Periodicity of Endocytosis in *Tetrahymena pyriformis*

T. R. Ricketts

Cell Biology Unit, Department of Botany, The University, Nottingham, England

Received June 8, 1971

Summary

Starved cells of *Tetrahymena pyriformis* show marked periodicity in the rates of uptake of digestive vacuoles when presented with a mixture of peptone and polystyrene latex particles. Rates of egestion also show some periodicity. The results show that the supply of membrane is not the sole factor (and possibly not even a main factor) restricting vacuolar uptake. The rates of egestion also do not appear to be greatly influenced by the amounts of digestive vacuole membrane to be reincorporated into the cell membrane. It is suggested that energy and lysosomal supplies may be more important than membrane supply for uptake and that reduction in the supply of uncombined lysosomes may in some way cause cessation of vacuole formation.

Fed cells of *Tetrahymena* also show variations in the rate of digestive vacuolar uptake with time. The maximum rate of uptake is about three times as great as that found in starved cells.

1. Introduction

It has been shown (Ricketts 1971) that fed cells of *Tetrahymena pyriformis* will rapidly endocytize digestible materials, such as peptone and yeast cells, and indigestible materials, such as latex or ink particles. Starved cells, on the other hand, will only endocytize the digestible materials. The latter type of cell, which contain no digestive vacuoles, are therefore very convenient for the investigation of rates of uptake of digestive vacuoles. When they are presented with peptone and polystyrene latex particles digestive vacuoles are rapidly formed in the cell and can be easily enumerated microscopically. The latex particles are not digested in the cell and are ultimately egested from the cell as discrete spherical globules or packages of latex particles, of similar size to the intracellular digestive vacuoles, which remain intact after egestion and can be enumerated. This system is therefore ideal for the investigation of uptake and egestion rates. The present paper reports the findings of such an investigation.
2. Materials and Methods

Axenic cultures of *Tetrahymena pyriformis* (Cambridge Culture Collection No. L1630/1 GL) were grown at 20°C as described in Ricketts (1970). After 4 days growth starved cell suspensions were prepared by sedimentation of the cells, resuspension of the deposit in an equal volume of mineral-salt medium (KCl, 6 mg; CaHPO₄, 4 mg; MgSO₄·7H₂O, 2 mg; per litre of water) and resedimentation. The final deposit was resuspended in an appropriate volume of mineral-salt medium and left at 20°C until required. Starvation periods are indicated in the text. For uptake experiments 15 ml of starved cell suspension were treated with 3 ml of 5g% w/v proteose peptone (Oxoid, L46) solution, pH 7, and 0.06 ml of Dow polystyrene latex particle suspension. The mixture was shaken at 100 oscillations per min at 20°C and 1 ml samples taken at intervals for microscopic examination, which were carried out after treatment with 0.5 ml of 25% formalin. The cell concentrations and egested latex globule concentrations were determined using Fuchs-Rosenthal haemocytometers. The cell concentrations did not change over the course of the experiments. Estimates of the uptake of digestive vacuoles were made microscopically by counting the number present per cell in 50 randomly selected cells at noted times after the start of the experiment. From the results it was possible to calculate the total numbers of intracellular digestive vacuoles and egested latex globules present per ml of culture at known time intervals. Standard deviations are given for the former. Summation of these two values at given times indicated how the total cellular uptake of digestive vacuoles varied with time. By division of the differences between successive values by time (min) and cell concentration, the mean rate of uptake of digestive vacuoles over this period could be determined and hence how this rate varied with time. Rates of egestion were calculated by dividing the differences between successive extracellular latex globule concentrations by the cell concentration and time. Vacuolar diameters were measured microscopically using a micrometer eyepiece.

3. Results

The variations in the average rates of formation of digestive vacuoles and the rates of egestion of latex globules by cells of *Tetrahymena pyriformis* are shown in Table 1. These had been starved for 52 hours before treatment with 1.1 μ diameter polystyrene latex particles (1.37 × 10¹¹ particles per ml) and proteose peptone.

Tables 2 and 3 show similar results of *Tetrahymena* which had been starved for 23 hours and 46.5 hours respectively before treatment with 0.5 μ diameter polystyrene latex particles (1.46 × 10¹² particles per ml) and proteose peptone.

Table 4 shows the results for unstarved *Tetrahymena* (which had been grown in peptone-yeast extract medium) after treatment with 0.5 μ diameter latex particles. It was not possible to study the uptake process for much longer than about 30 min because by this time there were so many intracellular digestive vacuoles present that it became very difficult to enumerate them with any accuracy. Even the 30 min value should be considered to be relatively inaccurate. The egestion of latex globules could however be followed for longer periods without difficulty.