Trichoplax adhaerens F. E. Schulze (Placozoa): The Formation of Swarmers

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Trichoplax adhaerens, Swarmer Formation

Flagellated swarmers representing an alternative mode of asexual reproduction are budded off as hollow spheres at the dorsal surface. They are covered by cells of the dorsal epithelium and the interior cavity is lined by ventral epithelium. Fiber cells are in the interspace. Early primordia in the interspace between both epithelia of the mother animal are cavities filled with granular material and surrounded by non-flagellated epithelium cells. No mitoses are seen during growth of the bud whose interior becomes densely flagellated as is characteristic for the ventral epithelium. Fiber cells are passively included. The bud at first forms a bulge at the dorsal surface which is then pinched off. The flattened shape of *Trichoplax* is attained *via* cup-shaped and C-shaped intermediate stages after opening of the hollow sphere.

Introduction

Two modes of asexual reproduction are known from the primitive metazoon *Trichoplax adhaerens,* binary fission and the formation of swarmers by budding. While binary fission normally leads to approximately equal sized daughter animals, the flagellated swarmers are much smaller than the mother individuum. They have been described on the basis of light microscopy as hollow ovoids or spheres covered by cells of the dorsal epithelium whose flagella are alone responsible for locomotion. The flagella of the ventral epithelium beat within the interior cavity. Contractile fiber cells are present in the space between both epithelia [1]. However, in an electron microscope study [2], a swarmer was pictured as a solid sphere of only about 12 μm diameter which contained all cell types but no differentiation of cell layers. The dorsal surface of *Trichoplax* is said to be the site of budding. By scanning electron microscopy [3], the swarmers were seen as spheres of 20–60 μm diameter covered by large cells which must represent dorsal epithelium. The pictures include a possible stage of transformation, *viz.* an indentation representing the ventral side with which they settle at the bottom. In the present study, we report in broad outlines on the origin of swarmers and their transformation to the flattened disk shape characteristic for *Trichoplax.*

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Materials and Methods

Trichoplax adhaerens was cultured as described by Grell and Benwitz [2]. Whole *Trichoplax* forming swarmers and free-floating swarmers were pre-fixed for 45 min in a mixture containing 5% glutaraldehyde, 5% paraformaldehyde and 10% dimethylsulphoxide in 0.1 M PIPES buffer, pH 7.0, and post-fixed for 5 min in 1% OsO_4 and 0.7% $K_3Fe(CN)_6$ in 0.05 M PIPES buffer at room temperature.

The material was stained en bloc in 2% uranyl acetate and dehydrated in ethanol. All samples were embedded in epon. Thin sections were stained with lead citrate for 5 min and viewed in a Philips EM 410 electron microscope at 80 kV.

Results and Discussion

Swarmer formation can occur at multiple sites. In the light microscope, they are recognizable as bright spots surrounded by dense tissue which have no fixed location within the mother animal. An early stage of swarmer formation is shown in Fig. 1. A cavity filled with granular material and surrounded by relatively large and dense cells appears in the interior. The fiber cells are not involved in its formation. It is not clear to what extent the two epithelia contribute to the cells which line the cavity. As yet, there is no bulge indicating budding from the dorsal surface. This is seen at a later stage when the cavity has enlarged and long flagella appear for the first time in its interior (Fig. 2). During further enlargement of the bud the number of flagella increases (Fig. 3, 4). Since each cell bears a single flagellum this means a corre-

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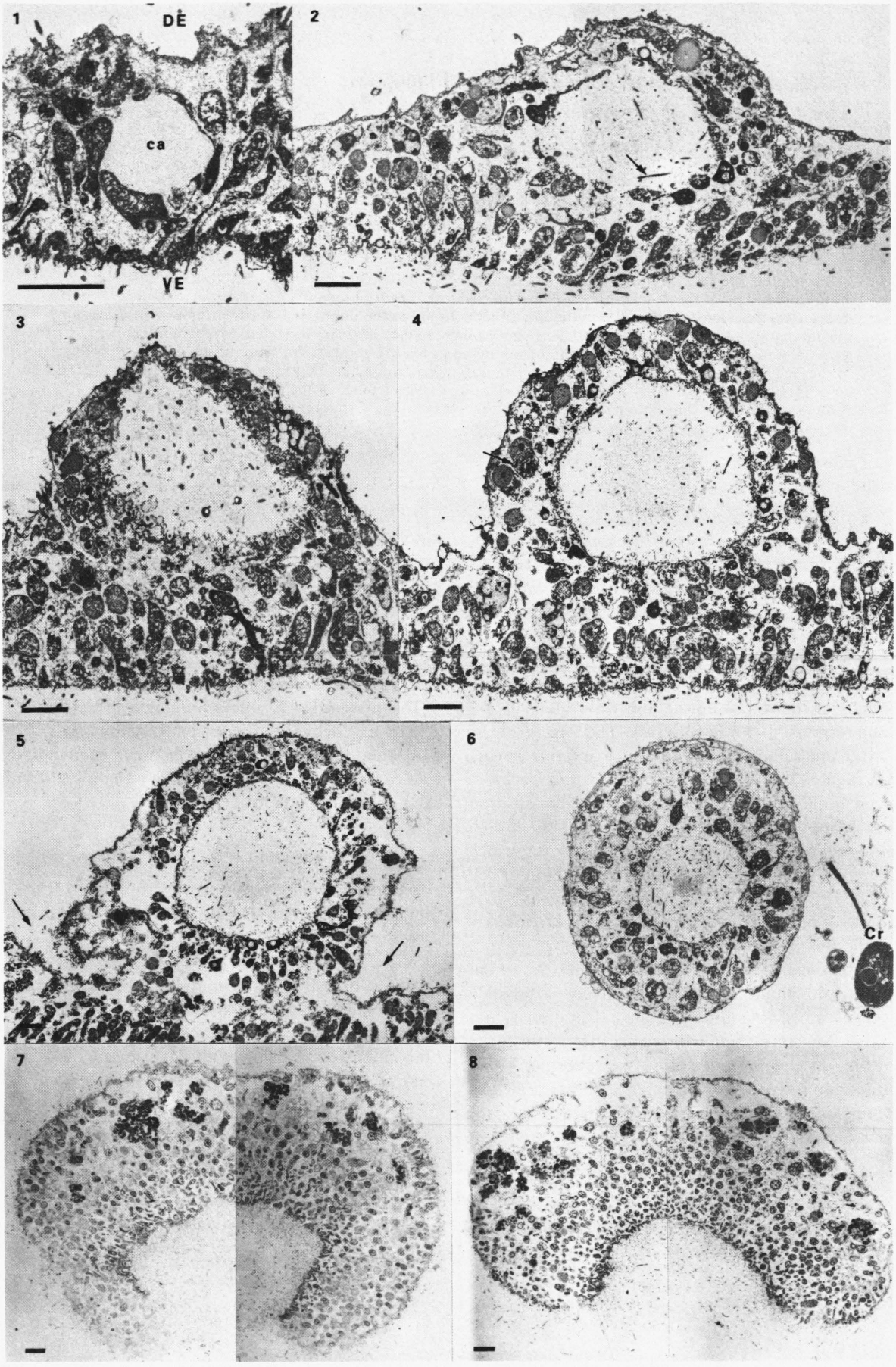
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sponding increase in the number of cells lining the cavity which also acquires a more smooth outline as development proceeds. Fig. 4 shows an advanced stage of swarmer formation. The sparsely flagellated outer covering is continuous with the dorsal epithelium while the interior is lined by ventral epithelium cells or by cells derived from it as indicated by the high flagellar density. Fiber cells, recognizable by their mitochondrial complex [2], are found between both layers. A bud which is about to be detached (Fig. 5) shows a deep circular infolding of the dorsal epithelium. The picture suggests that the swarmer is set free when the dorsal epithelium closes around it at the point of constriction but allows no conclusion regarding the mechanism involved in this process. Hollow, free-floating swarmers (Fig. 6) vary in size from 40 to 60 μm. Their formation takes about 24 h.

The transformation of the spherical swarmer to the flattened disk shape which is typical for *Trichoplax* involves an opening of the sphere at one point. An early stage is shown in Fig. 7. It is also evident that the cell number has increased and that the interspace between the two epithelia has enlarged. Further flattening leads from cup-shaped to C-shaped forms (Fig. 8) and finally to disks. Swarmers settle about one week after their formation at the bottom of the culture dish. Although the formation of swarmers involves an increase in cell number we have not observed any signs of mitosis. Either the cell divisions are synchronized and have been missed or growth is due to cell recruitment from the epithelia. Early stages such as Fig. 1 indicate that cells of both epithelia may contribute to the lining of the primordial cavity. Since reaggregation experiments with dissociated *Trichoplax* tissues [4] showed that the cell types are intermingled at first and are sorted out later with the ventral epithelium at the inside of the spherical aggregates, it may be assumed that a similar process can take place during swarmer formation. In some cases, these spherical aggregates became flattened, possibly like the swarmers, but this has not been studied.

Swarmer formation takes place sporadically and we have as yet no indication which factor or which combination of factors induces it. Especially the early stages of swarmer formation are accompanied by changes in cell structure which still have to be investigated. The epithelium cells including those which surround the initial cavity become unusually electron dense. They are at first without flagella. We are also uncertain whether the granular material is formed by the cells surrounding the cavity and which role it plays in bud formation. At the present time, available evidence indicates that the two epithelia play a major role in the morphogenesis of swarmers and that the fiber cells become passively included. The deepening constriction prior to the release of the swarmers must involve the dorsal epithelium only since fiber cells are not associated with it.

In addition to the hollow swarmers described above we have also found solid "swarmers" of an entirely different structure. They are much smaller (about 20 μm diameter). Approximately one half of their surface is covered by dorsal, the other by central epithelium cells while the fiber cells are in the interior. They may possibly be the result of a highly unequal fission. If so, they should be budded off at the margin.

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[1] K. G. Grell, Biologie in unserer Zeit **3,** 65 (1974).
[2] K. G. Grell and G. Benwitz, Cytobiologie **4,** 216 (1971).
[3] D. L. Ivanov, V. V. Malakov, and A. B. Tzetlin, Zool. Jb. **59,** 1765 (1980) (in Russian).
[4] A. Ruthmann and U. Terwelp, Differentiation **13,** 185 (1979).

Fig. 1. Early stage of swarmer formation. DE: dorsal, VE: ventral epithelium, ca: cavity. ×3000.
Fig. 2. The cavity has enlarged and long flagella appear for the first time in its interior. Arrow: flagellum. ×1600.
Fig. 3. Growing bud with still irregular contours. ×1600.
Fig. 4. Fully grown bud with smooth outlines. Arrow: Fiber cell. ×1350.
Fig. 5. A bud which is about to be detached. Note deep circular infolding (arrows) of the dorsal epithelium. ×800.
Fig. 6. Free-floating swarmer. Cr: *Cryptomonas* (food organism). ×1100.
Fig. 7. Cup-shaped swarmer after opening of the sphere. ×675.
Fig. 8. Transformation to the flattened disk shape. ×675. Scales Fig. 1–8: 5 μm.