JOURNAL OF BACTERIOLOGY, Mar. 1982, p. 977–984
0021-9193/82/030977-08$02.00/0

Vol. 149, No. 3

Cell Wall Turnover in Growing and Nongrowing Cultures of *Bacillus subtilis*

WIM R. DE BOER,† P. DIEDERICK MEYER, CORINNE G. JORDENS, FRED J. KRUYSSEN, AND JAN T. M. WOUTERS*

Laboratorium voor Microbiologie, Universiteit van Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands

Received 13 July 1981/Accepted 26 October 1981

Cell wall turnover was studied in cultures of *Bacillus subtilis* in which growth was inhibited by nutrient starvation or by the addition of antibiotics. Concomitantly, the synthesis of wall, as measured by the incorporation of radioactively labeled *N*-acetylglucosamine, was followed in some of these cultures. In potassium- or phosphate-starved cultures, growth stopped, but wall turnover continued at a rate slightly lower than that in the control cultures. Lysis of cells did not occur. In glucose-starved cultures, continued wall turnover caused lysis of cells, since wall synthesis apparently was inhibited. The same phenomenon was observed after growth arrest by the addition of wall synthesis inhibitors such as fosfomycin, cycloserine, penicillin G, and vancomycin. Growth arrest by the addition of chloramphenicol allowed the continuation of wall synthesis; therefore, the observed turnover generally did not cause cell lysis.

In a great variety of bacterial strains it has been shown that wall polymers are degraded and lost from the cell during growth (1, 3, 9, 11, 17, 23). This phenomenon is generally designated as wall turnover. In a previous paper (6), we reported on wall turnover in exponentially growing batch and chemostat cultures of *Bacillus subtilis* subsp. *niger* WM. An analysis of the kinetics by which radioactively labeled wall material was lost from the cells has led to a quantitative description of wall turnover in this organism.

Wall turnover is the result of the hydrolytic action of wall-bound autolytic enzymes on peptidoglycan in the outer wall layer in *B. subtilis* (17, 18). Little is known about the regulation of the autolysins in exponentially growing cells. Inhibition of these enzymes in the deeper, membrane-located wall layers may be effected by membraneous compounds such as phospholipids and lipoteichoic acid (5, 22). Autolysin activity in the turnover-sensitive outer wall layers may be regulated only by the amount and activity of the enzymes produced by the cells; the presence of more subtle regulatory mechanisms, however, cannot be ruled out.

Obviously, the maintenance of a stable balance between the rates of wall synthesis and turnover is of great importance for the cell, to prevent an undue weakening of its supporting structures. This system is particularly tested when the rate of growth is lowered abruptly; in this case, an uneven slowing down of the rates of wall synthesis and turnover may result in lysis of the cells.

Lysis of bacilli during growth inhibition, however, occurs only when wall synthesis is directly affected, e.g., by the addition of wall-directed antibiotics (19) or by the omission of the carbon source from the medium (2). Inhibition of protein (10) or RNA synthesis (16) generally does not result in lysis.

In the present report, we examined the response of the hydrolytic autolysin activity (wall turnover) together with the stability against lysis of *B. subtilis* subsp. *niger* WM towards an abrupt downward shift of the growth rate, which was caused either by starving the cells for essential nutrients or by adding antibiotics. Furthermore, the synthesis of wall polymers under these conditions was followed. The results indicate that the rate of turnover is not always directly coupled to the rate of wall growth. (These results were partly taken from a thesis submitted by W.R.d.B. to satisfy the requirements of the doctorate degree at the University of Amsterdam.)

MATERIALS AND METHODS

Strain. *B. subtilis* subsp. *niger* WM, as described previously (14) was used.

Media and growth conditions. Batch cultures and potassium-limited and phosphate-limited chemostat cultures were grown as described before (6). In addi-

† Present address: Gist Brocades NV, Microbiology Research Laboratory, Postbus 1, 2600 MA Delft, The Netherlands.

tion, batch cultures in the minimal medium of Evans et al. (8), with all the nutrients in nonlimiting concentration and enriched with 0.2% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) and with 100 mM glutamic acid instead of ammonium chloride, pH 7.0, were used (EMM).

Turnover experiments. Cells were pulse or continuously labeled or both in their walls while growing exponentially in chemostat or batch cultures, by using *N*-acetyl-D-[^{3}H]glucosamine ([^{3}H]GlcNAc) or *N*-acetyl-D-[^{14}C]glucosamine ([^{14}C]GlcNAc). After the labeling period, the remaining radioactive substrate was removed by a centrifugation procedure (6).

Cells labeled in batch cultures were suspended in the same medium at an E_{540} of ca. 0.2 and reincubated. To parallel flasks containing a sample of the same cell suspension as the growing control culture, either penicillin G (25 μg ml^{-1}; Gist-Brocades, NV, Delft, The Netherlands), fosfomycin (10 μg ml^{-1}; a generous gift of Merck & Co., Inc., Rahway, N.J.), cycloserine (250 μg ml^{-1}; Fluka, AG, Buchs, Switzerland), vancomycin (10 μg ml^{-1}; Sigma Chemical Co., St. Louis, Mo.), or chloramphenicol (50 μg ml^{-1}; Sigma) was added.

Cells labeled in chemostat cultures were resuspended after centrifugation in prewarmed minimal medium as used in the chemostat which had been buffered with imidazole-hydrochloride (50 mM, pH 7.0) but was lacking glucose and the previously growth-limiting nutrient. This suspension was diluted by using the same medium to an E_{540} of 0.2 and divided into several flasks. Nutrients and antibiotics were added as follows to appropriate flasks containing 15 ml of this basal suspension. Control cultures; glucose (final concentration, 2.5 mg ml^{-1}) and the previously growth-limiting nutrient at a nonlimiting concentration (see reference 8), i.e., potassium (2.5 mM) or phosphate (2.5 mM) were added. Glucose starvation; only potassium (2.5 mM) or phosphate (2.5 mM) was added to the basal suspension. Potassium or phosphate starvation; only glucose (2.5 mg ml^{-1}) was added to the basal suspension. Incubations with the presence of antibiotics; nutrients as in the control cultures and antibiotics at the following concentrations (micrograms per milliliter) were added: penicillin G, 100; fosfomycin, 250; cycloserine, 250; or chloramphenicol, 50.

Wall turnover was measured in the cultures by following the decrease in the amount of cell-bound radioactivity as described previously (6). During some turnover measurements the concomitant wall synthesis was followed. In these experiments, cells that had been pulse labeled with [^{3}H]GlcNAc in batch culture were reincubated in the same medium supplemented with [^{14}C]GlcNAc (ca. 0.2 μCi ml^{-1}) and 1 mM carrier GlcNAc. The ^{14}C label taken up by the cells during this incubation could be solubilized to more than 90% by lysozyme treatment of the cells, indicating that it was almost exclusively incorporated into peptidoglycan. During incubation, 250-μl samples were mixed with 250 μl of 10% trichloroacetic acid at 0°C, containing 1 mM GlcNAc and approximately 0.3 mg of heat killed carrier cells ml^{-1}. After 5 min at 0°C, 400 μl of this mixture was filtered over a glass fiber filter (Whatman GF/A). The filter was washed twice with 1 ml of 5% trichloroacetic acid at 0°C, dried, and counted as described previously (6).

The wall turnover parameters were calculated as described before (6). The decrease of cellular label per milliliter of culture shows first-order kinetics after a lag time of approximately 1 generation time (see Fig. 1–3). The rate constant λ of this decrease is expressed as decrease per hour. The fraction of the total wall material which is lost per hour is called κ (h^{-1}), whereas *k* is the percentage of the wall lost per generation time. X_B is the percentage of wall material which is susceptible to the random action of hydrolysis by autolytic enzymes. For further details and the derivation of these parameters refer to de Boer et al. (6).

RESULTS

Control cultures. Batch cultures grown with a relatively high rate in EMM showed wall turnover kinetics as described previously (6). Pulse labels were lost from the cells with first-order kinetics only after a lag period of approximately 1 generation time (Fig. 1). The concomitant

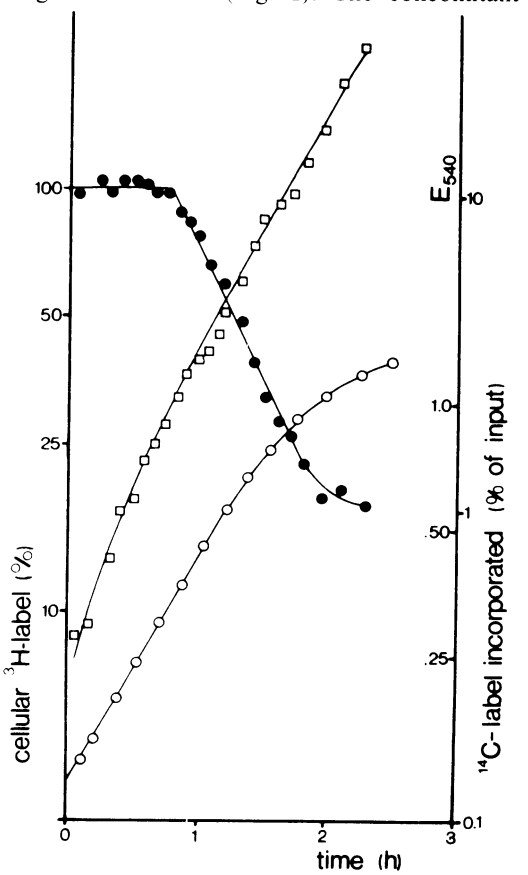

FIG. 1. Wall turnover and synthesis in EMM. Bacteria were grown to an E_{540} of 0.7 in EMM and pulse labeled with [^{3}H]GlcNAc. Cells were isolated by centrifugation and reincubated at an E_{540} of 0.15 in EMM containing [^{14}C]GlcNAc. The loss of cellular ^{3}H label (●), the incorporation of [^{14}C]GlcNAc (□), and E_{540} (○) were followed as described in the text. A 100% radioactivity was 6.7 × 10^4 dpm/ml for ^{3}H and 3.1 × 10^5 dpm/ml for ^{14}C.

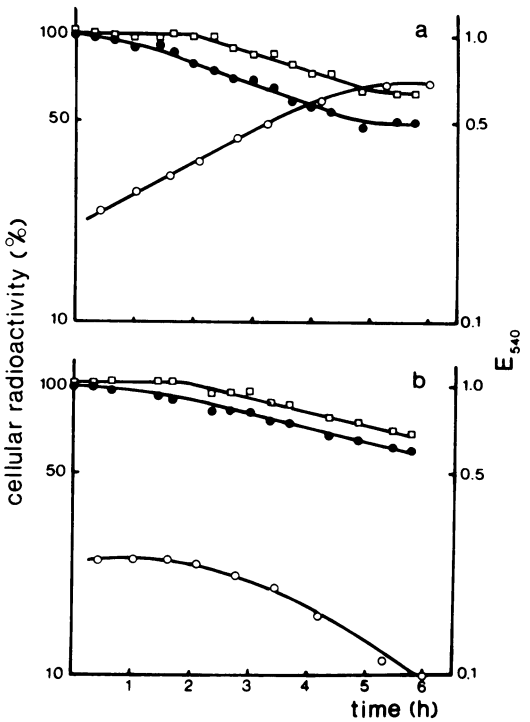

FIG. 2. Wall turnover in pulse and continuously double-labeled cells from a potassium-limited chemostat culture during nonlimited growth in batch culture (a) and inhibition of wall synthesis by fosfomycin (b). Bacteria growing in potassium-limited chemostat culture (μ [specific growth rate, see reference 6], 0.22 h^{-1}; E_{540}, 3.2) were labeled continuously for 15 h with [^{3}H]GlcNAc and pulse labeled (20 min) with [^{14}C]GlcNAc. After centrifugation, the cells were resuspended in imidazole-buffered medium lacking potassium and transferred to two flasks. Additions were (a) potassium and (b) potassium and fosfomycin. Cellular ^{3}H label (●), ^{14}C label (□), and E_{540} (○) were followed as described in the text. The initial radioactivity was 1.8×10^4 dpm/ml for ^{3}H and 3.2×10^3 dpm/ml for ^{14}C.

synthesis of peptidoglycan in this culture, as measured by the incorporation of differently labeled *N*-acetyl-glucosamine, is shown also in Fig. 1.

Wall turnover in cultures growing in minimal media at considerably lower rates was measured on cells inoculated in these media after labeling in chemostat cultures. The use of chemostat cultures enabled the investigation of cells containing either teichoic acid (potassium limitation) or teichuronic acid (phosphate limitation) as the anionic polymer in their walls (14). Moreover, cells with continuously labeled walls could be obtained by using a very low *N*-acetyl-glucosamine concentration in the medium, which would prevent aspecific incorporation of the precursor (4) and result in an evenly labeled culture. It was known from preliminary experiments that cells from chemostat cultures continue to grow without a lag after centrifugation and resuspension in imidazole-hydrochloride-buffered (pH 7.0), nonlimiting minimal medium. Exponential growth was observed for several hours at a rate slightly higher than that imposed on the culture in the chemostat. Wall turnover in such cultures of cells taken from potassium-limited and from phosphate-limited chemostats proceeded with the same kinetics as seen in previous experiments, in which the cells were regrown in chemostats after the chase of the radioactive precursor (6). Thus, pulse-labeled wall material was retained in the cells for approximately 1 generation time (Fig. 2a), whereas continuously labeled wall was lost from the start of the chase at an increasing rate (Fig. 2a and 3a) according to the kinetics described earlier (6). In Fig. 2a it is clearly seen that the decrease of both pulse and continuous labels in the same culture attained similar first-order kinetics. Wall turnover stopped when the culture approached the end of the exponential growth phase (Fig. 1, 2a, and 3a).

The different turnover parameters of the vari-

TABLE 1. Wall turnover parameters in control batch cultures of *B. subtilis* subsp. *niger* WM

Expt	Growth condition during[a]		Label[b]	Growth rate μ (h^{-1})[c]	Turnover parameters[d]					Figure[e]
	Labeling	Chase			λ (h^{-1})	Θ (h)	κ (h^{-1})	k (%)	X_B (%)	
1	EMM	EMM	Pulse	1.30	1.46	0.76	0.32	17	22	1
2	K-lim	MM	Pulse	0.24	0.15	2.42	0.064	18	44	2a
			Continuous		0.18	2.25	0.078	22	44	
3	P-lim	MM	Continuous	0.35	0.39	1.81	0.14	26	35	3a

[a] Labeling and chase were as described in the text. EMM, Enriched minimal medium batch culture; K-lim, potassium-limited chemostat culture; P-lim, phosphate-limited chemostat culture; MM, minimal medium with imidazole buffer (pH 7.0) batch culture.

[b] Pulse, Pulse label for 0.1 generation time; continuous, continuous label for ca. 5 generation times.

[c] For definition of growth rate see de Boer et al. (6).

[d] For definitions and derivations of the parameters, see de Boer et al. (6).

[e] Corresponding figure numbers.

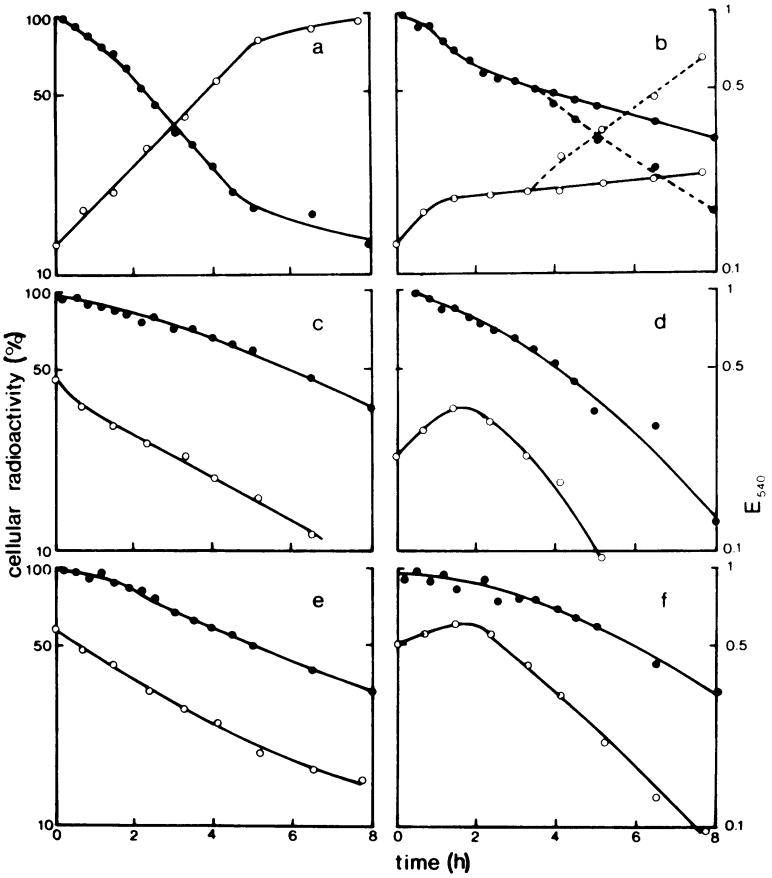

FIG. 3. Wall turnover in continuously labeled cells from a phosphate-limited chemostat culture during nonlimited growth in batch culture (a), starvation for phosphate (b) or glucose (c), and inhibition of wall synthesis by fosfomycin (d), cycloserine (e), or penicillin G (f). Bacteria were grown under phosphate limitation in a chemostat (μ, 0.23 h^{-1}; E_{540}, 2.9) and labeled continuously for 15 h with [^{3}H]GlcNAc. After centrifugation, cells were resuspended in imidazole-buffered medium lacking phosphate and glucose and were transferred to several flasks. The following additions were made: (a) phosphate and glucose; (b) glucose and phosphate were added after 3.5 h to a duplicate flask (dashed lines); (c) phosphate; (d) phosphate, glucose, and fosfomycin; (e) phosphate, glucose, and cycloserine; (f) phosphate, glucose, and penicillin G. Cellular radioactivity (●) and E_{540} (○) were followed as described in the text. The initial radioactivity was 1.9×10^4 dpm/ml in Fig. 3a and b, 7.6×10^4 dpm/ml in Fig. 3c, e, and f, and 3.8×10^4 dpm/ml in Fig. 3d.

ous batch cultures, as estimated by the method described in de Boer et al. (6), are compiled in Table 1. The fraction of the total cellular wall lost per hour (κ) of the slow-growing cultures (growth rates between 0.2 and 0.3 h^{-1}) varied considerably between 0.03 and 0.15 h^{-1}, but was lower than that in fast-growing cultures (not all experiments shown). However, the fraction of the total cellular wall lost per generation time (k) was around 20% for both slow- and fast-growing cells. Calculation of the fraction of the wall sensitive to turnover (X_B) yielded values of around 20% in cultures growing rapidly in complex media. In the slow-growing cultures in minimal medium, the X_B values varied between 25 and 44%.

Starvation for potassium or phosphate. Wall-labeled cells obtained from potassium-limited and phosphate-limited chemostat cultures were subjected to a downward shift of the growth rate by omitting potassium or phosphate, respectively, from the resuspension medium. After a short period in which the E_{540} showed a slight increase, growth was largely inhibited as judged from the optical density readings. Wall turnover continued for several hours and then declined slowly (Fig. 3b). Pulse labels were still lost only after a lag (6), whereas continuously labeled wall

material was released immediately from the start of the chase (Fig. 2b and 3b). The fraction of the initially present radioactivity lost per hour was taken as a measure for the turnover rate in the inhibited cultures; this parameter should be compared with the value of κ calculated from the experimental data of the growing control culture (Table 1). The values of the turnover rates found in the starved cultures are contained in Table 2. The rate of wall turnover in the starved cells was hardly suppressed during the first hours of the experiment, notwithstanding the large depression of the increase of the optical density in those cultures. A large proportion (over 60%) of the wall material present at the start of the starvation period (represented by the continuous wall label) was released during the experiment, yet the cells did not lyse. This fact made the occurrence of wall synthesis in the starving cultures plausible, at a rate at least matching the rate of turnover. The readdition of the growth-limiting nutrient after a 3.5-h deprivation period resulted in growth of the cells without a lag; the loss of radioactive wall accelerated (Fig. 3b).

Starvation for glucose. In cultures starved for the carbon source (glucose), presumably no wall synthesis was possible. These conditions approximated those often used for the assessment of the lytic activity of a culture ("incubation in a buffer"). The rate of lysis was not similar in the several incubations, but correlated with the rate of turnover exhibited by the growing control culture, which was a variable quantity in the different cultures (6, 7, 15). The loss of wall material occurred during glucose starvation at a rate somewhat lower than that in the control culture (Table 2, Fig. 3c).

Inhibition of wall synthesis. If wall synthesis was inhibited either at the cytoplasmic level (fosfomycin or cycloserine) or by preventing the incorporation of newly made peptidoglycan chains (penicillin G or vancomycin), lysis occurred with the antibiotic concentration used (Fig. 2b, 3d, 3e, 3f, and 4a). A small increase of the E_{540} preceded lysis in some cultures. Wall synthesis was severely inhibited after the addition of the antibiotic (Fig. 4a); however, during the initial increase of the E_{540} some label was incorporated. As was found with the glucose-starved cultures, lysis was more rapid and started after a shorter lag in cells for which a high turnover rate was found in the growing controls.

TABLE 2. Wall turnover in nongrowing cultures of *B. subtilis* subsp. *niger* WM

Expt[a]	Growth condition[b]		Treatment[c]	Rate of turnover[d] (h^{-1})	ΔE_{540}[e]	Figure[f]
	Labeling	Chase				
1	EMM	EMM	Cycloserine	0.10	111	4a
			Chloramphenicol	0.15	125	4b
2	K-lim	MM	Potassium-starved	0.07	180	—
			Glucose-starved	0.03	75	—
			Fosfomycin	0.06	48	2b
			Chloramphenicol	0.03	114	—
3	P-lim	MM	Phosphate-starved	0.14	144	3b
			Glucose-starved	0.08	33	3c
			Fosfomycin	0.12	31	3d
			Cycloserine	0.10	33	3e
			Penicillin G	0.09	38	3f
			Chloramphenicol	0.09	24	—
4	NB	NB	Control	0.23	480	—
			Vancomycin	0.29	16	—
			Chloramphenicol	0.07	135	—

[a] The numbers correspond with those in Table 1, where control cultures are represented, except for experiment 4.

[b] For explanation of growth conditions, see Table 1; NB, nutrient broth, batch culture.

[c] After labeling, cells were transferred to the medium specified, either containing antibiotic or lacking limiting nutrient or lacking glucose; for antibiotic concentrations, see text.

[d] The rate of turnover in nongrowing cultures was determined as the decrease of cellular radioactivity per hour. These values must be compared with the κ value for growing control cultures in Table 1.

[e] The change of the E_{540} after 2 h (experiments 1 and 4) or 6 h (experiments 2 and 3) was recorded. The initial optical density was set at 100 in all the incubations (actual initial E_{540} was between 0.07 and 0.5). In growing cultures the value of ΔE_{540} was between 400 and 800.

[f] Corresponding figure numbers; —, figure not shown.

The rate of wall turnover was only slightly lowered in the inhibited cultures (Table 2, Fig. 4a); no significant differences were found between cultures treated with fosfomycin, cycloserine, penicillin G, or vancomycin. Pulse and continuous wall labels were released at identical rates, the former only after a lag (Fig. 2b).

Inhibition of protein synthesis. During inhibition of protein synthesis, wall synthesis is still possible for some time in *B. subtilis* (10, 21). We found in our cultures that, indeed, incorporation of new peptidoglycan precursors continued after the addition of chloramphenicol, but with a somewhat lower rate than in the control (Fig. 4b). Generally, optical density increased slowly, and lysis occurred only after extended incubation. In some experiments, lysis was observed soon after the start of the chloramphenicol treatment (Table 2, experiment 3), suggesting by the lack of new protein synthesis that wall synthesis had deterioriated more rapidly than the turnover activity.

DISCUSSION

In *B. subtilis* subsp. *niger*, the percentage of the wall lost per generation time was found to be approximately the same in rapidly (generation time 40 min) and slowly (generation time 4 h) growing cells (6). These findings indicated that a relation exists between the rate of wall turnover and the rate of growth. The data presented in this paper show that such a relation does not hold in cells after a sudden downward shift of the growth rate, as caused by starving potassium- or phosphate-limited cells for their respective limiting nutrient. Under these circumstances, wall turnover continued at a rate comparable to that in the control cultures and declined only after several hours. This slow reaction argues against regulation of the autolysin activity by an immediate adjustment (e.g., by the rapid secretion of inhibitors into the wall) to the new growth rate. Instead, it may be assumed that only a gradual adaptation of the rate of wall turnover to the new growth rate will occur. One

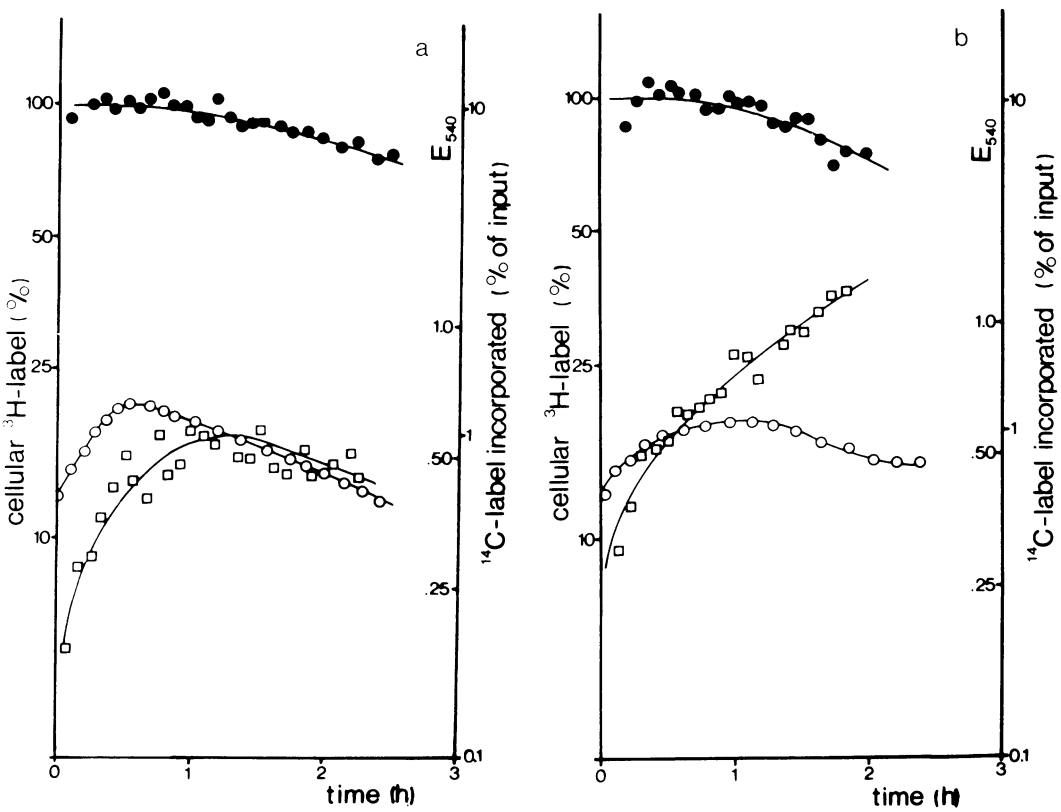

FIG. 4. Wall turnover and synthesis in EMM during inhibition of wall synthesis by cycloserine (a) and inhibition of protein synthesis by chloramphenicol (b). Bacteria were grown and pulse labeled with [^{3}H]GlcNAc as described in the legend to Fig. 1. Cells were isolated by centrifugation and reincubated at an E_{540} of 0.45 in EMM containing [^{14}C]GlcNAc. The loss of cellular ^{3}H label (●), the incorporation of [^{14}C]GlcNAc (□), and E_{540} (○) were followed as described in the text. A 100% radioactivity was 2.1×10^5 dpm/ml for ^{3}H and 4.6×10^5 dpm/ml for ^{14}C in both cultures.

might envisage that the wall material originally present is gradually diluted out by wall made at the new growth rate and containing a lower complement of autolytic enzymes. Since a large fraction of the radioactive wall could be lost in this period without the occurrence of cell lysis (Fig. 3b), it may be assumed that wall synthesis is adapted to the new growth rate in a coordinate fashion together with wall turnover. Under carbon-source starvation, however, the wall synthesis apparently was not adapted to the prevailing turnover rate, since extensive cell lysis occurred. Glaser and Lindsay (10) also examined some of the kinetics of cell wall turnover in *B. subtilis* 168. Using a labeling technique essentially identical to the one used by us, they showed that amino acid deprivation resulted in an adaptation of the turnover rate to the lowered growth rate. They generally found a faster adaptation rate than we found. However, the respective experiments are not entirely comparable, since we used phosphorus and potassium starvation and generally lower growth rates in the control cultures.

We obtained variable results when growth was inhibited with chloramphenicol. Generally, it is observed that inhibition of protein synthesis results in wall thickening (13), although lysis in the presence of 50 μg of chloramphenicol ml^{-1} has been reported as well (20). In our experiments, we found a correlation between the stability of the culture with respect to lysis after the addition of chloramphenicol and the rate of wall turnover. This rate has been shown to be rather variable between duplicate cultures (6, 7). In cultures showing a high rate of turnover, cell lysis occurred during chloramphenicol treatment, whereas cultures exhibiting a low rate of wall turnover remained stable for many hours. Presumably, both wall synthesis and autolysin activities decline during the inhibition of protein synthesis by the lack of new enzyme synthesis, but not always in an equivalent way. Therefore, the final status of the culture (lysis or stable culture, eventually with cells showing wall thickening) will be determined by the ratio of decreases of these respective activities.

The incubation of *B. subtilis* cells in the presence of wall synthesis inhibitors stopped the incorporation of new wall precursors, but hardly influenced the rate of loss of old (labeled) wall material. As a consequence, cell lysis occurred. The loss of pulse-labeled wall (being the last wall material made by the cells) after a short lag also showed that the most-underneath wall layers had become susceptible to hydrolytic attack by the autolysins. In growing cells, newly made wall layers are resistant towards turnover, possibly because of the presence of membraneous compounds (phospholipids and lipoteichoic acids), which prevent hydrolytic activity of the autolytic enzymes (5, 22). It was found in *B. subtilis* 168 that a rapid outflow of lipoteichoic acid occurs during incubation of the cells in the presence of penicillin G (12), which might result in the exposure of the underlying wall layers to hydrolytic attack by the autolysins. Our experiments showed that from the moment of antibiotic addition on, only a small fraction of the radioactively labeled wall had to be released to effect total lysis of the culture. Thus, when synthesis of new wall material is inhibited by starvation or by antibiotic action, the continuation of wall turnover disturbs the painstaking (essential for balanced growth of bacilli) tuning of these processes and causes the cells to lyse.

ACKNOWLEDGMENTS

F.J.K. was supported by the Foundation for Fundamental Biological Research, which is subsidized by the Netherlands Organization for the Advancement of Pure Research.

LITERATURE CITED

1. **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.
2. **Chaloupka, J.** 1967. Synthesis and degradation of surface structures by growing and non-growing *Bacillus megaterium*. Folia Microbiol. (Prague) **12**:264–273.
3. **Chaloupka, J., L. Říhova, and P. Křečková.** 1964. Degradation and turnover of bacterial cell wall mucopeptides in growing bacteria. Folia Microbiol. (Prague) **9**:9–15.
4. **Clarke, J. S., and C. A. Pasternak.** 1962. The regulation of amino sugar metabolism in *Bacillus subtilis*. Biochem. J. **84**:185–191.
5. **Cleveland, R. F., J.-V. Höltje, 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.
6. **de Boer, W. R., F. J. Kruyssen, and J. T. M. Wouters.** 1981. Cell wall turnover in batch and chemostat cultures of *Bacillus subtilis*. J. Bacteriol. **145**:50–60.
7. **de Boer, W. R., F. J. Kruyssen, and J. T. M. Wouters.** 1981. Cell wall metabolism in *Bacillus subtilis* subsp. *niger*: accumulation of wall polymers in the culture supernatant of chemostat cultures. J. Bacteriol. **146**:877–884.
8. **Evans, C. G. T., D. Herbert, and D. W. Tempest.** 1970. The continuous cultivation of micro-organisms. II. Construction of a chemostat, p. 277–327. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 2. Academic Press, Inc., New York.
9. **Frehel, C., and A. Ryter.** 1979. Peptidoglycan turnover during growth of a *Bacillus megaterium* Dap^- Lys^- mutant. J. Bacteriol. **137**:947–955.
10. **Glaser, L., and B. Lindsay.** 1977. Relation between cell wall turnover and cell wall growth in *Bacillus subtilis*. J. Bacteriol. **130**:610–619.
11. **Hebeler, B. H., and F. E. Young.** 1976. Chemical composition and turnover of peptidoglycan in *Neisseria gonorrhoeae*. J. Bacteriol. **126**:1180–1185.
12. **Horne, D., R. Hakenbeck, and A. Tomasz.** 1977. Secretion of lipids induced by inhibition of peptidoglycan synthesis in streptococci. J. Bacteriol. **132**:704–717.
13. **Hughes, R. C., P. J. Tanner, and E. Stokes.** 1970. Cell wall thickening in *Bacillus subtilis*. Comparison of thickened and normal walls. Biochem. J. **120**:159–170.
14. **Kruyssen, F. J., W. R. de Boer, and J. T. M. Wouters.** 1980. Effects of carbon source and growth rate on cell wall composition of *Bacillus subtilis* subsp. *niger*. J.

Bacteriol. **144:**238–246.
15. **Kruyssen, F. J., W. R. de Boer, and J. T. M. Wouters.** 1981. Cell wall metabolism in *Bacillus subtilis* subsp. *niger*: effect of changes in the phosphate supply to the culture. J. Bacteriol. **146:**867–876.
16. **Mauck, J., L. Chan, and L. Glaser.** 1971. Turnover of the cell wall of gram-positive bacteria. J. Biol. Chem. **246:**1820–1827.
17. **Pooley, H. M.** 1976. Turnover and spreading of old wall during surface growth of *Bacillus subtilis*. J. Bacteriol. **125:**1127–1138.
18. **Pooley, H. M.** 1976. Layered distribution, according to age, within the cell wall of *Bacillus subtilis*. J. Bacteriol. **125:**1139–1147.
19. **Rogers, H. J., and C. W. Forsberg.** 1971. Role of autolysins in the killing of bacteria by some bactericidal antibiotics. J. Bacteriol. **108:**1235–1243.
20. **Rogers, H. J., and C. Taylor.** 1978. Autolysins and shape change in *rodA* mutants of *Bacillus subtilis*. J. Bacteriol. **135:**1032–1042.
21. **Tynecka, Z., and J. B. Ward.** 1975. Peptidoglycan synthesis in *Bacillus licheniformis*. The inhibition of crosslinking by benzylpenicillin and cephaloridine *in vivo* accompanied by the formation of soluble peptidoglycan. Biochem. J. **146:**253–267.
22. **Wicken, A. J., and K. W. Knox.** 1977. Biological properties of lipoteichoic acids, p. 360–365. *In* D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.
23. **Wong, W., F. E. Young, and A. N. Chatterjee.** 1974. Regulation of bacterial cell walls: turnover of cell wall in *Staphylococcus aureus*. J. Bacteriol. **120:**837–843.