ENERGY OF MAINTENANCE IN *ESCHERICHIA COLI*

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ABSTRACT

McGREW, SARAH B. (Pennsylvania State University, University Park) AND M. F. MALLETTE. Energy of maintenance in *Escherichia coli*. J. Bacteriol. **83**:844–850. 1962.—Relatively dense populations of *Escherichia coli* B in log phase were used to detect utilization of exogenous glucose for maintenance without growth. Turbidity at 400 mμ was used as the measure of growth, since it should reflect changes in either cell number or size. A threshold level of glucose was observed below which turbidity did not change during short-time experiments. Repeated additions of glucose during prolonged incubation at 37 C either increased the turbidity slowly or maintained it, depending on the amount of glucose. Plate counts to follow viability showed slow decreases for 10 days, while the unfed controls lost viability quite rapidly. From these results it was concluded that *E. coli* specifically utilized exogenous glucose for maintenance, without growth. The conflict of this opinion with that of earlier workers is discussed and some implications suggested.

Many of the molecules of biological systems are both thermodynamically and kinetically unstable with respect to hydrolysis. Netter (1953) discussed the problems thus raised, including a need for sources of free energy, endogenous or exogenous, in preserving or replacing such unstable materials. In developing a similar concept, Rahn (1932) suggested that endogenous metabolism could be regarded as chemical wear and tear and that this gradual self-destruction might be reversed by addition of suitable exogenous nutrients. Free energy thus provided to maintain the status quo is referred to as *energy of maintenance*.

Although the properties of the metabolic intermediates and macromolecules lead one to expect a maintenance level of energy just sufficient to prevent their destruction or to replace them, there could be biological adaptations circumventing the instability. Compartmentalization of cells to prevent attack by degradative enzymes, location of the sensitive elements in organized solid phases, and the occurrence of high energies of activation could combine to diminish or eliminate the energy of maintenance in living cells. Because the information is insufficient for a conclusive theoretical analysis, solution of the problem must be experimental.

In general, theoretical opinions (Buchanan and Fulmer, 1928; Rahn, 1932; Netter, 1953; Sugita, 1955; Clifton, 1957) assume the existence of energy of maintenance, though not always under such a name. Friedlein (1928) found that very low levels of energy sources were not effective in subculturing bacteria, but the data do not eliminate the possibility of slight growth. Individual organisms may have enlarged without cell division. Kandler (1955) demonstrated at least the possibility of such energy of maintenance when he showed that not all of the free energy from exogenous materials goes into growth. However, his study did not identify the role of this extra energy. More recently, Windisch and Nordheim (1957) emphasized that starvation depletes cells of reserves, preventing their growth unless supplemented. This idea again suggests an energy of maintenance. On the other hand, most of the relatively direct experimental approaches have failed to offer support for the concept.

If energy is utilized specifically for maintenance, one would predict that, at sufficiently low rates, exogenous materials could not produce cell growth. Hence, plots of dry weight, or some related property, against amount of energy source at low levels ought not extrapolate to the origin. A positive value for the energy source at zero growth would correspond to the energy of maintenance. Using this approach, Maze (1902) plotted yield of fungus against invert sugar or ethanol consumed and extrapolated his lines through the origins, thus concluding that energy of maintenance was undetectable. The results actually do not appear to be clear-cut, partly

because the experimental points were not presented. Later, Rottier (1936) obtained a similar linear relationship between total growth of *Polytoma uvella* and the amount of peptone or asparagine in the medium. In one case, however, the data extrapolated to a negative intercept on the substrate axis.

With somewhat more sensitive methods, Monod and Tessier (1936) found that the total population of *Glaucoma piri formis* increased linearly with food supply over the range studied. They concluded that a given amount of food was necessary per new cell regardless of the exogenous energy level. Thus, one must infer that there was no perceptible energy of maintenance. Later, Monod (1942) restudied the problem more exhaustively, using *Escherichia coli* and *Bacillus subtilis*, and again concluded that any energy for maintenance was essentially zero. In addition to the usual comparison of population (turbidity) with energy source, Monod also studied the influence of variations in agitation of the cultures. He assumed justifiably that factors delaying the attainment of maximal growth ought to require more prolonged diversion of energy to maintenance and thus reduce the yield. Within the experimental uncertainties, prolonging the growth period by 50 to 100% did not affect the yield of microorganisms.

However, in another series of experiments, Monod varied the temperature and did find a decrease in total population at the higher temperatures. This rather generally observed phenomenon might be anticipated on the basis of increased energy requirements needed to offset the more rapid degradation of cellular components. On the other hand, such an effect could be offset partly or completely by the increase in time required to reach the maximal populations at lower temperatures. Monod concluded that there were not sufficient data to resolve the problem of the effect of temperature on the maximal population with a limited energy supply.

Most recently, Battley (1960) observed a linear relationship between the ultimate turbidity and limiting energy sources for *Saccharomyces cerevisiae*, with the plots extrapolating to the origin. In this work as in the others for which the necessary data are given, it appears that the quantity of substrate exceeds the dry weight of the cells produced at the lowest levels studied. Thus, it is conceivable that energy of maintenance was not detected simply because of insensitivity of the extrapolation under such conditions.

In reinvestigating the possibility of an energy of maintenance, the problem becomes one of designing an experiment of sufficient sensitivity to permit comparison of growth with exogenous energy source at low relative levels of the latter. High sensitivity is especially important since the relationship may not be linear for small amounts of substrate. Data presented below indicate that a maintenance level of energy does exist for *E. coli* B.

MATERIALS AND METHODS

E. coli B was obtained from the Department of Bacteriology, Pennsylvania State University. The organism was subcultured weekly on nutrient agar (Difco) slants. Inocula from these slants were grown in culture tubes (22 by 175 mm) to populations of 5×10^8 cells per ml in a glucose-salts medium by shaking at 200 cycles per min and an amplitude of 2 cm at 37 C. This medium contained 0.1% NH_4Cl, 0.05% NaCl, 0.041% $MgSO_4 \cdot 7H_2O$, 0.6% Na_2HPO_4, 0.3% KH_2PO_4, and 0.4% glucose. Samples (1.0 ml) of this culture were subcultured by addition to 10.0-ml lots of the glucose-salts medium, were grown to populations of 2.5×10^8 cells per ml, and were stored cold overnight. Just prior to the actual experiments, growth was continued to 5×10^8 cells per ml (still in the exponential phase and insuring physiologically active and relatively uniform organisms).

For the experiments, cells were harvested by transferring to small capped tubes and centrifuging for 15 min at $800 \times g$ at 2 to 4 C. The cells were all combined by suspending the pellets in 10 ml of a solution of the above salts with the glucose omitted. This suspension was centrifuged as above and the cells rewashed three times by suspending in 5 ml of the same salt solution and centrifuging 10 min. Finally, the organisms were suspended in the salt solution, using 1.0 ml for each tube of the final culture stage. At every step, the cells were kept as cold as possible by keeping suspensions in an ice bath and using cold solutions to minimize endogenous processes. Samples (1.00 ml) of this final concentrated suspension were added to experimental systems consisting of the same salt solution with or without glucose. Salt concentrations were kept constant throughout to avoid appreciable changes in osmotic concentration and any accompanying

changes in cell size. In preparing the final experimental suspensions, particular care was used in pipetting. All pipettes were read to marks; none was blown out. In all except the last relatively long-term study, cell suspensions were quickly brought to 37 C by shaking in a water bath, then transferred to the 37 C room for the duration of the incubation.

Plate counts were made on suspensions in quadruplicate with 100-mm petri dishes containing 30 ml of 2.3% nutrient agar (Difco) and 0.5% NaCl; 1.00-ml samples, read between marks on 2-ml serological pipettes, were used in diluting cell suspensions into 0.154 M NaCl in half-pint milk bottles. The necessary subdilutions were made in the same way after thorough mixing for several minutes at each step. A final dilution of 0.50 ml was made with a 1-ml pipette into 9.5 ml of diluting agar (one part plating agar to two parts nutrient broth). Diluting agar was melted in a hot-water bath, then cooled quickly to 37 C just before adding the cells. This suspension was mixed rapidly and 2.0 ml (pipette blown out) run onto the surface of a plate, previously warmed to 35 to 37 C, and the liquid spread by tipping the covered plate back and forth. After allowing the diluting agar to harden at room temperature, the plates were inverted to avoid subsequent streaking by drops of condensate, and were incubated 18 to 20 hr at 37 C. Dilutions were chosen to provide colony counts of 100 to 300 to permit easy counting and to limit the uncertainties of the averages to about ±10%.

Cell populations of cultures were determined by turbidity readings with a Coleman model 14 Universal spectrophotometer at 650 mμ calibrated against both plate and direct microscopic counts. Turbidity data in the growth experiments were obtained with a Beckman model DU spectrophotometer, reading all suspensions in the same cuvette and recording the averages of three readings on each suspension. Turbidity data were taken at 400 mμ to provide a maximal instrumental response, and at 600 mμ for comparison to ascertain that light absorption did not contribute to the data at the shorter wave length. Data taken at the two wave lengths always revealed the same effects; hence only those at 400 mμ are presented.

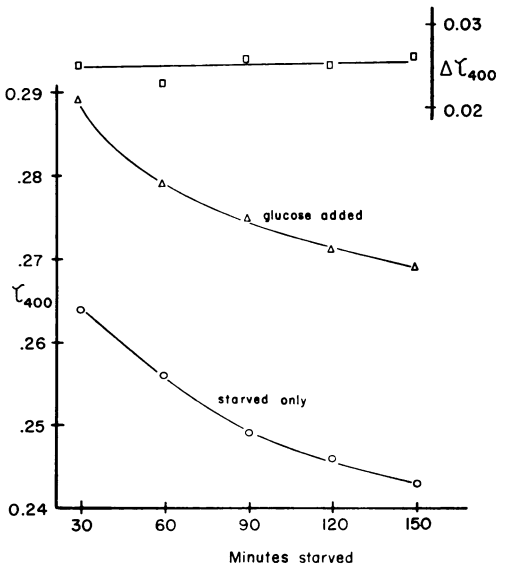

FIG. 1. *Decrease in turbidity of cell suspensions during starvation and its reversal with glucose. Cells were starved at 37 C on the shaker, and tubes were removed at the times indicated on the abscissa; 2.24 μmoles of glucose were added to the cells of the middle curve, and incubation then was continued for another 30 min to allow growth on the glucose. The top curve shows the differences between the other two at each pair of points. Each tube contained 4.9 × 10⁸ log-phase cells/ml in 10.0 ml of the salts medium. Turbidities were read at 400 mμ.*

RESULTS

In the first attempts, plate counts were used to determine populations and thus compare growth (change in population), with the amount of glucose at low levels. Exercise of all the technical care applicable nevertheless failed to provide the degree of reproducibility necessary for plotting and extrapolating the small changes. Although the use of turbidity as a measure of population had proved insensitive for the earlier workers, application in a different way was considered instead of plating procedures. In place of a small inoculum and extensive growth over an extended period, a large inoculum was employed to reduce the ratio of glucose to cells.

Figure 1 shows that the turbidities of cultures of starving cells decrease with time (after 30 min but perhaps not before), as shown by the bottom curve. Presumably, the refractive index of cells decreases or the cells shrink, or both

occur during the endogenous utilization of cellular material. Addition of a small amount of glucose at the time on the abscissa less 30 min increased the turbidity relative to the corresponding suspension under continuous starvation. With this limited feeding, either the population increased, the effects of starvation were avoided, or some combination of the two occurred. The two curves were essentially parallel as shown by the differential plot using $\Delta\tau$. Therefore, the addition of the glucose offset the effects of continuing starvation to the same relative extent, but not to the same original turbidity. Changes during the first 30 min could not be evaluated in this experiment, since data were taken in that interval only with the Coleman spectrophotometer, and these values were not reproducible enough for interpretation. Nevertheless, the observed decreases in turbidity and the effect of glucose suggested that some level of glucose might be found to maintain a uniform turbidity.

The turbidity data in Fig. 1 (measured with the Beckman spectrophotometer) showed consistent trends but did not really assess the reproducibility of the system. Therefore, a series of duplicate suspensions was prepared and the turbidities measured. Under these ideal conditions, the turbidity difference between the extreme values for ten tubes was 0.002, with a standard deviation of 0.002. When more individual volume measurements were required, as in adding glucose to suspensions, variations were greater but within about ± 0.003 turbidity units for the experiments in which all turbidities were measured consecutively in a short period. Such a degree of uncertainty seemed small enough to serve in this study.

According to Fig. 1, the turbidity of suspensions decreased after 30 to 60 min and perhaps before. To check this point specifically, the experiment plotted in Fig. 2 was conducted. As usual, each point represents a separate tube of cells, each incubated at 37 C for the time shown. Within experimental error there was no turbidity change after starvation for 30 min, but decreases occurred later. At the same time, a small amount of glucose was added to each tube of another series to discover the time required for completion of the turbidity increase. As shown, growth was completed in 15 min. As a result, the suspensions used in Fig. 3 were incubated for 20 min, long enough for completion of growth before starvation proceeded far enough to reduce turbidity measurably.

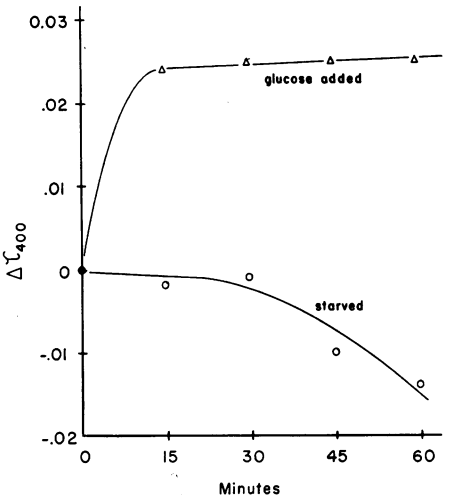

FIG. 2. *Time required for starvation to diminish turbidity and the time required for growth after one addition of glucose. Glucose (2.2 μmoles) was added at zero time to each tube of the upper curve. Suspensions were shaken at 37 C for the times shown on the ordinate. Each system contained 5.0 × 10^8 cells ml in 10.0 ml of medium. Differences in turbidity were calculated by subtracting the values for the nonincubated suspensions from those for the incubated suspensions.*

Figure 3 relates turbidity changes with the quantity of glucose added. Three different experiments are presented, demonstrating the general reproducibility. Although the plot obviously could contain a short curved segment, it appears that certain levels of glucose did not affect turbidity; hence growth did not occur. Thus, the 1.5 mg of cells present (dry basis) required more than about 0.1 mg of glucose for growth. Since the cells were in log phase and could at least increase in size, given enough of an exogenous energy source, it can be assumed that a threshold level of glucose was required for growth. Lesser quantities contributed only to maintenance of the cells or were below a threshold level necessary for penetration by the substrate. The exact value of such an energy level might be difficult to fix because the extent of washing and handling and the temperatures involved could alter the slight metabolic depletions occurring before the actual experiment was begun. In this case, all three experiments were very

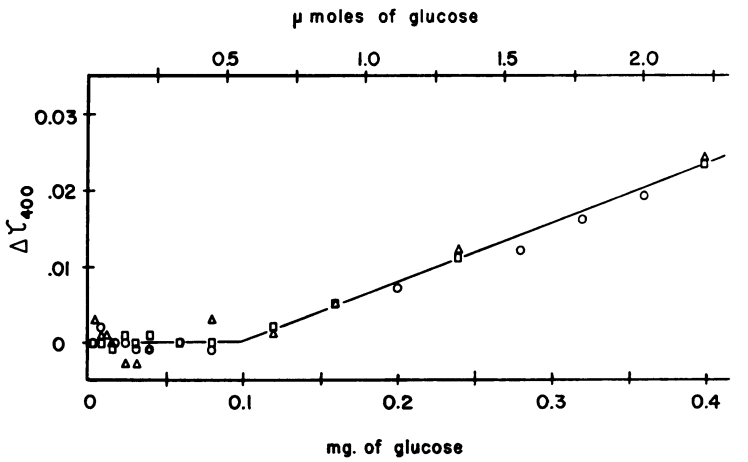

FIG. 3. *Threshold level of glucose for growth of E. coli. Glucose was added at zero time, and the suspensions shaken 20 min at 37 C. Turbidity differences were obtained by subtraction of the turbidities of control tubes, devoid of glucose but incubated with the others. The three different symbols for points represent three different experiments using fresh solutions. The triangular points were obtained 1 year after the others, suggesting some degree of biological stability for the property. Each tube contained 5.0 × 10^8 cells/ml in 10.0 ml of medium. The cells had a dry weight of 3.24 × 10^9 cells/mg (Palmer and Mallette, 1961).*

similar, probably because care was taken to use the same times, temperatures, and techniques at every step. Modifications in detail might lead to different threshold values.

Since turbidity depends upon the refractive index differential between particle and medium, addition of glucose should alter somewhat the intensity of the scattered light. Using a Phoenix differential refractometer, the glucose-salts solutions were compared with the salts solution alone. No difference in refractive index was detected beyond the experimental limit of less than $\pm 1 \times 10^{-5}$. Therefore, the low concentrations of glucose could not have influenced the turbidity data. Similarly, after incubating the cells and removing them by centrifugation, the medium after growth could not be distinguished from the salts solution.

Observation of an intercept in Fig. 3 suggested that the effects of starvation might be alleviated by the periodic addition of glucose to incubated cell suspensions. The level interpolated from Fig. 3 was chosen for a preliminary experiment in which turbidity increased slowly. This apparent growth led to use of an additional level in later studies.

The effect on turbidity of four daily additions of 0.556 μmoles of glucose is shown in Fig. 4. Data were corrected for the volume changes accompanying the additions of glucose (0.10 ml for each addition). Tightly capped tubes were used to avoid evaporation, which was checked by measuring the volumes of the three suspensions held for 10 days. Finding the expected volume of 14.0 ml in each tube substantiated the use of simple volume ratios in adjusting the turbidities to the original basis.

The steady rise in turbidity at this level of glucose indicated growth in cell size or number. Perhaps the multiple addition of glucose or some other change in detail permitted limited growth at the level derived from Fig. 3. Use of half as much glucose (Fig. 4) maintained an essentially constant turbidity, and growth did not occur. The tendency for the points on this middle curve to scatter was observed in repetitions at this level of glucose but not at the others shown. At present, no explanation can be offered. Turbidities of suspensions decreased markedly when glucose was not added. These controls began to stabilize when most of the cells had died.

The feeding program in this experiment demonstrated the feasibility of controlling the turbidity of *E. coli* suspensions for 10 days by adding limited amounts of glucose. However, the viability of the individual cells might become low with incubation, and additional data were needed to check this point. Therefore, samples of these same cell suspensions were diluted and plated for population counts, with the results

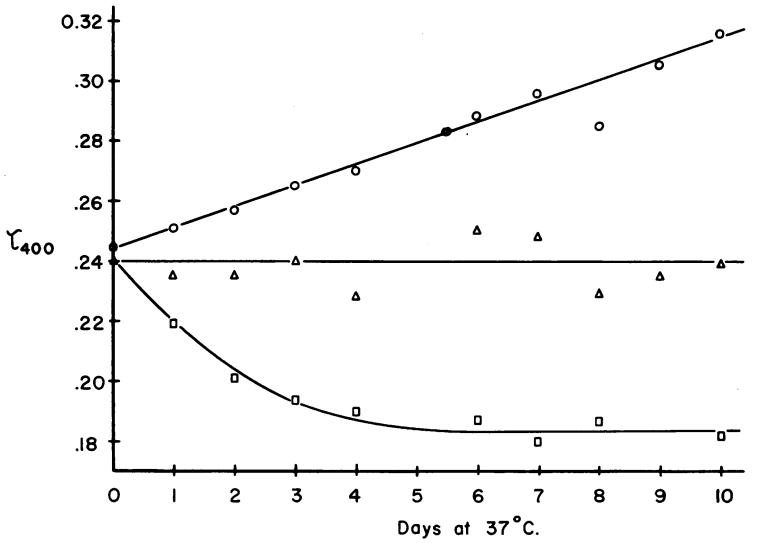

FIG. 4. *Effect of repeated additions of glucose on turbidity during prolonged incubation at 37 C. The lowest curve represents change due to complete lack of exogenous energy sources, the upper that due to 0.55 μmole of glucose added every 6 hr, the middle that due to 0.28 μmole of glucose also added every 6 hr. Data adjusted to the initial volume of 10.0 ml as indicated in the text. Each tube contained 5 × 10^8 cells/ml.*

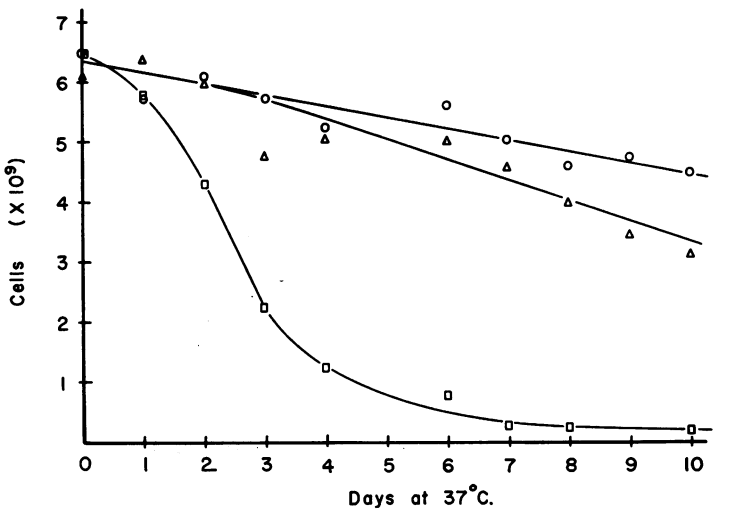

FIG. 5. *Effect of repeated additions of glucose on viable cell populations during prolonged incubation at 37 C. The individual points were obtained from samples taken from the suspensions of Fig. 4. Population counts were made by plating.*

shown in Fig. 5. Plate counts diminished with time in all three series, that without glucose dropping markedly. The other two decreased slowly by 30 to 50% at the end of 10 days. At least part of this change might have been due to clumping not prevented by the continuous shaking nor reversed by mixing during dilution for the plate count. On the other hand, loss of viability could have occurred, of course, without loss of turbidity if the refractive index of the nonviable cells equaled that of the living cells.

In the series with the larger amount of glucose, plate count and turbidity changed in opposite directions. Some cells may have died while

others divided, to account for the opposing effects. Perhaps some cells may have died while others increased in size or refractive index. As another possibility, a combination of growth and clumping could account for the observed changes.

Since tubes on the middle curve of Fig. 5 contained many more viable cells than did the starved controls, addition of glucose improved viability without inducing growth. This protective action could have occurred only if the cells utilized the glucose. Therefore, a threshold for cellular penetration by the glucose was not a factor in these experiments. As the remaining possibility, glucose below the levels inducing growth was utilized for energy of maintenance.

DISCUSSION

The above findings show that a single addition of a sufficiently small amount of glucose did not induce growth, and that multiple additions over a relatively long period maintained turbidity and at least partially preserved viability. Hence, the concept of energy of maintenance seems justified. This conclusion differs from that of earlier workers, probably because the latter used small inocula and grew the cultures for relatively long periods of time through a number of generations. The ratio of the weight of energy source initially present to the weight of cells at the end of the experiment was relatively high. Hence, extrapolations could not be expected to detect low positive threshold values. In the present work, the ratio of glucose to cell weight was much lower, by a factor of over 100 to 1000 compared to those studies where the necessary weight data are given. The difference thus appears to be one of sensitivity and is quantitative rather than qualitative. In the one series of suspensions, the glucose added four times daily totaled 1.1 μmoles or 0.20 mg per day for 1.5 mg of cells. This amount is quite appreciable but not detectable by earlier techniques.

It is conceivable that the energy of maintenance has important effects on the lag period when cells are subcultured, on the ecology of mixed cultures, on aerosols of bacteria, and on other problems of viability at temperatures in the range of growth. In fact, this principle may extend to other nutritional factors than energy sources. For example, a small amount of phosphate, sulfur, or nitrogen might be required to replace that lost in metabolic end products. Taken together, all such factors should be as important to viability in microorganisms as they obviously are to viability in adult higher animals.

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