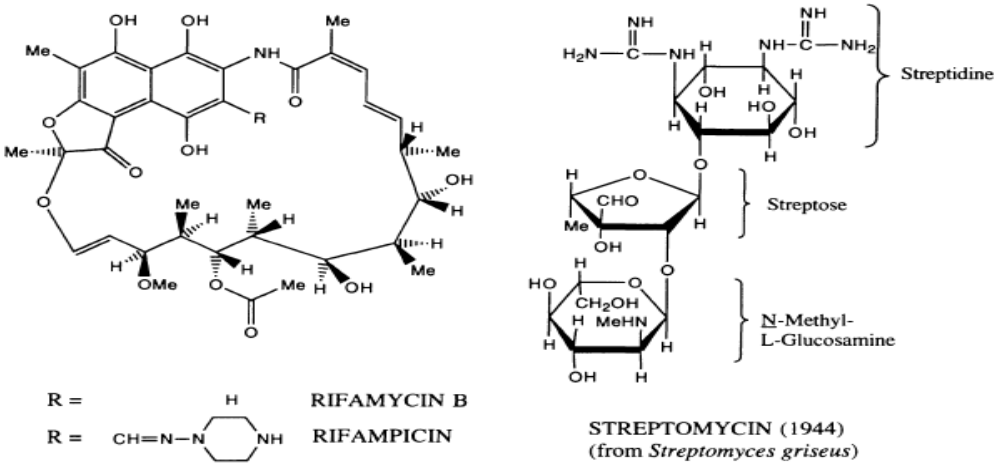
Antibacterial agents which impair protein synthesis

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.

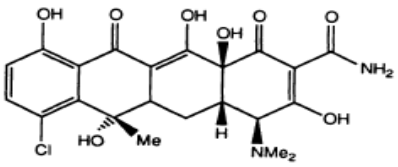
One of the best known tetracyclines is **chlortetracycline** (**Aureomycin**). It is a broad-spectrum antibiotic, active against both Gram-positive and Gram-negative bacteria. Unfortunately, it does have side-effects due to the fact that **it kills the intestinal flora that make vitamin K- a vitamin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

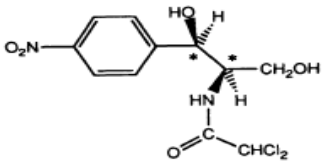
There is no reason why tetracyclines should not attack protein synthesis in mammalian cells as well as in bacterial cells? In fact, they can. Fortunately, bacterial cells accumulate the drug far more efficiently than mammalian cells and are therefore more susceptible

Chloramphenicol

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



Chlortetracyclin (aureomycin).



Chloramphenicol (from *Streptomyces venezuela*).

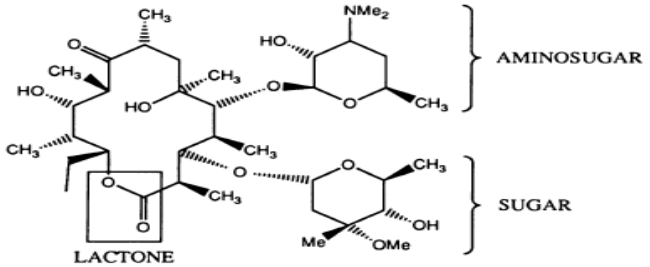
* Chiral centres

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.

Resistance of inhibitors of protein synthesis

Inhibition of protein synthesis is the antibacterial mechanism shared by most groups of antibiotics, though the exact action differs.

***Aminoglycoside-aminocyclitol group*(AGAC)**

Alteration of the antibiotic molecule is plasmid- or transposon-encoded. Three classes of enzyme can alter the AGAC molecule. **Aminoglycoside adenylyltransferases (AADs)** use adenosine triphosphate (ATP) as a cofactor in modifying certain hydroxyl groups in the antibiotic molecule by adenylylating them. **Aminoglycoside phosphotransferases (APH)** also use ATP to modify certain hydroxyl groups by phosphorylating them. **Aminoglycoside acetyltransferases (AACs)** use acetyl CoA as a cofactor and acetylate susceptible amino groups on the molecule. These three classes of enzyme have been further subdivided according to which site on the AGAC molecule is modified. For example, APH(6) phosphorylates the 6-hydroxyl group on the aminohexose group of streptomycin. Most AGAC antibiotics are susceptible to more than one modification reaction. Relatively small amounts of the antibiotic are modified, implying that resistance is determined by the relative rates of drug uptake and modification. A less efficient modifying enzyme will permit unmodified antibiotic to reach its ribosomal quantity. A more efficient enzyme, or greater quantities of the enzyme, will result in resistance.

AGAC-modifying enzymes are active outside the cytoplasmic membrane, in the periplasmic space in Gram-negative bacteria and extracellularly in Gram-positives.

A second mechanism of resistance to the AGACs involves an **alteration of the ribosomal target site**. Mutations in the gene coding for ribosomal protein S12 *(rpsL* in *E. coli)* prevent the antibiotics from binding to their

target. In mycobacteria, which possess only one ribosomal RNA operon, mutations in *rpoB,* coding for 16S rRNA, also inhibit binding of the drugs.

Tetracyclines

Plasmid- or transposon-encoded tetracycline efflux proteins. These efflux proteins are thought to span the cytoplasmic membrane and are dependent on the proton-motive force for their action. It is thought that **the efflux proteins bind tetracyclines and initiate proton transfer**, although no functional domains have been identified. Eight distinct tetracycline efflux proteins have been identified thus far.

Plasmid- or transposon-encoded ribosomal protection factors are a second mechanism of resistance to the tetracyclines. *These proteins are believed to alter the tetracycline binding site on the 30S ribosomal subunit, lowering the affinity for the drugs*.

Chloramphenicol

Plasmid- or transposon-encoded **chloramphenicol acetyltransferases (CATs)** are responsible for resistance by inactivating the antibiotic. **CATs convert chloramphenicol to an acetoxy derivative which fails to bind to the ribosomal target**.

Three other mechanisms of chloramphenicol resistance have been described. **First**, a transposon-encoded chloramphenicol efflux protein has been identified in *E. coli*. **Second**, some bacterial strains have been found to possess drug-resistant ribosomes, and **third**, low level resistance can arise by chromosomal mutations which reduce the amount of porins and therefore impair uptake. This last mechanism is essentially that described for the AGAC antibiotics.

Fusidic acid

Gram-negative bacteria are intrinsically resistant to low levels of fusidic acid (a steroid) **due to exclusion by the outer membrane**. Nevertheless,

acquired resistance does occur which has the effect of increasing the level of resistance to the antibiotic. Acquired resistance also occurs in Gram-positive bacteria normally susceptible to fusidic acid.

Plasmid-mediated resistance in Gram-positive bacteria is thought to **involve decreased uptake of the drug**, although the precise mechanism is unknown. Resistance to fusidic acid in Gram-negative bacteria is also poorly understood.

Chromosomal mutations have also been described which produce a modified translocation factor protein with lowered affinity for fusidic acid.

will become largely ineffective against bacterial infections