The Oxidative Pathway of Carbohydrate Metabolism in *Escherichia coli*

3. GLUCOSE 6-PHOSPHATE DEHYDROGENASE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE IN CELLS GROWN UNDER DIFFERENT CONDITIONS*

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Optimum conditions for the assay of glucose 6-phosphate dehydrogenase (G 6-PD) and phosphogluconate dehydrogenase (PGD) have been determined for extracts of *Escherichia coli* (Scott & Cohen, 1953a). In the second paper of this series (Scott & Cohen, 1953b) it was estimated that about 40% of carbohydrate might be metabolized by these enzymes *in vitro*. During these experiments it was noted that the use of optimum conditions for preparation of the extracts gave values for activities of these two enzymes which were remarkably constant on the basis of units of activity per mg. of protein in the extracts. In this paper experiments are described whose results indicate that this is the case under various conditions of growth, with several different carbon sources, and also under conditions of infection with bacteriophage. Further correlations between glucose utilization by the cells and the content of G 6-PD and PGD are also presented.

METHODS

The organisms investigated were *Esch. coli* strain B, for all substrates except arabinose, where strain B_{A15} was used (Cohen, 1953). Strain B is unable to adapt itself to arabinose; strain B_{A15} is a mutant of B selected for growth on arabinose and grown on it for 15 passages. Conditions for growth for aerobic cultures with glucose or gluconate were the same as previously described (Scott & Cohen, 1953b). The cultures were grown in rotating flasks or in cylinders equipped with aerators and with side arms which would fit into the Klett photocolormeter. Anaerobic cultures were incubated in stationary deep culture in Erlenmeyer flasks, or were treated with bubbling N₂ (Cohen & Barner, 1955). Growth was followed, both by turbidity measurements at 420 mμ., and by plate counts of the number of viable bacteria. To examine the effect of virus infection on the production of G 6-PD and PGD *Esch. coli* B was grown in roller flasks, and when the cells were growing in their exponential phase they were infected with bacteriophage. The lysis-inhibiting r+ strains of the T-even series of bacteriophages were used so that longer periods of infection could be obtained than with rapid-lysing r strains. The cultures were allowed to incubate

with aeration for varying lengths of time, and then chilled and harvested. Extracts were prepared as indicated below.

To determine the glucose removed from the medium by the cells during growth, samples were removed at intervals, and either chilled in an ice bath or placed in a boiling-water bath for 2 min. and centrifuged and glucose was determined by the Nelson (1944) method.

Extracts were prepared from the cells, and the activities of the glucose 6-phosphate dehydrogenase and phosphogluconate dehydrogenase were measured according to the procedure previously described (Scott & Cohen, 1953a). A unit of activity is defined as that which produces an increase of 1 in density at 340 mμ. and is caused by the reduction of 0.16 μmole of triphosphopyridine nucleotide (TPN).

RESULTS

A typical experiment for determining the rate of removal of glucose from the medium by the aerated cells growing in glucose is shown in Fig. 1A. Table 1 gives results of a different experiment and includes figures for the molecules of glucose taken up per cell/min.

$$\left(\frac{\text{g. of glucose (by difference) removed from medium in } y \text{ min.} \times 6.06 \times 10^{23}}{180 \times \text{mean no. of cells} \times y \text{ min.}} \right),$$

and also the activities of extracts of cells harvested during, and at the end of, the experiment. In four such experiments, the mean number of molecules of glucose taken up per cell/min. during the early logarithmic phase of growth was 7.3×10^7 .

A similar experiment in which cells were grown anaerobically with nitrogen bubbling is shown in Fig. 1B and Table 2. During the first hour there was a lag phase in growth, and from 20 to 30×10^7 molecules of glucose were removed per cell/min. During the next 2 hr. the rate of growth was exponential, and the pH value decreased by 0.4. Glucose was removed from the medium at a rate of 8 – 12×10^7 molecules/cell/min. At a high turbidity of 210 at 420 mμ. and a count of 1.7×10^9 cells/ml., and at pH 7.3 there was a break in the curves. For the next 2 hr. the rates of increase of turbidity and

* Part 2: Scott & Cohen (1953b).

Table 1. *Glucose uptake during aerobic growth and enzyme activities of extracts*

Esch. coli strain B grown in a roller flask in '52' medium containing 4 mg. of glucose/ml. Units of activity ($\Delta D/\text{mg. of protein/min.}$) = increase in density at 340 m μ . produced by reduction of TPN.

$$\text{Molecules of substrate oxidized/cell/min.} = \frac{\Delta D/\text{ml. of extract} \times \text{total ml. of extract} \times 6.06 \times 10^{23}}{6.22 \times 10^6 \times \text{total no. of cells extracted}}$$

Time (min.)	Count (cells/ml.) $\times 10^{-9}$	Glucose in medium (mg./ml.)	Glucose uptake (molecules/cell/min.) $\times 10^{-7}$	G 6-PD		PGD		Ratio: G 6-PD / PGD
				($\Delta D/\text{mg. of protein/min.}$)	(molecules/cell/min.) $\times 10^{-7}$	($\Delta D/\text{mg. of protein/min.}$)	(molecules/cell/min.) $\times 10^{-7}$	
0	0.224	4.05	7.5	—	—	—	—	—
60	0.305	3.68	5.6	—	—	—	—	—
90	0.385	3.51	10.0	—	—	—	—	—
110	0.80	3.15	4.2	—	—	—	—	—
130	1.06	2.92	4.6	1.50	5.0	0.71	2.3	2.1
150	2.1	2.47	4.1	—	—	—	—	—
170	2.15	1.96	—	1.47	3.6	0.67	1.6	2.2

Fig. 1. Growth and glucose utilization. *A*, Aerobic: bacteria were grown in a roller flask in '52' medium containing 3 mg. of glucose/ml.; *B*, anaerobic: bacteria were grown in a Klett side-arm cylinder with bubbled N_2 . The medium was '52' containing 7.17 mg. of glucose/ml. *T*, turbidity with 420 m μ . filter on Klett colorimeter; *C*, plate count $\times 10^{-7}$; *G*, glucose uptake from the medium (mg. of glucose/100 ml. of medium).

of cell number, and the rate of glucose removal, were lower, until the limit of growth was reached at a turbidity of 400–450. The pH decreased by 1.0 in the 2 hr. The glucose was removed at the rate of $3\text{--}5 \times 10^7$ molecules/cell/min.

The turbidity at which the break occurred varied with different experiments, in Fig. 1*B* at 220, but

Table 2. *Glucose uptake during anaerobic growth*

The culture was grown under the conditions described in the legend to Fig. 1*B*.

Time (min.)	Count (cells/ml.) $\times 10^{-9}$	pH	Glucose content of medium (mg./ml. of medium)	Glucose utilized (molecules/cell/min.) $\times 10^{-7}$
0	0.165	7.8	7.17	30
60	0.16	—	6.32	15
120	0.50	—	5.40	0
140	0.64	7.6	5.47	23
165	1.0	7.45	4.84	5.0
180	1.5	7.33	4.56	10.3
200	1.8	7.1	3.54	3.7
220	2.0	7.0	3.12	3.4
240	2.75	6.7	—	3.4
260	2.4	6.6	2.18	4.1
280	3.2	6.7	1.45	2.4
320	—	6.4	0.53	1.8
350	3.2	6.4	0	—

usually between 300 and 400. In two experiments cells were harvested before the break, in two at the break, and in the others after it.

The results of anaerobic experiments with glucose, and the mean activities of the enzymes in the extracts of cells grown on glucose, both aerobically and anaerobically, are shown in Table 3. The time of harvest in relation to the break in the growth curve had no significant effect on the enzyme activities of the extracts.

When the cells were grown under nitrogen with potassium gluconate as the carbon source, they were adapted to growth on gluconate in air before anaerobic conditions were established. In Expt. 1 of Table 4 the inoculum had been grown on gluconate in air, to a turbidity of 180. The inoculum (50 ml.) was added to 112 ml. of medium containing potassium gluconate, to give a concentration of 4.5 mg./ml. Nitrogen was bubbled and growth was logarithmic after a lag of 30 min., with a doubling time of 85 min. The cells were harvested at a

turbidity of 280, and the plate count of viable cells was 1.26×10^9 /ml. For Expt. 2, 50 ml. of an inoculum grown anaerobically on glucose was added to 150 ml. of medium containing potassium gluconate, to give a concentration of 12.2 mg./ml. The culture was aerated, and after a 60 min. lag phase logarithmic growth was established. After a further 60 min. nitrogen was bubbled. Doubling time was 75 min., and there was a break in the growth rate at a turbidity of 250. The cells were harvested at a turbidity of 315, and the number of cells was estimated to be 1.6×10^9 /ml. The activities of the extracts obtained in these two anaerobic experiments, and the mean data on aerobic gluconate cells, are shown in Table 4. There was no significant difference between these values.

When D-arabinose was the carbon source for growth (Expts. 3 and 5, as shown in Table 5) an inoculum of stock culture of strain B_{A15} was grown overnight in synthetic medium containing 0.5 mg. of glucose/ml. and 1.0 mg. of D-arabinose/ml. An overnight culture (100 ml.) was diluted with 100 ml. of synthetic medium with D-arabinose, so that the

culture contained 7 mg. of D-arabinose/ml. Growth in the roller flask was logarithmic, with a doubling time of 95 min. The cells were harvested when the turbidity was 350, and the number of viable cells 1.7×10^9 .

When lactate was the carbon source, cells of strain B were grown overnight in synthetic medium containing 3 mg. of sodium lactate/ml. To 100 ml. of this culture were added 200 ml. of synthetic medium in a roller flask, and sodium lactate to a concentration of either 3.6 or 6 mg./ml. The doubling time was 120 min. Cells were harvested during exponential growth in Expts. 7 and 9, and 17 hr. after growth had ceased in Expt. 8. When cells were grown on either D-arabinose or lactate, the extracts obtained had activities which were within the same range as with other substrates (Table 5). There was no growth of these cultures of *Esch. coli* on the latter two substrates under anaerobic conditions, even when cells were pre-adapted to the substrate in air. It was noted by Stephenson & Whetham (1924) that no growth of *Esch. coli* occurred on lactate or acetate, glycerol or succinate in an atmosphere of nitrogen.

Table 3. Activities of enzymes from bacteria grown on glucose under anaerobic conditions

Time of harvest	Gas phase	Turbidity at 420 m μ .	Count (cells/ml.) $\times 10^{-9}$	Enzyme activities of extracts				Ratio: G 6-PD PGD
				G 6-PD		PGD		
				(ΔD /mg. of protein/min.)	(molecules/cell/min.) $\times 10^{-7}$	(ΔD /mg. of protein/min.)	(molecules/cell/min.) $\times 10^{-7}$	
Before break	N ₂	260	1.8*	1.87	2.6	0.93	1.3	2.0
Before break	N ₂	310	1.8†	1.45	2.6	1.02	1.7	1.4
After break 300	N ₂	400	3.0*	1.67	2.6	0.95	1.17	1.8
At break	N ₂	400	3.0*	1.54	2.1	0.85	1.15	1.8
After break	N ₂	420	2.0†	1.33	2.4	0.66	1.24	2.0
Break at 300	N ₂	315	1.8†	1.40	2.9	0.93	1.85	1.5
After break	No aeration	340	2.0†	1.30	2.5	0.99	1.8	1.3
After break	No aeration	234	1.8*	1.50	2.6	0.75	1.3	2.0
Break at 400	No aeration	455	3.1†	1.93	2.4	0.92	1.2	2.1
Mean	Anaerobic	—	—	1.55	2.5	0.87	1.36	1.8
Standard deviation	—	—	—	± 0.27	± 0.2	± 0.12	± 0.26	1.9
Mean	Air	—	—	1.67 (24)‡	4.7 (18)	0.87 (12)	2.75 (12)	—
Standard deviation	—	—	—	± 0.48	± 1.2	± 0.15	± 0.63	—

* From standard curve relating turbidity to count.

† Plate count.

‡ Number of experiments in brackets.

Table 4. Activity of extracts of cells grown on gluconate under anaerobic conditions

Expt. no.	Gas phase	Turbidity at harvest (galvanometer readings)	Mean S.D.	G 6-PD		PGD		Ratio: G 6-PD PGD
				(ΔD /mg. of protein/min.)	(molecules/ cell/min.) $\times 10^{-7}$	(ΔD /mg. of protein/min.)	(molecules/ cell/min.) $\times 10^{-7}$	
	Air	—		1.54 (13)* ± 0.45	3.35 (9) ± 1.3	0.765 (13) ± 0.27	1.81 (9) ± 0.52	2.0 —
1	N ₂	280		1.32	4.0	0.80	2.4	1.65
2	N ₂	315		1.90	4.7	0.96	2.1	2.0

* Number of tests in parentheses.

Experiments with bacteriophage

Experiment with T-6r+. Cells were grown in 300 ml. of synthetic medium containing glucose in two roller flasks in the incubator at 36° to a turbidity of 460 and 475 indicating 1.8×10^9 cells/ml. The contents of one flask were removed, and chilled and harvested as usual. To the other 300 ml. were added 15 ml. of a suspension of T-6r+ which contained 7.4×10^{11} infectious particles/ml. and 3 ml. of 0.5% (w/v) solution of tryptophan, the absorption co-factor for T-6. The multiplicity or virus to cell ratio was 20. Samples were removed at intervals for the determination of turbidity. After continued aeration for 30 min., 150 ml. was removed. The remaining 120 ml. was harvested at 90 min. The cells were centrifuged, washed with saline and weighed as a wet pellet.

Each pellet was ground with alumina and was extracted three times with 5 vol. of 0.1M amino-tris(hydroxymethyl)methane (tris) buffer at pH 8.0. The extracts of the infected bacteria were very viscous and cloudy. Centrifuging for 2 hr. at

10 000 g was not sufficient to clear the extracts, although pellets of virus were deposited at the bottom of the tubes. The protein contents and the enzyme activities of the first extracts are shown as Expt. 1 in Table 6. Computed on the basis of protein content the enzymic activities of the extracts so obtained were the same as those of extracts prepared from normal cells.

Experiments with T-2r+. Cells were grown in 300 ml. of synthetic medium containing glucose in the roller flask at 37°. At a turbidity of 335, and a plate count of 1.08×10^9 cells/ml., 100 ml. were removed and harvested. To the rest of the culture were added 2 ml. of a suspension of T-2r+ with a plaque count of 7.5×10^{11} infectious particles/ml. This allowed infection of each cell with seven virus particles. After 15 min., 100 ml. was removed, and after 60 min., 68 ml. remained. Both portions were chilled and centrifuged. The infected cells were neither washed nor weighed, but immediately ground with alumina and extracted once with 10 vol. of 0.05M tris. As shown in Expt. 2 of Table 6, the protein content of the extracts of infected cells

Table 5. *Activities of enzymes of extracts from cells grown with aeration on D-arabinose and lactate*

Expt. no.	Substrate	Turbidity (galvanometer readings)	Count (cells/ml.) $\times 10^{-9}$	Enzyme activities of extracts				Ratio: G 6-PD PGD
				G 6-PD		PGD		
				(Δ D/mg. of protein/min.)	(molecules/ cell/min.) $\times 10^{-7}$	(Δ D/mg. of protein/min.)	(molecules/ cell/min.) $\times 10^{-7}$	
3	D-Arabinose	335	(1.6)*	1.31	(2.3)*	0.61	(1.10)*	2.1
4	D-Arabinose	—	—	1.86	—	0.74	—	2.5
5	D-Arabinose	350	1.7	1.08	3.5	0.83	2.90	1.3
6	D-Arabinose	—	—	1.32	—	0.47	—	2.8
7	Lactate	495	2.4	1.43	2.66	0.77	1.41	1.9
8	Lactate	680†	5.6	1.52	0.96	0.73	0.46	2.0
9	Lactate	405	2.4	1.34	1.96	0.81	1.17	1.7

* Estimated.

† Resting cells.

Table 6. *Enzyme activities of extracts of virus-infected cells*

Expt. no.	Virus type	Virus per cell	Time (min.)	Protein (mg./ml.)	Enzyme activities of extracts		Ratio: G 6-PD PGD
					G 6-PD (Δ D/mg. of protein/min.)	PGD (Δ D/mg. of protein/min.)	
1	T-6r +	20	0	27.2	1.29	0.59	2.2
			30	11.0	1.78	0.93	1.9
			90	16.0	1.25	0.44	2.85
2	T-2r +	7	0	9.8	1.37	0.98	1.4
			15	6.7	2.24	1.19	1.9
			60	5.3	1.77	1.66	1.1
3	T-2r +	5	0	10.3	1.22	0.69	1.8
			20	10.6	1.50	0.85	1.8
			60	8.5	1.32	0.61	2.2
4	T-2r +	7	0	10.5	2.14	0.91	2.2
			40	13.0	1.31	0.62	2.1
Mean	0	—	—	—	1.50	0.79	1.9
Mean	All virus	—	—	—	1.60	0.90	1.8

was lower than that of extracts of normal cells, and enzyme activities of the extracts were higher than normal, especially the phosphogluconate dehydrogenase. The total activity of the extracts of the infected cells was the same as, or slightly higher than, that of the uninfected cells.

In Expt. 3, cells at a turbidity of 300 were infected with five particles of T-2r + per bacterial cell and harvested at 20 and 60 min. The cells were neither washed nor weighed, but were ground and extracted twice, with 10 and 5 vol. of tris respectively. The ratios are normal.

The cells in Expt. 4 were harvested 40 min. after infection with T-2r + : the pellet was extracted once with 0.1 M tris, the uninfected cells with 10 vol., and the infected cells with about 7 vol. The activities of the extracts of infected cells were somewhat lower than the control, but the ratio was unchanged.

DISCUSSION

The experiments on glucose uptake by the growing bacteria were undertaken to verify the validity of the application to our system of *Esch. coli* B of Monod's (1942) findings that the limit of growth of bacteria was proportional to the amount of glucose supplied in the medium. On this basis, it had been calculated that glucose was used at a mean rate of 8×10^7 molecules/cell/min. (Scott & Cohen, 1953b). By determination of the glucose removed from the medium at intervals, a mean rate of 7.3×10^7 molecules of glucose taken up per cell/min. was obtained during early exponential phase of growth in air. This agrees fairly well with the value calculated on Monod's hypothesis. During the last hour the rate decreased. When aeration was sufficient, the logarithmic rate of increased turbidity was constant until growth stopped through exhaustion of either the carbon source or the nitrogen source. The pattern of anaerobic growth on glucose (measured turbidimetrically), either in deep stationary cultures or with bubbled nitrogen, was different from that of aerobic cultures. The rate of growth changed at some point between a turbidity of 200 and 400, and growth finally ceased at 400–450. The rate of glucose removal showed the same shape of curve, and growth ceased before either glucose or the nitrogen source had been exhausted. Although the pH fell faster after the break occurred, it had not fallen far enough to inhibit growth. Addition of NaOH to bring the pH back to 7.0 after growth had ceased did not allow further growth. According to Gale & Epps (1942) *Esch. coli* has the same rate of growth between pH 5.5 and 8.2. It may, therefore, be conjectured that some metabolic product was inhibiting growth. Dagley & Dawes (1953) found a similar cessation of growth with *Aerobacter aerogenes* grown in un-aerated cultures on citrate,

when both citrate and excess ammonium sulphate were present, and the pH was 7.0. Acetate was the metabolic product responsible for inhibition of further growth.

For determination of the significance of the difference between values for activity of extracts of cells grown aerobically or anaerobically Student's *t* test was applied. The activities of G 6-P, determined by the change in optical density at 340 m μ ., were calculated in terms of μ moles of TPN reduced by G 6-P per mg. of protein/min., with 6.22×10^6 as the molecular extinction coefficient of TPN (Horecker & Kornberg, 1948). The values obtained were 0.268 μ mole for aerobic conditions, and 0.247 μ mole for anaerobic conditions. The probability was 0.6 that this difference was due to chance, and therefore the difference is not significant. When calculated as molecules of G 6-P/cell/min., the values were 4.75×10^7 and 2.55×10^7 respectively, and the probability was less than 0.015. These appear to be significant differences. Under anaerobic conditions differences of rate between stationary deep culture and cultures bubbled with nitrogen, calculated on either basis, are not significant; nor are those between cultures harvested before, at, or after the break in the growth curve.

PGD activities in terms of μ moles of TPN reduced by PG per mg. of protein of the extracts from bacteria grown under different conditions, and with different carbon sources, were all of the same order (0.139 ± 0.024), and showed no significant differences. However, on the single-cell basis the value 1.36×10^7 molecules of TPN reduced by PGD per cell/min. under anaerobic conditions with glucose as the carbon source were significantly lower than the value 2.75×10^7 under aerobic conditions ($P = 0.01$ by *t* test).

The constancy of the activities of the extracts per mg. of protein under all conditions of growth and with different substrates is not in contradiction to the significant difference between the activities in molecules of substrate oxidized per cell. This latter may be ascribed to the difference in size of the bacteria under different conditions. The cells harvested during the logarithmic phase of growth on glucose under aerobic conditions are larger than cells harvested from any other cultures (Henrici, 1928; Hershey & Bronfenbrenner, 1938).

In some of our experiments there was a partially synchronized pattern of growth, so that at one time most of the cells were large, and 10 min. later most cells had divided and were small. This pattern of synchronized cultures will be discussed in a later paper.

From the two experiments with gluconate under anaerobic conditions, it would seem that the lack of oxygen does not decrease the size of the cells as it does when glucose is the carbon source. There was no

significant difference in the activity of G 6-PD per mg. of bacterial protein or in molecules oxidized per cell/min. whether the cells were grown with N_2 or with air. The same was true of PGD, which again showed half the activity of the former enzyme.

The bacteria grown on arabinose and lactate contained both enzymes with activities which fall within the normal range. In the lactate experiment, where cells were grown to high turbidity and left overnight in the cold room, the cells were small and the activities on a cell basis were very low.

The difference between the activities of the extracts on G 6-P and 6-PG is highly significant. The former was oxidized at twice the rate of the latter. This is in agreement with previous data (Scott & Cohen, 1953b).

It is interesting to compare the rates of glucose removed from the media by the cell and the extractable G 6-PD activities. During the early phase of aerobic growth more glucose (up to 10×10^7 molecules/cell/min.) is removed and a portion is probably incorporated into the cells as glycogen (Dagley & Dawes, 1949; Levine, Stevenson, Tabor, Bordner & Chambers, 1953) while the cell is enlarging. During the later phase of growth glucose is removed at a rate of $4-5 \times 10^7$ molecules/cell/min. and the activity of G 6-P varies from 4 to 6×10^7 molecules oxidized/cell/min. The correspondence is striking. However, at the same time PGD activity is only one-half of G 6-P activity. During anaerobic growth glucose is removed during the initial phase at a higher rate, which falls during the later phase to $2-4 \times 10^7$ molecules/cell/min., while the G 6-PD and PGD activities are 2.5 and 1.4 molecules/cell/min. respectively. The measurement of isotopic CO_2 derived from the first C atom of glucose gives an indication of PGD activity *in vivo* which would seem to be a maximum of less than 40% of the glucose used (Cohen, 1951). Thus we are faced with the alternatives, either that measurement of the G 6-PD in cell-free extracts represents only the capacity of cell enzyme which is not fully utilized in the cell and that this is less true for the PGD, or that the G 6-PD activity accounts for the full use of G 6-P at least during the later phase of aerobic growth and that the excess of PG is metabolized in some way other than by PGD, perhaps by the enzyme system which MacGee & Doudoroff (1954) found in *Pseudomonas* which produces 2-oxo-3-deoxy-6-phosphogluconate and then splits it into two 3-carbon fragments.

Anaerobic utilization of glucose might be carried on with a small constant activity of G 6-PD and PGD presumably to supply the ribose for the synthesis of ribonucleic acid and for other compounds derived from ribose 5-phosphate and the rest by another pathway, presumably the Embden-Meyerhof aldolase splitting of fructose-1:6-diphos-

phate. Or the dehydrogenases may be present as constitutive enzymes and not active at all. So far we do not yet have the data to derive any valid conclusions in this matter.

The glucose 6-phosphate-dehydrogenase activity of the extracts of infected cells was not significantly lower than the mean activity of extracts from normal *Esch. coli*, strain B. In fact, cells extracted up to 30 min. after infection tended to have a higher activity than the uninfected controls or the cells infected for longer periods. The phosphogluconate-dehydrogenase activity showed a similar pattern in infected cells except in one experiment (Expt. 2, Table 6) where the activity of the extract, 60 min. after infection, was increased by 6 times the standard deviation from the mean, that is, the value was 1.7 times the control.

Thus infection with bacteriophage does not decrease the extractable activity of these enzymes nor does phage present in the extracts interfere with the functioning of these enzymes *in vitro*. Lysis with phage could be used for preparation of these dehydrogenases from *Esch. coli* as well as for other enzymes (Pardee & Kunkee, 1952; Sher & Mallette, 1953).

It may be concluded therefore that the bacteriophage exerts its effects in changing the metabolism of the cell from self duplication to formation of phage, not by decreasing the amount of G 6-P and PG dehydrogenases, but by other enzymic changes. The dehydrogenases are still present, but by isotopic evidence do not appear to be as active in the cell during infection by bacteriophage (Lanning & Cohen, 1954).

The most noteworthy result of these experiments is the constancy of these two dehydrogenases in terms of activity/mg. of bacterial protein under all the conditions tested in *Esch. coli*.

SUMMARY

1. *Escherichia coli* has been grown in synthetic medium with glucose or gluconate under aerobic and anaerobic conditions, and with arabinose and sodium lactate under aerobic conditions.

2. Glucose 6-phosphate dehydrogenase in extracts from cells grown under all conditions tested reduced triphosphopyridine nucleotide (TPN) at a mean rate of $0.26 \mu\text{mole/min./mg. of protein}$.

3. Phosphogluconate dehydrogenase in the same extracts reduced TPN at a mean rate of $0.14 \mu\text{mole/min./mg. of protein}$.

4. Activity of these enzymes in terms of molecules of substrate oxidized/min./cell were calculated, and compared with the uptake of glucose from the medium as experimentally determined.

5. Infection with T-6r+ or T-2r+ bacteriophage did not change the extractable activity of glucose 6-phosphate dehydrogenase or phosphogluconate dehydrogenase in *Esch. coli*.

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The Oxidative Pathway of Carbohydrate Metabolism in *Escherichia coli*

4. FORMATION OF ENZYMES INDUCED BY 2:4-DINITROPHENOL

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The constancy of the extractable activity of the enzymes of the oxidative pathway of hexose phosphate metabolism was demonstrated in the previous papers of this series (Scott & Cohen, 1953b; Scott, 1956). An investigation into the effects of various enzyme inhibitors revealed that *Escherichia coli* grown in the presence of 2:4-dinitrophenol (DNP) had increased amounts of extractable activity of glucose 6-phosphate dehydrogenase (G 6-PD), while the extractable phosphogluconate dehydrogenase (PGD) activity was unchanged (Scott, 1954). An enzyme was found in these extracts which degrades phosphogluconate (PG) without the need for triphosphopyridine nucleotide (TPN). The conditions under which the DNP acts and some preliminary data on the PG-degrading enzyme are reported here.

METHODS

The bacteria, *Esch. coli* strain B, were harvested and extracted, and the enzyme activities were determined as previously described (Scott & Cohen, 1953a; Scott, 1956). Growth was followed by plate counts of viable bacteria and by turbidity, which was read on a Klett-Summerson photometer, with a 660 m μ . filter instead of the 420 m μ . filter to avoid the effect of the colour of the DNP. The DNP was added to the medium as 0.02M solution neutralized with NaOH. Glucose was determined by the anthrone

method (Morris, 1948; Dreywood, 1946; or Trevelyan & Harrison, 1952). Pyruvate was determined by the method of Friedemann & Haugen (1943).

RESULTS

Effect of DNP on the enzyme activity in vitro. DNP in concentrations from 1×10^{-6} to 1×10^{-3} M in the reaction mixture in which the activities of G 6-PD or PGD were determined had no significant effect on the activity of either enzyme.

Effect on respiration. DNP had the usual effect of increasing the total oxygen uptake of the bacteria respiring in the presence of glucose. Oxygen uptake was prevented if DNP was added before the glucose to resting cells.

Effect on glucose uptake. There was no significant effect of DNP at a concentration of 2×10^{-4} M upon the rate of removal of glucose from the medium by the growing bacteria (Fig. 1). The mean rate was 7×10^7 molecules of glucose removed per cell/min. Bacteria which were in the resting state, i.e. which were suspended in aerated medium containing glucose but with no nitrogen source, removed glucose at the rate of 4×10^6 molecules/cell/min., in the absence or presence of DNP.

Effect on growth. Patterns of bacterial growth in the presence of DNP were not reproducible. Under aerobic conditions, 2×10^{-4} M DNP usually had no