Growth of *Chlorella vulgaris* in the Dark

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During metabolic studies on *Chlorella vulgaris*[2] now in progress in this laboratory, it was found desirable to make use of very small inocula for starting liquid cultures. The culture medium consisted of a complete Hoagland solution to which 1% glucose was added. It was observed early in this work that such cultures when kept in a dark chamber did not show any visible signs of growth. However, when they were brought out into ordinary daylight, even after as long as 70 days in the dark, they grew in the same manner as do freshly inoculated cultures. In view of the numerous statements in the literature that *Chlorella* species and other algae grow and produce chlorophyll in the dark (*1*, *4*), it appeared desirable to investigate this phenomenon further.

TABLE 1

EFFECT OF PERIODS OF DARKNESS AND LIGHT ON THE GROWTH OF *Chlorella vulgaris*

Total weeks of growth	Weeks in dark	Followed by weeks in light	Cells/mm³
1	1	0	±*
1	0	1	100
3	1	2	400
3	0	3	4,000
6	3	3	1,200
6	0	6	39,000
10	10	0	±*

* Cultures water-clear; too few cells to count.

The initial trials were simply repetitions of the first experiments to make sure that what we had observed was not an artifact. A number of 250-ml culture flasks arranged for continuous aeration were each filled with 150 ml of complete Hoagland solution containing glucose and inoculated with small inocula of *Chlorella vulgaris* (2,000–10,000 cells per flask). A few of these were left in the ordinary daylight; the rest were placed in a dark chamber. Periodic obesrvations indicated that while the flasks in the light were detectably green after about 5–6 days, those in the dark did not show any signs of growth for as long as they remained in the dark, even up to 10 weeks.

At intervals, two flasks from the dark chamber were removed and placed in the light, where their growth was observed. At the same time periodic hemocytometer cell counts made on the cultures remaining in the dark revealed that these water-clear cultures, even after 10 weeks, contained too few cells to make a count possible. The results of the experiment are summarized in Table 1.

Other cultures were exposed for 10 days to the light, permitting a visibly green culture to develop, and were then placed in the dark. At 6 weeks from the time of inoculation they showed a count of 2,400 cells/mm³; the count was roughly the same at 10 weeks. Then when the cultures were exposed to light, they again started growing, although after a relatively long lag period.

It should be noted here that cells that have been in the dark for a long time lose practically all their chlorophyll, become much larger than their normal size, and appear to be granular. Such cells seem to have the capacity to recover if exposed to light. However, a few of the dark-grown cultures that were retained in the dark for 12 weeks became completely devoid of chlorophyll and did not recover the ability to resynthesize chlorophyll or show any signs of growth after exposure to light.

A preliminary attempt was made to find whether certain simple substances added to the medium would affect growth in the dark. To cultures which were grown in regular culture flasks as described, and which were started with small inocula, there was added, besides glucose, one of the following substances: yeast extract, indole acetic acid, glucoseamine, asparagine, vitamin B_1, and ascorbic acid. In all these cases the results with respect to growth in the dark were the same as in the Hoagland solution plus glucose, i.e., there was no observable growth. The controls in the light grew normally.

The results of these experiments indicate that this particular strain of *Chlorella vulgaris* seems to require for growth a factor (other than carbohydrate) which is either photochemically synthesized or activated and which is depleted in darkness. The possibility of a growth inhibitor adversely affected by light is also being considered in experiments now in progress.

The apparent discrepancy between the observations here reported and those reported in the literature may be explained by one of two suggested possibilities. First, we may have a strain of *Chlorella vulgaris* that has lost the capacity to grow or produce chlorophyll in the dark. This possibility is now being tested by growing a number of other strains under similar conditions. The other possibility is that in order to observe this phenomenon as strikingly as it is reported here, it is essential to use extremely small inocula and to observe the cultures for long periods of time. It is not evident that such conditions prevailed in experiments on growth of algae in darkness which have been described in the literature.

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[2] The original cultures of these algae were obtained from Dr. Robert Emerson in 1931 and have been cultured in this laboratory since then.

References

1. ALGEUS, SVEN. *Physiologia Plantarum*, 1948, **1**, 56, 236.
2. MYERS, JACK. *Plant Physiol.*, 1940, **15**, 575.
3. PEARSALL, W. H. and BENGRY, R. P. *Ann. Bot.*, 1940, **4**, 365.
4. VAN NIEL, C. B. *Adv. Enzymol.*, 1941, **1**, 263.