(r = .059), indicating independent genetic determination of the two characters.

Finally, an independent example of the nonadditive nature of resistance components comes to us from the results of an investigation in which the aims were quite different from our own. Hill et al. (1), disturbed by the possibility that residual congenital passive immunity might influence inheritance studies, undertook to employ a partially purified toxic fraction isolated from S. typhimurium. As in our experiments, marked response to selection occurred. The survival rates among toxin-injected mice (combining 9th and 10th generations) were 64.2% for the selected group compared to 14.0% for the control group. When live organisms were employed on similar mice, either per os or intraperitoneally, the results were "very surprising." The genetically toxin-resistant mice were on the average uniformly more susceptible to the living organism than were the controls. Unfortunately the experiment was terminated by the outbreak of the war, but the parallel with our independent results concerning leucocytes and blood pH is striking.

To test the suggestive hypothesis that a physiological relationship between leucocyte count and toxin resistance might exist, mice of LCH, LCL, and T strains have been subjected to massive intraperitoneal injections of vaccine. The results have been negative in terms of the hypothesis. In the first experiment (Test 9) employing a dose of 2,000,000,000 killed organisms, survival rates were: LCH, 28%; LCL, 29%; T (inbred), 34%. In Test 10, with a dose of 1,500,000,000, survival rates were: LCH, 49%; LCL, 54%; T (inbred), 61%. Nearly all deaths occurred within 48 hr of inoculation. Although the mouse strains reacted in the same manner to toxins as to live organisms, there still existed a possibility that there might be strain differences in ability to acquire immunity. Accordingly, mice of the 3 strains were immunized by sublethal doses of vaccine and subsequently challenged with 200,000,000 live organisms. Survival rates were: LCH, 76%; LCL, 79%; T (outbred), 77%; T (inbred), 84%. This experiment has not been repeated, but the test involved 209 mice with approximately equal numbers per strain. Agglutination tests failed to show consistent strain differences in antibody titer.

Some degree of genetic resistance to a naturally occurring mouse disease, as a result of past natural selection, is to be expected in any population of mice. Moreover, components of resistance are likely to be balanced in some manner. Artificial selection, based on ability to survive infection, will accomplish essentially the same result as a naturally occurring epidemic and should also lead to balanced gene combinations. This will not be true in the case of selection experiments directed toward altering a single character unless this character is directly adaptive. If leucocyte number, for example, affected resistance in general, then selection for high count should lead to enhanced resistance. Our investigation supports the

view that resistance to mouse typhoid is dependent on complex interactions of characters which, taken individually, do not confer resistance to the host.

Nonspecific mechanisms of resistance to bacterial diseases are likely to be common to mice in general, so that isolation of genetic components will be possible only in those rare cases which involve a mutation in an essential gene. Lack of complement in guinea pigs, resulting from a defective recessive gene, affords an example (12). Complement-deficient animals were found so susceptible to a variety of diseases that the line could not be maintained even under laboratory conditions.

#### References

- 1. HILL, A. B., HATSWELL, J. M., and TOPLEY, W. W. C. J. Hyg., 40, 538 (1940).
- GOWEN, J. W. Scientia, 44, 145 (1950).
   GOWEN, J. W., and CALHOUN, M. L. J. Infectious Diseases, 73, 40 (1943).
- 4. WEIR, J. A. Ibid., 84, 252 (1949).
- 5. GOWEN, J. W. In L. C. Dunn (Ed.), Genetics in the 20th Century. New York: Macmillan, 401 (1951).

- сеничту. New 10гк: Macmillan, 401 (1951).
  6. MacARTHUR, J. W. Am. Naturalist, 78, 142 (1944).
  7. WEIR, J. A. Records Genetics Soc. Am., 18, 118 (1949).
  8. BELL, A. E. J. Infectious Diseases, 85, 154 (1949).
  9. ОАКВЕRG, E. F. Ibid., 78, 79 (1946).
  10. BLOOM, W. L., et al. Ibid., 80, 41 (1947).
  11. CLARK, R. D. Selection of Strains of Mice for High and Lown Elode and and the analysis. Low Blood pH and Strain Reaction to Mouse Typhoid. M.S. thesis. Library, Univ. Saskatchewan (1950).

12. RICH, F. A. Vermont Agr. Expt. Sta. Bull., 230, 1 (1923).

Manuscript received August 8, 1952.

# A High-Temperature Strain of Chlorella

## Constantine Sorokin and Jack Myers

Department of Zoology, The University of Texas, Austin and Carnegie Institution of Washington, Stanford, California

Various strains of the green alga Chlorella have been used extensively for study of photosynthesis and other physiological processes. Such work has been restricted almost exclusively to temperatures at or below  $25^{\circ}$  C. The choice of lower temperatures, probably made on the basis of qualitative experience, is consistent with the ecological observation that "many algae do not survive a rise in temperature and thrive only in cold waters" (1).

Recent investigations on the mass culture of algae have demonstrated the difficulties of maintaining temperatures as low as 25° C in dense suspensions under sunlight illumination (e.g., [2]). An alga with a higher temperature optimum would be of obvious usefulness for both practical application to mass culture and experimental application to physiological studies. We are now able to report the isolation of a number of strains of Chlorella with higher temperature optima and the salient characteristics of one of the strains.

Samples from warm local surface waters were used to supply inocula for test tube cultures grown in a Knops solution at pH 6.8; the medium was provided with microelements and 0.5 g/liter of a chelating



FIG. 1. Growth rate as a function of temperature.  $\triangle$ , Chlorella pyrenoidosa (Emerson's strain) at 1600 ft-c; x, Tx 71105 at 1600 ft-c; O, Tx 71105 at 500 ft-c;  $\phi$ , Tx 71105 at 2800 ft-c.

agent, ethylene diamine tetracetic acid, as previously described (3). The cultures were aerated with 4%carbon dioxide in air, illuminated at 500 ft-c by fluorescent lamps, and incubated at  $32^{\circ}$  C. After several serial transfers, samples were plated out on agar medium. Bacteria-free cultures were obtained from isolated colonies.

Preliminary study of 12 strains isolated at 32° C indicated that one of these (Tx 1105) would show rapid and continued growth at 39° C. After repeated culture at 39° C, a second plating-out yielded a second series of isolates. One of these (Tx 71105) was selected for further study, preliminary results of which are presented here. Subsequently a third series of isolates was obtained via accumulation cultures at 39° C. Among 61 original strains established there are significant differences in growth rate and in the tendency of the cells to clump in liquid cultures. Cells of the various strains are not readily distinguishable from the Emerson strain of Chlorella pyrenoidosa in size, morphology, or mode of reproduction; at this time we prefer to regard them as strains of a species of Chlorella.

Tx 71105 and the Emerson strain of *C. pyrenoidosa* have been submitted to a comparative study of the effect of temperature on the growth rate at light saturation. Growth in test tube cultures in photo-thermostats (as described above for the accumulation cultures) was determined in terms of the optical density measured by an Evelyn colorimeter with 600 mµ filter. The logarithm of the optical density plotted against time yields a straight line, the slope of which (k) is the specific growth rate. For *C. pyrenoidosa* the method yields a value for k of  $0.85 \pm 0.05 \log_{10} u/day$  at 25° C, as compared to a value of 0.87 determined by the more elegant method of an automatic dilution device.

Light-saturation of growth of *C. pyrenoidosa* could be achieved by two banks of two 20-w daylight fluorescent lamps placed on each side of the photothermostat and delivering about 500 ft-c to opposite sides of the test tube cultures. This arrangement was adequate also for Tx 71105 at temperatures below  $26^{\circ}$  C. At higher temperatures two banks of tungsten lamps delivering 1600 ft-c or 2800 ft-c to opposite sides of the cultures were used to obtain light-saturation.

Cultures for the growth measurements were inoculated from 3 units of a continuous-culture apparatus which contained Tx 71105 at  $25^{\circ}$  and  $39^{\circ}$  C and C. *pyrenoidosa* at  $25^{\circ}$  C. Test tube cultures held at the experimental temperature were continued by serial transfer until the growth rate remained constant over two successive attempts.

The results are presented in Fig. 1. The less extensive data for *C. pyrenoidosa* indicate a temperature optimum at  $25^{\circ}-26^{\circ}$  C. In spite of repeated attempts we were unable to train *C. pyrenoidosa* to maintain a stable growth rate at temperatures higher than  $30^{\circ}$  C. The temperature optimum for Tx 71105 lies at about  $39^{\circ}$  C. A stable growth rate for this strain could not be obtained above  $41.2^{\circ}$  nor below  $25.5^{\circ}$  at 1600 ft-c. Reduction of the light intensity to 500 ft-e permitted a stable growth rate down to at least  $21.5^{\circ}$  C.

Preliminary manometric studies on Tx 71105 at  $39^{\circ}$  C have yielded maximum values of about 5 mm<sup>3</sup> O<sub>2</sub>/mm<sup>3</sup> cells/hr for respiration and 100 mm<sup>3</sup> O<sub>2</sub>/mm<sup>3</sup> cells/hr for photosynthesis, although in the latter case we are not certain that light-saturation was attained. This is the highest rate of photosynthesis per unit quantity of cell material of any organism so far observed.

### References

- 1. PRINGSHEIM, E. G. Pure Cultures of Algae. Cambridge: University Press, 82 (1946).
- FISHER, A. W., JR., WALSH, W. A., and PETTENGILL, K. H. In J. S. Burlew (Ed.), Algal Culture: From Laboratory to Pilot Plant, Carnegie Institution of Washington Pub. 600, Chap. 17 (in press).
   MYERS, J., PHILLIPS, J. N., and GRAHAM, J. R. Plant
- MYERS, J., PHILLIPS, J. N., and GRAHAM, J. R. Plant Physiol., 26, 539 (1951).

Manuscript received August 14, 1952.

## Metabolism and Removal of Ca<sup>45</sup> in Man<sup>1</sup>

## Judith Bellin and Daniel Laszlo<sup>2</sup> Division of Neoplastic Diseases, Montefiore Hospital, New York

This report deals with the metabolism and removal of  $Ca^{45}$  in man, as studied by the administration of a single tracer dose of high specific activity radiocalcium. Although such data are available for animals (1-9), none has been reported for man. From the rate of disappearance of  $Ca^{45}$  from the blood stream, its excretion, and its uptake and removal from bone, information on calcium metabolism may be obtained without disturbing the calcium homeostasis.

The sodium salt of ethylene diamine tetracetic

<sup>1</sup> This study has been supported in part by a grant from the National Cancer Institute of the National Institutes of Health, USPHS, and in part by a Contract AT(-30-1)-880 with the U. S. Atomic Energy Commission.

<sup>2</sup> The assistance of Leonard Woidowsky and Agnes Hausinger is gratefully acknowledged.