FINE STRUCTURE OF MEMBRANOUS
AND MICROFIBRILLAR SYSTEMS IN THE
CORTEX OF PARAMECIUM CAUDATUM

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ABSTRACT

An electron microscope study of the cortex of Paramecium caudatum has revealed new details pertinent to several unresolved problems. The lateral boundaries of the alveoli do not regularly follow the crests of the polygonal ridges and thus their staining with silver cannot account for the external lattice seen by light microscopists. A granulo-fibrillar material is present, however, within the peaks of the ridges, which would account for the external lattice if so stained. Perforations are present between adjacent alveoli which make the whole mosaic of alveolar sacs within the cell's cortex continuous—both the membranes and the lumen. A microfibrillar system exhibiting a cross-striated pattern lies in the superficial cortex. These bands are inserted at their ends in the epiplasm and have a fine structure and arrangement suggesting a muscular function. The infraciliary lattice is a branching system of fibers with electron-opaque posts at the center of each branching locus. This system is distinct from the striated bands in morphology and in space. The epiplasm is discontinuous along the crests of the ridges, which may account for the pellicles' disposition to tear along these lines. A three-dimensional drawing is presented to show the interrelationships between the above membranous and microfibrillar systems.

INTRODUCTION

Attempts to describe the complex structure located within the cortex of Paramecium have resulted both in clarification and in confusion, at first for the light microscopist and later for the electron microscopist. This has been due to the complicated interrelationships existing between the several membranous, fibrous, and microtubular structures found in this outer ectoplasmic zone, and to the apparent inconsistencies between the observations arising from the use of different techniques.

In his paper correlating evidence obtained by light microscopists who used silver staining techniques with evidence obtained by electron microscopists, Parducz (1962) recognized four fibrous structures present in the cortex of Paramecium. These are (a) the external lattice or indirect connecting silverlines which form a network of polygons at the surface of the cell, (b) the interciliary fibrils or direct connecting silverlines, lines which connect the basal bodies along each kinety, (c) the kinetodesmos or group of overlapping kinetodesmal fibers lying to the right side of the basal bodies of a kinety, and (d) the infraciliary lattice, a fibrous network lying in the plane of and surrounding the proximal ends of the basal bodies. The first two fibrous structures are now generally thought to be, in reality, junction lines between contiguous membrane-limited alveoli, or flattened vesicles, lying just under the plasma membrane (Dippell, 1962; Parducz, 1962; Pitelka,
A similar explanation had previously been suggested by Ehret and Powers (1959) for the external lattice, although Sedar and Porter (1955) had earlier advanced the notion that the "dense" material in the ridges of the ectoplasm presumably stained with silver and that this was what light microscopists had named the external lattice. Parducz (1962) has enumerated several observations which strongly suggest that the direct and indirect connecting silverlines are different in their material basis and are, in fact, spatially separated. The cortical structure which stains with silver to form the external lattice thus remains in doubt (see Pitelka, 1965). Why does the infraciliary lattice as seen by Pitelka (1965) will be shown to consist of two separate microfibrillar systems. Fibrous connections will be shown between the anterior of the pair of basal bodies of a territory and both the posterior basal body and its attached kinetodesmal fiber. The epiplasm will be shown to be discontinuous at the crests of the ridges, thus presumably causing the pellicle to be weaker along the polygonal margins. Also, both the membranes and the internal space of adjacent alveoli will be shown to be continuous via membrane-bounded pores. Consequently, all of the units of the mosaic of alveolar vesicles which underlie the surface membrane of Paramecium caudatum will be shown to be continuous.

**MATERIALS AND METHODS**

This investigation was carried out on Paramecium caudatum obtained from Dr. Roger Eckert, University of California, Los Angeles.
Preparation for Electron Microscopy

*Paramecium* was cultured in a hay infusion at room temperature. Organisms from 1-wk old cultures were concentrated by centrifugation and the growth medium was decanted. Most of the organisms were then bathed in an aqueous solution containing 1 mM CaCl₂ and 1 mM KCl for 1 hr prior to fixation (see Figs. 1, 3–15, 17–20, and 22) while others were transferred directly from the culture to the fixative solution (Fig. 2). (This prefixation bath was originally used before the preparation of *Paramecium* for electron microscope study, in order to maintain handling procedures identical with those used by Eckert and Naitoh (1970) in their electrophysiological study. At that time we were attempting to correlate structure with ciliary functions in *Paramecium* (Allen and Eckert, 1969). The present study is an elaboration and an extension of some observations made during the above study.) A 1.5–2% solution of glutaraldehyde (Sabatini et al., 1963) buffered with a 0.05 M cacodylate solution at pH 7.4 (Figs. 2 and 6–8) or with a 0.034 M collidine solution at pH 7.3 (Figs. 1, 3–5, and 9–22), was used at room temperature to fix the concentrated cells. This fixation was carried out for 25 min. This was followed by a wash in the appropriate buffer and a second fixation of 45 min in 1% OsO₄ buffered in the same way as the glutaraldehyde. The collidine-buffered preparations were then placed for 30 min in a 0.5% aqueous uranyl acetate solution to stain the cells prior to emedment. Dehydration was done in a graded series of ethanol followed by 100% propylene oxide. The cells were “flat”-embedded in a mixture of Epon-Araldite (Möllenhauer, 1964) or Epon (Luft, 1961). After a period of time in an evacuated desiccator, the above embedding mixtures were polymerized in an 80°C and a 60°C oven, respectively.

Individual cells were cut out of the block and sectioned on a Sorvall MT-2 ultramicrotome with diamond knives (Ivan Sorvall, Inc., Norwalk, Conn.). Sections were mounted on un supported 300-mesh copper grids or on slotted copper grids and stained 2–5 min with uranyl acetate (Watson, 1958) followed by 5 min with lead citrate (Reynolds, 1963). These were viewed in a Philips 300 electron microscope operated at 60 kv. (Philips Electronic Instruments, Mount Vernon, N.Y.)

Glycerination Procedure

A preliminary attempt was made to make “model” systems of the fibrillar structures of *Paramecium* to see if their integrity could be adequately preserved to allow future studies on their possible contractility. Other contractile and motile organelles, e.g. myofibrils and flagella, have previously been studied in this way (see Hoffman-Berling, 1960 and Szent-Györgyi, 1951). Producing models involves a gradual glycerina-}

ification of the cells which results in the cells’ death but leaves the motile systems structurally intact and functional, providing an energy source is supplied to the model. Although the initial attempts were only partially successful, the results did give some useful morphological information which contributed significantly as supporting observations in the present study.

A glycerol gradient was prepared by using the techniques of Kinoshita and Yazaki (1967). A stock solution of 50% glycerol, which also contained 0.08 M KCl, and 0.01 M Tris-HCl buffer adjusted to pH 7.4, was used to make the gradient. Concentrated *Paramecium* were layered onto the top of the gradient which was held at 4°C, and the cells were allowed to settle to the bottom of the column overnight. The glycerol solution was siphoned off and the cells were collected in 50% glycerol. These cells were then washed in a 25% glycerol solution for 30 min followed by a 5% glycerol wash for 30 min before being fixed and prepared for electron microscopy as described above.

RESULTS

Membranes of the Somatic Cortex

This study has confirmed the presence in *Paramecium* of most of the cortical patterns described by other authors (for a summary of these patterns, see Pitelka and Child, 1964 and Pitelka, 1965, 1969). The outer surface is sculptured into a system of rectangular or polygonal ridges approximately 0.1–0.3 µ thick. Cilia arise from the depressions enclosed by these ridges. Fig. 1 is a grazing section through the surface of *P. caudatum* which shows segments of four rows of polygons.

The plasmalemma or plasma membrane covers the outer surface of the depressions and ridges, and extends over the surface of the cilia (Figs. 1, 2, and 7). This membrane appears to have a thin external coating of fibrous material (Fig. 2). Interior to this membrane are the two membranes of the alveolar system. The outer of these two membranes (Figs. 1, 2, and 9) parallels the plasma membrane with a constant separation of 75–100 Å. Thus, the plasma membrane and outer alveolar membrane, which are each 80 Å thick, together with the intermembrane distance, constitute a layer approximately 200 Å thick covering the organism’s surface. The inner alveolar membrane (Figs. 2, 8, and 15) lies in close contact with the epiplasm. The outer and inner alveolar membranes break and fuse, the one with the other, along a line connecting the basal bodies and trichocysts of a kinety (see Figs. 1, 3, and 5). This line is in the same location as the in-
FIGURE 3  A tangential transverse section through the cortex. The more anterior end of the cell is toward the top of the picture. The continuous nature of the alveolus (a) passing from one septum (s) over the longitudinal ridge (lr) to the adjacent septum can be traced. This is particularly clear where an electron-transparent gap (arrows) is found between the crest of the ridge and the outer alveolar membrane. It is also clear from this picture that the membranes of the septa separate to allow the basal bodies to pass between the alveoli but do not directly touch the fibers of the basal bodies. Deeper in the cortex are the striated bands (sb), infraciliary lattice (il), kinetodesmal fibers (k), and posterior microtubules (pt). Tips of trichocysts (t) are also present. X 30,000.

FIGURE 4  A tangential longitudinal section through the cortex. In this case no septa may be seen running laterally across the alveolus. (The more anterior end of the animal is toward the right of the picture.) The alveolus (a) is continuous over the tips of the transverse ectoplasmic ridges (tr). The septa (s) present lie along meridians and can be seen at one point between the two basal bodies of the same depression. A kinetodesmal fiber (k) is also labeled. X 40,000.
terciary fibril or direct connecting silverline described by light microscopists (see Parducz, 1962).

The alveoli are the only morphological structures which are obviously altered by the initial treatment of the cells with the CaCl₂ and KCl bath. In the controls, which were not so treated, the inner and outer alveolar membranes usually lie close to each other with only a thin layer of fibrous material separating them (Fig. 2). In cells subjected to the initial bath, the space between these two membranes is greatly distended (Fig. 6).

In Fig. 1 a septum (alveolar margin) is present along the meridian in every cortical polygon. This septum is composed of the two membranes of adjacent alveoli bordering an interalveolar space of 50-60 A. Transverse views of several alveoli of a small portion of the surface of Paramecium can be seen in Fig. 3. The meridional septa, one per kinety, are evident in Fig. 3, but no other longitudinally directed septa between the meridional lines can be seen. Usually, the alveolar membranes appear to be uninterrupted from one meridional line laterally to the next. The inner alveolar membrane at the tips of the ectoplasmic ridges lies close to the outer alveolar membrane (Fig. 3), but these membranes do not join here as frequently as they do along the meridian. Electron-transparent gaps may often be seen between the crest of the ridge and the outer 26 mµ layer (Figs. 3 and 15). In alveoli sectioned longitudinally (Fig. 4) one sees lateral alveolar margins only infrequently. The crests of the transversely directed ridges come close to the outer 26 mµ layer but, as was true of the longitudinal ridges, the two opposing membranes do not usually fuse (Figs. 2, 4, and the sides of Fig. 1). The membrane surfaces which face the lumen of the distended alveolus are lined with an irregular coating of fibers. This is particularly evident along the inner alveolar membrane (Figs. 1, 5, and 13).

Electron-transparent profiles with round, elliptical, or kidney shapes are a constant feature of side views of alveolar margins (Figs. 6–8). These have been seen in both control cells which have expanded alveoli and cells subjected to the prefixation bath. These profiles vary in length from 30 to 60 mµ and in depth up to 40 mµ with an average depth of approximately 23 mµ. Their outer limits always lie along the inside surface of the 26 mµ.
layer. The depth of the alveolar margin of expanded alveoli varies along the length of the meridian. This depth in Figs. 6-8 varies from 60 to 175 µm. The electron-transparent regions never appear to be deeper than half the depth of the septum. In sections cut perpendicular to the plane in which the septum lies, discontinuities of from 20 to 40 µm long can be seen in the septum next to the outer alveolar membrane (Figs. 1, 3, 5, and 9). In this plane of sectioning, the two membranes of the two adjacent alveoli appear to bulge out slightly at these breaks, turn towards each other, and fuse along the edges of these discontinuities (Fig. 9). These thickened rims can also be detected when the "perforations" are viewed from the side (Figs. 7 and 8), since they are slightly more electron-opaque than the rest of the alveolar margin. The outer alveolar membrane is continuous through the discontinuities and thus across the meridian at these points (Fig. 9). Perforations are also present in the infrequent lateral septa (Fig. 3).

Transparent regions may sometimes be seen in meridional septa somewhat removed from the outer alveolar membrane (Fig. 10). This apparent distance from the outer membrane is probably a result of the orientation of the section and, if followed in serial sections, the transparent region would in all probability be found to lie next to the outer alveolar membrane.

The perforations described appear to be empty of any fibrous or membranous material. In side view and in many sections cut perpendicular to a septum they have approximately the same density as the embedding matrix. Those sections cut perpendicular to a septum which include perforations containing some opaque material probably result from the sections being cut partly through trans-

**Figure 11** A cross-section of a basal body at the level of the terminal plate. The basal body is being viewed from the distal end toward the proximal end; thus, the parasomal sac (ps) appears in this picture to the left of the meridian. Electron-opaque material, presumably an extension of the epiplasma, lies within the ring formed by the alveolar membranes and contacts the nine triplet fibers of the basal body. An electron-opaque sheet also crosses the lumen of the basal body. The meridional septum (s) can be seen both anterior (top of picture) and posterior to the basal body with a branch extending out to the parasomal sac. A fibrous material coats the cytoplasmic surface of the parasomal sac. A band of posterior microtubules (pt) is present. ×100,000.

**Figure 12** A cross-section of another basal body-cilium complex at a level immediately distal to the level shown in Fig. 11. This is at the plane where the triplet fibers of the basal body have become the doublet fibers of the cilium. The fibers to the left show some indication of being triplet in nature while those on the right are clearly double. Electron-opaque spurs arise from the midpoints of the exterior surfaces of these fibers and extend toward the alveolar membranes. Whether these spurs actually contact the membranes is not clear. s, meridional septum; pt, posterior microtubules; ps, parasomal sac. ×100,000.

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FIGURE 13 A section cut tangentially through the surface and oriented so that the more anterior end of the cell is toward the right side of the micrograph. The two microfibrillar systems, the striated bands (sb) and the infraciliary lattice (il), can be distinguished as being quite separate systems by their different morphology and their different position in the cortex. The striated bands are inserted at their ends in the epiplasm which underlies the bottoms of the surface depressions. The band identified by the bracket has three electron-opaque stripes interdigitating with two medium opaque stripes. Electron-opaque posts can be seen at the center of each branching locus of the infraciliary lattice. Granulo-fibrillar (g) material fills the peaks of the ridges. Parasomal sacs (ps) are indicated. The lower parasomal sac has a cytoplasmic coating of fibrous material. tt, transverse microtubules; k, kinetodesmal fibers. × 40,000.
parent regions and partly through adjacent membranes.

The two alveolar membranes of a meridional septum pass around the basal bodies and trichocyst tips. These organelles penetrate between these membranes to the plasma membrane (Figs. 3, 4, 11, and 14). When two basal bodies are found in the same depression the membranes of the septum involved have their normal 50-60 Å separation along a short distance of 50-80 mµ between the two bodies (Figs. 3 and 4). An electron-transparent perforation has also been seen in the septum between the two basal bodies (right side of Fig. 6).

To the right (animal's right) of each basal body or pair of basal bodies is a short invagination of the plasma membrane called a parasomal sac (Figs. 11, 12, 13, and 15). This sac is 60-75 mµ in diameter and has a 33 Å thick fibrous coating on its cytoplasmic surface similar to that found on coated pits (Roth and Porter, 1964). The right membrane of the septum extends out and surrounds this invagination (Figs. 3, 11-13), forming a short septum 50-100 mµ long between the basal body and the parasomal sac. Perforations are sometimes present across this branch also (top right of Fig. 13).

The space between the alveolar margins through which the cytoplasm can make contact with the plasma membrane, a contact which would have to extend through a continuous epiplasm, is effectively reduced by the membrane-limited perforations through the septa. In the left-hand ridge of Fig. 6, from the crest of the ridge down its right side to the edge of the basal body, this space is reduced by these perforations and their limiting membranes by approximately one-half.

The membranes of the alveolus do not come into contact with the nine triplet fibers of the basal body but are separated from them by a distance of some 30-50 mµ (Figs. 3, 4, and 11-14). However, at the level of the alveolar margin and the terminal plate of the basal body, the epiplasm which coats the cytoplasmic surface of the inner alveolar membrane extends into this 30-50 mµ space, passes around the fibers, and becomes continuous with the terminal plate (Figs. 6, 11, and 14). Additional electron-opaque filaments are located within a plane just distal to the level of the terminal plate which is approximately the same plane as that where the triplet fibers of the basal body change to the doublet fibers of the cilium (Fig. 12). These filaments extend toward the alveolar membrane from the outside margins of the nine fibers.

![Figure 14](image1.png)

**Figure 14** A striated band lying between two basal bodies. The band is composed of microfibrils oriented parallel to each other. Five stripes may be identified perpendicular to the long axis of the band. The most electron-transparent stripes lie closest to the epiplasm in which the band is attached. Electron-opaque stripes lie next to these transparent ones and, finally, a medium-opaque stripe lies across the middle of the band. The band is parted longitudinally by a parasomal sac (ps). The inset is an enlargement of the portion of the band indicated. Bridges may be seen between adjacent fibrils in this region. st, transverse microtubules; pt, posterior microtubules. × 80,000. Inset × 160,000.

![Figure 15](image2.png)

**Figure 15** A portion of a cell fixed in collidine buffer, cut in transverse section. Units of the striated bands (sb) and infraciliary lattice (il) appear in cross-section. The striated bands have a more open structure and their 60-A microfibrils are easily identified. The microfibrils of the infraciliary lattice, which are 30-40 Å in diameter, are partially obscured by the compactness of the arms. Extrafibrillar material may also be present between the microfibrils of this last system. Granulo-fibrillar (g) material is found within the tip of the ridge. k, kinetodesmal fibers; ps, parasomal sac; pt, posterior microtubules. × 67,000.

![Figure 16](image3.png)

**Figure 16** The striated band after glycerination of the cell. The body of the band, which is usually lost during the glycerination process, consists of relatively heavy fibrils whereas thinner, branching fibrils seem to connect these to the epiplasm. × 80,000.

![Figure 17](image4.png)

**Figure 17** The granulo-fibrillar meshwork in the tip of a surface ridge. Silver staining of this material could account for the external lattice pattern seen by light microscopists. × 80,000.
Microfibrillar Systems of the Somatic Cortex

Underlying the inner alveolar membrane is a sheet of amorphous, moderately electron-opaque epiplasm. This sheet continues into the ectoplasmic ridges but appears to be absent for a width of about 20 μm along the crests of the ridges (Figs. 2, 3, and 13). The only other perforations in the epiplasm are at the sites of the parasomal sacs and at the basal bodies. In this latter location the fibers of the basal body penetrate through the epiplasm. Besides the fibers, nine small electron-transparent regions forming a broken cylinder may be seen in the terminal plate located interior to the cylinder of basal body fibers (Figs. 11 and 14). The epiplasm may also be thin or absent in nine regions which are spaced equidistantly, and, as a group, form a broken circle around the outside of the basal body fibers. One of these electron-transparent zones lies adjacent to each opening between two peripheral fibers (Figs. 11 and 14). This is also suggested in published micrographs of negatively stained pellicular fragments of *Paramecium aurelia* (see Fig. 22 of Hufnagel, 1969).

Within the cortex are three distinct and separate systems of microfibrils. The most peripheral of these is a granulo-fibrillar meshwork lying only within the crests of the polygonal ridges (g in Figs. 1, 13, and 15). Its fine structure appears to be composed of electron-opaque granules about 10 μm in diameter from which short, 30–40 μm long, microfibrils radiate toward other surrounding nodes (Fig. 17). This meshwork fills the upper, narrow, one-half to one-third of the ridges, and is in contact along the sides of the ridges with the epiplasm. This material, like the other two filamentous systems, is more easily observed following fixation with collidine, rather than cacodylate, as the buffer (compare Fig. 2 with Fig. 15).

Below the granulo-fibrillar meshwork is a second system of microfibrils which form distinct bands. These bands, which vary in width from around 100 to 500 or so μm, are oriented in a plane parallel to the organism's surface (σ in Figs. 3 and 13). Groups of bands, which are always unbranched, radiate out from the bottom or side of one surface depression and extend to all other depressions surrounding it. In so doing the bands pass under both lateral and longitudinal ridges. An individual band appears to have its ends inserted in the epiplasm lining two adjoining depressions. There is no indication that the bands are in contact with the basal bodies, except through the extension of the epiplasm to the terminal plate as already described. A unique feature of these bands is their straightness; they appear as one would expect them to appear if they were under tension.

At higher magnification the bands are seen to be composed of fine microfibrils lying essentially parallel to each other (Fig. 14). It was not possible to determine the length of the individual microfibrils. In cross-section (Fig. 15) the bands are roughly circular bundles of solid-appearing filaments. These are not tightly packed together but are separated from each other by electron-transparent interstices. No regular pattern of filament arrangement was detected. The cross-sections of microfibrils show them to have a diameter of 60 Å. In longitudinal sections the bands appear to have an over-all cross-striated appearance (Figs. 13 and 14), suggesting an overlapping of fibrils in the more electron-opaque striae. However, these regions could also contain extra material surrounding a single complement of fibrils. The morphological basis for this striated pattern was not resolved. Lying next to the epiplasm is a stripe of least opacity (Fig. 14). This stripe seems to be composed of branching fibrils which appear to be more stable when subjected to the glycerination procedure than the fibrils of the more opaque stripes. Next to these stripes are the most opaque regions which are separated by another stripe of medium opacity. Some of the longer bands have three opaque stripes interdigitating with two regions of medium opacity (bracket, Fig. 13). The most opaque stripes are approximately 200 μm wide, the least opaque stripes are 100 μm wide, while the width of the region between the opaque stripes is approximately 150 μm. It is also possible to see small bridges of material linking fibrils which are lying next to each other (inset, Fig. 14). These bridges are about 100 Å long and, when several consecutive bridges are found along a fiber, they have a center-to-center spacing of about 150 Å. Fig. 16 shows the appearance of this band of fibrils following the glycerination procedure.

The third fibrous system appears to be the one comparable to the infraciliary lattice as first described by Gelei (1937) and later seen in the electron microscope studies of Sedar and Porter (1955) and others. This system is composed of bundles of compact microfibrils forming an elaborate reticulum in a plane passing through the proximal
half of the basal bodies (\(\phi\) in Figs. 3, 13, and 18). This reticulum forms a series of irregular polygons as it branches throughout the ectoplasm. The fibers of the reticulum may come in contact with the basal bodies (Fig. 18), but usually they are separated from the basal bodies by a distance of at least 100 \(\mu\mu\). The individual microfibrils making up the fibers are 30–40 \(A\) thick (Fig. 15) and are oriented approximately parallel to the long axis of the fiber (Fig. 19). It was not possible to determine the length of an individual microfibril. There is some indication that the microfibrils may branch or be attached to each other at intervals along their lengths. In glycerinated \textit{Paramecium} the microfibrils of the infraciliary lattice appeared as a loose network resembling a crumpled mat of yarn (Fig. 21). The microfibrils appeared to be thicker, about 70–90 \(\AA\) wide, in the glycerinated cells.

The arms of this reticulum have regular margins and are 70–100 \(\mu\mu\) wide. At the corners of the reticulum the fibers may be as much as 400 \(\mu\mu\) thick when viewed in the plane of the reticulum (Fig. 18). In the center of each branching region is an electron-opaque post which is oriented perpendicular to this plane. When the lattice is viewed in planar section as in Fig. 19, this post, which is 20 \(\mu\mu\) thick, often appears to have a triangular shape. The microfibrils which approach the post appear to bend away from the post and extend beyond it, forming an angle of about 120°. Thus, three arms extend away from the post in the plane of the reticulum. When the organism is cut in transverse section the post appears to extend across the lattice and to be lined with a relatively electron-transparent zone on either side. The posts vary in length with the thickness of the lattice and so may be 140–200 \(\mu\mu\) long (Fig. 20, and inset in Fig. 21). In cross-sections of these fibers the individual microfibrils are not as obvious as in the striated fibrous system described above (Fig. 15). The fibrils of the infraciliary lattice appear to be embedded in an amorphous matrix which makes the whole fiber more electron-opaque in cross-section than bands of the striated fibrous system sectioned in the same manner.

Besides the microfibrillar systems, \textit{Paramecium caudatum} also has cortical microtubules. Associated with each single basal body, or with the posterior basal body of a pair, are two small bands of microtubules, a band of posterior (right radial) microtubules (\(pt\) in Figs. 3, 11, 12, 14, and 18) which passes radially and posteriorly from the proximal end of the basal body over the band of kinetodesmal fibers to the right of the kinety, and a small band of transverse (left tangential) microtubules (\(tt\) in Figs. 13 and 22) which arises from the anterior proximal surface of the basal body and passes to the left of the kinety and toward the surface of the organism. A kinetodesmal fiber (\(k\) in Figs. 3, 4, 13, 18, and 22) also arises from the anterior right side of the proximal end of the basal body and passes anteriorly in line with similar fibers from other basal bodies (Fig. 15) in the same kinety. This is a banded fiber with a major periodicity of 23–27 \(\mu\mu\).

Other cytoplasmic microtubules are occasionally seen in the ectoplasm (Figs. 3 and 13). They are usually observed alone and do not appear to follow any regular pattern. They have a diameter of about 25 \(\mu\mu\). No basal microtubules (Allen, 1967) or longitudinal microtubular bands, as reported for \textit{Tetrahymena} (Pitelka, 1961; Allen, 1967), were observed in \textit{Paramecium caudatum}.

Another observation made in this study which has not been previously reported is the complement of fibrous connections that exist between the anterior basal body of a pair and the posterior basal body and its kinetodesmal fiber. Two thin filaments extend from an electron-opaque node along the surface of the kinetodesmal fiber to a similar node along the facing surface of the anterior basal body (right pair in Figs. 18 and 22). Another coarsely striated fiber crosses from the base of the kinetodesmal fiber to the posterior surface of this anterior basal body (right pair in Fig. 22). Finally, a third coarsely striated fiber extends from the anterior proximal surface of the posterior basal body and fans out or branches so that it attaches to the anterior basal body over the latter’s posterior left quadrant (Fig. 3 and left pair in Fig. 22). This last fiber is apparently what Pitelka (1965) saw and referred to as a “delicate, arched bridge,” and what Jurand and Selman (1969) refer to as a “bridge.” These three filaments or fibers are all located at the level of the “cartwheel” portion of the basal bodies.

A cutaway, three-dimensional drawing has been made of a small portion of the somatic cortex of \textit{Paramecium caudatum} (Fig. 23). The top end of a line drawn through the cilia of a kinety would lie toward the anterior end of the organism. Since the organism is being viewed by the observer from the outside, the left and right sides of the kineties are to the observer’s right and left, respectively. This
DISCUSSION

External Lattice—Morphological Basis

Light microscopists using silver staining techniques observed that the cortex of various ciliated protozoans exhibited darkly staining lines which were organized into patterns unique for different ciliates. These patterns are thought to be formed by the deposition of silver along the contiguous...
Figure 23 A three-dimensional drawing depicting the location and interrelationships of those membranous and microfibrillar systems discussed in this paper which are located in the somatic cortex of Paramecium caudatum. The anterior end of the cell is toward the top of the drawing. The right side of a given kinety is toward the observer's left when the cell is viewed from the outside, as is true here, and when looking along a kinety from the posterior to the anterior end. The various components are labeled as follows: pm, plasma membrane; a, alveolus; p, perforations connecting adjacent alveoli; ep, epiplasm; g, granulo-fibrillar meshwork; ps, parasomal sac; c, cilium; t, trichocyst tip; sb, striated bands; il, infraciliary lattice; k, kinetodesmal fiber; pt, posterior microtubules; ti, transverse microtubules; b, bridge connecting cartwheel portions of a pair of basal bodies.

Margins of adjacent alveoli (Ehret and Powers, 1959; Dippel, 1962; Pitelka, 1961, 1965, 1969; Parducz, 1962). In Paramecium, silver is sometimes deposited along the lines of the polygonal lattice and more frequently along the interciliary meridional lines. Parducz (1962) has called attention to the difference in the staining properties and the physical properties of the external lattice and of the meridional system. Yet, as Pitelka (1965) points out, these two systems are still regarded as having an identical morphological basis, a hypothesis which seems inconsistent with available evidence.

In that same paper Pitelka (1965) states that the alveolar margins appeared only erratically along the lines of the polygonal lattice in Paramecium multimicronucleatum and that, as was previously shown by Sedar and Porter (1955), the intrapellicular space is sometimes continuous over the ridges between polygons. According to these observations, alveoli bounded by the kinetal rows of cilia and trichocysts may thus extend longitudinally through several polygons without interruption. However, Pitelka also reported seeing the compartmentalization of some of these flattened vesicles into polygonal units as was previously intimated by Ehret and Powers (1959) for all polygonal units.

Such compartmentalization into polygonal units
of the alveoli was never convincingly seen in the present study. The only system of alveolar margins that is consistently present in *Paramecium caudatum* is that found connecting the trichocysts and lateral bodies of kinetics. Infrequently, margins running laterally between these meridians can be seen. However, they are often seen in regions other than at the crests of the ridges (Fig. 5), and there are often distances of several polygons between lateral margins. Margins running along the crests of ridges paralleling the meridional lines are also infrequent. It thus appears that the alveoli in *P. caudatum* are elongated flattened sacs extending from one end of a kinety to the other with their lateral margins running along the adjacent kinetics. Such an alveolus is indicated in Fig. 1 by the numeral 1 in the various spaces of this continuous sac. These alveoli are then partitioned at irregular intervals by laterally directed margins which are not necessarily associated with the crests of ectoplasmic ridges. However, it does appear that, even though the inner and outer alveolar membranes do not fuse at the crests of the ridges, a closer association may be maintained between these two membranes at these crests than at other points along the sides of the depressions. At least this is true of organisms which have expanded alveoli (Figs. 3 and 4). On the other hand, this apparently stable association may be an artifact, since these two membranes would be expected to be closer together at this point due to the projection of the ridge with its overlying inner alveolar membrane into the inflated sac. Also, in the uninflated condition, as in Fig. 2, the inner and outer alveolar membranes are actually farther from each other than they are along the sides of the depressions. In any case, the above-described arrangement of alveolar margins and their subsequent staining cannot account for the observations of the regular external lattice as seen in some silver-stained preparations.

However, another unique structure does exist which follows the pattern of the external lattice. This is the granulo-fibrillar material which is located within the tips of the ridges. Silver staining of this material would account for a very regular polygonal pattern coinciding with the sculptured surface patterns of *Paramecium*. This explanation which seems to best account for the external lattice reaffirms the earlier hypothesis for this pattern presented by Sedar and Porter (1955).

Staining of both the granulo-fibrillar material and the alveolar margins which are shown here to lie principally along the interciliary meridians would give rise to both the direct and indirect connecting silverlines and would be consistent with the observations about these two systems made by light microscopists as summarized by Parducz (1962). Without correlating each observation with the above explanation, it seems sufficient to say that this explanation for the above two systems satisfies the conclusion of Parducz (1962) that the external lattice and interciliary fibrils are different in their material basis and are probably separated in space. In further support of this hypothesis it can be pointed out that, in the published light micrographs of these two systems (Gelei, 1934 and Parducz, 1962), the external lattice appears wider than the interciliary fibrils. The granulo-fibrillar material seen in electron micrographs of *P. caudatum* can be, in fact, some 10 times the width of the meridional septum formed by the two alveolar membranes and their intervening space. Finally, one often sees in the published light micrographs of silver-stained *Paramecium*, lateral extensions of the interciliary fibrils which pass part or all of the way to the next adjoining interciliary fibrils in regions other than along the external lattice. These appear to be identical to the laterally directed margins in the present study which do not necessarily follow the tips of the ridges.

From all available evidence, then, it seems that the explanation first proposed by Sedar and Porter (1955) as the morphological basis of the external lattice should be accepted rather than the presently held view of contiguous alveolar margins.

**The Continuous Nature of the Alveolar System**

This study has shown, not only that the alveolar margins do not generally follow the crests of the polygonally shaped ridges, but also that adjacent alveoli are continuous through membrane-lined perforations which pass through both longitudinal and lateral septa. Thus, the whole mosaic of alveoli lying just under the plasma membrane is one continuous space enveloped by a continuous membrane. The functional implications of this observation are not entirely clear. Pitelka (1965) supported the concept that the alveolar mosaic served as an insulating layer to protect the underlying cytoplasm from physical and chemical assault. Allen and Eckert (1969) reported that this system superficially resembles the sarcoplasmic reticulum.
(SR) of striated muscle and, like the SR, might accumulate calcium which when released could play a role in some ciliary activity. Although no function can be conclusively ascribed to this system at present, it does appear to be an important system for future study.

Perforations between alveolar units are not unique to Paramecium. The author has observed similar perforations between alveoli in the ciliates Tetrahymena pyriformis and Coleps hirtus in unpublished micrographs. It seems likely that such perforations may be a constant feature of ciliates which have alveoli.

**The Discontinuous Epiplasm**

Pitelka (1965) observed that during fragmentation procedures the pellicle of Paramecium preferentially tears along the crests of the pellicular ridges. This phenomenon may be accounted for by the discontinuities in the epiplasm at the tips of the polygonal ridges (Figs. 2, 3, 13, and 15) which may weaken the pellicle along these lines.

**Distinguishing Between the Infraciliary Lattice and the Striated Microfibrillar Bands**

Underlying the alveoli and epiplasm are two systems of microfibrils. The deeper of these two is the infraciliary lattice as first described by Gelei (1937) and later by Sedar and Porter (1955), Roth (1958), Pitelka (1965), Hufnagel (1969), and Jurand and Selman (1969). The more superficial fibrils have also been seen by Schneider (1959), Pitelka (1965), and Jurand and Selman (1969), but their distinctiveness in morphology and spatial separation was not clearly established by these authors.

It seems correct to view these two kinds of microfibrils as two separate systems and to view only the deeper system as the infraciliary lattice. The infraciliary lattice can be distinguished from the more superficial bands by (a) being a branched system with small electron-opaque posts at the center of each branching locus, (b) having a more compact structure and consequently being more electron-opaque when viewed in either longitudinal or cross-section, (c) having no insertions on or firm connections with the pellicle or any organelles, (d) having microfibrils of a diameter, when viewed in cross-section, smaller than those of the striated bands, (e) exhibiting no broad striations across the bands, (f) exhibiting a different appearance following glycerination, and finally (g) having a location internal to, and separate from, that of the striated bands.

**The Infraciliary Lattice**

The structure and location of the infraciliary lattice pose several problems: What is it for, why does it remain in the ectoplasm, and how does it grow and branch as the animal grows? One function of the infraciliary lattice must be to help maintain order in the outer layer of this cell by surrounding and thereby maintaining the separation between the basal bodies and trichocysts. In Fig. 18 each basal body or pair of basal bodies, as well as each trichocyst tip, is isolated from other basal bodies and trichocyst tips by the meshwork of the infraciliary net. What holds the net in place around the basal bodies is another problem. One explanation is that the lattice would probably be prevented from moving very deeply into the cytoplasm since the dimensions of the mesh would be too small for it to be able to pass over the body of the trichocyst. Growth of the net presents a third problem. In order to grow with the organism, a new locus of branching would appear to require a new post around which the microfibrils can become oriented to form the dichotomous branches. What role does this post play, if any, in the formation of new branches? How does a new post arise? Also, how does a new branch get between the parent and daughter basal bodies as they move apart along a kinety in conjunction with the growth and division of the whole cell? These are intriguing and exciting problems for future studies on growth and development of single cells.

**Striated Microfibrillar Bands**

The more superficial microfibrillar system which will be referred to as the "striated bands" seems easier to correlate with known physiological processes. Paramecium have been shown to be able to change the shape of their pellicles. This phenomenon can be produced experimentally by electrical shock (Miller et al., 1968) or by placing the organisms in isosmotic solutions of SrCl$_2$ and CaCl$_2$ (Kamada and Kinoshita, 1945). Such a shape change is undoubtedly partially dependent upon some morphological system in the organism's cortex. The most obvious structure fitting this function would appear to be the striated bands.
which are described in detail here for the first time. Reasons for making this hypothesis may be given as follows. First, the straightness and regular outline of the bands as well as the straightness of the individual microfibrils making up the bands suggest that the bands are under tension. Second, the apparent firm insertion of the bands at both of their ends in the epiplasm underlying the pellicle would allow for a shape change upon shortening of the bands. Also, the patterns formed by the bands radiating in several directions from the base of a depression would allow for shape changes of a complex nature if individual bands were able to contract independently of surrounding bands. Finally, the striated appearance of these bands can be interpreted as suggesting that two sets of fibrils overlap within the more opaque stripes, a relationship necessary for a sliding-filament model of contraction. (For a review of the kinds of movement for which this model has been invoked, see Jahn and Bovee, 1969.) In cross-section the microfibrils appear much more distinct than those of the infraciliary lattice and thus resemble fibrils of other known contractile systems. Also, bridges are frequently seen between individual fibrils within the body of the bands (inset, Fig. 14). These observations do not constitute proof that these bands are contractile but they do appear to be sufficient, when accompanied by physiological and biochemical evidence (reviewed by Jahn and Bovee, 1969) from previous studies on Paramecium, to justify such an hypothesis until further work can be done.

Hufnagel (1969) has shown the presence of 85-A wide fibrils in the cortex of Paramecium which are held together at their centers in groups of three or four. These “bow tie-like” structures are consistently 425-465 mµ long. From a close study of the electron micrographs of the various fibrillar systems found in P. caudatum, it appears that Hufnagel’s bow tie fibers were isolated units of the striated bands. The distance from the epiplasmic edge of one opaque stripe to the epiplasmic edge of the next adjacent opaque stripe in P. caudatum (Fig. 14) is usually 500 mµ, or slightly less when measured in sections where all five stripes are visible. This length is close to that of the bow tie fibers and suggests that the fibrils stretching across one opaque stripe, through the medium opaque stripe, and across a second adjacent opaque stripe are composed of compound units such as those reported by Hufnagel (1969). The ends of these bow tie fibers may then interdigitate in the most electron-opaque zones with a set of fibrils extending out from the epiplasm, or in the case of longer bands, with another set of 500-mµ long fibrils (see upper part of Fig. 13). At this point the evidence favors two spatially and possible morphologically, but not necessarily biochemically, different sets of microfibrils making up these bands: one set attached to the epiplasm, and another set spanning the region between. This is suggested by two observations. First, the stripes nearest the epiplasm are of lowest electron opacity, suggesting that the fibrils in this region are either more sparsely present than, or are morphologically distinct (possibly thinner) from, the fibrils in the medium electron-opaque zone. Also, in glycercinated cells a network of thin fibrils is consistently found extending out from the sides of the ridges, i.e. in the same region as the most electron-transparent stripes in nonglycerinated cells, but only rarely are the fibrils which form the body of the bands in place (as in Fig. 16). Thus, the fibrils in the body of the bands are affected in a different manner by glycercination than those fibrils next to the epiplasm and, when lost, the fibrils in the body appear to be lost as a unit, leaving only the network of thin fibrils next to the epiplasm.

In summary, on morphological grounds, it appears that striated bands consist of a complex system of microfibrils which may have overlapping fibrils in the most electron-opaque stripes of the system. The function of these bands may be contractile, providing the organism the morphological machinery necessary to alter the shape of its surface.

Fibers Linking Paired Basal Bodies and the Associated Kinetodesmal Fiber

Hufnagel (1969) noted that when paired basal bodies were isolated from fragmented pellicles of Paramecium the two basal bodies and the kinetodesmal fiber of a single territory stay together. We have noted a set of three fibers which link the anterior basal body to the posterior basal body and its attached kinetodesmal fiber. Only one of these fibers has been previously reported (Pitelka, 1965 and Jurand and Selman, 1969), i.e., the arched fiber which runs directly between the two basal bodies. These three fibers apparently bind the anterior basal body to the other two structures so firmly that it does not become separated from them.
during the fragmentation process. Fibers have been reported between the paired somatic basal bodies of other ciliates (see Grain, 1969), as well as the basal bodies of flagellates (e.g., Ringo, 1967; and Outka and Kluss, 1967).

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