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Relation of hydrogen-ion concentration to growth of *Chilomonas* and *Chlorogonium*.

By

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(With 5 figures in the text.)

Introduction.

Many investigators have pointed out that hydrogen-ion concentration is an important factor in the ecology of protozoa. PRUTHI (1926) and DARBY (1929) have correlated p_H of the medium with a definite sequence of forms. With bacteria-free cultures of protozoa it is possible to study more accurately the relationship between this physico-chemical factor and growth, and recent investigations on a number of Euglenida (DUSI, 1930, and others cited below) have shown a specific correlation between the two factors.

In preliminary experiments on bacteria-free cultures of *Chlorogonium euchlorum*, *C. elongatum* and *Chilomonas paramecium* (LOEFER, 1931, 1932 a and b) it was shown that there was a marked difference in growth of these forms at different hydrogen-ion concentrations. Information concerning the growth- p_H relationship of members of the orders Chlamydomonadida and Cryptomonadida is extremely scanty. For this reason the following experimental observations were made in order to study and compare growth of these three flagellates over a given p_H range in various types of media. The writer wishes to express his gratitude to Professor R. P. HALL for numerous suggestions concerning the investigation.

Material and methods.

The pure strains of *Chlorogonium euchlorum*, *C. elongatum* and *Chilomonas paramecium* were the same as those used in earlier investigations (LOEFER, 1934 a, b and c). A more detailed description of the method is given in the first of the above papers. In general the procedure was as follows: An amount of standard medium of the following formula was made up in sufficient quantity (usually several liters) for an entire series.

KNO ₃	0.5 grams
KH ₂ PO ₄	1.5 "
MgSO ₄	0.1 "
NaCl	0.1 "
FeCl ₃	trace
Bacto-tryptone	2.5 grams
Distilled water	. . .	1 liter

Portions of it were titrated with N/1 HCl and N/1 NaOH to various points ranging from p_H 3.5 to 9.5, colorimetric determinations being made with a LA MOTTE-Comparator. These portions of medium of the above formula, or as modified for the particular experiment in question, were measured into 16 by 150 mm Pyrex culture tubes in equal amounts (9.5 ml). The tubes were plugged with cotton and autoclaved. Twenty-four hours later, six to twelve tubes of media at each p_H value were inoculated with a given species by means of a 1.0 ml sterile pipettes, from a flask culture in which the concentration of organisms was known. Initial concentration of the flagellates (x_0) was generally about 500 per ml, as in earlier investigations. After a period of incubation at 28° C, unless otherwise mentioned, in a thermostat-controlled water bath under constant light, the number of organisms per ml (x) was determined with a SEDGWICK-Rafter counting-cell, and the growth increase (x/x_0) calculated and plotted. In all cases the mean count of several samples of at least three tubes (or flasks when these were used) was computed. Determinations of p_H were made after inoculation and again after incubation. The two figures were seldom observed to vary appreciably during the relatively short culture periods.

Bacterial tests for purity of the cultures were made during the course of experimentation, test cultures being incubated both at room temperature and at 37° C for a time sufficient to determine their sterility.

Sodium acetate was obtained from the Eastman Kodak Company, Rochester, N. Y., Bacto-peptone, Bacto-dextrose broth, Bacto-tryptone etc. from the Difco Laboratories at Detroit, Michigan.

Experimental results.

Series I. *Chlorogonium euchlorum*.

A quantity of 0.5 % Bacto-tryptone medium (other ingredients the same as in the preceding formula) was divided into three portions, which were titrated to p_H 5.5, 6.8 and 7.9, respectively. One hundred milliliter quantities of each were measured into 250 ml ERLLENMEYER flasks and sterilized. Six flasks at each p_H value were inoculated with *Chlorogonium euchlorum* ($x_0 = 1,000$) from a six-day culture. One half of the flasks were incubated in darkness; the remaining three at each p_H value were incubated in light at room temperature. Samples were drawn from each flask at three-day intervals and counted. The average concentration of several samples from each of the flasks in light is recorded in thousands per ml in table 1.

Table 1.
Chlorogonium euchlorum.

Time	p_H 5.5	p_H 7.0	p_H 7.6
3 days	17.6	40.9	49.6
6 "	26.8	81.6	196.0
9 "	36.4	195.2	544.6
12 "	41.8	327.0	713.0
15 "	72.8	383.0	922.0

Growth in darkness is not recorded since the flagellates in all of the cultures decreased in number after the third day. It was later shown that this medium is not adequate for continued growth of *Chlorogonium* in darkness. In light, however, growth was greatest at p_H 7.6, the flagellates having increased to 922 thousand per milliliter in 15 days. At p_H 5.5 after 15 days there were 72,800 per ml, while at 6.8 the average density was 383 thousand. The same relative growth differences were evident after three, six, nine and twelve days, growth being most rapid in a slightly alkaline medium.

In a similar series of media at hydrogen-ion concentrations of p_H 6.0, 6.9 and 7.1, which contained NH_4NO_3 instead of KNO_3 , similar results were obtained. The initial count was 1,410. The final counts (table 2) indicate that a p_H above 7.0 is most favorable to growth. This is evident even after a three-day growth period. Slight in-

creases in p_H were noticed in the denser cultures after 15 days of incubation.

Table 2.
Chlorogonium euchlorum.

Time	p_H 6.0	p_H 6.9	p_H 7.1
3 days	14.8	19.7	34.5
6 "	31.8	42.0	49.8
9 "	66.6	90.5	115.9
12 "	95.5	193.4	222.7
15 "	127.9	289.6	359.8

In order to determine more accurately the optimum p_H for growth, the organisms were grown in culture tubes and the incubation period was shortened considerably in the following experiments. Sets of culture tubes containing media at each p_H value were made up as described above (page 2).

Series II. *Chlorogonium* and *Chilomonas*.

The purpose of this series was to determine the growth range of the three species in a 1.0% Bacto-peptone medium (substituted for Bacto-tryptone in the formula on page 2). Accordingly, sets of tubes of this medium were made up and inoculated, with the following p_H values after inoculation: 4.2, 4.6, 5.2, 6.0, 6.6, 7.1, 7.5, 7.9, 8.4 and 8.8. Six to twelve tubes at each p_H for each respective species were inoculated in the usual manner.

Figure 1, A shows the average growth increase (x/x_0) of *Chlorogonium euchlorum* at designated p_H values after an incubation period of 48 hours, the initial count having been 370. Growth occurred from p_H 5.1 to 8.1 (inclusive), with the most rapid growth at about p_H 7.5.

Results for *C. elongatum* after 120 hours (x_0 was 2,500) indicate a higher optimum p_H , as was reported previously (LOEFER, 1932 a), growth being best at p_H 7.9 (Fig. 1, B). Except for this difference, the results are relatively the same as those obtained for *C. euchlorum*. No growth was observed at 4.6 and 4.2. The point of least acidity in this series of cultures was p_H 8.8. It appears, however, that the alkaline growth limit of *Chlorogonium* is somewhat above this point.

Tubes of the same medium were inoculated with *Chilomonas* in a similar manner ($x_0 = 1,000$). After 68 hours incubation, average determinations were made and plotted (Fig. 1, C). The results are

somewhat different from those obtained for *Chlorogonium*. Growth was evident at p_H 4.6, which is below the minimum for *Chlorogonium*. Two growth maxima were observed, one at about p_H 5.0, and the other in the neighborhood of 7.0, the low intermediate point being about p_H 6.0.

Series III. *Chlorogonium*.

In order to check series II with respect to the optimum p_H for *Chlorogonium*, similar series of tubes containing the same medium were inoculated (x_0 for *C. euchlorum* = 476; x_0 for *C. elongatum* = 435). Growth was determined over the following range (p_H after inoculation): 4.6, 5.0, 5.5, 6.1, 6.5, 7.1, 7.4, 8.0, 8.4, 8.6 and 8.7. As in series II, no growth of either species occurred at p_H 4.6. From 5.0 to 7.4 growth of *C. euchlorum* had increased progressively with p_H after 125 hours of incubation. An optimum was observed at about p_H 7.4, while growth was progressively less at 8.0—8.7 (Fig. 1, D). Results for *C. elongatum* after 165 hours of incubation are similar, except that maximum growth occurred at a p_H nearer 8.0 (Fig. 1, E). These results, both as to range and optimum for growth, bear out those of series II. No p_H changes were observed during the culture periods recorded.

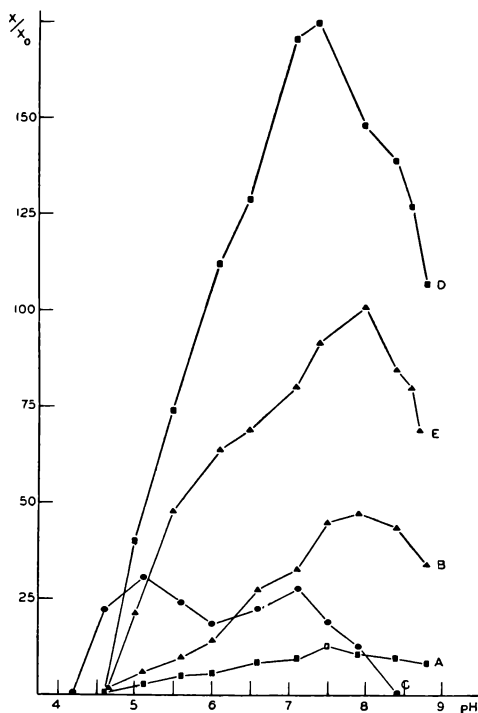


Fig. 1. Series II and III. Growth of *Chilomonas* and *Chlorogonium* in Bacto-peptone at different hydrogen-ion concentrations. A *Chlorogonium euchlorum* after 48 hours. B *C. elongatum* after 120 hours. C *Chilomonas* after 68 hours. D *Chlorogonium euchlorum* after 125 hours. E *C. elongatum* after 165 hours.

Series IV. *Chlorogonium* and *Chilomonas*.

The purpose of this experiment was, first, to compare growth of *Chlorogonium euchlorum* and *Chilomonas* in a Bacto-dextrose broth medium (substituted for Bacto-tryptone in the formula, page 2); and second, to compare growth of *Chilomonas* with respect to p_H in the same culture fluid, in Bacto-tryptone, and in the same concentration of Bacto-tryptone plus 0.5 % dextrin.

Tubes of Bacto-dextrose broth medium (0.25 %) at the following p_H values (after inoculation with *Chlorogonium euchlorum*, $x_0 = 198$) were incubated for 96 hours: 3.6, 4.1, 4.8, 5.3, 5.7, 6.1, 6.5, 6.7 and 7.1. Final determinations (Fig. 2, A) indicated positive growth from p_H 4.8 up to 7.1, increased growth being correlated directly with increased alkalinity.

Results for *Chilomonas* in tubes of the same medium after 100 hours incubation (x_0 was 112) are quite different from those for *Chlorogonium* (cf. Fig. 2, B and 2, A). A bimaximal graph was obtained with one growth maximum at about p_H 4.8 and the other slightly below the neutral point, with an intermediate low point at 6.1. No growth occurred at p_H 3.6, but at 4.1 growth was abundant. Thus it appears that an optimum for growth

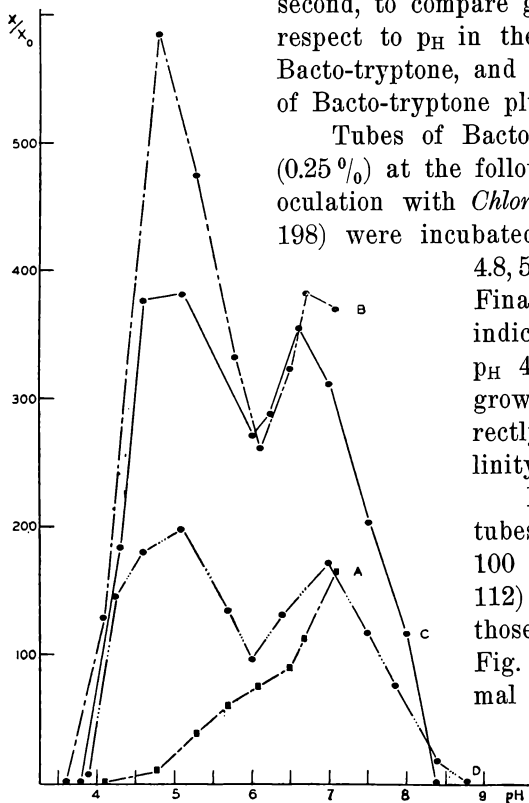


Fig. 2. Series IV. Growth of *Chlorogonium euchlorum* and *Chilomonas* in various media at different hydrogen ion concentrations. A *C. euchlorum* in Bacto-dextrose broth after 96 hours incubation. B *Chilomonas* in Bacto-dextrose broth after 100 hours. C *Chilomonas* in Bacto-tryptone after 72 hours. D *Chilomonas* in Bacto-tryptone plus dextrin after 54 hours.

of *Chilomonas* (though not for *Chlorogonium*) exists at about p_H 5.0. It is also apparent *Chilomonas* is more acidophilic than the green species.

A similar p_H series was next carried out with tubes containing Bacto-tryptone (formula page 2) to determine whether or not the bimaximal growth curve which was obtained for *Chilomonas* in Bacto-peptone and Bacto-dextrose broth would also obtain in this medium. A heavier inoculation ($x_0 = 458$) of *Chilomonas* was introduced and the incubation period was shortened to 72 hours. Growth occurred from 4.2 to 8.0, and a maximum was observed between 4.6 and 5.2. A low point was recorded at 6.0 and a second high at 6.6. Growth was progressively less at 7.0, 7.5 and 8.0 (Fig. 2, C).

A series of tubes at the same p_H values as those above but containing dextrin (0.2 %) in addition to the regular medium was given the same inoculation and incubated for 54 hours, at which time growth was determined (Fig. 2, D). The p_H -growth relationship is similar to that obtained above with Bacto-tryptone alone. It differs from the latter somewhat since slight growth is indicated at p_H 8.4 and the second growth optimum is nearer 7.0 than 6.6. From these results the following differences between *Chilomonas* and *Chlorogonium* are apparent: 1. *Chilomonas* is more acidophilic than *Chlorogonium*. 2. While *Chilomonas* has two maximal growth regions (p_H 4.6—5.1 and 6.6—7.0), *Chlorogonium* has but one maximum (above p_H 7.0). These differences are constant in the several types of media used.

Series V. *Chlorogonium*.

The purpose of this series was to compare, first, growth of *C. elongatum* with *C. euchlorum* in a Bacto-tryptone medium, and second, to compare the effect of maltose and sodium acetate on growth of *C. euchlorum* at different hydrogen-ion concentrations.

Similar sets of tubes of Bacto-tryptone medium (formula on page 2) at different p_H values were inoculated with *C. euchlorum* ($x_0 = 350$) and *C. elongatum* ($x_0 = 353$), and incubated 108 hours at 29° C. Growth at the following p_H values was recorded: 4.5, 5.0, 5.6, 6.0, 6.7, 7.0, 7.4, 7.6, 8.4, 8.6 and 8.7. No growth occurred at p_H 4.5. From 5.0 to 7.5 growth of *C. euchlorum* increased with p_H and then decreased to p_H 8.7. Growth of *C. elongatum* under similar conditions (Fig. 3, B) differed from that of *C. euchlorum* in several respects. First, a slightly higher optimum p_H was indicated after 108 hours, and second, rate of division was considerably lower, as was evident in the preceding experiments. The maltose (0.5 %) and sodium acetate (0.2 %) series on *C. euchlorum* were given the same inoculation ($x_0 = 350$) as the series with Bacto-tryptone. The maltose

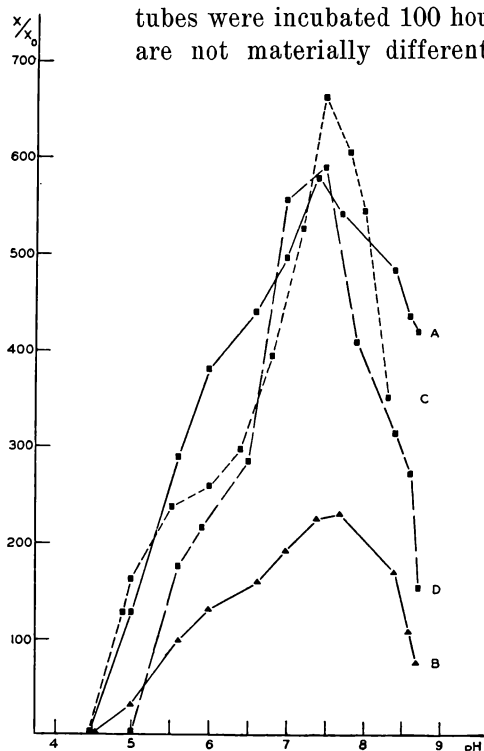


Fig. 3. Series V. Growth of *Chlorogonium* at different hydrogen-ion concentrations. A *C. euchlorum* in Bacto-tryptone after 108 hours. B *C. elongatum*, the same. C *C. euchlorum* with maltose after 100 hours. D *C. euchlorum* with sodium acetate after 65 hours.

tubes were incubated 100 hours and the results (Fig. 3, C) are not materially different from those obtained with Bacto-tryptone alone (cf. Fig. 3, A). Growth was accelerated by maltose, particularly at the optimal hydrogen-ion concentration (p_H 7.5).

The results of the series for *C. euchlorum* in which the medium contained sodium acetate were somewhat different. Growth at the optimal p_H after 65 hours was even better than growth after 108 hours with Bacto-tryptone alone (cf. Fig. 3, D and A). However, the acid range is definitely decreased, with no growth at p_H 5.0 as compared with growth at p_H 4.9 in the Bacto-tryptone medium without acetate (Fig. 4, A).

Series VI. *Chlorogonium*.

Limitation of growth range by sodium acetate was investigated further in the following experiments. A series of tubes containing the regular Bacto-tryptone medium with sodium acetate (0.2%) was made up at different hydrogen-ion concentrations and inoculated with *C. euchlorum*. Bacto-tryptone tubes without sodium acetate served as controls. Tubes of p_H values as recorded in figure 4 (after inoculation) were incubated 71 hours, when the average numbers of organisms at given p_H values were compared. In the control series no growth was recorded at p_H 4.4 (Fig. 5, A). Growth was positive and progressively better at 4.9, 5.7 and 6.0. No division occurred below p_H 5.4 in the tubes containing sodium acetate, although at

this hydrogen-ion concentration as well as at p_H 5.7 and 6.0, growth was much greater in the cultures with sodium acetate (Fig. 4, B).

An homologous series on *C. elongatum* ($x_0 = 530$; incubation time, 68 hours) showed similar sodium acetate growth limitation. Excellent growth resulted at p_H 6.9, 6.5, 6.4, 6.0, 5.7 and 5.5, but not below this point (Fig. 4, D), while the controls showed positive growth at p_H 4.9 and above (Fig. 4, C).

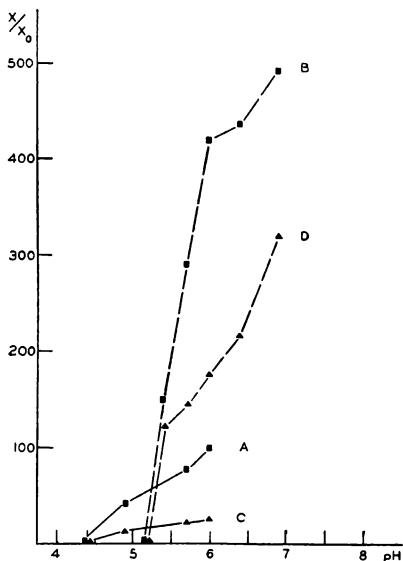


Fig. 4. Series VI. Growth of *Chlorogonium* with and without sodium acetate. A *C. euchlorum* after 71 hours without acetate. B Same, with acetate. C *C. elongatum* after 68 hours without acetate. D Same, with acetate.

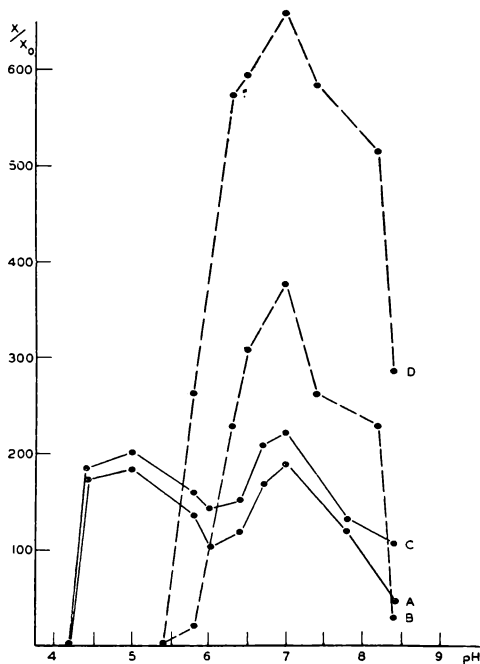


Fig. 5. Series VII. Growth of *Chilomonas* with and without sodium acetate at different hydrogen-ion concentrations. A Control, 45 hours. B With acetate, 45 hours. C Control, 68 hours. D With acetate, 68 hours.

Series VII. *Chilomonas*.

Since the threshold for acetate growth inhibition of *Chlorogonium* was at about p_H 5.3, a similar series of tryptone-acetate containing tubes was inoculated with *Chilomonas* to determine whether or not its range of growth below this point would be decreased in a similar manner. The initial concentration was 400 organisms per ml. Cultures at p_H 4.2, 5.0, 5.8, 6.0, 6.4, 6.7, 7.0, 7.8 and 8.4 were incubated for 68 hours. Tubes containing Bacto-tryptone without sodium acetate covering a similar p_H range served as controls.

A semi-final count of the acetate-containing and control cultures was made after 45 hours (Fig. 5, B and 5, A, respectively). Growth occurred in the controls over a p_H range of 4.4—8.4. with growth maxima at about 4.9 and 6.9, respectively, as observed in preceding experiments. However, growth in the acetate medium did not occur below p_H 5.8. After 66 hours the control series (Fig. 5, C) showed additional growth proportional to that observed at 45 hours. Cultures with sodium acetate, however, showed relatively more growth, especially at p_H 6.0—8.2. That the time element is important in comparing growth effects even in the same medium, is shown by comparing growth at p_H 5.8 and 8.4 in the experimental series and controls at 45 and 68 hours. At p_H 5.8 after 45 hours, growth with sodium acetate is less than in the control medium (cf. Fig. 5, B and A). After 68 hours, on the other hand, growth with sodium acetate is considerably better than in the controls (cf. Fig. 5, D and C). The same is true at p_H 8.4.

Discussion.

While numerous scattered observations have been made correlating growth of certain protozoa with p_H , relatively few species have been studied with respect to their p_H -growth range in a given medium. Such investigations have been made chiefly with Euglenida (cf. table 3) and they show that there are specific variations both as to range of growth and optimum for division. For instance, DUSI (1930) found that *Euglena gracilis* was more acid tolerant in a peptone medium than were any of the other species which he observed under the same conditions. Growth of *E. gracilis* over a relatively wide p_H range has been observed by JAHN (1931) and ALEXANDER (1931). It is also evident from the results of DUSI that *E. stellata* has a lower optimum p_H in light (5.5) than any of the other green forms which he observed. Most favorable growth of the other Euglenida occurs about the neutral point. Similar results were obtained for *Chlorogonium*, the single optimum for growth being slightly on the alkaline side of neutrality (p_H 7.4 for *C. euchlorum*) in a number of solutions which were used. The experiments with *C. elongatum* indicate a higher optimum p_H and a consistently lower growth rate, which may be correlated with its relatively greater size.

A bimaximal curve was obtained for *Chilomonas* in most of the media which were used, one optimum being at about p_H 4.9, and a second at about neutrality, with an intermediate low point at approximately p_H 6.0. In the original experiments on this form (LOEFER,

1932 b) only 0.05 % KH_2PO_4 was used as a buffer. After 3.5 days of incubation, cultures about the acid optimum had become slightly alkaline. In the experiments here outlined a heavier buffer was used and the time of incubation was considerably less so that changes greater than 0.2 p_H were not observed. From the results shown in table 3 it is seen that *Chilomonas* differs from *Chlorogonium* and the Euglenida. *C. paramecium* grows at p_H 4.2, while *Chlorogonium* did not multiply below 4.8. Also, the latter species grows well at p_H 8.7, while 8.4 marked the upper limit for *Chilomonas* in these experiments. These results are not in agreement with the findings of MAST and PACE (1933), who state (p. 342), "the process of division is largely independent of the . . . hydrogen-ion concentration between p_H 5.3 and 7".

Chilomonas resembles *Colpidium striatum* (ELLIOTT, 1933) more closely than it does either *Chlorogonium* or the Euglenida. The range of growth under similar conditions in a Bacto-tryptone medium is almost the same, although the optimum p_H for the ciliate appears to be somewhat higher (cf. table 3).

The minor discrepancies which are observed when one compares the reports of several investigators who have used the same species, are perhaps accounted for by temperature and time of incubation, culture medium, and other variables. That time of incubation is an important factor, even when other conditions are the same, is well illustrated in figure 5. After 45 hours incubation in a tryptone-acetate medium (Fig. 5, B) one might conclude that at p_H 5.8 and 8.4 sodium acetate was unfavorable for growth. After 68 hours, however, on comparing growth at the same hydrogen-ion concentration (Fig. 5, D and C) it is seen that sodium acetate is quite favorable for growth. If this difference is due to acclimatization, it is seen that *Chilomonas* can adapt itself to live at a lower p_H with sodium acetate than can *Colpidium striatum*, which does not multiply below p_H 6.2 in a similar medium, while *Chlorogonium* is capable of growth in the same medium at p_H 5.4. Time of inoculation after autoclaving is also a factor which must be carefully controlled since characteristic changes in oxidation-reduction potential are known to occur following this method of sterilization. The presence of certain salts in the medium also may influence the growth range of an organism. Such an effect has been demonstrated on bacteria by SHERMAN and HOLM (1922) and WINSLOW and FALK (1923). These investigators found that certain concentrations of NaCl limit the growth range of *B. coli*, although the optimum for growth remains unchanged. The writer's preliminary investigations on a bacteria-free strain of *Paramecium bursaria* indi-

Table 3.

Growth of various protozoa in bacteria-free cultures at different hydrogen-ion concentrations. Figures in parentheses indicate points of maximum growth.

Species	Investigator	Medium	Growth range observed PH
Euglenida:			
<i>Euglena gracilis</i>	DUSI, 1930 a	Beef peptone (Vaillant)	3.5—9.0
" <i>anabaena</i>	" 1930 b	" " "	6.5—8.0
" <i>deses</i>	" "	" " "	6.5 (7.0—7.5) 8.0
" <i>klebsii</i>	" "	" " "	5.5 (6.5) 7.5
" <i>pisciformis</i>	" "	" " "	6.0 (6.5—7.5) 8.0
" <i>stellata</i>	" "	" " "	4.5 (5.5) 8.0
" <i>gracilis</i>	ALEXANDER, 1931	" " "	3.0 (6.7) 7.7
" "	JAHN, 1931	Bacto-peptone	3.9 (6.6) 9.9
" <i>pisciformis</i>	HALL, 1931	Partially hydrolyzed casein	5.4 (6.8) 7.5
" <i>anabaena minor</i>	" 1933	Difco tryptophane broth	4.5 (6.9) 8.3
" <i>deses</i>	" "	" " proteose peptone	5.5 (6.9) 8.3
" <i>klebsii</i>	" "	" " tryptophane broth	5.3 (7.0) 8.0
" <i>klebsii</i>	R. H. HALL, MSS.	" " proteose peptone	5.7 (7.0) 8.0
" <i>klebsii</i>	R. H. HALL, MSS.	Bacto-tryptone	4.4 (7.0) 8.4
Phytomonadida:			
<i>Chlorogonium euchlorum</i>		Bacto-dextrose broth	4.8 (7.1)
		Bacto-peptone	5.0 (7.4) 8.7
		Bacto-tryptone	4.9 (7.4) 8.7
		" " and maltose	5.0 (7.5) 8.3
		" " and Na acetate	5.4 (7.4) 8.7
" <i>elongatum</i>		Bacto-peptone	5.0 (7.9) 8.7
		Bacto-tryptone	4.9 (7.6) 8.7
		" " and Na acetate	5.4 (6.9)
Cryptomonadida:			
<i>Chilomonas paramecium</i>		Bacto-dextrose broth	4.1 (4.8 and 6.8) 7.1
		Bacto-peptone	4.6 (5.1 and 7.1) 7.9
		Bacto-tryptone	4.2 (4.9 and 7.0) 8.4
		" " and Na acetate	5.8 (7.0) 8.4
Ciliata:			
<i>Colpidium striatum</i>	ELLIOTT, 1933	Bacto-dextrose broth	(5.5 and 7.5)
		Bacto-tryptone	4.5 (5.5 and 7.4) 8.5
	" 1935 a	" " and Na acetate	6.2 (7.0) 8.5
	" "	" " mannose	4.9 (5.5) 8.0
	" "	" " starch	4.5 (5.5 and 7.4) 8.5
	" "	" " maltose	4.5 (5.5) 8.5
	" "	" " levulose	4.5 (5.5) 8.5
	" 1935 b	Bacto-peptone	4.0 (5.7) 8.8
		Proteose-peptone	4.0 (5.7) 8.6
		Bacto-protone	4.0 (5.7) 8.0
		Bacto-veal	4.2 (6.5) 8.9
<i>Colpidium campylum</i>	" 1935 a	Bacto-tryptone	4.5 (5.5 and 7.4) 8.5
		" " and starch	4.5 (5.5) 8.5
		" " maltose	4.5 (5.5) 8.5
		" " mannose	4.9 (5.5) 8.0
		" " melezitose	4.5 (5.5) 7.4
		" " levulose	4.5 (5.5) 7.7
	" 1935 b	Bacto-peptone	4.0 (6.5 and 7.4) 8.8
		Proteose-peptone	4.0 (5.7) 8.6
		Bacto-protone	4.0 (6.5) 8.0
		Bacto-veal	4.2 (6.5) 8.9
<i>Glaucoma piriformis</i>	JOHNSON, MSS.	Bacto-tryptone	3.8 (5.3 and 6.6) 9.1

cate a similar result. HOPKINS and WANN (1925—1927) demonstrated that iron is a controlling factor in the growth of *Chlorella* sp. Addition of sodium citrate to the inorganic medium which they used prevented precipitation of iron above p_H 5.7, and thus permitted growth to occur. It is probable that tryptone, in relation to iron, acts in a manner similar to sodium citrate, since there was no evidence of inhibited growth of *Chlorogonium* above p_H 5.7.

In an earlier paper (LOEFER, 1934 a) it was pointed out that various carbohydrates and fatty acids accelerated growth at a hydrogen-ion concentration of p_H 7.0 or thereabouts. That the same relationship with sodium acetate does not hold over the entire growth range is clearly evident from the results of Series V, VI and VII (Figs. 3, D; 4 and 5). Although this compound greatly accelerated growth above p_H 6.0, it is quite toxic at greater hydrogen-ion concentrations. The fact that similar results obtain with *Chlorogonium*, *Chilomonas* and *Colpidium* (ELLIOTT, 1933) indicates that the toxicity is non-specific, though varying in degree as previously pointed out. CRUESS and RICHERT (1928) observed an analogous effect of sodium benzoate on food spoilage organisms. Retardation of the multiplication rate was always stronger below p_H 5.0 than above this value. Alcoholic fermentation of yeasts was similarly inhibited. The investigations of CRANE (1921) and BRESSLAU (1926) on *Paramecium* and *Colpidium campylum*, respectively, correlate toxicity with dissociation phenomena at different hydrogen-ion concentrations. An explanation for the observed effect of sodium acetate has not been advanced.

Summary.

The p_H -growth relationship of *Chilomonas paramecium*, *Chlorogonium euchlorum*, and *C. elongatum* has been determined for several types of media. *Chilomonas* has a range of p_H 4.1 to 8.4 and exhibits two growth optima (4.9 and 7.0) with an intermediate low point at p_H 6.0. Growth of *Chlorogonium euchlorum* increases progressively with p_H from 4.8 to an optimum at about 7.4, then decreases uniformly, growth still occurring at p_H 8.7. *C. elongatum* exhibits similar growth, the optimum being nearer p_H 7.8. Sodium acetate prevented growth of *Chlorogonium* below p_H 5.4 and growth of *Chilomonas* below p_H 5.8. Growth of all three species is greatly accelerated by sodium acetate about the neutral point. Comparative growth range and optimum p_H for various species in bacteria-free cultures is tabulated.

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