CHAPTER I

INTRODUCTION
Leprosy in ancient medical writings:

The origin of Leprosy is lost in the midst of antiquity although some of the oldest known records refer to skin diseases which have been thought by some to refer to this disease. In most of the ancient medical literature the definite recognition of the disease now called 'Leprosy' is difficult or impossible partly because - words which now mean Leprosy were sometimes used in ancient writings either with a much wider meaning or else with different meaning. So the original home of Leprosy, whether Africa or India can not be said with certainty. Bergmann (1897) noted that "the cradle of one of the oldest scourges of human races - Leprosy - is, according to Brugsch, Egypt where Leprosy was observed and correctly described thousands of years before the birth of Christ. Whether Leprosy was passed on from Egypt to India or whether it was in existence in both the countries at the same time, cannot be decided with certainty, but it is sufficient to note that India, East Africa and China, also are ancient homes of Leprosy."
Rogers states in his 'Croonian Lecture' (1924) that "Munro in an article in the Edinburgh Medical Journal 1377-79 refers to an Egyptian record of 1350 BC of leprosy among Negro slaves from the Sudan and Dafur during the reign of Rameses II. It has also been stated that Leprosy has been described in the Ebers Papyrus, written about 1550 BC." The authority of this statement has been disputed as Ruffer found no evidence of the occurrence of mutilation of Leprosy among a large number of mummies examined, but yet the tradition is going on that the cradle of Leprosy is to be found in the upper reaches of the Nile, for Lucretius in his De Natura Rerum makes reference to the birth of the disease:

1 High up the Nile midst Egypt's Central Plain
   Springs the dread leprosy and there alone."

Pacha (1914) and recently Baron (1957) state that home of Leprosy is India. Authentic references are only found in India where 'Kushtha' has been described in the Atreya's Rig Veda Samhita - 1400 BC. In Sushruta Samhita (600 BC), Leprosy has been described under Vata Rakta or Vata Shonita and Kushtha. Vata Rakta or Vata Shonita is characterised by the presence of hyperaesthesia, anaesthesia and deformities without skin patches. The term Kushtha is used for skin diseases in general, but one variety - known as Aruna Kushtha - corresponds to Leprosy. Two kinds of Kushtha have been described; in one the prominent symptoms among them being anaesthesia and deformities, etc., and
in other - ulceration, falling of fingers, etc. (Bhisagratna -1911). Reference has been found in earlier Indian literatures - Manusamhita.

In the literature of ancient China, there does not appear to be any clear evidence of existence of Leprosy except the report in Analects (Wong and Wu, 1932) during Chou Dynasty that a disciple of Confucius died of leprosy is found in the writings of the 3rd Century AD and it is only in the literatures of the 7th Century AD that clinical symptoms of the disease are found. Leprosy was present in Japan in the 7th Century, before Christ.

The disease was mentioned and described by Greek writers - Lucretius, Celsus in the first Century BC and by numerous later authors in Greco-Roman times. With the collapse of the Greco-Roman civilisation, the science of medicine retrogressed and was largely forgotten in Europe but the Greek medical knowledge was kept alive by the Arabians who studied Greek and probably the Indian writings on leprosy and wrote extensively on the subject. The Greek writings on leprosy were recovered in Europe first indirectly from Arabian writers. During this time the terminology of leprosy became confused and it took many centuries to clear up this confusion. The Greeks used the term lepra for psoriasis while for leprosy they used the word Elephantiasis which is still nowadays called 'Elephantiasis Graecorum' for leprosy, whereas the Arabians had translated 'lepra' for leprosy and Elephantiasis for filariasis and in some Arabic writings it is known as 'djudsum'. The medical writings on leprosy in Europe in the Middle ages were dominated very largely by the Greek writings and
contain little original material. Under the impression, possibly a mistaken one, that the terms Zaraath of Old Testament and the Lepra of New Testament indicated leprosy, the disease then common in medieval Europe, the word 'leprosy' was used in translating the Bible into some (but not all) European languages and the Mosaic Law relating to Zaraath was applied to the medieval leprosy. MacArthur (1953) states that in English the word 'Leper' originally signified the disease itself and not as at present the diseased person. This word, in a variety of related forms, runs through the Aryan languages. The basic meaning is, sometimes that peels off; and for this reason it was early applied to the inner bark of trees. The Latin form of the word was liber and as this bark was used to write on, liber came to mean a book, so it is interesting to remember that the modern 'library' and 'leprosy' are, in origin, the same word.

The spread of leprosy to Europe:

Upto the conquest of Egypt by Cambyses in 525 BC that country was closed to the Greeks and soon after came the conquest of Darius and then in 480 BC that of Xerxes, who according to Herodotus, led six million people into Europe from all the nations of Asia and Africa under his rule and left thousands behind him when he retired, which would account for the first introduction of leprosy in Greece and Asia Minor with a slow spread at first and gradually increasing. The further progress in Europe can be traced as leprosy was unknown in Italy until the return of Pompey's soldiers from the East in 62 BC while Galen wrote of the disease
in Germany in 180 AD and four centuries later it had become so common and widespread. In the fifth and sixth centuries Spain was infected by the Roman troops and it was common there by the tenth century, after the fall of Rome. The conquests of Alaric and others further disseminated the disease. Two centuries later, it was common in France, having been introduced in the seventh century in Pyrenees, whence it spread north. G. Newman states that the first known leper hospital in England was established in Nottingham in 625 or 638 AD and in Ireland almost certainly in 869 while James Y. Simpson of Edinburgh showed that leprosy had reached Wales by 950 AD. The disease later spread over the other parts of Europe, including Holland, Denmark, Swedens, Germany, accessible parts of Russia on the Baltic and the Black Sea etc. It was at its height in Europe in the 12th Century and from the 13th Century however leprosy began to decline in Europe and by the 17th Century it had almost died out with the exception of a few persistent foci where the disease exists till today. Many of the reasons for the decline of leprosy in Europe are obscure but it is possible that prophylactic measures adopted in the middle ages and improvement in diet and living conditions played an important part in this connection. Epidemics of the disease are also considered by some to be responsible for the reduction of leprosy. The disease will begin to disappear from our Country when circumstances will improve the social condition of the people that there is less overcrowding and opportunity of close contact between individuals. It appears that if
the communication between the skin of healthy person and that of open cases of leprosy can be cut, the disease slowly dies out of a community.

**Leprosy in the New World**:

While leprosy died out from Europe it was introduced into American Continent first by the Columbus' soldiers and much later by the slaves from West Africa and by the immigrants from Europe and China. It is interesting to note that there is no evidence of leprosy among the Eskimos of the Arctic Circle, neither is there any evidence that leprosy was introduced into America across the Bering Strait from what is now known as Siberia. In North America itself leprosy is an imported disease and as far as evidence goes there are no indigenous foci in any of the States in America, except in the Southern and Western States of Texas, Louisiana, Florida and California. Nevertheless as in Great Britain so in Northern States of America, the influx of large number of people from highly endemic areas can not be viewed with complacency, for if conditions arose which resulted in close skin-to-skin contact with such individuals. There is no inherent reason why new foci of the disease should not appear. In Central and South America, leprosy has steadily increased and been found in certain areas particularly in Argentina, Brazil, Columbia, Guianas and Paraguay.

More recently, during the last 100 years, leprosy has been introduced into several of the previously unaffected islands
in the Pacific, Chinese immigrants have played an important part in the spread of leprosy in these areas. The disease was recognised among the natives of Hawaii in 1835 and the disease spread slowly for 10 - 15 years and after that very rapidly affected a large population. Similarly a large number of cases was also found in Nauru and New Caledonia.
In Sushruta Samhita, it was mentioned that leprosy is carried from the diseased to the healthy persons in the following ways: by touch and breath of the patients, sharing the same bed, eating and drinking from the same vessel and using the same apparel etc. All these methods of transmission mentioned by Sushruta imply close contact with the patients. The view about the infectiousness of leprosy and about the necessity of isolation are also found in Chinese writings of the 7th Century and are very well illustrated in the severe measures taken against the spread of the disease during its prevalence in Europe in the Middle ages.

So from the ancient times leprosy was universally considered to be a communicable disease until from the Seventeenth to the later part of Nineteenth Century the hereditary theory of its origin gradually superseded the contagious view in European medical circle and was strongly supported on very inconclusive evidence by the Norwegian authors- Danielssen and Boeck in their work of 1848.

In the absence of any real evidence, the belief in the contagiousness of the disease began to lose ground. Observations made during the studies with individual cases and families failed to lend support to the idea of spread of the disease by contact. The hereditary, as opposed to infectious, theory of origin of leprosy received most influential support from the Report of the
Royal College of Physicians of London, prepared in 1362 at the request of the Secretary of States for Colonies by a Special Committee, none of whom appear to have had any experience of the disease. Their anticontagionist view had been much strengthened by the latter Report - namely the disease is never a communicable one, and that leprosy is essentially a constitutional disorder, indicative of a cachexia or depressed condition of the general system - precisely the view of Danielsson who described leprosy as due to blood dyscrasia.

In China, Japan and North Africa the belief in hereditary transmission still exists. In India, it is not unusual for a patient who has been told that he is suffering from leprosy to raise the objection that no such disease has been present in his family for generations; thus voicing the wide-spread belief that leprosy is a hereditary disease.

However, even before the discovery of Leprosy bacillus, convincing epidemiological evidence in favour of infections and against hereditary theory was forthcoming. Drognant - Landre in 1369 gave much evidence of the communicability of the disease, pointing out that affected adult Europeans had mostly lived in familiar relation with Negresses and European children had been allowed through the negligence of their parents to come into contact with native lepers. In 1376 all the thirteen medical men reporting to the British Guina Leprosy Commission favoured its contagious origin. It will suffice
to mention the most important subsequent papers which gradually brought about the general acceptance of the communicability of leprosy from sick to the healthy such as the 300 pages book of Brousse of 1879, recording many years' experience of Trinidad Leper Asylum, Hills' book on leprosy of 1881 based on years of study while in charge of the British Guiana Leper Asylum and Leloir's book on leprosy and Hawaii Report published in 1886.

Another theory which attracted some attention, was the fish theory of Sir Jonathan Hutchinson in 1863 (Dharmendra, 1960). It was believed that consumption of fish gave rise to leprosy in one of the following ways: (i) Excessive use of fish or use of fish in association with articles of diet such as milk, (ii) use of putrid and decomposed fish, and (iii) consumption of fish suffering from some disease allied to human leprosy. The theory, however, did not remain popular for long, because it was found that leprosy could sometimes be found in people who never have eaten fish and on the other hand, it had disappeared from the place where fish continues to form an important article of diet.

It is now accepted that leprosy is an infective disease caused by *Mycobacterium leprae* and more popularly known as Hansen's bacillus or lepra bacillus. It was discovered by Dr. G. Armauer Hansen in 1863 (though his published account did not appear till 1874), who stated the teaching that bacteria cause disease, was in its infancy and no disease was known to be of bacterial origin. Few leprologists appreciated the significance of Hansen's remarkable discovery but Danielssen and Boeck (1848),
the foremost leprologists of that era, who attributed leprosy to multiple and separate origin, firmly opposed the Hansen's view that the 'rods' which had been observed by him, were the cause of the disease. The acceptance of Hansen's bacillus as specific agent was supported in Germany by the work of Neisser, 1879, and in France by Brock in 1885, Leloir in 1886 and Besnier in 1887. Jeanselme (1934) remarked that the discovery of specific bacillus of leprosy by Hansen ruined many a cherished hypothesis and redeemed to the status of secondary causes many aetiological factors to which previously a preponderant role had been attributed.

A considerable long period has elapsed since the discovery of the causative organism and an immense amount of research work has been done all over the world without having yet fully elucidating the manner in which the organism passes from the diseased to infect healthy and yet the organism does not fulfil the Koch's postulate. That Myco. leprae is the causative agent of leprosy is supported by the following factors: (1) The constant presence of the bacillus in leprous lesions; (2) the characteristic cellular reaction provoked by Myco. leprae or its chemical fractions, and by (3) the fact that Myco. leprae cannot be found in healthy individuals.

Bacteriology of Myco. leprae:

The organism is a Schizo mycete of the order Actinomycetales and of the family Mycobacteriaccae and of the genus Mycobacterium.
The organisms of this genus are called acid-fast because of the property of resisting decolourisation by dilute acids, once they have been stained with strong basic dyes such as carbolfuchsin. The bacilli vary in size and shape but are usually straight or slightly curved rods varying from 1 to 3 μ in length and from 0.2 to 0.5 μ in width with parallel sides and rounded or blunt ends and stain evenly. Instead of staining solidly and evenly, they may resemble diphtheroids and show granular staining, confined to the poles or distributed throughout their length. Clubbed forms are not unusual, the organism often appearing fragmented or beaded. Lateral buds or branches are occasionally observed or the bacilli may be seen as oval body with its greater diameter from 0.8 to 1.6 μ. The leprosy bacilli are usually found in agglomeration termed globi which have the appearance of tightly packed bundles of cigars. Denny (1934) suggested that the globi are colonies growing within an unidentified restraining membrane. The clumps of bacilli appear to be found together by a lipid-like substance, the glia. The fact that *Mycobacterium leprae* appears in both intracellular and extracellular glial masses suggests that they are enclosed in a capsule-like membrane, Babes (1901) also describes a surrounding slime or capsule which has been recently confirmed by Hanks (1961). They are non-motile and non-sporing. The organism in size, morphology and in acid-fast staining reaction, closely resembles *Mycobacterium tuberculosis*, but it is more deeply stained with carbol fuchsin and with Gram's stain. It resists decolourisation with weak mineral acids to a lesser degree than *Mycobacterium tuberculosis*. *Mycobacterium leprae* is less granular,
has larger and more clearly defined granules and is more frequently observed intra-cellularly.

The morphology of *Mycobacterium leprae* as revealed by classical optics was described by Lohnis (1922) after a review of the literature from 1833 to 1913 and completed by Paldrock (1923).

**Electron microscopy:**

The electron micrograms of unsectioned bacilli, however, present a shadow image from which we have to deduce details of internal structure. More information can be obtained from ultrathin section of tissue or bacillus or cell. The first electron microscopical studies of Bishop *et al* of 1948 have shown that leprosy bacilli may appear as a filament, transparent in the electron beam, with denser polar ends and that it differs in appearance from tubercle bacillus grown in culture. Leprosy bacilli in smears of tissue juice often show this configuration (Brieger and Glauert, 1956). The dense condensation at the poles often appear as rod shaped inclusions and such inclusions are also seen in other parts of the bacillary cytoplasm. In other bacilli of the same preparation the cytoplasm appears vacuolized. Denny (1934) described a globus as a mass of bacilli contained in what appears to be a limiting membrane with the bacilli arranged concentrically around its edge. Rees *et al* (1953, 1960) demonstrated three distinct morphological types such as normal, degenerated and segmented, of which the degenerated forms are found in high proportion. deSouza- Arauja (1955, 1959) confirmed by electron microscopy the bacillary membrane, glia and granules, both internal and free and in gemmule forms what are seen by phase contrast microscopy. Similar findings on phase microscopy were also found by Richards and Wade (1948).
and Chatterjee et al (1955 a, b), Mo Fadzean and Valentine (1953, 1959, 1960) concluded that on an average, 56% of the leprosy bacilli are degenerated in untreated cases of lepromatous leprosy in contrast to about 5% degenerated form in rat leprosy. The percentage of degenerated forms increased after 6 months' treatment with sulphone and it was first noted by Malfati & Jonquires (1953) under electron microscopy. The treated bacilli show shrunken cytoplasm and empty cell membrane. These observations have been confirmed by other workers like Malfati (1952), Haedicke et al (1952), Terada (1953), Kooij (1953) and Immeda (1958) etc.

Yamamoto et al (1953) described the morphology of ultra-section of the bacillus. The cell wall of the organism is about 6 mμ and consists of outer and inner layers which are electron dense and a central layer which is less dense. Cytoplasm of the bacilli is moderately electron dense and has a relatively homogenous appearance. In treated cases only the cytoplasm shows condensation at the polar ends and sometimes granules (13 - 36 mμ) and these are believed by Bishop et al (1948) as nuclear structure. The nuclear apparatus is composed of moderately electron dense threads about 9 mμ wide which show distinct coiling along the long axis of bacillary bodies. The nuclear threads appear to be embedded in an electron-transparent matrix. In treated cases no clear threads are found. Brieger and Glauert (1956) also found dense threads and granules in transverse ultra thin section of leprosy bacilli. Chatterjee et al (1955) expressed the view that a slow phase of multiplication results in solid, homogenously dense bacilli while a rapid phase results in the forms possessing alternate light and dark regions.
Culture in vitro:

Innumerable attempts to cultivate *Mycobacterium leprae* have been made but yet no satisfactory proof exists that the real causative organism has been cultured in vitro. Many workers have reported the cultivation of one or more bacilli from the tissue of lepers. Diphtheroid like non-acid fast organisms have been isolated by Kedrowski (1901), Williams (1911), Bayon (1912), Duval and Wellman (1912) and Sarkar (1962). Chromogenic acid fast organism reported by Clegg (1909), Rost (1911), Williams (1911), Duval and Wellman (1912), McCoy (1914), Currie, Clegg and Hollman (1912) as well as many others. The nonchromogenic forms were observed by Duval (1910), Duval and Wellman (1912), Soule and McCoy (1932b), McCoy and Verder (1933), Soule (1934), McCoy and de Leon (1937), and the anaerobic forms were isolated by Ducrey, Campana and Serra (Cochrane, 1959). Most of the works were not reproduceable by many such as Schlossmann (1933), Duval and Holt (1934), Holt (1934a, b), Lowe and Dharmendra (1937), Dharmendra and Lowe (1933) and Chausin and (1947). In reviewing the literature on the bacteriology of leprosy, McCoy (1934), Soule and McCoy (1933) and Bechelli and Rotberg (1951) are of opinion that the organism isolated could not be proved to be *Mycobacterium leprae*. So the isolated organism may be either (i) contaminating organism that have nothing to do with the causation of leprosy or (ii) they are different stages in the life history of the true leprosy bacillus or (iii) they are the organism whose presence is in some way associated with that of the true leprosy bacillus which has not yet been cultivated.
It is certainly possible that as postulated by Hanks (1945) that Mycobacteria form a spectrum ascending from saprophyte through the commensals and intermediate form to higher species such as *Mycobacterium tuberculosis* which are pathogenic for different animals, from *Mycobacterium paratuberculosis* which has delicate growth in vitro and is highly selective. *Mycobacterium leprae murium* which cannot be cultivated in vitro and is found to infect only a few species of rodents and finally *Mycobacterium leprae*, uncultivable in vitro and infective only to man. In this series, the increasing selectivity of host species may well be related to an increased necessity of organism for intracellular existence, limiting the ability of the organism to gain energy from in vitro substrates and requiring protection afforded to it by the host's cell wall from humoral components or secretions. It is logical that in as much as intracellular survival is associated with low oxygen requirements, oxygen becomes increasingly toxic for the Mycobacteria at the upper end of the spectrum. This hypothesis may explain the difficulty encountered in cultivating *Mycobacterium leprae* and suggests numerous avenues of approach to the problem of cultivation. Many workers including Shepard (1957), Fjelde (1957) etc. studied the behaviour of different Mycobacteria using different types of tissue culture. Ranadive et al (1958) isolated an acid fast organism from cases of human lepromatous leprosy in a tissue culture system consisting of the SPG fibrocytic cell line derived from human foetal spinal ganglia. The organism designated the ICRC bacillus, can be grown and maintained in the conditioned fluid of stock.

Although generally the greatest numerical concentration of
organism in leprosy is within the cells of superficial tissue, in the neuritic form of the disease the bacillus seems limited to the nerve plexus. This predilection for nerve tissue in the presumably more resistant form of the disease might well furnish a clue to its optimal ambient which might be applicable to attempts at cultivation of the organism.

**Experimental inoculation:**

Many attempts to infect animals with organism have been made with unsatisfactory results. The injected bacillus can produce lesions at the site of inoculation and also elsewhere but there is usually no definite evidence of multiplication or of the generalisation of the disease. Similar lesions produced when the bacilli are killed previously by boiling. The bacilli will retain their morphological and staining characteristic for more than twelve months when inoculated into rats and may be found carried to their parts of the body such as liver or spleen. So it appears that, upto the present, the only animal found susceptible to the organism is man.

It is still a debated question whether or not infection can be induced by inoculation into skin. Klingmuller (1930) reviewed elaborately the evidence for and against experimental inoculation in man. Jeanselme (1934) cited the negative examples of Danielssen and Boeck (1843), Profeta (1884) Mouritz (1916) and concluded that there was no adequate proof of transmission by inoculation. Arning (1886) and Marchoux (1934) demonstrated the development of leprosy after inoculation. De Langen (1933) reported an accidental inoculation by a physician using for a hypodermic injection, a syringe...
which had been used on a person suffering from leprosy. Lagoudaky (1936, 1937) reported that repeated injections with blood of leprosy patient, developed cutaneous lesions. Porritt and Olsen (1943) described the development of leprosy over the site of tattoo marks in two men from the same community who were tattooed by the same person on the same day in Melbourne. So numerous attempts at experimental inoculation of human beings have been made, with positive results in a few but the conditions of the experiments have not been entirely satisfactory (Wade, 1943).

Normal animals are refractory to infection and almost every species of animals and every possible routes of inoculation have been investigated. The earlier literature on this subject have been well reviewed by Mc Kinley (1939). However, among the investigators who have reported some degree of success, the following are particularly noteworthy.

**Monkey:**

Nicolle (1905) inoculated two bonnet monkeys with reported successful results. Reenstierna (1926) and Soule and Mc Kinley (1932 a) found almost similar results in these animals. Collier (1940) reported success in the transmission of human leprosy to monkeys who had been fed on Colocasia or had received injection of sapotoxin prepared from Colocasia. Dharmendra and Mukerjee (1944) reported negative results in splenectomised monkeys. Cochrane et al (1945) did not find general dissemination of infection in (i) splenectomised monkeys; (ii) such animals put on Colocasia antiquorum; (iii) monkeys previously injected with Indian Ink. Lai (1955) concluded from his experiments with Taiwan
monkeys that suggestive signs of human leprosy can be induced in them.

Hamster:

Adler (1937) was successful in inoculating these animals intraperitoneally and by subcutaneous implantation of leprous nodules. Dharmendra and Lowe (1940) using a method similar to above, with 23 animals and extending the period of observation to one year, came to the conclusion that although bacilli could persist for long period but there was no multiplication. Burnet and Jadford (1940) reported successful infection of a syrian hamster by oral route. Burnet (1940) failed to infect more than one of the hamsters with human leprous material and stated that there was no clear evidence that hamsters are anymore susceptible to such inoculation than other rodents. He stressed the difficulty of excluding tuberculous infections (to which the hamsters are very susceptible), since some types of tuberele bacillus may fail to infect guineapigs. He also pointed out that only the development of typical mass infection of lepra cells or globi can be taken to prove infection with living bacilli. Chatterjee (1953 b) reported successful results by using any method of inoculation. Binford (1953 a, b) found histocytic granulomatous lesions in the testis and ears of the golden hamsters approximately 18 months after inoculation resembled human lepromatous leprosy in histologic pattern, number of intracellular Mycobacteria and the presence of bacilli within nerves. Thus isolated instances of infection, has been observed in hamsters, the majority of the workers did not find any clear evidence of general dissemination or progression of the infection.
Mice:

There is no unanimity of opinion about the success of inoculation of mice with human leprosy organism. While de Souza Arauja (1928, 1929), Shiga (1936), Nojima (1939), Yamamoto (1938) claim success in this direction with the possibility of passage to successive batches of animals. Sellard and Pinkerton (1936), Suzaki (1939), Burnet (1940), Nakagawa and Nakamura (1954) are of opinion that there is no definite sign of multiplication of the bacilli with general dissemination. Chatterjee (1958, b,c,d) demonstrated multiplication and generalisation of *Mycobacterium leprae* in specially selected hybrid black mice by using almost tissue free suspension of bacillus as an inoculum. He was successful by adopting any method of inoculation, and these findings require confirmation by other workers. So it appears that the problem of transmission of human leprosy to mice remains unsolved even now.

**Rat:**

Fite (1941 b) succeeded in producing a nodule in 6 out of 154 rats after an inoculation period of 18 months, at the site of injection of an emulsion of human leproma. Mucin suspension of the inoculum gave a larger proportion of positive results with more numerous bacilli. de Souza Arauja (1941) inoculated 3 white rats subcutaneously into the axilla with pus from groin gland of a patient rich in bacilli. Visceral infection was found in 2 of these after 15 and 17 months. The remaining one did not show such involvement after 13 months. Barman (1945) gave 56 - 76 inoculations over a period of 7 months or more to white rats 2 - 10 days old. A year after the last injection, non-specific inflammatory changes were found in various organs and...
many acid fast bacilli. The infected tissue were infective to small rats with only 4 injections. Lepromin prepared from the infected tissue gave a typical Mitsuda reaction in tuberculous cases and negative reaction in lepromatous cases. Fielding (1946) used dried faeces of patients containing leprosy bacillus to infect rat by repeated inoculation of scarified skin, in the skin shaft and by subcutaneous injections. The acid-fast organisms were subsequently found both locally and in small number internally in the rats.

Irradiated animals:

Feldman (1956) proposed animal transmission by whole X-ray-irradiation to reduce properdin level. Kelkar and Ranadive (1958) found evidence of survival, maintenance and limited multiplication of acid-fast organism (Mycob. leprae) in 50% of irradiated golden hamsters inoculated previously with material from leprosy, but Ghosh et al (1961) failed to demonstrate multiplication of Mycob. leprae in irradiated suckling white mice and Binford (1958 a, b) was not successful in cortisone treated or / and irradiated animals of different kinds such as golden hamsters.

Other animals:

Sato (1949) employed various methods of inoculation with gerbils, gold fish, frogs, toads, birds - such as paddy birds, canary, parrot and love birds, mus bacterianus, rattus norvegicus, caria coboya, rabbit, dogs, Japanese monkeys and hens. The results had been negative. Repeated injections of live bacilli into hens cause lesions similar to the changes of nodular leprosy but those lesions proved negative with successive inoculation. Tanimura and Nishimura (1953) inoculated fowls, white rat, guinea-pig, rabbit, golden hamsters.
producing only local reaction without any sign of multiplication. Mitsuda (1941) injected a bacillary emulsion into testicular substance of small pig and could produce only a tuberculoid lesion after 27 days. Wilkinson (1964) remarked that experimental transmission of guinea-pig is possible provided large number of bacilli and hyaluronidase are supplied. Nonaka (1940) failed to transmit Myco. leprae in chicken and Lobo and Carvalho (1946) came to a similar conclusion with respect to chicken and pigeon (1946). Ota (1941) succeeded in producing local lesions in breast muscles of hen in 50%. These persisted up to 6 to 12 months. Ota and Sato (1941) concluded that of all the laboratory animals, the fowl is the most susceptible and this could be transferred successively to seven generations (Ota and Nitto, 1941). Sato (1951) reported the occurrence of both local and visceral lesions in fowl and successful passage to two series. Chaussinand and Besse (1951) succeeded in infecting 4 rainbow perch.
PATHOLOGY OF LEPROSY

Uptil now there is no indication of the reasons why a specific sort of granuloma, in response to a particular microorganism was constituted. Nor could any clear idea be formulated regarding the mode of evolution of leprosy in man, and its spread in infected persons even after a careful microscopical study of the lesions from advanced cases of leprosy. The inability, artificially to cultivate the causative organism and to observe the evolution of the disease in experimental animals leave the gate widely open to theory and supposition and sometimes to the development of extravagantly fanciful ideas concerning the transmission and spread of the disease in human being. As here nature makes the experiment and we watch and understand them if we can and that these experiments supply a wealth of information on the fundamental question in human physiology while it would be difficult to glance even with the most clearly devised animal experiments.

As in the case of other mycobacterium, the most successful reaction to the primary lodgement of Myco. leprae at the site of introduction would be their complete suppression in so effective a manner that neither the histological picture, which morbid anatomists seek to recognize, nor the clinical lesions which the physicians strive to identify, are allowed to develop. This is observed in the case of those contacts of leprosy patients (Figueroeda and Desai, 1949) who escape histological or clinical evidence of infection although they show a temporary presence of acid-fast bacilli in the dermis and a graded, but more lasting, immunological transformation from a lepromin
negative to a lepromin positive condition. It is presumed that the leprosy bacilli enter into the body through breach of skin or breach of mucous membrane, but it was shown (Khanolkar, 1951) that infection may also occur through healthy skin. The bacilli float about as inert particle in the fluid watershed and the superficial lymphatic network of the dermis to all the parts of skin under the epidermis. Then the evolution of the diseased process depends on the susceptibility of the individual to leprosous infection and this depends on multiple factors, the knowledge of which is still imperfect such as natural immunity, acquired immunity or some secondary factors etc. when the suppression of the invading organisms is for a while incomplete - in other words, where there is an initial failure of the defence mechanism - the mycobacteria adapt themselves to the new environment in the dermis, slowly multiply and spread around in the outer layers of the cutis. The defence mechanism of the human host slowly and very gradually comes into operation. It is not yet known whether there is a very early and evanescent phase of tissue response comprising of changes in the blood-capillaries, fluid exudation, and a polymorphonuclear cell migration to the site of the deposition of the mycobacteria. The bacilli are ingested by wandering histiocytes but it takes some considerable time to completely digest the microorganisms. In the skin biopsies of certain presumably healthy contacts, it was noticed (Khanolkar, 1951) that such cells packed with acid-fast rods and broken-down bacilli, are called fuchsinophil cells because of the retention of basic dye in the cytoplasmic contents in spite of decolorization with weak acids. Presumably this stage may continue for months, even several years, and may finally terminate
without any clinical evidence of the disease. It may therefore be considered as a 'Silent phase' of infection. The further evolution of the disease, that is in those persons in whom spontaneous recovery does not take place, usually proceeds at a leisurely pace, with periods of quiescence, interspersed with spurts of abrupt activity.

The leprosy bacilli spread along the lymphatics into regional lymphatic gland or carried along the peri-neural lymphatics to the nerve trunk. Recently it has been shown by electron microscopy (Nishiura et al., 1957) that bacilli is taken up by the phagocytic activity of axon of the nerves. Recent tissue culture studies of the spinal ganglia (Nakai, 1955, 1956) have revealed pinocytosis and a tropistic reaction of the growth cones of the axons. From this finding, it is possible that growth cones of the regenerating axons catch leprosy bacillus and engulf them into axoplasm. In the pinocytosis of the growth cones of the axons, the vacuoles move in the centripetal direction in the axons. This finding suggests the presence of a centripetal stream of axosplasm which would transport the engulfed bacilli towards the spinal ganglion cells. It was also demonstrated by Nishiura et al. (1957) that cytoplasm of the Schwann cells which surrounds the normal nerve fibres did not show the presence of bacilli but only in Schwann cells of Bunher cord which probably phagocytosed the bacilli in the empty axon. The leprosy bacilli then multiply in the axoplasm of the nerve fibres and later phagocytosed by the Schwann cells and often remain there dormant in that sheltered location for long periods of time. Under the stimulus of certain supervening changes in the function of the body, as during adolescence, puberty, pregnancy and the onset of mild maladies, the bacilli begin to proliferate in the nerve fibres and appear in large numbers in the inter-
calated zones, from which they burst out in the endo and perineural tissues. There, they are taken up by the histiocytes which gradually become transformed either into lepra cells or into epithelioid cells depending upon the immunological response of the host to the presence of leprosy bacilli (Khanolkar, 1961). The pathological alterations and the subsequent signs which are observed in the nerves and in the skin are dependent upon the distribution of the inflammatory exudate in them and upon the functional impairment of the nerve supply to the cutaneous blood vessels and to the deeper cells of the epidermis.

The pathological changes in leprosy had been described by many workers and here an attempt has been made to summarise their findings of different kinds of tissues.

SKIN: Diffuse infiltration, localised patch with slight or marked infiltration, nodules may be present. Scaling and ulceration may be found.

NERVES: Both the cutaneous nerves and nerve trunks may be involved; the affected nerves are thickened sometimes markedly, often unilateral but bilateral and symmetrical in lepromatous cases. The thickening may be present over large parts of the course or in some limited parts only. The thickened nerve is hard and usually tender to touch. There may be round or oval swelling along the course of thickened nerve indicating the position of a nerve abscess. A nerve abscess is caused by localised area of necrosis and caseation in a tuberculous lesion of the nerve but very unusual in lepromatous cases. On opening, the nerve sheath is found thickened and the nerve bundles are often widely separated by whitish strands of chronic inflammatory
tissue, a nerve abscess results if the areas of caseation are large. In chronic long standing cases, instead of being thickened, the nerves may be thick and atrophic and consist of little more than bundles of fibrous tissue.

LYMPH GLANDS: Regional lymph glands may be enlarged and in some cases slight generalised glandular enlargement may be found. The changes are more marked in the superficial lymph glands, the glands in the thoracic and abdominal cavities show less marked changes. The enlarged lymph glands may burst, produce chronic ulcers and sinuses discharging pus.

BONES: The bone marrow may be infected and contain lepra bacilli. There may be leprous periostitis, osteomyelitis, enlargement of nutritive canals of the bones and even cyst formation in the bone. Degenerative changes may be present in the joints.

In the areas supplied by the affected nerves, trophic changes will be found in the bones. The changes in the bones are of trophic nature and results from the combined effect of neurocirculatory changes following on nerve involvement. As a result of pressure and injury on the affected part, there may be ulceration and secondary infection. The small bones of hands and feet are generally affected. The primary changes include decalcification, diffuse rarefaction, concentric atrophy and there may be complete absorption and destruction of bones. The most marked bony changes are found in secondary infection following an ulceration in the affected parts. These include periostitis, sclerosis, necrosis and complete or partial absorption of the bones and degenerative changes in the small joints.
EYES: Leprous lesions include episcleritis, conjunctivitis, keratitis, iritis, irido-cyclitis and panophthalmitis. When the eyes are involved due to affection of their nerve supply, there may be lagophthalmos, ectropion of the lower eyelid and the anaesthetic eye may be infected with secondary infection.

GASTRO INTESTINAL TRACT: There is leprous infiltration or nodulation of the mucous membrane of the lips, tongue and pharynx. Leprous changes have been reported in teeth, specially in the pulp; stomach and intestine do not usually show any gross changes but on microscopic examination may show presence of lepra cells and lepra bacilli. Nodules and ulcers in the intestine have also been found. The liver and spleen may show enlargement. Secondary amyloidosis of the spleen and liver may be present in advanced cases. The gall bladder is rarely affected.

RESPIRATORY TRACT: The upper part of respiratory tract, the nose, larynx may show infiltration, nodulation and ulceration, scarring etc. The affection may extend down to trachea, bronchi and bronchioles but lung parenchyma is usually not affected.

CIRCULATORY SYSTEM: The peripheral blood vessels, both the arteries and the veins may show sclerosis and the presence of leprosy bacilli in the walls. Proliferation of endothelium also are found. Heart and large vessels do not show any change.

UROGENITAL SYSTEM: The kidney, bladder, ovaries etc. do not show any macroscopic changes but microscopically may reveal the presence of leprosy bacillus and lepra cells. In advanced cases, secondary amyloidosis may be seen in the renal glomerules. Testis may show
microscopic involvement, hypertrophy and swelling later followed by atrophy; hyalin degeneration of the tubules may be present. In the case of testes, the affection may be marked enough to produce Gynaecomastia, i.e. enlargement of the male breast. The presence of leprosy bacilli in the placenta of infective cases of leprosy has been demonstrated but the bacilli are more common on the maternal side than on the foetal side.

GLANDS OF THE INTERNAL SECRETION: Microscopic evidence of infection is found in the glands of the internal secretion only in the case of testes, the affection is marked enough to produce symptoms such as gynaecomastia and loss of hair etc.

HISTOPATHOLOGY

The bacilli are carried away either by the blood or lymph when it becomes generalised to the distal organs. In the internal organs, the process appears to be self-limiting and it seems that without a continuous fresh supply of fresh bacilli and their ingestion by reticulo-endothelial cells, the lesions run a restricted and restrained course. The histological appearance of the productive granuloma in these organs presents a monotonously uniform picture. The histological reaction may be grouped under the following four types:-

(a) Lepromatous histology:-

In the very early stage there is usually only perivascular and perineural infiltration with loose granuloma consisting of
collection of small round cells and bacillary laden epithelioid cells with considerable amount of vacuolation, though well-formed foam cells may also be present. In the earlier stages the granuloma is loose and diffuse and forms a sheet of granuloma. The granulation tissue is held together in a very fine and rich tridimensional network of reticulinfibres. In well developed cases, the epidermis becomes thinned out and flattened, and clear subepidermal zone which is practically free from infiltration. There is distortion, even destruction of the hair follicles, the sweat and sebaceous glands. The nerves stand out prominently in the midst of granulomatous foci as nerves are free or comparatively free from infiltration although there may be perineural infiltration. Large number of acid fast bacilli are seen in the axon, Schwann cells and histiocytes. The bacillary laden cells are derived from (Khanolkar, 1955) inconspicuous non-phagocytic undifferentiated mesenchymal cells which are located in the proximity of blood capillaries and retain full potentialities of embryonic tissue for differentiating into phagocytic, supporting and blood cells. These cells were described by Danielssen and Boeck (1843) as oblong cells larger than usual inflammatory cells in leprous nodules. Later their morphological description was made by Virchow (1860, 1861), the father of modern pathologists in Germany and will bear the repetition even today as Virchow's cells or lepra cells, before the era of modern techniques and high power microscope. These cells consist of round, pale, finely acinous, easily perishable element with at most a moderately large and at the same time granular nucleus with nucleoli. In fresh state there is a particularly notable peculiarity, namely, their marked tendency to form a sort of vacuoles so that
they acquire a wholly physaliferous appearance. Virchow suggested that the lepra cells were derived from connective tissue cells in which he found nuclear division. Yamamoto et al (1958) and Nishiura (1960) described the morphology of lepra cells under electron microscope as cells which contain leprosy bacilli in the cytoplasm. Single bacillus and small group of bacilli are tightly surrounded with a limiting membrane which seems to be the cell membrane of the same lepra cells indented deeply during the phagocytic process along with various normal cytoplasmic components such as microsomes, mitochondria, lipoid granules and endoplasmic reticulum. Bacilli are often embedded in opaque droplet and the dense granular material of this opaque droplet in which the foamy structure develops from another inner limiting membrane. Each unit of foamy structure has a single layer of markedly electron-dense membrane (Brieger and Glauert, 1956), which seems to be a network of electron dense micells each about 20 μ long and 3 μ wide. The leprosy bacilli in foamy structure of lepra cells have the characteristic of lying side by side (observed under also light microscopy). The nucleus of the lepra cells is found aside by the foamy structure of the cytoplasm. The cell membrane is a continuous single structure but in a fully developed leproma it is often very hard to find the boundary of each lepra cell.

This type of histology is characteristic of lepromatous type and are found in the skin, nerve, mucous membrane, lymphatic gland, bone marrow and internal glands. When the disease regresses, either spontaneously or as a result of treatment, the lesions in the skin heal in a remarkable manner. There is often an absence of loss of
tissue or deep scarring which is so characteristic of other productive inflammatory conditions. The healed lepromatous lesions in the skin show a peculiar wrinkled crushed tissue paper appearance. Histologically it is seen that the granulation tissue gradually disappears, there is a collapse of the reticular frame work and a recession of the exuberant capillary network. This is accompanied by a regeneration of fine elastic and collagen fibres which bridge the gap left over by the absorption of the granuloma, but still the footprints of lepra cells may be found. These observations have also been confirmed by other workers, i.e. Henderson (1928), Mitsuda (1936), Bertellotti (1939), Fite (1943), Tilden (1945), Noel and Suzenee (1949).

(b) Tuberculoid histology:

The term has been derived from the structure 'tubercle' which is a constant finding in tuberculoid histology. Cases of tuberculoid leprosy were described by Arning (1884) and Jadassohn (1898) but its existence was not confirmed by other workers. It is amusing to read about the re-discovery of the type as an unusual occurrence from time to time (Betlley and Alpine, 1953).

Initially there is appearance of groups of epithelioid cells inside the fine nerve twig and the formation of sharply circumscribed foci of such cells surrounded by lymphocytes and histiocytes. The epithelioid cells show a tendency to coalesce and form under certain circumstances typical Langhans' giant cells. The architecture of the nerve twig in which this exudate gathers is progressively disrupted within the frame work of its epi and perineural sheath. The nerve fibres are frayed by the exudate, shrunk to knot-like vestiges and
and later with their complete obliteration have vague ghost outline of the original fibres. The reticulum network in which the granuloma develops is recognizably different from that of leproma. It is more open and irregular in its arrangements with wide gaps towards its middle, where epithelioid cells come to settle down. The fibres vary in thickness, but usually are coarse. The meshes of network become tighter towards the periphery where the reticulum fibres occasionally merge with the collagen in the adjacent area. This localised and compact granuloma spreads in the subepidermal zone and in the dermis in a cord like - usually along the neurovascular bundle. The epidermis at places is thinned out due to extension of the granuloma. These foci may later coalesce and become generalised, but still there remain indications of the foci having been separate. The granuloma composed of a central cone of large cells with faintly acidophilic, finely granular cytoplasm and indistinct cell margin and rarely necrosed tissue. Interspersed between these cells there are a few Langhan's giant cells. The central zone is surrounded by layers of variable thickness of lymphocytes, epithelioid cells, histiocytes and cells with acidophilic granules in the cytoplasm. The epithelioid cells do not show vacuolation. The most peculiar and most probably the most consistent feature of tuberculoid leprosy lies in changes in the nerves. The nerve shows endo, peri and epineurial infiltration. As a result of these cellular infiltration amongst the nerve fibres which causes pressure there may be slight to complete destruction of nerve fibres. Nishiura (1960) described the changes in the nerve under electron microscope as follows: (a) Zone of Wallerian degeneration, (b) zone of epithelioid tubercle formation, (c) zone of necrosis.
(d) zone of 

zone of necrosis. In the last zone, large cells containing many lipid droplets and onion-like bodies were found.

The degree of the tuberculoid change varies with the clinical activity and therefore with the thickness of lesions, the greater the thickening, the more marked the tuberculoid change. Bacilli are usually found in the axon, Schwann cells and there is extreme paucity of acid-fast bacilli in the cells of the inflammatory exudate.

C) Mixed, dimorphous or borderline histology:

This type shows all kinds of gradation, and combination of above types; for example, element of both tuberculoid and lepromatous histology may be found but none of them may be sufficiently well-defined for the section to be labelled as one or the other or one type of histology may be dominant but some features of the other types may also be present or else neither of the two elements may be definitely present. This is an unstable state.

D) Simple or uncharacteristic histology:

Nonspecific granuloma, mostly with small round cells which are found around the appendages of the skin and nerves. The granuloma consisted of small round cells, a few epithelioid cells found in the dermis. This type of histology may be found in any chronic skin diseases but endoneurial infiltration and the presence of acid-fast bacilli in the nerve are characteristic of this histology which is absent in other chronic skin diseases.

Histological observations have been made by many workers, i.e. Kedrowski (1914), Tebbut (1926), Henderson (1929), Muir and Chatterjee (1933), Wade (1934, 1937), Lowe (1936), Wade and Rodri-
guez (1937), Hughes (1938), Grieco (1938), Oberdoerffer and Collier (1939), Saenz and Palomino (1939), Bosq (1940), Fite (1943), Decotild (1948), Lowe et al (1948), Dharmendra (1949), Saikawa (1951) etc.

**REVIEW OF HISTOCHEMISTRY OF LEPROUS LESIONS.**

It was first claimed by Virchow (1860, 1861) that vacuoles in the lepra cells were due to a state of hydropsy but Mitsuda (1928) using the histochemical methods at that time for the demonstration of fatty substances, reported that the lepra cells contained lipoid and a by-product of destruction of leprosy bacillus. Herxheimer (1923) carried out detailed investigation of fat in lepra cell by means of various methods and demonstrated a mixture of cholesterol, glycerol and fatty acids.

Ueda (1949) reported that the chief component of lepra cell was lecithin like fat and that a large quantity of cholesterol is present when there is necrosis of lepra cells. Fite (1951) concluded that vacuoles in lepra cells were made up of a combination of neutral fats, fatty acids and their esters excluding cholesterol. Chatterjee (1953 a) and Chatterjee et al (1956 abc, 1957 ab, 1959) demonstrated by applying different histochemical technique, the localisation of various chemical constituents in the leprous lesions and in leprosy bacillus.

Harada (1955) applied Masson's methyl green-pyronin, PAS, Sudan III and black and Nile blue method to investigate the formation of lepra cells and concluded that phagocytosed bacilli and the cytoplasm of the lepra cells undergo fatty degeneration and the neutral
fat is discharged into the blood. Later, Harada (1956) tried to correlate the different histochemical findings with the stages of formation of lepra cells. Ortmann et al. (1956) applied Baker's method of lipoid demonstration and PAS in lepromatous leprosy and demonstrated that inclusions consisted mainly of lipids and polysaccharides and probably protein components. Sugai (1953) found phospholipids (Lecithin) fatty acids, a small amount of neutral fat and at times sterol is found but this can be interpreted as a process of cell degeneration.

Imaeda (1960) analysed physico-chemically the different kinds of lipoid in lepra cells by using electron stains of various purified lipids and concluded that opaque droplet of lepra cells contained lipoprotein. The minute vesicles, which arise from disintegration of opaque droplet is composed of phosphatide. The foamy structure, the terminal phase of opaque droplet, contains saturated fatty acids, their esters or lower molecular substances. The electron-transparent zone around leprosy bacilli does not consist of a lipoid phase but of a water phase in which are dissolved the metabolites of the bacilli.

Cutaneous nerves in different types of leprous lesions were studied (Gault et al., 1955; Mukerjee and Ghosal, 1957) by the acid phosphatase method of staining.

Wells (1957) demonstrated alkaline, acid phosphatase and non-specific esterase in different types of leprous lesions. In the tuberculoid type of leprosy, acid phosphatase was diffusely present with somewhat greater concentration in the giant cells and epithelioid cell but also present in lymphocytic mass. Strong esterase activity
was found in the cytoplasm of giant cells and other large branched cells of the infiltrate. No esterase was found in the epithelioid cells or lymphocytes or plasma cells. The heaviest concentration of esterase activity was found in lepromatous leprosy, especially in lepra cells. Imaeda (1953) found increased phosphatase activity in both lepromatous and tuberculoid lesions.

Pepler et al (1958) found more acid phosphatase in lepromatous leprosy than in tuberculoid type. So this enzyme may perhaps be of some importance in connection with lipid metabolism in the leprosy bacillus as in the case with the tubercle bacillus. Non-specific esterase, sulfatase was present in the infiltrate in both forms of leprosy. Neutral fat was demonstrated in Virchow Cells in every case of leproma as well as in a few epithelioid cells of tuberculoid cases. No phospholipids were ever found.

Histochemical methods have been used by some workers in the classification of leprosy. Jonquieres and Beramendi (1955) attempted to classify between lepromatous and borderline leprosy with the help of fat staining with Sudan III and later Davison et al (1960) evaluated the methods of fat staining in the classification of leprosy and concluded that the result of fat staining run parallel with the number of acid fast bacilli in the histological specimen and even with the results of bacteriological examination of smears and therefore does not give any more information for classification.

Bergel (1953) used methylene blue stain for classification as it stained strongly in vivo and vitro the lepromatous and yellow fat tissue but not other normal ones. Similar observation was also made by Convit et al (1960).
In addition to these publications, isolated works on this line have been found in the literature (Ogita, 1955; Dharmendra and Mukerjee, 1954; Azulay and de Andrade, 1952; Campos, 1950; De Souza and Alayon, 1942).

OBJECT AND SCHEME OF PRESENT INVESTIGATION

The object of the present investigation is to study the presence and localisation of different microscopical substances in the causative organism and the affected lesions in man.

This investigation has been aimed at the demonstration of particular chemical substance by standard histochemical methods. They are lipids viz. neutral fat, phosphatides, fatty-acids, cholesterol and its esters, carbohydrates - viz. polysaccharides in general, acid mucopolysaccharides, enzymes - viz. alkaline and acid phosphatase, G.nadi oxidase and DOPA oxidase reaction; protein viz. simple, DNA and RNA.

This study is expected to throw some light on the host-parasite relationship in leprosy, particularly the nature of changes induced at the cellular level. Histochemical features observed in polar types of leprosy may throw some light on their genesis.