Tuberculosis- Part. 8

Drugs used and regimens employed in the treatment of tuberculosis

13. Anti tuberculosis drugs

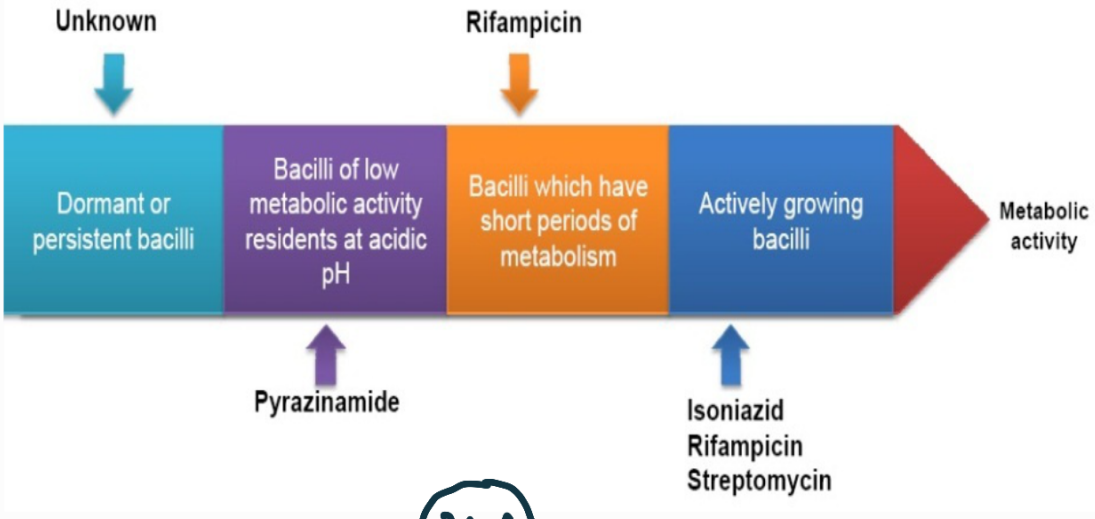
There are a number of drugs and combinations of drugs used in the therapeutic management of tuberculosis. Tuberculous disease caused by drug susceptible M.tuberculosis is called drug susceptible TB and the one caused by resistant bacilli is called drug resistant tuberculosis.

13.1. Are all bacteria in the bacterial population at the site of infection in the same metabolic state?

Ever since 1950s when first anti TB treatment was introduced, not much progress has happened in the treatment of tuberculosis in terms of realizing the objectives both in terms of treatment outcome and public health impact, even though several drugs have been added to the list of anti TB drugs, after an initial gap of 30 years. The objective of tb treatment is not only cure but also prevention of drug resistance. We have

made much headway in clinical cure of drug susceptible TB, but not in bacteriological cure or favourable treatment outcome in drug resistant TB. The drugs in use are bactericidal but poor sterilisers, not able to kill dormant bacilli which petsist in macrophage after the death of active bacilli. The drugs may ensure clinical cure but not bacteriological cure. Cultures of M.tb are sterilised in the culture plates in laboratory in a few days but it requires months to achieve the same result in host tissue. The persistence of bacilli is due to presence of different bacterial populations of different physiological states in the tissues. There is general agreement that we are in need of drugs which can shorten the period of treatment, which are safe, and potent, effective against resistant strains, and dormant or persistent forms and not interfere with anti HIV chemotherapy.

The bacteria at the site of infection are at different metabolic stage- some are active, some are multiplying, some others burst into activity in spurts and yet others are inactive and dormant. In tuberculosis lesions, M tb exists under four

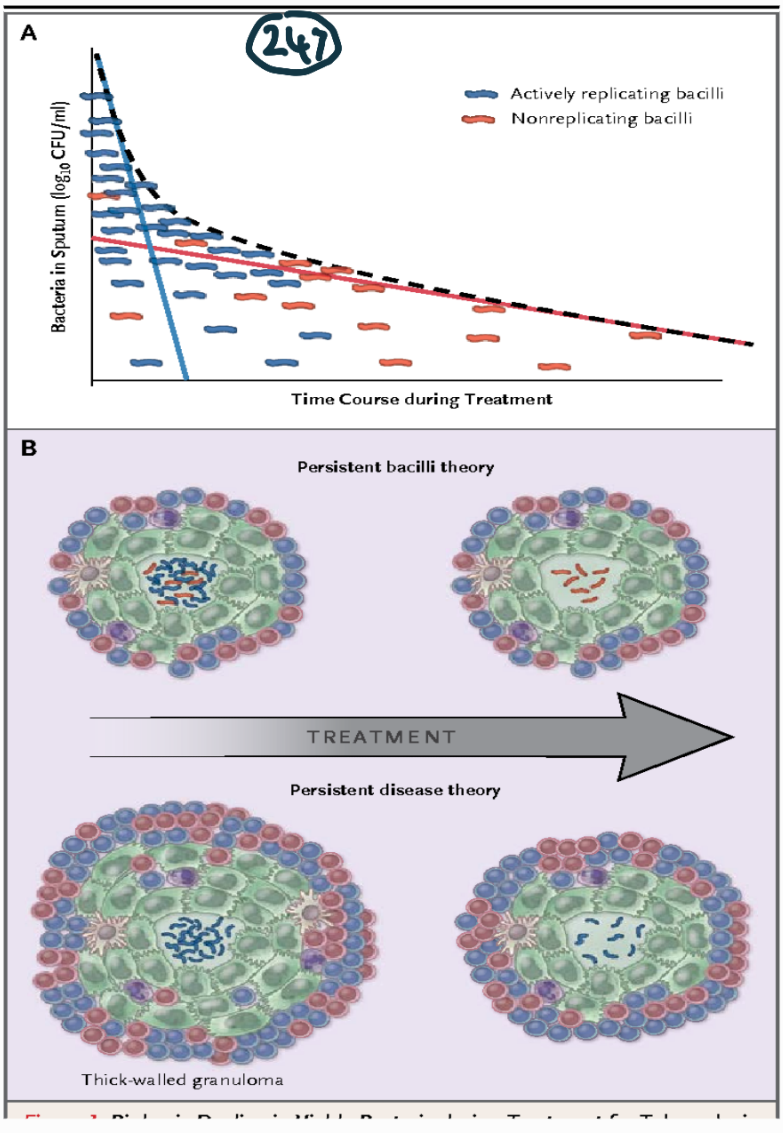


population stages (Fig.246). It would be easy if all are in the same state, is it not? (How can we expect them to be, in any population of living beings including microorganisms and humans?) While there are drugs which target the first 3 groups, there are no drugs which target the persistent or dormant bacilli. During the initial chemotherapy phase (2 months), actively dividing bacilli rapidly die mostly because of INH bactericidal activity. Thereafter bacilli of low metabolic activity suffer from a slow death under the effects of RIF and PZA. There is evidence that persistent bacillary population existing in the lesions usually determines the duration of therapy. The persistent bacilli have learnt the trick of residing in the dangerous hostile environment inside the macrophage because they are metabolically not active, not dividing, not producing any proteins. The enzymes involved in these activities are not active and so drugs cannot act on the quiescent bacilli. They are inside the belly of macrophages which do not sense the intruders.

All the antibiotics act on actively multiplying cells because they produce daughter bacilli which are still developing - cell wall is yet to be formed and proteins are yet to be synthesised- which can be killed easily or produce proteins which can be inhibited by enzymes activated by the antibiotics.

Therefore, efforts need to be made to target every physiological state of *M. tuberculosis* thus shortening the time of therapy and preventing the appearance of drug resistance.

Figure 247. Panel A shows the time course of decline of viable Mycobacterium tuberculosis in a sputum sample from a patient being treated for tuberculosis. The number of bacteria declines at a rapid rate during the early phase of therapy (blue curve), with a less rapid rate of decline during the later phase (red curve). The biphasic (two phases) pattern that is observed (black dashed curve) suggests that there are bacterial subpopulations that differ in their drug susceptibility. CFU denotes colony forming units of bacteria on culture media. Panel B shows two proposed explanations for this differential response: persistent bacilli and persistent disease. The first explanation is that bacteria in a replicating state (blue) are more susceptible to drugs than are bacteria in a nonreplicating or nonmultiplying state (red), which can persist despite drug treatment. The second explanation is that some bacilli are isolated or insulated in thick-walled granulomas, where antibiotics are not able to reach them, resulting in persistent disease.



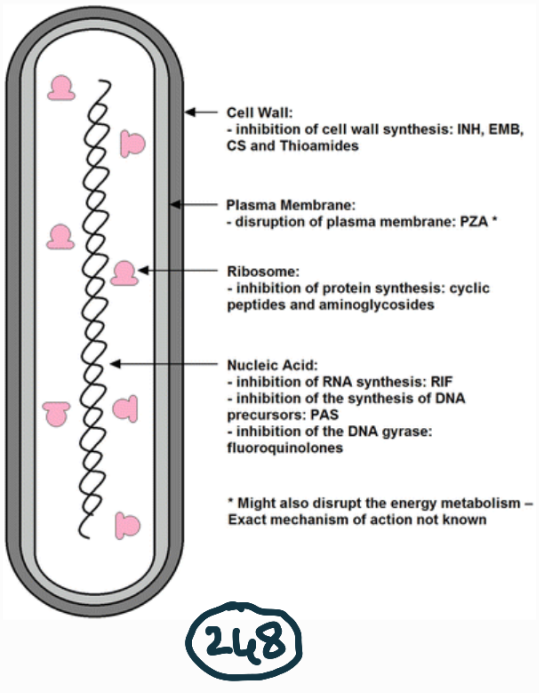
13.2. What are antibiotics?

Antibiotics (*against life)* are chemicals (metabolites) produced by microorganisms or synthesised in lab (sulphonamides and quinolones) and are used to kill or suppress the growth of other microorganisms. The first antibiotic, penicillin, was derived from a mold, *penicilium notatum*. Bacteria produce antibiotics as *secondary metabolites* to defend themselves against other competing bacteria. They are useful against other bacteria, but not against viruses. Bacteria develop resistance to antibiotics rapidly, because they produce these defensive metabolites routinely. For example, streptococcus produce metabolites against staphylococci. If you use this metabolite against streptococcus it would not work.

Bacterial infection can be countered by antibiotic or antibacterial agents. These are classified as either bactericidal, which kills the bacteria, or bacteriostatic, which inhibits any further growth of the bacteria. Generally antibiotics that inhibit the synthesis of cell wall or inhibit respiration of cell are cidal whereas those which inhibit protein synthesis are static. There is a general preference for bactericidal drugs, although evidence suggests bacteriostatic drugs can be equally effective without any of the bactericidal side effects, such as toxic shock syndrome. The type of infection will determine which class of antibiotic to use, or even a

combination of the two. Anti tuberculous drugs are antibiotics derived either from bacteria or synthesised.

13.3. How are anti tuberculous drugs grouped?



Antibiotics / anti TB drugs are grouped (Fig.248)into cell wall inhibitors (INH, EMB, ethionamide (ETH), and cycloserine (DCS); protein synthesis inhibitors(RIF), fluoroquinolones, STR, Kanamycin; and antibiotics which inhibit other metabo,ic processes including membrane energy metabolism (PZA).

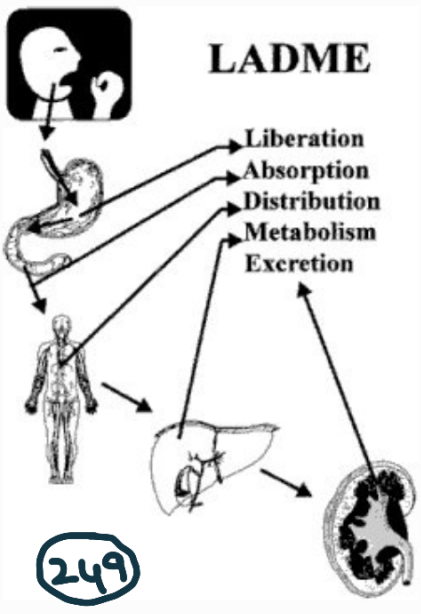
Drugs can also be classified in terms of whether they are used as first line drugs against drug susceptible Mycobacteria or drug resistant bacilli. They can also be categorized based on the class to which they belong.

Before we go into the details of each one of the drugs, let us try to recall some of the basic concepts which we have learned in chapter .. with some additions.

13.4. What makes antibiotics different from one another?

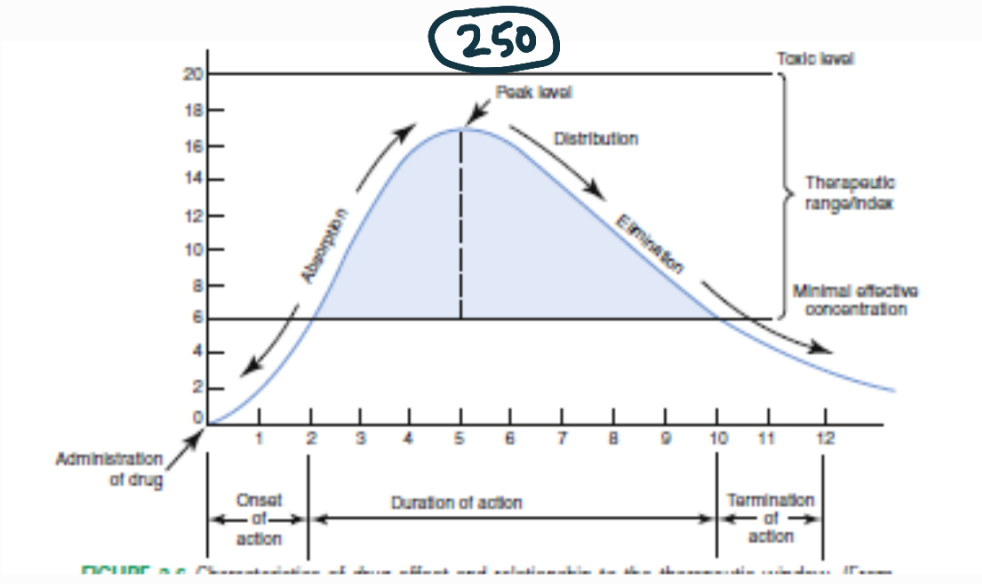
13.4.1.Basic concepts:

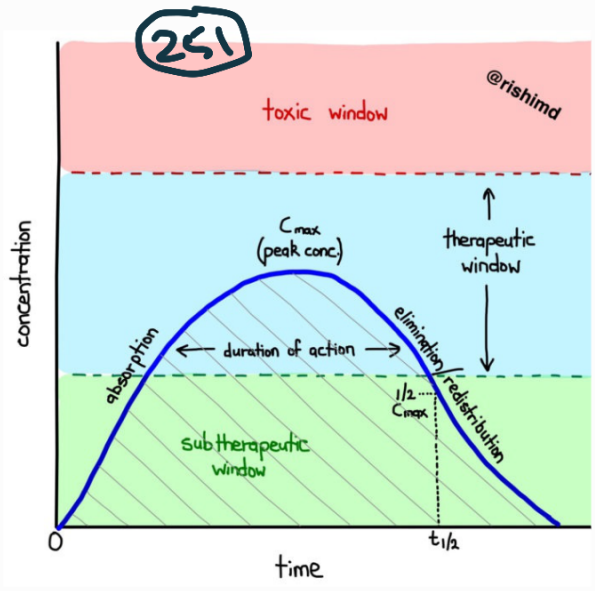
Antibiotics differ in the way they are absorbed, metabolised, transported, eliminated and bring about their biological effect at the target site of infection (Fig.249). These variations give us opportunity to choose approprite antibiotic for specific infections with specific bacteria. Bacteria can be gram positive, gram negative, or acid fast (*depending on whether they look purple or pink/red when sample is mixed with chemicals and observed under microscope*) or anaerobic (*grow under low oxygen conditions*). They are of different shapes and sizes. Antibiotics may work against a narrow or wide range of bacteria. For example, they may act against gram positive or gram negative or both or anaerobic or all the three. There are some antibiotics which are used only in tuberculosis like Isoniazid. When studying antibiotics, various terminologies like Half- life, MIC, MBC, MEC, bioavailability, therapeutic index, therapeutic window, are used. These are dealt with in chapter 12.1.7. Anyway, a brief summary is given. There are some more terms that are used that need some clarification. They are given brief mention here.

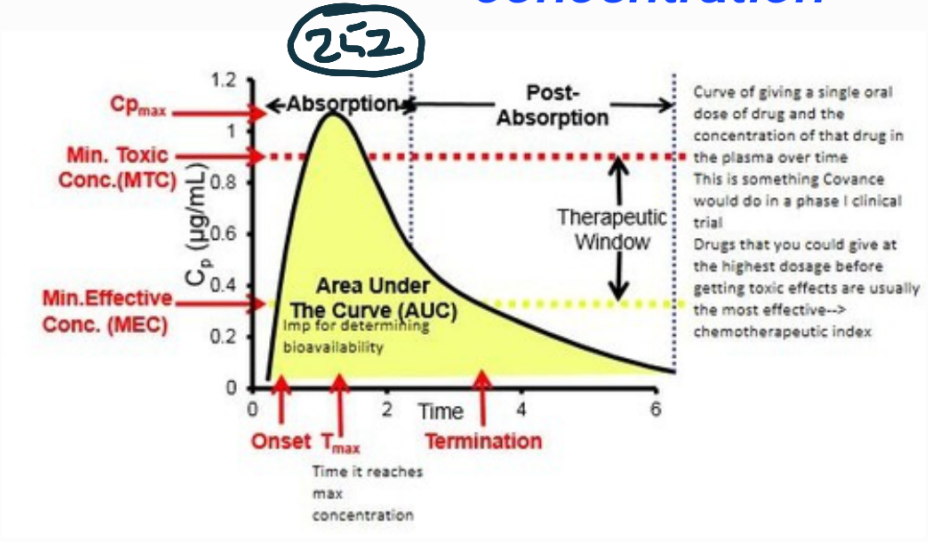


13.4.2. A few concepts revisited (Fig. 250,251,252)

When an antibiotic is administered, by whatever route, it appears in the blood after a certain time (the delay is minimal or nil in the case of Intravenous administration and maximum in the case of oral administration). There is a *minimum inhibitory concentration*







(MIC) - no growth of bacilii or *Minimum bactericidal concentration* (MBC)- 99.9% killed- of the drug. Minimum inhibitory concentration of an antibiotic cannot be determined inside the body, it can be measured only outside in bacterial cultures. There is

no effect on bacteria until this MIC is reached. It is expressed as *micrograms per ml*. The time between the appearance of the drug in the blood and the time it reaches the MIC or MBC or *Minimum Effective Concentration* (MEC) when it starts acting is called the *lag period*. Once the drug reaches the MIC there is *onset of effect*. The concentration of drug goes on increasing as it reaches the blood from the site of administration. This phase is called the *phase of absorption*. This phase can be slow or rapid depending on the route of administration. The drug concentration reaches the peak.

The concentration of the drug in blood is maximum and the effect is *peak effect*. It is called Cmax (*concentration maximum*). The drug concentration starts falling as it is no longer getting absorbed and the drug starts getting excreted in urine and feces. This descending curve is called the *phase of elimination*. This phase is slower than the phase of absorption. The phase can be slow or rapid depending on the rate of elimination of the drug. When the concentration falls to MIC the effect stops (sometimes the effect continues even below the MIC at a low level due to *postantibiotic effect*). The time interval between the onset and end of effect is called *duration of action (t)*. The time at which the maximum concentration of the drug is reached is

called Tmax. Drug concentration below the MIC does not produce effect- called *subtherapeutic*. There is also *minimum toxic concentration* (MTC) beyond which toxic effects manifest. The gap between the *minimum effective concentration* or *minimum inhibitory concentration* and *minimum toxic concentration* is the *safety range* and is called *therapeutic window.* Any dose within this range is less likely to produce toxicity. Wider the gap better is the safety of the drug. INH has the widest therapeutic window between the minimum inhibitory concentration and the minimum toxic concentration. The range between the minimum inhibitory concentration and the peak effect is called the *intensity of effect* which differs from drug to drug. *Area under the curve (AUC)*is the total drug exposure in 24 hours or between two doses (dosing inyerval). Typically, the area is calculated starting from the time the medicine is administered until the time when the concentration in plasma is insignificant. Expressed as micrograms per ml/ 24 hours.

MIC

Minimum inhibitory concentrations (MICs) (Fig. 250,251,252) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. This is the most important parameter. A resistant organism shows a raise in the MIC.

MBC

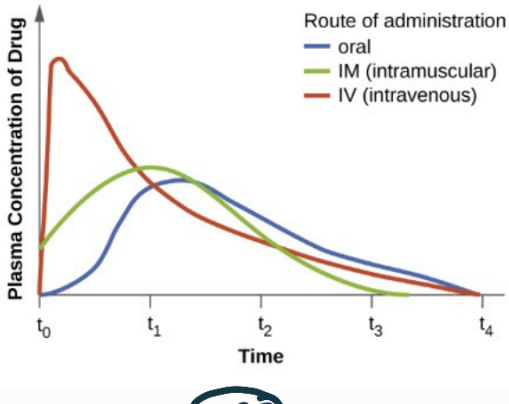
Minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. MICs are used by diagnostic laboratories mainly to confirm resistance

AUC

Total exposure to the drug per time (Fig.252). It combines both concentration of the drug and the time of exposure at the site of infection. AUC is the total amount of drug found in the blood from the time it appears up to the time it becomes almost zero. In essence, the AUC indirectly measures the two major factors for bacterial eradication and quantifies the amount of exposure of the organism to the antibiotic during any one dosing interval. Along with MIC it is an important parameter. The area under the serum concentration curve (AUC) after a dose of antibiotic measures how high (concentration) and how long (time) the antibiotic levels remain above the target MIC during any one dosing interval.

Bioavailability (Fig. 253)

The fraction of an administered drug that reaches the systemic circulation. If 100mg is dose given by intravenous injection and the blood concentration measured after a time shows 100mg. If 100 mg is given orally, it may be ,say, 70mg. Some of the drug is not absorbed, or metabolised in intestine and liver. The drug that finally reaches the blood is less than what is given. Bioavailability is 70%. It depends on the drug itself (ability to dissolve quickly), condition of the intestine, functional integrity of the liver.



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Minimum effective dose (Fig.254)

Concentration of a drug that produces a biological response.

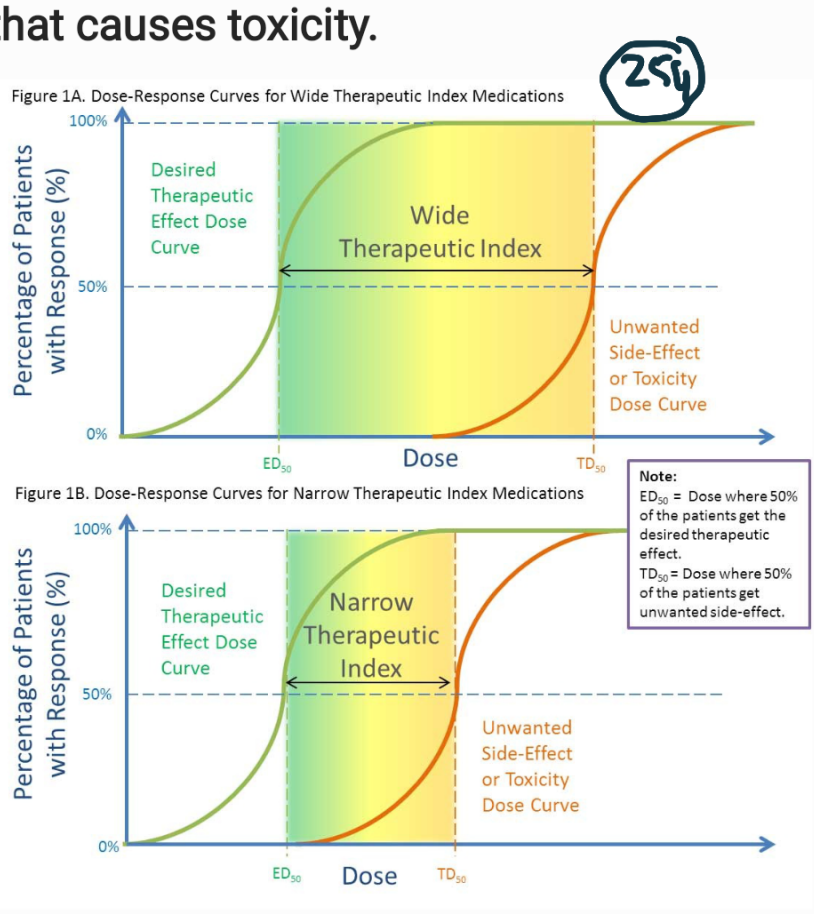
Maximum tolerable dose(Fig.254)

Similarly, the MTD is the highest possible but still tolerable dose level.

Therapeutic index (Fig.255)

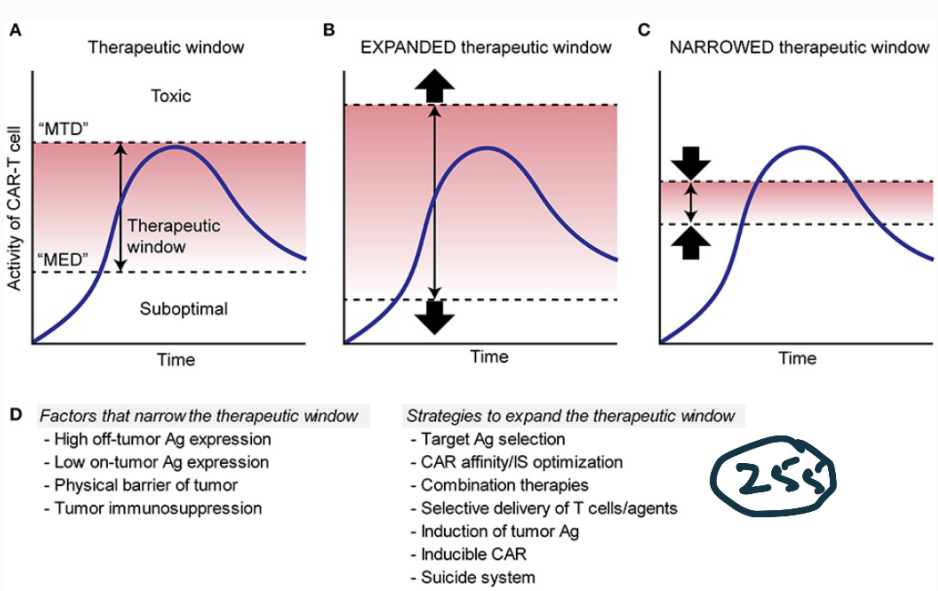
It is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity.

The therapeutic index compares the therapeutically beneficial dose to the toxic dose of a drug by using a simple ratio of the dose that produces toxicity to the dose needed to produce a therapeutic benefit. The convention is to use the dose that causes either beneficial or toxic effects in 50% of people (see Figure. 255).



By using this simple ratio it is relatively straightforward to see how a large TI means a drug is relatively safe because the amount it takes to cause harm is far greater than the amount it takes to get a benefit. In contrast, a low TI means that the potential for harm is greater if you take more than the directed amount. Drugs with TI's greater than 10 are considered relatively safe while those with TI's less than 3 typically require tighter controls on manufacturing (to ensure that the dosing is accurate) and patient monitoring.

Therapeutic window (fig.



254)

The dose range of a drug that provides safe and effective therapy with minimal adverse effects. It is the range between the minimum effective dose (MED) and the maximum tolerated dose (MTD). Wider the range or window greater us the safety of the drug.

cMax

Cmax is the maximum (or peak) (Fig.251)serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and before the administration of a second dose. Cmax is the highest concentration of a drug in the blood, cerebrospinal fluid, or target organ after a dose is given.

tMax

The time it takes for a drug to reach the maximum concentration (Cmax) (Fig.251) after administration of a drug that needs to be absorbed (e.g. an oral drug). Tmax is governed by the rate of drug absorption and the rate of drug elimination. At Tmax, these are equal.It is an important factor to consider when selecting dosing regimens and dosing intervals

LD 50

The value of LD_{50} for a substance is the dose required to kill half the members of a tested population after a specified test duration (Fig. 255).

TD 50

LD cannot be measured in humans. Instead TD or toxic dose is measured. It is the dose that produces toxic side effects in 50% of the population given the drug. (Fig. 255)

ED 50

It is the dose of a medication that produces a desired pharmacologic effect in 50% of the studied patient population that takes the medication. (Fig. 255)

13.4.3. What factors influence the killing capacity of antibiotics?

13.4.3.1 what is the basic mechanism of killing of bacteria?

Mechanism of action of drugs:

Through receptor interactions- (Fig. 256)there are certain protein receptors on the surface or inside a cell which have receptor binding site. A drug with matching configuration of the RBS binds to the receptor and and enhances the action of chemical connected to the receptor or blocks the normal chemical from binding to it and inhibits the action. The former is called agonist and the latter is called the antagonist.

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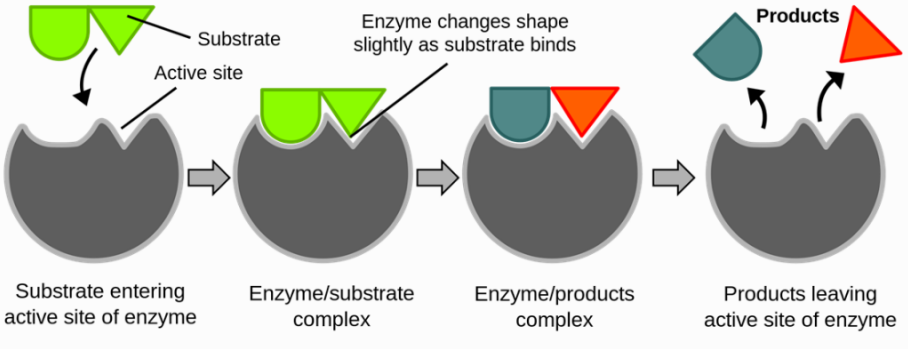
Enzyme interaction- (Fig. 257) there are several enzymes involved in different activities. A



drug binds to these enzymes and either enhance their activity or inhibit their activity. This is called selective interaction. They are called enzyme inducers or inhibitors.

Nonselective interaction- drugs bind to and nteract with cell wall components or metabolic processes inside the cell and bring about change.

13.4.3.2. What is the pharmacological basis of bacterial killing?

A. Concentration dependent and time dependent killing of bacteria

There are two pharmacological terms which are used in the treatment of a disease: pharmacokonetics and pharmacodynamics.

Pharmacokinetics (kinetics is movement) deal with the whole process involved in the movement of a drug from its site of administration to the place of its pharmacologic activity and its elimination from the body.

Pharmacodynamics deals with the process of interaction of the drug at the site and its biologic effect on the target (with antibiotics the target is the bacteria

and the effect is either killing or inhibiting growth).

There are various factors that affect the movement (kinetics) and fate of a drug in the body (LADME: Liberation Absorption Distribution Metabolism Elimination Fig. 249):

1. *release from the dosage form (tablet, capsule or injection, for example);*
2. *absorption from the site of administration into the bloodstream (from intestine or muscle or subcutaneous tissue, for example);*
3. *distribution to various parts of the body, including the site of action (remember, it is the free form , not protein bound form that is effective)and*
4. *rate of elimination from the body via metabolism and/ or excretion of unchanged drug.*

It is difficult to measure drug concentration directly at the site of infection. It can be measured in the serum over a period of time and the level in serum and other tissues over time is used as surrogate marker to measure the minimum concentration of the drug that is necessary to inhibit (MIC) or kill (MBC) organisms. Most bacteria are extracellular and are exposed to fluid outside the cell. Drug concentration in the extracellular fluid drives the antibiotic into the cell and finally to the

binding site. So, plasma concentration of drug correlates with bacterial eradication.

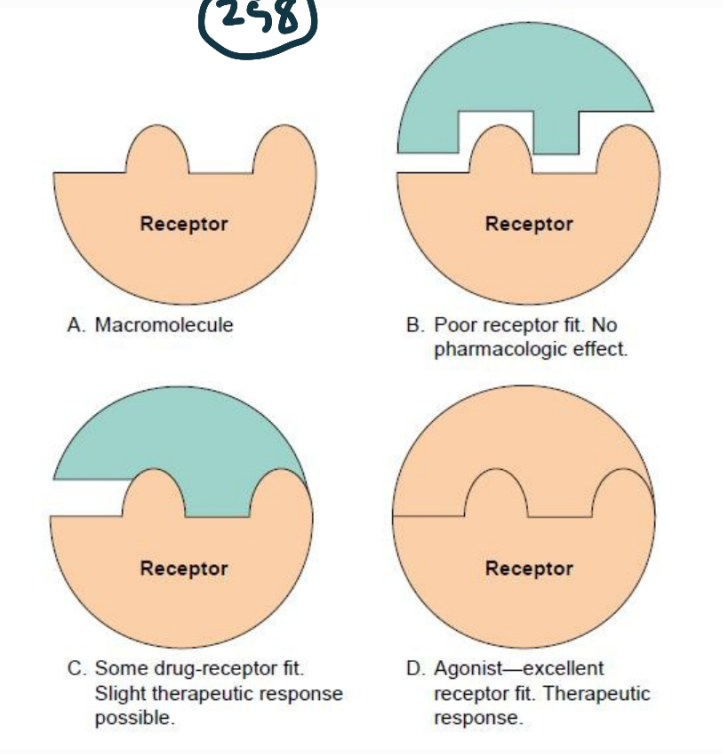
Eventhough MIC or MBC against a bacterium is the most important parameter it is important to remember that's not the only factor to be used to choose the best drug for an infection. It is one of the many factors that determine the best drug to cure an infection. These other factors such as protein binding, pharmacokinetics, distribution into the site of infection, the adequacy of the patient's host defenses and the amount of exposure of an organism to an antibiotic needed for its eradication should be kept in mind.
MIC or MBC is one of the important factors that determine the choice of a drug for an infection.

The effect of the drug is to inhibit growth or to kill. Pharmacodynamics tries to correlate the concentration of the drug especially the MIC with the effect of the drug. Antibiotics act by binding to a specific protein either outside on the membrane or envelope or inside to bring about the killing or growth inhibition of bacteria.

For an antibiotic to eliminate an organism, three major steps (Fig. 258) are required.

First, the antibiotic must bind to its target site(s) in the

bacterium. To reach the binding site is no easy matter. It has to cross several hurdles- must penetrate the outer membrane of the organism (penetration resistance), avoid being pumped out of the membrane (efflux pump resistance), and remain intact as a molecule (e.g., avoid degradation by enzymes like beta-lactamases). Once the target is reached, the antibiotic can still be useless if the binding site (receptor binding site) has changed its molecular configuration and no longer allows the drug to attach. A range of different binding sites has been identified including ribosomes, penicillin-binding proteins, DNA topoisomerase/gyrase, and the cell membrane itself. The crucial binding site will vary with the antibiotic class. These binding sites are the places where critical biochemical reactions occur which help the organism to carry out various functions, survive and multiply. Thus, by binding to these sites, the antibiotic interferes with the chemical reaction, prevents the action resulting from the reaction leading to the death of the bacterium.



Second, the drug must not only bind to the target but

also must occupy an adequate number of binding sites. These binding sites are receptors and there are hundreds and thousands of such receptors. A drug which binds to maximum number of receptors is considered to have high affinity compared to a drug which binds to less number of receptors. The affinity to bind depends on the concentration of the drug at the site. When all the receptor binding sites are occupied, we say, a saturation point has been reached.

The antibiotic needs some time to act. *Third*, for an antibiotic to work effectively, the antibiotic should remain at the binding site for a sufficient period of time in order for the metabolic processes of the bacteria to be sufficiently inhibited.
Penetration of bacterial cell and binding to the target site in sufficient concentration for sufficient period of time.

The three pharmacodyamic properties of antibiotics that best describe killing activity are *time-dependence, concentration-dependence, and persistent effects.* The rate of killing is determined by either the length of time necessary to kill (time-dependent), or the effect of increasing concentrations (concentration-dependent). Persistent effects include the Post-Antibiotic Effect (PAE). PAE is the persistant suppression of bacterial

growth following antibiotic exposure.

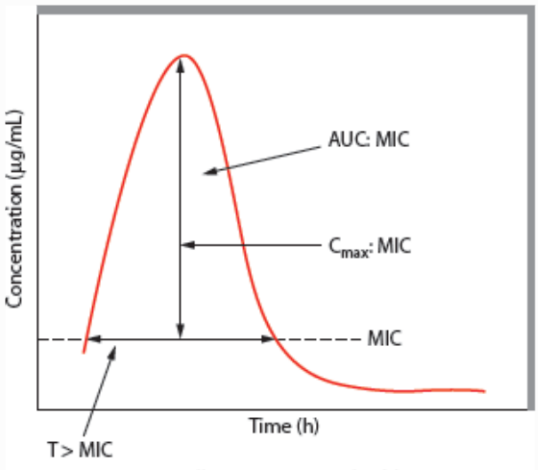
The major killing effect of an antibiotic against an organism is produced by either the time or the concentration of the drug at the binding site or by PAE. The first is said to be time dependent for killing and the second , concentration dependent for killing. The third may be due to reasons which are not clear. The primary measure of antibiotic activity is the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of an antibiotic that completely inhibits the growth of a microorganism in vitro. While the MIC is a good indicator of the potency of an antibiotic, it indicates nothing about the time course of antimicrobial activity. Other parameters are therefore used, in addition.

PK parameters quantify the serum levels over time of an antibiotic and the three pharmacokinetic parameters that are most important for evaluating antibiotic efficacy are the peak serum level (Cmax), the trough level (Cmin), and the Area Under the serum concentration time Curve (AUC). While these parameters quantify the serum level time course, they do not describe the killing activity of an antibiotic. It is MIC or MBC which describes the killing activity.

PD parameters: MIC or MBC.

Integrating the PK parameters with the MIC gives us three PK/PD parameters (Fig. 259)which quantify the activity of an antibiotic:

- *the Peak/MIC ratio- Cpmax divided by MIC (used mainly for concentration dependent killing)*
- *the T>MIC (time above MIC)- percentage of a dosage interval in which the serum level exceeds the MIC (used for time dependent killing)*
- *the 24h-AUC/MIC ratio- determined by dividing the 24-hour-AUC by the MIC (used for concentration dependent with moderate time dependent killing)*.



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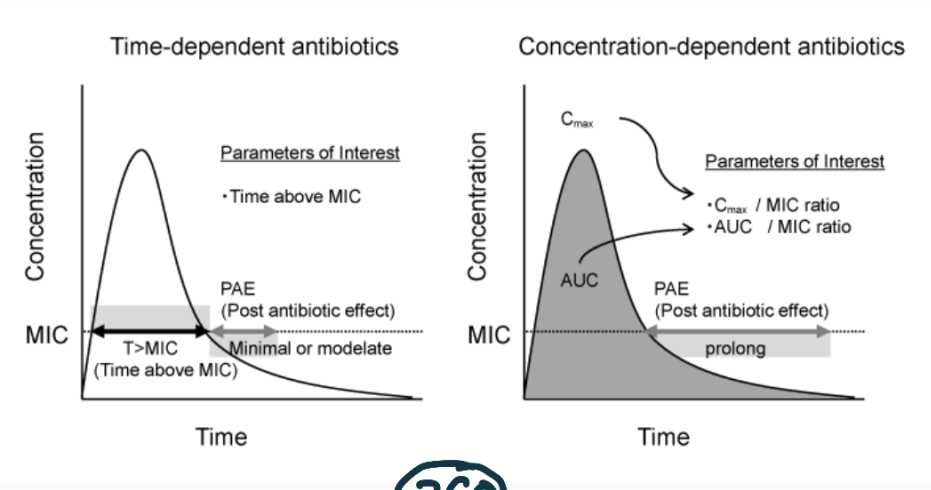
A.1. Time-dependent Killing:

Antibiotics, like beta-lactams (beta-lactams are called so because of the presence of a beta-lactam ring in the structure) penicillins, cephalosporins, carbapenems, monobactams), clindamycin, macrolides (erythromycin, clarithromycin), oxazolidinones (linezolid), are effective because of the length of time they bind to the

microorganism. Longer they stay at the site greater is the effect (up to a certain time limit). The effect is good because during the time they bind to the site their concentration exceeds the MIC or MBC for the bacteria. It does not work if concentration is less than MIC or MBC. Hence, these antibiotics are referred to as time-dependent antibiotics (Fig. 260). For time-dependent drugs, the pharmacodynamic parameter that is used is the time the serum concentrations remain above the MIC during the dosing interval (t>MIC) (between doses). For instance, for antibiotics with time-dependent killing, the optimal responses occur when the time that the drug remains above the MIC is equal or greater than 50% of the dosing interval. For example, if the dosing interval is 8 hours, at least 4 hours the concentration of drug should be above MIC. Linezolid is an antibiotic with time dependent killing and minimal PBE. Time dependent killing with moderate PBE- azithromycin, oxazolidinones, tetracyclines.



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A.2. Concentration-dependent Killing:

Other classes of antibiotics, such as aminoglycosides and quinolones, have high concentrations at the binding site which eradicates the microorganism and, hence, these drugs are considered to have a different kind of bacterial killing, named concentration-dependent killing(Fig.260). For concentration-dependent agents, the pharmacodynamic parameter can be simplified as a peak/MIC ratio.

For agents with concentration-dependent killing, the best responses occur when the concentrations are ≥ 10 times above the MIC for their target organism (s) at the site of infection. For example , if the MIC for an antibiotic is 10 microgram/ml, the best response is obtained if the peak is 100 microgram/ml. There are some antibiotics which are concentration dependent and they have prolonged persistent effect (PBE) like aminoglycosides and fluoroquinolones. *This is the reason why these antibiotics are given once a day.*

What is the reason for this difference between concentration and time dependent killing?

It is related to the location of each agent's target receptor. Both aminoglycoside and fluoroquinolone antibiotics have receptor targets that are intracellular (inside cell, in cytoplasm). It helps to have high concentration to penetrate the cell membrane and wall and reach the inside of cell. Higher the concentration better is the binding to receptors and better is the effect. Accordingly, these

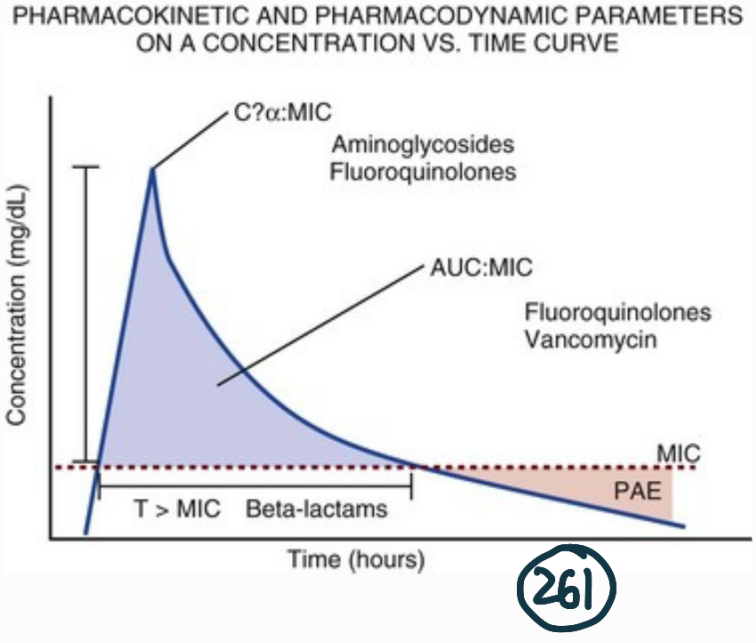
agents are classified as concentration-dependent antibiotics. Conversely, β-lactams inhibit the formation of bacterial cell wall via inhibition of penicillin-binding protein (PBP). This protein is located on the bacterial cell surface, allowing effective binding at lower concentrations. In fact, nearly all available PBP targets become saturated at concentrations that are four to five times the bacteria's MIC. Above this level, the action of β-lactams is relatively independent of concentration, making the duration of time that concentrations remain above the MIC the parameter most predictive of effect.
Thus the goal should be to maintain a serum concentration greater than the MIC for the longest duration possible—at least 60% (penicillins) to 70% (cephalosporins) of the dosing interval. Strategies to achieve this effect include increasing the dosing frequency, giving β-lactams with a long serum half-life, or giving the drug as a continuous infusion. Ultimately, when β-lactams are used in the ICU, combining higher doses with increased frequency of administrations will maximize antibiotic effect without increasing the risk of developing resistance.

It must be emphasized that once these target AUC/MIC ratios, Peak/MIC ratio and t/MIC are achieved there is no evidence that higher ratios result in more rapid killing or less emergence of bacterial resistance. In fact, excessive AUC/MIC ratios may produce unwanted adverse reactions by disrupting the normal gastrointestinal flora ("collateral damage") and producing organ dysfunction.

B. What is post antibiotic effect (PAE) and

how is it useful?

Another important observation from in vitro models is the persistent inhibition of bacterial growth after drug concentration falls below the MIC. This phenomenon, known as the postantibiotic effect (PAE) (Fig.261), is common to all antibiotics, although the magnitude varies depending on the specific antibiotic and pathogen being analyzed. PAE is usually prolonged (3-6 hours) for agents that inhibit nucleic acid and protein synthesis such as the aminoglycosides. Most cell wall active agents such as the β-lactams have a short PAE for gram-positive bacteria and complete absence of PAE against gram-negative bacteria. As a result, bacterial regrowth occurs immediately as concentration falls below the MIC. Carbapenems are an exception to this as they are cell wall active agents and have a prolonged PAE. Bacterial growth may be inhibited following exposure to an antibiotic even after the drug concentration has fallen below the MIC. This is known as the postantibiotic effect (PAE) and is determined *in vitro* (



experiments are called in vitro when they are done

outside in the lab on artificial medium. They are called in vivo when they are fone in live animals (often, including human) by observing bacterial growth on culture medium after drug removal. Animal models have been described which measure PAE *in vivo*. Postantibiotic effects can vary by drugs and micro-organism. For example, prolonged PAEs have been reported after aminoglycoside or fluoroquinolone exposure of Gram-negative *L. bacilli*, whereas most β-lactam antibiotics exhibit shorter PAEs.

The *mechanism* of the PAE is unknown. Possible explanations include:

- nonlethal bacterial damage induced by the antimicrobial agent
- persistence of the agent at the site of action.
- recovery of organism after reversible damage by antibiotic is slow
- new antibacterial enzymes have to be synthesised before their regrowth

How can this be used, theoretically?

- an agent with a long PAE can be dosed less frequently than an antimicrobial agent lacking a PAE; alternatively,

- an agent with little or no PAE may be most effective if it is given as a continuous infusion so that the serum concentration always exceeds the MIC.

This is the reason why certain antibiotics are given once a day, certain other antibiotics are given more than once a day and yet others are given in continuous infusion.

Antimicrobial Patterns

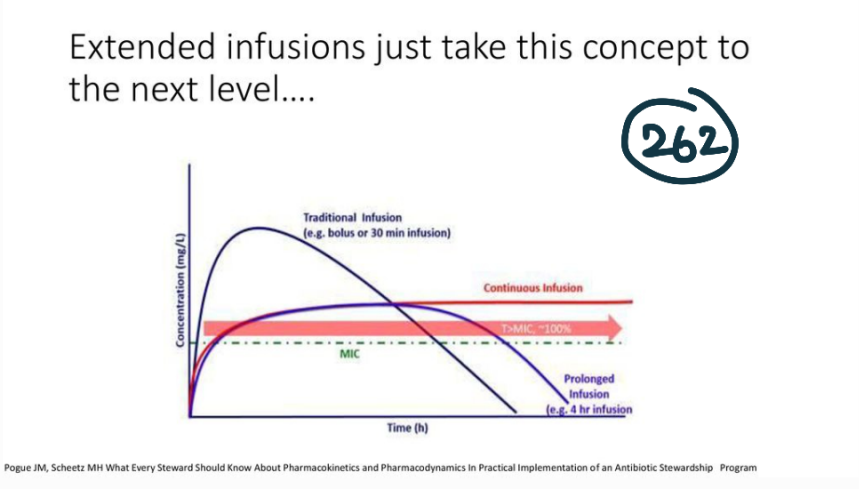
For Type I antibiotics (AG's, fluoroquinolones, daptomycin and the ketolides), the ideal dosing regimen would maximize concentration, because the higher the concentration, the more extensive and the faster is the degree of killing. Therefore, the Peak/MIC ratio is the important predictors of antibiotic efficacy. For aminoglycosides, it is best to have a Peak/MIC ratio of at least 8-10 to prevent resistence.

Type II antibiotics (beta-lactams, clindamycin, erythromcyin, and linezolid) demonstrate the complete opposite properties. The ideal dosing regimen for these antibiotics maximizes the duration of exposure. The T>MIC is the parameter that best correlates with efficacy. For beta-lactams and erythromycin, maximum killing is seen when the time above MIC is at least 70% of the dosing interval.

Type III antibiotics (vancomycin, tetracyclines, azithromycin, and the dalfopristin-quinupristin combination) have mixed properties, they have time-dependent killing and moderate persistent effects. The ideal dosing regimen for these antibiotics maximizes the amount of drug received. Therefore, the 24h-AUC/MIC ratio is the parameter that correlates with efficacy. For vancomycin, a 24h-AUC/MIC ratio of at least 400 is necessary for MRSA.

C. How can the time of action be extended without producing toxicity?
Continuous or extended infusion:

The importance of time dependent killing (T > MIC) has gained increasing attention in the last decade as a result of increasing bacterial resistance. Resistant bacteria have elevated MICs, making it more difficult to achieve adequate T > MIC. Increasing the dose or concentration may work up to a certain extent but beyond that it may reach levels of toxicity. One alternative is to change the shape of the concentration-time curve using continuous or extended infusions (Fig.262). As seen in fig. 262 extending the infusion duration changes the shape of the concentration-time curve to promote longer T > MIC. Several studies have confirmed that these alternative dosing strategies can increase T > MIC without increasing the size of the dose. One study found that T > MIC following a 2-g dose of meropenem was increased 15% by extending the infusion duration from 0.5 hour to 3 hours.T > MIC to be the important factor predicting efficacy.



D. What is the advantage of giving large doses less frequently?
Extended interval dosing

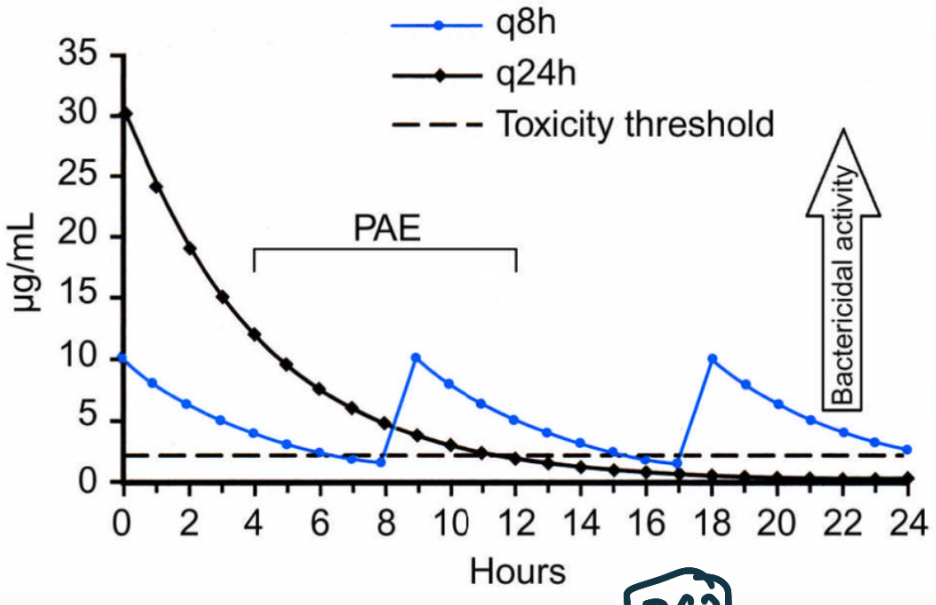
The traditional approach to aminoglycoside use was to give frequent small doses. This was believed to result in less toxicity but the killing rate was low .The likelihood of having a positive clinical response is greater than 90% when peak concentrations are 8 to 10 times the infecting organism's MIC.

A later study found that time to normalization of leukocytosis (immune cells) was greater than 90% when peak:MIC ratio was 10 or greater.

Aminoglycosides also exhibit a prolonged PAE. The duration of PAE varies from 1 to 8 hours and is a function of the peak:MIC ratio. Higher ratios produce longer PAE. In addition, data suggest that PAE may be enhanced in patients with an intact immune system.

Based on the combination of concentration-dependent activity (90% when concentration was 8 to 10 times the MIC) and a prolonged PAE (1 to 8 hours) the efficacy of

drugs like aminoglycosides could be maximized by giving large doses less frequently. This strategy is known as extended interval dosing (EID) (Fig.263).



Because aminoglycosides have short half-lives, the drugs are completely cleared from serum near the end of a 24-hour dosing interval in patients with normal renal function.

- Although the absence of drug may be concerning for the regrowth of bacteria, this is prevented by the PAE.
- In addition, a drug-free period near the end of the dosing interval minimizes the phenomenon known as *adaptive resistance* (*adaptive resistance* refers to the diminished rate of bacterial killing after initial exposure to aminoglycosides). This effect is caused by up-regulation of membrane-bound efflux pumps, which decrease the amount of drug that reaches the site of action inside the cell. When the bacteria are free from drug exposure for a sufficient amount of time the adaptive resistance is lost and

the bacteria will become fully sensitive again.

- Nephrotoxicity is not concentration dependent. High peak is as safe as low peak. Kidney toxocity depends on duration of treatment.

E. What is early bactericidal activity (EBA):

Early Bactericidal Activity (EBA) is one of the fundamental parameters to determine the clinical efficacy of antibiotics in the treatment of tuberculosis. In a large trial conducted on patients recently diagnosed with pulmonary TB and never treated before with any of the drugs, they were treated with antibiotics or combinations for a period of 2 to 14 days. Patients must not have used anti-TB drugs previously. During the treatment period, the amount of viable bacilli appearing in sputum samples was determined quantitatively. The traditional EBA unit is the logarithmic decrease of colony forming units (CFU/mL sputum/day during the first 48 hours). EBA studies showed that there were differences between the fall of viable bacteria counts in the first two days of treatment in comparison with the

Drug	n	Mean EBA (log_{10} cfu/mL/day)	SD*	95% CI†
INH 300 mg once daily	10	0.67	0.35	0.42 – 0.91
Linezolid 600 mg twice daily	9‡	0.26	0.42	-0.06 – 0.59
Linezolid 600 mg once-daily	10	0.18§	0.27	-0.01 – 0.37

*SD – standard deviation

†95% C.I. – 95% confidence interval.

‡One patient in the linezolid 600 mg twice-daily arm withdrew after randomization before receiving any doses of study drug.

following twelve . Differences among several treatments were also more significant during the first two days. In the early therapy, the activity of INH was superior and dominant regarding the other drugs administered in combination (Fig.264,265))Any addition of INH to a regime led to an increase of EBA but never higher than INH on its own. The addition of PZA to a regime of STR, INH, and RIF increased 0-2 days EBA from 0.415 to 0.472 . The greatest disadvantage of determining the EBA is its inability to detect sterilizing activity. Some researchers have concluded that extended EBA trials (2 to 14 days) do not correlate to the sterilizing activity . For example, the potent sterilizing activity of PZA was not detected in an extended EBA trial. STR showed potent activity in extended EBA, and it is known it has a very low sterilizing activity in randomized clinical trials. In extended EBA, EMB appears as antagonist; however, there is no clinical evidence that this drug interferes with the sterilizing effects of RIF and PZA.

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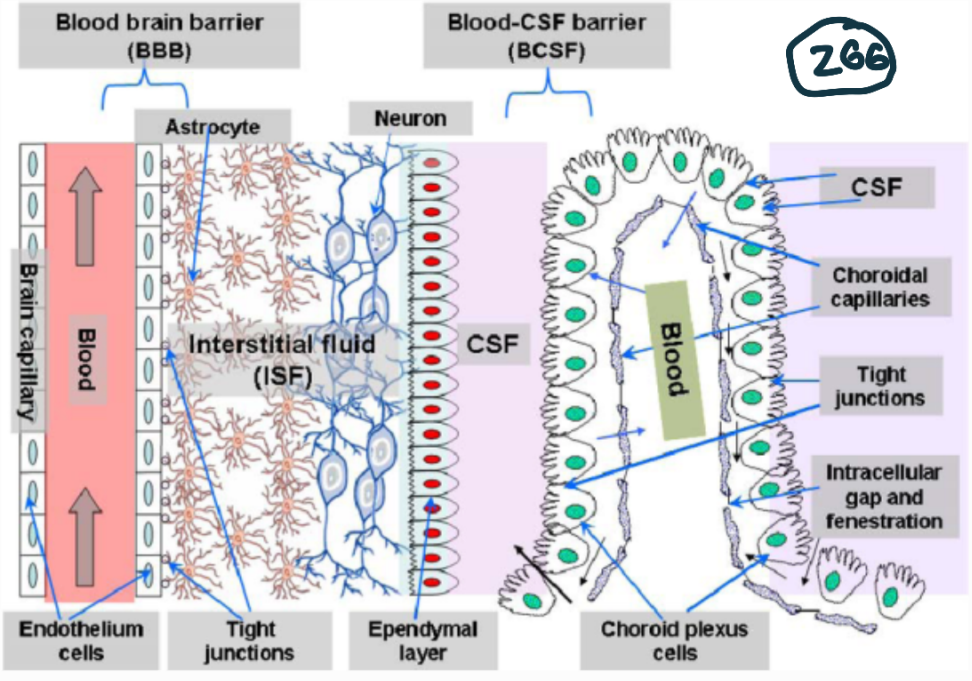
Antituberculosis Drug Activity

Drug	Early bactericidal activity	Preventing drug resistance	Sterilizing activity
Isoniazid	++++	+++	++
Rifampicin	++	+++	++++
Pyrazinamide	+	+	+++
Ethambutol	++ - +++	++	+

Highest ++++ High +++ Intermediate ++ Low +

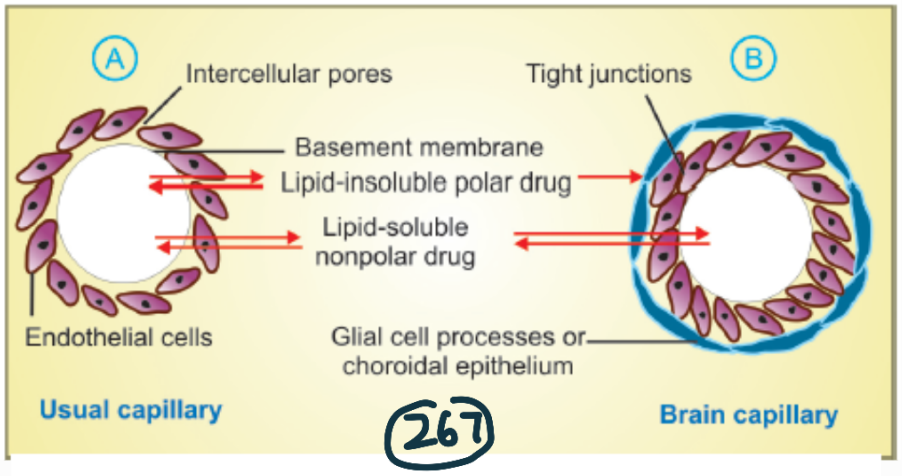
13.5.Blood brain barrier

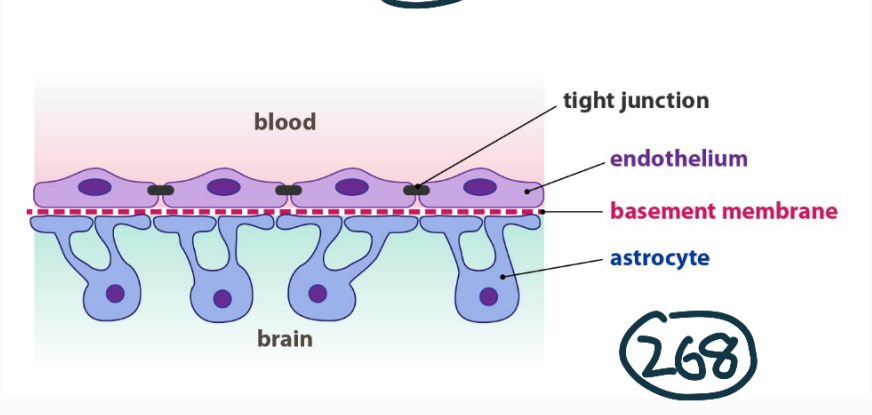
The most important organ in the body, the brain, is well protected physically, chemically, biologically and immunologically. The most important cell in the brain is neuron or nerve cell and the neurons are connected to each other through synapses or gaps where transmission of impulses from one neuron to another is conducted by chemical neurotransmitters like dopamine. The neurons are surrounded by capillaries carrying blood and support cells called pericytes and astrocytes.

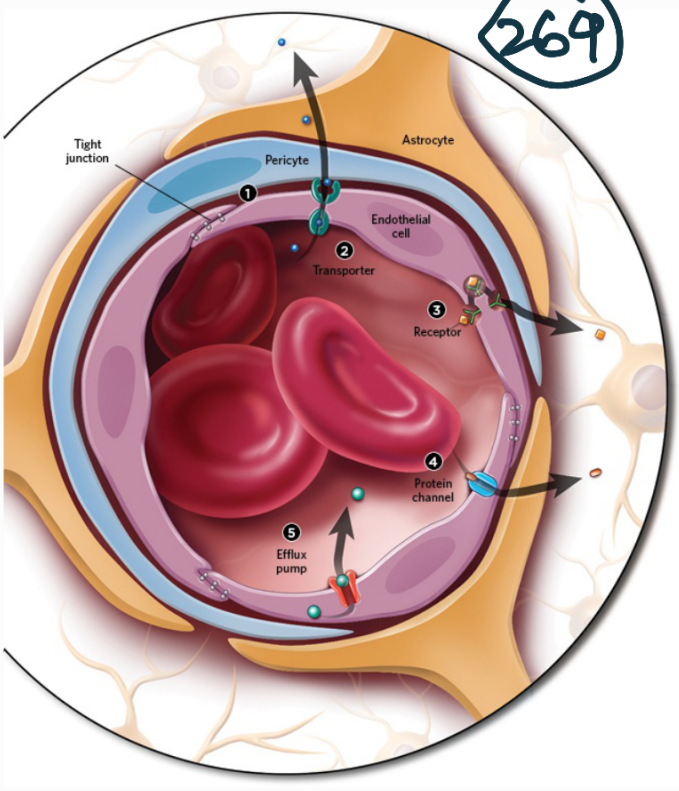


The blood-brain barrier (BBB) (Fig.266)separates the blood circulating through our bodies from the central nervous system (CNS), which includes the brain and the spinal cord. This arrangement is needed to protect the brain from harmful substances like bacteria and toxins. It acts as a control, ensuring that substances that are needed for normal brain function, such as glucose, amino acids and electrolytes, are present in appropriate levels.

So how does it do this? Like blood vessels in the rest of the body, those in the CNS are lined with a thin layer of so-called endothelial (endo- inside, thelia- nipple like) cells, which are in direct contact with the blood inside the vessels. The endothelial cells are arranged side to side with gaps in between through which substances can cross the cell. Movement of substances through gaps in between cells is called trancellular diffusion. But blood vessels in the brain differ in that their endothelial cells are packed tightly side by side, with their membranes zipped together by specialised protein structures that form tight junctions (Fig.267,268), with no gap in between . Unlike the blood vessels in the rest of the body where nutrients in the blood have to pass through single layer of endothelial cells, in the brain they have to pass through a single layer of endothelial cells plus a single layer of pericytes and astrocytes (Fig.268,269), supportive cells which give structural support to brain cells. These

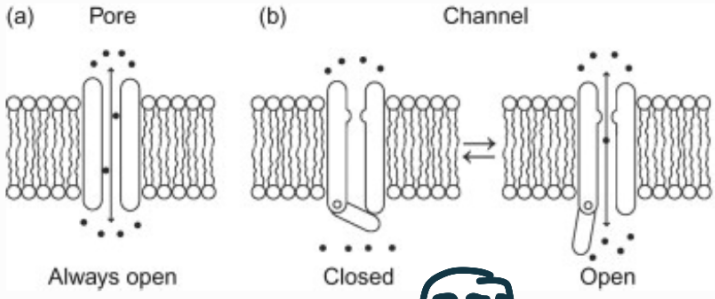


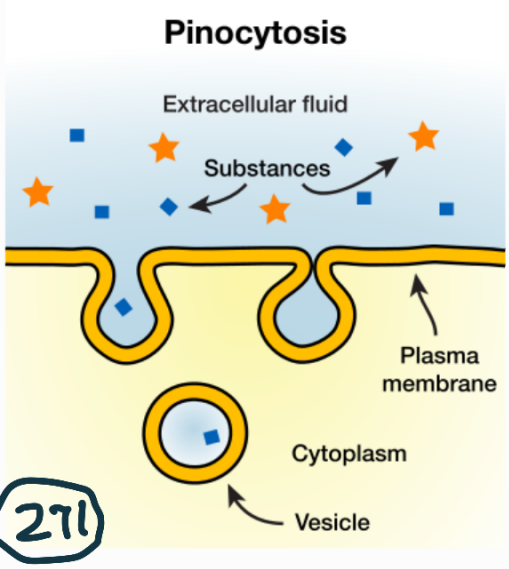




restrict the flow of substances from the blood to the CNS (central nervous system), allowing only small molecules such as water, some gases like oxygen, carbon dioxide, ammonia and compounds that can pass unaided through cell membranes (called lipid-soluble molecules- ethanol, steroid hormones) to pass freely into the brain.

The membranes also contain *efflux* pumps, which kick potentially harmful compounds and drugs back out of the brain. Small lipophilic substances move by passive diffusion, larger lipophylic or hydrophilic substances move by facilitated transfusion (carrier mediated) , diffusion through aqueous channel (Fig. 270), or active transport through transport proteins (glucose, aminoacids and neurotransmitters). Big molecules enter through pinocytosis (Fig.271)





Because of the BBB antibiotics have a difficult time penetrating the blood brain barrier and the blood-CSF barrier, leading to difficulty of some antimicrobials to achieve therapeutic concentration levels in the CSF to properly treat an infection of the brain and spinal cord.

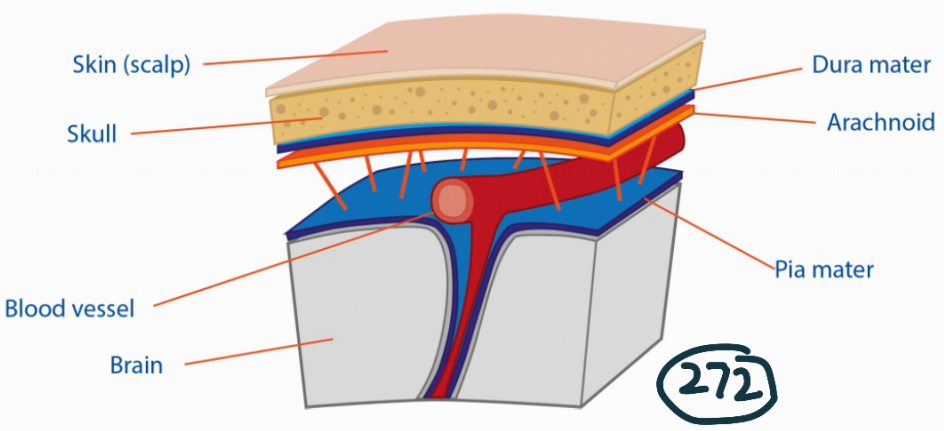
Antimicrobials can be broken down into 3 rough categories based on their capacity to penetrate the blood brain barrier:

Excellent/Good penetration of the CSF *(cerebrospinal fluid is formed by choroid plexus, a vascular tissue, jutting into the ventricles in the brain and it circulates in the subarachnoid space (space beneath the arachnid mater of meninges) of brain and spinal cord.)*

Fluoroquinolones
INH
Pyrazinamide
Zidovudine

Good penetration only in inflamed meninges (Fig.272)

(three layered membrane protecting the brain- dura mater is



the outermost, arachnoid is the middle and pia mater is the innermost. The cerebrospinal fluid is in the space between arachnoid and pia mater. Blood vessels are found in the subarachnoid space.)

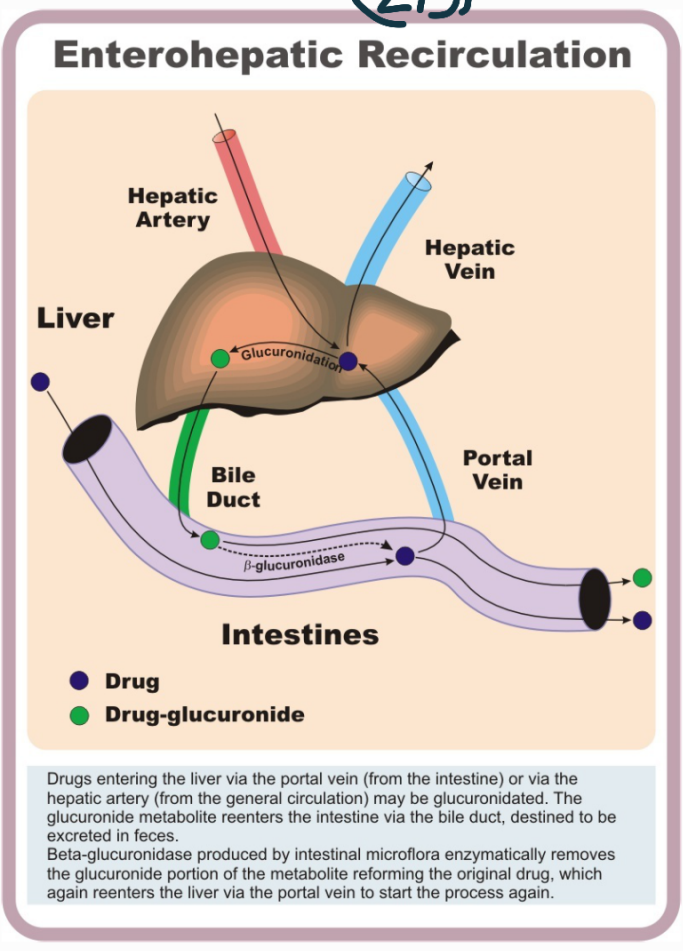
Vancomycin
Azithromycin
Rifampicin
Ethambutol

Poor penetration of the CSF

B-lactam antibiotics (amoxicyllin a d clavulanic acid)
Aminoglycoside
Tetracycline
Clindamycin

13.6. Enterohepatic recirculation (Fig.273):

Enterohepatic recirculation" means circulation between intestine and liver. When orally administered drug is ansorbed, it goes to liver through portal venous system not onto the general circulation (the body is careful. It wants to make sure thst substances other than food are first taken to the liver where it gets metabolised and transformed so that they are non toxic. They are conjugated (bound to) with glucuronic acid so that they become water soluble and are absorbed into the general circulation. Some of the conjugated drug is excreted along with bile into the small intestine where the

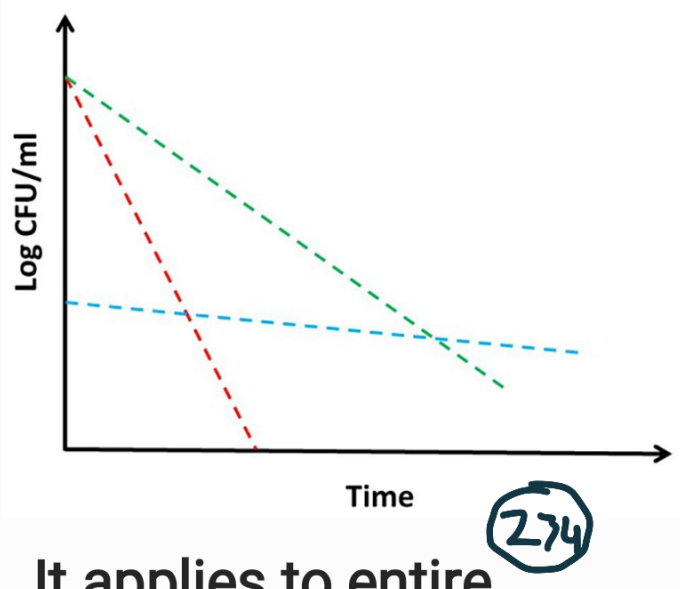


resident bacteria deconjugate the drug or remove glucuronic acid and the free drug is reabsorbed in the small intestine back to liver and from there to the general circulation. And some of it is excreted in the faeces. For many drugs that undergo this process, lower doses of drugs can be therapeutically effective because elimination is reduced by the 'recycling' of the drug. This has to be kept in mind while deciding on the dose of the drug. But for a small number of drugs that are very toxic to the intestine , these molecules which would not otherwise be very toxic can become so because of this process, and therefore inhibition of this step can be protective. For the majority of drugs which undergo enterohepatic recirculation that are not toxic to the intestine, inhibition of this process leads to a reduction of the levels of drug and reduced therapeutic effect. For example, antibiotics that kill gut bacteria often reduce enterohepatic drug circulation and this requires a temporary increase of the drug's dose until the antibiotic use is discontinued and the gut gets repopulated with useful bacteria.

13.7. TB drug tolerance and resistance

13.7.1.Tolerance

Drug tolerance, persistance resistance are three of the traits of bacteria which may hamper successful treatment of tuberculsis.

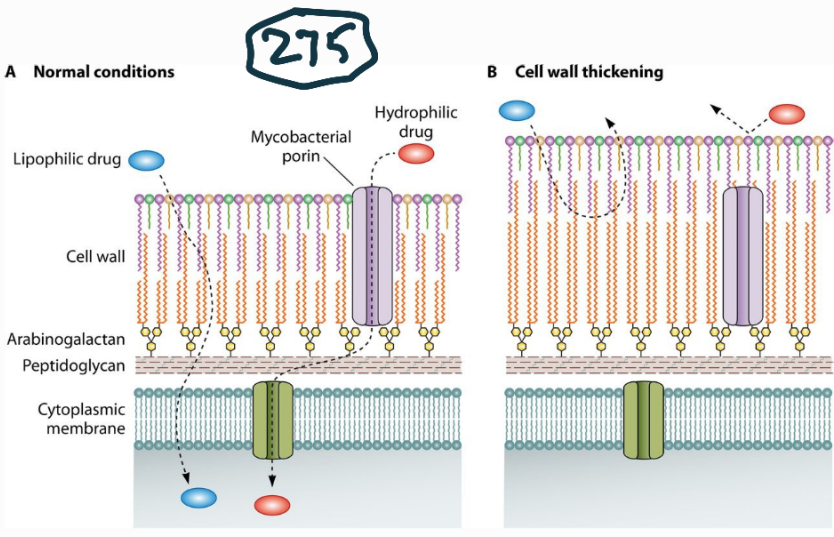


Tolerance (blue line in the figure, red line is susceptible) (Fig.274) is the ability of mycobacterial populations to survive antibiotics without developing mutations to confer resistance. It applies to entire bacterial population when a hostile environment is encountered by bacteria (exposure to drug). It is rarely genetically encoded, and does not affect the MIC but changes the MDK99, the minimum duration of treatment that kills 99% of the mycobacterial population . Upon exposure to bactericidal drugs, tolerant mycobacteria are thus killed at a lower rate than the fully susceptible population from which they arose .

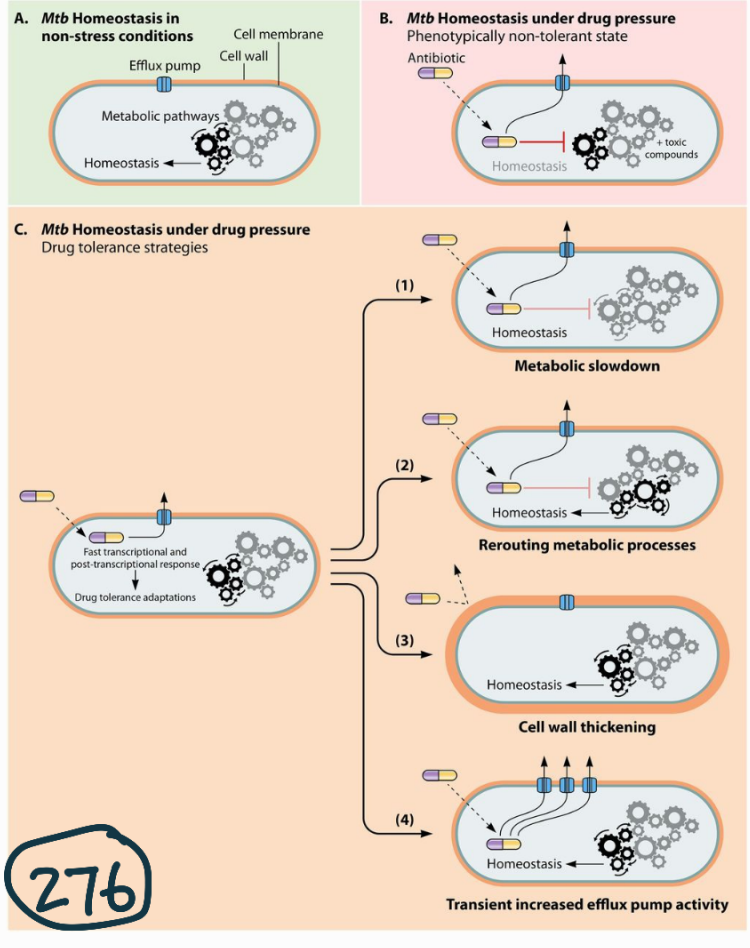
On the other hand, *persistence (green line in fig)* is characterized by the onset of bacterial subpopulations, which are extremely resistant to drug concentrations

several times higher than the MIC, with the rest of the population remaining sensitive.

Drug resistance, or the ability to replicate in the presence of a drug, is most often specific to a single drug or drug class, is inheritable, and increases the MIC, the lowest concentration of a specific antibiotic needed to prevent growth. Drug tolerance is due to the following mechanisms (Fig.275,276):



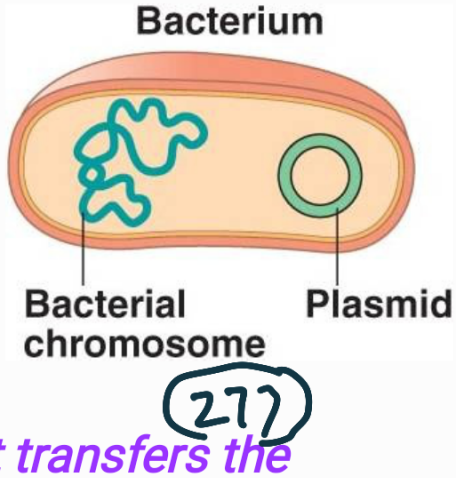
- *reduced metabolism of the bacteria;*
- *shifting of metabolic processes. It may be redirected to building fatty acids and mycolic acids or upregulation of genes associated with the drug target- DNA dependent RNA polymerase in rifampicin and ATP in bedaquiline.*
- *thickening of cell wall (Fig.275,276)due to increased production of mycolic acid making cell wall more impermeable; and*
- *promotion of efflux pump activity and increased expulsion of drug.*



13.6.2.Drug resistance

Drug resistance in tuberculosis is becoming an increasing threat to the efforts to eradicating tuberculosis.

Mycobacterium tuberculosis is naturally resistant to many antibiotics, limiting the number of compounds available for treatment. The common method among bacteria of horizontal transfer of resistance through plasmids is rare in Mycobacteria because they lack *plasmids* (Fig.277) (*pieces of DNA or viruses residing inside bacteria which may help in tranfering resistance from one to another bacteria. First they transfer resistant genes to the host bacteria and when the host bacteria multiplies it transfers the traits to daughter cells*). The intrinsic resistance is due to a number of mechanisms including a thick, waxy, hydrophobic cell envelope and the presence of drug degrading and modifying enzymes. Resistance to the drugs which are active against M. tuberculosis is, in the absence of horizontally transferred resistance determinants, conferred by mutations.

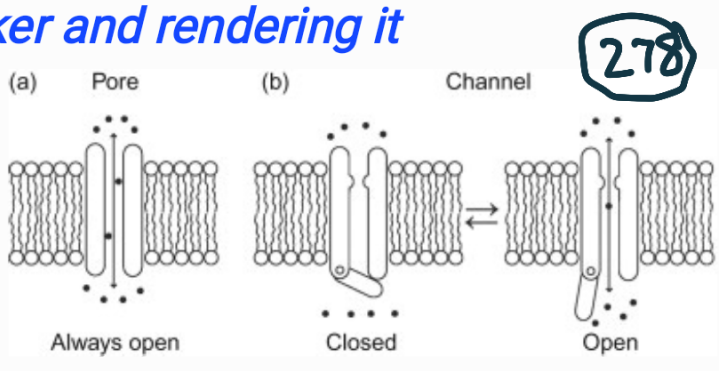


13.6.2.1. Mechanisms of drug resistance in Mycobacterium tuberculosis:

The drug has to pentrate the cell wall and get inside. The cell wall may prevent the permeability to the drug. Or once the drug enters the cell, it may be broken by enzymes or altered structurally to make them ineffective.

A. Cell wall and drug penetration

The cell wall of M. tuberculosis is unusually thick . This is because of the presence of a thick layer of mycolic acid and outer glycolipids can with supporting layers- arabinogalactan and peptidoglycon. This makes the cell wall extremely water hating and prevents the diffusion of water loving compounds. One of the mechanisms by which M.tuberculosis may develop reistance is by rendering its wall considerably thicker and rendering it impenetrable to antibiotics.

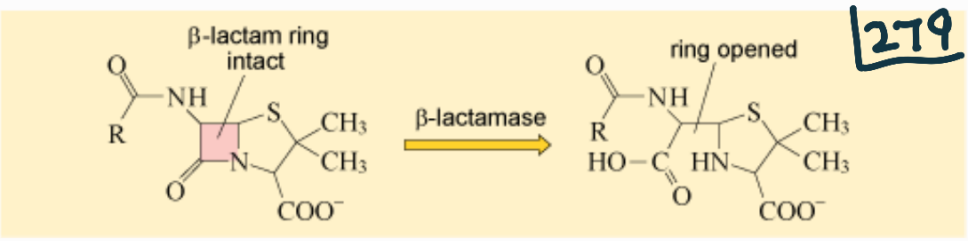


Secondly, It is thought that small (not large) hydrophilic compounds, including many antibiotics active against M. tuberculosis, can only traverse the cell wall via water-filled porins (Fig. 278)(porins are protein channels on the cell membrane whose inside is waterloving. They allow only small molecules- not large molecules or enzymes. Each porin has a certain size threshold. They are always open). But the number of porins is small when compared to gram negative bacteria. If the drug is hydrophilic it has to make an extra effort to reach the inside of the cell.

B. Drug inactivation by Mycobacterium tuberculosis

Enzymatic breakdown

After penetrating the cell wall , the initial defence layer, antibiotics may be broken enzymatically to render them ineffective. *One of the most prominent examples is the*



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enzymatic degradation of β-lactam antibiotics by β-lactamases, which hydrolyse the β-lactam ring of the antibiotics (Fig. 279). The M. tuberculosis β-lactamase shows broad substrate specificity and is considered an extended-spectrum

β-lactamase. It is irreversibly inhibited by the β-lactamase inhibitor clavulanate. That's why in extreme cases where bacteria are resistant to several drugs including second line, amoxacillin-clavulanic acid is included in the regimen.

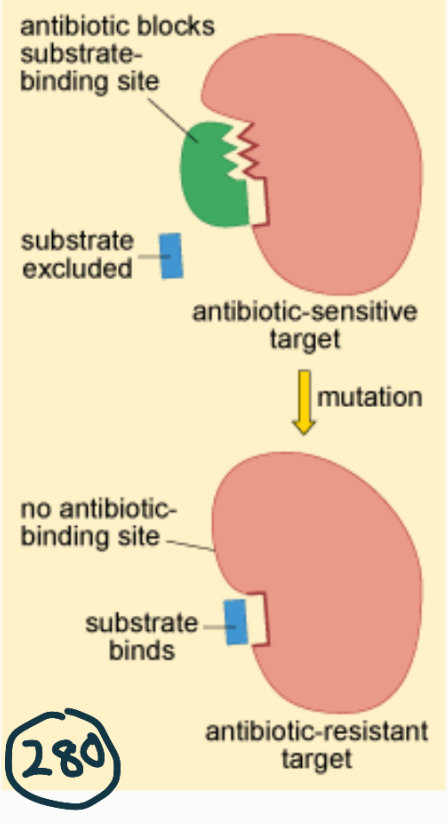
Modification of the drug

Second method of inactivation is by modification of the drug- e.g. by methylation or acetylation- by adding methyl or acetyl group to the drug so that it loses its efficacy. *Acetylation of various aminoglycoside (kanamycin, not amikacin) /cyclic peptide (capreomycin) antibiotics used for the treatment of MDR TB is by the 'enhanced intracellular survival protein' (Eis). Overexpression of Eis therefore might be the reason for the evolution of high-level aminoglycoside/cyclic peptide*

resistance.

C. Enzymatic drug target modification

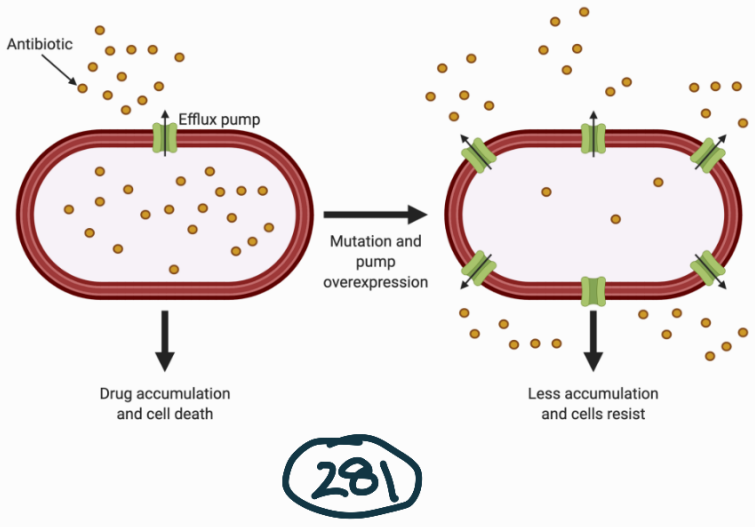
Many antibiotics in use are natural products produced by bacteria, which requires the producing bacteria to be resistant to these compounds; some of the mechanisms used by these bacteria are conserved in mycobacteria. Bacteria produce metabolites, primary and secondary. Primary metabolites are produced within the cell and are essential for the growth and survival of the organism. The secondary metabolites are not essential, produced and travel outside and are used in interactions with external environment. They are lipophilic because they have to cross the cell membranes. Generally the genes producing secondary metabolites are silent (silent genes) becoming active when faced with threats. If streptomyces produces a secondary metabolite, a macrolide like erythromycin, it is naturally resistant to it. It will not be affected by macrolide produced by other bacteria. They do this by changing the binding site (Fig. 280)on ribosome using enzyme called methyltransferase.



D. Drug efflux in Mycobacterium tuberculosis

Efflux pumps (Fig. 281) are transport proteins involved in the expulsion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment. These proteins are found in both Gram-positive and

-negative bacteria as well as in eukaryotic organisms (animals and humans). Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds (including antibiotics of multiple classes); such pumps can be associated with multiple drug resistance (MDR). There are five families of efflux pumps in bacteria.



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Efflux systems are important part of bacteria. The genome of M. tuberculosis encodes a multitude of different efflux systems,

The relevance of drug efflux for generating clinically relevant drug resistance in M. tuberculosis is controversial but has gained more attention in recent years. The observation that about 30% of isoniazid and 3% of rifampicin resistant clinical M. tuberculosis isolates do not show any known resistance mutation might be explained by drug efflux.

Furthermore, efflux systems have been shown to be essential in M. tuberculosis for intracellular growth in macrophages . Mycobacterial efflux pumps are able to extrude nearly all antituberculous drugs, including streptomycin, rifampicin, isoniazid, clofazimine, bedaquiline, fluoroquinolones and ethambutol. Expression of genes is modified by mutational pressure or nonmutational process. Expression levels of genes controlling efflux pumps are modified via non-mutational processes upon changes in the environment (when a specific environmental cue (e.g. antibiotics or the intracellular environment of a macrophage) is present).

13.6.3. Evolution of drug resistance in *Mycobacterium tuberculosis.*

Evolution of drug resistance in Mycobacteria may be *natural* or de nevo or *acquired* due to selection pressure. The latter is due to inappropriate or inadequate use of antibiotics- use of single antibiotics rather than a combination of more than one, subtherapeutic concentration of antibiotic (below MIC) for a long time. These are some of the reasons for exerting pressure on Mycobacteria to develop resistance. *As a natural process supposing a bacteria during multiplication develops a mutant with resistance to a drug. This is a random process. When you give an antibiotic all the bacteria susceptible to the antibiotic are killed. The few resistant ones multiply finally giving rise to a colony consisting mainly of drug resistant mutants (Fig.282).*

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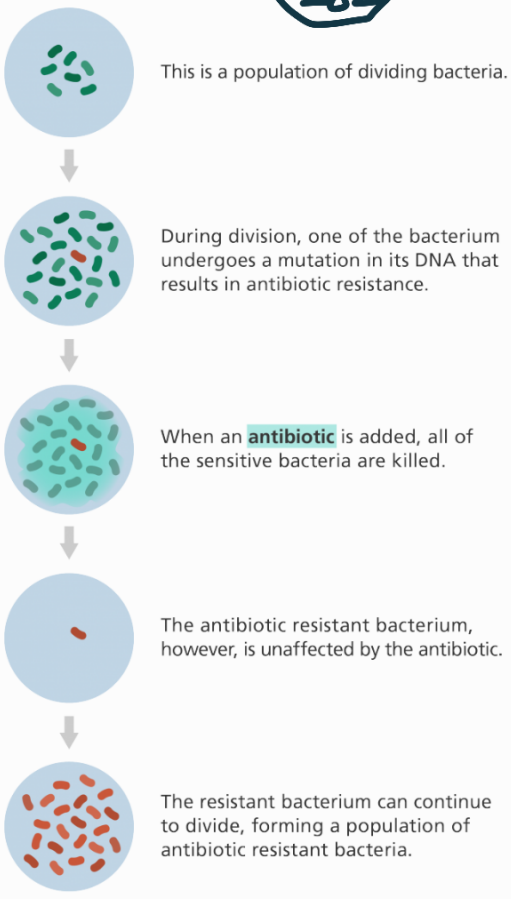
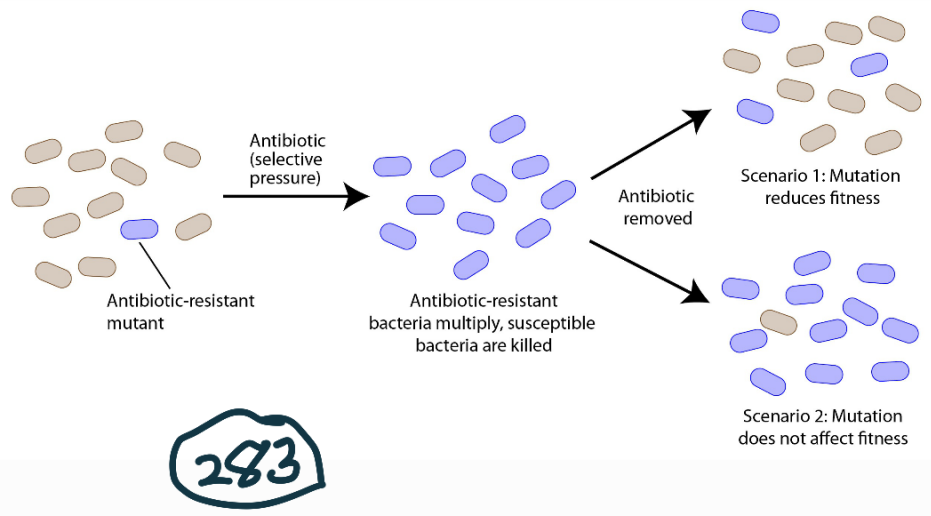


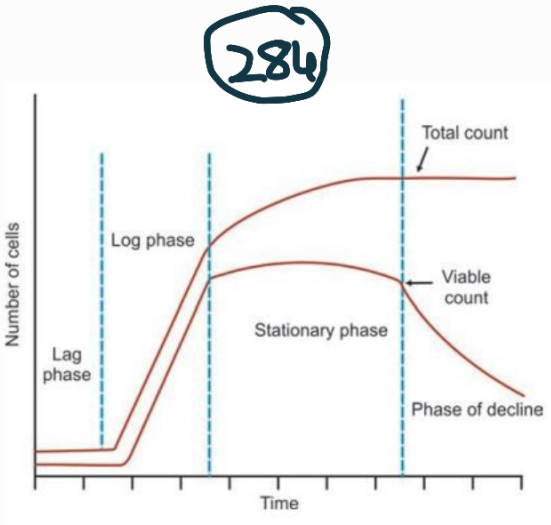
With mycobacteria there is very little influence of selection pressure. The number of organisms necessary to infect is small, the bacilli can exist in the host and activate later (it is not necessary always to transmit to suffer from the disease), a mutant organism is less fit to

infect and survive (Fig.283) (*Drug resistance was long believed to be universally associated with a reduction in the drug-resistant organism's fitness in the absence of antibiotics. Several studies have demonstrated, firvexample, a marked reduction in virulence of certain, but not all, isoniazid resistant strains in guinea pigs and mice).* If the incidence of drug resistance is growing over time it is mainly due to: a) transmission of infection (susceptible and drug resistant); and inappropriate use of mycobactericidal drugs exerting selection pressure. The former is more common than the latter.



13.6.3.1.Natural evolution of drug resistance

Bacterial growth curve phases (Fig.284):



Bacteria undergo different phases of growth. The first is lag phase when bacteria are quiet, sensing the environment and preparing to multiply. The second is log phase when logarithmic multiplication occurs and the number increases on exponential scale (2 becomes 4 becomes 8 becomes 16, and so on). The rate of growth depends on the integrity of the bacilli and environmental conditions. Soon the rate of growth becomes stationary in the

sense the number of bacilli dying will be equal to the number that is produced. Then the number dying will be more than thenumber produced and soon there will be no bacilli left. The stationary and decline phasesare due to exhaustion of nutrients or accumulation of toxic metabolites. The target for antibiotics is the log phase when they can easily atta k and kill the newborn cells by inhibiting their cell wall synthesis or by acting on important enzymes and killing them. Resistance development is also related to the log phase. As the bacteria multiply and produce daughter cells they can transfer resistant genes to the offspring.

There are three important factors influencing the de novo or natural evolution of drug resistance: the population size, as it relates to the number of binary fission or multiplication events the population

has undergone; the mutation rate; and the mutational target size.

Bacterial Population size

In a series of iconic experiments, Luria and Delbrück demonstrated that, for simple traits (e.g. most bacteriophage/antibiotic resistance), bacterial populations which undergo a sufficient number of doubling events or multiplication inevitably harbour resistant variants, following what is now called a Luria-Delbrück distribution. The larger the population, the more cell division events the population experienced, and therefore the larger probability for a drug resistance mutation to arise. Furthermore, if a resistance-conferring mutation evolves early during population expansion, the vast majority of the population will be resistant to a given drug even before treatment onset. The number of bacteria present in a single lesion is estimated to be in the order of 10-8

bacterial cells per lesion. In cavity walls, the bacillary load was estimated to range from 10^{-8} to 10^{-9} in contrast with only 10^{-2} to 10^{-4} in areas of caseous necrosis. For INH 2.56×10^{-8} and for Rifampicin 2.25×10^{-10}. The combined resistance will be 2 per 10^{-18}. This cannot occur naturally because the cavity has only 10^{-8} bacilli. The only way it can occur is by exerting selection pressure on the bacilli through the inadequate use of drugs. It is possible that first the bacilli develop resistance against one antibiotic and then after replication they develop resistance against second antibiotic, not simultaneously.

Mutation rates

In the case of antibiotic resistance, the mutation rate is frequently defined as the frequency (in culture medium or in vitro) at which detectable mutants arise in a bacterial population in the presence of a given antibiotic concentration.

Example: Even if there is only a single bacteria that makes its way into a skin wound, it would take only 10 generations for that single cell to grow into a colony of more than 1,000 (210 = 1,024), and just 10 more generations for it to erupt into a colony of more than 1 million (220 = 1,048,576). For a bacterium that divides about every hour, that is a lot of bacteria in 20 hours. Suppose the bacteria has about 2.8 million nucleotide base pairs in its genome. At a rate of, say, 10^{-10} mutations per nucleotide base, that amounts to nearly 300 mutations in that population of bacteria within 10 hours!

To better understand the impact of this situation, think of it this way: With a genome size of 2.8×10^{6} and a mutation rate of 1 mutation per 10^{10} base pairs, it would take a single bacterium 30

hours to grow into a population in which every single base pair in the genome will have mutated not once, but 30 times! Thus, any individual mutation that could theoretically occur in the bacteria will have occurred somewhere in that population—in just over a day.

The mutation rate is thought to be largely defined by the replication capacity of the mycobacterial enzymes. The mutation rate has been determined to be 4.52×10^{-10} ($2.95–7.35 \times 10^{-10}$ 95% confidence interval) per bp and generation, which is on the lower end of the spectrum compared to other bacteria.

Mutational target size

Given a mutation rate, the number of potential sites which may be mutated to confer drug resistance is an important factor involved in determining the rate of drug resistance evolution. As mentioned earlier, the mutational target size varies depending on resistance mechanism. The mutational target size for prodrugs activated by non-essential enzymes is much larger compared to the target sizes for mutational drug target alteration. Larger the target size the higher the number of potential sites and less is the potential. Two enzymes at two different sites the mutation is additive (a+b) and two enzymes and at the same site the mutation is a product of the two (a×b).

We have spent enough time trying to get an insight into the basic principles and processes involved in the action and impact of antibiotics in the management of infections in general and with M.tuberculosis in particular. Let us now try to understand the individual

drugs. First, we will discuss the drugs used in the treatment of tuberculosis which act on the cell wall.