Analysis of Growth Rate in Sucrose-Supplemented Cultures of \textit{Streptococcus mutans}

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In the presence of sucrose, \textit{Streptococcus mutans} grows in large glucan-containing aggregates. Because of reports of linear rather than exponential growth of sucrose-grown cultures, the kinetics of growth of sucrose-grown cultures of \textit{S. mutans} strain OMZ-176 were compared with those of glucose-grown cultures. Culture turbidity measurements indicated that growth of sucrose cultures was slower, did not follow exponential kinetics, and slowed and stopped at lower absorbance values than did glucose-grown cultures. However, measurements of the rates of accumulation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein using fully equilibrated radioactively labeled precursors of each of these macromolecular species in sucrose and glucose-grown cultures showed that: (i) for glucose cultures the synthesis of each of the three informational molecules occurred at the same exponential rate, which was identical to the rate of turbidity increase; (ii) for sucrose cultures each macromolecular species was synthesized at the same exponential rate and these rates were identical to the rate of increase of turbidity of the glucose-grown culture for periods of up to 7 h. Furthermore, the ratios of DNA to RNA, RNA to protein, and protein to DNA for the sucrose cultures were identical to those for the sucrose cultures for up to 10 doublings. From these data it was concluded that in the presence of sucrose \textit{S. mutans} grows in a balanced fashion at the same exponential rate as it does in glucose. The deviation from an exponential growth model of the absorbance in sucrose cultures was attributed to an optical artifact due to the formation of large glucan-containing aggregates of cells. The addition of dextranase to sucrose cultures resulted in cultures which increased in turbidity at the same exponential rate as glucose-grown cultures, without affecting the rate or extent of macromolecular synthesis.

When cultures of \textit{Streptococcus mutans} are grown in culture media containing sucrose as an energy source, large amounts of extracellular glucan are formed, which results in cells in large aggregates. Studies of the kinetics of growth were interpreted to indicate that growth in sucrose-containing media was linear as compared with exponential growth in media containing carbohydrates other than sucrose (14). The inference was made that some sort of diffusion barrier was present around the glucan-containing cell aggregates, thereby preventing the expression of exponential growth kinetics in the entire cell population. This finding, if true, has far-reaching consequences in studies of the physiology of plaque-forming, cariogenic streptococci. Linear growth implies that the cell clumps are heterogeneous, perhaps consisting of an assortment of growing and dividing cells responsive to the external environment and of some internal, nongrowing cells. Since large differences are observed in response to antimicrobial agents between growing and nongrowing streptococci (11, 12, 13), any therapeutic approach directed against glucan and plaque-forming streptococci would have to take into account the possibility of such a heterogeneity of cell populations. The view that oral streptococci grow linearly in sucrose was reinforced in the study mentioned above, by the suggestion that in media supplemented with sucrose linear kinetics were also obtained for intact in vitro plaque deposited on glass rods (14).

In this study, we have reexamined the hypothesis of Tanzer et al. (14) using \textit{S. mutans} strain OMZ-176. The growth kinetics of this organism do differ when sucrose is substituted for glucose in a chemically defined medium. With strain OMZ-176, the rate of increase of culture turbidity is slower and is relatively more limited in extent in sucrose- than in glucose-containing media. However, visible cell aggregates appear in sucrose-containing media, which makes it difficult to interpret growth
determinations based on optical absorbance measurements. In the present study we have added labeled specific precursors of macromolecules to the media and find that the kinetics of growth in sucrose are exponential and undistinguishable from those obtained in glucose for up to 10 doublings. Thus, the results presented here fail to confirm the findings of Tanzer et al. (14) for S. mutans strain OMZ-176 growing in liquid media.

**MATERIALS AND METHODS**

Organism and growth conditions. S. mutans strain OMZ-176 (Bratthall serotype d [1]) was obtained from B. Guggenheim (University of Zurich, Zurich, Switzerland) and was maintained in the lyophilized state. Experiments were initiated by first growing the lyophilized cultures on Todd-Hewitt agar overnight and then inoculating the organisms into a tube containing chemically defined medium (15) which was again incubated overnight. This overnight culture was then inoculated (0.25 ml) into a second tube (15 by 250 mm) containing 10 ml of chemically defined medium, and this culture was grown to an adjusted optical density at 675 nm (AOD; 17) of 300 to 600. Such a culture is one which is well into the exponential growth phase (15). AOD units are used to correct measurements of bacterial turbidity to agree with Beer’s law (17). As measured, 1 AOD unit corresponds to 0.39 μg of bacterial dry weight per ml of culture (15). Cultures were grown using either glucose or sucrose as an energy source at concentrations indicated in the various experiments. Unless otherwise stated, cultures were mixed vigorously for about 3 s in a Lab-line Super Mixer (Arthur H. Thomas Co., Philadelphia, Pa.) before each sampling or absorbance determination (about every 20 to 30 min).

Dextranase (EC 3.2.1.11, from Penicillium sp.) was purchased from Worthington Biochemical Corp. Standard methods were used for statistical analyses which were performed with the PDP-8 minicomputer.

Incorporation of isotopically labeled compounds. For all experiments, fully equilibrated labeling conditions were used in order that rates of increases in radioactivity would fully reflect rates of synthesis of cellular macromolecules (8). Thus, the overnight cultures described above were grown in the presence of a well-defined specific activity of 14C- and/or 3H-labeled precursors and transferred to culture tubes containing exactly the same specific activity of that compound. For measuring the incorporation of radioactive precursors into cellular macromolecules, 0.5-ml aliquots were withdrawn at intervals and added to tubes containing 5 ml of ice-cold 10% trichloroacetic acid. The tubes were mixed and left standing on ice for at least 30 min and no more than 24 h. Trichloroacetic acid precipitates were collected on glass-fiber filter disks (Reeve Angel 984-H) and the filters were washed twice with 5 ml of cold 10% trichloroacetic acid and twice with 5 ml of absolute ethanol. The dried filters were placed in scintillation vials, 0.5 ml of 90% NCS (Amersham/Searle) was added, and the vials were incubated overnight at 37 C. A toluene-based scintillation fluid (5 ml) was added (8), and the samples were counted in a Nuclear Chicago Mark I scintillation counter set for dual labels and yielding efficiencies of about 50% for 14C and 20% for 3H. A barium external standard was used to correct for quench and an appropriately programmed Digital Equipment Co., PDP 8/m computer was used to convert counts per minute to disintegrations per minute. All labeled compounds were obtained from Amersham/Searle and were used at the following levels and specific activities: L-[3H]leucine, 0.5 μCi/ml, 0.025 μCi/μg; [3H]uracil, 0.25 μCi/ml, 0.0125 μCi/μg; [14C]uracil, 0.1 μCi/ml, 0.005 μCi/μg; [14C]thymidine, 0.2 μCi/ml, 0.013 μCi/μg. The specificity of each labeled precursor was determined and found to be as follows: [3H]leucine into protein, 78 to 80% resistant to 90 C for 30 min in 10% trichloroacetic acid; [3H]- or [14C]uracil into ribonucleic acid (RNA), 85 to 87% solubilized at 90 C for 30 min in 10% trichloroacetic acid, or by incubation at room temperature in 0.3 M KOH for 17 h; [14C]thymidine into deoxyribonucleic acid (DNA), 95 to 98% solubilized at 90 C for 30 min in 10% trichloroacetic acid, and 98% resistant to incubation at room temperature in 0.3 M KOH for 17 h.

**RESULTS**

Turbidimetric measurements of growth of glucose- and sucrose-grown cultures. The growth of cultures of S. mutans OMZ-176 in chemically defined culture medium containing 2% glucose (glucose culture) or 2 or 4% sucrose (sucrose cultures) as measured by increase in turbidity (AOD) are shown plotted on both linear (Fig. 1A) and semilogarithmic (Fig. 1B) scales. In our hands, turbidity measurements of sucrose cultures were somewhat more erratic than those of glucose cultures, and were attributed to the presence of grossly visible aggregates of cells (Fig. 2B and C) after about 180 min in the sucrose cultures. These aggregates were absent in parallel glucose cultures (Fig. 2A). The turbidity of the glucose culture increased in good agreement with an exponential growth model (Fig. 1B), at a doubling time (Tb) of 68 min. In contrast, the observed turbidity increases of both sucrose cultures were at substantially slower rates and appeared to agree with either a linear (Fig. 1A) or exponential (Fig. 1B) growth model.

The statistical analyses of this (Fig. 1) and another similar experiment (Fig. 3) are shown in Table 1. The analyses indicate that for the glucose cultures a clear choice can be made.
 Volunteers for the two experiments was substantially the error provide probability level. Thus, the statistical sucrose cultures, a termination for the somewhat less definitive.

The coefficients of determination (9) were high (137.2 and 129.4, respectively), indicating that the two models differ from each other at the 0.1% probability level. For the linear growth model, the variance ratio (5.02) did not indicate a significant difference at the 0.1% probability level. Thus, the statistical analyses provide a clear choice in favor of an exponential growth model for the sucrose cultures and a less clear choice favoring an exponential growth model for the 2% sucrose culture, but they cannot distinguish between the two growth models for the 4% sucrose culture.

In the experiments shown in Fig. 1, sucrose cultures were inoculated with cells which were in exponential growth in glucose media for 290 min. Transfers of cells from glucose to sucrose cultures at earlier times yielded qualitatively similar results but were difficult to interpret quantitatively because of variations in lag times before resumption of growth in either glucose or sucrose media.

Often a clear-cut distinction may not be made easily between linear and exponential growth kinetics when observations are limited to one to two doublings (7). With batch cultures, dilutions of growing cultures can be used to prolong the exponential growth phase for an additional time interval. In the experiment shown in Fig. 3, 2% glucose and 2% sucrose cultures were inoculated from a glucose culture and grown as described above. At 200 min a portion of each culture was diluted (1:7) into fresh medium containing the same sugar. After dilution, the glucose culture continued to increase in turbidity at essentially the same rate as the first culture (T_D = 65 to 68 min). Both the initial and the secondary sucrose cultures increased in turbidity at a relatively rapid rate to an AOD of 200 to 250, after which the rate of turbidity rise slowed substantially. A similar decrease in rate of turbidity increase was seen in the experiment shown in Fig. 1B. Such observations raised the possibility that the marginal fit of the sucrose culture data to an exponential growth model was due to some change in the culture that occurs at a rather constant cell density level. The result would be an anomalous response to turbidity measurements. In this respect, it is also worthwhile to note that the 1:7 dilutions of both the glucose culture (at AOD 630) and the sucrose culture (at AOD 380) resulted in nearly the same initial turbidity reading (AOD 95 and 97, respectively) after dilution (Fig. 3A).

Measurements of the rate of incorporation of labeled precursors into DNA RNA and protein. In the same experiment (Fig. 3) the rates of incorporation of [3H]uracil and [14C]thymidine into trichloroacetic acid-precipitable material were measured in both the primary cultures and their dilutions. In both the glucose and sucrose cultures, the rate of incorpo-
Fig. 2. Comparison of cells growing in 2% glucose (A), 2% sucrose (B), and 4% sucrose (C). Dextranase treatment (50 µg/ml) of a 2% sucrose culture gave the results shown in (D) at 225 min.

The incorporation of [3H]uracil into RNA was clearly (i) exponential with a rate constant (λ) of 0.69/h; (ii) the same in both cultures and their dilutions; and (iii) the same as the rate of turbidity increase of the glucose culture (Fig. 3A). Therefore, the data indicate that despite the deviations in turbidity measurements, sucrose cultures synthesize RNA at a constant and relatively rapid rate for a period of nearly 7 h and for six doublings of RNA content.

A plot of the incorporation of [3H]uracil against turbidity increase (Fig. 3C) shows that for the glucose-grown culture and its dilution 125 dpm of [3H]uracil were incorporated per AOD unit. A much faster rate of [3H]uracil increase per AOD unit was seen in the 2% sucrose cultures. The calculated ratio for the sucrose culture was 245 dpm/AOD unit. However, the data showed a poor fit to a linear equation, presumably due to the biphasic nature of the absorbance curve (Fig. 3A). The value of such coordinate plots is discussed below.

The incorporation of [14C]thymidine into
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FIG. 2. C and D
Table 1. Comparison of linear to exponential growth models in glucose- and sucrose-supplemental cultures

<table>
<thead>
<tr>
<th>Plot</th>
<th>Expt 2 (Fig. 1)</th>
<th>Expt 2 (Fig. 3)</th>
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<td>2% Sucrose</td>
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<tr>
<td>No. of observations</td>
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<td>16 16</td>
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<td></td>
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<td>99.8 96.4</td>
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<td>134.65 11.48</td>
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<td>log 0.05 1.01 1.08</td>
<td>1.04 1.11</td>
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<tr>
<td>Variance ratio&lt;sup&gt;a&lt;/sup&gt;</td>
<td>lin 137.15&lt;sup&gt;b&lt;/sup&gt; 10.61&lt;sup&gt;b&lt;/sup&gt; 5.02</td>
<td>129.47&lt;sup&gt;b&lt;/sup&gt; 10.34&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>log 0.05 1.01 1.08</td>
<td>1.04 1.11</td>
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<sup>a</sup> See reference 9.
<sup>b</sup> Significantly different at the 0.1% probability level.

Fig. 3. (A) Effects of dilution on absorbance increase (○) and on incorporation of uracil into RNA (●) in cultures containing 2% glucose. (B) Absorbance (△) and RNA (▲) in cultures containing 2% sucrose. At 200
DNA followed the same pattern as \(^{[\text{H}]}\)uracil into RNA. This is shown in the graph of thymidine incorporation against uracil incorporation for both glucose and sucrose cultures (Fig. 3D). From this experiment it is evident that in both primary cultures and their dilutions RNA and DNA synthesis occurred coordinately.

From the results presented above it seemed likely that turbidity measurements of sucrose culture were affected by factors unrelated to cellular mass and were, therefore, not truly an index of increase in cellular mass. However, it remained possible that despite the rapid, exponential, and coordinate increases in RNA and DNA, other macromolecular parameters, which contribute significantly to cellular mass, do not increase in the same manner. In this way cellular mass could increase at a slower rate than the RNA or DNA content. Therefore, glucose and sucrose cultures were grown as described above, but in the presence of \(^{[\text{H}]}\)leucine and \(^{[\text{C}]}\)uracil or \(^{[\text{C}]}\)thymidine, so that the relationship between protein synthesis and RNA and DNA synthesis could be determined.

For these experiments, plotting the incorporation of \(^{[\text{H}]}\)leucine against incorporation of \(^{[\text{C}]}\)uracil (Fig. 4B) and \(^{[\text{H}]}\)leucine against \(^{[\text{C}]}\)thymidine incorporation (Fig. 4D) clearly shows that, for both glucose and sucrose cultures, (i) leucine was incorporated into protein at the same differential rate as uracil into RNA and thymidine into DNA, and (ii) these differential rates were the same for both cultures and their 1:5 dilutions. In contrast, differential plots of \(^{[\text{H}]}\)leucine versus AOD (Fig. 4A) and \(^{[\text{C}]}\)thymidine versus AOD (Fig. 4C) showed that the incorporation of both \(^{[\text{H}]}\)leucine and \(^{[\text{C}]}\)thymidine per AOD unit were higher (about 3 and 1.4 times, respectively) in the sucrose culture than they were in the glucose culture. Growth of cultures in media containing 4 and 6% sucrose yielded results essentially identical to those shown in Fig. 4 for 2% sucrose cultures.

**Growth and macromolecular synthesis in undisturbed and in anaerobic cultures.** In the experiments described above, cultures were mixed before each sampling and turbidity measurement. Such mixing would enhance dispersal of cells growing in large aggregates in sucrose cultures and could have promoted penetration of precursors into large aggregates of cells. Therefore, experiments were also performed with undisturbed cultures. For this purpose glucose-grown exponential-phase cells were inoculated into 40 ml of 2% sucrose medium containing \(^{[\text{H}]}\)leucine and \(^{[\text{C}]}\)thymidine. Immediately after inoculation and mixing, a series of 0.5-ml samples was withdrawn and each was placed into a tube (18 by 150 mm) and incubated without further disturbance and in parallel with the master culture. One series of 0.5-ml aliquots was incubated aerobically while the other series was incubated anaerobically (95% \(\text{N}_2\), 5% \(\text{CO}_2\)). In both series of undisturbed samples large, apparently glucan-associated clumps of cells adhering to the bottom or side of the tube were observed as growth proceeded. At intervals, a 0.5-ml sample was removed from the master culture and added to 5 ml of cold 10% trichloroacetic acid, and 5 ml of 10% trichloroacetic acid was added to one tube containing 0.5 ml of the aerobic culture and to one tube containing the anaerobic culture. The relative rates of incorporation of the two labeled precursors into protein and DNA, respectively, were identical for both the mixed master culture and the undisturbed anaerobic culture versus an undisturbed aerobic culture (Fig. 5B) and did not significantly deviate from an expected regression line with a slope of 1.0. In another experiment disturbed aerobically grown cultures showed the same rate of leucine incorporation as undisturbed aerobic cultures (Fig. 5B).

**Effect of dextranase on turbidity increase and macromolecular synthesis of sucrose cultures.** The major difference between glucose and sucrose cultures appeared to be only for increase in culture turbidity. Since cells aggregate into large clumps in sucrose but not in glucose cultures (Fig. 2A, B, and C), the effect of adding dextranase to the cultures was examined. Dextranase had no effect on either turbidity increase or \(^{[\text{H}]}\)leucine incorporation of glucose cultures (Fig. 6A). With the sucrose cultures, however, dextranase increased the rate of turbidity increase to a rate that was clearly

\[ \text{min dilutions were made from each culture into 2% glucose and 2% sucrose, respectively. Lines were obtained by regression analysis with an exponential growth model. The doubling time for RNA synthesis in the glucose cultures was 71 min. The doubling times for RNA synthesis in the sucrose cultures were 72 and 70 min for the primary culture and its dilution, respectively. See Table 2 for statistical analysis of the absorbance data for the first portion of the absorbance curves. (C) Coordinate plot relating uracil incorporation into trichloroacetic acid precipitates to increase in absorbance for glucose- (•) and sucrose- (△) supplemented cultures. The line for the sucrose culture is the best linear fit through all points. The slope of the regression line was 125 dpm of uracil/AOD for the glucose and 245 dpm of uracil/AOD for the sucrose-supplemented culture. (D) Coordinate plot relating uracil incorporation into trichloroacetic acid precipitates to thymidine incorporation into trichloroacetic acid precipitates. Symbols as in (B). The slope of the regression line was 12.5 dpm of uracil/dpm of thymidine for the glucose, and 11.6 dpm of uracil/dpm of thymidine for the sucrose-supplemented culture.} \]
Fig. 4. Coordinate plots of leucine incorporation versus absorbance (A), and leucine incorporated versus uracil incorporated (B). Symbols are as in Fig. 3B. The culture was followed for a total of 430 min. (A) The slope of the regression line is 192 dpm of leucine/AOD for the glucose-supplemented culture and 676 dpm of leucine/AOD for the sucrose-supplemented culture. (B) The slope of the regression line is 4.17 dpm of leucine/dpm of uracil for the glucose-supplemented culture and 4.07 dpm of leucine/dpm of uracil for the sucrose-supplemented culture. Coordinate plots of thymidine incorporation versus AOD (C) and thymidine incorporation versus leucine incorporation (D). Symbols are as in Fig. 3B. The lines were determined by regression analysis. The cultures were diluted once and were observed for 270 min equivalent to four exponential mass doublings. (C) The slope of the regression line was 144 dpm of thymidine/AOD for the glucose-supplemented culture and 176 dpm of thymidine/AOD for the sucrose-supplemented culture. (D) The slopes were 6.2 and 5.8 dpm of thymidine/dpm of leucine for the glucose and sucrose cultures, respectively.

Fig. 5. (A) Analysis of leucine (○) incorporation in 2% sucrose-supplemented cultures sampled every 20 min (disturbed) versus cultures distributed into 0.5-ml aliquots at the time of transfer to sucrose (undisturbed). The slope of the regression line is 0.931. (B) Comparison of leucine (○, ■) and thymidine (○, □) in-
exponential (Fig. 6B) and the same as the rate of turbidity increase of the glucose culture. Phase microscopy showed that the dextranase-treated culture no longer contained the very large aggregates (Fig. 2D) of cells present in the untreated culture (Fig. 2B).

**DISCUSSION**

At low culture densities, sucrose cultures appeared to increase in turbidity at the same rate as glucose cultures. However, when sucrose cultures reached densities of between 160 and 250 AOD units, the rate of increase in turbidity decreased. Since in individual experiments the phenomenon was observed at about the same culture density both in primary cultures (inoculated from glucose cultures) and in subcultures (dilutions from sucrose cultures into fresh sucrose media), the data rule out the possibility of a phenomenon related to prior growth in glucose (Fig. 1A, Fig. 3A, and Fig. 5B). From the size of the inoculum used, the primary 2% sucrose culture would contain 0.25 µg of glucose/µl, and the dilution would contain 0.05 µg of glucose/µl. Glucose concentration limits the growth rate of strain OMZ-176 in this medium in a linear manner at concentrations below 10 µg/µl (unpublished observations). Further, in many repeated experiments inoculation of cells into media containing 2 to 6% sucrose did not show any immediate absorbance change (see for example Fig. 1A).

The reduction of rate of increase in turbidity of sucrose cultures was accompanied by the appearance of cell aggregates, presumed to be due to the synthesis of extracellular glucan (Fig. 3B and C). However, the presence of large aggregates of cells did not impair either the rate or the levels of RNA synthesis in sucrose-supplemented cultures (Fig. 3A). These results therefore suggest that during a period of observation ranging from 4 to 10 doublings in RNA content, all of the cells in sucrose cultures are capable of RNA synthesis. If some cells were impaired or unable to synthesize RNA, the rate and eventually the extent of RNA synthesis would be affected. This point will be discussed in detail below.

The sudden appearance of "dead" cells in a culture can be readily estimated (3). With a large inoculum over a short period of observation the synthetically inactive cells \((n_D)\) will reduce the rate of any macromolecular synthesis \((k)\) expected from live, active cells \((n_L)\) to a new rate of synthesis \((k_{obs})\), as follows:

\[
n_D/n_L = (1 - k)/k_{obs} \times 100
\]

In the experiments presented here, sucrose cultures exhibited the same rate and levels of RNA synthesis throughout any experiment (Fig. 3A). Thus, in terms of RNA synthesis, sucrose cultures do not contain a detectable level of "dead" cells.

The production throughout the experiment of any inactive fraction of cells in sucrose but not in glucose cultures can also be ruled out. Such an event would lower net rates of macromolecu-

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Fig. 6. Increase in absorbance (O) and leucine incorporation (●) in the absence and presence of dextranase (100 U/ml). (A) 2% glucose- and (B) 2% sucrose-supplemented cultures. Cultures were diluted once at 70 min. The solid lines were drawn by regression analysis with an exponential growth model.
lar synthesis in sucrose cultures as compared to glucose cultures. The rates of incorporation of uracil into RNA in both glucose and sucrose cultures were identical over a period of 7 h (Fig. 3A). The rates of incorporation of thymidine into DNA were also the same in the two cultures, as indicated by the coordinate plot (Fig. 3C). Since both the glucose and the sucrose-supplemented cultures were obtained from an identical inoculum, identical rates of RNA and DNA synthesis indicate that the same proportion of cells continue to synthesize these macromolecules in the glucose- and sucrose-supplemented cultures throughout the 7 h of observation. This conclusion is unexpected in view of the large aggregates observed in sucrose-supplemented cultures (Fig. 2B and C). These aggregates could be thought to provide a restriction to the diffusion of precursors into cells. It is entirely possible that over long periods of observation (not studied here) sucrose-supplemented cultures may eventually contain an inactive fraction of cells.

In spite of some apparent deviation in rate of absorbance increase in sucrose cultures, the evidence presented here for RNA, protein, and DNA synthesis demonstrates that the exponential growth rate constant (λ) of the glucose and sucrose cultures is the same. A formal proof of this statement is given immediately below.

Figures 3C, 4B, and 4D show that the RNA/DNA, protein/RNA, and DNA/protein ratios are essentially identical for glucose and sucrose culture. It has been shown by Koch (6) that the RNA/protein ratio is proportional to λ. This finding is a consequence of the constant ribosome efficiency hypothesis of Schaechter et al. (10). In a balanced culture in exponential growth:

\[ d[p]/dt = \lambda[p] \]  
(2)

where \( d[p]/dt \) represents the rate of protein synthesis per unit time and \( [p] \) represents protein content. By definition (2), in a balanced culture all extensive parameters increase at the same rate, therefore in terms of RNA content, \([r]\):

\[ d[p]/dt = k_p[r] \]  
(3)

where \( k_p \) is a proportionality constant representing the rate of protein synthesis per unit RNA. From equations 2 and 3:

\[ [r]/[p] = \lambda/k_p \]  
(4)

where \( k_p \) represents the ribosome efficiency in protein synthesis and is constant at all growth rates (10). For the experiment shown in Fig. 4B, \([r]/[p]\) is identical for both the glucose and sucrose cultures, indicating that \( \lambda \) is also identical for the two cultures.

The same treatment applied to the data in Fig. 3C demonstrate that the DNA/RNA (\([d]/[r]\)) ratio is also identical for glucose and sucrose cultures, so that the rate of RNA synthesis/DNA \((k_r)\) is also the same for both cultures.

Last, the DNA/protein (\([d]/[p]\)) ratio is thought to be constant at various growth rates in bacterial cultures (10). If the assumption is made that in this organism, as in many other bacteria, protein synthesis is required for the initiation of chromosome replication, then

\[ [d]/[p] = \lambda/k_d \]  
(5)

where \( k_d \) is a proportionality constant representing the rate of DNA synthesis/unit of protein. The \([d]/[p]\) ratios are identical for glucose and sucrose cultures as shown in Fig. 4D; therefore, \( k_d \) is also the same for both cultures. This argument rests on the assumption that sucrose cultures are in balanced growth, defined as a situation where all measurable extensive properties increase at the same rate (2). The data shown here are restricted to demonstration of balanced synthesis of DNA, RNA, and protein, which represent about 60% of the dry weight in an average streptococcus (16).

Comparison of the rate of macromolecular synthesis in sucrose and glucose cultures appears to provide an index of the proportion of actively metabolizing ("growing") cells. Where no substantial difference is found, the conclusion is that essentially all cells are "growing." Further characterization of the culture can be obtained from a determination of the RNA/protein ratio at any one point, since if all cells are growing, then this ratio uniquely describes the growth rate constant (\( \lambda \)) of the culture.

If the growth rate constant \( \lambda \) is the same in glucose and sucrose cultures, the slower rate of turbidity increase in sucrose cultures can not be representative of mass, and may be due to formation of cellular aggregates in the cultures. Koch (5) has observed that the most important curvature in a standard curve relating absorbance to mass for bacterial suspensions is due to light scattering. All other conditions being equal, the larger the particle, the greater is the deviation from Beer's law, expressed as a bending of the standard curve. In the present context, the simplest hypothesis is that after some time in sucrose media, cell clumps begin to appear due to the formation of extracellular glucans. These clumps grow in size with time and increase the deviation from Beer's law for the absorbance measurements. In agreement with this view, dilution of sucrose cultures appears to reduce the deviation between the absorbances of glucose- and sucrose-supplemented cultures (Fig. 3A and 4A).
This hypothesis was tested by the addition of dextranase. In agreement with the results of Gibbons and Fitzgerald (4) there was a loss of clumps after addition of the enzyme (Fig. 6). Concomitantly, with disappearance of clumps, the rate of absorbance increase in sucrose culture increased and was found to parallel the rate of leucine incorporation into protein. Therefore, these data show that in S. mutans a true index of mass increase can be obtained by dextranase treatment to prevent the formation of aggregates.

These studies also show that vigorously mixed aerobic cultures, stationary aerobic cultures, and anaerobic cultures all incorporate thymidine and leucine at the same rates. Therefore, over the time of observation of these experiments, all cells under all three conditions exhibit exponential rates of protein and DNA synthesis, and presumably also of RNA synthesis.

The present findings indicate that aggregates can contain cells capable of balanced exponential growth. Further, we show that under our conditions all the cells of the aggregates are growing. Thus, aggregation per se does not preclude the possibility for all cells to grow in a balanced manner. It is entirely possible for a bacterial culture to contain a heterogeneous cell population (consisting of growing and non-growing cells), or to contain unbalanced cells, either when dispersed or in aggregates. Studies of cells growing in large aggregates for long time intervals are in progress.

The findings presented here indicate that a glucan-producing, plaque-forming strain of S. mutans grows exponentially in sucrose with an exponential growth rate constant (and therefore with a doubling time) identical to that obtained in glucose. In preliminary experiments, similar results were obtained with S. mutans strain OMZ-61 (unpublished data). The abnormally slow rate of absorbance increase in sucrose cultures appears to be due to a physical artifact. The present findings in broth cultures of OMZ-176 contrast with those of Tanzer et al. (14). Since turbidity measurements only were used by these investigators, it is entirely possible that their observed "linear" growth in broth cultures represent an extension of the physical artifact described here. Our findings using the incorporation of macromolecular precursors do not apply to their long-term observation of "linear" growth in plaques growing on glass rods, since both absorbance and DNA content were determined in those experiments (14).

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LITERATURE CITED