Transepithelial Cytophagy by *Trichoplax adhaerens* F. E. Schulze (Placozoa) Feeding on Yeast

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After accidental observations of unicellular algae trapped in the intermediate layer of *Trichoplax*, dead yeast cells were offered systematically as a food. Some of them could be found several hours later engulfed by the big tetraploid fibre cells closely beneath the dorsal epithelium of the animal. This thin epithelial sheet not only allows the expulsion of accumulated waste substances by temporal separation of the cell connections but obviously gives also access to food particles collected previously on the dorsal surface by flagellar movements. Thus, the uppermost cell layer of *Trichoplax* permits the passage of corpuscles of limited size between its cells.

Introduction

Trichoplax is a primitive multicellular animal that is found world-wide in the littoral areas of tropical and subtropical seas. It has a plate-like appearance and is only 0.2–2 mm wide. There are three cell layers: A flat dorsal epithelium, equipped with flagella; a ventral epithelium of columnar cells, also bearing flagella; an intermediate layer of rather large and contractile "fibre cells" with long interconnecting appendices, leaving between them and also under the dorsal epithelium an irregular fluid-filled space.

Grell, Ruthmann and coworkers [1–5], and later on Ivanov [6] extensively described the morphology of *Trichoplax* while several physiological problems remain to be solved, though some peculiarities of locomotion, reproduction, and regeneration are well documented [4, 7–9]. But up to now little is known about feeding mechanism and digestion. The theory is favoured that *Trichoplax*, gliding over food particles lying on the ground, digests them extracellularily and absorbs the products through the ventral epithelium. Rassat and Ruthmann [10] showed scan micrographs with unicellular algae closely attached to the dorsal surface of *Trichoplax*. The authors suspected this finding as related to feeding.

The fact that small particles are collected on the animal's upper surface raised the question of the biological significance of this phenomenon. In earlier experiments unicellular algae were occasionally encountered within the *Trichoplax* body and in close contact with the fibre cells [11], and Grell and Benwitz [3] noted starch granules derived from the food organism, *Cryptomonas*, within the concrement vacuoles of fibre cells. Feeding experiments with latex beads and human erythrocytes were unsuccessful though particles were regularly collected on the dorsal epithelium (Fig. 1). So other material that could serve as a food was sought for and yeast was tried. (Grell [12] had already cultured *Trichoplax* on dead nauplia larvae of *Artemia salina* (Crustacea)).

Material and Methods

The *Trichoplax* clone used stemmed from the Red Sea and has been kept for years, feeding on *Cryptomonas* algae, in the Zoological Institute of the University of Tübingen. Samples of this *Trichoplax* strain brought to Bochum thrived here in plastic Petri dishes on the same food at 22 °C and were exposed to artificial daylight for 12 of the 24 hours. A sea water suspension of commercial bakers' yeast *(Saccharomyces cerevisiae)*, killed by heating in a water bath (10 min at 95 °C), was prepared. There were approximately 200 cells/µl, comparable with the density of the algal population in the cultures mentioned above. Within a few minutes the yeast cells settled. Then about 50 *Trichoplax* individuals, after several washings, were added. Care was taken not to stir up the yeast sediment. After 10 to 24 hours the animals were removed with a Pasteur pipette and washed three times in sterile sea water. Since yeast cells cannot easily be identified in semithin sections in the light microscope, some *Trichoplax* specimens were squashed between two slides, fixed in meth-

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anol, and Giemsa-stained. The other animals were fixed for 90 min at 4 °C in a freshly prepared mixture of 1 ml OsO_4 (4 per cent in aq. dest.), 1 ml glutaraldehyde (10 per cent in aq. dest.) and 2 ml 0.2 M piperazin-N,N′-bis (2-ethanol-sulfonic acid) = PIPES zwitterionic buffer, pH 7.5. The fixed specimens were washed 3 times in the same buffer and dehydrated by increasing ethanol concentrations and propylene oxide. Epon embedding was performed in the routine manner. Ultrathin sections were cut with glass knives on a Reichert OMU-2 ultramicrotome. After contrasting the sections with 4 per cent uranyl acetate (15 min) and Reynolds' lead citrate (5 min) they were viewed and photographed with a JEM 100 B or a Philips EM 410 electron microscope.

Results

In the Giemsa-stained squash preparations yeast cells were readily detected as large basophilic elements amidst the sheet of cohering *Trichoplax* cells, often in contact or within big cells, distinguished by their cytoplasmic and nuclear size (Fig. 2). Electron microscopy displays phagocytosed yeast cells unequivocally, characterized by their size and their thick three-layered wall, sometimes also by their protoplasmic structure. The single yeast elements always lay in big fibre cells, mostly within a phagocytotic vacuole (Figs. 3, 5, 6). Now and then ongoing digestion was suggested through beginning desintegration of the cell wall (Fig. 5).

Discussion

While the regular accumulation of many individuals of the mobile alga *Cryptomonas* on the dorsal surface of *Trichoplax* may be explained by chemotaxis, the collection of immobile particles like vertebrate erythrocytes or yeast cells must be based upon another mechanism. Indeed dissecting microscope observations of living *Trichoplax* distinctly proved that human erythrocytes are quickly conveyed to the midst of the animal's dorsum (Fig. 1) as soon as the peripheral flagella have touched them while *Trichoplax* moves on the ground. This clearly directional transport by flagella takes place disregarding the kind of corpuscles, provided that they are not too big. The matter collected and finally settled on the dorsal plane seems to be protected by a mucous cap ("ciliated mucous field" [13]) resisting removal by ambient water currents as can be demonstrated by a thin water jet directed obliquely against the animal. This cap, besides the flat form of the body, helps *Trichoplax* to maintain its microhabitat in spite of the moving water, even when turbulent.

Before potential food particles are accepted for entry into the *Trichoplax* body they must be sorted according to their quality. Any material not convenient is rejected (*i.e.* latex beads). Suitable cells probably are recognized by receptors as "non-self" but as possible food [14] and allowed to pass the dorsal epithelium. Yeast cells are not a usual food of *Trichoplax* but seem to have surface qualities that

Fig. 1. Successive accumulation (a–c) of human erythrocytes (dark areas) by flagellar movements of the dorsal surface of *Trichoplax* (a suspension of erythrocytes was chosen because they give a better photographic contrast than the colourless yeast cells). ×25. a) After 8 min of incubation; b) after 20 min; c) after 45 min.

Fig. 2. Squash preparation of *Trichoplax* after feeding on a yeast cell suspension for 10 h; Giemsa staining. Y, yeast cell in vacuole; N, nucleus of the vacuole containing fibre cell; C, concrement vacuoles regularly occurring in many *Trichoplax* cells. ×2500

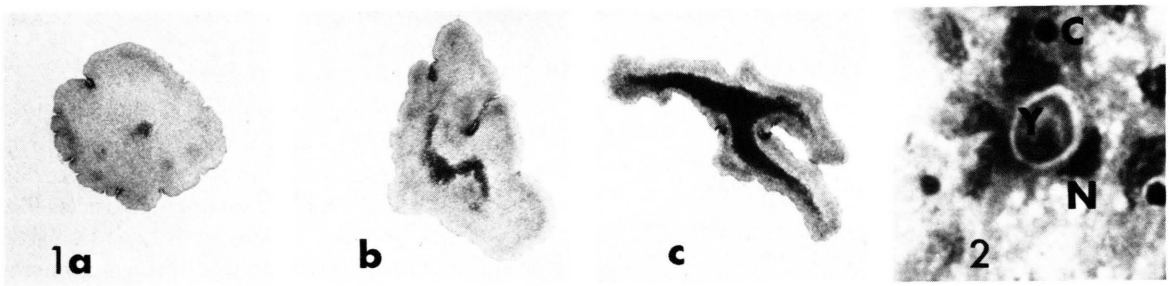
Fig. 3. Perpendicular section through *Trichoplax* after feeding on a yeast suspension for 10 h. Arrowhead: phagocytosed yeast cell in food vacuole of a large fibre cell touching the inner surface of flat dorsal epithelium (D). Arrow: Refractile body (waste material) in vacuole before being expelled through a temporal gap in the dorsal epithelium. V, ventral epithelium. ×6000. Inset: Same yeast cell; the three-layered cell wall is clearly visible. ×12000.

Fig. 4. Endocytosed alga with starch deposits, remnants of thylacoids, and disintegrating cell wall. ×8000.

Fig. 5. Endocytosed yeast cell in food vacuole. Inside the yeast cell various cytoplasmic structures, *i.e.* vacuole (V); probably beginning digestion of the cell wall. ×12500.

Fig. 6. Two yeast cells in the same food vacuole; the cell on the left side is cut near one end. ×10000.

Fig. 7. Yeast cell (right) endocytosed in large fibre cell with nucleus (upper part), mitochondrial complex (centre), Golgi apparatus, and cross-sectioned symbiotic bacteria surrounded by endoplasmic reticulum. A food vacuole around the yeast cannot be distinguished. On the right lower end of the yeast cell one sees a "budding scar" from the last fission. ×16000.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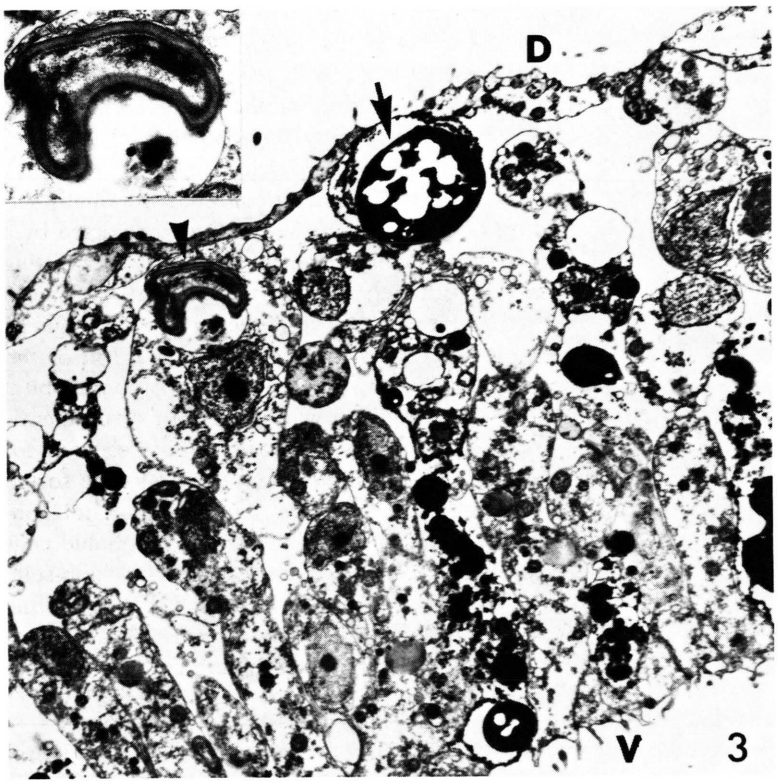
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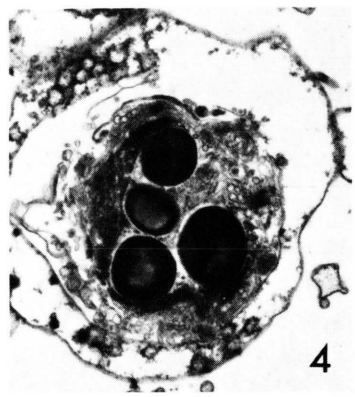
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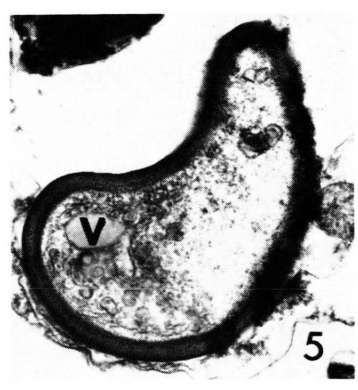
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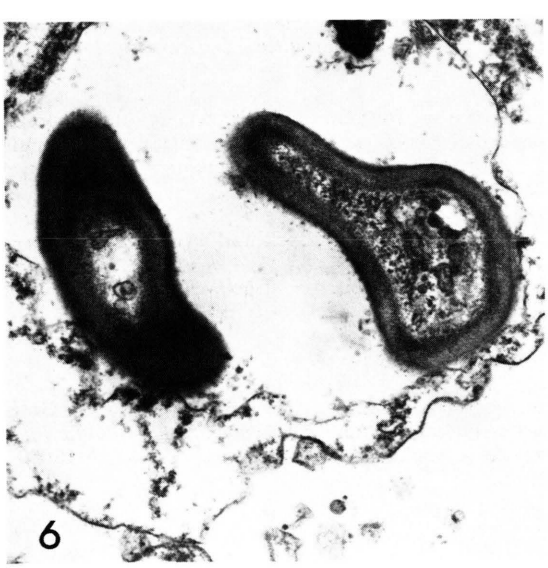
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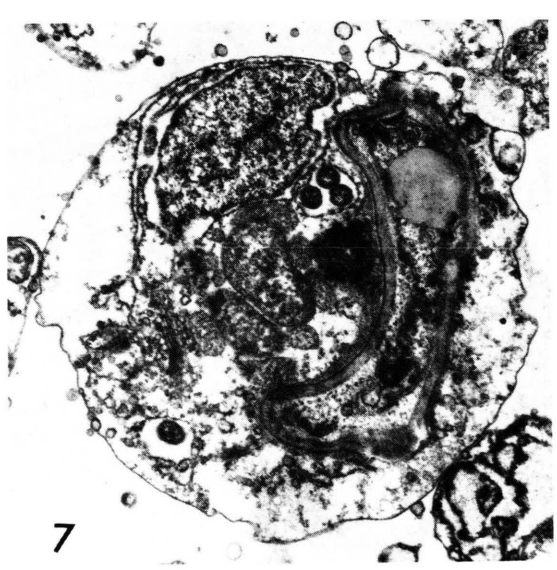
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make them attractive, though it is not clear at all how the entry of a dead organism functions. It may be discussed whether – after opening of the dorsal cell layer – a mobile fibre cell gets in immediate contact with the yeast cell and draws it into the subdorsal fluid space with phagocytosis following. However, the passage of immobile food particles through the dorsal epithelium – in spite of the intensive interdigitation of its cells and their belt desmosomes – is an uncommon functional trait, observed only in *Trichoplax* as is known so far. Entry of food particles into the mesogloea of Porifera is described but the external epithelia of these animals have preformed and never closing openings [15]. Active transepithelial migration of vertebrate granulated leukocytes [16] has nothing to do with the act of food uptake in *Trichoplax*. Since no yeast specimen was found interlocked in the epithelium or halfways engulfed by a fibre cell it must be guessed that *Trichoplax* incorporated its food rather quickly. In *Amoeba* and *Dictyostelium* phagocytosis of yeast cells is complete in a couple of minutes [17, 18]. Obviously, in *Trichoplax* the dorsal epithelium on the one hand allows the expulsion of large osmiophilic globules ("defecation") and on the other hand lets enter food particles.

The phagocytosed yeast elements are slightly deformed, probably by fluid loss in the hypertonic sea water. Most of the incorporated yeast cells lie in separated food vacuoles within the cells which seem to be specially enabled to digest food. Provided with a mitochondrial complex and symbiotic bacteria, these big tetraploid [19] cells are probably very active in metabolism. Some yeast cells were found lying directly in the cytoplasm of the fibre cells without surrounding vacuole (Fig. 7), perhaps in a later stage of phagocytosis which would agree with the observations by Steinman [20]. The internal structure of the engulfed yeast is not well preserved due to the preparation chosen. However, in several yeast cells one can recognize endoplasmic reticulum and vesicles, vacuole, and filaments, possibly representing DNA of the probably single mitochondrion [21]. Owing to the chitin component in the thick cell wall [22] yeasts withstand digestion remarkably well. Renwrantz *et al.* [23] could trace yeast in phagocytes up to 50 hours. Fig. 5 shows a yeast cell with partially fading cell wall differentiation in an advanced state of digestion.

The histological structure of *Trichoplax* suggests that nutrition is mostly through the ventral epithelium: There are cylindrical cells, built like similar ones in other phyla and known for absorptive faculties. Special cells, probably secreting enzymes, are located between the cylindrical elements [1]. Digestion of food by the ventral epithelium is obviously extracellular as is stated by many investigators [1, 8, *et al.*]. Since *Trichoplax* is a suspension feeder, usually relying on floating material sinking to the ground, one may argue whether the animal feeds in any other way than by grazing. The feeding experiments with yeast reported here show that uptake of formed nutrients from the dorsal surface is possible as well and constitutes a part of the normal feeding behaviour of *Trichoplax*. This assumption is confirmed by the accidental finding of phagocytosed algae (Fig. 4). The fact that two different kinds of feeding exist in the same species is well known (other examples of simultaneous intra- und extracellular digestion are cited by Gomme [24]).

If one follows LaBarbera's [25] classification, *Trichoplax* is a facultatively active polyphageous suspension feeder. The activity is documented by the ciliary transport of food particles to the dorsal surface, followed by cytophagy as shown in the yeast experiments described above. Better understanding of feeding mechanisms and digestion in *Trichoplax* may be provided by further investigations planned, using different food materials, enzyme assays, and autoradiography.

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