Turnover of the Cell Wall Peptidoglycan of Lactobacillus acidophilus

THE PRESENCE OF A FRACTION IMMUNE TO TURNOVER*

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SUMMARY

Exponentially growing cultures of Lactobacillus acidophilus strain 63AM Gasser were previously shown to lose about one-third of their cell wall peptidoglycan per generation via turnover (BOOTHBY, D., DANEO-MOORE, L., HIGGINS, M. L., COYETTE, J., AND SHOCKMAN, G. D. (1973) J. Biol. Chem. 248, 2161-2169). We now show that 20 to 30% of the \([\text{H}]\)lysine initially present in insoluble peptidoglycan fractions was retained after 4 or more generations of continued exponential growth of cultures in the absence of label. Treatment of peptidoglycan fractions, before and after 6 or 8 generations of chase with lysozyme (EC 3.2.1.17), released soluble products containing \([\text{H}]\)lysine which had electrophoretic mobilities identical with the disaccharide-peptide derivatives obtained from the wall peptidoglycan of this species. Because protein is known to contaminate peptidoglycan residues, the double labeled technique was used to show that one-half or less of the label lysine present after 6 or 8 generations of chase could be attributed to protein contamination. This then left a minimum fraction of 10 to 20% of the peptidoglycan that was immune to turnover. The absence of turnover of peptidoglycan labeled during short pulses has now been quantitated to show that pulses shorter than 12% of a generation (6 to 7 min) did not turn over. This turnover-immune fraction is in reasonably good agreement with the immune fraction of 10 to 20% observed after long periods of chase of extensively labeled peptidoglycan.

Evidence consistent with turnover of bacterial cell wall peptidoglycans has been obtained with Bacillus megaterium K12 (1-3), Bacillus cereus (3), Bacillus subtilis (2, 4), and more recently with Lactobacillus acidophilus strain 63AM Gasser (5). On the other hand, a variety of bacteria, including a mutant of B. megaterium (6), Escherichia coli (7, 8), and Streptococcus faecalis ATCC 9790 (5) do not exhibit peptidoglycan turnover. This lack of consistency suggests that peptidoglycan turnover may not be essential to peptidoglycan biosynthesis. Furthermore, peptidoglycan turnover does not appear to be required, either for enlargement of the cellular surface area, or for the normal reactions leading to wall thickening in a variety of bacteria (9).

In earlier work from this laboratory, several anomalous properties of peptidoglycan turnover in L. acidophilus were noted. (a) Peptidoglycan labeled for less than 0.2 generation did not turn over, whereas longer pulses resulted in increasingly faster rates of turnover (5). (b) When peptidoglycan labeled for 6 or more generations during exponential growth was chased, turnover was preceded by a lag period equivalent to 0.8 to 2 generations in length (5). (c) At no time did peptidoglycan turnover appear to go to completion. That is, after relatively prolonged chases, the rate of turnover appeared to slow and then cease.

We now present evidence supporting the view that a fraction of the peptidoglycan of L. acidophilus is immune to turnover. In addition, analysis of data from pulse-labeling experiments is used to more clearly delineate the relationships of pulse time to the occurrence of peptidoglycan turnover.

EXPERIMENTAL PROCEDURE

The methods used for growth, radioactive labeling, and determination of incorporation of \([\text{C}]\)- or \([\text{H}]\)lysine into the peptidoglycan fraction have been described in detail (5, 10). Peptidoglycan determinations were made by modification of the Park and Hancock procedure (11) with the use of glass fiber filter discs (12).

The presence of \([\text{H}]\)lysine in the disaccharide-peptide and peptide cross-linked oligomers of the cell wall peptidoglycan of L. acidophilus (13) was ascertained by high voltage paper electrophoresis of lysozyme hydrolysates of pronase-treated peptidoglycan residues (12) before and after extended periods of chase. Peptidoglycan residues on glass fiber discs were treated with 1 mg of hen egg-white lysozyme (EC 3.2.1.17) in 0.08 M ammonium acetate, pH 6.5, for 16 hours. The supernatant fractions were removed, clarified by centrifugation, and lyophilized to remove.
most of the volatile salts. The residues were taken up in a small volume of water, applied to paper strips (Whatman No. 3MM), and subjected to high voltage electrophoresis (60 volts per cm in 0.5 M formic acid, pH 2, for 2 hours, followed by 2 hours at 45 volts per cm in the same buffer system). The strips were dried, cut into 1.5-cm bands, and each band was placed into a vial, solubilized, and counted (12).

On one strip internal standards were used. These standards were added to a zero generation $[^3H]$lysine- and $[^{14}C]$valine-labeled sample before electrophoresis and consisted of a mixture of (a) the disaccharide-peptide $N$-acetylglucosaminyl-$N$-acetylmuramyl-$N$-(L-Alanyl-0-isoglutaminyl)-$N$-(o-isosparaginyl)-$N$-lysyl-$N$-[L-lysyl-$N$-[L-$^{14}C$]alanine], (b) the peptide cross-linked dimer of this disaccharide-peptide, and (c) a mixture of cross-linked trimers and oligomers of the same disaccharide-peptide. These products were isolated and purified from an autoytic $N$-acetylmuramidase digest of walls from an exponential phase culture of L. acidophilus grown for about 8 generations in the presence of $N$-$[^{14}C]$alanine.

Amino acid analysis of whole cells was performed on a Phoenix KS200 amino acid analyzer with the use of custom spherical resins, after hydrolysis of the sample in 6 N HCl for 18 hours at 110-120°C. The asymptotic regression program was designed to yield a least squares fit of data points thought to reflect a curve which approaches a line asymptotically. The equation is of the form $y = \alpha + \beta x$, where $\alpha$ is the asymptote, $\beta$ is a parameter which expresses curvature, and $\alpha + \beta$ is the $y$ intercept. In the case of this particular application, $\beta$ was limited to any value between 0 and 1, inclusive. The program was created and written in Focal-8 and was run on a Digital Equipment Corporation PD/P6e computer.

RESULTS

Extent of Wall Peptidoglycan Turnover—The extent of peptidoglycan turnover was investigated by chasing a culture previously labeled for an extended period, while maintaining conditions permitting continued exponential growth (Fig. 1). A culture of L. acidophilus was grown in the presence of a high specific activity of $[^3H]$lysine (100 $\mu$Ci, 20 $\mu$g of lysine per ml) for about eight generations and chased by a 1:10 dilution into fresh medium containing unlabeled lysine (300 $\mu$g per ml). After two doublings in turbidity, a volume of fresh medium equal to that remaining in the growth tube was added. After addition of fresh medium, an equal volume of the cell suspensions was removed in order to maintain a nearly constant volume. This process of dilution was repeated a total of seven times, so that growth of the culture was maintained within one doubling of turbidity (i.e., between turbidities equivalent to 40 to 80 $\mu$g per ml, dry weight). At intervals, samples were removed, and the radioactivity in the peptidoglycan fraction was determined. As shown in Fig. 1, peptidoglycan turnover began after a lag of about 60 min, continued with a $t_1/2$ of 68 min and then rapidly slowed and stopped at about 225 min, after about 31/2 generations of growth. At this time, and for several generations thereafter, about 20% of the initially present $[^3H]$lysine in the peptidoglycan fraction remained present. In a separate experiment, with a lag before turnover of 75 min and a $t_1/2$ of 83 min, turnover stopped at 280 min (or about 41/2 generations), when about 19 to 21% of the $[^3H]$lysine in the peptidoglycan fraction remained present.

The observation of a slowing down and cessation of loss of labeled lysine from the peptidoglycan fraction was rather unexpected. However, since cellular proteins, which also contain $[^3H]$lysine, showed little or no turnover during exponential growth, it seemed possible that after long periods of chase a larger than expected fraction of the peptidoglycan residue consisted of protein. If the residual labeled lysine is due partially or entirely to contaminating protein, then (a) protein contamination should be detectable by means of a labeled amino acid not present in the peptidoglycan (e.g. L-valine or L-leucine), (b) the lysine remaining in the peptidoglycan fraction should be resistant to lysozyme digestion with the lysozyme-sensitive portion of the $[^3H]$lysine in the peptidoglycan residues being an index of their true peptidoglycan content, and (c) after lysozyme treatment, little or no $[^3H]$lysine should be present in disaccharide-peptide fragments of the peptidoglycan.

Extent of Contamination of Peptidoglycan Fractions with Protein—Cells were grown in the presence of $L$-$[^3H]$leucine, $L$-$[^3H]$valine, or $L$-$[^{14}C]$valine for 6 or more generations and then chased for 5 to 6 generations of exponential growth. Peptidoglycan residues isolated before and after the period of chase were found to contain comparable levels of contamination with the two non-peptidoglycan amino acids. For example, in two separate experiments, at the start of the chase the peptidoglycan fractions contained 4% of the total $L$-$[^3H]$valine and 2% of the total $L$-$[^{14}C]$valine, respectively. The corresponding figures after 6 generations of chase were 5 and 3%, respectively. Since these values are not relative to labeled peptidoglycan lost by turnover, both the levels of protein contamination and the amounts of lysine incorporated into peptidoglycan were determined in a single experiment. Cells were grown exponentially for 8 generations in the presence of $L$-$[^3H]$lysine (100 $\mu$Ci, 20 $\mu$g per ml) and $L$-$[^{14}C]$valine (25 $\mu$Ci, 40 $\mu$g per ml). The culture was centrifuged (3,000 $\times$ g, 10 min) and the cells resuspended in 10 times the volume of complete medium containing 300 $\mu$g of $L$-lysine and 400 $\mu$g of $L$-valine per ml. This chased culture was grown for 3 more generations, diluted 1:8, grown for an additional 3 generations, diluted again, and grown for 2 more generations (a total of 8 generations). Samples were taken at zero time and after 6 and 8 generations of chase. The results from two such experiments are shown in Table 1. Similar to the experiment shown in Fig. 1, in Experiment 1, Table I, 23% of the $[^3H]$lysine remained in the peptidoglycan fraction after 6 generations of chase (Column 2),
TABLE I
Presence of L-[3H]lysine and L-[14C]valine in peptidoglycan and non-peptidoglycan fractions of Lactobacillus acidophilus

| Experiment | L-[^3H]lysine | L-[^14C]valine | Corrected lysine (as % of total) | Lysine:valine |
|------------|---------------|----------------|---------------------------------|--------------|
|            | dmol x 10^{-3} | % of | % of | in protein | In peptidoglycan | % of | ratio |
|            | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Experiment 1 | | | | | | | | | | | |
| 0 generation | | | | | | | | | | | |
| Total TCA ppt. | 3990 | (100) | (100) | 232 | (100) | (100) | 2.2 | - | - | 17.2 | (1.0) |
| Peptidoglycan | 698 | (100) | 17.5 | 5.7 | (100) | (100) | 3.4 | 14.1 | (100) | 122 | 7.1 |
| Peptidoglycan after lysozyme treatment | 68 | (100) | 1.7 | 3.6 | (100) | (100) | 2.2 | - | - | 17.9 | 1.0 |
| 5 generations | | | | | | | | | | | |
| Total TCA ppt. | 3320 | 83.2 | (100) | 268 | 115 | (100) | - | - | - | 17.6 | (1.0) |
| Peptidoglycan | 162 | 23.2 | 4.9 | 6.6 | 115 | 2.5 | 3.4 | 1.5 | 10.6 | 24.5 | 2.0 |
| Peptidoglycan after lysozyme treatment | 48 | 70.5 | 1.6 | 4.9 | 199 | 1.8 | 2.5 | - | -1.1 | 9.8 | 0.8 |
| 8 generations | | | | | | | | | | | |
| Total TCA ppt. | 3363 | 84.3 | (100) | 230 | 99 | (100) | - | - | - | 14.6 | (1.0) |
| Peptidoglycan | 176 | 25.2 | 5.2 | 6.5 | 114 | 2.8 | 3.8 | 1.4 | 9.9 | 27.0 | 1.8 |
| Peptidoglycan after lysozyme treatment | 47 | 69.1 | 1.4 | 4.2 | 110 | 1.8 | 2.5 | - | -1.1 | 11.2 | 0.8 |
| Experiment 2 | | | | | | | | | | | |
| 0 generation | | | | | | | | | | | |
| Total TCA ppt. | 3408 | (100) | (100) | 130 | (100) | (100) | - | - | - | 26.1 | (1.0) |
| Peptidoglycan | 609 | (100) | 17.9 | 3.4 | (100) | (100) | 3.6 | 14.3 | (100) | 180 | 6.9 |
| Peptidoglycan after lysozyme treatment | 24 | (100) | 0.7 | 1.0 | (100) | 0.8 | 1.0 | - | -0.3 | - | 25 | 1.0 |
| 6 generations | | | | | | | | | | | |
| Total TCA ppt. | 2890 | 84.8 | (100) | 131 | 100.1 | (100) | - | - | - | 21.9 | (1.0) |
| Peptidoglycan | 191 | 31.3 | 6.6 | 3.6 | 106 | 2.7 | 3.7 | 2.9 | 20.3 | 53.1 | 2.4 |
| Peptidoglycan after lysozyme treatment | 23 | 95.9 | 0.8 | 1.4 | 140 | 1.0 | 1.3 | - | -0.5 | - | 16.5 | 0.8 |

a All values are the mean of two duplicate samples corrected for dilution, each counted to minimize error (less than 2%) and deviate from each other by a maximum of 10%.
b Valine values are multiplied by 1.37 to correct for differences in cellular content of valine (297 nmol/mg) and lysine (408 nmol/mg) and are the equivalent to "protein" lysine.
c Negative values and 0 are most likely a function of the overcorrection referred to in the text as well as rather variable solubilization of protein during the lysozyme treatment.

and this level of [3H]lysine was maintained for the next 2 generations. In Experiment 2, Table 1, a somewhat higher fraction of the initial [3H]lysine (61%, Column 2) remained in the peptidoglycan fraction after 6 generations of chase.

The lysine and valine content of L. acidophilus was found to be 408 nmol and 297 nmol per mg (dry weight), respectively. The molar ratio of lysine to valine (1.37) of whole cells can be used to estimate the amount of lysine in the peptidoglycan fraction which can be attributed to protein (Table I, Column 7). This calculated fraction of lysine (Table I, Column 7) can then be subtracted from the percentage of total lysine found in the peptidoglycan fraction (Table I, Column 3) to correct observed values of lysine content in the peptidoglycan residues for lysine due to protein (Table I, Column 8). Correcting lysine found in peptidoglycan fractions for contaminating protein lysine in terms of percentages of total obviates the need for accounting for differences in the specific activities of the two labeled compounds. The factor used may overcorrect, since it is based on lysine in whole dried cells (i.e. in both protein and peptidoglycan) rather than only in protein, whereas the required correction should use only lysine in cellular protein. However, the correction does provide an estimate of the maximum contamination of the peptidoglycan fractions with protein lysine. Comparison of these corrected values indicated that in Experiment 1, Table I, 10 to 11% of the [3H]lysine present in the peptidoglycan fraction at zero time remained in the peptidoglycan fraction after 6 and 8 generations of chase (Column 9). In Experiment 2, Table I, the same type of correction resulted in a value of 20% (Column 9) of the initially present lysine in peptidoglycan after 6 generations of chase. In Experiment 2, Table I, the uncorrected [3H]lysine content of the peptidoglycan fraction after 6 generations of chase was somewhat higher than usually observed and is therefore considered to represent an upper limit.

The extent of contamination of peptidoglycan fractions by protein can be calculated by dividing the calculated fraction of lysine in protein (Table I, Column 7) by total lysine in the peptidoglycan fraction (Table I, Column 3). This calculated value takes into account lysine lost via peptidoglycan turnover and shows that in Experiment 1, Table I, 19.4, 69.4, and 73% of lysine in peptidoglycan fractions could be attributed to protein after 0, 6, and 8 generations of chase. For Experiment 2, Table I, the corresponding values for contaminating protein in peptidoglycan fractions were estimated to be 20% at zero time and 59% after 6 generations of chase. The differences between these calculated values and the much lower values determined directly from valine concentrations (Table I, Column 6) arise from the
occurrence of peptidoglycan turnover and a very low rate of protein turnover in exponential phase cells (5).

Double label experiments possess an added advantage, that is, the ratio of \(^{3}H:^{14}C\) can be compared in individual samples. In this way all comparisons are internal and independent of variations of sample size, and of dilutions. Furthermore, setting the ratio of lysine to valine (\(^{3}H:^{14}C\)) in the hot trichloroacetic acid precipitates (total) for each series to a value of 1.0 permits a direct comparison of ratios between sample series. Thus, samples with a relative lysine to valine ratio over 1.0 contain less protein than the corresponding hot trichloroacetic acid precipitates. In both experiments (Table I, Column 11) the relative ratios of lysine to valine in the peptidoglycan fractions at zero time were about 7. As expected, loss of \(^{3}H\)lysine from peptidoglycan, accompanied by little or no loss of protein, resulted in a decrease in the \(^{3}H:^{14}C\) ratio of peptidoglycan fractions after 6 and 8 generations of chase (Table I, Column 10). However, in all cases, after the chases the relative ratio remained at 1.8 to 2.4 (Table I, Column 11), about twice the level of the \(^{3}H:^{14}C\) ratio of the hot trichloroacetic acid-precipitable material. Consequently, by a somewhat independent method it was established that after prolonged periods of chase the peptidoglycan fractions remained enriched for \(^{3}H\)lysine. The observation that after 6 or 8 generations of chase the ratio of \(^{3}H:^{14}C\) remained at twice that of the hot acid precipitate indicates that about one-half of the \(^{3}H\)lysine in the peptidoglycan fraction is, in fact, truly in peptidoglycan. These estimates suggest that 10 to 15% of the initially labeled peptidoglycan is immune to turnover.

Lysozyme Sensitivity of Peptidoglycan Fractions Before and After Chase—Isolated cell walls of this species are relatively resistant to dissolution by hen egg white lysozyme (13). However, lysozyme treatment removed \(^{3}C\)-or \(^{3}H\)lysine from peptidoglycan fractions. Increased susceptibility of the peptidoglycan fractions to lysozyme could be due to loss of neutral and acid polysaccharides (13) during the hot trichloroacetic acid treatment.

In a number of experiments, lysozyme treatment of peptidoglycan fractions (2 mg of lysozyme in 2 ml of 0.1 m sodium phosphate, pH 6.7, for 30 to 60 min at 37°) resulted in the loss of 95 to 97% of the \(^{3}H\)lysine present in the start of the chase, and 92 to 99% after 5 or 6 generations of chase. In each case, after lysozyme treatment the relative ratio of lysine to valine remained approximately the same characteristic of the hot trichloroacetic acid precipitates (Table I, Column 11). The solubilization and consequent loss of \(^{3}H\)lysine is presumed to be due solely to lysozyme hydrolysis of peptidoglycan and could be considered to be good evidence for the presence of peptidoglycan in the chased samples. However, at least in these cases these data must be viewed with considerable caution. Specifically, it was found that the lysozyme treatment also resulted in the loss of a substantial amount of leucine (data not shown) or valine from peptidoglycan fractions (Table I, Column 3). From experiment to experiment, loss of protein labels was highly variable, ranging from a low of 30% to a high of about 75% of the labeled leucine or valine found in peptidoglycan fractions of both zero time and chased samples. Chased samples did not consistently lose either more or less labeled valine or leucine than did zero time samples. The observed loss of protein labels following lysozyme treatment was surprising. Possibly protein or peptides resistant to the pronase treatment remain associated with the peptidoglycan during the fractionation procedure. Although not rigorously excluded, the variable amounts of protein label lost do not support the idea that a protein(s) is covalently bound to peptidoglycan in this organism.

High Voltage Electrophoresis of Products of Lysozyme Digestion of Peptidoglycan Fractions—High voltage paper electrophoresis at pH 2 of lysozyme hydrolysates from labeled peptidoglycan fractions obtained after different periods of chase. Cells of Lactobacillus acidophilus were labeled for 8 generations. Samples taken at zero time and after 6 and 8 generations of chase were acid-precipitated and treated for isolation of the peptidoglycan. Peptidoglycan fractions were hydrolyzed with lysozyme, and the products were separated by paper electrophoresis at pH 2. The products, deposited 40 cm from the anode, migrated toward the cathode. A, profile of L-alanine-labeled disaccharide peptide standards (as described in the text) subjected to coelectrophoresis with the zero time sample. The low level of \(^{14}C\) valine present in the zero time sample permitted the inclusion of \(^{14}C\)-labeled internal standards without interference. B, C, and L \(^{3}H\)lysine profiles of the zero time, 6, and 8 generations, respectively. E, \(^{14}C\)valine profile for the 6-generation chase sample. Zero on the abscissa represents the origin, while numbers toward the right on the axis reflect distances, in centimeters, toward the cathode.

![Fig. 2](http://www.jbc.org/)
had electrophoretic mobilities identical with soluble peptidoglycan fragments isolated from walls of this species. In all three samples (panels B, C, and D in Fig. 2 for 0, 6, and 8 generations of chase, respectively) "H-containing peaks with the same mobilities as authentic disaccharide-peptide monomer, dimer, trimer, and higher oligomers (Panel A, Fig. 2) were seen.

The relatively high level of background counts observed could have been due either to the presence of "H-labeled peptides of protein origin or to peptidoglycan fragments covalently attached to a residue of other (non-peptidoglycan) wall polymers which were not completely removed by the hot trichloroacetic acid treatment. In all cases, little radioactivity remained near the origin where large molecular weight polypeptides would be expected. Since lysine is required for growth of this organism, and during the labeling phase lysine was present in the chemically defined medium at a relatively low concentration (20 pg per ml) compared with large excesses (100 to 300 pg per ml) of all other amino acids (10), it seems extremely unlikely that lysine was converted to another amino acid by this lactic acid bacterium.

Labeling Kinetics—It was shown previously (5) that ["H]lysine pulses of less than 20% of a generation did not show peptidoglycan turnover. With longer pulses the rate of turnover appeared to increase with increasing pulse time. Fig. 3 shows the results of an experiment in which ["H]lysine was used as the pulse label for the indicated fraction of a generation, while exposure to ['"C]lysine was initiated 6 to 7 generations prior to the pulse. The rates of peptidoglycan turnover, derived from data from four similar experiments (including the experiment shown in Fig. 3), were plotted against pulse time (in generations) and compared with the first order decay constants (in generations⁻¹) derived with a computer program (Fig. 4). Ideally, such a line should be asymptotic and approach a value of decay constant that is equal to the steady state value of the fully equilibrated label. Fig. 4 shows the actual data points as well as the best curve through those points, as determined by the computer program. The requirement for a computer program is apparent when the scatter of the data points is considered, especially at those pulse times which do not result in turnover. Rather than averaging the values of the points where k = 0, we chose to allow the values of k derived from longer pulse times to influence the value for the x intercept. The intercept on the x axis is the derived point for which a longer pulse time would yield turnover, while a shorter pulse will not yield turnover. The value is 0.122 or about 12% of a generation, corresponding to 6 to 6.6 min in these experiments.

DISCUSSION

The results presented strongly suggest that a fraction of the cell wall peptidoglycan of L. acidophilus strain GaSSM Gasser is immune to turnover. The data on extended chases of cells grown in the presence of labeled precursors of peptidoglycan and protein indicate that at least 10% of the peptidoglycan present at the beginning of the chase fails to turn over. In some experiments a somewhat larger immune fraction was observed ranging to a maximum of about 20% of the peptidoglycan.

An entirely different methodological approach resulted in nearly the same conclusion. Quantitation of the pulse time required to observe peptidoglycan turnover showed that turnover was only observed after a pulse exceeding 12% of a generation (6 to 7 min). Thus, by this method the amount of cellular peptidoglycan synthesized in 12% of a generation is immune to turnover, a value in excellent agreement with that obtained from the data on extended periods of chase of extensively labeled peptidoglycan.

The immunity to turnover may be restricted to a specific, chemically different, portion of the wall, such as that fraction more closely associated with one or both of the covalently attached non-peptidoglycan wall polymers (13), to a particular layer (e.g. innermost), or to a topographical portion (e.g. the cell poles) of the wall. It should be noted that the amount of pepti-
doglycan remaining after completion of turnover could be visualized as enough to cover the poles of this cylindrically shaped species. Beyond this, there is no real basis for any of the above explanations. Moreover, it should be noted that peptidoglycan made during valine starvation (when little surface enlargement occurs and walls primarily undergo thickening) is as susceptible to turnover during subsequent regrowth as is wall made during exponential growth, even though during valine starvation turnover of peptidoglycan is not observed (5). To explain some of the anomalies of peptidoglycan turnover in L. acidophilus, a model was suggested in which precursors could be directed toward two peptidoglycan products, one which was rapidly labeled but exhibited little if any turnover, and a second which was labeled somewhat slower but turned over at a high rate. An association of either of these two products with a non-peptidoglycan polymer could possibly result in reduced incorporation or increased degradation rates or both, as required in the above scheme. In this respect, the association of newly incorporated teichoic acid chains with newly synthesized peptidoglycan in B. subtilis (14) may be of significance. Also worth noting are the observations of Mirelman et al. (15) that two different mechanisms, a penicillin-sensitive transpeptidation and a penicillin-resistant transglycosylation, appear to be involved in inserting newly synthesized peptidoglycan chains into the wall of Micrococcus luteus. A substantial portion of the incorporation into the wall appears to occur via the transglycosylation reaction. In this connection it is of interest to note that in numerous rod-shaped organisms, low concentrations of penicillin permit surface elongation and surface thickening, but affect septation (16).

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