Studies on the question of autotrophic nutrition in *Chlorogonium euchlorum, Euglena anabaena* and *Euglena deses.*

By

R. P. Hall and **H. W. Schoenborn**
(Biological Laboratory, University College, New York University).

Introduction.

The chlorophyll-bearing euglenoid flagellates have usually been considered plant-like, or at least facultatively autotrophic organisms. Although ZUMSTEIN (1899) and TERNETZ (1912) concluded that inorganic media, while not prohibitive, are unfavorable to growth of *Euglena gracilis*, PRINGSHEIM (1912) reported that this organism grew almost as well in suitable inorganic solutions as in media containing organic food. Likewise, MAINX (1928) concluded that the species of *Euglena* which he investigated are capable of autotrophic nutrition. JAHN (1929) also maintained a pure line of *Euglena* sp. in inorganic media for more than a year, although the cultures were not bacteria-free. DUSI (1933, 1933 a), however, was unsuccessful in carrying *E. deses* and *E. pisciformis* through successive transfers in inorganic media, although *E. gracilis*, *E. klebsii*, *E. stellata* and *E. anabaena* grew fairly well. More recently, HUTNER (1936) has failed to grow either *E. anabaena* or *E. gracilis* with an inorganic source of nitrogen.

In an attempt to assist in the solution of these problems, the writers have tried to grow *Euglena anabaena*, *E. deses*, and the phytomonad flagellate, *Chlorogonium euchlorum*, in certain media containing only an inorganic nitrogen source. *C. euchlorum* was used primarily as a control, since LOEFER (1934) had previously reported that this species is facultatively autotrophic.

There are several possible sources of error in investigations dealing with the autotrophic nature of flagellates. In the first place, the organisms must be carried through enough transfers to eliminate, through serial dilution, the peptone from the original stock culture. HUTNER (1936) has suggested that the successful results obtained by other workers may have depended upon peptone carried over into the 'inorganic' media. That such a factor may be important is indicated by the findings of HALL and LOEFER (1936), who reported that *Chilomonas paramecium* will grow in a salt solution containing peptone in a dilution of 1:100,000. In addition to elimination of peptone through serial dilution, chemical cleanliness of all glassware is absolutely essential. Furthermore, the transfers should not be incubated for periods long enough to permit the cultures to reach their peak and death of the organisms to begin. Decomposition of dead organisms would result in the addition of suitable organic materials to the medium. The writers have attempted to eliminate, as nearly as possible, such errors in the experiments described below.

Material and methods.

The bacteria-free strains of *Euglena anabaena* var. *minor* (MAINX strain), *E. deses* and *Chlorogonium euchlorum* were obtained in 1931 from Professor E. G. PRINGSHEIM of Prag, and have since been maintained in stock cultures in our laboratory. Stock media which have proven useful are described in detail elsewhere (HALL, 1937).

For our experiments the following media were used, as specified in the different series:

Medium D:

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 EA:

NH_4NO_3	0.5	gm
KH_2PO_4	0.5	gm
$MgSO_4 \cdot 7H_2O$	0.1	gm
NaCl	0.1	gm
$FeCl_3$	0.0025	gm
Distilled water	1.0	liter

Medium O:

$(NH_4)_2HPO_4$	1.0	gm
K_2HPO_4	0.2	gm
$MgSO_4 \cdot 7H_2O$	0.1	gm
$FeCl_3$	0.0025	gm
Distilled water	1.0	liter

The hydrogen ion concentration was adjusted so that the media, after sterilization, would approximate the optimal p_H (Dusi, 1933a; Hall, 1933; Loefer, 1935) for the species concerned. The medium was then tubed and sterilized in the autoclave.

The method of successive transfers in inorganic media was applied to each species, the technique being similar to that used in earlier investigations (Lwoff, 1932; Dusi, 1933, 1933a; Loefer, 1934; Hall and Loefer, 1936). In most of the experimental series, a stock flask containing the appropriate medium was seeded from a peptone stock culture of the species concerned. Tubes of the first transfer received, in different series, either 0.5 or 1.0 cc inocula from the stock flask. After incubation of the first transfer, the tubes of the second transfer were inoculated from a tube of the first, either directly or after dilution with the medium in use. The third transfer, in turn, was inoculated from a tube of the second transfer after incubation, and the same procedure was followed in subsequent transfers. In a few experiments, indicated below, the stock flask was inoculated from an agar slant culture by means of a platinum loop. Otherwise, the procedure was the same as that just described. In the first method, serial dilution reduced the peptone from the stock culture to an insignificant amount after several transfers. In the second method, the concentration of peptone was very low even in the tubes of the first transfer. In addition, utilization of organic materials by the flagellates further reduced the peptone concentration in successive transfers. Hence, the calculated peptone dilutions (grams per cubic centimeter) listed below are liberal estimates of the peptone present.

In the experiments, 14—16 tubes of medium were used for each transfer series. After inoculation, four tubes were fixed for the initial counts (number of flagellates per cubic centimeter), one was used in determining initial p_H, and the remainder were incubated. After incubation, four tubes were fixed for the final counts, one was used for determination of final p_H, another for inoculation of tubes of the next transfer, and the remainder were retained for later observations.

In order to approximate natural conditions of daylight and fluctuating temperature, all cultures were incubated at room temperature near a window. Conditions were uniform for the tubes of any given series, but quantitative comparison of one series with another is of value only in certain paired series designated below. In other cases the differences in time of incubation and prevailing weather conditions invalidate direct comparison. All p_H determinations were made with a LAMOTTE roulette comparator. Initial and final counts (number of flagellates per cubic centimeter) were made with a WHIPPLE ocular micrometer and a SEDGWICK-Rafter counting cell, as described elsewhere (HALL, JOHNSON and LOEFER, 1935).

Growth in Medium D.

Medium D is simpler than the medium used by HUTNER (1936), and yet is comparable in that it contains sodium acetate, magnesium sulphate, an ammonium salt and a buffer. MAST and PACE (1933) reported that this medium would support growth of bacteria-free *Chilomonas paramecium*. HALL and LOEFER (1936), however, failed with their strain of *C. paramecium* after the peptone from the original stock culture was diluted to 5×10^{-6}. Seven series were carried out in this medium, and the results are summarized in table 1.

Series I. *Chlorogonium euchlorum*. This series was carried through five transfers. The tubes of the first transfer were inoculated from a culture in medium EA at the ninth transfer, in which the peptone dilution had already reached 8×10^{-17}. In the five transfers in medium D, the dilution was carried to 2.5×10^{-22}. The initial p_H in different transfers ranged from 6.7 to 6.9, and no significant changes were noted after incubation periods of 5—7 days. Initial counts in successive transfers were: 14,200, 12,866, 3,266, 866, and 153. The results (table 1) show that medium D is less satisfactory than media EA and O for *C. euchlorum*. In a companion series, a duplicate of series I except for differences in initial counts, growth was observed in six successive transfers, after which the series was discontinued. As in series I, growth of *C. euchlorum* was comparatively slow.

Series II. *Euglena anabaena*. Initial p_H was 6.5, 6.7 and 6.9, and the initial counts were 3100, 176, and 80, respectively, in three successive transfers. Calculated peptone dilutions were 5×10^{-5}, 2.5×10^{-6}, and 1.2×10^{-7}. Incubation periods were 14, 21 and 21 days, and no significant p_H changes were noted after incubation. The

results (table 1) showed no growth in the first transfer, a very slight increase in number in the second, and a decrease in the third.

Series III. *Euglena anabaena.* Initial p_H was 6.8 in the first and second transfers, and 6.9 in the third. No significant p_H changes occurred after incubation for 13, 21 and 21 days, respectively. Initial counts were 750, 70 and 65. Calculated peptone dilutions were the same as in series II. The apparent increases in number (table 1) in the first and second transfers are of doubtful significance, and in the third transfer a decrease in number was apparent. This series was run concurrently with series II.

Series IV. *Euglena anabaena.* In this case, the stock flask for the first transfer was inoculated from an agar slant culture. Calculated peptone dilutions were 2.5×10^{-6} in the first transfer, 1.2×10^{-7} in the second, and 6×10^{-9} in the third. Initial p_H was 7.0 in the first, and 6.8 in the second and third transfers. Initial counts were 2,980, 590, and 52, and the incubation periods were 16—17 days. The results (table 1) show slow growth in the first transfer, very little in the second, and a decrease in number in the third. The evidence from series II—IV thus indicates that medium D is inadequate for continued growth of *E. anabaena.*

Table 1.

Series I—VII. Growth of *C. euchlorum, E. anabaena* and *E. deses* in medium D. Growth in the successive transfers of each series is expressed as x/x_0 (ratio of final to initial concentration of flagellates per cubic centimeter).

Series	x/x_0 in successive transfers				
	First	Second	Third	Fourth	Fifth
I	13.1	6.4	1.3	3.4	1.5
II	0.9	1.6	0.25	—	—
III	1.1	1.4	0.3	—	—
IV	2.1	1.6	0.2	—	—
V	0.3	—	—	—	—
VI	1.1	0.7	—	—	—
VII	1.0	0.6	—	—	—

Series V. *Euglena deses.* The stock flask for the first transfer was inoculated from an agar slant culture, and the calculated peptone dilution in the culture tubes was 2.5×10^{-6}. The initial p_H was 7.0 and the initial count 640. After 17 days of incubation a decrease in number (table 1) was apparent.

Series VI. *Euglena deses.* Initial p_H was 6.5 in the first transfer and 6.8 in the second. Initial counts were 386 and 87, and the

incubation periods were 14 and 21 days. Calculated peptone dilutions were the same as in series II. No growth occurred in either transfer (table 1).

Series VII. *Euglena deses.* Initial p_H in the first and second transfers was 6.8, and the initial counts were 360 and 93. The first transfer was incubated for 13 days and the second for 21 days. Calculated peptone dilutions were practically the same as in series VI. No growth occurred in either transfer. This series was run concurrently with series VI. From the results obtained in series V—VII (table 1), it is apparent that medium D is inadequate for continued growth of *E. deses.*

Growth in medium EA.

Medium EA was used by Loefer (1934) for growth of *Chlorogonium euchlorum* during a period of ten months, in which transfers were made at monthly intervals. Six series were carried out in this medium, and the results are recorded as x/x_0 in table 2.

Series VIII. *Chlorogonium euchlorum.* This series was carried through 15 transfers and then discontinued. The initial p_H of different transfers ranged from 6.7 to 6.9, with no significant changes after incubation. The first transfer was incubated for 9 days, and the others for 5 or 6 days. Initial counts for the first nine transfers were as follows: 1,215, 9,825, 12,240, 14,540, 12,467, 680, 2,546, 5,366, 6,800. Growth in the first five transfers is recorded in table 2; in the sixth to ninth transfers, x/x_0 values were: 13.9, 46.0, 32.9, and 12.5, respectively. Counting was discontinued after the ninth transfer, but growth was still vigorous in the fifteenth. In the ninth transfer the calculated peptone dilution had been carried to 8×10^{-17}, and in the fifteenth transfer to 1.2×10^{-23}. It seems obvious, therefore, that medium EA is adequate for growth of *C. euchlorum*, as previously reported by Loefer (1934).

Series IX. *Chlorogonium euchlorum.* This series was discontinued after the twelfth transfer. In this case, medium EA was modified by the addition of manganese chloride ($MnCl_2 \cdot 4H_2O$) in a concentration of 5×10^{-7} molar. Initial p_H ranged from 6.7 to 7.1 in different transfers, and incubation periods from 5 to 9 days. Initial counts for the first six transfers were: 687, 1,667, 15,546, 3,200, 2,333, 4,200. Calculated peptone dilutions were similar to those in series VIII, reaching 10^{-20} in the twelfth transfer. Counting was discontinued

after the sixth transfer (x/x_0, 14.8), but growth was macroscopically evident in every case thereafter. Hence, this modified medium EA also seems adequate for growth of *C. euchlorum*.

Series X. *Euglena anabaena*. Initial p_H of different transfers ranged from 6.7 to 6.9. Incubation periods were 9, 12, 9, and 22 days; initial counts: 5,440, 790, 135, 240. Calculated peptone dilutions were: 5.2×10^{-5}, 3.3×10^{-6}, 1.6×10^{-7}, and 8×10^{-9}, respectively. The results (table 2) show that, under the conditions described, medium EA is inadequate for growth of *E. anabaena*.

Table 2.

Series VIII—XIII. Growth of *C. euchlorum*, *E. anabaena* and *E. deses* in medium EA. Growth in the successive transfers of each series is expressed as x/x_0 (ratio of final to initial concentration of flagellates per cubic centimeter).

Series	x/x_0 in successive transfers				
	First	Second	Third	Fourth	Fifth
VIII	21.8	7.3	9.6	10.5	6.9
IX	3.0	90.0	6.4	17.7	19.4
X	1.4	1.8	1.0	0.2	—
XI	1.1	0.6	0.8	—	—
XII	0.3	0.2	—	—	—
XIII	1.0	0.3	0.9	—	—

Series XI. *Euglena anabaena*. In this series medium EA was modified as for series IX. Calculated peptone dilutions were: 5×10^{-5}, 2.5×10^{-6}, and 1.2×10^{-7} for three transfers. Initial p_H was 7.1, 6.9 and 7.0, and the initial counts were: 6,413, 730, and 70. This medium also was unsatisfactory for growth of *E. anabaena* (table 2).

Series XII. *Euglena deses*. Initial p_H was 7.1 in the first and 6.8 in the second transfer, and the initial counts were 400 and 130. Calculated peptone dilutions were 5×10^{-5} and 2.5×10^{-6}. The first transfer was incubated for 10 days and the second for 22. No growth occurred in either case (table 2).

Series XIII. *Euglena deses*. In this case, manganese chloride was added to medium EA, as in series IX and XI. In the three transfers, initial p_H was 7.1, 6.9 and 7.0; initial counts: 340, 320, and 53; incubation periods: 9, 16, and 21 days. Calculated peptone dilutions were approximately the same as in series X. As in series XII, no growth occurred in any transfer (table 2).

Growth in medium O.

This medium is the one which Dusi (1933 a) reported as supporting growth of *Euglena anabaena* through a number of successive transfers. It was used in series XIV—XX, the results of which are summarized in table 3.

Series XIV. *Chlorogonium euchlorum.* This series was carried through five transfers and then discontinued. Initial p_H was 6.9—7.0 in different transfers, and initial counts were: 198, 798, 2,403, 14,070, 3,175. Incubation periods were 11, 12, 8, 5 and 5 days, respectively. No p_H change greater than 0.1 was noted. The calculated peptone dilution in the first transfer was 1.8×10^{-4}; in the fifth, 8×10^{-10}. The medium appears to be quite satisfactory for growth of *C. euchlorum* (table 3).

Series XV. *Chlorogonium euchlorum.* In this series medium O was made up without ammonium phosphate. Initial p_H was 7.0, 6.9, and 7.0 in successive transfers, and initial counts were 170, 540, and 180. Incubation periods were 11, 12 and 8 days. Calculated peptone dilutions were the same as in series XIV. This series, without ammonium phosphate, may be compared directly with series XIV, since the initial counts for the first and second transfers are comparable and the time and other conditions of incubation were practically identical. It is apparent that peptone carried over from the original stock culture supported growth in the second transfer (table 3) at a dilution of 1.6×10^{-6}. In the third transfer (peptone dilution, 1.6×10^{-7}) the flagellates decreased in number and the majority were etiolated or otherwise abnormal in appearance.

Series XVI. *Chlorogonium euchlorum.* This series was carried through six transfers and then discontinued. The first transfer was started from a culture in medium EA at the tenth transfer, in which the calculated peptone dilution had already been carried to 4.1×10^{-18}. In the six transfers in medium O, this dilution was increased to 6×10^{-25}. Initial p_H was 6.9—7.1 in different transfers, and incubation periods ranged from 5 to 7 days. No counts were made, but growth was macroscopically evident in every transfer.

Series XVII. *Euglena anabaena.* In this series initial p_H was 7.0 in the first and second transfers, and 6.9 in the third; initial counts were 240, 555, and 53, respectively. The first two transfers were incubated 14 days, and the third 21 days. Good growth (table 3) was observed in the first transfer, very little in the second, and none in the third. Calculated peptone dilutions were: 1.4×10^{-4},

Table 3.

Series XIV—XX. Growth of *C. euchlorum*, *E. anabaena* and *E. deses* in medium O. Growth in the successive transfers of each series is expressed as x/x_0 (ratio of final to initial concentration of flagellates per cubic centimeter).

Series	x/x_0 in successive transfers				
	First	Second	Third	Fourth	Fifth
XIV	169.3	20.5	24.3	4.2	13.5
XV	89.9	3.7	0.6	—	—
XVI	No counts; growth macroscopically evident				
XVII	20.4	1.4	1.0	—	—
XVIII	46.2	1.5	0.9	—	—
XIX	0.7	0.8	—	—	—
XX	0.7	0.9	—	—	—

7×10^{-6}, and 7×10^{-7}. In spite of Dusi's earlier findings, this medium appeared to be inadequate for growth of *Euglena anabaena.*

Series XVIII. *Euglena anabaena.* This series was started in medium O without ammonium phosphate. Initial p_H was 7.0, 6.9 and 7.0 in successive transfers, and initial counts were 383, 958, and 123. Incubation times and calculated peptone dilutions were the same as in series XVII. It is interesting to compare this series with the preceding one. In the first transfer (table 3) growth was greater in series XVIII without ammonium phosphate, and in the second and third transfers the results were almost identical. It seems obvious, therefore, that growth of *E. anabaena* in both series XVII and XVIII was dependent upon the peptone carried over from the stock culture. The difference in growth in the first transfers was unexpected and, since it indicated a partial inhibition of growth by ammonium phosphate, further tests were begun. While these are still in progress (Hall and Schoenborn, MSS), some of the results show a definitely inhibitory effect of ammonium phosphate in one of our peptone stock media. Our completed findings will be described elsewhere.

Series XIX. *Euglena anabaena.* In the first transfer, the initial count was 6,933; initial p_H, 7.1; calculated peptone dilution, 4.4×10^{-5}. In the second, the count was 313; initial p_H, 7.0; calculated peptone dilution, 2.2×10^{-6}. The first transfer was incubated for 17 days, and the second for 19 days. No growth occurred in either transfer (table 3). The difference in growth in the first transfers of series XVII and XIX is probably to be correlated with the difference in peptone concentrations.

Series XX. *Euglena deses.* The initial p_H, calculated peptone dilutions, and periods of incubation were the same as in series XIX. The initial count was 1,246 in the first transfer and 67 in the second. No growth was observed in either transfer (table 3).

Viability tests.

As a check on the survival of *Euglena anabaena* and *E. deses* in organic media, tubes of stock peptone medium were inoculated in 1.0 cc amounts from tubes of various transfers in inorganic media. The peptone cultures were then incubated at room temperature and examined at intervals. No attempt was made to determine maximal length of survival, but it was found that *E. anabaena* was still viable after 51 days in first transfer tubes of medium EA, after 36 days in the second, and after 19 days in the third transfer. Comparable results were obtained for *Euglena deses.* It is obvious, therefore, that both species may survive for several weeks in an inorganic medium, although they cease to divide when the concentration of peptone from the original stock culture reaches approximately 1.6×10^{-7} for *E. anabaena* and 5×10^{-5} for *E. deses.* It is interesting to note that the cryptomonad flagellate, *Chilomonas paramecium* (Hall and Loefer, 1936), failed to grow in medium D when the peptone dilution reached 5×10^{-6}, although growth was observed at a dilution of 10^{-5}.

Discussion.

The results described above show that *Chlorogonium euchlorum* is capable of continued growth in media containing an inorganic source of nitrogen, as reported previously by Loefer (1934). Our technique was more exacting than that used by Loefer, since our transfers were made at shorter intervals in order to reduce the possible contribution of organic nitrogen from death of the flagellates. Yet this species grew vigorously through fifteen transfers in one medium and was carried for shorter series in others. On the other hand, both *Euglena anabaena* and *E. deses* failed to grow beyond the first or second transfer in these media, although one of the solutions (medium O) was found by Dusi (1933 a) to be satisfactory for *E. anabaena.* Our results with *E. anabaena* thus corroborate the findings of Hutner (1936).

Viability tests showed that both species of *Euglena* could survive for several weeks in an inorganic medium, even when the concentration of peptone derived from the stock culture was too low to permit

cell division. Hence, their failure to multiply is obviously dependent upon the lack of some factor essential for growth. This factor may be either organic or inorganic. However, if it is assumed that the missing substance is inorganic, there is the difficulty of explaining the growth of *Chlorogonium euchlorum* in the same media. Hence, it is not easy to avoid the conclusion that lack of an organic nitrogen source in the solutions tested prevented growth of *E. anabaena* and *E. deses*. This is the conclusion reached by Dusi (1933 a) with respect to *E. deses*.

On the other hand, there remains the fact that Dusi (1933 a) carried *E. anabaena* through eight successive transfers in the solution listed above as medium 0. These findings reversed an earlier report (Dusi, 1930) that *E. anabaena* is not autotrophic. It is difficult to question the ability of *E. anabaena* to utilize inorganic nitrogen salts, since Hall (1937 a) reported that nitrate is reduced by this species at a rate more or less comparable to that of the alga, *Chlorella* (Beckwith, 1929). However, negative nitrite tests were obtained with *E. deses*.

Assuming that Dusi's technique produced an adequate serial dilution of peptone from the stock culture and that his glassware was always chemically clean, there remains a possibility that differences in the purity of his chemicals and of ours might explain the contradiction in results. In other words, some essential substance might have been present as an impurity in Dusi's chemicals and absent in ours. The observations of Hall (1938), that growth of *E. anabaena* is accelerated by manganese chloride in certain concentrations, suggested the addition of this substance to an inorganic medium. The results (series XI, XIII), however, were not appreciably different from those obtained with the other media. Dusi (1933 a) found it necessary to add calcium to his inorganic media for best growth of *E. stellata* but not for *E. anabaena*. It is probable, as Dusi suggested, that this represents a quantitative rather than a qualitative difference in growth requirements, and that the trace of calcium necessary for *E. anabaena* was already present in the chemicals which he used. Furthermore, Mast and Pace (1936) have reported, for *Chilomonas paramecium*, that elimination of calcium from the medium has the same effect as elimination of magnesium — cell division is prevented. These results, together with Dusi's findings, suggest the possible importance of calcium for growth of *Euglena anabaena* and *E. deses*. However, none of our media was free from

calcium, since several salts — ammonium chloride, sodium chloride and magnesium sulphate — each contained a trace of calcium oxide.

According to the present findings, *E. anabaena* and *E. deses* are incapable of growth through successive transfers in several inorganic media which do support growth of *Chlorogonium euchlorum*. These observations may indicate that, in spite of the possession of chlorophyll, physiological specialization of these Euglenidae has progressed to such an extent that they have lost the plant-like characteristic of autotrophic nutrition.

Literature cited.

Beckwith, T. D. (1929): The metabolism of Chlorella. Proc. Soc. Exper. Biol. a. Med. **27**, 1—3.

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

— (1933): Recherches sur la nutrition de quelques Euglènes. I. Euglena gracilis. Ann. Inst. Pasteur **50**, 550—597.

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

Hall, R. P. (1933): On the relation of hydrogen-ion concentration to the growth of Euglena anabaena var. minor and E. deses. Arch. Protistenkde **79**, 239—248.

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

— (1937a): Certain culture reactions of several species of Euglenidae. Trans. Amer. Micr. Soc. **56**, 285—287.

— (1937): Effects of manganese on the growth of Euglena anabaena, Astasia sp. and Colpidium campylum. Arch. Protistenkde **90**, 178—184.

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

Hall, R. P. and J. B. Loefer (1936): On the supposed utilization of inorganic nitrogen by the colorless cryptomonad flagellate, Chilomonas paramecium. Protoplasma (Berl.) **36**, 321—330.

Hall, R. P. and H. W. Schoenborn: Effects of certain ammonium salts on growth of Euglena anabaena. (MSS.)

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

Jahn, T. L. (1929): Studies on the physiology of the euglenoid flagellates. I. The relation of density of population to the growth rate of Euglena. Biol. Bull. Mar. biol. Labor. Wood's Hole **57**, 81—106.

Loefer, J. B. (1934): The trophic nature of Chlorogonium and Chilomonas. Ibid. **66**, 1—6.

Lwoff, A. (1932): Recherches biochimiques sur la nutrition des protozoaires. Le pouvoir synthese. Monographies de l'Institut Pasteur (Paris, Masson & Cie.), 58 pp.

Mainx, F. (1928): Beiträge zur Morphologie und Physiologie der Eugleninen. II. Untersuchungen über die Ernährungs- und Reizphysiologie. Arch. Protistenkde **60**, 355—414.

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

— (1936): The effects of magnesium and calcium on growth in Chilomonas paramecium. Anat. Rec. **67** (Suppl. 1), 121.

Pringsheim, E. G. (1912): Kulturversuche mit chlorophyllführenden Mikroorganismen. II. Zur Physiologie der Euglena gracilis. Beitr. Biol. Pflanz. **12**, 1—47.

Ternetz, C. (1912): Beiträge zur Morphologie und Physiologie der Euglena gracilis. Jb. Bot. **51**, 435—514.

Zumstein, H. (1899): Zur Morphologie und Physiologie der Euglena gracilis. Jb. Bot. **34**, 149—196.