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Site of Initiation of Cellular Autolysis in *Streptococcus faecalis* as Seen by Electron Microscopy

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Low concentrations of glutaraldehyde (0.1% or higher) blocked cellular and wall autolysis. The site of autolytic activity was studied by allowing cell autolysis to proceed for very short periods (0 to 15 min) before addition of glutaraldehyde. Electron microscopy of ultrathin sections showed that the primary site of autolytic activity was the leading edge of the nascent cross wall. The base of the cross wall seemed more resistant than the tip. Evidence supporting the involvement of autolysin activity in continued wall extension and in cell separation as well as in the initiation of new sites of wall extension was obtained. In cells exposed for 10 min to chloramphenicol, wall dissolution was very much slower but occurred at the same cross wall site.

Streptococcus faecalis ATCC 9790 has been previously shown to contain an autolysin (a β 1,4 *N*-acetylmuramide glycanhydrolase) which exists in the cell in both latent and cell wall-bound, active forms. Exponential-phase cells (LOG cells) are prone to rapid autolysis. Both biochemical (9, 17) and electron microscopic (16) evidence suggest that the wall-bound active form is located at, or very near, the most recently synthesized portion of the wall. With the electron microscope, wall autolysis, resulting from the action of the active form of the enzyme in situ, was shown to result in the release of concentric ribbons of walls, beginning at the coccal equator and proceeding toward the poles. In contrast, the action of egg-white lysozyme, or isolated, partially purified autolysin on detergent-inactivated walls, or latent autolysin in situ (after trypsin activation) resulted in random wall dissolution. We now report on electron microscopic observations of the action of the active form of the autolysin in whole streptococci, particularly during the early stages of cell autolysis. This proved to be possible since a variety of observations strongly suggest that autolysis of LOG cells in buffers is due to the action of the active and not the latent form of the enzyme on the walls (9, 16, 17). Evidence for this includes the following: (i) Trypsin, which activates the latent form of the autolysin in isolated walls, does not affect the rate of autolysis of whole cells unless they have been first damaged (e.g., by a mild heat treatment). (ii) In growth reinitiation experiments, the specific activity of the latent form of the autolysin in isolated walls begins to increase immediately, whereas that of the active form is delayed and parallels the ability of cells to autolyze (9). The present studies of ultrathin sections indicate that the major site of action of the active form of the autolysin is along the leading edge and side of the growing cross wall. Although chloramphenicol treatment results in a rapid decrease in the rate of cellular autolysis, autolysin action, at a much slower rate but at the same location, could still be seen in cells exposed to chloramphenicol for short periods.

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MATERIALS AND METHODS

Effects of fixatives on cellular autolysis. LOG cells were harvested on membrane filters (0.65 μm pore size; Millipore Corp.), washed three times with 5 ml of ice-cold deionized distilled water, suspended in 0.01 M phosphate (*p*H 6.5), and allowed to autolyze at 37 C. Similar samples of cells were first treated for 1 hr with various concentrations of calcium carbonate-neutralized glutaraldehyde, neutralized Formalin, or buffered osmium tetroxide (*p*H 6.5) and treated as above. Autolysis of cells was followed as previously described (15, 17). As treatment with glutaraldehyde (4%) seemed to stop cell autolysis in a most rapid manner (*see below*), this treatment (for 1.5 hr) was selected for morpho-

logical studies. Fixed cells were harvested by centrifugation (15,000 × *g*, 20 min) and placed into 2% agar [made with 0.01 M phosphate (*p*H 6.2) to which 0.08 M potassium chloride and 0.01 M magnesium acetate were added]. Cubes of agar-embedded cells were cut and washed overnight in the above phosphate buffer, followed by three more changes of phosphate and one change of Veronal-acetate buffer. The cells were postfixed in osmium tetroxide and uranyl acetate by the method of Kellenberger et al. (6), embedded in Epon 812 (*7a*), and sectioned. The sections were stained for 20 min with saturated uranyl acetate in 50% ethanol and lead citrate (11) for 5 min. LOG cells were also treated with chloramphenicol (50 μg/ml) at a turbidity equivalent to 0.4 mg (dry weight) per ml, for 10 min after allowing autolysis to take place. Such cells were fixed and sectioned as described above.

RESULTS

Effects of fixatives on cellular autolysis. Initially it was necessary to find fixation conditions which would, as far as possible, rapidly and completely stop cellular autolysis. Figure 1A shows that fixation for 1 hr with 3% glutaraldehyde almost completely stopped cellular autolysis, whereas 10% Formalin and 0.1% osmium tetroxide were much less effective. (The concentration of OsO_4 commonly used as a prefixative in the Kellenberger et al. method is usually 0.1%.) After 16 hr at 37 C, glutaraldehyde-fixed cells lost less than 2% of their initial turbidity. This same level of protection from autolysis was observed even when the time of exposure to glutaraldehyde was reduced to 5 min and when the concentration was reduced to 0.1%. When the concentration of osmium tetroxide was increased to 1%, the interfering effect of the black reduction products made comparative turbidimetric measurements of doubtful value. In spite of this, it was judged that 1% osmium tetroxide was probably equal to glutaraldehyde in stopping cellular autolysis. Glutaraldehyde (1%) was also effective in rapidly stopping the autolysis of isolated walls (Fig. 1B). On the basis of its rapid effect, even at low concentration, glutaraldehyde (4%) was used as a prefixative for these studies.

Observations of cells undergoing autolysis. During cellular autolysis, it appears that dissolution of the growing cross wall takes place well before dissolution of peripheral wall can be observed. Figure 2 shows a central section of a cell undergoing autolysis (from a suspension at 74% of its initial turbidity). It is obvious that much of the cytoplasmic contents of the cell shown in Fig. 2 has already been lost. This indicates an opening somewhere in the wall of this cell, perhaps at a point visible in another section. However, the peripheral wall in this section appears to be in-

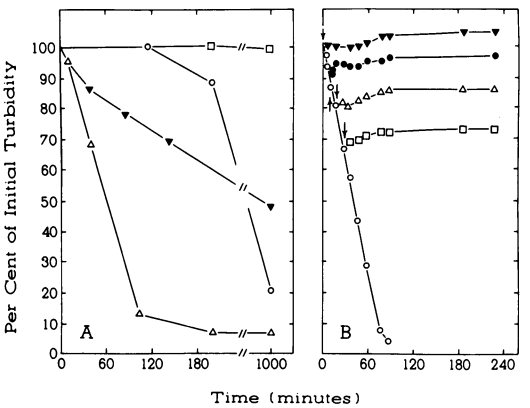


FIG. 1. *Inhibition of cellular autolysis (A) and trypsin-activated wall autolysis (B) by various fixatives. A, Cellular autolysis. Exponential-phase cells were harvested, washed by filtration, and placed in 0.01 M sodium phosphate, pH 6.5. The cells were exposed for 1 hr at room temperature to 0.1% OsO_4 (▼), 10% Formalin (○), or 3% glutaraldehyde (□). The untreated control(△) was incubated for 1 hr at 0 C. B, Wall autolysis. LOG walls were suspended in 0.01 M sodium phosphate, pH 6.5 (0.7 mg/ml); trypsin (1 μg/ml) was added, and the wall suspensions were incubated at 37 C. Glutaraldehyde (1%) was added to one tube at zero time (▼) and to other tubes at the times indicated by the arrows. One tube (○) remained as a control. Cellular and wall turbidity were followed as previously described (9, 18).*

tact and maintains the typical dark-light-dark tribanded structure seen in the wall on central sections of *S. faecalis* cells (5). Only at the area of the cross wall can considerable, localized wall dissolution be seen. To obtain information concerning the stages of wall (and cell) dissolution which preceded that shown in Fig. 2, LOG cells at still earlier stages of autolysis (100 to 90% of the initial turbidity, 0 to 15 min of autolysis) were examined. Areas of the wall involved with cross wall formation, typified by that shown within the rectangle in Fig. 2, were of particular interest. Figure 3 shows an area of cross wall formation from a typical section of a LOG cell control (before autolysis). At this stage of centripetal cross wall growth, the nascent cross wall is nearly twice as thick as the nearby peripheral wall and is tightly sheathed by the septal membrane. Figures 4 to 9 show corresponding areas from cells in the very early stages of autolysis. As cellular autolysis proceeds, the septal membrane appears to maintain its position as the sides and leading tip of the cross wall become less electron dense (e.g., Fig. 4–7). Initially, autolysis seems to take place at the leading edges of the nascent cross wall ("zone of solubilization," Fig. 14). The base of the cross wall seems to be somewhat less rapidly hydrolyzed

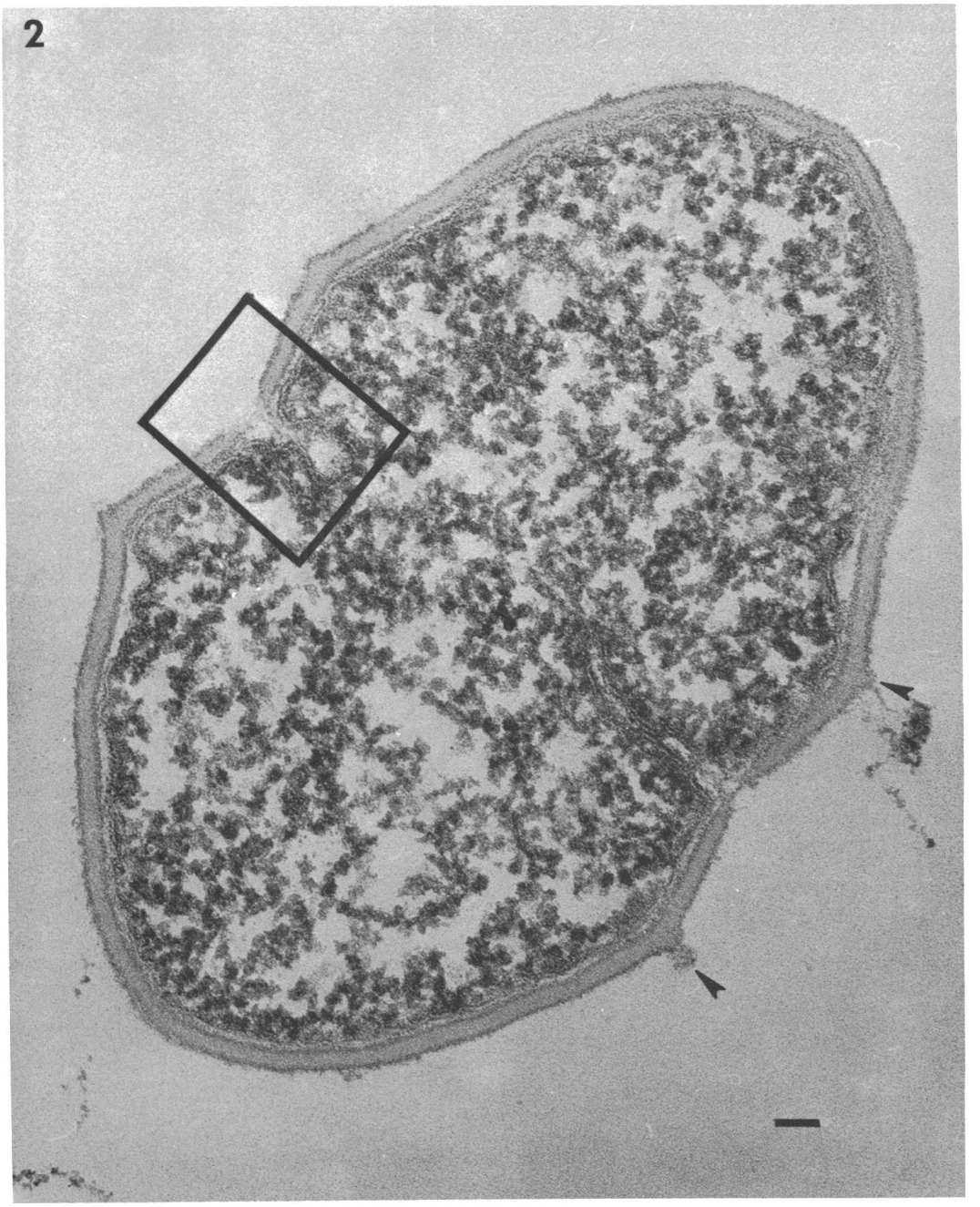


FIG. 2. *Central, longitudinal section of a cell 60 min after the beginning of autolysis (at 74% of the initial turbidity). Note that the entire cross wall is no longer visible (rectangular area), but that peripheral wall seems to be intact. The wall bands (arrows) mark the separation of "new" equatorial, peripheral wall from "old" polar wall (5). Bar equals 0.1 μm.*

so that the base of the remaining cross wall is then broader than the tip (Fig. 4 and 6 and "zone of inhibition," Fig. 14). In some cases, a rather narrow zone of lesser electron density can be seen running up the center of the cross wall (e.g., Fig. 5 and "zone of separation," Fig. 14). As autolysis proceeds, the cross wall begins to be dissolved from its tip (Fig. 6) toward the base (Fig. 7), and finally perforation of the wall seems to occur frequently through the central portion of the cross wall (Fig. 7). This point of rupture can be seen occasionally to allow the membrane to extrude through the resulting opening (Fig. 8). Such an extrusion of membrane would appear to result in a localized rupture of the wall, releasing cytoplasmic contents to the medium, tending to equalize the internal and external osmotic pressures (Fig. 9). Such an initial localized perforation could then account for the observation that, at most points along the cross wall annulus, the septal membrane retains its invaginated position even after apparent cross wall dissolution (Fig. 2). Cords of ribosomes and membrane-associated ribosomes remain in partially autolyzed cells and, in fact, are even more prominent in such cells (Fig. 2). It appears that the removal of soluble cytoplasmic material and some small particulates increases the definition of the remaining ribosomes. Figure 10 shows the cross wall area of a pair of cells that have just about completed their cross wall. In such cells autolysin action is very similar to that of cells in an earlier stage of division, except that the "zone of inhibition" extends deeper into the cross wall. In such cases, dissolution of a central line down the cross wall can be seen. This line would correspond to the outer surface of the walls of the two daughter cells. Such observations lead to at least an inference for a requirement for autolysin action in cell separation.

In dividing cells, a completed cross wall will separate to form one of the two polar regions of the new daughter cells. Autolysin activity at the one pole but not the other can be seen to occur for some time in a few cells. Figure 12 shows such an example, where dissolution of the wall at one pole, probably the one resulting from the most recent division, of the cell can be seen.

After dissolution of the cross wall, autolysin then continues to attack the peripheral wall adjacent to the cross wall area (Fig. 13), and the wall lytic action moves toward the coccal poles. The stage of wall dissolution shown in Fig. 13 probably corresponds to the early stages of dissolution of isolated LOG walls previously seen by the negactive staining technique (16). Even after extensive leakage of the cytoplasmic contents, the plasma membrane usually continues to maintain its integrity and is often seen to be most closely associated with the wall band area of the cell (e.g., Fig. 13).

Effect of chloramphenicol on cellular autolysis. The addition of chloramphenicol (50 μg/ml) to an exponentially growing culture results in cessation of exponential growth and a rapid inhibition of cellular autolysis. In this way chloramphenicol mimics the effect of amino acid starvation (10). Cells treated for 10 min with chloramphenicol will still autolyze, but very slowly. Also, morphologically, the location and pattern of initial autolysin attack appears to be virtually identical (Fig. 11) except that the period of incubation must be much longer (60 to 120 min versus 0 to 15 min for LOG cells). It was noted, however, that the resistant core of the cross wall was more persistent (Fig. 11) than those observed in LOG cells.

DISCUSSION

The present observations add a new dimension to earlier results from this laboratory. Not only is the active form of the autolysin located in recently synthesized portions of the wall (9, 17), but we now show that its action, in exponential-phase cells undergoing autolysis, appears to begin at the leading tip and edge of the centripetally growing cross wall. On the basis of electron microscopic observations of exponentially growing cells, we recently proposed (5) that both centripetal cross wall growth and peripheral wall extension result from wall synthetic activity occurring at or near the leading edge of the septum—the same area at which cellular autolysis appears to be initiated.

It would then seem that this cellular location would be ideal for a role for the autolysin in cell wall synthesis. Cleavage of the β1,4 linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine by the enzyme (18) could certainly lead to new acceptor sites for insertion of peptidoglycan disaccharide monomer units according to the known wall biosynthetic mechanism (19). The role for such a wall lytic enzyme in the initiation of new sites of wall growth in the middle of old wall was relatively obvious. Association of autolysin with the growing tip of the cross wall in cells that have almost completed a division (e.g., Fig. 10) suggests a continuing role for the enzyme during centripetal cross wall growth. Autolysin action appears to continue at one pole of a recently divided cell (Fig. 12) and a narrow zone of decreased electron density, running up the center of a growing cross wall, can be seen occasionally (e.g., Fig. 5 and 10). The eventual separation or weakening of a common cross wall into polar,

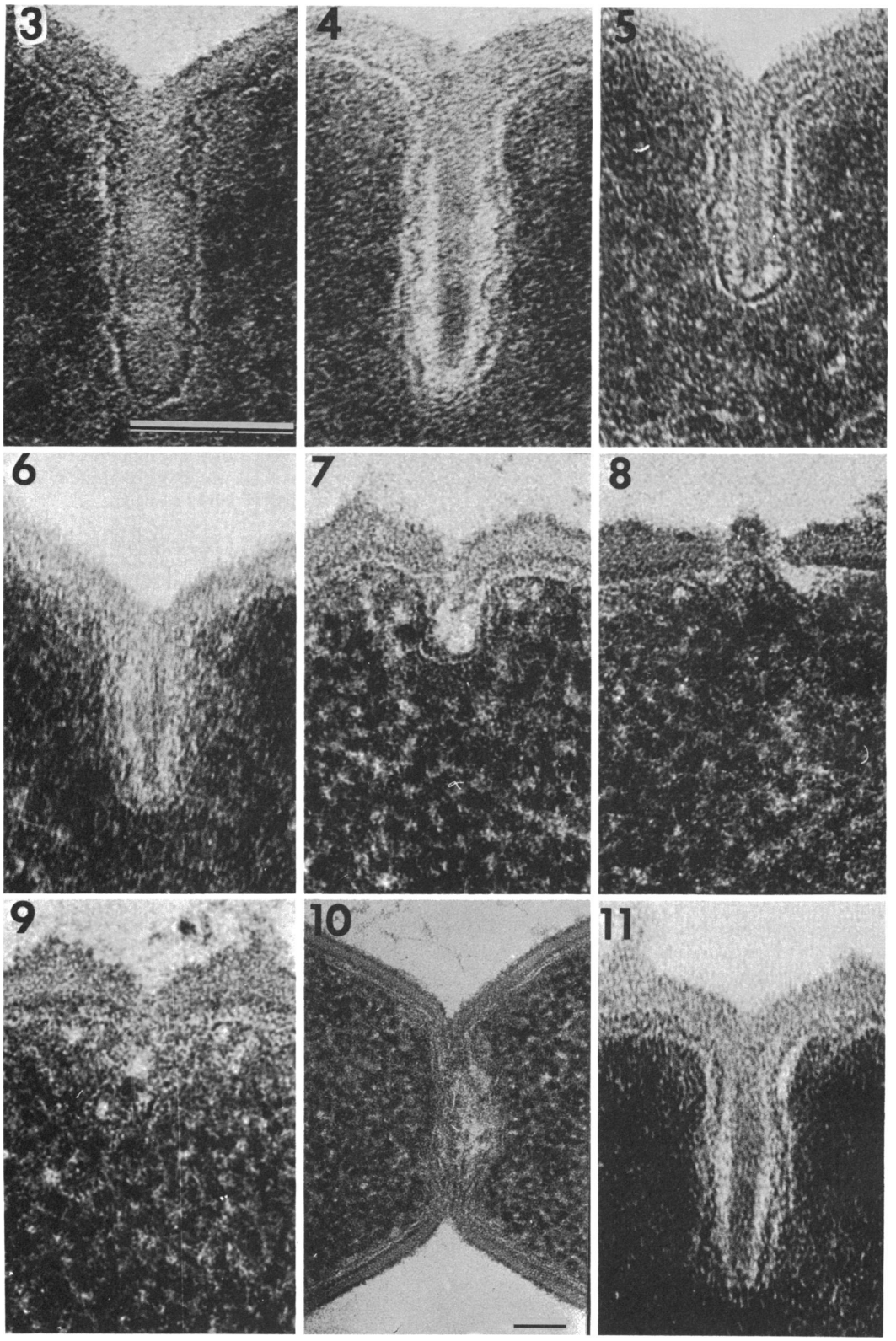
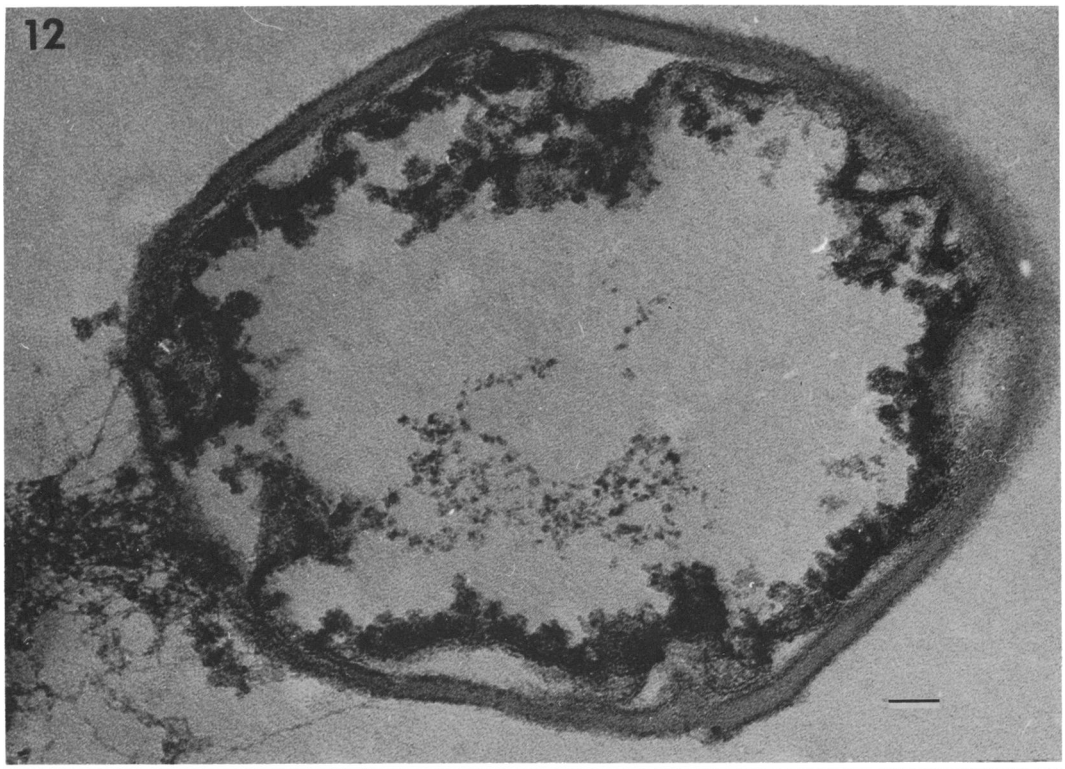


FIG. 3–11. *Stages of cross wall dissolution.* FIG. 3. *Control (nonautolyzing cross wall).* FIG. 4. *Primary attack at the leading edge of the cross wall. The base of the cross wall remains intact and the septal membrane maintains its original invaginated position.* FIG. 5. *Appearance of a central "channel" or "zone of separation" after partial cross wall dissolution.* FIG. 6. *Degradation of cross wall proceeding from the tip toward the base.* FIG. 7. *Beginning of wall perforation.* FIG. 8. *Membrane eversion, probably occurring at the first site of wall perforation in this cell.* FIG. 9. *Beginning of cytoplasmic leakage.* FIG. 10. *Autolytic attack of a completed cross wall.* FIG. 11. *Autolytic*



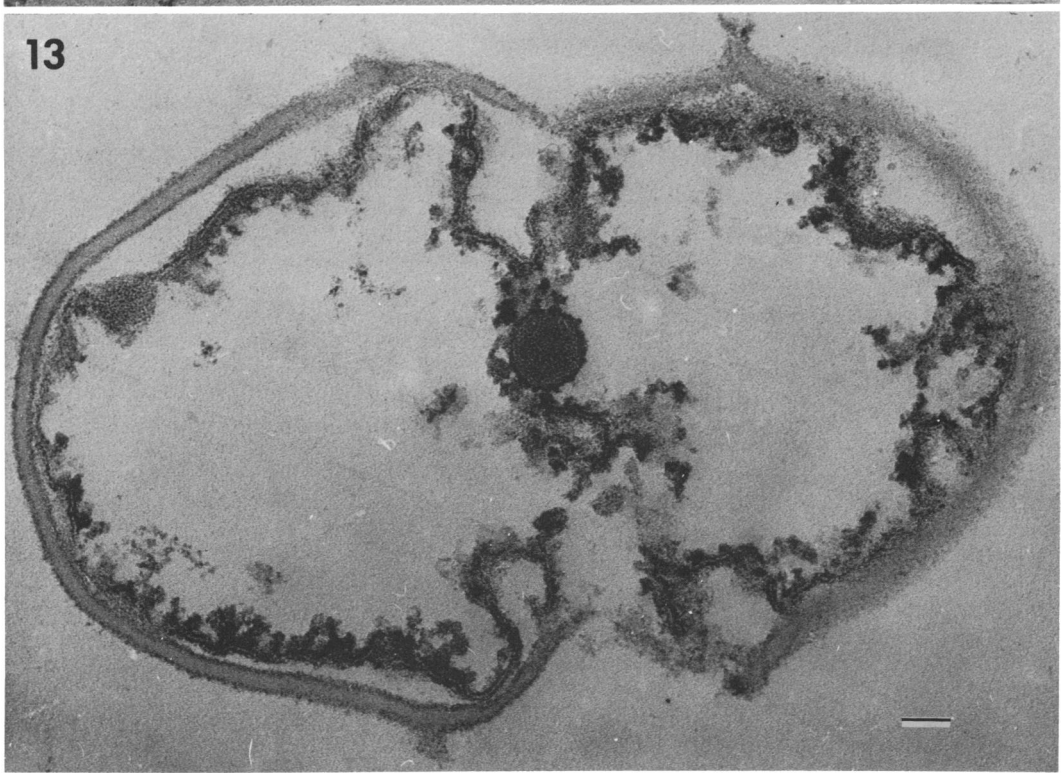


FIG. 12. *Autolysis occurring at a single pole of a cell. As a result of centripetal equatorial wall synthesis, one pole of each cell is one or more generations newer than the other. Autolytic activity is frequently retained at the apparently newer pole well after subsequent cross wall completion and cell separation. Bar equals 0.1 μm.*

FIG. 13. *After cross wall removal, the peripheral wall is attacked. Note membrane associated with the wall band areas of the cell wall. Bar equals 0.1 μm.*

activity in a cell previously treated for 10 min with chloramphenicol (50 μg/ml). Fig. 3 to 9, and 11 are at the same magnification which is indicated by the bar in Fig. 3. Fig. 10 is at a lower magnification, indicated by the bar in the figure. The bars equal 0.1 μm.

peripheral wall of the two daughter cells could also involve autolysin action, although this may represent merely separation of two cell wall layers synthesized separately. These last observations suggest that the factors thought to be involved in the chaining and unchaining of streptococci (2, 7) may be identical with the autolysin. At present we are attributing all three of these roles (in initiation of new wall synthetic sites, in continued wall elongation, and in cell separation) to the sole wall lytic activity that we have been able to detect in this organism (18).

After dissolution of the cross wall and cell lysis, the autolysin continues to act on the nearby peripheral wall (Fig. 13). This type of "creeping" action can be attributed to the high affinity of autolysin for the wall (9, 14, 17, 18). Such a high degree of affinity would appear to be helpful in restricting the area of action of the enzyme. However, Coyette and Ghuysen (1*a*) have recently found that the autolysin of *Lactobacillus acidophilus* (also an *N*-acetylmuramidase) does not have the same degree of affinity for its own walls.

The diagrammatic representation of the initial stages of cellular autolysis (Fig. 14) summarizes our current ideas. Cellular autolysis begins in the "zone of solubilization," perhaps starting at the nascent cross wall tip and working back towards the base. This is the area likely to be involved in wall extension. Activity in the "zone of separation" would be involved with the process of cell separation.

Treatment of LOG cells with chloramphenicol results in cell wall thickening (12) and in a rapid loss in the ability of cells to autolyze, but in a much less rapid decrease in content of the active form of the autolysin (10). However, the present electron microscopic observations show that the initial stages of cellular autolysis occur at the identical place in both LOG and chloramphenicol-treated cells, that is, the leading tip and edge of the nascent cross wall. However, after chloramphenicol treatment, the rate of dissolution of the cross wall is strikingly reduced, and the central core of the cross wall seems to be particularly resistant to dissolution. The "semi-resistant core" and "zone of inhibition" (Fig. 14) reflect, in part, our ideas concerning resistance to cellular dissolution after chloramphenicol treatment. In addition, these regions of the cross wall may reflect an unknown mechanism whereby the growing and dividing cell can limit autolysin action to pertinent areas of the wall.

Chloramphenicol not only has little effect on the location of the initial stages of autolysis, but there is little or no change in the overall susceptibility of walls isolated from chloramphenicol-treated cells to the action of isolated autolysin (10).

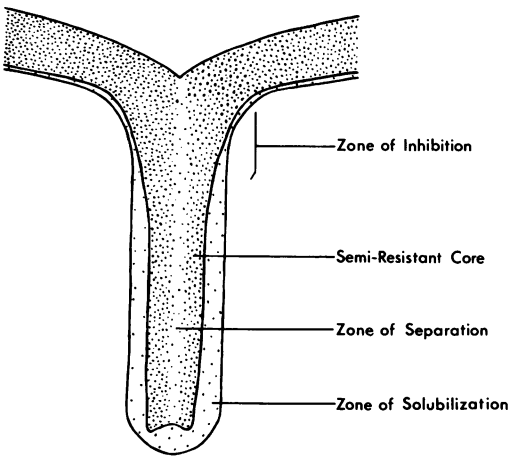


FIG. 14. *Diagrammatic view of cellular autolysis at the cross wall. The "zone of solubilization" represents the area between the autolyzing cross wall and the membrane and is probably filled with hydrolyzed wall material. The "semi-resistant core," and especially the base of the cross wall, may be more resistant to dissolution than the leading edges. The "zone of inhibition" extends into and includes the peripheral wall, which dissolves quite slowly as compared to the cross wall.*

The relative resistance to autolysis of chloramphenicol-treated cells could be due in part to the increased thickness of the walls, but it would seem that the increased resistance to autolysis of the core and base of the cross wall is of greater significance. At present, we have no information concerning the precise nature of this change, but it does seem likely that localized differences in the extent of cross-linking of the peptidoglycan could be a factor. Tipper (20) proposed that newly synthesized wall is less cross-linked than older wall in *Staphylococcus aureus*. Also, it seems likely that the cross-linking transpeptidation reaction takes place outside the permeability barrier of the cell, whereas synthesis of the linear glycan chains takes place, at least partially, in the membrane fraction (21). This physical separation suggests that the processes may also be separated in time. Chloramphenicol treatment, which appears to result in a rapid decrease in the rate of cross wall and peripheral wall extension, may allow the cross-linking reactions to occur closer to the growing edge of wall extension. At present we do not know whether the degree of local cross-linking affects the rate of autolysin action, although we can speculate that it may be necessary to break a greater number of glycan bonds to solubilize a more highly cross-linked wall.

We currently favor the idea of a common cross wall which later thickens and becomes separated into the walls of the two daughter cells. This situ-

ation is not as clearcut as the one seen in *Bacillus cereus* (1) and *B. megaterium* (3), where a cross wall of single thickness is completed and then thickens during cell separation to a width equal to that of the two daughter cells. In *S. faecalis*, centripetal cross wall growth begins at a thickness approximately equal to that of the nearby peripheral wall and gradually doubles in thickness during cross wall closure (5). It remains possible, however, that the cross wall is made up of two separate layers initially of half thickness, and each rapidly attains full thickness. Despite the tribanded (dark-light-dark) staining consistently seen on antitangential cuts through the wall, we have no evidence of layering of the wall components in this organism. It does seem likely that the charged phosphate groups of the teichoic acid-like polymers present (14) would tend to repel each other and to be concentrated on the outer wall surfaces. The presence of these polymers in the wall favorably affects the rate of hydrolysis of the wall substrate by the autolysin (18) without significantly affecting the nature of the binding of the enzyme to the wall (14). Thus, local concentrations of teichoic acid in the wall could "channel" autolysin action. For example, an increased concentration of teichoic acid down the middle of a centripetally growing cross wall could direct autolysin action up this "track," thus favoring cell separation. The role of teichoic acids in cell separation is supported by the findings of Mirelman et al. (Bacteriol. Proc., p. 47, 1969) and of Tomasz (21). The former observed that a mutant of *S. aureus* H, which contains a greatly decreased amount of teichoic acid in its walls, failed to divide into separate cells. Tomasz (21) observed that the substitution of ethanolamine for choline in the wall teichoic acid (8) of *Diplococcus pneumoniae* resulted, among other things, in growth in extremely long chains.

With *B. megaterium*, Fitz-James and Hancock (4) observed a similar localized distortion or partial dissolution of cross wall 10 min after penicillin addition to "fully recovered, rapidly dividing cells" in a sucrose-stabilized growth medium. These investigators observed the accumulation of fibrous wall-like material between the septal membrane and the nascent cross wall, suggesting to them that it represented unorganized wall material. We did not observe a fibrous accumulation in autolyzing cells of *S. faecalis*. At early stages of cellular autolysis, before rupture of the cytoplasmic membrane and release of cytoplasmic contents, the septal membrane maintains its configuration against the high internal osmotic pressure of the cell (e.g., Fig. 4 to 7). Thus it is likely that the products of wall dissolution remain in this less electron-dense area between the septal membrane and the remaining cross wall to counteract the high internal osmotic pressure. This is especially likely since the wall of this organism is highly cross-linked (18) and complete autolytic digestion of isolated walls did not result in the release of detectable quantities of low-molecular-weight products (18).

Penicillin inhibits the transpeptidation reaction leading to cross-bridging of the wall peptidoglycan (17). Thus, this unorganized fibrous material observed by Fitz-James and Hancock (4) has been interpreted to be a result of inhibition of cross-linking (19). Penicillin inhibition results in cell lysis or spheroplast formation. In fact, penicillin at relatively low concentration results in lysis of *S. faecalis* when placed in a medium which permits wall synthesis but not a net increase in protein (13). This has been considered to be due to continued action of the autolytic enzyme system. Thus the fibrous material seen in penicillin-treated *B. megaterium* could result, in part, from action of a wall lytic system.

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LITERATURE CITED

1. Chapman, G. B., and J. Hiller. 1953. Electron microscopy of ultra-thin sections of bacteria. I. Cellular division in Bacillus cereus. J. Bacteriol. **66**:362–373.
1a. Coyette, J., and J.-M. Ghuysen. 1970. The wall autolysin of *Lactobacillus acidophilus* strain 63 A. M. Gasser. Biochemistry, *in press*.
2. Ekstedt, R. D., and G. H. Stollerman. 1960. Factors affecting the chain length of Group A streptococci. I. Demonstration of a metabolically active chain-splitting system. J. Exp. Med. **112**:671–686.
3. Ellar, D. J., D. G. Lundgren, and R. A. Slepecky. 1967. Fine structure of *Bacillus megaterium* during synchronous growth. J. Bacteriol. **94**:1189–1205.
4. Fitz-James, P., and R. Hancock. 1965. The initial structural lesion of penicillin action in *Bacillus megaterium*. J. Cell Biol. **26**:657–667.
5. Higgins, M. L., and G. D. Shockman. 1970. A model for cell wall growth of *Streptococcus faecalis*. J. Bacteriol. **101**:643–648.
6. Kellenberger, E., J. Sechaud, and A. Ryter. 1959. Electron microscopical studies of phage multiplication. IV. The establishment of the DNA pool of vegetative phage and the maturation of phage particles. Virology **8**:478–498.
7. Lominski, I., J. Cameron, and G. Wyllie. 1958. Chaining and unchaining *Streptococcus faecalis*—a hypothesis of the mechanism of bacterial cell separation. Nature (London) **181**:1477.
7a. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409–414.
8. Mosser, J., and A. Tomasz. 1970. Choline-containing teichoic acid as a structural component of pneumococcal cell wall

and its role in sensitivity to lysis by an autolytic enzyme. J. Biol. Chem. **245**:287–298.

9. Pooley, H. M., and G. D. Shockman. 1969. Relationship between the latent form and the active form of the autolytic enzyme of *Streptococcus faecalis*. J. Bacteriol. **100**:617–624.
10. 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.
11. Reynolds, E. S. 1963. The use of lead citrate at high *p*H as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
12. Shockman, G. D. 1965. Symposium on the fine structure and replication of bacteria and their parts. IV. Unbalanced cell-wall synthesis: autolysis and cell-wall thickening. Bacteriol. Rev. **29**:345–358.
13. Shockman, G. D. 1959. Reversal of cycloserine inhibition by D-alanine. Proc. Soc. Exp. Biol. Med. **101**:693–695.
14. Shockman, G. D., and M. C. Cheney. 1969. Autolytic enzyme system of *Streptococcus faecalis*. V. Nature of the autolysin-cell wall complex and its relationship to properties of the autolytic enzyme of *Streptococcus faecalis*. J. Bacteriol. **98**:1199–1207.
15. Shockman, G. D., M. J. Conover, J. J. Kolb, P. M. Phillips, L. S. Riley, and G. Toennies. 1961. Lysis of *Streptococcus faecalis*. J. Bacteriol. **81**:36–43.
16. Shockman, G. D., and J. T. Martin. 1968. Autolytic enzyme system of *Streptococcus faecalis*. IV. Electron microscopic observations on autolysin and lysozyme action. J. Bacteriol. **96**:1803–1810.
17. Shockman, G. D., H. M. Pooley, and J. S. Thompson. 1967. Autolytic enzyme system of *Streptococcus faecalis*. III. Localization of the autolysin at the sites of cell wall synthesis. J. Bacteriol. **94**:1525–1530.
18. Shockman, G. D., J. S. Thompson, and M. J. Conover. 1967. Autolytic enzyme system of *Streptococcus faecalis*. II. Partial characterization of the autolysin and its substrate. Biochemistry **6**:1054–1065.
19. Strominger, J. L., K. Izaki, M. Matsuhashi, and D. J. Tipper. 1967. Peptidoglycan transpeptidase and D-alanine carboxypeptidase: penicillin-sensitive enzymatic reactions. Fed. Proc. **26**:9–22.
20. Tipper, D. J. 1969. Mechanism of autolysis of isolated cell walls of *Staphylococcus aureus*. J. Bacteriol. **97**:837–847.
21. Tomasz, A. 1968. Biological consequences of the replacement of choline by ethanolamine in the cell wall of pneumococcus: chain formation, loss of transformability, and loss of autolysis. Proc. Nat. Acad. Sci. U.S.A. **59**:86–93.