Studies on the Growth and Encystment of *Polytomella agilis*¹

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Summary

Changes in cell population density, cell protein and cell carbohydrate levels of the flagellate *Polytomella agilis* during growth in batch cultures on a complex medium at 25°C, 18°C and 9°C were examined. At 25°C, cell protein and carbohydrate levels fell markedly during exponential population growth. At 18°C, cell protein values remained fairly constant, while cell carbohydrate increased. During growth at 9°C, both cell protein and carbohydrate increased. Changes in these cellular parameters were related to marked differences in the rates of population growth at these temperatures. The cellular locus of changes observed at 25°C was examined by centrifugal fractionation of disrupted cells on density gradients.

Encystment was studied in cultures grown at 25°C. The maximum number of cysts produced was 5–10 per cent of the peak population density; the rate and degree of encystment were not increased by growth on conditioned medium or starvation. Cysts isolated from mixed populations by a centrifugal procedure remained viable, even after storage at —5°C for several weeks. The soluble proteins of separately disrupted motile and encysted forms of *P. agilis* were examined and compared by disc electrophoresis.

1. Introduction

The logarithmic phase of growth of cells cultured in flasks is characterized by a constant rate of cell proliferation. Other culture parameters, such as cell dry weight per ml, protein per ml and carbohydrate per ml, also increase exponentially during this phase (KEMPNER and MILLER 1965, SHEELER et al. 1968 a). In view of this constancy (often referred to as “balanced growth”), logarithmic phase cells are frequently selected for quantitative metabolic and physiological studies. However, a series of recent reports indicate that this constancy does not necessarily apply to events occurring in the individual cells of the culture (BUETOW and LEVEDAHL 1962, SUMMERS 1963, WILSON and LEVEDAHL 1964). In cultures of *Euglena gracilis* a marked decline in cell protein and carbohydrate occurs during the logarithmic phase of the

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growth curve (Buetow and Levedahl 1962, Wilson and Levedahl 1964). Similar changes in cell protein and starch occur in Polytomella agilis (Sheeler et al. 1968 a). In Tetrahymena pyriformis (Summers 1963) and P. agilis (Sheeler et al. 1968 a), average cell size decreases during the logarithmic phase of growth. Declines in cellular parameters have also been observed in other logarithmic phase microorganisms including yeast (Johnson 1968) and bacteria (Burleigh and Dawes 1967). Thus, there is a distinction between measurements made on the total population and on individual cells.

In cultures of Chilomonas paramecium, the rate of cell proliferation and average size and dry weight of cells in the logarithmic phase depend on the temperature of incubation (Mucibabic 1956, Johnson and James 1960). We have noted that the growth rate of P. agilis during the logarithmic phase increases with incubation temperature in the range 9-25°C. Accordingly, experiments were conducted to examine the influence of temperature on the magnitude and direction of changes in cell protein and carbohydrate levels over the growth curve. We have also attempted to identify those subcellular components in which changes occur.

Unlike species of Euglena, Tetrahymena, and Chilomonas, the life cycle of Polytomella includes a cyst stage (Aragao 1910). Consequently, this organism is also well suited for studying the biochemical and morphological aspects of cellular reorganization which occur during this form of differentiation. Although cyst stages are common to many protozoa, the conditions causing encystment are extremely variable, often contradictory, and in many cases simply unknown (van Wagendonk 1955). The only previous study of encystment in the genus Polytomella is that of Kater and Burroughs (1926), who employed starvation, temperature shifts and growth on used medium but were unable to define a single procedure for consistently inducing encystment. They concluded that the same conditions which favored the rapid growth and reproduction of motile cells (trophs) also resulted in the production of cysts. Adverse environmental conditions resulted in the death of cultures rather than encystment. Basic to investigations of the process of encystment are methods for producing the requisite numbers of cysts as well as the separation and isolation of the two forms for independent study. We have followed the kinetics of encystment in batch cultures of P. agilis and have reexamined some of the conditions used previously in an effort to induce encystment in this genus. In addition, methods for separating the encysted and trophic forms were developed and the soluble proteins of disrupted cells examined and compared by disc electrophoresis.

2. Materials and Methods

P. agilis was grown in Erlenmeyer flasks in batch cultures at 9°C, 18°C, and 25°C in a complex medium containing 0.2 per cent (w/v) tryptone, 0.1 per cent (w/v) yeast extract, and 0.2 per cent (w/v) sodium acetate. Inocula, which consisted of cells from early