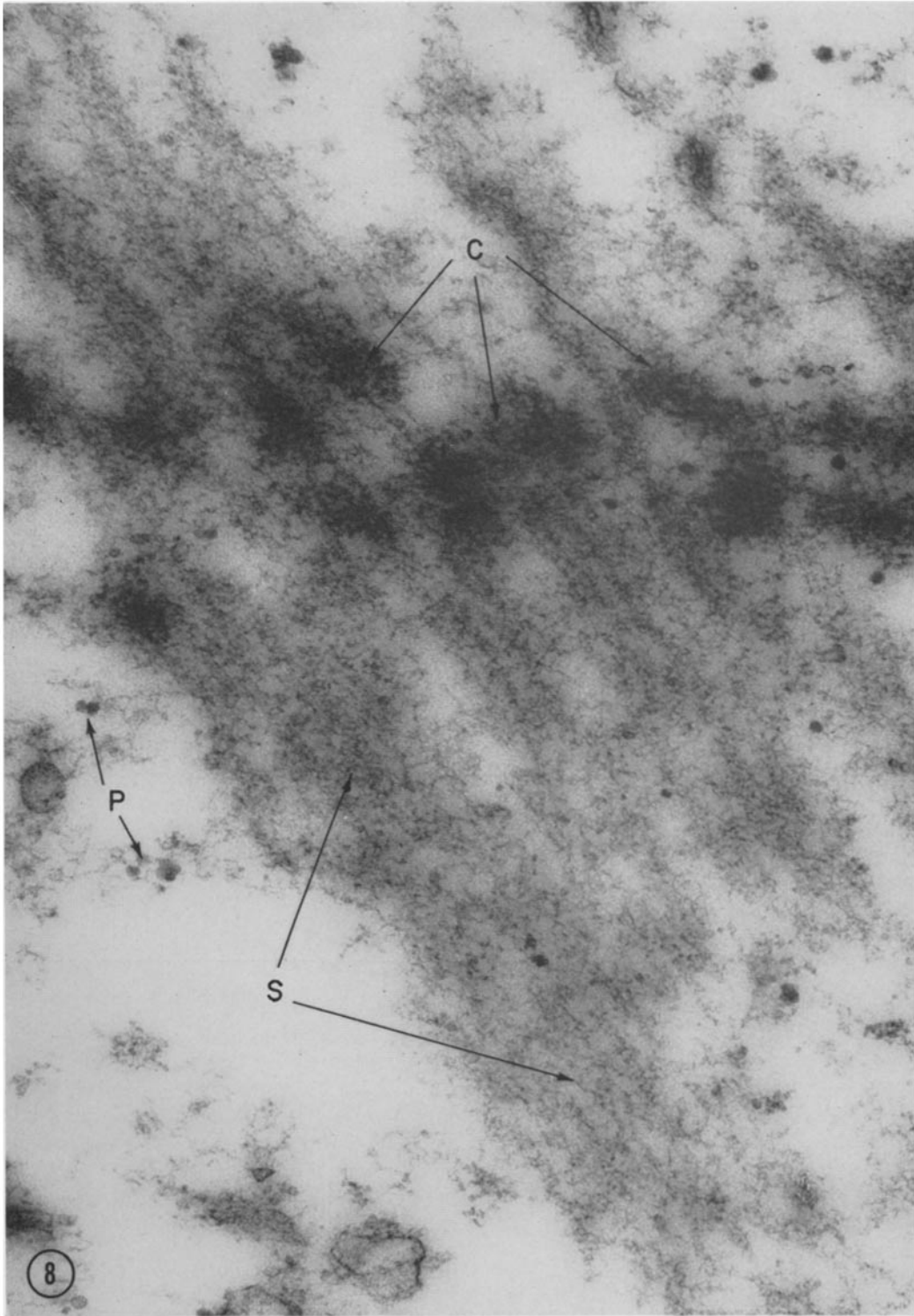
The micrographs described provide new information on the structure of the achromatic apparatus. The evidence shows that conventional preparation methods preserve the structures of the achromatic apparatus sufficiently well for profitable electron microscopic studies.

The Spindle

Two types of spindle morphology have been reproducibly demonstrated depending upon the

FIGURE 8

Part of a metaphase nucleus showing spindle components (*S*) and chromosomes (*C*) in an organism fixed without a divalent cation in the osmium tetroxide solution. Ribosome-like particles (*P*) are present. $\times 63,000$.



presence or absence of a divalent cation in the osmium tetroxide fixing solution. The probable explanation of such variation is that the cation reached the proteins first, affecting their binding, and that osmium arrived slightly later, reacting with but not destroying the cation-effected structure. From the information available, we should attempt to decide which of the structures observed represents the truer picture of the mitotic spindle.

Greenberg (14) states that the alkali earth cations probably combine with the free carboxyl groups of the dicarboxylic acid and the hydroxyl groups of the hydroxyamino acid residues in proteins.² Correlating well with this is the finding by Mazia (27) that, in isolated sea urchin spindles, aspartic acid and glutamic acid account for 25 per cent of the amino acid residues, and serine and threonine account for another 15 per cent.

Mazia has shown that isolated sea urchin spindles observed by phase microscopy are irreversibly affected by treatment with a 2.5×10^{-3} M calcium chloride solution; they become "extremely stable" and the fibers and chromosomes become more obviously visible than they were immediately after isolation (28). Magnesium solutions cause similar changes that are, however, partially reversible (29).

The possible role of polyvalent cations in the spindle has been discussed in a stimulating paper by Anderson (3). He proposes that the protein molecules are held in an extended shape

²This hypothesis has not been revised by later investigators (16) although the carboxyl-calcium-carboxyl binding is favored and the hydroxyl-calcium-carboxyl binding is believed to be less important.

and formed into fibrils by positive charges, e.g., from calcium, magnesium, or especially the widely distributed organic bases such as spermine. Fibril contraction would be effected by neutralization of these charges. Gross (15) emphasizes that all processes that Anderson considers to be initiated by organic polycations can probably be initiated as well by calcium ions.

Thus the involvement of polyvalent cations with proteins and in particular with spindle protein is known. However, the degree and significance of the involvement *in vivo* in the spindle is still open to considerable question. As long as such a question remains, further microscopic and biochemical studies will be necessary, and it is not possible to describe spindle fibril structure adequately because the conditions of fixation cannot be specified well enough.

It is notable that our fixations in osmium tetroxide solutions containing divalent cations do not result in short fibrillar segments randomly oriented around the spindle fibrils, despite the prevalent physicochemical concept of spindle fibril structure which suggests that this perifibrillar zone is composed of non-oriented molecules of spindle protein. Similarly, fibrils are not observed in prophase nuclei fixed in calcium-containing osmium tetroxide solutions despite the indication that late prophase cells have already synthesized a high percentage of their spindle protein (60). We may conclude that these divalent cations do not themselves cause the formation of fibrils from non-oriented protein, and suggest that cation binding can be only partially responsible for the fibril structure observed in this study.

Nevertheless, the tentative statements may be

FIGURE 9

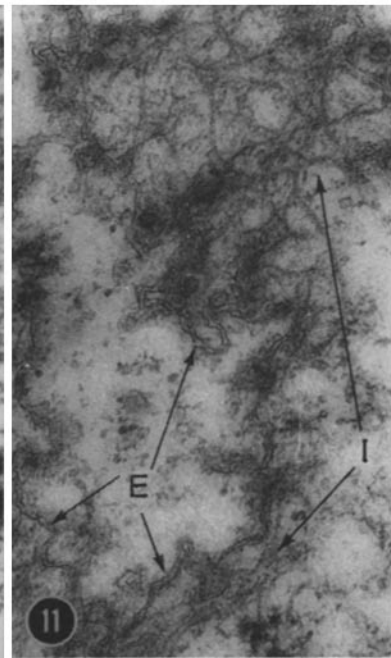
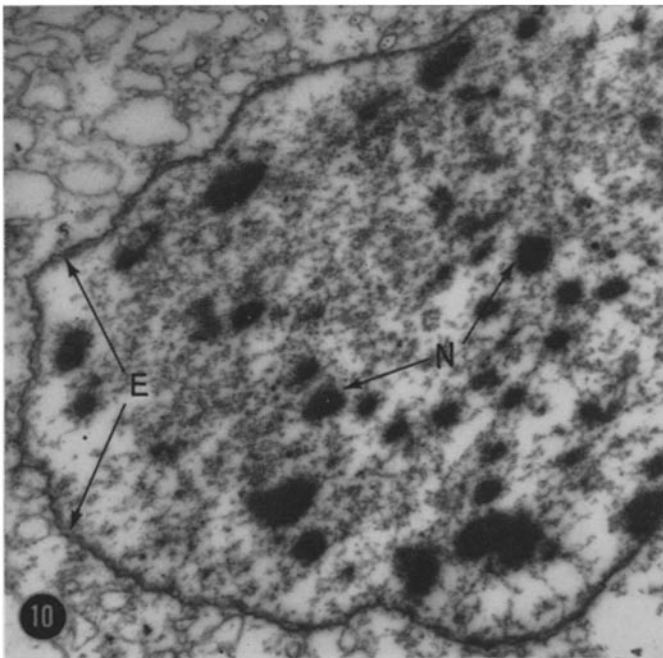
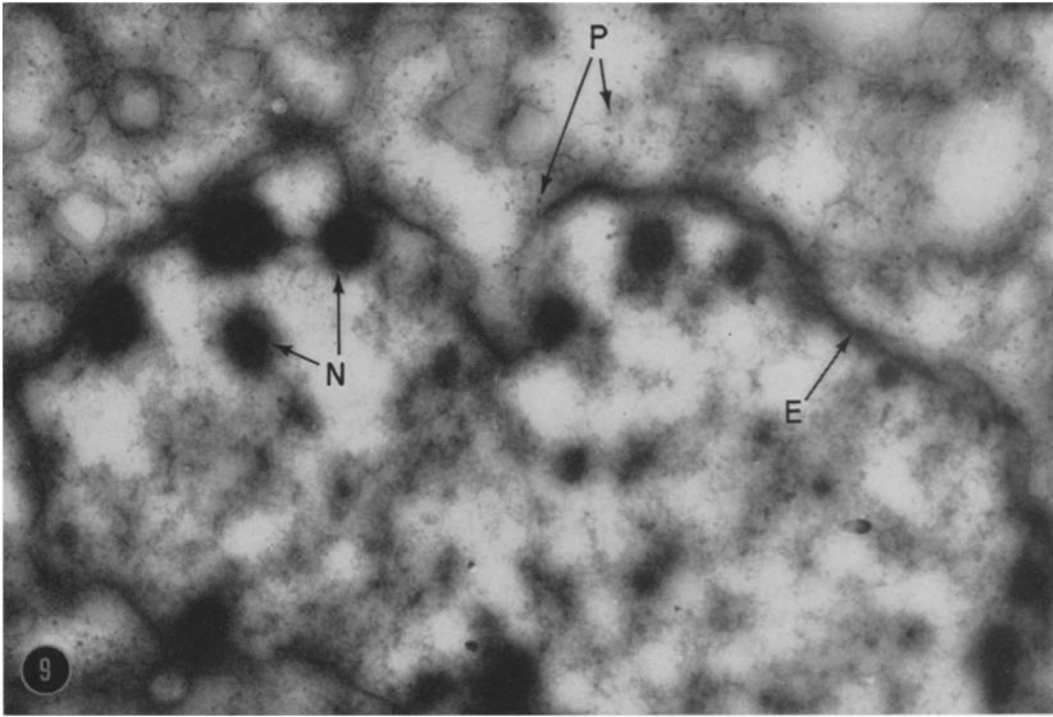
The interphase nucleus is characterized by a simple envelope (*E*) and numerous, peripheral nucleoli (*N*). Ribosome-like particles (*P*) are present in large numbers in the cytoplasm unassociated with membrane systems and are lacking in the nucleoplasm. $\times 14,500$.

FIGURE 10

In the prophase, the envelope (*E*) has a thin layer of material on its inner surface and the nucleoli (*N*) have lost their peripheral position. $\times 8200$.

FIGURE 11

Higher magnification of the prophase nuclear envelope (*E*), showing that the material (*I*) on the inner surface is fibrillar in nature. $\times 41,000$.



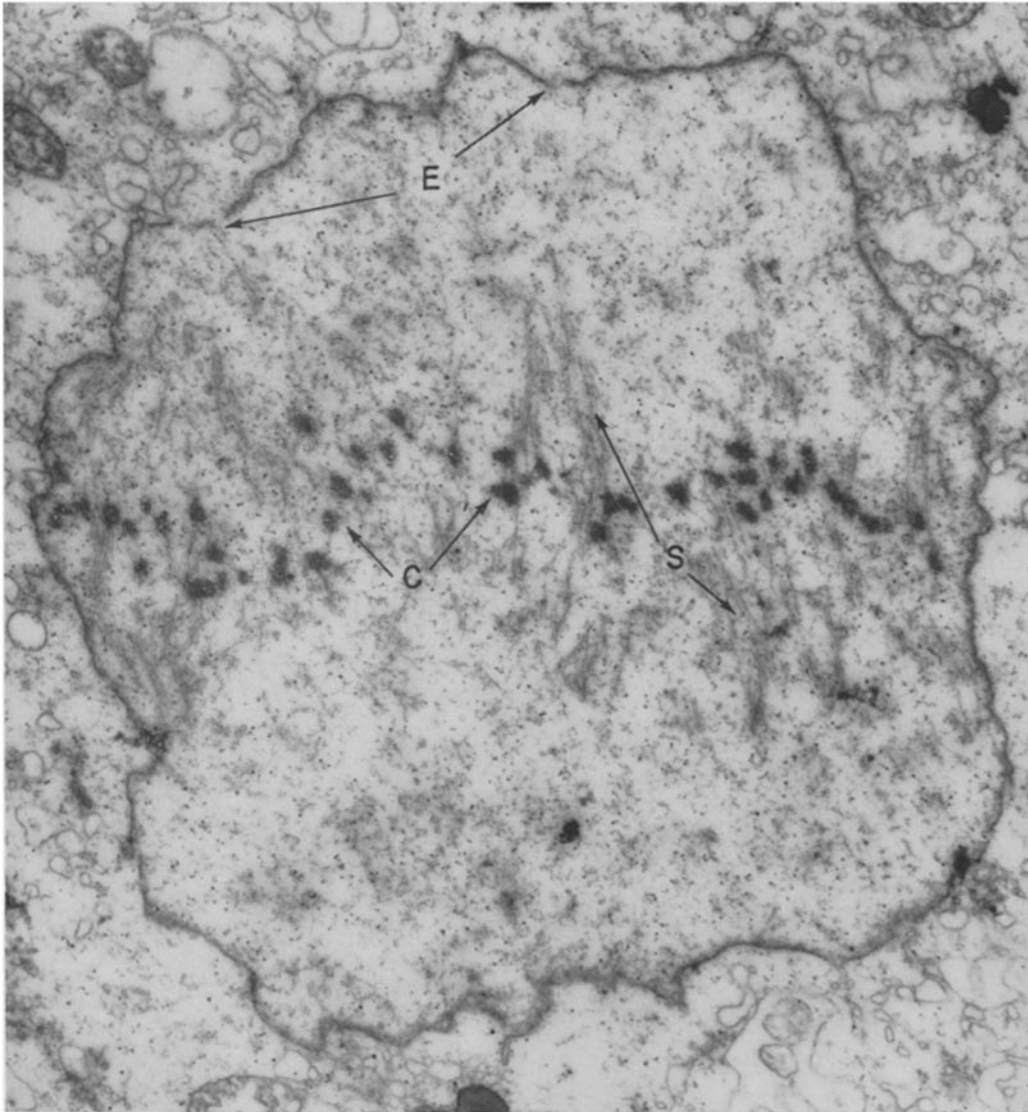


FIGURE 12

Early metaphase: spindle fibrils (*S*) can be seen extending from chromosomes (*C*) about half the distance to the nuclear envelope (*E*). The following four figures were taken from different nuclei in the same organism as that used for this figure. $\times 8400$.

made that our observations show little or no morphological change in spindle fibrils during mitosis and that only one morphological type of fibril is present. Thus, the evidence agrees with the current hypothesis that chromosome movements are caused by equilibrium changes acting to alter molecular charges or configurations in oriented, birefringent array.

The Nuclear Envelope and Spindle Formation

The breakdown of the nuclear membranes should be regarded as an event of crucial importance in mitosis for nearly all cells. No enclosing membrane or noticeable phase boundary was observed in these mitotic figures as Wada (57, 58) has suggested; the spindle is in free con-

tact with the remainder of the cell content. Wasermann (59) stresses that the dissolution of the membranes should be considered an important event signifying the conclusion of the prophase, and that the formation of a "Mixoplasma der Mitose" results. Yatsu (65) and Wilson (61) state that cytaster formation can take place only after the germinal vesicle has faded. Brachet (10) states: "In eggs, mixing of this [nuclear] sap with the cytoplasm seems to be an absolute prerequisite for the formation of an achromatic apparatus." Bajer (4, 5) stresses that the rupture of the envelope in endosperm cells initiates the "contraction stage" when the spindle is first manifested and the guiding role of kinetochores in chromosome movements is first observed.

The formation of a mixoplasm is an important feature in terms of the whole mitotic process regardless of whether the nuclear envelope disappears entirely or is only interrupted by openings that are larger than the "pores." The significant prometaphase event would seem to be the mixing of cytoplasm and nucleoplasm rather than a complete disappearance of the envelope. The observation here of spindle fibrils in early metaphase gives evidence that even though the envelope would still have appeared to be continuous in the light microscope, a mixoplasm could already have been formed when the spindle fibrils were first observed.

Thus, discussions of whether a spindle is of nuclear or cytoplasmic origin seem to be less significant (see *e.g.* 21, 24, 46, 57).³ These observations probably indicate that the spindle was formed in what had previously been nucleus or cytoplasm but was filled with a mixoplasm at the time of spindle formation. Thus, the formation of the spindle would seem usually to require chemical substances from both the cytoplasm and the nucleus, and the implication is that these substances do not usually pass through envelope "pores."

However, spindle fibrils in *Pelomyxa*, in *Lilium* (46), and in other cells (49) seem to arise from the chromosomal plate or specifically from individual kinetochores and extend outward. It is obvious that the spindle does not form simply because a mixoplasm forms, for then fibrils would

³ A special case is known in some cells, *e.g.* some ciliate protozoan micronuclei, where the nuclear envelope is thought not to rupture at division and yet a somewhat typical mitotic separation of chromosomes takes place.

be expected to originate at the points of cytoplasmic and nucleoplasmic contact. Rather, it is indicated that an influence of the kinetochores or chromosomes on the components of the mixoplasm causes fibrils to originate at the chromosomes (49).

Electron microscopy is necessary for determining the earliest time at which the envelope has perforated and the mixoplasm has begun to form; light microscopy gives an erroneously late impression. For example, Richards and Bajer (39) from their interference microscopic study on endosperm report that the nuclear mass is reduced during the prophase just before the envelope disappears, and Bajer (4, 5) reports from the analysis of his cinematographic data that a "clear zone" is formed around the prophase nucleus. We suspect that these changes result from the movement of compounds through envelope fissures that could not yet be detected by light microscope techniques. Some rethinking may likewise be necessary for Schrader's discussion (49), which is climaxed as follows: "Interaction between the prophase chromosomes and the centers must take place through or across the nuclear membrane."

The Ribosome-like Particles in Mitosis

Transfer of materials from the nucleus to the cytoplasm is an important aspect of mitosis particularly as it involves ribose nucleic acid. Evidence indicates that RNA is lacking in the early telophase nucleus of *Amoeba proteus* (38), whose nuclei, morphologically similar (42) to those shown in this study, are free of ribosome-like particles. The achromatic apparatus in many cells is strongly basophilic (31, 44, 50, 51); similarly, the interzonal space between separating chromosome plates is strongly basophilic (44) and may be more so than the other parts of the cell (9). This may also be regarded as the basophilia of the mixoplasm, to which both the nucleoplasm and the cytoplasm have contributed.

The possibility has been expressed that ribosomes participate directly in the assembly of the spindle (15). Ribosomes are usually implicated in protein synthesis, however, and Went (60) has shown that the spindle protein has largely been formed before fertilization and cleavage in sea urchin eggs. An alternate hypothesis is that ribosomes are formed in association with the mixoplasm, and are liberated into the cytoplasm

by being left in the interzonal space during the anaphase. This change of form of RNA could perhaps be a factor in accounting for the reduction of RNA that has been reported during mitosis (62), and adhering ribosomes could be a factor in

accounting for the demonstrated presence of RNA in mitotic chromosomes (55).

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FIGURE 13

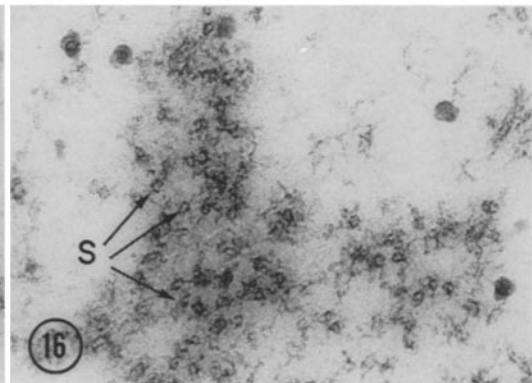
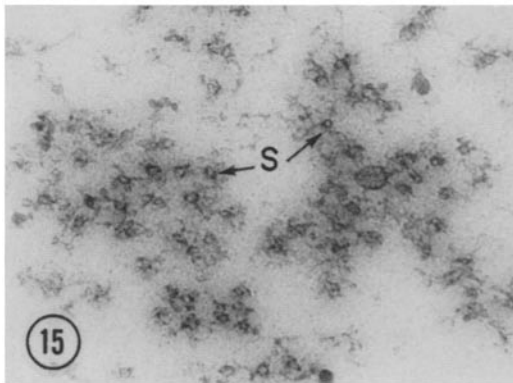
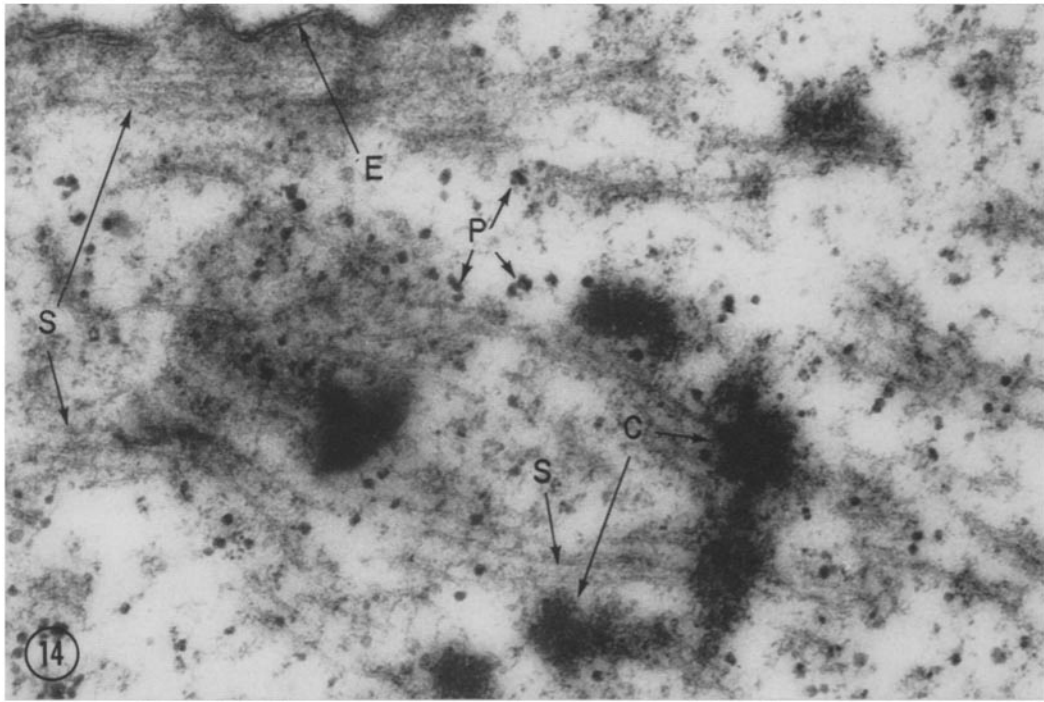
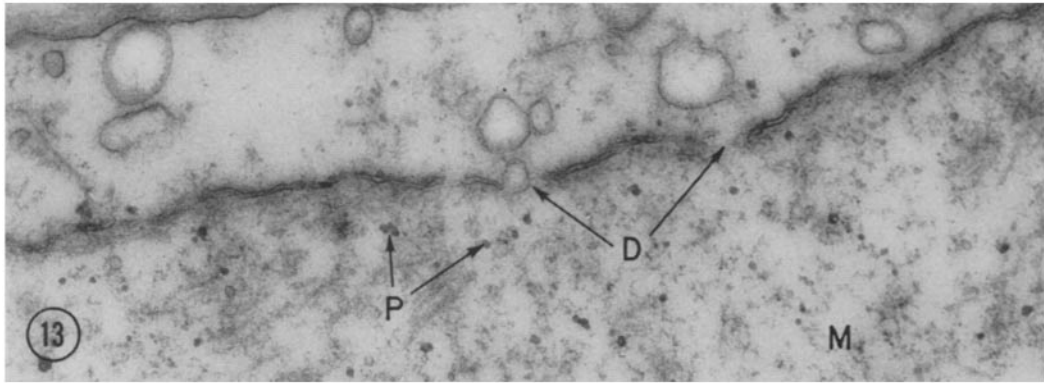
Early metaphase: In the earliest stages of fibril formation observed, discontinuities (*D*) have already appeared in the nuclear envelope, and the resulting mixoplasm (*M*) contains ribosome-like particles (*P*). Nucleoli are no longer present. $\times 24,000$.

FIGURE 14

Longitudinal section of early fibrils (*S*) including chromosomes (*C*) and many ribosome-like particles (*P*). The periphery of the chromosome plate is pictured, so that remnants of the envelope (*E*) are included. $\times 50,000$.

FIGURES 15 AND 16

Cross-sections of early metaphase spindle fibrils (*S*) and the fine material closely associated with them. Compare with Figs. 3 and 4, where the circular contours are somewhat more obvious. $\times 65,000$.



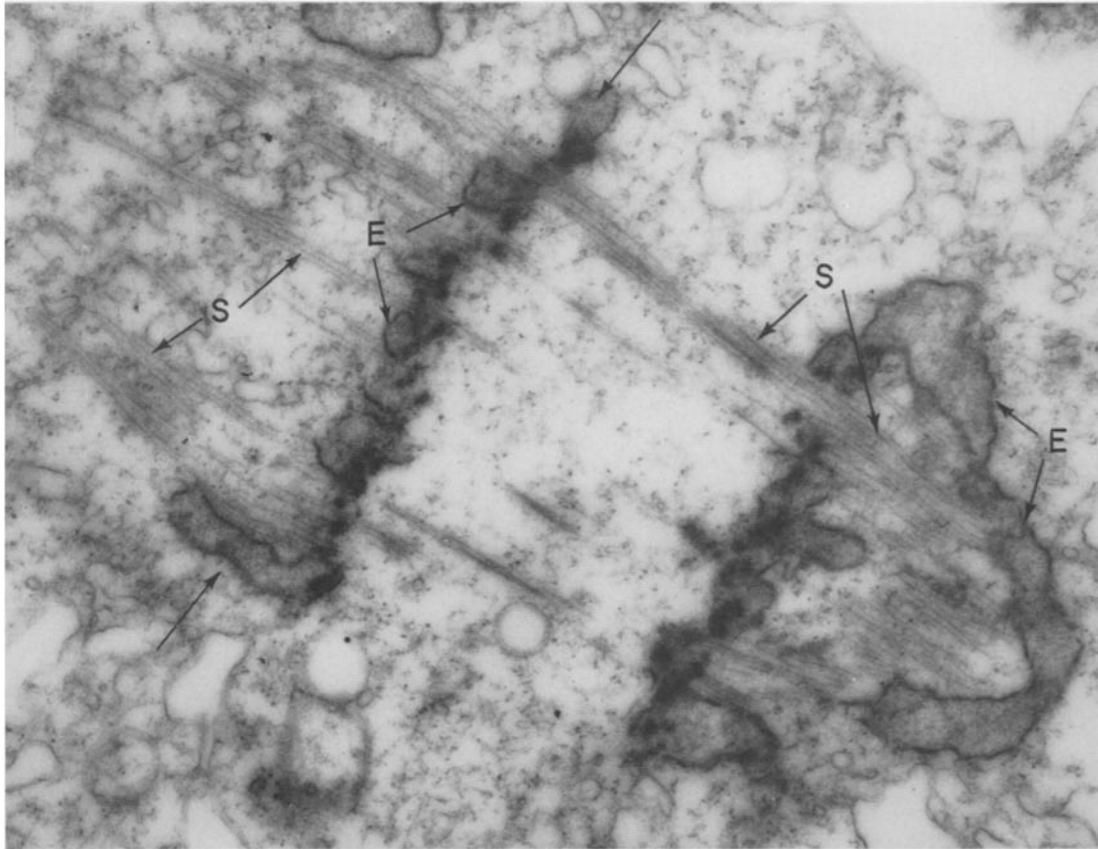


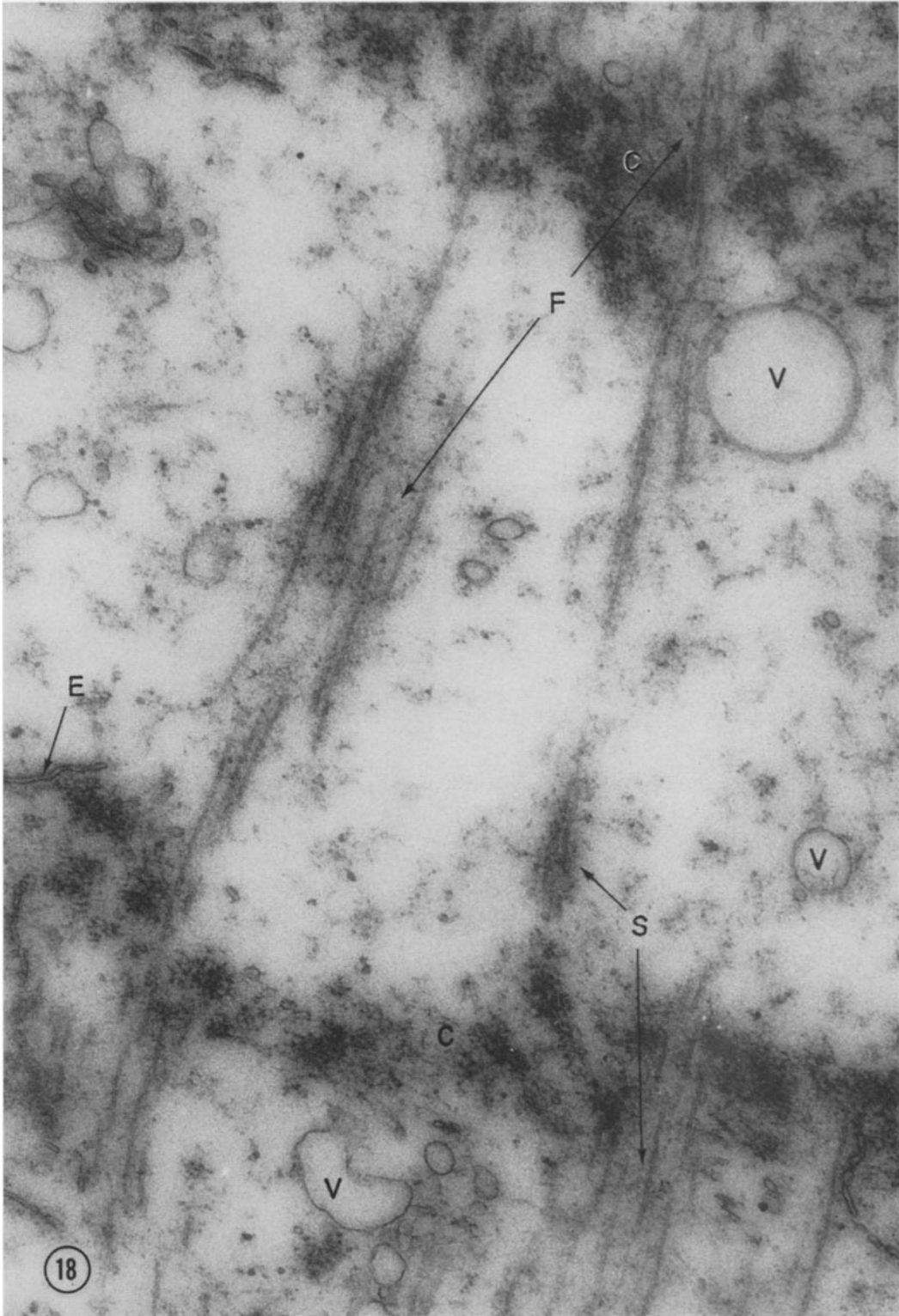
FIGURE 17

Nucleus in a piece cut from an organism and placed in a calcium-containing osmium tetroxide solution 5 to 6 minutes after the anaphase began. The separation of chromosome plates is now 4 to 5 μ . Notice that large remnants of the envelope (*E*, right) are still present and are preceding the chromosomes poleward while other remnants (*E*, left) are closely applied to the chromosomes. A slight convergence of the spindle fibrils (*S*) can now be seen. This is a slightly oblique section, so that the left plate is sectioned nearly centrally while the right one is sectioned closer to its periphery. The two unlettered arrows denote the plane of the cross-section in Fig. 19. $\times 11,000$.

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FIGURE 18

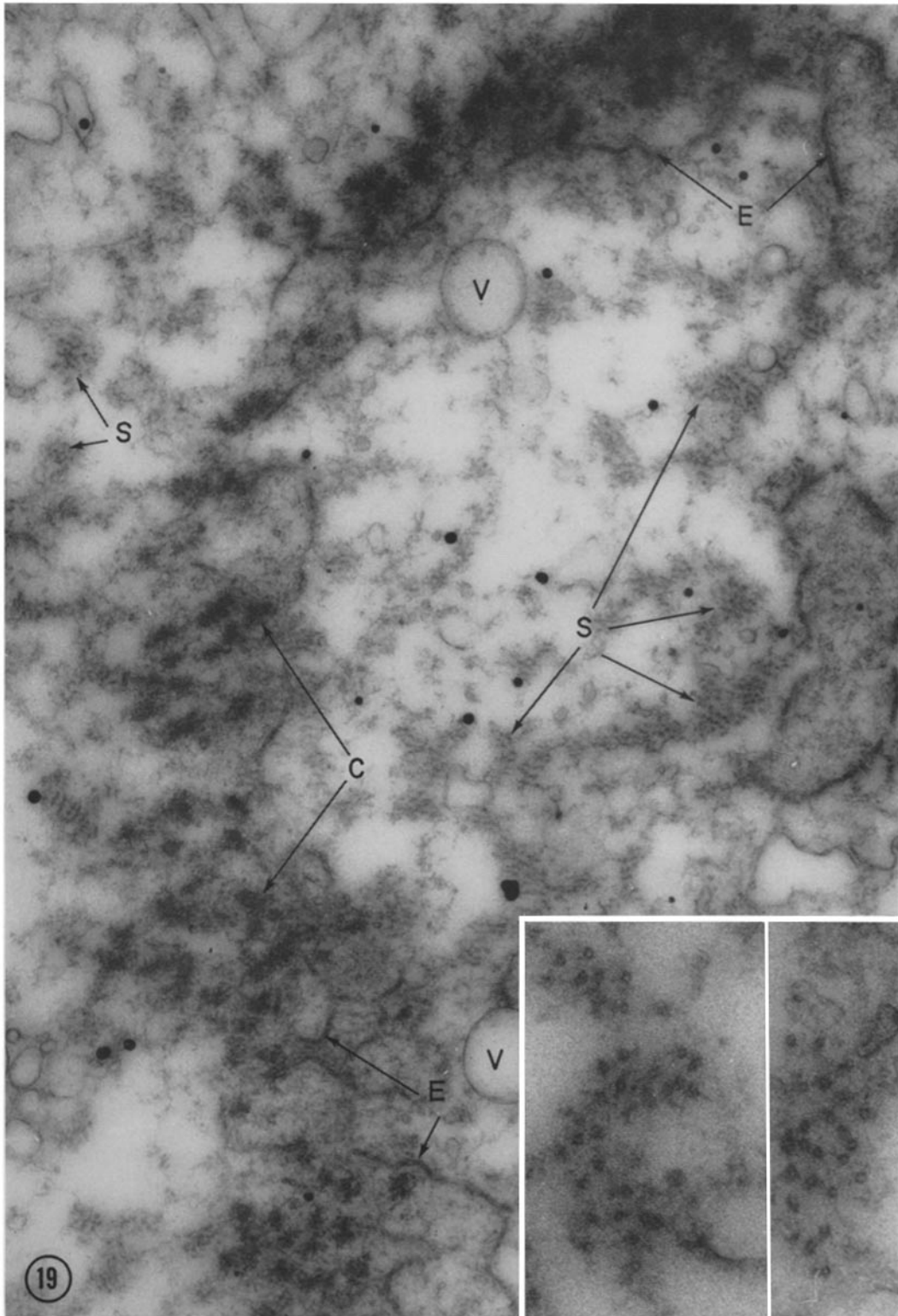
Early anaphase: fibrils (*S*) are present extending poleward (some of these are probably chromosomal fibrils) and between the chromosome (*C*) plates (probably continuous fibrils). Notice that fibril morphology and the presence of fine material (*F*) around the fibrils appear to be the same for both chromosomal and continuous fibrils. Vacuoles (*V*) are frequently included in the spindle. The anaphase began 2 or 3 minutes before this nucleus was placed in a calcium-containing osmium tetroxide solution; the separation of chromosome plates is now about 3 μ . *E*, nuclear envelope remnants. $\times 44,000$.



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FIGURE 19

Cross-section of an anaphase nucleus from the same piece of an ameba as that in Fig. 17, showing bundles or sheets of fibrils (*S*), chromosomes (*C*), remnants of the nuclear envelope (*E*), and vacuoles (*V*). The inset is a higher magnification of fibril cross-sections. Although the contrast is lower than that in other stages because these sections were not stained with permanganate, it is still possible to see fine material surrounding the fibrils. $\times 24,000$; inset, $\times 76,000$.



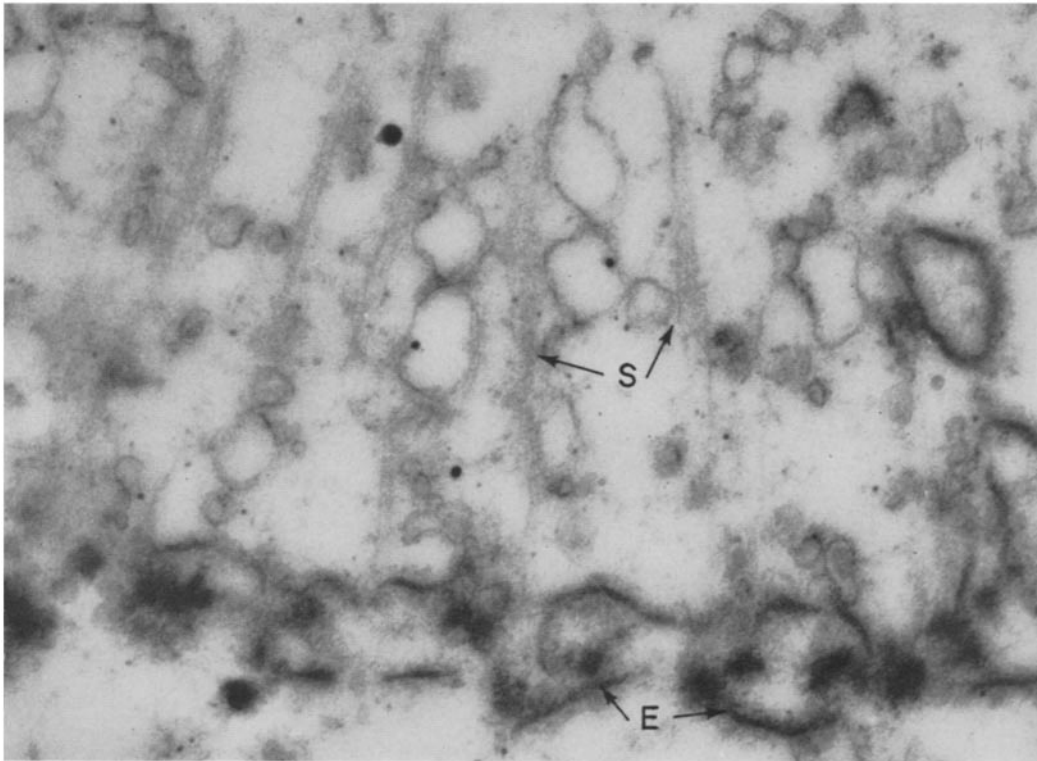


FIGURE 20

The anaphase: chromosomal plate with envelope remnants (*E*) closely applied. Chromosomal fibrils (*S*) are grouped in bundles which show some convergence. Fixed in an osmium tetroxide solution that had no divalent cations present. $\times 19,500$.

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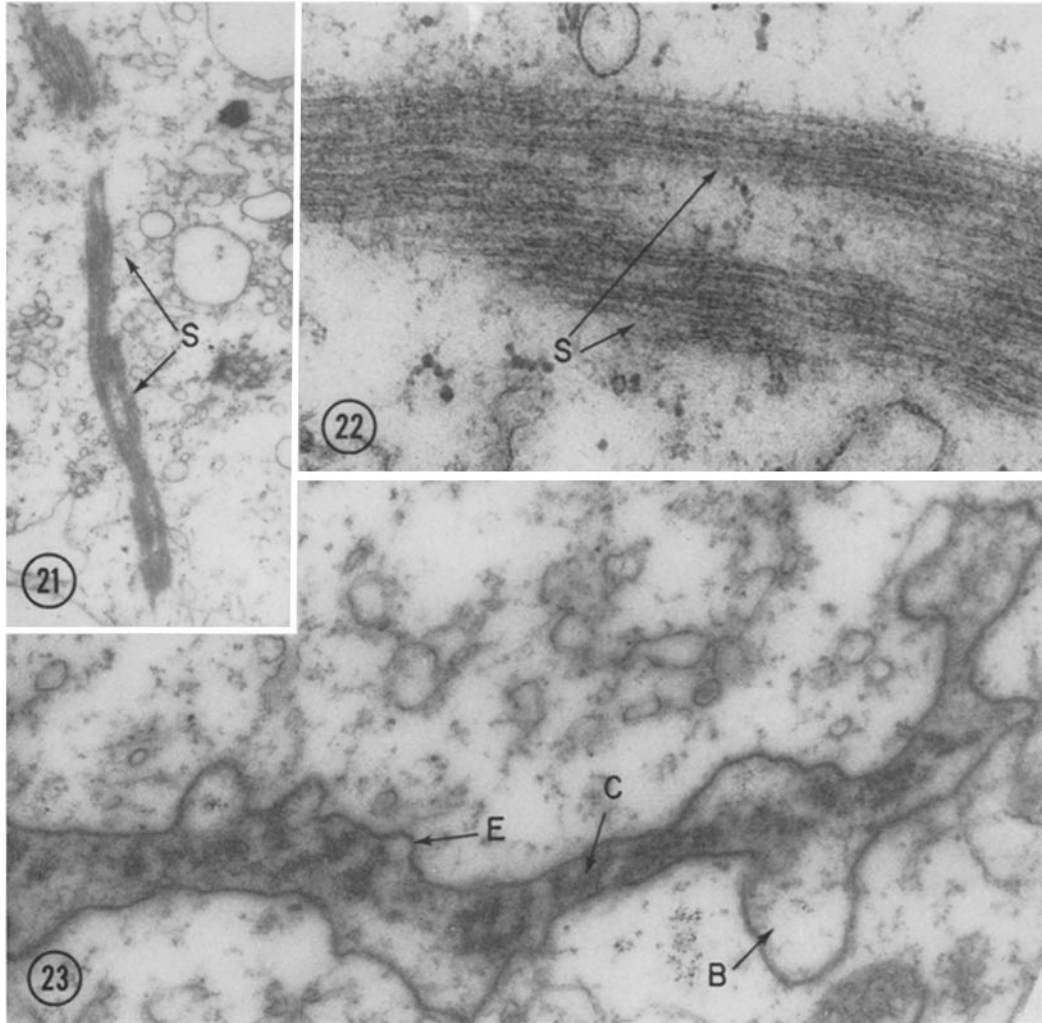


FIGURE 21

In early telophase, fibril bundles (*S*) can be found in the cytoplasm just after the nuclei have re-formed continuous envelopes. $\times 10,000$.

FIGURE 22

At higher magnification, the fibril (*S*) structure and diameter are seen to be the same as at other stages, but the separation of fibrils and the amount of fine adhering material is much less. $\times 58,000$.

FIGURE 23

Early telophase: the nucleus (from the same organism as that shown in Figs. 21 and 22) now has a continuous envelope (*E*) that is closely applied to the chromosomes (*C*) except for characteristic swellings (*B*). This is a cross-section of a nucleus which is still plate-shaped. $\times 19,000$.

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