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

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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

be considered an obligate autotroph, absolutely requiring both light and carbon dioxide for growth. This is analogous to the situation in *Thiobacillus thiooxidans* (*9*), where sulfur and carbon dioxide are required for growth and can be replaced by no other energy or carbon source, respectively.

Possibly some simple compound that we have overlooked will suffice to support growth in darkness. It may be, however, that for the growth of some organisms, such as *Chl. Moewusii* and *Th. thiooxidans*, one type of energy source is not replaceable by another. Herein lies the problem of obligate autotrophy.

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A Coenzyme of Spleen β-Glucuronidase[1]

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The preparation of an electrophoretically homogeneous β-glucuronidase from calf spleen has been described recently (*1*). The activation of β-glucuronidase by nucleic acids (*2*) and the existence of naturally-occurring inhibitors of β-glucuronidase (*3*) have been pointed out.

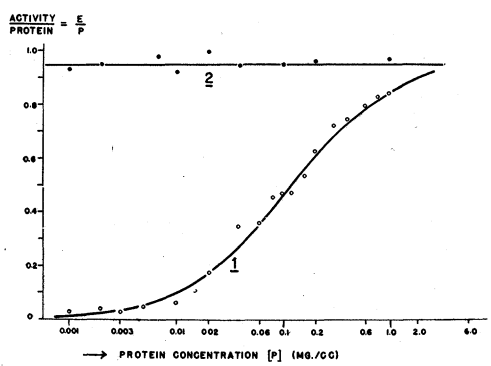

FIG. 1.

It has now been found that the activity of pure spleen β-glucuronidase is not proportional to the enzyme concentration, in contrast to the behavior of less pure preparations. The ratio of enzyme activity to protein concentration (E/P) drops greatly with dilution. Our experimental activity values (circles, Fig. 1) with increasing dilution coincide with a theoretical curve (Curve 1, Fig. 1) obtained from the usual dissociation expression:

$$\frac{(P-E)(C-E)}{(E)} = K, \qquad (1)$$

where P = total protein, C = total coenzyme, E = active enzyme, $\frac{P}{C}$ = constant, and K, a constant.

However, the addition of a boiled and filtered solution of the pure enzyme to dilute β-glucuronidase produces a strong activation (Table 1). This behavior is consistent with the view that spleen β-glucuronidase contains a thermostable coenzyme that dissociates on dilution. Furthermore, in the presence of 0.3% desoxyribonucleate the activity of pure β-glucuronidase is proportional to the protein concentration; i.e., E/P becomes independent of the protein concentration (black dots, Fig. 1). The distance on the ordinate between open circles and black dots indicates the activation by DNA for each protein concentration. This is very considerable at low protein concentrations.

TABLE 1

Protein concentration (mg/ml)	$\frac{\text{Activity}}{\text{Protein}} = \frac{E^*}{P}$	$\frac{E}{P}$ in the presence of boiled enzyme†	Activation by boiled enzyme
0.08	0.46	...	.....
.025	.23	0.41	1.8 fold
.02	.17	.44	2.6 "
.015	.11	.37	3.4 "
0.01	0.064	0.38	6 "

* The ratio E/P is given the value 1 when 0.165 mg phenolphthalein glucuronide is hydrolyzed in 1 min at 37° C by 1 mg protein in a digest of 1 ml.

† The concentration in the digest of the thermostable matter added with the boiled and filtered enzyme corresponds to 0.08 mg protein/ml before boiling.

When the activity of a dilute solution of pure glucuronidase (0.01 mg protein per ml) is determined in the presence of increasing amounts of desoxyribonucleic acid (DNA), and when the ratio of the enzyme activity to the protein concentration is plotted against the log of the DNA concentration, another S-shape curve is obtained. This experimental curve is in accordance with equation (1) when C becomes $(C+N)$, and N = concentration of DNA. This generalized equation also explains why E/P becomes independent of the protein concentration in the presence of a large excess of DNA (Curve 2, Fig. 1).

Accordingly, the addition of DNA in this system has the same effect as if more of the coenzyme were being added. However, we have no evidence that DNA is indeed the coenzyme. Yeast ribonucleic acid (RNA) gives a similar but somewhat smaller effect. For both DNA and RNA the activating effect is independent of their degree of polymerization.

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