# THE EFFECT OF pH ON PURE CULTURES OF EUGLENA MUTABILIS[1]

HERMAN VON DACH,
Department of Physiology, Georgetown University
School of Medicine, Washington, D. C.

The extraordinary acid-tolerance of *Euglena mutabilis* Schmitz has recently been pointed out by Lackey ('38). He found this green flagellate in abundance in numerous pools and streams polluted by acid drainage from coal mines, at acidities as high as pH 1.8. He stated that this one organism was the most characteristic of the highly acid streams.

Stock cultures of *Euglena mutabilis*, contaminated by fungi and bacteria, have been maintained in the protozoology laboratory of Ohio State University for several years at pH 3 or 4, in a medium devised by Kostir and Lotze. The present paper gives the results of a quantitative study on the effect of pH on survival and growth of this organism in pure culture.

For accurate experimental work a pure culture was of course indispensable. A single cell of *Euglena mutabilis* was washed free of bacteria and fungi by migration in a capillary pipette (a micro-modification of the "migration-pipette" method of Glaser and Coria, '30). A pure clone culture was then established for use in the experiments which followed.

## METHODS

The following culture medium, a slightly modified form of the Kostir-Lotze medium, was employed in all experiments:

| | |
|---|---|
| $KH_2PO_4$ | 0.25 gm. |
| KCl | 0.25 gm. |
| $NaHCO_3$ | 0.25 gm. |
| $Ca(NO_3)_2$ | 1.50 gm. |
| $MgSO_4$ | 0.50 gm. |
| $FeCl_3 \cdot 6H_2O$ | 0.0004 gm. |
| $MnSO_4$ | 0.0002 gm. |
| Aminoids[2] | 0.50 gm. |
| Triple-distilled water | 1000 cc. |

Adjustment of pH to the various desired levels was made by adding strong solutions of NaOH or $H_2SO_4$; then the medium was measured into Pyrex tubes (20 x 150 mm.) and autoclaved.

Three separate experiments were performed. In each experiment, all cultures were inoculated from a single source culture of pH 3.4 whose cell concentration was known. The culture tubes were then incubated at room temperature near a north window. After incubation, three cultures at each pH value were used for making cell counts, microscopic examinations of the cells, and pH determinations. The amount of growth was determined by comparing the concentration of cells per cubic centimeter after incubation (X) with the initial cell concentration ($X_o$), and expressed as ratio of growth ($X/X_o$).

The customary bacteriological techniques and tests for purity of cultures were employed. Cell counts were made with a Whipple ocular micrometer and a Sedgwick-Rafter counting cell. Determinations of pH were made after inoculation and after incubation, by means of a Hellige colorimetric comparator and a Leeds and Northrup glass electrode. During the experiments the pH of the various culture series remained constant within 0.1 of a pH unit.

---

[1]Contributions from the Department of Zoology and Entomology, Ohio State University, No. 115.

This work was done while the writer held a Muellhaupt Scholarship at the Ohio State University, 1939–40.

[2]A casein digestive product of the Arlington Chemical Co., Yonkers, N. Y.

## RESULTS

Table I gives the results of three separate experiments. These results show that *Euglena mutabilis* survived for at least 12 days over a range of pH 1.4 to pH 7.9. The total range of growth was from pH 2.1 to pH 7.7, with maximum growth between pH 3.4 and pH 5.4.

In Exp. III, cells from the various culture series were examined at one- or two-day intervals. At pH 0.9 all cells were dead within 24 hours. At pH 1.4 (equivalent to approximately N/25 sulfuric acid) some cells remained alive for as long as 12 days, remaining bright green and showing slight but unmistakable movements during this period. At pH 1.9 some cells remained alive for 13 days.

*Euglena mutabilis* tolerates a greater hydrogen-ion concentration for survival and for growth than any protozoan in pure culture previously reported. (See tables of pH-growth relationships, Loefer, '35, p. 220; Calkins and Summers, '41, p. 540.)

TABLE I. EFFECT OF pH ON EUGLENA MUTABILIS CULTURES

$X_o$ = initial number living cells per cc.
X = number living cells per cc. after incubation.
$X/X_o$ = ratio of growth.
$X_o$ in Exp. I = 100 cells per cc.
Exp. II = 30 cells per cc.
Exp. III = 335 cells per cc.

| pH | $X/X_o$ AT 12 DAYS | | | $X/X_o$ AT 24 DAYS | | |
|---|---|---|---|---|---|---|
| | Exp. I | Exp. II | Exp. III | Exp. I | Exp. II | Exp. III |
| 0.9 | ..... | —* | —* | ..... | —* | —* |
| 1.4 | <1 | —* | <1 | —* | —* | —* |
| 1.9 | <1 | ..... | <1 | —* | ..... | —* |
| 2.1 | ..... | ..... | 20 | ..... | ..... | 90 |
| 2.4 | 16 | 42 | ..... | 74 | 372 | ..... |
| 3.4 | 62 | 210 | ..... | 165 | 644 | ..... |
| 4.0 | ..... | 218 | ..... | ..... | 680 | ..... |
| 4.2 | 65 | ..... | ..... | 158 | ..... | ..... |
| 4.8 | ..... | 193 | ..... | ..... | 793 | ..... |
| 5.4 | ..... | 144 | ..... | ..... | 704 | ..... |
| 6.0 | 4.8 | ..... | ..... | 4.6 | ..... | ..... |
| 7.7 | 1.9 | 2.9 | ..... | 2.2 | 2.1 | ..... |
| 7.9 | ..... | <1 | ..... | ..... | —* | ..... |
| 8.2 | ..... | —* | ..... | ..... | —* | ..... |

* No living cells present.

## SUMMARY

In pure clone culture in an organic medium, *Euglena mutabilis* survived for at least 12 days over a range of pH 1.4 to pH 7.9. Its total range of growth was from pH 2.1 to pH 7.7, with maximum growth between pH 3.4 and pH 5.4.

## LITERATURE CITED

**Calkins, G. N.,** and **F. M. Summers.** 1941. Protozoa in biological research. New York.
**Glaser, R. W.,** and **N. A. Coria.** 1930. Methods for the pure culture of certain protozoa. Jour. Exp. Med., vol. 51, p. 787.
**Kostir, W. J.,** and **J. C. Lotze.** Unpublished data.
**Lackey, J. B.** 1938. The flora and fauna of surface waters polluted by acid mine drainage. Public Health Reports, vol. 53, p. 1499.
**Loefer, J. B.** 1935. Relation of hydrogen-ion concentration to growth of Chilomonas and Chlorogonium. Arch. f. Protistenk., vol. 85, p. 209.