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Effect of Penicillin G on Release of Peptidoglycan Fragments by *Neisseria gonorrhoeae*: Characterization of Extracellular Products

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The effect of penicillin G (penG) on the turnover and release of peptidoglycan (PG) by *Neisseria gonorrhoeae* RD_5 was investigated. We previously showed that exponentially growing gonococci (labeled in the glycan moiety with glucosamine and muramic acid and in the peptide with diaminopimelic acid) turn over ca. 35% of their PG per generation. In current studies, addition of penG accelerated the rate of PG hydrolysis by more than twofold and resulted in a corresponding increase in the amount of soluble PG fragments found in the medium. This increase in soluble PG was accounted for mainly by the release of nonreducing (anhydro-muramyl-containing) disaccharide peptide dimers (molecular weight, about 2,000) and trimers that were composed of subunits, linked not by peptide cross-linking bonds, but probably only by glycosidic bonds. The enhanced release of these products suggested that penG, directly or indirectly, stimulates the activity of a glycan-splitting, gonococcal PG:PG-6 muramyl transferase (transglycosylase). PG monomers that were released in the presence of penG were identical, both qualitatively and quantitatively, to the monomers released as a result of turnover in the absence of penG and consisted of anhydro-muramyl-containing disaccharide tripeptides and tetrapeptides. PenG-treated bacteria consistently released slightly less free disaccharide and free peptides than did control cultures, implying a penG-associated depression in the activity of the gonococcal *N*-acetylmuramyl-L-alanine amidase. In addition to stimulating the release of PG fragments, penG was also associated with greatly enhanced release from cells of glucosamine-containing non-PG macromolecule(s).

Gonococci appear to be unusual among gram-negative bacteria in that they exhibit extensive turnover of macromolecular peptidoglycan (PG) during exponential growth (4, 6, 8, 14) and release soluble PG fragments into the culture medium (14, 18). Our previous analysis (14, 18) of the radiolabeled soluble PG in supernatants revealed four major types of soluble PG fragments detected by gel filtration. These were identified as forms of bisdisaccharide peptide dimer (PGI; 14), disaccharide peptide monomer (PGII, the principal extracellular PG product), free peptides (PGIIIa), and free disaccharide (PGIIIb). The presence of these fragments suggested that turnover and release of PG by growing gonococci was mediated by both glycan-splitting (hexosaminidase) and peptide-splitting (*N*-acetylmuramyl-L-alanine amidase) activities.

Further radiochemical characterization has recently revealed that the predominant monomer fraction was a mixture of two distinct disaccharide peptides (18). The major (80%) component was *N*-acetylglucosaminyl-β-1$\rightarrow$4,1,6-anhydro-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-diaminopimelic acid; the remainder was the corresponding anhydro-*N*-acetylmuramyl-containing disaccharide tetrapeptide containing a C-terminal D-alanine. The novel feature of these and all other glycan-containing fragments in supernatants was the presence of the nonreducing (anhydro-muramyl) end. This reflected the activity of a gonococcal PG:PG-6-muramyl transferase (transglycosylase), apparently identical to a hexosaminidase activity previously detected in *Escherichia coli* (2, 12, 20). The structure of these extracellular PG products appeared to be otherwise identical to the reducing fragments typically derived from gram-negative PG (chemotype I; 17) by the action of lysozyme and bacterial muramidases.

Our ultimate objective is to characterize and purify these (and other) gonococcal PG fragments that conceivably might gain the opportunity to interact with the host in vivo and to

assess the effects of these soluble fragments on the host response to gonococcal infections. The current study characterizes the PG fragments that are released by gonococci upon addition of penicillin G (penG) to cultures.

MATERIALS AND METHODS

Growth and radiolabeling of bacteria. *Neisseria gonorrhoeae* RD_5, a highly autolytic strain (7) kindly supplied by F. E. Young, University of Rochester School of Medicine and Dentistry, Rochester, N.Y., was used throughout these studies. The organism was maintained by serial passage on clear solid medium (19), and colonies were typed by the criteria of Swanson (19). Bacteria used in all experiments were nonpiliated (p−) and transparent (tr) according to this scheme (19). The minimal inhibitory concentration of penG for strain RD_5 was 0.007 to 0.015 μg/ml.

Gonococci were cultured at 37°C in liquid medium ($LGCB^+$; pH, 7.3 ± 0.1) containing 0.4% (wt/vol) pyruvate as the carbon source as described (15, 16). To radiolabel the peptide and glycan moieties of gonococcal PG, gonococci were grown in the presence of DL-[*G*-^{3}H]diaminopimelic acid (DAP; Amersham Corp.) or D-[6-^{3}H]- or [^{14}C]glucosamine ($glcNH_2$; ICN Pharmaceuticals Inc.), respectively, as described (14, 18). In purified PG, $glcNH_2$ labels both sugars of the glycan backbone, exclusively; DAP labels specifically the peptide (14).

For pulse-chase experiments, labeled gonococci were washed with fresh medium at 37°C to remove unincorporated label and to deplete cytoplasmic pools of labeled PG precursors (14, 18). Equal portions of the washed labeled bacteria were inoculated into fresh radioactive-free $LGCB^+$ containing penG (2 μg/ml; Sigma Chemical Co.) or into $LGCB^+$, alone. At the time of inoculation, all of the labeled $glcNH_2$ was present in bacteria as acid-precipitable (macromolecular) material (18). For all experiments, penG (when present) was added at 0 min. Cultures were inoculated to an initial density of approximately 10^8 bacteria/ml and, at intervals, the turbidity at 540 nm of cultures was measured with a Spectronic 20 spectrophotometer (Bausch & Lomb). Samples were also taken to (i) assess PG turnover and (ii) test for soluble PG in supernatants after centrifugation at 12,000 × *g* for 10 min.

Isolation and analysis of PG and PG fragments. Procedures described in earlier publications (14, 16, 18) were used for the purification of intact insoluble PG and determination of the rate of turnover of radiolabeled PG in cells (both utilizing a sodium dodecyl sulfate extraction procedure; 8, 14), for fractionation of soluble PG fragments in supernatants using gel filtration on Sephadex G-50 and G-25, and for preparation of purified disaccharide peptide monomers (and higher oligomers) obtained from Chalaropsis B muramidase (Miles Laboratories, Inc.) digests of purified intact PG. The methods used for radiochemical analysis of PG (and non-PG) fractions have also been described previously: (i) labeled amino sugars, e.g., $glcNH_2$ and muramic acid, were identified by paper chromatography of acid hydrolysates in butanol-acetic acid-water (5:1:2, vol/vol) (14, 18); (ii) reducing sugar analysis was performed using the borohydride method (3, 18); (iii) free amino group analysis of labeled DAP residues was performed with fluorodinitrobenzene (FDNB) (16, 18); and (iv) disaccharide peptide monomers that contained a reducing muramic acid end or a nonreducing (anhydro-muramyl) end were identified by paper chromatography (18).

Radioactivity determinations for aqueous samples and for samples dried on paper were as described (14, 16). All data were corrected for quenching and reported as disintegrations per minute (dpm).

RESULTS

Effect of penG on release of PG fragments. We previously determined the rate of PG turnover in exponentially growing *N. gonorrhoeae* RD_5 and characterized the soluble PG fragments which appeared, concurrently, in culture supernatants (14, 18). Under our growth conditions employing liquid medium at pH 7.3, the turnover rate of both the peptide and the glycan moieties followed first-order kinetics and was approximately 35% of existing PG per generation (ca. 63 min). This rate of PG turnover was confirmed in current experiments (Fig. 1). The principal $glcNH_2$-labeled PG fragments released as a result of turnover were disaccharide peptide monomers (Fig. 2), which were previously shown to contain an unusual anhydromuramyl (nonreducing) end (18).

Addition of penG (2 μg/ml) to cultures had little or no effect on culture turbidity for the first 20 min after inoculation of washed labeled bacteria into fresh unlabeled medium. Thereafter, turbidity dropped by 25 to 50% during the 60-min incubation. Throughout the entire incubation period, the rate of hydrolysis of $glcNH_2$-labeled PG was much higher in penG-treated than in control (minus penG) cultures (Fig. 1).

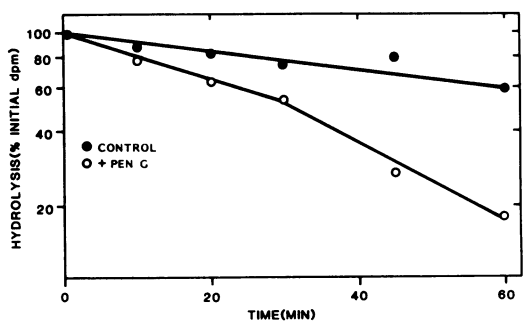

FIG. 1. *Hydrolysis of $glcNH_2$-labeled PG in N. gonorrhoeae RD_5 cultured in $LGCB^+$ media with or without penG (2 μg/ml). PG was measured as sodium dodecyl sulfate-insoluble material. PenG was present in the designated culture at 0 min.*

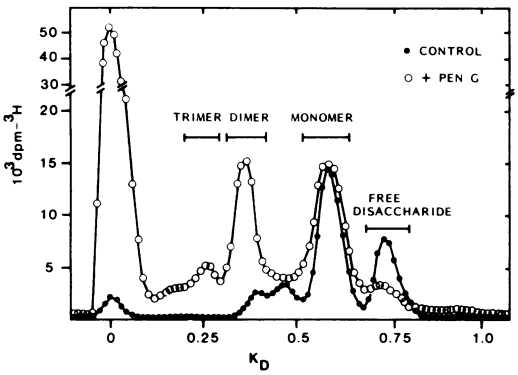

FIG. 2. *Gel filtration on connected columns of Sephadex G-50 and G-25 of supernatant fluids obtained from [^{3}H]glcNH$_2$-labeled RD$_5$ after 60 min of incubation in LGCB$^+$ (control) or in LGCB$^+$ plus penG (2 μg/ml). The supernatants were harvested from the same cultures as those used to determine PG turnover in Fig. 1. Included fractions were found to contain PG fragments as designated. Void peaks contained little or no PG (see Results).*

PenG enhancement of PG hydrolysis in gonococci grown under conditions that were different from ours has been reported previously (4, 22).

The enhanced PG hydrolysis in penG-treated cultures of glcNH$_2$-labeled cells was accompanied by a corresponding net increase in the release of soluble products having K_d values indistinguishable from PG fragments released by control cultures (Fig. 2). This penG-mediated increase in the release of PG fragments was accounted for almost exclusively by material in the size range of disaccharide peptide dimers (ca. 2,000 daltons) and trimers. There was essentially no net change in the release of glcNH$_2$-labeled fragments with the K_d of disaccharide peptide monomers. Curiously, the level of free disaccharide (reflecting, in part, the activity of the gonococcal amidase; 9, 14, 18) was reduced compared with that of untreated cultures (Fig. 2). This is consistent with the fact that penG-treated bacteria that had been pulsed with [^{3}H]DAP released low levels of free peptides relative to control cultures (data not shown). In addition, there was no indication in penG cultures of the peak (K_d = 0.48) intermediate in size to monomer and dimer (Fig. 2), which we previously suggested (18) might be a glycan-linked tetrasaccharide containing a peptide substituent on only one of the two muramic acid residues. Generation of this product, similar to that of free disaccharide, would also seem to require, in part, the activity of a gonococcal amidase. It should be emphasized that we used a common source of labeled cells and inoculated equal portions into medium with or without penG. Therefore, the amounts of each product (assessed as total dpm under the specified peak) in penG-treated and control cultures can be compared directly (Fig. 2).

Perhaps the most dramatic effect associated with penG was the greatly enhanced release of glcNH$_2$-labeled macromolecular material in the void volume (K_d = 0). This voided material appeared to contain little or no PG (see below). In each of three separate experiments that were performed identically to that in Fig. 2, the direction and magnitude of the effect of penG on release of individual PG fragments and of the glcNH$_2$-labeled material at K_d = 0 were similar.

Characterization of glcNH$_2$-labeled material released in the presence of penG. To confirm that the soluble fragments released by RD$_5$ in the presence of penG and included in the Sephadex G-50 and -25 columns (Fig. 2) were PG, we identified the radiolabeled amino sugars in acid hydrolysates of isolated fragments. All of the included fragments, ranging in size from that of free disaccharide through that of disaccharide peptide trimers, contained both [^{3}H]glcNH$_2$ and [^{3}H]muramic acid, and in the same relative amount as intact PG isolated from the parent culture. The large void peak liberated from penG-treated cultures, on the other hand, contained mostly glcNH$_2$, and little or no muramic acid. In addition, the total dpm in the included (PG) fragments (Fig. 2) accounted for all of the sodium dodecyl sulfate-insoluble dpm that were solubilized as a result of PG hydrolysis (Fig. 1). Together, these data indicated that the included fractions were PG fragments, as expected, and that the voided material(s) were non-PG macromolecule(s). Consistent with this latter conclusion, the voided material was not degraded by Chalaropsis B muramidase. An internal control of intact PG in the same tube, however, was digested completely to the expected low-molecular-weight PG fragments.

We previously reported (18) that borohydride treatment of isolated glycan-containing PG fragments released by untreated RD$_5$ yielded only glcNH$_2$ and muramic acid. Control (internal) samples of PG fragments obtained by Chalaropsis B enzyme digestion of intact PG yielded mainly glcNH$_2$ and muramicitol, as expected for the digestion products of a muramidase (18). In current experiments, the monomer and dimer fractions released by penG-treated cultures of RD$_5$ were found to be similar to fragments released by untreated exponentially growing gonococci in that they yielded glcNH$_2$ and muramic acid and little or none of the corresponding alcohols after borohydride treatment (Fig. 3). This indicated that, like PG fragments from untreated bacteria, the soluble PG released in

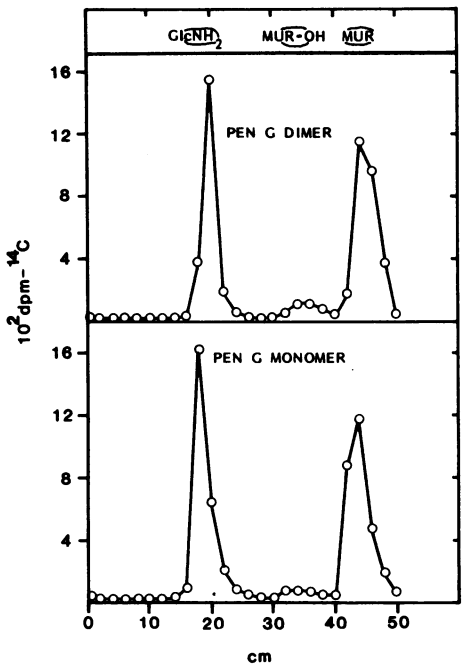

FIG. 3. *Paper chromatography in butanol-acetic acid-water (3:1:1; vol/vol) of acid hydrolysates of borohydride-treated PG fragments (monomer and dimer) isolated from the culture supernatant of [^{14}C]-glcNH$_2$-labeled, penG-treated gonococci. Reference compounds, i.e., glcNH$_2$, muramic acid (mur), and muramicitol (mur-OH) were detected using ninhydrin.*

the presence of penG contained no reducing ends. To confirm this, monomers isolated from control and penG cultures and from muramidase digests of intact PG were subjected to paper chromatography in butanol-acetic acid-water (5:1:2; vol/vol). This was previously employed (18) as one of several methods used to identify the monomer fraction released from untreated exponentially growing gonococci as a mixture of the anhydro-muramyl-containing disaccharide tripeptide (ca. 80%) and the corresponding disaccharide tetrapeptide (ca. 20%). On this basis, the composition of the monomer fraction released from penG-treated gonococci was identical to that released from untreated bacteria (data not shown).

FDNB analysis of PG fragments released by untreated RD$_5$ indicated previously (18) that the majority of each oligomer fraction was peptide cross-linked. A minor amount appeared to represent subunits linked by bonds other than peptide bonds involving DAP residues; these were thought to be glycosidically linked oligomers. Interestingly, the dimer and trimer fractions from penG-treated bacteria appeared to contain no peptide cross-linking bonds, i.e., these yielded only 2,4-dinitrophenyl (monoDNP)-DAP after treatment with FDNB (Fig. 4). These components may represent oligomers of the disaccharide peptide repeating subunit linked by glycosidic bonds, exclusively.

In summary, penG markedly stimulated hydrolysis of gonococcal PG concomitant with the enhanced release of certain soluble PG fragments and of a non-PG macromolecule(s). The increased level of soluble PG in supernatants of penG-treated cultures was accounted for by the appearance of large amounts of disaccharide peptide dimers and trimers that consist of subunits linked not by the usual peptide cross-linking bonds but probably by glycosidic bonds.

DISCUSSION

Previous work from Morse's (22) and Tomasz' (4) laboratories indicated that penG stimulates the hydrolysis of macromolecular gonococcal PG. One of these groups (4) examined the turn-

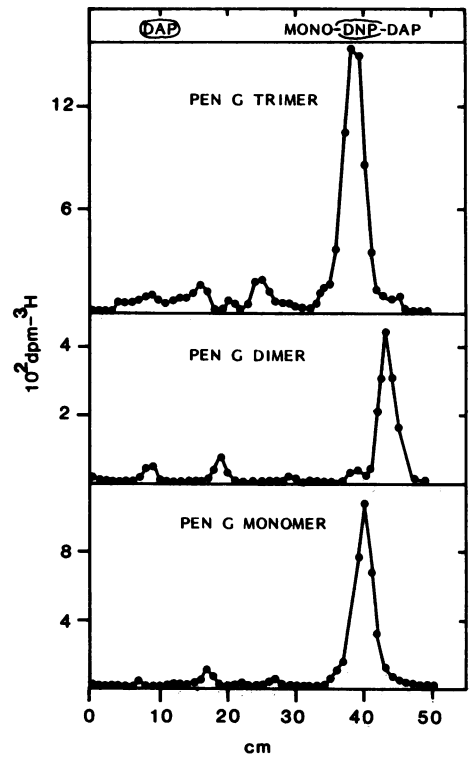

FIG. 4. *Free amino group analysis with FDNB of [^{3}H]DAP residues in soluble PG fragments isolated from the culture supernatant of penG-treated gonococci. Samples were treated with FDNB, acid hydrolyzed, and chromatographed on paper in butanol-acetic acid-water (5:1:2; vol/vol) to separate monoDNP-[^{3}H]DAP and free [^{3}H]DAP. The migrations of reference compounds (detected with ninhydrin) are indicated.*

over of the DAP-labeled peptide and $glcNH_2$-labeled glycan moieties of PG from gonococci grown in "lysis-protective" (pH 6.4, 0.05 M Mg^{2+}) and "lysis-permissive" (pH 8.0) medium. Because we employed pH 7.3 medium exclusively, their data (4) and ours are not directly comparable. Nevertheless, our results confirm the general finding of penG enhancement of PG hydrolysis (4, 22) and extend the observations to include characterization of the soluble PG fragments released, concurrently, into the medium. This characterization allows assessment of the role of specific PG hydrolases in the process and has implications for the pathobiology of gonococcal disease.

The effects of penG on gonococci appear to be complex and to involve opposite net effects on two principal PG hydrolase activities (transglycosylase and amidase). Yet, it should be emphasized that our analyses of soluble PG in the medium assess the influence of penG on only one expression of PG hydrolase activity, i.e., that involved in release of PG fragments, per se. One should not infer that the effects of penG on the total cellular activity of a given PG hydrolase would necessarily be similar to the effects on the enzymatic activities actually responsible for the release of soluble PG. For example, a select fraction of glycan-splitting activity could conceivably occupy a domain in the cell envelope where it may cleave internal linkages in the glycan backbone, but at restricted sites, which do not lead to removal of any soluble PG products from the insoluble macromolecular matrix. Localized restriction of enzyme activity of this sort could relate to the local spatial relationship between the enzyme and an appropriate PG substrate or to the presence of bacterial inhibitors of PG hydrolase activity. Our results do not necessarily reflect such an activity, i.e., one that does not result in release of the PG products from cells. In short, gonococci may possess additional PG hydrolases (that may or may not be affected by penG), the detection of which is selectively discriminated against in these studies. In fact, recent experiments (E. Gubish and T. Buchanan, personal communication) suggest that gonococci do possess a glucosaminidase capable of hydrolyzing bonds in gonococcal PG although there is no evidence that such an activity is involved in the release of PG fragments to the external environment.

In RD_5, penG directly or indirectly (i) enhances release of PG fragments, e.g., disaccharide peptide dimers and higher oligomers, that could be accounted for solely by stimulation of gonococcal transglycosylase activity, and (ii) concurrently depresses release of fragments, e.g., free peptide and free disaccharide, that requires amidase activity for their generation. The apparent stimulation of transglycosylase activity is intriguing in several ways. First, why does penG cause greatly enhanced release of oligomers although it has little or no net effect on the release of monomers? After all, it seems likely that a common glycan-splitting activity would be involved in the generation of both. Second, why do the oligomers from penG cultures (unlike control cultures) consist of subunits that appear to be linked via glycosidic bonds, exclusively, and are not cross-linked by DAP-containing peptide bonds. Currently we can offer no satisfactory explanations for these phenomena. It is possible that they may relate to penG-associated alterations in substrate specificity of glycan-splitting enzyme(s). It is also conceivable that activation of a D-alanine-*meso*-DAP endopeptidase activity (which would split peptide cross-linking bonds) may be involved. To our knowledge such an activity has not been described in gonococci.

The accumulation of $glcNH_2$-containing, non-PG macromolecules in culture supernatants of penG-treated gonococci is also of potential interest in that it possibly may be related to penG-enhanced PG hydrolysis and release of transglycosylase-derived PG fragments. This datum is consistent with the earlier observations of Goodell et al. (4) who reported that gonococci release large amounts of non-PG material as a result of penG treatment (even under cultural conditions which protected the bacteria from penG-induced lysis); they suggested that this material was lipid and lipopolysaccharide. We are aware of no data demonstrating a cause and effect relationship between the loss of this lipid-containing substance(s) and enhanced PG hydrolysis in gonococci; at this time, there is only circumstantial evidence. Yet, it is interesting to speculate that such material may be related to the regulation of PG hydrolase activity in gonococci in a manner analogous to the suspected role of other lipid-containing substances as natural inhibitors of autolysis in some gram-positive bacteria (21).

Although we are pursuing the mechanisms which promote the release of soluble PG after addition of penG, there are two somewhat more immediate questions. First, could the generation of soluble PG fragments resulting from penG-enhanced hydrolysis of PG occur in vivo? And second, what is the possible biological relevancy of these products should they arise during the course of β-lactam treatment of gonococcal (or other bacterial) infections? As to the former, numerous complex factors could undoubtedly influence the extent of penG-mediated PG release in vivo; one of obvious importance is whether release of PG is induced by penG at

concentrations of the drug that are actually achievable in tissues. We have not yet tested the effects of low concentrations of the drug, e.g., 1 minimal inhibitory concentration, on PG release. However, even the rather large amount of penG used in these experiments (2 μg/ml; ca. 200 minimal inhibitory concentrations) is apparently readily achievable in the blood after standard penG therapy for gonorrhea (1). In regard to the second question, it may be significant that the principal PG fragments released as a result of penG action, like the products derived from control cultures, possess the unusual anhydromuramyl (nonreducing) end. As considered previously (18), this modification could conceivably potentiate the biological activities of soluble PG derivatives that may depend on interaction with cell membranes. In addition, the penG-induced fragments, unlike the major PG fragments (disaccharide peptide monomers) derived from supernatants of untreated gonococci, appear to be mainly glycosidically linked dimers and higher oligomers. This altered distribution in the size of PG fragments might also be significant in light of the evidence that some biological activities of PG, e.g., complement consumption (5) and arthritogenicity (13), appear to require glycosically linked polymers of the disaccharide peptide subunit for optimal activity.

For many infectious diseases, including gonococcal infections, β-lactam antibiotics represent the most effective therapy against the majority of offending strains. Yet any biological consequences of PG-host interactions (possibly promoted by this therapy) would not necessarily cease upon eradication of the infection, per se. As hypothetical examples (i) the sudden release of a large load of PG from penG-lysed bacteria, (ii) the long-term persistence of PG fragments that might be only slowly degraded by the host, and (iii) the possible penG-enhanced release of PG from gonococcal protoplasts (10, 11) that arise naturally or during β-lactam therapy represent factors that might promote a biologically relevant PG burden in the host.

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LITERATURE CITED

1. **Adams, H. G., M. Turck, and K. K. Holmes.** 1975. Comparison of aqueous sodium penicillin G in lidocaine and aqueous procaine penicillin G for the treatment of gonorrhea, p. 279–284. *In* D. Danielsson, L. Juhlin, and P. A. Mardh (ed.), Genital infections and their implications. Almqvist and Wiksell International, Stockholm.
2. **Ghuysen, J. M.** 1977. Biosynthesis and assembly of bacterial cell walls, p. 463–595. *In* G. Poste and G. L. Nicholson (ed.), Cell surface reviews, vol. 4. Elsevier/North-Holland Publishing Co., New York.
3. **Ghuysen, J. M., D. J. Tipper, and J. L. Strominger.** 1966. Enzymes that degrade bacterial cell walls. Methods Enzymol. **8**:685–699.
4. **Goodell, E. W., M. Fazio, and A. Tomasz.** 1978. Effect of benzylpenicillin on the synthesis and structure of the cell envelope of *Neisseria gonorrhoeae*. Antimicrob. Agents. Chemother. **13**:514–526.
5. **Greenblatt, J., R. J. Boackle, and J. Schwab.** 1978. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. Infect. Immun. **19**:296–303.
6. **Guymon, L. F., D. L. Walstad, and P. F. Sparling.** 1978. Cell envelope alterations in antibiotic-sensitive and -resistant strains of *Neisseria gonorrhoeae*. J. Bacteriol. **136**:391–401.
7. **Hebeler, B. H., and F. E. Young.** 1975. Autolysis of *Neisseria gonorrhoeae*. J. Bacteriol. **122**:385–392.
8. **Hebeler, B. H., and F. E. Young.** 1976. Chemical composition and turnover of peptidoglycan in *Neisseria gonorrhoeae*. J. Bacteriol. **126**:1180–1185.
9. **Hebeler, B. H., and F. E. Young.** 1976. Mechanism of autolysis of *Neisseria gonorrhoeae*. J. Bacteriol. **126**: 1186–1193.
10. **Hickman, R. K., and J. W. Lawson.** 1980. Isolation of a stable cell wall-defective form of *Neisseria gonorrhoeae* from a case of untreated gonococcal urethritis. J. Clin. Microbiol. **12**:603–605.
11. **Holmes, K. K., L. T. Gutman, M. E. Belding, and M. Turck.** 1971. Recovery of *Neisseria gonorrhoeae* from "sterile" synovial fluid in gonococcal arthritis. N. Engl. J. Med. **284**:318–320.
12. **Holtje, J. V., D. Mirelman, N. Sharon, and U. Schwarz.** 1975. Novel type of murein transglycosylase in *Escherichia coli*. J. Bacteriol. **124**:1067–1076.
13. **Kohasi, O., C. M. Pearson, Y. Watanabe, and S. Kotani.** 1977. Preparation of arthritogenic hydrosoluble peptidoglycan from both arthritogenic and non-arthritogenic bacterial cell walls. Infect. Immun. **16**:861–866.
14. **Rosenthal, R. S.** 1979. Release of soluble peptidoglycan from growing gonococci: hexaminidase and amidase activities. Infect. Immun. **24**:869–878.
15. **Rosenthal, R. S., R. S. Fulbright, M. E. Eads, and W. D. Sawyer.** 1977. Ethylenediaminetetraacetic acid-sensitive antiphagocytic activity of *Neisseria gonorrhoeae*. Infect. Immun. **15**:817–827.
16. **Rosenthal, R. S., R. M. Wright, and R. K. Sinha.** 1980. Extent of peptide cross-linking in the peptidoglycan of *Neisseria gonorrhoeae*. Infect. Immun. **28**:867–875.
17. **Schleifer, K. H., and O. Kandler.** 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36**:407–477.
18. **Sinha, R. K., and R. S. Rosenthal.** 1980. Release of soluble peptidoglycan from growing gonococci: demonstration of anhydro-muramyl-containing fragments. Infect. Immun. **29**:914–925.
19. **Swanson, J.** 1978. Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. Infect. Immun. **19**:320–331.
20. **Taylor, A., B. C. Das, and J. van Heijenoort.** 1975. Bacterial-cell-wall peptidoglycan fragments produced by phage λ or Vi II endolysin and containing 1,6 anhydro-*N*-acetylmuramic acid. Eur. J. Biochem. **53**:47–54.
21. **Tomasz, A.** 1979. From penicillin-binding proteins to the lysis and death of bacteria: a 1979 view. Rev. Infect. Dis. **1**:434–467.
22. **Wegener, W. S., B. H. Hebeler, and S. A. Morse.** 1977. Cell envelope of *Neisseria gonorrhoeae*: penicillin enhancement of peptidoglycan hydrolysis. Infect. Immun. **18**:717–725.