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Factors Limiting Bacterial Growth. II. Growth Without Lag in *Bacterium coli* Cultures.

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The continuation of studies already reported[1] has brought out two facts bearing on the question of bacterial lag. First, it has been found that from the moment of seeding and throughout the early hours of growth, the oxygen-uptake of cultures of *Bacterium coli* increases logarithmically, irrespective of the age of the organisms used for inoculation. Secondly, it was possible to show that the oxygen-consumption calculated per unit of bacterial nitrogen is constant during all phases of growth, when measured under uniform conditions.[2] From these observations, it was inferred that a plausible explanation of the phenomenon of lag was to be found in the changes in average size of cells during the early development of the culture. The experiment to be reported here served, therefore, both to confirm the manometric findings, and to provide direct evidence of the nature of the lag phase.

The data required were obtained by simultaneous determinations of bacterial numbers by plating, and of total bacterial nitrogen by micro-Kjeldahl analysis of the centrifugate,[3] at intervals throughout the growth-period. Beef-extract broth in 200 ml. amounts, containing M/100 phosphate buffer of pH 7.4, was seeded at 37°C. with aliquots of a 24-hour culture of *Bact. coli* grown in the same medium. The number of cells and the bacterial nitrogen represented by the inoculum were obtained by analysis of additional aliquots of the parent culture. At intervals, one of the seeded flasks was sampled for enumeration, and immediately killed either by addition of 0.5% formalin or, in other experiments, by heating at 60°C. for 10 minutes. Nitrogen was determined after centrifuging and washing the killed bacterial cells. Blank determinations were made by suspending small amounts of a bacterial suspension of known nitrogen-content in sterile broth, and recentrifuging. Typical results are shown in Fig. 1.

[1] Hershey, A. D., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 556.

[2] In preparation.

[3] Mueller, J. H., *J. Bact.*, 1935, **29**, 383.

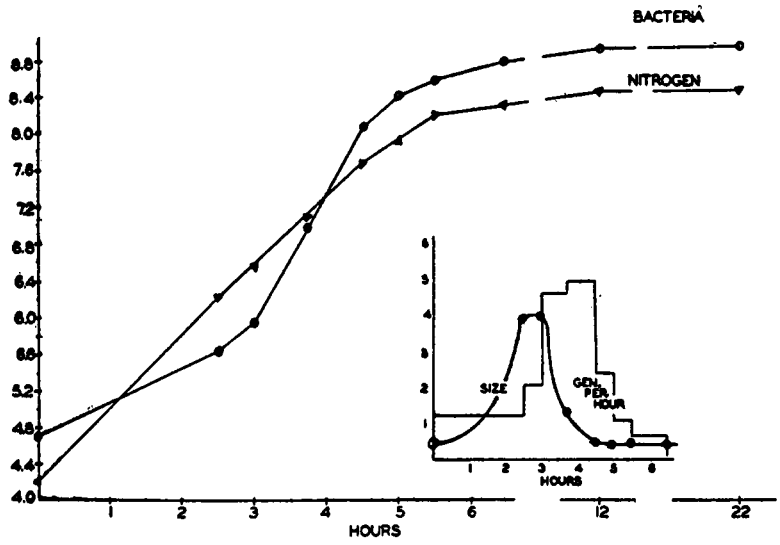

FIG. 1.

Increase of Population and of Bacterial Nitrogen in a *Bacterium coli* Culture. The logarithms of numbers and of nitrogen in mg. $\times 10^{10}$ per ml. are plotted against time. The insert shows the changes in average size expressed as nitrogen in mg. $\times 10^{-10}$ per cell, and in the average rate of growth as reciprocal of generation time during the intervals observed.

It is clear that the rate of growth represented as increments of bacterial nitrogen is highest during the early hours of growth, and is already diminishing before the period of rapid multiplication has ended. The nitrogen-per-cell quotient shows a ten-fold increase during the initial period of retarded multiplication, and a corresponding decrease while the multiplication-rate is at its peak.

It is suggested that in view of these findings, the terms "phase of cell-enlargement" and "phase of rapid cell-division" better characterize the early events of growth under these conditions, than do the terms "phase of bacterial lag" and of "logarithmic increase."

The application of these facts to the problem of growth rates in artificial media is obvious. It is unnecessary any longer to postulate obscure physiologic states corresponding with the varying activity of *Bact. coli* at different times during the life of the culture. It is evident that mechanisms underlying the observed changes in the rate of growth are to be sought primarily in the analysis of environmental influences.