# STRUCTURAL VARIATIONS DURING MITOSIS IN THE CHICK EMBRYO

ALLAN L. ALLENSPACH and L. E. ROTH

From the Microscopy Laboratory, Department of Biochemistry and Biophysics, Iowa State University, Ames. Dr. Allenspach's present address is the Department of Zoology and Physiology, Miami University, Oxford, Ohio

ABSTRACT

Selected tissues from chick embryos were fixed in 2% glutaraldehyde and 1% $OsO_4$, both buffered at pH 7.6 with Veronal-acetate, and were embedded in Maraglas or Araldite. Two types of cell division have been noted. Generally, epithelial cells divide predominantly by a shortening of the chromosome-to-pole distance rather than by spindle elongation; mesenchymal cells undergo extensive spindle elongation. The presence of numerous continuous microtubules in cells that undergo extensive spindle elongation functionally implicates these tubules in the elongation process. In most embryonic epithelia, the cleavage furrow converges to a fixed site forming a mid-body near the anchoring desmosomes at the free surface; symmetrical furrow formation is typical of mesenchymal cells which lack desmosomes. The hypothesis of cleavage furrow formation and the fate of the mid-body that is formed during cytokinesis are discussed.

## INTRODUCTION

One of the phenomena commonly observed by developmental biologists is the accumulation of embryonic epithelial cells at various free surfaces during mitosis. The chick neural tube provides an excellent source of dividing cells of a "typical" embryonic epithelium. The classic and elegant work of Sauer (21) described mitosis in the neural tube and emphasized the role of the terminal web to anchor dividing cells. Our observations, which extend this accurate piece of work, are largely possible only because of superior preservation of structure and the resolution obtainable by electron microscopy.

Although the general problem of cell division has intrigued investigators for many years, numerous, recent papers on mitosis are accumulating in the literature. The primary source material for most of these reports has been the cultured cell (see references 1, 3, 19), although several references to dividing cells in embryonic epithelial tissue have appeared (5, 7). By extending our work to include mesenchymal cells, a number of significant differences can be compared quite directly. Mesenchymal cells, generally, are surrounded by large extracellular spaces and are not interconnected by desmosomal complexes, in contrast to the arrangement of epithelium. Comparison of mitotic apparatuses in epithelial and mesenchymal cells thus allows observations of differences in cleavage patterns, spindle lengths, and their morphology.

Briefly, the evidence presented suggests that desmosomes at epithelial-free surfaces are fixed sites which influence the cleavage pattern, that spatial confinement prevents spindle elongation, and that continuous spindle tubules may be involved in spindle elongation.

## MATERIALS AND METHODS

Tissues of White Leghorn chick embryos ranging from 2½ to 16 days of incubation (stages 16–42; reference 6) were used in this study. Selected tissues were dissected from whole embryos, immersed in 2% glutaraldehyde buffered at pH 7.6 with Veronal-acetate, and removed to fresh fixative for final trimming with microknives or tungsten needles (4). After a total fixation time of 15–40 min in glutaraldehyde, tissues were rinsed briefly in a buffer and postfixed for 1 hr in 1% $OsO_4$ similarly buffered. Since selective dissection greatly facilitated electron microscope observations, blocks of tissues 0.3–0.5 mm in diameter were used routinely. Precise localization of dividing epithelial cells was accomplished by repeated check of thick sections by phase-contrast microscopy. The epithelium-associated mesenchymal cells provided the material for studies on mesenchymal mitosis.

Two embedding media were used in the study. Dehydration in ethyl alcohol and propylene oxide preceded embedment in Araldite (11). Tissues embedded in Maraglas were dehydrated in acetone.

Sections showing pale gold-to-gray interference colors were cut on the Porter-Blum MT-1, LKB, or Reichert microtomes, by using either glass or diamond knives, and mounted on uncoated 300-mesh or parlodion-coated 200-mesh grids. Sections were stained with both uranyl acetate (24) and lead citrate (18).

Observations were made with an RCA EMU-3F electron microscope, operated at 50 or 100 kv, equipped with 10-mil and 30-$\mu$ condenser and objective apertures, respectively. Photographs were taken at direct magnifications up to 24,000.

## OBSERVATIONS

Our initial plans were to restrict our study to the neural tube since extremely large numbers of dividing cells are located along the neural canal, but interesting and striking differences in other embryonic tissues prompted us to extend the work. The following observations are the result of studies of neural, esophageal, and tracheal epithelia and their associated mesenchymes. Although the neural epithelium received the most attention and is described extensively in this paper, the mitotic phenomena have been confirmed in other epithelia.

### *Interphase*

The oval-shaped, interphase cell body in the embryonic neural tube is located in the mantle layer and extends to the neurocoel as a long, narrow, cytoplasmic process that contains numerous microtubules. Properly oriented sections confirm this observation, which was reported many years ago by Sauer (21). This slender cell process (Fig. 1, *P*) is attached to adjacent cells by dense desmosomes (Figs. 1 and 2, *D*) which are seen only near the luminal surface.

The large oval nucleus (Figs. 1 and 2, *N*) with its typically regular, smooth envelope occupies most of the cell body in neural epithelia. The nucleoli are discrete, irregularly shaped bodies that are granular around their periphery (Fig. 2, *Nu*) and lie in a homogeneous nucleoplasm of dense, finely textured material which includes chromatin and its surrounding matrix (Fig. 2, *N*). Interphase nuclei of mesenchymal cells have identical features.

The cytoplasm of interphase cells in neural epithelium contains both microvesicular and lamellate Golgi components (Fig. 2, *G*) in the perinuclear zone, usually on the canal side (Fig. 1, *G*). Mitochondria, some of typical morphology and others with internal lamellar whorls (Fig. 2, *M*), are located either in the cell body or in the narrow cytoplasmic process subjacent to the free surface. Centrioles, observed either singly or in obliquely oriented pairs, commonly are located beneath the free surface and frequently serve both as basal bodies from which cilia sprout at the free surface (Fig. 1, *Ce*) of this and other embryonic epithelia and as structures from which cytoplasmic microtubules radiate. With the exception of a few, small, vesicular profiles with attached ribosomes, the cytoplasm contains free ribosomes in polysome configurations (Fig. 2). There is little well-developed rough-surfaced endoplasmic reticulum (RER) in 3 day chick-embryo neural epithelium. For further descriptions on cytoplasmic differentiations in neuroblasts, the reader is referred to the excellent paper by Lyser (12).

The cytoplasm of the mesenchymal interphase cell differs somewhat from that in epithelial cells. In addition to free ribosomes, it may have a well-developed RER, particularly in older mesenchyme, which undoubtedly synthesizes materials for extracellular use.

### *Prophase*

The prophase cell body in neural epithelium always is located in the mantle layer, an observation substantiated repeatedly in radioautographic studies (9, 14, 22, 23). Prophase nuclei have condensations of coarsely granular material

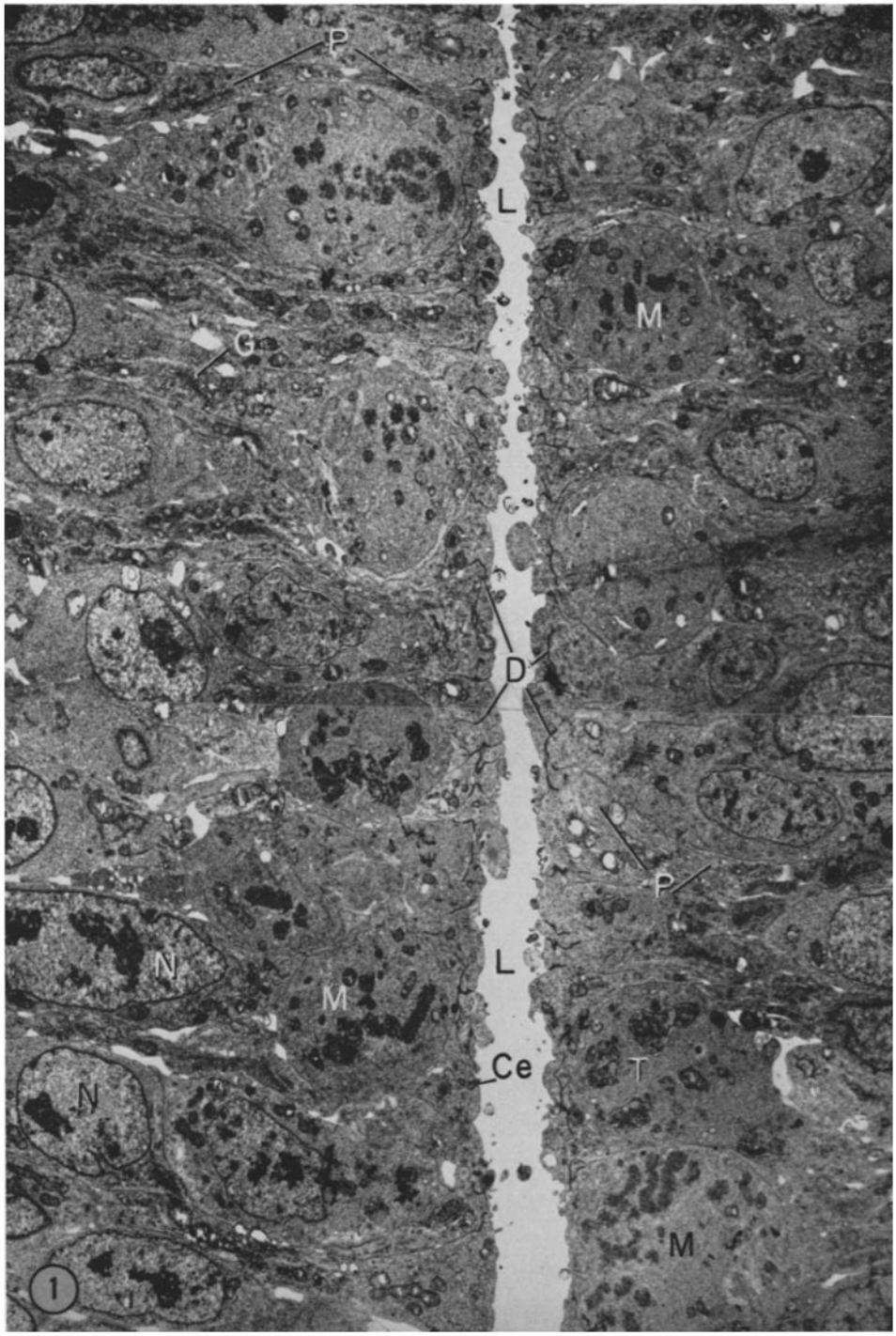



FIGURE 1 Survey montage of the neutral tube including about 75 $\mu$ of the lumen (*L*). The orientation is a frontal section with the vertical dimension of the micrograph coinciding with the anterior-posterior axis of the embryo. Desmosomes (*D*) are present only near the luminal surface, and centrioles (*Ce*), some of which are now basal bodies of short cilia, are usually at this surface. Cells migrate to and divide at the luminal surface by using long, thin cytoplasmic processes (*P*). The result is that only cells in or near division are located at the lumen; 12 dividing cells are shown, including those in metaphase (*Me*) and telophase (*T*). *N*, interphase nucleus; *G*, golgi material. × 4000.

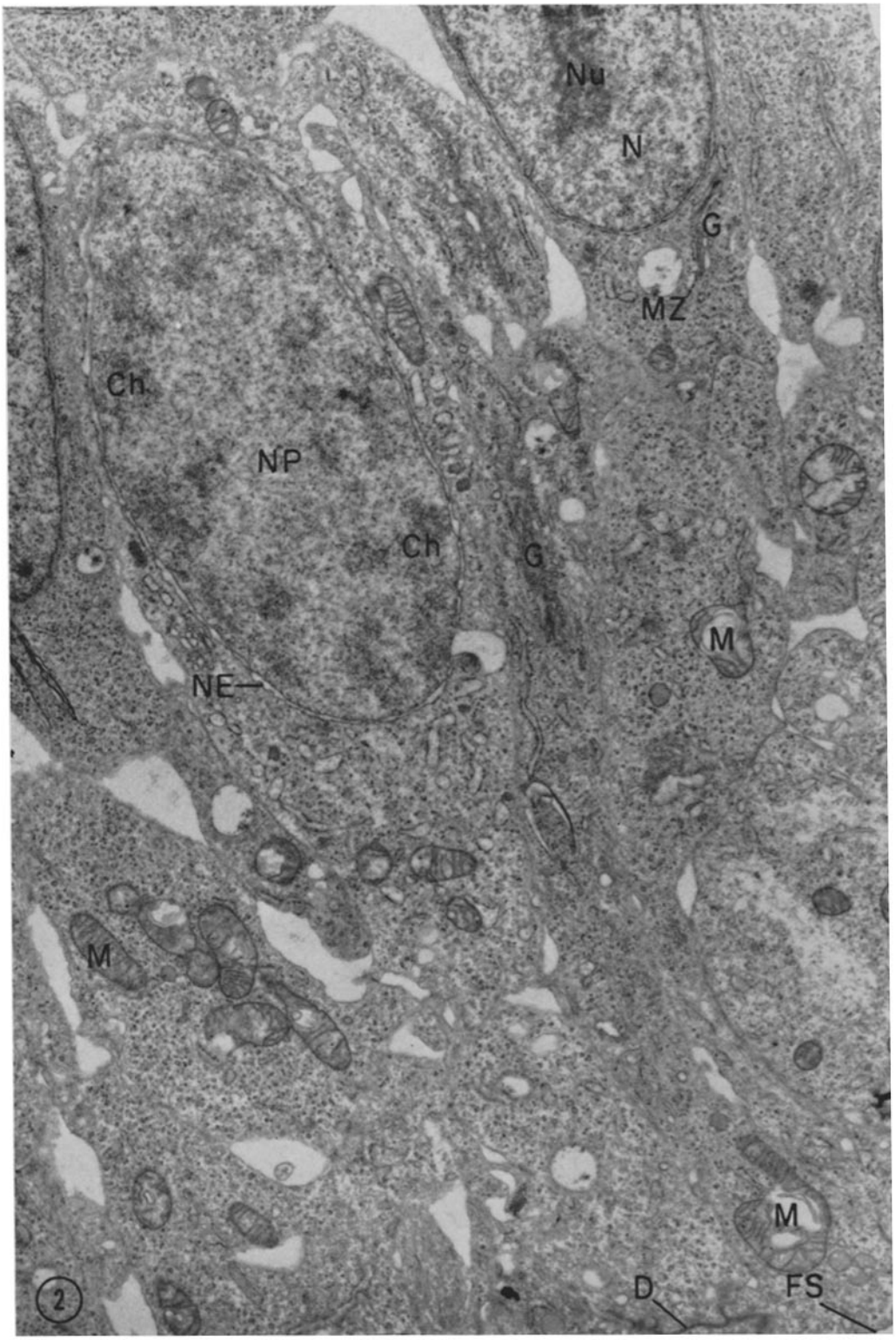



FIGURE 2 A portion of the neural tube wall showing the free surface (*FS*) and the mantle zone (*MZ*). Prophase nuclei (*NP*) have condensed chromatin (*Ch*) and an irregular nuclear envelope (*NE*), and the interphase nucleus (*N*) has a typical nucleolus (*Nu*) and nuclear envelope. Golgi material (*G*) and mitochondria (*M*), the latter with vesicular inclusions, are frequent. *D*, desmosome. × 9000.

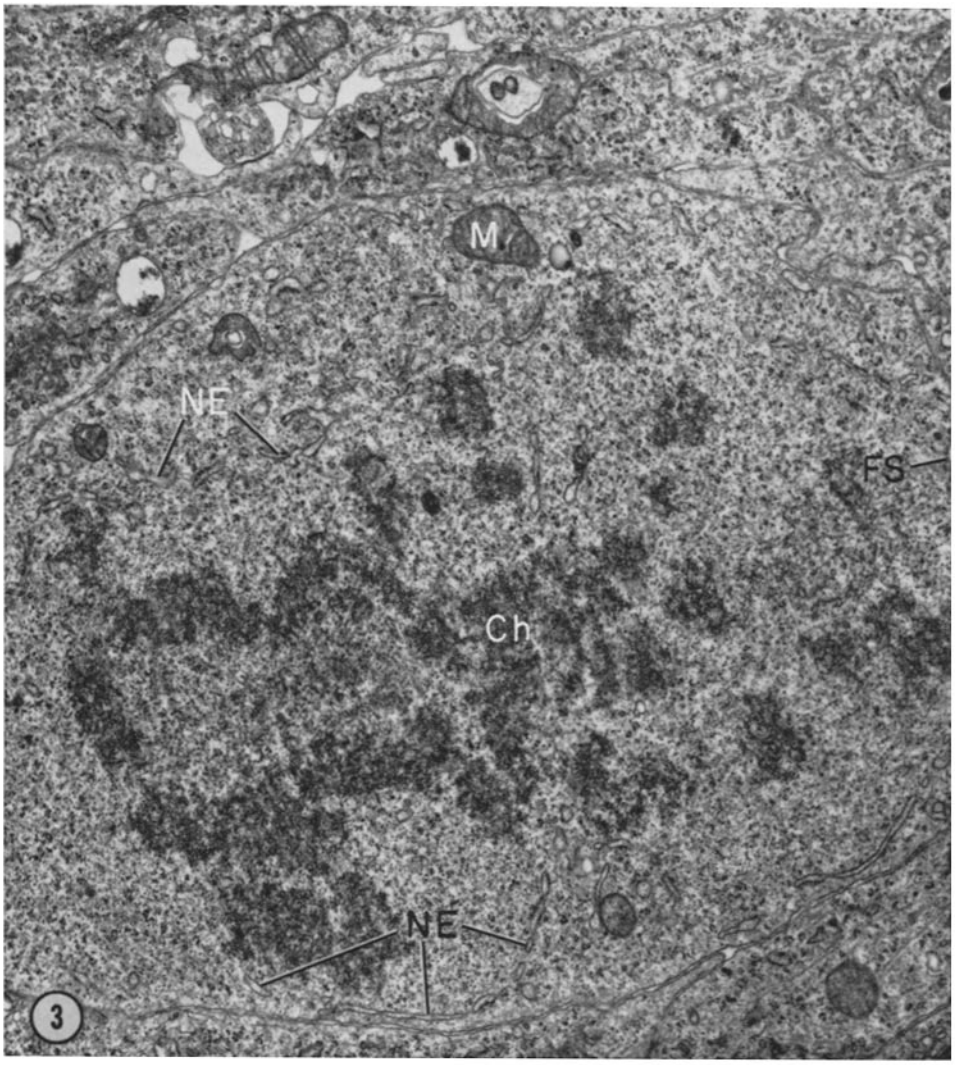



FIGURE 3 Prometaphase cell in the neural tube. Remnants of the nuclear envelope (*NE*) circumscribe the condensed chromosomes (*Ch*), and cytoplasmic organelles such as mitochondria (*M*) have been peripherally displaced. The spherically shaped cell occupies a position on the free surface (*FS*). × 15,500.

either scattered throughout the nucleus or closely apposed to the inner nuclear membrane (Fig. 2, *Ch*). Epithelial prophase nuclei remain oval during prophase, whereas mesenchymal prophase nuclei are consistently lobulated. In both types of cells, the two nuclear membranes characteristically become separated irregularly (Fig. 2, *NE*), and have numerous, obvious pores before the impending breakdown at prometaphase. Prophase cells in mesenchyme are distributed randomly, but those in epithelia are close to the luminal surfaces.

No marked change in cytoplasmic morphology has been observed at prophase. Many small vesicles, apparently Golgi bodies, are scattered indiscriminately throughout the cytoplasm of mesenchymal cells. Occasionally, a centriole and associated cytoplasmic tubules are observed between the nucleus and the free surface in epithelial cells.

### *Prometaphase*

With progressive condensation of chromatin and organization of the mitotic spindle, the epithelial cell assumes a spherical shape and is located at the free surface. Prometaphase cells are recognized by the breakdown of the nuclear envelope into many smooth-surfaced vesicles (Fig. 3, *NE*) which are scattered uniformly over the periphery of the cell and, immediately after fragmentation, form a circular profile around the cluster of chromosomes (Fig. 3, *Ch*). The envelope fragments first occupy a cortical position (Fig. 3, *NE*), but eventually they mix indiscriminately and indistinguishably with other smooth-surfaced profiles. The numerous dense chromosomes take their position at the equatorial plate, which occupies almost the entire diameter of the cell, and force the organelles to poleward cortical positions. Occasionally small vesicular profiles and simple membranous whorls are trapped within the mitotic apparatus (MA), but mitochondria and smooth endoplasmic reticulum (SER) are relegated to peripheral positions (Fig. 3). Microtubules first are observed in prometaphase after nuclear envelope breakdown but before chromosomes become aligned at the equatorial plate.

### *Metaphase*

Only those mitotic figures showing convergent spindle tubules at both ends of the equatorial plate were considered to be in metaphase. Precise identification of this stage became necessary since it was observed that epithelial cells, for the most part, lack or contain very few microtubules in the interzone, but mesenchymal MA contain larger quantities of tubules in the interzone. Thus, careless observation of a chromosomal plate with convergent microtubules on one side and parallel microtubules on the other side of the chromosomes could lead to erroneous interpretations. The actual number of metaphase cells observed was limited because of the short duration of the stage and the scarcity of precisely oriented cells.

Metaphase cells in most embryonic epithelia characteristically become spherical and occupy a position at the free surface, to which they are bound by desmosomes (Fig. 1, *D*). Mesenchymal cells, on the other hand, need not round up, but often have generous amounts of polar cytoplasm in which are found numerous, irregularly shaped vesicles (Fig. 4). Many of these profiles are intracellular vacuoles, but the possibility that cortical profiles are invaginations in the cell surface cannot be ignored.

In their morphology, the MA's in the two cells are essentially identical at metaphase. The spindle tubules converge toward and terminate in a densely osmiophilic pericentriolar zone; we have been unable to observe connections of the tubules with the centriole (Figs. 4 and 6, *Ce*). Cross-sections of mesenchymal metaphase plates reveal large numbers of microtubular cross-sections, usually in small groups of twelve or less (Fig. 5, *Mt*). They usually are embedded in amorphous, ground substance, although tubules surrounded by small clear halos have been observed at the periphery of chromatin masses. Our present methods fail to reveal any new details in the chromosomes.

The cytoplasmic organelles are displaced to the cortex of the cell by the MA. Whereas only a few, very small vesicles are observed within the MA (Fig. 4), mitochondria, electron-opaque lysosome-like bodies, and large smooth-surfaced vesicles consistently lie outside the MA. Ribosomes are abundant in the MA, but polysomal configurations usually can be seen only in rather peripheral positions. Fragments of RER have been observed in dividing mesenchymal cells of 8-day embryos, but Golgi components have not been observed with certainty at metaphase.

### *Anaphase*

The earliest detectable separation of chromosomes signifies the onset of anaphase (Fig. 7). It is in this stage that we see striking differences between epithelial and mesenchymal cells.

The epithelial cell generally remains virtually spherical through anaphase because of the spatial confinement by juxtaposed cells; the mitotic spindle always is aligned parallel to the free surface. Most mesenchymal cells, on the other hand, are not confined spatially, but have variable, elongated shapes.

One of the most striking differences between epithelial and mesenchymal cells is that, in the latter cells, there is an extensive increase in pole-to-pole length during anaphase (Figs. 10 and 11), whereas in the former cells chromosome separation is accomplished predominantly by shortening of the chromosome-to-pole distance, not by pole-to-pole elongation. Selected measurements of

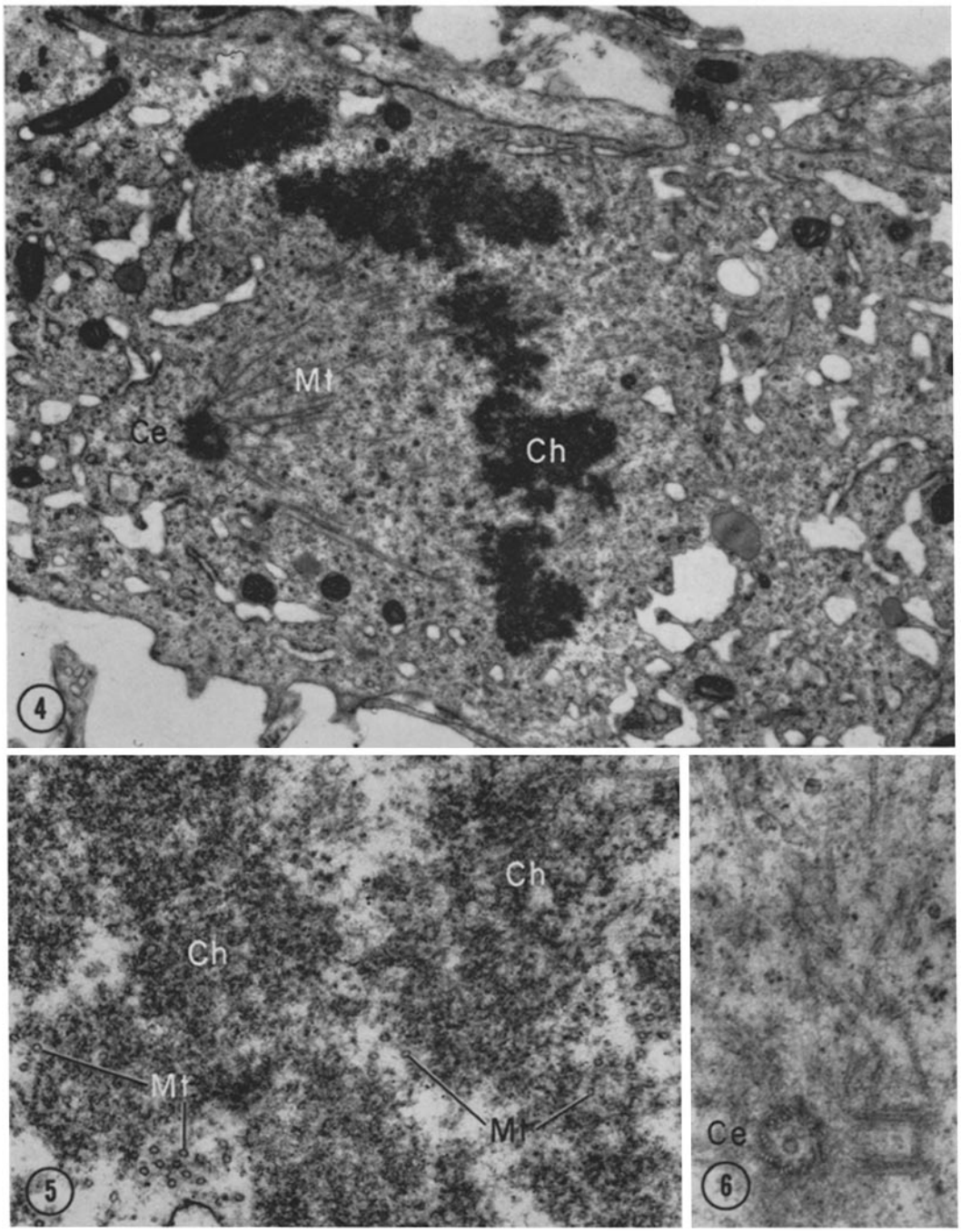



FIGURE 4 A low magnification view of a mesenchymal cell at metaphase. Spindle tubules (*Mt*) converge toward and terminate in the dense pericentriolar zone on one side and in chromosomes (*Ch*) on the other. Numerous vesicles appear in the cortical cytoplasm. *Ce*, centriole. × 15,000.

FIGURE 5 A micrograph of a transversely sectioned equatorial plate at metaphase. Chromosomes (*Ch*) are densely granular aggregates between which can be seen numerous spindle microtubules in small bundles (*Mt*). × 62,000.

FIGURE 6 A pair of centrioles (*Ce*), perpendicularly oriented, with short fragments of spindle tubules extending radially. × 46,500.

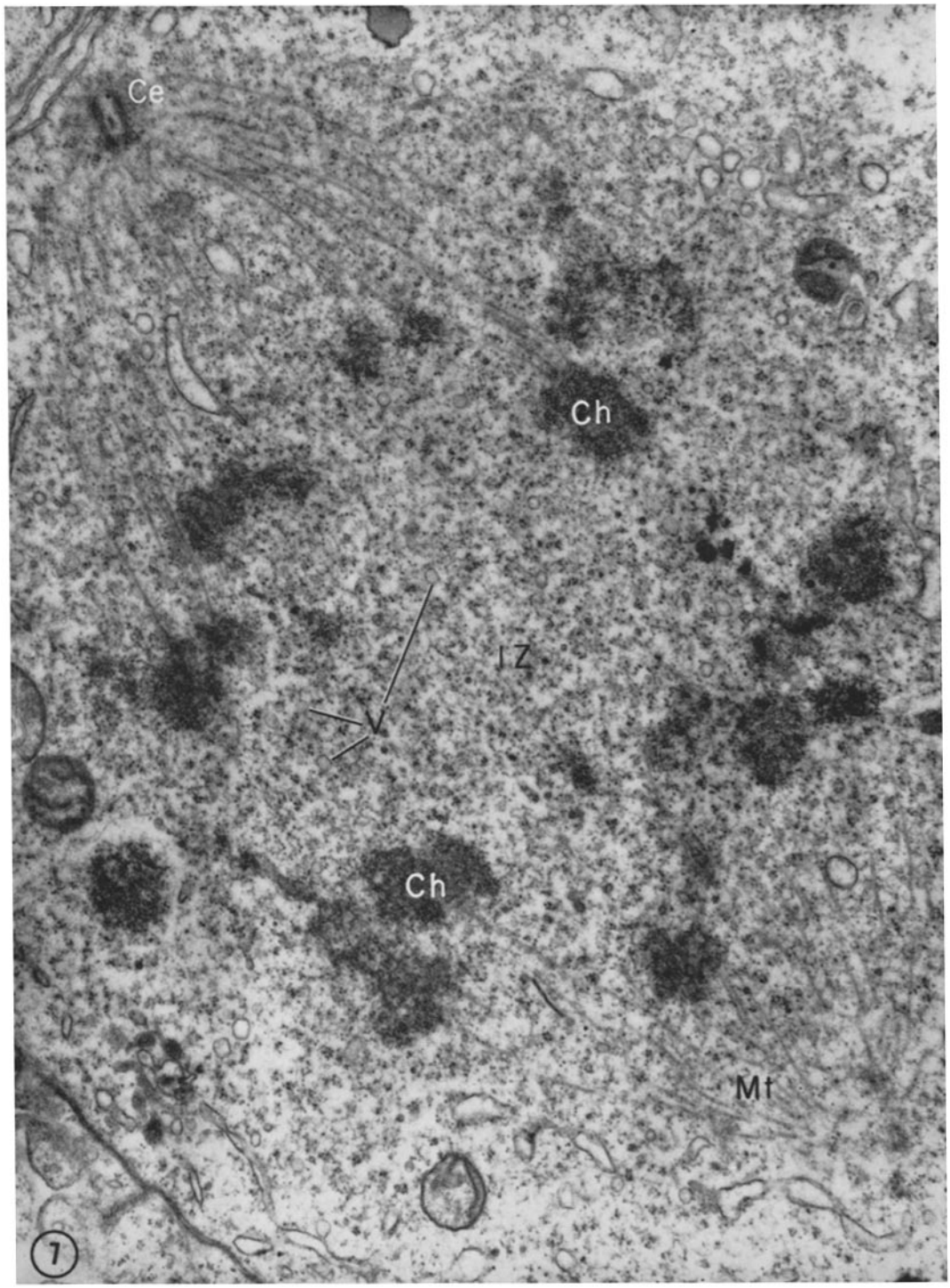



FIGURE 7 A full section of a typically spherical mid-anaphase cell adjacent to the neural canal. No continuous spindle tubules but numerous vesicles (*V*) are usually seen in the interzone (*IZ*) in these cells which are spatially confined and divide without spindle elongation. The chromosomes (*Ch*) have many microtubules (*Mt*) extending poleward to centrioles (*Ce*) that are very near the plasma membrane. × 21,500.

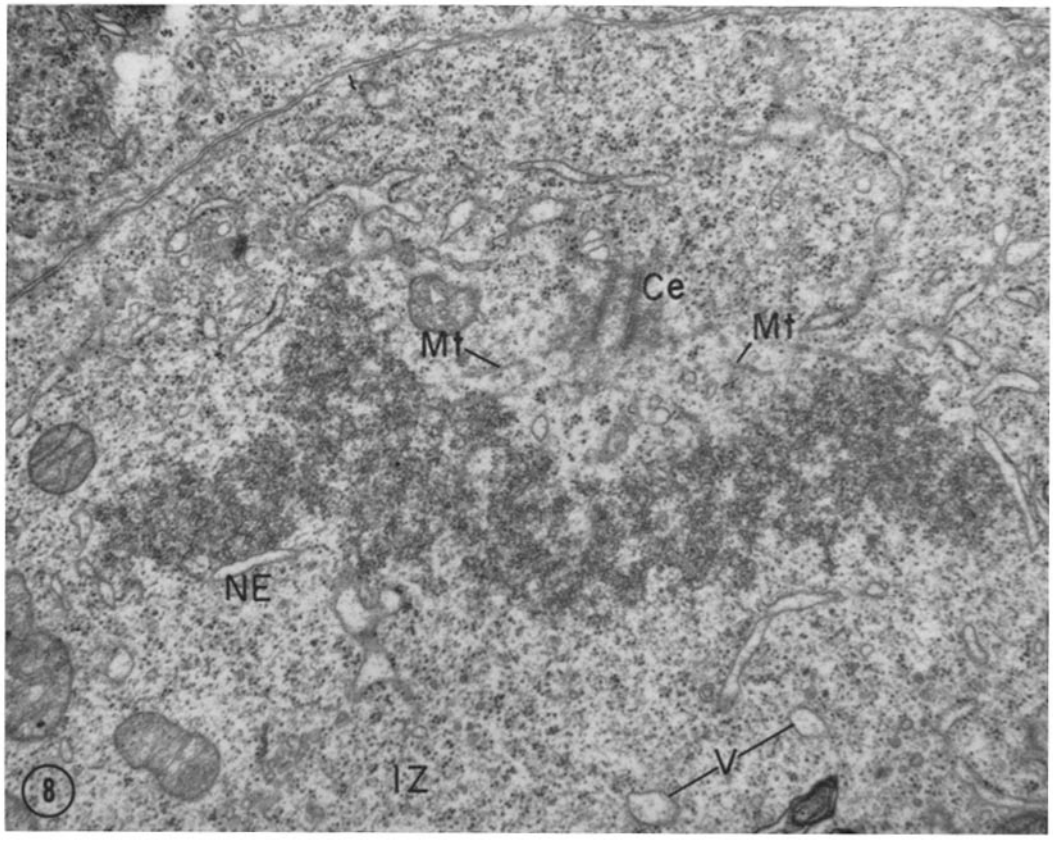



FIGURE 8 A portion of a late anaphase spindle in the neural tube. Chromosome movement toward the centriole (*Ce*) is completed, and short fragments of microtublues (*Mt*) remain in the pericentriolar zone. The first signs of nuclear envelope (*NE*) formation are visible; small vesicles are present (*V*). *IZ*, interzone. × 29,500.

anaphase spindles indicate a final length of about 5 $\mu$ for epithelial cells and 8–10 $\mu$ for mesenchymal cells.

Correlated with the changes in cell shape are differences in numbers of continuous spindle tubules. Epithelial cells have many chromosome-to-pole tubules (Figs. 7–9, *Mt*), but few continuous tubules can be seen in the interzone in the micrographs we have examined (Figs. 7–9, *IZ*). The interzone is a composite of granular and amorphous substance with interspersed sparsely populated microvesicles of about 50–100 m$\mu$ in diameter (Figs. 7–9, *V*).

Mesenchymal cells are marked by the presence of numerous, continuous spindle tubules, in addition to the chromosome-to-pole tubules. The continuous tubules, which are identical in morphology to the chromosomal tubules, are identified more positively in late anaphase cells (see Fig. 13).

In their morphology the spindle tubules in all chick embryo cells observed appear to be identical. Fortuitous sections show some spindle tubules spanning the distance from chromosomes to pole (Fig. 7). The spindle tubules vary from 17 to 26 m$\mu$ in diameter, with the majority measuring the higher figure. The spindle tubules converge to and terminate in the pericentriolar zone, an area up to about 300 m$\mu$ in diameter.

Important differences in chromosome movements at late anaphase deserve special consideration. Separation of chromosomes in most epithelial cells is primarily the result of a shortening of the chromosome-to-pole distance. The centrioles (Figs. 7–9, *Ce*) move only short distances and are seen very near the plasma membrane.

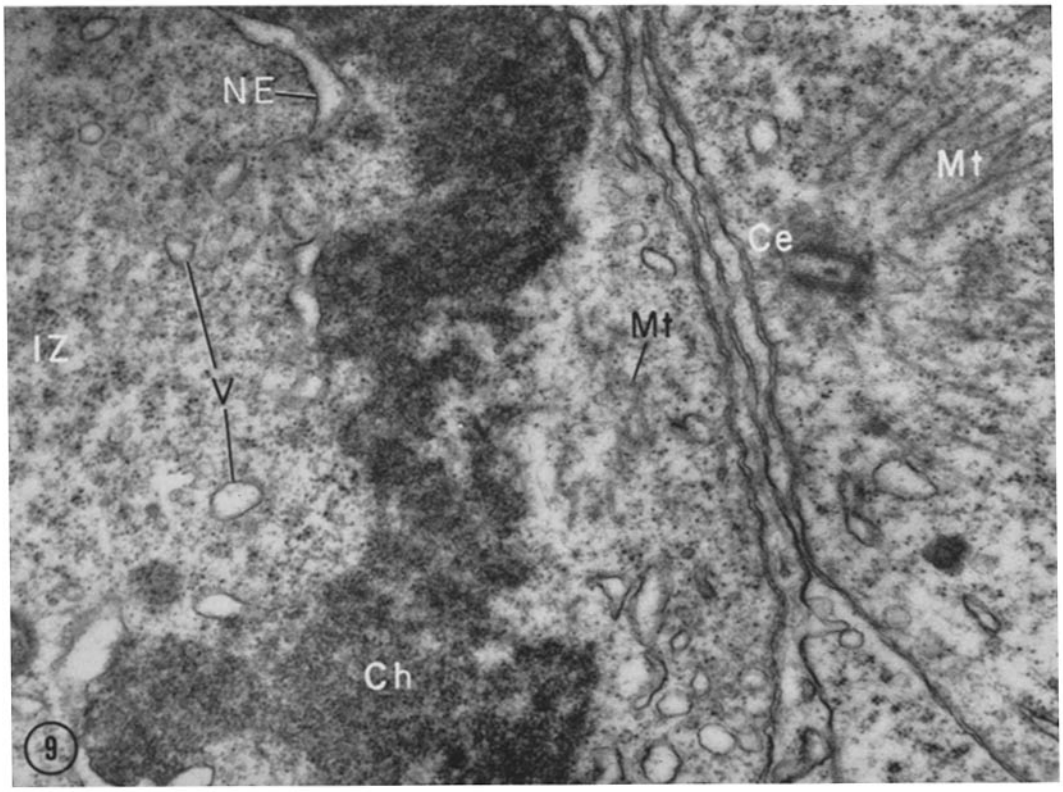
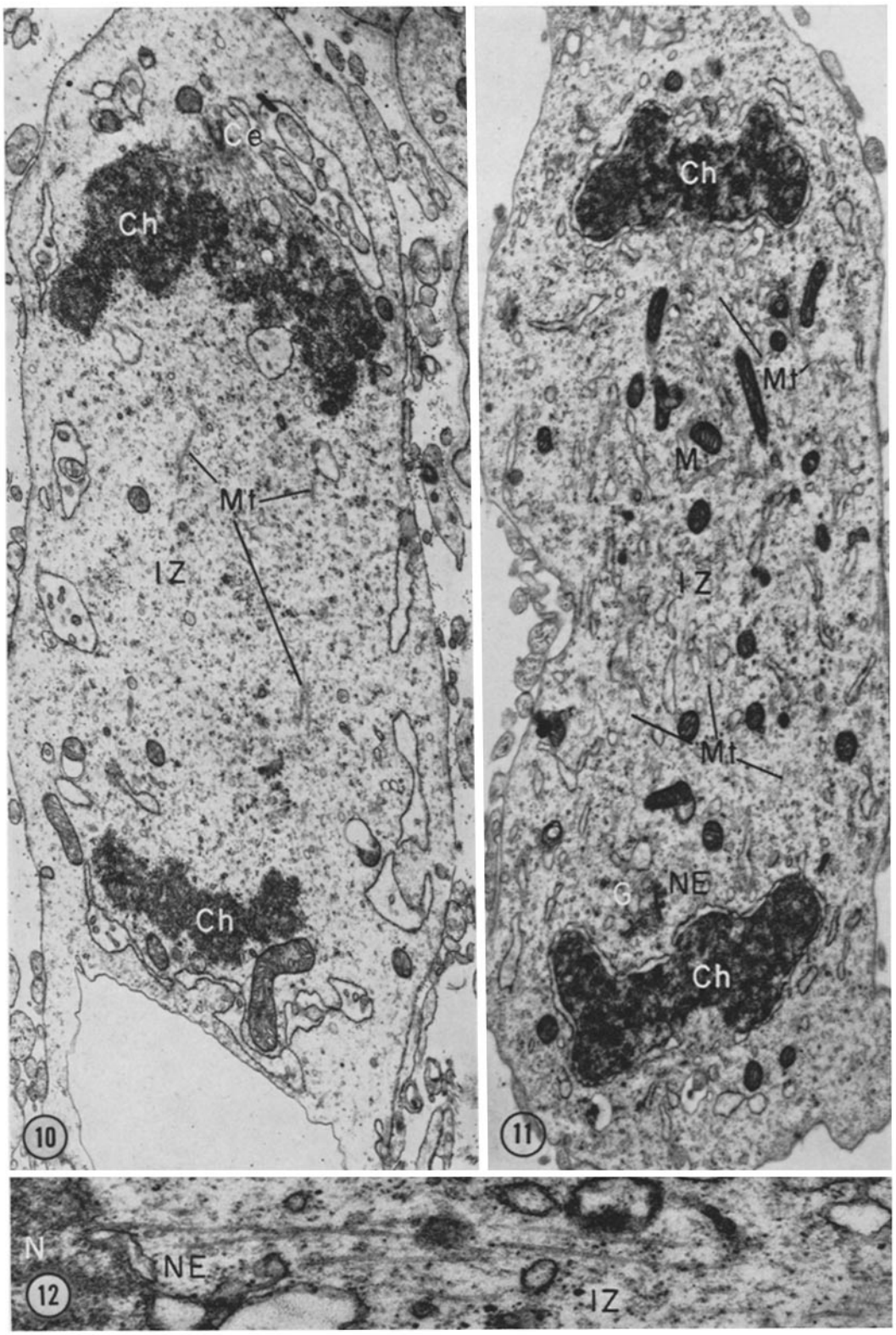



FIGURE 9 A high magnification view of centrosomal regions in adjacent dividing epithelial cells; the centriole at right is an enlargement of that in Fig. 6. This micrograph demonstrates the similar morphology of spindle tubules (*Mt*) in mid and late anaphase. No spindle tubules are observed in the interzone (*IZ*). The nuclear envelope (*NE*) is being reformed by vesicle fusion at the chromatin (*Ch*) surfaces. *V*, vesicles; *Ce*, centriole. × 26,500.

From selected measurements it is estimated that the pole-to-pole distance in epithelial cells increases by about 1.4 times. Conversely, reduction of the chromosome-to-pole distance and remnants of the spindle are apparent in epithelial cells; poleward movement of the chromosomes is virtually complete, and small fragments of the spindle tubules are observed (Figs. 8 and 9, *Mt*).

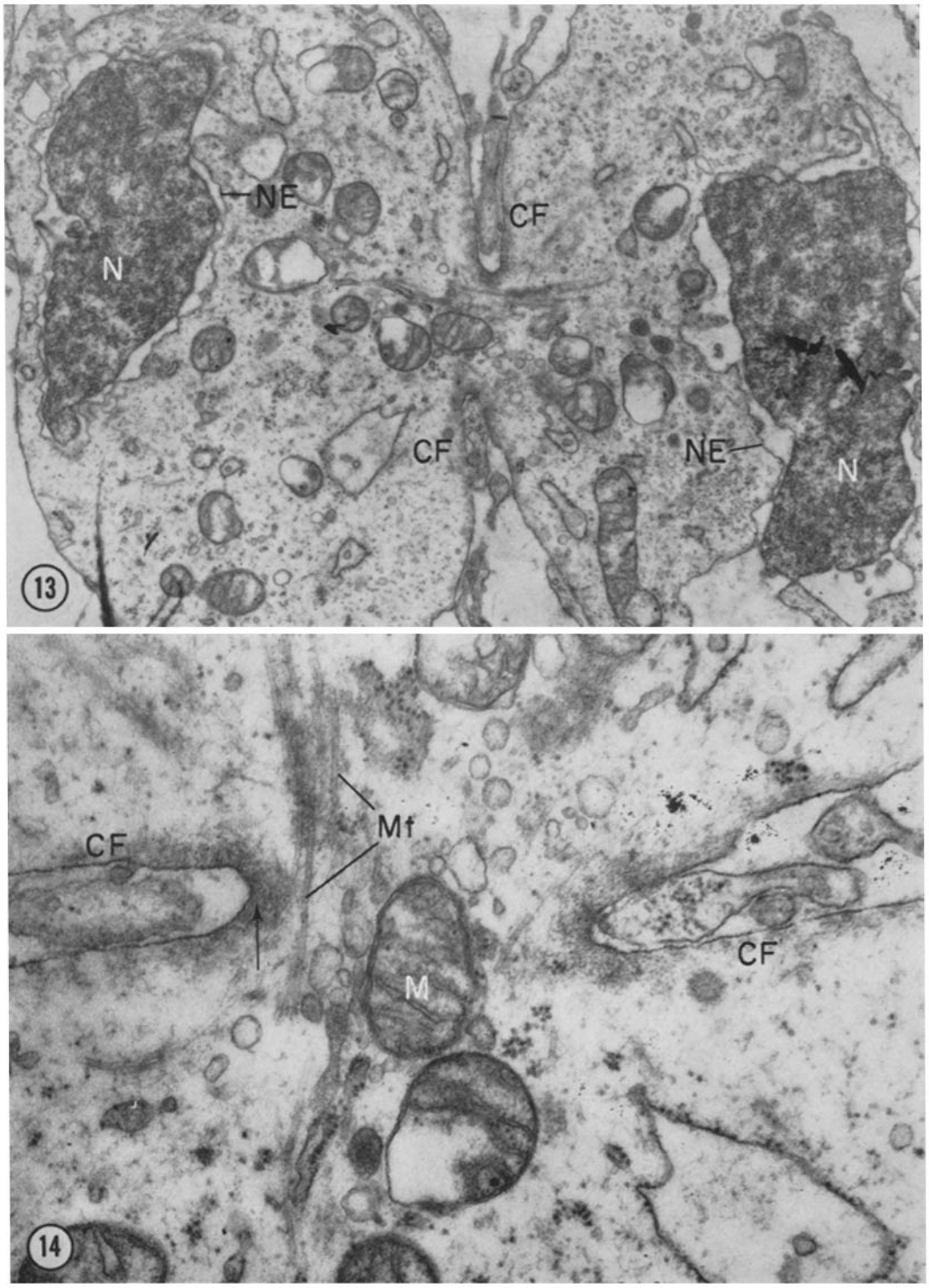
By contrast, the separation of chromosomes in mesenchymal cells is apparently the result not only of shortening of the chromosome-to-pole distance, *but also of a substantial increase* in the pole-to-pole distance. Pole-to-pole measurements of selected metaphase and anaphase cells indicate an increase in spindle length of approximately four times. It is noteworthy that most mesenchymal cells in early chick embryos are surrounded by vast extracellular spaces; the cell in Fig. 10 contacts a basement membrane, but the one in Fig. 11 has little surface contact with adjacent cells. Both cells have undergone extensive elongation. Furthermore, the interzone of the dividing mesenchymal cell contains numerous, continuous spindle tubules. These microtubules can be observed at low magnification in the interzones (Figs. 10–12, *Mt*). Microtubules also are present poleward at this time but are short and demonstrate that chromosome-to-pole shortening also has occurred (Fig. 10). The chromatin is covered closely by the reconstituting nuclear envelope, and numerous organelles enter progressively the interzone as constriction begins (Figs. 10 and 11).

Some similarities in morphology of the two types of cells may be noted. To enumerate, the chromosomal microtubules, both chromosomal




FIGURES 10 and 11 Survey electron micrographs of late anaphase in mesenchymal cells. Chromosomes (*Ch*) have moved toward the poles, so that the centriole (*Ce*)-to-chromosome distance is reduced. Numerous microtubules (*Mt*) are observed in the interzone (*IZ*) of these cells which show extensive spindle elongation. The nuclear envelope (*Ne*) is well formed in Fig. 11. *M*, mitochondrion; *G*, Golgi zone. Fig. 10, × 14,500; Fig. 11, × 12,500.

FIGURE 12 A high magnification electron micrograph of the nucleus (*N*) and interzone (*IZ*) of a late anaphase mesenchymal cell. Microtubules are commonly observed coursing through the chromatin and interzone. *NE*, nuclear envelope. × 50,500.




FIGURES 13 and 14 Electron micrographs of late telophase in embryonic mesenchyme. Nuclear envelopes (*NE*) are now continuous, but typically irregular. Microtubules (*Mt*) are being concentrated in the mid-body which also contains mitochondria (*M*) and ribosomes. Dense, amorphous material (arrow in Fig. 14) is observed on the leading edge of the cleavage furrow (*CF*) which characteristically has small processes included extracellularly. *N*, nucleus. Fig. 13, × 17,000; Fig. 14, × 52,000.

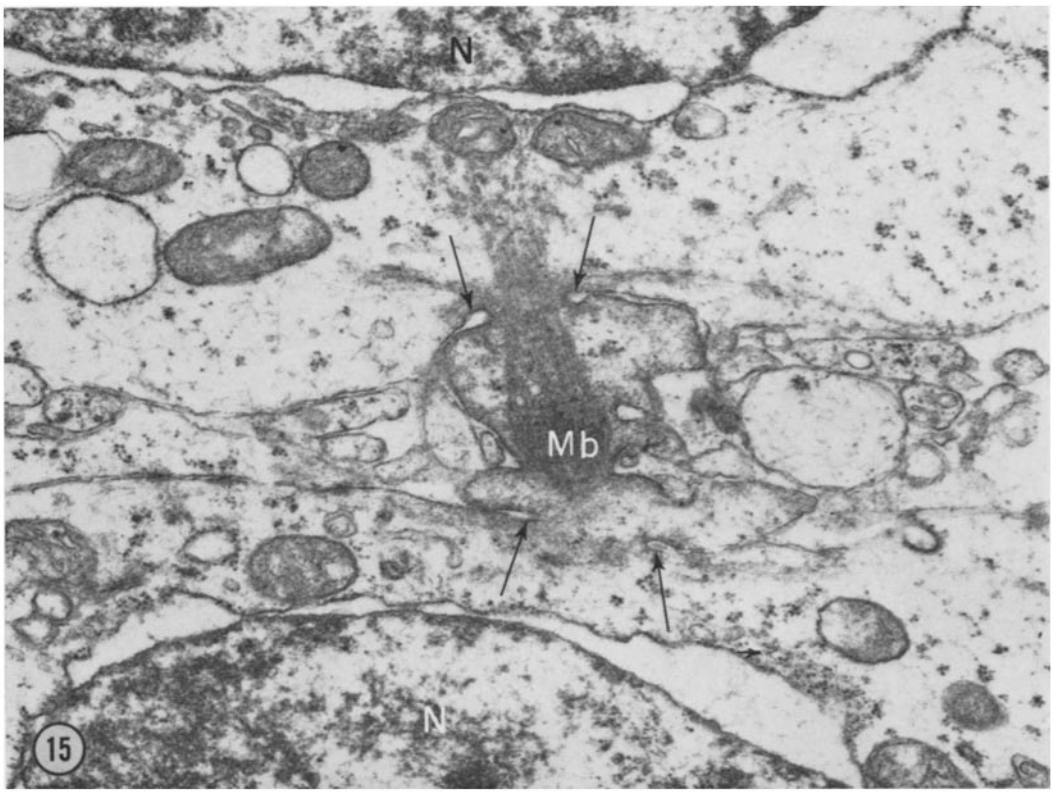


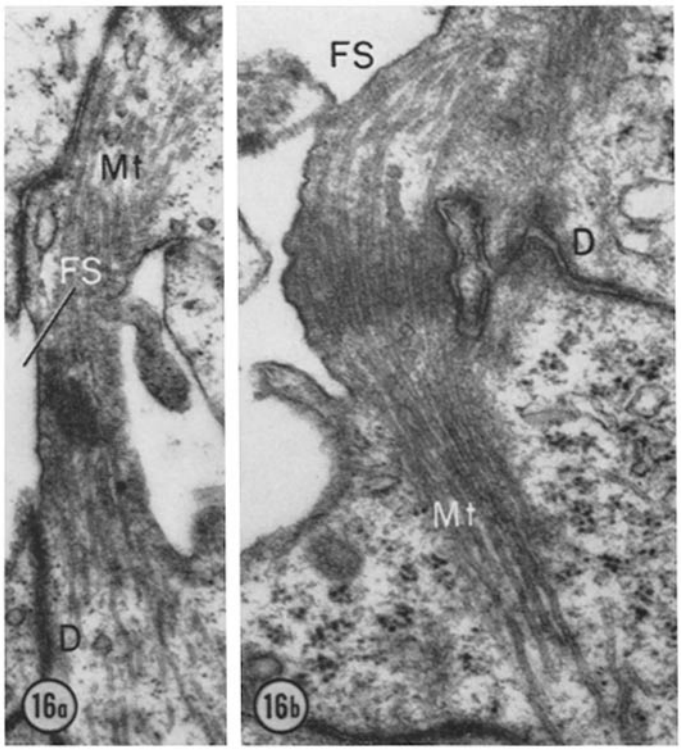

FIGURE 15 Telophase in a mesenchymal cell. Microtubules are concentrated into a mid-body (*Mb*) by the cleavage furrow (arrows) which now seems to be separated into two parts. Daughter nuclei (*N*) have continuous but irregular envelopes. × 20,500.

and continuous, are identical in morphology. Also, reconstitution of the nuclear envelope appears to be similar; that is, by vesicular fusion starting on the equatorial side (Figs. 8, 9, and 11), the nuclear membranes gradually circumscribe the entire chromatin mass and form lastly in the centriolar area (Fig. 11).

### *Telophase*

Those cells in which the nuclear envelope is reconstituted fully and in which cleavage furrows form have been classified as telophase cells. Important differences in the formation of the cleavage furrow have been observed. Furrowing in those epithelial cells in contact with the free surface is asymmetrical; that is, the furrow converges through the equatorial zone from the region of the mantle zone toward a fixed site on the lumen of the neural canal; the free surface does not participate in the furrowing process. The result is a spectacle-shaped pair of cells with a reduced cytoplasmic connection near their free surfaces. The remaining cytoplasmic connection is referred to as the mid-body. There is no indicative ultrastructural change in the equatorial cytoplasm during furrowing.

Cytokinesis in mesenchymal cells is usually symmetrical, with the cleavage furrow constricting nearly equally from all sides (Fig. 13). Numerous cytoplasmic processes, presumably from neighboring cells, typically occupy the furrow space. Within the narrow cytoplasmic connection, a number of microtubules, smooth-surfaced vesicles, ribosomes, and mitochondria can be seen (Fig. 14). At a more advanced stage of furrow formation, the tubules lose their identity in the dense mid-body but can be traced into the cytoplasm as separate entities (Fig. 15). The cytoplasm beneath the plasma membrane at the leading edge of the furrow is more dense than medullary




FIGURES 16 *a* and *b* Longitudinal views of mid-bodies typical of embryonic epithelia. Some microtubules (*Mt*) are seen compacted into the structure which lies at the free surface (*FS*) and near desmosomes (*D*). Fig. 16 *a*, × 55,500; 16 *b*, × 36,500.

cytoplasm (Fig. 14, *CF*), and, upon very close observation, circular profiles can be seen within the dense, amorphous substance on the advancing front of the furrow (Fig. 14, arrow).

By virtue of its formation, the cleavage furrow concentrates certain interzonal structures in the mid-body. Figs. 16 *a* and *b* illustrate, in longitudinal view, mid-bodies of epithelial cells at the free surface. The mid-bodies contain a small number of compressed microtubules that are located near the desmosomes concentrated at the lumen of the neural canal. Cross-sections of the mid-bodies reveal closely arranged circular profiles. Not all the microtubules are well preserved, but it is estimated that there are about 100–150 continuous tubules in the mid-body of the neural epithelial cell (Fig. 17). Cross-sections of mid-bodies of mesenchymal cells have not been observed.

During cleavage furrow formation, the plasma membrane is impeded by and flattens out alongside the densely organized mid-body. Fig. 16 *a* illustrates the transient checking of a cleavage furrow in an epithelial cell. A similar phenomenon is observed in mesenchymal cells (Fig. 15) where the plasma membrane has been diverted along the length of the mid-body in both directions. The dense material previously located in one zone under the plasma membrane now is separated into two zones (Fig. 15, arrows).

The nuclear morphology of telophase cells is characterized by virtually complete formation of the nuclear envelope (Fig. 13). The inner and outer nuclear membranes are separated by large irregular spaces which diminish progressively. Concomitantly, the chromatin begins to disperse and changes the nucleus from a dense chromatin mass (Fig. 13) to a diffuse combination of granular chromatin and amorphous nucleoplasm (Fig. 15).

Identification of newly formed cells is quite certain in epithelia because of the orientation of the nuclei and more rigidly controlled changes in cell shapes. There are, however, particular,

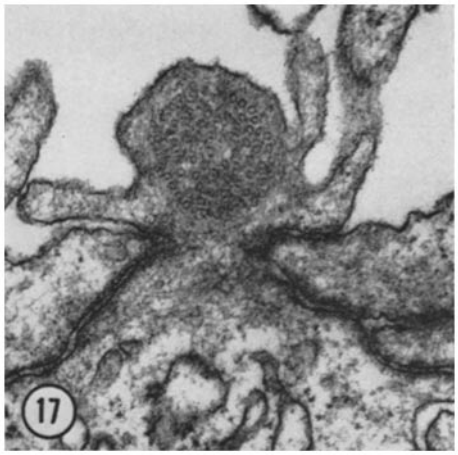


FIGURE 17 Transverse section of a mid-body like that in Fig. 16, showing the microtubules surrounded by dense material. × 46,500.

identifiable, ultrastructural features of daughter cells. The nuclear envelope of the daughter cell gradually is characterized by irregular, nonparallel arrangements of its inner and outer membranes. Whether this is an in vivo effect or an artifact has not been determined, but in this study the feature is constant for telophase cells. The outer membrane is frequently coated with ribosomes, and points of fusion of inner and outer membranes possibly indicate the presence of pores (Fig. 18).

The chromatin material within the envelope continues to disperse. The daughter cells in the neural epithelium illustrate dissolution of dense chromatin when compared to the compactly aggregated chromatin observed in late telophase nuclei of mesenchymal cells in Figs. 13 and 15.

Certain cytoplasmic irregularities, which may not be necessarily typical features of mitosis, have been observed. Occasionally, delicately textured fibrillar regions can be observed scattered diffusely throughout the cytoplasm (Fig. 18, *R*). Free ribosomes along with mitochondria comprise the remainder of the cytoplasm. Little Golgi material is identifiable in the cells, although small amounts can be seen in some anaphase cells (Fig. 11, *G*).

## DISCUSSION

The observations reported here provide ultrastructural details of mitosis in the chick embryo and point out two types of cell division.

### *MA Structures Associated with Cleavage and Anaphase Movements*

Distinct differences in spindle elongation during anaphase and telophase have been noted. In general, epithelial cells, being confined spatially, divide predominantly by a shortening of the chromosome-to-pole distance rather than by spindle elongation. The small increase in spindle length (1.4–1.7 times) may be attributed to centriole transposition within the cytoplasm since, at late anaphase, centrioles are located almost immediately beneath the plasma membrane and the shape of the cell is unaltered. This interpretation was presented by Hughes and Swann (8) to account for similar increases in spindle length (1.4–1.8 times) in cultured chick-embryo osteoblasts that also are arranged compactly. Recently published micrographs of dividing rat thymocytes in vivo (15) also demonstrate limited spindle elongation.

On the other hand, one conspicuous feature of embryonic mesenchymal cells is that they are surrounded by large, extracellular spaces which apparently allow for extensive spindle elongation. Selected measurements of spindle length at anaphase indicate about a 3–4-fold increase, which is at least double the spindle length in epithelial cells at anaphase.

Recent evidence suggests, however, that spindle elongation may occur without concomitant cell elongation; that is, elongation also depends on spindle orientation within the cytoplasm. In cytoplasm-filled elongate, meristem cells of wheat, the spindle, oriented parallel with the greatest cytoplasmic axis, lengthens about four times to occupy virtually the entire protoplasmic space, without increase in cell length (16). These cells, like embryonic mesenchymal cells, also have numerous microtubules in the interzone. If the metaphase spindle is oriented along the shorter cytoplasmic axis, as in stomatal epithelial cells, little elongation occurs, even though *some* microtubules are found in the interzone (17). It appears that a spindle will elongate if it is oriented properly and if it is provided the cytoplasm in which to do so.

The frequency with which continuous tubules occur in the interzone of these two types of cells, therefore, is noteworthy because of the directness of the comparisons that may be made. Longitudinal sections of spindles in epithelial cells revealed

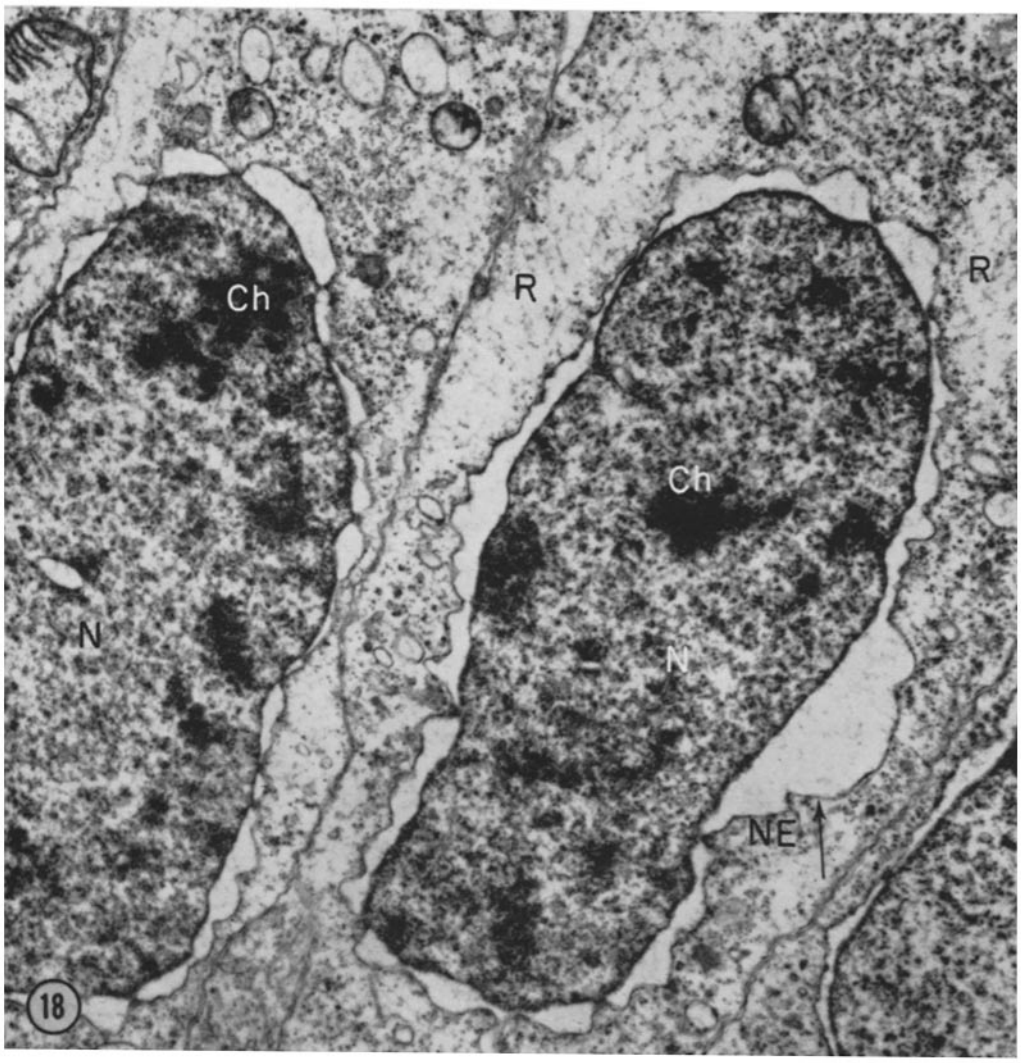



FIGURE 18 Daughter cells in the neural tube epithelium. The nuclear envelopes (*NE*) are irregular, but continuous; chromatin (*Ch*) is dispersing. The cytoplasm is composed of free ribosomes and some delicately fibrous regions (*R*). *N*, nucleus. × 17,000.

small numbers of continuous tubules; cross-sections of the mid-body demonstrate fewer than 150 microtubules. In contrast, numerous continuous tubules are observed in longitudinal sections of mesenchymal cells. Although we have no actual count, it is likely that the number of these tubules in mesenchymal cells compares favorably with the 500–600 reported by Krishan and Buck (10) for the mid-body of a cultured L-strain fibroblast.

These observations strongly suggest that the continuous tubules are involved closely in elongation of the spindle. This hypothesis should be tested by attempts to demonstrate the localized assembly of tubule macromolecules within the interzone region during anaphase. However, it is clear that intussusception of molecules within an already formed structure is implicated strongly; such a concept, at the present time, is accepted readily only in regard to membrane dynamics. Nevertheless, this study provides evidence that cells characterized by a large elongation of the anaphase spindle have relatively high numbers of microtubules in their interzones. Such a con-

clusion was inferred previously (20), but the study reported here provides a firmer basis for the concept because comparisons have been made in the same species, organism, and thin section.

### *Formation of the Cleavage Furrow*

The two patterns of cleavage formation observed in the chick embryo apparently are also dependent upon cell interactions. The symmetrical, equiconvergent furrow observed in mesenchymal cells, similarly observed in L-strain fibroblasts (10) and Chinese hamster cells (1), is not observed in epithelial cells which are attached firmly by desmosomes near their free surface. In epithelial cells, the cleavage furrow converges asymmetrically to a fixed site at the free surface.

Nevertheless, in both cases the furrow forms as an advancing front of amorphous cortex and plasma membrane, similar to that described by Robbins and Gonatas (19). Furrow formation does not occur by vesicle formation and fusion as typified by some mammalian cells (see references 3, 15).

Despite the fact that glutaraldehyde is the best fixative available for spindle preservation, its use fails to preserve structures that would provide direct support for the hypothesis of furrow formation by an intracellular contraction (13). Close examination of the leading cytoplasm reveals little evidence of contractile elements, unless the thin layer beneath the plasma membrane serves this function.

The remaining hypothesis to be advanced (2) is that the furrow is formed by regionally specific synthesis of new plasma membrane. During cleavage, cells are involved in many synthetic reactions, not the least of which must be plasma membrane synthesis. Our observations suggest that the furrow is formed by specific synthesis, at least in epithelial cells, of the plasma membrane on the side opposite the desmosome-containing free surface. Buck and Krishan were able to show that desmosomes are stable structures and that membrane synthesis occurred specifically at the equatorial zone. The synthesis and assembly of molecules in this membrane is not preceded by cytoplasmic membrane formation, as is the case in reports mentioned previously (see references 3, 15). Consideration of cell membrane dynamics in chick embryo epithelial cells suggests that, in order for the desmosomes, presumably fixed structures, to "move" as they do from their original positions to the site of the mid-body, molecules must be removed from the free surface. Thus, a flux exists in which molecules removed from membranes at the free surface may be used in furrow formation.

### *Fate of the Mid-Body*

Frequent conjectures have been made regarding the fate of the mid-body. Buck and Tisdale (3) report that the mid-body is relegated to one of the daughter cells in which its fate has not been traced. Our observations suggest that the mid-body, being dense and obstructive, becomes enclosed by the furrow membrane and is discarded. Figures 16 *a* and *b* and 17, representing epithelial cells, and Fig. 15, representing a mesenchymal cell, illustrate how the furrow membranes, after being impeded, enclose the structure. Robbins and Gonatas (19) and Murray et al. (15) have published photographs suggesting similar membrane activity. In addition, we have not seen remnants of the mid-body in daughter cells. The observation of "free" membrane-limited bodies containing tubule-like structures within the neural canal strengthens the hypothesis, although it is possible that these bodies are not unattached completely.

Dr. Allenspach is indebted warmly to Dr. R. A. Jenkins for his technical assistance. A major portion of this work was performed during a leave of absence from Albright College, Reading, Pennsylvania, which Dr. Allenspach recognizes for interest and support.

This investigation was supported in part by a Public Health Service Special Fellowship 1-F3-CA-24,731-01 from the National Cancer Institute to Dr. A. L. Allenspach and by grants HD 1260 and HD 2585 to Dr. L. E. Roth from the National Institute of Child Health and Human Development.

*Received for publication 16 August 1966.*

## REFERENCES

1. Brinkley, B. R., E. Stubblefield, and T. C. Hsu. 1967. The effects of colcemid inhibition and reversal on the fine structure of the mitotic apparatus of Chinese hamster cells *in vitro*. *J. Ultrastruct. Res.* In press.
2. Buck, R. C., and A. Krishan. 1965. Site of mem-

brane growth during cleavage of amphibian epithelial cells. *Exptl. Cell Res.* **38**:426.

3. BUCK, R. C., and J. M. TRISDALE. 1962. An electron microscopic study of the development of the cleavage furrow in mammalian cells. *J. Biol.* **13**:117.
4. DOSSEL, W. E. 1958. Preparation of tungsten microneedles for use in embryological research. *Lab. Invest.* **7**:171.
5. GONATAS, N. K., and E. ROBBINS. 1964. The homology of spindle tubules and neurotubules in the chick embryo retina. *Protoplasma.* **59**:25.
6. HAMBURGER, V., and H. L. HAMILTON. 1951. A series of normal stages in the development of the chick embryo. *J. Morphol.* **88**:49.
7. HERMAN, L., and S. L. KAUFFMAN. 1966. The fine structure of the embryonic mouse neural tube with special reference to cytoplasmic microtubules. *Develop. Biol.* **13**:145.
8. HUGHES, A. F., and M. M. SWANN. 1948. Anaphase movements in the living cell. A study with phase contrast and polarized light on chick tissue cultures. *J. Exptl. Biol.* **25**:45.
9. KÄLLÉN, B., and K. VALMIN. 1963. DNA synthesis in the embryonic central nervous system. *Z. Zellforsch. Mikroskop. Anat.* **60**:491.
10. KRISHAN, A., and R. C. BUCK. 1965. Structure of the mitotic spindle in L strain fibroblasts. *J. Cell Biol.* **24**:433.
11. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:49.
12. LYSER, K. M. 1964. Early differentiation of motor neuroblasts in the chick embryo as studied by electron microscopy. I. General aspects. *Develop. Biol.* **10**:433.
13. MARSLAND, D. 1956. Protoplasmic contractility in relation to gel structure: temperature-pressure experiments on cytokinesis and amoeboid movement. *In* International Review of Cytology. G. H. Bourne and J. F. Danielli, editors. Academic Press Inc., New York. **5**:199.
14. MARTIN, A., and J. LANGMAN. 1965. The development of the spinal cord examined by autoradiography. *J. Embryol. Exptl. Morphol.* **14**:25.
15. MURRAY, R. G., A. A. MURRAY, and A. PIZZO. 1965. The fine structure of mitosis in rat thymic lymphocytes. *J. Cell Biol.* **26**:601.
16. PICKETT-HEAPS, J. D., and D. H. NORTHCOTE. 1966. Organization of microtubules and endoplasmic reticulum during mitosis and cytokinesis in wheat meristems. *J. Cell Sci.* **1**:109.
17. PICKETT-HEAPS, J. D., and D. H. NORTHCOTE. 1966. Cell division in the formation of the stomatal complex of the young leaves of wheat. *J. Cell Sci.* **1**:121.
18. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
19. ROBBINS, E., and N. K. GONATAS. 1964. The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**:429.
20. ROTH, L. E., H. J. WILSON, and J. CHAKRABORTY. 1966. Anaphase structure in mitotic cells typified by spindle elongation. *J. Ultrastruct. Res.* **14**:460.
21. SAUER, F. C. 1935. Mitosis in the neural tube. *J. Comp. Neurol.* **62**:377.
22. SAUER, M. E., and B. E. WALKER. 1959. Radioautographic studies of interkinetic nuclear migration in the neural tube. *Proc. Soc. Exptl. Biol. Med.* **101**:557.
23. SIDMAN, R. L., I. L. MIALE, and N. FEDER. 1959. Cell proliferation and migration in the primitive ependyma zone; an autoradiographic study of histogenesis in the nervous system. *Exptl. Neurol.* **1**:322.
24. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4**:475.