Relaxation of Catabolite Repression in Streptomycin-Dependent *Escherichia coli*

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Acetohydroxy acid synthetase, which is sensitive to catabolite repression in wild-type *Escherichia coli* B, was relatively resistant to this control in a streptomycin-dependent mutant. The streptomycin-dependent mutant was found to be inducible for β -galactosidase in the presence of glucose, although repression of β -galactosidase by glucose occurred under experimental conditions where growth of the streptomycin-dependent mutant was limited. Additional glucose-sensitive enzymes of wild-type *E. coli* B (citrate synthase, fumarase, aconitase and isocitrate dehydrogenase) were found to be insensitive to the carbon source in streptomycin-dependent mutants: these enzymes were formed by streptomycin-dependent *E. coli* B in equivalent quantities when either glucose or glycerol was the carbon source. Two enzymes, glucokinase and glucose 6-phosphate dehydrogenase, that are glucose-insensitive in wild-type *E. coli* B were formed in equivalent quantity on glucose or glycerol in both streptomycin-sensitive and streptomycin-dependent *E. coli* B. The results indicate a general decrease or relaxation of catabolite repression in the streptomycin-dependent mutant. The yield of streptomycin-dependent cells from glucose was one-third less than that of the streptomycin-sensitive strain. We conclude that the decreased efficiency of glucose utilization in streptomycin-dependent *E. coli* B is responsible for the relaxation of catabolite repression in this mutant.

Streptomycin-dependent micro-organisms excrete L-valine during growth. Tirunaryanan, Vischer & Renner (1962) observed valine excretion with several streptomycin-dependent strains of *Escherichia coli* as well as with a strain of *Mycobacterium*. Bragg & Polglase (1962) found that a streptomycin-dependent strain of *E. coli*, growing on glucose-salts medium, excreted 10% of the substrate carbon as L-valine. This apparent regulatory imbalance was correlated with an augmented specific activity of AHA* synthetase in several streptomycin-dependent strains of *E. coli* (Coukell & Polglase, 1965). However, the normal biosynthetic feedback controls of AHA synthetase, i.e. end-product inhibition and repression by biosynthetic end products, were not significantly different in streptomycin-dependent and streptomycin-sensitive cultures (Polglase, 1966). Coukell & Polglase (1969) found that AHA synthetase was sensitive to catabolite repression in wild-type *E. coli* B. Therefore in the present work we investigated catabolite repression in streptomycin-dependent *E. coli*. The results indicate that there is a general decrease or relaxation of this type of regulation in streptomycin-dependent mutants,

and this is correlated with decreased efficiency in the utilization of glucose.

METHODS AND MATERIALS

Organisms. Two wild-type (streptomycin-sensitive) strains of *E. coli* were used, *E. coli* B (A.T.C.C. 11303) and *E. coli* E, the latter obtained from the Department of Bacteriology, University of Laval, Montreal, Que., Canada. The corresponding streptomycin-dependent mutants were isolated as described by Coukell & Polglase (1965). In the present paper, the strains are designated SB and SE (streptomycin-sensitive) and DB and DE (streptomycin-dependent).

Culture conditions. All cultures were grown on a minimal medium based on that described by Davis & Mingioli (1950) with the omission of citrate. The carbon source (glucose, glycerol or lactose) was autoclaved separately in a concentrated solution and was added as indicated in each experiment. Streptomycin-dependent cultures were grown on minimal medium supplemented with 1 mg. of dihydrostreptomycin/ml. (Merck, Sharp and Dohme, Montreal, Que., Canada). For cultures that were to be grown overnight without agitation, the pH of this medium was adjusted to 7.6 by the addition of NaOH solution.

The methods used for the growth of cultures and for the preparation of the crude ultrasonic extracts were described by Coukell & Polglase (1969). Growth was followed by

* Abbreviation: AHA, acetohydroxy acid.

measurement of E_{420} in a 1.0 cm.-light-path cuvette with a Beckman B spectrophotometer. Under these conditions, extinction was found to be directly proportional to total cellular protein and to cell mass (dry wt.) up to an extinction of 1.0.

Chemical analyses. Glucose was determined by the commercial preparation Glucostat (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) as follows: to portions (0.5 ml.; containing 0–300 μ g. of glucose/ml.) were added 2.5 ml. of 0.1M-potassium phosphate buffer, pH 7.0, and 2.0 ml. of the Glucostat reagent. This mixture was incubated at 37° until the reaction had reached completion (30 min.). Two drops of 6N-HCl were added to each tube, followed by 5.0 ml. of water; E_{420} was measured and converted by a standard curve into μ g. of glucose.

Protein was determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951) with bovine γ -globulin (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.) as a standard.

Enzyme assays. β -Galactosidase was determined in samples (1.0 ml.) of culture by the method of Pardee, Jacob & Monod (1959). Toluene (1 drop) and β -mercaptoethanol (final concentration 15 mM) were added to the cell suspensions. The suspensions were lysed by agitation on a Vortex mixer for 30 sec., followed by incubation at 37° for 30 min. One unit of enzyme is defined as the amount of enzyme that catalyses the hydrolysis of 1 μ mole of *o*-nitrophenyl β -D-galactopyranoside/min. at 28° and pH 7.0.

All other enzyme activities were determined on the crude ultrasonic extracts. Extracts not assayed the day they were prepared were stored at –20° and assayed within 48 hr. AHA synthetase activity was measured as described by Coukell & Polglase (1969). One unit of activity is defined as the formation of 1 μ mole of acetoin/hr. at 37°.

The condensing enzyme citrate synthase was determined spectrophotometrically by measuring the change in E_{233} (Srere & Kosicki, 1961). Each assay contained in a volume of 1.0 ml.: tris-HCl buffer, pH 8.0, 0.10 M-mole; oxaloacetate, 0.40 μ mole; acetyl-CoA, 0.16 μ mole. The reaction was initiated by the addition of crude extract (25–35 μ g. of protein). The change in E_{233} was recorded continuously against a blank containing all the ingredients except extract. At this wavelength (233 m μ) the non-specific absorption is high, and greater accuracy can be obtained by using a cuvette with a relatively short light-path (0.2–0.5 cm.). The decrease in E_{233} is due both to the cleavage of the thiol ester bond and to the utilization of oxaloacetic acid. The combined molar extinction coefficient of 5400 determined by Srere & Kosicki (1961) was used in this work. A unit of enzyme activity is defined as the formation of 1 μ mole of citrate/min.

Fumarase and aconitase activities were determined spectrophotometrically at 240 m μ as described by Hanson & Cox (1967). Aconitase was determined only on fresh undiluted extracts, since this enzyme loses activity on storage or dilution. A unit of activity for each enzyme is defined as an extinction change of 0.001/min.

Isocitrate dehydrogenase was determined by measuring at 340 m μ the rate of reduction by isocitrate of NADP⁺. Each reaction mixture contained, in a volume of 1.0 ml.: tris-HCl buffer, pH 7.5, 50 μ moles; MgCl₂, 10 μ moles; sodium DL-isocitrate, 10 μ moles; NADP⁺, 0.3 μ mole; extract (50–70 μ g. of protein). A unit of activity is defined as the formation of 1 μ mole of NADPH/min.

The reaction mixture used for determining glucose 6-phosphate dehydrogenase contained, in 1.0 ml.: glucose 6-phosphate, 5 μ moles; MgCl₂, 10 μ moles; NADP⁺, 0.14 μ mole; glycylglycine buffer, pH 7.5, 50 μ moles; crude extract (0.25–0.35 mg. of protein). The change in E_{340} was recorded as a function of time. A unit of activity is defined as the formation of 1 μ mole of NADPH/min.

Glucokinase activity was determined by an assay described by Coukell & Polglase (1965). One glucokinase unit is defined as that amount of enzyme that forms 1 μ mole of NADPH from NADP⁺/min.

All spectrophotometric determinations were made with a Cary 15 recording spectrophotometer at 25°. Specific activities were calculated as units of enzyme/mg. of protein.

Diauxic experiments. The cells were grown on a shaker overnight (16 hr.) at 37° in minimal medium supplemented with 0.2% of glucose. To remove the residual constituents of the medium the cultures were harvested by centrifugation and resuspended in one-half of the original culture volume of 0.1M-potassium phosphate buffer, pH 7.4, at 37°. The suspensions were again centrifuged and the washed cells were resuspended in fresh medium to give E_{420} approx. 0.1. After the addition of glucose (0.025%) and lactose (0.20%) the cultures were vigorously aerated by means of a sparger during agitation in a Gilson (Omni) shaking water bath at 37°. The extinction of the culture was followed during growth, and at intervals samples (1.0 ml.) of culture were removed for determination of β -galactosidase activity. Growth was generally expressed as μ g. of total protein/ml. of culture. Preliminary experiments established that, under these conditions, the glucose was consumed when the protein concentration reached 85–95 μ g./ml. (see Figs. 2a and 2b).

β -Galactosidase induction in pseudo-resting cells. Owing to the extreme sensitivity of β -galactosidase induction to catabolite repression, the effects of different carbon sources on induction could not be evaluated reproducibly in cells starved of nitrogen. To overcome this problem, a 'pseudo-resting' cell system was devised in which both a carbon source and a nitrogen source were added to cells previously starved for both of these compounds.

Cultures grown aerobically on glycerol (0.2%) at 37° were harvested by centrifugation at an extinction of 1.0 and were washed once in 0.1M-potassium phosphate buffer, pH 7.4. The washed cells were resuspended in buffer of the same composition and were shaken vigorously at 37° for 30 min. At the end of this incubation period, the extinction of the culture was adjusted to 1.0 with buffer and the cells were transferred to a 30° water bath. After 5 min., a carbon source (glycerol, 0.05%), a nitrogen source (ammonium sulphate, 0.1%) and the inducer lactose (0.2%) were added, and β -galactosidase formation was followed during a 2 hr. induction period. Cell suspensions treated in this manner showed less than 15% increase in extinction during the 2 hr. induction period, and were referred to as 'pseudo-resting' cells.

RESULTS

Relaxation of the 'glucose effect' on AHA synthetase in streptomycin-dependent *E. coli* B. Coukell & Polglase (1969) reported that the biosynthetic acetolactate-forming system of wild-type *E. coli* B is repressed by growth on a rapidly metabolizable carbon source such as glucose. The results of a

similar experiment with the streptomycin-dependent mutant of *E. coli* B are shown in Fig. 1. After a slight decrease in specific activity at glucose or glycerol concentrations greater than 0.05% the activity remained constant during a tenfold increase in carbon-source concentration. Unlike the AHA synthetase of the wild-type organism (Coukell &

Polglase, 1969) the specific activity of this enzyme in the streptomycin-dependent mutant was not significantly altered by changing the carbon source from glucose to glycerol (Fig. 1). These results suggest that in the streptomycin-dependent mutant, in contrast with the wild-type, there is at least partial relaxation of repression by the carbon source of AHA synthetase.

Modification of diauxic growth in streptomycin-dependent mutants. For a more detailed investigation of the mechanism of relaxation of catabolite repression in the streptomycin-dependent mutants, the β -galactosidase system was chosen as the experimental tool (Jacob & Monod, 1961).

The classical experiment that demonstrates the 'glucose effect' (Epps & Gale, 1942) is the occurrence of a biphasic growth curve in a bacterial culture growing on a mixture of glucose and lactose. This biphasic growth pattern was named the 'diauxic effect' by Monod (1947). Wild-type *E. coli* B (Fig. 2a) exhibits a classical diauxic curve owing to the complete repression of β -galactosidase during growth on glucose. The corresponding streptomycin-dependent culture (Fig. 2b), however, showed only a slight diauxic effect. The partial elimination of diauxic growth in the streptomycin-dependent strain appeared to result from initiation of induction of β -galactosidase at least 1½ hr. before the exhaustion of glucose (3½ hr.). Varying the lactose:glucose ratio did not prevent early induction or restore typical diauxic growth.

To confirm the differences in the diauxic response of streptomycin-sensitive and streptomycin-dependent organisms, another strain of *E. coli* was examined. The growth curves are given in Fig. 3 for wild-type *E. coli* E (strain SE) and the streptomycin-dependent derivative of this (strain DE),

Fig. 1. Effect of increasing the initial concentration of the carbon source on the formation of AHA synthetase in streptomycin-dependent *E. coli* strain DB. The minimal salts medium was supplemented with either glucose (●) or glycerol (○). The results of an identical experiment (----) with the wild-type parent of this mutant, grown on glucose, were taken from the preceding paper (Coukell & Polglase, 1969; Table 1). Specific activity is expressed as μ moles of acetoin formed/mg. of protein/hr. at 37°.

Fig. 2. Growth (○) and β -galactosidase induction (●) in streptomycin-sensitive (strain SB) and streptomycin-dependent (strain DB) *E. coli* B on glucose-lactose-salts medium, showing differences between the mutants in the diauxic effect. (a) Strain SB; (b) strain DB.

Fig. 3. Growth ($\circ$) and β -galactosidase induction ($\bullet$) in streptomycin-sensitive (strain SE) and streptomycin-dependent (strain DE) *E. coli* E on glucose-lactose-salts medium, showing differences between the mutants in the diauxic effect. (a) Strain SE; (b) strain DE.

Fig. 4. Difference between streptomycin-sensitive (strain SB) and streptomycin-dependent (strain DB) *E. coli* B in the effect of glucose on β -galactosidase induction. Cells were induced with lactose (0.2%) while growing exponentially on glycerol-salts (0.1%) medium ($\circ$). Glucose (0.05%) was added, as indicated by the arrows ($\bullet$). (a) Strain SB; (b) strain DB.

grown under identical conditions. Strain SE, like strain SB, exhibited diauxic growth (Fig. 3a), whereas the streptomycin-dependent mutant DE (Fig. 3b) did not even show the slight diauxic effect obtained with mutant DB. Thus growth on glucose completely failed to repress β -galactosidase induction in strain DE. This was confirmed by an examination of the induction curve (Fig. 3b).

Differences between streptomycin-sensitive and streptomycin-dependent E. coli in the 'glucose effect' on β -galactosidase synthesis. A more direct approach to the measurement of catabolite repression is to

study the effect of various carbon sources on the differential rate of enzyme synthesis (Cohn & Horibata, 1959). Fig. 4(a) shows the lactose-induced formation of β -galactosidase in cultures of strain SB growing exponentially on glycerol, a carbon source that exerts little catabolite repression owing to its relatively low rate of metabolism (Magasanik, Magasanik & Neidhardt, 1959). The concentration of enzyme/ml. is plotted as a function of total cellular protein (Fig. 4a). The addition of glucose (final concentration 0.05%) resulted in a rapid decrease in the differential rate of β -galactosidase synthesis.

Fig. 5. Difference between streptomycin-sensitive (strain SE) and streptomycin-dependent (strain DE) *E. coli* E in the effect of glucose on β -galactosidase induction. Cells were induced with lactose (0.2%) while growing exponentially on glycerol-salts (0.1%) medium (○). Glucose (0.05%) was added as indicated by the arrows (●). (a) Strain SE; (b) strain DE.

In this strain, the rate of enzyme synthesis on glucose was approximately 70% less than the rate on the glycerol control.

The results of an identical experiment with strain DB are given in Fig. 4(b). In this case, the addition of glucose evoked a sharp but short-lived repression of β -galactosidase synthesis, which rapidly gave way to a differential rate only 15% less than that of the control.

Experiments identical with those described for strains SB and DB were carried out with strains SE and DE. Addition of glucose to cultures of strain SE growing exponentially on glycerol caused an initial sharp repression of enzyme that was transient in this strain and gave way to a steady-state rate about 40% less than the rate observed on glycerol alone (Fig. 5a). In contrast, addition of glucose to strain DE had no effect on the rate of β -galactosidase synthesis (Fig. 5b).

Both streptomycin-dependent strains thus showed resistance towards catabolite repression compared with the parent streptomycin-sensitive strains. However, there were quantitative differences between the two dependent strains both in diauxic growth (Figs. 2b and 3b respectively) and in β -galactosidase induction (Figs. 4b and 5b respectively). Thus strain DE appeared completely resistant to catabolite repression, but strain DB was somewhat less resistant to this effect.

β -Galactosidase induction in pseudo-resting cells. The question now may be asked: do streptomycin-

dependent mutants lack the mechanism for the formation of the catabolite co-repressor, as suggested by the results with strain DE, or is the co-repressor synthesized at a rate insufficient to cause repression, the situation indicated by results with strain DB? To answer this question, β -galactosidase induction was examined in 'pseudo-resting' cell suspensions of strains SE and DE prepared as described in the Methods and Materials section. These experimental conditions resulted in a limitation of anabolism without limiting to the same degree the corresponding catabolic processes, thus permitting an accumulation of co-repressor molecules (Mandelstam, 1961). Results of a typical experiment are presented in Fig. 6. When glucose at a final concentration of 0.075% was added to cultures of strain SE or strain DE after 90 min. of lactose-induced synthesis of β -galactosidase, enzyme synthesis was rapidly inhibited in both cultures. Hence the mechanism for formation of co-repressor was operative in streptomycin-dependent mutants, but, under the conditions of exponential growth, apparently the rate of formation of co-repressor in strain DE was insufficient to cause a 'glucose effect'. Only when conditions were modified so as to favour the accumulation of high concentrations of co-repressor was catabolite repression demonstrable in this streptomycin-dependent organism.

In addition to lactose, the gratuitous inducers, methyl β -D-thiogalactopyranoside and isopropyl β -D-thiogalactopyranoside were used as inducers of

Fig. 6. Effect of the addition of glucose on β -galactosidase induction in 'pseudo-resting' cultures of *E. coli*. (a) Strain SE; (b) strain DE. Experimental conditions are described in the Methods and Materials section. Glucose was added as indicated by the arrows, to final concentration 0.075%. O, Glycerol control; ●, glucose added.

β -galactosidase in many of these studies. Results with methyl β -D-thiogalactopyranoside generally agreed with those obtained with lactose. Isopropyl β -D-thiogalactopyranoside, however, was unsatisfactory in this study; β -galactosidase induction by this powerful inducer (Jacob & Monod, 1961; Moses & Prevost, 1966) was completely resistant to repression by glucose under the conditions used.

Relaxation of catabolite repression in pathways of carbohydrate metabolism. If the de-repression of AHA synthetase in the streptomycin-dependent mutant (Fig. 1) was the result of a general decrease in the concentration of catabolite co-repressor in the cells, then other enzymes subject to repression by carbon source should also reflect this loss of control. Many of the enzymes of intermediary metabolism are known to be sensitive to catabolite repression (Magasanik, 1961; Gorini & Gundersen, 1961; Hanson & Cox, 1967). For example, the activities of the Krebs-cycle enzymes are lower in micro-organisms grown on a salts medium containing glucose than in cells grown on a poor carbon source such as glycerol or lactate (Hanson & Cox, 1967; Gray, Wimpenny & Mossman, 1966). To determine whether or not the apparent decrease in concentration of catabolite co-repressor in streptomycin-dependent mutants influences the control of other enzymes, the specific activities of several Krebs-cycle enzymes and certain other enzymes involved in carbohydrate metabolism were determined in crude extracts of strains SB and DB grown on glucose and glycerol. The results are

summarized in Table 1. Examination of the results for the wild-type organism (strain SB) indicates that the four Krebs-cycle enzymes investigated, as well as AHA synthetase, exhibit a 'glucose effect' as demonstrated by (specific activity with glycerol)/(specific activity with glucose) ratios greater than unity (Table 1, column *b/a*): this effect was particularly marked for fumarase and aconitase. In the corresponding streptomycin-dependent mutant, the repression of glucose-sensitive enzymes observed in the wild-type organism was absent (Table 1, column *d/c*), permitting many of the enzymes to reach specific activities equal to or even exceeding those obtained by growth of the wild-type parent strain on glycerol. The relationship between carbon source and streptomycin phenotype with respect to catabolite repression is summarized by the ratios of the glucose-sensitive enzymes in strain DB to those in strain SB (Table 1). After growth of cells on glucose, the ratios for all five enzymes exceeded 2 (Table 1, column *c/a*), and for fumarase and aconitase ratios greater than 6 were observed. In extracts of cells grown on glycerol, the ratios of most enzymes approached unity (Table 1, column *d/b*), except the ratio for aconitase, which was relatively high.

The specific activities of two other enzymes, glucose 6-phosphate dehydrogenase and glucokinase, were essentially the same in the streptomycin-sensitive and the streptomycin-dependent organisms, and were not affected by changing the carbon source (glucose or glycerol) (Table 1). These

Table 1. *Specific activities of enzymes from wild-type E. coli strain SB and streptomycin-dependent E. coli strain DB grown on glucose or glycerol*

Glucose and glycerol, when present, were added at an initial concentration of 0.4%. Enzymes were assayed in ultrasonic extracts of cells harvested at E_{420} 0.8. Specific activities are mean values obtained from two or more separate experiments.

Strain	Carbon source	Enzyme	Specific activity (units/mg. of protein)				Ratios			
			SB		DB		b/a	d/c	c/a	d/b
			Glucose (a)	Glycerol (b)	Glucose (c)	Glycerol (d)				
AHA synthetase			4.42	9.32	9.64	9.52	2.11	0.99	2.18	1.02
Citrate synthase			172	440	493	349	2.56	0.71	2.87	0.80
Fumarase			396	2070	2545	1885	5.23	0.74	6.42	0.91
Aconitase			111	460	789	1317	4.14	1.67	7.10	2.86
Isocitrate dehydrogenase			365	629	880	943	1.73	1.07	2.41	1.49
Glucose 6-phosphate dehydrogenase			130	109	137	137	0.85	1.00	1.05	1.25
Glucokinase			55.4	68.9	67.0	64.2	1.22	0.96	1.20	0.93

Fig. 7. Yield of cells from glucose during growth of streptomycin-sensitive (strain SB) and streptomycin-dependent (strain DB) strains of *E. coli* B. Exponential cells were washed and resuspended in minimal salts medium containing 400 µg. of glucose/ml. During aerobic growth, samples of the culture were removed, rapidly cooled and centrifuged at 16000g for 10 min., and the supernatant solutions were assayed by the Glucostat method. ●, Strain SB; ○, strain DB.

growth on limiting glucose was recorded for streptomycin-sensitive *E. coli* B and for the streptomycin-dependent mutant (Fig. 7). The yield of cells was approximately 35% lower for the streptomycin-dependent strain. This observation of decreased efficiency of carbohydrate metabolism in the streptomycin-dependent mutant was confirmed in several similar experiments.

DISCUSSION

The first indication of altered regulation in streptomycin-dependent micro-organisms was the observation (Tirunaryanan *et al.* 1962; Bragg & Polglase, 1962) that L-valine was an extracellular product of these mutants. Subsequent work (Coukell & Polglase, 1965; Desai & Polglase, 1965) showed that the regulatory enzyme for valine biosynthesis, AHA synthetase, was de-repressed in streptomycin-dependent *E. coli* even though end-product control could be demonstrated in these organisms (Polglase, 1966). The finding (Coukell & Polglase, 1969) that AHA synthetase can be repressed by growth on glucose prompted the present investigation of catabolite repression in streptomycin-dependent *E. coli*. The results with AHA synthetase (Fig. 1) indicate that glucose repression of this enzyme was relaxed in streptomycin-dependent *E. coli* B. Studies on catabolite repression of the inducible β -galactosidase in *E. coli* B and in *E. coli* E showed that control of this enzyme by the carbon source also was relaxed in streptomycin-dependent mutants. Both the classical diauxic growth pattern (Figs. 2 and 3) on glucose-lactose-salts medium and the response of the differential rate of β -galactosidase synthesis to the addition of glucose (Figs. 4 and 5) were modified in streptomycin-dependent strains in such a way as to

results indicate that only those enzymes that respond to changes in the concentration of some general catabolite co-repressor are elevated in streptomycin-dependent *E. coli*.

Differences between streptomycin-sensitive and streptomycin-dependent *E. coli* B in cell yield from glucose. The weight of cells obtained during aerobic

Table 2. *Growth rates of E. coli strains*

All cultures were grown on the basal salts medium supplemented with a carbon source at an initial concentration of 0.4%. Growth rate is expressed as the doubling time in minutes.

Strain	Carbon source	Doubling time (min.)
SB (streptomycin-sensitive)	Glucose	56
	Glycerol	60
DB (streptomycin-dependent)	Glucose	85
	Glycerol	102
SE (streptomycin-sensitive)	Glucose	54
	Glycerol	66
DE (streptomycin-dependent)	Glucose	85
	Glycerol	110

indicate a diminished 'glucose effect'. However, β -galactosidase induction in streptomycin-dependent mutants was sensitive to glucose repression under conditions where the anabolic rate was limited (Figs. 6a and 6b), thus indicating that the mechanism concerned with catabolite co-repressor synthesis is operative in the streptomycin-dependent mutant. In addition, certain enzymes of the Krebs cycle that were sensitive to catabolite repression in wild-type *E. coli* B were de-repressed in the streptomycin-dependent mutant (Table 1).

Any mutant that has lost its sensitivity to glucose for a number of glucose-sensitive enzymes should have either (1) an impaired glucose dissimilation or (2) an improved overall anabolic capacity (Neidhardt, 1960). Since the growth rate of streptomycin-dependent *E. coli* B on glucose-salts medium was lower than that of the wild-type strain (Table 2), the decreased catabolite repression observed in the streptomycin-dependent mutant cannot be attributed to a higher anabolic rate. On the other hand, the efficiency of glucose utilization in streptomycin-dependent *E. coli* B was only two-thirds that of the parent strain (Fig. 7). This observation provides an explanation for the decreased catabolite repression in streptomycin-dependent *E. coli* B. The many observations referred to above that indicate loss of regulatory control in streptomycin-dependent *E. coli* appear to be explicable solely on the basis of impaired carbohydrate metabolism in this mutant.

Goodman & Spotts (1967) reported that both the inducible and the constitutive synthesis of β -galactosidase in cultures of streptomycin-dependent *E. coli* were inhibited by streptomycin deprivation. They attributed part of this inhibition to an increase in catabolite repression in these deprived cells, which in turn may have resulted from an alteration in the structure of the ribosomes sufficient to impair normal protein synthesis. Related studies in our Laboratory on several glucose-

sensitive enzymes in streptomycin-starved cultures of streptomycin-dependent *E. coli* B support their conclusions (M. B. Coukell & W. J. Polglase, unpublished work). Thus there are two distinct and contrasting observations on streptomycin-dependent *E. coli* to be understood: first, the impaired carbohydrate metabolism, which decreases catabolite repression; secondly, the effect of streptomycin deprivation, which increases catabolite repression. The latter observation can be understood in terms of a decreased rate of protein synthesis resulting from impaired ribosomal function, but the former observation can be understood only if one postulates a second mutation resulting in a decreased efficiency in energy metabolism. Alternatively, the presence of streptomycin, although permitting maximal anabolic activity in the streptomycin-dependent cells, may simultaneously interfere with carbohydrate metabolism.

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