enzyme generally believed to require no –SH group for its function. Slater (*6*) has also noted that *p*-chloromercuribenzoate "has some effect on cytochrome oxidase." It has been shown that phenylmercuric nitrate can inactivate other nonsulfhydryl enzymes (*7*). It should be emphasized that, although it is possible to protect cytochrome oxidase against mercurial inhibition by means of cysteine or other sulfhydryl compounds, it is not possible to reverse the inhibition by such means (*1, 8*). When enzyme inhibition by mercurials is due to combination with essential –SH groups, such reversal can normally be accomplished (*5*).

Details of these investigations and additional data concerning the mechanism of inactivation will be published elsewhere.

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Differentiation of Bacterial Species and Variation within Species by Means of 2,3,5-Triphenyltetrazolium Chloride in Culture Medium[1]

I. Forest Huddleson and Betty Baltzer

Department of Bacteriology and Public Health, Michigan State College, East Lansing

When different species or varieties of bacteria are grown as separate colonies on a clear agar medium, such as tryptose agar, containing 0.01% or less of triphenyltetrazolium chloride, the colonies of each will develop either a different shade of red coloring or a similar shade of red along with a different pastel color in the borders of the colonies. The latter colors, often the most pronounced differences seen, are tints of green, blue, yellow, or red.

The following procedures are recommended for use in the detection of colonial differences in bacteria: A 1% solution of tetrazolium chloride is prepared in distilled water and sterilized at 115° C for 15 min. One ml of this solution is added to 100 ml of melted tryptose agar and mixed well just before pouring on Petri plates. The depth of the agar in the plates should be approximately 0.5 cm. Since a solution of the salt is colorless in the oxidized form, the agar, after pouring, will remain colorless. The poured plates should be incubated at 37° C for about 20 hr to evaporate the excess moisture from their surfaces.

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The surface of the agar plate should be inoculated in such a way that the colony population that arises will not be crowded. The bacterial suspension should be prepared in sufficient dilution so that when 1 cm is spread over the surface of the agar, from 200 to 400 colonies will arise on incubation. The period of incubation at 37° C before examination for differences in color will depend upon the rapidity of growth of the microorganisms under study. Those falling in the genera *Salmonella, Shigella, Escherichia, Alcaligenes,* and *Streptoccus* should be incubated for 48 hr. Agar plates inoculated with *Brucella* should be incubated for 4 days. For the differentiation of *Staphylococci* and certain aerobic spore-bearing *Bacilli,* the concentration of the salt in the agar medium should be reduced to 0.0025%, as higher concentrations inhibit their growth.

The color patterns and differences in color patterns of bacterial colonies can best be seen by examining the growth on agar Petri plates under a low power (×12) dissecting microscope, illuminated by reflected, oblique light from the new Spencer microscope lamp. The light is focused at an angle of 40° on a small concave mirror placed in front of the microscope. The rays are reflected at an oblique angle from the mirror and strike the under surface of the Petri plate resting on the glass stage.

Thus far 15 different species or varieties of bacteria of 7 different genera have been studied. The bacterial cultures examined were from a culture collection. Each had been previously identified as to its genus, species, and variety by standard laboratory methods. The colonial variants of the species of *Brucella* used were from a large collection previously separated and identified by the senior author. With the exception of *Brucella,* no previous attempt had been made to bring about dissociation of the cultures studied.

All the varieties of *Salmonella* cultures examined, with the exception of *S. typhimurium,* showed two or more easily distinguishable colonial growth phases. Each phase could be distinguished from the other by differences in size, shape, the shade of red in the central area, and pronounced differences in delicate pastel tints in the border. The culture of *S. typhimurium,* showed only one growth phase. The color of the colonies was a light shade of red extending from the center to the border. Each of two cultures of *E. coli* showed two growth phases, differing in shades of red in the central area and pastel tints in the borders of the colonies. One culture of *S. sonnei* showed two distinct colonial growth phases, one of which was larger and more irregular in shape than the other. The larger colonies contained irregular deposits of deep red color superimposed on a lighter shade of red; the border was tinted light yellow. The smaller colony was more circular; the central area was colored light red and the border a light yellow-green.

Colonies of the smooth phase and closely related phases of the 3 *Brucella* species can be distinguished from each other more easily on agar medium containing tetrazolium chloride than on regular tryptose agar. Colonies of the 3 species and all growth phases of each are circular in shape. The central circular area of S phase colonies of *Br. suis* is stained a darker (almost black) shade of red

than that of *Br. abortus*. The same area in colonies of *Br. melitensis* is stained a lighter shade of red than that of *Br. abortus*. Another marked difference is seen in the circular borders of the colonies. The borders of *Br. suis* colonies are colored a light opaque yellow; those of *Br. abortus* a light, almost transparent bluish green; and those of *Br. melitensis* a light opaque pink.

The color differences in colonies of various species of bacteria and in their colonial variants do not change rapidly after the maximum color differences first develop. The most marked change that occurs with age takes place in the colony borders, which become more opaque. Color differences exhibited by colonies of distinct species of bacteria or their different phases of growth on a medium containing tetrazolium chloride are due to variations in the ability of their enzyme systems to reduce the compound to insoluble red triphenyl formazan (*1*).

The results obtained thus far by this method indicate that the procedure can be used as a means of identifying bacterial species or variations that occur within a species. It is highly useful as a means of detecting colonial variations in a supposedly pure growth phase culture of bacteria.

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Obligate Autotrophy in *Chlamydomonas Moewusii* Gerloff

Joyce Chismore Lewin
Osborn Botanical Laboratory, Yale University, New Haven, Connecticut

In a recent issue of SCIENCE, Finkle, Appleman, and Fleischer (*1*) reported that they have been unable to grow a certain strain of *Chlorella vulgaris* in the dark. Although many species of algae, including other strains of *Chl. vulgaris*, can be grown readily in the dark on simple organic substrates, other very closely related species apparently cannot be grown at all under these conditions. This presents a fascinating problem in the investigation of autotrophic and heterotrophic nutrition of algae—e.g., the species of *Euglena* studied by Dusi (*2*).

Some species of *Chlamydomonas* may be grown heterotrophically. Klebs (*3*) was able to grow *Chl. media* in darkness on sucrose media. Out of 7 species investigated by Lucksch (*4*), 6 (*Chl. dorsoventralis, monoica, pulchra, pseudagloë, pseudococcum,* and *subglobosa*) could be grown in darkness on media containing various sugars or acetate. *Chl. humicola* appeared to require light for growth, although carbon dioxide was not essential. It was deduced, from experiments with various algae, including *Chl. pseudococcum*, that light exerted some beneficial effect other than its action on photosynthesis (*5*). Jacobsen (*6*) did not obtain dark growth with 3 species of *Chlamydomonas* (*Chl. Ehrenbergii, intermedia, variabilis*).

Gerloff (*7*), in his description of *Chlamydomonas Moewusii*, stated that cells of this species are unable to multiply in darkness. Experiments to overcome the "dark block" in *Chl. Moewusii* (strains isolated by L. Provasoli in 1948) have been carried out here during the past year. No cell multiplication has been obtained in mineral media containing, or enriched with, any of the following organic compounds. (At the concentrations used, the growth of controls in the light was not inhibited.) All media were adjusted to pH 7. Sterilization was by autoclaving or by Seitz filtration.

Acids	*Hexoses*	*Phosphate Compounds*
Acetate	Glucose	Glucose-1-phosphate
Citrate	Galactose	ATP (animal)
Isocitrate	Fructose	Glycerophosphate
Fumarate	Mannose	Phosphoglycerate
α-Keto-glutarate		Adenylic acid
Lactate	*Pentoses*	Creatine
Malate	L-Arabinose	
Malonate	D-Arabinose	*Nitrogen Compounds*
Oxalo-acetate	Rhamnose	Alanine
Pyruvate	Ribose	Asparagine
Succinate	Xylose	Glutamine
Oxalate	Sorbose	Urea
Formate		
Thioglycolate	*Disaccharides*	*Various*
Propionate	Sucrose	Soluble starch
Valerate	Trehalose	α-Methyl glucoside
Butyrate	Maltose	Peptone
	Lactose	Tryptone
Alcohols	Melibiose	Coconut milk
Ethanol	Cellobiose	Yeast extract
Butanol		Liver extract
Glycol	*Trisaccharides*	Casein hydrolysate
Glycerol	Raffinose	Synthetic vitamin mixture
Mannitol	Melezitose	Gelatin
Dulcitol		Ascorbic acid
Sorbitol		Thiamine

A 5% carbon dioxide-air mixture was found equally ineffective when used supplementary to many of these carbon sources. Cell extracts, hydrolysates, and filtrates of light-grown cells do not support growth of cells in darkness; there seems to be no photostable toxic substance, produced by cells stored in the dark, inhibitory to cells growing in the light. Since *Chl. Moewusii* can perform the oxyhydrogen reaction in the dark (*8*), it was thought that energy derived from such a reaction might be utilized for growth. In other words, a chemoautotrophic mechanism might replace photosynthesis. However, an attempt to obtain growth in the dark in the presence of a gas mixture of 5% air, 5% carbon dioxide, and 90% hydrogen was unsuccessful.

It seemed possible that the cell membrane might constitute a barrier preventing the entry of organic substrates that could support growth. The problem, however, is apparently not one of permeability, since Warburg manometric experiments have shown that compounds such as acetate, pyruvate, or succinate enter the cells and are readily oxidized in the dark. Energy released by oxidation of these substrates does not appear to be available for the multiplication of the organism. Possibly some energy-transfer mechanism is lacking.

In the absence of carbon dioxide, no growth has been observed on any substrates in light or darkness. Until further experiments prove otherwise, *Chl. Moewusii* must