PATHWAYS OF GLUCOSE OXIDATION IN DIVIDING AND NONDIVIDING CELLS OF *ESCHERICHIA COLI*[1]

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Cohen (1951) reported that under oxidative conditions resting cells of *Escherichia coli* dissimilated glucose by means of the Embden-Meyerhof pathway, while rapidly growing cells used chiefly the hexosemonophosphate pathway. Heath and Koffler (1956) with *Penicillium chrysogenum* also showed that actively growing cells metabolized glucose by a C-1 preferential pathway and that the fraction of glucose oxidized by this pathway increased with the rate of growth. Beevers and Gibbs (1954), however, observed that nonproliferating yeast cells oxidized glucose by a C-1 preferential pathway. Research was undertaken to determine whether a shift in metabolic pathways is associated with a change in growth rate or cessation of cell division.

A test system was needed in which growth and cell division could be separated. Loveless *et al.* (1954) reported that 5-diazouracil at low concentrations would inhibit cell division in cultures of *E. coli* while having no effect on growth. Filamentous cells were formed. Katchman *et al.* (1955) stated that no inhibition of synthesis of ribonucleic or desoxyribonucleic acid occurred. Results of our studies also show that during the first 30 min treatment with diazouracil does not alter the rate of growth, respiration or assimilation of substrates though it prevents cell division. We thus have a test system for comparing metabolic pathways in dividing and nondividing cells that are growing at the same rate.

The yields of $C^{14}O_2$ from both glucose-1-C^{14} and glucose-6-C^{14} were compared and the C-1:C-6 ratios determined (Beevers and Gibbs, 1954). Theoretically, a ratio of 1.0 indicates glucose breakdown by the Embden-Meyerhof pathway while higher ratios indicate the operation of other pathways, usually the hexosemonophosphate pathway. Though Scott and Cohen (1951) have demonstrated the enzymes of the hexosemonophosphate pathway in *E. coli*, we have not proved their presence in our strain and have taken a high C-1:C-6 ratio to indicate that oxidation of glucose occurred via a C-1 preferential pathway. The results of this study indicate that a shift from a C-1 preferential pathway toward the Embden-Meyerhof pathway attends a decrease in the rate of growth but does not necessarily accompany cessation of cell division.

MATERIALS AND METHODS

Conditions for growth. The organism used was *Escherichia coli* strain 61. In all experiments, cells were grown in either the synthetic medium of Dagley *et al.* (1951) or a modification of this medium in which $(NH_4)_2HPO_4$ was substituted for $(NH_4)_2SO_4$, the concentration of $MgSO_4 \cdot 7H_2O$ was lowered from 0.4 g per L to 0.2 g per L, and the glucose concentration was 0.15 per cent. This modified medium prevented a sharp drop in the pH of the culture during growth.

Before all experiments, the inoculum was prepared by making two serial transfers in synthetic medium. Growth of the inoculum was at 37 C in liquid medium in Erlenmeyer flasks shaken on a Burrell wrist-action shaker. A 12 hr culture was taken as the inoculum for the test cultures. It was adjusted to an optical density of 0.20 at 655 mμ (Coleman model 14 spectrophotometer) to give approximately 3×10^8 cells per ml.

Cultures were grown in Dixon-Keilin type, double-sidearm flasks for determination of oxygen uptake and carbon dioxide evolution, including $C^{14}O_2$ from radioactive glucose. Conventional double-sidearm Warburg flasks were used to measure increase in cell mass and substrate up-

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take, as well as to ascertain relatively the rates of growth in different flasks by means of manometric determinations. Growth and respiraton appeared to proceed at the same rate in both types of flasks, as measured by total manometric change during incubation. For other experiments in which only growth and substrate uptake were measured cultures were grown in 50 ml of synthetic medium in 500 ml Erlenmeyer flasks. The temperature of incubation was 37 C, and the Warburg flasks were shaken at a rate of 120 strokes per min.

Growth in Erlenmeyer flasks was followed by optical density and direct microscopic counts but in Warburg flasks only by direct microscopic counts. One ml aliquots of cultures were placed in 2.0 ml of 6 per cent formaldehyde solution and total counts were made later with a Petroff-Hausser counting chamber. This chamber was examined with a bright high phase contrast, oil immersion objective and 15 × oculars. In some experiments measurement of cell lengths was made on the same preparations, with a calibrated ocular micrometer.

Manometric procedures. Flasks for determination of O_2 uptake and CO_2 evolution were run in duplicate and flasks to determine growth were generally run in duplicate. The total O_2 uptake and CO_2 evolution from the start of the experiment were measured in each Dixon-Keilin flask; to obtain the changes for a 30 min period, the total values obtained from separate flasks at the start and end of the interval were subtracted. In experiments in which the C-1:C-6 ratios were determined, glucose-1-C^{14} or glucose-6-C^{14} was tipped into the main compartment of separate flasks 30 min prior to the addition of acid used to stop the reaction. Standard methods of manometry were employed; details are given by Allen (1957).

Radiochemical methods. After complete absorption of the $C^{14}O_2$ the NaOH solution was quantitatively removed from the center well of the stopcock of the Dixon-Keilin flasks and the carbonate collected was precipitated with saturated $Ba(OH)_2$-10 per cent $BaCl_2$. Unlabeled $NaHCO_3$ was added as carrier when necessary. The $BaCO_3$ was washed twice with water, resuspended in 95 per cent ethanol and plated on microporous porcelain discs (Harshaw Scientific Company, Cincinnati, Ohio). The discs were counted with a Nuclear model 192 windowless gas flow counter (Nuclear, Chicago). The counts were corrected for self-absorption (Schweitzer and Stein, 1950). The glucose-1-C^{14} and glucose-6-C_{14} were obtained from the National Bureau of Standards through the courtesy of Dr. H. S. Isbell.

Other analytical techniques. Aliquots of glucose-C^{14} were combusted to $C^{14}O_2$ by the persulfate method described by Calvin *et al.* (1949). Residual glucose was determined by the anthrone test (Loewus, 1952) as modified by Niss (1958). Residual ammonia nitrogen was determined by the phenol hypochlorite method of Niss.

RESULTS

Escherichia coli showed a shift from the glucose-C-1 preferential pathway toward the Embden-Meyerhof pathway at the onset of the stationary phase of growth. When glucose was the substrate that limited growth, the C-1:C-6 ratio dropped from 7.2 during the late logarithmic phase to 1.4 in the early stationary phase. In the late logarithmic phase 86 per cent of the glucose was oxidized by a C-1 preferential pathway. In the early stationary phase only 29 per cent of the residual glucose was oxidized by this pathway. When the concentration of ammonia nitrogen limited growth the C-1:C-6 ratio was 4.9 in the late logarithmic phase; in the early stationary phase the ratio was 2.1. In the logarithmic phase 80 per cent of the glucose and in the stationary phase 53 per cent of the glucose was oxidized by a C-1 preferential pathway. Whether cessation of cell division, an altered growth rate, or both were the factors responsible for the shift in pathways was not clear from these experiments. In order to learn the significance of the shift, the C-1:C-6 ratios were tested in a system in which growth and cell division could be separated.

The effect of 5-diazouracil on substrate uptake, respiration and growth. A concentration of 1 to 3 μg per ml of 5-diazouracil (Nutritional Biochemicals) was found to inhibit cell division when added to logarithmically growing cultures of *E. coli*. In experiments to study glucose metabolism the culture was prepared by inoculating 50 ml of synthetic medium in a 500 ml Erlenmeyer flask to an optical density of 0.20. The flask was then agitated on a wrist-action shaker at 37 C. In 90 min when the optical density of the culture was 0.40 (4.0×10^8 cells per ml) the culture was divided into two equal parts. To one part was

added 1.0 ml of 5-diazouracil solution and to the other 1.0 ml of sterile distilled water. Three ml aliquots of each culture were placed in Warburg flasks for further growth. When this transfer was made quickly and with care to maintain the culture at 37 C, growth continued logarithmically. Samples and readings were taken at the time of the addition of the inhibitor and at 30 min intervals thereafter. The growth curves for these cultures are shown in figure 1. With the concentration of 5-diazouracil used (3 μg per ml), the cells overcame the inhibition and began to divide again 1½ to 2 hr after treatment.

With this technique a comparison of the uptake of glucose and ammonia nitrogen by treated and control cells was made (table 1). During the first 30 min after addition of 5-diazouracil the treated cells continued to utilize both glucose and nitrogen at the same rate as the control cells. However, after this period variable rates of uptake were noted with the treated cells, and values

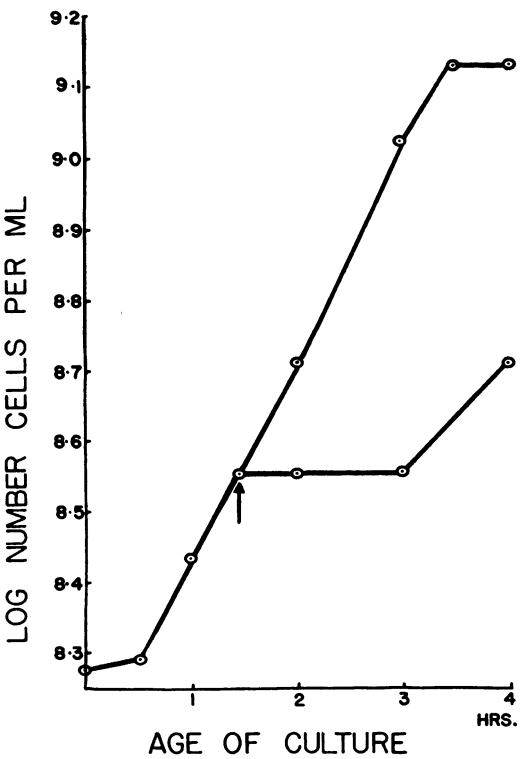

Figure 1. The effect of 5-diazouracil (3 μg per ml) on cell division of *Escherichia coli*. The cultures were grown in Warburg flasks and the inhibitor added at 1½ hr (arrow). The lower curve is the one for the treated culture.

from the treated cells in general appeared to be lower than the controls. The values from different experiments presented in table 1 varied considerably; the variation probably is related to the fact that the lag periods and therefore the total cell populations at the same test interval were different in each experiment. For example, the total counts at 30 min for experiments 1 and 3 were 12.4 and 5.74 $\times$ 10^8 cells per ml (control cultures).

For a study of the effect of 5-diazouracil on the oxygen uptake and carbon dioxide production of the cells during the first 30 min of treatment a modified growth technique was developed. The freshly inoculated test medium was placed directly in Warburg flasks, and the cultures then were grown into the logarithmic phase. After 90 min, when the cells were dividing rapidly, in some flasks 0.5 ml of a 5-diazouracil solution was tipped into the culture from a sidearm. The final concentration of inhibitor in each flask was 3 μg per ml. In other flasks an equal volume of sterile distilled water was added. During the first 30 min of treatment no changes in the oxygen uptake and carbon dioxide production were detected (table 1).

Measurement of cell lengths gave a more direct test of the effect of 5-diazouracil on growth. Lengths only were measured, because the cells were too small to permit accurate comparisons of diameters. However, the diameters of all the cells appeared to remain in the same range, i. e. slightly smaller than the smallest division on the ocular micrometer (0.7 μ). Treated cultures did not increase appreciably in numbers of cells, but growth occurred as elongation of cells. Of course, growth of the control cultures was manifested by an increase in numbers of cells of a fairly constant average length. For comparison of the growth rates of these cultures the term relative growth was applied; it is defined by the following formula:

Relative growth = N_1L_1/N_0L_0.
N_0 = number of cells per ml at the initial time, t_0
L_0 = average length of cells at t_0
N_1 = number of cells per ml at the final time, t_1
L_1 = average length of cells at t_1.

Table 2 presents a comparison between the relative growth values of treated and control cells. No significant difference in the average rate of growth of the two cultures was observed.

Pathways of glucose oxidation during growth in the absence of cell division. In experiments to measure the C-1:C-6 ratio before and after addition of 5-diazouracil the same growth technique was employed. Duplicate flasks were used to measure the amount of $C^{14}O_2$ liberated from both glucose-1-C^{14} and glucose-6-C^{14} at each period studied. The total cell count at the time of the addition of 5-diazouracil was 3.66×10^8 cells per ml; 30 min later in the treated culture the value was 3.78×10^8 cells per ml and in the control flasks 5.25×10^8 cells per ml. The difference between the total cell counts of the zero hour cultures and treated cultures is not significant since the deviation in the counting technique was 6 per cent. According to the results (table 3) no marked shift in C-1:C-6 ratios occurred when cell division was selectively inhibited. Although the trend for the values suggests a slight shift in glucose metabolism by the treated cells, the difference in C-1:C-6 ratios is not statistically significant. Other experiments indicated that about 15 per cent of the treated cells might not be viable (Allen, 1957). Other experiments showed that the ratios remained high throughout the 1½ hr period of inhibition.

TABLE 1

A comparison of the glucose and ammonia-nitrogen uptake and respiration of treated and control cultures during the first 30 min after addition of 3 μg per ml of 5-diazouracil (DAZU)

Expt* No.	Treatment	Glucose Uptake†	Ammonia-N Uptake†	Oxygen Uptake‡	Carbon Dioxide Evolution‡
		μg/ml	*μg/ml*	*μL/flask*	*μL/flask*
1	None	215	42		
	DAZU	448	66		
2	None	705	63		
	DAZU	770	70		
3	None	124	12		
	DAZU	179	23		
4	None	223	12	141	151
	DAZU	248	12	140	147

* Values of different experiments are not comparable since growth conditions varied.

† Average maximum deviation = 12 per cent.

‡ Average maximum deviation = 4 per cent.

TABLE 2

The effect of treatment with 5-diazouracil (3 μg per ml) on relative growth rate

Expt No.	Age of Culture	Length of Cells*		Total Count		Relative Growth†	
		Control	Treated	Control	Treated	Control	Treated
	min	*μ*		*cells/ml* $\times 10^8$			
5	0	2.7		5.49		1.0	
	30	2.8	4.0	8.71	5.47	1.6	1.5
	60	2.7	5.3	12.90	5.08	2.4	1.8
	90	2.1	4.6	15.50	6.62	2.2	2.1
6	0	2.8		3.79		1.0	
	30	2.8	3.8	5.44	3.90	1.4	1.4
	60	2.8	5.2	7.50	3.97	2.0	2.0
	90	2.6	6.3	11.00	4.67	2.7	2.8

* Average of 50 to 55 cells.

† Defined in text.

TABLE 3

*Yields of $C^{14}O_2$ and C-1:C-6 ratios during logarithmic growth with and without cell division**

Age of Culture	Treatment	$C^{14}O_2$ from Glucose-1-C^{14}†	$C^{14}O_2$ from Glucose-6-C^{14}‡	C-1:C-6 Ratio
min		*cpm*	*cpm*	
60–90	None	3101 3232	338 328	11.2 ±0.5
90–120	None	5682 7008	635 599	12.2 ±1.7
90–120	3 μg/ml, 5-diazouracil	4939 6017	691 731	9.1 ±1.2

* Experiment 4.

† Total cpm glucose-1-C^{14} added was 69,421.

‡ Total cpm glucose-6-C^{14} added was 81,959.

DISCUSSION

The results show that a shift in pathways of glucose oxidation occurs at the onset of the stationary phase of *E. coli*. This shift occurs when growth is limited by either the glucose or nitrogen concentration in the medium. Decrease in C-1:C-6 ratios was more marked when glucose limited growth. This observation might be attributed to the degree of metabolic activity at the onset of the stationary phase. A limited supply of glucose, the sole energy source of the medium, might more effectively restrict cellular activity than a limited nitrogen supply. Assimilation of glucose and other carbon sources by cells

deprived of exogenous nitrogen sources has often been observed (Clifton, 1946). Also, Holme and Palmstierna (1955) report that a polyglucose of glycogenic nature accumulates in the cells of *E. coli* when growth is limited by the nitrogen concentration of the medium.

The addition of 5-diazouracil, an inhibitor of cell division, to a rapidly growing culture of *E. coli* causes no significant changes in substrate uptake, respiration and growth during the first 30 min of treatment. After this period some inhibition is noted. With this inhibitor a test system is available for the study of the reactions related to cell division.

Preliminary experiments (Allen and Powelson, 1957) had given the impression that the fraction of labeled CO_2 from glucose-1-C^{14} was significantly lower in the treated as compared with the control cultures. Also it appeared that this inhibition increased as the filaments became longer. However, further experimentation and calculation have shown that these decreases are probably not related to the process of cell division. They can be accounted for either by the lowered growth rate and metabolic activity at least of some of the cells, especially noted after 30 min of treatment, or by variations within the experimental error of the test used.

Results reported here show that both dividing and nondividing cells growing at the same rate oxidize glucose primarily by a C-1 preferential pathway. The decrease in the activity of the C-1 preferential pathway at the onset of the stationary phase of growth probably is related to the decreased rate of growth. The role of this pathway during growth of *E. coli* is still unknown.

Since the hexosemonophosphate pathway can supply pentose units such as ribose which are an essential part of the nucleotides, an active C-1 pathway may be related to growth through synthesis of the nucleic acids. Synthesis of RNA and DNA does not appear to be inhibited by 5-diazouracil during growth, and nuclear division continues after addition of the inhibitor (Katchman *et al.*, 1955). A study of inhibitors of nucleic acid synthesis and their effect on the glucose metabolism of growing cells might be pertinent.

SUMMARY

A comparison of the amounts of labeled carbon dioxide liberated by metabolism of glucose-1-C^{14} and glucose-6-C^{14} was made to judge the relative activity of the Embden-Meyerhof-Parnas pathway and a C-1 preferential pathway of glucose oxidation by *Escherichia coli*. A shift from a glucose-C-1 preferential pathway toward the Embden-Meyerhof pathway was observed at the onset of the stationary phase of growth in cultures when either glucose or nitrogen limited growth and cell division.

The compound 5-diazouracil was used to inhibit cell division selectively. Both nondividing and dividing cells growing at the same rate oxidize glucose primarily by a C-1 preferential pathway. The role of this pathway appears to be closely related to growth and not to the process of cell division.

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