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On the relation of hydrogen-ion concentration to the growth of *Euglena anabaena* var. *minor* and *E. deses.*

By

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(With 5 figures in the text).

In regard to the relation of hydrogen-ion concentration to the growth of euglenoid flagellates, several investigators have obtained results which are qualitative rather than quantitative in nature. MAINX (1928), for example, has determined that certain species are best adapted to growth in basic media, some in neutral media, and others in slightly acid media. DUSI (1930, 1930 a), determining the relative amounts of growth by macroscopic methods, has worked out the p_H range and approximate optimum for each of several species. More recently, ALEXANDER (1931) and JAHN (1931), using quantitative methods for measuring growth, have determined an optimum p_H and p_H range for growth of *Euglena gracilis.* In the present investigation, the writer has determined the p_H range and optimum p_H for growth of *Euglena anabaena* var. *minor* and *E. deses.*

Material and methods.

The bacteria-free strains of the two species investigated were obtained from the laboratory of Prof. E. G. PRINGSHEIM. In some of the experimental series, as indicated below, 1 % peptone solution (Difco proteose peptone) has been used as a medium. In other series the following medium (JAHN, 1931) has been used:

KNO_3 .	0.5 gm
KH_2PO_4	0.5 „
$MgSO_4$.	0.25 „
NaCl	0.10 „
$FeCl_3$ . . . . . (10 % sol.) . .	3 drops
Difco dehydrated tryptophane broth .	5.0 gm
Distilled water . . .	1.0 litre

A Lamotte "Roulette" colorimetric set was used in adjusting media to the desired p_H.

Experimental series were started in pairs, the tubes of one series being incubated in darkness while those of the other series were exposed to light. Each series consisted of sets of four or more tubes at each p_H. Stock flask cultures in the appropriate medium were used in inoculating the paired series. In each series two extra tubes were inoculated at each p_H for determination of initial concentration of organisms and the p_H after inoculation. The method of counting used in the investigation was described by JAHN (1929), and the same author has described, in a later paper (JAHN, 1931), the constant temperature water bath and general culture methods.

Euglena anabaena var. *minor*.

Series I and II

In these two series JAHN's (1931) medium was used, the p_H of the different sets of tubes being adjusted as follows: 4.5, 4.7, 5.2, 6.2, 6.6, 6.9, 7.4, 7.7 and 8.3. Each tube received a 0.5 cc. inoculation from a 14-day flask culture in the same medium (p_H 6.7). The initial count was 1740 per cubic centimeter. In series I the tubes were incubated in the water bath in light at a constant temperature of 29.5° C. for 13 days.

The final counts (fig. 1) ranged from 2030 at p_H 4.5 to 7340 at 6.9, and 1860 at p_H 8.3. Maximum growth occurred at p_H 6.9. The final count at p_H 8.3 indicates that very little, if any, growth occurred, and that this point lies near the upper limit of the p_H range for the species. This agrees closely with DUSI's (1930 a) observation that p_H 8.5 is the upper limit. The results of the counting method indicate that the optimum lies near p_H 6.9, whereas DUSI described an optimum of 6.5—8.0. DUSI observed growth at p_H 6.0 as a lower limit, while the present results indicate that there is perhaps slight growth at 4.7 and 4.5, although the apparent increase

over the initial count is not particularly significant from the statistical standpoint.

Except for the addition of a set of tubes at p_H 8.0, series II duplicates series I in p_H. The tubes received the same inoculation from the stock culture used in series I, but were incubated in darkness at room temperature for 24 days. The temperature, registered by a recording thermometer, ranged from 17 to 26 ° C. during the period of incubation. Final counts (fig. 1) ranged from 1890 at p_H 4.5 to 3330 at 6.6, and 1890 at p_H 8.3; maximum growth occurred at p_H 6.6, rather than at 6.9. This apparent difference in optimum

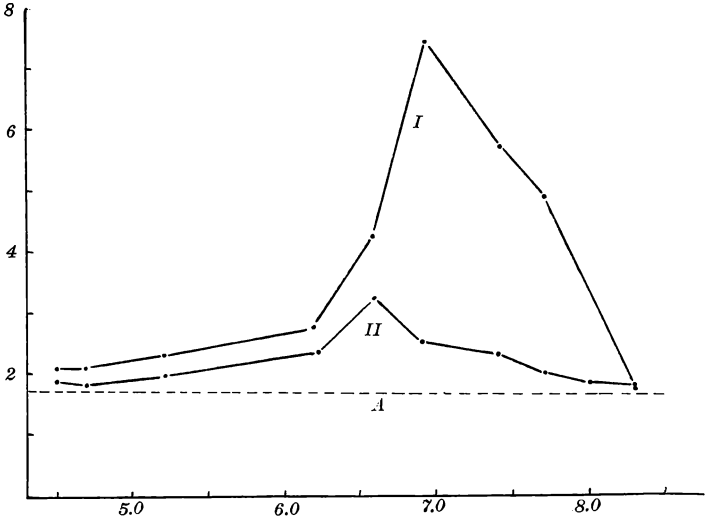

Fig. 1. *Euglena anabaena* var. *minor*, series I and II; numbers of organisms (thousands per cubic centimeter) plotted against p_H; initial count indicated by horizontal broken line, A.

might be due to a real difference between the optimum p_H for photosynthesis and for saprozoic nutrition in darkness. On the other hand, other factors might be involved, since series II was incubated at a lower temperature and for a longer time than series I. At p_H 4.5, 4.7, and 8.3, the final counts are not significantly greater than the initial count, and the occurrence of growth is doubtful. Comparing series II as a whole with series I, it is seen that in every case except at p_H 8.3 the final counts are lower in the former series. There is thus no evidence for any photodynamic effect in the lower part of the p_H range, as was reported by ALEXANDER (1931) for *Euglena gracilis*.

Series III and IV.

In these series 1 % peptone solution (Difco proteose peptone) was used with p_H as follows: 4.7, 5.5, 6.2, 6.9, 7.7, 8.4. The initialcount was 174. In series III the tubes were incubated in light in the water bath for a period of 12 days. Final counts ranged from 170 at p_H 4.7 to 167 at p_H 8.4, with a maximum of 365 at 6.9. Growth apparently occurred from p_H 5.5 to 7.7, but the results indicate that 1 % peptone is a less satisfactory medium than that used in series I and II.

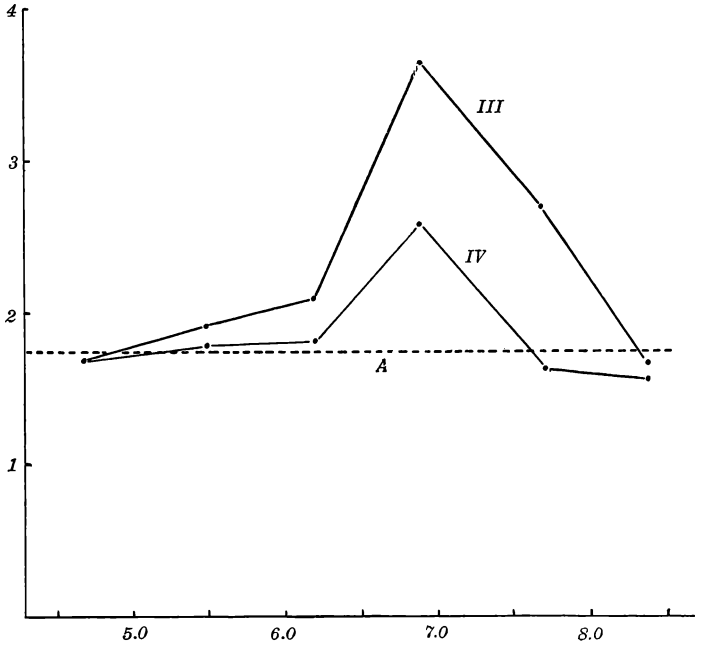

Fig. 2. *Euglena anabaena* var. *minor*, series III and IV; numbers of organisms (hundreds per cubic centimeter) plotted against p_H; initial count indicated by horizontal broken line, A.

Series IV (fig. 2) is a duplicate of series III in p_H range, initial count, and time and temperature of incubation. This series was incubated in darkness, however. The final counts indicate that growth occurred only at p_H 6.9.

Euglena deses.

Series I and II.

Difco proteose-peptone in 1 % solution was used in these series, with the following p_H after inoculation: 4.9, 5.7, 6.4, 7.0, 7.5, 8.0. Each tube received a 0.5 cc. inoculation from a 7-day stock culture

in the same medium, the initial count being 1200. Series I was incubated in the light in the water bath for 12 days. Final counts (fig. 3) showed 1097 at p_H 4.9, 1219 at 8.0, and a maximum of 3250 at p_H 7.0.

Series II is a duplicate of series I, except that the tubes were incubated in darkness in the water bath. Final concentrations

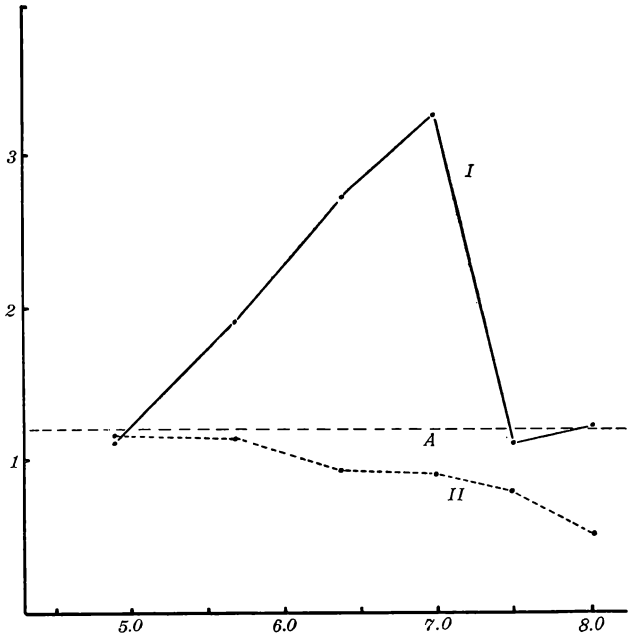

Fig. 3. *Euglena deses*, series I and II; numbers of organisms (thousands per cubic centimeter) plotted against p_H; initial count indicated by horizontal broken line, A.

(fig. 3) were low, ranging from 1180 at p_H 4.9 to 513 at p_H 8.0. No growth occurred at any p_H, and the decrease in number of organisms varied inversely with the p_H.

Series III and IV.

In series III and IV JAHN'S (1931) medium was used in the following range: 4.7, 5.3, 6.1, 6.5, 6.7, 7.0, 7.4, 7.6, 7.8. Each tube received a 0.5 cc. inoculation from a 7-day stock culture in the same medium (p_H 6.7). The initial count was 2500. In series III the tubes were exposed to a constant light source, while those of series IV were kept in darkness; both series were incubated for 13 days in the water bath.

In series III (fig. 4) the final counts ranged from 2160 at p_H 4.7 to 19,965 at 7.0 and 13,460 at p_H 7.8. These results are in general agreement with the findings of Dusi (1930 a), who placed the optimum p_H for growth at p_H 7.0—7,5. Dusi, however, mentioned a p_H range of only 6.5 to 8.0, while the present observations indicate that growth occurs below p_H 6.1 in the medium used.

In series IV, incubated in darkness, there was no evidence for growth except at p_H 6.7 and 7.0, with final counts of 3,013 and

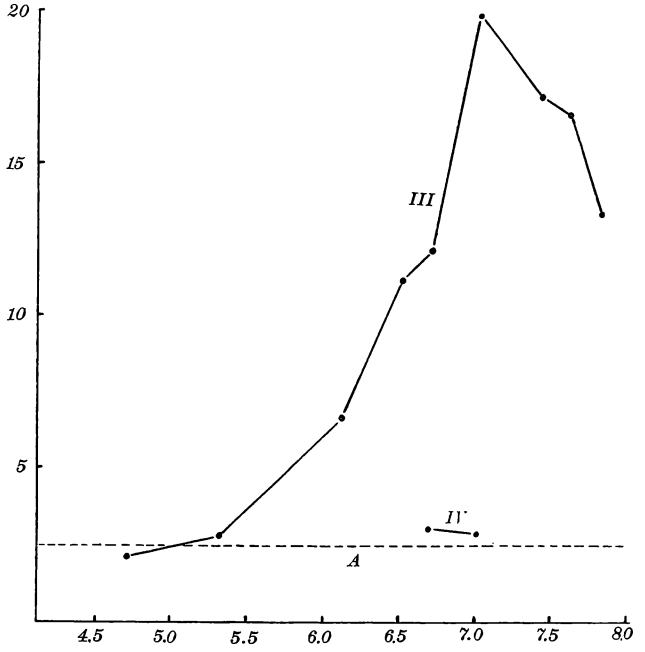

Fig. 4. *Euglena deses*, series III and IV; numbers of organisms (thousands per cubic centimeter) plotted against p_H; initial count indicated by horizontal broken line, A.

2,920, respectively (fig. 4). In darkness the growth range of *E. deses* is thus more restricted than that of *E. anabaena* var. *minor* in the same medium.

Division rate in relation to p_H.

Concerning the relation of hydrogen-ion concentration to the division rate of Protozoa, conflicting opinions are to be found in the literature. Darby (1929) has shown that, under the conditions of his experiments, the division rates of *Paramecium caudatum* and

P. aurelia vary widely in media of different p_H. At p_H 5.3, for example, *P. caudatum* showed a division rate of 0.37, as compared with 2.35 at the optimum (p_H 7.0) and 1.016 at p_H 8.1. Similar differences were observed in the case of *P. aurelia.* PHELPS (1931), on the other hand, states that in cultures of *P. aurelia* fed only on *Erythrobacillus prodigiosus*, "the division rate is practically unaffected by change in p_H between the limits of p_H 5.9 and 7.7." PHELPS attributed DARBY'S different results to the latter's use of an uncontrolled mixture of bacteria as food for the ciliates.

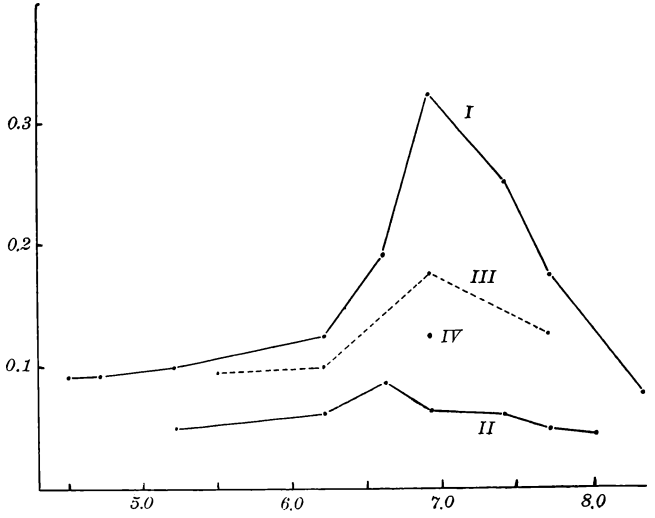

Fig. 5. *Euglena anabaena* var. *minor*, series I—IV; calculated average division rates (divisions per day per organism) plotted against p_H; in series IV, growth occurred only at p_H 6.9.

In bacteria-free cultures of *Euglena* the diet of the flagellates may be controlled as carefully as in PHELPS' cultures of *Paramecium aurelia.* Plotting the average division rate (divisions per day per organism, against p_H (fig. 5), it may be noted that in *Euglena anabaena* var. *minor* the division rate varies definitely with the p_H of the medium. In series I the division rate at p_H 6.9, for example, was 0.324, while at 6.6 and 7.4 it was 0.191 and 0.253, respectively. From p_H 6.2 to p_H 7.7, the differences in division rate were quite marked. In series II, grown in darkness, the differences in division rate are smaller, although that at p_H 6.6 is obviously higher than the rates for p_H 6.2 and 6.9. In series III the division rate is distinctly higher at p_H 6.9 than at 6.2 and 7.7.

In the case of *Euglena deses*, division rates in series I were as follows: at p_H 5.7, 0.131; at 6.4, 0.192; and at 7.0, 0.225. In series II, incubated in darkness, no growth occurred. In series IV growth occurred at p_H 6.7 and 7.0 only; corresponding division rates were 0.0898 and 0.093. Division rates in series III are tabulated below (Table 1):

p_H	5.3	6.1	6.5	6.7	7.0	7.4	7.6	7.8
Division rate	.088	.207	.344	.376	.614	.532	.515	.414

Thus the division rates of *E. anabaena* var. *minor* and *E. deses* vary distinctly with the p_H of the culture medium within the p_H range (5.9 to 7.7) mentioned by Phelps (1931).

Discussion [1]).

Jahn (1929, 1930) had found that the Sedgwick-Rafter counting-cell method was well adapted to the measurement of growth in *Euglena*, and hence in a later paper (Jahn, 1931) he applied this method to the study of growth of *Euglena gracilis* in relation to hydrogen-ion concentration. His results showed that the counting-cell method is more accurate than the methods used by earlier workers, and that appreciable differences in amount of growth may not be detected by the previously used macroscopic methods. In the same year Alexander (1931) published the results of a similar investigation on the same species. While his results agree in some respects with those of Jahn, the two investigations are not entirely comparable. For example, Alexander's cultures were incubated for only 48—72 hours, whereas Jahn's counts were made after longer periods of incubation.

It is possible also that differences in culture media may be of some significance in the interpretation of results. Alexander used a 1% peptone medium (Difco proteose peptone), while the medium used by Jahn is the one cited above (Jahn, 1931). Sherman and Holm (1922) have shown that there is an "accelerating effect of certain salts upon the growth of *B. coli*". They have concluded, furthermore, that "the limiting H-ion zone of growth may be modified by the addition of NaCl to the medium"; and, while the optimum p_H for growth seems to be about the same, beyond the range for optimum growth there seems to be a decided retardation for each small change of hydrogen-ion concentration. In comparing series I

[1]) Reviews of earlier investigations will be found in the papers of Darby (1929), Jahn (1931) and Mainx (1928).

and III of *E. anabaena* var. *minor*, differences in division rate are observed which may be attributed to differences in culture media. Series I was grown in JAHN's medium, while ALEXANDER's 1% peptone medium was used in series III. Comparable division rates are as follows: at p_H 6.2 — series I, 0.123 and series III, 0.099; at p_H 6.9 — series I, 0.324 and series III, 0.174; at p_H 7,7 — series I, 0.172 and series III, 0.129. The two series, I and III, were incubated for 13 and 12 days respectively, so that the age of the cultures could scarcely be a factor. The two series do differ in initial concentration, series I having an initial count of 1740 and series III only 174. JAHN (1929, 1930), however, has found that in *Euglena* sp. the division rate is higher in cultures of low concentration than in those of high concentration. On this basis a higher division rate would be expected in series III, whereas the reverse is true.

The variation in division rate with change in p_H is also more marked in series I than in series III. Thus, in series I the rate at p_H 6.9 is 1.8 times that at p_H 7.7, and in series III it is only 1.3 times as great; similarly, the division rate in series I is 2.6 times as great at p_H 6.9 as at 6.2, while in series III it is only 1.7 times that at 6.2. Hence, it seems that the observations of SHERMAN and HOLM (1922) on *B. coli* are, to some extent at least, applicable also to cultures of *Euglena*, in that the presence of various salts in the culture medium may accentuate the effect of changes in p_H on the division rate.

In darkness, growth of *E. anabaena* var. *minor* apparently occurred between p_H 5.2 and 7.7 in JAHN's (1931) medium, while slow growth of *E. deses* apparently occurred at p_H 6.7 and 7.0. In ALEXANDER's 1% peptone medium, *E. anabaena* grew only at p_H 6.9, while *E. deses* was unable to grow at all in darkness. The results indicate that these two species of *Euglena* can grow without photosynthesis in JAHN's medium, but within a shorter p_H range than that in which growth occurs in light.

In cultures of *Euglena gracilis* at low p_H ALEXANDER (1931) has reported a "photodynamic effect", which appears as a depression of division rate in cultures exposed to light as compared with cultures incubated in darkness. In the present investigation, however, the writer has been unable to detect such a photodynamic effect. In those cases in which growth occurred, the division rate of cultures incubated in darkness was lower than that of the corresponding cultures exposed to light.

Alexander (1931) has suggested that the typical "euglenoid movement" (metaboly) of *Euglena gracilis* is a modified avoiding reaction associated with transfer of the "organisms from the culture in which they have been living to one in which the p_H is markedly different". Since metaboly may also be observed in Euglenida transferred merely from a culture dish to a slide, the writer made a few observations on freshly inoculated cultures of *E. anabaena* var. *minor*. It was noted that flagellates transferred from a stock culture at p_H 6.7 to fresh media at p_H 6.7 and 7.0 showed metaboly as distinctly as those transferred to media of lower and higher p_H. Hence, it would seem that a distinct change in p_H is not an essential factor in inducing "euglenoid movement" of *E. anabaena*.

Literature cited.

Alexander, G. (1931): The significance of hydrogen ion concentration in the biology of Euglena gracilis Klebs. Biol. Bull. Vol. 61 p. 165—184.

Darby, H. H. (1929): The effect of hydrogen-ion concentration on the sequence of protozoan forms. Arch. f. Protistenk. Bd. 65 p. 1—37.

Dusi, H. (1930): Les limites de la concentration en ions H pour la culture d'Euglena gracilis Klebs. C. R. Soc. Biol. T. 103 p. 1184—1185.

— (1930a): Limites de la concentration en ions H pour la culture de quelques Euglènes. Ibid. T. 104 p. 734—736.

Jahn, T. L. (1929): Studies on the physiology of the euglenoid flagellates. I. The relation of the density of population to the growth rate of Euglena. Biol. Bull. Vol. 57 p. 81—106.

— (1930): II. The autocatalytic equation and the question of an autocatalyst in the growth of Euglena. Ibid. Vol. 58 p. 281—287.

— (1931): III. The effects of hydrogen-ion concentration on the growth of Euglena gracilis Klebs. Ibid. Vol. 61 p. 387—399.

Mainx, F. (1928): Beiträge zur Morphologie und Physiologie der Eugleninen. Arch. f. Protistenk. Bd. 60 p. 305—414.

Phelps, A. (1931): Effect of hydrogen-ion concentration on the division rate of Paramecium aurelia. Sc. Vol. 74 p. 395—396.

Sherman, J. M. and Holm, G. E. (1922): Salt effects on bacterial growth. II. The growth of Bact. coli in relation to hydrogen-ion concentration. J. Bact. Vol. 7 p. 465—470.