

Functional Capacities and the Adenylate Energy Charge in *Escherichia coli* Under Conditions of Nutritional Stress

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Functional capacities in *Escherichia coli* cells starved for glucose were examined by comparing protein synthesis, utilization of new substrates, and maintenance of viability with the adenylate energy charge of the culture. When growth ceased because of glucose exhaustion in an *E. coli* culture, the energy charge dropped from 0.90 to about 0.80. During this time, the viable-cell count and the capacity for protein synthesis and for induction of new enzymes were maintained only if other substrates were available in the medium. The culture could be maintained for many hours without growth or death if glucose was added slowly; the energy charge in this case stabilized at about 0.80. A consistent transient decrease in the energy charge to around 0.80, accompanied by a decrease in protein synthesis, was also observed during the adaptation from glucose to other substrates during diauxic growth on glucose and glycerol or lactose.

The amount of metabolically available energy in the adenylate pool is expressed by the adenylate energy charge (1, 3). This parameter, (adenosine 5'-triphosphate [ATP] + $\frac{1}{2}$ adenosine 5'-diphosphate [ADP])/(ATP + ADP + adenosine 5'-monophosphate [AMP]), is useful in determining the effect of the energy state of the cell on metabolic regulation. Measurements of the energy charge of a cell can be compared with the responses of enzymes *in vitro*. The activities of some major regulatory enzymes *in vitro* as a function of energy charge have been reviewed (2). As the energy charge increases, adenylate-regulated enzymes in ATP-regenerating sequences decrease in activity and adenylate-regulated enzymes in ATP-utilizing sequences increase in activity. Response curves of adenylate-regulated enzymes to changes in the energy charge show the sharpest response to changes in energy charge at values above 0.7. The half-maximal responses for enzymes of both types occur between energy charge values of 0.8 and 0.9, which indicates that the energy charge should be tightly regulated in this range. As predicted by the response curves of adenylate-regulated enzymes as a function of energy charge *in vitro*, growing and actively metabolizing cells of *Escherichia coli* and other species (4, 7, 18) have been found to maintain energy charge values near 0.9.

When growth ceases as a result of carbon starvation in *E. coli*, a definite decrease in energy charge has been observed (7, 18). In this

paper we examine the relationship between the adenylate energy charge of the cell and its ability to adapt to new substrates and to survive nutritional stress. The capacities of a glucose-starved *E. coli* culture to synthesize protein, induce new enzymes, and maintain viability have been determined and compared with the energy charge of the culture.

MATERIALS AND METHODS

Growth conditions. *E. coli* B was grown in a medium (final pH, 7.0) containing (per liter): KH_2PO_4 , 10 g; K_2HPO_4 , 26 g; $(\text{NH}_2)_2\text{SO}_4$, 2 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2 g; and ion supplement solution (14), 5.0 ml. Dow silicone Antifoam A (0.15 ml/liter) was added to prevent foaming. Cultures were grown with forced aeration at 37°C.

Culture apparatus and sampling. The culture vessel was a modified 500-ml gas-dispersion flask. The culture was mixed with a stirring bar; baffles extended from the walls into the flask to increase turbulence. In the glucose maintenance experiments, glucose was pumped continuously into the flask with a Sage syringe pump (model 355) through sterilized silicone rubber tubing. The tubing extended below the culture level to just above the stirring bar. The growth flask contained a sampling port in the lower part of the flask. Samples were removed with a sterile syringe through a serum cap placed in the port. Culture samples could also be withdrawn through a sampling tube held in place by a rubber stopper which fitted into the sampling port. In the latter case the sampling tube was flushed before a sample was taken.

Cell growth and viable-cell count measurements. Cell growth was monitored by measurement of optical absorbance at 540 nm. Viable cells were counted

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by plating 1 ml of culture (diluted 10^7 - to 10^8 -fold) in 10 ml of Difco standard methods plate count agar medium. Colonies were counted after incubation at 30°C for 4 days.

Determination of β -galactosidase inducibility. β -Galactosidase was induced by rapidly removing 10 ml of culture and placing it in a 125-ml Erlenmeyer flask with the non-metabolizable β -galactosidase inducer isopropyl- β -D-thiogalactoside (IPTG) at a final concentration of 0.5 mM. The sample was incubated at 37°C in a reciprocating water-bath shaker for 1 h. The incubation was terminated by adding toluene and deoxycholate according to the method of Revel, Luria, and Rotman (15). The activity of β -galactosidase increased linearly with time from zero level during the 1-h induction period.

The β -galactosidase assay was a modification of that described by Pardee, Jacob, and Monod (13). A 0.2-ml volume of 13.3 mM *o*-nitrophenyl- β -D-galactoside in 250 mM NaH_2PO_4 , pH 7.0, was added to 1.0 ml of toluene-treated cells. Samples were incubated at 30°C for various periods of time (0 to 1.5 min). The reaction was then stopped by adding 5.0 ml of 1 M Na_2CO_3 , and absorbance was measured at 420 nm. One unit of activity is defined as the amount of enzyme that will catalyze the cleavage of 1 μmol of *o*-nitrophenyl- β -D-galactoside per min at 30°C, pH 7.0.

Determination of total protein. Protein was determined by the modified biuret procedure developed by Klungsoyr (11) in the glucose starvation and maintenance experiments, whereas the method of Lowry et al. (12) was used in the diauxic experiments. The two methods gave similar results.

Determination of labeled leucine incorporation. A 1-ml sample of culture was incubated with 2.0 μCi of [^3H]leucine and 2.0 μg of leucine (final specific activity of the [^3H]leucine was 130 $\mu\text{Ci}/\mu\text{mol}$) for 3 min at 37°C. In the glucose plus glycerol diauxic experiment, 0.2 μCi of [^{14}C]leucine was substituted (final specific activity was 13 $\mu\text{Ci}/\mu\text{mol}$). The incorporation of labeled leucine was stopped by adding 1 ml of 20% (wt/vol) trichloroacetic acid containing 0.5% (wt/vol) leucine at 0°C. The trichloroacetic acid-precipitable material was collected on membrane filters (1.2- and 0.45- μm pore size, Millipore Corp.) with a Millipore sampling manifold (model 3025). After collection of the precipitates, the filters were dissolved in Bray's solution (5), and radioactivity was measured with a liquid scintillation spectrometer.

Extraction of the adenine nucleotides. A 1.0-ml volume of culture was added to 0.2 ml of cold 35% (wt/vol) HClO_4 . This procedure was completed within 10 to 12 s. The cell extract was incubated in HClO_4 at 0°C for 30 min and then frozen at -70°C. Within 24 h of sampling, the extract was thawed, mixed, and centrifuged at $10,000 \times g$ (4 min at 0 to 4°C) to remove acid-precipitable proteins. An 0.8-ml portion of the supernatant solution was then neutralized by adding 0.3 ml of a solution containing 2.6 M KOH and 0.58 M KHCO_3 . After 5 min, the neutralized samples were centrifuged in a clinical centrifuge for 3 min to remove KClO_4 , and the samples were immediately stored at -70°C.

Determination of adenine nucleotides in the culture medium. About 2.5 ml of bacterial culture was rapidly removed from the culture flask and placed in a 5-ml syringe attached to a Swinnex-25 filter holder containing two membrane filters (1.2- μm pore size filter on top of a 0.45- μm pore size filter). The culture sample was then filtered, and 1.0 ml of the filtrate was added to cold HClO_4 . The entire sampling process was completed within 30 s. The filtrate was treated as described above for total culture samples.

Sample preparation for the luciferase assay. The cell extract samples and adenine nucleotide standard samples were prepared for luciferase assay of ATP by the method described by Ball and Atkinson (4). All samples were assayed within 10 h after preparation.

Procedure for the luciferase assay. The amount of ATP in cell samples was determined by means of the luciferase reaction (17) using a Luminescence Biometer (E. I. DuPont de Nemours & Co.). The luciferin-luciferase mixture used in the assay was prepared as described by Ball and Atkinson (4). The sum $\text{ADP} + \text{ATP}$ was determined similarly after incubation of the extract with pyruvate kinase and phosphoenolpyruvate to convert ADP to ATP, and the sum $\text{AMP} + \text{ADP} + \text{ATP}$ was determined after incubation with pyruvate kinase, phosphoenolpyruvate, and adenylate kinase to convert both AMP and ADP to ATP. Concentrations of AMP and ADP were determined by difference.

Calculation of the energy charge. The concentrations of intracellular nucleotides were determined by subtracting the nucleotide concentrations of the medium samples from the concentrations of the total culture samples. The standard error of the mean for the energy charge determinations, calculated as described by Swedes, Sedo, and Atkinson (18), was between 0.01 and 0.02 energy charge unit.

RESULTS

The adenylate energy charge of *E. coli* cells growing on limiting (5.0 mM) glucose was around 0.90 to 0.93. When the glucose was exhausted and growth ceased, the energy charge dropped below 0.90 and remained between 0.80 and 0.90 for 5 to 7 h (Fig. 1). Then there was a large drop in energy charge to a value below 0.5, where the energy charge remained for many hours. The total pool of adenine nucleotides ($\text{ATP} + \text{ADP} + \text{AMP}$) fell by about 50% when growth ceased and remained low (not shown). These results are generally consistent with the energy charge measurements in *E. coli* reported by Chapman and Atkinson (7) and Swedes et al. (18).

The number of viable cells (colonies formed on plates) more than doubled after the optical absorbance had become constant. The reason for this is not known; perhaps a round of division followed the cessation of actual growth. The number of viable cells remained constant

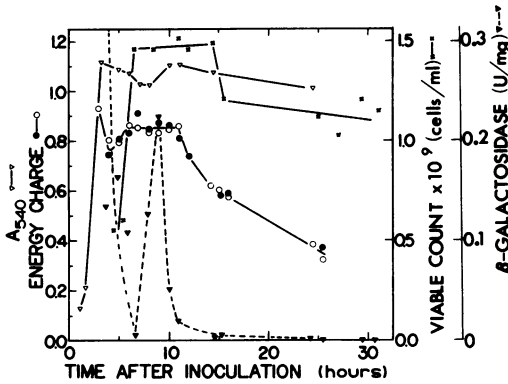


FIG. 1. Energy charge, viable-cell count, and β -galactosidase inducibility of *E. coli* B grown on limiting glucose. Cells were grown aerobically in a 5 mM glucose medium, and absorbance was followed at 540 nm. β -Galactosidase inducibility was measured by removing 10 ml of culture and incubating the separate sample with IPTG as described in Materials and Methods. The energy charge values of the parent culture are shown by the open circles and those of the separate β -galactosidase-induced samples are shown by the closed circles. The number of cells forming colonies when plated on Standard Methods plate count agar is shown by crosses. Inducibility is reported as enzyme units per milligram of protein and is plotted in the figure at the time the sample was removed from the parent culture. Inducibility at 3 h was 14.8×10^2 U/mg of protein.

until after the energy charge fell below 0.8; there was then a 25% decrease followed by a more gradual decline.

The ability of *E. coli* cells to synthesize new protein, particularly the inducible enzyme β -galactosidase, was examined in cells starved for glucose and was compared with the energy charge of the culture (Fig. 1). Samples were removed from the culture for induction with IPTG, so that the parent culture was never exposed to the inducer. The energy charge of the separately induced samples was very similar to that of the parent culture. β -Galactosidase inducibility increased during the last hours of the exponential phase as the concentration of glucose diminished (not shown). When the cells became limited for carbon and growth ceased, β -galactosidase inducibility declined. However, after 3 to 4 h of stationary phase, β -galactosidase inducibility increased sharply and then dropped shortly before the energy charge started to decrease. There was no apparent change in the total amount of protein during the peak in inducibility. The peak in β -galactosidase inducibility was repeatedly found to occur 3 to 4 h after the end of exponential growth in *E. coli* B and *E. coli* K-12 (ATCC 23716) starved for glucose.

In glucose-limited cultures of *E. coli*, there is a large increase in the concentration of adenosine 3',5'-monophosphate as growth ceases (6). Adenosine cyclic monophosphate was not measured in this study. Its addition at 3 mM to cultures of glucose-starved cells did not affect the pattern of decrease and subsequent peak in β -galactosidase inducibility.

An estimate of new protein synthesis in the culture starved for glucose was obtained by measuring the uptake of [3 H]leucine into trichloroacetic acid-precipitable material (Fig. 2). The patterns of [3 H]leucine incorporation and β -galactosidase inducibility were similar, with a simultaneous peak in each activity about 3 to 4 h after growth stopped.

β -Galactosidase inducibility decreased rapidly with no peak when *E. coli* cells were centrifuged and resuspended in fresh medium without glucose (Fig. 3). This result suggested that early stationary *E. coli* cells might be utilizing exogenous substrates in the medium in the synthesis of β -galactosidase and other proteins. The energy charge and β -galactosidase inducibility were compared in cells filtered at the end of exponential growth and resuspended in their own medium and in cells filtered and resuspended in fresh medium without glucose. Three identical *E. coli* B cultures were grown in medium containing limiting (5 mM) glucose. Just as the cultures exhausted the glucose in the medium and growth ceased, two of the cultures

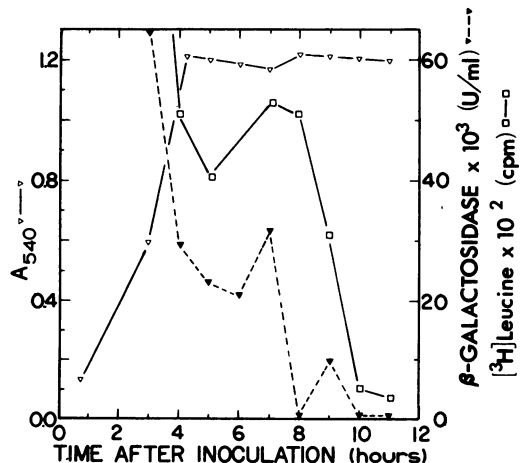


FIG. 2. β -Galactosidase inducibility and [3 H]leucine incorporation in *E. coli* B grown on limiting glucose. Cells were grown in 5 mM glucose medium and β -galactosidase inducibility was measured as described in the legend for Fig. 1. [3 H]leucine incorporation into trichloroacetic acid-precipitable material was measured as described in Materials and Methods and is reported in counts per minute.

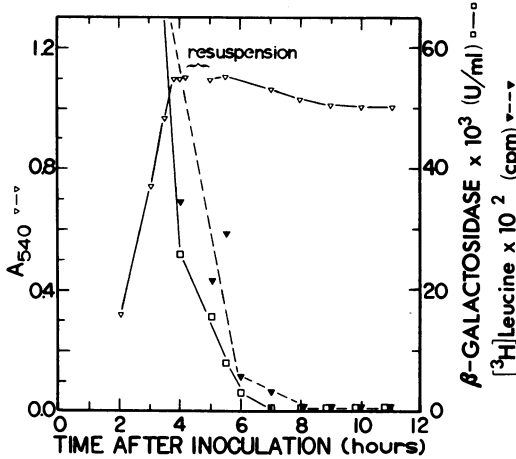


FIG. 3. β -Galactosidase inducibility and $[^3\text{H}]$ leucine incorporation in *E. coli* B resuspended in medium without glucose. *E. coli* B was grown in medium containing 5 mM glucose. At 4.25 h, the culture was centrifuged for 5 min at $10,000 \times g$ at 15°C . The cells were then resuspended in fresh minimal salts medium without glucose.

were rapidly filtered; the third was allowed to go into stationary phase. Filtered cells from the first culture were resuspended in fresh medium without glucose, and filtered cells from the second culture were resuspended in their own medium. The energy charge of the culture resuspended in its own medium recovered to above 0.80 for 6 h and then gradually fell (Fig. 4). Similar results were obtained with the culture that was not filtered (not shown). The energy charge of the culture resuspended in fresh medium without glucose (Fig. 5) was recovered to a value of 0.77, but this value was not maintained. The charge soon began to fall and reached a value of 0.37 within 10 h after resuspension. The viable count of the culture resuspended in fresh medium without glucose decreased much more rapidly than that in the two cultures resuspended or kept in their own growth media.

After the glucose has been exhausted and exponential growth has ended, the cells appear to utilize some compound(s) in the medium that enables them to maintain their energy charge at 0.80 to 0.85 for several hours, and also enables them to adapt to new substrates such as lactose through the synthesis of β -galactosidase when induced to do so. The observations of Holms et al. (9, 10) suggest that the main substrate in the medium of glucose-grown *E. coli* cultures is acetate. When exponentially growing cells were filtered and resuspended in 2 mM acetate, we observed a peak in β -galactosidase inducibility similar to that observed in early

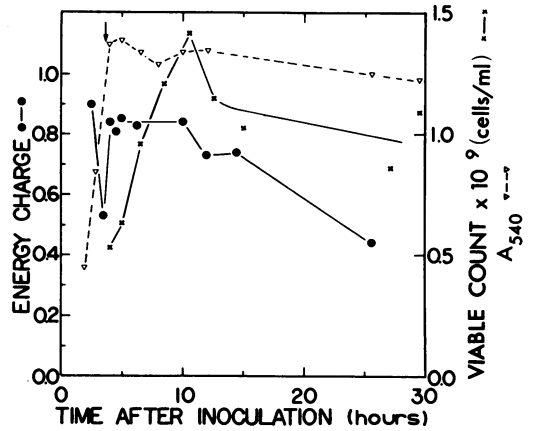


FIG. 4. Energy charge and viable-cell count of an *E. coli* B culture resuspended in its own medium. Cells were grown in 5 mM glucose medium. Just as the culture reached its maximal absorbance of 1.2 at 3.75 h after inoculation, the culture was filtered and resuspended in its own medium from exponential growth. The arrow in the figure indicates the time that the culture was resuspended.

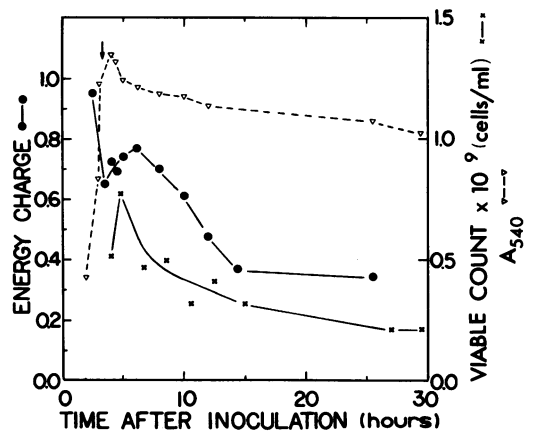


FIG. 5. Energy charge and viable-cell count of an *E. coli* B culture resuspended in fresh minimal salts medium without glucose. The experiment was identical to that of Fig. 4, except for the resuspension medium.

stationary phase of a culture grown in limiting glucose (Fig. 6).

Our observations indicate that the energy charge is maintained between 0.80 and 0.85 and the number of viable cells remains constant as long as there are exogenous substrates in the medium that can be utilized by *E. coli* starved for glucose. We were able to extend this maintenance period for days by continuous slow feeding of glucose to glucose-limited cells. Two identical *E. coli* cultures were grown in limiting (5 mM) glucose. Starting 0.5 h after the

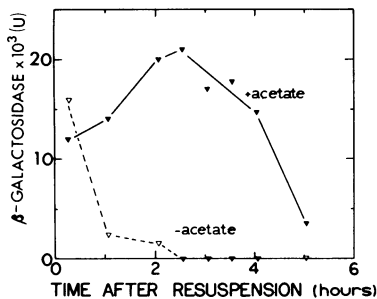


FIG. 6. β -Galactosidase inducibility in *E. coli B* resuspended in fresh minimal salts medium with and without 2 mM acetate. Cells were grown in 5.7 mM glucose medium. Just after the culture reached its final absorbancy at 540 nm of 1.12 at 3.75 h after inoculation, the culture was divided and filtered. Half of the filtered cells were resuspended in fresh minimal salts medium and the other half were resuspended in the same medium plus 2 mM acetate.

cessation of growth, glucose in minimal salts medium was added to one of the cultures at a rate of 0.15 μ mol of glucose/mg of protein per h. The second culture received minimal salts medium at the same rate. During exponential growth, the energy charge was slightly above 0.90 (Fig. 7). When the glucose from the original batch culture was exhausted, the energy charge declined to about 0.88 for the first 5 h of stationary phase in both cultures. Then the energy charge of the culture receiving only minimal salts medium started to fall and reached a value below 0.5 after 20 h of glucose starvation. In the culture receiving glucose, the energy charge declined to below 0.88 after 8.5 h of stationary phase and then leveled off at around 0.80 (± 0.01) for the next 60 h of glucose maintenance. The number of viable cells reached 1.8×10^9 cells/ml in each of the cultures (Fig. 8). After the energy charge had fallen below 0.80 in the control culture receiving only medium salts, viability began to decline, and only about 40% of the cells were able to form colonies on plate count agar after 50 h of glucose starvation. In the glucose-maintained culture the number of viable cells remained constant for over 75 h. The viable-cell count results were corrected for dilution (M. W. Simmons, Ph.D. thesis, Univ. of California, Los Angeles, 1974).

Maintenance of the energy charge at around 0.80 was achieved when glucose was added at a rate of 0.15 μ mol of glucose/mg of protein per h. The energy charge could be stabilized only within a relatively narrow range of values around 0.80 without cell growth or death. When glucose was added at a rate of 0.19 μ mol/mg of protein per h the number of viable cells in-

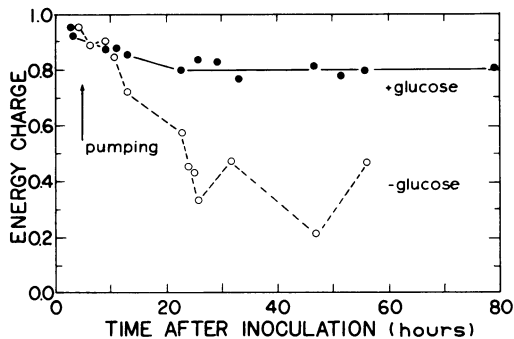


FIG. 7. Maintenance of the energy charge in *E. coli B* by slow addition of glucose. Two identical cultures in medium containing 5 mM glucose reached a final absorbancy at 540 nm of 1.20 and became stationary. Minimal salts medium containing glucose was pumped into one of the cultures, starting 30 min after the end of growth, at such a rate as to add 0.15 μ mol of glucose/mg of protein per h. Minimal salts medium lacking glucose was pumped into the second culture at the same flow rate. Intracellular energy charge values are reported.

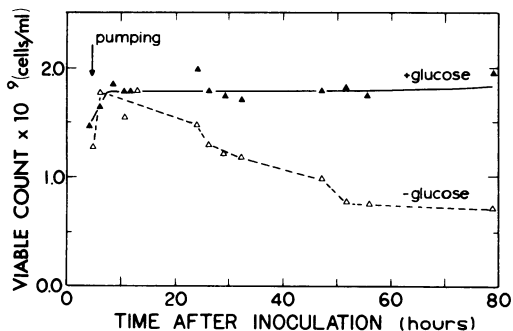


FIG. 8. Maintenance of the viable-cell count in *E. coli B* by slow addition of glucose. Two *E. coli B* cultures were maintained with glucose and minimal salts medium or minimal salts medium only, as described in the legend of Fig. 7. Viable-cell counts (corrected for dilution) are reported as in Fig. 1.

creased 153% during 25 h and the energy charge averaged 0.84. When the rate of glucose addition was lowered to 0.094 μ mol/mg of protein per h, there was no increase in the number of viable cells during glucose addition, and the energy charge decreased to 0.73 after 25 h of glucose addition.

When growth on glucose ceased in cultures grown in medium containing glucose and lactose (Fig. 9), the energy charge declined to about 0.86 for about 15 min and then increased to a value of about 0.91. A simultaneous fall in the rate of incorporation of [3 H]leucine into protein was observed during the transition from glucose to lactose utilization. β -Galactosidase activity appeared just as the energy charge be-

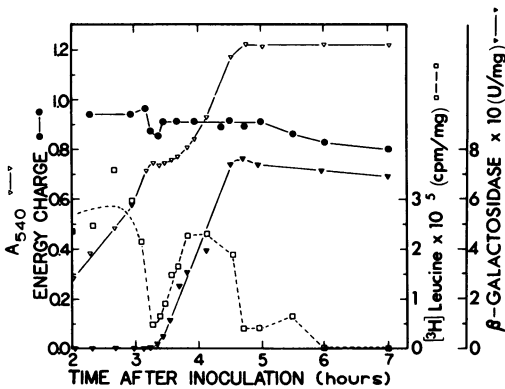


FIG. 9. Energy charge, specific activity of β -galactosidase, and [^3H]leucine incorporation in *E. coli B* during diauxic growth in glucose and lactose. Cells were inoculated into minimal salts medium containing 3 mM glucose and 1 mM lactose. Descriptions of curves are as in Fig. 1 and 2.

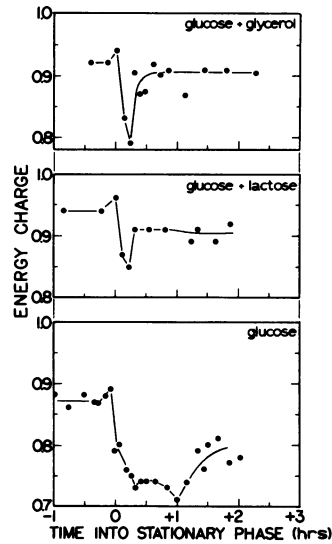


FIG. 10. Comparison of the energy charge fall during diauxic growth in glucose + glycerol and glucose + lactose, and in starvation after growth on glucose. Energy charge values are shown during late exponential growth on 3 mM glucose and during adaptation to 4 mM glycerol or 1 mM lactose. Total energy charge values (not corrected for adenylates in the medium) are reported for the culture grown in only 5 mM glucose. The energy charge values are shown on a time scale with zero at the time that growth on glucose ceased.

gan to recover, and the activity increased until growth on lactose had ceased. A similar drop in energy charge and in the rate of incorporation of leucine was observed in cultures grown on glucose and glycerol. The energy charge fell to 0.83 to 0.79 for 10 to 15 min at the end of growth on glucose during adaptation to utilization of glycerol. The fall in energy charge was accompanied by a decrease in rate of incorporation of labeled leucine into protein. As growth resumed because of glycerol utilization, the energy charge and rate of protein synthesis readjusted to values similar to those for exponential growth on glucose.

The decreases in energy charge during adaptation from glucose to glycerol or lactose, and from glucose to exogenous substrates in the medium during glucose starvation, are compared in Fig. 10. (The energy charge values of the glucose-starved culture have not been corrected for adenylates in the medium, which lowers these values about 0.03 energy charge unit.)

DISCUSSION

The results reported here demonstrate that *E. coli* cells subjected to nutritional stress are able to maintain their viability and capacity for protein synthesis and for adaptation to new substrates as long as the energy charge can be stabilized at 0.80 or above. Such stabilization is dependent on the availability of exogenous substrate.

Several reports (8-10, 16) have indicated that approximately 15% of the glucose metabolized by aerobically grown *E. coli* or *Aerobacter aerogenes* is excreted as acetate into the medium

during growth on glucose. Holms et al. (9, 10), studying *E. coli* ML 308, found that 17% of the carbon supplied as glucose accumulated in the medium as acetate during growth on limiting glucose, and that the acetate was utilized within 3 h after the end of exponential growth. They also observed a peak in O_2 consumption after the end of exponential growth, similar in timing to the peak in β -galactosidase inducibility that we have observed. We found that a culture resuspended in minimal salts medium also containing acetate at about 12% of the amount of carbon originally supplied as glucose behaved much like a stationary-phase culture in its own medium.

In this study the energy charge of an *E. coli* culture limited for glucose could be maintained for days by the continuous slow addition of glucose. The energy charge could be stabilized without cell growth or death only within a relatively narrow range of values near 0.80. When an *E. coli* culture is starved for glucose, once the energy charge drops below 0.80 there follows a large and rapid drop in energy charge to a level between 0.2 and 0.5, accompanied by large decreases in functional capacities and finally by cell death.

The extreme sensitivity of ATP-consuming sequences to decreases in the value of the energy charge is reflected in the small magnitude of the fall in energy charge that occurs when glucose is exhausted (Fig. 10). Since metabolic intermediates and biosynthetic starting materials will also be limited, the decrease in energy charge cannot be the only immediate cause of the large decrease in metabolic rates that must necessarily occur at this time. However, in view of the rapid turnover of ATP and the participation of the adenine nucleotides in all metabolic sequences, only very effective kinetic controls could limit the decrease in energy charge to 20% or less at the onset of starvation.

When an alternative energy source was available, the energy charge was restored to its normal value within a rather short time (Fig. 10, top two panels). When lactose was the alternate substrate, the rise in energy charge coincided in time with the appearance of a detectable level of β -galactosidase, and resumption of growth followed quickly (Fig. 9). When no alternative substrate was available except for materials excreted or lost from the cells during growth (probably mainly acetate), the energy charge recovered weakly and much more slowly (Fig. 10, bottom panel).

Taken together with the ability of *E. coli* cells to survive apparently indefinitely at an energy charge of 0.8 when glucose is supplied at a low rate, these results suggest that the response of this species to absence of a metabolizable energy source may be as suggested in Fig. 11. Under normal growth conditions, metabolic sequences in which ATP is regenerated should respond to variation in the adenylate energy charge as shown by curve R, and the response of sequences in which ATP is utilized should resemble curve U. When the carbon and energy

source is exhausted or severely limited, as in our experiments in which glucose was added slowly, the rates of all reaction must decrease. The primary responses in most cases are variations in affinities of enzymes for substrates, and the actual responses of enzymes in sequences that regenerate ATP should still resemble curve R. However, the rate of a reaction depends on substrate concentration as well as on the affinity of the enzyme for substrate, so when substrates are severely limited the reaction rates will be limited even if the enzyme is fully activated (has its maximal affinity for substrate). Accordingly, the actual flux through catabolic or R-type sequences must at all values of energy charge be limited by substrate concentrations, as indicated by curve R'. Similarly, even when interactions between energy charge and product feedback effects lead to maximal affinity for substrate of regulatory enzymes in biosynthetic sequences, the biosynthetic rates will be limited by the concentrations of substrates, and response curves like U' should result. Homeostasis of course depends on equality of rates of regeneration and utilization of ATP. As a consequence of severe limitation of the carbon and energy source, the homeostatic state should shift, for example, from intersection *a* to intersection *b*.

Figure 11 illustrates why a small change in the value of adenylate energy charge should be expected to be associated with a large change in metabolic fluxes. It also may help to explain our failure to stabilize the charge at values below 0.8. As substrate limitation becomes increasingly severe, the maximal heights, and thus the slopes, of both R-type and U-type curves must decrease. As the slopes at the intersection become smaller, the stabilizing effect must decrease. Figure 11 does not, however, explain why the charge falls to 0.8 and stabilizes at that value; according to the simplified model illustrated, the stabilizing tendency would be expected to decrease steadily with decrease in availability of carbon and energy. Stabilization at 0.8 demands that in effect one or both curves become steeper at this point than at higher energy charge values. An actual increase in steepness is extremely unlikely; probably the stabilization depends on a specific effect at some key regulatory branch point, such as the partitioning of phosphoenolpyruvate between carboxylation to yield precursors for biosynthetic sequences and conversion to pyruvate for use in ATP regeneration. If the availability of biosynthetic starting materials were rather sharply curtailed at this point, the result would be a further drop in the U' curve, which could have the same effect on metabolic stabilization

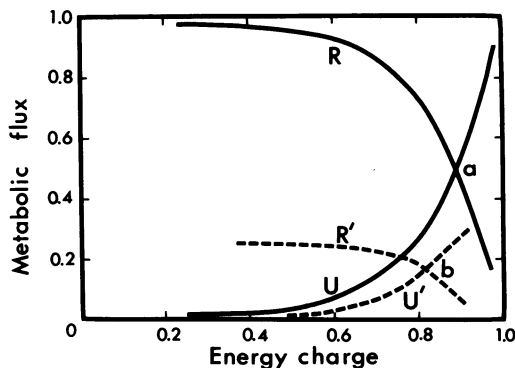


FIG. 11. Suggested changes in metabolic flux response curves as a result of limitation of the carbon and energy source.

as a region of increased steepness of that curve. In any case, the stabilization of energy charge at about 0.8 is not a general phenomenon; in starving yeast cells the charge drops rapidly far below the physiological range (4). Different species clearly have evolved quite different strategies for dealing with energy deprivation.

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LITERATURE CITED

- Atkinson, D. E. 1968. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry* 7:4030-4034.
- Atkinson, D. E. 1969. Regulation of enzyme function. *Annu. Rev. Microbiol.* 23:47-68.
- Atkinson, D. E., and G. M. Walton. 1967. Adenosine triphosphate conservation in metabolic regulation. Rat liver citrate cleavage enzyme. *J. Biol. Chem.* 242:3239-3241.
- Ball, W. J., and D. E. Atkinson. 1975. Adenylate energy charge in *Saccharomyces cerevisiae* during starvation. *J. Bacteriol.* 121:975-982.
- Bray, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* 1:279-285.
- Buettner, M. J., E. Spitz, and H. V. Rickenberg. 1973. Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. *J. Bacteriol.* 114:1068-1073.
- Chapman, A. G., L. Fall, and D. E. Atkinson. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. *J. Bacteriol.* 108:1072-1086.
- Hadjipetrou, L. P., J. P. Gerrits, F. A. G. Teulings, and A. H. Stouthamer. 1964. Relation between energy production and growth of *Aerobacter aerogenes*. *J. Gen. Microbiol.* 36:139-150.
- Holms, W. H., and P. M. Bennett. 1971. Regulating isocitrate dehydrogenase activity in *Escherichia coli* on adaptation to acetate. *J. Gen. Microbiol.* 65:57-68.
- Holms, W. H., I. D. Hamilton, and A. G. Robertson. 1972. The rate of turnover of the adenosine triphosphate pool of *Escherichia coli* growing aerobically in simple defined media. *Arch. Mikrobiol.* 83:95-109.
- Klungsoyr, L. 1969. Quantitative estimation of protein. *Anal. Biochem.* 27:91-98.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β -galactosidase by *E. coli*. *J. Mol. Biol.* 1:165-178.
- Powell, E. O., and F. P. Errington. 1963. Generation times of individual bacteria: some corroborative measurements. *J. Gen. Microbiol.* 31:315-327.
- Revel, H. R., S. E. Luria, and B. Rotman. 1961. Biosynthesis of β -D-galactosidase controlled by phage-carried genes. *Proc. Natl. Acad. Sci. U.S.A.* 47:1956-1967.
- Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. 1955. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Washington Publ. 607, p. 16.
- Strehler, B. L. 1968. Bioluminescence assay: principles and practice, p. 99-181. In D. Glick (ed.), *Methods of biochemical analysis*, vol. 16. John Wiley & Sons, Inc., New York.
- Swedes, J. S., R. J. Sedo, and D. E. Atkinson. 1975. Relation of growth and protein synthesis to the adenylate energy charge in an adenine-requiring mutant of *Escherichia coli*. *J. Biol. Chem.* 250:6930-6938.