REVIEW
OF
LITERATURE
Leprosy: Historical aspects:

Leprosy is a disease of antiquity. Mankind as a disease knows it since ages. It has been prevalent since ancient times in India, China & Africa. Egyptians accounts tell of a disease resembling leprosy as early as 4,600 B.C.

Leprosy is mentioned at several places in the "BIBLE". In Biblical times lepers were treated as outcasts. People used to fled from them. They were segregated from the rest of the inhabitants & pronounced unclear.

Leprosy has been described as 'UCHEDA' in 'EBER’S. PAPYRUS' written about 1555 B.C.

Leprosy is mentioned in 'RIGVEDA' about 1500 B.C. It was then called as 'KUSHTHA' meaning leprosy as well as other skin diseases.

"SUSHRUTA SAMHITA" presents earliest description about leprosy under the term "VAT-RAKTA" & "VAT-SAMHITA". It recognises two types of leprosy, one neural & other with ulceration. He also mentions "TUVARKA" as a potent remedy against leprosy. This "TUVARKA" may be identical with Hydnocarpus oil.

Around 300 B.C. "HUC TO" of China recognised leprosy by giving vivid descriptions of lepers. Much work was done by Chinese Scientists to emphasize the nature, cause & treatment of leprosy in the subsequent years. They showed that the leprosy was a communicable disease.

Discovery of gold in California (1848) caused a great influx of Chinese, which resulted in introduction of leprosy in "HAWAII" by middle
The microscopic structure of leprosy lesion was first described by "RUDOLPH VIRCHOW" in 1864. He first described lepra cells or brown pigmented bodies.

Aetiology :-

In 1873 Dr. Gerhard Henrick Armauer Hansen, a Norwegian scientist, discovered Mycobacterium Leprae, the causative organism of leprosy. After the discovery of Mycobacterium Leprae leprosy is also termed as "Hansen's disease".

Leprosy can be defined as "Leprosy is a chronic granulomatous immunological disorder caused by M. leprae primarily affecting the peripheral nerves & secondarily involving skin & mucosal membrane etc., possibly leading to a variety of disabilities as sequelae which are not necessarily ascribable to the causative agent." Other visceral organs are also involved.

Hansen's bacilli (Mycobacterium leprae) are stained by the Ziehl-Neelsen method & appear pink/red. They are arranged in small or large groups (globi) or may occur singly. They are less toxic & less pathogenic in nature than many other organisms. The generation time is believed to be around 13-14 days. Because of these properties, as well as host factors, 2-5 years may elapse before clinically detectable disease is apparent. The incubation period of the disease is thus much longer than that of most other infectious diseases.

Leprosy occupies a special position amongst communicable diseases because of long duration of the disease, the high frequencies of disabilities it produces & social as well as economical consequences it has [Dharmendra (1)]. It is not a hereditary disease, can occur at any age. Young children are at extreme risk. It is a significant public health
problem. Hansen's disease is much behind with its study due to the lack of method of cultivation of Mycobacterium leprae [Mayama A. (11)].

**Transmission :-**

A severe degree of bacillaemia is observed mainly in untreated lepromatous leprosy. The major exit points of M. leprae from untreated lepromatous patients are the nose, mouth & in some cases abraded skin lesions. It has been estimated that an untreated lepromatous patient could discharge upto $2.4 \times 10^8$ bacilli through nasal discharge in a day [Davey & Rees (12)]. About $1.6 \times 10^6$ AFB may be discharged while speaking & coughing [Hubscher et al (13)]. Schaffer (14) demonstrated that large number of AFB upto 1,85,000 could be projected to a distance of 1.5 metres in 10 minutes while coughing, sneezing & during normal speech. Experimental studies have shown that these bacilli can live for 7-9 days after their exit from human body [Desikan (15)].

Most likely sites of entry of the organism into human body are skin and nasal mucosa [Machin (16), Pallin and Mc Dermott (17)].

**Pathogenesis :-**

Schwann cells are the target for M. leprae. The organisms remain unrecognized for a long period in the Schwann cells and multiply gradually, depending upon resistance, i.e. cell-mediated immunity determined by T lymphocytes. Once the cells recognize the presence of M. leprae in the Schwann cells, host-parasite interaction takes place; further growth is checked and disease resolves to a subclinical level in most cases. Susceptible subjects are unable to mount an immune response and cannot overcome infection at subclinical levels. They may present with various types of leprosy depending upon the levels of cell-mediated immunity that they exhibit.
Role of M. Leprae in leprosy:

The majority of patients approach clinicians with immunoclinical problems rather than with bacteriological problems as in the case of other chronic diseases such as tuberculosis. The proportion of multibacillary forms of leprosy, with bacterial loads between $10^{11}$ & $10^{12}$ is about 20-25% ; the remainder are paucibacillary types. Even if lepromatous patients with multibacillary leprosy are left untreated, no gross systemic disturbances may be observed except at a very advanced stage. Complications affecting the kidney, eye or nerves are due primarily to deposition of antigen-antibody complex during the host reaction, rather than directly to live M. leprae. In the subcutaneous nodular reaction the nodules are situated on the small joints of the hands or tendons. If not managed properly, such patients are prone to develop a typical reactional hand or a swan-neck deformity of the hands.

The role of M. leprae is limited to a triggering of the disease pathology. The subsequent pathological & clinical changes are attributable to immunological responses leading to a spectrum of different presentations of leprosy with rare combinations of different types in some patients e.g. a patient with nodular & BT-BB lesions. It is characteristic of leprosy that even after bacterial death, the pathological process continues & complications continue to appear until mycobacterial antigens totally disappear. In other bacterial diseases including tuberculosis, once the causative agent is tackled, pathology halts & complications generally do not appear.

Epidemiology:

On the basis of clinical description, leprosy appears to be one of the oldest diseases to have afflicted human & from ancient times was universally considered to be a communicable disease. Leprosy is one of six main tropic communicable diseases in developing & underdeveloped
countries. Leprosy is considered important mainly because of its potential to cause permanent and progressive physical deformities with serious social & economic consequences.

Approximately 1.3 billion people on earth live in areas where leprosy is an important problem i.e. where the estimated prevalence is over 1 case per 1000 persons, and thus may be considered at significant risk of contracting the disease [WHO, 1988 (18)].

There are twelve million cases of Hansen's disease worldwide approximately, India is a leading country having three million lepers. Out of which twenty five percent are of infectious type. In India Andhra state has maximum leprosy patients. The other countries of the world with a very high incidence are Central & South America, Africa, Philippines. With the widespread application of multidrug therapy (MDT) in leprosy control the prevalence of leprosy declined significantly in the last decade of the twentieth century.

In countries where leprosy is endemic, the prevalence rates of registered cases show marked variations with rates ranging from below 1 per 1000 to over 30 or more per 1000. The uneven nature of the distribution of the disease appears to be a characteristic of leprosy. In areas where leprosy is dying out, the few cases that occur do have a predominance of lepromatous leprosy.

Leprosy is known to occur at all ages ranging from early infancy to very old age. The major factor that determines age distribution appears to be opportunities for exposure rather than age per se.

In most parts of the world males are affected more frequently than females, often in the ratio 2:1. The male preponderance is much more pronounced in lepromatous leprosy than in tuberculoid leprosy. In areas where females showed low prevalence, they not only suffer from less serious disease, but their chances of developing deformities were also
very much less, mainly due to less frequent involvement of nerve trunks among the females. The relatively low prevalence of leprosy among females may be due to environmental or biological factors. Because of their lifestyle males are more exposed to infection.

The uniqueness of leprosy is that the pattern of the disease is determined not by bacteria but by the host’s cell mediated immune responses. Tuberculoid leprosy, one polar form is characterised by high cellular immunity to the bacteria. Lepromatous leprosy, another polar form is characterised by suppressed (impaired) cellular immunity to the bacteria. Leprosy rarely kills in a short period, rather it progressively cripples, mutilates, disfigures.

The classification forms a very important part of diagnosis, treatment & prognosis of leprosy. Two schemes of classification based on advances in clinical, bacteriological, histopathological, immunological & chemotherapeutic knowledge are commonly used.

A) The Ridley – Jopling classification:

This Classification is based essentially on histopathological, immunological and clinicobacteriological findings. The spectrum is divided into five groups.
1) TT- Tuberculoid Tuberculoid.
2) BT- Borderline Tuberculoid.
3) BB- Borderline Borderline or Midborderline.
4) BL- Borderline Lepromatous
5) LL- Lepromatous Lepromatous.

B) The Indian Association of Leprologists Classification:

This classification [Indian Association of Leprologists, 1982 (19)] is based on clinical & bacteriological criteria & the whole spectrum is divided into five groups, i.e. I,T,B,L & P.

1) I - Indeterminate leprosy.
2) T - Tuberculoid leprosy.
3) B - Borderline leprosy.
4) L - Lepromatous leprosy
5) P - Pure neuritic leprosy.

As pure neuritic leprosy is seen fairly often in India, it has been given a special position in this classification. More recently, finer differentiation of group B into BT (three lesions or more) & BL (ten lesions or more) has been recommended by the Indian Association of Leprologists.

C) Classification for chemotherapy:

From the viewpoint of chemotherapy, the spectrum of leprosy is divided into two groups (i.e. multibacillary-MB & paucibacillary-PB) based on bacterial load & clinical picture. According to World Health Organization (1982), (10) patients with a bacteriological index (B.l.) of 2 or more at any site (BB-BL-LL types) are classified as multibacillary and those with a B.l. of less than 2 (I,TT & BT types) are designated as paucibacillary. However the Indian group designates all skin smear positive cases as multibacillary (EB,BL,LL) & all those who are negative on skin smears (I,TT, & BT) as paucibacillary.

**Bacterial Index (B.l.) :-**

The bacterial index (B.l.) is frequently used in leprosy as a guide to the numbers of bacilli in slit skin smears or biopsies. A negative B.l. means that no bacilli can be seen. B.l. + to ++++ + + + reflects increasing numbers of visible acid-fast bacilli (M. Leprae).

- + -- 1-10 bacilli in 100 1/12-inch objective fields.
- + + -- 1-10 bacilli in 10 1/12-inch objective fields.
- + + + -- 1-9 bacilli in an average 1/12-inch objective field.
- + + + + -- 10-100 bacilli in an average 1/12-inch objective field.
- + + + + + -- more than 100 bacilli in one 1/12-inch objective field.
Common Clinical Presentations In Leprosy :-

Leprosy patients may present with one or more of the following clinical features.

a) Circumscribed skin lesions, hypopigmented or erythematous.
b) Shiny, diffusely erythematous lesions, especially on the earlobes & face.
c) Sparseness or loss of eyebrows.
d) Anaesthesia of hands & feet.
e) Tingling & numbness of limbs.
f) Clawing of fingers & toes.
g) Weakness & wasting of small muscles of the limbs.
h) Foot drop, lagophthalmos, wrist drop.
i) Recurrent burns (while cooking or smoking etc.)
j) Area(s) of anaesthesia unaccompanied by cutaneous pigmentary changes (nonvisible anaesthetic lesions).
k) Eruption of erythematous lesions with or without fever.
l) Sudden swelling of face, hands & feet.
m) Pain due to neuritis or joint pains.
n) Thickening of superficial nerves or nerve trunks, or rarely nerve abscesses.
o) Non-healing plantar ulcers.
p) Gross mutilation of limbs, depressed nose, gynaecomastia.

Careful examination of skin lesions as well as limbs to demonstrate loss or impairment of sensation to temperature, pain (to pinprick) or light touch as well as palpation of nerves for evidence of thickening gives clinical indications & may settle the diagnosis. If the patient belongs to the
multibacillary type, demonstration of acid-fast bacilli in smears from multiple sites confirms the diagnosis.

**Treatment Of Leprosy :-**

The outlook for persons with leprosy has been remarkably altered by successful chemotherapy. Patients with leprosy can be classified as "infectious" or "non-infectious" on the basis of the type, duration & effects of therapy. Therapy, when effective, heals ulcers & mucosal lesions in months. Cutaneous nodules respond more slowly & it may take years to eradicate bacteria from mucous membranes, skin & nerves. The degree of residual pigmentation or depigmentation, atrophy & scarring depends upon the extent of the initial involvement.

The drugs available for the treatment of leprosy can be divided into three groups: primary, secondary & investigational.

1) The primary drugs include dapsone, clofazimine & rifampicin.
2) Secondary drugs consist of ethionamide/prothionamide, thiacetazone, thiambutosine, the long acting sulfonamides & certain aminoglycosides.
3) Investigational drugs currently undergoing clinical trials include several fluoroquinolones (primarily ofloxacin), minocycline & one of the newer macrolides, clarithromycin.

Patients with tuberculoid leprosy may develop "reversal reactions" which are manifestations of delayed hypersensitivity to antigens of M. leprae. Cutaneous ulcerations & deficits of peripheral nerve function may occur. Early therapy with corticosteroids or clofazimine is effective.

Reactions in the lepromatous form of the disease (erythema nodosum leprosum - ENL) are characterized by the appearance of raised, tender, intracutaneous nodules, severe constitutional symptoms & high fever. This reaction may be triggered by several conditions but is often associated with therapy. It is thought to be an Arthus-type reaction.
related to release of microbial antigens in patients harboring large numbers of bacilli. Treatment with clofazimine or thalidomide is effective.

Leprosy patients were treated with dapsone monotherapy since 1941. But the gradual increase in the incidence of drug resistance (primary & secondary) & drug persisters now necessitated the recommendation of dapsone plus one or two additional drugs for the treatment of leprosy.

The World Health Organization recommended multidrugtherapy (MDT) for all patients with leprosy [WHO study group, 1982, (10)]. The reasons for using combinations of agents include:

i) Reduction in the development of resistance,

ii) The need for adequate therapy when primary resistance already exist &

iii) Reduction in the duration of therapy.

Treatment regimens recommended by WHO:

1) Patients with a small population of bacteria (smear negative cases) (paucibacillary form – PB) including TT & BT groups the regimen suggested is: dapsone -100mg daily (unsupervised) + rifampicin – 600mg once a month (supervised) for 6 months. Relapses are treated by repeating the regimen.

2) Patients with large population of bacteria (smear positive cases) (Multibacillary form – MB) including BB, BL & LL groups the regimen suggested is: Dapsone -100mg daily (unsupervised) + Rifampicin-600mg once a month (supervised) + Clofazimine-300mg once a month (supervised) & 50mg daily (unsupervised).

All medications are continued for at least 2 years & to B.I. negativity on skin smears.
Dapsone - 4,4’ – diamino diphenyl sulphone

Dapsone is bacteriostatic, but not bactericidal for M.leprae & the estimated sensitivity to the drug is between 1 & 10ng/ml for microorganisms recovered from untreated patients. M. leprae may become resistant to the drug during therapy.

**Mechanism of action:**

Dapsone is structural analog & competitive antagonist of paraaminobenzoic acid (PABA) & thus prevents normal bacterial utilization of PABA for the synthesis of folic acid (pteroylglutamic acid). They are competitive inhibitors of dihydropteroate synthase, the bacterial enzyme responsible for the incorporation of PABA into dihydropterioic acid, the immediate precursor of folic acid. Sensitive microorganisms are those that must synthesize their own folic acid, bacteria that can utilize preformed folate are not affected. Bacteriostasis induced by dapsone is counteracted by PABA competitively.

Dapsone - resistant strains of M. leprae are termed “Secondary”, if they emerge during therapy. Secondary resistance is usually seen in lepromatous patients treated with a single drug.

**Untoward Effects:** The most common side effect is hemolysis of varying degree. Methemoglobinemia is also common and Heinz-body formation may occur. Diminished red-cell survival usually occurs during the use of sulphones and is presumed to be a dose related effect of their oxidising activity. Anorexia, nausea and vomiting may follow the oral administration of sulphones. Isolated instances of headache, nervousness, insomnia, blurred vision, drug fever, paresthesia, hematuria, psychosis and a variety of skin rashes are reported.

**Rifampicin**

This antibiotic is the most powerful bactericidal drug for M. leprae and the minimal inhibitory concentration is less than 1μg/ml. Infectivity of
patients is rapidly reversed by therapy that includes rifampicin in multidrug therapy.

**Mechanism of action:**

Rifampicin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex leading to suppression of initiation of chain formation (but not chain elongation) in RNA synthesis. More specifically, the beta subunit of this complex enzyme is the site of action of the drug, although rifampicin binds only to the holoenzyme. Nuclear RNA polymerase from a variety of eukaryotic cells does not bind rifampicin & RNA synthesis is correspondingly unaffected. While rifampicin can inhibit RNA synthesis in mammalian mitochondria, considerably higher concentrations of the drug are required than for the inhibition of the bacterial enzyme. High concentrations of rifampicin antibiotics also inhibit viral DNA-dependent RNA polymerases and reverse transcriptases. Rifampicin is bactericidal for both intracellular and extracellular microorganisms.

**Untoward Effects:** The most common side effects are rash, fever, nausea and vomiting. The most notable problem is the development of jaundice. Incidence of severe hepatic problems increase in patients of chronic liver disease, alcoholism and old age. Heavy doses of rifampicin causes a flu-like syndrome with fever, chills and myalgias, eosinophilia, interstitial nephritis, thrombocytopenia, hemolytic anemia and shock.

Rifampicin is a potent inducer of hepatic microsomal enzymes.

[3] **Clofazimine:**

**Mechanism of action:**

Clofazimine may inhibit the template function of DNA by binding to it. It is weakly bactericidal against M. leprae. The drug also exerts an anti-inflammatory effect and prevents the development of erythema nodosum leprosum. It is also useful for the treatment of chronic skin ulcer (Buruli
ulcer) produced by M. ulcerans. A component of MDT gives good results with dapsone-resistant bacilli from human leprosy.

Clofazimine, a phenazine dye, gets deposited in various tissues and its accumulation inside macrophages leads to its action on intracellular organisms.

**Untoward Effects** :- Common side effects are coppery red pigmentation of body, ichthyotic changes in the skin of extremities. Red colouration of conjunctiva and occasional acute abdominal pain with vomiting and diarrhoea may occur.

**Mycobacterium Leprae (Hansen's Bacillus)**

**General Characteristics :-**

Taxonomically M. leprae is classified under the order of Actinomycetals and the family Mycobacteriace. It is a straight or slightly curved rod-shaped organism with parallel sides and rounded ends, 1.8 μm long and 0.3 μm in diameter. Like other species of mycobacteria, M. leprae divides by binary fission, and is gram positive and strongly acid fast following staining with carbol-fuchsin, although the staining is irregular in most of the organisms.

Characteristically, acid-fastness is lost following extraction with pyridine. Distinctively, M. leprae is a not-cultivable acid-fast bacterium capable of limited multiplication locally following mouse footpad inoculation, and possessing a phenoloxidase. It is an obligate intracellular parasite, predominantly in macrophages, where the organisms commonly occur in clumps or 'globi' which may become very large, containing hundreds of bacteria. In smaller clumps the organisms characteristically occur in parallel array, resembling 'bundles of cigars'. M. leprae is the only species of mycobacteria to infect peripheral nerves, and specifically Schwann cells. Heated suspension of bacteria from the skin lesions of
lepromatous patients (Mitsuda lepromin) produce a positive skin response (lepromin reaction) in patients with tuberculoid leprosy (TT,BT) but no response in patients with lepromatous leprosy.

**Structure of M. leprae :-**

A structural model of M. leprae is shown in figure 1.

**Capsule :-**

Like other mycobacteria, the outer surface of M. leprae is characterized by the presence of copious amounts of lipid components which are probably partly responsible for the 'electron transparent zone' and for the foamy material seen in macrophages of lepromatous patients. The two most prominent capsular lipids are related in structure and contain a long-chain diol esterified with two long chain methyl-branched fatty acids. One of the lipids is the wax ester phthiocerol dimycocerosate (PDIM). PDIM of M. leprae is chemically distinct from PDIM from other mycobacteria. The second prominent capsular lipid, phenolic glycolipid-I (PGL-I) contains a phenol group glycosylated with a characteristic trisaccharide apparently unique to M. leprae. (Fig. 1).

Both PDIM and PGL-I are found in vast amounts in infected armadillo and human tissues, indicating that these lipid components probably persist for long periods after the intact bacteria have been degraded and eliminated. The terminal 3,6-di-o-methyl glucose residue of PGL-I has not been found in any other natural molecule and this moiety is the key to the highly specific antibody response induced to PGL-I during infection with M. leprae. PGL-I can react with free radical compounds (20,21), the lipid capsule may protect bacteria from the toxic effects of lysosomal enzymes and reactive oxygen metabolites produced by host macrophages during infection.
Fig: 1  Schematic model showing the structure of M. leprae. Key aspects of the structure of M. leprae are shown with a cross-section of the cell wall and membrane depicted in the circular inset. The capsular lipids are shown as interacting with cell wall mycolic acids to form an 'outer membrane' structure. Structural features which are characteristic of M. leprae are identified by numbers as follows:

1. The capsular phenolic glycolipid (PGL-I) has a unique pattern of sugar residues.
2. The phthiocerol moiety of M. leprae phthiocerol dimycocerosate (PDIM) differs in chain length from that of M. tuberculosis.
3. The pattern of cell wall mycolic acids differs between M. leprae and most other cultivated mycobacteria.
4. L-alanine is replaced by glycine in M. leprae peptidoglycan.
5,6,7. Distinctive proteins have been identified in cell wall, membrane and cytoplasmic fractions from M. leprae
8. The 16S rRNA molecule of M. leprae contains species-specific sequences
9. The genome of M. leprae is lower in G+C content than that of other mycobacteria. Several M. leprae specific DNA sequences have been identified, including repetitive element.
Cell wall:

The cell wall of M. leprae resembles other mycobacteria in consisting of a cross-linked peptidoglycan, covalently attached to an arabinogalactan polymer modified by addition of branched chain mycolic acids, shown as forming a loosely defined 'outer membrane' structure in figure (1). In the M. leprae peptidoglycan, glycine rather than L-alanine is found at the amino terminus of the tetrapeptide side chain and the pattern of cell wall associated mycolic acids distinguishes M. leprae from most mycobacteria.

A second important mycobacterial component containing the same arabinofuranose motif is lipoarabinomannan (LAM). LAM consists of a membrane 'anchor' segment related to the phosphatidyl inositol mannosides, with a mannan core, supporting arabinose structures including the common serologically dominant motif. LAM may be anchored in the plasma membrane and permeate the wall; it can also be readily isolated in a soluble form in the supernatant of cultivable mycobacteria and in leprosy infected tissues. LAM shares many of the properties of lipopolysaccharides from gram negative bacteria, including their immunoregulatory functions, and may play an important role in pathogenesis and intracellular survival of mycobacteria.

Cell wall preparations of M. leprae contain a substantial amount of tightly associated protein material, which has been identified as a major target of T-cell immunogenicity. A protein doublet with molecular weight of 17 kilodaltons (kD) was found to be prominent in sodium dodecyl sulfate (SDS) extracts from cell wall preparations and analysis with human T-cell clones showed that mother of the cell wall components is the 14 kD protein found also as a major cytoplasmic constituent and corresponding to the Gro ES heat shock protein of E. Coli. Previous reports had suggested a cell wall location for the 65 kD Gro EL protein in
M. leprae (22), although more rigorous extraction procedures indicated that this association is less tenacious than that seen with Gro ES. In addition to contributing to cell wall structure, wall associated proteins may be important in mediating uptake of nutrients into mycobacteria and in light of the proposed function of Gro EL and Gro ES as polypeptide ‘Chaperones’, it is perhaps also attractive to speculate on a role for such molecules in secretion of proteins from M. leprae.

**Cell membrane:**

The lipid composition of the M. leprae membrane shows the presence of phospholipids characteristic of the membrane of cultivable mycobacteria, including members of the serologically active family of phosphatidylinositol mannosides (PIM). The diversity of PIMs found in M. leprae is less than that seen in cultivable mycobacteria. Although trehalose dimycolate (‘cord factor’) was not detected in M. leprae, small amounts of trehalose monomycolate have been found.

Biochemical fractionation studies identified two major polypeptides—MMP-I and MMP-II associated with the cell membrane of M. leprae. MMP-I is a 35 kD protein independently identified as a serologically active component recognized by murine monoclonal antibodies specific to M. leprae. The molecular weight of MMP-II is 22 kD as determined by SDS-polyacrylamide gel electrophoresis and the sequence of the 14 amino acids from the N-terminus of the purified protein has been established. While these two proteins may represent major constituents, it is clear that many more proteins—cytochromes, transport systems, etc. must be present in the membrane.

**Cytoplasm:**

Cytoplasm of M. leprae is dominated by three major proteins. Analysis of extracts from cultivable mycobacteria demonstrates a very much broader range of ‘major’ proteins. The strikingly simple profile from
M. leprae is influenced by a bias in favour of proteolytically stable components persisting within dead organisms. Of the three major proteins, one was found as a doublet at 28 kD, a second had molecular weight of 17 kD and the third corresponded to the Gro ES heat shock protein found also in cell wall fractions.

**Genome :-**

The M. leprae genome is striking in its low guanine + cytosine (G+C) content in comparison to other mycobacteria. The G + C content of M. leprae DNA estimated ranges from 54-58 %, with estimates for most other mycobacteria ranging from 65-69 %.

**Lipid Synthesis :-**

In addition to degradation to release CO₂ (23) radiolabelled precursors such as glycerol and palmitate can be incorporated into lipids by M. leprae suspensions. Incorporation has been demonstrated into bulk lipid fractions [Wheeler, (24)] and into the species-specific phenolic glycolipid [Harris et al (25)]. Acetate and pyruvate were found to be less efficient precursors in such experiments. Assay of enzyme activities in disrupted bacteria indicated that pathways suitable for utilization of preformed fatty acids are more active in M. leprae than are those responsible for de novo fatty acid biosynthesis, prompting the suggestion that M. leprae may rely on scavenging of host cell derived fatty acids to provide precursors for lipid synthesis in vitro [Wheeler et al, (26)]. In order to obtain free fatty acids, it can be anticipated that M. leprae will secret hydrolytic enzymes (lipases, phospholipases) to carry out digestion of host cell lipids.

**Survival mechanisms :-**

In order to survive within macrophages M. leprae has to be able to withstand the toxic effects of host-derived reactive oxygen metabolites and attention has been given to the characterization of enzymes involved
in this process. Kusunose et al (27) partially purified superoxide dismutase and catalase from armadillo-derived M. leprae and reported preliminary biochemical characteristics of the manganese dependent superoxide dismutase enzyme. The gene encoding M. leprae superoxide dismutase (the 28 kD antigen identified by monoclonal antibodies) has been cloned (28) and it can be anticipated that future studies based on the recombinant enzyme will allow a detailed characterization of the biochemical properties and regulation of the enzyme. Wheeler and Gregory (29) also detected superoxide dismutase activity in M. leprae extracts but, although they found a weak peroxidase activity, they were unable to detect an M. leprae catalase. Similarly Katoch et al (30) found no evidence for a bacterial catalase in M. leprae extracts and Lygen et al (31) could detect neither catalase nor peroxidase. Superoxide activity in the absence of catalase - leading to accumulation of hydrogen peroxide would represent a rather inefficient defense mechanism.
II) LIPIDS

Principal sites affected in leprosy are peripheral nerves and skin, followed by reticulo-endothelial organs like liver, spleen, lymph nodes, bone marrow and other visceral organs. Amongst various systemic effects produced by leprosy hepato-biliary system is most commonly affected. The hepatic involvement is seen in early stage of the disease. The liver being an organ peculiar in its position, blood supply and function, it seems to be having its share of damaging agents. Even tuberculoid leprosy patients have shown hepatic involvement. The studies on functional status of the liver in leprosy have revealed minimum dysfunction in tuberculoid leprosy and marked dysfunction in lepromatous leprosy (1).

Hepatic damage is a frequent finding at autopsy or biopsy studies in leprosy patients (32, 33, 34, 35, 36, 37). In these studies the involvement of liver was found to variable extents.

Liver plays a central role in the metabolism of lipids, proteins, carbohydrates, vitamins and minerals. It has an ability to synthesize and utilize different lipids and proteins. Under normal conditions liver derives various lipid components from the diet and has the capacity to synthesize various lipid classes and to distribute these through plasma to other extrahepatic tissues. However these processes appear to be disturbed in leprosy.

Many interrelationships exist between lipid metabolism and host responses to infection. Basic mechanisms leading to observed effects of infection on lipid metabolism of the host are as follows.

A) Effects associated with the presence of invading microorganisms. :-

1) Direct effects :-

a) Utilization of host lipids required by replicating microorganisms.
b) Localized destruction of fat cells at sites of an infectious process.
c) Disruption of host cell metabolism by intracellular microorganisms.

2) Indirect effects :-

a) Alterations in host lipid metabolism caused by bacterial exotoxins, endotoxins or enzymes.
b) Activation of lipase or other lysosomal enzymes within host phagocytes.
c) Release of mediator substances from host cells, i.e. endogenous pyrogen.

B) Effects secondary to development of generalized illness due to infection :

1) Decreased dietary intake due to loss of appetite.
2) Minimal interference with intestinal absorption of fats.
3) Altered lipid metabolism within host cells :-
   a) Altered rates of hormone mediated lipolysis within fat depots to supply increased metabolic demands.
   b) Altered rates of lipid synthesis within the liver.
   c) Altered rates of fat utilization by peripheral tissues.
   d) Changes related to localization of infection within specific tissues, i.e. liver, pancreas, brain.
4) Alterations in lipid transport :-
   a) Changing concentrations of serum lipids and their transport proteins.
   b) Altered activities of non-hormone mediated lipases.
5) Participation of specialized lipids in the coagulation mechanism or in anti-inflammatory and allergic responses.
6) Ill-defined effects related to the prior nutritional status of the host.
7) Terminal effects associated with shock and overwhelming septicaemia.
Lipid metabolism may be intimately associated with defense mechanisms of the host. On the other hand it may be involved in pathogenic events of a harmful nature [Beissel et al, (38)].

Plasma lipids originate largely from liver which secretes them as lipoproteins. Under normal conditions the rate of synthesis of lipids in liver is usually proportional to their secretion into plasma. If any modified secretary function is brought about by any manipulation, the levels of lipids are altered in both liver and plasma [Malhotra et al, (39)].

The concentration of lipid in serum may vary in either direction depending upon an algebraic summation of multiple factors related to its uptake or release from adipose stores, from sites of hepatic metabolism or from sites of peripheral utilization [Beisel et al, (38)].

Alterations in serum moieties during infection are varied and complex [Nishishita, (40)]. Different studies have indicated that body fat stores were mobilized during the infectious process (41, 42).

Hypocholesterolemia and diminution in the concentration of other blood lipids is observed frequently during the course of acute infectious diseases.

In 1925, Balbi (43) observed hypocholesterolemia in leprosy patients.

In 1928, Boyd and Roy (44) found hypocholesterolemia in patients with leprosy.

In 1936, Villela et al (45) observed low values of cholesterol and high values of total lipids in leprosy patients.

In 1938, Mitsuda K. (46) reported the presence of a certain kind of lipid (phospholipid or glycolipid) and fatty acids and absence of neutral fat and cholesterol in lepra cells.
In 1939, Dharmendra et al (47) found decreased serum cholesterol levels in leprosy patients which were increased with the clinical improvement, with the treatment of hydnocarpous oil.

Contrary to the above general consensus of opinion, in 1939, Hopkins et al (48) observed increased levels of serum cholesterol in leprosy patients.

In 1955, Harada K. (49) found that neutral fat tends to be present in lepra cells with large fat globules or globi in advanced stages of lepromatous leprosy.

In 1956, Kusaka T. (50) observed an increase in total cholesterol and total phosphatides (especially sphingomyelin) in all tissues of rats with leprosy whereas neutral fat decreased. He remarked that these changes were pronounced in peripheral tissues than in the internal organs.

In 1957, Tarabini et al (51) reported low cholesterol levels in leprosy patients with lepra reactions.

In 1957, Gokhale S.K. and Godbole S.H. (52) studied serum lipid levels and lipolytic enzyme activities in 165 normal subjects and 105 leprosy patients. There was a significant increase in the levels of serum total cholesterol and phospholipids in leprosy patients as compared with the control subjects. Total fatty acids and total lipids were increased while serum lipolytic enzyme activities, viz; the esterase and lipase were decreased in the leprosy patients.

In 1958, Kusaka T. (53) observed low serum cholesterol levels in leprosy, in contrast to normal total lipid and phospholipid content in normal subjects.

In 1960, Chekherdernian M. (54) reported low serum cholesterol levels but marked hyperlipemia in leprosy patients as compared with normal subjects.
In 1961, Dhople and Magar (55) reported higher levels of polyunsaturated fatty acids and lower percentage of polyenoid fatty acids in leprosy sera.

In 1961, Ramu G. and Nagarajan V. (56) found higher values of serum lipids and low cholesterol in leprosy patients during reactive stages. Serum lipids dropped after a reactive stage but serum cholesterol showed a slight increase.

In 1962, Nath R.L. and Chatterji Anjali (57) estimated levels of cholesterol and phospholipids in red cells and plasma of 35 normal subjects and 39 leprosy patients. The levels of cholesterol in red cells were significantly lowered in lepromatous and tuberculoid leprosy and plasma cholesterol levels were significantly decreased in lepromatous leprosy only as compared with normal subjects. The levels of phospholipids in red cells and plasma were significantly lowered in tuberculoid leprosy and lowering of phospholipids in red cells and plasma were not statistically significant in lepromatous leprosy as comparing with phospholipids levels in red cells and plasma of normal subjects.

In 1964, Dhople A.M. and Magar N.G. (58) studied the levels of serum cholesterol and phospholipids in 8 normal subjects and about 200 patients of leprosy. Lepromatous patients had lower values of total cholesterol and phospholipids while there was little variation from the normal in the tuberculoid (both major and minor) and maculoanaesthetic types as compared with the lepromatous type. Reverse was the case as regards the sphingomyelin values among the patients of advanced stages. Whereas the levels for the early cases were a little below those of the normal. There was a significant difference in the values of both total cholesterol and phospholipids between lepromatous and tuberculoid (and maculoanaesthetic) types, but there was no significant difference between tuberculoid and maculoanaesthetic types. There was a
significant change in levels of blood contents due to the DDS treatment
the serum levels were raised as compared with those of untreated subjects.

In 1964, Misra U.K. and Venkitasubramanian T.A. (59) examined
the status of different serum lipid fractions in normal subjects and leprosy
patients. A general but uniform decrease in total lipids, total cholesterol
and total phospholipid phosphorus was observed in the serum of patients
with lepromatous leprosy as compared with normal values. This decrease
in serum lipid components, however, did not change the percentage
composition of cholesterol and phospholipid phosphorus in serum lipids
as compared with the normal. Reductions in all lipid components were
observed except for esterified cholesterol, which remained unchanged
and triglycerides showing a slight increase.

In 1965, Balkrishnan S. (60) determined blood lipids in 20 normal
subjects, 28 cases of acute, 10 cases of subacute and 8 cases of chronic
reaction during reactive and subsided phases. The most consistent
change that he found was the lowering of cholesterol levels in every form
of reaction and in both the phases. In the acute form of reaction, there
was a tendency for the levels to return to normal or near normal during
the subsided phase while in chronic reaction, the levels in the two phases
were practically same. The levels of total lipids were seem to be little
lowered in the subsided phases. Similarly there was a significant
decrease in serum phospholipids in all leprosy subjects.

There was a significant lowering in the ratio of cholesterol/total
lipids and phospholipids/total lipids in all leprosy subjects.

In 1966, Ramu G. and Nagarajan V. (61) analysed serum lipids in
12 controls and 12 lepromatous cases with lepra reaction. They noticed a
significant increase in the total lipids during the lepra reaction and
significant decrease in the cholesterol levels during reaction and also after the subsidence of the lepra reaction.

In 1971, Sakurai Isamu and Skinsnes Olaf K. (62) studied tissue lipids in leprosy by chromatographic analysis. Young adults mice were used for study. They observed that the lipids were composed mainly of triglycerides derived from the subcutaneous fat cells. The tissue of human tuberculoid leprosy had more total lipids than those of the lepromatous cases employed in that study although it is well known that lepromatous leprosy shows much lipid substance in the lesions. There was a significant increase in the total lipids, phospho and glycolipid, free cholesterol, free fatty acids of the lepromatous and tuberculoid skin tissues. Along with these fractions was a significant lowering in triglycerides, cholesteryl ester and phospholipids in the leprosy patients as compared with control subjects.

In 1971, Hariprasad C.H. et al (63) quantitated serum beta lipoprotein levels by turbidimetric method in 13 normal subjects, 36 tuberculoid and 11 lepromatous leprosy patients. They found a significant lowering of beta lipoproteins in the tuberculoid type as compared with controls.

In 1972, Kokrady S. et al (64) analysed total glycolipids (cerebrosides + sulphatides) in peripheral nerve biopsy samples obtained from leprosy patients and compared with the peripheral nerves obtained at autopsy from patients with non-neurological disorders. The glycolipid content decreased markedly as the disease progressed. The results suggested that there was loss of myelin glycolipids as well as the degeneration of the peripheral nerve as observed histologically.

In 1974, Kapoor K.K. and Gupta S.C. (65) examined sera from 25 normal healthy subjects and 193 patients from different forms of leprosy. Serum cholesterol levels were found to be depleted significantly
from the normal in all types of leprosy. No correlation between the reduced levels of serum cholesterol and severity of the disease was observed.

In 1976, Gupta R. K. and Gupta Sushma (66) determined serum cholesterol and lipoproteins in 50 patients suffering from various types of leprosy and 20 age and sex matched healthy controls. In lepromatous leprosy a significant decrease in cholesterol, serum alpha lipoproteins and its cholesterol content was seen. In dimorphous leprosy reduction in serum total cholesterol, alpha lipoproteins and its cholesterol content while increase in beta lipoproteins and beta lipoprotein cholesterol was observed. In tuberculoid leprosy a significant reduction in serum cholesterol, alpha lipoproteins and alpha lipoprotein cholesterol content with a consequent rise in beta lipoproteins was observed. In polynervitic leprosy serum cholesterol values were unaltered with reduction in alpha lipoproteins and increase in beta lipoproteins.

In 1977, Verma K.C. et al, (67) estimated tissue lipids in leprosy patients. Skin sections of lepromatous cases showed large number of lepra bacilli whereas all the smears and skin sections from tuberculoid cases were negative for lepra bacilli. The neutral fats were seen prominently at the sites of granulomatous collections. Phospholipids and the fatty acids were observed in lepra bacilli found in or outside the lepra cells. Cholesterol and its esters could not be demonstrated in the lepromatous granulomas. At the same time this study showed the absence of any type of lipids in the tuberculoid granuloma.

In 1979, Sritharan V. et al (68) studied serum lipid profile in 11 healthy persons and 79 leprosy patients. They observed a generalised decrease in total lipid concentration in leprosy as compared with normal subjects. Total cholesterol was less than normal in all types of leprosy and it was well marked with lepromatous leprosy (active) and in
lepromatous reaction. Free cholesterol was significantly higher in active lepromatous leprosy and in reactions of LL. Triglycerides were significantly decreased in all reactive states and also in active LL cases. Significant decrease in phospholipids was noted in all types of reactive states and also in active LL and BL cases. Beta lipoproteins showed a decrease in all types of leprosy and the decrease was well marked in LL reaction and in active LL. Lipase showed significant decrease in LL reaction and also in active LL cases. Serum lipids showed a gradual increase in the order LL (reaction), BT (reaction), BB (reaction).

In 1980, Robins K. et al (69) carried out liver function tests in 20 controls and 79 leprosy patients. Out of which 28 patients were being treated and 42 patients were untreated and the rest 9 patients were clinically quiescent. The most important abnormality observed in the patients was a reversal of albumin/globulin ratio, which was more common in the lepromatous group than in the tuberculoid group. Treatment with DDS had resulted in the reduction of the serum globulin. The mean serum cholesterol values of the patients were lower than those of the controls.

In 1980, Bhushan Kumar et al (70) tested sera of 58 patients of various types of leprosy for total fat, phospholipids, cholesterol and alpha and beta lipoproteins. Total fat and both fractions of lipoproteins were found to have values comparable to normal in all types of leprosy. Serum phospholipids levels were significantly reduced in LL type of leprosy as compared to normal and TT group of patients. Similarly cholesterol levels were found to have significantly no value in LL group compared to that of the controls.

In 1981, Young DB (71) used thin layer chromatography to compare lipid extracts from lepromatous skin biopsies with those from normal skin and from Mycobacterium leprae purified from armadillo.
spleen. Several lipids were found in infected skin which were absent from normal skin but corresponded to lipids present in the purified M. leprae. These included mycolic acids, a 6-deoxyhexose-containing lipid (glycolipid 1) and a wax ester (possibly related to the Mycobacterium tuberculosis wax, phthiocerol dimycocerosate). Unlike mycobacterium lepraemurium, M. leprae contained no C-type mycosides. In terms of lipid profile, M. leprae from armadillo spleen showed the same characteristics as bacilli from human skin samples. Quantitative analysis of mycobacterial lipids in lepromatous skin biopsies indicated that their concentrations were much higher than would be predicted from the number of acid fast bacilli present. Accumulation of lipid debris from dead M. leprae could provide a protective environment in infected cells for remaining viable bacilli.

With an aim to study the cholesterol biosynthetic capacity of the leprosy patients, Kannan K. B. et al (72) in 1982 indirectly determined the levels of the enzyme Beta hydroxy methyl glutaryl Co A reductase (HMG Co A reductase) in 33 lepromatous leprosy patients, 15 non lepromatous patients and 18 control subjects by assaying the circulating levels of HMG Co A and mevalonate and finding out the ratio between two. The ratio was around 1 in leprosy patients indicating a normal HMG CO A reductase activity and approximately the same values were obtained in healthy controls. The cholesterol level was significantly low in lepromatous group as compared with non lepromatous and the control groups. The results suggested that cholesterol biosynthetic capacity of leprosy patients was normal.

In 1983, Kher J. R. et al (73) studied serum total cholesterol and the lipoprotein fractions in 40 lepromatous leprosy patients and age matched 35 control subjects. The study revealed a significant decrease in serum cholesterol in the disease group as compared to control group. An
alteration in serum lipoprotein fractions was observed in disease group. The beta-lipoprotein fraction showed a significant decrease along with a rise in alpha-lipoprotein fraction. A positive correlation was also observed between total cholesterol and beta-lipoproteins.

In 1984, Sritharan V. et al (74) estimated the levels of circulating serum high density lipoprotein (HDL) cholesterol and total cholesterol in control subjects and in lepromatous leprosy patients before and after drug therapy. The subjects were distributed in three groups on the basis of their age. HDL cholesterol to total cholesterol ratio was significantly raised in both treated and untreated patients in all three groups as compared to healthy controls.

In 1984, Khandke L. et al (75) analyzed lipids of skin infected with M. leprae, obtained from lepromatous leprosy patients. That showed changes in lipid composition as compared to the skin from normal individuals. The infected skin had several M. leprae specific lipids and also showed higher amount of phospholipids and glycolipids.

In 1984, Nair Ishwari and Mahadevan P. R. (76) studied cholesterol metabolism of macrophages in presence of M. leprae. Macrophages from mice in the presence of phagocytosed M. leprae showed a preferential accumulation of cholesterol ester. Such an increase in the ester level was not seen in the presence of dead bacteria.

In 1985, Wright S. (77) investigated plasma phospholipid essential fatty acids in 40 leprosy patients and 40 controls. A significant reduction in linoleic acid was found in the leprosy patients, with an increase in its metabolite dihomo-γ-linolenic acid. No difference was found between patients with multibacillary and paucibacillary leprosy. Patients treated for less than 6 months were found to have low levels of linoleic acid and high levels of dihomo-γ-linolenic and arachidonic acid compared with patients treated for more than 6 months.
1986, Chung et al (78) estimated the levels of serum high density lipoprotein cholesterol and total cholesterol in 22 non lepromatous and 24 lepromatous leprosy patients. A slight decrease in serum total cholesterol was observed in both types of leprosy patients. Significantly decreased values of high-density lipoprotein cholesterol were found in leprosy patients in comparison with healthy control subjects.

In 1988, Kumar N. et al (79) quantitated high density lipoprotein cholesterol (HDL-C) levels in 96 lepromatous leprosy patients of which 50 were under treatment & 46 untreated & 84 matched control subjects. The study revealed that HDL cholesterol levels in lepromatous leprosy patients were raised & significantly different when compared with control group. It was opined that a negative test would be mainly useful in excluding the diagnosis of lepromatous leprosy.

In 1989, Mukherjee A. et al (80) studied the dermal lymphatic vessels in lepromatous and tuberculoid leprosy lesions by light & electron microscopy. In the lepromatous patients, lymphatic vessels were seen in both intra & peri granulomatous areas. The lymphatic lining cells contained lipid droplets, lysosomes and numerous pinocytotic vesicles. Cells bearing bacilli were only occasionally seen. In the tuberculoid cases, lymphatic vessels were seen only along the edges of the granulomas & the lining cells were less prominent. Inflammatory cells, both lymphocytes & histiocytes, were found traversing the walls of lymphatic vessels in both groups of patients. The results of the study confirm the continued & increased functioning of the lymphatic drainage system in dermal leprosy lesions and indicate that it may be a major route for the clearance of lipids from the lipid-rich bacilliferous lesions in the lepromatous patients. The lymphatic pathway appears to be a minor pathway for the dissemination of Mycobacterium leprae in comparison with the blood vascular system.
In 1991, Wright S. et al (81) investigated the fatty acid composition of plasma phospholipids in 61 patients with leprosy of various clinical types with either a short or long duration of treatment. All patients had significantly decreased levels of linoleic acid & alpha-linoleic acid, the parent fatty acids of the n-6 & n-3 families, respectively. Patients with a treatment duration of more than 6 months had significantly low levels of arachidonic acid & eicosapentaenoic acid compared to controls or to patients with a treatment duration of less than 6 months. There were no differences in the fatty-acid composition between multibacillary patients & paucibacillary patients. They concluded that dietary supplementation with essential fatty acids should be indicated in patients with leprosy, particularly in those with a long treatment duration.

In 1991, Sen R. et al (82) investigated lipid-laden macrophages in bone marrow of leprosy patients. A study was planned to observe the occurrence & morphological characterization of these macrophages in various types of leprosy. Bone marrow records from 48 cases of paucibacillary & 72 cases of multibacillary leprosy were analysed. The macrophages accounting at the most for 3.5 % of marrow cells were observed in 5 cases of paucibacillary & 43 cases of multibacillary leprosy with a maximum incidence being observed in patients with ENL (16/17). The lipid present in the cytoplasm of these cells could be derived from the lipid of the cell wall of Mycobacterium leprae. It was the first report about there cells leprosy.

In 1992, Ahaley S.K. et al (2) carried out a comprehensive study of serum lipids & lipoproteins in leprosy spectrum. It was the first report about the quantitation of very low density lipoprotein (VLDL) in leprosy patients. The study included 60 leprosy & 40 age & sex matched healthy controls. The study subjects included cases of LL with reactions, LL without reactions, BL with reactions, BL without reactions, BT & TT types

36
of leprosy. The levels of serum phospholipids, triglycerides, total cholesterol, LDL & VLDL fractions were significantly decreased in leprosy patients as compared to control subjects. The levels of serum HDL cholesterol & HDL fraction were significantly elevated in leprosy patients. Maximum elevation in serum HDL cholesterol level & HDL fraction & maximum reduction in the levels of serum phospholipids, triglycerides, total cholesterol & LDL & VLDL fractions were observed in lepromatous leprosy (LL) patients with reactions. (P<0.001).

In 1992, Chakrabarty A.N. & Dastidar S.G (83) in their study observed that the lipid profiles of all the chemoautotrophic nocardioform (CAN) bacteria derived from human & animal leprosy tissues were identical with each other & closest to or identical with the most probable profile of M. leprae.

In 1992, Chattopadhya D. et al (84) assessed nutritional status of children of urban leprosy patients staying at preventoria based on biochemical parameters. The study included 60 healthy children (group A) whose parents suffered from leprosy & who had been separated at the age of 4 years & brought up in preventoria & a group of healthy children from the same poor socioeconomic class (group B). In both groups the serum concentrations of cholesterol & triglycerides were well below those found in Western populations.

In 1992, Diamantopoulos E.J. et al (85) investigated coronary heart disease risk factors in 293 Hansen's disease (HD) sufferers which might explain the high prevalence of coronary heart disease (CHD) among HD patients. The patients, after having completed WHO adopted questionnaire, were given a complete physical examination, a resting & exercise electrocardiogram, & biochemical as well as hematological examinations. Coronary HD patients, when compared to noncoronary, HD patients, showed statistically significant differences in the following
parameters. 1) Mean age, 2) mean concentration of the electrophoretic fraction of alpha-lipoproteins, 3) deviation from mean weight, 4) prevalence of hypertension, and 5) prevalence of borderline lepromatous form of HD. However, the differences found when comparing other parameters, such as blood pressure, smoking, diabetes mellitus, total cholesterol, triglycerides, pre-beta & beta-lipoproteins, uric acid, erythrocyte sedimentation rate, ABC blood groups, etc. did not reach the level of significance. These findings suggest that HD sufferers are a special population subgroup with reference to CHD risk factors, differing in many ways from the general population.

In 1994, Lee S.J., et al (86) investigated lipid composition of the stratum corneum (SC) of the sole in patients with leprosy by thin layer chromatography. Extraction of the SC lipids with a methanolchloroform-H$_2$O mixture (4:2:1.6, v/v/v, Bligh-Dyer solvent) was carried out after shaving of the SC from the sole TLC was performed & the composition of lipids was quantitated by photodensitometry. The composition of SC lipids in the anaesthetic lesions of leprosy patients was higher in cholesterol sulphate & triglycerides & lower in sphingolipids & cholesterol esters than that of normal subjects.

In 1996, Chauhan S.L. et al (87) studied calcification of peripheral nerves in leprosy. In the study 74 TT/BT patients, with gross thickening of nerves together with nerve abscess, showed calcification in 8 patients. Calcification was most common in the ulnar nerve followed by the lateral popliteal nerve. All eight patients were males with significantly longer duration of illness before start of treatment. Patients with late onset of nerve abscess were found to be more prone to calcium deposition in the nerves. Caseous pus of the abscess had high lipid content with raised cholesterol & cholesterol ester ratio to total lipids suggesting a dystrophic nature of calcification.
In 1996, Memon R.A. et al (88) conducted a study to assess alterations in serum lipids in lepromatous leprosy patients with & without ENL reactions & their relationship to acute phase proteins. The concentrations of serum lipids, serum amyloid A (SAA) & C-reactive protein (CRP) were measured in patients with lepromatous (LL/BL) leprosy & erythema nodosum leprosum (ENL). LL/BL patients had significantly higher serum triglyceride & lower HDL cholesterol concentrations compared to the endemic controls. ENL patients had significantly lower total, HDL & LDL cholesterol levels compared to the endemic controls. The levels of all lipid metabolites also were significantly lower in ENL patients compared to LL/BL patients. The concentrations of SAA & CRP were markedly elevated in ENL patients but were not statistically different in LL/BL patients compared to control subjects. There was a significant negative correlation between SAA & HDL-cholesterol levels in both stable lepromatous & reactional (ENL) patients; there was no statistically significant correlation between CRP & HDL-cholesterol levels. SAA levels also had a significant negative correlation with total & LDL-cholesterol levels. These results suggested that an increase in SAA levels might divert the metabolism of lipoproteins from hepatocytes towards macrophages, resulting in a decrease in serum lipoprotein levels.

In 1997, Srikanth N.C. et al (89) made an attempt to characterize leprosy patients belonging to different spectra using nasal flushings in the waxy layer. Thin layer chromatography (TLC) analysis of this layer, using chloroform-methanol-water system, showed different spots when sprayed with acid alcohol & heated at 160 degrees C. the TLC profile of lipids of lepromatous & borderline (MB according to the WHO terminology) leprosy patients was distinctly different from that of tuberculoid leprosy patients & normal human volunteers.
In 1997, Memon R.A. et al (90) determined the concentrations of serum lipids & tumor necrosis factor (TNF) in leprosy patients across the spectrum of the disease and in erythema nodosum leprosum (ENL) patients at the onset of the reaction & after the reaction had clinically subsided. Lepromatous/borderline lepromatous (LL/BL) patients had significantly higher serum triglyceride & lower HDL-cholesterol levels; there was no such change in the tuberculoid/borderline tuberculoid (TT/BT) patients. The household contacts of the LL/BL patients also had significantly lower serum HDL levels. ENL patients during the acute phase of the reaction had significantly lower total, LDL, HDL-cholesterol levels compared to the stable LL/BL patients, and these changes were reversible to pre-ENL levels after the reaction had subsided. Serum TNF levels were significantly higher in household contacts and in LL/BL patients but were not statistically different in TT/BT patients. Serum TNF levels were also significantly higher during the acute phase of ENL, and declined after the clinical remission of the reaction to levels comparable with those of LL/BL patients. There was a significant negative correlation between serum TNF & HDL-cholesterol levels during & after ENL reaction. However, there was no such correlation between TNF & total or LDL-cholesterol levels in ENL patients. The changes in the HDL-cholesterol metabolism were a specific part of the host response to lepromatous leprosy & to the ENL reaction & might be mediated by increased TNF production.

In 1997, Bansal S.N. et al (91) studied serum lipids & lipoproteins in 40 patients of leprosy & 20 age & sex matched healthy controls. The levels of serum triglycerides were low in both paucibacillary & multibacillary cases but the difference was not statistically significant. Total cholesterol was significantly decreased only in paucibacillary group. HDL cholesterol levels were significantly increased in both the groups.
LDL-cholesterol showed a significant decrease in both groups but VLDL cholesterol levels did not show any significant change in both groups of leprosy cases.

The change in the lipid profile was not associated with any change in liver functions as the values of SGOT, SGPT & A/G ratio were within normal limits in all cases.
III) LIPID PEROXIDATION

A “Free radical” is defined as any atom, group of atoms or molecule in a particular state with one unpaired electron occupying an outer orbital. A few common molecules such as nitrous oxide (NO) & nitrogen dioxide (NO₂) contain an unpaired electron in an outer orbital in their normal state & by definition therefore, are free radicals (92).

Lipid peroxidation is an autocatalytic process initiated & sustained by free radicals (93). An oxidative, oxygen dependant deterioration of fats, particularly the unsaturated fatty acids is commonly described as lipid peroxidation (94).

The one electron reduction of O₂ results in the formation of the superoxide anion radical, O₂⁻ (The superscript dot • denotes an unpaired electron). Other terms commonly used for O₂⁻ include superoxide, superoxide anion & superoxide radical. The two electron reduction product of O₂ in the fully protonated form is hydrogen peroxide, H₂O₂ while the three electron reduction product of O₂ is the hydroxyl radical, OH⁻ (92). This univalent pathway for reduction of molecular oxygen is shown in following figure.

Fig :- The univalent pathway for reduction of molecular oxygen.

The lone electron present in the outer orbital of a free radical endows it with very unusual chemical reactivity & physical characteristics. Free radical reactivity is accounted for by the strong tendency of the unpaired electrons to interact with other electrons to form an electron pair & thus a chemical bond. Radicals, however, vary greatly in reactivity with
 Radical reactions can be divided conveniently into three types - initiation, propagation & termination reactions and these tend to occur as chain reaction (92).

An initiation reaction is a reaction in which free radicals are formed.

\[
\begin{align*}
R:R & \rightarrow R^\cdot + R^\cdot \\
\text{Reaction (1)}
\end{align*}
\]

Free radicals may be initiated by radiolysis, proteolysis, homolysis & during oxidation-reduction reactions (95,96).

A propagation reaction is a free radical transfer reaction in which the site of the free radical is altered but a free radical always results.

\[
\begin{align*}
R^\cdot + O_2 & \rightarrow RO_2^\cdot \\
\text{Reaction (2)}
\end{align*}
\]

\[
\begin{align*}
RO_2^\cdot + RH & \rightarrow ROOH + R^\cdot \\
\text{Reaction (3)}
\end{align*}
\]

The hydroperoxides formed during the initial phases of autooxidation decompose by several different pathways & a complex mixture of products is encountered.

Termination reaction occurs in free radical systems resulting in the removal of the free radical from the propagating pool (92).

\[
\begin{align*}
RO_2^\cdot + RO_2^\cdot & \rightarrow ROOR + O_2 \\
\text{Reaction - (4)}
\end{align*}
\]

\[
\begin{align*}
RO_2^\cdot + R^\cdot & \rightarrow ROOR \\
\text{Reaction - (5)}
\end{align*}
\]

\[
\begin{align*}
R^\cdot + R^\cdot & \rightarrow RR \\
\text{Reaction - (6)}
\end{align*}
\]
After initiation of any free radical, the number & complexity of possible reactions increases since each system under investigation usually contains other reactive constituents as well as solvent (92).

Various potential sources of superoxide anion radical exist and have been reviewed (97,98,99). A group of cellular enzymes, which are involved in catalyzing oxidation reactions, result in the univalent reduction of O₂ to O₂⁻. Well studied examples of these are xanthine oxidase (100), aldehyde oxidase, dihydro-orotic dehydrogenase, flavin dehydrogenase (97) and peroxidases (101).

The chemistry of O₂⁻ has been extensively studied (102), O₂ may react as a reducing agent, donating its extra electron, or as an oxidizing agent in which case it is reduced to H₂O₂. A radical may also join to a non-radical. Whichever of these three types of reaction occurs, the non-radical species becomes a radical. A feature of the reactions of free radicals with non-radicals is that they tend to proceed as chain reactions where one radical begets another (103). Superoxide anion radical can also oxidize molecules such as ascorbic acid (104) and adrenalin (105). Although O₂⁻ is generally considered not to react with lipids, Thomas et al (106) have demonstrated that O₂⁻ may react with lipid hydroperoxides to form alkoxy radicals (RO·).

The radiation causes one of the oxygen-hydrogen covalent bonds in water to split, leaving a single electron on hydrogen and one on oxygen, thus creating two radicals.

\[
\begin{array}{c}
H - O - H \\
\text{Intermediate} \\
\text{Stages} \\
\rightarrow \\
H^* + OH^* \\
\rightarrow \\
\text{Reaction-(7)}
\end{array}
\]

H* is a hydrogen radical and OH* is a hydroxyl radical. The latter is the most reactive radical known to chemistry. It can attack and damage...
almost every molecule found in living cells at a diffusion controlled rate, i.e. OH \* reacts as soon as it comes into contact with another molecule in solution. Since it is so reactive, OH \* generated in vivo does not persist for even a microsecond and rapidly combines with molecules in its immediate vicinity (103).

**Properties of free radicals:**

- High reactivity with a consequent extremely short life span.
- Self-perpetuating (auto catalytic) and diverse chemical reactivity.
- Low chemical specificity.
- Generated both in vivo and in vitro.

Reactions of OH \* with biologic molecules, most of which are non radicals, set off chain reactions. Reactions of OH \* include its ability to interact with the purine and pyrimidine bases, thiols etc. leading to radicals that have a number of possible chemical fates.

Perhaps the best characterized biologic damage caused by OH \* is its ability to stimulate the free radical chain reaction known as lipid peroxidation. This occurs when OH \* is generated close to membranes and attacks the fatty acid side chains of the membrane phospholipids. It preferentially attacks polyunsaturated fatty acid side chains, such as arachidonic acid. The OH \* abstracts an atom of hydrogen from one of the carbon atoms in the side chain and combines with it to form water.

\[
\text{—CH— + OH}^* \rightarrow -\text{C—} + \text{H}_2\text{O}
\]

Reaction (8)

This reaction removes the OH \*, but leaves behind a carbon centered radical (—C—\*) in the membrane. Carbon centered radicals formed from polyunsaturated fatty acid side chains usually undergo
molecular rearrangement to give conjugated diene structures, which can have different fates. Thus, if two such radicals are collided in the membrane, cross-linking of fatty acid side chains could occur as the two electrons join to form a covalent bond. Reaction with membrane proteins is also a possibility. However, under physiologic conditions, the most likely fate of carbon-centered radicals is to combine with oxygen, creating yet another radical, the peroxyl radical (peroxy radical).

\[
-C^- + O_2 \rightarrow \text{Reaction (9)}
\]

Peroxyl radicals are reactive enough to attack adjacent fatty acid side chains, abstracting hydrogen -

\[
\text{Reaction (10)}
\]

Another carbon-centered radical is generated, and so the chain reaction (reactions 9 & 10) continues. One OH can result in the conversion of many hundred fatty acid chains into lipid hydro-peroxides. Accumulation of lipid hydro-peroxides in a membrane disrupts its function & can cause it to collapse. Lipid hydroperoxides can also decompose to yield a range of highly cytotoxic products, among the most unpleasant of which are aldehydes (107).

A great deal of attention in the literature has been focussed on malonaldehyde (malondialdehyde), but this is much less noxious than such products as 4 hydroxy nonenal (107,108). Peroxyl radicals &
Cytotoxic aldehydes can also cause severe damage to membrane proteins, inactivating receptors & membrane bound enzymes (109).

The demonstration that \( \text{OH}^- \) is generated in vitro by polymorphonuclear leukocytes, monocytes & macrophages (110) & that it may have bactericidal properties (111) suggests that it may be generated extracellularly at sites of inflammation. Since \( \text{OH}^- \) is one of the primary products of irradiation, its properties have been studied extensively(112).

The Haber – Weiss reaction (Reaction 11) has been suggested as mechanism for the generation of \( \text{OH}^- \) from \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) (113).

\[
\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^+ \quad \text{Reaction (11)}
\]

Another reaction for \( \text{OH}^- \) generation has been \( \text{O}_2^- \) mediated reduction of metal chelates (reaction 12) followed by a Fenton Type reaction (reaction 13).

\[
\text{Me}^{n+} \text{ Chelate} + \text{O}_2^- \rightarrow \text{Me}^{(n-1)+} \text{ Chelate} + \text{O}_2 \quad \text{Reaction (12)}
\]

\[
\text{Me}^{(n-1)+} \text{ Chelate} + \text{H}_2\text{O}_2 \rightarrow \text{Me}^{n+} \text{ Chelate} + \text{OH}^- + \text{OH}^+ \quad \text{Reaction (13)}
\]

There is considerable evidence that oxygen radicals or species resembling them, are responsible in large measure for the induction of lipid peroxidation. Such evidence fostered a unifying concept for lipid peroxidation, on that incorporates reactions involving free or chelated
transition metals, oxygen radicals, a variety of reducing species &
unsaturated fatty acids (94).

During the course of many redox reactions, oxygen can serve as
the immediate electron acceptor, either forming radical species of oxygen
or adding to the radical in question. If such reactions involve only lipid
radicals and oxygen, the term ‘auto-oxidation’ is applied (94).

The lipid peroxidation process is readily initiated by an abstraction
of a hydrogen atom from the methylene between a cis double bond pair
of an unsaturated fatty acid (114).

Polyunsaturated fatty acids (PUFA) are particularly vulnerable to
free radical attack. The presence of unsaturated lipids in cells is a
potential threat because of the extreme lability of such lipids in the
presence of oxygen, and their wide spread distribution in the cell
membranes (115).

Animal tissues contain large amounts of PUFA distributed primarily
in the phospholipids of cellular membranes. Membrane phospholipids
include several phosphatides, with phosphatidyl choline, phosphatidyl
ethanolamine, phosphatidyl serine, sphingomyelin & inositol
phosphatides being the most prominent. The amount of each differs in
various tissues as well as in the various subcellular membranes within a
single tissue. These phospholipids contain a variety of unsaturated fatty
acids with linoleic (18:2), arachidonic (20:4) & decosahexaenoic (22:6)
predominating (15). These PUFA can undergo peroxidative breakdown.
This results in the destruction of the original lipid. In cells this destruction
could lead to the loss of integrity of the membranes.

When lipid peroxidation occurs in biological membranes, there may
be gross disturbances in structural organisation & in associated enzyme
function. Numerous examples are now known where lipid peroxidation
produces irreversible damage to membrane systems, which often results in the death of the affected cells.

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules & which are generally very reactive. They are produced continuously in cells either as accidental by products of metabolism or deliberately during phagocytosis (Fig. 2).

One of the most important mechanisms of phagocytic killing of ingested microorganisms by leukocytes is the generation of toxic oxygen products. During phagocytosis, neutrophils, as well as monocytes & macrophages, display a strongly increased cell respiration. Quantitatively the most important product of this reaction is hydrogen peroxide. Superoxide is also generated in large amounts, probably as on an intermediate in the formation of hydrogen peroxide. Indications exist that singlet oxygen & hydroxyl radicals are also formed in this process. Some of these oxygen products have microbicidal properties by themselves. The effect of hydrogen peroxide is greatly enhanced by the enzyme myeloperoxidase.
Fig.: 2 The production of superoxide and hydrogen peroxide in polymorphonuclear leucocytes and macrophages during phagocytosis.
Several dysfunctions of this system are known. In chronic granulomatous disease (CGD) the enzyme system that produces superoxide is not operative. Thus no $O_2$ or $H_2O_2$ is generated, leading to a severely decreased bacterial killing capacity. The exact molecular defects in the X-linked & the autosomal form are yet undefined. Two variants are also known: lipochrome histiocytosis, with different clinical & histological manifestations, & a ‘triggering defect’ where only strongly opsonized particles trigger the respiratory burst. Myeloperoxidase deficiency leads to slightly decreased killing capacity, especially for yeast. In glucose-6-phosphate dehydrogenase deficiency no oxygen radicals or $H_2O_2$ are produced because no equivalents for oxygen reduction can be generated in HMP shunt. Deficiencies in the glutathione redox system also result in impaired phagocyte function, probably because the cells have to be protected against their own toxic oxygen products.

The generation of free radicals by polymorphonuclear cell, provides protection from infection. Polymorphonuclear cells (PMNS) are normally found in the blood circulation in an active state. Cell membrane enzymes such as NADPH oxidase also exist in an inactive form. In his setting polymorphonuclear cell may encircle or even ingest bacteria, but they are incapable of damaging or killing them (116,117). Exposures to immunoglobulin coated bacteria, immune complexes, complement 5a, or Leukotriene, however, activate the enzyme NADPH-oxidase (Fig. 3).

This activation initiates a respiratory burst at the cell membrane to produce superoxide. Activation of NADPH oxidase occurs at pH 7.0 to 7.5 and therefore is effective in infection sites with low oxygen tension (118). The pH within the vacuoles of polymorphonuclear cell initially rises, plateaus & then quickly becomes acidotic. Apparently, most of the killing action by superoxide occurs during the elevated pH phase because
Fig. 3 An overview of how polymorphonuclear cells (PMNS) are activated to produce hydroxyl ion or hypochlorous acid (HOCL) as important bactericidal agents and the possible role these reactive oxygen species play in leprosy.
neutrophils produce much less superoxide at acidic pH than at neutral pH.

Once the polymorphonuclear cell encircles the bacteria, granules released by the cell generate reactive oxygen species (ROS) within 15 to 60 seconds to destroy the bacterial membrane (119). Actually killing of bacteria depends on increased pH & the presence of superoxide or its reduced components H₂O₂, hydroxyl ions of HOCl as well as number of bactericidal peptides such as defenses & cathepsin. Subsequent events working through G proteins, phospholipase C, and inisitol 1,4,5-triphosphate produce an increase in cytosolic calcium & bacterium death (120).

In recent days, reactive oxygen species, lipid peroxidation have drawn much attention both from fundamental & clinical point of view. Stress, pathological conditions induce the auto oxidation of polyunsaturated fatty acids, which leads towards the formation & accumulation of lipid peroxides. Free radicals are produced during normal metabolism & are normally efficiently scavenged. Oxidative stress occurs when there is an imbalance between production & scavenging of these free radicals. Reactive oxygen species such as superoxide radical, hydrogen peroxide & hydroxy radical are important mediators of cellular and extracellular injury via the destruction of membrane phospholipids, lipoproteins & alteration of critical enzyme system (Fig. 4).

**Immunity in Leprosy: Cell Mediated Immunity:**

A large body of evidence suggests that in lepromatous leprosy (LL) there is a selective unresponsiveness (anergy) of T cell response to Mycobacterium leprae antigens and therefore, the host in unable to mount an adequate cell mediated immunity (CMI) which could protect the host from the infection. In this form of the disease macrophages primarily
Fig.: 4 Free radical damage to membranes (in lower panel) including disulfide crosslinking of membrane surface proteins, protein strand scission, lipid-lipid crosslinking and fatty acid peroxidation (lipid peroxidation). The latter leads to release of derivatives such as malondialdehyde.
in the nerves, skin & mucous membranes get heavily infiltrated with the bacilli.

**Cellular interactions in CMI**: 

The phagocytic cells (macrophages, Langerhans cells, dendritic cells), known to engulf & process the invading pathogens & their soluble products & also designated as antigen presenting cells (APCs) are capable of presentation of the processed antigens to the T cells through their receptors (121). At the initial stages of CMI response, T cells cluster around the surface of the APCs before transforming into blast cells. Thereafter, APCs release interleukin (IL-1) which induces the production of interleukin-2 (IL-2) by T cells along with expression of IL-2 receptors for multiplication by replication of these antigen specific lymphocytes for clonal expansion (122). While such a clonal expansion goes on, the cellular interaction further liberates a variety of other interleukins (IL-1, IL-3, IL-4 to 8) & lymphokines [granulocyte monocyte colony stimulating factors, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α)] which influence the morphological & functional behaviour of various CMI inducing cells.

In general, all these cells consisting of activated mononuclear phagocytes, cytotoxic T cell, natural killer (NK) cells & lymphokine activated killer (LAK) cells create an environment of cell mediated immune effector population. Effector molecule like IFN-γ & TNF-α are known to activate macrophages to make them more armed with enhanced production of toxic reactive oxygen intermediates (ROI), superoxide anions & also by generation of reactive nitrogen intermediates (RNI) for more effective killing of both intracellular & extracellular microbial agents (123, 124). Further, IL-2 & IL-6 influence T-, NK-and LAK-cells to become cytolytic by increasing the perforin (pore forming
protein) contents & leukolexin in them (125). These interleukins are also known to modify the functions of other cells like endothelial cells, keratinocytes & Langerhans cells.

Reduction in CD4+ cells with an increase in CD8+ cells simply indicates a state of immunosuppression. Considering that lepromatous patients are not prone to get other infections which are known to inflict immunosuppressed individuals, it is more rational to assume that in lepromatous leprosy there is no real alteration in the CD4+:CD8+ ratios in the peripheral blood.

The immunopharmacology of antileprosy agents:

Unlike other acute & chronic bacterial infections, the antimicrobial chemotherapy of leprosy is complex due to the occurrence of adverse immunological reactions. The role played by antimicrobial agents in precipitating or exacerbating these reactions is controversial. Various mechanisms of induction of adverse immunological reactions exist in this disease.

Immunological status of untreated LL patients:

Acquired specific immunological unresponsiveness (tolerance, anergy) to Mycobacterium leprae antigens is found in individuals with LL & may be total or partial according to the state of advancement of the disease & bacillary load. This specific anergy develops as a consequence of the extremely high antigen load which occurs in LL. Apparently, the high antigen concentrations in vivo reach a threshold at which the host immune system detects that sustained immune reactivity against M. leprae is to the continued detriment of the host. In this situation the immune response is ineffective in eradicating the antigen, but continues to inflict damage on bystander tissues in the vicinity of the antigen. Immunologically mediated tissue damage occurs by the release of toxic
oxygen radicals & proteolytic enzymes, such as elastase & collagenase, from phagocytic cells which have been mobilized & activated by pro-inflammatory lymphokines released from antigen-activated T-lymphocytes.

Predisposition to the development of this chronic, infective inflammatory response which leads to LL may be genetically determined or acquired (e.g. in nutritional deficiency states). Induction of tolerance is mediated by recruitment of antigen-specific suppressor T-lymphocytes which suppress specific cell-mediated immunity (CMI) to M. leprae (126). Other mechanisms, which decrease CMI responses to M. leprae are also operative such as, generalized anergy & humoral factors with immunosuppressive activity. Paradoxically, the induction of suppression of specific CMI is probably beneficial by reducing the degree of immunologically mediated tissue damage. However, damage to tissues is an on-going process due to non-specific & antibody mediated immune mechanisms & immunologically uncontrolled growth of M. leprae.

The situation in individuals with LL prior to the commencement of antimicrobial chemotherapy is that they have a) an extremely high antigen load & b) specific immunological tolerance to M. leprae. Activation of immune reactivity in LL following antimicrobial chemotherapy:

Antimicrobial agents may contribute to the development of adverse immunological reactions by either or both of two possible mechanisms:

a) As a consequence of the antibacterial activity of the drugs with activation of latent or hitherto suppressed immunological reactions.

Antimicrobial agents cause disintegration of the bacterial cells with release of antigens which form circulating or localized immune complexes. These complexes cause regional or generalized complement activation with mobilization of granulocytes, which migrate to sites of
immune complex deposition. Binding of granulocytes to the immune complexes with subsequent phagocytosis or exocytosis causes release of toxic oxygen radicals & proteolytic enzymes which damage surrounding tissues. It is likely that granulocyte activation by the interaction of immune complexes & complement is responsible for the development of erythema nodosum leprosum (ENL) and its complications in individuals with a high bacillary load (BL-LL).

Antigen-elimination during antimicrobial chemotherapy causes a decrease in the antigen load with a consequent reduction in the extent of antigen - induced immunosuppression & recovery of specific CMI to M. leprae. Reactivation of CMI leads to the development of an adverse immunological reaction (reversal immunity reaction) caused by the induction of production of pro-inflammatory lymphokines, which mobilize, attract & activate granules, macrophages & T- lymphocytes. These highly reactive cells release toxic oxidants & proteases which, although important in the intracellular destruction of microorganisms, are also released extracellularly & may mediate the tissue damage, which accompanies reversal immunity reactions. These reactions may occur anywhere in the leprosy spectrum except the polar groups.

b) The second mechanism by which antimicrobial agents may contribute to the development of adverse immunological reactions is by possession of intrinsic immunostimulatory activity, i.e. direct drug-mediated enhancement of cellular immune responsiveness independent of antimicrobial activity. Such a mechanism is probably less important than antigen release mechanisms related to antimicrobial activity. However, a drug such as dapsone which has been reported to increase granulocyte motility & lymphocyte proliferation (127) could be expected to potentiate ENL & reversal immunity reactions in susceptible individuals.
Effects of antileprosy drugs on cellular – immune reactivity:

The three widely used antimycobacterial agents rifampicin, dapsone and clofazimine may regulate cellular immune functions by antigen elimination mechanisms as described above.

1) **Rifampicin** :- Rifampicin is an antimicrobial agent which is an inhibitor of lymphocyte responses to mitogens & antigens & of PMNL migration in vitro. Animal studies have also shown that rifampicin is immunosuppressive in vivo causing inhibition of both antibody & cell-mediated immune responses. However, studies have shown that rifampicin at concentrations of 0.01-100 μg/ml had no effects on human monocyte migration in vitro (128). No effects of rifampicin could be demonstrated on parameters of humoral or cellular immunity. In other two studies no inhibitory effects of rifampicin intake on polymorphonuclear leucocyte (PMNL) migration were observed (129, 130) over a 1-month period in individuals with LL & actually observed improved lymphocyte responsiveness to mitogens. The effects of rifampicin on humoral & cell-mediated immune responses appear to be variable according to the response studied & the in vitro model used.

2) **Dapsone** :- It is reported that dapsone per se causes stimulation of PMNL motility in normal adults & individuals with LL in vitro (127). Ingestion of the drug over short periods was associated with increased PMNL migration & lymphocyte responsiveness to mitogens in the control & LL groups (127, 129, 130). These effects of dapsone were related to the anti-oxidant activity of the drug & not to its antimicrobial properties. Antioxidants sustain & enhance cellular immune reactivity by preventing the auto-oxidative loss of migratory responsiveness of PMNL & mitogen & antigen-induced lymphocyte proliferation (131). A second possible mechanism of dapsone-mediated
immunostimulation, also related to an anti-oxidant mechanism, may be inhibition of the synthesis of immunosuppressive prostaglandins (PGs). It has been indicated that PGs released by monocytes induce suppressor cell activity which may be the cause of the impaired CMI observed in diseases such as Hodgkin's disease (132). It has been reported that this PG-dependent suppression is operative in individuals with the BT & TT forms of the disease but not in the BL & LL forms. However, it is possible that during antimicrobial chemotherapy associated recovery CMI in BL-LL cases that T-lymphocytes may become more responsive to PG-mediated suppression. Inhibition by dapsone of this mechanism may therefore possibly contribute to enhanced CMI & development of reversal immunity reactions.

These observations suggest that dapsone is pro-inflammatory & may contribute to ENL & reversal immunity reactions by stimulating PMNL motility & lymphocyte responsiveness to antigens respectively. However, the drug has well documented anti-inflammatory activity in a variety of dermatological conditions (133), which is probably related to its ability to inhibit phagocyte degranulation (134). It is suggested that dapsone may confer a measure of protection against the development of reversal immunity reactions in individuals with BL. This may seem difficult to reconcile with the proposed pro-inflammatory activity of the drug in LL. However, in individuals with LL & a high antigen load it is possible that the immunostimulatory, pro-inflammatory activities of the drug are dominant since the anti-inflammatory effect on degranulation may be negated as a result of increased leucocyte infiltration & high concentrations of immune complexes.

3) Clofazimine: Clofazimine, (or lamprene (R) or B 663) has no documented immunostimulatory properties & on the contrary has been
reported to be useful in controlling both ENL & reversal immunity reactions whilst conferring antimicrobial chemotherapy (135, 136, 137).

It has been shown that clofazimine inhibits the motility of PMNL & mitogen-induced transformation of lymphocytes from normal adults & individuals with LL in vitro, similar effects were observed following ingestion of the drug (138, 139). These observation suggest that the most probable mechanisms of clofazimine – mediated anti-inflammatory activity are inhibition of PMNL migration & T-lymphocyte responsiveness to antigens which may control ENL & reversal immunity reactions respectively.

In 1967, Kher & Salan ki (140) reported that the incidence of glucose-6-phosphate dehydrogenase (G-6-P -D.) deficiency in 125 patients with leprosy & 100 with tuberculosis in Nagpur was 22% & 8% respectively as against 9.4% in the general population in Nagpur & surrounding areas. This deficiency may possibly predispose the patient to infections & colonisation with lepra bacilli. The association between leprosy & erythrocyte G-6-P.D. deficiency is of significant clinical importance for therapeutic agents like sulphones, that may lead to hemolysis in patients with G-6-P.D. deficiency.

Mycobacterium leprae, an intracellular parasite, survives & multiplies in the melieu provided by the macrophages of lepromatous leprosy patients, while it disintegrates & dies in the macrophages of tuberculoid leprosy. This difference in behaviour is probably due to the result of functional variation in the macrophages in the two forms of disease (141).

monocytes challenged with live M.leprae. The macrophages from
can be attributed to the incapacity of lysing live M. leprae. Live M. leprae injected into
the foot pad of Wistar strain of rats evoked similar responses on the tenth
day in normal & protein deficient animals.

Superoxide anion, hydrogen peroxide, hydroxyl radicals, singlet
oxygen & the myeloperoxidase –hydrogen peroxide– halide system (110,
143) are the main oxidative bactericidal intermediates of phagocytic
cells, and their appropriate levels & activities are responsible for their
adequate function. In the extreme case of the clinical condition known as
chronic granulomatous disease (X-linked), the polymorphonuclear
leukocytes (PMNL) are deficient in their overall ability to undergo the
phagocytosis induced oxidative changes that characterize their normal
phagocytic function (144), the result being an enhanced host
susceptibility to severe infections by germs that under normal conditions
are mildly, or nonpathogenic (145,146). Circulating leukocytes from
lepromatous patients did not differ from those of normal subjects with
respect to their levels of β-glucuronidase, β-galactosidase, acid & alkaline
phosphatase & lipase activities. There was not a significant difference in
the “diaphorase” activity of both lepromatous & normal groups as
determined by the nitro-blue tetrazolium (NBT) test. Complementary
results were obtained by other groups with respect to lysosomal enzymes
(147) & NBT reduction.

In 1978, Oscar Rojas-Espinosa (148) studied the ability of
polymorphonuclear leukocytes to produce superoxide ($O_2^-$) anion while
phagocytosis in lepromatous leprosy patients & normal subjects. No
differences were found between lepromatous & normal groups. No
difference was found either when the comparison was made between
patients showing any form of leprosy reaction & patients without leprosy

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complications at the time of the study. These findings, with other results reported previously, suggested that PMNL from lepromatous patients were not different from PMNL from healthy individuals with respect to their ability to generate & show the metabolic oxidative changes (as measured by the NBT & the O$_2$ tests) induced by in vitro phagocytosis of latex particles. These metabolic activities remained normal even when PMNL might show alterations at other levels of the phagocytic process.

In 1979, Meny Bergel (149) suggested to investigate the in vitro antioxidant activity of biologic as well as an industrial antioxidants by using as substrate, that is, the fatty material, a synthetic mixture of fats quite similar to the human subcutaneous fat of leprosy patients, or of normal persons living in countries where leprosy is highly endemic. From the most powerful antioxidants found to act upon such fats it would be advisable to test their antileprosy activity in patients.

In 1982, Miyachi & Niwa (150) reported the inhibitory effects of dapsone on PMNL-derived oxygen intermediates (OIs) including CL.

In 1984, Niwa et al (151) investigated the effect of dapsone on the generation of OIs compared with several OI scavengers with different action sites. They found a dissociation of the inhibitory effect of dapsone, i.e. dapsone markedly reduced the generation of H$_2$O$_2$, OH$^-$ & CL but slightly increased O$_2^-$ levels. It was concluded that dapsone molecules appear to act like scavengers, presumably reacting directly with H$_2$O$_2$ with resultant decrease in hydroxy ions & O$_2$. Therefore, dapsone may exert its beneficial clinical effects by removing potent OIs as well as by inducing the inhibition of the myeloperoxidase-H$_2$O$_2$-halide system. As for a slight increase in O$_2^-$ levels by dapsone, it is considered that removal of later components of the OI generation pathway (H$_2$O$_2$, Hydroxyl & O$_2$) increase the levels of an earlier component (O$_2^-$).
In 1984, Webster et al (152) showed a dose-dependent dapsone-induced inhibition of opsonized zymosone-induced human polymorphonuclear leucocytes (PMN) chemiluminescence (CL) in vitro, suggesting that inhibition of myeloperoxidase may be the mechanism by which dapsone inhibits PMN CL.

Macrophages are one of the primary sites of multiplication of Mycobacterium leprae in susceptible hosts. Attempts to grow the organism in cultures of macrophages from normal mice, athymic mice & armadillos & man have all failed (153, 154, 155, 156). This failure might be due to a lack of essential growth requirements or susceptibility to intracellular killing mechanisms in macrophages in vitro. Hydrogen peroxide is thought to be a major component of the antibacterial activity of the macrophage (157, 158) & may be of particular relevance in the intracellular killing of M. leprae given the inability to detect catalase activity associated with this organism (29).

In 1985, Sharp A.K. et al (159) investigated susceptibility of Mycobacterium leprae to the bactericidal activity of mouse peritoneal macrophages & to hydrogen peroxide. Macrophages from athymic nude mice were infected in vitro with Mycobacterium leprae to study the intracellular fate of this organism. Using the proportional bactericidal test, they showed that the viability of M. leprae declined rapidly within these macrophages; although results of clearance experiments demonstrated that live & killed organisms were cleared at comparable rates. They also showed that M. leprae was susceptible to the bactericidal effects of hydrogen peroxide and suggested that hydrogen peroxide generated by macrophages was responsible for the killing of intracellular M. leprae.

Competent CMI is known to require the co-operation of both T lymphocytes & macrophages (160) & a number of studies have
attempted to identify a defect in these cell types in lepromatous leprosy. Gamma interferon, a macrophages activating factor, is produced in lower levels by lepromatous T lymphocytes than by T lymphocytes from tuberculoid patients or controls (161). It is possible that as result of this depressed gamma interferon production, lepromatous macrophages are not activated in the manner required to control intracellular growth of M. leprae.

Activation of macrophages results in the induction or augmentation of a number of cellular functions including production of hydrogen peroxide ($H_2O_2$) & superoxide ($O_2^-$). The production of these potentially bactericidal metabolites was correlated with the intracellular killing of M.microti (157), M.tuberculosis (162). M. leprae has been shown to be susceptible to $H_2O_2$ in vitro (159).

In 1985, Sharp & Banerjee (163) examined peripheral blood monocytes from a range of leprosy patients to identify a possible defect in macrophage function. The ability of these cells to produce hydrogen peroxide & superoxide, two bactericidal metabolites of the monocyte/macrophage was measured. Monocytes from leprosy patients were found to be capable of producing normal amount of hydrogen peroxide & superoxide, and no differences in production were found between tuberculoid, lepromatous & control monocytes. These results suggest that macrophages in leprosy are competent, and that probably a T lymphocyte defect contributes to susceptibility to this disease.

In 1985, Mori T. et al (164) carried out a study to understand respiration in Mycobacterium leprae. Fairly pure leprosy bacilli were easily collected from nude mouse footpad lepromas. Catalase activity was not found in whole cells, the cell-free extract, or particle fractions of M. leprae. Any catalase activity associated with M. leprae suspensions is a tissue contaminant. NAD-peroxidase activity was also not detected in
the cell free extract of the leprosy bacillus. These results would indicate that leprosy bacilli can not degrade hydrogen peroxide.

In 1986, Mistry N.F. et al (165) demonstrated abnormal phagocytosis of M. leprae by macrophages of lepromatous patients under various conditions. The largest proportion of macrophages with an excessive bacterial load belonged to the lepromatous group of patients. Lepromatous macrophages treated with Cytochalasin B, an inhibitor of phagocytosis, exhibited a significantly lower degree of ingestion of heat-killed organisms whereas uptake of ‘viable’ organisms was not affected to the same extent. Regulation of phagocytosis was studied by noting the rate of phagocytosis of M. leprae after the ingestion of a primary particle, viz; carbonyl iron. Solely in lepromatous macrophages, phagocytosis of carbonyl iron did not result in a decreased uptake of M. leprae implying aberrant phagocytic activity. Lastly, excessive phagocytosis was always noted in macrophages of familial contacts of leprosy patients who displayed decreased Fc receptor expression after M. leprae ingestion. This is of interest since phagocytosis is a membrane dependant event.

Mycobacterium leprae is an intracellular pathogen that is ingested by & proliferates within cells of the monocyte/macrophage series. Mechanisms by which intracellular pathogens resist destruction may involve failure to elicit a phagocyte “respiratory burst” or resistance to toxic oxygen derivatives & lysosomal enzymes. In 1986, Holzer TJ, et al (166) studied the ability of M. leprae & M. bovis BCG to stimulate the generation of superoxide anion (O$_2^-$) in vitro by human blood neutrophils & monocytes & murine peritoneal macrophages. M. leprae bacteria failed to stimulate significant O$_2^-$ release except at high bacteria-to-cell ratios (>50:1) whether or not they were pretreated with normal serum or serum from patients with lepromatous leprosy. Either viable or irradiated BCG; on the other hand stimulated the three cell types to release significant
amounts of $O_2^-$ when challenged with as few as 10 organisms per cell. Serum pretreatment enhanced the release of $O_2^-$ by the three cell types. Preincubation for 18 hours with viable M. leprae did not inhibit the ability of monocytes to respond with an oxidative burst to phagocytic stimuli. The failure of M. leprae to stimulate phagocyte $O_2^-$ generation may be an important factor in its pathogenicity.

In 1989, Marolia & Mahadevan (167) demonstrated a comparative study of the level of hydrogen peroxide & superoxide produced by peripheral blood phagocytes from normal healthy individuals & lepromatous leprosy patients which showed a deficiency in superoxide production in the patients. In the phagocytes from normal healthy individuals, there was good release of superoxide ions, and this mediated the killing of M. leprae. The lack of superoxide production allowed the viability of M. leprae inside the macrophages from leprosy patients. This deficiency could be rectified by the use of an immunomodulator. The delipidified cell wall of M. leprae. This modulation resulted in the ability of the patients' phagocytes to respond to M. leprae, to produce reactive oxygen intermediates such as superoxide, and also to kill the bacteria. These observations indicate that delipidified cell wall could have significant potential to positively modulate the immune deficient cells of leprosy patients.

In 1989, Desai S.D. et al (168) carried out a study to assess the killing of M. leprae by resting & gamma interferon (IFN-gamma)- activated macrophages in normal subject & leprosy patients. Resting macrophages from normal individuals demonstrated the ability to kill M. leprae. For macrophages from tuberculoid patients, killing of M. leprae was only achieved in the presence of IFN-gamma, suggesting that initial T-cell activation occurs prior to the killing of M. leprae. In contrast, though activation with IFN-gamma rendered the lepromatous macrophage...
microbicidal, it failed to induce lymphocyte proliferation, suggesting a
defect at either antigen-presenting cell or the lymphocyte level or both.
The concept that T - cell anergy is primarily due to lack of lymphokine
generation was ruled out by these results, since responsiveness was
restored in only a small proportion of lepromatous patients after
exogenous lymphokine addition. Thus this study demonstrated that killing
& antigen presentation are two independent events. It appears that the
ability of the macrophages per se to kill M. leprae may be of greater
importance than lymphocyte-mediated activation for protection against M.
leprae infection.

In 1990, Vachula M. et al ( 169 ) determined the effect of
Mycobacterium leprae's Phenolic Glycolipid - I on Interferon - gamma
augmentation of monocyte oxidative responses. Peripheral blood
monocytes were pretreated with phenolic glycolipid- I (PGL-I),
dimycocerosyl phthiocerol (DIM) or mycoside A, then cultured in the
presence or absence of interferon - gamma (IFN-γ). Their oxidative
responses to Mycobacterium leprae phorbol myristate acetate (PMA),
and opsonized zymosan were evaluated. In response to M. leprae,
monocytes pretreated with PGL-I released less O2⁻ than non lipid -
treated control cells. The IFN-γ augmentation of oxidative responses was
suppressed only in PGL-I pretreated monocytes & only when the stimulus
was M. leprae. This suggested that PGL-I, by afflicting the IFN-γ
enhancement of phagocytic cell oxidative responses, aids further the intra
cellular survival of M. leprae.

In 1990, Vachula M. et al ( 170 ) conducted a study including
comparison of monocyte of oxidative responses in leprosy patients &
healthy subjects as influenced by mycobacterial lipid pretreatment.
Superoxide anion (O2⁻ ) release by monocytes from leprosy patients in a
paired study was lower than that released by monocytes from healthy.
Pretreatment of healthy control monocytes with phenolic glycolipid – I (PGL-I) of Mycobacterium leprae resulted in the release of less \( \text{O}_2^- \) than released by buffer – treated cells or cells pretreated with structurally similar lipids.

However, pretreatment of patients' monocytes with PGL-I did not affect the \( \text{O}_2^- \) generation, perhaps because the cells already had a lower capacity to produce \( \text{O}_2^- \). Upon further examination of the data from the patient population, monocytes from lepromatous patients released significantly less \( \text{O}_2^- \) than cells from normal controls, while tuberculoid patient cells released \( \text{O}_2^- \) in amounts similar to that generated by cells from normal controls. In addition monocytes, from patients with a high bacterial index had a lower capacity to generate \( \text{O}_2^- \) when compared to cells from healthy individuals.

In 1991, Vachula M. et al (171) investigated the effect of glucocorticoids & \( \gamma \)-interferon on the oxidative responses of monocytes from leprosy patients & normal donors. Leprosy patients suffering from erythema nodosum leprosum are frequently treated with glucocorticoids. Monocytes from leprosy patients receiving prednisone therapy responded to lower concentrations of \( \gamma \)-IFN in vitro with enhanced superoxide anion release when challenged with M. leprae or M. bovis BCG than did monocytes from healthy subjects & other leprosy patients. Although the number of patients was small & the population heterogenous, the data suggested that prednisone could alter \( \gamma \)-IFN efficacy & led to the examination of the effect of glucocorticosteroids on \( \gamma \)-IFN activation of monocytes. \( \gamma \)-IFN treatment following in vitro dexamethasone pretreatment of monocytes from healthy subject resulted in a greater enhancement of superoxide anion generation than that observed with \( \gamma \)-IFN treatment alone.
In 1992, Santos D.O. et al (172) examined the status of reactive oxygen intermediates in the phagocytes from reactional & non reactional leprosy patients. They measured monocyte activation by luminol-dependent chemiluminescence and lymphocyte proliferation in response of M. leprae. Resting monocytes from nonreactional BL & LL patients showed lower background chemiluminescence than monocytes from healthy controls. On the other hand, monocytes from BL & LL patients with reactions showed higher resting chemiluminescence than cells from healthy controls. Upon stimulation with PMA there was an increase in chemiluminescence with monocytes from the healthy controls & from BT patients. In nonreactional LL patients there was no chemiluminescence with PMA stimulation. In contrast, monocytes from LL patients with ENL showed activation with PMA. In patients with ENL reactions being treated with thalidomide, chemiluminescence induced by PMA was lower than that seen in patients with untreated ENL.

As expected, lymphocyte proliferation in vitro in response to M. leprae was positive in some of the healthy controls & BT patients, but was essentially negative in BL & LL patients.

The results suggested that during ENL the monocytes of LL patients could respond to PMA although their lymphocytes remained unresponsive to M. leprae.

In 1993, Damle & Mahadevan (173) studied in vivo effect of delipidified cell component (DCC) of Mycobacterium leprae in relation to infection with leprosy bacteria in mice. The delipidified cell component (DCC) of M. leprae was used as an immunomodulatory agent in Swiss white mice. The peritoneal macrophages of these mice were activated to produce increased amount of reactive oxygen intermediates like hydrogen peroxide (H₂O₂) & superoxide. These macrophages also attained the ability to kill M. leprae in vitro as shown by several assay
systems including the conventional mouse foot-pad technique. The increased levels of superoxide seem to be responsible for the killing of M. leprae as addition of the enzyme superoxide dismutase, which breaks down O\textsubscript{2}, resulted in the survival of these bacilli inside the macrophages. The increased production of H\textsubscript{2}O\textsubscript{2} does not seem to be responsible for killing M. leprae. The results indicate that the DCC of M. leprae acts as an effective immunomodulator in mice leading to the activation of macrophages with increased production of H\textsubscript{2}O\textsubscript{2} & superoxide as well as enabling them to kill M. leprae via the action of superoxide anions.

In 1995, Agnihotri N. et al (174) investigated role of reactive oxygen species (ROS) in causation of renal damage in mice infected with Mycobacterium leprae. At least six animals from each group (control & infected) were killed at 0 day, 3, 6 & 9 months postinfection. The results showed a significant increase in the chemiluminescence (CL) response of peritoneal macrophages, which was maximum between 3 & 6 months. No significant increase was observed in CL response of blood neutrophils. A significant increase in lipid peroxidation was observed at 3 & 6 months as evident by an increase in malondialdehyde (MDA) levels. The increased ROS production might be the cause of lipid peroxidation. The renal damage was evident by decrease in the activity of renal brush border membrane enzymes, namely, alkaline phosphatase, leucine aminopeptidase & \( \gamma \)- glutamyl transpeptidase. Thus ROS might play a role during early stages of M. leprae infection but in the later stages other immunological mechanisms may overpower the effect of ROS.

The production of reactive nitrogen intermediates (RNI) by macrophages is critical to host defense, particularly for exerting the bactericidal & tumoricidal properties. In 1997, Khare S. et al (175) reported about the release of reactive nitrogen intermediates from the peripheral blood-derived monocytes/macrophages of leprosy patients.
stimulated in vitro by tuftsin. Nitric oxide (NO) were measured in the peripheral blood-derived monocytes/macrophages of normal & leprosy patients (BT/TT & BL/LL) in the presence & absence of 'tuftsin' as a function of in vitro culture age (on 1,3,7 days). Macrophages from both groups of leprosy patients were able to produce NO during the unstimulated state but only BL/LL macrophages could be activated by tuftsin to produce significantly high levels of NO. This increase was highest on day 1, then gradually decreased with in vitro culture age. Surprisingly tuftsin was unable to enhance the NO production in normal macrophages above the basal level. Further, normal & BT/TT macrophages had only Cu-Zn derived superoxide dismutase (SOD) activity whereas BL / LL cultures has Cu-Zn & Mn derived SOD activity. These studies indicated that in BL/LL cultures a)apart from tuftsin, some additional signal was required to activate nitric oxide synthase (NOS) gene for NO production & b) Mn-SOD produced by M. leprae was playing a defensive role against toxic-free radicals. The final outcome of this mechanism was the survival of M. leprae inside the macrophages.
IV – ANTIOXIDANTS

Antioxidant is a substance or chemical compound that inhibits oxidation. But in 1992, Norman Krinsky modified that definition by considering biological antioxidants as "antioxidants are the compounds which protect biological system against the potentially harmful effects of process or reactions that can cause excessive oxidation." Various types of biological antioxidants, their locations within the cell & mechanism of actions can be described by using this definition.

In order to survive, aerobic organisms have to develop antioxidant systems to protect themselves against the deleterious effects of free radical species. Body has evolved antioxidant defenses to protect against free radicals. Tissues have an effective antioxidant defense system. Under normal circumstances this defense system is able to cope up with a free radical in the tissue by the antioxidant donating an electron to stabilize the free radical, in doing so it can harmlessly decay itself or regenerate into another antioxidant. Earlier it was shown that human plasma is a powerful antioxidant.

Antioxidants can be classified by various ways. -

1) Depending on their source, antioxidants can be grouped as a) Naturally occurring fat soluble or water soluble vitamins like alpha-tocopherol (vitamin E), Retinoic acid (vitamin A), ascorbic acid (vitamin C) & Beta-carotene. These guard the intra or extra – cellular fluids against oxidant injuries. B) Intracellular essential enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase etc. which scavenge the peroxide radicals produced during the mitochondrial respiratory process. C) Synthetic antioxidants include drugs like allopurinol, oxypurinol, calcium channel blockers & many mores. Above mentioned enzymes require micronutrients such as zinc, cooper, iron, manganese, selenium etc.
2) In healthy individuals, three classes of antioxidants have been identified.

a) Primary antioxidants – They prevent the formation of new free radical species. e.g. superoxide dismutase, glutathione peroxidase, ceruloplasmin, transferrin, ferritin.

b) Secondary antioxidants – They remove newly formed free radicals before they can initiate chain reactions. These chain reactions can lead to cell damage & further free radical formation e.g. vitamin E, vitamin C, beta carotene, uric acid, albumin & bilirubin.

c) Tertiary antioxidants – These repair cell structures damaged by free radical attack. e.g. DNA repair enzymes, methionine sulphoxide reductase.

Deficiencies in the antioxidants system can develop due to a number of reasons. Low intake of dietary antioxidants, diseases of GIT that reduce the absorption of antioxidant nutrients from food e.g. malabsorption syndrome, idiopathic steatorrhoea, coeliac disease, sprue, Crohn's disease etc. In this situations the antioxidant system struggles to protect the body from the free radical attack & as a result the risk of free radical – mediated disease increases.

A considerable amount of evidence is available about the endogenous defense systems evolved by organisms to protect their biologic integrity from free radical destruction (176). A hierarchy of mechanisms has been evolved to deal with the deleterious reactive oxygen intermediates. These protective & controlling mechanisms can be divided into enzymatic, hydrophobic, hydrophilic & structural groups.
A) Enzymatic:

A variety of enzymatic mechanisms, which can bypass the electron spin restriction of $\text{O}_2$ & accomplish the divalent & tetravalent reduction of $\text{O}_2$ to $\text{H}_2\text{O}$ have evolved (Fig. 5).

![Enzymatic Defense Mechanisms Diagram]

The majority of $\text{O}_2$ reduced by a aerobic cells is carried out tetravalently by cytochrome oxidase (177) which prevents the release of $\text{O}_2, \text{H}_2\text{O}_2$ & $\text{OH}$ into the cellular milieu.

The contribution of the univalent pathway of $\text{O}_2$ reduction in aerobic cells, although small (98, 178, 179), is important. The $\text{O}_2$ flux in aerobic cells appears to have necessitated the evolution of a variety of superoxide dismutase whose function is to catalytically scavenge $\text{O}_2^\cdot$.

Superoxide dismutase (SOD) was discovered by McCord & Fridovich (180). Superoxide dismutase (SOD) catalyzes the dismutation of $\text{O}_2^\cdot$ to $\text{H}_2\text{O}_2$ & $\text{O}_2$ (reaction-I).

$$\text{O}_2^\cdot + \text{O}_2^\cdot + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$  Reaction (I)
Selenium dependent enzyme glutathione peroxidase & a haem containing enzyme catalase maintain the concentration of hydrogen peroxide within the range of $10^{-9} - 10^{-7}$ M.

Glutathione peroxidase, which is abundantly present in heart, lung brain, liver & erythrocytes, is located in the cytosol & mitochondria & catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GS-SG), simultaneously utilising $H_2O_2$ (reaction - II).

\[
H_2O_2 + 2 \text{GSH} \rightarrow \text{GS-SG} + 2H_2O \rightarrow \text{Reaction (II)}
\]

GS - SG is converted back to GSH by NADPH enzyme, glutathione reductase.

Catalase, found in most aerobic cells in the cytosol, mitochondria & other subcellular organelles, such as peroxisomes & which is especially abundant in liver & erythrocytes, decreases the intracellular concentration of hydrogen peroxide by promoting the following reaction (reaction - III).

\[
2H_2O_2 \rightarrow 2H_2O + O_2 \rightarrow \text{Reaction (III)}
\]

Formation of reactive oxygen species and antioxidant mechanisms in biological systems has been shown in fig.6.
Fig. 6: Shows formation of reactive oxygen species and antioxidant mechanisms in biological systems.
B) Hydrophobic:

Lipid membranes with their large content of PUFA are the only sustained hydrophobic regions associated with cells (181). The peroxidation of lipids due to homolytic mechanisms may occur at low rates in all aerobic cells (95). However, they are normally controlled by hydrophobic cavengers. A compound, alpha-tocopherol, often called vitamin E, intercalated in cellular membranes by providing hydrogen atoms may prevent chain propagating reactions in lipid membranes (95, 99) as shown in reaction - IV.

\[
\begin{align*}
\text{ROO}' + \text{AH} & \rightarrow \text{ROOH} + \text{A}' \\
\text{Lipid peroxyl} & \quad \text{vit. E} \\
\text{radical} & \\
\end{align*}
\]

Vitamin E itself is converted to a weakly reactive radical (A'), thus interrupting the chain reaction of peroxidation (182).

Each molecule of vitamin E stops two oxidation chains because the vitamin E radical (A') is too unreactive to continue the chain.

\[
\begin{align*}
\text{ROO}' + \text{A}' & \rightarrow \text{ROOA} \\
\text{Reaction (V)} & \\
\end{align*}
\]

Vitamin E can be regenerated by ascorbic acid (183) (Fig. 7).

Alpha-tocopherol & beta-carotenes may also quench O\(_2\) (\(^1\Delta g\)) & thus prevent lipid hydroperoxide (99).
Fig. 7 Membrane lipid peroxidation, only one leaflet of the bilayer is represented.

a) Initiation of the peroxidation by an oxidizing radical, $X$, forming pentadienyl radical.

b) Oxygenation to form a peroxyl radical and a conjugated diene.

c) Repair of peroxyl radical at water membrane interface by tocopherol.

d) The peroxyl radical is converted to a lipid hydroperoxide and the resulting tocopheroxyl radical repaired by ascorbate.

e) Tocopherol recycled by ascorbate, the resulting ascorbate radical can be recycled by enzyme systems – Phospholipase A$_2$ (PLA$_2$), Phospholipid hydroperoxide - glutathione peroxide (PH - GPx), Glutathione peroxidase (GPx), and fatty acyl - coenzyme A (FA - CoA) cooperate to detoxify and repair the oxidized fatty acid chain of the Phospholipid.
C) Hydrophilic :

Hydrophilic protective mechanisms are those chemical substances involved in controlling radical reactions which occur in the ionic or water components within the cell. Molecules which may have scavenging potential in ionic environments are compounds such as ascorbic acid, cysteine & reduced glutathione (95, 104, 184). Plasma constituents such as ceruloplasmin & transferrin also appear to have scavenging capability (185, 186, 187) & ceruloplasmin is an ‘acute phase’ reactant whose concentration increases during inflammation (188).

Ascorbic acid is a powerful reducing agent (electron donor) & antioxidant (189). It reacts with superoxide, peroxide & hydroxyl radicals to form, via a semidehydroascorbate intermediate, its metabolite, dehydroascorbic acid (DHA) (190).

\[
\text{AA} + 2\text{H}^+ + 2\text{O}_2 \rightarrow 2\text{H}_2\text{O}_2 + \text{DHA} \quad \text{Reaction (VI)}
\]

Under certain physiological conditions ascorbate can promote lipid peroxidation by the reduction of ferric iron (Fe$^{+3}$) to ferrous iron (Fe$^{+2}$).

\[
\text{Fe}^{+3} + \text{AA} \rightarrow \text{Fe}^{+2} + \text{DHA} \quad \text{Reaction (VII)}
\]

The ferrous iron can then take part in a Fenton reaction to generate the damaging hydroxyl species.

AA is regenerated by glutathione in a reaction catalyzed by dehydroascorbate reductase.
D) Structural

The protective mechanisms appear to be intimately associated with the structural integrity of living cells. Disruption of this structural integrity leads very quickly to rancidification, that is, the peroxidative free radical induced reactions with PUFA (186). Cholesterol, by its structure & size, intercalated into biomembranes may protect the fatty acid double bonds from peroxidative injury (191). The localization of certain reactions to peroxisomes & mitochondria & their special scavenging mechanisms also play a role in controlling radical reactions.

The purpose of these protective mechanisms is to prevent & repair the biochemical injury induced by a variety of O₂ derived reactive species.

The regulation of the oxidative environment of cells is a highly complex process in which there is a fine balance between free radical production via metabolism & the activity of a multilevel antioxidant system which serves to limit the concentration of these toxic chemical species.

**Vitamin E** *(α-tocopherol)*

**Biochemical Significance:**

a) Vitamin E is involved in the removal of free radicals & prevents their peroxidative effects on unsaturated lipids of membranes & thus helps to maintain the integrity of cell membrane. Free radicals like OH, superoxide anion O₂⁻ are formed during the action of some oxidoreductases such as the microsomal NADPH oxidase. Toxic substances are converted to free radicals. A free radical with an unpaired electron may take away hydrogen from a methylene group of polyunsaturated fatty acid & convert it into a fatty acid radical, which
binds with $O_2$ to give a fatty acid peroxy radical. This then changes into a fatty acid hydroperoxide by accepting hydrogen the methylene group of another polyunsaturated fatty acid, converting it into yet another free radical of fatty acid. Thus the free radicals peroxidate the unsaturated fatty acids of the cell membranes, mitochondrial membranes etc & cause their rupture. Vitamin E prevents this peroxidation. Chromanol ring of tocopherols donates its phenolic hydrogen to reduce the free radical & is itself oxidized to the quinone form.

b) Antioxidant action of vitamin E along with other factors prevents the peroxidative effects of $O_2^-$, $H_2O_2$ & $NO_2$ on respiratory membrane thus prevent their damage.

c) Factor 3 has been identified as a selenium compound (selenoprotein) which gives a complete protein against necrosis. Vitamin E helps to protect selenide at the active sites of membrane selenoproteins against the effects of free radicals.

d) Vitamin E prevents the peroxidative changes in membranes of mitochondria & helps in maintaining the smooth translocation of phosphate ions into mitochondria. Thus the oxidative phosphorylation is enhanced. In addition, the oxidation of sulphhydryl enzymes is also prevented by tocopherols.

e) Vitamin E prevents oxidation of vitamin A & carotenes & reduces their wastage (sparing action).

Vitamin E despite its low molar concentration in membranes effectively serves as the major lipid soluble chain breaking antioxidant. It is well accepted as the first line of defense against lipid peroxidation protecting membrane phospholipid PUFA through its free-radical quenching activity in biomembranes at an early stage of free radical attack. (192, 193).
Selenium containing glutathione peroxidase destroys lipid peroxides before they can damage cell membrane (194).

Fig. 8 shows the various modes of action attributed to vitamin E in membranes.

Trace elements (Fe, Zn, Cu) and vit. E in leprosy:

The nutritional studies are the most neglected pathological & biochemical studies in leprosy. The interaction between undernutrition & leprosy is potentially complex, poorly documented & barely understood. There are very few reports about the status of vitamin E in leprosy spectrum. The human immune system is now known to be profoundly affected by trace elements. Trace elements, although required in very minute quantities, are the micronutrients, which play pivotal role in metabolic pathways & cellular functions. However little is known about the role of trace elements in leprosy.

Gynaecomastia & testicular atrophy occur in 10-20% of patients with leprosy. In 1968, Martin F.I.R. et al (195) studied urinary total gonadotrophins, urinary oestrogens & plasma testosterone in patients with and without gynaecomastia & testicular atrophy. Patients with gynaecomastia & testicular atrophy had significantly raised levels of gonadotrophins & lowered plasma testosterone.

In 1969, Chitre & Balasubrahmanyan (196) reported that serum copper & PPD-oxidase (copper oxidase) levels were elevated in leprosy patients than those of control subjects. Patients from lepromatous leprosy with gynaecomastia showed more elevation in the levels of serum copper & PPD-Oxidase than those of lepromatous leprosy without gynaecomastia.

In 1971, Gupta S.C. et al (197) studied serum copper & PPD oxidase (purified protein derivative) in different types of leprosy. Serum copper & PPD oxidase levels were found to be increased in tuberculoid,
Fig. 8 Vitamin E as a biological – response modifier.
dimorphous & lepromatous leprosy. The degree of hypercupraemia was found to be directly proportional to the severity of the disease process. A definite & increasing correlation between serum copper & P.P.D. oxidase was observed in all types of leprosy with increase in severity of the disease except in the lepromatous type.

In 1972, Khaire & Magar (198) examined haemolytic effects of DDS in leprosy patients. Glycolytic & pentose phosphate pathway enzymes were determined in red blood cells of leprosy patients. The enzyme levels decreased significantly in all types of leprosy patients. Red blood cell content of glutathione & glutathione reductase were lowered significantly in leprosy patients. The haematinic indices, i.e. Hb, MHB & RBC levels of blood were measured during the usual DDS treatment sufficient for the lepraebacilli suppression. In the leprosy patients having less G-6-P.D. activity an initial DDS induced anemia was observed but later on the anemia corrected itself.

In 1972, Khaire & Magar (199) estimated plasma & urinary copper in leprosy patients. Plasma copper (free, bound & total) & ceruloplasmin levels were higher in non-treated leprosy patients than in the controls. There was an increase in copper excretion through urine in all patients. The urinary copper excretion was higher in lepromatous than in non-lepromatous type. Result indicated that above copper components in all types of leprosy patients decreased significantly after DDS treatment thereby showing a tendency to attain normal values.

In 1974, Oon B.B. et al (200) measured skin & serum zinc in patients with leprosy with & without trophic skin ulceration. Serum zinc concentrations were decreased in leprosy irrespective of the presence or absence of skin ulceration. Serum zinc concentrations in leprosy were also unrelated to smears positive for Mycobacterium leprae & to the
clinical type of leprosy. Skin zinc concentrations in leprosy did not differ significantly from the corresponding value in control subjects.

In 1974, Bergel M. (201) demonstrated that sulfones were biological antioxidants with vitamin activity.

In 1974, Curnette J.T. et al (202) associated the inadequate production of superoxide dismutase with chronic granulomatous disease in humans.

In 1976, Dass J. et al (203) studied the androgenic status of 12 normal healthy males & 24 lepromatous leprosy male patients, 12 with & 12 without gynaecomastia. Plasma testosterone levels were significantly diminished in the gynaecomastia group as compared to levels in either of the control group. The patients without gynaecomastia showed values not significantly lower than those of normal males. Histopathologic examination of the leprous testes showed a variable mixture of inflammatory, degenerative & fibrotic changes which were more severe & more frequent in the patients with gynaecomastia than in those without it.

In 1977, Sheskin & Zeimer (204) investigated trace elements in the skin of leprous patients at various stages of the disease by diagnostic X-ray spectrometry. In the active stage raised iron & slightly raised zinc levels were found.

In 1978, Bharadwaj V.P. et al (205) estimated serum iron and total iron binding capacity in 45 leprosy patients & 15 healthy subjects. Hypoferraemia was observed in lepromatous leprosy & was particularly marked during the reactive phase.

In 1981, Sheskin J. et al (206) quantitated the concentrations of copper, zinc & iron in the skin of healthy individuals & of patients suffering from lepra reaction at sites of the lesions before & after treatment with thalidomide. No significant changes in the copper level of leprous skin were found. The zinc levels showed significant elevations in some lesions.
with no apparent trend. The iron level in the affected areas had highly elevated values in all cases of lepra reaction. However, in contrast to the fast clinical improvement, which followed the treatment with thalidomide, the iron levels did not increase for prolonged periods.

In 1981, Sher R. et al (8) determined levels of serum zinc, copper, iron & vitamin A in healthy controls & leprosy patients. Leprosy patients were divided into two main groups as tuberculoid consisting of BT patients & lepromatous consisting of BL & LL patients. The lepromatous group was found to have significantly lower serum levels of zinc & iron & elevated levels of copper. Vitamin A levels were also significantly lower in the lepromatous groups than in the tuberculoid group. Vit.A levels were significantly lower in LL patients than those of BL patients.

In 1982, Soderberg T. et al (6) treated 90 leprosy patients with a total of 128 ulcers on the soles of their feet with local applications of adhesive zinc tape or gauze soaked in Eusol. The average healing time was shorter for the zinc tape – treated ulcers compared to the gauze – treated ulcers.

In 1983, Venkatesan K. et al (207) assessed serum copper & zinc levels in 15 healthy controls, 15 LL, 10 BT, 15 LL/BL patients during the reactional as well as the subsided states. Hypercupraemia associated with hypozincæmia was observed in active LL & LL/BL reactive patients as compared to controls & BT patients. 12 patients in BL/LI reaction with blistering & ulcerations including varying degrees of arthritis & 12 active LL patients were given oral zinc sulphate therapy for two weeks & then subjected to copper- zinc analysis at the end of the therapy. Zinc sulphate therapy was found to restore the initial low zinc level while the copper level was lowered from the initial higher level.

In 1983, Mathur N.K. et al (208) reported that oral zinc sulphate was tried in 8 cases of recurrent Erythema Nodosum Leprosum (ENL)
reaction. To control ENL, they required high dosage of clofazimine & steroids for prolonged periods. After instituting oral zinc, the dose of clofazimine could be reduced to 100 mg twice a week & steroids could be withdrawn completely. Four out of five patients, who were not tolerating dapsone earlier, started tolerating dapsone. Incidence & severity of subsequent ENL was also reduced.

Role of zinc in immunomodulation is well established (209, 210). Zinc also inhibits complement dependent immune complex reactions (211, 212) & polymorphonuclear leucocyte chemotaxis (213).

In 1984, Mathur N.K. et al (5) estimated serum zinc levels in 146 patients with different types of leprosy & 40 control subjects. Tuberculoid patients were found to have normal serum zinc levels. A gradual reduction in serum zinc levels was observed from TT to LL. Serum zinc levels were not significantly higher in-patients treated with dapsone for approximately 18 months. These findings suggest that there are correlations among bacillary load, immune status & serum zinc.

In 1984, Mathur N.K. et al (214) tried oral zinc in 15 cases of multibacillary leprosy as an immunostimulant in addition to conventional antileprosy drugs. Results were compared with those in ten similar cases treated with dapsone alone. Cases treated with zinc showed faster clinical improvement, regrowth of eyebrows, rapid fall in the bacterial index (BI) & in the bacterial index in the granuloma (BIG), early & greater influx in lymphocytes in the granuloma & neovascularization. Upgrading occurred in 6 out of 15 patients taking zinc but in only 1 out of 10 patients in the control group. Five out of six patients who showed upgrading in the treated group became lepromin positive. Only one patient (BL) showed lepromin conversion in the control group.

In 1984, Kelly J.W. et al (215) studied vitamin E & dapsone-induced hemolysis in 16 leprosy patients, each receiving 100 mg of
dapsone per day. Vitamin E (dl-alpha tocopherol acetate), 800 mg/day, was then administered for up to 3 months and dapsone therapy was continued at the same dose. Haemolysis factors were reexamined immediately prior to cessation of vitamin E therapy. No substantial change was demonstrable for levels of hemoglobin, reticulocyte count & haptoglobin at the end of vitamin E therapy, despite a significant rise in serum vitamin E levels. Erythrocyte survival measured in 4 patients before & at the end of vitamin E therapy also showed no substantial change. Erythrocyte Heinz body count, however, fell in 9 of 15 patients studied, none showed an increase in this measurement while receiving vitamin E. This indicated that in patients receiving dapsone at 100 mg/day, vitamin E therapy at 800 mg/day could not substantially ameliorate the hemolytic effect of dapsone.

In 1985, Rao K.N. et al (9) estimated serum zinc, copper, calcium & magnesium levels in 42 normal subjects & 56 leprosy patients comprising 14 BT, 12 BB, 11 BL & 19 LL by atomic absorption spectrophotometry. A significant elevation of serum copper & depletion of zinc, calcium & magnesium were noted throughout the leprosy spectrum.

In 1986, Kumar & Lakshmanam (216) studied the operational feasibility of ordinary adhesive zinc tape treatment of ulcers under field conditions on 89 uncomplicated superficial ulcers amongst 50 leprosy outpatients. Deep ulcers with or without sinus & purulent discharge were not considered fit for tape treatment. All the 13 hand ulcers & 62 plantar ulcers healed in 3.8 ± 2.1 and 9.5± 7.6 weeks of tape treatment while patients were ambulatory. The tape treatment was found to be effective, economical, acceptable & convenient to patients & operationally feasible.

In 1986, Rao & Saha (217) investigated undernutrition in lepromatous leprosy patients. They found that the diet of leprosy patients was not deficient in dietary zinc, copper, calcium & magnesium this was
comparable to that of healthy individuals. However these patients showed significantly low levels of zinc, calcium and magnesium but increased copper in comparison to that in healthy control subjects.

In 1987, Rao K.N. et al (218) determined the status of micronutrients & their transport proteins in healthy control subjects & lepromatous leprosy patients. Serum zinc, vitamin A & vitamin E were significantly reduced in lepromatous leprosy patients. However serum iron levels were found to be comparable in both the groups. Also concentrations of vitamin A transport proteins such as retinol binding protein & prealbumin in sera of the lepromatous patients were significantly decreased in comparison with the control subjects. Of the two zinc–binding proteins, i.e. serum albumin & alpha – 2 macroglobulin, only albumin was significantly reduced in the patients group. Surprisingly, though serum iron, transferrin & ferritin levels were similar in both the patients & control groups, the hemoglobin levels were significantly reduced in the lepromatous patients.

In 1988, Rao & Saha (219) measured levels of serum vitamins A & E in 55 control subjects & 67 leprosy patients comprising 9 BT, 10 BB, 15 BL, 27 LL, including 12 histoid cases. Serum levels of vitamins A & E were significantly reduced in leprosy groups as compared to normal controls.

In 1988, Saxena N. et al (220) studied serum zinc level in 15 control subjects & different types of 75 leprosy patients comprising 15 each of TT, BT, BB, BL & LL groups. Serum zinc level was observed to be significantly low in all types of leprosy except tuberculoid leprosy (TT). No significant difference was observed in serum zinc levels before & after 90 days of dapsone therapy.

In 1988, Foster R.L. et al (221) in their review on nutrition in leprosy have indicated that the dietary factors that appear to influence the
etiopathogenesis of Hansen's disease include vitamin A, vitamin B group, vitamin C, vitamin D, vitamin E, calcium & zinc.

In 1988, Cherjan E. et al ( 222 ) investigated the effect of three types of dressings on bacterial flora in ulcers. Debrisan served to be more effective than zinc tape & collagen sheet in reducing the number of bacterial pathogens.

In 1988, Anderson R. et al ( 223 ) studied the involvement of phospholipase A₂, but not protein kinase C, in the pro-oxidative interactions of clofazimine with human phagocytes. Clofazimine at concentrations of 0.1-5 micrograms / ml caused a dose – related, stimulus non-specific (N-formyl-methionyl-leucyl-phenyalanine, calcium ionophore, opsonised zymosan, arachidonic acid & phorbol myristate acetate) potentiation of superoxide generation by human neutrophils in vitro without affecting basal oxidative responses. The pro-oxidative interactions of clofazimine with neutrophils were eliminated by the phospholipase A₂ inhibitor 4- p – bromophenacyl bromide but not by the protein kinase C (PKC) inhibitor H-7. In support of these observations clofazimine promoted the release of radiolabelled arachidonic acid from neutrophil membrane phospholipids but did not influence the activity of PKC in cytosolic extracts of neutrophils or of purified PKC from rat brain. Pro-oxidative interactions of clofazimine with human phagocytes may contribute to the intraphagocytic antimycobacterial activity of this agent.

In 1989, Saha & Rao ( 224 ) reported about the comparison of status of macro & micronutrients in control subjects, patients with lepromatous leprosy, lepromatous leprosy coinfected with pulmonary tuberculosis & pulmonary tuberculosis. Levels of serum albumin, prealbumin & retinol binding protein were significantly decreased in all 3 groups of patients. Immunoglobulins were raised in all groups of patients as compared with controls. Serum transferrin levels were decreased only
in the tuberculosis patients with or without lepromatous leprosy, but not in-patients with leprosy alone. While hemoglobin levels decreased in all patients group, serum iron concentrations were reduced most in lepromatous patients concomitantly infected with pulmonary tuberculosis. Serum ferritin levels increased in pulmonary tuberculosis & lepromatous leprosy patients, but were severely reduced in lepromatous patients with associated pulmonary tuberculosis. Serum zinc levels were decreased & serum copper levels were increased in all groups of patients as compared with healthy controls.

During an inflammatory process in the body, there is a fall of serum iron levels & a release of various acute phase reactants (225). Ferritin behaves as an acute phase reactant & it is a measure of the body's iron stores (226).

Hyperferritinemia has been observed in inflammatory conditions such as Hodgkin's disease, carcinomas, osteomyelitis, tuberculosis & AIDS. (226, 227)

In 1989, Saha K. et al (228) reported that serum ferritin concentrations were significantly elevated in leprosy patients as compared to age & sex matched healthy control subjects. The induction in ferritin levels was more pronounced in-patients with lepra reactions (type 1 & 2) than those in nonreactional lepromatous patients.

In 1990, Saxena N. et al (229) quantitated serum iron & total iron binding capacity in 20 control subjects & 40 patients with different types of leprosy. Significantly low serum iron & total iron binding capacity were observed in lepromatous leprosy patients.

In 1991, Foster R. et al (230) determined blood levels of 40 elements in 14 leprosy patients & 5 control subjects in Zambia. Serum zinc levels were marginally depressed in the tuberculoid & elevated in the lepromatous patients. Serum copper levels were increased in leprosy
Serum selenium levels were lowered in the whole blood of patients as compared to controls. In 1991, George J. et al (231) studied serum zinc & copper levels & zinc/copper ratios in 86 healthy controls, 45 cases of BT, 31 cases of BL, 117 cases of LL, 16 cases with severe ENL reaction & 16 cases with severe ENL reaction receiving oral zinc therapy. A significant reduction in serum zinc levels was noticed in all types of leprosy, the maximum decrease was seen in cases with ENL reaction. Conversely, the copper levels were significantly increased from BT to LL cases with ENL reaction in a progressive manner. A very good negative correlation ($r = -0.998$) was noticed between serum zinc & copper levels from healthy controls to active LL cases with ENL reaction. After oral zinc therapy, the serum zinc levels were significantly increased in all of the 16 LL patients with ENL reaction. In contrast the copper levels were not decreased, indicating that oral zinc therapy can restore normal zinc levels in leprosy patients but is unable to reduce the increased copper levels.

In 1991, Sen R. et al (232) investigated 128 leprosy patients for the morphological type of anaemia, the underlying disturbances in iron metabolism & patterns of erythropoiesis & other cytomorphological changes in the bone marrow. In paucibacillary (PB) leprosy, the anaemia was mild to moderate degree, while in multibacillary (MB) leprosy it was of a severe degree. Iron deficiency was observed in only a few patients. Impaired iron utilization as observed in anaemia of a chronic disorder was a common finding in MB leprosy & more so in new cases. Megaloblastic erythropoiesis was also more frequent in MB leprosy as compared to PB leprosy, accounting for the severe degree of anaemia in the former type. In 17.2% of the total patients (MB-21.4%; PB - 9%) both megaloblastic erythropoiesis & features of impaired iron utilization were observed in bone marrow. Disturbances in iron metabolism & erythropoiesis were
also observed but to a lesser degree in-patients receiving specific antileprosy treatment. Irrespective of the type of the disease and duration of treatment, increasing frequency of acid – fast bacilli (AFB) positivity & granulomas was observed in the bone marrow with an increasing severity of anaemia.

In 1991, Overbeek & Tham (233) studied the effectiveness of adhesive zinc oxide tape as additional therapy to the usual application of povidone iodine (10%) on plantar ulcers in leprosy patients. They found that there was no statistically significant difference in wound healing between the two therapies indicating no statistical significance of the effectiveness of adhesive zinc oxide tape in leprosy patients.

In 1992, Momotani E, et al (234) brought about immunohistochemical identification of ferritin, lactoferrin & transferrin in leprosy lesions of human skin biopsies. Granulomatous lesions of human leprosy contained ferritin & lactoferrin but little or no transferrin. Lactoferrin was found in the neutrophils. These results suggested that the cells of the host mononuclear phagocyte system in leprosy granulomas provide an adequate nutritional environment for iron acquisition by M. leprae.

In 1992, Lapinsky S.E. et al (235) investigated the mechanisms responsible for anaemia in leprosy prior to the institution of therapy in 56 patients with active disease. Hematological indices, iron – related measurements were assessed. Anaemia was more common in the patients with lepromatous leprosy than it was in the rest of the group. The lepromatous group exhibited the disordered iron transport of the anaemia of chronic disorders in that they had a significantly lower serum iron level & a mildly raised serum ferritin concentration.

In 1992, Chattopadhya D. et al (84) reported nutritional status of children of urban leprosy patients staying at preventoria based on
biochemical parameters. Almost all children in both groups (test group & control group) were anaemic, but serum iron & ferritin levels were satisfactory. Folate & vitamin B₁₂ levels in test group were significantly lowered. In test group one third of the children had low levels of serum albumin & zinc. Fifteen percent of test group children showed vitamin A deficiency, but none were deficient in vitamin E.

In 1993, Mennen U. et al (237) estimated serum zinc levels in 86 control subjects & 64 leprosy patients by means of atomic absorption spectrophotometry. Leprosy patients were of TT (5), BT (6), BB (10), BL (13), LL (14) & burnt out leprosy (BO) (16) types. Serum zinc levels were significantly low in the leprosy group than the control group.

In 1994, Mahajan P.M. et al (238) studied the effect of oral zinc as an immunomodulator in patients with recurrent ENL over a period of one year. In the study, 40 leprosy patients with chronic ENL, requiring more than 30 – 40 mg of prednisolone/day for the control of their reactions, were given oral zinc sulphate for a period of four months and marked improvement in the frequency, duration & severity of reactions was observed after zinc therapy. Also evident was marked reduction in the steroid requirement after oral zinc therapy.

In 1995, Jain A. et al (239) investigated biometals in skin & sera of leprosy patients & their correlation to trace element contents of M. leprae & histological types of the disease. Levels of iron, zinc & copper in dermal granuloma were significantly low in comparison with those of healthy contralateral skin of leprosy patients. Among leprosy patients, patients with ENL showed maximum levels of iron zinc & copper in dermal granulomas. Concentrations of serum iron & zinc were significantly decreased while serum copper levels were significantly increased in leprosy patients as compared to normal controls. Serum levels of zinc & iron tended to decrease as the disease went from TT pole to the LL pole.
of the leprosy spectrum while serum levels of copper tended to increase. During ENL episode there was hypercupremia.

In 1996, Sethi N.C. et al (240) estimated serum zinc, copper, magnesium, total proteins, albumin – globulin fractions & superoxide dismutase (SOD) in 40 controls & 80 untreated patients with TT, BT, BL & LL types of leprosy. The investigations were repeated on day 30, 60 & 120 after starting multidrug therapy (MDT-WHO) on the patients. Serum zinc was significantly lowered in all types of leprosy on days 0 & 30. Serum copper was significantly raised in all types of leprosy. This was not significant in BT, TT cases on 60, and 120 days. There was correlation between serum zinc & copper levels & the severity & type of leprosy. The lowering of serum magnesium values was not significant. With therapy, there was a shift of all the three elements towards normal values. Serum total proteins reduction was not significant. Serum albumin was significantly lowered in all types of leprosy. Serum globulin was significantly raised in all types of leprosy. This rise in TT, BT was not significant on day 60 & 120 after starting treatment. Serum SOD was significantly reduced in all the untreated cases. It gradually increased with the clinical improvement under MDT.

Leprosy is a disease of immune aberration. Multibacillary (MB – BB, BL, LL) patients are clinically, immunologically, bacteriologically more severe than paucibacillary (PB – TT, BT) patients.

In 1999, Gupta A. et al (241) investigated inhibition of apoptosis by ionomycin & zinc in peripheral blood mononuclear cells (PBMC) of leprosy patients. PBMC from tuberculoid (BT, TT) & lepromatous leprosy (BL, LL) patients showed spontaneous apoptosis when cultured in the absence of mitogen for 24 hrs. which was inhibited by anti-tumour necrosis factor – alpha (TNF-alpha) antibodies. Apoptosis was also inhibited by ionomycin & zinc, which also increased IL – 2 & decreased
TNF – alpha production. The increase in IL – 2 production suggested a mechanism whereby dietary supplements with zinc might alter the cell mediated immunity response in leprosy patients.
V) MEMBRANE PROFILE

The cell membrane consists of a lipid-protein mosaic made up of a bimolecular layer of phospholipids and globular proteins embedded within the lipid bilayer (242). An intact plasma membrane is essential to the maintenance of normal cell permeability & volume. Loss of volume regulation, increased permeability to extracellular molecules and demonstrable plasma membrane ultrastructural defect occur in the earliest stages of irreversible cell injury. Several biochemical mechanisms have been identified as contributors to such membrane damage (Fig-9) (243).

Figure: 9 Possible Mechanisms of membrane Damage in Irreversible cell injury

- Progressive loss of phospholipids
- Increased degradation ↑
- Decreased synthesis ↓
- Cytoskeletal alterations
- Activation of proteases ↑+
- Damage to cytoskeletal membrane connections
- Physical effects of cell swelling
- Free radical – induced injury ↑
- Lipid breakdown products
One important mechanisms of membrane damage is injury induced by free radicals particularly by activated oxygen species (244, 245, 246). It is now accepted that the major site of oxidative damage to tissues is in the membranous system of the cell (247). Biological membranes are rich source of unsaturated fatty acids because of which they are easily susceptible to peroxidative attack (248, 249).

To a greater degree than most other cells, erythrocytes are exposed to radicals with the capacity to initiate the peroxidation of membrane phospholipids (250). Liability of RBC membrane to lipid peroxidation can be influenced by a large number of biochemical components. RBCs are highly sensitive to oxidative damage. They are rich in highly unsaturated lipids & are continuously exposed to a high oxygen concentration. Hemoglobin & iron in RBCs are powerful catalysts of oxidative reactions (251).

The mature mammalian RBC is devoid of a nucleus but is highly specialized and metabolically active. The three main functional constituents of the RBC are the cell membrane, the Hb complex & the intracellular metabolic apparatus which consists of enzymes, cofactors and substituents. These metabolic systems are closely inter-related to each other. This interaction is directed towards the primary function of the cell i.e. the transportation of oxygen to tissues.

The life span of a mature RBC in circulation is about 120 days. During this period the red cell undergoes a lot of beating, passing through the capillaries having literally to squeeze through them & hurtling through the blood vessels at tremendous speed & being buffeted about at the same time. Lacking a nucleus & organelles, the erythrocyte is incapable of protein synthesis & oxidative phosphorylation & thus of self-repair. But it still maintains its discoid shape, helps in the transport of cations against
ionic gradients & provides the reduction potential to protect its Hb against methaemoglobin formation & oxidative denaturation (252).

In the past few years, due to the work done on oxidative damage to cellular components by McCord & Fridovich (253) & Del Maestro (92), our insight to the biological production & scavenging of free radicals has increased tremendously. With the identification of SOD activity in 1969 by McCord & Fridovich (180), oxidative damage caused to RBCs by ‘Oxygen Free Radicals’ came into focus. Dormandy (254) demonstrated the importance of oxidation in the ageing & cellular breakdown of erythrocytes. The red cell is at risk of damage due to free oxygen radicals. Free radicals are produced in RBCs. Extracellularly generated radials can also cross over into RBC through the cell membrane via the anion channel. These may react with intracellular Hb & cause oxidative damage (255, 256).

The RBC membrane is physically a minor component of the cell & comprises a mere 2% of the total, red cell mass. The RBC membrane consists of a lipid bilayer with integral membrane protein spanning it. A filamentous meshwork of protein is also found along the cytoplasmic surface (peripheral membrane protein). These form a membrane skeleton for the red cell. There are 4,000,000 protein molecules contained within 140 square micron surface area of the red cell membrane along with 2,40,000 phospholipid & 1,90,000 cholesterol molecules (257).

Cell membrane proteins are of two types –
1) Those found on the surface (peripheral proteins or extrinsic proteins) and 2) Integral proteins (intrinsic proteins) found between the lipid bilayer.

Peripheral proteins like spectrin can be solubilized by simple mild treatment. But integral proteins like core proteins & drug & hormone receptors require powerful hydrophobic bond breaking treatment (258).
A majority of peripheral proteins in RBC membrane are organised into a filamentous network along with the cytoplasmic surface of the bilayer. Skeletons of membrane ghosts predominantly contain spectrin, ankyrin, phospholipids & some minor protein components (257). The major skeletal protein is spectrin. It exists as a dimer & accounts for 75% of the skeletal mass. It is a long coiled flexible molecule capable of establishing vertical & horizontal connections with the membrane skeleton. Spectrin dimers bind head to head to form spectrin tetramers (257).

A major portion of the lipid contents of a mature erythrocyte is found in its membrane (259). Variations reported in literature probably arise due to extraction techniques, fractionation & analysis.

Red cell phospholipids possess a distinct asymmetry with the unusual matrix of the membrane lipid bilayer. Evidence over the years shows that the conservation of acidic lipids along the inner surface of lipid is protective & their appearance on the outer surface promotes activation of the clotting cascade (260). This may lead to enhanced red cell endothelium interaction (261) and may even cause removal of red cells from circulation by the reticulo-endothelial system (262).

Murphy (263) noted that cholesterol appears to be concentrated at the periphery of the cell membrane. According to him due to its asymmetrical deployment interfacial tensions might be present in the membrane resulting in the red cell being biconcave disc rather than a spherical one. Any changes in membrane cholesterol content may affect cell shape & the surface area (264). The alteration occurring due to the action of serum LCAT indicated this. This enzyme esterifies cholesterol present in serum or which passes into the serum from membrane. The net result is reduction of membrane cholesterol & decrease in surface area of the cell.
The red cell susceptibility to oxidative injury is a function of the overall balance between the magnitude of oxidative stress & its capability to neutralize it. Any potential build up of activated oxygen due to increased production or deficient antioxidant potential results in an increased threat of oxidative damage to cellular components.

Erythrocyte deformability or the capacity of the cell to change its shape under applied stress is important for erythrocyte function. Because the small vessels of the microcirculation have a diameter less than that of the resting erythrocyte, cells must deform markedly as they circulate (265). The capacity of the erythrocytes to survive in the circulation is generally a consequence of factors that affect their mechanical property i.e. cellular deformability. Determinants of cellular deformability include extrinsic properties e.g. cell shape & the surface area/cell volume ratio and intrinsic properties e.g. internal viscosity and membrane mechanical behaviour (266,267). Among the biologic processes likely to affect the mechanical behaviour of the plasma membrane are those which involve peroxidation of endogenous membrane phospholipids (268). Alterations in bulk lipid fluidity consequent to membrane lipid peroxidation may be associated with the formation of fluorescent amino-phospholipid complexes & high molecular weight protein polymers derived from spectrin (269). An agent that might be responsible for such polymerization reactions during lipid peroxidation is malonaldehyde (MDA) (270). This reactive aldehyde damages enzymes & proteins in membrane. Pfafferott et al (268) have demonstrated that addition of malonaldehyde to erythrocytes, in the absence of lipid peroxidation, results in decreased cellular deformability. Their experiments are consistent with a view that the polymerization of membrane components may alter cellular deformability & survival through effects on membrane mechanical behaviour.
These effects of lipid peroxidation on erythrocyte membranes are schematically represented in figure 10.

**Figure : 10 Effects of Lipid Peroxidation on Erythrocyte Membranes**

Human erythrocytes have two types of ATPase activity both located within the membrane. The sensitive ATPase requires Na\(^+\) & K\(^+\). Energy from ATP is utilized by this enzyme for cation transport (252). The peroxidation of membrane phospholipids & the accumulation of MDA may cause inhibition of membrane Na\(^+\), K\(^+\) - ATPase & the accumulation of intracellular Na\(^+\). Such a series of events could result in an increase in cellular volume & a decrease in cellular deformability (268).
Under aerobic conditions autooxidizable substances such as phenyl-hydrazine may react with molecular oxygen to form a variety of reactive species, e.g. $\cdot O_2^+$, $H_2O_2$ & $OH^-$, each with the capacity to initiate the peroxidation of unsaturated fatty acids in endogenous membrane phospholipids (97). These reactions may give rise to alterations in red cell membrane function & structure, which in turn affect the survival of cells in the circulation. Inhibition of membrane ATPases & the polymerization of both lipid & protein membrane components subsequent to the peroxidation of membrane lipids has been observed (270, 271). These latter events are presumably a consequence of the formation of malonaldehyde (MDA) formed during the breakdown of peroxidised fatty acids. MDA has the capacity to cross-link the amino groups of both phospholipids & proteins through Schiff's base formation (271).

Rice–Evans & Hochstein (271) in their study have given evidence that the treatment of erythrocyte ghosts with phenylhydrazine causes striking change in the physical state of these membranes as measured with fluorescent probes. They found that the peroxidation of membrane lipids in induced by phenylhydrazine treatment leads to the increased lipid microviscosity as measured by diphenylhexatriene (DPH).

This reflects an overall increase in lipid–lipid interaction and lipid packing density & a diminution of protein–lipid interaction (272). In this connection it has been demonstrated that aggregation of spectrin polypeptide is increased during peroxidation of membrane lipids (271).

Work done by Rose & Gyorgy (273) & confirmed by other workers (274, 275) shows that in vitamin E deficiency, erythrocytes are very susceptible to hemolysis under oxidizing conditions. Tocopherols & other lipid antioxidants afford protection to the cells under these conditions. In the absence of such protection oxidation of unsaturated lipid components of the cell membrane occurs & this leads to the observed hemolysis.

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either directly as a result of structural changes or indirectly through the release of a hemolytic agent.

Evidence supporting this hypothesis has come from studies with the thiobarbituric acid test used as a measure of lipid oxidation (275,276).

Activated oxygen & its free radical derivatives are highly toxic & thus a threat to cellular integrity. Their removal is essential to maintain cell viability.

NADPH provided by HMP pathway in RBCs provides reducing potential in the cell to prevent or reverse reactions that give rise to oxidized (GSSG) glutathione and methaemoglobin.

Glutathione (GSH) is a tripeptide discovered by Hopkins in 1921. The –SH group of the cystine residue is the chemically active moiety of this molecule. It is readily oxidized by a variety of compounds to give GSSG. Neither GSH nor GSSG can enter red cells from extra cellular fluid (277, 278). The main role of the –SH group is in the membrane where it helps to preserve red cell deformability (279). GSH helps to protect –SH group & it possibly also acts as a free radical scavenger. Failure to maintain GSH leads to oxidation of protein –SH group. This is followed by protein degradation in the cell. Other unseen oxidative degradation occurring to enzymes & membrane proteins can eventually lead to red cell lysis. Depletion to GSH leads to leak cells, increased ATP utilisation resulting in diminished red cell flexibility (280).

The glutathione system & hemoglobin act as oxidant traps within the red cell but when they are exhausted, or by passed, in the course of a study production of oxidant, then membrane components are at risk. Attention has mainly focussed on peroxidative damage to cell lipids though protein structural components & enzymes are also susceptible particularly if they contain sulphydryl groups. The damage may not
necessarily be in the oxidation of free sulphydryls but in the splitting of essential disulphide bonds.

Primary oxygen radicals produced in cells & their secondary lipid radical intermediates can modify & fragment proteins. The products are often more susceptible to enzymatic hydrolysis & so radical fluxes may accelerate proteolysis inside & outside the cells. Proteins are present inside and outside cells in very high concentrations and, because many are catalytic, modification by free radicals may have an amplified effect. Proteins may thus be critical targets (281). Free radicals such as OH possibly alkoxy (RO') intermediates of lipid peroxidation can fragment & crosslink protein (282). In presence of oxygen fragmentation is much more pronounced (281).

Lynch & Fridovich (283) studied effects of superoxide on the erythrocyte membrane. They observed that superoxide moved across the stromal membrane with surprising ease & was able to attack & lyse this membrane. They also found that histidine, azide & amines, which do not react with superoxide, prevented lysis. So they concluded that the superoxide must have functioned as a precursor of more reactive species. Preloading the membranes with lipid hydroperoxides, by exposure to a photochemical flux of singlet oxygen, markedly sensitized them to the lytic attack of enzymically generated superoxide. These authors concluded that superoxide attacks the membranes.

Rosen G.M. et al (284) have demonstrated irreversible hanges in erythrocyte membrane organization following exposure to superoxide.

Singer (285) has suggested that fluidity & protein mobility appear to be common properties of biological membranes & that fluidity is decreased following formation of ordered complexes within the membrane or with membrane attached structures. Certain functions of
membranes are dependent upon the maintenance of a specific degree of protein mobility.

Superoxide radicals may be generated in erythrocytes subsequent to the autooxidation of intracellular haemoglobin or a variety of chemicals which find their way into the blood, e.g. metals such as Cu^{2+} & phenolic and hydrazo compounds. The failure of systems to adequately detoxify O_2^\cdot & H_2O_2 may result in their interaction to form hydroxyl (OH^-) radicals. Alternatively the decomposition of H_2O_2 in the presence of endogenous hemoglobin or nucleotide pyrophosphate- Fe complexes may yield OH^- in either event the formation of lipid hydroperoxides & their ultimate decomposition result in the formation of several volatile products, hydrocarbon radicals & MDA. This dialdehyde has the capacity to cross-link amino phospholipids & membrane spectrin. These polymerization reactions contribute to the alterations in membrane bulk lipid fluidity, to the decreased deformability of affected cells, & to the eventual entrapment of cells in the microcirculation. (250) (Figure 10).

Formation of polymers of lipid & protein can be mimicked by the addition of MDA to intact erythrocytes (270). The peroxidation of erythrocytes has also been found to be associated with decreased cellular deformability (286). The peroxidation of erythrocyte membrane causes an increase in bulk lipid microviscosity (271). Such an increase may be the consequence of increased lipid – lipid interaction or decreased lipid – protein interaction (272). Either of these latter events might result directly from the formation of fluorescent chromolipid polymers or from the formation of spectrin aggregates with diminished interaction with phospholipids. The decreased deformability of cells subsequent to lipid peroxidation may be a consequence of these polymerization reactions that lead to alter membrane mechanical behaviour.
Figure: 11 Effects of Lipid Peroxidation on Erythrocytes
Cellular rigidity might well be enhanced by these alterations in membrane properties. Although the mechanical properties of cells are also a function of surface to volume ratios & the viscosity of internal contents, alterations in membrane properties described above may contribute to a loss of erythrocyte deformability & to enhanced sequestration. Lipid peroxidation may be a key factor in determining the contribution of membrane fluidity to the mechanical properties of red cells (271).

Shiga et al (287) have found decreased membrane fluidity for in vivo aged erythrocytes & have correlated this with impaired functional properties, including increased oxygen affinity and decreased deformability. Results obtained by Rosen et al (284) further demonstrate that exposure of erythrocyte ghosts to hydroxyl radicals produces a decrease in the W/S ratio indicating a corresponding reduction in membrane fluidity. This is presumably due to lipid peroxidation, which effectively causes a polymerization of the lipid components leading to decreased fluidity & protein mobility.

In 1939, Ham & Castle (288) proposed that erythrostasis was an important feature of red cell destruction in diseases of man. One component responsible for this change in osmotic fragility is a decrease in cell surface are due to loss of membrane lipids (289).

Selective loss of membrane cholesterol is a physiologic event secondary to an altered state of serum lipids. Conjoint loss of phospholipid & cholesterol, however, results from intrinsic injury to the red cell membrane (289).

A specific role for cholesterol in the red cell membrane has not been defined although the membrane of the red cells is particularly rich in cholesterol as compared to other membranes (290). Cholesterol appears to play a major role in determining cell shape. Murphy has
provided data demonstrating a high concentration of cholesterol along the convexity of normal red cell (263). Its loss from the membrane causes a closely correlated loss of surface area as manifested by increased osmotic fragility (291, 264).

The loss of cholesterol & phospholipid from membrane has been attributed to membrane fragmentation by Weed & Reed (292). However, Langley & Axell (293) have shown that a loss of membrane protein does not accompany this lipid loss, thus indicating that pieces of membrane are not lost but rather that the membrane affinity for lipids is altered.

The overall effect of lipid peroxidation is to decrease membrane fluidity, increase cellular rigidity, destabilizing membrane receptors, & induce antiphospholipid antibodies. Lipid peroxidation products, and particularly the aldehyde derivatives, can inhibit protein synthesis, block macrophage action & cause changes in chemotaxis & enzymic activity (249).

The products cause polymerization of membrane phospholipids & proteins, increase membrane rigidity & decrease cellular deformability.

The gene for a 28 – kDa Mycobacterium leprae protein Ag, a major target of antibodies from patients with lepromatous leprosy, was cloned from a lambda – gt 11-M. leprae DNA expression library & sequenced. Antibodies to this protein were detected in the serum of the majority of 15 individual lepromatous patients that were tested. The predicted amino acid sequence of the 28 – kDa protein suggested that it be localized to the bacterial plasma membrane or cell wall [Cherayil & Young (294)].

In 1990, Hunter S.W. et al (295) investigated the major native proteins of the leprosy bacillus. The study addressed a major obstacle to vaccine development for leprosy, the isolation & characterization of the native protein antigens of the leprosy bacillus. Mycobacterium leprae
harvested from armadillos was subjected to a simple fractionation protocol to arrive at the three major subcellular fractions, cells walls, cytoplasmic membrane, & soluble cytoplasm. The application of extensive detergent phase separations to membrane fractions allowed removal of lipoarabinomannan & the mannosyl phosphatidylinositol, and the recognition & purification of two major membrane proteins (MMP) of molecular mass 35 kDa (MMP-I) & 22 kDa (MMP – II); recovery of these proteins was about 0.5 mg each per g of M. leprae. MMP – I is N-blocked & is perhaps a lipoprotein. End group analysis on MMP – II indicated a new protein. There major cytoplasmic proteins ( MCP) of molecular mass 14 kDa (MCP – I), 17 kDa (MCP – II), & 28 kDa (MCP – III) were also recognized. MCP – I, the most abundant protein in M. leprae, represents 1% of the bacterial mass. End group analysis of the first 30 residues & immunoblotting studies demonstrated sizeable structural homology to a protein from Mycobacterium tuberculosis but immunological distinctiveness. MCP – I, which also occurs in highly immunogenic peptidoglycan – bound form, is a primary candidate for future vaccine development. The cells walls of M. leprae are also characterized by one major extractable protein also of molecular mass 17 kDa.

However no reports are available in the literature about the quantitation of membrane proteins & membrane lipids, viz; triglycerides, phospholipids, and cholesterol in leprosy spectrum.