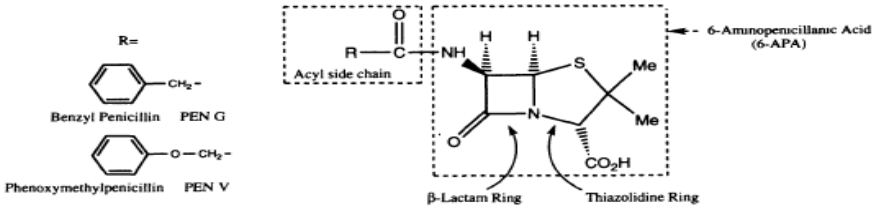
Antibacterial agents which inhibit cell wall synthesis

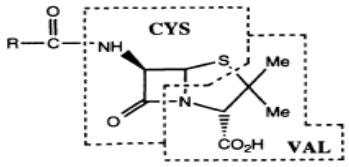
There are two major classes penicillins and cephalosporins.

Penicillins

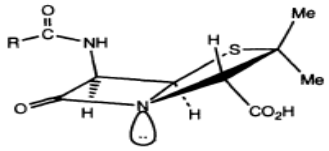
Penicillin contains a highly unstable-looking bicyclic system consisting of a four membered (**β-lactam ring**) fused to a five-membered **thiazolidine ring**.



The structure of penicillin.



Penicillin appears to be derived from cysteine and valine.



Shape of penicillin.

Properties of penicillin G (the first penicillin to be isolated)

1- Active versus Gram-positive bacilli (e.g. *staphylococci*, *meningitis*, and *gonorrhoea*) and many (but not all) Gram-negative cocci.

2- Non-toxic**:** penicillins are amongst the safest drugs known to medicine.

3- Not active over a wide range (or spectrum) of bacteria.

4- Ineffective when taken **orally** since it breaks down in the acid conditions of the stomach. Penicillin G can only be administered by **injection**.

5- Sensitive to all known (***β*-lactamases**). These are enzymes produced by penicillin resistant bacteria which catalyze the degradation of penicillins.

6- Allergic reactions are suffered by some individuals.

Clearly, there are several problems associated with the use of penicillin G :

A. The acid sensitivity of penicillins

There are three reasons for the acid sensitivity of penicillin G :

1-Ring strain.

The bicyclic system in penicillin consists of a four-membered ring and a five membered ring. As a result, penicillin suffers large angle and torsional strains. Acid-catalyzed ring opening relieves these strains by breaking open the more highly strained four-membered lactam ring.

Ring opening.

2-A highly reactive (β-lactam carbonyl group).

The carbonyl group in the (β -lactam ring) is highly susceptible to nucleophiles and as such does not behave like a normal tertiary amide which is usually quite resistant to nucleophilic attack. This difference in reactivity is due mainly to the fact that stabilization of the carbonyl is possible in the tertiary amide, but impossible in the (β -lactam ring).

3-Influence of the acyl side-chain (neighboring group participation).

The neighboring acyl group can actively participate in a mechanism to open up the lactam ring. Thus, penicillin G has a self-destruct mechanism built into its structure.

Solving the problem of acid sensitivity

Nothing can be done about the first two factors since the β-lactam ring is vital for antibacterial activity. Without it, the molecule has no useful biological activity at all.

Therefore, only the third factor can be solved. The task then becomes one of reducing the amount of neighboring group participation to make it difficult, if not impossible, for the acyl carbonyl group to attack the β-lactam ring.

Penicillin V has an electronegative oxygen on the acyl side-chain with the electron withdrawing effect required. **The molecule has better acid stability than penicillin G and is stable enough to survive the acid in the stomach.** *Thus, it can be given orally*. However, Penicillin V is still sensitive to **penicillinases** and is slightly less active than penicillin G.

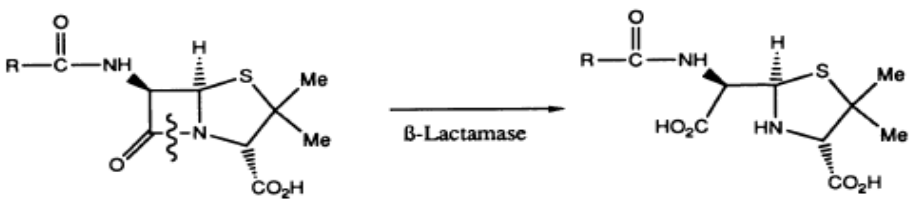
A range of penicillin analogues which have been very successful are penicillins which are disubstituted on the alpha-carbon next to the carbonyl group. As long as one of the groups is electron withdrawing, *these compounds are more resistant to acid hydrolysis and can be given orally* (e.g. **ampicillin** and **oxacillin**).

To conclude, the problem of acid sensitivity is fairly easily solved by having an electron withdrawing group on the acyl side-chain.

B. Penicillin sensitivity to (β-lactamases)

β-Lactamases are enzymes produced by penicillin-resistant bacteria. The same ring opening and deactivation of penicillin which occurred with acid hydrolysis.

The problem of β-lactamases became critical in 1960 when the widespread use of penicillin G led to an alarming increase of *Staph. aureus* infections. These problem strains had gained the lactamase enzyme and had thus gained resistance to the drug.



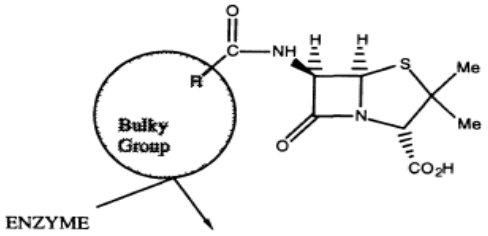
β-Lactamase deactivation of penicillin.

Solving the problem of β-lactamase sensitivity

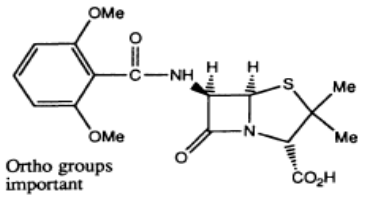
The strategy is to block the penicillin from reaching the penicillinase active site. One way of doing that is to place **a bulky group** on the side-chain. This bulky group can then act as a '**shield**' to ward off the penicillinase and therefore prevent binding.

Methicillin was the first semisynthetic penicillin unaffected by penicillinase and was developed just in time to treat the *Staph. aureus* problem already mentioned.

However, methicillin is by no means an ideal drug. **Since there is no electron withdrawing group on the side-chain, it is acid sensitive**, and so has ***to be injected***.



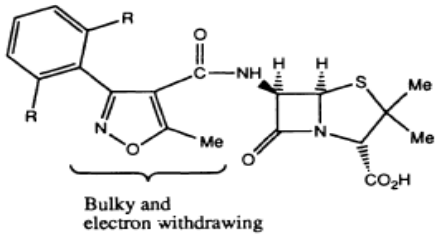
Blocking penicillin from reaching the penicillinase active site.



Methicillin.

These compounds (**oxacillin**, **cloxacillin**, and **flucloxacillin**) are acid-resistant and penicillinase-resistant, and are also useful against *Staph. aureus* infections.

The only difference between the above three compounds is **the type of halogen substitution** on the aromatic ring. Cloxacillin is better absorbed through the gut wall than oxacillin, whereas flucloxacillin is less bound to plasma protein, resulting in higher levels of the free drug in the blood supply.



OXACILLIN R = R' = H
CLOXACILLIN R = Cl, R' = H
FLUCLOXACILLIN R = Cl, R' = F

Incorporation of a five-membered heterocycle.

C.Narrow spectrum of activity

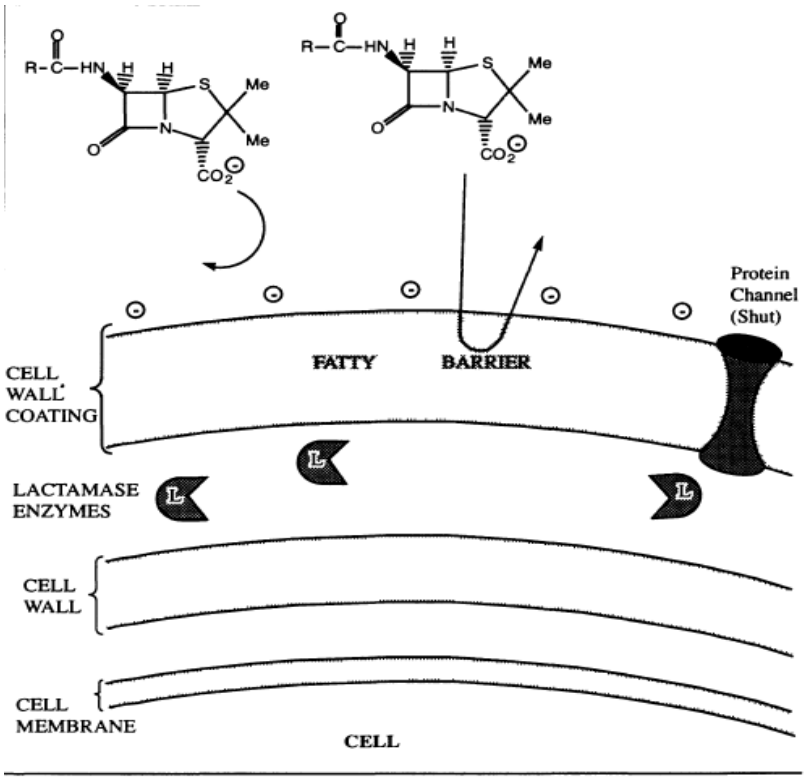
One problem has cropped up in everything described so far; most penicillins show a poor activity against Gram-negative bacteria. There are several reasons for this resistance:

1- Permeability barrier.

The outer surface may have an overall negative or positive charge depending on its constituent **triglycerides**. An excess of **phosphatidylglycerol** would result in an overall **anionic** charge whereas an excess of **lysylphosphatidylglycerol** would result in an overall **cation** charge. Penicillin has a free carboxylic acid which if ionized would be repelled by the former type of cell membrane.

Alternatively, the fatty portion of the coating may act as a barrier to the polar hydrophilic penicillin molecule.

The only way in which penicillin can negotiate such a barrier is through protein. channels in the outer coating. Unfortunately, most of these are usually closed.



Permeability barrier of a Gram-negative bacterial cell.

2- High levels of transpeptidase enzyme produced.

The **transpeptidase** enzyme is the enzyme attacked by penicillin. In some gram negative bacteria, a lot of transpeptidase enzyme is produced, and the penicillin is incapable of inactivating all the enzyme molecules present.

3- Modification of the transpeptidase enzyme.

A mutation may occur which allows the bacterium to produce a transpeptidase enzyme which is not antagonized by penicillin.

4- Presence of β-lactamase.

We have already seen that (β-lactamases) are enzymes which degrade penicillin. They are situated between the cell wall and its outer coating.

5- Transfer of the (β-lactamase enzyme).

Bacteria can transfer small portions of DNA from one cell to another through structures called **plasmids**. These are small pieces of circular bacterial DNA. If the transferred DNA contains the code for the (β-lactamase enzyme), then the recipient cell acquires immunity.

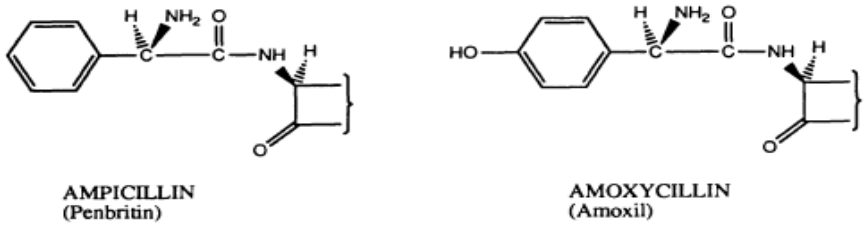
Solving the problem of narrow activity spectrum

Enhancement of Gram-negative activity is found to be greatest **if the hydrophilic group (e.g. NH_2 , OH, CO_2H) is attached to the carbon, alpha to the carbonyl group on the side-chain.**

Those penicillins having useful activity against both Gram-positive and Gram negative bacteria are known as **broad-spectrum antibiotics**. There are two classes of broad-spectrum antibiotics. Both have an **alpha-hydrophilic group**. However, in one class the hydrophilic group is an **amino function** as in **ampicillin** or **amoxicillin**, while in the other the hydrophilic group is an **acid group** as in **carbenicillin**.

Class I broad-spectrum antibiotics—ampicillin and amoxicillin.

Ampicillin is the second most used penicillin in medical practice. Amoxicillin differs merely in having a **phenolic group**. It has similar properties, **but is better absorbed through the gut wall**.



AMPICILLIN (Penbritin)

AMOXYCILLIN (Amoxil)

Class I broad spectrum antibiotics.

Properties:

• Active versus Gram-positive bacteria and against those Gram-negative bacteria which do not produce penicillinase.

• Acid-resistant **due to the NH_2 group**, and is **therefore orally active**.

• Non-toxic.

• Sensitive to penicillinase (no 'shield').

• Inactive against *Pseudomonas aeruginosa* (a particularly resistant species).

• Can cause diarrhea due to poor absorption through the gut wall leading to disruption of gut flora.

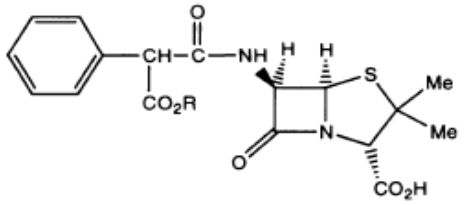
The last problem of poor absorption through the gut wall is due to the dipolar nature of the molecule since it has both a free amino group and a free carboxylic acid function. This problem can be alleviated by using a prodrug where one of the polar groups is masked with a protecting group. This group is removed metabolically once the prodrug has been absorbed through the gut wall.

Class II broad-spectrum antibiotics—carbenicillin

In general, **carbenicillin** is used against penicillin-resistant Gram-negative bacteria. The broad activity against Gram-negative bacteria is **due to the hydrophilic acid group** (ionized at pH 7) **on the side-chain**. It is

particularly interesting to note that the stereochemistry of this group is important. The alpha-carbon is chiral and only one of the two enantiomers is active. This implies that the acid group is involved in some sort of binding interaction with the target enzyme.

Carfecillin is the prodrug for carbenicillin and shows an improved absorption through the gut wall.



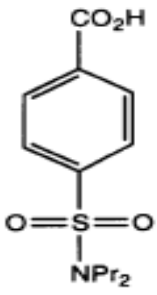
R = H CARBENICILLIN

R = Ph CARFECILLIN

Synergism of penicillins with other drugs

There are several examples in medicinal chemistry where the presence of one drug enhances the activity of another. In many cases this can be dangerous, leading to an effective overdose of the enhanced drug. In some cases it can be useful. There are two interesting examples whereby the activity of penicillin has been enhanced by the presence of another drug.

One of these is the effect of **clavulanic acid**, The other is the administration of penicillins with a compound called **probenecid**. Probenicid slows down the rate at which penicillin is excreted by competing with it in the excretion mechanism. As a result, penicillin levels in the bloodstream are enhanced and the antibacterial activity increases—a useful tactic if faced with a particularly resistant bacterium.



Probenicid.