KINETIC STUDIES OF TEMPERATURE EFFECTS ON THE CELLULAR LEVEL*

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SUMMARY

Temperature and illuminance dependence of growth was studied in the low- and high-temperature strains of the green unicellular alga, *Chlorella pyrenoidosa*. The developmental and metabolic diversity of cells in steady-state microbial populations was evaluated. The effects of temperature and light were considered separately on the accumulation of cell material and on cell division. It was concluded that the values of activation energy for growth are characteristic of neither of these two processes but are influenced by both of them. The implications of the master reaction theory and of the statistical approach on the cellular and reaction levels in biochemical and biophysical studies were critically discussed.

INTRODUCTION

Kinetic studies of temperature effects on simple reactions or complex biological processes, *in vitro* or *in vivo*, have been invariably evaluated on the reaction level. The enormous volume of the literature in this field precludes discussion here of even the most significant publications. A reference is made to several books and reviews in which the effects of temperature on life processes, the kinetics of these processes and the theoretical aspects of the reaction rates are discussed[1–5].

A treatment of the temperature data on a cellular level, attempted in this report, may add a new approach in kinetic studies of biological processes. By using unicellular organisms all complications arising from the structural and physiological relationships of the cells in multicellular organisms were avoided. Photoautotrophic properties of these organisms permitted studies of temperature effects in connection with another environmental factor, light. The availability of the strains of different temperature preference allowed an extention of the temperature range not readily obtainable with only one organism.

MATERIAL AND METHODS

The studies involved comparative investigations of two strains of green unicellular alga: *Chlorella pyrenoidosa*, Emerson strain, representative of the low-temperature

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group, and *Chlorella* 7-11-05 representing the high-temperature algae. Descriptions of the strains and details of the technique have been published elsewhere[6,7].

RESULTS AND DISCUSSION

Values of activation energy for growth

The dependence of growth in two strains on temperature is described at two light intensity levels: 440 foot candles, fluorescent lamps, and 1600 foot candles, incandescent lamps (Fig. 1). In an attempt to evaluate the temperature curves the ascending portions of the curves in Fig. 1 are replotted as logarithms of the rates against the reciprocals of absolute temperature (Fig. 2). Although these curves were plotted in Fig. 2 with the abrupt breaks in the slopes, these sharp breaks are considered not to be real but to be caused by the relatively small number of measurements along the temperature scale, the actual changes in the slopes being gradual.

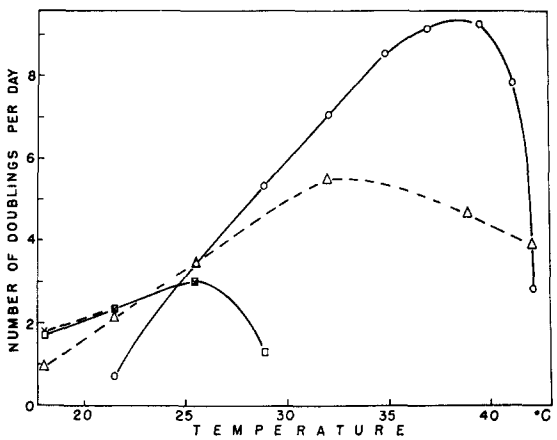

Fig. 1. Multiplication rates of *Chlorella* 7-11-05 and the Emerson strain as a function of temperature. The symbols are as follows: circles, *Chlorella* 7-11-05 at 1600 foot candles, incandescent light; triangles, *Chlorella* 7-11-05 at 440 foot candles, fluorescent light; squares, Emerson's strain at 1600 foot candles, incandescent; and, crosses, Emerson's strain at 440 foot candles, fluorescent.

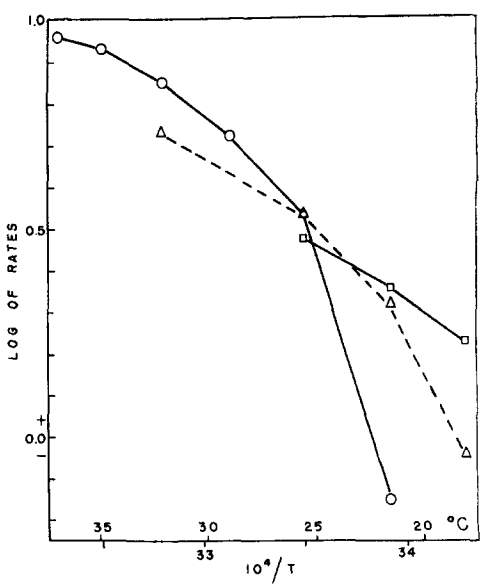

Fig. 2. Logarithms of the multiplication rates for *Chlorella* 7-11-05 and the Emerson strain plotted against the reciprocals of absolute temperature. Circles, *Chlorella* 7-11-05 at 1600 foot candles; triangles, *Chlorella* 7-11-05 at 440 foot candles; and squares, Emerson's strain at 1600 and 440 foot candles.

A comparison of the logarithmic curves indicates that their slopes are steeper and consequently the values of activation energy, μ, calculated from these slopes (Table I), are higher for *Chlorella* 7-11-05 than for the Emerson strain, compared in the same temperature ranges. For *Chlorella* 7-11-05 they are greater at 1600 foot candles than at 440 foot candles. Since growth curves are downwardly concave, the values of μ increase with the decrease in temperature. For *Chlorella* 7-11-05 in the temperature range of 21.5–25.5° at 1600 foot candles μ is equal to 68,000.

TABLE I

VALUES OF ACTIVATION ENERGY (μ) FOR GROWTH

Temperature interval, °C	*Emerson at 440 and 1600 f.c.*	*7-11-05 at 440 f.c.*	*7-11-05 at 1600 f.c.*
18.0–21.5	15,000	41,000	
21.5–25.5	12,000	21,000	68,000
25.5–28.9		13,000	23,000
28.9–32.1			17,000
32.1–34.9			12,000
34.9–37.0			6,000

Far-reaching conclusions could be drawn from the kind of data presented in Fig. 2 and in Table I. The conclusions and speculations could bear upon the pronounced difference in the levels of activation energy for growth between the low- and high-temperature strains and upon the higher requirements for activation energy at higher light intensity clearly demonstrated for *Chlorella* 7-11-05. From the inconsistency of the slopes of the logarithmic curves in different temperature intervals it could be deduced, as it has been often done, that different reactions limit the rate of the over-all process at different temperatures. An attempt to determine the number of these reactions and to formulate their thermodynamic and possibly some other characteristics could also be made.

The inconsistency and differences in the steepness of the slopes of the logarithmic curves require, however, special consideration. Given a sufficient temperature range any curve bends at the upper end. The cause of the phenomenon is probably a competing process, or possibly a number of competing processes, which interferes with the increase in metabolic rates with the increase in temperature. Such processes can operate at temperatures considerably lower than those for optimal growth. But this is hardly adequate to explain the gradual decrease in the logarithmic growth rates all the way to temperatures at which the growth rate is minimal. An examination of the effects of temperature and light on cell division may provide such an explanation.

The effect of temperature and light on cell division

The effect of temperature on cell division is indicated by the following observation. If a suspension of synchronized cells of *Chlorella* 7-11-05, grown in the light and brought to readiness for cell division, is placed in the dark at 39°, cell divisions in individual cells are completed within 3 to 4 h. If the same algal suspension is placed in the dark at 15°, cell division proceeds so slowly that even after 24 h in darkness one finds only a few cells divided into daughter cells. Between 15° and 39° the rate of cell division is a

function of temperature. At lower temperatures the over-all growth of an algal suspension is greatly retarded by the effect of low temperature on cell division. This explains why the logarithmic curve of the growth rates (Fig. 2) is steeper and the value of μ higher in the lower temperature range.

Another factor contributing to the change in the slopes of the curves plotting logarithms of the growth rates against temperature (Fig. 2) is the effect of light intensity on cell division. In a suspension of synchronized cells of *Chlorella* 7-11-05 cell divisions usually start after 9 to 10 h of growth under optimal conditions of temperature and light intensity. However, when the time for cell division arrives all cells do not divide simultaneously. Individual cells divide after different periods of growth, and, in an algal suspension as a whole, cell divisions are generally spread over 8 to 9 h if the cell suspension is left in the light. If a suspension of cells ready to enter cell division is transferred into darkness, then the time necessary for all cells to complete division is shortened to 3–4 h.

The effect of light intensity on cell division is reflected in higher requirements for minimal temperatures at higher light intensities if cell division and over-all growth are to take place. In Fig. 3 the lower portions of the growth curves of Fig. 1 are extrapolated to limiting temperatures at which multiplication rates have their minimal values. For *Chlorella* 7-11-05 the limiting temperature for growth is at 1600 foot candles at 20.5° and at 440 foot candles it is at 15.3°. For the Emerson strain the minimal temperatures for growth at these light intensities are, respectively, 7.8° and 6.6°. As a result the starting points for growth are higher on the temperature scale at higher light intensities and the temperature curves become steeper. This causes values of μ to rise at higher light intensities (Table I).

Master reaction theory

An attempt to interpret the effects of temperature and light intensity on growth can be advantageously connected with the understanding of growth as consisting of two processes—cell division and accumulation of cell material. A conception of the dependence of the processes of accumulation of cell material and cell division on temperature and light intensity is given in Fig. 4 in which logarithms of the hypothetical rates of these two processes at two levels of light intensity are plotted against the reciprocals of absolute temperature.

In photoautotrophic organisms the process of accumulation of cell material is largely dependent on photosynthesis. Both the increase in temperature and in light intensity, within limits, favorably affect the photosynthetic rate, and, at higher temperatures, the light saturation point is moved to a higher light intensity. For this reason the dependence of the rate of the accumulation of cell material on temperature is depicted (Fig. 4) at two light intensities by two diverging lines indicating a favorable effect of higher light intensity at higher temperatures.

It has been shown that the increase in temperature, within limits, favorably affects the rate of cell division, but the increase in light intensity, at least at low temperatures, has an unfavorable effect on this process. For this reason the lines indicating the rate of cell division at two light intensities are drawn far apart at lower temperatures. The suggestion that these two lines are converging, as they approach higher temperatures, needs further experimental verification.

The difference in the slopes of the temperature curves for the accumulation of cell

material and cell division results in a crossing of the curves at some temperature. With regard to the effects of temperature one is tempted to discern two master reactions governing the over-all process of growth in the lower and higher temperature ranges. Each of these reactions is connected with one of the two different processes—one, cell division, limiting the over-all process of growth in the lower temperature range; another, accumulation of cell material, the rate of which is largely responsible for the rate of the over-all process of growth in the higher temperature range.

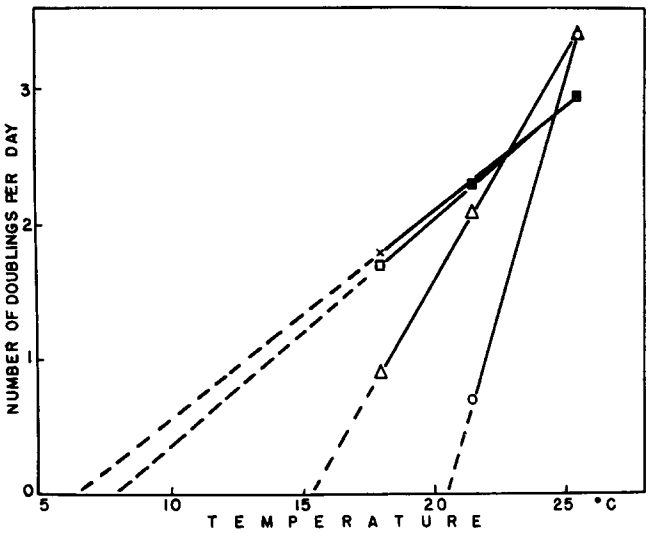

Fig. 3. Multiplication rates of *Chlorella* 7-11-05 and the Emerson strain extrapolated to minimal temperatures. The symbols are as follows: circles, *Chlorella* 7-11-05 at 1600 foot candles; triangles, *Chlorella* 7-11-05 at 440 foot candles; squares, Emerson's strain at 1600 foot candles; and, crosses, Emerson's strain at 440 foot candles. Solid lines are based on data obtained from measurements; broken lines are results of extrapolation of experiment data to minimal temperatures.

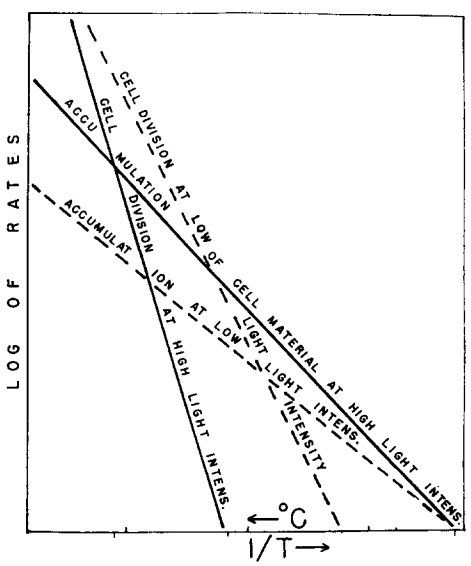

Fig. 4. Hypothetical dependence of the accumulation of cell material and cell division on temperature and light intensity.

From Fig. 2 one can see that the most drastic changes in the slopes of the logarithmic curves for growth coincide with the temperature interval of 25–26°. These changes are reflected in differences of μ values below and above this point. For *Chlorella* 7-11-05 at 1600 foot candles μ for the temperature range of 21.5–25.5° is

68,000, and for that of 25.5–28.9°, 23,000; at 440 foot candles the values of μ are, respectively, 21,000 and 13,000. In accordance with the view that a master reaction can be characterized by the value of activation energy, one logically concludes that for *Chlorella* 7-11-05 the temperature region of 25–26° is a breaking point at which a change from one master reaction to another takes place.

The master reaction theory worked out by BLACKMAN[8] and PÜTTER[9] and expanded by CROZIER[10] is a speculative theory as applied to complex biological processes. It has not been proved or disproved for the simple reason that no master reaction has been isolated and studied as such in any complex biological process. The speculations reflected in Fig. 4 have the advantage of being based on the factual knowledge of the processes of the accumulation of cell material and cell division. The participation of these two processes in the over-all process of growth is not a speculation. Several of the characteristics, particularly the dependence of these two processes on temperature and light intensity, are well established.

The participation of these two processes in the over-all process of growth represents probably the most tangible model for the master reaction concept and such a model is not readily available for any other complex biological process. Though the discussion of the data here is on a cellular level it could probably serve as a pattern for the treatment of similar data on a reaction level. Yet the limitations of the master reaction concept should be clearly comprehended.

Evaluation of the master reaction theory

In a succession of generations of cells growth is not an uninterrupted process. Cell division and the accumulation of cell material succeed each other in time. Depending on the environmental factors the time distribution of these two processes in the life cycle of a cell is such that at the beginning of cell development the accumulation of cell material is predominant or largely expressed. By the end of the life cycle the processes leading to and indicating cell division become dominant, to the complete suppression of the process of the accumulation of cell material. In substituting for each other the accumulation of cell material and cell division are consecutive and interdependent steps in the over-all process of growth.

During a more or less extended period of the life cycle these two processes proceed simultaneously and compete with each other. Competition between these two processes is conditioned by factors inherent in the genetical constitution of the organism. It is aggravated by differences in the sets of conditions favoring each of these processes. As far as an individual cell is concerned, the processes of the accumulation of cell material and of cell division proceed interdependently, consecutively, and competingly. In a growing cell any characteristic is a resultant of the interplay of these two processes.

The conclusions reached that the over-all process of growth in the lower temperature ranges depends largely on the rate of cell division, and in the higher temperature ranges depends on the accumulation of cell material may be useful in broad qualitative terms. However, there is no reason to believe that at any temperature one of these two processes assumes an exclusive role as a limiting factor in the over-all growth process. The slopes of the temperature curves and the values of μ are characteristic of neither of these processes but are influenced by both of them. The breaks in the slopes of temperature curves are uncertain indications of the temperatures at

which one of these processes assumes a predominant role in conditioning the rate of growth.

For photoautotrophic organisms temperature dependence is complicated by the complementary effects of light. For organisms growing in the dark, such as *Escherichia coli*, temperature curves were obtained giving a linear Arrhenius plot over an extended temperature range[2]. However, this observation does not indicate that in heterotrophic organisms the processes of cell division and of the accumulation of cell material are not affected differentially by temperature. Experiments on the synchronization of bacterial cultures indicate that also in these organisms cell division and the accumulation of cell material have different temperature characteristics[11]. Thermodynamic information obtained in growth studies of heterotrophic organisms is therefore no more characteristic of any single reaction or of any simple process than it is for photoautotrophic organisms.

So far the discussion of the relationships of the processes of accumulation of cell material and of cell division has been limited to the status in a single cell or in a succession of a few generations of its progeny. In multicellular organisms as well as in steady-state suspensions of microorganisms the situation is much more complex.

Studies on synchronized algal suspensions[12–14] have revealed considerable differences in metabolic activity during the life cycle of a cell. Small daughter cells emerging from cell division have the lowest rates of metabolic activity. Then, as the cells grow, the rates of metabolic processes increase. The peak of photosynthetic activity in *Chlorella* 7-11-05 at 39° coincides with cells about 3 h old after which time the photosynthetic capacity decreases to the time of cell division. The peak in respiration activity occurs much earlier in the life cycle—about half an hour after the start of growth.

Studies on the formation of nucleic acids[15,16] indicated considerable differences in the time course of the accumulation of the ribonucleic and desoxyribonucleic acids during the life cycle of the cells. The accumulation of chemical elements as well as effect of the deficiency in different elements, particularly in sulfur, also has been shown to be dependent on the stage of cell development[17,18].

Biochemical and biophysical studies on the effects of environmental factors on cellular characteristics have been based on a tacit assumption that these effects on the studied cellular characteristics are the same in sign and more or less the same in degree in different cells. No proof for this assumption can be found in the experimental data. As was pointed out, cell division and accumulation of cell material have different optimal temperature, illuminance, and nutritional requirements. It has been shown that cells at different stages of the life cycle have different rates of metabolic activity. Since a steady-state cellular population consists of cells in different developmental stages, it can be expected that individual cells differ widely in their responses to environmental factors. Any cellular characteristic obtained for such material is an average of characteristics of individual cells and, as in any average, may or may not be intrinsic to any portion or, strictly speaking, even to a single cell of a given population. Studies on synchronized microbial cultures reveal the metabolic and biochemical differences in cells of different developmental stages and emphasize the futility of the statistical approach in measurements of cellular characteristics for steady-state populations.

All this poses a problem in the calculation of theoretical curves from kinetic data

for complex biological processes. Calculations of the theoretical curves involve reliance upon one of the theories of reaction rates. However, it should be pointed out that whatever major theory—the EYRING theory[19] of absolute reaction rates, or the MICHAELIS-MENTEN theory[20] of the kinetics of enzymic reactions—is laid as a basis for calculations of the reaction rates, the member of the equation indicating temperature dependence of the process is obtained in practice from kinetic data derived from measurements of the rate of the process at different temperatures. Any agreement of the theoretical curve with experimental data is often considered a proof of the validity of the theoretical curve. Thus, from both the theoretical and the experimental point of view a proper evaluation of kinetic data is of considerable interest.

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