FINAL REPORT

Effects of Environment on Cell Modulations

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from the Army Research Office

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During the grant period, funds were used for partial support of a number of related studies of cell modulation in bacteria. The principal support, however, was for the work of graduate student Robert P. Moore. This work was reported in Dr. Moore's thesis ("Influence of Temperature on Macromolecular Composition of Escherichia coli", Ph.D. Thesis, Iowa State University, Ames, Iowa, 1969). Experimental findings and conclusions from that study are summarized in this report. A detailed report of the findings is being prepared for publication; publications will acknowledge DA-ARO support, and reprints will be furnished.

The objective of this research was to determine the relationship between temperature and the physiological state of Escherichia coli during balanced growth. This problem has been studied by asking the questions: 1) to what extent does temperature affect the rate of balanced growth, 2) does the chemical environment alone regulate the rate of growth and determine the physiological state of an organism, or 3) are both the chemical and physical environments responsible for a particular physiological state?

The relationship between incubation temperature and the physiological state of Escherichia coli ATCC 9367 was studied in batch and chemostat cultures. Chemostat cultures were grown in Brain Heart Infusion broth at two different temperatures and at three different rates of growth. Both physical and chemical methods were employed to characterize quantitatively the macromolecular composition of the steady state populations for the purpose of determining the extent to which the chemical and physical environments were responsible for a particular physiological state.

The optimum growth temperature is usually considered to be that temperature at which the growth rate of an organism is at its maximum. Below the optimum growth temperature, the rate of growth is reduced. Variations in the rate of growth of batch cultures with temperature have been shown to conform to the Arrhenius equation over the growth temperature range of an organism. None of the theoretical considerations of the continuous cultivation of bacteria has considered temperature as a variable in controlling rates of balanced growth. Rates of growth were shown to be a function of the composition of the growth medium, and presumably temperature was expected to affect rates of growth in the same manner as observed in batch cultures.

In batch cultures of E. coli, the growth rate at 25 C was a little more than half the 37 C rate. From these results it was expected that the maximal

rate of balanced growth in a chemostat at 25 C would be half that at 37 C. Temperature was found to affect the maximal growth rate of E. coli in chemostat cultures, but not to the extent observed in batch cultures. The maximal growth rate of E. coli at 37 C in chemostat culture was approximately 2.25 generations/hr. while in batch culture the maximal rate was 2 generations/hr. At 25 C, the maximal rate was 2.1 generations/hr in the chemostat and 1.27 generations/hr in batch culture. Earlier workers had observed similar differences in maximal growth rates between batch and chemostat cultures. They suggested that growth at values higher than the critical dilution rate could result from (1) the growth rate in continuous culture being higher than in batch culture, or (2) from the washout rate being less than predicted by the growth equation for continuous cultivation of bacteria. The results obtained in our balanced growth studies with E. coli support the idea that the growth rate attainable in the chemostat is faster than the growth rate in batch cultures.

As the rate of dilution is increased in a chemostat, the concentration of organisms is decreased by dilution and the substrate concentration increases. Thus when the dilution rate is fixed, the substrate concentration and population density come to a level that establishes an equilibrium between the growth rate and the dilution rate. The results obtained in our balanced growth with E. coli indicate that for a given medium, the ratio of nutrients/cell required for a particular growth rate is determined by temperature. The difference in steady state populations at the two incubation temperatures indicates that a higher nutrient/cell ratio is required at 25 C for growth at a given rate, but when the proper ratio exists the rate of growth is independent of temperature. The increased nutrient requirement probably reflects an increase in the maintenance energy requirement for organisms growing at the lower temperature. In a batch culture system, the equilibrium between nutrient concentration and cell population cannot be effectively established, so a lower maximal growth rate is observed.

Chemical analyses of steady state populations supported the concept that cells are characterized by a unique macromolecular composition at each growth rate. Although the amount of RNA, DNA and protein per ml of culture decreased with an increase in growth rate, there was an increase in total nucleic acids per unit mass associated with an increase in growth rate. As reported by earlier workers, the amount of DNA/unit mass did not vary significantly with either the rate of growth or the temperature of incubation. Earlier investigators had demonstrated that the cellular content of RNA, on the other hand, increases with an increase in growth rate and varies with the nature of the nutrient medium. In addition to such increases in RNA content, however, we observed also a change in RNA content associated with the incubation temperature. At any given rate of growth in chemostat cultures, cells grown at 25 C contained significantly more RNA/unit mass than did cells grown at 37 C. Ultracentrifugation analyses of cell contents showed that the temperature-associated change in RNA content occurred in the form of an increase in the ribosomal RNA content. This observation raised the question: Are the rates of macromolecular synthesis the same at both temperatures, or does the increase in RNA content indicate a compensatory mechanism for maintaining the level of protein synthesis required at a given growth rate?

It is well established that the amount of protein/cell and the rate of protein synthesis are proportional to the RNA content of a cell. Thus an increase in RNA content is generally observed to effect an increase in the rate of protein synthesis. But in a ''temperature-shift'' experiment, in which steady-state cultures growing at 37 C were shifted to 25 C without a change in dilution (growth) rate, temperature had differential effects on RNA and protein synthesis. During the shift period before establishment of the new cell composition characteristic of the 25 C steady state, rates of protein synthesis were markedly reduced while RNA synthesis continued at the previous rate. These

differential rates of synthesis eventually produced a new steady state, with a characteristically high ratio of RNA (mostly ribosomal) to protein. Evidently, temperature affects the efficiency with which ribosomes function in protein synthesis.

The differential effect of temperature on RNA and protein synthesis can be explained by the fact that RNA synthesis is controlled by the internal concentration of amino acids, while protein synthesis is controlled by the functional activity of ribosomes. Amino acids act as inducers of RNA, thereby relieving the repressive effect of the latter on RNA synthesis. Since the concentration of amino acids is fixed by the medium composition and by the dilution rate, temperature does not affect the rate of RNA synthesis in chemostat cultures. Ribosomes function in the synthesis of protein until they run off the messenger-RNA molecule, then to function again they must be activated (possibly phosphorylated by ATP). Lowering the incubation temperature appears to increase the amount of energy required for ribosome activation. In our balanced growth studies, this increase in energy requirement was reflected in a lower rate of protein synthesis and the maintenance of a lower population density in 25 C cultures.

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Variations in the rate of growth of batch cultures of Escherichia coli shown to conform to Arrhenius equation over the growth temperature range. Temperature effects on growth rate in batch cultures was not directly predictable of the situation for continuous culture under conditions of balanced growth. Evidence was adduced for temperature affecting the efficiency of protein synthesis by ribosomes. Lowering incubation temperature increased the amount of energy required for ribosome activation. In balanced growth this increase in energy requirement is reflected in the lower rate of protein synthesis, and reduced population density.

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