Preface
Tuberculosis is the single largest infectious killer disease of the world (Raviglione et al., 1995). The causative organism is *Mycobacterium tuberculosis*, an acid fast bacilli, transmitted primarily via respiratory route. Surveys with purified protein derivative (PPD) or tuberculin skin tests suggest that one third of the world's population is infected with *M. tuberculosis* (Dye et al., 1999). Far from being under control tuberculosis is on the rise in both developing and industrialized countries (Raviglione et al., 1995). A vast majority of the individuals infected with *M. tuberculosis* are latent carriers and these individuals represent slow release reservoirs.

The latent state is characterized by evidence of an immune response against the bacterium (a positive tuberculin skin test) without clinical signs of active infection. Latent *M. tuberculosis* infection presents one of the major obstacles in gaining control over tuberculosis worldwide. Latent carriers harbour a 2-23% life time risk of developing reactivation tuberculosis and the risk for individuals immunosuppressed by HIV infection is estimated to be as high as 10% per year (Bloom & Murray, 1992). In addition, the front line antibiotics are by and large ineffective in eliminating *M. tuberculosis* during latent stages of infection. The reasons for this discrepancy are currently unclear, but it may be a result of the ability of *M. tuberculosis* to enter a quiescent state during periods of persistence. The widespread emergence of drug resistant strains of *M. tuberculosis* has led to tuberculosis becoming a threat to world health.

Much of the success of *M. tuberculosis* as a pathogen and our failure to control this microorganism are due to the ability of this organism to survive in an apparently latent state for long periods within caseous or closed lesions (Wayne, 1994). This clinical latency can persist throughout the person's lifetime. However, in some circumstances the
host immune response is perturbed, and reactivation of latent infection results. This
process can occur, for example, through HIV infection, malnutrition, use of steroids or
other immuno-suppressive medications, or advanced age (Flynn & Chan, 2001).

During the latent infection bacilli persist for years before they reactivate and
cause active tuberculosis (Wayne, 1994; Parrish et al., 1998). How the tubercle bacilli
survive during the latent state of infection is largely unknown. Following the initial
infection, the bacilli typically replicate inside host macrophages until an effective
immune response is mounted and the bacilli become restricted to the characteristic
tuberculous lesions or granuloma and the progression of disease is halted. The bacilli can
survive in the caseous necrotic center of these lesions, but it can not multiply because of
oxygen deprivation and other adverse conditions (Dannenberg, 1994). Thus, granuloma is
presumed to be the hypoxic environment which is responsible for holding bacterial
replication in check and the mycobacterial dormancy probably establishes within this
anaerobic environment of the caseous necrotic material (Wayne & Sohasky, 2001). The
physiological state in which *M. tuberculosis* survives in the lesions is not known. In the
literature, this stage of the disease has been referred to as latency or dormancy, and
dormancy has also been used to describe “the physiological state in which the bacteria
exist” (Cunningham & Spreadbury, 1998; Gangadharam, 1995; Wayne, 1994). In
bacterial physiology, the term dormancy is used to define “a reversible state of low
metabolic activity, in which cells can persist for extended periods without division” (Kell
et al., 1995). It is suggested that an altered physiological state of persistent *M.
tuberculosis* accounts for its tolerance to drugs as well as the ability to survive in the host
for many years. Persistence is likely to be a combined effect of both the immune system
and bacterial physiology, resulting in what is generally referred to as a latent state (Bloom & Mckinney, 1999).

Examination of surgically removed human lung tissue from tuberculous lesions has demonstrated that bacilli are present in blocked airways of tuberculosis patients for years after conversion to sputum negative status, but such bacteria do not appear to be metabolically active (Wayne, 1960). Maintenance of non replicating state is thought to be mediated by reduced oxygen tension and nutrient limitation within a caseous granuloma (Wayne, 1994; Dannenberg, 1993)

There are a number of important questions that remain to be answered with respect to latent tuberculosis. How does the bacteria evade host antimicrobial defenses and survive in the face of a strong immune response? What immune factors contribute to establishment of a latent infection?

Latent M. tuberculosis infection presents one of the major obstacles in gaining control over tuberculosis world wide. Lack of information about the state of the bacilli during clinical latency hinders our ability to model latent tuberculosis in laboratory settings. However, both in vitro (Wayne & Hayes, 1996; Betts et al., 2002) and in vivo systems have been developed which contribute to our current understanding of latency. Clinical and experimental evidences suggest that mycobacterial persistence might arise in response to different stress responses, such as oxygen deprivation (Wayne & Lin, 1982), nutrient starvation (Betts et al., 2002), pH, temperature etc. Wayne showed that oxygen depletion in vitro indeed triggered dormancy response of the bacilli (Wayne, 1994; Wayne & Hayes, 1996). In addition, oxygen starved bacilli are rapidly killed by the drug metronidazole, which is usually used for the treatment of infections caused by anaerobic
microbes (Wayne & Sramek, 1994). It is recently reported that Rv 3133c, alphacrystallin homolog, Rv2623 and Rv2626c were upregulated during dormant state in *M. bovis* BCG (Boon *et al.*, 2001).

Animal models of infected mice which develop a chronic infection similar to latent human tuberculosis are being exploited (McCune *et al.*, 1956). These models were helpful to identify new drug targets for persistent *M. tuberculosis* in the recent past (Cunningham & Spreadbury, 1998; Hu & Coats, 1999; Boon *et al.*, 2001). Isocitrate lyase, an enzyme of the glyoxylate shunt was shown to be required by *M. tuberculosis* for survival in macrophages and during persistent infection in vivo (Mckinney *et al.*, 2000). Using *M. marinum*-frog model, genes required in persistent infection and granuloma formation have been identified (Ramakrishnan *et al.*, 2000).

However, the genetic factor(s) responsible for dormancy are still not known. The ability to persist in a host is a complex process involving the coordinated expression of mycobacterial genes and adaptive processes. Defining these factor will lead to understanding of biology of latency, drug targets and development of new drugs. The mechanisms by which *M. tuberculosis* establishes a latent metabolic state, eludes immune surveillance and responds to triggers that stimulate reactivation are high priorities for the future control of TB. Understanding these mechanisms should open up new avenues for improving the control and treatment of tuberculosis in the future.

On the basis of available evidences, it can be hypothesized that the study of the metabolic state during persistence and the bacterial factors that contribute to latency or persistence of mycobacteria, will give insight into both the mechanisms by which mycobacteria persists and the means of elimination of latent infection.
Hence the present study was planned with the following aims and objectives:

1) Simulation of an *in vitro* model of non replicating persistent state.

2) Analysis and identification of the proteins differentially expressed during non replicating persistent state of *M. bovis* BCG.

3) Identification of the *M. tuberculosis* genes induced during non replicating persistent state of mycobacteria.

4) Analysis of the metabolites and total lipids of replicating and non replicating persistent mycobacteria by $^1$H NMR.

It is hoped that the data obtained and described in this thesis will add new knowledge which will perhaps throw light on the survival strategies of mycobacteria during persistent state of infection. Identification of proteins and genes differentially expressed or induced during non replicating persistent state of mycobacteria, will provide lead to the understanding of biology of latency and design of control measures such as drug targets and development of new vaccines.

The thesis is divided into the following chapters:

**Chapter 1:** It describes the relevant review of the literature.

**Chapter 2:** It describes materials and methodology employed in this investigation.

**Chapter 3:** The chapter gives an account of total results of this investigation.

**Chapter 4:** The results have been discussed.

**Chapter 5:** All the literature referred to in this thesis are listed. This is followed by appendix.