JOURNAL OF BACTERIOLOGY, Feb. 1978, p. 822-829
0021-9193/78/0133-0822$02.00/0

Vol. 133, No. 2
Printed in U.S.A.

Cellular Autolytic Activity in Synchronized Populations of *Streptococcus faecium*

ROBERT P. HINKS, L. DANEO-MOORE, AND G. D. SHOCKMAN*

Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Received for publication 2 September 1977

The autolytic capacity of *Streptococcus faecium* (*S. faecalis* ATCC 9790) varied during synchronous cell division. This phenomenon was initially observed in rapidly dividing populations (T_D = 30 to 33 min) synchronized by a combination of induction and size selection techniques. To minimize the problems inherent in studies of cells containing overlapping chromosome cycles and possible artifacts generated by induction techniques, the autolytic capacities of slowly dividing populations (T_D = 60 to 110 min) synchronized by selection only were examined. Although the overall level of cellular autolytic capacity was observed to decline with decreasing growth rate, sharp, periodic fluctuations in cellular autolytic capacity were seen during synchronous growth at all growth rates examined. On the basis of similar patterns of cyclic fluctuations in autolytic capacity of cultures synchronized by (i) selection, (ii) amino acid starvation followed by size selection, and (iii) amino acid starvation followed by inhibition of DNA synthesis, a link of such fluctuations with the cell division cycle has been postulated.

Bacterial cell division obviously requires coordination and regulation of many processes, including those related to chromosome replication and to various aspects of cell surface enlargement. Although the factors which are directly responsible for the regulation of cell division have not yet been precisely identified or, for that matter, sorted out from the very large number of factors which can indirectly affect morphogenesis and division, it seems highly likely that the bacterial cell surface is a key element in bacterial cell division. Certainly, the regulation of the assembly, modification, and degradation of the principal shape-maintaining wall component, peptidoglycan (PG), is essential for surface enlargement and division.

Many bacterial species contain PG hydrolase activities which, under appropriate conditions, can cause cellular lysis (5a). The presence of such activities in rapidly growing cultures led to the long-standing idea that precisely regulated PG hydrolase activities may play a role(s) in surface growth and division (18, 23, 28), and a number of potential functions for PG hydrolase activities have since been proposed (5a). Recently, data have been obtained which suggest that enzyme activities involved in PG hydrolysis, assembly, and/or modification can vary with respect to the cell division cycle in *E. coli* (1, 7, 8, 14, 16, 17, 30).

Streptococcus faecium (*S. faecalis* ATCC 9790) is particularly amenable to studies on PG hydrolase activity and wall growth. In this species, cell division occurs in only one plane (12); wall expansion results from localized equatorial assembly of precursors (11-13); the wall components do not turnover during growth (2); and only a single, autolytic PG hydrolase activity, an *N*-acetylmuramoylhydrolase, seems to be present (26). This PG hydrolase activity is primarily associated with newly synthesized wall (25) and provokes cellular lysis by dissolution of wall at nascent septa (10, 15). In this study, we have examined the capacity of synchronized, slowly growing and dividing populations of cells to autolyze when placed in an appropriate buffer. Slowly growing cells were used to minimize overlaps in replicating chromosomes and in the initiation of new septal wall growth sites. Evidence is presented that cyclic variations in cellular autolytic capacity occur during the cell division cycle of *S. faecalis*.

MATERIALS AND METHODS

Growth of cultures. For all experiments, *S. faecium* (*S. faecalis* ATCC 9790) was grown at 37°C in a chemically defined medium (22) containing L-tryptophan at 20 μg/ml to reduce chain formation (27). The medium was filter sterilized before use (0.45-μm-diameter pore size). Specific growth rates were obtained by adjusting the glutamic acid concentration in the absence of glutamine (28). With *S. faecium*,

growth rate in this medium is proportional to glutamate concentrations of up to 50 μg/ml (28). Furthermore, growth of glutamate-limited cultures, under the conditions used, is balanced in terms of DNA, RNA, protein, PG, and lipid synthesis (P. Lancy, Jr., Ph.D. dissertation, Temple University, Philadelphia, Pa., 1976).

Exponential-phase cultures grown at a doubling time (T_D) of 31 to 33 min were kept at 0 to 4°C and maintained as inocula by weekly transfer. Slowly growing cultures were obtained by transferring a 1:10,000 dilution of the inoculum into growth medium containing the desired quantity of glutamate. This culture was then grown at the desired rate to mid-exponential phase, chilled, and used for 1 week.

Growth was monitored turbidimetrically at 675 nm in a Coleman 14 Universal spectrophotometer (Coleman Instruments, Maywood, Ill.) in optically calibrated tubes (22). Optical density readings were adjusted to agree with Beer's Law according to Toennies and Gallant (22) and expressed as adjusted optical density (AOD). One AOD unit of exponential-phase cells (T_D = 31 to 33 min) is equivalent to 0.39 μg of cellular dry weight per ml.

Cultures were inoculated with freshly grown, mid-exponential-phase cells to an initial AOD of 1 to 5 and underwent at least five to six doublings in turbidity before the beginning of an experiment. Occasionally, growing cultures were diluted with prewarmed medium to allow an additional one to two doublings before the beginning of an experiment. Cultures growing at T_D values longer than 31 to 34 min were generally maintained between 150 and 300 AOD by the periodic addition of prewarmed medium containing the same glutamate concentration as originally present in the culture. This was done to insure maintenance of balanced exponential growth of these cultures (L. Daneo-Moore, unpublished observations).

Gradient selection. Cells were fractionated by centrifugation on sucrose gradients to obtain the smallest, presumably youngest cells in the population using a modification of the technique of Mitchison and Vincent (19). Discontinuous, exponential sucrose gradients were prepared 1 h before use as follows. A filter-sterilized solution of 35% (0.98 M) sucrose (Pfanstiehl Laboratories, Waukegan, Ill.) in 0.1 M sodium phosphate, pH 6.9, was diluted with 0.1 M sodium phosphate, pH 6.9, to obtain six solutions of sucrose: 15, 19, 22, 24.5, 25.5, and 26.5%. Twelve-milliliter layers of each concentration were pipetted gently and sequentially, beginning with the highest concentration, onto 12 ml of a 35% sucrose cushion into cellulose nitrate or polycarbonate centrifuge tubes (30 by 160 mm).

Cells from 250 ml of exponential-phase (250 to 260 AOD) or valine-starved (70 min; about 300 AOD; see below) cultures were chilled to 0°C, sedimented (3,000 × *g*, 15 min), and resuspended in 2 ml of 0.1 M sodium phosphate, pH 6.9 (28,000 to 30,000 AOD/ml), mixed vigorously with a Vortex mixer for 1 min, further disaggregated in a Sonicator water bath (30 to 45 s), and then layered on a gradient. Gradients were centrifuged in an International no. 269 head at 2°C at 3,000 rpm for 7 min and then 2,000 rpm for 11 min. After removal of the top 10 to 15 ml of clear sucrose, the next 5 ml of the visible cell band was removed and added to 50 ml of fresh, prewarmed growth medium. For exponential-phase cultures, the 5-ml fraction usually contained about 14 to 16% of the cellular turbidity added to the gradient (8,000 to 9,000 AOD), yielding an initial AOD of 145 to 155 in the resulting 55 ml of culture. In many experiments, larger initial cultures and multiple gradient tubes were used.

Valine starvation. Cultures were grown at a T_D (turbidity) of 70 to 80 min to about 300 AOD; the cells were harvested by filtration (0.65-μm-diameter pore size), washed twice with 5.0 ml of prewarmed growth medium lacking valine, and resuspended in the same final volume of medium lacking valine as that used for growth. The entire procedure took 2 to 5 min, with recovery of 80 to 95% of the initial cellular turbidity.

Inhibition of DNA synthesis following valine starvation. For these experiments, after incubation for 70 to 75 min in minus-valine medium, hydroxyphenylhydrazinopyrimidine (HPUra) (0.5×10^{-5} M, final concentration) and valine (200 μg/ml) were added, and the incubation at 37°C was continued for 25 min. This concentration of HPUra selectively prevented DNA synthesis for 30 to 40 min (unpublished results). The culture was again filtered, washed twice with 10.0-ml portions of prewarmed fresh growth medium containing valine and lacking HPUra, resuspended in twice the volume of fresh growth medium (150 to 200 AOD), and incubated at 37°C.

Cell counts. At intervals, 0.1-ml samples of cultures were added to 0.4 ml of 10.4% Formalin at room temperature. Cells were always counted immediately after an experiment was completed. After thorough agitation, 0.05 ml was added to a 30-ml plastic cup, and 10.0 ml of 0.9% sterile, nonpyrogenic saline was pipetted rapidly into the cup. At least two counts (and in many cases a second dilution and two more counts) were taken and averaged on each sample, using a Coulter Counter model B (Coulter Electronics, Chicago, Ill.) equipped with a 30-μm-diameter orifice and settings of 1/aperture current = 0.707, 1/amplification = ½, lower threshold = 6, and upper threshold = 105. The number of particles was maintained between 5,000 and 15,000 by dilution with a known volume of saline to avoid coincidence as particles passed through the orifice. The net number of counts was multiplied by the dilution factor (10,000) to obtain the approximate number of cell units per milliliter of culture.

Determination of cellular autolytic capacity. As described previously (21), samples yielding 250 to 300 AOD/ml in 6 ml of buffer were pipetted into an approximately equal volume of double-distilled water at 0°C. After chilling for 15 to 30 s, the cells were filtered (0.65-μm-diameter pore size), washed three times with distilled water at 0 to 4°C, resuspended in 6.0 ml of 0.3 M sodium phosphate, pH 6.5, with Vortex mixing, and placed in a 37°C water bath. Turbidity at 675 nm was monitored and the rate of cellular autolysis was determined from a logarithmic plot of turbidity loss with time. The slope of the exponential curve was used as the (pseudo) first-order reaction rate constant (k) in reciprocal hours.

RESULTS

Relationship of the turbidity of exponentially growing cultures to the expression of cellular autolytic capacity. Previous studies (24) suggested that the rate of autolysis of cells from rapidly growing (T_D = 33 min) exponential-phase cultures varied with the turbidity at which they were harvested. This phenomenon was reinvestigated with exponential cultures growing at doubling times close to, and somewhat longer than, the duration of the chromosome replication (C) and division (D) cycle (C + D = 75 to 77 min) in this organism (5a). For the five growth rates examined, rates of cellular autolysis remained constant between turbidity values of 150 and 300 AOD (Fig. 1).

Effect of growth rate on cellular autolytic capacity. Cells from cultures growing at a T_D = 95 min autolyzed more slowly than those growing at the faster rates (Fig. 1), and cells from all cultures examined in these studies autolyzed more slowly than those growing at faster rates studied previously (23, 24, 26). Comparisons of the autolytic rates of cells from balanced exponential-phase cultures growing at a series of different rates (Fig. 2) showed that cellular autolytic capacity decreased with decreasing growth rate. This relationship appeared to be biphasic, with a more pronounced exponential decrease in autolytic rates at T_D values longer than 85 or 95 min. Although a decreasing capacity of more slowly growing and dividing cells to autolyze suggests a relationship between these two factors (see Discussion), at present we have no explanation for the apparent biphasic exponential relationship. The changes in cellular autolytic rate with growth rate were carefully taken into account in all subsequent experiments

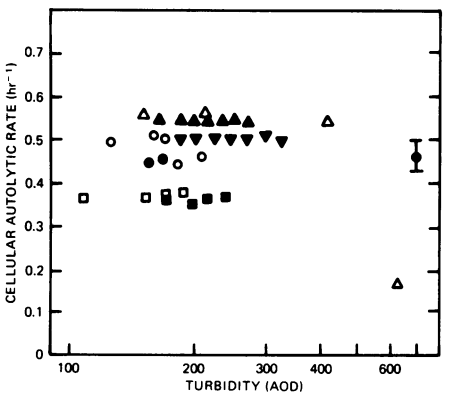

FIG. 1. *Rates of cellular autolysis as a function of culture turbidity (AOD) during asynchronous exponential growth. The symbols represent different experiments at various slow growth rates (determined from turbidity [AOD]). T_D = 70 min (△); 77 min (▲); 75 min (▼); 80 min (○, ●); 95 min (□, ■). In the latter two experiments, the open symbols represent the cellular autolytic rate prior to a dilution, and the closed symbols represent the rate after dilution. The bar defines the variability in cellular autolytic rate within the worst experiment* (○, ●).

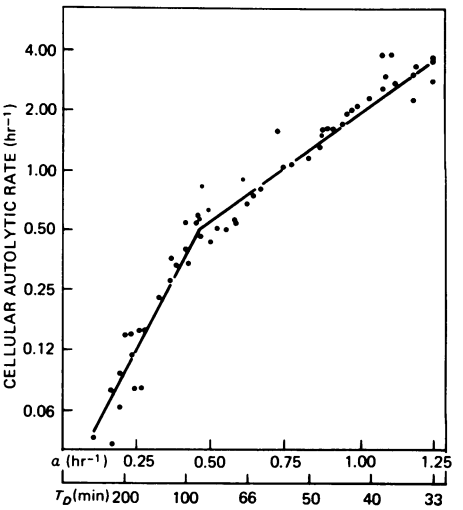

FIG. 2. *Relationship of rate of cellular autolysis to the rate of growth* (α) *of balanced exponential-phase cultures. The biphasic line was determined by exponential regressions through the points for T_D values between 33 and 100 min as well as 100 and 300 min. In each culture, growth rate was limited by the glutamate concentration as described previously (28).*

Rates of cellular autolysis during growth of synchronized cultures. Stepwise increases in the capacity of cells to autolyze were consistently observed in rapidly growing cultures recovering from 60 to 90 min of valine starvation. Such cultures were also partially synchronized for cell division as demonstrated by stepwise increases in cell numbers. Synchrony of cell division was improved when the small cells of valine-starved cultures were selected after gradient centrifugation and permitted to regrow (Fig. 3A). Again, stepwise increases in the capacity of cells to autolyze were seen (Fig. 3A). The pauses in increase in rate of cellular autolysis were observed at about the same time that the increase in cell number showed a plateau and commenced to increase again when cell division occurred. Such observations suggested a possible relationship between cellular autolytic capacity and cell division.

The chromosome replication time (C) in this species was determined to be 50 to 53 min (10); it is clear that cultures growing at a T_D of 32 min

contain overlapping chromosome cycles. To differentiate between chromosome cycles, cultures were grown at a T_D of 55 min, starved of valine for 70 min, and then regrown at a T_D of 55 min. Measurements of the capacity of cells to autolyze during regrowth clearly showed a sharp decrease in cellular autolytic capacity at about the time that cell numbers plateaued (Fig. 3B). Decreases in cellular autolytic capacity were noted during both the first and second synchronous divisions.

The decrease in cellular autolytic capacity accompanying valine starvation (20), and which accounted for the low rate of cellular autolysis of the initial samples in the experiments shown in Fig. 3, is also accompanied by numerous other metabolic changes (24). Consequently, it was deemed desirable to examine the autolytic capacity of cells synchronized simply by gradient selection from exponential-phase populations.

Small cells selected from sucrose gradient-fractionated cultures increased in cell number in a synchronized fashion (Fig. 4). Cells taken at various times during growth of such cultures consistently and reproducibly showed cyclic fluctuations in rates of autolysis which were far in excess of those expected from chance variability. A small portion of the variability could be attributed to the handling and centrifugation of cells through sucrose gradients (R. Hinks, Ph.D. dissertation, Temple University, Philadelphia, Pa., 1976). For example, samples taken during the first few minutes of growth recovery consistently autolyzed at slow rates. Similarly, cells fractionated by sucrose gradient centrifugation and recombined before regrowth also showed an initially depressed capacity to autolyze rapidly followed by an increase to the rate expected for cultures growing exponentially at that rate (R. Hinks, Ph.D. dissertation). After reaching their maximum rate, cells from reconstituted cultures (T_D = 73 min) failed to exhibit fluctuations in cellular autolytic rates of the magnitude shown in Fig. 4. Therefore, interpretations of results are all based on observations made after the initial rise in autolytic rates, and emphasis has been placed on events that occur in the second, presumably less disturbed, cell division cycle. After the initial rise in autolytic rate, fluctuations in cellular autolytic capacity correlated extremely well with the cyclic increases in cell numbers in cultures growing at a series of different growth rates (Fig. 5; T_D = 60 to 110 min) and with other events in the cell cycle (R. Hinks, L. Daneo-Moore, and G. D. Shockman, manuscript in preparation). Furthermore, other methods of synchronization of cell division were also accompanied by fluctuations in rates of cellular autolysis. Although rap-

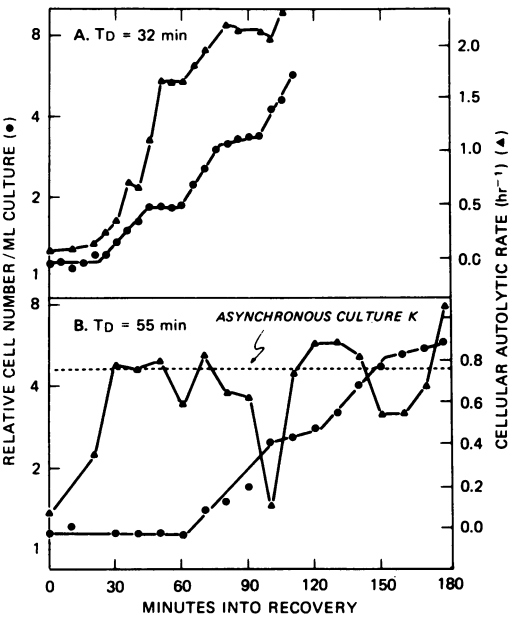

FIG. 3. *Cellular autolytic rate in cultures synchronized by valine starvation (70 min) and then selection of small cells after sucrose gradient centrifugation. (A) Cells growing in complete chemically defined medium at a T_D of 32 min were starved for valine for 70 min, and small cells selected by gradient centrifugation were regrown in complete medium. (B) Cells growing at a T_D of 55 min in a glutamate-limited medium were starved for valine and otherwise treated as indicated above. The dashed line indicates the rate of cellular autolysis (k) of an asynchronous exponential culture growing at a T_D of 55 min. The typical rate of cellular autolysis for a mid-exponential-phase culture growing at a T_D of 32 min is not shown but would be about 3.5 h^{-1}.*

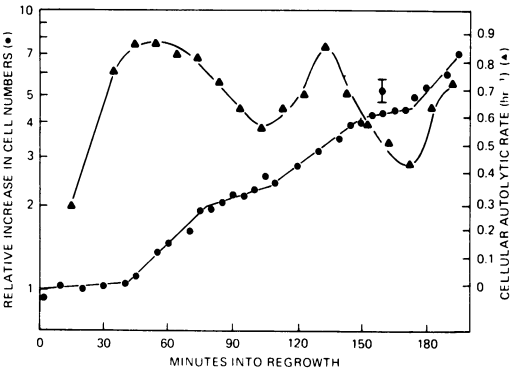

FIG. 4. *Cellular autolytic capacity of a gradient selected synchronous population growing at a T_D = 68 min. The bars denote the limits of variation possibly attributable to experimental error; the midpoint of the bars is set at the average cellular autolytic rate for this growth rate as estimated from Fig. 2.*

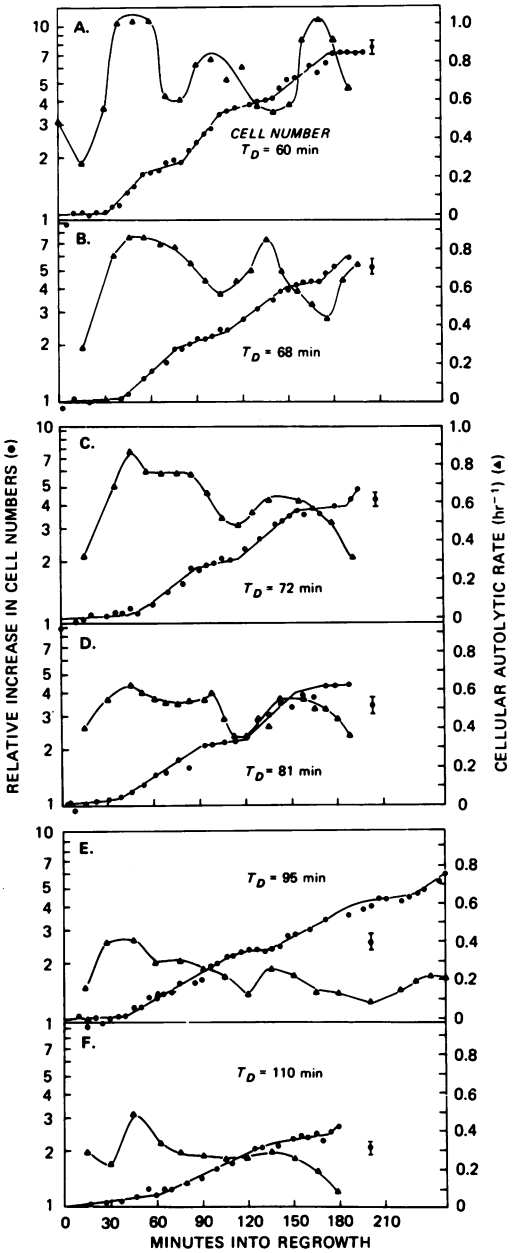

FIG. 5. *Patterns of autolytic capacity of gradient-selected, synchronous populations growing at a series of different rates. The cell number doubling times are indicated on each panel. All other conditions are identical to those of the experiment shown in Fig. 4.*

idly growing cultures (T_D = 33 min) could be partially synchronized by 70 min of valine starvation (a required amino acid; 22), slowly growing cultures required additional treatments.

Reasonably well synchronously dividing populations at T_D values of 70 to 90 min were obtained after valine starvation followed by gradient fractionation (Fig. 6B), or valine starvation followed by inhibition of DNA synthesis with HPUra (Fig. 6A). In both cases, cyclic variations in cellular autolytic rates closely resembled those observed for the exponential-phase, gradient-selected cells. Although some quantitative differences were noted for synchronized cultures obtained by all three methods, a generalized picture of the relationship of cellular autolytic capacity and cell division was observed. Generally, cellular autolytic activity declined sharply during the time that cell numbers were not increasing significantly (plateau), then increased at the time of rapid rise in cell numbers, and reached a maximum at about the beginning of the next plateau in cell numbers.

DISCUSSION

A distinct and consistent pattern of cyclic fluctuations in the capacity of cells to autolyze

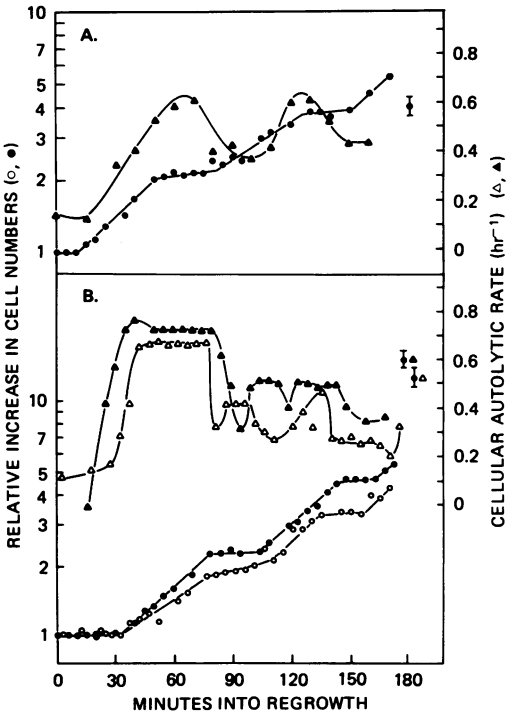

FIG. 6. *Patterns of cellular autolysis of: (A) cultures regrown after 70 min of valine starvation followed by 25 min of DNA inhibition with HPUra (turbidity [AOD] T_D = 75 min, cell number T_D = 68 min); and (B) culture regrown after 70 min of valine starvation followed by gradient selection. Two separate experiments are shown in B: closed symbols (turbidity [AOD] T_D = 73 min, cell number T_D = 55 to 73 min); open symbols (turbidity [AOD] T_D = 83 min, cell number T_D = 55 to 68 min).*

during the cell division cycle was observed. Cellular autolytic capacity generally increased during the rise in cell numbers and declined during the plateau. This pattern was observed not only for synchronized populations selected by sucrose gradient centrifugation grown at several different growth rates (Fig. 4 and 5), but also for cells synchronized by induction techniques (Fig. 3 and 6), one of which did not involve gradient centrifugation (Fig. 6A). It therefore seems unlikely that the overall pattern was due to artifacts induced by pretreatments. Furthermore, the constancy of this oscillatory pattern suggested a close coordination of autolytic capability with other events in the cell division cycle. The relationship of autolytic activity to wall growth during the cell cycle will be examined and discussed in a subsequent paper (R. Hinks et al., in preparation).

Although the mechanism of this apparently precise and rapid regulation of cellular autolytic activity is currently unknown, several possibilities can be considered. First, and least likely, is the idea that these cycles result from changes in rates of synthesis and degradation of the autolytic enzyme itself. Such a series of events seems unlikely because of the speed of the observed changes and because of previously demonstrated maintenance of levels of autolytic enzyme activity in disrupted cell preparations when the capacity of intact cells to autolyze decreased (20, 21). Second, and probably equally unlikely, is regulation at the stage of proteinase-catalyzed conversion of latent enzyme to the active form. Again, the speed of responses and observations that relative levels of the active and latent forms both remain at previous levels when the capacity of cells to autolyze decreased substantially (20) are not consistent with proteinase activation being the sole mechanism of regulation. Furthermore, although cellular autolytic capacity in the presence of 0.4 μg of trypsin per mg of cellular dry weight at three doubling times (33, 48, and 83 min) were each about twice the rates observed in the absence of trypsin, approximately proportional decreases in rates of autolysis with increased doubling times, similar to those shown for the same T_D values in the absence of trypsin, were seen (not shown). Third is the possibility that some cyclic mechanism may permit the more rapid hydrolysis of the wall PG substrate or may permit the hydrolysis of fewer bonds to result in destruction of cellular integrity. A topologically localized change in the structure or thickness of the PG which is later modified or repaired or in non-PG wall polymers which can affect hydrolytic activity could account for the observations. Evidence that increases in the extent of peptide cross-linking of PG of *S. faecium* occur after assembly (6) and that cyclic changes in the activity of enzymes considered to be involved in PG assembly, modification, and hydrolysis occur in *Escherichia coli* (8, 14, 16) are consistent with such a hypothesis. Fourth, and perhaps most likely, would be the cyclic influence of effector molecules which influence the activity of the autolytic enzyme. Such a mechanism is particularly attractive because of the rapidity and ease of reversibility of any action which affects the activity of an enzyme as well as, in this case, the necessity to regulate an activity which resides outside of the cellular permeability barrier (15). The speed of inhibition of cellular autolytic capacity while cells retain full levels of autolysin activity upon inhibition of protein synthesis (20), and the very rapid recovery of cellular autolytic capacity upon relief of inhibition of protein synthesis (20), are consistent with such a mechanism. Significant in this respect are the observations that two classes of membrane-associated compounds, lipoteichoic acids (3–5) and certain lipids, notably diphosphatidylglycerol (3, 5), have been shown to inhibit the action of the *N*-acetylmuramoylhydrolase of *S. faecium*.

Regulation of cellular autolytic capacity via the action of effector ligands on the activity of the *N*-acetylmuramoylhydrolase has an additional attribute. Multiple roles for this enzyme activity in surface expansion and modification as well as in the final stages of daughter cell separation have been postulated (2, 4, 5a). During the final stages of cell division, effector-mediated inhibition of *N*-acetylmuramoylhydrolase activity tightly bound to the inner wall surface could result in a change from a surface expansion mode of wall assembly to a mode resulting in cross wall closure (24) without inhibiting enzyme molecules bound to the outer wall surface that are dedicated to a residual role in the final stages of cell separation.

One or more of several factors could contribute to the observed decrease in cellular autolytic capacity with decreasing growth rate. These factors include: (i) as growth rate decreases, total autolysin content could be constant but a larger fraction could be in the latent (proteinase-activatable) form, resulting in the expression of the activity of fewer molecules in the active form. This does not appear to be the case for cultures growing at T_D values between 33 and 83 min, since rates of cellular autolysis in the presence and absence of trypsin decreased in parallel (not shown). (ii) If cellular autolytic capacity reached a fixed maximum level for a constant time interval in the cell cycle, then in an asynchronous population, as the doubling time and the length

of the cycle increased, a smaller fraction of cells would contain the elevated level so that the overall rate of cellular autolysis would decline. The studies of populations dividing synchronously at several rates (Fig. 5) showed that this does not seem to be the case. Although cyclic fluctuations were seen in all cultures, both maximum and minimum rates decreased with growth rate. A short period of a high rate of autolysis was not observed. (iii) The decrease in cellular autolytic rate with decreasing growth rate could be due to a change in amount or susceptibility of wall substrate. Lancy et al. (manuscript submitted for publication) showed that the relative amount of cellular dry weight accounted for as cell wall material increased as growth rate decreased. Thus, it is possible that the decreased rates of autolysis of cells might be accounted for by a need to hydrolyze more bonds to lyse each cell. Such a change in cellular composition, accompanied by a slower rate of overall protein synthesis and the consequent slower synthesis and accumulation of autolysin molecules, could account for the relationship observed. (iv) The relative amounts of inhibitory effector molecules in cells could increase as growth rate decreases. Data (Lancy et al., manuscript submitted for publication) consistent with increased levels of both cellular lipoteichoic acid and lipids as growth rate is decreased have been obtained. The relationship, if any, of the mechanism regulating levels of autolytic activity with growth rate to the mechanism of regulating activity in the cell division cycle is, at present, unknown. The evidence obtained so far suggests that such a relationship would be, at best, indirect.

ACKNOWLEDGMENTS

This research was supported by Public Health Service research grant AI-05044 from the National Institute of Allergy and Infectious Diseases. R.P.H. was a a predoctoral trainee supported by Public Health Service training grant GM-00983 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Beck, B., and J. T. Park.** 1976. Activity of three murein hydrolases during the cell division cycle of *Escherichia coli* K-12 as measured in toluene-treated cells. J. Bacteriol. **126**:1250–1260.
2. **Boothby, D., L. Daneo-Moore, M. L. Higgins, J. Coyette, and G. D. Shockman.** 1973. Turnover of bacterial cell wall peptidoglycans. J. Biol. Chem. **248**:2161–2169.
3. **Cleveland, R. F., L. Daneo-Moore, A. J. Wicken, and G. D. Shockman.** 1976. Effect of lipoteichoic acid and lipids on lysis of intact cells of *Streptococcus faecalis*. J. Bacteriol. **127**:1582–1584.
4. **Cleveland, R. F., J.-V. Holtje, A. J. Wicken, A. Tomasz, L. Daneo-Moore, and G. D. Shockman.** 1975. Inhibition of bacterial wall lysins by lipoteichoic acids and related compounds. Biochem. Biophys. Res. Commun. **67**:1128–1135.
5. **Cleveland, R. F., A. J. Wicken, L. Daneo-Moore, and G. D. Shockman.** 1976. Inhibition of wall autolysis in *Streptococcus faecalis* by lipoteichoic acid and lipids. J. Bacteriol. **126**:192–197.

5a. **Daneo-Moore, L., and G. D. Shockman.** 1977. The bacterial cell surface in growth and division, p. 597–715. *In* G. Poste and G. Nicholson (ed.), Cell surface reviews, vol. 4. Elsevier/North Holland Publishing Co., Amsterdam.

6. **Dezellee, P., and G. D. Shockman.** 1975. Studies of the formation of peptide cross-links in the cell wall peptidoglycan of *Streptococcus faecalis*. J. Biol. Chem. **250**:6806–6816.
7. **Hakenbeck, R., and W. Messer.** 1974. Activity of murein hydrolases and membrane synthesis in synchronized *Escherichia coli* B/r. Ann. Microbiol. Inst. Pasteur **125B**:163–166.
8. **Hakenbeck, R., and W. Messer.** 1977. Oscillations in the synthesis of cell wall components in synchronized cultures of *Escherichia coli*. J. Bacteriol. **129**:1234–1238.
9. **Higgins, M. L., L. Daneo-Moore, D. Boothby, and G. D. Shockman.** 1974. Effect of inhibition of deoxyribonucleic acid and protein synthesis on the direction of cell wall growth in *Streptococcus faecalis* (ATCC 9790). J. Bacteriol. **118**:681–692.
10. **Higgins, M. L., H. M. Pooley, and G. D. Shockman.** 1970. Site of initiation of cellular autolysis in *Streptococcus faecalis* as seen by electron microscopy. J. Bacteriol. **103**:504–512.
11. **Higgins, M. L., H. M. Pooley, and G. D. Shockman.** 1971. Reinitiation of cell wall growth after threonine starvation of *Streptococcus faecalis*. J. Bacteriol. **105**:1175–1183.
12. **Higgins, M. L., and G. D. Shockman.** 1971. Procaryotic cell division with respect to wall and membranes. Crit. Rev. Microbiol. **1**:29–72.
13. **Higgins, M. L., and G. D. Shockman.** 1976. Study of a cycle of cell wall assembly in *Streptococcus faecalis* by three-dimensional reconstructions of thin sections of cells. J. Bacteriol. **127**:1346–1358.
14. **Hoffman, B., W. Messer, and U. Schwarz.** 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli*. J. Supramol. Struct. **1**:29–37.
15. **Joseph, R., and G. D. Shockman.** 1976. Autolytic formation of protoplasts (autoplasts) of *Streptococcus faecalis* 9790: location of active and latent autolysin. J. Bacteriol. **127**:1482–1493.
16. **Mirelman, D., Y. Yashouv-Gan, and U. Schwarz.** 1976. Peptidoglycan biosynthesis in a thermosensitive division mutant of *Escherichia coli*. Biochemistry **15**:1781–1790.
17. **Mirelman, D., Y. Yashouv-Gan, and U. Schwarz.** 1977. Regulation of murein biosynthesis and septum formation in filamentous cells of *Escherichia coli* PAT 84. J. Bacteriol. **129**:1593–1600.
18. **Mitchell, P., and J. Moyle.** 1957. Autolytic release and osmotic properties of "protoplasts" from *Staphylococcus aureus*. J. Gen. Microbiol. **16**:184–194.
19. **Mitchison, J. M., and W. S. Vincent.** 1965. Preparation of synchronous cell cultures by sedimentation. Nature (London) **205**:987–989.
20. **Pooley, H. M., and G. D. Shockman.** 1970. Relationship between the location of autolysin, cell wall synthesis, and the development of resistance to cellular autolysis in *Streptococcus faecalis* after inhibition of protein synthesis. J. Bacteriol. **103**:457–466.
21. **Sayare, M., L. Daneo-Moore, and G. D. Shockman.** 1972. Influence of macromolecular biosynthesis on cellular autolysis in *Streptococcus faecalis*. J. Bacteriol. **112**:337–344.
22. **Shockman, G. D.** 1962. Amino acids, p. 567–673. *In* F. Kavanagh (ed.), Analytical microbiology. Academic Press Inc., New York.
23. **Shockman, G. D.** 1965. Symposium on the fine structure and replication of bacteria and their parts. IV. Unbal-

anced cell-wall synthesis: autolysis and cell-wall thickening. Bacteriol. Rev. **29**:345–358.

24. **Shockman, G. D., L. Daneo-Moore, and M. L. Higgins.** 1974. Problems of cell wall and membrane growth, enlargement and division. Ann. N.Y. Acad. Sci. **235**:161–197.
25. **Shockman, G. D., H. M. Pooley, and J. S. Thompson.** 1967. The autolytic enzyme system of *Streptococcus faecalis*. III. The localization of the autolysin at the sites of cell wall synthesis. J. Bacteriol. **94**:1525–1530.
26. **Shockman, G. D., J. S. Thompson, and M. J. Conover.** 1967. The autolytic enzyme system of *Streptococcus faecalis*. II. Partial characterization of the autolysin and its substrate. Biochemistry **6**:1054–1065.
27. **Toennies, G., L. Iszard, N. B. Rogers, and G. D. Shockman.** 1961. Cell multiplication studied with an electronic particle counter. J. Bacteriol. **82**:857–866.
28. **Toennies, G., and G. D. Shockman.** 1958. Growth chemistry of *Streptococcus faecalis*. Proc. 4th Int. Congr. Biochem. Vienna **13**:365–394.
29. **Weidel, W., and H. Pelzer.** 1964. Bag-shaped macromolecules—a new outlook on bacterial cell walls. Adv. Enzymol. **26**:193–232.
30. **Wolf-Watz, H., and S. Normark.** 1976. Evidence for a role of *N*-acetylmuramyl-L-alanine amidase in septum separation in *Escherichia coli*. J. Bacteriol. **128**:580–586.