Decline in the Cellular Content of RNA, Protein and Dry Weight during the Logarithmic Growth of *Euglena gracilis*

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SUMMARY

Determinations of the cellular content of RNA, total protein and dry weight of *Euglena gracilis* at different times during logarithmic growth indicated that these parameters did not reflect the constant reproductive rate of these organisms during this period of growth. During exponential growth, the RNA content was reduced by 37 %, the total protein by 45 % and the dry weight by about 76 %. In addition, the RNA and protein content of *Euglena* showed a similar pattern of decline as growth progressed; the DNA content, however, remained constant throughout the logarithmic growth phase.

INTRODUCTION

Various growth phases are observed when micro-organisms are grown in culture flasks. One of these growth phases, the logarithmic phase, is characterized by a constant reproductive rate, and it is often assumed that this constancy is reflected in other cellular properties. Consequently, this period of growth has been used when cells in the same physiological state are required for biochemical and biophysical experimentation. The question arises whether or not this assumption of constancy in cellular properties other than the reproductive rate is valid. The problem can be investigated by determining the content/cell of various constituents at different times during logarithmic growth. The constituents to be studied would logically include deoxyribosenucleic acid (DNA), ribosenucleic acid (RNA) and proteins, especially since these are involved in the synthesis of other cellular materials (Brachet, 1957, 1960; Chargraff & Davidson, 1955). The present paper reports such an analysis of the DNA, RNA, total protein content and dry weight of the flagellate protozoan *Euglena gracilis* at different times in its logarithmic phase of growth.

METHODS

Growth. Experimental and stock cultures of a streptomycin-bleached strain, SM-L1, of *Euglena gracilis* var. *bacillaris* were grown in the dark on a defined medium (Cramer & Myers, 1952) with Na acetate (0.061 M) as sole carbon source. All growth studies were carried out at 25° and pH 6.8. The generation time of *Euglena* on this medium was about 22 hr. (Buetow & Levedahl, 1960).

Dry-weight determination. Triplicate samples of 1 to 2×10^6 organisms were dried

to constant weight at 105° on previously weighed planchets. The weight of the dried organisms was determined on a Sartorius-Werke microbalance.

Total protein determination. Total protein was first determined by the biuret reagent described by Knights, MacDonald & Ploompuu (1957). Each sample contained approximately 10⁶ organisms. In later studies, total protein was determined in quadruplicate samples by the Folin phenol reagent method of Lowry, Rosebrough, Farr & Randall (1951). The Lowry method allows the use of fewer organisms (i.e. 10⁵ organisms) per sample. The two methods yielded comparable results.

Nucleic acid determination. The method of Schmidt & Thannhauser (1945) was considerably modified to determine the RNA and DNA content of the *Euglena*. The modified method was as follows: duplicate samples 2–3 × 10⁶ organisms were concentrated by centrifugation and successively extracted with 1 ml. absolute ethanol, twice with 1 ml. of a 50-50 mixture absolute ethanol-ether (v/v), and then with 1 ml. absolute ethanol. The organisms were concentrated by centrifugation between each extraction and only the resulting pellets saved. Acid-soluble materials were extracted twice in ice for 15 min. with 1 ml. cold 0.2 N-HClO₄ and the samples were centrifuged for 1 min. at 2°–4°. The resulting pellet was incubated with 0.5 ml. N-NaOH at 30° for 16 hr., neutralized with 0.5 ml. N-HCl and chilled in ice for 10 min. The samples were next extracted twice with a total of 3 ml. cold N-HClO₄ and centrifuged at 2°–4° for 5 min. The resulting supernatant fluids were read at 260 and 315 mμ in a Beckman DU spectrophotometer against a N-HClO₄ blank. The difference in readings (i.e. OD₂₆₀ – OD₃₁₅) gives the RNA fraction.

The pellet was twice incubated with a total of 1.3 ml. N-HClO₄ at 90° for 10 min., cooled in ice and centrifuged for 4 min. The resulting supernatant fluids were read in a Beckman spectrophotometer at 267 and 315 mμ. The difference in readings (i.e. OD₂₆₇ – OD₃₁₅) gives the DNA fraction (DeDeken-Grenson & DeDeken, 1959). It was found that a single extraction with HClO₄ for the DNA fraction gave variable results with *Euglena*, whereas double extractions with HClO₄ gave consistent results. The DNA values reported in the present paper agree with those reported by Neff (1960) for colourless *Euglena*. The amounts of RNA and DNA present in a sample were determined by using the optical density conversion factors derived by DeDeken-Grenson & DeDeken (1959); these values were converted to μg. RNA or μg. DNA/10⁶ organisms.

RESULTS

During logarithmic growth, both the dry weight and total protein content of the *Euglena* decreased as growth progressed (Table 1). There was an early threefold decrease, a levelling-off, and then further decline in the dry-weight measurements. The total protein content per million organisms decreased from about 500 to 285 μg. in Expt. 1 and from 536 to 295 μg. over a comparable growth range (Expt. 2). This latter measurement represents a 45 % decrease in the total protein content per average organism during the logarithmic phase of the growth cycle.

The RNA and DNA contents of the *Euglena* were also determined at various points in their logarithmic growth phase. Results of these experiments are presented in Table 2. Expt. 1 indicated a decrease in the RNA content as growth progressed. Expt. 2 was so planned that measurements were taken at *Euglena* concentrations

intermediate between those reported in Expt. 1. In Expt. 2, the RNA content/million *Euglena* declined from 34.7 to 21.9 $\mu\text{g.}$, a decrease of 37 % during logarithmic growth. The DNA content of the *Euglena* remained constant during the exponential growth phase in all experiments. However, at the beginning of the stationary phase of the growth cycle (i.e. 335,000–340,000 *Euglenas*/ml.), both the RNA and

Table 1. *Total protein and dry weight of Euglena during logarithmic growth*

Expt.	Concentration (organisms/ml. medium)	Total protein* ($\mu\text{g.}/10^6$ organisms)	Dry weight (mg./ 10^6 organisms)
1	20,200†	498 ± 34.8	3.57 ± 0.04
	36,600	—	1.19 ± 0.01
	123,000	—	1.06 ± 0.02
	158,000	348 ± 26.0	0.84 ± 0.02
	170,000	329 ± 29.9	—
	237,000	285 ± 24.1	—
2	13,000	551 ± 9.5	—
	23,400	536 ± 26.9	—
	38,100	375 ± 17.6	—
	64,500	378 ± 11.5	—
	120,000	386 ± 13.3	—
	259,000	295 ± 11.2	—
	335,000‡	302 ± 21.0	—

* Measured by the method of Knights *et al.* (1957) in Expt. 1 and by the method of Lowry *et al.* (1951) in Expt. 2. Values given are the mean $\pm$ standard deviation.

† End of the lag phase, beginning of the logarithmic growth phase.

‡ Beginning of stationary phase of growth cycle.

Table 2. *RNA and DNA content of Euglena during logarithmic growth*

Expt.	Concentration; organisms/ml. medium	RNA* ($\mu\text{g.}/10^6$ organisms)	DNA* ($\mu\text{g.}/10^6$ organisms)
1	42,800	30.2	4.34
	87,700	30.2	4.34
	175,000	27.8	4.08
	290,000	26.9	4.14
	340,000†	32.3	4.72
2	23,400	34.7	4.17
	38,100	30.2	4.63
	64,500	29.2	4.60
	120,000	27.7	4.39
	259,000	21.9	—
	335,000†	31.0	5.12

* Mean of duplicate samples; in these experiments, the average deviation between duplicate samples was 1.1 $\mu\text{g.}/10^6$ organisms for RNA and 0.4 $\mu\text{g.}/10^6$ organisms for DNA.

† Beginning of the stationary phase of the growth cycle.

the DNA content increased (Table 2). Such a finding probably results from the fact that a culture in the stationary phase of the growth cycle is essentially a non-dividing one. Under such conditions, the cell constituents would be expected to 'pile up', at least initially. Of further interest is the similar decrease of RNA and total protein of *Euglena* during exponential growth. Figure 1 presents the

results of an experiment in which both RNA and protein were measured in the same culture during its exponential growth phase. Although the protein initially decreased faster than the RNA, its overall pattern of decline was similar to that of the RNA. After an initial sharp decrease, there is a levelling-off of in the decline of RNA and protein during the middle of logarithmic growth, followed by another sharp decline late in the exponential growth period. This observation appears to be in line with the often demonstrated influence of RNA and protein syntheses on each other (Brachet, 1957).

Fig. 1. Decline in mean RNA and total protein content per average *Euglena* cell during logarithmic growth. Zero time represents the beginning of logarithmic growth. The generation time of *Euglena* is about 22 hr. In order to show the similarity in decline, both the RNA and total protein content per average cell are given in arbitrary units. (See Tables 1 and 2 for actual values.)

DISCUSSION

The constant reproductive rate of *Euglena gracilis* during logarithmic growth is not reflected in the RNA or total protein contents or dry weight of these organisms. The authors believe the present observations are the first to demonstrate a variability in such cellular parameters during the logarithmic growth of protozoa. These results are reminiscent of those obtained with bacteria and mammalian cell lines maintained in culture. The HeLa cell (Salzman, 1959) as well as other mammalian cells of carcinogenic origin (Swaffield & Foley, 1960) show systematic fluctuations in cellular protein, RNA and DNA content and protein composition (Kruse, Schooler &

White, 1960) during logarithmic growth. Morse & Carter (1949) showed a rapid decline in the RNA content of *Escherichia coli* during the early part of its logarithmic growth phase. The data of Dean & Hinshelwood (1959) on *Aerobacter aerogenes* and of Belozersky (1960) on *Azotobacter agile* indicate a continuous decline in the RNA content of these organisms during exponential growth. The cell size of *A. aerogenes* also decreased during this period of growth (Dean & Hinshelwood, 1959). The decrease in dry weight as well as in the cellular content of RNA and protein during the exponential growth of *Euglena gracilis* examined here suggests that these constituents are synthesized and accumulated during the lag phase of growth. Such a synthesis and accumulation of nucleic acid and protein during the lag phase was shown for bacteria such as *A. agile* (Belozersky, 1960), *A. aerogenes* (Dean & Hinshelwood, 1959; Neidhardt & Magasanik, 1960) and *E. coli* (Morse & Carter, 1949) and various mammalian cells (Salzman, 1959; Swaffield & Foley, 1959). These observations indicate that the lag phase is a period of intense metabolic activity related primarily to protoplasmic growth rather than cell division. The decline in RNA, protein and, especially, dry weight during the exponential growth of *Euglena gracilis* suggests that other cell parameters, i.e. lipids, carbohydrates, etc., may also change during this period of growth. Therefore, before any such parameter is used as an indication of the amount of cell population, its measurement should be completed throughout the growth cycle.

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