Chapter 2

REVIEW OF LITERATURES

2.1 Aetiology of TB

The causative organism of TB, the tubercle bacillus, was isolated and described by Robert Koch in 1892. It was subsequently included in the genus Mycobacterium and named *M. tuberculosis*. A closely related species isolated from cattle which is also able to cause human TB is termed *M. bovis*. Mycobacterial strains with rather variable properties principally encountered in Equatorial Africa are collectively termed *M. africanum*. In addition, a rarely encountered type forming unusual smooth colonies on solid culture media has been named *M. microti*. A rare cause of TB in small mammals but of very low virulence in humans, is also closely related. Strictly speaking, these bacilli are all members of a single species which is usually termed the *M. tuberculosis* complex. Members of this complex are obligate pathogens and thus distinct from almost all other mycobacteria, of which there are over 80 species (Grange and Zumla, 2002 ; Reichman and Hershfield, 2000).

Tubercle bacilli are aerobic, non-motile, non-sporing, often slightly curved rods 2-4 μm in length and 0.3-0.5 μm in diameter (Figure 2.1). In common with other mycobacteria, they retain arylmethane dyes on treatment with mineral acids, a property termed acid-fastness. This property is widely used to detect mycobacteria in clinical specimens by light microscopy (the Ziehl-Neelsen method) or by fluorescent microscopy. Tubercle bacilli grow slowly on conventional solid culture media and

colonies take from 2 to 6 weeks to appear (Blanc and Nunn, 1999 ; Grange and Zumla, 2002 ; Reichman and Hershfield, 2000).

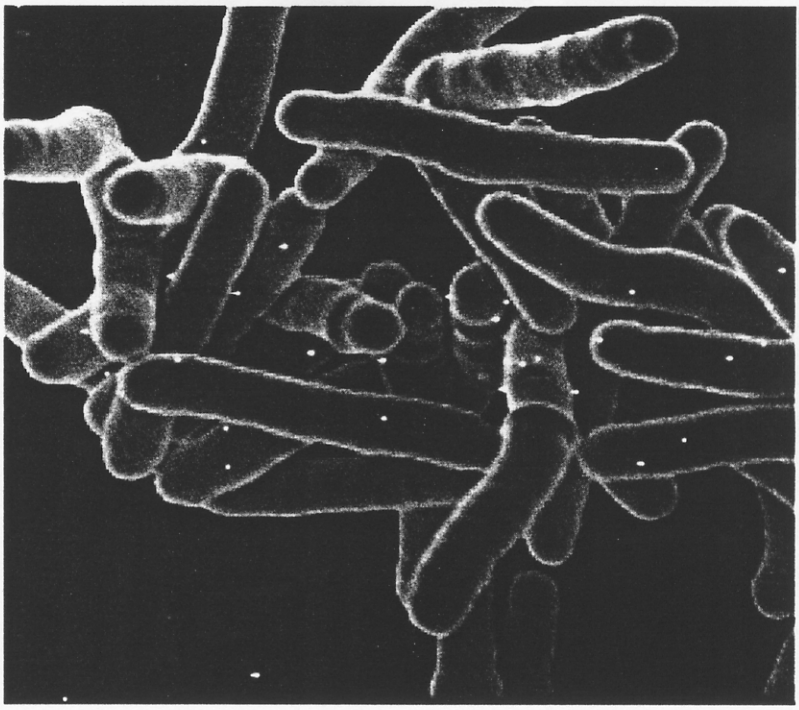

Figure 2.1 Scanning electron microscope of *M. tuberculosis* (Adapted from http://www.niaid.nih.gov/dir/labs/lhd/barry.htm)

The cell envelope is unusually thick and waxy. It has an elaborate structure (Figure 2.2) comprising four classes of polymer: peptidoglycan; arabinogalactan; the mycolic acids; and lipoarabinomannan. The basic structure of the envelope has been known for some time, but the biosynthetic processes involved in its construction have only recently begun to be determined.

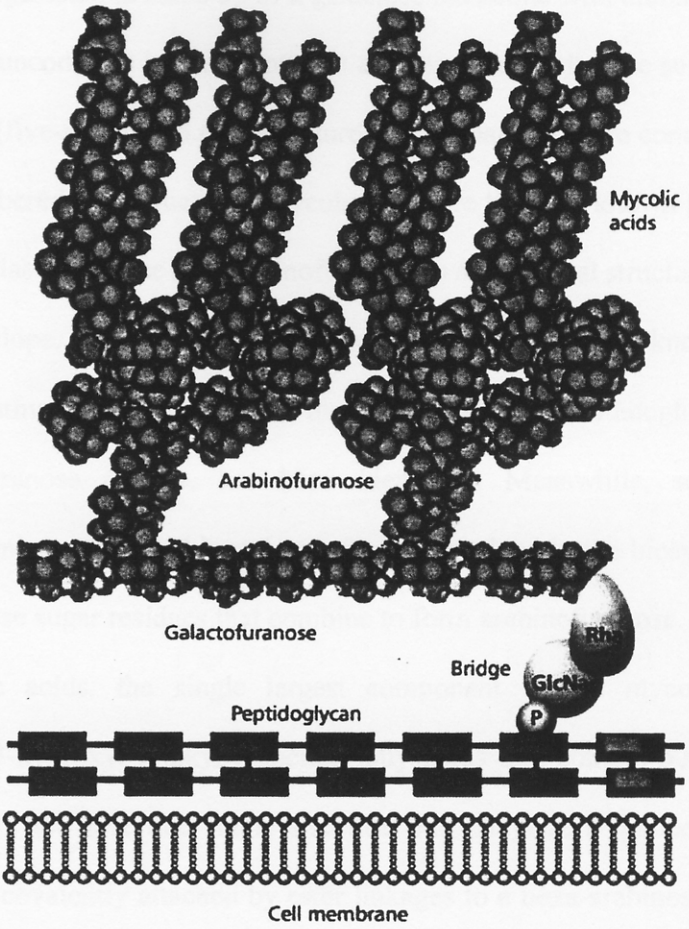

Figure 2.2 Mycobacteria cell envelope (does not show LAM)
(From http://www.chemsoc.org/chembytes/ezine/1998/evans.htm)

The peptidoglycan found in *M. tuberculosis* is generally similar to that found in other bacteria and consists of linear polysaccharide chains, extensively cross-linked by short peptides. Peptidoglycan is linked to the next polymer in the cell envelope, arabinogalactan, by a unique diglycosylphosphoryl bridge, containing rhamnose and *N*-acetyl glucosamine. Recent research has isolated one of the enzymes involved in the synthesis of this linkage, rhamnosyl transferase.

Arabinogalactan is made up of a galactose backbone with arabinose branches. Its structure is uncommon because both the arabinose and galactose sugars are in the furanose form (five-membered ring structure) rather than the more common pyranose form (six-membered ring structure). Mycobacteria are the only known pathogens that contain both galactofuranose and arabinofuranose in an essential structural component of the cell envelope. Galactofuranose is seldom found in nature and knowledge of the biosynthetic pathways involved in its formation is limited, although one enzyme, UDP-galactopyranose mutase, has been identified. Meanwhile, scientists have identified a number of the arabinosyl transferases involved in the biosynthesis of the various arabinose sugar residues that combine to form arabinofuranose.

Mycolic acids, the single largest component of the mycobacterial cell envelope, are 3-hydroxy, 2-alkyl-branched fatty acids that contain 60 to 90 carbon atoms. They do not have one single structure but comprise a number of different forms, and are covalently attached by ester linkages to a hexa-arabinose motif found at the terminus of the branched arabinogalactan. A number of the enzymes involved in the various stages of mycolic acid biosynthesis have been identified. These include: enoyl reductase, cyclopropanated mycolic acid synthase; the three fibronectin-binding proteins that form the so-called antigen 85 complex; and mycolyl transferase, which catalyses the exchange of mycolic acids between trehalose, trehalose monomycolate, trehalose dimycolate and the cell envelope.

The glycolipid lipoarabinomannan (LAM) is the final part of the cell envelope. Its complex structure is built around a core of mannose residues, to which are attached multiple, branched, mannose-capped arabinofuranosyl side chains and a phosphatidyl-inositol unit, which may be used as a linkage to the other elements of the cell

envelope. One of the enzymes involved in LAM biosynthesis, polyisoprenolphosphate mannosyltransferase, has recently been isolated and partially purified.

2.2 Epidemiology of TB

TB continues to cause an enormous global problem. Despite a steadily declining infection rate in the 1980's, there has been a resurgence of this disease during the last decade. The global prevalence of TB was 32% (1.86 billion people) and the global fatality rate was 23%. The number of estimated new cases of TB was 7.96 million in 1997, with 80% of all incident TB cases being found in 22 countries and more than 40% in five Southeast Asian countries (Blanc and Nunn, 1999 ; Grange and Zumla, 2002 ; Reichman and Hershfield, 2000 ; Storey, 2004).

In the year 2000, around 8 million new cases of TB arose from the infected pool, 95% of them in the developing nations. Around 1.5 million cases occurred in sub-Saharan Africa, nearly 3 million in Southeast Asia and over a quarter of a million in Eastern Europe. Figure 2.3 illustrates the estimated rates of TB incidence by continent. Owing to the chronic nature of the disease and the limited resources for effective diagnosis and treatment in many countries, there are at any given time around 20 million people with active TB. Around half of these have infectious forms of the disease and infect some 100 million people annually. Between 2 and 3 million people, principally young adults, die of TB each year, with 98% of deaths occurring in the developing nations.

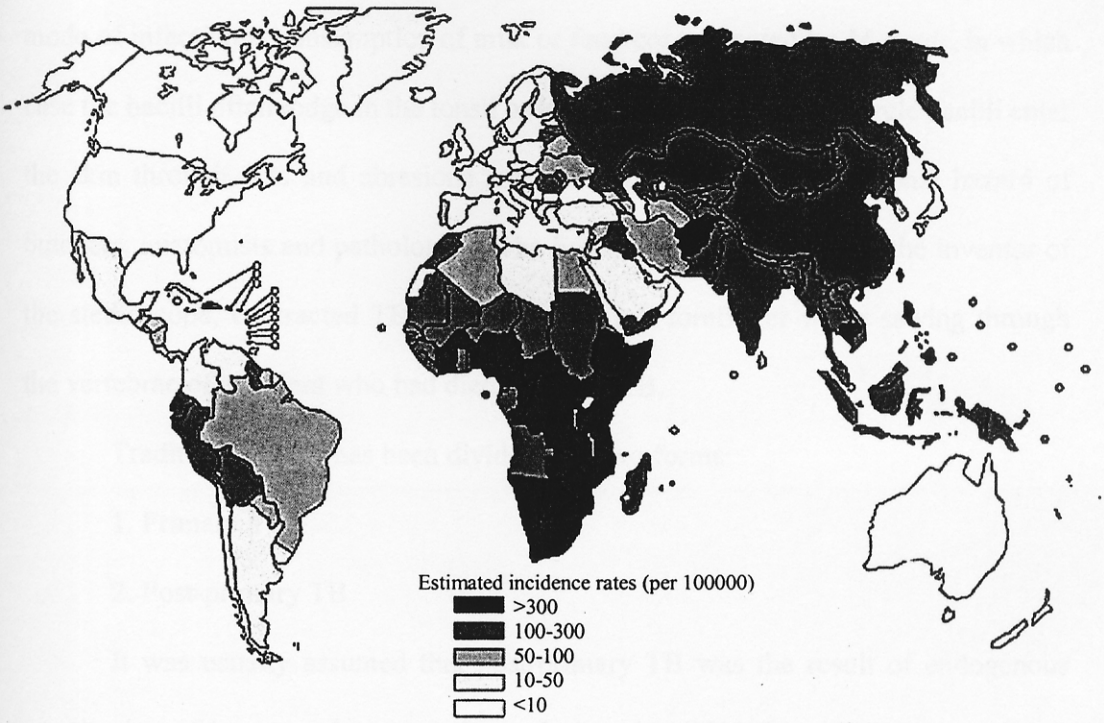

Figure 2.3 Estimated TB incidence rate

2.3 Pathogenesis

Infection of humans with *M. tuberculosis* can occur via several routes:

1. Inhalation
2. Ingestion
3. Inoculation

Congenital transmission is extremely rare. The usual route of infection is via inhalation of small droplets of cough spray containing bacilli. These spray particles, around 5 μm in diameter and containing a few bacilli, lodge in the alveolae or small airways, usually in the lower regions of the lung. The usual sources of such infectious particles are other human beings with open pulmonary TB but agricultural workers

may be infected by *M. bovis* in the cough spray of diseased cattle. A less frequent mode of infection is consumption of milk or food contaminated by *M. bovis*, in which case the bacilli often lodge in the tonsil or intestinal wall. Rarely, tubercle bacilli enter the skin through cuts and abrasions and TB is formerly an occupational hazard of butchers, anatomists and pathologists. The French physician Laenecc, the inventor of the stethoscope, contracted TB by injuring his left forefinger while sawing through the vertebrae of a patient who had died of spinal TB.

Traditionally, TB has been divided into two forms:

1. Primary TB

2. Post-primary TB

It was usually assumed that post-primary TB was the result of endogenous reactivation of latent or dormant primary lesions but DNA fingerprinting has shown that many cases, particularly among immunosuppressed persons, are due to exogenous reinfection.

The majority of people infected by tubercle bacilli do not develop clinically evident TB and the primary infection may go unnoticed. Their bodies mount an effective immune response that encapsulates the organism and contains it for the rest of their lives. As a general rule around 2-5% of persons infected develop clinically evident primary TB and a further 2-5% subsequently develop post-primary disease. Little is known of the early events following initial infection and our limited understanding derives principally from experimental observations in animals. In the case of pulmonary infection, the bacilli are initially engulfed by alveolar macrophages in which they multiply, eventually killing the cell. Additional blood-borne phagocytic cells, both macrophages and polymorphonuclear leucocytes, aggregate around the

focus of infection and form a foreign body granuloma termed the *primary focus*. Some bacilli are transported to the regional lymph nodes (the mediastinal, paratracheal and the supraclavicular nodes when the primary focus is in the lung) where secondary lesions develop. The combination of the TB primary focus, with lymphangitis and lymphadenitis, is termed the *primary complex*. Some bacilli may subsequently enter the bloodstream and lodge in various organs of the body and cause the various non pulmonary forms of primary TB. In the majority of cases, the immune response enables the primary complex to contain the infection; the lesions (lung, tonsil, gut or skin) become fibrotic and may subsequently become calcified but tubercle bacilli may persist within these dormant lesions for years or decades. Bacilli may also persist outside lesions as *in situ* nucleic acid amplification techniques have revealed the presence of DNA specific to the *M. tuberculosis* complex in various cells of normal lung tissue derived post mortem from persons in areas with a high incidence of TB. The nature and form of these "persisters" have generated much speculation. Some researchers postulate that they are truly dormant and that their reactivation involves a "wake-up gene" producing a resuscitation-promoting cytokine factor (Rpf), while others suggest that they replicate, though slowly, but are destroyed by immune mechanisms at roughly the same rate (Grange and Zumla, 2002 ; Reichman and Hershfield, 2000).

Primary infection is often self-limiting and resolves itself in a large majority of cases but in a minority of cases it manifests as clinical disease in a number of ways and local or systemic spread may occur. The primary foci at the periphery of the lung may rupture into the pleural cavity, causing a self-limiting pleural effusion or a much more serious empyema. Diseased mediastinal lymph nodes may rupture into the

pericardial cavity, causing tuberculous pericarditis, or into a bronchus, causing a spreading endobronchial infection and bronchopneumonia. Enlarged mediastinal lymph nodes may press on the major bronchi, causing partial or total obstruction and pulmonary collapse. The primary lesion may progress to tuberculous pneumonia with tissue destruction, especially when immunity is compromised. Alternatively, the primary lesion may gradually enlarge to form a circular "coin lesion" which may progress to a characteristic post-primary lesion or may heal with calcification. Concentric rings of calcification, resulting from alternating periods of progression and healing, may be seen. Primary lesions in the tonsils may spread to cervical nodes, from which local and systemic spread may occur.

Haematogenous dissemination following infection leads to serious, often fatal, non-pulmonary disease, principally involving the central nervous system, bones and kidneys. Observations in the pre-chemotherapy era, notably by Wallgren (Grange and Zumla, 2002) revealed a sequence of events, or "timetable", of primary TB, as shown in Table 2.1. This is only a rough guide and many individual variations occur. Young children are very prone to overt disease following infection but those between the age of 5 years and the onset of puberty appear to be relatively protected the "safe school age".

Table 2.1 The "time table" of primary TB

Stage	Duration	Features
1	3-8 weeeks	The primary complex develops. Conversion to tuberculin positivity occurs.
2	About 3 months	Life threatening forms of disease due to haematogenous dissemination occur, i.e. tuberculous meningitis and miliary TB.
3	3-4 months	Tuberculous pleurisy may be the result of either haematogenous spread or direct spread from an enlarging primary focus.
4	Up to 3 years	The stage lasts until the primary complex resolves. More slowly developing extrapulmonary lesions, particularly in the bones and joints, may appear.
5	Up to 12 years	Genitourinary TB may occur as a late manifestation of primary TB.

Within 3-8 weeks of initial infection conversion to dermal reactivity to tuberculin occurs. Since the description of the tuberculin skin test by the Austrian physician Clemens von Pirquet in the early 1900s, there has been considerable speculation as to the nature and significance of the positive tuberculin test, particularly its relevance to protective immunity. It now appears that the dermal induration seen in a positive tuberculin reaction is due to tissue oedema resulting from a number of immune processes, some associated with protection and some not. Thus a positive test is an indicator of recent or past infection by a tubercle bacillus or BCG vaccination but not of the immune status of the infected person.

Post-primary TB differs from primary disease in several important features. It may develop directly from a primary lesion *progressive primary TB* but more often there is a latent phase of several years or even decades before the disease becomes apparent. As mentioned above, post-primary TB may be the result of endogenous reactivation of latent foci of infection or to exogenous reinfection. In the case of the lung, post-primary lesions often develop, for poorly understood reasons, in the upper

regions of the lung. The characteristic feature of post-primary pulmonary TB is gross tissue necrosis which is attributable to cytokines, thought to be secreted by Th2 helper T cells, which render infected tissue very susceptible to killing by tumour necrosis factor. As a result, large lesions containing abundant caseous necrotic tissue develop and, as they radiologically resemble tumours, they are termed tuberculomas. The centre of the tuberculoma is anoxic and acidic and is a hostile environment to tubercle bacilli, so that relatively few viable bacilli are present. The caseous material is softened and eventually liquefied by proteases secreted by activated macrophages. The enlarging tuberculoma may eventually erode into a bronchus so that the softened or liquefied caseous material is discharged into the bronchial tree and a cavity, a characteristic feature of post-primary pulmonary TB is formed. The environment of the cavity is quite different from that of a closed tuberculoma. Air enriched with carbon dioxide enters the cavity, providing oxygen for the bacilli and neutralizing the acidity. The tubercle bacilli are then able to replicate freely and huge numbers line the cavity well. These bacilli gain access to the bronchi and are expectorated in the sputum, the patient becomes infectious and is said to have open TB. Bacilli escaping from the cavities also infect other parts of the same, and often the other, lung via the bronchial tree. A typical radiological appearance of post-primary pulmonary TB is of one or more apical cavities and numerous smaller lesions in the other lung fields. Bacilli in the sputum may also lodge in the larynx, causing tuberculous laryngitis, or may be swallowed and cause indurating ulcers in the intestinal tract and, rarely, anal fistulae. In contrast to primary TB, the post-primary lesions are usually so walled off by fibrosis that lymphatic and haematogenous dissemination of disease is unusual.

Both cavity formation and the localization of disease are due to immune processes and, as described below, are compromised in immunosuppressed patients.

2.4 Treatment of TB

Highly effective drug treatments for TB have been available for half a century. These treatments, when used within the WHO DOTS (Directly Observed Treatment, Short-courses) strategy, form the basis of the modern management of TB.

Chemotherapy

The three aims of antituberculosis chemotherapy are:

1. To cure the patient.

2. To render the patient rapidly non-infectious.

3. To prevent the emergence of drug resistance.

From the point of view of therapy, the tubercle bacilli may be thought of as being in three different "compartments": those replicating rapidly on the walls of the cavities, those replicating less rapidly in anoxic and acidic solid lesions and those in a dormant or near dormant state within dense lesions or macrophages. It is important to kill all bacilli as the immune responses cannot be relied on to deal with any remaining bacilli.

Effective cure of the patient is ensured by using agents able to kill bacilli in all three physiological compartments. In those with open or infectious pulmonary TB, the great majority of bacilli are freely replicating in the cavity walls and are rapidly killed by isoniazid, thereby rendering the patient non-infectious. Isoniazid is less active against slowly replicating bacilli in closed, acidic lesions but rifampicin and

pyrazinamide are effective against this population. There are two phases in the drug treatment of TB:

1. *An initial phase* lasting for 2 months where three (rifampicin, isoniazid, pyrazinamide) or four (plus ethambutol) antituberculosis drugs are given. This intense attack reduces the load of mycobacteria and allows sensitivity patterns to be established.

2. *A continuation phase* during which rifampicin and isoniazid are continued for a further 4 months at least. There are other continuation regimens recommended by the WHO in use in low-income countries.

If the patient regularly receives at least two drugs to which the bacilli are susceptible, the chance of the emergence of drug resistance is very small. In view of the increasing prevalence of resistance to one or two drugs, a fourth drug, usually ethambutol, is now routinely given in the intensive phase of treatment. An alternative to ethambutol is streptomycin but, as this must be given by intramuscular injection, there is a risk of transmitting HIV and other viruses by use of inadequately sterilized needles.

The best drug for the destruction of near-dormant persisting bacilli is rifampicin, which is therefore given during the continuation phase. Although isoniazid has little activity against near-dormant bacilli, it is included in the continuation phase to destroy any rifampicin resistant mutants that commence active replication.

Modern short-course regimens have the added advantages of low toxicity and low cost. In most regimens, all the drugs are given orally. As four drugs are used in the intensive phase, resistance to one of the drugs used does not render the regimen ineffective. Combination tablets are available, usually isoniazid + rifampicin or

isoniazid + rifampicin + pyrazinamide. The WHO recommends that only those combination tablets that have been shown in human studies to yield bactericidal levels of the constituent drugs should be used. Table 2.2 is shows the WHO-recommended short-course for TB treatment regimen.

Table 2.2 The WHO-recommended short-course antituberculosis drug regimens

Duration	Initial phase	Continuation phase
1. For 6 months treatment	Initial 2 months	4 months later
Isoniazid	5 mg/kg/day	5 mg/kg/day
Rifampicin	10 mg/kg/day	10 mg/kg/day
Pyrazinamide	35 mg/kg/day	
Plus		
Streptomycin	15-20 mg/kg/day	
or Ethambutol	25 mg/kg/day	
2. For 8 months treatment	Initial 2 months	6 months later
Isoniazid	5 mg/kg/day	5 mg/kg/day
Rifampicin	10 mg/kg/day	10 mg/kg/day
Pyrazinamide	35 mg/kg/day	
Thioacetazone	4 mg/kg/day	4 mg/kg/day
Plus		
Streptomycin	15-20 mg/kg/day	
or Ethambutol	25 mg/kg/day	
3. For 12 months treatment	Initial 2 months	10 months later
Isoniazid	5 mg/kg/day	5 mg/kg/day
Rifampicin	15-20 mg/kg/day	
Plus		
Thioacetazone	4 mg/kg/day	4 mg/kg/day
or Ethambutol	15 mg/kg/day	10 mg/kg/day

2.5 Physiology of the Lungs

2.5.1 Histological features of the lungs

The respiratory tract can be divided into upper airways (the nose, mouth, larynx, and pharynx) and lower airways (from the trachea to the alveoli). The average weight of human lungs is 0.6 kg. Because the lungs receive the entire cardiac output, their blood flow is as high as 5,700 ml/min, more than five times that of the portal system (1,125 ml/min), including the stomach and the small and large intestines. Airway diameter decreases and surface area increases according to the successive branching of the airways. The cross sectional area of the trachea is about 2.5 cm^2, while that of the respiratory zone is much wider. The total cross sectional area of the alveoli is about 10^4 cm^2. The total surface area of the airway tubes also increases and that of alveoli is more than 100 m^2 as large as that of the small intestine. The epithelial layer of the trachea is composed mainly of columnar ciliated cells. The thickness from the airway surface to blood vessels is on the order of 30 to 40 μm. Particulates deposited in the upper airways are rapidly carried away by mucociliary transport, resulting in a short of residence time. The alveolar surface is populated by two major epithelial cell types: the terminally differentiated type I cell and its progenitor type II cell. The alveolar epithelium is quite thin. In the alveoli drugs have to travel only 0.5 to 1.0 μm to enter the blood stream. Total fluid volume in the human lungs is approximately 10 ml. Lung pH at the site of drug absorption has been estimated at about 6.6 (Okamoto *et al.*, 2002). The alveolar surface is lined by a surface-active material called the lung surfactant, which is a mixture of lipids, proteins, and carbohydrates. Phospholipids account for 75-80% of the total weight,

and dipalmitoyl phosphatidylcholine (DPPC) accounts for nearly half of that. The lung surfactant reduces alveolar surface activity and stabilizes alveolar structure. Lavage of a normal adult lung yields a cell count that is 93% macrophages, 7% lymphocytes, and less than 1% neutrophils, eosinophils, or basophils. Alveolar macrophages interact with microorganisms or particulates, act as effector and accessory cells in inflammatory and immune reactions, and protect alveolar structures to form a protease attack (Okamoto *et al.*, 2002).

2.5.2 Drug absorption through the lungs

The pulmonary absorption of small molecules basically obeys pH-partition theory, *i.e.*, drugs are likely to be absorbed by diffusion across a lipid membrane. *In vivo* rat lung absorption data for saccharides of various molecular weights (122 to 75,000) showed that the absorption rate constants were inversely related to molecular weight and directly related to the diffusion coefficients of the compounds. The transport of dextrans (4 to 150 kDa) across rat alveolar epithelial cell monolayers suggested that macromolecules with a radius under 5 μm transported across paracellular pathways, while macromolecules with a radius of 6 μm or larger across the barrier via other pathways such as pinocytosis. The penetration of hydrophilic compounds through excised rabbit trachea sacs also inversely correlates to molecular weight. When several hydrophilic and lipophilic drugs were administered through rat trachea as aerosols, the absorption rates were roughly twice as rapid as when administered by the intratracheal injection of drug solutions. These results suggest that drug absorption is more rapid in the alveolar region than in the tracheobronchial region of the lungs. The distal or deep lung is the optimal site for the

high absorption of proteins. Inhaler systems should be designed to maximize deposition in this region. In general, the metabolic activity of the lungs is much lower than that of the intestinal wall and liver. In the lungs there is no first-pass conjugation of some drugs (Okamoto *et al.*, 2002).

2.5.3 Particle characteristics

The size of the particles is a critical factor affecting the site of their deposition, since it determines operating mechanisms and extent of penetration into the lungs. Aerosol size is often expressed in terms of aerodynamic diameter. The aerodynamic diameter is defined as the equivalent diameter of a spherical particle of unit density having the same settling velocity from an air stream as the particle in question. Thus, particles having density higher than one will have actual diameters smaller than their aerodynamic diameter. Conversely, particles with small density (< 1g/ml) will have geometric diameters larger than their aerodynamic diameter. Aerosol size distributions may be characterized as practically monodisperse (uniform size, geometric standard deviation (GSD) of < 1.2) or polydisperse (nonuniform sizes, GSD ≥ 1.2) (Suarez and Hickey, 2000).

In mammals, respiratory anatomy has evolved in such a way as to actively prevent inhalation of airborne particulates. The upper airways (nose, mouth, larynx and pharynx) and the branching anatomy of the tracheobronchial tree act as a series of filters for inhaled particles. Thus, aerosol particles > 100 μm generally do not enter the respiratory tract and are trapped in the naso/oropharynx. Particles > 10 μm will not penetrate the tracheobronchial tree. Particles must generally be < 5 μm in order to reach the alveolar space. On the other hand, particles < 0.5 μm in diameter

penetrate the lung deeply, but have a high tendency to be exhaled without deposition (Hickey, 1992 ; Suarez and Hickey, 2000).

The traditional particle size analysis methods for medical inhalers in the US and European Pharmacopoeias require some forms of drug assay be undertaken of the collected size fractions, so that there is a direct link between measured particle aerodynamic size and the mass of active pharmaceutical ingredient (API). This link is especially important when excipients, such as surfactant particles, are present together with API. Non-invasive techniques based on light scattering or the time-of-flight (TOF) principle cannot distinguish between API and non-volatile, non-pharmaceutically active substances in the formulation. They will measure an overall particle size distribution which may not be a reflective of the actual API based size distribution. The current compendial techniques listed in both USP and Ph.Eur. are summarized in Table 2.3. They are all based on the principle of inertial impaction that size-fractionates the incoming aerosol weight in terms of API mass. In addition, this table also includes the Next Generation Pharmaceutical Impactor (NGI, MSP Corp., Shoreview, MN, USA), as this equipment will be adopted as apparatus 5 and 6 in the USP and also as apparatus E in the European Pharmacopoeia. It should be noted that apparatuses A and B of the Ph.Eur. are the glass Twin Impinger and single-stage metal impactor respectively, both of which are likely to be shortly withdrawn, as although they are useful for rapid quantification of fine particle fraction (FPF), they provide insufficient size resolution in the critical range from 0.5 to 5.0 μm aerodynamic diameter. In the case of DPI testing, a fixed volume of air is drawn through the inhaler with the valve to the pump downstream of the inertial impactor or impinger operated at critical flow, so that the resistance of the inhaler determines the

precise flow rate-time profile once the solenoid valve is actuated to begin the process of sampling from the DPI. The final flow rate is achieved rapidly, and is therefore used to define the size-separating performance of the particle size analysis equipment. This process is closer to actual use by a patient, as it simulates the inhalation process, but there is no attempt to replicate actual inhalation flow rate-time profiles. In contrast, the impactor or impinger is always operated at constant flow rate (Mitchell and Nagel, 2004).

Table 2.3 Particle size analysis methods for medical aerosols listed in the current USP and Ph. Eur.

Apparatus Description	USP	Ph. Eur.*
Andersen 8-stage impactor /no preseparator	Apparatus 1 for pMDIs	Apparatus D
Marple-Miller model 160 5-stage impactor	Apparatus 2 for DPIs	-
Andersen 8-stage cascade impactor/preseparator	Apparatus 3 for DPIs	Apparatus D
Multi-stage liquid impinger (4-stages)	Apparatus 4 for DPIs	Apparatus C
Next Generation Pharmaceutical Impactor (7-stages)	Apparatus 5 for DPIs Apparatus 6 for MDIs	Apparatus E

* 1. Apparatus A is the glass Twin Impinger and apparatus B is the Metal Impinger that are both anticipated to be withdrawn in a future revision of Ph. Eur.
2. All apparatuses listed in the table utilize the USP/Ph. Eur. Induction port (throat)

Table 2.4 contains a summary of the particle size analysis techniques that are currently in widespread use for the evaluation of medical inhalers, showing the operating principle, size range and inhaler types for which they are most applicable. Most particle sizing methods used for inhaler aerosol assessments are invasive, in that they require either a sample or the entire aerosol produced on actuation to be collected by the measurement equipment. Techniques that are based on either inertial impaction or TOF both determine aerodynamic diameter. However,

only multi-stage cascade impactors or liquid impingers directly provides the mass distribution data that are more relevant than the corresponding count/number size distribution results in predicting the mass of API likely to be delivered to different parts of the respiratory tract (Mitchell and Nagel, 2004).

Table 2.4 Summary of particle sizing methods used to characterize medical aerosols from inhalers

Technique	Operating principle	Size Range (μm)	Assay for API	Direct Measure of Aerodynamic Diameter
Cascade impactor, multi-stage liquid impinger	Inertial size separation in laminar flow	0.1-15 μm overall range, but varies from one instrument to another. Cascade impactors typically have 7-8 stages. The current multi stage liquid impinger has 4 stages + back-up filter.	YES	YES
Single stage impactors / Twin Impinger	Inertial size separation in laminar flow	Cut size chosen to separate coarse from fine particles likely to penetrate the lower respiratory tract (*e.g.* 6.4 μm for twin impinger at 60 l/min)	YES	YES
Particle time-of-flight	Particle acceleration in ultra-Stokesian flow; transit time between two detectors	Aerosizer® (no longer available : 0.2-200 μm, extendable to 700 mm with larger nozzle. TSI 3603 PSD analyzer is successor :0.2-700 mm TSI 3321 APS® with 3306 impactor inlet : 0.5-20 μm.	NO	YES
Laser diffractometry	Low angle laser light scattering	0.5 μm-3mm overall range, but varies from one instrument to another.	NO	NO
Phase-Doppler particle size analysis	Phase shift observed by several detectors observing particle interaction with interference fringes formed from intersecting laser beams	0.3-several hundred μm. Precise range depends upon the optical configuration chosen. Particle velocity can also be measured in 1-, 2- or 3-components of direction, depending on sophistication of measurement system.	NO	NO

Ideally, the measurement technique should not perturb the inhaler aerosol being evaluated, since the process of moving the aerosol to the measurement instrument may alter the size distribution by enhancing processes that cause particles to deposit prematurely, or may result in droplet size reduction as a result of evaporation of volatile species, if care is not taken to minimize such behavior. So-called noninvasive methods, which are all based on some form of particle-light interaction process, have not yet been refined to the point at which simultaneous chemical assay of the measured particles takes place to link the size measurement with mass of API delivered from an inhaler. However, a new generation of noninvasive instruments, such as the Ultra-Violet Aerodynamic Particle Sizer® aerosol spectrometer (TSI Inc., Shoreview, MN, USA) offers the potential for combining particle sizing with an assay procedure (Mitchell and Nagel, 2004).

2.6 New Emerging Alternative Methods of TB Therapy

2.6.1 Antituberculosis drugs formulated for inhalers

For over 30 years no antituberculosis agents with new mechanism of action have been developed. Conventional antituberculosis drugs are low efficacy for treatment of TB. The development of antituberculosis drugs delivered to the lung directly is one of the most promising methods of administration. The direct lung delivery of antituberculosis drugs can reduce drug dose, dose frequency and toxicity. Hence, improvement in patient compliance is expected. Most importantly, targeting the drug to the alveolar macrophage may improve efficacy and potentially reduce

systemic toxicity. In addition, giving a high local drug concentration may reduce the duration of treatment and prevent the multi-drug resistance of TB.

The delivery of drugs to the lower airway can be formulated in several drug delivery systems such as a metered dose inhaler (MDI), nebuliser and DPI (Hickey, 1992 ; Sethuraman and Hickey, 2002).

A nebuliser (Figure 2.4) is an atomiser which can generate small droplets of condensable fluids. The droplets produced range up to 10 μm, which are much smaller than those produced by the spray atomiser (>100 μm). The atomised droplets move into an evaporation section, where they are almost completely vaporised. However, a tiny residue of particles remain which serve as nuclei in the vapour and these nuclei provide a steady supply of monodisperse aerosols of approximately submicron size. Either solutions or suspensions may be aerosolised with nebulisers. One of the main problems associated with nebulisers is that large residue of the drug formulation remains in the device. Also a long nebulisation time can lead to patient non compliance.

The example of MDI products was shown in Figure 2.5. The main advantages of the MDI are that they are reliable, portable and convenient to use. However, the major problems with MDI are high oral deposition. The rapidly moving propellant droplets form an aerosol, which travels at least 20 cm from the valve before they completely evaporate. Consequently, the use of pressurised aerosols results in extensive drug impaction at the oropharynx and relatively little penetration further to the lungs. Various reservoirs, holding chambers or spacers have been designed in

order to reduce the velocity of the droplets and give a greater chance of obtaining a uniformly small size.

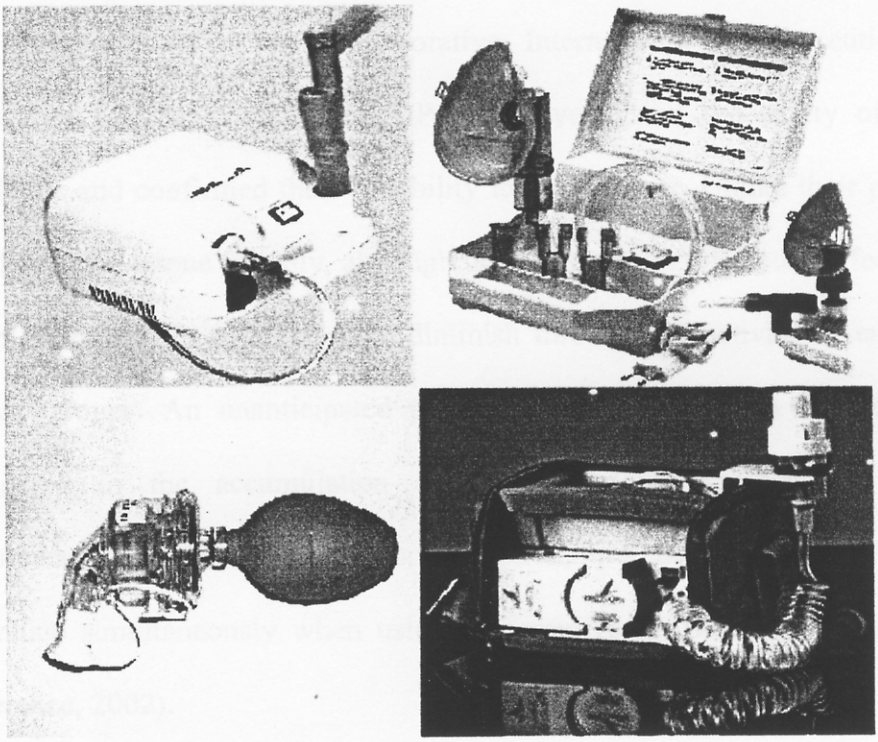

Figure 2.4 Examples of nebuliser on the market today (From http://www. medisave. co.uk/images/omcxp.jpg ; http://www.childcare.ie /images/magazine/ articles/ nebulizer.jpg ; http://wwwmedilinkeast. com/pages/img/ nebuliser.jpg ; http://www. westonsinternet. co.uk/images/bneb92m.jpg)

Despite the MDI popularity, it is currently under threat due to the concern over the detrimental effect of chlorofluorocarbon (CFC) to the ozone layer. Since this time the casual role of CFC in ozone layer thinning has gained support culminating in the signing of the Montreal Protocol in 1987 making a commitment to cease production of the CFC by 1996. Specific exemptions were granted for essential use including the use in MDI. However the pharmaceutical industry faced with the

prospect of a diminishing, expensive supply and the possibility of being overtaken by an equally effective alternative inhalers. As a result, new alternative propellants were developed, the most important group being the hydrofluoroalkanes (HFAs) such as HFA 134a and 227. The Collaborative International Pharmaceutical Aerosol Consortium for Toxicity Testing (IPACT) investigated the safety of these new propellants and confirmed their suitability for human use. Unlike their predecessors, these HFAs are ozone friendly, although they do exert a greenhouse effect, for which reason their use in the future may diminish through the activity of environmental pressure groups. An unanticipated potential problem for HFA has recently been highlighted in the accumulation of the breakdown product of HFA 134a, trifluoroacetic acid, in wet land areas. In addition, up to 80% of patients fail to actuate and inhale simultaneously when using a pressurized MDI (British Pharmaceutical Conference, 2002).

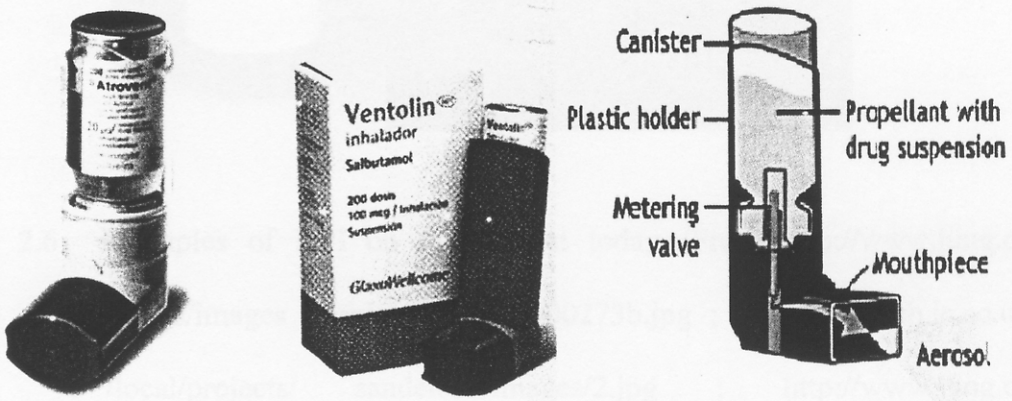

Figure 2.5 Examples of MDI on the market today (From http://www.lung.ca/ drugs/ images /respiratory /img 000047b.jpg ; http://www.gsk. com.ve/attach /156/default/ventoloin.jpg;http://www.siamhealth.net/ Disease/Respirator/ asthma1 /Inhaler.gif)

The DPIs on market today such as Turbuhaler®, Diskhaler®, Diskus® (Accuhaler), Clickhaler®, Pulvinal®, and Easyhaler® (Smith and Parry-Billings, 2003) are shown in Figure 2.6.

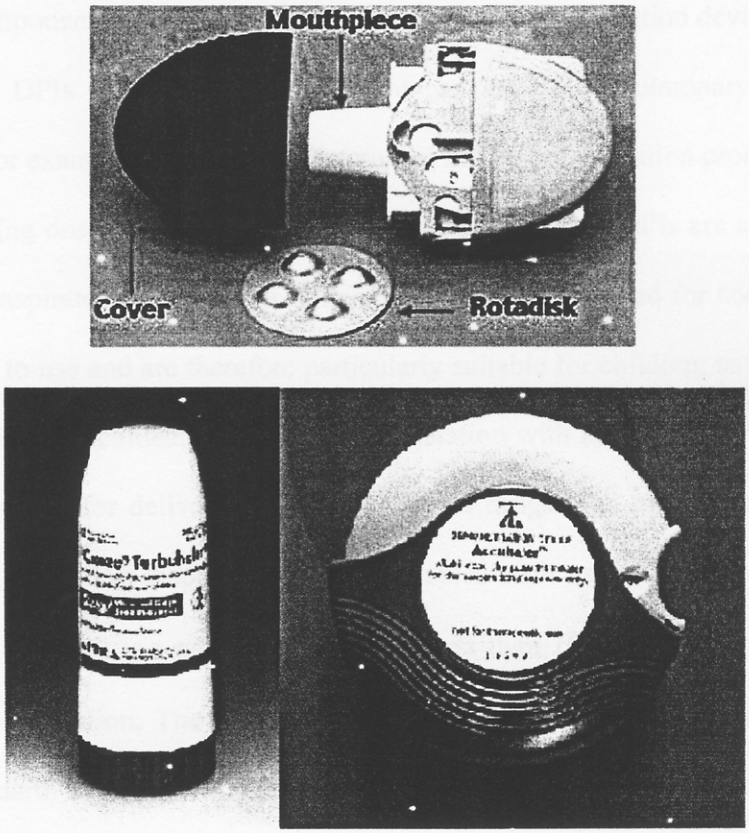

Figure 2.6 Examples of DPI on the market today (From http://www.lung.ca /drugs/images /respiratory /img000273b.jpg ; http://www.ch.ic.ac.uk /local/projects/ sanderson/images/2.jpg ; http://www.lung.ca /drugs/images/respiratory /img000215b.jpg http://www. asthmaeducatorsvic. org.au/images/ Photographs /Accuhaler %20counter.jpg)

DPIs appear to be the most promising of lung delivery for future use because the device is small and relatively inexpensive (Okamoto *et al.*, 2002). These

combine powder technology with device design in order to disperse dry particles as an aerosol using the patient's inspiratory effort as an energy source. All DPIs have four basic features: (1) a dose metering mechanism (2) an aerosolisation mechanism (3) a deaggregation mechanism (4) an adaptor to direct the aerosol into the patient's mouth. The major components of a DPI are the formulation and the inhalation device.

DPIs represent a significant advance in pulmonary delivery technology. For example, they are breath-actuated and so co-ordination problems such as synchronizing dose discharge with inhalation are overcome. DPIs are actuated by the patient's inspiratory effort in his or her own time with no need for coordination. DPIs are easy to use and are therefore particularly suitable for children, as well as for adults who have difficulties in coordinating inhalation with actuation. DPIs are also potentially suitable for delivering a wider range of drugs than pressurized metered dose inhalers, including biopharmaceutical therapies such as peptides and proteins. Indeed, DPIs can deliver a range of doses from less than 10 μg to more than 20 mg via one short inhalation. The disadvantage of DPI such as irritation caused by the powder (Bisgaard, 1998 ; British Pharmaceutical Conference, 2002 ; Malcolmson and Embleton, 1998).

In theory, using inhaled antibiotics for treatment of pulmonary infections should have several advantages over the same compounds given by more conventional oral routes. For tracheobronchial infections in particular, the deposition of inhaled aerosol particles on the surface of the airways can result in peak concentrations at the infection site that are several orders of magnitude higher than those can be achieved by oral or intravenous delivery. The topical administration of an inhaled aerosol to the airways can generate high pulmonary concentrations while

simultaneously minimizing both peak serum levels and the exposure of non-involved tissue to the drug. Thus inhalation can provide a way to circumvent the dose-limiting systemic toxicity of an antibiotic. Finally, for compounds with low oral availability, inhalation is a cost-effective alternative to intravenous delivery for out patient use (Challoner, 2002).

There are a few studies of antituberculosis drugs as dry powder formulations for delivery to the lower airway. Reverchon *et al.* (2002) formulated rifampicin as microparticles produced by supercritical antisolvent precipitation obtaining particles with mean diameters ranging from 0.4 and 1 μm and 2.5 to 5 μm depending on the pressure operated in the production process. This method was suitable for producing DPIs. According to Falk *et al.* (1997), who prepared rifampicin microspheres by precipitation with a compressed antisolvent using poly(L-lactide) for controlled release. The results suggest that drug/polymer particles were spherical in shape and between 0.2 and 1.0 μm in diameter but rifampicin cannot be used for controlled release in this formulation.

Para-aminosalicylic acid was formulated into large porous particles by spray drying technique for direct delivery into rat lungs via inhalation. The results show these particles less than 3.4 μm and have physical stability over 4 weeks at elevated temperatures. *Para*-aminosalicylic acid delivered by insufflation had very high concentration in the lung and was cleared within 3 hours from the lung lining fluid and plasma but was still present at therapeutic concentrations in the lung tissue. These results suggest that inhalation delivery of *para*-aminosalicylic acid can potentially allow for reduction in the total dose delivered while providing for higher local and lower systemic drug concentrations (Tsapis, 2003).

Dutt and Khuller (2001b) formulated isoniazid and rifampicin as liposome and microparticles using poly(DL-lactide-co-glycolide) as carrier. The results indicate that antituberculosis drugs exhibited a sustained release up to 4 weeks in the lungs. Saurez *et al.* (2001) encapsulated rifampicin in microspheres and delivered it to alveolar macrophage that host cells of *M. tuberculosis* by insufflation or nebulization directly to rat lungs. The results suggest that these formulations reduced inflamation and lung trauma compared with control groups. Similarly the study of Sharma *et al.* (2001), who formulated isoniazid and rifampicin as microparticles using poly(DL lactic acid) targeted to murine alveolar macrophages for the treatment of TB. The results suggest that the inhalation of microparticles containing multiple antituberculosis drugs offer a promising reduction of dose and dosing-frequency, toxicity alleviation and targeted macrophage resident persistent mycobacteria. In addition, Vyas *et al.* (2004) designed liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. The results showed that after delivery to the rat lungs rifampicin had an immediate high concentration and maintained high drug concentration for a long period.

2.6.2 Carrier for DPIs

The excipient, or "carrier", is used to aid dispersion of drug form the inhaler device and ease handling of bulk powder during manufacturing and filling processes (French *et al.*, 1996). DPIs must be used for formulation with or without carriers for the delivery of active drugs to the lower airways. Lactose and mannitol are US FDA-approved carriers for inhalation. Many studies formulated DPIs using lactose as carrier were suitable for lung delivery (Flament *et al.*, 2004 ; Gilani *et al.*,

2004 ; Larhrib *et al.*, 2003a ; Larhrib *et al.*, 2003b ; Sham *et al.*, 2004 ; Steckel *et al.*, 2004 ; Tee *et al.*, 2000 ; Tsapis *et al.*, 2002). However, lactose cannot be used for compounds that interact with the reducing sugar function of the lactose such as formoterol, budesonide or peptide and proteins. For these results, mannitol and glucose monohydrate showed potential as drug carrier to be used in DPIs instead of lactose (Steckel and Bolzen, 2004). Some compounds can be used for carriers in DPIs such as D-mannitol, D-trehalose monohydrate (Bosquillon *et al.*, 2004), γ-cyclodextrin (Srichana *et al.*, 2001), sorbitol (Tee *et al.*, 2000), polyethyleneglycol (Gilani *et al.*, 2004 ; French *et al.*, 1996), poly(DL-lactide-co-glycolide) (Dutt and Khuller, 2001a ; Dutt and Khuller, 2001b ; Sethuraman and Hickey, 2002). In addition, endogenous materials such as albumin, DPPC and protein stabilizers such as trehalose are used as excpients for carriers to improve aerosolization properties (Bosquillon *et al.*, 2001).

Mannose, the disaccharide, was found to be an interesting as a carrier for the delivery of antituberculosis drugs because of its biodegradable properties which may help in the uptake of alveolar macrophage that host cells of *M. tuberculosis* and results in killing the mycobacteria. Opanasopit *et al.* (2001) studied and formulated liposomes which coated with serum mannan binding protein. They found that macrophages attracted mannosylated liposome by the mannose receptors that recognized D-mannose, *N*-acetyl-D-glucosamine, or L-fucose on their surface.