Georgia Journal of Science

Volume 75 *No. 3 Notes, Letters, Editorials, and Errata* Article 2

2017

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Acknowledgements
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RESEARCH NOTE

DO ORGANIC SOLUTES TRIGGER PARTICLE INGESTION IN THE CILIATE *Tetrahymena pyriformis*?

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The filter-feeding ciliate *Tetrahymena pyriformis* is a popular organism for the study of phagocytosis. Nevertheless, fundamental questions remain as to how ingestion is triggered. Some have found that a particle's surface properties or chemical composition influence food preference or feeding rate (Thurman et al. 2010; Dürichen et al. 2016). Those findings suggest that phagocytosis is preceded by molecular recognition. Other results are consistent with the hypothesis that ciliates feed indiscriminately (Mueller et al. 1965; Boenigk and Novarino 2004; Perez et al. 2016; Menta et al. 2017). There is also uncertainty as to whether *Tetrahymena* will feed on inert particles in the absence of dissolved organic solutes to trigger ingestion. Mueller et al. (1965) found the *T. pyriformis* consumed polystyrene beads while in a starvation medium just as readily as when the cells were in a culture solution. In contrast, Seaman (1961) and Ricketts (1972) found very little phagocytosis unless dissolved organics were available. Ricketts (1972), however, used such high concentrations of organic compounds that they might activate receptors which normally mediate the recognition of particles prior to ingestion. Since molecular recognition is an area of current study, we set out to determine if *T. pyriformis* feeds on inert particles in the absence of organic solutes.

Axenic cultures of *Tetrahymena pyriformis* were obtained from Carolina Biological Supply Company (Carolina) and maintained in Carolina's growth medium (proteose peptone, 5 g/l; tryptone, 5 g/l; K_2HPO_4, 1.1 mmol/l). The day before testing, *Tetrahymena* cells were centriguged and resuspended in Prescott's medium ($MgSO_4$, 0.011 mmol/l; KCl, 0.022 mmol/l; $CaCl_2$, 0.030 mmol/l; K_2HPO_4, 0.030 mmol/l, pH 7.0) three times to remove organic compounds and to starve the cells. The next day, 75 µl of *Tetrahymena*-containing Prescott's medium was placed into each of three microcentrifuge tubes. An additional 75 µl of Prescott's medium was added to one tube, another got the same but with 1.6% glucose, and the third received 75 µl of Carolina's *Tetrahymena* medium (Figure 1). Investigators were unaware of which tube contained which solution. Finally, 3 µm diameter, hydrophobic polystyrene beads (Magsphere) were added to each

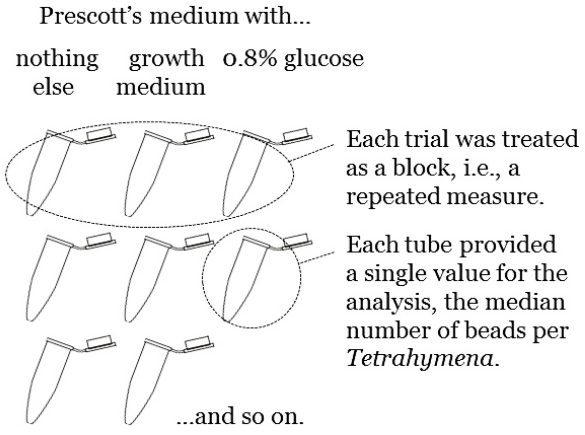

Figure 1. Experimental design. Microcentrifuge image courtesy of clker.com (permissions: clker.com/disclaimer.html)

tube to a final concentration of 9.75×10^6 beads/ml.

Ten minutes later, 75 µl of medium from each tube was combined with 75 µl of 5-fold diluted Lugol's iodine (Carolina) to kill the cells. Slides were viewed in dark field (Figure 2) and scanned systematically to ensure that there was no bias in selecting *Tetrahymena* for examination. The median number of beads in 20 *Tetrahymena* cells per microcentrifuge tube was determined. Each median was treated as a single datum, or replicate, in the analysis (Figure 1). Each set of three microcentrifuge tubes constituted one trial. Data from each trial were treated as a single block, or repeated measure, in a one-way ANOVA. There were 21 trials.

The number of beads consumed was nearly the same when *Tetrahymena* cells were placed in proteose peptone as when they were in the starvation medium, Prescott's solution (Figure 3). The number of beads was lowest when cells were in a 0.8% glucose solution. We cannot exclude chance as the cause of any of the differences in the sample means (one-way ANOVA for repeated measures, $F = 2.253_{2.40}$, $P = 0.118$).

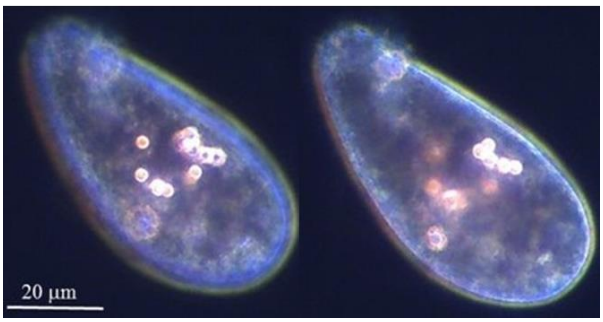

Figure 2. Two images of the same *T. pyriformis* at different focal planes. The bright circles are polystyrene beads.

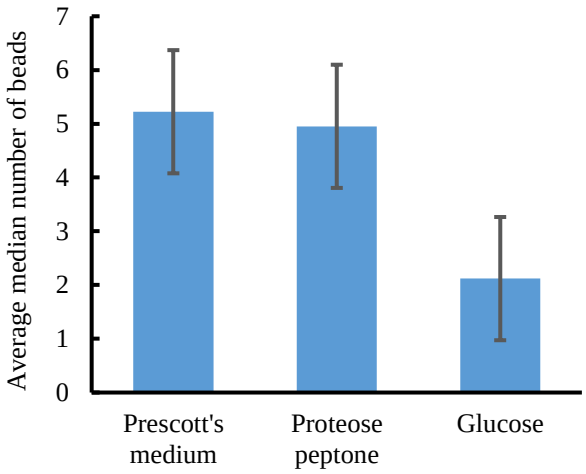

Figure 3. Average median number of beads per *Tetrahymena* per trial after a 10 min incubation. Each average is derived from 21 replicates in each group. Each replicate consists of the median number of beads in 20 *Tetrahymena* cells from one microcentrifuge tube. Bars show standard error of the mean, which was calculated according to Loftus and Masson's (1994) recommendations to account for the use of repeated measures.

Our results contrast markedly with those of others (Seaman 1961; Ricketts 1972). Out of 100 cells in starvation medium (Prescott's solution), Ricketts (1972) found light uptake of polystyrene beads in four *Tetrahymena* cells and no uptake in the rest. When 0.45% (w/v) proteose peptone was present, 88 cells showed heavy uptake, 10 medium, and two light. Our final concentration of proteose peptone was nearly the same as his, but we found no evidence that *Tetrahymena* ingests more beads in proteose peptone than in Prescott's solution. Both we and Ricketts found that *Tetrahymena* feeds in 0.8% glucose, but we found a nonsignificant trend towards less feeding in glucose than in Prescott's solution. Ricketts (1972) found hardly any feeding in Prescott's medium at all. Overall, we failed to confirm earlier findings that *Tetrahymena* will not feed on particles without the presence of dissolved organics. Instead, our results parallel those of Mueller et al. (1965). They observed that *Tetrahymena* consumed polystyrene beads while in a starvation medium just as effectively as when the cells were in a growth medium.

It is unclear why our results and those of Mueller et al. (1965) differ so much from Ricketts's (1972), whose data show large treatment effects. Ricketts, however, may have engaged in pseudreplication, *sensu* Hurlbert (1984). For example, if all the cells in a treatment group were tested together in one container, like one microcentrifuge tube, data from those cells would not be independent of each other. Even so, he provides two sets of data, one in Table I and another in the text, and similar results in Ricketts (1971).

Although Ricketts published some 45 years ago, fundamental questions remain today as to what triggers phagocytosis in ciliates. If organic solutes do trigger ingestion, the high concentrations that Ricketts used could conceivably allow the compounds to activate receptors which would normally be involved in the molecular recognition of food particles. Our results allow that such recognition might take place, but they are also consistent with indiscriminate feeding.

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