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Altered End-Product Patterns and Catabolite Repression in *Escherichia coli*[1]

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ABSTRACT

DOBROGOSZ, WALTER J. (North Carolina State University, Raleigh). Altered end-product patterns and catabolite repression in *Escherichia coli*. J. Bacteriol. 91:2263–2269. 1966.—End products formed during growth of *Escherichia coli* ML30 on glucose were examined under various conditions known to promote or prevent catabolite repression of the inducible β-galactosidase system in this organism. Cultures were grown under these conditions in the presence of C^{14}-glucose or C^{14}-pyruvate. The products formed were assayed isotopically after separation on columns of silicic acid. Under conditions known to promote catabolite repression, glucose was degraded primarily to acetate and CO_2. When repression was turned off by anaerobic shock, glucose metabolism was characterized by the accumulation of ethyl alcohol in addition to acetate and CO_2. The results presented in this report indicate that oxidative decarboxylation of pyruvate may markedly affect the amount of energy that can be derived from glucose catabolism. In turn, the amount of energy derived from catabolic processes may play a key role in the mechanism of catabolite repression.

Under aerobic conditions, growth of *Escherichia coli* on glucose results in catabolite repression of inducible or constitutive β-galactosidase formation. Cohn and Horibata (2) found that this repression by glucose could be temporarily eliminated if aerobically growing cultures were made anaerobic, i.e., subjected to an anaerobic shock. Conversely, catabolite repression is resumed when nonrepressed, anaerobically growing cultures are exposed to air. Thus, by appropriate transitions from an aerobic environment to an anaerobic environment, or vice versa, catabolite repression by glucose can be readily "switched on" or "switched off." More recently, it was found that repression in *E. coli* obtained under aerobic conditions is not "switched off" by anaerobic shock if nitrate is included in the growth medium (4). If nitrate is added to anaerobic cultures after repression is "switched off" by anaerobic shock, catabolite repression is again resumed.

These findings indicate that catabolite repression of β-galactosidase formation in this organism is dependent in some manner on certain metabolic oxidation-reduction reactions. It is contended that the mechanism responsible for catabolite repression will become more understandable if the role played by these reactions is clarified. The ability to turn catabolite repression "on" or "off" by the varied manipulations described above has provided suitable test systems for obtaining information along these lines. In this regard, the following question has been asked. What alterations in glucose metabolism occur during the various "repression on" and "repression off" conditions, and how are these changes involved in the mechanism of catabolite repression of β-galactosidase formation in *E. coli*? The present report is the first in a series of studies designed to answer this question. Alterations in glucose metabolism under the varied conditions that produced ("on") or prevented ("off") repression were studied as reflected by concomitant changes in end-product patterns.

MATERIALS AND METHODS

Organism and growth conditions. E. coli ML30, inducible for β-galactosidase, was used throughout this study. The culture was obtained through the courtesy of J. L. Ingraham (University of California, Davis). The growth medium consisted of the following (grams per liter): K_2HPO_4, 28.0; KH_2PO_4, 8.0;

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$MgSO_4 \cdot 7H_2O$, 0.10; $(NH_4)_2SO_4$. 1.0; *p*H, 7.2. In all cases, 0.25% casein hydrolysate (acid-hydrolyzed, vitamin-free; Nutritional Biochemicals Corp., Cleveland, Ohio) was added to the medium prior to incubation. By incorporating a high level of phosphate buffer in the medium, the *p*H did not drop below 6.8 under the conditions employed.

Other conditions concerning growth of the organism, induction of β-galactosidase with 2.5×10^{-3} M isopropyl-β-D-thiogalactoside (IPTG), or assay of β-galactosidase activity by use of *o*-nitrophenyl-β-D-galactoside (ONPG) as substrate and toluenized cells as enzyme source were as previously described (4). The differential rates of β-galactosidase formation were calculated from plots relating units of enzyme activity per milliliter of culture versus the micrograms (dry weigh) per milliliter of culture. The slope of this plot is expressed as the *P* value (2, 9). One unit of β-galactosidase activity is defined as that amount of enzyme that hydrolyzes 1 μmole of ONPG per hr at 30 C in the presence of 2×10^{-3} M substrate, 1.24×10^{-4} M glutathione (reduced), and 0.05 M sodium phosphate buffer, *p*H 7.5.

Determination of end products. Nongaseous end products of glucose or pyruvate metabolism were determined by column chromatography on silicic acid with various concentrations of *t*-butanol in chloroform as solvents. The technique used for preparation and packing of the silicic acid and for preparation of solvents was essentially that of Ramsey (10). Samples (1 ml) were mixed into 1.8 g of dry silicic acid, quantitively transferred to the top of prepared columns, and layered with 5 ml of benzene. The columns were developed by elution with 50 ml of chloroform, followed by 600 ml of a solvent gradient consisting of 300 ml of chloroform in the mixing chamber and 300 ml of 5% *t*-butanol in chloroform in the addition chamber of the gradient apparatus. After gradient elution was completed, an additional 50 ml of 5% *t*-butanol in chloroform was added, followed by 100 ml of 10% *t*-butanol in chloroform. All solvents were passed through the columns at a rate of 2 ml/min. To maintain this flow rate, a solution metering pump (Beckman Instruments, Inc., Fullerton, Calif.) was operated between the solvent chambers and the columns. Fractions (10 ml) were collected with the use of a siphon assembly attachment to an automatic fraction collector.

Sample preparation. Cultures were grown aerobically overnight, harvested, washed, and inoculated into fresh medium containing unlabeled substrates. Aerobic incubation was then continued for 2 hr. At the end of this period, the exponentially growing cultures contained 40 to 100 μg (dry weight) per ml. C^{14}-labeled substrates plus other additions were then added as indicated in the text, and incubation continued under either aerobic or anaerobic conditions for not more than 3 hr. At the end of the incubation period, 5 ml of culture was added to 5 ml of a mixture of cold carrier compounds containing sufficient H_2SO_4 for a final *p*H of 1.5 to 1.8. This step served to stop any further reactions in addition to providing cold carrier materials. The cold carrier mixture contained the following components dissolved in growth medium: ethyl alcohol, acetic acid, pyruvic acid, formic acid, fumaric acid, lactic acid, succinic acid, and citric acid. All concentrations were 0.1 M, except fumaric acid which was 0.05 M. The cold carrier-culture mixture was centrifuged at 10,000 × *g* for 10 min, and the supernatant fraction was frozen until used.

Known samples of C^{14}-labeled ethyl alcohol, acetate, pyruvate, formate, lactate, and succinate were treated in the above manner to determine the recovery of each compound from the columns, and to determine their elution position. In all cases, 90 to 100% of label was recovered, and consistent elution profiles were obtained.

Sample counting. All samples were counted with the use of a Packard Tri-Carb liquid scintillation counter (Packard Instruments Co., La Grange, Ill.). Samples (1 ml) of column fraction were mixed with 1 ml of *t*-butanol in counting vials, followed by 15 ml of scintillation fluid [0.4% PPO (2,5-diphenyloxazole) and 0.01% POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene) in toluene]. Corrections were made for activity quenching by chloroform. When aqueous samples were counted, samples of no more than 0.10 ml were dissolved in 2 ml of absolute ethyl alcohol before the addition of scintillation fluid.

In some experiments, radioactivity released as $C^{14}O_2$ was determined. In these cases, cultures were grown in the usual manner but in radiorespirometer vessels (14), and the evolved $C^{14}O_2$ was trapped in alcoholic solutions of hyamine or ethanolamine.

Assimilated radioactivity was determined by collection of cells on membrane filters (Bac-T-Flex, type B-6, Schleicher & Schuell Co., Keene, N.H.). After being washed and dried, the filters were placed in scintillation fluid and counted as described above.

Miscellaneous. Glucose was determined enzymatically with the Glucostat reagents obtained from Calbiochem.

Chemicals. Glucose-C^{14} (uniformly labeled), sodium pyruvate-*3*-C^{14}, ethyl alcohol-*1*-C^{14}, sodium acetate-*2*-C^{14}, sodium formate-C^{14}, sodium lactate-*1*-C^{14}, and succinic acid-*2,3*-C^{14} were purchased from New England Nuclear Corp. (Boston, Mass.). The silicic acid was a grade suitable for chromatography, and was purchased from Mallinckrodt Chemicals (St. Louis, Mo.).

RESULTS

As shown in Fig. 1, a repressed rate ($P = 0.20$) of β-galactosidase formation results when *E. coli* is grown aerobically on glucose (curve 1). If the culture is subjected to anaerobic shock (curve 2), β-galactosidase is formed at a nonrepressed rate ($P = 0.66$) for 90 to 120 min (approximately 1.5 generations of growth). Thereafter, catabolite repression again commences. The nonrepressed rate is identical to the rate observed when cultures are grown in a minimal medium on substrates such as succinate or α-ketoglutarate (4). If pyruvate is the substrate (curve 5) in these experiments, a nonrepressed rate of enzyme

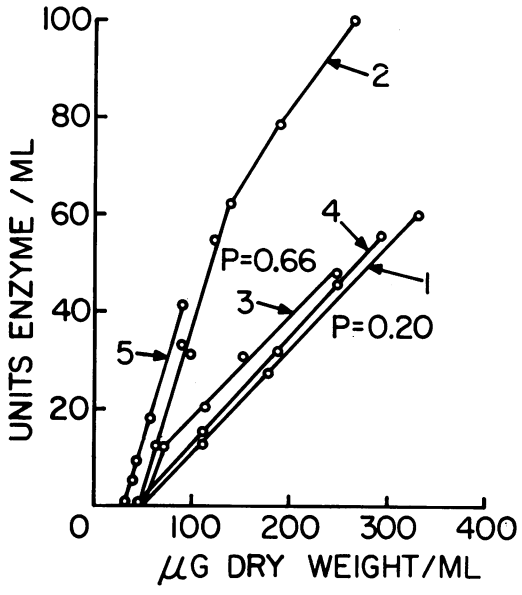

FIG. 1. *Effect of anaerobic shock, nitrate, and pyruvate on catabolite repression by glucose. To cultures growing exponentially under aerobic conditions with 0.04 M pyruvate (curve 5) or 0.02 M glucose (curves 1, 2, 3, and 4), 2.5 × 10^{-3} M IPTG was added at the beginning of the experiments shown above. In each case, the differential rate of β-galactosidase formation was measured after inducer addition. The subsequent conditions of incubation were as follows: curve 1, glucose culture grown under continued aerobic conditions; curve 2, glucose culture made anaerobic at time of IPTG addition [50 μg (dry weight) per ml]; curve 3, glucose culture made anaerobic with 8 × 10^{-3} M nitrate added; curve 4, glucose culture made anaerobic with 0.04 M pyruvate added; curve 5, pyruvate culture made anaerobic.*

formation is observed during anaerobic growth. It had been established previously that addition of nitrate (curve 3) or nitrite to cultures grown on glucose prevented the anaerobically induced reversal of catabolite repression (4). It was found later that addition of pyruvate to anaerobic cultures growing on glucose also prevented reversal of repression by anaerobic shock (curve 4). Catabolite repression by glucose can thus be easily "switched off" by anaerobic shock, or "switched on" by aerobic shock. Inclusion of nitrate or pyruvate to anaerobically shocked cultures (repression "off") results in repression being "switched on."

To determine the mechanism responsible for these changes in catabolite repression by glucose, it was necessary to determine the concomitant changes in glucose metabolism that occur under these varied conditions. The first approach employed in this regard was to determine what changes in distribution of end products resulted from these changes in glucose metabolism. For this purpose, a rapid but specific method for determining *E. coli* end products was needed. This was provided by appropriate modification of the silicic acid column chromatographic system of Ramsey (10). Nongaseous C^{14}-labeled end products accumulating during growth on C^{14}-labeled substrates were detected isotopically after appropriate elution from columns of silicic acid. The suitability of this procedure was determined by a series of inventory studies, wherein added and recovered C^{14} was accounted for in all culture fractions as shown in Table 1. All the C^{14}-labeled glucose added to these cultures could be satisfactorily accounted for in the cell, CO_2, or supernatant fractions. When activity in the supernatant fraction was corrected for unused glucose, the activity remaining was entirely accounted for as products detected through silicic acid chromatography.

Figure 2 shows the end-product patterns resulting from growth of *E. coli* on uniformly labeled C^{14}-glucose under aerobic conditions (repression "on"), anaerobic conditions (repression "off"), anaerobic conditions with nitrate added (repres-

TABLE 1. *Distribution and recovery of C^{14} after growth of Escherichia coli ML30 on uniformly labeled glucose-C^{14}*

Distribution of C^{14}	Growth conditions*		
	Anaerobic	Anaerobic plus 0.04 M pyruvate	Anaerobic plus 8 × 10^{-3} M nitrate
In culture fraction			
Total added	30.8†	30.8	30.8
Cell fraction	2.3	2.4	3.4
CO_2 fraction	2.1	1.1	1.8
Supernatant fraction	24.0	23.3	25.4
Per cent recovery	92.2	87.3	99.3
In supernatant fraction			
Supernatant	24.0	23.3	25.4
Calculated as unused glucose‡	2.3	5.0	0.0
Recovered as column effluent	20.9	19.2	22.2
Per cent recovery	96.4	104	87.5

* All cultures were grown anaerobically for 180 min in medium containing uniformly labeled 0.015 M glucose-C^{14} (1.03 × 10^5 counts per min per μmole of glucose). A 20-ml amount of each culture grown under these conditions was inventoried for label distribution as indicated.

† Results expressed as counts per minute (× 10^{-6}), with the exception of per cent recovery.

‡ Determined with the use of the Glucostat reagents (Materials and Methods).

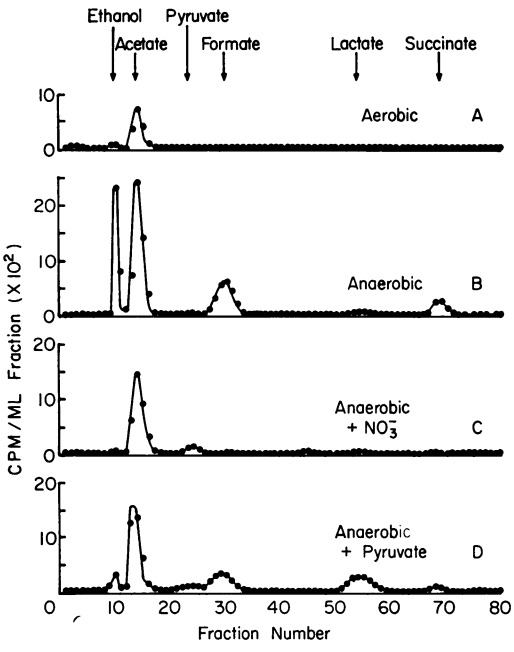

FIG. 2. *Effect of aerobiosis, anaerobiosis, nitrate, and pyruvate on end product formed during growth of Escherichia coli ML30 on uniformly labeled glucose. All cultures were pregrown aerobically for 120 min in basal medium containing 0.015 M glucose and 0.25% casein hydrolysate. Uniformly labeled glucose-C^{14} (0.5 μc/ml) was then added, and incubation was continued for 180 min under the following conditions: profile A, aerobic conditions; profile B, anaerobic conditions; profile C, anaerobic conditions plus 8 × 10^{-3} M KNO_3; profile D, anaerobic conditions plus 0.04 M pyruvate. The arrows on the top of the graph identify the elution region for the indicated products.*

sion "on"), and anaerobic conditions with pyruvate added (repression "on") (A, B, C, and D respectively, in Fig. 2). Under aerobic conditions (profile A), acetate is the only nongaseous end product; whereas, under anaerobic conditions (profile B), glucose carbon accumulates mainly as acetate and ethyl alcohol with lesser amounts of formate and succinate. In these experiments, the cultures were grown for 180 min. Similar product-accumulation patterns were obtained when the cultures were examined after only 90 min of growth. When nitrate is included in the anaerobic system (profile C), acetate is the predominant product, with some pyruvate accumulation also detected. Ethyl alcohol formation is essentially eliminated. When pyruvate was added to these cultures containing glucose (profile D), acetate was again the major product found. Ethyl alcohol accumulation was severely repressed, whereas some lactate production was noted. These data show that nitrate and pyruvate are capable of altering the flow of glucose carbon under anaerobic conditions, and that they do so presumably by competing for electrons that would otherwise result in ethyl alcohol accumulation.

The fate of the added nitrate under these conditions had been previously established. Electrons generated by glucose fermentation reduced nitrate to nitrite, followed by a reduction of nitrite to the level of ammonium ion (4). The fate of the added pyruvate in these experiments is shown in Fig. 3. Cultures were grown in media containing the usual level of glucose (0.02 M), but with the further addition of 0.04 M pyruvate-3-C^{14} under aerobic conditions (profile A), anaerobic conditions (profile B), and anaerobic conditions with 8 × 10^{-3} M nitrate (profile C). Under aerobic conditions or anaerobic conditions with nitrate added, the pyruvate was degraded primarily to acetate. Under anaerobic conditions alone, ace-

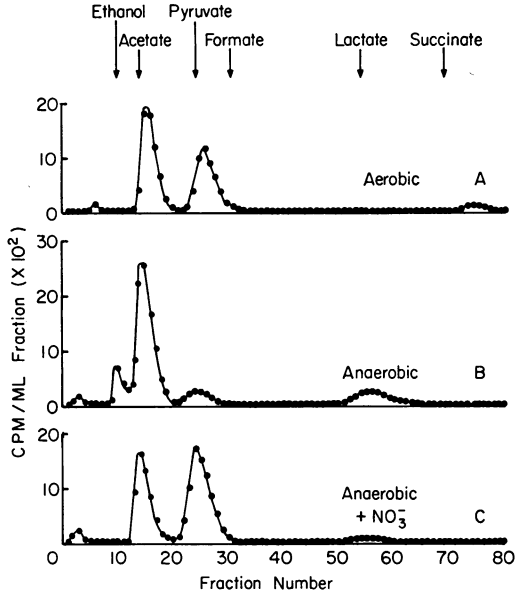

FIG. 3. *End products of pyruvate metabolism with cultures grown on a combination of glucose and pyruvate. Conditions were the same as described in profile D of Fig. 2, except that the pyruvate rather than the glucose was labeled. The initial pyruvate concentration was 0.04 M, and included approximately 0.50 μc of pyruvate-3-C^{14} per ml of culture. End products were determined after 180 min of growth. Profile A, 0.015 M glucose plus 0.04 M pyruvate-3-C^{14}, aerobic incubation; profile B, 0.015 M glucose plus 0.04 M pyruvate-3-C^{14}, anaerobic incubation; profile C, 0.015 M glucose plus 0.04 M pyruvate-3-C^{14} and 8 × 10^{-3} M KNO_3, anaerobic incubation.*

tate was again the principal product of pyruvate degradation. In this case, however, appreciable quantities of pyruvate were converted to the reduced products lactate and ethyl alcohol.

When identical experiments were conducted with cultures grown on 0.04 M pyruvate-3-C^{14} in the absence of glucose (Fig. 4), acetate accumulation accounted for essentially all of the degraded pyruvate under all the conditions employed.

Glucose fermentation by *E. coli* usually results in lactic acid accumulation. In the present experiments, only traces of lactate were detected during fermentation of glucose. It is known, however, that lactate accumulation is repressed when a high *p*H is maintained during anaerobic growth (12). The medium used in the present study contained sufficient phosphate buffer to maintain a *p*H of 7.1 to 6.8 during the entire course of these experiments. If cells were grown anaerobically in a poorly buffered medium, lactate accumulation could be demonstrated as shown in Fig. 5 (profile A). In these experiments, the phosphate

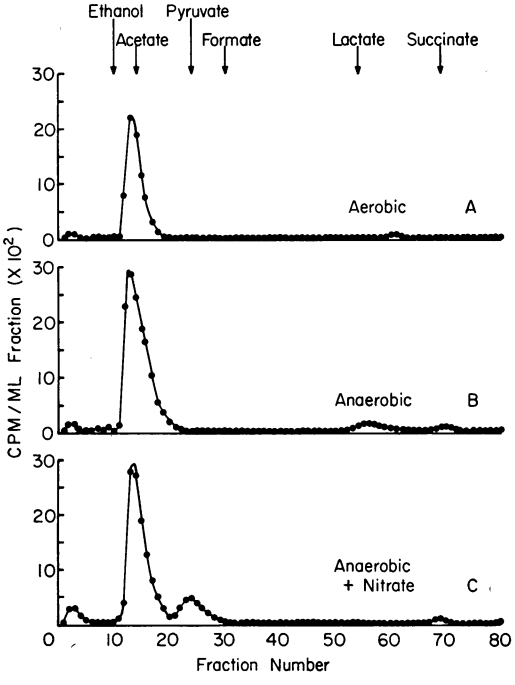

FIG. 4. *End products of pyruvate metabolism in Escherichia coli ML30. The conditions used in these experiments were identical to those described in Fig. 2 and 3, except that the above cultures were grown with 0.04 M pyruvate as sole substrate. Each culture was grown for 180 min in the presence of 0.5 μc of pyruvate-3-C^{14} per ml. Profile A, aerobic incubation; profile B, anaerobic incubation; profile C, anaerobic incubation plus 8 × 10^{-3} M KNO_3.*

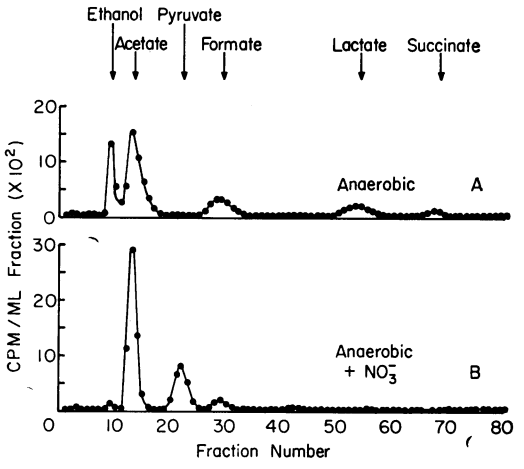

FIG. 5. *End products of glucose metabolism formed during growth in low buffer medium. Conditions were identical to those described in Fig. 1, except that the growth medium had a low buffer capacity as described in the text. Profile A, 0.02 M glucose-C^{14} (uniformly labeled), anaerobic conditions, initial pH 7.0, final pH 5.2; profile B, 0.02 M glucose-C^{14} (uniformly labeled), anaerobic conditions plus 8 × 10^{-3} M KNO_3, initial pH 7.0, final pH 5.8.*

buffer concentration was reduced to one-eighth of its normal level, and the *p*H dropped during the growth period from 7.0 to 5.2. When nitrate was included in this poorly buffered system (profile B), ethyl alcohol and lactate accumulation was inhibited and pyruvate formation was again detected—a situation comparable to that observed in the highly buffered culture (Fig. 2, profile C).

DISCUSSION

Some previous studies on the β-galactosidase system of *E. coli* have suggested that certain oxidation-reduction reactions are closely associated with the mechanism by which glucose or other readily metabolized substrates can regulate formation of this enzyme, i.e., the mechanism of catabolite repression. This is based on the findings that catabolite repression by glucose is temporarily eliminated when aerobically growing cultures are subjected to anaerobic shock (2), and that nitrate can prevent the reversal of repression caused by anaerobic shock (4). It was reasoned that support for this hypothesis might be obtained by examining the end product formed under conditions known to promote or prevent expression of catabolite repression. It was also considered that such studies might yield clues as to the metabolic site of these reactions. Thus, the present study was made of the alteration in end-

product patterns during growth of *E. coli* ML30 on glucose under aerobic conditions (repression on), anaerobic conditions (repression off), anaerobic conditions plus nitrate (repression on), and anaerobic conditions plus pyruvate (repression on).

There is, of course, no paucity of information on end products of glucose metabolism in *E. coli*. Under aerobic conditions, acetate and CO_2 are the major products formed (11), whereas during anaerobic growth glucose is converted predominantly to acetate, CO_2, ethyl alcohol, lactate, and succinate, with minor amounts of 2,3-butanediol, acetoin, and glycerol. Hydrogen evolution is also characteristic of *E. coli* fermentations (1, 3, 12, 15). The amount of lactic acid accumulating during glucose fermentation is known to be dependent on the *p*H of the medium (12). By growing cultures on uniformly labeled glucose-C^{14} and through use of silicic acid chromatography of end products, all of the above characteristics of aerobic and anaerobic glucose metabolism were substantiated for the *E. coli* ML30 strain used in the present study.

Nitrate reduction coupled with anaerobic glucose metabolism is known to alter the pattern of end products in *E. coli* (13), and has been inferred to yield 3 moles of adenosine triphosphate (ATP) per mole of nitrate reduced in *Aerobacter aerogenes* (7). A recent study along this line by Forget and Pichinoty (5) with *A. aerogenes* is most pertinent to our present consideration. Anaerobic control cultures produced primarily ethyl alcohol, acetate, and formate, with smaller amounts of CO_2, H_2, succinate, and lactate. With nitrate included in the medium, formation of ethyl alcohol, hydrogen, succinate, and lactate was virtually eliminated. Pyruvate accumulation was noted under these conditions, with acetate, formate, and CO_2 accumulation accounting for the bulk of the end products. Under aerobic conditions, acetate and CO_2 were the major end products. These effects of nitrate on *A. aerogenes* fermentations are identical to the results reported in the present study with *E. coli* ML30. Nitrate was effective in competing for electrons generated by glucose metabolism that would otherwise have resulted in ethyl alcohol formation. Pyruvate accumulation was also noted under these conditions. The involvement of the hydrogen-generating system in relation to altered glucose metabolism and catabolite repression will be considered in a later report.

At this point, however, it is clear that nitrate reduction does alter carbon flow during anaerobic glucose metabolism, and that this alteration is accompanied by a concomitant change in catabolite repression. Whether or not these two changes have a specific cause-effect relationship remains to be shown.

The addition of exogenous pyruvate to an anaerobic glucose culture also turns on the repression mechanism (Fig. 1). The pyruvate addition can also alter the carbon flow of glucose metabolism, as shown by a significant inhibition in formation of labeled ethyl alcohol from glucose (profile D, Fig. 2). It is considered likely, however, that the ability of exogenous pyruvate to augment catabolite repression by glucose is not solely founded, as in the case of nitrate, on its capacity to act as a hydrogen acceptor. Although some of the added pyruvate does become reduced to lactate (profile B, Fig. 3), it is primarily decarboxylated to acetate. It is also known that (data not presented) the addition of pyruvate to cultures growing anaerobically on glucose stimulates the rate of growth.

On this basis, the following situation can be visualized. When oxygen is available or when nitrate is available under anaerobic circumstances, the pyruvate formed during glucose metabolism is readily decarboxylated to acetate, and the concomitant energy gain is high. In the absence of external electron acceptors, some pyruvate carbon becomes reduced and the energy gain is decreased accordingly (6, 7). This situation is circumvented when an exogenous supply of pyruvate is added to these anaerobic glucose cultures. The availability of ATP in these cultures would then be implicated as the primary regulator for catabolite regulation. It should be noted at this point that involvement of ATP in the mechanism of catabolite repression has been suggested by Mandelstam (8).

These speculations are based largely on the indirect evidence obtained from analysis of end-product profiles. Currently in preparation are studies of a more direct nature, including radiorespirometric analyses of glucose degradation under repression "on" and repression "off" conditions and the role of hydrogen evolution and pyruvate accumulation in catabolite repression.

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