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Effect of hydrogen-ion concentration on the growth and morphology of *Paramecium bursaria*.

By

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With 3 figures in the text.

Introduction.

A series of investigations involving problems pertaining to the cultivation of protozoa is in progress, using a bacteria-free strain of *Paramecium bursaria* EHRENBURG as the experimental organism. Some of the specific problems arising from the association of zoochlorellae with *Paramecium bursaria* are discussed and an historical review of the work on this well known 'symbiote' is presented in an earlier paper (LOEFER, 1936). The importance of the concentration factor is demonstrated for certain organic and inorganic constituents of media and is discussed in relation to the growth of other protozoa. In another series of experiments (LOEFER, 1936 a) a number of protein media and carbohydrates were tested as to suitability for culturing this particular form. Bacto-tryptone and Proteose-peptone support indefinite growth if the cultures are transferred to fresh medium regularly; addition of dextrose to either of these media results in better growth.

While the above-mentioned investigations have dealt with the effect of various factors on growth as measured by numbers of organisms, there have been no extensive studies reported for any protozoa on the relationship of cultural conditions to growth and morphological variations. In the present paper not only growth, but

the morphology of *Paramecium bursaria*, i. e., its size and shape, is considered in relation to hydrogen-ion concentration of the cultures. The findings indicate a distinct relationship between p_H and morphology, and it appears to be independent of the p_H effect on growth.

Material and methods.

The bacteria-free strain of organisms used is one which has been maintained by frequent subculturing for more than two years (LOEFER, 1934) in this laboratory, and used in previous studies (LOEFER, 1936, 1936 a). Bacto-Tryptone (Difco), 0.5%, or Proteose-peptone (Difco), 0.75%, were added to the following mineral solution, portions of which were titrated to the p_H desired with N/1 NaOH or HCl before tubing and autoclaving:

NaCl	0.003 gm.
CaSO ₄	0.015 "
MgSO ₄	0.0045 "
KNO ₃	0.0013 "
FeCl ₃	0.0002 "
Glass distilled water to .	1 liter.

Twenty-four to thirty-six hours later the Pyrex tubes of each series (each tube containing 9 ml of media) were inoculated from a stock culture and then incubated at a north window (temperature, 19—26° C). After 12—21 days incubation, depending upon the series in question, the cultures were fixed by the addition of several drops of BOUIN's fluid. Before fixation final hydrogen-ion concentration was determined colorimetrically with a LA MOTTE Comparator. Growth determinations were made with the SEDGWICK-RAFTER counting-cell previously adapted for use with an ordinary dissecting binocular (LOEFER, 1936). The ratio of final to initial concentration of ciliates (x/x_0) was calculated and represented graphically. Size measurements were made with an ocular micrometer under high power magnification. The measurements represent averages of one hundred to two hundred ciliates selected at random from each type of culture. Calculations were made according to the following formulae: Standard deviation,

$$\sigma = \sqrt{\frac{\sum (fd^2)}{N}} \text{ where } f = \text{frequency, } d = \text{deviation, and } N = \text{total number of organisms measured. Probable error, } E_m = \frac{\sigma \times 0.6745}{\sqrt{N}}.$$

Coefficient of variation, C.V. = $\frac{100 \sigma}{m}$, where m = mean. The figures

were reconstructed from camera lucida drawings and average measurements. A haemocytometer was used to determine the concentration of zoochlorellae in the same cultures.

Growth in relation to p_H .

The purpose of series I was to determine the effect of p_H on growth of *Paramecium bursaria* in a tryptone medium. Eight-tube sets of media titrated to p_H values ranging from 4.4—8.9 were made up and autoclaved. Later they were given 1.0 ml inoculations from a tryptone stock culture containing 60 ciliates per ml. One tube of each set was used to check p_H , and cultures at the following p_H values were then incubated: 4.6, 5.1, 5.5, 6.0, 6.5, 6.9, 7.4, 7.7, 8.2 and 8.6. Examination of the cultures after 21 days indicated growth as shown in figure 1. Growth is accompanied by a rise in p_H , cultures below p_H 7.4 changing as much as 0.3 p_H . The greater changes occurred in those cultures with most ciliates. Cultures on the alkaline side of neu-

trality did not change as much as those below p_H 7.0 (Fig. 1). The results indicate a growth range for *Paramecium bursaria* from p_H 5.1—8.2, with a single optimum at 6.5—6.8 in a tryptone medium. Cultures at the optimal p_H just mentioned showed an average increase of more than 39 times. Those at p_H 4.6 still contained living organisms, although their number had not increased. These results are typical of several series with Bacto-tryptone.

Series II was similar to series I, except that Proteose-peptone was used instead of tryptone. Hydrogen-ion concentrations ranged from p_H 4.0—9.4, with the following p_H series after inoculation: 4.2,

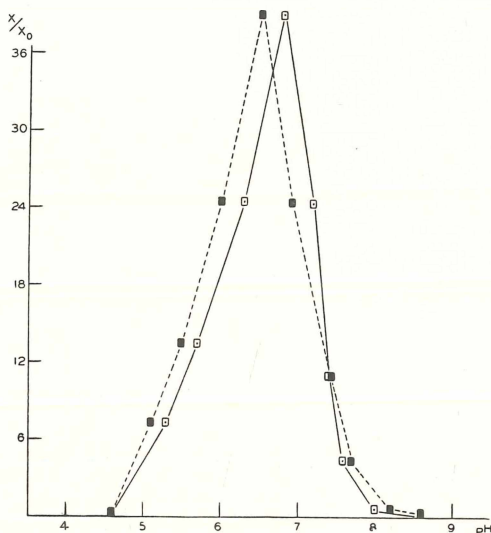


Fig. 1. Series I. Growth of *Paramecium bursaria* at different hydrogen-ion concentrations in a Bacto-tryptone medium. x/x_0 = ratio of final to initial concentration of ciliates per ml. p_H of cultures at the beginning of incubation (■-----■); p_H after 21 days growth (□——□).

4.5, 4.9, 5.4, 6.0, 6.4, 6.8, 7.2, 7.4, 7.8, 8.4, 8.7 and 9.0. The inocula were obtained from a 10-day Proteose-peptone culture and the initial count of ciliates was 37 per ml. After 12 days of incubation, growth determinations were made and plotted graphically as shown in figure 2. Growth was best in cultures at about p_H 6.8, although occurring over a range of 4.9—7.8. As in the preceding series, slight changes in p_H took place during the period of culture. Although cultures at p_H 4.2, 4.5, 8.2 and 8.4 contained living organisms, a decrease in number had occurred in each case.

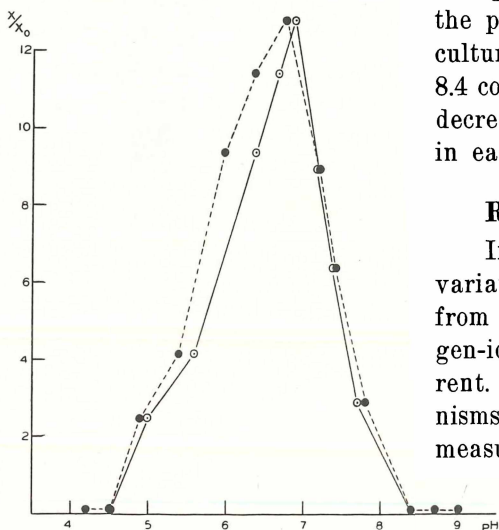


Fig. 2. Series II. Growth of *Paramecium bursaria* at different hydrogen-ion concentrations in a Proteose-peptone medium. x/x_0 = ratio of final to initial concentration of ciliates per ml. p_H of cultures at the beginning of incubation (●-----●); p_H after 12 days growth (○——○).

Relation of p_H to size.

In counting series I a marked variation in the size of ciliates from cultures at different hydrogen-ion concentrations was apparent. A large number of organisms from the series were measured with an ocular micrometer. Averages of length and width indicate a considerable size range in cultures at different hydrogen-ion concentrations (table 1). Organisms grown at p_H 6.0—6.3 were the longest in the series.

They averaged 129.3 microns in length, while those in more acid or alkaline media were considerably shorter. The shortest organisms had an average length of 86—87 microns and they were obtained from cultures at p_H 7.6—8.2. Ciliates grown at either the acid or alkaline extreme of the p_H range showed a relatively greater width as well as reduced length, although the greatest variation from the maximum is observed in the alkaline media. Individual variation in size is greatest at the extremes of the growth range. It is to be noted that no increase in number of ciliates occurred at p_H 4.6. This would account for the fact that no appreciable p_H change was observed during the culture period. The organisms were more spherical than normally, indicating a

Table 1.
Average size of *Paramecium bursaria*.

Initial p _H	Final p _H	Length in microns	Coefficient of variation-length	Width	Remarks
4.6	4.6	97.5 ± 3.32	31.5	51.4 ± 1.03	no growth growth " " " " " "
5.1	5.3	110.6 ± 1.04	10.7	48.4 ± 0.462	
5.5	5.7	126.6 ± 0.687	6.2	43.2 ± 0.280	
6.0	6.3	129.3 ± 0.829	7.7	44.4 ± 0.350	
6.5	6.8	115.8 ± 0.729	7.3	44.4 ± 0.448	
6.9	7.2	102.9 ± 0.632	7.0	44.2 ± 0.428	
7.4	7.4	100.2 ± 0.786	9.0	44.7 ± 0.611	
7.7	7.6	86.0 ± 0.784	9.8	46.0 ± 0.561	
8.2	8.0	87.0 ± 0.780	9.4	54.7 ± 0.781	
—	—	Avg. = 106.2	—	Avg. = 46.8	—

disturbance in metabolism. That p_H 4.6 is actually very near the lethal p_H is shown by the fact that the organisms do not increase in number. There is very little growth in the cultures at p_H 7.6—8.2,

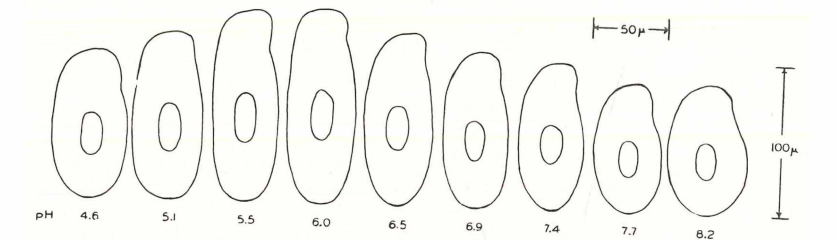


Fig. 3 Relation of p_H to size of *Paramecium bursaria*. Diagrammatic reconstructions from camera lucida drawings and size measurements shown in Table 1. Organisms were grown in a Bacto-tryptone medium. The numbers designate initial p_H of the cultures from which the ciliates were taken.

although the length of these ciliates is reduced to less than that of those at the acid limit. In figure 3 are shown diagrams drawn to scale to illustrate length and width of *Paramecium bursaria* grown at different hydrogen-ion concentrations. The longest and most typical forms are found in cultures ranging from p_H 5.4 to 6.8.

Discussion.

The growth series (I and II) illustrate that proteose-peptone is a better medium than tryptone for *Paramecium bursaria*. This was also shown in an earlier series of experiments (LOEFER, 1936 a). The reason for this difference is uncertain, although a possible explanation is suggested by the relative abundance of the amino acid tryptophane in Bacto-tryptone. HALL and ELLIOTT (1936) reported

that this amino acid is distinctly unfavorable to growth of *Colpidium*, even when present in low concentrations.

The p_H -growth range, however, is essentially the same in the two types of media, growth occurring over a range of 4.9–8.0. In cultures below p_H 4.9 the inoculated organisms were always found in a good state of preservation, although above p_H 8.0 the ciliates were completely disintegrated. The reason for this is probably found in MALM'S (1930) explanation that free hydrogen ions harden, while hydroxyl ions soften the pellicles of ciliates. In both series the optimum hydrogen-ion concentration for growth is near p_H 6.8. In this connection DARBY (1929) observed optimum growth of *Paramecium caudatum* at a p_H near neutrality. A similar optimum for growth is characteristic of a number of forms which have been studied under bacteria-free conditions (cf. LOEFER, 1935, Table 3).

It may be suggested that *Paramecium bursaria* lives entirely on zoochlorellae and that near the ends of the p_H growth range the food supply is curtailed, which would account for the smaller number of ciliates observed per ml. However, if one compares the number of ciliates in series I at p_H 6.3 with those at p_H 7.2, they are about equal. The algae in the latter cultures, however, number five to six times as many as in the more acid medium. So if it were merely a matter of abundant food supply (algae?) the cultures at p_H 7.2 might be expected to have relatively more ciliates per unit volume than those at p_H 6.3, which however, is not the case.

Measurements of *Paramecium bursaria* vary from an average of 86 or 87 microns in media of p_H 7.6–8.2 to 129 microns at p_H 6.0–6.3. These lengths differ somewhat from the figures given by WENRICH (1928), who lists 120–160 microns as the average length of the species; KUDO (1931) lists 100–200 microns as the length and 50–60 microns as the average width. Reference to table 1 shows that the average width of this strain of *Paramecium bursaria* is less than the limits given by KUDO, while the average length is less than that given by WENRICH. At p_H 4.6 there was no increase in number of organisms during the period of culture, but there is much variation in the length and width of the ciliates, as seen by the large coefficient of variation. The variation is much less pronounced in all the other cultures in which growth occurred.

The optimal p_H for growth and length is not the same. The longest and most typical forms were observed in cultures at p_H 6.0–6.3 (Fig. 3), while the optimum for growth was in the vicinity of p_H 6.8. The smaller size cannot be correlated with more rapid division, since

cultures at p_H 6.0—6.3 and 6.9—7.2 contained about the same number of ciliates, although their lengths varied from 129 to 103 microns, respectively. In this connection, ELLINGSON (1930) found that population density of *Amoeba proteus* was greatest at p_H 7.8—8.4 and that size also was greatest in this range.

The problem of morphological variation as determined by medial factors is one which has not yet been extensively investigated, although it appears frequently as a secondary problem in protozoological literature. The investigations of VOLKONSKY (1930) have shown that the reserves of *Polytoma uella* are determined by medial constituents, e. g., in young sodium-acetate cultures starch was stored, while protein reserves were accumulated in a butyrate-containing medium. The effect of temperature on STENTOR was studied by ZINGHER and FISIKOW (1931), who reported a seasonal variation of 19—25 microns in size. ZINGHER, NARBUTT and ZINGHER (1932) observed an average fluctuation of 62 microns between starved and normal strains of a large race of *Paramecium*, as well as a marked variation in *Stylonychia* in similar experiments. They conclude: "Die Leibesform von *Stylonychia pustulata* und *Paramaecium caudatum* verändert sich scharf (besonders bei den Stylonychien) im Zusammenhang mit verschiedenen Nahrungsmengen im Milieu", p. 91.

The importance of induced variation in species diagnosis can hardly be overestimated. Certain findings on *Glaucoma* and *Colpidium* (mss.) show that in certain media these ciliates are as much as 25 % larger than controls in tryptone. Since size is often used as a specific criterion and since species have been described from all sorts of 'hay' infusions, this factor is of importance from a taxonomic standpoint, and the variations of a given species should be known before a positive identification is made. LEFÈVRE (1931) demonstrated the variability of single characteristics in *Euglena* and *Phacus*. Changes in hydrogen-ion concentration caused variation in "cell metabolism" and cell form. He expressed the opinion that size variations are more numerous than generally believed and that the forms described as "major" and "minor" should no longer be considered valid. LEFÈVRE (1932) found that *Euglena deses* practically lost its striations after a year of cultivation, and that *Euglena spirogyra* lost its stripes when conditions were unfavorable, although they did not disappear in forms grown under favorable conditions.

These findings concerning induced morphological variations indicate the necessity for uniform procedures in taxonomic work on

protozoa. Obtaining bacteria-free cultures is only one step in that direction, and more must be known about the general physiology of many protozoa before procedures regarding physical and chemical conditions of culture can be standardized.

Summary.

Growth of *Paramecium bursaria* in relation to p_H of the medium was determined in a Bacto-tryptone and in a Proteose-peptone medium. Growth was observed from p_H 4.9—8.0, with a maximum at about p_H 6.8. Measurements of the ciliates in the tryptone series were taken. They varied from an average length of 86 or 87 microns at p_H 7.6—8.0 to 129 microns at p_H 6.0—6.3. Greatest variation was observed in cultures at p_H 4.6, in which no growth had taken place. The shortest organisms, at the acid and alkaline extremes of growth, were the widest, while those in cultures from p_H 5.7—7.4 were narrowest, having an average width of 44 microns. The optimum for size therefore does not correspond with the p_H most favorable for multiplication. Induced morphological variation is discussed and the need for a standardized procedure for culturing protozoa is pointed out.

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