

THE COMPARATIVE RESISTANCE OF DIFFERENT SPECIES OF EUGLENIDAE TO CITRIC ACID.*

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In the course of his experiments on the nutrition of *Euglena gracilis*, Zumstein (1900) discovered that this species is able to tolerate surprisingly high concentrations of certain organic acids. He made use of this fact to obtain cultures free, or nearly free, from bacteria. Adding various proportions of the acid to the culture medium employed, he found that while the multiplication of bacteria was effectively inhibited, rich cultures of apparently normal Euglenæ could be obtained. Ternetz (1912) working with the same species, also made use of this method.

Of several organic acids experimented with, Zumstein found citric acid to be the least harmful to this organism. He reports that solutions of 0.5 to 2% are "not injurious," and that cultures to which these percentages of the acid had been added were quite successful. Whether all the individuals used in inoculating these cultures survived the transfer to the acid medium is unfortunately not clear from Zumstein's account. In the case of "3 and 4%," he states that many individuals remained living even after 88 hours. In "4 and 5%" many were actively moving after five days. In "5 and 6%" a few remained alive even after 17 days. Higher concentrations were apparently found to be uniformly fatal, though Zumstein makes no definite statement as to the lowest percentage sufficient to kill all the individuals exposed to it.

Unfortunately, the results of these experiments have been interpreted by the writers of certain text-books and manuals in a way not at all justified by the facts. Results obtained on a single species have been made the basis for generalizations concerning the whole genus *Euglena*, and even the family Euglenidæ. Prowazek, in his "Einfuehrung in die Physiologie der Einzelligen" (1910), Lemmermann, in the section on the Euglenidæ in "Die Suesswasserflora Deutschlands, Oesterreichs, und der Schweiz" (1913), and Doflein, in the fourth

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edition of his text-book (1916) all either definitely state or imply that a high degree of resistance to organic acids is characteristic of *Euglenæ* (or even of *Euglenidæ*) in general.

The present writer became interested in this matter when, having successfully cultivated *Euglena gracilis* in strongly acid media, he attempted to use similar methods with *Euglena deses*. These attempts met with failure in every case, and even relatively weak solutions of citric acid proved fatal to this species.

How was this to be interpreted? Is the case of *Euglena deses* merely a striking exception to the general rule for the group? Or is *Euglena gracilis* the exceptional form? Or, lastly, may it be that no general rule for the group is justified?

Good cultures of several species of *Euglena* and of one species of the closely related genus *Phacus* were at hand at the time, and it occurred to the writer that a comparatively simple series of experiments with them might throw light on these questions. Such a series of experiments was actually planned and carried out, as described in the following paragraphs.

A quantity of clear surface water was procured from a near-by pond (known to be a habitat of several species of *Euglena*), and filtered. Tests showed it to be decidedly alkaline in reaction. By careful titration, using neutral red as an indicator, it was found that 1 c. c. of 1% HCl (chemically pure) was needed to bring 190 c. c. of this water to the point of neutrality corresponding to that of distilled water. Accordingly, the original quantity of filtered water was now divided into two parts, one of which was left unchanged, while the other was made neutral by the addition of the appropriate quantity of 1% HCl. With this neutralized pond water the following solutions of citric acid were then made up (chemically pure citric acid being employed): .025%, .05%, .1%, .25%, .5%, 1%, 2%, 3%, 4%, 5%.*

As seven different species were available for the experiment, seven series of these solutions were now put into Syracuse watch

*It may be noted that the hydrogen ion concentration of these solutions is by no means proportional to the concentration of the acid, since the degree of ionization decreases as the concentration of the acid becomes greater. But the work of Collett (*Jour. Exp. Zool.*, 1919) and others has shown that other factors besides hydrogen ion concentration are involved in the toxicity of organic acids; hence, for the purposes of these experiments, known concentrations of the acid are preferable to known concentrations of hydrogen ions. Per cent solutions were used rather than molar solutions in order to make the results directly comparable to those of Zumstein.

glasses, 5 c. c. of liquid in each watch glass. To each series were added, in similar glasses, both neutralized and unneutralized pond water without citric acid, these to serve as controls; and each series was then inoculated with a different species, about five or six individuals being introduced into each watch glass. The quantity of culture medium introduced with these was so slight as to be negligible. As the watch glasses were kept stacked (close to a north window and away from the direct rays of the sun) except when actually being handled, it is evident that evaporation was also a negligible factor.

Since the object was purely to get a measure of the *comparative* resistance to the acid, no attempt was made to determine the length of time that the organisms could live in a given concentration of the acid. Instead, all the watch glass cultures were very carefully examined just 24 hours after inoculation, and the condition of every individual was noted. In every case all the organisms in the controls (both neutralized and unneutralized pond water) were found to be alive and normal in every way, showing that the results observed in the other solutions were actually due to the addition of the citric acid, and not to anything in the original pond water, nor to the HCl introduced in neutralizing it.

The results from the citric acid solutions are concisely expressed in the accompanying table. In each case where all the individuals in the watch glass were still alive after 24 hours, the proper space has been left blank. Where part of the individuals were dead, an asterisk (*) is used; and where all were found dead, a large X. In the case of each species, therefore, the column with the last blank space (counting from left to right) indicates the highest percentage of the acid successfully withstood by one hundred per cent of the individuals tested; while the column with the first X indicates the lowest percentage causing the death of one hundred per cent.

The criterion used in determining whether an individual was dead or alive was the unmistakable change in color and appearance which ensues shortly after death has occurred. Movement can not be used as a criterion in this group, since individuals may temporarily remain perfectly motionless for a considerable length of time.

It will readily be seen from the table that the tolerance of different individuals of the same species is not necessarily the same. Some of the *Euglena deses*, for example, succumbed to the .05%, while the rest were killed by the .1%. In *Euglena gracilis* the variability in this regard is very great, a fact shown by Zumstein's results also. Similar physiological variability among the individuals of a species and even in the same culture is a phenomenon which the writer has repeatedly observed in connection with factors other than acidity. Its significance remains to be shown.

TABLE I.
PERCENTAGES OF CITRIC ACID CAUSING DEATH WITHIN 24 HOURS.

*—Some, but not all, dead.
×—All dead.

	.025%	.05%	.1%	.25%	.5%	1%	2%	3%	4%	5%
<i>E. deses</i> Ehrenb.		*	×	×	×	×	×	×	×	×
<i>E. acus</i> Ehrenb.			×	×	×	×	×	×	×	×
<i>E. geniculata</i> Duj. (?)			×	×	×	×	×	×	×	×
<i>E. ehrenbergii</i> Klebs			*	×	×	×	×	×	×	×
<i>E. oxyuris</i> Schmarda				×	×	×	×	×	×	×
<i>Ph. anacoelus</i> Stokes				×	×	×	×	×	×	×
<i>E. gracilis</i> Klebs							*	*	*	×

The most striking fact brought out by a perusal of the table is the remarkable difference between *Euglena gracilis* and all the other species. Thus the species first studied in this connection, and assumed by various writers to be typical of the other species of its group, is seen to be decidedly exceptional when compared with the six other forms that have been tested.

One discrepancy between these results and those of Zumstein may be noted. All the individuals of *Euglena gracilis* in the 5% solution were found dead; while Zumstein states that a few remained alive in 5 and 6%. In view of the great variability which we have noted in this species, and the relatively small

numbers of individuals tested, this difference is undoubtedly without significance.

Further study on the physiology of these interesting organisms, to include, it is hoped, a more detailed investigation of their behavior toward acids, is in progress.

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