JOURNAL OF BACTERIOLOGY, Jan. 1972, p. 229-235

Vol. 109, No. 1
Printed in U.S.A.

Response of *Escherichia coli* B/r to High Concentrations of Sucrose in a Nutrient Medium

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Received for publication 23 August 1971

Escherichia coli B/r was subjected to sucrose concentrations up to 1 M in the presence of Nutrient Broth. Plasmolysis seldom was evident 2 min after this treatment. The subsequent response was characterized by transient decreases in optical density as well as changes in appearance as seen under phase optics. No transient effects were detected in the synthetic rates or in the division of the survivors.

Escherichia coli cells are expected to plasmolyze when subjected to high concentrations of sucrose (4, 6, 7, 24). Deplasmolysis generally follows after some minutes, but can be delayed if nutrients are removed from the medium before the sucrose is added (6). Cellular activities subsequent to deplasmolysis have received little attention. Reports that do exist are based on conditions under which either nutrients were removed for plasmolysis and then resupplied (17, 22) or the high concentrations of sucrose were diluted before measurements were made (3).

Almost no information is available on the response of these cells to high concentrations of sucrose under conditions that otherwise would permit optimal synthesis and growth. Some aspects of such a response are described in this report. Visible evidence of plasmolysis was found to be absent 1 to 2 min after the cells were placed in contact with the sucrose. However, subsequent transient changes in appearance as well as transient changes in optical density (OD) were observed. At the same time, no corresponding effects were detected in the synthetic rates or in the division rates of the surviving cells.

MATERIALS AND METHODS

E. coli B/r obtained from Stanley Person of this laboratory were grown in 10-ml volumes of air-bubbled Nutrient Broth (Difco) at 37 C. During log phase, at concentrations near 2×10^8 cells/ml, 3 ml of such cultures were pipetted into 7 ml of warmed, air-bubbled Nutrient Broth containing appropriate amounts of dissolved, reagent-grade sucrose. Various means were then used to monitor the response of the cell. The uniqueness of the response was checked by use of other strains of *E. coli*, B_{s-1} and $15T^-$ (supplemented with thymidine at 0.4 μg/ml), also obtained from Dr. Person, as well as a different species, *E. intermedia* (ATCC 21073).

Microscopic observation. Observations were carried out at room temperature with medium dark-contrast phase microscopes at magnifications of 1,000 to 2,000. Samples were removed and placed on microscope slides at various times after the cells were subjected to sucrose solutions. Vaseline or paraffin was placed around the cover slip to prevent evaporation. Photomicrographs were obtained with a ×90 normal-contrast objective on a Leitz microscope with Kodak Contrast Copy film.

OD measurement. The OD of cell suspensions was measured at 425 nm with a Bausch & Lomb Spectronic-20 spectrophotometer. This information was used to determine growth rates and to follow changes subsequent to subjecting cells to sucrose. The test tube containing the entire culture of interest was transferred to the spectrophotometer for each measurement, a procedure that required less than 20 sec per reading.

Survival. Colony-forming ability was determined by diluting the cells through warm (37 C) blanks containing the same medium as the culture being examined, and then plating them in triplicate on nutrient agar plates containing the same sucrose concentration as the culture and blanks. The plates were incubated at 37 C, and colonies were counted when they became easily visible. This required incubation for 3 or 4 days at the higher sucrose concentrations.

Incorporation of radioactive macromolecule precursors. Log-phase cells were grown for approximately four division times in a Nutrient Broth medium containing the labeled compound to be investigated. For thymine, this included 0.093 μg of ^{14}C-thymine/ml (New England Nuclear Corp.) at a specific activity of 0.04 μCi/ml, 0.4 μg of ^{12}C-thymine/ml, and 250 μg of deoxyadenosine/ml. When such a culture reached about 10^8 cells/ml, 6 ml was added to 14 ml of a similar solution containing, in addition, the appropriate concentration of sucrose. For the control, 3 ml of the culture was

added to 7 ml of the medium lacking sucrose. At specified times, 1-ml samples were removed and placed in 9 ml of cold 5% trichloroacetic acid for at least 2 hr. These suspensions were then filtered (0.45-μm membrane filters; Millipore Corp.) and washed with 15 ml of 5% trichloroacetic acid. The filters were glued to planchets, dried, and counted with a Nuclear-Chicago gas-flow Geiger counter (model 1105). Incorporation was assumed concomitant with deoxyribonucleic acid (DNA) synthesis.

A similar procedure was followed for investigating the incorporation of uracil and proline into ribonucleic acid (RNA) and protein. For uracil incorporation, the medium contained 0.074 μg of ^{14}C-uracil/ml at 0.02 μCi/ml; for proline incorporation, it contained 9 μg of ^{14}C-proline/ml at 0.005 μCi/ml.

RESULTS

Appearance of cells. Cells were observed under phase contrast 1 to 2 min after being placed in a medium containing Nutrient Broth and sucrose. Very few signs of plasmolysis were evident, even in sucrose concentrations of 1 M, whereas in the absence of nutrients plasmolysis was evident for several minutes at 0.2 M sucrose (24). Figures 1b and e show typical phase micrographs of *E. coli* B/r cells shortly after they were immersed in 0.5 and 1.0 M sucrose, respectively. Deplasmolysis apparently had already occurred.

It is important to note that both a 1 M sucrose solution and normal *E. coli* cells have a refractive index near 1.38 (28). Hence, the fact that the cells, when placed in 1 M (and higher) sucrose, still appeared quite dark in positive phase contrast indicates that the refractive index of the cells had increased (2). This is consistent with the belief that deplasmolysis occurred as sucrose got into the cell.

The cells began to change their appearance as a function of time in the sucrose. Internal regions, often near the center of the cell, began to exhibit a decrease in contrast, indicating that the refractive index of this central portion of the cell was approaching that of the me-

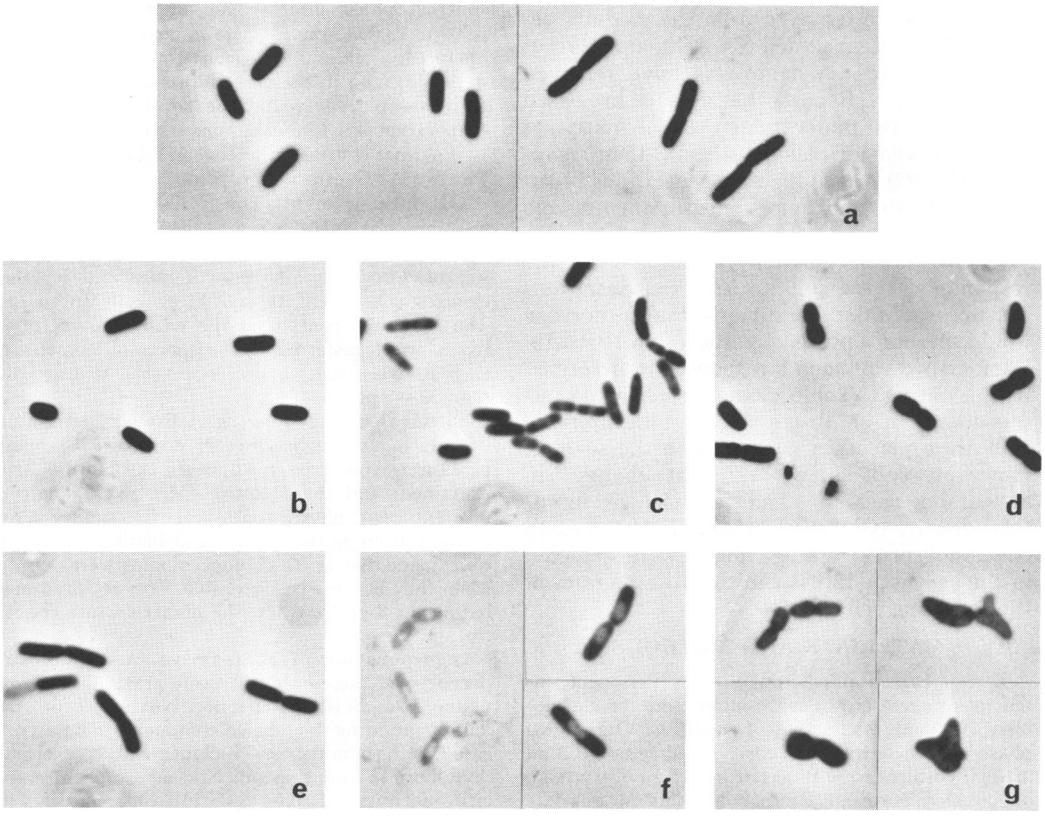

FIG. 1. *Phase micrographs of E. coli B/r cells (1 mm = 0.49 μm). (a) Normal log-phase cells. (b) Log-phase cells shortly after being placed in a broth medium containing 0.5 M sucrose. (c) Log-phase cells placed in broth with 0.5 M sucrose for approximately 15 min. (d) Cells in log phase grown in broth with 0.5 M sucrose. (e) Log-phase cells shortly after being placed in broth with 1 M sucrose. (f) Log-phase cells after being in broth with 1 M sucrose for 30 min. (g) Cells grown in broth with 1 M sucrose.*

dium. Examples of this are shown in Fig. 1c and f. The regions of low contrast will be referred to as light centers. Light centers required a longer time to develop and became more pronounced with increased sucrose concentrations. A difference in contrast between cells can be noted in both Fig. 1e and f. This is believed to have been the result of a differential accessibility to the sucrose.

Certain orientations of plasmolyzed cells could present somewhat similar appearances; however, the appearance referred to here took several minutes to develop and did not change in tumbling cells as different views were presented, nor was more than an occasional cell observed that had the more familiar features of plasmolyzed cells, in particular, the sharp difference in contrast between cytoplasm and periplasmic space.

Eventually, much of the original contrast returned to cells placed in concentrations up to about 0.5 M sucrose, and although they may have become shorter they still maintained their rodlike shapes (Fig. 1d). On the other hand, many cells in 1 M sucrose, after several days of incubation, were found to have lost their normal shape; these cells usually showed less overall contrast than normal cells (Fig. 1g). The decrease in contrast of normal cells suggests that their internal environments had been at least partially restored to that for normally grown cells, whereas the swollen and distorted appearance of other cells suggests that sucrose may have interfered with normal construction of the rigid layer in the cell envelopes. It is not known whether cells with distorted shapes were capable of reverting to normal or of forming colonies.

The light centers resembled those shown in other phase micrographs of *E. coli* placed in media of raised refractive index (13, 23, 27). In those instances, the high refractive index was achieved with material such as serum albumin, gelatin, or polyvinylpyrrolidone whose osmotic activities, on a weight basis, are much less than that of sucrose. The light regions were purported to represent the positions of the nuclear material. Similar results could not be obtained by placing the cells used here in a medium containing 20 to 30% bovine serum albumin. Perhaps in these *E. coli* B/r cells the DNA normally was spread throughout the cell and the response to sucrose included a temporary rearrangement of the DNA to a more compact configuration. This is consistent with electron micrographs of normal B/r cells (25) as well as with other reports that nuclear material can exhibit altered appearance when cells are subjected to various treatments either before or during the fixation process (11, 14, 15).

Several other factors were investigated for possible influence on this response. Light centers were observed when sucrose was replaced with similar concentrations of glucose or lactose (but not with NaCl), when Nutrient Broth was replaced with Roberts' C-minimal salts (20), when the culture in sucrose was bubbled with nitrogen, or when the culture was not bubbled at all. Pretreatment of the cells with chloramphenicol (0.1 mg/ml) to halt protein synthesis did not prevent the formation of light centers, but did prevent their subsequent disappearance. Both KCN (0.85 mg/ml) and low temperature (0 C) inhibited the formation of light centers. When cold cells in sucrose and broth were rewarmed, the light centers began to appear. When cells having light centers were centrifuged and resuspended in broth without sucrose, no light centers remained.

These results indicated that some metabolic activity was required for the appearance of light centers, that protein synthesis was required for the return to normal contrast in sucrose-containing media, and that formation of light centers was reversed rapidly when the sucrose was removed.

Changes in OD. OD at 425 nm showed an initial value near or slightly higher than that which would be expected by the dilution factor. The OD then underwent a transient decrease, the duration and amplitude of which increased with increasing concentrations of sucrose (Fig. 2). Light centers were most prevalent at times corresponding to the minimum of OD. These times for reaching the minimum OD are shown as a function of sucrose concentration in Fig. 3. The factors previously mentioned that affected the development of the observable light centers affected the decrease in OD in a similar manner.

Myers et al. (16) showed that cells placed in 0.6 osmolal sucrose should produce a 40% decrease in OD as a result of the increased refractive index of the medium. The shrinkage associated with plasmolysis was believed to increase the OD so that the OD which they measured was not much different from that of the control cells. The almost unchanged initial OD in even higher sucrose concentrations as reported here may indicate a similar cancellation of effects. The increased refractive index of plasmolyzed cells would be partially maintained as sucrose entered the cells during deplasmolysis. Rubenstein et al. (22) also reported a temporary decrease in OD in cells

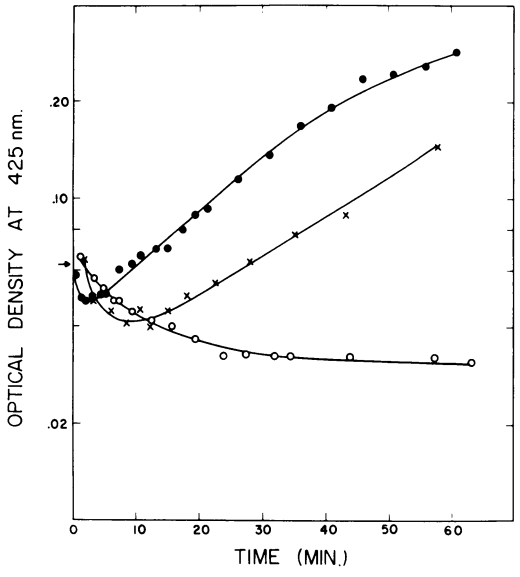

FIG. 2. *Changes in optical density after E. coli B/r cells were subjected to sucrose-containing broth: ●, 0.2 M sucrose; ×, 0.5 M sucrose; ○, 1 M sucrose. The arrow indicates the expected initial value on the basis of dilution.*

that had been washed before being subjected to sucrose. Other investigators have reported time-dependent optical effects at sugar concentrations of 0.2 M or less. These effects included increases in OD found in nongrowth media and attributed to cell shrinkage (1, 12), as well as decreases in OD attributed to swelling accompanying sugar uptake (18, 26) or to the sugar becoming associated with, and raising the refractive index of, the cell membrane (21). All of these effects could have been present when higher concentrations were used.

Dips in OD and the development of light centers also were observed at 0.5 M sucrose with *E. coli* B_{s-1}, *E. coli* $15T^-$, and *E. intermedia*. Plasmolysis vacuoles were evident in *E. intermedia* for several minutes.

Survival of colony-forming ability. It was desirable to determine whether the changes in appearance and in OD were accompanied by changes in colony-forming ability. Figure 4 shows the survival of colony-forming ability for cells subjected to, diluted through, and plated on sucrose-containing media. Decreased survival became appreciable above 0.6 M sucrose as reported for conditions conducive to more long-lasting plasmolysis (24). Since light centers were observed in cells at all concentrations above 0.2 M, the qualitative change observed in appearance was not correlated in any obvious fashion with cell death. Most cells in 0.5 M sucrose developed light centers and also formed colonies with no evidence of a detrimental effect. In 1 M sucrose, light centers were as plentiful, but colony formation was depressed by 90%. It is possible that only cells

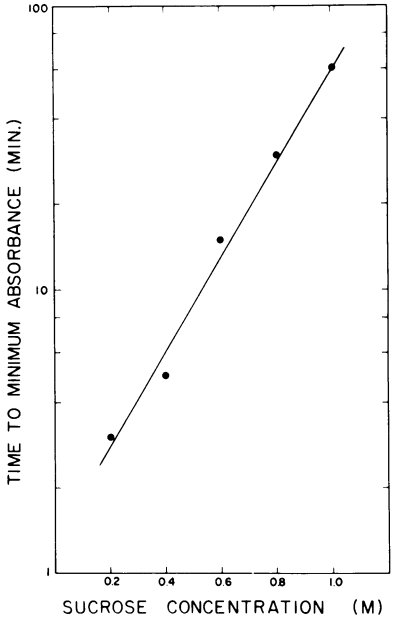

FIG. 3. *Time required for the optical density to reach a minimum for E. coli B/r placed in broth containing various concentrations of sucrose.*

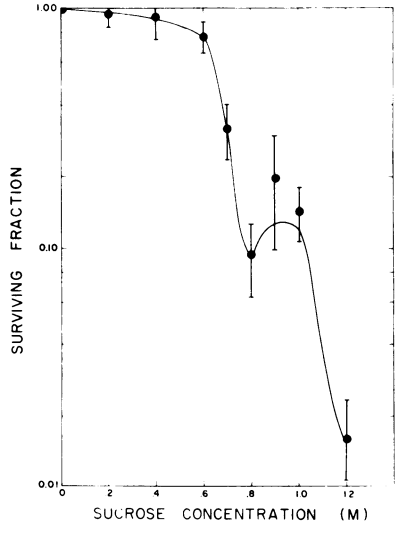

FIG. 4. *Survival of E. coli B/r colony-forming ability in broth containing various concentrations of sucrose. Error bars represent standard deviations for three or more experiments.*

not having light centers survived at the higher concentrations. The survival curve does hint that there may have been different lethal factors operating above and below a concentration of 1 M, although another interpretation is offered in the next section. Other evidence has shown survival of *E. coli* B to be near 90% when subjected to > 1 M sucrose if the sucrose is then diluted slowly (3).

Growth and rates of synthesis. Growth rates finally attained by survivors in various concentrations are shown in Fig. 5. Small amounts of sucrose actually enhanced growth in broth, but an upper limit seems likely to have existed near 1.6 M if a linear extrapolation is valid.

The transient changes observed in OD and in the appearance of cells were expected to be reflected in transient changes in the major metabolic processes, the synthesis of DNA, RNA, and protein. This was not the case. Figure 6 presents representative results of plating cells, on sucrose-containing plates, as a function of time after contact with sucrose. Extrapolations to zero time gave values corresponding to the survival shown in Fig. 4. The final slopes for 0.2 and 0.4 M sucrose were consistent with the growth rates at these concentrations (Fig. 5) and, clearly, these new division rates were achieved rapidly.

The behavior in 0.8 M sucrose (Fig. 6c) illustrates an effect that was not present at concentrations of 0.6 M and lower. An impressive decrease in survival was evident for cells diluted and plated within 20 min of being placed in the sucrose. Temperature shock was ruled out as a cause, because similar results were obtained when the experiment was performed in a room maintained at 37 C. Although the basis for this behavior has not been determined, we suggest that it might be the cause of the strange survival data in Fig. 4. It is possible that a physical factor, such as shear during pipetting, added to the lethality, or that a chemical factor, carried from the growth culture to the sucrose but absent in the dilution blanks and plates, was necessary for the initial stages of successful adaptation. A somewhat similar anomaly was noted by Rubenstein et al. (22), who suggested that it was the result of decreased ability of plasmolyzed cells to generate colonies on solid medium.

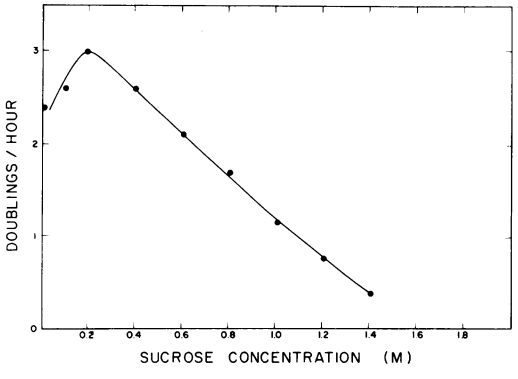

FIG. 5. *Growth rates for E. coli B/r grown in broth with various concentrations of sucrose.*

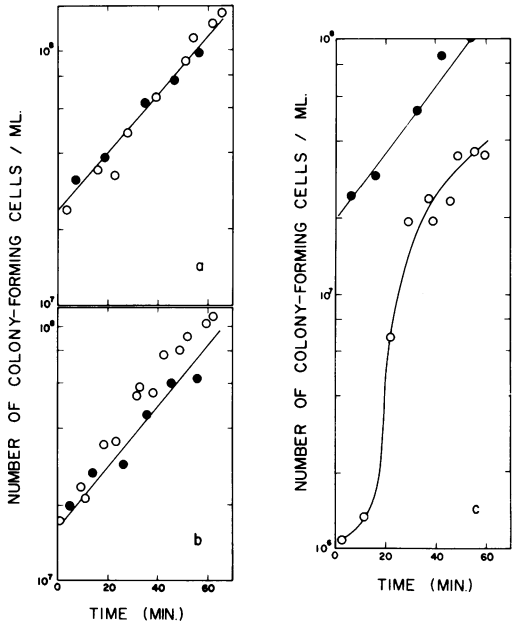

FIG. 6. *Representative results showing colony-forming ability of E. coli B/r as a function of time after subjection to a sucrose-broth medium: (a) 0.4 M sucrose; (b) 0.6 M sucrose; (c) 0.8 M sucrose. Controls are represented by filled circles.*

No transient effects were detected in the uptake of thymine, uracil, or proline for sucrose concentrations up to 0.8 M. Uptake curves at 0.6 M are presented in Fig. 7. Most of what appeared to be delays could be accounted for by assuming that only the surviving fraction shown in Fig. 4 participated in the uptake. This would imply a rapid cessation of the uptake machinery in nonsurvivors and may also account for a similar delay mentioned by Roberts et al. (20) and Rubenstein et al. (22). At the same time, it should be pointed out that Henneman and Umbreit found little depression in the respiration of glucose by *E. coli* B at 0.8 M sucrose (9).

DISCUSSION

Inferences based on these results may be

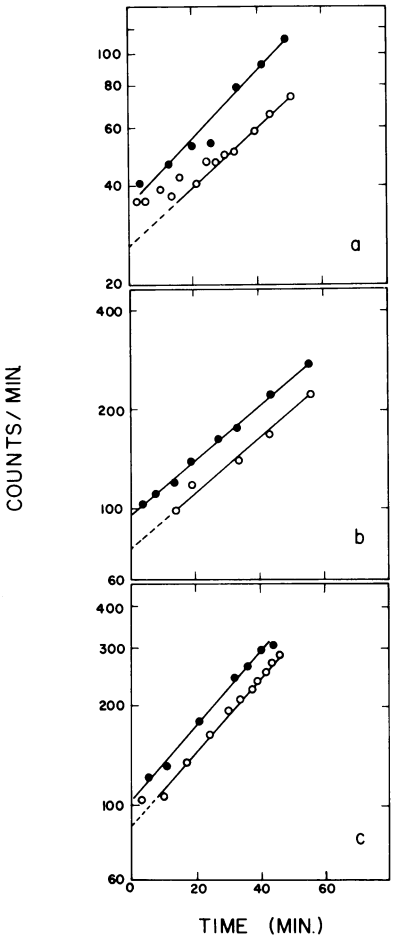

FIG. 7. *Representative results showing incorporation of radioactive precursors into E. coli B/r cells exposed to broth with 0.6 M sucrose. Controls are represented by filled circles. (a) ^{14}C-thymine. (b) ^{14}C-uracil. (c) ^{14}C-proline.*

made regarding several characteristics of *E. coli* cells. The apparently rapid deplasmolysis implies that the cells were not very impermeable to high concentrations of sucrose when nutrients were present. What is not clear is whether this is a normal state of affairs or whether plasmolysis caused a temporary alteration in the semipermeable barrier. It has been reported difficult to obtain plasmolysis in 100% of a population of cells (6, 24). This suggests that some cells are freely permeable to sucrose and the plasmolytic step may be omitted. It supports other evidence showing a high permeability of *E. coli* to sucrose (5, 10). On the other hand, it also has been reported that plasmolysis resulted in leaky cells with increased permeability to other molecules such as uridine nucleotides (8).

A second point concerns the operation of the cell with the sucrose inside. Myers et al. (16) reported that maximal plasmolysis reduced the cell volume by 20%. Presumably, this represents the portion of a deplasmolyzed cell which is filled with the external concentration of sucrose. If 30% of the cell volume is external to the plasma membrane, as Myers et al. also reported, then the 20% figure represents about 30% of the internal volume. Sucrose in this volume might be expected, in addition to lowering the water activity, to increase the viscosity (0.5 M sucrose has a viscosity 50% higher than water) and decrease the dielectric constant of the cell sap. These changes in turn would decrease the expected rate of diffusion and increase the effects of ions and exposed charge groups on their surroundings. Processes for which these are rate-limiting should have been altered. One can note that diffusion rates in water have been shown to be only marginally compatible with the synthetic rates observed in bacteria when the cell is assumed to consist of enzymes in solution (19). Perhaps some internal organization exists and the synthetic processes measured in this study took place in environments other than those containing the sucrose.

The transient decrease in OD as well as transient changes in appearance may have been a combination of the cells changing volume as sucrose leaked in, together with some internal rearrangements as osmoadaptation took place. Electron microscopy could, perhaps, provide some necessary evidence for this.

The cause of the loss in viability at the higher concentrations of sucrose is unknown. It is interesting that the decrease in colony-forming ability began near 0.6 M sucrose, whether or not nutrients were present when sucrose was added (24). This concentration also represents the point at which Myers et al. (16) claimed that the plasmolytic volume decrease reached a maximum. Perhaps at higher osmolarities normally bound water is removed and leads to irreversible changes in macromolecular structure.

ACKNOWLEDGMENTS

Assistance in the early stages of this investigation was provided by Fred Henry, Kathy Hird, and Robert Goldstein.

This work was supported by grants to the department by the National Aeronautics and Space Administration (NGR 39-009-008 to E. C. Pollard) and by Public Health Service training grant GM-01015 from the National Institute of General Medical Sciences.

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