CHAPTER III

STUDY OF LEPROMATOUS LEPROSY
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MATERIAL

The material for this study was collected from cases of lepromatous leprosy. The patients were selected from the outpatients' clinic of the Leprosy Department of the School of Tropical Medicine, Calcutta. The patients with lesions over the forearm were selected because biopsies were taken from the lateral aspect of forearm so that histopathological pattern would be similar.

The leprosy cases were all 'active' and attended for the first time, receiving no specific treatment previously for leprosy. The patients selected for the investigation were all male Indians (Figs. 1—4), the age varying from 12 to 57 years. The complaints which brought the patients to the hospital, were variable, i.e., loss of sensation, patch, nodules, ulcers, etc. over different areas of skin surface. Duration of the disease varied from one month to seven years. History of contact with infectious leprosy cases in his own family was elicited in one patient only.

Diagnosis of leprosy was made in all the cases after a thorough and careful clinical examination and was confirmed and classified by the laboratory examinations. Clinical examination revealed the morphology of leprous lesions including sensory
disturbances and the nerve thickening. Laboratory investigations included bacteriological and immunological (lepromin) examinations. For the bacteriological examination, smears were taken from the skin lesions and the nasal mucous membrane by the 'slit-scrape' method. When the smears were positive, degrees of positivity (Dharmendra, 1960) were noted. For the lepromin test, the refined standardised lepromin (Dharmendra, 1947) was used and the reaction was read 24 hours after the intradermal injection.

Detailed summary of the findings of these cases are given in tabular form in the appendix "A".

A small piece of skin (12 mm. x 5 mm.) including subcutaneous tissues was excised from the radial side of the upper end of the forearm and usually cut into two pieces longitudinally. The pieces were fixed in different fixatives as required for particular histochemical methods adopted (discussed previously).

HISTOLOGICAL OBSERVATION

Normal structural design of the epidermis was lost. The rete pegs were flattened (Fig. 5) and thinned out in 12 out of 15 cases. Hyperkeratosis and dyskeratosis were found in 9 cases. Clear subepidermal zone noticeable in all cases except in three where there was no change in the epidermis and the subepidermal zone was infiltrated with cells at places.

In most cases massive but loose and neat granuloma was found in the dermis. Focal arrangement of granuloma was found
in 5 cases only but even in these, there was a tendency of generalised spread deep in the dermis. Large empty spaces, round or ovoid in shape (Figs. 5 & 6), were seen in the granuloma in 11 cases. The predominant cells in the granuloma were histiocytes and most of them showed vacuolation (Fig. 7) in the cytoplasm. They were of different shapes and sizes. In most of these cells the nucleus was found in the centre; in some it appeared in the periphery and in the rest nucleus was not seen in the particular plane of the section. These cells were recognised as Virchow's cells, foamy cells or lepra cells. Giant cells were found in 5 cases. These were multinucleated and the nuclei were distributed irregularly in the cytoplasm but giant cells of Langhans' type were also found in one case (Fig. 7a). The other cells found were lymphocytes and plasma cells. A few epithelioid cells were found in a few cases especially where the granuloma was not generalised.

Nerves were found in all the sections examined. In most cases transverse sections of nerve were seen but in a few cases some longitudinal sections of nerve were noted. Perineural infiltration was seen but there was no endoneural inflammatory reaction. Vascular changes were prominent in all cases of this group except one. Smaller arterioles showed proliferation of endothelial cells and some of these cells were vacuolated (Fig. 8) containing AFB. Thickening of the tunica media was found in all cases. Reduplication of elastic lamina was observed in most cases (Fig. 9). Periarteritis was found in 13 cases of this group.
Sebaceous and sweat glands were found to be of normal appearance and embedded in the granuloma.

With acid-fast stain, the mycobacteria were found in large numbers. They occurred as globi, clumps, sheaves, tufts, or singly. They were mostly intracellular and a few appeared extracellular. The bacilli were found not only in lepra cells, but in nerves and in the empty looking (in H. & E. preparation vide supra) spaces as well. Most of the empty looking spaces were almost filled up with large number of acid-fast bacilli. (Figs. 9A, 9B, and 9C).

**HISTOCHEMICAL OBSERVATIONS**

**Lipoids:**

Neutral fat, as demonstrated by Fettrot and Sudan III & IV method, was prominently seen at the sites of granulomatous collection even under the low power view of the light microscope. Histiocytes of different shapes and sizes were well stained showing the presence of neutral fat (Fig. 10). The intensity of staining reaction for fat was variable in different histiocytes (Fig. 11). The variation of the staining was proportional to the number of bacilli within the histiocytes as was evident from the serial sections stained by the Ziehl-Neelsen method of staining. Staining for neutral fat was faint and distributed in dot like manner where only a few bacilli were demonstrable (Figs. 12 & 13) but it was intense and diffuse throughout the cytoplasm, the nucleus being pushed to one side (Fig. 14) when the cells were packed up with Myco. leprae.
The distribution of neutral fat rigidly confined with the arrangement of the bacilli, and there was no extra-bacillary lipid droplet in the cytoplasm of these histiocytes (Fig. 15). Other cells of granuloma viz. plasma cells, lymphocytes etc. did not contain neutral fat demonstrable by this method. Besides, the centrally located cells in the fundus of the acini of sebaceous glands contained lipoid globules. In the preductal regions, lipoids filled up the excretory duct. Epidermal cells showed fine lipoid granules in the perinuclear zone.

Phospholipids were found only in the lepra cells and not in any other cells by the Baker’s acid-haematein method (Fig. 16). This was confirmed by pyridine extraction after which the staining reaction for phospholipids was no more discernible. No other skin structure showed the phospholipids except the cells of the sebaceous glands.

Fischler’s method for the demonstration of fatty acids was employed and a reaction was noted in the leprosy bacilli whether found in a cell or outside (Fig. 17). The fatty acids were not demonstrable in other cells. This reaction was positive.

Cholesterol and its esters could not be demonstrated in lepromatous histology, with the Schultz – Smith method and Okamoto’s method. The localisation of cholesterol in the epidermal cells, glands, was in agreement with that found in normal skin.

Carbohydrates:

The periodic acid Schiff (PAS) reaction was positive in
areas of the cells where the bacilli were present, in a remarkably consistent fashion in all the specimens examined. The reaction was unaffected by ptyalin digestion indicating that the polysaccharide was not glycogen. The cytoplasm of the cell containing no bacilli did not show positive reaction with PAS technique. *Mycobacterium leprae* were clearly seen (Fig. 18) in the cells only when present in limited number. The PAS positive material was found in masses and in irregular collections (Fig. 19) where the cells were packed up with acid-fast bacilli, its accumulation being proportional to the bacillary concentration.

With the help of acidic alcian blue stain, hyaluronic acid type of polysaccharide was not demonstrable either in the cells or in the *Mycobacterium leprae*. Following chloro-zinc iodine method, cellulose could not be detected. The reaction with iodine did not reveal the presence of starch. After pyridine extraction, the PAS reaction was still positive and the bacilli were seen clearly. It could thus be concluded that the PAS positive substance in *Mycobacterium leprae* was probably not glycolipid nor acid mucopolysaccharide, but neutral mucopolysaccharide.

**Enzymes:**

**Alkaline phosphatase:** The marked activity of the enzyme alkaline phosphatase as detected by the revised technique of Gomori was noted over granulomatous areas. Lepra cells showed positive reaction for alkaline phosphatase in the cytoplasm and the bacilli were stained. The reaction was strongly positive for endothelium of blood vessels and positive to some extent for the nuclei of
various cells and nerve structures. The basement membrane of sweat gland showed positive reaction. It was also demonstrable in the hair follicles, the greatest activity was in the papillae. No enzyme was demonstrable within the sebaceous glands.

**Acid phosphatase**: The localisation of the enzyme acid phosphatase was determined by the technique of Gomori. The reaction was quite conspicuous in the nerves and it was also positive in all the cells of granuloma.

**Dopa oxidase reaction**: Dopa oxidase reaction was revealed by the method of Becker et al. and did not represent any variation from the normal.

**Nucleic acid**: With pyronin-methyl-green stain, the stratum corneum was stained pale yellowish pink, cytoplasm of the epidermal cells, histiocytes and lymphocytes was light red. The epithelial lining of sweat ducts stained slightly darker red. Nuclei of all cells were stained green or purplish green. The collagen fibres were stained pale pink. After hydrolysis, cytoplasmic pyroninophilia was removed and the nuclei were stained red; the latter effect is known to be due to depolymerisation of DNA.

On staining with toluidine blue, it was found that a large number of mast cells were present. They were chiefly distributed in subepidermal region, around the blood vessels, in the granuloma and in the adjacent connective tissue. They were of different shapes and sizes. The cells were spindle-shaped, round
polygonal or filiform in shape. The granules of the mast cells were discrete, prominent and round and about 0.2 μ in diameter. The granules were distributed intracellularly in most of the cells but in a few they were found in the clear space outside the cells where the cell outline was lost as if the granules were discharged by the cells in the clear extracellular space. The granules of the mast cells were stained red with Fite-Faraco's method which demonstrated the presence of fuchsinophilic substance in the cells. They were PAS positive and were diastase-fast. With Feulgen reaction, the granules were unstained, but with Pyronin-methylgreen stain, they appeared to have a pyroninophilic component. On hydrolysis in 2 M HCl overnight at 4°C, the pyroninophilia was partially removed from the granules; faint pink colour could be detected and the nuclei were stained red. This indicated that the granules contained ribonucleic acid in addition to other substance, the nuclear DNA was depolymerised by acid hydrolysis. On staining with alcian blue, the granules were positive.

Discussion

The observations recorded above would seem to make it evident that the morbid changes occurring in the skin were proportional to the extent of the granuloma found in the lesions. Where the granuloma was extensive and generalised, the changes in the epidermis were marked. Hyperkeratosis and dyskeratosis were found only in those 9 cases where the superficial skin showed scale formation following local application of irritant drugs of indigenous origin.
The empty spaces in the dermis having endothelial lining and filled with acid-fast organism, partially or completely, were probably lymph spaces. This is in agreement with the accepted postulate of the lymphatic spread of the organism. Nevertheless, the site of predilection and as such the commonest site of detection of the bacilli are known to be perineural lymphatics. The localisation of the bacilli in large number in the dermal lymphatic spaces, as has been recorded here, were probably detected for the first time. It remains to be seen if the regional localisation of the organism is in any way related to the stage and severity of the disease.

Vacuolated cells or lepra cells were found to contain neutral fat. The intensity of staining reaction for neutral fat was proportional to the concentration of bacilli in the cells. The presence of neutral fat was also demonstrated by several workers (Fite, 1951; Ogata, 1953; Sugai, 1958 and Pepler et al., 1958). But Imaeda (1960) showed by electron micrographic study that lepra cells did not contain neutral fat.

It has been recorded here that these cells also contained phospholipids which were described by Ueda (1949) and Sugai (1958). Pepler et al. (1953), however, could not demonstrate phospholipids in lepra cells. It is not known whether the negative findings of Pepler et al. (1953) were due to the smaller concentration of leprosy bacilli in these cells. The present investigation showed that the staining reaction for phospholipids was discernible only in the Hansen's bacilli, and not in any extra-bacillary cytoplasmic area.
The distribution of the fatty acid found in the lepra cells also appeared to be confined to the bacilli. Herxheimer (1923), Fite (1951) and Sugai (1953) had also revealed the presence of fatty acid in lepra cells. Recently Imaeda (1960) demonstrated saturated fatty acid in the opaque droplet by using electron stain of various purified lipoid.

Cholesterol and its esters were not demonstrable in any of the cells of the granuloma, but Herxheimer (1923) and Sugai (1953) demonstrated cholesterol in lepra cells. The present study, however, supports the observation of Fite (1951), who denied the presence of cholesterol in these cells.

Dible (1950) remarked that abnormal presence of fat in cells indicates a condition of disordered metabolism or disease of the cells. Virchow thought that fatty changes might be due to infiltration or degeneration. The fat in lepra cells may originate by accumulation of pinocytic fat droplet from surrounding fatty tissue (Ogata, 1953; Azulay and de Andrade, 1952). This type of formation is usually found in fatty changes in the liver cells. In acute infection or in phosphorus and other chemical poisoning, the fatty change found only in the liver cells is due to accumulation of fat from depot fat and never found in Kupffer's cells (Dible, 1950).

It was reported by several workers (Ueda, 1949; Mitsuda, 1936; Harada, 1955; Chatterjee, 1953) that fatty degeneration or metamorphosis of cell-protein of the lepra cells was caused by the invasion of the leprosy bacillus. The findings recorded here,
however, do not support these views.

It is well known that histiocytes have got the property of phagocytosis and they engulf the leprosy bacilli in the cytoplasm. The leprosy bacilli multiplied and died in these cells. These bacilli contain lipoid material in them. When the cells are filled up with the bacilli in globi, or large masses, they stain deeply, intensely and somewhat diffusely showing the presence of lipids. The different kinds of lipoid material which were found in lepra cells were also found in leprosy bacilli histochemically. So it may be concluded that fatty materials in lepra cells were not due to infiltration of fat from fat depot or it was not due to degeneration of cell protoplasm. So it appears that fatty material in lepra cells is primarily due to the presence of Hansen's bacilli; because lipoid material is not present in histiocytic cells where bacilli were absent. Similar observation was also made by Davison et al (1960).

That the lipoids found in the lepra cells and that in the lepra bacilli were histochemically similar and that the lipoid was absent in the bacilli-free histiocytic cells, or in the extra-bacillary areas of the lepra cells would seem to combine to make it probable that accumulation of fatty substance in the lepra cells is rather an expression of bacillary concentration, than any indication of fatty infiltration or degeneration.

It may be pointed out that at the present time there is no certain evidence that fat is ever produced from protein in mammalian body or unmasking of fat is possible (Dible, 1950).
The PAS positive material was found in the body of the bacilli, irrespective of their site of lodgement, intracellular or extracellular, appeared to be a neutral mucopolysaccharide by the histochemical methods employed. Chatterjee et al (1956 c) concluded that PAS positive material was acid-mucopolysaccharide as the Myco. leprae and macrophages showed 'gamma' metachromasia with Brachet's toluidine blue. Their conclusion, however, would seem to require a revision, because, the interpretation of 'gamma' metachromasia then accepted is no longer held valid (Pearse, 1960). Glycogen could not be demonstrated in the lepromatous histology, which confirmed the work of Ogita (1955).

Marked alkaline phosphatase activity was noted in the granulomatous areas. All the cells of the granuloma showed the positive reaction for alkaline phosphatase. Most of the Myco. leprae were not found to contain this enzyme, except a few. This enzyme is known to be concerned with the carbohydrate, protein and possibly fat metabolism. Its activity is regulated in part by the endocrine system. In the skin physiology, the role of alkaline phosphatase remains for the most part an enigma, but it serves a variety of process which require the mobilisation of phosphate ions or entails dephosphorylation as steps in the metabolism and transport of a large group of phosphate containing compounds.

The presence of different microchemical substances including the enzyme, alkaline phosphatase, as has been observed there, however, does not justify any conclusion about their probable
significance in the bacterial physiology or any speculation about the quality of the host response.