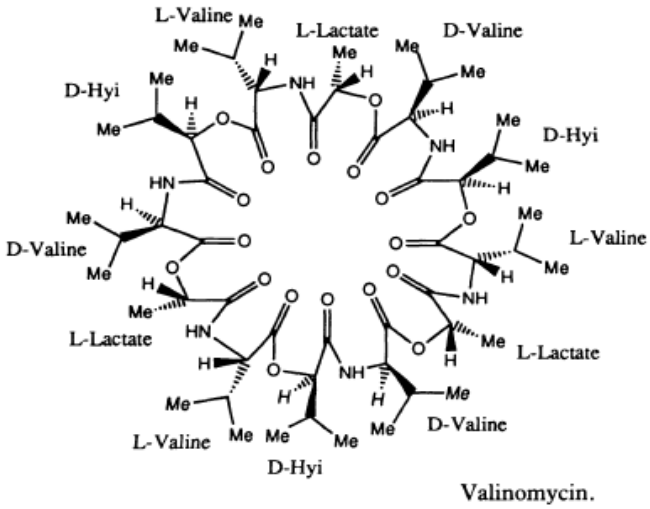
Antibacterial agents which act on the plasma membrane structure

The peptides V**alinomycin** and **Gramicidin A** both act as **ion conducting antibiotics and allow the uncontrolled movement of ions across the cell membrane**. Unfortunately, both these agents *show no selective toxicity for bacterial over mammalian cells* and are **therefore useless as therapeutic agents**.

Valinomycin is a cyclic structure containing three molecules of **L-valine**, three molecules of **D-valine**, three molecules of **L-lactic acid**, and three molecules of **D-hydroxyisovalerate**. These four components are linked in an ordered fashion such that there is an alternating sequence of ester and amide linking bonds around the cyclic structure. This is achieved by the presence of a lactic or hydroxyvaleric acid unit between each of the six valine units. Further ordering can be observed by noting that the L and D portions of valine alternate around the cycle, as do the lactate and hydroxyisovalerate units.



Valinomycin.

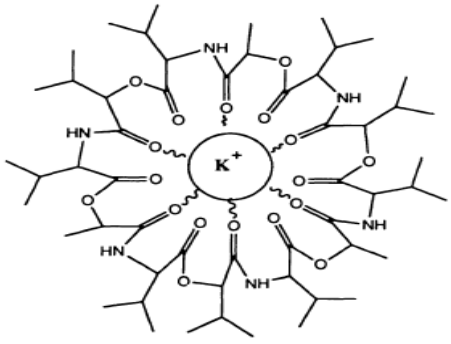
Valinomycin acts as an **ion carrier** and in some ways could be looked upon as an inverted detergent. Since it is cyclic, it forms a doughnut-type structure where the polar carbonyl oxygens of the ester and amide groups face inside, while the hydrophobic side-chains of the valine and

hydroxyisovalerate units point outwards. This is clearly favored since the hydrophobic side-chains can interact via van der Waals forces with the fatty lipid interior of the cell membrane, while the polar hydrophilic groups are clustered together in the Centre of the doughnut to produce a hydrophilic environment.

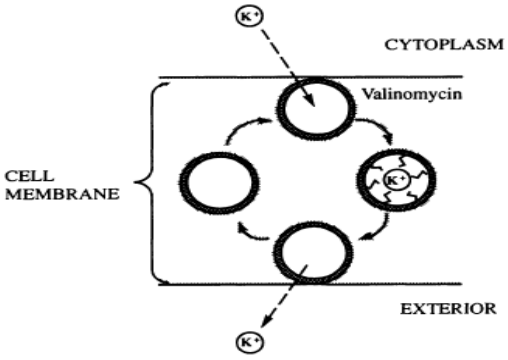
This hydrophilic centre is large enough to accommodate an ion and it is found that a 'naked' potassium ion (i.e. no surrounding water molecules) fits the space and is complexed by the amide carboxyl groups.

Valinomycin can therefore 'collect' a potassium ion from the inner surface of the membrane, carry it across the membrane and deposit it outside the cell, thus disrupting the ionic equilibrium of the cell. **Normally**, cells have a high concentration of potassium and a low concentration of sodium. The fatty cell membrane prevents passage of ions between the cell and its environment, and ions can only pass through the cell membrane aided by specialized and controlled ion transport systems. Valinomycin introduces an uncontrolled ion transport system which proves fatal.

Valinomycin is specific for potassium ions over sodium ions. *One might be tempted to think that sodium ions would be too small to be properly complexed. However, the real reason is that sodium ions do not lose their surrounding water 'coat' very easily and would have to be transported as the hydrated ion. As such, they are too big for the central cavity of valinomycin.*



Potassium ion in the hydrophilic centre of valinomycin.

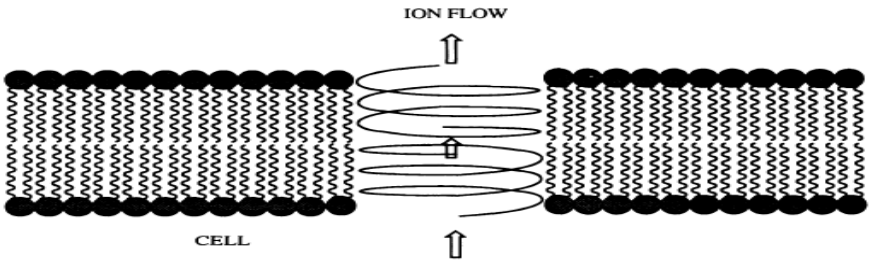


Valinomycin disrupts the ionic equilibrium of a cell.

Gramicidin A is a peptide containing 15 amino acids which is thought to coil into a helix such that the outside of the helix is hydrophobic and interacts with the membrane lipids, while the inside of the helix contains hydrophilic groups, thus allowing the passage of ions. Therefore, gramicidin A could be viewed as an escape tunnel through the cell membrane.

Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NH-CH_2-CH_2-OH

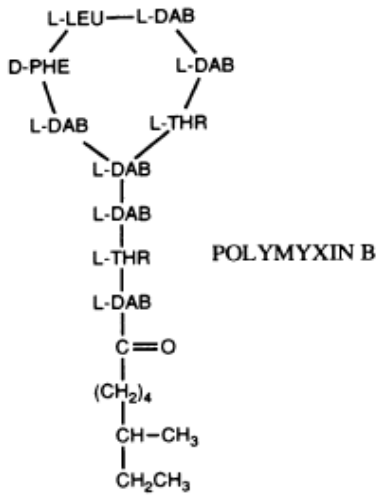
Gramicidin A.



Gramicidin helices aligned end-to-end traversing membrane.

The polypeptide antibiotic **polymyxin B** also operates within the cell membrane. It shows selective toxicity for bacterial cells over animal cells, which appears to be related to the ability of the compound to bind selectively to the different plasma membranes.

Polymyxin B acts like valinomycin, but it causes the leakage of small molecules such as nucleosides from the cell. The drug is injected intramuscularly and is useful against *Pseudomonas* strains which are resistant to other antibacterial agents.



POLYMYXIN B

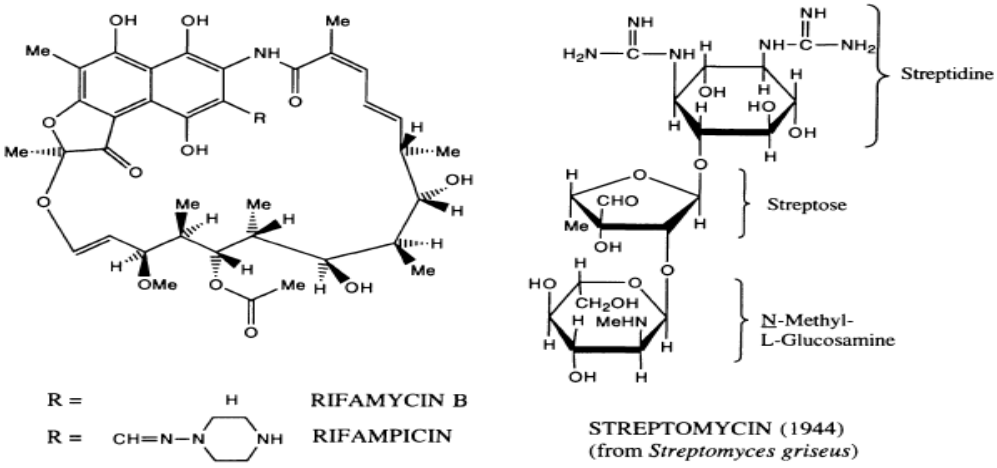
Antibacterial agents which impair protein synthesis

Examples of such agents are the **rifamycins** which act against RNA, and the **aminoglycosides**, **tetracyclines**, and **chloramphenicol** which all act against the ribosomes.

Selective toxicity is due to either different diffusion rates through the cell barriers of different cell types or to a difference between the target enzymes of different cells.

Rifamycins

Rifampicin inhibits Gram-positive bacteria and works by binding non-covalently to **RNA polymerase** and inhibiting RNA synthesis. The DNA-dependent RNA polymerases in eukaryotic cells are unaffected, since the drug binds to a peptide chain not present in the mammalian RNA polymerase. It is therefore highly selective.



Antibacterial agents which impair protein synthesis.

The drug is mainly used in the treatment of **tuberculosis** and **staphylococci infections** that resist penicillin. It is a very useful antibiotic, showing a high degree of selectivity against bacterial cells over mammalian cells. Unfortunately, it is also expensive, which discourages its use against a wider range of infections.

The selectivity of this antibiotic is interesting since both bacterial cells and mammalian cells contain the enzyme RNA polymerase. However, as

we have seen, **the enzyme in bacterial cells contains a peptide chain not present in mammalian RNA polymerase**.

Aminoglycosides

Streptomycin effectives against the lethal disease tuberculous meningitis. The drug works by inhibiting protein synthesis. It binds to the 30 S ribosomal subunit and prevents the growth of the protein chain as well as preventing the recognition of the triplet code on mRNA.

Aminoglycosides are fast acting, but they can also cause ear and kidney problems if the dose levels are not carefully controlled.

Tetracyclines

The tetracyclines as a whole have a broad spectrum of activity and are the most widely prescribed form of antibiotic after penicillins. They are also capable of attacking the malarial parasite.

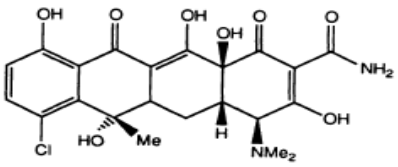
One of the best known tetracyclines is **chlortetracycline** (**Aureomycin**). It is a broad-spectrum antibiotic, active against both Gram-positive and Gram-negative bacteria. Unfortunately, it does have side-effects due to the fact that **it kills the intestinal flora that make vitamin K- a vitamin which is needed as part of the clotting process**.

Chlortetracycline inhibits protein synthesis by binding to the 30 S subunit of ribosomes and prevents the aminoacyl-tRNA binding to the A site on the ribosome. This prevents the codon-anticodon interaction from taking place. Protein release is also inhibited.

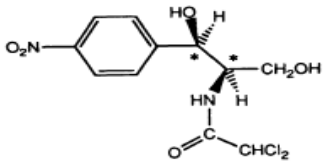
There is no reason why tetracyclines should not attack protein synthesis in mammalian cells as well as in bacterial cells? In fact, they can. Fortunately, bacterial cells accumulate the drug far more efficiently than mammalian cells and are therefore more susceptible

Chloramphenicol

Chloramphenicol was originally isolated from *Streptomyces venezuela.,* but is now prepared synthetically.



Chlortetracyclin (aureomycin).



* Chiral centres

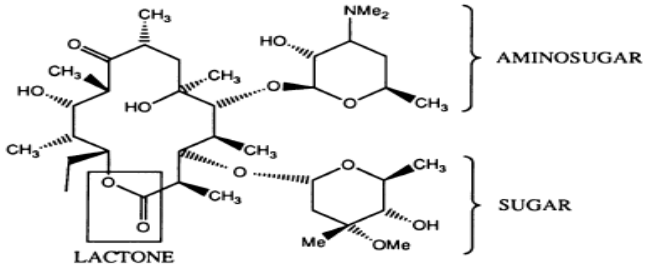
Chloramphenicol (from *Streptomyces venezuela*).

Chloramphenicol binds to the **50 S** subunit of ribosomes and appears to act by inhibiting the movement of ribosomes along mRNA, probably by inhibiting the **peptidyl transferase** reaction by which the peptide chain is extended. Chloramphenicol is the drug of choice against **typhoid** and is also used in severe bacterial infections which are insensitive to other antibacterial agents. It has also found widespread use against **eye infections**.

Macrolides

The best known example of this class of compounds is **erythromycin** - a metabolite produced by the microorganism *Streptomyces erythreus.* The structure consists of a macrocylic lactone ring with a sugar and an aminosugar attached. The sugar residues are important for activity.

Erythromycin acts by binding to the 50 S subunit by an unknown mechanism. It works in the same way as chloramphenicol **by inhibiting translocation**, where the elongated peptide chain attached to tRNA is shifted back from the aminoacyl site to the peptidyl site. Erythromycin was used against penicillin-resistant staphylococci, but newer penicillins are now used for these infections. It is, however, the drug of choice against 'legionnaires disease'.

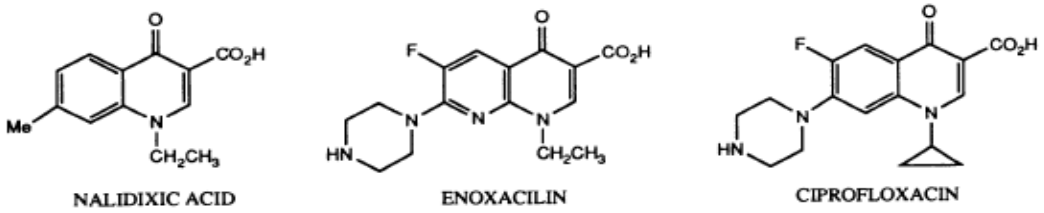


Erythromycin.

Agents which act on nucleic acid transcription and replication

Quinolones and fluoroquinolones

The quinolone and fluoroquinolone antibacterial agents are relatively late arrivals on the antibacterial scene, but are proving to be very useful therapeutic agents. They are particularly useful in **the treatment of urinary tract infections** and also for the treatment of infections which prove resistant to the more established antibacterial agents. In the latter case, microorganisms which have gained resistance to penicillin may have done so by mutations affecting cell wall biosynthesis. Since the quinolones and fluoroquinolones act by a different mechanism, such **mutations** provide no protection against these agents.



Quinolones and fluoroquinolones.

Nalidixic acid was the first therapeutically useful agent in this class of compounds. It is active against Gram-negative bacteria and is useful in the short-term therapy of urinary tract infections. It can be taken **orally**, but unfortunately, bacteria can rapidly develop resistance to it.

Further adjustments led to **ciprofloxacin**, now the agent of choice in treating travellers' **diarrhoea**. It has been used in the treatment of a large range of infections involving the urinary, respiratory, and gastrointestinal tracts as well as infections of skin, bone, and joints. It has been claimed that ciprofloxacin may be the most active broad-spectrum antibacterial agent on the market. Furthermore, bacteria are slow in acquiring resistance to ciprofloxacin, in contrast to nalidixic acid.

The quinolones and fluoroquinolones are thought **to act on the bacterial enzyme deoxyribonucleic acid gyrase (DNA gyrase)**. *This enzyme*

catalysis the supercoiling of chromosomal DNA into its tertiary structure. A consequence of this is that replication and transcription are inhibited and the bacterial cell's genetic code remains unread. At present, the mechanism by which these agents inhibit DNA gyrase is unclear.

Aminoacridines

Aminoacridines such as **proflavine** are topical antibacterial agents which were used in the Second World War for the treatment of surface wounds.

Proflavine.