(From the Department of Bacteriology, University of Kansas, Lawrence.)

Correlation of Immunologic and Physiologic Types of *Euglena gracilis* Klebs [1].

Von

Mary Elmore Sauer.

The present paper describes reactions which distinguish two groups within the species *Euglena gracilis* Klebs.

Ternetz (1912) has shown that *Euglena gracilis* can assume four distinct forms:

1. The normal green form encountered in nature.
2. The hyaline dark form. If the green form is put in the dark and supplied with organic nutrient materials, the chloroplasts lose their chlorophyll and the euglenas become perfectly colorless; however, they revert to the green form in the light.
3. The hyaline light form, a variant lacking chloroplasts, hence, permanently colorless even in the light.
4. An unstable form intermediate between the first and third.

Materials and Methods.

Six strains of *Euglena gracilis* of widely different origin were used. Each was a pure culture originating from a single cell and maintained free from bacteria. All were maintained as the normal green form. The hyaline dark modification of each could be produced at will by growth in the dark. The hyaline light form of one strain was available. In addition to the cultures in the light,

[1]) Submitted to the Department of Bacteriology and the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

cultures of this strain had also been kept in the dark from the time of its first isolation. After more than five years of continuous cultivation in the dark, it was found upon bringing transfers into the light that one of the tubes remained colorless in the light.

The medium used in the cultivation of *Euglena gracilis* was that adopted by the writer (1928 a) in a study of the preparation and effect of immune serum, and consisted of a 0.25 Proz. peptone broth to which inorganic salts had been added. This paper showed that the serum of rabbits immunized to *Euglena gracilis* contained a specific cytotoxin, in the presence of which the euglenas discard their flagellum and settle to the bottom of the tube.

Experimental work.

The strains of *Euglena gracilis* cultivated fell into two distinct groups as to their growth in the liquid medium used. Jahn's (1931) partially hydrolyzed casein medium for *Euglena gracilis* was tried, with the same results. These are tabulated below, the names attached to the strains being merely convenient designations:

Luxuriant growth	Delicate growth
Noland	Prague
Turtox	Michigan
Jewell	Rochester

No morphological differences were noted in living or stained preparations. All of the strains were submitted to Dr. L. B. Walton, who replied that he was quite certain that they should be referred to *Euglena gracilis* Klebs. The Prague strain had been received from Dr. Felix Mainx as a pure culture of *Euglena gracilis*. He stated in a personal communication concerning the Noland strain which had been sent to him that it was entirely identical physiologically with his own cultures of this species. He uses a different medium, and did not give any basis for this opinion. It is hardly thought that this difference in manner of growth is an adaptive change on the part of the organism to prolonged cultivation, for this difference has been noted since their first isolation in pure culture, and with continued cultivation the delicate forms have shown no change. It is thought rather that this is one indication of at least two distinct gracilis types. In case this is true, immunological tests should bring out this difference even more clearly.

Accordingly, suspensions containing approximately the same number of organisms from cultures of the same age were prepared

of each of the various strains and forms of *Euglena gracilis* available. Various dilutions of anti-Noland (normal green) serum were prepared. This was an immune serum obtained as previously described by the writer (1928 a), and active against the Noland strain in 1 : 1000 dilution. Each dilution of serum was mixed with an equal volume of *euglena* suspension in a small glass cell, and the reaction observed under low magnification. The results were distinct within an hour. They are summarized in the accompanying table, where plus and minus signs are used to indicate the effect of the serum upon the euglenas. A minus sign denotes that no change could be observed, the euglenas continuing to swim actively, a plus-minus combination that any effect was doubtful, one-plus denotes slight effect, and four plus complete sedimentation of the euglenas, no motile forms being present.

Table 1.

Cytotoxin reaction with various strains and forms of *Euglena gracilis* KLEBS, using anti-Noland (normal green) serum, titer 1 : 1000.

Organism	Serum dilution					
	1 : 50	1 : 250	1 : 500	1 : 1000	1 : 5000	saline
Noland						
green	++++[1]	++++	++++	++++	—	—
hyaline light	++++	++++	++++	++++	—	—
hyaline dark	++++	++++	++++	++++	—	—
Turtox						
green	++++	++++	++++	++++	—	—
hyaline dark	++++	++++	++++	++++	—	—
Jewell						
green	++++	++++	++++	++++	—	—
hyaline dark	++++	++++	++++	++++	—	—
Prague						
green	+	+—	—	—	—	—
hyaline dark	—	—	—	—	—	—
Michigan						
green	+[2]	+—	—	—	—	—
hyaline dark	—	—	—	—	—	—
Rochester						
green	—	—	—	—	—	—
hyaline dark	—	—	—	—	—	—

[1]) ++++ indicates no motile forms, complete sedimentation.

[2]) Fewer organisms in suspension used.

It is seen from the table that the strains fall into two groups which are exactly correlated with the two groups tabulated above in connection with the growth characteristics. The Turtox and Jewell strains react to the titer of the serum, while the serum is

practically without effect upon the Prague, Michigan, and Rochester strains. The one hyaline light form available and the hyaline dark form of each strain react just like the corresponding green form.

Discussion.

The division of *Euglena gracilis* into two groups by means of the cytotoxin antibody as well as by means of the hitherto unnoticed physiological difference in manner of growth is so distinct that it would seem to be of fundamental significance. The additional correlation in growth characteristics would indicate that the groups are fully as distinct as the serologic types common among the bacteria.

That at least the hyaline dark form is not identical antigenically with the normal green form has been shown in another place (Elmore, 1928 b), but that the various forms (normal green, hyaline light, hyaline dark) of one strain are more closely related serologically than corresponding forms of different strains is indicated by the above results.

Summary and Conclusions.

Euglena gracilis Klebs is not a homogeneous group, but consists of at least two distinct types. This is demonstrated by the cytotoxic effect of an immune serum, and is correlated with the growth characteristics. The various forms (normal green, hyaline light, hyaline dark) within one type show a closer antigenic relationship to each other than they show to corresponding forms of the other type.

References.

Elmore, Mary E. (1928 a): Antigenic properties of Euglena gracilis. Journal of Immunology Vol. 15 pp. 21—32.

— (1928 b): The production of anaphylaxis with Euglena gracilis and certain other unicellular chlorophyll-bearing organisms. Journal of Immunology Vol. 15 pp. 33—36.

Jahn, Theo. L. (1931): Studies on the physiology of the euglenoid flagellates. III. The effect of hydrogen ion concentration on the growth of Euglena gracilis Klebs. The Biological Bulletin Vol. 61 pp. 387—399.

Ternetz, Charlotte (1912): Beiträge zur Morphologie und Physiologie der Euglena gracilis Klebs. Jahrb. f. wiss. Bot. Bd. 51 pp. 435—514.