REGULATION OF MICROTUBULES IN *TETRAHYMENA*

I. Electron Microscopy of Oral Replacement

NORMAN E. WILLIAMS and JOSEPH FRANKEL

From the Department of Zoology, University of Iowa, Iowa City, Iowa 52240

ABSTRACT

The coupled resorption and redifferentiation of oral structures which occurs in *Tetrahymena pyriformis* under conditions of amino acid deprivation has been studied by transmission electron microscopy. Two patterns of ciliary resorption have been found, (*a*) *in situ*, and (*b*) after withdrawal into the cytoplasm. No autophagic vacuoles containing cilia or ciliary axonemes have been seen. Stomatogenic field basal bodies arise by a process of rapid sequential nucleation, with new ones always appearing next to more mature ones, even though the latter may not be fully differentiated. Accessory radial ribbons of microtubules develop immediately adjacent to oral field basal bodies as a late step in their maturation. It can be seen that the formation of basal bodies and their orientation within the oral complex are separate processes. This is true for about 130 of the approximately 170 oral basal bodies; the remaining 40 or so form within the patterned groups of ciliary units as a later event. Clusters of randomly oriented thin-walled microtubules are found surrounding oral basal bodies at all times during stomatogenesis. They may either represent stores of microtubule subunit protein, or serve as effectors of basal body movement during their orientation into pattern.

INTRODUCTION

Oral replacement in *Tetrahymena*, which is the resorption of old feeding structures and the simultaneous redevelopment of new feeding structures, is an advantageous system for the study of the regulatory mechanisms by which cells control both the assembly and dissociation of cellular structures. The oral apparatus (OA) of *Tetrahymena* is essentially a microtubular complex, consisting of many ciliated basal bodies organized into a specific pattern and interconnected within the cell by other microtubular structures (4, 16, 22). Differentiation and regression of the OA therefore presents the general problem of the regulation of microtubule structures within cells. This problem is of general interest, being of central importance in the understanding of the formation and regression of mitotic spindles, neurotubules, axopodia, and various other structures occurring in a wide variety of cells. The present paper is the first in a series designed to explore this problem in the ciliate *Tetrahymena*.

The occurrence of oral replacement in the genus *Tetrahymena* has recently been reviewed (9). In *T. pyriformis*, used in the present study, oral replacement occurs in nutrient medium lacking amino acids and can be synchronized by intermittent or continuous 34°C heat shocks (6, 7). The sequence of developmental events observed within the developing OA during oral replacement appears to be the same as that observed in the formation of new oral structures during cell division; only the site is different. In division, the new OA forms in the midequatorial region of the

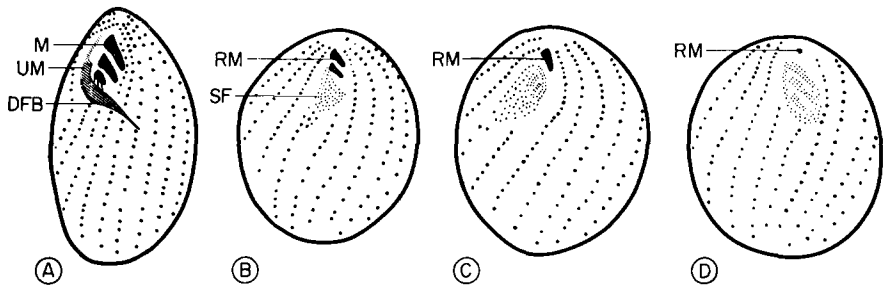


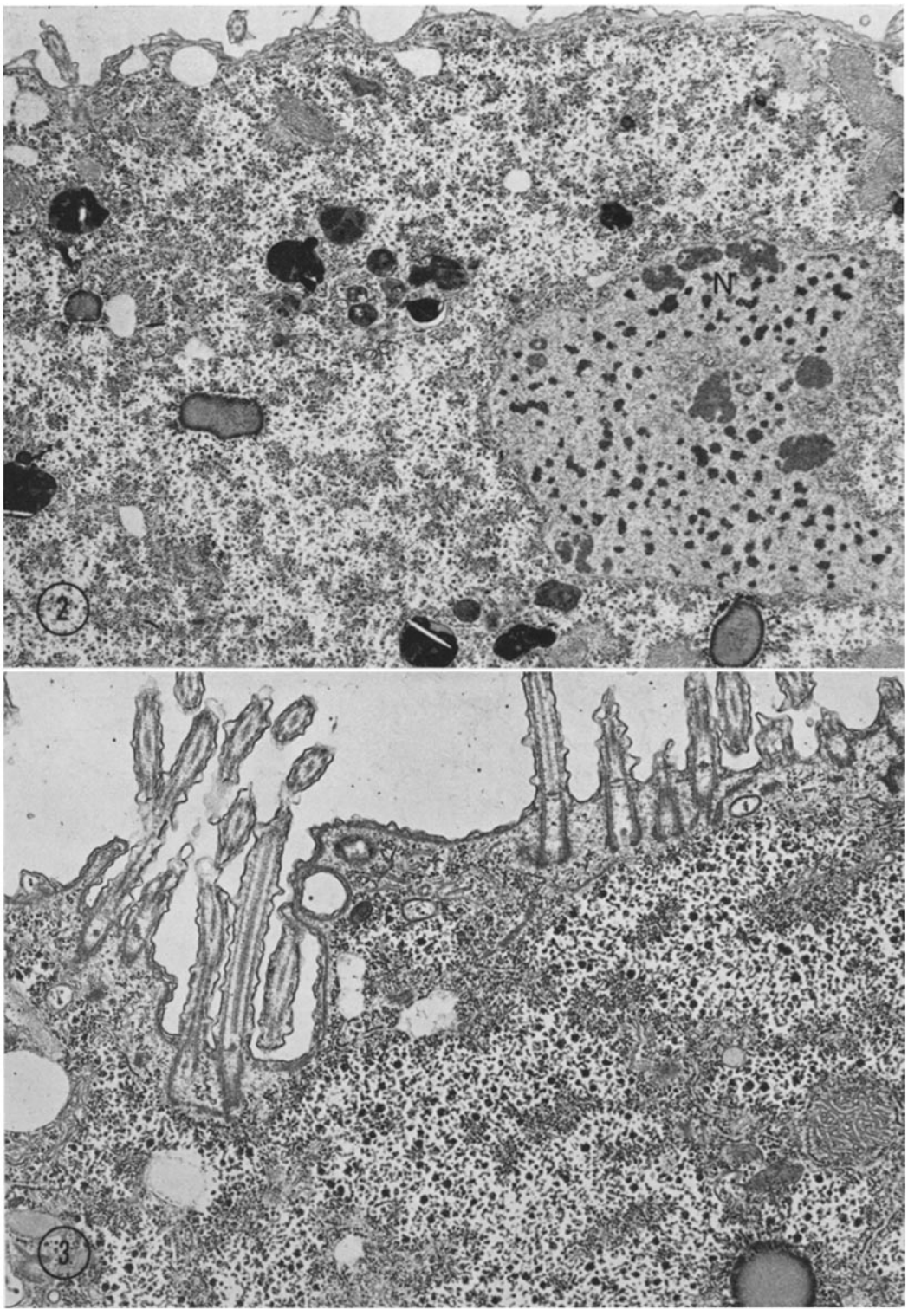
FIGURE 1 Oral replacement in *T. pyriformis*, strain GL-C. Diagram A (left) shows a normal nondeveloping cell. The oral area includes three membranelles (*M*), an undulating membrane (*UM*), and a microtubular deep fiber bundle (*DFB*). Membranelle one is on the cell's left, membranelle three is closest to the *UM*, and membranelle two is in the center. Diagrams B, C, and D show stages of oral replacement. In each of these the regressing membranelle remnants (*RM*) are diagrammed as homogenous masses, though in fact composed of separate basal bodies. In the developing oral primordium, situated posterior to the regressing old structures, the basal bodies are represented individually by dots. Initially the basal bodies of the developing oral structures constitute a stomatogenic field (*SF*) that has no obvious spatial organization (diagram B). The basal bodies of this field then begin to undergo mutual alignment to form double-rowed membranelle rudiments (diagram C). These organized areas subsequently extend posteriorly, a third row begins to appear at the anterior end of each membranelle, and the new UM becomes organized (diagram D). The new oral structures move anteriorly, simultaneously undergoing completion of ciliary structures and formation of a deep fiber bundle. This returns the cell to the configuration shown in diagram A.

parent cell cortex and passes to the posterior division product. In oral replacement, the new OA forms immediately behind the old OA and ultimately takes the exact position of the old OA after the latter's resorption is complete. The cells temporarily round up but do not divide.

The present study is an electron microscope investigation of oral replacement in synchronized *T. pyriformis*, strain GL-C. The regulation of oral structures is more easily studied at the ultrastructural level in this system than in synchronized dividers for a number of reasons. First, every dividing cell has a nondeveloping OA as well as a developing one, whereas all OAs are in some stage of development or regression during oral replacement. This greatly facilitates the interpretation of randomly sectioned material. Secondly, cells in oral replacement are smaller than are synchronized dividers, whereas there is no difference in the size of the OA; this means greater informational content per unit area of sectioned material. Finally, both formation and regression of microtubular structures can be studied simultaneously in oral replacement. Criteria have been discovered by which developing and regressing structures can often be distinguished from each other in sections of cells undergoing oral replacement. Some of the major events in both processes are described and discussed, including the formation of basal bodies and associated microtubule ribbons, the development and dissolution of cilia, and the orientation of ciliary units into the complex associations characteristic of the *Tetrahymena* OA.

FIGURE 2 Survey micrograph of *Tetrahymena* nucleus and cytoplasm after 19 h in amino acid-free medium. The heat-shock synchronizing treatment was given over the latter part of this interval and the sample was fixed at 60 min after EST. Visible effects of this treatment include the formation of nucleolar fusion bodies (*N*), deposition of large amounts of lipid (dense bodies in cytoplasm) and glycogen, and formation of autophagic vacuoles. × 9,000.

FIGURE 3 Longitudinal section through the oral region of a cell approximately in the stage of oral replacement indicated in Fig. 1 C. The regressing structures are in a depression in the cell surface (at the left in the micrograph), and the developing OA is on the surface of the cell posterior to this (on the right in the micrograph). × 16,000.


N
2
3

MATERIALS AND METHODS

T. pyriformis strain GL-C stock cultures were maintained at 28°C in 5-ml quantities of Frankel's medium (5) contained in 25-ml screw-capped culture tubes. The cells were kept in logarithmic growth by subculturing daily. In order to induce oral replacement, cells were first grown at 28°C in 150 ml of Frankel's medium for 20–24 h. These cultures were inoculated from day-old stocks, the size of the inoculum being adjusted so that the cell density at the end of the growth period was about 1×10^5 cells/ml. The cells were then washed twice by gentle centrifugation with amino acid-free medium and resuspended in 150 ml of this medium at a density of about 80,000 cells/ml. The deficient medium was Frankel's medium minus the amino acid source (tryptone).

The cells were kept in amino acid-deficient medium at 28°C for 8–12 h before the synchronizing treatment. During the first 5 h in deficient medium, about a 50% increase in cell number occurred, after which time there was no further increase in cell number. After 8–12 h, the heat-shock synchronization treatment began. This consisted of 7 (sometimes 8) half-hour periods at 34.0°–34.3°C separated by half-hour intervals at 28°C. After the end of the synchronizing treatment (EST) the culture was maintained at 28°C.

Cell samples were withdrawn at frequent intervals after EST and prepared for light microscope study by the silver impregnation method. The silver preparations were made according to a modified Chatton-Lwoff procedure (8). 100 cells from each sample were then tallied according to stage of oral development (6). The course of oral replacement could then be inferred from the changes in stage distributions with time.

Cell samples were also withdrawn at various times after EST and prepared for electron microscopy. The cells from 5-ml samples were fixed in 2.4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 20 min. The cells were then rinsed in one change of phosphate buffer and postfixed for 30 min in 1% osmium tetroxide in 0.1 M phosphate buffer. After this the cells were rinsed in 50% alcohol and embedded in 2% agar. Cut agar blocks were then dehydrated and embedded in Epon in the standard manner (15). Sections were cut on a Reichert ultramicrotome (American Optical Corp., Buffalo, N. Y.) and observed with an RCA-EMU-3F electron microscope.

RESULTS

Light Microscope Observations

The course of oral replacement in *T. pyriformis* as seen at the level of light microscopy has been described in detail by Frankel (6, 7), and is outlined and described in Fig. 1. Silver preparations were made at each time a sample was fixed for electron microscopy. Nearly all of the micrographs presented here are from cells fixed at EST plus 50 min, at which time the silver preparations indicated that all of the developmental stages were present. The largest percentage of cells was in the stage indicated in Fig. 1 D (49%), 6% were as indicated in Fig. 1 C, 6% as in Fig. 1 B, 36% were not developing (Fig. 1 A), and 3% were at a slightly later stage than indicated in Fig. 1 D. Samples fixed at EST plus 20, 30, 40, 60, and 70 min were also studied.

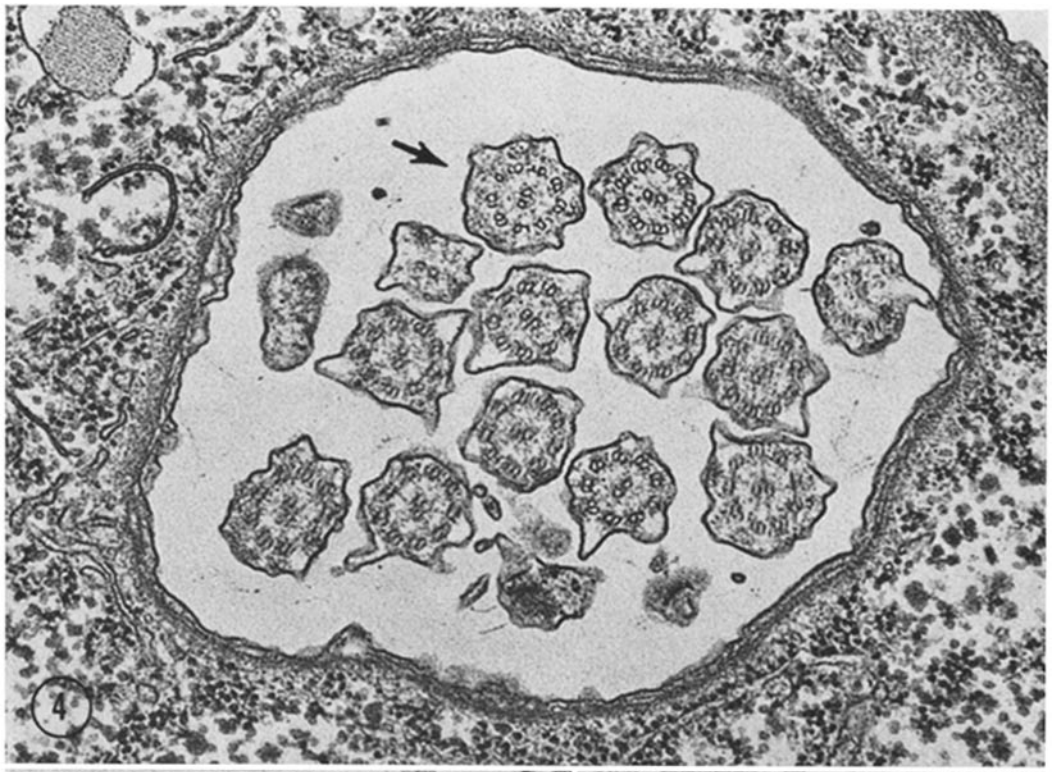
Electron Microscope Observations of Regressing OAs

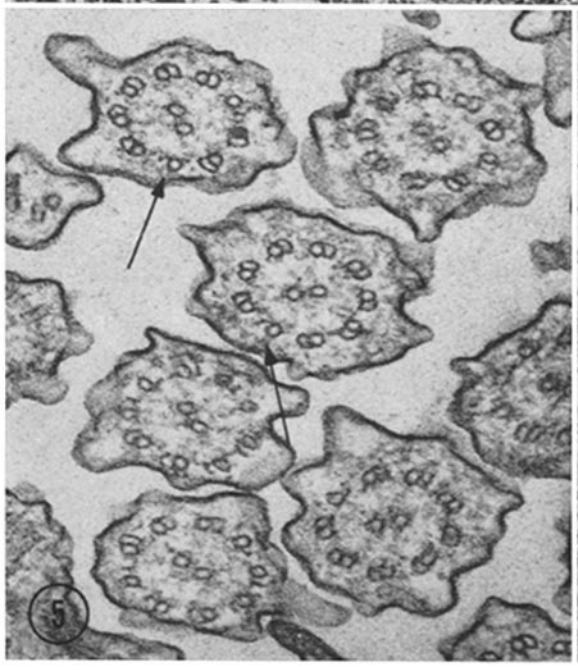
The general condition of cells subjected to the starvation and synchronizing treatments is indicated in Fig. 2. The fixation appears adequate, although these cells show more clumping of cyto-

FIGURE 4 Cross section through a depression in the cell surface containing regressing oral cilia. This depression, seen in longitudinal section in Fig. 3, is characteristic of the regressing OA and serves to identify cilia in the process of regression. The breakdown of ciliary axonemes can be seen in the cilium marked with the arrow. × 42,500.

FIGURE 5 Several oral cilia undergoing *in situ* regression of ciliary axonemes during oral replacement. Outer doublets typically regress by first losing the outer wall of the B tubule. This can be seen in six cilia in this single field, most clearly at those places indicated by the arrows. The inner wall of the B tubule is lost subsequently, then the A tubule regresses. × 72,000.

FIGURE 6 A swollen cilium in the regressing OA. This configuration is encountered frequently and may represent a stage in the breakdown of all oral cilia. Note the three single microtubules within. These are probably A tubules from the outer doublets persisting at this stage. × 52,000.





plasm than is typical for unstarved cells. We have found, in confirmation of Levy and Elliott's previous report (14), that *Tetrahymena* cells starved for amino acids but not for a carbon source show (*a*) autophagic vacuoles in the cytoplasm, (*b*) massive deposition of fat and glycogen, and (*c*) altered nucleoli.

Regressing OAs typically are found in a depression in the cell surface (Figs. 3, 4), at least in the later stages of this process. In sections where this can be seen, it is thus possible to be certain about which ciliary structures belong to the regressing and which to the developing OAs. The developing OA lies on the surface of the cell just posterior to the regressing one (Fig. 3).

The regression of oral cilia proceeds in two ways, *in situ* (Figs. 4–6) and intracytoplasmically (Figs. 7, 8). The *in situ* pattern appears to be the most common. The outer doublet microtubules regress first by losing the outer wall of the B tubule (Fig. 5), then the inner wall, followed by loss of the A tubule. This is not synchronous within each individual cilium, for doublet microtubules in various stages of dissolution are frequently encountered in the same cilium. The central pair of microtubules similarly regresses at different times in relation to the time of doublet regression. Swollen cilia were also frequently seen (Fig. 6) and this may represent a late stage in *in situ* regression. No stages in the regression of basal bodies have yet been found.

In the other pattern of ciliary regression, oral cilia and basal bodies are withdrawn into the cytoplasm (Figs. 7, 8), after which the axonemes regress as described above. No membranes have ever been found around the intracytoplasmic cilia (Fig. 8). This suggests that the ciliary axonemes regress free in the cytoplasm without being first incorporated into autophagic vacuoles. The fate of the ciliary membrane is unknown.

Electron Microscope Observations of Developing OAs

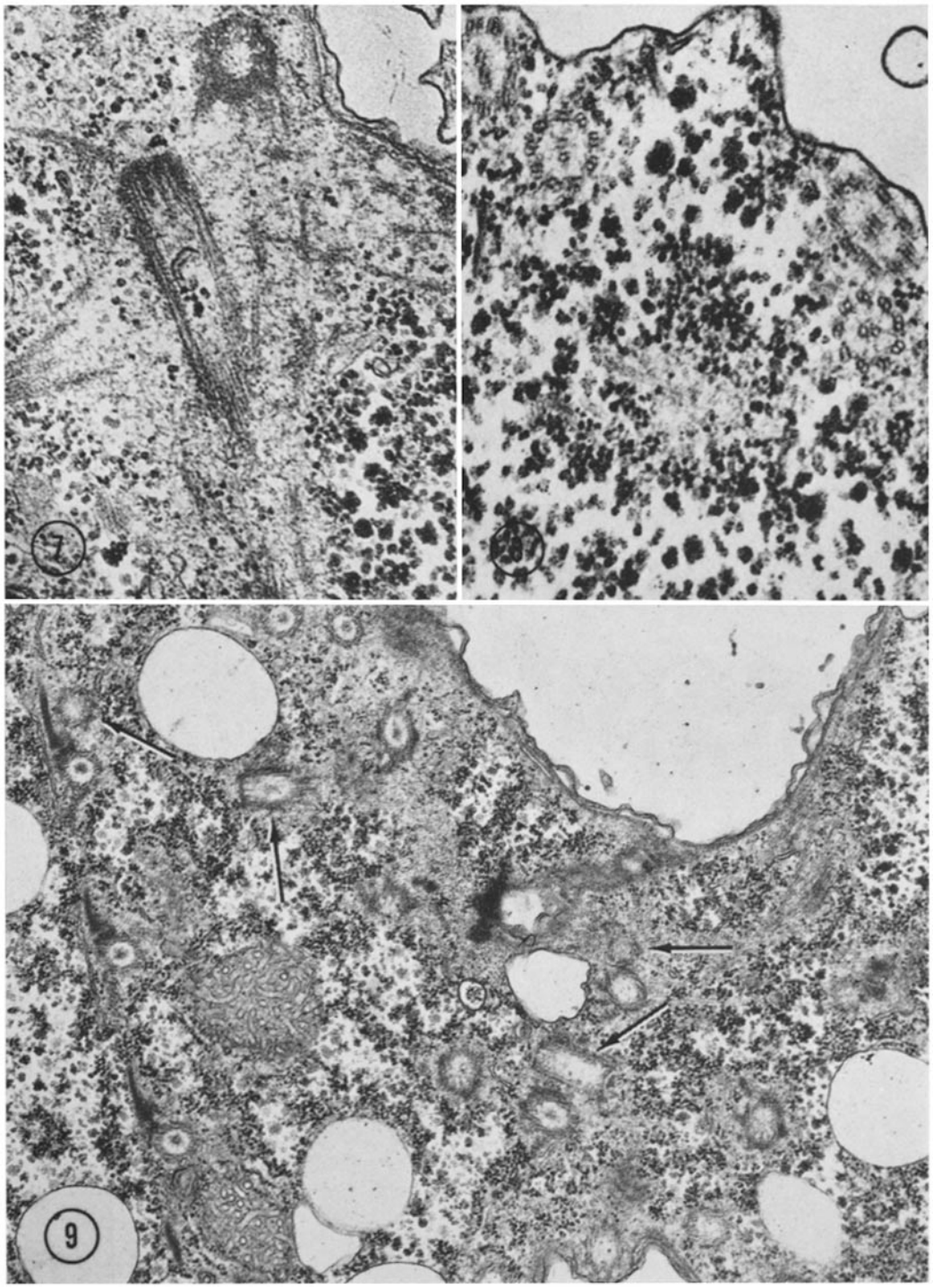
The site of formation of the new OA during oral replacement is the region of cortex immediately posterior to the regressing structures. This is seen to be a slightly elevated region in a number of sections.

Basal body proliferation occurs rapidly in this region. The earliest stage of oral development found in the present study is shown in Fig. 9. Several basal bodies can be seen which can be presumed to be nascent ones because of their size and orientation (arrows). From the beginning, oral basal bodies are situated in a finely granular matrix from which ribosomes and glycogen bodies are excluded (see also Fig. 20). The pattern of origin of new basal bodies of the oral field appears to be the same as that described previously for ciliate somatic basal bodies (1, 2) in that each new basal body forms adjacent to a more mature basal body. Dippell (2) described a "generative disc" of dense fibrous material as the first stage in formation of new basal bodies. Fig. 10 shows the only configuration seen in the present study which might be interpreted as a generative disc, although the identification is not certain. Dippell (2) found that short singlet microtubules form within such a disc, then they become doublets and triplets. These stages were not seen in the present study, although they probably occur. The

FIGURE 7 Intracytoplasmic regression of oral ciliary units during oral replacement. Oral cilia and basal bodies are sometimes withdrawn into the cytoplasm where they later break down. This intracytoplasmic regression does not involve autophagic vacuoles, however, as no membranes have ever been seen surrounding these ciliary axonemes. Note the membranous and granular components within the longitudinally cut ciliary unit. These elements, presumably derived from the cytoplasm, are never seen in nonregressing cilia. × 38,000.

FIGURE 8 Cross section showing four oral cilia after being withdrawn into the cytoplasm. Note the absence of membranes around these axonemes. This represents a stage in the intracytoplasmic regression of oral cilia during oral replacement in *Tetrahymena*. × 47,000.

FIGURE 9 Section through the stomatogenic field at an early stage in the process of oral replacement (see Fig. 1 B for orientation). Four developing basal bodies are indicated by the arrows, although the one on the left of the micrograph may not be an oral basal body. This is suggested by its position adjacent to a mature basal body within a somatic ciliary row. × 24,000.


7
8
9

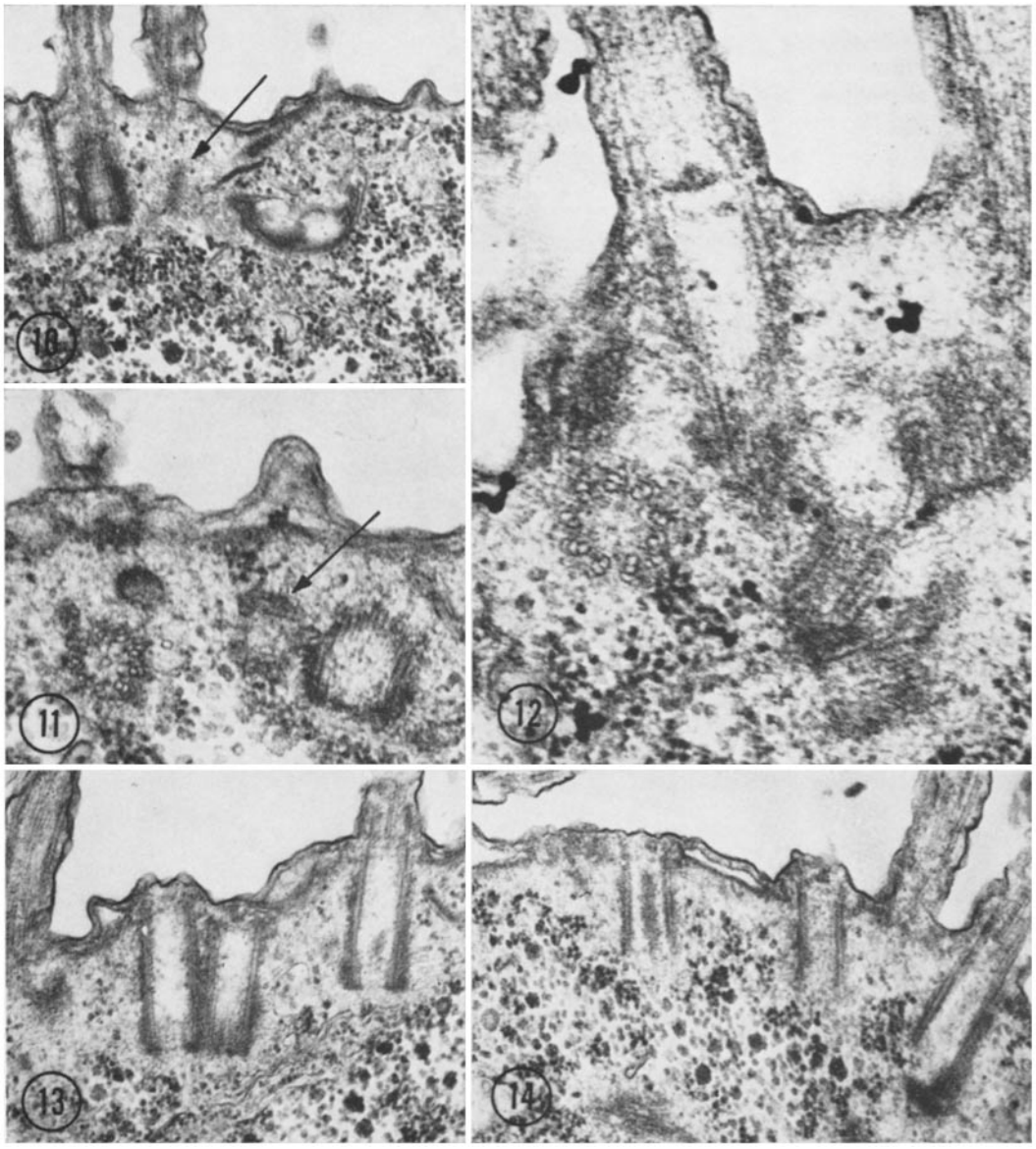


FIGURE 10 Section through dense plaque adjacent to mature basal bodies in the developing OA (arrow) which may represent the earliest recognizable stage in the formation of new oral basal bodies. × 25,500.

FIGURE 11 Developing basal body (arrow) during stomatogenesis. This developing basal body is adjacent and at right angle to a mature basal body, is shorter than a mature structure, and therefore might be termed a probasal body. × 38,000.

FIGURE 12 Section through stomatogenic region showing three basal bodies with positions and orientations suggestive of immature stages in development. The close proximity of these developing structures suggests rapid proliferation. × 63,000.

FIGURE 13 Two immature basal bodies in the stomatogenic field. The close proximity and the slight difference in stage of development of the two suggests that the less mature basal body may have formed adjacent to and at right angle to the more mature one while the latter was in an early stage of development. × 30,000.

FIGURE 14 Late stages in basal body development. The one on the left, which has the dense core and horizontal plates near the ciliary region, shows no ciliary shaft. The one on the right, which appears to be just beginning to grow a ciliary shaft, has the dense ball which is typically found at the base of the central pair of ciliary singlet microtubules. × 25,500.

next stage, the "probasal body" is clearly seen in Fig. 11. Subsequently the probasal body elongates further, tips, and rises to the surface adjacent to the more mature structure with which it is associated (Fig. 13). During the latest stages of development, the central mass within the basal bodies can be seen (Fig. 14). The formation of these basal bodies, therefore, appears to be entirely conventional, with one exception. In contrast with basal bodies of the somatic region, stomatogenic field basal bodies proliferate in large numbers in a limited area in a short space of time (Fig. 12). This is apparently accomplished by the formation of new basal bodies adjacent to others which are themselves still in immature stages of development (Fig. 13).

At some time late in the above-described maturation of oral field basal bodies, an accessory ribbon of dense-walled microtubules forms at one side of each basal body in a manner such as to suggest that it is a lateral extension of one of the triplets, though composed of singlet microtubules (Figs. 15–18). Early stages show one or two accessory singlets, more mature ones show about tight. These accessory ribbons show definite curvature (arrow, Fig. 15) and this curvature is opposite that found in somatic accessory ribbons (the "radial" or "postciliary" ribbons of various authors). The latter can be seen marked by the arrows in Fig. 16. The difference is illustrated and described in Fig. 17. The pattern on the left in this figure, termed *divergent*, is found in the somatic ribbons whereas the pattern on the right, termed *convergent*, is a distinguishing feature of the oral field basal bodies. The oral accessory ribbons associated with basal bodies not yet incorporated into the patterned groups of developing membranelles or undulating membrane (UM) are randomly oriented with respect to the anterior-posterior axis of the cell (Figs. 15, 16).

These basal bodies next move into rows, and the earliest such configurations always consist of rows of two. The orientation begins first at the site of the first membranelle, followed by the second and third membranelles in turn, and lastly in the UM area. At this stage, and subsequently, only the inside row of basal bodies in each of these incipient structures has the accessory ribbons (Fig. 20). "Inside" here is with reference to the site of the future cytostome (mouth opening into the cytoplasm), which lies between the third membranelle and the UM. The outside row of basal bodies at the two-rowed stage may have (*a*) formed in place in the aggregates without accessory ribbons, (*b*) arisen earlier in the field without accessory ribbons, or (*c*) arisen in the field with accessory ribbons and resorbed the ribbons during migration into the aggregations. The latter interpretation is most consistent with the observations made with both the light and electron microscope.

The UM remains composed of two rows of basal bodies, but all three membranelles next add a third row to the outside. This is well illustrated in Fig. 19, where a probasal body can be seen adjacent to a basal body of the outside row of a two-rowed membranelle. It is also indicated in Fig. 20 (arrow). These basal bodies are formed at the final site they will occupy in the OA, unlike those formed previously, and they develop without associated accessory ribbons.

The persistent accessory ribbons of the inside row of basal bodies of the first and second membranelles (Fig. 21) take up a position which bridges between the three membranelles and can now be identified as the "membranellar connectives" (16, 22). They later grow in length as the membranelles move further apart. The fate of the accessory ribbons to the inside of membranelle three is less certain. They could participate in formation of the "deep fiber bundle" although this would require some major position changes; accessory ribbon cross sections are typically seen beside basal bodies, whereas the deep fiber bundle, at least in its fully developed state, comes directly off from the bases of the third membranelle basal bodies and runs down deep into the cytoplasm (Fig. 24). The accessory ribbons of the incipient UM are believed to develop into the ribbed wall microtubules (Fig. 22) by extension of their length.

Throughout oral development there are additional microtubules to be found in great numbers surrounding the developing basal bodies and accessory ribbons (Fig. 18). These microtubules are "thin-walled," i.e., do not have the dense material around the outer surface which is seen in basal body and ribbon microtubules, and seem to be randomly oriented around basal bodies but attached to them. Tubules of this type are rarely seen in the fully differentiated OA. The possible functional significance of these thin-walled microtubules will be considered in the Discussion.

Cilia begin to grow out from the oral basal bodies even before the basal bodies begin to assume the ordered pattern of the membranelles, contrary to the reports in earlier light microscope studies. This has been seen in sectioned material in the

present study, but is most clearly demonstrated by scanning electron micrographs (Dr. Howard E. Buhse and Dr. John Ruffolo, personal communications). Stages equivalent to that shown in Fig. 1 B show short ciliary shafts in the anterior portion of the stomatogenic field but no ciliary growth yet in the posterior basal bodies. Slightly later stages show cilia throughout the field with a gradient of lengths such that the longest cilia are present in the anterior field and the shortest are in the most posterior part of the field. Growth of these cilia continues throughout the stages of membranelle and UM formation until they reach their final length. This pattern of ciliary growth is not followed in two regions. First, the inner row of basal bodies of the UM remains without cilia permanently. Secondly, the final (third and outside) row of basal bodies of each membranelle develops late and grows cilia later than all the rest. This is seen in Fig. 19, where cilia are present in a two-rowed membranelle at the time the third row of basal bodies is not yet fully developed, and in Fig. 23, where the cilia on the third row are shorter than those of the other two rows of basal bodies in the same membranelle. These cilia apparently complete their growth later than all the rest, although it is not known precisely at what stage of oral development this is.

A number of steps may be characterized as late developmental events, although the precise time and sequence of these events is not established. After the stage indicated in Fig. 1 D the buccal cavity forms and the following events occur, apparently in association with this process. The microtubule bundles known as "cross connectives" form (seen in Fig. 23, arrow). The presence of the buccal cavity is indicated by the two membranelles being situated at different levels in this micrograph, unlike the situation earlier which is shown in Fig. 21. The formation of the ribbed wall is also detected about this time (Fig. 22), and the deep fiber bundle can be identified (Fig. 24). The filamentous reticulum also probably forms late in development. The portion of this network which comes to lie below the UM basal bodies was seen only after the UM basal bodies were arranged in rows and after the ribbed wall developed. Other parts of the filtamentous reticulum are difficult to find in sectioned material (22) and have not been seen in developing OAs as yet.

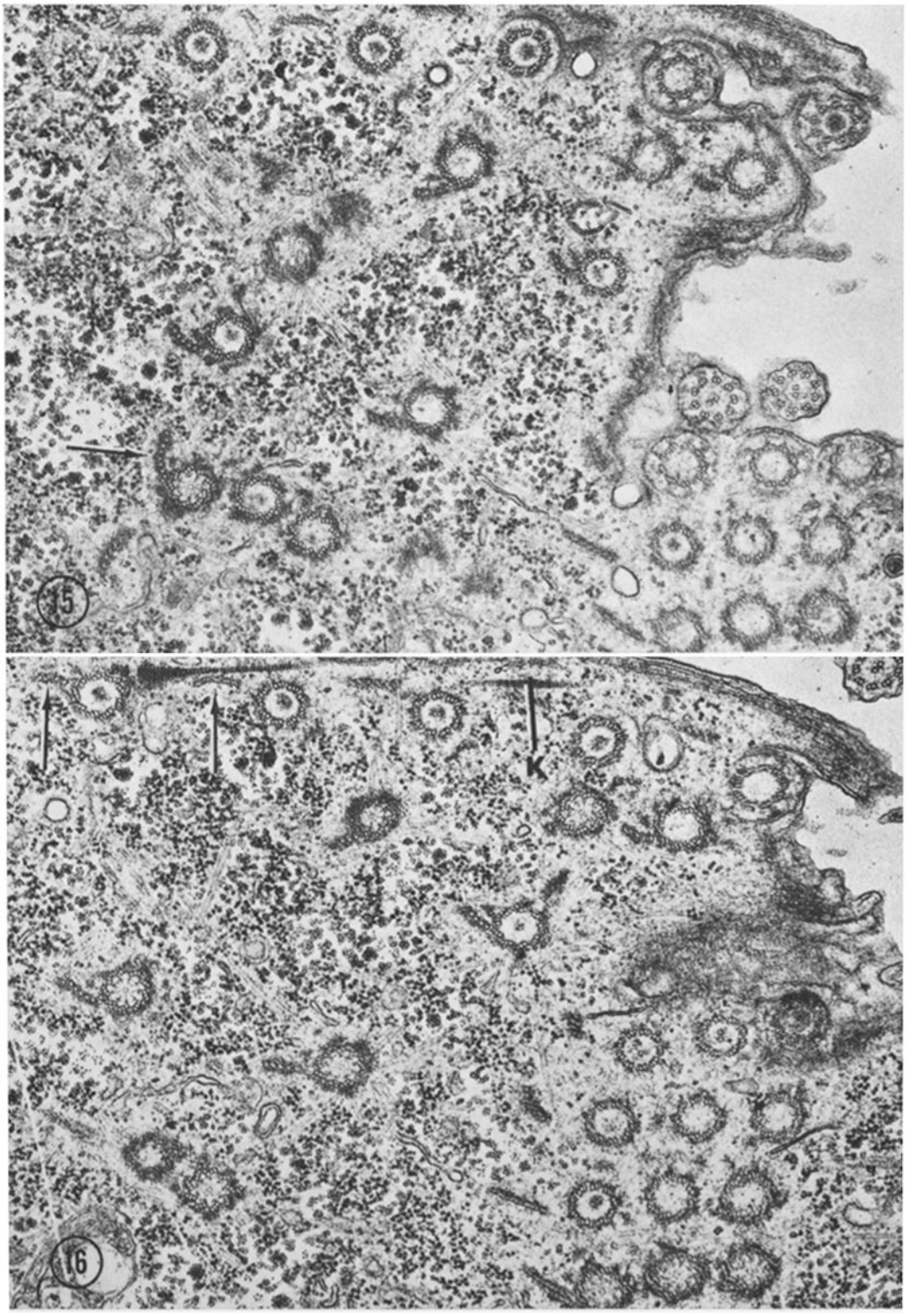
DISCUSSION

Oral replacement in *Tetrahymena* provides a demonstration of the existence and precision of spatial controls in cellular morphogenesis. While one OA is resorbing, a second OA is forming just a few microns away from it in the same cell. The mechanisms behind this precise spatial regulation of assembly and dissociation of presumably identical molecular components present important problems for the understanding of cellular development. The problem has been discussed recently by Tucker (20), who presents evidence for local intracytoplasmic controls of formation and dissolution of microtubular structures in the ciliate *Nassula*.

New basal bodies of the stomatogenic field in *Tetrahymena* form adjacent to old basal bodies. Descriptions available to date indicate that this is the way somatic basal bodies are formed in a wide range of ciliates. The single unique feature in oral

FIGURE 15 Ten basal bodies from an unpatterned segment of the stomatogenic field are found in this micrograph below a row of somatic basal bodies (top) and to the left of a portion of a developing membranelle (rows of three at the right). This is a section through a developmental stage close to that shown in Fig. 1 C. The developmental state of the membranelle can be inferred from the lack of a ciliary shaft in one of the three rows. The free basal bodies typically have accessory ribbons of dense-walled microtubules associated with them (arrow). A distinct curvature is present in the accessory ribbons with constant relation to the associated basal bodies. The pattern seen here, called convergent, is different from that seen in somatic accessory ribbons (described in Fig. 17). × 30,000.

FIGURE 16 Section adjacent to that seen in the previous figure. All eight free stomatogenic field basal bodies seen are also seen sectioned at another level in Fig. 15. The associated accessory ribbons impart polarity to the basal bodies. Examination shows that at this stage in development, the free basal bodies are not oriented with any constant relation to the anterior-posterior axis of the cell. The latter axis is indicated by the somatic kinetodesmal fiber (*K*). The arrows indicate somatic accessory ribbons showing divergent curvature (see Fig. 17). × 30,000.


15
K
16

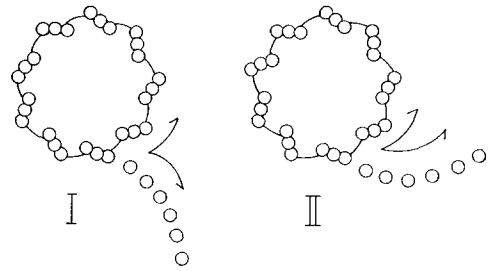


FIGURE 17 Two orientations of accessory microtubule ribbons in relation to ciliary basal bodies. In orientation I (left) the direction of curvature of the accessory ribbon is opposite to that of the basal body, as schematically indicated by the curved arrows; this pattern is here termed divergent. In orientation II (right), the ribbon curves in the same direction as the basal body; this pattern is termed convergent. These topological relations are independent of the direction in which the sectioned basal body is viewed, whether from the interior (as in this diagram) or from the exterior, and are also independent of the direction of the accessory ribbon in relation to the cellular coordinates, whether anterior or posterior, left or right. Orientation I is characteristic of the postciliary (right radial) microtubular ribbon of basal bodies within kineties of *Tetrahymena* (e.g., reference 1), while orientation II is a special distinguishing feature of basal bodies of developing oral primordia.

field basal body proliferation seems to be that a rapid sequential proliferation occurs in a restricted area by means of new basal bodies forming in association with other basal bodies which are still in the process of formation (Fig. 13). Basal bodies proliferating in this way were first seen in logarithmically grown *Tetrahymena* cells by Allen (1), who guessed (correctly) that these might be OA basal bodies because this pattern of proliferation was not seen in (known) somatic regions. Our study of oral replacement in synchronized cells, where developing oral areas can be more readily found and identified, has substantiated this interpretation. In a recent review, Fulton (11) has commented that in situations where cells make many "centriolar pinwheels" (includes basal bodies) from a few, they do so through intermediate structures (cf. Dirkson [3]) instead of through several rounds of sequential production of procentrioles from centrioles. The formation of oral primordium basal bodies in *Tetrahymena* is apparently an exception to this generalization.

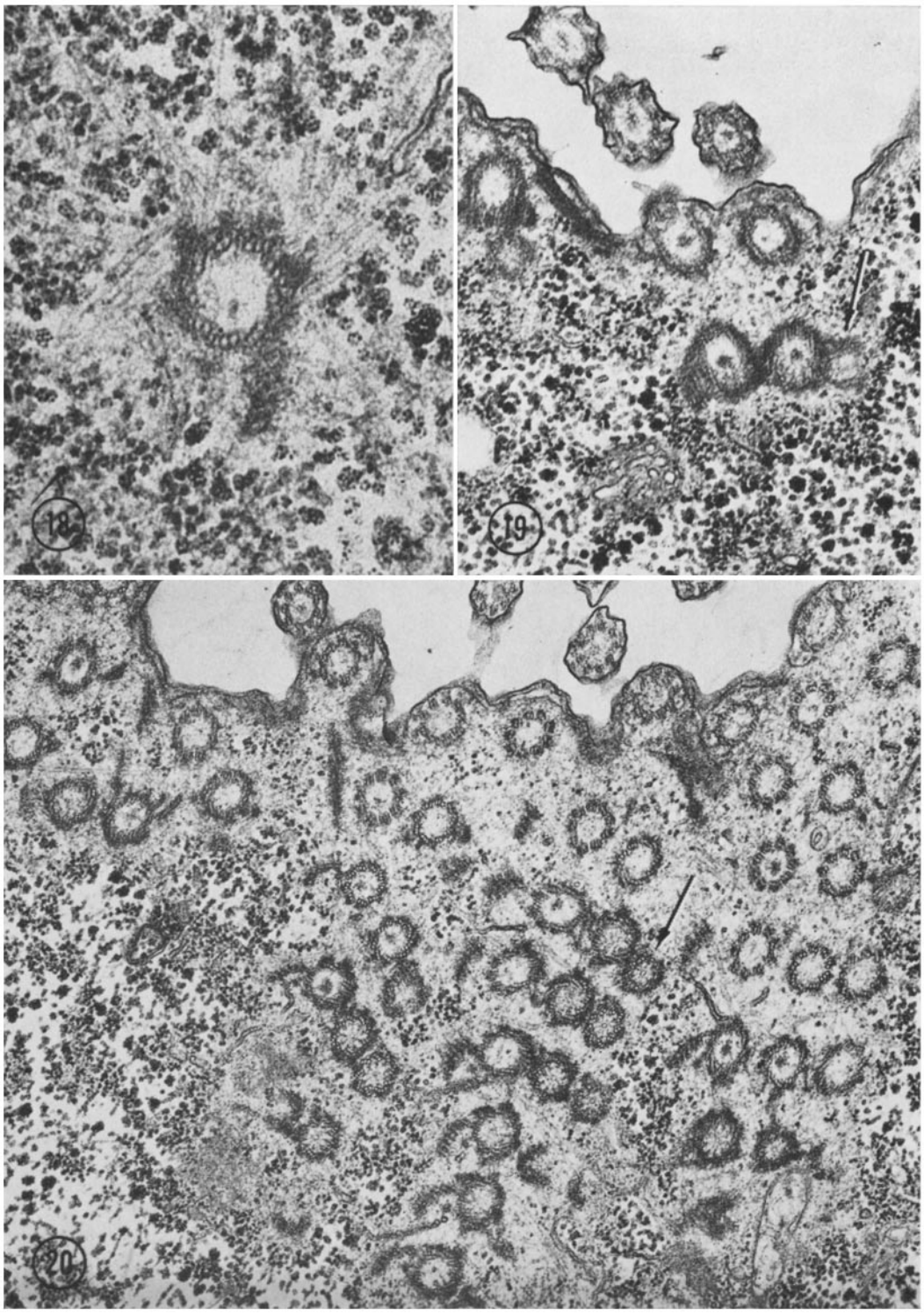
Whereas most oral field basal bodies develop in association with other oral field basal bodies, the problem of the origin of the first basal bodies in the field has not been clarified by the present electron microscope study. Earlier light microscope studies suggest that the earliest basal bodies form adjacent to mature basal bodies of the UM of the old OA and the anterior region of the somatic row in this region (6). It remains undecided whether or not any basal bodies of the old OA persist and become incorporated into the new structure. However, if this occurs, the number must be small, because large numbers of proliferating basal bodies are found in the region of the new OA throughout most of the developmental sequence.

Accessory radial ribbons of microtubules form in association with stomatogenic field basal bodies (Figs. 15, 16, 18). There is a curvature within these ribbons which is different from that found in somatic radial (postciliary) ribbons. The pattern in oral

FIGURE 18 Enlargement from Fig. 15 of a basal body in the stomatogenic field showing an abundance of randomly oriented thin-walled microtubules in the surrounding cytoplasm. These thin-walled microtubules are typically associated with basal bodies in the developing primordium. × 63,000.

FIGURE 19 Section showing an early stage of membranelle development. At this stage only two rows of mature basal bodies are present and the outside row of the membranelle is in the process of formation (arrow). The basal bodies of the outside row are apparently nucleated adjacent to the mature basal bodies of the middle row and mature in position by conventional means. × 34,000.

FIGURE 20 Intermediate stage in the organization of basal bodies into pattern in the oral primordium of *Tetrahymena*. The three rows of basal bodies at the right are from the first membranelle, and the groups of basal bodies to the left of this are, in sequence, the second membranelle, the third membranelle, and the UM. Notice the gradient of organization from right to left in the picture (the animals left to right). The final pattern for adult structures is realized in membranelle one, membranelle two shows immature basal bodies of the outside row in process of formation, membranelle three is more disorganized and incomplete, and the UM pattern does not yet resemble the final pattern of organization of this structure at all. × 27,000.


18
19
20

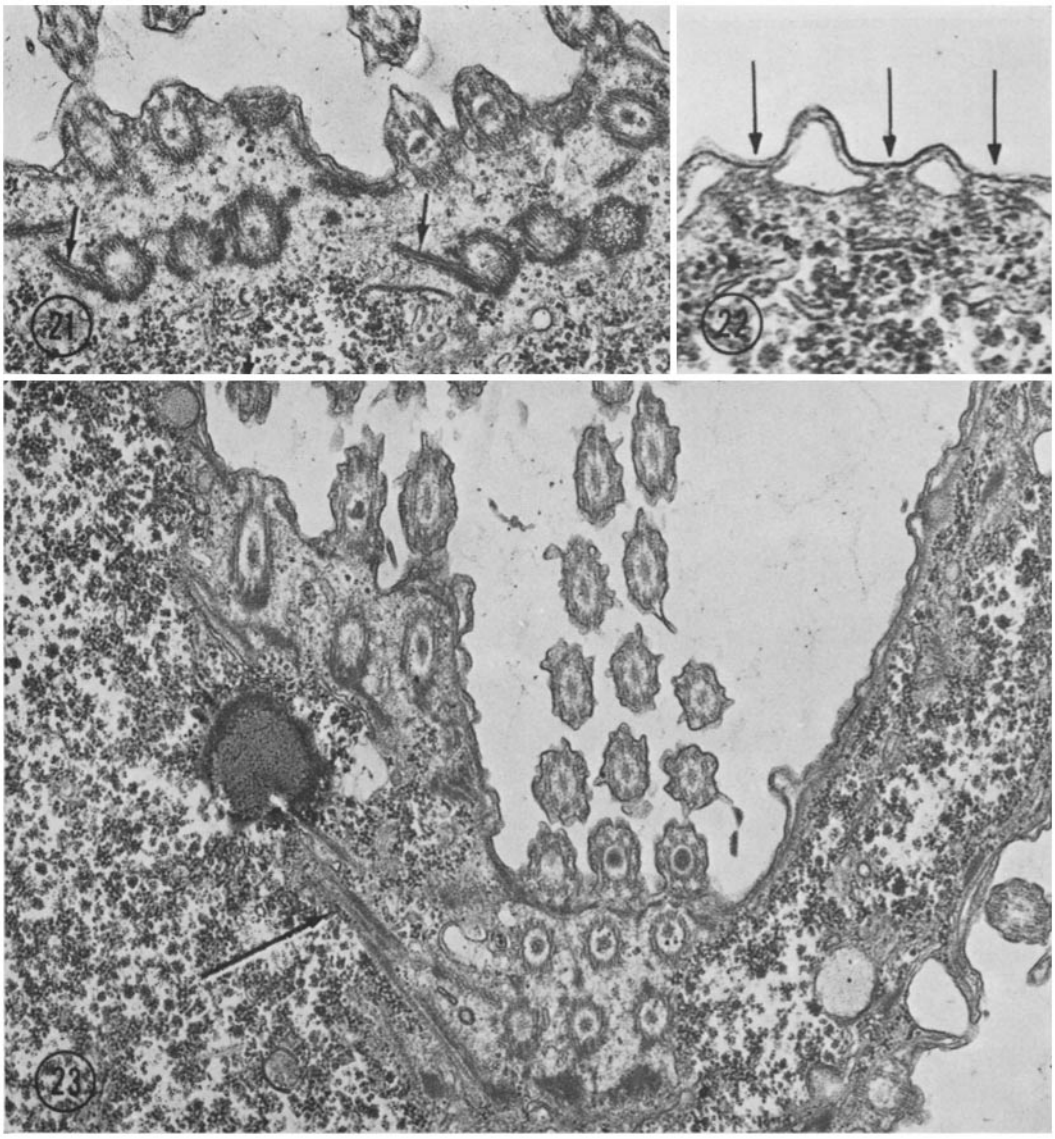


FIGURE 21 Later stage in membranelle formation. Note that only the inside row of basal bodies of each membranelle now has an associated microtubule ribbon (arrows). This can also be seen to advantage in Fig. 20. These ribbons develop into the membranellar connectives. × 27,000.

FIGURE 22 Earliest stage observed of the developing ribbed wall. The arrows indicate the sites of future ridges. The microtubules which underlie the ridges are already present. The regions between the arrows will ultimately sink in to form the troughs of the ribbed wall. The pellicular alveoli between the microtubule bundles of the ribbed wall are more easily seen at this stage than in the fully differentiated structure. × 50,000.

FIGURE 23 Late stage in membranelle formation. The outside row of basal bodies of each membranelle grows cilia at a later time than do the other two rows. This is reflected in the smaller number of ciliary cross sections adjacent to the basal bodies of this row in this grazing section through a posterior membranelle. At this time the microtubule bundles of the cross connective fibers can be found (arrow). × 21,000.

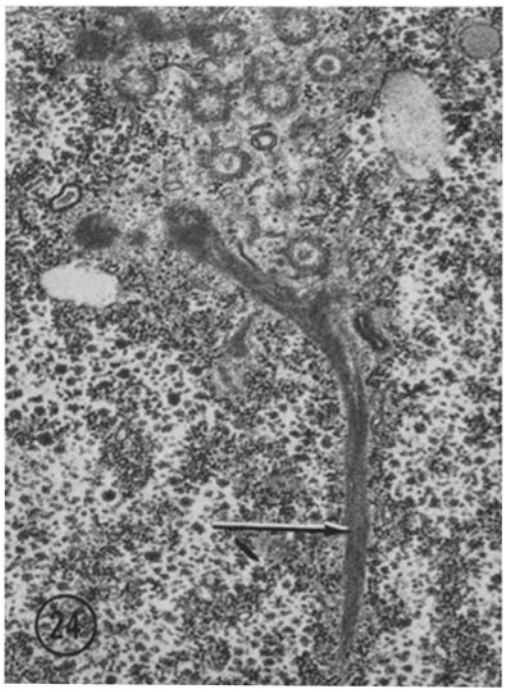


FIGURE 24 Section through the base of the third membranelle showing the developing deep fiber bundle (arrow) attached to the basal bodies. This fiber bundle forms late in oral morphogenesis and passes deep into the cytoplasm. × 26,000.

accessory ribbons is called convergent whereas the pattern found in somatic postciliary ribbons is called divergent (Fig. 17). The divergent pattern of somatic ribbons can also be seen in electron micrographs published by Allen in *Tetrahymena* (1) and Dippell in *Paramecium* (2), although neither author called attention to this regularity. Comparison of Allen's morphogenetic study with our own allows the conclusion that both somatic and oral field basal bodies develop accessory radial ribbons at a point late in maturation before growth of the cilium. Once the accessory ribbon is formed, it is possible to distinguish between somatic and oral basal bodies by the curvature of the ribbon. This will be of use in electron microscope studies of cortical morphogenesis. It is not clear, however, whether all stomatogenic field basal bodies in *Tetrahymena* develop accessory ribbons. It has been reported that some basal bodies are formed in *Stentor* without accessory ribbons (17).

Formation of the accessory ribbons imparts polarity to the basal body-ribbon complexes in the stomatogenic field. It is seen that before pattern formation (alignment of basal bodies into rows to form membranelles and UM) the orientation of each basal body-ribbon complex is random with respect to the anterior-posterior axis of the cell. The randomness is lost during pattern formation. This has also been seen in *Stentor* by Paulin and Bussey (17). An analogous situation was described by Frisch and Farbman (10), who noted that the orientation of developing ciliary units in mouse embryo nasal epithelium was also random (as evidenced by central pair ciliary microtubules and basal body "feet") before the alignment into rows. The above authors concluded that the differentiation of the structures and their orientation into patterned groups are independent processes. This conclusion also applies to the behavior of the majority of the developing ciliary units in the OA of *Tetrahymena*, although many individual ciliary units become oriented into membranellar pattern while still growing a cilium. Within the developing system as a whole, there is no separation of differentiation and orientation of ciliary units, since new ciliary units are still being added to the OA late in the process of pattern formation.

Several events demonstrate an anterior-posterior morphogenetic gradient in the developing OA. Cilia begin to grow first in the anterior part of the stomatogenic field. A little later, about the time of initiation of membranellar pattern formation, an anterior-posterior gradient of ciliary lengths is found. The orientation of ciliary units into rows also begins in the anterior portion of the stomatogenic field (to the left of center) and proceeds posteriorly (and toward the right).

Of the approximately 170 basal bodies of the developing OA (16), about 130 form in positions other than those in which they are found in mature structures. Subsequent morphogenetic movements are involved before the final pattern of basal body-basal body associations is realized. The basal bodies first align into double files, as reported in previous light microscope studies (9). The UM remains in this condition, but each of the three membranelles later adds another row of basal bodies and cilia to the outside. Thus, about 40 ciliary units develop within the membranellar pattern and no subsequent positioning movements are required for these. The movement of stomatogenic field basal bodies into double files involves both a translation of position in the cortex and a rotation which brings the accessory ribbons into register. The rotation in the membranelles brings

the ribbons to the right side and in the UM to the left.

The significance of the thin-walled microtubules (Fig. 18) may be in relation to these morphogenetic movements of basal bodies. From the beginning of stomatogenesis these randomly oriented microtubules are associated with the developing oral basal bodies. One possible explanation of these structures is that they represent a kind of storage form for the great quantities of microtubule protein which are likely to be in this region. An alternative possibility is suggested by analogy with centrioles. Such clusters of randomly oriented microtubules have been described surrounding centrioles by Robbins et al. (18). It has been suggested that these microtubules may be the effectors of centriole movement, in particular the separation of elements of the diplosome. The extensive movements which the oral field basal bodies undergo during pattern formation similarly suggest that their morphogenetic movements may be effected by their associated thin-walled microtubules (compare our Fig. 18 with Fig. 8 *b* in Robbins et al. [18]).

Subsequent to membranelle and UM organization, the oral rib microtubules and the membranellar connectives develop from the aligned accessory radial ribbons by lengthening of these microtubules. The buccal cavity begins to form, with the UM coming to lie on the right rim and the three membranelles along the left wall of the cavity. It is possible that growth of certain of the bundles of microtubules in the developing OA might be involved in buccal cavity formation. However, no direct evidence is available for this. Other late events in oral morphogenesis are the formation of the UM ribbed wall, the cross connectives, and the deep fiber bundle. The latter two attach to the bases of oral basal bodies and together form a structural underpinning of the OA which may be presumed to contribute to its strength. Development of the filamentous reticulum also appears to be a late event, and this too may add strength to the structure. The late appearance of the filamentous reticulum excludes the possibility that it plays any role in orientation of the basal bodies during pattern formation.

Resorption of the old OA during oral replacement in *Tetrahymena* involves both *in situ* and intracytoplasmic regression of cilia. Both types of resorption have recently been noted in different species of Suctoria by Kormos (13), but never both types in the same species of ciliate. Both types occur in *Tetrahymena* and at the same time within a single cell. This has also been seen by Moore,[1] who studied pressure-induced resorption of oral cilia in growing *Tetrahymena*. Intracytoplasmic resorption is preceded by withdrawal of whole cilia, minus the ciliary membranes, into the cytoplasm (Fig. 8). Subsequently, the ciliary axonemes are resorbed, but they have never been seen contained within vacuoles of any kind. This suggests that autophagy is not involved in resorption of these structures. These observations are consistent with (but do not prove) the idea that ciliary microtubules are simply disassembled without loss of the protein subunits. Experimental evidence that the ciliary microtubule subunits may be conserved and reutilized in formation of the new OA is presented in the next report in this series (23). Resorption of cilia within vacuoles has been reported in rumen ciliates (19), but this apparently does not occur in *Tetrahymena*.

Observations on *in situ* resorption of cilia in the present study have provided more detailed information on the dissolution of ciliary axonemes. These observations are confirmed by Moore,[1] who has in addition described the dissolution of basal bodies. Each ciliary outer doublet breaks down in the same way. First, the outer wall of the B tubule is lost (Fig. 5), then the inner wall disappears, followed by loss of the A tubule, the latter apparently in a single step. This is precisely the reverse of the sequence by which ciliary doublets form, as described by Warner (21) in blowfly spermatid differentiation. It is, in addition, the same sequence of dissolution observed in vitro by Witman (24) in differential solubilization experiments with detergent.

A lack of synchrony in the dissolution of the nine outer doublets within a single cilium was consistently found. Such a lack of integration has also been seen in the formation of doublet microtubules in spermatid differentiation by Warner (21). This suggests that each sector of the cilium containing a doublet is to some extent a separate morphogenetic field. In addition, no correlation between the time of central pair resorption and the time of outer doublet resorption has been found, suggesting separate controls for these elements. A late stage in ciliary resorption in *Tetrahymena* appears to be swelling of the cilium (Fig. 6). A similar change has been noted in resorbing flagella in

[1] Moore, K. 1973. *J. Ultrastruct. Res.* In press.

Chlamydomonas by Johnson and Porter (12). The fate of the ciliary membrane after the swollen stage has not been determined, but there is evidence that the microtubule subunit protein is conserved and used again (23).

This investigation is dedicated to Professor Waldo H. Furgason in recognition of his pioneering taxonomic, morphological, and developmental studies on the oral apparatus of various species of *Tetrahymena*, the genus which he established as one result of these studies. The authors wish to thank Miss Paula Williams for expert technical assistance throughout the course of this investigation.

This work was supported by grants from the National Science Foundation to Dr. Williams (GB-24901) and from the National Institutes of Health to Dr. Frankel (GM-11012).

Received for publication 19 May 1972, and in revised form 2 October 1972.

REFERENCES

1. ALLEN, R. D. 1969. *J. Cell Biol.* **40**:716.
2. DIPPELL, R. 1968. *Proc. Natl. Acad. Sci.* **61**:461.
3. DIRKSEN, E. R. 1971. *J. Cell Biol.* **51**:286.
4. FORER, A., J. R. NILSSON, and E. ZEUTHEN. 1970. *C. R. Trav. Lab. Carlsberg.* **38**:67.
5. FRANKEL, J. 1965. *J. Exp. Zool.* **159**:113.
6. FRANKEL, J. 1969. *J. Protozool.* **16**:26.
7. FRANKEL, J. 1970. *J Exp. Zool.* **173**:79.
8. FRANKEL, J., and K. HECKMANN. 1968. *Trans. Am. Microsc. Soc.* **87**:317.
9. FRANKEL, J., and N. E. WILLIAMS. 1973. *In* The Biology of *Tetrahymena*. A. M. Elliott, editor. In press.
10. FRISCH, D., and A. I. FARBMAN. 1968. *Anat. Rec.* **162**:221.
11. FULTON, C. 1971. *In* Origin and Continuity of Cell Organelles. J. Reinert, editor. Springer-Verlag, New York. 170.
12. JOHNSON, U. G., and K. PORTER. 1968. *J. Cell Biol.* **38**:403.
13. KORMOS, J. 1971. *Acta Biol. Acad. Sci. Hung.* **22**:245.
14. LEVY, M. R., and A. M. ELLIOTT. 1968. *J. Protozool.* **15**:208.
15. LUFT, J. H. 1961. *J. Biochem. Biophys. Cytol.* **9**:409.
16. NILSSON, J. R., and N. E. WILLIAMS. 1966. *C. R. Trav. Lab. Carlsberg.* **35**:119.
17. PAULIN, J., and J. BUSSEY. 1971. *J. Protozool.* **18**: 201.
18. ROBBINS, E., G. JENTZSCH, and A. MICALI. 1968. *J. Cell Biol.* **36**:329.
19. ROTH, L. E., and Y. SHIGENAKA. 1964. *Z. Zellforsch. Mikrosk. Anat.* **64**:19.
20. TUCKER, J. B. 1971. *Nature (Lond.).* **232**:387.
21. WARNER, F. D. 1971. *J. Ultrastruct. Res.* **35**:210.
22. WILLIAMS, N. E., and J. H. LUFT. 1968. *J. Ultrastruct. Res.* **25**:271.
23. WILLIAMS, N. E., and E. M. NELSEN. 1973. *J. Cell Biol.* **56**:458.
24. WITMAN, G. B. 1970. *J. Cell Biol.* **47**:229 a.