Technical Papers

Conjugation in *Tetrahymena*[1]

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Ten wild strains of *Tetrahymena* sp. have been derived from single cell isolations, and in all of them conjugation occurs readily within each clone. Following isolation from various natural sources near Ann Arbor, Mich., the cells were grown in a medium of boiled and filtered Cerophyll (1.5 g/l) which had been inoculated the previous day with *Aerobacter aerogenes*. Conjugation occurs consistently soon after the nutrient medium has been exhausted. Seven of the ten strains have been established in chemically defined media.

During conjugation the cells are attached only at their oral surfaces, though temporary attachments may occur elsewhere. Since the oral surface is near the anterior end of the cell, the conjugating pairs characteristically flare at a wide angle (Fig. 1). In

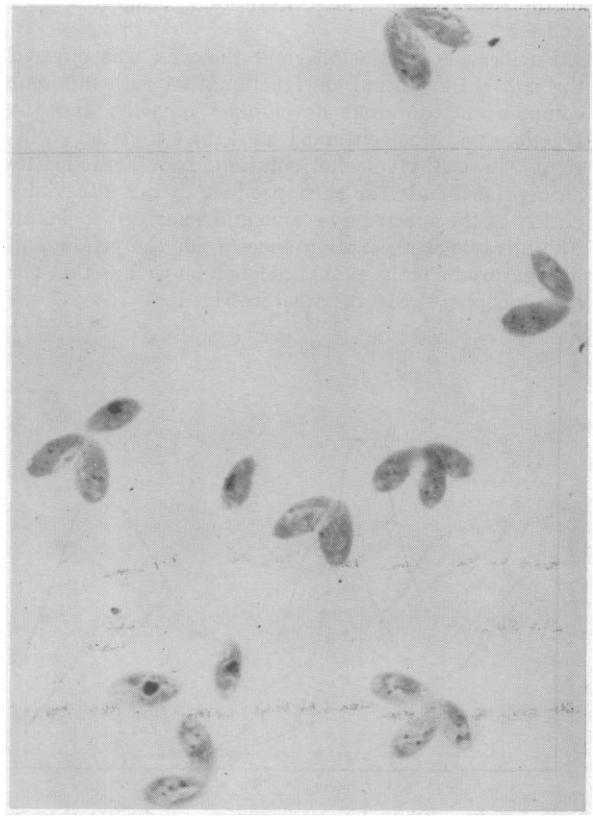

FIG. 1.

[1] Waldo Furgason has tentatively identified these organisms as previously undescribed species of the genus *Tetrahymena*. His observations have been confirmed, by Corliss, who in a preliminary morphological study, cautions, however, that differences between this ciliate and the type species of the genus are so slight that discovery of intermediate forms may make inadvisable their separation into two species.

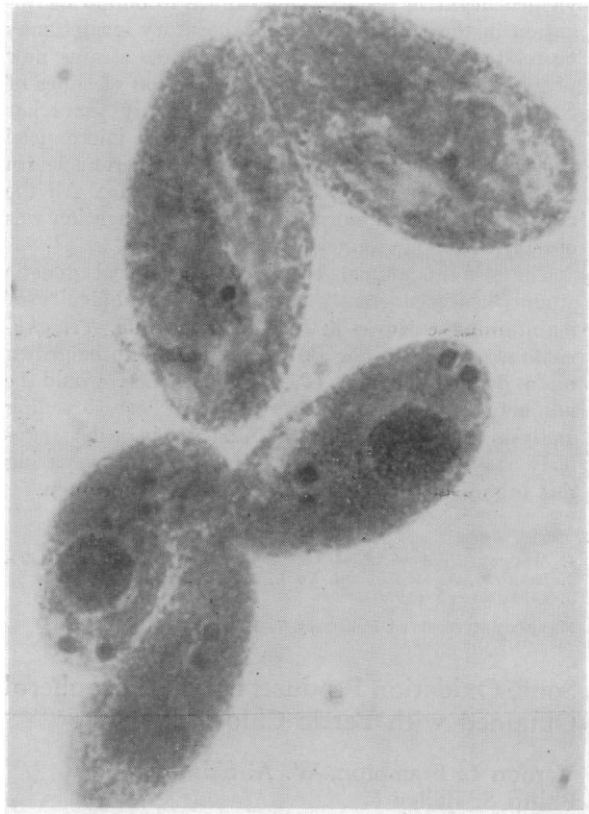

FIG. 2.

cultures in which most of the cells are conjugating, the union of three cells is often observed (Fig. 2). In this case the three cells are firmly attached by their oral surfaces. Preliminary observations would indicate that tripolar fertilization takes place, but genetic proof would be necessary to establish this conclusion. The details of the conjugation process and some of the interstock variations will be presented in a later paper. The general features of the conjugation process resemble those reported by Maupas (*1*) for *Leucophrys patula*.

Observations thus far indicate that conjugation within a clone results in death of the conjugants. Following an exchange of nuclei, the cells fail to separate, and after a number of hours coalesce, become vacuolated, and finally lyse. Attempts are now being made to find conditions under which the conjugants will survive. It has not yet been determined whether conjugation between cells of different origin also results in death, or indeed whether such conjugations occur.

Repeated attempts by one of the authors (A. M. E.) to observe conjugation during the past 19 years with a particular bacteria-free strain of *Tetrahymena geleii* (E) have uniformly met with failure. Mixing various bacteria-free strains (E, W, GL, H) and fixing them at 30-sec intervals over the entire growth period (6

days) showed no evidence for conjugation or other types of nuclear reorganization. Bacterizing the same cultures and mixing them in all combinations have yielded negative results. This failure to obtain conjugation in the long-maintained laboratory strains may be related to the fact that none of these strains now possesses micronuclei. Recent examination of slides of Strain E prepared at intervals for many years has shown that this strain has not possessed micronuclei since 1936, though it may have had micronuclei in 1932 soon after it was isolated from nature. All the newly isolated wild cultures in which conjugation was observed have micronuclei.

The use of animal cells for biochemical genetic studies has been greatly retarded by the difficulty of maintaining cultures in a defined medium. *Tetrahymena* is one of the few animal cells that can be grown on a defined medium (*2, 3*) and, hence, would be uniquely suited for such studies if crossbreeding analysis were possible. Obtaining conjugation regularly may be considered the first step in preparing this organism for increased use in the laboratory.

References

1. MAUPAS, E. *Arch. Zool. exptl. gen.*, Ser. 2, **7**, 149 (1889).
2. ELLIOTT, A. M. *Physiol. Zoöl.*, **22**, 337 (1949).
3. *Ibid.*, **23**, 85 (1950).

Manuscript received February 7, 1952.

Some Oxidation Products of DL-α-Tocopherol Obtained with Ferric Chloride

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Interest in the products obtained upon the mild oxidation of the tocopherols is aroused in a consideration of their fate during the development of oxidative rancidity in vegetable oils, in which they occur, and in consideration of their possible role in muscle metabolism.

This communication is to record the isolation of five products, four colored oils and a colorless wax, formed upon the oxidation of DL-α-tocopherol with ferric chloride in methyl alcohol. Some of these same products are also obtained when gold chloride is used as the oxidizing agent. (Ferric and gold chlorides are used in the more popular methods for tocopherol assay [*1, 2*], and their usefulness for this purpose is based on the fact that the chief product obtained at room temperature with both these salts is the well-known tocoquinone [*3*].)

The oxidation products were resolved chromatographically on a zinc carbonate column when the chromatogram was developed with petroleum ether (b. 60°–75°). The bands, in descending order, were blue-gray, purple, orange, and yellow in color, and the colorless wax was found in the effluent liquid; when the chromatogram was developed with diethyl

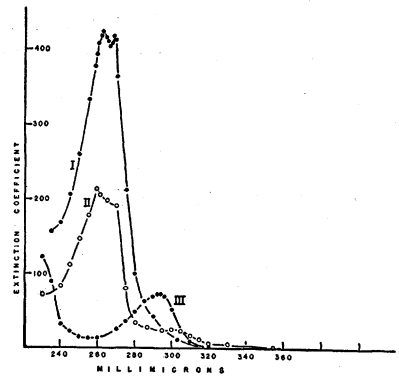

FIG. 1. Ultraviolet absorption spectra for the yellow tocoquinone (I), the red ortho quinone (II), and for DL-α-tocopherol. The ordinate is $E^{1\%}_{1\ cm}$, and the abscissa is in mμ.

ether, the positions of the purple and the blue-gray bands were reversed. The relative proportion of each product formed is a function of the conditions under which the oxidation was carried out; when the oxidation was carried out for 3 hr with ferric chloride in great excess, and at 50° in methyl alcohol, the yields, in the order given above, were 14.5, 32.5, 42.0, 10.4 and 0.5%.

Purification of the oils and the wax was effected through chromatographic fractionation with different columns and different developing solvents, and the fractionation was continued until the extinction coefficients, in methyl alcohol solution, for characteristic absorption bands for each product in the ultraviolet region of the spectrum became and remained constant. The ultraviolet absorption spectra for the yellow and orange products, in methyl alcohol, as well as that for DL-α-tocopherol, are recorded in Fig. 1.

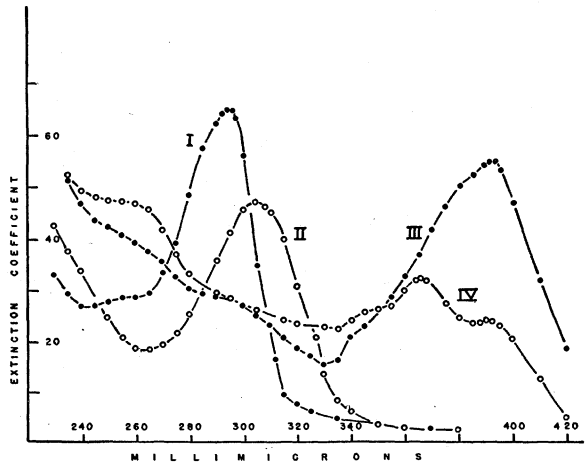

FIG. 2. The ultraviolet absorption spectra for (I) the acid form and (II) the basic form of the purple oil and for (III) the basic and (IV) the acid form of the blue-gray oil. The ordinate is $E^{1\%}_{1\ cm}$, and the abscissa is in mμ.

Both the blue-gray and purple oils have indicator properties, being blue-gray and purple, respectively,