CORTICAL ULTRASTRUCTURE OF *PARAMECIUM AURELIA*

Studies on Isolated Pellicles

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ABSTRACT

Two methods have been devised for the isolation of large quantities of purified pellicles (cortical layers) of *Paramecium aurelia*. Pellicles isolated by both procedures, when examined by electron microscopy, were found to contain ciliary basal bodies, two types of cortical membranes, ribbons of microtubules, kinetodesmal fibers, and elements of the infraciliary lattice system. By electron microscopy, the extent of preservation of the various cortical structures when pellicles are isolated by each method has been characterized.

Pellicles isolated in both ways have been utilized to investigate cortical morphology of *Paramecium*. Both phase-contrast and electron microscopic observations have been made. Many new ultrastructural features were observed and are reported herein.

An interesting result of this study is the discovery in stock CD that the structure of cortical territories (the territory is the functional unit of cortical morphogenesis and physiology) may vary within a single organism. Features which show variation include number of parasomal sacs, microtubular ribbons, and basal bodies (and therefore cilia) per territory, number of microtubules per ribbon, and length of kinetodesmal fibers. The possible significance of these variations, with respect to territory replication, is discussed.

In addition, preliminary observations on the solubility of various cortical organelles in the presence of a number of protein-denaturing agents are reported.

INTRODUCTION

Ciliated protozoa are unicellular eucaryotes whose most interesting differentiations occur within a thin layer of cortical cytoplasm commonly known as "pellicle." Recently, it has been established for the ciliate, *Paramecium*, by Sonneborn and Dippell (Sonneborn, 1963), that in animals reproducing by division the old cortical pattern influences the organization of the newly forming cortex. The studies of Sonneborn and his coworkers (see also Beisson and Sonneborn, 1965) and of others (Tartar, 1961; Nanney, 1966a, b, c, 1967; Lwoff, 1950) have aroused interest in the ciliate cortex as a morphogenetically active system inherently worthy of study. There have also been suggestions that events which occur in the ciliate cortex in a very diagrammatic fashion are only superficially different from events occurring in or between other eucaryotic cells. Thus an understanding of mechanisms governing ciliate morphogenesis could have general application.

In addition, there are the phenomena of mating type interactions and serotypes (for summaries see

Preer, 1957a; 1957b, 1969; Beale, 1959, 1964), both of which are closely related to cortical function. While a great deal is known genetically about these properties of ciliates, the mechanisms of serotypic and mating type interactions are little understood.

A better understanding of the functions of cortical structures of ciliates requires detailed knowledge of their ultrastructural organization, within a single organism and within genetically differing stocks. In addition, an understanding of the relationship of the cortex to the molecular mechanisms of gene action, cellular motility, and excitability is needed, and this necessitates a chemical characterization of cortical structures.

A useful way to study single organelles or groups of related organelles is to isolate them from other cellular constituents. While methods for isolating cortical structures from ciliates have previously been reported (*Tetrahymena*: Child and Mazia, 1956; Argetsinger, 1965; Satir and Rosenbaum, 1965; Hoffman, 1965; rumen ciliates: Olpin, 1967; *Euplotes*: Grim, 1966), a method for use with *Paramecium* would be especially interesting in light of all that is known of its genetics and biology (Beale, 1954; Wichterman, 1953). Methods have now been devised for isolation of pellicles from *Paramecium aurelia* and will be reported in this paper.

In order to determine which cortical organelles are present in isolated pellicles, and to estimate the degree of preservation of these organelles, observations on pellicular ultrastructure were made, by using electron microscopy of negatively stained material. These observations will be described, and comparisons will be made with previous observations on cortical fine structure in *Paramecium* (Ehret and Powers, 1959; Metz et al. 1953; Pitelka, 1965; Sedar and Porter, 1955; Stewart and Muir, 1963).

Isolated pellicles should be useful for the eventual purification of individual cortical structures, since pellicular fractions represent an enrichment of such organelles as ciliary basal bodies, kinetodesmal fibers, cortical membranes, cortical microtubular ribbons, and infraciliary lattice. Toward this end, some preliminary observations on the differential solubility of various cortical structures will be reported.

In addition, an examination of the over-all chemical composition of isolated pellicles would be desirable. In a separate paper, observations on the DNA associated with pellicles will be reported (Hufnagel, in preparation).

Sone of the observations reported here have been briefly described in earlier publications (Hufnagel, 1965, 1966).

MATERIALS AND METHODS

Stocks

Stocks 7K (syngen 2) and 51K (syngen 4), of *Paramecium aurelia*, were utilized in developing isolation techniques and for some studies involving the solubility of pellicles. Detailed electron microscopic examination of pellicles was carried out on stock CD (a syngen 1 derivative isogenic with stock 90 except for the presence in homozygous condition of the genes *cl*, *dp*, *mt*, d^{60}, and g^{60} (Kimball and Gaither, 1955), kindly supplied by Dr. R. Kimball. Stock CD was used because it is virtually free of crystals, which were found to be a major contaminant of pellicles isolated from crystal-containing stocks.

Culture, Harvesting, and Storage

Stock cultures were maintained in test tubes on sterile, buffered Scottish grass (Anderson et al., 1964), inoculated with *Aerobacter aerogenes* 1 day prior to feeding. Mass cultures were grown in 5-gal jugs and were harvested as described by Preer and Preer (1959). Harvested animals were regularly stored before use for 1–7 days at 15°C, at a concentration of 10^7 animals (1 g packed cells) per 100 ml exhausted culture fluid (approximately 500 ml per 2 liter flask). Therefore, pellicles were usually isolated from starved, nondividing animals.

Electron Microscopy

Two methods were employed for examination of isolated pellicle preparations with the electron microscope. Some pellicles were negatively stained with 4% phosphotungstic acid (PTA), adjusted to pH 7.0 with NaOH (0.4% sucrose added; Yamamoto and Anderson, 1961), with or without prior fixation (5 min) in osmium tetroxide vapors. Fixing and staining were carried out after a drop of pellicle suspension had been placed on a carbon-coated Formvar grid.

In addition, pellicles were prepared by Anderson's critical point method (Anderson, 1952, 1954), to preserve three-dimensional structure. Stereo pictures were taken by means of the special stereo specimen holder for the Akashi Seisakusho microscope (Hardco Scientific Division, Fieldstone Corporation, Cincinnati, Ohio) and were mounted in pairs for stereoscopic viewing.

Material which had been negatively stained or treated by the critical point method was examined, and photographs were taken with an Akashi Trono-

scope, model TRA-50EI, a Siemens Elmiskop I (at the Institute for Cancer Research, Fox Chase), and a Philips model 200 electron microscope (Philips Electronics and Pharmaceutical Industries Corp., New York). Micrographs, taken on Ilford and Kodak high-contrast plates and on Kodak fine grain positive 35 mm film, were developed with D-19 or Microdol X.

Isolation of Pellicles

A variety of homogenization media, involving different sugars, alcohols, pH, presence of calcium, and EDTA, have been examined. Two media were found to give good preservation of pellicular structure and to produce only a little clumping of other cellular organelles. The two media are given below and will be designated "raffinose medium" and "ethanol medium," respectively.

I. 0.2M raffinose
0.01M sodium phosphate buffer, pH 6.8

II. 12% ethanol in distilled water (pH approximately 5.5)

PROCEDURE: Animals were collected by centrifugation for 1 min at top speed (700 *g*) in an International oil centrifuge (Model H). (International Equipment Co., Needham Heights, Mass.). The supernatant was discarded. The packed cells were resuspended in approximately 9 volumes of ice-cold homogenization medium. The suspension was transferred to a Potter-Elvehjem homogenizer of suitable size, having a close-fitting Teflon or glass pestle, and was homogenized by hand or mechanically (for large quantities of cells). Homogenization was monitored by examination of a sample under a dissecting microscope, and was stopped when most, but not all, of the animals were broken open.

The homogenate was transferred to precooled 12 or 40 ml centrifuge tubes, diluted to a depth of about 7 cm with homogenization medium, and spun in a Sorvall RC-2 refrigerated (0–2°C) centrifuge (Sorvall, Ivan, Inc., Norwalk, Conn.) for 5 min at 2000 rpm (480 *g*), with an SS-34 rotor. The precipitate was collected, resuspended in homogenization medium, and centrifuged as before. The second precipitate was then examined for whole cells and, if any were present, was rehomogenized by hand in a very close-fitting glass homogenizer. Rehomogenization also helped to free small particulate contaminants which had lodged within nearly intact pellicles.

After rehomogenization, the pellicles were washed as before through four more changes of homogenization medium (for the last two washes, raffinose medium was sometimes replaced by 0.01M phosphate buffer, pH 6.8).

Studies on Solubilization

Several techniques were used for treating pellicles with solubilizing agents. The most satisfactory method was to sediment a small quantity (0.01 ml) of pellicles by centrifuging about 0.6 ml of pellicle suspension at 2000 rpm (515 *g*) for 5 min in 6 by 50 mm glass tubes, with the swinging bucket (HS) rotor of the Sorvall refrigerated centrifuge. The supernatant fluid was removed by pipette and replaced by a similar quantity of test solution, which was mixed with the precipitated pellicles. The mixture was then examined by phase-contrast microscopy and recentrifuged to concentrate the pellicles, if their integrity had not been altered. Another method was to mix a concentrated pellicular suspension with 10–20 parts of the test solution. For some tests of pH effects, pellicles were suspended in phosphate buffer, and 1 N HCl or 1 N NaOH was added dropwise. The pH was continuously monitored with a Beckman pH meter (Beckman Instruments, Inc., Fullerton, Calif.), while progressive changes in pellicular structure were monitored by phase-contrast microscopy.

OBSERVATIONS

Microscopic Observations

Pellicles have been isolated from stock 7 (syngen 2), stock 51 (syngen 4), and stock CD (syngen 1). When raffinose medium was employed, a yield was obtained of 1.8–15 mg (dry weight) of pellicles per g of packed cells (stock CD), but usually between 2 and 5 mg. The yield of pellicles isolated in ethanol has not been determined. Pellicles isolated by both methods, when examined by phase-contrast microscopy (Figs. 5, 7), were found to consist of large fragments, some representing the entire cell surface. Gullets usually remained attached to a portion of the surrounding pellicle. Cilia and trichocysts were not present, both having been detached. The exact level at which cilia were broken is not known; however, no portion of a ciliary stump was ever recognized in negatively stained preparations.

A rough estimate of the purity of the preparations was made by phase-contrast microscopy. In stocks 7 and 51, crystals constituted the major contaminant of pellicles. In stock CD, a mutant having relatively few small crystals, there was no detectable contamination from crystals. Other particulate contaminants were trichocysts, mitochondria, cilia, and bacteria.

A tendency was observed for pellicles isolated in raffinose medium to undergo slow degradation.

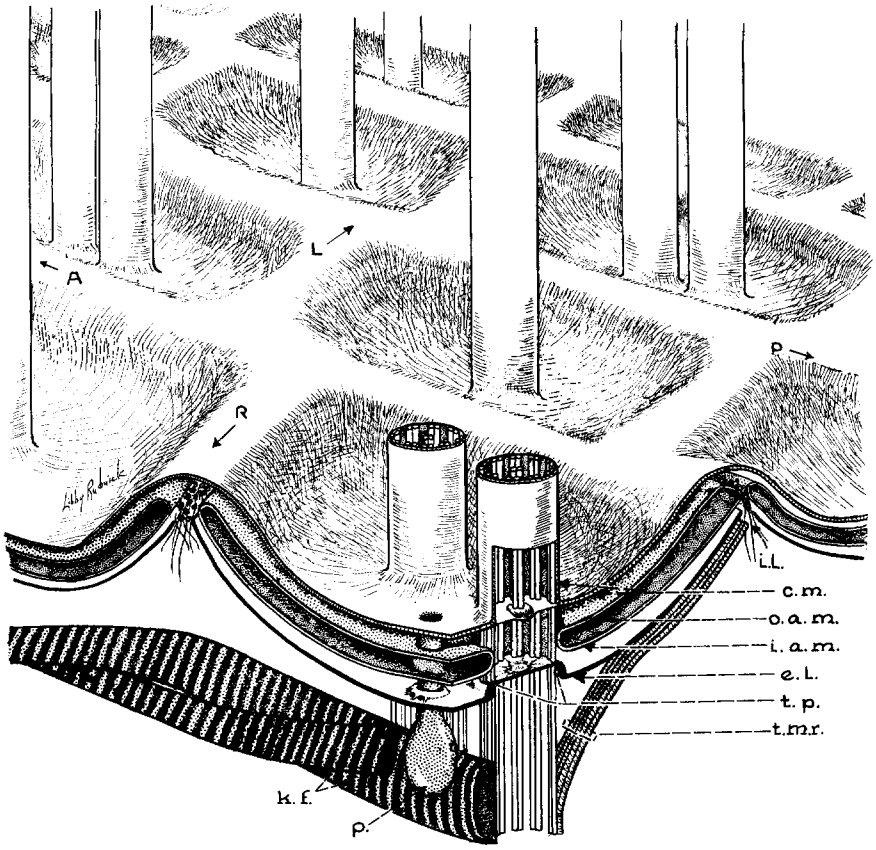


FIGURE 1 Schematic representation of a portion of a pellicle of *Paramecium*, illustrating the associations between membranes, basal body, kinetodesmal fiber, and microtubules and based on previously published observations of other investigators and on the present study. *L*, *R*, *A*, *P*: animal's left, right, anterior, and posterior; *k.f.*, kinetodesmal fiber; *p.*, parasomal sac; *i.l.*, infraciliary lattice; *c.m.*, cell membrane; *o.a.m.*, outer alveolar membrane; *i.a.m.*, inner alveolar membrane; *e.l.*, epiplasmic layer; *t.p.*, transverse plate; *t.m.r.*, transverse microtubular ribbon.

Pellicular breakdown involved detachment of kinetodesmal fibers through dissolution of the kinetosomes, swelling of the membranous portions of the pellicle, and separation of pellicular fragments into many smaller pieces (Fig. 6). Degradation could be minimized by use of fresh solutions and ice-cold temperatures throughout pellicular isolation. Progressive pellicular breakdown was never observed when ethanol medium was employed.

Raffinose-isolated pellicles of stock 7 and stock CD and ethanol-isolated pellicles of stock CD were examined by electron microscopy. For a diagrammatic portrayal of the organization of structures within the cortical region of *Paramecium*, the reader may refer to Fig. 1.

Pellicles fixed in osmium tetroxide vapors and stained with phosphotungstic acid (Fig. 8) were observed to consist of longitudinal rows (kineties) of repeating units corresponding to the "ciliary corpuscles" of Ehret and Powers (1959) or the "territories" of Pitelka (1969). Each territory is roughly 2 μ long and 1.7 μ wide. Width was found to be quite constant but length can vary by a factor of almost two. Each territory consists of a basal body (or pair of basal bodies), attached kinetodesmal fiber, and associated membranes. Each territory also contains one or more parasomal sacs and an assortment of microtubules and microfibrils.

In the present studies, it was found that if pellicles are not fixed before being stained with

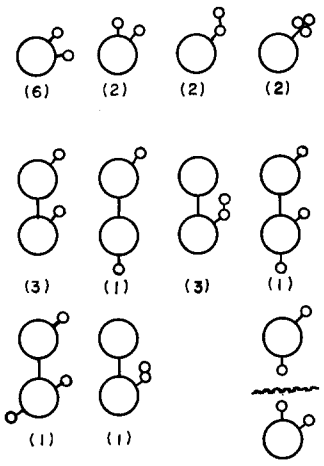


FIGURE 2 Associations between normal and supernumerary parasomal sacs and kinetosomes, observed in pellicles of stock CD. In each case, the number of observed cases is indicated in brackets. The last example represents adjacent territories in the same kinety.

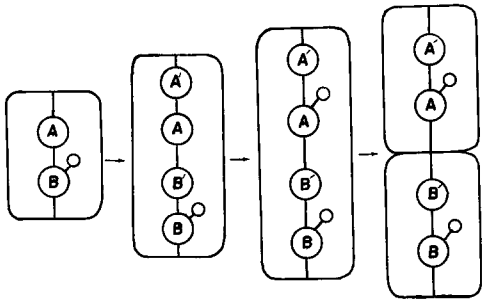


FIGURE 3 The author's interpretation of cortical territory replication, as described by Dippell, 1965. Note elongation of territory and formation of new structures within the original territory, followed by splitting of one territory into two. A, B, old kinetosomes; A′, B′, new kinetosomes.

PTA, kinetosomes, kinetodesmal fibers, microtubules, and some membranous materials are solubilized by the stain. Such preparations are useful for examining the fine structure of remaining parts of the pellicle. The information presented below will be a summary of observations on both fixed and unfixed material. An attempt to summarize those observations which are entirely new will be made later.

The following structures were observed in pellicles isolated in the presence of both raffinose and ethanol: kinetosomes, kinetodesmal fibers,

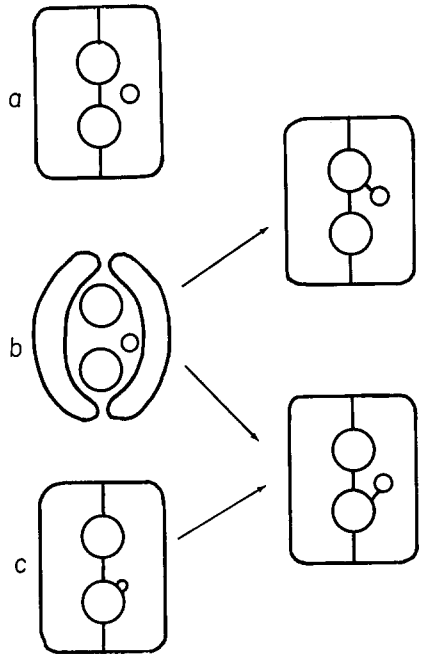


FIGURE 4 Three alternative hypothetical territory configurations whose occurrence would depend on the mode of formation of parasomal sac and pellicular alveoli. In case (*a*) the sac develops apart from the kinetosomes and follows the development of alveoli. In case (*b*), formation of the sac precedes formation of the alveoli. In case (*c*) the sac and alveoli develop concurrently, with the sac first appearing near the posterior kinetosome. Both (*b*) and (*c*) could account for the observed relationship between sac and posterior basal body.

cortical alveoli, epiplasmic layer, parasomal sacs, cortical microtubular ribbons, and infraciliary lattice. The appearance of each of these components will now be discussed:

KINETOSOMES: Each cortical territory may contain either one or two kinetosomes (*k.*), depending on its location in the cortex (distribution of singlets and doublets will be presented later). Each kinetosome measures about 0.5 μ in length and 0.3 μ in diameter. Kinetosomes are present in both raffinose- and ethanol-isolated pellicles. However, they appear to be more stable after isolation by the ethanol method, since in negatively stained ethanol-isolated kinetosomes (Fig. 10) uncollapsed microtubules (thought to be uncollapsed because of their stain-containing lumens) may be seen, whereas the microtubules of raffinose-isolated kinetosomes (Fig. 9) appear to be collapsed and

partially disintegrated. Due to kinetosomal thickness, the finer structure of the intact negatively stained kinetosome cannot be seen.

Single collapsed kinetosomal microtubules have been observed (Fig. 11). They are composed of longitudinal microfibrils about 45 A wide, having a beaded appearance. The total number of microfibrils per tubule could not be determined; at least six fibrils were visible in some tubules.

KINETODESMAL FIBER: The kinetodesmal fiber (*k.f.*) is a long, tapering fiber, which is attached by its broad end to the right side of the proximal end of the kinetosome or to the posterior kinetosome of a pair (Figs. 1, 8, 22). In the latter case, the kinetodesmal fiber lies to the right side of the anterior kinetosome and is attached to it quite securely, in a manner which is not clear from negatively stained specimens. This attachment is evidenced by the fact that mechanically freed kinetodesmal fibers frequently have two attached kinetosomes. Each kinetodesmal fiber projects anteriorly, parallel to kinetodesmal fibers emanating from other kinetosomes of the same longitudinal row (kinety). Together, these fibers form a loose bundle. However, near the gullet this bundle seems more tightly knit and its fibers may twist very slightly about each other, as Ehret and Powers (1959) have described for *P. bursaria*. However, no evidence of twisting has been reported in sectioned *P. aurelia* (Dippell, personal communication).

The kinetodesmal fiber is about 410 mμ wide at the point where it attaches to the kinetosome. About 700 mμ away from the kinetosome it begins to taper rapidly, then more slowly again (Fig. 23). At the point where greatest change in diameter occurs, the kinetodesmal fiber is slightly bent. This possibly occurs when the fiber, rising from the level of the proximal end of the kinetosome toward the cortical membranes, changes course to run parallel to the membranes (Fig. 1). Length of the kineotodesmal fiber may vary, depending on the part of the cortex in which it is found. Near the mouth, fibers may extend a distance of at least six territories, while on the dorsal surface the length is somewhat less. Thickness of the fiber may also vary.

As Pitelka (1965) has observed, negatively stained kinetodesmal fibers exhibit a repetitive substructure along their lengths (Figs. 19–21). The periodicity of raffinose-isolated kinetodesmal fibers from stock CD varies from 350 to 400 A Periodicities of ethanol-isolated kinetodesmal fibers are smaller: 290–340 A. Pitelka has reported periods of 300–350 A for *P. multimicronucleatum* (1965). The cross-striations are not always at right angles to the axis of the fiber, but make an angle with it (Fig. 19) which decreases in size progressively distal to the kinetosome. Measurement of this angle gives values varying from 75 to 90°.

As described before by Pitelka (1965) for *P. multimicronucleatum*, the major period is divided into two minor bands (Fig. 21). In *P. aurelia*, the paler

FIGURE 5 Pellicles of *P. aurelia* isolated in buffered raffinose; phase contrast. × ~800.

FIGURE 6 Pellicles isolated in buffered raffinose and treated with 1/2% sodium deoxycholate for several minutes; phase contrast. × ~800.

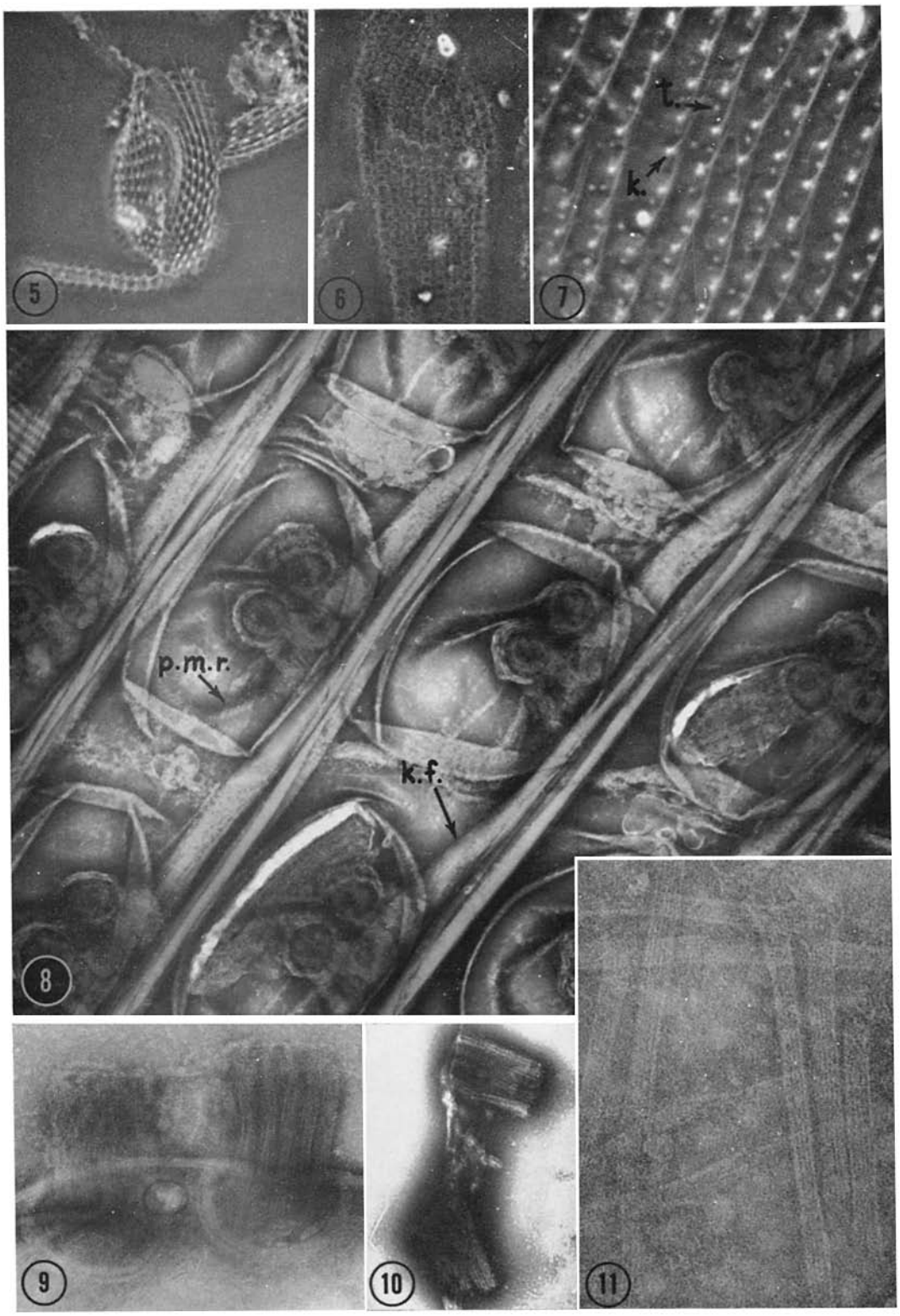
FIGURE 7 Pellicle isolated in ethanol medium; flattened by Sato's method (personal communication) between oil and glass. *t.*—trichocyst attachment site; *k.*—kinetosome. Phase contrast. × 2,300.

FIGURE 8 Raffinose-isolated pellicle, circumoral region; OsO_4 fixation; PTA staining; *p.m.r.*, posterior microtubular ribbon; *k.f.*, kinetodesmal fiber. × 28,000.

FIGURE 9 Raffinose-isolated kinetosome, attached to cortical membranes; note partial distortion of microtubular components. OsO_4; PTA. × 49,000.

FIGURE 10 Ethanol-isolated kinetosomes, still connected by remnants of shared kinetodesmal fiber. Note that their tubular elements are visible. OsO_4; PTA. × 110,250.

FIGURE 11 Separated kinetosomal microtubules, identified by their association (not shown) with pellicular membranes. Note 45 A subfibrils. Raffinose; OsO_4; PTA × 116,250.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band measures about 180 A in width and was observed to contain five minor bright lines (Pitelka observed only three lines). The central line is slightly narrower than the four others, although the space it occupies appears to be about the same. The darker minor band is about 135 A wide and contains a fuzzy bright band which appears slightly off-center. The subdivisions of the major period can be seen clearly only in small areas of some kinetodesmal fibers.

The kinetodesmal fibers of isolated pellicles were observed to be composed of longitudinal fibrils having a center to center period of 47 to 51 A (Fig. 21). These fibrils present a beaded appearance. The dimensions of the subunits could not be determined. In raffinose preparations the subfibrils become evident only when the fiber is partly destroyed (Fig. 19). The fibrillar nature of ethanol-isolated fibers can be seen even when the fibers are relatively intact (Fig. 21).

MEMBRANES: Pitelka (1965) has demonstrated that the pellicle of *Paramecium* contains two separate membranous systems (see Fig. 1). These are (*a*) the cell membrane and (*b*) the membranes of the pellicular alveoli. Only the alveolar membranes are recognizable in negatively stained, isolated pellicles. The cell membrane may be present, but there is no absolute way of identifying it. An invagination of the cell membrane, the parasomal sac, is present, and will be discussed later.

The alveoli (*a.m.*), originally described in *Paramecium* by Ehret and Powers (1959), are flattened unit membrane-bounded vesicles which are closely applied to the undersurface of the cell membrane. There are two such vacuoles in each territory, each forming a half-moon configuration around the centrally located basal bodies (Figs. 1, 3, 12, 13). The two vacuoles are contiguous along a line extending from the basal bodies forward to the anterior margin of the territory and backward to the posterior margin. At the points at which this line of contiguity meets the margins of the territory, there are specially differentiated attachment structures for the trichocysts (Figs. 22, 25).

The line of contiguity between alveoli (*l.c.*) may be seen in raffinose-isolated pellicles prepared by the critical point method; it is a single fine line directly connecting kinetosomes of a single kinety (Fig. 22). In PTA-stained, raffinose-isolated pellicles which have experienced partial degradation of fibrous structures (Fig. 12), the line appears to

FIGURE 12 Portion of a cortical territory from which basal bodies and kinetodesmal fiber have been removed. Note that the line of contiguity (*l.c.*) between alveoli is composed of two closely spaced membranes. (*p.s.*, parasomal sac) Raffinose; OsO_4; PTA. × 37,370.

FIGURE 13 Portion of a territory containing two kinetosomal attachment sites. Note the presence of a transverse plate at each kinetosomal site and the association of parasomal sac with the posterior-most kinetosome, (*a.m.*, alveolar membrane; *p.s.*, parasomal sac; *e.l.*, epiplasm). Raffinose; OsO_4; PTA. × 51,290.

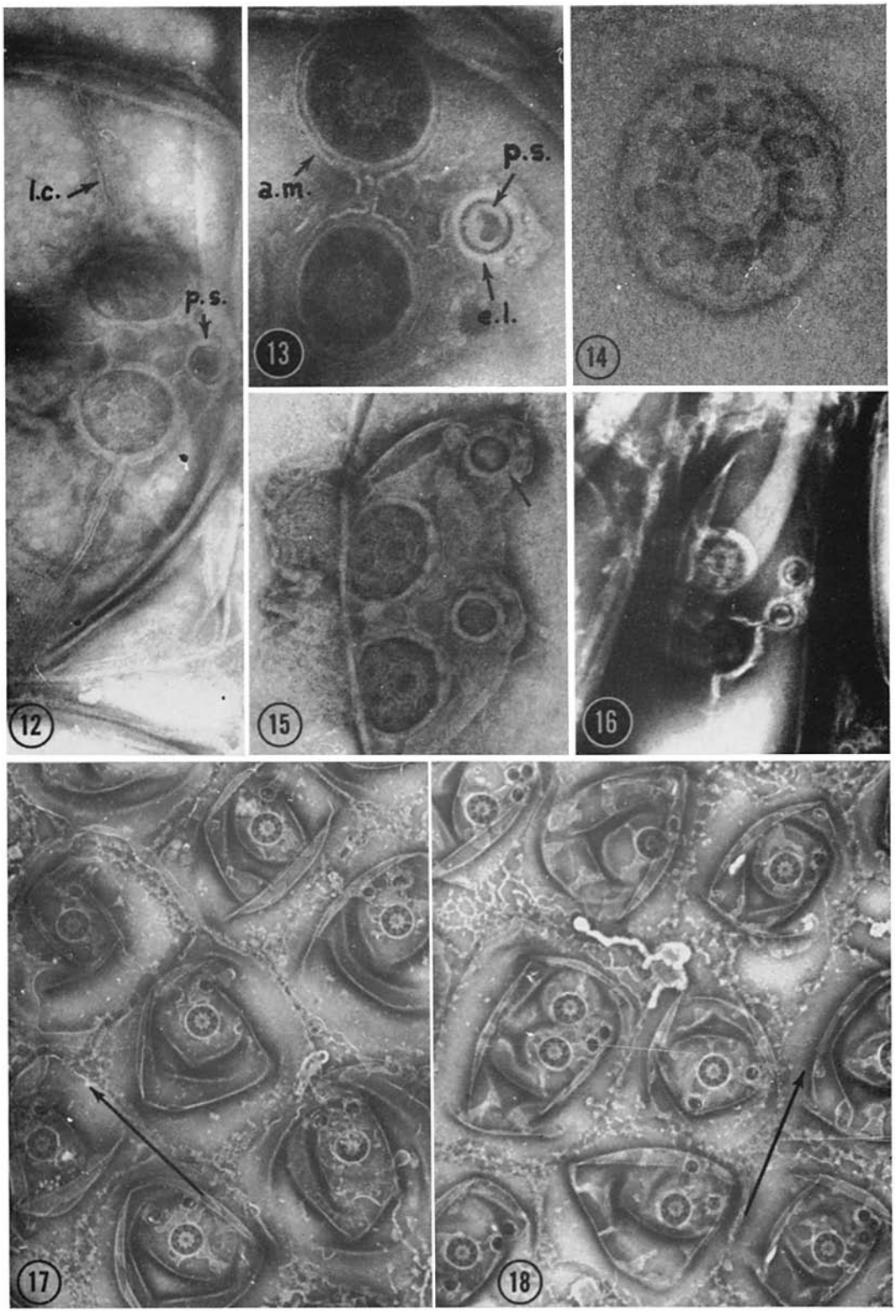
FIGURE 14 Transverse plate, separated from rest of epiplasm. Raffinose; PTA. × 91,890.

FIGURE 15 Two parasomal sacs in one territory, in stock CD. (arrow indicates extra sac). Raffinose; OsO_4; PTA; × 38,100.

FIGURE 16 Two parasomal sacs in one territory in stock CD, both associated with only one kinetosome. Raffinose; OsO_4; PTA. × 20,500.

FIGURE 17 Extra sacs are present in some but not all territories (arrow indicates axis of kinety). Note that each territory contains only one kinetosomal site; therefore this pellicular fragment is from the dorsal region of the animal. Ethanol; no fixation; PTA. × 15,170.

FIGURE 18 Same as Fig. 17. Arrow indicates axis of kinety. Two territories are nearly mirror images (near arrow). Granular-fibrillar portions of the infraciliary lattice surround each territory. Note in the lower right-hand and upper left-hand corners the presence of territories containing three parasomal sacs; in each case one sac is slightly smaller than the others. × 15,170.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be composed of two closely spaced membranes (width: 110 A), separated by a width of 100 A. PTA-stained, ethanol-isolated pellicles exhibit the line most clearly (Fig. 25). The membranes are very closely applied and there may be nodular thickenings where they meet. Nodules are also observed in material prepared by the critical point method (Fig. 22).

The right alveolus of each territory is molded around the parasomal sac opening in such a way that it meets itself along a line extending from sac aperture to the attachment site of the posterior kinetosome (Figs. 12, 13, 15, 16). The significance of this observation will be discussed later.

There is some discussion in the literature about whether the alveolus is flat or inflated in vivo (Pitelka, 1965). In the present studies, under conditions which destroy basal bodies and kinetodesmal fibers and separate territories, the territories often appear to swell or round up (Fig. 6). This suggests that the alveoli are flat when the pellicles are first isolated and later can inflate. The osmotic properties of the alveoli should be subjected to more thorough studies.

EPIPLASM (*e.l.*): Pitelka (1965) has shown that a thin amorphous layer, which she considers analogous to the epiplasm (Fauré-Fremiet et al., 1962) of other ciliates, underlies the pellicular alveoli. Her thin sections of *Paramecium multimicronucleatum* indicate that the epiplasm forms a dense ring around the base of each cilium and is continuous across the top of the basal body. Thus, the basal body is separated from the cilium by a transverse epiplasmic plate ("transverse plate"), except where the outer fibers of the cilium are continuous with microtubular elements of the basal body (Fig. 1).

The present studies agree with these findings. The epiplasm is best seen in raffinose- or ethanol-isolated pellicles which have been stained with PTA without prior fixation with OsO_4 (Figs. 13, 14, 17, 18). From this material, it is evident that the epiplasm is not continuous over a large area of the animal, but is interrupted at the margins of each territory. High resolution micrographs have not revealed any oriented substructure in the epiplasm.

Around each ciliary base and parasomal sac opening the epiplasm thickens to form a ring (Figs. 12, 13). The ring around the parasomal sac is about 225 A wide and is finely serrated (170 A spacing) around its inner margin. There are four or five large (335 A) notches along its right margin. There appears to be a dense granule in each notch.

The epiplasm continues across the ciliary attachment site as the transverse plate (Figs. 12–14). This plate is a stellate structure consisting of a central hub, nine radiating spokes, and an outer rim. Diameter of the transverse plate is 0.4 μ; diameter of the ring of holes through which the kinetosomal microtubules pass is about 0.3 μ, corresponding to the diameter of the kinetosome. On occasion, the transverse plate has been observed to become separated from the rest of the epiplasm (Fig. 14).

PARASOMAL SAC: First recognized in electron micrographs by Ehret and Powers (1959), the parasomal sac (*p.s.*) is an invagination of the cell membrane (see Fig. 1). Each cortical territory usually contains one parasomal sac. If there is

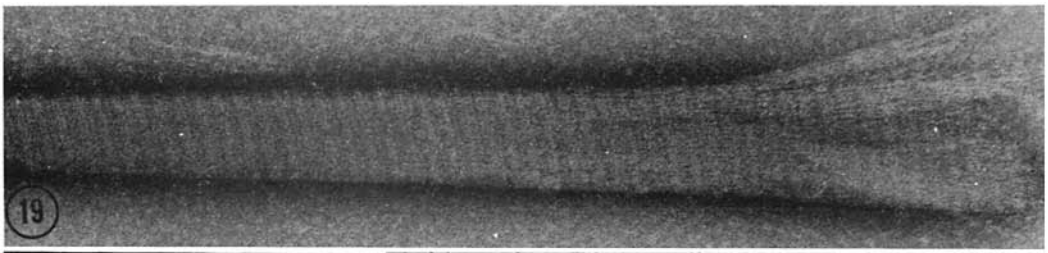
FIGURE 19 Kinetodesmal fiber. Note that cross striations are not perpendicular to fiber axis. Note fraying at one end of fiber. Raffinose; OsO_4; PTA. × 68,250.

FIGURE 20 High resolution micrograph of kinetodesmal fiber shown in Fig. 23. Lines indicate repeat distance. Ethanol; OsO_4; PTA. × 154,350.

FIGURE 21 High resolution micrograph of a kinetodesmal fiber showing longitudinal microfibrils. Ethanol; OsO_4; PTA. × 17,810.

FIGURE 22 Raffinose-isolated pellicle prepared by Anderson's critical point method. Note thickened rings around kinetosome and line of contiguity (*l.c.*) between alveoli. (*t.*, trichocyst attachment site). × 80,850.

FIGURE 23 Proximal end of kinetodesmal fiber, with associated kinetosome (*k.*) and some fibrous material. Note bend in fiber and change in its diameter. Ethanol; OsO_4; PTA. × 154,350.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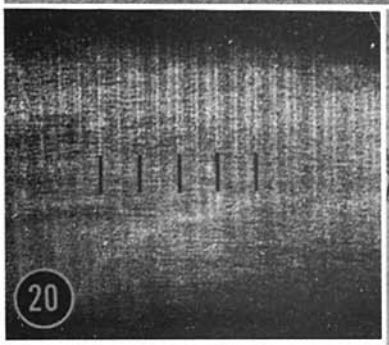
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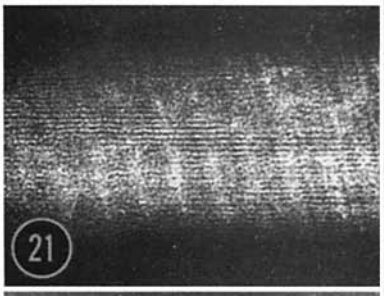
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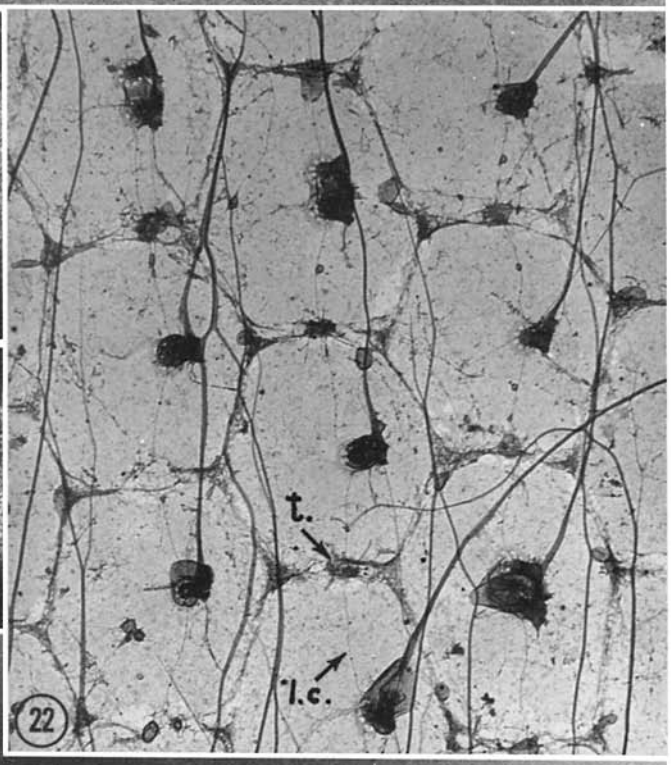
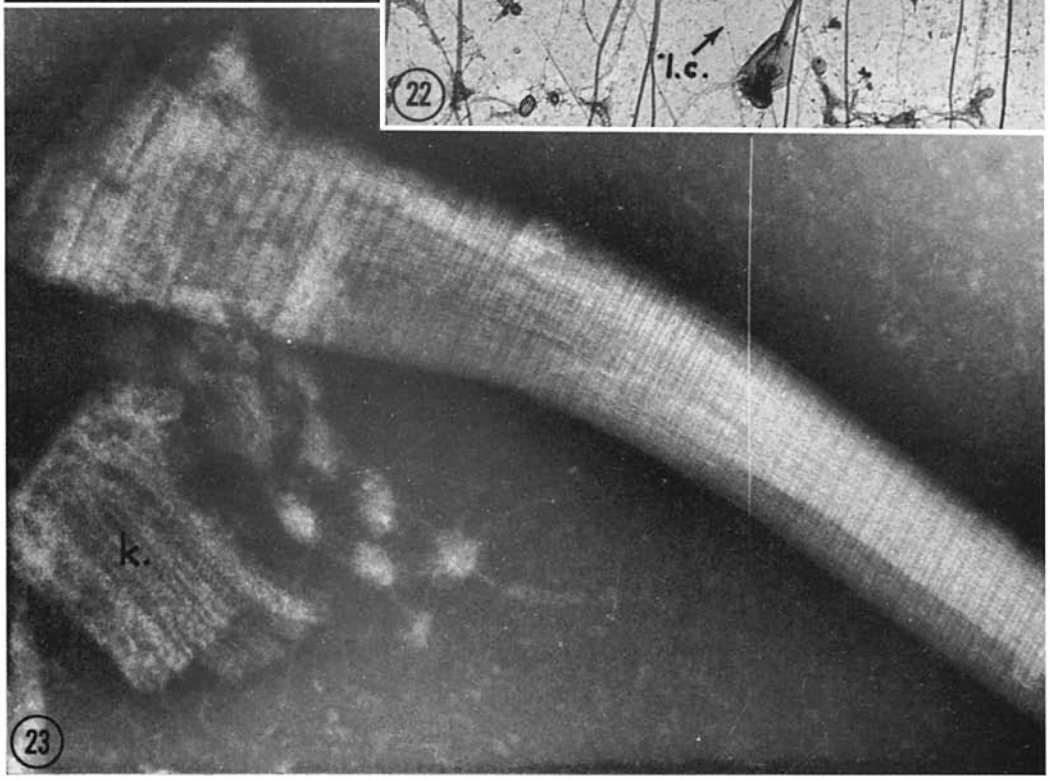

t.
l.c.
22


k.
23

one kinetosome, the sac is located to the right and slightly anterior to the kinetosome. When there are a pair of kinetosomes, the sac is associated with the posterior kinetosome (Figs. 12, 13).

In stock CD, but not in stock 7, as many as two super-numerary parasomal sacs have been observed in some territories (Figs. 15–18). A summary of the types of configurations which have been seen is given in Fig. 2. No consistent relationship can be drawn between these observed configurations and the possible mode of replication of cortical territories suggested by Dippell (1965) and shown in Fig. 3. Territories containing more than one sac do not appear to be significantly larger than territories with only one sac, as might be true if presence of extra sacs indicated stages in growth and duplication of territories. The frequency of territories having extra sacs ranged from 0.10 (3/31) to 0.26 (8/31), for three separate pellicular fragments which were photographed. However, the frequency for whole pellicles may actually be less since the fragments were photographed because they contained abnormal configurations.

Frequently, territories having similar sac configurations were found to occur in groups. Sometimes the repetition took place along a kinety (Fig. 17). At other times it occurred between adjacent kineties (Fig. 17). In one case, territories in the same kinety were seen to have configurations which are nearly mirror images (Fig. 18). In two other cases, an extra sac was observed to be slightly smaller and in close contact with the regular sac (Fig. 18, lower right). The possibility that these smaller sacs are preparation artifacts cannot be overlooked. The possibility that these unusual configurations have a bearing on territory replication will be discussed below.

MICROTUBULES: At least two groups of microtubules (identified as tubules in sections by Pitelka [1965]) are closely associated with the membranes of each cortical territory, and have been seen in both raffinose- and ethanol-isolated pellicles. The "posterior microtubular ribbon" (*p.m.r.*, Figs. 8, 25) begins near the posterior right margin of the basal body or posterior kinetosome of a pair and extends posteriorly at least part of the distance to the margin of the territory. The "transverse microtubular ribbon" (*t.m.r.*, Figs. 24–26) inserts near the left edge of the kinetosome (or posterior kinetosome of a pair) and extends to the left edge of the territory, curving slightly posteriorly. The tubules vary in number from one to six.

A third group of microtubules may occur in some territories. It was observed in ethanol-isolated pellicles only (Fig. 24) and in only a few territories, apparently in territories containing two basal bodies. The group consists of one or a few tubules, originates at the kinetosomes, and extends to the anterior left corner of the territory. Because of its location this group of tubules will be referred to as the anterior microtubular ribbon (*a.m.r.*). The negatively stained *a.m.r.* closely resembles the *t.m.r.* when the *t.m.r.* is only composed of one or two tubules.

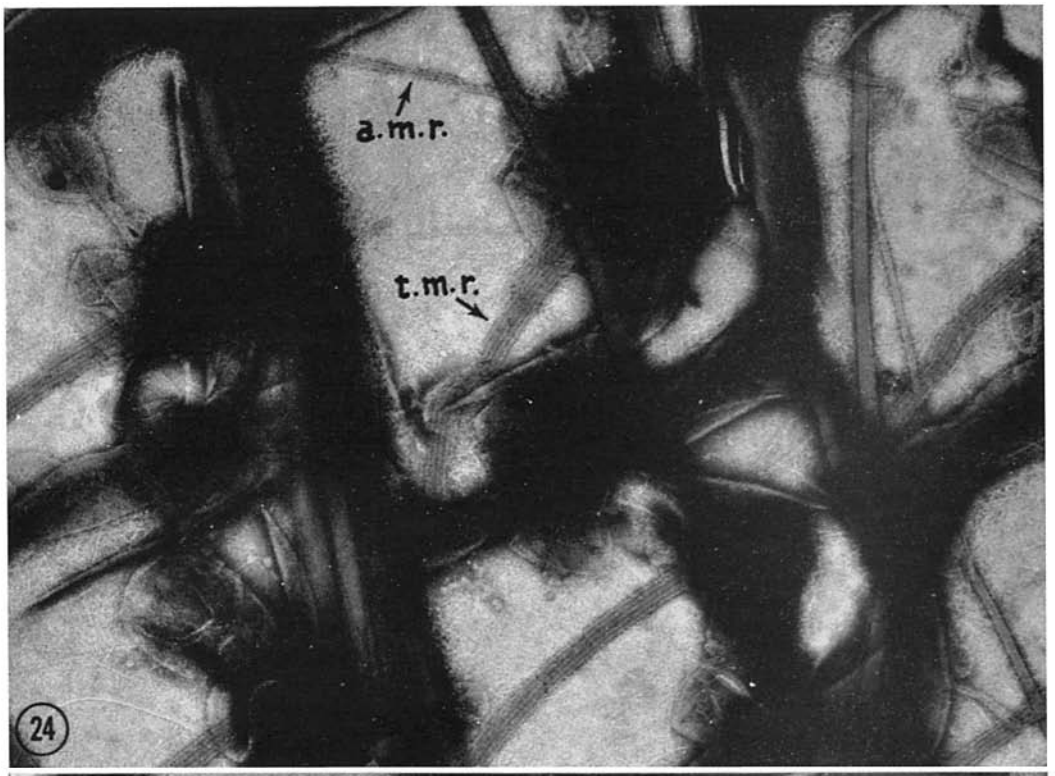
Each group of microtubules is a ribbon, one tubule thick. The tubules of ethanol-isolated pellicles measure 307–328 A wide and have walls 100 A thick and lumens about 110 A wide. Tubules

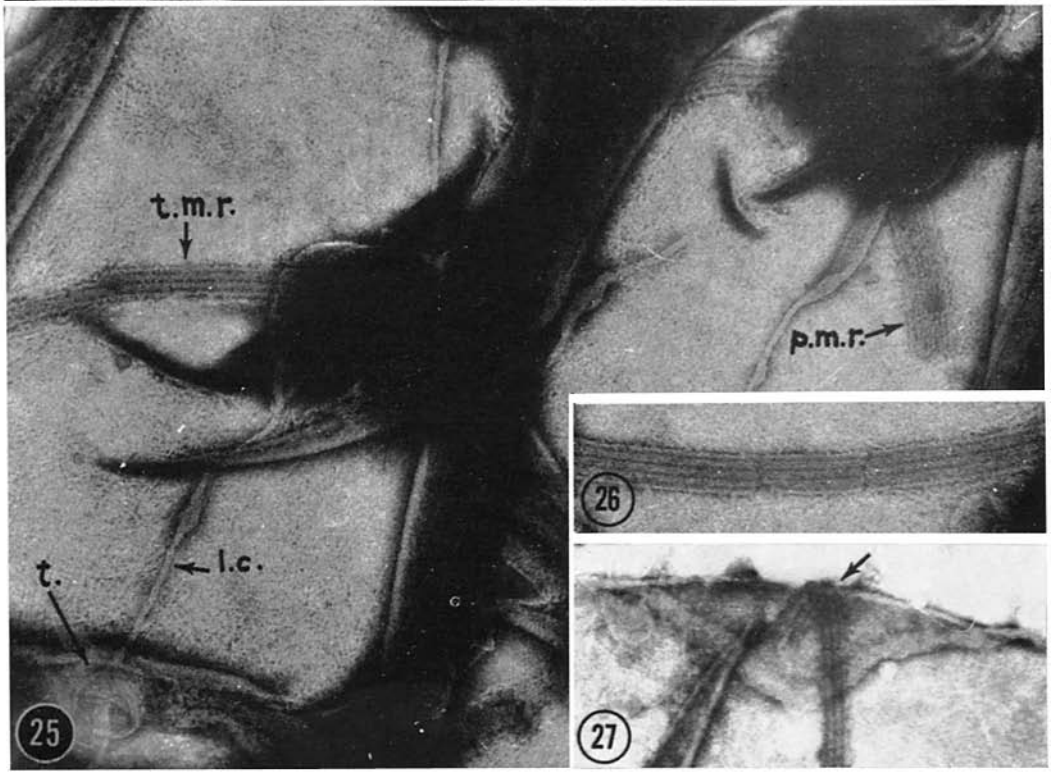
FIGURE 24 Pellicle isolated in ethanol medium. Note in particular the microtubular ribbons which extend from the vicinity of basal bodies (dark masses). Two territories contain anterior microtubular ribbons (*a.m.r.*, anterior microtubular ribbon; *t.m.r.*, transverse microtubular ribbon). OsO_4; PTA. × 39,000.

FIGURE 25 A single territory of an ethanol-isolated pellicle. Note transverse microtubular ribbon (*t.m.r.*), posterior microtubular ribbon (*p.m.r.*), and line of alveolar contiguity (*l.c.*). Note also fibrous mat underlying the membranes. (*t.* trichocyst attachment site). OsO_4; PTA. × 42,250.

FIGURE 26 Transverse microtubular ribbon. Wide dark lines are lumens of tubules. Narrow dark lines are spaces between tubules. Ethanol; OsO_4; PTA. × 68,250.

FIGURE 27 This picture illustrates the suggestion that microtubular ribbons closely adhere to the cortical membranes: where membranes fold (arrow), ribbons also fold. Ethanol; OsO_4; PTA. × 39,900.


a.m.r.
t.m.r.
24


t.m.r.
p.m.r.
t.
l.c.
25

26

27

are closely spaced, fitting together by means of a tongue-and-groove arrangement (Fig. 26). Fig. 27 shows that the tubular ribbons parallel folds in the membranes, which suggests that the ribbons are very closely applied to the membranes.

INFRACILIARY LATTICE (*i.l.*): The *i.l.* is a subcortical network of fibrils which was first observed in electron micrographs by Sedar and Porter (1955) and was later seen also by Roth (1958) and Pitelka (1965). In unfixed, negatively stained raffinose- or ethanol-isolated pellicles, material tentatively identified as part of the infraciliary lattice appears to consist of two elements, one globular, the other fibrillar (Figs. 17, 18, 28, 29).

In OsO_4-fixed, ethanol-isolated pellicles only, the fibrillar element seems continuous with a disorganized fibrillar layer which underlies and perhaps attaches to the epiplasm (Figs. 24, 25, 28). It is interesting to note that Ehret and Powers (1959) earlier suggested that a close relationship exists between the fibrils of the *i.l.* and the bottom of the pellicular membranes. The individual fibers are composed of three or four 85 A wide fibrils, fused at their centers to form bow-tie-like structures (Fig. 30). The bow-ties are consistently from 425 to 465 mμ in length. The granular component is irregular in size and shape.

Differences Among Cortical Territories in P. Aurelia

In stock CD, several aspects of territorial fine structure have been observed to vary from territory to territory. For example, the number of kinetosomes per territory may be one or two. As previously observed in *P. nephridiatum* (not an acceptable species, according to Wichterman, 1953) and *P. caudatum* by von Gelei (1925, 1934) and in *P. calkinsi* by Metz et al. (1953), territories located near the mouth almost always contain two kinetosomes. In *P. aurelia*, two kinetosomes are also found, usually, in territories near the anterior end of the animal, whereas on the dorsal surface and toward the posterior end there is usually one kinetosome per territory. Between the various areas, types of territories may be mixed.

Parasomal sacs and microtubular ribbons also vary in number in different territories. Sac variation has already been described above at length. Microtubule ribbons of different territories may contain different numbers of tubules. In addition, territories differ with respect to the number of ribbons they contain. Length of the kinetodesmal fibers is also variable.

Organelles of the Buccal Cavity

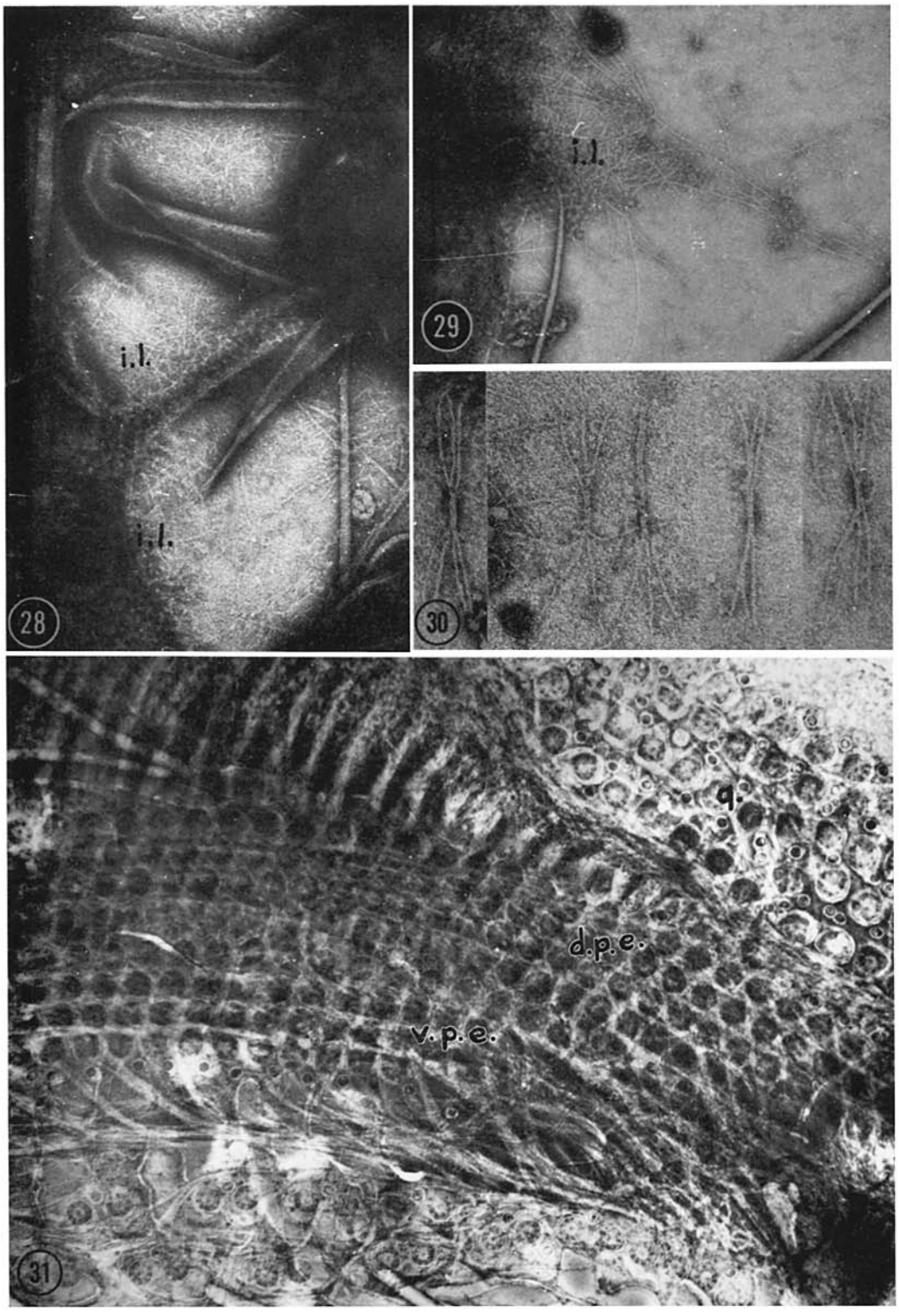
Observations on the isolated buccal cavity (gullet) are meager. Figs. 31 and 32 are of different regions of a single negatively stained, raffinose-isolated gullet. Observations on this gullet are consistent with the interpretation that it is from an animal which was in some stage of division preceding formation of the oral anlage. The peniculus (p.) consists of two groups of kinetosomes, each divided into four parallel kinetosomal rows. A row of parasomal sacs lies between the dorsal and ventral peniculi and a single row borders each peniculus. Between the peniculi and the left vestibular kineties (paired kinetosomes at bottom of Fig. 31), fibers emanating from the penicular region form a posteriorly-directed bundle. The bundle is joined by other fibers originating among the left vestibular kineties. These fibers appear unrelated to the kinetodesmal fibers found elsewhere in the cortex, but probably correspond to fibers described by Ehret and Powers (1959; p. 125) as "kinetodesmata." The quadrulus (*q*; Fig.

FIGURE 28 Ethanol-isolated pellicle showing portions of the infraciliary lattice (fibrous and granular material at margins of territories, *i.l.*) OsO_4; PTA. × 35,150.

FIGURE 29 Infraciliary lattice material (*i.l.*), pulled away from pellicle. Ethanol; OsO_4; PTA. × 23,750.

FIGURE 30 Composite picture of individual fibers of infraciliary lattice. Ethanol; OsO_4; PTA. × 80,850.

FIGURE 31 Organelles of left wall of buccal cavity. Note fiber bundles emanating from penniculus and from left vestibular kineties. (*q.*, quadrulus; *d.p.e.*, dorsal peniculus; *v.p.e.*, ventral penniculus). Raffinose; OsO_4; PTA. × 9,000.


i.l.
i.l.
28
i.l.
29
30
q.
d.p.e.
v.p.e.
31

31) could not be clearly discerned, except that there appear to be many parasomal sacs interspaced among the kinetosomes. The ribbed wall (*r.*) consists of 0.5 μ wide ribs which exhibit a vague cross-striated appearance and contain fibrous knots of material (Fig. 32).

An anarchic field (*a.*), which may give rise to a new mouth for the posterior daughter during division (Porter, 1960), is also present (Figs. 32–36). Anarchic fields were usually observed in electron micrographs of mouths, although the isolations were made from slowly dividing populations of cells. The probability of encountering dividing animals with such frequency suggests that anarchic fields might occur throughout the interphase period. To further examine this possibility, the isolated buccal cavities of animals from slowly multiplying cultures were examined by phase-contrast microscopy. In every case an anarchic field could be seen (Figs. 34, 35). Such fields may form during or soon after division and remain present throughout the interphase period. The size of the anarchic field is somewhat variable, perhaps indicating progressive changes preceding cell division.

The anarchic field in Fig. 32 contains pairs or small groups of transverse plates, each group associated with a parasomal sac opening. Similar groupings may be seen by phase-contrast microscopy (Fig. 34). Kinetosomes, as well as transverse plates, are present in the anarchic field (Figs. 33, 35). The kinetosomes and transverse plates are not arranged into orderly rows, although they may become so arranged at a later time, if the anarchic field generates basal bodies for the new buccal cavity (Porter, 1960). Therefore, the transverse plates may not have a role in the organization of kinetosomes into orderly rows by acting as templates for insertion of preformed kinetosomes.

Several incomplete transverse plates (arrows, Fig. 32) are also visible along the edge of the ribbed wall, in the position of the "endoral membrane" (*e.*) seen in light micrographs (Fig. 34). In light of this observation, it is of interest that Roque (1957) considers the endoral membrane of the related holotrich, *Disematostoma*, to be a stomatogenic kinety.

Solubility Studies

Raffinose-isolated pellicles of stock 7 and stock CD were treated with solubilizing agents in hopes of finding differential solubilizing effects which could be used later in relating chemical composition of pellicles to structural components. The agents chosen included detergents, lipid solvents, enzymes, and hydrogen bond- and S—S bond-breaking substances. Effects of varying ionic strength and pH were also examined.

In order to determine which pellicular structures were being affected, action of the reagents was monitored by phase-contrast microscopy. The reagents which produced promising results were further studied by electron microscopy. Some treatments, negative by phase-contrast criteria, might have given interesting results had electron microscopic observations been made (for example, 0.6 M to 0.8 M KCl may cause solution of microtubules or an effect on kinetosomes, since this agent is a useful solvent for spindle fibers (Sakai, 1966; Mazia et al., 1961), ciliary microtubules (Gibbons, 1965), and microtubules from the oral apparatus of an oligotrich (Olpin, 1967). These studies were of a preliminary nature. A summary of results is given in Table I.

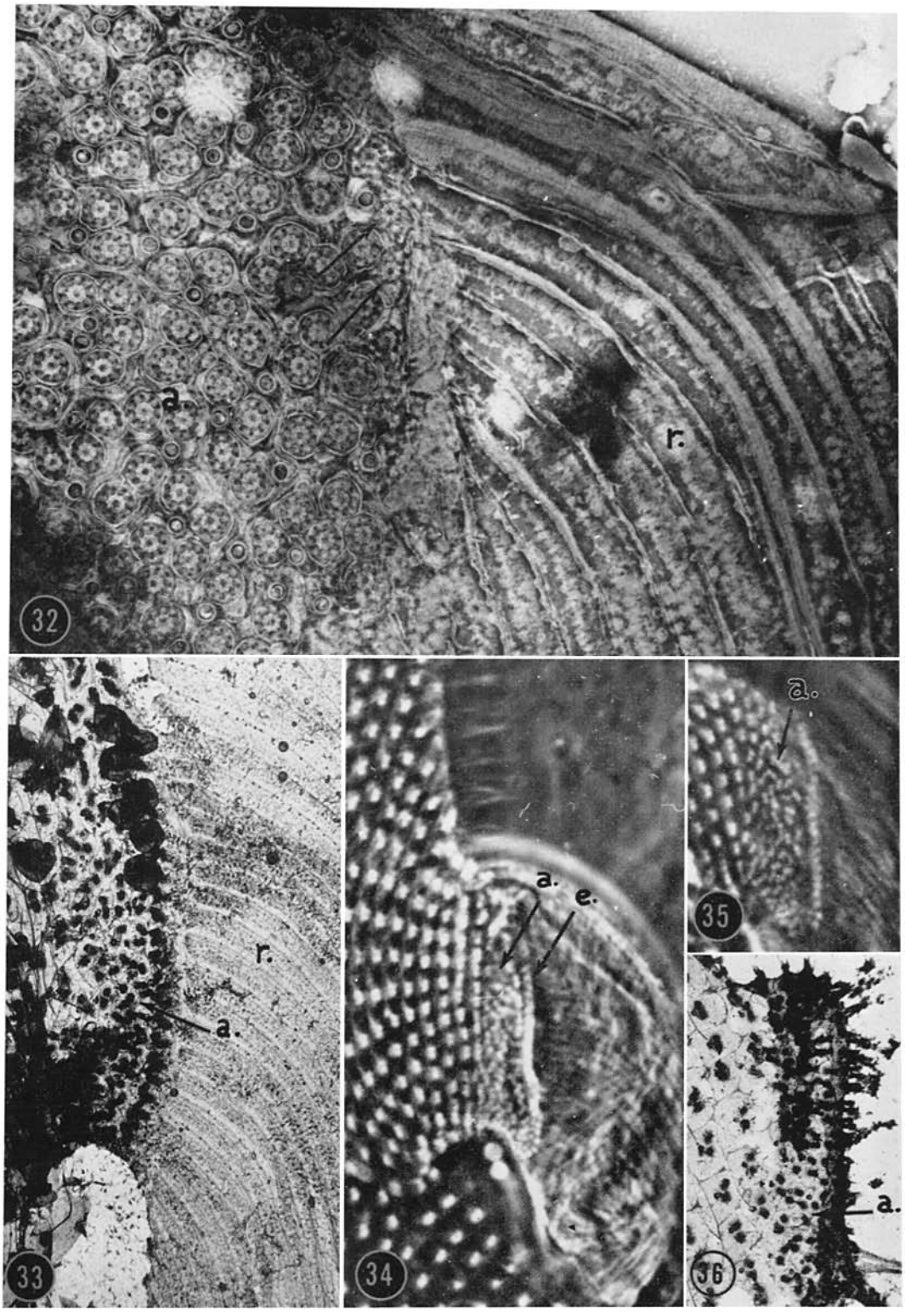
FIGURE 32 Organelles of right wall of buccal cavity. Note incomplete transverse plates lying near ribbed wall (arrows) (*a.*, anarchic field; *r.*, ribbed wall). Raffinose; OsO_4; PTA. × 14,625.

FIGURE 33 Anarchic field (*a*) and ribbed wall (*r.*). Raffinose; critical point method. × 10,000.

FIGURE 34 Phase-contrast micrograph of right wall of buccal cavity (*a.*, anarchic field; *e.*, endoral membrane; *r.*, ribbed wall). Ethanol. × 2690.

FIGURE 35 As in Fig. 34. × 2690.

FIGURE 36 Anarchic field (*a*) and portions of right vestibular kineties. Note the presence of basal bodies in the anarchic field. Raffinose; critical point method; × 8,050.


a.
r.
32
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33
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34
a.
35
a.
36

TABLE I

Action of Solubilizing Agents on Pellicular Structures

Agent	Method of observation	K*	Kf	Am	E	Mic	Il
1. Detergents							
½% sodium deoxycholate	PC‡ EM‡	S§	S	P§	P?	S	P
¼% sodium dodecyl sulfate	PC EM	S	S	S	S		P
1% digitonin	PC	I§	I	I	I		
2. Breaks H-bonds							
8M urea	PC EM	S	S	S	S	S	S
3. Breaks S-S bonds							
0.1M mercaptoethanol	PC EM	I	I	I	I	I	I
4. Chelating agent							
0.1M EDTA in 1.0 mM tris buffer, pH 8.3	PC EM	S	P	Swollen	I	?	I
5. Lipid solvents							
Ether	PC	?	?	P	P		
Benzene	PC	I	?	P	P		P
6. Enzymes							
Trypsin	PC EM	S	S	S	S	S	S
7. Ionic strength							
0.6M KCl	PC EM	I	I	I?	I	?	I
Distilled water	PC EM	I	I	I	I	?	I
8. pH							
HCl 0.0001M, pH 4.0	PC EM	G	G	G	I	?	S
0.001M, pH 3.0	PC EM	S	S	S	I	?	S
0.01M, pH 2.0	PC EM	S	S	S	I	?	S
0.01M Phosphate buffer, pH 6.8	PC EM	G	I	I	I	P	I
9. Miscellaneous							
Phosphotungstic acid, pH 7.0	PC EM	S	S	P	I	S	P
Phosphotungstic acid, pH 4.0	PC	I	I	I	I	?	I

* K, kinetosome; Kf, kinetodesmal fiber; Am, alveolar membranes; E, epiplasm; Mic, microtubules; Il, infraciliary lattice.

‡ PC, phase contrast microscopy; EM, electron microscopy.

§ S, soluble; I, insoluble; P, partly soluble; G, gradually soluble.

In general, three categories of effect were observed: (*a*) solvation of certain structures only; (*b*) solvation of a small portion of all structures; (*c*) complete destruction of all structures, leaving a small insoluble residue of amorphous nature. The second case occurred only in the presence of EDTA, and its significance is unclear. The third case occurred with trypsin, 8 M urea and ¼% sodium dodecyl sulfate; these agents later proved to be useful in chemical studies.

The most interesting results were those obtained with PTA at pH 7.0, high and low pH, and ½% sodium deoxycholate. These agents dissolve kinetosomes, kinetodesmal fibers, microtubules and alveolar membranes, leaving the epiplasm relatively untouched. The effect of PTA has been described above; the effects of PTA at pH 7.0 (Figs. 17, 18) and of low pH appear identical in electron micrographs, while deoxycholate has a somewhat different result in that it also tends to attack the epiplasm to some extent. As the pH was gradually lowered with HCl, the substance holding pellicles together (infraciliary lattice) was affected first (at pH 6.4 and below). Somewhere between pH 4.0 and 3.4, kinetosomes and kinetodesmal fibers were dissolved. As the pH was raised with NaOH, dissolution of kinetosomes, kinetodesmal fibers, and infraciliary lattice appeared to occur simultaneously around pH 8.0. At very high (11.0) and very low (1.5) pHs, some amorphous material could still be sedimented by centrifugation at 10,000 rpm for 10 min.

Because the method of isolating pellicles in 12% ethanol was only recently developed, ethanol-

isolated pellicles have not been used for solubility studies. However, during the course of routine electron microscopic studies, it was observed that 4% PTA at pH 7.0 has exactly the same solubilizing effects on ethanol-isolated as on raffinose-isolated pellicles.

DISCUSSION

Isolation Methods

Two methods were described for the isolation of pellicles of *Paramecium*: one method utilizes a homogenization medium consisting of buffered raffinose and would appear to be quite gentle in that no protein-denaturing agents are present; the other method utilizes a medium consisting of 12% ethanol and therefore introduces the possibility of artifact through protein denaturation or lipid removal. Pellicles isolated by both methods have been shown by electron microscopy to contain kinetosomes. However, kinetosomes isolated in the presence of raffinose show greater signs of breakdown than those isolated in ethanol. On the basis of these observations it is possible that both media may be useful, depending on the particular studies one would like to make. The raffinose medium was chosen for studies on pellicle-associated DNA (Hufnagel, in preparation) because it seemed suitable at the time and because the ethanol method had not yet been developed. The electron microscopic studies reported here lead one to conclude that pellicles isolated in the presence of ethanol might be more suitable for extraction of DNA. On the other hand, protein studies might be more successfully carried out on raffinose-isolated pellicles.

It seems likely that raffinose acts partly by preventing the breakdown of lysosomes, through control of osmolarity. However, when sucrose was substituted for raffinose, isolation of pellicle was unsuccessful. This suggests that raffinose also may have a more specific action: inhibiting "stickiness" on the part of other cellular organelles. Raffinose may also act by increasing the stability of the pellicles. However, two aspects of the studies suggest that pellicular structure is not highly stable in the presence of raffinose medium. First, variation in the quality of pellicle preservation occurs within a single pellicle preparation and from preparation to preparation. Secondly, in high resolution photographs, raffinose-isolated pellicles always appear slightly fuzzy and are usually surrounded by particulate debris, suggesting flaking off of protein subunits from pellicular structures (Figs. 9, 19).

Ethanol medium, on the other hand, seems to have a stronger stabilizing effect, judged by the sturdiness of pellicles during and after washing and by their crisp appearance in electron micrographs (Figs. 20, 21, 24, 25). It is of interest that ethanol was also present in the medium utilized by Gibbons and his coworkers (Gibbons, 1965; Renaud et al., 1968) for the characterization of proteins of isolated ciliary microtubules of *Tetrahymena*.

Electron Microscopy

Electron microscopic observations show that kinetosomes and most other cortical organelles, except cilia and trichocysts, are present in pellicles isolated by the use of both raffinose and ethanol. It is not clear whether the cell membrane is preserved.

In general, the observations on isolated pellicles reported here are in agreement with those of other workers (Pitelka, 1965; Ehret and Powers, 1957, 1959). In addition, new observations have been made. These include the following:

1. Kinetosomal microtubules consist of 45 A wide subfibrils. This size agrees closely with the value of 40–50 A found for the diameter of the subfibrils of sectioned *Chlamydomonas reinhardi* basal bodies by Ringo (1967). It also lies within the range (30–70 A) reported by a number of other workers for the diameters of subfibrils of cytoplasmic tubules from a variety of organisms and organelles (André and Thiéry, 1963; Pease, 1963; Gall, 1966; Ledbetter and Porter, 1964; Burton, 1966; Barnicot, 1966; Behnke and Zelander, 1966).

2. In stock CD, cortical territories may sometimes contain a group of microtubules ("anterior microtubular ribbon") which begin near the kinetosome or kinetosomal pair and extend anteriorly and to the left, probably to the edge of the territory. The number of tubules in this ribbon may vary from one to a few. In *P. multimicronucleatum*, according to Pitelka (1968), territories which contain two basal bodies also contain two transversely oriented tubular ribbons, one associated with each basal body. It is likely that the *a.m.r.* and *t.m.r.* described here correspond to Pitelka's anterior "left tangential ribbon" and posterior "left tangential ribbon". Jurand and Selman (personal communication) report observations on sections of *P. aurelia* which support this interpretation.

Pitelka (1969) has suggested that these microtubular ribbons may serve to regulate cortical territory dimensions during territory formation. The present observation that some microtubular ribbons extend to the margin of the territory is consistent with this hypothesis.

3. In stock CD, one or two extra parasomal sacs may occur in some cortical territories. The significance of this observation will be discussed later.

4. Membranes of the cortical alveoli are molded around the parasomal sac opening in such a way that, if there are two kinetosomes, the sac is always linked to the posterior one by alveolar membranes.

This association between sac and kinetosome points to a morphogenetic relationship between the two organelles. If the sac had formed by invagination of the cell membrane at the sac's usual position with respect to the kinetosomes, a round hole should have been punched through the right alveolus (see Fig. 4a). This situation has never been observed. Nor has the sac ever been observed to connect to the anterior kinetosome, except when extra sacs are present in a territory. Two explanations remain which can account for the connection between sac and posterior kinetosome: (*a*) the sac forms first in its normally observed position (Fig. 4b), and the alveolus, in growing around it, is influenced by some consistent asymmetry in the forces determining direction of growth of different regions of the alveolus; (*b*) the sac develops near the posterior kinetosome (Fig. 4c) by invagination of the cell membrane, and later migrates to its usual position, forcing a channel through the alveolus.

Either of these explanations is consistent with Dippell's (1965) description of territory reproduction (Fig. 3) based on her light and electron microscopic observations. According to Dippell, the territory increases in size while new structures begin to form within it. A new kinetosome appears just anterior to each old one, and a new parasomal sac forms adjacent to the old anterior kinetosome; then the unit divides equatorially to form two. In general, new organelles appear in the same relative positions that they will later occupy.

The arrangements of supernumerary sacs in stock CD, however, do not seem to reflect normal stages in territory reproduction, if Dippell's description of this process is correct. Supernumerary sacs may arise through deviations in normal territory replication.

5. The epiplasm forms a thickened ring around the parasomal sac opening. The ring had been described previously by Metz et al. (1953), but its epiplasmic nature was not recognized by them; nor did they observe its fine structure. The ring is serrated along its inner edge and has four or five notches along one portion of its outer edge.

In a consideration of the formation of a parasomal sac, the configuration of alveolar membranes which develops around the sac, the process of cell membrane invagination, and the formation of a hole encircled by a dense ring of epiplasm must be taken into account. Coordination of these events may involve an influence of one membrane component upon another or mutual interactions. It will be of great interest to elucidate the nature of these processes.

6. The epiplasmic transverse plate is separable from the rest of the epiplasm and consists of an outer rim, a central granule, and nine spokes with nine spaces between them to allow passage of the outer ciliary fibers into the kinetosome. It is possible that this plate may form a barrier to passage of materials from the lumen of the kinetosome to the lumen of the cilium. Whether or not it is present during growth of the cilium would be worth ascertaining. It would also be of interest to examine whether (*a*) plate formation is induced by prior presence of the kinetosome, (*b*) the reverse induction occurs, or (*c*) both structures develop independently and later become associated. Further electron microscopic examination of the anarchic field may shed light on this question.

7. Periodicity of the kinetodesmal fibers of *P aurelia* is 350–400 A for raffinose-isolated pellicles and 290–340 A for ethanol-isolated pellicles (for *P. multimicronucleatum*, Pitelka [1965] has previously reported a period of 300–350 A and Sedar and Porter earlier reported variations between 300 and 500 A).

8. Kinetodesmal fibers consist of bundles of longitudinal microfibrils having a center to center spacing of 47–51 A and a beaded substructure. The similarity between these measurements and those of the microfibrils composing the kinetosomal microtubules (*#1*, above) raises the possibility that both kinetodesmal and kinetosomal microfibrils may be composed of identical subunits associated in different ways. An alternative hypothesis is that the subunits are similar but not identical. There is no evidence to support either contention, but the latter seems more plausible.

One can imagine a series of mutations of the structure of kinetosomal fibrillar subunits which would not affect their longitudinal association into fibrils but which would greatly alter their lateral association with each other or with other kinds of subunits. Of course, a third alternative is that kinetosomal and kinetodesmal subunits are entirely dissimilar. Isolation and characterization of the proteins of kinetosomes and their derivatives should help to distinguish between these alternatives.

9. Portions of the infraciliary lattice seem to remain attached to isolated pellicles and consist of two elements, one fibrous and the other globular. The fibers are each 425–465 mμ long and consist of three or four 85 A wide fibrils which are fused at their centers, giving the appearance of cat's whiskers. Pitelka (1965) proposes that the infraciliary lattice performs a contractile function. The role that the globular element performs in this process is unclear. However, involvement of fibrillar structures in the contractile process is well-established in the case of muscle (Huxley and Hanson, 1954; Huxley and Niedergerke, 1954). It may be feasible to isolate the fibrous component of the infraciliary lattice and compare its properties with those of muscle proteins.

10. A dense fibrous bundle occurs between the peniculus and the left vestibular kineties, and is contributed to by smaller fibers emanating from the peniculus and the vestibular kineties. Pitelka (1963) suggests a relationship between gullet fibers and the fibers comprising the infraciliary lattice. Other than their filamentous nature, however, little similarity exists between the organization of the two structures. Williams and Zeuthen (1966) have suggested that in *Tetrahymena* the proteins of the fibers associated with oral structures may have a critical role in the control of cell division.

11. Anarchic fields are present in all interphase animals.

12. Epiplasmic transverse plates are found in the anarchic field; some plates are incompletely formed.

13. A number of structural differences between cortical territories were described for the first time. These variations involve number of kinetosomes and parasomal sacs per territory, presence or absence of the "anterior microtubular ribbon," number of microtubules per ribbon, and kinetodesmal fiber length.

In earlier reports on the cortical structure of *Paramecium* (e.g. Ehret and Powers, 1959; Ehret et al., 1964) the similarities between territories were stressed. These similarities include presence of kinetosomes, kinetodesmal fiber, parasomal sac, alveolar membranes, epiplasm, and cell membrane, all in essentially the same relationship with one another in every territory. This concept of the replicate nature of territories is consistent with the observation of Beisson and Sonneborn (1965) that the territory has a great deal of autonomy; when its polarity is reversed, with respect to the polarity of the whole cell, it continues to propagate with reversed polarity. That is, its polarity is not influenced by its environment.

However, other observations of Sonneborn and his coworkers concerned with formation of the cytopyge suggest that the structure of a territory is to some extent under the influence of its environment. This view is further supported by Tartar's (1961) studies on *Stentor* and by Nanney's (1966a, 1966b, 1966c, 1967) observations on *Tetrahymena*, showing that kinetosomal function may be determined by environmental conditions and be dependent upon the particular environment in which the kinetosome finds itself. The present observation of structural heterogeneity among territories also favors environmental induction of heterogeneity for three reasons: (*a*) some of the observed variations are directly related to area of cortex, e.g. kinetosomal fiber length and number of kinetosomes per territory; (*b*) the territories of a single kinety may be heterogeneous in structure; and (*c*) some variations in the structure of territories are found to extend to neighboring kineties, although territories are thought to multiply, usually, in a longitudinal direction. Lateral replication of territories ("délineation") does occur in ciliates (Lwoff, 1950), but it usually gives rise to new kineties intercalated between old ones, rather than to add to kineties already present. The nature of the sensitive site in the territory and of the active agents influencing territory form are a mystery, but the ultimate events must occur at the level of aggregating protein subunits.

In the future, it would be of value to investigate further the distribution of variations over the ciliate surface. Similar studies on dividing cells are also desirable, since some of the variations described above could occur at specific stages of territory replication. Finally, the occurrence of territorial heterogeneity suggests the need for comparative electron microscopic studies on nega-

tively stained, ethanol-isolated pellicles of various stocks, syngens and species of *Paramecium*. If enough information were acquired on structural differences between strains, genetic studies might be feasible.

Solubility Studies

Solubility studies indicate that a number of agents, notably 4% PTA at pH 7.0, may be of use in isolating the chemical components (particularly the proteins) of specific pellicular structures.

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