Effects of Growth Rate and Limiting Substrate on Glucose Metabolism in Escherichia coli

D. N. WRIGHT AND W. R. LOCKHART

Department of Bacteriology, Iowa State University, Ames, Iowa

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ABSTRACT

WRIGHT, D. N. (Iowa State University, Ames), AND W. R. LOCKHART: Effects of growth rate and limiting substrate on glucose metabolism in Escherichia coli. J. Bacteriol. 89:1082-1085. 1965.—Escherichia coli was grown in continuous culture at various rates in a defined medium with either glucose of (NH₄)₂SO₄ as the rate-limiting substrate. Cellular content of polysaccharide ("glycogen") is greater in cells grown under nitrogen limitation with glucose available in excess, and is greater in rapidly grown than in slowly grown cells. The ability of cells to carry on endogenous respiration, as measured by tetrazolium reduction, can be correlated with their glycogen content. In carbon-limited cultures, the proportion of substrate glucose diverted to glycogen production is least for cells grown slowly, which may reflect greater energy requirements for cell maintenance in such cultures. The activity of glucose-6-phosphate dehydrogenase (indicating function of a C-1 preferential pathway for glucose degradation) is greater in rapidly grown cells, confirming earlier observations in batch cultures. Activity of this enzyme is also greater in nitrogen-limited than in carbon-limited cells, suggesting that there may be catabolic repression of the Embden-Meyerhoff pathway when glucose is available in excess.

Cohen (1951) reported that, under oxidative conditions, resting cells of Escherichia coli metabolize glucose by means of the Embden-Meyerhoff pathway, whereas rapidly growing cells use chiefly the hexosemonophosphate (C-1) pathway. Subsequent work with Penicillium chrysogenum by Heath and Koffler (1956) indicated that the proportion of glucose oxidized by the C-1 pathway increases with the rate of growth. Allen and Powelson (1958) showed that a shift in relative emphasis from the C-1 to the Embden-Meyerhoff pathway occurs at the onset of the stationary-growth phase. Ribbons and Dawes (1963) noted that the endogenous respiration rate of E. coli varies with the phase of growth from which cells are harvested, and can be correlated with a cellular carbohydrate (glycogen) which is the major source of endogenous energy. Holme (1957) studied the synthesis and degradation of glycogen by E. coli grown in continuous culture limited by the available nitrogen, and pointed out that yield determinations are very simple when factors other than the energy source are growth-limiting. Work in this area had been done mostly in batch culture; we have extended it in a continuous growth system and examined the interactions of carbohydrate pathways in terms of cellular growth rate and substrate limitation.

MATERIALS AND METHODS

E. coli K-12 was grown in a defined medium containing: 0.7% K₂HPO₄, 3H₂O, 0.3% KH₂PO₄, and 0.01% MgSO₄•7H₂O in deionized water at pH 7.0, to which were added 400 μg of (NH₄)₂SO₄ per ml, and either 500 μg of glucose per ml (for carbon-limited cultures) or 4,000 μg of glucose per ml (for nitrogen-limited cultures). The continuous-culture system employed to obtain balanced growth at various rates, and techniques for sampling and for preparing cell extracts, have been described (Wright and Lockhart, 1965b).

Total carbohydrate and carbohydrate as glucose were determined by the anthrone method of Loe-wus (1962), as modified by Ecker and Lockhart (1961a).

To determine the reduction of triphenyl tetrazolium chloride (TTC) by whole cells, a 10-ml sample of cold cell suspension (cells kept at -2°C showed no change in TTC-reducing ability for up to 6 hr) was centrifuged at 3,100 × g for 10 min. The cells were mixed with 2 ml of phosphate buffer at pH 7, and incubated at 37°C for 3 min. After the

1 Based on a thesis submitted by the senior author to Iowa State University in partial fulfillment of requirements for the Ph.D. degree.

2 Present address: University of California, Naval Biological Laboratory, Naval Supply Center, Oakland, Calif.
primary incubation, 1 ml of freshly prepared 0.1% TTC was added to each sample. The samples were thoroughly mixed and again incubated at 37 C for 15 min, and a convenient volume of acetone was then added to extract the reduced TTC. The amount of reduced TTC was determined photometrically at 450 m\( \mu \) by the technique outlined by Kopper (1962).

Bacterial glycogen was determined by a modification of the methods of Good, Kramer, and Symogyi (1933) and Palmstierna (1956). To the cells from 10 ml of culture was added 0.2 ml of diethyl ether. The cell-ether mixture was boiled and cooled to room temperature, and 0.2 ml of 30% KOH was added. The mixture was again boiled for 30 min, and 2 drops of 0.1% sodium sulfate were added to the extract, followed by the addition of 0.5 ml of ethyl alcohol. The suspension was boiled for 30 sec and allowed to cool to room temperature. The samples were centrifuged, and the sediment was hydrolyzed by refluxing in 0.6 N HCl for 3 hr. The carbohydrates were then determined as described by the workers cited.

Glucose-6-phosphate dehydrogenase activity was determined by the procedure outlined by Scott and Cohen (1953). The enzyme reaction mixture contained, in 3 ml: 0.1 M tris(hydroxymethyl)-aminomethane (Tris) buffer at pH 8.5, 1.85 ml; MgCl\(_2\) at 3 \( \times 10^{-4} \) M, 0.2 ml; glucose-6-phosphate (G-6-P), 2 umoles; nicotinamide adenine dinucleotide phosphate (NADP), 0.4 umoles; NADP and G-6-P were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio). The reaction was started by adding 0.05 ml of cell-free extract at pH 8 (Wright and Lockhart, 1965a) to the reaction mixture. The change in absorbancy at 340 m\( \mu \) was measured against a reference containing the same reagents, except that water was substituted for the cell extract. Each reaction was allowed to proceed at 25 C for 10 min, or until the transmission was less than 15%. The enzymatic activity was calculated as defined by Lohr and Waller (1963).

**RESULTS**

The values for TTC reduction shown in Table 1 indicate that the ability to carry out endogenous respiration after removal from a growth medium is directly related to the rate at which cells have been growing and metabolizing. The nutrient environment also affects the endogenous metabolic state; cells limited by nitrogen (with excess carbon in the medium) reduce more tetrizolium than carbon-limited populations.

Wilkinson (1963) showed that a polyglucose, called glycogen, serves *E. coli* as a reserve energy depot when exogenous energy sources are not available. The glycogen found in carbon- and nitrogen-limited cell populations (Table 1) agrees well with the reducing activity of the cells. However, glycogen content appears to be affected more by the rate at which cells are grown than by the limiting substrate. This finding was confirmed by infrared-absorption spectra of the cells (Wright and Lockhart, 1965b). The efficiency of glycogen synthesis is increased at high growth rates, at least when glucose is the growth-limiting substrate.

There is considerably more activity of glucose-6-phosphate dehydrogenase in nitrogen-limited than in carbon-limited cells. The values shown in Table 2 are based on a constant protein concentration in the reaction mixture. Since there is approximately five times as much protein per cell in populations grown at 0.5 generations per hr as in those grown at 0.1 generations per hr (Wright and Lockhart, 1965b), greater enzyme activity per cell is found in populations grown at fast rates with either carbon or nitrogen limiting. Enzyme activities in cells grown at intermediate rates (0.23 and 0.38 generations per hr) were intermediate

**TABLE 1. Endogenous reduction of triphenyl tetrazolium chloride (TTC), and glycogen content of Escherichia coli K-12 grown at various rates in continuous cultures limited by glucose or (NH\(_4\))\(_2\)SO\(_4\).**

<table>
<thead>
<tr>
<th>Limiting substrate</th>
<th>Growth rate (generations/hr)</th>
<th>TTC reduced per 10 ml of culture</th>
<th>TTC reduced per cell* (( \mu )g ( \times 10^{-6} ))</th>
<th>Glycogen content per cell (( \mu )g ( \times 10^{-7} ))</th>
<th>Amt of limiting substrate used per ( \mu )g of glycogen formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>0.10</td>
<td>10</td>
<td>0.3</td>
<td>0.80</td>
<td>21.2</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.23</td>
<td>32</td>
<td>7.1</td>
<td>1.50</td>
<td>12.9</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.38</td>
<td>66</td>
<td>21</td>
<td>3.02</td>
<td>10.0</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.50</td>
<td>93</td>
<td>46</td>
<td>4.43</td>
<td>5.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.10</td>
<td>94</td>
<td>16</td>
<td>2.49</td>
<td>1.3</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.23</td>
<td>171</td>
<td>66</td>
<td>3.80</td>
<td>1.6</td>
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<tr>
<td>Nitrogen</td>
<td>0.38</td>
<td>240</td>
<td>110</td>
<td>5.87</td>
<td>1.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.50</td>
<td>275</td>
<td>130</td>
<td>7.61</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Values per cell based on viable counts.
† Glycogen was determined in terms of glucose.
TABLE 2. Activity of glucose-6-phosphate dehydrogenase in Escherichia coli K-12 grown at different rates in carbon- and nitrogen-limited cultures

<table>
<thead>
<tr>
<th>Limiting substrate</th>
<th>Growth rate (generations/hr)</th>
<th>Units of enzyme activity/ml*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>0.1</td>
<td>792</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.5</td>
<td>540</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.1</td>
<td>1,840</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.5</td>
<td>2,060</td>
</tr>
</tbody>
</table>

* All cell extracts were adjusted to a constant protein concentration of 3.47 mg/ml.

between those shown here for the maximal and minimal growth rates.

**Discussion**

Cells grown in nitrogen-limited medium (i.e., in the presence of excess glucose) accumulate about twice as much glycogen as those grown in carbon-limited cultures. Holme (1937) found that the greatest accumulation of glycogen occurs in nitrogen-limited cultures, and Ribbons and Dawes (1963) suggested that glycogen synthesis is inhibited by NH$_4^+$, a view consistent with the data reported here. As Clifton (1963) pointed out, the growth medium and culture conditions influence subsequent metabolic activity of the cells; we found that both substrate and growth rate influence the metabolic capabilities of cells after removal from the growth environment. A cell is best equipped to carry out endogenous metabolism when it has been grown at high rates in a medium containing excess glucose, thus providing an intracellular store of carbon compounds that is available on demand.

Growth rate has a greater influence on endogenous ability to reduce TTC than does the composition of the medium. This is predictable from the measured glycogen changes, which show a three- to fourfold increase in cellular glycogen concentration with a rate increase from 0.1 to 0.5 generations per hr. Of particular interest is the fact that cells growing rapidly in glucose-limited cultures produce much more glycogen per unit of glucose utilized than do slow-growing cells. Observations (Ecker and Lockhart, 1961b) that large cell populations show reduced efficiency in producing protoplasm from a limiting substrate support the contention (Rahn, 1932) that part of a cell's available resources must be diverted to "energy of maintenance." More recently, Mallette (1963) and Marr, Nison, and Clark (1963) demonstrated that a portion of the energy derived by a cell from its medium is indeed used to maintain cellular integrity. It thus is possible that the reduced efficiency of glycogen production by cells growing slowly in carbon-limited media results from a need for larger proportions of energy directed toward cellular upkeep. This change in efficiency of glycogen production would not be seen in nitrogen-limited cultures, where glucose is available in quantities beyond those required for growth, division, and cell maintenance.

The observation that activity of glucose-6-phosphate dehydrogenase is greater in rapidly growing cells confirms the idea that there is an increase in the activity of the hexosemonophosphate (C-1) pathway with an increase in cellular growth rate. Such increased use of the primary pathway leading to pentose synthesis might be expected to accompany the increased demand for nucleic acids in rapidly growing cells (Schechter, Maalej, and Kjeldgaard, 1958).

The much greater activity of glucose-6-phosphate dehydrogenase found in nitrogen-limited cells may be a reflection of the "glucose effect." Magasanik (1961) pointed out that this ability of glucose to inhibit synthesis of certain enzymes might more properly be termed catabolic repression, because of the relationship between some catabolic products of glucose and the enzymes they inhibit. The increased activity of glucose-6-phosphate dehydrogenase in nitrogen-limited cultures, with glucose present in excess, may result from catabolic repression of enzymes necessary for maximal function of the Embden-Meyerhoff system. This repression would increase the demand on the C-1 pathway, resulting in a corresponding increase in the activity of enzymes associated with it. The shift in glucose metabolism seen at the onset of the stationary phase in batch culture may then be caused either by a decrease in growth rate resulting from exhaustion of essential metabolites, or by decreased repression of enzymes of the Embden-Meyerhoff pathway as the supply of glucose is depleted.

**Acknowledgment**

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**Literature Cited**


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