SMITHSONIAN MISCELLANEOUS COLLECTIONS
VOLUME 92, NUMBER 5

Arthur Fund

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(WITH THREE PLATES)

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(PUBLICATION 3256)

CITY OF WASHINGTON
PUBLISHED BY THE SMITHSONIAN INSTITUTION
OCTOBER 8, 1934

The Lord Baltimore Press
BALTIMORE, MD., U. S. A.

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COLONIAL FORMATION OF UNICELLULAR GREEN ALGAE UNDER VARIOUS LIGHT CONDITIONS[1]

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INTRODUCTION

Light of certain ranges of wave lengths and intensity is generally considered essential for the formation of chlorophyll in green plants. A number of green algae, mosses, and pine seedlings prove to be exceptions to this generalization. For example, *Scenedesmus obtusiusculus* Chodat and *Scenedesmus chlorelloides* Chodat develop and maintain their green chlorophyll better in darkness than in light. Different green algae, however, vary in their reactions to light conditions just as different higher plants vary in their reactions to temperature and other environmental conditions. *Chlorella rubescens* Chodat forms chlorophyll in the dark but not so vigorously as in the light, while the cells of *Scenedesmus quadricauda* are dark green in diffuse light and pale green in direct light.

The ability of these plants to form chlorophyll without the aid of natural or artificial light is generally attributed to the presence of assimilable carbohydrates in the nutrient solution in which they are growing. Chodat (1913) has shown in the case of *Stichococcus bacillaris* that when a carbohydrate is assimilated with difficulty or not assimilated at all by an alga in the dark as demonstrated by its decoloration or complete lack of growth, the growth and development of chlorophyll by the same alga in the light is not prevented in the slightest degree. Chodat carried on a long series of experiments to determine the type of sugar best assimilated by certain algae growing in darkness. For that reason, further discussion of the necessary nutriments will not be treated here.

[1] This paper reports investigations made under a grant from the National Research Council to the author as National Research Fellow in the Biological Sciences from July 1931 to 1933.

The experiment described below is one carried out preliminary to an elaborate piece of research under definitely controlled conditions on the effect of light intensity and wave length on algae. A number of different algae from my collection in pure culture were studied to determine their general reaction to natural and artificial light and to total absence of light.

I wish to express my appreciation to Dr. Earl S. Johnston, Assistant Director of the Division of Radiation and Organisms, Smithsonian Institution, for his aid and suggestions. I am also very grateful to Dr. W. T. Swingle, of the United States Department of Agriculture, for his cooperation in obtaining the excellent color plates of the algal cultures made by Marcel L. F. Foubert, of the Photographic Division of the Department of Agriculture.

DISCUSSION OF LITERATURE

Numerous workers have studied the effect of varying day lengths on higher plants. The recent work by Arthur (1930), Garner and Allard (1925), and Shirley (1929) covers the field very well, and their long lists of literature references are an indication of the work done on this subject from the time of Hervé Mangon (1861), who found that grain planted in darkness turned yellow while that in electric light was green and thriving, up to the present when the beneficial amount of artificial light that should supplement winter daylight has been ascertained for various plants. References to the effect of light and darkness on lower plants are not as abundant.

Klebs (1896) studied the effect of light and darkness on gamete formation in *Chlamydomonas media* Klebs and demonstrated that only vegetative division takes place in darkness. A 2 percent sugar solution aided growth but did not entirely replace the light.

Etard and Bouilhac (1898) recorded the presence of chlorophyll in a *Nostoc* cultivated in the dark in a nutrient solution to which glucose had been added. They extracted the chlorophyll with alcohol and found the resulting yellow-green solution showed a red fluorescence and had the following absorption bands: 6900 to 6500 A, 6310 to 6060 A, 5890 to 5680 A, and 5480 to 5360 A.

Artari (1899, 1900, 1902) studied *Pleurococcus* and *Scenedesmus* in media containing peptone, glucose, maltose, beet sugar, or mannite and found growth in conjunction with chlorophyll formation taking place not only in the light without carbon dioxide but also in absolute darkness. He also reported chlorophyll formation in the dark for

Stichococcus bacillaris, Chlorococcum infusionum, Chlorella vulgaris, Raphidium polymorphum, and the gonidia of certain lichens. He showed that the formation and quantity of chlorophyll was dependent on nitrogenous conditions and carbon sources in the solution. However, different algae vary in this respect.

Radais (1900) grew *Chlorella vulgaris* on steamed potato slices and malt extract in light and darkness between 12° and 38° C. (25° C. optimum). The multiplication of the alga was similar in light and in darkness, and when both sets of cultures were dissolved in alcohol and examined spectroscopically, their absorption spectrum at 1/500 concentration was found to be 6910 to 6450 A, 6280 to 6040 A, and 5920 to 5670 A. A carbon bisulphide solution of the chlorophyll gave the same absorption spectrum but with a shift toward the red and very slight differences in the borders of the bands. Both dark and light cultures gave identical absorption bands. By dilution, the two bands of shorter wave lengths disappeared, but the band 6910 to 6450 A was still visible at a concentration of 1/100000.

Matruchot and Molliard (1902) reported green cells in *Stichococcus bacillaris major* Naegeli growing in darkness.

Grintzesco (1903), experimenting with *Chlorella vulgaris* Beyerinck, found that too much light—that is, direct sunlight—is unfavorable and injures the cell membrane. The algae developed well in electric light, but no intensity data are given. The cultures growing in darkness on agar with an addition of 2 percent glucose were a beautiful green and presented a better growth than those in flasks placed in light. His cultures of *Scenedesmus acutus* Meyen were 3 to 4 times smaller in darkness than in light, but they were green.

Muenscher (1923) grew a *Chlorella* in diffuse light and in total absence of light for 105 and 235 days in a nutrient solution to which nitrogen was supplied either as calcium nitrate or ammonium sulphate. He states that *Chlorella* can synthesize proteins in total darkness when nitrogen is supplied in inorganic combination.

Colla (1930) found that the chloroplasts of *Chlorella* were discolored when grown on flint stone in a petri dish of Detmer solution for 35 days. He does not mention the presence of glucose in his solution. He then irradiated the alga for 2 hours daily, and the chloroplastids became intensely green the third day. He repeated the experiment with *Elodea canadensis* which had become etiolated after growing for 1 month in darkness on dampened cotton. After 2 days of irradiation of 7 hours daily the chlorophyll reappeared in the cells of the plant. He found very little variation in the chlorophyll absorption bands of the normal and the irradiated plants.

Meier (1929) proved that light is not necessary for the formation of chlorophyll and carotin in the cells of *Chlorella rubescens* Chodat, *Scenedesmus obtusiusculus* Chodat, and *Scenedesmus chlorelloides* Chodat when an abundant quantity of nitrate and glucose are present in the medium upon which they are growing.

METHOD

The medium on which the algae were grown for these experiments was Detmer solution made up in the following proportions:

	Grams
Calcium nitrate	1.
Potassium chloride	0.25
Magnesium sulphate	0.25
Potassium acid phosphate	0.25
Distilled water	1000.
Ferric chloride	0.002

The solution was diluted to $\frac{1}{3}$ for this experiment, then 2 percent dextrose and 2 percent difco-bacto agar were added.

Nine series of 18 different algae were inoculated in 125-cc Erlenmeyer flasks each containing 75 cc of the above medium. Three inoculations were made on the surface of the solid medium in each flask by means of a platinum needle. The necessary precautions were taken for strict sterilization. All the algae used were from pure cultures, the majority of which had been isolated by the author.

The following set of nine treatments was given to each alga:

1. Control in intermittent light (daylight) during 2 months.
2. Intermittent light for 1 month, then placed in continuous light (electric light) for 1 month.
3. Intermittent light for 1 month, then placed in continuous darkness for 1 month.
4. Continuous darkness during 2 months.
5. Continuous darkness for 1 month, then placed in intermittent light for 1 month.
6. Continuous darkness for 1 month, then placed in continuous light for 1 month.
7. Continuous light during 2 months.
8. Continuous light for 1 month, then placed in intermittent light for 1 month.
9. Continuous light for 1 month, then placed in continuous darkness for 1 month.

The schematic outline of the treatments is as follows:

	Intermittent light	Continuous darkness	Continuous light
Day 1 (Inoculation)			
Day 2	1 2 3	5 4 6	7 8 9
Day 30	1 5 8	3 4 9	7 2 6
Day 60 (End of experiment)			

The continuous light was supplied by four 300-watt Mazda daylight lamps so placed that there was a distance of 92 centimeters from the filament to the top of the table on which were the flasks containing the algae. The intensity as measured by the thermocouple was 60 microwatts per square millimeter per second, which is about the same as 1/10 of noon sunlight in the summer.

The continuous darkness was provided by placing the flasks of algae in a tightly closed closet in a concrete pier in a basement room that as a rule is kept dark continually, and if lighted is illuminated by a red lamp.

The intermittent light and darkness were natural day and night conditions in March and April in a north window of the flag tower of the Smithsonian Institution.

RESULTS

All the results have been tabulated on pages 9-12 for convenient reference.

CHLOROPHYLL FORMATION

Eleven varieties were equally green in all nine treatments at the end of 30 days. However, in the following cultures a variation in chlorophyll content was indicated by a difference in color: *Oocystis naegelii* and *Chlorella vulgaris* var., in all nine treatments; *Scenedesmus quadricauda,* very evident change in the cultures in continuous darkness, slight differences in color in the cultures in intermittent and continuous light; *Chlorococcum viscosum* in intermittent light and in continuous darkness; *Cystococcus irregularis* in continuous light; *Coccomyxa viridis* in continuous light and in intermittent light; and *Palmellococcus protothecoides* in all cultures except treatment 5.

After 60 days seven algae showed abundant chlorophyll in all nine treatments. This included the following varieties: *Scenedesmus chlorelloides* var., *Heterococcus viridis, Chlorella viscosa, Chlamydomonas intermedia, Oocystis naegelii, Cystococcus cohaerens* var., and *Chlorococcum viscosum.*

Six varieties had very little or no chlorophyll in any of the cultures at the end of 60 days. Among these were *Chlorella vulgaris, Chlorella vulgaris* var., *Palmellococcus variegatus, Scenedesmus flavescens, Palmellococcus protothecoides,* and *Coccomyxa viridis.* But after the 30-day period, the following were still green: *Palmellococcus variegatus* and *Scenedesmus flavescens* in the cultures that had been kept in intermittent light and in continuous darkness, *Palmellococcus proto-*

thecoides in the culture kept in continuous darkness, and *Chlorella vulgaris* in the culture kept in intermittent light.

Two varieties, *Scenedesmus quadricauda* and *Coccomyxa viridis,* were all slightly off-color, being a more or less pale or mottled green. *Cystococcus irregularis* cultures in treatments 2, 6, and 7 were also off-color.

Cystococcus irregularis had green colonies in treatments 1, 5, 8, 3, 4, and 9.

Coccomyxa simplex retained chlorophyll in treatments 1, 5, 8, 3, and 2. At the end of 30 days all the cultures had been equally green.

Haematococcus pluvialis was green only in treatments 1, 5, and 8. At the end of the 30-day period it had also been green in 4, 5, and 6.

Scenedesmus quadricauda formed a green culture in intermittent light.

Stichococcus bacillaris was greenest in 3, 4, and 9 at the end of 60 days; at the end of 30 days the cultures in 4, 5, and 6 were a little greener than those in the other six treatments.

DECOLORATION AT THE END OF 60 DAYS

Decoloration was evident in all the cultures of *Chlorella vulgaris* var.; in *Chlorella vulgaris* in 3, 4, and 9 and in 2, 6, and 7; in *Coccomyxa simplex* in 4, 9, 6; in *Palmellococcus protothecoides;* and in *Cystococcus cohaerens* in 1, 8, 4, 9, 2, 6, and 7. Decoloration (rose color) was beginning to appear in *Palmellococcus variegatus* under conditions 8, 3, 9, 2, 6, and 7; and in *Coccomyxa simplex* in 7.

CAROTIN FORMATION

Five of the 18 algae formed carotin. *Chlorella vulgaris* and *Scenedesmus flavescens* had orange-colored colonies in all the treatments. The greatest amount of carotin was formed for *Chlorella vulgaris* in intermittent light and for *Scenedesmus flavescens* in intermittent light and in continuous light. Beads of carotin were also formed for *Chlorella vulgaris* var. in 1, 8, 3, 4, 2, 6, and 7: for *Palmellococcus variegatus* in 1, 5, 3, 4, and 2; and for *Palmellococcus protothecoides* in 4.

HAEMATOCHROME

Haematochrome was formed in *Haematococcus pluvialis* in 2, 6, 7, 4, and 9. The greatest amount was in treatment 2, the culture kept in intermittent light the first month and in continuous light the second month.

GROWTH AND GENERAL DEVELOPMENT

In the majority of the varieties studied, the growth and development of the colony attained its maximum at the end of 30 days, and with the exception of the algae listed below, the colonies showed no increase in size after the first month. These exceptions are *Chlorella vulgaris* in 5, 1, 2, 7, 8 and 9; *Palmellococcus variegatus* in 5, 2, 3, 7, 8, and 9; *Chlorella vulgaris* var. in all nine treatments; *Chlorococcum viscosum* in all nine treatments; *Scenedesmus quadricauda* in 7, 8, 9; *Oocystis naegelii* in 4 and 6; *Heterococcus viridis* in 2 and 5; *Scenedesmus chlorelloides* var. in 2.

In general, the colonies made the same amount of growth and were approximately the same size in all nine treatments. There were striking exceptions, however, in the following cases as indicated in the table and plates 2 and 3. *Palmellococcus variegatus* in 2 and 5; *Scenedesmus chlorelloides* var. in 2; *Chlamydomonas intermedia* in 4; *Heterococcus viridis* in 5 and 2; *Cystococcus cohaerens* var. in 2 and 3; *Chlorococcum viscosum* in 1, 2, 3, and 9; *Chlorella vulgaris* var. in 2, 4, 5, and 6; and *Chlorella vulgaris* in 1 and 5.

Those cultures that showed remarkably poor growth were *Chlorococcum viscosum* in 6; and *Chlorella vulgaris* in 4, 6, and 3.

There were striking morphological differences in some colonies of the same alga subjected to different conditions. The disks of *Chlorella vulgaris* in 1, 5, 8 were firm and sectored; in 3, 4, 9 they had a moist appearance; and in 2, 6, 7 they appeared to be very dry. *Coccomyxa viridis* had very firm colonies in 1, 5, 8 and in 2, 6, 7, but those in 3, 4, 9 were very fluid. *Cystococcus irregularis* in 1, 3, 2 and 6 were characteristically wrinkled, while those in 7, 8 and 9 were smooth little peaks. For *Scenedesmus quadricauda* in 1, 4, 6 all the three colonies were run together in brilliant liquid masses, in 2 and 7 the colonies were finely nerved; while in 8 and 9 they were mottled and distinct. *Chlorococcum viscosum* in 2, 6 and 7 had dull disks while the other six treatments caused glistening and brilliant colonies. The colonies of *Stichococcus bacillaris* were firm, and rough with tiny excrescences, shining brightly with the exception of those in 3, 4, 9 and 2 which were dull. The cultures of *Oocystis naegelii* were smooth, brilliant, and fluid with the exception of 6 and 7, which were wrinkled, dry, and dull, and 2, which had smooth, dry, and dull colonies. The colonies of *Chlamydomonas intermedia* were brilliant in three treatments but in 2, 6, 7 were dull, while 3, 4, 9 caused a curdled appearance. *Heterococcus viridis* had dry, dull disks in 1, 8, 2, 6, and 7 but brilliant, moist ones in 5, 3, 4, and 9. *Chlorella viscosa* in 2, 6, 7 had wrinkled disks but brilliant, smooth ones in the other six treatments. *Scenedesmus chlorelloides* var. had moist colonies in 1, 5, 8, and dull, dry ones in the other six treatments.

SUMMARY

Of 18 varieties of unicellular green algae, 11 developed chlorophyll while growing for 30 days in continuous light, in natural conditions of intermittent light and dark, and in continuous darkness. The other 7 varieties showed individual reactions to the different treatments; 3 showed poor development in all of the conditions; 2 algae had the best chlorophyll formation in continuous darkness and in intermittent light; 1 grew best in continuous light; and 1 variety developed best in continuous darkness. Seven of the 11 algae still maintained their chlorophyll at the end of 60 days, although the growth and development of each colony had attained its maximum at the end of 30 days. Decoloration was manifested in all the cultures of 1 alga and in some treatments of 6 other algae.

Five of the 18 algae formed carotin, 2 of the 5 in all the treatments, the greatest amount being formed in intermittent light.

Haematochrome was formed in 1 variety to the largest extent in continuous light and least in those cultures exposed to intermittent light during the last 30 days of their development.

The colonies differed greatly in morphological appearance.

TABLE I.—*Appearance of the Colonies after Two Months*

Symbols: I, intermittent light (daylight) and darkness (night); CD, continuous darkness (dark cupboard); CL, continuous artificial illumination. The arabic numbers in parentheses indicate the relative size of the colonies, l being the largest. b, brilliant; c, curdled appearance; d, dry; du, dull; f, firm; fl, fluid; m, moist; n, finely nerved; p, peaked; s, smooth; se, sectored; w, wrinkled.

Treatment number First month Second month	1 I I	5 CD I	8 CL I	3 I CD	4 CD CD	9 CL CD	2 I CL	6 CD CL	7 CL CL
ALGA *Chlorella vulgaris* var.									
Waxlike with characteristic beading 3 mm high (12 to 20 mm diam.)	. . . .	Green border with green and white beads on murky green center (3)	White with green beaded border and orange beaded center (3)	Green border and small white center with orange beads (4)	Dirty white with orange beads (1)	Green rim and flesh-colored border and white center (5)	White with orange beads (1)	White with orange beads (2)	White with orange and murky green beads (3)
Palmellococcus variegatus									
Firm, waxlike with slight excrescences and beads (3 to 15 mm diam.)	Brilliant green rim about a pale rose center (4)	Green with orange beads (1)	Rose center with a murky green rim (4)	Orange with a rose center (3)	Green with yellow beads (5)	Apple-green with a rose center (4)	Rose and green with some orange beads (2)	Murky green border with pale rose center (4)	Murky green border with rose center (4)
Chlorella vulgaris (pl. 2)									
Beaded peaks (5 to 20 mm diam.)	Deep orange with green border (1) f se	Red-orange with green border (2) f se	Red-orange with green border (3) f se	Orange with green border (7) m	Orange with green border (8) m	Orange with white beads and green border (7) m	Orange-pink with white beads and green border (4) d	Smoky-pink with white beads and green border (6) d	Orange-pink with white beads and green border (5) d
Scenedesmus flavescens									
Uniform, brilliant mounds, slight green border and striking green roots, 2–3 mm high	Orange	Brilliant orange	Brilliant orange	Green-yellow	Yellow-green	Yellow-orange	Brilliant orange	Brilliant orange	Brilliant orange

TABLE 1.—*Appearance of the Colonies after Two Months* (continued)

Treatment number First month Second month	1 I I	5 CD I	8 CL I	3 I CD	4 CD CD	9 CL CD	2 I CL	6 CD CL	7 CL CL
Haematococcus pluvialis									
Tiny specks in the agar	Dark green (1)	Green (2)	Dark green (2)	....	Red-brown (3)	Olive-green (2)	Deep red (2)	Red (2)	Red (3)
Palmellococcus protothecoides									
Characteristic zonation, conspicuous depressed center, and ruffled rim. Uniform in size, firm, 1 mm high (12 to 16 mm diam.)	Soft green with gray center	Dark green with pale green center	Dark green rim about green with gray center	Slight orange rim about apple-green with pale green center	Green with orange and white beads in center	Olive rim about green with gray center	Olive rim with white center	Olive rim with white center	Brown rim with green spots and white center
Coccomyxa viridis									
Brilliant, all slightly off-color, smooth, slightly raised above surface except fluid ones which cover surface of agar (10 x 15 mm to 15 x 12 mm)	Faded green f	Faded green f	Faded green f	Pale green center surrounded by yellow fluid fl	Pale green center surrounded by yellow fluid fl	Pale green center surrounded by yellow fluid fl	Gray-green center with yellow rim f	Gray-green center with yellow rim f	Gray-green center with yellow rim f
Cystococcus irregularis									
Brilliant, firm (15 to 20 mm diam.)	Green (1) w	....	Apple-green with tiny yellow border (3) p	.... (2) w	....	Apple-green with yellow border (3) p	Gray-green (off-color) (2) w	Yellow-green (off-color) (2) w	Yellow-green (off-color) (3) p
Scenedesmus quadricauda									
All slightly off-color (15 x 10, 20 x 15, 40 x 40 mm)	Green (3) fl	....	Mottled green (2)	....	Pale brown (1) fl	Mottled green (2)	Green with discolored border (2) n	Very pale green (2) fl	.... (2) n

TABLE 1.—*Appearance of the Colonies after Two Months* (continued)

Treatment number First month Second month	1 I I	5 CD I	8 CL I	3 I CD	4 CD CD	9 CL CD	2 I CL	6 CD CL	7 CL CL
Chlorococcum viscosum (pl. 3)									
Uniform, glistening, brilliant (7 x 10 mm)	Dark green center with apple-green rim (1)	Dark green center with apple-green rim (1)	Dark green center with apple-green rim (2)	Dark green center with yellow rim (1)	Green center with apple-green border and yellow-green rim (1)	Yellow-green (2)	Dark green (2) d	Dark green (2) d	Dark green (2) d
Stichococcus bacillaris									
Firm, shining, rough with tiny excrescences (2 x 3 to 15 x 5 mm)	Dark green with trace of decoloration (1)	Dark green with trace of decoloration (1)	Dark green with trace of decoloration (1)	Green (2) d	Green (2) d	Green (2) d	Gray-green (3) d	Tiny gray specks (5)	Brown-gray (4)
Cystococcus cohaerens var. (pl. 1)									
Characteristic dull vermiform masses 1.5 mm high, uniform (10 x 20 mm)	Tendency toward green-yellow	....	Green	Green-yellow	Green-yellow	....	Green	Green	....
Coccomyxa simplex									
Smooth, cone-shaped mounds .5 mm high (7 x 7 to 10 x 10 mm)	Yellow-green c	Dark green with apple-green sectors	....	Yellow-green c	Green with trace of decoloration in center	Green with trace of decoloration in center	Yellow-green c	Green with white center	Flesh-colored center with tiny green border
Oocystis naegelii									
Brilliant, smooth, liquid colonies except 2, 6, 7 which are wrinkled, dry, and dull (25 x 15 to 20 x 20 mm)	Green with light green border s fl b	Green with light green border s fl b	Green with light green border s fl b	Green with light green border s fl b	Green with light green border s fl b	Green with light green border s fl b	Dark green center and light green border with yellow-green dots s d du	Dark green 1 mm high w d du	Dark green 1 mm high w d du

TABLE 1.—*Appearance of the Colonies after Two Months* (concluded)

Treatment number First month Second month	1 I I	5 CD I	8 CL I	3 I CD	4 CD CD	9 CL CD	2 I CL	6 CD CL	7 CL CL
Chlamydomonas intermedia									
Smooth plateaus 1 mm high (3 x 3 to 20 x 4 mm)	Dark green (3) b	Dark green (3) b	Dark green (4) b	Brilliant apple-green (2) b c	Brilliant apple-green (1) b c	Brilliant apple-green (4) b c	Dark green center with green rim (4) du	Dark green center with green rim (4) du	Dark green center with green rim (4) du
Heterococcus viridis									
Finely powdered excrescences, 2 mm high (5 mm diam.)	Dark green d du	Dark green m b	Dark green d du	Green m b	Green m b	Green m b	Dark green d du	Green d du	Dark green d du
Chlorella viscosa									
Fairly uniform (10 to 15 mm diam.)	Dark green with tiny yellow rim b s	Dark green b s	Dark green b s	Dark green with green border and yellow rim b s	Dark green b s	Dark green b s	Dark green w	Dark green w	Dark green w
Scenedesmus chlorelloides, var.									
Small beaded colonies 0.5 mm high (5 x 5 to 10 x 6 mm)	Dark green m	Dark green m	Dark green m	Dark green d	Dark green d	Dark green d	Dark green d	Dark green d	Dark green d

EXPLANATION OF PLATES

Plate 1

Cystococcus cohaerens var. (× approx. ¾) grown on Detmer ⅓-agar 2 percent-dextrose 2 percent in intermittent light for one month. The algae in plates 2 and 3 were cultured in a similar manner.

Plate 2

Chlorella vulgaris, × approx. ½.

Plate 3

Chlorococcum viscosum, × approx. ½.

(Figure numbers refer to both plates.)

Fig. 1. Intermittent light for 3 months.
2. Intermittent light for 1 month, continuous light for 2 months.
3. Intermittent light for 1 month, continuous darkness for 2 months.
4. Continuous darkness for 3 months.
5. Continuous darkness for 1 month, intermittent light for 2 months.
6. Continuous darkness for 1 month, continuous light for 2 months.
7. Continuous light for 3 months.
8. Continuous light for 1 month, intermittent light for 2 months.
9. Continuous light for 1 month, continuous darkness for 2 months.
10. Continuous darkness for 3 months.
11. Continuous light for 3 months.

Fig. 1-9. Colonies on Detmer ⅓-agar 2 percent-dextrose 2 percent.
Figs. 10-11. Colonies on Detmer ⅓-agar 2 percent.

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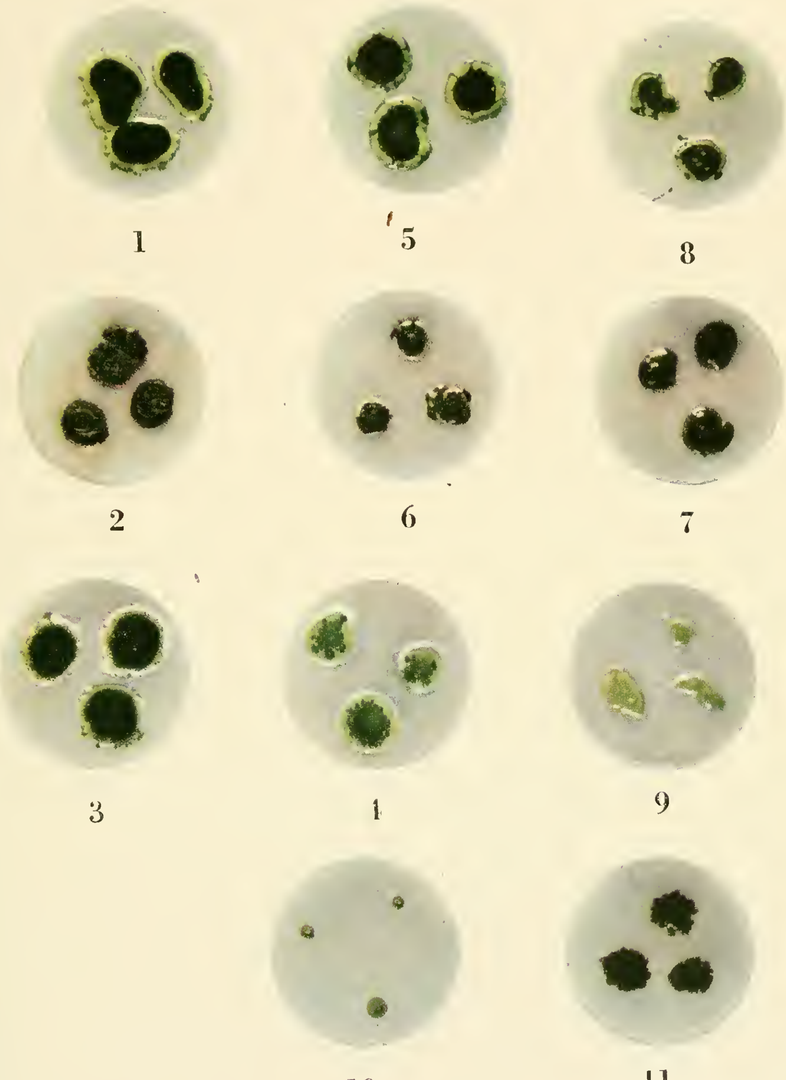
CYSTOCOCCUS COHAERENS VAR., X APPROX. 3/4

(For explanation, see page 13.)

CHLORELLA VULGARIS, X APPROX. ½

(For explanation, see page 13.)



CHLOROCOCCUM VISCOSUM, X APPROX. ½

(For explanation, see page 13.)