The Physiological Ecology of *Cyanidium caldarium*

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SUMMARY

The distribution and physiology of *Cyanidium caldarium* in its natural habitat in acidic hot springs and hot soils have been studied. This eucaryotic alga was the sole photosynthetic organism in habitats with pH less than 5 and temperatures greater than 40°. The upper temperature limit of the alga was 55° to 56° and the optimum temperature for growth was 45°. Temperature strains such as are found in blue-green algae of alkaline thermal habitats were not found for *Cyanidium caldarium*. In aquatic habitats the lower temperature limit was about 35° to 36°, the organism apparently being unable to compete at temperatures below this with other algae. In soils the alga was found at temperatures as low as 10°, apparently because in terrestrial habitats competition with other algae was less significant. The pH range at which the alga has been found in nature was from 0.05 to 5.0 and growth in culture occurred over this whole range. The optimum pH for growth was between 2 and 3. In nature the alga was found in habitats of widely varying light intensity, up to 7000 ft-candles. The alga became adapted to reduced light intensity by increasing its photopigment concentrations. Photosynthesis in populations adapted to reduced light intensities was inhibited by high light intensities. The alga grew well on glucose in the dark, and the concentration of photosynthetic pigments was reduced. When such bleached cells were transferred to the light in the presence of glucose, pigments were not synthesized and heterotrophic growth continued; when glucose was omitted, pigment synthesis occurred and photosynthetic growth resumed. Glucose did not inhibit pigment synthesis when added to cells growing in the light.

INTRODUCTION

The organism which is now called *Cyanidium caldarium* was first described by Tilden (1898) in the acid thermal areas of Yellowstone National Park. Since Tilden’s initial observation, this alga has been called various names and has been characterized as a member of the Rhodophyta (Hirose, 1958; Geitler, 1958), the Chlorophyta (Tilden, 1898; West, 1904; Hirose, 1950; Allen, 1952, 1954, 1959), the Cryptophyceae (Fogg, 1956; Dougherty & Allen, 1960; Lewin, 1961; Silva, 1962); and the Cyanophyta (Tilden, 1898, 1910; Collins, Holden & Setchell, 1901; Geitler & Ruttner, 1936; Copeland, 1936; Drouet, 1943; Negoro, 1944; Smith, 1950; Gusev, 1961). The confusion surrounding the taxonomy of this alga arises because although it contains phycocyanin (Allen, 1959), a characteristic pigment of the blue-green algae, it has a morphology and cytoplasmic organization similar to Chlorella. Klein & Cronquist (1967) have therefore proposed that *Cyanidium caldarium* is a distinct evolutionary form which arose during the transition from the blue-green algae to either the Chlorophyta or the Rhodophyta.

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Two environmental factors, low pH and high temperature, which favour the growth of *Cyanidium caldarium* (Fukuda, 1958; Allen, 1959; Ascione, Southwick & Fresco, 1966), exclude other photosynthetic organisms. Certain blue-green algae such as *Synechococcus* sp. can grow at temperatures exceeding 40° (Brock & Brock, 1966; Castenholz, 1969), but do not exist at pH values much below 5.0 (Brock & Brock, 1970). Alternatively other eucaryotic algae such as *Chlamydomonas acidophila* (Fott, McCarthy & McCarthy, 1964), *Euglena mutabilis* (Hein, 1953), *Zygogonium* sp. (Lynn & Brock, 1969) and *Chlorella* sp. (Kessler, 1967) can grow in environments with a pH less than 5.0 but cannot grow at temperatures exceeding 40°. Although *Cyanidium caldarium* does not exist at temperatures exceeding 56° to 57° (Doemel & Brock, 1970) it is the only photosynthetic organism existing at temperatures above 40° in acid thermal habitats and can grow at pH values as low as 0 (Allen, 1959). In this paper we present results of studies on the temperature, pH, and light relationships of *Cyanidium caldarium* in acidic hot springs and hot soils. Most of this work was carried out in Yellowstone National Park, but confirmatory studies were carried out in other thermal areas of western United States, Italy, New Zealand, Japan, Iceland and Central America.

**METHODS**

**Media.** Two basal media were used: Allen’s medium (Allen, 1959) and Ascione’s medium (Ascione et al. 1966). These media were adjusted to various pH values with 10 N-H₂SO₄ or 1 N-NaOH. When needed, glucose was added to 1 % (w/v).

**Isolation of pure cultures.** Screw-cap tubes containing Allen’s medium at pH 2.0 were inoculated with natural material and incubated at 25° to 55° with 400 to 600 ft-candles of light. To promote growth, the tubes were initially flushed with pure CO₂. These initial isolates were passed through a series of transfers in liquid medium and then streaked on Allen’s medium containing 1.5% Difco agar and 1% (w/v) glucose. The small yellow colonies which arose after incubation for several days at 37° in approximately 400 to 600 ft-candles of light were reinoculated in Allen’s medium, pH 2.0.

The purity of these cultures was established by inoculating material into nutrient broth, peptone yeast extract, and Allen’s medium containing 1 % glucose at pH 2.0 and pH 7.0, incubating for 2 weeks at 37° in the dark, and examining the medium microscopically.

**Growth in culture.** Autotrophic cultures of *Cyanidium caldarium* in Allen's medium were aerated in 20 × 150 mm. tubes by passing 5% CO₂ in air aseptically through capillary tubes inserted through Morton closures. These culture tubes were incubated in glass aquaria illuminated by eight 20 W fluorescent bulbs giving light intensities of about 600 to 800 ft-candles, the aquaria being heated either with immersion heaters or with an external circulating heater.

Heterotrophic cultures were grown in 500 ml. flasks containing Allen’s or Ascione’s medium with 1 % (w/v) glucose in a New Brunswick metabolite shaker operated at 120 rev./min. Either the flasks were covered with black tape or the water bath was covered with a black cloth to exclude light.

**Growth in situ.** Growth in situ was measured by monitoring the apparent increase of cells on wooden channels placed in the stream. The population density was determined by placing a 5.45 cm. I.D. brass tube firmly on the surface of the substrate. The algal population was then removed with a brush and suction, and the organisms collected in a vacuum flask.

These algal suspensions were transferred to bottles and then made to a known volume with tap water and a sufficient amount of 38 % formalin to give a final concentration of 4 % formalin. These suspensions were then either briefly sonicated or homogenized to break up
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The physiological ecology of *Cyanidium caldarium*. (The cells were not apparently damaged by either procedure.) The density of organisms was then determined with a Petroff Hauser counting chamber.

**Identification of *Cyanidium caldarium* in the field.** In earlier studies at Yellowstone National Park, California, and Nevada, the presence of *Cyanidium caldarium* was established solely by the coloration of the natural populations and the morphology and coloration of the cells. *Cyanidium caldarium*, however, is difficult to distinguish microscopically from species of Chlorella, but can be distinguished by its ability to grow at 45° and by the presence of phycocyanin. Therefore, samples of the natural populations were inoculated into Allen’s medium, pH 2.0, incubated at 35° and 45°, and the extinction spectra of the resulting cultures were then determined.

**Chlorophyll and protein.** Chlorophyll was extracted with *N,N*-dimethyl formamide (DMFA) (Volk & Bishop, 1968) since extraction with 90% acetone, absolute methanol or acetone: methanol was not quantitative. The concentration of chlorophyll was calculated by means of the extinction coefficient reported by Volk & Bishop (1968) for chlorophyll in DMFA.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

**Environmental measurements.** The temperature of the environment was measured with a Yellow Springs Instrument Co. model no. 42SC Telethermometer with a model no. 408 ‘large banjo’ probe. The probe was periodically checked with a standard mercury thermometer.

The pH of the aquatic environment was determined *in situ* either with a portable Sargent model PL pH meter or an Orion model 401 pH meter with a combination glass electrode. In the laboratory, pH measurements were made with a Corning pH meter with a similar electrode. The meters were standardized with two buffers at the same temperature as the samples.

The pH of the soil was determined by measuring the pH of various dilutions of the soil in deionized or distilled water; these values were plotted as H⁺ activity as a function of dilution, and then extrapolated to no dilution. The pH of the soil water, defined as water lost upon heating at 110° to constant weight, was then calculated by correcting the dilutions of soil to dilutions of soil water and again extrapolating to no dilution. Details of this method have been described by Doemel & Brock (1971).

Light intensity was measured with a Pilot light meter. The reading was converted to foot-candles with a conversion table provided by the manufacturer.

**¹⁴CO₂ incorporation.** Photosynthesis of natural populations was measured by adapting methods developed by Brock & Brock (1967). Portions of a natural population from a known temperature were harvested and resuspended in a container of spring water. These cell suspensions were agitated vigorously, clumps of cells were permitted to settle, and the supernatant was strained into a second container. To a 5.0 ml clear glass, screw-cap vial with a Teflon-lined cap, previously rinsed twice in spring water, 4 or 5 ml. of this suspension was added while maintaining the homogeneity of the suspension with continual stirring. The vials were sealed and placed horizontally into the stream water to equilibrate; uptake of [¹⁴C]bicarbonate was linear throughout the experiment. For most experiments vials were incubated for 30 min. with 0.02 μCi/ml. (10 μg./μCi) and incorporation was stopped by adding 0.5 ml. of 40% formaldehyde. Dark fixation of ¹⁴CO₂ was determined by darkening vials with aluminum foil. The ¹⁴CO₂ incorporated was then determined by the method of Brock & Brock (1967).

**Habitats.** ‘Cyanidium Creek’ (unofficial name), ‘Nymph Creek’ (unofficial name) and an effluent on the southern edge of Roaring Mountain in Yellowstone Park were chosen for
the study of the physiology of natural populations. At Cyanidium Creek and Nymph Creek permanent stations were established at all sources and along the channel at about 15 m.

Cyanidium Creek is a minor effluent in the Norris Geyser Basin, adjacent to the old Grand Loop Road bed, about 0.2 miles southwest of Pearl Geyser. It was under observation from 1967 to the end of 1970. Water arising from the primary source joins with water from a secondary source and flows into a third deep pool. From this latter the water flows about 150 m. through a narrow channel and drains into the south fork of Tantalus Creek. A thermal gradient had established along this effluent which was relatively stable over long periods of time. Data on seasonal temperature stability were given by Doemel (1970). This effluent was not greatly affected during periods of heavy precipitation. However, during the summer of 1969 a number of mud flows arising from the third pool covered the mat, decreasing the cell concentration and making further growth studies impossible.

Nymph Creek, a small stream which originates from a group of springs, is just west of the Grand Loop Road, about 2.2 miles north of Norris Junction. The stream flows east for about 150 m. where it drains into Nymph Lake. The small springs near the base of the road provide about 50% of the water; most of the remainder of the water comes from a cluster of six springs on the northern bank of the stream about 30 m. south of the road. During the latter part of the summer, most of the flow originates from these latter springs. Unlike Cyanidium Creek, this effluent is extensively shaded by lodgepole pine (*Pinus contorta*).

An acid effluent on the southern edge of Roaring Mountain, located 4.5 miles north of Norris Junction, provides a broader range of temperatures, 90° to 20°. Although the volume of the flow is less, the velocity is greater, because the stream flows down the steep western slope of Roaring Mountain.

Some aspects of the chemistry of these springs is given in Table 1.

A few studies were also done on Lemonade Creek, an acidic stream in the Amphitheatre Springs area; however, no chemical data are available for this effluent.

Table 1. Aspects of the chemistry of selected acid springs

<table>
<thead>
<tr>
<th>Effluent</th>
<th>Cyanidium Creek</th>
<th>Nymph Creek</th>
<th>Roaring Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.95 to 3.10</td>
<td>2.70 to 2.80</td>
<td>2.0 to 2.3</td>
</tr>
<tr>
<td>Particulate carbon (mg./l.)*</td>
<td>4.50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soluble carbon (mg./l.)*</td>
<td>9.20</td>
<td>15.13</td>
<td>7.22</td>
</tr>
<tr>
<td>PO₄³⁻(mg./l.)†</td>
<td>0.70± to 1.14</td>
<td>0.00 to 0.707</td>
<td>0.379 to 0.788</td>
</tr>
<tr>
<td>SO₄²⁻(mg./l.)‡</td>
<td>113</td>
<td>256</td>
<td>528</td>
</tr>
<tr>
<td>NH₄⁺(mg./l.)§</td>
<td>1.20</td>
<td>1.42</td>
<td>1.58</td>
</tr>
<tr>
<td>Silica (mg./l.)§</td>
<td>271</td>
<td>220</td>
<td>N.D.</td>
</tr>
<tr>
<td>Conductivity (µmhos × 10⁻¹/cm.²)∥</td>
<td>1.59 to 1.64</td>
<td>1.26 to 1.28</td>
<td>2.85</td>
</tr>
</tbody>
</table>

N.D. = not determined.

* Determinations made by Wilson, Montana State University, 1969.
† Determination according to Strickland & Parsons (1968).
‡ Determination according to American Public Health Association (1965).
§ Determinations made by Coller, Indiana University, 1968.
∥ Measured with a Yellow Springs conductivity meter.
Fig. 1. The temperature and pH parameters for *Cyanidium caldarium* in aquatic habitats. Each solid circle represents a pH and temperature at which *C. caldarium* was observed in nature.

Fig. 2. The temperature and pH parameters for *Cyanidium caldarium* in terrestrial habitats. Each solid circle represents a temperature and a soil pH and each solid square represents a temperature and a soil water pH at which *C. caldarium* has been observed in nature. Soil water pH was determined by the method of Doemel & Brock (1971).
RESULTS

Field observations on the distribution of Cyanidium

Cyanidium caldarium exists in two distinct kinds of habitats: aquatic and terrestrial. Aquatic habitats include pools and drainways from springs. Terrestrial habitats include steam-drenched soil and rock and warm dry soil in solfatara areas. To define the habitats of C. caldarium in nature, an extensive survey was made of more than 200 thermal areas in Yellowstone National Park, Wyoming; Mt Lassen National Park, California; The Geysers, Sonoma Co., California; Steamboat Springs, Washoe Co., Nevada; an area near Beowawe, Nevada; Hawaiian Volcanoes National Park, Hawaii; Pozzuoli (Solfatara), Italy; North Island, New Zealand; Gunma Prefecture, Japan; Krisuvik, Iceland; Geysir, Iceland; Myvatn (Namaskold), Iceland; and Los Ausoles near Auachapan, El Salvador. Fig. 1 shows the temperature and pH conditions at which C. caldarium was found in aquatic habitats and Fig. 2 shows the data for terrestrial habitats.

The temperature range in most aquatic habitats was between 30° to 35° and 53° to 57°. At temperatures exceeding about 40°, Cyanidium caldarium was the only photosynthetic organism. Bacteria and fungi were observed but constituted less than 10% of the population in most instances. At temperatures below 40°, other algae were present, including Euglena mutabilis, Chlorella sp., Chlamydomonas sp., diatoms, photosynthetic flagellates, and Zygogonium sp. Presumably C. caldarium was not found at lower temperatures because it was unable to compete with these other algae.

On the other hand, the temperature range in terrestrial habitats was considerably broader, between 10° and 55° to 57°. In these terrestrial habitats Cyanidium caldarium existed at considerably lower temperatures and other algal species were less common in these cooler habitats than they were in aquatic habitats. Conceivably, the absence of other algae in cooler terrestrial habitats permitted the growth of C. caldarium. Although it is difficult to determine unambiguously the upper temperature limit for C. caldarium in terrestrial habitats because of the temperature variability of the habitat, C. caldarium was never observed in areas where the temperature exceeded 60° at any time.

In both aquatic and terrestrial environments, Cyanidium caldarium was restricted to habitats with pH less than 5.0. The lower pH limit could not really be defined as C. caldarium was found in the most acidic habitats studied (pH 1.8 in aquatic environments and pH 0.05 in terrestrial environments). In every geographical region except Hawaii, C. caldarium was found in acid thermal habitats. In Hawaii, only a Chlorella species with an upper temperature of 36° was found, even though habitats apparently suitable for C. caldarium were present.

Effect of temperature on photosynthesis and growth of Cyanidium

The effect of temperature on photosynthesis by suspensions of the alga collected from known temperatures is shown in Fig. 3. Twenty-two experiments were done to measure the temperature optimum for photosynthesis of samples taken from habitats of different temperature. The optimum was between 45° and 50°, irrespective of the source of the sample (Doemel, 1970). Thus natural populations of Cyanidium caldarium had a temperature optimum for photosynthesis independent of the temperature at which these algae existed in their natural habitat. These observations suggested that strains adapted to various temperatures did not exist.

However, the measurement of photosynthesis considers only the optimum temperature of one component of the system and it was felt desirable to observe the effect of temperature on growth. To estimate the growth rate of natural populations, alga-free pine channels
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were placed at various temperatures in Nymph Creek and in Cyanidium Creek. The increase of the *Cyanidium caldarium* populations with time was monitored by removing cores of a known area with suction and then enumerating chlorophyll-containing cells with fluorescence microscopy. Light was required for full colonization, as was shown by measurements of growth on two portions of a channel, one portion of which was darkened (Fig. 4). The population density on the undarkened portion increased at a logarithmic rate until a stable population was obtained whereas on the darkened channel colonization occurred at a much slower rate and the maximum population density was about 10% of the uncovered channel. These wooden channels appeared to be neutral substrates since similar growth rates were obtained on a channel constructed from concrete and on a wooden channel lined with plastic (Saran; Dow Chemical Co.). Since the true growth rate is equal to the observed growth rate plus the washout rate minus the settling rate (Brock & Brock, 1968), and since neither washout rates nor settlement rates were determined for all channels at all temperatures, only the apparent growth rate could be determined. Growth on channels placed at different temperatures was measured and Fig. 5 shows a summary of the data. The optimum temperature for population growth was about 43°C.

Another measure of optimum conditions is the density of the maximum population which a stream will support. Assuming that throughout the thermal gradient of a stream effluent all conditions except temperature are equivalent, then the effect of temperature can be
measured. Fig. 6 shows that in the three thermal effluents maximum population densities were observed at temperatures between 40° and 50°.

Peary & Castenholz (1964) isolated several strains of the thermophilic blue-green alga *Synechococcus lividus* which had temperature optima for growth that were similar to the temperatures at which they were isolated. Similar attempts were made to isolate temperature strains of *Cyanidium caldarium*. Because of the instability of acid agar at high temperature, unialgal cultures were obtained by a dilution procedure rather than by plating on agar.

Therefore, the isolates may not necessarily have arisen from single organisms. All of these isolates had a temperature optimum of 45° (Fig. 7), which, although lower than the optimum for photosynthesis, were similarly independent of the temperature of isolation. We conclude that temperature strains of *C. caldarium* do not exist and that over the complete temperature range in which it exists in aquatic environments, 35° to 56°, only a single strain is present with a temperature optimum of about 45°.

**Effect of pH on growth and photosynthesis of Cyanidium**

As indicated in Fig. 1 and 2, *Cyanidium caldarium* appeared to be restricted to habitats having a pH of less than 5.0. However, axenic cultures of *C. caldarium* have been reported
The physiological ecology of *Cyanidium caldarium* by Allen (1959) to grow at pH 7.0 and conversely by Ascione et al. (1966) and Fukuda (1958) not to be able to grow at pH values greater than 5.0. Both heterotrophically and autotrophically grown *C. caldarium* had broad pH optima for growth between 1 and 4 and no growth occurred above 5.0 (Fig. 8). At an initial pH of 7.0, the pH of the medium rapidly decreased and growth began only after the pH had decreased to less than 5.0. However, when the pH was maintained at 7.0, growth did not occur.

The restriction to pH values less than 5.0 appeared not to be a result of the alga's inability to photosynthesize at pH values greater than 5.0. *Cyanidium caldarium* taken from a

habitat at pH around 2 incorporated $^{14}$CO$_2$ equally well at pH 7.0 as at pH 2.0 with an apparent optimum at pH 4.0 (Fig. 9). Experiments on the effect of pH on photosynthesis of cultures gave similar results. The possibility that the end-products of photosynthesis might be different at various pH values or that high pH may have long-term effects on photosynthetic ability was not investigated.
Effect of light on growth and photosynthesis of Cyanidium

Growth of *Cyanidium caldarium* in axenic cultures appears to be inhibited by high light intensities (Halldal & French, 1958; Brown & Richardson, 1968). However, in its natural habitat *C. caldarium* is frequently subjected to light intensities exceeding 6000 ft-candles at midday during the summer. Therefore the method of Brock & Brock (1969) was modified for the determination of the effect of light intensity on the photosynthesis of natural populations of *C. caldarium* in Cyanidium Creek. Low-density populations adapted to various light intensities were produced in a wooden algal-free channel (temperature, about 48°). The channel was divided into four sections. One section was left uncovered, while the three other sections were covered with one, two and three 46 x 61 cm. plates of 0.57 mm. thick neutral density glass (Gray Lite no. 52; Pittsburgh Plate Glass Co.) reducing the light intensity by 53, 83 and 92% respectively (Brock & Brock, 1969). The light reduction may have ultimately been somewhat greater than these values since the glass plates gradually became covered with deposits of silica. No significant differences in temperature developed.

![Graph](image1)

**Fig. 9.** The pH optimum for photosynthesis of a terrestrial population of *Cyanidium caldarium*. An aqueous suspension of a terrestrial population of *C. caldarium* from Roaring Mountain was incubated with 14CO₂ at various pH values. The pH did not change significantly during the experiment.

![Graph](image2)

**Fig. 10.** The photosynthesis of natural populations of *Cyanidium caldarium* adapted to different light intensities as a function of the light intensity. Natural populations of *C. caldarium* from a naturally shaded channel in Nymph Creek (▲) and from portions of a channel in Cyanidium Creek which were uncovered (●) and covered by one (○, 53% light reduction), two (■, 83% light reduction) and three (▲, 92% light reduction) neutral density filters were harvested with suction and diluted with spring water. The photosynthesis of these populations as a function of the light intensity was determined.
under the portions of the channel subjected to different light intensities. A second channel was also placed at 49° in Nymph Creek where the light intensity was considerably reduced by the shading of pine trees on either side of the stream.

After 1 month a layer of *Cyanidium caldarium* had developed. The populations of *C. caldarium* which developed under the lowest light intensity (92% reduction) had significantly higher chlorophyll concentration (3×10^{-7} \mu g./organism) than populations which developed under intermediate and high light intensities (1 to 1.8×10^{-7} \mu g./organism).

The response of these light-adapted populations to various light intensities was then measured *in situ* with [14C]bicarbonate. To avoid self-shading, a relatively dilute suspension of the light-adapted cells was prepared and then distributed to 5.0 ml. vials. These vials were then placed in nylon mesh bags of various thicknesses which reduced the light intensity by 30, 56, 86 and 93% (Brock & Brock, 1969).

Populations which developed under high or intermediate light intensities contained similar concentrations of chlorophyll and were not inhibited by high light intensities (Fig. 10). Populations adapted to low light intensities contained either significantly greater amounts of chlorophyll or were a darker blue-green colour and were inhibited by high light intensities, although they showed higher photosynthetic efficiency at optimum light intensity. The darker blue-green colour suggested higher concentrations of phycocyanin as well as chlorophyll but procedures to measure phycocyanin quantitatively were not available.

**Heterotrophy in Cyanidium**

*Cyanidium caldarium* grows heterotrophically in the dark with a variety of carbon sources (Allen, 1952). Also, Ascione et al. (1966) have grown *C. caldarium* in the light with galactose rather than with 5% CO2 in air. These observations suggest that *C. caldarium* may have three modes of growth – autotrophic, heterotrophic and mixotrophic. The ability of *C. caldarium* to grow heterotrophically at a rate (D.T. 12 h.) approximately equivalent to the maximal autotrophic rate (D.T. = 8 h.) suggests that heterotrophically growing *C. caldarium* may exist in nature. Fluorescence microscopy can distinguish between fluorescing and non-fluorescing cells, but the non-fluorescing cells may be either growing heterotrophically or may be dead. Although *C. caldarium* could be isolated heterotrophically (G. Darland, personal communication), bacteria and fungi were the predominant organisms in glucose enrichments incubated in the dark. These observations were strengthened by the observations of the flora on a darkened channel. Here the primary organisms were again yeast, bacteria, and fungi and only a few apparently moribund *C. caldarium* were observed.

Natural populations of *Cyanidium caldarium* from Nymph Creek incorporated [14C]-glucose. However, since autotrophic, mixotrophic and heterotrophic cultures of the alga, as well as bacteria and fungi, were all able to incorporate glucose, the existence of heterotrophic *C. caldarium* cells in natural populations could not be distinguished by isotopic methods.

Although glucose and other presumably utilisable carbon sources could be incorporated by *Cyanidium caldarium* during growth in the light, no enhancement or inhibition of growth by organic compounds such as glucose, galactose or sucrose was observed. Furthermore, chlorophyll synthesis by *C. caldarium* incubated in the light was not repressed by organic carbon sources.

When grown on glucose in the dark, *Cyanidium caldarium* lacked chlorophyll and phycocyanin. When transferred to the light in the presence of glucose, the synthesis of chlorophyll and the photosynthetic apparatus continued to be repressed (Fig. 11). In contrast, dark grown populations of *C. caldarium* which were transferred to the light without glucose
ceased to divide immediately, but began to synthesize chlorophyll and to assimilate CO₂ at a continually increasing rate. Growth resumed at an apparently slower rate after 20 to 30 h. The initiation of the synthesis of the photosynthetic apparatus was light-dependent, since organisms transferred to a similar medium without glucose but maintained in the dark neither grew nor increased their rate of CO₂ fixation. Therefore glucose appeared to repress the synthesis of the photosynthetic apparatus when dark grown algae were transferred to the light but not in organisms previously grown in the light.

Fig. 11. The effect of glucose on the development of the photosynthetic capability of *Cyanidium caldarium*. Cells of *C. caldarium* strain YS670206 were previously grown in the dark for several generations in Allen's medium with 1 % (w/v) glucose at 45°, pH 3.5. While in exponential growth the cells were harvested, washed and then resuspended in Allen's medium, pH 3.5 with and without glucose: control, no additions made (○); same as control but incubated in the dark (□); glucose added to medium to 1 % (w/v) (■); glucose also added to medium to 1 % (w/v), but incubated in the dark (▲). These cultures were incubated at 45°, 400–600 ft-candles of light. Dark conditions were maintained by covering the incubation tubes with aluminium foil. At various intervals of time, samples were withdrawn and the ability to incorporate ¹⁴CO₂ and the extinction determined.
DISCUSSION

*Cyanidium caldarium* was the sole photosynthetic organism in habitats with pH value less than 5 and temperature greater than 40°. Our studies of photosynthesis in natural populations confirm previous cultural studies of Allen (1959), Ascione et al. (1966) and Fukada (1958), and the field observations of Schwabe (1942, 1944), Negoro (1944) and Rigano & Taddei (1967). *Cyanidium caldarium* is found in both aquatic and terrestrial habitats of appropriate temperature and pH. However, aquatic habitats of suitable temperature and pH are much less extensive than appropriate terrestrial habitats. The predominant habitat for *C. caldarium* was not the relatively stable acid thermal stream but rather the unstable fumaroles and acid thermal soils. In these terrestrial habitats the populations experience not only relatively high light intensities and low pH, but also wide temperature fluctuations. Even the relatively stable acid thermal springs are subject to a greater temperature fluctuation than alkaline effluents because of the greater dependence on ground water supply (Allen & Day, 1935). In such unstable thermal environments, the evolution of strains adapted to a specific temperature range would be of little advantage, and this idea is in keeping with the lack of temperature strains found in our studies. These observations lend support to the thesis of Slobodkin & Sanders (1969) that unpredictable and severe environments are characterized by (1) a low species diversity and (2) by organisms able to adapt to environmental fluctuations by physiological rather than genetic changes. On the other hand, alkaline thermal streams provide an extensive and stable habitat for the blue-green alga *Synechococcus lividus* and temperature strains of this alga are found (Peary & Castenholz, 1964; Brock, 1967).

The pH of the environment also places a restriction on the diversity of species, for the inhabitants must either be acid-tolerant or acidophilic. It is striking that all of the algae found in habitats with pH less than 4 are eucaryotes (Brock & Brock, 1970; Brock, 1971), as is *Cyanidium caldarium*. The presence of acid-labile molecules such as chlorophyll, DNA and ATP suggest that H+ must be excluded from the cell. However, the mechanism of this exclusion has not been elucidated. For the acidophilic organisms, not only the resistance but also the dependence upon H+ remains to be explained. Conceivably the presence of a chloroplast in eucaryotic algae is of benefit in excluding H+ ions from the acid-labile chlorophyll of the photosynthetic apparatus.

*Cyanidium caldarium*, like many other algae, can grow heterotrophically as well as autotrophically. In the dark on glucose, photopigment synthesis was significantly repressed, although there was a low basal amount of chlorophyll present even in cultures maintained in the dark with glucose for long periods of time. When transferred from the dark to a basal medium without glucose in the light, the cells began to synthesize chlorophyll and assume a phototrophic mode of growth. However, when transferred from the dark to the light, but with glucose still present, photopigment synthesis continued to be repressed and the cells apparently continued to grow heterotrophically. If light-grown cells were transferred from a basal medium without glucose to a basal medium with glucose and incubated in the light, chlorophyll synthesis was not repressed. Although the mechanism of repression of chlorophyll is not understood in this alga, in other eucaryotic algae inhibition of chlorophyll synthesis is thought to arise from the lack of either an essential nitrogenous metabolite or reserve material which either represses essential genes or inhibits biosynthetic enzymes (Aoki, Matsuka & Hase, 1965; Shihira-Ishikawa & Hase, 1965; Harris & Kirk, 1969).

In considering the ecological advantage of heterotrophy, it might be noted that the mats of *Cyanidium caldarium* which existed in the aquatic habitats were quite dense, $10^{12}$ cells/m², with a thickness approaching 1 cm. Although *C. caldarium* was present throughout the mat,
the mat had various colour horizons. At the surface the mat was a light green, immediately under the surface the mat was a dark blue-green, and the bottom portion of the mat in most dense populations was yellow-brown. Within the mat there was a light as well as a nutrient gradient. At the surface the cells were exposed to full sunlight but in the lower levels of the mat the effective light intensity may have been limiting. In the water of Nymph Creek, the soluble carbon was quite low (Table I) and may have been insufficient to support a dense heterotrophic population (Fig. 4), but within the mat the soluble carbon concentration may have been increased sufficiently by photosynthesis to support either mixotrophic or heterotrophic growth in the depths of the mat. This is supported by the observed absence of a rapid washout in the population of C. caldarium at Nymph Creek when the channel was darkened.

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REFERENCES


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