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Nuclear Elongation and Cytokinesis in *Drosophila montana*

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We have used time-lapse cinematography and electron microscopy to study *Drosophila montana* embryos during formation of the cellular blastoderm, which occurs shortly after the twelfth mitotic division of the nuclei.

At the end of this division, the 3500 spherical nuclei in the peripheral cytoplasm elongate to 2.6 × their diameter, increasing their volume 2.4-fold. Bundles of microtubules, oriented parallel to the direction of elongation, surround the elongating nuclei. We propose that the tubules serve to constrain the enlarging nuclei so that they elongate rather than becoming larger spheres. Cytokinesis begins as the nuclei elongate, resulting in the simultaneous division of the embryo into 3500 peripheral cells surrounding the internal yolk mass. We observe that some cleavage furrows form through spindles, but most form between asters belonging to adjacent nuclei. These observations support the ideas of Rappaport that furrow initiation results from the interaction of pairs of asters with the egg surface. Some evidence is given for the existence of a system of contractile fibers which may be responsible for furrowing. We demonstrate that there are both slow and fast phases of furrowing, and we present the possibility that there are at least two sources of membrane for furrow growth.

INTRODUCTION

After fertilization of the *Drosophila* egg, twelve synchronous mitotic divisions occur to form the syncytial blastema. After several divisions the nuclei become distributed throughout the length of the egg, and after the eighth and ninth divisions the peripheral cytoplasm surrounding the internal yolk mass becomes populated with nuclei. Three more synchronous divisions take place before the egg transforms from this syncytial blastema stage into a cellular blastoderm.

At this time there is a dramatic simultaneous elongation of the 3500 peripheral nuclei to approximately 2.6 times their original central-peripheral dimension. Simultaneously the nuclei retract 1–2 μ deeper from the surface of the egg. It is during early nuclear elongation that nucleoli first become visible in the nuclei. Prior to nuclear elongation, folds appear in the plasma membrane and continue to increase in number and complexity until cleavage of the cytoplasm begins. Cleavage furrows grow inward from the egg surface between the elongating nuclei, eventually delimiting a single peripheral layer of cells which are separated from the internal yolk mass by a yolk membrane. Formation of the blastoderm is now complete, and morphogenetic movements of the blastoderm immediately ensue.

Time-lapse cinematography of early *Drosophila* embryogenesis has revealed that a number of events prior to blastoderm formation involve rearrangement or restructuring of egg components. Cytoplasmic movements which occur during the first twelve nuclear divisions have been studied by Kinsey (1967) and Wilson (1970) in this laboratory. These movements may effect distribution of the cleavage nuclei throughout the length of the egg and their subsequent migration into the peripheral cytoplasm. The ensuing events of nuclear elongation and cytokinesis are the subjects of this investigation. The *Drosophila* egg is particularly appropriate for a study of cytokinesis, since the peripheral cytoplasm is divided into 3500 cells at the same time. We have investigated these events with the electron microscope, correlating their timing with information obtained from

time-lapse movies made by J. C. Wilson.

Detailed descriptions of early *Drosophila* development are given by Poulson (1950) and Sonnenblick (1950). Bodenstein (1953) and Counce (1961) reviewed experimental analyses of the egg.

The development of cleavage furrows and the ultrastructure of the blastoderm have been described in some detail by Mahowald (1963a,b). Improved methods of aldehyde fixation (Sabatini *et al.*, 1963) have allowed us to undertake a more complete analysis of the ultrastructural changes during the processes of nuclear elongation and cytokinesis.

MATERIALS AND METHODS

We chose *Drosophila montana* for this study because, unlike some other *Drosophila* species, they do not retain fertilized eggs in the uterus. Consequently precisely timed eggs could be obtained easily.

Most eggs were fixed with 2.5% glutaraldehyde in 0.062 *M* sodium cacodylate buffer, pH 7.2, with 0.2 *M* sucrose added. This fixative allowed easy visualization of microtubules (cf. Fig. 5). However, the cytoplasm was clumped, and mitochondria were often quite swollen. A much better preservation of the cytoplasm was obtained with the following fixative: 2% glutaraldehyde, 2% acrolein in 0.125 *M* sodium cacodylate, pH 7.2, plus 0.2 *M* sucrose and 0.0009 *M* $CaCl_2$. Microtubules were less visible, partly because the surrounding cytoplasm was more evenly fixed and therefore more dense.

Adult flies obtained from the Genetics Foundation of the University of Texas were allowed to lay on yeasted banana agar. When the eggs reached the desired age, they were transferred to the fixative and a hole was made in each egg with a fine glass needle. After 10 min to 1 hr the chorion and vitelline membrane were removed with glass needles. The eggs remained in the fixative at room temperature for 1–2 hr. They were then washed in buffer, post-fixed in 2% OsO_4 in the appropriate buffer, and bulk-stained in 0.5% uranyl acetate overnight. Washing and poststaining were carried out at 4°C.

The material was dehydrated in a graded series of alcohols and embedded in an Epon–Araldite mixture (Mollenhauer, 1964). Sections were stained 15 min in uranyl acetate (Watson, 1958) and 15 min in lead citrate (Reynolds, 1963). The sections were observed and photographed using an RCA EMU-3G or EMU-3F electron microscope.

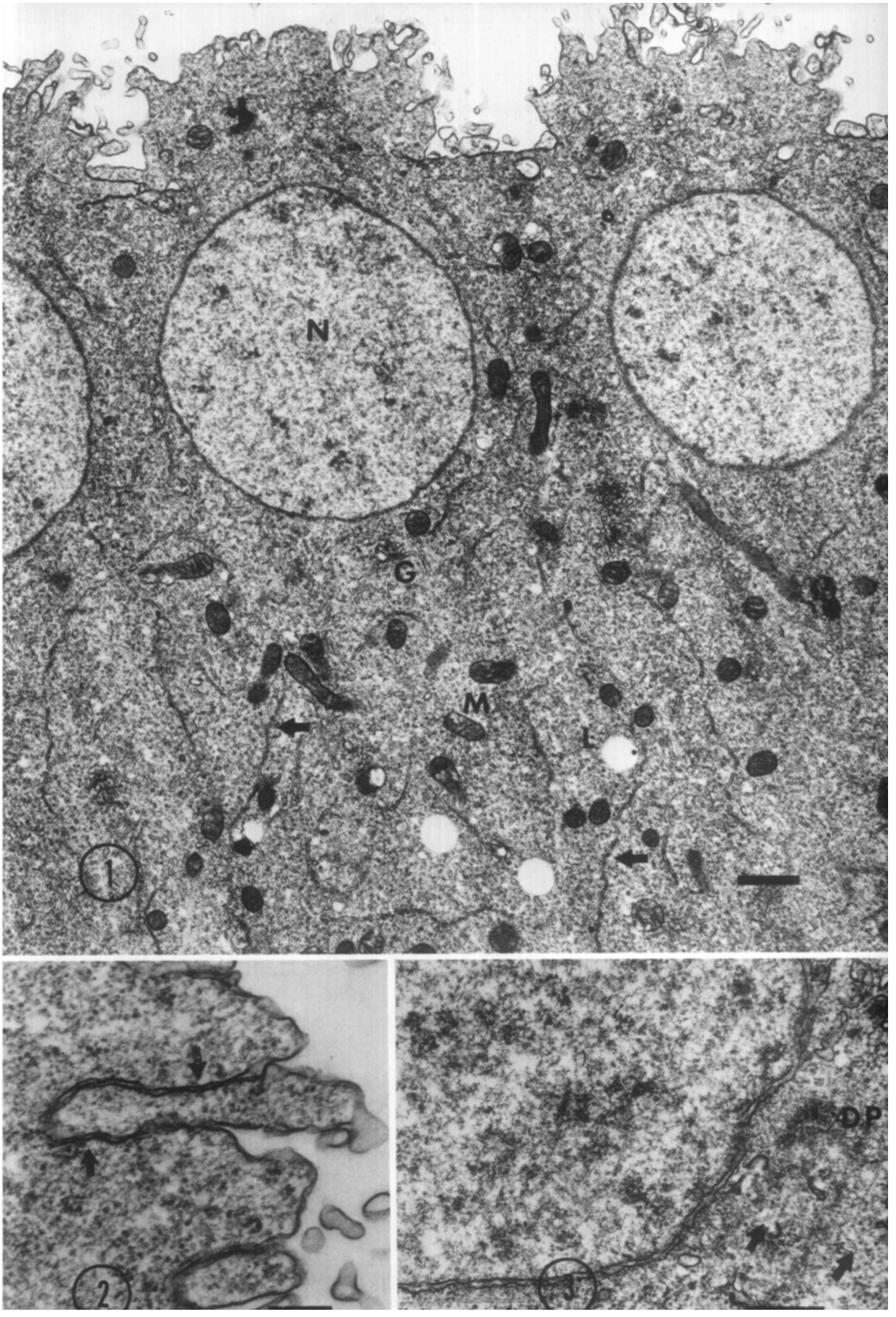
OBSERVATIONS

Nuclear Elongation

From time-lapse movies it was determined that nuclear elongation in *D. montana* begins at approximately 3.5 hr after the onset of development. Within a period of 35–40 min the spherical nuclei with a diameter of 4.9 μ elongate to 12.7 μ in length (measurements are from electron micrographs). At the same time their diameter decreases only slightly to about 4.7 μ. During the process of elongation nuclear volume increases 2.4-fold (from 63 μ^3 to 151.3 μ^3).

Figures 1–3 demonstrate features of the egg after the twelfth mitotic division prior to the beginning of nuclear elongation. At this time blastema nuclei are located 1–2 μ from the egg surface (Fig. 1). They have well-formed membranes and typical nuclear pores. Nucleoli are not visible until the nuclei have begun to elongate. A few short segments of microtubules are evident in random positions near the nuclei (Fig. 3).

After the nuclei have begun to elongate, the most striking cytoplasmic inclusions are bundles of microtubules oriented perpendicular to the surface of the egg and parallel to the direction of nuclear elongation (Figs. 4 and 5). The tubules extend from points near the surface of the egg to the base of the elongating nuclei (the ends of the nuclei nearest the yolk). The tubules have never been observed to extend below the base of the nuclei, which indicates a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polarity in at least some phases of tubule growth. They apparently extend toward the yolk at approximately the same time that the nuclei elongate in this direction, rather than growing toward the egg surface or fully polymerizing *in situ* before the nuclei begin to elongate. These points are significant to later discussions of how the microtubules are organized and of their role in the process of nuclear elongation.

The longest segments of tubules, measuring several microns in length, appear to follow the curvature of the nuclear surface while maintaining a distance of at least 650 Å from the outer nuclear membrane. Two different arrangements of tubules occur. In one configuration the bundles of tubules parallel the nuclei and continue in a straight path into the peripheral cytoplasm just beneath the egg surface (Fig. 5). In the other, the tubules curve over the apical ends of the nuclei in what has been described elsewhere (Pearce and Zwaan, 1970) as an "inverted basket" arrangement (Fig. 6). The two configurations may serve different purposes, as will be discussed later.

In cross sections it is evident that the microtubules are arranged at random around the circumference of the nuclei. They may occur singly or in bundles of 4 or 5 at a given point near the nucleus, and frequently they appear at some distance from the nuclei.

Each microtubule is surrounded by a "halo" 100–300 Å thick, which is less electron dense than the rest of the cytoplasm and is free of structural elements (Figs. 5 and 6). A distance of at least 200 Å separates the tubules from each other. This is equal to the radii of two adjacent "halos," so that although no connections or bridges between tubules or between tubules and the nuclei have been seen, the tubules may be spaced by material in the halo region which is not preserved by the fixative or made visible by the stain.

In many sections a cytoplasmic differentiation is associated with the microtubules apical to the nuclei. Circular or semicircular arrangements of dense particles, surrounded by a mass of less dense but similar particles, lie near the apical surface of the nucleus (Figs. 3 and 6). While the nuclei are still spherical, the clusters are situated at about the center of the distal surface of the nucleus (Fig. 3). Later they usually appear off center (Fig. 6). Short segments of microtubules radiate from or into these clusters of particles.

Centrioles have frequently been found in this same position above elongated nuclei (Fig. 7). These have distinct satellite bodies, which are similar to the clusters of particles seen above other nuclei. Microtubules radiate also from these centriolar regions. Centrioles have not been found lateral to the nuclei.

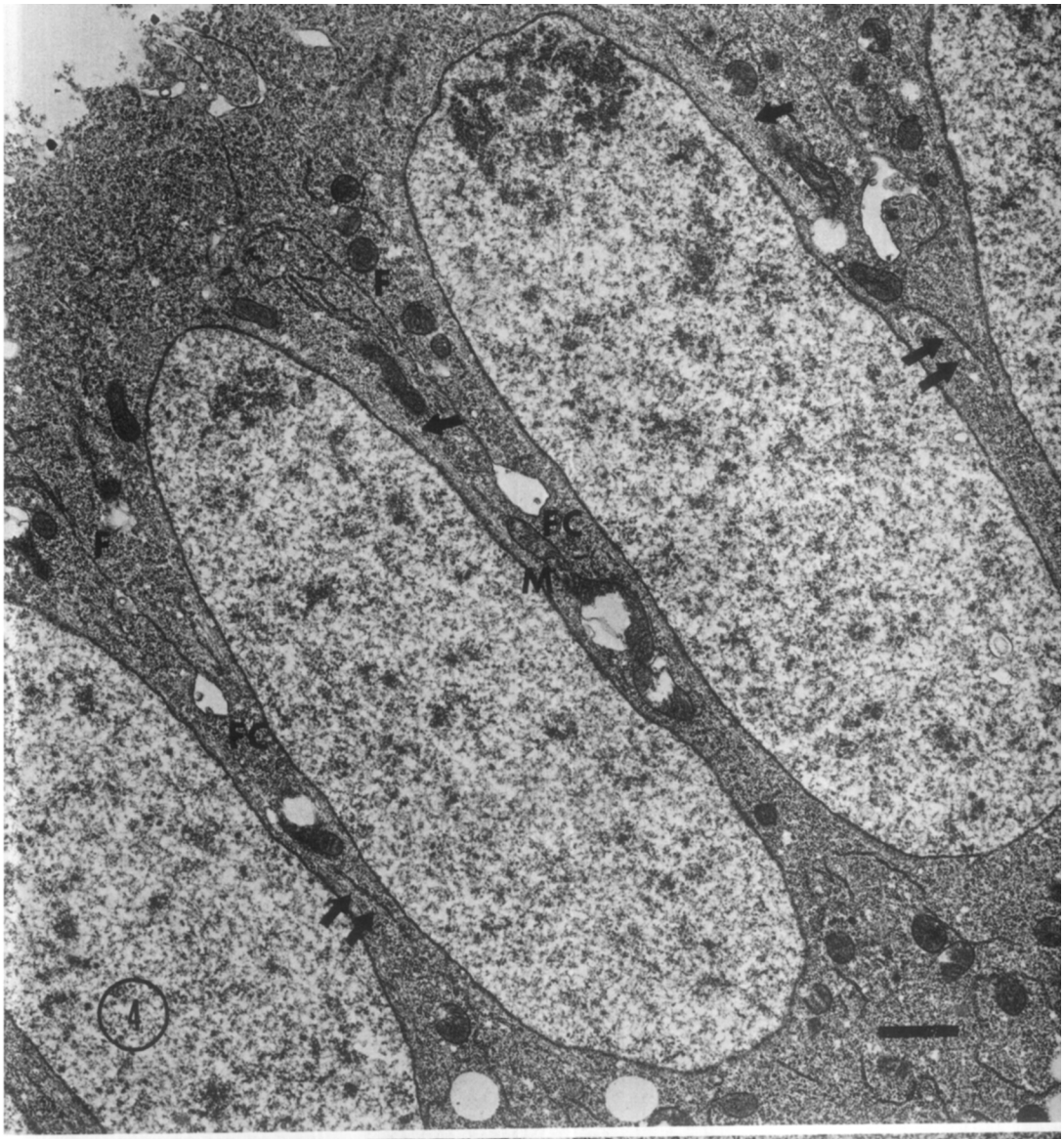
Cleavage

Time-lapse observations. From high-magnification movies taken of the posterior pole of the egg it was calculated that cytoplasmic cleavage was completed in 1.5–1.75 hr at 25°C. The movies were taken through Nomarski optics, and the events were observed in an optical section that

FIG. 1. Periphery of *Drosophila montana* egg seen in cross section. The egg was fixed at 3.5 hr post-deposition, just prior to cytokinesis. The egg surface is raised into hillocks covered with villi over each nucleus (*N*). The hillocks are separated by flattened areas of surface membrane in positions where the cleavage furrows will form. Paired membranes (arrows) are predominantly oriented perpendicular to the egg surface and parallel to the path of cleavage furrow extension. *M*, mitochondrion; *G*, Golgi-like clusters of smooth vesicles; *L*, extracted lipid droplets. Bar represents 1 μ.

FIG. 2. Higher magnification of villous projections occurring on a hillock such as those shown over nuclei in Fig. 1. Spacing of unit membranes covering villi in contact with one another at arrows is 150–200 Å. Bar represents 0.2 μ.

FIG. 3. Peripheral nucleus of egg fixed at 3.5 hr post-deposition. Microtubules (arrows) are arranged at random around the nucleus, and several appear near dense particles (*DP*) located above the nucleus. The egg surface is to the upper right of the figure. Bar represents 0.5 μ.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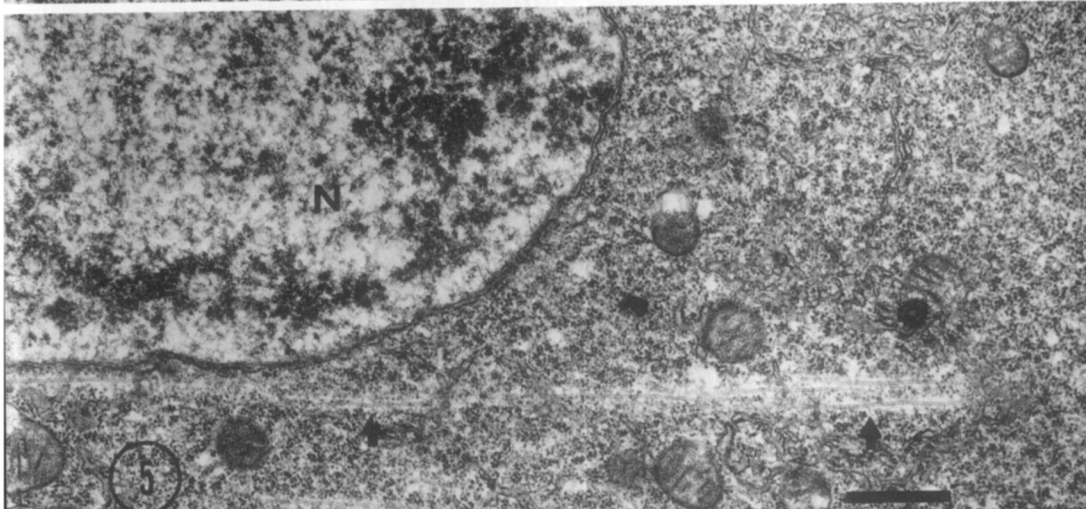
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corresponds to a sagittal section. Indentations of the surface membrane formed, disappeared, and reformed between divisions 10 and 11 and between 11 and 12, as reported previously (Mahowald, 1963a). When the cleavage furrows themselves formed, wide, shallow indentations were visible between the nuclei, creating a scalloped appearance of the egg surface (Fig. 1). After the furrows had progressed several microns into the egg they were observable in optical section as a light band, which corresponded to the advancing base of the furrows.

We have confirmed the previous observation (Mahowald, 1963a) that the first phase of cleavage, which lasts until the furrows have reached the base of the nuclei, proceeds slowly. Cleavage of the remainder of the cytoplasm proceeds twice as rapidly as the first phase. Table 1 shows data on the rate of cleavage of three eggs filmed at 25°C. The duration of cytokinesis varies among eggs and with environmental conditions. The cell height at the end of cytokinesis differs from one region of the egg to another. However, the rate of furrowing during phase 1 proportional to the rate during phase 2 is quite constant.

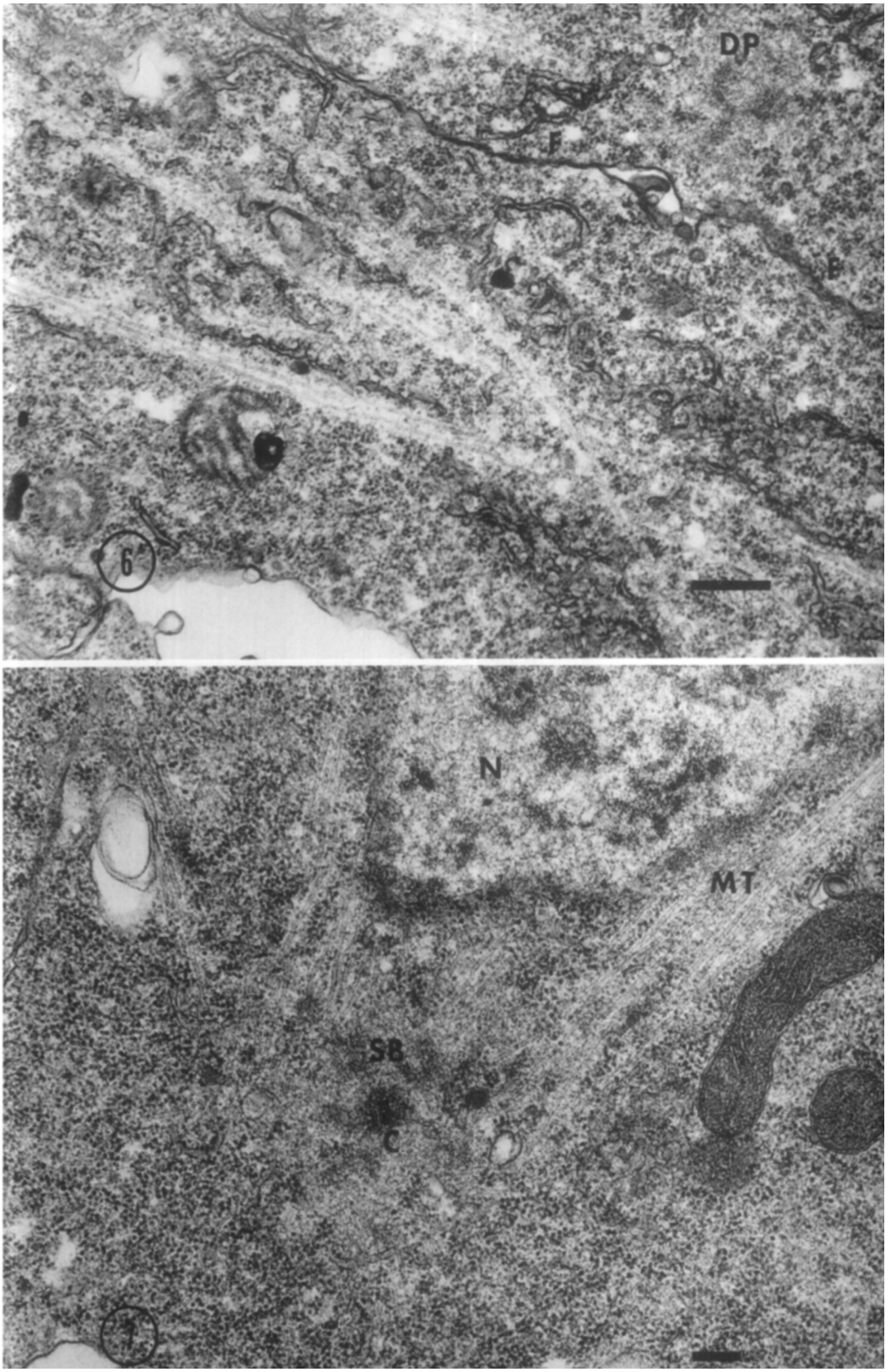
At the end of the twelfth nuclear division a tremendous amount of activity begins in the heretofore quiescent peripheral cytoplasm. This activity, seen in time-lapse cinematography, seems to involve movement of cytoplasmic components back and forth in a direction perpendicular to the surface of the egg. Since this "dancing" activity is so obvious, at the relatively low magnification of the movies, it must involve the larger formed elements in the cytoplasm such as endoplasmic reticulum or mitochondria.

Ultrastructural observations. The twelfth nuclear division is tangential to the egg surface (Fig. 8). This division results in an hexagonal packing of the blastema nuclei, such that each nucleus has six (or, infrequently, five) neighbor nuclei (Fig. 9). This relationship is maintained until the end of cleavage, when the newly formed blastoderm cells are also hexagonally or pentagonally packed. In cross section the cells are roughly six-sided (Fig. 10). Along their "sides" the adjacent cell membranes are closely apposed, but at the angles of each hexagonal cell boundary, where three cells are contiguous, a gap occurs.

Figure 1 shows a typical cross section of an egg fixed just prior to the beginning of cytokinesis. The surface membrane of the egg above each nucleus is raised into a hillock from which a number of villi project. Where the membranes covering adjacent villi are in contact with one another, a fairly consistent spacing of 200 Å is maintained between the membranes (Fig. 2). This spacing is similar to the usual intercellular distance in many cell types. Between nuclei the surface is flattened and contains few villous projections. We believe the flattened area represents the beginning of the "furrow vesicle." This structure, described by Mahowald (1963a) and seen in cross section in Figs 4, 11, and 12, represents a widening of the space between adjacent membranes of the cleavage furrow. It is found at the base of the cleavage furrow and is seen in this position in nearly all sections through the furrow. For this reason we consider furrow "vesicle" to be a misnomer: if it were a "vesicle" it would occur in only a limited number of sections. We believe that these are cross sections through an intercommunicating canal which is formed by a widening of the base of the cleavage furrows and which

FIG. 4. Periphery of an egg fixed at 3.75 hr post-depostion as seen in cross section. Nuclear elongation is maximal. Bundles of microtubules run parallel to the nuclei at arrows. *F*, cleavage furrows; *FC*, furrow canals at base of cleavage furrows. Some mitochondria (*M*) are "exploded" by the fixative, while others seem to be intact. Strands of paired membranes are abundant in the cytoplasm below the nuclei, and two such membranes are located between the nuclei (double arrows). Bar represents 1 μ.

FIG. 5. Microtubules (arrows) run beside the nucleus (*N*) of a 3.75-hr egg and terminate in the peripheral cytoplasm. The surface of the egg is just out of the field to the right. Bar represents 1 μ.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forms a hexagonal network surrounding all the nuclei. The relationships among cleavage furrows, furrow canal, and nuclei are diagrammed in Fig. 13.

Since the furrow canal is found at the base of the cleavage furrows it must be the first portion of surface membrane to move inward when cleavage begins. Therefore it is appropriate to search for mechanisms of cleavage initiation in conjunction with a search for the origin of this basal portion of the furrow which persists as a wide space between the furrow membranes. At the end of cleavage the membranes of the furrow canals fuse to form the basal portion of the cell membrane and the yolk membrane, which separates the newly formed cells from the yolk (Mahowald, 1963a).

Another structure which must be mentioned, since it may play a role in the cleavage process, is a coated pit found attached to the surface membrane (Fig. 14). These flask-shaped structures are coated with short fibers on their cytoplasmic surfaces and usually contain a relatively dense amorphous material on their extracellular surfaces. Coated vesicles have been found in the cytoplasm near the egg surface, and occasionally flask-shaped coated structures are attached to membranous, Golgi-like elements.

The coated pits are found at the surface of eggs as early as 1.5 hr after fertilization. In the egg shown in Fig. 1, they were never found attached to the flattened surface membrane, but were often seen at the edges of the flattened areas. After cleavage has begun they are frequently attached to the furrow canal (Fig. 12), less frequently to the sides of the cleavage furrow (Fig. 11), and only rarely to the membrane at the surface of the egg.

DISCUSSION

Nuclear Elongation

Recently microtubules have been found in various cell types in many different organisms where the cells are undergoing changes in shape (Branson, 1968; Byers and Porter, 1964; Gibbins *et al.*, 1969; Overton, 1966; Porter, 1966; Tilney and Gibbins, 1969). In all cases the tubules are oriented parallel to the direction of elongation of the cells. If the tubules are disrupted by treatment with cold temperatures, colchicine, or hydrostatic pressure, the elongation of the cells does not occur, or in some cases an elongated state is not maintained (Tilney, 1968). There is, then, evidence that microtubules are causative agents in cellular and organelle elongation.

Only a few examples of microtubules associated with nuclear elongation have been described. These are instances involving spermatid formation (Kessel, 1966, 1967; McIntosh and Porter, 1967). Kessel found in the grasshopper that tubules oriented in the direction of nuclear elongation were positioned obliquely during early stages of elongation and later became straight (Kessel, 1967). McIntosh and Porter (1967) demonstrated that in the elongating spermatid nucleus of the domestic fowl the microtubules ran around the nucleus in helical fashion, the "coils" of tubules circumscribing the short dimension of the elongating nuclei. These authors proposed a "squeezing" action by the tubules to effect elongation of the nuclei.

In the *Drosophila* blastema the appearance of bundles of microtubles is correlated in time with the elongation of the peripherally arranged nuclei. Furthermore, the orientation of the tubules close to the nuclei

FIG. 6. A cleavage furrow (*F*) separates a future cell in the upper half of the figure from that in the lower half of the figure. Microtubules in the cell at the bottom of the figure curve toward one another over the apex of a nucleus which is out of the plane of sectioning. Dense particles (*DP*) near the apex of the upper nucleus resemble centriolar satellite bodies. The surface of the egg is to the right of the figure. Bar represents 0.5 μ.

FIG. 7. Apex of a nucleus (*N*) of an egg fixed at 4.5 hr post-deposition. Cytokinesis of this egg was still incomplete, although nuclear elongation was maximal. Microtubules (*MT*) seem to be converging toward a centriole (*C*) and its satellite bodies (*SB*). *S*, surface membrane. Bar represents 0.2 μ.

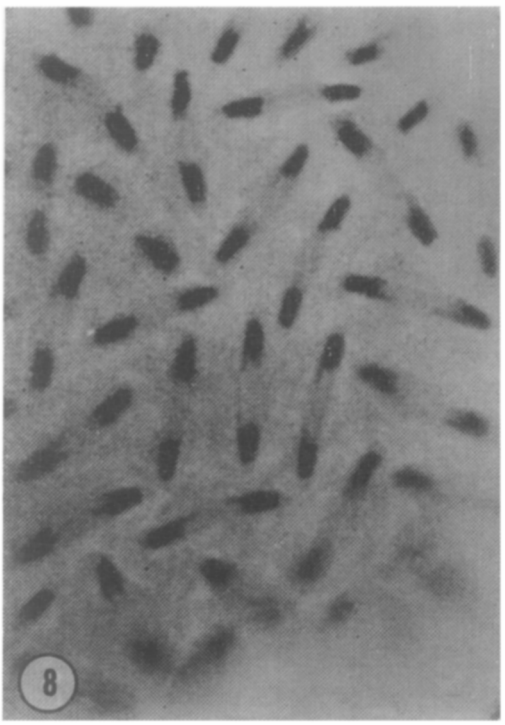
and parallel to the path of nuclear elongation strongly suggests that these organelles play a role in the elongation process. It is possible that they may be actively involved in the elongation, in some way stretching the nuclei out to their maximum length. However, since the nuclei increase their volume 2.4-fold from the spherical pre-elongation state to the fully elongated conformation, bundles of rigid microtubules may passively force the enlarging nuclei to elongate rather than becoming larger spheres.

TABLE 1
RATE OF FURROWING DURING FORMATION OF CELLULAR BLASTODERM[a]

Film	Phase 1 (μ/min)	Phase 2 (μ/min)	Final cell depth (μ)	Total cytokinesis time (min)
I (P)	0.20	0.44	23.5	87
II (P)	0.18	0.44	30.5	112
III (A)	0.23	0.44	27.5	87

[a] Rate of cell furrow growth determined from films of anterior (A) or posterior (P) portions of eggs filmed at × 400 magnification. Phase 1: growth of furrows from egg surface to base of nuclei. Phase 2: growth of furrows from base of nuclei to edge of yolk.

The fact that some tubules are crossed over the apical ends of the nucleus (Fig. 6) while others run straight into the peripheral cytoplasm (Fig. 5) suggests different functions for the tubules in the two arrangements. During the period of elongation, the nuclei move inward from the egg surface a distance of several microns. The crossed tubules may push the nuclei away from the surface, as Pearce and Zwaan (1970) have suggested in proposing a mechanism for the interkinetic nuclear movement in developing lens cells. If the



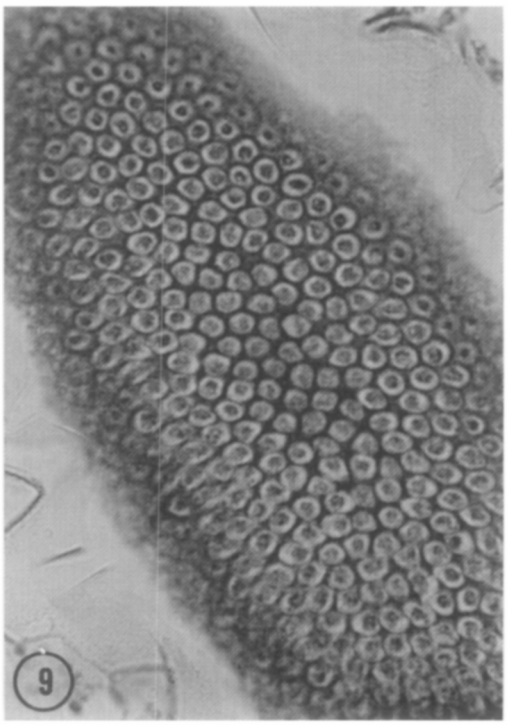


FIG. 8. A tangential section of *Drosophila melanogaster* egg during the twelfth nuclear division. Division spindles are parallel to the surface of the egg. Taken from Sonnenblick (1950) Fig. 17 with permission.

FIG. 9. Tangential section of a *Drosophila montana* egg fixed while nuclear elongation was in progress. Nuclei become hexagonally packed around the curved surface of the egg as a result of the twelfth nuclear division shown in Fig. 8. × 740.

tubules have the ability to undulate, as some evidence indicates (Clermont, 1964), such a motion may not only push the nuclei from the egg surface but also may squeeze the nuclei into an ellipsoidal shape at the same time. The straight, noncrossed tubules may then serve simply to constrain the enlarging nuclei and to direct their path of elongation.

The available evidence from electron micrographs indicates that the tubules begin to form in nonoriented fashion. After the last (12th) synchronous division of the nuclei, only a few short fragments of tubules are seen. These are arranged randomly around the nuclei. However, before the nuclei begin to elongate, clusters of finely granular material and coarser particles are often found apical to the still-sperical nuclei. No centrioles have been observed at this stage, but this could well be due to the chances of sectioning. Centrioles have been seen above elongating nuclei, surrounded by satellite bodies similar to the clusters of particles often found when no centriole is visible. These clusters of particles may be involved in microtubule organization, and they may in turn have a relationship to the centriole and its associated structures.

Centriolar satellite bodies have been implicated as the organizing sites of microtubules in other tissues and organisms (de-Thé, 1964; Gibbins, *et al.*, 1969; Kessel, 1967; Robbins and Gonatas, 1964; Szollosi, 1964). If they perform a similar function in the *Drosophila* egg, the question arises why microtubular segments are seen at random positions around the nuclei prior to nuclear elongation (Fig. 3). Tubules have been found near nuclei in eggs as early as 1.5 hr after fertilization. Possibly they polymerize at multiple sites and later are oriented by an association with the centriolar region. Or perhaps they are remnants of the mitotic apparatus. More will be said of the latter possibility in the discussion of cleavage.

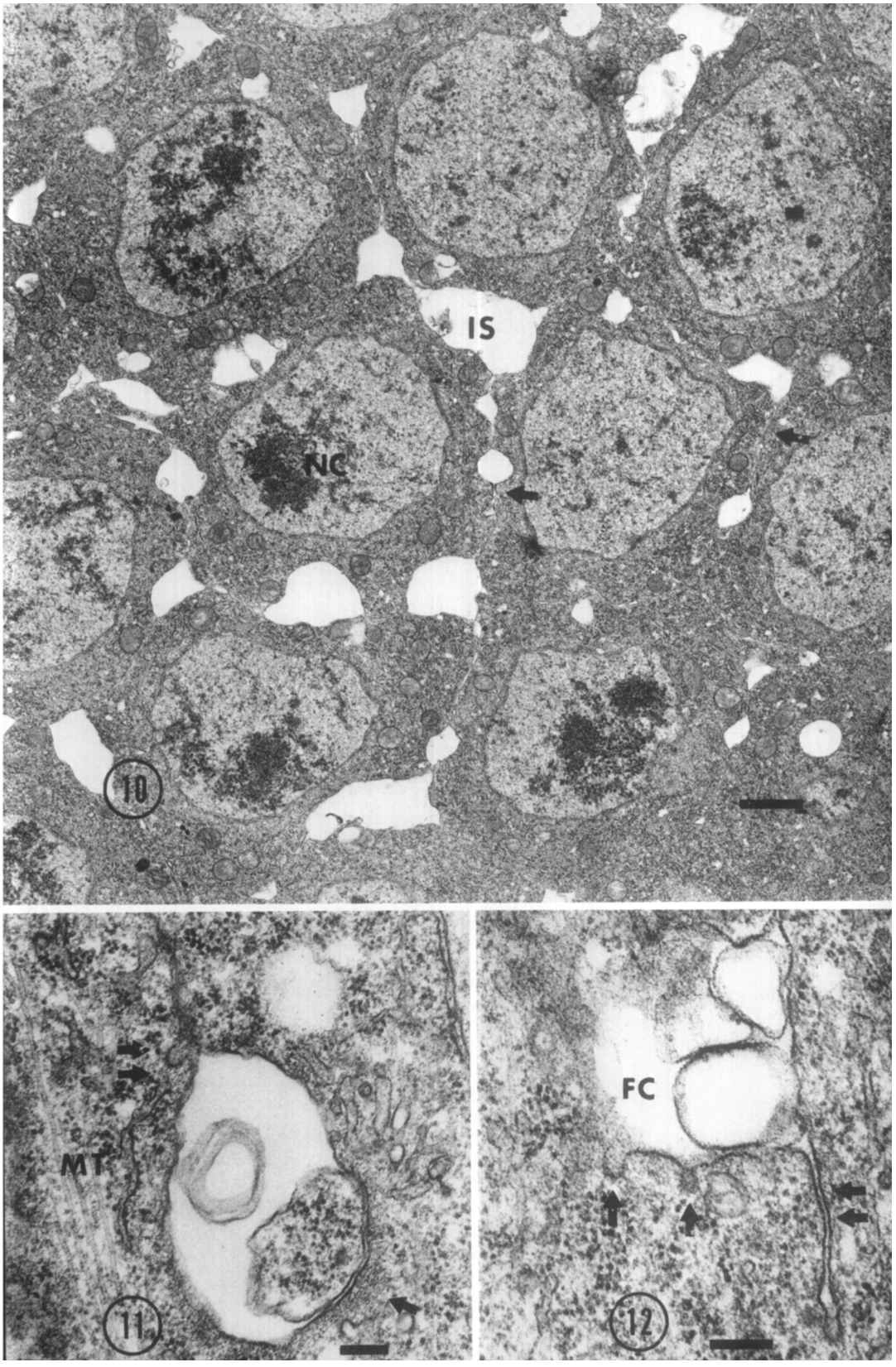
In summary, the microtubules associated with elongating nuclei of the *Drosophila* blastema are probably directly involved in the elongation process. It is most likely that they form a rigid framework which passively forces elongation of the nuclei as the nuclei increase in volume. The tubules may also push the nuclei away from the surface of the egg during the period of elongation.

Cytokinesis

Two principal questions may be asked about cytokinesis: (1) What are the mechanical forces accomplishing the division of the cytoplasm and the establishment of a pair of apposed cell membranes which separate the resulting cell units; and (2) What controlling factors determine when and where cytoplasmic division occurs? Cleavage of a single egg cell can be considered a special type of cytokinesis, since in various kinds of eggs the cleavage process must take into account the presence of yolk, the component unique to this single cell type.

We have begun to answer the first question in the *Drosophila* egg by observing the events occurring during cleavage of the living egg and by describing the ultrastructure of the egg fixed at intervals during cleavage. We are exploring the possibilities of experimental approaches to the problem, which are complicated by the impenetrability of the living egg to chemical agents.

To answer this first question concerning the mechanism of cleavage, three points must be considered. The first is the force responsible for the initial indentation of the egg membrane at sites which demarcate the surface dimensions of the future cells. In *Drosophila* the location of areas of flattened membrane in micrographs of an egg just prior to cleavage (cf. Fig. 1) and the packing of the blastema nuclei (cf. Fig. 9) have led us to conclude that the flattened areas are arranged in an hexagonal or honeycomb pattern over the curved egg surface. We suggest that the membrane in these areas corresponds to the membrane of the furrow canals at the base of the cleavage furrows, and that this membrane is the first to move inward from the surface in cytokinesis. To account for the flattening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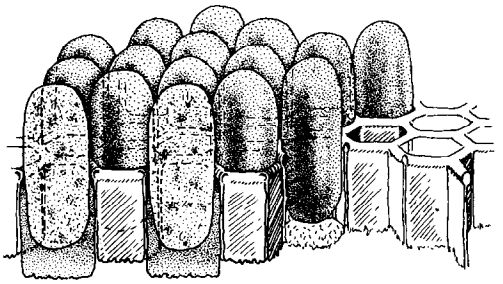

FIG. 13. Schematic representation of a longitudinal section through an egg during cytokinesis. The bottom of the drawing represents the surface of the egg. Cleavage furrows partially surround the nuclei as they extend inward from the surface. The furrows are preceded into the interior of the egg by the advancing tubular furrow canal which forms an interconnecting hexagonal network around the nuclei. On the right side the section has been peeled away to reveal a nucleus within its sheath of plasma membrane and the arrangement of the forming plasma membranes as they would appear empty of nuclei.

of the surface membrane in these locations and for the initiation of the cleavage process, we have considered the possibility that microfibrils are involved.

The simultaneous cleavage of the syncytial *Drosophila* egg into 3500 blastoderm cells implies a controlling system over the entire egg surface. The simplest system we can conceive of to accomplish this is a continuous array of microfibrils arranged in an hexagonal network that would define all the furrow canals and deepen them as the network of fibrils contracts.

Contractile microfibrils have been implicated as agents involved in cleavage in several egg types (Arnold, 1969, 1971; Goodenough *et al.*, 1968; Schroeder, 1968, 1969; Selman and Perry, 1970; Szollosi, 1968) and in cell division (Schroeder, 1970). If such a contractile system existed in the *Drosophila* egg just beneath the surface membrane and oriented in an hexagonal

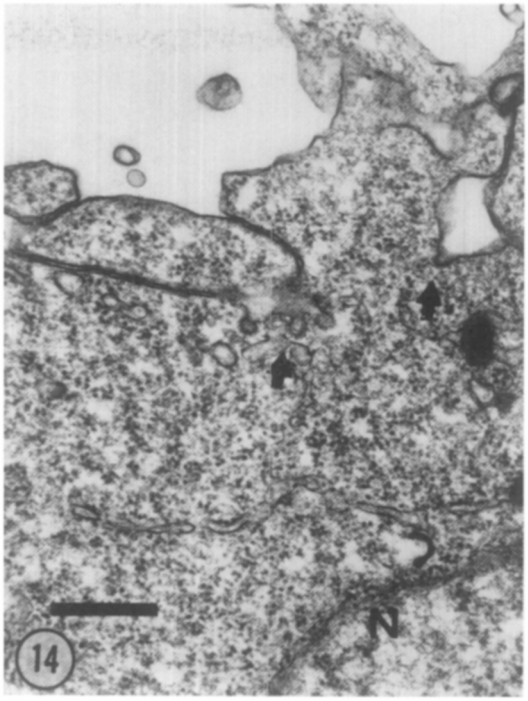

FIG. 14. Surface of an egg fixed at 3.5 hr postdeposition. Coated vesicles are attached to the surface membrane at arrows above a spherical nucleus (*N*). Bar represents 0.5 μ.

array at the sites of cleavage furrow initiation, the contraction of the fibers could be responsible for the flattening of the surface membrane surrounding the nuclei, with the resultant "bunching up" of the surface above the nuclei into hillocks and villi as seen in Fig. 1. Further contraction might be the force responsible for the initial movement of the furrow canal into the cytoplasm, thereby initiating cleavage. Both we (Fig. 11) and Mahowald (1963a) have observed fibrillar material near the furrow canals, although such a network has not yet been demonstrated beneath the flattened surface membrane prior to cytokinesis.

FIG. 10. Cross section of nuclei of a newly formed blastoderm. Nucleoli (*NC*) appear in some nuclei. The cell in the center is surrounded by six cells. Cells adhere tightly together in some places (arrows) and are separated by large intercellular spaces in others (*IS*). Bar represents 1 μ.

FIG. 11. Beaded fibrous material appears beneath the furrow canal at the arrow. A coated vesicle appears to be attaching to the furrow (double arrows). *MT*, microtubules. Bar represents 0.2 μ.

FIG. 12. Coated vesicles attached to membrane of the furrow canal (*FC*) at arrows. A long paired membranous extension of the furrow canal (double arrows) also terminates in a coated vesicle. This furrow canal is located at the base of a cleavage furrow which extends half-way down the length of an elongated nucleus. Bar represents 0.2 μ.

Careful investigation of the area beneath the flattened surface of the egg has led us to believe that bundles of straight fibers do not exist there. However, it is quite possible that a fibrillar network such as that occurring in the microspikes of axons (Yamada *et al.*, 1970) does occur there and may be demonstrated by fortuitous sectioning tangential to the surface of another egg at precisely the right moment before cleavage begins.

The second point to consider is the extension of the cleavage furrow, which involves the third consideration, that of the origin of new membrane for the forming cells. In other systems the extension of the cleavage furrow seems to involve progressive contraction of a fibrous network beneath the membrane of the advancing furrow, thereby pulling the furrow membranes together in the complete cleavage of coelenterate eggs (Schroeder, 1968) and cytokinesis of HeLa cells (Schroeder, 1970) or slicing through the cytoplasm in the meroblastic cleavage of the squid egg (Arnold, 1969). In the former systems a ring of contractile fibers provides the force for cleavage, whereas in the latter, the anchor points for the contractile fibers are the membrane at the ends of the furrow. Thus meroblastic cleavage, at least in squid, depends on the curvature of the blastodisc.

In the *Drosophila* egg, where 3500 cells are formed at the same time, insertion points for contractile fibers could be the edges of the areas of flattened surface membrane just prior to cleavage, and the membrane on either side of the base of the furrow canal after cleavage has begun. Continued contraction of such fibers, together with the addition of new membrane to the furrow as discussed below, would result in extension of the cleavage furrows toward the yolk.

In considering the origin of new membrane for furrow growth, we must mention two obvious points about the furrows and furrow canals, namely, that the apposed membranes of the cleavage furrows maintain the 150–200 Å spacing characteristic of completely formed cells, while the membranes comprising the walls of the furrow canal are not mutally adherent, a condition that may be established prior to the beginning of cleavage. This difference in membrane properties may be partially responsible for the appearance of the egg surface in Fig. 1, where the wide flattened surface area surrounding the nuclei represents the membrane destined to form the furrow canal. The surface membrane covering the hillocks and villi, which already exhibits the ability to adhere closely to the membrane of adjacent villi, could contribute to the apposed membranes of the cleavage furrow moving inward from the surface behind the advancing furrow canal. The furrow membranes formed from these regions would be mutually adhesive and would assume the characteristic 150–200 Å spacing as they were brought into apposition by being "pulled," or otherwise moved, into the furrows.

The tremendous amount of new membrane necessary for the formation of 3500 cells can be accounted for partly by the membrane which appears in the villi of the egg just prior to cytokinesis. Approximately $2.3 \times 10^6\ \mu^2$ of new membrane is needed to form 3500 cells measuring 5.8 μ in diameter by 30 μ in length, the average dimensions of the blastoderm cells. This figure also includes the yolk membrane which forms simultaneously with the basal portion of the cell membranes. From linear measurements of the amount of surface membrane in sections such as pictured in Fig. 2, we estimated that it takes five times as much membrane to cover the villous projections as it would take to cover the same amount of smooth egg surface. Prior to cytokinesis we considered the egg a prolate spheroid whose average dimensions are 170 μ (minor axis) by 560 μ (major axis) and whose surface area is 229,823 μ^2. If five times this amount of membrane is provided by the villi, there are approximately $1.15 \times 10^6\ \mu^2$ of available surface membrane prior to cytokinesis. Although

not enough to account for the entire amount of new membrane needed to form the blastoderm cells, this amount of membrane is sufficient for the extension of the cleavage furrows almost to the base of the nuclei. By that time the apical surfaces of the forming cells are flat. It is feasible that the cleavage furrows progress at the expense of membrane from the surface during the first slow phase of cleavage, until the furrows have reached the base of the nuclei. At this time there may be another source of preformed membrane which accounts for the second rapid phase of furrow growth.

Two observations bear upon a second possible source of preformed membrane for the growing cleavage furrows. The first is the active directional movement of formed elements in the cytoplasm during cleavage which we have observed in time-lapse movies. We were later led to consider that this motion could be due to sheets of paired membranes which we found in abundance in electron micrographs (Fig. 1). These paired membranes, often with ribosomes attached, are oriented quite predominantly parallel to the axis of cleavage furrow growth. The appearance and orientation of these membranes in our Fig. 1 and those in Mahowald's Fig. 1 (Mahowald, 1963a) are strikingly similar. Possibly these sheets of membranes attach directly to the advancing cleavage furrow, and it is their motion toward (and away from) the furrows which is seen in the films. We have seen a few instances in which closely apposed membranes extend toward the yolk from sites where they connect to the furrow canal (Fig. 12). A study of carefully timed stages during the second phase of cleavage is in progress to determine whether the sheets of membrane are attaching to the cleavage furrow and, if so, how the canal is maintained at the advancing edge of the furrow.

Another source of at least a small amount of membrane for the developing furrows and furrow canals is the coated pits found attached to the egg surface before cytokinesis begins and to the furrows and furrow canals themselves during cytokinesis. These pits are reminiscent of structures of similar morphology which have been seen in oocytes (Anderson, 1962; Kessel and Beams, 1963; Roth and Porter, 1962; Stay, 1965). In these cases the coated pits have been involved in micropinocytosis. Recently similar structures have been described by Sheffield (1970) in reaggregating chick neural retina. He proposed that the coated pits represented precursors of *macula adhaerentes diminuta*, junctions between associating cells.

There is no evidence that the coated pits contribute to the formation of specialized cell junctions in the *Drosophila* blastema. However, it is quite likely that they contribute to the surface properties of the egg and to the growing cleavage furrow. They may be involved in creating the differences in surface membrane which account for the distinction between furrow canal and the furrow itself. Their possible contribution to the microfibrils at the base of the furrow canals is discussed below.

The second question pertaining to control of when and where cleavage occurs is particularly intriguing when asked of the *Drosophila* egg, in which so many cells are formed simultaneously. The sites of cleavage furrow initiation, whether a network of contractile fibers is involved or not, are arranged in a honeycomb pattern over the entire surface of the egg.

The theory of Rappaport (1965, 1969) which explains localization of the cleavage furrow in sea urchin eggs offers an explanation for cleavage furrow formation in *Drosophila*. According to Rappaport, the furrow forms as a result of the combined influence of two asters on the egg surface at the site of furrowing. Both the distance between the asters and the distance between the asters and the surface are critical: if one distance is increased experimentally, the other must be decreased in order that a furrow be produced between the two asters. This leads to the conclusion that furrowing of the membrane takes place at the point of overlap of the influence of two asters.

If a similar mechanism is operating in cytokinesis of the *Drosophila* blastema one would expect furrowing to be initiated on the egg surface above the midpoint of each twelfth division spindle and between adjacent amphiasters belonging to different spindles. The spatial relationship among asters which fulfills the conditions necessary for them to influence furrowing of this type is demonstrated by measurements obtained from Fig. 8 and given in Fig. 15.

Although the number of measurements is too small to allow us to attach a statistical significance to the difference in the distances between asters, we have included the measurements to demonstrate that amphiasters of adjacent nuclei are at least as close to one another as are amphiasters situated across spindles. The proximity of all asters to one another creates conditions that could determine the positions of the cleavage furrows in multiple cytokinesis, where many cells are formed simultaneously, by the same mechanism proposed by Rappaport for furrowing in sea urchin eggs. Since the *Drosophila* egg is not amenable to micromanipulation, other means will have to be devised for testing this hypothesis.

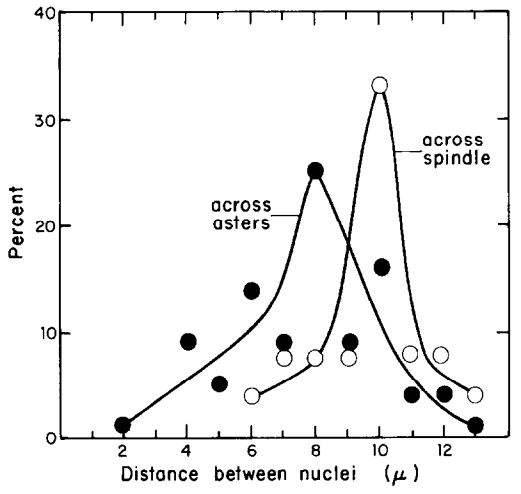

FIG. 15. Distribution of distances between nuclei as represented by the chromosomal masses of the division figures in Fig. 8. A total of 76 measurements were made across adjacent asters; 24 measurements were made across spindles. Measurements are expressed as percentage of the total number in each category.

The extremely tight packing of the nuclei at the end of the twelfth nuclear division may cause the shifting of the asters and centrioles to positions between the nuclei and the egg surface, where centrioles have been observed. Since the twelfth division is tangential to the surface, one would expect the centrioles to be located lateral to the nuclei, but they have never been seen in this position in eggs fixed after nuclear division has been completed.

This shifting of centrioles and, presumably, asters may be accomplished by the movement of these organelles themselves or possibly by rotation of the nuclei. A result of this change in polarity could be the outgrowth of microtubules from the astral region in a direction 90° from the spindle axis of the preceding nuclear division, that is, in the direction in which the nuclei elongate. The position of the asters above the nuclei could also result in the exclusion of certain formed elements, e.g., coated vesicles, from the surface directly above the nuclei. The vesicles would then attach to the surface membrane only where the overlap of two asters occurred. In this way the surface precisely where the cleavage furrows were destined to form could be rendered "different" from the remainder of the surface membrane lying directly above the nuclei. Perhaps the fibrous material on the cytoplasmic surface of the coated vesicles contributes to the elaboration of the proposed contractile fibers, and the attachment of the vesicles and subsequent elaboration of the contractile system constitutes the "difference" imbued to this region of the surface where the cleavage furrows are initiated. Such a mechanism has been suggested and backed up with supporting evidence for cleavage in the squid egg (Arnold, 1969, 1971).

Figure 16 summarizes the processes of nuclear elongation and cytokinesis in the egg of *Drosophilia montana*.

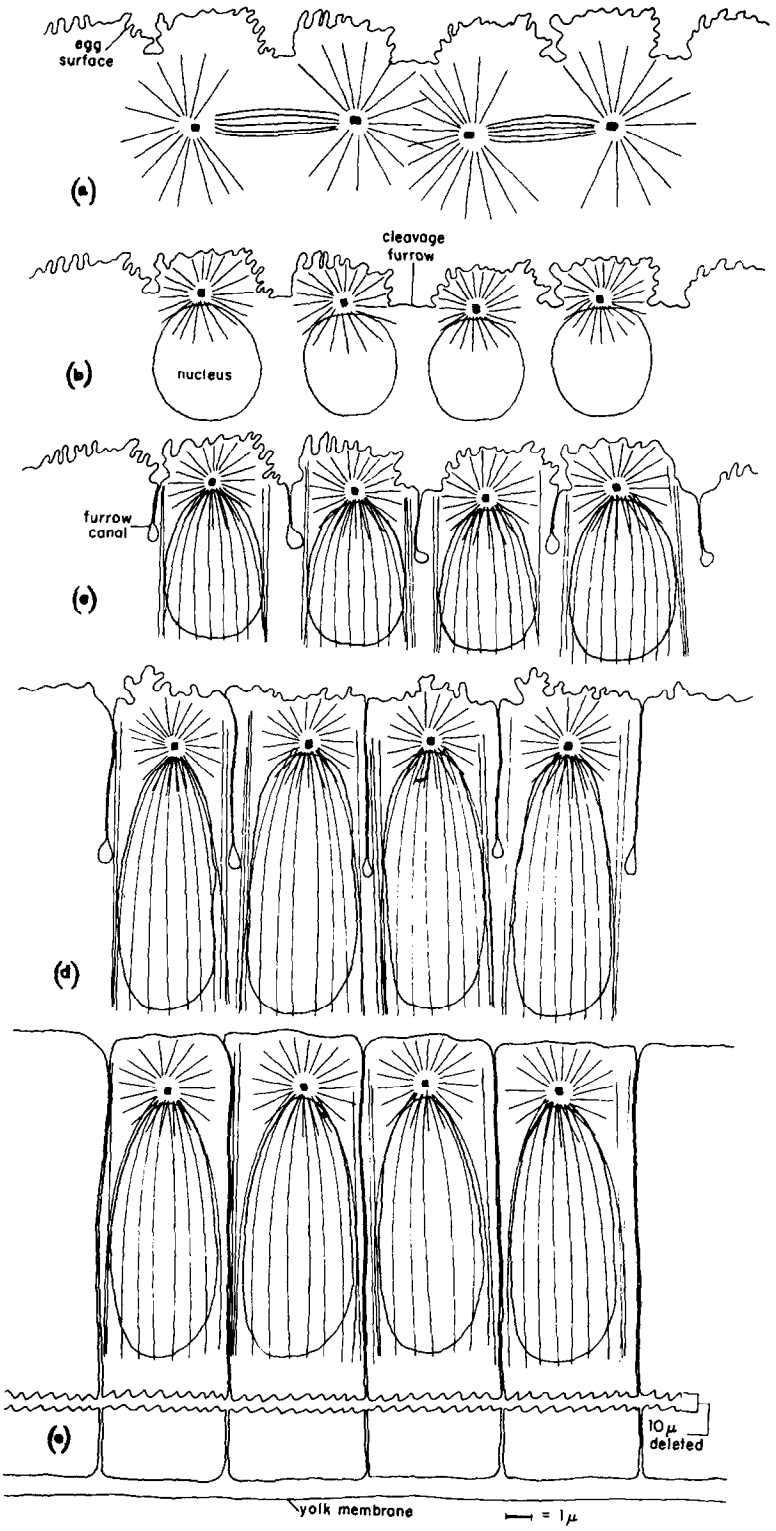

FIG. 16. Schematic representation of the 12th nuclear division, nuclear elongation, and cytokinesis in *Drosophila montana*.

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