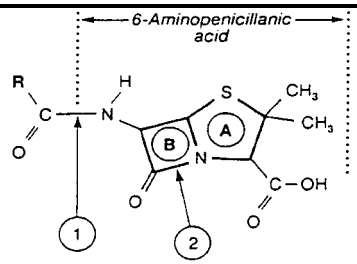
Alice Prince

PENICILLINS

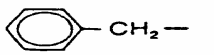
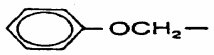
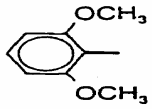
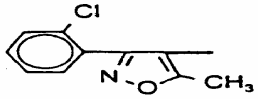
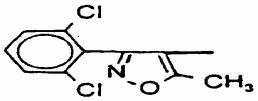
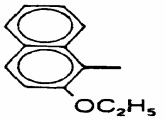
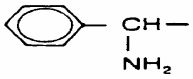
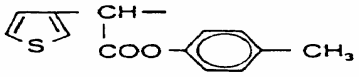
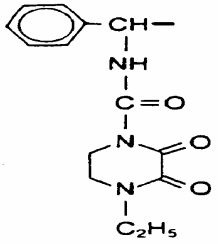
I. CHEMISTRY

A basic structure of penicillins consists of a nucleus with three components: a thiazolidine ring, a β-lactam ring and a side chain. The side chain determines, in large part, the antibacterial spectrum and pharmacologic properties.

STRUCTURE OF COMMONLY USED PENICILLINS



Structure of penicillin nucleus: *A* = thiazolidine ring; *B* = beta-lactam ring. Sites of penicillinase action: (1) = amidase; (2) = beta-lactamase.

R SIDE CHAIN	CHEMICAL NAME	NONPROPRIETARY NAME
	Benzyl penicillin	Penicillin G
	Phenoxymethyl penicillin	Penicillin V
	Dimethoxyphenyl penicillin	Methicillin
	5-Methyl-3-*o*-chloro-phenyl-4-isoxazolyl penicillin	Cloxacillin
	5-Methyl-3-(2,6-dichloro-phenyl)-4-isoxazolyl penicillin	Dicloxacillin
	2-Ethoxy-1-naphthyl penicillin	Nafcillin
	α-Aminobenzyl penicillin	Ampicillin
	α-*p*-Cresylcarbonyl-3-thienylmethyl penicillin	Ticarcillin
	α-[(4-Ethyl-2,3-dioxo-1-piperazinyl)-carbonyl-amino] benzyl penicillin	Piperacillin

Chemical structures of R side chains and names of various penicillins.

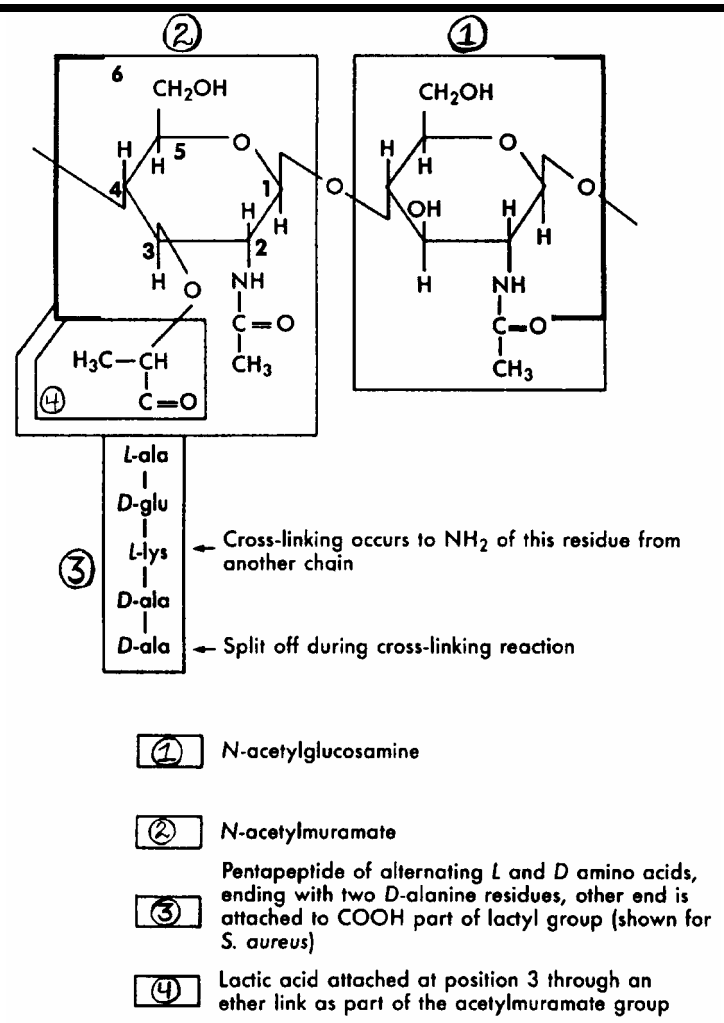
From Kalant and Roschlau, Principles of Medical Pharmacology. B.C. Decker Inc. 5th edition, 1989.

II. MECHANISMS OF ACTION

The penicillins cause the lysis of growing bacteria. They bind to the enzymes involved in the biosynthesis of the bacterial cell wall. Since eukaryotes do not have cell walls, this is a particularly convenient and safe target for antimicrobial chemotherapy. Thus, the β-lactam antibiotics, as a group, are the most widely prescribed antibiotics.

Penicillins bind to a number of receptor proteins, transpeptidases and carboxypeptidases called penicillin binding proteins (PBPs). Different microorganisms vary in the affinity of their PBPs for penicillin. In addition, some organisms, particularly gram-positive bacteria, are able to mutate their PBPs to provide targets with significantly less affinity (resistance) for penicillin binding. Some of the PBPs are essential, and are present in low amounts. Other PBPs are not essential, and thus, are less desirable targets for antibiotics.

Differences in the "activity" - that is, the amount of a particular penicillin needed to kill an organism - are also related to the ability of the penicillin to go through the outer wall of a bacterium. This usually depends on charge properties of the molecule and the affinity of that penicillin for PBPs involved in cell wall biosynthesis.



Repeating glycan portion of peptidoglycan matrix (shown as n units), consisting of the disaccharide *N*-acetylmuramate plus *N*-acetylglucosamine connected through b-1, 4-link, and with the lactyl and pentapeptide attached as shown.

III. BACTERIAL RESISTANCE TO PENICILLINS

A. β-lactamase production

Even before there was widespread use of the penicillins, microbiologists had found organisms which were able to destroy the antibiotic. The best understood mechanism of resistance is the production of β-lactamases. These enzymes cleave the β-lactam ring of the drug, which comprises the binding site to the PBP, effectively inactivating it.

1. Gram-positive organisms

In bacteria such as Staphylococcus aureus, β-lactamase production is generally constitutive and may be plasmid- or chromosomally-mediated. The enzyme is excreted into the milieu around the cell, thus, large amounts of enzyme are produced. Most of these enzymes are penicillinases, and a cephalosporin drug, with a somewhat different structure to the β-lactam ring, is usually stable to these enzymes. However, more recently, Staphylococci have been isolated which produce large amounts of β-lactamases which can hydrolyze some cephalosporins as well as penicillins.

To overcome the problem of β-lactamase producing Staphylococci, side chains were added to the penicillin nucleus to STERICALLY inhibit the binding of the enzyme. These drugs include oxacillin, methicillin, nafcillin, cloxacillin, and dicloxacillin.

2. Gram-negative organisms

a. Chromosomal β-lactamases

Many gram negative organisms such as Pseudomonas aeruginosa, Citrobacter species and Enterobacter, have inducible chromosomal β-lactamases. The enzymes are strategically located in the periplasmic space, thus, molecules of a β-lactam drug must first traverse the outer cell wall via a porin, cross the periplasmic space and find a receptor, a penicillin-binding protein. Low level expression of these enzymes is constitutive and contributes to resistance to many β-lactam antibiotics. Mutations in the regulatory genes can cause de-repression of the enzymes and high level expression.

b. Plasmid-mediated enzymes

Gram-negative organisms may also constitutively express plasmid-mediated β-lactamases. The plasmid encoded enzymes are often encoded by transposons and are usually penicillinases. As the expression of β-lactamases is quite common, there are many genes which are involved, and thus, many potential sites for mutation. Plasmid-mediated cephalosporinases have been reported which were unheard of a few years ago.

The accumulation of multiple individual mutations, in the presence of selective pressure, allows for the stable maintenance of broad spectrum β-lactamases. As shown, the first well-characterized penicillinase was the plasmid-encoded "TEM-1" enzyme which had activity against penicillin and ampicillin. TEM-24 and TEM-26 have broad spectrum activity against both penicillins and cephalosporins.

Some of these TEM-mutants can escape the inhibition of the commonly used β-lactamase inhibitors sulbactam and clavulanic acid.

The amino acid changes and their locations within the protein sequence identified in the development of extended-spectrum β-lactamases.

β-lactamase	Amino acid at position							
	39	104	164	205	237	238	240	265
TEM-1*	Gln	Glu	Arg	Gln	Ala	Gly	Glu	Thr
TEM-2*	Lys							
TEM-13*	Lys						Met	
TEM-3	Lys	Lys				Ser		
TEM-4		Lys				Ser	Met	
TEM-5			Ser			Thr	Lys	
TEM-6		Lys	His					
TEM-7	Lys		Ser					
TEM-8	Lys	Lys	Ser			Ser		
TEM-9		Lys	Ser				Met	
TEM-10			Ser				Lys	
TEM-11		Lys		His			?	
TEM-12			Ser					
TEM-14		Lys	Lys				Ser	Met
TEM-15			Lys				Ser	
TEM-16		Lys	Lys	His				
TEM-17			Lys					
TEM-18		Lys	Lys					
TEM-19							Ser	
TEM-24		Lys	Lys	Ser		Thr	Lys	
TEM-26			Lys	Ser				
SHV-1*	Gln	Asp	Arg	Arg	Ala	Gly	Glu	Leu
SHV-2					Ser			
SHV-3			Leu		Ser			
SHV-4			Leu		Ser	Lys		
SHV-5					Ser	Lys		

* These are parental types, lacking extended-spectrum activity.

c. Strategies to deal with β-lactamase producing gram-negative bacteria have included :

i. The development of cephalosporins which are stable to the plasmid-mediated β-lactamases.

ii. The addition of β-lactamase inhibitors (sulbactam and clavulanic acid) which have high affinity for the plasmid-mediated enzymes, sopping them up while enabling the antibiotic to reach its target:

ampicillin + sulbactam => Unasyn
amoxicillin + clavulanate => Augmentin
ticarcillin + clavulanate => Timentin
piperacillin + tazobactam => Zosyn

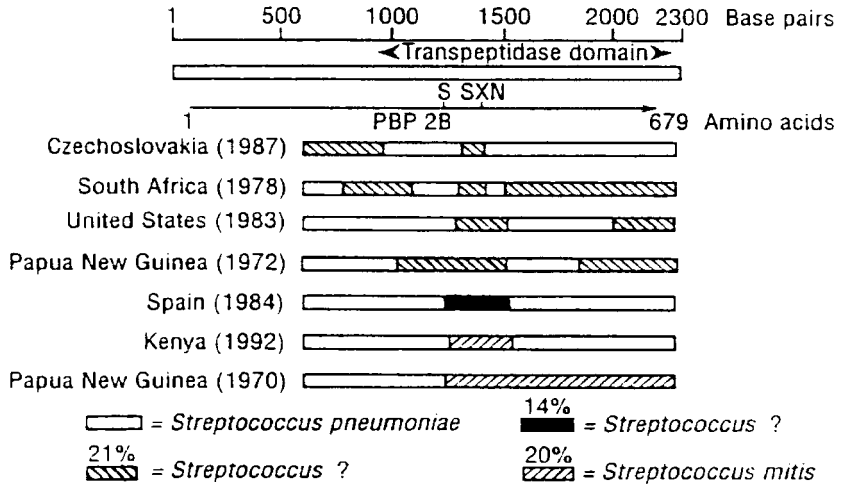
(Note: These drugs would also have good activity against gram-positive organisms.)

iii. The development of similar drugs which do not have β-lactam rings, and are, thus, inherently resistant to these enzymes.

B. Resistance may also due be due to failure of the drug to reach a receptor site. This is mainly a problem in gram-negative bacteria, where the permeability through the porin channels may be altered. In addition, some gram-negatives, particularly Pseudomonas aeruginosa, can express EFFLUX pumps which actually pump the drug out of the periplasm, preventing it from reaching the target sites.

C. Resistance can also develop due to the alteration of the targets. Mutants which synthesize altered PBPs which do not bind penicillins are selected from the population. This is a major problem in gram-positive organisms.

In S. pneumoniae there are increasing numbers of penicillin-resistant isolates. These organisms spontaneously take up DNA and can incorporate sequences from related species into their genomes by homologous recombination. As shown below, acquisition of DNA into the locus coding for one of the major transpeptidases, PBP 2B results in the expression of an enzyme which has reduced affinity for penicillin and results in clinical penicillin resistance.



Mosaic PBP 2B genes in penicillin-resistant pneumococci. The divergent regions in the PBP 2B genes of seven resistant pneumococci from different countries are shown. These regions have been introduced from at least three sources, one of which appears to be S. mitis. The approximate percent sequence divergence of the divergent regions from the PBP 2B genes of susceptible pneumococci is shown.

IV. GENERAL PHARMACOLOGIC PROPERTIES OF ALL PENICILLINS

A. Absorption

1. Several penicillins have been developed for oral use including phenoxymethylpenicillin, amoxicillin (a derivative of ampicillin which has increased absorption), the anti-staphylococcal drugs dicloxacillin, cloxacillin, and the combinations of amoxicillin and clavulanic acid.
2. Most penicillins that are orally absorbed yield peak serum levels 1-2 hours after ingestion.
3. Repository forms of penicillin G, but not of other agents, are available. These forms are absorbed more slowly from intramuscular sites (benzathine penicillin).
4. Penicillins are bound to serum proteins in varying degree, from 17% for ampicillin to 97% for dicloxacillin. Only free drug exerts its antibacterial activity.

B. Metabolism

Penicillins are metabolized only to a minor degree, but this may affect serum half-life in renal failure since drugs will be minimally metabolized and will accumulate.

C. Excretion

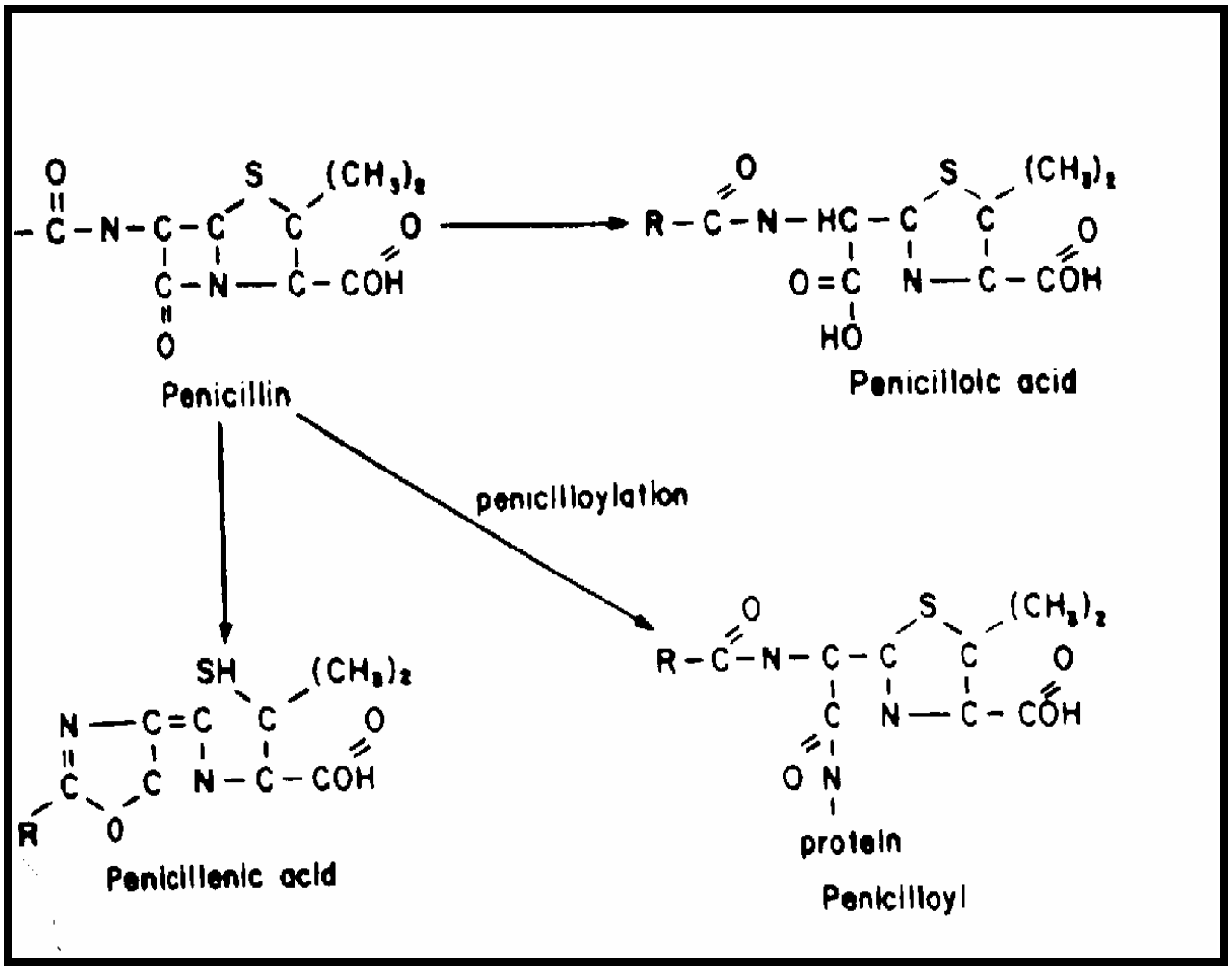
1. The major mechanism of excretion of all penicillins is as intact molecules via the kidney. Most excretion is via tubular secretion. The rate and amount of excretion by this mechanism varies for each agent.
2. Probenecid blocks tubular secretion of penicillins.
3. Penicillins accumulate in the body only in the presence of markedly reduced renal function, i.e. newborns, individuals with Ccr<30 ml/min.
4. Biliary excretion of all penicillins occurs, but is important only for nafcillin and the ureidopenicillins.

D. Distribution

1. Penicillins are well distributed to lung, liver, kidney, muscle, bone and placenta.
2. Penicillins do not penetrate cells, including white cells.
3. Urinary concentrations of all penicillins are high except when renal function is markedly reduced, Ccr<10ml/min.
4. Concentration of penicillins in bile are in excess of those in serum, but are reduced in presence of common duct obstruction.

E. Untoward reactions to penicillins

Major adverse effect of the penicillins are hypersensitivity reactions, which range from rash to immediate anaphylaxis. Penicillins act as haptens to combine with human proteins. There are a number of antigenic components. The major, or penicilloyl, determinant is produced by the cleavage of the β-lactam ring, thus allowing an amide linkage to the body proteins. The major determinants, however, are less important in causing anaphylactic and accelerated reactions than are the minor determinants, i.e., benzylpenicilloate.


Penicillin
Penicilloic acid
penicilloylation
Penicillenic acid
protein
Penicilloyl

PENICILLIN INDUCED HYPERSENSITIVITY REACTIONS

Type of Reaction	Responsible Antibody Class	Responsible Antigen
Immediate (2-30 minutes)		
urticaria, laryngeal edema, hypotension, shock	IgE	Minor determinants BP (rarely)
Accelerated Urticarial	IgE	BP*
urticaria, pruritis, wheezing		Minor determinants
Late reactions, 72 h		
morbilliform eruption,	IgM	BP
urticarial, Arthus-like	IgG	BP
Immune hemolysis	IgM	BP
Serum sickness, vasculitis	IgG	BP

*BP = benzylpenicilloyl (major determinants)

F. Determination of allergy

1. Skin testing with benzylpenicilloyl-polylysine (PrePen) will detect major determinants, and decayed benzylpenicillin can be used to detect minor determinants. Development of wheal and flare reaction to intradermal injection of the two reagents predicts that the patient is highly likely to have an immediate reaction. Negative skin tests do not mean that late reactions, i.e., rash, will not occur.

2. Penicillins should not be given to patients who have had immediate reactions. Desensitization by repeated administration of increasing doses can be used, but alternative agents should be sought.

V. INDIVIDUAL PENICILLINS

A. **Penicillin G**

This is the prototypic penicillin with activity against a variety of organisms such as the streptococci, treponemes, meningococci. As pen G is NOT β-lactamase stable, and organisms are developing altered targets (PBPs), it is less likely to remain the drug of first choice for many infections.

1. Administration
   a. Is usually given parenterally: Available as Na^+ or K^+ salts for IV, IM use.
   b. Repository form - Procaine penicillin G - delayed absorption, thus levels persist for 12 hrs.
      Benzathine penicillin - long-lasting depot preparation, so levels persist for 12-28 days.
   c. Phenoxymethyl penicillin (Penicillin V) - Oral substitute for penicillin G.

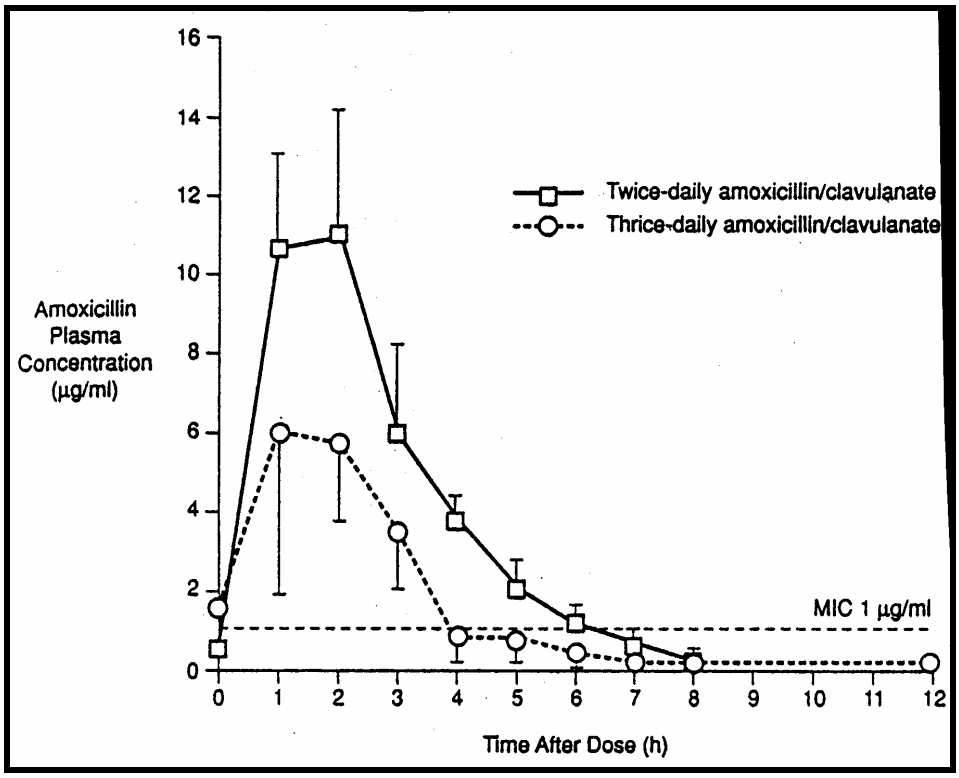
B. Anti-staphylococcal penicillins

1. Used primarily against staphylococci, but they inhibit hemolytic streptococci, pneumococci but not enterococci. Their bulky side chains inhibit the action of staphylococcal β-lactamases, but their size prevents them from getting through the porins of gram-negative bacteria.
2. Available agents include:
   a. Methicillin - least protein bound of this group
   b. Nafcillin - high biliary excretion
   c. Isoxazolyl penicillins
      i. parenteral - oxacillin
      ii. oral - cloxacillin and dicloxacillin
      These drugs differ in their oral absorption and protein binding.

C. Aminopenicillins

1. Ampicillin
   a. Spectrum - gram positive organisms - streptococci, enterococci (unless they express β-lactamases), gram negative organisms such as Hemophilus species, E. coli, Salmonella, and Shigella. Note that many of the gram negative organisms express plasmid mediated β-lactamases and have become resistant to ampicillin limiting its use.
   b. Oral, IV use.
   c. Rash - common.

2. Amoxicillin
   a. Oral form of ampicillin with increased absorption. Well absorbed in the presence of food - can be given on a bid or tid schedule.
      Better absorption - less effective against lumenal pathogens such as Shigella.
   b. Pharmacology



Overall mean (±SD) steady state plasma concentration-time curve for amoxicillin after oral suspension administration of 22.5 mg/kg twice daily or 13.3 mg/kg three times daily. Data obtained from four children between the ages of 2 and 12 years in each dose group.

D. Broad-spectrum penicillins

1. Ticarcillin (+ clavulanic acid (Timentin))
   Ampicillin derivative with increased activity against many gram negative organisms given WITH a β-lactamases inhibitor - clavulanic acid (see below).
   a. Parenteral - high dosage required for Pseudomonas aeruginosa, activity against Bacteroides, Proteus.
   b. Longer half life - accumulation in renal failure
   c. Adverse effects - same as all penicillins -- rash, hypersensitivity. Binds to ADP site on platelets, produces hypokalemia (Na-salt).
   d. Broad spectrum gram positive (including Staphylococci - with clavulanate) gram negative, and anaerobic activity.

2. Piperacillin (+ tazobactam (Zosyn))
   a. Structure
      This is the R group on the penicillin nucleus seen in Figure 1.
   b. Parenteral ureido derivative of ampicillin. Not stable to beta-lactamases, more active than other penicillins against *Pseudomonas aeruginosa* and inhibits 50% klebsiella.
   c. Nonlinear pharmacokinetics, kidney and liver excretion
   d. Most active against pseudomonas
   e. Combined with beta-lactamase inhibitor => Zosyn (pip and tazobactam)