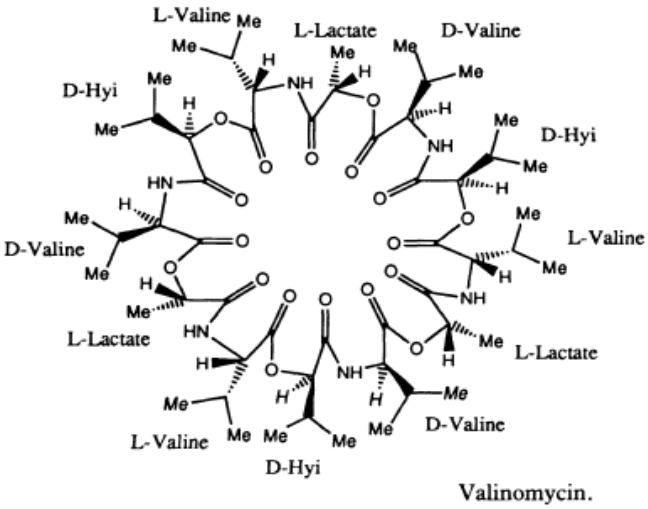
## Antibacterial agents which act on the plasma membrane structure

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.

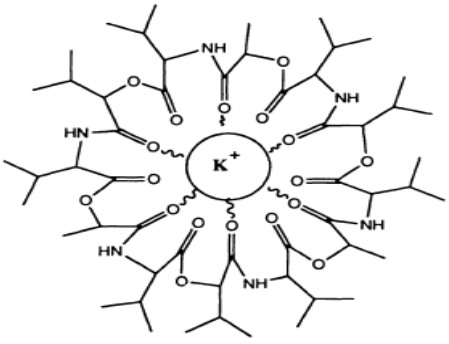
Valinomycin acts as an **ion carrier** and in some ways could be looked upon as an inverted detergent. Since it is cyclic, it forms a doughnut-type structure where the polar carbonyl oxygens of the ester and amide groups face inside, while the hydrophobic side-chains of the valine and

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.

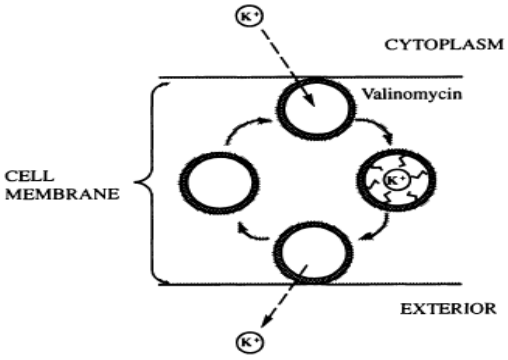
This hydrophilic centre is large enough to accommodate an ion and it is found that a 'naked' potassium ion (i.e. no surrounding water molecules) fits the space and is complexed by the amide carboxyl groups.

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.*

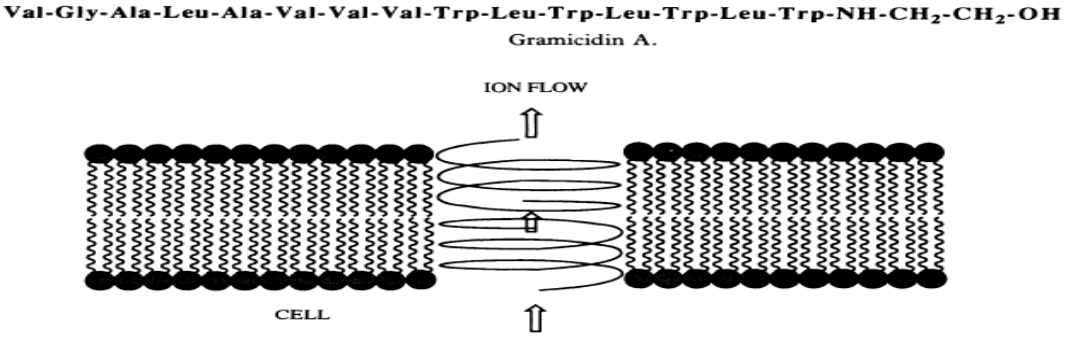



Potassium ion in the hydrophilic centre of valinomycin.



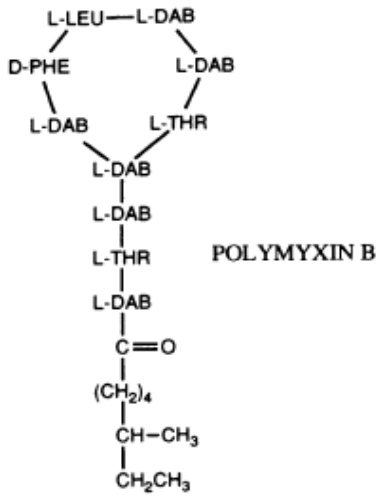

Valinomycin disrupts the ionic equilibrium of a cell.

**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.




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.



## Resistance of membrane-active antibiotics

### *Polymyxins*

Polymyxins are a group of antibiotics which disrupt bacterial cell membranes. Two mechanisms of acquired resistance to the polymyxins have been identified.

Acquired resistance to polymyxins in *E. coli* occurs because of **chromosomal mutations which cause incorporation of aminoethanol and aminocarabinose in lipopolysaccharide (LPS) in place of phosphate groups.** The altered LPS has a decreased ionic charge which results in lowered binding of polymyxin and thus an increase in resistance to this group of antibiotics.

The mechanism of acquired resistance in *Pseudomonas aeruginosa* is different. **Chromosomal mutations result in the increase of a specific outer membrane protein with a concomitant reduction in divalent cations.** Polymyxins bind to the outer membrane at sites normally occupied by divalent cations, and therefore it is thought that a reduction in these sites will lead to decreased binding of the antibiotic with a consequent decreased susceptibility of the cell.

## Multidrug resistance pumps

Acquired low-level resistance to many unrelated antibiotics by efflux has also increased in prominence in recent years. For example, **MAR** (multiple antibiotic resistance) mutants were first described in the early 1990s in *E. coli* and were resistant to low levels of chloramphenicol, tetracyclines, rifampicin, penicillins and quinolones, due to impaired uptake of the antibiotics. **Increased active efflux** of the drugs has been shown to be important in this type of resistance. A number of multidrug resistance pumps (**MDRs**) have been identified and are widespread among bacteria. For example, seven distinct MDRs have been described in *E. coli* alone.

The most common type belongs to a group of proteins involved in membrane translocation. This type of MDR is closely related to **specific efflux proteins** such as that responsible for tetracycline resistance. The origins of MDRs are unknown but a number of factors suggest that they may have arisen by mutations in specific drug efflux pumps causing a loss of specificity. These factors include **the similarity of some MDRs to specific drug efflux pumps such as tetracycline**, and **the high incidence of apparently independent evolution of MDRs.**