Developmental Biology 451 (2019) 97–133

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Commentary

Commentary and tribute to Antone Jacobson: The pioneer of morphodynamics

Antone Jacobson, a committed embryologist in the age of genetics, passed away in 2017. In this issue of *Developmental Biology,* we honor his legacy by re-publishing two seminal papers. Antone and his lab made major contributions to our understanding of a wide swath of developmental biology, so it is fitting that the two papers tackle radically divergent problems, the first examining cell and tissue movements during amphibian neural tube closure (Burnside and Jacobson, 1968) and the second, the mechanism of *Drosophila* cellularization (Fullilove and Jacobson, 1971). These papers represent the starting point for his most lasting legacy: Working in a time when serious computation required feeding paper cards into room-sized computers, Antone foresaw by decades that highly interdisciplinary studies blending biology with mathematics and computer simulation would be crucial for understanding tissue morphogenesis in animals.

Antone was born in Salt Lake City, Utah in 1929, and both his parents were teachers. After graduating at the top of his class in rural Utah, Antone received his bachelor's degree with Honors from Harvard in 1951. As a PhD student with Dr. Victor Twitty at Stanford, Antone developed a passion for embryology and anatomy that would define him from his first paper (on induction of the optic vesicle), to his last (on gill slits in a fossil vertebrate) (Dominguez et al., 2002; Jacobson, 1955). He was drafted after completing his PhD, and always considered his time at Walter Reed Army Institute for Research as a "weird post-doc." In 1957, he joined the faculty of the University of Texas at Austin, where he remained for the rest of his career, contributing his insights to several generations of trainees. He rose through the ranks and was promoted to Professor in 1968.

The same year, Antone and Beth Burnside published a paper in *Developmental Biology* that through the lens of hindsight might be considered to mark the beginning of our modern, quantitative understanding of tissue morphogenesis (Burnside and Jacobson, 1968). Cinematography had been sporadically applied to embryonic development since the invention of the medium (Landecker, 2006; Wellmann, 2018). What made the paper by Burnside and Jacobson so revolutionary was its systematically quantitative approach. From time-lapse movies of amphibian neural tube closure, the authors generated high-resolution data on the speed and direction of individual cells' movements while at the same time systematically quantifying the deformation of the entire tissue using D'Arcy Thompson grids. By complementing these temporal data with spatial information from histological sections, the paper presented an unprecedented quantitative description of tissue morphogenesis across multiple length- and time-scales (Burnside and Jacobson, 1968).

In subsequent years, Antone and Richard Gordon would use the data generated from this and similar studies to develop a computational model of neural morphogenesis. In a landmark paper in *The Journal of Experimental Zoology* in 1976, Antone put forth his notion that morphogenesis research should proceed as a closed loop of experiment, formal mathematics, and computer modeling, whereby modeling generated predictions that could be experimentally validated and also inform further experiments in order to improve the model (Jacobson and Gordon, 1976). With over 50 figures (and an appendix that is remarkable even with today's insatiable appetite for supplemental data), this paper coined the term "morphodynamics" and laid out what remains the standard blueprint today for studies of embryonic morphogenesis.

Antone continued these pursuits for over a decade, collaborating with leading mathematical biologists George Oster and Garrett Odell to develop formal mathematical models of cell behaviors (Jacobson et al., 1986). He also and made efforts to explain the mechanics of neural morphogenesis, positing roles for eulerian buckling of the elongating tissue and for fluid pressure inside the closed neural tube (Desmond and Jacobson, 1977; Jacobson, 1978). The long shadow of those studies can be glimpsed in the recent surge of interest in embryo mechanics (e.g. (Mongera et al., 2018; Neumann et al., 2018; Savin et al., 2011; Sedzinski et al., 2016).

In addition to his crucial contributions to the study of morphogenesis, Antone maintained a strong interest in the inductive interactions underlying specification and commitment of vertebrate tissues. With a focus on the lens, embryonic placodes, and heart, Antone spent decades interrogating the spatial positioning of induced tissues (Jacobson, 1963; Sater and Jacobson, 1989, 1990; Zhang and Jacobson, 1993). Most of his studies of induction were carried out before we had any understanding of the molecular language of cell-cell interactions, or of the mechanisms underlying cell fate specification. Thus, his questions addressed the spatial and temporal dynamics of inductive interactions, often at a relatively fine scale. He viewed these tissue interactions as an ongoing "conversation", with a cumulative impact, in which the eventual establishment of individual cell fates emerged as a result of a sequence of inductive signals; the spatial restriction of these signals allowed for progressive refinement and fine tuning of the placement of the fully committed tissue (Jacobson and Sater, 1988). It is nearly unthinkable now for us to envision these interactions without any sense of their molecular

* Corresponding author.

https://doi.org/10.1016/j.ydbio.2019.04.019

identities or the underlying mechanisms, as Antone did.

Over the span of 50 years at UT Austin, Antone taught over 8500 undergrads, graduated 13 PhDs, 11 postdoctoral fellows and 9 MS students. He was funded by the NIH continually for 38 years. He was an astute and remarkably generous mentor, who valued independence in his trainees. A beginning graduate student could be greeted with: "Here's your dissecting scope and your incubator. There's your lab bench. We get chick eggs on Mondays and Thursdays. Let me know when you come up with a project." Nonetheless, he was also attentive and happy to provide guidance and feedback. Antone remained active in his lab even as a full professor, and he drew a distinction between his own projects and those of his trainees. The latter group's work spanned a broad range of developmental processes and organismal systems, ranging from *Drosophila* and the ghost crab to the full spectrum of vertebrate model systems: fish, frogs, newts, lizards, chick, quail, and mouse.

An outstanding example of the breadth of Antone's work -and of his commitment to his trainees-is provided by his work on *Drosophila* cellularization. As early as the mid-1960's, Antone's PhD student Janice Kinsey was using time-lapse cinematography to follow up on John Patterson's studies of lethal hybrids of *Drosophila* (Kinsey, 1967). The project was Kinsey's, so Antone was not an author on these papers. Shortly thereafter, another PhD student, Susan Fullilove combined time-lapse imaging with electron microscopy to perform a comprehensive analysis of cellularization of the blastoderm, which was published in *Developmental Biology* and is reprinted here (Fullilove and Jacobson, 1971). The work suggested a key role for actin in the initiation of cellularization and formalized the problem of membrane addition to the elongating furrows. Roughly 30 years later John Sisson's lab would take a genetic and biochemical approach to the very same problem (Papoulas et al., 2005), just two floors below the old Jacobson Lab in Patterson Laboratories at UT, Austin. Sadly, John Sisson passed away too soon, leaving much of his work unfinished. But the dynamics of *Drosophila* cellularization remains a key paradigm in developmental cell biology, and the questions Antone and his students asked are still being answered today (e.g. (Figard et al., 2016; Figard et al., 2013).

Even outside of UT, Antone was a powerful influence on generations of developmental biologists. Notably, as faculty at the MBL Embryology Course in Woods Hole in 1969, Jacobson discussed his work on morphogenesis with one curious young student in particular: Eric Wieschaus, now a Nobel Laureate, who was a pioneer of quantitative studies of morphogenesis in *Drosophila* (e.g. (Irvine and Wieschaus, 1994; Sweeton et al., 1991), and he counts Burnside and Jacobson's 1968 paper in *Developmental Biology* as a major influence. He continues this work today (e.g. (Martin et al., 2009; Streichan et al., 2018)), even asking the kinds of physical/mechanical questions that Antone was so enthusiastic about (e.g. (Doubrovinski et al., 2017; Polyakov et al., 2014).

Antone likewise had an outsized impact on Ray Keller, who as much as anyone else who charted the course of quantitative cell biology and mechanics in vertebrate embryo morphogenesis. Ray interviewed for a job at UT, Austin almost exactly 40 years ago, in February of 1979. He recalled how, with Antone as his host, he was ferried around in a Volkswagen "California Hippie Bus" and drank his first Pearl beer (in a shortneck bottle). Ray would start his lab in Berkeley, though, and he recalled Antone's visit in 1984 as the beginning of "one of the most interesting periods, lasting through the 1980's, in developmental biology and morphogenesis." During that period, Ray's lab laid the foundation for our current understanding of diverse collective cell movements, including epiboly by radial intercalation (Keller, 1980), invagination by apical constriction (Hardin and Keller, 1988), and convergent extension by mediolateral intercalation (Shih and Keller, 1992a,b). This work continues in the Keller lab today.

Antone retired and became Emeritus Professor at UT in 1997, but continued to attend seminars and interact with colleagues in Patterson Labs until only a few months before his death. In 2018, a symposium entitled "*Changes in the Shape*" (taken from the title of his 1976 paper) was held in his honor at UT Austin, with talks from Ray Keller, Lance Davidson, Bob Goldstein, Ondine Cleaver, Danelle Devenport and others. Beth Burnside, who rose through the ranks at U.C. Berkeley and retired recently as Provost, attended and spoke about life in the Jacobson lab back in the day. Acknowledging Antone's lifelong support of women in biology, Dr. Burnside has established the Antone Jacobson Memorial Fund, which supports the Women in Sciences Program at UT, Austin. The symposium and the memorial fund highlight the remarkable durability of Antone's vision for developmental biology, and this despite a constitutive resistance to anything relating to molecules!

Indeed, Antone's decidedly old-fashioned approach to embryonic anatomy still resonates, and he even contributed TEM data on eye development to a paper published after his death (Hocking et al., 2018). He refused authorship on that paper, which is notable because he might also be considered as a silent co-author for this piece. Several years ago, he walked into my (JBW's) office and said, "I'm going to die … Oh, not anytime soon, but eventually." He went on to explain that, no doubt, someone would ask us to write something about him. "I know y'all are really busy" he said, "so I took the liberty of writing a draft for you. It's on this thumb drive."

He always was ahead of his time.

Acknowledgements

We thank Ray Keller, Beth Burnside, Janice Grebe (née Kinsey), Eric Wieschaus, Seema Agarwala, and Ophelia Papoulas for sharing stories and memories of Antone.

References

Burnside, M.B., Jacobson, A.G., 1968. Analysis of morphogenetic movements in the neural plate of the newt Taricha torosa. Dev. Biol. 18, 537–552.
Desmond, M.E., Jacobson, A.G., 1977. Embryonic brain enlargement requires cerebrospinal fluid pressure. Dev. Biol. 57, 188–198.
Dominguez, P., Jacobson, A.G., Jefferies, R.P., 2002. Paired gill slits in a fossil with a calcite skeleton. Nature 417, 841.
Doubrovinski, K., Swan, M., Polyakov, O., Wieschaus, E.F., 2017. Measurement of cortical elasticity in Drosophila melanogaster embryos using ferrofluids. Proc. Natl. Acad. Sci. Unit. States Am. 114, 1051–1056.
Figard, L., Wang, M., Zheng, L., Golding, I., Sokac, A.M., 2016. Membrane supply and demand regulates F-actin in a cell surface reservoir. Dev. Cell 37, 267–278.
Figard, L., Xu, H., Garcia, H.G., Golding, I., Sokac, A.M., 2013. The plasma membrane flattens out to fuel cell-surface growth during Drosophila cellularization. Dev. Cell 27, 648–655.
Fullilove, S.L., Jacobson, A.G., 1971. Nuclear elongation and cytokinesis in Drosophila Montana. Dev. Biol. 26, 560–577.
Hardin, J., Keller, R., 1988. The behaviour and function of bottle cells during gastrulation of Xenopus laevis. Development 103, 211–230.
Hocking, J.C., Famulski, J.K., Yoon, K.H., Widen, S.A., Bernstein, C.S., Koch, S., Weiss, O., Agarwala, S., Inbal, A., Lehmann, O.J., 2018. Morphogenetic defects underlie Superior Coloboma, a newly identified closure disorder of the dorsal eye. PLoS Genet. 14, e1007246.
Irvine, K.D., Wieschaus, E., 1994. Cell intercalation during Drosophila germband extension and its regulation by pair-rule segmentation genes. Development 120, 827–841.
Jacobson, A.G., 1955. The roles of the optic vesicle and other head tissues in lens induction. Proc. Natl. Acad. Sci. U. S. A. 41, 522.
Jacobson, A.G., 1963. The determination and positioning of the nose, lens and ear. I. Interactions within the ectoderm, and between the ectoderm and underlying tissues. J. Exp. Zool. 154, 273–283.
Jacobson, A.G., 1978. Some forces that shape the nervous system. Zoon 6, 13–21.
Jacobson, A.G., Gordon, R., 1976. Changes in the shape of the developing vertebrate nervous system analyzed experimentally, mathematically and by computer simulation. J. Exp. Zool. 197, 191–246.

Jacobson, A.G., Oster, G.F., Odell, G.M., Cheng, L.Y., 1986. Neurulation and the cortical tractor model for epithelial folding. J. Embryol. Exp. Morphol. 96, 19–49.
Jacobson, A.G., Sater, A.K., 1988. Features of embryonic induction. Development 104, 341–359.
Keller, R.E., 1980. The cellular basis of epiboly: an SEM study of deep-cell rearrangement during gastrulation in Xenopus laevis. J. Embryol. Exp. Morphol. 60, 201–234.
Kinsey, J.D., 1967. Studies on an embryonic lethal hybrid in Drosophila. Development 17, 405–423.
Landecker, H., 2006. Microcinematography and the history of science and film. Isis 97, 121–132.
Martin, A.C., Kaschube, M., Wieschaus, E.F., 2009. Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457, 495–499.
Mongera, A., Rowghanian, P., Gustafson, H.J., Shelton, E., Kealhofer, D.A., Carn, E.K., Serwane, F., Lucio, A.A., Giammona, J., Campàs, O., 2018. A fluid-to-solid jamming transition underlies vertebrate body axis elongation. Nature 561, 401.
Neumann, N.M., Perrone, M.C., Veldhuis, J.H., Huebner, R.J., Zhan, H., Devreotes, P.N., Brodland, G.W., Ewald, A.J., 2018. Coordination of receptor tyrosine kinase signaling and interfacial tension dynamics drives radial intercalation and tube elongation. Dev. Cell 45, 67–82 e66.
Papoulas, O., Hays, T.S., Sisson, J.C., 2005. The golgin Lava lamp mediates dynein-based Golgi movements during Drosophila cellularization. Nat. Cell Biol. 7, 612.
Polyakov, O., He, B., Swan, M., Shaevitz, J.W., Kaschube, M., Wieschaus, E., 2014. Passive mechanical forces control cell-shape change during Drosophila ventral furrow formation. Biophys. J. 107, 998–1010.
Sater, A., Jacobson, A.G., 1989. The specification of heart mesoderm occurs during gastrulation in Xenopus laevis. Development 105, 821–830.
Sater, A., Jacobson, A.G., 1990. The role of the dorsal lip in the induction of heart mesoderm in Xenopus laevis. Development 108, 461–470.
Savin, T., Kurpios, N.A., Shyer, A.E., Florescu, P., Liang, H., Mahadevan, L., Tabin, C.J., 2011. On the growth and form of the gut. Nature 476, 57.
Sedzinski, J., Hannezo, E., Tu, F., Biro, M., Wallingford, J.B., 2016. Emergence of an apical epithelial cell surface in vivo. Dev. Cell 36, 24–35.
Shih, J., Keller, R., 1992a. Cell motility driving mediolateral intercalation in explants of Xenopus laevis. Development 116, 901–914.
Shih, J., Keller, R., 1992b. Patterns of cell motility in the organizer and dorsal mesoderm of Xenopus laevis. Development 116, 915–930.
Streichan, S.J., Lefebvre, M.F., Noll, N., Wieschaus, E.F., Shraiman, B.I., 2018. Global morphogenetic flow is accurately predicted by the spatial distribution of myosin motors. Elife 7, e27454.
Sweeton, D., Parks, S., Costa, M., Wieschaus, E., 1991. Gastrulation in Drosophila: the formation of the ventral furrow and posterior midgut invaginations. Development 112, 775–789.
Wellmann, J., 2018. Model and movement: studying cell movement in early morphogenesis, 1900 to the present. Hist. Philos. Life Sci. 40, 59.
Zhang, J., Jacobson, A.G., 1993. Evidence that the border of the neural plate may be positioned by the interaction between signals that induce ventral and dorsal mesoderm. Dev. Dynam. 196, 79–90.

John B.Wallingford*
Department of Molecular Biosciences, University of Texas at Austin, USA
* *Corresponding author.*

Amy K.Sater
Department of Biology, University of Houston, USA

DEVELOPMENTAL BIOLOGY 18, 537–552 (1968)

Analysis of Morphogenetic Movements in the Neural Plate of the Newt *Taricha torosa*[1]

M. B. BURNSIDE AND A. G. JACOBSON

Department of Zoology, The University of Texas, Austin, Texas 78712

Accepted August 13, 1968

INTRODUCTION

During gastrulation of salamander embryos, all the ectodermal cells move away from the animal pole toward the blastopore in the movement called epiboly. As the chordamesoderm involutes around the dorsal lip of the blastopore and then underlies the ectoderm, a dramatic change in cell behavior becomes visible on the dorsal surface of the embryo. Those cells that will form the neural plate change their patterns of movement. Some reverse their course and move back toward the animal pole; others move toward the midline and anteriorly; still others stop movement; some continue to move toward the blastopore. These complex deformations of the forming neural plate occur mostly before those movements in which the plate rolls into a tube.

These movements are interesting both as an early consequence of primary induction, and as a particularly good example for study of orderly morphogenetic deformation of a sheet of cells. We have made careful qualitative and quantitative analyses of these movements as an essential prelude to investigations into the mechanics of the underlying processes.

The movements have been described to some extent in previous studies, using vital stains, by Goerttler (1925), Vogt (1929), and

[1] Supported in part by U. S. Public Health Service Research Grant HD00725-07 and previous grants from the National Institute of Child Health and Human Development to A. G. Jacobson, in part by a Public Health Service Predoctoral Fellowship Number 5-F1-GM-30, 828-03 from the National Institute of General Medical Sciences to M. B. Burnside, and in part with funds for equipment supplied through the Graduate School of The University of Texas at Austin from an NIH Biomedical Sciences Support Grant.

Manchot (1929); and by Jacobson (1962), using time-lapse cinematography and vital staining. Unpublished studies of these cell movements using time-lapse cinematography were made in this laboratory by Epstein (1963). None of these previous studies provided sufficiently detailed descriptions and measurements of the cell movements to serve for more penetrating analysis of the mechanisms of the movements. We have attempted to do this.

There are advantages in choosing for study the salamander neural plate. It is formed from a sheet of cells just one cell thick. Since the forming neural plate occupies the uppermost surface of the embryo, cell movements in the neural plate may be easily observed while the embryo is oriented in its normal manner. Several species of salamanders (including the West Coast newt, *Taricha torosa*, which we have chosen for this study) have variegations in the egg pigmentation so that spots, confined to one cell, or to a few cells that remain associated during neurulation, may be followed without staining to trace cell movements. The relatively large cells of salamanders make it possible to follow cell associations through this period of development. There is very little cell division or change in embryo size and shape during the time of early neural plate formation, so complications from these variables are not serious. The period of time in which these movements occur is quite short, about 13 hours at 17°C in *Taricha torosa*.

METHODS

Embryos of the newt *Taricha torosa* were collected at early developmental stages near Palo Alto, California, and shipped by air in cool water to our laboratory in Texas. Embryos were transferred on arrival to constant-temperature boxes at 17°C. All subsequent procedures were carried out at 17°C, which is optimal for development in this species.

A synopsis of events during the stages of development under study follows. Stage numbers refer to the staged series for *Taricha torosa* of Twitty and Bodenstein in Rugh (1962). Gastrulation occurs between stages 9 and 12. By stage 13 the neural plate has started forming, the yolk plug is small, and the archenteron is fully formed. The notochord has not yet taken shape from the roof mesoderm. The mesoderm is firmly attached to the neural ectoderm, and the two tissues are difficult to separate. At stage 14 the adhesion between the

mesoderm and neural ectoderm is decreasing, neural folds are first apparent, and the notochord becomes cylindrical. By stage 15 the neural folds are converging toward the midline and beginning to lift.

Embryos to be analyzed were removed from their jelly, then photographed by time-lapse cinematography in cooled boiled tap water in an operating dish which rested in a metal jacket through which cooled water circulated. The water temperature was regulated to keep the egg at 17°C. A thermistor near the egg in the operating dish was connected to a recorder and the temperature in the operating dish was recorded throughout the period of time-lapse cinematography. The temperature never deviated from 17°C more than 0.5°C. Exposures of 0.25 sec were made at 1-min intervals on 16-mm black and white movie film using a 50-mm lens and reflected light from two microscope lamps. The lights were on only during the brief exposure and were equipped with heat filters. In these movies at low magnifications the image of the entire embryo just filled the frame and the magnification was about 5-fold on the film.

For movies at higher magnifications, portions of the neural plate were photographed through a Leitz Ortholux microscope equipped with Ultrapak water immersion incident light optics. The images of the cells on the film were magnified between 50 and 200 times. The boundaries of individual cells were easily distinguishable at these magnifications.

After completion of photography, embryos were maintained at 17°C in constant temperature boxes until swimming larval stages were reached. If an embryo failed to develop to larval stages, or looked unhealthy, films recording its development were discarded.

Cell movements were mapped by projecting low-magnification time-lapse pictures onto large sheets of paper frame by frame and marking pathways of pigmented spots with pencil lines. The position of the embryo did not vary from frame to frame. It was ascertained from higher magnification films that the pigmented spots that were followed represented single pigmented cells or small clusters of pigmented cells that remained associated.

Because of the extensive movements we observed between stages 14 and 15 and the irregularity of speeds during this period, we have distinguished an additional stage, 14½, for this study. The morphological appearances used to define developmental stages persist for extended periods of time, too long to provide sufficiently accurate

timing for comparison of our films. We have chosen an easily recognized developmental event, the first contact of the closing neural folds, and denote our stages by times prior to that event. On each time-lapse film the frame in which the folds first made contact was located and stage 13 was defined as 1157 frames (19 hours 17 min) prior to that frame. A frame of the film designating each subsequent stage was marked. Stage 14 is designated as 345 frames (and minutes) after stage 13. Stage 14½ is 126 min after stage 14; stage 15 is 295 min after stage 14½.

After locating and marking each stage on a time-lapse film, cell movements between the stages were then analyzed. The points traced were those at the intersections of a Cartesian coordinate system superimposed on the image of a stage 13 embryo. The pigmented spots at these intersections were followed to stage 15, and as each marked frame denoting stages 14, 14½, and 15 was projected, the position of each displaced spot was marked on an outline drawing of the embryo at that stage. The spots were connected with lines drawn with French curves to give transformed coordinates. Only the points at the intersections of the grid represent observed spots.

Tracings on both sides of the midline of the neural plate were made for stages 13 to 14 and found to be mirror images. Consequently only the right side was traced for the subsequent stages and the left side was copied from it.

The average length of stage 13 embryos (2.4 ± 0.1 mm) was determined by measurement of 10 embryos.

Embryos were fixed for light microscopy in Kahle's (Guyer, 1953) or Ammerson's (Humason, 1962) fixatives, and stained with a Janus green and neutral red technique for embryos (Jones, 1964).

RESULTS

The tracing of pigmented spots permitted precise description of the morphogenetic movements that occur during early formation of the neural plate, in terms of pathways and rates of movement of groups of cells, and in terms of area changes of regions of the surface of the plate.

The actual pathways of the cells as traced appear in Fig. 1. The time elapsed was known for each interval so that speed along the indicated pathways could be calculated. The lengths of the pathways were measured by stepping off with dividers. Table 1 shows

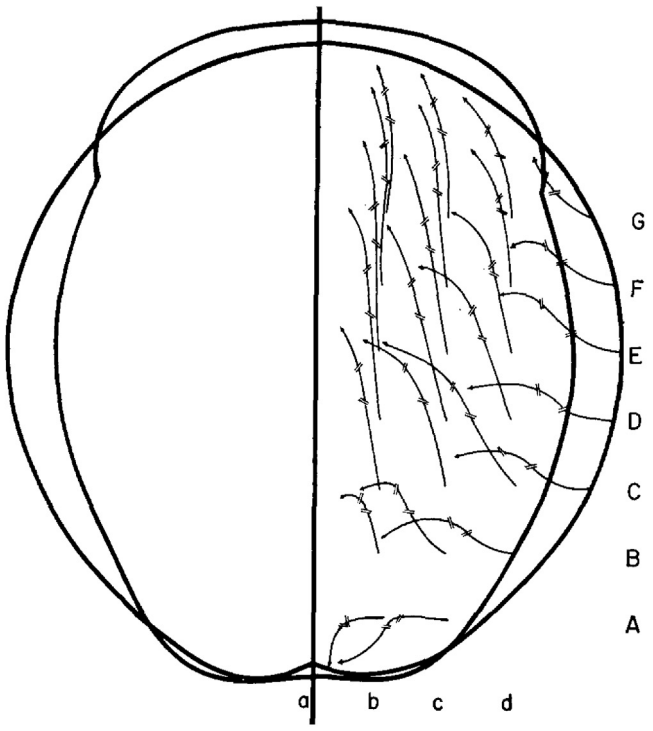


FIG. 1. Actual pathways of displaced cells traced from time-lapse films. Cells followed were at the origins of the arrows at stage 13, at the first hash mark at stage 14, at the second hash mark at stage 14½, and at the points of the arrows at stage 15. The wider contour represents stage 13, the starting point, and the narrower contour stage 15.

average speeds of cell groups during each interval studied and also the average speeds for the entire interval from stage 13 to stage 15.

Area changes in regions of the neural plate are indicated by the series of deformed coordinates in Fig. 2. Measurements made of the indicated regions at each stage are given in Table 2 in terms of percentage increase or decrease from the original size at stage 13. Since the curvature of the embryo distorts observations in marginal regions, only the shaded areas of Fig. 2 are considered. The anterior shaded square is deleted since it moves forward into the curvature of the embryo during the period studied.

It must be noted again that the cell groups were followed only at the intersections of the coordinate grid and the lines connecting these

TABLE 1

AVERAGE SPEEDS OF DISPLACEMENT OF CELLS (μ/HR)

Cell locations coordinates (Figure 2)	Average speeds between stages			Overall average stages 13–15 12.8 hr
	Stages 13–14 5.8 hr	Stages 14–14½ 2.1 hr	Stages 14½–15 4.9 hr	
Ga	76	48	17	48
Gb	63	53	18	47
Gc	58	53	25	44
Gd	46	53	32	42
Fa	69	65	49	60
Fb	68	66	47	62
Fc	60	60	23	46
Fd	50	39	43	46
Ea	79	70	49	65
Eb	77	73	55	68
Ec	75	53	58	64
Ed	51	35	58	50
Da	95	75	32	68
Db	77	79	55	69
Dc	75	66	52	64
Dd	58	58	56	57
Ca	94	76	37	68
Cb	58	66	32	53
Cc	58	66	40	53
Cd	58	68	63	62
Ba	46	27	9	26
Bb	24	33	14	22
Bc	33	53	29	34
Bd	33	40	60	45
Aa	7	60	49	19
Ab	24	4	34	25
Ac	29	33	47	33

points were drawn in with French curves to give the transformed grids. The area changes are an estimation to the extent that they depend on the assumption that cell movements between measured points are smooth and orderly. They appear so in the time-lapse movies.

To illustrate the remarkable consistency of cell movements observed at each stage in different embryos, the observed spots at the intersections of the grid between stages 14 and 14½ are shown for three

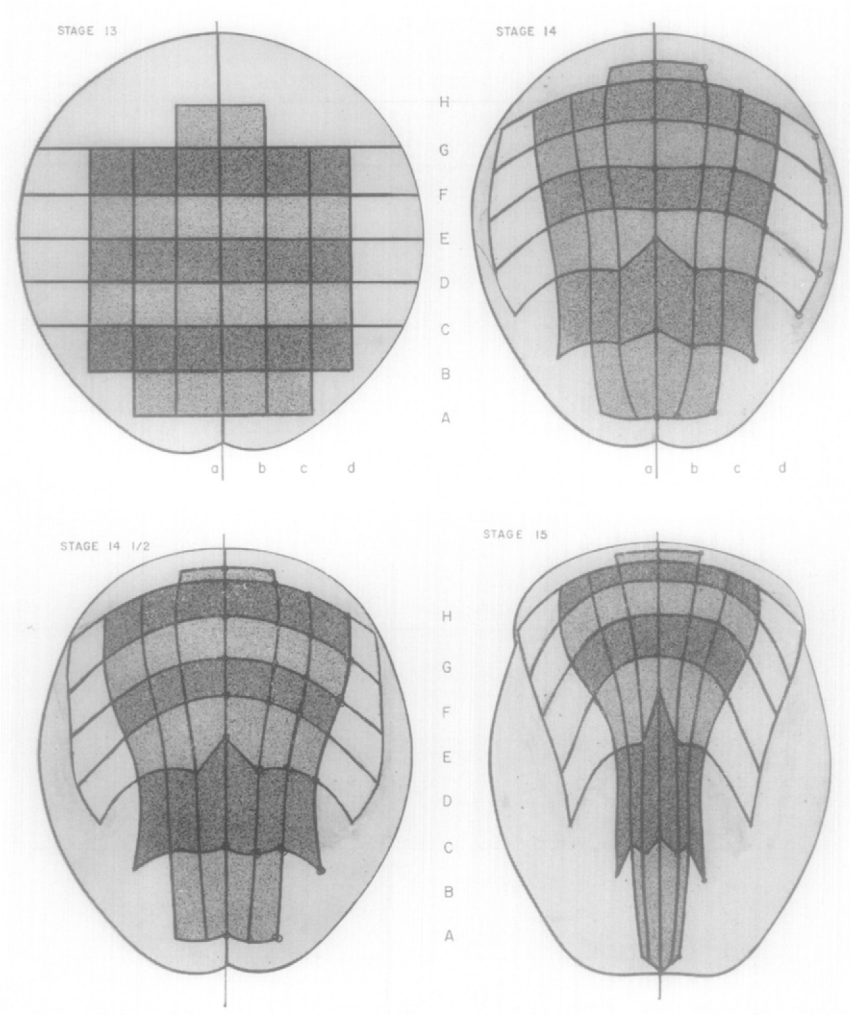


FIG. 2. Pigmented cells at the intersections of the lines of the coordinate grid on the stage 13 embryo were traced by time-lapse cinematography through subsequent stages shown. The transformation of the coordinate grid is shown at each stage. Three replicate cases are plotted (open circles) on the right half of stage 14½ and the traced cells virtually overlie each other, indicating the regularity of these movements among different embryos. At stage 13, the total area of the shaded squares in the right half of the embryo was 1.24 mm^2. This total area decreased 4.2% by stage 14, 6.0% by stage 14½, and 38% by stage 15. Each square at stage 13 was 0.69 mm^2 in area. See text for additional explanation.

TABLE 2

PERCENTAGE CHANGES IN AREA, COMPARED TO STAGE 13, OF REGIONS OF THE NEURAL PLATE INDICATED BY GRID COORDINATES FROM FIG. 2

	a	b	c	d	
H					H
Stages					
14	−54				
14½	−60				
15	−76				
G					G
14	+12	−29	−28		
14½	−6	−36	−14		
15	−65	−72	−54		
F					F
14	+11	−40	−17		
14½	+11	−31	+7		
15	−40	−46	−28		
E					E
14	0	−41	−4		
14½	−20	−30	−20		
15	−43	−49	−13		
D					D
14	−17	−11	+1		
14½	−2	−20	+1		
15	−42	−14	−35		
C					C
14	+54	+8	+22		
14½	+58	+12	+27		
15	+4	−31	−20		
B					B
14	+23	+16			
14½	+22	+8			
15	0	−53			
A					A
	a	b	c	d	

embryos on Fig. 2, stage 14½. The points at each intersection overlie each other so closely that they are difficult to distinguish from one another.

If the cell movements observed are the result of changes in the shapes of individual cells, then regions that change in surface area between stages 13 and 15 might be expected to show inversely correlated changes in the height of the columnar epithelium that composes the forming neural plate.

The height of the simple columnar epithelium was measured along the midline of the neural plate in sagittal sections of stage 13 and stage 15 embryos. Sections of these stages are illustrated in Figs. 3

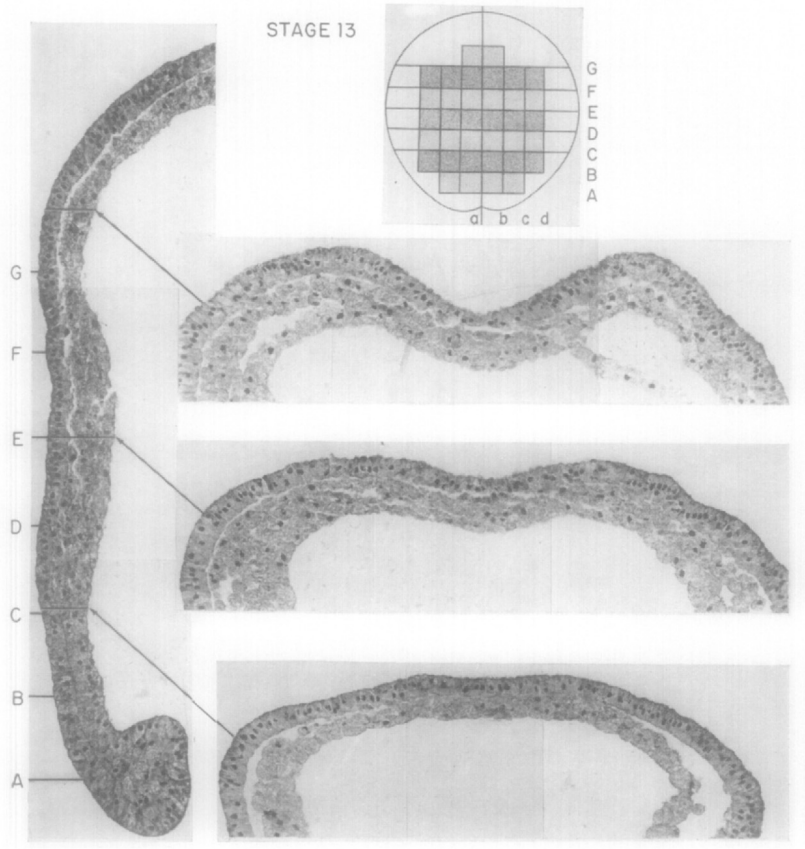


FIG. 3. Mid-sagittal section and transverse sections of embryos at stage 13. Regions of the sagittal section corresponding to the horizontal lines (A-G) of the coordinate grid are indicated. ×136.

and 4 and the measurements are given in Table 3. The percentage change in epithelium height is compared to the percentage change in area of regions along the midline in Fig. 5. A rough inverse correlation is evident.

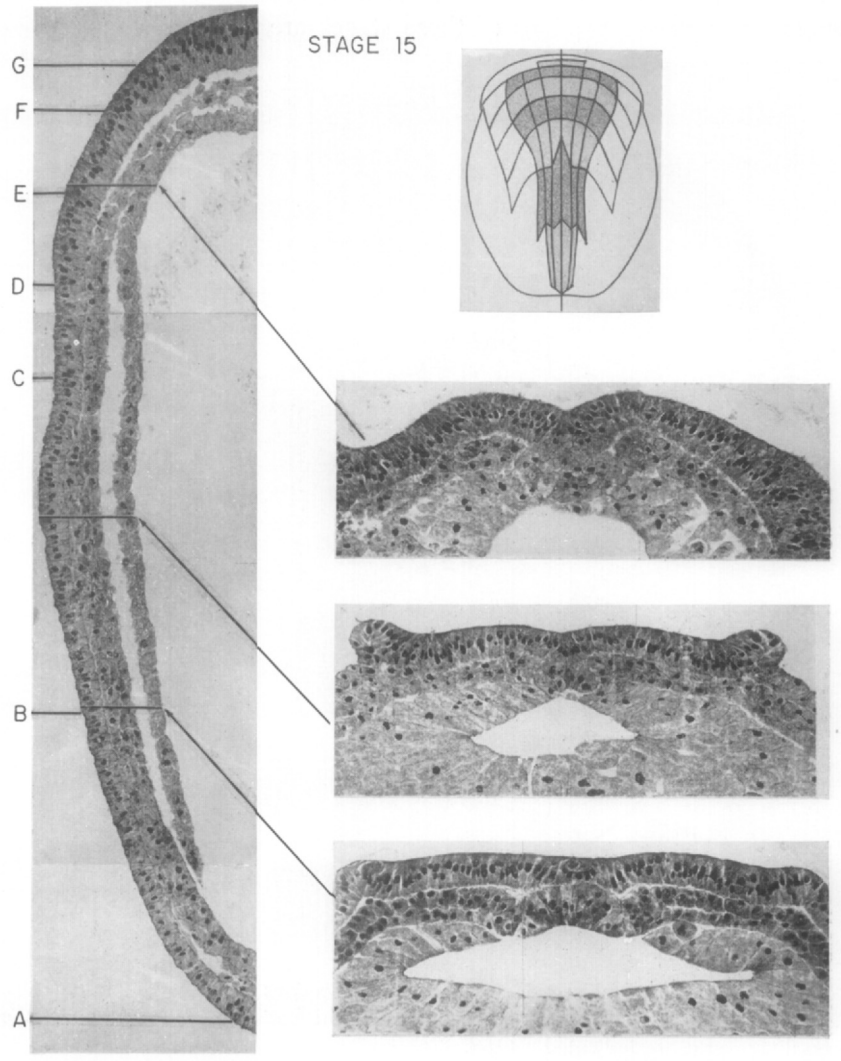


FIG. 4. Mid-sagittal section and transverse sections of embryos at stage 15. Horizontal coordinate grid lines are indicated on the sagittal section. Compare changes in height of the neural epithelium with regional area changes indicated by the transformed coordinate grid and by the values in Table 2 and Fig. 5. ×136.

TABLE 3

MEASUREMENTS OF HEIGHT OF THE NEURAL EPITHELIUM IN THE REGIONS INDICATED IN FIGS. 3 AND 4[a]

Region	Stage 13, av. height (μ)	SD	Stage 15, av. height (μ)	SD	Percent change in height from stage 13–15	Percent change in area for regions in column a–b	Region
A–B	55.4	1.6	51.1	1.2	-8 ± 10	± 0	A–B
B–C	53.2	0.4	51.8	0.4	-3 ± 4	$+4$	B–C
C–D	47.5	0.4	63.4	0.2	$+33 \pm 2$	-42	C–D
D–E	54.7	0.3	86.4	0.3	$+58 \pm 2$	-43	D–E
E–F	51.1	0.5	86.4	0.2	$+69 \pm 3$	-40	E–F
F–G	51.1	0.2	93.6	0.5	$+83 \pm 3$	-65	F–G

[a] The percentage change in height was calculated from average heights of samples from the two stages; the indicated range is for the extremes within two standard deviations (SD).

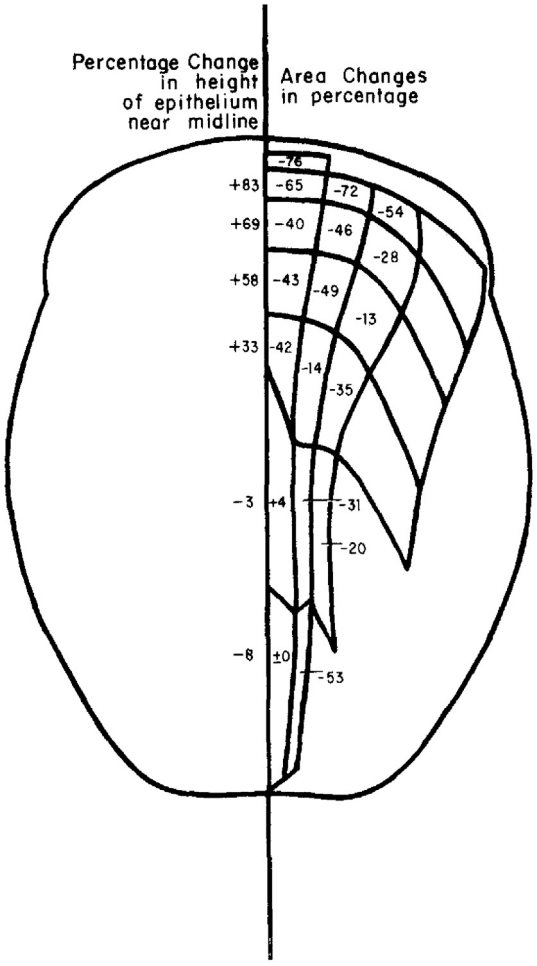


FIG. 5. On this outline of a stage 15 embryo, percentage changes in area compared to stage 13 are indicated in selected squares of the transformed coordinate grid on the right. A rough inverse correlation with percentage changes in height of the epithelium can be noted near the midline (see Table 3).

These measurements are rough since any comparison between even well-fixed material and living systems is somewhat distorted by whatever shrinkage or swelling may occur during preparation of the tissue for sectioning and microscopy. However, this variable is probably consistent in all regions. More important is the consideration that the sagittal section comprises only a small part of the region making up the quadrilateral involved in the area measurement. Examination of

cross-sections at various regions (see Figs. 3 and 4) supported the inverse correlation indicated by the sagittal sections and the changes in cell shape may indeed account for the surface area changes, and thereby for the visible relocations of the traced cells.

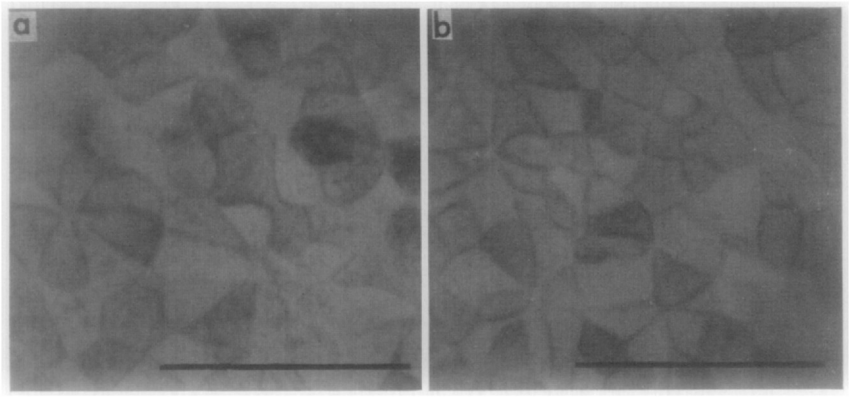


FIG. 6. Photographs of the dorsal surface of early stage 13 embryo (a) and stage 14 embryo (b) showing the appearance of cells in the higher magnification time-lapse films. Note visibility of cell boundaries that made possible the tracings shown in Fig. 7. The bar in both a and b represents 100 μ.

From time-lapse movie films made at higher magnifications (Fig. 6) it was possible to trace the dorsal outlines of individual cells engaged in the movements mapped from lower magnification films. Drawings of cells photographed from stage 14 to 14½ (approximately) at 12.5-min intervals (Fig. 7) show that no cell established contact with a new neighbor during the period considered. Although the relative amounts of contact of adjacent cells varied, no new contacts were established. Area measurements of 10 cells showed an increase in area of 5% for the region photographed (approximately region EFbc in Fig. 2). This change is in keeping with the 9% increase between stages 14 and 14¾ obtained for this area from the tracings at lower magnifications (Table 2).

These higher magnification movie films also made it quite clear that the pigmented spots followed in the lower magnification films represented single pigmented cells, or small groups of pigmented cells that remained in association. The darkly pigmented cells retain their color throughout the observation period and no lightly pigmented cells become darkly pigmented. Divisions among such cells result in similarly colored daughter cells that remain in close proximity.

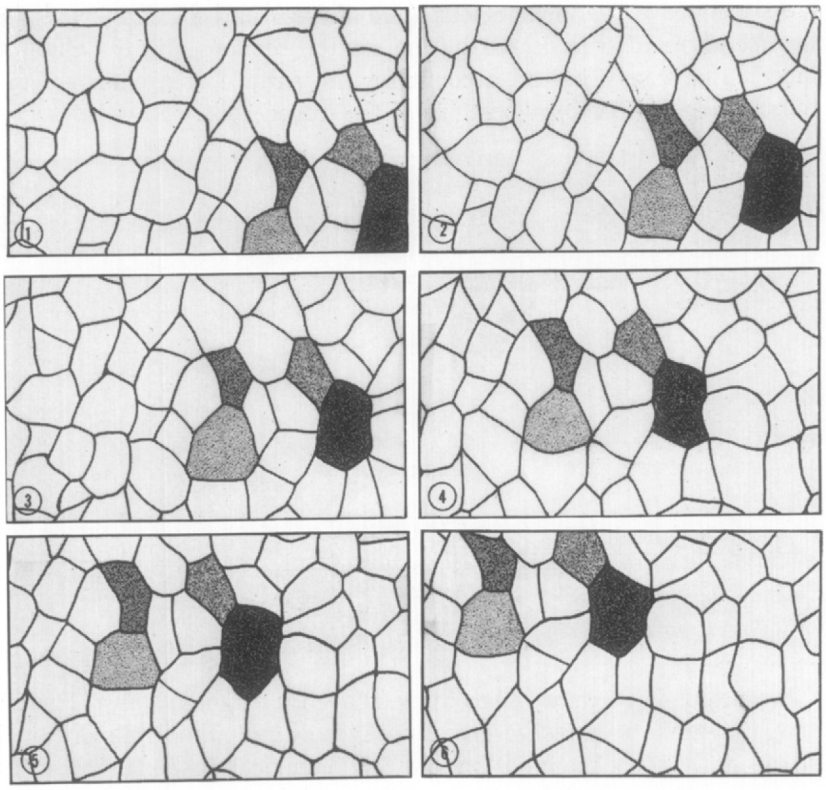


FIG. 7. Tracings of time-lapse films of a region in square EFbc (Fig. 2) at 12.5-min intervals between stages 14 and 14½ approximately (since the end point of closing neural folds used for analysis of low magnification films is impractical with these high magnification films, approximate staging by morphological appearance is required). The visible surface area of the cells increases slightly (about 5% by measurement of 10 cells with an Adisco area measurer). This increase is in accord with measurements made from Fig. 2 of the region involved (square EFbc) which showed an increase in area of 9% during this period. Note the maintenance throughout the period of the original cell contact relationships. No cells were seen to establish contact with a new neighbor.

DISCUSSION

We have proposed that the morphogenetic movements during early neural plate formation may be accounted for by observed changes in the shapes of the cells in the neural epithelium. Jacobson (1962) also noted correlation between neural plate cell height increase and

dorsal surface area decrease. The regions showing the greatest amounts of displacement are those near the center of the neural plate (Fig. 1), where the cells are displaced toward the midline and anteriorly in a convergent pattern. The region where least displacement was observed (midline in A-B region in Fig. 2) appeared as a "dead-point" in the time-lapse films. Cells moved away from this point both anteriorly and posteriorly. The columnar epithelial cells of this region became flattened between stages 13 and 15 (sections in Figs. 3 and 4), and the cells viewed from the dorsal surface appeared to move away from the center of the thinning region, both anteriorly and posteriorly.

If changes in cell shape are responsible for the movements that give the early neural plate its form, then the amount of change in height of the epithelium, the rate of change in height, and the timing of initiation and stopping of these changes are all important factors in the control of these morphogenetic movements.

Defining a mechanism for initiating and controlling these changes is at present only conjecture. The time when these changes begin, however, corresponds to the time when the advancing archenteron roof comes to underlie the anterior neural plate region. The initiation of changes in cell shape could possibly be one consequence of embryonic induction.

SUMMARY

These time-lapse cinematographic studies provide measurements and descriptions of the pathways and speeds of displacement of cell groups in the forming neural plate of a newt. At speeds ranging from 4 to 95 μ per hour, cell groups are displaced almost as much as 1 mm (896 μ) on a 2.5 mm embryo during plate development prior to neural tube formation. Changes in area of regions of the surface of the neural plate are presented and are found to correlate inversely with changes in height of the neural plate epithelium. Observations with high magnification time-lapse films establish that cells retain their contact relationships with neighboring cells throughout the period of study.

The newt neural plate is a sheet of cells just one cell thick. Observed displacements of groups of neural plate cells are the consequence of deformations of the sheet. Observations and correlations indicate that these deformations are the result of regional differences in the amounts of change in cell shape of the constituent cells.

Thus one consequence of primary embryonic induction is a patterned change in the height of the cells in the forming neural plate with concomitant displacements of cell groups and regional changes in area that give the neural plate its characteristic shape.

REFERENCES

EPSTEIN, M. J. (1963). Mechanism and control of cell movement during amphibian neurulation. M. A. Thesis, University of Texas at Austin, unpublished.

GOERTTLER, K. (1925). Die Formbildung der Medullaranlage bei Urodelen. *Arch. Entwicklungsmech. Organ.* **106,** 503–541.

GUYER, M. G. (1953). "Animal Micrology," p. 236. Univ. of Chicago Press, Chicago, Illinois.

HUMASON, G. L. (1962). "Animal Tissue Techniques," p. 16. Freeman, San Francisco, California.

JACOBSON, C.-O. (1962). Cell migration in the neural plate and the process of neurulation in the Axolotl larva. *Zool. Bidr. Uppsala* **35,** 433–449.

JONES, R. M. (1964). *In* "McClung's Handbook of Microscopical Technique," pp. 180–181. Hafner, New York.

MANCHOT, E. (1929). Abgrenzung des Augenmaterials und andere Teilbezirke in der Medullarplatte; die Teilbewegungen während der Auffaltung. *Arch. Entwicklungsmech. Organ.* **116,** 689–708.

RUGH, R. (1962). "Experimental Embryology," p. 90, Burgess, Minneapolis, Minnesota.

VOGT, W. (1929). Gestaltungsanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Arch. Entwicklungsmech. Organ.* **120,** 384–706.

DEVELOPMENTAL BIOLOGY 26, 560–577 (1971)

Nuclear Elongation and Cytokinesis in *Drosophila montana*

SUSAN L. FULLILOVE AND ANTONE G. JACOBSON

Department of Zoology, University of Texas, Austin, Texas 78712

Accepted September 1, 1971

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, postfixed 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.

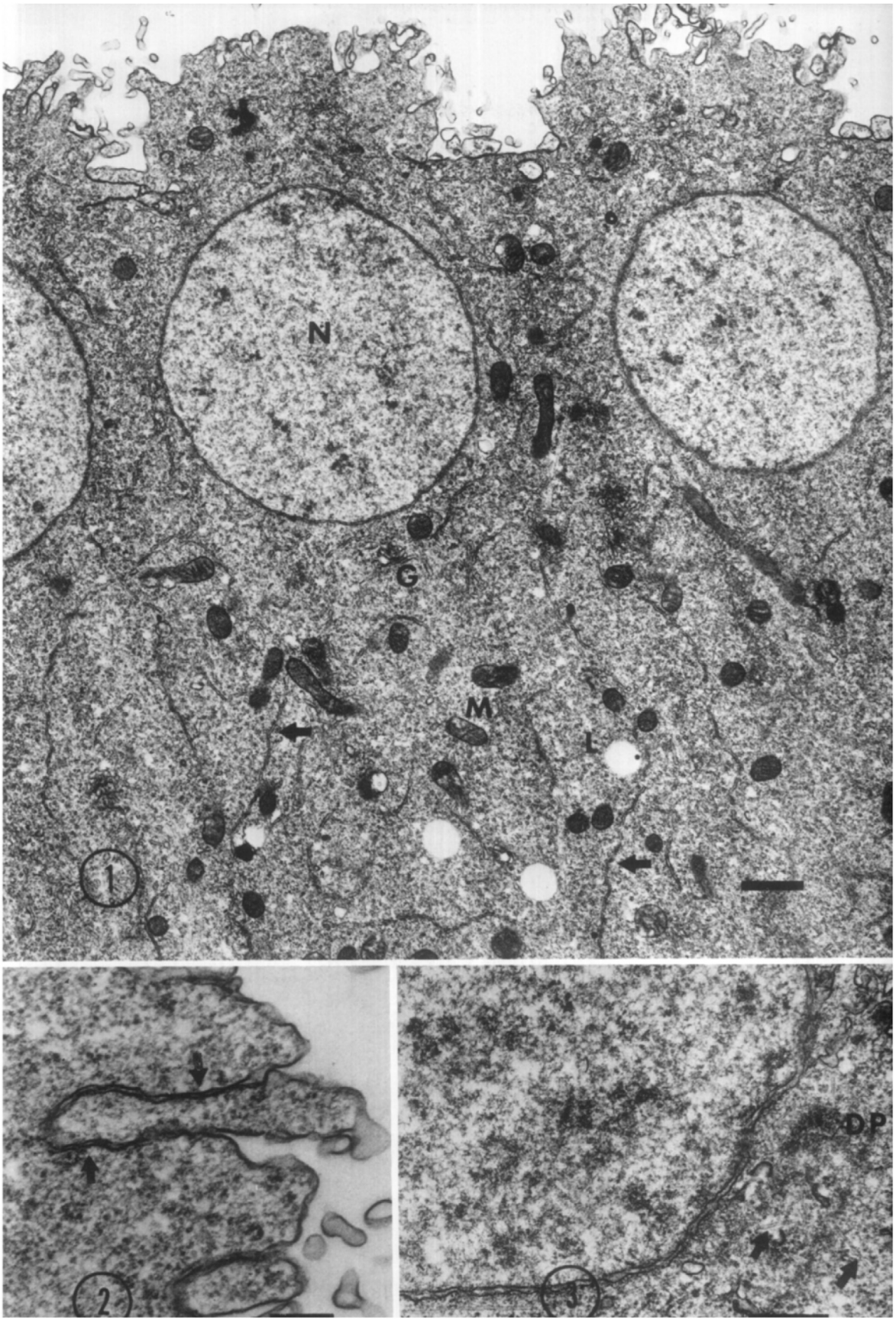
OBSERVATIONS

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


N
G
M
L
1
2
3
DP

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.

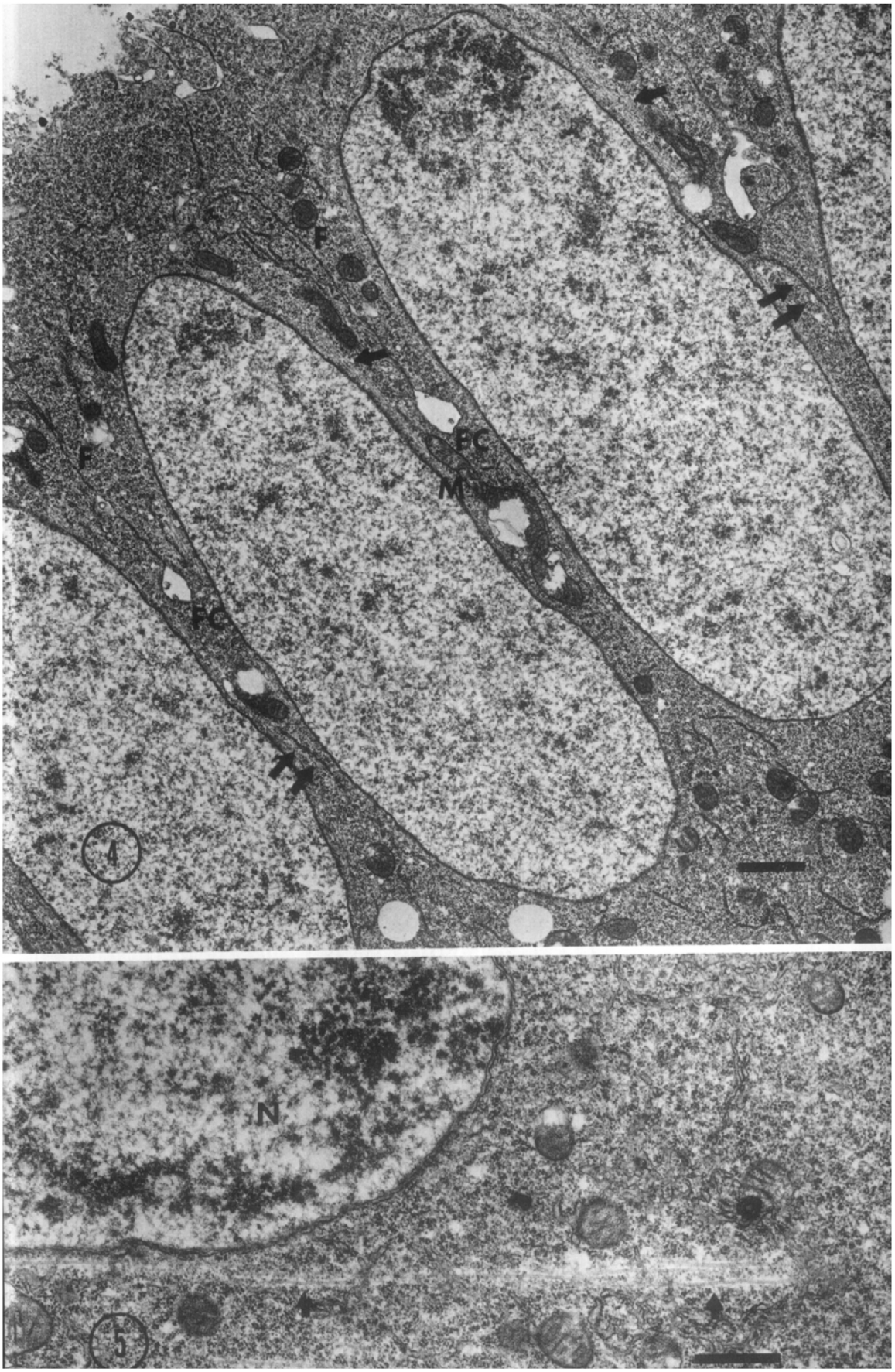
Cleavage

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.


F
F
BC
M
FC
1
N
5

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.

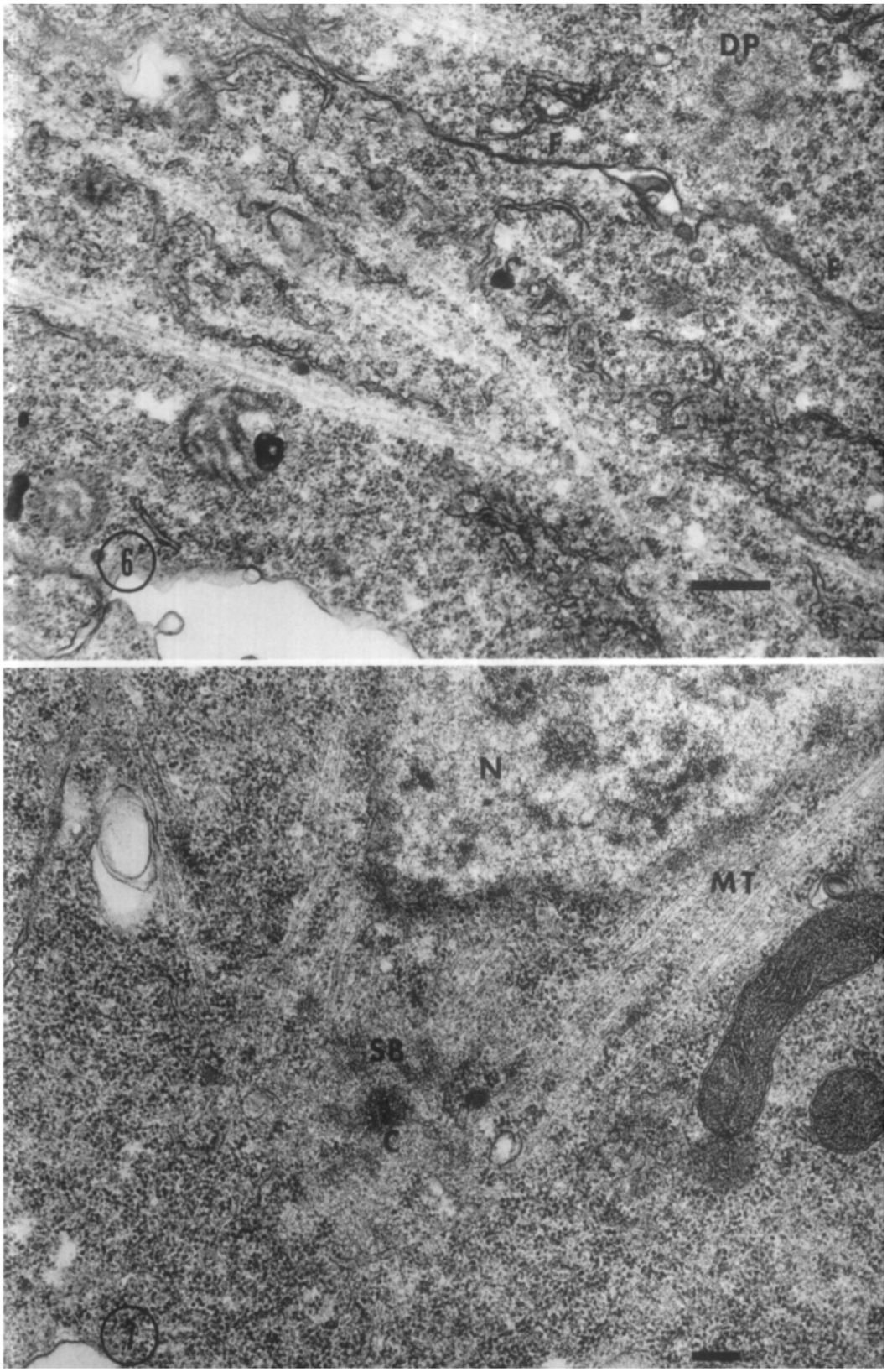
At the end of the twelfth nuclear division a tremendous amount of activity begins in the heretofore quiescent peripheral cytoplasm. This activity, seen in time-lapse cinematography, seems to involve movement of cytoplasmic components back and forth in a direction perpendicular to the surface of the egg. 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.


DP
F
6
N
MT
SB
C
1

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.

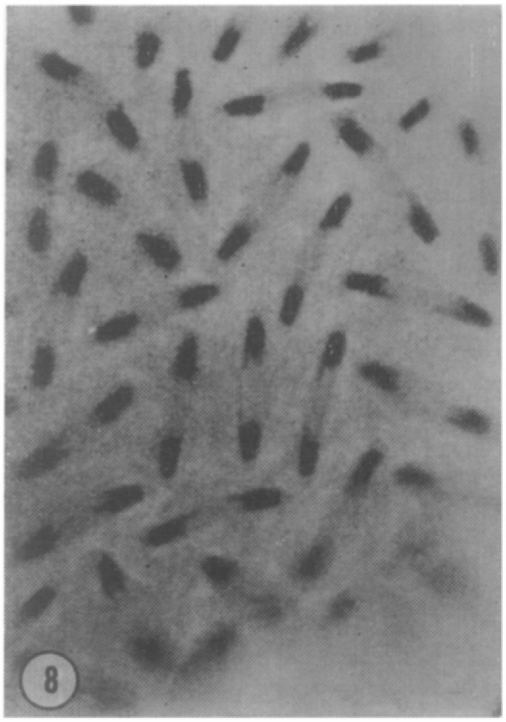
and parallel to the path of nuclear elongation strongly suggests that these organelles play a role in the elongation process. It is possible that they may be actively involved in the elongation, in some way stretching the nuclei out to their maximum length. However, since the nuclei increase their volume 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



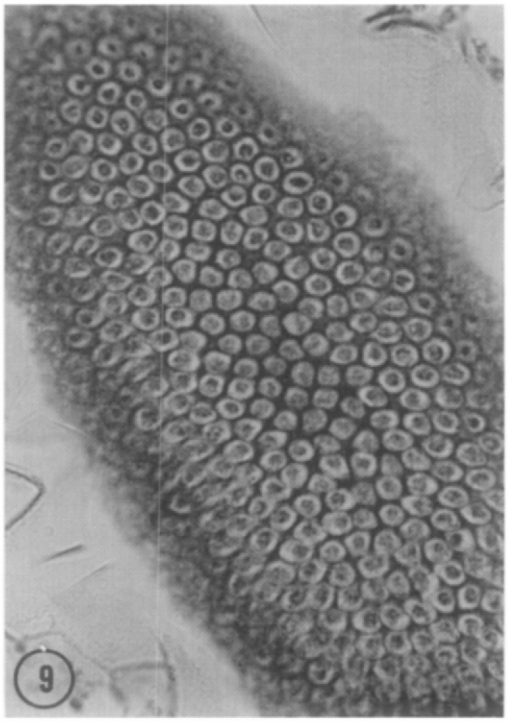


FIG. 8. A tangential section of *Drosophila melanogaster* egg during the twelfth nuclear division. Division spindles are parallel to the surface of the egg. Taken from Sonnenblick (1950) Fig. 17 with permission.

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.

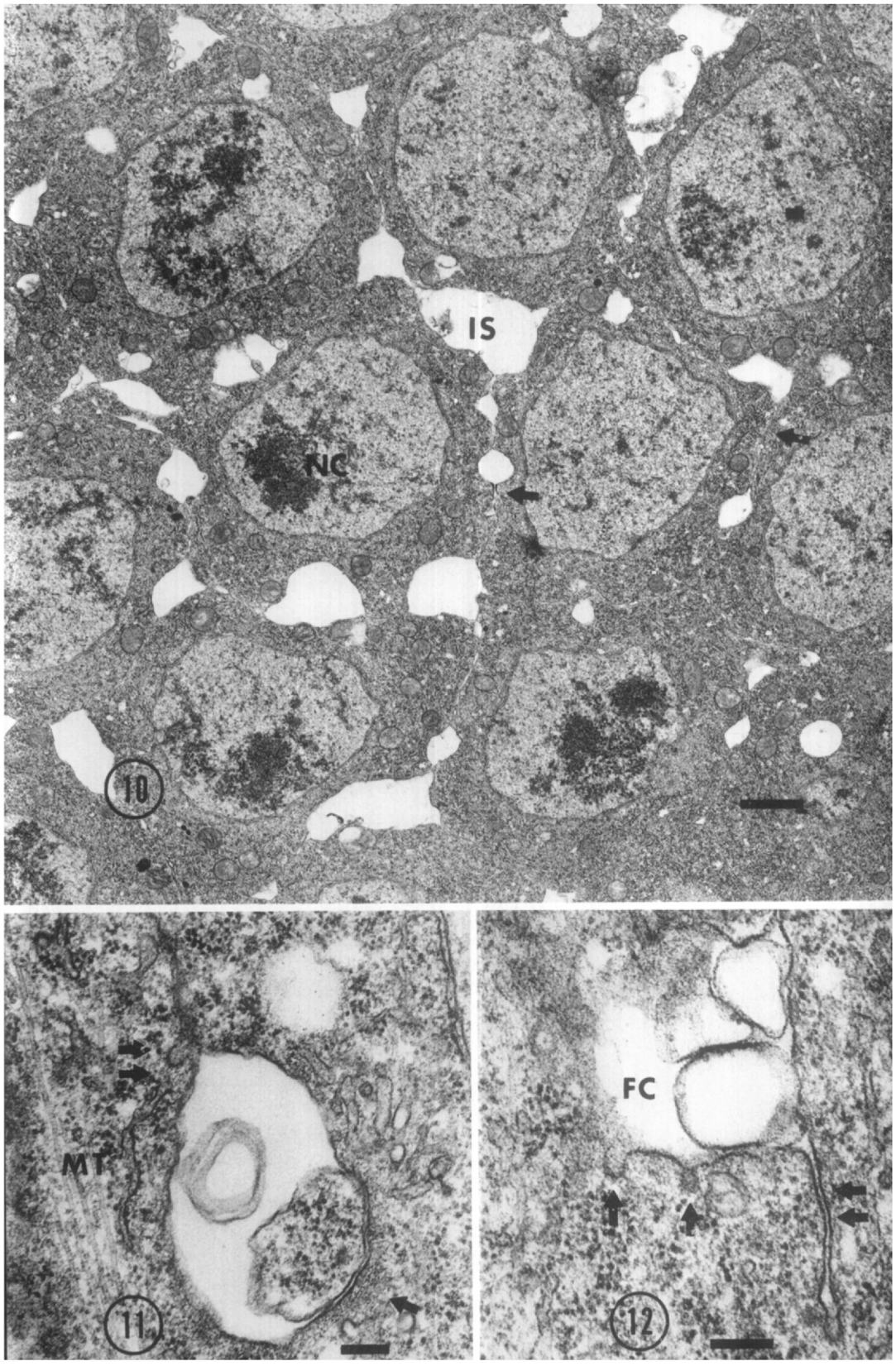
In summary, the microtubules associated with elongating nuclei of the *Drosophila* blastema are probably directly involved in the elongation process. It is most likely that they form a rigid framework which passively forces elongation of the nuclei as the nuclei increase in volume. The tubules may also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


IS
NC
10
MT
11
FC
12

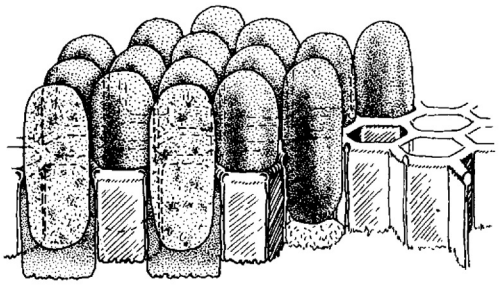


FIG. 13. Schematic representation of a longitudinal section through an egg during cytokinesis. The bottom of the drawing represents the surface of the egg. Cleavage furrows partially surround the nuclei as they extend inward from the surface. The furrows are preceded into the interior of the egg by the 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

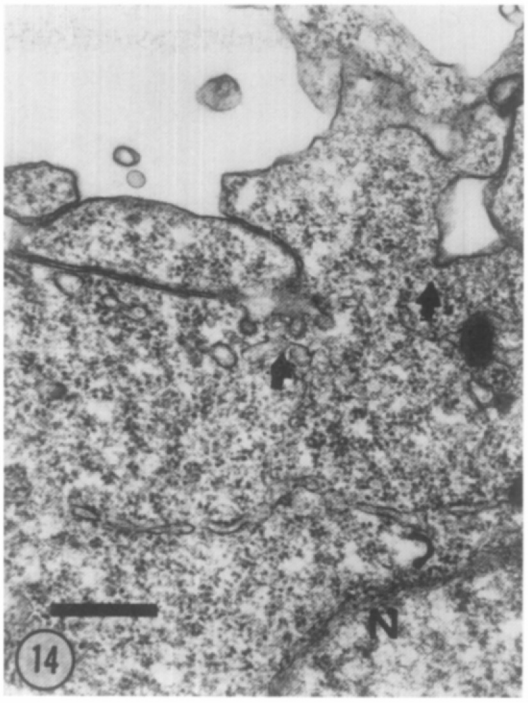


FIG. 14. Surface of an egg fixed at 3.5 hr post-deposition. Coated vesicles are attached to the surface membrane at arrows above a spherical nucleus (*N*). Bar represents 0.5 μ.

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.

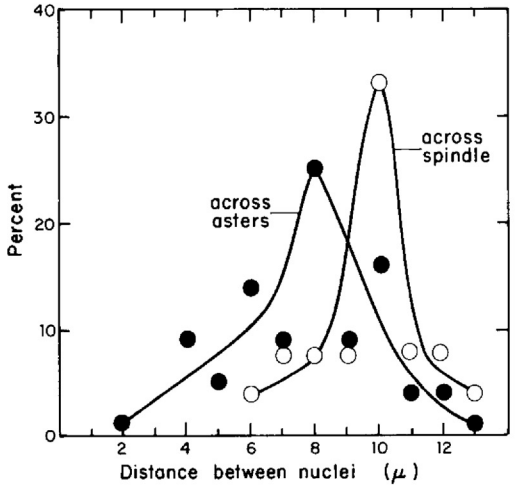


FIG. 15. Distribution of distances between nuclei as represented by the chromosomal masses of the division figures in Fig. 8. A total of 76 measurements were made across adjacent asters; 24 measurements were made across spindles. Measurements are expressed as percentage of the total number in each category.

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*.

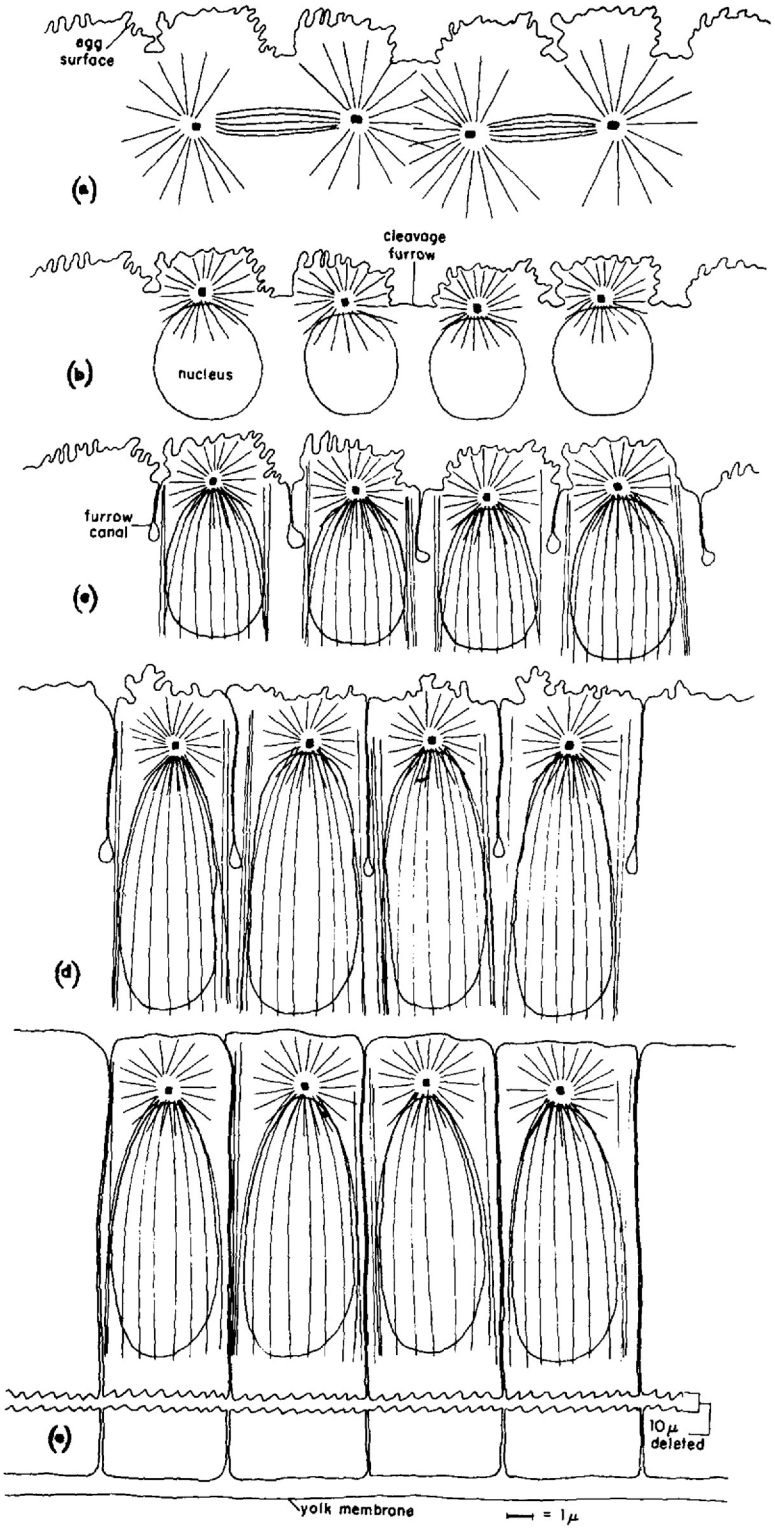


FIG. 16. Schematic representation of the 12th nuclear division, nuclear elongation, and cytokinesis in *Drosophila montana*.

This investigation was supported by U.S. Public Health Service Research Grant No. HD 03803-03 from the National Institute of Child Health and Human Development.

REFERENCES

ANDERSON, E. (1962). The ovary of *Periplaneta americana*. Second Ann. Meeting Am. Soc. Cell Biol., San Francisco, p.2.

ARNOLD, J. M. (1969). Cleavage furrow formation in a telolecithal egg (*Loligo pelii*) I. Filaments in early furrow formation. *J. Cell Biol.* **41**, 894–904.

ARNOLD, J. M. (1971). Cleavage furrow formation in a telolecithal egg (*Loligo pelii*). II. Direct evidence for a contraction of the cleavage furrow base. *J. Exp. Zool.* **176**, 73–86.

BODENSTEIN, D. (1953). Embryonic development. *In* "Insect Physiology" (K. D. Roeder, ed.), pp. 780–821. John Wiley, New York.

BRANSON, R. (1968). Orthogonal arrays of microtubules in flattening cells of the epidermis. *Anat. Rec.* **160**, 109–113.

BYERS, B. and PORTER, K. R. (1964). Oriented microtubules in elongating cells of the developing lens rudiment after induction. *Proc. Nat. Acad. Sci. U. S.* **52**, 1091–1099.

CLERMONT, Y. (1964). Motility of the flagellum and cytoplasmic membranes of the rat spermatids. *Anat. Rec.* **148**, 271 (Abstract).

COUNCE, S. J. (1961). The analysis of insect embryogenesis. *Ann. Rev. Entomol.* **6**, 295–312.

DE-THÉ, G. (1964). Cytoplasmic microtubules in different animal cells. *J. Cell Biol.* **23**, 265–275.

GIBBINS, J. R., TILNEY, L. G., and PORTER, K. R. (1969). Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. I. The distribution of microtubules. *J. Cell Biol.* **41**, 201–226.

GOODENOUGH, D. A., ITO, S., and REVEL, J. P. (1968). Electron microscopy of early cleavage stages in *Arbacia punctulata*. *Biol. Bull.* **135**, 420–421.

KESSEL, R. G. (1966). The association between microtubules and nuclei during spermiogenesis in the dragon fly. *J. Ultrastruc. Res.* **16**, 293–304.

KESSEL, R. G. (1967). An electron microscope study of spermiogenesis in the grasshopper with particular reference to the development of microtubular systems during differentiation. *J. Ultrastruc. Res.* **18**, 677–693.

KESSEL, R. G. and BEAMS, H. W. (1963). Micropinocytosis and yolk formation in oöcytes of the small milkweed bug. *Exp. Cell Res.* **30**, 440–443.

KINSEY, J. D. (1967). Studies on an embryonic lethal hybrid in *Drosophila*. *J. Embryol. Exp. Morphol.* **17**, 405–423.

MCINTOSH, J. R. and PORTER, K. R. (1967). Microtubules in the spermatids of the domestic fowl. *J. Cell Biol.* **35**, 153–173.

MAHOWALD, A. P. (1963a). Electron microscopy of the formation of the cellular blastoderm in *Drosophila melanogaster*. *Exp. Cell Res.* **32**, 457–468.

MAHOWALD, A. P. (1963b). Ultrastructural differentiations during formation of the blastoderm in the *Drosophila melanogaster* embryo. *Develop. Biol.* **8**, 186–204.

MOLLENHAUER, H. H. (1964). Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39**, 111–114.

OVERTON, J. (1966). Microtubules and microfibrils in morphogenesis of the scale cells of *Ephestia kühniella*. *J. Cell Biol.* **29**, 293–305.

PEARCE, T. L. and ZWAAN, J. (1970). A light and electron microscopic study of cell behavior and microtubules in the embryonic chicken lens using Colcemid. *J. Embryol. Exp. Morphol.* **23**, 491–507.

PORTER, K. R. (1966). Cytoplasmic microtubules and their functions. *Ciba Found. Symp., 1965, Principles Biomol. Organ.* p. 308.

POULSON, D. F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster*. *In* "The Biology of *Drosophila*" (M. Demerec, ed.), pp. 168–274. Wiley, New York.

RAPPAPORT, R. (1965). Geometrical relations of the cleavage stimulus in invertebrate eggs. *J. Theoret. Biol.* **9**, 51–66.

RAPPAPORT, R. (1969). Aster-equatorial surface relations and furrow establishment. *J. Exp. Zool.* **171**, 59–67.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

ROBBINS, E. and GONATAS, N. K. (1964). The ultrastructure of a mammalian cell during the mitotic cycle. *J. Cell Biol.* **21**, 429–463.

ROTH, T. F. and PORTER, K. R. (1962). Specialized sites on the cell surface for protein uptake in the oöcyte of the mosquito *Aedes aegypti*. *Proc. Int. Congr. Electron Microsc. 5th, Philadelphia, 1962*, LL-3.

ROTH, T. F. and PORTER, K. R. (1964). Yolk protein uptake in the oöcyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* **20**, 313–332.

SABATINI, D. D., BENSCH, K. C., and BARRNETT, R. J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**, 19–58.

SCHROEDER, T. E. (1968). Cytokinesis: Filaments in the cleavage furrow. *Exp. Cell Res.* **53**, 272–276.

SCHROEDER, T. E. (1969). The role of "contractile ring" filaments in dividing *Arbacia* eggs. *Biol. Bull.* **137**, 413–414.

SCHROEDER, T. E. (1970). Functional and biochemical aspects of contractile ring filaments in HeLa cells. *J. Cell Biol.* **47**, 183a.

SELMAN, G. G. and PERRY, M. M. (1970). Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. *J. Cell Sci.* **6**, 207–227.

SHEFFIELD, J. B. (1970). Studies on aggregation of embryonic cells: Initial cell adhesions and the formation of intercellular junctions. *J. Morphol.* **132,** 245.

SONNENBLICK, B. P. (1950). The early embryology of *Drosophila melanogaster*. *In* "The Biology of *Drosophila*" (M. Demerec, ed.), pp. 62–167. Wiley, New York.

STAY, B. (1965). Protein uptake in the oocytes of the Cecropia moth. *J. Cell Biol.* **26,** 49–62.

SZOLLOSI, D. (1964). The structure and function of centrioles and their satellites in the jellyfish, *Phialidium gregarium*. *J. Cell Biol.* **21,** 465–479.

SZOLLOSI, D. (1968). A cytoplasmic filament array associated with the cleavage furrow. *Anat. Red.* **160,** 437 (Abstract).

TILNEY, L. G. (1968). The assembly of microtubules and their role in the development of cell form. *Develop. Biol.*, Suppl. 2, 63–102.

TILNEY, L. G., and GIBBINS, J. R. (1969). Microtubules in the formation and development of the primary mesenchyme in *Arbacia punctulata*. II. An experimental analysis of their role in development and maintenance of cell shape. *J. Cell Biol.* **41,** 227–250.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4,** 4751.

WILSON, J. C. (1970). Analysis of pre-blastoderm cytoplasmic and nuclear movements in *Drosophila montana*. M. A. Thesis, University of Texas at Austin.

YAMADA, K. M. SPOONER, B. S., and WESSELLS, N. K. (1970). Axon growth: Roles of microfilaments and microtubules. *Proc. Nat. Acad. Sci. U.S.* **66,** 1206.