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REFERENCES

- Cohen, S. S. (1951). *Nature, Lond.*, **168**, 746.
 Cohen, S. S. (1953). *J. biol. Chem.* **201**, 71.
 Cohen, S. & Barner, H. (1955). *J. Bact.* **69**, 59.
 Dagley, S. & Dawes, E. A. (1949). *Biochem. J.* **45**, 331.
 Dagley, S. & Dawes, E. A. (1953). *J. Bact.* **66**, 259.
 Gale, E. F. & Epps, H. M. R. (1942). *Biochem. J.* **36**, 600.
 Henrici, A. T. (1928). *Morphologic Variation and the Rule of Growth of Bacteria*. Springfield, Ill.: Charles C. Thomas.
 Hershey, A. D. & Bronfenbrenner, J. (1938). *J. gen. Physiol.* **21**, 721.
 Horecker, B. L. & Kornberg, A. (1948). *J. biol. Chem.* **175**, 385.
 Lanning, M. & Cohen, S. S. (1954). *J. biol. Chem.* **207**, 193.
 Levine, S., Stevenson, H. J. R., Tabor, E. C., Bordner, R. H. & Chambers, L. A. (1953). *J. Bact.* **66**, 664.
 MacGee, J. & Doudoroff, M. (1954). *J. biol. Chem.* **210**, 617.
 Monod, J. (1942). *Recherches sur la croissance de bactériennes*. Paris: Hermann et Cie.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Pardee, A. B. & Kunkee, R. E. (1952). *J. biol. Chem.* **199**, 9.
 Scott, D. B. M. & Cohen, S. S. (1953a). *Biochem. J.* **55**, 23.
 Scott, D. B. M. & Cohen, S. S. (1953b). *Biochem. J.* **55**, 33.
 Sher, I. H. & Mallette, M. F. (1953). *J. biol. Chem.* **200**, 257.
 Stephenson, M. & Whetham, M. D. (1924). *Biochem. J.* **18**, 498.

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

inhibitory action on growth with glucose, as measured by the increase in turbidity, although the plate counts of viable cells were usually lower than normal (Fig. 1). Above this concentration DNP inhibited growth, almost completely at 2×10^{-3} M. When cells were growing under anaerobic conditions, the extent and pattern of inhibition seemed to depend on the previous history of the cell population. The inoculum for the experiment shown in Fig. 2A was a culture which had ceased growing under anaerobic conditions. The growth pattern obtained when the inoculum consisted of a suspension of cells from an actively growing anaerobic culture is shown in Fig. 2B. When the same inoculum was used for a culture which was aerated the pattern was similar, with early inhibition and later recovery, and the rate of growth after recovery was the same as the anaerobic rate without DNP (Fig. 2C). The effect of DNP on adaptation to anaerobic conditions was tested in one experiment. DNP (2×10^{-4} M) was added to an aerated culture. Change of the gas phase from air to nitrogen inhibited growth for about 30 min.; growth was resumed at an anaerobic rate.

The effect of 2×10^{-4} M DNP on the growth on gluconate of cells previously adapted to gluconate is shown in Fig. 3A. The growth of aerated cultures was depressed to a rate close to that of normal anaerobic cultures. Under anaerobic conditions, DNP decreased the rate about one-half.

One observation was made for which the mechanism is not yet apparent. In the experiments shown in Tables 3 and 5 it was found that a substance analysing as pyruvate appeared in the medium, at a concentration of $170 \mu\text{g./ml.}$ at 15, 30 and 60 min. after the addition of DNP (2×10^{-4} M). At 120 min. the pyruvate was no longer detectable. The pyruvate was isolated as the 2:4-dinitrophenylhydrazone, with m.p. 220° (uncorr.) and an absorption peak at $430 \text{ m}\mu$, identical with authentic dinitrophenylhydrazone of pyruvic acid prepared in the same way. (A similar pattern of appearance of pyruvate in the medium of growing *Streptomyces griseus* has been noted by Hockenhull, Fantes & Herbert (1954). See also Quastel, Stephenson & Whetham (1925) for production of pyruvate by *Esch. coli* grown with lactate and nitrate.)

Fig. 1. Effect of DNP on growth and glucose uptake. Cultures were grown in two rotated flasks at 36° . Each held 200 ml. of 52 medium containing 4 mg. of glucose/ml. At 85 min. DNP was added to one to a concentration of 2.5×10^{-4} M. At 110 and 140 min., one-half of the contents of each flask was removed and harvested for enzyme analysis. (Because of the removal of samples, the roller speed was variable, and evidently not great enough for full aeration.) O, plate counts ($\times 10^{-7}$) obtained without additions; ●, plate counts ($\times 10^{-7}$) obtained with added DNP; ×, turbidity measured at $660 \text{ m}\mu$; Δ, glucose removed from medium (mg. $\times 10^3$); ▲, glucose removed from medium with added DNP (mg. $\times 10^3$). A = growth; B = glucose utilization.

Fig. 2. Effect of concentration (M) of DNP on growth of anaerobic cultures with glucose. A: inoculum was grown overnight under N_2 to a turbidity of 330, and was in a resting state; 1 ml. was put into 21 ml. of 52 medium containing 10 mg. of glucose/ml., and DNP as indicated. The cultures stood for 30 min. before N_2 was bubbled without change of turbidity; temp. 37° . B: inoculum was grown in deep stationary culture, and was still growing; 5 ml. was put into 15 ml. of 52 medium containing 15 mg. of glucose/ml. and DNP as indicated. N_2 was bubbled through. C: the same as B except that air was bubbled through.

Esch. coli strain B, grown on glucose, would not adapt to growth on sodium lactate in the presence of DNP. When the inoculum was adapted to lactate, growth was slightly inhibited by $2 \times 10^{-4} \text{ M}$ DNP. Growth on D-arabinose was not inhibited by $5 \times 10^{-5} \text{ M}$ DNP (Fig. 3B).

Effect of growth in the presence of DNP on extractable-enzyme activity

Dehydrogenases for G 6-P and PG. Cells were harvested after varying periods of growth in the presence of DNP and the extracts were prepared. The rate of reduction of TPN was measured in buffer solutions containing samples of the extracts and G 6-P or PG. The activities in terms of the increase in density at $340 \text{ m}\mu/\text{min.}/\text{mg.}$ of protein in the extract, and the ratio between the two dehydrogenase activities, are shown in the tables. The values shown in Table 1 were obtained with extracts of aerated cultures, either resting or growing with glucose in the medium. When there was no ammonium sulphate in the medium and hence no

growth, there was no change in the enzyme activities after the addition of DNP. With a nitrogen source in the medium growth occurred and the extracts were found to have an increased rate of reduction of TPN in the solutions of G 6-P; with PG the rate was unchanged. This accelerated G 6-P activity was directly proportional to the length of time the cells were grown in the presence of DNP up to at least 120 min. (Fig. 4). G 6-PD was also increased by DNP when the cells were grown under anaerobic conditions (Tables 2 and 3). The development of increased G 6-PD took a longer time with gluconate than with glucose (Fig. 5). The effect of different concentrations of DNP is demonstrated in Fig. 6. There was no change at $2 \times 10^{-5} \text{ M}$; $5 \times 10^{-5} \text{ M}$ to $1 \times 10^{-3} \text{ M}$ concentrations were effective; $2 \times 10^{-3} \text{ M}$ DNP inhibited growth markedly and G 6-PD formation was not stimulated.

If arabinose or lactate was supplied to the cells as growth metabolite no significant effect of DNP on extractable enzyme activity was perceptible (Table 4).

Fig. 3. Effect of DNP ($2 \times 10^{-4} \text{ M}$) on growth with gluconate as carbon source. A: Inoculum was grown overnight in aerated culture containing 2 mg. of potassium gluconate/ml. The aerated cultures were grown in roller flasks in 150 ml. of 52 medium containing 5 mg. of potassium gluconate/ml. The DNP concentration in one was $2 \times 10^{-4} \text{ M}$. The anaerobic cultures were grown in Klett side-arm cylinders with an aerator through which N_2 was bubbled. One hundred and fifty ml. of 52 medium contained 5 mg./ml. of K gluconate. DNP concentration in one was $2 \times 10^{-4} \text{ M}$. B: effect of DNP ($2 \times 10^{-4} \text{ M}$) on growth on lactate and DNP ($5 \times 10^{-5} \text{ M}$) on growth on D-arabinose. The cultures were prepared and grown as indicated in Table 4.

Fig. 4. Dehydrogenase activities of extracts of cells grown on glucose with DNP ($2 \times 10^{-4} \text{ M}$). The cultures were prepared and grown as indicated in Table 1.

Fig. 5. Dehydrogenase activities of extracts of cells grown on potassium gluconate with DNP ($2 \times 10^{-4} \text{ M}$). The cultures were prepared and grown aerobically as indicated in Table 3.

Reversal of DNP effect on G 6-PD. Cultures were grown in the presence of 2×10^{-4} M DNP for 1 or 2 hr. Part of the culture was harvested and extracts were prepared. The other half was washed in medium containing no ammonium sulphate. A sample of the bacteria suspension was inoculated into complete 52 medium for the growing culture. The rest was incubated without a nitrogen source as a resting culture. Then both cultures were harvested and extracts prepared. It was found that the extracts of

the resting bacteria still showed increased G 6-PD activity. The extracts of the bacteria which had continued growth after removal from the medium containing DNP lost the excess of G 6-PD when they were allowed to grow from a turbidity of 22 to 140, as in the glucose experiment, but not when the growth was only to a turbidity of 97 from 31, as in the gluconate experiment. A greater dilution of the excess of enzyme had occurred in the more rapidly dividing bacteria. These results are summarized in Table 5.

Table 1. *Effect of DNP on dehydrogenases of aerobic cells grown on glucose*

For resting-cell cultures, the inoculum was grown overnight on glucose and with bubbled air. The cells were centrifuged, washed once with saline and suspended in 52-N medium containing no $(\text{NH}_4)_2\text{SO}_4$. Glucose (2 mg./ml.) was added, of which 1 mg./ml. was removed by the cells in 3 hr. The culture was aerated at 37°. For growing cultures, a similar inoculum was put into complete 52 medium containing glucose. DNP was added to the cultures and the times of incubation were as indicated. Activity is expressed as increase in absorption at 340 m μ ./mg. of protein/min. One ΔD /mg./min. is equivalent to the reduction of 0.16 μ mole of TPN/mg. of protein/min. (Values italicized are significantly greater than mean values without DNP.)

Expt. no.	Growth phase	DNP		Enzyme activities of extracts		
		Concn. (M)	Time (min.)	G 6-PD (ΔD /mg./min.)	PGD (ΔD /mg./min.)	Ratio: G 6-PD / PGD
Mean*	All	0	—	1.67	0.87	1.92
1	Resting	0	—	2.44	1.32	1.85
		2×10^{-4}	180	2.40	1.06	2.26
		1×10^{-3}	180	2.04	1.04	1.96
		2×10^{-4}	180	2.50	1.26	1.98
2	Resting	2×10^{-4}	180	2.50	1.26	1.98
3	Growing	0	—	1.50	0.71	2.12
		2×10^{-4}	30	3.26	0.76	4.3
4	Growing	0	—	2.14	0.91	2.36
		2×10^{-4}	45	4.24	0.85	5.0
5	Growing	0	—	1.47	0.67	2.20
		2×10^{-4}	80	4.55	0.77	5.9
6	Growing	2×10^{-4}	120	8.24	1.02	8.0
7	Growing	0	180	1.48	0.92	1.61
		2×10^{-4}	180	5.90	0.93	6.35
8	Growing	0	—	0.96	0.70	1.37
		1×10^{-5}	180	2.10	1.25	1.67
		2×10^{-4}	240	3.30	0.68	5.0
9	Growing	2×10^{-5}	180	2.90	1.10	2.46
		2×10^{-4}	180	12.00	1.41	8.5

Table 2. *Effect of DNP on dehydrogenases of anaerobic cells growing on glucose*

The cultures were prepared and grown as indicated in Table 1, except that nitrogen was bubbled through the aerator tubes. Values italicized are considered to be significantly greater than mean values.

Expt. no.	DNP		Enzyme activities of extracts		Ratio: G 6-PD / PGD
	Concn. (M)	Time (min.)	G 6-PD (ΔD /mg./min.)	PGD (ΔD /mg./min.)	
Mean*	0	—	1.55	0.87	1.78
13	0	—	1.45	1.02	1.42
	2×10^{-4}	120	4.15	1.02	4.03
11	0	—	1.87	0.93	2.01
	5×10^{-5}	180	3.08	0.85	3.62
12	0	—	1.33	0.66	2.00
	1×10^{-4}	210	3.03	0.64	4.73

* Mean for extracts of cells grown aerobically or anaerobically taken from Scott (1956).

PG-degrading enzyme. Experiments were undertaken to determine whether PG was split without oxidation by TPN. Extracts (0.02–0.2 ml.) were incubated at 37° with limited amounts of PG (0.1 μ mole) in 0.05M buffer (total volume 1 ml.) at pH 7.4. After an interval of time TPN was added to measure the amount of PG remaining. No bivalent cations were added since it was found that Ca^{2+} , Mg^{2+} , and Mn^{2+} inhibited the degrading enzyme. These cations also inhibit the re-oxidation of TPN. Therefore determinations of PG were corrected for this re-oxidation by extrapolation as shown in Fig. 7.

Destruction of PG was proportional to the amount of extract in the mixture (Fig. 8), as well as to the time it was allowed to act. The enzyme was very labile and lost up to 80 % of the activity within a few hours at 0°. It was extremely sensitive to Ca^{2+} concentration; 0.0067M inhibited the reaction completely and 0.00083M inhibited it by 50 %. Preliminary tests indicated maximal activity from pH 5.2 to 7.6. Optimum conditions for stability and for action of the degrading enzyme have not been determined. From the data so far obtained, rates on non-oxidative disappearance of PG were

Table 3. *Effect of DNP with potassium gluconate as carbon source*

The cultures were prepared as in Table 1 except that potassium gluconate (4 mg./ml.) was the carbon source. Values italicized are considered to be significantly larger than control values.

Expt. no.	Condition	Gas phase	DNP		Turbidity count		Enzyme activities of extracts		Ratio: G 6-PD / PGD
			Concn. (M)	Time (min.)	At 660 m μ .	($\times 10^{-9}$)	G 6-PD (ΔD /mg./min.)	PGD (ΔD /mg./min.)	
14	Resting*	Air	0	—	280	—	2.18	1.16	1.88
14	Resting	Air	2×10^{-4}	180	226	—	2.18	1.06	2.06
14	Resting	Air	1×10^{-3}	180	212	—	2.58	1.18	2.20
15	Growing	Air	0	—	167	2.5	2.16	0.79	2.74
15	Growing	Air	2×10^{-4}	180	167	1.5	10.3	0.86	12.0
16	Growing	Air	5×10^{-4}	120	87	0.48	7.8	0.78	10.0
15	Growing	Air	1×10^{-3}	210	122	0.95	10.2	0.86	11.9
19	Growing	Air	0	—	245	1.7	1.42	0.84	1.69
19	Growing	Air	2×10^{-4}	165	132	1.4	6.11	0.91	6.7
19	Growing	N ₂	0	—	185	1.3	1.32	0.80	1.65
19	Growing	N ₂	2×10^{-4}	270	80	0.5	4.45	0.70	6.3

* Not previously adapted to growth on gluconate.

Fig. 6. Effect of concentration of DNP on enzyme activities of cells grown on potassium gluconate. The cultures were prepared as indicated in Table 3 and grown aerobically for 120 min.

Table 4. *Effect of DNP during aerobic growth on lactate and arabinose*

Cultures were prepared as in Table 1 except that D-arabinose (5 mg./ml.) or sodium lactate (6 mg./ml.) was added to the media instead of glucose.

Expt. no.		DNP		Enzyme activities of extracts		Ratio: G 6-PD / PGD
		Concn. (M)	Time (min.)	G 6-PD (ΔD /mg./min.)	PGD (ΔD /mg./min.)	
20	D-Arabinose	0	—	1.31	0.61	2.16
		5×10^{-5}	240	1.71	0.61	2.8
21	Lactate	0	—	1.43	0.77	1.86
		2×10^{-4}	360	1.60	0.74	2.16

Table 5. *Effect of transferring cells grown in the presence of DNP to medium without DNP*

A 60 min. 100 ml. of *a* was harvested and extracted. The other 100 ml. of *a* culture was centrifuged and washed and suspended in 120 ml. of 52 medium with no $(\text{NH}_4)_2\text{SO}_4$. For *b* cultures 20 ml. of this suspension was added to 100 ml. of complete 52 medium and substrate (2 mg./ml.). To the remaining 100 ml. of suspension substrate was added at 2 mg./ml. for *c* culture.

Expt. no.	DNP		Condition	Substrate	Turbidity at 660 m μ .	Count $\times 10^{-8}$	Pyruvate ($\mu\text{g.}/\text{ml.}$ of medium)	Extract activities		Ratio: G 6-PD / PGD
	Concn. (M)	Time (min.)						G 6-PD ($\Delta D/\text{mg.}/\text{min.}$)	PGD ($\Delta D/\text{mg.}/\text{min.}$)	
26 <i>a</i>	2×10^{-4}	60	Growing	Glucose	131	1.4	30.0	4.5	0.88	5.10
<i>b</i>	0	120	Growing	Glucose	140	1.8	3.4	2.6	1.17	2.2
<i>c</i>	0	140	Resting	No N, glucose	147	2.8	31.5	5.0	1.15	4.33
27 <i>a</i>	2×10^{-4}	120	Growing	Gluconate	155	1.8	11.0*	4.45	0.82	5.4
<i>b</i>	0	120	Growing	Gluconate	97	1.2	4.7	3.75	0.81	4.65
<i>c</i>	0	120	Resting	No N, gluconate	142	1.5	47.4	7.45	1.22	6.1

* Pyruvate was 39 $\mu\text{g.}/\text{ml.}$ at 30 min.

calculated in terms of μmoles degraded per min./mg. of protein. The most active extract had activities of 0.012 unit of degrading enzyme, and 0.16 unit of the PG dehydrogenase.

An increase in the extractable activity of the degrading enzyme occurred under the influence of DNP and was greater the longer the time of exposure to the drug (Tables 6, 7).

The products of the reaction have not yet been isolated. However, in two reaction mixtures, where 0.09 μmole of PG had been destroyed, 0.03 and 0.05 μmole of pyruvate were indicated by the formation of a 2:4-dinitrophenylhydrazone. The hydrazone spectrum had a peak at 430 m μ ., as does the 2:4-dinitrophenylhydrazone of pyruvic acid. The new intermediate 2-oxo-3-deoxyphosphogluconate, reported by MacGee & Doudoroff (1954), may be present in the reaction mixture, but has not yet been identified.

TPN oxidase. The characteristics of this enzyme have not been investigated fully. It was most active between pH 6.4 and 7.4, and was inhibited by $1.5 \times 10^{-3} \text{ M-Ca}^{2+}$. The activity was not strictly proportional to the amount of extract in solution, but was greater in the more concentrated state. Reduced triphosphopyridine nucleotide (TPNH) was oxidized at about one-twentieth the rate at which it was produced by PGD. That TPNH was oxidized and not destroyed could be demonstrated:

Fig. 7. Destruction of PG by extracts before addition of TPN. Extract made of bacteria grown on gluconate in air in the presence of DNP ($2 \times 10^{-4} \text{ M}$). Each 1 ml. cuvette contained 0.092 μmole of PG in 0.05 M glycylglycine (pH 7.4) and 0.05 ml. of extract. Total volume was 0.98 ml. Temp. 37°. At times indicated by arrows 0.02 ml. of TPN (0.18 μmole) was added to a cuvette.

if to a solution in which TPNH had been completely re-oxidized, further PG was added, there was an immediate increase in the density at 340 m μ . The activity of the oxidase did not seem to be affected by the presence of DNP in the growth medium. Ascorbic acid or oxidized glutathione and cysteine increased the rate of re-oxidation of TPNH.

Effect of sodium azide. Experiments similar to those with DNP were undertaken, using azide in the growth medium. A concentration of 1×10^{-3} M inhibited growth about 10 % and 2×10^{-3} M about 20 %. The results in Table 8 show slight increases in G 6-PD which are of questionable significance. There were variable effects on the degrading enzyme activity; inhibition was usual after 30 min. and later the enzyme activity was recovered.

Fig. 8. Effect of enzyme concentration on degradation of PG. Each 1 ml. cuvette contained 0.088 μ mole of PG in 0.05 M aminotrihydroxymethylmethane (pH 7.2) and amounts of enzyme indicated. TPN was added after 15 min. incubation at 36°. Total volume was 1 ml. A, 0.05 ml. of extract contained 0.26 mg. of protein; B, 0.10 ml. of extract contained 0.52 mg. of protein; C, 0.15 ml. of extract contained 0.78 mg. of protein; D, 0.05 ml. of extract was added after TPN.

DISCUSSION

The evidence seems unequivocal that the phenomena observed represented the induced increase of one or more enzymes. The G 6-PD is an enzyme which is normally present in the *Esch. coli* cells with activity which is constant in terms of mg. of protein, under all tested conditions of growth. The increased formation of enzyme has been brought about by a drug which has no structural relationship to the natural substrate of the enzyme. DNP has been found to block the synthesis of adaptive enzymes in many systems. It is possible that DNP blocks other pathways of metabolism of G 6-P with the result that substrate is piled up, and that more enzyme is formed in response to the increased G 6-P concentration.

If the increased extractable enzyme were indicative of greater activity *in vivo*, it would be expected that more PG would be formed. However, no increase of PGD could be demonstrated, either in cells grown in the presence of DNP, or when gluconate was the carbon source for growth. So other

Table 6. Increase in degrading enzyme with time of incubation of extracts with DNP (2×10^{-4} M)

In Expt. 9 extracts were prepared from cells grown aerobically on glucose. In Expt. 10 extracts were prepared from cells grown aerobically on potassium gluconate.

Expt. no.	Time (min.)	PG degraded (μ moles/min./mg. of protein)
9	15	0.0018
	30	—
	60	0.0039
	120	0.0044
10	15	0.0010
	30	0.0019
	60	0.0061
	120	0.0068

Table 7. Activities of degrading enzyme in extracts of cells grown in presence of DNP

In Expts. 7 and 8 extracts were prepared from cells grown aerobically on glucose for 180 min. In Expts. 17 and 15 cells were grown aerobically on potassium gluconate for 180 min.

Expt. no.	Concn. DNP (M)	μ mole of PG/min./mg. of protein
17	0	0.0046
	1×10^{-4}	0.0080
	5×10^{-4}	0.0082
	2×10^{-3}	0.0037
7	0	0.0003
	2×10^{-4}	0.0020
	1×10^{-3}	—
8	0	0.0007
	2×10^{-4}	0.0030
15	0	0.0030
	2×10^{-4}	0.0036
	1×10^{-3}	0.0041

Table 8. *Dehydrogenase activities of cells grown in the presence of sodium azide*

Values italicized are considered to represent significant increases over control values.

Expt. no.	Carbon source	Azide		Extract activities		Ratio: G 6-PD PGD
		Concn. (M)	Time (min.)	G 6-PD (ΔD /min./mg. of protein)	PGD (ΔD /min./mg. of protein)	
22	Glucose	0	—	1.72	0.86	2.0
		2×10^{-5}	110	2.01	1.13	1.78
		2×10^{-4}	110	1.98	0.97	2.04
		2×10^{-3}	110	1.68	1.02	1.65
23	Gluconate	0	—	1.8	1.01	1.8
		1×10^{-5}	120	2.8	0.98	2.9
		2×10^{-5}	120	2.4	0.96	2.5
24	Gluconate	0	—	2.5	1.00	2.5
		1×10^{-4}	90	2.7	1.02	2.5
		2×10^{-4}	90	3.42	1.13	3.02
		1×10^{-3}	90	2.9	0.74	3.9
25	Gluconate	2×10^{-4}	15	1.91	0.66	2.86
		2×10^{-4}	30	2.20	0.77	2.86
		2×10^{-4}	60	2.70	0.88	3.07
		2×10^{-4}	120	2.30	0.88	2.62

enzymes were sought which might be able to act on PG. Entner & Doudoroff (1952) described an enzyme occurring in *Pseudomonas saccharophila* which brought about the non-oxidative cleavage of PG. This organism respiring on glucose or gluconate in the presence of DNP or arsenite produced approximately 2 molecules of pyruvic acid/molecule of substrate. One molecule of pyruvate was formed by way of glyceraldehyde phosphate. Extracts of organisms grown on glucose produced pyruvate from excess PG at 30° at a rate of 0.06 μ mole/min./ml. of extract. If it is assumed that the extracts contained about 10 mg. of protein/ml. the rate would be 0.006 μ mole/min./mg. of protein. In the experiments described in this paper pyruvate appeared in the medium during growth of *Esch. coli* in the presence of DNP and, in extracts prepared from such organisms, an enzyme was present which was able to degrade PG in the absence of pyridine nucleotides. Extracts of bacteria grown on glucose alone had slight activity. Extracts of bacteria grown on glucose in the presence of DNP, or on gluconate, caused significant destruction of PG. Of the extracts investigated, the most active was prepared from cells grown on gluconate, and this caused the disappearance of 0.012 μ mole of PG/min./mg. at 37° and pH 7.4. The rates of reaction of the extracts were of the same order as that reported by Entner & Doudoroff (1952) for extracts of *Ps. saccharophila*. These rates are less than one-tenth that of the PG oxidation by the dehydrogenase. Since the products of this reaction and the optimum conditions for its occurrence have not been determined for *Esch. coli*, it is as yet impossible to estimate the importance of the non-oxidative degradation of PG to the total cell metabolism of *Esch. coli*.

SUMMARY

1. When *Escherichia coli* was grown in the presence of 2:4-dinitrophenol, there was a two- to six-fold increase in the activity of glucose 6-phosphate dehydrogenase in the cell-free extracts. There were no changes in the phosphogluconate-dehydrogenase activity of these extracts.

2. When *Esch. coli* was grown in the presence of sodium azide, the changes in the extractable activities of either enzyme were of questionable significance.

3. Extracts of *Esch. coli* were capable of degrading 6-phosphogluconate slowly in the absence of pyridine nucleotides. This activity was greater in cells grown with gluconate in the medium than with glucose, and was increased by the presence of DNP.

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REFERENCES

- Dreywood, R. (1946). *Industr. Engng Chem. (Anal.)*, **18**, 499.
 Entner, N. & Doudoroff, M. (1952). *J. biol. Chem.* **196**, 853.
 Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
 Hockenull, D. J. D., Fantes, K. H. & Herbert, M. (1954). *J. gen. Microbiol.* **10**, 353.
 MacGee, J. & Doudoroff, M. (1954). *J. biol. Chem.* **210**, 617.
 Morris, D. L. (1948). *Science*, **107**, 254.
 Quastel, J. H., Stephenson, M. & Whetham, M. D. (1925). *Biochem. J.* **19**, 304.
 Scott, D. B. M. (1954). *Fed. Proc.* **13**, 402.
 Scott, D. B. M. (1956). *Biochem. J.* **63**, 587.
 Scott, D. B. M. & Cohen, S. S. (1953a). *Biochem. J.* **55**, 23.
 Scott, D. B. M. & Cohen, S. S. (1953b). *Biochem. J.* **55**, 33.
 Trevelyan, W. E. & Harrison, J. S. (1952). *Biochem. J.* **50**, 298.