

ANTIMICROBIAL AGENTS

I. INTRODUCTION

A. DEFINITIONS

B. IMPACT OF ANTIMICROBIALS ON HEALTH CARE

C. HISTORY

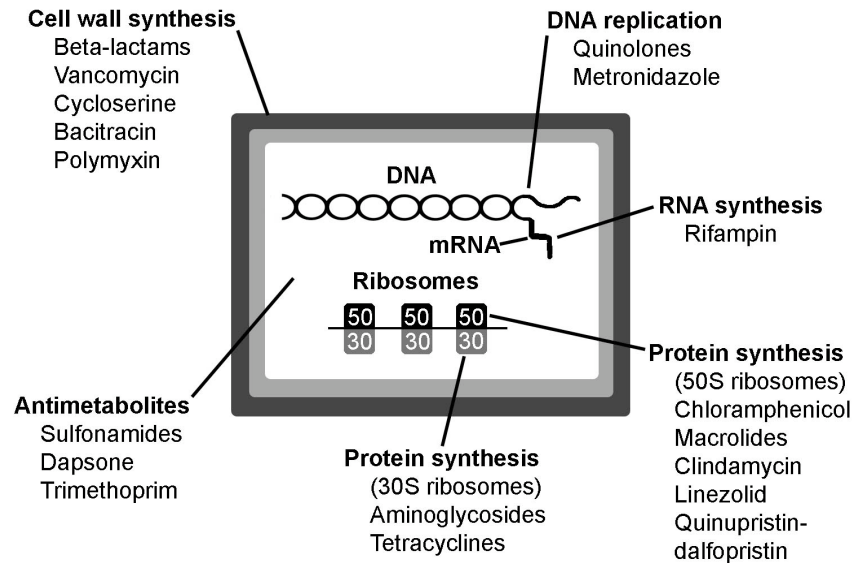
D. PRODUCTION, ISOLATION, PURIFICATION OF ANTIBIOTICS

E. THERAPY GUIDELINES

1. Antibiotic must reach site of infection
2. Bacteriostatic drug may work if host defenses are adequate
3. Bacteriocidal drug may be needed in medically compromised patients or in severe infections

F. FUTURE

II. COMMON MECHANISMS OF ACTION OF ANTIMICROBIALS



Basic sites of antibiotic activity

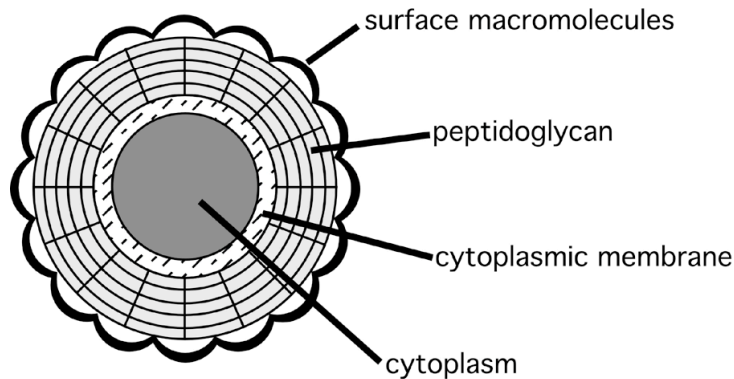
Adapted from Murray, Rosenthal, Pfaller. Medical Microbiology, 5th Edition, Elsevier-Mosby 2005.

A. INHIBITORS OF BACTERIAL CELL WALL SYNTHESIS

e.g., Penicillins, Cephalosporins, Vancomycin

1. Bacterial cell walls and membranes

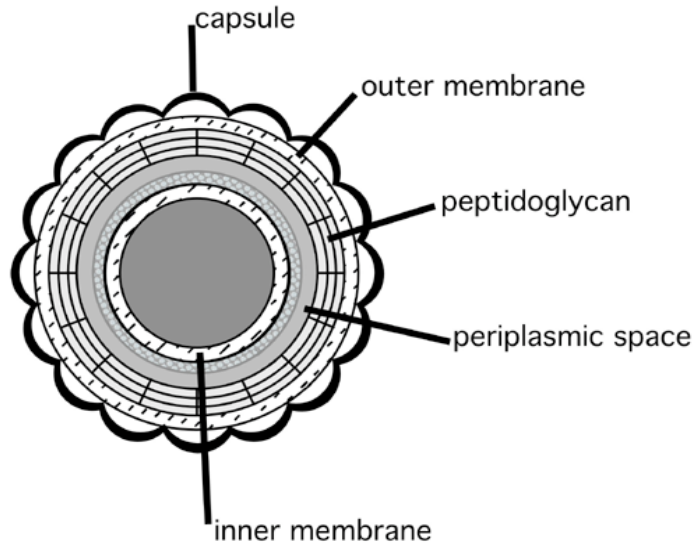
a) Gram-positive bacteria



b) Characteristics of Gm (+) bacteria

- 1) less developed biosynthetic capability
- 2) high internal osmolality
- 3) relatively simple cell wall

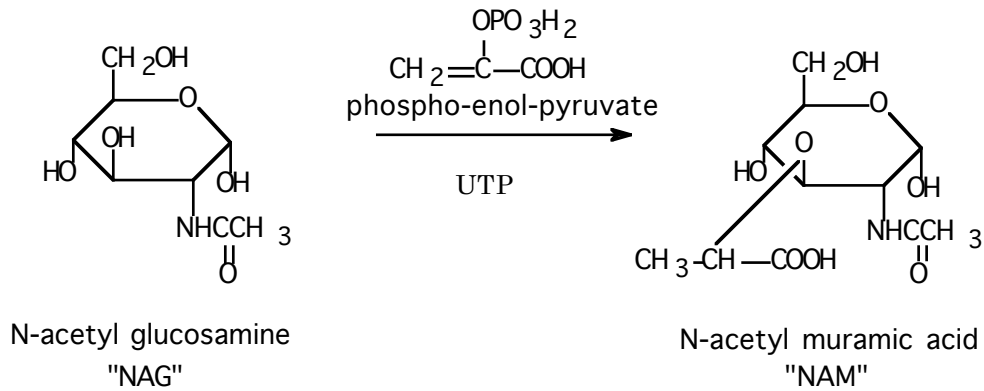
c) Gram-negative bacteria



d) Characteristics of Gm (-) bacteria

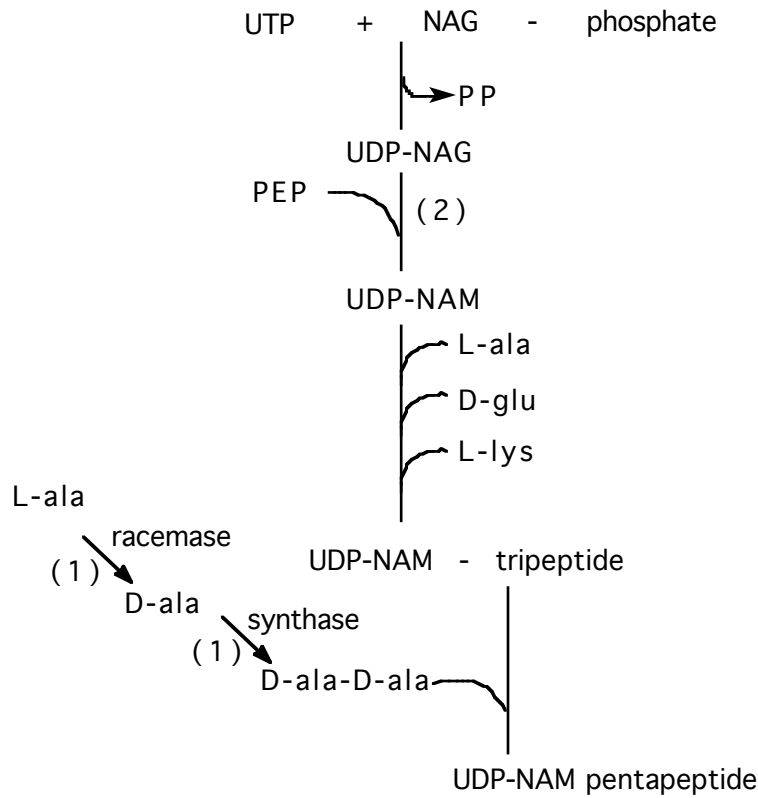
- 1) highly developed synthetic capacity
- 2) highly adaptive
- 3) low osmolality
- 4) complex 5 layered cell wall; thin peptidoglycan layer
- 5) penetration of antibiotic is often restricted - porin channels used for transport of hydrophilic molecules including some antibiotics through the outer membrane

2. Basic Building Blocks of Peptidoglycan layer.

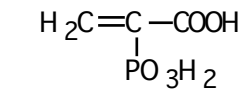
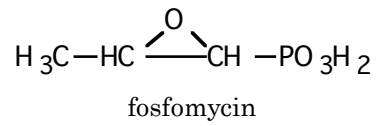
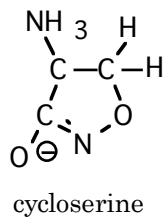
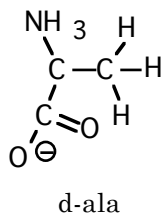


3. Biosynthesis of Glycopeptide

a) Precursor Formation (in cytoplasm)

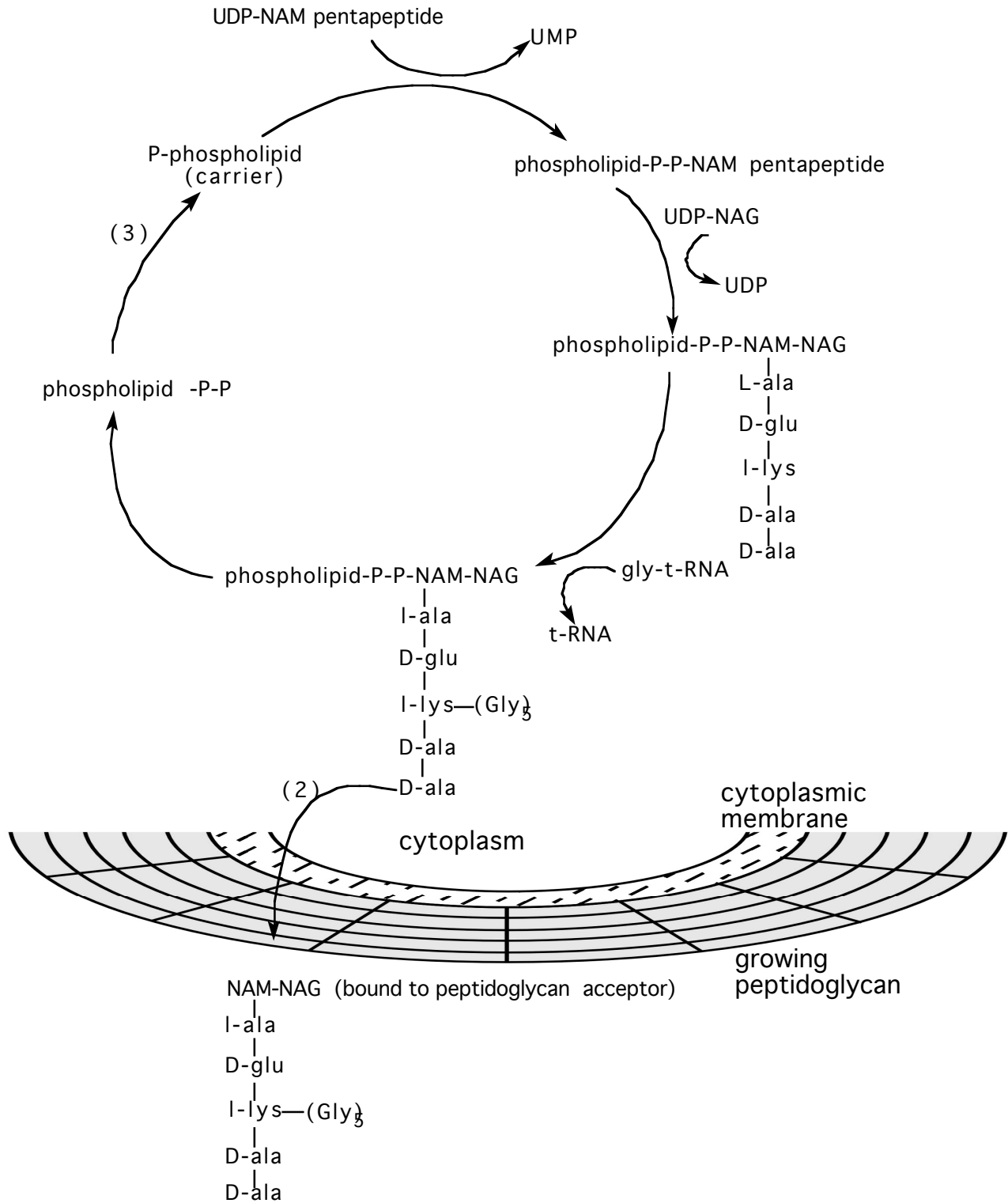


- (1) inhibited by cycloserine
- (2) inhibited by fosfomycin



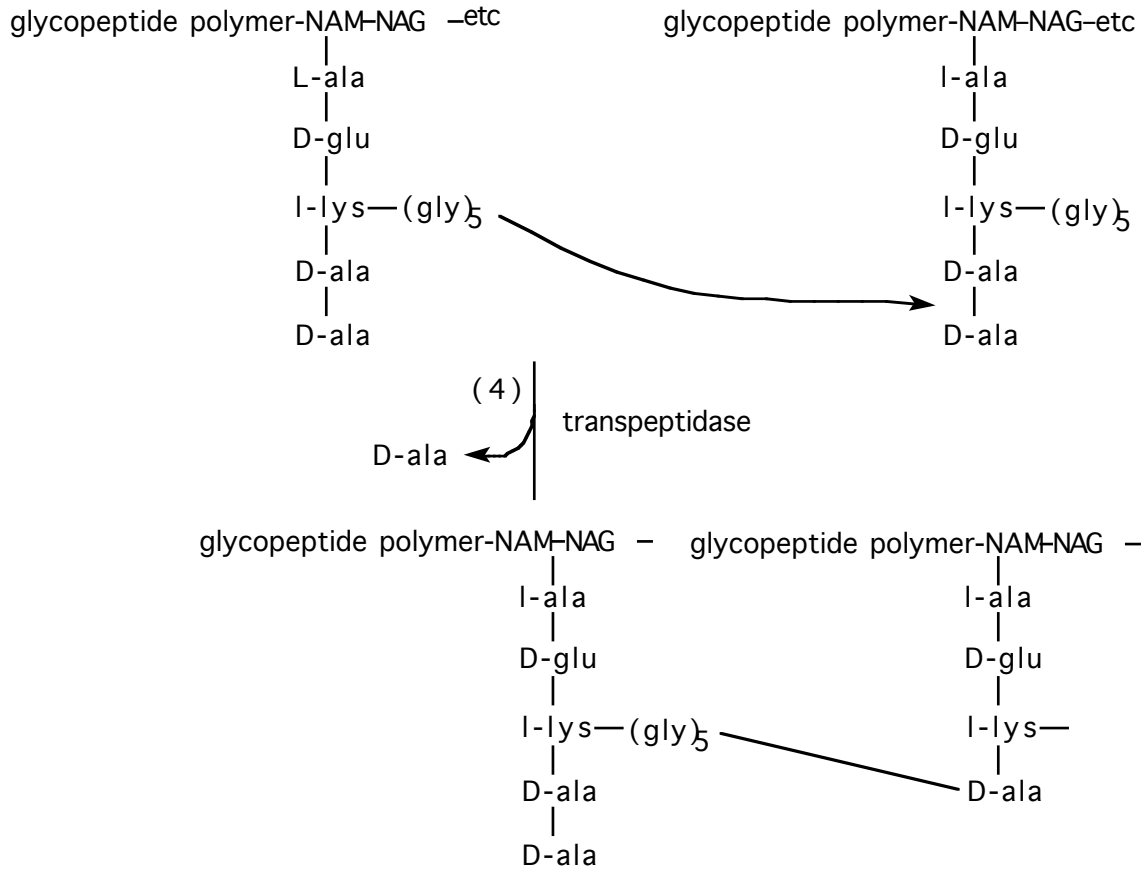
phosphoenol pyruvate

b) Formation of Linear Peptidoglycan
(on cytoplasm side of membrane surface)

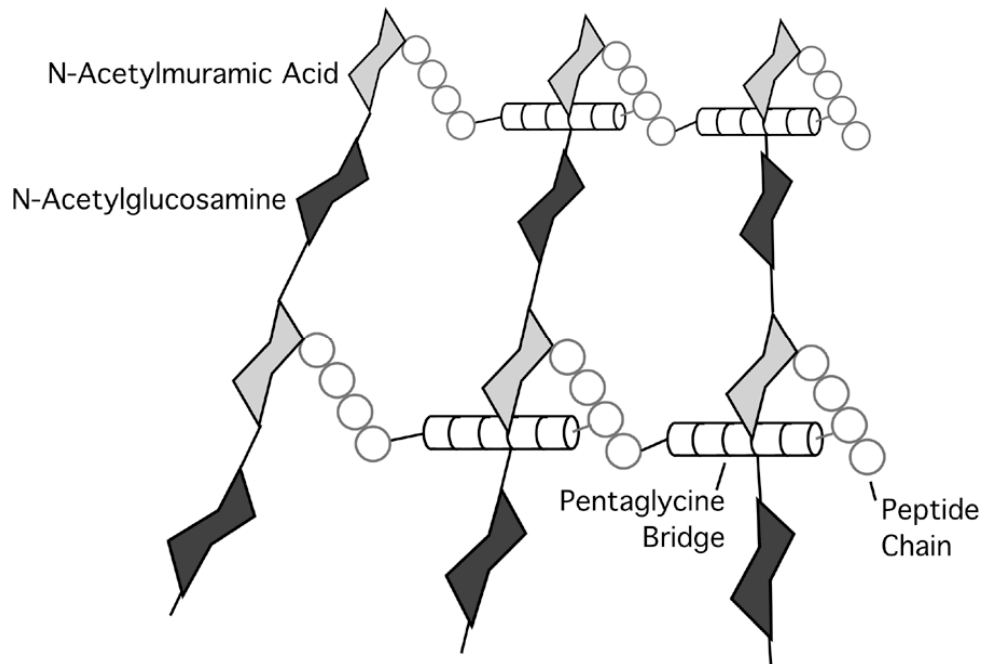


- (2) inhibited by vancomycin (binds to d-ala-d-ala)
- (3) inhibited by bacitracin

c) Cross-linking of the Peptidoglycan



(4) inhibited by penicillins and cephalosporins



5. Penicillin binding proteins (PBP's) of E. Coli

<u>PBP</u>	<u>% of total PBP</u>	<u>Function</u>	<u>inhibition lethal?</u>
1a } 1b }	8	transpeptidases	yes
2	0.7	maintenance of rod shape	yes
3	2	septum formation	±
4	4	D-alanine carboxypeptidase	no
5	65		
6	2		

Figure 3-6. Stereomodels of penicillin (A) and of the D-alanyl-D-alanine end of the peptidoglycan strand (B). The arrows indicate the CO-N bond in the β -lactam ring of penicillin and the CO-N bond in the D-alanyl-D-alanine at the end of the peptidoglycan strand. (From Strominger et al.⁹)

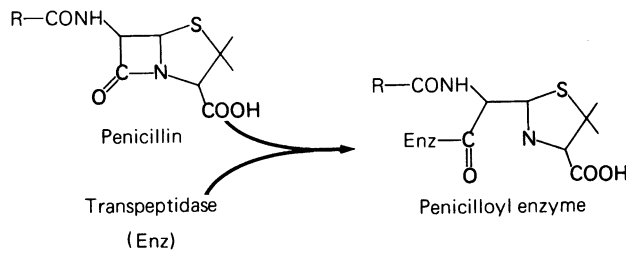
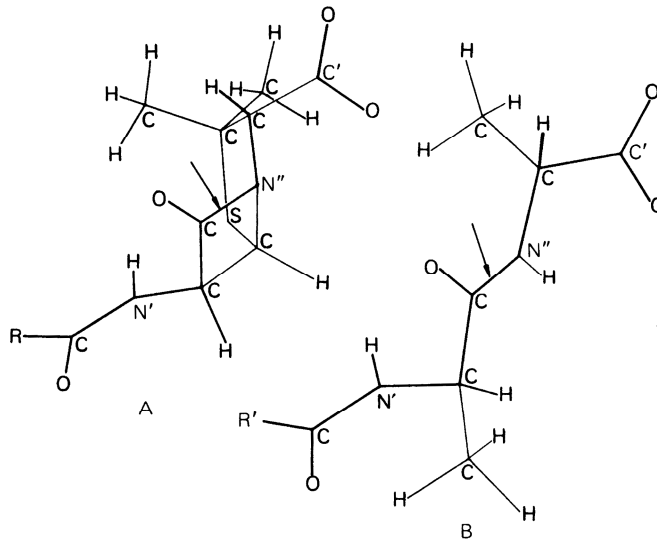


Figure 3-7. Proposed mechanism of transpeptidase inhibition by penicillin. Penicillin occupies the D-alanyl-D-alanine substrate site of transpeptidase, the reactive four-membered (β -lactam) ring is broken by cleavage at the CO-N bond, and the antibiotic becomes linked to the enzyme by a covalent bond. (From Tipper and Strominger.³⁷)

6. Autolysis

Lysis of peptidoglycan occurs and accounts for bacteriocidal effect of Beta lactam antibiotics when synthesis of new wall is inhibited.

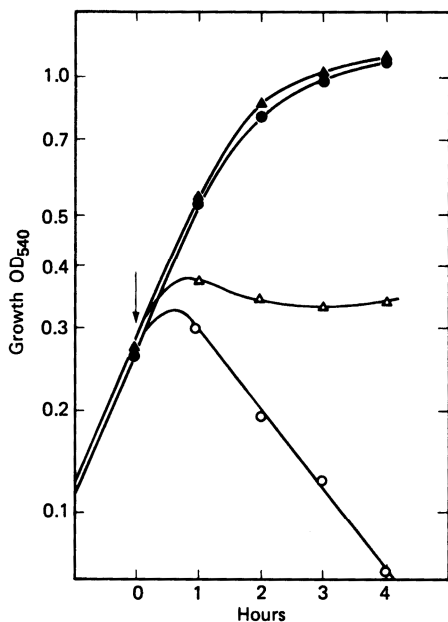


Figure 3-9. Effect of penicillin on the growth of a parent strain of *Bacillus subtilis* with normal autolytic enzyme activity and an autolysin-deficient mutant. Penicillin was added to cultures of *B. subtilis* in the middle log phase of growth and growth was assayed by turbidity at 540 nm. The arrow indicates the time of drug addition. ●, parent strain; ○, parent strain plus penicillin; ▲, autolysin-deficient mutant; △, mutant plus penicillin. The parent strain was lysed, whereas the growth of the mutant was halted without lysis. The same phenomenon was observed upon the addition of cycloserine, vancomycin, or bacitracin. (From Ayusawa et al.⁶³)

Table 3-3. Susceptibility of 60 strains of *Staphylococcus aureus* to oxacillin. The minimum concentration of oxacillin required to inhibit growth (MIC) and the minimum concentration required to kill 99.9% of organisms (MBC) were determined for 60 randomly selected clinical isolates of *S. aureus*. The penicillin was bactericidal against 27 strains and only bacteriostatic against the other 33. It should be noted that the "tolerant" organisms will die very slowly if kept in the presence of a high concentration of oxacillin for many hours. The data represent the mean MIC and MBC for each group with the range of values shown in parenthesis.

Isolates	MIC ($\mu\text{g/ml}$)	MBC ($\mu\text{g/ml}$)
Sensitive group	0.30 (0.1-1.6)	2.2 (0.2-12.5)
"Tolerant" group	0.33 (0.1-0.8)	>100 (50->100)

Source: From Mayhall et al.,⁶⁵ Table 1.

7. Penetration

- a) Antibiotic must reach site of peptidoglycan synthesis i.e., the outside of the inner cytoplasmic membrane. For Gm (+) this is relatively easy. For Gm (-) this may be difficult due to lipid bilayer outer membrane. Porin channels through outer membrane may allow relatively polar antibiotics to pass through, however.

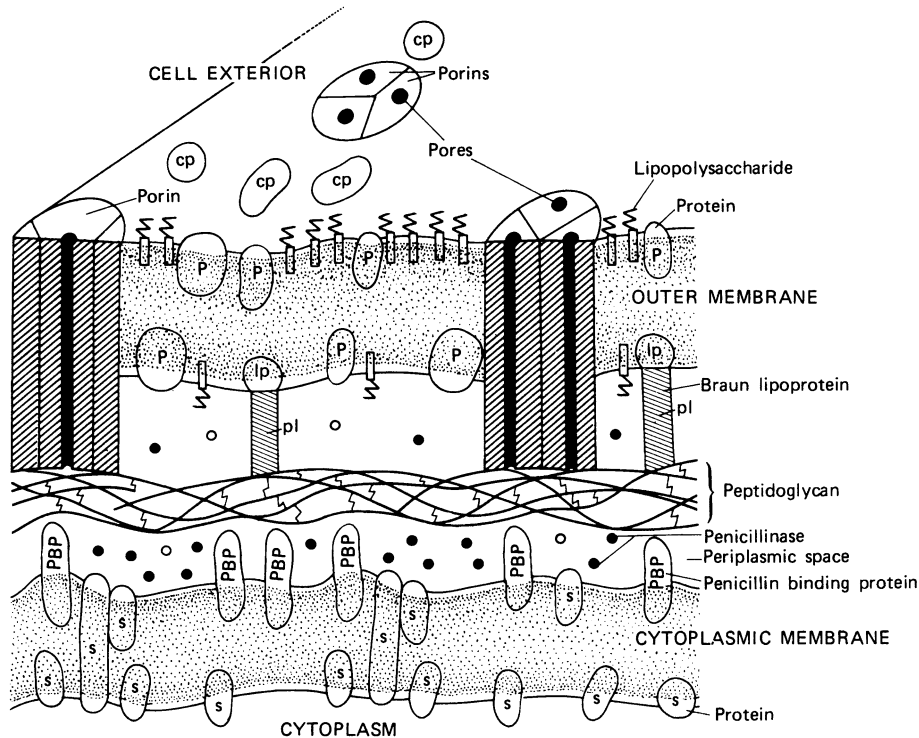


Figure 3-10. Diagram of the gram-negative cell envelope. The three-layered gram-negative envelope consists of two membranes separated by the periplasmic space containing the cross-linked peptidoglycan. Hydrophobic regions are stippled. Each membrane has protein as well as phospholipid and lipopolysaccharide components: s and p designate the protein components of these membranes. There are also extra-membrane proteins, some of which are capsular proteins (cp) and others are located in the periplasmic zone (O). β -Lactamase-producing gram-negative bacilli retain most of the enzyme (●) in the periplasmic space. The outer membrane is attached to the peptidoglycan by bridges of Braun lipoprotein; pl and lp refer to the protein and lipid portions of these bridging units. The penicillin binding proteins (PBP) are attached to the cytoplasmic membrane and extend into the periplasmic space. Antibiotics traverse the outer membrane by passing through pores formed by proteins called porins, which extend from outside the cell to the peptidoglycan. In *E. coli* and some other enterobacteria the porins are arranged as trimers with each monomeric unit contributing a channel. (Adapted from Costerton and Cheng,⁷³ Fig. 2; Nikaido and Nakae,⁷⁵ Fig. 11.)

- b) Ability of an antibiotic to affect Gm (-) pathogens depends on ability to penetrate, ability to bind to one or more PBP's, and the ability to resist degradation by enzymes (e.g. beta lactamases) in the periplasmic space.

B. INHIBITORS OF BACTERIAL PROTEIN SYNTHESIS

1. Bacterial Protein Synthesis

- a) Ribosomes - eukaryotes (have nuclear membrane, mitochondria or chloroplasts, other organelles)
 - 1) 80s ribosomes
 - 2) subunits are 40s and 60s
 - 3) are ~50% larger than bacterial
 - 4) bound to rough endoplasmic reticulum and are fewer in number
- b) Ribosomes - prokaryotes (bacterial)
 - 1) 70s
 - 2) 30s and 50s subunits
 - 3) not bound
- c) General Points
 - 1) intricate process and many opportunities for inhibition
 - 2) selective effect due to lack of 70s ribosomes in eukaryotes and different structural and functional proteins on the subunits
 - 3) generally some effect on mammalian systems that may result in toxicity when higher doses are used

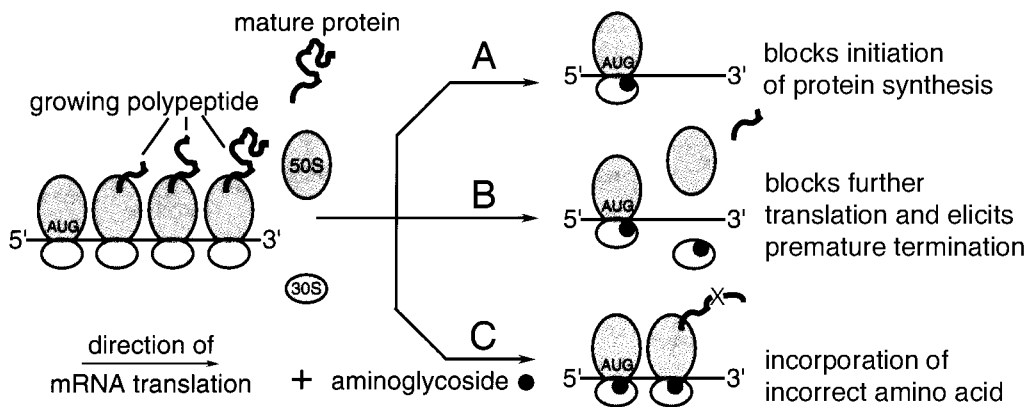


Figure 46-2. Effects of aminoglycosides on protein synthesis.

A. Aminoglycoside (represented by closed circles) binds to the 30 S ribosomal subunit and interferes with initiation of protein synthesis by fixing the 30 S-50 S ribosomal complex at the start codon (AUG) of mRNA. As 30 S-50 S complexes downstream complete translation of mRNA and detach, the abnormal initiation complexes, so-called streptomycin monosomes, accumulate, blocking further translation of message. Aminoglycoside binding to the 30 S subunit also causes misreading of mRNA, leading to **B.** premature termination of translation with detachment of the ribosomal complex and incompletely synthesized protein, or **C.** incorporation of incorrect amino acids (indicated by the "X"), resulting in the production of abnormal or nonfunctional proteins.

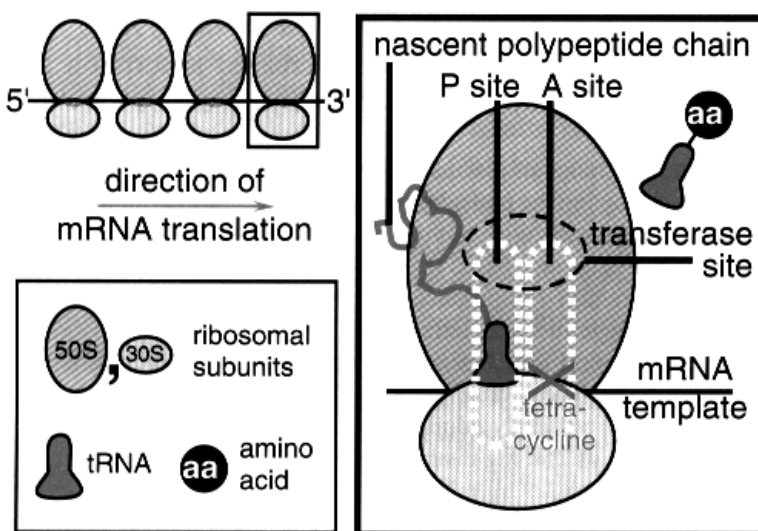


Figure 47-1. Inhibition of bacterial protein synthesis by tetracyclines.

Messenger RNA (mRNA) becomes attached to the 30 S subunit of bacterial ribosomal RNA. The P (peptidyl) site of the 50 S ribosomal RNA subunit contains the nascent polypeptide chain; normally, the aminoacyl tRNA charged with the next amino acid (aa) to be added to the chain moves into the A (acceptor) site, with complementary base pairing between the anticodon sequence of tRNA and the codon sequence of mRNA. Additional details of bacterial protein synthesis are given in Chapter 46. *Tetracyclines* inhibit bacterial protein synthesis by binding to the 30 S subunit, which blocks tRNA binding to the A site.

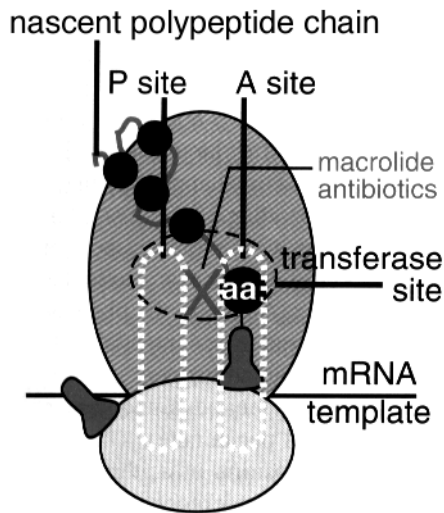


Figure 47-3. Inhibition of bacterial protein synthesis by the macrolide antibiotics erythromycin, clarithromycin, and azithromycin.

Macrolide antibiotics are bacteriostatic agents that inhibit protein synthesis by binding reversibly to the 50 S ribosomal subunits of sensitive organisms. Erythromycin appears to inhibit the translocation step wherein the nascent peptide chain temporarily residing at the A site of the transferase reaction fails to move to the P, or donor, site. Alternatively, macrolides may bind and cause a conformational change that terminates protein synthesis by indirectly interfering with transpeptidation and translocation. See Figure 47-1 and its legend for additional information.

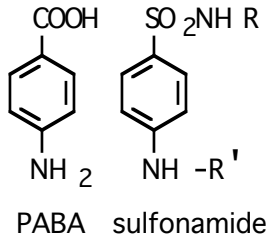
C. ANTIMICROBIAL INHIBITORS OF BACTERIAL PROTEIN SYNTHESIS

1. Aminoglycosides - bind to 30s ribosomal subunit to decrease initiation - e.g., streptomycin - binds to P12 protein on 30s ribosomal subunit to decrease binding of phenylalanine-t-RNA but to substitute isoleucine-t-RNA. Code misread resulting in death of cell; bacteriocidal.
2. Linazolid - also inhibits initiation and is mostly bacteriocidal
3. Tetracycline - bacteriostatic
decrease binding of 2nd amino acid-t-RNA to 30s ribosomal subunit.
4. Chloramphenicol - bacteriostatic - inhibit peptidyl transferase on 50s ribosomal subunit.
5. Erythromycin - bacteriostatic - binds to 50s subunit to decrease translocation.
6. Clindamycin - bacteriostatic - similar to chloramphenicol in inhibition of protein synthesis.
7. Synercid® - contains 2 streptogramin antibiotics
dalfopristin - blocks peptidyl transferase similar to erythromycin
quinupristin - blocks addition of t-RNA to peptide similar to tetracycline
combination is mostly bacteriocidal

D. INHIBITION OF NUCLEIC ACID SYNTHESIS AND FUNCTION

1. Rifampin - binds to DNA dependent RNA polymerase
2. Fluoroquinolones - blocks DNA gyrase (topoisomerases II and IV)
3. Antivirals - discuss later

E. ANTIMETABOLITES



Sulfonamides - are PABA antimetabolites to inhibit folic acid biosynthesis.

F. AGENTS AFFECTING MEMBRANE PERMEABILITY

peptide antibiotics (e.g., polymyxin B), amphotericin B (antifungal), and daptomycin

III. MECHANISMS OF TOXICITY

A. EXTENSION OF ANTIBIOTIC ACTION

e.g., some inhibitors of bacterial protein synthesis effect mammalian protein synthesis

B. HYPERSENSITIVITY

e.g., sulfa drugs, penicillins

C. TOXICITY UNRELATED TO ANTIBIOTIC MECHANISM

e.g., hepatotoxicity due to trovafloxacin

D. INDIRECT EFFECT - due to perturbation of the normally protective microflora

e.g. overgrowth of candida albicans

e.g. overgrowth of other intestinal microbes to result in antibiotic-associated diarrhea

e.g. overgrowth of *Clostridium difficile* to result in antibiotic-associated diarrhea, antibiotic-associated colitis or pseudomembranous colitis.

