After 18 days of heating, T_{sa} had declined almost 2°C, and power input had steadily climbed to approximately 35 percent above its original level. On the other hand, T_{sp} had declined only 0.8°C, and power input had increased only 18 percent over the original level. Thus an initial surface temperature of 45°C appeared to promote a much greater response than the stimulus of 42°C did.

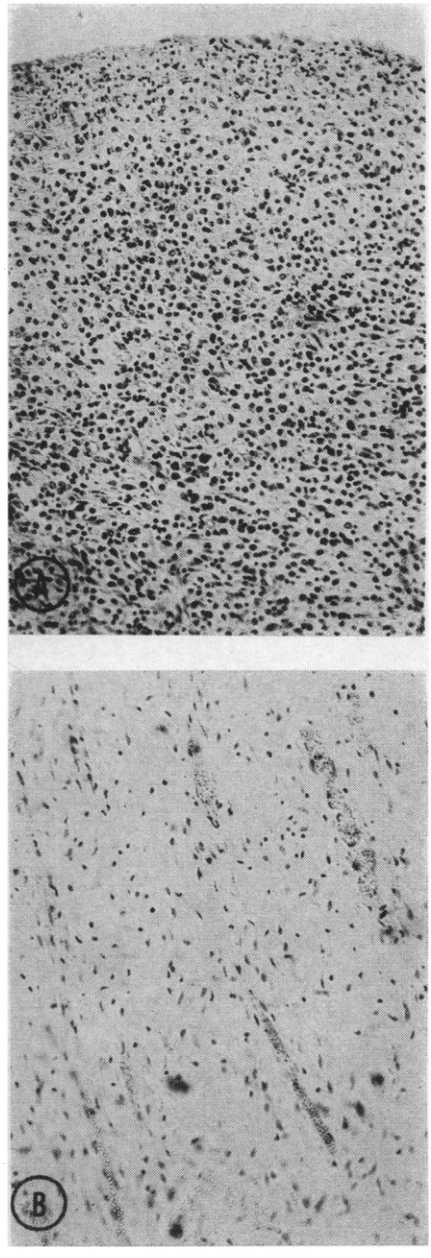

Fig. 2. Histological differences between encapsulating tissue formed at body temperature (A) and that formed in the presence of additional heat (B). (A) Densely packed nuclei in relatively avascular connective tissue. (B) Less-dense fibrous sheath penetrated by numerous vascular channels containing erythrocytes (× 126; hematoxylin and eosin).

The phenomenon observed could result from increased local convective heat transfer by the blood, caused by increased cardiac output, or from increased local effective blood flow, or both. Since it is unlikely that the former underwent such a significant increase, the thermal data suggest a significant change in local vascularization.

At autopsy on the 29th day, a gross histological survey revealed enlarged venous channels radiating from the sites of implantation. The heaters were encased in highly vascularized connective-tissue envelopes that appeared deep red. The tissue around the anterior heater was darker red and appeared more vascular than that around the cooler, posterior heater.

At 60 days, the unheated heaters in the control were found encapsulated in tough, fibrous, relatively avascular tissue like that usually associated with implanted prosthetic devices. This tissue, unlike that around the heated implants, was pale yellow. This finding supports the suggestion that the vascularization of the encapsulating tissue was a result of the heat stimulus. Histological examination of the tissues revealed a marked vascularization of capsular tissue around the heated implant compared to the relative avascularity of that around the unheated one (Fig. 2).

The findings suggest that, in visceral tissue, temperatures high enough to be ordinarily considered damaging to cell function can be used to stimulate vascularization, and at the same time to increase effective local blood flow. Such a response occurs in heated skin of humans (*2*).

The response of visceral tissue in our experiment points up the curious phenomenon that tissue within the body cavity, which is never subjected to noxious temperatures under natural circumstances, exhibits adaptive, compensatory capabilities against such high temperatures.

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5 September 1967

New High-Temperature Chlorella

Abstract. *Growth characteristics of* Chlorella *1-9-30, a new strain of green, high-temperature algae, are compared with those of the widely used* Chlorella *7-11-05. Under comparable conditions,* Chlorella *1-9-30 has the same temperature range for growth as has* Chlorella *7-11-05, but it generally has a higher growth rate. It also differs from* Chlorella *7-11-05 morphologically. Because of its high capacity for organic synthesis,* Chlorella *1-9-30 may be useful in biochemical, biophysical, and physiological research.*

Strains of the green, high-temperature algae were first isolated in 1951 and introduced in 1953. One of those strains, *Chlorella* 7-11-05 (*1*), is now extensively used in physiological and biochemical research. In studies of the adaptation of unicellular algae to mass production, this strain has also been used as a source of organic material for food and for industrial purposes or as a photosynthetic gas-exchanger in closed ecological systems. The growth characteristics of *Chlorella* 7-11-05 have been reported (*1–4*).

I now report on the growth characteristics of another strain of green, high-temperature alga, *Chlorella* 1-9-30, which seems to be superior in its capacity for organic synthesis. *Chlorella* 7-11-05 was isolated from a sample collected in 1951 from Waller Creek on the campus of the University of Texas in Austin, and *Chlorella* 1-9-30 was isolated from another sample collected from the same stream in 1954. The techniques for obtaining the growth characteristics of these two strains have been described (*2*). The capacity for growth is depicted for *Chlorella* 1-9-30 in Fig. 1.

Temperature range (limits) for continuous growth is practically the same for the two strains. Optimum temperature for growth is 38° to 39°C, maximum temperature is about 42°C, and minimum temperature (depending on light intensity) is somewhere between 15° and 20°C. Minimum temperature suitable for growth extends generally to lower temperatures at lower light intensities; that is, the capacity to grow at lower temperatures is limited in the high-temperature strains by increase in light intensity (*5*).

Under comparable conditions, growth rate for *Chlorella* 1-9-30 is higher than for *Chlorella* 7-11-05. Thus, at 38°C

and 17,600 lumen/m², other conditions being optimum, the number of cells of *Chlorella* 1-9-30 doubles 11.3 times compared to 9.9 times for *Chlorella* 7-11-05 over a 24-hour period. When the amount of growth is plotted against the light intensity, the resulting curve asymptotically approaches a plateau indicative of light saturation, and the growth rate may slightly increase with a further increase in light intensity. At 29,700 lumen/m² and 38°C, the growth rate may reach 10.5 doublings per 24-hour period for *Chlorella* 7-11-05, and 11.8 doublings for *Chlorella* 1-9-30.

The growth rates for *Chlorella* 7-11-05 are somewhat higher than previously reported for this strain (*3*). The feasibility of such an increase, with the improvement in growth conditions, was, however, foreseen (*3*). The improvements in experimental conditions, which made higher growth rates possible, consisted of more frequent transfers of the batch cultures into fresh media and use of a fluorescent, instead of an incandescent, light source.

These growth rates were measured on nonsynchronized populations of cells. The reaction of a cell to strong light varies in the course of the life cycle (*6*). Light-saturating intensities and the capacity to use strong light for photosynthesis are higher for cells which have not yet approached the end of the growth division cycle in their development. This capacity becomes greatly impaired as the cell draws near cell division. At that time, strong light is detrimental to photosynthesis, growth, and cell division. Consequently, growth rates for synchronized populations of algal cells may be considerably higher than for the random nonsynchronized cultures (*7*).

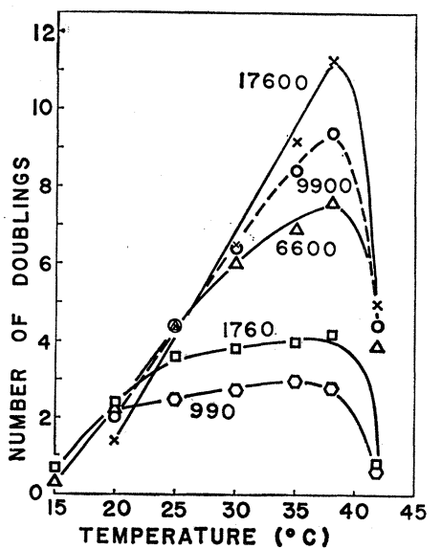

Fig. 1. Growth rates (number of times the cell material doubled over 24-hour period, measured as optical density) of *Chlorella* 1-9-30 as dependent on temperature and light intensity. Light intensities (in lumen/m²) are indicated on the curves.

With decreases in temperature and light intensity, growth rates decline (Fig. 1). However, at any light intensity, the capacity for organic synthesis is higher in *Chlorella* 1-9-30 than in *Chlorella* 7-11-05. The maximum growth rates (in doublings of cell material per 24-hour period) for the two strains were, respectively, at 9900 lumen/m², 9.4 and 8.0; at 6600 lumen/m², 7.5 and 6.0; at 1760 lumen/m², 4.2 and 3.6; and at 990 lumen/m², 3.0 and 2.7.

Morphologically, *Chlorella* 1-9-30 differs from *Chlorella* 7-11-05 in having somewhat larger cells. Cell size in *Chlorella* is variable depending on temperature, light intensity, nutritional factors, population density of cell suspensions, and the history of the cells and on their physiological condition (determined by developmental stage). For the young daughter cells, the cell size in both strains may range from 1.5 to 3.0 μ; just before the release of the daughter cells from the wall of the mother cell, the cell size of the mother cells may reach 8 to 10 and even 12 μ in diameter. The distinction between the cell size in two strains can only be made under strictly comparable experimental conditions.

Taxonomically, *Chlorella* 7-11-05 at the time of its introduction (*1*) was assigned to the species *C. pyrenoidosa*. On the basis of biochemical investigations, *Chlorella* 7-11-05 was later elevated to the rank of a species, *Chlorella sorokiniana* (*8*). Recently, using different assemblage of *Chlorella* strains and different biochemical characteristics, Kessler suggested that *Chlorella* 7-11-05 be united with several other strains in one species designated at *C. III* (*9*). The taxonomic identification of *Chlorella* 1-9-30 will depend on special comparative investigations.

In comparison to low-temperature algae, the usefulness of high-temperature algae lies in their capacity to grow at higher temperatures and in their increased efficiency at higher light intensities. Metabolic activity and growth rate, as an integrated indicator of this activity, are much greater under optimum conditions in the high-temperature strains than in the low-temperature strains (Fig. 2). Greater metabolic activity permits measurements of metabolic and biochemical characteristics with higher accuracy and within a shorter period of time.

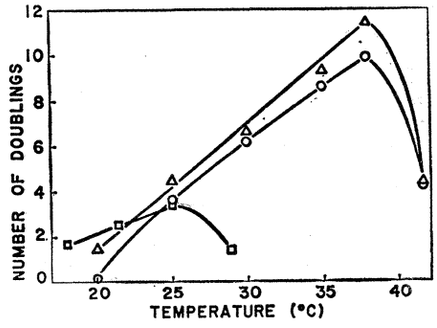

Fig. 2. Growth rates of the green, high-temperature algae, *Chlorella* 7-11-05 and *Chlorella* 1-9-30, in comparison with the growth rates of the low-temperature Emerson strain. Circles, *Chlorella* 7-11-05; triangles, *Chlorella* 1-9-30; squares, Emerson strain. Light intensity, 17,600 lumen/m².

An extended range of temperature and light intensity makes high-temperature algae especially suitable for studies of the effects of temperature and light on photosynthesis, growth, cell division, and other physiological processes. A critical problem in illuminated cultures is the generation of heat. Due to greater endurance of higher temperatures by high-temperature algae, temperature control in algal cultures is more manageable and more economical than in cultures of low-temperature algae. The newly discovered member of the high-temperature group, *Chlorella* 1-9-30, shares all of the above characteristics with *Chlorella* 7-11-05. The superior capacity for growth of *Chlorella* 1-9-30 may be of advantage in biochemical, biophysical, and physiological research.

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