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THE ULTRASTRUCTURE OF THE SOMATIC CORTEX OF *PSEUDOMICROTHORAX DUBIUS*: STRUCTURE AND FUNCTION OF THE EPIPLASM IN CILIATED PROTOZOA

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SUMMARY

The ultrastructure of the somatic cortex of the ciliate *Pseudomicrothorax dubius* is studied with emphasis on the epiplasm layer which lies immediately under the inner alveolar membrane and is continuous with the terminal plates of cortical basal bodies.

In addition to a clearly demonstrable cytoskeletal role, the epiplasm appears to function as a cementing substance which integrates numerous cortical fibres and membranes. The kinetodesmal, postciliary and transverse fibre systems which originate at the proximal ends of basal bodies extend toward the cell surface and end at or in the epiplasm. Inner alveolar membranes and trichocyst membranes are attached to the epiplasm. Basal bodies are anchored into the epiplasm *via* their terminal plates.

The epiplasm appears to be morphogenetically important as a matrix into which newly formed basal bodies insert. Electron-opaque arms occur at the terminal plate level of new basal bodies, and these arms fuse with the epiplasm when basal body insertion occurs. The position of trichocysts in the cortex is specified by the epiplasm.

Evidence from numerous other ciliates tends to confirm both structural and morphogenetic roles of the epiplasm.

INTRODUCTION

Many studies of the ciliate cortex have reported an epiplasm layer; however, we possess neither a precise definition of this structure, nor an account of information currently available about it. The present paper extends the observations of Fauré-Fremiet & André (1967) on the cortical ultrastructure of the ciliate *Pseudomicrothorax dubius*, and demonstrates that the 'lamina corticalis' described by these workers in *P. dubius* is equivalent to the epiplasm in other ciliates. A definition of the epiplasm is proposed based upon comparative cortical analysis. Particular attention is given to the association between the epiplasm and other cortical structures in *P. dubius* and other ciliates, and to its possible functions in the cortex.

MATERIALS AND METHODS

Pseudomicrothorax dubius strain F was cultured initially in Millipore-filtered pond water and later in a medium consisting of 0·5 mM KCl, 2·0 mM NaCl, 0·2 mM $MgCl_2$, 0·8 mM $CaCl_2$ and 0·43 mM NaH_2PO_4, with the pH adjusted to 7·0 by addition of NaOH. The ciliate was fed either *Oscillatoria formosa* or *Phormidium autumnale*. The former was grown at 30–32 °C in a modified

Hughes medium (Allen & Stanier, 1968) and the latter at 26–28 °C in 0·42 mM $Ca(NO_3)_2$, 0·99 mM KNO_3, 0·12 mM $MgSO_4$, 0·23 mM K_2HPO_4, 0·01 mM $Fe(ClO_4)_3$, 2 % (vol./vol.) Sphagnum moss extract and 4 % (vol./vol.) earth extract. Both cyanophytes were illuminated with Osram-L-Fluora bulbs in a 12-h light/dark cycle.

Epiplasm isolation procedures were performed at room temperature (*ca.* 20 °C) except where otherwise specified. Epiplasm ghosts were isolated by placing cells previously rinsed twice in distilled H_2O into 1·5 ml of distilled H_2O and adding 0·3 ml of freshly prepared 0·4 % (wt/vol.) sodium dodecyl sulphate (SDS).* The suspension was agitated for 3 min by rapid pipetting and then 4·0 ml of freshly prepared 2 % (wt/vol.) Triton X-100 were added and agitation was continued intermittently for 15 min. The suspension was then diluted with 2·0 ml of distilled H_2O and centrifuged at 1500 **g** for 10 min in the swing-out rotor of an MSE Super Minor centrifuge in a 1 °C cold room. The pelleted ghosts were rinsed 3 times with distilled H_2O. To produce epiplasm fragments, cells in culture medium were ultrasonicated at the no. 5 setting of a Bronson Sonifer B-12 for 40–60 s until all cells were broken open. Specimen heating was controlled by use of an ice-water bath. The resulting suspension was made 0·8 M in sucrose, placed in 15 ml polycarbonate centrifuge tubes and centrifuged at 2300 **g** for 10 min in the swing-out rotor of an MSE Super Minor centrifuge in a 1 °C cold room. The supernatant was poured off and each pellet was rinsed twice by resuspending it in 0·8 M sucrose and centrifuging as above. The pellet was then resuspended in 17·5 ml of distilled H_2O, and 1·5 ml of freshly prepared 0·4 % (wt/vol.) SDS were added. The suspension was mixed for 5 min and then 20 ml of freshly prepared 2 % (wt/vol.) Triton X-100 were added and agitation was continued intermittently for 20 min. The suspension was then centrifuged as above at 2300 **g**. The supernatant was removed by aspiration and the epiplasm fragments were rinsed 3 times with distilled H_2O.

Techniques for transmission electron microscopy (TEM) have been described elsewhere (Peck, 1974). Material was prepared for scanning electron microscopy (SEM) by the technique of Small & Marszalek (1969). A 400–500-nm-thick layer of gold was evaporated on to freeze-dried specimens in a Balzers Sputtering Device prior to observation in a Siemens Autoscan electron microscope. For light microscopy, epiplasm ghosts were air dried on microscope slides and stained for 15 min in a 0·1 % (wt/vol.) solution of bromophenol blue in saturated $HgCl_2$ at 45 °C. Slides were subsequently rinsed in 0·1 M Sorensen's phosphate buffer at pH 7·0 for 1–2 min until the epiplasm became blue (controlled with a dissecting microscope) and then slides were briefly rinsed sequentially in fresh buffer and distilled H_2O and rapidly air dried. Protargol staining was performed by the Bodian technique (Kirby, 1950).

Basal body triplets are numbered according to Antipa (1971).

RESULTS

Light microscopy; SEM

Pseudomicrothorax dubius is a dorsoventrally flattened, rigid, ciliated cell. Most of kinety 1 and the 2 short kinetal segments of the primordial field (*pf*) are composed of paired basal bodies (Fig. 3). Kineties occur in cortical grooves which are visible in

* Serva purissima (> 99 %) SDS was recrystallized from 75 % ethanol before use.

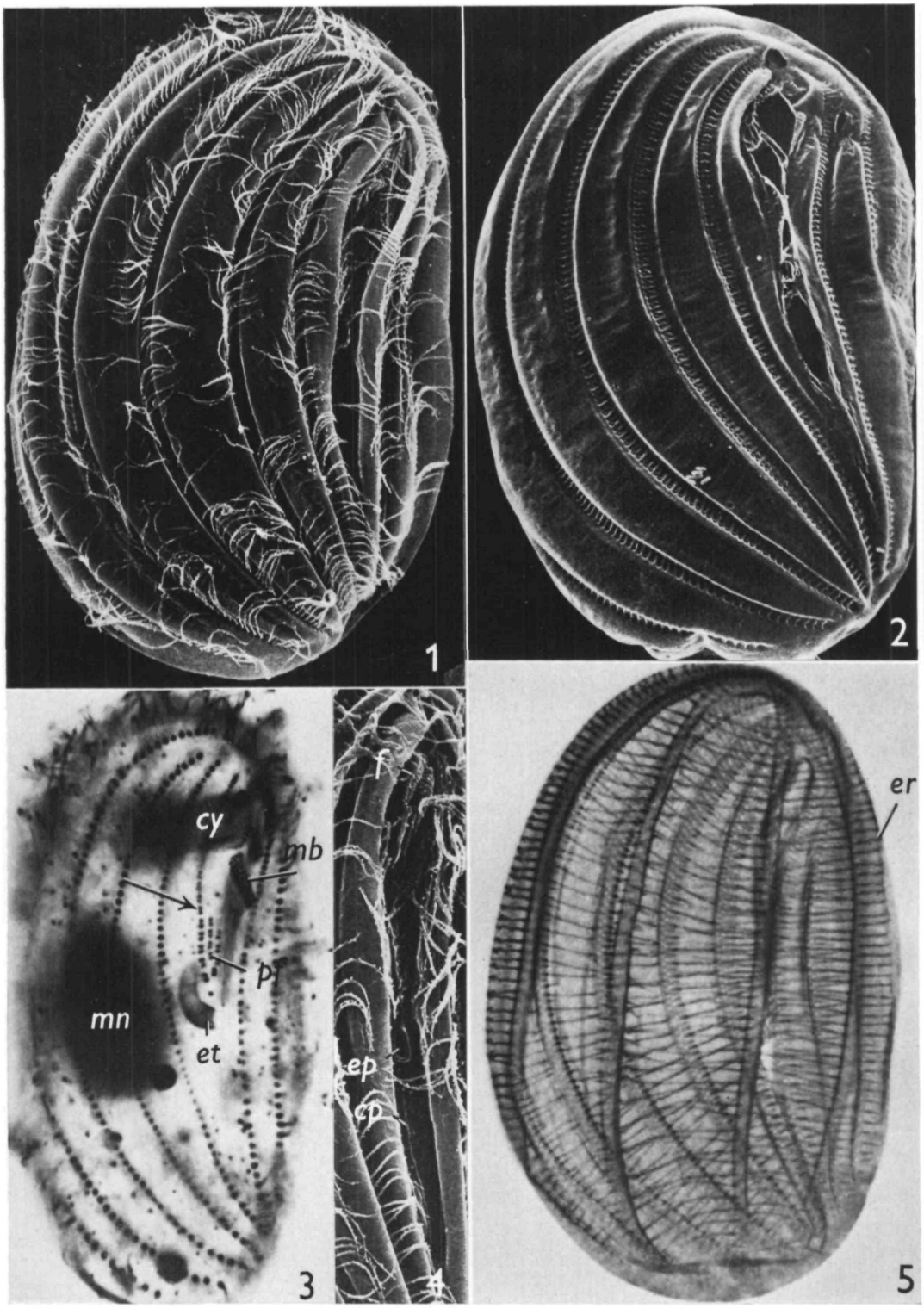
Fig. 1. SEM of entire cell. × 800.

Fig. 2. SEM of epiplasm ghost. × 1100.

Fig. 3. Protargol-stained cell showing a membranelle (*mb*), the cytopharynx (*cy*), paired basal bodies of the primordial field (*pf*) and kinety 1 (arrow), the expulsion vacuole tube (*et*), and the macronucleus (*mn*). × 1300.

Fig. 4. SEM showing the corrugations (*f*) of the cytostome, the expulsion vacuole pore (*ep*) and the cytoproct (*cp*). × 1600.

Fig. 5. Mercuric bromophenol blue-stained epiplasm ghost showing the epiplasm ridges (*er*). × 900.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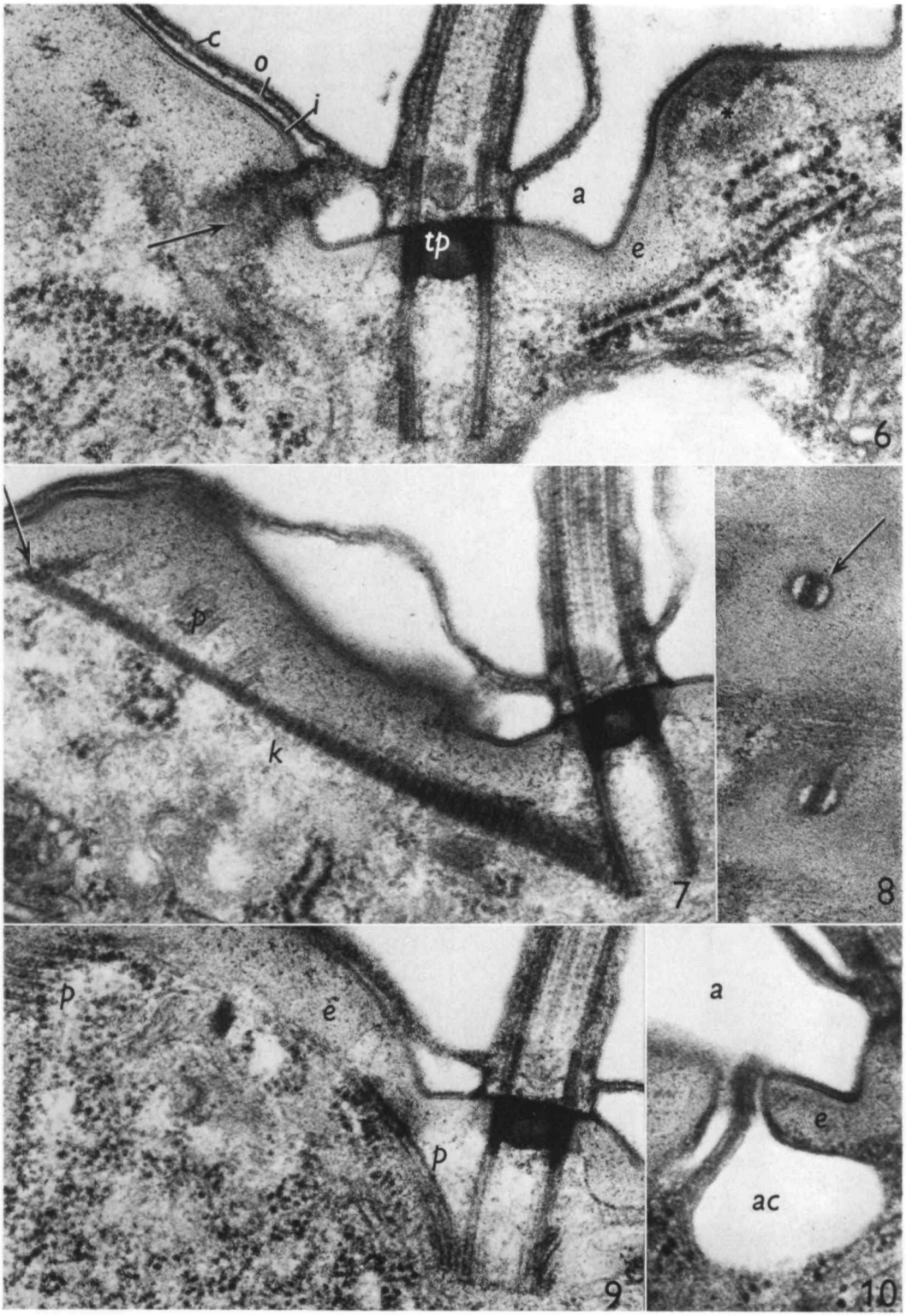
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both the intact cell and the epiplasm ghost which retains the original cell shape (Figs. 1, 2). Mercuric bromophenol blue staining of the epiplasm ghost reveals localized thickenings called the epiplasm ridges (*er*) (Fig. 5). The buccal system consists of 3 membranelles (*mb*) and an underlying cytopharynx (*cy*). The cytostome is located at the distal end of the cytopharynx and is surrounded by thickened corrugations (*f*) (Fig. 4). The cytoproct (*cp*) is a narrow ridge posterior to the expulsion vacuole pore (*ep*) (Fig. 4). Protargol staining reveals the expulsion vacuole tube (*et*) which leads from the pore to the expulsion vacuole (Fig. 3).

TEM–somatic cortex

Membrane systems and epiplasm. The cell membrane (*c*) surrounds the entire cell including the cilia. The outer alveolar membrane (*o*) and inner alveolar membrane (*i*) are located below the cell membrane and circumscribe an alveolar space (*a*) (Fig. 6). The alveolar space is inflated along the kineties but flattened interkinetally, and it appears to lack septa. The outer and inner alveolar membranes join around basal bodies, parasomal sacs and the tips of trichocysts (Figs. 6, 19).

The epiplasm (*e*) lies immediately below the inner alveolar membrane and is continuous with the terminal plates (*tp*) of basal bodies in both the intact cell (Figs. 6, 11) and epiplasm fragments (Fig. 25). The terminal plates and the epiplasm are both *ca.* 150 nm thick. The periphery of each terminal plate is perforated by 9 holes. One hole occurs exterior to and between each adjacent pair of axonemal microtubules A and B (Figs. 11, 12). A very thin electron-translucent layer and an equally thin electron-opaque layer form the external portion of the epiplasm below the inner alveolar membrane (Figs. 6, 19). The remainder of the epiplasm stains inhomogeneously, showing electron-opaque specks on a more electron-translucent background. The inner alveolar membrane is firmly bound to the epiplasm, since it remains attached to the epiplasm following ultrasonication (Fig. 24). The cytoplasmic surface of the epiplasm is differentiated into ridges (*er*) which run between adjacent kineties (Fig. 17).

The parasomal sac is an invagination through the alveolus and the epiplasm, and is

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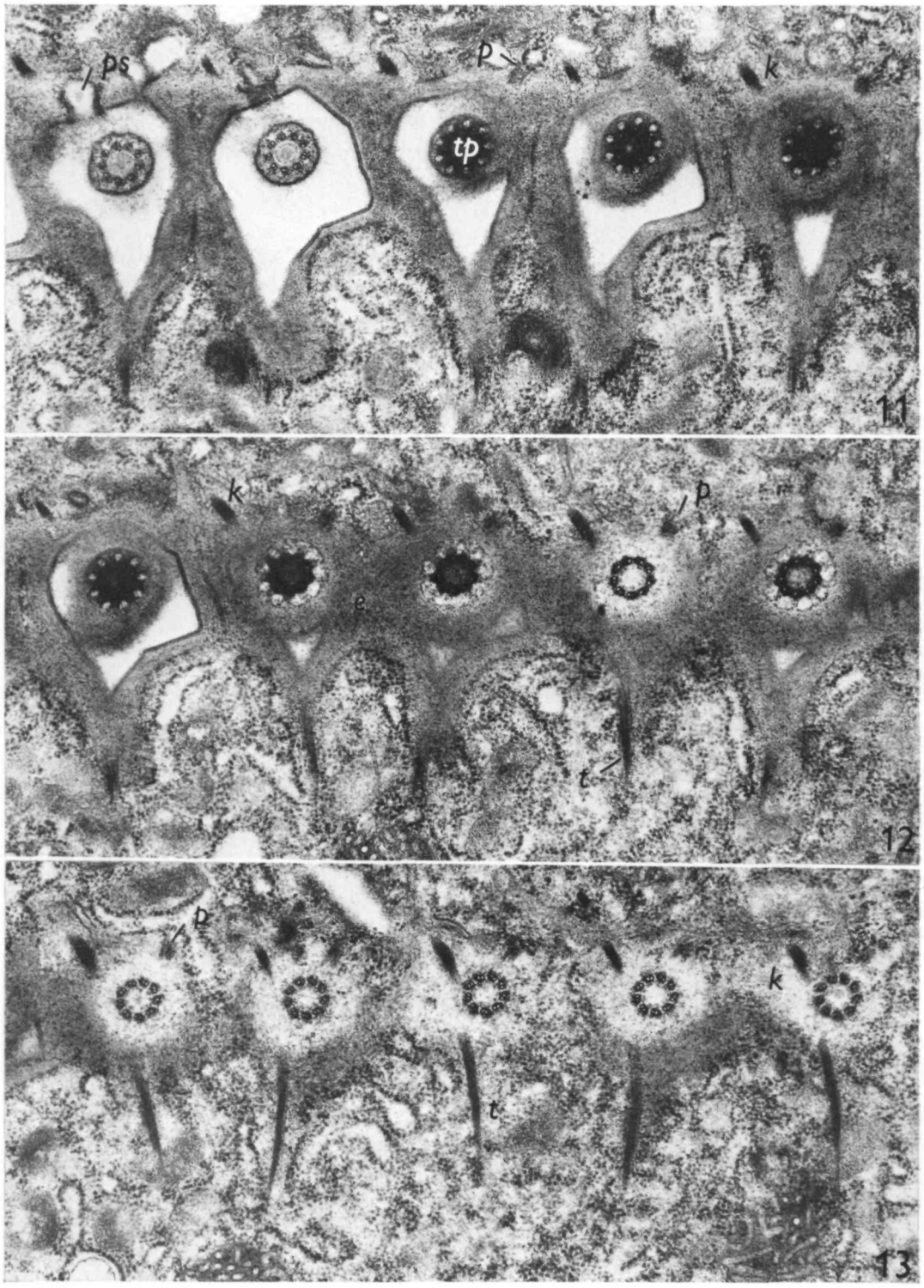
Fig. 6. Transverse section of the cortex showing continuity of the epiplasm (*e*) with the terminal plate (*tp*). The cell membrane (*c*) covers the outer (*o*) and inner (*i*) alveolar membranes which surround an alveolar space (*a*). The membrane of the parasomal sac (arrow) is sectioned superficially. The asterisk indicates filamentous material in an unoccupied trichocyst site. × 63 000.

Fig. 7. Transverse section of the cortex showing the postciliary microtubules (*p*) and the contact (arrow) of the kinetodesmal fibre (*k*) with the epiplasm. × 63 000.

Fig. 8. Longitudinal section of the epiplasm showing orifice (arrow) of an alveolocyst. × 102 000.

Fig. 9. Transverse section of the cortex showing postciliary microtubules (*p*) which arc up from the proximal end of the basal body and contact the epiplasm (*e*). × 62 500.

Fig. 10. Transverse section of an alveolocyst (*ac*) showing its relationship to the alveolar space (*a*) and the epiplasm (*e*). × 75 000.



Figs. 11–15. Sequential longitudinal sections of the cortex showing the origin of the kinetodesmal fibre (*k*), postciliary (*p*) and transverse (*t*) microtubules and the postciliary accessory fibre (*pc*) around the basal body, and the relationship of these fibres to the epiplasm (*e*). Nine electron-translucent perforations are observed at the periphery of each terminal plate (*tp*). *ps*, parasomal sac. Figs. 11–13, ×32 500;

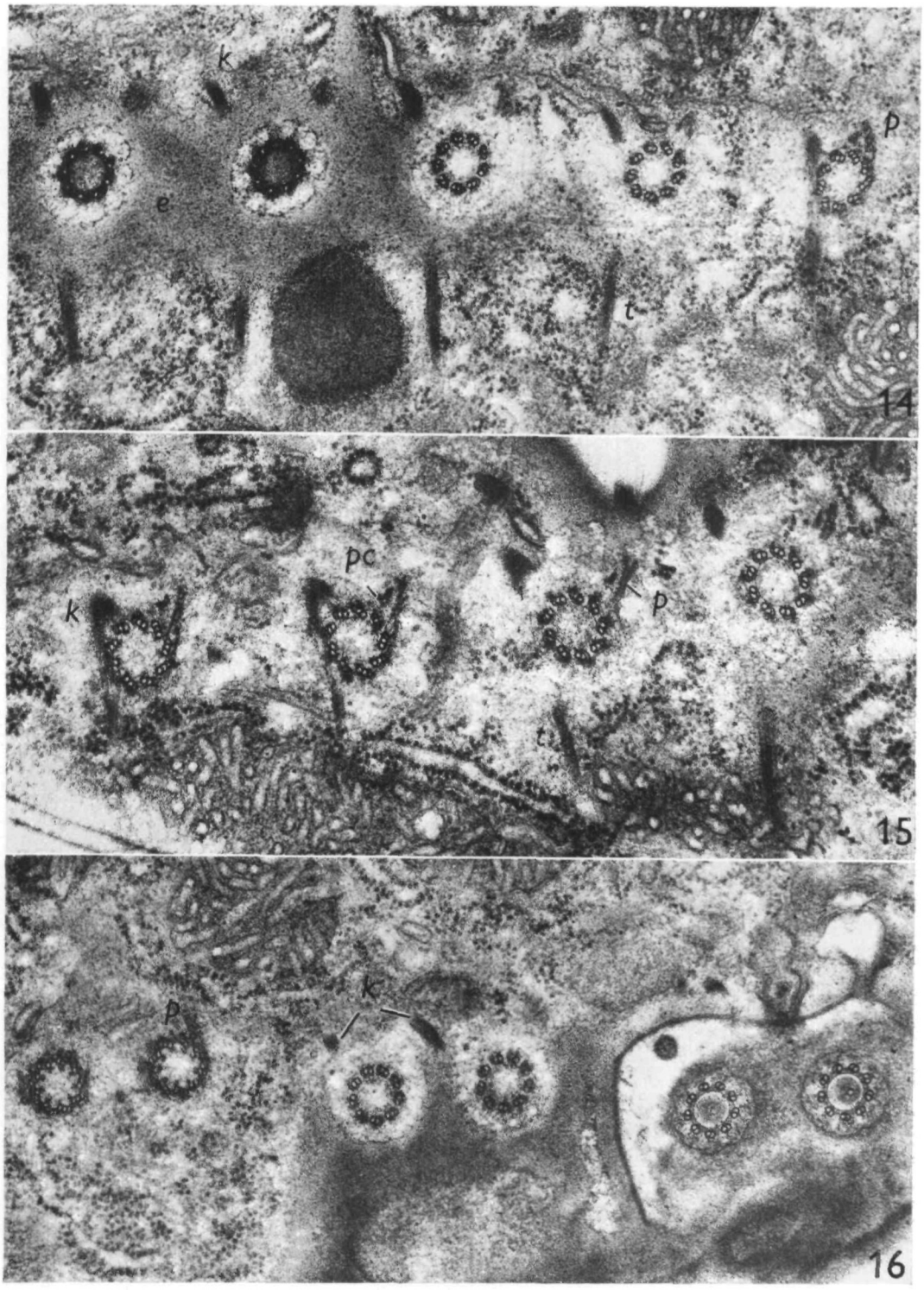
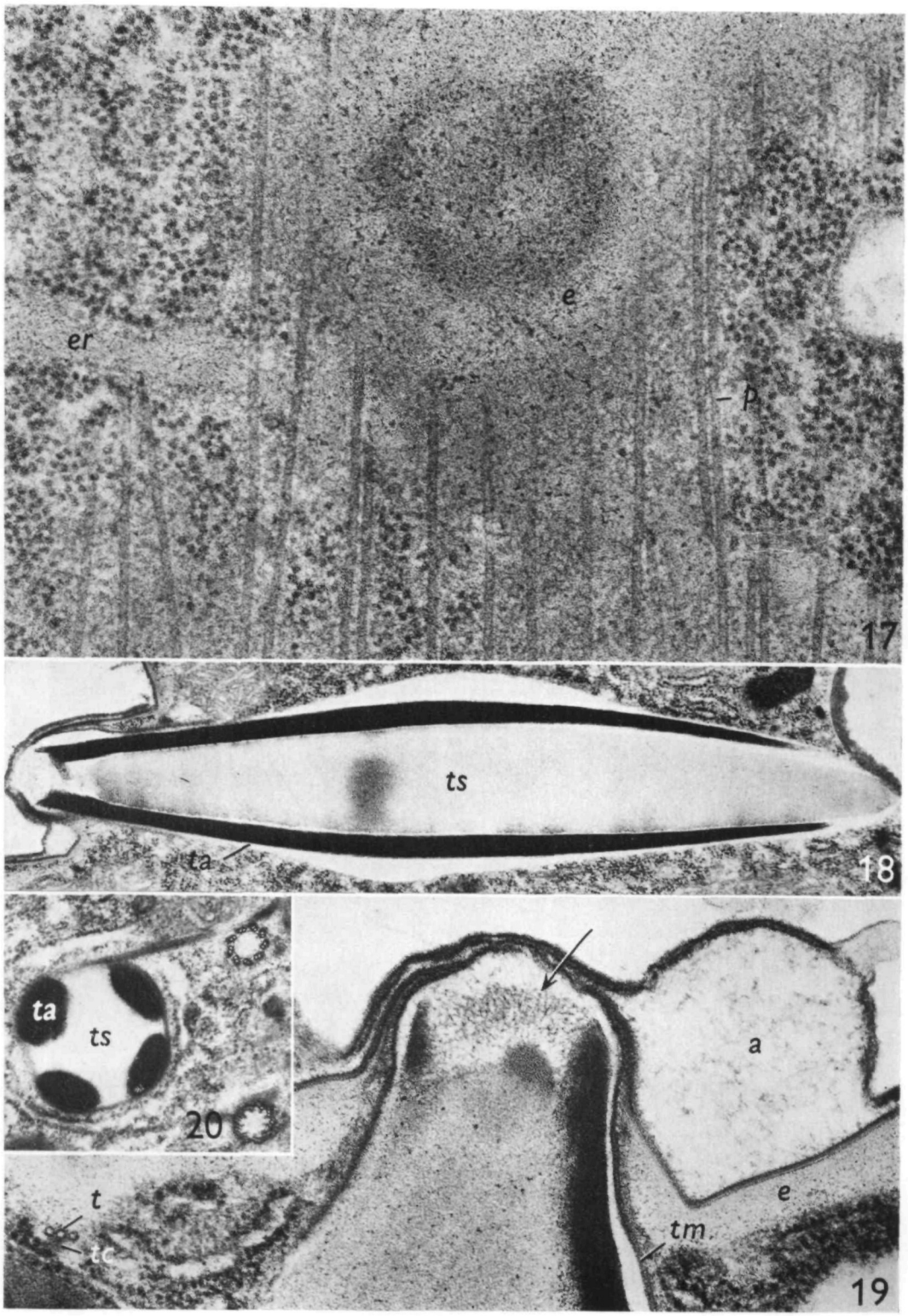


Fig. 14, ×39500; Fig. 15, ×52000.
Fig. 16. Longitudinal section of part of kinety 1 showing 3 pairs of basal bodies with their complement of kinetodesmal (*k*) and postciliary (*p*) fibres. ×42000.


er
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lined by the cell membrane. Parasomal sacs are located in the wall of the kinetal groove to the right and slightly anterior to the basal bodies (Figs. 6, 11).

The alveolocyst (*ac*) is an invagination of the inner alveolar membrane through the epiplasm. The lumen of the alveolocyst is confluent with the alveolar space. The alveolocyst is partitioned in two by an evagination from its bottom or side (Fig. 10) which extends up to the orifice of the alveolocyst in such a way that cross-sections of the orifice show 2 openings (Fig. 8).

Fibre systems. Description of fibres associated with single basal bodies will be given first, followed by that for paired basal bodies.

The kinetodesmal fibre (*k*) arises in the vicinity of basal body triplets 5–7 and runs sharply toward the surface where it terminates at the epiplasm, slightly anterior to and dextrally from the basal body (Figs. 7, 11–15). The kinetodesma has a major periodicity of 25 nm.

The postciliary microtubular ribbon (*p*) arises adjacent to basal body triplet 9 and is composed of 4–5 microtubules that are each 30 nm in diameter (Figs. 14, 15). Electron-opaque accessory material (*pc*) is associated with the postciliary microtubules at the proximal end of the basal body and accompanies the microtubules for a short distance distally (Fig. 15). The microtubules extend posteriorly, dextrally and toward the surface, where they become embedded in an epiplasm ridge and then run posteriorly with a slight dextral inclination for a long distance, intersecting successive epiplasm ridges (Figs. 9, 17, 26).

Three to four transverse microtubules 30 nm in diameter arise anterior to basal body triplets 3 and 4 and extend to the left where they contact the epiplasm (Figs. 11–15). The transverse microtubules are accompanied by transverse accessory material (*tc*) which arises from the electron-opaque material at the proximal end of the basal body (Fig. 19).

When paired, both basal bodies possess a kinetodesmal fibre, but only the posterior basal body possesses a postciliary microtubular ribbon. Transverse microtubules are not present on either basal body (Fig. 16).

Trichocysts. When trichocysts are present, they are located to the left and between single basal bodies (Fig. 20). The 4 electron-opaque trichocyst arms (*ta*) surround the trichocyst shaft (*ts*); arms and shaft are of approximately equal length (Fig. 18). At their distal ends, the arms and shaft are interconnected by a loose filamentous material

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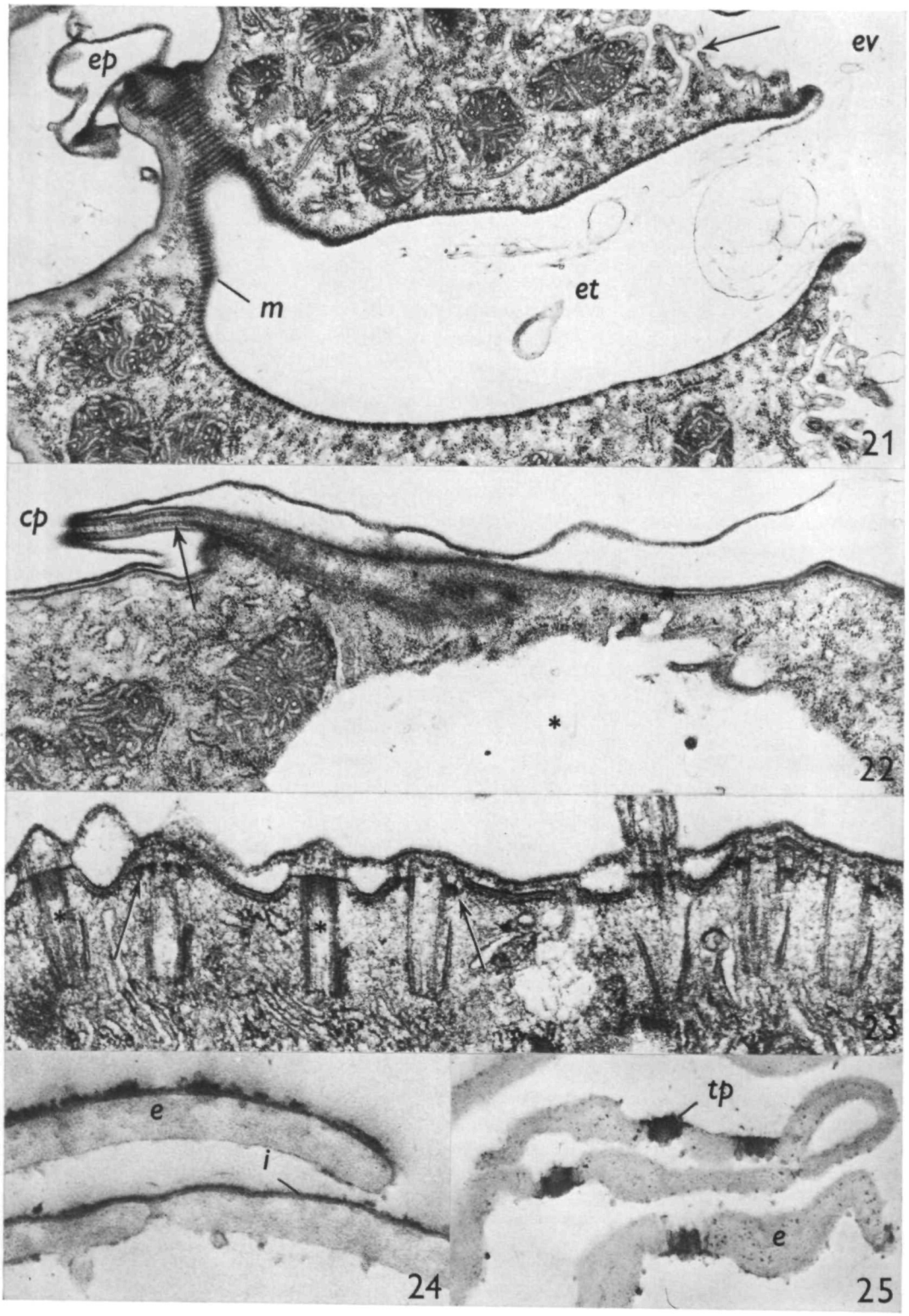
Fig. 17. Slightly oblique section of the cortex showing relative positions of the postciliary microtubules (*p*), the epiplasm layer (*e*) and the epiplasm ridges (*er*). × 69000.

Fig. 18. Longitudinally sectioned trichocyst showing the trichocyst shaft (*ts*) and arms (*ta*). × 25500.

Fig. 19. Transverse section of the cortex showing the epiplasm (*e*), the alveolar space (*a*) and the trichocyst membrane (*tm*) at the site of trichocyst insertion. Arrow indicates filamentous material interconnecting the distal ends of the trichocyst arms and shaft. The transverse microtubules (*t*) are accompanied by transverse accessory material (*tc*). × 60000.

Fig. 20. Cross-section of a trichocyst showing its 4 arms (*ta*) and the shaft (*ts*). The trichocyst occurs to the left and between adjacent basal bodies of a kinety. × 30000.


ep
ev
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cp
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tp
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(Fig. 19). The trichocyst is surrounded by a single membrane (*tm*) which touches the epiplasm where the latter is penetrated by the trichocyst. When trichocysts are not present, a depression is seen in the epiplasm where a trichocyst would normally occur. The epiplasm is not interrupted at this site, but is electron-opaque and linked to underlying filamentous material (Fig. 6).

Specialized cortical areas. The expulsion vacuole pore (*ep*) is the external opening to the expulsion vacuole tube (*et*), the expulsion vacuole (*ev*), and the nephridioplasm (Fig. 21). The nephridioplasm is a reticulum of smooth membrane channels which open into the expulsion vacuole (Fig. 21). A single layer of microtubules (*m*) 30 nm in diameter lies under the membrane of the expulsion vacuole tube. These microtubules are enmeshed in a fine, filamentous material.

The cytoproct is a narrow cortical ridge with alveoli on both sides but not at the apex (Fig. 22). Microtubules occur beneath the membrane at the apex and descend toward the permanent cytoproct vacuole (Fig. 22).

During cell division, newly formed basal bodies possess arm-like projections which fuse to the epiplasm layer (Fig. 23). Basal bodies bearing such projections do not show a clearly recognizable terminal plate, which develops following fusion of the basal body with the epiplasm. In the oral primordium region of basal body proliferation, the epiplasm is reduced to about one-third of its original thickness.

DISCUSSION

Defining the epiplasm; ultrastructural comparisons

Many structures that lie under the cortical membranes have been called epiplasm since Fauré-Fremiet (1962) first employed this term. There is considerable confusion, however, about exactly what the epiplasm is. For instance, in *Didinium nasutum*, Wessenberg & Antipa (1968) named a 1-μm-thick, finely filamentous layer underlying the alveoli as the 'outer fibrous layer'. This layer strongly resembles a diffuse filamentous layer called the epiplasm in the peritrich *Epistylis anastatica* (Fauré-Fremiet,

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Fig. 21. Longitudinal section of the expulsion vacuole tube (*et*) showing part of the expulsion vacuole (*ev*), the expulsion vacuole pore (*ep*), and the microtubules (*m*) lining the tube. Note the smooth membrane channels (arrow) leading into the expulsion vacuole. × 24000.

Fig. 22. Cross-section of the cytoproct ridge (*cp*) showing microtubules (arrow) which descend into the cytoplasm toward the cytoproct vacuole (asterisk). × 24000.

Fig. 23. Cross-section of the oral primordium showing new basal bodies inserting into the cortex. At the presumptive terminal plate level, arms (arrows) occur which fuse with the surrounding epiplasm, thus inserting the basal body into the cortex. Asterisks indicate 2 new basal bodies which are inserted in the cortex and contain terminal plates; however, cilia have not yet formed. × 34000.

Fig. 24. Section of the epiplasm (*e*) and attached inner alveolar membrane (*i*) after ultrasonication. × 60000.

Fig. 25. Section of epiplasm (*e*) after ultrasonication and detergent treatment showing presence of terminal plates (*tp*). × 40000.

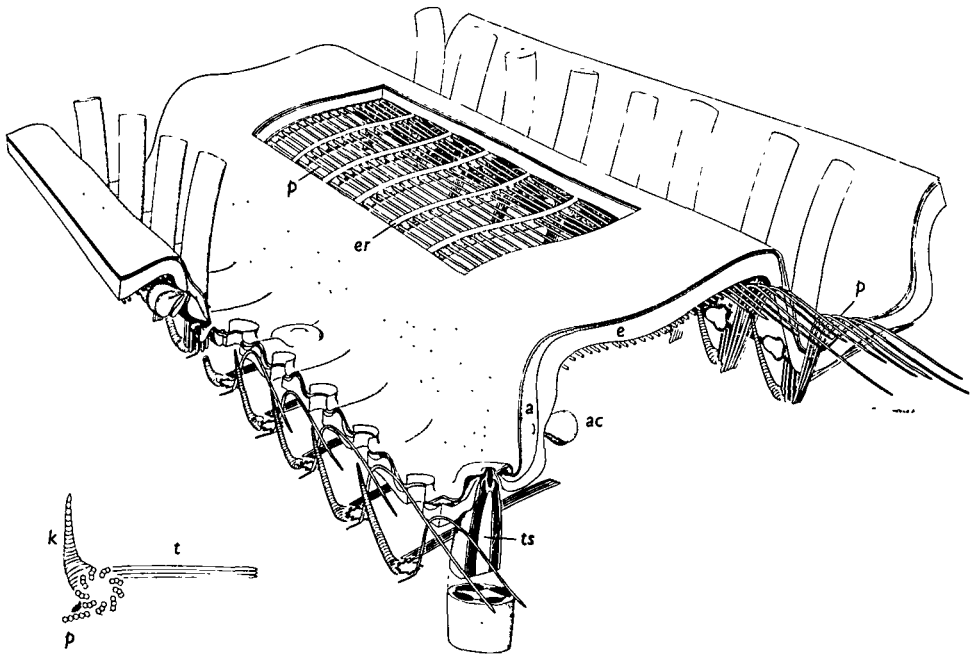

Fig. 26. Reconstruction of the somatic cortex. *a*, alveolar space; *ac*, alveolocyst; *e*, epiplasm; *er*, epiplasm ridge; *k*, kinetodesmal fibre; *p*, postciliary microtubules; *t*, transverse microtubules; *ts*, trichocyst shaft.

Favard & Carasso, 1962) and also resembles the epiplasm reported in the suctoria *Acineta tuberosa* (Bardele, 1968) and *Discophrya piriformis* (Mignot & de Puytorac, 1968). However, are all or any of these structures epiplasm?

Fauré-Fremiet (1962) originally used the term epiplasm to designate an electron-opaque area under the alveolar space of *Paranassula brunnea*. This area was only described as being of complex structure similar to that which is seen in *Tetrahymena*, *Paramecium* and *Colpidium*. Studies on *Paramecium multimicronucleatum* (Pitelka, 1965) and *Tetrahymena pyriformis* (Tokuyasu & Scherbaum, 1965) later showed that the area comparable to what Fauré-Fremiet called epiplasm was actually composed of the inner alveolar membrane plus an electron-opaque layer. Following verification of the existence of this layer by a number of investigators, Pitelka (1969) labelled it the epiplasm. Continuity of the epiplasm with the terminal plates of basal bodies has been clearly shown in numerous ciliates: *Paramecium* spp. (Allen, 1971; Ehret & McArdle, 1974; Hufnagel, 1969; Jurand & Selman, 1969; Pitelka, 1965), *T. pyriformis* (Allen, 1967; Munn, 1970), *Conchophthirus curtus* (Antipa, 1971), *Dexiotricha* spp. (Peck, 1977), *Cyclidium* sp. (Beams & Kessel, 1973), *Colpoda steinii* (Lynn, 1976), *Nassula aurea* (Tucker, 1971), *Chilodonella cucullulus* (Soltynska, 1971), *Chilodochona quennerstedti* (Grain & Batisse, 1974), *Leptopharynx costatus* (Prelle, 1965), *Trichodinopsis paradoxa* (Favard, Carasso & Fauré-Fremiet, 1963), *Tokophrya infusionum* (Millecchia & Rudzinska, 1972), *Hyalophysa chattoni* (Bradbury & Pitelka, 1965), *Coelophrya* sp. and *Dicoelophrya* sp. (Grain & de Puytorac, 1974), and *Climacostomum virens* (Peck,

Pelvat, Bolivar & de Haller, 1975). Based upon its structure in the above organisms the epiplasm may be defined as a layer of material of variable thickness that lies immediately under the inner alveolar membrane and/or cell membrane, and which is continuous with the terminal plates of cortical basal bodies. According to this definition, some structures formerly regarded as epiplasm, for example in *E. anastatica* and *D. piriformis*, are not epiplasm. Likewise, the 'outer fibrous layer' in *D. nasutum* is exactly what the name implies and cannot be construed as epiplasm.

Fauré-Fremiet & André (1967) described a layer 120–150 nm thick lying under the cortical membranes of *P. dubius* and called it the 'lamina corticalis'. I have shown that this layer is continuous with the terminal plates and lies immediately under the inner alveolar membrane in this ciliate, and thus it is epiplasm.

The fine structure of the epiplasm differs among ciliates. It is variously described as being of amorphous or microfibrillar substructure. The epiplasm of *N. aurea* (Tucker, 1971) consists of 4 laminae of different electron opacity following staining and resembles the epiplasm of *P. dubius*. In both ciliates a thin electron-translucent layer and a thin electron-opaque layer, respectively, underlie the inner alveolar membrane. The other 2 deeper, thick layers in *N. aurea* correspond to a single, thick layer which includes the epiplasm ridges in *P. dubius*; in both ciliates these layers comprise the bulk of the epiplasm. With the proper plane of section laminae are visible in *P. aurelia* (Allen, 1971) and *C. cucullulus* (Soltynska, 1971) epiplasm, since it stains darker near its 2 surfaces than in the centre.

One or more of the epiplasm laminae of *P. dubius* may actually be part of the inner alveolar membrane. Distinguishing which lamina belong to the membrane and which to the epiplasm would be feasible where these structures separate; however, the only such region in *P. dubius* is the perforated zone at the periphery of the terminal plate, which is structurally very different from the remainder of the cortex and thus comparisons are impossible.

Association with other cortical structures

The epiplasm and terminal plates of *P. dubius* are pierced by ciliary microtubules, the terminal plate perforations, parasomal sacs, alveolocysts and trichocysts. Additionally, the epiplasm is thin or absent at the cytostome, cytopyge and expulsion vacuole pore.

The peripheral ciliary microtubules penetrate the epiplasm at the terminal plate, which is the transition zone from the microtubular doublets characteristic of the cilium to the microtubular triplets of the basal body (Allen, 1969, 1971; Antipa, 1971; Dippell, 1968; Munn, 1970; Pitelka, 1965, 1974; Sattler & Staehelin, 1974). The terminal plate may be considered part of the epiplasm. This conclusion is based upon (1) the epiplasm-terminal plate structural continuity, and (2) the fact that epiplasm and terminal plates are isolated together following different isolation procedures (Hufnagel, 1969; Munn, 1970; Vaudaux, 1972; Wolfe, 1970). The epiplasm of *T. pyriformis* and *P. aurelia* is thickened into a 'circumciliary ring' around the basal body at the terminal plate level (Hufnagel, 1969; Munn, 1970; Vaudaux, 1972). From the circumciliary ring, the epiplasm extends towards the basal body as 'cross-

bridges' (Wunderlich & Speth, 1972), 'spokes of the rosette' (Munn, 1970) or 'linkers' (Sattler & Staehelin, 1974) which directly connect to the basal body microtubules. The 'linker' or 'spoke' region corresponds to the perforated zone of the terminal plate described in *P. dubius* and visible in electron micrographs of *N. aurea* (Tucker, 1971) and *C. cucullulus* (Soltynska, 1971).

A distinct, compact epiplasm does not occur at the bottom of parasomal sacs as is especially apparent in organisms with a thick epiplasm layer such as *P. dubius*, *N. aurea* (Tucker, 1971) and *C. cucullulus* (Soltynska, 1971). However, a 'fuzzy' or 'fibrous' coat lines the cytoplasmic surface of the parasomal sac membrane in numerous ciliates (Allen, 1967, 1971; Franke, 1971; Noirot-Timothée, 1968; Peck, 1971; Soltynska, 1971; Tucker, 1971), and it is continuous with the epiplasm layer in at least one case (Franke, 1971).

The 'alveolocysts' of *P. dubius* were thus named because their orifices open into the alveolar space. The alveolocyst is probably the structure Fauré-Fremiet & André (1967) called a 'pertuis' (opening) in the epiplasm layer, but its fine structure and relationship to the alveolus were not shown. The function of the alveolocyst is not known, nor have similar structures been reported for other ciliates.

The trichocysts of *P. dubius* are a non-membranous type and show complex differentiation into 4 arms surrounding a central shaft. A similar anatomy has been shown for the trichocysts of 2 other microthoracines, *Leptopharynx costatus* (Prelle & Aguesse, 1968) and *Drepanomonas dentata* (Prelle, 1968; Hausmann & Mignot, 1975), and has also been previously reported for *P. dubius* (Fauré-Fremiet & André, 1967).

As in *P. dubius*, interruption of the epiplasm layer occurs around the tips of trichocysts in *P. aurelia* (Plattner, Miller & Bachmann, 1973) and around the tips of mucocysts in *T. pyriformis* (Allen, 1967; Tokuyasu & Scherbaum, 1965). Coordinate use of thin-sectioning, freeze-fracturing and freeze-etching techniques shows that the epiplasm surrounds mucocysts and trichocysts in the latter 2 organisms at a level corresponding to an 'annulus' of fine granules located within the mucocyst or trichocyst membrane (Plattner *et al.* 1973; Satir, Schooley & Satir, 1973; Wunderlich & Speth, 1972). Since these granules protrude from the trichocyst membrane and attach to the adjacent alveolar membranes in *P. aurelia* (Plattner *et al.* 1973), they must come in contact with, or be part of, the epiplasm. In discussing mucocyst discharge in *T. pyriformis*, Wunderlich & Speth (1972, p. 266) state that: 'In this discharging process the "particle ruff" ["annulus" of granules] at the top of the mucocyst membrane may be also involved. It is conceivable that this structure hinders a complete ejection of the mucocyst membrane by becoming continuous with the pellicular membrane, and then forming a stable ring around the discharged mucocysts which may be anchored at the relatively stable epiplasmic layer. Indeed, such a stabilizing particle ring can occasionally be revealed on the fractured inner face of the pellicular membrane around the aperture of discharged mucocysts.'

All of the cortical fibres of *P. dubius* arise at precise positions around the proximal portion of the basal bodies, and extend distally to make contact with the epiplasm. The positioning of the fibre systems around the basal bodies of *P. dubius* is similar to a pattern widely recognized in the ciliates (Allen, 1967; Grain, 1969; Lom & Corliss,

1971; Pitelka, 1969). Attachment of these fibres to the epiplasm is commonly seen in the cortex. Pitelka (1969) noted the attachment of postciliary and transverse microtubules to the epiplasm in *Paramecium* spp. and *T. pyriformis*. Allen (1967) showed that the kinetodesmal fibre as well as the postciliary and transverse microtubular ribbons of *T. pyriformis* terminate within or just under the epiplasm. Franke (1971) discussed in detail the direct contact between cortical microtubules and the epiplasm of *T. pyriformis*. Antipa (1971) demonstrated that the kinetodesmal fibre, transverse and postciliary microtubules and also a non-microtubular transverse fibre all end at, or in, the epiplasm of *C. curtus*. Longitudinally running microtubules, probably extensions of the postciliary microtubular ribbons, lie flat against the epiplasm in *N. aurea* (Tucker, 1971). Cortical fibres which do not originate from basal bodies may also be attached to the epiplasm, as for example the longitudinal microtubular ribbons in *T. pyriformis* (Tokuyasu & Scherbaum, 1965; Franke, 1971) and the 'striated bands' of *P. caudatum* (Allen, 1971). Certain microtubules in specialized cortical regions are also connected to the epiplasm. In *P. caudatum*, cytoproct microtubules arise at the epiplasm and extend deep into the cytoplasm (Allen & Wolfe, 1974), and arm-bearing microtubules are attached to the epiplasm at the tentacle tips of the suctorian *T. infusionum* (Tucker, 1974).

Function

Evidence exists for both structural support and morphogenetic roles of the epiplasm.

Two types of structural support exist: *direct support*, where the epiplasm is a cytoskeleton; and *indirect support*, where the epiplasm is a cementing substance which integrates various cortical elements into a structurally coherent ensemble. The cytoskeletal role of the epiplasm of *P. dubius* is demonstrated by isolated epiplasm ghosts which retain the overall shape and surface sculpturing characteristic of the intact cell. This has previously been shown by Fauré-Fremiet & André (1967). Electron microscopy reveals that epiplasm ghosts consist uniquely of the epiplasm and terminal plates, like epiplasm fragments after detergent treatment. In *P. aurelia* and *T. pyriformis*, the terminal plates and the thickened epiplasm ring surrounding each basal body and parasomal sac opening are skeletal elements, since they retain their form following cortical fragmentation and isolation (Hufnagel, 1969; Munn, 1970). Allen (1971) proposed that the discontinuity observed in the epiplasm at the cortical ridges of *Paramecium* spp. weakens the cortex and accounts for the preferential tearing of the isolated cortex along the ridges, as observed by Pitelka (1965). The epiplasm of the adhesive disk in *T. paradoxa* is modified into an 'anneau lamellaire' which may participate with the skeletal plates to make the disk rigid (Favard *et al.* 1963).

The complex organization of the cortex requires structural integration of its diverse elements. The concept of the epiplasm as a cementing substance permitting such integration arises from its association with not only the basal bodies, cortical fibres, and mucocyst and trichocyst membranes, as discussed above, but also with the alveolar membranes surrounding the cell. The inner alveolar membrane adheres to the epiplasm strongly enough to resist ultrasonic disruption in *P. dubius*. Franke (1971)

states: 'The manifold direct...continuity between alveolar membrane and epiplasm strongly suggests a tight linkage of both components' in *T. pyriformis*. Direct contact between the epiplasm and the inner alveolar membrane or cell membrane is seen in all ciliates possessing an epiplasm. Due to the direct contact between the epiplasm and nearly all cortical structures, one can hypothesize that it contributes to their integration.

One of the intriguing problems in ciliate morphogenesis is to understand what factors influence the insertion of newly formed basal bodies into the cortex. The cortical epiplasm may provide a framework for this process: the terminal plate of a new basal body could fuse with the epiplasm, thus inserting and properly orienting the basal body into the cortex. In *P. dubius*, electron-opaque arms occur at the presumptive terminal plate level of newly formed basal bodies and these arms fuse with the epiplasm as the basal body becomes inserted in the cortex. An electron-opaque collar is visible at the periphery of the distal end of the developing basal body of *T. pyriformis* at the presumptive terminal plate level. Allen (1969) remarks that the collar 'comes in contact with the pellicle'. A nascent basal body with a similar collar has been shown as the collar fuses with the epiplasm in *P. aurelia* (fig. 4 in Dippell, 1976). New basal bodies of *N. aurea* (Tucker, 1971) possess 'epiplasmic cuffs' similar to the collars cited above. The saucer-shaped cuff forms at the terminal plate level of each new basal body and later, the rim of the cuff fuses with the cortical epiplasm layer, forming a closed 'epiplasmic chamber'. Finally, the cortical epiplasm wall of this chamber breaks down and the cuff and the basal body become level with the surrounding cortical epiplasm. Results of heat-shock experiments also indicate that the cuffs and cortical epiplasm are necessary for insertion of new basal bodies. Cortical morphogenesis is abnormal following heat shocks in *N. aurea*. Tucker (1971, p. 550) states that: 'When shocks take effect new basal bodies which already bear cilia remain in position. Those which bear cuffs fuse with the epiplasmic layer, the outer wall of the epiplasmic chamber often breaks down, but cilia do not form. Basal bodies which have not formed cuffs are unable to do so or to fuse with the epiplasmic layer. Nevertheless, ciliary fibres and various rootlet fibres still develop against them.'

The epiplasm appears to play a role in positioning of cortical structures in *P. dubius*. In cells grown for many generations under conditions which completely inhibit trichocyst production, the trichocyst position is nonetheless faithfully reproduced adjacent to all basal bodies and is visible as a depression of the inner surface of the epiplasm similar to that seen in Fig. 6. Thus, although the trichocysts are not present, their site is specified and continually reproduced in the epiplasm. Following trichocyst induction, newly formed trichocysts insert in the cortex at these sites (Peck & de Haller, unpublished results).

The epiplasm may be contractile in the tentacles of suctoria. Tucker (1974) suggested that the tentacle epiplasm of *T. infusionum* contracts during feeding and is responsible for the observed outward and downward bending of microtubules attached to the epiplasm at the tentacle tip, and also for tentacle shortening. After treatment with muscle heavy meromyosin, the tentacle epiplasm of *Dendrocometes paradoxus* shows organized arrays of filaments which may indicate the presence of contractile

proteins (Curry & Woolley, 1975; Geldard & Butler, 1975). Experiments with the effects of the anaesthetic Halothane on the contractile tentacles of *Heliophrya erhardi* indicate that the epiplasm and/or microfilaments are the contractile elements (Hauser & Van Eys, 1976). To test directly for epiplasm-mediated contractility, it would be interesting to produce epiplasm ghosts of Suctoria and attempt to induce contraction with an ATP medium similar to that used for glycerol models.

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