Synthesis of Macromolecules by *Escherichia coli* near the Minimal Temperature for Growth

MAXWELL K. SHAW1 AND JOHN L. INGRAHAM

Department of Bacteriology, University of California, Davis, California 95616

Received for publication 17 March 1967

When a culture of *Escherichia coli* ML30 growing exponentially at 37 C in a glucose minimal medium was shifted abruptly to 10 C, growth decreased for about 4.5 hr. There was no net synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. The cells, however, respired at a rate characteristic of cells growing in the steady state at 10 C and were able to accumulate a-methyl-D-glucoside. When growth recommenced at 10 C, protein synthesis started at 4 hr, RNA synthesis, with a burst at 6 hr, and DNA synthesis, with a burst at 7 hr. One synchronous division occurred at about 11 hr after shifting to 10 C. There was no alteration in the steady-state RNA to protein ratio. The results are discussed in relation to other reported effects of shifts in environmental conditions. The lag at 10 C was dependent on prior conditions of growth at 37 C. Growth at 37 C under conditions giving catabolite repression were necessary for the lag to be established on shifting to 10 C.

It has been shown (11) that when a culture of *Escherichia coli* ML30 growing exponentially at 37 C is shifted to 10 C, a lag period in growth results.

Possibly, this lag accounts for the existence of the minimal growth temperature. It is known that the lag period increases as the temperature to which the shift is made is decreased (H. Ng, Ph.D. Thesis, Univ. of California, Davis, 1963). The minimal temperature for growth might well be that temperature at which the lag becomes infinite. The generality of this phenomenon has already been shown (14). When a culture of a mesophlic or psychrophilc yeast growing exponentially at a “moderate” temperature is shifted abruptly to a temperature just above the minimum for growth, the initial result is a complete cessation of growth for a number of hours. The length of this lag period is directly related to the magnitude of the shift; i.e., the wider the spread of temperatures over which the shift is made, the longer the lag (14). A study of this lag period seemed essential to an understanding of the factors controlling the minimal temperature for growth of microorganisms.

At the outset of the investigation, the facts known about this lag period are: optical density and the viable count remain constant (11), and the fatty acids of the cell become more highly unsaturated during the 1st hr of this lag (15); however, adjustment of the degree of unsaturation of the cellular fatty acids is not necessary for resumption of growth at 10 C (15).

The experiments reported here are planned to define the lag period in biochemical terms. The effect of a shift in temperature from 37 to 10 C on the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein has been investigated, as well as the effect on the increase in numbers of cells, on the ability to respire exogenous glucose, and on the ability to accumulate solutes.

The lag period, however, is related to conditions giving catabolite repression. The establishment of the lag period is dependent on prior growth at 37 C under conditions giving catabolite repression.

**MATERIALS AND METHODS**

*Organism. Escherichia coli* ML30 obtained from Jacques Monod was used in these experiments.

**Techniques of cultivation.** Media, culture techniques, and measurements of growth rate were the same as reported previously (11). Glucose was normally used as the carbon source at a concentration of 0.1% (w/v). In the anaerobic experiments, 0.3% (w/v) glucose was added. Succinate, pyruvate, and a-methylglucoside were added in certain experiments to give a final concentration of 0.3% (w/v). Cultures were made anaerobic by sparging continuously with a mixture of 95% N₂ and 5% CO₂.
Protein analysis. Samples (5 ml) of the culture were filtered through membrane filters (27 mm in diameter, 0.45-μm pore size; Millipore Corp., Bedford, Mass.). The pad was washed twice with cold distilled water and was then placed in 2.5 ml of cold distilled water. The cells were removed from the pad by vigorous agitation with a Vortex mixer. Protein content was estimated by a modified method of Lowry et al. (8). A sample (0.5 ml) of the resuspended cells was added to 0.5 ml of 0.2 N NaOH and was allowed to stand overnight at room temperature; 5 ml of alkaline copper reagent (1 volume of 2% CuSO4·5H2O), 1 volume of 4% sodium tartrate, and 48 volumes of 3% Na2CO3 were then added. After 10 min, 0.5 ml of diluted (1:3 in water) Folin-Ciocalteau reagent was added. After 30 min, the absorbancy at 660 μμ was read in a Beckman DU spectrophotometer against a water blank containing the reagents. Standards of crystallized bovine albumin were run with each experiment.

RNA and DNA analysis. Samples (5 ml) of the culture were filtered through membrane filters (27 mm in diameter, 0.45-μm pore size). The pad was washed twice with cold distilled water, and the cells were suspended in 2.5 ml of cold distilled water by vigorous agitation with a Vortex mixer. A sample (1.0 ml) of the resuspended cells was added to 2.0 ml of distilled water. RNA was determined by the orcinol method (13) with D-ribose as the standard (4.9 μg of RNA was equivalent to 1 μg of ribose).

DNA was determined by the diphenylamine method (2) with 0.5 ml of the resuspended cells added to 0.5 ml of 10% (w/v) trichloroacetic acid.

Estimation of cell numbers. Total numbers of cells were estimated by use of a model B Coulter Counter with a 30-μ aperture. A sample (0.1 ml) of the culture was diluted to 100 ml with a solution of 0.85% NaCl in 0.1% Formalin. The maximal number per milliliter of diluted suspension was 6 x 108. The conditions of counting were window settings, 10 to 100; aperture current, 1/2, and 1/amplification, 1/4.

Respiratory activity. Respiration rates were determined by use of a rotating platinum electrode respirometer. To determine respiration rate, 10 ml of the culture was injected into the respirometer vessel. The rate of oxygen uptake (microliters of O2 per milligram per hour or QO2) is given by the following equations

\[
rate = \frac{x \times \mu lits of dissolved O_2/ml}{span \times mg (dry weight)/ml} \times \frac{60}{time}
\]

\[
= \mu lits of O_2 \ per \ mg \ per \ hr \ or \ QO_2,
\]

where \(x\) = decrease on recorder chart; \(span\) = oxygen saturated level — nitrogen level; and \(time\) = number of inches traveled on the recorder (set to travel 1 inch/min).

Permeability experiments. The α-methyl glucoside permease assay (4) was used to investigate the ability to take up solutes during the lag. Washed cells were resuspended in a volume of the reaction mixture (see below) to give 200 to 1,000 μg (dry weight) per ml. The suspension was incubated at the particular temperature for 10 min, filtered, and washed with 0.1 M phosphate buffer (pH 7.4); the pad was placed in 15 ml of Cab-o-sil scintillation fluid (10) and was counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

The reaction mixture was the following: KH2PO4, 0.3%; KH2PO4, 0.7%; MgSO4·7H2O, 0.01%; glycerol, 0.2%; sodium malate, 0.2%; chloramphenicol, 50 μg/ml; and 2 x 10-5 M α-methyl-d-glucoside-U14C, 0.033 μC/ml).

The specific activity of the added α-methyl-d-glucoside-U14C was 1.48 mc/mmcue; thus, 0.033 μC of 14C-labeled α-methyl-d-glucoside added to the reaction mixture was equivalent to 0.05 μmc of α-methyl-d-glucoside. The count for 1 ml of the reaction mixture was 4.13 x 104 counts per min. Thus, 1 μmc was equivalent to 8.2 x 106 counts per min. The results are expressed as micromoles of uptake per 10 min per gram (dry weight).

Incorporation of radioactive precursors. The incorporation of uracil-2-14C and L-leucine-4,5-14H was measured simultaneously by use of a liquid scintillation system (Packard Instrument Co., Inc.). Samples (5 ml) of the culture were added to 5 ml of 10% (w/v) trichloroacetic acid at 0 C. After 30 min at 0 C, the cells were deposited on a membrane filter (27 mm, 0.45 μμ) and washed five times with 5% trichloroacetic acid (w/v) containing 10 μg of uracil per ml and 20 μg of leucine per ml. The filter was placed in the bottom of a counting vial, and 15 ml of Cab-o-sil liquid scintillation counting fluid was added (10). Counts were performed under conditions giving discrimination between 14H and 13C emissions.

Chemicals. Scintillation chemicals were purchased from Packard Instrument Co.; uracil-2-14C and α-methyl-d-glucoside-U14C, from Calbiochem, Los Angeles, Calif.; and L-leucine-4,5-14H, from New England Nuclear Corp., Boston, Mass.

All other chemicals were reagent grade.

Shift in temperature. Temperature shifts were performed by transferring the culture vessel to the new temperature and sparging vigorously with air saturated with water vapor at the new temperature.

RESULTS

Effect of a rapid temperature shift on growth. When there was a shift in temperature to 10 C of a culture of E. coli ML30 growing exponentially at 37 C on 0.1% glucose minimal medium (specific growth rate \(k = 0.9 \text{ hr}^{-1}\) , growth (in terms of dry weight) stopped for about 4.5 hr (Fig. 1). When growth resumed, the initial rate \(k = 0.07 \text{ hr}^{-1}\) was faster than the steady state rate at 10 C \(k = 0.04 \text{ hr}^{-1}\) for about one doubling in mass.

Effect of a rapid temperature shift on the synthesis of macromolecules. (i) Chemical determinations. Figure 2(A) shows the effect on protein content (μg/ml) of a shift in temperature from 37 to 10 C compared to the effect on optical density and dry weight. Protein, dry weight, and optical
FIG. 1. Effect on growth of a shift in temperature to 10°C of a culture of Escherichia coli ML30 growing exponentially at 37°C. Growth was followed by measuring optical density at 420 nm and converting to dry weight by use of a standard curve of optical density against dry weight.

density all remained constant for about 4 to 4.5 hr. Fig. 2(B) shows the effect on DNA (μg/ml) and RNA (μg/ml) as compared to that on dry weight and optical density. RNA content showed a slight initial drop and did not begin to increase until about 6 hr when there was a period of rapid synthesis. DNA was constant for about 7 hr, at which time there began a period of rapid synthesis. At the time of shifting, the ratio of RNA to protein was 0.64, and, after 12 hr at 10°C, it was 0.67.

(ii) Incorporation of radioactive precursors.

The synthesis of RNA and protein after the shift to 10°C was studied by following simultaneously the incorporation of L-leucine-4,5-3H and uracil-2-14C into the cold trichloroacetic acid insoluble fraction. Measurement of L-leucine-4,5-3H (0.05 μC/ml) incorporation was used as a measure of total protein synthesis, and the incorporation of uracil-2-14C (0.05 μC/ml), as a measure of total RNA synthesis. Figure 3 shows there was little or no incorporation of uracil-2-14C and L-leucine-4,5-3H for the first 4 hr. Protein (3H incorporation) started to increase at 4 hr, and RNA (14C incorporation) showed a period of rapid synthesis at about 6 hr. At the time of shifting to 10°C, the

FIG. 2. (A) Effect of a shift in temperature from 37 to 10°C on the synthesis of protein, increase in optical density, and dry weight of Escherichia coli ML30. Symbols: △ dry weight (μg/ml); ○, optical density at 420 nm; ◆, protein content (μg/ml). (B) Effect of a shift in temperature from 37 to 10°C on the synthesis of RNA and DNA, increase in optical density, and dry weight of Escherichia coli ML30. Symbols: △, dry weight (μg/ml); ○, optical density at 420 nm; ◆, RNA content (μg/ml); ◆, DNA content (μg/ml).
ratio of $^3$H to $^{14}$C was 0.11, and, after 20 hr at 10°C, 0.13.

**Effect of a rapid temperature shift on cell numbers.** Figure 4 shows the effect of the shift in temperature to 10°C on the increase in cell numbers. The respiration rates of cells in samples of a culture shifted from 37 to 10°C are listed in Table 1. At 37°C, the respiration rate was constant at about 259 μl per mg per hr (Q₀₂); at 10°C, the rate was constant at about 65 μl per mg per hr (Q₀₂). The phase of growth at 10°C (lag, transient fast rate, or steady-state rate) had little or no effect on the rate of respiration of the cells.

**TABLE 1.** Respiratory activity of Escherichia coli ML30 after a shift of exponentially growing cells from 37 to 10°C

<table>
<thead>
<tr>
<th>Time after shifting (hr)</th>
<th>OD₆₅₀</th>
<th>Dry wt (μg/ml)</th>
<th>Respiration ratea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0b</td>
<td>0.515</td>
<td>119.5</td>
<td>250.8</td>
</tr>
<tr>
<td>1</td>
<td>0.515</td>
<td>119.5</td>
<td>69.6</td>
</tr>
<tr>
<td>2</td>
<td>0.514</td>
<td>119.0</td>
<td>63.6</td>
</tr>
<tr>
<td>3</td>
<td>0.516</td>
<td>119.7</td>
<td>58.8</td>
</tr>
<tr>
<td>4</td>
<td>0.515</td>
<td>119.5</td>
<td>60.0</td>
</tr>
<tr>
<td>5</td>
<td>0.519</td>
<td>120.4</td>
<td>63.6</td>
</tr>
<tr>
<td>6.5</td>
<td>0.545</td>
<td>126.0</td>
<td>82.8</td>
</tr>
<tr>
<td>10b</td>
<td>0.600</td>
<td>143.0</td>
<td>76.8</td>
</tr>
<tr>
<td>21.5</td>
<td>0.450</td>
<td>104.0</td>
<td>69.6</td>
</tr>
<tr>
<td>26b</td>
<td>0.573</td>
<td>135.0</td>
<td>62.4</td>
</tr>
<tr>
<td>46, 5c</td>
<td>0.420</td>
<td>94.0</td>
<td>63.6</td>
</tr>
<tr>
<td>96</td>
<td>0.600</td>
<td>143.0</td>
<td>62.4</td>
</tr>
</tbody>
</table>

a Expressed in microliters of O₂ per milligram per hour or Q₀₂.

b Respiration rate determined at 37°C.

c Cultures diluted 1:2 with fresh medium at 10, 26, and 46.5 hr after shifting.

**TABLE 2.** Uptake of α-methyl-D-glucoside-U-14C by Escherichia coli ML30 prior to and after shifting from 37 to 10°C

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>OD₆₅₀</th>
<th>Dry wt (μg/ml)</th>
<th>Dry wt (μg) in assay</th>
<th>Assay temp (°C)</th>
<th>Uptakea</th>
</tr>
</thead>
<tbody>
<tr>
<td>−60</td>
<td>0.116</td>
<td>24.5</td>
<td>245</td>
<td>37</td>
<td>22.1</td>
</tr>
<tr>
<td>0b</td>
<td>0.263</td>
<td>56.3</td>
<td>563</td>
<td>10</td>
<td>2.8</td>
</tr>
<tr>
<td>60</td>
<td>0.262</td>
<td>56.0</td>
<td>560</td>
<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>135</td>
<td>0.260</td>
<td>55.8</td>
<td>558</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>190</td>
<td>0.260</td>
<td>55.8</td>
<td>558</td>
<td>10</td>
<td>12.8</td>
</tr>
<tr>
<td>240</td>
<td>0.260</td>
<td>55.8</td>
<td>558</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>300</td>
<td>0.275</td>
<td>59.2</td>
<td>592</td>
<td>10</td>
<td>14.0</td>
</tr>
<tr>
<td>540</td>
<td>0.330</td>
<td>72.5</td>
<td>725</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>770</td>
<td>0.408</td>
<td>91.0</td>
<td>910</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>1440</td>
<td>0.643</td>
<td>155.2</td>
<td>776</td>
<td>10</td>
<td>10.4</td>
</tr>
</tbody>
</table>

a Expressed in micromoles per gram per 10 min.

b Time of shifting to 10°C. Sample at zero-time was taken before the culture was shifted to 10°C and assayed for permease at 10°C.
Effect of a rapid temperature shift on the permeability to α-methyl-glucoside. The α-methyl-glucoside permease assay (4) was used as an index of the permeability characteristics of the cells. The uptake of α-methyl-glucoside-\textsuperscript{14}C is expressed in terms of micromoles of α-methyl-glucoside taken up per gram (dry weight) every 10 min. The uptake at 37 C was 20 μmoles per g per 10 min. On shifting to 10 C, there was an initial inhibition of uptake (2.8 μmoles per g per 10 min), but this rate of uptake reached a level of about 10 μmoles after 1 hr and remained relatively constant for the rest of the time at 10 C (Table 2). The sample showing the initial inhibition at 10 C was of cells grown at 37 C and assayed for permease at 10 C.

Effect of anaerobiosis. In all previous experiments, cultures were grown under aerobic conditions with glucose as the carbon source. When \textit{E. coli} ML30 was grown under anaerobic conditions at 37 C and then at 10 C, there was no lag or transient rate after the shift (Fig. 5). The kinetics did not resemble those observed under aerobic conditions.

To determine whether this anaerobic effect was the result of anaerobic growth at 37 C or at 10 C, the temperature of a culture growing aerobically at 37 C was shifted to 10 C, and the culture was made anaerobic. In a parallel experiment, the temperature of a culture growing anaerobically at 37 C was shifted to 10 C, and the culture was made aerobic. The establishment of the lag resulted only in cultures grown aerobically at 37 C (Fig. 6), but the state of aerobiosis at 10 C had no effect on the lag period. In contrast, the period of rapid growth always occurred when conditions were aerobic at 10 C, and it was independent of the state of aerobiosis at 37 C. Shifting the temperature of a culture growing aerobically at 37 C to 10 C and imposing anaerobic conditions resulted in a lag, but in no period of rapid growth rate. Immediately after the lag, the culture grew at a constant growth rate ($k = 0.03 \text{ hr}^{-1}$), characteristic of a culture growing in the steady state anaerobically at 10 C.

Effect of the carbon source. All previous experiments were performed with glucose as the carbon source. The result of a shift from 37 to 10 C in a medium with succinate as the carbon source is shown in Fig. 7. The lag period was markedly

---

**Fig. 5.** Effect of previous growth under aerobic and anaerobic conditions on the establishment of the lag on shifting from 37 to 10 C. \textit{Escherichia coli} ML30 was grown aerobically in minimal medium containing 0.1% glucose. Under anaerobic conditions, 0.3% glucose was added. The vertical line indicates the time of transfer to 10 C. Symbols: ○, aerobic; ●, anaerobic.

**Fig. 6.** Effect of prior condition of growth of \textit{Escherichia coli} ML30 at 37 C on the establishment of the lag at 10 C. Symbols: ○, aerobic at 37 C and aerobic at 10 C; ●, aerobic at 37 C and anaerobic at 10 C.
decreased, and the steady-state growth rate at 10°C was faster than growth on glucose at 10°C ($k = 0.07$ hr$^{-1}$ on succinate; $k = 0.04$ hr$^{-1}$ on glucose). There was no complete cessation of growth and no transient fast rate.

**Relationship between the lag and catabolite repression.** When *E. coli* ML30 was grown anaerobically in glucose minimal medium, there was little catabolite repression; i.e., the differential rate of synthesis of β-galactosidase was similar to the differential rate of synthesis by a culture growing aerobically on succinate. This confirms the results of Piper et al. (Bacteriol. Proc., p. 81, 1965) who also showed that the addition of pyruvate to a culture of ML30 growing anaerobically on glucose restores catabolite repression.

To test further the apparent relationship between catabolite repression and the growth lag at 10°C, *E. coli* ML30 was grown anaerobically at 37°C on minimal medium containing glucose and pyruvate and the temperature was shifted to 10°C (Fig. 8). The lag period of 4.5 hr was observed.

When the pyruvate was added at the time of shifting to 10°C, no lag resulted. Conversely, washing the cells free of pyruvate at the time of shifting to 10°C did not remove the lag period (Fig. 9). Again, the conditions of growth at 37°C determined the establishment of the lag period at 10°C.

Similarly, when *E. coli* ML30 was grown aerobically at 37°C on minimal medium containing 0.2% succinate and 0.2% α-methyl glucoside and when the temperature was shifted to 10°C with a dry weight of 90 μg/ml, the lag period was observed and the growth rate was lower at 10°C in the presence ($k = 0.04$ hr$^{-1}$) than in the absence ($k = 0.07$ hr$^{-1}$) of α-methyl glucoside (Fig. 10). Thus, all growth conditions at 37°C which resulted in the growth lag on shifting to 10°C are also those conditions which caused the establishment of catabolite repression at 37°C.

**Discussion**

The simultaneous stoppage of synthesis of all macromolecules in a medium capable of supporting growth is a unique situation. Most of the known cases of inhibition of macromolecular synthesis do not result in the immediate inhibition of synthesis of all macromolecules. Starvation for a required amino acid (12) immediately stops further increases in RNA and protein, but some increase in DNA and in numbers of cells does.
occur. Starvation for thymine with a thymineless strain of *E. coli* (15T-) results in the immediate inhibition of DNA but not of RNA and protein synthesis (3). Addition of chloramphenicol to a growing culture specifically inhibits protein but not RNA (1) synthesis.

Possible reasons for this stoppage are: (i) a sudden loss of permeability; (ii) the need to dilute or destroy a toxic product produced at 37 C; and (iii) the need to synthesize a specific requirement for growth at 10 C.

The first reason seems unlikely. Although there was an initial inhibition of permeation of α-methyl-D-glucoside on a shift to 10 C, and although the growth lag lasted for about 4.5 hr, full permeation was resumed after 1 hr.

The present experiments do not distinguish between the other possibilities.

However, the fact that the cells respired during the lag at a rate characteristic of cells growing in the steady state at 10 C establishes that there is no inhibition of respiratory activity.

After the lag at 10 C, there was a period of unbalanced synthesis of macromolecules. Syntheses recommenced in the order of protein, RNA, DNA, and number of cells. This is similar to the order of increase of rate of synthesis of macromolecules during the "up-shift" experiments of Kjeldgaard, Maaløe, and Schaechter (6), except that RNA precedes protein in the Kjeldgaard et al. experiments. However, unlike the situation studied by Kjeldgaard et al., the period of unbalanced growth did not result in a change in macromolecular composition of the cells in their steady-state of growth at 10 C. The final ratio of RNA to protein was not altered by growth at 10 C. Leucine and uracil incorporation experiments showed a \(^{3}H\) to \(^{14}C\) (protein to RNA) ratio of 0.11 at 37 C which returned to 0.13 after 20 hr at 10 C.

The synchronous division produced by this shift in temperature from 37 to 10 C is not a unique occurrence. Hotchkiss (5) was able to synchronize cultures of *Pneumococcus* species by a double shift in temperature from 37 to 25 C and back again to 37 C. Lark and Maaløe (7) were able to obtain a single synchronous division by shifting a culture of *Salmonella typhimurium* from 25 to 37 C.
The causal relationship between the lag at 10 C and the minimal temperature for growth of *E. coli* does not seem to exist. The lag was only present when conditions of growth at 37 C were those that also established catabolite repression.

Marr, Ingraham, and Squares (9) have shown with *E. coli* that catabolite repression of β-galactosidase synthesis was greatest at 10 C and decreased as the temperature was increased. The results presented here described a unique physiological state related circumstantially to catabolite repression but differing in effect. All macromolecular synthesis is inhibited, whereas permeability and respiration are apparently unaffected. The results appear to establish the existence of a master control process of macromolecular synthesis.

Shaw (14) has shown that, when mesophilic and psychrophilic yeasts are shifted from a "moderate" to a "low" temperature, they also respond with an initial cessation of growth. Thus, the phenomenon described here might be a general one.

Acknowledgments

This investigation was performed while one of us (M. K. S.) was the holder of a C.S.I.R.O. (Australia) Overseas Postgraduate Traineeship. This investigation was supported by U.S. Public Health Service grant AI-05526 from the National Institute of Allergy and Infectious Diseases.

Literature Cited