THE ULTRASTRUCTURE OF A MAMMALIAN
CELL DURING THE MITOTIC CYCLE

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ABSTRACT

With a technique of preselecting the mitotic cell in the living state for subsequent electron microscopy, it has been possible to examine the ultrastructure of the various stages of mitosis with greater precision than has been reported previously. The early dissolution of the nuclear envelope has been found to be preceded by a marked undulation of this structure within the nuclear "hof." This undulation appears to be intimately related to the spindle-forming activity of the centriole at this time. Marked pericentriolar osmiophilia and extensive arrays of vesicles are also prominent at this stage, the former continuing into anaphase. Progression of the cell through prophase is accompanied by a disappearance of these vesicles. A complex that first makes its appearance in prophase but becomes most prominent in metaphase is a partially membrane-bounded cluster of dense osmiophilic bodies. These clusters which have a circumferential distribution in the mitotic cell are shown to be derived from multivesicular bodies and are acid phosphatase-positive. The precise selection of cells during the various stages of anaphase has made it possible to follow chronologically the morphological features of the initiation of nuclear membrane reformation. The nuclear membrane appears to be derived from polar aggregates of endoplasmic reticulum, and the process begins less than 2 minutes after the onset of karyokinesis. While formation of the nuclear envelope is initiated on the polar aspects of the chromatin mass, envelope elements appear on the equatorial aspect long before the polar elements fuse. Apparently interfering with this fusion are continuous spindle tubules which traverse the chromatin mass in striking density at characteristic points. Several cortical changes, also most pronounced in anaphase, have been described, as has the kinetochore which is seen to good advantage only in this stage. The Golgi complex has been found to disappear both morphologically and histochemically during mitosis and to reappear rapidly in telophase. Evidence is presented which implicates the continuous spindle tubules in certain phases of chromosome movement.

Although the light microscopy literature on the morphology of cell division is voluminous, the number of electron microscope investigations on the subject, excepting the chromosomes, is relatively small (1–8; also see 1 for additional references). Circumscribed descriptions of one phase or another have been given. Most studies, however, have been limited by their reliance on chance for obtaining cells in the phase of mitosis it was desired to examine, as well as by inadequate fixation techniques. Porter and Machado (1), Harris (2), and Sato (8) have circumvented the problem of cell selection to some extent by choosing as their test material onion root tips, fertilized sea-urchin eggs, and Lily pollen mother cells, respectively, which provide some degree of synchrony. Even here, however, comment has been made (1) on the difficulty of locating cells in the

electron microscope in each of the stages of mitosis. We have recently described a simple means of localizing a selected cell in vitro and preparing it for subsequent electron microscopy (9). With this technique, which is considerably more convenient than one with a similar objective reported by Bloom (10), it has been possible to prepare and study large numbers of serial sections of cells fixed in a predetermined phase of the mitotic cycle. The present preliminary paper serves to introduce some previously unreported observations on dividing mammalian cells made with this technique and also to emphasize some of the ways in which various details of mammalian mitosis differ from those of mitosis in certain other species in which it has been more extensively studied.

MATERIALS AND METHODS

Electron Microscopy

HeLa cells (S3 strain) cultivated in Eagle's basal medium (11) supplemented with 15 per cent fetal calf serum were used throughout these studies. All ultrastructural observations were carried out using the Siemens Elmiskop I.

The method used for the preparation of selected cells for electron microscopy has been detailed elsewhere (9). Briefly it consists of cultivating monolayers of HeLa cells on heavily carbon-coated slides, locating the desired cell, fixing it, and encircling it using a Leitz diamond slide marker which in turn produces a circular break in the carbon coat on the slide. Following dehydration and embedding, an epoxy resin-filled gelatin capsule is inverted over the cell on the carbon-coated slide. Upon polymerization it is found that the carbon coat and embedded cells have become an adherent unit. When the polymer is removed from the slide, the carbon coat follows along with the cells, and the small circular break in the coat is readily seen under the dissecting microscope. The block may then be trimmed of all cells except those which remain within the circle (4 to 5 cells). It is then relatively simple to section the cells and to distinguish the desired cell from its 4 or 5 neighbors in the electron microscope.

The fixation and embedding procedures used in these studies also have been previously described (9) but here are slightly modified. Glutaraldehyde (5.5 per cent) (12) in Tyrode's solution at pH 7.3 was used as an initial fixative for 5 minutes. This was followed by 1 per cent OsO_4 in one-half isotonic Tyrode's solution at pH 7.3 for 15 minutes. Finally, to a one-half isotonic, calcium-free Tyrode's solution at pH 8.3, OsO_4, ethyl alcohol, and formaldehyde were added to a concentration of 1 per cent, 2 per cent, and 1 per cent, respectively. Fixation time in this last solution was 35 minutes. This fixation schedule has been highly effective in preserving the fine structure of the mitotic figure especially the labile structure of the spindle. The ultrastructure of the chromosomes, however, remains elusive.

Specimens were dehydrated in methyl Cellosolve and embedded in Araldite. (Ciba, Duxford, Cambridge, England). Thin sections were stained first with 4 per cent uranyl acetate in 40 per cent ethanol (40 minutes at 45° C) and then with lead hydroxide according to Millonig (13, 14).

Light Microscopy and Histochemistry

For observing the mitotic cycle in living cells, HeLa cells were grown on coverslips within a perfusion chamber (15); a cell in early prophase was located and then photographed every few minutes until division was completed. Zeiss phase contrast optics were used. For histochemical studies, cells were grown on coverslips, fixed with 5.5 per cent glutaraldehyde in isotonic Tyrode's solution for 5 minutes, rinsed in distilled water, and stained for acid phosphatase or thiamine pyrophosphatase reaction products (16, 17). The distribution of these, the former considered specific for lysosomes and the latter for the Golgi apparatus, was followed in the various stages of the mitotic cycle. Incubation times for both enzyme reactions was 4 to 8 hours. All solutions were prepared fresh each time the test was run; in order to avoid non-specific precipitation, the preparations were incubated with the cells supported just below the surface of the substrate medium and facing downward.

RESULTS AND DISCUSSION

Figs. 1 a to 1 k show the mitotic phases of the HeLa cell life cycle; they are offered to facilitate orientation since reference is made to the various substages of mitosis in the following pages. Cells in several of these phases were selected in the light microscope and subsequently studied in the electron microscope.

INTERPHASE: The interphase HeLa cell has often been used in electron microscope studies, and descriptions of it are numerous (18, 19). In our studies we have not seen anything not previously noted, and therefore Figs. 2 and 3 are mainly for comparative purposes. This is especially relevant to Fig. 3 since the centriole and its surrounding osmiophilic halo pass through several apparently distinct transitions during mitosis.

Most of the major inclusions in the HeLa cell are present in Figs. 2 and 3; endoplasmic reticulum (ER) is relatively scarce, which frequently is the case in undifferentiated cells. Two centrioles

with their long axes perpendicular to each other are visible in Fig. 3; the 9 triplets of rods of one of the centrioles are seen in cross-section. A diffuse, osmiophilic, unstructured zone extends about 0.09 micron beyond the centriole.

PROPHASE: The initial condensation of chromatin at the nuclear membrane and the eventual disappearance of the latter during prophase have been exhaustively documented in the light microscope and to some extent in the electron microscope (1, 20): of interest to us here are some of the less apparent transformations occurring concomitantly. Along with intranuclear chromatin condensation is the shifting of the nucleus to a less eccentric position. Fig. 4 is an electron micrograph of a cell which was fixed when it had progressed in prophase to the stage shown in Fig. 1 b. The nuclear "hof" with its contained centriole is seen, and in this particular section the nuclear envelope appears intact. Fuzzy segments of the envelope are judged to be regions which have been cut tangentially. This is more convincingly demonstrated in Fig. 5, a higher magnification of the same cell. Details of the centriole, somewhat unfavorably sectioned, are revealed within the invagination. The pericentriolar osmiophilic zone is extensive, compared to its appearance at interphase, and is more dense but without obvious structure. Spindle tubules extending in every direction apparently pass to intranuclear chromosomes, although no kineto-chore has been seen in this stage. Also noted are many small, circular profiles or vesicles arranged radially, and present only in this phase of mitosis.

An adjacent section of this same cell. (Fig. 6) shows centriolar triplets; this conforms with descriptions of the structure already published (21, 22). However, of interest is the pronounced pericentriolar osmiophilia which extends about 0.16 micron beyond the boundary of the centriole in contrast to the 0.09 micron noted routinely in interphase. The irregular, almost wave-like character of the nuclear membrane in the vicinity of the centriole, in this section, is exaggerated. In this section an atypical sharp projection (NEP) of the envelope is directed towards the centriole and may manifest an interaction between these two structures. Light microscopic studies by Izutsu (22 a) made on orthopteran spermato-cytes suggest a similar phenomenon and indicate that this is a wide-spread occurrence. Fig. 7 shows the details of this phenomenon. The impression is

that the envelope is intact around the entire periphery of the projection; however, it is discontinuous at the lower right of Fig. 6 where a chromosome appears to be making its way through the gap. Several spindle tubules pass towards this chromosome, and even into it, but no direct point of attachment is visible. We have never observed fragmentation of the nuclear envelope and subsequent passage of the endoplasmic-reticulum-like remnants to the poles as has been described in plant cells by Porter and Machado. Figure 7 a which is another section through the same prophase cell as shown in Figs. 5 to 7 illustrates the fact that the centriole is duplicate; two additional centrioles were seen at the opposite pole of this cell, making a total of 4, and indicating that centriole replication is one of the earliest events occurring in the mitotic cycle.

Fig. 8 is an electron micrograph of a cell fixed in late prophase as shown in Fig. 1 e; the spindle tubules have markedly increased in length, compared to those in Fig. 6, but reveal no change in diameter. While the centriole does not undergo any noticeable change between early and late prophase, conspicuous in their absence from the vicinity of the centriole in late prophase (Fig. 9) are the circular profiles seen earlier. Also absent in prophase are the randomly arranged spindle tubules which are seen in almost every interphase cell prepared with glutaraldehyde fixation (see Fig. 2).

METAPHASE: The HeLa cell in metaphase characteristically displays clusters of structurally complex, membrane-bounded bodies (MBOB) at scattered points in the cytoplasm (Fig. 10). They are usually found relatively close to the periphery, outside the mitotic apparatus. Each cluster contains from 3 or 4 to as many as 15 or 20 subunits, the exact number obviously depending in part on the level of the section. The bodies are seen occasionally in interphase as isolated structures; the formation of clusters begins in early prophase (Fig. 4). It is during metaphase, however, that they are most prominent. Within any one cluster the bodies show considerable polymorphism. Each body is surrounded by a unit membrane and contains a dense core separated from the membrane by a less dense halo. They most commonly take the shape of a sphere or ellipsoid with an upper size limit of about 0.8 micron. Figs. 11 and 12 offer convincing evidence that these bodies represent transformations of multivesicular bodies
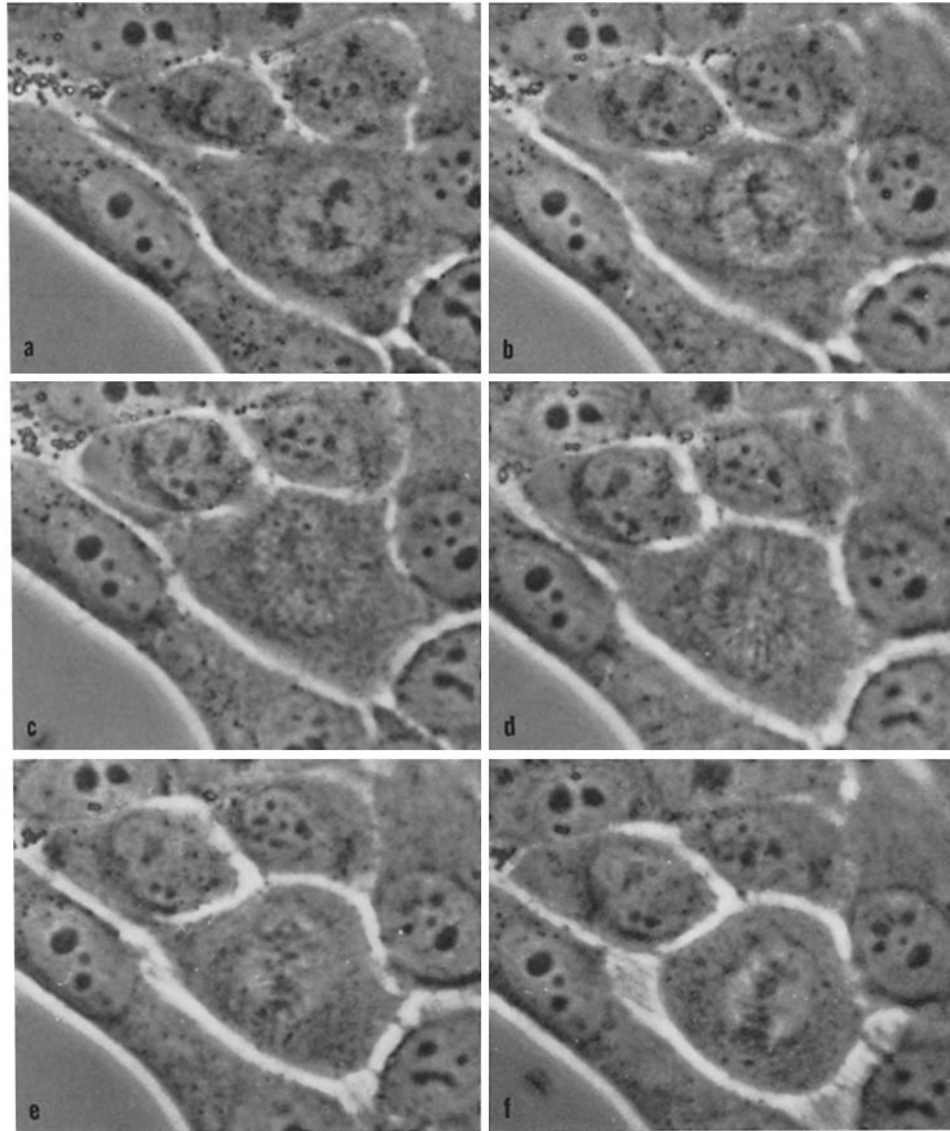
Key to Abbreviations

BLV, bleb vesicles	*M*, mitochondria
BL, bleb	*MB*, membrane-bounded osmiophilic bodies (in text referred to as *MBOB*)
C, centriole	*MVB*, multivesicular bodies
CP, circular profiles	*N*, nucleus
Cr, chromosome	*NE*, nuclear envelope
Cs, centrosome	*NEP*, nuclear envelope projection
EC, elongated cisternae	*S*, spindle tubule
K, kinetochore	*um*, unit membrane
G, Golgi apparatus	*MdB*, mid-body

FIGURE 1a–1k   Mitotic cycle of the HeLa cell. Phase contrast. × 640.

  1 a. time (t) = 0. Chromosome condensation has begun. Early prophase.
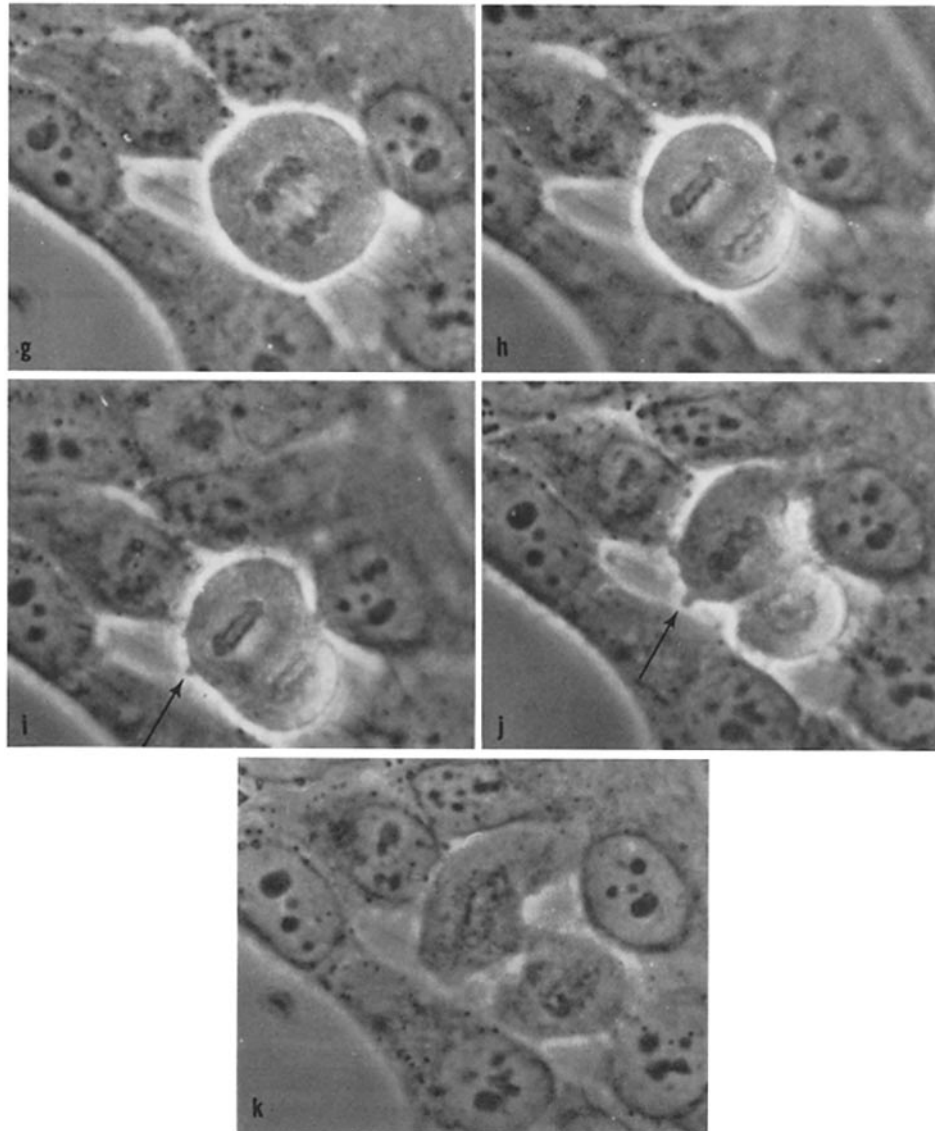
b. t = 3 minutes.

c. t = 4 minutes.

d. t = 10 minutes. Nucleolus and nuclear membrane have disappeared; chromosomes have started moving towards metaphase plate.

e. t = 18 minutes. Chromosomes have almost completed their alignment on the metaphase plate.

f. t = 21 minutes. Metaphase.

g. t = 57 minutes. Anaphase.

h. t = 58 minutes. Equatorial constriction is visible.

i. t = 61 minutes. Surface "blebs" are indicated (arrow).

j. t = 70 minutes. Equatorial constriction is deepened; "blebs" are more prominent (arrow) although they have passed beyond the point of maximum size by this time. Chromosomes have begun to uncoil.

k. t = 80 minutes. Nuclear reconstruction is almost complete.

(MVB). In Fig. 11 a typical MVB, the matrix of which shows a slightly increased osmiophilia, is in close association with the membrane bounded osmiophilic bodies. In Fig. 12 this association is established unequivocally by the image of a body containing several typical MVB vesicles, with varying degrees of osmiophilia, embedded in a characteristic MBOB matrix. In general these bodies are the most dense structures in the cell. Commonly and, perhaps, more than coincidentally associated with the clusters is a peripheral or juxtaposed, but incomplete, double membrane (Fig. 13). Also noteworthy is the myelin figure formation seen in one of these bodies, in Fig. 14 since myelin figures are frequently found in lysosomes.

Although the metaphase centriole illustrated in Fig. 15 is not surrounded by the osmiophilic zone prominent in prophase, evidence to be presented elsewhere (23) indicates that the absence of this zone here is a result of sectioning and that the extensive pericentriolar osmiophilia persists until anaphase.

Another common characteristic of HeLa cell in metaphase is what appears to be the undeviated passage of spindle tubules through condensed chromosomes (Fig. 16). In many cases these tubules have been determined to be "continuous tubules" and the conclusion that these, as well as "direct tubules," may play a role in karyokinesis becomes more plausible with this evidence (see below).

The dissolution of the nuclear envelope in prophase is followed by a massive elaboration of smooth membranes towards the periphery of the metaphase cell (Fig. 17). These aggregates are, in general, polar in distribution, but extend about halfway to the equator. Their morphologic resemblance to the ER cisternae is clear, and the part they play in nuclear envelope reconstruction (see below) indicates that they represent pre-
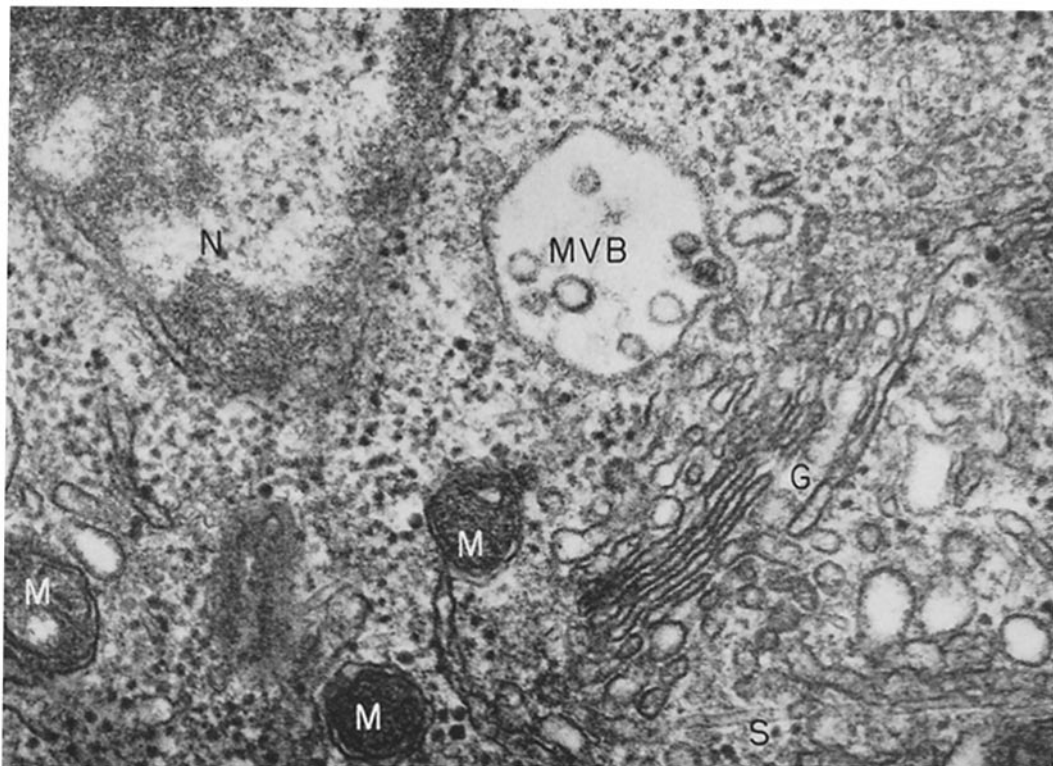
FIGURE 2   Portion of interphase HeLa cell showing Golgi apparatus, multivesicular body, 250. A diameter spindle tubule, mitochondria, nuclear envelope, and dense particles (possibly glycogen) scattered throughout the cytoplasm. × 80,000.

cursors of this envelope as suggested by Porter and Machado (see also references 24, 25).

ANAPHASE: The chromosomes in early anaphase are still distinguishable as discrete entities devoid of a nuclear envelope, as shown in Fig. 18. The polar aggregations of membranous cisternae alluded to above are prominent, as are clusters of MBOB. Spindle tubules may be seen converging towards the centriole, although the section does not pass through this organelle. Fig. 19 is a section through another part of the same cell and was chosen because of its clear visualization of the crescentic, extremely osmiophilic kinetochore which is separated from the chromosome proper by a band about 150 A wide. This band appears to have no affinity for osmium, but it is assumed to be the means of junction between the kinetochore and the chromosome. Several spindle tubules are attached to a single kinetochore (8).

Hardly 2 minutes after the initiation of karyokinesis (Fig. 1 g) nuclear envelope reconstruction has begun. Arrays of elongated cisternae have approached the still discrete chromosomes without yet establishing contact (Fig. 20). One to 3 minutes later the chromosomes have fused into a continuous mass (Fig. 21), and in many areas the discontinuous nuclear envelope is juxtaposed to this mass; at the arrow in Fig. 21, 4 ribosomes are attached to the outer surface of an isolated segment of envelope, further supporting the Porter-Machado concept of nuclear envelope reformation from cisternae of endoplasmic reticulum (see also references 24, 25). Unlike the situation in the sea-urchin (2), the individual chromosomes in HeLa cells do not acquire envelope during anaphase. The figure also suggests that the presence of the nuclear envelope is incompatible with spindle tubule contact with chromatin; we have noted this incompatibility quite consistently (also see Fig. 25 and below). However, Fig. 22 shows an example of a spindle tubule traversing an intact envelope and disappearing into the chromatin mass. While the appearance of such a phenomenon is certainly not the rule, it does occur; we believe it is not due to superposition within the section, although superposition cannot be absolutely ruled out.

Four to 6 minutes after the onset of anaphase (Fig. 1 i), an equatorial constriction is initiated and densities which herald the formation of the mid-body appear on the interzonal spindle fibers (Fig. 23). Mid-body formation has been described
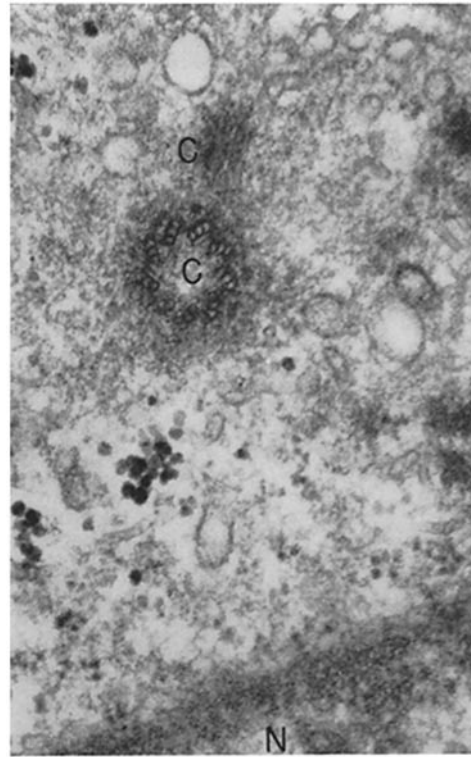

FIGURE 3 Interphase centrioles. One is barely clipped; in the other one the nine groups of triplets are clearly discernible; peripheral osmiophilia extends approximately 0.1 micron beyond centriole. X 54,500.

in detail by Buck and Tisdale (26). To this description we may add that formation of the mid-body is instituted less than 4 minutes after the cell enters this phase and is simultaneous with the onset of equatorial constriction. The dense material which constitutes the mid-body anlage encompasses both the spindle tubule "wall" and the immediate surrounding protoplasm and makes its initial appearance where bundles of spindle tubules are in closest approximation. Although the interzonal region where the mid-body forms is relatively free of mitochondria and most other cytoplasmic organelles, MBOB are sometimes seen within this area (Fig. 24).

Concurrent with anaphase equatorial constriction is an increase in cortical density at the equator which extends about one-quarter of the way around the cell periphery; the remainder of the cell surface frequently manifests irregular protoplasmic protrusions or "blebs," often described in the light microscope (27, 28). The electron micro-
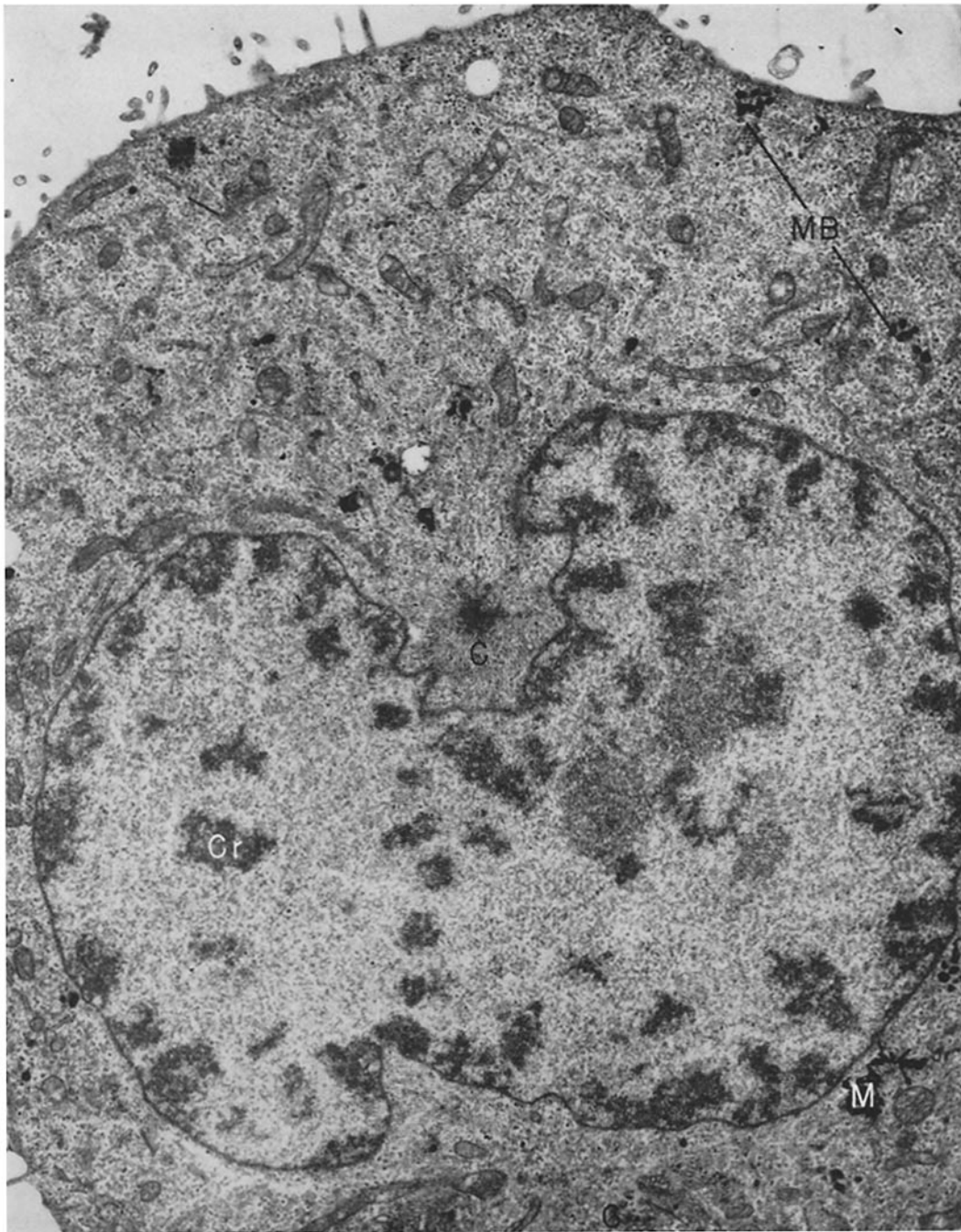
FIGURE 4 Early prophase. Peripheral condensed chromatin is juxtaposed to intact nuclear envelope; diffuse nucleolus is still present. Centriole, mitochondria, and Golgi apparatus are indicated. Membrane-bounded osmiophilic bodies are seen in small clusters. Also note early dissolution of the nucleolus in close association with the chromosomes. × 7,000.

scopic evidence indicates that the blebs do not extend beyond the junction between normal cortex and the more dense cortex of the cell. These changes are discussed in more detail below, since they are seen to better advantage later in anaphase.

Fig. 25, a higher magnification of Fig. 23, shows the duplicate anaphase centriole and a later phase of nuclear envelope formation. Several cisternae have coalesced although several discontinuities remain. In one extensive region where spindle tubules enter the chromatin mass and, in some cases, perforate it, the envelope is absent. This region, which is invaginated, is characteristically located in the HeLa cell and is visible in the light microscope in all cells in anaphase (Fig. 1 *i*). In other words, the HeLa cell has a predetermined pattern of nuclear envelope formation which is initiated at the polar aspect of the chromosomes but is not completed until a fair amount of envelope elements is formed on the equatorial aspect as well.

The late anaphase cell in Fig. 26 provides a good example of the cortical changes already mentioned. While the cell has progressed far in its ultimate division (between Figs. 1 *i* and 1 *j*), the increased cortical density extending along the cell periphery from the equatorial constriction has not enveloped any additional fraction of the periphery beyond approximately the one quarter noted earlier in anaphase. Protoplasmic blebs are prominent in this stage of anaphase; two blebs are shown in detail in Fig. 27. The plasma membrane surrounding them appears intact except where it is hazy due to its being cut tangentially. No characteristic fine structure is found within the blebs themselves, but at the base of one of them four circular profiles with a thickened osmiophilic membrane and a lightly osmiophilic matrix are seen. While these profiles are most conspicuous and frequently multiple at the base of the bleb in anaphase, we have seen single circular profiles in other phases. They may represent either pinocytotic vesicles or vesicles that may possibly be causally related to the blebs. The lightly osmiophilic matrix which is characteristic of the vesicles particularly in anaphase suggests a possible subtle distinction between these vesicles and those in other phases.

Fig. 28 gives details of the intercellular bridge in the late anaphase cell. Mitochondria, spindle tubules, mid-body anlage, and membrane-bounded osmiophilic bodies are all conspicuous and intermingled, but no elements of ER are seen. While the classical view that the spindle is free of organelles applies for the most part in early anaphase, it loses its validity later in this stage of division. In the upper right and lower left parts of the micrograph the increased cortical density which we have discussed is seen to be composed of an unstructured osmiophilia extending about 0.1 micron below a normal plasma membrane.

TELOPHASE: As the cell enters telophase, nuclear envelope reconstruction is completed and the uncoiling of chromosomes commences. Fig. 29 depicts this phase in a thin section. Also seen are the details of the mid-body. The figure suggests that mid-body elimination is finally effected by symmetrical invagination of the plasma membrane in a sort of pincer movement. This process of mid-body elimination is at variance with that which Buck has described in erythroblasts (5, 26) where the mid-body appears to be extruded rather than pinched off although the apparent extrusion phenomenon has also occasionally been observed. In addition, the cleavage of the HeLa cell, again unlike the erythroblast, is not accompanied by equatorial vesicle formation; Fig. 26, which typifies the rule, shows that separation of the two daughter cells is effected by a wedge-shaped constriction of the equatorial region rather than by the formation of a vesicular cell plate. Missing from the HeLa cell in telophase is the cortical thickening which was so prominent earlier.

Telophase is also the time in the division cycle when the clusters of MBOB begin to decrease in number and disperse. Fig. 30 is thus of interest since elongated cisterns (EC) are seen in close association with Golgi components. This finding is not an infrequent one and may represent a functional association between these two organelles.

The Golgi Apparatus During Mitosis

The Golgi complex becomes vesicular during early prophase, and as this phase progresses the typical parallel saccules become more difficult to find (Fig. 31). By metaphase, the organelle is not present in recognizable form. Studies of large numbers of serial sections have convinced us that this absence of Golgi complex is not due to a sampling error. Although we have seen rudimentary Golgi vesicles in late anaphase, it is not until telophase that one finds the full-fledged complex (Figs. 29, 32).
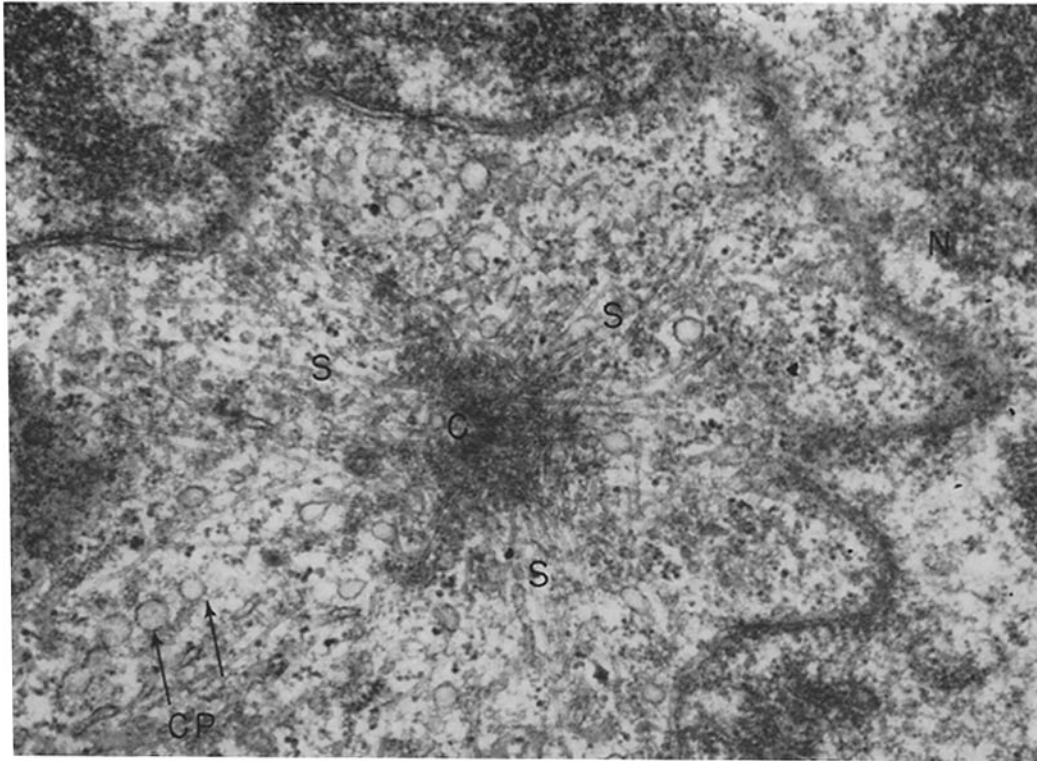
FIGURE 5   Detail of part of Fig. 4. Section has clipped centriole; spindle tubules pass and apparently arise multidirectionally from this structure. Numerous circular profiles (cp) are associated with the centriole-centrosome complex. × 36,000.

Histochemistry:

The histochemical studies, carried out to obtain ancillary data, confirm the morphological observations. Figs. 33 *a* and 33 *b* show that acid phosphatase-positive granules aggregate circumferentially in packets during metaphase, contrary to their polarized distribution as individual granules in interphase. Correlation of these data with the data on the distribution of MBOB in Fig. 10 leaves little doubt that the MBOB are acid phosphatase-positive, and hence a type of lysosome.

Thiamine pyrophosphatase activity during the mitotic cycle is shown in Figs. 34 to 36. By the middle of prophase the enzyme reaction is almost completely negative; in metaphase it is totally absent, consistent with morphological findings, and in late anaphase it begins to reappear as granules which are undoubtedly aggregations of vesicles which we have sometimes seen in the electron microscope. In each of the stages of division illustrated, numerous neighboring interphase

cells with their full complement of Golgi canaliculi are discerned and serve as an internal control of the staining reaction.

DISCUSSION

The brief description presented here of the mitotic process in HeLa cells followed in the electron microscope is not complete, but it is an attempt to provide an integrated account of several morphological transformations that occur. It is of particular interest to compare some of our observations with those made by other investigators on different material. With reference to the centriole-pericentriolar osmiophilic zone complex, Kawamura and Dan, using histochemical techniques, reported the presence in the centrosphere of sulfhydryl (SH) groups in high concentration during prophase and metaphase, with gradual disappearance of these groups during anaphase (29). Taking into account the plane of sectioning as described for Fig. 15, our results are consistent

with the histochemical data, and the pericentriolar osmiophilia would thus appear to coincide with the location of SH groupings. The duplication of the centriole by early anaphase (Fig. 27) is consistent with the findings in species as divergent as *Pseudotrichonympha* (30) and sea urchin (31); however, that this process may begin as early as prophase in mammalian cells is somewhat surprising.

In a recent paper, Rebhun (32) has commented on the aggregation of multivesicular bodies around asters in marine eggs during mitosis. We have not found any comparable behavior in HeLa cells; on the other hand, marine eggs do not display the multivesicular body transformations we have described. It is interesting to note that Palay (33) has seen similar transformations, including the appearance of intrabody myelin figures during the formation of neurosecretion droplets in the gold-fish.

As indicated in Fig. 30, there is a close association between the MBOB and the Golgi complex. The work of Novikoff (17 *a*) who reports the apparent formation of acid phosphatase-positive inclusions from Golgi constituents provides a precedent for drawing a conclusion concerning a relationship between these inclusions and the Golgi complex, but here the conclusion is reversed. In other words, the decrease in MBOB and their close association with the Golgi complex may indicate that MBOB have transformed to Golgi elements.

With respect to the phenomenon of nuclear dissolution and reconstruction, it is of some interest to note that we have not seen the extensive lamellar systems of cytoplasmic membranes described by Ito (34) in *Drosophila* spermatogenic cells and by Buck (35) in tumor cells. These systems, suggested to be the origin of the nuclear envelope in the daughter cells, may, however, be analogous to the polar aggregates of ER cisternae which we have documented in the HeLa cell.

With regard to the Golgi apparatus in mitosis, Ludford states that a transformation from canaliculi to granules occurs at this time (36); we would expect, however, that any granules that were visible in the light microscope would be recognizable as structures of appreciable size in the electron microscope. It is conceivable that the silver-reducing substance noted during mitosis by early investigators was in reality the transformed MVB (MBOB) which, as noted, have such a pronounced capacity for reducing OsO_4. However,

while we have not checked this point, the suggestion is weakened somewhat by Dalton's convincing observations on the absence, during mitosis, of a positive Kolatchev-Nassonov reaction for Golgi elements in several tumor cells which he has studied (37). Thus the discrepancy again may be due to species variation. Other aspects of the Golgi apparatus which we have observed is its sometimes equatorial proliferation during telophase (Fig. 29) and its sometimes polar location (Fig. 32). These observed variations may conceivably be due to sectioning, but this appears unlikely at present, and their reality gains support from histochemical observations in which the three-dimensional localization of the organelle is preserved. In connection with such morphological variability of position of the Golgi apparatus, it is frequently found that anaphase chromosomes which have passed poleward reverse their direction and to some extent return towards the equator as anaphase progresses (compare Figs. 23 and 26). This behavior of chromosomes may be the result of intermeshing of the continuous spindle tubules with the chromatin. When these tubules are being gathered together by equatorial constriction, the chromosomes may be pulled equatorially. However, failure to observe such chromosome reversal in all cases might be due to a block to the movement of chromosomes by the mass of Golgi vesicles which are sometimes reformed on the equatorial plane.

Although undoubtedly the mammalian cell cortex (ectoplasm) undergoes extensive modifications during mitosis (38), the only ultrastructural changes which we have consistently seen are the formation of anaphase blebs and an increase in the density of the equatorial cortex during cleavage. It is quite possible that bleb formation may be related to solation and that the increased density of the cortex is due to gelation. Perhaps these changes in the dividing HeLa cell are manifestations of these two processes, deemed by Marsland and Swann (39, 40) to be essential for cleavage.
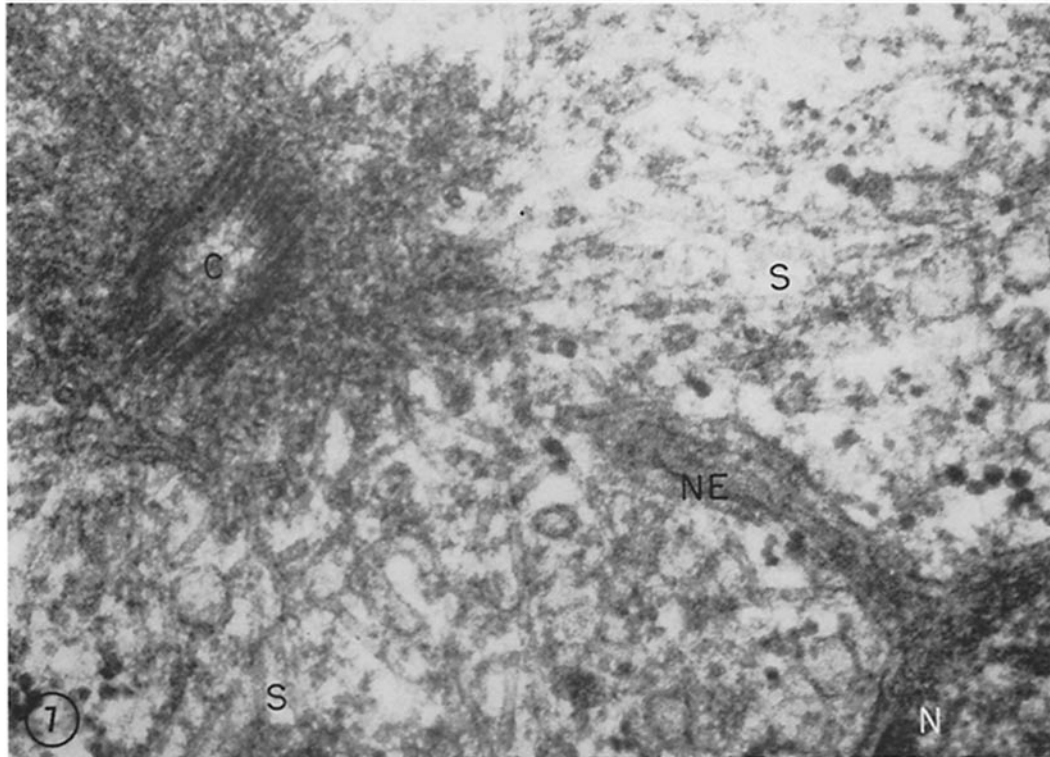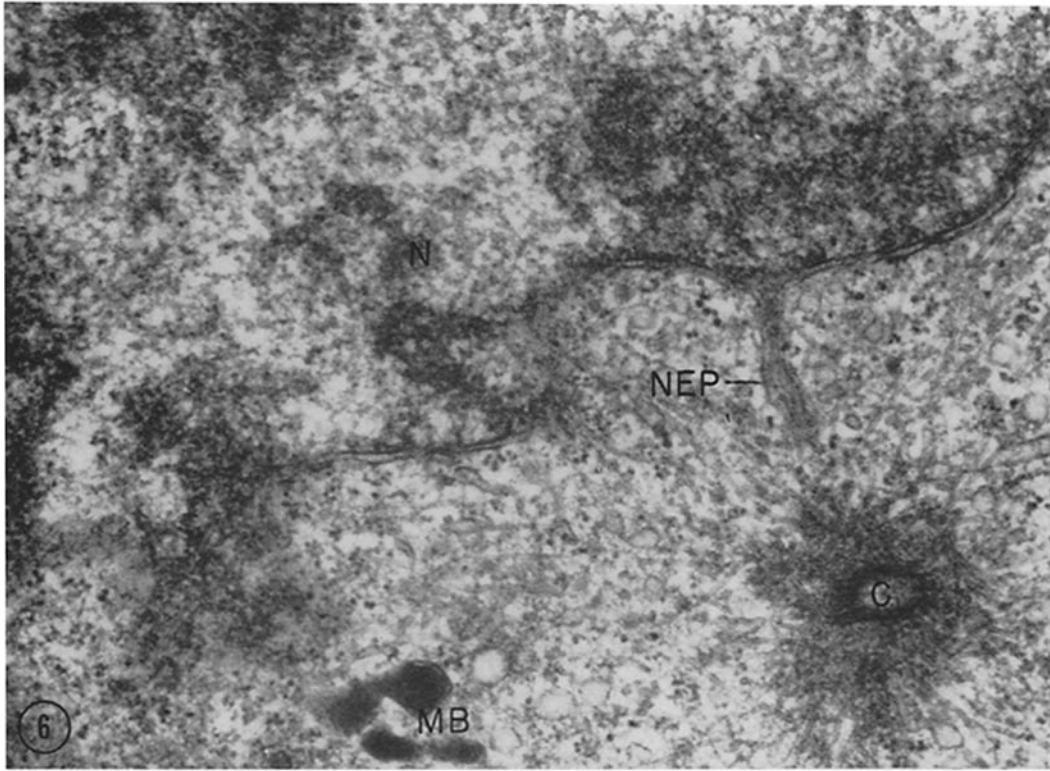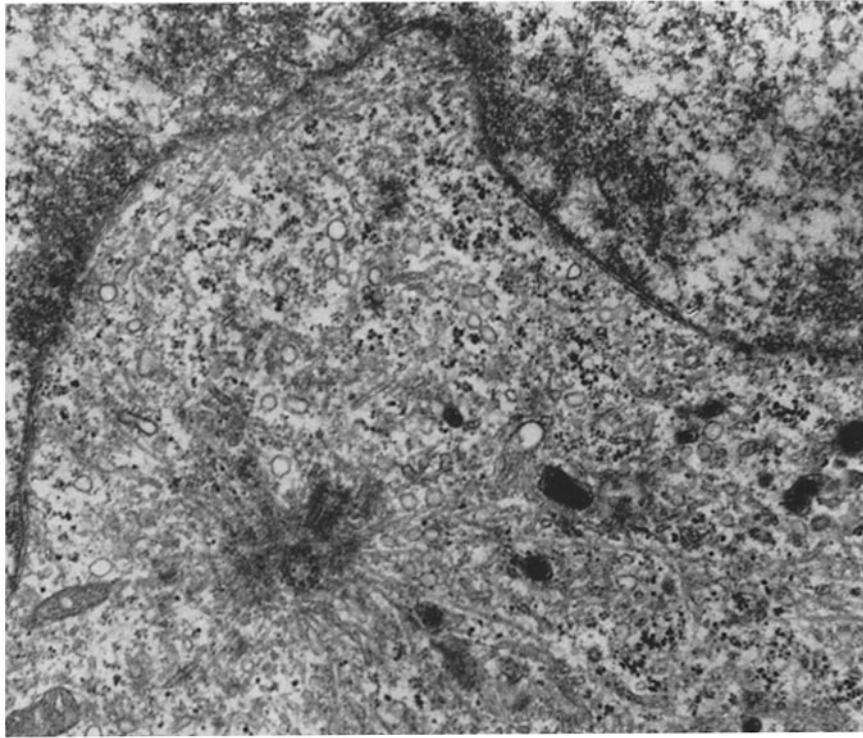
FIGURE 7 a Same cell as in previous figure, adjacent section, showing duplicate centriole. × 19,500.

For References, see following page.

FIGURE 6 Same cell as in previous figure, but adjacent section. Typical longitudinal section of centriole and extensive pericentriolar osmiophilia are indicated, as are membrane-bounded osmiophilic bodies. × 36,000.

FIGURE 7 Detail of Fig. 6. An abrupt projection of the nuclear envelope and included nucleoplasm is seen directed toward centriole. × 85,000.

REFERENCES

1. PORTER, K. R., and MACHADO, R. D., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 167.
2. HARRIS, P., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 419.
3. SELBY, C. C., *Exp. Cell Research*, 1953, **5**, 386.
4. DE HARVEN, E., and BERNHARD, W., *Z. Zellforsch*, 1956, **45**, 378.
5. BUCK, R., *in* The Cell in Mitosis, (L. Levine, editor), New York, Academic Press, Inc., 1963, p. 55.
6. YASUZUMI, G., *Z. Zellforsch*. 1959, **50**, 110.
7. SATO, S., *Cytologia*, 1959, **24**, 98.
8. SATO, S., *Cytologia*, 1960, **25**, 119.
9. ROBBINS, E. and GONATAS, N. K., *J. Cell Biol.*, 1964, **20**, 356.
10. BLOOM, W., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 191.
11. EAGLE, H., *J. Exp. Med.*, 1955, **102**, 595.
12. SABATINI, D. D., BENSCH, K., and BARNETT, R. J., *J. Cell Biol.*, 1963, **17**, 19.
13. MILLONIG, G., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 736.
14. HUXLEY, H. E., and ZUBAY, G., *J. Cell Biol.* 1961, **11**, 273.
15. ROBBINS, E., *J. Gen. Physiol.*, 1960, **43**, 853.
16. GOMORI, G., *Stain Technol.*, 1950, **25**, 81.
17. NOVIKOFF, A. B. and GOLDFISHER, S., *Proc. Nat. Acad. Sc.*, 1961, **47**, 802.
17 a. NOVIKOFF, A. B., ESSNER, E., GOLDFISHER, S., and HUES, M., *in* Symposium of the International Society for Cell Biology, New York, Academic Press, Inc., 1962, **1**, 149.
18. EPSTEIN, M. A., *J. Biophysic. and Biochem Cytol.*, 1961, **10**, 153.
19. JOURNEY, L. J., and GOLDSTEIN, M. N., *Cancer Research*, 1961, **21**, 929.
20. MOSES, M., *Proc. 4th Int. Cong. Electron Micr.*, Berlin, 1958, **2**, 230.
21. BERNHARD, W., and DE HARVEN, E., *Proc. 4th Int. Cong. Electron Micr.*, Berlin 1958, **2**, 217.
22. GALL, J. G., *J. Cell Biol.*, 1961, **10**, No. 4 suppl., 163.
22 a. IZUTSU, K., *Cytologia*, 1959, **23**, 485.
23. ROBBINS, E., and GONATAS, N. K., data in preparation.
24. BARER, R., JOSEPH, S., and MEEK, G. A., *Proc. 4th Internat. Cong. Electron Micr.* Berlin, 1958, **2**, 233.
25. MOSES, M. J., *Proc. 4th Internat. Cong. Electron Micr.*, Berlin, 1958, **2**, 230.
26. BUCK, R., and TISDALE, J., *J. Cell Biol.*, 1962, **13**, 109.
27. BOSS, J., *Exp. Cell Research*, 1955, **8**, 181.
28. HUGHES, A. W., The Mitotic Cycle, New York, Academic Press, Inc., 1952, 145.
29. KAWAMURA, N. and DAN, K., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 717.
30. CLEVELAND, L. R., *in* The Cell in Mitosis, (L. Levine, editor), New York, Academic Press, Inc., 1963, 3.
31. MAZIA, D., HARRIS, P. and BIBRING, T., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 1.
32. REBHUN, L. I., *Ann. New York, Acad. Sc.*, 1960, **90**, 357.
33. PALAY, S. L., *Anat. Rec.*, 1960, **138**, 417.
34. ITO, S., *J. Biophysic. Biochem. Cytol.*, 1960, **7**, 433.
35. BUCK, R., *J. Cell Biol.*, 1961, **11**, 227.
36. LUDFORD, R. J., *Proc. Roy. Soc. London, Series B*, 1924, **97**, 50.
37. DALTON, A. J., *Ann. New York Acad. Sc.*, 1951, **51**, 1295.
38. ROBBINS, E., work in preparation.
39. MARSLAND, D. A., and LANDAU, J. V., *J. Exp. Zool.*, 1954, **125**, 507.
40. SWANN, M. M., and MITCHISON, J. M., *Biol. Rev.*, 1958, **33**, 103.
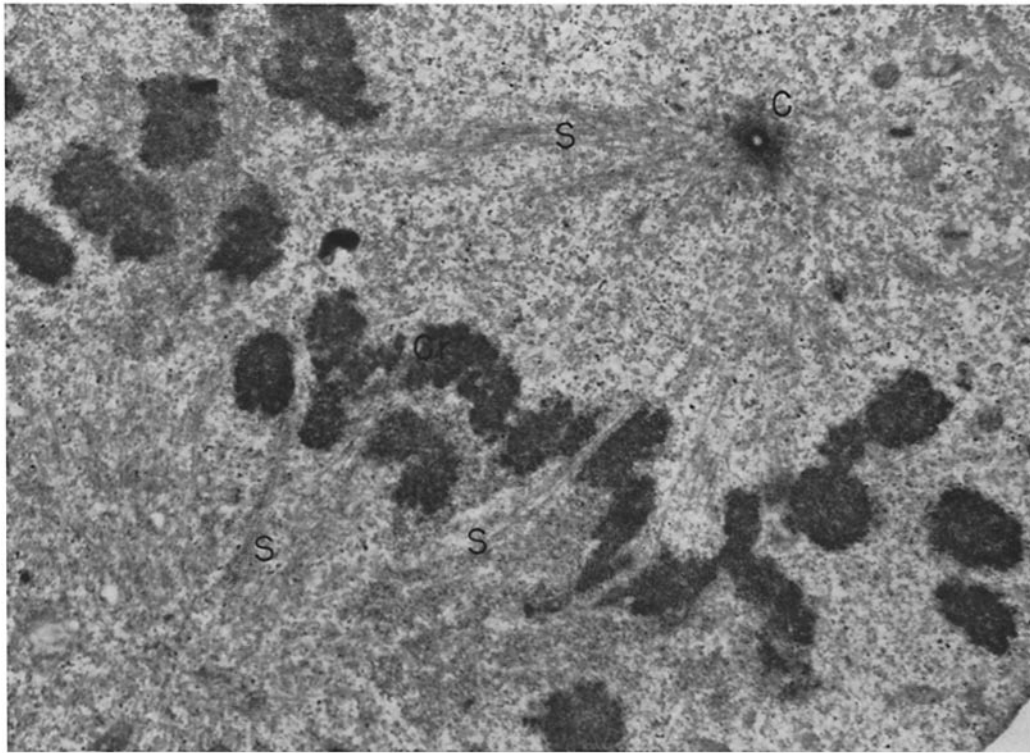
FIGURE 8   Late prophase. Chromosomes have progressed towards equatorial plate, and spindle tubules have elongated (compare Fig. 5). Pericentriolar region has maintained its extensive osmiophilia. × 15,000.
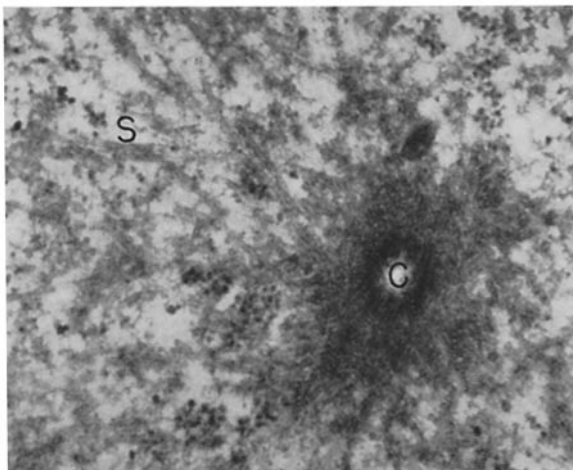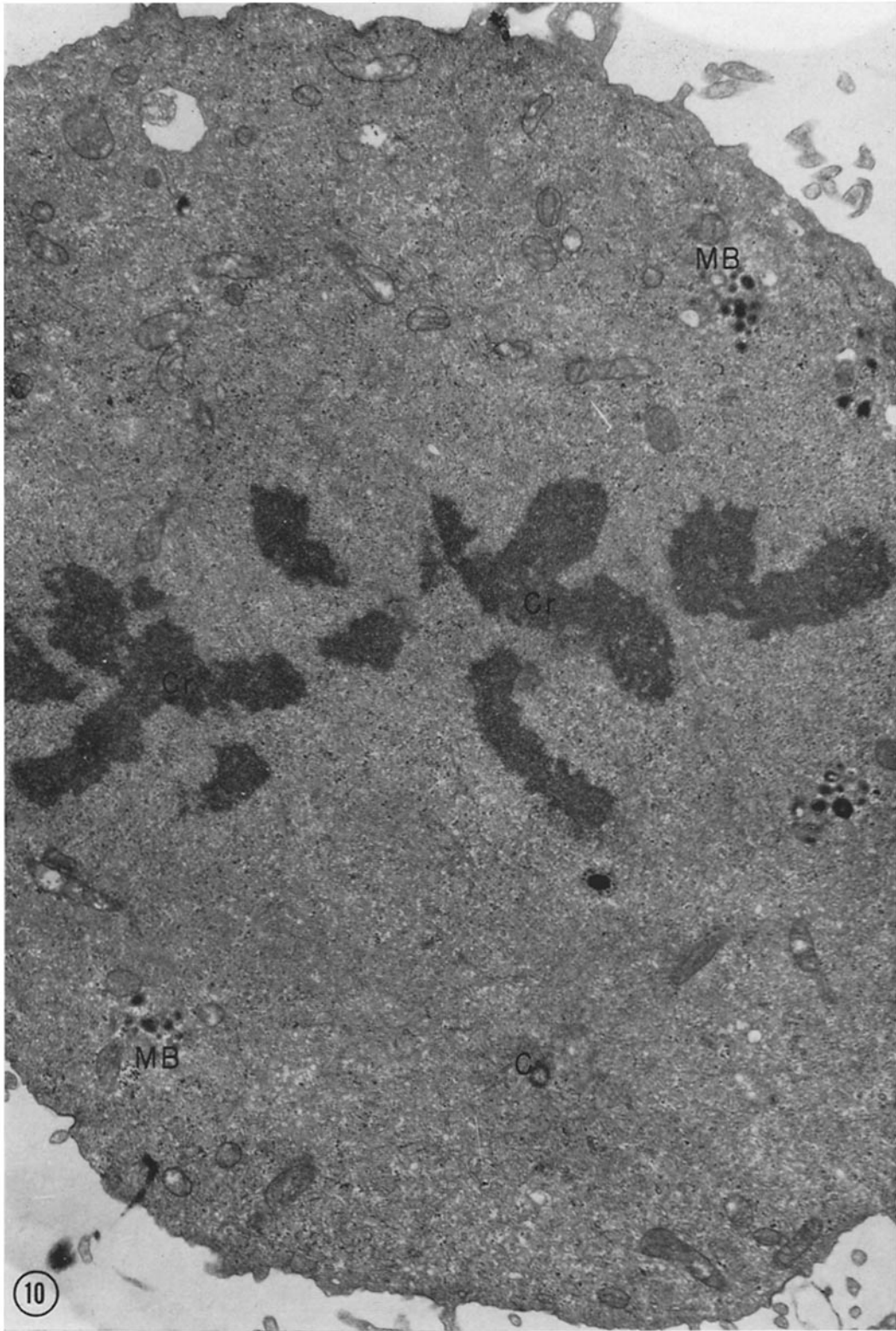

FIGURE 9   Detail of centriole complex from Fig. 8. Associated circular profiles noted in early prophase are absent. × 47,000.
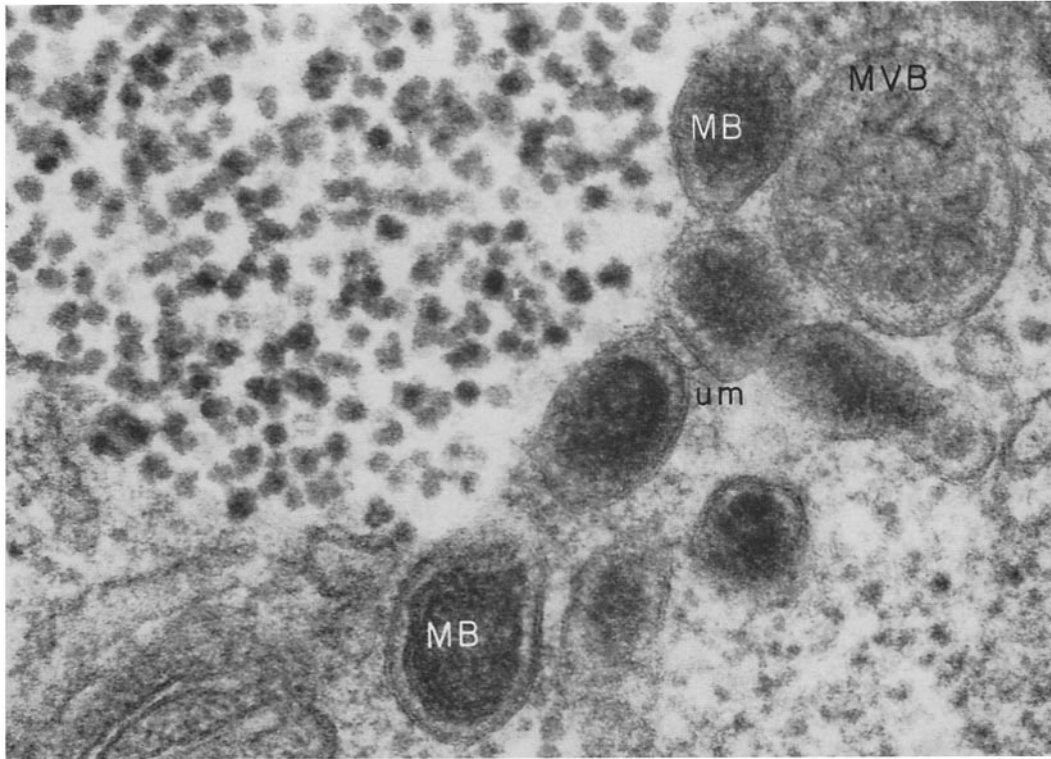
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FIGURE 11 Cluster of *MB*'s in close proximity to a multivesicular body (*MVB*). The vesicles within the *MVB* are somewhat fuzzy, and the matrix is more osmiophilic than normal (compare Fig. 2). Each unit of the *MB* cluster is surrounded by a unit membrane. Immediately juxtaposed is an extensive aggregation of dense particles. These aggregates, which are composed of the same particles described in Fig. 2, may assume dimensions of as much as 3 microns. They do not show any predilection for the interphase or dividing cells. × 115,000.

FIGURE 10 Metaphase. Chromosomes are aligned on metaphase plate. One centriole is caught in the plane of the section. Clusters of *MB* are scattered about the cell periphery. × 12,000.
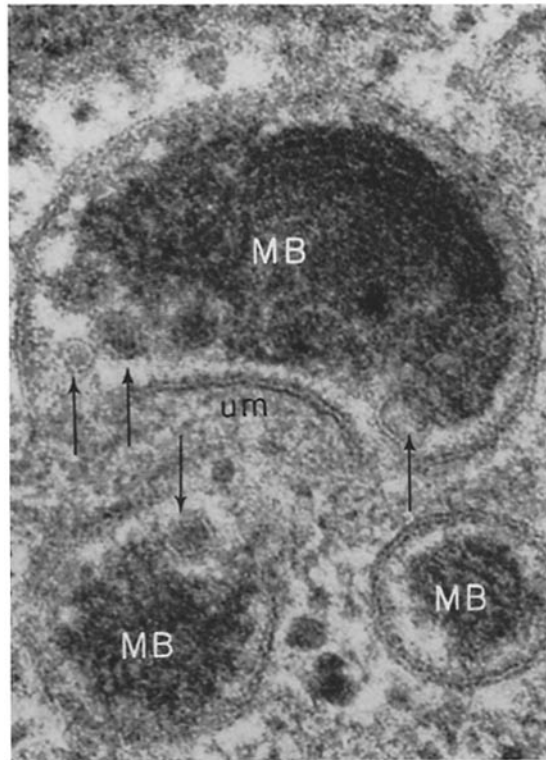
FIGURE 12 *MVB* partially transformed to *MB*'s, showing typical *MVB* vesicles. × 153,000.
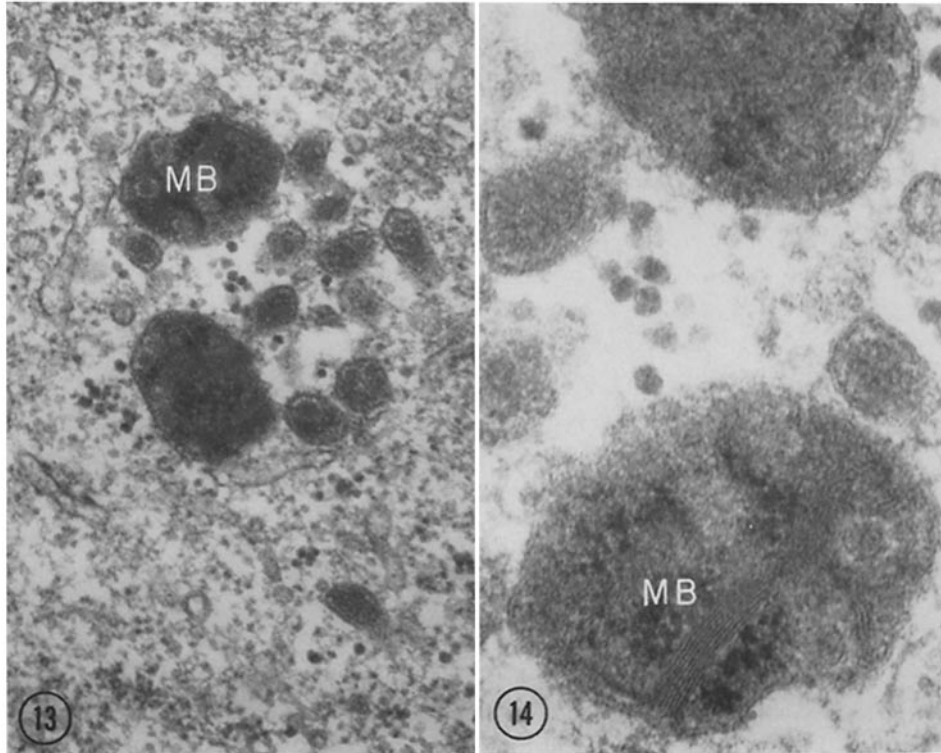
FIGURE 13   Cluster of *MB* with closely associated membranous cisterns. × 61,500.

FIGURE 14   Higher magnification of the same cluster shown in Fig. 13 illustrating intrabody myelin figure with 42 A periodicity. × 161,500.
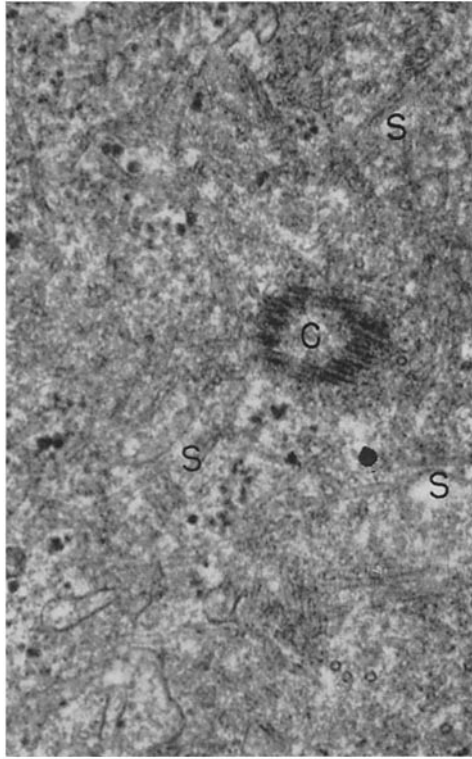
FIGURE 15 Detail of metaphase centriole. Peri-centriolar osmiophilia is absent due to level of section. Centriole is cut inlongitudinal section. Multidirectional arrays of astral and spindle fibers are seen in longitudinal and cross section. × 60,000.

FIGURE 16 Metaphase chromosomes pierced by continuous spindle tubules. × 46,000.

FIGURE 17 Polar aggregation of small, polymorphic membranous cisterns in metaphase. × 25,000.
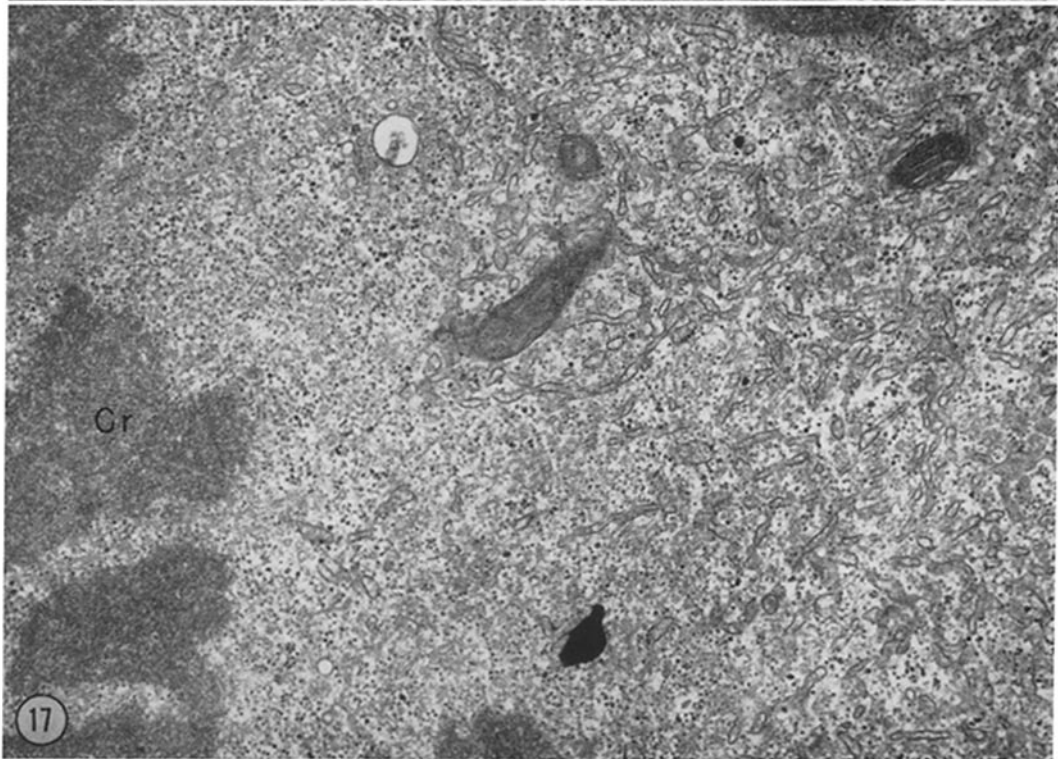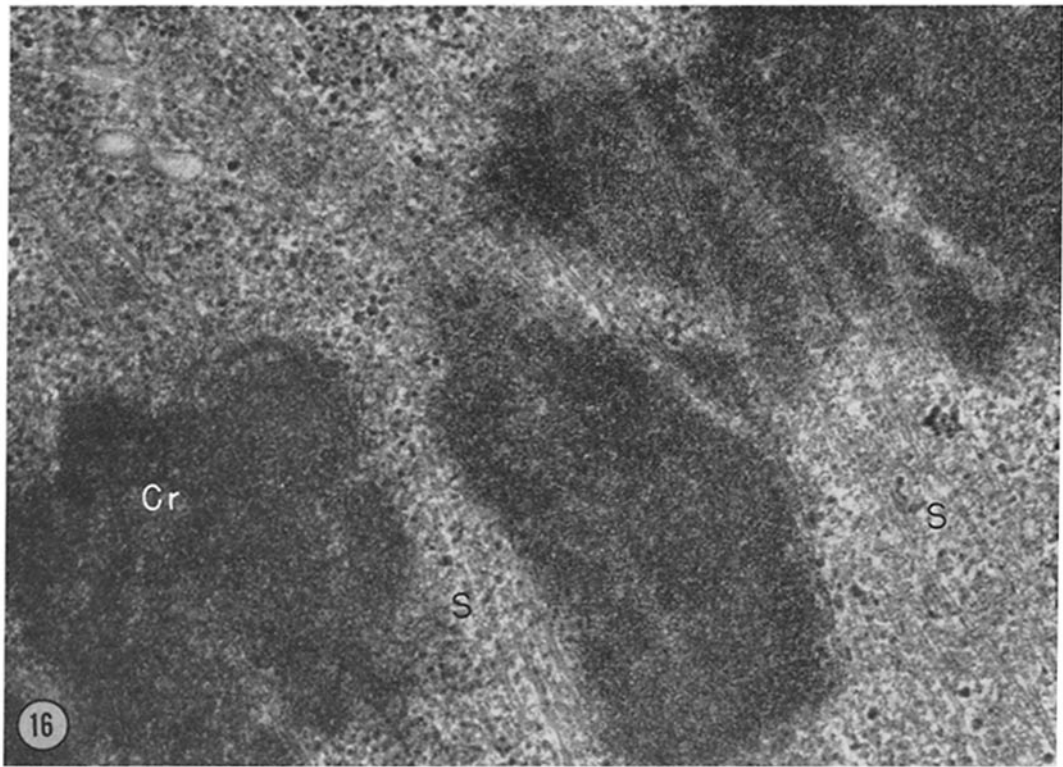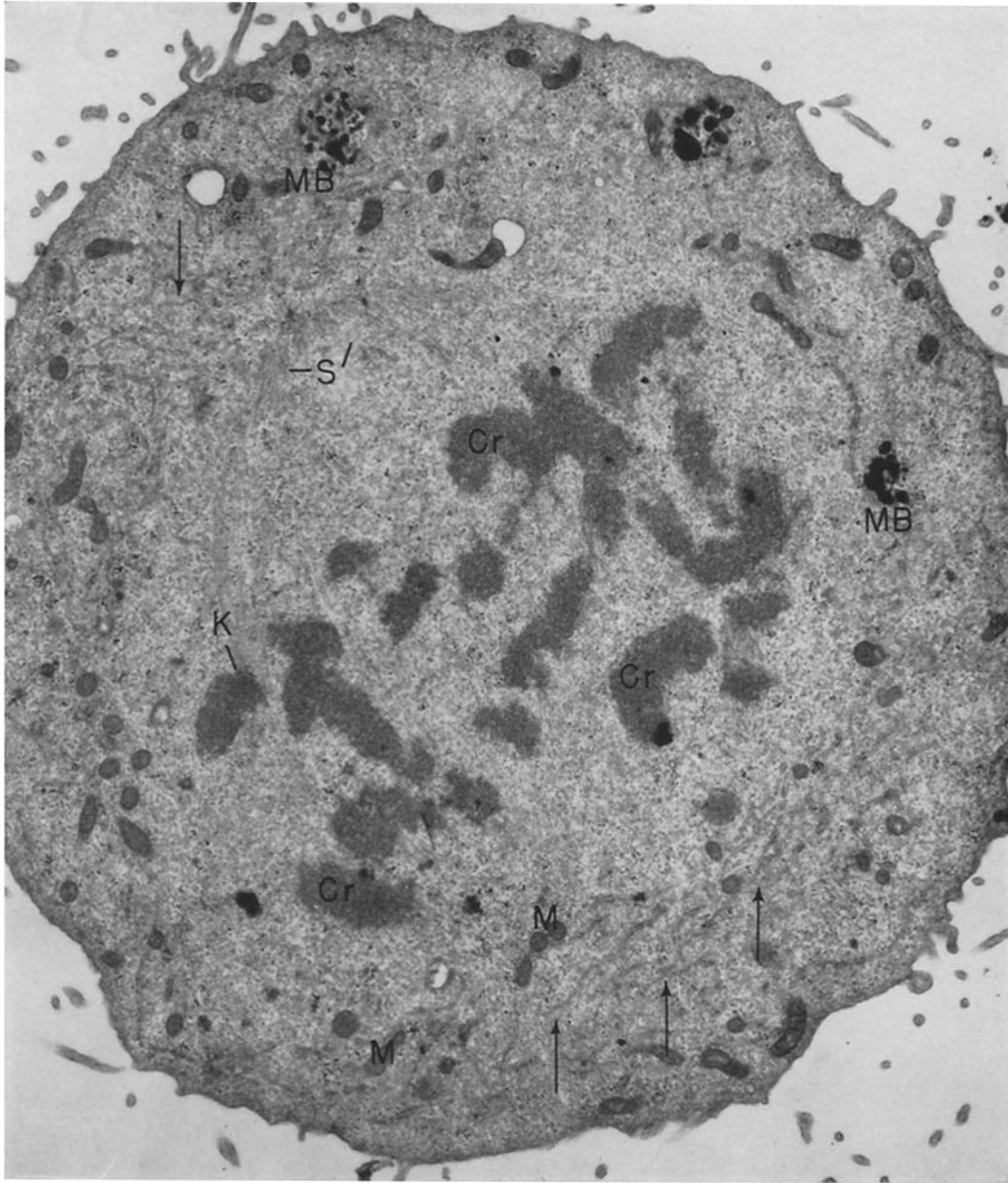
FIGURE 18   Early anaphase, 1 minute after commencement of karyokinesis. Membrane-bounded osmiophilic bodies and polar arrays of membranes (arrows) are visible. Also note kinetochore and the continuous spindle tubules that pass between chromosomes and converge on centriole. × 10,250.
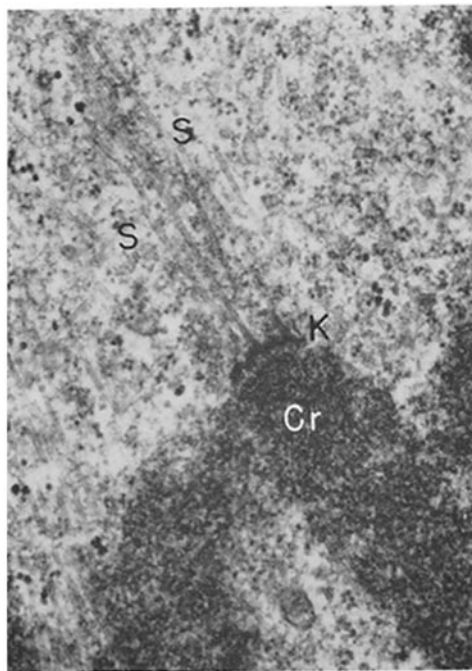
FIGURE 19 Higher magnification of the kinetochore, showing details of its interrelationship with the chromosome. × 38,000.
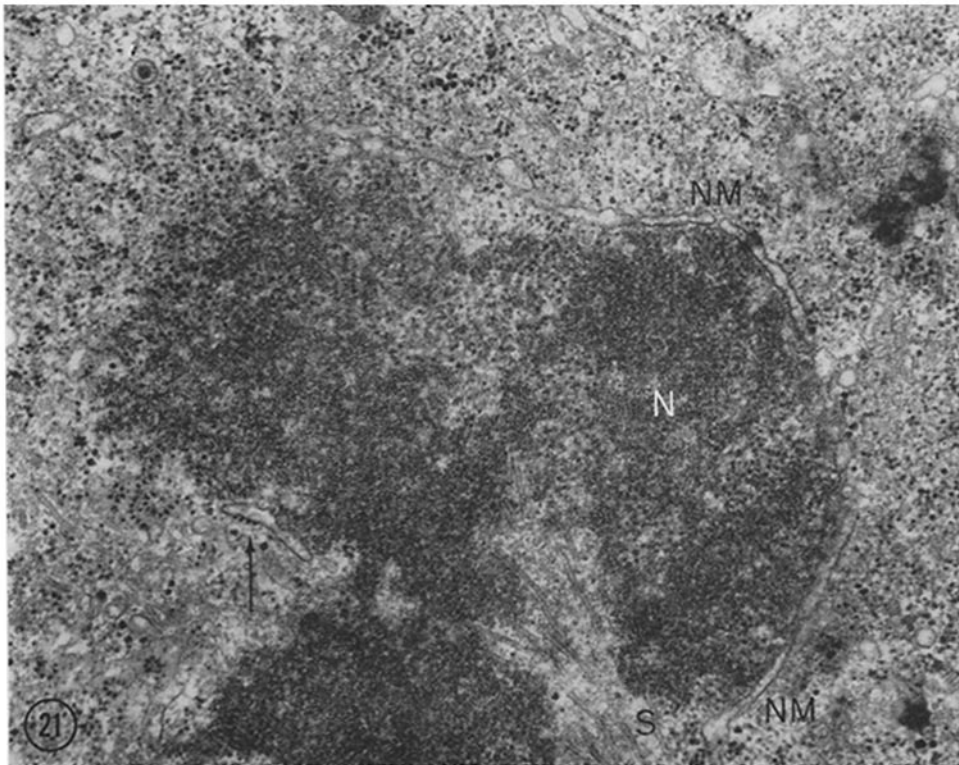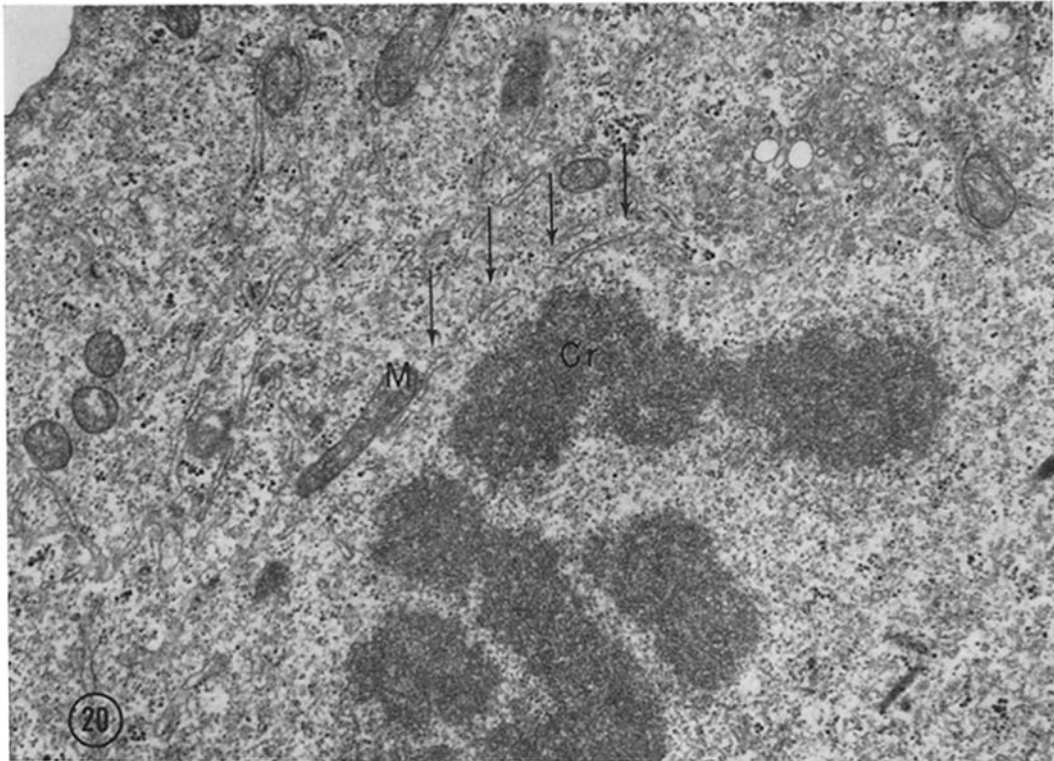
FIGURE 22   Anaphase cell. Spindle tubule is observed passing through intact segment of nuclear envelope (arrow). × 47,000.

FIGURE 20   Anaphase cell, 2 minutes after commencement of karyokinesis, depicting approach of elongated cisternae (arrows) on polar aspect of chromosomes, as early stage in the process of nuclear envelope formation. × 21,500.

FIGURE 21   Anaphase cell, 4 minutes after commencement of karyokinesis. Newly opposed segments of nuclear envelope have appeared on the partially fused chromatin mass. Membrane is absent where spindle tubules enter chromatin. An isolated segment of membrane with 4 attached ribosomes is present (arrow). × 35,000.
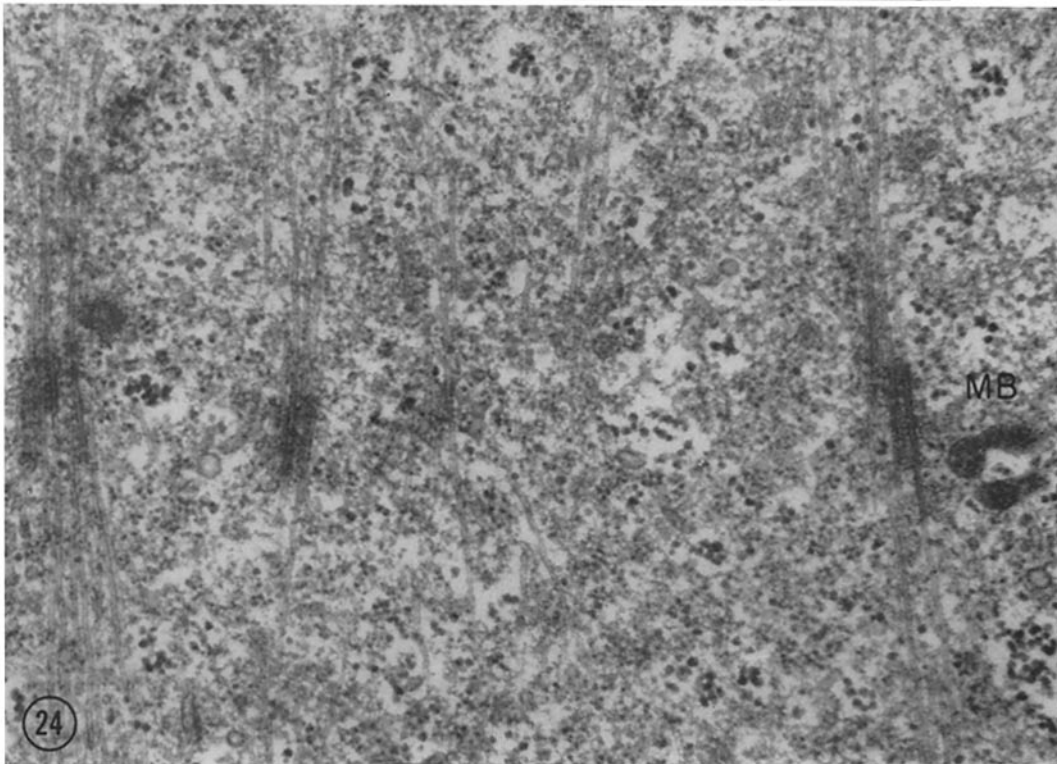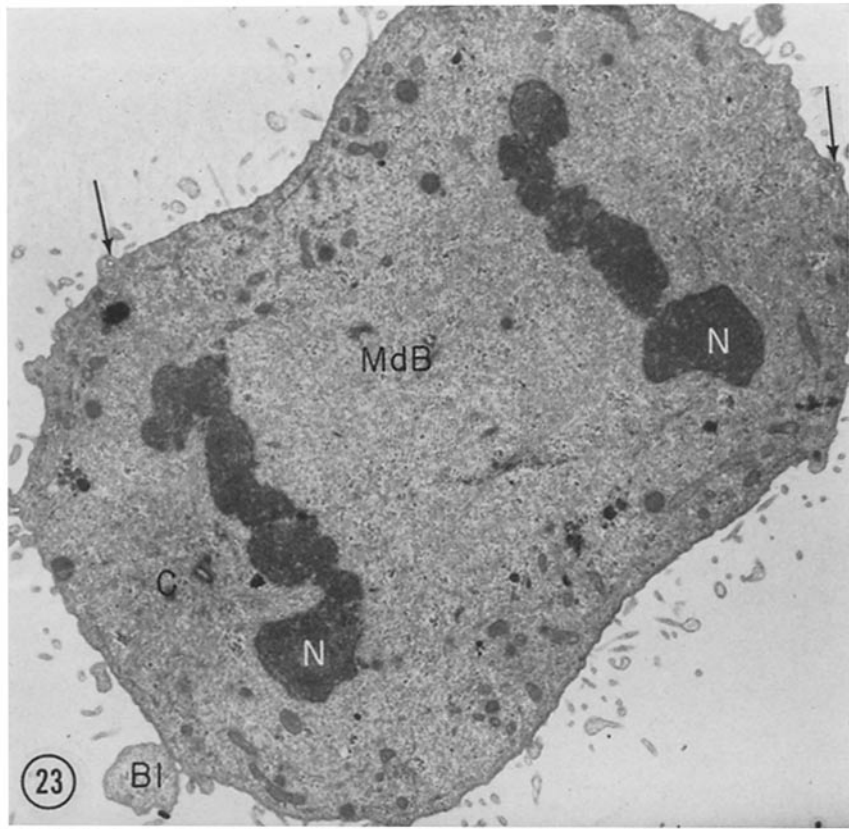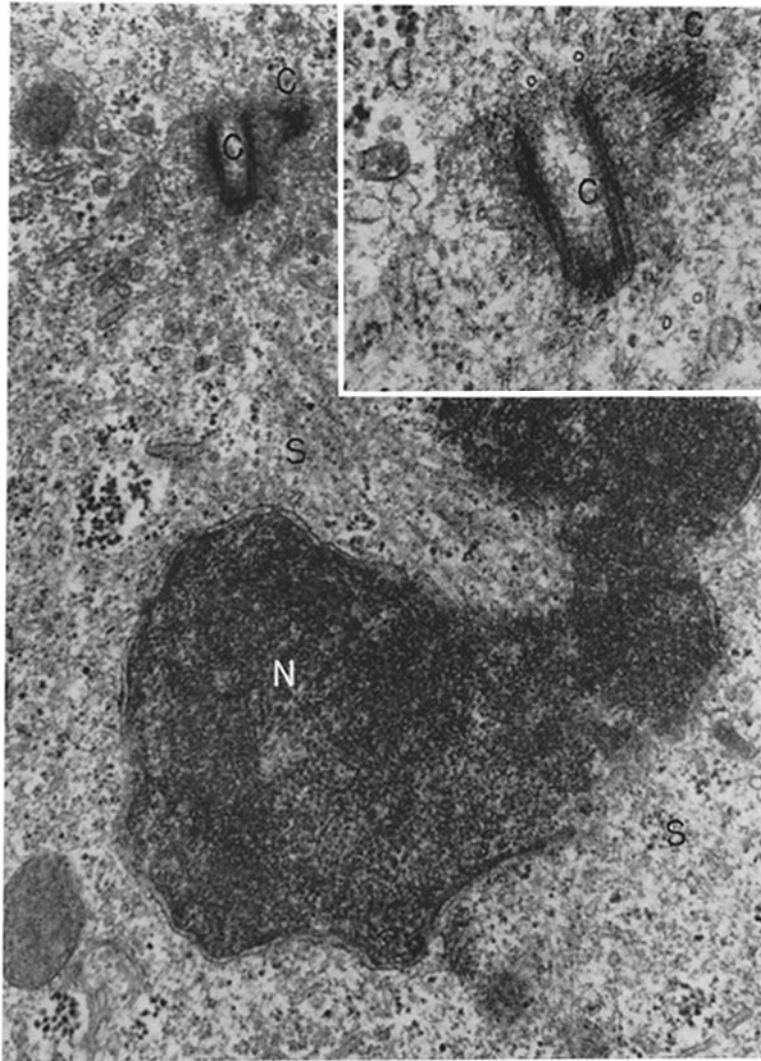
FIGURE 25   Detail of centriole in cell shown in Fig. 23. Note orthogonal arrangement of daughter centrioles and slight centrosomal osmiophilia. Spindle tubules and chromatin mass form junction at points still devoid of nuclear envelope. Insert shows higher magnification of centriole. × 25,000; insert, × 56,000.

FIGURE 23   Anaphase cell, 4 minutes after commencement of karyokinesis. The centriole is duplicate. Mid-body formation, equatorial constriction, and polar "blebs" (arrows) have been initiated. × 6,000.

FIGURE 24   Detail of interzonal region in cell shown in Fig. 23. Spindle tubules show localized regions of increased density (early mid-body formation). Except for two MB and the ever-present dense particles, no other organelles are present. The increase in density on the segments of spindle tubules occurs characteristically where groups of tubules make their closest approach. × 41,500.
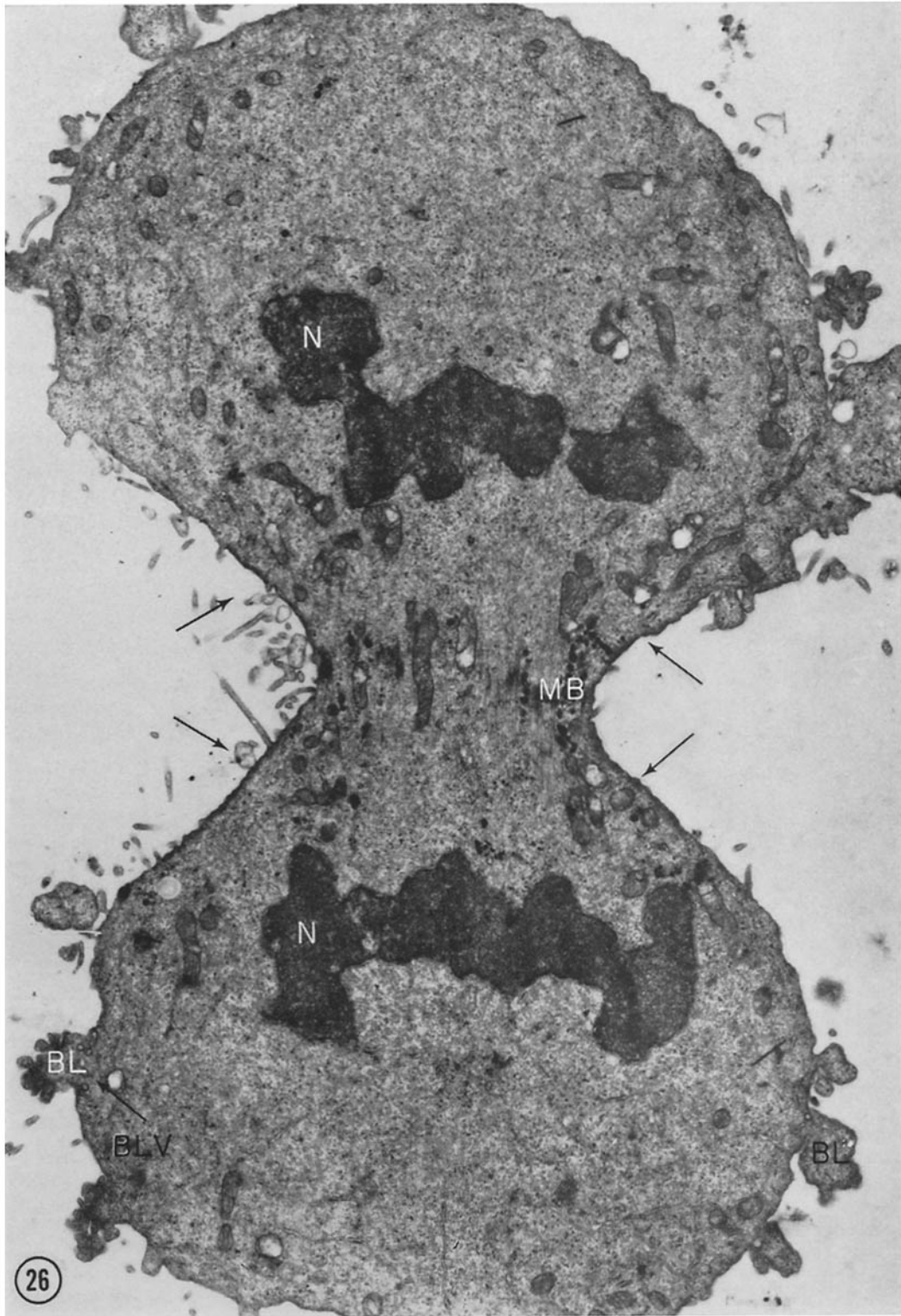
FIGURE 27 Detail of protoplasmic "bleb" on the surface of the anaphase cell shown in Fig. 26. Although extremely irregular, the plasma membrane appears intact. Four circular profiles with thickened osmiophilic membrane and lightly staining matrix are located at the base of the "blebs" (arrows). × 27,000.

FIGURE 26 Anaphase cell, 8 minutes after commencement of karyokinesis. Surface "blebs" are prominent. Note increased density (arrows) of cell cortex extending in "V"-shaped fashion from equator towards, but not necessarily to, junction with surface "blebs." Intercellular bridge contains various cellular organelles including *MB* and mitochondria. × 9,500.
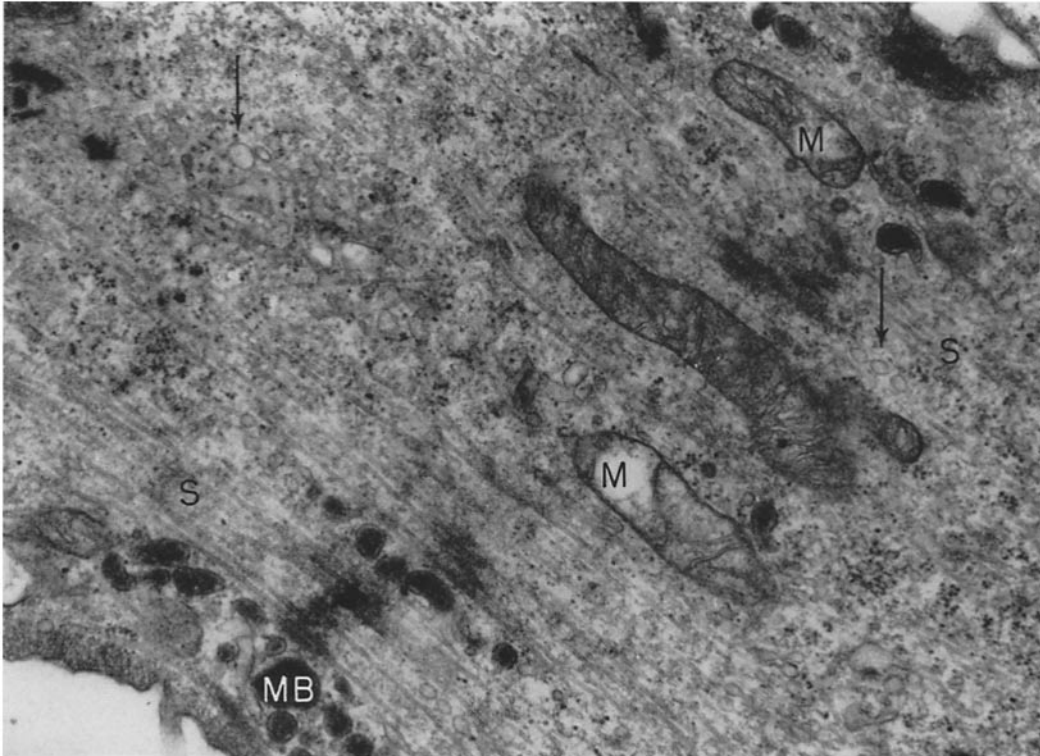
FIGURE 28   Intercellular bridge in the anaphase cell shown in Fig. 26. Spindle tubules, *MB*, and mitochondria are prominent, as are circular profiles (arrows) of the same character noted around the centriole complex in early prophase (Fig. 5). At the lower left and upper right the cytoplasm immediately beneath the normal plasma membrane shows increased density. × 28,500.

FIGURE 29   Cell in late telophase. Note prominent Golgi complex in upper part of figure. Insert shows details of mid-body and intercellular bridge at this stage. Symmetrical bilateral invaginations of plasma membrane (arrows) suggest that mechanism of mid-body elimination may be the pincer-like behavior of the invaginations. Spindle tubules are continuous through dense mid-body. Numerous circular profiles remain associated with intercellular bridge, as noted in Fig. 28. A single *MB* also is seen. × 25,000; insert, × 30,500.
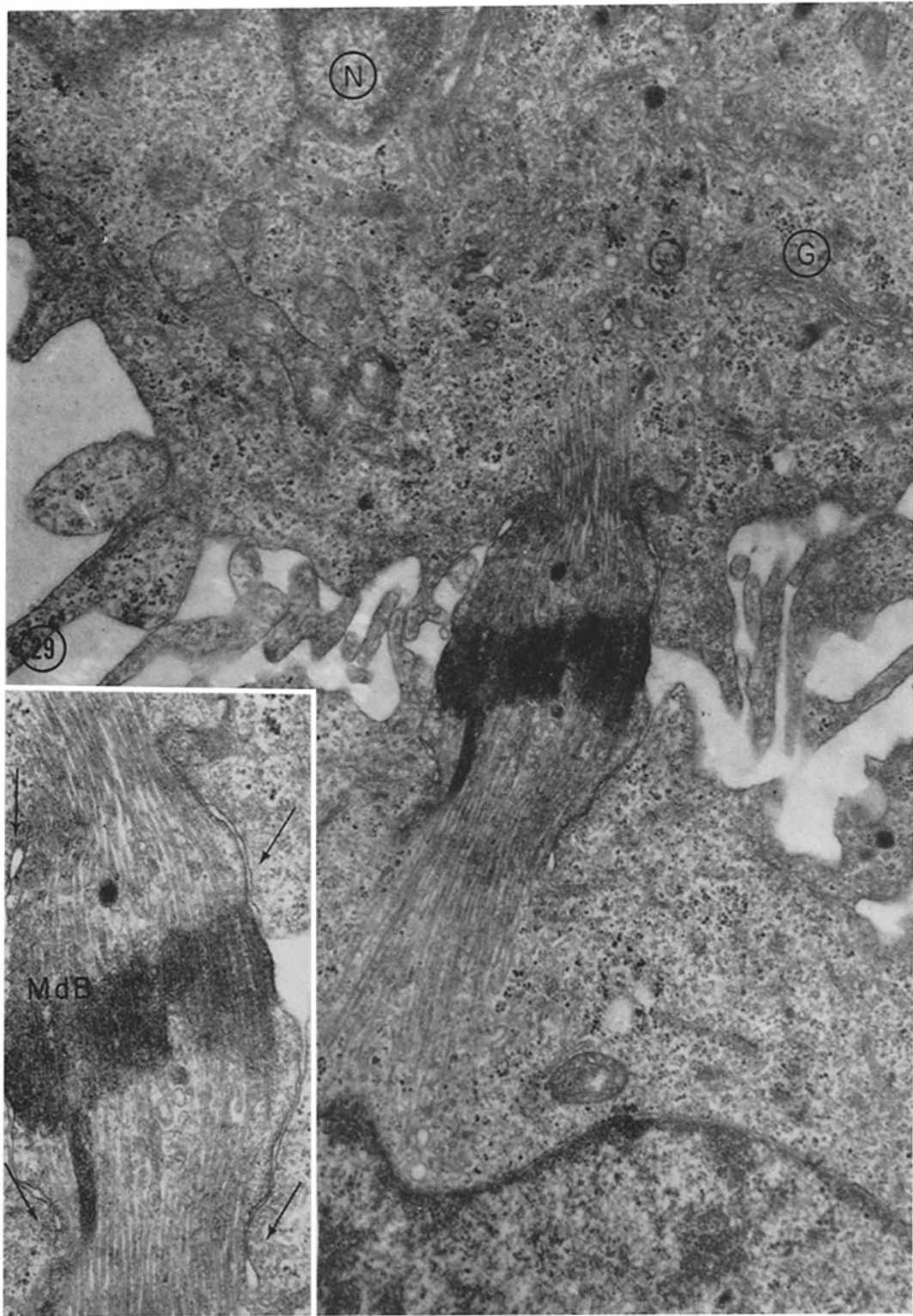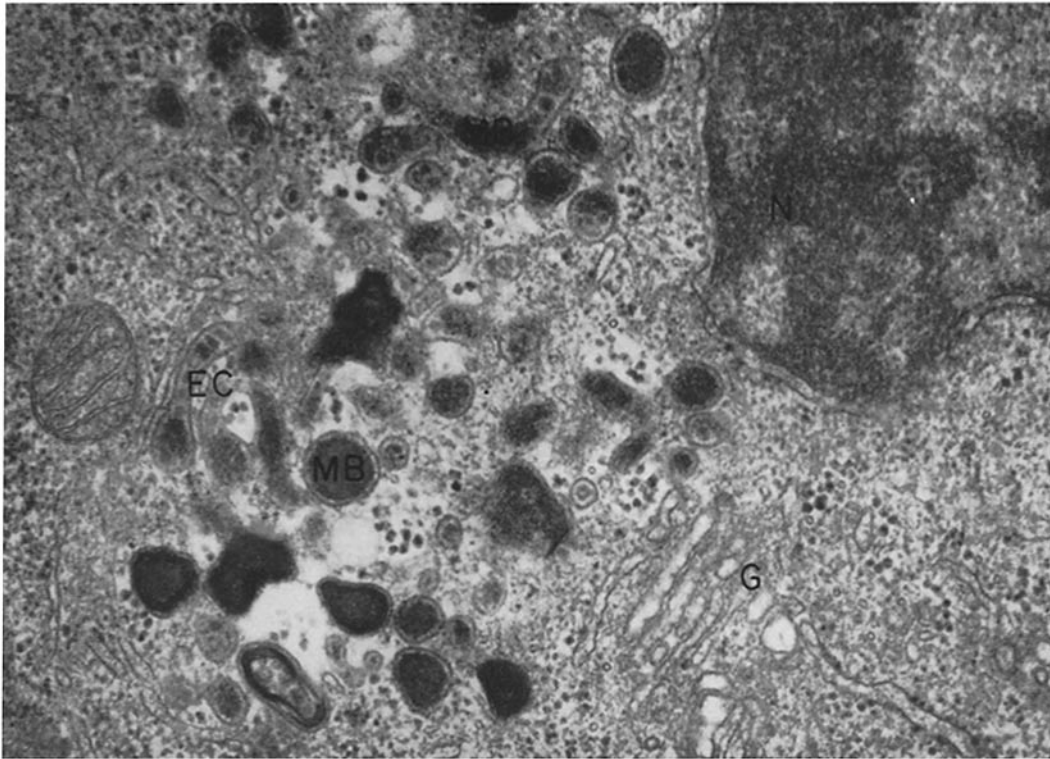
FIGURE 30   Cluster of *MB* in telophase, showing elongated cisterns and their contained osmiophilic particles in close association with the Golgi complex. × 45,000.

FIGURE 31   Detail of part of same cell shown in Fig. 4, illustrating Golgi vesicles in this early prophase cell. × 33,000.

FIGURE 32   Late telophase showing extensive Golgi apparatus in nuclear "hof" and "medial" displacement of nucleus in cell on left. Note cluster of *MB* which is less compact than the clusters ordinarily seen in metaphase or anaphase. × 9,000.
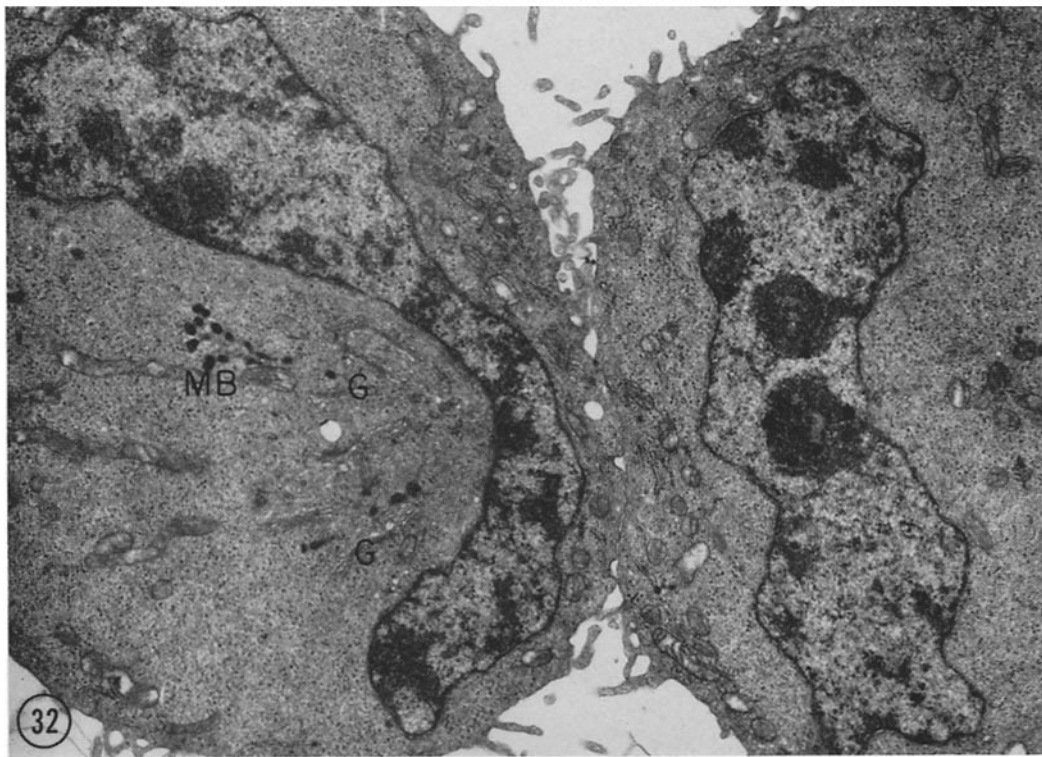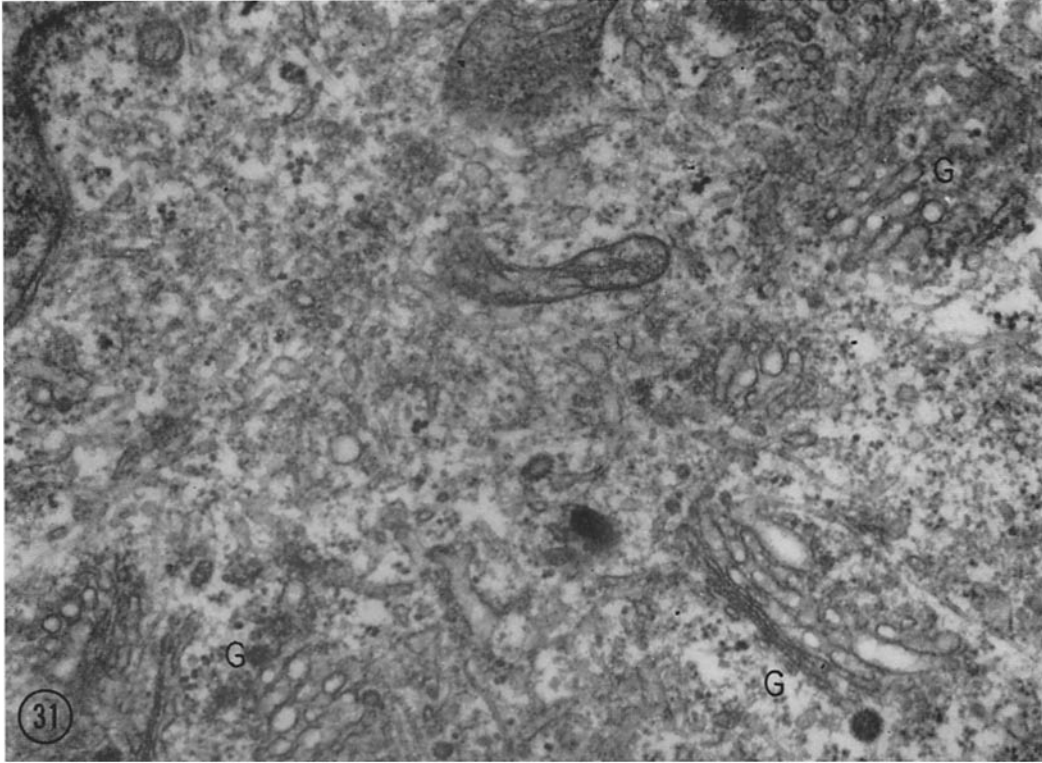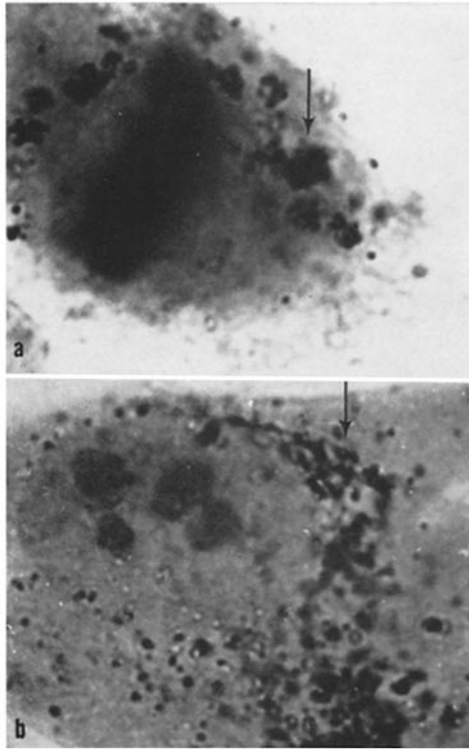
FIGURE 33 a Light micrograph of metaphase cell stained for acid phosphatase. Packets of acid-phosphatase positive granules are prominent (arrow). × 1,400.

FIGURE 33 b Light micrograph of interphase cell stained for acid phosphatase. Note that positive granules are usually singular (arrow) rather than in packets. × 1,400.
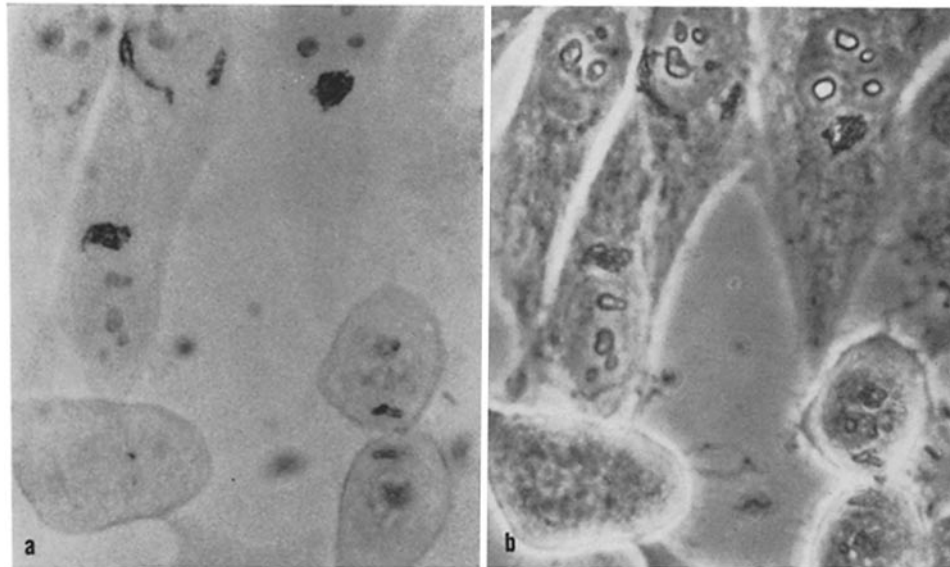

FIGURE 34 a Prophase cell stained for thiamine pyrophosphatase activity to localize the Golgi apparatus. Small specks of lead sulfide indicate remnant of enzyme activity. Surrounding interphase cells are positive for activity. × 560.

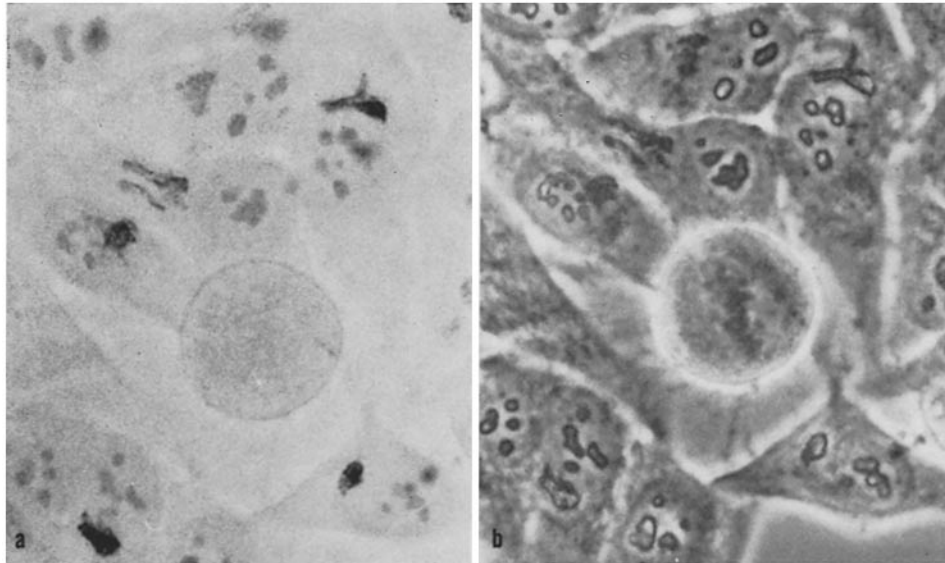FIGURE 34 b Same cells confirming that cell of interest is in prophase. Phase contrast. × 560.

FIGURE 35 a   Thiamine pyrophosphatase activity is completely absent from round metaphase cell. × 560.

FIGURE 35 b   Same cells showing metaphase cell more clearly. Phase contrast. × 560.

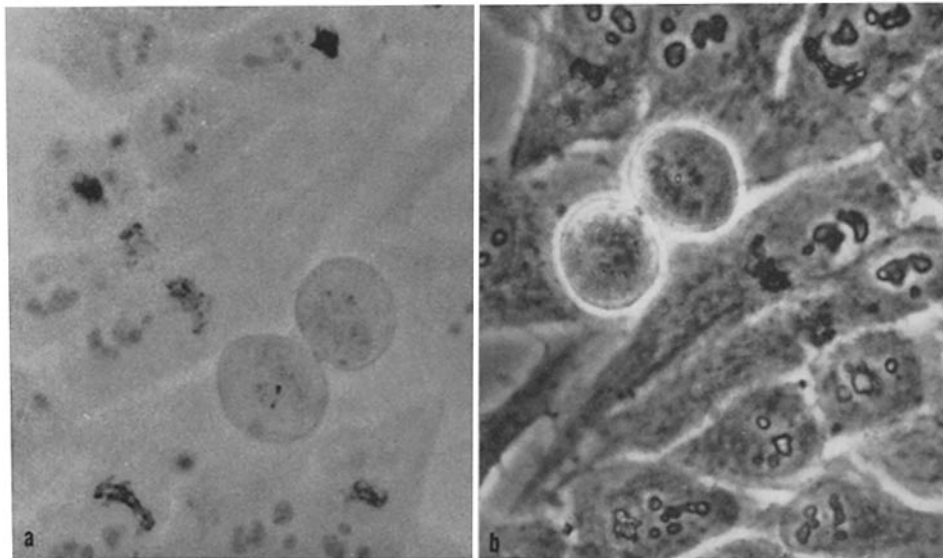FIGURE 36 a   Return of thiamine pyrophosphatase activity in early telophase cells. × 560.

FIGURE 36 b   Same cells. Phase contrast. × 560.