The Oxidative Pathway of Carbohydrate Metabolism in *Escherichia coli*

6. ADAPTATION OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE TO GROWTH IN COMPLEX MEDIA*

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(Received 23 October 1958)

In a previous paper of this series (Scott, 1956*a*) we reported that the activities of the two dehydrogenases of the hexose monophosphate-oxidation pathway, which were obtained in cell-free extracts of *Escherichia coli*, varied within a narrow range both of levels and of the ratios of one to the other under all conditions tested. This constancy was evident when the activities were calculated as rate of reduction of triphosphopyridine nucleotide/mg. of protein of the extract. If the calculation was for activity per cell there was a variation which depended on the size of the bacterial cells (Scott & Cohen, 1953*b*). The conditions tested included rest or growth, and aerobiosis or anaerobiosis. The medium was synthetic, containing trace salts, ammonium sulphate for a nitrogen source, phosphate and a carbon source. The carbon sources tested were glucose, D-arabinose, potassium gluconate and sodium lactate.

It was also reported that under these same conditions of growth the presence of 2:4-dinitrophenol would increase the extractable activity of the glucose 6-phosphate dehydrogenase as much as sixfold without affecting the level of activity of the phosphogluconate dehydrogenase (Scott, 1956*b*).

The present paper shows that certain changes in the medium involving both the nitrogen and the carbon source can produce changes in the levels of both enzymes and in the ratios between them.

METHODS AND MATERIALS

The bacterial cultures of *E. coli*, strain B, were carried on nutrient-agar slants from which a loopful was inoculated into 10 ml. of salts and glucose medium (Cohen & Arbogast, 1950). This culture was allowed to grow with aeration until very turbid and is called the 'weekly culture'. From it was taken 0.01 ml. for inoculation into 100 ml. of salts and glucose medium or nutrient broth. This culture was allowed to grow with aeration overnight at 37° and constituted the 'overnight culture'. According to the size of the inoculum and the time of incubation the culture had either finished growing and was in a resting or stationary phase, or it was still growing logarithmically. In other

experiments the cultures were grown in towers of 200 ml. capacity, each of which had a Klett tube as side arm and a tube for aeration and was kept in a water bath at 37°.

The salts medium of Cohen & Arbogast (1950) contained $(\text{NH}_4)_2\text{SO}_4$ as a source of nitrogen and glucose (2 mg./ml.) as a source of carbon. Other media were made up as indicated, with the following: nutrient broth, a mixture of Bacto-beef extract and Bacto-peptone (Difco Laboratories, Detroit, Mich., U.S.A.), 8 mg./ml.; casein hydrolysate, an acid hydrolysate of casein (General Biochemicals Inc., Chagrin Falls, Ohio, U.S.A.), 1 mg./ml.; Difco yeast extract, the water-soluble portion of autolysed fresh yeast, 1 mg./ml.; yeast ribonucleic acid (Schwarz Laboratories Inc., New York, N.Y., U.S.A.), 1 mg./ml.

E. coli ribonucleic acid (RNA) was prepared from the bacteria by the method of Kirby (1956) and added to the media at a concentration of 0.16 or 0.13 mg./ml.

The methods used for extraction of the cells and measurement of the enzyme activities were as described previously (Scott & Cohen, 1953*a*). A unit of enzyme activity is defined as that which produces an increase of 1 in extinction at 340 m μ in 1 min. and is caused by the reduction of 0.16 μ mole of triphosphopyridine nucleotide (TPN).

The number of doublings of cell mass undergone by a culture is indicated by $\log_2 T/T_0$, where T is the turbidity at the time under consideration and T_0 is the initial turbidity.

RESULTS

Two experiments with inocula grown in salts and glucose medium which were transferred to nutrient broth are shown in Table 1. The first inoculum was taken from a resting stationary-phase culture and the second from the logarithmic stage of growth. After varying periods of growth in the nutrient broth, some of the culture was harvested and extracted and the enzyme activities were determined. During the first doubling of turbidity, the glucose 6-phosphate dehydrogenase (G 6-PD) activity decreased to approximately half the initial value in units/mg. of protein in the extract. In the same two extracts the phosphogluconate dehydrogenase (PGD) value was unchanged; thus the ratio was halved. Such results may be explained by the formation of new PGD at the same rate as the total cell substance, while there was no or slight new synthesis of G 6-PD.

* Part 5: Scott & Cohen (1957).

Table 1. *Effect of transfer to different media*

Medium	Time (min.)	$\log_2 T/T_0$	Enzyme activities of extracts		
			G 6-PD (units/mg. of protein)	PGD (units/mg. of protein)	G 6-PD/PGD
I. Inoculum-salts and glucose (resting) Nutrient broth	Overnight	—	1.88	0.85	2.22
	75	0.76	1.20	0.86	1.40
	111	1.5	0.84	0.99	0.85
	136	3.6	0.74	0.92	0.80
	243	4.2	0.83	0.90	0.92
II. Inoculum-salts and glucose (log phase) Nutrient broth	Overnight	—	1.88	0.99	1.90
	64	1.72	0.96	0.97	0.99
	120	2.33	0.89	1.24	0.72
	180	3.5	0.94	1.02	0.92
	245	3.6	0.98	1.07	0.92
III. Inoculum-nutrient broth (resting) Salts and glucose	Overnight	—	1.14	1.13	1.0
	245	1.2	1.02	0.84	1.21
	300	2.2	1.35	0.78	1.73
	420	4.0	1.56	0.95	1.64
IV. Inoculum-nutrient broth (resting) <i>a.</i> Nutrient broth (log phase) <i>b.</i> Salts and glucose (from <i>a</i>)	Overnight	—	0.86	0.96	0.90
	120	1.5	0.72	1.0	0.72
	125	1.0	0.95	0.72	1.32

Table 2. *Enzyme activities of extracts of cells grown in various media*

Figures in parentheses indicate the numbers of individual experiments.

Medium	Enzyme activities of extracts		
	G 6-PD (units/mg. of protein)	PGD (units/mg. of protein)	G 6-PD/PGD
Salts and glucose (6) { Mean Range	1.60	0.86	1.90
	1.3-2.0	0.6-1.1	1.5-2.6
Including previous experiments (36) $\pm$ s.d. (Scott, 1956 <i>a</i>) (mean)	1.72 $\pm$ 0.42	0.90 $\pm$ 0.14	1.93 $\pm$ 0.37
Salts and glucose + yeast extract	1.23	0.74	1.65
	1.70	0.63	2.7
	1.22	1.03	1.2
Salts and glucose + yeast RNA	1.60	1.14	1.40
	1.66	0.93	1.72
Salts and glucose + <i>E. coli</i> RNA	1.10	0.61*	1.74
	1.37	0.76	1.81
Salts and glucose + casein hydrolysate	1.51	1.10	1.37
Salts and glucose + casein hydrolysate + <i>E. coli</i> RNA	2.03	0.97	2.10
	1.25	0.76	1.64
	0.58*	0.90	0.65*
Salts + yeast extract	0.89*	0.96	0.92*
	0.95	0.80	1.18*
	0.91*	0.87	1.04*
Salts + casein hydrolysate + yeast extract	0.86*	0.72	1.20*
	1.08	0.61*	1.78
Salts + casein hydrolysate + yeast RNA	0.91*	0.90	1.0*
	0.64-1.19	0.60-1.13	0.7-1.3
Nutrient broth (5) { Mean Range	0.72*	0.70	1.05*
Nutrient broth + <i>E. coli</i> RNA			

* The probability that values for G 6-PD below 0.91 or 0.62 would occur by chance was 5 and 1% respectively. For PGD, 95% of the values would be expected to lie between 1.18 and 0.62.

During the second and third doubling the G 6-PD activity decreased still further but was sufficiently high to indicate the formation of new enzyme. At the same time, the PGD activity increased and in Expt. II reached a level of 1.2 units. Thus the ratios fell from 1.9 or 2.2 to 0.7 and 0.8.

In the reverse experiment (Fig. 1) the inoculum was grown overnight in nutrient broth and was a resting culture. The activity of G 6-PD was 1.14 units, that of PGD 1.13 units and the ratio was 1.00. Salts and glucose medium was inoculated with washed cells. The culture grew very slowly without cell division for 3 hr. and then the cell numbers increased at the same rate as the turbidity in two cultures. The third culture happened to be synchronized for two divisions. During the period of adaptation to growth on the synthetic medium, both enzymes decreased markedly in activity/mg. of protein. Since the decrease was not to half the inoculum value, it seems that by the time the cells had doubled in mass they had synthesized some new enzymes, more of G 6-PD than of PGD, so that the ratio increased. During the next doubling period this relationship between amounts of new enzyme synthesized continued and the ratio rose to 1.7. Thereafter the enzymes were synthesized at a fairly constant ratio, fluctuating slightly at the lower limit, which is usual for cultures grown in synthetic medium and glucose.

An attempt was then made to analyse in more detail the effect of some of the different components of the nutrient broth (Table 2). Nutrient broth is a complex product of animal tissues, the main components of which are peptones and nucleic acids and their products of hydrolysis.

Casein hydrolysate was added to the salts and glucose medium and the enzyme activities were not significantly different from those of bacteria grown in salts and glucose medium. In three experiments when glucose was omitted and the casein hydrolysate was the only source of carbon in the medium, the G 6-PD activity was decreased to 0.58, 0.89 and 0.95; the PGD values were not changed, so the ratios were 0.65–1.2.

Addition of yeast extract, either with the casein hydrolysate or with glucose, did not change the enzyme values appreciably.

Other differences are not significant, although addition of *E. coli* RNA, but not yeast RNA, to media containing glucose tended to give cultures in which PGD activity was at the lower limit of the salts-and-glucose medium range, except for one experiment when casein hydrolysate was also present. In this experiment 0.13 mg./ml. was the concentration of *E. coli* RNA used, about two-thirds of that used in the other experiments and one-tenth of the amount of yeast RNA used. The effect of this low concentration of *E. coli* RNA on

the rate of increase of turbidity had ceased before the cells were harvested for preparation of the extract.

These nutrients all had effect on the growth of the bacteria. Growth in nutrient broth is faster than in salts-and-glucose medium but equal rates could be attained by addition to the salts-and-glucose medium of casein hydrolysate, yeast extract and, even better, of smaller concentrations of the nucleic acid from *E. coli*. Yeast nucleic acid was inhibitory when used alone but not in combination with casein hydrolysate.

Fig. 1. Two bottles containing 100 ml. of nutrient broth were inoculated with 0.01 ml. of a weekly culture suspension of *E. coli* B, and incubated with aeration at 37° overnight. One bottle was harvested. The culture in the other bottle was centrifuged, washed and suspended in salts medium. An inoculum of 4.2 ml. was added to each of three towers containing 174 ml. of salts medium with glucose (2 mg./ml.). The lag period was 120 min., which has been omitted on the graph. Turbidity curves were approximately the same for the three towers: only the curve for tower III is shown (●). Cell counts of towers I, II and III are indicated by ○, △ and □ respectively. Cultures were harvested at the times indicated by the arrows. Enzyme activities of the extracts of the washed pellets are shown in the bar graphs, cross-hatched for PGD and filled in for G 6-PD. The number of doublings of turbidity (measured with a 420 mμ filter in the Klett photometer) at the time of harvest is indicated by $\log_2 T/T_0$.

DISCUSSION

We might interpret these data on an adaptive-enzyme basis, a teleological basis or on feed-back inhibition. The fact that casein hydrolysate produced no decrease in G 6-PD and PGD when glucose was also present with salts medium would indicate that the amino acids do not exercise the role of feed-back control of these dehydrogenases. More experiments, especially kinetic ones, are needed to show whether RNA prepared from *E. coli* can act as feed-back control.

The experiments of Fowler & Cohen (1948) on the effect of the medium on bacteriophage synthesis by *E. coli* B are relevant to the present work. When bacteria which had been grown on nutrient broth were infected in a synthetic medium containing either lactate or glucose the formation of new virus was delayed and the burst size was less than one-tenth of that in nutrient broth. Addition of amino acids and nucleic acid derivatives to the mineral medium allowed a burst size approximately the same as that in broth. The formation of the virus involves synthesis of protein and of DNA at a rate faster than that of the normal bacterial synthesis. On transfer from nutrient broth to mineral medium time is required for the production of the enzymes which synthesize the components previously supplied by the nutrient broth, and virus infection may inhibit this new enzyme production. However, the cell can use preformed amino acids and purines for virus synthesis. Extracts of cells grown in salts-and-glucose medium and infected with virus in the same medium had shown no significant change in the ratio of G 6-PD to PGD (Scott, 1956a). These were cells infected with Tr⁺ strains of bacteriophage, by which early lysis was inhibited.

Re-interpretation of these data from a kinetic viewpoint shows that at 15, 20 and 30 min. after infection with phage there was an increase in both dehydrogenases. After that the enzyme activities usually decreased in the extracts of these infected cells. The decrease of enzyme which was found to occur after 30 min. may well have been due to loss of enzyme into the medium from the injured cells, especially when there had been an attempt to wash them. These data, though not definitive, may indicate an increased synthesis of enzyme in response to the need for more of the components of the virus, or no increase of the G 6-PD and PGD but a loss of other protein from cells which were made more permeable by the virus infection.

There is also the phenomenon of increased formation of G 6-PD in bacteria exposed to dinitrophenol (Scott, 1956a) and some heterocyclic compounds with a nitro group. This may be in response to a need for reduced triphosphopyridine nucleotide, the other product of G 6-PD activity, for reactions involved in detoxication of nitro compounds. On the other hand, supply of glucose to cells which have not been using glucose can also lead to adaptive increases of hexokinase in a glucose-utilizing mutant of *Pseudomonas putrefaciens* (Klein & Doudoroff, 1950), and in *E. coli* of at least one enzyme dealing with glucose 6-phosphate, the dehydrogenase. Adaptive increase of enzymes dealing with galactose and galactosides is well known, even to produce substances which cannot be used in further metabolic reactions (Cohn, 1957).

Perhaps it is possible that there is a time or a function for both mechanisms, adaptive enzyme formation in response to the presence of inducer and adaptive increase in response to need for the products.

SUMMARY

1. The activity of glucose 6-phosphate dehydrogenase in cell-free extracts is decreased during the growth of *Escherichia coli* in medium containing nutrient broth or casein hydrolysate without glucose. The ratios of glucose 6-phosphate dehydrogenase to 6-phosphogluconate dehydrogenase ranged between 0.6 and 1.3.

2. When glucose is present in the medium, the activity of glucose 6-phosphate dehydrogenase is increased relative to 6-phosphogluconate dehydrogenase and the ratios range between 1.4 and 2.7.

This investigation was supported by grant CY 2189 from the National Institute of Health, Public Health Service of the U.S.A.

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