INTRODUCTION
CHAPTER I
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Tuberculosis takes a heavy toll of life in India and according to the results of the sample survey conducted by the Indian Council of Medical Research (1955-58), the prevalence rate for 'active' and 'probably active' tuberculosis varies from 13 to 25 and the rate of bacteriologically positive cases varies from 2 to 8 per 1000 population. Further, the problem of tuberculous infection exists in cattle, swine and birds. In recent years, the prevalence of atypical or anonymous mycobacteria as causative agent of disease in human-beings has also attracted much attention.

The classification of mycobacteria has thus great importance in relation to the epidemiology of the disease, and the problems associated with their typing into human, bovine, avian, murine, atypical or 'anonymous' and saprophytic mycobacteria, are of far reaching importance.

Very little basic information on the mycobacterial phage-host relationship is available at present. While the study of bacteriophages in some other groups of bacteria have proceeded intensively for nearly 35 years, the interest in mycobacterial phages is of recent origin and to-date there have been no reports of the study of mycobacterial phages in India.

The mycobacteriophage studies offer an opportunity to solve taxonomic problems and to establish a phage typing system for the classification of acid-fast bacilli. In addition, the study of host-phage relationships and antibiotic-bacteria complexes is important in providing information of intimate
relationship of one biological unit to another and in unifying biological concepts. The studies on basic problems like lysogeny, antibiosis, adaptation to host-range, and the relationship of phage activity to drug resistance and virulence, are likely to lead to a deeper understanding of these biological relationships.

Attempts at 'phage typing' mycobacteria have been carried by Froman et al. 1961; Hnatko, 1955; Takeya et al. 1960; Penso & Ortali, 1949; Tokunaga et al. 1962; Murohashi & Tokunaga, 1965; Redmond, 1965; Cater & Redmond, 1960 and Redmond & Ward, 1966. The progress in the earlier attempts has been hampered due to the isolation of phages mainly lytic to saprophytic mycobacteria and the few phages lytic to pathogenic mycobacteria were non-specific and had a wide spectrum of activity. Recently, the method has been used with some success due to the availability of a few phages with sufficient specificity and has attracted attention at the proceedings of the Second Symposium on isolation, classification and world-wide distribution of mycobacteria (Prague, 1965). At present, however, the number of such available phages is very limited, and there are several species which are resistant to the available phages. Moreover, its use on a wide-scale as a reliable method remains to be established.

This study has, therefore, been initiated with the object of isolating bacteriophages from different sources in India and to observe their characterization, including their phage susceptibility patterns. It is also the aim of this study to develop suitable methods of typing and to
determine if the mycobacteria prevalent here can be adequately differentiated and identified by phage typing using the phages isolated in India and also those obtained from different workers from abroad, and to carry out a comparative study of phage susceptibility of Indian strains of mycobacteria, with reference to their cultural and biochemical characteristics.

There are no reports of mycobacterial phages isolated in India and it was thought that these may have their own specificity and characterization. Only fragmentary information is available on the mycobacterial strains present in India, so far, and phage typing may be of great help either alone or in association with other tests, in differentiating these strains.

Many methods have been used for the classification and identification of members within the genus *Mycobacterium*. Such diverse features as cultural properties, biochemical tests, pathogenicity to laboratory animals and sensitivity to dyes, have been employed. All these methods have their limitations and the classification of mycobacteria still present shortcomings and no single test is adequate for identification of a strain of mycobacteria. The characters of the colonies are sometimes misleading and recently it has been shown (Mitchison *et al.* 1960; Bhatia *et al.* 1961) that identification of Indian cultures of tubercle bacilli as *Mycobacterium tuberculosis* var *hominis*, based on their virulence for the guinea-pigs may be misleading. Some of
the biochemical procedures have been found to be helpful but these sometimes give erroneous results. Further virulence tests are not of much help in the classification of atypical strains and isoniazid resistant typical strain which display attenuated virulence for guinea-pigs and mice.

The knowledge about the prevalence of bovine strains, avian strains and atypical or 'anonymous' organisms, is very meagre. The establishment of reliable and comparatively easy methods of typing the strains would be of great help in the understanding of the epidemiological problems. The assessment of human type infection in cattle, the bovine disease in human-beings causing known pulmonary and extra-pulmonary lesions, the role of avian tubercle bacilli causing disease in human-beings, cattle and swine and the incidence of atypical or anonymous organisms, would greatly help in the control measures to be taken and to deal with these problems expeditiously. The subject of bovine tuberculosis is of particular importance in this country, since a few studies carried out have revealed only very rare pulmonary or extra-pulmonary lesions caused by bovine organisms unlike that in other western countries.

An attempt has been made to classify human strains on the basis of their phage patterns into different groups. These have an important bearing regarding their use as a valuable epidemiological tool in contact-index studies and investigations regarding dissemination of the disease.

It has also been considered of great interest to compare the phage susceptibility of drug sensitive strains and their
resistant mutants developed in the laboratory.

The bacteriophage studies offer an opportunity to obtain knowledge of fundamental importance and initiate research in diverse fields. The biological features like adsorption behaviour, burst size and the latent period of the phages isolated here have been determined and their serological and phage-host relationships have been examined.

The study of relationship between bacteria and temperate bacteriophages is of great importance and it has been determined that Corynebacterium diphtheriae is not toxigenic unless it is lysogenized with certain bacteriophages (Freeman & Morse, 1952; Groman, 1955).

The genetic characters, like drug resistance, have been reported to be transferred from certain strains of bacteria to recipient bacteria viz. bacteriophages (Zinder & Lederberg, 1952, Lennox, 1955). Hewitt (1953) has postulated that temperate bacteriophages have an important bearing on bacterial evolution and epidemiology. It has, therefore, been considered of interest to study lysogeny amongst Indian strains of mycobacteria.

Antagonism between closely related bacteria has often been observed and bacteriocin-like substances are seen to have striking resemblance to bacteriophages. An attempt has, therefore, been made to observe bacteriocinogenic strains amongst Indian strains of mycobacteria, and study the characters of bacteriocins thus isolated.

Further, the adaptation of phages isolated here to lyse resistant strains has been attempted. Besides the
fundamental interest, the subject has a practical application in obtaining phages which would lyse hitherto resistant strains and thus increase the utility of phage typing methods.

These studies are, therefore, aimed at deeper understanding of the above-mentioned problems and their interrelationship and to fill important lacunae in the knowledge that exists at present and the results of these studies are presented in this thesis. It has also been possible, during the course of these studies, to isolate phages specific for human and bovine strains and this is a significant development in this field since the discovery of phage active on human tubercle bacilli. These phages should be of inestimable value in epidemiological studies, a phase of work which has not developed largely because no adequate method was available. These findings should give a stimulus to phage typing of mycobacteria and further understanding of the role of lysogeny and bacteriocinogeny in mycobacteria.