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.

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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

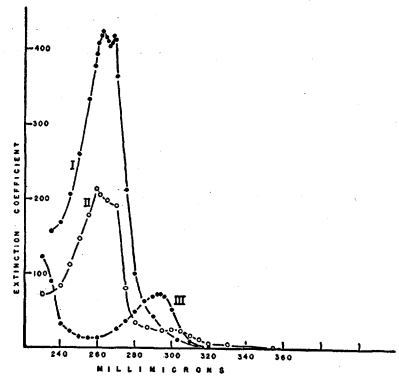

FIG. 1. Ultraviolet absorption spectra for the yellow tocoquinone (I), the red ortho quinone (II), and for DL-α-tocopherol. The ordinate is $E^{1\%}_{1\ cm}$, and the abscissa is in mμ.

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.

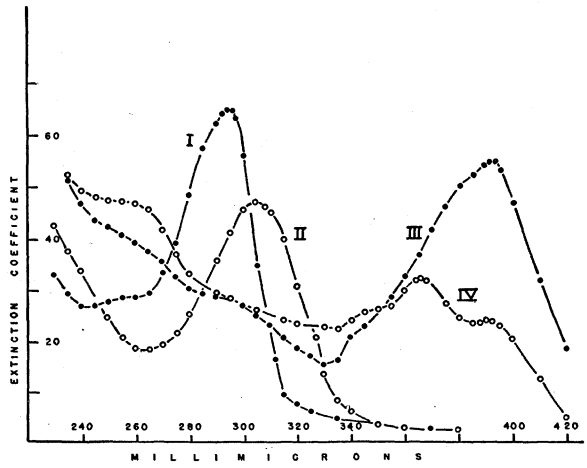

FIG. 2. The ultraviolet absorption spectra for (I) the acid form and (II) the basic form of the purple oil and for (III) the basic and (IV) the acid form of the blue-gray oil. The ordinate is $E^{1\%}_{1\ cm}$, and the abscissa is in mμ.

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

in alkaline media and yellow in acid media. The color change with the purple oil, serving as an indicator in aqueous acidimetric titrations, occurs in the pH range 3.8 to 4.4; the color is yellow at pH 3.8, pink at 4.0, and purple at pH 4.4. The color transition with the blue-gray oil, in methyl alcohol, occurs at an apparent pH of 8.8 to 9.2. The ultraviolet absorption spectra for both forms of each of these indicators, in methyl alcohol solution, are recorded in Fig. 2.

The orange and yellow oils and the yellow forms of the purple and blue-gray oils all show a green fluorescence in ultraviolet light.

Although proofs of structure of these products will be reported elsewhere, it may be stated here that the evidence is that the purple and blue-gray oils are hydroxy paraquinones, the chroman ring in the tocopherol molecule has been ruptured in the case of the purple oil, that the orange is an ortho quinone, and that, with the exception of the yellow product, which is the well-known tocoquinone, the production of the quinones involved the elimination of methyl groups on the aromatic ring of the tocopherol molecule.

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Manuscript received February 4, 1952.

An Egg Encrusted with Protoporphyrin

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The egg shown in the accompanying illustrations was found by a farmer's wife in the oviduct of a hen being prepared for the table. Fortunately it was saved and eventually turned in to one of us for an explanation of its nature and origin. The voluminous literature on abnormal eggs was reviewed by Davaine (*1*) in 1860, and again recently by Romanoff and Romanoff (*2*), but neither in these compilations nor elsewhere have we been able to find any record of an egg like this one. A brief report therefore seems desirable.

When received, some three weeks after its discovery, the egg was completely covered with a rough, granular, dark purplish-brown material. The coating was smoothest around the middle and was rough and pebbled at both ends. The egg measured 6.54 × 4.69 cm and weighed 70 g. When shaken, it sounded like an addled one.

Inquiry revealed that it had been removed from the uterine region of the oviduct of a 4-year-old hen, a hybrid from the cross Barred Rock ♀ × Rhode Island Red ♂. The hen had been a good layer and was in good health when killed for the table about Oct. 1, 1951. Because of the age of the hen and the season of the year, one would expect the ovary to be inactive at that time. The owner reported that the only other eggs in the hen were small follicles, attached to the ovary. When freshly removed, the abnormal egg was more purple in color than it appeared after drying.

Contents of the egg were carefully blown out by A. A. Allen. On drilling the hole, nothing could be blown out until water was forced in through the blowpipe. The first material to come out was yellow and caseous. Eventually, by blowing in water and air alternately, the contents were removed. No bad odor was noticeable.

The soaking necessary to blow out the egg loosened the pigmented coat, which cracked in many places as it dried (Fig. 1). It became clear that under the dark encrustation there was a light-colored eggshell, calcareous in nature and apparently normal. Overlying it, the dark material formed a discrete crust composed of two layers (Fig. 2). The inner of these was dense, almost glassy, and about 1 mm thick. The outer layer was thinner and less dense. The average thickness of the crust, including both layers, was 1.6 mm.

FIG. 1. The encrusted egg, showing cracks that developed in the crust after it was blown and dried out. The white spot at the top shows the underlying shell where a fragment of the crust was removed.

Because the hen was derived from two breeds that normally lay brown-shelled eggs, it was suspected that the crust might be composed of protoporphyrin, the pigment causing the brown color of the shell in the eggs of many birds (*3*). Chemical tests by one of us (J. B. S.) verified this assumption. The amount of iron was only 0.007%, which showed that hemoglobin was not present, and hence that there was no blood in the crust. Fragments of the crust were finally dissolved in 25% HCl. When the insoluble material was

[1] We wish to thank Arley Bever for determining the location of the three absorption bands.