THE BIOCHEMICAL BASIS OF ANTIMICROBIAL AND BACTERIAL RESISTANCE*

HAROLD C. NEU, M.D.

Professor of Medicine and Pharmacology
College of Physicians and Surgeons, Columbia University
Chief, Division of Infectious Diseases—Epidemiology
Columbia-Presbyterian Medical Center
New York, New York

T HE past 50 years have seen the continued development of new antimicrobial agents of natural, semisynthetic, and synthetic nature, and it has been possible to elucidate the biochemical basis of their action. The basic mechanisms of antimicrobial activity have been demonstrated to be due to inhibition of cell walls, damage to cytoplasmic membranes, inhibition of ribosome function, and inhibition of nucleic acid synthesis. Although many antimicrobial agents are available, bacteria over the past four decades have developed many mechanisms to overcome the action of antimicrobial agents. These mechanisms have involved the modification or duplication of target enzymes, prevention of access of antimicrobial agents to the targets, and synthesis of enzymes that modify or destroy the antimicrobial agents. Bacterial resistance has become a serious problem in many parts of the world because resistance genes exist on plasmids and transposons that can be widely disseminated. Mechanisms to overcome resistance have included molecular modification of antimicrobial agents and use of combinations of antimicrobial agents. Understanding how antimicrobial agents affect bacteria and how resistance develops is extremely important if we are to prevent bacteria from overwhelming man during the coming decades.

Man and microorganisms coexist in a very tenuous relationship just as nations exist in delicately balanced relations. Some organisms are naturally pathogenic, some organisms produce disease only when host defences are disrupted, and some microorganisms protect us from the deleterious effects of other bacteria or fungi. In considering the role of antimicrobial chemother-
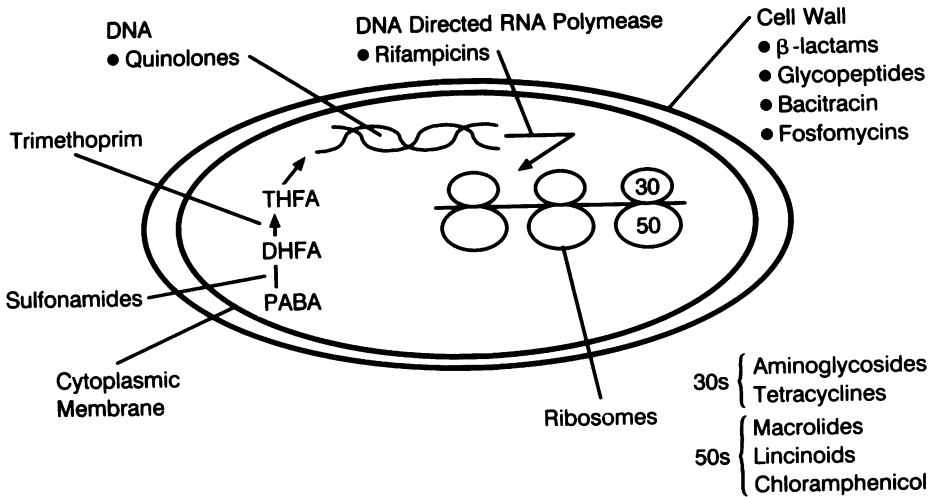
Fig. 1. Sites of action of various antimicrobial agents

apy one must remember that chemotherapeutic agents may affect not only the intended pathogens, but they may have profound effects on the micro-ecology of the environment.

Improvements in fermentation techniques and advances in medicinal chemistry have provided many new chemotherapeutic agents that are novel molecular modifications of existing compounds. Great progress has been achieved in the development of new and novel antibacterial agents.

BIOCHEMICAL BASIS OF ANTIMICROBIAL ACTION

As bacterial cells multiply and divide, they make new molecules of DNA, RNA, and protein, and obtain from their environment the smaller units such as amino acids or sugars present in their walls and membranes. Antimicrobial agents have specific targets (Figure 1), which can be separated into such groups as inhibitors of cell walls, distorters of cytoplasmic membranes, inhibitors of nucleic acid synthesis, inhibitors of ribosome function, and inhibitors of intermediary cell metabolism (Table 1). Antimicrobial agents may be either bactericidal or bacteriostatic. Ideally, bacteria should be killed, but even agents which only inhibit growth of bacteria can be extremely beneficial since they permit the normal defenses of the host to destroy the microorganisms.

TABLE I. MECHANISMS OF ACTION OF ANTIMICROBIAL AGENTS

A) *Inhibition of bacterial cell wall synthesis*

   1) Inhibition of biosynthetic enzymes
      Fosfomycin
      Cycloserine
   2) Antibiotics which combine with carrier molecules
      Bacitracin
   3) Antibiotics which combine with substrates of wall
      Vancomycin
   4) Inhibition of polymerization and attachment of new peptidoglycan to cell wall
      Penicillins
      Cephalosporins
      Thienamycins
      Monobactams

B) *Inhibitors of cytoplasmic membranes*

   1) Drugs disorganizing cytoplasmic membrane
      Tyrocidins
      Polymyxins
   2) Drugs producing pores in membranes
      Gramicidins

C) *Inhibitors of nucleic acid synthesis*

   1) Agents which impair DNA template function: intercalating agents
      Chloroquin (parasites)
   2) Inhibitors of DNA replication
      Nalidixic acid
      Ciprofloxacin, ofloxacin, norfloxacin, enoxacin
      Nitroimidazoles
   3) Inhibitors of RNA polymerase
      Rifampin

D) *Inhibitors of ribosome function*

   1) Inhibitors of 30S units
      Streptomycin
      Kanamycin, gentamicin, tobramycin
      Spectinomycin
      Tetracyclines
   2) Inhibitors of 50S units
      Chloramphenicol
      Lincomycins
      Erythromycin
      Fusidic Acid
   3) Inhibition of folate metabolism
      Inhibition of pteroic acid synthetase
      Sulfonamides
      Inhibition of dihydrofolate reductase
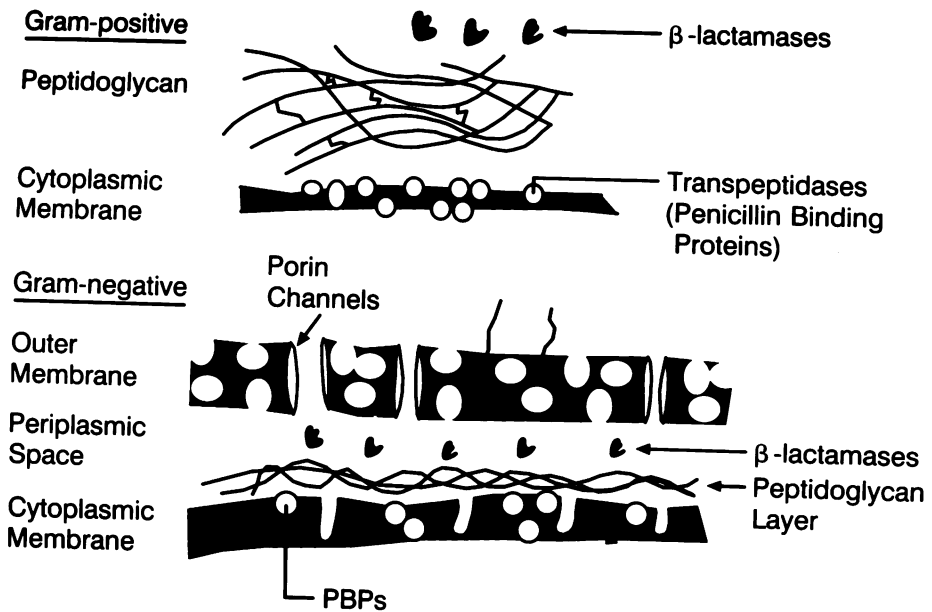   Trimethoprim

Fig. 2. Characteristics of Gram-positive and Gram-negative bacteria

BACTERIAL CELL WALL INHIBITORS

Bacteria are divided into Gram-positive and Gram-negative organisms on the basis of staining characteristics. Gram-positive bacterial cell walls contain peptidoglycan, teichoic, or teichuronic acid. They may or may not be surrounded by a protein or polysaccharide envelope. Gram-negative bacteria contain peptidoglycan, lipopolysaccharide lipoprotein, phospholipid, and protein (Figure 2). The critical attack site of anticell wall agents is the peptidoglycan layer, which is essential for bacterial survival.

Peptidoglycan synthesis occurs in three stages.[1] The first stage occurs within the cytoplasm where low molecular weight precursors UDP-GlcAc and UDP-MurNAc-L-ala-D-glu-meso-Dap-D-ala-D-ala are synthesized. A number of antimicrobial agents interfere with early steps in cell wall biosynthesis. UTP and GluNAcl-P are converted to UDP-GluNAc, which is subsequently converted to UDP-MurNAc by the enzyme phosphoenolpyruvate: UDP-GlcNAc-3-enol-pyruvyl transferase. Fosfomycin and fosmidomycin block this transfer by direct nucleophilic attack upon the enzyme.[2] Human enolase, pyruvate kinase, and carboxykinases and shikimate enoloases are not inhibited by these compounds, hence fosfomycins have no effect on mammalian metabolic pathways.

The dipeptide D-alanyl-D-alanine is synthesized from two molecules of D-alanine by the enzyme D-alanyl-D-alanine synthetase. D-alanine is produced from L-alanine by an alanine racemase. Cycloserine is a compound which inhibits both alanine racemase and D-alanine-D-alanine synthetase due to the structural similarity of cycloserine to D-alanine.[3]

The second stage of cell wall synthesis is catalyzed by membrane bound enzymes. The non-nucleotide portion of precursor molecules are transferred to a carrier in the cytoplasmic membrane which is a phosphorylated un-decaprenyl alcohol. Bacitracin is a peptide antibiotic that specifically inter-acts with the pyrophosphate derivate of the undecaprenyl alcohol prevent-ing transfer of the muramylpentapeptide from the precursor nucleotide to the nascent peptidoglycan.[4]

The third stage of the synthesis of cell wall involves polymerization of the subunits and attachment of nascent peptidoglycan to the cell wall by a trans-peptidase reaction that involves peptide chains in both polymers. The trans-peptidase enzyme cleaves the peptide bond between two D-alanyl residues in the pentapeptide and becomes acylated via the carbonyl group of the penul-timate D-alanine residue. This reaction is inhibited by beta-lactam antibi-otics, penicillins (penems), cephalosporins (including oxacephems and cephamycins), penems, thienamycins (carbapenems), and aztreonam (monobactams) (Figure 3).[5] These drugs, by binding to the enzymes in-volved in this final process of cell wall formation, are called penicillin-binding proteins since they were discovered by use of radioactive penicil-lin G. The enzymes markedly differ in Gram-positive and Gram-negative bacteria and in anaerobic species.[6] These differences in penicillin-binding proteins explain differences in antibacterial activity of the beta-lactam an-tibiotics. The penicillin-binding proteins to which a particular beta-lactam antibiotic binds also affects the morphological response of the bacterium to the agent. Some antibiotics bind to a penicillin-binding protein involved in septum formation producing long filaments which eventually die. Binding to another penicillin-binding protein results in rapid lysis of a bacterium be-cause the wall bulges due to weakened peptidoglycan and the bacterium bursts.

Vancomycin and other glycopeptides such as teichoplanins also interfere with cell wall synthesis by combining with the D-ala-D-ala termini of grow-ing peptidoglycan attached to the undecaprenyl pyrophosphate and prevent interaction of muramidases with the glycan chain. The specificity of van-comycin and other antibiotics of the teichoplanin class for the acyl-D-ala-D-ala site explains the lack of resistance to these antibiotics.[7]
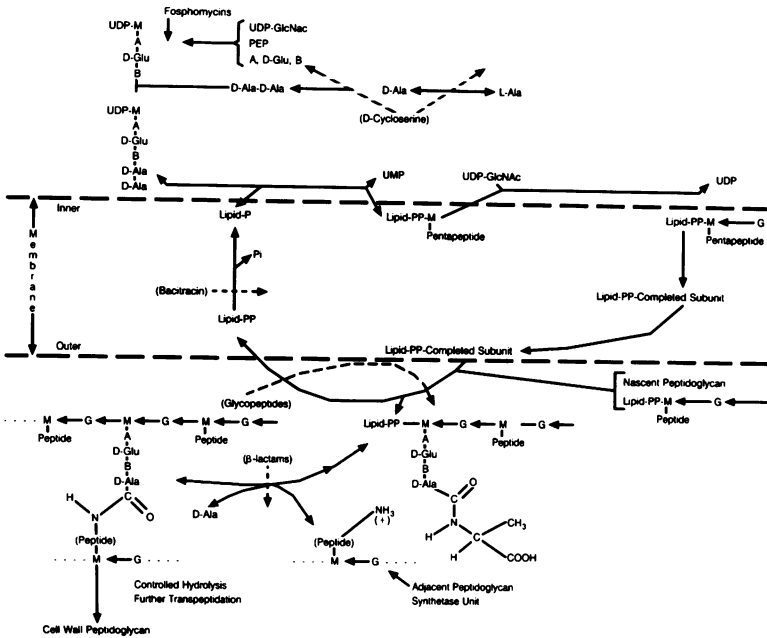
Fig. 3. Synthesis of bacterial cell walls

ANTIBIOTICS AFFECTING FUNCTION OF CYTOPLASMIC MEMBRANES

Bacterial cytoplasmic membranes. Membranes are composed of lipid, protein, and lipoprotein. The cytoplasmic membrane acts as a diffusion barrier for water, ions, and nutrients, as well as for transport systems. Membranes are a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bylayer and partly through the aqueous component of the membrane. Some antibaterial agents such as the polymyxins, polymyxin B, and colistemethate (polymyxin E) can cause disorganization of the membrane. These compounds are octapeptides characterized by high molecular weights. The agents inhibit Gram-negative bacteria which have negatively charged lipids at the surface. Since the activity of the agents can be antagonized by cations Mg^{2+} and Ca^{2+}, it is probable that the polymyxins competitively displace Mg^{2+} or Ca^{2+} from the negatively charged phosphate groups present on membrane lipids.[8] Basically polymyxins disorganize membrane permeability so that nucleic acid material and cations leak out of the bacterial cell.

Inhibition of DNA directed DNA polymerase. Rifamycins are a class of antibiotics which inhibit DNA-directed RNA polymerase activity.[9] RNA

polymerase is an enzyme that possesses four alpha and beta subunits. Poly-peptide chains attach to the component which confers specificity for the recognition of the correct promoter sites to initiate transcription of the DNA. Rifampin binds to a beta subunit and interferes specifically with the initiation process, but has no effect after polymerization has begun.

Inhibition of DNA replication. DNA gyrase is an enzyme that changes negative supercoiled strands of DNA into closed circular duplex DNA, and it is essential for replication of circulation chromosomes. It also is involved in breakage and reunion of DNA strands. The gyrase consists of two components A and B, with the A subunit more abundant. Nalidixic acid, a quinolone, binds to a component of DNA gyrase and inhibits its action. Recently other drugs that belong to the quinolone class but which are fluorinated carboxy derivatives have been synthesized. Nalidixic acid inhibited only Gram-negative species whereas the newer agents, ciprofloxacin, ofloxacin, enoxacin, and norfloxacin inhibit Gram-positive species as well, and some of the new agents also inhibit anaerobic bacteria and mycobacteria.[10] The DNA gyrase β subunit can be inhibited by other agents, but none have clinical utility at present.

Nitroimidazoles and metronidazole inhibit anaerobic bacteria and protozoa. The nitro group of the nitrosohydroxyl amino group of the compound is reduced by bacteria which use anaerobic metabolism.[11] The drugs diffuse into bacteria where they are concentrated, reduced by an electron transport protein, and cause strand breaks in the DNA. Mammalian cells are unharmed because they lack the enzymes to reduce the nitro group of these agents.

ANTIMICROBIAL INHIBITORS OF RIBOSOME FUNCTION

Bacterial ribosomes contain 65% protein and 35% RNA. They can be dissociated into subunits referred to as 50S and 30S ribosomal subunits. Since it is possible to dissociate ribosomes into subunits, it is possible to localize the action of antibiotics to one or both units (Figure 1). It is also possible to isolate specific proteins in the ribosome units to which the agent binds and to isolate mutants of bacteria which can be shown to lack a specific ribosomal protein and thereby be resistant to a particular agent.

Aminoglycosides are complex sugars connected in glycosidic linkage. The compounds differ by virtue of both the nucleus, which can be streptidine or 2-deoxystreptidine, and the aminohexoses linked to this nucleus. Essential to the activity of these agents are free NH_4 and -OH groups by which aminoglycosides bind to specific ribosomal proteins.

Streptomycin binds to a specific S12 protein in the 30S ribosome.[12] The

outcome of in vitro binding is achieved at much higher concentrations than are possible in vivo. Many textbooks continue to discuss the misreading of the genetic code caused by aminoglycosides. Although this occurs in vitro, it is unlikely to occur in vivo.

Other aminoglycosides such as gentamicin, tobramycin, and amikacin bind to the S12 protein of the 30S ribosome, but they also bind to the L6 protein of the 50S ribosome.[13] This later binding is quite important in respect to the resistance of bacteria to aminoglycosides. Aminoglycosides probably have multiple binding sites on the 30S ribosomes and ultimately cause death of bacteria by formation of aberrant initiation complexes sequestering the ribosomes from the ribosome pool.

Tetracyclines inhibit binding of aminoacyl tRNA on the 30S bacterial ribosome, but the binding is a transient affair and tetracyclines are bacteriostatic.[14]

Three important classes of drugs affect the larger 50S ribosome subunit. Chloramphenicol inhibits peptide bond formation by binding to a peptidyl transferase enzyme on the 50S ribosome. Macrolides and lincinoids bind to 50S ribosomes and impair a peptidyl transferase reaction, translocation, or both reactions.[15] Both of these classes of antibiotics are bacteriostatic and only inhibit the formation of new peptide chains although both macrolides and lincinoids can be bactericidal for some Gram-positive species and will kill some intracellular bacteria. Chloramphenicol also is bactericidal for *S. pneumoniae* and *H. influenzae*.

DRUGS WHICH INHIBIT OTHER BIOCHEMICAL TARGETS

Both trimethoprim and sulfanomides interfere with folate metabolism in the bacterial cell by competitively blocking biosynthesis of tetrahydrofolate, a precursor of folinic acid (Figure 4).[16] Unlike mammals, bacteria and protozoan parasites usually lack a transport system which would enable them to use preformed folic acid abundant in their environment. Most organisms must synthesize folates, although some are capable of using exogenous thymidine and circumventing the need for folate metabolism.

Sulfonamides competitively block the conversion of pteridine and para-aminobenzoic acid (PABA) to dihydrofolic acid. Sulfonamides have a greater affinity for the enzyme that performs the conversion than does PABA. Trimethoprim has an affinity for the enzyme dihydrofolate reductase and inhibits synthesis of tetrahydrofolate. This latter compound acts as a co-factor for carriers of 1-carbon fragments and is necessary for the ultimate synthesis of DNA, RNA, and bacterial cell wall proteins.
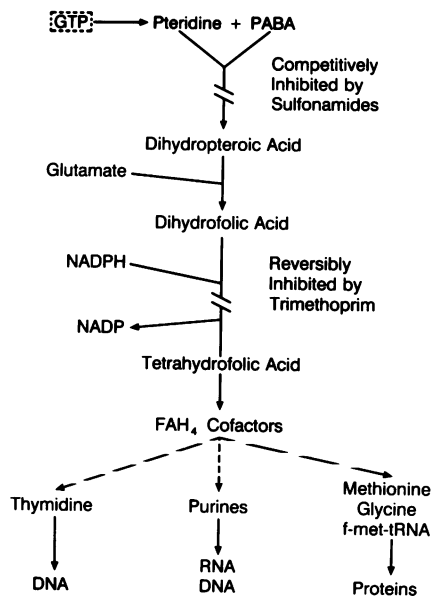
Fig. 4. Mode of action of sulfamethoxazole and trimethoprim within the bacterial cell

BACTERIAL RESISTANCE

Bacteria have proved particularly adept at becoming resistant to each new antimicrobial agent that is discovered in nature or synthesized by medicinal chemists. There are several ways whereby bacteria can be or become resistant to antimicrobial agents (Table II).[17] Early studies of bacterial resistance focused on single step mutational events of chromosomal origin. Sulfonamide resistance resulted from a single amino acid change in the enzyme dihydropeteroic synthetase that caused sulfonamides to bind less well than para-aminobenzoic acid. A mutational event resulted in a single step mutation that altered a ribosomal protein, and bacteria were able to resist the action of streptomycin. However, in the late 1950s Japanese workers found that enteric bacteria such as *Shigella dysenteriae* had become resistant not only to sulfonamides but to the tetracyclines and chloramphenicol. This resistance was not a chromosomal change, but was due to the presence of extrachromosomal DNA which was transmissible. This resistance is referred to as plasmid resistance, and was formerly called R-factor, that is, resistance factor resistance.

Resistance conferring plasmids have been identified in virtually all bacteria (Table III),[18] and have been widely dispersed in nature, appearing in *Haemophilus influenzae* in 1974 and subsequently in 1976 in *Neisseria gonor-*

TABLE II. RESISTANCE MECHANISMS

1) Modification of a target enzyme so that it is insensitive to an inhibitor but still functions

2) Reduction in physiological importance of a target

3) Duplication of a target enzyme

4) Prevention of access to the target

5) Depression of metabolic activity that normally converts an inert agent into an active agent

6) Synthesis of enzymes that:
   a) Inactivate an antimicrobial agent
   b) Modify the agent to alter entry or binding to a receptor

TABLE III. R-PLASMID-MEDIATED RESISTANCE

Antibiotic	Mechanism	Organisms
Penicillin, ampicillin carbenicillin, etc.	Beta-lactamase hydrolysis	Gram (+), (−)
Oxacillin, methicillin, etc.	Beta-lactamase hydrolysis	Gram (−)
Cephalopsorins	Beta-lactamase	Gram (+), (−)
Chloramphenicol	Acetylation	Gram (+), (−)
Tetracyclines	Permeability block	Gram (+), (−)
Aminoglycosides Streptomycin Neomycin Kanamycin Gentamicin Tobramycin Amikacin	Acetylation, Phosphorylation, or Adenylation— Alters binding to ribosomes and uptake of drug	Gram (+), (−)
Macrolides-Lincinoids Erythromycin Clindamycin	Altered 23S RNA	Gram (+), (−)
Trimethoprim	Altered dihydro- folate reductase	Gram (−)
Sulfonamides	Altered tetrahydro- pteroic synthetase	Gram (−)
Nitrofurans	Unknown	Gram (−)
Fosfomycin	Altered glucose transport system	Gram (+), (−)

rhoeae. Unfortunately, many bacteria contain transposons, so-called jumping genes which can enter plasmids or the chromosome. It is possible that plasmids will pick up chromosomal genes of resistance and transfer these genetic elements to species not currently resistant.

Whether anitmicrobial agents are the major selective pressure upon the development of both chromosomal- and plasmid-mediated resistance is discussed elsewhere in this issue. The use of antibiotics in an environment, whether the hospital as a unit or an individual patient as a small ecosystem, will destroy antibiotic-susceptible bacteria and permit the proliferation of bacteria which are intrinsically resistant or which have acquired extrachromosomal resistance. Plasmid resistance, from an epidemiological viewpoint, is the most important resistance because it is transmissible, usually highly stable, confers resistance to many different classes of antibiotics simultaneously, and often is associated with other characteristics that enable a microorganism to colonize and to invade a susceptible host. But, as noted elsewhere in this issue, resistance also can be a deleterious factor since growth is slower.

MECHANISMS OF BACTERIAL RESISTANCE

The basic mechanisms by which microorganisms become or are resistant to antimicrobial agents are development of altered receptors for a drug, decrease in the amount of a drug that reaches the receptor by altering entry or increasing removal of a drug, destruction or inactivation of a drug, and synthesis of resistant metabolic pathways. Bacteria can possess one or all of these mechanisms simultaneously.

RESISTANCE BASED ON ALTERED RECEPTORS FOR A DRUG

Beta-lactams. Analysis of changes in receptors for beta-lactams by competition experiments in which ^{14}C penicillin is inhibited from binding to penicillin-binding proteins has explained a number of cases of resistance of bacteria to penicillins and cephalosporins. In 1977 *Streptococcus pneumoniae* resistant to penicillin G were encountered in South Africa.[19] These organisms appeared in ill patients in Johannesburg and Durban and did not possess beta-lactamases but had altered penicillin-binding proteins.[20,-22] The newly synthesized penicillin-binding proteins have a decreased affinity for penicillins, but interestingly the aminothiazolyl cephalosporins such as cefotaxime, etc. bind to the new penicillin-binding proteins at concentrations

that can be achieved in blood, tissue, and cerebrospinal fluid. Resistance of
S. pneumoniae to penicillin has been increasing, and there are relatively resis-
tant isolates (MIC 0.1-lμg/ml) in many parts of the world because of altered
penicillin-binding proteins.

Altered penicillin-binding protein receptors are why some *Staphylococcus
aureus,* the so-called methicillin-resistant,[23] are resistant to beta-lactamase
stable penicillins and cephalosporins. Methicillin-resistant *Staphylococcus
aureus* contain a new penicillin-binding protein 2.[1] This new enzyme is in-
duced by beta-lactams such as nafcillin, methicillin, oxacillin, etc.[24-27] The
cephalosporins also induce the new penicillin-binding protein 2 but less ef-
fectively and hence the laboratory may inadvertently report a *S. aureus* resis-
tant to oxacillin but susceptible to cephalothin. This is an artifact of the an-
timicrobial test system. If the agar medium contains 2.5 to 5% NaCl,
incubation is performed at 30°C and the plates not read before 24 hours,
the true resistance will be noted. Only *S. aureus* or *S. epidermidis* with a
cephalothin MIC of >1 μg/ml should be considered resistant. Semisynthetic
penicillin susceptibility should be determined with oxacillin, not nafcillin,
since nafcillin MICs may be falsely below 8 μg/ml. The precise mechanism
of methicillin resistance in *S. epidermidis* has not been worked out, but it
seems probable that the beta-lactam resistance of *S. epidermidis* is also the
result of altered penicillin-binding protein.

The resistance of enterococci of the group D streptococci to beta-lactam
antibiotics also results from lower affinity of the penicillin-binding protein
for the penicillins and particularly for cephalosporins which do not inhibit
these species.[28,29,] *Neisseria gonorrhoeae* resistance which is chromosomally
mediated can be correlated with diminished affinity of the target penicillin-
binding proteins.[30] Altered penicillin-binding proteins in *Enterobacteriaceae*
and *Pseudomonas aeruginosa* can produce resistance, but isolation of such
organisms in nature is extremely uncommon.[31,32]

Macrolide-lincinoid resistance. The occurrence of macrolide-lincinoid-
streptogramin resistance in clinical isolates of staphylococci and streptococci
has been recognized for the past several decades. The mechanism of resis-
tance is methylation of two adenine nucleotides in the 23S component of 50S
RNA, the genetic basis of the resistance is plasmid, and the resistance is pres-
ent on transposons.[33] A methylating enzyme normally repressed in nonresis-
tant bacteria is induced by the compounds. Methylated ribosomal RNA binds
macrolide-lincomycin-type drugs less well than does unmethylated
RNA.[33-36] Induction of resistance varies by bacterial species and by inducer.
For example, erythromycin is a more effective inducer of resistance in most

Gram-positive species than is clindamycin. There is extensive structural similarity of the plasmids in streptococci and staphylococci that mediate macrolide-lincinoid resistance, indicating that these plasmids readily pass between these species.[37-39]

Rifampin resistance. Resistance of bacteria to rifampin is on the basis of an altered DNA directed RNA polymerase.[9] Change of one amino acid in the beta subunit of DNA directed RNA polymerase alters the binding of rifampin to the enzyme. The degree of resistance is related to the degree that the enzyme is changed, but does not correlate strictly with enzyme inhibition. This form of resistance exists at a low level in any population of bacteria so that resistance is seen to develop during therapy. Appearance of such resistance is not due to a mutational event, but rather to selection of a subset of the bacterial population which possessed an RNA polymerase with poor affinity for rifampin. Such organisms are more common among the *Enterobacteriaceae*, explaining why organisms causing urinary tract infection rapidly became resistant to rifampin. Resistance of *Neisseria meningitidis* to rifampin appeared in closed military settings in which rifampin was used as prophylaxis at the time of outbreaks.[40]

Sulfonamide-trimethoprim resistance. The presence of an altered or new dihydropeteroic synthetase that binds PABA better than sulfonamides is the basis of resistance to these compounds.[41] Sulfonamide resistance of this type can result from a point mutation or from the presence of a plasmid which causes synthesis of a new enzyme.[42] Resistance to trimethoprim is plasmid and transposon mediated[43-45] and due to production of an altered dihydrofolate reductase which has markedly reduced affinity for trimethoprim.

Since the genetic information for synthesis of the enzymes resides on transposons, we can anticipate even greater resistance to trimethoprim in the future in developing countries.[46]

Quinolone resistance. Resistance of bacteria to older quinolone antibiotics such as nalidixic acid, cinoxacin, and oxalinic acid probably was due to either altered DNA gyrase or, in some bacteria, to failure of entry of the agent.[47] This is not a plasmid-mediated form of resistance but a mutational event or selection of such strains from the bacterial population similar to the rifampin resistance. The mechanism of the resistance of bacteria to the new fluorinated carboxyquinolones such as norfloxacin, ofloxacin, and ciprofloxacin has not yet been elucidated. These agents are active against DNA gyrase A mutants resistant to nalidixic acid, but the concentration of the drug needed to inhibit naladixic acid-resistant bacteria is much greater. It is probable that resistance of some isolates is due to permeability, but it may also

be due to other factors such as increased inhibition of quinolone action by higher intracellular Mg^{2+} concentrations or by failure to activate DNAses which cleave excision fragments produced by quinolone binding.

DECREASED ENTRY OF A DRUG

Tetracycline uptake by *Enterobacteriaceae* is a biphasic process with an initial energy-independent rapid phase thought to represent binding of the drug to cell surface layers with passage by diffusion through the outer layers of the cell wall. The second phase of uptake is energy dependent as the tetracycline crosses the cytoplasmic membrane, probably by means of a proton motive force. The precise transport system has not been identified.

Tetracycline resistance is common in both Gram-positive and Gram-negative bacteria and is plasmid encoded and inducible. Chromosomal, constitutive resistance to tetracyclines is present in some species such as *Proteus*.[48,49] So far, five plasmid-specified tetracycline resistance determinants have been found in enteric bacteria and one of these determinants, Tet B, the most common, is also present in *H. influenzae* and some *N. gonorrhoeae*. Tetracycline resistance in *S. aureus* is primarily due to small plasmids that exist in multiple copies, and chromosomal resistance is rare. Tetracycline resistance is found in *S. faecalis* on nonconjugative plasmids and in the chromosomes of *S. pneumoniae*, *S. agalactiae* (group B streptococci), and oral streptococci such as *S. mutans*. *Clostridium* sp. such as *C. difficile* harbor chromosomal genes for resistance to tetracycline.

Basically, tetracycline resistance is due to a decrease in drug accumulation because of a drug efflux mechanism.[50,51] Probably decreased uptake and efflux occur simultaneously. Tetracycline-resistant bacteria bind less tetracycline, and the tetracycline they do accumulate is lost by an energy-dependent process that pumps the drug out when bacteria are in a drug-free milieu.

Plasmid resistance to tetracyclines can be partially overcome in Gram-positive species by molecular modification of the tetracycline nucleus. Thus, minocycline and doxycycline in particular will inhibit at achievable concentrations some streptococci such as *S. pneumoniae* and some *S. aureus*. Molecular modification has not been successful in overcoming tetracycline resistance of *Enterobacteriaceae* and *Pseumdononas* nor of most *Bacteroides* sp.

Tetracycline resistance has been a major concern since tetracycline resistance is located on plasmids near insertion sites, and it appears that tetracycline plasmids can readily acquire other genetic information to be resistant

to other antibiotics. Although other forms of resistance, such as altered binding site on 30S ribosomes, can occur, this is much less frequent.

In *Enterobacteriaceae* and *Pseudomonas* sp., the aminoglycosides pass through the cell wall by going through channels through which cationic molecules enter the periplasmic space. Aminoglycosides which reach the inner cytoplasmic membrane are moved across the membrane by a proton motive force to enter the cytoplasm where they bind to ribosomes situated just below the membrane. The initial uptake of aminoglycoside into the membrane is energy dependent. Aminoglycosides which enter the cell will bind only to ribosomes actively engaged in protein synthesis. Binding to the ribosomes induces a protein involved in the uptake of the aminoglycosides.[54]

Bacteria may contain enzymes in the periplasmic space that will acetylate, phosphorylate, or adenylate aminoglycosides to varying degrees depending upon the molecular configuration of the molecule. Whether the enzymes are free in the periplasmic space or bound to the cytoplasmic membrane is not completely clear. Aminoglycosides acetylated, phosphorylated, or adenylated do not bind well to ribosomes, and hence uptake is poor or does not occur since there is no induction of the transport protein.

Aminoglycoside modifying enzymes have been found in Gram-positive species such as *S. aureus, S. faecalis, S. pyogenes,* and *S. pneumoniae.* These enzymes are particularly prevalent in *Enterobacteriaceae* and *P. aeruginosa.*[55]

Anaerobic species such as *Bacteroides* sp are resistant to aminoglycosides because of the lack of an oxygen-dependent transport system to move the drug into and across the cytoplasmic membrane.[56] Although most resistance of *S. aureus* to aminoglycosides is due to modifying enzymes, small colony variants of staphylococci are resistant, and the resistance appears to be due to a defect in adenyl cyclase or in cyclic-AMP binding proteins so that those bacterial cells which have a reduced growth rate do not transport aminoglycosides into the cytoplasm.[56] Finally, some *Enterobacteriaceae* and *P. aeruginosa* appear to be resistant because of altered porin channels, and no drug reaches the periplasmic space or cytoplasmic membrane to be transported within the cell.[57]

DESTRUCTION OR INACTIVATION OF A DRUG

Chloramphenicol resistance. Many Gram-positive and Gram-negative bacteria, including recently some *Haemophilus influenzae,* are resistant to chloramphenicol because they possess the enzyme chloramphenicol transacetylase.[58] This enzyme, unlike the aminoglycoside inactivating enzymes and
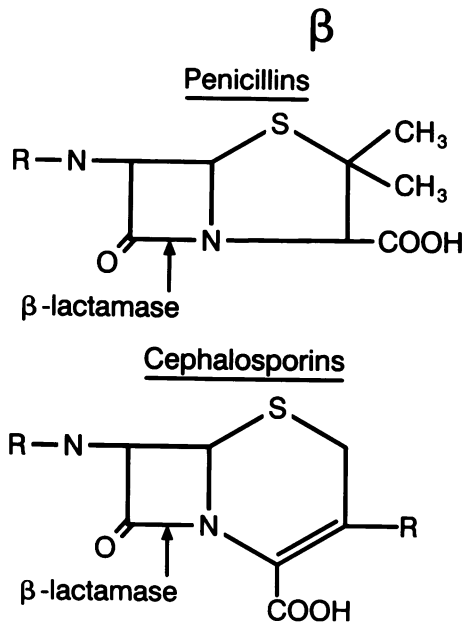
Fig. 5. Site of action of beta-lactamases

beta-lactamases, is an intracellular enzyme of larger molecular weight and of subunit structure. It is in most instances plasmid mediated. The chloramphenicol acetyltransferase acetylates hydroxyl groups. Basically, acetylated chloramphenicol binds less well to the 50S ribosome and protein synthesis continues normally.

Beta-lactam resistance. The best known mechanism of bacterial resistance is that of the beta-lactamases. In the 1940s resistance of staphylococci was shown to be due to a penicillinase.[59] With the advent of other beta-lactam compounds such as cephalosporins, carbapenems, and monobactams, it is more appropriate to designate these enzymes as beta-lactamases since their attack on the beta-lactam nucleus is the most important aspect of their activity (Figure 5). Beta-lactamases are widely distributed in nature and can be classified in various ways, but usually are referred to on the basis of the principal compounds they destroy, hence they are penicillinases or cephalosporinases.[60-62] Beta-lactamases can be chromosomally or plasmid mediated, constitutive, or inducible enzymes.

In Gram-positive species beta-lactamases are exoenzymes, excreted into the milieu around the bacteria (Figure 2). Virtually all hospital isolates of staphylococci, both *S. aureus* and *S. epidermidis*, possess beta-lactamases,

and 50 to 80% of community staphylococcal isolates produce beta-lactamases. In Gram-negative species, beta-lactamases are contained in the periplasmic space (Figure 2).

At present 10 to 35% of *H. influenzae* in the United States produce beta-lactamase. The beta-lactamase enzyme of *Haemophilus* is the same as that in *E. coli, Salmonella, Shigella,* and *N. gonorrhoeae.* The enzyme has generally been called the TEM enzyme, so named after the Greek girl from whom an *E. coli* which contained a plasmid beta-lactamase was isolated by Datta and Kontomichalou in 1964[63] These enzymes are also called Richmond-Sykes class IIIa enzymes from a classification proposed by Richmond and Sykes in 1973.[60] This beta-lactamase is also present in *N. gonorrhoeae.* By far the most common plasmid beta-lactamase found in nature is the TEM-1 enzyme which has been reported to account for 75-80% of plasmid mediated beta-lactamase resistance worldwide.[64-67] There are at present some 22 plasmid mediated beta-lactamases.

Chromosomally mediated beta-lactamases are present in many *Enterobacter, Citrobacter, Proteus-Providencia,* and *Pseudomonas.* All *Klebsiella* sp. possess a beta-lactamase which acts primarily as a penicillinase and which is chromosomally mediated. Constitutively produced beta-lactamases are also present in most *Bacteroides* sp.

Table IV illustrates the major beta-lactamases of clinical importance. Beta-lactamases vary in their ability to destroy penicillins and cephalosporins. It is extremely important to realize that beta-lactamase activity studies with isolated or purified beta-lactamases may not reflect the activity of a compound against Gram-negative bacteria since resistance of Gram-negative bacteria to beta-lactams is a combination of decreased entry, B-lactamase stability, and affinity of the compound for penicillin-binding proteins.

SYNTHESIS OF RESISTANT METABOLIC PATHWAY

No synthesis of a new type of cell wall resistant to beta-lactams has occurred, but some bacteria, particularly some streptococci, lack hydrolytic enzymes necessary to form a new cell wall, and thus beta-lactams do not cause lysis of these bacteria. An altered cell wall hydrolytic system thus converts a bactericidal antibiotic into a bacteriostatic agent. Whether such resistance occurs in Gram-negative species is not clear.

Some thymidine-requiring streptococci are not inhibited by trimethoprim and sulfonamides. These organisms cause some urinary tract infections, albeit rare, but such organisms fail to undergo thymineless death that occurs normally with bacteria exposed to these agents. Other bacteria produce adequate

TABLE IV. CLASSIFICATION OF B-LACTAMASES

Amino acid composition	Richmond-Sykes classification	Bacteria	Trivial names	Preferred substrate	Genome	Inhibitor susceptibility
A	—	S. aureus	PC	Penicillins	Plasmid	Cloxacillin, clavulanate
		B. licheniformis			Chromosomal	
	IIIa	Enterobacteriacea	TEM-1, TEM-2	Penicillins, some cephalosporins	Plasmid	Cloxacillin, clavulanate
		Haemophilus				not PCMB* Clavulanate
		Neisseria				
	V	Klebsiella	SHV-1	Penicillins, some cephalosporins	Plasmid	Clavulanate
		E. coli	OXA		Plasmid	
B	—	P. aeruginosa	PSE	Penicillin + caropenems	Chromosomal	Chelators of Zn^{2+}
		B. cereus	II			
		B. fragilis	(Zn^{2+} stimulated)			
		P. maltophilia				
C	Ia	Enterobacter Morganella,	P99	Cephalosporins	Chromosomal	Cloxacillin, PCMB, not clavulanate
	Ic	P. vulgaris		Cephalosporins	Chromosomal	Clavulanate
	Id	P. aeruginosa	S-A	Cephalosporins	Chromosomal	Cloxacillin, not clavulanate
	IV	Klebsiella	K-1	Penicillins	Chromosomal	Clavulanate, not cloxacillin

*PCMB=1
From Ambler: The structure of beta-lactamases. *Philos. Trans. R. Soc.* (Biol.) 289:321-31. 1980.

TABLE V. MECHANISMS TO REDUCE ANTIBIOTIC RESISTANCE

1) Improved hygiene in hospitals and among hospital personnel and reduced movement of patients might eliminate the dissemination of resistant organisms within hospitals.

2) Avoid topical use of antimicrobial agents with the exception of silver-sulfadiazine.

3) Use antibiotics chemically modified not to select plasmid resistance.

4) Use antimicrobial agents in a pharmacologically proper way to reduce rapidly the number of bacteria.

5) Adjust therapy to prevent loss of normal flora when the infecting pathogen has been identified.

dTMP by alternate methods and as a result survive exposure to these folate inhibitors. Some rare anaerobic bacteria do not convert imidazoles to their metabolic derivate that can damage DNA.

CONCLUSION

Bacteria continue to evolve new mechanisms of resistance to old and to new antimicrobial agents. Some bacteria such as *P. aeruginosa* are particularly adept at utilizing a number of different mechanisms simultaneously to become resistant to agents in virtually every class and agents which have such diverse sites of action as cell wall, protein biosynthesis, or DNA and RNA synthesis. It is probable that the developments in other areas of medicine will keep patients alive who become nosocomially infected by resistant pathogens.

Proper selection of new antibiotics will be a major force in slowing the development of antimicrobial resistance. Proper hygienic practices will reduce plasmid transfer and the establishment of multiply drug resistant bacteria in the hospital and delay the appearance of such species in the community (Table V). The health care provider must be continually alert to the appearance of antibiotic resistance within the hospital and community. A better understanding of the mechanisms of action and mechanisms of resistance will permit us to avoid some of the problems of resistance which have occurred in the past four decades. But the advances in health care delivery will make it impossible to avoid bacterial resistance completely. In many community situations, as noted elsewhere in this issue, most bacteria are susceptible to many antibiotics. Hospitals dealing with patients with markedly deranged immunological and white cell defenses or with patients who are immunologically depressed by chemotherapy will develop infection due to resistant bacteria. Stopping use of antibiotics is not the answer, but better hygenic practices will keep the problem controlled.

SUMMARY

Over the past 45 years a large number of antimicrobial agents have been found in nature or synthesized. Antibacterial agents inhibit cell wall formation, disrupt cytoplasmic membrane function, prevent DNA synthesis, interfere with protein synthesis and halt folate synthesis. Resistance to antibiotics has been due to three major mechanisms: prevention of the antibacterial agent from reaching its receptor site, production of altered targets or destruction or modification of the agents. Bacterial resistance has been on the basis of chromosomal changes or the presence of plasmids and transposons. Resistance to beta-lactams is the result of beta-lactamases, the production of altered penicillin-binding proteins and due to altered cell wall permeability. Important examples of these resistance forms occur in staphylococci with altered penicillin-binding proteins and in *Enterobacter, Pseudomonas, Serratia,* which produce chromosomally mediated beta-lactamases. Resistance to aminoglycosides is due to enzymes that acetylate, adenylate, or phosphorylate the compounds causing poor binding to ribosomes. Tetracycline resistance is due to plasmids which cause efflux of the agent from the cytoplasm. Macrolide resistance is the result of an altered 23S ribosomal component of the 50S ribosome. Sulfonomide and trimethoprim resistance is due to production of altered synthetase and reductase enzymes in the folate cycle. Although resistance is a major problem, production of molecularly modified compounds, beta-lactamase inhibitors, or novel agents has provided compounds that provide agents to treat highly resistant bacteria.

REFERENCES

1. Tipper, D.J. and Wright, A.: The Structure and Biosynthesis of Bacterial Cell Walls. In: *The Bacteria,* Sokatch, J.R. and Ornstein, L.A., editors. New York, Academic, 1979, pp. 291-426.
2. Kahan, F.M., Kahan, J.S., Cassidy, P.J., and Kropp, H.: The mechanism of action of fosfomycin. *Ann. N.Y. Acad. Sci. 235:*364-86, 1974.
3. Neuhaus, F.C. Carpenter, C.V., Lambert, M.P., and Wargel, R.J.: D-Cycloserine as a Tool in Studying the Enzymes in the Alavine Branch of Peptidoglycan Synthesis. In: *Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes,* Manoz E., Ferrandiz, F., and Vasquez, D.,

editors. Amsterdam, Elsevier, 1972, pp. 339-87.
4. Stewart, G. and Strominger, J.L.: Bacitracin, an inhibitor of the dephosphorylation of lipid pyrophosphate, an intermediate in biosynthesis of the peptidoglycan of bacterial cell walls. *Proc. Nat. Acad. Sci. 57:*767, 1967.
5. Waxman, D.J. and Strominger, J.L.: B-Lactam Antibiotics: Biochemical Modes of Action. In: *Chemistry and Biology of B-Lactam Antibiotics,* Morin, R.B. and Gorman, M., editors. New York, Academic, 1982, pp. 210-86.
6. Spratt, B.G.: Biochemical and genetical approaches to the mechanism of action

of penicillin. *Philos. Trans. R. Soc. Lond.* (Biol.) *289*: 273-83, 1980.

7. Perkins, H.R. and Nieto, M.: The chemical basis for the action of the vancomycin group in antibiotics. *Ann. N. Y. Acad. Sci. 235*:348, 1974.

8. Storm, D.R., Rosenthal, K.S., and Swanson, P.E.: Polymyxin and related antibiotics. *Ann. Rev. Biochem. 46*:723, 1977.

9. Wehrli, W.: Rifampin: mechanisms of action and resistance. *Rev. Inf. Dis. 5*:407-11, 1983.

10. Wolfson, J.S. and Hooper, D.C.: The fluoroquinolones: structures, mechanisms of action, resistance, and spectra of activity in vitro. *Antimicrob. Agents Chemother. 28*:581-86, 1985.

11. Chrystal, E.J.T., Koch, R.L., McLafferty, A., and Goldman, P.: Relationship between metronidozole metabolism and bactericidal activity. *Antimicrob. Agents Chemother. 18*:556, 1980.

12. Brimacombe, R., Stoffler, G., and Wittman, H.G.: Ribosome structure. *Ann. Rev. Biochem. 47*:217, 1978.

13. Misumi, M., Nishimura, T., Komai, T., and Tanaka, N.: Interaction of kanamycin and related antibiotics with the large subunit of ribosomes and the inhibition of translocation. *Biochem. Biophys. Res. Commun. 84*:358-63, 1978.

14. Tritton, T.R.: Ribosome-tetracycline interactions. *Biochemistry 16*:4133, 1977.

15. Peska, S.: Antibiotics as probes of ribosome structure: binding of chloramphenicol and erythromycin to polyribosomes; effect of other antibiotics. *Antimicrob. Agents Chemother. 5*:255, 1974.

16. Hitchings, G.H.: Mechanisms of action of trimethoprim-sulfamethoxazole-1. *J. Infect. Dis. 128*:S433, 1973.

17. Neu, H.C.: Changing mechnisms of bacterial resistance. *Am. J. of Med. 77*:11-23, 1984.

18. Levy, S.B.: Microbial resistance to antibiotics: An evolving and persistant problem. *Lancet 2*:83-88, 1982.

19. Jacobs, M.R., Koornhof, H.J., Robins-Browne, R.M., et. al.: Emergence of multiply resistant pneumonococci. *N.*

Engl. J. Med. 299:735-40, 1978.

20. Hackenbeck, R., Tarpay, M., and Tomasz, A.: Multiple changes of penicillin-binding proteins in penicillin resistant clinical isolates of Streptococcus pneumoniae. *Antimicrob. Agents Chemother. 17*:364-68, 1980.

21. Williamson, R., Hackenbeck, R., and Tomasz, A.: In vivo interaction of beta-lactam antibiotics with penicillin-binding proteins of Streptococcus pneumoniae. *Antimicrob. Agents Chemother. 18*:629-37, 1980.

22. Percheson, P.B. and Bryan, L.E.: Penicillin-binding components of penicillin-susceptible and resistant strains of Streptococcus pneumoniae. *Antimicrob. Agents Chemother. 18*:390-96, 1980.

23. Hayes, M.V. et. al.: Decreased affinity of a penicillin-binding protein for beta-lactam antibiotics in a clinical isolate of Staphylococcus aureus resistant to methicillin. *FEMS Microbiol. Lett. 10*:119, 1981.

24. Yokota, T.: Methicillin and cephem-resistant Staphylococcus aureus. *Infect. Inflam. Immunol. 14*:87, 1984.

25. Chambers, H.F. et. al.: Increased amounts of a novel penicillin-binding protein in a strain of methicillin-resistant Staphylococcus aureus exposed to nafcillin. *J. Clin. Invest. 76*:325, 1985.

26. Ubukata, K. et. al.: Occurrence of a beta-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. *Antimicrob. Agents Chemother. 27*:851, 1985.

27. Rossi, L. et. al.: Regulation of penicillin-binding protein activity: description of methicillin-inducible penicillin-binding protein in Staphylococcus aureus. *Antimicrob. Agents Chemother. 27*:828, 1985.

28. Fontana, R., et. al.: Identification of a streptococcal penicillin-binding protein that reacts very slowly with penicillin. *J. Bacteriol. 155*:1343, 1983.

29. *Williamson, R., et. al.: One or two low affinity penicillin-binding proteins may be responsible for the range of susceptibility of Enterococcus faecium to benzylpenicillin. J. Gen. Microbiol.*

*131:*1933, 1985.

30. Dougherty, T.J., Koeller, A.E., Tomasz, A.: Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant Neisseria gonorrhoeae. Antimicrob Agents Chemother 1980; 18:730-737.

31. Godfrey, A.J. and Bryan, L.E.: Mutation of Pseudomonas aeruginosa specifying reduced affinity for penicillin to antimicrobial agents. *Antimicrob Agents Chemother. 21:*218-23, 1982.

32. Godfrey, A.J., Bryan, L.E., and Rabin, H.: Beta-lactam-resistant Pseudomonas aeruginosa with modified penicillin-binding proteins emerging during cystic fibrosis treatment. *Antimicrob. Agents Chemother. 19:*705-11, 1981.

33. Lai, C.J. and Weisbaum, B.: Altered methytation of ribosomal RNA in an erythromycin-resistant strain of Staphylococcus aureus. *Proc. Nat. Acad. Sci. USA 68:*856-60, 1971.

34. Saito, T., Hashimoto, H., and Mitsuhashi, S.: Drug resistance of staphylococci. Decrease in formation of erythromycin-ribosomes complex in erythromycin-resistant bacteria. *Jap. J. Microbiol. 13:*119-21, 1969.

35. Lai, C.J., Weisblum, B., Fahrestock, R., and Nomura, M.: Alteration of 23s ribosomal RNA and erythromycin-induced resistant to lincomycin and spiramycin in Staphylococcus aureus. *J. Mol. Biol. 74:*67-72, 1973.

36. Allen, N.E.: Macrolide resistance in Staphylococcus aureus inducers of macrolide resistance. *Antimicrob. Agents Chemother. 11:*669-74, 1977.

37. Clewell, D.B.: Plasmids, drug resistance and gene transfer in the genus streptococcus. *Microbiol. Rev. 45:*409-36, 1981.

38. Horinouchi, S., Byeon, W.H., and Weisblum, B.: A complex attenuator regulates inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics in streptococcus sanguis. *J. Bacteriol. 154:*1252-62, 1983.

39. Engle, H.W., Soedirman, N., Rost, J.A., et. al.: Transferability of macrolide, lincoamide and streptogramin resistances between group A, B and D

streptococci, Streptococcus pneumoniae and Staphylococcus aureus. *J. Bacteriol. 142:*407-13, 1980.

40. Beaty, H.N.: Rifampin and minocycline in meningococcal disease. *Rev. Infect. Dis. 5:*451-58, 1983.

41. Pato, M.L. and Brown, G.M.: Mechanisms of resistance of E. coli to sulfonamides. *Arch. Biochem. Biophys. 103:*443-48, 1963.

42. Skold, 0.: R-factor mediated resistance to sulfonamides by a plasmid-borne, drug-resistant dihydropteroate synthetase. *Antimicrob. Agents Chemother. 9:*49-54, 1976.

43. Amyes, S.G.B., and Smith, J.T.: R-factor trimethoprim resistance mechanism: an insusceptible target site. *Biochem. Biophys. Res. Commun. 58:*412-18, 1974.

44. Datta, N., Nugent, M., and Richards, H.: Transposons encoding trimethoprim and gentamicin resistance in medically important bacteria. *Cold Spring Harbor Symp. Quant. Biol. 45:*45-52, 1981.

45. Towner, K.J., Venning, B.M., and Pinn, P.A.: Occurrence of transposable trimethoprim resistance in clinical isolates of Escherichia coli devoid of self-transmissible resistance plasmids. *Antimicrob. Agents Chemother. 21:*336-38, 1982.

46. Murray, B.E., Rensimen, E.R., and duPont, H.L.: Emergence of high-level trimethoprim resistance in fecal Escherichia coli during oral administration of trimethoprim-sulfamethoxazole. *N. Engl. J. Med. 306:*130-35, 1982.

47. Gellert, M., Mizunchi, K., O'Dea, M.H., et. al.: Malidixic acid resistance a second genetic character involved in DNA gyrase activity. *Proc. Nat. Acad. Sci. USA 74:*4772-76, 1977.

48. McMurry, L. and Levy, S.B.: Two transport systems for tetracycline in Escherichia coli: critical role for an initial rapid uptake system insensitive to energy inhibitors. *Antimicrob. Agents Chemother. 14:*201-09, 1978.

49. Levy, S.B.: The Tetracyclines: Microbial Sensitivity and Resistance. In: *New Trends in Antibiotics: Research and Therapy,* Grassi G.G. and Sabath, L.D.,

editors. Amsterdam, Elseviere North Holland, 1981; 27-44.

50. Ball, P.R. Shales, S.W., and Chopra, I.: Plasmid mediated tetracycline resistance involves increased efflux of the antibiotic. *Biochem. Biophys. Res. Commun.* 93:74-81, 1980.

51. Chopra, I. and Eccles, S.J.: Diffusion of tetracycline across the other membrane of Escherichia coli K 12 involvement of protein la. *Biochem. Biophys. Res. Commun.* 83:550-57, 1978.

52. Medonza, M.C., Garcia, J.M., Lianeza, J., et. al.: Plasmid determined resistance to fosphomycin in Serratia marcescens. *Antimicrob. Agents Chemother.* 18:215-19, 1980.

53. Hancock, R.E.: Aminoglycosides uptake and mode of action, with special reference to stereptomycin and gentamicin, I. Antagonists and mutants. *J. Antimicrob. Chemother.* 8:249-76, 1981.

54. Bryan, L.E., van Den Elzen, H.M.: Effects of membrane energy mutations and cations of streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin in susceptible and resistant bacteria. *Antimicrob. Agents Chemother.* 12:163-77, 1977.

55. Shannon, K. and Phillips, I.: Mechanisms of resistance to aminoglycosides in clinical isolates. *J. Antimicrob. Chemother.* 9:91-102, 1982.

56. Bryan, L.E., Koward, S.K., and van Den Elzen, H.M.: Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: Clostridium perfringens and Bacteroides fragilis. *Antimicrob. Agents Chemother.* 15:7-13, 1979.

57. Miller, M.H., Edberg, S.C., Mandel, L.J., et. al.: Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of Staphylococcus aureus. *Antimicrob. Agents Chemother.* 18:722-29, 1980.

58. Bryan, I.E., Haraphonge, R., and van Den Elzen, H.M.: Gentamicin resistance in clinical isolates of Pseudomonas aeruginosa associated with diminished gentamicin accumulation and no detectable enzymatic modification. *J. Antibiot.* 29:743-53, 1976.

59. Shaw, W.V.: Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria. *Methods Enzymol.* 43:737-55, 1975.

60. Kirby, W.M.M.: Extraction of highly potent penicillin inactivator from penicillin-resistant staphylococci. *Science* 99:452-53, 1944.

61. Richmond, M.H. and Sykes, R.B.: The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. *Adv. Microb. Physiol.* 9:31-88, 1973.

62. Sykes, R.B., Matthew, M.: The beta-lactamases of gram-negative bacteria and their role in resistance to beta-lactam antibiotics. *J. Antimicrob. Chemother.* 2:115-57, 1976.

63. Hamilton-Miller, J.M.T.: Beta-lactamases and their clinical significance. *J. Antimicrob. Chemother.* 9:11-19, 1982.

64. Datta, N. and Kontomichalou, P.: Penicillinase synthesis controlled by infectious R-factors in Enterobacteriaceae. *Nature* 208:239-41, 1965.

65. Matthew, M.: Plasmid-mediated beta-lactamases of gram-negative bacteria: properties and distribution. *J. Antimicrob. Chemother.* 5:349-58, 1979.

66. Simpson, I.N., Harper, P.B., and O'Callaghan, C.H.: Principal beta-lactamases responsible for resistance to beta-lactam antibiotics in urinary tract infections. *Antimicrob. Agents Chemother.* 17:926-36, 1980.

67. Roy, C., Fox, A., Segura, C., et. al.: Plasmid-determined beta-lactamases identified in a group of 204 ampicillin-resistant enterobacteriaceae. *J. Gen. Microbiol.* 12:507-10, 1983.