

## COMPLEX SURFACE INVAGINATIONS IN FROG OOCYTES

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### INTRODUCTION

The increased penetrating power of high voltage electron microscopes (2, 5) permits thick rather than thin slices of embedded biological tissues to be examined. This capability has created a means for effective examinations of three-dimensional intracellular structures and relationships within *in situ* specimens (e.g., 2, 7, 8, 12, 14). The practical problem of getting around the confusion of small details in these images, that is due to the superimposed projections of the several levels of object structure within each thick section, is less restrictive with regard to the surface features of cells that are not immediately apposed to other cells (e.g. 7). In certain cases, reconstruction of the third dimension of surface topography from serial thin sections is also an effective, though technically quite demanding, approach (e.g. 13).

The components and topographical organization of the surface of amphibian oocytes have been previously studied, especially by means of the electron microscopy of individual thin sections (reviewed in 18). During the later stages of vitellogenesis, frog oocytes over 1 mm in diameter have surface features consisting of numerous microvilli projecting into the vitelline envelope, micropinocytotic invaginations, and localized desmosomal attachments to the macrovillar extensions from their follicle cells (4, 18). Additional surface specializations, such as the cryptlike invaginations (17), the microvillar bases (3), and the localized pits that precede the formation of coated micropinocytotic vesicles (17), can develop and serve to make the surface topography even more complex. The placement of these varied surface components is highly irregular, and hence the spatial architecture of the surface of these cells has been difficult to describe analytically. Neither scanning non-transmission microscopy nor the examination of surface replicas can be used for such studies since no methods appear to be known for removing the vitelline envelope from the underlying plasmalemma without damaging and changing many of the surface features. The present report considers the presence of two specializations in the surface

architecture of frog oocytes that had not been anticipated from previous examinations of many single thin sections.

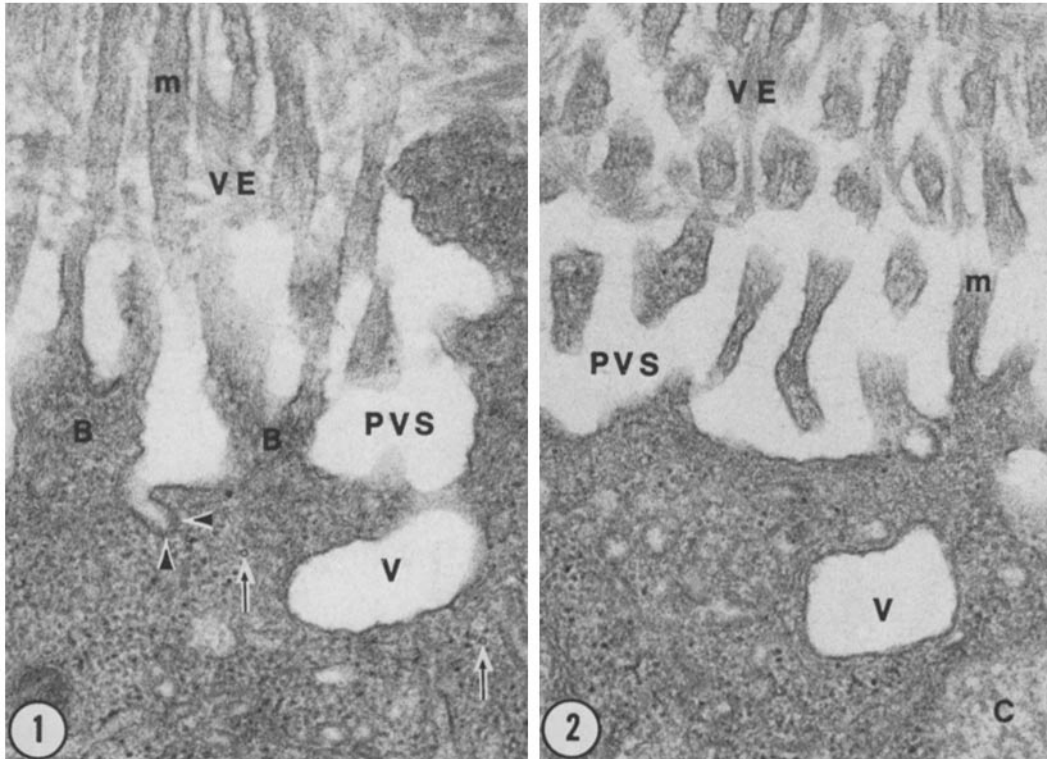
### MATERIALS AND METHODS

Ovarian oocytes from adult green tree frogs, *Hyla cinerea*, and from canyon tree frogs, *Hyla arenicolor* (Arizona Reptiles, Tempe, Ariz.), were fixed with glutaraldehyde and OsO<sub>4</sub> as has been previously reported (10). Tissue blocks were stained before dehydration by immersion in a buffered aqueous solution of uranyl acetate (6). Sections with a nominal thickness of 1–2 μm were cut with glass knives on a Porter-Blum MT-1 ultramicrotome (Ivan Sorvall, Inc., Newtown, Conn.), and were further processed as previously described (11); the mounted thick sections were restained with aqueous uranyl acetate and then with lead citrate (16). Single and serial thin sections from the same specimen blocks were cut with a diamond knife (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.) and were also restained.

All thin sections were examined at 80 kV with a JEOL JEM-100B electron microscope. Ink tracings of the path of the oocyte plasma membrane across each of the serial sections were made from enlarged prints onto transparent plastic sheets. Thick sections were examined at 1,000 kV (11) with the 1.5 megavolt instrument at Toulouse (5); a 30 μm objective aperture was used. High voltage images were recorded upon Special Lantern Contrasty glass plates (Ilford, Ltd., Ilford, Essex, England). Stereoscopic visualization, as well as the inspection of single images, has shown that the stains had penetrated through the entire thickness of the thick sections.

### RESULTS

The course of the oocyte plasma membrane (oolemma) in larger oocytes is quite contorted in thin sections (Figs. 1 and 2). At certain stages, the basal regions of the oolemmal contour are free from the vitelline envelope, whereas the outer portions of the evaginated surface are enmeshed by its substance (Figs. 1 and 2). Some thin sections oriented perpendicularly to the surface show vacuoles or large vesicles in the peripheral cytoplasm (Figs. 1 and 2); these elements can appear to be separated from the oolemma and the extracellular (perivitelline) space only by a tenuous layer of



FIGURES 1 and 2 Surface of large oocytes of *Hyla arenicolor*, as seen in individual thin sections (80 kV). The course of the oolemma is convoluted and complex. Note the apparent vacuoles (*V*) in the peripheral cytoplasm. Perivitelline space (*PVS*), vitelline envelope (*VE*), microvillus (*m*), microvillar base (*B*), micropinocytotic invagination (arrowheads), cortical granule (*C*), microtubule (arrow).  $\times 45,500$ .

ectoplasm (Fig. 1), or by a considerably thicker zone (Fig. 2). Thin sections oriented obliquely or tangentially to the curved surface reveal the very irregular placement of the varied surface structures. Microtubules are not prominent in the ectoplasm at this time (Fig. 1).

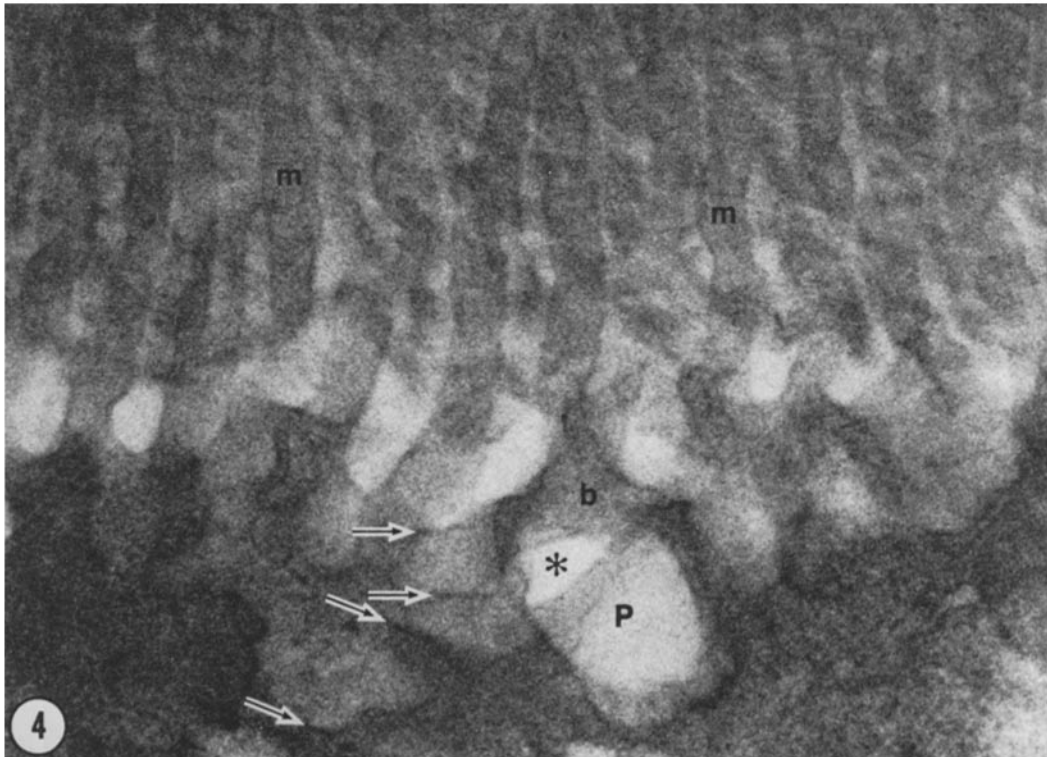
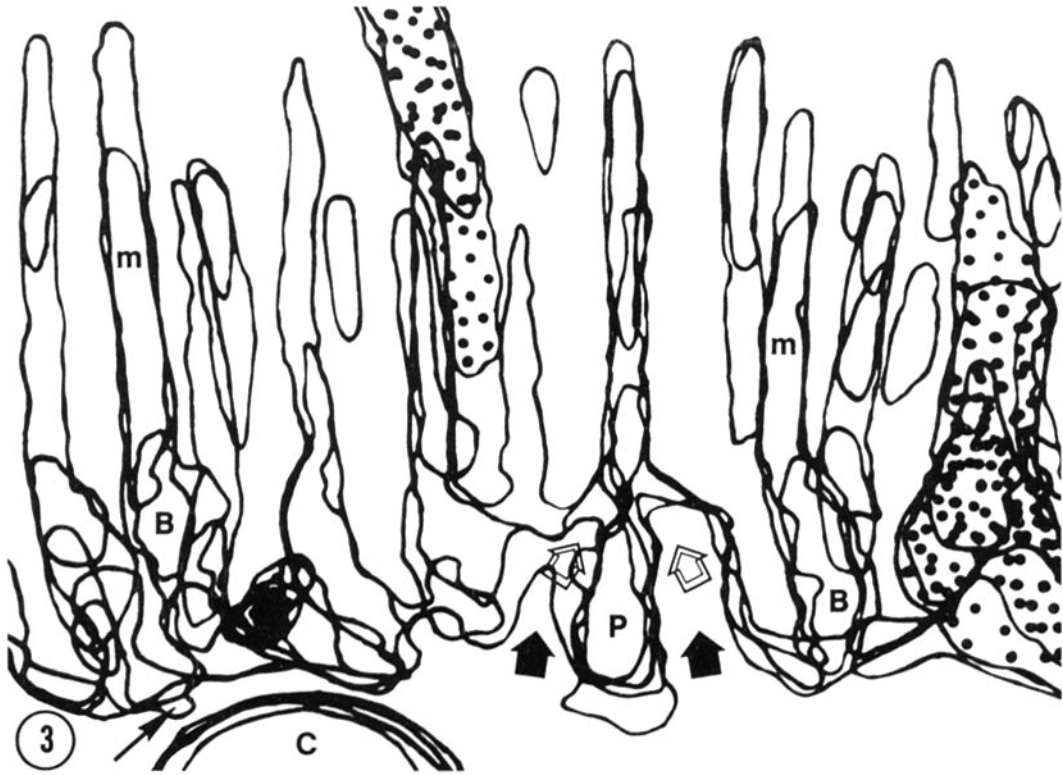
Study of the three-dimensional contour of frog

oocyte surfaces by means of serial thin sectioning is made difficult by the numerous invaginations and evaginations of the surface contour, and by the fact that many of the irregularly placed features have sizes similar to that of the thickness of the sections. For clarity, tracings of only four serial thin sections are presented in Fig. 3; the tracings

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FIGURE 3 Superimposed tracings from four serial thin sections (80 kV), representing a  $0.3 \mu\text{m}$  thickness from a large oocyte of *H. cinerea*; compare with image in Fig. 4. Macro villar projections from follicle cells have been stippled, and the substance of the vitelline envelope has not been represented within the perivitelline space. Superficial protrusions of ectoplasm (large arrows) meet and form a bridge that loops over a subsurface passageway (*P*). Some of the microvilli (*m*) sprout from microvillar bases (*B*). Cortical granule (*C*), micropinocytotic invagination (small arrow).  $\times 55,000$ .

FIGURE 4 Thick section ( $2 \mu\text{m}$ ) from a *H. cinerea* oocyte, imaged at 1,000 kV; compare with tracings in Fig. 3. Bridge (*b*) loops over a subsurface passageway (*P*) that runs obliquely through the section. The asterisk denotes an area where the electron beam has only passed through the embedding plastic; arrows designate linear densities representing oolemma seen edge on due to its undulating course. Microvillus (*m*).  $\times 66,000$ .



have been superimposed, without inserting intervening spacers to represent the thickness of the sections, in order to emphasize the several levels of surface structure that are present in this 0.3  $\mu\text{m}$  of material. Two protrusions of the peripheral ectoplasm are seen to meet and fuse (Fig. 3), thus forming a bridge about 1,400  $\text{\AA}$  in vertical thickness, that passes over a subsurface passageway that is 1,500–2,000  $\text{\AA}$  in width. The tunnel-like passageway (Fig. 3) is oriented perpendicularly to the plane of sectioning; a microvillus can be seen sprouting from the bridge. Superimposing tracings of the next three serial thin sections, representing a total thickness of around 0.5  $\mu\text{m}$ , reveals the subsurface passageway with difficulty due to the other surface contours that become superimposed upon this area. Examination of the individual micrographs of these seven sections shows that the bridge was not evident in any single section, but that the two protrusions appear to approach each other closely; the tunnel could be followed as it became continuous with the perivitelline space in the later sections.

High voltage images of thick sections from these same oocytes show the bridges and subsurface passageways with much greater clarity. The passageway in Fig. 4 is proceeding obliquely to the plane of sectioning, which is oriented approximately perpendicularly to the cell surface. The gray area around the letter *P* represents part of its wall, where this surface is curving within the 2  $\mu\text{m}$  thickness of this section. The very light areas in Fig. 4, such as at the asterisk, represent projections through a full thickness of just the plastic embedment; the darkest regions represent a full thickness of the oocyte cytoplasm. The proximal portion of a microvillus appears to extend from the top of the bridge. In this same micrograph, one can recognize certain spatial details without needing to use stereoscopic visualization. The linear densities denoted by the arrows in Fig. 4 represent regions where the oocyte surface is curving within the thickness of this section to become parallel to the electron beam, and hence the oolemma is seen edge on. This indicates the existence of localized steplike undulations in the surface contour; these topographical features are not readily perceived or appreciated by the study of individual thin sections. Another bridge and subsurface tunnel are evident at the upper left in Fig. 5; stereoscopic visualization has shown that the extracellular space is continuous around the

microvillar base, which is located in front of this bridge, and then continues into the passageway.

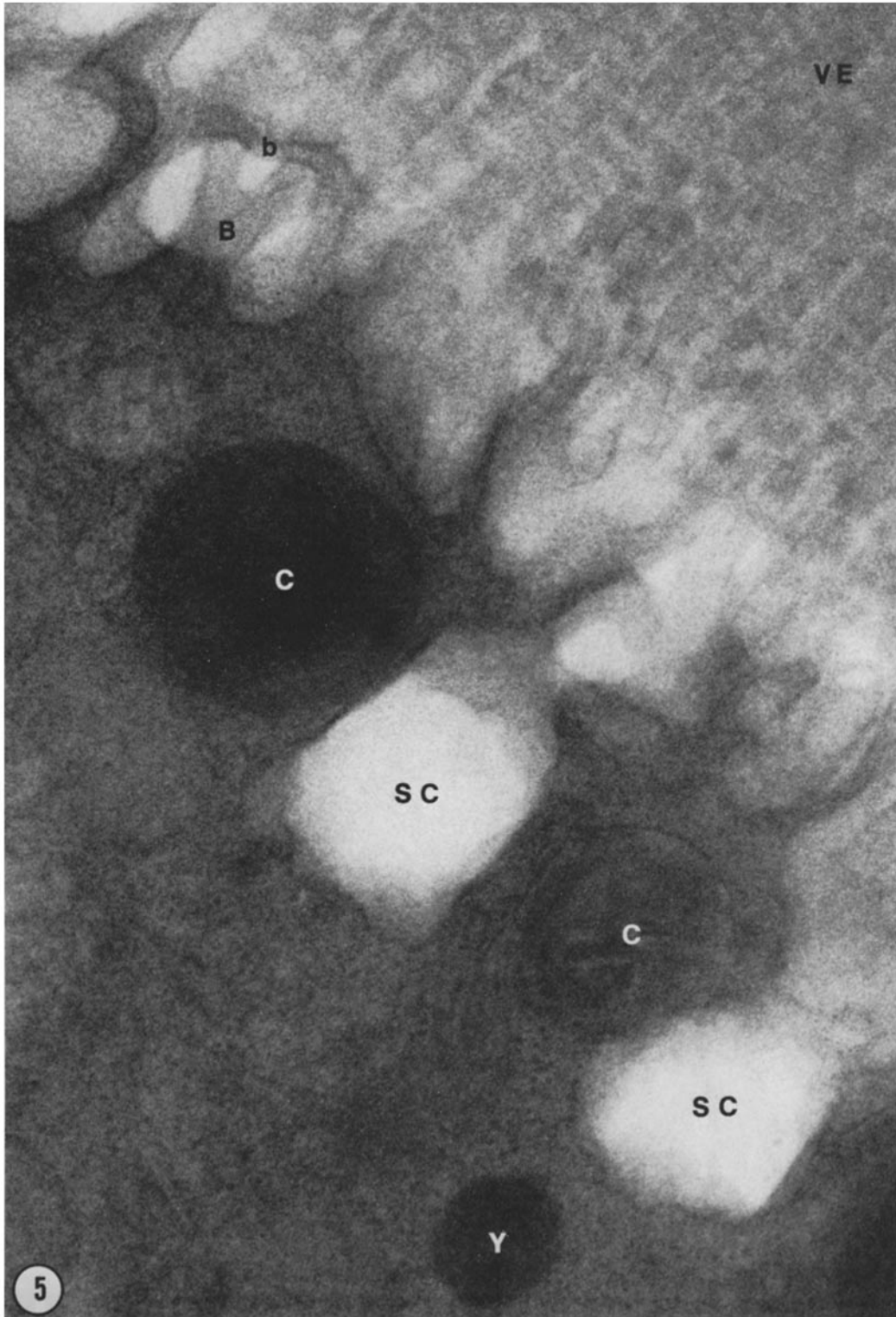
Two large invaginations into the surface contour are prominent in the 1  $\mu\text{m}$  thick section shown in Fig. 5. These subsurface caverns are situated at the same level as the peripheral layer of cortical granules; hence, they are more deeply placed than the bridged tunnels which are usually at or even superficial to the basal level of the oocyte surface. The extracellular space is continuous with the interior of the cavern near the center of Fig. 5 through a very narrow (about 1,000  $\text{\AA}$  in diameter) necklike opening; the rear wall of this opening has been included within the thickness of this section. The luminal spaces of the two cavernous invaginations depicted here appear to extend farther into the third dimension from both surfaces of this slice which is oriented approximately perpendicularly to the cell surface. The material of the vitelline envelope does not enter either of these large invaginations. Subsurface passageways and caverns also have been recognized in thick sections of large oocytes from frogs of the genus *Rana*.

#### DISCUSSION

The unexpected existence of several complex specializations in the surface contour of frog oocytes has been revealed by the study of greater thicknesses of surface than are included within single thin sections. The bridged subsurface passageways were revealed both by serial thin sectioning and by the high voltage study of thick sections.

The bridges appear to be fairly narrow in width; thus, thin sections oriented across the path of a bridge would show a roundish profile not readily distinguishable from some of the other surface projections. The subsurface passageways extending under the looping bridges probably could account for some observations of large vesicles in thin sections through the peripheral ectoplasm. The peripheral "vacuoles" that have been seen in thin sections (e.g., 4, 15, and the present report) are probably actually intersections through portions of the cavernous invaginations from the extracellular compartment; the narrow connections of some of these caverns with the perivitelline space only would be intersected very infrequently by single thin sections.

Both types of surface invaginations described in this report have lumens that are continuous with the perivitelline space. A section that was oriented



**FIGURE 5** Thick section ( $1\ \mu\text{m}$ ) from a *H. cinerea* oocyte, imaged at 1,000 kV. Bridge (*b*) and subsurface passageway are behind a microvillar base (*B*) at the upper left. Two subsurface caverns (*SC*) are invaginated from the extracellular space. Half of the wall bounding the narrow opening leading into the cavern at the center has been included within the thickness of this section. Vitelline envelope (*VE*), cortical granule (*C*), yolk sphere (*Y*).  $\times 76,000$ .

tangentially to the curved surface, and that included one of the narrow necklike openings of the cavernous invaginations, could produce an image similar to that given by the bridged passageways. However, in most instances, the bridges and tunnels are being observed in sections that are oriented almost perpendicularly to the cell surface (see Fig. 5). In addition, one must recognize that the bridged tunnels appear to be more numerous and more superficially situated than are the cavernous invaginations. Thus, it seems likely that the two surface invaginations are separate and distinct entities.

Considerable changes in the three-dimensional surface contour occur throughout the developmental phases of amphibian oogenesis (e.g., 3, 4, 18), and during ovulation and the cortical reaction that follows fertilization (e.g., 1, 9, 15). Some of the surface convolutions can be related to defined functions, as especially in the case of the prominent micropinocytotic uptake of yolk precursors that develops in vitellogenic oocytes of some species (17). The micropinocytotic invaginations presumably are in continuity with the oolemma only for fairly short terms (17), and this serves to indicate a certain physiological turnover in the plasma membrane of these cells. The formation of vacuoles by means of a ruffling activity or invaginations of the oolemma is not generally recognized as occurring in frog oocytes (however, see reference 15). Thus, the subsurface passageways and cavernous invaginations, as well as the microvilli and microvillar pedestals, probably have a greater temporal stability. A variety of physicochemical factors, including the microvillar microfilaments and the more compacted state of the peripheral ectoplasm, must be involved in maintaining the stability of this complex surface architecture (12). While the functions of the localized surface specializations found during the present study are not yet known, it is possible that these structures develop in response to a need for increased surface area to allow even greater uptake of small molecular weight metabolites, or, for a reservoir to reconstitute the surface boundary during active micropinocytosis or during the volume change of postvitellogenic growth.

#### SUMMARY

The spatial architecture of the surface of large frog oocytes was investigated by means of the high voltage electron microscopy (1,000 kV) of thick sections and by the study (80 kV) of serial thin

sections. The resultant three-dimensional perspectives revealed the unexpected presence of looping bridges of peripheral ectoplasm that pass over tunnel-like passageways, and of subsurface caverns that are continuous with the extracellular compartment through narrow necklike openings.

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