THE FINE STRUCTURE OF CORTICAL COMPONENTS OF PARAMECIUM MULTIMICRONUCLEATUM*

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INTRODUCTION

The cytoplasm of *Paramecium* is differentiated into a narrow peripheral region, the cortex (also called ectoplasm or ectosarc) and an inner, more fluid zone of greater mass, the endoplasm (also called endosarc). What is generally defined as cortex includes the fine fibrillar systems, the cilia with their basal bodies or kinetosomes, the trichocysts, and a few mitochondria embedded in a gelatinous cytoplasmic matrix. Externally the cortex is limited by a covering, the pellicle; internally the limits are less definite. The endoplasm contains the macronucleus, micronuclei, food and contractile vacuoles, mitochondria, and other inclusions.

The extraordinarily complex and highly organized arrangement of the more prominent elements of the cortex has attracted considerable study. It appears, for instance, that the ciliary basal bodies or kinetosomes are self-perpetuating units of the cytoplasm, not unlike the centrioles, and that a division product of these bodies can develop into a quite different structure, the trichocyst (30). It is further apparent that the system of fibrils within the cortex is important to the metachronal motion of the cilia. These and other phenomena which take place in this highly differentiated region of the protozoon are as little understood as they are remarkable and so become particularly inviting processes to explore with the higher resolution available in electron microscopy. A few such studies have heretofore been undertaken but for the most part they utilized preparation procedures which in their destructiveness somewhat nullified the advantages of the resolutions attained. It is quite evident that acquiring a thorough understanding of the kinetics of the morphogenesis and

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function of cilia and all related components will depend upon a knowledge of the fine structure of the intact cortex. The present study which is directed toward this end attempts to analyze this structure with improved preparation procedures. It is judged from the order and reproducibility of structure observed in these specimens and from earlier studies of OsO_4 fixation (46) that the structure resolved is closely descriptive of the native state.

In order not to complicate unduly the description, the more complex arrangement of the components of the oral region, as opposed to the body proper, is not taken up in this report. Attention rather is focused mainly on the fine structure and spatial relationships of the main cortical components of the body in non-dividing organisms, although some preliminary observations on animals approaching fission will be included.

Materials and Methods

The materials used were derived from a clone of *Paramecium multimicronucleatum* maintained in a timothy hay infusion plus *Aerobacter aerogenes* as a source of food. Most of the observations to be reported were made on non-dividing organisms from starved cultures. In some instances, however, the populations of fixed and partially dehydrated cells (70 per cent alcohol) were segregated with the aid of a dissecting microscope into various stages approaching fission[1] and treated thereafter as separate samples.

The preparation procedure usually employed was as follows: Living animals were concentrated by centrifugation at approximately 400 *g* for about 3 minutes, after which the supernatant fluid was decanted quickly and replaced with a solution of the fixing reagent—in this case a 1 per cent OsO_4 buffered with acetate-veronal to a pH 8.2–8.5. Fixation extended over periods of from 30 minutes to 3 hours[2] at a temperature of approximately 2°C. The fixed animals were thereafter washed rapidly in one change of veronal-acetate buffer solution (diluted 1:1 with distilled water), dehydrated with ethanol and embedded in *n*-butyl methacrylate (36). Low centrifugal forces were used throughout the dehydration and embedding procedure to concentrate the cells. Sections, 250 to 500 A thick, were cut from the plastic blocks with the Porter-Blum microtome (45). They were mounted either on copper mesh, Philips slit grids, or Philips slit grids cut to fit the holder of an RCA electron microscope. All grids were previously covered with a thin supporting film of parlodion. The sections (in some cases serial on slit grids) were examined without removing the plastic. Micrographs were taken at original magnifications of 1,500 to 10,000 diameters and enlarged photographically as required.

OBSERVATIONS

The Pellicle and Plasma Membrane.—Hyman (22) and Kudo (28) describe the pellicle of *Paramecium* as the outermost layer of ectoplasm forming a pattern of polygons. According to Shuberg (54) and Lund (29) the pellicle and the outer fibrillar system are one and the same, the fibrillar system forming the pattern. This usually appears as a system of regular hexagons on the dorsal side of the animal and less regular quadri-

[1] The work of previous investigators has established that the ciliary apparatus is duplicated at this stage of the life cycle (17, 11).

[2] Shorter periods (30 minutes to 1 hour) appear to give more satisfactory fixation with fewer effects ascribable to extraction.

laterals in the region of the oral groove, sutures, and cytopyge. Other protozoologists claim, however, that the outer fibrillar system is subpellicular in location and describe it as the "*Indirekt Verbindungs System*" (25) or the "*Stützgitter System*" (18).

Electron micrographs of vertical sections through the cortex of the organism (Figs. 1, 2, and 4) show the cortex to be limited or covered externally by a thin shealth (*p*). This, at higher magnification, is seen to be made up of two closely apposed membranes, together having a thickness of about 250 A. Beneath this double membrane or pellicle (*p*) the micrographs show a space and below that the surface of the organism's cortex. This is molded into ridges that form a polygonal pattern and it is these ridges which give the cell surface its characteristic configuration (Fig. 3). The cilia (*c*) project from the centers of the depressed areas defined by the ridges, whereas the tips of the trichocysts (*tt*) are located in the ridges at points in line with the cilia (Fig. 3). The material within the peaks of the ridges appears more dense in sections than the underlying cytoplasm and is presumably the material which is stained by silver techniques and corresponds to the outer fibrillar system of the light microscope image.

The relation between the pellicle (*p*) and the surface (*pm*) of the protoplast is fairly complex (Figs. 2 and 4). It can be demonstrated that the continuity of the double membrane structure (the pellicle) is interrupted at the cilia and the trichocysts. At the cilium (*c*), the outer membrane (*op*) of the pair is continuous with the membrane surrounding the cilium (*cm*) and the inner membrane (*ip*) is continuous with the plasma membrane (*pm*) of the cortex and cytoplasm. At the trichocyst (*t*), essentially the same relationship holds. The outer membrane goes over the tip, the inner one follows down the side of the ridge and continues as the plasma membrane (Fig. 9). The doubleness is also interrupted along a line connecting cilia and trichocysts (Fig. 3). The spaces between the outer double membrane (or pellicle) and the surface of the cortex are therefore in the nature of long blisters or vesicles running along the surface of the organism between the rows of cilia and trichocysts. Each blister is limited by the inner membrane of the pellicle and the plasma membrane over the outer surface of the protoplast. Where two adjacent blisters meet, as along the rows of cilia and trichocysts, the partition between them is double (Fig. 3).[3]

[3] It is evident from the electron micrographs (Figs. 2 and 4) that the pellicle and plasma membrane should not be regarded as two distinct and separate structures. The inner of the two membranes constituting the pellicle is continuous with the plasma membrane over the cortex. The outer of the two, which in no obvious regard differs from the inner or from the designated plasma membrane, is continuous with the membrane which covers the cilia. Since the latter are obvious extensions of the protoplast it is reasonable to regard their covering as a plasma membrane and so also the outer membrane of the pellicle. The blisters or vesicles, therefore become special structures of the cortex limited by a separate membrane. In spite of these obvious relationships the designations in the text have been retained to avoid conflict at this time with the established terminology.

The Cilium and Basal Body.—Light microscope studies describe the cilia as slender protoplasmic processes found in great numbers on the surface of ciliates. They are generally arranged in parallel rows, with one cilium in each pellicular hexagon. Previous studies with the electron microscope (24, 19) have shown them to contain 10 or 11 slender fibrils surrounded by a membrane and there is evidence to indicate that of these fibrils, 9 are arranged in a ring around a single central fibril or pair of fibrils.

It has long been recognized that the cilium has at its proximal end a small spherical granule or basal body. These bodies, also called kinetosomes, are described as self-reproducing and the progenetors of new cilia. Lwoff (30) claims in addition that they may secrete fibers within the ectoplasm and that certain of their division products give rise to trichocysts. They are clearly structural elements of considerable interest.

In electron micrographs of thin sections of *Paramecium multimicronucleatum* cut both tangentially and vertically to the cortex, the cilia (*c*) are observed to extend, as expected, from the depressed centers of the polygons. Where the plane of section splits the cilium longitudinally, it is seen to have two central filaments (*cf*) with lateral and parallel filaments (*pf*) on each side (Fig. 4). These are embedded in a matrix of some density and the whole is surrounded by a very thin membrane (*cm*). These same components are also evident in cross section (Fig. 6). Here, however, it is further evident that the lateral or peripheral filaments (*pf*) number 9 and that these are evenly spaced in a circle around the central pair (*cf*). Each of the peripheral filaments is double and each component of the doublet is equivalent in size to one filament of the central pair. It appears, therefore, that in actual fact, the cilium contains 10 pairs of very slender filaments, the central pair differing from the peripheral 9 only in having a greater space between the individual filaments. The cross sectional image of the so called filaments or fibrils suggests that they have a tubular structure (Fig. 6), for each appears as a dense ring around a less dense center. The density of the ring, could, however, reflect simply a greater reduction of OsO_4 at the interphase between filament and matrix. The average cilium is 270 mμ in diameter and each individual filament or microtubule within the cilium measures 150 to 200 A.

At its proximal end the cilium is continuous with a root or basal body that extends for a short distance (370 mμ) vertically into the cortex of the cell (Figs. 1, 2, and 4). The structure differs somewhat from that of the cilium. Its distal or outer limits are defined by a tranverse plate or ring (*tp*) at the level of the plasma membrane (*pm*) (Fig. 4). The 9 peripheral filaments of the cilium pierce this transverse partition (*tp*) and extend into the cortex. The peripheral filaments, together with the matrix material which tends to condense around them form the sheath of the basal body (*k*). At its innermost limits the central part or medulla appears to be continuous with the cortical matrix of the organism. It is obvious from the micrographs (Figs. 2 and 4) that the basal body or kinetosome is tube-shaped and that therefore the terms "basal corpuscle or granule" are descriptively inappropriate.

The Accessory Basal Body.—It is perhaps reasonable to expect that if the basal bodies or kinetosomes divide as Lwoff (30), among others, has claimed, some evidence of the division should be apparent in these preparations. Thus far, however, nothing in the nature of a budding basal body has been encountered. The earliest stage in the development of a new cilium encountered in this material is evident as an accessory basal body (*ak*). This has been observed in a number of non-dividing specimens where the section cuts the cortex obliquely (Figs. 3, 10, and 11). Its appearance in cross section, 9 filaments around a central space, identifies it as a basal body. It is only slightly removed (200 mμ) from the ciliary basal body and is on the same meridional line as the cilia and trichocysts. At this stage it is confined to a shallow depth of the cortex and has, in other words, no connection with a cilium. It appears, moreover, to have no neuromotor fiber connections. The origin of the new basal body is presently in doubt, but the absence of images showing a connection with the basal body seems of some significance. The light microscope image of it budding from the basal body can be easily explained by the small, essentially unresolvable distance (0.2 μ), between them.

Kinetosomal Fibers.—Besides the "outer fibrillar system" mentioned above, students of the ciliates also define an "inner fibrillar system" which corresponds to Klein's "*Direkt Verbindungs System*" (*Meridian II Ordnung*) (25), von Gelei's "*Neuroneme System*" (16), or Chatton and Lwoff's "infraciliature" (30). It is composed of longitudinal fibers (neuronemes, kinetodesmata, silverline fibers, neuromotor fibers) interconnecting the basal bodies (kinetosomes) of the cilia. This inner fibrillar system is subpellicular in position, lying below the outer "polygonal-fibrillar complex." According to the terminology of Lwoff (30), the "infraciliature" is composed of all the "kineties" in the organism; each "kinety" is, in turn, made up of a longitudinal row of cilia, a longitudinal row of kinetosomes (basal bodies), and the kinetodesma interconnecting the kinetosomes. In addition to the inner and outer fibrillar complex mentioned above, a third fibrillar system has been described by von Gelei (15) at the level of and below the basal bodies of the cilia, roughly parallel to the surface of the animal. Its fibers form a polygonal newwork similar to the outer fibrillar complex but with smaller meshes and greater irregularities in pattern. The demonstration of these fiber systems in light microscopy depends on the successful use of silver reduction techniques. These provide an over-all picture of the fiber patterns and little more.

In earlier electron microscope studies of the kinetodesmal system on preparations of cortical fragments Pitelka and Metz (42) have demonstrated that the kinetodesma is made up of a number of fibrils, each of which arises independently from a kinetosome to join the kinetodesmal array. Each fibril has a limited length corresponding to 5 or 6 interkinetosomal intervals and tapers gradually to terminate in the main bundle. Therefore in forming a kinetodesma the fibrils overlap shingle-like.

In preparations of thin sections, such as have been used in the present study, the kinetodesmal arrays are readily identified just beneath the plasma membrane along each row of cilia. Portions of four groups of kinetodesmal

fibrils (*kf*) in longitudinal section are depicted in Fig. 3. These fibrils are polarized with respect to the axis of the animal assuming a longitudinal course lateral to the kinetosomal and ciliary meridians. Each kinetodesmal fibril (*kf*) curves laterally from its point of origin (Fig. 3 referred to below) to run parallel to other kinetodesmal fibrils arising from neighboring basal bodies of a particular meridian. It is difficult to obtain anterior and posterior reference points in addition to dorsoventral orientations in thin sections of paramecia, so that it is impossible to ascertain in Fig. 3 whether the kinetodesmal fibrils travel in an anterior or posterior direction. However, if the rule of desmodexy[4] of Lwoff (30) is applied, the kinetodesmal fibrils are coursing anteriorly from their points of origin on the kinetosomes.

Cross sections of the individual kinetodesmal fibrils (*kf*) are depicted in Figs. 1 and 2. In a transverse section of any one group of fibrils belonging to a meridian, the individual diameters of the fibrils vary in size; the fibril of least diameter is the furthest laterally from the adjacent kinetosome (see Fig. 2 for dimensions). To account for 6 kinetodesmal fibrils seen in a given transverse section, each of the individual kinetosomal fibrils must have a length of 5 interkinetosome intervals before tapering to a point and terminating. There appears to be no evidence in the micrographs for a sheath-like structure enclosing the kinetodesmal fibrils in a compartment. The individual fibrils seem, for the most part, to travel parallel to one another and to the surface of the animal. Figs. 3 and 11 show sections through individual kinetodesmal fibrils at the point where they originate from the ciliary basal body or kinetosome. The fibrils do not start directly from the anterior or posterior face of the kinetosomes but slightly to one side and follow a course at a slight angle to the meridian of the cilia. In this way the fibril (*kf*) bypasses the accessory basal body (*ak*) and joins the kinetodesmal system or ribbon-like array. At the point of origin of the fibril there is evident on the kinetosome (*k*) a spur-shaped structure (Figs. 3 and 11). Whether this is part of the kinetodesmal fibril origin, or is an associated structure of the basal body (*k*) giving it an asymmetrical shape, is difficult to determine from the micrographs.

In favorable longitudinal sections of kinetodesmal fibrils (*kf*) it is possible to discern in them a periodicity of structure (Figs. 3 and 5). This takes the form of uniformly spaced cross bands or striae of greater density separated by intervening bands of lesser density. In their structure the fibrils therefore resemble a number of protein fibers (53, 20). The cross bands in Fig. 5 measure ~250 A, and the space between is ~100 A. Thus each repeating unit of structure measures ~350 A. Some variation has been noted in the length of the intervening light band but whether this is related to their function is not in-

[4] The rule of desmodexy states that the basal bodies are located to the right of the associated kinetodesma when a ciliate is seen in normal orientation.

dicated. The dense striae likewise vary in appearance and occasionally seem to be composed of two or more narrower bands.

The Infraciliary Lattice System.—Tangential sections at the level of the ciliary basal bodies show a fibrous polygonal network (*ils*) (Figs. 10 and 11). Portions of this network (lattice system) are seen also in transverse sections of the animal (Fig. 2). This system, which will be referred to as the *infraciliary lattice system*, courses in a plane roughly parallel to the body surface at approximately the level of the kinetosomes. In the micrographs the lattice network shows an irregular arrangement because of variation in size and shape of the polygons (compare Figs. 10 and 11). The fibers comprising the sides of the individual polygons consist of a bundle of delicate filaments (Fig. 10) in which no evidence of periodicity has been seen. It should be emphasized that this infraciliary lattice system is separate and distinct from the outer lattice structure demonstrated by various Ag reduction techniques to be associated with the pellicle. It doubtless represents the third fibrillar system of Von Gelei referred to above.

The Trichocysts.—The structure of the trichocysts and mitochondria has been reviewed by Wichterman (67). Trichocysts are located in the cortical zone of cytoplasm and oriented perpendicular to the body surface in the centers of the anterior and posterior walls of the polygons of the "outer fibrillar complex." They are fusiform or carrot-like in shape and measure approximately 4 μ in length. With a variety of stimuli trichocysts are extruded and the basal or shaft portion assumes a much longer and more slender form. The fine structure of trichocysts, especially extruded trichocysts, has been studied perhaps more than any other part of the organism. The observations of Jakus (23) are particularly noteworthy. Her micrographs show the trichocyst to have two distinct parts, a sharp dense tip and a long shaft. The tip has a characteristic morphology, resembling a golf tee, which has been noted to vary slightly in different species of paramecia (12). In the extruded trichocysts the shaft is regularly striated at repeated intervals of $\sim$ 550 A (23).

Thin sections of the *Paramecium* cortex are particularly useful for exploring the fine structure of trichocysts. As noted above the distal tips of the unextruded trichocyst are located in the centres of the anterior and posterior ridges of the system of cortical folds forming the polygonal pattern (*tt*, Fig. 3). The individual tip is enclosed in a hood-shaped cap (*tc*) and both are considerably more dense to the electron beam than the material of the subjacent shaft (Figs. 9 and 13). The extreme distal end of the tip is covered over by the outermost only of the two membranes constituting the pellicle (referred to as plasma membrane in footnote). Around the surface of the tip and under the cap there are usually a number of small dense granules ($\sim$250 A in diameter) like those which are frequently found in the proximal ends of cilia (compare Figs. 9 and 13 with Fig. 4). A membrane appears to limit the whole trichocyst, passing

over the cap and around the shaft. In Fig. 13, this membrane looks extremely thin and discontinuous but both qualities are suspected as being products of poor fixation or embedding.

The shaft portion of the unextruded or "resting" trichocyst is a carrot-shaped body proximal to the tip (Fig. 13). It is about 3 μ long and 0.75 μ wide. The material constituting it appears homogeneous with a surprising low density. It is in fact, only slightly more dense than the surrounding plastic matrix. Under the stimulation of fixation the trichocyst may attempt to discharge but instead of the tip being propelled outward, the shaft is extended inward into the body or endoplasm of the organism. After this event the shaft takes on the striated appearance (*dt*, Fig. 12) of the normally extruded trichocyst (compare Jakus (23)). Dense bands alternate with less dense in a regular repeating pattern. Each repeating period measures approximately 550 A. It is to be noted further that the material of the extended shaft is much more dense than that of the unextruded even though there is no appreciable reduction in volume or condensation of material (compare Figs. 12 and 13). This density may reflect a chemical combination with osmium but if so, it must be that the instantaneous shift in molecular organization accompanying the change in shape exposes or creates reactive groups by virtue of which the structure depicted is fixed. The swollen appearance of the unextruded shaft, its lower density, and the absence of order in its structure suggest that good fixation was not achieved and that some of the materials composing it have been lost during washing and dehydration.

Mitochondria.—It has recently been demonstrated by Palade (37, 38) that the mitochondria of plant and animal cells are remarkably similar in possessing an internal system of ridges or folds. His observations did not, however, extend to protozoans and so part of the interest in examining these organelles here is to learn whether or not the structure is similar to that found in Metazoa. A suggestion that they are somewhat similar is derived from a brief report of Powers, Ehret, and Roth (48). These investigators observed that the mitochondria of *P. aurelia* consist of "an osmiophilic continuum interrupted by small tubular spaces." They made the additional interesting observation that the mitochondria appeared not to have an external membrane (as in those of higher forms) and that the cavities of the internal tubular spaces are frequently continuous with the surrounding cytoplasm.

The mitochondria of *P. multimicronucleatum* are particularly satisfactory objects for electron microscopy following the procedures employed in this study. In a section of a portion of the cortex, they (*m*) appear as oval, circular, or rod-shaped profiles randomly distributed in the cytoplasm (Fig. 1). The oval profiles predominate since in thin sections there is a greater probability of cutting mitochondria obliquely than transversly or longitudinally. The mitochondria possess a fine structure similar in general features to that described for a number of animal somatic cells (37, 38, 61), but different in certain

finer structural details. They have a distinct external membrane about 125 A thick (Figs. 2 and 14). The structures corresponding to cristae mitochondriales in mammalian somatic cells appear as finger-like projections or microvilli (*mv*) extending into the interior of the organelle from an internal mitochondrial membrane (*imm*) (Fig. 14). The finger-like shape of the projection is indicated by (*a*) the occurrence of circular, oval, and elongated profiles in each mitochondrial section, (*b*) the predominance of oval profiles, and (*c*) the analysis of serial sections in which the oval profiles can be traced through successive sections. In a few instances the microvilli are seen to branch but this condition appears to be the exception rather than the rule. These microvilli follow a sinuous course within the mitochondrion (Fig. 14) and terminate freely in the mitochondrial matrix (*mm*) without making a second contact with the inner mitochondrial membrane. The lumen of each element appears to be continuous with the space separating the outer and inner mitochondrial membranes and has not been seen to connect directly with the outside.

Vesicular and Small Particulate Components.—Porter (43, 44) and Palade and Porter (39) have identified and described in a number of cell types a finely divided vacuolar system which has come to be known as the endoplasmic reticulum. In addition, Palade (40) has presented evidence for the existence of a dense particulate component, 100 to 150 A in diameter, in the ground-substance of the cytoplasm in a number of mammalian and avian cell types. In many cell types, though not all, this particulate component is in close association with the membrane surfaces of the endoplasmic reticulum. Recently, Palade and Siekevitz (41) have shown that the microsome fraction of rat liver is composed of fragments of the endoplasmic reticulum, particularly of fragments bearing attached small particles. Further analysis has demonstrated that the particles consist of nucleoprotein with a high ribonucleic acid content (30 to 60 per cent). The findings indicate that cytoplasmic basophilia resides in the small particulate component.

Electron micrographs of the cortical cytoplasm of *Paramecium* exhibit a number of elements of different shapes and sizes, enclosed individually by membranes which can be identified with the endoplasmic reticulum (Figs. 2, 3, and 10). These elements, for the most part, assume the form of vesicles, although flattened sacs, vacuoles, and canaliculi are sometimes encountered. In serial sections, portions of these elements may be traced through consecutive sections indicating that some at least are part of a continuous reticular system.

A dense particulate material is also seen scattered widely throughout the cortical cytoplasmic matrix (Figs. 3, 10, 11, and 12). These particles appear to be discrete macromolecular units ranging in size from 100 to 300 A and are identified with the dense particulate component of Palade. The particles rarely show a preferred association with the membranes of the vesicular component although in many animal cell types such a preferential association is usual (40).

Paramecia Approaching the Fission Stage.—The light microscope observations of von Gelei (17) and Downing (11) have shown that the body cilia are duplicated at the stage when the paramecia increase noticeably in width. Von Gelei (17) has emphasized that during this stage, new basal bodies and accessory basal bodies (*Nebenkörner*) originate from old ones by a budding process which is subsequently followed by separation, and growth until finally, the young and old basal bodies cannot be distinguished on the basis of size. The cilia were found to originate in the same pattern as the new basal granules: where the basal granules are double, the cilia are double.

Electron micrographs of paramecia in stages approaching fission confirm the light microscope observations of von Gelei (17) and Downing (11) as well as the earlier electron microscope observations of Sedar (58, 59), that the cilia are duplicated during this stage. In Fig. 7 where the section plane passes longitudinally through the ciliary apparatus, it is seen that the cilia (*cc*) are double, two cilia protruding from the center of the depression in the plasma membrane. These paired cilia (*cc*) are continuous with their associated kinetosomes (*kk*).

If the basal bodies (kinetosomes) and accessory basal bodies divide as von Gelei (17), among others, has observed, some evidence of the division should be apparent in preparations of animals approaching the fission stage. Thus far nothing in the nature of a budding basal body has been encountered. Instead, only paired cilia and their associated kinetosomes have been found. These paired cilia are often observed to touch one another while the distance separating their basal bodies is greater (0.14 μ) (Fig. 7). The latter spacing is similar to the distance separating the ciliary and accessory basal bodies in non-dividing paramecia (see Fig. 3). It is tempting to speculate therefore that the newly formed cilium originated from the accessory basal body very early in the approaching fission stage. However, until more specimens in stages approaching fission as well as during and after fission are carefully studied, the morphogenesis of the cilium will remain obscure.

DISCUSSION

It has been recently established that cilia and flagella of protozoa and plants, sperm flagella and cilia of metazoan cells all show a characteristic fine structure consisting of longitudinally oriented filaments embedded in a protoplasmic matrix and surrounded by a membrane (see Fawcett and Porter (14) for a review of the literature on the subject). There exist in the literature estimates of the number of filaments or fibrils per cilium or flagellum which fall in the range of from 9 to 12. Using dried unsectioned material, Jakus and Hall (24) counted approximately 11 fibrils in the cilia of *Paramecium* while Krüger and Wohlfarth-Bottermann (27) counted 10 to 12. Improvements in the techniques for preparation of biological material for electron microscopy have enabled investigators to describe more precisely the number as well as the pattern of organization of the filaments in cilia or flagella (64, 8, 14, 60, 47).

For the most part these workers are in agreement that the peripheral filaments of a given cilium or flagellum form a cylindrical array of 9 fibrils around a central pair although some investigators have reported a single central filament (19, 10, 51, 3). Studying non-sectional material, Manton and her coworkers (31, 33) have come to the conclusion that the number and arrangement of the filaments is essentially the same in cilia and flagella of plants. Fawcett and Porter (14) have shown in addition that while the two axial filaments of the cilium are single, the 9 peripherally located filaments are clearly double in molluscs, amphibia, mouse, and man. This was also observed independently by Manton and her colleagues (32) in plant material. The results of the present investigation on the body cilia of *Paramecium multimicronucleatum* are in agreement with these findings. Potts and Tomlin (47) have recently published similar results for the cilia of the cytopharynx in *Paramecium*.

The electron micrographs of thin sections of the kinetosome in *Paramecium* have revealed new information in regard to shape, internal structure, and manner of association of the kinetosome with its cilium. The kinetosome, instead of having the shape of a granule or small spherical corpuscle (67), has a cylindrical form. The longitudinal peripheral filaments of the cilium penetrate a transverse partition at the level of the plasma membrane, continue into the cortex, and form a major portion of the substance of the kinetosome. The cilium therefore is structurally continuous with its basal body by way of its peripheral longitudinal filaments. The ciliary membrane which encloses the filaments and matrix material is continuous with the outer member of the paired pellicular membrane contrary to the observations of Bretschneider (5, 6) the cilium sheath was described as continuing into the ectoplasm to form the cilium root (see footnote 3).

The structural relationship of the cilium to the basal body described for *Paramecium* does not precisely parallel that evident in material from certain other phyla in the animal kingdon. In Mollusca and Amphibia, for example, the filaments of the cilium terminate in a dense transverse plate which is separated by a narrow space from a similar plate delimiting the distal surface of the basal body. The cilium and basal body are held together by their common enclosure in the ciliary membrane. In mammals, on the other hand, there is no transverse plate at the junction of cilium and basal body, and the filaments continue directly into the basal body (14). There are, in addition, a number of differences in shape of the basal body among different species (14). No obvious functional differences are evident to correlate with these morphological variations.

Investigators of this material are not in agreement regarding the morphology of the basal apparatus of the cilia in *Paramecium*. Von Gelei (16) described a basal ring surrounding a basal granule and an accessory granule (*Nebenkorn*)

lying to the right of the junction of the basal ring with the neuroneme; only the basal granule was associated with the cilium. Klein (25) pictured the basal apparatus as formed by three granules in a ring (*Zirkularfibrille*). The middle one of these he designated as the basal granule and the others as "*Nebenkörner*." Because of the variation in number which he found among these accessory granules, Lund (29) was convinced that they were artifacts produced by deposits of silver at points where two fine fibrils intersected. Earlier electron microscope studies of Sedar (58, 59) confirmed the existence of an accessory basal body although Metz, Pitelka, and Westfall (34) in later studies found no evidence of it.

In the micrographs presented here the existence of an accessory kinetosome is confirmed. It is however, very doubtful whether this accessory kinetosome is similar to the *Nebenkorn* of von Gelei or is related to the *Nebenkörner* of Klein mentioned above since it is confined to the same meridian as the ciliary kinetosome and is separated from it by a distance of only 0.2 μ or less. It is presumed that the accessory basal body represents a newly formed kinetosome which gives rise to a new cilium during the approaching fission stage of the life cycle in *Paramecium*.

Thin sections of the cortex both in tangential and transverse planes reveal that (*a*) the kinetodesma connecting the ciliary kinetosomes of the light microscopist is a compound structure composed of a number of discrete fibrils and (*b*) there is a precise three dimensional disposition of these elements in the organism's cortex. A single kinetodesmal fibril is observed to originate from a ciliary kinetosome and curve laterally before turning longitudinally to join 4 or 5 other kinetodesmal fibrils originating from ciliary kinetosomes immediately posterior in a given longitudinal row. The observation that the kinetodesma is a compound structure has already been described earlier from preparations of fragmented paramecia (42, 58, 34). Recently Metz and Westfall (35) have observed in fragmented preparations that the kinetodesmata of *Tetrahymena* are also made up of associated fibrils. The ciliary fiber root associated with each basal body in fragments of *Colpidium* studied by Wohlfarth-Bottermann (68) is similar in structure to the individual kinetodesmal fibril observed in *Tetrahymena*. It is reasonable to suppose from these various observations that the kinetodesmata of other ciliates are also composed of fibrils.

The number of kinetodesmal fibrils comprising a given kinetodesma is different in *Paramecium* and *Tetrahymena*. Pitelka and Metz (42) first observed 3 to 4 fibrils per kinetodesma in *Paramecium*. Later Metz, Pitelka, and Westfall (34) found that each kinetodesmal fibril extends for about 5 interciliary intervals as a member of the array before tapering to a point. The observations reported here on *Paramecium multimicronucleatum* show as many as 6 kinetodesmal fibrils in transverse sections indicating that each fibril may extend 6 interkinetosomal intervals before tapering to a point and terminating. In

Tetrahymena, on the other hand, Metz and Westfall (35) found that the kinetodesmal fibrils are short and extend only 1.5 ciliary intervals so that there is only a single overlap. In this case the kinetodesma can appear in transverse section either as a single fiber or as a pair of fibers. This seems also to be the case in Wohlfarth-Bottermann's observations on *Colpidium* (68).

The kinetodesmal fibrils associated with the ciliary kinetosomes in *Paramecium*, *Tetrahymena*, and *Colpidium* are comparable to the ciliary rootlets found associated with the basal bodies in the ciliated cells of a number of metazoans. These ciliary rootlets have been described with the electron microscope by a number of investigators (57, 70, 14). Fawcett and Porter (14) observed for the first time that the ciliary rootlets in molluscs and amphibia possessed cross striations. The rootlet fibers were poorly developed or absent in mammalian ciliated epithelia, an observation confirmed by Rhodin and Dalhamm (51). The cilitate kinetodesmal fibrils also show a striated structure. The spacing evident between striae appears to vary considerably. Pitelka and Metz (42) obtained a period of 400 A for the kinetodesmal fibrils in paramecia. Wohlfarth-Bottermann reported a period of 420 A for the ciliary fiber roots in *Colpidium* (68). In the present study on sectioned paramecia the spacing was observed to vary between 300 and 500 A, although the width of the dense striae themselves tends to be regular. The periodic structure in rootlet fibers associated with the basal bodies in metazoan cells is more complicated than in the protozoans thus far studied, finer striae being present in the interval between the major bands (14).

In their cross striated appearance the kinetodesmal fibrils are similar to a number of protein fibers which play a supportive rather than contractile role in tissues. Whether the kinetodesmata perform a similar function within the cortex of *Paramecium multimicronucleatum* is unknown. Variations in periodicity that have been encountered could be a result of stretching and not an indication of active contraction. Metz, Pitelka, and Westfall (34) apparently favor the possibility that the kinetodesmal fibrils fulfill a role in the coordination of ciliary activity in *Paramecium*, and cite the microdissection studies of Worley (69) as support for this view. There is nothing in the observations recorded in the present study that conflicts with assigning a coordinating role to the kinetodesmal system. It is true as pointed out by Metz, Pitelka, and Westfall (34) that the individual fibrils do not connect directly in the kinetodesmal array, but run parallel to one another. There is moreover no evidence of organization or connecting structure in the intervening ectoplasm. The separating space is, however, small and it is not difficult to believe that a wave of activity in one fibril would induce a similar wave in an adjacent fibril, thus providing for propagation along the kinetodesmata. In associating fiber systems with ciliary motion, however, it must be recognized that there is at least one other system, described here as the infraciliary fiber lattice which runs through

the cortex at the level of the basal bodies. This system does not have as definite a structural continuity with the basal bodies as do the kinetodesmal fibrils, but a less prominent connection has not been excluded. It seems appropriate in this connection to call attention to observations on the presence of acetylcholine and acetylocholinesterase in the cortex of *Tetrahymena* (55, 56) as well as in ciliated cells of *Mytilus* gill plates (7) and esophageal and tracheal mucosae of frog and rabbit respectively (26). Applications of acetylcholine and eserine to isolated preparations of these tissues were observed to affect the rate of ciliary motion. Presumably, these observations can be taken to suggest that some cortical structure involved in ciliary motion has a neuromotor function.

The present observations here reported provide the first electron microscope evidence that an infraciliary lattice system, noted above, exists in the cortex of *Paramecium multimicronucleatum*. It is difficult to confuse this system with either the outer polygonal system or the inner fibrillar system for the following reasons: (*a*) the infraciliary lattice system forms a characteristic pattern consisting of an irregular polygonal network at the level of the ciliary kinetosomes and accordingly, is well separated from the pellicle and ridges in the plasma membrane, (*b*) the kinetodesmata, composed of overlapping kinetodesmal fibrils, also have a well defined distribution, coursing in longitudinal rows to one side of the ciliary basal bodies, (*c*) the fine structure of the fibers comprising the infraciliary lattice shows no evidence of periodicity and is otherwise quite distinct from that of the kinetodesmal fibrils associated with the ciliary kinetosomes, (*d*) in all cases, portions of these systems are seen in transverse and oblique sections and are easily distinguished. This is not the first time an infraciliary lattice system has been described in *Paramecium*. Von Gelei (15) discovered a new infraciliary lattice system in *Paramecium* at approximately the level of the basal bodies coursing roughly parallel to the surface of the animal. The photomicrographs showed that the infraciliary lattice system was distinct from the outer polygonal lattice system. The role of this infraciliary lattice system in the life and reproduction of the animal is, at present, unknown.

Although the outer fibrillar system in *Paramecium* has been demonstrated by investigators using silver impregnation techniques (25, 18) and recently with the electron microscope (59, 34), the present study has failed to confirm the existence of such a system. The electron micrographs of thin sections show no evidence of any fibrillar structures in the pellicle or associated with it. Instead the surface of the animal is folded into ridges to which the pellicle is closely applied. It is these ridges that give the cell surface its characteristic polygonal pattern and coincide with the outer fibrillar system of the light microscopists. The pattern of cortical ridges has been interpreted as a fiber system because in silver impregnated preparations it has the appearance of a network of lines or fibers. The reason for this appearance is not easily understood from electron

micrographs. The ridges do not contain fibrous structures of appropriate dimensions, they consist only of an apparently homogeneous material having a density (as defined by the electron beam) similar to that of the material constituting the fibers of the kinetodesmal and infraciliary systems. Presumably this material represents the firmly gelled layer of the ectoplasm which maintains the form of the ridges. It is conceivable that, like the fibers, this is impregnated and rendered visible by the silver techniques. Lund (29) has provided a similar interpretation from light microscope observations. The description of an outer fibrillar lattice associated with the pellicle made from electron microscope studies of cortical fragments of the organism (58, 59, 34) requires some explanation. It is reasonable to suppose that with these relatively thick fragments the observer could be misled into interpreting the ridges as fibers. They have a greater mass and density than the surrounding cortex and consequently produce a more intense shadow in the electron beam. Whether the structures are fibrous or not would, moreover, be hidden by the extraneous material over and around them in the dried preparations.

Mitochondria of a wide variety of mammalian cell types have been described as possessing a limiting membrane (37, 38), which in favorable sections is double (61, 62, 50, 63) and a system of paired membranes in the interior. The latter structures are interpreted by Palade (38) as representing profiles of folds (ridges or cristae) which project perpendicularly from the mitochondrial membrane into the interior. Sjöstrand (61) preferred to interpret the internal structure of the mitochondrium as consisting of "double membranes" not necessarily continuous with the limiting membrane. Mitochondria of cell types present in amphibian and mollusc (14) as well as insect (9) tissues, possess an internal structure similar to that described by Palade. The mitochondria of *Paramecium multimicronucleatum* also show internal projections of the mitochondrial membrane. In this organism, however, these internal structures have the form of finger-like projections or microvilli. Similar intramitochondrial entities have also been found in *Tetrahymena* and *Tokophrya* (52). Other instances of filamentous intramitochondrial structures have been reported in mammalian cells of the adrenal cortex and kidney (38) as well as of the liver (13), while in insect tissues both double membranes and tubular structures have been described (4, 2). Still another variation in mitochondrial internal structure is found in the germ cells of *Helix* (1) which possess concentric lamellae. From the data already accumulated it is apparent that, contrary to an earlier conclusion (38), a characteristic pattern of mitochondrial fine structure is not found in all types of animal mitochondria. It should be pointed out, however, that although the structural device varies in different cell types, the end result—an increase in intramitochondrial surface area—is a constant feature. In favorably oriented thin sections it is seen clearly that the mitochondrial membrane in *Paramecium multimicronucleatum* is double and that

the walls of the microvilli which protrude into the interior of the mitochondrion are continuous with the inner of the two limiting membranes. These projections which end freely in the mitochondrial matrix thus resemble the "cristae" of other mitochondria. The diameters of the microvilli in *Paramecium multimicronucleatum* are however on the average greater than the width of the "cristae" or "double membranes" in mammalian cell types.

Lately, the generalization that mitochondria possess limiting membranes has been challenged (66, 48, 49). Powers, Ehret, and Roth (49), working with *Paramecium*, observed that if the plastic is not removed before electron microscopic examination of the sections, "it may appear" that the mitochondrion is bounded by a continuous membrane. These investigators feel, however, that only if the sections are soaked in toluene, which removes most of the plastic, and then examined with the electron microscope, is the true structure of the mitochondrion revealed. Under such conditions the image of the mitochondrion lacks a limiting membrane distinct from the dense material of the walls of the "tubules" and the matrix substance in the interior. It is difficult to concur with the interpretation of these investigators because a structural alteration most probably occurred during the technical procedure. Hillier and Gettner (21) have already pointed out that removal of the embedding material from a section plus the surface tension effects during drying introduces serious distortion. To account for these investigators' results, the following explanation is offered: Distinct visualization of a membrane in the electron microscope requires that the plane of the membrane be normal to the plane of section and parallel to the beam so that it is viewed edge on. It is possible that after treatment of the section with toluene to remove the plastic, followed by drying, the membranes (limiting membranes and membranes of the microvilli) of the mitochondrion change their orientation, no longer being supported by the plastic. The narrow ribbon of membrane included in the section might after such preparation procedures come to lie more nearly in the plane of section rather than perpendicular to it. If then examined with the electron microscope, it would not be obvious as a thin dense line. The increase in density of the matrix material also reported by these investigators may be due, in part, to the reorientation of the membranes. It is interesting to note, in this connection, that if the plastic is removed by a method of electron extraction (65) little or no distortion can be observed. In fact, if Watson's electron micrographs of identical fields of lung tissue are examined before and after electron extraction, it is readily seen that the mitochondrial membranes are intact even after almost complete removal of the embedding material.

The present report has shown that current techniques of preparation of biological material for electron microscopy are applicable to *Paramecium*. The results have demonstrated that a number of the cortical components are comparable and in many cases similar in fine structure to corresponding elements

in metazoan cell types. A number of discrepancies in interpretation of cortical structures appear to have been resolved. In addition the fine structure of existing components has been amplified and their three dimensional relationships established.

Clarification of the fine structure of the cortex of *Paramecium* in non-dividing animals provides a point of departure for studies on the morphogenesis of a number of the cortical components. Research is now in progress studying the morphogenesis of the kinetosomes and cilia at various stages in the life cycle of *Paramecium multimicronucleatum*.

SUMMARY

The electron microscope was used to study the structure and three dimensional relationships of the components of the body cortex in thin sections of *Paramecium multimicronucleatum*.

Micrographs of sections show that the cortex is covered externally by two closely apposed membranes (together ~250 A thick) constituting the pellicle. Beneath the pellicle the surface of the animal is molded into ridges that form a polygonal ridgework with depressed centers. It is these ridges that give the surface of the organism its characteristic configuration and correspond to the outer fibrillar system of the light microscope image. The outer ends of the trichocysts with their hood-shaped caps are located in the centers of the anterior and posterior ridges of each polygon. The cilia extend singly from the depressed centers of the surface polygons. Each cilium shows two axial filaments with 9 peripheral and parallel filaments embedded in a matrix and the whole surrouned by a thin ciliary membrane. The 9 peripheral filaments are double and these are evenly spaced in a circle around the central pair. The ciliary membrane is continuous with the outer member of the pellicular membrane, whereas the plasma membrane is continuous with the inner member of the pellicular membrane. At the level of the plasma membrane the proximal end of the cilium is continuous with its tube-shaped basal body or kinetosome. The peripheral filaments of the cilium, together with the material of cortical matrix which tends to condense around them, form the sheath of the basal body. The kinetodesma connecting the ciliary kinetosomes (inner fibrillar system of the light microscopist) is composed of a number of discrete fibrils which overlap in a shingle-like fashion. Each striated kinetosomal fibril originates from a ciliary kinetosome and runs parallel to other kinetosomal fibrils arising from posterior kinetosomes of a particular meridional array. Sections at the level of the ciliary kinetosomes reveal an additional fiber system, the infraciliary lattice system, which is separate and distinct from the kinetodesmal system. This system consists of a fibrous network of irregular polygons and runs roughly parallel to the surface of the animal.

Mitochondria have a fine structure similar in general features to that de-

scribed for a number of mammalian cell types, but different in certain details. The structures corresponding to cristae mitochondriales appear as finger-like projections or microvilli extending into the matrix of the organelle from the inner membrane of the paired mitochondrial membrane. The cortical cytoplasm contains also a particulate component and a system of vesicles respectively comparable to the nucleoprotein particles and to the endoplasmic reticulum described in various metazoan cell types.

An accessory kinetosome has been observed in oblique sections of a number of non-dividing specimens slightly removed from the ciliary kinetosome and on the same meridional line as the cilia and trichocysts. Its position corresponds to the location of the kinetosome of the newly formed cilium in animals selected as being in the approaching fission stage of the life cycle.

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EXPLANATION OF PLATES

Abbreviations

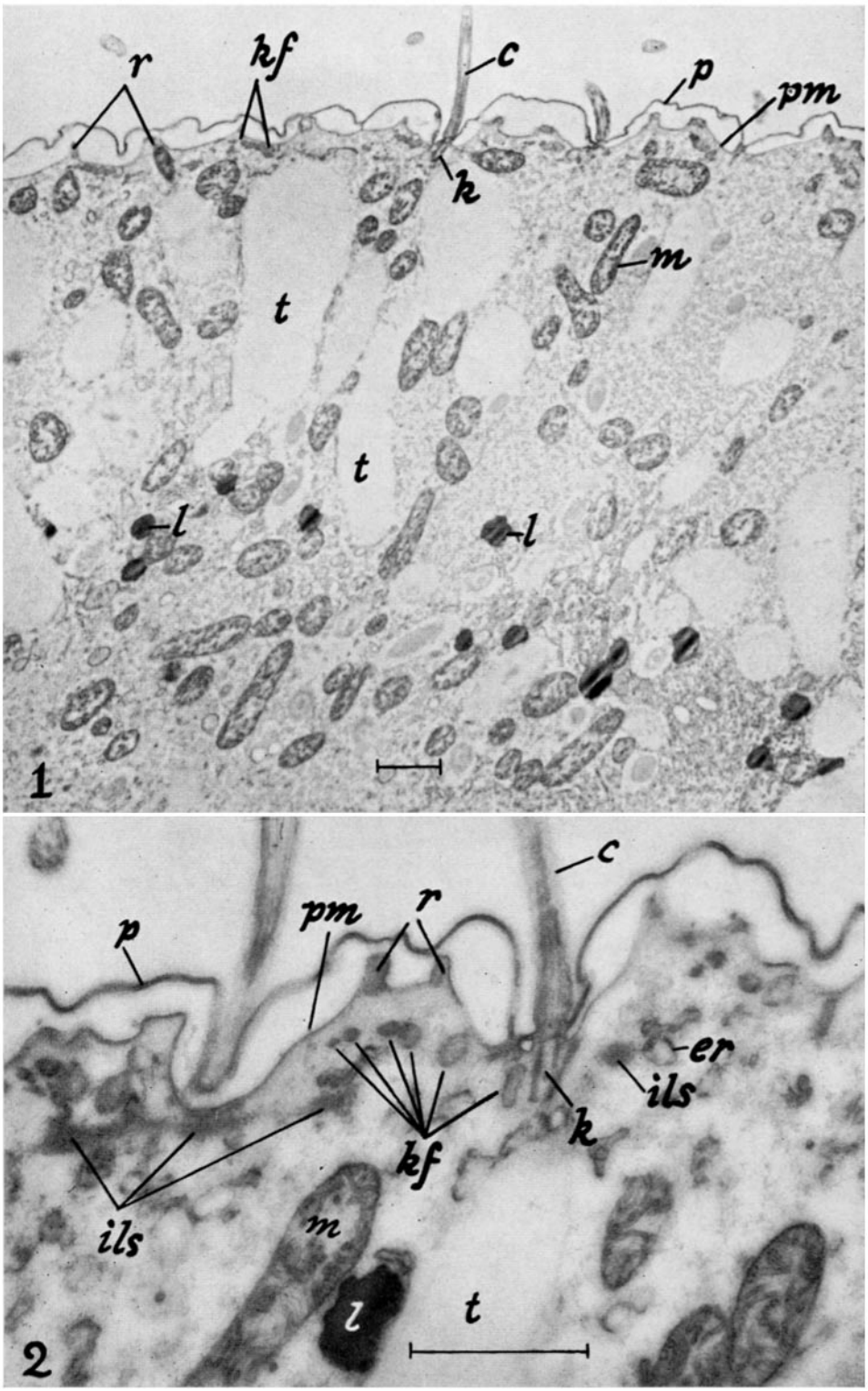
ak, accessory kinetosome.
c, cilium.
cc, paired cilia.
cf, central filaments of cilium.
cm, ciliary membrane.
dt, discharged trichocyst shaft.
er, vesicles of the endoplasmic reticulum
ils, infraciliary lattice fiber.
imm, internal mitochondrial membrane.
ip, inner member of pellicular membrane.
k, kinetosome (basal body).
kf, kinetodesmal or kinetosomal fibrils.
kk, kinetosomes of paired cilia.
l, lipide granule.
m, mitochondrion.
mm, mitochondrial matrix.
mv, microvilli of mitochondria.
omm, outer mitochondrial membrane.
op, outer member of pellicular membrane.
p, pellicle.
pc, particulate component.
pf, peripheral filaments of cilium.
pm, plasma membrane.
r, ridge in surface polygonal ridgework.
t, trichocyst.
tb, trichocyst body.
tc, trichocyst hood-shaped cap.
tm, trichocyst membrane.
tp, transverse partition defining upper limit of kinetosome.
tt, trichocyst tip.

The scale mark drawn on each micrograph is equivalent to one micron unless otherwise indicated.

PLATE 151

FIG. 1. A relatively low power electron micrograph of a transverse section through a portion of the cortex and underlying endoplasm of *Paramecium multimicronucleatum*. The cortex is covered externally by the pellicle (*p*). Beneath the pellicle the surface of the organism, limited by the plasma membrane (*pm*), is molded into ridges (*r*). A cilium (*c*) protrudes from a depression in the organism's surface defined by the ridges. A ciliary basal body or kinetosome (*k*) is located at the proximal end of the cilium (*c*). To the left of this kinetosome a number of kinetodesmal fibrils (*kf*) are pictured in cross section and such groups of fibrils make up the kinetodesmata (neuroneme, neuromotor fibers, silverline fibers) which, in light microscope studies, are observed to interconnect the basal bodies in cilia in each longitudinal row. Other structures recognized as part of the cortex are profiles of mitochondria (*m*), unextruded, trichocyst bodies (*t*), and lipide granules (*l*). × 9,000.

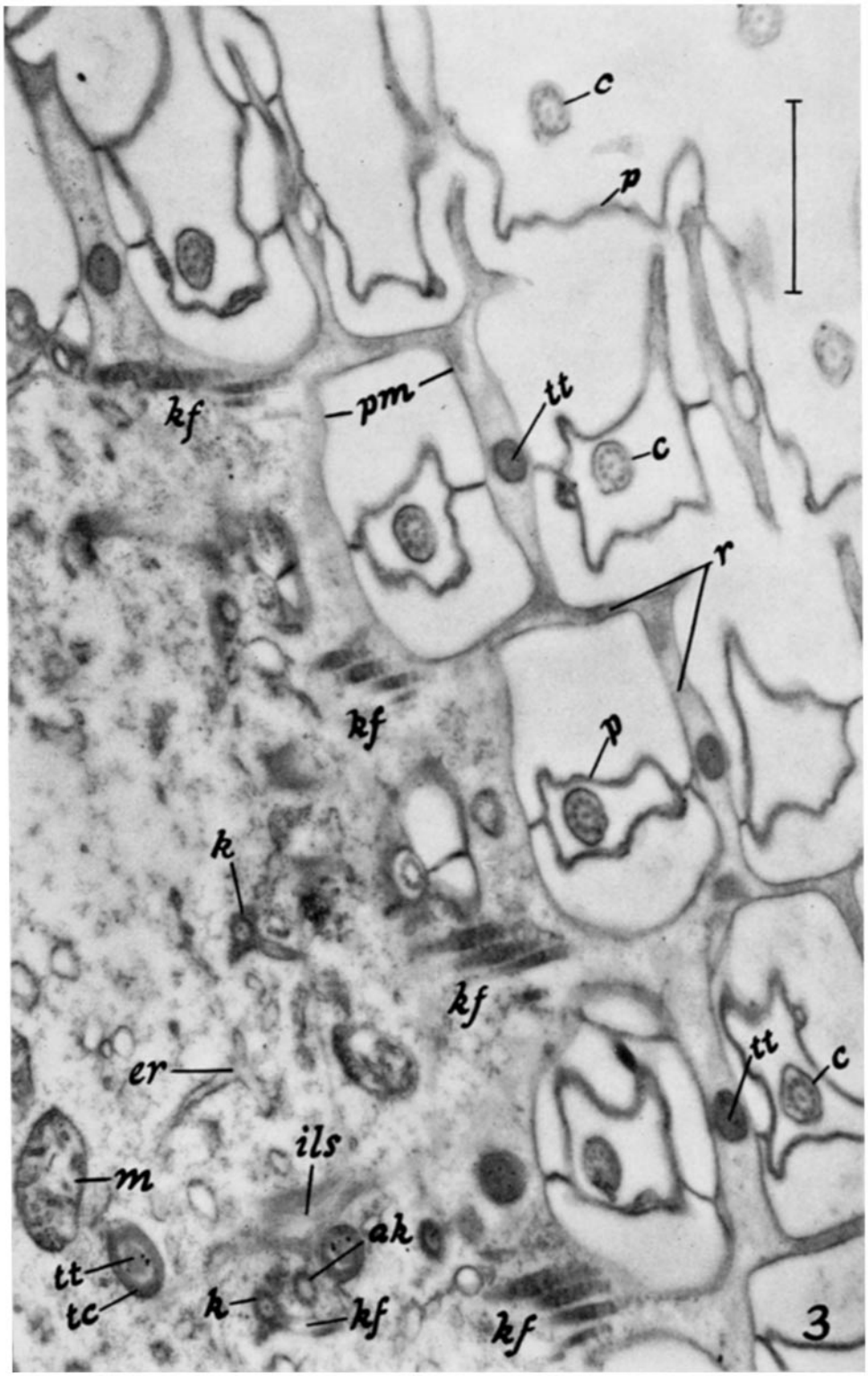
FIG. 2. Electron micrograph of a transverse section through a small portion of the cortex of *Paramecium multimicronucleatum* showing cortical components in greater detail. The pellicle (*p*) consists of two closely apposed membranes (see also Fig. 4). The clear space beneath the pellicle corresponds to the cavity of the system of blisters or vesicles which extends over the surface of the organism and separates, except at the cilia and trichocysts, the pellicle from the surface of the protoplast (*pm*). This surface is molded into a system of ridges (*r*), which gives the organism its characteristic surface pattern. Cilia (*c*) project from the centers of depressions defined by the ridges and each cilium is continuous proximally with a basal body or kinetosome (*k*). To the left of the basal body, in this figure there is an array of 6 kinetodesmal fibrils (*kf*) in cross section. The diameters of these diminish from right to left, descriptive of the fact that the individual fibrils of the array are tapered, and that each extends over only 5 interkinetosome intervals from its origin. Portions of fibers belonging to the infraciliary lattice system (*ils*) are shown within the cortex of the organism at approximately the level of the kinetosome. This system of fibers lies in a plane parallel to the surface of the organism and is separate and distinct from the inner fibrillar system to which the kinetodesmal fibrils belong. Vesicles of the endoplasmic reticulum (*er*), profiles of mitochondria (*m*) showing microvilli internally, a lipide body (*l*), and a portion of an unextruded trichocyst (*t*) occupy the rest of the field. × 25,300.



(Sedar and Porter: Cortex of *Paramecium*)

PLATE 152

FIG. 3. Electron micrograph of a tangential-oblique section through the surface and cortex of *Paramecium multimicronucleatum*. Cross sections of cilia (*c*), showing internal structure, and arranged in parallel rows are evident to the right when the plane of section leaves the organism. The clear areas defined by the plasma membrane (*pm*) and the pellicle (*p*) represent the spaces beneath the pellicle (*p*), *i.e.*, the cavities of the system of cortical vesicles or blisters. (Compare this figure with Figs. 1, 2, and 4). The outer ends of the trichocysts (*tt*) are located along the same meridian as the cilia, in the centers of the anterior and posterior ridges (*r*) the surface polygonal ridgework. To the left of the micrograph in line with the cross sections of the cilia and in a deeper plane of the section, cross sections of the ciliary kinetosomes (basal bodies) (*k*) are evident. At the bottom center of the micrograph a portion of a kinetodesmal fibril (*kf*) is seen to depart laterally from the kinetosome and curve to by-pass the accessory kinetosome (*ak*). At the point of origin of the kinetodesmal fibril there is a spur-shaped structure of unknown significance. Portions of four groups of kinetodesmal fibrils (*kf*) in longitudinal section and showing periodicity of structure, are seen coursing parallel to the kinetosomal and ciliary meridians. A cross section of a trichocyst showing the tip (*tt*) surrounded by the hood (*tc*) (and with dense granules between) is depicted in the lower left hand corner of the figure. A part of the infraciliary lattice system (*ils*), mitochondria (*m*), and vesicles of the endoplasmic reticulum (*er*) are evident in the cortical cytoplasm. × 27,700.



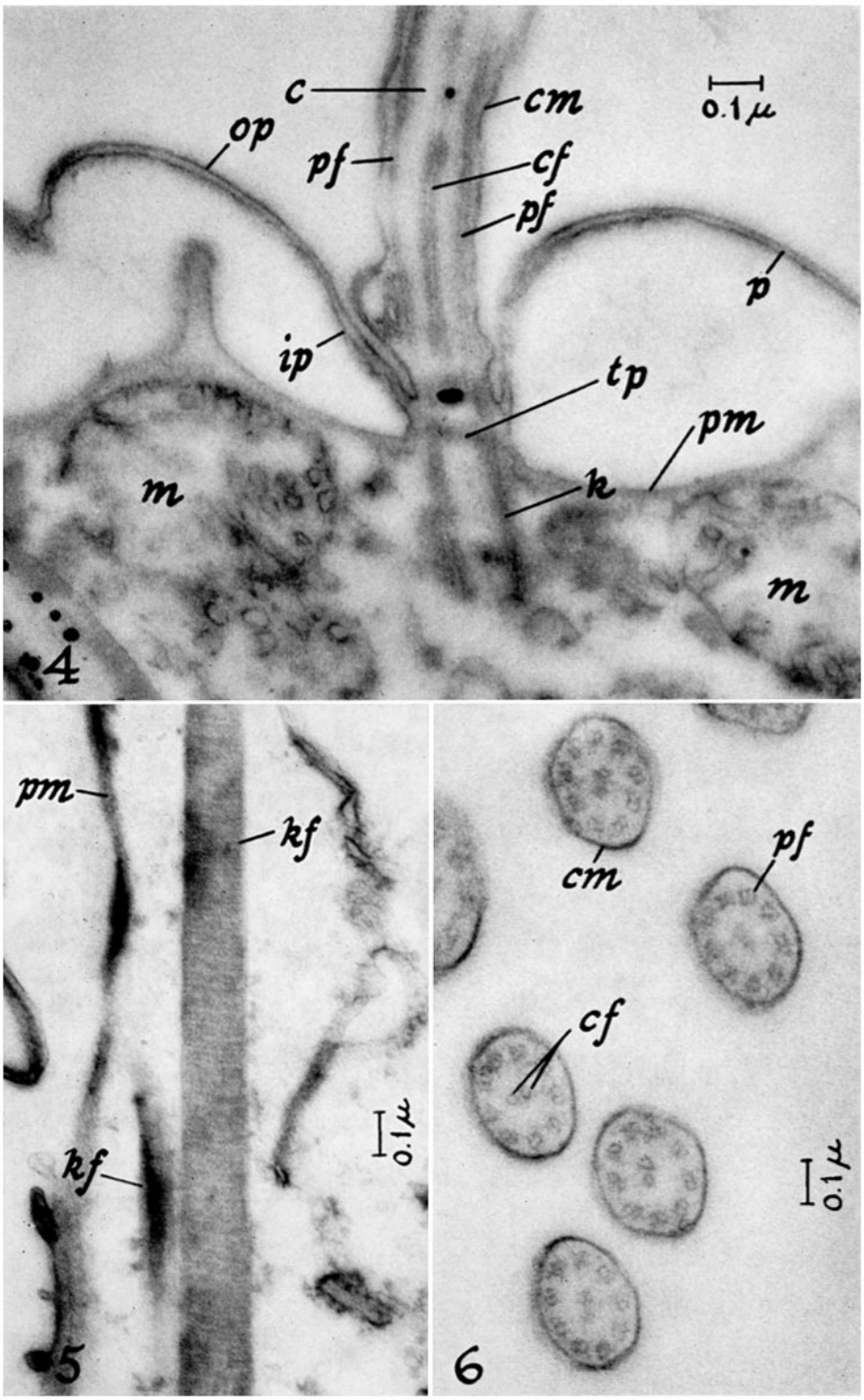
(Sedar and Porter: Cortex of *Paramecium*)

PLATE 153

FIG. 4. Electron micrograph of a vertical section of a portion of the cortex of *Paramecium multimicronucleatum*. The external covering or pellicle (*p*) of the organism's surface is composed of two closely approximated membranes, an outer membrane (*op*) and an inner membrane (*ip*). The outer member of the pair (*op*) is continuous with the membrane (*cm*) surrounding the cilium (*c*); the inner membrane (*ip*) is continuous with the plasma membrane (*pm*) of the cortex and cytoplasm. The two central filaments (*cf*) are seen clearly in this section of the cilium; two of the peripheral filaments (*pf*) pierce a transverse partition (*tp*) and extend into the cortex. Such extensions of the peripheral filaments of the cilium together with the matrix material which tends to condense around them form the sheath of the ciliary basal body or kinetosome (*k*). It is to be noted that central or medullary portion of the kinetosome is continuous, proximally, with the cortical matrix. Mitochondria (*m*) showing both longitudinal and cross sections of microvilli are evident just beneath the plasma membrane (*pm*). × 75,000.

FIG. 5. Micrograph of a longitudinal section of a portion of two kinetodesmal fibrils (*kf*) in *Paramecium multimicronucleatum* showing in them a periodicity of structure. The cross bands in this figure measure ~250 A and the space between is ~100 A. Thus each repeating unit of structure measures ~350 A. Some variation has been observed in the width of the intervening light band in other kinetodesmal fibrils, but the width of the dense band is relatively constant. × 65,000.

FIG. 6. Micrograph showing 5 body cilia of *Paramecium multimicronucleatum* in cross section. The cilia have been compressed slightly in the thin-sectioning process giving them the oval outline depicted. Each cilium shows two central (*cf*) and 9 peripheral filaments (*pf*) embedded in a matrix. The whole is surrounded by a membrane (*cm*). This micrograph shows to good advantage that the 9 peripheral filaments are evenly spaced in a circle around the central pair. Each of the 9 peripheral elements is is itself double with each component of the doublet measuring 150 to 200 A; this is equivalent in size to one of the central filaments. The cilium, therefore, contains 10 pairs of filaments, a central pair and 9 peripheral pairs. Each filament appears in cross section as a tiny tubule. × 63,600.



(Sedar and Porter: Cortex of *Paramecium*)

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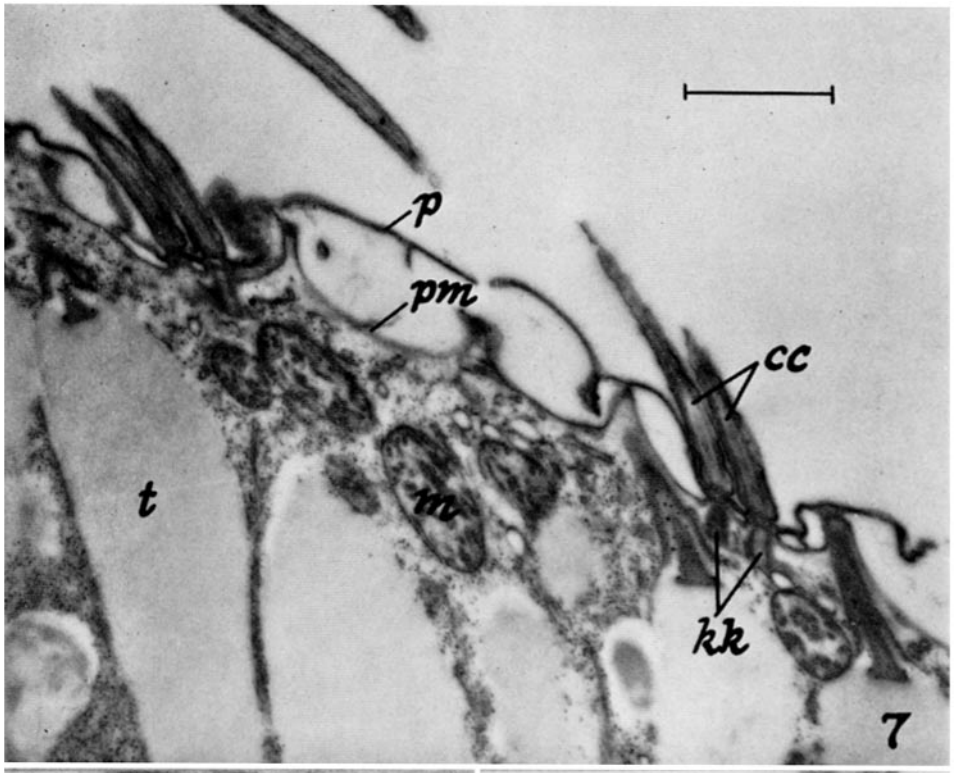
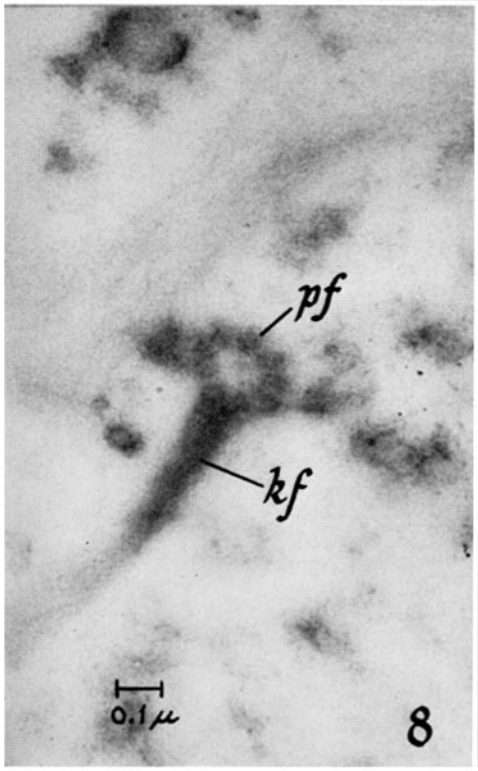
FIG. 7. Micrograph of a sagittal section through the cortex of an organism approaching the fission stage. It is obvious that the cilia have recently duplicated. Instead of only one cilium protruding from the center of the depressions in the cortex there are now two (compare with Fig. 3). These paired cilia (*cc*) are continuous with their associated kinetosomes (*kk*). The pellicle (*p*), plasma membrane (*pm*), mitochondrial profiles (*m*), and portions of unextruded trichocysts (*t*) are also seen in the micrograph. × 19,400.

FIG. 8. Micrograph of a transverse section through a ciliary kinetosome of *Paramecium multimicronucleatum*. It is evident that the wall of the basal body is composed of filaments (*pf*) with a condensation of matrix material around them. These are continuous distally with the peripheral filaments of the cilium. A portion of a kinetodesmal fibril (*kf*) is seen originating from the kinetosome. At right angles to the origin of the kinetodesmal fibril (*kf*) portions of additional osmiophilic material are seen associated with the wall of the basal body. Whether these structures belong to the basal body, or are associated with the origin of the kinetodesmal fibril, cannot be determined from the micrograph. Compare with kinetosomes shown in Fig. 3. × 63,200.

FIG. 9. Micrograph showing details of the anterior end of an unextruded trichocyst. The trichocyst tip (*tt*), enclosed in a hood-shaped cap (*tc*), is located on the blunt end of the body (*tb*). The anterior end of the cap (*tc*) appears in close association with the pellicle (*p*). Both the hood and tip of the trichocyst are clearly more dense to the electron beam than is the body. A number of small dense granules are present between the tip and the cap of the unextruded trichocyst. × 53,700.





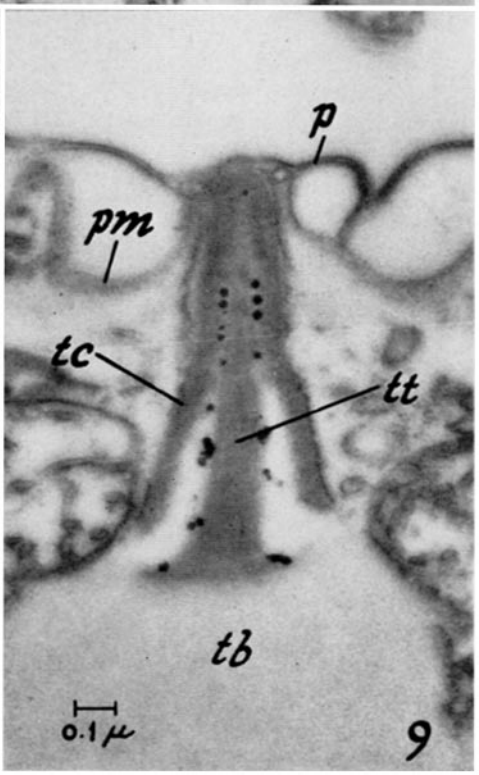


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FIG. 10. Micrograph of a tangential and slightly oblique section through the cortex of *Paramecium multimicronucleatum* at the level of the ciliary kinetosomes (*k*) showing the infraciliary lattice system. A portion of this system is depicted in the form of an incomplete fibrous polygon (ils) with portions of fibers defining adjacent polygons. The fibrous lattice comprising the system lies in a plane roughly parallel to the surface of the body at the level of the kinetosomes. For this reason it is difficult to obtain more than a portion of it in any one thin section. However in thicker sections (see Fig. 11) a more extensive display of the system is occasionally apparent. The infraciliary lattice system should not be confused with the outer lattice structure demonstrated by various silver impregnation techniques to be associated with the pellicle. A mitochondrion (*m*), an accessory kinetosome (*ak*), kinetodesmal fibril (*kf*), cilium (*c*), cross sections of trichocysts (*t*), vesicles of the endoplasmic reticulum (*er*), and the dense particulate component of the cytoplasm are also evident in the micrograph. × 42,000.

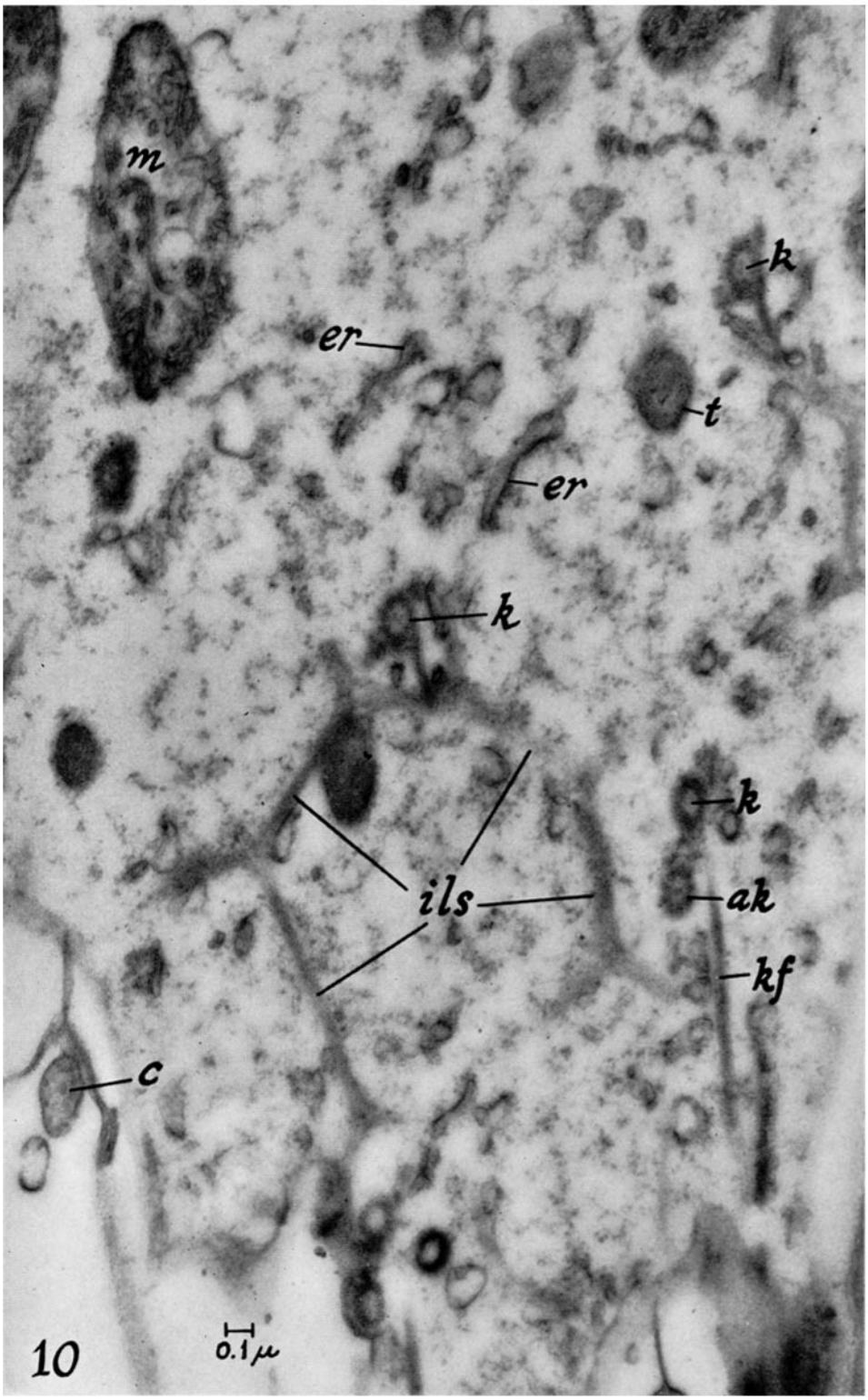

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FIG. 11. Micrograph of a relatively thick tangential section through the cortex of *Paramecium multimicronucleatum* at the level of the ciliary basal bodies (*k*). The infraciliary lattice system is depicted more prominently than in the previous figure. The micrograph shows, in addition, the irregular nature of the system due to the variation in size and shape of the polygons outlined by the fibers. Cross sections of the anterior ends of trichocysts (*t*), with tip (*tt*), associated dense granules and hood (*tc*); an accessory kinetosome (*ak*), kinetodesmal fibrils (*kf*), and a mitrochondrion (*m*), are also evident in the micrograph. × 38,000.

FIG. 12. Micrograph showing the appearance of the shaft of an internally discharged trichocyst in the cortex of *Paramecium multimicronucleatum*. The shaft (*dt*) has a striated appearance with dense bands alternating with less dense in a periodic pattern. Each repeating period measures approximately 550 A. The material of the discharged trichocyst shaft is clearly more dense than that of the undischarged (compare with Fig. 13). A portion of a mitochondrion (*m*) and undischarged trichocyst (*t*), as well as the particulate component (*pc*) are also evident in the micrograph. × 39,000.

FIG. 13. Micrograph of a vertical section through the cortex of *Paramecium multimicronucleatum* to show the shape and structure of the unextruded trichocyst. The trichocyst shows a carrot-shaped body (*tb*) surrounded by a thin membrane (*tm*). The tip (*tt*), resembling a golf tee in shape, enclosed in a hood-shaped cap (*tc*), is located on the blunt end of the body. Both the hood and point of the trichocyst are more dense than the body. The tip of the undischarged trichocyst always has a number of extremely small dense bodies associated with it. Portions of cilia, and the pellicle with the folded surface of the organism beneath it are also shown in the micrograph. × 29,500.

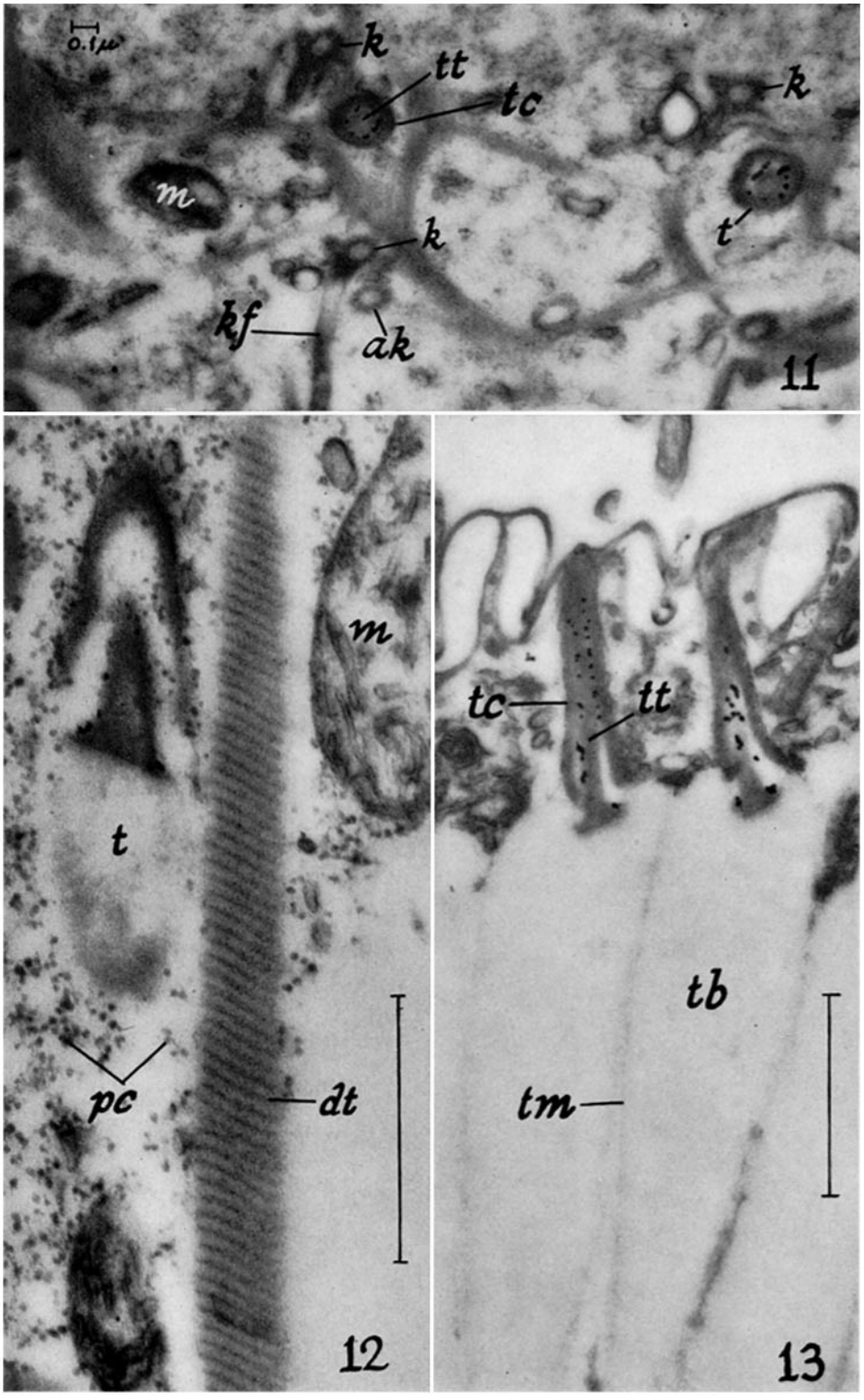

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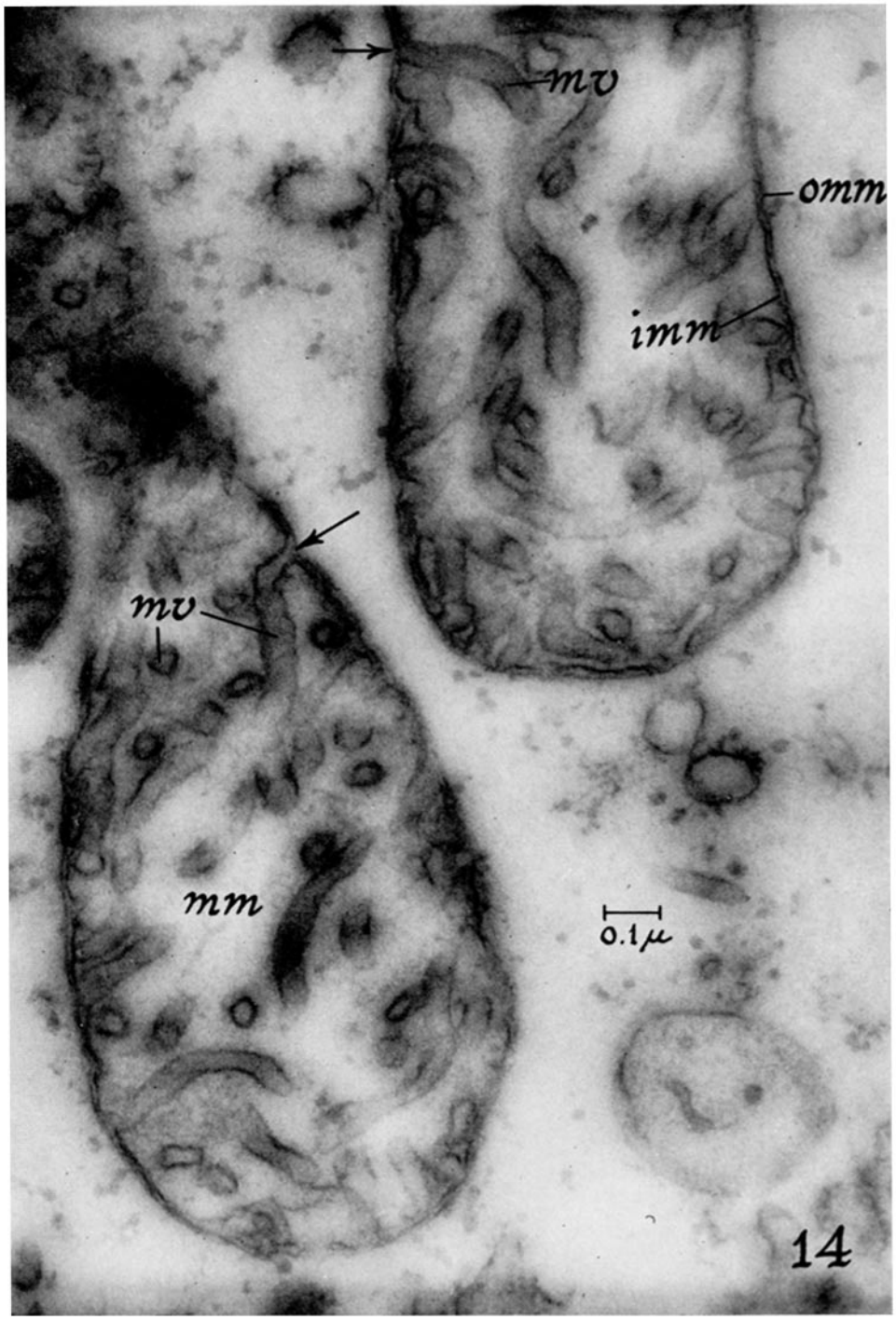
FIG. 14. Micrograph depicting portions of two mitochondria as found in the cortex of *Paramecium multimicronucleatum*. The limiting membrane of the mitochondrion is seen clearly to be double, consisting of two closely apposed membranes, an outer mitochondrial membrane (*omm*) and an internal mitochondrial membrane (*imm*). The structures corresponding to cristae mitochondriales in mammalian somatic cells appear as microvilli (*mv*) protruding into the matrix of the organelle from the internal mitochondrial membrane (*imm*). At points indicated by arrows the lumen of a microvillus appears continuous with the space separating the inner and outer mitochondrial membranes. Both oval and circular profiles as well as longitudinal sections of the microvilli are shown within the mitochondrial profiles indicating that these elements are true villi and not lamellae or cristae. The diameters of the microvilli (40 to 50 mμ) are greater than the thickness of the cristae found in the mitochondria of somatic cells of higher animals. $\times$ 80,000.



(Sedar and Porter: Cortex of *Paramecium*)