Antibiotics

Volume V/Part 1

Mechanism of Action of Antibacterial Agents

Edited by Fred E. Hahn

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Preface

The first volume of *Antibiotics* was published in 1967 and contained a series of review papers on antibiotic actions. The editors, Drs. GOTTLIEB and SHAW, were aware of the rapid development of this field of study and provided a number of addenda in an effort to keep knowledge up to date while the book was in production.

One year after the publication of *Antibiotics I*, this editor had a conference with Dr. KONRAD F. SPRINGER in which it became clear that another volume on actions of antibiotics would be necessary. For a variety of reasons, this was delayed until 1975 and became *Antibiotics III*. It did not contain addenda since it was recognized by the editors, Drs. CORCORAN and HAHN, that still another volume would have to follow and that in a moving field, such as the study of the actions of antibacterial drugs, no publication can be definitive or remain current, except for a limited period of time.

The editors of Volume III grouped the contributions into sections: 1. Interference with nucleic acid biosyntheses, 2. Interference with protein biosynthesis, and 3. Interference with cell wall/membrane biosynthesis, specific enzyme systems, and those in which the mode of action was not known with certainty.

We now present Volume V, Part 1 of *Antibiotics*, the third volume on the actions of antibacterial compounds, with contributions selected either because the active compounds have not previously been reviewed in this series, or because further progress has been made in knowledge of such actions during the intervening years.

The problem of grouping the contributions into logically coherent sections has again arisen. Realizing that no set of criteria can satisfactorily organize the knowledge, this editor finally resigned himself to presenting the contributions alphabetically.

Another problem in assembling and editing such a series of articles lies in the disparity of the existing knowledge. For some substances, either new or studied only to limited extents, the knowledge does not significantly exceed a level which is conventionally referred to as *mode of action*, i.e., a documentation that a particular physiological process or overall biochemical event is affected. For other substances, for example chloramphenicol, aminoglycosides, or nalidixic acid, just to name a few, the *mechanisms* of their actions at the molecular level approach resolution in detail. The field no longer possesses the relative homogeneity in the level of knowledge which still existed in 1967 when *Antibiotics I* was published. The Editor of a future volume may have to cope with this emerging problem through categorizing articles as to (1) mode of action and (2) mechanism of action.

A review of the contents of this Volume V, Part 1 might suggest that certain topics have been omitted. This is especially true for the lactam antibiotics,

the penicillins, and cephalosporins. They have not been reviewed for the following reasons: In terms of enzymology, the inhibition of peptidoglycan biosynthesis is known and has been reviewed on numerous occasions. There has emerged, however, an element of doubt that the anatomical changes induced by these drugs in bacteria, as well as the attendant bactericidal effects, can be fully explained by known enzyme inhibitions. The physiological/biochemical basis of the bacterial destruction by these antibiotics is currently under study, but has not yet attained a level of understanding at which it would lend itself to a systematic review. This editor felt, therefore, that such updated treatment of the actions of the lactam antibiotics should be deferred to a future volume.

In *Antibiotics I*, Drs. GOTTLIEB and SHAW made a highly successful beginning, and a deliberate effort has been made in this Volume V, Part 1 to maintain their editorial style and approach to the treatment of subject matter. The most important feature in this striving for continuity has been that only such scientists have been invited to authorship who have materially and originally contributed to the bodies of knowledge which they were asked to review. Every contribution is based on first-hand knowledge of the treated subject matter. The editor hopes that such a policy has provided for an element of actuality and currency which should distinguish Volume V, Part 1 from academic textbooks.

There remains the pleasant task of thanking my publisher, Dr. KONRAD F. SPRINGER, for his unwavering interest in, and support of, this undertaking, and for having once again provided for the production of such a well-appointed and handsome book.

Washington, D.C., Spring 1979 FRED E. HAHN

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Contents of Part 1

Bacitracin

D.R. STORM and W.A. TOSCANO, JR.

Introduction

Since their discovery in 1945 (JOHNSON et al., 1945), the bacitracin peptides have been extensively studied by microbiologists, biochemists, and chemists. Although the major effort has been directed toward elucidation of the mechanism for the antimicrobial activity of bacitracins, the peptides have also served as useful tools for studying various biochemical processes and the chemistry of lipid peptide interactions. The primary goal of this article is to review the literature concerning the mechanism of action of bacitracin. Although this problem has been studied for over twenty years, the mechanism for bacitracin's biological activities has not been unambiguously defined. Indeed, the bacitracins apparently affect a number of biochemical processes and it is not clear that the peptides inhibit bacterial growth by virtue of a single effect on bacterial metabolism. The timing of this review was dictated by the rapid proliferation of research in this area, and the existence of several different proposals in the literature for the mechanism of action of bacitracin.

Isolation and Separation

Bacitracin was first isolated in 1945 from a strain of *Bacillus licheniformis* obtained from tissue taken from a wound of a patient named Tracy (JOHNSON et al., 1945). The antibiotic was readily extracted from culture fluids at pH 7.0 and redisolved in aqueous solutions at pH 3.0. It was soon discovered that bacitracin isolated from culture media was a heterogeneous family of related peptides. A number of different separation techniques have been used in an attempt to resolve this mixture. The most widely used technique for this purpose has been counter-current distribution (CRAIG et al., 1952, 1969; NEWTON and ABRAHAM, 1953). When commercial bacitracin is submitted to counter-current distribution at least 9 different components are obtained. Ion exchange chromatography has also been employed for partial separation of bacitracin peptides; however, this method has not been as successful as counter-current distribution (KONIGSBERG and CRAIG, 1959; STORM and STROMINGER, 1973). For example, carboxymethyl cellulose was used to resolve commercial bacitracin and only 6 fractions were obtained (STORM and STROMINGER, 1973). Bacitracins A and

B were not separated by this method. The most effective method for separation of the bacitracin peptides is high-pressure liquid chromatography (TSUJI et al., 1974). This technique has been used to isolate more than 22 components of bacitracin in less than 40 min. Although the composition of commercial bacitracin varied considerably between manufacturers, it was relatively constant for each supplier.

It is not totally understood why bacitracin isolated from *B. licheniformis* is so heterogeneous. One of the major reasons this question has not been answered is because the structures of all the bacitracin peptides have not been determined. Heterogeneity is due in part to breakdown of the peptides but it is also evident that several bacitracin species differ only by one or two amino acids. The latter fact may reflect mistakes in the synthesis of bacitracin since this process is carried out by a multienzyme complex (BERNLOHR and NOVELLI, 1960; FROYSHOV and LALAND, 1974; ISHIHARA and SHIMURA, 1974) which may not dictate the degree of sequential specificity exhibited by normal protein synthesis on ribosomes.

Structures of the Bacitracin Peptides

The major and most active component of commercial bacitracin is bacitracin A, which comprises from 60% to 80% of the mixture. The structure of bacitracin A is shown in Fig. 1 (GALARDY et al., 1971). The antibiotic contains a seven-membered peptide ring with a tail of five amino acids. The bacitracin A molecule has a number of novel compositional and structural features including the thiazoline ring formed between the terminal isoleucine and cysteine residues and an amide bond between the ε-amino group of lysine and asparagine. In addition, like many peptide antibiotics, bacitracin A contains a number of amino acids not normally found in proteins which include D-amino acids and the ornithine residue. The only ionizable functions in bacitracin A are the N-terminal amino group, glutamic and aspartic carboxyls, the ε-amino group of ornithine and the histidine imidazole. It is notable that the ratio of hydrophobic to polar amino acids is significantly greater than one, which may partially account for the affinity of bacitracin A for specific membrane lipids.

The structures of the other bacitracin peptides have not been described in detail. It has been observed that in neutral or slightly alkaline solutions, bacitracin A is slowly transformed to bacitracin F (ABRAHAM, 1957; REGNA, 1959; WEINBERG, 1967). Bacitracin F is a derivative of bacitracin A in which the thiazoline ring has been oxidatively deaminated. Bacitracin F exhibits some antibiotic activity against *Micrococcus lysodeikticus*; however, it is approximately 20- to 30-fold less active than bacitracin A (STORM and STROMINGER, 1973). Bacitracin B is quite similar to bacitracin A, but they differ by the replacement of an isoleucine residue in bacitracin A by a valine residue in bacitracin B (ABRAHAM, 1957). There may be other structural differences between bacitracins A and B; however, the complete sequence of bacitracin B is not available. In addition, there are a number of other bacitracin peptides (e.g., E and D) which have low "amide" nitrogen relative to bacitracin A, and they may repre-

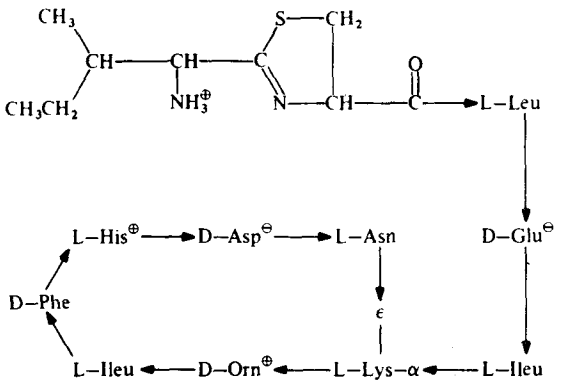

Fig. 1. Structure of bacitracin A (GALARDY et al., 1971)

sent deaminated forms such as desamido bacitracin in which the asparagine residue has been deaminated. It is clear that a great deal of structural work remains to be done and this information will undoubtedly be valuable for structure-function correlations.

A limited amount of detailed information concerning the secondary and tertiary structure of bacitracin A is available. The peptide has not been crystallized, therefore X-ray crystallographic data have not been obtained. However, evidence for the existence of intramolecular hydrogen bonds in bacitracin A has been reported (GALARDY et al., 1971). The kinetics for tritium-hydrogen exchange of bacitracin A were examined as a function of pH. The 11 peptide hydrogens exchanged in 3 kinetically distinct classes. Other protons including the free amino protons, the C_2 proton of the thiazoline ring, the C_2 proton of the histidine, and the two primary amide protons were not observed to exchange during the time course of these experiments. A single, abnormally slow-exchanging proton was observed. Oxidation and hydrolysis of bacitracin A at the aspartic-asparagine bond gave a linear peptide which did not contain the slow-exchanging proton. In addition, the exchange rate for all other protons was affected by this treatment. These data indirectly suggest the existence of secondary structure in bacitracin A and the occurrence of at least one intramolecular hydrogen bond. It was proposed that the slow-exchanging proton was either an intramolecular hydrogen bond or the ε lysine amide proton, which might be intrinsically slow-exchanging. CRAIG has also suggested, on the basis of thin film dialysis studies, that bacitracin A has a compact structure with the peptide tail folded over the ring (GALARDY et al., 1971).

Other evidence suggesting secondary or tertiary structure for bacitracin has been obtained by carbon-13 NMR (LYERLA and FREEDMAN, 1972). The spectrum of bacitracin was tentatively assigned on the basis of spectra obtained from linear peptides and individual amino acids. The chemical shifts of most of the residues did not deviate significantly from those found in the constituent amino acids or model peptides. However, the isoleucine resonances in the peptide ring and side-chain deviated somewhat from their expected values, indicating

that these residues had unique microenvironments. These observations may reflect interactions between the peptide ring and side-chain which restrain the isoleucine methyl groups in specific orientations. It is obvious that much more information concerning the three-dimensional structure of bacitracins is required in order to rationalize the biological properties of the peptides in terms of detailed molecular interactions.

Biosynthesis of Bacitracin

The biosynthesis of bacitracin is intrinsically interesting since it occurs by a process distinct from normal protein synthesis and does not require ribosomes, tRNA, or mRNA. The synthesis of bacitracin is catalyzed by a multienzyme complex which has been partially purified in several different laboratories (FROYSHOV and LALAND, 1974; ISHIHARA and SHIMURA, 1974). The complex apparently contains specific activating enzymes for each of the amino acids of bacitracin. These activating enzymes catalyze the exchange of ATP with inorganic pyrophosphate only in the presence of constituent amino acids, which suggests the involvement of intermediate aminoacyl adenylates. There is also evidence that the amino acids and intermediate peptides are covalently attached to the bacitracin synthetase complex by thioester bonds (FROYSHOV, 1975). FROYSHOV has identified a number of peptide intermediates covalently attached to the complex, which include Ile-Cys, Ile-Cys-Leu, and Ile-Cys-Leu-Glu; these are clearly intermediates in bacitracin synthesis. It is also apparent that bacitracin synthesis proceeds in the C-terminal direction.

Regulation of bacitracin synthetase is an interesting problem which is currently being studied in a number of laboratories. In general, production of bacitracin parallels bacterial growth in synthetic media lacking glucose (SNOKE and CORNELL, 1965; HAAVIK, 1974). Glucose inhibits formation of bacitracin; however, this effect is apparently not due to catabolite repression but is caused by a decrease in the pH of the growth medium in the presence of glucose (HAAVIK, 1974). The ultimate purification of the bacitracin synthetase complex will make it possible to study directly the effects of various metabolites on this process. Although it was originally suggested that bacitracin is a structural component of the spore coat of *B. licheniformis*, analysis of hydrolysates of purified spore coats showed only trace amounts of ornithine, indicating that bacitracin is not a significant component of the spore coat (MARSCHKE and BERNLOHR, 1970). Furthermore, the existence of a mutant, deficient in bacitracin synthesis, which sporulated normally further suggests that bacitracin is not an essential spore component (HAAVIK and FROYSHOV, 1975).

Antimicrobial Spectrum

Bacitracin is a potent antibiotic against many gram-positive bacteria but is relatively ineffective against gram-negative bacteria (WEINBERG, 1967; JAWETZ,

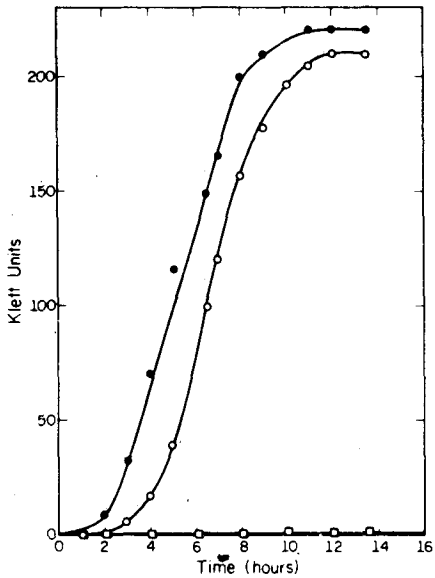

Fig. 2. Effect of polymyxin-agarose and bacitracin on the growth of *E. coli* SC 9251. Synthetic medium was innoculated with 5×10^6 cells/ml at time zero and with zero or 200 μg/ml bacitracin (●—●), 200 μg/ml polymyxin-agarose (○--○), or 20 μg/ml bacitracin and 200 μg/ml polymyxin-agarose (□—□)

1970). The insensitivity of gram-negative bacteria to bacitracin is somewhat surprising since the target sites for bacitracin action are apparently present in the inner membranes of gram-negative bacteria. There is, however, some evidence that insensitivity may be due to the outer membrane system of gram-negative bacteria, which functions as a barrier for some antibiotics (BROWN and RICHARDS, 1965; LEIVE, 1974). The antibiotic activity of bacitracin against *Escherichia coli* SC 9251 was greatly enhanced in the presence of polymyxin B covalently attached to agarose (ROSENTHAL and STORM, 1977). Polymyxin B is known to disrupt the structure of gram-negative outer membranes (STORM et al., 1977) and apparently retains this property when covalently attached to agarose (LAPORTE et al., 1977). Growth curves illustrating synergism between bacitracin and polymyxin-agarose are shown in Fig. 2. Bacitracin at concentrations greater than 200 μg/ml had no effect on the growth of *E. coli* SC 9251. A combination of polymyxin-agarose and 20 μg/ml of bacitracin completely inhibited *E. coli* growth for at least 14 h. Thus, the activity of bacitracin was increased at least ten-fold by polymyxin-agarose. These results suggest that the insensitivity of gram-negative bacteria to bacitracin may be due to the inability of the peptide to penetrate the outer membrane.

An important question concerning the antibiotic activity of bacitracin is the susceptibility of the producing strain, *B. licheniformis*, particularly since formation of bacitracin parallels growth of the bacteria (SNOKE and CORNELL, 1965; HAAVIK, 1974). Although the producing strain is sensitive to bacitracin,

its sensitivity is approximately 50-fold less than that of other gram-positive bacteria. In addition, inhibition of *B. licheniformis* growth was only observed if the antibiotic was initially present, i.e., when the medium was innoculated (SNOKE and CORNELL, 1965). Addition of bacitracin 6 h after the culture was initiated had little effect on growth. Thus, cultures of this strain can accumulate large quantities of bacitracin because its growth inhibitory effects appear restricted to early growth stages. In contrast, HAAVIK has reported that *B. licheniformis* is sensitive to bacitracin both in early and late growth phases, but only in the presence of Mn^{2+} (HAAVIK, 1975). It was proposed that bacitracin participates in the transport of Mn^{2+} into the bacteria at sufficiently high levels to inhibit bacterial growth.

Mechanism of Action

General Considerations

Bacitracin has been reported to affect a number of biochemical processes in bacteria, including the synthesis of inducible enzymes (CREASER, 1955; GALE and FOLKS, 1955; SMITH and WEINBERG, 1962), peptidoglycan biosynthesis (ABRAHAM and NEWTON, 1958; PARK, 1958, 1960; MANDELSTAM and ROGERS, 1959; SIEWERT and STROMINGER, 1967), selective membrane permeability (CRAWFORD and ABRAHAM, 1957; SMITH and WEINBERG, 1962; SNOKE and CORNELL, 1965), and metal ion transport (HAAVIK, 1976). It has been difficult to distinguish primary effects of the antibiotic from secondary effects which may result from inhibition of bacterial growth. Those processes most sensitive to the antibiotic appear to be selective membrane permeability and peptidoglycan biosynthesis. Although bacitracin may indeed have multiple actions, it is possible that its influence on membrane permeability and peptidoglycan biosynthesis has a common molecular basis (STORM and STROMINGER, 1974).

Interactions with Divalent Cations

The antibiotic activity of bacitracin requires the presence of a divalent cation. For example, its activity against *Staphylococcus aureus* was completely eliminated in the presence of EDTA (ADLER and SNOKE, 1962). Antibiotic activity was restored by various divalent cations, with Cd^{2+}, Mn^{2+}, and Zn^{2+} being most effective in this respect. Other cations including Hg^{2+}, Ba^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, and Mg^{2+} were relatively ineffective. Weinberg has also reported a divalent metal ion requirement for bacitracin activity (WEINBERG, 1958). However, the highest activity was seen in the presence of Zn^{2+}, whereas ADLER and SNOKE (1962) observed that both Cd^{2+} and Mn^{2+} were more effective than Zn^{2+}. HAAVIK has proposed that bacitracin may function in the transport of essential divalent cations from the surface of *B. licheniformis* to transport

systems in the cytoplasmic membrane (HAAVIK, 1974). A number of divalent cations (Mn^{2+}, Zn^{2+}, and Cd^{2+}) are toxic at high levels and bacitracin enhanced the toxicity of these cations. In contrast, a bacitracin-negative mutant was not affected by Mn^{2+} at concentrations which inhibited the growth of the bacitracin-producing strain (HAAVIK and FROYSHOV, 1975). The proposal that bacitracin participates in metal ion transport with *B. licheniformis* is an attractive hypothesis, but it remains to be directly established that the peptide actually catalyzes the uptake of divalent cations by bacteria.

Direct interaction between bacitracin and divalent metal ions has been detected by a number of techniques including potentiometric titrations, optical absorbance changes and NMR. The apparent order of binding from titration studies was $Cu^{2+} > Ni^{2+} > (Co^{2+}, Zn^{2+}) > Mn^{2+}$ (GARBUTT et al., 1961). Titration data have strongly implicated the imidazole function in bacitracin as a site for metal ion complexation. All of the metals examined, with the exception of Mn^{2+}, complexed with a group titrating between pH 5.5 and 7.5. The histidine imidazole would be the most likely candidate for this interaction. In addition, the same authors examined the influence of divalent cations on the ultraviolet spectrum of bacitracin. The spectrum of bacitracin exhibits a weak maximum at 253 nm. Addition of Cu^{2+} or Ni^{2+} enhanced this absorption considerably, whereas Zn^{2+} and Co^{2+} had lesser effects upon the ultraviolet spectrum.

The Zn^{2+}-bacitracin complex has also been studied using ORD and proton NMR in order to identify specific residues of the peptide directly interacting with the divalent cation (CORNELL and GIUNEY, 1970). The ORD spectrum of bacitracin exhibits two UV Cotton effects, one at 250 nm and another at 200 nm. The former was assigned to the thiazoline ring. Addition of Zn^{2+} in tenfold excess over bacitracin perturbed both Cotton effects. CORNELL and GIUNEY proposed that the thiazoline ring provides one coordination site for Zn^{2+}. In addition, it was shown that in the presence of Zn^{2+}, the bacitracin imidazole C-4 and C-2 hydrogens in the NMR spectrum shifted 0.18 to 0.20 ppm downfield. It was proposed that the Zn^{2+} also coordinates through the N-3 of histidine. These NMR studies were done in CD_3OD as a solvent. Similar, but somewhat different, results were obtained when the NMR spectrum of pure bacitracin A was examined in D_2O (STORM, 1974). Addition of Mg^{2+} to metal-free bacitracin caused a shift of approximately 10 cps in the imidazole C-2 hydrogen with little effect on the C-4 hydrogen. These results implicated the N-1 nitrogen of histidine. This apparent discrepancy with CORNELL's NMR results may reflect either the difference in solvent systems or divalent cations used in the two studies. Interactions between bacitracin and Mn^{2+} and Cu^{2+} have also been studied using ^{13}C NMR (WASYLISHEN and GRAHAM, 1975). These paramagnetic ions cause broadening of specific resonances to an extent which depends upon the distance between the metal and specific carbon atoms. This study implicated that the aspartic acid, glutamic acid, histidine and thiazoline residues may directly interact with the divalent action. The coordination scheme for binding of metal ions to bacitracin is not adequately defined. However, there is a consensus that the histidine and thiazoline ring are probably directly involved in complexation of divalent metal ions, a proposal first advocated by Lyman CRAIG (CRAIG et al., 1969).

Effects on Membrane Permeability

The two proposals for the mechanism of action of bacitracin which have received the most attention are perturbation of selective membrane permeability and inhibition of peptidoglycan biosynthesis. There is substantial evidence that bacitracin affects the permeability of protoplast membranes prepared from gram-positive bacteria. Bacitracin caused a rapid lysis of protoplasts prepared from *B. licheniformis* and *M. lysodeikticus* which required the presence of either cadmium or zinc ions (SNOKE and CORNELL, 1965). The ratio of antibiotic to protoplasts required for this lytic action was comparable to the ratio of antibiotic per cell necessary to inhibit bacterial growth. However, it should be emphasized that protoplast preparations are often inherently unstable and susceptible to surface-active compounds. For example, bacitracin had little or no effect on *B. megaterium* protoplasts if they were allowed to stabilize for some period of time (REYNOLDS, 1971). This is consistent with the observation that freshly prepared protoplasts only carried out peptidoglycan synthesis after an incubation period in growth media.

The growth of protoplasts does not require peptidoglycan biosynthesis and, as expected, penicillin and D-cycloserine do not inhibit the growth of *B. megaterium* protoplasts (HANCOCK and FITZ-JAMES, 1964). However, bacitracin did inhibit protoplast growth at concentrations comparable to the minimal inhibitory concentration for whole cells. Similar results have been reported for *Streptococcus faecalis* protoplasts (SHOCKMAN and LAMPEN, 1962), and L-forms of some *S. aureus* strains were as sensitive to bacitracin as the corresponding whole cells (WILLIAMS, 1963; MOLANDER et al., 1964). In addition, it has been reported that bacitracin did not induce L-forms of *S. aureus* whereas methicillin, oxacillin, and cephalothin did (MOLANDER et al., 1964). In contrast, L-forms indistinguishable from those produced by penicillin were formed when group A Streptococci were exposed to bacitracin (ROTTA et al., 1965). More direct evidence for bacitracin-induced structural changes in plasma membranes has been obtained by electron microscopy (SLEYTR et al., 1976). Freeze-fractured plasma membranes treated with bacitracin revealed pronounced morphological changes in the form of rods 25–35 nm in diameter. The above evidence, taken collectively, strongly suggests that bacitracin affects membrane structure and permeability. However, the relationship between these phenomena and the biological properties of bacitracin is not clearly defined.

MACDONALD et al. (1974) have observed that bacitracin at concentrations between 10^{-3} and 10^{-4} M stimulated the release of low molecular weight markers trapped in phospholipid liposomes. This activity was enhanced specifically by cadmium ions. However, the physiological significance of these results is open to question since the concentrations of bacitracin employed were several orders of magnitude higher than those required for inhibition of bacterial growth. Bacitracin at 5×10^{-4} M did, however, lower the conductance of planar lipid bilayers. In contrast, MUELLER and RUDIN (1969) observed no influence of bacitracin on the conductance of planar bilayers. It is clear that further model membrane studies are required in order to draw definitive conclusions concerning the biological significance of bacitracin-induced permeability changes.

Inhibition of Peptidoglycan Biosynthesis

Bacitracin has been shown to inhibit peptidoglycan biosynthesis in a number of different studies. For example, the antibiotic caused the accumulation of uridine nucleotides (ABRAHAM and NEWTON, 1958; PARK, 1958, 1960) and inhibited the incorporation of radioactive labeled amino acids into peptidoglycan at concentrations of the peptide which did not affect incorporation of amino acids into cellular protein (PARK, 1958, 1960; MANDELSTAM and ROGERS, 1959). In addition, bacitracin induced the formation of protoplasts from *S. aureus* (ABRAHAM, 1957) and L-forms from strains of Group A Streptococci (ROTTA et al., 1965). In the latter study, L-forms obtained either by penicillin or bacitracin treatment had quite similar morphological and bacteriological properties. Bacitracin has also been reported to inhibit *B. megaterium* peptidoglycan biosynthesis (REYNOLDS, 1971). In general, inhibition of peptidoglycan biosynthesis occurred at concentrations of the antibiotic comparable to minimum inhibitory concentrations. The significance of these observations is difficult to evaluate since it has been proposed that bacitracin has multiple effects on bacterial metabolism and peptidoglycan biosynthesis could not be completely inhibited in vivo at any concentration of bacitracin. The inability to completely inhibit peptidoglycan biosynthesis by bacitracin was clarified when the specific bacitracin-sensitive step in cell wall biosynthesis was identified.

In 1967, the bacitracin-sensitive step of peptidoglycan biosynthesis was first identified (SIEWERT and STROMINGER, 1967). Using (^{14}C-pentapeptide)-^{32}PP-C_{55}-isoprenol as a substrate, SIEWERT and STROMINGER demonstrated that ^{14}C-labeled peptidoglycan was synthesized in vitro in the presence of *M. lysodeikticus* membranes. In the presence of bacitracin, peptidoglycan was still synthesized; however, ^{32}P-labeled inorganic phosphate was not released and a ^{32}P-labeled lipid, assumed to be the C_{55}-isoprenyl pyrophosphate, accumulated at higher levels.

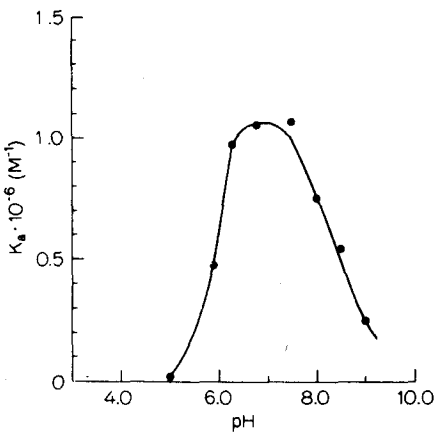

Fig. 3. pH dependency for binding of bacitracin A to C_{55}-isoprenyl pyrophosphate. The buffers contained 1 mM $MgCl_2$ and ionic strength was constant at 0.081 M (STORM and STROMINGER, 1973)