Penicillin
Historical view:

Fleming in 1928 observed that a mold contaminant in a culture plate containing Staphylococcus aureus had caused the dissolution of some adjacent colonies. He isolated the mold in a pure culture; studied the antibacterial properties of broth cultures and the crude extract and concluded as a result of his investigations, that the antibacterial substance, which he named penicillin after the genus of the mold, was relatively nontoxic to experimental animals, to leucocytes and to conjunctiva of the human eye.
• Reid (1935) reported the antibacterial activity of broth filtrates that contained penicillin.

• In (1938) Florey and his associates undertook an extensive study of antibiotics at Sir William Dunn School of Pathology at Oxford University. Penicillin was obtained as a brown powder in fairly good condition and used for investigations on animals and human beings. The results of these researches were reported by Chain and his associates (1940) and by Abraham and his colleagues (1941).
Properties of Penicillin:

- Penicillin is active against Gm+ve bacteria and certain Gm-ve bacteria in the presence of blood, pus and body fluids.
- It is nontoxic.
- In a small percentage of cases, individuals allergic to penicillin are found, urticaria, hives and itching are some of the responses observed in sensitive persons.
- There is partial inhibition of phagocytosis by penicillin.
- Soluble in water, acetone, amyl-acetate, cyclohexane, dioxane, ethyl acetate, ethyl alcohol
• Less soluble in benzene, chloroform and carbon-tetra-chloride.
• Decomposed by strong acids and alkalies.
• Inactive in oxidizing agents.
• Contact with certain heavy metals such as copper, silver, lead, mercury lead to destroy it.
• Zinc salts may inactivate penicillin.
• The basic structure of penicillin is destroyed by methanol, by sulfhydryl compounds, by heat and by repeated freezing and thawing.
• Penicillin is destroyed by penicillinase, an enzyme secreted by a large number of bacteria, molds and yeasts.
• The crystalline salts of penicillin are stable over a period of several months.
• Aqueous solutions of penicillin are unstable and must be stored under refrigeration conditions.
• Penicillin is most stable in the pH range of 6- 6.5.
• The half lives of penicillins F, G, K and X are 11, 18.5, 7 and 11 minutes, respectively, at pH 2 and 24°C.
General formula:

- \( C_9H_{11}O_4SN-R \)
- \( S \)
- \( R-CONH—CH—CH \quad C—(CH_3)_2 \)
- \( O=C—N—CH—COOH \)
- \( R: \) represents the radical or group that is different for each type.

Most penicillins are a mixture of F, G, and K types, but penicillin G is produced in the largest quantities and considered to be the most practical one. Crystalline sodium penicillin G must contain at least 90% of G.
Sources:

- Penicillin F, G, X and K are produced by strains of *P. Chrysogenum* strains.
- Flavicidin (flavicin) produced by *Aspergillus flavus* strains.
- Dihydro F penicillin (gigantic acid) produced by *Aspergillus giganteus*.
## Chemical Structure of Some Sodium Penicillins

<table>
<thead>
<tr>
<th>Type (American)</th>
<th>British designation</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin F (delta-penteyl-penicillin)</td>
<td>I</td>
<td><img src="image" alt="Structure of Penicillin F" /></td>
</tr>
<tr>
<td>Penicillin G (benzyl-penicillin)</td>
<td>II</td>
<td><img src="image" alt="Structure of Penicillin G" /></td>
</tr>
<tr>
<td>Penicillin X (p-hydroxy-benzyl-penicillin)</td>
<td>Penicillin K (n-heptyl-penicillin)</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>III</strong></td>
<td><strong>IV</strong></td>
<td></td>
</tr>
</tbody>
</table>

**III**

$$C_6H_5—CH_2—CONH—CH—CH \quad C—(CH_3)_2$$

$$| \quad | \quad |$$

$$OH \quad O=C——N—— \quad CH—COONa$$

**IV**

$$CH_3—(CH_2)_5—CH_2—CONH—CH—CH \quad C—(CH_3)_2$$

$$| \quad | \quad |$$

$$O=C——N——CH—COONa$$
<table>
<thead>
<tr>
<th>Type (American)</th>
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<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydro F Gigantic acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

```
S
/ \ 
CH₃—(CH₂)₃—CH₂—CONH—CH—CH C—(CH₃)₂
|   |   |
O=C—N—CH—COONa
```

| Flavicidin | | 

```
S
/ \ 
CH₃—CH=CH—CH₂—CH₂—CONH—CH—CH C—(CH₃)₂
|   |   |
O=C—N—CH—COONa
```
Penicillin Production:

Two methods can be used for cultivation of P. chrysogenum, submerged and surface methods.
# Medium Composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn extract</td>
<td>2g/L</td>
</tr>
<tr>
<td>Lactose</td>
<td>30g</td>
</tr>
<tr>
<td>Glucose</td>
<td>30g</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>3g</td>
</tr>
<tr>
<td>KHPO$_4$</td>
<td>0.5g</td>
</tr>
<tr>
<td>MgSO$_4$. 7H$_2$O</td>
<td>0.25g</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>0.0045g</td>
</tr>
<tr>
<td>MnSO$_4$</td>
<td>0.00045g</td>
</tr>
</tbody>
</table>
Sterilization for 20 min. at 1.5 pound/mm$^2$. In industry large containers with very thousands gallons of medium are used. The fungus inoculums must be used as 5% as starter. Fermentation method is achieved at 4 days. Most penicillin is secreted in the broth as exo-product.
Penicillin Extraction:

After incubation period, the mycelia mat was separated by filtration or by centrifugation.

The mycelia mat was washing several times, and then this washing is added to filtrate.

Two methods can be used for extraction of penicillin from filtrate.
By Ethyl acetate:-

- The filtrate mixed with ethyl acetate at pH 2-2.5 using phosphoric acid.
- Shake well at low temperature, then penicillin transfer into solvent layer.
- Separate the solvent containing penicillin using separating funnels.
- Mixed the solvent with distilled water at pH 7-7.5, then penicillin transferred into aqueous layer in sodium salt after addition of sodium bicarbonate.
- Separate the ethyl acetate from aqueous layer.
- To obtain pure penicillin, repeat using different solvents under low temperature.
- Dry the drug under low temperature and pressure.
By Charcoal:-

• Mixed the filtrate with charcoal 2-2.5%wt, then penicillin adsorbed on charcoal surface.
• Separate the drug by filtration, then washed by distilled water.
• Penicillin separated from charcoal by adding solution 80% acetone and 20% distilled water.
• Another solvent is added must be homogenous with acetone and not with water.
• Then, sodium bicarbonate solution is added, penicillin is separated in sodium salt form.
• Dry under low temperature and low pressure
Uses of Penicillin:

• In overcoming many bacterial diseases such as diseases caused by Staphylococcus and Streptococcus.
• Also, anthrax and tuberculosis, and in curing diphtheria and syphilis diseases. Also, it is used after surgical operations to avoid microbial contamination.