Nachdruck verboten.
Übersetzungsrecht vorbehalten.

Nitrogen requirements of *Euglena anabaena* var. *minor*.

By

R. P. Hall

(Biological Laboratory, University College, New York University).

With 4 figures in the text.

It has been pointed out elsewhere (Hall, 1939), in a discussion of the trophic nature of Phytomastigophora, that several types of nutrition may be distinguished in Euglenidae grown in light. Photoautotrophic nutrition may be exhibited by chlorophyll-bearing species capable of continued growth in inorganic media. In photomesotrophic nutrition amino acids are utilized instead of ammonium compounds or nitrates, and in photometatrophic nutrition peptones or more complex substances may be utilized. Certain species have been considered obligate photometatrophs, others are capable of photomesotrophic as well as photometatrophic nutrition, and a few species have been recognized as facultative photoautotrophs.

Several workers have grown *Euglena anabaena* var. *minor* for long periods in peptone media, and Dusi (1933) has obtained growth on a variety of single amino acids. These results indicate that the species may show either photomesotrophic or photometatrophic nutrition under appropriate conditions. Hutner (1936), however, has been unable to grow this flagellate in salt solutions to which gelatin, casein, mixtures of amino acids, or single amino acids were added. These findings suggest a rather restricted type of nitrogen metabolism, and are in disagreement with certain results obtained by Dusi and also with earlier observations on stock cultures in our laboratory. The ability of *E. anabaena* var. *minor* to carry on photo-

autotrophic nutrition has likewise been disputed. DUSI (1930) reported negative results, but later (DUSI, 1933) concluded that the species is photoautotrophic. More recently, HUTNER (1936) and HALL and SCHOENBORN (1938) have failed to grow the same strain in several inorganic media. HUTNER's media supported growth of *Chlorella*, while those of HALL and SCHOENBORN were adequate for growth of *Chlorogonium euchlorum*.

In view of the disputed trophic status of *E. anabaena* var. *minor*, the writer has attempted to carry this flagellate through successive transfers in media containing gelatin (photometatrophic nutrition), asparagin and glycocoll (photomesotrophic nutrition), and an inorganic nitrogen source (photoautotrophic nutrition). Demonstration of photoautotrophic nutrition was attempted with a medium which supports growth of *E. gracilis* (HALL and SCHOENBORN, 1939) but had not been tried previously for *E. anabaena* var. *minor*.

Material and methods.

The bacteria-free strain of *E. anabaena* var. *minor* (MAINX strain) was obtained from Professor E. G. PRINGSHEIM in 1930, and has since been maintained in pure culture in our laboratory. Culture media which have proven satisfactory are described elsewhere (HALL, 1937). In the experiments described below, the following media were used:

Medium D (MAST and PACE, 1933):

NH_4Cl	0.497 gm
$MgSO_4$ (anhydrous)	0.048 gm
K_2HPO_4	0.209 gm
Sodium acetate	1.148 gm
Distilled water	1.0 liter

Medium DO: The same formula as medium D, except for the omission of NH_4Cl.

Medium EF:

NH_4NO_3	1.0 gm
$MgSO_4 \cdot 7H_2O$	0.2 gm
KH_2PO_4	0.2 gm
$CaCl_2$	0.1 gm
$FeCl_3$	0.0025 gm
$MnCl_2 \cdot 4H_2O$	0.0001 gm
Distilled water	1.0 liter

Medium EFO: Same formula as medium EF, except for the omission of NH_4NO_3.

Organic nitrogen compounds, as specifically indicated, were added to one of the solutions listed above. In the attempt to demonstrate photoautotrophic nutrition, medium EF was used. Each medium was adjusted, when necessary, to allow for a drop in p_H during sterilization. Tubes and flasks were filled with measured amounts of medium and then sterilized in the autoclave. In certain experimental series one flask was inoculated from an agar slant stock culture and then incubated at room temperature for several days. A second flask was then inoculated from the first by means of a calibrated pipette, and the former was used as stock in inoculating tubes of the first transfer. In this way, the organic materials carried over from the original agar slant were diluted considerably, even before inoculation of the first transfer. In other series, a culture in peptone medium was substituted for the agar slant culture, and only one stock flask was used in inoculating the first transfer. Fourteen or more tubes of medium were prepared for each transfer in a series. After inoculation, four were fixed for determination of the initial count (number of flagellates per cubic centimeter), one was used for determination of initial p_H, and the rest were incubated at room temperature near an east window. After incubation of the first transfer, four of the tubes were fixed for the final counts, one was used for determination of final p_H, another for inoculation (0.5 cc inocula) of the second transfer, and the remainder were retained for later observations. Similarly, the third transfer was inoculated from a tube of the second, and so on in subsequent transfers. Counts of the flagellates were made with a Sedgwick-Rafter counting cell, as described previously (Hall, Johnson and Loefer, 1935). A LaMotte roulette comparator was used for p_H determinations.

Photometatrophic nutrition.

In series I, medium D contained Difco granulated gelatin in a concentration of 0.5 %. Medium D alone had previously failed to support growth of *E. anabaena* var. *minor* (Hall and Schoenborn, 1938). The initial count in the first transfer was 290, and the initial p_H was 6.8. The procedure of inoculation diluted the organic materials from the agar-slant stock culture to approximately 1.3×10^{-7} grams per cubic centimeter in the first transfer. After incubation for 12 days, final counts showed no apparent increase in number (Fig. 1). The remaining tubes were incubated for several weeks until growth of the flagellates was macroscopically evident. One of

these tubes was then used for inoculation of the second transfer, in which the initial count was 4,726 and the initial p_H 7.4. After incubation for 19 days, heavy growth was apparent (Fig. 1). In the third transfer, the initial count was 5,933 and the initial p_H 6.9. The cultures were incubated for 13 days, when growth was again evident. Initial counts in the next three transfers were: 3,053; 1,493; and 1,980; the initial p_H: 6.7, 6.9 and 7.1. Incubation periods ranged from 17 to 20 days. Growth occurred in each transfer (Fig. 1).

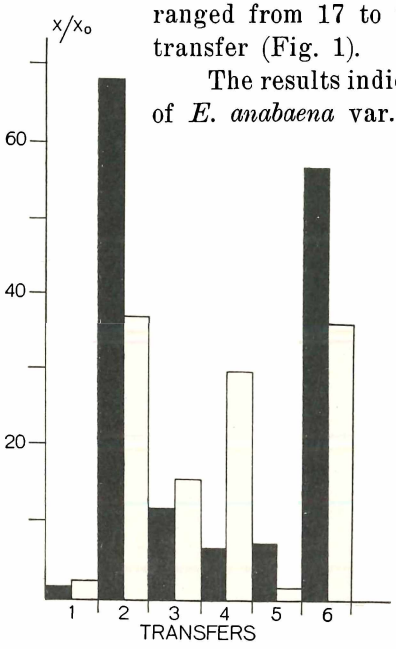

Fig. 1. Growth (x/x_0) of *E. anabaena* var. *minor* in gelatin media through six successive transfers; series I (black blocks); series II (white blocks).

The results indicate that gelatin will support growth of *E. anabaena* var. *minor* in medium D, whereas this medium alone had previously failed (HALL and SCHOENBORN, 1938) to support growth after the peptone dilution reached 10^{-7} grams per cubic centimeter. In the sixth transfer of series I, serial dilution had reduced the organic materials from the original stock culture to less than 2.5×10^{-15} grams per cubic centimeter. Hence, it is unlikely that material carried over from the agar-slant stock had any bearing on growth in the later transfers of this series.

In series II medium DO was substituted for medium D. Otherwise the procedure was the same as in series I. In the first transfer, the initial count was 260 and the initial p_H 6.9. After 12 days of incubation, there was a slight, although statistically significant, increase in number (Fig. 1). In the second to sixth transfers, initial p_H was 7.4, 7.3, 7.3, 6.8 and 7.5; initial counts: 4,726; 3,740; 3,093; 7,113; and 6,247. Fairly good growth was evident (Fig. 1) except in the fifth transfer, where little increase in number occurred. Incubation periods, as in series I, were 19, 13, 17, 18 and 20 days, respectively. These results indicate that gelatin is adequate for growth of the flagellate in medium DO.

In series III, gelatin (0.5%) was added to medium EFO. Tubes of the first transfer were inoculated from a stock peptone culture.

Initial counts in eleven successive transfers were: 707; 9,367; 6,400; 12,667; 9,400; 8,133; 8,667; 3,813; 3,207; 2,360; 4,527. Initial p_H ranged from 6.9 to 7.5 in different transfers, and incubation periods varied from 11 to 21 days. Rapid growth (Fig. 2) occurred in the first transfer, and moderate growth in the ten subsequent transfers. The peptone carried over from the stock culture was reduced from 10^{-4} grams per cubic centimeter in the first transfer to less than 1.3×10^{-17} in the eleventh transfer. The series is now in the sixteenth transfer, with no marked change in rate of growth.

Series IV was run concurrently with series III and was identical with it except that the first transfer was inoculated from a tube of series II at the sixth transfer. Initial counts in the first eleven transfers were: 15,653; 11,027; 8,933; 10,467; 4,667; 4,200; 3,133; 1,200; 1,340; 1,147; 1,900.

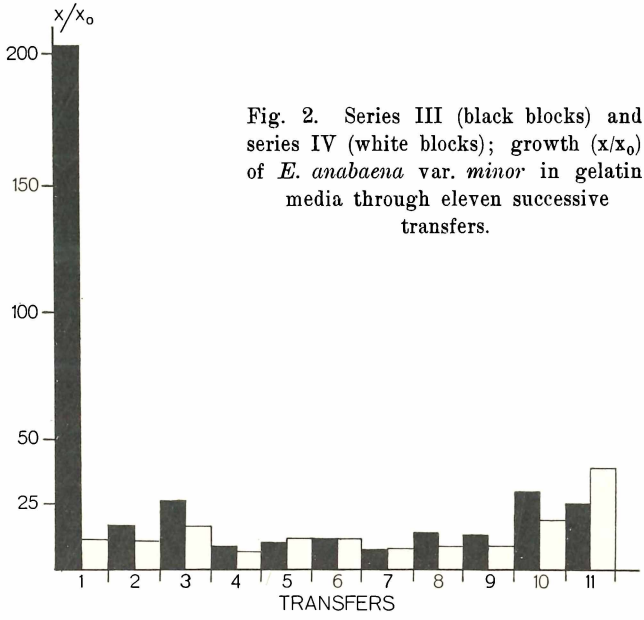

Fig. 2. Series III (black blocks) and series IV (white blocks); growth (x/x_0) of *E. anabaena* var. *minor* in gelatin media through eleven successive transfers.

Initial p_H ranged from 6.9 to 7.5 in different transfers, and incubation periods were the same as in series III. Except for the first transfer, growth (Fig. 2) was more or less comparable to that in series III. This series, like series III, is also in the sixteenth transfer, and has shown no marked change in growth rate since the eleventh transfer. Since the concentration of peptone had already been reduced to 2.5×10^{-15} in the sixth transfer of series II, it is obvious that growth of *E. anabaena* var. *minor* in series IV was dependent upon utilization of gelatin.

Photomesotrophic nutrition.

In series V Difco asparagin (0.1%) was added to medium EFO. Tubes of the first transfer were inoculated from a tube of series IV

at the third transfer. The initial p_H ranged from 6.9 to 7.3 in different transfers, and each transfer was incubated for 21 days. The initial counts in nine successive transfers were: 12,400; 4,127; 760; 440; 467; 600; 587; 807; 393. Growth (Fig. 3) was slow in the first and second transfers, but more rapid in most of the subsequent ones, and it is apparent that asparagin will support growth of *E. anabaena* var. *minor* in medium EFO.

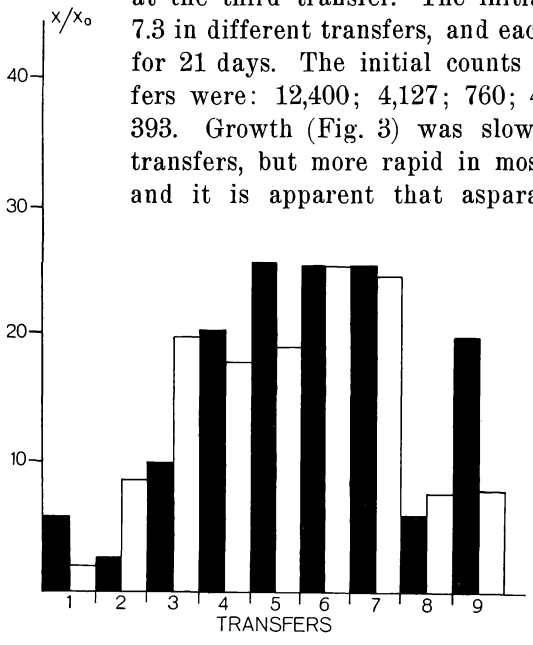

Fig. 3. Series V (black blocks), growth of *E. anabaena* var. *minor* in an asparagin medium in nine successive transfers. Series VI (white blocks), growth of *E. anabaena* var. *minor* in a glycocoll medium in nine successive transfers.

Series VI. Medium EFO contained 0.1% glycocoll (EIMER and AMEND, C. P.), but in other respects this series was similar to series V. The initial p_H ranged from 6.9 to 7.6 in different transfers, and initial counts in nine successive transfers were: 11,200; 1,220; 533; 693; 640; 533; 607; 880; 280. Each transfer was incubated for 21 days. Growth was slow in the first transfer (Fig. 3), better in the second, and more rapid in most of the later transfers.

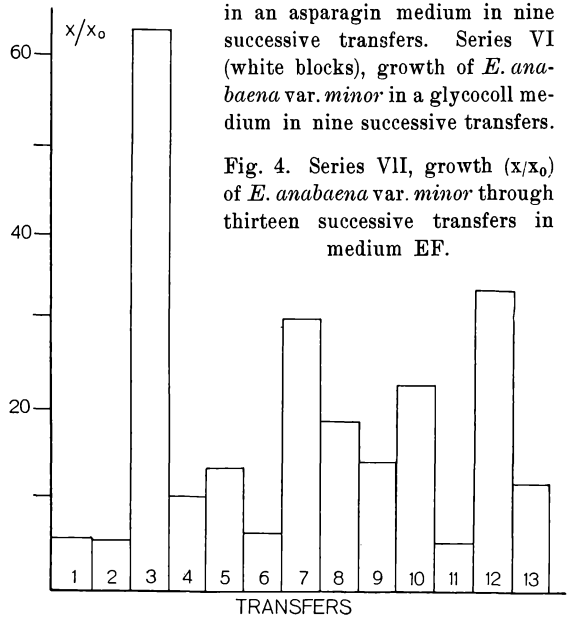

Fig. 4. Series VII, growth (x/x_0) of *E. anabaena* var. *minor* through thirteen successive transfers in medium EF.

Both series V and VI were started from a strain which had reached the ninth successive transfer in gelatin media. In the first transfer of series V and VI the gelatin concentration was reduced to 2.5×10^{-4}

grams per cubic centimeter, and in the ninth transfer to less than 2.3×10^{-13}. If growth had been affected appreciably by this decrease in gelatin concentration, a lower growth rate would have been expected in the later transfers rather than the early ones. Thus, there is no evidence that the gelatin carried over into the asparagin and glycocoll media exerted any significant effect on growth, and the results clearly indicate that *E. anabaena* var. *minor* is capable of carrying on photomesotrophic nutrition in such media.

Photoautotrophic nutrition.

Series VII in medium EF was started from a peptone stock culture, and is now in the fifteenth transfer. The initial p_H ranged from 6.7 to 7.3 in different transfers, and the initial counts in the first thirteen transfers were as follows: 6,980; 2,427; 287; 1,093 713; 673; 293; 507; 540; 480; 600; 440; 1,067. Each transfer was incubated for 21 days at room temperature near an east window. The peptone carried over from the stock culture was reduced to less than 1.2×10^{-20} grams per cubic centimeter in the thirteenth transfer. Growth was slow in the first and second transfers, rapid in the third, and slow to moderate in later transfers. The evidence thus indicates that *E. anabaena* var. *minor* is capable of photoautotrophic nutrition in medium EF.

Interesting morphological changes were noted in the first three transfers. In the first transfer many of the flagellates, and in the second transfer the majority, were etiolated and non-motile and appeared to be moribund. In the third transfer, on the other hand, practically all of the flagellates were green and apparently healthy, and this type has been predominant in later transfers. Comparable morphological changes have been observed in *Euglena gracilis* (Hall and Schoenborn, 1939). These phenomena have been interpreted as indicating a selective action of inorganic media, in which many less resistant or less adaptable flagellates die in the first few transfers. The survivors appear to be facultative photoautotrophs capable of continued growth in an inorganic medium.

Discussion.

Dusi (1933) was able to carry *Euglena anabaena* var. *minor* through four successive transfers in inorganic solutions to which one of the following nitrogen compounds had been added: glycocoll, alanine, valine, leucine, phenylalanine, proline, aspartic acid, and asparagin. Hutner (1936), on the other hand, was unable to grow this

species on single amino acids, various mixtures of amino acids, or in protein hydrolysates to which amino acids had been added. Dusi's findings suggest that, in photomesotrophic nutrition, the organic food requirements of this flagellate are easily satisfied, while Hutner concluded that the species „could not be grown in any of the simplified media effective for *E. gracilis* and barely survived in complex media, such as peptone."

Bare survival in peptone media is contrary to the experience of the writer, who has grown the Mainx strain of *E. anabaena* var. *minor* in such media since 1930 and has failed to note any necessity for delicate adjustment of the food supply. Preliminary observations (Hall and Schoenborn, 1939) indicate that the basic solution, to which Hutner added various organic materials, may not be favorable to growth of the flagellates. The results obtained by the writer show that gelatin, asparagin and glycocoll, when added to a suitable salt solution, will each support growth of *E. anabaena* var. *minor* through a series of successive transfers. Hence, the present evidence agrees entirely with the findings of Dusi (1933), and there seems to be no doubt that this species is capable of either photomesotrophic or photometatrophic nutrition, depending upon the nature of the culture medium.

The failures of Dusi (1930), Hutner (1936), and Hall and Schoenborn (1938) to grow *E. anabaena* var. *minor* in inorganic media probably resulted from the use of solutions which were not particularly suitable for this flagellate. The experience of Hall and Schoenborn (1939) with *E. gracilis* indicates the difficulty of growing Euglenidae in inorganic media, and shows that successful results are dependent upon satisfactory media. In both *E. gracilis* and *E. anabaena* var. *minor*, the technical difficulties have been aggravated by an apparent process of selection. Observations on *E. gracilis* (Hall and Schoenborn, 1939) and the present findings indicate that transfer of a strain of flagellates from peptone media to favorable inorganic solutions is followed by a process of selection and adaptation in which many of the organisms lose their chlorophyll and die, while a few more resistant or more adaptable ones survive to give rise to an autotrophic strain. Such an autotrophic strain of *E. anabaena* var. *minor* is now in the fifteenth transfer in an inorganic medium.

Literature cited.

Dusi, H. (1930): Pouvoir de synthèse de quelques Euglènes: Euglènes autotrophes et Euglènes hétérotrophes. C. r. Soc. Biol. Paris **105**, 837—839.

—, (1933): Recherches sur la nutrition de quelques Euglènes. II. Euglena stellata, klebsii, anabaena, deses et pisciformis. Ann. Inst. Pasteur **50**, 840—890.

Hall, R. P. (1937): „Growth of free-living Protozoa in pure culture", in: Culture Methods for invertebrate Animals (Ithaca, Comstock Publ. Co.), 51—59.

—, (1939): The trophic nature of the plant-like flagellates. Quart. Rev. Biol. **14**, (In press).

Hall, R. P., Johnson, D. F. and J. B. Loefer, (1935): A method for counting Protozoa in the measurement of growth under experimental conditions. Tr. Amer. Micr. Soc. **54**, 298—300.

Hall, R. P. and H. W. Schoenborn, (1938): Studies on the question of autotrophic nutrition in Chlorogonium euchlorum, Euglena anabaena and E. deses. Arch. Protistenkde **90**, 259—271.

—, (1939): The question of autotrophic nutrition in Euglena gracilis. Physiol. Zool. (In press).

Hutner, S. H., (1936): The nutritional requirements of two species of Euglena. Arch. Protistenkde **88**, 93—106.

Mast. S. O. and D. M. Pace, (1933): Synthesis of protoplasm from inorganic compounds in the colorless animal, Chilomonas paramecium. Protoplasma **20**, 326—358.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Archiv für Protistenkunde

Jahr/Year: 1938

Band/Volume: 91_1938

Autor(en)/Author(s): Hall Richard P.

Artikel/Article: Nitrogen requirements of Euglena anabaena var. minor. 465-473