CHAPTER I
GENERAL INTRODUCTION
The existence of the actinomycetes has been recognised for over a hundred years. For much of this time they were regarded as an exotic group of organisms with affinities to both bacteria and fungi. However, determinations of their fine structure and chemical composition, started in the 1950s, and confirmed their prokaryotic nature. They now constitute the order Actinomycetales (Buchanan, 1917) and their removal from the mycologist's sphere of influence has been completed. Their change of status paralleled that of the blue-green algae to the cyanobacteria but it was accepted more rapidly and less acrimoniously. It is not easy to give a short, accurate definition of actinomycetes. They are frequently described as bacteria which have the ability to form branching hyphae at some stage of their development. However, this attribute is tenuous and it often requires imagination to believe in it (Gottlieb, 1973). The exact composition and boundaries of the order Actinomycetales are still open to question and modification by the application of new taxonomic techniques which have also led to the improvements in the classification and identification of actinomycetes. Despite their relation to a single order of the kingdom Prokaryotes, their biological attributes, their importance to man and their history have ensured that actinomycetes are still generally studied as a group, distinct from other bacteria.
As with many microbes, the study of actinomycetes was initiated in the late 19th century by workers examining diseased materials from humans, animals and plants. The first unambiguous description of actinomycete was probably that of Cohn (1875) who observed filamentous growth in concretions from lachrymal ducts and named the organism *Streptomyces foersteri* but this generic name has been used by Corda (1839) for a group of fungi and was invalid.

The actinomycetes have been traditionally considered to be prokaryotic bacteria with elongated cells or filaments that usually showed some degree of true branching. Although the morphology of these organisms ranges from simple to complex, most strains of most of the species can be assigned to one of the two broad morphological groups, nocardiform — and sporo-actinomycetes (Prauser, 1970, 1976, 1978,). Nocardioform bacteria form hyphae which eventually fragment into coccoid or rod-like elements that give rise to new mycelia (Locci, 1976, 1978, 1981). The genera *Caseobacter*, *Mycobacterium* and *Rhodococcus* are generally included in this group, though all of them contain strains that exhibit little if any, branching, growing merely as rod or coccoid elements. The *Sporoactinomycetes* encompass a greater morphological complexity that includes the formation of spores in or on definite parts of the mycelium. (Locci, 1976; Williams *et al.*, 1976; Williams and Wellington 1980). A third level of organization is presented by *Dermatophilus*.
and *Geodermatophilus* which form a substrate mycelium that divides both transversely and longitudinally to give a primitive multilocular sporangium. Coccoid elements are released which may gain mobility and eventually germinate into filaments or hyphae (Cross and Goodfellow 1973). *Frankia* shows some of the morphological traits associated with *Dermatophilus* and *Geodermatophilus* (Callaham et al., 1978; Becking, 1981).

Bacterial systematics has undergone revolutionary change in the last 20 years. The application of new and reliable biochemical, chemical, genetical, numerical and molecular biological techniques have been responsible for rapidly changing views on how bacteria ought to be classified and identified (Goodfellow and Board, 1980; Berkeley and Goodfellow, 1981). These techniques have generally been applied to the greatest effect on taxa where dependence on form and function proved most unsatisfactory and they have provided a framework for revised classification of both cornyeform (Bousefied and Callely, 1978; Stackebrandt et al., 1980a,b, Keddie and Jones, 1981; Dopfer et al., 1982) and nocardioform bacteria (Bradley and Mordarski, 1976, Lechevalier, 1976; Goodfellow and Minnikin, 1977, 1978, 1981a,b, 1983; Mordarski et al., 1977, 1978 a,b, 1981, a,b, Stackebrandt and Woese, 1981a,b; Minnikin and Goodfellow, 1976, 1980, 1981).
Ribosomal RNA cistron similarity data show that the acid-fast actinomycetes are phylogenetically close (Mordarski et al.; 1980, 1981b) and indicate that sporoactinomycetes fall into at least three major homology groups:— a) Actinoplanes, Amorphosphorangium, Ampullariella b) Micromonospora, Planobispora, Planomonospora and c) Streptosporangium (Stackebrandt et al., 1981). 16S-rRNA cataloguing data show that Gram-positive bacteria form a distinct phyletic line that can readily be divided into two branches on the basis of DNA base composition (Stackebrandt and Woese, 1981a). The actinomycete-cornyeiform line includes bacteria with a guanine (G) plus cytosine (C) content about above 55 mol.% and can be separated from the low G+C content of (below 50 mol%) Clostridium-Bacillus-Streptococcus branch. Several taxa previously associated with the actinomycetes clearly belong to this second evolutionary branch. The genus Eubacterium is phylogenetically related to Clostridium, Kurthia to the Lactic acid bacteria and, perhaps most surprisingly of all, Thermoactinomyces to the Bacillacea (Ludwig et al., 1981; Tanner et al., 1981; Stackebrandt and Woese, 1981a).

The classification of actinomycetes is still confusing, morphology which has always featured prominently in the recognition and definition of actinomycetes and their classification into families and genera does not hold true. In 1973, Gottlieb considered that the actinomycetes
consisted of varied groups of bacteria whose common feature is the formation of hyphae at some stages of development but Gottlieb said that in some organisms, hyphal formation was tenuous and that it required imagination to believe in it. In the following year, in the current edition of Bergey's Manual of Determinative Bacteriology (Gottlieb, 1974), organisms classified in the order Actinomycetales were considered to be 'bacteria that tend to form branching filaments which in some families develop into a mycelium'. It was, however, conceded that the filaments might be short, as in members of the families Actinomycetaceae and Mycobacteriaceae, and in certain taxa they underwent fragmentation and consequently could only be observed in some stages of growth cycle.

The relatively simple morphology of most mycobacteria partly explains why these organisms were sometimes omitted from classification of the actinomycetes (Waksman, 1961, 1967). Other workers questioned the collocation of the actinomycete as a convenient but artificial taxon (Sneath, 1970; Prauser, 1970) and the difficulty of distinguishing between nocardioform actinomycetes and coryneform bacteria was widely recognised (Williams et al, 1976; Locci, 1981). It was also conceded that an overreliance on morphological criteria had blurred the boundaries between Corynebacterium, Mycobacterium and Nocardia so that section of these genera
were more or less interchangeable (Bousefield and Goodfellow, 1976).

The morphological concept of an actinomycete can now be considered critically in the light of information derived from the application of genetic and chemical methods.

It is already quite apparent from 16S-rRNA cataloguing studies that morphological features are poor tokens of phylogenetic relationships and that traditional morphological definition of an actinomycete can not be sustained. It is perhaps not too surprising that the morphologically simple Corynebacteria show a close evolutionary relationship with the more elaborate Mycobacteria, Nocardiae and Rhodococci as this grouping is consistent with the results of a most of chemical (Minnikin and Goodfellow, 1980, 1981a) comparative immunodiffusion (Lind and Ridell, 1976, 1982) and numerical phenetic studies (Goodfellow and Minnikin, 1981b,c: Goodfellow and Wayne, 1982). Indeed the traditional practice of separating the more highly differentiated actinomycetes from the morphologically simple coryneform bacteria no longer holds as strain of Actinomyces, Oerskovia and Promicromonospora shows a closer phylogenetic affinity to Arthrobacter, Brevibacterium, Cellulomonas, Curtabacterium and Microbacterium than to mycelium-forming organisms such as Nocardia and Streptomyces. Further, the mycelium forming
Thermoactinomyces must now be classified in the family Bacillaceae with the aerobic, endospore forming bacilli. It is evident from these findings that the possession of branched hyphae should not automatically place a strain in the actinomycetes. Conversely, the inability of a bacterium to produce branching filaments does not necessarily exclude it from this group of bacteria.

It is perhaps premature to specify the unifying characters for defining this expanded group of bacteria but it is a task that will be expected and shall be attempted, albeit with some trepidation knowing the fate of the many previous attempts.

The actinomycetes are Gram-positive bacteria with a high G+C content in their DNA (above 55 mol%) which are phylogenetically related from the evidence of 16S rRNA oligonucleotide sequencing and nucleic acid hybridization studies. The actinomycetes include genera exhibiting a very wide range of morphology extending from the coccus e.g. Micrococcus, though fragmenting hyphal forms to genera with a permanent and highly differentiated branched mycelium e.g. in Streptoverticillium. There are no clear encompassing chemotaxonomic characters but the genera are linked in a series on the evidence of wall structure and lipid composition. Some, but not all, genera form spores which include motile zoospores and specialised structures that
resist desiccation and mild heat but do not display the organization and extreme resistance properties of endospores (so excluding the genus *Thermoactinomyces*).

The thermophilic genus *Thermoactinomyces* has long been regarded as an actinomycete which shows gross appearance of its powdery white or yellow colonies on agar and branching hyphae which carry lateral single spores on both substrate and aerial hyphae. Several taxonomic studies have shown that the genus *Thermoactinomyces* can be clearly distinguished from actinomycete genera with single spores, for example *Micromonospora*, *Saccharomonospora* and *Thermonospora* by characters shown in table (1.1). It was

**TABLE 1.1**

Differential Characters of the genus *Thermoactinomyces* and other monosporic genus

<table>
<thead>
<tr>
<th>Character</th>
<th><em>Thermoactinomyces</em></th>
<th><em>Saccharomonospora</em></th>
<th><em>Micromonospora</em></th>
<th><em>Thermonospora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial mycelium</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Endospores</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycine in cell wall</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sugar in whole cell hydrolysate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Galactose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tr>
</tbody>
</table>
suggested by Cross and Goodfellow (1973) that it should be placed in a distinct family because of its ability to produce endospores.

**Thermoactinomyces** are Gram-positive bacteria with extensive branching hyphae forming compact colonies differentiated into substrate and aerial mycelium. Single spores are formed endogeneously and have the typical structure of endospores (Cross et al; 1968; Dorokhova et al, 1968). The genus Thermoactinomyces was one of the earliest actinomycete genera to be named but it is now becoming increasingly evident that Thermoactinomyces should no longer be classified within the Actinomycetales. Their menaquinones, wall composition, dipicolinic acid containing endospores, low mol% G+C content and 16S RNA sequence data all suggest that the genus be reclassified with in the family Bacillaceae. However, till now their typical actinomycete like morphology has, caused them to be studied and enumerated along with the sporoactinomycetes.

**Micropolysporas** form a group of sporoactinomycetes with a peptidoglycan containing meso DAP and wall associated arabino-galactan polymers but which do not contain mycolic acids. This aggregate group contains the currently recognised genera Actinopolyspora, Pseudonocardia, Saccharomonospora and Saccharopolyspora together with species that have been misclassified in the genera Nocardia.
and *Streptomyces* as a result of undue weight being given to morphological characters. The transfer of *Micropolyspora brevicatena* to the genus *Nocardia* (Goodfellow and Pirouz, 1982) has left the remaining *Micropolyspora* species without a type species but the proposal to conserve the generic name *Micropolyspora* would provide a genus for some of these organisms and a convenient umbrella name for the group (McCarthy *et al*; 1983). It is not yet clear whether all of the genera in the aggregate group really merit generic status or if they collectively form a distinct evolutionary branch. They do however, have a number of properties in common. They are all aerobic, gram positive, non motile and catalase positive but they are morphologically somewhat heterogenous. Thus, single or short chains of spores can be present either on the aerial mycelium or on both the aerial and the substrate mycelium. Fragmentation is generally much less pronounced than in nocardioform actinomycetes and may be due to localazied areas of autolysis (Williams *et al*, 1976). They have wall Chemotype IV. Probably the most detailed studies of the members of this genus have been directed towards *Micropolyspora faeni* (Cross *et al* 1968), the main causative agent of farmer’s lung and previously named ‘Thermopolyspora polyspora’ (Corbaz *et al* 1963), and *Micropolyspora rectivirgula* (Krasilnikov and Agre, 1964). *Micropolyspora faeni* and *Micropolyspora rectivirgula* have recently been shown to be synonomous (Arden-Jones *et al*, 10
M. faeni was recommended as a nomen conservandum after a study proving synonymy with M. rectivirgula.

Saccharomonospora is a monosporic actinomycete having cell wall of meso DAP and arabinogalactan. Originally the single species Saccharomonospora viridis had been named Thermoactinomyces viridis (Schuurmane et al., 1956) but it was transferred to Thermomonospora and cited as "Thermomonospora virida" (Kusteer and Locci, 1963b) in the eighth edition of the Berly's Manual of Systematic Bacteriology. The reclassification of this species has been supported by numerical phenetic studies (Goodfellow and Pirouz, 1982, McCarthy and Cross, 1984). In the more recent classification of actinomycete, genus Saccharomonospora was classified in an aggregate group name Micropolyspora (Goodfellow and Cross, 1984). These organisms have a similar wall composition and lack mycolic acid but relationship between genera have yet to be determined.

ECOLOGY AND PHYSIOLOGY OF ACTINOMYCETES

One of the first truly saprophytic actinomycetes to be detected was Streptothrix chromogena, which was isolated from soil by Beijerinck (1900). The widespread occurrence of actinomycetes (particularly streptomycetes) in soil was demonstrated by Krainsky (1914) and Waksman and Curtius (1916, 1918). Over the next 20 years, knowledge of the
ecology of actinomycetes in soil, composts and other habitats was considerably extended by Waksman, Jensen and other soil microbiologists. They are free living organisms, and usually very high concentration of actinomycetes are found in soils of all types. In the top soil, $5 \times 10^6$ colony forming units/g of soil were exposed (Lacey 1973, Tabor, 1980), whereas in the deeper layer of soil, the number of actinomycetes decreases. Most actinomycetes have been reported in slightly alkaline to neutral soils, whereas high acidity seem to affect their survival adversely (Lacey, 1973).

Members of the actinomycetes are associated with various natural processes in the environment (Gottlieb, 1973). They decompose organic matter and a soil conducive to crop production. The economic importance of actinomycetes is well recognised and includes production of life saving antibiotics and useful metabolic products. (Gottlieb, 1973). The first purified antibiotic to be obtained from an actinomycete was actinomycin (Waksman and Woodruff, 1940), which was soon superceded by that of streptomycin (Schatz et al, 1944) which is best known for its use in the control of tuberculosis. Although most of these antibiotics originated from *Streptomyces*, other genera such as *Actinoplanes*, *Actinomadura* and *Micromonospora*, also produce useful or potentially useful antibiotics.
However, some of the species are established plant pathogens, capable of infecting and destroying valuable crops. The first plant pathogen *Streptomyces* (nee *Gospora*) *scabies*, was isolated from potato scab by Thaxter (1891). Potato scab is a major disease that affects the crop in all parts of the world and periodically causes economic disaster (Large and Honey, 1952).

Several members of actinomycetes are capable of producing diseases in humans and animals (Gordan, 1980). These include some widespread and intensively studied diseases, such as diphtheria (*Corynbacterium diptheriae*), tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*), but it must be noted that inclusion of such microbes in the actinomycetes has been a matter for debate.

However, there is a wide range of actinomycetes infection which are less widely known (Slack and Gerencser, 1975; Lloyd and Sellers, 1976). Many of these are proving to be more clinically significant than previously thought, partly due to improvements in procedures for their diagnosis. Diseases of impairment such as actinomycosis, lacrimal canaliculis, periodontal disease and caries may be caused or initiated by *Actinomyces*, *Archina*, *Bifidobacterium* "Bacterionema" or *Rothia* strain. All these are commonly present on the mucosal surfaces of healthy individuals, but
may also become invasive or harmful as endogenous pathogens.

Since Bollinger’s first description of bovine actinomycoses involved animals, the disease which is also called lumpy jaw resembles human actinomycoses. The principal causative agent invading the tissue endogenously is \textit{Actinobovis} (Bollinger, 1877; Slack and Gerencser; 1975) but infection with \textit{Actin israelii} have been reported in cattle (King and Meyer, 1957, Cummins and Harris, 1958; Pine et al, 1960). Pulmonary and systemic nocardiosis, localised cutaneous or subcutaneous nocardiosis and actinomycetoma are mainly caused by \textit{Nocardia}, \textit{Actinomadura} and \textit{Streptomyces}. Interstitial pneumonitis (Extrinsic allergic alveolitis or Hypersensitivity pneumonitis) is caused by certain thermophilic actinomycetes.

THERMOPHILIC ACTINOMYCETES:

Thermophilic actinomycetes grow well in situations in which the temperature exceed 50°C either by artificial heating or microbial fermentation. Large number of spores are liberated from the luxuriant growth of thermophilic actinomycetes on such substrates. The thermophilic actinomycetes cause allergic respiratory diseases in humans and animals (Kurup, 1984). These diseases are commonly known as extrinsic allergic alveolitis (EAA) in U.K. and hypersensitivity pneumonitis (hypersensitivity pneumonitis) in U.S.A.. Human diseases result from inhalation of
actinomycetes from the genera Faeni, Saccharomonospora and Thermoactinomyces (Kurup, 1984). These three taxa contain species unique in their ability to grow at higher temperatures than most other organisms could survive. On exposure for prolonged periods of time to an environment contaminated with these organisms, hypersensitivity pneumonitis may develop in susceptible individuals. The four major diseases caused by these organisms are Farmer’s lung, Mushroom worker’s lung, Bagassosis and Ventilation system pneumonitis. The five commonly occurring thermophilic actinomycetes are Faenia rectivirgula (F. rectivirgula), Thermoactinomyces candidus (T. candidus), Thermoactinomyces sacchari (T. sacchari), Thermoactinomyces vulgaris (T. vulgaris) and Saccharomonospora viridis (S. viridis) (Hollick and Kurup, 1983, Kurup et al (1984), Kurup and Fink, (1975).

Specialised environments particularly those of closed buildings and artificial air handling systems, have contributed to the selective growth and exposure related health impact of several thermophilic actinomycetes.

Intense exposure of these organisms leads to hypersensitivity pneumonitis. In other environments such as hay grain, compost, bagasse, humidifiers and other materials proliferation of bacteria and fungi occurs when sufficient moisture is present (Lacey, 1973).
During their growth, some microbes ferment the substrate, resulting in elevation of its temperature. Thermophilic actinomycetes grow very rapidly in such conditions and release abundant spores into the atmosphere, these spores and vegetative fragments may reach very high levels in such closed environments, and the resulting exposures eventually lead to the development of hypersensitivity pneumonitis (Brummund et al, 1988). In the lungs the size of the particles inhaled determines the type as well as the location of hypersensitivity reaction. Actinomycete spores, being mostly about 1μm in diameter, are small enough to penetrate to the alveoli where they are deposited to sensitize an individual. Constitutional predisposition is unimportant, but heavy exposure is necessary (Lacey, 1981).

**HYPERSENSITIVITY PNEUMONITIS**

Hypersensitivity pneumonitis is an allergic lung disease resulting from the sensitization and recurrent exposure to any of the wide variety of organic dusts containing thermophilic actinomycetes from mouldy hay, mouldy sugar compost, forced air heating, cooling or humidification systems, and avian antigens. The disease is a diffuse, predominantly mononuclear inflammation of the lung parenchyma, particularly the terminal bronchioles interstitium and alveolus, the inflammation often organises
As clinicians have become aware of the possibility that hypersensitivity pneumonitis may be the result of inhalation, exposure and sensitization to organic dusts encountered in a variety of occupations or environments. Suberosis has been described in Portugal as occurring in the cork manufacturing industry (Avila and Villar 1968) and most likely is the result of the inhalation of the spores of *Pencillium frequentans* contaminating the cork dust (Avila and Lacey, 1974). Sequoiosis has been described in the red wood lumber industry (Cohen et al, 1971), and spores of *P. Caseii* present on cheese have caused cheese workers lung (Deweck, et al 1969). Another hypersensitivity pneumonitis has been described in individuals with diabetes insipidus who used heterologus pituitary powder as snuff to control their disease (Mahon et al, 1967). More recently volatile chemicals such as phthalic anhydride and toluene disocyanate, widely used in the plastic industry, have also been associated with hypersensitivity pneumonitis (Fink and Schluter et al, 1978; Schlueter et al, 1974). Other common organism involved in hypersensitivity pneumonitis include fungi such as *Alternaria, Penicillium* and *Aspergillus*.

**EPIDEMIOLOGIC STUDIES**

There are no definitive studies delineating the
prevalence of hypersensitivity pneumonitis. Studies of farmers in the British Isles have indicated an attack rate of approximately 7% (Boyd, D.H.A., 1971; Grant et al; 1972). In an office studied by Banaszak et al (1970) 15% of the workers exposed to a contaminated air-conditioning system had pulmonary disease caused by sensitization to thermophilic actinomycetes. Studies of pigeon breeder club members indicate that between 6% and 21% of exposed breeders may develop disease (Caldwell et al, 1973; Christensen, et al, 1975). Clarification of the frequency of disease in an exposed population will require additional surveys.

**ETIOLOGIC AGENTS AND ENVIRONMENTAL FACTORS**

The penetration of microbial spores into the airways has been studied in the past (Austwick, 1966). The largest spores and aggregated particles tend to be deposited on the surface of nasopharynx and trachea, whereas the smaller ones (< 5 um) may penetrate into the alveolar spaces (Austwick PKC, 1966). Gregory (1961) estimated that in Great Britain during June to September, the number of spores in the outdoor may vary from 12,000 to 15,000 per m³. However, in enclosed areas, the number may be much larger, that is, in the neighbourhood of $1.6 \times 10^{19}$ per m³. This will result in the inhalation of $4.8 \times 10^8$ spores in 30 min and a retention of $2 \times 10^8$ spores in the lower respiratory tract, (Lacey and
Gregory and Lacey (1963) have reported studies showing the presence of up to $1.6 \times 10^9$ actinomycetes spores in the air after disturbing mouldy hay. Since particle sizes are smaller than 6 microns, it has been estimated that a farmer working in this environment might inhale and retain in his lung as much as 750,000 spores per minute (Lacey and Lacey 1964).

Although thermophilic actinomycetes grow abundantly in compost, they are ubiquitous and can be found in soil, food, fresh water, the atmosphere, and many other natural sources. Protein derived from feathers, serum and excrement of several avian and rodent species are also important causes of hypersensitivity pneumonitis.

Organic dusts producing the disease have been found to exert a variety of biological effects. In addition to acting as sources of antigen and eliciting immunological reactions, they can act as adjuvants (Bice et al. 1977) and thus promote the development of humoral and cell mediated immunity. They may also activate alveolar macrophages (Stankus et al.; 1978) and directly activate the alternative complement pathway (Edward et al. 1974) providing the necessary stimuli for increased permeability and chemotactic migration of polymorphonuclear leukocytes and macrophages to
the lungs. These materials also contain endotoxin (Rylander, R. et al 1978) and histamine releasers (Burell and Polarney 1977). The inflammatory consequences of these non-specific injurious effects and those modulated by complement and macrophages could be important factors in the pathogenesis of hypersensitivity pneumonitis.

ENVIRONMENTAL FACTORS

Exposure to the offending agents are usually related to occupations or hobbies. The concentration of these agents in a given environment varies significantly according to the climatic, meteorologic and local conditions. For example, actinomycetes grow in hay or bagasse under conditions of high humidity and temperature. For this reason the concentration of actinomycetes per pound of hay or bagasse is significantly lower if the material has not been wet or submitted to high temperatures.

Contamination of humidifiers is very likely related to the humidifiers intrinsic water dispersal system and particularly the frequency of cleaning of the system. Exposure in the work setting may vary among different workers depending on the place at work in relation to the source of the antigenic material.
The earliest description of a disease resembling hypersensitivity pneumonitis probably was presented by Ramazzine in 1713 among grain sifters and measures, and was attributed to weevils and molds present in the grain. The first report of a well characterised hypersensitivity pneumonitis, namely farmer's lung, came from Great Britain in 1932 (Campbell, 1932). Subsequently, other occupationally linked hypersensitivity pneumonitides were recognised from several parts of the world (Dickie and Rankin 1958; Lacey 1971b). Mushroom worker's lung, caused by the inhalation of compost dust containing thermophilic actinomycete was reported by investigators in the United States and the United Kingdom (Bringhurst et al 1959; Runyon et al 1980; Sakula A., 1967.) Mouldy bagasse has been described as the source of antigens responsible for bagassosis whereas air-handling systems contaminated with thermophilic actinomycetes, have been responsible for ventilation system-induced pneumonitis in exposed susceptible individuals (Banaszak et al 1970; Fink et al 1971; Lacey; 1971b).

CLINICAL FORMS OF HYPERSENSITIVITY PNEUMONITIS

The clinical manifestation of these respiratory disorders may be present in number of forms, depending on
the immunologic response to the inhaled antigens, the antigenicity of the dust, and the frequency and intensity of exposure. In general, the manifestations are similar regardless of the organic dust inhaled, and hypersensitivity pneumonitis may be considered as a syndrome with a spectrum of clinical features; even though each specific disease may be due to different organic dust. The atopic individual may demonstrate typical, bronchospasm or rhinnorrhea immediately, following inhalation of the dust, this reaction may be followed hours later by the clinical features of hypersensitivity pneumonitis. The non atopic patients on the other hand, usually will respond with late type reaction on long exposures characteristic of these disorders.

Acute Form

Within 4-6 hours of exposure the sensitized patients develop respiratory and systemic symptoms of cough, dysnea, fever, chills, myalgia and malasie resembling systemic viral or bacterial infection. Symptoms persist for 8-12 hours, but the patient recovers spontaneously. Numerous attacks may be associated with weight loss and anorexia. Between the acute attacks and in the absence of further antigen exposure, the patients often feels quite normal.

Subacute form

Some patients have a more insidious type of disease,
with more acute attacks, These individuals are usually exposed to small amounts of antigen over long periods. The symptoms resemble those of progressive bronchitis with dyspnea, chronic productive cough with scanty sputum, anorexia, fatigue and weight loss.

CHRONIC FORM

In some cases of hypersensitivity pneumonitis, chronic irreversible lung damage may occur. This may take the form of irreversible fibrosis and pulmonary insufficiency. These persons have symptoms of progressive dyspnea and may develop irreversible pulmonary function abnormalities or restriction diffusion defects, and stiff lungs that do not respond to corticosteroids.

RADIOGRAPHIC FEATURES

Hypersensitivity pneumonitis can not be distinguished radiographically from other non-immunologic interstitial disorders. Rotengenograms may be normal as they may show recurrent interstitial nodular infiltration or fibrotic changes, depending on the stage of the disease (Bringhust et al 1959, Pepy, 1969).

PATHALOGICAL FEATURES :

The histologic features of the lung in hypersensitivity pneumonitis depends on the stage of the
disease at the time of biopsy. In early stages of farmer’s lung, bagassosis, mushroom picker’s disease and hypersensitivity pneumonitis caused by antigens other than thermophilic actinomycetes, the alveolar walls are infiltrated with lymphocytes, plasma cells and histocytes, containing foamy cytoplasm may also be seen with in the alveolar spaces. Later, the interstitium becomes infiltrated with mononuclear and scattered giant cell granulomata. In later stages, fibrosis of these areas occurs and bronchiolitis obliterans may be seen.

In biopsy specimens from cases of pigeon breeder’s disease, similar interstitial and alveolar granulomatous and infiltrative changes may be seen (Hensley et al. 1969).

In addition, foamy macrophages, possibly derived from alveolar macrophages, may be found in the interstitial areas as well as in the alveoli. The interstitial position of these foamy cells may be unique for pigeon breeder’s disease because this feature has not been described in other hypersensitivity pneumonitis, peripheral destruction of alveoli in some chronic cases of farmer’s lung, bagassosis or pigeon breeder’s disease. The interstitial and intra alveolar infiltrate in these cases is less distinctive, and the foam laden macrophages are less frequent than in the other forms of the disorder.
IMMUNOLOGIC FEATURES

The characteristic immunologic feature of these disorders is the presence of precipitins against offending antigens in the sera of affected individuals. These antibodies may be demonstrated by gel diffusion technique using the patient's serum and the suspected antigen. Immunoelectrophoresis has shown these precipitating antibodies to be of the IgG class although other studies have demonstrated antibody activity in other classes of immunoglobulins as well (Patterson et al. 1976). Up to 50% of symptomatic individuals exposed to the same antigens may also have precipitins, but usually of lesser titre.

The presence of cell-mediated immune response to offending antigens has been detected in the majority of patients and in minority of asymptomatics, but exposed individuals. Increased number of lymphocytes also have been shown to be present in the alveolar lavage fluids from cellular immunity to specific organic dust antigens, as well as the enumeration of cells obtained from alveolar lavage are current research tools that ultimately may be of clinical importance in evaluating suspected individuals.

Skin tests with suspected thermophilic antigens have been shown to be unreliable because of non-specific irritation type reactions. However, in the disorders caused by inhalation of serum proteins, such as pigeon breeder's
Disease, skin test may be of value. Both immediate wheal and flare and late (4-6 hrs) skin reactions may be observed.

The immediate reactions are the same type as seen with common inhalant allergens, but the late reaction resemble the Arthus phenomenon, indicative of vasculitis because of precipitin antigen reaction. The late reaction begins with a variable edema and erythema of the injected site. It can progress to central necrosis, but it usually subsides in 24 hrs unless necrosis has occurred. Histologic examination of biopsies of such skin reactions has demonstrated lesions, consistent with Arthus type reaction, with a mild vasculitis consisting of polymorphonuclear and plasma cell infiltration of the vessels in the area (Pepys 1969). This type of late reaction also may be occurring in the lungs after inhalation, but as yet there is no clear evidence that precipitating antibody participates in the genesis of hypersensitivity pneumonitis.

DIFFERENTIAL DIAGNOSIS

Diagnosis of a typical case of a hypersensitivity pneumonitis can be made by evaluating the environmental history, appropriate laboratory and serologic studies, and a trial of avoidance of re-exposure.

Following are some of the diagnostic criteria for
hypersensitivity pneumonitis

**History**

**Exposure to antigen**
cough, fever, dyspnea

clinical features
diffuse nodular, infiltrate

Chest roentenogram
restrictive pattern

Lung function test

Immunological changes

skin test positive Arthus
reaction serum precipitin
antibody present using
antigenic extracts of the
causative organism.

Lung biopsy

granulomatous alveolitis

Inhalation challenge
cough, fever, dyspnea

Bronchial Lavage

T-suppressor cell Lymphocytosis

**THERAPY**

As in all other allergic disorders, the primary therapy suggested in avoidance of the offending antigen once it is known, because many of these disorders are occupational, certain measures may be necessary, such as the use of masks with filters capable of removing the antigen, appropriate ventilation of working areas, or even a change of occupation.

Drug therapy may be needed in the acute or subacute forms of these disorders, where avoidance can not be carried
out immediately. Although antihistamine or bronchodilators have no effect on the pattern of symptoms. Patients usually respond to the administration of corticosteroids.

Moderate doses of these drugs may be necessary for prolonged periods, along with the avoidance, to determine if reversibility of the clinical abnormalities is possible. Hyposensitization should be avoided, since toxic immune complexes may be formed when the injected antigen combines with the precipitating IgG, and systemic vasculitis or serum sickness may result.

DISEASES CAUSED BY THERMOPHILIC ACTINOMYCETES

Farmer’s Lung

Farmer’s lung results from the inhalation of mouldy hay dust contaminated with thermophilic actinomycetes and fungi (Campbell, 1932; Dickie and Rankin, 1958; Pepys, 1969). Although Farmer’s Lung was described by Cambell in 1932, the exact cause of the disease was determined only in recent years (Cross et al, 1968, Dickie and Rankin, 1958) when dust containing thermophilic actinomycetes are inhaled, susceptible individuals develop the disease. The organisms implicated are F. rectivirgula, T. vulgaris, T. candidus and S. viridis (Kurup 1984, Kurup and Fink 1975; Pepys 1969) Precipitins to the sensitizing antigens can be demonstrated in the sera of affected patients and, additionally, in 20 per cent of exposed individuals without any disease. (Wenzel
et al., 1974). When very sensitive methods such as biotin-avidin-linked enzyme immunoassay (BALISA) or radio immunoassays are used, antibodies are detectable in all the sera, although quantitative differences have been noted between patients and normals (Kurup et al. 1987).

Cellular immune response to specific antigens also have been reported, with published reports differing considerably in details (Brummund et al. 1988, Cormier et al. 1984, Leatherman et al. 1984, Mormex et al. 1984). Skin test reactivity demonstrates both late and delayed type reactivity. Brummund (1988) indicated that in a majority of well-characterized patients, immediate skin test reactions can also be demonstrated. No major lymphocytes phenotype differences have been demonstrable between patients and exposed individuals. (Brummund et al. 1988). Pulmonary function changes and radiologic findings are consistent with hypersensitivity pneumonitis as described previously (Banaszak et al., 1970).

Bagassosis

Bagasse, the sugar-cane fiber left after extraction of the juice, promotes the growth of thermophilic antinomycetes when it is stacked. These sugar remains contain some residual sugar that also will promote the growth of bacteria and fungi, process that leads to heating. Thermophilic
actinomycetes will supersede the normal thermolabile organisms soon after the substrate temperature is raised to as high as 50 to 70°C owing to the fermentation of moldy bagasse. The commonly isolated emerging organisms are *T. sacchari*, *T. candidus* and *T. vulgaris* (Kurup, 1981, Lacey, 1971). The disease is predominantly caused by the inhalation of *T. sacchari*, and patients show high levels of specific serum precipitins. The disease has been reported from all parts of the world where sugar cane is grown and processed.

**Ventilation System Pneumonitis**

Closed air systems in the work area and homes support the growth of several microorganisms including fungi and actinomycetes. Thermophilic actinomycetes associated with hypersensitivity pneumonitis often are prominent in this flora, and exposed persons frequently have detectable antibodies to antigens of thermophilic actinomycetes (Kurup et al, 1976, Wenzel et al 1974). However, patients occasionally may not show antibodies against standard antigens available commercially or from other sources. The first report of hypersensitivity pneumonitis due to contaminated heating system was studied by Banaszak et al (1970) almost 20 years ago. Since then, several reports, including multiple cases from contaminated building appeared in the literature (Arnow et al 1978, Fink et al, 1976). The most common organisms implicated in these cases are *T.*
Mushroom Worker's Lung

Mushroom compost, a pasteurized mixture of horse and chicken manure and vegetable matter, is used for edible mushroom cultivation. Several thermophilic actinomycetes have been isolated from the compost and the associated environmental air (Kleyn et al., 1981; Kurup et al. 1976). Because mushroom farming is carried out in dark and humid enclosed places, without active ventilation, airborne spore concentration readily reach very high levels following disturbance of the compost. The first case of mushroom worker's lung was reported by Bringhust and colleagues in 1959 and since then, many reports have appeared from other parts of the world (Sakula 1967, Stewart, 1974). During the spawning, a large number of spores are liberated into the air. The common organisms associated with disease are T. candidus, T. vulgaris and S. viridis. Another thermophilic actinomycete, Thermomonospora fusca, has been frequently isolated from the compost and air samples of mushroom farms, precipitins to all these organisms are present in the sera of patients and exposed, healthy individuals. The role of Thermomonospora in the disease is not yet understood. It is also suggested that nitrogen dioxide, liberated during compost processing might also have contributed additional lung damage in reported cases (Campbell, 1932).
Since the recognition that inhalation of avian antigens can result in sensitization and subsequent hypersensitivity pneumonitis, bird breeder's disease has been studied in pigeon breeders (Hendrix et al., 1978), chicken (Elman et al., 1968) and turkey farmers (Boyer et al., 1974).

SCOPE OF WORK

Repeated exposure of certain organic dust sensitizes some people and further exposure with these organic substances can cause an immunological reaction with the specific IgG antibodies in the serum. This allergic reaction belongs to type III hypersensitivity, accompanied with the formation of immune complex of antigen and specific IgG antibodies. Most of the organic dusts contain a variety of microorganisms such as thermophilic actinomycetes Mucor, Penicillium, Aspergillus fumigatus and avian proteins.

Prevalence of thermophilic actinomycetes and their involvement in hypersensitivity pneumonitis has been reported from most parts of Europe, U.S.A. and Canada. Various methods which were used for the detection of antibodies in hypersensitivity pneumonitis by various workers ranged from ouchterlony double diffusion, counter immuno electrophoresis to more sensitive techniques such as
hemagglutination, immunoflourescence, radio-immuno assay and immunoenzymatic assays. Dot immunobinding and western blots were also used for the detection of antibodies. Practical methods of all these tests mainly depend on the availability of suitable antigen preparation and should cover whole range of possible organisms to which the recurrent exposure in environment is possible, but the definitive diagnosis of the disease is not made using commercially available antigens because there are lots of variations in the characteristics of antigen/allergenic components of thermophilic actinomycetes obtained from different places. As the chemical and immunological characters of antigens vary from batch to batch and among laboratories and countries, so a characterised potent antigen is required for reproducible and dependable results.

Only some of the forms of hypersensitivity pneumonitis are so far etiologically well characterised because the diagnostic antigens are not as yet available and commercial antigen preparations are limited to extracts of \textit{M. faeni} and \textit{T. vulgaris} and are often impure as they contain components of \textit{T. candidus} and \textit{T. thalphophilus}. There are very few scattered reports of prevalence of actinomycetes and their involvement in hypersensitivity pneumonitis are available in India, by Jindal \textit{et al} (1982) in which he
reported antibodies in patients of chronic specific bronchitis in India against *M. faeni* antigens whereas Khan *et al* (1985) reported antibodies against *M. faeni* in animals suffering from hypersensitivity disorders. Gangwar *et al* (1989) and Singh *et al* (1990) also reported the prevalence of thermophilic actinomycetes in north western India and Amritsar respectively. However, typical survey for the prevalence of these thermophilic actinomycetes, their involvement in hypersensitivity pneumonitis and their antigenic characters has not been studied properly in India. India being a tropical agricultural country, higher prevalence of thermophilic actinomycetes is expected and could be a dominant factor in many cases of interstitial lung diseases. It was therefore thought necessary to survey different parts of the country to evaluate prevalence of these microorganisms and their involvement in hypersensitivity pneumonitis. In order to develop immunochemical methods to aid in the diagnosis of these diseases, it is necessary to study and characterize the antigens from prevalent dominant species of thermophilic actinomycetes and study their role in the suspected cases of interstitial lung diseases in India and to prepare and evaluate the use of appropriate antigens in the diagnosis of these diseases.